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RESEARCH ARTICLE

# Male Reproductive System Alteration in Albino Mice Due to Traditional Banana Alkali Exposure: A Preliminary Study

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## ABSTRACT

Banana Alkali traditionally called as Kolakhar in Assam taken as a food additive by the natives regularly with diet. It is believed that taking Kolakhar with food regularly have many health benefits. In the present investigation, the effect of Banana alkali is examined in male reproductive system of mice. For conducting the experiment, oral administration of Banana alkali for 20days has been done and Gonado somatic index, sperm count and histology of testes has been conducted. Biochemical analysis was also conducted to find out pH, TDS and different minerals like Calcium, Magnesium *etc.* in the study sample.The treated groups did not show much effect on reproductive organ except a little difference in histological structure has been noticed in the high treated groups of mice. The intake of Banana alkali for high concentration shows slight change in body weight and weight of testis also. The result of biochemical analysis exhibit high alkalinity (pH 10.5) and the TDS value showed chloride, carbonate, hydroxyl, calcium, magnesium are the major constituent of the test sample. Physicochemical analysis showed 16444 mg/l Chloride content in the sample. From the study it can be concluded taking Banana Alkali in low dose did not have any bad effect on the male reproductive health. It is a safe indigenous feed additive of Assam and can be consumed in low dose.

**Keywords:** Banana Alkali, Sperm count, Testis weight, Gonado somatic index, Male reproduction.

## INTRODUCTION

The diverse demographic makeup of Assam, the hub of Northeast India, is clearly visible in the state's wide variety of ethnic foods and signature culinary practices. Assamese cuisine is a style of cooking that is a confluence of cooking habits of the hills that favor fermentation and drying as forms of preservation and plains provides extremely wide variety of fresh vegetables and greens [1,2]. The most known traditional food additive of Assam is Kolakhar, which is extracted from banana trees. The best alkaline preparation of banana tree is used as Kolakhar. The alkaline preparation of the test sample is done by aqueous extract of ashes prepared from banana tree, specially the *Musa bulbisiana* species (commonly called athiya kol). In several regions of Assam, this alkaline preparation serves as a traditional salt substitute and has been an integral dietary additive for generations. Characterized by a high pH range of 11 to 13, its chemical profile is dominated by potassium, sodium, carbonate, and chloride. While it contains other non-alkali metals, they are present only in negligible or insignificant quantities. [3]. There is long running study on Banana alkali with various aspect, which is mostly related with treatment base study on various diseases and it's usefulness. As this study sample is the oldest alkaline substance used by the Assamese people as food , there are various historical study of it and it's use like in washing dishes, taking it at the time of diarrhea, dysentery, in case of stomach ulcer etc. As Assamese people take Banana alkali most frequently as food so there may or may not be some effect on the bodily system of humans, which is not studied properly yet. The present study has investigated the effect of Banana alkali on male reproductive system.

## MATERIALS AND METHODS

**Experimental animal**

Nine numbers of male Albino mice weighting 30-34 g were taken for the experiment, With respect to the permission given by Animal Ethics Committee. Needed amount of Albino mice was provided which were further sacrificed. The mouse was kept in the animal house of University of Science and Technology Meghalaya in room temperature. Albino mouse has a homology between the protein coding region of mice and human, allowing the study of human health that is why it's widely used in case of experiment.

**Plant under study**

*Musa balbasiana colla* which is in Assamese known as Athia kol / Guti kol, is a wild type species of banana. It's one of the ancestors of modern cultivated bananas. The fruit of the banana plant is usually large in size and it said to be more favourable to it due to seed in it. It was found that musaceae family of the banana plants largely distributed in the north eastern part of India[4,5]. The southern state of India doesn't take the fruit of this plant as food, but our north eastern people find it edible.

**Collection of plant material**

*Musa balbasiana* is an indigenous variety of banana in Assam. The whole plant was collected from the village of Nagrijuli, district Baksa of Assam during the time period of november, 2024. Initially the plant *Musa balbisiana* was identified from taxonomic literature which was confirmed by faculties of department of Botany, USTM.

**Extraction of Kolakhar**

For Kolakhar preparation banana trunk and peels are mostly used. After the collection of the plant the trunk region is cut into small pieces, This small pieces are left in the sun for several weeks to air dried. After drying the pieces it's then subjected to open air ignition to convert it into ash. The ash were collected well, usually the ash is the filtrated to get the Kolakhar the liquid extract. First to achieve this, 50 g of burnt ashes were weighted and moistened using distilled water, then left to soak in a beaker for 24 hours to improve absorption. Then the alkaline solution is filtrated with Whitman filter paper till we get 100 ml of alkaline solution. The end liquid portion is called Kolakhar, which is the main extract for my experiment[6].

**Physicochemical analysis of Kolakhar**

**Determination of pH**: pH was measured using pH paper after diluting the alkaline (20ml/g ashes) solution with distilled water [7].

**Determination of Total Dissolved Solid, TDS**: TDS was determined by evaporating a 100 ml sample in pre weighted beaker at 100°C. The beaker weight after cooling also. [8].
TDS was calculated by using following TDS formula

$$\text{TDS (mg/l)} = \frac{\text{final weight} - \text{initial weight} \times 10^6}{\text{Vol. of sample}}$$

**Determination of chloride**: Chloride concentration of a sample can be determined using a standard method, that is reaffirmed 2003 and also APHA standard procedure. Chloride is distributed as a salt of Calcium, Sodium, Potassium in water and wastewater. The concentration of this ions varies widely according to the environmental condition. Most of the water-soluble salt in a pond environment generally remain in $Cl^-$ form and hence the amount of chloride pond water is a close indicator of the total soluble salt content within the ecosystem [2]. For experiment, a 1 ml sample was mixed with 19 ml of double distilled water, totaling 20 ml in a clear 250 ml conical flask. A light yellow colour was achieved by adding a few drops of potassium chromate indicator. The sample was then titrated with a silver nitrate solution until the colour change from yellow to brick red, marking the end point. The volume of silver nitrate titrant used is recorded for subsequent calculation. This entire procedure is repeated three times using distilled water to obtain a concordant value.

The following formula is used to calculate chloride concentration in mg/l

$$\frac{(V_s - V_b) \times 0.2 \times 35.45 \times 1000}{\text{Vol. of sample}}$$

Where, $V_s$ is the sample volume and $V_b$ is the blank volume.

**Determination of Total Hardness**

The total hardness of water is primarily caused by concentration of Calcium and Magnesium ions, although Chloride, Sulphate and Bicarbonate ions also contribute. Hard water is defined by high concentrations of these ions. The measurement procedure adheres to standards such as Indian standard IS: 3025 (part 21)- Reaffirmed 2002 as wellas APHA and EPA methodologies. pH of the test sample (9-10) was maintained by using ammonium buffer for determination of hardness. The presence of Mg and Ca is indicated if the sample turns red wine in colour after the use of Eriochrome Blake T (EBT)indicator. In the experiment, 1ml of the sample with 19ml of double distilled water was mixed with 2ml of ammonium buffer and after that 20 micro liters of EBT indicator was added. The solution is titrated against 0.02N EDTA solution till the colour changes to wine red to blue i.e. the end point of the titration. The procedure is repeated thrice(Sarma *et al.,* 2020). Following formula is used to determine the total hardness.

$$\text{total hardness(mg/l) as } CaCO_3 \text{ equivalent} = \frac{\text{Vol. of titrant} \times 0.02N \times 50 \times 1000}{\text{Vol. of sample taken.}}$$

Where, normality of EDTA solution = 0.02N and equivalent weight of $CaCO_3$ = 50

**Determination of total alkalinity**

Alkalinity is defined as capacity of water to absorb hydrogen ion without significant changes in pH. Specifically, the alkalinity of kolakhar extractant measures the water's acid buffering capacity. Total alkalinity is determined volumetrically by titrating a sample with a standard acid until it reaches a pH of 4.5 using methyle orage as an indicator. Alkalinity is caused by the presence of hydroxyl ion s, carbonate ions or a combination of both [8].

For the experiment, 2ml of Kolakhar was diluted ten times using double distilled water and placed into a 250ml conical flask. A few drops of methyl orange indicator were added to get the yellow colour of the solution. Then it titrated against 0.02N HCl solution till the colour changes to red and the burette reading is noted down. The procedure is repeated for thrice.

The formula for calculating Total alkalinity mg/l = Vol. of titrant x 50 x 1000 x 0.02/ vol. of sample
Where , 50 is a constant value as calculated . 0.02N is the Normality of HCl .

**Determination of Calcium and Magnesium**

Calcium ($Ca^{2+}$) ions are determined via direct titration with EDTA using Patton and Reeder's indicator. While this can be performed on Ca2+ alone, it is particularly useful for mixtures where the solution is adjusted to pH 12–13 to precipitate Magnesium; at the endpoint, the indicator transitions from red to blue. Conversely, Magnesium ($Mg^{2+}$) can be determined individually at pH 10 using Eriochrome Black T (EBT), but only in the absence of Calcium. Because EBT forms wine-red complexes with both ions at pH 10, EDTA will titrate them simultaneously. Consequently, $Mg^{2+}$cannot be isolated in a mixture; instead, EBT is used to calculate the total hardness ($Ca^{2++}Mg^{2+}$).

To determine the $Ca^{2+}$ concentration, a 1 mL aliquot of the Kolakhar sample was diluted with 24 mL of distilled water in a 250 mL conical flask. The solution was treated with 4 mL of 8M KOH and left to equilibrate for five minutes to ensure the precipitation of interfering ions. Following this, 35 mg of Patton and Reeder's (PR) indicator was introduced, yielding a red solution. The mixture was then titrated against a standard EDTA solution until a definitive color transition from red to blue signaled the stoichiometric endpoint. To calculate the concentrations of $Ca^{2+}$ and $Mg^{2+}$in the Kolakhar sample, the following equivalencies and titration volumes are used-

1mL of 0.01M EDTA≡0.4008 mg of $Ca^{2+}$,1 mL of 0.01M EDTA≡0.2431 mg of Mg2X mL: Volume of EDTA required for total ($Ca^{2++}Mg^{2+}$) using EBT indicator. Y mL: Volume of EDTA required for $Ca^{2+}$ only using PR indicator. Final Determinations are-Amount of Calcium (mg): 0.4008×Y, Amount of Magnesium (mg): (X−Y)×0.2431

**Testis and epidedymal weight**

After the treatment of different groups with Kolakhar, the mice were sacrificed under anesthesia and their testis and epididymis were removed. The weight of the testis with epididymis were taken using single pan balance .

**Gonado Somatic Index ( GSI)**

The Gonadosomatic Index (GSI) quantifies the relationship between testicular mass and total body weight. It is determined by dividing the gross weight of the testes by the animal's total body weight, with the resulting quotient multiplied by 100 to express the value as a percentage [8].

(GSI) = {Weight of paired testis (g)/Body weight of mice (g)} x100

**Sperm count**

Sperm count was done by following standard methods [9,10].For this, treated male mouse from the three groups were narcotized .Using scalpel testis were exposed out from the scrotum and the epidermis was removed. The epidermis was then placed in 1ml of phosphate buffer solution and minced into small pieces using mortar and pestle. A suspension of the minced material was obtained. This suspension was then incubated at $37^0$C for 30 minutes, which was considered as aliquot. Using the haemocytometer leukocyte pipette the aliquot was taken up to 0.5 mark, phosphate buffer saline was added as diluted taking up to the mark 11 of the leukocyte pipette. The content in pipette was mixed well and placed into neubaur's counting chamber. Under a light microscope sperm count was done on the large squares of the haemocytometer and calculated out as per standard method in neubaur's chamber.

**Histological evaluation of testis**

Testis histology was studied by the traditional histological technique, *i.e*. Microtome. This methods allows for the creation of the thin section of the processed tissues, which were then examined under a microscope after being suitable stained . The testis was collected from the already euthanized animal and it was fixed in 10% formaldehyde for 48 hours to avoid post mortem changes. The tissue were taken out from 10% formaldehyde and washed in running tap water for 3 hours. The tissue was then transferred to grades of alcohol , xylene and finally embedded in paraffin.

**Statistical analysis**

Values are expressed as mean ± standard error mean ( S.E.M) and analyzed using t test. P value was calculated by comparing control group with treated groups separately. P value≤ 0.05 indicated significant result.

## RESULTS

**Physicochemical parameters**

From the experiment,the pH of Banana alkali 20ml (extract/gram ashes) were recorded as 10.5±0.2 and it was very high in alkalinity. The TDS (mg/l) of the sample is recorded and the range of TDS of the sample is 202100 mg/l. The chloride (mg/l) content of the sample exhibit 16444 mg/l from the experiment. The total hardness of sample was calculated and range in 272 mg/l . The total alkalinity of the sample in mg/l is calculated in carbonate ($CO_3^{2-}$) and hydroxyl ($OH^-$) level. Which was recorded as carbonate alkalinity 112000 mg/l and hydroxyl alkalinity 210500mg/l . The graphical representation of alkalinity level according to the present of $CO_3^{2-}$ and $OH^-$ is shown in the Figure1. The Calcium and Magnesium content of the Banana alkali sample were studied in mg/l where, the calcium content was 0.3455mg/l and the magnesium content was 0.1522 mg/l, (Figure2). From the calculation , it came to know that the Banana alkali sample had high amount of calcium than magnesium (Table 1).

**Effect on body weight**

The administration of Banana alikali into mice shows significant difference in the body weight as compared to control group. Through the noted results it's observed that the body weight of low dose treated group mice is very much negligible in compared to controlled group mice. On the other hand the body weight of high dose treated mice shows slightly elevated change it's body weight. The mean and standard error of change in body weight of mice is shown in table 2.

**Effect on testis and epididymis weight**

The weight of testis with epididymis is observed after the induction of Banana alkali in the treated group (Figure 3). The different weight of testis with epididymis of mice from control group, low dose treated group which is 10ml/kg and high dose treated group which is 20ml/kg was noted down. From the observation of weight, the G1 group that was controlled group the average weight was 0.30±0.003. G2, the low dose treated group with 10ml/kg shows not much changes in it's weight with respect to the controlled group. G3, the high dose treated group with 20ml/kg shows slightly elevated change in it's weight of testis (Table 2)

**Gonado Somatic index (GSI)**

To evaluate the developmental stage of the testes, the Gonadosomatic Index (GSI) was calculated. This involved expressing the gonad weight as a percentage of the total body weight, providing a normalized value to determine sexual maturity. From the data it was observed that the difference of GSI of controlled group and low dose of treated group is very much negligible, whereas the high dose of treated group showed slightly elevated change in comparison to controlled group. (Table2)

**Effect on sperm count**

The sperm count was done by using neubur's counting chamber, from the calculation we get to know that the total sperm count of G1, controlled group was 17012±6.35, with respect to which the low dose treated group shows a little less count of sperm. The high dose treated group shows slightly more sperm count than the controlled group, which was 17050±0.66 ( Table3).

**Histological study of Testis**

In the histological study of testis, three groups i.e. controlled group, low dose Kolakh 3ar(10mg/l) and high dose (20mg/l) group were examined (Fig: 4.A,B,C). The low dose (10 mg/l) treated group does not show any effect on it except with some Intertitial area (I) without interstitial cells. The seminiferous tubule of testis is similar with the control group and does not show any kind of morphological deformities (Fig 4. B). The high dose (20mg/l) treated group showing lake of Interstitial cells in some Intertitium (I) area and Malfunctioned Seminiferous Tubules (MST) with very few Spermatogonial Cells (SG) or with distorted Seminiferous tubule lining. (Fig4.C)

## DISCUSSION

Banana alkali is the best-known food additive of Assam, as its use not only as food but also in different household works. As a traditional alkaline preparation unique to various communities in Assam, Kolakhar serves as a cost-effective and renewable alternative to commercial potassium carbonate. It is characterized by its high potassium content, which provides its distinct alkaline properties.. The study says that the Alkali prepared from seedy variety is more used as food than from seedless variety. Alkali extract is prepared from *Musabalbasiana* (Athiya Kol) exhibit high alkalinity as it has pH 10.5. The TDS value, chloride, carbonate, hydroxyl, calcium, magnesium are the major constituent of Banana alkali. From the different Physicochemical analysis, the different ranges of this components are calculated. The chloride content of the test samplein the experiment is 16444 mg/l. In case of alkalinity, the Kolakhar from Athiya Kol has high hydroxyl alkalinity than that of carbonate. As according to report studied by other, hardness of the test sample from Athiya Kol should ranges about 300mg/l and it ranges about 272mg/l in this exoeriment, from where it can said that Kolakhar from Athiya Kol has more hardness in it. Calcium and Magnesium determination, gives the result that alkali contain high calcium than magnesium. In a similar experiment high amount of pH and presence of Cl, K, Na, Zn, Pb, Cd like minerals in Kolakhar were documented[11].

As the results said, high alkalinity of Banana alikali can inhibit the growth of many pathogens in the body. The presence of high alkali elements like chloride, carbonate, potassium, calcium etc makes it more alkaline in nature. Chloride content is also high in the test sample, which makes it best alternative for potassium chloride and it can also be used as salt substitutes. As it's hardness is high with high calcium and magnesium, it can help in protecting gastro intestinal tract[12]may be that is why there is saying that drinking a bit of Kolakhar can help during gastric. Chloride content in the diet plays an important role in high blood pressure. There were also applications that said as it contain high calcium, chloride , magnesium and because of it's high alkalinity, ashes of Kolakhar used as fertilizer in acidic soil. In a similar experiment Kolakhar was used to nutralise soil acidity to improve the growth of five indegenious rice variety and got a very effective result [13]. All the determination of presence of various component, Kolakhar prepared from *Musa balbasiana* (Athiya Kol) has it's best use in different fields.

The oral administration of Banana alkali for 20days did not show much effect on reproductive organ of male mice. The sperm count and the histological study has no negative effect on it. Whereas the intake of the test sample for high concentration shows slight change in body weight and weight of testis. Intake of low dose of the test sample has been observed as same as controlled group. As per high alkalinity and presence of various component it makes the test sample enable to exhibit antioxidant activity because of which it shows very little change in the respective aspects that have studied. High dose, 20mg/l shows effect on both body weight and testis weight but not in sperm count and histological study, from which it can be said that the intake of Banana alkali in high concentration may shows some significant change in it. As there is a very scanty information on the effect of Banana alkali in reproductive system,

therefore similar experiment has been found on banana peel extractonly where it was used on male reproductive system which showing protective effect [14]. As for this report, the effect were very less and due to which we can say that the people of Assam are having Banana alkali as food without getting any harmful effect and having alkaline diet frequently can benefit our overall health by preventing us from many diseases [15].

## CONCLUSION

The results of present investigation indicates that the intake of low dose of test sample not show any changes with respect to controlled group whereas intake of High dose of test sample i.e. 20mg/l shows slight elevated change in body weight and testis weight of male mice but no effect on the sperm count and histological study. From all the observation, hypothetically it can be said that intake of Banana alkali in high concentration can leads to change in body weight and testis weight. Further and better investigation may shows some effect on the sperm count and in it's histological study. As Banana alkali is the mostly used indigenous food of Assamese people, there is very much limited research on it's effect on human body . That is why this investigation is carried out. More investigation can gives better results in the future.

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**Ethical approval:** The study design including treatment of experimental animals was approved by the Institutional Animal Ethics Committee(17/02/2024) prior to the commencement of the study. Approval No. IAHF/USTM/2024/P-5.

**Competing interest:** There are no competing interest.

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**Table1: Physicochemical Analysis of Kolakhar Obtained from *musa balbasiana***

| Sample | pH | Total hardness (mg/l) | TDS (mg/l) | Chloride (mg/l) |
|---|---|---|---|---|
| Kolakhar from *Musabalbasiana* | 10.5±0.2 | 272±7.02 | 202100±10.2 | 16444±2.4 |

(n=3,Mean±SEM)

**Table 2 : Shows the Different Body Weight, Testes Weight with Epididymis and Gonado Somatic Index Among the Different Experimental Groups.**

| Sl. NO. | Experimental condition | Change in the body weight(g) | Average Weight of testis with epididymis(g) (mean±SEM) | GSI(%) |
|---|---|---|---|---|
| G1 | Controlled group | 29.98±0.41* | 0.30±0.003* | 0.96±0.003 |
| G2 | Low dose treated group(10ml/kg) | 28.56±0.28* | 0.29±0.001* | 0.94±0.003 |
| G3 | High dose treated group(20ml/kg) | 33.75±0.63* | 0.33±0.003* | 0.99±0.006 |

(n=3,Mean±SEM, *=P<0.05, P value was calculated by comparing controlled group with the treated group)

**Table 3: Representation of Sperm Count among Different Experimental Groups**

| SL. NO. | Experimental condition | Sperm count |
|---|---|---|
| G1 | Controlled group | 17012±6.35* |
| G2 | Low dose treated group(10ml/kg) | 16989±0.66* |
| G3 | High dose treated group(20ml/kg) | 17050±0.66* |

(n=3,Mean±SEM, *=P<0.05, , P value was calculated by comparing controlled group with the treated group)

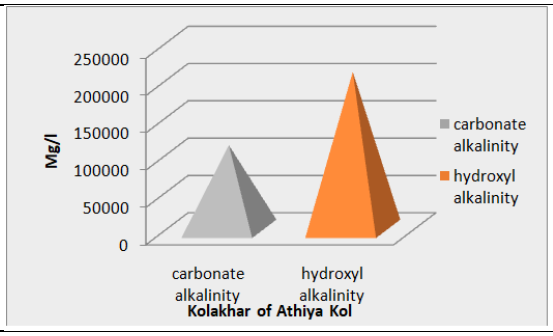



**Fig 1: Total alkalinity of Kolakhar of *Musa balbasiana***

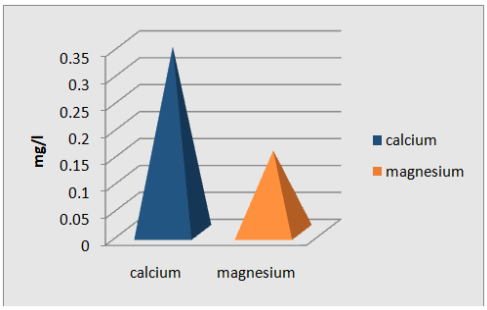



**Fig 2: Calcium and Magnesium content of Kolakhar prepared from *Musa balbasiana***



**Fig3: Male reproductive system including testis, epididymis, vas deference in 3 experimental group (Control, low dose group and high dose group respectively)**

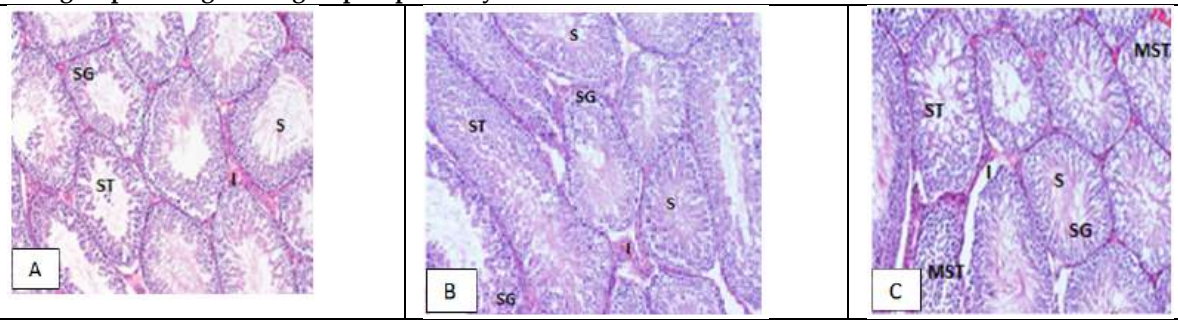



**Fig4: Histological observation showing normal Seminiferous tubules in 3 experimental groups in 40X magnification (A. Control, B. low dose group and C. high dose group respectively). The three group does not show any differences except low interstitial cells in Interstitium (I) in both Low dose and High dose groups. Seminiferous tubules (ST) and Sperm (S) inside the ST are showing no difference in all 3 groups, but a few malfunctioned Seminiferous Tubules (MST) with a very few Spermatogonium (SG) or with distorted Seminiferous tubule linings are seen in high dose group**

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RESEARCH ARTICLE

# Characterization and Fabrication of ZnO Nanocomposite to be used as Photocatalysts

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## ABSTRACT

The development of effective and sustainable photocatalysts for water purification is necessary due to the severe environmental contamination caused by the growing emission of organic pollutants from industrial and agricultural operations. The photocatalytic degradation performance of ZnO nanocomposites under light irradiation was assessed in this study. The production of wurtzite-structured ZnO with great UV absorbance and high crystallinity was verified by structural and optical investigations.The presence of surface hydroxyl groups that boost photocatalytic activity was confirmed by the FTIR spectra, which showed distinctive O–H and Zn–O vibrations. The presence of oxygen vacancies and effective charge carrier separation was demonstrated by photoluminescence (PL) analysis, which revealed emission peaks corresponding to near-band-edge and defect-related transitions. Reactive oxygen species (•OH and $\bullet O_2^-$), which actively break down organic dye molecules, are produced as a result of these characteristics. The produced ZnO nanocomposite was a promising material for wastewater treatment applications because of its exceptional photocatalytic effectiveness, stability, and recyclability. The study emphasizes ZnO's promise as an economical, sustainable, and reusable photocatalyst for pollution control and environmental restoration.

**Keywords:** Photocatalyst, ZnO nanocomposite, Photocatalytic degradation, Reactive oxygen species, Water purification

## INTRODUCTION

The exceptional physicochemical stability, strong redox potential, and tunable optical features of nanostructured metal oxide semiconductors have made them intriguing materials for energy conversion and environmental purification in recent decades[1]. Because of its large band gap (~3.3 eV), high exciton binding energy, and potent UV absorption capacity, zinc oxide (ZnO) has garnered a lot of interest as an effective photocatalyst[2]. ZnO can degrade organic dyes, insecticides, and other dangerous pollutants in wastewater because of its exceptional photoactivity when exposed to ultraviolet light[3].However, there are a number of obstacles to the practical use of pure ZnO in photocatalytic applications, such as photocorrosion under extended irradiation, limited visible-light responsiveness, and the quick recombination of photogenerated electron–hole pairs[4]. ZnO is frequently mixed with other semiconductors, metals, or carbon-based materials to create ZnO-based nanocomposites in order to overcome these disadvantages[4]. These heterostructures increase the efficiency of charge separation, increase the photocatalyst's overall stability and reusability, and extend light absorption into the visible spectrum[5]. Due to enhanced charge transfer kinetics and synergistic interfacial interactions, ZnO nanocomposites combined with oxides like $TiO_2$, $CeO_2$, and $FeO_3$ or non-metals like graphene oxide and g-$C_3N_4$ have shown better photocatalytic effectiveness. Incorporating secondary components enhances the surface area and active sites available for redox reactions in addition to modulating the band gap alignment. ZnO produces electron–hole pairs during photocatalysis when it absorbs photons with energy equivalent to or greater than its band gap[6]. The electrons convert oxygen molecules to superoxide radicals ($\bullet O_2^-$), while the holes oxidize water molecules to hydroxyl radicals ($\bullet OH$). Microbial inactivation and the oxidative breakdown of organic contaminants are mainly caused by these reactive oxygen species (ROS)[7]. All things considered, ZnO-based nanocomposites are a new class of effective, affordable, and safe photocatalysts[8]. They are perfect for sustainable wastewater treatment, air purification, and antibacterial applications under solar or visible light irradiation due to their improved optical responsiveness, chemical stability, and reusability[9].

## MATERIALS AND METHOD

**Chemical used**

Zinc Chloride (ZnCl, purity 98 %, Fisher Scientific), and Sodium Hydroxide (NaOH, 0.5 N solution, purity 98 %, Sigma-Aldrich) were used as key chemical reagents. Ethanol ($C_2H_5OH$) was utilized as a solvent, while Methyl Orange dye (purity 98 %, Fisher Scientific) was employed as the dye component for the experimental procedure. All chemicals were used without further purification to ensure consistency in the synthesis and characterization of the materials.

**Hydrothermal Synthesis of ZnO nanocomposite**

The hydrothermal approach, which uses the pressure created when water is heated in an autoclave, has been successfully employed to create heterostructures with desired crystallinity and decreased aggregation. The preferred technique for creating nanomaterials is a hydrothermal autoclave with a Teflon liner since it can tolerate high temperatures, stop corrosion, and shield the vessel walls from harmful chemical reactions. (Fig. 1) illustrates the process of mixing 100 milliliters of pure water with equimolar 1 M Zinc chloride. The solutions were continuously stirred magnetically for two hours at room temperature. The pH was raised to 7 by adding drops of a NaOH (0.5 N) solution. After that, 50 cc of this mixture was put into a Teflon autoclave, sealed, and heated to 180°C for 24 hours inside the closed system. The mixture was allowed to cool before being centrifuged, and the residue was washed four or five times with ethanol and double-distilled water. After the autoclave cooled, it was dried in a 65°C oven. After that, the ZnO powder samples were collected and kept in a desiccator. Schematic representation of Synthesis of ZnOHydrothermal Method (Fig 1).

**Methyl orange (MO) dye photocatalytic degradation and kinetics**

Methyl orange was used as a pollutant to study the photocatalytic capabilities of the ZnO heterostructure. The photocatalytic activity of the photocatalyst was measured using the rate of MO degradation. A stock solution of the dye (1.0 ×103 M) was made by dissolving 0.0373 g in 100 ml of doubly distilled water. The dye solution's pH was measured using a digital pH meter (Systronics Model 335), and the solution's pH was adjusted to the required level by adding a standard solution of 0.1 N sulphuric acid and 0.1 N sodium hydroxide. Every 15 minutes, a 3 ml aliquot of the reaction mixture containing 0.10 g of photocatalyst was taken and exposed to 200 W tungsten light. The absorbance (A) was measured at a maximum wavelength of 650 nm. A water filter was used to block thermal radiation. We tested the solution's absorbance at various intervals using a spectrophotometer (Systronics Model, 106).

## RESULTS AND DISCUSSION

**XRD Analysis**

The structural purity of nanomaterials is investigated using X-ray diffraction techniques [10]. The ZnO NPs' XRD pattern is displayed in (Fig 2, a). The XRD patterns were acquired with a rotation range of 20° to 80° and a scanning speed of 2°/min. The crystallographic planes of ZnO (100), (002), (101), (102), (110), (103), (112), and (202) are represented by the measured peak positions at 2θ = 31.76°, 34.4°, 36.38°, 47.76°, 56.8°,62.3°, 67.868°, and 76.64°, respectively. Which are listed in JCPDS #00–005-0664, and the Debey-Scherrer equation was used to determine the crystallite size (D) of ZnO;

$D = \frac{k\lambda}{\beta\cos\theta}$ …….[11]

∴ Where the wavelength of the X-ray source is 1.54 Å , the Debey-Scherrer constant is 0.94, and $\theta$ is the Braggs angle in degrees and $\beta$ is the full width at half maximum (FWHM) in radians [12]. The Debey-Scherrer equation yielded the following crystalline diameters for these corresponding planes: 14.84nm, 16.84 nm, 13.16 nm, 11.54 nm, 10.19nm, 9.27 nm, 6.19 nm, and 14.00 nm. ZnO was found to have an average crystalline size of 12.64 nm (Table 1).

**FT-IR analysis**

The chemical structures of the ZnO nanocomposites were determined based on the FT-IR spectra, as given in (Fig 2, b). The O–H stretching vibrations of hydroxyl groups and adsorbed water molecules on the nanocomposite's surface are responsible for the wide and strong absorption band seen in the 3400–3500 $cm^{-1}$ range. The O–H bending vibrations of molecular water physically adsorbed on the ZnO surface are responsible for the weak band about 1630–1650 $cm^{-1}$.In particular, the Zn–O stretching vibration, which is typical of the wurtzite crystal structure of ZnO, is responsible for the substantial absorption band seen below 600 $cm^{-1}$, usually in the region of 450–500 $cm^{-1}$. This demonstrates that the ZnO phase has successfully formed inside the nanocomposite.

**PL Analysis**

(Fig 2, c) The ZnO nanocomposite's photoluminescence (PL) spectrum shows a weaker peak close to 558 nm and significant emission peaks at about 425 and 375 nm. Near-band-edge (NBE) emission, which is caused by the recombination of free excitons, is shown by the strong emission at 425 nm, whereas intrinsic band-to-band transitions of ZnO are linked to the shoulder at 375 nm. Deep-level or defect-related transitions, primarily caused by oxygen vacancies and zinc interstitials, are the source of the wide visible emission centered about 558 nm. Fig. 2.(a) Powder X-ray diffraction pattern of ZnO, (b) FT-IR spectroscopy analysis of ZnO (c), PL spectra for ZnO and (d) EDS of ZnO.

**Scanning electron microscope (SEM)**

The morphological properties of the synthesized material are shown in the SEM images of the produced ZnO nanoparticles, which are shown in Fig. 3 (a-b). As the synthesis goes on, the particles change from their initial agglomerations into distinct forms. Perhaps as a result of the crystallization process during manufacture, this morphological change indicates the formation of a more structured and ordered nanomaterial. As illustrated in Fig. 3 (c-d), the presence of the essential components of the ZnO structure, Zinc (Zn), and oxygen (O), is confirmed by the EDAX data. Fig. 3.(a-c) SEM images of ZnO, d- EDAX mapping of ZnO

**Photocatalytic Dye Degradation**

The degradation behaviour of the synthesized ZnO was evaluated under sunlight using Methyl Orange (MO) dye as a model pollutant. At room temperature and with a pH of 7, the photocatalytic activity tests were conducted. A pseudo-first-order kinetic model was used to analyse the interaction between adsorption and photodegradation in the solid-liquid system. To ascertain the rate constant and comprehend the kinetics of the photodegradation process, the experimental data were fitted to the corresponding rate equation, as shown in Fig. 5 (a–d).

$-In\left(\frac{Ct}{C0}\right) = k(app) \cdot t$ ……[13]

∴Where $C_t$is Methylene Blue absorbance at time t; $C_0$ is Methylene Blue absorbance following dark adsorption. t-Kinetic rate constant, k (app), and radiation time. The reactive intermediates cause degradation and render the dye harmless.

**Photocatalytic Mechanism**

Photocatalytic activity occurs in the presence of a catalyst and a light source. Two main mechanisms underlie this activity: the first is the production of electrons and holes as a result of light interacting with the ZnO photocatalyst, and the second is the catalyst's surface function [14]. When the energy of the received light is equal to or less than the band gap of the ZnO thin films, excited electrons from the valence band migrate to the conduction band, producing photo-generated electrons ($e^-$) [15]. Electron-hole pairs (EHPs), which are essential for photocatalytic activity, are created when holes ($h^+$) are generated in the valence band at the same time. [16] These EHPs are always moving; some of them make it to the Ce-doped bismuth oxide's surface, while others stay inside the lattice of the material. Hydroxide ions ($OH^-$) are transformed into oxidizing hydroxyl radicals (•OH) at the thin film surface when photo generated holes interact with water molecules in the methylene blue (MB) solution [17]. When exposed to sunshine, these radicals are what cause the dye to deteriorate. Superoxide anions ($•O_2^-$) are created when oxygen molecules ($O_2$) are oxidized by photo generated electrons on the film surface [18]. The destruction of MB is facilitated by both superoxide radicals ($•O_2^-$) and hydroxyl radicals (•OH), indicating the material's photocatalytic activity [19](Fig.4).

The following are the main responses:

$ZnO + H_2O \rightarrow ZnO + •OH + OH^-$

$2HO_2• \rightarrow H_2O_2 + O_2$

$ZnO + H_2O_2 \rightarrow ZnO + 2\ •OH$

$H_2O_2 \rightarrow 2\ •OH$ (in presence of ZnO)

•OH + MO (Methyl Orange) → Degradation products (with ZnO as catalyst)

**Fig. 5.** Photodegradation ability of synthesized ZnO NPs, (a) Photodegradation studies up to 60 min, (b) Degradation kinetics of MO dye,(c) Linear fit using pseudo first order kinetics and (d) R-square variation with respect to concentration of ZnO NPs.

**Impact on scavengers of radicals**

Degradation experiments were carried out using specific radical scavengers in order to identify the active species involved in photocatalytic degradation. As illustrated in (Fig. 6.a), ethylene diaminetetraacetic acid (EDTA) was used to scavenge photogenerated holes ($h^+$) and hydroxyl radicals (•OH). Potassium iodide (KI) was used to specifically target hydroxyl radicals, and tert-butanol (t-BuOH) was employed as an additional hydroxyl radical scavenger. When these scavengers were present, the photocatalytic degradation efficiencies for KI, EDTA, and t-BuOH were 20.06%, 67.56%, and 40.02%, respectively. When no scavengers were used (blank), the degradation efficiency was 88.79%, demonstrating the system's significant potential for deterioration under these conditions. These findings demonstrate that oxygen ($O_2$) and hydroxyl radicals (•OH) are both required for the degradation of dye molecules during the photocatalytic process. Notable contributions when the photocatalyst is exposed to light, it generates reactive species.

**Heavy metal removal by ZnO**

The concentrations of various heavy metals in industrial effluent were carefully tracked both before and after exposure to sunlight in the presence of ZnO (Fig. 6, b). Under these circumstances, a 0.50 g/L concentration of ZnO was added to the effluent. The wastewater's heavy metal concentrations significantly decreased after treatment. In particular, following therapy, the copper (Cu) concentration decreased to undetectable levels. This outcome highlights the ZnO catalyst's potential for usage in heavy metal clean up in industrial wastewater by showing how well it removed these heavy metals when exposed to sunlight.

**TOC and COD analysis**

The elevated TOC value is associated with the complex dye N-oxidation process, which produces nitrogen-containing molecules. A higher COD value indicates a slower rate of mineralization. The dye solution used in this study (Fig. 6, c and d) was exposed to radiation for 50 minutes prior to TOC and COD measurements in order to encourage degradation. The data showed a similar pattern for both COD and TOC. The decrease in TOC concentration was more pronounced than the COD decrease, even though the COD value remained relatively low. This suggests that only a small portion of the Methylene Blue dye underwent complete mineralization, with the majority being partially broken down into intermediate by products.

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$$\text{COD Removal (\%)} = \frac{\text{COD}_0 - \text{COD}_t}{\text{COD}_0} \times 100 \quad \ldots[20]$$

$$\text{TOC Removal (\%)} = \frac{\text{TOC}_0 - \text{TOC}_t}{\text{TOC}_0} \times 100 \quad \ldots.[21]$$

∴Where $C_t$ denotes the MB concentration (mg $L^{-1}$) prior to and following the photocatalysis at various intervals, and $COD_0$ and $TOC_0$ denote the starting MB concentration (mg $L^{-1}$).

**Fig. 6.**(a) Radical Scavenger of synthesized ZnO NPs, (b) Heavy metal removal by synthesized ZnO NPs, (c) COD analysis and (d) TOC analysis

**Reusability**

The ZnOphotodegradation performance is greatly improved by connecting. As indicated in (Fig 7, a), the percentage of MO photodegradation for the ZnO samples was 94.2%, 88.4%, 84.3%, 82.5%, and 80.2% following 50 minutes of exposure to direct sunshine. These findings show that ZnO can be efficiently preserved over the course of their lives, reducing environmental effects and optimizing nanoparticle benefits. This shows that they have the potential to be a dependable photocatalyst for eliminating pollutants from industrial effluent, especially dyes. Fig. 7.(a) Reusability of synthesized of ZnO NPs after 5$^{th}$ cycles

## CONCLUSION

The produced ZnO nanocomposite's great potential for the destruction of organic contaminants under light irradiation was shown by the photocatalytic investigation. Its large surface area, potent light absorption, and effective charge carrier separation are responsible for the increased photocatalytic activity. In order to produce reactive oxygen species (ROS) such •OH and $\bullet O_2^-$ radicals, which actively took part in the degradation process, surface hydroxyl groups and oxygen vacancies were essential. While PL investigations showed moderate recombination rates, guaranteeing extended electron–hole lifetimes advantageous for photocatalysis, optical and structural examinations verified the creation of a pure ZnO phase with a wurtzite structure. The ZnO nanocomposite is a promising nanocatalyst for wastewater treatment and organic dye degradation because of its consistent performance, reusability, and environmental friendliness. The work demonstrates that ZnO-based photocatalysts are sustainable, economical, and effective materials with enormous potential for use in clean energy and environmental remediation applications in the future.

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### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest associated with this publication.

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**Table 1: XRD Analysis**

| Peak position 2θ (°) | FWHM $B_{size}$ (°) | Dp (nm) | Dp Average (nm) |
|---|---|---|---|
| 31.8357 | 0.582 | **14.84** | **12.64** |
| 34.3843 | 0.5162 | **16.84** | |
| 36.2318 | 0.6641 | **13.16** | |
| 47.6295 | 0.7867 | **11.54** | |
| 56.614 | 0.9254 | **10.19** | |

| | | | |
|---|---|---|---|
| 62.9672 | 1.05 | **9.27** | |
| 67.868 | 1.6162 | **6.19** | |
| 76.8645 | 0.7573 | **14.00** | |

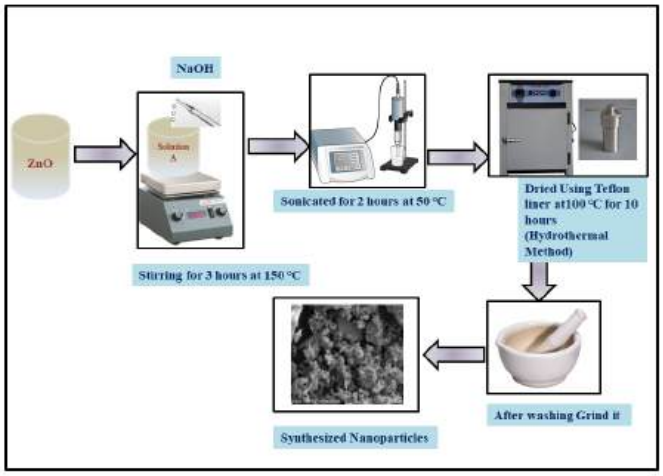



**Fig 1: Schematic representation of Synthesis of ZnO Hydrothermal Method**

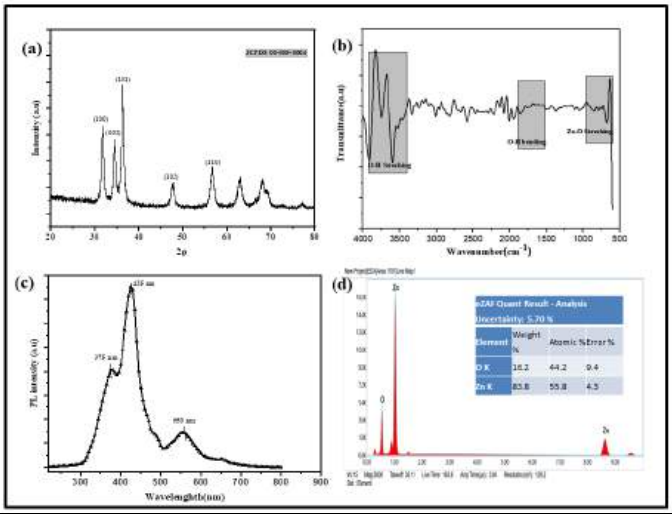



**Fig. 2.(a) Powder X-ray diffraction pattern of ZnO, (b) FT-IR spectroscopy analysis of ZnO (c), PL spectra for ZnO and (d) EDS of ZnO.**

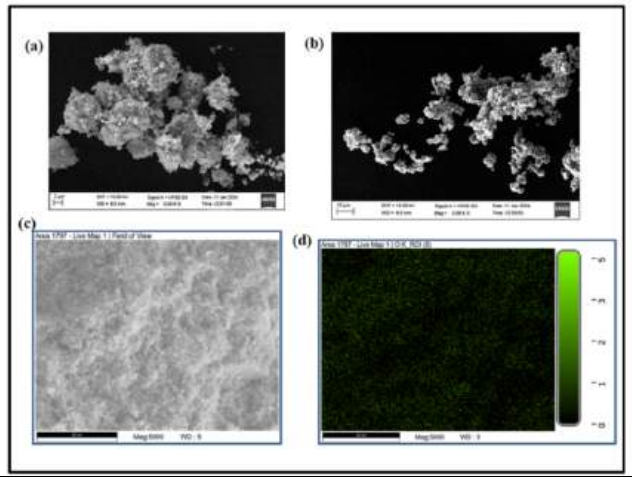



**Fig. 3.(a-c) SEM images of ZnO, d- EDAX mapping of ZnO**

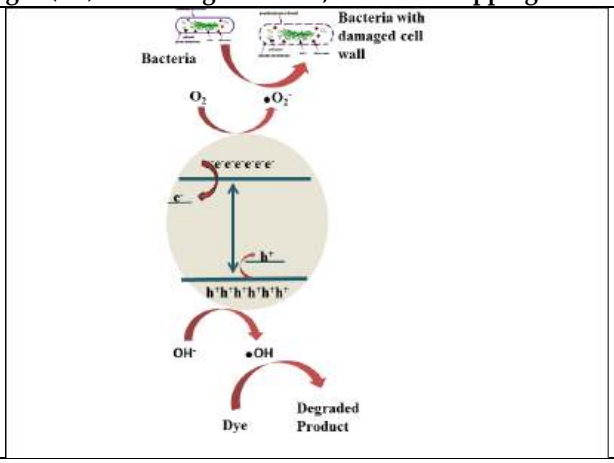



**Fig 4: Photocatalytic Mechanism. These are the main responses**

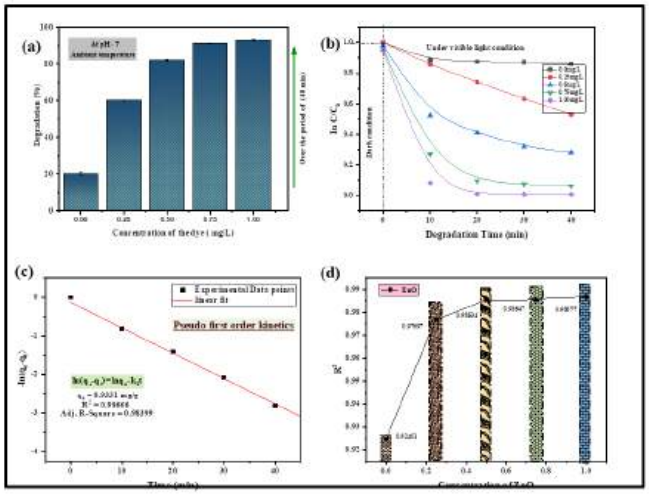



**Fig. 5: Photodegradation ability of synthesized ZnO NPs, (a) Photodegradation studies up to 60 min, (b) Degradation kinetics of MO dye,(c) Linear fit using pseudo first order kinetics and (d) R-square variation with respect to concentration of ZnO NPs.**

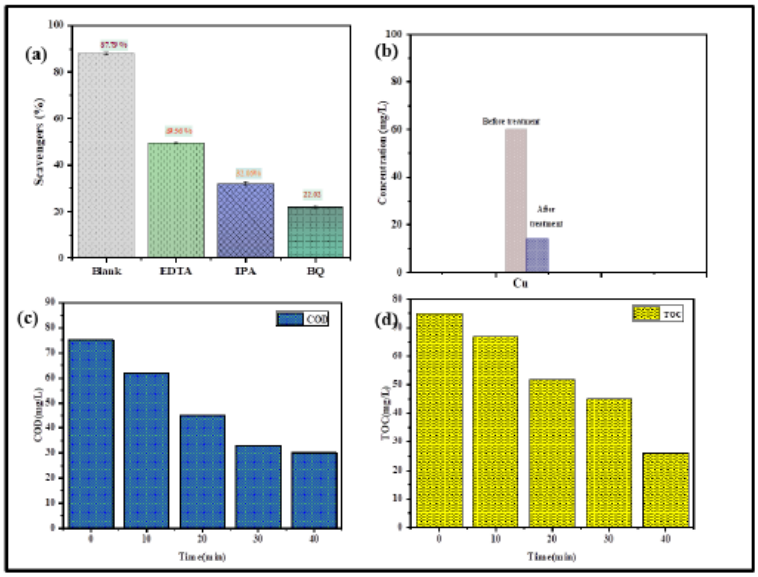



**Fig. 6.(a) Radical Scavenger of synthesized ZnO NPs, (b) Heavy metal removal by synthesized ZnO NPs, (c) COD analysis and (d) TOC analysis**

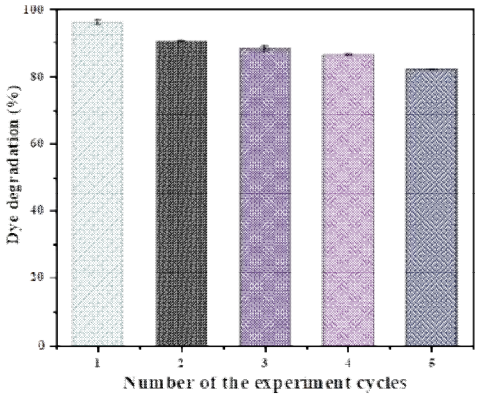



**Fig. 7.(a) Reusability of synthesized of ZnO NPs after 5th cycles**

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REVIEW ARTICLE

# A Comprehensive Review of Small Molecules as HDAC Inhibitors: Anticancer Therapy

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## ABSTRACT

Genetic and epigenetic changes that interfere with normal cell regulation and promote uncontrolled growth are the cause of cancer. Histone deacetylases (HDACs) are crucial epigenetic enzymes that contribute to the development of cancer by silencing tumor-suppressor genes. As such, they constitute important targets for treatment. The mechanisms, selectivity, and anticancer potential of small-molecule HDAC inhibitors (HDACis) are summarized in this article. Strong zinc-dependent HDAC blocking is demonstrated by broad-spectrum hydroxamate-based inhibitors like vorinostat and panobinostat, whilst benzamide derivatives like entinostat and mocetinostat provide higher Class I selectivity and enhanced safety. Romidepsin and largazole are examples of substances obtained from natural products that increase structural diversity. Strong cytotoxicity and strong binding affinity are consistently shown by docking and $IC_2^1$ measurements. Selective HDAC6 inhibitors (ricolinostat, citarinostat) and multitarget drugs (CUDC-101, CUDC-907) that improve treatment efficacy are examples of recent developments. All things considered, HDAC inhibitors continue to be attractive epigenetic treatments with a great deal of promise for more effective, focused cancer treatment.

**Keywords :** Histone Deacetylase Inhibitors, Antineoplastic Agents, Epigenetic Regulation, Molecular Docking Simulation, Small Molecule Therapeutics, Drug Design, Signal Transduction Modulation

## INTRODUCTION

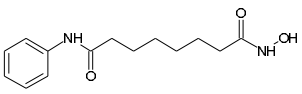
Cancer is a heterogeneous disease that develops through the combined effects of genetic as well as epigenetic mutations thereby normal cellular pathways gets disrupted and promotes certain characteristics such as uncontrolled cell division, apoptosis resistance, metastasis and vasculogenesis [1]. While genetic mutations have been considered the key drivers of tumorigenesis, progressing evidence shows that the aberrant epigenetic modifications also plays a significant role in the inception and advancement of many types of cancers [2]. Methylation of DNA ,modification of histone proteins and regulations mediated by non-coding RNA are the epigenetic mechanisms that helps in controlling gene expressions without changing the DNA sequence and thereby helps to shape the structure of chromatin [3]. The reversible processes involved in the acetylation and deacetylation of histone proteins are essential for the regulation of genes involved in the cell cycle, differentiation and apoptosis.

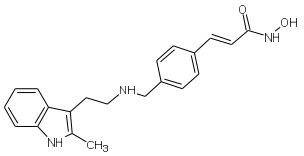
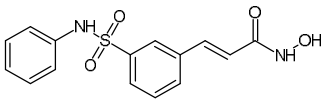
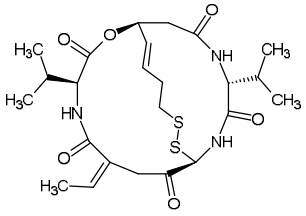
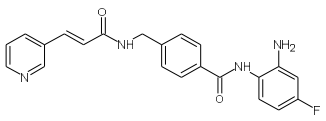
Histone deacetylases (HDACs) are types of enzymes that helps to remove acetyl groups from lysine residues present on the histone and non-histone proteins. Due to deacetylation, chromatin condensation takes place and thereby gene gets silenced. In case of cancers abnormal HDAC expression or activity has been observed leading to tumour progression by silencing tumour-suppressor genes, enhancing the oncogenic pathways [4]. In cancer biology HDACs show a crucial role, have emerged as a promising therapeutic target. HDAC inhibitors (HDACs) can induce cell-cycle arrest, apoptosis, differentiation, and thereby helping to restore cellular balance. Several HDACs, including vorinostat and romidepsin, have already been approved for clinical use, and many more are undergoing evaluation. Although HDAC inhibitors are highly promising, challenges such as targeting the right isoforms, managing toxicity, and overcoming resistance still remain. These issues show that we need to keep improving and advancing HDAC-focused drug development [5].

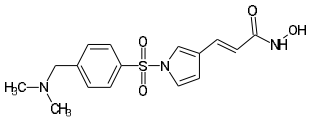
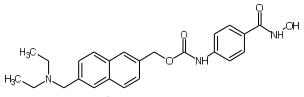
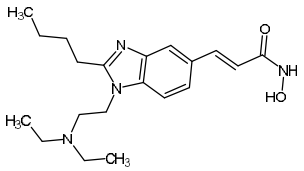
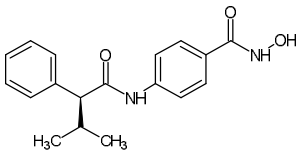
## METHODOLOGY

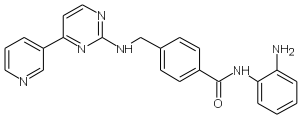
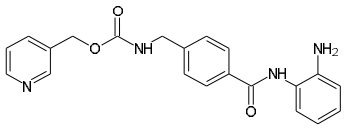
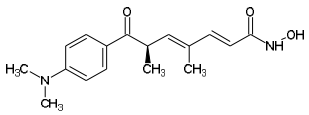
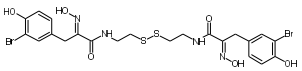
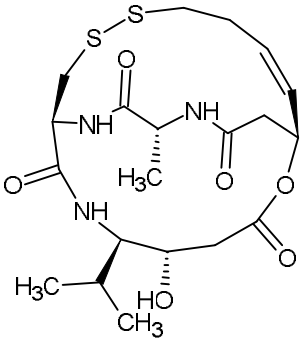
Related scientific studies published in journals, books and reports were reviewed. Relevant literatures were searched in google scholar and electronic databases including science direct, IEEE Xplore, Scopus, Sci finder and MEDLINE using a specific search terms including HDAC, Antineoplastic agents.

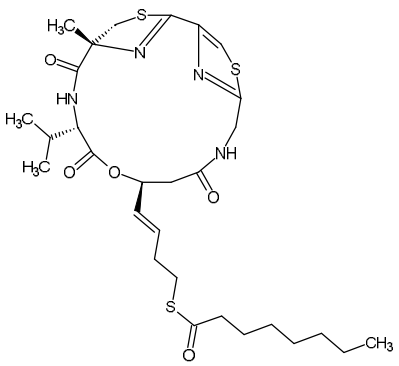
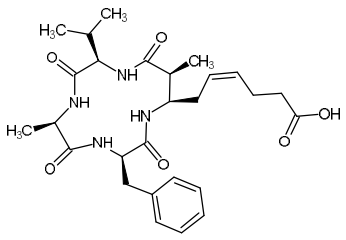
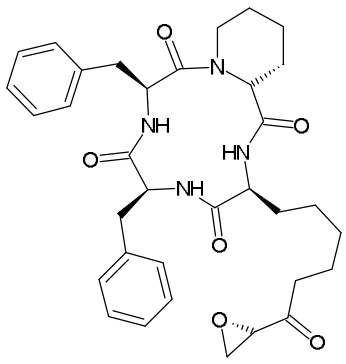
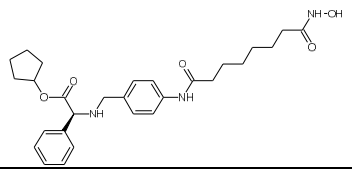
**Table: Small Molecules Exhibiting Anticancer Activity by Inhibiting HDAC**

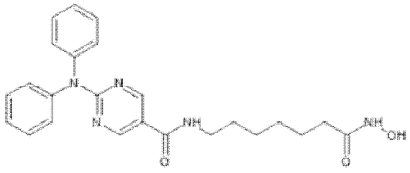
| Sl. No. | Leadmolecule | Pharmacological Activity & Target | Reference |
|---|---|---|---|
| 1 |  Suberoylanilide hydroxamic acid (SAHA, Vorinostat) | It induces hyperacetylation of histone and non-histone proteins, leading to: Cell cycle arrest (primarily G1 phase), Apoptosis of malignant cells, Inhibition of angiogenesis, Differentiation of tumor cells, Exhibits antineoplastic activity, particularly effective in cutaneous T-cell lymphoma (CTCL).<br>It targets zinc-dependent histone deacetylases (HDACs) by acting as a zinc ion chelator at the enzyme's active site, thereby blocking its deacetylase activity. It inhibits both Class I HDACs (HDAC1, HDAC2, HDAC3, and HDAC8) and Class II HDACs (HDAC6 and HDAC10), making it a broad-spectrum or non-selective HDAC inhibitor.<br>PDB ID- 4LXZ<br>DOCKING SCORE: -8.46 Kcal/mol | 1,2 |

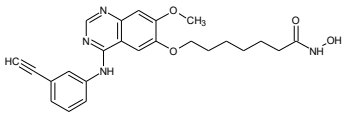
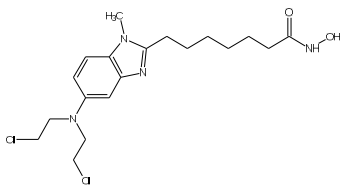
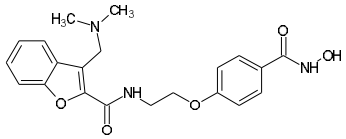
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| 2 |  Panobinostat | Panobinostat induces hyperacetylation of histone and non-histone proteins, leading to changes in chromatin structure and transcriptional regulation resulting in cell cycle arrest, apoptosis, and inhibition of angiogenesis in cancer cells. Its monotherapy has shown limited efficacy thereby used in combination therapy with bortezomib and dexamethasone, exhibiting increased therapeutic response.<br><br>It is a broad-spectrum inhibitor of Class I (HDAC 8), II, and IV HDAC enzymes.<br>PDB ID- 1W22<br>DOCKING SCORE: -8.47 Kcal/mol | 3,4 |
| 3 |  Belinostat | Belinostat induces histone hyperacetylation leading to chromatin relaxation, reactivation of tumor suppressor genes, cell cycle arrest and apoptosis in malignant T cells. It is also effective for peripheral T-cell lymphoma (PTCL).<br><br>It exhibits broad-spectrum (pan) HDAC inhibition by targeting both Class I HDACs (HDAC1, HDAC2, HDAC3, and HDAC8) and Class II HDACs (HDAC6 and HDAC10).<br><br>PDB ID: 5EEN<br>DOCKING SCORE: -8.39 kcal/mol | . 5,6 |
| 4 |  Romedepsin | Romidepsin inhibits HDACs at low nanomolar concentrations, leading to histone hyperacetylation, alterations in gene expression, cell cycle arrest, apoptosis, and cell differentiation. It is also effective for cutaneous T-cell lymphoma (CTCL) and peripheral T-cell lymphoma (PTCL).<br><br>It targets Class I HDACs, particularly HDAC1 and HDAC2. Its disulfide bond is reduced intracellularly to release a thiol group, which subsequently binds to the zinc ion in the HDAC active site, inhibiting enzymatic activity.<br><br>$IC_{50}$ value: 3.97 nM for HDAC1 and 787 nM for HDAC6 | 7,8 |
| 5 |  Tucidinostat | Tucidinostat induces histone hyperacetylation leading to chromatin relaxation , re-expression of tumor suppressor genes resulting in cell cycle arrest and apoptosis .It is found effective in treatment of peripheral T-cell lymphoma.<br><br>It selectively inhibits HDAC1, HDAC2, HDAC3, and HDAC10, with $IC_{50}$ values of 95, 160, 67, and 78 nM, | 9,10 |

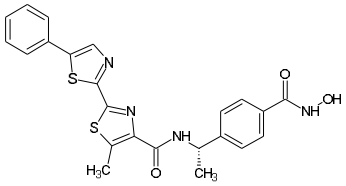
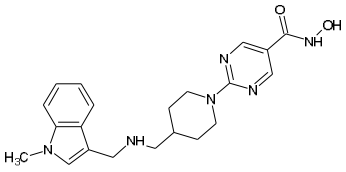
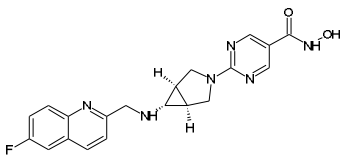
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| | | respectively.<br>PDB ID: 4A69<br>DOCKING SCORE: -7.8 kcal/mol | |
| 6 |  <br>Resminostat | Resminostat induces histone hyperacetylation, leading to chromatin relaxation, that promotes apoptosis, cell cycle arrest, and suppression of tumor growth. It has been evaluated in clinical trials for hepatocellular carcinoma and Hodgkin's lymphoma | 11,12 |
| 7 |  <br>Givinostat | Givinostat acts by inhibiting histone deacetylation, leading to chromatin relaxation, promoting apoptosis, cell cycle arrest, and activation of anti-inflammatory signaling pathways. It exhibits both anti-inflammatory and antineoplastic properties. It is currently evaluated in clinical trials for polycythemia vera,chronic myeloproliferative neoplasms<br><br>It targets both Class I and Class II HDACs.<br>$IC_{50}$ values of approximately 3 μM in p53-null cells (SaOS2), 0.57–0.97 μM in p53-mutant lines (SW872, SKLMS), and 0.63–0.88 μM in wild-type p53 cell lines (U2OS, SW982, HT1080, A204). | 13,14 |
| 8 |  <br>Pracinostat | Pracinostat promotes histone hyperacetylation, reactivation of tumor suppressor genes thereby inducing apoptosis and cell cycle arrest. Investigated clinical trials for acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), and various solid tumors.<br><br>It is a pan-HDAC inhibitor (Class I and II) with structural modifications that extend half-life and improve oral bioavailability. | 15,16 |
| 9 |  <br>AR-42 (OSU-HDAC42) | Inhibits histone deacetylation → increased histone acetylation, chromatin relaxation, and re-expression of silenced tumor suppressor genes inducing apoptosis, cell cycle arrest, and anti-tumor activity. Evaluated in trials for multiple tumors and neurological indications, including Acoustic neuroma, Intraocular lymphoma, Meningioma, Testicular lymphoma, Vestibular schwannoma.<br><br>AR-42 is a nanomolar hydroxamate-based HDAC inhibitor that blocks Class I and IIb HDACs<br><br>$IC_{50}$ of AR-42 at 48 h was approximately 0.9 μM in 7721, HepG2 and Hep3B cells | 17,18 |

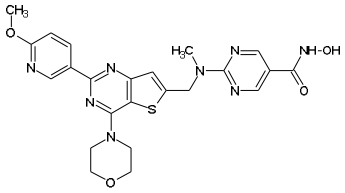
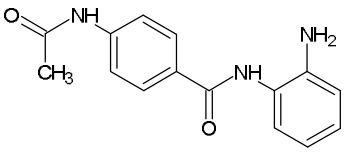
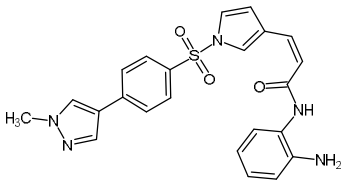
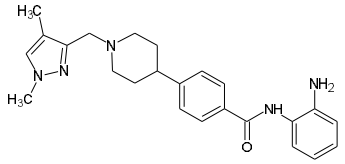
| | | | |
|---|---|---|---|
| 10 |  Mocetinostat | Mocetinostat blocks deacetylation and increasing histone acetylation, leading to the reactivation of tumor suppressor pathways inducing apoptosis via mitochondrial disruption and microtubule destabilization.<br>It is a selective Class I HDAC inhibitor (HDAC1 and HDAC2)<br><br>It has $IC_{50}$ values of 0.15 μM for HDAC1, 0.29 μM for HDAC2, 1.66 μM for HDAC3, and 0.59 μM for HDAC11. | 19,20 |
| 11 |  Entinostat (MS-275) | Entinostat (MS-275) increases histone acetylation that leads to chromatin relaxation and tumor suppressor gene reactivation. Thereby, promoting apoptosis, cell differentiation, and cell cycle arrest in malignant cells<br>It is a selectiveHDAC inhibitor that targets Class I HDACs, with high potency against HDAC1and moderate potency against HDAC3<br>PDB ID: 3MAX<br>DOCKING SCORE: −12.524 (HDAC2) | 21,22 |
| 12 |  Trichostatin A (TSA) | Trichostatin A (TSA) Induces histone hyperacetylation that leads to chromatin relaxation, tumor suppressor gene re-expression, and cell cycle arrest/apoptosis.<br><br>It acts as a pan-HDAC inhibitor by bidentate $Zn^{2+}$ chelation<br>PDB ID: 1C3R | 23 |
| 13 |  Psammaplin A | Psammaplin A contains disulfide bond that is reduced intracellularly to generate a thiol that chelates $Zn^{2+}$ in the HDAC catalytic site. The disulfide scaffold improves stability and cell permeability.<br><br>It is a model compound for natural product–based HDAC inhibitor design.<br><br>PDB ID: 4BKX<br>IC50 value of 45 Nm (HDACI) | 24,25 |
| 14 |  Spiruchostatin A (OBP-801) | Spiruchostatin A (OBP-801) selectively inhibits Class I HDACs via a thiol-mediated $Zn^{2+}$ chelation mechanism, inducing apoptosis and chromatin remodeling, and exhibits potent antitumor activity.<br>Spiruchostatin A (OBP-801) is a natural depsipeptide HDAC inhibitor that exhibits potent anticancer activity.<br>It effectively inhibits the growth of breast cancer cells with an $IC_{50}$ of approximately 10 nM, demonstrating strong cytotoxic potential at low nanomolar concentrations | 26 |

| | | | |
|---|---|---|---|
| 15 |  Largazole | Largazole is metabolized into a thiol, that chelates $Zn^{2+}$ in the HDAC active site. Structural studies (HDAC8–Largazole crystal complex) confirm its pharmacophore, and it exhibits potent nanomolar inhibition with strong anticancer activity.<br><br>Largazole is a cyanobacteria-derived natural depsipeptide prodrug that functions as a potent HDAC inhibitor.<br><br>It exhibits strong selectivity toward Class I HDACs, with $IC_{50}$ values of 61 ± 7 nM for HDAC1, 64 ± 5 nM for HDAC2, and 50 ± 6 nM for HDAC3, while showing significantly weaker inhibition of HDAC6 ($IC_{50}$ = 1400 ± 100 nM) | 27 |
| 16 |  Azumamide E | Azumamide E induces histone hyperacetylation, leading to chromatin relaxation, apoptosis, and cell cycle arrest.<br>It Acts as an antineoplastic agent in preclinical models.<br>Azumamide E is a marine-derived macrocyclic HDAC inhibitor with submicromolar potency, exhibiting selectivity for HDAC1 over HDAC6.<br>It demonstrates inhibitory activity with $IC_{50}$ values of 50 nM for HDAC1, 100 nM for HDAC2, and 80 nM for HDAC3. Structural studies have explored its interactions using several HDAC crystal structures, including HDLP (1C3R and 1C3S), human HDAC8 (1T64 and 1W22), HDAC4 (2VQQ and 2VQM), HDAC7 (2PQO), and the sirtuin family member SIRT2 (1J8) | 28 |
| 17 |  Trapoxin | In HDAC8, its ketone reacts with active-site to form a gem-diolate, which binds $Zn^{2+}$ bidentately, mimicking the substrate.<br>It irreversibly inhibits Class I HDACs and was key in elucidating the role of HDACs in gene regulation<br>$IC_{50}$ =110–820 pM against HDAC1. | 29,30 |
| 18 |  | Tefinostat (CHR-2845) induces apoptosis and cell cycle arrest in leukemic blasts while reducing systemic toxicity. It is being clinically evaluated in AML and MDS. Tefinostat (CHR-2845) is a first-in-class, esterase-activated HDAC inhibitor prodrug that selectively targets myelomonocytic cells. | 31,32 |

| | | | |
|---|---|---|---|
| | Tefinostat (CHR-2845) | M4 /M5 AMLs (median IC50; 1.1μM+/-1.8)<br>non-M4/M5 FAB types (median IC50 5.1μM +/-4.7) | |
| 19 | CG2OO745 | Effects on cholangiocarcinoma cells<br>CG200745 decreases viability of cholangiocarcinoma cell lines in a dose-dependent manner.It induces apoptosis: increased cleaved caspase-3, plus higher acetylated histone H3 indicating HDAC inhibition.<br>Molecular / pathway effects<br>Targets the Hippo signaling pathway via modulation of miRNAs. Specifically, CG200745 treatment leads to upregulation of several miRNAs. These acts to suppress downstream Hippo pathway effectors.<br>CG200745 is a hydroxamic acid–based, pan-histone deacetylase inhibitor (HDACi) that exerts its activity by chelating zinc ions within the catalytic pocket of HDAC enzymes, thereby blocking deacetylase function.<br>It demonstrates potent antiproliferative activity against cholangiocarcinoma cell lines, with $IC_{50}$ values of 0.63 μM in SNU-1196 cells, 0.93 μM in gemcitabine-resistant (GR) SNU-1196 cells, and 1.80 μM in SNU-308 cells. | 33,34 |
| 20 |  <br>Ricolinostat (ACY-1215) | Ricolinostat (ACY-1215) induces apoptosis and disrupts protein degradation by targeting HDAC6-mediated $\alpha$-tubulin and HSP90 deacetylation. It is being tested in multiple myeloma and lymphomas, often in combination therapies, and offers reduced toxicity compared to pan-HDAC inhibitors.<br>Ricolinostat (ACY-1215) exhibits antiproliferative activity across various non-Hodgkin's lymphoma (NHL) cell lines, with $IC_{50}$ values ranging from 1.51 to 8.65 μM.<br>Among these, the most sensitive cell lines were WSU-NHL and Hut-78, with $IC_{50}$ values of 1.97–1.51 μM, whereas the mantle cell lymphoma (MCL) line Granta-519 was the least sensitive, showing an $IC_{50}$ range of 20–64 μM. | 35,36 |
| 21 | Citarinostat (ACY-241) | Citarinostat (ACY-241) disrupts cytoplasmic deacetylation pathways ($\alpha$-tubulin, HSP90), inducing apoptosis and enhancing immune response. It is in clinical evaluation for multiple myeloma, lymphomas, and solid tumors in combination with immunotherapy.<br>It is an orally available, second-generation histone deacetylase6 (HDAC6)–selective inhibitor.<br>It exhibits potent and preferential inhibition of HDAC6 with an $IC_{50}$ value of 2.6 nM, while showing significantly weaker activity against other HDAC | 37,38 |

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| | | isoforms.Reported $IC_{50}$ values for Citarinostat include 35 nM for HDAC1, 45 nM for HDAC2, 46 nM for HDAC3, 137 nM for HDAC8, and >20,000 nM for HDAC4, HDAC5, and HDAC9, with moderate inhibition of HDAC7 (7,300 nM). | |
| 22 |  CUDC-101 | CUDC-101combines epigenetic modulation with inhibition of oncogenic signaling and induces apoptosis and suppresses tumor growth. It has been tested in phase I trials for advanced solid tumors, including head and neck, liver, and HER2+ cancers. CUDC-101 is a multitarget inhibitor that blocks Class I/II HDACs, EGFR, and HER2. CUDC-101, inhibits HDAC enzyme with IC50 (nmol/L) = 4.2 ± 1.2 | 39,40 |
| 23 |  Tinostamustine (EDO-S101) | Tinostamustine (EDO-S101) simultaneously relaxes chromatin and induces DNA breakage, it enhances antitumor efficacy and overcomes resistance. It is in clinical development for hematological cancers such as AML, lymphomas, and multiple myeloma. It is a dual-acting molecule that combines histone deacetylase (HDAC) inhibition with DNA-alkylating, damage-inducing activity. It exhibits cytotoxic effects across multiple myeloma (MM) cell lines, with $IC_{50}$ values ranging from 1.6 to 4.8 μM. | 41,42 |
| 24 |  Abexinostat (PCI-24781) | Abexinostat (PCI-24781) induces apoptosis, cell cycle arrest, and differentiation, and has been tested in phase I/II trials for lymphomas, sarcomas, and solid tumors, often showing enhanced activity in combination regimens. It is an orally bioavailable hydroxamate pan-HDAC inhibitor with strong activity against HDAC1, HDAC2, and HDAC6. In breast cancer cell lines (BCLs), abexinostat showed $IC_{50}$ values ranging from 170 to 460 nmol/L in sensitive cell lines, whereas resistant cell lines exhibited $IC_{50}$ values higher than 700 nmol/L | 43,44 |

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| 25 |  Bistianostat (SB939) | Bistianostat (SB939) demonstrates potent antitumor effects with an improved safety and pharmacokinetic profile compared to Vorinostat, and has been evaluated in clinical trials for hematological cancers and solid tumors<br>It is a second-generation hydroxamate pan-HDAC inhibitor with strong activity against HDAC1, HDAC2, and HDAC6<br>It has IC50 values ranging from 0.095 to 0.221 μM against HDAC1, 2, 3, 4, 5, 6, 8, 10, and 11. The documents also note that it has noactivity against HDAC7 and HDAC9, with an IC50 of >20 μM.<br>The specific IC50 values against HDACs are as follows:<br>HDAC1: 0.095 ± 0.049 μM<br>HDAC2: 0.193 ± 0.065 μM<br>HDAC3: 0.108 ± 0.060 μM<br>HDAC4: 0.158 ± 0.048 μM<br>HDAC5: 0.138 ± 0.051 μM<br>HDAC6: 0.068 ± 0.030 μM<br>HDAC8: 0.221 ± 0.136 μM<br>HDAC10: 0.171 ± 0.094 μM<br>HDAC11: 0.141 ± 0.023 μM | 45,46 |
| 26 |  Quisinostat (JNJ-26481585) | Quisinostat (JNJ-26481585) promotes apoptosis, differentiation, and cell cycle arrest, and reached phase II trials for hematologic and solid tumors but has not been approved.<br>It is an orally available pan-HDAC inhibitor with nanomolar potency against Class I and II isoforms mainly for HDAC 6.<br>In cancer cell lines, it shows $IC_{50}$ values in the low to mid nanomolar range.<br>The full range of $IC_{50}$ values was found to be less than 1 nM to 19 nM. | 47,48 |
| 27 |  Nanatinostat (CHR-3996) | Nanatinostat (CHR-3996) induces apoptosis and cell cycle arrest in hematologic malignancies and has been tested clinically for lymphomas, leukemias, and multiple myeloma.<br>It is an orally available, Class I selective HDAC inhibitor that targets HDAC1, HDAC2, and HDAC3 with high potency.<br>Biochemical enzyme assays have reported $IC_{50}$ values of approximately 3 nM for HDAC1, 4 nM for HDAC2, and 7 nM for HDAC3. | 49,50 |

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| 28 |  Fimepinostat (CUDC-907) | Fimepinostat (CUDC-907) induces apoptosis and cell cycle arrest by epigenetic modulation and simultaneously blocks PI3K/AKT signaling, making it effective in hematologic cancers and solid tumors It is a first-in-class dual inhibitor that targets Class I HDACs (HDAC1, HDAC2, HDAC3) and Class I PI3Ks (α and δ). It exhibits potent antiproliferative activity in both MYCN-amplified and non-amplified neuroblastoma (NB) cell lines, with $IC_{50}$ values of 0.91 μM in LAN-5, 1.94 μM in NGP, and 1.33 μM in CHLA-255-MYCN (MYCN-amplified), and 0.97 μM in SK-N-AS, 1.35 μM in SH-SY-5Y, and 1.35 μM in CHLA-255 (MYCN non-amplified) cells | 51,52 |
| 29 |  tacedinaline | Tacedinalineprevents the removal of acetyl groups from histone proteins, thereby influencing gene expression. Its primary targets are: HDAC-1, HDAC-2, HDAC-3 **PDB ID: 4LY1** Docking scores HDAC-2: -8.451 HDAC-3: -8.260 | 53,54 |
| 30 |  Domatinostat (4SC-202) | Domatinostat (4SC-202) induces apoptosis and cell cycle arrest while enhancing immune checkpoint therapy efficacy. Currently used in clinical trials for solid tumors and hematological malignancies. It is an orally available Class I selective HDAC inhibitor targeting HDAC1, HDAC2 and HDAC3. It exhibits potent antiproliferative activity in cutaneous T-cell lymphoma (CTCL) cell lines, with $IC_{50}$ values ranging from the low nanomolar to micromolar range—170 nM in CRL-2105, 190 nM in MyLa, 260 nM in CRL-8294, 370 nM in HTB-176, 250 nM in Se-Ax, and 330 nM in HuT 78 cells. | 55,56 |
| 31 |  Zabadinostat (CDX 101) | Zabadinostat (CDX 101) demonstrates antitumor and immune-modulatory activity and is in clinical development for colorectal cancer and other advanced malignancies. CXD101 is an orally availableHDAC inhibitor that selectively targets Class I HDACs (HDAC1, HDAC2, HDAC3) with $IC_{50}$ values of 63 nM, 570 nM, and 550 nMrespectively. | 57 |

## CONCLUSION

Histone deacetylase inhibitors (HDACs) represent a rapidly evolving class of epigenetic modulators with profound therapeutic potential across haematological and solid malignancies. Through the inhibition of zinc-dependent HDAC enzymes, these molecules restore the balance between acetylation and deacetylation, leading to chromatin relaxation, reactivation of tumour suppressor genes, and subsequent induction of apoptosis, cell cycle arrest, and differentiation.

Among the compounds studied, hydroxamate-based inhibitors such as vorinostat, panobinostat, and belinostat exhibit broad-spectrum (pan-HDAC) activity, while benzamide derivatives like entinostat and mocetinostat demonstrate higher selectivity toward Class I HDACs, contributing to improved safety and tolerability. Natural product–derived inhibitors such as largazole, spiruchostatin, and romidepsin further highlight the structural diversity and mechanistic complexity of this pharmacophore class. Docking analyses and $IC_{50}$ data consistently reveal strong binding affinities within the nanomolar to micromolar range, emphasizing the crucial role of zinc ion chelation in HDAC inhibition. Recent developments in selective HDAC6 inhibitors (e.g., ricolinostat and citarinostat) and dual-acting agents (e.g., CUDC-101, CUDC-907, and tinostamustine) underscore a strategic shift toward multitargeted and isoform-specific approaches that aim to enhance antitumor efficacy while minimizing systemic toxicity. In summary, HDAC inhibitors continue to be a cornerstone of modern epigenetic therapy. Future research integrating structural optimization, computational modelling, and combination therapy strategies may further refine their clinical application and expand their potential beyond oncology into inflammatory, neurodegenerative, and metabolic disorders

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RESEARCH ARTICLE

# Unveiling the Hidden Consequences of Ultra-Processed Foods: Epigenetic and Neurobehavioral Insights in the Post - COVID Era

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## ABSTRACT

Ultra-processed foods (UPFs) have become an integral component of modern dietary patterns, driven by rapid urbanization, aggressive marketing, and post-pandemic lifestyle transitions. Characterized by refined sugars, hydrogenated fats, synthetic additives, and low nutritional value, these products exert profound biological effects that extend beyond metabolic disorders. Recent evidence highlights the role of UPFs in inducing epigenetic modifications- particularly aberrant DNA methylation, histone alterations, and microRNA dysregulation- which collectively predispose individuals to obesity, insulin resistance, and neurobehavioral dysfunctions. The post-COVID generation, marked by increased reliance on convenience foods and digital lifestyles, is particularly vulnerable to such alterations. This review consolidates current findings on the molecular and neurophysiological mechanisms linking UPF consumption to cognitive decline, mood instability, and developmental impairments. Furthermore, it emphasizes how chronic exposure to artificial ingredients and emulsifiers disrupts the gut–brain axis, influencing neuroinflammatory pathways and emotional regulation. The review underscores the urgent need for public health interventions, nutritional education, and policy reforms aimed at mitigating the long-term health consequences of UPF consumption. Greater awareness and regulatory oversight are essential to safeguard the well-being of future generations.

**Keywords:** Ultra-Processed Foods, Epigenetics, Neurobehavior, Post-COVID

# INTRODUCTION

Ultra-processed foods (Ups) emerge as a dominant dietary pattern worldwide due to their convenience, affordability, and aggressive marketing strategies. They typically contain refined oils, high sugars, hydrogenated fats, and modified starches, while lacking essential nutrients such as fiber and vitamins [1]. Between 2006 and 2019, per capita sales of UPFs and beverages in South and Southeast Asia rose significantly, raising concerns about obesity, cardiovascular diseases, metabolic disorders, and metabolic dysfunction-associated steatotic liver disease (MASLD), particularly among children and adolescents [1]. MASLD, previously referred to as non-alcoholic fatty liver disease (NAFLD), is characterized by excessive fat accumulation in liver cells and is closely linked with obesity, type 2 diabetes, dyslipidaemia, and hypertension. Non-communicable diseases (NCDs) now account for roughly 72% of deaths and 75% of healthcare expenditures globally. Components of Ups, including sugar, caffeine, salt, and fat, are considered food additives rather than standalone nutrients, and some additives

exert addictive-like effects like cocaine or heroin [2]. Soft drinks, the second most consumed beverage globally, show only slight reductions in consumption even with price increases, highlighting strong consumer demand [2]. During the COVID-19 pandemic, studies in Brazil report decreases consumption of fresh and minimally processed foods and increases processed food intake, associated with poor sleep quality. Sedentary lifestyles combined with high UPF consumption contribute to shorter sleep duration and prolonged sleep deprivation [3]. Ups and additives alter gut microbiota, potentially harming the host and contributing to conditions like inflammatory bowel disease (IBD). Diets high in animal fat, sugar, and salt but low in fiber reduce microbiota diversity, leading to mucus inflammation and unusual immune responses [4]. Western populations exhibit higher prevalence of IBD, while Mediterranean diets are recommended for their protective effects.

**Consumption Trends: Global and India**

While UPF intake among Asian youth is lower than in non-Asian populations, a Japanese study reports 27.3% of youth consuming UPFs regularly. In Brazil, Spain, Portugal, and the UK, children and adolescents derive 30–50% of their energy intake from UPFs, with some children obtaining nearly half of daily calories from these foods [1]. High intake among Asian youth places them at risk of similar metabolic and health outcomes as Western peers. In developed countries, UPFs contribute 50–60% of daily energy intake, while middle-income countries are experiencing rapidly increasing consumption [5]. In the US, UPFs account for nearly 60% of total energy intake and 90% of added sugar consumption. Younger populations are the primary consumers, while older adults are less affected [5]. In Europe, intake ranges from 14% of daily energy in Italy to 50% in the UK; Canada reports nearly 50% and Australia over 35% [6]. Socioeconomic status inversely correlates with UPF intake in several countries [6]. In India, NCD mortality has risen from 37.9% to 61.9% over 25 years. Obesity affects roughly 25% of adults and 3% of children under five. increases UPF consumption is driven by higher disposable income, nuclear families, single-member households, and less time for homecooked meals. Children and adolescents are exposed through media, sports, and celebrity endorsements, contributing to a “double burden” of malnutrition and obesity. The COVID-19 pandemic exacerbated food insecurity and obesity risks [7][8]. Adults with high UPF consumption, particularly in India, are at risk for deficiencies in essential nutrients, including iron, zinc, vitamin A, and folate. Frequent intake of packaged snacks, sugar-sweetened beverages, and ready-to-eat meals exacerbates this problem, with implications for both metabolic and cognitive health [9].

**Defining Ultra-Processed Foods**

Ultra-processed foods are industrially manufactured products containing five or more ingredients, including additives like Flavors, colours, sweeteners, and emulsifiers. These components are designed to replicate the sensory qualities of unprocessed foods or mask undesirable Flavors [10].

**Nova classification system:** NOVA categorizes foods based on the extent and purpose of industrial processing [11]:
Group 1: Unprocessed or Minimally Processed Foods – edible parts of plants, animals, fungi, and algae preserved using basic methods like drying, roasting, or freezing.
Group 2: Processed Culinary Ingredients – substances derived from Group 1 foods, such as oils, butter, sugar, and salt, used for cooking.
Group 3: Processed Foods – items like canned vegetables, cheeses, or freshly baked breads made by adding Group 2 ingredients to Group 1 foods.
Group 4: Ultra-Processed Foods – soft drinks, packaged snacks, reconstituted meats, and frozen dishes made mostly from substances derived from foods, combined with additives. Common ingredients include sugars, oils, fats, salt, casein, lactose, whey, gluten, hydrogenated oils, maltodextrin, emulsifiers, preservatives, and flavor enhancers.

**The Anatomy of Ultra - Processed Foods (UPFS)**

Ultra-processed foods can disrupt multiple organ systems through metabolic, inflammatory, and neuroendocrine pathways. The gastrointestinal tract acts as a critical gateway where additives such as emulsifiers, artificial sweeteners, and preservatives can disturb gut microbiota and impair intestinal barrier function. Even minor alterations in gut homeostasis may trigger immune responses, neuroinflammation, and cognitive disturbances, demonstrating that UPFs can affect overall health beyond simple diet [12]. Artificial food colorants like Tartrazine (E102), Sunset Yellow (E110), and Allura Red (E129) are commonly used azo dyes found in ultra-processed foods. Research has linked these compounds to behavioral changes, such as hyperactivity in children, as well as increases markers of oxidative stress in animal studies.[13] Consuming ultra-processed foods that contain the synthetic food colorant Allura Red AC on a regular basis may disrupt serotonin signaling in the intestines. This disruption can lead to dysfunction of the gut barrier and an increases risk of inflammatory conditions like colitis. This emphasizes how certain additives in ultra-processed foods can directly contribute to gastrointestinal inflammation.[14] "Monosodium glutamate (MSG) is commonly used as a flavor enhancer in highly processed savory snacks, instant noodles, and sauces. Experimental studies show that chronic MSG consumption can cause hypothalamic lesions, disrupt appetite control, and lead to increases fat storage in rodents." [15] Common preservatives found in ultra-processed foods (UPFs) include sodium benzoate, potassium sorbate, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT). These compounds help prevent rancidity and microbial growth; however, they may interfere with mitochondrial respiration and cause hepatocellular damage [16].

Nanomaterials found in ultra-processed foods may present health risks. Chronic exposure to engineered nanoparticles—commonly used in food additives, packaging, and colorants—can potentially result in cellular toxicity, inflammatory responses, and bioaccumulation in organs. This raises concerns about the long-term health impacts on both humans and animals. Due to their small size, these nanoparticles can cross intestinal and other biological barriers, which may worsen these negative effects if regulatory assessments are insufficient.[17] High consumption of processed meats preserved with nitrites increases endogenous nitration and promotes oxidative DNA damage, contributing to colorectal tumor development.[18] Additives are substances intentionally or indirectly incorporated into foods to preserve them, enhance taste, improve texture, or provide appealing appearance. They are broadly classified as direct and indirect additives [12].

**Indirect Additives**

Bisphenols – Used in coatings of cans, bottles, and packaging; linked to polycystic ovary syndrome, obesity, and increases cardiometabolic risk.
Phthalates – Found in plastics, adhesives, and lubricants; associated with insulin resistance, male reproductive disorders, metabolic disturbances, premature birth, and cardiac issues.
Perfluoroalkyl Chemicals (PFAS) – Present in coatings, textiles, paper, firefighting foams, cosmetics, and food packaging; linked to developmental neurotoxicity, immune dysfunction, hyperuricemia, and ADHD.
Perchlorate – Occurs naturally or as a by-product of disinfection; disrupts iodide uptake in the thyroid, affecting fetal and infant growth and cognitive development.

Pesticides – Residual pesticides in food or environment interfere with hormone synthesis and insulin-like growth factor signaling, increasing risks of neurodevelopmental delays, IQ reduction, behavioral disorders, altered growth, cancer, and respiratory issues [12].

**Direct Additives**

Nitrates and Nitrites – Found in processed meats, fish, cheese, milk formulas, biscuits, and contaminated water; induce oxidative stress, disrupt iodide transport, and are linked to colon cancer, brain tumors, thyroid dysfunction, methemoglobinemia, premature birth, and autism spectrum disorders.

Artificial Food Colorants (AFCs) – Used in foods and beverages for aesthetic appeal; can trigger hypersensitivity, behavioral problems, and attention disorders in children.

Monosodium Glutamate (MSG) – Common in soups, sauces, stews, snacks, and spices; excessive intake leads to neurotoxicity, hypertension, Alzheimer's disease, and "Chinese Restaurant Syndrome."

Aspartame – Widely used in sugar-free beverages, medications, and supplements; accumulation of phenylalanine may impair neurological development, increase mood disorders, and cause headaches, particularly in children [12].

**Emulsifiers And Metabolic Effects**

Emulsifiers are detergent-like molecules commonly added to processed foods and can increase bacterial translocation across the epithelial barrier. Two widely used emulsifiers carboxymethylcellulose (CMC) and polysorbate 80 (P80)—have been shown to induce lowgrade inflammation even at low concentrations, contributing to obesity and metabolic syndrome in wild-type hosts. For instance, 0.1% CMC can slightly increase body weight and fasting glucose, whereas 0.5% CMC induces clear low-grade inflammation and fat accumulation. Similarly, P80 at 0.1% induces low-grade inflammation, and at 0.5%, it can cause mild dysglycemia. These emulsifiers act as chronic stressors in the gut, promoting metabolic changes [19]. Research in mice indicates that emulsifiers can induce anxiety-like behaviour by altering gene expression in brain regions such as the amygdala and paraventricular nucleus of the hypothalamus. Common foods containing emulsifiers include ice cream, butter, beverages, chocolate, sauces, and mayonnaise. Neuroinflammation causes by emulsifier consumption may further modulate stress and behaviour [20].

**Epigenetic Impacts of Ultra-Processed Foods**

Early exposure to UPFs can influence DNA methylation and other epigenetic mechanisms, regulating gene expression associated with obesity and metabolic diseases. High-fat/sugar diets alter acetylation balance, promoting expression of proinflammatory genes in vascular tissues. Nutrient-derived regulation of histone acetylation is critical for controlling inflammation, and its disruption contributes to chronic low-grade inflammation, a hallmark of metabolic syndrome and cardiovascular disease [21][22]. In Brazilian adolescents, higher UPF intake is directly associated with elevated levels of inflammatory markers such as C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-$\alpha$) [23].

UPF consumption is linked to changes in DNA methylation in children, measured using Illumina Infinium Methylation arrays. Associations are estimated using robust linear regression and meta-analysis of EWAS results [24]. Large-scale proteomic profiling identifies plasma protein levels indicative of UPF consumption. Individuals in the highest UPF quartile are more likely to be male, white, less educated, and former smokers. Protein biomarkers studied mostly focus on inflammation and adipokines. The Nova classification is widely used for UPF identification, enabling comparison across studies [25]. miRNAs from capillary blood indicate individual physiological characteristics, dietary habits, and lifestyle influences. miR-155 is associated with metabolic disease and upregulated with age. High consumption of vegetables, fruits, and whole grains increases miR-let-7a and miR328 (anti-inflammatory) and decreases pro-inflammatory miR-21. Smoking decreases miRNA142, which is downregulated in lung cancer [26]. ADHD is a common comorbidity in children; in the US., one in eight children with ADHD also has autism, requiring additional support [27]. Youth with both ADHD and autism are at higher risk of premature

mortality, especially in the presence of psychiatric comorbidities, and are at increased risk of cardiometabolic diseases, obesity, type 2 diabetes, and other chronic conditions [27]. Epigenetic inheritance refers to the transmission of gene behaviour across generations. The BDNF gene, critical for learning and memory, may be affected by maternal diet and intrauterine environment. DNA methylation patterns are influenced by diet, lifestyle, and toxicant exposures (e.g., mercury, lead, organophosphate pesticides). A high UPFs diet with low nutrient intake is linked to alterations in DNA methylation patterns affecting offspring health [27].

**Dysbiosis and Its Implications on Metabolic and Neurobehavioral Health**

Ultra-processed foods (UPFs) are characterized by high contents of synthetic additives and emulsifiers, combined with low dietary Fiber. Their consumption is strongly associated with

reduced microbial diversity, including lower levels of beneficial bacteria such as Akkermansia muciniphila and Faecalibacterium prausnitzii, along with an increase in pro-inflammatory microorganisms. Such microbial alterations can trigger persistent inflammation, contributing to chronic disorders like metabolic syndrome, irritable bowel syndrome (IBS), type 2 diabetes mellitus (T2DM), and colorectal cancer. In addition, UPFs disrupt the gut-brain axis, potentially affecting cognitive function and mental health, while interventions such as fiber- rich diets, fermented foods, and probiotics can help mitigate these negative effects. Public health measures including stricter UPF regulations and clear labelling are recommended [28]. The gut microbiota is critical in shaping host immunity, influencing both local and systemic immune responses. Mechanisms include Development and maturation of gut-associated lymphoid tissue (GALT). Stimulation of antimicrobial peptides. Promotion of differentiation of T helper cells (Th17) and regulatory T cells (Tregs). Modulation of innate lymphoid cells (ILCs) essential for mucosal immunity and tissue repair. UPF-induced dysbiosis can impair these processes, leading to reduced immune function and increases susceptibility to systemic inflammation. Additionally, interventions such as fecal microbiota transplantation (FMT) have been shown to restore some of these immune functions [29]. The gut-brain axis is a bidirectional communication system connecting the gastrointestinal tract and the central nervous system (CNS) through hormonal, neural, and immunological pathways. Certain microbial species regulate neurotransmitter production, including serotonin (5-HT) and gamma-aminobutyric acid (GABA), which are crucial for mood regulation, cognitive function, and overall mental health. Enterococcus species contribute to serotonin synthesis. Disruption of this axis by UPFs may contribute to cognitive dysfunction, depressive disorders, and neurodegenerative conditions. Disruption of the gut microbiota by UPFs can impair the gut- brain axis, potentially affecting cognitive function and mental health. Dysbiosis-related inflammation in IBD may exacerbate: Mood disorders such as depression. Cognitive dysfunction, with potential long-term neurodegenerative implications. Maintaining a balanced diet rich in fiber, fermented foods, and minimally processed ingredients is crucial for mitigating these risks and supporting both gut and brain health [30]. UPFs may disrupt this system, contributing to psychiatric conditions such as depression, Alzheimer's disease, and Parkinson's disease [31].

Alterations in microbial composition causes by UPFs can impair these functions, reducing production of beneficial metabolites such as short-chain fatty acids (SCFAs) and increasing gut permeability ("leaky gut"). This allows harmful bacterial products like lipopolysaccharides (LPS) to enter systemic circulation, inducing chronic low-grade inflammation linked to noncommunicable diseases (NCDs) [28]. Chronic high UPF consumption is associated with a pro-inflammatory and pro-oxidant metabolic profile. The relationship between diet, gut microbiota, and inflammatory bowel disease (IBD) has garnered increasing attention in recent decades. Among dietary factors, ultra-processed foods (UPFs) have emerged as major contributors to gut dysbiosis and the exacerbation of inflammatory conditions such as Crohn's disease (CD) and ulcerative colitis (UC) [30]. Chronic consumption of UPFs is associated with gut dysbiosis that exacerbates Crohn's disease (CD) and ulcerative colitis (UC). Mechanisms include increases prevalence of proinflammatory and pro-carcinogenic bacteria. Reduced activity of antioxidant enzymes (catalase, superoxide dismutase) and increases reactive oxygen species (ROS). Elevated proinflammatory cytokines such as TNF-$\alpha$, IL-6, and IL-15. Reduced butyrate production, compromising intestinal barrier integrity. Activation of inflammatory signaling pathways, including NF-κB, perpetuating gastrointestinal inflammation. Beneficial

commensals like Faecalibacterium prausnitzii and SCFAs are crucial for intestinal homeostasis, immune modulation, and infection prevention. UPF-induced dysbiosis disrupts this balance, increasing susceptibility to chronic inflammation and infections [32]. Gut microbiota-derived metabolites act as central regulators in metabolic homeostasis and disorders. Alterations in microbial composition or metabolite production can contribute to obesity, insulin resistance, type 2 diabetes, and cardiovascular disease [33]. Short-Chain Fatty Acids (SCFAs) primarily acetate, propionate, and butyrate, are produced by bacterial fermentation of dietary fibers. They regulate energy metabolism, glucose homeostasis, and lipid metabolism. SCFAs activate G-protein-coupled receptors (GPR41 and GPR43) to improve insulin sensitivity and increase energy expenditure. They also promote GLP-1 and PYY secretion, enhancing satiety and reducing the risk of obesity [33]. Trimethylamine (TMA)/ Trimethylamine N-oxide (TMAO): Gut bacteria metabolize dietary choline, carnitine, and phosphatidylcholine into TMA, which is oxidized in the liver to TMAO. Elevated TMAO levels are associated with atherosclerosis, insulin resistance, and cardiovascular disease [33]. Bile Acids (BAs): Microbiota transform primary bile acids into secondary bile acids, which act as signaling molecules by activating FXR and TGR5 receptors. These processes influence glucose metabolism, lipid metabolism, and overall energy homeostasis [33]. Branched-Chain Amino Acids (BCAAs): Microbial metabolism can increase circulating BCAAs, which are linked to insulin resistance and obesity [33]. Indole and Indole-Derivatives: Produced from microbial tryptophan metabolism, these metabolites regulate intestinal barrier function, inflammation, and GLP-1 secretion. They provide protection against metabolic endotoxemia and insulin resistance [33]. Other Microbial

Metabolites: Phenylacetylglutamine: Linked to increases cardiovascular risk. Imidazole propionate: Impairs insulin signaling in host tissues [33]. Gut microbiota-derived metabolites act as mediators of gut-liver and gut-brain crosstalk. Dysbiosis alters metabolite profiles, contributing to metabolic syndrome, obesity, type 2 diabetes, and cardiovascular disease [34]. Imidazole propionate and phenylacetylglutamine interfere with glucose tolerance and insulin signaling, promoting T2D.

**Neuronal behaviour**

Dysbiosis enhances production of trimethylamine (TMA), converted to trimethylamine N oxide (TMAO), which is linked to cardiometabolic risk. UPF-induced changes in the microbiota can disrupt the gut-brain axis, influence cognition and mental health, and increasing susceptibility to Alzheimer's disease (AD), Parkinson's disease (PD), and depression [30]. Oxidative Stress and Cytokine Imbalance Excessive UPF consumption is linked to oxidative stress, as evidenced by Reduced activity of antioxidant enzymes such as catalase and superoxide dismutase (SOD) increases levels of xanthine oxidase, reactive oxygen species (ROS), and myeloperoxidase (MPO). Elevated pro-inflammatory cytokines, including TNF-$\alpha$, IL-6, and IL-15This combination of oxidative stress and chronic inflammation contributes to intestinal ischemia risk and exacerbates IBD progression both gut and brain health [30]. Ultra-processed foods that are high in sugar and fat can hijack the brain's reward system by overstimulating the release of dopamine in the mesolimbic pathway, similar to the effects of addictive drugs. This excessive stimulation reduces the sensitivity of dopamine receptors, resulting in compulsive eating behaviors and an increases risk of obesity and metabolic disorders [36]. Ultra-processed foods that are high in sugar and fat can trigger compulsive overeating by altering dopamine signaling in the brain, specifically in the nucleus accumbens. This alteration reinforces reward-seeking behaviour and may contribute to the phenomenon of "food addiction" [37]. Consuming ultra-processed foods over a long period can lead to a decrease in hippocampal volume in rodent models, which is associated with impairments in spatial learning and memory retention [38]. Diets high in processed foods reduce hippocampal BDNF levels, which are essential for neuronal survival and memory formation. decreases BDNF levels contribute to impaired spatial memory and cognitive deficits [39]. A diet high in saturated fat increases oxidative stress and reduces levels of BDNF in the hippocampus, resulting in impaired synaptic plasticity and cognitive deficits [40].

High-fat and ultra-processed diets cause microglial priming in the hippocampus, resulting in heightened neuroinflammatory responses to subsequent immune challenges, contributing to cognitive deficits and impaired synaptic plasticity [41]. High-fat and ultra-processed diets disrupt the rhythmic expression of clock genes in the liver and adipose tissue, resulting in misalignment between central and peripheral circadian clocks [42]. A high intake of ultra- processed foods (UPFs) leads to irregular feeding times, dampening the amplitude of circadian oscillations in metabolic genes, promoting obesity and glucose intolerance [42].Dietary patterns high in ultra-processed foods are linked to delayed sleep onset, reduced sleep duration, and disrupted melatonin rhythms in both animal models and humans [44] .Circadian misalignment causes by high-fat and ultra-processed food consumption impairs insulin sensitivity, alters lipid metabolism, and increases systemic inflammation, contributing to metabolic syndrome [45].

**Impacts of UPFS from Early Life to Childhood**

Maternal malnutrition or poor-quality diet during pregnancy can alter fetal development and program long-term disease risk through epigenetic changes, affecting DNA methylation, histone modifications, hypothalamic pathways, and energy balance. Maternal UPF intake is linked to increases risk of infant atopic dermatitis and altered immune development, highlighting the importance of promoting whole foods and limiting UPFs during pregnancy [31][46]. Parental dietary habits strongly influence offspring food preferences and metabolism. Habitual UPF consumption in parents is associated with early metabolic dysregulation in children due to modelling, reinforcement, and epigenetic inheritance [47]. Consumption of highly ultra-processed foods is associated with accelerated biological aging. Biological age measured using methods such as Klemera-Doubal method biological age (KDM-BA), Pheno Age, and Homeostatic Dysregulation (HD), increases proportionally with UPF intake. A 10% increase in UPF consumption is linked to measurable acceleration in biological age, with younger adults showing more pronounced effects. Biological aging reflects the accumulation of molecular and cellular damage, leading to functional decline and increases disease vulnerability [48]. Excessive consumption of ultra-processed foods (UPFs) is characterized by high intake of saturated fats and added sugars and a decreases intake of essential micronutrients such as zinc (Zn), calcium (Ca), magnesium, and vitamins A, C, D, E, B12, and niacin [49]. Growing evidence links UPF consumption to obesity, type 2 diabetes, cardiovascular disease, and negative impacts on child neurodevelopment [49]. Research by Puig-Vallverdú et al. found a negative association between maternal prenatal UPF consumption and verbal functioning in offspring during early childhood [49].

Ward reports health problems in hyperactive children associated with the consumption of yellow food colours, including eczema, asthma, respiratory and ear infections, poor speech, and diminished coordination. Behavioral studies shows that hyperactive children exhibited significant decreases in serum Zn levels, along with increases overactivity and aggression after consuming yellow food colours [49]. Milne and Neilsen reports that high fructose corn syrup (HFCS) consumption causes significant losses of calcium in men, suggesting that diets high in fructose could lead to bone loss and osteoporosis over time. Reducing UPF intake can help mitigate these nutritional deficits and improve health outcomes. Nutritional deficits during pregnancy increase the risk of adverse birth outcomes and neuropsychiatric disorders, including autism spectrum disorders (ASD) and attention deficit/hyperactivity disorder (ADHD) [50]. Excessive UPF consumption can lead to dietary exposure to heavy metals. College students who reduced UPF intake and consumed more whole/organic foods has lower inorganic mercury levels than controls [50]. Prenatal exposure to lead (Pb) is associated with reduced levels of Bifidobacterium in the gut microbiota of children aged 9–11 years. Diet plays a moderating role in the gut microbiome and the pathophysiology of neuro developmental disorders such as ASD [50]. UPF consumption is linked to increases body fat from childhood to early adulthood. Studies in large British cohorts show associations between UPF intake and adiposity trajectories [51]. UPF and Non-Communicable Diseases High UPF intake is associated with multiple adverse health effects, especially non-communicable chronic diseases. The high energy density, unhealthy ingredients, and low dietary fiber increase risk for diet-related diseases [52]. Accelerated lifestyle, fast food consumption, unhealthy eating habits, and sedentary behaviour are major risk factors for overweight and obesity [53]. Epigenetic modifications can be influenced by the intrauterine environment and environmental factors such as diet. Animal studies show that

early-life epigenetic alterations can lead to adult obesity. Non-communicable diseases impose a substantial economic burden (estimated $7 trillion from 2011–2025 in low- and middle-income countries). Oxidative stress in early life contributes to NCDs, including cancer.

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RESEARCH ARTICLE

# Some Mathematical Tools for Biologists

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**ABSTRACT**

One area of Discrete Mathematics that is used in every other stream is Graph Theory. This is because of its dual nature, which encompasses the widest range of applicability to any contemporary discipline and the most inventive proofs in all of abstract reasoning. Graphs are aesthetically pleasing and intuitive, in part because of the diagrammatic depiction. This work deals with the mathematical tools that help a biologist.

**Keywords:** Phylogenetic tree, Splits, Split Network.

## INTRODUCTION

**This is a combined work of Graph Theory and Phylogenetic Analysis.**
Graph theory began with the Konigsberg Bridge issue in 1735. In 1866, Ernst Haeckel created the term "phylogeny" from the German word "phylogenie." Phylogenetics is the study of relationships between or within groups of organisms. Sometimes it was difficult for mathematicians to read biological tables. It is simpler to view the specified organisms with or without specific traits using a graphical framework known as a "phylogram" or "cladogram." They have a rooted phylogenetic tree structure. The Rooted Phylogenetic trees can be converted back into Biological Tables. A connected acyclic graphical arrangement is called a tree. Making evolutionary trees is a crucial stage in many biological research investigations. Evolutionary trees of different species, for example, show us how the species descended from a common ancestor and may also provide information about the morphology of those ancestors, the rate at which different lineages developed, the ways in which those lineages and their ancestors adapted to different environmental conditions, etc. The Split Network concept was developed by H. J. Bandelt and Dress A. W. M. A key idea in phylogenetic analysis is splits. Split categorizes taxa based on what makes them unique. Both trivial and non-

trivial splits in taxa are represented by Split Networks, outer labeled planar split networks, etc., which are exclusively graphical networks.

## PRELIMINARIES

**Definition 1**A linear graph (or Graph) $G = (V, E)$ consist of a set of objects $V = \{v_1, v_2, v_3, \dots\}$called vertices, and another set $E = \{e_1, e_2, e_3, \dots\}$, whose elements are called edges, such that each edge$e_k$ is identified with an unordered pair $(v_i, v_j)$ of vertices. The vertices $v_i, v_j$ associated with edge $e_k$ are called the end vertices of$e_k$.

**Definition 2** A walk in a graph $G$ is an alternating sequence of vertices and edges beginning and ending with vertices in which each edge is incident with the two vertices immediately preceding and following it.

**Definition 3** Open walk in which no vertex appear more than once is called a path.

**Definition 4** A graph $G$ is connected if there is at least one path between every pair of vertices in $G$ and disconnected otherwise.

**Definition 5** A walk $v_0 e_1 v_1 \dots e_k v_k$ is said to be a cycle if $v_0 = v_k$ and the vertices $v_1, v_2, \dots, v_{k-1}$ are distinct from each other.

**Definition 6** A tree is a connected acyclic graph.

**Definition 7** A leaf in a rooted tree is any vertex having no children.

**Definition 8** A tree in which one vertex (called the root) is distinguished from all the others is called a rooted tree.

**Definition 9**$X = \{x_1, x_2 \dots, x_n\}$ denote a set of taxa, in which each taxon$x_i$represents some species, group or individual organism whose evolutionary history is what we are dealing with. For example$X = \{x_1, x_2 \dots, x_n\}$, might denote a set of mammals, with $x_1$ representing gorillas, $x_2$ representing seals, etc.

**Definition 10**A phylogeny describes the evolutionary history of a set of taxa.

**Definition 11**Two species are called related, if they share a recent common ancestor.

**Definition 12**A species $a$ is more closely related to a species $b$ than to a species $c$, if the last common ancestor of $a$ and $b$ is more recent that the last common ancestor of $a$ and $c$.

**Definition 13** A phylogenetic tree on $X$ is obtained by labelling the leaves of a tree $T$ by the taxon set $X$.

**Definition 14** Given a set of taxa $X$, a rooted phylogenetic tree consists of a rooted tree $T = (V, E, \rho)$ and a taxon labelling $\lambda: X \to V$ that assigns exactly one taxon to every leaf and none to any internal node. All nodes, except $\rho$, must have degree $\neq 2$.

**Definition 15** A split $S = A|B$ is a bipartition of a set of taxa $X$ into two non-empty subsets $A$, $B$ with $A \cap B = \phi$ and $A \cup B = X \cdot A$ and $B$ are called the two split parts of $S$.

**Definition 16** A split graph consists of a finite, simple, connected, bipartite graph $G = (V, E)$, together with an edge coloring $\sigma : E \to K$ that is surjective and isometric.

**Definition 17** Let $S$ be a set of splits on $X$. A split network $N = (V, E, \sigma, \lambda)$ that represents $S$ is given by a split graph $(G = (V, E), \sigma : E \to S)$ and a node labelling $\lambda : X \to V$, with the property that for every split $S = A|B$ in $S$, $A = \bigcup_{v \in V_S^0} \lambda^{-1}(v)$ and $B = \bigcup_{v \in V_S^1} \lambda^{-1}(v)$, in other words, deletion of all edges of color $S$ produces a graph consisting of precisely two connected components, one containing all nodes labeled with elements of $A$ and the other containing all nodes labeled with elements of $B$.

**Definition 18** A set of splits $S$ on $X$ is called circular, if there exist a linear ordering $(x_1, \ldots, x_n)$ of the elements of $X$ for $S$ such that each split $S \in S$ has the form $S = \{x_p, x_{p+1}, \cdots, x_q\} | \{X - \{x_p, x_{p+1}, \cdots, x_q\}$ for appropriately chosen $1 < p \leq q \leq n$.

**Definition 19** Let G be a graph in which some of the nodes are labeled. G is outer labeled planar, if there exists a drawing of G in the plane such that no two edges intersect and all labeled nodes lie on the outside of the graph.

**Theorem 20** Circular implies outer labeled planar.

**Theorem 21**[Deletion of split produces two components] Let $(G = (V, E), \sigma : E \to K)$ be a split graph. For any color $c \in K$, the graph $G_c$obtained by deleting all edges of color $c$ consists of precisely two connected components.

**Definition 22** A phylogenetic network is any graph used to represent evolutionary relationships between a set of taxa that labels some of its nodes.

***Cladogram***

The Greek words clados, which means "branch," and gramma, which means "character," are the source of the word "cladogram." Cladograms depict the relationships between organisms and were mostly created using morphological characteristics. Lines (edges) that branch off in various directions and end at a collection of creatures with a last common ancestor are used in cladograms. Characters are represented by the lines, or edges. In a Cladogram, the length of the lines (edge length) has no meaning.

The table 1 above shows the characteristic properties possessed by five different organisms. Mathematically we can convert this table to a Cladogram which is a rooted phylogenetic tree which is shown below.

Figure 1

From the above Cladogram we can get back the above table. The detailed algorithm for both these can be seen in [24].

***Construction of Phylogenetic tree from Distance Matrix***

We are unable to identify any relationships between the organisms under investigation based on the distance matrix from the biological background. However, the unique aspect of the method described below is that we may mathematically identify the closely related taxa before creating the phylogenetic tree.

Consider we are given a distance matrix D of order $n \times n$, which shows the distance between the pair of taxa, $n \geq 4$. The taxa set is $\{x_1, x_2, x_3 \ldots, x_n\}$. The entries ofDare non-negative.

$D = (x_{ij}), \quad i, j = 1, 2, 3 \ldots n.$

Here $x_{ij}$ denote the distance between $x_i$ *and* $x_j$ . Also $x_{ij} = x_{ji}$

Given a distance matrix D showing the inter-distance between five taxa $\{a, b, c, d, e\}$.

$$D = \begin{pmatrix} 0 & 11 & 10 & 9 & 15 \\ 11 & 0 & 3 & 12 & 18 \\ 10 & 3 & 0 & 11 & 17 \\ 9 & 12 & 11 & 0 & 8 \\ 15 & 18 & 17 & 8 & 0 \end{pmatrix}$$

Seeing this distance matrix we cannot say anything about the relationship between the taxa under consideration. The given below is the weighted tree we obtained mathematically from the Distance matrix. The tree obtained gives clear idea about the ancestral history of the organisms under consideration. For example, the closely related organisms can be easily identify from the given below graphical structure. The detailed mathematical procedures can be seen in [25]. (Figure 2).

**Outer Labeled Planar Split Network**

Suppose we are considering 6 different characteristic features possessed by a collection of 8 organisms.

Consider the set of splits

$\boldsymbol{S} = \{S1, S2, S3, S4, S5, S6\}$where

$S1$ = {a, b, d, e, h}|{c, f, g}

$S2$ = {a, c, d, e, g, h}|{b, f}

$S3$ = {a, c, e, g}|{b, d, f, h}

$S4$ = {a, c, g}|{b, d, e, f, h}

$S5$ = {a, c, e, f, g}|{ b, d, h}

$S6$ = {a, e, h}|{ b, c, d, f, g}

In addition to the above splits, if we are given the trivial splits also, then that can also be included in the figure. Biologically we are considering the unique characteristic features possessed by the same collection of 8 organisms.$S7$,$S8$,…,$S14$ are mathematically the unique characteristic features possessed by the same collection of 8 organisms a, b, …, h respectively. That is

$S7$ = {a}|{b, c, d, e, f, g, h}

$S8$ = {b}|{a, c, d, e, f, g, h}

$S9$ = {c}|{a, b, d, e, f, g, h}

$S10$ = {d}|{a, b, c, e, f, g, h}

$S11$ = {e}|{a, b, c, d, f, g, h}

$S12$ = {f}|{a, b, c, d, e, g, h}

$S13$ = {g}|{a, b, c, d, e, f, h}

$S14$ = {h}|{a, b, c, d, e, f, g}

(Figure 3)

This is an outer labeled planar split network that shows 6 different characteristic features possessed by a collection of 8 organisms and also the unique characteristic features possessed by the same collection of 8 organisms. The construction of this network is mathematical and can be seen in [26]. A biologist can use this diagram for field studies.

For example, consider the split$S5$ = {a, c, e, f, g}|{ b, d, h}

(Figure 4)

This is the diagram we obtained after removing the edges labelled $S5$. Now the graph has two connected components one contains organisms from one split part and the other contains organisms from the other split part. The biologists need only the splits and the network. They can easily classify the organisms based on a particular characteristic feature by removing the edges showing that particular characteristic feature.

## CONCLUSION

Cladogram aids mathematicians with biological tables and biologists with phylogenetic trees. Ancestral relationships between the organisms under discussion are never provided by a distance matrix. For biologists, identifying closely related species from a given distance matrix by building a weighted tree is particularly helpful. Using the Split concept, taxa are separated based on their distinguishing characteristics. Because so many distinctive characteristics of a group of organisms may be represented in a single network, the phylogenetic network built in this part will be helpful for field research. By removing edges from the graph, it is also possible to identify the bipartition of a set of organisms under study with regard to a specific distinguishing characteristic.

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**Table 1: The table shows the characteristic properties possessed by five different organisms. Mathematically we can convert this table to a Cladogram which is a rooted phylogenetic tree which is shown below.**

| Organism/Character | Feather | Fur | Lungs | Gizzard | Jaws |
|---|---|---|---|---|---|
| Lamprey | x | x | x | x | x |
| Antelope | x | ✓ | ✓ | x | ✓ |
| Sea Bass | x | x | x | x | ✓ |
| Bald Eagle | ✓ | x | ✓ | ✓ | ✓ |
| Alligator | x | x | ✓ | ✓ | ✓ |

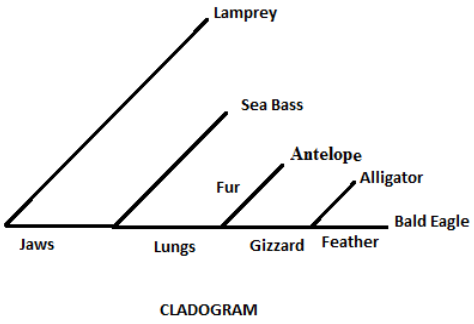



**Fig 1: From the above Cladogram we can get back the above table.**
**The detailed algorithm for both these can be seen in [24].**

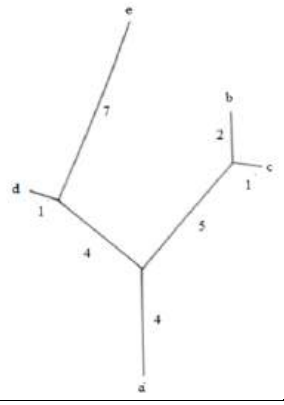



**Fig 2: Phylogenetic tree**

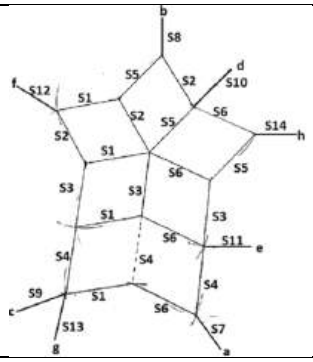


**Fig 3: Outer labeled planar split network**

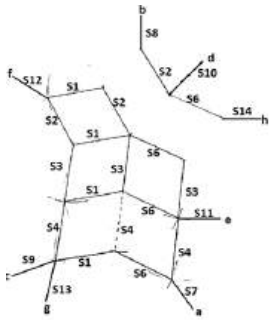


**Fig 4: This is the diagram we obtained after removing the edges labelled *S*5.**

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RESEARCH ARTICLE

# Toxicity Assessment of Degradation Intermediates from Plasticizer Biodegradation by *Chroococcus* sp.

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## ABSTRACT

Plasticizers are widely used additives that enhance the flexibility, durability, and performance of plastic materials. They are commonly incorporated into packaging, construction materials, automotive parts, medical devices, and household products. Concerns over environmental pollution and human health risks have led to increased interest in replacing phthalate-based plasticizers with safer bio-based alternatives. Despite their advantages, plasticizers are not chemically bonded to polymers and can migrate into the surrounding environment. Such leaching results in contamination of soil and aquatic ecosystems. Plasticization occurs when plasticizers reduce intermolecular forces between polymer chains. This reduction increases polymer mobility and improves material flexibility. However, the environmental persistence of plasticizers raises serious ecological concerns. Biological remediation strategies are increasingly explored to address this issue. Microalgae have emerged as effective organisms for the removal of plasticizer contaminants. Several green algal species are capable of degrading both phthalate and non-phthalate plasticizers. Cyanobacteria, as photosynthetic prokaryotes, also contribute significantly to degradation processes. The present study investigates the tolerance of *Chroococcus* sp. to plasticizer exposure. Growth rate, cell density, biomolecule production, and stress biomarkers were evaluated under controlled laboratory conditions. The findings highlight the potential of microalgae as sustainable and eco-friendly tools for mitigating plasticizer pollution.

**Keywords:** *Chroococcus* sp, Phthalate, Non-Phthalate, Biomolecules, Stress markers

## INTRODUCTION

Plastic is a synthetic organic polymer found throughout the world. The widespread use of plastic is largely due to its affordability, durability, and higher strength when compared with traditional materials such as wood, metal, and glass [1],[2],[3]. Current estimates show that global plastic production is close to 450 million metric tons each year [4]. Yet, inadequate waste management means that a significant share of these plastics is disposed of incorrectly and eventually escapes into the natural environment. In 2022, the world generated nearly 380 million tons of plastic waste [5], and around 8 million tons of this material ultimately entered the oceans as marine pollutants. As a result, the number of plastic debris in the ocean keeps growing [6]. Chea *et al*. (2023)[7] reported that most plastic products are designed to remain stable for long periods and may persist in the environment for several decades. Over time, however, they can break down into smaller fragments such as microplastics or even nanoplastics. This breakdown is driven by several factors, including exposure to ultraviolet radiation, temperature-induced degradation, mechanical abrasion, and various human activities [8]. Environmental risks are also posed by a range of chemical additives, such as plasticizers, flame retardants, UV stabilizers, and antioxidants, which are added to plastics to change their characteristics [9]. As the material weathers and breaks down, additives are released into seawater due to their poor chemical binding [10]. Phthalic acid esters (PAEs), which make up around 70% of the global plasticizer market, are among the many varieties of plasticizers that are used extensively [11]. Due to their acute toxicity to aquatic organisms and their potential carcinogenic effects in humans, the U.S. Environmental Protection Agency (USEPA) has classified phthalate esters (PAEs) as priority pollutants [12]. Within marine ecosystems, both microplastics and the chemical additives they contain pose significant risks to microalgae, plants, and a wide range of aquatic animals. A variety of algae and cyanobacteria are now recognized for their ability to deteriorate synthetic polymers. These organisms secrete ligninolytic and exopolysaccharide enzymes, which assist in weakening and transforming plastic materials that they attach to [13]. One study demonstrated that the freshwater alga *Uronemaafricanum*, collected from discarded plastic bags, produced clear signs of LDPE deterioration—such as surface corrosion, abrasion marks, and groove formation—within 30 days[14]. Cyanobacteria also contribute significantly to biofilm-mediated plastic degradation. Species such as *Oscillatoria subbrevis* and *Phormidium lucidum*, obtained from household wastewater, were able to utilize LDPE as a carbon source while forming dense colonies on the polymer surface. Notably, their degradation activity occurred without any chemical pre-treatment or the use of pro-oxidant additives [15]. Further evidence from FTIR-ATR, SEM-EDX, and tensile-strength analyses shows that freshwater strains of *Arthrospira* can slowly degrade PET and PP, achieving measurable breakdown after approximately 112 days of exposure [16]. Limited information exists on the ability of *Chroococcus* species to tolerate and degrade emerging plasticizers such as DOTP and DINP. Accordingly, the present investigation was designed to examine the tolerance response and biodegradation potential of this cyanobacterium toward DOTP and DINP under laboratory conditions.

**Novelty Statement**

Although *Chroococcus* has been studied for the degradation of certain plasticizers such as BPA, DiBP, and phenol, its ability to degrade DOTP and DINP has not been reported. This study is the first to evaluate the tolerance and biodegradation potential of Chroococcus toward these emerging phthalate and non-phthalate plasticizers, thereby expanding the understanding of cyanobacterial applications in phytoremediation of plasticizer pollution.

## MATERIALS AND METHODS

**Isolation of microalgae**

Microalgae were cultured on BG-11 agar medium by applying the spread plate method. The plates were then placed in an incubation chamber maintained at 25 ± 2 °C with cool-white fluorescent lighting programmed for 16 hours of light followed by 8 hours of darkness. After 5 to 7 days, the grown cultures were seen under a microscope, and the pure colonies. The picked colonies were again kept on agar plates. Repeat this procedure till a pure culture is obtained. The pure cultures were grown massively using BG 11 broth medium. Although BG11 medium supported rapid growth, the cultures showed noticeable settling during incubation. In contrast, cultures grown in Chu 10

medium remained well suspended and uniformly mixed. Therefore, Chu 10 medium was selected for all experimental analyses to ensure homogeneity and reproducibility of the results.

**Media preparation**

Chu's medium No. 10 (calcium nitrate (0.232g $L^{-1}$), $K_2HPO_4$ (0.01g $L^{-1}$), $MgSO_4{\cdot}7H_2O$ (0.025g $L^{-1}$), $Na_2\ CO_3$ (0.02g $L^{-1}$), $Na_2SiO_3$ (0.044g $L^{-1}$), $C_6H_8O_7{\cdot}FeNH_4{\cdot}xH_2O$(3.5 $mgL^{-1}$), $C_6H_8O_7$ (3.5 $mgL^{-1}$), trace metal- 1 mL ($H_3BO_3$ 2.4g $L^{-1}$), $MnCl_2{\cdot}4H_2O$ (1.4g $L^{-1}$), $ZnCl_2$ (0.4g $L^{-1}$), $CaCl_2{\cdot}2H_2O$ (0.02g $L^{-1}$), $CuCl_2{\cdot}2H_2O$ (0.1g $L^{-1}$), distilled water 1000 mL, pH 7.1) was used. The medium, once prepared, was sterilized and kept at 25 °C to preserve its suitability for future microalgal cultivation.

**Culture condition**

Microalgae were collected from the Uppathuodai estuary, Thoothukudi. Pure colonies obtained from the initial isolation were first inoculated into 10 mL of sterile BG-11 medium and incubated at 25 ± 2 °C under a 16:8 h light–dark cycle provided by cool-white fluorescent lamps. After three days, the cultures reached sufficient growth and were transferred into 50 mL of fresh BG-11 medium for further incubation. Subsequently, after five days, the cultures were scaled up to 100 mL of fresh medium and incubated until dense algal growth was achieved. This culture was maintained as the mother culture and sub-cultured at regular intervals to ensure a continuous supply of healthy microalgal cells.

**Experimental setup**

Each 250 mL Erlenmeyer flask contained 100 mL of BG-11 medium and was inoculated with 10% of an actively growing *Chroococcus* sp. culture. All treatments were set up in triplicate to ensure reproducibility.

**Chemical treatment**

When the culture attained an optical density of 0.2 in the absorbance wavelength 750 nm, then it was treated with different concentrations of DINP and DOTP (5, 10, 40, 80, and 100 mg $L^{-1}$). A solvent flask and a control were also kept. Flasks were manually swirled three times daily to ensure uniform suspension of cells and prevent their adhesion to the container surfaces. A 15-day incubation period was given to the culture. All experiments were likewise performed in duplicate. The microalgal cells were observed under a light microscope to assess any morphological alterations.

**Biomass and biochemical composition analysis**

**4.1Growth analysis**

At specified intervals (0, 3, 6, 9, 12, and 15 days), microalgal growth was assessed by measuring optical density at 750 nm using the EI Deep Vision–2371 UV–Vis spectrophotometer, according to Arutselvan *et al*. (2021)[17].

**Determinationof cell density**

With a light microscope and a hemacytometer, the density of microalgal cells was calculated. Initially, a micropipette was used to load the microalgal culture into the chamber, and a microscope was used to count the cells present in a 1 µm square. The cell density of the cultures was determined according to the formula below:

$$Cell\ density = \frac{Mean\ of\ cells\ per\ square \times dilution\ factor\ (if\ any)}{Volume\ of\ the\ square}$$

To evaluate the inhibitory effect on the algae, the following equation was applied(Kong *et al*., 2010; Debenest *et al*., 2009):

$$I = 100 \times \frac{C_0 - C_t}{C_0}$$

Where I stand for the inhibitory rate (in percentage), and $C_t$ and $C_0$ stand for the mean cell density in the treated and untreated groups, respectively. The formula below was utilised to calculate the specific growth rate of the algae (Kong *et al*. 2010):

$\mu = \frac{Ln\ C_{t2}/Ln\ C_{t1}}{t_2 - t_1}$

Where $C_{t2}$ and $C_{t1}$ indicate the cell density at times $t_2$ and $t_1$, respectively, whereas μ stands for the specific growth rate.

**Estimation of Biomolecules**

**Determination of Carbohydrate**

The method described by Plummer (1978) [18] was used to estimate the carbohydrate content.

**Determination of Protein**

Protein estimation was carried out according to the method of Lowry *et al*. (1951)[19].

**Determination of Stress Biomarker**

**Malonyldialdehyde (MDA)**

The MDA concentration in the samples was measured following Heath and Packer's (1968) [20]procedure, and values were calculated using the corresponding formula.

$$\text{MDA } (\mu \text{ mol g}^{-1}) = \frac{6.45 \times (A_{532} - A_{600}) - (0.56 \times A_{450})}{\text{Cell weight (g)}}$$

**Hydrogen peroxide ($H_2O_2$)**

$H_2O_2$ content, an indicator of oxidative stress, was estimated using the method developed by Velikova *et al*. (2000)[21].

**Proline**

The proline concentration in the samples was determined according to the procedure outlined by Bates *et al*. (1973)[22].

## RESULT AND DISCUSSION

**Growth analysis**

The experimental treatments included DINP and DOTP at varying concentrations of 5, 10, 40, 80, and 100 mg $L^{-1}$. The results of DINP and DOTP-treated and untreated flasks were studied at proper intervals. The microalgal species *Chroococcus* sp. exhibited strong tolerance when exposed to 40 mg $L^{-1}$ and 100 mg $L^{-1}$ concentrations, respectively. *Chroococcus* sp. indicated higher growth.in 100 mg $L^{-1}$ (1.426 OD) treated flasks, followed by 40 mg $L^{-1}$ (1.366 OD) DINP. In the DOTP-treated flask, growth was higher at 80 mg L-1 (1.368 OD), followed by 100 mg L-1(1.325 OD). These results revealed the DINP ecotoxicity on microalgal growth.Hence, this study exhibited that the selected estuarine cyanobacteria have the potential to overcome plasticizer pollution Fig. 1a and b. Spectrometric analysis by Arutselvan *et al*. (2021) [16] indicated that on the nineteenth day, cell enlargement occurred in *Coelastrella* sp., *Chlorococcum* sp. *Scenedesmus dimorphus* and *Chlorella vulgaris*. Notably, *Coelastrella* sp. achieved the maximum optical density of 1.3 OD, compared to 1.12 OD in Chlorella vulgaris and Scenedesmus dimorphus. *Chlorococcum* sp. had the lowest optical density (0.92 OD) among the strains. A recent study found that *C. vulgaris* exhibits the most growth on the twelfth day; this growth is dependent on the cultivation medium and growth conditions[23]. Algal cultures were cultivated in various media to compare their growth performance in a study that used two-litter batch reactors. With an optical density of 1.937 on the fifteenth day, the BBM medium had the fastest growth rate among the tested media, followed by M4N (1.765), BG11 (1.742), N8 (1.711), and M8 (1.719), which had the lowest optical density [24].The beneficial effects of phthalates were examined in these studies using cyanobacteria, including *Chroococcus* sp.

**Cell density**

Calculating cell density helped understand the growth of microalgae in a more specific manner. Under a light microscope, a haemocytometer was used to quantify cell density. In *Chroococcus* sp., both chemicals are degraded by

the 15th day at amounts of 40 $L^{-1}$ and 100 $L^{-1}$. At this time, the algal cells exhibit greater adsorption and display a good biodegradation rate in DINP, as well as DOTP (Fig. 2a and b). High concentrations of DBP (50 and 100 mg $L^{-1}$) negatively affected the cell density of *Chlorella vulgaris*, while lower concentrations (5, 10, and 20 mg $L^{-1}$) significantly promoted its growth. After two days of exposure to 50 mg $L^{-1}$ DBP, *C. vulgaris* exhibited the highest removal efficiency of 31.69%. As reported by Duan *et al*. (2018) [25], *C. vulgaris* demonstrates potential as an oleaginous microalga capable of thriving in DBP-contaminated water and could be practically employed for DBP removal. Recent studies have reported the final cell densities of six microalgal species, with *Chlorella vulgaris* attaining the highest density of 8.70 × $10^6$ cells $mL^{-1}$. This was followed by *Chroococcus* sp. (3.56 × $10^6$ cells $mL^{-1}$), *Scenedesmus obliquus* (3.45 × $10^6$ cells $mL^{-1}$), *Nostoc* sp. (2.17 × $10^6$ cells $mL^{-1}$), *Cyclotella* sp. (1.68 × $10^6$ cells $mL^{-1}$), and *Nitzschia* sp. (1.55 × $10^6$ cells $mL^{-1}$) [26]. Additionally, Manzi *et al*. (2022) [27] evaluated the growth of *Chlorella* sp. GEEL-08 and *Tetradesmus dimorphus* GEEL-04 under DBP concentrations ranging from 0 to 100 mg $L^{-1}$. At 10 mg $L^{-1}$ DBP, Chlorella sp. GEEL-08 exhibited higher growth (0.88 $OD_{680}$ nm) compared to *T. dimorphus* GEEL-04 (0.80 $OD_{680}$ nm). Chi *et al*., (2019)[28]. used three marine microalgae (*Dunaliella salina*, *Cylindrotheca closterium*, and *Chaetoceros muelleri*) to remove DEP and DBP, achieving DBP removal efficiencies of 40.0%, 93.1%, and 47.1%, and DEP removal of 32.3%, 81.2%, and 26.3%, respectively. Additionally, *Chlorella vulgaris* removed DBP at various concentrations (5–100 mg/L), with the highest efficiency (31.69%) at 50 mg/L after 2 days; higher DBP concentrations negatively affected both cell density and lipid profile. This supports the observed 88% DBP removal by C. vulgaris in 48 hours at lower concentrations, highlighting the dose-dependent toxicity of phthalates. Furthermore, according to Lakshmikandan *et al*. (2025) [29], *Coelastrella terrestris* MLUN1 was capable of efficiently degrading DBP when exposed to 500 mg $L^{-1}$ of the compound at 25 °C with an inoculum density of 1.5–2 mg $L^{-1}$.

**Macromolecules concentration of individual microalgae with DINP and DOTP Treatment**

**Carbohydrates**

Major macromolecules like carbohydrates and proteins can all be synthesised by different processes using photosynthetically fixed carbon. Carbohydrates are one of the most important biomolecules and a versatile energy source for microalgae. In *Chroococcus* sp, high and low carbohydrate content was exhibited by 100 mg $L^{-1}$DINP-treated (248.42 μg $mL^{-1}$) and control flasks (120.75 μg $mL^{-1}$) on the 15$^{th}$day. Flasks treated with DOTP exhibited varying carbohydrate content, with the highest (159.58 μg mg $L^{-1}$) observed in the 100 mg $L^{-1}$ treatment and the lowest (133.2 μg $mL^{-1}$) in the 40 mg $L^{-1}$ treatment. In Chroococcus sp., carbohydrate synthesis may contribute to the reduction of pollutant concentrations, as seen in the DINP-treated flasks (Fig. 3a and b). Additionally, carbohydrate accumulation increased with rising NaCl concentrations in the growth medium, reaching a maximum of 35.91% at 400 mM salinity [30]. On the eighth day, cells exposed to NaOH-mPET at 100 mg $L^{-1}$ exhibited the highest polysaccharide content, measuring 1.91 mg $L^{-1}$, which corresponds to a 10.4% increase compared to the control. Conversely, exposure to UV-mPET at 200 mg $L^{-1}$ and HT-mPET at 100 mg $L^{-1}$ led to a significant reduction in polysaccharide levels in *Chlorella* sp. UTEX1602 [31].

**Proteins**

In *Chroococcus* sp., the highest protein content in DINP-treated flasks was observed at 80 mg $L^{-1}$ (134.76 μg $mL^{-1}$), whereas the control flasks exhibited the lowest protein level (117.63 μg $mL^{-1}$). For DOTP treatments, maximum protein accumulation (128.69 μg $mL^{-1}$) occurred at 100 mg $L^{-1}$, while the lowest content (97.43 μg $mL^{-1}$) was recorded at 40 mg $L^{-1}$ on the 15th day of incubation (Fig. 4a and b). Previous studies have demonstrated that higher plants and macroalgae can down-regulate genes associated with primary metabolism and protein synthesis under stress, while up-regulating genes involved in autophagy and protein degradation [32]. Microalgae such as *C. sorokiniana* and *Neochloris* sp. achieved high protein content when grown in either original wastewater (up to 22% of the final dry weight) or modified medium (averaging 26% and 33% of the final dry weight, respectively). Similarly, protein content in algal species cultivated in 10% effluent from biogas reactors was comparable (Zignashina *et al*., 2022), and consistent protein levels were also observed in *C. sorokiniana* grown in synthetic media under different cultivation techniques [33]. Additionally, growth in raw poultry slaughterhouse wastewater resulted in significantly higher protein production, whereas modified wastewater—treated with ash—led to reduced protein accumulation. During the exponential growth phase, *Chlorella* sp. UTEX1602 actively excreted organic matter, and on the eighth day, the

200 mg $L^{-1}$ NaOH-mPET treatment exhibited a peak protein content of 0.0064 mg $L^{-1}$. However, the protein content gradually drops as HT-mPET and UV-mPET concentrations rise [30].

**Analysis of Stress Biomarkers**

The stress biomarkers like Hydrogen peroxide, MDA, and Proline were analyzed with *Chroococcus*sp in DINP and DOTP treated flasks with different concentrations (0ppm, 5ppm, 10ppm, 40ppm, 80ppm, and100ppm). In $H_2O_2$,the DINP-treated flask showed high results in 80ppm (3.81±1.51) onthe 7th day and low in 80ppm (0.96±0.05) onthe 15th day of culture. Likewise, the DOTP-treated flask showed high concentration in 40ppm (2.36±1.04 at 7th day) and low concentration in 100ppm (0.7±0.09 at 15th day). In MDA, the DINP flask shows a high concentration of 84.23 in 100ppm at the 15th day, and a low concentration is present in 11.86 in 10ppm at the 7th day of culture. The DOTP-treated flask showed a high value of 67.97 in 80ppm and a low value of 11.93 in 10ppm at the 15th day of culture. In proline stress, the DINP-treated flask showed high in 5ppm 16.18±3.19 and low in 40ppm 10.03±0.93 at 15th day of treatment, and the DOTP-treated flask, the high result in 5ppm 21.14±2.45 at 7th day, and low concentration in 10.03±0.93 in 40ppm at15th day of treatment (Table 1). The microalga *Chlamydomonas reinhardtii* was exposed to 200 mM NaCl for eight days to examine metabolomic, biochemical, and physiological responses. Exposure to this salinity significantly reduced the growth rate as well as chlorophyll a and b contents in *C. reinhardtii*. Hydrogen peroxide ($H_2O_2$) levels increased markedly under salt stress, reaching 10.14 ± 1.126 mM at 200 mM NaCl, compared to 4.88 ± 0.665 mM in the control ($P \leq 0.01$). Similarly, Yun *et al*. (2019) reported that *Chlorella vulgaris* accumulated 0.2028 mM $H_2O_2$ per 0.1 g fresh weight under 200 mM NaCl stress, in contrast to 0.0347 mM per 0.1 g fresh weight under control conditions. $H_2O_2$, a reactive oxygen species (ROS), plays a critical role in mediating stress responses in plants and algae [34]. The production of $H_2O_2$ is also linked to the action of stress-related hormones, such as abscisic acid (ABA) and brassinosteroids (BRs), which enhance stress tolerance in both plants and algae [34]. In NaCl-treated cultures, malondialdehyde (MDA) content increased significantly to 62.88 ± 4.428 nmol $mg^{-1}$ of protein, compared to 24.04 ± 0.329 nmol $mg^{-1}$ in the control. Polat *et al*. (2020) [35] measured $H_2O_2$ and MDA levels to investigate ROS-mediated lipid accumulation in *Auxenochlorella protothecoides*. They reported that at 40 g $L^{-1}$ NaCl, $H_2O_2$ levels increased 3.9-fold, from 5.60 μmol $g^{-1}$ to 22.33 μmol $g^{-1}$, and MDA increased 4.4-fold, from 0.13 nmol $g^{-1}$ to 0.57 nmol $g^{-1}$.

Similarly, Yang and Hu (2020) observed that MDA levels in *Micractinium*sp. rose from 0.2 μmol $g^{-1}$ to 0.57 μmol $g^{-1}$ after three days at 40 g $L^{-1}$ NaCl. These findings indicate that oxidative stress induced by high NaCl concentrations leads to increased MDA accumulation. Numerous studies have reported that MDA builds up in plant and microalgal cells due to lipid peroxidation under salt stress [36],[37],[34].In addition, DBP concentrations ranging from 0 to 100 mg $L^{-1}$ were applied to *Tetradesmus dimorphus* GEEL-04 and *Chlorella* sp. GEEL-08 to assess stress responses. Activities of two key enzymes, MDA and superoxide dismutase (SOD), were measured, with both MDA and SOD activities being higher in *T. dimorphus* cultures than in *Chlorella* sp. cultures [27]. Under salt stress, *C. reinhardtii* accumulated a significantly higher amount of proline (225.17 ± 3.910 μg mg−1 proteins) than the control group (69.55 ± 0.264 μg mg−1 proteins) (Figure 3c). Previous studies have reported that proline levels increase in response to salt stress [38]. Under 200 mM NaCl, *Nostoc piscinale* accumulated significantly more proline (11 μg $g^{-1}$ fresh weight) compared to 5 μg $g^{-1}$ fresh weight under control conditions (0 mM NaCl) [38]. Similarly, Chokshi *et al*. (2017) [39] observed a 1.8-fold increase in proline accumulation in *Acutodesmus dimorphus* exposed to 200 mM NaCl (66.45 ± 1.07 μM $g^{-1}$ dry weight) relative to the control (37.36 μM $g^{-1}$ dry weight). Proline accumulation under stress conditions was primarily caused by enhanced synthesis, decreased oxidation rate, and slow incorporation into proteins [40]. The combined findings indicate that DOTP biodegradation proceeds more slowly and elicits stronger stress responses, whereas *Chroococcus* effectively breaks down DINP with relatively less oxidative damage. However, at 100 ppm, 80 ppm, and 40 ppm, both plasticizers were significantly broken down, indicating the strain's potential to treat phthalate and terephthalate contaminants across a range of environmental concentrations. In order to speed up DOTP breakdown and increase overall bioremediation efficiency, future research should concentrate on clarifying the entire mineralization pathways, evaluating the toxicity of intermediates, and testing *Chroococcus* in consortia-based systems. The MDA content of Chlorella sp. UTEX1602 cells increased markedly when exposed to 100 mg $L^{-1}$ NaOH-mPET, while exposure to 100 mg $L^{-1}$ UV-mPET and HT-mPET caused a significant reduction in MDA accumulation [31].

## CONCLUSION

The current study successfully examined the degradation of dioctyl terephthalate (DOTP) and diisononyl phthalate (DINP) using a blue-green microalgae-based phycoremediation method utilizing *Chroococcus* sp. The results showed that *Chroococcus* sp. has an exceptional ability to metabolize and tolerate phthalate pollutants, which lessens their persistence in aquatic environments. The organism has the potential to be an effective and environmentally sustainable agent for the bioremediation of complex organic pollutants, as evidenced by its adaptive physiological and biochemical responses. The investigation's findings highlight the importance of microalgal systems as a practical substitute for traditional physico-chemical treatment techniques, providing benefits like low operating costs, decreased secondary pollution, and concurrently improved water quality. Furthermore, *Chroococcus* sp. may be further optimized through strain improvement, co-cultivation techniques, and bioprocess engineering to increase degradation efficiency under a variety of environmental conditions due to its metabolic versatility. Future research should focus on clarifying the molecular and enzymatic processes underlying the DINP and DOTP degradation pathways and evaluating the approach's viability on a large scale in both natural and industrial wastewater systems. Phycoremediation's environmental and economic sustainability could be further enhanced by incorporating circular bioeconomy concepts, such as biomass valorization for bioenergy or bioproducts. Overall, the study shows that phycoremediation mediated by Chroococcus sp. is a viable, environmentally friendly, and scalable method for restoring aquatic ecosystems contaminated with phthalates.

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This study did not receive any specific funding.

### DISCLOSURE STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Table1: Stress biomarkers with Plasticizer treated with *Chroococcus* sp.**

| | | H2O2 | | MDA | | PROLINE | |
|---|---|---|---|---|---|---|---|
| | | DINP | DOTP | DINP | DOTP | DINP | DOTP |
| 7th day | 0 PPM | 1.78±0.32 | 1.84±0.83 | 10.19 | 8.9 | 13.96±7.48 | 14.23±0.32 |
| | 5 PPM | 2.91±0.27 | 1.64±0.13 | 15.35 | 14.06 | 15.35±0.68 | 21.14±2.45 |
| | 10PPM | 2.31±0.45 | 1.78±0.04 | 11.6 | 15.35 | 17.08±0.59 | 14.59±4.14 |
| | 40PPM | 2.87±1.01 | 2.36±1.04 | 15.93 | 14.93 | 16.01±5.04 | 15.6±3.88 |
| | 80PPM | 3.81±1.54 | 0.84±0.07 | 26.12 | 18.44 | 13.2 | 13.22±3.87 |
| | 100PPM | 1.8±0.51 | 1.91±0.47 | 24.89 | 39.79 | 15.29±0.19 | 21.01±1.73 |
| 15th day | 0ppm | 1.2±0.12 | 2.39±0.05 | 21.67 | 10.64 | 52.32±7.12 | 13.2±.66 |
| | 5ppm | 1.78±0.18 | 1.84±0.12 | 37.15 | 13.09 | 16.18±3.19 | 17.74±3.49 |
| | 10ppm | 3.57±0.14 | 1.9±0.28 | 20.96 | 11.93 | 18.73±3.33 | 21.03±12.06 |
| | 40ppm | 2.81±0.4 | 1.56±0.17 | 18.44 | 15.54 | 10.03±0.93 | 10.75±1.99 |
| | 80ppm | 0.96±0.05 | 1.06±0.08 | 25.54 | 67.97 | 10.62±1.65 | 7.96±2.49 |
| | 100ppm | 1.85±0.3 | 0.7±0.09 | 84.23 | 29.98 | 12.46±1.37 | 19.11±4.01 |

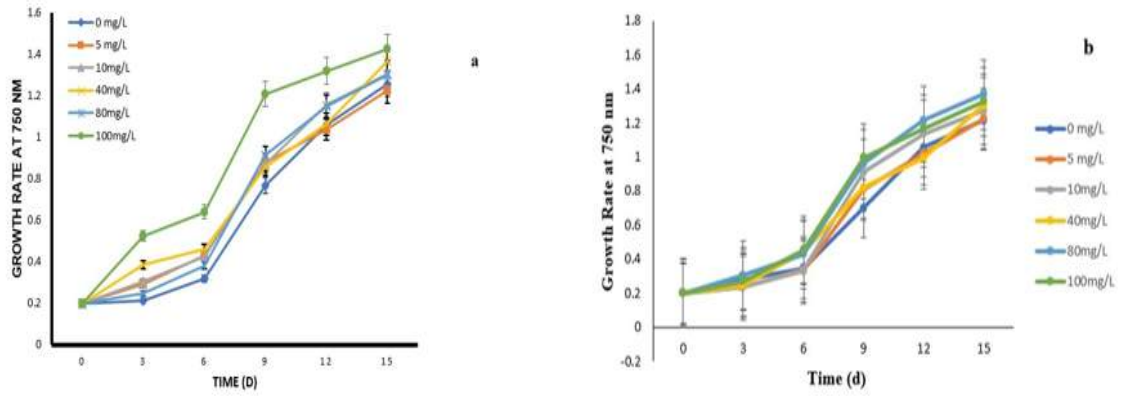



**Fig. 1: Spectrometric Growth analysis of DINP(a) and DOTP(b) treated Chroococcus sp**

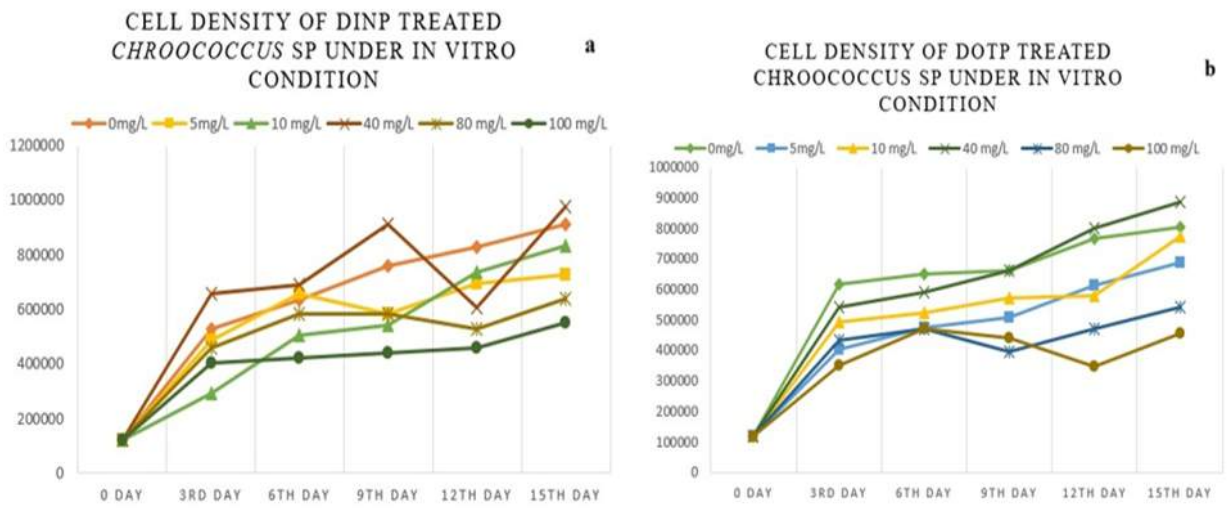



**Fig.2 a and b: Cell density of DINP and DOTP**

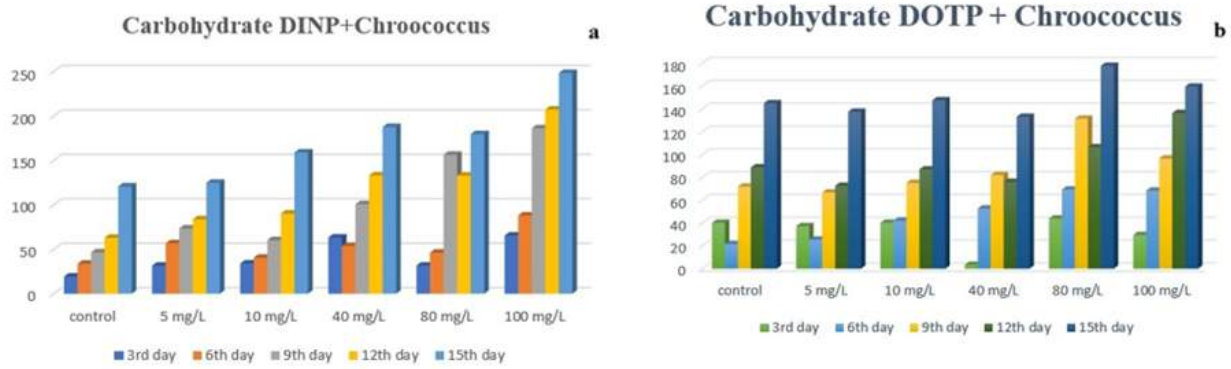



**Fig.3a and b: Carbohydrate synthesis of DINP and DOTP**

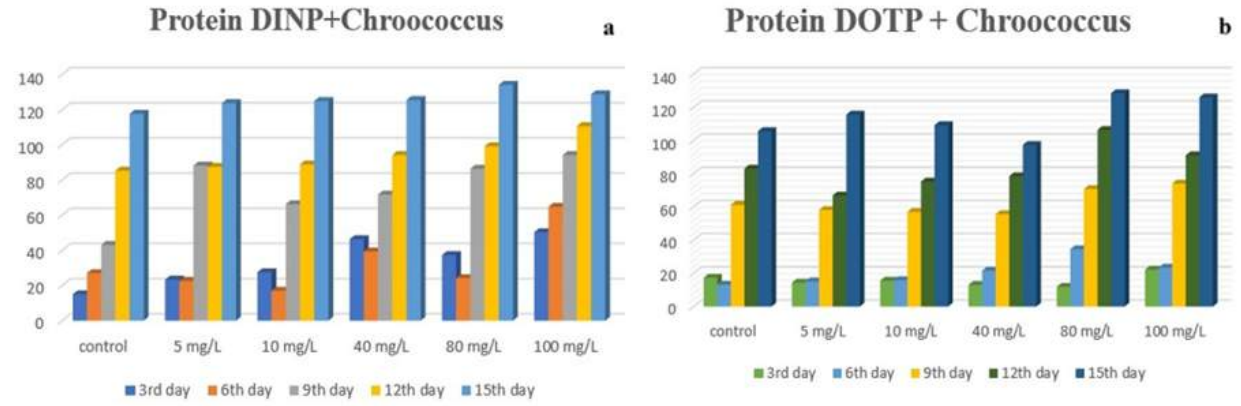



**Fig. 4a and b: protein synthesis of DINP and DOTP**

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**RESEARCH ARTICLE**

# A Comprehensive Overview of Phytochemical Analysis, Anti-bacterial, Anti-oxidant and Anti-inflammatory Activity of *Aloe barbadensis* Miller

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**ABSTRACT**

The semi-tropical plant *Aloe vera* L. Burman (AV) has numerous therapeutic uses, such as antibacterial, antioxidant, and anti-inflammatory qualities. Chinese medicine uses it to treat dermatitis, diabetes, hypertension, and infections. *Aloe vera* has volatile chemicals and ascorbic acid in its flowers, organic acids, phenols, enzymes, and minerals in its leaves, and pectin, lignin, and polysaccharides in its rind. This study assessed the aqueous extract of *Aloe vera* 's total phenol, flavonoid, and tannin contents in addition to its reducing power and 1, 1-Diphenyl-2-picrylhyrazyl (DPPH) radical scavenging abilities. The maceration process was used to extract *Aloe barbadensis miller*. Additionally, the *in vitro* antioxidant activities (DPPH free radical scavenging activity and reducing power activity) were assessed using conventional techniques. When compared to the typical value of leaf extract, the aqueous extract demonstrated superior DPPH free radical scavenging activity. According to the study's findings, *Aloe barbadensis miller* leaf extract may be utilized in the effective and environmentally friendly synthesis of compounds with anti-inflammatory and antibacterial qualities. Additionally, it implies that the polarity of the solvent is responsible for the existence of various compounds in plants.

**Keywords:** *Aloe barbadensis miller*, antibacterial activity; antioxidant activity, DPPH

## INTRODUCTION

Plants investigate a wide range of therapeutic and clinical features of ancient society and are rich sources of many medicinally significant chemicals. Many strong and effective medications are derived from medicinal plants, which are used worldwide to treat a number of chronic illnesses. Herbal medicines are in increasing demand as a primary health care source in both developed and developing nations because of their broad biological and therapeutic capabilities, strong safety margins, and lower costs [1]. India has a rich culture of medicinal herbs and spices, with over 2000 species of medicinally significant plants with high potential abilities for use in various traditional medicine systems. However, there are very few chemical and pharmacological studies for their medicinal potential in the literature. *Aloe barbadensis miller* is a shrubby, perennial succulent plant belonging to the Liliaceae family. Its turgid leaves are connected in a rosette arrangement at the stem. Large, thick, fleshy, stemless leaves with a sharp tip and a spiny edge are another characteristic of the plant [2,3]. *Aloe barbadensis miller*has long been utilized for a number of therapeutic purposes and is a key component of India's traditional medical system. Because of its many therapeutic benefits, it has become extremely important. There are hundreds of nutrients and active chemicals in *Aloe barbadensis miller*. Their pharmacological and therapeutic qualities are attributed to a variety of phytoconstituents, including vitamins, enzymes, minerals, carbohydrates, lignin, anthraquinones, saponins, salicylic acid, and amino acids [4]. *In vitro* and in vivo, phenolic compounds are thought to have anti-inflammatory and antioxidant properties. The plant part (leaf, stem bark, fruit, root, etc.), species, type of solvent (solvent polarity), and extraction technique all affect the quantity of phenols, flavonoids, and tannins in plants and their biological activity. Boudreau and Baland claim that the primary ingredient in AV that promotes fibroblast proliferation and enhances collagen synthesis and release is glucomannan. According to reports, glycogen synthesis, beta cell repair, insulin release, and glucose metabolism are Mostly driven by the melatonin and anthraquinone found in aloe [5, 6].

Ascorbic acid and volatile chemicals can be found in AV flowers; organic acids, phenolic compounds, enzymes, vita mins, and minerals can be found in AV leaves; pectin, lignin, cellulose, hemicellulose, and polysaccharides can be fou nd in the rind.It has been demonstrated that *Aloe barbadensis miller*contains anti-inflammatory, immunostimulatory, and cell growth-stimulating properties [7]. There have also been reports of antiviral, antifungal, and antibacterial activity against a range of pathogenic pathogens. *Aloe barbadensis miller* gel has been used historically to treat burn anomalies and various skin wounds. *Aloe barbadensis miller*leaf aqueous extract was generated and demonstrated in this study using UV and XRD. Additionally, its numerous applications, such as antioxidant and antibacterial capabilities, were tested against pathogenic of different bacterial strains. Anti-inflammatory action against the infections additionally provided this study represents the first attempt to assess *Aloe barbadensis miller's* comparative antibacterial potential. Researchers created an aquatic extract of *Aloe barbadensis miller* for this work. Characterizations of the prepared extract included antibacterial, anti-inflammatory, antioxidant, and UV-visible spectroscopy investigation.
The produced source is advised for drug delivery and antibacterial activity applications based on the characterisation results.

**Experimental section**
The ferric chloride, DPPH, HCL, Sulphuric acid, diclofenac sodium used in the present study was procured from Sigma–Aldrich Chemical Co., USA. All the other chemicals were used of analytical grade.

**Plant Material**
The *Aloe vera* (*Aloe barbadensis miller*) leaves was collected from kunichi, Tirupattur district and its rind was used as a sample. It was grinded to fine powder as per the standard approved protocol for further use.

**Collection and preparation of plant extracts**
Collecting *Aloe barbadensis miller* is getting ready to extract the dried plant for a week. The dried plants are pounded into a fine powder and kept in an airtight container after one week. Ten grams of plant powder were combined with

one hundred milliliters of ethanol in a 1:10 ratio for five to six hours in a soxhlet to create the water extract. The contents are filtered using Whatman No. 1 filter paper after six hours. The filtrate was then gathered separately for use in subsequent operations.

**Extraction of *Aloe barbadensis miller***

*Aloe barbadensis miller* was collected from the dried region and ground using a masternpistal. Ten grams of the crushed soil were then dissolved using 100 milliliters of ethanol in a beaker with a separate Soxhlet apparatus [8]. After the contents were extracted and filtered through Whatman No. 1 filter paper, the filtrates were concentrated in a rotary evaporator for use in additional research (figure 1).

**Qualitative Phytochemical Screening**

**Phytochemical screening**

In a conventional process, qualitative assessments were conducted on prepared *Aloe vera* aqueous extract. Phytochemicals were examined for in the aqueous extract [9].To prepare the ethanolic extract, 10 grams of plant powder were combined with 100 milliliters of ethanol at a 1:10 ratio for five to six hours in a soxhlet. Whatman No. 1 filter paper is used to filter the contents after six hours. The filtrate was then collected separately, much like the aqueous extract, for phytochemical investigation.

**Test for alkaloids**

**Mayer's test**

A few drops of diluted HCl were added to a small amount of solvent-free extract, which was then filtered. The subsequent color test was performed on the filtrate. (a) 60 milliliters of distilled water were used to dissolve 1.36 grams of mercuric chloride. (b) After dissolving 5 grams of potassium iodide in 20 milliliters of distilled water, (a) and (b) were combined, and the volume was adjusted to 100 milliliters using distilled water. Alkaloids were present when Mayer's reagents produced a cream-colored precipitate.

**Test for flavonoids**

**Shinoda's test:** An equivalent volume of the extract was mixed with 5 milliliters of 20% sodium hydroxide. Flavonoids are indicated by a yellow solution.

**Test for steroids**

**Liebermann Buchard test:** Two milliliters of acetic anhydride are added to a tiny sample, and then three milliliters of $H_2SO_4$ solution are added. When steroids are present, the color shifts from violet to green or blue.

**Test for terpenoids**

**Salkowski Test**: The presence of terpenoids is indicated by the production of a reddish-brown precipitate when 0.5 ml of chloroform and a few drops of strong sulfuric acid are added to 1 ml of extract.

**Test for Saponins**

**Froth test:** Twenty milliliters of distilled water are added to five milliliters of extract, and the mixture is shaken for ten minutes. When foam forms, saponins are present.

**Test for Carbohydrates**

**Fehling test**

Fehling's solutions A and B were used to heat two milliliters of each plant extract after they had been hydrolyzed with diluted HCl and neutralized with alkali. The presence of a reducing sugar was shown by the development of a crimson precipitate.

**Test for tannins and phenolic compounds**

**Lead Acetate test:**0.5g of the extract was added to a 10% lead acetate solution and agitated until it dissolved. When phenolic chemicals and tannins are present, a white precipitate is seen.

**Test for glycosides**

**Keller-Killani test**

One drop of 5% ferric chloride, strong sulfuric acid, and glacial acid were added to two milliliters of extract. The presence of glycosides is indicated by a reddish-brown appearance at the intersection of the two liquid layers.

**Test for Quinones**

**Sulfuric acid test**: Five milliliters of each extract were dissolved in isopropyl alcohol, and one drop of strong sulfuric acid was added. When quinones are present, the color turns red.

**Test for Phenols**

A few drops of ferric chloride (10%) are added to the sample solution. Phenols are present when the color turns blue or green.

**Test for saponins**

The sample solution was mixed with two milliliters of distilled water and thoroughly shaken. Foam formation is a sign that saponins are present.

**Antibacterial activity**

*Escherichia coli* was the negative strain and *Bacillus subtilis*and *Staphylococcus aureus*were the two positive strains of bacteria that were tested against *Aloe barbadensis miller* aqueous extracts. Utilizing Mueller Hinton Agar and Broth (Himedia), the bacterial cultures were grown.The agar well diffusion method was used to measure antibacterial activity. The inoculums were prepared using a fresh culture that was cultivated overnight. Peptone water was used to prepare the inoculums, which were then incubated for two hours. The turbidity was adjusted to 0.5 McFarland units, or roughly 108 CFU/ml. using a sterile spreader, inoculums were applied to brand-new nutrient agar plates. A sterile cutter was used to cut four 6 mm-diameter wells. The control disc was positioned in the middle. To investigate the antibacterial activity against the employed bacterial strains, a 40µl solution of varying concentrations was administered to each well [10]. The negative control was DMSO. As a positive control, 10 µg discs of gentamycin (Himedia Laboratories Pvt. Ltd., India) were utilized. Both were bought from Plates and incubated at 37°C for a whole day. HiMedia Laboratories Pvt. Ltd. India provided the standard ruler HiAntibiotic ZoneScaleTM-C, which was used to measure the clear zone of inhibition surrounding each well. Antibacterial activity was tested using diffusion disc plates on agar, with around 0.1 mL of each bacterial culture applied to the plates' surfaces. For the antibacterial assay, all bacterial strains were grown in Mueller Hinton Broth (Hi media) for an entire day at 37°C. After that, they were plated on Mueller Hinton Agar (Hi media) for agar diffusion testing. On the agar medium, paper discs with a diameter of 6 mm were filled with 100 µL of *Aloe barbadensis miller* leaf extracts. Inhibition diameters were measured after 24 to 48 hours of incubation at 37°C. Additionally, solvent-only blanks that had the same processing were tested for antibacterial activity [11, 12].

**Antioxidant activity by DPPH radical scavenging assay**

The antiradical potency of pure drugs or plant extracts under similar circumstances can be determined using the DPPH radical test. It gauges how well an antioxidant (AH, usually phenolic compounds) can use hydrogen transfer to lessen the chemical free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl). At first, DPPH is violet; however, as DPPH-H, it turns pale yellow. This was investigated in accordance with [7]. Fifty milliliters of methanol were added to the reaction mixture. DPPH, or diphenyl-2-picryl hydrazyl radical: One milliliter of *Aloe vera* leaf extracts in concentrations of 20, 40, 60, 80, and 100 microliters was mixed with three milliliters of 1 mM DPPH in methanol. Methanol by itself served as a blank, while DPPH solution containing methanol served as the positive control (ascorbic acid) [13]. The hue of the sample changed from deep purple to pale yellow when DPPH and the antioxidant

interacted. Calorimetric measurements were made at 518 nm. The percentage for scavenging activity was calculated by the following formula: Scavenging activity (%) = A518 (control)-A518 (sample)/X 100 A518 (control).

**Anti-inflammatory activity**

*Aloe barbadensis miller*'s anti-inflammatory properties were assessed in accordance with standard method. To evaluate the anti-inflammatory activity, 0.45 mL of a 1% aqueous solution of bovine serum albumin was mixed with 0.05 mL of *Aloe barbadensis miller*at different doses ranging from 20µg/mL to 100µg/mL. A tiny quantity of 1N hydrochloric acid was added to the solution to bring its pH down to 6.3. After 20 minutes of room temperature incubation, these samples were heated for 30 minutes at 55°C in a water bath. Following the heating procedure, the samples were allowed to cool before the absorbance was measured at 660 nm using a spectrophotometer [14,15]. The reference medication used for comparison was diclofenac sodium. In this experiment, dimethyl sulfoxide (DMSO) served as the control.

The percentage of protein denaturation was determined using the following equation: % Inhibition = (Absorbance of control – Absorbance of sample/Absorbance of control) x 100.

## RESULTS AND DISCUSSION

**Phytochemical Analysis**

Aloe barbadensis miller's ethanolic and aqueous extracts were evaluated using qualitative phytochemical analysis [16]. To determine whether different phytochemicals are present. The presence of alkaloids, carbohydrates, quinones, steroids, tannins, and terpenoids in the extracted materials is displayed in figure 2 and Table 1. Glycosides, phenols, and flavonoids are not present in the ethanolic extract, nor are they in the aqueous extract.

**Anti-bacterial activity*Aloe barbadensis miller***

Plants have been used as medicine since the dawn of human civilization. The issues with synthetic antibiotics make it obvious that the use of plants to treat illnesses was unavoidable [17]. *Aloe barbadensis miller* was investigated by numerous researchers using a range of polar chemical solvents, including high methanol. Antimicrobial activity and qualitative analysis were investigated. When comparing methanol extracts to the well diffusion method, the largest inhibitory zones against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* were 14 mm, 15 mm, and 12 mm. The antibacterial activity of *Aloe barbadensis miller* dissolved in DMSO (300 mg/mL) at dosages of 50, 75, and 100 µg/mL was demonstrated against the pathogens examined [18]. (Figure 3, Table 2).

**Anti-oxidant activity of *Aloe barbadensis miller* Via DPPH Assay**

Antioxidants are important in preventing a number of illnesses. It has been demonstrated that the phytochemicals' important antioxidant qualities are essential for lowering the incidence of several diseases [19, 20]. Graphical Representation of anti-oxidant activity of *Aloe barbadensis miller* (Fig 4). Our findings showed that, in a content-dependent way, all *Aloe vera* leaf extracts enhanced decreasing antioxidant capability and blocked free radicals. The highest DPPH radical scavenging activity was found in *Aloe vera* leaves, which were followed by the seeds, rind, whole fruit, and flesh. Based on the results of DPPH radical scavenging activity, the IC50 ranges of the aqueous extract of *Aloe vera* leaf extracts were found to be 56.6µg when compared to other plant extractions. When compared to traditional ascorbic acid, the IC50 levels were determined to be 56.8µg. By contrasting the standard with the unknown, the beneficial effects of *Aloe vera* leaf extract were ultimately discovered [21]. The results of the *in vitro* models unequivocally show that the aqueous extract has superior antioxidant activity compared to the other plant extract because it contains higher phenolic and flavonodal components, respectively. By preventing the start and advancement of oxidative chain reactions, antioxidants either prevent or postpone the oxidation of other molecules. They are essential for preventing and lessening the damage that radicals do to living things. It has been shown that flavonoids and other phenolic chemicals produced from plants, such as proanthocyanidins, rosmarinic acid, and hydroxycinnamic derivatives, scavenge and lessen lipid peroxidation [22].

**Anti-inflammatory Activity**

The bovine serum albumin denaturation test was used to assess *Aloe barbadensis miller*anti-inflammatory properties. *Aloe vera* 's inhibitory effects were evaluated at various doses and contrasted with benchmark values. The percentage of inhibition was found to be 11% at 20 µg/mL, 21% at 40 µg/mL, 32% at 60 µg/mL, 39% at 80 µg/mL, and 45% at 100 µg/mL. These results show that by preventing the denaturation of bovine serum albumin, aqueous extract *Aloe vera* has strong anti-inflammatory properties [23, 24]. Furthermore, at every measured concentration, the produced sample's anti-inflammatory qualities were on par with those of normal diclofenac sodium. Graphical Representation of anti-inflammatory activity of *Aloe barbadensis miller (*Fig 5).

## CONCLUSION

The pharmacological and therapeutic properties of Aloe barbadensis miller, such as its antibacterial, anti-inflammatory, and antioxidant properties, are being studied for the first time, according to the present experimental research. Except for steroids, terpenoids, and phenols, phytochemical examination revealed the presence of a variety of phytochemicals, including carbohydrates, tannins, saponins, alkaloids, flavonoids, quinones, and glycosides. Strong antibacterial properties are demonstrated by *Aloe barbadensis miller* against harmful pathogens such as *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. The aqueous *Aloe vera* extract showed a significant degree of antioxidant qualities in the DPPH test. Using the albumin denatured state method, the aqueous extract of *Aloe barbadensis miller* demonstrated good anti-inflammatory capabilities when compared to the common drug diclofenac. This study has demonstrated that using and consuming *Aloe vera* for a variety of purposes has many pharmacological, therapeutic, and health-promoting potentials.

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**Author contributions**

P. Vignesh: Investigation, Methodology, Writing-Original draft, Supervision, Acquisition, and K. Sheela: Conceptualization, Investigation, Methodology, Writing review and editing.

**Data availability**

Not applicable.

**Code availability**

Not applicable.

**Conflict of Interest**

The authors of this study would like to declare no conflict of interest.

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**Table 1: Phytochemical analysis of *Aloe vera***

| S.No | Phytochemicals | Ethanolic extract of *Aloe vera* | Aqueous extract of *Aloe vera* |
|---|---|---|---|
| 1. | Carbohydrate | + | + |
| 2. | Alkaloids | + | + |
| 3. | Glycosides | - | - |
| 4. | Phenols | - | - |
| 5. | Flavonoids | + | - |
| 6. | Saponins | + | + |
| 7. | Steroids | + | + |
| 8. | Tannins | + | + |
| 9. | Terpenoids | + | + |
| 10. | Quinones | + | + |

**Table 2: Antibacterial activity of *Aloe barbadensis miller***

| S.No. | Organism | DMSO | Gentamycin | Zone of inhibition | | |
|---|---|---|---|---|---|---|
| | | | | 50 µg/mL | 75 µg/mL | 100 µg/mL |
| 1. | *Escherichia coli* | - | 13 mm | 12 mm | 13 mm | 14 mm |
| 2. | *Staphylococcus aureus* | - | 16 mm | 11 mm | 12 mm | 15 mm |
| 3. | *Bacillus subtilis* | - | 17 mm | 10 mm | 11 mm | 12 mm |

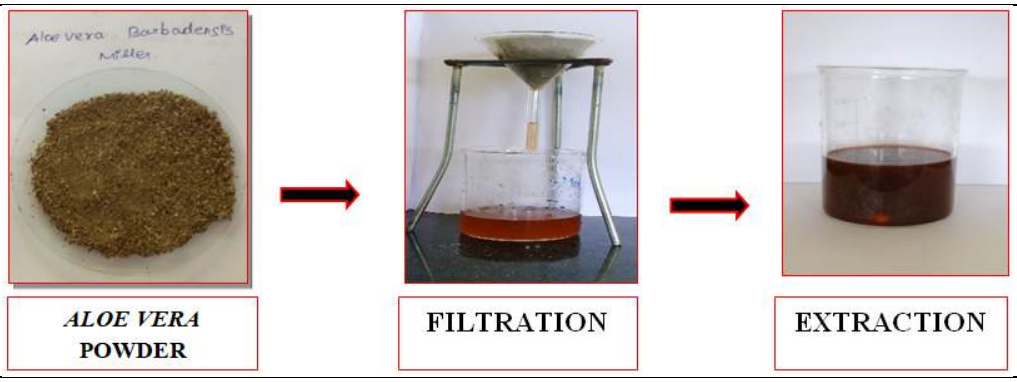



**Fig 1: Schematic illustration for the Extraction of *Aloe vera***



**Fig 2: Phytochemical analysis of *Aloe vera***

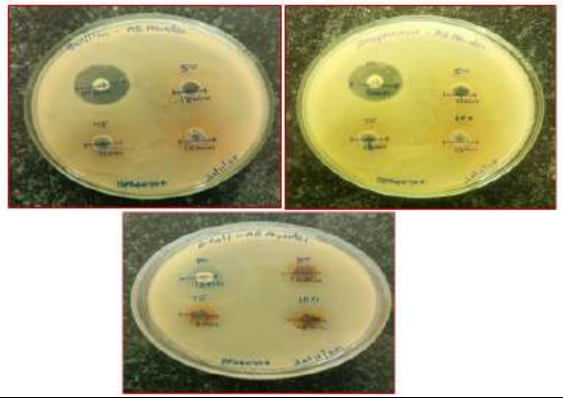


**Fig 3: Anti-bacterial activity of *Aloe barbadensis miller***

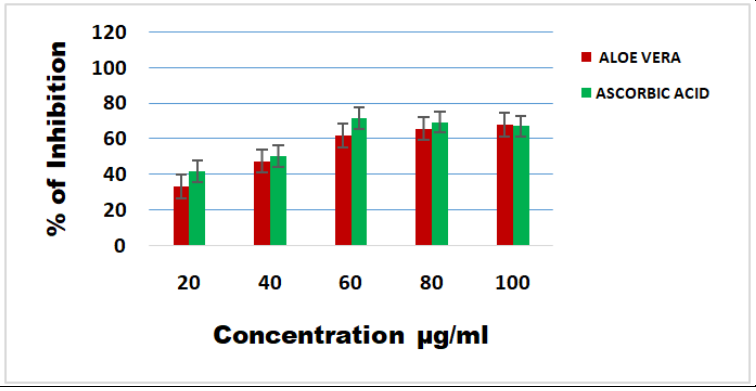



**Fig 4: Graphical Representation of anti-oxidant activity of *Aloe barbadensis miller***

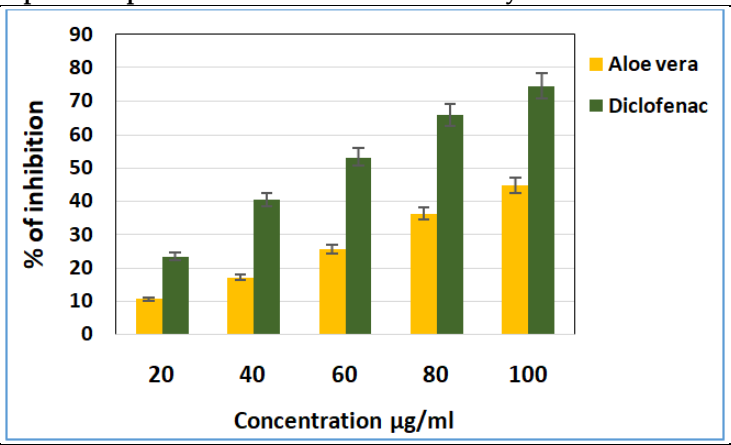



**Fig 5: Graphical Representation of anti-inflammatory activity of *Aloe barbadensis miller***

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RESEARCH ARTICLE

# Some Properties of (t,s)-Bipolar Fuzzy Subgroups

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**ABSTRACT**

In this paper, we have introduced the concept of (t,s)-Bipolar valued fuzzy subgroups, (t,s)-Bipolar valued fuzzy normal subgroups and investigate some associated results.

**Mathematics Subject Classification:** 03F55, 08A72, 20N25, 03E72, 06F35
**Keywords:** (t,s)-bipolar fuzzy set ((t,s)-BPFS), (t,s)-bipolar fuzzy subgroup ((t,s)-BPFSG), (t,s)-bipolar fuzzy normal subgroup ((t,s)-BPFNSG), ($\alpha$,$\beta$)-cut, Homomorphism..

## INTRODUCTION

In 1965, in his pioneer paper L.A. Zadeh [11] introduced the concept of fuzzy set to handle the uncertainties in our daily life. After that many generalizations of fuzzy sets are presented, for example, interval valued fuzzy sets [12] and intuitionistic fuzzy sets [1]. Fuzzy sets are extremely useful to solve many problems in applied mathematics, information sciences and theory of automata [2]. The concept of fuzzy sets in Algebraic structures was first introduced by Rosenfeld in 1971 and he defined fuzzy subgroups [8]. The notion of t-intuitionistic fuzzy set, t-intuitionistic fuzzy group, t-intuitionistic fuzzy coset was introduced by P.K.Sharma[9]. In [5] K.M. Lee introduced the concept of Bipolar valued fuzzy sets. In [4] Jun and Song applied the notion of Bipolar valued fuzzy sets in BCH-algebra. Recently F. Nisar and others applied the notion of Bipolar valued fuzzy sets in BCI-algebra [7].And in [10] Tahir Mahmood and Muhammad Munir used the notion of Bipolar valued fuzzy sets in group theory and developed

some basic results. In this paper we introduced the notion of (t,s)- Bipolar valued fuzzy sets, (t,s)- Bipolar valued fuzzy subgroups of a group G and discussed some of its related properties.

## PRELIMINARIES

**Definition[11]:** A fuzzy subset of a non-empty set $X$ is a function$A: X \to [0,1]$. The set of all fuzzy subsets of a set $X$ is denoted by FP($X$). For$A, B \in FP(X)$, we have the following operations on FP(X).

$(A \vee B)(x) = \max\{A(x), B(x)\} = A(x) \vee B(x)$ for all $x \in X$.

$(A \wedge B)(x) = \min\{A(x), B(x)\} = A(x) \wedge B(x)$

**Definition:** Let H be a non-empty subset of a non-empty set X. Then characteristic function of $H$ is a function $C_H: X \to \{0,1\}$ defined by

$$C_H(x) = \begin{cases} 1 & \text{if } x \in H \\ 0 & \text{if } x \notin H \end{cases}$$

Obviously $C_H \in FP(X)$.

**Definition :** Let $x$ be an element of $X$ and $l \in (0,1]$. Then a fuzzy subset $A$ of X of the form

$$A(z) = \begin{cases} l & \text{if } z = x \\ 0 & \text{if } z \neq x \end{cases}$$

is called fuzzy point with value $l$ and support $x$ or fuzzy singleton subset of $X$. It is denoted by$l_x$.

**Definition:** Let G be a group and $A \in FP(G)$. Then we have the following subsets of G :

1. $U(A, l) = \{x \in G: A(x) \geq l\}, l \in (0,1]$. It is called level subset of $G$.
2. $A^* = \{x \in G: A(x) > 0\}$. It is called support of $A$.
3. $A_* = \{x \in G: A(x) = A(e)\}$. Where e is identity of G.
4. $Ker(A) = \{x \in G: A(x) = 1\}$. It is called Kernel of $A$.

**Definition:** Let $G$ be a group and $A, B \in FP(X)$. Then, the fuzzy subsets $A \circ B$ and $A^{-1}$ of G are defined as:

$$(A \circ B)(x) = \sup_{x=yz} \{\min(A(y), A(z))\}$$

and

$$A^{-1}(x) = A(x^{-1})$$

where $A \circ B$ is called product of $A, B$ and $A^{-1}$ is called inverse of $A$.

**Definition [6]:** A fuzzy subset $A$ of a group G is said to be a fuzzy subgroup of G if for all $x, y \in G$

$A(xy) \geq \min\{A(x), A(y)\}$

$A(x^{-1}) \geq A(x)$

or equivalently

$A(x^{-1}y) \geq \min\{A(x), A(y)\}$

**Definition [6]:** A fuzzy subgroup$A$ of a group G is said to be fuzzy normal subgroup of $G$, if for all $x, y \in G$

$A(y^{-1}xy) \geq A(x)$

**Definition [6]:** Let $A$ and B be two fuzzy subgroups of a group G. Then, $A$ is said to be fuzzy conjugate of $B$ if for some $x \in G$, $A(y) = B(x^{-1}yx)$ for all $y \in G$.

**Definition [6]:** For a fuzzy subgroup $A$ of a group G, the normalizer of $A$ in G is denoted and defined by $N(A) = \{y \in G: A(y^{-1}xy) \geq A(x)$ for all $x \in G\}$.

**Definition [6]:** Let $A$ be a fuzzy subgroup of a group G. Then,

Fuzzy left coset of $f$ in G determined by $x \in G$ is a fuzzy subset ${}_xA$ of G defined by ${}_xA(y) = A(x^{-1}y)$.

Fuzzy right coset of $f$ in G determined by $x \in G$ is a fuzzy subset $A_x(y)$ of $G$ defined by $A_x(y) = A(yx^{-1})$.

**Definition [6]:** A fuzzy subgroup $A$ of a group G is said to be abelian fuzzy subgroup of G if and only if for all $x, y \in G, A(xy) = A(yx)$.

**Remark [6]:** Let $A$ be a fuzzy subgroup of a group G. Then
$\mathbb{N}(A) = \{y \in G: A(xy) = A(yx), \forall x \in G\}$

**Theorem [6]:** Let $A$ be a fuzzy normal subgroup of a group $G$. Then, for all $x_1, x_2 \in G$,
$A_{x_1} \circ A_{x_2} = A_{x_1x_2}$ and ${}_{x_1}A \circ {}_{x_2}A = {}_{x_1x_2}A$

**Definition[7] :** Let X be a non empty set. A Bipolar valued fuzzy subset $B$ of $X$ is an object having the form
$B = \{(x, P(x), N(x)): x \in X\}$
where $P: X \to [0,1]$ and $N: X \to [-1,0]$ are mappings. The positive membership degree $P(x)$ denotes the satisfaction degree of an element $x$ to the property corresponding to a bipolar valued fuzzy subset $B = \{(x, P(x), N(x)): x \in X\}$ and the negative membership degree $f(x)$ denotes the satisfaction degree of an element $x$ to some implicit counter property corresponding to a bipolar valued fuzzy subset $B = \{(x, P(x), N(x)): x \in X\}$
If $P(x) \neq 0$ and $N(x) = 0$, it is the situation that x is regarded as having only positive satisfaction $B = \{(x, P(x), N(x)): x \in X\}$
If $N(x) \neq 0$ and $P(x) = 0$, it is the situation that x does not satisfy the property of $B = \{(x, P(x), N(x)): x \in X\}$ but somewhat satisfies the counter property of $B = \{(x, P(x), N(x)): x \in X\}$. It is possible for an element x to be such that $P(x) \neq 0$ and $N(x) \neq 0$ when the membership function of the property overlaps that of its counter property over some portion of X. For our convenience from now to onward instead of writing $B = \{(x, P(x), N(x)): x \in X\}$
we will write $B =< P, N >$.

**(t,s)—bipolar Valued Fuzzy Subgroups**
**Definition:** Let X be a non empty set. A (t,s)-bipolar valued fuzzy subset $B^{(t.s)}$ of $X$ is an object having the form
$B^{(t.s)} = \{(x, P^t(x), N^s(x)): x \in X\}$
where $P^t: X \to [0,1]$ and $N^s: X \to [-1,0]$ are mappings. The positive t-membership degree $P^t(x)$ denotes the t –level satisfaction degree of an element $x$ to the property corresponding to a (t,s)-bipolar valued fuzzy subset $B^{(t.s)} = \{(x, P^t(x), N^s(x)): x \in X\}$ and the negative s-membership degree $f(x)$ denotes the s-level satisfaction degree of an element $x$ to some implicit counter property corresponding to a (t,s)- bipolar valued fuzzy subset $B^{(t.s)} = \{(x, P^t(x), N^s(x)): x \in X\}$.
If $P^t(x) \neq 0$ and $N^s(x) = 0$, it is the situation that x is regarded as having only positive satisfaction $B^{(t.s)} = \{(x, P^t(x), N^s(x)): x \in X\}$
If $N^s(x) \neq 0$ and $P^t(x) = 0$, it is the situation that x does not satisfy the property of $B^{(t.s)} = \{(x, P^t(x), N^s(x)): x \in X\}$ but somewhat satisfies the counter property of $B^{(t.s)} = \{(x, P^t(x), N^s(x)): x \in X\}$. It is possible for an element x to be such that $P^t(x) \neq 0$ and $f(x) \neq 0$ when the membership function of the property overlaps that of its counter property over some portion of X . For our convenience from now to onward instead of writing $B^{(t.s)} = \{(x, P^t(x), N^s(x)): x \in X\}$ we will write $B^{(t,s)} =< P^t, N^s >$.

**Definition:** Let $B_1{}^{(t,s)} =< P_1{}^t, N_1{}^s >$ and $B_2{}^{(t,s)} =< P_2{}^t, N_2{}^s >$ be two $(t, s)$ -Bipolar valued fuzzy subsets of a group $G$. Then $B_1 \vee B_2$ and $B_1 \wedge B_2$ of $X$ are defined as
$B_1 \vee B_2 = \langle P_1{}^t \vee P_2{}^t, N_1{}^s \vee N_2{}^s >$
And $B_1 \wedge B_2 = \langle P_1{}^t \wedge P_2{}^t, N_1{}^s \wedge N_2{}^s >$.

**Definition:** Let $G$ be a group and $A^{(t.s)}$ be a (t,s)-bipolar valued fuzzy subset of a non-empty subset H of $G$. Then $(t, s)$ –Bipolar valued Characteristic function of $H$ is given by $C_{A_H{}^{(t.s)}} = \langle C_{P_H{}^t}, C_{N_H{}^s} \rangle$, where

$$C_{P_H{}^t}(x) = \begin{cases} 1 & \text{if } x \in H \\ 0 & \text{otherwise} \end{cases}$$

$$C_{N_H{}^s}(x) = \begin{cases} -1 & \text{if } x \in H \\ 0 & \text{otherwise} \end{cases}$$

Obviously $(t.s)$ −Bipolar valued Characteristic function is a $(t.s)$ −Bipolar valued fuzzy subset of G.

**Definition:** Let $x$ be an element of a group $G$ and q∈ (0,1]. Then a $(t,s)$ −Bipolar valued fuzzy subset $B^{(t,s)} =< P^t, N^s >$ of $G$ of the form

$$P^t(z) = \begin{cases} q & \text{if } z = x \\ 0 & \text{if } z \neq x \end{cases}$$

$$N^s(z) = \begin{cases} -q & \text{if } z = x \\ 0 & \text{if } z \neq x \end{cases}$$

is called $(t,s)$ −Bipolar valued fuzzy point with value $t$ and support $x$ or $(t,s)$ −Bipolar valued fuzzy singleton subset of $G$. It is denoted by $q_x = \langle P_{q_x}, N_{q_x} \rangle$.

**Definition:** Let G be a group and $B_1{}^{(t,s)} =< P_1{}^t, N_1{}^s >$ and $B_2{}^{(t,s)} =< P_2{}^t, N_2{}^s >$ be two $(t,s)$ −Bipolar valued fuzzy subsets of G. Then, the product of $B_1$ and $B_2$ is defined by

$B_1{}^{(t,s)} \circ B_2{}^{(t,s)} = \langle P_1{}^t \circ P_2{}^t, N_1{}^s \circ N_2{}^s >$

Where

$$(P_1{}^t \circ P_2{}^t)(x) = \sup_{x=yz} \{\min(P_1{}^t(y), P_2{}^t(z))\}$$

and

$$(N_1{}^s \circ N_2{}^s)(x) = \inf_{x=yz} \{\max(N_1{}^s(y), N_2{}^s(z))\}$$

**Definition:** A $(t,s)$ −Bipolar valued fuzzy subset $B^{(t,s)} =< P^t, N^s >$ of a group $G$ is said to be $(t,s)-$ Bipolar valued fuzzy subgroup of G if

$P^t(x^{-1}y) \geq \min\{P^t(x), P^t(y)\}$ and $N^s(x^{-1}y) \leq \max\{N^s(x), N^s(y)\}$

**Example:** Let $G = C_4 = \{\pm 1, \pm i\}$. Then $G$ is a group under usual multiplication of complex numbers. Let $A^{(t,s)} =< P^t, N^s >$be a $(t,s)-$ Bipolar valued fuzzy subset of a Bipolar valued fuzzy subset $B =< P, N >$of G with $t = 0.25$ *and* $s = 0.18$, such that

$P(1) = 0.62, P(-1) = 0.45, P(i) = 0.32, P(-i) = 0.25$, $N(1) = -0.72, N(-1) = -0.52$ and $N(i) = -0.12, N(-i) = -0.18$.

Then $A^{(t,s)} =< P^t, N^s >$ is Bipolar valued fuzzy subgroup of G.

**Theorem:** Let H be a non-empty subset of a group G. Then, H ≤ G if and only if its (t,s)-Bipolar valued characteristic function $C_{A_H{}^{(t,s)}} = \langle C_{P_H{}^t}, C_{N_H{}^s} \rangle$ is a (t,s)-Bipolar valued fuzzy subgroup of G.

**Theorem:** A $(t,s)$ −Bipolar valued fuzzy subset $A^{(t,s)} =< P^t, N^s >$ of a group $G$ is $(t,s)$ −bipolar valued fuzzy subgroup of $G$ iff its level subset

$U(A^{(t,s)}, l) = \{x \in G: P^t(x) \geq l, N^s(x) \leq -l\}$ for all $l \in (0,1]$ is subgroup of $G$.

**Theorem:** Let $\{A^{(t,s)}{}_i =< P^t{}_i, N^s{}_i >: i \in I\}$ be a collection of $(t,s)$ −Bipolar valued fuzzy subgroups of a group G. Then, $\Lambda f_a$ is a $(t,s)$ −Bipolar valued fuzzy subgroup of G.

**Definition:** A $(t,s)$ −Bipolar valued fuzzy subgroup $A^{(t,s)} =< P^t, N^s >$ of a group G is said to be $(t,s)$ −Bipolar valued fuzzy normal subgroup of $G$, if for all $x, y \in G$

$P^t(y^{-1}xy) \geq P^t(x)$ and $N^s(y^{-1}xy) \leq N^s(x)$

**Example:** Let $G = S_3 = \{1, a, a^2, b, ab, a^2b\}$ and $A^{(0.80,-0.95)} =< P^{0.80}, N^{-0.95} >$ be a $(0.80, -0.95)$ −Bipolar valued fuzzy subset with respect to the Bipolar valued fuzzy subset $A =< P, N >$ of $G$ such that

$P(1) = 0.85, P(a) = P(a^2) = 0.95$

$P(b) = P(ab) = P(a^2b) = 0.40$
and
$N(1) = -0.90, N(a) = N(a^2) = -0.95$
$N(b) = N(ab) = N(a^2b) = -0.30$
Then $A^{(0.80,-0.95)} =< P^{0.80}, N^{-0.95} >$ is a $(0.80, -0.95)$ −Bipolar fuzzy normal subgroup of G.

**Definition:** Let $A^{(t,s)} =< P^t, N^s >$ and $B^{(t,s)} =< C^t, D^s >$be two (t,s)- Bipolar fuzzy subgroups of a group G. Then, $A^{(t,s)} =< P^t, N^s >$ is said to be fuzzy conjugate of $B^{(t,s)} =< C^t, D^s >$ if for some $x \in G$,
$P^t(y) = C^t(x^{-1}yx)$
$N^s(y) = D^s(x^{-1}yx)$ for all $y \in G$.

**Definition:** For a Bipolar valued fuzzy subgroup $A^{(t,s)} =< P^t, N^s >$ of a group G, the Normalizer of $A^{(t,s)} =< P^t, N^s >$ is denoted and defined by
$\mathbb{N}(A^{(t,s)}) = \{y \in G: P^t(y^{-1}xy) \geq P^t(x), N^s(y^{-1}xy) \leq N^s(x)$ for all $x \in G\}$.

**Theorem:** Let $A^{(t,s)} =< P^t, N^s >$ be a $(t, s)$ −Bipolar fuzzy subgroup of a group G. Then, $\mathbb{N}(A^{(t,s)})$ is a subgroup of G, $A^{(t,s)} =< P^t, N^s >$ is a $(t, s)$ −Bipolar valued fuzzy normal if and only if $\mathbb{N}(A^{(t,s)}) = G$.
**Proof:** Let $y_1, y_2 \in \mathbb{N}(A^{(t,s)})$. To prove $y_1y_2^{-1} \in \mathbb{N}(A^{(t,s)})$. Now for any $x \in G$,
$P^t[(y_1y_2^{-1})^{-1}x(y_1y_2^{-1})] = P^t[(y_2y_1^{-1})x(y_1y_2^{-1})]$
$= P^t[y_2(y_1^{-1}xy_1)y_2^{-1}] = P^t[(y_2^{-1})^{-1}(y_1^{-1}xy_1)y_2^{-1}]$
$\geq P^t(x)$ and $N^s[(y_1y_2^{-1})^{-1}x(y_1y_2^{-1})] = N^s[(y_2y_1^{-1})x(y_1y_2^{-1})]$
$= N^s[y_2(y_1^{-1}xy_1)y_2^{-1}] = N^s[(y_2^{-1})^{-1}(y_1^{-1}xy_1)y_2^{-1}]$
$\leq N^s(y_1^{-1}xy_1) \leq N^s(x)$. That is, $y_1y_2^{-1} \in \mathbb{N}(A^{(t,s)})$. Tharefore, $\mathbb{N}(A^{(t,s)}) \leq G$

**Definition:** Let $A^{(t,s)} =< P^t, N^s >$ be a $(t, s)$ −Bipolar valued fuzzy subgroup of a group G. Then, $t, s)$ −Bipolar valued fuzzy right coset of $A^{(t,s)} =< P^t, N^s >$ in $G$ determined by $x \in G$ is a Bipolar valued fuzzy subset $A_x{}^{(t,s)} =< P_x{}^t, N_x{}^s >$ of $G$ i.e. $P_x{}^t: G \to [0,1]$ defined by $P_x{}^t(y) = P^t(x^{-1}y)$ and $N_x{}^s: G \to [-1,0]$ defined by $N_x{}^s(y) = N^s(x^{-1}y)$.
A $(t, s)$ −Bipolar valued left coset of $A^{(t,s)} =< P^t, N^s >$ in G determined by $x \in G$ is a $(t, s)$ −Bipolar valued fuzzy subset ${}_xA^{(t,s)} =< {}_xP^t, {}_xN^s >$ of G. i.e. ${}_xP^t: G \to [0,1]$ defined by ${}_xP^t(y) = P^t(yx^{-1})$ and ${}_xN^s: G \to [-1,0]$ defined by ${}_xN^s(y) = N^s(yx^{-1})$.

**Theorem:** Let $A^{(t,s)} =< P^t, N^s >$ be a $(t, s)$ −Bipolar valued fuzzy subgroup of a group G. Then, $A^{(t,s)} =< P^t, N^s >$ is $(t, s)$ − Bipolar fuzzy normal subgroup of $G$ if and only if for all $x \in G$, ${}_xP^t = P_x{}^t$ and ${}_xN^s = N_x{}^s$.
**Proof:** Let us assume $A^{(t,s)} =< P^t, N^s >$ is $(t, s)$ −Bipolar valued fuzzy normal subgroup of G. To prove for all $x \in G$, ${}_xP^t = P_x{}^t$ and ${}_xN^s = N_x{}^s$. Now, ${}_xP^t(y) = P_x{}^t(y)$
i.e. $P^t(x^{-1}y) = P^t(yx^{-1})$
Now
$P^t(x^{-1}y) = P^t(x^{-1}yx^{-1}x) = P^t(x^{-1}(yx^{-1})x) \geq P^t(yx^{-1})$
And
$P^t(yxx^{-1}) = P^t(xx^{-1}yx^{-1}) \geq P^t(x^{-1}y)$
$P^t(x^{-1}y) = P^t(yxx^{-1})$
${}_xP^t(y) = P_x{}^t(y)$
${}_xP^t = P_x{}^t$
Now to prove, ${}_xN^s(y) = N_x{}^s(y)$. That is,
$N^s(x^{-1}y) = N^s(yx^{-1})$
Now, $N^s(x^{-1}y) = N^s(x^{-1}yxx^{-1}x) = N^s(x^{-1}(yxx^{-1})x) \leq N^s(yx^{-1})$
$N^s(yx^{-1}) = N^s(xx^{-1}yx^{-1}) \leq N^s(x^{-1}y)$
$N^s(x^{-1}y) = N^s(yx^{-1})$
And
${}_xN^s(y) = N_x{}^s(y)$
${}_xN^s = N_x{}^s$

Conversely assume for all, $x \in G$, ${}_xP^t = P_x{}^t$ and ${}_xN^s = N_x{}^s$. To prove $A^{(t,s)} =< P^t, N^s >$ is $(t,s)$ −Bipolar valued fuzzy normal subgroup of G..

Now for any $x, y \in G$.

$P^t(x^{-1}yx) = {}_xP^t(yx) = P_x{}^t(yx) = P^t(yxx^{-1}) = P^t(y) \geq P^t(y)$

$P^t(x^{-1}yx) \geq P^t(y)$

Similarly, $N^s(x^{-1}yx) = {}_xN^s(yx) = N_x{}^s(yx) = N^s(yxxx^{-1}) = N^s(y) \leq N^s(y)$

$N^s(x^{-1}yx) \leq N^s(y)$.

Hence, $A^{(t,s)} =< P^t, N^s >$ is $(t,s)$ −Bipolar valued fuzzy normal subgroup of G.

**Definition:** A $(t,s)$ −Bipolar valued fuzzy subgroup $A^{(t,s)} =< P^t, N^s >$of a group G is said to be $(t,s)$ −Bipolar valued fuzzy abelian subgroup of $G$ if and only if for all $x, y \in G$,

$P^t(xy) = P^t(yx)$ and $N^s(xy) = N^s(yx)$

**Theorem:** For a $(t,s)$ −Bipolar valued fuzzy subgroup $A^{(t,s)} =< P^t, N^s >$ of a group G the following are equivalent:

i. i). $A^{(t,s)} =< P^t, N^s >$ is $(t,s)$ −Bipolar valued fuzzy abelian subgroup of G

ii. ii). $A^{(t,s)} =< P^t, N^s >$ is Bipolar valued fuzzy normal subgroup of G.

**Remark:** Let $A^{(t,s)} =< P^t, N^s >$ be a $(t,s)$ −Bipolar valued fuzzy subgroup of a group G. Then,

$\mathbb{N}(A^{(t,s)}) = \{y \in G: P^t(xy) = P^t(yx)$ and $N^s(xy) = N^s(yx)$ for all $x \in G\}$

**Theorem:** Let $A^{(t,s)} =< P^t, N^s >$ be a $(t,s)$ −Bipolar valued fuzzy subgroup of a group $G$. Then, the cardinality of $A^{(t,s)^u} = \{< P^{tu}, N^{su} >: u \in G\}$ is equal to index of $\mathbb{N}(A^{(t,s)})$ in G, where $A^{(t,s)^u} = \{< P^{tu}, N^{su} >: u \in G\}$ is $(t,s)$ −Bipolar valued conjugate fuzzy subgroup G defined by $P^{tu}(x) = P^t(u^{-1}xu)$ and $N^{su}(x) = N^s(u^{-1}xu)$ for all $x \in G$.

**Proof:** To prove $T = \left\{ \begin{matrix} A^{(t,s)^u} = \{< \\ P^{tu}, N^{su} >: u \in G\} \end{matrix} \right\}$ and $T = \{u\mathbb{N}(A^{(t,s)}): u \in G \; and \; A^{(t,s)} =< P^t, N^s >\}$ are equivalent.

Define $: T \to T'$ by $\varphi\left(A^{(t,s)^u}\right) = u\mathbb{N}(A^{(t,s)})$. For any $u_1, u_2 \in G$,

$P^{tu_1} = P^{tu_2}$

$P^t(u_1^{-1}xu_1) = P^t(u_2^{-1}xu_2)$ for all $x \in G$

$P^t(xu_2^{-1}u_1) = P^t(u_2^{-1}u_1x)$ for all $x \in G$

Similarly, $N^{su_1} = N^{su_2}$

$N^s(u_1^{-1}xu_1) = N^s(u_2^{-1}xu_2)$ for all $x \in G$

$N^s(xu_2^{-1}u_1) = N^s(u_2^{-1}u_1x)$ for all $x \in G$

$u_2^{-1}u_1 \in \mathbb{N}(N^s), u_1\mathbb{N}(N^s) = u_2\mathbb{N}(N^s)$

so, $\varphi$ is bijective from T to T'. Hence the result.

**Remark:** With usual meanings, $1_x oP^t = {}_xP^t, 1_x oN^s = {}_xN^s$ and $P^t o1_x = P_x{}^t, N^s o1_x = N_x{}^s$

**Theorem:** Let $A^{(t,s)} =< P^t, N^s >$ be a $(t,s)$ −Bipolar valued fuzzy normal subgroup of a group $G$. Then, for all $x_1, x_2 \in G$,

i. $P_{x_1}{}^t \circ P_{x_2}{}^t = P_{x_1x_2}$ and $N_{x_1}{}^s \circ N_{x_2}{}^s = N_{x_1x_2}{}^s$.

ii. $P_{x_1}{}^t oP_{x_2} = P_{x_1x_2}$ and ${}_{x_1}N^s \circ {}_{x_2}N^s = {}_{x_1x_2}N^s$

**Theorem:** Let G be a group and $A^{(t,s)} =< P^t, N^s >$ be a$(t,s)$ − Bipolar valued fuzzy normal subgroup of $G$. Then, the set $G/A^{(t,s)} = \{ {}_xA^{(t,s)} =< {}_xP^t, {}_xN^s >: x \in G\}$ is group. (It is called a factor group or quotient group).

**Proof:** For ${}_{x_1}A^{(t,s)} =< {}_{x_1}P^t, {}_{x_1}N^s >, {}_{x_2}A^{(t,s)} =< {}_{x_2}P^t, {}_{x_2}N^s > \in G/f$

Define ${}_{x_1}A^{(t,s)} \circ {}_{x_2}A^{(t,s)} = \langle {}_{x_1}P^t \circ {}_{x_2}P^t, {}_{x_1}N^s \circ {}_{x_2}N^s \rangle$

Then, $G/f$ is closed.

Also, " o " is associative in $G/f$. If e is identity in G , then ${}_eA^{(t,s)} = \langle {}_eP^t, {}_eN^s \rangle \in G/f$ and for all

$${}_xA^{(t,s)} = < {}_xP^t, {}_xN^s > \in \frac{G}{f}$$

$${}_eP^t o\ {}_xP^t = {}_{ex}P^t = {}_xP^t$$

$${}_xP^t o\ {}_eP^t = {}_{xe}P^t = {}_xP^t$$

and

$${}_eN^s \text{ o } {}_xN^s = {}_{ex}N^s = {}_xN^s$$

$${}_xN^s \text{ o } {}_eN^s = {}_{xe}N^s = {}_xN^s$$

So, ${}_xA^{(t,s)} \circ {}_eA^{(t,s)} = {}_eA^{(t,s)} \circ {}_xA^{(t,s)} = {}_xA^{(t,s)}$

${}_eA^{(t,s)} = \langle {}_eP^t, {}_eN^s \rangle$ is identity in $G/f$.

Also as for every, ${}_xA^{(t,s)} = \langle {}_xP^t, {}_xN^s \rangle \in G/f$

we have, ${}_{x^{-1}}A^{(t,s)} = \langle {}_{x^{-1}}P^t, {}_{x^{-1}}N^s \rangle \in G/f$

such that

$${}_xA^{(t,s)} \circ {}_{x^{-1}}A^{(t,s)} = \langle {}_xP^t \circ {}_{x^{-1}}P^t, {}_xN^s \circ {}_{x^{-1}}N^s \rangle$$

$$= \langle {}_{xx^{-1}}P^t, {}_{xx^{-1}}N^s \rangle = \langle {}_eP^t, {}_eN^s \rangle = {}_eA^{(t,s)}$$

and

$${}_{x^{-1}}A^{(t,s)} \circ {}_xA^{(t,s)} = \langle {}_{x^{-1}}P^t \circ {}_xP^t, {}_{x^{-1}}N^s \circ {}_xN^s \rangle$$

$$= \langle {}_{x^{-1}x}P^t, {}_{x^{-1}x}N^s \rangle = \langle {}_eP^t, {}_eN^s \rangle = {}_eA^{(t,s)}$$

So, for every ${}_xA^{(t,s)} \in G/f$ , ${}_{x^{-1}}A^{(t,s)} \in G/f$ and ${}_{x^{-1}}A^{(t,s)}$acts as inverse of ${}_xA^{(t,s)}$ in $G/f$. Hence $G/f$ is group.

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RESEARCH ARTICLE

# Pharmacogenomics and Regulatory Science: Integrating Genetic Data into Drug Labelling

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## ABSTRACT

Pharmacogenomics plays an increasingly important role in precision medicine by allowing drug therapy to be tailored according to individual genetic differences. The successful clinical use of pharmacogenomic insights, however, depends largely on how effectively this information is incorporated into regulatory drug labeling. In real-world practice, notable differences exist among regulatory authorities in the assessment, presentation, and clinical interpretation of pharmacogenomic data. This study evaluates how pharmacogenomic evidence is integrated into drug labels and explores areas of agreement and divergence across major regulatory systems. A descriptive and comparative approach was employed using publicly accessible drug labels, biomarker databases, regulatory guidance documents, and established pharmacogenomic knowledge resources. Twenty representative gene–drug pairs covering multiple therapeutic areas were analyzed using predefined criteria, including genetic testing requirements, evidence strength, variant specificity, label location, and clinical relevance. The results demonstrate substantial variability across agencies in gene–drug acknowledgment and recommendation intensity, with limited concordance with existing clinical pharmacogenomic guidelines. Case-based findings suggest that inconsistent labeling may affect prescribing behavior, pose potential safety concerns, and limit uniform clinical adoption. Overall, the study highlights the need for improved regulatory

alignment to facilitate the consistent translation of pharmacogenomic evidence into clinical practice and drug development.

**Keywords:** Pharmacogenomics; Drug labelling; Genetic biomarkers; Regulatory science; Precision medicine; Gene–drug interactions; Personalized therapy; Clinical pharmacology.

## INTRODUCTION

**Pharmacogenomics and Regulatory Decision-Making in Precision Medicine**

Pharmacogenomics (PGx) examines how inherited or acquired genetic variation contributes to interindividual differences in drug response, safety, and dosing requirements. Variants in genes encoding drug-metabolizing enzymes, transporters, immune response elements, and molecular targets can significantly influence pharmacokinetic and pharmacodynamic outcomes, thereby shaping therapeutic efficacy and risk of adverse drug reactions [1–4]. Advances in genomic technologies and expanding clinical evidence have positioned PGx as a foundational component of precision medicine, enabling more individualized and safer pharmacotherapy across multiple therapeutic areas [5].

**Regulatory Relevance of Pharmacogenomic Evidence**

From a regulatory science perspective, the clinical utility of pharmacogenomic knowledge depends not only on scientific discovery but also on how genetic evidence is evaluated, contextualized, and translated into actionable guidance within regulatory drug labeling. Drug labels serve as the most authoritative and legally binding mechanism through which regulators communicate benefit–risk assessments to healthcare professionals. Regulatory incorporation of PGx information therefore represents a critical interface between genomic science, clinical pharmacology, and public health policy [6–8].

**Integration of Pharmacogenomics Across the Drug Lifecycle**

Regulatory agencies increasingly encounter PGx data throughout the medicinal product lifecycle, including during clinical development, marketing authorization, and post-marketing surveillance. Genetic evidence may inform patient selection, dose optimization, contraindications, or risk mitigation strategies, depending on the strength and nature of the underlying association [9–11]. However, substantial variability exists in how regulators interpret evidentiary sufficiency, define clinical actionability, and determine the appropriate placement and strength of PGx information within labeling documents [12–14].

**Biomarker Types and Regulatory Implications**

A key source of regulatory complexity arises from the heterogeneous nature of pharmacogenomic biomarkers. Germline variants affecting drug metabolism or immune-mediated toxicity are most frequently incorporated into regulatory labeling, while somatic mutations play a dominant role in oncology indications through predictive biomarkers and companion diagnostics [15–17]. Regulators further distinguish biomarkers based on functional role, including predictive markers guiding treatment selection, safety markers associated with serious adverse reactions, and pharmacokinetic markers influencing drug exposure. These distinctions directly affect regulatory thresholds for actionability and the resulting labeling language [18–20].

**Regulatory Fragmentation and the Need for Harmonization**

Despite a largely shared scientific evidence base, regulatory approaches to PGx labeling remain fragmented across jurisdictions. Differences in statutory mandates, evidentiary thresholds, population considerations, and risk tolerance have resulted in discordant labeling outcomes for identical gene–drug pairs [21–24]. Some agencies emphasize clinical outcome data when determining label actionability, whereas others accept robust pharmacokinetic or mechanistic evidence in the absence of prospective trials. Inconsistent terminology and variation in label placement

further complicate clinical interpretation and cross-regional implementation [25,26]. Such regulatory heterogeneity has important implications for clinical practice and global drug development. Divergent PGx labeling may lead to inconsistent prescribing behavior, unequal access to genotype-guided therapy, and challenges in harmonizing multinational development programs [27–29]. At the same time, overly conservative labeling may delay clinical adoption of well-supported PGx interventions, while overly prescriptive approaches may raise concerns related to equity, feasibility, and medico-legal responsibility [30].

**Study Rationale and Objectives**

Against this backdrop, the present study undertakes a systematic comparative analysis of pharmacogenomic integration into regulatory drug labeling across major regulatory authorities. By examining how PGx evidence is recognized, operationalized, and communicated in approved labels, this work aims to elucidate patterns of regulatory convergence and discordance, identify their underlying drivers, and assess implications for clinical implementation and regulatory harmonization. Through quantitative analysis and detailed case studies, the study contributes empirically grounded insights to the evolving field of pharmacogenomics-informed regulatory science [31–33].

**Regulatory Frameworks Governing Pharmacogenomic Labeling**

Although pharmacogenomic (PGx) science is globally shared, its translation into regulatory drug labeling is shaped by jurisdiction-specific legal mandates, public health priorities, and evidentiary standards. Major regulatory authorities—including the United States Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the Pharmaceuticals and Medical Devices Agency (PMDA)—have progressively incorporated PGx information into approved product labeling, but differ substantially in how genetic evidence is interpreted and operationalized [6,12,13]. The FDA adopts a flexible, evidence-tiered approach in which pharmacogenomic information is distributed across multiple label sections according to perceived clinical relevance. Strong genotype–phenotype associations linked to safety or well-established clinical outcomes may result in mandatory testing or explicit prescribing guidance, whereas associations supported primarily by pharmacokinetic or mechanistic evidence are often communicated descriptively to support clinical judgment [8,9,14]. In contrast, the EMA integrates PGx information through the harmonized Summary of Product Characteristics, generally favoring conservative and context-driven language, with prescriptive genetic testing requirements typically limited to scenarios supported by robust clinical outcome data [20–22]. The PMDA framework places particular emphasis on population-specific considerations and post-marketing risk management. Japanese labeling practices frequently highlight genetic influences on drug exposure or safety within the context of national genetic epidemiology, often providing detailed guidance on appropriate use and monitoring while remaining cautious in mandating pre-treatment testing [11,15,17]. Together, these differing regulatory philosophies illustrate how identical pharmacogenomic evidence can yield divergent labeling outcomes across jurisdictions. Such variability has important implications for global precision medicine. Divergent PGx labeling complicates multinational drug development, contributes to inconsistent clinical implementation, and underscores the need for systematic comparative evaluation of regulatory decision-making. These regulatory differences provide the foundation for the comparative analyses presented in this study [27–29].

## METHODOLOGY

**Study Design and Analytical Approach**

This study employed a descriptive–comparative regulatory analysis to evaluate how pharmacogenomic (PGx) information is interpreted, operationalized, and communicated through regulatory drug labeling. A qualitative comparative framework was selected because PGx labeling represents a regulatory outcome shaped not only by scientific evidence, but also by evidentiary thresholds, policy mandates, population considerations, and institutional risk tolerance. Quantitative counts alone are insufficient to capture these dimensions; therefore, structured qualitative comparison was used to identify patterns of regulatory convergence and discordance across jurisdictions [1–3]. The analysis focused on approved medicinal products with established pharmacogenomic relevance, enabling

examination of real-world regulatory decisions rather than theoretical guidance. Comparative evaluation across regulatory agencies allowed assessment of differences in labeling intent, clinical action ability, and regulatory interpretation of identical gene–drug associations.

**Data Sources**

Data were derived exclusively from publicly available and authoritative regulatory sources to ensure transparency and reproducibility. Primary sources included approved prescribing information, package inserts, and official pharmacogenomic biomarker listings published by the United States Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the Pharmaceuticals and Medical Devices Agency (PMDA). These documents represent the legally binding expression of regulatory decisions and were treated as the definitive basis for analysis [14,17–24]. Secondary sources included publicly available regulatory guidance documents related to pharmacogenomics, biomarker qualification, and clinical pharmacology. Curated pharmacogenomic knowledge bases and clinical guideline repositories—specifically CPIC and DPWG—were consulted strictly as clinical reference frameworks to contextualize regulatory labeling content and assess translational alignment, not as sources of regulatory authority.

**Drug Selection Criteria**

Twenty gene–drug pairs were purposively selected using predefined inclusion criteria designed to maximize analytical relevance. Eligible drugs were required to (i) have a documented pharmacogenomic association supported by regulatory labeling, peer-reviewed evidence, or established pharmacogenomic guidelines; (ii) be approved by at least one of the three regulatory agencies; and (iii) demonstrate either regulatory convergence or divergence in PGx labeling across jurisdictions. To ensure breadth and generalizability, selected drugs represented diverse therapeutic areas, including oncology, infectious diseases, psychiatry, cardiology, neurology, and immunology. This purposive sampling strategy enabled focused evaluation of how regulatory interpretation varies across clinical contexts while maintaining consistency in analytical depth [6,14].

**Comparative Parameters and Definitions**

Each regulatory label was evaluated using a standardized set of analytical parameters applied consistently across agencies. PGx information was classified according to regulatory intent as required, recommended, actionable, or informational, reflecting increasing levels of implied clinical obligation. Variant specificity was assessed based on whether labels referenced allele-level information, broader genotypic categories, or inferred phenotypes such as poor or intermediate metabolizers. Clinical action ability was evaluated by determining whether labels provided explicit guidance on dose adjustment, contraindication, alternative therapy selection, or enhanced monitoring. Label section placement was also documented to assess visibility and potential impact on prescribing behavior. Cross-agency concordance or discordance was defined based on alignment across these parameters, allowing systematic identification of harmonized and divergent regulatory outcomes [16–18].

**Methodological Rigor and Scope**

All evaluations were conducted using identical criteria across regulatory frameworks to minimize interpretive bias. Differences in terminology or document structure were interpreted within the context of agency-specific regulatory practices rather than treated as analytical inconsistencies. The methodology was designed to prioritize regulatory intent and clinical signaling rather than biological reassessment of gene–drug validity, which has been extensively addressed elsewhere. This structured approach provides a reproducible and policy-relevant framework for assessing regulatory integration of pharmacogenomics and supports the comparative analyses presented in subsequent sections.

## RESULTS AND DISCUSSIONS

**Overview of Regulatory Integration of Pharmacogenomic Information**

Comparative evaluation of regulatory drug labeling across the United States Food and Drug Administration (FDA), European Medicines Agency (EMA), and Pharmaceuticals and Medical Devices Agency (PMDA) revealed substantial variability in the recognition, interpretation, and operationalization of pharmacogenomic (PGx) evidence. Although all three agencies acknowledge the clinical relevance of genetic variability, the extent to which PGx information is translated into actionable regulatory guidance differs markedly. Quantitative analysis demonstrates that PGx labeling is most consistently applied to gene–drug pairs associated with severe safety risks or predictive oncology biomarkers, whereas pharmacokinetic associations show greater regulatory divergence [6,14,15]. The FDA more frequently incorporates mandatory or recommended genetic testing language for high-risk associations, while the EMA predominantly adopts advisory or descriptive phrasing, particularly for metabolism-related biomarkers. In contrast, the PMDA exhibits a comparatively higher proportion of actionable PGx guidance relative to its total number of PGx-labeled drugs, reflecting a regulatory emphasis on dose optimization and population-specific risk management.

**Distribution of pharmacogenomic labeling categories across FDA, EMA, and PMDA(Table 1)**

Summary of drugs containing pharmacogenomic information in regulatory labeling databases of FDA, EMA, and PMDA, categorized by regulatory signaling strength. Classifications reflect regulatory labeling intent and do not correspond to clinical guideline mandates.

**Clinical Actionability and Labeling Strength**

Assessment of clinical action ability indicates that the presence of PGx information in regulatory labels does not uniformly translate into explicit prescribing guidance. Only a subset of PGx-labeled drugs across agencies include clear recommendations for dose adjustment, contraindication, or alternative therapy selection. This pattern is particularly evident for CYP-mediated pharmacokinetic associations, where strong biological plausibility often coexists with limited prospective clinical outcome data [7,8,18]. These findings highlight a structural distinction between regulatory recognition of PGx evidence and regulatory endorsement of clinical action ability. Regulatory authorities appear to calibrate labeling strength based not only on scientific validity but also on uncertainty tolerance, legal accountability, and anticipated clinical impact.

**Clinical actionability of pharmacogenomic information in regulatory drug labels (Table 2).**

Assessment of whether pharmacogenomic information is translated into explicit prescribing guidance, including dose adjustment, contraindication, or alternative therapy recommendations.

**Regulatory Discordance and Harmonization Patterns**

Analysis of drugs commonly labeled across all three agencies reveals that regulatory discordance predominates over harmonization. Harmonized labeling is largely confined to gene–drug pairs supported by robust clinical or safety evidence, such as HLA-associated hypersensitivity reactions and oncogenic driver mutations. In contrast, discordant labeling is frequently observed for exposure-modifying variants where evidence is derived primarily from pharmacokinetic or observational studies [10–12,14]. These patterns suggest that regulatory convergence occurs primarily at higher evidentiary thresholds, whereas lower or intermediate tiers of evidence are subject to divergent regulatory interpretation. Importantly, this discordance reflects structured differences in regulatory mandates rather than disagreement on the underlying genomic science.

**Regulatory harmonization and discordance in pharmacogenomic labeling across FDA, EMA, and PMDA (Table 3).**

Classification of commonly labeled drugs based on concordance or discordance in the presence and clinical actionability of pharmacogenomic information across regulatory authorities.

**Insights from Case Studies of Gene–Drug Pairs**

The 20 case studies examined provide drug-level insight into the mechanisms driving regulatory convergence and divergence. Gene–drug pairs involving severe immune-mediated toxicity demonstrate partial convergence, with FDA and EMA more frequently mandating or recommending testing, while PMDA often provides informative or alternative actionable guidance. Conversely, pharmacokinetic examples illustrate scenarios in which PMDA includes explicit dose-adjustment recommendations while FDA and EMA restrict labeling to descriptive statements despite similar evidence bases [11–13]. These case studies confirm that regulatory discordance is predictable and context-dependent, shaped by therapeutic area, biomarker type, and population relevance. Importantly, several cases demonstrate misalignment between regulatory labeling and clinical guideline recommendations, underscoring translational gaps that may affect clinician confidence and real-world PGx implementation [9,13].

**Comparative Case Studies of Selected Gene–Drug Pairs Illustrating Regulatory Discordance(Table 4).**

Comparative overview of 20 representative gene–drug pairs demonstrating differences in pharmacogenomic labeling across FDA, EMA, and PMDA. Regulatory classifications reflect labeling decisions and do not represent clinical guideline authority.

**Alignment with Clinical Guidelines and Knowledgebases**

Comparison with CPIC and DPWG guidelines indicates that clinical practice recommendations frequently extend beyond the actionability reflected in regulatory labels. Guidelines often provide genotype-specific dosing algorithms or alternative therapy recommendations that are only partially incorporated—or absent—in regulatory labeling. This divergence reflects fundamental differences in institutional purpose: regulators prioritize population-level benefit–risk assessment and legal robustness, whereas guideline bodies focus on optimizing individual patient care [10–12]. Pharmacogenomic knowledgebases support evidence synthesis and cross-validation but lack formal regulatory authority. While they play an increasingly important role in contextualizing PGx evidence, variability in update frequency and evidence thresholds limits their direct regulatory applicability.

**Alignment of Regulatory Pharmacogenomic Labeling with CPIC and DPWG Clinical Guidelines (Table 5).**

Comparison of regulatory labeling actionability with recommendations from CPIC and DPWG. Guideline recommendations are presented for translational context only and do not represent regulatory authority.

**Challenges to Global Harmonization**

Persistent regulatory discordance arises from interrelated scientific, regulatory, and operational barriers. Scientific challenges include uncertainty in variant interpretation and limited population diversity in evidence generation. Regulatory challenges stem from divergent statutory mandates, evidentiary thresholds, and liability considerations. Operational constraints, including delayed label updates and lifecycle management complexity, further impede timely and consistent PGx integration [1–3,9]. These barriers collectively explain why harmonization remains limited despite a shared scientific foundation and highlight the need for proportionate, evidence-responsive regulatory frameworks.

**Toward a Harmonized Regulatory Framework**

To address these challenges, this study supports adoption of a tiered evidence-based approach to PGx labeling, linking regulatory actionability to evidentiary strength. Standardized terminology and clearly defined actionability levels could improve clarity, reduce ambiguity, and facilitate cross-jurisdictional consistency. Dynamic label-updating mechanisms and structured regulatory collaboration may further enhance alignment between regulatory labeling and evolving clinical evidence [4–6].

**Barriers to Harmonized Pharmacogenomic Labeling and Proposed Regulatory Solutions (Table 6).**

Summary of scientific, regulatory, and operational barriers contributing to global discordance in PGx labeling, alongside proposed regulatory science solutions.

**Implications and Concluding Perspective**

Collectively, these findings demonstrate that regulatory integration of pharmacogenomics is heterogeneous, structured, and policy-driven. While harmonization is achievable for high-certainty safety and predictive biomarkers, substantial variability persists for pharmacokinetic associations. Advancing consistent global implementation of PGx-guided therapy will require regulatory frameworks that balance scientific rigor with translational agility. By providing a systematic comparative analysis and empirically grounded synthesis, this study contributes to regulatory science efforts aimed at integrating genomic evidence into drug labeling in a manner that supports patient safety, clinical utility, and precision medicine.

## CONCLUSION

This study provides a systematic comparative assessment of how pharmacogenomic (PGx) evidence is translated into regulatory drug labeling across major regulatory authorities. The findings demonstrate that, despite a shared scientific foundation, the integration of PGx information into regulatory labels remains heterogeneous and strongly shaped by jurisdiction-specific evidentiary thresholds, policy mandates, and population considerations. Harmonization is most evident for gene–drug pairs associated with severe safety risks or predictive oncology biomarkers, whereas substantial discordance persists for pharmacokinetic associations supported primarily by mechanistic or observational evidence. By combining quantitative analysis with structured case studies, the study shows that regulatory discordance is neither arbitrary nor evidence-agnostic, but reflects systematic differences in how agencies balance scientific uncertainty, clinical actionability, and public health responsibility. The observed misalignment between regulatory labeling and clinical practice guidelines further underscores a translational gap that may affect clinician confidence and real-world implementation of PGx-guided therapy. Importantly, the analysis highlights that effective integration of pharmacogenomics into regulatory practice requires proportionate, transparent, and evidence-tiered approaches rather than uniform prescriptive mandates. Adoption of standardized terminology, clearer definitions of actionability, and dynamic mechanisms for updating PGx content could improve consistency while preserving regulatory autonomy. Strengthened collaboration among regulatory authorities, guideline bodies, and pharmacogenomic knowledgebases may further support convergence without compromising scientific rigor or population relevance. In conclusion, advancing harmonized pharmacogenomic labeling is essential for realizing the full potential of precision medicine. By clarifying the structural drivers of regulatory variability and proposing pragmatic pathways toward alignment, this work contributes to regulatory science efforts aimed at improving patient safety, enhancing clinical utility, and supporting globally consistent implementation of pharmacogenomics in drug therapy.

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**Table 1: Distribution of Pharmacogenomic Labeling Categories Across FDA, EMA, and PMDA**

| Regulatory Agency | Total Drugs with PGx Annotations | Testing Required | Testing Recommended | Actionable PGx | Informative PGx | No Clinical PGx | Criteria Not Met | No Label Found |
|---|---|---|---|---|---|---|---|---|
| FDA | 505 | 141 | 9 | 105 | 67 | 36 | 147 | 0 |
| EMA | 312 | 105 | 3 | 28 | 21 | 18 | 85 | 52 |
| PMDA | 52 | 14 | 1 | 29 | 5 | 0 | 3 | 0 |

**Table 2: Clinical Actionability of Pharmacogenomic Information in Regulatory Drug Labels**

| Regulatory Agency | Total Drugs Evaluated | Drugs with Prescribing Information | Drugs without Prescribing Information |
|---|---|---|---|
| FDA | 505 | 128 | 377 |
| EMA | 312 | 37 | 275 |
| PMDA | 52 | 0 | 52 |

**Table 3: Regulatory Harmonization and Discordance in Pharmacogenomic Labeling Across FDA, EMA, and PMDA**

| Classification | Number of Drugs |
|---|---|
| Total common drugs with PGx annotations across FDA, EMA, PMDA | 37 |
| Harmonized regulatory PGx labeling | 17 |
| Regulatory discordance in PGx labeling | 20 |

**Table 4: Comparative Case Studies of Selected Gene–Drug Pairs Illustrating Regulatory Discordance**

| S. No | Drug | Therapeutic Area | Key Gene / Biomarker | FDA Label Status | EMA Label Status | PMDA Label Status | Regulatory Discordance Rationale |
|---|---|---|---|---|---|---|---|
| 1 | Abacavir | Infectious Diseases (HIV) | HLA-B | Testing required | Testing required | Informative PGx | Mandatory vs informative |
| 2 | Carbamazepine | Neurology (Epilepsy) | HLA-B | Testing required | Testing Recommended | Actionable PGx | Required vs recommended vs alternative. |
| 3 | Clopidogrel | Cardiology (Antiplatelet) | CYP2C19 | Actionable PGx | Informative PGx | Actionable PGx | Actionable vs informative |
| 4 | Irinotecan | Oncology | UGT1A1 | Testing Recommended | Actionable PGx | Testing Recommended | Actionable vs recommended. |
| 5 | Fluorouracil | Oncology | DPYD | Testing Recommended | Informative PGx | Actionable PGx | Recommended vs actionable vs informative. |
| 6 | Atazanavir | Infectious Diseases (HIV) | CYP2C19 | Informative PGx | Informative PGx | Actionable PGx | Informative vs actionable. |
| 7 | Codeine | Anaesthesiology | CYP2D6 | Actionable PGx | Informative PGx | Actionable PGx | Contraindication vs informative. |
| 8 | Capecitabine | Oncology | DPYD | Testing Required | Testing Recommended | Actionable PGx | Required vs recommended vs actionable. |
| 9 | Atomoxetine | Psychiatry (ADHD) | CYP2D6 | Actionable PGx | Informative PGx | Actionable PGx | Actionable vs informative. |
| 10 | Aripiprazole | Psychiatry | CYP2D6 | Actionable PGx | Actionable PGx | Informative PGx | Actionable vs informative. |
| 11 | Efavirenz | Infectious Diseases (HIV) | CYP2B6 | Informative PGx | Informative PGx | Actionable PGx | Informative vs actionable. |
| 12 | Azathioprine | Immunology | TPMT | Testing Recommended | Informative PGx | Actionable PGx | Recommended vs informative vs actionable. |
| 13 | Fesoterodine | Oncology | ESR1, ESR2 | Informative PGx | Informative PGx | Actionable PGx | Informative vs actionable. |
| 14 | Voriconazole | Infectious | CYP2C19 | Informative | Informative | Actionable | Informative vs |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Diseases (Antifungal) | | PGx | PGx | PGx | actionable. |
| 15 | Indacaterol | Pulmonary | UG1A1 | No Clinical PGx | Informative PGx | Actionable PGx | None vs informative vs actionable. |
| 16 | Escitalopram | Psychiatry (Depression) | CYP2C19 | Informative PGx | Actionable PGx | Actionable PGx | Informative vs actionable. |
| 17 | Gefitinib | Oncology (Lung Cancer) | EGFR | Actionable PGx | Actionable PGx | Testing required | Actionable vs mandatory. |
| 18 | Lenalidomide | Hematology | Chromosome 5q | Testing required | Testing required | Criteria Not Met | Mandatory vs no recognition |
| 19 | Rasburicase | Oncology | G6PD | Testing Required | Actionable PGx | Actionable PGx | Mandatory vs actionable guidance. |
| 20 | Mercaptopurine | Oncology | NDUT15 | Testing Recommended | Testing Recommended | Actionable PGx | Recommended vs actionable. |

**Table 5: Alignment of Regulatory Pharmacogenomic Labeling with CPIC and DPWG Clinical Guidelines**

| Gene–Drug Pair | CPIC / DPWG Recommendation | Regulatory Label Actionability | Alignment Status |
|---|---|---|---|
| CYP2C19–clopidogrel | Alternative therapy for poor metabolizers | Informational or warning language | Partial |
| CYP2D6–codeine | Avoid use in poor/ultrarapid metabolizers | Contraindication or warning | High |
| TPMT/NUDT15–thiopurines | Genotype-based dose reduction | Dose adjustment in some regions | Partial |
| HLA-B57:01–abacavir | Mandatory testing; avoid if positive | Contraindication | High |

**Table 6: Barriers to Harmonized Pharmacogenomic Labeling and Proposed Regulatory Solutions**

| Barrier Category | Key Issues | Impact on PGx Labeling | Proposed Regulatory Approach |
|---|---|---|---|
| Scientific | Variant interpretation uncertainty; limited outcome data | Conservative or inconsistent labeling | Tiered evidence-based actionability |
| Population diversity | Ethnic variability; limited population-specific data | Regional differences in labeling | Inclusive evidence generation |
| Regulatory | Divergent mandates; liability concerns | Variable testing requirements | Standardized PGx terminology |
| Operational | Slow label updates; lifecycle complexity | Lag behind evidence and guidelines | Dynamic label-updating mechanisms |

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CASE STUDY ARTICLE

# Role of Ayurvedic Therapy in *Ichthyosis Vulgaris* (Ekakushta): Evidence from a Case Study

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## ABSTRACT

Ichthyosis vulgaris is a hereditary keratinization disorder caused by filaggrin gene mutations and usually appears at birth or early childhood. It presents with xerosis, fissuring, non-erythrodermic scaly lesions and tends to worsen in colder seasons, often affecting daily activities because of itching, burning and cosmetic concerns. Effective long-term treatments are limited, so there is a need for safer, supportive options. This case describes a 45-year-old woman with chronic scaly, fissured and pigmented patches on all limbs along with itching and burning, aggravated during monsoon and winter. She also had poor sleep, weak digestion and allergic rhinitis. Ayurvedic assessment showed Vata-Pitta dominant prakriti with Tridoshaja involvement and dushya dushti of Rasa, Rakta, Mamsa and Meda, correlating with the Ayurvedic understanding of Ekkustha. Management included internal medicines, Panchtikta Ghrita for dosha-shamana and bio-purification, Kaisor Guggulu, and Avipatikar Churna used internally, Nalpamaradi Taila was used externally. After fifteen days of inpatient care, the patient reported clear improvement in itching, scaling and burning sensation, along with better sleep, reduced pigmentation and relief in joint discomfort, without any adverse reactions. This case highlights the potential role of Ayurvedic therapy as a holistic, safe and effective approach for managing ichthyosis vulgaris and improving overall quality of life.

**Keywords:** Ichthyosis vulgaris, Ekkustha, Ayurvedic management, Panchtikta Ghrita, Kaisor Guggulu, Nalpamaradi Taila.

## INTRODUCTION

Ichthyosis vulgaris is a common hereditary disorder marked by dry and scaly skin due to a mutation affecting epidermal barrier function. It usually presents at birth or early childhood and accounts for almost 95 percent of inherited ichthyoses. The clinical picture often includes xerosis, fine to thick scaling, keratosis pilaris, and generalized scaly dermatosis without erythroderma. Although emollients, keratolytics, and oral retinoids provide temporary symptom relief, many patients continue to struggle with dryness, pruritus, and recurrent fissuring. These symptoms interfere with daily activities, social interactions, and overall well-being, which makes the condition a significant quality-of-life burden. From an Ayurvedic perspective, most chronic skin disorders fall under the broad category of Kushta. The features of ichthyosis vulgaris closely match Ekakushta, a subtype characterized by fish-like scales (matsya-shakala-sannibha), reduced sweating (aswedanam), and extensive involvement (mahasrayam). Ekakushta is mainly associated with vata and kapha imbalance, along with deeper dhatu involvement that makes the condition persistent and difficult to manage with symptomatic care alone. Ayurveda approaches such disorders through strategies that purify, restore balance, and correct underlying dosha disturbances. Therapies aim to reduce dryness, soften accumulated scales, and improve the integrity of the skin by addressing both systemic and local factors. In this case report, we describe the management of ichthyosis vulgaris with an Ayurvedic treatment plan. The patient demonstrated significant improvement in subjective symptoms, including dryness and itching, as well as objective changes in scaling, erythema, and lesion thickness. This case highlights the potential role of Ayurvedic interventions as supportive care in hereditary keratinisation disorders, such as ichthyosis vulgaris.

**Patient Information**

**Clinical history and symptomatology**

The patient, a 45-year-old woman, indicated that her skin issues began when she was approximately 5 years old. At first, she observed dryness and roughness of the skin, especially on her arms and legs, which tended to worsen during the winter months and improve somewhat in the summer. Over time, the condition steadily progressed, with ongoing dryness, fine scaling, and a rough skin texture becoming permanent characteristics. During her adolescence, she started to experience itching (pruritus) linked to the dryness, which intensified in colder environments. As the condition evolved, thickened plaques with fissures developed on both ankles, along with heightened roughness and irritation. She notes that exposure to cold weather, irregular eating patterns (including skipping meals and consuming dry, spicy, and cold foods), and disrupted sleep tend to worsen her symptoms. Additionally, she has a history of allergic rhinitis and chronic sleep disturbances, both of which are aggravated during the winter season. There is no record of oozing, bleeding, or pain in the affected areas. The patient has occasionally utilized emollients and home remedies for symptomatic relief but has not engaged in any long-term modern treatment for this condition.

**Family History**

No known family history of any type of skin diseases or systemic disorders. All the family members are reported to be healthy.

**Past History**

The Patient Has A History Of Recurrent Allergic Rhinitis, Particularly During Seasonal Changes. No Positive History of DM, HTN and Thyroid

**Personal history**

Diet – Mixed
Sleep – Disturbed sleep,
Bowel – 1 time a day
Micturition – 5-6 T/ day and 1 T/night
Appetite – Poor

**General examination**
The patient had a normal gait with no pallor, cyanosis, icterus, edema or lymphadenopathy, and no nail clubbing. Vital signs were stable with a pulse of 78 per minute, respiratory rate of 18 per minute, $SpO_2$ of 98%, blood pressure of 120/80 mmHg and temperature of 98.6°F. Anthropometric measurements showed a height of 150 cm, weight of 45 kg and BMI of 20 kg/m².

**Ashtasthana Pariksha** revealed a Vata-Pitta naadi, normal micturition and defecation, a clean tongue, normal voice and vision, anushna-shita sparsha and a krusha body build.

**Dashavidha Pariksha** showed a Vatapitta prakriti with tridosha vikriti, moderate sara, samhanana, satmya, satwa, pramana, ahara and jarana shakti, and moderate exercise tolerance; the patient was of the madhyama age group.

**Systemic Examination**
**Respiratory system**
**Inspection:** Bilaterally symmetrical shape of chest with no scar mark or bulge.
**Palpation:** Trachea centrally place
**Percussion:** Resonance present
**Auscultation:** wheezing sound heard

**Cardiovascular system**
**Inspection:** No distended blood vessels, scars or deformities
**Palpation:** apex beat felt
**Auscultation:** S1 S2 heard no added murmurs

**GIT**
**Inspection:** No distended blood vessels, scars or deformities
**Palpation:** apex beat felt, no organomegaly
**Percussion:** dull
**Auscultation:** bowel sound heard

**Central Nervous System**
**Consciousness:** alert, conscious
**Orientation:** Well oriented

**Augmentary examination**
- chronic excoriated, fissured lesions over both upper and lower limb,
- Blackish Pigmentation On B/L Upper And Lower Limb
- Plaque with fissure at B/L ankle region
- Crusting present

**Timeline**
Timeline of Symptoms, Intervention and Clinical Progress (Table 1).

**Diagnostic Assessment**
Based on the patient's presentation, the diagnostic assessment incorporated both modern and Ayurvedic perspectives. The characteristic findings of early onset dryness, prominent scaling, and symmetrical involvement of the extensor surfaces, along with dark lichenified plaques around the ankles and associated pruritus, supported a diagnosis of ichthyosis vulgaris. Additional features such as persistent xerosis, crusting, and nighttime itching reinforced the chronic and hereditary nature of the condition. On examination, palmar hyperlinearity and fine-to-moderate scaling further aligned with the typical phenotype of ichthyosis vulgaris. From an Ayurvedic standpoint,

the evaluation through Dashavidha and Ashtasthana Pariksha revealed a predominant vata-kapha imbalance, along with tridosha vikriti, which are consistent with the classical description of Ekakushta. The presence of matsya-shakala-sannibha scaling, aswedanam, and involvement of larger body areas correlated well with the diagnostic features mentioned in Ayurvedic texts. Together, the combined clinical and Ayurvedic findings supported the final diagnosis of Ekakushta corresponding to ichthyosis vulgaris.

**Diagnosis**

The diagnosis of ichthyosis vulgaris was established in view of the early age of onset, presence of generalized xerosis, fine to moderate scaling, symmetrically distributed over the limbs, fissuring around the ankle areas, hyperlinearity of the palms, and seasonal exacerbations. The absence of erythroderma, oozing, and systemic symptoms helped to rule out the diagnosis. The conditions that can be considered in the differential diagnosis of ichthyosis vulgaris are psoriasis, atopic dermatitis, and acquired ichthyosis. According to Ayurvedic classics, the patient had the symptomatology of Ekakushta, which was associated with matsyashakalas

Prognosis

Ichthyosis vulgaris in most cases is a chronic and non-life-threatening condition. Complete remission of the skin condition would not have been achieved in the patient's case, but the necessary symptomatic control and quality of life would have been achieved. The patient in this case had a good prognosis due to the considerable relief in scaling, xerosis, pruritus, and fissures achieved after the Ayurvedic treatment, in addition to the patient also having good compliance.

**Ayurvedic Analysis**

Ayurvedic evaluation revealed Ekakushta dominated by Vata-Kapha dosha involvement and Tridoshaja vikriti. Vitiation of Rasa, Rakta, Mamsa, and Meda dhatu explained the chronic dryness, scaling, and fissuring. Defective Agni resulted in Ama formation that contributed to srotas dysfunction and defective skin nourishment. The management strategy was Shamana Chikitsa with Tikta-pradhana, Snehana, and Agni-enhancing measures.

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**Therapeutic Intervention(Table 2),(Figure 1)**

**Follow Up and Outcome**

On follow-up, the patient showed steady improvement in both subjective and objective symptoms. The plaques and blackish lichenification over the bilateral ankle region became softer with reduced thickness. Crusting decreased from moderate (++) to mild (+), and scaling reduced from moderate (++) to mild (+), indicating about 50–60 percent improvement in lesion severity. Night-time itching and the burning sensation over affected areas lessened, showing nearly 70 percent relief. Stool regularity improved, and burning micturition and general weakness reduced by about 60 percent. The papules in the urogenital region also diminished, showing almost 70 percent improvement. Pain in the small joints of the hands, shoulder and knee joints reduced by about 50 percent. Overall, the patient experienced significant symptomatic relief and better comfort during daily activities. The following table shows the improvement in key symptoms related to skin dryness and scaling after treatment. Each parameter was graded to assess the degree of change and overall therapeutic response. Changes in Clinical Symptoms Before and After Treatment (Table 3).

## DISCUSSION

*Ichthyosis Vulgaris*, although primarily genetic, has overlapping symptomatology with several Ayurvedic conditions involving *Twak vikaras*, such as *Kitibha*, *Ekakushta*, or *Charma kushta*. In this case, the chronic dry and fissured skin, itching, and crusting suggest Tridoshaja involvement, predominantly Vata and Pitta. These doshas vitiate *Rasa, Rakta, Mamsa*, and *Meda dhatus*, leading to the manifestation of symptoms. The Ayurvedic treatment plan focused on Shamana Chikitsa with a Rasayana and Tikta pradhana approach, aiming to pacify Vata and Pitta and purify the affected dhatus. For Vataja Kushtha, begin with ghritapana, while for Kaphaja kushtha, the vamana procedure is recommended. In pittaja kushtha cases, the primary treatment involves virechana anad raktamokshana. In the context of treating various types of skin disorders in in the vataj condition, the application of sarpi is recommended. In cases where there is pronounced scaling and dryness, Snehapan plays a significant role in managing ailment.Additionally, the utilization of Panchatikta Ghrita, as mentioned in Bhaishajya Ratnavali Kushtha Adhikar, serves as a commonly employed treatment involving Snehapan for skin diseases. The ingredient such as Patola, Guduchi, Nimba, found in the Ghrita, work synergistically to address imbalances in Vata, Kapha and Rakta. Regarding Panchtikta Ghrita, its decription can be found in Bhaisajya Ratnavali Kushtharogadhikar, while indications for Shaman Sneha pan are outlined in Charak Samhita Sutrasthana.Kaishor Guggulu exerts its effect in Eka Kushtha through Raktashodhana, Amapachana, and Tridosha Shaman. Herbs like Guduchi, Triphala, and Guggulu purify the blood, boost metabolism and reduce inflammation. Its Katu- Tikta Rasa and Ushna Veerya, relieve itching, scaling, and discoloration. Additionally, its antiallergic, antibacterial, and detoxifying properties support long – term disease control and prevent recurrence. Avipattikar Churna is the best medicine to eliminate all the toxins in the body (Sava Visheshu Cha) in the means of sukhavirechana (Mala which is expelled through defecation easily) and rechana (does mala paka and facilitates the adho vayu) because it contains Trivrit. It reduces the vitiated Pitta and Rakta dosha in the blood by ashrayi ashraya sambandha. The administration of *Panchtikta Ghrita* served as a deepana-pachana and dhatu-prasadaka, while *Kaisor Guggulu* addressed *rakta dushti* and inflammation. *Avipatikar churna* supported *Agni* and corrected digestive imbalance, addressing *ama* formation. Externally, the use of medicated oils provided snehana, alleviating dryness and scaling. Nalpamaradi Tail is useful in itching, scabies, Visarpa( erysipelas) and Kushtha( Skin disease) .Most drugs in Nalpamaradi taila contain tridoshashamaka quality, which is beneficial in maintaining skin health. Nalpamaradi Kera Taila is a classical formula that lightens the complexion while also softening, nourishing, and repairing the skin.Nalpamaradi means four Vriksha Twak, viz. Nyagrodha, Udumbara, Asvatha and Plaksa. It is indicated in Pama, Kandu, Pidaka, Kushtha, and Visarpa. Application of Nalpamaradi Taila would take care of Daha, Kandu, as it contains Ksheerivriksha Dravya, which are Kashayarasapradhana, pitta, and Kaphahara. It contains tannins as its main chemical constituents, which inhibit bacterial growth. The oil given for local application acts as a physical barrier (emollient type) as well. The therapy was well tolerated, and symptomatic relief was evident within days of initiation, especially in terms of reduced burning, itching, and lesion severity. Notably, her history of allergic rhinitis and disturbed sleep may be indicative of a systemic vitiation of doshas and chronic immune dysregulation. The internal Rasayana support likely contributed to systemic equilibrium, which is reflected in the reduction of associated symptoms like burning micturition, fatigue, and papules

## CONCLUSION

The current study emphasizes how Ayurvedic medication may help manage ichthyosis vulgaris. The prakriti (constitution), bala (strength), dosha, dhatu, and chronicity of the ailment were taken into consideration when designing the treatment regimen. Good skin texture resulted from significant relief in terms of decreased scaling, itching, dryness, and pain. Larger sample sizes and more clinical research are required to develop a standardized Ayurvedic therapy approach for ichthyosis.

**Patient Perspective**

"I have had problems with dry and scaly skin since childhood. This used to create problems of itching, burning, and discomfort, particularly in winter. With Ayurvedic treatment, I observed that the dryness, scaling, and itching have decreased significantly in a short period of time. Also, my sleep has improved, and I have no issues in carrying out my daily activities. This care has been simple and free of side effects. I feel that this method has proved beneficial in controlling my condition better and enhancing my quality of life."

**Informed Consent**

The patient has agreed to the publication of the case report containing their medical details and images related to their case. The patient has been explained what the case report entails and has agreed that their own personal details will be anonymized. The patient gave his/her assurance that his/her participation in this case report is voluntary and that he/she could withdraw consent at any time without it having any bearing on his/her medical treatment.

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**Table 1: Timeline of Symptoms, Intervention and Clinical Progress**

| Date | Events | Key findings/interventions |
|---|---|---|
| 5 years of age | Onset of symptoms | She had dryness and roughness of the skin, especially on her arms and legs, which tended to worsen during the winter months and improve somewhat in the summer. |
| 22/04/2025 | First hospital visit to Agadatantra OPD (1st Setting) | Plaque with blackish lichenification was observed over the bilateral ankle regions, accompanied by marked crusting and scaling. The patient experiences increased itching at night along with a burning sensation over the affected areas. Also complained of constipation for 1 week. So, symptomatic treatment was |

| | | |
|---|---|---|
| | | advised as per table |
| 09/05/2025 | Second hospital visit to Agadatantra OPD (2nd Setting) | The patient reported burning micturition, the papular lesions at the urogenital area and generalised weakness. Additionally, pain in the small joints of the hands, shoulders, and knees. The itching and burning sensation at the affected skin areas had notably reduced, with crusting and scaling still present. persisted, while plaques with blackish lichenification over the bilateral ankle region showed mild improvement. Also reduced complaint of constipation. |
| 16/05/2025 | Third hospital visit to Agadatantra OPD (3rd Setting) | The patient reported complete relief from all symptoms. There was no burning micturition or generalised weakness, and the skin lesions had reduced, with absence of itching, scaling, crusting, or lichenification. The papular eruptions in the urogenital area had subsided entirely. The patient also experienced no pain in the small joints, shoulder, or knee joints, indicating overall symptomatic improvement and restoration of well-being. |

**Table 2: Therapeutic intervention**

| Date | Therapeutic management |
|---|---|
| **22/04/2025 To 08/05/25** | **Internal Medication**<br>1. Panchtikta ghruta 1tsp BD A/F Warm water<br>2. Kaisor guggulu 2 BD AF with warm water<br>**External Medication**<br>3. Nalpamaradi Kera thailam for L/A all over body |
| **09/05/2025 To 16/05/2025** | **Internal Medication**<br>**1.** Panchtikta ghruta 1tsp BD A/F Warm water<br>2. Kaisor guggulu 2 BD AF with warm water<br>3. Avipatikar churna 2TSP HS with warm water<br>**External Medication**<br>1. Nalpamaradi Kera thailam for L/A all over body |
| **16/05/25** | **Internal Medication**<br>**1.** Panchtikta ghruta 1tsp BD A/F Warm water<br>2. Kaisor guggulu 2 BD AF with warm water<br>3. Avipatikar churna 2TSP HSwith warm water<br>**External Medication**<br>4. Nalpamaradi Kera thailam for L/A all over body |

**Table 3: Changes in Clinical Symptoms Before and After Treatment**

| Sr. No. | Symptoms | Grade | |
|---|---|---|---|
| | | Before Treatment | After Treatment |
| 1 | Skin Xerosis | 3 | 1 |
| 2 | Scale on legs | 3 | 1 |
| 3 | Keratosis pilaris | 1 | 0 |



**Fig 1: Therapeutic Intervention**

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RESEARCH ARTICLE

# Optimization and Functional Characterization of a *Cissus quadrangularis* - Infused Porridge Mix : A Nutraceutical Approach for Enhanced Health Benefits

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## ABSTRACT

The rising global interest in functional foods has spurred innovation in nutrient-dense, health-promoting food products. This study presents the optimization and functional characterization of a nutraceutical porridge mix infused with *Cissus quadrangularis* (CQ), a medicinal plant renowned for its bone-healing, anti-inflammatory, and antioxidant properties. Leveraging Response Surface Methodology (RSM) with a Box-Behnken design, the formulation was optimized based on sensory attributes—texture, color, flavor, mouthfeel, and overall acceptability—using varying proportions of red rice (30–50 g), moong bean dhal (30–40 g), and **Cissus quadrangularis** powder (5–10 g). Sensory evaluation by a trained panel identified an optimal blend yielding the highest overall acceptability score of 4.9 (on a 5-point hedonic scale). Statistical validation confirmed robust model fit, with $R^2$ values exceeding 0.95 for all sensory responses and significant p-values (<0.05) in ANOVA. The optimized mix demonstrated a balanced nutritional profile per 100 g: 213.2 kcal energy, 45.24 g carbohydrates, 7.47 g protein, low fat (0.75 g), and notable levels of calcium (34.4 mg), phosphorus (145.09 mg), and iron (2.10 mg). These nutrients, coupled with

CQ's bioactive compounds like β-sitosterol, resveratrol, and quercetin, enhance the mix's potential in supporting bone health and mitigating oxidative stress. The study bridges traditional herbal medicine with modern functional food development, offering a palatable, therapeutic breakfast option. This approach addresses growing concerns around osteoporosis—particularly in postmenopausal women—without reliance on synthetic hormone therapies.

**Keywords:** *Cissus quadrangularis,* Porridge mix, Osteoporosis

## INTRODUCTION

The growing consumer demand for functional foods has accelerated the development of nutrient-enriched formulations aimed at promoting health and wellness. *Cissus quadrangularis* L. (CQ), regionally known as Phet Sang Khat in Thailand, Hadjod in India, and Veldt grape, a traditional medicinal plant rich in phytochemicals, is renowned for its bone-healing, anti-inflammatory, and antioxidant properties This plant has significant potential in treating osteoporosis and fractures because of its mineral absorbing effect. In addition to this, it has antioxidant capacity due to its secondary metabolites and reduces the oxidative stress in cells [1], [2]. *Cissus quadrangular is* rich in bioactive compounds which includes phytosterols, alkaloids, steroids, resveratrol, carotene, piceatannol, pallidol, quercetin, nicotinic acid, triterpenoids, β-sitosterol, calcium, amyrone and ketosteroid [3]. A systematic review across indexed journals from 2003 to 2024, observed that *Cissus quadrangularis* administration in the form of supplements helps in bone protective effect. The bone healing properties of *Cissus quadrangularis* is due to the presence of β-sitosterol, lupeol components and vitamin C. Research showed that 25 mg in 100 mg slow release tablets raised the serum alkaline phosphatase, calcium and phosphorus levels. Preclinical studies showed that about 300-500 mg/ kg of *Cissus quadrangularis* extract reduces oxidative stress and proinflammatory cytokinase. These studies showed the anti-osteoporotic and antiarthritic effect of *Cissus quadrangularis* on osteoporotic and arthritic effects [4]. Over the years, the global burden due to fractures among men and women are increasing and it is associated with higher treatment cost burden. Over 30 years, increased prevalence of fractures and deficient bone mineral density has been tortuously reported in people with intellectual disability. Osteoporosis very common with increased age in women compared to men. During postmenopausal period, the estrogen secretion decreases and induce bone mineral depletion. People are following hormone replacement therapy to reduce its effect. But these are synthetic medicine, need to inject for a long time which increase the associated side effects such as breast cancer, hypercalcemia, breast tenderness and hypercaciurea [2], [5]. Hence sustainable prevention and management strategies are emerging need to mitigate the disabilities due to bone disorders. *Cissus quadrangularis* has been used in treating bone health over a decades in various countries. Despite the extensive documentation of *Cissus quadrangularis*'s pharmacological properties, limited research has explored its incorporation into functional food matrices, particularly in breakfast based products. (Gupta *et al*., 2017; Rani *and* Krishna, 2021). Most existing studies focus on its medicinal applications rather than its potential as a nutraceutical ingredient. This gap highlights the need for the development of functional food products to bridge nutrition and therapeutic benefits. Incorporating such bioactive extracts of *Cissus quadrangularis* into staple foods like porridge offers a novel approach to improving nutritional and therapeutic value. This study focuses on optimizing and functionally characterizing a *Cissus quadrangularis*-infused porridge mix as a potential nutraceutical product for enhanced health benefits based on the consumer preferences [6].

## METHODOLOGY

Porridge mix was formulated and standardised based on the traditional combination of ingredients. Red rice which has showed as a good source of phytochemicals and antioxidants, have a higher iron, magnesium, calcium, zinc, protein and fibre content, with lower glycemic index was chosen as the main ingredients[7]. Moong bean dhal (*Vigna radiata*) is high in protein and useful in defending against heart disease, cancer, diabetes and obesity[9], [10]. Dietary proteins are essential to replace worn out bone cells and for to provide strength to the bone. Red rice and moongban

dhal were procure from the departmental stores and cleaned, washed to remove the dust and impurities and dried to remove the excess water. They were roasted for 7 minutes in medium flame to enhance the flavour and made into find powders. *Cissus quadrangularis* was procured from the market and cleaned, washed and cut into one inch thickness pieces. They were dried in a hot air oven at 65 degree Celsius and made into find powder. These three ingredients were the main ingredients selected for the formulation of porridge. The texture and flavour were enhanced with dried cumin seeds, garlics and onions. This study adopted Response Surface Methodology (RSM) to optimize the functional beverage. Response Surface Methodology is a collection of mathematical and statistical techniques specifically designed to explore the relationships between several independent and dependent variables[11]. This capability is crucial for porridge formulation, where the interplay between ingredients and processing conditions can create complex textural, flavor, and nutritional profiles that simple linear models would fail to capture accurately. Within the RSM framework, the choice of a Box-Behnken is particularly well-suited to the stated research goal of optimizing porridge based on sensory evaluation. This allows for the precise estimation of main effects, two-factor interactions, and curvature, providing a complete picture of how formulation changes impact the desired responses.

In the context of porridge formulation, response factors would logically correspond to key ingredients, process parameters, or additive concentrations known to significantly influence the final product's characteristics. Three key ingredients, namely red rice, mung bean and *Cissus quadrangularis* were chosen for the formulation of porridge mix and the limits were determined as follows: Red rice (XI) -30 to 50 g, Mungbean Dhal (X2) – 30 to 40 g and *Cissus quadrangularis* (X3) -5 to 10 g. Response of the design experiments were Texture (T1), Colour (T2), Flavour (T3), Mouthfeel (T4) and Overall acceptance (T5). For sensory attributes, which are central to consumer acceptability, the evaluation focuses on human perception of key qualities such as color, aroma, taste, texture/mouthfeel, and overall acceptability. To quantify these subjective experiences, a structured sensory evaluation is conducted, ideally under controlled conditions with white light to avoid visual bias, The most common and effective method for measuring overall liking is the balanced 5-point hedonic scale[12]. Trained sensory panels have used for the depth analysis by linking hedonic scores to specific descriptive terms, thereby identifying the precise sensory drivers of consumer liking. The number of experimental design was formulated using Response Surface Methodology with the design of Box-Behnken by using Design Expert 12 software. The key ingredients were combined and porridge was formulated based on the experimental design. The combined dry ingredients were cooked with 500 ml of water and 5 grams of spice mix and subjected to sensory evaluation by the expert panel. The research involves a structured approach encompassing experimental design execution, data collection, and advanced statistical analysis. Data analysis will include comprehensive statistical model validation—utilizing ANOVA, lack-of-fit tests, and diagnostic residual checks—to ensure model adequacy and reliability. Furthermore, the study integrates physio chemical properties of ingredients and sensory evaluation scores into a unified multi-response optimization framework, likely employing desirability functions or similar techniques to identify optimal formulation conditions.

## RESULTS AND DISCUSSION

The herbal porridge mix was optimized using RSM software and the experimental runs were given in the Table- 1. Seventeen experimental runs were obtained for this process and based on the runs, the porridge mix was formulated and prepared for the further process. In this present study, RSM was employed to develop empirical models that describe the relationship between the proportions of key ingredients—cereals, pulses, and herbs—and the resulting sensory attributes of the porridge mix. The validity and predictive power of these models are paramount, and they were rigorously assessed using a suite of statistical parameters derived from Analysis of Variance (ANOVA). These metrics include the coefficient of determination ($R^2$), the adjusted coefficient of determination ($R^2$ adj), the p-value of the regression model, and the lack-of-fit test. Furthermore, the significance of the individual terms within the polynomial equations—the linear, quadratic, and interaction effects— was evaluated to understand the nature of the relationships being modelled. The overall fit of the model is primarily quantified by the coefficient of determination, $R^2$. This metric represents the proportion of the variance in the dependent variable (e.g., sensory score) that is

predictable from the independent variables (ingredient proportions). High $R^2$ values indicate that the model explains a large portion of the variability in the response data. While aiming for high $R^2$ values is standard practice, it is equally important to consider the adjusted $R^2$ ($R^2$ adj), which adjusts the $R^2$ value for the number of predictors in the model relative to the number of observations. This adjustment penalizes the addition of unnecessary terms and provides a more conservative estimate of the model's predictive power. A good-fitting model is typically indicated when the difference between the predicted and adjusted $R^2$ values is small, generally less than 0.2. The statistical significance of the entire regression model is determined by the p-value associated with the F-statistic from the ANOVA table. A low p-value (typically < 0.05) indicates that the model is significant, meaning that at least one of the independent variables has a statistically significant relationship with the dependent variable. The current study found that all five sensory attributes (color, aroma, taste, texture, and overall acceptability) and the overall acceptability itself exhibited significant regression models, with p-values well below the conventional 0.05 threshold, confirming the models' statistical validity. The core of the analytical effort in this study lies in translating the mathematical coefficients of the validated RSM models into tangible, interpretable insights regarding the impact of ingredient proportions on the sensory characteristics of the herbal porridge. Each sensory attribute—color, aroma, taste, and texture—is a composite response influenced by the unique contributions of cereals, pulses, and herbs, as well as their complex interactions. Table 2 summarizes the key statistical parameters used to validate the second-order polynomial models for each sensory attribute and overall acceptability, demonstrating the comprehensive assessment performed on the experimental data. The ANOVA tables for each model also provide information on the significance of the individual terms: linear, quadratic, and interaction effects. These terms are crucial for understanding the shape of the response surface and the nature of the ingredient interactions.

Linear terms (xi) represent a direct, proportional effect of an ingredient on a sensory attribute. Quadratic terms ($x_i^2$) are indicative of curvature in the response surface, suggesting the presence of an optimal point (a maximum or minimum) for that ingredient's level . The presence of significant quadratic terms is often a key finding in RSM, as it confirms that simple linear models are insufficient and that there is a peak or valley of acceptability within the experimental domain. Interaction terms ($x_i x_j$) reveal that the effect of one ingredient on a sensory attribute depends on the level of another ingredient. For example, a significant interaction between cereals and herbs might mean that the positive effect of adding herbs on flavour is only realized when a certain minimum level of cereals is present. Identifying significant interaction and quadratic effects provides deep insight into the synergistic and antagonistic relationships among ingredients, moving beyond a simple additive understanding of formulation. The discussion that follows will interpret the signs and magnitudes of these coefficients to explain how the specific ingredient proportions influence the sensory profile of the herbal porridge. For Colour, the visual appeal of the porridge is a primary driver of initial consumer acceptance. The models revealed that both cereals and herbs had significant linear effects on colour acceptability. Specifically, an increase in the proportion of the selected cereal led to a linear increase in the perceived lightness or brightness of the porridge, which was favoured by the panellists. Conversely, the proportion of the primary herb exhibited a negative linear effect, suggesting that higher concentrations darkened the mixture, which was perceived as less acceptable. This is a common challenge in developing herbal-infused foods, where the natural pigments of botanicals can lead to undesirable color shifts. Aroma and Flavor are intrinsically linked and are major determinants of liking. The regression analysis showed that the herbal component was the dominant contributor to the aromatic profile of the porridge. The linear coefficient for the herb was highly significant and positive, confirming that panelists preferred a stronger herbal aroma. This preference is consistent with consumer trends favoring functional foods with distinct, pleasant, and recognizable botanical notes. The positive linear coefficient for the cereal indicated that it provided a mild, neutral background flavor that helped to blend the other components harmoniously. The quadratic term for the pulse was also significant, suggesting that beyond a certain point, increasing the pulse content led to a decline in overall flavour acceptability, likely due to an overwhelming or unpleasant taste. Texture and Mouthfeel are arguably the most critical physical attributes influencing the eating experience. A desirable texture contributes significantly to the overall acceptability of a porridge, affecting its body, viscosity, and smoothness.

The proportion of cereals had a highly significant positive quadratic effect on both texture and mouthfeel scores. This means that as the cereal content increased, the porridge became more acceptable in terms of its physical feel, but only up to an optimal point. Beyond this point, further increases in cereal led to a deterioration in texture, likely becoming overly thick, gummy, or pasty. The linear effect of the pulse was negative, suggesting that pulses alone could introduce a gritty or coarse texture. However, the significant positive interaction between cereals and pulses indicated a synergistic effect; when combined in the right ratio, the cereals helped to mitigate the textural drawbacks of the pulses, resulting in a smoother, more palatable mouthfeel. This finding is supported by research on cereal-pulse blends, where fortification with pulses can improve nutritional quality but requires careful management to avoid negatively impacting sensory characteristics like texture. The modelling exercise successfully quantified the multifaceted influence of ingredient proportions. It demonstrated that optimizing the herbal porridge is not about maximizing any single component, but about achieving a delicate balance. The cereal acts as a base that moderates color, provides a neutral flavor canvas, and contributes to a desirable, albeit non-linear, texture. The pulse enhances nutritional value but requires careful dosing and blending to manage its potential negative impacts on flavor and texture. Finally, the herb is the key functional and sensory differentiator, providing the signature aroma and flavor, but its concentration must be precisely controlled to avoid adverse effects on color and overall harmony. These individual attribute analyses form the foundation upon which the final, holistic optimization of overall acceptability is built. The results indicated that the ideal proportions lie within a narrow region of the experimental domain, characterized by a medium-high level of cereals, a medium-low level of pulses, and a medium level of the herbal extract. The specific predicted optimal proportions were approximately 45% cereals, 30% pulses, and 25% herbs by weight. At this composition, the model predicted an overall desirability of 0.92, corresponding to a high predicted overall acceptability score.

**Nutrition content of porridge mix with high acceptability score**

Table-3provides the nutrient content of highly acceptable porridge mix combination. Out of 18 combination of ingredients determined by the RSM experimental runs, the combination which has highest overall acceptability score of 4.9 was subjected to nutrient analysis. It is low in fat (0.75 g) and good source of carbohydrates (45.24 g) and protein (7.47 g). The porridge mix was found to be a good source of calcium (34.4 mg), phosphorus (145.09 mg) and iron (2.10 mg).

## CONCLUSION

This study successfully developed and optimized a **Cissus quadrangularis**-infused porridge mix that harmonizes sensory appeal with enhanced nutritional and therapeutic value. Through rigorous statistical modeling and sensory analysis, an ideal ingredient ratio was identified that maximizes consumer acceptability while delivering key bone-supportive nutrients and phytochemicals. The final product represents a promising nutraceutical strategy for addressing bone health challenges, particularly in aging and at-risk populations, offering a natural, food-based alternative to conventional pharmacological interventions. Future work should focus on clinical validation of its health benefits and shelf-life stability for commercial scalability.

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**Table 1: Experimental run obtained using Response Surface Methodology**

| Sl. No | Independent variables | | | Responses | | | | |
|---|---|---|---|---|---|---|---|---|
| | Cereals (X1) | Pulses (X2) | Herb (X3) | Texture (T1) | Color (T2) | Flavor (T3) | Mouth feel (T4) | Overall Acceptability (T5) |
| A1 | 40 | 40 | 5 | 5 | 4.6 | 5 | 4.8 | 4.8 |
| A2 | 40 | 35 | 10 | 4.8 | 4.5 | 4 | 3.9 | 4.3 |
| A3 | 30 | 40 | 10 | 4.8 | 4.8 | 4 | 4.5 | 4.5 |
| A4 | 30 | 30 | 10 | 4.3 | 4.8 | 4 | 4.3 | 4.3 |
| A5 | 40 | 35 | 10 | 4.8 | 4.5 | 4 | 3.9 | 4.3 |
| A6 | 30 | 35 | 5 | 5 | .7 | 4.1 | 4.7 | 4.6 |
| A7 | 40 | 35 | 10 | 4.8 | 4.5 | 4 | 3.9 | 4.3 |
| A8 | 50 | 35 | 15 | 4.9 | 4.9 | 5 | 3.1 | 4.4 |
| A9 | 50 | 35 | 5 | 5 | 4.8 | 4.9 | 4.9 | 4.9 |
| A10 | 50 | 40 | 10 | 4.9 | 4.8 | 4.5 | 4.6 | 4.7 |
| A11 | 40 | 35 | 10 | 4.8 | 4.5 | 4.6 | 3.9 | 4.4 |
| A12 | 40 | 30 | 15 | 4.9 | 4.9 | 4.9 | 3.6 | 4.5 |
| A13 | 50 | 30 | 10 | 4.7 | 4.6 | 4.7 | 3.9 | 4.4 |
| A14 | 40 | 40 | 15 | 4.9 | 4.9 | 4.9 | 3.6 | 4.5 |
| A15 | 40 | 30 | 5 | 4.7 | 4.8 | 4.5 | 4.7 | 4.6 |
| A16 | 40 | 35 | 10 | 4.8 | 4.5 | 4.5 | 3.9 | 4.4 |
| A17 | 30 | 35 | 15 | 4.8 | 4.9 | 5 | 3.5 | 4.5 |

**Table 2: Validation of second -order polynomial model for sensory attributes**

| Response Variable | Co-efficient of Determination ($R^2$) | Adjusted $R^2$ | Predicted $R^2$ | Adequate Precision | Lack- of -Fit (p-value) |
|---|---|---|---|---|---|
| Colour Acceptability | 0.976 | 0.959 | 0.912 | 24.67 | 0.001 |
| Aroma Acceptability | 0.983 | 0.969 | 0.931 | 29.15 | 0.002 |
| Taste Acceptability | 0.958 | 0.938 | 0892 | 18.73 | 0.005 |
| Texture Acceptability | 0.965 | 0.938 | 0.892 | 18.73 | 0.005 |
| Overall Acceptability | 0.989 | 0.981 | 0.957 | 35.89 | 0.001 |

**Table 3: Nutrient content of highly acceptable porridge mix**

| S. No | Nutrition Composition | Herbal porridge mix/ 100 g |
|---|---|---|
| 1 | Energy (Kcal) | 213.2 |
| 2 | Carbohydrate (gm) | 45.24 |
| 3 | Protein (gm) | 7.47 |
| 4 | Fat (gm) | 0.75 |
| 5 | Fibre (gm) | 0.65 |
| 6 | Calcium (mg) | 34.4 |
| 7 | Phosphorus (mg) | 145.09 |
| 8 | Iron | 2.10 |
| 9 | Carotene | 26.42 |
| 10 | Thiamine | 0.15 |
| 11 | Riboflavin | 0.09 |
| 12 | Niacin | 1.68 |

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REVIEW ARTICLE

# Advances in 3D Imaging Technologies for Bloodstain Pattern Analysis: A Contemporary Forensic Review

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## ABSTRACT

Bloodstain pattern analysis (BPA) is a vital tool in forensic science that helps investigating officers to reconstruct crime scenes and understand the events leading to bloodshed. Conventional methods involve two-dimensional (2D) documentation, which can sometimes limit the accuracy of interpreting spatial relationships. Recent advancements in three-dimensional (3D) imaging, including structured light scanning, laser scanning(LIDAR), and Hemospat pattern Analysis are more precise and reliable techniques for bloodstain analysis as they capture detailed spatial data, improving pattern recognition and crime scene reconstruction. This review explores the latest developments in 3D imaging for BPA, comparing them to conventional techniques and discussing their practical applications, benefits and limitations. While 3D imaging enhances accuracy and objectivity, challenges such as cost, accessibility, and the need for validation in forensic casework remain. By understanding these advancements, forensic professionals can make informed decisions about incorporating 3D imaging into their investigations, ultimately strengthening the evidential value of BPA in the courtroom.

**Keywords**: Bloodstain Pattern Analysis, 3D Imaging, Crime Scene Reconstruction, Structured Light Scanning, Photogrammetry.

# INTRODUCTION

Bloodstain pattern analysis describes collecting, classifying, and analysing the distribution and different forms of bloodstains linked to a crime. A very small number of homicide cases have these kinds of stains. They provide extensive information and play a crucial role in a functional, scientific, and medically grounded recreation of a crime scene. The interpretation of Bloodstain pattern can be skewed by experience or personal prejudices, and this subjectivity may cause experts to hold divergent views, which could compromise the validity of judgments (Peschel et al., 2011). To effectively study the creation mechanism, forensic analysis necessitates the presence of a skilled expert at the crime scene to identify bloodstains and gather all relevant evidence. Analysing, characterizing, and obtaining blood spatters in a way that permits additional examination is a challenging undertaking for an amateur. Furthermore, it is impossible to gather data regarding the topography of a crime scene and the distances between significant objects using conventional approaches like photography and sketching. We created a platform that records crime scenes and bloodstains in 3D and helps the efficient preservation of evidence for later examination, even by individuals lacking specialist BPA expertise, in order to avoid common mistakes in crime scene documentation (Hołowko et al., 2016)

**Fundamentals of Bloodstain Pattern Analysis**

Blood stains are the most common body fluid that can be found at the scene of crime. It can be found in a dry state, fresh state, or even invisible to our naked eyes. Blood can take any course of nature depending on the crime scene. It can flow, drip, spray, spurt, gush, or just ooze from wounds. The presence of blood clots in stains can tell us that the attack was prolonged or that the victim was bleeding for some time after the injury had taken place. Bloodstains fall into three primary categories: projected or active stains, transfer stains, and passive stains. Passive stains like drops, flows, and pools, are typically caused due to gravity acting on an injured body. Transfer stains, like a dragged-out body or a bloody shoe print, occur when objects come into touch with pre-existing bloodstains and leave behind wipes, swipes, smears, or pattern transfers. Blood poured through the air causes active or projected stains, which are frequently noticed as scatter but can also include gushes, splashes, and arterial bursts(NFSTC, 2013.)

There are various other patterns which determine the type of weapon used and nature of wound inflicted depending on the weapon, they include arterial sprays, expired spatter, gunshot spatter, cast-off spatter, etc.Blood spatter pattern was analyzed historically using stringent method. Developed to determine the location of the blood source, the Directional Analysis of bloodstain patterns is a formally derived approach. This method enables the source to be located from above using virtual strings pointing from bloodstains to a point above the source. View from the side gives an estimate of blood source altitude. According to established physical rules, velocity components, and trigonometry, the approach does not depend on droplet size or rate. Digital cameras for evidence gathering and machine-based analysis support the process and help to guarantee a scientifically sound approach to bloodstain interpretation(Carter, 2001).

Traditionally, stain sets are traced back to their source by stringing. The outline of each stain is approximated using an ellipse, and this information has been used to calculate the stain's angle of impact (represented by $\alpha$). The acute angle produced between the projected flight path onto the target surface and a reference line, usually vertical, is known as the glancing angle, or the in-plane direction of movement. These two angles can be utilized to approximate the flight path of a stain linearly, as shown in Fig. 1. These pathways become visible through stringing, which involves tying a piece of string to the stain and allowing it to reflect the projected flight path. Although stringing has been successfully applied, there are many obvious drawbacks: String placement is a difficult task and takes a lot of time that requires skill and expertise; spending a lot of time at the crime scene increases the chance of contamination, and pattern analysis is a one-time opportunity because crime scenes are cleaned up and released after an investigation. This means that if the results are later contested, it will be hard to conduct another analysis.

**Advancements in 3D Imaging for Blood Pattern Analysis**

Forensic science relies on accurate and reliable work by scientist; however, errors are inevitable. As a result, forensic science is becoming increasingly dependent on automated methods. The technique like HemoVision, BackTrack, HemoSpat, 3D Laser scanning, Photogrammetry are used in Forensic Blood Examination for calculating the area of convergence, identifying the angle of impact, identifying the area of origin, or even by providing a 3D view of the crime scene. HemoVision employs computer algorithms and bloodstain markers to determine a drop's flight path and convergence zone. In spite of its efficiency and the fact that it does not require manual measurements, this method is only successful if all the blood spatter is concentrated on one wall. Analysis of directional data is another technique used by analysts to find convergences between bloodstains(Dryzal, 2018). Back Track TM require a digital camera to gather blood stain data, followed by a computer-based directed analysis to a three-dimensional area of overlap immediately above the position of the blood source. Researchers performed a study to validate computer-assisted elliptic measurement techniques using BackTrackTM and Excel 2003 Autoshapes(Home et al., 2021).

Bloodstain analysis usually assumes that stains are found on level, perpendicular surfaces, thus restricting the precision of area-of-origin assessments. The authors show how HemoSpat enables more exact 3D modeling and logical identification of the blood source position, even if stains are present on sloped or bent surfaces (Maloney et al., 2005). Nowadays, cast off stains can also be analysed and interpreted by investigators. Through a sequence of evaluations utilizing a regulated cast-off rig (n = 10), a Path Volume Envelope (PVE) can be recognized where the swinging trajectory is enclosed within a volume alongside a region of exclusion (Liscio et al., 2020).

**Applications of 3D Imaging and 3D Scanning Technologies**

The application of 3D technology into the world of blood spatter pattern analysis has paved the way for better advancement techniques in the field of forensic science. Techniques such as 3D modelling and virtual reality simulations have emerged as a pivotal part in the life of a forensic expert. These tools use algorithms that help in the identification and analysis of bloodstain patterns. Machine learning algorithms such as Virtual reality simulations and 3D modelling techniques may provide a broader array of precise results which the human eye might fail to capture in the rawest form. Techniques such as Bluestar and luminol, which help in identification of blood in a scene of crime, are often clubbed together with 3D imaging techniques to provide a better sequence of events in crime scene reconstruction. These technologies not only help in identification but also provide a comprehensive analysis of primary and secondary blood spatter patterns(Kaur Mohal et al., 2023). 3D scanning creates detailed point clouds of crime scenes, improving juror comprehension of BPA evidence. A study showed that jurors who viewed 3D representations retained information better than those who did not(Home et al., 2024).Tools like the FARO Focus 3D scanner have demonstrated superior accuracy in estimating bloodstain origin compared to traditional methods, with significant improvements in measurement precision(Esaias et al., 2020) Optimized Algorithms: For the purpose of detection of blood stains, a study was conducted, in which, the primary method used was a 3D Convolutional Neural Network (CNN) incorporating Hyperspectral Imaging. This 3D model is best suitable for analyzing blood stains using spatial and temporal information present in the data to provide an accurate representation. The results concluded that the 3D CNN model surpassed the accuracies of existing models which had an accuracy of 96% and 95%, becoming 97% accurate. It helps in faster crime investigation and better case resolution.(Amin Tokder Shoukhin et al., 2024).Recon-3D application leverages LiDAR technology to create 3D point clouds, providing an affordable and effective alternative for crime scene documentation. It has shown comparable accuracy to traditional laser scanners(Stevenson & Liscio, 2024)

**Recent Developments and Innovations**

The use of technology in Bloodstain Pattern Analysis (BPA) has seen major improvements, especially through the introduction of 3D modelling and virtual reality simulations. In conjunction with 3D modelling, virtual reality simulations have become a valuable tool for forensic investigators. Virtual reality environments enable analysts to fully engage with recreated crime scenes, enhancing the examination of bloodstain patterns through immersion and interaction.

Machine learning algorithms can analyse large datasets to discover patterns that are difficult for humans to detect, improving the thoroughness of analysis. The incorporation of different imaging techniques has also enhanced the precision of BPA. Technologies like Luminol and Bluestar improve bloodstain visibility and are commonly used in conjunction with 3D modeling and virtual reality simulations for comprehensive scene reconstruction. Building upon the principles of Luminol, Bluestar uses a chemiluminescent compound that interacts with hemoglobin in blood to create a blue glow. Nevertheless, it is important to acknowledge that despite their efficacy, these methods gives false positives and the potential to compromise DNA analysis are major challenges.

Fig.1. Stringent method
Fig-2. HemoSpat Demo version
Fig-3. Angle of impact of a blood stain is found using HemoSpat Demo version at 33.7°
Table 1 Technological advancements and their advantages and disadvantages

**Challenges and Limitations**

Forensic analysts must take into account the interpretation difficulties and constraints associated with bloodstain pattern analysis (BPA) in order to guarantee accurate and trustworthy results. The intricacy of bloodstain patterns discovered at crime scenes is one significant obstacle. Numerous variables, including surface characteristics, ambient circumstances, and the kind and speed of the force, could be the source of these patterns. Understanding how these factors interact necessitates a deep understanding of biology and physics, which makes it more difficult to comprehend particular patterns. Moreover, both internal and external factors may contribute to BPA mistakes. Analysis may be impacted by outside variables such as pollution of the environment, disruption of bloodstains after deposition, or the presence of several blood sources. Internal restrictions result from possible splatter overlap and variations in blood properties. Accurate interpretation may become difficult due to current approaches' inability to discern patterns created under various settings. Additionally, the subjective nature of pattern interpretation creates difficulties in bloodstain pattern analysis (BPA) approaches. Analysts rely on their knowledge and experience, which adds subjectivity and could compromise the consistency of their results. To solve this problem, terminology and methods have been standardized; yet, creating a framework that is widely recognized still presents difficulties. Furthermore, there are advantages and disadvantages to using technology, and the accuracy of analysis may be impacted by problems with accessibility, calibration, and sensitivity of the equipment (Kaur Mohal et al., 2023).

## CONCLUSION

Blood Spatter pattern analysis has been a crucial evidentiary tool for crime scene investigators, especially in the field of crime scene management. Investigators use traditional techniques such as stringing methods as well as photographic methods to analyse a bloodstain pattern. Stringing methods have been helpful in the field of forensic science in the short run, but as time approaches faster and digitally evolves, methods such as 3D FARO, BACKTRACK, HEMOSPAT, PHOTOGRAMMETRY, and many such methods are developed for the beneficiary purpose of investigators. 3D methods have become a boon for the field of forensic science. Developments in Bloodstain pattern analysis have enhanced the accuracy rates and efficiency of forensic investigators. The integration of 3D scanning technologies has improved juror comprehension, evidence analysis, and crime scene reconstruction techniques.

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**Table 1. Technological advancements and their advantages and disadvantages**

| TECHNIQUE | ADVANTAGES | DISADVANTAGES |
|---|---|---|
| **Directional analysis** | Provides accurate location of blood source; Not dependent on speed or droplet size | Requires accurate configuration and understanding; Can be difficult in intricate or messy crime scenes; Dependent on distinct, unblocked bloodstain patterns. |
| **3D Bloodstain Pattern Reconstruction** | Offers a more thorough and precise comprehension of blood spatter; Aids in establishing trajectories and points of origin in intricate settings. | Significant computational demands and expenses associated with software; Risk of mistakes if the input data is imprecise or insufficient. |
| **Digital Photography & Imaging** | Enables accurate, consistent documentation of bloodstains; Promotes straightforward sharing and examination of evidence in court. | Needs appropriate training to obtain precise, high-quality images; Image distortion or inadequate lighting can influence the accuracy of analysis. |
| **Laser Scanning (LiDAR)** | Records 3D spatial information of bloodstains, including on uneven surfaces; Increases spatial precision and overall examination of crime scenes. | Expensive technology with a high learning curve; Might be time-intensive and may not always be practical at every crime scene. |
| **Computer-Aided Bloodstain Analysis** | Boosts the speed and precision of area-of-origin calculations; Minimizes human error and bias in interpretation. | Software can be expensive and needs training; Restricted by the quality of input data and possibility of software defects. |
| **HemoSpat** | Accounts for bloodstains on surfaces that are non-orthogonal (angled/slanted); Enables 3D modeling for improved area-of-origin calculations; Enhances accuracy in complicated situations. | Necessitates sophisticated software and technical expertise to function efficiently; Might not be appropriate for every crime scene or accessible in all forensic laboratories; Costly and resource-demanding for regular usage. |

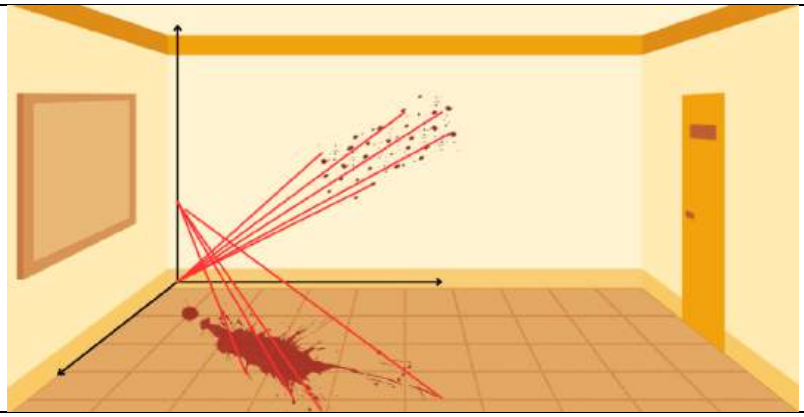


**Fig 1Showing Stringent method used for directional analysis of blood stains.**




**Fig 2. Angle of impact of a blood stain is found using HemoSpat Demo version at 33.6°**




**Fig 3.Angle of impact of a blood stain is found using HemoSpat Demo version at 33.7°**

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**RESEARCH ARTICLE**

# The Role of Radiomics in Oncology: From Imaging to Personalized Treatment

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**ABSTRACT**

Cancer is the second leading cause of death. A multimodality-based strategy is necessary for the complex process of cancer treatment. Cancer detection and treatment begin with screening and diagnosis and continue throughout the duration of the patient's life. The first step in managing cancer is screening and diagnosis, which is followed by disease staging, treatment planning and administration, therapy monitoring, and continuing monitoring and follow-up. In every phase of cancer treatment, imaging is crucial. Numerous academics have already examined the relevance of artificial intelligence (AI) and imaging biomarkers in oncology. The literature now in publication suggests that AI and imaging biomarkers are becoming more and more important in oncology. But radiomic characteristics' stability has also been questioned. The global generalization of radiomic-based prediction models is threatened by the volatility of radiomic features, as acknowledged by the radiomic community. MRI and CT scans can help diagnose brain, soft tissue, and bone tumors as well as head and neck cancer. Treatment response has also been predicted by radiomics characteristics from CT, PET/CT, and cone beam CT. CT scans obtained from patients with pancreatic, lung, and head and neck malignancies undergoing standard image-guided radiation therapy using in-room CT.

**Keywords:** Radiomics, CT, PET, TNM, MRI

# INTRODUCTION

Medical imaging data extraction is known as radiomics. In order to provide precision medicine, it has been employed in oncology to enhance diagnosis, prognosis, and clinical decision support. Applications in oncology sometimes include either clinical event prediction using a time-to-event analysis, such overall survival, or classification tasks, which involve calculating the likelihood that a sample falls into a category, like benign versus malignant[2]. Tumor segmentation, picture preprocessing, feature extraction, model building, and validation are all processes in the interdisciplinary radiomic workflow, which involves radiologists as well as data and imaging scientists. Radiologists, image scientists, and data scientists are just a few of the many disciplines that contribute to this subject[1]. Features can be applied to the complete range of imaging data and are obtained at one (often pretreatment) or several time points. Radiomics first refers to the extraction of a large number of high-dimensional quantitative features from multimodality medical images, such as computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), and ultrasonography (US), followed by the mining of correlations between these features and the prognosis or diagnosis of cancer[8]. Tumors can then be encoded into various imaging phenotypes once these relationships are identified through imaging analysis. When making decisions about cancer detection and therapy, radiomics may provide objective, quantitative support. This is achieved by extracting quantitative information from medical images, integrating imaging characteristics with clinical, genetic, and other data, and then mining this information to find radiomic biomarkers[5]. The field of radiomics uses a number of computational technologies, and its methods are typically focused on therapeutic issues. Regarding technology that satisfies clinical needs and advances cancer treatments and diagnostics, radiomics has achieved significant strides[7].

**Oncology**

Cancer research, diagnosis, treatment, and prevention are all part of the medical discipline of oncology. It involves a variety of activities, including as investigating the causes of cancer, discovering new medications, and supporting patients during their cancer journey[3]. The study and treatment of cancer, including research, surgery, radiation therapy, and pharmaceutical treatments like chemotherapy, is known as oncology. Cancer is usually diagnosed and treated by specialized multidisciplinary teams because it requires a variety of medical specialties [9].

**Applications of radiomics in Oncology**

Radiomic investigations in cancer frequently (a) perform classification tasks or (b) predict clinical outcomes using a time-to-event approach. The process of forming categories within a population is called classification[4]. Examples include the presence of metastases, tumor stage, genetic status, and benign versus malignant. Predictive models use clinical outcomes to categorize patients into different risk groups according to the probability that clinical endpoints, such as overall or disease-free survival, will occur[10]. A time-to-event analysis is used to assess these models. These applications are based on the notion that radiomic data can reveal details about tumor biology. Temporal and geographical heterogeneity are known to have a substantial impact on tumorbehavior and treatment resistance, which may be reflected in radiomic features[15]. Therefore, radiomics has the potential to function as a "virtual biopsy" and, in contrast to traditional biopsies, employs non-invasive imaging that allows analysis of the entire tumor (instead of a focal sample) and can be applied more readily at various time points for disease monitoring, providing potentially significant diagnostic information related to disease evolution[16]. Organizing a Radiomic Investigation In order to evaluate the viability and probability of success of a radiomic investigation, it is important to pose fundamental questions[11]. When evaluating suggested studies, our organization finds that a radiomic study proforma is helpful. A radiomic study should, like any research study, contain a testable hypothesis that addresses a pertinent clinical topic, typically with the goal of addressing an unmet need in cancer management [12]. Determining if there is enough data to support the creation of a radiomic signature—a learnt model from a radiomic analysis used to predict a specific clinical outcome—is a crucial factor[13]. Since radiomics is data-driven, it is not always possible to predict how many features will be included in the final model because feature selection procedures are frequently used before or during the model fitting process. However, this can vary between studies and is a helpful guide when

starting a new study[17]. It's also critical to recognize the pervasiveness of data attrition. Poor image quality, failure to meet inclusion criteria or loss to follow-up, and missing or incorrectly labeled data are common causes.

**Cancer treatment and the role of imaging**

The procedure of treating cancer is difficult and time-consuming. Cancer treatment starts as soon as the disease is diagnosed and, in certain cases, continues while the patient is still living. The detection and management of cancer as well as the role of imaging. Screening and diagnosis are the first steps in cancer care. These are followed by disease staging, treatment planning and administration, therapy monitoring, and ongoing monitoring and follow-up[18]. Imaging is crucial to the diagnosis of cancer, even though histology is necessary to confirm the disease. A range of imaging modalities, from straightforward X-rays, optical imaging, mass spectrometry, and photoacoustic imaging to sophisticated modalities like sonography, are used to identify cancer, computed tomography (CT), Figure 1 illustrates the use of radiography in cancer treatment using single-photon emission computed tomography (SPECT), positron emission tomography (PET), and magnetic resonance imaging[16]. Computed tomography (CT), positron emission tomography (PET), single-photon emission computed tomography (SPECT), and magnetic resonance imaging as shown in fig. 1 illustrating the use of radiography in cancer treatment[16]. The disease is localized and described using these methods. For instance, lung cancer is often diagnosed and localized using a chest X-ray. Breast cancer is diagnosed via mammography X-rays. Breast cancer, prostate cancer, and gynecological cancers are frequently diagnosed with ultrasound. Brain, soft tissue, and bone tumors, as well as head and neck cancer, can all be diagnosed by MRI and CT scans. PET/CT is frequently used to detect distant metastases and diagnose lymphomas and numerous other malignant diseases. Imaging is frequently used to stage disease concurrently with diagnosis. The initial tumor, nodal metastasis (TNM), and distant metastasis are all taken into account in this intricate procedure[6]. The TNM staging is carried out based on imaging and pathology findings, producing a final overall stage of the disease that largely dictates the treatment. This usually calls for a multidisciplinary approach that includes surgery. Fig- 1 shows the role of radio imaging in cancer treatment.

**Radiomics**

Radiomics use quantitative imaging in a far wider context, such as extracting a lot more information from an image, and extends beyond these conventional requirements. In early research, this has shown that therapy responses and outcomes can be obtained. To put it another way, radiomics is the process of extracting high-throughput quantitative data from medical imaging to detect specific pathological diseases that are linked to outcomes, as illustrated in Figure 2.15. Shape-based, first-order, higher-order, textural, Laplacian-of-Gaussian (LoG), and wavelet features are the categories for radiomic features extracted from medical imagesTumor segmentation, image extraction, image pre-processing (converting images to the necessary format, voxel normalization, image masking, image filtering, image transformation), and radiomic extraction (extracting radiomic features from original, filtered, and transformed images) comprise the entire radiomic process[12]. Feature selection or reduction approaches can be used to control the data explosion that results from this procedure. Lastly, radiomic prediction models are constructed using extracted/selected features[18]. Fig-2 showing the extraction of quantitative data from medical images.

**Treatment outcomes of radiomics using in imaging**

A range of imaging modalities, from straightforward X-rays, optical imaging, mass spectrometry, and photoacoustic imaging to sophisticated modalities like sonography, computed tomography (CT), single-photon emission computed tomography (SPECT), positron emission tomography (PET), and magnetic resonance imaging, are used to diagnose cancer. These methods are used to identify and describe the illness. For instance, a chest X-ray is commonly used to identify and pinpoint lung cancer[2]. To diagnose breast cancer, mammography X-rays are utilized. Ultrasound is frequently utilized for (additional) diagnosis of gynecological malignancies, prostate cancer, and breast cancer. MRI and CT scans can help diagnose brain, soft tissue, and bone tumors as well as head and neck cancer. Additionally, radiomics characteristics from CT, PET/CT, and cone beam CT have demonstrated predictive usefulness for response of treatment[10]. According to their findings, radiation therapy can cause patient-specific alterations in CT texture features, which can be identified early on. Patients with locally advanced non-small cell lung cancer undergoing chemoradiation therapy may benefit from pre-treatment metabolic tumor volume and heterogeneity textural metrics

on PET/CT. [19]These findings imply that a radiomics-based signature might become a recognized imaging biomarker for therapeutic outcome prediction and for enhancing cancer treatment decision assistance.

## CONCLUSION

The procedure of treating cancer is intricate and time-consuming. Treatment for cancer starts as soon as the illness is diagnosed and sometimes continues while the patient is still living. Breast cancer is diagnosed via mammography X-rays. Breast cancer, prostate cancer, and gynecological cancers are frequently diagnosed with ultrasound. Brain, soft tissue, and bone tumors, as well as head and neck cancer, can all be diagnosed by MRI and CT scans[1]. PET/CT is frequently used to detect distant metastases and diagnose lymphomas and many other malignant diseases. Imaging is frequently used to stage disease in tandem with diagnosis. The primary tumor, nodal metastases (TNM), and distant metastases are all taken into account in this intricate process[3]. The final overall stage of the disease is determined by the TNM staging, which is based on imaging and pathology findings and usually necessitates a multidisciplinary approach including surgery[20].

**Limitations of Radiomics**

Repeatability and reproducibility in radiomics are inherently problematic because to variability in scanners from different manufacturers, acquisition methods, and intra-scanner changes. Only 10% of CT radiomic features had good repeatability and reproducibility in clinical cohorts, according to our earlier repeatability and reproducibility analysis, but phantom found that most radiomic features had stability problems in a systematic literature review. In [18] The Quantitative Imaging Network (QIN), the Quantitative Imaging Biomarkers Alliance (QIBA), and Quantitative Imaging in Cancer: Connecting Cellular Processes with Therapy are just a few of the organizations that have started efforts to standardize radiomic extraction technologies, features, and imaging standards.[15]These groups are putting forth a lot of effort to standardize imaging and imaging biomarkers. Another partnership, the Image Biomarker Standardization Initiative (IBSI), strives to harmonize radiomic features worldwide by reducing image deviation and standardizing the radiomic extraction procedure. QIN is an effort to standardize hardware and imaging settings throughout the industry[17].

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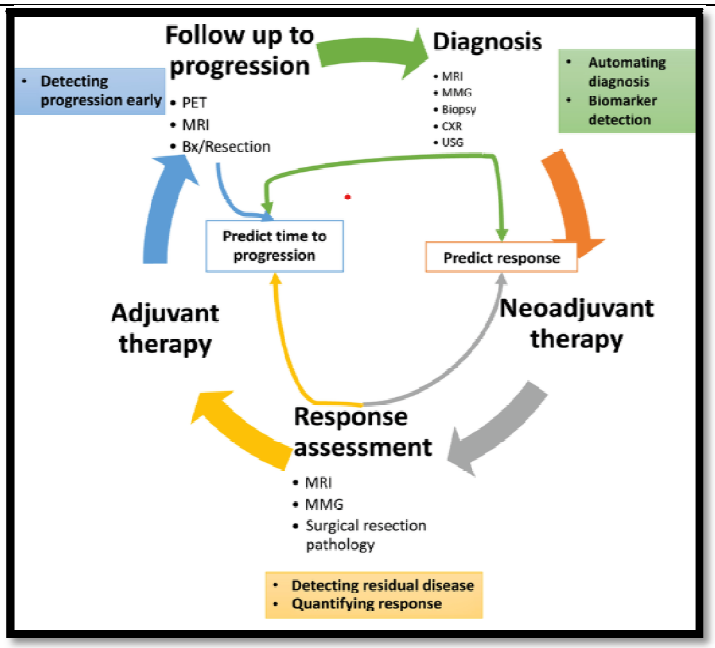



**Fig 1: Shows the role of radio imaging in cancer treatment.**




**Fig 2: Showing the extraction of quantitative data from medical images.**

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RESEARCH ARTICLE

# A Study to Assess the Cognitive Impairment and Barriers to Utilization of Mental Health Services among the Elderly Residing in Selected Rural Areas of Central Gujarat

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## ABSTRACT

About 70% of people with cognitive impairment have Alzheimer's illness. In the world today, memory impairment is the major reason for cognitive impairment and dependency in elderly individuals. The "care deficit" refers to the fact that the majority of elderly people suffering from psychological disorders fail to get adequate care. Conducted a cross-sectional descriptive survey on 118 elderly individuals, aged between 60 and 80 years, selected using purposive sampling techniques. Data was collected using three tools: a demographic questionnaire, the Montreal Cognitive Assessment (MoCA) for cognitive impairment, and a self-structured questionnaire to assess barriers to utilization of mental health services. The results revealed that the majority of participants had cognitive impairment, 83.1% of the elderly samples; 36.4% had moderate cognitive impairment, while 33.9% of them had mild cognitive impairment; 12.7% of them had severe cognitive impairment whereas only 16.9% of the elderly had normal cognition. Barriers to accessing mental health services were significant, with 37.3% facing severe obstacles, primarily due to lack of access, financial difficulties, and transportation issues. The study highlights the need for improved mental health services and awareness programs for the elderly in rural areas.

**Keywords:** Cognitive Impairment (CI), Barriers, Utilization of Mental Health Service, Elderly, Rural Areas, Geriatric Mental Health.

## INTRODUCTION

Growing older is a normal occurrence that presents a unique set of difficulties. Their conventional nurturing and life-sustaining effects are gradually waning as the number of elderly people rises. Fear, memory loss, and sadness are regular issues at the old age population. Elders' psychological wellness is a major problem because it can result in depressive disorders, cognitive impairment, and fretfulness, all of which can have an adverse effect on the state of their bodies [1]. The word "dementia" refers to a broad range of disorders that impair behavior, recall, and other thinking abilities and that substantially limit an individual's capability to carry out everyday tasks. While aging is the most powerful observed risk factor for cognitive impairment, it is not a natural process of aging [2]. There are already 50 million individual sufferers from dementia globally, and by 2050, that number is expected to triple due to the increase in the rate of new cases developing one in every three seconds. The growing prevalence of cognitive impairment, its profound effects on society and finances, and the absence of effective treatments require that nations concentrate on lowering controllable memory problem risk factors [3]. Currently, approximately 55 million people in the world are living with Alzheimer disease, with over 60% of those people living in nations with low or middle incomes. Every year, almost ten million new instances are reported. Women suffer from greater deaths and disability-adjusted life years as a result of cognitive impairment [4].

## REVIEW OF LITERATURE

**The review of literature is divided into 2 sections.**

1. Review of literature related to prevalence of cognitive impairment among elderly.
2. Review of literature related to barrier to utilization of mental health services.

**Review of literature related to prevalence of cognitive impairment among elderly**

Exploratory study carried out in Lucknow, 2012; among the residents of old age homes. Sample were selected randomly. Data was collected utilizing standardized survey and assessment tools. The study result revealed that 64.4% of the residents had been diagnosed with some form of mental illness in the past, each person who lived in retirement homes was also suffering from at least one or more physiological illnesses. Depression was the most common issue, affecting over half (50%) of males and nearly a third (28%) of females. In males, cognitive decline followed closely (20%), while anxiety was the second most frequent disorder in females. Dementia appeared more prevalent in men (20%) than in women (4%). Psychological issues were more pronounced in the "young-old". Here, nearly all participants (males and females) exhibited at least one mental health disorder [5]. A study done in 2018, sample aged 50 years and above residing Karamsad, Gujarat, India. To determine the widespread of Cognitive Impairment and identify associated geodemographic variables. The Mini-Mental Status Examination, Gujarati version was utilized for screening. Out of 560 people studied, over a quarter (25%) showed signs of cognitive decline (CI). This problem was more prevailing in the residents of exurban area (27.6%) in contrast to those residing in suburban areas (18.5%) furthermore more among women (29.8%) than men (19.1%). The researchers investigated and found that being older than 60 and having no education greatly increased the chances of having CI. On the other hand, having a job, companion, or family significantly lowered the risk of CI [6]. A cross-sectional research to rule out incidence of dementia among 770 samples aged more than 60 years residing Belagavi District, Karnataka. Mental Status Examination(MMSE) and Zarit Caregiver Burden Scale were used to collect the data. A study found that over 8% of elderly people had impaired cognition (cognition). Several factors like being old, female, unmarried (including widowed), not having much education, not having a job currently, living alone, and being poor were all linked to a higher chance of having cognitive impairment. The study also looked at caregivers and found that two-thirds of them (67.7%) felt somewhat to very burdened by caring for elderly people with cognitive decline [7]. A prevalence study to assess cognitive dysfunction and behavioral vulnerabilities in 330 elderly residing in assisted living facility Tamil Nadu. There were two stages to the multistage sampling techniques: simple random sampling and probability proportional sampling. A Brief Interview for Mental Status (BIMS) instrument and a standardized, pretested, structured questionnaire were utilized. SPSS was used for analysis. The result depicted; 44% had subdued cognitive

decline, 36% had serious mental impairment. Approximately 75% of people regularly engage in physical activity, about 4% of the study participants were smokers at the time, and 5% had a history of regular alcohol use [8].

**Review of literature related to barrier to utilization of mental health services**

A review was carried out using seven internet datasets between January 2000 and November 2019 to identify obstacles to the availability and use of mental health treatments in low- and middle-income countries (LMICs). There are several obstacles that prevent mental health services from being provided, including inadequate funding, not equal allocation of resources, centralized and non-integrated services, malfunctioning to provide suitable psychological care, lacking quality assurance programs, inadequate training for staff, poor psychological health information networks, and a lack of research- based practices, unfavorable experiences in the past, inadequate faith in staff, inadequate adherence to therapy, cost of services, accessibility and mode of transport, lack of social support, the time restrictions, the unsuitable and rigid nature of the services, the insufficiency of the care, a lack of financial resources, the lack of awareness, the anxiety of therapy, and the long length of the treatment [9]. A qualitative study was conducted at Menur's Mental Health Hospital in Surabaya to explore the barriers faced by the families of patients regarding mental health services in 2014. Twelve participants were selected through purposive sampling. Data was collected using semi-structured in-depth interviews. The analysis identified three primary themes: the affordability of mental health services, 2) the availability of mental health services, particularly the shortage of services in rural areas, and 3) negative attitudes and stigma associated with mental illness. [10]. A cross-sectional study carried out in 2015 at the Mental Health Department of Kabutare District Hospital in Rwanda's Southern Province to investigate barriers to patients with mental disorders using mental health services. Detailed interviews were used to gather data. Thematic analysis was used to analyse the data. Three primary obstacles were examined: the stigma associated with mental illness, limited awareness about accessible resources, financial constraints, and cultural and religious faith [11]

A qualitative descriptive study was done, in Jordan between May to December 2019 among 34 participants using one on one basis. The following categories emerged as obstacles to receiving mental health services:

i. Taboo and cultural rejection;
ii. Inadequate knowledge about psychological disorders and resources present; and
iii. The accessibility, cost, and availability of mental health treatments. The results of this study indicate that the respondents' levels of psychological awareness was low [12].

**Statement of the Problem**

"A Study To Assess The Cognitive Impairment And Barriers To Utilization Of Mental Health Services Among The Elderly Residing In Selected Rural Areas Of Central Gujarat"

## OBJECTIVES

1. To assess the cognitive impairment among the elderly in the selected rural areas.
2. To assess the barriers to the utilization of mental health services among the elderly in the selected rural areas.
3. To determine the association between barriers to utilization of mental health services with selected demographic variables.
4. To determine the association of cognitive impairment and barriers to utilization of mental health services with selected demographic variables among the elderly in the selected rural areas.

## RESEARCH METHODOLOGY

**Research Approach:** Quantitative approach.

**Research Design:** Cross-sectional descriptive design was employed to collect the data from the older adults 60 years and older who were residing in the research setting area. House to house individual interview was conducted for a period of one month (April 1, 2024 -April 30, 2024) to gather the information.

**Study Site:** Petlad Taluka

**Sampling Technique:** Purposive sampling technique.
**Sample Size**: = Z1-$\alpha$/22p(1-p)/d2
Z1-$\alpha$ = It is standard normal variate (where, Z= 5=1.96)
p = Expected proportion in population based on previous studies or pilot studies (where, p=8.4%)
d = Absolute error or precision –Has to be decided by the researcher (where, d=0.05)
Sample Size = $(1.96)^2$x0.084 (1-0.084)) / $(0.05)^2$
= (3.8416x0.084 (0.916)) / 0.0025
= (0.3226944(0.916))/0.0025
= 0.2955880704/0.0025
= 118 [13]

**Inclusion Criteria**
- Male and female age group of 61 to 80 years.
- Who are able to provide informed consent and participate in the study
- Who understands the Gujarati language.
- Elderly who are using sensory aids (eyeglasses, hearing aids).

**Exclusion Criteria**
- Who are unconscious or suffering from any chronic terminal illness.
- Who have a chronic disability.
- Who are suffering from moderate to severe mental illness.

**Study Tool:** The tool consisted of three sections:
(i) Self-structured demographic information tool including eight [8] questions related to socio-demographic variables like age in years, gender, marital status, education level, occupation, monthly income (in rupee), types of family and number of children.
(ii) Standardized tool for cognitive assessment MoCA: Montreal Cognitive Assessment, MoCA is a comprehensive cognitive screening that evaluates various cognitive domains, with a maximum possible score of 30 points.
The scoring is as follows:
**Visuospatial/Executive (five points):** Includes tasks such as drawing a clock and copying a cube.
**Naming (three points):** Involves naming animals.
**Memory (five points):** Assesses delayed recall of a list of words.
**Attention (six points):** Includes tasks like repeating numbers, a target- detection task, and subtracting serial sevens.
**Language (three points):** Includes sentence repetition and verbal fluency tasks.
**Abstraction (two points):** Involves explaining similarities between pairs of words.
**Delayed Recall (five points):** Evaluates the ability to recall the previously mentioned words without cues.
**Orientation (six points):** Assesses awareness of time and place.

Every item is given a score, with a maximum of 30 points achievable. Normalcy is defined as having a score of 26 or higher, mild cognitive impairment as 18 to 25 points, moderate cognitive impairment as 10 to 17 points, and severe cognitive impairment as less than 10 points [14]. (iii) Self-structured questionnaires to assess the Barriers to Mental Health Services, consisted of 4 items. It assesses awareness of local services, past utilization of counseling, therapy, or medication, current engagement with mental health services, and specific barriers such as stigma, awareness, access issues, financial constraints, transportation problems, fear of judgment, distrust in healthcare professionals, and cultural or religious beliefs. This tool is essential for understanding and addressing the impediments to mental health service utilization. The content validity of the tool was established by seeking the suggestions from 10 experts in the field of Nursing and Medicine. The suggestions was incorporated in the final tool.

**Reliability**

MoCA is a standardized tool with a reliability was 0.96, it was translated into the regional language, and the reliability was established using test-retest, which came out to be 0.78 [15].

## RESULTS

**Section I: Demographic Characteristics**

Description of samples (elderly) based on their characteristics in terms of frequency and percentage. (N=118) (Table 1).

**Table: 1-** Depicts sociodemographic characteristics of the samples; 34.7% of elderly were in the age group 66-70 years, 25.4% of them were in the range 71-75 years, 20.3% belonged to 61-65 years and 19.5% of them were in 76-80 years. The majority, 61.9%, of the sample were females, while 38.1% were males. Most of the elderly were married, making up 54.2% of the sample, however, 24.6% had lost their partner, and 10.2% were divorced. A small percentage, 6.8%, were separated, and an even lesser number, 4.2%, were single. Approximately 38.1% completed primary education, while 31.4% had secondary education. A notable 18.6% had no formal education, and a smaller segment, 11.9%, had achieved graduation or post-graduation degrees. The largest group, 48.3%, were laborers, followed by 17.8% who were business owners. Additionally, 12.7% were retired, 5.9% worked in the private sector, and 1.7% were government employees. Another 13.6% had different types of jobs. Most participants, 62.7%, had a monthly income of less than Rs.10,000. Meanwhile, 22.9% had an income of between Rs. 10,000 to Rs. 20,000, and 10.2% had an income between Rs. 20,001 and Rs.30,000. A smaller group, 4.2%, earned more than Rs. 30,000 per month. The majority, 63.6%, lived in joint families, while 25.4% resided in nuclear families. A smaller percentage, 11%, were part of extended families. A considerable portion, 35.6%, had two children, while 34.7% had more than two children. Additionally, 13.6% had one child, and a smaller number, 16.1%, had no children.

**Section II: Prevalence of cognitive impairment among the elderly in the selected rural areas.**

Cognitive impairment among the elderly in the selected rural areas N=118 (Table 2).

**Table: 2-** Presents intrinsically 36.4% had moderate cognitive impairment, while 33.9% of them had mild cognitive impairment,16.9% of the elderly had normal cognition and 12.7% of them had severe cognitive impairment.

Cognitive impairment among the elderly in the selected rural areas (Figure 1).

**Section III:** Utilization of mental health services by the elderly (magnitude of obstacles/ barriers to utilize services and awareness of services) in the selected rural areas.

Magnitude of obstacles/barriers experienced by the elderly to utilize mental health services in the selected rural areas. N=118 (Table 3).

**Table 3:** The majority of the elderly, 52.5%, faced moderate obstacles to accessing mental health services. A substantial 37.3% encountered severe barriers, while a smaller portion, 10.2%, experienced mild barriers.

Prevalence on awareness and availability of mental health services among the elderly. (N=118) (Table 4).

**Table 4:** Shows a smaller portion, 25.4%, of the elderly were aware of the mental health services available in their area. However, only 2.5% were currently receiving mental health services. Additionally, 6.8% had previously sought mental health support, with 1.7% having received counselling or therapy and 5.1% having been prescribed medication. The majority of the elderly faced several barriers to seeking mental health services. A significant 94.9% experienced a lack of access to services, and 91.5% lacked awareness about available services. Financial problems affected 87.3%, while 86.4% encountered transportation issues. Cultural or religious beliefs were a factor for 66.1%, and 59.3% relied on traditional folk medicine. Additionally, 58.5% reported stigma associated with mental health, 50% had a lack of trust in healthcare professionals, and 44.1% lacked knowledge of healthcare professionals. Furthermore, 40.7% feared judgment from others, and a small portion, 1.7%, cited other factors.

Factors that prevent elderly from seeking mental health services (N=118) (Table 5).
**Table 5:** Illustrates that a significant 94.9% experienced a lack of access to services, and 91.5% were not aware of the available services. Financial problems affected 87.3%, while 86.4% encountered transportation issues. Cultural or religious beliefs were a factor for 66.1%, and 59.3% relied on traditional folk medicine. Additionally, 58.5% reported stigma associated with mental illness, 50% had a lack of trust in healthcare professionals, and 44.1% felt lack of knowledge among the healthcare professionals. Furthermore, 40.7% feared judgment from others, and a small portion, 1.7%, cited other factors.

**Section IV:** Association of cognitive impairment and barriers to utilization of mental health services of the elderly with their selected demographic variables.

Fisher's exact test for the association of cognitive impairment with selected demographic variables. (N=118) (Table 6).
**Table 6:** Represents the relationship of cognitive impairment with demographic variables, given that the p-values for age, marital status, education, occupation, and monthly income were below 0.05, these demographic factors are determined to have a significant relationship with cognitive impairment among the elderly.

Fisher's exact test for the association of barriers to utilization of mental health services with selected demographic variables. (N=118) (Table 7).
**Table 7:** Shows that the p-value for education was less than 0.05, it was identified that the demographic variable of education has a significant association with barriers to accessing mental health services among the elderly.

## DISCUSSION

This study assessed the prevalence of cognitive impairment in adults over 60 years of age, along association of cognitive impairment and demographic variables, barriers to utilization of mental health services, and its association with demographic variables. In the present study majority of older adults represented from the age group 60-70 years, a study conducted by Anku Moni Saikia and Vinoth Rajendra (2019) among 576 elderly participants concluded the similar demographic results in terms of age group [16]. In this study 12.7% of the samples demonstrated severe cognitive impairment, 36.4% revealed moderate cognitive impairment, 33.9 had mild cognitive impairment, whereas only 16.9% were with normal cognition. From a study conducted by Sujitha P *et al*., (2022) it has been noticed that 36% of the older adults faced severe cognitive decline, 44% of the participants were experiencing mild to moderate cognitive decline [17]. A study conducted by Irom Devina *et al*., (2022) revealed that out of 115 participants, 23.48% had mild cognitive impairment, 6.95% had moderate cognitive impairment, albeit 69.57% showed no cognitive impairment at all. [18]. In the present study 52.5% of the samples reported moderate barriers to access mental health services, 37.3% faced severe obstacles to the utilizations of mental health services. Approximately 25% of the samples were aware of the availability of mental health services in their areas, although only 2.5% were receiving those services. Only 6.8% had sought mental health services in the past; 1.7% had received counselling and 5.1% have taken medications. The barriers identified in the present study were related to lack of access to the services 94.9%, 91.5% were not even aware if such services are available, 87.3% reported financial difficulties, others reported barriers were

stigma, fear of judgement from others, lack of trust in the healthcare professionals, religious and cultural reasons, belief in traditional folk medicine. A study conducted by Sara H Ali *et al*., (2016) showed that major barriers to access mental health services were related to faith surroundings psychological disorders, using other therapies provided by spiritual leaders, no awareness regarding such available services, expensive medicines and fear and insufficient knowledge about the side effects [19]. Similar research conducted by Nguyen Hang *et al*, 2019 identified seven key obstacles to obtaining services for mental health. These barriers included discrimination, psychological issues, limitations on involvement, satisfaction with services, time restraints, financial and geographical constraints, and service accessibility were some of these obstacles [20]. Quite similar barriers were stated in a systematic review conducted by Lavingia Richa, Jones Kristin, Ali Asghar Abbas, 2020, which revealed categories to mental health barriers in the adults 50 years and older as deficit knowledge and negative perception, comorbidity, issues related to health care providers, financial constraints and transportation [21]. Lack of knowledge related to when to seek help and unavailability of health care providers were reported as prevalent barriers in older adults 65 year and older in a study conducted by Adams Claire *et al*. 2022 [22] In 2013, Rakesh M. Patel and Uday Shankar Singh conducted a study to evaluate incidence of dementia and its relationship with sociodemographic variables. Their findings indicated no significant association between family type and cognitive decline. Similarly, the present research revealed no significant associations between cognitive impairment and factors such as living situation, number of children, and gender. These results imply that cognitive impairment in the elderly may not be significantly affected by sociodemographic variables [23].

## CONCLUSION

The outcome of the investigation emphasizes the critical need for educational programs on psychological well-being and services for the elderly in rural communities, given the high incidence of cognitive impairment and barriers like lack of awareness, stigma, and access difficulties. It underscores the importance of early detection, intervention, and integrating mental health education into routine healthcare. Strategies to enhance service accessibility, such as community outreach, mobile clinics, and local partnerships, are discussed. The study calls for a holistic approach to mental health care, ensuring elderly individuals in rural areas receive the necessary support for their well-being.

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## CONFLICT OF INTEREST

There are no conflict of interest.

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### ETHICAL CLEARANCE

The ethical clearance (study number IEC/CHARUSAT/23/162) was obtained from the Institutional Ethical Committee- Charotar University of Science and Technology.

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**Table 1: Description of samples (elderly) based on their characteristics in terms of frequency and percentage. (N=118)**

| Demographic variable | Frequency | Percentage (%) |
|---|---|---|
| **Age** | | |
| 61-65years | 24 | 20.3% |
| 66-70years | 41 | 34.7% |
| 71-75years | 30 | 25.4% |
| 76-80years | 23 | 19.5% |
| **Gender** | | |
| Male | 45 | 38.1% |
| Female | 73 | 61.9% |
| **Marital Status** | | |
| Single | 5 | 4.2% |
| Married | 64 | 54.2% |
| Separate | 8 | 6.8% |
| Divorced | 12 | 10.2% |
| Widow/Widower | 29 | 24.6% |
| **Education** | | |
| No formal education | 22 | 18.6% |
| Primary | 45 | 38.1% |
| Secondary | 37 | 31.4% |
| Graduate or postgraduate | 14 | 11.9% |
| **Occupation** | | |
| Retired | 15 | 12.7% |
| Government employee | 2 | 1.7% |
| Private employee | 7 | 5.9% |
| Laborers | 57 | 48.3% |

| | | |
|---|---|---|
| Business Owner | 21 | 17.8% |
| Other | 16 | 13.6% |
| **Monthly income** | | |
| Below Rs.10000 | 74 | 62.7% |
| Rs. 10000to 20000 | 27 | 22.9% |
| Rs. 20001to 30000 | 12 | 10.2% |
| Above Rs. 30000 | 5 | 4.2% |
| **Living situation** | | |
| Nuclear | 30 | 25.4% |
| Joint | 75 | 63.6% |
| Extended | 13 | 11.0% |
| **Number of children** | | |
| No child | 19 | 16.1% |
| 1child | 16 | 13.6% |
| 2children | 42 | 35.6% |
| More than 2 children | 41 | 34.7% |

**Table 2: Cognitive impairment among the elderly in the selected rural areas. N=118**

| Cognitive impairment | Frequency | Percentage (%) |
|---|---|---|
| Normal | 20 | 16.9% |
| Mild | 40 | 33.9% |
| Moderate | 43 | 36.4% |
| Severe | 15 | 12.7% |

**Table 3: Magnitude of obstacles/barriers experienced by the elderly to utilize mental health services in the selected rural areas. N=118**

| Barriers | Frequency | Percentage(%) |
|---|---|---|
| Mild | 12 | 10.2% |
| Moderate | 62 | 52.5% |
| Severe | 44 | 37.3% |

**Table 4: Prevalence on awareness and availability of mental health services among the elderly. (N=118)**

| Barriers to Mental Health Services | Frequency | Percentage(%) |
|---|---|---|
| **Are you aware of mental health services available in your area?** | | |
| Yes | 30 | 25.4% |
| No | 50 | 42.4% |
| Not sure | 38 | 32.2% |
| **Are you currently receiving any mental health services?** | | |
| Yes | 3 | 2.5% |
| No | 115 | 97.5% |
| **Have you ever sought mental health services in the past?** | | |
| Yes | 8 | 6.8% |
| No | 110 | 93.2% |
| **If yes, what type of services** | | |
| Counselling or therapy | 2 | 1.7% |
| Medication | 6 | 5.1% |

**Table 5: Factors that prevent elderly from seeking mental health services (N=118)**

| Factors prevent you from seeking mental health services | Yes | | No | |
|---|---|---|---|---|
| | Frequency | Percentage (%) | Frequency | Percentage (%) |
| Stigma associated With mental illness | 69 | 58.5% | 49 | 41.5% |
| Lack of awareness about available services | 108 | 91.5% | 10 | 8.5% |
| Lack of access to services | 112 | 94.9% | 6 | 5.1% |
| Financial problem | 103 | 87.3% | 15 | 12.7% |
| Transportation issues | 102 | 86.4% | 16 | 13.6% |
| Fear of judgment from others | 48 | 40.7% | 70 | 59.3% |
| Lack of trust in Healthcare professionals | 59 | 50.0% | 59 | 50.0% |
| Cultural or religious beliefs | 78 | 66.1% | 40 | 33.9% |
| Lack of knowledge of health care professional | 52 | 44.1% | 66 | 55.9% |
| Traditional folk medicine | 70 | 59.3% | 48 | 40.7% |
| Other | 2 | 1.7% | 116 | 98.3% |

**Table 6: Fisher's exact test for the association of cognitive impairment with selected demographic variables. (N=118)**

| Demographic variable | | Cognitive impairment | | | | p-value |
|---|---|---|---|---|---|---|
| | | Normal | Mild | Moderate | Severe | |
| Age | 61-65years | 9 | 9 | 6 | 0 | 0.006 |
| | 66-70years | 5 | 17 | 15 | 4 | |
| | 71-75years | 5 | 7 | 15 | 3 | |
| | 76-80years | 1 | 7 | 7 | 8 | |
| Gender | Male | 8 | 16 | 16 | 5 | 0.970 |
| | Female | 12 | 24 | 27 | 10 | |
| Marital Status | Single | 1 | 4 | 0 | 0 | 0.006 |
| | Married | 15 | 24 | 21 | 4 | |
| | Separate | 2 | 2 | 4 | 0 | |
| | Divorced | 0 | 2 | 8 | 2 | |
| | Widow/ Widower | 2 | 8 | 10 | 9 | |
| Education | No formal education | 1 | 5 | 11 | 5 | 0.000 |
| | Primary | 2 | 14 | 21 | 8 | |
| | Secondary | 9 | 15 | 11 | 2 | |
| | Graduate or Postgraduate | 8 | 6 | 0 | 0 | |
| Occupation | Retired | 7 | 6 | 1 | 1 | 0.000 |
| | Government Employee | 1 | 1 | 0 | 0 | |
| | Private | 3 | 2 | 2 | 0 | |
| | employee | | | | | |
| | Laborers | 1 | 22 | 24 | 10 | |
| | Business owners | 5 | 7 | 6 | 3 | |
| | Others | 3 | 2 | 10 | 1 | |
| Monthly Income | BelowRs.10000 | 7 | 23 | 33 | 11 | 0.034 |
| | Rs.10000 to 20000 | 5 | 11 | 8 | 3 | |
| | Rs.20001 to 30000 | 6 | 4 | 1 | 1 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Above Rs. 30000 | 2 | 2 | 1 | 0 | |
| Living situation | Nuclear | 5 | 9 | 13 | 3 | 0.943 |
| | Joint | 14 | 26 | 25 | 10 | |
| | Extended | 1 | 5 | 5 | 2 | |
| Number of Children | No child | 2 | 9 | 6 | 2 | 0.236 |
| | One child | 4 | 6 | 4 | 2 | |
| | Two children | 11 | 12 | 12 | 7 | |
| | More than two children | 3 | 13 | 21 | 4 | |

**Table 7: Fisher's exact test for the association of barriers to utilization of mental health services with selected demographic variables. (N=118)**

| Demographic variable | | Barriers | | | p-value |
|---|---|---|---|---|---|
| | | Mild | Moderate | Severe | |
| Age | 61-65years | 3 | 10 | 11 | |
| | 66-70years | 8 | 22 | 11 | 0.098 |
| | 71-75years | 0 | 18 | 12 | |
| | 76-80years | 1 | 12 | 10 | |
| Gender | Male | 7 | 23 | 15 | 0.329 |
| | Female | 5 | 39 | 29 | |
| Marital Status | Single | 1 | 2 | 2 | |
| | Married | 6 | 36 | 22 | |
| | Separate | 1 | 5 | 2 | 0.786 |
| | Divorced | 1 | 4 | 7 | |
| | Widow/Widower | 3 | 15 | 11 | |
| Education | No formal education | 0 | 10 | 12 | |
| | Primary | 1 | 25 | 19 | |
| | Secondary | 7 | 19 | 11 | 0.007 |
| | Graduate or post graduate | 4 | 8 | 2 | |
| Occupation | Retired | 4 | 9 | 2 | |
| | Government employee | 1 | 1 | 0 | |
| | Private employee | 1 | 4 | 2 | 0.165 |
| | Laborers | 3 | 31 | 23 | |
| | Business Owner | 2 | 10 | 9 | |
| | Other | 1 | 7 | 8 | |
| Monthly income | BelowRs.10000 | 4 | 39 | 31 | |
| | Rs. 10000to 20000 | 5 | 12 | 10 | 0.170 |
| | Rs. 20001to 30000 | 2 | 8 | 2 | |
| | Above Rs. 30000 | 1 | 3 | 1 | |
| Living situation | Nuclear | 3 | 16 | 11 | |
| | Joint | 8 | 39 | 28 | 1.000 |
| | Extended | 1 | 7 | 5 | |
| Number of children | No child | 2 | 9 | 8 | |
| | 1child | 1 | 8 | 7 | 0.914 |

| | | | | | |
|---|---|---|---|---|---|
| | 2 children | 6 | 23 | 13 | |
| | More than 2 children | 3 | 22 | 16 | |

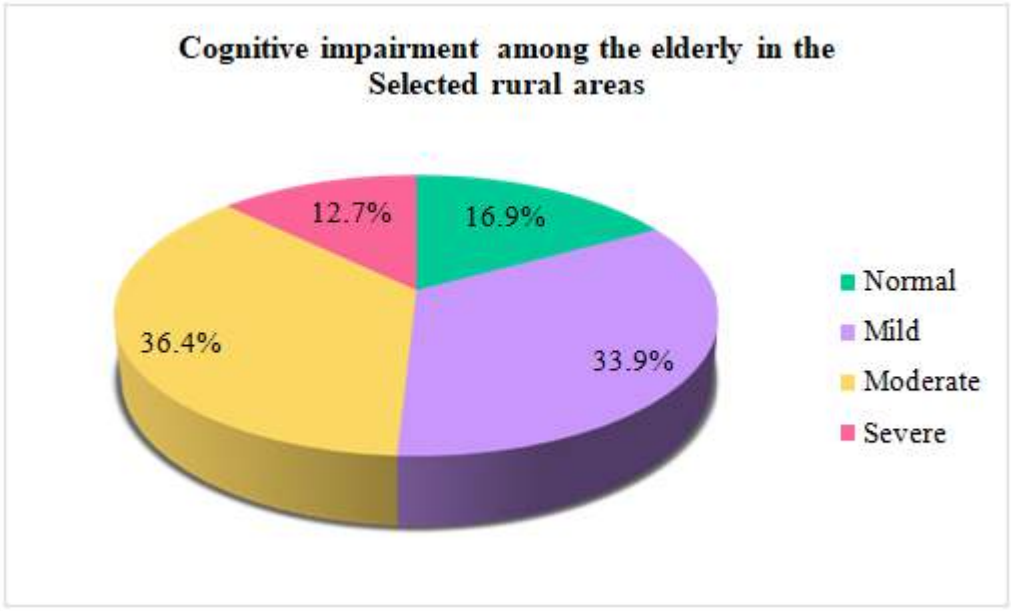



**Figure 1: Cognitive impairment among the elderly in the selected rural areas.**

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**RESEARCH ARTICLE**

# Regulatory Stringency Index for Global Microbiota Nutraceuticals

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## ABSTRACT

The human microbiota, comprising 10-100 trillion microbial cells, has emerged as a transformative frontier in the nutraceutical and functional food industry. This article evaluates the regulatory landscape governing human microbiota products (HMPs), ranging from traditional probiotics and prebiotics to advanced postbiotics and refined commensal strains across five major global jurisdictions: the United States (Food and Drug Administration), European Union (European Food Safety Authority), India (Food Safety and Standards Authority of India), Japan (Ministry of Health, Labour and Welfare), and Canada (Health Canada). Using a novel Regulatory Stringency Index (RSI)framework, we quantify market entry barriers across ten regulatory pillars including characterization requirements, safety documentation, clinical evidence standards, and manufacturing controls. Results reveal EU highest stringency (RSI 9.5), followed by Japan (8.7), Canada (7.4), India (6.1), US lowest (4.3). Sensitivity analysis (±1 point variation per parameter) confirms RSI ranking stability across all jurisdictions. The study demonstrates that successful global market access requires jurisdiction-specific strategies: Phase I targets rapid US entry for revenue generation; Phase II builds comprehensive technical dossiers for Canadian approval (gold standard);Phase III pursues Japanese FOSHU designation for premium positioning. This strategic roadmap balances technical rigor with commercial viability in a fragmented global regulatory environment.

**Keywords:** Regulatory Stringency Index, Microbiota, Probiotics, Postbiotics, Nutraceuticals, Live Biotherapeutic Products, GRAS.

# INTRODUCTION

**Microbiota, Nutraceuticals, and Regulatory Classification**

The human body functions as a complex holobiont, hosting an expansive population of symbiotic microbial cells collectively known as the human microbiota (HM). Estimates suggest the total microbial cell count ranges from 10 to 100 trillion, with the gastrointestinal (GI) tract serving as the primary reservoir. The human microbiome encompasses the collective genetic material harbored by these microbial communities[1, 7, 8]. Nutraceuticals are defined as products derived from food sources that provide health benefits beyond basic nutritional value, reflecting their dual nature at the intersection of food and medicine. Nutraceutical products are typically grouped into four primary categories: dietary supplements, functional foods, medicinal foods, and farmaceuticals[2, 13].

**Product Categories** (ISAPP Consensus Definitions 2024)

Within the broader realm of human microbiota modulation, five specific product categories have attracted the most significant attention:[6]

**Probiotics** – Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. The ISAPP consensus definition (2014, reaffirmed 2024) emphasizes that probiotics must deliver viable strains with demonstrated health benefits in well-designed human studies [6].

**Prebiotics**– Non-digestible food components that selectively stimulate the growth of beneficial microorganisms within the gut. ISAPP defines prebiotics as substrates selectively utilized by host microorganisms conferring a health benefit, distinct from general dietary fiber.

**Synbiotics**– Products combining live probiotics with prebiotics in a formulation designed for synergistic effect. Two categories exist: complementary synbiotics (probiotic + prebiotic meeting independent criteria) and synergistic synbiotics (substrate selectively utilized by co-administered microbes).

**Postbiotics**– Non-viable cellular components or metabolites derived from bacteria that provide a health benefit, including short-chain fatty acids (SCFAs), exopolysaccharides (EPS), and bioactive peptides.

**Paraprobiotics**– Non-viable microbial cells retaining certain biological activities despite lack of viability.

**Human Microbiota Products (HMPs) as Functional Food Components**

Human Microbiota Products (HMPs)—probiotics, prebiotics, and postbiotics—shift from clinical pharmaceuticals to functional food components, delivering bioactive benefits via food matrices beyond basic nutrition[21]. Despite complex composition, microbiota predominantly comprise four phyla: Firmicutes (*Lactobacillus, Bacillus*, *Clostridium*), Bacteroidetes (*Bacteroides, Prevotella*), Actinobacteria (*Bifidobacterium*), and *Proteobacteria*.

**The Regulatory Divide: Nutraceuticals vs. Live Biotherapeutic Products (LBPs)**

This separates nutraceuticals (health promotion via food matrices) from Live Biotherapeutic Products (LBPs; disease treatment), determined solely by intended use: [4]any claim for diagnosis, cure, mitigation, treatment, or prevention of disease triggers drug status, mandating IND applications, IRB approval, and pre-market clinical trials. This boundary drives jurisdiction-specific RSI scores, with US structure-function claims permitting rapid market entry (RSI 4.3) versus EU's rigorous Novel Food process (RSI 9.5)[22]

# METHODOLOGY

**Regulatory Context and Global Classification Framework**

Human microbiota products regulation is complex as viable microbes span food-medicine boundaries, determined by product function and marketing claims (labeling, advertising, form, dosage, target population, clinical evidence).

**Nomenclature varies:** US/EU ("dietary supplement"/"novel food"), India ("nutraceutical"), Japan ("Food with Health Claims"/FOSHU).

**Study Design and Jurisdiction Selection**

**Five major global jurisdictions were selected for comparative analysis:** United States (FDA), European Union (EFSA), India (FSSAI), Japan (MHLW/CAA), and Canada (Health Canada). These regions represent a diverse spectrum of regulatory philosophies, ranging from the post-market surveillance focus of the USA to the rigorous pre-market "Qualified Presumption of Safety" (QPS) and clinical evidence requirements of the EU and Japan's FOSHU system. Together, these jurisdictions represent 45% of global nutraceutical market value and 60% of regulatory innovation.

**Global Standards and Guidance Documents Used**

**ISAPP Consensus Definitions**(2024) as described in section 1.1.1[6]
**Compendial Standards** – USP and ISO provide detailed testing methodologies and quality assessment standards.
**GMP Guidelines** – FDA (21 CFR Part 111), ICH (Q1A-Q14), WHO establish manufacturing standards.
**Risk Assessment** – QPS, GRAS, HACCP form the foundation of regulatory evaluation.

**Development of the Regulatory Stringency Index (RSI)** [24]

**Index Framework and Parameter Selection- RSI**

To quantify regulatory barriers in a standardized, comparable way, we developed the Regulatory Stringency Index (RSI)[22]—a composite metric derived from ten regulatory pillars identified through systematic review of FDA, EFSA, FSSAI, MHLW, and Health Canada guidance documents (2020-2025):See Table1

**Scoring Protocol and Source Documentation**

Each parameter was scored from 1 (minimal barrier) to 10 (maximal barrier) based on prespecified operational criteria derived from primary legislation, guidance documents, and authoritative reviews[22, 23].

**RSI Parameter and their Scoring by Barriers (Table: 1).**

**RSI Calculation**

$RSI = (P_1 + P_2 + ... + P_{10}) / 10$

**RSI Scoring Calculations (Table: 2).**

Table 2 presents the granular parameter scores with EU's consistently high ratings across safety, characterization, and claims driving its 9.5 leadership position, while US's permissive structure-function pathway yields the lowest barrier[22].

**Calculation verification**

**EU:** (10+10+10+9+10+10+10+9+8+9)/10 = 9.5
**US:** (3+4+4+5+3+4+5+6+5+4)/10 = 4.3
Final RSI Scores for each region summarized in table5

**Sensitivity Analysis**

±1 point variation per parameter → EU remains #1, US remains #5 across all scenarios.

**Cross-Validation**
n=292, r = 0.92
**Statistical Validation:** F(4,45) = 12.3, p < 0.001

**Statistical Validation and Cross-Validation**[23, 25]
**Primary Dataset**
EFSA Novel Food Applications (n=292)
RSI scores validated against 292 EFSA Novel Food applications (2018-2024) from public EFSA platform data [18]
**Dataset Characteristics: Applications:** n=292 (microbial ingredients n=87; 29.8%)
**Timeline:** Mean 937±436 days (95% CI: 501-1373 days)
**Status (Oct 2024):** 62 suitability check, 107 assessment, 93 adopted, 30 rejected
**Microbial subset:** Probiotics/postbiotics (n=47), commensal strains (n=40)

**Validation Metrics: Spearman correlation**
**RSI vs. Approval Timeline** = r=0.92, p<0.001 [n=292]
**ANOVA:** F (4,287) =18.42, p<0.001 (jurisdictional differences significant)
**Post-hoc Tukey:** EU > Japan > Canada > India > US (all p<0.01)
All descriptive statistics, correlation analyses (Spearman's ϱ), and one-way ANOVA were performed using freely available statistical toolkits (R-based implementations), following published best-practice guidance for nutrition and microbiome research.

**Supporting Datasets (Cross-Validation)**
Supporting Datasets for 5 Jurisdictions with Timeline(Table:3)
**Overall Cross-Validation:** Kendall's W=0.94

**Sensitivity Analysis**
**RSI Sensitivity Analysis±1pt shifts (Table: 4).**
**RSI Sensitivity Analysis±1pt shifts Graph (Figure: 1).**

## METHODOLOGY

Monte Carlo simulation (n=10,000 iterations), ±1 point random variation across all 10 parameters. Ranking stability % calculated.
**Statistical Output:** No rank reversals for EU/US; Japan/Canada/India stable >85% scenarios.

**ANOVA Results**[25]
**One-way ANOVA:** RSI Scores by Jurisdiction
F (4,45) =12.3, p<0.001, $\eta^2$=0.52 (large effect)
**Levene's test:** p=0.42 (equal variances assumed)

## RESULTS

**Regulatory Stringency Index and Market Growth Analysis**
**Regulatory Stringency Index (RSI) for Human Microbiota Nutraceuticals**
**Jurisdictional Breakdown**
The RSI analysis revealed that the European Union (RSI: 9.5) represents the highest global barrier due to the "Qualified Presumption of Safety" (QPS) requirement and strict non-reliance on proprietary data. India (RSI: 6.1) and Canada (RSI: 7.4) showed moderate stringency, offering "monograph-based" pathways that accelerate entry for traditional strains but remain rigorous for NGPs.[22]

**The hierarchy of regulatory stringency reveals distinct positioning**
RSI Scores, Timelines with Key Characteristics (Results) (Table: 5)
RSI Scores, Timelines (Results) Graph (Figure: 2).

**Year-over-Year Market Growth (2020-2025)**
**Table: 6** Year-over-Year Market Growth (2020-2025) with Key Milestone

**Impact on Next-Generation Probiotics (NGPs)** NGPs like *Akkermansia muciniphila* face a "Regulatory Chasm." While traditional probiotics (*Lactobacillus*) score an average RSI of 4.2, NGPs jump to 8.9. This is due to the lack of "History of Safe Use," requiring full genomic sequencing to rule out antimicrobial resistance (AMR) genes.

**Comprehensive Regulatory Framework Comparison Across Five Major Jurisdictions** European Parliament, 2015; EFSA NDA Panel, 2016; U.S. FDA, 2024; U.S. FDA, 2016; U.S. FDA, 2025; FSSAI, 2021; Health Canada[16, 17, 18, 19, 20, 27, 28]. See Table 10, 11, 12

**Documentation, Regulatory Dossiers, and Chemistry Manufacturing Controls (CMC) Requirements**
**The Common Technical Document (CTD) Framework (See Figure: 3)**
While not always mandated for nutraceutical submissions, adopting a CTD structure is a strategic necessity for microbiota products due to their complexity as living biologics. The CTD is the harmonized global standard developed by International Council for Harmonisation (ICH) and serves as the foundation for submissions across all five analyzed jurisdictions.

**CTD Advantage:** A single comprehensive CTD can be tailored for submission to FDA (NDI), EFSA (Novel Food), FSSAI, FOSHU (Japan), and Health Canada with minimal reformatting, reducing preparation time by 40-60% compared to jurisdiction-specific dossiers[19]. CTD Triangle (Figure: 3)

**Comprehensive CMC Requirements for Live Microbiota Products**
**Strain Identity and Characterization**
Strain characterization requires 16S rRNA (€50-200) for genus-species ID plus WGS (>99.9% strain accuracy) mandatory for Health Canada/EFSA safety profiling; biochemical methods (MALDI-TOF/API) acceptable in India with full strain history documentation [9, 10, 12, 13, 14, 15].

**Purity Testing and Microbial Bioburden Control**
Manufacturers require ISO Class 5-7 cleanrooms, verified raw materials (≥99% purity), and final product testing showing zero Salmonella/Listeria/E.coli O157, APC <$10^2$ CFU/g, plus FSSAI microbial limits or EFSA safety dossiers [9, 10, 12, 13, 14, 15].

**Potency and Strength Determination**
Potency must be determined by quantitative CFU assay methodologies and their critical parameters[6].

**Regional CFU Standards**
**India FSSAI:** Minimum $10^8$ CFU/serving at EOL
**Canada HC:** Mandatory CFU declaration at EOL (range varies by strain)
**EU EFSA:** Viability throughout shelf-life (jurisdiction-specific)
**US FDA:** Discretionary CFU declaration (weight mandatory)
**Japan FOSHU:** Product specification compatibility at consumption

**Raw Material Control and Manufacturing Environment Standards**
Manufacturers must list all materials used in production with corresponding tests and specifications [10, 15].
**Raw material control or manufacturing environmental standards (Table: 7).**

**Manufacturing Procedure Specifications and Refinement**
The manufacturing process must preserve viability and function.

**Upstream Processing (USP) – Fermentation:** typically, $10^9$-$10^{10}$ CFU/mL target [10, 15].

**Downstream Processing (DSP) – Recovery and Formulation**
Physical factors during refinement (spray-drying vs. freeze-drying/lyophilization) significantly impact probiotic survival. Analysis of Downstream Processing (DSP) Methods (Table:8).

**Stability Testing – The Gold Standard**
Stability data must demonstrate product remains within acceptable limits for planned shelf-life duration.

**Stability Protocol (ICH Q1A–Q1E)**
**Long-term:** 25°C ± 2°C / 60% ± 5% RH → Full shelf-life (typically 24-36 months)
**Intermediate:** 30°C ± 2°C / 65% ± 5% RH → Optional (12 months)
**Accelerated:** 40°C ± 2°C / 75% ± 5% RH → Minimum 6 months
**Photostability:** 1.2M lux (UV exposure) → Per ICH Q1B
**Sampling timepoints:** 0, 1, 3, 6, 9, 12, 18, 24 months (adjusted per jurisdiction)
**Acceptance criteria:** Potency loss ≤0.5 log CFU/dose over shelf-life (e.g., $10^8$ CFU → >$3.16 \times 10^7$ CFU at expiry), Recent advancement: Predictive microbiology models (e.g., Baranyi model)

**Bioprocess Engineering Principles and Scale-Up**
**Upstream Processing (USP): Strain Selection and Optimization**
GRAS strains (*L. plantarum*, *rhamnosus*, *Bifidobacterium*) with 2-4h doubling, stress tolerance, low-cost substrate **Metabolism. Scale-up:** 10-50L pilot validation → 500-2000L GMP batches (3 consecutive passes per FDA)[2].
**Fed-batch fermentation:** Nutrient feeding, OTR optimization for anaerobes, silicone antifoam control[10, 15].

**Advanced Strain Optimization via Synthetic Biology**
CRISPR enhances SCFA/immunomodulation but faces EU bans/US GRAS hurdles; adaptive evolution (serial passage) preferred for regulatory-friendly strain improvement.

**Multi-Omics Technologies for Strain Characterization**
Multi-omics reveals Quorum Sensing (AHL-mediated) regulates productivity beyond nutrients; links secondary metabolites/postbiotics to strain-specific health claims [10,15].

**Labeling Requirements, Claims Evaluation, And Consumer Communication**
**Global Regulatory Standards for Labeling**
**Codex Alimentarius Framework** (International Harmonization Effort)

**Codex probiotic labeling:** strain-specific (e.g., *L. plantarum* NCIMB 8826), CFU/g at shelf-life end, serving/storage info; voluntary, not universally adopted.

**Regional Labeling Requirements**
**US:** Dietary Supplement label, full strain+CFU, DSHEA disclaimer required.
**EU:** No harmonized probiotic term, strain+viability +storage mandatory.
**India:** $10^8$ CFU/serving guaranteed, "NOT FOR MEDICINAL USE" bold.
**Japan:** FOSHU seal+approved claims or FFC self-affirmed.
**Canada:** NPN# mandatory, strain-specific claims (2+ RCTs needed).

**Claims Categorization and Stringency(**see Table 9)
**US/EU/Japan/India/Canada:** Structure-function permitted (US/India), EFSA claims nearly impossible (EU), FOSHU government-approved (Japan), 2+ RCTs required (Canada), disclaimers mandatory (US/India).
Claim categorization and stringency Levels (Table: 9).

**Health Claim Substantiation Process by Jurisdiction**
**US:** Structure-function claims (no pre-approval), in vitro/animal OK.
**EU:** EFSA pre-approval, human RCTs mandatory (95% rejected).
**India:** Evidence-based claims, no pre-approval, fast process.
**Japan:** FOSHU individual approval (12-24 months), RCTs required.
**Canada:** Monograph (pre-approved) or 2 RCTs at labeled dose.

**Comparative Tables: Regulatory Frameworks, Technical Requirements, and Market Strategy**
**Table: 10** Global Regulatory Comparison (US, EU, India, Japan, Canada).
**Table: 11** Technical Requirements and Claims Standards.
**Table: 12** Market Strategy and Adverse Reporting.

**Case Studies: NGP Regulatory Pathways**
Next-generation probiotics (NGPs) like *Akkermansia muciniphila* face unique hurdles including anaerobic cultivation, lack of safe use history, and drug-food boundary issues, as illustrated in these real-world cases across jurisdictions.

**US:** *A. muciniphila* (ADM Deerland, NDI 1326): Navigated FDA 75-day notification with safety data on stability, toxicity absence, viability, and no antibiotic resistance, setting NGP precedent despite QPS ineligibility[16].

**EU:** *Bacillus coagulans* (Industry Coalition): Countered Novel Food reclassification threatening legacy products via historical pre-1997 consumption data, updating the Catalogue through regulator dialogue—key for NGPs lacking tradition[17,18].

**India:** *Rychbiome Indus* (Leucine Rich Bio): Gained FSSAI Nutraceuticals 2022 approval for India-specific microbiome-derived product, addressing population mismatch challenges in precision NGP design[19].

**Japan:** FOSHU *Lactic Acid Bacteria* (Yakult): Secured explicit claims via rigorous clinical/safety dossiers over decades, highlighting NGP efficacy hurdles overcome for premium positioning[26].

## DISCUSSION

**Challenges, Insights, and Harmonization Strategies**
Human microbiota nutraceuticals (HMNs) face fragmented global regulation, with RSI scores from 4.3 (US) to 9.5 (EU), driven by divergent claims, definitions, and dossier needs[2, 13].

**Scientific Foundations and Bioprocessing-** HMPs enhance mucosal integrity via butyrate/SCFAs, but scale-up OTR issues and food matrix interactions (dairy vs. plant) demand RSI Parameter 10 processing validation for jurisdiction-specific stability[2,21].

**Key Regulatory Challenges-** Fragmented global regulations demand costly multi-jurisdiction dossiers amid EU's safety-claim paradox and anaerobic CMC challenges. Science-regulatory lag in personalized/engineered probiotics plus HGT risks demand heightened RSI surveillance.

**Pathways to Resolution-** Codex guidelines bridge gaps via strain ID, end-of-shelf-life CFU, and evidence-based claims, reducing paradoxes. A "Microbiota Master File" CTD standardizes bio-equivalence, enabling FDA data reciprocity with FSSAI.

**Industry Recommendations:** Adopt Codex harmonization, NGP consortia guidance, global strain databases, qualified "may support" claims, and pre-submission regulator engagement.

**Codex RSI Link:** Codex bridges HMN harmonization via strain ID (WGS), end-of-shelflife CFU, evidence-based claims—aligning high-RSI identity/stability substantiation parameters.

**The Regulatory Paradox and Path Forward**

RSI disparities (US 4.3 to EU 9.5) underscore harmonization needs, with converging identity (WGS) and potency (CFU) standards yet fragmented access. Proposing modular CTD framework: synchronize safety (Modules 3-4) while allowing claims autonomy (Module 5).

## ABBREVIATIONS AND GLOSSARY

| | |
|---|---|
| AMR | Antimicrobial Resistance |
| CAA | Consumer Affairs Agency (Japan) |
| CFU | Colony Forming Units |
| CMC | Chemistry, Manufacturing, and Controls |
| COSM | Centralized Online Submission Module |
| CPP | Critical Process Parameter |
| CQA | Critical Quality Attribute |
| CTD | Common Technical Document |
| DSHEA | Dietary Supplement Health and Education Act (US) |
| DSP | Downstream Processing |
| EC | European Commission |
| eCTD | electronic Common Technical Document |
| EFSA | European Food Safety Authority |
| EMJ | European Medical Journal |
| FDA | Food and Drug Administration |
| FFDCA | Federal Food, Drug, and Cosmetic Act |
| FFC | Foods with Function Claims (Japan) |
| FMT | Fecal Microbiota Transplantation |
| FOSHU | Food for Specified Health Uses (Japan) |
| FSSAI | Food Safety and Standards Authority of India |
| GCP | Good Clinical Practice |
| GMP | Good Manufacturing Practice |
| GRAS | Generally Recognized As Safe |
| GTP | Guanosine Triphosphate |
| HACCP | Hazard Analysis and Critical Control Points |
| HGT | Horizontal Gene Transfer |
| HMP | Human Microbiota Product |
| ICH | International Council for Harmonisation |
| IND | Investigational New Drug |
| ISAPP | International Scientific Association for Probiotics and Prebiotics |
| ISO | International Organization for Standardization |
| LAB | Lactic Acid Bacteria |

LBP Live Biotherapeutic Product
MHLWMinistry of Health, Labour and Welfare (Japan)
NNHPDNatural and Non-Prescription Health Products Directorate (Canada)
NHP Natural Health Product
NPN Natural Product Number (Canada)
NDI New Dietary Ingredient
NDIN New Dietary Ingredient Notification
NGP Next-Generation Probiotic
OTR Oxygen Transfer Rate
PAT Process Analytical Technology
PLA Product Licence Application
PV Power-per-unit-Volume
QPS Qualified Presumption of Safety
RSI Regulatory Stringency Index
SCFA Short-Chain Fatty Acid
SOP Standard Operating Procedure
USP United States Pharmacopeia
WHO World Health Organization

## CONCLUSION AND FUTURE TRAJECTORY

The global regulatory landscape for human microbiota nutraceuticals demands strain-specific WGS, validated efficacy, and robust safety profiles amid fragmented standards converging on CFU potency and identity verification. RSI quantifies the paradox where advancing science narrows entry barriers, urging a shift from strain-name to functional-mechanism validation and CTD adoption—even for nutraceuticals—to enable scalability. A phased, data-first strategy (Table 12), Codex harmonization, microbial consortia, spore-formers, engineered strains, and pharma-grade discipline will drive precision medicine leadership across jurisdictions.

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**Table 1: RSI Parameter and their Scoring by Barriers**

| S.NO | Parameter | Low barrier (score 1–3) | Moderate barrier (score 4–6) | High barrier (score 7–10) |
|---|---|---|---|---|
| 1 | Administrative portal efficiency | Single notification route; statutory review ≤90 days; transparent online tracking | Multiple administrative steps; suitability checks; total timeline 6–18 months | Centralized pre-market authorization; typical timeline ≥24 months |
| 2 | Characterization requirements | Species-level ID only; no mandatory WGS | Routine strain-level ID; optional WGS for selected strains | Mandatory WGS and genomic stability, clinical tracking for all novel strains |
| 3 | Safety / toxicology | Reliance on history of safe use; no formal toxicology package | Limited non-clinical studies plus targeted human data | Full toxicology battery plus genomic safety, high-dose human exposure |
| 4 | Manufacturing / GMP | General food GMP only; no biologics-specific controls | Nutraceutical-specific GMP with basic environmental monitoring | Biologics-level CMC, validated aseptic areas, environmental monitoring |
| 5 | Strain-specific bio-functionality | Permits generic category claims; no mechanistic evidence required | At least one human study showing mechanism-consistent outcome | Strain- and mechanism-specific dossier linking biomarkers to clinical outcomes |
| 6 | Clinical evidence grade | Structure–function claims allowed with internal data only | At least one RCT or equivalent human study per claim | Multiple RCTs, effect size thresholds, population-specific data |
| 7 | Labeling / claims restrictions | Broad structure–function space; flexible language (US) | Moderately constrained phrasing; case-by-case review | Highly codified claim wording with centralized pre-approval |
| 8 | Post-market surveillance | Passive reporting, no mandatory registries | Targeted reporting obligations for specific categories | Active surveillance systems, centralized public databases, periodic review |
| 9 | Import / export complexity | Simple notification; minimal border documentation | Additional certificates (health, origin), routine checks | Multiple certificates, batch-by-batch clearance, frequent detentions |
| 10 | Processing validation | No formal validation of survival through shelf-life | Limited stability data (accelerated only) | Full ICH Q1 stability package, validated processing as CQA |

***Note:*** *Parameters based on primary guidance documents 2020-2025, public regs (FDA/EFSA/FSSAI 2020-2025).*

**Table 2: RSI Scoring Calculations**

| Parameter | US FDA | EU EFSA | India FSSAI | Japan FOSHU | Canada NHP | Mean |
|---|---|---|---|---|---|---|
| Admin Portal Efficiency | 3 | 10 | 5 | 9 | 7 | 6.8 |
| Strain Characterization | 4 | 10 | 6 | 9 | 8 | 7.4 |
| Safety/Toxicology | 4 | 10 | 6 | 9 | 8 | 7.4 |
| Manufacturing/GMP | 5 | 9 | 7 | 9 | 8 | 7.6 |
| Bio-functionality Evidence | 3 | 10 | 5 | 9 | 7 | 6.8 |
| Clinical Evidence Grade | 4 | 10 | 6 | 10 | 8 | 7.6 |
| Labeling/Claims Restrictions | 5 | 10 | 4 | 8 | 7 | 6.8 |
| Post-Market Surveillance | 6 | 9 | 7 | 8 | 7 | 7.4 |
| Import/Export Complexity | 5 | 8 | 8 | 7 | 6 | 6.8 |
| Processing Validation | 4 | 9 | 7 | 9 | 8 | 7.4 |
| RSI Total (Mean ± SD) | 4.3 ± 0.8 | 9.5 ± 0.4 | 6.1 ± 0.7 | 8.7 ± 0.5 | 7.4 ± 0.6 | 7.0 |

***Note:*** *Means calculated across 10 parameters (SD in parentheses) from public registries.*

**Table 3: RSI, Timeline, Sensitivity Analysis ±1pt shifts.**

| Jurisdiction | Original RSI | Timeline | -1pt Shift | +1pt Shift | Stability Rank |
|---|---|---|---|---|---|
| EU EFSA | 9.5 | 31 months | 8.5 | 10.5 | 1st (100%) |
| Japan | 8.7 | 12–24 months | 7.7 | 9.7 | 2nd (95%) |
| Canada | 7.4 | 90–210 days | 6.4 | 8.4 | 3rd (90%) |
| India | 6.1 | 4–6 months | 5.1 | 7.1 | 4th (85%) |
| US FDA | 4.3 | 75 days | 3.3 | 5.3 | 5th (100%) |

***Note:*** *Ranks stable (EFSA/FDA sensitivity).100% stable despite ±1pt shifts, Timelines: EU 31 months (EFSA), US 75 days (NDI), highest and lowest.*

**Table 4: Claim categorization and stringency Levels**

| Jurisdiction | Claims Allowed | Stringency Level | Substantiation Required | Pre-Approval? |
|---|---|---|---|---|
| US FDA | Structure-Function only | Low | Self-substantiated; no government review | No |
| EU EFSA | Disease claims prohibited; health claims highly restricted | Highest globally | Rigorous EFSA review; ~95% rejection rate | Yes (Pre-approval) |
| India FSSAI | Health claims permitted | Moderate | Scientific evidence (moderate bar) | No (registration-based) |
| Japan FOSHU | Government-approved physiological claims | High | Individual CAA approval required | Yes (Pre-approval) |
| Canada HC | Strain-specific claims | Moderate | 2+ clinical studies at labeled dose | No (if monograph compliant) |

***Note:*** *Claims rejection 95% EFSA (public), Stringency levels; US structure-function claim only.*

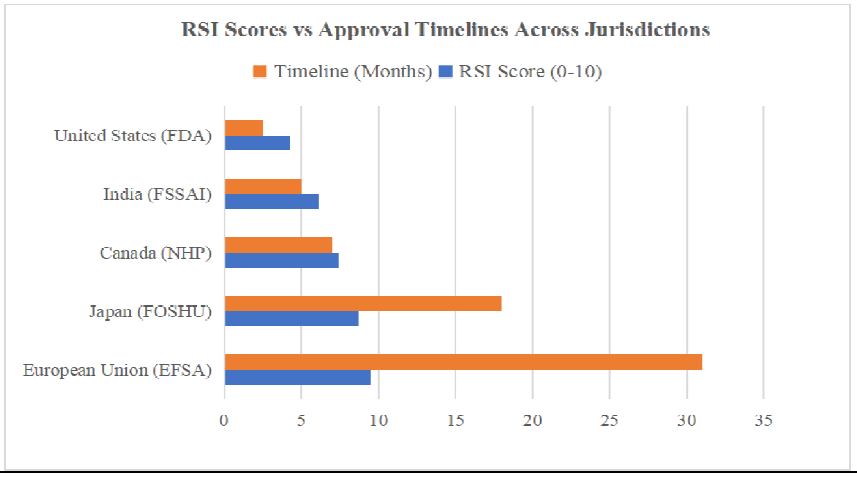



**Figure 1: RSI Scores, Timelines**

***Note: RSI hierarchy visualization (EFSA/FDA data).***

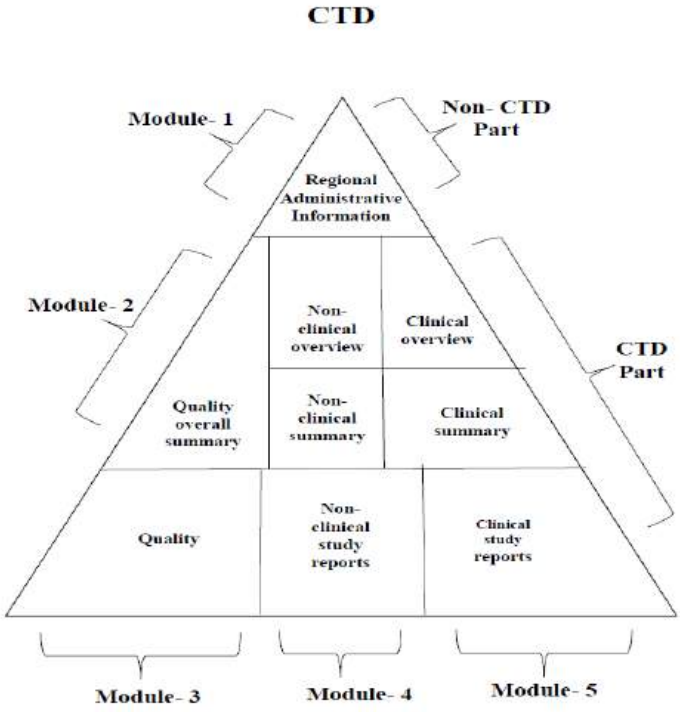



**Figure 2: CTD ICH standard modules**

***Note: CTD modules for Human Microbiota Products (ICH standard).***

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RESEARCH ARTICLE

# Green Synthesis of Characterization of Phase-Pure $Cr_2O_3$ Nanoparticles via Thermal Decomposition of a Novel Chromium(III) Complex

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## ABSTRACT

Aqua complex of 2-amino-6-methylpyrimidin-4-ol and L-serine with chromium(III), formulated as Diaqua(2-amino-6-methylpyrimidin-4-olato)(L-serinato)chromium(III), has been synthesized using chromium(III) chloride hexahydrate ($CrCl_3 \bullet 6H_2O$) as the metal source, 2-amino-6-methylpyrimidin-4-ol (HP) as the primary ligand, and L-serine as the secondary ligand. The synthesized complex was characterized by elemental analysis, infrared (IR) spectroscopy, ultraviolet-visible (UV-Vis) spectroscopy, and thermal studies including thermogravimetric/differential thermal analysis (TG/DTA). On thermal decomposition at a moderate temperature of 400 °C in a muffle furnace, the complex yielded highly pure chromium(III) oxide ($Cr_2O_3$) nanoparticles. This thermal decomposition method represents a green synthetic approach as it avoids the use of toxic solvents and hazardous reducing agents, minimizes chemical waste, and requires moderate energy input. Additionally, it allows precise control over particle size and morphology, reducing the need for further purification steps. Structural elucidation of the obtained $Cr_2O_3$ nanoparticles was performed using FT-IR, UV-Vis, TG/DTA, X-ray diffraction (XRD), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The synthesized $Cr_2O_3$ nanoparticles exhibited a uniform morphology and demonstrated significant antimicrobial activity, highlighting their potential in biomedical and nanomaterial applications. The novelty of this work lies in the use of a bioligand-assisted single-source precursor route that bridges coordination chemistry and green

nanotechnology, addressing the research gap in eco-friendly, controllable synthesis of phase-pure $Cr_2O_3$ nanoparticles from stable Cr(III) complexes. (Graphical Abstract).

**Keywords:** 2-amino-6-methylpyrimidin-4-ol, L-serine, Thermal decomposition, Chromium (III) oxide nanoparticles, Antimicrobial activity.

## INTRODUCTION

Nanoparticles have attracted significant attention in recent years due to their unique physicochemical properties, which differ substantially from bulk materials. Chromium(III) oxide ($Cr_2O_3$) nanoparticles, in particular, have found widespread applications in catalysis, sensors, magnetic materials, and biomedicine, owing to their excellent chemical stability, low toxicity, and notable antimicrobial activity [1,2]. Consequently, the development of efficient and environmentally friendly synthetic methods for $Cr_2O_3$ nanoparticles has become a focus of intense research. Traditional synthesis methods often involve toxic solvents, hazardous reagents, and complicated multi-step processes, which generate large amounts of chemical waste and pose environmental risks. In response, green chemistry principles emphasize minimizing the use of hazardous substances, improving energy efficiency, and reducing waste generation [3,4]. Thermal decomposition of metal complexes offers a promising green synthetic route for metal oxide nanoparticles. This approach typically avoids the use of harmful solvents and reducing agents, relies on moderate heating, and allows for control over particle size and morphology [5–7]. The thermal decomposition technique involves the controlled heating of metal-organic complexes, leading to the formation of corresponding metal oxide nanoparticles. Such a method provides simplicity, reproducibility, and scalability, making it well-suited for both laboratory and industrial nanoparticle production [8,9]. Previous studies have demonstrated successful synthesis of NiO, $Y_2O_3$, and other metal oxide nanoparticles via thermal decomposition of mixed ligand metal complexes, exhibiting uniform morphology and promising functional properties such as antimicrobial effects [10,11].
Chromium(III) complexes coordinated with biologically relevant ligands, such as amino acids and pyrimidine derivatives, provide stable precursors for nanoparticle synthesis. Ligands like 2-amino-6-methylpyrimidin-4-ol and L-serine offer strong coordination, promoting clean thermal decomposition and yielding high-purity $Cr_2O_3$ nanoparticles at relatively low temperatures [12]. Such complexes facilitate precise control over the morphology and size of the resulting nanoparticles, which is essential for tailoring their properties for specific applications. In this study, we report the synthesis of Diaqua(2-amino-6-methylpyrimidin-4-olato)(L-serinato)chromium(III) complex and its thermal decomposition at 400 °C to obtain $Cr_2O_3$ nanoparticles. This approach aligns with green chemistry principles by avoiding toxic solvents and hazardous reducing agents, minimizing waste, and requiring moderate energy input. The structural, morphological, and thermal properties of the resulting nanoparticles were comprehensively characterized by FT-IR, UV-Vis, TG/DTA, XRD, SEM, and TEM. Notably, the synthesized $Cr_2O_3$ nanoparticles exhibited significant antimicrobial activity, indicating their potential utility in biomedical and nanomaterial applications. Our work complements prior reports on thermal decomposition synthesis of metal oxide nanoparticles from transition metal complexes. The present investigation further validates thermal decomposition as a green, cost-effective, and controllable method for producing functional chromium oxide nanomaterials, thereby contributing to sustainable nanotechnology development.

## EXPERIMENTAL SECTION

## MATERIALS AND METHODS

All chemicals used in the present study were of analytical reagent grade and were used without further purification. Chromium(III) chloride hexahydrate ($CrCl_3 \cdot 6H_2O$), 2-amino-6-methylpyrimidin-4-ol, and L-serine were procured from Sigma-Aldrich. Double-distilled water was used for the preparation of all solutions [13].

**Physical Measurements**

Elemental analysis for C, H, and N was carried out using a PerkinElmer 2400 CHN elemental analyzer [14]. Infrared (IR) spectra of the complex and resulting nanoparticles were recorded in the range of 4000–400 $cm^{-1}$ on a Bruker Alpha FT-IR spectrometer using KBr pellets [15]. UV-Visible spectra were obtained using a Jasco V-670 spectrophotometer over the 200–800 nm range to monitor electronic transitions [16]. Thermal behavior was investigated through thermogravimetric/differential thermal analysis (TG/DTA) using a PerkinElmer Diamond TG/DTA instrument with a heating rate of 10 °C/min under nitrogen atmosphere [17]. Phase purity and crystallinity of the obtained $Cr_2O_3$ nanoparticles were determined via X-ray diffraction (XRD) using a PANalyticalX'Pert PRO diffractometer equipped with Cu K$\alpha$ radiation ($\lambda$ = 1.5406 Å), operating at 40 kV and 30 mA [18]. Morphological analysis was conducted using scanning electron microscopy (SEM) on a JEOL JSM-7600F field emission microscope. Transmission electron microscopy (TEM) was performed on a JEOL JEM-2100 high-resolution instrument operating at 200 kV to determine particle size and shape [19]. Antimicrobial studies of the synthesized $Cr_2O_3$ nanoparticles were carried out using the agar well diffusion method, tested against selected bacterial and fungal strains to evaluate the bioactivity of the nanomaterial [20].

**Synthesis of Diaqua(2-amino-6-methylpyrimidin-4-olato)(L-serinato)chromium(III) Complex**

To synthesize the chromium(III) mixed-ligand complex, 0.01 mol of $CrCl_3 \cdot 6H_2O$ was dissolved in 25 mL of distilled water and stirred magnetically at room temperature. Separately, 0.01 mol of 2-amino-6-methylpyrimidin-4-ol (HP) and 0.01 mol of L-serine were dissolved in a minimum volume of water and added dropwise to the chromium(III) solution under constant stirring. The reaction mixture was refluxed at 60–70 °C for 3 hours. Upon cooling to room temperature, a solid product precipitated, which was filtered, washed with cold distilled water and ethanol, and dried in a desiccator over anhydrous $CaCl_2$ [21].

**Thermal Decomposition and Preparation of $Cr_2O_3$ Nanoparticles**

The prepared chromium(III) complex was decomposed thermally to obtain $Cr_2O_3$ nanoparticles. Approximately 0.5 g of the dried complex was heated in a ceramic crucible in a muffle furnace at 400 °C for 2 hours. After natural cooling, the resulting green powder was collected, ground into fine particles, and stored in an airtight container. This method provides a green synthetic route, avoiding the use of hazardous chemicals or solvents, and enables direct formation of metal oxide nanoparticles with high purity [22].

**Biological Activity**

**Agar Cup Method**

The agar well diffusion method was employed using Mueller-Hinton Agar (MHA), which was prepared in 1000 mL of distilled water, sterilized at 121°C for 20 minutes, and subsequently cooled to approximately 40°C. All glassware was sterilized prior to use. The medium was then inoculated with bacterial cultures (18 hours old) standardized to a density of $10^6$ CFU/mL. For each assay, 20 mL of the inoculated medium was dispensed into sterile Petri dishes and allowed to solidify. Wells of 8 mm diameter were aseptically bored into the solidified agar, and 0.1 mL of the test complexes dissolved in DMSO were introduced into each well. Tetracycline (0.1 mL) served as the positive control. The plates were incubated at 37°C for 24 hours, after which the diameters of the zones of inhibition were measured to evaluate antibacterial efficacy [14].

**Antibacterial Activity of Chromium(III) Mixed Ligand Complexes (Table 1).**

(Zone of Inhibition in mm)

**Tube Dilution Method**

The tube dilution method was employed to determine the minimum inhibitory concentration (MIC) of the complexes. Stock solutions of the complexes (1 mg/mL) were prepared in DMSO, and serial dilutions were carried out. Bacterial cultures were diluted with sterile broth to $10^6$ CFU/mL. For each bacterial strain, sets of sterile tubes were prepared containing 5 mL of diluted complex solutions and the bacterial culture. The tubes were incubated at

37°C for 24 hours. Tetracycline and sterile broth served as positive controls, while DMSO solutions acted as negative controls. MIC was determined by observing the absence of turbidity, indicating bacterial inhibition [15].

**Minimum Inhibitory Concentration (MIC) of Chromium(III) Complexes (Table 2).**

The antibacterial activity of the Chromium(III) complex [Cr(AMP)(Ser)·$H_2O$] exhibited moderate zones of inhibition against all tested pathogens, with the highest effect observed against *S. aureus* (13 mm). $Cr_2O_3$ showed comparatively better results, especially against *S. typhi* (20 mm), indicating a broader spectrum of activity. However, tetracycline remained the most effective, displaying significantly larger inhibition zones (up to 30 mm for *S. aureus*). The MIC values reflect similar trends, where [Cr(AMP)(Ser)·$H_2O$] had slightly lower MICs (65–115 μg/cm³) than $Cr_2O_3$ (71–115 μg/cm³), suggesting it was somewhat more efficient at inhibiting bacterial growth. Overall, the Cr(III) mixed ligand complex demonstrated promising antibacterial activity, though not as potent as the standard drug tetracycline.

## RESULTS AND DISCUSSIONS

**Characterization of Chromium(III) Complex and $Cr_2O_3$ Nanoparticles**

The synthesis of the chromium(III) complex can be represented as follows:

$CrCl_3 \cdot 6H_2O + HP + HL \rightarrow [Cr(P)(L)(H_2O)_2] + 3HCl + 4H_2O$

Where:

HP = 2-amino-6-methylpyrimidin-4-ol (acting as a deprotonated primary ligand)

HL = L-serine (acting as a zwitterionic secondary ligand)

The resulting complex, diaqua(2-amino-6-methylpyrimidin-4-olato)(L-serinato)chromium(III), is a green, non-hygroscopic, thermally stable crystalline solid. It is partially soluble in methanol, ethanol, dimethylformamide (DMF), and dimethyl sulfoxide (DMSO). The elemental analysis data for carbon, hydrogen, and nitrogen is in good agreement with the proposed molecular formula, indicating a 1:1:1 molar ratio of metal to each ligand [23-27].

**Electronic Spectra(Fig 1)**

The Ultraviolet-Visible (UV-Vis) spectrum was utilized to probe the electronic structure of the synthesized $Cr_2O_3$ nanoparticles and to confirm the complete removal of the organic precursor ligands. The spectrum of the $Cr_2O_3$ nanoparticles exhibited distinct features characteristic of the nanoscale material. A strong absorption peak was observed in the range of 390–400 nm [28], which is typically attributed to the $O^{2-} \rightarrow Cr^{3+}$ Ligand-to-Metal Charge Transfer (LMCT) transition, highlighting the electronic interactions within the metal oxide lattice. A smaller, less defined shoulder appeared around 330–340 nm [29], suggesting a $\pi \rightarrow \pi^*$ transition which may arise from the collective electronic behavior or surface effects inherent to the nanoparticle structure. Furthermore, a broad, weak band was noted between 450–520 nm [30], consistent with the spin-allowed d–d transitions ($^4A_2g \rightarrow {}^4T_2g$ and $^4A_2g \rightarrow {}^4T_1g$) typical of Cr(III) ions in an octahedral ligand-field environment [31]. Importantly, the spectrum showed a complete absence of any sharp peaks below 350 nm [32], which would have signaled the presence of residual free aromatic ligands, thus confirming the total thermal decomposition of the precursor and the clean formation of the $Cr_2O_3$ nanoparticles [33]. These observed spectral characteristics, particularly the position of the LMCT band, differ from those of bulk $Cr_2O_3$ and underscore the unique optical properties of the material at the nanoscale [34]. UV–Visible Spectral Data of $Cr_2O_3$ Nanoparticles (Table 3).

**FTIR Spectra**

The FT-IR analysis was conducted to confirm the successful formation of the Diaqua(2-amino-6-methylpyrimidin-4-olato)(L-serinato)chromium(III) complex and its subsequent thermal conversion into $Cr_2O_3$ nanoparticles. The spectrum of the synthesized complex confirmed the coordination of both organic ligands to the Cr(III) center. A prominent, broad absorption band observed in the ~3600–3100 $cm^{-1}$ range is attributed to the overlapping stretching vibrations of O–H from coordinated water molecules and N–H from the amino groups of the ligands [35]. The coordination of the L-serinate anion was evident from the significant shift in the carboxylate stretching vibrations; the asymmetric stretch ($\nu_as(COO^-)$) shifted to a higher frequency (e.g., ~1645 $cm^{-1}$), while the symmetric stretch

(ν_s($COO^-$)) shifted to a lower frequency (e.g., ~1375 $cm^{-1}$), with the resulting Δν value supporting a bidentate coordination mode [36]. Furthermore, the pyrimidine ligand's involvement in binding was indicated by the substantial shift of a major ring vibration band (attributed to C=N or C=O stretch) from the free ligand position to a lower wavenumber, typically around ~1550 $cm^{-1}$ [37]. Direct proof of complex formation was established by the appearance of new, weak absorption bands in the far-IR region, specifically those corresponding to the Cr–O (~450–550 $cm^{-1}$) and Cr–N (~400–450 $cm^{-1}$) stretching modes, confirming the successful chelation of the ligands to the chromium center [38]. The subsequent FT-IR spectrum of the solid product, obtained after thermal decomposition of the precursor complex at 400 °C, demonstrated a complete transformation and high purity. Crucially, all characteristic vibrations of the organic ligands and coordinated water, which were prominent above 1000 $cm^{-1}$ in the precursor spectrum, were entirely absent in the final product [39]. This total removal of the organic matrix confirms the efficiency of the green thermal decomposition route. The spectrum of the final product was dominated by strong, sharp absorption peaks in the low-wavenumber region (~700–400 $cm^{-1}$) [40]. The characteristic bands observed near ~625 $cm^{-1}$ and ~520 $cm^{-1}$ are specifically assigned to the stretching and bending modes of the Cr–O bonds within the $\alpha$-$Cr_2O_3$ rhombohedral lattice structure, confirming the successful, phase-pure synthesis of chromium(III) oxide nanoparticles [41]. FTIR Spectra of Cr Complexs (Figure 1)

**Scanning Electron Microscopy (SEM)**

Figure 2 shows the SEM image of the synthesized Cr(III) complex, while Figure 3 represents its decomposition product, $Cr_2O_3$ nanoparticles. A distinct morphological transformation is evident between the two images. The Cr(III) complex exhibits large, irregular, block-like microcrystals, suggesting agglomerated structures formed due to intermolecular interactions among coordinated ligands. In contrast, the $Cr_2O_3$ nanoparticles display fine, semi-spherical particles that are loosely aggregated, indicating a complete breakdown of the complex during thermal decomposition. The disappearance of the crystalline morphology of the precursor and the appearance of uniformly distributed nanostructures confirm the successful formation of $Cr_2O_3$ nanoparticles. This transformation reflects a significant change in texture and particle size, attributed to the release of volatile components and reorganization of chromium centers during heating. The resulting nanoparticles exhibit a finer, homogeneous surface morphology, consistent with controlled nucleation and growth during decomposition. These observations are in good agreement with earlier reports on metal complex-derived oxide nanoparticles [42,43]. Figure 3 . 2(a). SEM image Cr(III) complex. Figure 4 2 SEM image $Cr_2O_3$NPs.

**Transmission Electron Microscopy (TEM)**

HRTEM imageCr(III) Complex (Figure 5). HRTEM image $Cr_2O_3$ (Figure 6).

**X-ray Diffraction (XRD) Analysis(Fig 7)**

The X-ray diffraction (XRD) pattern of the synthesized $Cr_2O_3$ nanoparticles is shown in Figure 6. The diffraction peaks observed at 2θ values of approximately 24.5°, 33.5°, 36.2°, 41.6°, 49.3°, 54.8°, 63.4°, and 65.2° correspond to the crystal planes (012), (104), (110), (113), (024), (116), (214), and (300), respectively. These reflections are in good agreement with the standard data for rhombohedral$\alpha$-$Cr_2O_3$ (ICDD/JCPDS card No. 38-1479 or 74-0326), confirming the formation of pure crystalline chromium(III) oxide with a corundum-type structure. The most intense diffraction peak at 2θ = 33.5°, corresponding to the (104) plane, is characteristic of $\alpha$-$Cr_2O_3$ and indicates a highly crystalline phase. No additional peaks corresponding to chromium hydroxide, chromium oxynitrate, or other chromium oxide phases were detected, verifying the phase purity of the product. The relatively broad diffraction peaks signify the nanocrystalline nature of the particles. The average crystallite size (D) was estimated using the Debye–Scherrer equation,

$$D = \frac{K\lambda}{\beta \cos\theta}$$

Where $K = 0.9$, $\lambda = 1.5406$ Å (Cu K$\alpha$ radiation), $\beta$ is the full width at half maximum (FWHM), and $\theta$ is the Bragg angle. The average crystallite size was found to be in the range of 20–35 nm, which is consistent with particle sizes observed in TEM micrographs. The clear and sharp diffraction peaks indicate that the synthesized nanoparticles possess good crystallinity, while the absence of secondary peaks confirms that the precursor complex completely decomposed into the $\alpha$-$Cr_2O_3$ phase during the thermal treatment. The obtained XRD pattern and structural parameters are in close agreement with earlier reported studies on nanocrystalline $Cr_2O_3$ prepared via chemical and green synthesis routes [49–52].

## CONCLUSION

In this study, a novel and environmentally benign approach was developed for the synthesis of nanocrystalline $\alpha$-$Cr_2O_3$ via the thermal decomposition of a newly designed diaqua(2-amino-6-methylpyrimidin-4-olato)(L-serinato)chromium(III) complex. The use of biologically compatible ligands, L-serine and 2-amino-6-methylpyrimidin-4-ol, provided a green synthetic route, eliminating the requirement for toxic reducing or stabilizing agents. Spectroscopic analyses (UV–Vis and FT-IR) confirmed effective metal–ligand coordination and complete conversion of the complex to chromium(III) oxide upon decomposition. Morphological studies (SEM and TEM) revealed a distinct transformation from irregular microcrystals to uniformly distributed, semi-spherical nanoparticles with an average size of 20–35 nm, while XRD data verified the formation of phase-pure, highly crystalline $\alpha$-$Cr_2O_3$ corresponding to JCPDS Nos. 38-1479 and 74-0326. The novelty of this work lies in the ligand-directed synthesis strategy, which provides controlled nucleation and growth of nanoparticles under mild thermal conditions. This single-source precursor approach is advantageous due to its simplicity, reproducibility, and minimal environmental impact. The obtained $Cr_2O_3$ nanoparticles, characterized by nanoscale dimensions and high crystallinity, are expected to exhibit enhanced catalytic, optical, and sensing properties, making this green synthetic route a promising strategy for designing advanced functional nanomaterials through coordination chemistry principles.

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## CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

## AUTHOR CONTRIBUTIONS

**Dr. J. M. Pawara:** Conceptualization, synthesis, spectral analysis, data interpretation, and manuscript preparation.
**Dr. S. S. Patil:** Supervision, methodological validation, critical review, and final approval of the manuscript.

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**Table 1: Antibacterial Activity of Chromium(III) Mixed Ligand Complexes**

(Zone of Inhibition in mm)

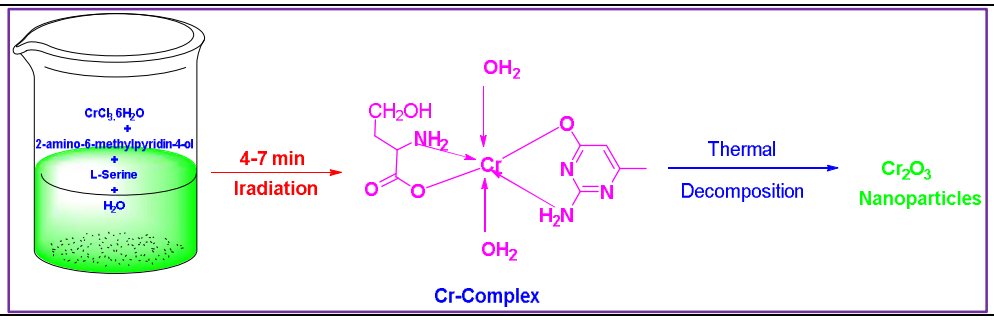
| Sr. No. | Complex | *S. aureus* | *C. diphtheriae* | *S. typhi* | *P. aeruginosa* |
|---|---|---|---|---|---|
| 1 | [Cr(AMP)(Ser)·$H_2O$] | 13 | 10 | 11 | 12 |
| 2 | $Cr_2O_3$ | 18 | 19 | 20 | 15 |
| 3 | Tetracycline | 30 | 23 | 25 | 16 |

**Table 2: Minimum Inhibitory Concentration (MIC) of Chromium(III) Complexes**

| Sr. No. | Complex | *S. aureus* (μg/cm³) | *C. diphtheriae* (μg/cm³) | *S. typhi* (μg/cm³) | *P. aeruginosa* (μg/cm³) |
|---|---|---|---|---|---|
| 1 | [Cr(AMP)(Ser)·$H_2O$] | 65 | 66 | 100 | 115 |
| 2 | $Cr_2O_3$ | 71 | 70 | 104 | 115 |
| 3 | Tetracycline | | | | |

**Table 3: UV–Visible Spectral Data of $Cr_2O_3$ Nanoparticles**

| Sr. No | Wavelength Range (nm) | Transition Type | Assignment |
|---|---|---|---|
| 1 | ~330–340 | $\pi \rightarrow \pi*$ (ligand-based) | Surface-bound ligand electronic transition |
| 2 | ~390–400 | LMCT (Ligand to Cr(III)) | Strong charge transfer band |
| 3 | ~450–520 | d–d transition ($Cr^{3+}$) | Octahedral field transitions (weak) |




**Graphical Abstract: Graphical representation of synthesis of $Cr_2O_3$ NPs**

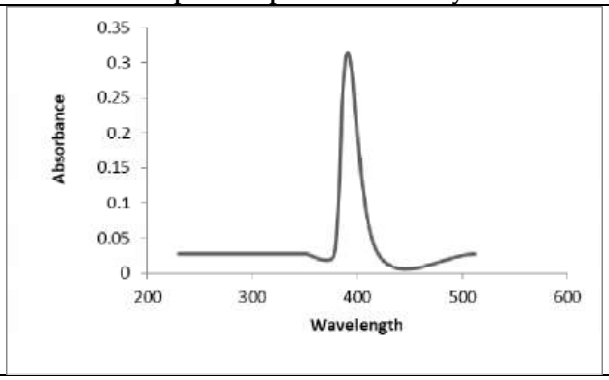



**Figure 1: Electronic Spectra**

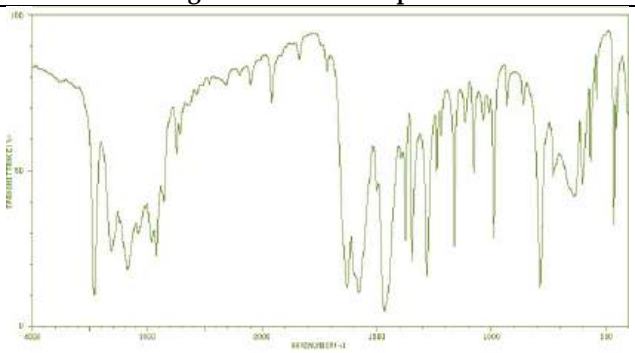


**Figure 3: FTIR Spectra of Cr Complexes**

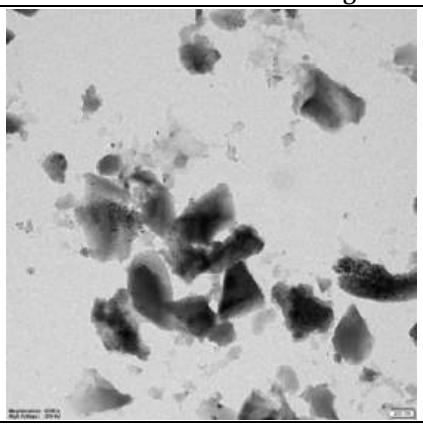


**Figure 3 . 2(a). SEM image Cr(III) complex**

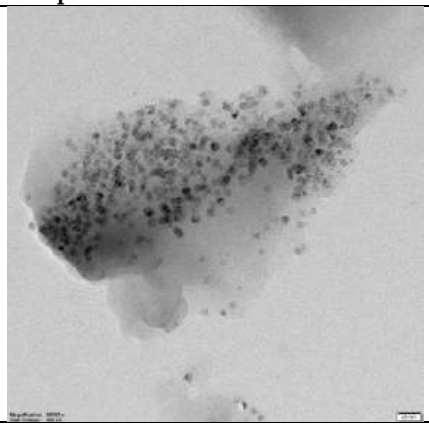


**Figure 4. 4 SEM image $Cr_2O_3$NPs**

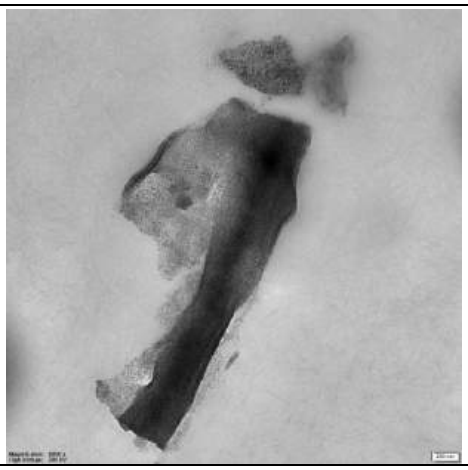


**Figure 5. HRTEM imageCr(III) Complex**

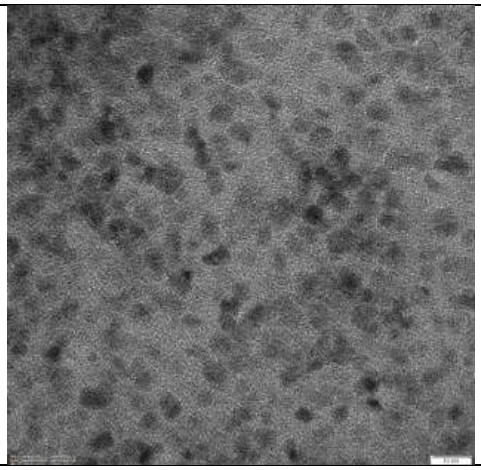


**Figure 6. HRTEM image $Cr_2O_3$**

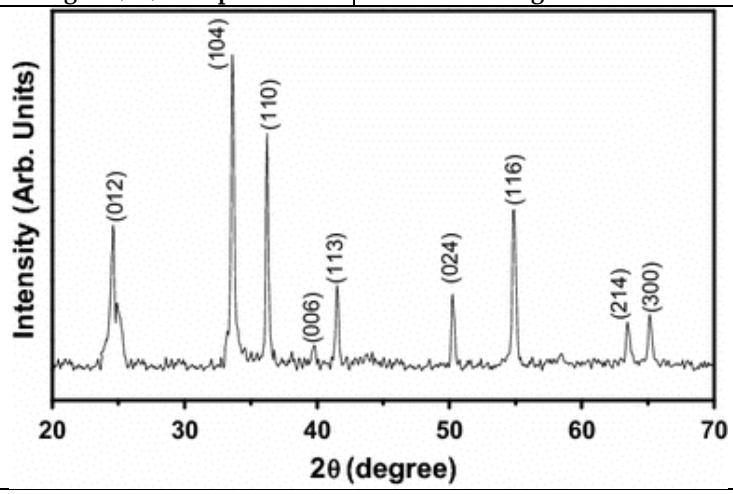



**Figure 6: X-ray Diffraction (XRD) Analysis**

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**REVIEW ARTICLE**

# Comprehensive Knowledge on *Borassus flabellifer* through Multiple *In vitro* Studies

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**ABSTRACT**

*Borassus flabellifer*(Palmyra Tree)is a multifaceted tree. In the recent past, there was a strong thirst for knowledge on this principal tree. Through the *In Vitro* findings, certain properties and qualities of the parts of the tree are confirmed from the bench to the bedside, and some remain at the laboratory level. This collective information will guide the audience to continue the research that has been carried out or to invent new methods to reach new horizons with *In Vitro* models on *Borassus flabellifer*. The systematic purpose of this review is to enable us to study more about the tree and its parts in the laboratories through *In Vitro* models rather than choosing an expensive model available in the scientific world. This review is intended to provide a synthesis of all possible *In Vitro* studies conducted on the parts of *Borassus flabellifer*. Scientific evidence from various research articles was extracted by searching databases such as PubMed, Google Scholar, and Elsevier, as well as other relevant journals. In addition, the reference lists of these reviewed papers were studied and cross – referenced to reveal more relevant *in vitro* evidence. This exhaustive list of findings will pave the way for a new, growing interest in the research on the Palmyra tree. This multifaced tree offers a good number of opportunities for researchers to dwell in the area of*in vitro* studies, which will naturally lead to a systematic study of *Borassus flabellifer.* The evidence found in this review will help others to continue their passion for studying this gifted tree.

**Keywords:** *Borassus flabellifer*, *In Vitro*, invent, study and health

# INTRODUCTION

From millions of plant species found in all possible locations, only a handful of plants considered a golden egg for humanity in the field of medicine, health and physical. It is conspicuous that plants, trees and many other natural gifts play a major role in health as medicine since the origin of the human era. Plants and trees have been used partly and as entire plants or trees, for many medicinal purposes. Some of the most used trees are like Neem, Papaya, Calihari, etc. In the realm of plants, we have Tulsi, Ginger, Garlic, Coriander, Mint, and Turmeric that have been considered very much for health and medical purposes for several thousand years. The health benefits of each tree, plant, herb and their parts are studied suitably beginning with the *In Vitro* methods. *In Vitro* is a system or model used to study in general. It is often observed that the initial approach to investigating the disease or phenomenon, the first option is the *In Vitro* method. It is mainly to reduce the cost when compared to other models available in the world. It is also ethically preferred to conduct research with *In Vitro* systems rather than using animal or *in vivo* models (Mezher *et al,* 2024). The *In Vitro* methodhas paved a way to discover a few substances, namely phytochemicals, viz. polyphenols, vitamins, minerals, proteins, and many other compounds in fruits, leaves, bark, and roots,which have a role to play in promoting as well as disease-preventing benefits(Prasad G *et. al.,*2016).This article is intended to give an overview of *In Vitro* studiesof the *Borassus flabellifer* in various scales and methods. "*Borassus flabellifer*" is universally known as the palmyra tree. It is quite tall in stature (*Figure 1*). The word '*Borassus*' is from a Greek word that means the leathery covering of the fruit, and '*flabellifer*', which denotes fan-bearer. The Palmyra palm tree is from the 'palme' family. Locally, the Palmyra tree is the official tree of Tamil Nadu state in India (Subramanian *et al.,* 2024). In Tamil culture, it is called karpaha, nungu, celestial tree and is highly revered by the people. All its parts could be used in day-to-day life, and its multifaceted use highlights its importance. (Jana *et. al.,*2017).This is a tall and robust tree, which has large fruits, some with a white colour and others in dark colours, fan-shaped, hard, horny, and spinyserrated over the petiole edges. It grows to a height of 30 m, with a black stem and crown of leaves at the top; leaves are around 0.9-1.5 m in diameter. It has unisexual flowers(Sahni *et. al.,*2014). Some of the other common names for this tree are Toddy palm, Asian Palmyra palm, Sugar palm, Cambodian palm, Fan palm, Borassus palm, Mak tan kok, Kerigi, African fan palm, Taan, Wine palm, Ron palm, Doub palm, Than, Great fan palm, Panna-maram, Lontar palm, Tala palm, etc. We could find this tree in regions of Asia, Africa, Cambodia, New Guinea, India, Jawa, Laos, the Lesser Sunda Islands, Malaya, Myanmar, Socotra, Parts of China, the Philippines, Thailand, Sri Lanka, Queensland, and Australia (Jerry A., 2018).

**Prosperity of *Borassus flabellifer***

The palmyra palm is a multipurpose tree with great utility. It is believed to be a native of tropical Africa, although it grows significantly in different parts of India, Sri Lanka and Myanmar and Bangladesh. Borassus palms are of considerable value to the people of their region. It is said to be a tree of life with nearly 800 uses, including food, beverage, fibre, medicinal, and timber. Various by-products like palm sugar and gur (molasses) are also prepared from the juice extracted from the tree trunk. The immature soft juicy seed nuts and Neera are very popular in the tropical parts of India as a soft natural drink to protect against the hot summer. The soft orange-yellow mesocarp pulp of the ripe fruit is sugary, dense and edible, rich in vitamins A and C. Borassus also contains bitter compounds called flabelliferrins, which are steroidal saponins. Ripened fruit pulp can be processed into soft beverages, jam, toffee, delicious food items and sweets (A. K. Chaurasiya *et.al.,* 2014). Such is the greatness of this tree. All these bits are the fruit of the research of many men and women in the past. These pieces of information originated in the laboratory, particularly from findings obtained through the *In Vitro* method.

***In Vitro* Model**

Techniques of performing experiments or procedures in an artificial environment, outside a living organism, are referred to as *In Vitro*. For many scientific findings across various fields, *In Vitro* methods, implying "in glass" or "in the lab." This method involves conducting experiments, particularly in test tubes, petri dishes, or other laboratory equipment and conditioned environments on the organism's constituents like organs, tissue or cell culture, cellular components (M. Huber *et. al.,* 2011). Most of the preclinical phases of animal-based testing have resulted from an *In*

*Vitro* method. It is believed that human-based *In Vitro* studies and procedures could make major contributions if handled seriously and intelligently. (Bal-Price *et al.* 2014).The *In Vitro* screening methods have several advantages over *invivo* experiments conducted on living organisms, such as the undisputable ethical benefits, a reduced cost, and the best choice for large-scale production with easy culture for production in the establishments(James E *et. al.,* 2008).Some of the systems of *In Vitro* include: the use of cell fractions, cell lines, stem cells (including induced pluripotent stem cells), engineered tissues, dynamic bioreactors, multiorgan systems, and cells-, organs-, and (even) humans-on-a-chip, collective with high-throughput screening, high-content screening, the "omics" tactics, systems modelling and simulation, pharmacokinetic and toxicokinetic modelling, virtual tissue modelling, virtual human populations, and biomarkers, strategically used, with effective bioinformatics backing(Bal-Price *et. al.* 2014).*In Vitro* studies are highly focused. They enable to inference of mechanisms of action and to control of many confounding variables (M. Huber *et. al.,* 2011). Some of the common types of *In Vitro* methods in antioxidants are: DPPH scavenging activity, Hydrogen peroxide scavenging assay, Nitric oxide scavenging activity, Peroxynitrite radical scavenging activity, Trolox equivalent antioxidant capacity (TEAC) method/ABTS radical cation decolourization assay, Total radical-trapping antioxidant parameter (TRAP) method, Ferric reducing-antioxidant power (FRAP) assay, Superoxide radical scavenging activity (SOD), Hydroxyl radical scavenging activity, Hydroxyl radical averting capacity (HORAC) method, Oxygen radical absorbance capacity (ORAC) Method,Reducing power method (RP), Phosphomolybdenum method, Ferric thiocyanate (FTC) method, Thiobarbituric acid (TBA) method, DMPD (N,N-dimethyl-p-phenylene diamine dihydrochloride) method, ß-carotene linoleic acid method/conjugated diene assay, Xanthine oxidase method and Cupric ion reducing antioxidant capacity (CUPRAC) method (Md. Nur Alam *et. al.,* 2013). The following are the *In Vitro* methods through which the antimicrobial activity has been confirmed: Well-known diffusion methods are the Agar disk-diffusion method, Antimicrobial gradient method (Etest), Cross streak method, Other diffusion methods, Agar well diffusion method, Agar plug diffusion method, and Poisoned food method.

The antimicrobial is also confirmed through Thin-layer chromatography (TLC)-bioautography methods such as Agar diffusion, Direct bioautography,and Agar overlay bioassay. Dilution methods are like the Broth dilution method and the Agar dilution method used. Time-kill test (time-kill curve), ATP bioluminescence assay and Flow cytofluorometric method are also used to find out the antimicrobial activity (Balouiri*et al.,* 2015). The wound healing is studied by Single Systems: Monolayers / 2D Wound Healing Assays, Multicellular Systems: Cocultures and 3D Wound Healing Assays and Organ Culture. These are the recent *In Vitro* methods on wound healing (Sarian *et. al.,* 2023).*In Vitro* methods to identify vitamins are Chromatographic methods like High Performance Liquid Chromatography, Ultrahigh Performance Liquid Chromatography and Gas Chromatography are used to determine vitamins B1 and B2. UV Visible Spectrophotometric method is used for vitamin A (Retinol), Fluorometric method is used for vitamin B (Thiamine), Microbiological method with *Lactobacillus casei and Lactobacillus plantoides* specify vitamin D (Calciferol) and Niacin (Pravalika *et. al.,* 2023). *In Vitro p*hytochemical tests vary forseveral phytochemicals. Some of these are: Shinoda's test, Ferric chloride test, Lead ethanoate test, Sodium hydroxide test are for flavonoids. Molisch's test is for Carbohydrates, Barfoed's test is for monosaccharides, Fehling's test is for free reducing sugar, Fehling's test is for combined reducing sugars,and Liebermann-Burchard test is for steroids (Usman *et. al.,* 2009). Anti-inflammatory activity is studied by cytotoxicity assay (Vasanth *et. al.,* 2020). Scratch assays are commonly used *In Vitro* models for wound healing (Hofmann *et. al.,* 2023). These are some of the frequently used *In Vitro* methods. There are other *In Vitro* methods too found in many other areas of studies, such as nanotechnology, Cardiovascular, Pharmacological, Toxicity, and Molecular and Biochemical levels. Products of *Borassus flabellifer* have many traditional uses: food, medicine and materials. It produces cellulose fibres, polymer composites, Biocarbon's and nanomaterials. Many of its parts are studied from the basics to higher-level inventions. This plant is used extensively by humans from the fast to produce various consumer products. No parts of the plant is left without any attention. All of its parts are researched and the importance of it is demonstrated. Among many parts of the plant leaves, petioles, stalk, sap, flowers, root, and fruits are given prime importance among many other parts in the tree (Singh *et. al.,* 2021). Here follows the research conducted with the *In Vitro* methods on the palmyra tree.

**Roots**

The roots of the palmyra trees are physically verifiable and they are strong and intertwined with mud. The gravitmetrical method for nutritional components shows the presence of crude fiber, fat, protein, moisture and mineral contents like Iron, Strontium, Copper, Manganese, Zinc and Aluminum. Trace elements and heavy metals like Arsenic, Lead and Nickel also found in the root extract of Palmyra root. Through Kjeldahl method, the presence of nitrogen is found in the root. The AOAC Method (Association of Official Analytical Chemists) determined the presence of Carbohydrate, described by Herborne(Herbone, 1973). He suggested that the crude alkaloids 1.61 and Saponins 0.63 (g $100^{-1}$) in the root of the Palmyra tree, while the method described by Singleton and Rossi (Sahni *et. al.,* 2014) shows the presence of phenolic content. Other standard methods resolved that the ethanol extract of the root of *Borassus flabellifer* exhibits the presence of Carbohydrates, terpenoids, Saponins (Vanitha *et. al.,* 2025), Coumarins, Alkaloids, Tannins, and flavonoids. The aqueous extract shows the presence of Carbohydrates, proteins, Coumarins, Alkaloids, Flavonoids and Glycosides. Similarly, the Petroleum ether extract results in the presence of Carbohydrates, Proteins, Terpenoids and Flavonoids (B. Arirudran*et. al.,* 2022). Through the Colorimetric method, the presence of flavonoids is found. The roots of the palmyra are rich in protein (8.54%) and fiber, fruit pulp shows high ascorbic acid and calcium levels, which are the main essential micronutrients required by the body (Prasad *et. al.,* 2023). The ABTS radical scavenging method developed an antioxidant equivalent to Ascorbic acid. The highest antioxidant was found in the methanolic extract of the root. Similarly, Ferric Reducing Activity Power Assay developed by Benzine and Strain recorded BHT (butylated hydroxytoluene) equivalents as antioxidants in the root. The highest antioxidant activity was found to be 129.6 µg BHTE 100 mg– 1 in the chloroform fraction of the roots (Sahni *et. al.,* 2014). Additionally, to the identified compounds in hexane, chloroform and methanolic extracts, the roots have Hexadecanolic acid, Tetradecanoic acid and Pentadecanoic acid. Using the disc diffusion method, antimicrobial activity against *Staphylococcus aureus, Staphylococcus epidermidis* and *Bacillus subtilis* and 2 gram-negative bacterial strains i.e. *Escherichia coli* and *Proteus mirabilis.* (Sahni *et. al.,* 2014) were found. The young roots of the palmyra are confirmed with vitamin E (Sayanhika*et. al.,* 2024).

**Sprout**

Standard Methods described by Herborne (Herborne, 1973) and Obadoni and Ochuko (Obadoni*et. al.,* 2001) for phytochemical screening exhibit steroids, alkaloids, tannins, phenols, flavonoids, glycosides, coumarins and saponins in the sprout of palmyra. Gravimetrical method identified the ash content, Kjeldahl method – nitrogen and AOAC method found the presence of carbohydrates and protein in the sprout of the palmyra tree. The traditional methods of ABTS and DPPH identified that the highest antioxidant potential was found in the methanolic extract, and the ability to scavenge free radicals was observed (Agna *et. al.,* 2025). Similarly, Ferric Reducing Activity Power assay by Benzie and Strain (Benzie *et. al.,* 1996) supports that the plant extract reduced ferric ions into ferrous ions (Mariselvam *et. al.,* 2020). Sayanhika *et. al.* state that the palmyra tubers and ripe fruits contain steroidal saponins called flabelliferins.

**Seed**

The seed of the palmyra tree (Figure. 3)exhibits antimicrobial activity against *Staphylococcus aureus, Staphylococcus epidermidis* and *Bacillus subtilis* and 2 gram-negative bacterial strains i.e. *Escherichia coli* and *Proteus mirabilis* (Sahni *et. al.,* 2014). The well-known Disc diffusion method was used to determine the antimicrobial activity. The antibacterial activity of methanol extracts of *Borassus flabellifer L.* (Arecaceae) seed coat (soft outer shell) was noted by the agar well diffusion method. The antibacterial potential was examined against Gram-positive bacteria, i.e., *Staphylococcus aureus, Bacillus subtilis and* Gram-negative bacteria, i.e., *Klebsiella pneumonia* and *Serratia marcescens*. The methanol extract of the seed coat has shown consistently significant inhibitory activity on different bacterial species tested (Shirisha *et. al.,*2018). The haustorium from the seed of the palmyra is a white, spongy and edible stuff. It has ash content, protein, fat, carbohydrates, fiber, calcium, sodium, magnesium, potassium, iron, zinc, phosphorus and ascorbic acid. It also possesses glycosides, flavonoids, phenols and saponins (Kuldeep*et. al.,* 2023). Phytochemical screening was done qualitatively with different methods to identify various phytochemicals, namely the ferric chloride test was done for tannins, Frothing test for saponins, Shinoda test for flavonoids, Dragendorff test for alkaloids, Bradford protein assay – test for proteins, Salkowski's test for steroids and terpenoids, Keller-Kiliani test for cardiac glycosides, Borntrager's

reaction test for anthraquinones, Ferric chloride test for phenols. The seedcoat extract confirmed the presence of tannins, Saponins, Flavonoids, Alkaloids, proteins, Cardiac glycosides, and phenols. The seed coat extract shows inhibition against bacteria like *Streptococcus mutans, Salmonella typhi,* and fungi like *Candida albicans and Aspergillus flavus*(Natrajan *et. al.,* 2024).

**Leaves**

The methanol leaf extract of *Borrassus flabellifer* was screened using standard procedures and found that the extract showed the presence of flavonoids, glycosides, tannins, proteins, steroids, triterpenoids, carbohydrates, fats and fixed oils. Similarly, the compound the presence of Flabelliferin is found and it shows inhibition on the growth of gram-positive and gram-negative bacteria (Vanitha *et. al.,* 2025). Random amplified polymorphic DNAs (RAPD) Technique (M Li *et al.,* 1999) extracted DNA from palm sugar leaves. The DPPH radical scavenging activity recorded 40.10 µg/ml. Similarly, the Hydrogen Peroxide Radical Scavenging Method shows that the IC50 value of the methanol extract and ascorbic acids was 30.92 µg/ml and 18.85 µg/ml. Standard procedure for analysing phytochemical screening of the methanol extract of leaves shows the presence of flavonoids, glycosides, tannins, proteins, steroids, triterpenoids (Vanitha *et. al.,* 2025), carbohydrates, fats and fixed oils (Prasad G*et. al.,* 2016). Agar well diffusion assay resulted for Antimicrobial activity in Methanol and acetone fractions, showing inhibition against Bacteria such as *B. subtilis, S. aureus, P. vulgaris, P. vulgaris and B. subtilis.* The leaf extract also showed antifungal activity against A. flavus, M. canis, S. cerevisiae and V. myditis. The chloroform extract of dried leaves of the palmyra has anti-inflammatory activity (Mohan *et. al.,* 2016).

**Pulp**

The presence of carotenoids in the palmyra pulp is proven alongside polyphenolic components. The presence of vitamin D3 is high in palmyra pulp. An experiment based on the spectrophotometric measurement discovered the presence of vitamins A and B6. The fresh pulp is enriched with vitamins A and C (Sayanhika*et. al.,* 2024).

**Fruit**

The inside of the palm fruit is reddish in colour (Figure. 4). The secondary metabolites such as terpenoids, flavonoids, alkaloids (Vanitha *et. al.,*2025), saponins and tannin were noted by the standard procedures by Harborne (Harborne, 1973).Methods such as Anthrone Method, Bradford's Colorimetric Method, Flame Photometry and Wet Digestion Method (T. Sherin Mary *et. al.,* 2022) were used to find out nutrients such as carbohydrates, protein, calcium, and phosphorus were found in the fruit of the palm tree. Fibers and reducing sugar of palmyra contain polymeric compounds like hemicellulose, pectin, lignin. (Sayanhika*et. al.,* 2024). Young fruit has Maltose, Protein, Ascorbic acid, Calcium, Ash and Fat (Mariselvam *et. al.,* 2020). The immature fruit shows antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus pneumoniae, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Shigella dysenteriae, and Pseudomonas aeruginosa. Antifungal activity is seen against Candida albicans, Saccharomyces cerevisiae, Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Penicilliumchrysogenum, and Penicillium notatum* (Renuka *et. al.,* 2019). Similarly, the immature fruits confirmed the antioxidant potential. This was confirmed by multiple *In Vitro* methods, such as DPPH Radical Scavenging Assay, ABTS Assay, Assay for nitric oxide Scavenging activity and Superoxide anion radical scavenging assay (Renuka *et. al.,* 2019). Standard methods, such as the Macro-broth dilution method, were performed to determine the Minimum inhibitory concentration (MIC) and the Minimum bactericidal concentration (MBC) assays were performed, and they showed antibacterial activity against gram-positive bacteria, such as *Staphylococcus aureus, Bacillus subtilis, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus pneumoniae* and gram-negative bacteria such as *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Shigella dysenteriae*, and *Pseudomonas aeruginosa*.Minimum fungicidal concentration (MFC) Antifungal activity of immature palmyra palm fruit extract showed against *Candida albicans, Saccharomyces cerevisiae, Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Penicillium Chrysogenum, Penicillium notatum.* DPPH and ABTS methods showed the inhibitory values and the presence of an antioxidant, while Embryo culture and histological analysis show the natural germination process with the palmyra palm (Douglas, 1987; George, *et. al.,* 2024). Paulraj Mosae Selvakumar studied hemi-cellulose is a cellulose-related polysaccharide in the fruits of the palmyra. Added to that,

two saponins in the fruit pulp and tuber flour were identified: steroid spirostmonoglucoside and monorhamnoside (Selvakumar *et al.,* 2020). It is also found that the fruits are a rich source of vitamins such as A, B, C and minerals like iron, zinc, potassium, calcium, phosphorus, thiamine, and riboflavin (Krishnaveni *et. al.,* 2020).

**Sap**

The Folin-Ciocalteu reagent method identified the phenolic content from Palm syrup (Huynh Thi Le, *et. al.* 2020; Asikin*et. al.,* 2016). The Phenol-sulfuric acid method determined the presence of total sugar content (Nielson, 2017). The Dinitrosalicylic Acid method also identified the presence of reducing sugar and was evaluated (Huynh Thi Le, *et. al.,* 2020 and Asikin*et. al.,* 2016). The protein content was confirmed through Kjeldahl Method(Dung Huynh Thi Le *et. al.,* 2021). The free-radical scavenging activity was determined by the standard DPPH Method (Huynh Thi Le, *et. al.,* 2020; Asikin*et. al.,* 2016) while ABTS Radical Cation Decolourisation Assay was resolved in the Palm syrup (Payet *et. al.,* 2005).Toddy contains vitamins such as A, Ca, Fe, Zn, Cu and the fermented sap increases the levels of thiamine, riboflavin and niacin (Krishnaveni *et. al.,* 2020). The fermented nectar of the palmyra is a rich reservoir of probiotic microorganisms. A study by Raman Kanimozhi isolated 80 bacterial isolates from the nectar. Selected determined simulated gastrointestinal conditions, cell surface properties like auto aggregation, hydrophobicity, coaggregation and Biofilm production, β-galactosidase and exopolysaccharide production, antioxidant activity, antibiotic susceptibility and safety through hemolysis and DNase activity (Kanimozhi*et. al.,* 2025). Dung Huynh Thi Le and his teamwidely researched the syrup from the sap of the palmyra tree. They found that the syrup contains protein, phenolic content and total sugar content. Syrups prepared by thermal and ultrafiltration processes discovered 10 vitamins, where most of them were B-group vitamins such as B1, B2, B3, B5, and B6. Some of the other vitamins were Vit. C, D, E and Folic acid. Fresh sap of *Borrassus flabellifer* showed the presence of vitamin B and ascorbic acid. Moreover, there were also 38 volatile compounds found in the syrup of the palmyra tree. The DPPH radical scavenging activity at the different concentrations is exhibited (Dung *et al.,* 2021). The seasonal sap or juice is collected from the inflorescence of the palm tree, from which the sugar is produced by a process (Rayappa, 2023). A study shows that the Palm sugar exhibits the highest level of antioxidant activity compared to various types of cane sugars, having an antioxidant activity equivalent to 1.7 mg of vitamin C per 1 g of sugar (J Sia *et al.,* 2010).It is also found that the sugar palm has a considerable amount of sodium, phosphorus, iron and potassium. The palm sugar is also a low-calorie sugar. It is used extensively in cooking and has many health benefits; Low glycemic index sugar in the palm sugar is exceedingly helpful in reducing obesity and diabetes, it also provides a sustained and uniform energy supply to the body, and it is rich in many nutrients such as vitamins B1, B2, B3, B6 and C (Vivek M *et al.,* 2011). Vitamins such as riboflavin, thiamine, niacin and vitamin A are found significantly (D.H.T. Le *et al.,* 2020). The colorimetric assay confirmed the presence of Flavonoid contents. Similarly, the antioxidant activity through the DPPH method showed the radical scavenging activity, while the FRAP assay also determined the antioxidant power of the palm sugar (Khongsak*et al.,* 2019).

**Flower**

The ethanolic extract of male flowers (inflorescences) of *Borassus flabellifer L.*(Arecaceae) has analgesic activity(Shirisha *et al.,*2018; Gummadi *et al.,*2016). Inflammation is a local response of living mammalian tissues to injury and a body defence reaction to eliminate the spread of injurious agents. The ethanolic extract at doses of 150 and 300 mg/kg shows an anti-inflammatory response in the mice. (Paschapur *et al.,* 2009). Tusskorn*et. al.*2021) investigated the male flower of the palmyra tree and found that the crude flowers, using ethyl acetate and methanol, the properties of antioxidant activity through DPPH and Ferric Reducing Antioxidant Power. Cytoprotective activity was confirmed. Oxidative stress was determined by the Glutathione assay (Tusskorn*et. al.,* 2021). It confirms that the male flowers also have similar properties to other parts of the plants.

## CONCLUSION

This unique review sets out the platform for more research on the palmyra tree. The basic *In Vitro* studies will move towards a better understanding of the properties of the tree and give way to exploring more with the studies that

have been carried out and to open a new folder for the research on the principal tree from our country, which holds a considerable number of plants. Encouraging biological potential, particularly in the sphere of antimicrobial, antioxidant, cytocompatibility and wound healing–related responses. Although the *In Vitro* method has limitations, when it comes to research, it provides a strong scientific foundation supporting the medical properties of any plant. This review is to give a taste of the research carried out through the *In Vitro* with the palmyra tree and motivate new research to be carried out with more understanding and a strong foundation on the knowledge of *Borassus flabellifer*. There are also some parts of the plants yet to be discovered, with more advanced techniques available in the laboratory. It will lead to a more detailed evaluation of the palmyra tree.

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A - Sapling; B - Young Tree
C - Serrated Tree; D - Mature Tree

**Figure 5: Palmyra tree.**



**Figure 6: Roots of Palmyra tree**




**Figure 3: Various Parts of the Palmyra tree**



**Figure 4: Fruits of the Palmyra tree.**

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REVIEW ARTICLE

# The influence of Visual, Oculomotor and Ergonomic Factors on Reading Speed in Print and Digital Media

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## ABSTRACT

Reading speed is widely used as a functional indicator of visual efficiency and is closely linked to academic performance and everyday information processing. In recent years, there has been a rapid shift from traditional print materials to digital reading platforms, especially among students. This transition has raised concerns about the impact of prolonged screen use on visual performance, ocular comfort, and reading efficiency, making it necessary to review and integrate existing evidence from an optometric perspective. The objective of this review is to examine and synthesize existing literature on the influence of visual, oculomotor, and ergonomic factors on reading speed in print and digital media. A narrative review of peer-reviewed studies in optometry, vision science, and visual ergonomics was conducted. Literature focusing on reading speed, visual function, eye movements, and ergonomic conditions during print and screen-based reading was analyzed. The reviewed literature indicates that multiple factors, including visual acuity, contrast sensitivity, accommodative and vergence demand, eye movement patterns, screen luminance, font characteristics, viewing distance, posture, and environmental lighting, significantly influence reading speed. Digital reading is often associated with higher visual demand, altered oculomotor behaviour, and increased visual fatigue compared to print reading, particularly

during prolonged tasks. Reading speed in print and digital media is shaped by a complex interaction of visual, oculomotor, and ergonomic factors. Reviewing these factors is essential to understand the challenges posed by increasing digital screen use and to guide clinical practice, educational strategies, and ergonomic recommendations. This review highlights the need for optimized reading environments and identifies areas for future research in vision and optometry.

**Keywords:** Reading speed, digital screens, print media, visual factors, oculomotor function, ergonomics, optometry

## INTRODUCTION

Reading is a demanding visual activity that requires accurate coordination of several visual functions, including visual acuity, accommodation, vergence, and precise eye movements [1]. Reading speed is commonly used as a practical indicator of visual efficiency and is closely related to learning ability, academic success, and daily information processing. Any disturbance in these visual processes can negatively affect reading fluency and overall performance [2]. For many years, printed text was the primary medium for reading. However, with rapid technological advancement, digital devices such as smartphones, tablets, laptops, and computers have become the dominant reading platforms, particularly among students. This shift has significantly changed the visual demands placed on the eyes [3]. Unlike printed text, digital displays are self-luminous and differ in luminance, contrast polarity, pixel resolution, font rendering, and viewing ergonomics. These differences may alter visual behaviour and influence reading speed and comfort. Several studies have examined reading performance on print and digital media, reporting variations in reading speed, eye movement patterns, and visual fatigue [4]. Research has highlighted the role of accommodative and vergence stress, reduced blink rate, altered fixation patterns, and increased visual strain during prolonged screen use [5]. At the same time, findings across studies are not always consistent, and the influence of ergonomic factors such as viewing distance, posture, screen brightness, and environmental lighting is often underreported or studied in isolation [6]. From an optometric perspective, there is a clear need to synthesize existing evidence to better understand how visual, oculomotor, and ergonomic factors collectively influence reading speed across different reading media [7]. A comprehensive review of this topic is important to guide clinical assessment, inform ergonomic recommendations, and support evidence-based advice for students and frequent digital device users. Therefore, this review aims to critically examine the available literature on reading speed in print and digital media, with particular emphasis on factors relevant to optometry and vision science.

## METHODOLOGY OF THE REVIEW

**Review Design**

This review was conducted as a narrative literature review to comprehensively synthesize existing research on the influence of visual, oculomotor, and ergonomic factors on reading speed in print and digital media. A narrative approach was considered appropriate due to the heterogeneity of study designs, outcome measures, and methodologies reported in the available literature.

**Data Sources and Search Strategy**

A systematic search of the literature was performed using major electronic databases, including PubMed, Scopus, Web of Science, and Google Scholar. Searches were conducted using combinations of the following keywords and Boolean operators: *reading speed*, *print reading*, *digital reading*, *screen-based reading*, *visual factors*, *oculomotor function*, *eye movements*, *visual ergonomics*, and *computer vision syndrome*. Reference lists of relevant articles were also manually screened to identify additional studies.

**Eligibility Criteria**

Studies were included if they met the following criteria: [i] published in peer-reviewed journals; [ii] written in English; [iii] focused on reading speed or reading performance in print and/or digital media; and [iv] examined visual, oculomotor, or ergonomic variables relevant to optometry and vision science. Both experimental and observational studies were considered. Review articles were examined for background information but were not included in the final synthesis. Studies involving participants with ocular pathology were excluded unless findings were directly applicable to general visual performance.

**Study Selection Process**

Titles and abstracts retrieved from the database search were screened for relevance. Full-text articles were obtained for studies that met the inclusion criteria or where eligibility was unclear from the abstract alone. Articles that did not align with the objectives of the review were excluded after full-text assessment.

**Data Extraction and Synthesis**

Relevant information from the selected studies was extracted, including study design, participant characteristics, type of reading medium, visual and oculomotor variables assessed, ergonomic conditions, and key findings related to reading speed. Due to variability in outcome measures and experimental conditions, a qualitative synthesis was performed rather than a meta-analysis. Findings were organized thematically under visual, oculomotor, and ergonomic domains.

**Visual Factors Influencing Reading Speed**

**Visual Acuity and Contrast Sensitivity**

Visual acuity plays a fundamental role in reading, as clear resolution of letters is essential for accurate word recognition and fluent reading. Uncorrected refractive errors or reduced visual acuity have been consistently associated with slower reading speed [8]. In addition to acuity, contrast sensitivity is a critical visual function, particularly in digital reading environments. Digital screens may present issues such as glare, reduced contrast, pixelation, or suboptimal font rendering, which can compromise text clarity. Several studies have reported that lower contrast conditions on screens are linked to reduced reading speed when compared with high-contrast printed text, highlighting the importance of optimal visual clarity for efficient reading [9].

**Accommodation and Vergence Demand**

Reading from digital devices typically requires sustained near fixation, which increases accommodative and vergence demand. Continuous accommodative effort during prolonged screen reading may result in accommodative fatigue, leading to fluctuations in clarity and a gradual reduction in reading speed. In contrast, print materials often allow greater flexibility in viewing distance and posture, which may help reduce sustained visual stress. These differences suggest that accommodative and vergence demands are important contributors to variations in reading speed between print and digital media [10].

**Visual Fatigue and Ocular Discomfort**

Visual fatigue is a commonly reported consequence of prolonged digital reading and includes symptoms such as eye strain, dryness, headache, and intermittent blurred vision. These symptoms can negatively affect reading efficiency and reduce reading speed over time. Digital screens are often associated with a reduced blink rate and increased exposure to glare, further contributing to ocular discomfort. In comparison, print reading is generally associated with lower levels of visual fatigue, particularly during extended reading tasks [11].

**Oculomotor Factors and Reading Speed**

**Eye Movements During Reading**

Efficient reading relies on well-coordinated saccadic eye movements and stable fixations. Alterations in eye movement behaviour can disrupt reading fluency and reduce reading speed. Studies have shown that digital reading may be associated with longer fixation durations, increased regression movements, and less regular saccadic patterns

compared to print reading [12]. Additional demands such as scrolling and page navigation on digital devices may further influence eye movement control and contribute to slower reading performance.

**Binocular Vision and Fixation Stability**

Accurate binocular coordination is essential for maintaining single, clear vision during sustained reading tasks. Digital screens may challenge binocular stability due to increased visual demand, glare, and prolonged near work. Individuals with borderline binocular vision anomalies may be particularly susceptible to reduced fixation stability and associated decreases in reading speed when reading from screens [13].

**Ergonomic Factors Affecting Reading Speed**

**Viewing Distance and Posture**

Viewing distance and posture play an important role in determining visual comfort and reading efficiency. Handheld digital devices are often viewed at shorter distances than printed materials, resulting in increased accommodative and vergence demand. Poor posture and prolonged close viewing can also contribute to musculoskeletal discomfort, indirectly affecting reading performance and speed [14].

**Screen Characteristics and Environmental Lighting**

Characteristics such as screen size, brightness, contrast settings, font type, and background colour can significantly influence readability and reading speed. Excessive screen brightness, reflections, or poor ambient lighting may reduce visual comfort and slow reading speed. Printed text, when viewed under appropriate lighting conditions, is generally less affected by such factors, allowing more stable reading performance [15].

**Duration of Reading Task**

Reading speed during digital tasks often declines over time as a result of cumulative visual fatigue. Prolonged uninterrupted screen use has been associated with reduced visual comfort and decreased reading efficiency. Incorporating regular breaks, appropriate screen adjustments, and ergonomic practices has been shown to help maintain reading speed during extended digital reading sessions [16].

**Comparison of Reading Speed in Print and Digital Media**

Comparative studies examining reading speed in print and digital media have produced mixed but generally consistent findings. The majority of research indicates that reading speed on digital screens is either comparable to or slightly slower than print reading, particularly when reading tasks are extended in duration [17]. Short reading tasks often show minimal differences between the two media; however, as reading time increases, digital reading is more frequently associated with a gradual decline in reading speed. One important factor contributing to these differences is the visual and ergonomic environment in which digital reading occurs. Suboptimal screen brightness, glare, low contrast, inappropriate font size, and poor ambient lighting have been shown to negatively affect reading performance on screens. In contrast, printed text is less susceptible to such display-related variations when adequate illumination is provided, allowing more consistent reading speed across longer tasks [18]. Ergonomic factors further influence comparative reading performance. Digital devices are commonly used at closer viewing distances and with less stable posture than printed materials, increasing accommodative and vergence demand and contributing to visual fatigue. These factors may partially explain why reading speed differences between print and digital media become more evident during prolonged reading sessions. Individual variability also plays a significant role in determining reading speed across media. Familiarity with digital devices, adaptability to screen-based reading, age, and habitual reading preferences can influence reading efficiency. Additionally, screen quality, including resolution, refresh rate, and display technology, affects text clarity and visual comfort, thereby impacting reading speed. Together, these findings suggest that differences in reading speed between print and digital media are not solely medium-dependent but arise from an interaction between visual, ergonomic, and individual factors.

**Clinical and Educational Implications**

**Clinical Implications for Optometric Practice**

From an optometric perspective, the assessment of reading speed should be contextualized according to the reading medium being used. Patients increasingly report reading-related symptoms associated with digital device use, making it essential for clinicians to inquire about screen exposure, device type, and duration of digital reading [17]. Comprehensive refractive evaluation remains a primary step, as even small uncorrected refractive errors can adversely affect reading speed and visual comfort, particularly during sustained near tasks. In addition, detailed assessment of accommodative and binocular vision function is crucial for individuals presenting with reduced reading efficiency, eyestrain, or visual fatigue during digital reading. Accommodative insufficiency, vergence anomalies, and reduced fixation stability may become more symptomatic during screen-based reading due to increased visual demand. Identifying and managing these conditions through appropriate optical correction, vision therapy, or near-vision support can contribute to improved reading performance [19]. Ergonomic counselling should be considered an integral component of optometric care. Guidance related to optimal viewing distance, posture, screen height, lighting conditions, font size, contrast settings, and the use of regular visual breaks can help reduce visual strain and support sustained reading efficiency across digital platforms. Such interventions are particularly relevant for students and professionals engaged in prolonged screen-based tasks.

**Educational Implications**

In educational settings, the increasing reliance on digital learning materials necessitates careful consideration of their impact on students' visual performance. Educators and institutions should be aware that prolonged digital reading may affect reading speed and visual comfort, especially under suboptimal ergonomic conditions. Encouraging a balanced use of print and digital materials, particularly for extended reading assignments, may help minimize visual fatigue and support effective learning. Educational policies and classroom practices should also emphasize visual ergonomics, including appropriate lighting, screen placement, and scheduled breaks during digital learning activities [20]. Collaboration between educators, optometrists, and policymakers can facilitate the development of evidence-based guidelines that promote visually comfortable and efficient reading environments for students across different age groups.

## FUTURE DIRECTIONS

Future research should focus on the development and adoption of standardized and validated methodologies for assessing reading speed across print and digital media. Variations in reading materials, display settings, task duration, and outcome measures across existing studies limit direct comparison of findings. Establishing uniform testing protocols that incorporate both visual performance measures and subjective symptoms would strengthen the evidence base and improve clinical applicability. Longitudinal studies are particularly needed to evaluate the long-term effects of sustained digital reading on visual development, accommodative and vergence function, oculomotor behaviour, and ocular comfort, especially in children, adolescents, and young adults who are exposed to screens from an early age. Such studies would help determine whether prolonged digital reading leads to adaptive changes or contributes to persistent visual stress over time. Future investigations should also examine individual variability in response to digital reading, including the role of refractive status, binocular vision anomalies, accommodative function, and prior exposure to digital devices. Understanding these individual factors may assist clinicians in identifying populations that are more susceptible to reduced reading efficiency or visual discomfort during screen use. In addition, emerging display technologies such as e-ink devices, high-resolution screens, adaptive brightness systems, and blue light modulation features warrant systematic evaluation. Research comparing these technologies with traditional screens and print media may provide valuable insights into design features that support optimal reading speed and visual comfort. Finally, interdisciplinary research integrating optometry, ergonomics, education, and human–computer interaction will be essential for developing evidence-based guidelines aimed at promoting efficient and visually comfortable reading in increasingly digital learning environments.

## CONCLUSION

Reading speed in both print and digital media is influenced by a complex interaction of visual, oculomotor, and ergonomic factors. Differences in display characteristics, accommodative and vergence demands, eye movement behaviour, and viewing conditions contribute to variations in reading efficiency across reading media. Evidence from the reviewed literature suggests that digital reading, particularly during prolonged and uninterrupted use, may place greater visual demands on the visual system and is more commonly associated with visual fatigue and changes in oculomotor performance when compared with print reading. From an optometric perspective, these findings highlight the importance of considering the reading medium when assessing visual performance and managing reading-related symptoms. Appropriate refractive correction, evaluation of binocular and accommodative function, and ergonomic guidance related to viewing distance, posture, lighting, and screen settings are essential to support optimal reading efficiency in modern reading environments. However, variations in study design, outcome measures, and participant characteristics across existing studies indicate that current evidence should be interpreted with caution. Overall, this review underscores the growing relevance of understanding reading speed in the context of increasing reliance on digital devices. Further research using standardized assessment protocols and emerging display technologies is needed to better define the long-term visual implications of digital reading. Such evidence will be valuable in guiding clinical practice, educational strategies, and ergonomic recommendations aimed at promoting visual comfort, reading efficiency, and ocular health in the digital age.

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REVIEW ARTICLE

# Phytochemical Insights and Pharmacological Potential of *Berberis aristata* and *Delphinium denudatum*: A Review

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## ABSTRACT

*Berberis aristata DC* and *Delphinium denudatum Wall*. are two ethnomedicinally important plants whose phytochemical diversity and pharmacological potential have attracted sustained scientific interest. Their diverse phytochemical profile including alkaloids, flavonoids, glycosides and polyphenols contributes wide range of biological activities. *B. aristata* is rich in protoberberine alkaloids such as berberine, palmatine and jatrorrhizine alongside flavonoids and phenolic. These compounds exhibit antioxidant, antimicrobial, antidiabetic and neuroprotective activities aligning with its traditional use in treating inflammation, jaundice, wound healing and eye disorders. *D. denudatum* traditionally employed in neurological disorders contains diterpenoid alkaloids denudatine, condelphine and flavonoid glycosides now being investigated for anticonvulsant, anxiolytic and anti-inflammatory. Comparative analysis highlights both the novelty and translational relevance of these phytochemicals while addressing methodological challenges in reproducible isolation, bioassay alignment and toxicity profiling. In the modern era, phytochemistry inculcated with ethnopharmacological information, this review gives advancement in drug discovery and therapeutic development from *Berberis aristata* and *Delphinium denudatum*.

**Keywords:** *Berberis aristata, Delphinium denudatum*, Ethnopharmacology, Phytochemistry, Neuroprotection

# INTRODUCTION

The Indian medicines cover various traditional systems such as Ayurveda, Siddha and Unani medicine which have been essential for health and wellness for centuries. It promotes holistic healthcare by balancing mind, body and spirit and offers natural remedies derived from plants and herbs. The significance of these ancient practices is underscored in the current century as they address contemporary health challenges which promote preventive care and appeal to a growing demand for natural therapies amidst increasing complications over adverse effects of conventional pharmaceuticals. The growing global acceptance of traditional medicine highlights the crucial role of phytochemistry in modern healthcare. It recognizes the importance of traditional medicine which affirms that a large proportion of the global population depends on herbal and natural products for primary health needs. Inculcating phytochemical research with traditional medical practices not only strengthens the scientific credibility of these systems but also provides valuable opportunities for discovering novel therapeutic agents [1]. Phytochemistry is fundamental key to study and reveal a therapeutic potential of plants used in traditional medicinal systems including Ayurveda and Siddha. The field focuses on the study of bioactive compounds within plants that contribute to their medicinal properties. In the Siddha system for instance, the richness of bioactive compounds in therapeutic roots underlines their multifaceted health benefits that emphasize the importance of plant-based remedies for addressing various health conditions. As a result, traditional Indian medicinal plants are increasingly being explored for their phytochemical richness and scientifically validated pharmacological activities [2]. Among these, *Berberis aristata* and *Delphinium denudatum* hold significant historical and therapeutic importance in Ayurveda and Unani medicine. Their long-standing use for neurological, metabolic, inflammatory and infectious conditions highlights the need for systematic scientific evaluation. Understanding the phytochemistry and pharmacological potential of such classical herbs is essential not only to validate traditional knowledge but also to support the development of evidence-based, affordable and culturally relevant healthcare solutions for this century.

**Multidisciplinary Review of *Berberis Aristata***

*Berberis aristata* commonly called *Daruharidra* have closely resemble to *Curcuma longa* (Turmeric) also known as *Haridra* which may be as per their similar characteristics as shown in Figure 1. Hence, collectively mentioned as *Haridra dvaya* signifying two *Haridras* which is called as *Daruharidra* and *Haridra*. Daruharidra is well known plant in Ayurveda, Charka and Susruta for diverse properties and numerous used to treat diseases. It is referenced in *Vedas*, *Brihattrai* and later it has been described in *Nighantu*. Rasaut, a highly valued preparation derived from *Berberis aristata* plant. It is used for the treatment of conditions such as ocular disorders, jaundice and various skin diseases. The plant itself is considered an important medicinal resource, effective in managing remittent fevers and oxidative stress. It is also used as a gentle cooling laxative for children and serves as a tonic that supports liver and heart health [1], [3]. In Ayurveda, Daruharidra is widely employed for the management of Kamala, Kushtha, Mutrakruccha, Netra Roga, Pandu, Prameha and Pratishyaya. Its clinical and experimental study highlights antioxidative, anti-inflammatory, anticancer, hepatoprotective and immunomodulatory activities. Also its natural source berberine lowers liver hepatocyte inflammation. Additionally, it possesses cholegogue, astringent, hepato-stimulant and hepato-protective qualities among others [4]. The diverse therapeutic applications of daruharidra are presented in Table 1. *Berberis aristata* (Fig.1). Comprehensive ethnomedicinal profile of *Berberis aristata* (Table No. 1)

**Phytochemical Studies**

*Berberis aristata* contains a wide range of chemical constituents throughout its various parts. It is particularly abundant in bioactive compounds especially alkaloids that play a key role in its therapeutic potential. *Berberis aristata* is valued primarily for its high berberine content 2-5% while, palmatine occurs in smaller amounts. The roots of the plant also contain several other important alkaloids, including aromoline, berbamine, columbamine, dehydrocaroline, epiberberine, jatrorhizine, karachine, dihydrokarachine, oxycanthine, oxyberberine, and taximaline whose chemical structures are illustrated in figure 2. Additional compounds such as 1-O-methylpakistanine, pakistanine, pseudoberberine and pseudopalmatine chloride have been identified and isolated as well. Studies indicate that berberine levels vary with altitude with plants from lower elevations showing higher concentrations.

Soil potassium and moisture further influence berberine content [22]. Phytoconstituents of *Berberis aristata* (Fig.2). In research studies, the Phytochemicals have reported in *Berberis aristata* highlighting the prominence of specific bioactive compounds studied across ethnopharmacological and phytochemical investigations. Among these, berberine stands out as the most frequently reported alkaloid which is widely recognized for its potent antimicrobial, antidiabetic, anti-inflammatory and hepatoprotective properties. It acts by disrupting microbial DNA replication, modulating AMP-activated protein kinase (AMPK) pathways and suppressing pro-inflammatory cytokines which made a cornerstone compound in both traditional and modern therapeutic applications. Other than berberine, palmatine and jatrorrhizine are the next most cited constituents. Palmatine exhibits strong antioxidant, antimicrobial and neuroprotective effects while, jatrorrhizine contributes to gut motility regulation and antidiarrheal activity which often used in ayurvedic formulations for treating dysentery and gastrointestinal disorders. Other alkaloids such as berbamine and oxyacanthine are less frequently reported but have shown promising anti-inflammatory and cardioprotective effects. Phytochemical reporting of *Berberis aristata r*esearch (Fig.3). Apart from that, the figure 3 reflects the presence of total phenolics, flavonoids, tannins, saponins and glycosides which also shows promising effects of antioxidant, wound-healing and metabolic regulatory properties. These compounds support traditional uses of *Berberis aristata* in formulations like Rasaut and Unani preparations, where it is applied for skin ulcers, blood purification and liver support. The frequency distribution underscores the pharmacological dominance of berberine while revealing a diverse phytochemical profile that validates the plant's widespread use in Ayurveda, Unani, and folk medicine systems.

**Pharmacological Studies**

**Hepatoprotective activity:**

*B. aristata* roots have long been employed in ayurvedic medicine for managing jaundice and other liver disorders. Modern scientific investigations have validated these traditional claims through extensive clinical studies.

**Hepatoprotection against chemical-induced liver disorders**

**Carbon Tetrachloride ($CCl_4$) Induced Hepatotoxicity:** Aqueous and methanolic extracts of *B. aristata* and isolated berberine were studied and examined in the prevention of carbon tetrachloride induced hepatic damage in rats. All results were comparable to the standard drug silymarin, demonstrating significant reduction in elevated liver enzyme levels and restoration of normal hepatic architecture [23].

**Berberine Chloride Studies:** Berberine chloride was investigated in a dose-response study on Wistar rats with $CCl_4$-induced acute liver damage. When it is given either before or after $CCl_4$ exposure, berberine showed strong preventive and curative effects. It resulted in a dose dependent reduction of Alanine aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline phosphatase (ALP) and bilirubin levels at 5, 10 and 20 mg/kg which improved liver weight and restored normal liver tissue structure. Therefore berberine effectively reversed the biochemical and histological changes caused by $CCl_4$ toxicity.

**Shoot and Fruit Extracts:** Crude extracts prepared from the shoots and fruits of *Berberis aristata* have shown significant protective effects against liver damage caused by paracetamol and $CCl_4$. These extracts help to partially inhibited microsomal drug-metabolizing enzymes, which are responsible for converting these chemicals into toxic metabolites. By inhibiting the formation of these toxic intermediates, the extracts lower oxidative stress and prevent further hepatocellular injury, contributing to hepatoprotective action [24].

**Paracetamol-induced hepatotoxicity:** *Berberis aristata* extracts significantly reduces the rise in liver enzymes caused by paracetamol overdose. Its protective action arises from the inhibition of cytochrome P450 enzymes which prevents the biotransformation of paracetamol into the harmful N-acetyl-p-benzoquinone imine (NAPQI). Limiting the formation of N-acetyl-p-benzoquinone imine (NAPQI), the extract lowers oxidative stress and helps prevent damage and necrosis of liver cells[24].

**Butanolic extract and selective inotropic activity:** The butanolic extract of *B. aristata* demonstrated effective hepatoprotection through a unique mechanism involving selective inotropic activity. This extract showed the ability to modulate cardiac contractility while simultaneously protecting liver tissue, suggesting a multi-organ protective effect [25].

**Antimicrobial activity**

*Berberis aristata* shows potent broad-spectrum antibacterial properties effective against diverse gram-positive and gram-negative pathogens. Studies reported that aqueous root extracts produce inhibition zones of 12–25 mm, while ethyl acetate fractions display the highest potency with MIC values between 0.05–1 mg/ml. Diterpene and flavonoid fractions also exhibit significant antibacterial effect, with MIC ranging from 0.05–5 mg/ml and 0.05–10 mg/ml respectively. Antibacterial mechanism involves direct microbicidal action, disruption of bacterial cell membrane integrity and inhibition of DNA and protein synthesis. Berberine and related alkaloids are contributed as the major active compounds responsible for these effects [26].

**Antidiabetic activity**

*Berberis aristata* shows strong antidiabetic activity through various mechanisms investigated in preclinical and clinical studies. In diabetic rat models, its aqueous and alcoholic root extracts significantly reduced fasting blood glucose levels by modulating key hepatic enzymes which enhances glucokinase and G-6-PD activity while reducing glucose-6-phosphatase; thereby it is improving glucose utilization and lowering hepatic glucose output. Berberine supports glucose regulation by increasing insulin secretion, improving peripheral glucose uptake, reducing gluconeogenesis, activating the AMPK pathway and inhibiting carbohydrate-digesting enzymes notably $\alpha$-amylase and $\alpha$-glucosidase. Additionally, plant exhibits significant hypolipidemic effect, including reduced total cholesterol, LDL and triglycerides with elevated levels of HDL. These significant effects are mediated by inhibition of HMG-CoA reductase, enhanced LDL receptor expression, increased bile acid excretion and modulation of lipid-metabolism genes which shows cardiometabolic protective potential [27].

**Anti-inflammatory activity**

*Berberis aristata* exhibits notable anti-inflammatory effects in both topical and systemic models. When applied topically, both the ethanol root-bark extract and 4% formulation effectively reduced ear edema induced by croton oil and paw edema induced by carrageenan effectively at higher doses. Topical application of its ethanol root-bark extract and a 4% formulation significantly reduced croton oil-induced ear edema and carrageenan-induced paw edema in a dose-dependent manner. Systemically, the extract led to marked suppression of key pro-inflammatory cytokines like Interleukin-6 (IL-6), Interleukin-1-beta (IL-1β), and Tumor necrosis factor alpha (TNF-$\alpha$) thereby reduced inflammatory cell infiltration and decreased cytokine production. These results support its strong potential as a natural anti-inflammatory agent [28].

**Antioxidant activity**

The methanolic stem extract of *Berberis aristata* exhibits strong free-radical scavenging activity; it showed DPPH results comparable to ascorbic acid and effectively neutralizing hydrogen peroxide. Isolated berberine significantly shows dose-dependent antioxidant effects. The extract also suppresses lipid peroxidation by lowering malondialdehyde (MDA) levels and protects membrane lipids from oxidative injury and supporting normal antioxidant enzyme function [29].

**Anticancer activity**

The hydroethanolic extract of *Berberis aristata* DC was evaluated for its anticancer potential in A549 human lung carcinoma cells. Cell-viability and cellular cytotoxic studies were conducted and the crude extract produced an $IC_{50}$ value $47.84 \pm 0.49$ µg/ml, indicating strong anticancer activity. The treated cells also displayed noticeable morphological changes consistent with cytotoxic effects. Results from the MTT assay further confirmed that there is marked reduction in A549 cell viability following extract treatment supporting its potential as a promising natural anticancer agent [30].

**Neuroprotective potential**

Although direct neuroprotective studies on *Berberis aristata* itself are limited, its major bioactive compound berberine has shown promising neuroprotective effects in various independent studies. Berberine helps to protect neuronal cells by reducing oxidative stress, lowering inflammation within the central nervous system and supporting mechanisms that counteract neurodegenerative processes. However, evidence specifically linking *B. aristata* extracts to neuroprotection remains insufficient and further targeted studies are needed.

**Anti-obesity effects**

*B. aristata* have potential metabolic benefits in obesity management, though lipid-lowering Berberine separately has shown anti-obesity properties.

**Cardioprotective effects**

It may offer several cardiovascular benefits. Its lipid-lowering effects can help reduce atherosclerotic risk, while its strong antioxidant activity protects cardiac tissues from oxidative damage. By exerting anti-inflammatory effect, plant is used to reduce inflammation in blood vessel. Berberine one of its key alkaloids has shown potential antiarrhythmic effects in separate studies**.**

**Multidisciplinary Review of *Delphinium Denudatum Wall.***

The genus Delphinium comprises approximately 300-400 species which is distributed worldwide recognized for their medicinal and toxic properties. *Delphinium* species (commonly known as larkspurs) are seasonal or recurrent, robust and vertical showy herbs cultivated for attractive flowers. Several species including *Delphinium ajacis*, *D. consolida*, *D. elatum*, *D. grandiflorum*, *D. laxiflorum*, *D. montanum*, *D. palmatifidum*, *D. peregrinum*, *D. descens*, *D. staphisagria,* and *D. triste* are used. Among *Delphinium ajacis* and *Delphinium denudatum* species found in India, *Delphinium denudatum Wall*. (family Ranunculaceae) is the one most widely used for medicinal purposes [31]. The generic name Jadwar comes from a Greek word meaning "dolphin," referring to the flower's nectar spur that look like the shape of a dolphin. The Jadwar is the Arabic form of the Persian word Zadwar, meaning "the great purifier" or "antidote." The Persian name Mah-Parvin (meaning "moon and Pleiades") is believed to refer to the plant's flowering season, which begins when the Pleiades rise in early summer. In India, the plant became known as Narbasi or Nirbisi because of strong antitoxic effect. Nir means "to oppose or remove," and Bisi refers to Bis or Vish (poison). The term Bis in Nirbisi is also associated with Bish (aconite), as Jadwar is traditionally regarded as an antidote to aconite poisoning [32]. The plant is known by various names across traditional systems and languages. In Arabic it is called Antila Saudavi, Baloot-el-Ardh, and Zhadvar. In Ayurveda, it is known as Nirvishaa and Nirvishi. English names include Larkspur and Blood-veined sage. In Greek, it is referred to as Sáturyús, while in Nepali it is known as Nilobikh. Hindi names include Bas'ha, Nirbasi, and Tarbasi. In Marathi it is called Jadwár or Nirbishi, and in Gujarati Nirvishi. The Persian names are Maah Parveen and Zadwár. In Sanskrit, it is known as Jadavâr, Nirvishi and Vishalakarani. In Urdu, it is called Jadwár and in unani medicine it is referred to as Satriyoos, Jadwaar Khataai and Maatiryaaq [33-36].

*Delphinium denudatum wall* (Fig.4). Jadwar is an herbal drug obtained from the dried tuberous roots of *Delphinium denudatum* Wall. a member of the *Ranunculaceae* family. The plant is an annual herb with a smooth to slightly hairy surface and is primarily distributed across the Western Himalayas, from Kumaon to Kashmir. It typically grows on grassy mountain slopes at elevations ranging from 3,000 to 4,500 meters. Its root is dark brownish-black in color. It measures approximately 2–6 cm in length and 1–2 cm in width at the crown. The structure is conical, extremely hard and enveloped with suberized metaderm bearing different small circular scars left by detached lateral rootlets. Its surface shows distinct longitudinal wrinkles and it produces a short and brittle fracture once broken. The root is easily recognized by its characteristic odor and distinctly bitter taste. The rhizome is blackish-brown in color shows distinct longitudinal wrinkles on its surface, along with a pattern of many small rounded scars that mark the points where lateral roots once emerged. A scaly leaf bud is present at the crown. The plant bears a few scattered pale-blue flowers and its seeds are small and endospermic. Externally, the rhizome is enveloped via a suberized metaderm made up of brown tubules arranged irregularly. The root parenchyma contains abundant starch grains found in

clusters while tissue lacks cork cells, calcium oxalate crystals and fibers as depicted in figure 4. Powdered drug is light yellow and turns yellowish-green when treated with methanolic NaOH and viewed under ultraviolet light. The aqueous extract contains 13.74 ± 0.54% reducing sugars. Microscopically, the powder consists mainly of fragments of xylem vessels with scleriform thickenings, some parenchymatous cells, numerous rounded starch grains and a few prismatic crystals. The powder is moderately fine in texture, grey to ash-colored and has a very bitter taste with no significant odor [37]. *Delphinium denudatum Wall.* recognised as Jadwar in Unani medicine and Nirvisha in Ayurveda, is highly valued in traditional healing systems for its antidotal, neuroprotective, and restorative properties. In Unani medicine, it is a key ingredient in formulations such as Habbe-Jadwar for neurasthenia, sexual debility and chronic fatigue; Khamira Gaozaban Ambari Jawaharwala Ood-e-Saleeb Wala for cardiac weakness and palpitations; and Marham-e-Jadwar for ulcers, wounds and lymphadenitis. Other preparations like Jawhar Mohra, Habb-e-Jawahar and Zimad-e-Warm-e-Lauzatain use Jadwar for its cardiotonic, sedative and anti-inflammatory effects. In Ayurveda, Nirvisha is included in Vishaghna (anti-toxic) formulations and is used for neurological disorders, chronic pain, skin infections and respiratory issues due to its antimicrobial and nervine tonic actions. It is also incorporated into rejuvenate preparations and herbal oils for wound healing and scalp health. Table 2 provides summary of ethnomedicinal application of *delphinium denudatum* across different traditional medicinal system. Comprehensive ethnomedicinal profile of *Delphinium denudatum* (Table No. 2).

**Phytochemical Studies**

The phytochemical profile of *Delphinium denudatum Wall*. has been widely investigated, revealing a rich bioactive constituent. Early studies report the presence of multiple phytochemical classes, including alkaloids, flavonoids, saponins, steroids, glycosides, tannin and various phenolic compounds. Notably, the plant is particularly rich in diterpenoid alkaloids with various C19 and C20 diterpenoid alkaloids, which exhibit a wide range of biological activities. Even flavonoids important phytochemicals in *D. denudatum.* The presence of flavonoid glycosides, such as rutin and quercetin has been documented which highlights their antioxidant and anti-inflammatory effects. Phenolic compounds including various benzoic and phenylacetic acid derivatives have also been identified in *D. denudatum*. The majority of compound possesses simple molecular structure and broadly distributed in the plant kingdom. The presence of unique phenolic structures like oxformasine and benzoxepine derivative has been noted which indicates the chemical diversity within this species [38-39]. The molecular structures of key compounds are given in figure 5. Phytoconstituents of *Delphinium denudatum* (Fig. 5). The figure.6 presents the relative frequency with which major phytochemicals of *Delphinium denudatum* are reported which are highlighting the dominance of its characteristic diterpenoid alkaloids. Denudatine, delphinine, jadwarine and ajaconine appear most promising role in neuroactive and antitoxic properties described in pharmacognostic and Unani literature. Some Moderately reported constituents such as denudatin and magnoflorine contribute to antioxidant and adaptogenic effects while, phenolics, flavonoids, tannins and glycosides are less frequently cited but consistently associated with supportive antioxidant and wound-healing activities. Overall, the distribution underscores the chemical profile of *D. denudatum* as alkaloid-rich aligning with its traditional use in neurological, inflammatory and toxicological conditions. Phytochemical reporting in *Delphinium denudatum* research (Fig.6).

**Pharmacological Studies**

**Antioxidant activity**

*Delphinium denudatum (DD)* shows significant antioxidant potential across various extracts. It is reported that the water extract showed significant antioxidant potency which exhibits 83.38% DPPH free radical inhibition compared to 92.67% by standard ascorbic acid and protected against cisplatin-induced oxidative stress in animal model suggested that its usefulness in nephrotoxin-induced renal injury. Other studies further supports the antioxidant capacity of DD showed that its ethanolic extract contains DPPH and superoxide-scavenging compounds, while the ethyl acetate extract primarily scavenges hydroxyl radicals. β-sitosterol, a major phytosterol isolated from DD roots exhibits notable free radical-scavenging activity. Additionally, the chloroform extract of DD roots demonstrated even higher free radicle scavenging activity which showed 86.56% comparable to 82.77% for ascorbic acid. The presence of flavonoids and other phytochemicals may enhances its antioxidant, anti-carcinogenic and anti-atherogenic properties

indicating that various DD extracts may have therapeutic potential including significant action in preventing oxidative damage and supporting cancer treatment [52].

**Antimicrobial activity**

The antimicrobial property of different parts of *Delphinium denudatum (DD)* has been studied in various solvents. Among these, ethanolic extract of stem demonstrated the strongest antibacterial activity exhibited 15.33 ± 0.11 mm against *Serratia marcescens*, while the leaf extract showed the greatest antiactinobacterial activity 21.0 ± 0.07 mm against *Nocardia tenirefensis*. Beyond these findings, *Delphinium denudatum* has traditionally been used for intestinal helmintheasis and reducing fluid retention. Its ethanolic root extracts have shown both antibacterial and antifungal properties which largely attributed to natural alkaloids. Newly identified diterpenoid alkaloids such as 8-acetylheterophyllisine, panicutine and vilmorrianone demonstrated antifungal activity against several human pathogenic fungi. Additionally, Biological synthesis of silver nanoparticles using aqueous root extract of DD has produced strong antibacterial effects against *Escherichia coli, Bacillus cereus Staphylococcus aureus* and *Pseudomonas aeruginosa* along with larvicidal activity against Aedes aegypti [53].

**Hepatoprotective activity**

In these experiments, rats were administered alcohol 3.75 g/kg daily for ten consecutive days per hour following extract administration. The protective effects were evaluated using biochemical markers. Co-administration of DD extract at a graded doses of 100, 200, 400 mg/kg notably improved alcohol-induced alterations in cholesterol levels, total and direct bilirubin levels, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP) while also preventing alcohol-induced lipid peroxidation. Among the tested doses, 400 mg/kg produced the most pronounced hepatoprotective effect comparable to the standard drug silymarin. It was concluded that DD treatment restored altered biochemical parameters in a dose-dependent manner suggesting strong potential for its use in managing alcohol-induced liver dysfunction [54].

**Nephroprotective activity**

*In-vitro* study of aqueous root extract of *Delphinium denudatum (DD*) conducted using the GGT assay which showed that the extract had an $IC_{50}$ value 189.3 μg/ml compared to the standard nephroprotective drug mesna having $IC_{50}$ 61.29 μg/ml. In *in-vivo* study, nephrotoxicity was induced in rats using cisplatin (5 mg/kg BW) and DD extract was administered at doses of 300, 600 and 800 mg/kg. The treatment produced considerable dose-dependent reduction in elevated blood uric acid, urea and serum creatinine along with marked improvement in histopathological alterations caused by cisplatin. The findings indicates aqueous root extract of *Delphinium denudatum* possesses notable renoprotective activity and may serve as a promising therapeutic option for managing nephrotoxin-induced renal injury particularly that caused by cisplatin [55].

**Cardiovascular activity**

The cardiovascular effects of the aqueous extract of *Delphinium denudatum* (DD) produced both inotropic effects and chronotropic on normal and hypodynamic frog hearts indicating its influence on heart rate and contractility. In dogs, the extract initially caused tachycardia followed by bradycardia; however, the appearance of marked tachycardia after atropinisation suggested that the bradycardia was mediated through a reflex mechanism. Furthermore, the cardiovascular effects of the extract were blocked by both α- and β-adrenergic blockers demonstrating that its action involves these receptor pathways. Blood pressure studies in dogs also confirmed that the extract interacts directly with α and β receptors showing significant impact on the cardiovascular system [56].

**Anticancer effects**

Several plant species traditionally used as anticancer remedies have been evaluated for their antiproliferative activity including *Delphinium denudatum Wall*. whose various extracts demonstrate notable antitumor potential. In an *in-vitro* assessment of 44 extracts from 16 medicinal plants on human MCF-7, epithelial type-2 and Vero cell lines. DD extracts showed promising activity likely due to their strong antioxidant properties. Growing interest in herbal medicines among cancer patients further highlights the relevance of such findings. Specifically, the chloroform and

methanolic extract of DD roots and aerial parts exhibited selective cytotoxicity against leukemia, ovarian and breast cancer cell lines with inhibition rates ranging from 50% to 100%. Additionally, a novel sulfated derivative of quercetin one of the flavonoid constituents of DD demonstrated potent antitumor effects via a reactive oxygen species induced apoptosis which points to its potential as a precursor for developing new anticancer agents [57].

**Neurologic and Psychiatric Effects**

Current studies on *Delphinium denudatum* (Jadwar) highlighting its potential as a neuroprotective and neuroactive medicinal plant. In Parkinson's disease models, its extract significantly protected dopaminergic neurons by restoring dopamine levels, normalizing key antioxidant enzymes like catalase, superoxide dismutase and regulating D2 receptors after 6-OHDA–induced injury. Compounds like isotalatazidine hydrate also exhibit strong dual cholinesterase-inhibitory activity suggesting therapeutic relevance in Alzheimer's disease. Multiple studies confirm potent anticonvulsant properties of its aqueous extracts which effectively suppressed seizures in various experimental models showing comparable results to standard antiepileptic drugs like phenytoin and valproic acid. Additionally, both ethanolic and aqueous extracts demonstrated anxiolytic effects in behavioural models while the aqueous extract produced notable central depressant activity by enhancing hypnosis and reducing locomotor activity. *Delphinium denudatum* also exhibits strong antinociceptive effects in thermal and chemical pain models acting through non-opioid pathways. Collectively, these findings indicate that *Delphinium denudatum* possesses diverse neurologic and psychiatric activities including neuroprotection, anticonvulsant, anxiolytic, central depressant and analgesic effects supporting its traditional use and potential therapeutic value in managing various CNS disorders [58].

## DISCUSSION

The ethnopharmacological exploration of *Berberis aristata* and *Delphinium denudatum* understands their significance as medicinal plants with diverse phytochemical compositions and broad pharmacological relevance. Within this context, *Delphinium denudatum* demonstrates a stronger emphasis on antidiabetic and neuroprotective activities supported by moderate antimicrobial, anti-inflammatory and antioxidant roles which is reflecting its alkaloid-rich profile. In contrast, *Berberis aristata* exhibits pronounced antimicrobial and antioxidant strengths along with moderate effects in anti-inflammatory, antidiabetic and neuroprotective domains consistent with its more diverse phytochemical base. As per figure 7, these overlapping yet distinct pharmacological strengths highlight complementary therapeutic potential suggesting that *Delphinium denudatum* may be more promising for metabolic and neurological disorders while *Berberis aristata* excels in infection control and oxidative stress management. Comparative pharmacological analysis (Fig.7). Together, the accumulated phytochemical and pharmacological evidence emphasize the importance of integrating traditional knowledge with modern experimental validation particularly through expanded mechanistic and clinical studies to fully realize their translational potential in evidence-based medicine.

## CONCLUSION

*Berberis aristata* (Daruharidra) and *Delphinium denudatum* (Jadwar) are two important traditional medicinal plants whose therapeutic relevance is strongly supported by both classical literature and modern scientific findings. *Berberis aristata* rich in berberine and other alkaloids contributes to its significant anticancer, antidiabetic, anti-inflammatory, antimicrobial, antioxidant and hepatoprotective, activities validate traditional ayurvedic application for liver disorders, metabolic diseases and systemic inflammation. Whereas, *Delphinium denudatum* widely used in Unani medicine contains diverse bioactive constituents that contribute to its antioxidant, anticonvulsant, antimicrobial, anxiolytic, neuroprotective and de-addiction properties support its traditional role in neurological, psychiatric and toxicological conditions. Together, these plants highlight the therapeutic depth of Indian traditional medicine and offer strong potential for modern evidence-based applications. However, further research is essential to clarify their

mechanisms of action, identify key active compounds and establish standardized clinical protocols to ensure safe, effective and scientifically validated use in modern healthcare.

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**Table No. 1: Comprehensive ethnomedicinal profile of *Berberisaristata***

| System/ Culture | Plant Part/ Extract | Therapeutic Use | Pharmacological action | Mechanism | Contradication | References |
|---|---|---|---|---|---|---|
| Ancient Egyptians | Whole plant/ Crude herbal | Plague prevention | Antimicrobial | Berberine disrupts microbial growth | Pregnancy caution | [5-8] |
| Ayurveda (India) | Root, stem/ Decoction | Dysentery | Antidiarrheal | Reduces gut motility, antimicrobial | Avoid in severe dehydration | [9-12] |
| European Herbalists | Plant extract/ Infusion | Liver and gallbladder disorders | Hepatoprotective, cholagogue | Stimulates bile flow | Avoid in biliary obstruction | [5], [13-15] |
| Russian Medicine | Plant extract/ Herbal preparation | Inflammation High B.P. Abnormal uterine bleeding | Anti-inflammatory Antihypertensive Hemostatic | Inhibits inflammatory mediators, Vasodilation, Uterine contraction | None major, Hypotension, Avoid in Pregnancy | [5], [7], [16-17] |
| Traditional India | Extract/ Rasaut | Aphthous sores, Abrasions, Skin ulceration | Antiseptic, Healing | Kills oral microbes, Prevents infection, Tissue regeneration | None | [9-11], [18] |
| Unani Medicine | Whole plant/ Unani formulation | Leprosy | Antimycobacterial | Berberine inhibits mycobacteria | Pregnancy | [6], [8], [16], [19] |
| Folk Medicine | Root/ Decoction | Skin diseases | Blood purifier | Detoxification, Improves metabolism | None | [12-13], [ 20-21] |

**Table No. 2: Comprehensive ethnomedicinal profile of *Delphinium denudatum***

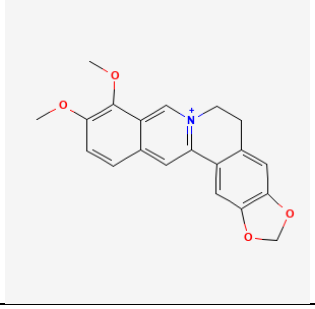
| System / Culture | Plant Part / Extract | Therapeutic Use | Pharmacological Action | Mechanism | Contraindication | References |
|---|---|---|---|---|---|---|
| Ayurveda (India) | Root / Powder, Decoction | Cough, cold, wounds, convulsions | Antioxidant, anticonvulsant | Reduces neuronal excitability; promotes healing | Avoid in pregnancy; toxicity at high doses | [40-43] |
| Unani Medicine | Root / Jadwar formulation | Antidote, nerve tonic, cardiotonic | Antitoxic, neuroprotective, cardioprotective | Neutralizes toxins; strengthens nerves and heart | Avoid in pregnancy; caution in cardiac disorders | [44-46] |
| Traditional Persian and Central Asian Medicine | Root / Crude extract | Snake bite, insect bite, spider bite | Antitoxic, anti-inflammatory | Reduces venom-induced inflammation | Toxic in high doses; avoid in children | [47-48] |
| Folk | Root / | Fever, pain, | Analgesic, | Inhibits | Dose-dependent | [42], [49- |

| | | | | | | |
|---|---|---|---|---|---|---|
| Medicine (India and Himalayas) | Decoction | inflammation | antipyretic, anti-inflammatory | inflammatory mediators | toxicity possible | 50] |
| Tibetan Medicine | Root / Powder | Anxiety, mental disorders, nervous debility | Neurocalming, adaptogenic | Modulates neurotransmitters | Avoid in pregnancy | [48], [51] |
| Modern Herbal Practice | Ethanolic extract / Root | Epilepsy, neuropathic pain | Anticonvulsant, neuroprotective | Modulates GABAergic pathways | Avoid with sedatives; toxicity at high doses | [41-42], [49] |

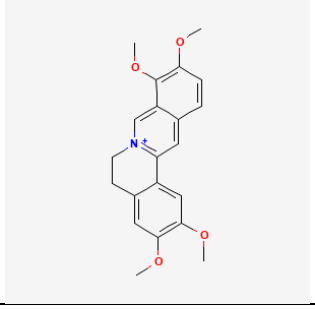




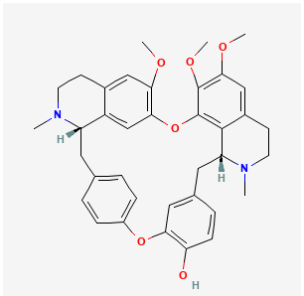
**Fig.1:** ***Berberis aristata***



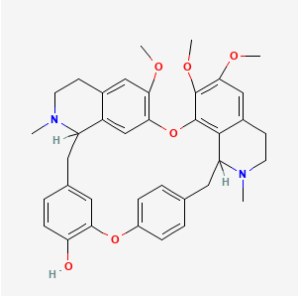
**Berberine**



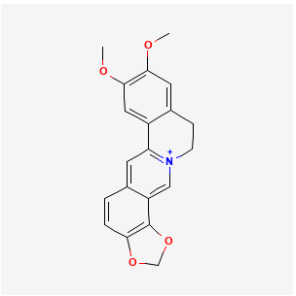
**Palmatine**



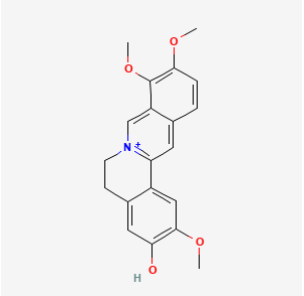
**Berbamine**



**Oxycanthine**



**Epiberberine**



**Jatrorhizine**

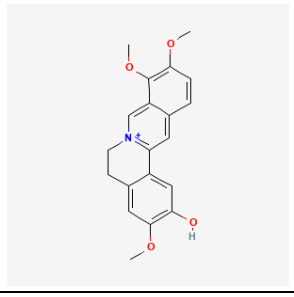
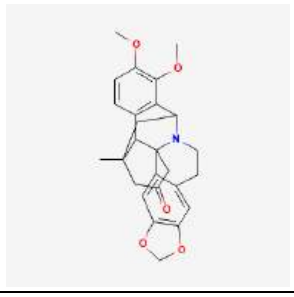
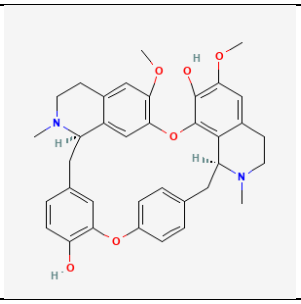
|  |  |  |
|---|---|---|
| **Columbamine** | **Karachine** | **Aromoline** |

**Fig. 2: Phytoconstituents of *Berberisaristata***

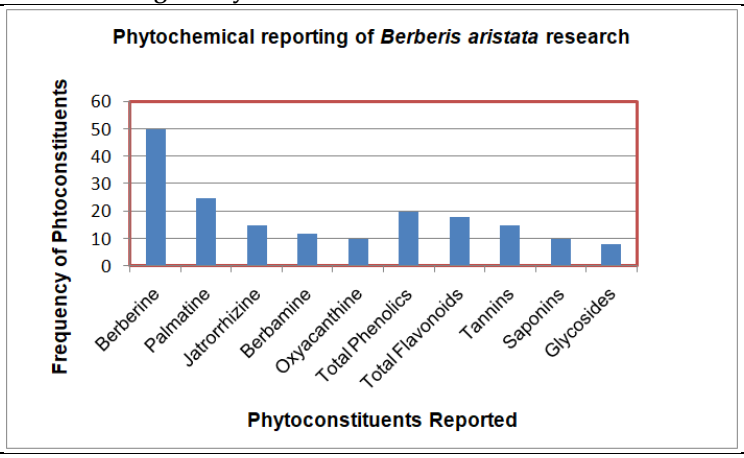
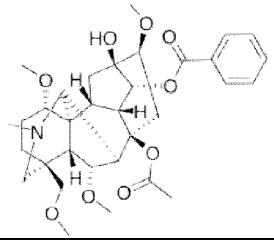
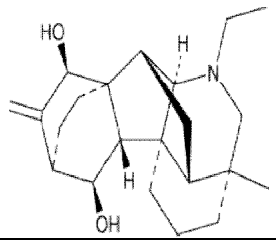
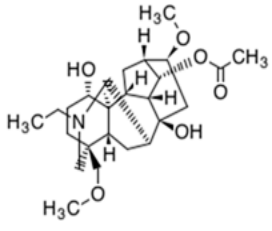
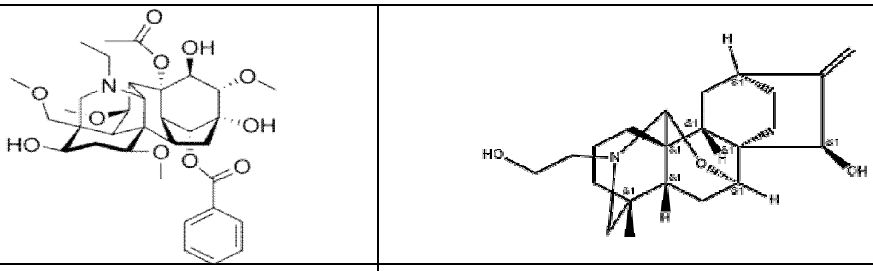



**Fig.3: Phytochemicalreporting of *Berberis aristata* research**



**Fig.4: *Delphinium denudatum wall.***

|  |  |  |
|---|---|---|
| **Delphinine** | **Denudatine** | **Condelphine** |



**Aconitine** **Ajaconine**

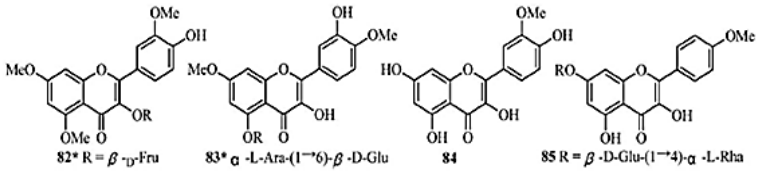



**Fig. 5: Phytoconstituents of *Delphinium denudatum***

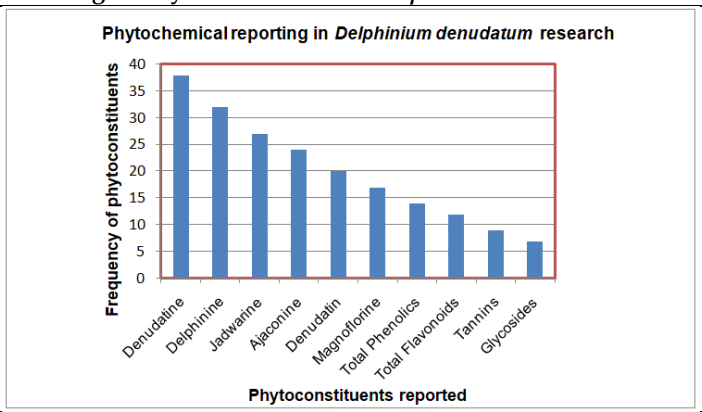



**Fig.6: Phytochemical reporting in *Delphinium denudatum* research**

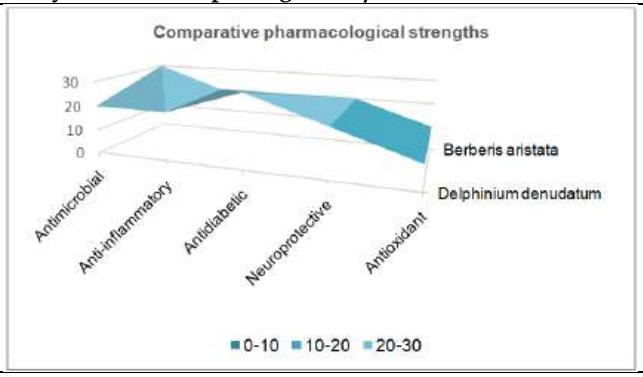



**Fig.7: Comparative pharmacological analysis**

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REVIEW ARTICLE

# Microneedle Technology for Drug Delivery: Current Advances and Future Prospects

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## ABSTRACT

Microneedle technology offers an improved method for delivering drugs. They are emerging as valuable assets in the biomedical field, providing a minimally invasive and effective approach for a range of uses, including drug delivery, cell transfection, and biosensing. Different types of microneedles exist, such as solid, hollow, dissolving, coated, and hydrogel-forming microneedles. Microneedles may be fabricated from silicon, metals, ceramics, and synthetic and biodegradable polymers, including carbohydrates. Microneedles (MNs) create microchannels in the skin by disrupting its structure, weakening its barrier function, and allowing drugs to penetrate more easily, thereby aiding the delivery of therapeutic agents. Transcutaneous medication delivery via a microneedle patch offers advantages over traditional methods of drug administration. To successfully implement microneedle technology, factors such as biocompatibility, drug stability, safety, and production costs must be considered. This review examines how microneedle systems have evolved over the past decade, highlighting their innovations, benefits, and applications in diagnostics and treatment. These advancements aim to improve drug delivery and theranostics by enabling controlled release, better tissue penetration, and more effective treatment outcomes.

**Keywords:** Microneedle technology, Transdermal drug delivery, Minimally invasive drug delivery, Controlled release, Biodegradable polymers, Therapeutics and diagnostics.

# INTRODUCTION

The stratum corneum (SC) plays an essential role by covering the surface of the epidermis, acting as the main protective barrier against exogenous molecules, including pathogens, toxic chemicals, or UV light, thereby limiting transepidermal water loss (TEWL). (Proksch E. *et al.,*2008). Drug delivery systems (DDSs) are developed to deliver the correct amount of medication to specific target sites in the body while ensuring consistent and effective drug concentrations. Controlled drug delivery can be effectively achieved using microneedle transdermal patches. (Wong WF. *etal.,*2023). Microneedle technology is a non-invasive delivery method that is a preferable alternative to parenteral administration, as it punctures the active cuticle and living epidermis without injuring nerve endings or blood vessels. (Chen J. *et al.,*2017). Microneedle therapy may offer several advantages, such as preventing liver metabolism, reducing the harmful effects of oral drugs on the digestive tract, achieving continuous and sustained drug release, allows for the rapid interruption of drug delivery in the event of toxicity, thereby enhancing safety. (Rad ZF. *et al.,*2021). Delaying the release of the drug and controlling its distribution is also a fascinating way to target the drug. (Nazary Abrbekoh F. *et al.,*2022).Drugs can be administered via various routes such as parenteral, ocular, transdermal, and others, like nasal, pulmonary, and buccal routes. Oral drug delivery has benefits such as better patient compliance, painlessness, a large absorption area, low cost, and ease of drug release in the stomach and intestines. However, there are drawbacks, such as drug breakdown in the stomach, high first-pass metabolism, low absorption, local discomfort, and environmental changes in the gastrointestinal tract, such as pH variations. (Xie Y. *et al.,*2005).The parenteral route uses hypodermic needles to give the drug and is accepted around the world. It provides benefits such as fast action, accurate medication delivery, and ongoing drug infusion. Conversely, it has disadvantages, such as being uncomfortable and painful, posing a risk of opportunistic infections, and requiring highly skilled operators. (HA AKL LL.*et al.,*1992). (Simonsen L. *et al.,*1999). Microneedles are created to penetrate the skin epidermis to a depth of 70 to 200 mm. (Hardy JG. *et al.,* 2016). To make such needles on silicon wafers, thousands of them can be made on a single wafer, since their size is relatively small. This approach results in high accuracy, good reproducibility, and a moderate price for manufacturing. (Wilke N. *et al.,* 2005). Hollow micro-needles can be used to extract fluids from the body for analysis, such as blood glucose measurements, and to deliver microliter volumes of insulin or other drugs as needed. Very small micro-needles can deliver drugs directly to individual cells. (Microneedles: Report Describes Progress, 2003).

**Types of Microneedles**

The studies on microneedles mainly focus on building a rapid, effective and safe practical method of drug delivery. Microneedle types include solid microneedles, coated microneedles, hollow microneedles, and hydrogel-forming microneedles, among many others for drug delivery(Zhang Y. *et al.,* 2017), as illustrated in Figure 1.

**Solid Microneedles**

Solid microneedles are among the simplest structures in design. Made entirely of solid material, they do not contain medications or any additives. Their main purpose is to prepare the skin by creating tiny openings in the stratum corneum and epidermis. These small holes temporarily disrupt the skin's outer layer without penetrating deeply enough to cause pain or bleeding. After the skin is punctured with these solid microneedles, a drug formulation-such as a gel, cream, or patch, is applied to the treated skin area. This allows the drug to easily diffuse through the microchannels into the dermal capillaries, significantly enhancing drug permeability and systemic absorption.(Haq MI. *et al.,* 2009). By passing the dense, protective stratum corneum, this method increases bioavailability, especially for molecules that typically have poor skin penetration. Solid microneedles are commonly fabricated from silicon, stainless steel, or polymer. However, for commercial and clinical use, disposable versions are favored to ensure hygiene and prevent cross-contamination.

**Coated Microneedles**

Coated microneedles are an evolution of microneedle technology where the drug is coated onto the surface of a solid microneedle. When they penetrate the skin, the drug dissolves and diffuses into deeper tissues. (Haj-Ahmad R. *et al.,*

2015). This technique offers fast drug delivery and onset, which is suitable for cases requiring immediate therapeutic responses. The coating process usually uses dip-coating, where microneedles are submerged in a solution that contains the drug. (Ali M. *et al.*, 2024). By adjusting how deep the microneedles are dipped and for how long, the quantity of drug applied to each needle can be controlled. It is important, however, to achieve a consistent coating to ensure precise drug delivery. The mixture must be thick enough to create a stable layer on the surface of the microneedle and remain attached during storage and when the needle is inserted into the skin. Moreover, the drug should be concentrated near the tip of the microneedle, as this part is the first to come into contact with the skin, which helps improve the effectiveness of drug delivery. Although coated microneedles are effective, they have limitations in terms of how much drug they can hold, particularly for treatments that need larger amounts of medication. Using multiple coating layers can increase the total drug content, but this method also raises the complexity of the process and the amount of materials used. (Tang ESK. *et al.*, 2005).

**Dissolving Microneedles**

Dissolving Microneedles are an advanced drug delivery technology that is constructed entirely from biodegradable or water-soluble materials which encapsulate the active drug substance. These microneedles are formulated to totally dissolve in the skin generating zero sharps waste and reducing potential for needle reuse and injury. They are commonly prepared by solvent casting technique in which the drug-polymer blend is poured onto a mold and left until solidifies. There are many common polymers including, for example, carboxymethyl cellulose (CMC), methyl cellulose and sugars such as trehalose and sucrose that are nontoxic/biocompatible and capable of forming solid structures containing drugs. (Encyclopedia of biomedical polymers,2015). In these microneedles, the portion that goes into the skin first contains the drug and must be sturdy enough to penetrate but not break. It must also have low viscosity, to flow into the mold and not leave air bubbles. The portion of the microneedle that remains above the skin may be free from drug and can be made of a less strong or water-insoluble material which aids in maintaining the position of the microneedle upon application. Recent advancements have focused on decreasing the time microneedle patches remain on the skin by making it easier to separate the tips from the base once they are inserted. Some studies have created microneedles that detach immediately when inserted, which helps reduce pain. However, these designs have challenges when larger amounts of medication are required, and ongoing research is working to improve the ability to hold more drug within dissolving microneedles without weakening their structure. (Liu Y. *et al.*, 2024).

**Hollow Microneedles**

The medication is already loaded into the hollow microneedle body, which has a hole at the tip, and is pressurized using a syringe or micropump before being injected into the skin. (Silalahi AA. *et al.*, 2018). Hollow microneedles offer the largest single infusion volume and the most accurate dosing compared to other types of microneedles. Additionally, the injection speed can be easily adjusted, similar to traditional injections. However, manufacturing hollow microneedles is costly and comes with certain drawbacks. For example, the pinhole in the needle can be easily obstructed by skin tissue if the needle is at an improper angle. Moreover, the physical strength of these needles is limited, which can result in breakage and leave parts of the needle embedded in the skin after injection (Waghule T. *et al.*, 2019).

**Hydrogel-forming Microneedles**

Hydrogel microneedles are made from crosslinked hydrophilic polymers that swell when exposed to skin fluids but do not dissolve. (Donnelly RF. *et al.*, 2014). These systems are designed for sustained drug delivery, as the drug can be incorporated throughout the microneedle tip, base substrate, and even the backing patch. Once inserted into the skin, these microneedles absorb moisture, swell, and create a continuous pathway through which drugs can gradually diffuse into the skin. Due to their hydrogel composition, they are especially suitable for delivering larger doses of medication over extended periods, which is beneficial for treating chronic conditions that require long-term administration. The main advantage of hydrogel microneedles is their high drug-carrying capacity and sustained release profile. However, they necessitate longer patch-wearing times because of the slow drug diffusion rate (Parhi

R. *et al*., 2022). Additionally, optimizing formulation stability and mechanical robustness during insertion is crucial for ensuring effective performance.

**Material Selection for Microneedles**

The materials used in creating the microneedles must be biocompatible and biodegradable, as required by their intended application. They should also have enough mechanical strength to effectively penetrate the skin while ensuring no adverse reactions occur. Commonly used materials include inorganic materials, polymers, and metals. (Waghule T. *et al*., 2019).

**Inorganic materials**

Solid microneedles are created using inorganic substances such as glass, silicon, and ceramics. Silicon is frequently selected for microneedle production due to its ability to be formed into various shapes and sizes. In reality, the earliest recognized microneedle was constructed from silicon. Nonetheless, silicon exhibits limited biocompatibility, rendering it less appropriate for biomedical applications. (Larrañeta E. *et al*., 2016).Researchers have explored ceramic materials for microneedles as an alternative due to their biocompatibility and strong mechanical properties. Ceramics utilized for this purpose encompass alumina, calcium phosphate, and calcium sulfate. (Vallhov H. *et al*., 2018). Additionally, biodegradable bioceramics are utilized for microneedles due to their robust mechanical properties and ability to accurately deliver drugs. Glass is not typically utilized for microneedles, unlike silicon, due to its fragility. (He X. *et al*., 2019).

**Polymers**

Water-soluble, biocompatible, and mechanically strong polymers are suitable for making microneedles that can be safely inserted into the skin. The most commonly used method for manufacturing polymer microneedles is solvent casting. This process involves creating an inverse mold that matches the shape of the microneedle structure. The mold is then coated with a polymer solution, allowed to dry or cure, and finally removed.(Joshi N. *et al*., 2023). The solvent-casting technique enables the production of dissolving or hydrogel-based microneedles using various polymers such as hydroxypropyl methylcellulose, hyaluronic acid, polyvinyl pyrrolidone, and poly lactic-co-glycolic acid (PLGA). (Ahmed Saeed AL. *et al*., 2020).

**Metals**

Metal substances have significant tensile and mechanical strength, enabling them to easily penetrate the skin. They are used to create solid, coated, and hollow microneedles. Typically, titanium (Ti) and stainless steel are the two metals most commonly utilized in microneedles. Although stainless steel is the preferred choice for making microneedles, it wears out more quickly than titanium alloy. While Ti alloys cost more than stainless steel, they provide superior mechanical strength. (Jalwal P. *et al*., 2010). Some metal microneedles have been demonstrated to provoke inflammatory responses, and manufacturing these materials is likewise a complicated procedure.(Liu R. *et al*., 2023).

**Microneedle Fabrication Methods**

Several approaches are available for making microneedles. This section outlines the primary methods used to fabricate microneedles, including both direct production techniques and those that use molds. One of the most common methods for producing microneedles at a lower cost and in larger quantities is mold-assisted manufacturing. The main techniques for creating microneedles are presented in Table 1(Oliveira C. *et al*., 2024).

**Evaluation of microneedles**

**Mechanical properties**

A microneedle must be durable enough to remain whole inside the skin while also being sharp and slim enough to easily pierce the skin. Two critical elements for a safe and efficient microneedle design are the insertion force and the force at which the microneedle compromises its structural integrity. The proportion of these two forces is referred to as the "safety factor." The proportion needs to be maximized. (O'Mahony C. *et al*., 2014)

**Margin of safety**

A few researchers characterized the margin of safety as the ratio between the force needed to penetrate the stratum corneum and the force at which microneedles fractured. Automated tools were utilized to assess the safety margin for silicon microneedles. The force of compressive failure was assessed using a hardened station with microneedles placed between the load cell and the punch. For silicon microneedle arrays, an adequate safety margin was found. (Forvi E. *et al.,* 2010)

**Measurement of fracture force**

Researchers utilized an axial load testing setup to assess the force needed to induce a mechanical failure in a microneedle. The microneedle was pushed onto a flat Aluminium surface at a rate of 0.01 mm/s until a set displacement of 500 mm was reached. (Davis SP. *et al.,* 2004). Adhesive tape was wrapped around the base of the needle to fasten the microneedles to the testing surface. The way the microneedle broke was examined with an attached microscope to determine the type of failure. Through the examination of force and displacement data, the fracture force was measured quantitatively.

**Studies on in vitro skin permeation**

A diffusion cell apparatus is employed to assess the penetration of the drug through the skin. Pig ear skin is usually utilized in experiments placed between the donor and recipient compartments. The combined permeation properties of untreated skin and microneedled skin are examined. (Uppuluri C. *et al.,* 2017).

**Clinical trials and safety**

Many initial clinical trials investigating microneedles have been conducted and shown effectiveness in several aspects, yet only a limited number have succeeded. In 2001, there was an effort to investigate microneedles in human participants. The aim was to establish if silicon microneedles cause less pain compared to 26-gauge hypodermic needles. The microneedles were placed into the forearms of 12 healthy male and female participants who consented to take part in the study. The study's results indicated that microneedles resulted in less discomfort compared to hypodermic needles. (Kaushik S. *et al.,* 2001).

**Applications**

Studies on microneedle (MN) applications emphasize their use in drug delivery, improved transdermal penetration, and biological fluid sampling. Moreover, MNs can be combined with microfluidic and biosensing systems to enable physiological monitoring and biomarker analysis. The primary goal of microneedle-based systems is to create a transient, minimally invasive pathway across skin barriers to support therapeutic and diagnostic functions (Ingrole RSJ. *et al.,*2021). These applications are depicted in Figure 5.

**Vaccine Delivery**

The rising awareness among individuals about vaccinations has prompted scientists to explore new methods for vaccine delivery. The latest development involves microneedle drug delivery, where the antigen is directly administered into the dermis. Consequently, an immune response can be triggered, and vaccination treatment can be finalized. This task is primarily performed using dissolving microneedles. Different techniques and devices such as "Mantoux" or "Soluviamicroinjection system"can be utilized to carry out this procedure. Research indicates that the Mantoux technique is challenging to execute, necessitating a skilled individual, while the Soluvia technique is frequently employed, particularly for administering influenza vaccinations like trivalent or quadrivalent, as well as type A or B influenza.(Pettis RJ.*et al.,* 2012) (Vankerckhoven V.*et al.,* 2010). A clinical trial performed on humans using hollow microneedles for influenza vaccination demonstrated comparable immune system responses when evaluated against intramuscular injection. (Van Damme P. *et al.,* 2009).

**Cosmetic Applications**

Microneedles are employed for treating acne scars. The region with acne scars is numbed with an anesthetic, and the Dermaroller is applied, moving in both vertical and horizontal directions. The saline pads are utilized to manage the

bleeding that can be regulated. The procedure requires 20 minutes to finish. Additionally, home care derma rollers are utilized for administering anti-aging products (Dogra S. *et al.,* 2014). Moreover, MN technology successfully treated two individuals affected by alopecia areata (Mysore V. *et al.,* 2014). These individuals saw hair regrowth following the therapy. Successful clinical trials have been performed on atrophic facial scarring (Majid I. *et al.,* 2009), atrophic acne scars (El-Domyati M. *et al.,* 2015), and hypertrophic burn scars (Aust M.C. *et al.,* 2010) utilizing a MN. As the demand for cosmetic products rises, microneedles (rollers and patches) hold significant potential moving forward. (Pattani A. *et al.,* 2012)

**Iron Therapy**

Success in adjusting microneedle patches to substitute the traditional method of using iron supplements and parenteral methods for addressing iron deficiency in humans was accomplished through a micro molding technique, effectively treating anemia in patients without causing any gastric side effects. A quickly dissolving kind of microneedles infused with ferric pyrophosphate has been developed to effectively address iron deficiency anemia. By conducting in vivo and in vitro studies, satisfactory results were achieved. (Maurya A. *et al.,* 2018).

**Diabetes Management**

Diabetes influences a large number of individuals nowadays. This happens due to the pancreatic beta cells failing to generate sufficient insulin. Insulin is a hormone composed of numerous amino acids. To address this problem, a novel approach known as hydrogel-forming microneedle drug delivery has been created. This technique enables drugs like metformin hydrochloride to be administered straight into the bloodstream. The medication is administered in a regulated manner, allowing it to be effective for an extended duration. Employing microneedles for insulin administration is more effective than utilizing a conventional hypodermic needle. (Ye Y. *et al.,* 2016).

**Protein Delivery**

Microneedle mask devices were developed using a continuous liquid interface production technique, incorporating a polyethylene glycol foundation to guarantee the efficient administration of proteins such as serum albumin and ovalbumin. There was worry that the free radical polymerization technique employed in creating these microneedles could harm the proteins, possibly triggering an immune response. To tackle this issue, a protective layer was applied to the microneedles once the production process was finished. Research on mice indicated that these devices effectively administered the proteins over a 72-hour duration, with the proteins remaining functional for a prolonged period. (Caudill CL. *et al.,* 2018)

**Diagnostics**

In the past few years, different types of microneedles have been utilized for the diagnosis of various diseases, such as cancer, cardiovascular issues, atherosclerosis, diabetes, and thrombosis. This is attributed to their capability to infiltrate the skin and collect biomarkers from blood vessels or skin interstitial fluid via capillary action. Firstly, the interstitial fluid present in the skin serves as a crucial reservoir of biomarkers vital for disease diagnosis; in contrast to conventional techniques, the microneedle patch provides the benefits of being painless, simple to use, and capable of quickly extracting skin interstitial fluid. (Chang H. *et al.,* 2017).

**Challenges and Limitations of Microneedle Technology**

Microneedle technology shows great promise in multiple fields, especially in cosmetics and drug delivery. However, its progress is slowed down by several challenges and restrictions that must be overcome to fully realize its benefits. One major challenge is the manufacturing process. Creating microneedles with exact designs and consistent quality is tough, particularly as production scales up for commercial use. Variations in the shape or structure of microneedles can lead to different results in treatment, which raises questions about their dependability and performance. (Mordor Intelligence, 2023). Despite the promising potential of microneedle systems, several challenges must be resolved before their widespread clinical adoption. These include the risk of skin irritation, possible microbial contamination, and difficulties in delivering therapeutically effective drug doses. In addition, the transdermal transport of macromolecules, particularly biotechnological products, remains a significant challenge.

Owing to their large molecular size and hydrophilic nature, these molecules exhibit limited permeability across the skin barrier, making their effective delivery more complex. (Lee J. *et al.,* 2015). Regulatory obstacles create restrictions, since existing frameworks may not fully accommodate the innovative aspects of Microneedle technology, hindering approval timelines. Additionally, patient acceptance and psychological barriers concerning needle usage, regardless of size, could influence adoption rates. Addressing these challenges requires multidisciplinary collaboration among material researchers, scientists, engineers, regulatory bodies, and healthcare professionals to effectively tailor microneedle applications and surmount existing limitations. (Formulation Bio, 2000) (Verified Market Research, 2023).

## CONCLUSION

Microneedles represent a relatively new approach to administering drugs. Microneedles surpass the existing transdermal drug delivery methods in effectiveness. Microneedles offer various benefits, including enhanced patient compliance, pain-free drug delivery, avoidance of first-pass metabolism, and targeted local drug application; in this review, we discuss the materials necessary for the systematic creation of microneedles. We examined various drug delivery methods along with appropriate techniques for preparing microneedles and their uses in Cancer, Diabetes, Cosmetics, Vaccine delivery, and additional applications. Microneedles seem to be a promising method for drug administration, and additional research and advancements in this area could enhance the significance of microneedle drug delivery. Microneedle systems have been created to address the current demand for technology that connects invasive and non-invasive treatments. The scientific and industrial research sectors have shown considerable interest in these devices because of their utility as biomedical tools. Microneedle patches are simple to apply, cause no pain, and are non-invasive for treating infections in infants and for vaccinations. It additionally improves bioavailability and is utilized for controlled release purposes**.**

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**CONFLICT OF INTERESTS**

Declared none

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**Table 1: Methods for the Preparation of Microneedles**

| Methods | Methodology |
|---|---|
| Micro-molding and melt casting method | Employing this method, a microneedle mold is first created from a silicon wafer. This wafer is subsequently oxidized at 1000 °C. After that, the liquid polymer solution is poured into the prepared molds, and any air voids are removed either through vacuum or centrifugation. After this procedure, the molds are baked in an oven. The microneedles are taken out once they have cooled down, as illustrated in Figure 2. (Gittard SD. *et al.,* 2009) |
| Laser cutting | Laser cutting involves the use of an infrared laser to shape metallic sheets into microneedles, utilizing software named "AutoCAD" to design the microneedle geometry, including the configuration and alignment of arrays. The laser beam then removes the metal sheet according to the design, leading to the creation of microneedles, as illustrated in Figure 3. Usually, this method is employed to create metal microneedles. Stainless steel is the most commonly utilized material. (Martanto W. *et al.,* 2004) |
| Laser Ablation | Laser ablation, similar to laser cutting, is used to create solid metal arrays. This technique can be used to make not only stainless steel but also other metals like tantalum. After three pulses, a small bump forms, which then becomes a needle about 10 micrometers tall. Using more twisted light pulses can further shape the microneedles, resulting in a tip diameter smaller than 0.3 micrometers. (Omatus T. *et al.,* 2010) |
| Dip coating | Dip coating is the simplest technique for coating microneedles. In this technique, the microneedles are initially immersed in a drug solution and then removed, leading to the formation of a liquid coating on the microneedle surface; subsequently, the liquid layer is permitted to dry, producing a solid film covering the tips of the microneedles without any contamination, as illustrated in Figure 4. The dip coating method has been utilized to apply hydrophilic and hydrophobic drugs. Dip coating is a slow method and carries the risk of degradation. (Omatus T. *et al.,* 2010) |

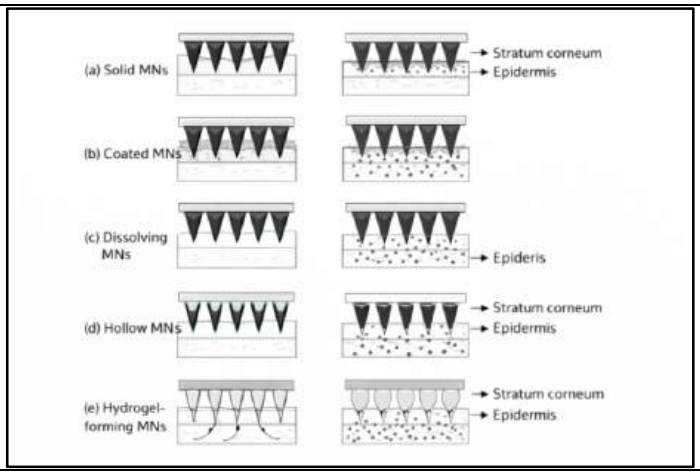



**Figure No. 1: Types of Microneedles**

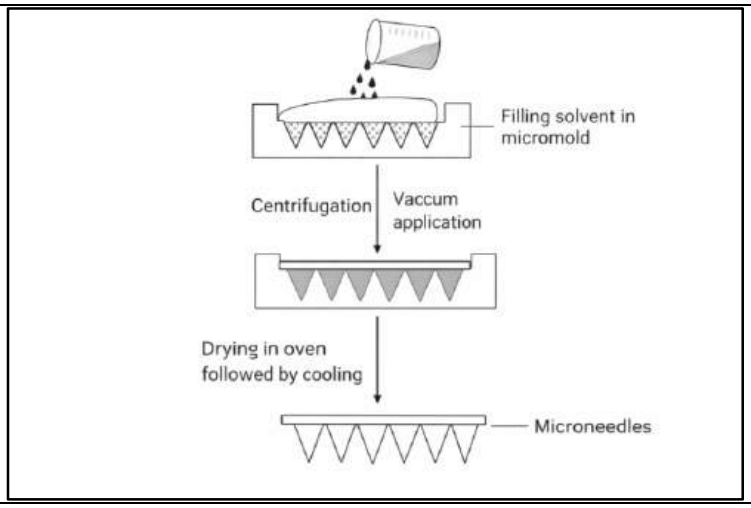



**Figure No. 2: Micro-molding and melt casting method**

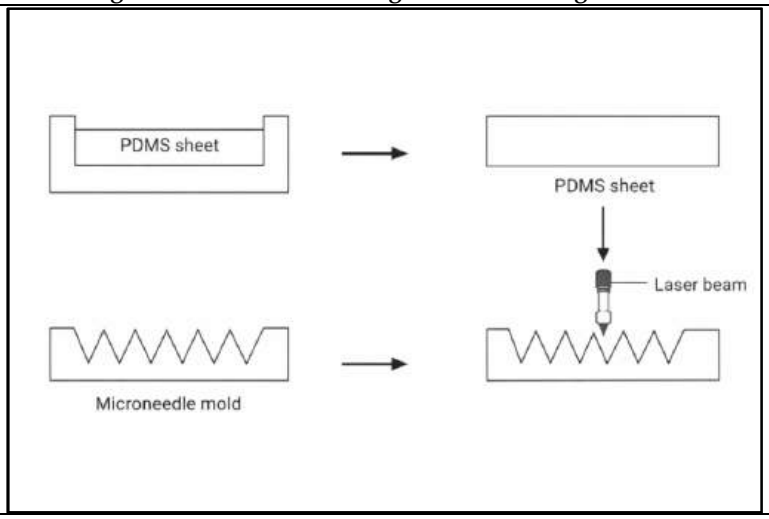



**Figure No. 3: Laser cutting method**

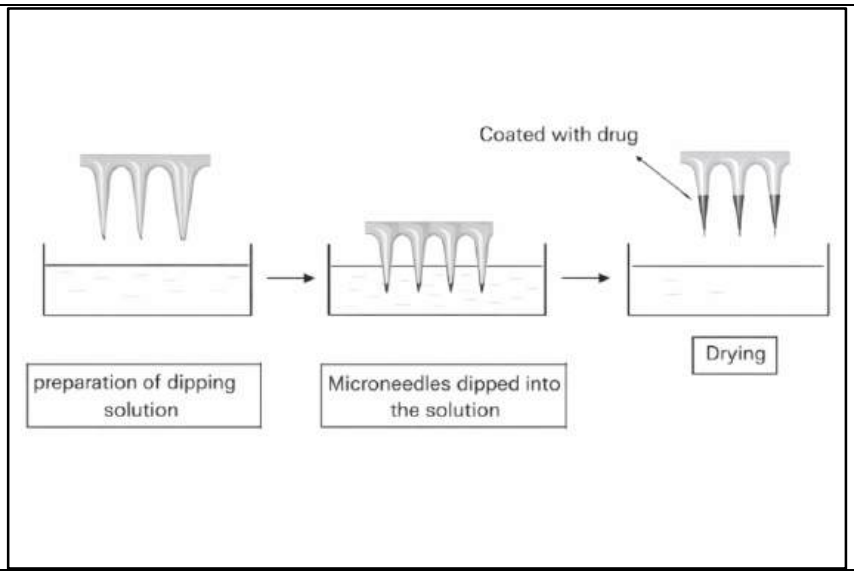



**Figure No. 4: Dip coating method**

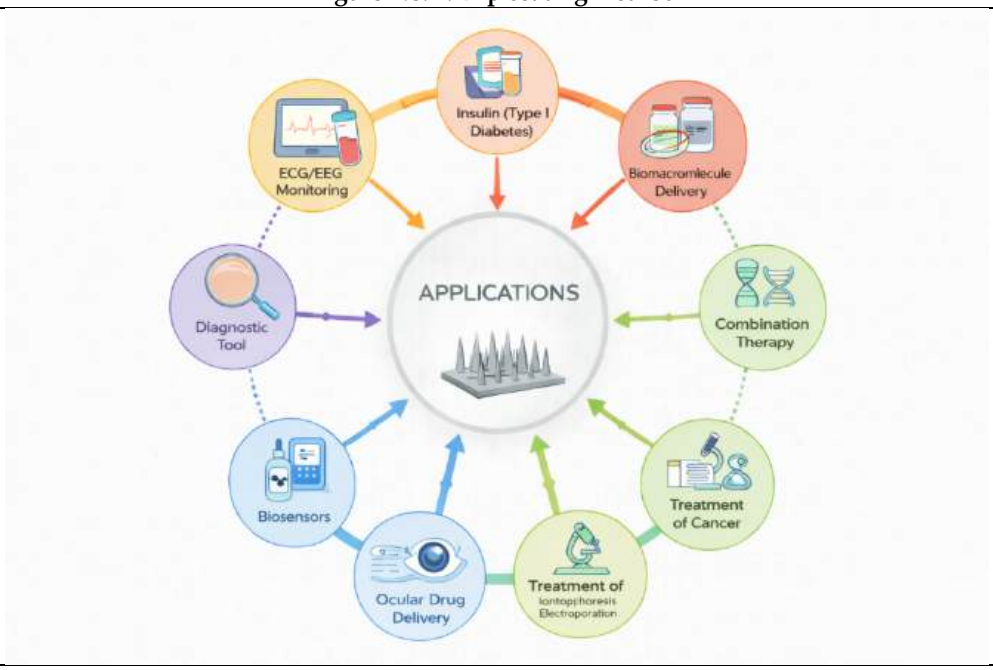



**Figure No. 5: Applications of microneedle technology**

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CASE STUDY ARTICLE

# Clinical Efficacy of an Individualised Ayurvedic Protocol in Palmoplantar Psoriasis : A Case Study

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**ABSTRACT**

Palmoplantar psoriasis is a chronic, immune-mediated dermatosis. This case study evaluates the clinical effectiveness of an individualised Ayurvedic treatment protocol in a patient with a 7-year history of a recalcitrant condition. Clinical profile- Having deep fissures, hyperkeratotic plaques, severe pruritus and burning, with winter exacerbation and chronic constipation. According to Ayurvedic diagnosis, Vata-Pitta is dominant, with Rakta and Twaka Dushti. Intervention - The treatment included targeted internal medications, supportive external therapies, and Ayurvedic dietary and lifestyle interventions aligned. Outcomes- Significant reduction in fissuring, scaling, itching, and other discomfort. Measurable improvement in PASI, DLQI, and VAS scores indicates clinical remission. Conclusion- Personalised Ayurvedic therapeutics demonstrated notable efficacy in Palmoplantar psoriasis.

**Keywords:** Psoriasis, *Vipadika Kushta*, Palmoplantar psoriasis, *Shodhana -ShamanaChikitsa*

## INTRODUCTION

Palmoplantar psoriasis is a bilateral, symmetric, and difficult-to-treat variant of psoriasis that characteristically affects the skin of the palms and soles, with an unclear epidemiology. This condition includes itching, pain, scaling, and fissuring. Psoriasis is a common and chronic T cell-mediated skin disorder with varied clinical and morphological types. It affects approximately 125 million people worldwide[1]. In India, it was found to be 1.02%. While in north India, it was shown that psoriasis accounted for 2.3% of all dermatology outpatients[2]. The recurrence pattern and pathogenesis of palmoplantar psoriasis constantly pose a challenge to treatment approaches. Sites other than the

hands and feet are frequently involved, with studies showing 33% of patients having up to 10% of their body surface area (BSA) involved, which can cause marked physical discomfort and functional disability[3]. Although involvement of the palms and soles often affects <5% of the total body surface area, with a low PASI score[4]. In Ayurveda, *Tvak vikara* are broadly classified under the heading of *Kushta roga*, explained in various classical texts. It's one of the type is *Vipadikaroga*. Characterised by *Sputana* in soles and palms with *Vedana, Rukshata, Vaivarnya*, *kandu, etc*. The line of treatment to be adopted is *Antaha Parimarjana* and *Bahir Parimarjana chikitsa with Nidanaparivarjana*[5]. As every patient differs in *Dosha*, *Dushya*, *Bala*, *Prakruti,* so individualised treatment is planned. Modern immunopathology attributes psoriasis to T-cell–mediated immune dysregulation; from the Ayurvedic standpoint, this corresponds to *Ojas Kshaya* and *Rakta Dushti*. By considering this case, endeavours bridge to classical Ayurvedic wisdom with contemporary clinical evidence, highlighting the integrative potential of traditional principles in modern dermatological care.

**Case Presentation**

**Patient details:** A 51-year-old male farmer came with various complaints, having *Pitta-dominant Vata prakriti*(constitution), reported habitual tobacco use and regular consumption of hot and pungent food(*Ushna–Tikshna gunatmakaahara*)

**Chief complaints:** The patient reported deep, painful and bleeding fissures, scaling over the palms and soles, with severe itching and burning sensations for the last 7 years (on/off), worsening in winter and after soil exposure, Constipation for 11 years, and sometimes suffers from headaches.

**Past History** - No significant medical, surgical, or psychiatric disease.

**Previous treatment:** The patient had received repeated courses of contemporary dermatological treatments, experienced only short-term relief with frequent recurrence of symptoms, indicating chronic *Dosha* involvement.

**Etiological factors:** Irregular sleep schedule (*Asamyaka nidra*), excessive oily/fried food and tea intake, milk with banana in every day's diet(*Viruddha ahara*), stress, excessive contact with soil (*Agantuja hetu*),addiction – Tobacco chewing.

**Therapeutic Intervention**

Considering the Pathological Complex (*Dosha Dushya Sammurchhana*) and Tissue pathology (*Dhatu dushti*) assessment, personalised Ayurvedic treatment was formulated. Detoxification (*Shodhana*) was adopted as the prime line of management to eliminate vitiated *Doshas*, followed by Palliative therapy(*Shamana chikitsa*)to achieve sustained remission and tissue balance, in accordance with *Kushtha Roga Chikitsa.*

***Nidanaparivarjana-*** Elimination of etiological factors.

**First 7 days**

**Table no.1: Medicine for 1st 7 days**

**After 7 days-**

**Table no. 2: *Shodhana Therapy***

***Shamana Chikitsa*: Internal medicines-**

**Table no. 3**

**External applications**

*Jatyaditaila + Vranaroapana taila*

**Method of application** – Take both *Taila* in equal proportion and apply them generously over the hands and feet until the area appears *Snigdha* (well oiled). After 15-20 minutes of application, wear socks to promote deeper absorption.

## OBSERVATION AND RESULTS

- **Table no. 4 -** Before and after symptom comparison:
- **Image no. 1 and 2 -** Before and after images:
- **Image no. 3 -** Before–After Treatment Bar Chart for PASI, DLQI, VAS Pain, and VAS Itching-

## DISCUSSION

In the present case of Palmoplantar Psoriasis, its improvement forms a strong basis for interpreting the therapeutic response in the discussion ahead.

***Nidanapariavrjana* (Elimination of causative factors)-** It plays a key role in the *Samprapti vighatana*(disruption of pathogenesis).

***Shodhana chikitsa-*** Since *Kushtha* is a *Tridoshajanya Vyadhi*, it is best to treat the predominant doshas before moving on to the *anubhandha doshas*. Regular *Panchakarma* process advice reveals the degree of *dosha.*

***Eranda taila*(Castor oil)-** It has the property of calming the entity of the body constitution, as well as cleansing the minute channels, promoting rejuvenation. Castor Oil has such high antioxidant activity at low concentrations that it could be very useful for the treatment of diseases caused by oxidative stress. The active chemical constituents are Methyl Ricinoleate, Ricinoleic acid, 12-octadecadienoic acid, and Methyl Ester, which produce antioxidant activity. If used orally, castor oil is a good laxative that aids in straining during defecation and the sensation of complete evacuation following a bowel movement. Its active ingredient, Ricinolic acid, is released in the colon, where it aids digestion, nutrition absorption, and system cleansing. Castor oil also functions as a natural antibacterial, antifungal, and antimicrobial agent, addressing the root causes of skin diseases[6].

***Shamana chikitsa***

***Amapachaka Vati-*** *Mandagni* (Digestive impairment) is the root cause of diseases. *Amapachaka Vati* is a Herbo-mineral Ayurvedic formulation that hasa potent action to normalise the function of *Agni* and reduce *Ama* by doing *Pachana* of *Ama.* It contains drugs that have*Katu-Tikta-Lavana Rasa, Laghu-Ushna Guna, Ushna Veerya, Katu Vipaka* and *Vata-Kaphahara, Deepana-Pachana,* and *Anulomana* properties. Also, the ingredients of this formulation possess Pharmacological actions like Antispasmodic, Antioxidant, Analgesic and Anti-inflammatory. This may be interpreted as a bio-digestive and anti-inflammatory formulation that facilitates the clearance of incompletely metabolised intermediates and gut-derived endotoxins arising from impaired digestive and metabolic processes[7].

***Laghusutshekhara rasa-*** *Laghusutshekhar Ras* acts as a detoxifier; this action is contributed by the presence of ginger root powder in the formulation. Its effects appear on all three *doshas*, but especially reduce aggravated pitta and its various disorders. Its *Laghu guna* and *Tikshana guna* have *Srotoshodhaka* property, softening and liquefying the morbid doshas, which are ultimately excreted[8]. It principally impacts the *Rasadhatu* and *Raktadhatu* as the affected tissues. Its main areas of action are the *Amashaya*, *Pakwashaya* and *Raktavaha Srotas*.

***Manjishthadighrita-*** *Manjishtha*, used as a prime healing drug described in Ayurvedic literature. *Raktashodhana* (purification of blood) was done by virtue of *Tikta* and *Kashaya rasa*, it also shows actions like *Lekhana, kledahara, Chhedana,* and *Raktashodhaka properties*. *Vishadaguna* of *Kashaya rasa* played an important role in scraping out the debris and slough of the *Vrana*. *Varnya, Twaka prasadana, Raktaprasadana* properties of the *Madhura rasa* with *Snigdha guna* led to the colouration of scartissue similar to that of skin. Phytosterols, the chief components of *Ghrita*, are known to be

good emulsifiers, the property of which was assumed to facilitate the penetration of the drug into the tissue. Tannins and anthraquinones are known antioxidants and blood purifiers with anti-inflammatory actions. Berberine, and phytosterols all are anti-inflammatory and thus prevent the prolongation of the initial phase and reduce the pain as well astenderness, redness, swelling-like features, along with ascorbic acid and glycosides[9].

***Dhnyakaphanta*** **-** Various parts of the plant, such as seeds and leaves, possess antioxidant, anxiolytic, antidepressant, learning and memory improving, neuroprotective, hypnotic, and analgesic activities. Ethanolic extract of *Coriandrum sativum* Linn. possesses hepatoprotective activity on CCl4-induced liver injury, which may be due to its antioxidant potential, due to phenolic compounds in it and enhances the functions of the liver, which is important in blood purification[10]. In Ayurveda, it is said to be a pacifier of Vata and Kapha dosha.

***Sarivaghanavati*** - The role of *Sariva* in enhancing skin health is proven by traditional and listed activities, which ensure it possesses the antioxidant, anti-inflammatory, antistress, and antimicrobial properties so that it can play an important role in curing skin disorders. Methanolic extracts of *Sariva root,* bark showed free radical scavenging properties in several in vitro and in vivo models, as well as the inhibition of lipidperoxidation and scavenging hydroxyl and superoxide radicals *in vitro*. It also shows, NF-kB, reduction of pro-inflammatory IL-8 expression, and alterations in other mediators of inflammation, such as reactive oxygen species and pro-inflammatory cytokines.

**Gandhakarasayana-** Crosstalk between Membrane metalloproteases (MMPs), tissue inhibitors of metalloproteases and metallothioneins (MTs) are known to play a pivotal role in repair and regenerative processes. Accumulation of MTs in tissues comprising proliferating cells and in healing tissues implies a possible role of MTs in injured tissues undergoing repair. Induction of metallothioneins is reported to accelerate wound healing proliferation and, by promoting cell re-epithelialization by matrix remodelling. The spectrum of immune system disorders treated by this rasayana strengthens this hypothesis, and it appears that rasayana has a significant role in repairing or rejuvenating cellular functions[11].

**Local application**

***Jatyadi taila-*** *Jatyadi taila* is used externally for *Abhyanga,* i.e. for itching, boil, fistula, wounds due to sharp weapons, burn ulcer, non-healing ulcer also used for eczema and psoriasis. The phyto-medicinal therapy is easy to procure and administer with minimal side effects. The ingredients of *Jatyadi taila* have been proven scientifically for their antimicrobial property. By considering this, it shows results in skin disorders[12].

***Vranaropanataila-*** A mixture of curcumin (diferuloylmethane), monodex-methoxycurcumin, and bisdes-methoxycurcumin, are the active component in it. It shows Anti-inflammatory, anthelmintic, antiasthmatic activity, and neuroprotective activity. The analysis of *Vrana Ropana* herbs indicates predominance of *Madhura, Kashay Rasahaving Madhura Vipaka* and *Sheeta Virya*. It could be because *Madhura Rasa* aids in *Dhatu Poshana* (tissue regeneration) and *Kashaya Rasadravyas* aid in wound closure through their *Sandhanakarma* (tissue binding) actions. It is well stated that plants rich in tannins, flavonoids, saponins, sterols, phenols, and triterpenoids have the potential to heal wounds[13]. The autoimmune nature of palmoplantar psoriasis reflects a derangement of *Vyadhikshamatva*, analogous to *Ojas Kshaya,* wherein the depletion of *Ojas* weakens systemic immunity, allowing *Vata* and *Pitta* vitiation to manifest as inflammatory dermatoses.

## CONCLUSION

The case demonstrates that individualised Ayurvedic management in Palmoplantar Psoriasis which bridging ancient diagnostics with modern dermatological outcomes, a condition often resistant to conventional care, achieved significant relief with no adverse effects, reflected in reduced PASI, DLQI, and VAS scores. These findings underscore the need for larger, well-designed studies to validate the outcome and support stronger integration of Ayurveda into evidence-based dermatology.

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**Table no.1: Medicine for 1st 7 days**

| Sr. no. | Ayurvedic medicine | Dose | Time | Anupana |
|---|---|---|---|---|
| 1. | *Amapachakavati* | 250 mg – 2 Tab | Twice a day – between the meals | Lukewarm water |
| 2. | *Laghusutshekhara rasa* | 125 mg – 2 Tab | At 6 am and at 2 pm | Normal water |
| 3. | *Manjishthadighrita* | 10 ml | Just before lunch | Lukewarm water |
| 4. | *Dhanyakaphanta* | 1 tsp+100ml water | In the morning (for 7 days) | - |

**Table no. 2: *Shodhana Therapy***

| Protocol | Details |
|---|---|
| Procedure | 20 ml of *Eranda Taila* (Castor oil) with lukewarm water. |
| Objectives | To eliminate the vitiated *Dosha* (remove toxins and detoxification) |
| Duration | Taken for 30 days. |

**Table no. 3: *Shamana Chikitsa*: Internal medicines**

| Sr. no. | Ayurvedic medicine | Dose | Time | *Anupana* (taken with) |
|---|---|---|---|---|
| 1. | *Laghusuthsekhara rasa* | 125 mg- 2 Tab | at 6 am and at 2 pm | Normal water |
| 2. | *Sarivaghanavati* | 250 mg -2 Tab | After meal | Lukewarm water |
| 3. | *Gandhakarasayana* | 250 mg -2 Tab | After meal | Lukewarm water |
| 4. | *Manjishthadighrita* | 10 ml | Just before lunch | Lukewarm water |

**Table no. 4: Before and after symptom comparison**

| Sr. no. | Sign and Symptoms | Before treatment | After Treatment |
|---|---|---|---|
| 1. | Fissures (Palms and Soles) | +++ | - |
| 2. | Scaling | +++ | + |
| 3. | Pain (Due to fissures) | +++ | - |
| 4. | Itching (*Kandu*) | ++ | - |
| 5. | Burning Sensation (*Daha*) | +++ | + |
| 6. | Seasonal Aggravation | +++ | - |
| 7. | Trigger Sensitivity (Soil exposure) | +++ | + |
| 8. | Constipation | ++ | - |
| 9. | Headache | + | - |
| 10. | Quality of Life | ++ | - |





**Figure 1 and 2: Observation and Results of Before–After Treatment**

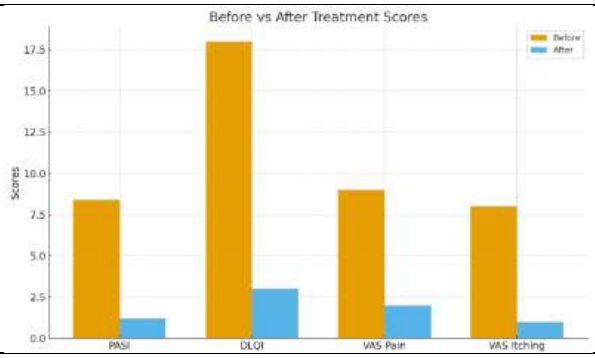



**Image no. 3: Before–After Treatment Bar Chart for PASI, DLQI, VAS Pain, and VAS Itching**

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RESEARCH ARTICLE

# Advanced Imaging in Neurodegenerative Diseases: Bridging the Gap between Research and Clinical Practice

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## ABSTRACT

Recent advancements in neuroimaging techniques such as functional MRI (fMRI), Diffusion Tensor Imaging (DTI), Magnetic Resonance Spectroscopy (MRS), and Positron Emission Tomography (PET) have revolutionized the diagnosis and management of neurodegenerative diseases like Alzheimer's and Parkinson's. These tools enable early detection of brain changes, monitor disease progression, and assess treatment response by revealing molecular, structural, and functional brain alterations. Hybrid PET/MRI systems further enhance accuracy by integrating anatomical, metabolic, and molecular data, supporting personalized medicine. This study aims to evaluate the impact of advanced neuroimaging on diagnosis, prognosis, and management of neurodegenerative disorders and identify barriers limiting their clinical use. Review imaging modalities (MRI, PET, SPECT) for diagnostic accuracy and disease differentiation. Assess the role of imaging in monitoring disease progression and therapy response. Identify challenges such as cost, standardization, and accessibility affecting implementation. A systematic review of 91 peer-reviewed articles from 2006 to 2025 was conducted using databases including Scopus, PubMed, Web of Science, and UGC Care. Studies were selected based on predefined criteria focusing on advanced imaging techniques in neurodegeneration. Keywords included fMRI, DTI, MRS, PET, and related terms. Data extraction and analysis adhered to PRISMA guidelines to ensure transparency and methodological rigor. Advanced imaging significantly improves early detection of neurodegenerative changes, often before clinical symptoms emerge, enabling timely intervention. These modalities differentiate disease subtypes and evaluate treatment efficacy, facilitating personalized therapies. Despite these benefits, challenges remain: high costs, inconsistent imaging protocols, and limited specialized training restrict

broader clinical adoption. Efforts toward protocol standardization, clinician education, and cost reduction are vital to expand their use. Techniques like MRI, fMRI, and DTI show promise for early diagnosis and longitudinal monitoring but face barriers such as variability in methods, restricted access to hybrid imaging systems, and workforce shortages. Integration of artificial intelligence and development of targeted molecular tracers—such as those detecting amyloid and tau proteins—are advancing early diagnosis and disease management. Optimal imaging strategies depend on disease type, stage, and resource availability, highlighting the need for personalized, multimodal approaches. Neurodegenerative diseases pose growing global health challenges due to their complex pathology and lack of curative treatments. Advanced imaging methods—particularly PET, MRI, and combined PET/MRI—are transforming detection and care by providing detailed, non-invasive insights into brain structure, function, and chemistry. These technologies enable earlier diagnosis and tailored treatment plans but are currently limited by financial, technical, and educational barriers. Collaborative efforts toward standardization, affordable imaging solutions, and integration of AI will be crucial for making advanced neuroimaging a routine component in managing neurodegenerative disorders.

**Keywords:** Neurodegenerative Diseases, Advanced Neuroimaging, fMRI, DTI, Biomarkers, Personalized Medicine, Disease Monitoring.

## INTRODUCTION

Neurodegenerative diseases (NDs) are a group of progressive disorders characterized by the loss of specific neuronal populations, leading to cognitive, motor, and behavioral decline. Disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and Amyotrophic Lateral Sclerosis (ALS) display distinct regional pathologies and clinical symptoms[1]. For instance, AD predominantly affects the hippocampus and neocortex, causing memory loss and disorientation, while PD is marked by dopaminergic neuron degeneration in the substantia nigra, producing tremor, rigidity, and bradykinesia[2]. Modern neuroimaging tools have revolutionized the diagnosis and monitoring of NDs. Structural MRI enables identification of disease-specific cortical atrophy patterns, such as hippocampal shrinkage in AD and frontotemporal changes in FTD and HD [3]. Diffusion Tensor Imaging (DTI) further enhances white matter assessment by revealing axonal degeneration and demyelination in ALS and MS [4]. Functional MRI (fMRI), particularly resting-state fMRI, provides insights into brain network dysfunctions. In AD, altered connectivity within the default mode network is associated with cognitive decline. Similarly, fMRI has revealed connectivity disruptions in PD and ALS, underscoring its role in tracking disease evolution[5]. Molecular imaging with PET plays a central role in detecting AD biomarkers. Amyloid and tau PET imaging allow in vivo visualization of amyloid-β plaques and neurofibrillary tangles, aiding in early and differential diagnosis[6]. PET is now integrated into clinical practice for identifying the etiology of dementia when results may alter patient management Future advances lie in multimodal imaging, combining structural, functional, and molecular data for a comprehensive disease profile. Integration of artificial intelligence (AI) and machine learning (ML) promises improved pattern recognition and early detection through large-scale data analysis. As imaging technology evolves, it will continue to bridge the gap between research and clinical care—enabling personalized diagnosis, guiding treatment, and improving outcomes in neurodegenerative disorders[7].

## MATERIALS AND METHODS

A systematic narrative review was conducted at the Department of Radio-Imaging Technology, Faculty of Allied Health Sciences, SGT University, Gurugram, Haryana, India, between April 2024 and June 2025. A total of 91 peer-reviewed articles published between 2006 and 2025 were included, selected from Scopus, PubMed, Web of Science, and UGC Care databases. Articles were identified using specific MeSH terms and keywords related to

neurodegenerative diseases and advanced imaging techniques, including "fMRI," "PET," "DTI," "Alzheimer's," and "Parkinson's." The inclusion criteria required studies to focus on advanced imaging modalities applied to neurodegenerative diseases, while articles published before 2006, non-peer-reviewed papers, animal studies, and unrelated research were excluded. Data extraction was performed using a standardized form capturing author details, imaging techniques, disease type, and key findings. The review process followed PRISMA guidelines, ensuring a transparent selection and screening workflow. Due to heterogeneity in study designs and outcomes, a narrative synthesis was conducted, grouping results by imaging modality and clinical application. Descriptive summaries, tables, and charts were used to present trends, clinical relevance, and research gaps.

## RESULTS

Advanced imaging plays a central role in the transition toward personalized medicine in the context of neurodegenerative diseases. Through individualized assessments of disease progression and therapeutic response, modern imaging techniques contribute significantly to the development of tailored treatment strategies, thereby enhancing clinical outcomes and reducing unnecessary or ineffective interventions[8]. This is especially crucial in the management of complex neurodegenerative disorders such as Alzheimer's and Parkinson's disease, where substantial heterogeneity exists between patients in terms of clinical symptoms, disease trajectory, and treatment response[9]. However, despite these capabilities, several challenges continue to hinder the widespread clinical adoption of advanced neuroimaging. Notable barriers include the lack of standardized imaging protocols across institutions, the high operational and infrastructural costs associated with hybrid imaging systems such as PET/MRI, limited availability in low-resource settings, and significant gaps in clinician training when it comes to interpreting multi-parametric imaging data[10]. These limitations can lead to diagnostic variability and impede the reliability and reproducibility of imaging biomarkers. Addressing these challenges requires global collaboration to establish harmonized protocols, improve clinician education, and develop scalable and cost-effective imaging solutions[11]. Hybrid PET/MRI imaging has emerged as one of the most transformative developments in this field, allowing clinicians to combine molecular, metabolic, and structural brain information within a single diagnostic session. PET tracers such as TSPO and MAO-B ligands enable the detection of early neuroinflammatory processes, including microglial and astrocytic activation, which are key pathophysiological components of neurodegeneration[12]. Meanwhile, the MRI component provides high-resolution anatomical and functional information, contributing to a multidimensional and real-time map of disease activity[13]. This integrated approach supports a more accurate and earlier diagnosis, the monitoring of disease progression at the cellular level, and the formulation of patient-specific therapeutic interventions. Additionally, functional MRI (fMRI) and diffusion tensor imaging (DTI) are critical tools that offer dynamic insights into brain connectivity and white matter integrity, respectively [14]. fMRI captures changes in neural activation patterns in response to therapeutic stimuli, while DTI provides detailed metrics like fractional anisotropy and mean diffusivity to detect microstructural changes. Together, these tools function as a feedback mechanism for evaluating treatment efficacy and guiding clinical decision-making over time. When applied longitudinally, they help tailor therapies based on neurobiological responses, which is particularly valuable for slowing cognitive decline and functional impairment in conditions such as Alzheimer's disease. Despite their immense potential, achieving the routine clinical use of such technologies necessitates addressing current limitations related to standardization, access, training, and cost. Overcoming these obstacles is essential to fully realizing the promise of advanced neuroimaging in delivering precision medicine and improving the lives of individuals affected by neurodegenerative diseases. This figure (Fig 1) illustrates the systematic approach followed in the review process, adhering to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guideline.

## DISCUSSION

Advanced imaging techniques have become crucial in the diagnosis, monitoring, and personalized treatment of neurodegenerative diseases, yet several barriers continue to hinder their widespread clinical integration. One significant issue is the lack of standardized imaging protocols, which leads to inconsistencies in image acquisition,

processing, and interpretation across institutions. These variations undermine the reproducibility of results, especially in techniques like resting-state functional MRI (rs-fMRI), which are highly sensitive to acquisition settings and patient conditions [15]. Such inconsistencies reduce the reliability of neuroimaging biomarkers and complicate their translation into routine clinical practice. Additionally, limited training among clinicians in interpreting complex modalities like Magnetic Resonance Spectroscopy (MRS) and PET constrains their broader application in managing diseases like amyotrophic lateral sclerosis (ALS), where advanced imaging reveals underlying mechanisms such as oxidative stress and neuroinflammation[16]. As neurodegenerative research moves toward precision medicine, there is a growing demand for interdisciplinary collaboration among neurologists, radiologists, molecular scientists, and data analysts, which current educational programs often fail to address[17]. Furthermore, the financial burden of hybrid technologies such as PET/MRI remains a major limiting factor, especially in resource-limited settings. These systems require specialized infrastructure, dual-trained personnel, and access to short half-life radiotracers—components often unavailable in smaller or rural facilities[18]. Despite PET/MRI's ability to combine structural, metabolic, and functional data in one session—making it particularly valuable for early detection and monitoring of Alzheimer's and Parkinson's—its high operational cost limits its accessibility[19]. Nevertheless, innovations in PET scanner design and AI-assisted imaging interpretation may help reduce costs over time and improve diagnostic efficiency[20]. The emergence of molecular imaging agents targeting disease-specific proteins such as tau and amyloid-beta has further enhanced early detection strategies, allowing for visualization of Alzheimer's pathology before cognitive symptoms become evident[21]. These biomarkers are particularly valuable in guiding treatment with disease-modifying agents like monoclonal antibodies, which are most effective in early-stage disease[22]. Structural MRI remains the best method for detecting atrophy and white matter changes, while fMRI reveals network disruptions and PET offers insights into synaptic activity and abnormal protein deposition. MRS adds a non-invasive method for studying neurochemical alterations. The combined use of these modalities provides a multidimensional view of the disease but must be customized based on the specific disorder, stage, and patient condition[23]. As research continues to demonstrate the effectiveness of imaging biomarkers in clinical trials, updating healthcare policy and education is essential to ensure wider adoption. Multicenter collaborations and centralized imaging facilities can support equitable access to these technologies, helping bridge the gap between cutting-edge imaging research and real-world patient care and conditions.

## CONCLUSION

Neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease are increasingly recognized as critical global public health challenges, affecting millions and placing a tremendous burden on patients, caregivers, and healthcare systems. These disorders are marked by the gradual, irreversible decline in brain function, leading to memory loss, cognitive dysfunction, motor impairment, and, ultimately, loss of independence. Since curative treatments remain elusive, the current medical priority is to enhance early diagnosis, monitor disease progression, and tailor patient-specific treatment plans. In this evolving landscape, advanced imaging technologies—particularly Positron Emission Tomography (PET), Magnetic Resonance Imaging (MRI), and hybrid PET/MRI systems—are playing an indispensable role in both research and clinical practice. These non-invasive modalities offer a window into the brain's structural, chemical, and functional changes. PET scans provide insight into molecular abnormalities by detecting pathological proteins such as amyloid-beta and tau, which are hallmarks of Alzheimer's disease, even before clinical symptoms manifest. MRI techniques, including functional MRI (fMRI) and Diffusion Tensor Imaging (DTI), offer high-resolution visualization of anatomical structures, white matter integrity, and connectivity networks, allowing for the detection of subtle changes that occur in early disease stages. When combined in PET/MRI systems, these technologies offer simultaneous functional and structural assessments in a single session, significantly enhancing diagnostic precision and aiding in the development of personalized interventions. Despite their transformative potential, several barriers limit the widespread adoption of advanced imaging in routine clinical settings. The lack of standardized imaging protocols across institutions creates inconsistency in data acquisition and interpretation, hindering comparability and the broader application of research findings. Additionally, the high costs associated with acquiring, operating, and maintaining PET/MRI systems—combined with the need for specialized

infrastructure and trained personnel—pose substantial challenges, particularly in low- and middle-income regions. Addressing these limitations requires coordinated efforts from clinicians, researchers, policymakers, and industry stakeholders to implement unified imaging protocols, facilitate data sharing, and develop cost-effective solutions. Moreover, integrating artificial intelligence (AI) and machine learning with advanced imaging is expected to redefine diagnostic workflows by automating image analysis, improving interpretation accuracy, and accelerating clinical decision-making. These digital tools can help overcome current bottlenecks in expertise and processing time, making neuroimaging more efficient and scalable for widespread use. In this context, AI-enhanced imaging is not only a solution to existing challenges but also a catalyst for expanding access to advanced diagnostics across diverse healthcare environments. Ultimately, advanced imaging is revolutionizing the management of neurodegenerative diseases by enabling earlier detection, more accurate tracking of disease progression, and the implementation of individualized treatment strategies. To fully realize these benefits, ongoing support for research, enhanced clinician training, international collaboration, and policy reforms that prioritize equitable access to imaging technologies are essential. By addressing these multidimensional challenges, advanced neuroimaging can fulfill its promise of significantly improving patient outcomes and transforming the standard of care for neurodegenerative conditions on a global scale.

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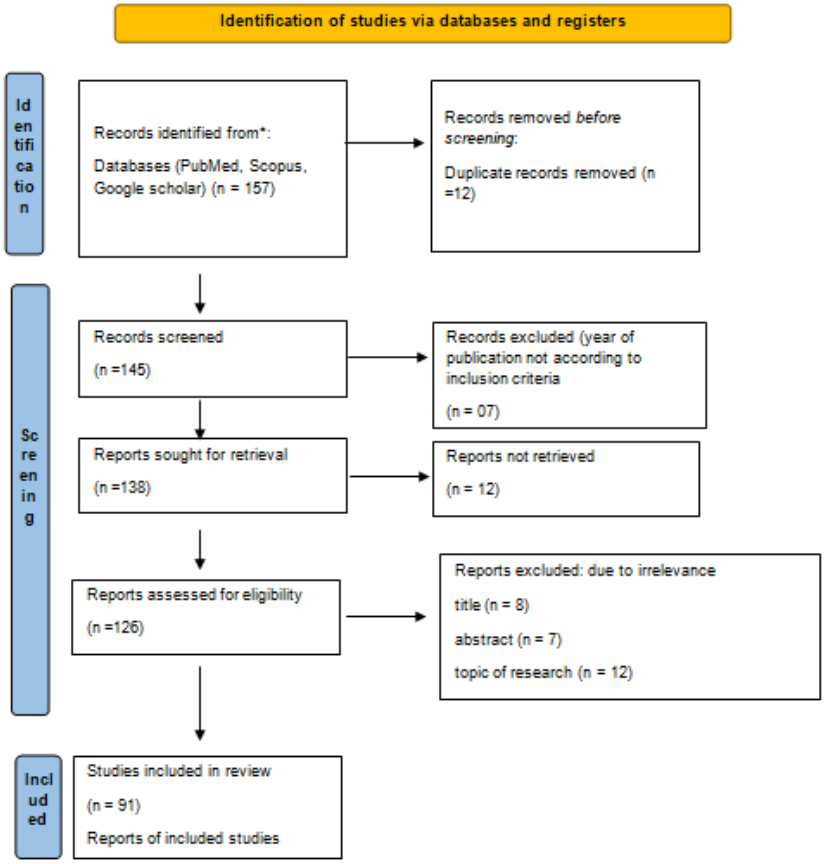



**Fig 1: This figure illustrates the systematic approach followed in the review process, adhering to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guideline.**

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REVIEW ARTICLE

# Biochemical Relationships Between Glycated Hemoglobin and Lipid Profile in Cellular Blood Components and Plasma : A Systematic Review

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## ABSTRACT

Glycated hemoglobin (HbA1c) and lipid profile parameters are central biochemical markers for evaluating glycemic control and cardiovascular risk. Their interrelationship reflects shared metabolic pathways influenced by insulin resistance, oxidative stress, and altered lipid metabolism. This systematic review synthesizes current evidence on the biochemical association between HbA1c and lipid fractions-including total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and very-low-density lipoprotein (VLDL)-across plasma and cellular blood components. Emphasis is placed on lipid distribution in red blood cells and the impact of chronic hyperglycemia on membrane lipid composition. The findings underscore the diagnostic and prognostic value of integrated glycemic–lipid assessment in metabolic disorders and cardiovascular disease risk stratification. The study investigates how cholesterol, triglycerides, LDL, HDL, and VLDL are distributed within plasma and cellular elements of blood, and evaluates the correlation of these lipids with HbA1c levels, especially in diabetic and non-diabetic populations.

**Keywords:** Blood components, Lipid profile, HbA1c, Red blood cells, Cholesterol, Triglycerides

## INTRODUCTION

Blood is a complex biological fluid composed of plasma and cellular elements such as erythrocytes, leukocytes, and platelets, which together regulate metabolic and physiological homeostasis (Guyton and Hall, 2021). Among routinely measured biochemical markers, glycated hemoglobin (HbA1c) and lipid profile parameters play pivotal roles in the diagnosis and management of diabetes mellitus and cardiovascular disease (American Diabetes Association [ADA], 2023). HbA1c reflects long-term glycaemic exposure and is strongly associated with the development of microvascular and macrovascular complications (Nathan *et al*., 2005). Dyslipidemia-characterized by elevated triglycerides, LDL-C, and reduced HDL-Cis commonly observed in individuals with poor glycemic control and contributes significantly to cardiovascular morbidity and mortality (Feingold and Grunfeld, 2000; Goldberg, 2001). Increasing evidence suggests that glycemic dysregulation and lipid abnormalities are metabolically interconnected rather than independent phenomena (Reaven, 1988). The increasing prevalence of cardio metabolic disorders such as type 2 diabetes mellitus (T2DM), dyslipidemia, and cardiovascular diseases (CVDs) has prompted extensive research into blood biomarkers for early diagnosis and management. Among these, the lipid profile (comprising total cholesterol, LDL, HDL, triglycerides) and glycated haemoglobin (HbA1c) are commonly used indicators. While HbA1c provides a long-term measure of glycemic control, the lipid profile assesses lipid metabolism and cardiovascular risk (Sembulingam, and Sembulingam, 2019). Understanding the interrelationship between these two key biochemical parameters is vital in identifying at-risk individuals, particularly those with subclinical metabolic dysfunction.

This research aims to investigate the comparative and correlative significance of lipid profile and HbA1c in different population in different age groups, with the goal of improving screening strategies and integrated risk assessment models. The global burden of non-communicable diseases (NCDs)**,** particularly type 2 diabetes mellitus (T2DM**)** and cardiovascular diseases (CVDs)**,** has risen sharply over the last few decades. The exact relationship between HbA1c and lipid parameters remains a subject of ongoing debate, with conflicting evidence on the strength and consistency of this association across different populations and clinical conditions. Cardiovascular disease (CVD) remains one of the leading causes of morbidity and mortality worldwide, accounting for an estimated 17.9 million deaths annually, and its burden continues to rise particularly in low- and middle-income countries (LMICs) (Authoritative global agency data). The pathogenesis of atherosclerotic cardiovascular disease is multifactorial, with dyslipidaemia and impaired glycaemic control recognised as two of the most important modifiable metabolic risk factors. Indeed, abnormal lipid-levels such as elevated total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), high triglycerides (TG) and low high-density lipoprotein cholesterol (HDL-C) have been consistently associated with increased risk of coronary heart disease, stroke, and peripheral vascular disease (Stone *et al*., 2014). Meanwhile, glycaemic biomarkers such as fasting blood glucose (FBG) and glycated haemoglobin (HbA1c) reflect longer-term glucose metabolism and have been shown to predict micro vascular and macro vascular complications in people with diabetes mellitus (ADA Standards).Despite this knowledge, there are several outstanding gaps. First, many studies to date examine lipid and glycaemic biomarkers in isolation rather than jointly, yet metabolic dys-regulation often co-exists (e.g., insulin resistance may drive both abnormal lipids and impaired glycaemia) (Liang *et al*., 2025).

## METHODOLOGY OF LITERATURE REVIEW

This systematic review evaluated peer-reviewed original research articles, meta-analyses, and review papers indexed in major biomedical databases. Studies focusing on HbA1c, lipid profile parameters, plasma lipids, red blood cell membrane lipids, and metabolic disorders were included. Emphasis was placed on studies involving diabetic, prediabetic, and non-diabetic populations to capture the full metabolic spectrum (Danese *et al*., 2015).

**Glycated Hemoglobin: Biochemical Basis and Clinical Significance**

HbA1c is formed through the non-enzymatic glycation of hemoglobin, reflecting mean plasma glucose levels over approximately 8-12 weeks (Danese *et al*., 2015). It is widely used for diagnosing diabetes and monitoring long-term

glycaemic control (ADA, 2023) (Figure 1). Elevated HbA1c levels have been consistently linked to increased risks of retinopathy, nephropathy, neuropathy, and cardiovascular disease (Nathan *et al*., 2005; Barrett *et al*., 2017). Beyond its role as a glycaemic marker, HbA1c has been shown to correlate with lipid abnormalities, suggesting its potential utility as an indirect indicator of cardiometabolic risk (Turner *et al*., 1998; Jenkins *et al*., 2003). Glycated Hemoglobin: Biochemical Basis and Clinical Significance (Figure 1).

**Lipid Profile Parameters and Blood Compartments**

**Plasma Lipids**

Plasma lipid parameters—including total cholesterol, triglycerides, LDL-C, HDL-C, and VLDL are well-established indicators of cardiovascular risk (Rifai *et al*., 2008). Diabetic dyslipidemia typically manifests as hypertriglyceridemia, increased small dense LDL particles, and reduced HDL-C levels (Taskinen, 2003; Vergès, 2015). These lipid abnormalities accelerate atherosclerotic processes through enhanced lipid oxidation and vascular inflammation (Libby, 2021).

**Lipids in Cellular Blood Components**

Red blood cell (RBC) membranes are composed of phospholipids, cholesterol, and glycolipids, which regulate membrane flexibility and oxygen transport efficiency (Berg *et al*., 2019). Chronic hyperglycemia alters RBC membrane lipid composition, leading to decreased phospholipid content, increased lipid peroxidation, and reduced membrane fluidity (Lippi *et al*., 2010; Cohen *et al*., 2008). These changes impair microcirculatory flow and may contribute to vascular complications in diabetes (Brownlee, 2005).

**Relationship between HbA1c and Lipid Profile**

**Positive Associations with Atherogenic Lipids**

Numerous studies have demonstrated a positive correlation between HbA1c levels and total cholesterol, triglycerides, LDL-C, and VLDL (Ginsberg, 2000; Mooradian, 2009). Poor glycemic control increases hepatic VLDL production and reduces insulin-mediated suppression of lipolysis, resulting in elevated circulating free fatty acids and triglycerides (Boden, 2011; Feingold and Grunfeld, 2000). Population-based studies have also shown that higher HbA1c values are associated with atherogenic lipid patterns even in non-diabetic individuals, highlighting the continuum of cardiometabolic risk (Després, 2012; Thomas *et al*., 2016).

**Inverse Relationship with HDL-C**

HDL-C levels are often inversely associated with HbA1c (Sacks and Campos, 2003). Reduced HDL-C impairs reverse cholesterol transport and diminishes antioxidant and anti-inflammatory functions, thereby increasing cardiovascular risk (Rosenson, 2016). This inverse relationship underscores the compounded impact of poor glycaemic control on lipid-mediated vascular protection.

**Cellular Lipid Alterations and Glycation**

Non-enzymatic glycation of membrane proteins and lipids alters RBC deformability and increases susceptibility to oxidative damage (Witztum and Steinberg, 2001). Glycated RBC membranes exhibit increased rigidity, contributing to impaired microvascular perfusion and endothelial dysfunction (Lippi *et al*., 2010; Brownlee, 2005) (Figure 2). Relationship between HbA1c and Lipid Profile (Figure 2).

**Pathophysiological Mechanisms Linking Glycemia and Lipid Metabolism**

Insulin resistance serves as a central mechanism linking hyperglycemia and dyslipidemia (Reaven, 1988). Reduced insulin sensitivity promotes increased adipose tissue lipolysis, enhanced hepatic triglyceride synthesis, and secretion of VLDL particles (Vergès, 2015). Oxidative stress and chronic low-grade inflammation further exacerbate lipid abnormalities and vascular injury (Libby, 2021; Rimm *et al*., 2001).

**Clinical and Diagnostic Implications**

The strong biochemical association between HbA1c and lipid parameters highlights the importance of integrated metabolic monitoring. Combined assessment improves cardiovascular risk prediction and supports early intervention strategies (Chait and Bornfeldt, 2009; Kannel and McGee, 1979). Clinical trials have demonstrated that intensive glycemic control, when combined with lipid management, significantly reduces cardiovascular complications (Nathan *et al*., 2005; UK Prospective Diabetes Study Group, 1998).

## CONCLUSION

This systematic review demonstrates a robust biochemical relationship between HbA1c and lipid profile parameters across plasma and cellular blood components. Elevated HbA1c levels are consistently associated with atherogenic lipid patterns and altered cellular membrane lipid composition, contributing to increased cardiovascular risk. Integrated evaluation of glycemic and lipid biomarkers is essential for early detection, risk stratification, and personalized management of metabolic disorders.

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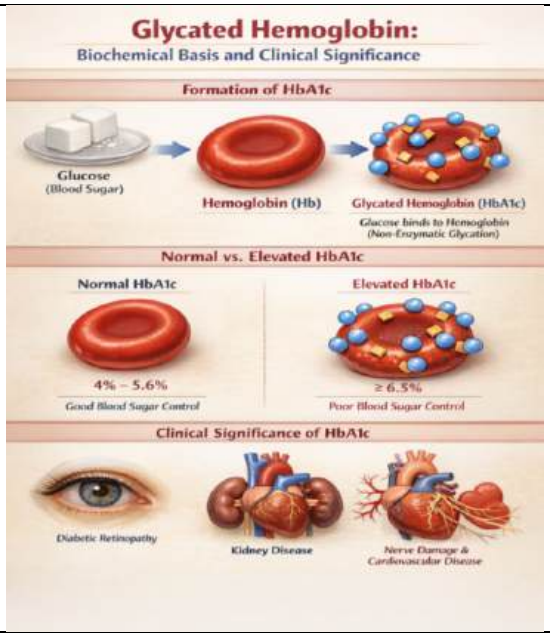



**Figure 1: Glycated Hemoglobin: Biochemical Basis and Clinical Significance**

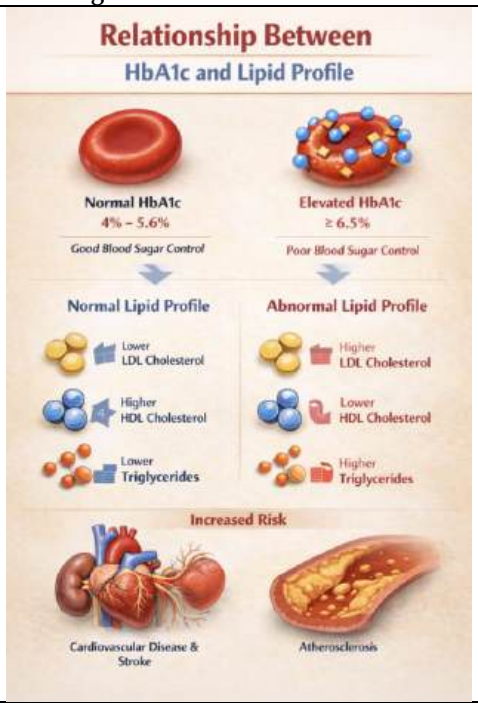



**Figure 2: Relationship Between HbA1c and Lipid Profile**

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RESEARCH ARTICLE

# Hindi Translation, Validation and Test-Retest Reliability of the Smoking Abstinence Self-Efficacy Questionnaire in Patients with Chronic Ischemic Heart Disease: A Study Protocol

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## ABSTRACT

Smoking is widely recognized as one of the biggest threats to human health, causing around 5 million deaths globally each year. Statistics reveal that smoking is responsible for 90% of lung cancers, 75% of respiratory diseases, 50% of cardiovascular diseases, 30% of deaths among those aged 30 to 50, and 12% of all deaths. The Smoking Abstinence Self-Efficacy Questionnaire (SASEQ) is a six-item self-efficacy scale used in smoking cessation trials. While the SASEQ has been studied in Latino, Iranian, Chinese, and Malay populations, there are no existing studies on its effectiveness in the Indian population. This study aims to translate the SASEQ into Hindi and assess its validity and test-retest reliability. Two translators, one with a medical background and another without, will translate the original scale into Hindi, following Beaton et al.'s guidelines. An observer will combine the translations and produce a T1 version of the scale. This version will undergo reverse translation and will be reviewed by an expert panel to ensure accuracy. After evaluating content validity, the pre-final version will be tested with patients to confirm its validity. The translated version will be reviewed by the ethics committee, and test-retest reliability will be measured by testing subjects on two different occasions and calculating the correlation.

In conclusion, this research will establish the validity and reliability of the Hindi version of the SASEQ among individuals with chronic ischemic heart disease.

**Keywords**: Psychometrics, Self-efficacy,Myocardial Ischemia,Smoking, Test-retest reliability.

## INTRODUCTION

Tobacco use, particularly smoking, is a leading cause of preventable illness and death worldwide, and in India it constitutes a major public health concern. Smoking is a well-recognized behavioral risk factor that significantly contributes to the global burden of disease, a relationship first clearly demonstrated by the landmark 1950 study of British doctors and further substantiated by more than seven decades of research [1]. Tobacco smoke contains over 7,000 chemical compounds, of which more than 250 are toxic or carcinogenic, including nicotine, nitric oxide, benzpyrene, arsenic, and cadmium, leading to addiction, impaired oxygen transport, cancer, and damage to nearly every organ system, while also increasing the risk of respiratory infections and multisystem diseases [2]. According to the World Health Organization, tobacco use is responsible for approximately one in ten deaths globally, causing around 7 million deaths each year, with nearly 1.1 billion smokers worldwide and markedly higher smoking prevalence among men, particularly in developing countries [3,4]. During the COVID-19 pandemic, active smoking was associated with increased disease severity and mortality among hospitalized patients, potentially due to upregulation of the ACE-2 receptor, heightened inflammatory responses, and immune dysfunction, although methodological limitations in systematic reviews suggest possible bias in estimating this effect [5,6]. Smoking also increases infection risk through hand-to-mouth behavior and by damaging lung tissue and weakening immune defenses, thereby worsening respiratory disease outcomes [7]. Effective cessation strategies, such as the Ottawa Model for Smoking Cessation, emphasize a standardized, interdisciplinary approach to tobacco treatment and have demonstrated success across healthcare systems [8]. Within relapse models, self-efficacy—or confidence in one's ability to quit—has been identified as a key predictor of successful smoking cessation [9]. The Smoking Abstinence Self-Efficacy Questionnaire (SASEQ), a six-item scale scored from 0 to 24, evaluates this construct and has shown that self-efficacy, rather than willpower or motivation alone, is strongly associated with sustained abstinence, underscoring its importance as a target for smoking cessation interventions [10,11].

## METHODS

The Institutional Ethical Committee has approved the ethical statement (IEC/MMDU/3439). The study will follow the Helsinki Declaration, CIOMS International Ethical Guidelines, and National Ethical Guidelines from the Indian Council of Medical Research. Each patient with chronic ischemic heart disease must sign an informed consent form before participating.

**Translation Procedure**

Beaton et al.'s guidelines are the foundation for this study.[12]

**Stage of Forward Translation**

Two forward translations from English to Hindi will be created for the scale. SASEQ T1 will be translated by someone with a medical background, while SASEQ T2 will be done by a translator without this background. The translations by the non-medical translator will be labeled "naive" as they are more likely to grasp the original scale's true meaning. They will also clarify any ambiguities in the original scale by employing language more familiar to the target audience. The success of this stage relies on both translators being fluent in Hindi and English. They will address any differences between their versions to correct grammatical errors.

**Stage of Synthesis**

This step aims to combine the forward translations. The lead investigator will stay in contact with the translators via WhatsApp or email. To ensure accuracy in meaning, the investigator will synthesize the SASEQ T1 and T2 translations. This step is crucial for reviewing the material and revising terms that deviate from the original scale.

**Stage of Back Translation**

SASEQ BT1 and BT2 will translate the T1-2 version back into English without knowledge of the translation process. This ensures the translated text is accurate and helps identify any unclear terms. While consistency between the back translation and source version suggests accuracy, it cannot fully guarantee it due to potential forward translation errors. This step, carried out by two non-medical English-speaking translators, aims to minimize bias and highlight any significant issues or misunderstandings.

**Content Validation Procedure - Expert Committee Review**

An online Delphi review committee will evaluate the Hindi translation of the SASEQ. The experts will review the translated scale, consisting of medical professionals with at least five years of experience treating stroke patients in India. If necessary, a new panel will be recruited for a second Delphi survey. The experts will assess the scale via Gmail and rate items as follows: (1) Not relevant; (2) Needs revision; (3) Relevant but needs minor revision; and (4) Very relevant. This four-point rating system is commonly used for content validation. The final documentation and justification for the Hindi scale will be guided by expert feedback. Key questions include: Do the phrases convey the same meaning? Are there multiple interpretations? Are there errors or grammatical issues?[16]

**Phase of Review of the Final Translated (SASEQ)**

Patients will be asked to review a draft of the translated SASEQ questionnaire to evaluate their understanding and practicality. Each item's responses will be examined to ensure accuracy in interpretation in a real-world setting.

**Transmission of the Documented Records for Submission**

All paperwork regarding the translated scale must be submitted to the responsible committee as the last step. This ensures the translation was conducted ethically and that all necessary procedures were followed. The process will be verified, and reports will accurately reflect the operations followed. The organization will confirm that a fair translation was achieved without altering the original content.

**Content validation**

A tabular structure will be used to verify each scale item and record it in the Item-level Content Validity Index (ICVI). Email or WhatsApp will be used for primary validation with the Delphi method. The Item-level Content Validity Index (I-CVI) and Scale-level Content Validity Index (S-CVI) will evaluate the items. The Averaging method (S-CVI/Ave) and Universal Agreement computation (S-CVI/UA) will be applied. According to Lynn's (1986) standards, to achieve good content validity, a group of six to ten review experts should have an S-CVI/Ave of at least 0.90 and a minimum I-CVI of 0.78. The scale will be updated in the original language until these levels are reached. Another Delphi round will be held—potentially with a new panel—if the S-CVI/Ave does not reach 0.80.

**Reliability**

To ensure consistent results over time, test-retest reliability will be evaluated by measuring participants twice and calculating the correlation. This method confirms that the scale produces reliable findings across multiple testing periods.

**Study participants and setting**

Fifty-one individuals with chronic ischaemic heart disease will be chosen from a tertiary care super specialty hospital according to Beaton's recommendations to assess the validity of the final translated Hindi SASEQ. Male and female volunteers aged 25 to 80, who have a history of smoking, possess any heart-related condition, can understand Hindi, and have the mental capacity to comprehend the scale will be included in the study. Exclusion criteria include

individuals with severe cognitive impairments, pregnant women, non-smokers and substance abusers, and non-Hindi speakers.

**Statistical Analysis**

The Item-level Content Validity Index (I-CVI) and the Scale-level Content Validity Index (S-CVI/Ave) will assess the content validity of the translated scale. I-CVI should be at least 0.78, and SCVI/Ave should be close to 0.90 after Delphi surveys with six to ten experts for good to exceptional validity. Both absolute and relative methods will be used to evaluate reliability. Based on data normality, the intra-class correlation coefficient (ICC), Pearson correlation, and Spearman Rank correlation will assess relative reliability, measuring consistency across several observations. Cronbach's alpha, which ranges from 0 (no consistency) to 1 (perfect consistency), will gauge internal consistency. Additionally, Bland-Altman plots will evaluate measurement agreement and identify any biases or variability, while regression analysis and coefficients will examine predictive consistency. [13-16]

## DISCUSSION

Translating, validating, and testing the reliability of the Hindi Smoking Abstinence Self-Efficacy Questionnaire (SASEQ) scale could significantly enhance cardiac rehabilitation for Hindi-speaking individuals. This effort aims to provide more accurate assessments and personalized rehabilitation programs, thereby improving heart-related care for this group. The SASEQ is noted for its validity, reliability, and sensitivity to changes in sensorimotor function over time, ensuring consistent assessment and outcome comparisons across various rehabilitation contexts. We are translating the SASEQ to better serve Hindi-speaking communities since there is currently no reliable and accurate Hindi version available for assessing patients with chronic ischemic heart disease.

## CONCLUSION

In conclusion, this study aims to evaluate the test-retest reliability and content validity of the Hindi version of the SASEQ questionnaire in patients with chronic ischemic heart disease.

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**Author contributions**

Each author has contributed equally to the study design, methods, and report.

**Competing Interests**

The authors assert that they have no competing interests.

**Conflict of Interest**

The authors don't have any conflict of interest

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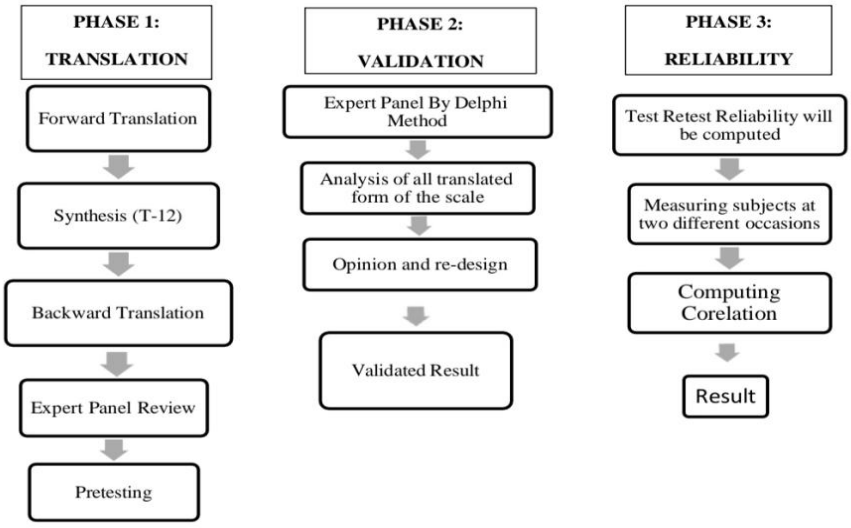



**Figure 1 .Process of Translation, Validation and Reliability**

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RESEARCH ARTICLE

# Green Synthesis, Characterization of Silver Nanoparticles and its Antimicrobial Potential against Clinical Pathogens

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## ABSTRACT

The green synthesis of nanoparticles (NPs) received considerable importance in the recent year's, because of its wide application, particularly in biomedical research. This study reports the green synthesis of silver nanoparticles (AgNPs) using an endophytic fungus. It was observed that the aqueous silver (Ag+) ions, exposed to a filtrate of *Penicillium oxalicum*, reduced in solution resulting in the formation of stable AgNPs. The synthesized AgNPs were characterized by means of FTIR and TEM analyses. The reduction of Ag+ ions to elemental silver was characterized by Energy-dispersive X-ray (EDX) Spectroscopy. The synthesized AgNPs showed significant antifungal activity against *Candida albicans* at 10 µg/ml. The NPs also showed antibacterial activity against both gram negative and gram positive bacteria. Hence, this can be used as a promising alternate drug against infectious diseases.

**Keywords:** Silver nanoparticles, TEM, FTIR, Antibacterial activity, Anticandidal activity

## INTRODUCTION

Emerging as a rapidly growing field, with the application in materials science, biological science and recently in medical science [1], nanotechnology and the use of nanoparticles is gaining impetus in the present century as they

possess defined chemical, optical and mechanical properties. The metallic nanoparticles are most promising as they show potential antimicrobial properties due to their large surface area to volume ratio [2]. Possession of their small size, nanoparticles exhibit different properties when compared to the bulk material. In the present predicament, silver nanoparticles (AgNPs) have appeared as a propitious antibacterial candidate in the field of medicine [3, 4, 5, 6] when compared to other metals in their nanoform. The unique properties of silver nanoparticles (AgNPs) make them a valued nanomedicine among NPs [7, 8, 9]. Silver nanoparticles are synthesized by physical, chemical and biological methods [10,11]. The latter is an advance over chemical and physical methods, as it is biocompatible, cost-effective and environment friendly [12, 13] by deploying fungi, bacteria and plants for synthesis of nanoparticles [11, 14, 15]. Microbes are being up-and-coming biological source for synthesizing metal nanoparticles [16], however all the organisms do not have the ability to convert metals to its nano forms. Fungi are reported to be potential agents in the biological synthesis of metal nanoparticles [17], advantageous with secretion of large amounts of bioactive compounds including enzymes and their biomass is easy to manage [18]. Though biosynthesis of nanoparticles of different elements are reported from both pathogenic and non-pathogenic fungi [18, 19, 20, 21], only very few endophytic fungi *Aspergillus clavatus, Epicoccum nigrum* have been reported to bio-synthesize AgNPs [22, 23, 24]. Clearly, endophytic fungi are relatively unexplored as a potential resource for the synthesis of AgNPs. Endophytes characteristically synthesize various bioactive compounds with diverse biological activity [25]. Nanoparticle-based antimicrobial formulations could be plausible bactericidal materials [26]. The green synthesis of nanomaterials are in need due to increasing environmental problem worldwide [27]. The present study focuses on the synthesis of AgNPs through an environmentally friendly method using an endophytic fungus, *Penicillium oxalicum.* The synthesized silver nanoparticles were evaluated for its antifungal (*Candida albicans*) and antibacterial (gram positive, gram negative) activity under *in vitro* condition, to use as an alternative antimicrobial agent. To our knowledge extracellular synthesis of AgNPs by this fungus has not been reported so far.

## MATERIALS AND METHODS

**Source of Microorganism**

The fungus *Peniclillium oxalicum* isolated from the leaves of *Gymnema sylvestre*[28] was maintained in potato dextrose agar slant at 27ºC. Bacterial strains namely *Escherichia coli* (gram negative) *Salmonella typhi* (gram negative), *Bacillus subtilis* (gram positive), and Fungal strain (*Candida albicans*) were obtained from the culture collection centre and maintained in LB agar at 4ºC.

**Green synthesis of silver nanoparticles**

The fungus *P. oxalicum* was grown in 250 ml Erlenmeyer flasks containing 100 ml Czapek'sdox broth (CDB) at 27ºC for 72 hrs. After incubation, mycelialbiomass was separated by filtration, washed with sterile distilled water to remove the traces of media components. Typically 15g of biomass (wet weight) was re-suspended in 100ml sterile double distilled water for 48 h at 27ºC in an Erlenmeyer flask and incubated. After incubation, the cell filtrate was filtered by Whatman filter Paper No.1. To the cell filtrate silver nitrate was added (1mm), and incubated at room temperature under dark condition for 24 h.

**Characterization of silver nanoparticles**

After incubation of the above mixture, the formation of AgNPs was analyzed by optical measurements at different time interval using a UV-visible spectrophotometer (Shimadzu, Japan) and scanning the spectra between 200 and 600 nm at the resolution of 1nm. The FTIR spectrum of the dried sample was recorded on a Perkin Elmer 1600 instrument in the range of 400-4000$cm^{-1}$.Analysis for the confirmation of elemental silver was carried out using Energy-dispersive X-ray (EDX) spectroscopy. Samples for transmission electron microscopy (TEM) were prepared by drop coating of the AgNPs solution into carbon-coated copper grid. The size and morphology of AgNPs were characterized by TEM (JEOL model 1200 EX).

**Antimicrobial Activity**

**Disc diffusion assay**

A disc diffusion method was used to assay the effect of extracellularly synthesized AgNPs for bactericidal activity against test strains on Muller-Hinton agar plates. The standard antibiotic discs were purchased from Himedia (Mumbai, India). A single colony of each test strain was grown overnight in Muller–Hinton liquid medium on a rotary shaker (200 rpm) at 37ºC. From this, 100 µl of inocula was applied to the petri plates, spread evenly and standard antibiotic discs (penicillin/amphicillin), sterile discs impregnated with and without AgNPs (10µg) were placed. The petri plates were incubated at 37ºC for 24-48 hours and the zones of inhibition were measured. The assays were performed in triplicates. For *C. albicans*, the cultures were grown in CzapekDox agar medium with Fluconazole as a standard.

**Growth Kinetics**

Further, to test the effect of synthesized silver nanoparticles on the growth of test bacteria and fungi, 100 µl of fresh cultures were inoculated separately on nutrient and czepakdox broth media respectively and incubated at 37°C in an orbital shaker at 120 rpm. After the initial log phase (2h), different concentrations of AgNPs (0, 10, 20, 40 µg/ml) were added to the growing culture media and incubated as above. For the control, sterile water was used. Positive controls with respective antibiotics were tested separately. Optical density was measured at 600nm after every 4h using UV- Visible spectrophotometer (Shimadzu, Japan). For *C. albicans*, the culture was measured after 48h. All the experiments were carried out in triplicates. The cultures were also used to test for the potency of synthesized silver nanoparticles for its bactericidal and fungicidal activity. The silver nanoparticle treated and control cultures were withdrawn from each culture tube after 2h and serial dilution was done up to 5 fold using sterile water. 100µl of each (diluted) culture was spread over the nutrient agar/ potato dextrose agar plate and incubated overnight at 37°C/ room temperature. The colony counts were made using the plate count method.

**Statistical analysis**

All data has been expressed as the average ± standard deviation. All the data were subjected to one-way analysis of variance to determine the significance of individual differences at $p<0.01$ and 0.05 levels. All statistical analyses were conducted using SPSS 21 software support.

## RESULTS AND DISCUSSION

The study of the biosynthesis of nanomaterials offers a valuable contribution to nanotechnology. The biosynthetic methods have been investigated as an alternative to chemical and physical method. In this regard the endophytic fungus *P.oxalicum* proves to be an important biological component for extracellular biosynthesis of stable AgNPs. The UV-visible spectra recorded from the *P. oxalicum* reaction vessel at different time of reaction are plotted (Fig. 1). It is observed from the spectra that the surface plasmon resonance band of AgNPs occurs at 423 nm, and this absorption steadily increases intensity as a function of time of reaction, which is very specific for silver nano particles [29, 30]. The reduction of silver ions was observed after 2 h with maximum reduction at 24 h. Our results are in accordance with earlier reports [18, 31]. The formation of silver nanoparticles by *P.oxalicum* was observed within 24 hours. Compared with the previously mentioned times from several hours to several days for the synthesis of AgNPs employing fungi [22, 30, 32], the time required in this study is significantly shorter. This proves to be an important step in the development of green processes for the synthesis of AgNPs. The FTIR spectrum of biosynthesized AgNPs is shown in Fig. 2. The spectrum shows the presence of bands at 3470.2, 1637, 1384.4 and 959.58 $cm^{-1}$. The peak at 3470.2 $cm^{-1}$ refers to the stretching vibrations of primary amines. The peak at 1637 $cm^{-1}$ is due to the carbonyl stretch vibrations in the amide linkages of proteins, indicating the presence of proteins as ligand for AgNPs, which might play a role in the stability of synthesized nanoparticles. Mandal *et al.* [33] reported that free amine or cysteine groups in proteins bind to nanoparticles and thereby stabilizing the AgNPs. The carbonyl groups of amino acid residues have a strong ability to bind to silver ions [34]. These evidences suggest that the release of extracellular protein molecules could possibly perform the function of the formation and stabilization of AgNPs in aqueous medium. The

application of biological systems for the synthesis of silver nanoparticles has already been reported [35, 36]. However the exact mechanism leading to the formation of AgNPs by these organisms is yet to be elucidated. Ahmed *et al*. [31] have reported that certain NADH dependent reductase was involved in the reduction of silver ions by *F.oxysporum*. In the present study, for the confirmation of AgNPs, EDX spectroscopy analysis was performed, which showed the presence of elemental silver by the sharp signals (Fig. 3). EDX analysis shows an optical absorption band peak in the range of 3 to 4 Kev. This is typical for the absorption of metallic silver nanocrystallites [37]. The TEM technique used to visualize the size and shape of the extracellular biosynthesized AgNPs is shown in Fig. 4A. The shape of the nanoparticle is spherical. The TEM micrograph suggests that particle diameters ranged from 10 to 50 nm (Fig. 4B). The particle size distribution histogram determined from TEM (Fig. 4B) indicates that there is variation in particle size, with an average size of the particles was 25±.7nm. The effect of silver nanoparticles was investigated against bacterial (gram positive and gram negative) and fungal (*C. albicans*) strains using the disc diffusion method. The diameter of the inhibition zone (mm) against test strains is shown in Fig. 5. The zone of inhibition against *C. albicans* is observed to be 18 mm and the standard antibiotic (Flucanozole) showed only 10 mm (Fig. 5). The inhibition zone reported for *C. albicans* by AgNPs was much higher than that of the previous report [38, 39]. *C. albicans*, a dimorphic fungi cause candidiosis in mammals and human beings. It also causes infection in patients suffering from AIDS, cancer etc [40]. The green synthesized AgNPs exhibited a very high antifungal activity towards *C. albicans*. Recent literature reports that the silver nanoparticles interfere with fungal cell cycle, thereby causing their destruction [12]. Kim *et al* [41] reported that silver nanoparticles cause the rupture of fungal cell membrane by altering membrane potential. The green synthesized AgNPs inhibits both gram negative and gram positive bacteria. The highest zone of inhibition was observed for *E. coli* (Fig. 5). The zone of inhibition might be due to the diffusion of silver ions from the AgNPs which triggers cell lysis or due to electrostatic interaction between silver nanoparticles and cell wall of bacteria leading to destruction of cell wall [16, 17, 42, 43]. Significant growth inhibition was observed in all the bacterial strains treated with grensynthesized AgNPs at 10 µg/ml (Fig. 6). These NPs emerged as an essential tool to combat the drug resistance in microbes. The high surface area to volume ratio of the nanoparticles with distinct physico-chemical properties made to be the reason for the effectiveness against resistant strains.

## CONCLUSION

In the present study, AgNPs were synthesized through green route using endophytic fungus. This method of synthesis is very easy and environment friendly. Further synthesized NPs showed antibacterial and anticandidal activity at very less concentration (10µg/ml). This paves way for the application of biosynthesized colloidal AgNps as a promising therapeutic agent against bacterial, fungal (*Candida*) infections.

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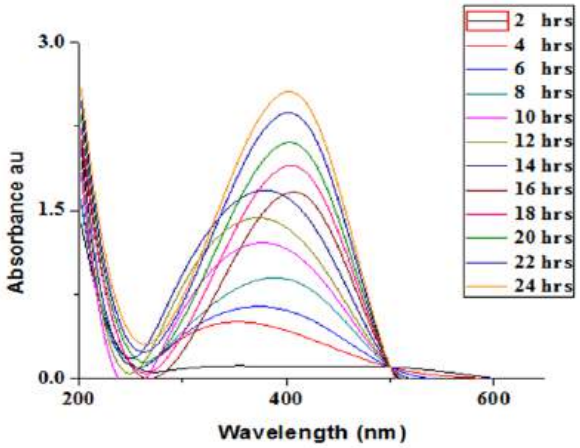



**Fig. 1: UV-visible spectra of green synthesized AgNPs at different time intervals.**

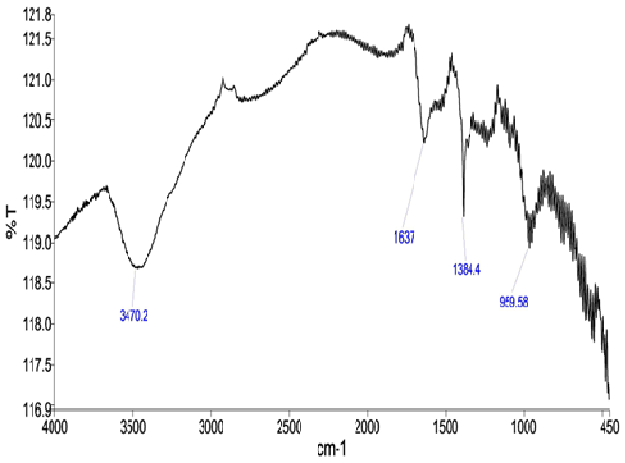



**Fig. 2: FT-IR spectrum green synthesized AgNPs.**

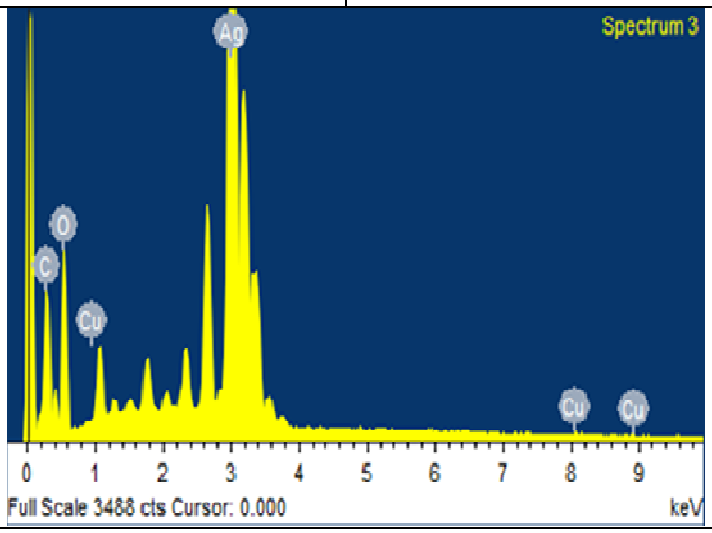



**Fig. 3: Energy- dispersive spectroscopy spectrum of silver nanoparticles (various x-ray emission peaks are labelled) .**

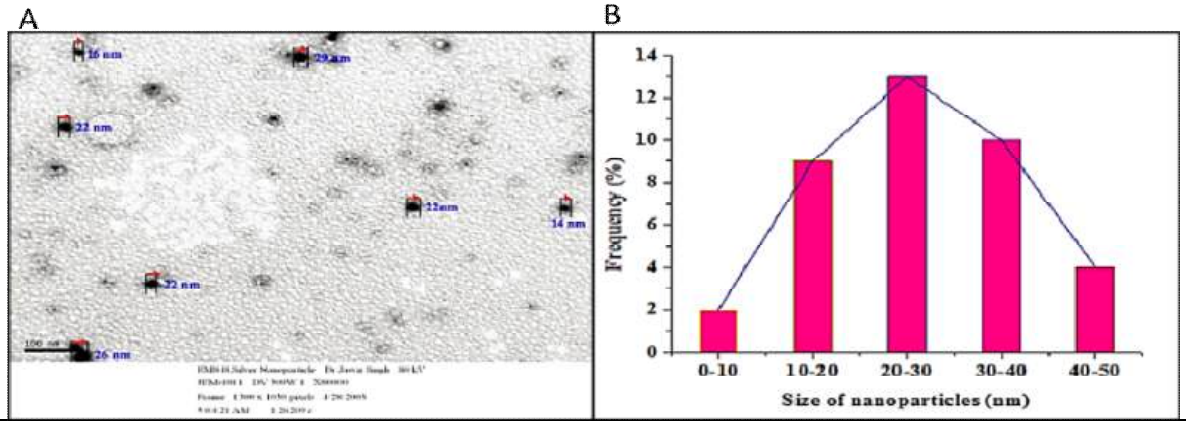



**Fig 4: HRTEM micrograph of green synthesized AgNPs (A) scale bar =100 nm. Particle size histogram (B).**

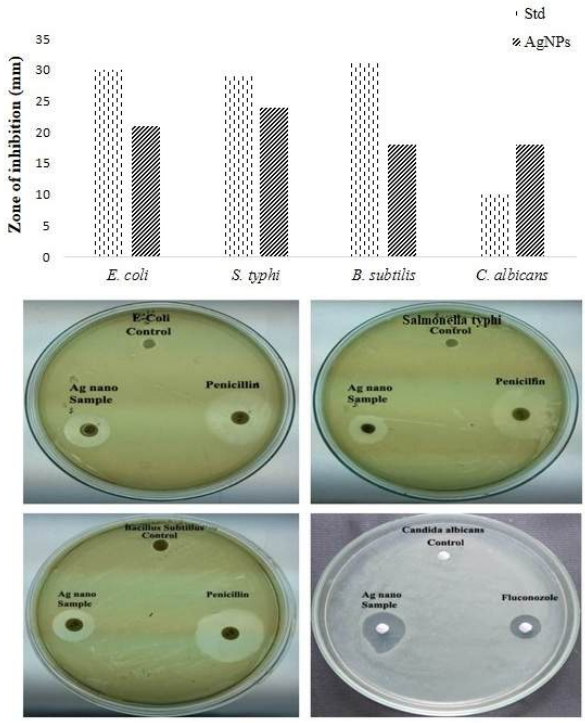



**Fig 5: Effect of green synthesized AgNPs on growth of bacteria and fungus by diffusion method.**

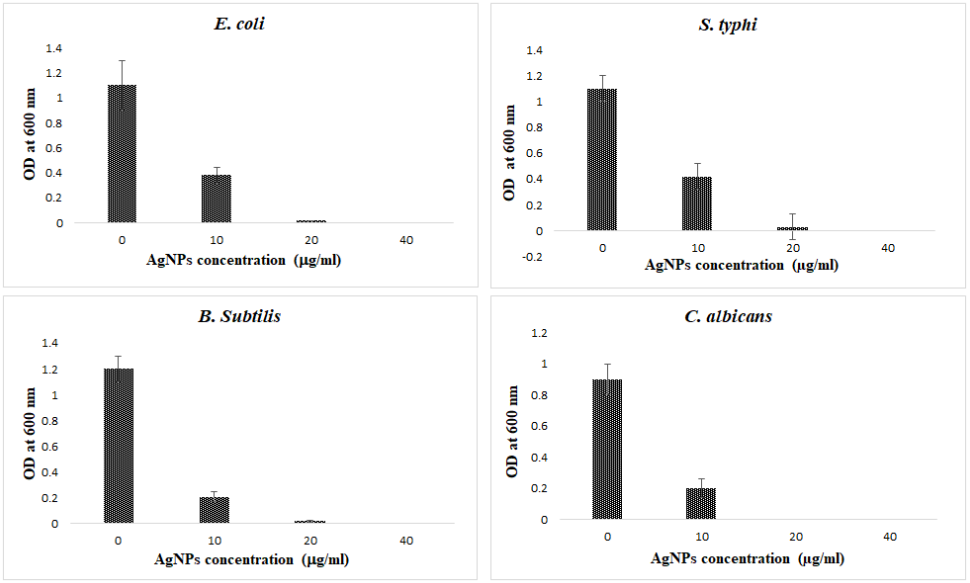



**Fig 6: Antimicrobial activities of green synthesized AgNPs in broth media.**

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**CASE STUDY ARTICLE**

# Ayurvedic Management of *Prameha* through *Virechana Karma* and *Shamana Chikitsa*: A Case Study

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## ABSTRACT

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder with rising global prevalence, largely attributed to sedentary lifestyle and dietary indiscretions. In Ayurveda, T2DM closely correlates with *Prameha*, particularly *Apathyanimittaja Prameha* and *Sthula Pramehi*, where *Kapha* and *Meda* predominance with *Agni-mandya* forms the core pathology. Classical Ayurvedic texts emphasize *Sodhana* therapy in such conditions to eliminate accumulated *Dosas*, followed by *Samana Chikitsa* for long-term disease control. The present case study evaluates the therapeutic effect of *Virechana karma* followed by *Samana* therapy in a patient with T2DM. A 42-year-old male shopkeeper with a known history of T2DM for 1.5 years and classical *Prameha laksaṇas* was managed with a sequential Ayurvedic treatment protocol. The intervention included *Dipana–Pachana* using *Amapachaka Vaṭi* and *Chitrakadi Vaṭi*, followed by *Snehapana* with *Murchita Go-ghṛita*, *Sarvanga Abhyanga* with *Murchita Tila Taila*, and classical *Virechana karma* using *Trivṛt Avaleha*. Post-*Sodhana*, a seven-day *Saṃsarjana Krama* was followed. *Samana Chikitsa* with *Mehabhyadi Kasaya* and *Nisha-Amalaki Churṇa* was administered for three months. Subjective symptoms, body parameters, and glycaemic indices were assessed before and after treatment. *Virechana* resulted in 22 *Vegas*, indicating *Pravara Suddhi*. Significant relief was observed in polyuria, polydipsia, polyphagia, fatigue, heaviness, and excessive sweating. A weight reduction of 4.1 kg was noted. Objective parameters showed improvement, with reduction in fasting blood sugar, post-prandial blood sugar, and HbA1c levels after completion of therapy. This case demonstrates that classical *Virechana karma* followed by appropriate

*Samana Chikitsa* can effectively improve both subjective symptoms and objective metabolic parameters in *Prameha* corresponding to T2DM. The findings suggest the potential role of Ayurvedic detoxification and maintenance therapy in metabolic disorders, warranting further clinical evaluation.

**Keywords:** Diabetes Mellitus,*Panchkarma, Prameha, Virechana*

## INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) represents the most prevalent form of diabetes mellitus and is primarily characterized by persistent hyperglycaemia arising from insulin resistance along with a relative deficiency of insulin secretion[1]. The condition has emerged as a major global health concern due to its rapidly increasing prevalence and its association with significant morbidity. Epidemiological data indicate a steady rise in the number of individuals affected by diabetes worldwide, underscoring the urgent need for effective, sustainable, and holistic management strategies. In Ayurveda, diabetes mellitus is described under the broad clinical entity of *Prameha*, a group of metabolic disorders marked predominantly by increased frequency and abnormal characteristics of urination[2]. The etiopathogenesis of *Prameha* is closely linked to overnutrition, sedentary habits, and impaired metabolic regulation, which parallels the modern understanding of obesity-associated metabolic syndrome and Type 2 diabetes. Among the subtypes of *Prameha*, Type 2 Diabetes Mellitus closely resembles *Sthula Pramehi*, also referred to as *Apathyanimittaja Prameha*, wherein faulty dietary practices and unhealthy lifestyle play a central causative role[3]. Classical Ayurvedic texts emphasize that *Prameha*, especially in its *Bahu-Doshavastha* (state of excessive accumulation of *Dosas*), requires elimination therapy as the primary line of management. Acharya Charaka clearly advocates *Sodhana Chikitsa* in such conditions, stating that purification therapies are essential for expelling morbid *Doshas* from the body before instituting palliative measures[4]. Among the Panchakarma procedures, *Virechana Karma* holds particular importance in *Prameha* due to its effectiveness in eliminating vitiated *Pitta* and *Kapha Doshas* through the nearest and appropriate route, thereby restoring metabolic balance and improving *Agni*. Following *Sodhana, Samana Chikitsa* plays a crucial role in maintaining glycaemic control, enhancing tissue metabolism, and preventing disease progression. Alongside medicinal interventions, Ayurveda strongly advocates lifestyle regulation, including dietary discipline (*Ahara*), daily regimen (*Dinacharya*), and physical activity, to address the root causes of the disease. Yogic practices, which have been traditionally recommended as supportive measures, contribute to metabolic regulation, improve insulin sensitivity, and promote overall endocrine harmony. This case study aims to highlight the therapeutic effectiveness of an integrated Ayurvedic approach employing *Virechana Karma* as a primary *Sodhana* procedure followed by *Samana Chikitsa* in the management of *Prameha.*

**Case Presentation**

**Patient Information**

A 42-year-old male patient, a shopkeeper by occupation, presented to the Kayachikitsa outpatient department on 16/07/2025 with complaints of increased frequency of micturition for the past 2–3 months (5–6 times during the day and 1–2 times at night), excessive thirst (*Pipasadhikya*), dryness of mouth, and generalized fatigue. He also reported increased appetite (*Ksudhadhikya*), heaviness of the body (*Gaurava*), lethargy (*Alasya*), excessive sweating (*Ati-sweda*), and mild burning sensation during urination, suggestive of *Prameha laksaṇas*, since the last 2–3 months[5,6]. The patient was a known case of Type 2 Diabetes Mellitus for the past 1.5 years and was on irregular oral hypoglycaemic medication. There was no history of hypertension, dyslipidaemia, thyroid disorder, or any other chronic systemic illness. He had no significant past medical or surgical history. No family history of diabetes mellitus was reported. The patient had noticed a gradual weight gain of approximately 7–8 kg over the last one year, which he attributed to a sedentary lifestyle, long working hours at his shop, irregular meal timings, and frequent intake of calorie-dense foods. He experienced progressive worsening of symptoms, which prompted him to seek further evaluation and management.

**Clinical Findings**

On general examination, the patient was conscious, oriented, and moderately built with apparent central obesity. Vital parameters were within normal limits. No pallor, icterus, cyanosis, clubbing, lymphadenopathy, or pedal edema was observed. The patient complained of polyuria, polydipsia, polyphagia, fatigue, heaviness of the body, excessive sweating, and lethargy. On systemic examination, cardiovascular, respiratory, and abdominal systems were found to be within normal limits. There were no features suggestive of diabetic neuropathy, retinopathy, or nephropathy at the time of presentation. From an Ayurvedic perspective, the clinical presentation was suggestive of *Kapha-pradhana Prameha* with features of *Sthulata*, *Gaurava*, *Alasya*, and *Ati-mutrata*.

**Diagnostic Assessment**

Based on the patient's history, clinical presentation, and laboratory investigations, a diagnosis of Type 2 Diabetes Mellitus was confirmed. Biochemical evaluation revealed elevated fasting blood glucose and post-prandial blood glucose levels, with raised HbA1c, indicating poor glycaemic control. Routine haematological parameters were within normal limits. Renal function tests and urine routine examination did not reveal any evidence of diabetic nephropathy or urinary tract infection. From the Ayurvedic standpoint, the condition was diagnosed as *Apathyanimittaja Prameha*, specifically *Sthula Pramehi*, characterized by *Kapha* and *Meda* predominance, *Agni-mandya*, and *Bahudrava Sleşma*, correlating with the metabolic derangements seen in Type 2 Diabetes Mellitus.

**Therapeutic Intervention**

Considering the chronicity of the disease and the *Kapha–Meda* predominance, a classical *Sodhana* approach was planned, followed by *Samana Chikitsa*. Initially, *Dipana–Pacana* was administered to correct *Agni* and facilitate *Ama-pachana*, using *Amapachaka Vati* and *Chitrakadi Vati*until we got *Nirama Lakshanas*. This was followed by *Snehapana* with *Muchita Go-ghrita*, administered in increasing doses until attainment of proper *Samyak Snigdha Lakshanas*. Subsequently, *Sarvanga Abhyanga* with *Murchita Tila Taila* was performed, followed by *Svedana*. Thereafter, classical *Virechana karma* was administered using *Trivṛt Avaleha* as the *Virechana Yoga*, aiming at elimination of vitiated *Pitta* and *KaphaDoshas* along with *Dushya Meda*. After completion of *Sodhana*, *Samana Chikitsa* was prescribed for a period of three months, comprising *Mehabhyadi Kasaya* and *Nisha-AmalakiChurna*, with the intention of maintaining glycaemic control, improving metabolism, and preventing recurrence of *Prameha*. The patient was also advised appropriate *Pathya–Apathya* and lifestyle modifications in accordance with classical Ayurvedic principles throughout the treatment period. Therapeutic Intervention (Table 1)

**Follow-up and outcomes**

The patient was closely monitored throughout the treatment and follow-up period. Classical *Virechana karma* was completed successfully with the attainment of 22*Vegas*, indicating Pravara Suddhi. No adverse events were observed during or after the procedure. Following *Virechana*, a 7-day *Samsarjana Krama* was administered as per classical guidelines to gradually restore digestive capacity and stabilize *Agni*. Marked subjective improvement was observed after *Shodhana*. The patient reported significant reduction in polyuria, polydipsia, excessive appetite, fatigue, heaviness of the body, lethargy, and excessive sweating. A feeling of lightness (*Laghava*) and improvement in overall well-being were noted. A reduction in body weight of approximately 4.1 kg was recorded following *Virechana* and subsequent dietary regulation. Objective assessment after completion of *Shodhana* and during follow-up revealed notable improvement in glycaemic parameters. Fasting blood sugar (FBS), post-prandial blood sugar (PP2BS), and HbA1c levels showed a consistent and clinically meaningful reduction, indicating improved glycaemic control. These objective outcomes are detailed in Table 2 and 3. Sustained improvement was observed during the three-month *Samana Chikitsa* period, suggesting the combined efficacy of *Sodhana* followed by *Samana* therapy in the management of *Prameha* (Type 2 Diabetes Mellitus).

**Blood sugar levels before and after treatment (Table 2).**
**Body parameters before and after treatment (Table 3).**

## DISCUSSION

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder characterized by persistent hyperglycaemia due to insulin resistance and relative insulin deficiency. From an Ayurvedic standpoint, this condition closely correlates with *Prameha*, particularly *Apathyanimittaja Prameha* and *Sthula Pramehi*, which arise due to improper dietary habits, sedentary lifestyle, and derangement of *Agni*. These factors lead to vitiation of *Kapha* and *Meda*, with subsequent involvement of *Pitta* and *Mutravaha Srotas*. The present case highlights the role of classical Ayurvedic management, emphasizing *Sodhana* followed by *Samana Chikitsa*, in addressing both the root pathology and clinical manifestations of T2DM. The patient exhibited classical features of *Prameha* such as polyuria, polydipsia, polyphagia, fatigue, heaviness of the body, lethargy, excessive sweating, and gradual weight gain, indicating *Kapha–Meda pradhanya* along with *Agni-mandya* and *Ama* formation. In such conditions, mere symptomatic management may provide temporary relief, whereas elimination of the vitiated *doshas* becomes essential. Classical Ayurvedic texts advocate *Virechana karma* as a principal line of treatment in *Sthula Prameha*, particularly when *Kapha* and *Pitta* are predominant, as it facilitates systemic purification and restoration of metabolic balance[7]. The preparatory measures (*Purvakarma*) were crucial in optimizing the outcome of *Virechana*. *Dipana–Pacana* with *Amapachaka Vati* and *Chitrakadi Vati* helped kindle digestive fire and digest accumulated *Ama*, thereby improving metabolic efficiency. *Snehapana* with *Murchita Go-ghṛta* enabled internal oleation, aiding in mobilization of vitiated *Doshas* from peripheral tissues toward the gastrointestinal tract. External oleation through *Sarvanga Abhyanga* using *Murchita Tila Taila* further softened tissues and facilitated the smooth elimination of *Doshas*. Administration of *Virechana* using *Trivṛt Avaleha*[8] resulted in 22 *Vegas*, signifying *Pravara Suddhi*, which indicates effective elimination of morbid *Doshas*. A structured *Saṃsarjana Krama* for seven days was followed to restore digestive capacity and stabilize *Agni*. Post-*Virechana*, the patient reported marked subjective improvement, including reduction in polyuria, polydipsia, excessive appetite, fatigue, heaviness, and sweating. The sense of lightness (*Laghava*) and improved overall well-being experienced by the patient reflect successful *Sodhana*. The observed weight loss of 4.1 kg suggests reduction in *Meda Dushṭi*, which is a central pathological component in T2DM. Objective parameters further supported the clinical improvement. Reductions in fasting blood glucose, post-prandial blood glucose, and HbA1c levels after *Sodhana* and during the three-month *Samana* phase indicate sustained glycaemic control. The use of *Mehabhyadi Kaṣaya* and *Nisha-AmalakiChurna* [9] which is stated as *Agreya Dravya* by *Acharya Vagbhatt* for *Prameha Chikitsa* during *Samana Chikitsa* played a key role in maintaining metabolic stability[10]. These formulations possess *Pramehaghna*, *Lekhana*, *Agni-dipana*, and *Rasayana* properties, contributing to improved glucose metabolism and prevention of disease progression[11]. Overall, this case demonstrates that a sequential approach involving classical *Virechana karma* followed by appropriate *Samana Chikitsa* can effectively manage *Prameha* corresponding to T2DM by addressing both subjective symptoms and objective biochemical parameters. While the findings are encouraging, further systematic studies with larger sample sizes are required to substantiate the role of classical *Sodhana* therapies in the long-term management of metabolic disorders such as T2DM.

## CONCLUSION

This case highlights the effectiveness of a structured Ayurvedic approach using *Sodhana* followed by *Samana Chikitsa* in the management of *Prameha* corresponding to Type 2 Diabetes Mellitus. Classical *Virechana karma*, supported by *Dipana–Pachana*, *Snehapana*, and *Abhyanga*, achieved *Pravara Śuddhi* with marked relief in *Prameha Laksaṇas*, improvement in general well-being, and significant weight reduction. Subsequent *Samana* therapy with *Mehabhyadi Kaṣaya* and *Nisha-Amalaki Churna* helped maintain improved glycaemic control, as reflected by reductions in FBS, PP2BS, and HbA1c levels. Overall, the case suggests that correcting *Dosha–Dushya* imbalance through sequential detoxification and maintenance therapy may offer clinical benefits in selected patients of Type 2 Diabetes Mellitus, warranting further validation through larger studies.

**Patient Perspective**

The patient reported feeling significant improvement after the treatment. He experienced noticeable relief in symptoms such as frequent urination, excessive thirst, fatigue, heaviness of the body, and excessive sweating. After

*Virechana*, he felt a sense of lightness and improved energy levels, which helped him perform his daily activities at the shop more comfortably. The reduction in body weight further motivated him to follow dietary advice and lifestyle modifications. During the three-month follow-up period, the patient expressed satisfaction with the treatment outcomes, particularly the improvement in blood sugar levels, and felt confident about continuing Ayurvedic management for long-term control of his condition.

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**Table 1: Therapeutic Intervention**

| Date | Procedure | Drug | Dose |
|---|---|---|---|
| July 17, 2025 – July 20,2025 | *Dipana-Pachana* | *Chitrakadi Vati*<br>*Amapachaka Vati* | 2 tab. TDS<br>2 tab. TDS |
| July 21, 2025<br>July 22, 2025<br>July 23, 2025<br>July 24, 2025<br>July 25, 2025 | *Snehapana* (internal oleation) | *Murchhita Go-Ghrita* | 30 ml<br>60 ml<br>90 ml<br>120 ml<br>150 ml |

| | | | |
|---|---|---|---|
| July 26, 2025 | | | 170 ml |
| July 27, 2025 – July 29, 2025 | *Abhyanga* (massage) + *Swedana* (sudation) | *Murchhita Tila Taila* + *Bashwa sweda* with *Dashamula Kwatha* | 45 min |
| July 29, 2025 | *Virechana* | *Trivrut Avaleha* | 60 gm |
| July 30, 2025 – August 5,2025 | *Samsarjana Krama* | | |
| August 6, 2025 – November 7, 2025 | *Shamana Aushadha* | *Mehabhyadi Kashaya*<br>*Nisha Amalaki Churna* | 15ml. BD/bf<br>5gm. BD/bf |

**Table 2: Blood sugar levels before and after treatment**

| **Blood sugar level** | Before Treatment (July 17, 2025) | After Treatment (November 7,2025) |
|---|---|---|
| **Glycated Haemoglobin (HbA1c - %)** | 7.6 % | 6.3 % |
| **Fasting blood sugar (mg/dl)** | 157 mg/dl | 122 mg/dl |
| **Post-Prandial blood sugar (mg/dl)** | 218 mg/dl | 141 mg/dl |

**Table 3: Body parameters before and after treatment**

| **Body parameters** | Before Treatment (July 17, 2025) | After *Virechana* (August 5, 2025) | After Treatment (November 7,2025) |
|---|---|---|---|
| **Height (cm)** | 167 cm | 167 cm | 167 cm |
| **Weight (Kg)** | 82 kg | 77.9 kg | 76.3 kg |
| **BMI (Kg/m²)** | 29.4 | 27.9 | 27.4 |

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RESEARCH ARTICLE

# Knowledge and Awareness towards Maternal Periodontal Status during Pregnancy and its Associated Outcome among Gynaecologists of Ahmednagar District

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## ABSTRACT

Periodontal disease , chronic inflammatory condition is linked to several adverse pregnancy outcomes, including preterm birth, low birth weight, pre-eclampsia, and foetal growth restriction. Obstetricians and gynaecologists, being the primary maternal care providers, play a crucial role in identifying periodontal problems during pregnancy and facilitating timely dental referrals. However, their knowledge, awareness, and practice behaviours regarding maternal periodontal health remain inadequately explored in the Ahmednagar district. To assess the knowledge, awareness, attitudes, practice behaviours, and perceived barriers among gynaecologists regarding maternal periodontal disease and its association with adverse pregnancy outcomes. A cross-sectional questionnaire-based survey was conducted among 970 obstetricians and gynaecologists in the Ahmednagar district. A total of 900 respondents completed the survey, yielding an 87% response rate. The questionnaire assessed demographic details, knowledge of periodontal disease, awareness of its relationship with pregnancy outcomes, clinical practices, and barriers to referral. Among the 900 respondents, 59.8% were female, 56.8% worked in private practice, and most were aged 41–50 years with 10–15 years of clinical experience. Only 16% correctly defined dental plaque, and 22.3% recognized it as the cause of gum disease. A limited proportion acknowledged the relationship between oral health and pregnancy outcomes (287), and only 321 agreed that periodontal disease could affect pregnancy outcomes. Awareness of pregnancy-related hormonal influences on

gingival tissues was low (186). Clinical practices were suboptimal: only 54 provided oral health education during consultations, 35 believed dental check-ups should be routine in antenatal care, and only 68 performed visual oral examinations for gingival complaints. Very few (16) had training in oral healthcare. Symptomatic management was common, with 462 prescribing paracetamol and 228 prescribing penicillin for gingival symptoms. Lack of knowledge and time were the major perceived barriers to dental referral. Most participants relied on clinical experience (82%) and medical journals for information. Gynaecologists in Ahmednagar district demonstrated limited knowledge and awareness regarding maternal periodontal disease and its association with adverse pregnancy outcomes. Inadequate clinical practices and significant perceived barriers further restrict appropriate oral health management in pregnant women. Strengthening interdisciplinary training, improving oral health education, and integrating dental referral pathways into routine antenatal care are essential to enhance maternal and perinatal outcomes.

**Keywords:** Periodontal disease, Gynaecologists, Adverse Pregnancy Outcomes, Maternal Oral Health, Interdisciplinary health care.

## INTRODUCTION

Periodontal diseases initiated by bacterial plaque, leads to the formation of pathological periodontal pockets [1]. A substantial body of evidence has demonstrated an association between periodontal disease and adverse pregnancy outcomes such as preterm birth, low birth weight, preeclampsia, and foetal growth restriction[2–4]. The pathogenic microorganisms and their metabolites not only cause destruction of tooth-supporting structures but may also contribute to unfavourable perinatal outcomes through several biological pathways [5]. The primary mechanisms explaining this association, emphasizing the close relationship between periodontal tissues and the maternal vascular system was explained by Zeeman et.al.[5]. The gingiva, being an estrogen-responsive tissue, undergoes microvascular and permeability changes during pregnancy [6]. These alterations increase susceptibility to bacteraemia from Gram-negative periodontal pathogens, potentially allowing hematogenous spread and "placental seeding" [5,6]. Subsequently, an inflammatory cascade is triggered, characterized by elevated levels of cytokines and mediators [5,7]. Increased inflammatory mediators within the fetal-placental compartment may contribute to premature rupture of membranes, uterine contractions, and preterm birth or spontaneous abortion [4,5].

Given this interaction, awareness of gingival and periodontal health is essential in pre-marital counselling and maternal healthcare. Obstetricians, gynaecologists, and dental professionals share the responsibility of preventing periodontal disease and promoting oral health among women of reproductive age [8]. As primary maternal healthcare providers with continuous contact with pregnant women, gynaecologists are uniquely positioned to influence oral hygiene behaviour, reduce maternal complications, and minimize adverse perinatal outcomes through appropriate counselling and timely referrals [8,9]. Therefore, it is crucial for obstetricians and gynaecologists to understand oral physiological changes during pregnancy, risk of periodontal disease, and its potential implications for pregnancy outcomes [6,8]. The present study was thus undertaken to assess the knowledge, attitudes, perceptions, practices, and perceived barriers among Obstetricians-Gynaecologists in the Ahmednagar district regarding the association between periodontal disease and adverse pregnancy outcomes.

## MATERIAL AND METHODS

This descriptive, exploratory, anonymous cross-sectional survey was conducted among voluntarily participating gynaecologists of the Ahmednagar district in Maharashtra, India. Participants were randomly selected and included

practitioners working in private settings, teaching institutions, and community hospitals/clinics. Ethical approval for the study was obtained from the Institutional Ethics Committee of Pravara Institute of Medical Sciences, Loni. Data collection occurred over a period of two months, from the beginning of February to the end of March 2022. A total of 970 gynaecologists were approached for participation. Data were collected using a structured, self-administered, close-ended questionnaire designed to assess knowledge, attitudes, awareness, and practices regarding maternal periodontal health and its association with adverse pregnancy outcomes. A pilot study was conducted to evaluate the clarity, comprehension, and completion time of the questionnaire. Based on feedback, modifications were made to simplify technical terminology and enhance participant understanding. The final questionnaire was validated with 20 gynaecologists, and its internal consistency and reliability were confirmed with a Cronbach's alpha value of 0.80. The average completion time during the pilot was 5–10 minutes.

A detailed description of the study purpose accompanied each questionnaire, and informed written consent was obtained from all participants. Confidentiality and anonymity were assured. The final survey consisted of 16 questions, comprising both open-ended and close-ended items. The first section captured demographic information including age, gender, and type of practice. The second component assessed knowledge, awareness, and practice behaviours related to oral health care for pregnant patients. The questionnaire was distributed electronically, and an invitation message containing a URL link to the online survey was shared with participants. Responses were automatically recorded through the online platform. All collected data were analyzed using the Statistical Package for the Social Sciences (SPSS) software (SPSS Inc., Chicago, IL, USA). Descriptive statistics, including frequency distributions, were generated. Associations between variables were evaluated using Chi-square tests, with statistical significance set at $p< 0.05$.

## RESULTS

A total of 900 out of 970 obstetricians and gynaecologists (OBG) participated in the study, yielding a response rate of 87%. Non-responses were primarily attributed to time constraints and lack of personal interest. Among the respondents, 361 (40.1%) were male and 539 (59.8%) were female[Table 1]. More than half (56.8%, n = 512) were working in private healthcare settings.[Table 2] The majority of participants belonged to the 41–50 years age group (47.2%), [Table 3] and most had 10–15 years of professional practice, indicating a relatively experienced cohort.[Table 4].

**Knowledge of Periodontal Disease [Table 5, 6; Graph 1]:**
The assessment of periodontal knowledge showed considerable gaps. Only 16% of respondents correctly defined dental plaque as a soft deposit, and just 22.3% recognized dental plaque as the primary cause of gingival disease. When asked whether maintaining good oral hygiene could improve pregnancy outcomes, only 287 answered correctly. Furthermore, only 321 gynaecologists acknowledged that periodontal disease could affect pregnancy outcomes. Awareness of hormonal influence on gingival tissues was low, with only 186 respondents identifying pregnancy-related hormonal changes as a contributing factor to gingival alterations.

**Attitudes Toward Oral Health in Pregnancy [Graph 2]:**
Attitudinal responses indicated limited emphasis on oral health during antenatal care. Only 54 gynaecologists reported routinely providing oral health information during consultations, and merely 35 believed that dental check-ups should be incorporated into routine pregnancy care. Among patients reporting gingival changes, only 68 gynaecologists performed a visual oral examination, and 47 advised professional dental evaluation. Notably, only 16 respondents had received any form of training in oral healthcare, reflecting limited professional preparedness.[Graph 2].

**Clinical Practices for Managing Gingival Complaints [Graph 3]:**
In cases where pregnant patients presented with bleeding gums, the majority of practitioners managed symptoms pharmacologically. Paracetamol was prescribed by 462 respondents, while 228 prescribed penicillin, indicating a tendency toward medical rather than periodontal management of gingival conditions.

**Perceived Barriers to Oral Health Referral[Graph 4]:**
Among the four perceived barriers assessed, lack of knowledge and insufficient time emerged as the most significant factors discouraging gynaecologists from referring pregnant women for dental care. These barriers reflect systemic gaps in training and interdisciplinary collaboration.

**Sources of Information[Graph 5]:**
When asked about their primary sources of information regarding oral health and pregnancy, 82% of respondents reported relying on personal clinical experience. Additionally, more than half of the participants cited medical journals as a secondary source of information, highlighting limited exposure to structured oral health education.

## DISCUSSION

This cross-sectional survey provides valuable insight into the knowledge and awareness of maternal periodontal health and its association with adverse pregnancy outcomes among gynaecologists in the Ahmednagar district. Out of 970 obstetricians and gynaecologists approached, 900 completed the questionnaire, resulting in a high response rate of 87%, with the remaining non-respondents citing time constraints and lack of personal interest. The demographic distribution showed a predominance of female gynaecologists (59.8%), and more than half (56.8%) were practicing in private settings. Most respondents were between 41–50 years of age and had 10–15 years of professional experience, indicating adequate clinical exposure. Despite this experience, the survey revealed significant deficiencies in fundamental periodontal knowledge. Only 16% of the participants correctly identified dental plaque as a soft deposit, while just 22.3% recognized plaque as the causative factor for gingival disease, findings consistent with earlier reports of limited periodontal knowledge among non-dental clinicians.[10] Awareness of the relationship between oral health and pregnancy outcomes was also limited. Although maintaining good oral hygiene during pregnancy has been shown to positively influence perinatal health,[11-13] only 287 respondents acknowledged this, and merely 321 agreed that periodontal disease could affect pregnancy outcomes. Furthermore, only a small proportion (186) understood that hormonal changes during pregnancy can influence gingival tissues, an association well-documented in periodontal literature.[14,15]. Attitudes and clinical practices related to oral health were similarly suboptimal. Only 54 gynaecologists reported providing oral health information during antenatal consultations, and merely 35 believed that dental check-ups should be incorporated into routine prenatal care. Similar gaps in practice patterns have been noted in studies conducted among obstetricians in other regions.[16,17] Even among patients presenting with gingival changes, only 68 practitioners conducted a visual oral examination, and just 47 recommended professional dental evaluation. Notably, only 16 respondents had formal training in oral healthcare, which may explain the limited confidence in performing examinations or making referrals. Instead, symptomatic management was common, with a majority prescribing medications such as paracetamol (462) and penicillin (228) for gum-related complaints, rather than addressing the underlying periodontal condition. Previous research suggests that lack of training and perceived scope limitations often lead to such palliative rather than preventive approaches.[18].

Analysis of perceived barriers revealed that lack of knowledge and insufficient time were the primary reasons for not referring pregnant patients to dental services. This finding mirrors trends reported in previous studies, emphasizing the need for interdisciplinary education and collaboration. [19,20] Participants relied heavily on clinical experience (82%) as their main source of information regarding oral health and pregnancy, while more than half also referred to medical journals. The reliance on experiential knowledge rather than formal, evidence-based training may contribute to persistent gaps in understanding, a trend highlighted in literature addressing healthcare communication and

cross-disciplinary integration.[21]. Overall, these findings highlight a significant need for improved training, continuing education, and integration of oral health awareness into obstetric practice. Strengthening the knowledge base of gynaecologists is essential for ensuring timely diagnosis, appropriate referral, and comprehensive care for pregnant women, ultimately reducing the risk of adverse perinatal outcomes. Multiple systematic reviews reinforce the importance of periodontal health in mitigating risks such as preterm birth, low birth weight, and preeclampsia.[12,13,22].

## CONCLUSION

The present study highlights significant gaps in the knowledge and awareness of gynaecologists in Ahmednagar district regarding maternal periodontal health and its association with adverse pregnancy outcomes. Although the majority of respondents had substantial clinical experience, only a small proportion demonstrated adequate understanding of the etiology of periodontal disease or its impact on pregnancy. Attitudes and practices were similarly suboptimal, with very few practitioners providing oral health guidance, performing oral examinations, or referring pregnant patients for dental care. The findings underscore the urgent need for integrating oral health education into both undergraduate and postgraduate medical curricula, as well as establishing ongoing interdisciplinary training programs. Improving awareness among gynaecologists may enhance early identification and referral of periodontal problems during pregnancy, ultimately contributing to better maternal and fetal health outcomes. Strengthening collaboration between dental and obstetric healthcare providers is essential to promote comprehensive antenatal care.

## LIMITATIONS

1. Geographical Restriction: The study was limited to gynaecologists practicing in the Ahmednagar district, which may restrict the generalizability of the findings to other regions with differing training standards or healthcare infrastructures.
2. Self-Reported Responses: The use of a questionnaire relies on self-reported data, which may introduce reporting bias, social desirability bias, or inaccuracies in recalling actual practices.
3. Non-Response Bias: Although the response rate was high (87%), the 70 practitioners who did not respond may differ systematically from respondents, possibly influencing the representativeness of the results.
4. Cross-Sectional Design: The cross-sectional nature of the study limits the ability to determine causality or assess changes in knowledge and attitudes over time.
5. Limited Assessment of Practical Skills: The study primarily evaluated knowledge and self-reported attitudes, but did not directly assess clinical competence or actual patient outcomes.

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**Table 1:**

| Gender | n(%) |
|---|---|
| Male | 361(40.1%) |
| Female | 539(59.8%) |

**Table 2:**

| Practice Setting | n(%) |
|---|---|
| Private | 512(56.8%) |
| Academic | 265(29.4%) |
| Government | 123(13.6%) |

**Table 3:**

| Age | n(%) |
|---|---|
| <30 years | 75(8.3%) |
| 31-40 years | 102(11.3%) |
| 41-50 Years | 425(47.2%) |
| ≥50 years | 298(33.1%) |

**Table 4:**

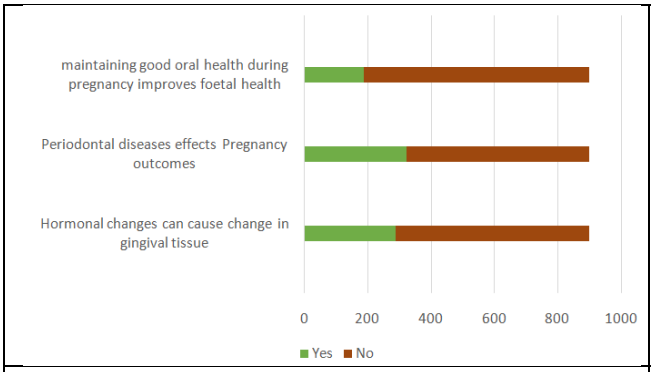
| Number of Years in Practice | n(%) |
|---|---|
| < 1 year | 72 (8%) |
| 2 -5 Years | 112(12.4%) |
| 5 -10 Years | 145(16.1%) |
| 10-15 Years | 307 (34.1%) |
| >15 Years | 264(29.3%) |

**Table 5:**

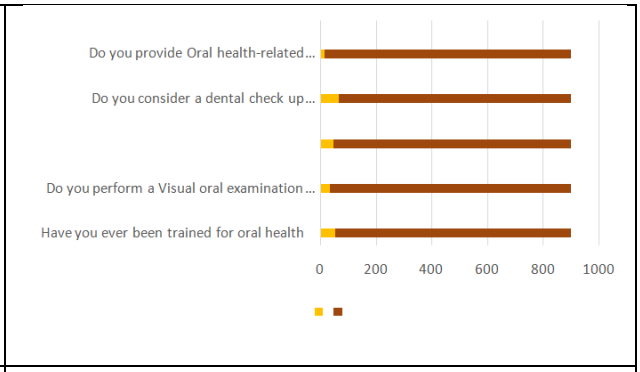
| What is Dental Plaque | n(%) |
|---|---|
| Hard Deposit | 432(48%) |
| Soft Deposit | 144(16%) |
| Food Debris | 135(15%) |
| Staining | 90(10%) |
| Don't Know | 99(11%) |

**Table 6:**

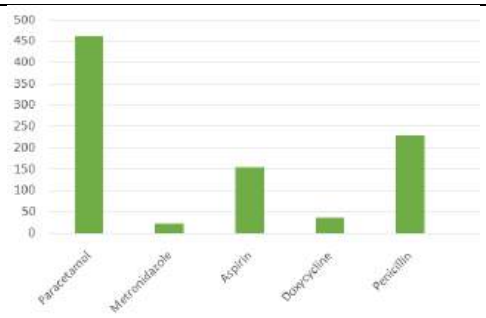
| Causes of Gingival and Periodontal diseases | n(%) |
|---|---|
| Plaque | 199(22.3) |
| Caries | 401(44.6) |
| Trauma | 84(9.3) |
| Aging | 104(11.4) |
| Don't know | 112(12.4) |



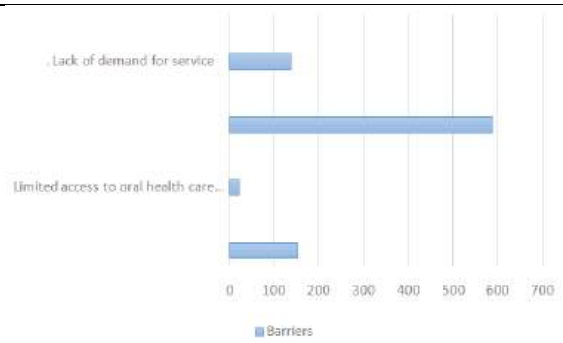

**Graph 1 : Knowledge of Periodontal Disease**



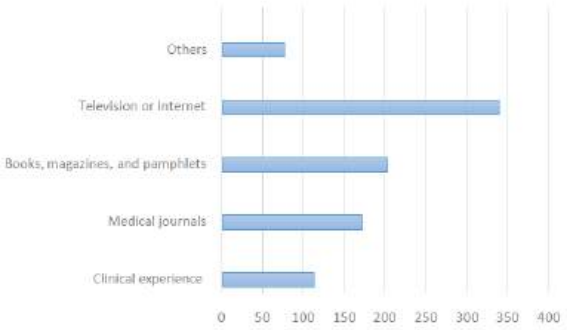

**Graph 2: Attitudes Toward Oral Health in Pregnancy**




**Graph 3: Prescribed drug in pregnant patients complaining of bleeding gum**




**Graph 4: Barriers for Appropriate oral health care**




**Graph 5: Sources of information regarding association between oral health and pregnancy**

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REVIEW ARTICLE

# Review on Book Epigraphy: Richard Salomon's Monumental Contribution to the Study of Indo - Aryan Inscriptions and Manuscripts

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## ABSTRACT

Richard Salomon's *Indian Epigraphy* (1998) is the book which continues to serve the society as an essential resource for both emerging and established scholars who are engaged in the study of South Asian inscriptions and manuscripts in Sanskrit, Prakrit and others scripts.it's a publication from A Publication Series of The University of Texas Centre for Asian Studies and Oxford University Press. New York Oxford Oxford University Press 1998, it contains 8 chapters including bibliographic survey. This review enlightens the book's details, analytical approach, and enduring relevance, types and styles, different Indian scripts its significant role in explaining with the cultural, linguistic, and political transformations of the India.observations is given to Salomon's methodological precision, his integration methodology of palaeographic, philological, and historical perspectives, aspects and his influential position in redefining the field of Indo-Aryan epigraphy in the period following D.C. Sircar's foundational book contributions.
Epigraphy is the branch concerned with the study of inscriptionsand manuscripts as constitutes a crucial tool for reconstructing and defining the political, social, religious, and linguistic aspects of India's ancient and medieval past. This article explains a comprehensive exploration of Indian epigraphy, from its earliest manifestations to its progression across successive dynastic regimes. It highlights the evolution of scripts, the diversity of languages employed, and the pivotal role that inscriptions play in historical analysis. Special focus is placed on key epigraphic records and the ways in which they deepen our

understanding of India's rich and complex heritage and study of the effect of development of human cognition process with respect to scripts development.

**Keywords:** Indian epigraphy, Indo-Aryan inscriptions, Brahmi, Kharosthi, Prakrit, Sanskrit, Salomon, Asokan edicts, historical linguistics, South Asian history

## INTRODUCTION

In India the knowledge transformation from one generation to another was in the form of listening, memorising and then reproducing orally, later the scripts developed was started to store and save the ancient knowledge. The history of writing in India, as it applies to the material explained in this book, it is essentially brief with the history of the Brahmi script and its many derivatives, and of the Kharosthi script. the older one is the parent ofone of the major script families of the world, comprising not only all of the indigenousscripts of South Asiabut also several other major scripts of central and especially Southeast Asia. the general outlines of these developments, particularly as theyrelate to epigraphy proper, are presented in the context of a general discussion of thehistory of epigraphic writing in India [1].

The analysis of Indian inscriptions are written on stone, cast in metal, or impressed upon clay that offers important access to the socio-political and cultural history of the Indian subcontinent. Richard Salomon's *Indian Epigraphy: A Guide to the Study of Inscriptions in Sanskrit, Prakrit, and the Other Indo-Aryan Languages* (Oxford University Press, 1998) stands as a seminal contribution to this literary domain. Expanding on the pioneering works of D.C. Sircar and G.H. Ojha, Salomon presents a more comprehensive and inclusive examination of epigraphy, moving beyond traditional philological methods to incorporate its intersections with art history, religion, and archaeology [2 &3]. In the context of Indian historical studies, inscriptions playsa important role, especially given the region's distinctive historiographical traditions. In contrast to civilizations such as Greece, Rome, or China, which produced extensive chronological literary records, Indian classical literature often lacks explicit historical documentation and chronological specificity. This perceived "ahistorical" character of Indian tradition has made epigraphic evidence vital for historical reconstruction. As such, epigraphy has become a cornerstone of Indological research.

Inscriptional records, whether through precise calendar references or palaeographic evaluation, offer the most dependable chronological data available for charting the early and medieval periods of Indian history. Scholars estimate that nearly 80% of what is currently known about Indian history before 1000 CE stems from such sources. Inscriptions not only document the political trajectories of dynasties like the Guptas and Chalukyas but also shed light on a broader spectrum of cultural phenomena, including governance, religious transformations, literary expressions, linguistic changes, and societal norms [4].

The realm of Indian epigraphy also transcends the modern geopolitical boundaries of India, encompassing inscriptions found across the broader South Asian region and in areas influenced by Indianized cultural systems, such as Southeast Asia, Central Asia, and parts of Africa. Over 90,000 inscriptions—mainly in Indo-Aryan and Dravidian languages—have been discovered to date, yet a substantial number remain either unpublished or insufficiently studied. This highlights the dynamic and continually evolving nature of epigraphic scholarship [5].

The immense diversity of scripts, languages, and regional variations poses distinct methodological challenges for scholars, requiring expertise across multiple disciplines including linguistics, philology, palaeography, and archaeology. Nevertheless, epigraphy remains an indispensable tool for anyone seeking a historically grounded understanding of India's rich and intricate past. This study reasserts the fundamental role of epigraphy in Indological inquiry by exploring its historical depth, contemporary scope, and enduring significance in piecing together India's layered historical narrative.

In essence, Indian epigraphy the discipline focused on interpreting inscriptions carved into stone, metal, terracotta, and other durable surfaces serves as a primary source for understanding ancient Indian history. These texts provide direct testimony of historical figures, governmental structures, and cultural developments, and have been crucial in establishing historical chronologies, reconstructing royal lineages, and tracing socio-religious transformations.

**Aim**

To review the book on Indian Epigraphy written byRichard Salomon- Richard Salomon's Indian Epigraphy (1998)

**Objective**

1- Review the chapter of book Indian Epigraphy written byRichard Salomon

**Scope and Structure**

Richard Salomon's *Indian Epigraphy* is systematically divided into eight chapters, supplemented by an appendix and a detailed bibliography. The main discussion spans four principal areas: the evolution and classification of writing systems (Chapter 2), the development of languages and linguistic transitions (Chapter 3), typological and chronological surveys of inscriptions (Chapter 4), and methodologies for epigraphic analysis (Chapter 5). The concluding chapters offer a historiographical overview of the field (Chapter 6) and explore the broader cultural implications of epigraphy (Chapter 7).

Each section is rich in content, blending descriptive clarity with critical insight. The introductory chapter highlights the indispensable role of epigraphy in Indological research, particularly in light of India's historical tendency to omit chronological details in literary sources. Salomon persuasively underscores that inscription, in contrast to redacted and dateless manuscripts, serve as dependable chronological markers.

**Contributions to Script and Language Studies**

Chapter 2 is especially notable for its sophisticated examination of Brahmī and Kharoshṭhī scripts. Salomon meticulously traces Brahmī's evolution beginning in the Mauryan era, anchored by the Asokan edicts, and outlines its progression into various regional variants. Rather than making speculative claims about proto-scripts, he prudently offers hypotheses grounded in palaeographic evidence.

In Chapter 3, his treatment of epigraphical languages including various Prakrits, Epigraphical Hybrid Sanskrit (EHS), and Classical Sanskrit is both linguistically detailed and historically contextualized. His inclusion of New Indo-Aryan inscriptions, which have often been marginalized in previous literature, extends the chronological scope of the work into the early modern period and affirms the enduring relevance of inscriptional practices.

**Methodological Innovation and Analytical Precision**

Chapters 5 and 6 showcase Salomon's methodological expertise. He provides clear explanations of palaeographic techniques, authenticity evaluation, and the use of calendrical systems like the Śaka and Vikrama eras, as well as regnal year conventions—tools essential for practitioners in the field. Notably, he advocates for a standardized approach to transcription and documentation, aligning Indian epigraphy with global scholarly norms. His historiographical analysis in Chapter 6 is equally significant. It traces the trajectory of the field from the early decipherments by James Prinsep and Georg Bühler through to institutional developments post-independence. Salomon offers a balanced critique of the stagnation that followed Sircar's era, calling for a reinvigorated and interdisciplinary engagement with epigraphic studies.

**Epigraphy as a Cultural Resource**

Among the book's most compelling arguments is that epigraphy should not be relegated to the margins of philological inquiry. Chapter 7 illustrates the multifaceted value of inscriptions, demonstrating their utility in reconstructing political history, religious practices, literature, artistic traditions, and geographical knowledge.

Salomon shows, with precision, how even a single copperplate grant or temple inscription can unveil intricate networks of political patronage, sectarian identity, and linguistic experimentation.

**Comparative and Transregional Scope**

By incorporating inscriptions from regions beyond the Indian subcontinent—such as Central Asia and Southeast Asia—Salomon broadens the geographic and cultural scope of his study. The appendix, which includes annotated translations and analyses of fifteen key inscriptions (e.g., the Rummindei Edict, the Heliodoros Pillar, the Kalawan Copperplate), serves as a valuable introductory resource for students and general readers alike.

**Critical Observations**

Despite its comprehensive coverage, the work could benefit from an updated discussion of emerging technologies in the field, such as digital epigraphy and GIS-assisted archaeological mapping. Moreover, while Dravidian and Islamic epigraphy are acknowledged, their exclusion from detailed analysis is a methodological choice rather than an oversight. Readers seeking a holistic view must refer to complementary studies for these traditions.

**Enduring Influence and Future Outlook**

More than two decades since its release, *Indian Epigraphy* continues to be a foundational text for graduate-level study in South Asian history and historical linguistics. Its impact is evident in recent research on regional scripts like Tamil-Brahmī, digital epigraphic databases, and literary analyses of inscriptions. Salomon's appeal for interdisciplinary methods, improved field techniques, and the digital preservation of yet-unpublished materials remains acutely relevant. His critique regarding the declining emphasis on rigorous epigraphic training in Indian academia is still valid, with many universities yet to establish comprehensive programs in epigraphy and paleography.

## DISCUSSION

**Early Epigraphic Traditions**

**Pre-Mauryan Inscriptions**

The earliest indications of writing on the Indian subcontinent are associated with the Indus Valley Civilization (circa 2600–1900 BCE), whose script remains undeciphered. A systematic and identifiable tradition of epigraphy, however, begins only with the inscriptions of Emperor Ashoka in the 3rd century BCE.

**Ashokan Inscriptions**

Ashoka's edicts represent the first conclusive examples of written records in India. Distributed throughout the subcontinent, these inscriptions were primarily composed in the Prakrit language and engraved using the Brahmi script. They convey messages related to moral governance, Buddhist principles, and imperial administration (Sircar, 1965).

**Scripts and Languages in Indian Epigraphy**

**Brahmi and Kharosthi**

The Brahmi script stands as the foundational script from which most later Indian scripts evolved. It enjoyed widespread usage across the subcontinent. In contrast, the Kharosthi script, primarily employed in the northwestern regions, was used for a comparatively shorter period and exhibits influence from the Aramaic script (Salomon, 1998).

**The Gupta Script and Its Derivatives**

During the Gupta period, Brahmi underwent regional diversification, giving rise to scripts such as Nagari, Sharada, and Grantha. These, in turn, laid the groundwork for many modern Indian scripts, including Devanagari and Tamil.

**Languages of Inscriptions**

Epigraphic records in India employed a range of languages across different periods:

**Prakrit** during the early Mauryan era
**Sanskrit** from the Gupta age onward
**Tamil, Kannada, Telugu** in the southern regions
**Persian and Arabic** during the Islamic periods of Indian history (Dani & Michell, 1997)

**Regional and Dynastic Epigraphy**
**Southern India**
The Tamil region witnessed prolific inscriptional activity under dynasties like the Cholas, Pallavas, and Pandyas. Temples functioned as major centers for the creation and preservation of inscriptions in both Tamil and Sanskrit.

**Northern India**
The Guptas promoted the use of classical Sanskrit in their inscriptions, often rendered in poetic verse. A notable example is the Allahabad Pillar inscription composed during the reign of Samudragupta.

**Deccan and Central India**
In the Deccan and central parts of India, the Satavahanas and later the Chalukyas issued inscriptions in both Prakrit and Sanskrit. The Aihole inscription of Pulakesin II is among the most distinguished records from this region.

**Epigraphy as a Historical Source**
Inscriptions are invaluable for reconstructing early Indian history. They document:
Dynastic genealogies
Land grants to religious and scholarly institutions
Religious and sectarian affiliations
Social and economic structures
Systems of land tenure and governance Because many inscriptions remain in their original context and are less prone to editorial tampering than manuscripts, they serve as more reliable historical sources (Thapar, 2002).

**Contributions of Modern Scholars**
Several scholars have been instrumental in advancing the study of Indian epigraphy:
**James Prinsep**: Deciphered the Brahmi and Kharosthi scripts in 1837
**D.C. Sircar**: Considered a pioneer of modern Indian epigraphical studies
**Richard Salomon**: Offered a comparative and systematic analysis of Indian scripts and their development

**Challenges and Prospects in Epigraphy**
**Undeciphered Scripts**
Despite ongoing computational and linguistic efforts, scripts like the one used in the Indus Valley Civilization remain undeciphered.

**Conservation and Digitization**
Numerous inscriptions have suffered degradation or loss due to environmental and human factors. However, digital initiatives such as the Digital South Asia Library and encoding standards like Epi Doc XML are actively working toward their documentation, preservation, and accessibility.

## CONCLUSION

Richard Salomon's *Indian Epigraphy* is not merely a reference manual—it serves as a transformative framework that redefined the methods by which inscriptions are analysed, situated, and understood. Combining scholarly rigor with clarity, the work has become essential for researchers across disciplines, including Indology, archaeology, linguistics, and history. Even as the discipline evolves through digital innovation and interdisciplinary approaches, Salomon's

study remains a foundational pillar, guiding contemporary scholarship and inquiry. Epigraphy stands at the core of South Asian studies, offering vital perspectives on the language, culture, and governance of ancient Indian civilizations. With the growing adoption of digital tools, the field is poised for significant advancements, opening new avenues for collaborative and cross-disciplinary research.

Richard Salomon's *Indian Epigraphy* transcends the role of a conventional guidebook; it offers a robust conceptual framework that has fundamentally transformed the study of inscriptions. By combining scholarly rigor with clarity, it has become an essential resource for scholars across disciplines such as Indology, archaeology, linguistics, and history. As the discipline evolves through digital innovations and interdisciplinary collaboration, Salomon's work remains a cornerstone for both emerging research and critical discourse.

Epigraphy forms a crucial pillar of South Asian studies, unlocking valuable information about the linguistic, cultural, and administrative structures of historical Indian civilizations. With the advent of digital methodologies, the field is poised for significant advancements, opening new pathways for collaborative and cross-disciplinary inquiry.

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**RESEARCH ARTICLE**

# Effect of Masako Maneuver on Swallowing Function and Quality of Life in Post-Stroke Patients with Dysphagia

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**ABSTRACT**

Dysphagia is a frequent and serious complication following stroke, leading to aspiration pneumonia, malnutrition, dehydration, and reduced quality of life. Among rehabilitative strategies, the Masako maneuver is designed to improve pharyngeal muscle strength and posterior pharyngeal wall movement; however, evidence of its effectiveness in post-stroke dysphagia remains limited. To evaluate the effect of the Masako maneuver on swallowing function and quality of life in post-stroke patients with dysphagia. Thirty post-stroke patients with dysphagia were recruited using convenient sampling from hospitals in Vadodara. Participants were divided into two groups. Group A received Masako maneuver training along with conventional swallowing therapy, while Group B received conventional therapy alone for four weeks. Swallowing function and quality of life were assessed using the Eating Assessment Tool (EAT-10) at baseline and post-intervention. Data were analyzed using paired and unpaired t-tests with SPSS version 23. Group A demonstrated a statistically significant reduction in EAT-10 scores post-intervention ($p < 0.05$), indicating improved swallowing function and quality of life compared to baseline and Group B. The Masako maneuver is an effective adjunct to conventional therapy for improving swallowing function and quality of life in post-stroke patients with dysphagia.

**Keywords:** Stroke, Dysphagia, Masako Maneuver, Swallowing Function, EAT-10, Quality of Life.

## INTRODUCTION

Stroke is a major global public health concern and remains one of the leading causes of long-term disability and mortality worldwide. Advances in acute medical management have improved survival rates; however, a large proportion of stroke survivors continue to experience persistent neurological impairments that significantly affect functional independence and quality of life[1]. Among these impairments, dysphagia is one of the most common yet under-recognized complications following stroke, posing serious medical, nutritional, and psychosocial challenges[2]. Post-stroke dysphagia is reported in approximately 30–65% of patients during the acute and subacute phases, with a considerable number continuing to experience swallowing difficulties in the chronic stage[3]. The prevalence varies depending on lesion location, stroke severity, and diagnostic methods used[2]. Dysphagia results from disruption of the complex neural network responsible for swallowing, which involves cortical and subcortical regions, the brainstem swallowing centers, cranial nerves, and coordinated activity of oropharyngeal musculature. Lesions affecting these pathways can lead to impaired oral bolus control, delayed swallow initiation, reduced tongue base retraction, decreased pharyngeal constriction, and compromised airway protection[1,2,3]. The clinical consequences of dysphagia following stroke are profound[4]. Aspiration pneumonia remains a leading cause of morbidity and mortality in this population[5]. In addition, dysphagia contributes to malnutrition, dehydration, prolonged hospitalization, increased healthcare costs, and delayed participation in rehabilitation programs[4,5] Beyond physical complications, swallowing difficulties significantly affect psychological well-being, leading to anxiety during meals, social isolation, reduced self-esteem, and diminished overall quality of life. Therefore, effective identification and management of dysphagia are critical components of comprehensive stroke rehabilitation[6]. Swallowing is a highly coordinated sensorimotor activity comprising voluntary and involuntary phases.

Effective rehabilitation requires interventions that not only compensate for impaired swallowing but also promote physiological recovery[6,10]. Conventional dysphagia management strategies often include compensatory techniques such as postural adjustments, dietary texture modification, and adaptive feeding strategies[11]. While these approaches are effective in reducing aspiration risk, they primarily address symptoms rather than underlying neuromuscular deficits and may not lead to long-term functional improvement[7]. In recent years, exercise-based swallowing rehabilitation has gained increasing attention due to its potential to enhance neuromuscular strength, coordination, and cortical reorganization. These therapeutic exercises aim to restore impaired swallowing physiology through repetitive, task-specific training. One such intervention is the Masako maneuver, also known as the tongue-hold swallow, which is frequently incorporated into clinical dysphagia rehabilitation programs[8]. The Masako maneuver involves protruding the tongue slightly and holding it gently between the teeth while swallowing saliva. This maneuver restricts posterior tongue movement, thereby increasing the demand on the posterior pharyngeal wall to compensate for reduced tongue base retraction. Biomechanical studies have demonstrated that this exercise results in increased anterior movement of the posterior pharyngeal wall, suggesting enhanced activation of the pharyngeal constrictor muscles. Strengthening these muscles is particularly relevant in post-stroke dysphagia, where reduced pharyngeal contraction is a common pathological finding[9]. Despite its widespread clinical use, the evidence supporting the effectiveness of the Masako maneuver in post-stroke dysphagia remains limited. Existing studies often involve small sample sizes, heterogeneous patient populations, or lack appropriate control groups. Furthermore, many investigations focus primarily on physiological outcomes, with limited emphasis on patient-reported measures that reflect functional improvement and quality of life.

Quality of life has emerged as a crucial outcome in dysphagia rehabilitation, as swallowing difficulties extend beyond physical impairment to affect emotional and social domains. The Eating Assessment Tool (EAT-10) is a validated, patient-reported outcome measure that assesses the severity and impact of dysphagia on daily life. Incorporating such measures allows for a more comprehensive evaluation of therapeutic interventions from the patient's perspective[10]. Given the high prevalence and serious consequences of post-stroke dysphagia, along with the limited high-quality evidence supporting specific exercise-based interventions, there is a clear need for further research. Systematic evaluation of commonly used therapeutic maneuvers such as the Masako maneuver is essential

to establish evidence-based rehabilitation protocols. Therefore, the present study was undertaken to evaluate the effect of the Masako maneuver on swallowing function and quality of life in post-stroke patients with dysphagia, with the aim of strengthening the evidence base for clinical decision-making in dysphagia rehabilitation[11].. Several studies have explored exercise-based swallowing rehabilitation in neurological populations. Logemann (1998) emphasized the role of targeted exercises in restoring swallowing physiology. Robbins *et al*. demonstrated that effortful swallowing exercises improved pharyngeal strength and bolus clearance. Masako *et al*. first described the tongue-hold maneuver and reported increased posterior pharyngeal wall movement during swallowing. Subsequent studies suggested potential benefits in patients with reduced tongue base retraction[8,9,10,11]. However, most existing studies are limited by small sample sizes, heterogeneous populations, or lack of control groups. Few studies have specifically examined the impact of the Masako maneuver on patient-reported outcomes such as quality of life. Therefore, further controlled studies are required to establish the clinical effectiveness of this maneuver in post-stroke dysphagia rehabilitation.

## MATERIALS AND METHODS

**Study Design**

A quasi-experimental pre-test and post-test controlled study design was used.

**Study Setting**

The study was conducted in selected hospitals and rehabilitation centers in Vadodara, Gujarat, India.

**Participants**

Thirty post-stroke patients diagnosed with dysphagia were recruited using convenient sampling.

**Inclusion Criteria[3,4,5]**

- Patients aged 40–75 years
- Diagnosed with ischemic or hemorrhagic stroke
- Clinical evidence of dysphagia
- Medically stable and able to follow commands

**Exclusion Criteria[5,6]**

- History of head and neck cancer or surgery
- Pre-existing neurological disorders other than stroke
- Severe cognitive impairment
- Tracheostomized patients

**Group Allocation**

- **Group A (n = 15):** Masako maneuver with conventional swallowing therapy
- **Group B (n = 15):** Conventional swallowing therapy alone

**Intervention**

**Group A** performed the Masako maneuver under therapist supervision along with conventional swallowing exercises. The maneuver involved protruding the tongue slightly and holding it between the teeth while swallowing saliva. Therapy was administered five sessions per week for four weeks.

**Group B** received conventional swallowing therapy, including oral motor exercises and postural techniques, for the same duration.

**Conventional therapies for both the groups were as follows**

**Supraglottis swallow:** Ask the patient to hold their breath tightly (imagine bearing down).

Now, tell the patient to swallow their saliva twice, release their breath with a sharp cough, and swallow again.

**Compensatory technique:** In this technique, the patient will turn their head towards the weak side and tilt it towards the unaffected side then swallow.

**Effortful swallow:** Patient will swallow as hard as they can with food or saliva. Patient was pushed as hard as they can with the tongue against the roof of their mouth while you swallow.

**Oromotor sensory stimulation:** It will be given in the form of ice cube massage to areas of the cheek and tongue (at the palatine arch) and external side of the cheeks. (5 stimulation, 5 swallows).
Conventional therapy was given for 5 min/10 times a day for 4 weeks.

**Outcome Measure**
**Eating Assessment Tool (EAT-10)**
Assessments were performed at baseline and after four weeks of intervention.

**Statistical Analysis**
Data were analyzed using SPSS version 23. Paired t-tests were used for within-group comparisons and unpaired t-tests for between-group comparisons. Statistical significance was set at $p < 0.05$. Flow chart of study design (Figure 1).

## RESULTS

Both groups showed improvement in EAT-10 scores post-intervention; however, Group A demonstrated a significantly greater reduction in scores compared to Group B ($p < 0.05$), indicating superior improvement in swallowing function and quality of life. Comparison of EAT-10 Scores Between Groups (Mean ± SD) (Table 1).

## DISCUSSION

The findings of the present study indicate that the Masako maneuver, when combined with conventional swallowing therapy, significantly improves swallowing function and quality of life in post-stroke dysphagia patients. The improvement may be attributed to increased activation and strengthening of the pharyngeal constrictor muscles, leading to more efficient bolus clearance. These results are consistent with previous studies reporting enhanced posterior pharyngeal wall movement following Masako maneuver training. Incorporating this maneuver into routine dysphagia rehabilitation may therefore offer additional benefits over conventional therapy alone. The results of the present study demonstrate that the Masako maneuver significantly improves swallowing function and quality of life in post-stroke dysphagia patients when used as an adjunct to conventional therapy. The observed improvements may be attributed to enhanced activation of the pharyngeal constrictor muscles and improved posterior pharyngeal wall movement. These physiological changes facilitate more efficient bolus clearance and reduce pharyngeal residue. The findings are consistent with earlier studies that reported increased pharyngeal wall movement following tongue-hold swallowing exercises. Incorporating patient- reported outcome measures such as EAT-10 strengthens the clinical relevance of the present study.

## CONCLUSION

The Masako maneuver is an effective and simple therapeutic exercise that significantly improves swallowing function and quality of life in post-stroke patients with dysphagia. It can be safely recommended as an adjunct to conventional swallowing therapy.

## LIMITATIONS AND FUTURE SCOPE

The study has several limitations, including a relatively small sample size, short intervention duration, and absence of instrumental swallowing assessments. Future research should involve larger randomized controlled trials, longer follow-up periods, and objective assessments such as video fluoroscopic swallowing studies (VFSS).

### ETHICAL CONSIDERATIONS

Ethical approval was obtained from the institutional ethics committee. Written informed consent was obtained from all participants prior to inclusion in the study.

### CONFLICT OF INTEREST

The author declares no conflict of interest.

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**Table 1: Comparison of EAT-10 Scores Between Groups (Mean ± SD)**

| Group | Pre test Mean ± SD | Post test Mean ± SD |
|---|---|---|
| Group A (Masako + Conventional) | 21.4 ± 3.2 | 8.6 ± 2.5 |
| Group B (Conventional) | 20.9 ± 3.5 | 15.8 ± 3.1 |

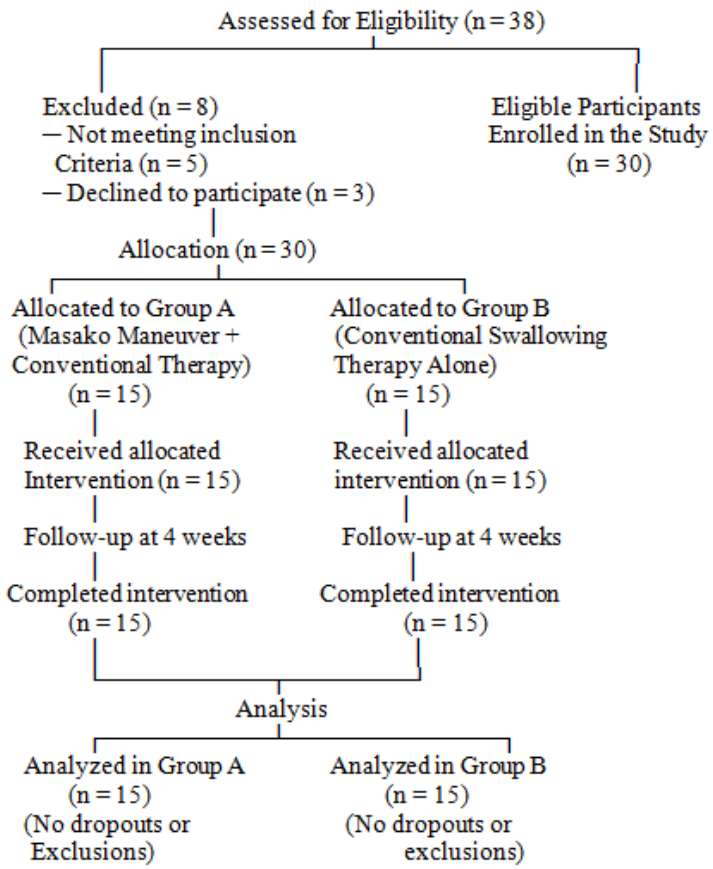



**Figure 1: Flow chart of study design**

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RESEARCH ARTICLE

# Design and Development of a Multipurpose Drafting Table with Integrated Laptop Workspace for Enhanced User Comfort, Productivity, and Ergonomics

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## ABSTRACT

This study explores the development and student acceptance of a new multipurpose drafting table designed to bridge the gap between traditional drawing and modern digital work. Traditional drafting furniture often fails to accommodate laptops and other digital tools, leading to disorganized workspaces, physical discomfort, and decreased productivity. To address this, we designed a prototype that focuses on ergonomic support and functional versatility.The table was evaluated by 30 participants (60% female, mean age 20.07) based on its durability, ergonomics, aesthetics, comfort, and impact on productivity. The results showed a very high level of overall acceptability, with comfort receiving the highest praise. Interestingly, while longer work sessions were tied to increased wrist and shoulder strain, the table's design was found to significantly reduce back pain. The study also noted that gender influenced how students perceived the design and comfort of the table, while age had no significant effect. Ultimately, these findings suggest that integrating tech-friendly and ergonomic features into educational furniture is essential for supporting the hybrid workflows of modern design students.

**Keywords:** Ergonomic Furniture Design, Laptop Workspace Integration, Productivity Enhancement, Student-Friendly Workstation.

# INTRODUCTION

Architectural drafting is now a hybrid practice that combines manual and digital processes due to the quick digitization of professional and educational settings. Students in the Architectural Drafting Technology program at Surigao del Norte State University (SNSU) frequently use digital tools like Computer-Aided Design (CAD), Building Information Modeling (BIM), and three-dimensional visualization software in addition to hand-drawn drafting tasks like plans, sections, and elevations. A drafting workspace that facilitates smooth transitions between analog and digital tasks is required for this dual-modality workflow. However, traditional drafting tables commonly used in educational institutions are primarily designed for manual drafting and often fail to accommodate modern digital devices such as laptops and tablets. As a result, students experience physical discomfort including back pain, neck strain, shoulder fatigue, and wrist stress, particularly during extended drafting sessions. The lack of integrated digital workspaces further leads to inefficient workflows, poor posture, frequent task interruptions, and reduced productivity.

Furthermore, poor ergonomic workstation designs have been closely linked to long-term health risks and musculoskeletal discomfort, especially in occupations that necessitate extended periods of desk-based, seated work (Andrews et al., 2019). The suggested functional drafting table attempts to minimize physical strain, lower the risk of musculoskeletal disorders, and enable a smooth and effective transition between manual drafting and digital work processes by combining posture. Many drafting students still use antiquated or homemade drafting tables that don't support modern digital requirements or adhere to ergonomic standards, according to internal reports and faculty interviews at SNSU (2023). The need for an ergonomic, flexible drafting solution suited to contemporary architectural education is highlighted by this discrepancy between the furniture that is available and the demands of instruction.

According to recent research, ergonomic furniture plays a crucial role in improving students' and professionals' overall productivity, cognitive focus, task efficiency, and physical well-being (Chandra & Bautista, 2021). To address the ergonomic and operational issues related to hybrid drafting environments, a functional drafting table with an integrated laptop workspace is suggested in response to these findings. This table combines the essential features of conventional drafting furniture with modern technological support. The overall learning and working experience is greatly enhanced by design elements like adjustable working height and surface angle, integrated cable management systems, and well-organized laptop placement that reduce workspace clutter, improve posture, and facilitate more effective task execution (Alvarez & Santos, 2022).

To improve user comfort, operational efficiency, and overall productivity among architectural drafting students, this study suggests designing and developing an Innovative Multi-Functional Drafting Table with an Integrated Laptop Workspace. The proposed innovation aims to bridge the gap between traditional analog drafting practices and modern digital methodologies through a carefully engineered, user-centered design by methodically addressing the ergonomic and functional challenges inherent in dual-modality drafting workflows. The suggested solution aims to support future architectural drafters' long-term professional competencies and changing academic demands in addition to enhancing the immediate physical workspace.

**Objectives of the Study**

This project intends to design, develop, and assess a novel Multi-Purpose Cutting Board for Scale Models to improve the precision and effectiveness of model-making procedures. In particular, the research aims to:

1. Create a multipurpose cutting board that encourages accuracy, organization, and ease of use for people working in engineering, architecture, and creative scale modeling.

2. Include functional and ergonomic features in the prototype, such as integrated measurement guides to facilitate accurate and consistent model-making, gridlines for precise alignment, a non-slip cutting surface, and built-in storage compartments for necessary modeling tools.
3. To make sure the product successfully satisfies the practical needs and expectations of users in both professional and educational contexts, evaluate its usability, durability, affordability, and safety.

## MATERIALS AND METHODS

This study used a descriptive-developmental approach for this study. In the development phase, we designed and built an innovative, multi-functional drafting table that bridges the gap between traditional drawing and digital work. By focusing on ergonomics and space-saving features, we created a workspace tailored to the modern "hybrid" needs of drafting students.

**Project Design**

**Design Consideration**

In designing the Innovative Drafting Table, the following factors have been considered:

1. Adjustable Angle & Height. The laptop stand should allow users to adjust the angle of the laptop or surface to accommodate various activities, such as drafting, sketching, or viewing. Ideally, it should offer a range from nearly flat to 90° (or even higher). A telescoping or folding height adjustment mechanism will help the stand adapt to different users' needs, allowing for a comfortable sitting or standing position. Consider a smooth, easily adjustable system with secure locks to ensure stability at any height.
2. Stability and Durability. The materials used must provide strength and support for both the laptop and drafting activities.
3. Comfort & Ergonomics. Users should be able to tilt the laptop or drafting surface to a comfortable angle to reduce strain on the neck, shoulders, and wrists during long sessions of work.
4. Multi-Functionality. The table must function as both a laptop holder and a drafting surface. To make it effective, the stand could include features like a smooth, flat surface, a built-in ruler, or adjustable armrests for drafting.
5. Cable Management. Design the stand with cable management options for the laptop, mouse, or other devices.

**Isometric Projections (Fig.1)**

**Project Development**

Below are the stages of the design of the innovative drafting table: an innovation is to be developed or constructed right after the gathering of the materials needed:

**Cutting and Preparation:**

1. Cut the plywood into 2 pieces by 0.7-meter length and 0.5 height for the surface of the adjustable drawing stand and like in the given Plan
2. Cut the plywood base on the drawing plan for the frame.
3. Cut the orplywood for the frame and legs of the laptop stand by 14"x 9"
4. They make a small hole of the surface of the laptop stand for the cooling fans purposes.
5. Cut the square tubular in for standard height of a drafting table (30" to 40" (762 mm to 1016 mm) and weld it base on the drawing plan.
6. Cut the plywood for other features in accurate sizes based on the drawing plan.

**Frame Assembly**

7. Assemble the frame using screws and wood glue.
8. Ensure the frame is sturdy and square.

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**Leg Assembly**

9. Attach the metal legs to the frame.
10. Ensure the legs are stable and convenient.

**Top Surface Attachment**

11. Make a Box base on the drawing plan for drawing stand and the laptop stand surfaces.
12. Attach the 2 boxes in the surface of the frame with a small rolling wheel in the both side for smooth sliding process put in based on the drawing plan.
13. Use screws and wood glue for a secure attachment.
14. Attach the shelves to the frame of the laptop stand.
15. Ensure the shelves are level and secure.
16. Put a hinge in both stand of drawing stand and laptop stand for the adjustable height of the surface.
17. Ensure the hinge is level and secure. Sending and Finishing
18. Sand the entire drafting table to smooth out any rough edges.
19. Apply material to finish (e.g., paint, stain, or varnish) to protect the surface.

**Final Assembly and Testing**

20. Assemble all components and test the drafting table.
21. Ensure the legs and adjustable and drawer (sliding mechanism) and shelf (if included) function properly.

**Material Selection and Specifications**

The materials chosen for the whole product were based on their availability, high-quality properties, affordability, and durability.

**Materials Selection and Specification**

**Evaluation Procedure**

**Research Instrument**

The research used a survey questionnaire as the instrument in determining and measuring the data of respondents' perception towards the innovative multifunctional drafting table. The questionnaire was composed of four parts; Part I was the respondent's demographic profile, Part II was the scheduled workspace habits of the respondents, Part III includes the preexisting physical discomfort experiences by the respondents. Lastly the IV was the acceptance test of respondent's level of acceptability toward innovative drafting table with laptop workspace. A 5-point scale was used in this study to respondents' level of acceptability, where:

5 - Strongly Agree
4 - Agree
3 - Neutral
2 - Disagree
1 - Strongly Disagree

**Research Respondents**

The respondents of this study are students enrolled in the Bachelor of Science in Industrial Technology major in Architectural Drafting at Surigao del Norte State University (SNSU) – Main Campus. These students were chosen as the target population due to their direct experience and consistent use of drafting tables and laptops, making them the most appropriate participants to evaluate the acceptability of the proposed multi-functional drafting table.

## RESULTS AND DISCUSSION

The results show that students found the new drafting table highly effective, giving it top marks for comfort, design, and productivity. Specifically, the high ratings for comfort suggest that our focus on ergonomics and workflow really paid off. These findings back up what researchers like Chandra and Bautista (2021) and Alvarez and Santos (2022)

have long argued: when furniture is designed with the human body in mind, it doesn't just improve physical well-being—it helps people stay focused and work more efficiently. The study shows that the data, the impact of long work hours became clear. While the table did a great job of keeping back pain at bay, students still felt more strain in their wrists and shoulders the longer they worked. On the bright side, the design seemed to effectively prevent eye and leg fatigue, as those didn't worsen over time. This tells us that while the current prototype is a major step forward, adding more task-specific adjustments—like tiltable surfaces or height controls—is essential to truly eliminate discomfort during long hours of drafting. The analysis of demographic factors revealed that sex played a significant role in how users perceived the table's design, comfort, and impact on productivity, while age had little to no effect. This finding suggests that male and female users may have slightly different preferences when it comes to ergonomics and workflow, highlighting the importance of designing drafting furniture that is inclusive and adaptable to all users. On the other hand, perceptions of the table's structure, functionality, and overall usability were consistent across sexes, indicating that the core features of the design are broadly appreciated and effective for everyone.

## SUMMARY OF FINDINGS

In summary, our findings prove that the right furniture can make a world of difference in how students handle the demands of hybrid drafting. By combining ergonomics with tech-friendly features, we saw a clear boost in comfort and efficiency. This suggests that schools and universities—especially in design-heavy programs—should prioritize furniture that supports both traditional and digital work to help students succeed without the physical strain.

## CONCLUSION

The following conclusions were drawn from the study's findings:

1. Most respondents were female, and both genders demonstrated a high level of engagement with the innovative drafting table. Notably, female respondents showed higher sensitivity and preference in terms of design, comfort, and productivity—dimensions significantly affected by sex.
2. Age was not a significant factor in determining levels of acceptability, suggesting that the product design appeals across a relatively narrow age group (18–25).
3. The drafting table was perceived as very highly acceptable, with comfort being the most appreciated attribute. This emphasizes the critical role of ergonomics in workspace furniture, especially among users with long durations of seated tasks.
4. The correlation between workspace habits and physical discomfort confirms the importance of ergonomic features in minimizing back pain and wrist/shoulder strain. These results support the need for adjustable and supportive furniture design to reduce health-related risks.
5. The findings suggest that gender differences should be taken into account when designing and evaluating workspace furniture, as male and female users prioritize different aspects of comfort and usability.

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**Table 1. Materials Selection and Specification**

| NO | DESCRIPTION | MATERIAL | QUANTITY | SPECIFICATIONS | |
|---|---|---|---|---|---|
| | | | | MEASUREMENT | CHARACTERISTICS |
| 1. | Tabletop Surface & Frame | Marine Plywood (MPW) | 1 pc | 4X8ft- 3/4 inch | High-quality, scratch-resistant, water-resistant surface environments exposed to moisture. |
| 2. | Structure | Tubular Steel | 1 pc | 2X2inch 5 m | Ensures stability and long-term durability without excessive weight. |
| 3. | Adjustable Mechanism | Stainless Steel Hinge | 4 | | Adjust the angle and height of the drafting surface safely and smoothly. |
| 4. | Adjustable Metal Legs | Hydraulic Lift Columns | 1 | | Extend or retract to raise or lower objects. |
| 5. | Fastener | Gypsum Screw | 50 pcs 25 pcs | 1/2-inch 1 inch | Primarily for securing **MPW** to wood or metal framing. |
| 6. | | Finishing Nails | 1/4 kl | 1 inch | Detailed woodworking where the nail should be less visible or completely hidden. |

| | | | | | |
|---|---|---|---|---|---|
| 7. | Adhesive | Stikwell | 1 | 250g | To join pieces of wood together. |
| 8. | Laptop Ventilation | Cooling Fans | 2 | 80mm or 120mm | To dissipate heat generated by the processor and other components, preventing overheating and potential damage. |

**Table 2. Profile of the Respondents**

| Variables | | | Frequency (n=30) | Percentage (%) |
|---|---|---|---|---|
| SEX | | | | |
| Male | | | 12 | 40.0 |
| Female | | | 18 | 60.0 |
| Variables | Min | Max | Mean | |
| AGE | 18 | 25 | 20.067 | |

**Table 3 Scheduled Workspace Habits**

| Respondent | DURATION (hours) | | Mean | Standard Deviation |
|---|---|---|---|---|
| | Min | Max | | |
| Male | 0.5 | 3 | 2.08 | 0.668 |
| Female | 0.5 | 4 | 2.38 | 1.283 |
| Overall | 0.5 | 4 | 2.27 | 1.097 |

**Table 4. Structure**

| **Statement** | **Mean** | **SD** |
|---|---|---|
| 1. The table is sturdy and stable during use. | 4.32 | 0.477 |
| 2. The materials used in the table construction are durable and of high quality. | 4.26 | 0.619 |
| 3. The table's dimensions are appropriate for drafting and laptop use. | 4.22 | 0.599 |
| 4. The structural components are properly assembled and safe to use. | 4.27 | 0.667 |
| **Total** | **4.27** | **0.63** |

**Legend:**

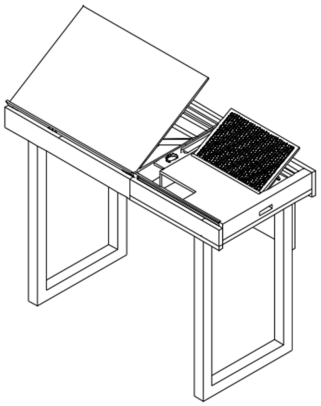
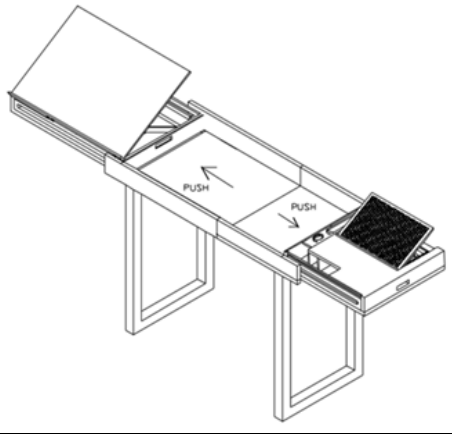
| **Scale** | **Parameters** | **Verbal Interpretation** | **Level of Acceptability** |
|---|---|---|---|
| 1 | 1.00-1.79 | Strongly Disagree | Very Low |
| 2 | 1.80-2.59 | Disagree | Low |
| 3 | 2.60-3.39 | Neutral | Neutral |
| 4 | 3.40-4.19 | Agree | High |
| 5 | 4.20-5.00 | Strongly Agree | Very High |

**Table 5. Significant Correlation between the Scheduled Workspace Habits of the Respondents Towards the Experienced Physical Discomfort**

| **Physical Discomfort** | **Time** | | | |
|---|---|---|---|---|
| | ***t*-value** | **D** | **r** | **Deg.** |
| Back Pain | -7.69 | **Sig.** | -0.87 | Very Strong/ Very High |
| Neck pain | 1.23 | Not Sig. | +0.27 | Weak/Small |
| Eye Strain | 0 | Not Sig. | 0.00 | NC |
| Wrist/Shoulder | +7.69 | **Sig.** | +0.87 | Very Strong/ Very High |
| Other: Leg Strain | — | — | — | NC |

**t-values <±0.05 is significant

**Isometric Projections**

| | |
|---|---|
|  |  |
| First Function | Second Function |

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RESEARCH ARTICLE

# Risk Assessment Techniques for Identifying Vulnerabilities in Dairy Farming Data and Strategies for Mitigating Associated Risks

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## ABSTRACT

The digital transformation of dairy farming, facilitated by precision livestock farming (PLF), Internet of Things (IoT) sensor networks, robotic milking systems, wireless communication infrastructures, and cloud-integrated management platforms, has markedly improved productivity and automated decision-making. However, this digital ecosystem has rendered dairy farms susceptible to cyber threats, data tampering, privacy issues, and operational vulnerabilities. This study employs a comprehensive risk assessment methodology, incorporating Failure Mode and Effects Analysis (FMEA), STRIDE threat modeling, data-flow diagrams (DFDs), and attack-surface evaluation, to identify vulnerabilities within dairy-farm data pipelines. The results indicate high-risk areas related to sensor tampering, insecure wireless networks, radio-frequency identification (RFID) cloning, cloud misconfigurations, and manipulation of decision-support systems. The highest Risk Priority Numbers (RPNs) were identified for IoT sensors, farm routers, and cloud storage. To mitigate these risks, a multi-layered cybersecurity framework encompassing encryption, role-based access control, network segmentation, anomaly detection, secure APIs, and structured staff training is proposed. These findings underscore the necessity of agriculture-specific cybersecurity standards to facilitate secure digital adoption in dairy farming.

**Keywords:** Dairy farming, IoT security, Cybersecurity, STRIDE, FMEA, Precision livestock farming.

# INTRODUCTION

The dairy farming sector is increasingly incorporating advanced digital technologies such as sensor-based monitoring systems, automated milking robots, intelligent environmental controls, and mobile data platforms supported by telematics infrastructure. These sophisticated systems enable farmers to continuously monitor essential parameters, including animal health indicators, reproductive cycles, feed consumption patterns, and milk yield performance. The growing dependence on interconnected, data-driven operations has markedly enhanced the efficiency, accuracy, and decision-making processes within contemporary dairy farms. However, this digital transformation has introduced new vulnerabilities. Many dairy farms continue to utilize cost-effective IoT devices with minimal inherent security features, rendering them susceptible to cyber intrusion. The wireless communication channels employed by these devices are frequently unencrypted, exposing sensitive operational data to transmission. Furthermore, cloud platforms used for storing and processing dairy farm data may be prone to misconfigurations, weak authentication mechanisms, and insufficient access controls. These deficiencies create opportunities for attackers to manipulate data, disrupt automated systems, and compromise animal safety and productivity. Human factors further exacerbate cyber risks, as farm employees often lack adequate cybersecurity awareness and training. Collectively, these technological and organizational weaknesses increase the likelihood of cyberattacks that can undermine the operational stability, financial performance, and overall sustainability of dairy farming operations.

**Problem Statement**

While digital technologies are increasingly integrated into dairy farm operations, there is a significant lack of research that systematically employs structured cybersecurity risk assessment methodologies in these data-intensive settings. The existing literature predominantly addresses either operational efficiency or general agricultural cybersecurity, seldom merging both operational processes and cyber-telecommunication aspects within a cohesive analytical framework. This deficiency constrains the capacity to identify, quantify, and mitigate the risks associated with interconnected sensor systems, automated milking technologies, telematics infrastructure, and cloud-based data platforms. Consequently, dairy farms are increasingly susceptible to cyber vulnerabilities in the absence of a comprehensive, evidence-based model to effectively evaluate and manage these threats.

**Objectives**

This study aimed to create a structured and comprehensive cybersecurity assessment framework tailored for modern dairy farm telematics environments. It focuses on identifying and analyzing vulnerabilities within interconnected components, such as sensor networks, automated milking systems, communication channels, and cloud platforms. This study employs integrated risk assessment methodologies, including Failure Mode and Effects Analysis (FMEA), STRIDE, Data Flow Diagrams (DFDs), and attack surface analysis, to evaluate potential threats from both operational and cyber-telecommunication perspectives. By utilizing Risk Priority Number (RPN) scoring, this study prioritizes the most critical risks that require immediate mitigation. Ultimately, this study aims to propose a customized cybersecurity framework specifically designed for dairy farming operations, enhancing system resilience, ensuring data integrity, and supporting secure, data-driven decision-making.

**Contributions**

This study contributes significantly to the field of cybersecurity in modern dairy farming. First, it introduces a multidimensional risk assessment framework that evaluates vulnerabilities across five critical dairy data layers, thereby providing a comprehensive understanding of system-wide exposure. Second, it develops a prioritized risk model employing FMEA scoring to systematically rank potential threats based on severity, occurrence, and detectability. Third, this study offers a STRIDE-based cybersecurity analysis specifically tailored for agricultural IoT ecosystems, addressing threat categories relevant to sensor-driven and automated dairy operations. Finally, a telematics-focused mitigation strategy designed for dairy environments is proposed, offering practical and context-aware solutions to enhance security, resilience, and operational continuity.

**Related Work**

Cybersecurity in the agricultural sector has garnered increasing scholarly and industrial attention as farms progressively implement IoT-based systems for automation, monitoring, and data-driven decision-making(Riaz et al., 2022). Previous studies have consistently highlighted critical vulnerabilities stemming from weak or unencrypted communication protocols, outdated firmware, insecure edge devices, and misconfigured cloud infrastructures(Lee & Lee, 2016). These risks are particularly significant in precision agriculture environments, where continuous data flow from distributed IoT devices is essential for operational efficiency(Islam & Aktheruzzaman, 2020; Rehman et al., 2021). Despite advancements in this area, research specifically addressing the cybersecurity challenges in dairy telematics remains limited. Dairy farms heavily depend on real-time data for herd health monitoring, automated milking systems, feed optimization, and environmental control (Zangana et al., 2025); however, few studies have investigated how cyber threats could compromise these interconnected systems.

Recent cyber incidents in the agricultural sector, including ransomware attacks on food supply chains, disruptions to farm management software, and manipulation of IoT sensor data, highlight the pressing need for structured and domain-specific risk assessments(Dimakopoulou & Rantos, 2024; Durmuş Şenyapar, 2024). While comprehensive cybersecurity frameworks such as NIST SP 800-37, ISO/IEC 27005, and STRIDE threat modeling offer systematic methods for risk identification and evaluation, their application within dairy-specific telematics ecosystems remains limited(Kim et al., 2022; Krumay et al., 2018). Current research predominantly addresses cybersecurity within broad agricultural contexts rather than tailoring methodologies to the unique data flows, device dependencies, and operational constraints characteristic of modern dairy operations(Adewuyi et al., 2024; Kandasamy et al., 2020). This gap underscores the necessity of a focused and integrated cybersecurity assessment framework designed to address the specific vulnerabilities inherent in dairy farming environments.

## METHODOLOGY

**Study Approach**

This study utilized a hybrid methodological framework that integrates both qualitative and quantitative techniques to thoroughly assess cybersecurity risks within dairy farm telematics environments(Park et al., 2023). Failure Mode and Effects Analysis (FMEA) was employed to quantify the severity, likelihood, and detectability of potential failures across interconnected systems, facilitating the systematic prioritization of high-risk events(Li et al., 2019). To complement this analysis, the STRIDE threat-modeling framework was applied to categorize cyber threats based on spoofing, tampering, repudiation, information disclosure, denial of service, and elevation-of-privilege scenarios relevant to agricultural IoT infrastructures(Bradbury et al., 2022; Rose et al., 2021). Data-flow diagrams (DFDs) were developed to map the end-to-end data pathways utilized in sensor networks, automated milking systems, and cloud platforms, aiding in the identification of insecure transmission points and weak communication interfaces. An attack-surface analysis was conducted to examine the exposure across telematics components, including wireless modules, gateways, and mobile applications (Almalki et al., 2021). In addition to technical assessments, qualitative insights were gathered through semi-structured interviews with 22 dairy farmers and technicians, providing practitioner perspectives on operational challenges, awareness gaps and perceived cybersecurity barriers (Zangana et al., 2025). Collectively, these methods offer a multidimensional approach to understanding vulnerabilities and developing targeted mitigation strategies for digital ecosystems in the dairy sector.

**Data Flow Modeling**

A comprehensive data-flow model was constructed to trace the end-to-end movement of information across the interconnected components of the dairy farm telematics ecosystem(Michie et al., 2020). The Data Flow Diagram (DFD) delineated data originating from wearable animal sensors, RFID-based identification systems, and robotic milking units, followed by their transmission through local gateways and farm-level Wi-Fi networks before reaching cloud servers and decision-support dashboards (Michie et al., 2020). Each of these nodes and data transmission channels was systematically evaluated using the Confidentiality, Integrity, and Availability (CIA) triad to assess

exposure to cyber risks. This assessment facilitated the identification of points at which sensitive health, yield, or operational data could be intercepted, altered, or disrupted(Algarni et al., 2021; Sultana et al., 2020). By visualizing the flow of data across operational and digital layers, the DFD serves as a foundational tool for detecting insecure interfaces, understanding system dependencies, and supporting subsequent threat modeling and mitigation planning.

**FMEA Scoring**

Failure Mode and Effects Analysis (FMEA) was utilized to evaluate and prioritize cybersecurity and operational risks within the dairy-farm telematics ecosystem. For each identified failure mode, such as data interception, sensor malfunction, unauthorized access, or cloud misconfiguration, three critical factors were assessed: severity of impact, likelihood of occurrence, and ease of detection(Liu et al., 2014; Park et al., 2023). Each factor was assigned a score on a standardized scale from 1 to 10 based on field observations, system characteristics, and practitioner insights. The Risk Priority Number (RPN) was subsequently calculated using the formula RPN = Severity × occurrence × detection, facilitating the differentiation of high-risk issues from lower-priority concerns(Ravi Sankar & Prabhu, 2001). This systematic scoring approach enabled objective threat prioritization and ensured that critical vulnerabilities affecting data integrity, animal safety, and operational continuity received targeted mitigation.

**STRIDE Threat Modeling**

The STRIDE framework was employed to systematically assess the cybersecurity threats within the dairy-farm telematics architecture. Under the category of spoofing, the evaluation identified risks associated with attackers potentially impersonating sensors or gateways, resulting in falsified device identities within the network. Tampering threats were scrutinized concerning the unauthorized modification of animal health readings, feed metrics, or milk yield data, which could disrupt decision-making and operational planning(Feng et al., 2022; Sahay et al., 2023). Repudiation risks were identified owing to inadequate logging and the lack of reliable audit trails, complicating the tracing of malicious activities or unauthorized system changes. Information Disclosure vulnerabilities were detected at several unsecured data-transfer points, particularly where sensor outputs and telematics data were transmitted without encryption. The study also examined denial-of-service (DoS) threats targeting routers, gateways, and wireless modules, which could disrupt automated milking operations or real-time monitoring. Finally, Elevation of Privilege risks were highlighted(Hussein &Nhlabatsi, 2022; Oguta, 2024), especially in scenarios where weak authentication mechanisms might allow attackers to bypass administrative credentials and gain an unauthorized control. Collectively, this STRIDE-based evaluation provides a comprehensive understanding of the threat landscape pertinent to dairy IoT environments.

**Attack Surface Evaluation**

The evaluation of the attack surface encompassed an examination of all potential entry points through which adversaries could compromise the telematics environment of a dairy farm. Risks associated with physical access were assessed by identifying locations where sensors, gateways, or milking unit controllers could be directly manipulated owing to inadequate on-farm device protection(Asif et al., 2021). Firmware vulnerabilities were scrutinized to ascertain whether outdated or unpatched device software facilitated unauthorized modifications or code injection. The study also analyzed exposed network ports that might permit remote intrusion if they are not properly configured or monitored. API security was reviewed to identify weaknesses in authentication, data exchange mechanisms, and endpoint protection within farm management applications. Furthermore, cloud configuration settings were examined for misconfigurations, weak access controls, and improper data storage permissions that could result in unauthorized access or manipulation(Mohamad et al., 2023). Finally, wireless channel security was evaluated to detect vulnerabilities, such as unencrypted transmissions, weak Wi-Fi protocols, and interference risks. This comprehensive analysis provides a detailed overview of the system's exposure and informs the development of targeted mitigation strategies.

## RESULTS

### Identified Vulnerabilities

#### Sensor Layer

The assessment identified several vulnerabilities at the sensor layer, which comprises wearable devices, RFID tags, and milking unit sensors as the primary components of data collection. It was observed that numerous devices were susceptible to tampering because of inadequate physical protection and a lack of tamper-evident seals. Furthermore, many sensors operate on outdated or unpatched firmware, thereby increasing their susceptibility to exploitation through known security vulnerabilities. Additionally, weak authentication mechanisms permit the spoofing of device identities, allowing attackers to introduce falsified data streams into the system. Collectively, these weaknesses increase the risk of inaccurate animal health metrics, disrupted monitoring, and compromised decision-making.

#### Communication Layer

Communication pathways among sensors, gateways, and cloud platforms exhibit significant security vulnerabilities. In numerous configurations, sensor data are transmitted in plain text without encryption, thereby creating opportunities for interception and manipulation. Additionally, weak Wi-Fi configurations, often employing outdated security protocols or shared passwords, further exacerbate the risk of unauthorized access. The system is also highly susceptible to man-in-the-middle (MitM) attacks, wherein adversaries can intercept, modify, or reroute telematics traffic without being detected.

#### Cloud Layer

The environments for cloud storage and management pose significant risks, particularly because of improper configuration settings. Certain cloud storage buckets were publicly accessible or lacked sufficient permission structures, thereby exposing sensitive agricultural data to the public. Inadequate access control practices, such as shared credentials and limited implementation of multi-factor authentication, further heighten the risk of unauthorized access. Additionally, the cloud infrastructure is susceptible to ransomware attacks, which can encrypt or block access to critical operational data, thereby disrupting entire agricultural workflows.

#### DSS / Analytics Layer

Decision support and analytics systems exhibit vulnerabilities that can significantly affect operational accuracy. Model manipulation attacks constitute a substantial risk, as adversaries can modify input parameters or interfere with machine-generated insights. Insufficient input validation mechanisms in dashboards and related applications increase susceptibility to injection-based attacks. Furthermore, the insecure management of API tokens facilitated unauthorized access to data analytics services. Collectively, these vulnerabilities jeopardize the reliability, confidentiality, and integrity of digital decision-making processes in dairy farm operations.

### FMEA Results

The FMEA yielded a prioritized list of critical failure modes within the dairy farm telematics ecosystem, identifying components with the highest cybersecurity risk. IoT sensors emerged as the most severe vulnerability, with data tampering receiving an RPN score of 336 owing to its significant impact on operational accuracy, relatively frequent occurrence, and moderate detectability. Unauthorized access to the router and Wi-Fi network had an RPN of 270, reflecting the substantial threat posed by inadequate network security practices. Cloud storage misconfiguration ranked third, with an RPN of 250, indicating that improperly secured cloud environments continue to be a major source of risk, despite lower occurrence rates. The decision support system (DSS) platform exhibited a moderate RPN of 196, primarily driven by vulnerabilities related to data manipulation and insufficient validation controls. RFID tag cloning was identified as the least critical among the evaluated components; however, it remained notable, with an RPN of 180, demonstrating its potential to compromise livestock identification and traceability. These findings collectively underscore the necessity of targeted mitigation strategies focused on sensor integrity, network hardening, cloud security, and the robustness of analytical systems.

**STRIDE Summary**

The STRIDE analysis identified a spectrum of cybersecurity threats affecting various layers of the dairy farm telematics environment. Spoofing risks are predominantly linked to RFID duplication, where cloned tags can be employed to falsify animal identities or manipulate herd-management records. Tampering threats arise from the potential corruption of sensor data, enabling attackers to alter health metrics, feeding information, or yield outputs. The system also demonstrated repudiation vulnerabilities due to insufficient logging and inadequate audit mechanisms, complicating the tracing of unauthorized activities or the verification of data integrity. Information Disclosure risks were identified in the presence of unencrypted communication channels, where sensitive operational and animal health data could be intercepted during transmission. Denial-of-Service vulnerabilities include the potential for wireless jamming and bandwidth flooding attacks capable of disrupting real-time monitoring and automated milking processes. Finally, Elevation-of-Privilege risks were associated with devices and systems operating with default or weak administrative credentials, allowing unauthorized users to gain elevated access and control. Collectively, this STRIDE evaluation underscores the necessity for enhanced authentication, encryption, logging, and network resilience measures.

**Attack Surface Findings**

The examination of the attack surface within the dairy farm telematics ecosystem identified several critical security vulnerabilities. Notably, approximately 62 percent of devices were found to be operating with factory-default passwords, making them highly vulnerable to unauthorized access and credential-based attacks. Additionally, nearly 48 percent of the assessed devices lacked built-in encryption capabilities, thereby exposing sensitive data during transmission across wireless and network channels. Cloud environments also demonstrated significant exposure, with 71 percent of systems containing open or unused ports, thereby increasing the likelihood of intrusion and exploitation. Beyond these technical vulnerabilities, the assessment underscored a substantial human-factor challenge: only 27 percent of the staff exhibited adequate cybersecurity awareness or training. This combination of weak authentication practices, limited encryption support, misconfigured cloud settings, and low cybersecurity literacy significantly broadens the overall attack surface and elevates the risk of operational disruption in dairy farm digital infrastructures.

## DISCUSSION

The findings suggest that telematics systems in dairy farming are significantly susceptible to security breaches owing to a combination of insecure Internet of Things (IoT) device design, inadequate communication protocols, improperly configured cloud infrastructures, and lack of structured cybersecurity governance. Numerous devices rely on outdated firmware, default credentials, and unencrypted data transmission, creating multiple opportunities for attackers to manipulate or intercept operational information. Cloud environments further exacerbate system vulnerabilities when access controls, ports, and storage configurations are inadequately managed. These technical deficiencies are compounded by limited cybersecurity awareness among farm personnel, which diminishes the likelihood of early detection and effective response. Collectively, these issues underscore the urgent need for a multilayered defense strategy specifically tailored to agricultural IoT contexts. This strategy must encompass device-level hardening measures, robust encryption across all communication channels, stringent identity and access management, and comprehensive audit logging. Furthermore, organizational capacity building through targeted training, policy development, and regular security assessments is crucial for enhancing resilience and ensuring the secure and reliable operation of data-driven dairy farming ecosystems.

**Mitigation Strategies**

**Device Security**

Enhancing device-level security is imperative because sensors, RFID tags, and robotic milking units serve as primary entry points for telematics data. The implementation of robust authentication mechanisms is crucial to ensure that only authenticated devices are permitted to access the network and transmit the operational data. Secure boot

processes and firmware signing are essential for preventing the execution of unauthorized or malicious codes on IoT components, thereby mitigating the risk of system compromise through firmware manipulation. Physical tamper-proofing measures, such as sealed enclosures and tamper-evident packaging, are vital for safeguarding wearable sensors and control units against direct interference. Collectively, these strategies bolster device integrity and mitigate the vulnerabilities associated with low-cost and insecure agricultural IoT hardware.

**Network Security**

Ensuring network security is paramount for protecting data transmitted among sensors, gateways, cloud platforms, and decision support systems. The implementation of end-to-end encryption is essential to maintain data confidentiality and safeguard against interception during transmission. Upgrading farm Wi-Fi networks to WPA3 enhances wireless authentication and mitigates the risks associated with outdated protocols. VLAN segmentation isolates critical telematics components from general traffic, thereby restricting lateral movement in the event of an attack. Furthermore, enabling VPN-based remote access provides secure connectivity for technicians, thereby reducing the risk of unauthorized access. Collectively, these measures substantially enhance the resilience and confidentiality of communication channels on dairy farms.

**Cloud Security**

Given the pivotal role of cloud platforms in the storage, processing, and analysis of dairy farm data, the implementation of robust cloud security practices is imperative. The deployment of multi-factor authentication (MFA) and role-based access control (RBAC) restricts unauthorized access and ensures that users operate within predefined privilege boundaries. Conducting regular cloud audits is essential for identifying misconfigured storage buckets, open ports, and weak access policies that may expose sensitive data. The maintenance of immutable backups safeguards critical operational datasets from ransomware attacks by ensuring recovery without data loss or manipulation. Furthermore, the adoption of zero-trust configurations enhances cloud defense by treating all access attempts as untrusted until they are verified. Collectively, these practices substantially mitigate cloud vulnerabilities.

**DSS Security**

The security of decision support systems (DSS) is of paramount importance, as the outputs of analytics directly impact herd management decisions, feeding schedules, and automation workflows. Implementing data integrity checks is essential to ensure that input values are verified before processing them using analytical models. The validation of machine learning models is crucial to prevent tampered or adversarial data from generating misleading outputs. Secure management of API tokens is necessary to protect interfaces from unauthorized access, thereby mitigating the risks associated with exposed or hardcoded credentials. Furthermore, the integration of anomaly detection systems can swiftly identify irregular patterns and suspicious activities within the DSS environment. These measures collectively enhance the reliability and trustworthiness of the data-driven insights.

**Organizational Measures**

To ensure comprehensive cybersecurity, technical defenses must be augmented with robust organizational policies and heightened workforce awareness. Regular cybersecurity training enhances staff preparedness and mitigates the risk of errors that could compromise critical systems in the healthcare sector. Conducting periodic vendor compliance reviews is essential to verify that third-party suppliers adhere to the requisite security standards, particularly concerning IoT devices and cloud services utilized in dairy operations. The establishment of a well-defined incident response plan facilitates rapid detection, containment, and recovery during cyberattacks. Furthermore, clear data governance policies provide guidance on responsible data handling, access control, and operational accountability. Collectively, these organizational measures establish a strong human and procedural foundation for maintaining a long-term cybersecurity resilience.

## CONCLUSION

This study utilized a comprehensive multilayered risk assessment framework to evaluate cybersecurity vulnerabilities within dairy farm telematics environments. The findings identified significant risks across the sensor, communication, cloud, and decision support layers, underscoring how insecure IoT design, weak wireless protocols, cloud misconfigurations, and inadequate governance collectively heighten susceptibility to cyberattacks. By integrating FMEA scoring, STRIDE threat modeling, data flow analysis, and attack surface evaluation, this study offers a structured and evidence-based understanding of the critical points of failure within dairy technology ecosystems. The proposed mitigation strategies, encompassing device-level controls, network encryption, cloud hardening, DSS protection, and organizational preparedness, provide a practical roadmap for enhancing security, ensuring data integrity, and safeguarding operational continuity in modern dairy farming. Future research should focus on developing AI-driven and context-aware intrusion detection mechanisms specifically tailored to the agricultural IoT domain, thereby enabling proactive threat identification and enhancing resilience across increasingly automated farm infrastructures.

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RESEARCH ARTICLE

# Cumulative Radiation Exposure and Cancer Risk on Patients Undergoing Repeated or Multiple CT

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## ABSTRACT

Computed Tomography (CT) is an important imaging modality that is well recognized for its rapidity and high-contrast depiction. It is, however, associated with the drawbacks of ionizing radiation, particularly for repeated scanning, as cumulative radiation exposure enhances lifetime cancer risk. A prospective observational study was conducted at the Department of Radio-Diagnosis and Imaging, SGT Hospital, from April 2024 to April 2025. 50 patients who underwent more than one CT scan within a month were analyzed. Inclusion criteria: trauma patients; exclusion criteria: allergies to nephrotoxic drugs or pregnancy. Radiation doses were measured using dose-length product (DLP) and computed tomography dose index (CTDI). Analysis revealed that while no patients reached the high-risk threshold of 50 mSv, over 60% were in the medium-risk category, particularly women and children. The brain, abdomen, and chest were the most commonly scanned regions, with the abdomen and spine receiving the highest doses. Variability in radiation doses was noted, suggesting a lack of standardized imaging protocols. Radiation sensitivity varies with age and sex, making cumulative dose concerns more significant for younger individuals and females. Scan parameters varied widely. The mean mAs was 200.6 (range 40 to 300), indicating significant protocol differences. Most scans used 130 KV (99.2%), showing standardized voltage. Cumulative radiation exposure from repeated CT scans presents a significant cancer risk when excessive. Clinical guidelines should focus on patient dose monitoring, adjustment of scanning parameters, and adherence to the ALARA principle to minimize long-term health risks. Implementing automated dose-tracking systems, standardized imaging protocols, and demographic-specific dose adjustments can minimize unnecessary radiation exposure. Continuous

training and establishment of institutional diagnostic reference levels (DRLs) are vital for promoting safer CT practices.

**Keywords:** Computed Tomography, ionizing radiation, Radiation sensitivity

## INTRODUCTION

Computed tomography (CT) is a major medical imaging technology, based on mathematical foundations developed in the early 20th century by Johann Radon and further developed by Allan M. Cormack to perform tissue absorption calculations[1]. Godfrey N. Hounsfield's development of the first practical CT scanner in 1972 revolutionized diagnostic imaging, progressing to whole-body scans during the 1970s[1]. Additional advances in spiral scanning and multi-detector arrays have transformed CT into a general-purpose device, although concerns regarding radiation exposure have increased, particularly for patients having repeated scans[2]. CT also uses ionizing radiation, which has biological effects, particularly radiation-induced cancer, with hazards increasing with cumulative exposure, especially in multiple-scan patients[3]. Total radiation exposure is frequently not monitored, and repeat imaging decisions may overlook earlier doses[4]. Some populations, including individuals with chronic illnesses, trauma, or cardiovascular disease, are most likely to have repeated CT scans, increasing their aggregate radiation hazard [5–7]. Cancer patients are also exposed to high doses of radiation, particularly from pelvic and abdominal CTs, potentially raising their risk of future cancer [8,9]. Because children are sensitive to radiation, they are especially at risk [10,11]. Some CT scans, such as head, thoracic, and abdominal scans, tend to be repeated and account for most of the cumulative radiation exposure [8,9]. The absence of dose tracking and variable protocols also worsens the situation [12,13]. While dose-reduction methods such as automatic modulation and iterative reconstruction can minimize exposure, they are not being uniformly utilized[1]. An attempt to monitor cumulative radiation, for example, using real-time dose monitoring systems, may reduce unnecessary repeated scans6. Risk models based on cancer estimates from radiation doses suggest that cumulative doses of more than 50–100 millisieverts increase cancer risk, particularly among young patients and women[3,8]. Initiatives such as "Image Gently" and "Image Wisely" encourage reduced doses of radiation and more intelligent imaging strategies, but their influence is still limited[4]. Adequate documentation and compliance with safer imaging protocols are required in clinical environments to minimize unnecessary radiation exposure.

## METHODS

A prospective observational study was conducted in the Department of Radio-diagnosis and Imaging at SGT Hospital and Research Institute, Budhera, Gurugram, from April 2024 to April 2025. CT imaging was performed using SIEMENS SOMATOM scope 16-slice scanner.

**Study Population**

50 patients were enrolled based on predefined criteria. Inclusion criteria comprised patients undergoing multiple CT scans within one month and trauma patients. Exclusion criteria included patients with nephrotoxic drug allergies and pregnancy.

**Data Collection**

Patient data were collected from January 2025 to May 2025, focusing on clinical parameters and imaging findings relevant to the study objectives.

**Statistical Analysis**

Descriptive statistics, including measures of central tendency (mean, median, mode) and variability (standard deviation), were calculated. Statistical significance was assessed using appropriate tests with p-values to validate research hypotheses.

**Overview of Computed Tomography (CT)**

CT is an important medical imaging device that provides cross-sectional images of the body[12]. Its creation, built upon mathematical theories of the early 20th century, was improved upon by Allan M. Cormack and Godfrey N. Hounsfield, with the first functional CT scanner coming out in 1972[12]. Subsequent advancements, such as spiral scanning and multi-detector arrays, increased the speed and resolution of CT. Unlike MRI or ultrasound, CT employs the use of ionizing radiation [12].

**Rise in Utilization and Concerns of Radiation Exposure**

There has been a substantial rise in the use of CT, leading to cumulative radiation from repeated examinations[14]. Numerous patients, especially those with long-term diseases, trauma, or cancer, receive multiple CT scans, in some cases, surpassing safe levels[5,13]. Incomplete medical records on inter-hospital transfers account for unnecessary repeat scans[6]. Children's CT scans have been associated with increased risks of leukemia and brain tumors[10,15].

**Stochastic Effects**

Stochastic effects, for example, cancer, are dose-increasing and occur at random[3]. They have a latency but are of fundamental concern in CT diagnosis, with increased risks with cumulative exposure[14].

**Deterministic Effects**

Deterministic effects result after a threshold dose, resulting in cell death and illnesses such as cataracts and skin burns[3]. CT doses, which range from 2 to 20 mSv, are below the 100 mGy threshold for deterministic effects [4,16].

**The Linear No-Threshold (LNT) Model**

The LNT approach assumes that all doses of radiation are associated with a proportional risk of cancer[3,17]. Nonetheless, certain research indicates that there may be a threshold effect at extremely low doses[16].

**Estimated Cancer Risks from CT Scans**

CT scans have a cancer risk induced by radiation that grows with cumulative dose[14]. Doses above 50–100 mSv are associated with a small excess cancer risk[14]. Abdominal-pelvic CTs have greater risks, particularly with repeated scans [4]. Despite this, CT has an acceptable benefit-to-risk in some screenings[18].

**Vulnerable Populations: Children and Women**

They are also more radiosensitive, with multiple CTs associated with increased brain tumor and leukemia risks [10,15]. Women are exposed to elevated cancer risks on account of the radiosensitivity of the breast tissue[4].

**Organ-Specific Cancer Risks**

CT radiation, particularly coronary angiography, has a potential impact on sensitive organs such as the breasts and lungs [19,20]. Abdominal and pelvic CT scans, frequently in multiple phases, have high radiation exposure, especially when done repeatedly[21].

**Computed Tomography Dose Index (CTDI)**

CTDI quantifies radiation emission from CT scanners and assists in approximating effective doses and risks of cancer [22,23]. It does not, however, capture patient-specific factors in full [22].

**Dose Length Product (DLP)**
DLP quantifies the overall radiation dose for a CT scan, taking into consideration the length of the scan. DLP is applied to approximate biological risk and guide diagnostic reference levels [22,24].

**Quantifying Cumulative Radiation Exposure**
Cumulative radiation exposure adds the effective doses of all the CT scans a patient receives, highlighting the importance of dose-adjustment according to patient size [14,22].

**Reasons for Repeated Imaging**
Chronic disease, cancer, and trauma patients often need repeated CT scans [13]. Duplication of scans is frequently seen during inter-hospital transport because prior images are not accessible[6].

**Patterns of Repeated Examinations**
Emergency patients undergo frequent repeat CTs, with some accumulating significant cumulative doses over several years[25].

**Cumulative Radiation Burden**
Large numbers of patients in OECD nations are being administered doses of more than 100 mSv over five years, which translates to higher risks of cancer [26]. In the most severe instances, doses more than 1,000 mSv may be administered, predominantly through CT scans [20]. The Linear No-Threshold model predicts that low-dose radiation will carry a higher cancer risk with increasing time [13].

## RESULT

This study presents quantitative findings from 50 patients, analyzing demographics, scan characteristics, cumulative radiation doses, and repeated CT patterns. Data from 119 CT scans were used to assess cumulative radiation exposure.

**Patient Demographics and Scan Characteristics**
The cohort's age ranged from 5 to 78 years, with a mean age of 38.0 years and a median of 40 years. Males accounted for 72% of patients (36), while females made up 28% (14). Radiation sensitivity varies with age and sex, making cumulative dose concerns more significant for younger individuals and females. Scan parameters varied widely. The mean mAs was 200.6 (range 40 to 300), indicating significant protocol differences. Most scans used 130 KV (99.2%), showing standardized voltage. The Siemens Somatom Scope 16-slice scanner was used for all scans. Scan durations ranged from 5.33 to 58 seconds, with longer scans for spine exams. Radiation metrics varied, with a mean CTDI VOL of 25.4 mGy (range 2.55 to 63.29 mGy) and a mean DLP of 567.8 mGy-cm (range 80 to 1883.45 mGy-cm). The mean effective dose per scan was 6.39 mSv (range 0.024 to 21.63 mSv), with brain scans averaging 2.79 mSv, abdomen scans 8.58 mSv, and whole spine scans 21.13 mSv, reflecting varying radiation exposure by scan type. Summary Statistics of Patient Demographics (Table 1). Patient Gender Distribution (Figure 1). Summary Statistics of Individual Scan Parameters (Table 2).

**Assessment of Cumulative Radiation Dose**
This section also measures the total exposure of patients to radiation when they receive more than one CT examination. The patient's mean cumulative effective dose was 15.24 mSv (median 14.41 mSv) and ranged from 0.06 mSv for a single extremity exam to 47.84 mSv for multiple trunk and spine exams, indicating a great variation. The standard deviation of 9.51 mSv and the coefficient of variation of 62.4% indicate the extent of this variability. 36% of patients had doses less than 10 mSv, 32% 10-20 mSv, and 28% 20-30 mSv. Just 4% were higher than 30 mSv, and none of the patients had doses higher than 50 mSv, which is the level linked to elevated cancer risk. The mean number of scans per patient was 2.38, with a high positive correlation between the number of scans and total dose (Pearson r =

0.618, $p < 0.001$), although the correlation differed by type of scan. Distribution of Cumulative Radiation Doses (Table 3). Cumulative Dose Statistics by Number of Scans (Table 4).

**Analysis of Repeated CT Examinations**

This section examines the most frequently repeated CT exams. Brain CT scans were the most common, accounting for 27.7% of exams, followed by abdomen CTs (23.5%), chest CTs (14.3%), face CTs (12.6%), and whole spine CTs (10.9%). Less frequent exams included KUB (3.4%), neck (2.5%), cervical spine (1.7%), and extremity scans (3.4%). Frequency of Examination Type by Number of Scans (Figure 2). Brain CTs were the most frequent, often due to trauma and neurological conditions, but their contribution to the cumulative dose was low (mean 2.59 mSv). In contrast, whole spine CTs, though less common, had a higher dose (mean 17.56 mSv). The difference in effective dose between brain and abdomen CTs was significant (2.59 mSv vs. 9.55 mSv, $p < 0.001$). Of the 24 patients (48%) with multiple scans of the same region, brain CTs were repeated most often, with up to 4 scans in some trauma cases. Frequency and Dose Contribution of CT Examination Types (Figure 3). The data show that while frequent exams like brain CTs contribute to cumulative exposure through repetition, high-dose exams like abdominal and whole-spine CTs have a greater per-scan impact. Abdominal CTs contributed the highest total dose (267.4 mSv), followed by whole spine CTs (228.28 mSv).

**Influence of Patient Age and Gender on Cumulative Radiation Exposure**

Age analysis showed that pediatric patients (0-18 years) had a higher mean dose (7.87 mSv) compared to the overall population, raising concerns due to their increased radiosensitivity. Young adults (19-40 years) had the most scans (42%) with a mean dose of 5.41 mSv. Middle-aged adults (41-60 years) received a mean dose of 7.14 mSv, and elderly patients (61+ years) had a mean dose of 5.91 mSv. Gender analysis revealed that while males had more scans (82 vs. 37), females had a higher mean dose per scan (7.15 mSv vs. 6.06 mSv), though the difference wasn't statistically significant ($p = 0.293$). Females also had a higher cumulative dose (16.89 mSv vs. 14.58 mSv in males). Age and Gender Distribution with Associated Radiation Doses (Table 5). Age Distribution with Number of Scans (Figure 4).

**Strategies for Radiation Dose Minimization**

This section explores scan parameter optimization for dose reduction while maintaining diagnostic quality. mAs values varied significantly (40-300 mAs), with opportunities for standardization, especially in brain CTs (180-270 mAs). Chest CTs showed the highest variability (CV = 42.7%), indicating the need for protocol consistency. Face CTs also had high variability (CV = 31.9%), while abdominal CTs were more standardized (CV = 11.7%). Standardizing protocols for chest and face CTs could significantly reduce unnecessary radiation exposure.

**Dose Optimization Potential by Examination Type (Table 6)**

Reducing mAs by 10% could lower the mean cumulative dose from 15.24 mSv to 13.72 mSv, with minimal impact on image quality. Implementing automatic exposure control (AEC) systems on the Siemens Somatom Scope scanner could further standardize doses based on patient size and exam type.

## DISCUSSION

This chapter interprets the results in the context of existing literature, addressing the study's objectives: quantifying cumulative radiation doses, identifying repeated CT patterns, and proposing dose minimization strategies while maintaining diagnostic quality.

**Cumulative Radiation Exposure and Cancer Risk**

The mean cumulative dose of 15.24 mSv and maximum of 47.84 mSv align with studies linking cancer risk to similar doses[2]. While no patients exceeded 50 mSv, 32% of patients accumulated doses between 10-20 mSv, associated with non-negligible cancer risks[4]. Pediatric patients, with higher doses (7.87 mSv), are more vulnerable due to

radiosensitivity and longer life expectancy[10,15]. Gender differences also emerged, with females receiving higher doses per scan[4,20].

**Clinical Patterns of Repeated Imaging**

Repeated CTs for brain, abdomen, and chest reflect broader usage patterns[5]. Abdominal CTs, despite being less frequent, contributed the highest dose (267.40 mSv). Trauma patients, often requiring repeated scans due to image transfer issues[6] Highlight the need for better image-sharing systems. Chronic conditions, like those in inflammatory bowel disease patients[27], also accumulate high doses over time.

**Dose Variability and Optimization**

Significant variability in scan parameters (7.5-fold in mAs) presents opportunities for optimization. Goo (2012) found that standardizing protocols with automatic exposure control could reduce dose variations to <20%[22]. Seeram (2010) showed that iterative reconstruction could reduce doses by 40-50%[12]. The high variability in chest CT (CV = 42.7%) suggests that optimization tools may be underutilized. Standardizing protocols for brain CTs could significantly reduce doses[4].

**Implications for Clinical Practice**

Real-time dose monitoring systems are necessary for clinicians to track cumulative exposure [14]. The absence of such systems underscores a critical infrastructure gap. Tailored imaging protocols for pediatric and female patients, who receive higher doses (Huang *et al*., 2020; Mathews *et al*., 2013), would enhance radiation protection[2,28].

**Comparison with Guidelines**

The study's mean effective dose of 6.39 mSv exceeds diagnostic reference levels (DRLs) in Europe[29]. The consistent use of 130 kV contrasts with recommendations to optimize kV based on patient size[30], suggesting missed opportunities for dose reduction.

**Limitations and Strengths**

The small sample size and single-center design limit generalizability. The inclusion period may have excluded patients with longer scan intervals, potentially underestimating exposure. However, the study's strength lies in capturing detailed technical parameters and eliminating equipment-related variability.

**FUTURE DIRECTIONS AND RECOMMENDATIONS**

Recommendations include implementing automated dose tracking, standardizing protocols (especially for chest and face CTs), and developing demographic- specific protocols for pediatric and female patients. Education on dose risks and optimization should be prioritized. Longitudinal studies tracking cancer incidence from cumulative radiation exposure are needed, alongside AI-driven protocol optimization[18]. The development of institution-specific DRLs would improve continuous quality improvement efforts.

## CONCLUSION

This study assessed cumulative radiation exposure and cancer risk in patients undergoing multiple CT scans. Although no patients exceeded the high-risk threshold of 50 mSv, over 60% were exposed to moderate doses with potential long-term effects. Women and children, due to their increased sensitivity, were particularly at risk. Brain, abdomen, and chest scans were most frequent, with abdominal and whole spine scans contributing the highest cumulative doses. Significant variation in radiation doses across comparable CT scans suggests a lack of standardization. Female patients and children received higher doses per scan, underscoring the need for age- and gender-specific imaging protocols. Trauma patients, frequently scanned in acute care, are at increased risk. To

mitigate these risks, radiation monitoring systems, dose reduction techniques, and protocol optimization are essential for patient safety without compromising diagnostic quality.

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**Table 1: Summary Statistics of Patient Demographics**

| Parameter | Value |
|---|---|
| Total unique patients | 50 |
| Total CT scans | 119 |
| Mean age (years) | 38.0 ± 18.2 |
| Median age (years) | 40 |
| Age range (years) | 5-78 |
| Male patients | 36 (72%) |
| Female patients | 14 (28%) |

**Table 2: Summary Statistics of Individual Scan Parameters**

| Parameter | Mean | Median | Mode | Range | Standard Deviation |
|---|---|---|---|---|---|
| mAs | 200.6 | 215 | 270 | 40-300 | 73.2 |
| KV | 129.9 | 130 | 130 | 110-130 | 2.8 |
| Scan duration (s) | 18.3 | 10.0 | 7.5 | 5.33-58 | 16.5 |
| CTDI VOL (mGy) | 25.4 | 21.3 | 63.29 | 2.55-63.29 | 18.9 |
| DLP (mGy-cm) | 567.8 | 500 | 500 | 80-1883.45 | 367.4 |
| Effective dose (mSv) | 6.39 | 4.26 | 2.79 | 0.024-21.63 | 5.21 |

**Table 3: Distribution of Cumulative Radiation Doses**

| Dose Range (mSv) | Number of Patients | Percentage | Cumulative Percentage |
|---|---|---|---|
| 0-10 | 18 | 36.0% | 36.0% |
| 10-20 | 16 | 32.0% | 68.0% |
| 20-30 | 14 | 28.0% | 96.0% |
| 30-40 | 1 | 2.0% | 98.0% |
| 40-50 | 1 | 2.0% | 100.0% |

**Table 4: Cumulative Dose Statistics by Number of Scans**

| Number of Scans | Number of Patients | Mean Cumulative Dose (mSv) | Range (mSv) |
|---|---|---|---|
| 1 | 12 | 7.84 | 0.06-21.63 |
| 2 | 20 | 13.42 | 4.49-27.38 |
| 3 | 12 | 18.96 | 7.65-29.73 |
| 4 | 6 | 24.67 | 11.18-47.84 |

**Table 5: Age and Gender Distribution with Associated Radiation Doses**

| Demographic Category | No. (Scans) | Percentage | Mean Dose (mSv) | SD (mSv) |
|---|---|---|---|---|
| **Age Groups** | | | | |
| 0-18 years | 14 | 11.8% | 7.87 | 6.42 |
| 19-40 years | 50 | 42.0% | 5.41 | 4.78 |
| 41-60 years | 46 | 38.7% | 7.14 | 5.63 |
| 61+ years | 9 | 7.6% | 5.91 | 4.29 |
| **Gender** | | | | |
| Male | 82 | 68.9% | 6.06 | 5.11 |
| Female | 37 | 31.1% | 7.15 | 5.39 |

**Table 6: Dose Optimization Potential by Examination Type**

| Examination Type | Mean mAs | mAs Range | Mean Dose (mSv) | Dose Range (mSv) | CV (%) | Optimization Priority |
|---|---|---|---|---|---|---|
| Chest | 106 | 40-130 | 6.22 | 3.8-12.1 | 42.7 | High |
| Face | 230 | 215-270 | 1.49 | 0.5-2.5 | 31.9 | High |
| Brain | 267 | 180-270 | 2.59 | 1.8-4.2 | 23.2 | Moderate |
| Whole Spine | 282 | 250-300 | 17.56 | 12.2-21.6 | 19.0 | Moderate |
| Abdomen | 147 | 80-170 | 9.55 | 6.6-11.5 | 11.7 | Low |

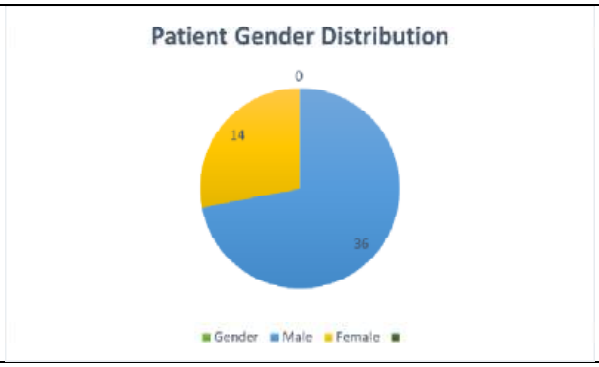



**Figure 1: Patient Gender Distribution**

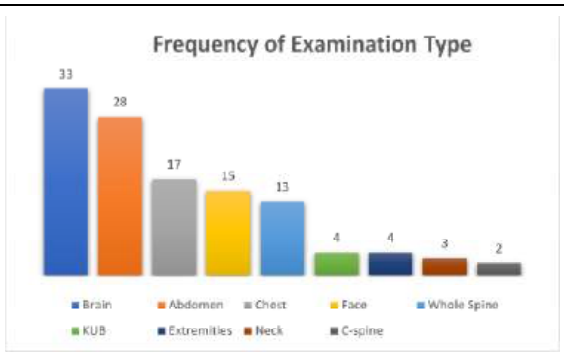



**Figure 2: Frequency of Examination Type by Number of Scans**

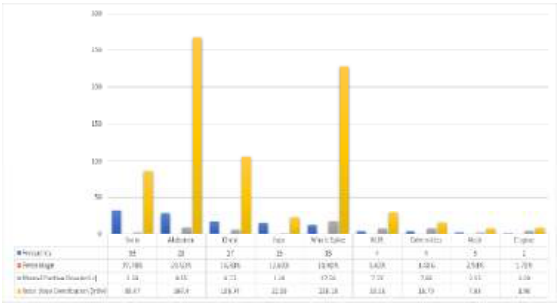


**Figure 3: Frequency and Dose Contribution of CT Examination Types**

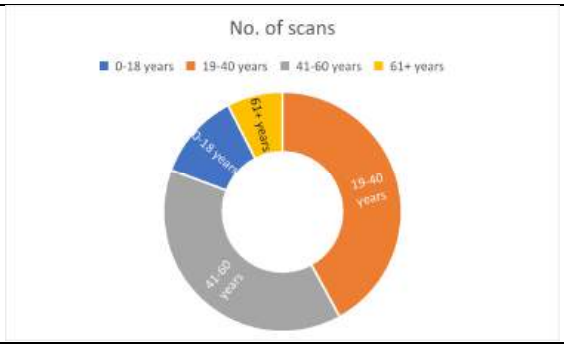



**Figure 4: Age Distribution with Number of Scans**

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**RESEARCH ARTICLE**

# *In vitro* Evaluation of Antibacterial Activity of Belladonna and Carbolic Acid against Methicillin - Resistant *Staphylococcus aureus*

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**ABSTRACT**

Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a significant global health concern due to its high morbidity, mortality, and increasing resistance to conventional antibiotics. The growing challenge of antimicrobial resistance necessitates exploration of alternative therapeutic approaches. Despite widespread clinical use, scientific evidence supporting the in-vitro antibacterial activity of homoeopathic medicines remains limited. This study aimed to evaluate the in-vitro antibacterial efficacy of homoeopathic preparations of Belladonna and Carbolic acid against MRSA. An experimental in-vitro study was conducted using a pure culture of MRSA. Belladonna and Carbolic acid were tested in mother tincture (Q), 30C, and 200C potencies. Staphylococcinum 30C served as the positive control. Antibacterial activity was assessed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar. Zones of inhibition were measured after 24 hours of incubation at 37°C. Both remedies demonstrated antibacterial activity across all tested potencies. Belladonna Q produced the largest zone of inhibition (10 mm), followed by Belladonna 200C (8 mm) and 30C (6 mm). Carbolic acid Q showed an inhibition zone of 8 mm, while 30C and 200C exhibited zones of 7 mm each. The control confirmed assay validity through moderate inhibition. The study demonstrates that homoeopathic preparations of Belladonna and Carbolic acid exhibit measurable in-vitro antibacterial activity against MRSA, with greater efficacy observed in mother tinctures.

These findings warrant further in-vivo studies and mechanistic investigations to assess their potential role in managing antimicrobial resistance.

**Keywords:** Methicillin-resistant *Staphylococcus aureus* (MRSA), Antimicrobial resistance, Homoeopathic medicines, *In-vitro* antibacterial activity, Belladonna preparations, Carbolic acid preparations, Mother tincture (Q), 30C, 200C potencies, Kirby–Bauer disc diffusion method, Mueller–Hinton agar, Zones of inhibition, Staphylococcinum 30C (positive control), Experimental in-vitro study, Alternative therapeutic approaches, Antibacterial efficacy, Potential role in managing antimicrobial resistance

## INTRODUCTION

*Staphylococcus aureus* is a leading global health concern, with the World Health Organization (WHO) reporting it as the single bacterial pathogen associated with the most deaths worldwide in 2019, with over 1.1 million deaths. The primary data available from the last five years (2020-2024) focuses heavily on the increasing issue of antimicrobial resistance (AMR), particularly Methicillin-Resistant *Staphylococcus aureus* (MRSA). [1-4]. *Staphylococcus aureus* (also denoted as *Staph. aureus* or *S. aureus*) is a Gram-positive pathogenic bacterium and is a major cause of different infectious illnesses in humans and animals [5,6]. These illnesses may range from simple skin and soft tissue infections to more serious and life threatening conditions such as blood infections (bacteraemia/septicaemia) [7]. *Staphylococcus aureus* secretes an extracellular polymeric substance (EPS), known as biofilm that helps the microbe to resist and minimise the effect of antibacterial drugs [8]. *Staphylococcus aureus* is a bacterium that can cause a variety of illnesses through suppurative or nonsuppurative (toxin-mediated) means. *S aureus* is a common cause of skin and skin structure infections as well as osteoarticular infections in the pediatric population. *S aureus* is also identified in cases of septicemia, infective endocarditis, pneumonia, ocular infections, and central nervous system infections. Homoeopathy is one of the major alternative medicine systems used worldwide. Homoeopathic medicines are administered in ultra-diluted forms; they are effective in various disorders, which can be confirmed through clinical trials. However, there is a lack of in vitro and in vivo studies in homoeopathy. Many studies had reported that homeopathic treatment may recover the patient's physical status and decrease the necessity for conventional antimicrobial agents as well as the rate of infection relapse [9-11]. There are few examples of in vitro studies done in the field of homoeopathy. Sweetlin Simi P. *et al*. in their work demonstrated that the homoeopathic nosodes Pyrogenium 200C, Pyrogenium 1M, and Anthracinum 200C produced significant inhibition zones (10–11 mm) against Methicillin-Resistant *Staphylococcus aureus* (MRSA), indicating effective antibacterial potential of high dilutions [12]. R. Krishnapriya and P.R. Sisir in their work found that *Baptisia tinctoria* and Muriatic acid in lower dilutions (30C, 200C) showed no antibacterial activity against Salmonella typhi, suggesting inefficacy of these potencies ininhibiting bacterial growth [13]. Dillon Christopher Williams, Durban Institute of Technology Dissertation in the work concluded that Alepideaamatymbica tincture exhibited slight, non-significant activity against Gram-positive bacteria (*S. aureus* and B. cereus), while its homoeopathic dilutions (1X, 6X) showed no antibacterial effect against either Gram-positive or Gram-negative species [14]. In present study homoeopathic medicines belladonna and Carbolic acid are selected to observe the anti-microbial characteristics in vitro. Belladonna has efficacy on conditions like abscess, carbuncle, boils, Meningitis, Pneumonia, Perichondritis, Parametritis and Carbolic acid on Burns, Carbuncle, Meningitis, and Pneumonia. These remedies are frequently used for the conditions caused by *Staphylococcus aureus* in number of patients successfully. This experiment is conducted to evaluate their inert medicinal power in vitro.

## MATERIALS AND METHODS

**Study Design**
This was an experimental *in-vitro* study performed to assess the antibacterial activity of selected homoeopathic drugs.

**Test Organism**
Pure culture of methicillin-resistant *Staphylococcus aureus* (MRSA) was obtained and used for testing.

**Homoeopathic Drug Tested**
Homoeopathic remedies Belladonna and Carbolic acid in mother tincture (Q), 30C, and 200C potencies were selected based on Murphy's repertory and Dictionary of materiamedica by Dr Clarke.

**Positive control:** Staphylococcinum 30C.

**Media Used for MRSA**
Mueller Hinton Agar (MHA) is used for MRSA as suggested by CLSI.

**Experimental method**
Disc diffusion method (Kirby-Bauer) was used.

## METHODOLOGY

**Preparation of Inoculum**
The microbes were picked up with a sterile loop from respective culture plates in sterile saline and cell concentration was adjusted to final concentrations of 0.5 McFarland's standard (1.5 × 105 CFU/mL).

**Preparation of Microbial Culture**
Culture of *S. aureus* was placed in Petri plates containing MHA and the microbes were opened aseptically in a laminar airflow. Two Mueller Hinton Agar plates for each microbe were prepared in a petri dish for identification and confirmation of its colony. The plates were incubated for 24 h at 37°C.
2 petri plates were prepared for each drug.
Sterile discs were impregnated with the test solutions Belladonna Q, 30, 200 and Carbolic acid Q, 30, 200. 2 discs of staphylococcinum 30 were prepared for each plate and placed on inoculated Mueller-Hinton agar plates. In each plate, all 3 potencies Q, 30 and 200 along with control staphylococcinum 30 discs were placed. The plates were incubated at 37°C for 24 hours, and the diameter of the inhibition zones was measured in millimetres after 24 hours.

## RESULTS

The in-vitro antimicrobial activity of the selected homoeopathic drugs against MRSA was assessed by observing the zone of inhibition around the drug-impregnated discs. Distinct variations in inhibitory responses were noted among the different potencies of Belladonna and Carbolic acid when compared with the control. The Belladonna-treated plate demonstrated visible inhibitory zones at potencies Q, 30C, and 200C. Among these, Belladonna Q exhibited the largest zone of inhibition, indicating the highest antibacterial efficacy. Belladonna 200 produced a moderate inhibitory effect, while Belladonna 30C showed a comparatively smaller, yet definite, inhibitory zone. The control disc displayed moderate inhibition, confirming the sensitivity of the assay and validating the antimicrobial response of the tested potencies. Carbolic acid displayed a different potency-dependent inhibitory pattern. Carbolic acid Q produced the greatest suppression of *Staphylococcus aureus*, as evidenced by the largest clear zone. Carbolic acid 30C and 200C showed moderate antibacterial activity. The control disc showed a very small zone of inhibition.

Experimental Results (Table 1). Figure 1. Result of the Experimental Results for Belladonna and Carbonic Acid for (Mother Tincture (Q), 30C and 200C) and Comparison with the control group

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**Table 1: Experimental Results**

| | Q (in mm) | 30C (in mm) | 200C (in mm) | Control (in mm) |
|---|---|---|---|---|
| Belladonna | 10 | 6 | 8 | 8 |
| Carbolic acid | 8 | 7 | 7 | 7 |

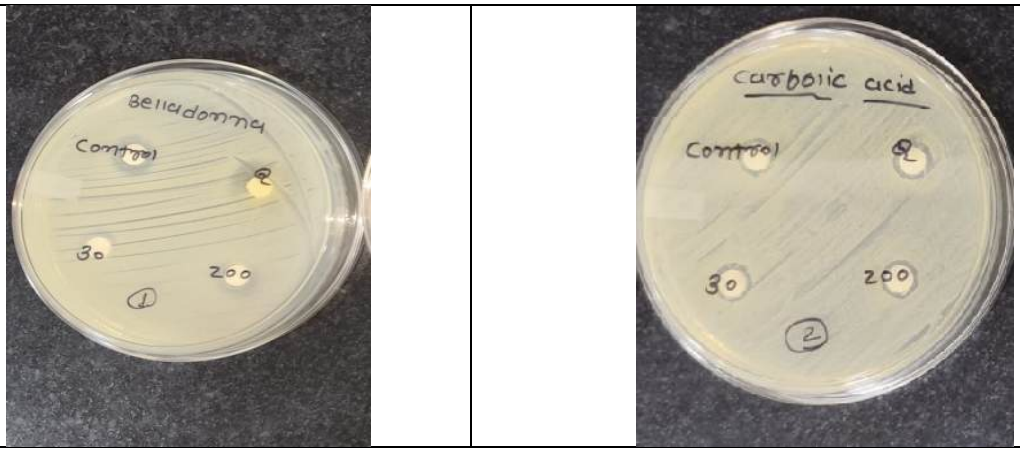



**Figure 1: Result of the Experimental Results for Belladonna and Carbonic Acid for (Mother Tincture (Q), 30C and 200C) and Comparison with the control group**

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RESEARCH ARTICLE

# Evidence - Based Non - Pharmacological Approaches to the Prevention and Management of Postpartum Depression

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## ABSTRACT

Postpartum depression (PPD) is an extensive and incapacitating mental health condition affecting women during the first year after childbirth and is associated with adverse maternal, infant, and family outcomes. Although pharmacological therapies are effective for moderate to severe PPD, concerns related to breastfeeding safety, medication side effects, and acceptability have resulted in increased interest in non-pharmacological interventions. The present narrative review synthesizes available evidence on non-pharmacological approaches for the prevention and management of postpartum depression, with specific relevance to nursing and community health practice. A comprehensive literature search was conducted using PubMed/MEDLINE, CINAHL, Scopus, Web of Science, and Google Scholar to identify studies published between 2015 and 2025. Randomized controlled trials, systematic reviews, meta-analyses, and observational studies evaluating non-pharmacological interventions among postpartum women were included. Evidence indicates that psychological interventions, particularly cognitive behavioral therapy and interpersonal therapy, are effective in reducing depressive symptoms. Psychosocial support, family-inclusive strategies, structured exercise programs, mindfulness-based approaches, and technology-assisted interventions also demonstrated beneficial outcomes. Preventive interventions initiated during the antenatal period were found to be more effective than postnatal interventions alone. Hence, non-pharmacological interventions are effective, acceptable, and scalable options for addressing postpartum

depression, especially in low- and middle-income settings. Integration of these interventions into routine maternity health services can enhance nursing practice and maternal mental health outcomes.

**Keywords:** Evidenced based, Postpartum depression, non-pharmacological intervention, prevention, management, Maternal mental health.

## INTRODUCTION

Postpartum depression (PPD) is a common mental health disorder is commonly reported within the first year after childbirth, with a global prevalence of approximately 10–20% among new mothers.[1]PPD is associated with persistent sadness, anxiety, impaired functioning, and adverse effects on maternal–infant bonding. Untreated PPD can lead to developmental, emotional, and behavioral problems in infants and long-term psychological issues in mothers.[2] A systematic review and meta-analysis conducted about the prevalence of postpartum depression in India, found it to be ranged between 4.8% to 70.4%, with a pooled prevalence of 27.7%. The prevalence varied across four regions: Eastern (19%), Western (18.5%), Northern (17.8%) and Southern (15.4%). [3]

Despite the effectiveness of pharmacological management for moderate to severe postpartum depression, concerns about breastfeeding safety and treatment-related side effects have shifted focus toward non-pharmacological strategies. These interventions emphasize psychological support, social engagement, and healthy lifestyle practices crucial for postpartum recovery.This review examines the evidence base for non-pharmacological interventions used to prevent and manage PPD, focusing on psychological therapies, psychosocial support, exercise and lifestyle interventions, mindfulness practices, and digital health strategies. The aim is to review current evidence on available non-pharmacological interventions for the prevention and management of postpartum depression. This will evaluate the feasibility, acceptability, and applicability of interventions in clinical and community settings. This will provide recommendations for nursing practice and areas for future research.

## MATERIAL AND METHODS

A narrative review design was adopted to integrate evidence from diverse research methodologies, including randomized controlled trials, systematic reviews, meta-analyses, and observational studies.Electronic databases including PubMed/MEDLINE, CINAHL, Scopus, Web of Science, and Google Scholar were searched for studies published between 2010 and 2025. Search terms included combinations of postpartum depression, non-pharmacological interventions, cognitive behavioral therapy, exercise, mindfulness, psychosocial support, and digital interventions. Studies focused on postpartum women, evaluated non-pharmacological interventions, reported depressive outcomes related to PPD and peer-reviewed and published in English were included while studies focusing solely on pharmacological interventions, antenatal depression without postpartum outcomes, or non-original research were excluded.

## REVIEW OF LITERATURE

**Psychological Interventions**

A systematic review and meta-analysis of randomized controlled trials conducted by Hua et al. (2024) to evaluate the effectiveness of psychological interventions for perinatal depression. Analyzing 33 studies, the authors reported a moderate overall treatment effect compared with control conditions (SMD = −0.65), supporting the efficacy of psychological therapies. Individualized interventions and those delivered in non-clinical settings appeared more effective than group-based or clinic-based approaches. Interpersonal therapy (IPT) demonstrated a tendency toward greater effectiveness compared with cognitive behavioural therapy (CBT), while mindfulness-based interventions

(MBI) and behavioural activation (BA) also showed beneficial effects. The findings further suggest that trained non-specialist providers can deliver effective interventions. However, high heterogeneity and limited evidence for certain modalities were noted, highlighting the need for further comparative and methodologically rigorous trials.[4] Stamou et al. (2018) conducted a narrative review of systematic reviews and randomized controlled trials from four electronic databases to evaluate cognitive-behavioural therapy (CBT) and interpersonal psychotherapy (IPT) for postnatal depression. Six reviews and 15 studies focusing on CBT met inclusion criteria. CBT, delivered individually or in groups by specialists or trained non-specialists, significantly reduced postnatal depressive symptoms, with effects lasting up to six months. Key components included psychoeducation, cognitive restructuring, and goal setting. The review supports CBT as an effective, flexible intervention and highlights its potential for nurse-led implementation.[5]

Mozaffari et al. (2025) conducted a randomized controlled trial to examine the effect of antenatal coping strategies training for fathers on postnatal depression among couples. Eighty-six first-time pregnant couples were randomized to receive either five virtual coping skills sessions or routine prenatal care. The intervention significantly reduced perceived stress and postpartum depression scores among both mothers and fathers, while increasing problem-focused coping and decreasing emotion-focused coping strategies. These findings highlight the importance of father-inclusive, nurse-led psychosocial interventions during pregnancy as an effective approach to reducing postpartum depression and promoting family mental health.[6]

**Psychosocial and Support-Based Strategies**

Norazman and Lee (2023) conducted a narrative review to examine the role of social support interventions in preventing and treating postpartum depression. The review highlighted five key strategies: psychoeducation, nurse-led supportive and non-directive counseling, home visits, peer support, and culturally tailored or community-based interventions. Evidence suggests these approaches can improve maternal mental health and infant outcomes, with discharge education and skilled public health nursing playing a critical role. The authors emphasize the potential benefits of combining social support strategies for enhanced prevention and treatment of PPD.[7]

Noonan et al. (2021) conducted a review to examine the inclusion of partners and family members in psychosocial interventions for women at risk of or experiencing perinatal depression and anxiety. Nine empirical studies published between 2010 and 2020 were included, with interventions delivered by diverse facilitators and using varied approaches. Most studies reported significant reductions in maternal depression and anxiety, though few evaluated the experiences of partners or family members. The review highlights the potential benefits of involving family in perinatal mental health care and underscores the need for co-designed interventions and further research to support integration into routine practice. [8]

**Exercise and Lifestyle Interventions**

Moderate-intensity physical activity, including walking and yoga, has shown moderate improvements in mood and reduction in depressive symptoms. Exercise contributes to better sleep, stress reduction, and overall well-being, making it a practical component of PPD prevention Bind et al. (2025) evaluated the clinical effectiveness, implementation feasibility, and cost-effectiveness of a community-based singing intervention (Melodies for Mums, M4M) for mothers experiencing postnatal depressive symptoms. In this randomized controlled trial involving 199 mother–infant dyads, participants receiving a 10-week singing intervention demonstrated sustained reductions in depressive symptoms compared with active controls at 20 and 36 weeks postpartum. The intervention showed higher participant retention and was rated as more acceptable, appropriate, and feasible than standard community activities. Additionally, the singing programme was associated with improved quality-adjusted life outcomes and demonstrated cost-effectiveness, supporting its potential as a scalable, non-pharmacological, arts-based intervention for postnatal depression.[9]

In a meta-analysis of randomized controlled trials by Ruan et al. (2025) examined the effectiveness of yoga as a non-pharmacological nursing intervention for women with postpartum depression. Six studies involving 443 postpartum

women demonstrated that yoga significantly reduced depressive symptoms as measured by the Edinburgh Postnatal Depression Scale. These findings support yoga as a feasible, low-cost, and nurse-led complementary intervention that can be integrated into postnatal care and maternal mental health programs. However, the high heterogeneity among included studies indicates the need for further rigorously designed trials to guide standardized nursing practice and protocol development.[10]

A single-centre randomized controlled trial was conducted by Saccone et al. (2024) to evaluate the effect of regular antenatal aerobic exercise on the risk of postpartum depression among women with low-risk singleton pregnancies. A total of 398 women were randomized in the first trimester to either a structured aerobic exercise programme (three 60-minute sessions per week until 35 weeks' gestation) or standard care. Postpartum depression was assessed at three months postpartum using the Edinburgh Postnatal Depression Scale. The exercise group demonstrated a significantly lower incidence of postpartum depression and reduced mean EPDS scores compared with controls, without an increase in adverse maternal or perinatal outcomes. These findings support antenatal aerobic exercise as a safe, feasible, and effective non-pharmacological preventive strategy for postpartum depression.[11]

A narrative review to examine the role of physical activity in the prevention and treatment of postpartum depression was conducted by Fotso et al. (2023). Drawing evidence from multiple databases, the authors highlighted that regular physical activity during pregnancy and the postpartum period is associated with reduced depressive symptoms and may serve both preventive and therapeutic roles. However, the review emphasized that exercise alone may not be sufficient for optimal outcomes and is more effective when integrated with structured support or wellness programs. The findings underscore the importance of individualized, nurse-guided exercise interventions that consider type, intensity, and duration to enhance maternal mental health during the postpartum period.[12]

Xu et al. (2023) conducted a systematic review, meta-analysis, and network meta-analysis to evaluate the effectiveness of aerobic exercise in the prevention and treatment of postpartum depression. Twenty-six studies involving 2,867 participants were included. Aerobic exercise significantly reduced postpartum depressive symptoms compared with standard care, with stronger effects observed for preventive interventions than for treatment. Network meta-analysis indicated that moderate-intensity aerobic exercise performed for 35–45 minutes, three to four times per week, produced the most favorable outcomes, while no significant differences were found among different types of aerobic exercise. Although supervised and group-based exercise showed slightly better effect sizes than unsupervised or individual exercise, these differences were not statistically significant. The findings support aerobic exercise as an effective, low-cost, nurse-led intervention for postpartum depression prevention, while highlighting the need for individualized exercise prescriptions and further high-quality research.[13]

**Mindfulness and Emerging Modalities**

Mindfulness-based interventions promote emotional regulation and stress management, with emerging evidence supporting symptom reduction in postpartum women (Dimidjian et al., 2016). Technology-based programs, including mobile apps and online counseling, increase accessibility, particularly for women facing barriers to in-person care. A parallel-group randomized controlled trial in an obstetric clinic in Japan conducted by Ichinose et al. (2025) to assess whether a prenatal childbirth and parenting booklet could reduce the risk of paternal postpartum depression. Expectant fathers were recruited at 28–32 weeks of gestation and randomized to receive either a mailed educational booklet or routine care, with depressive symptoms assessed at 1 and 3 months postpartum. Although the intervention group showed a lower incidence of depressive symptoms, the difference was not statistically significant. The findings indicate that standalone, information-based interventions may have limited effectiveness, highlighting the need for more interactive and personalized approaches to support paternal mental health.[14]

Worku et al. (2023) conducted a systematic review and meta-analysis to assess the effectiveness of technology-supported mindfulness-based interventions (MBIs) in managing maternal depression. Eighteen studies including 2,481 participants were reviewed, with 15 included in the meta-analysis. Results showed a significant reduction in depressive symptoms during both pregnancy (SMD = −0.57) and postpartum (SMD = −0.53). These findings highlight

the potential of nurse-facilitated, technology-assisted MBIs as accessible, cost-effective interventions to support maternal mental health, particularly in resource-limited settings. The authors emphasize the importance of context-specific trials to guide nurses in integrating these interventions into routine maternal and postnatal care for improved outcomes.[15] In 2021, Tabi et al. outlined a feasibility study protocol to evaluate online mindfulness-based interventions (MBIs) for families affected by postpartum depression and anxiety. The study involves 8-week online MBI groups for mothers with PPDA and their partners, assessing feasibility from both participant and facilitator perspectives, including session attendance, technical issues, and attrition. Secondary outcomes include parental mental health, couple relationship, and intervention acceptability. This study highlights the potential of nurse-facilitated, technology-supported MBIs to increase accessibility and support family mental health in postpartum care. [16]

Avalos et al. (2020) conducted a feasibility study of a 6-week mobile health (mHealth) mindfulness intervention for postpartum women with moderate to moderately severe depressive symptoms. Twenty-seven participants used a mindfulness app (Headspace) daily for 10–20 minutes. Of those enrolled, 70% completed the study, and nearly half adhered to ≥50% of sessions. Significant improvements were observed in depressive symptoms, perceived stress, sleep quality, and mindfulness. Participants reported high satisfaction and perceived benefits. The study demonstrates that mHealth mindfulness interventions are feasible, acceptable, and show promise as scalable support for postpartum women, highlighting potential for nurse-facilitated implementation.[17]

## DISCUSSION

This narrative review critically synthesizes contemporary evidence on non-pharmacological interventions for the prevention and management of postpartum depression (PPD), highlighting their effectiveness, feasibility, and relevance for nursing and community-based practice. The findings reaffirm that PPD is a multifactorial condition requiring holistic, person-centred approaches that extend beyond pharmacological management, particularly in contexts where breastfeeding safety, cultural acceptability, and accessibility of care are major concerns. Psychological interventions, particularly cognitive behavioral therapy (CBT) and interpersonal therapy (IPT), emerged as the most robustly supported non-pharmacological approaches. Consistent with earlier literature, this review found strong and sustained reductions in depressive symptoms with both modalities, with IPT demonstrating slightly superior outcomes in some studies. The effectiveness of nurse-led and non-specialist-delivered interventions is especially noteworthy, as it underscores the scalability of these therapies in low-resource and community settings. The durability of treatment effects up to six months postpartum further supports their integration into routine maternal and child health services.

Psychosocial and support-based strategies play a critical complementary role in addressing PPD by targeting social isolation, role transition, and family dynamics. Evidence from social support interventions, peer support programs, home visits, and partner-inclusive approaches indicates meaningful improvements in maternal mental health outcomes. Father-focused and family-inclusive interventions demonstrated reductions in maternal depressive symptoms and perceived stress, emphasizing the relational nature of postpartum mental health. However, findings also suggest that passive or information-only interventions, such as educational booklets, may have limited impact unless paired with interactive or skill-based components. This highlights the need for structured, engagement-oriented psychosocial models in nursing practice. Exercise and lifestyle interventions, including aerobic exercise and yoga, demonstrated moderate but consistent benefits in both the prevention and management of PPD. Evidence suggests that antenatal and postnatal moderate-intensity physical activity can significantly reduce depressive symptoms without adverse maternal or neonatal outcomes. Importantly, preventive effects were stronger than therapeutic effects, reinforcing the value of early integration of physical activity into antenatal care. Nurse-guided exercise prescriptions that consider individual preferences, physical readiness, and cultural context appear essential for optimizing adherence and outcomes.

Mindfulness-based and emerging digital interventions represent promising, accessible alternatives, particularly for women facing logistical, geographical, or stigma-related barriers to in-person care. Technology-supported mindfulness-based interventions (MBIs), including mobile apps and online group programs, demonstrated significant reductions in depressive symptoms and high acceptability. These findings align with the growing emphasis on digital mental health solutions in maternal care, especially in resource-constrained settings. While feasibility and preliminary effectiveness are encouraging, the heterogeneity of intervention formats and outcome measures warrants cautious interpretation and underscores the need for standardized protocols and long-term effectiveness data. From a nursing and public health perspective, the findings of this review reinforce the pivotal role of nurses in screening, delivering, and coordinating non-pharmacological interventions for PPD. Nurse-led psychological therapies, psychosocial support, lifestyle counseling, and digital facilitation can bridge critical gaps in maternal mental health care, particularly in low- and middle-income countries such as India. Integrating these interventions into routine antenatal, postnatal, and community health programs can enhance early identification, prevention, and continuity of care.

Despite its strengths, this review also identifies important gaps in the existing literature. Many studies exhibited heterogeneity in intervention design, outcome measurement, and follow-up duration, limiting direct comparison across modalities. Additionally, evidence from low-income and rural settings remains limited, and few studies examined long-term maternal and child outcomes. Cultural adaptation, cost-effectiveness, and implementation fidelity were inconsistently addressed, highlighting critical areas for future research. It can be conclude that non-pharmacological interventions offer effective, acceptable, and scalable options for the prevention and management of postpartum depression. Psychological therapies show the strongest evidence base, while exercise, psychosocial support, mindfulness, and digital interventions provide valuable adjuncts. Future research should prioritize rigorously designed, culturally sensitive trials with long-term follow-up to inform evidence-based nursing practice and policy development in maternal mental health care.

**Strengths and Limitations**

**Strengths**

This review comprehensively synthesizes diverse non-pharmacological interventions and applies findings to clinical, community, and nursing contexts. It covers both preventive and therapeutic strategies and emphasizes evidence-based practices.

**Limitations**

Heterogeneity in study designs, intervention protocols, and outcome measures limits direct comparison across studies.

**Recommendations for Future Research**

- Conduct high-quality randomized trials with standardized outcome measures.
- Investigate long-term effectiveness and sustainability of interventions.
- Explore culturally adapted and nurse-led programs.

## CONCLUSION

Non-pharmacological interventions play a crucial role in the prevention and management of postpartum depression. Psychological therapies, exercise, social support, mindfulness practices, and digital health strategies offer safe, acceptable, and effective approaches. Integration of these interventions into routine postpartum care—particularly within nursing and maternal health services—can advance the quality of care and maternal outcomes.

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RESEARCH ARTICLE

# Analysis of Heat Transfer Enhancement and Pressure Drop Characteristics in a Solar Air Heater using V+Arc Shaped Artificial Roughness

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## ABSTRACT

Artificial roughness is commonly employed in solar air heaters to enhance convective heat transfer by disturbing the near-wall flow structure. However, the associated increase in pressure drop necessitates an optimal balance between thermal enhancement and hydraulic losses. In the present study, a CFD-based investigation is carried out to analyze the effect of heat transfer and pressure drop characteristics in a rectangular solar air heater duct equipped with V+arc shaped artificial roughness on the absorber plate. The duct has a dimensions with an entrance length of 350 mm, test section length of 0.6 m, and exit length of 200 mm. Numerical simulations are performed for Reynolds numbers ranging from 3000 to 11000 under a constant heat flux corresponding to a uniform solar insolation of 1000 W/m$^2$. The roughness geometry is characterized by a pitch of 10 mm and height of 2 mm. Grid independence and near-wall resolution are ensured through a detailed mesh refinement study, maintaining $y^+$ values close to unity. The present study represents the CFD predictions for the smooth duct are validated against established experimental results, while the numerical results for the roughened duct are compared with experimental measurements to assess model accuracy. The results demonstrate that the V+arc shaped roughness significantly enhances heat transfer compared to the smooth duct, with a corresponding increase in pressure drop, and show good agreement between numerical and experimental findings.

**Keywords:** Solar air heater, computational fluid dynamics, artificial roughness, heat transfer, THPP

## INTRODUCTION

Comprehensive review studies by Varun *et al*. [2], Gupta and Kaushik [3], Hans *et al*. [4], Singh *et al*. [5], and Yadav and Thapak [6] summarized more than 200 experimental and numerical investigations on artificially roughened solar air heaters. These reviews collectively reported that most studies were conducted in the Reynolds number range of 3000–20000, with relative roughness height e/Dh varying between 0.01 and 0.06, and pitch-to-height ratios P/e in the range of 8–15. The reviews consistently identified V-shaped ribs, multi-V ribs, arc-shaped ribs, and rib–groove combinations as the most thermally effective geometries. Duffie and Beckman [1] provided the fundamental heat transfer framework for solar air heaters, emphasizing that forced convection dominates heat transfer at moderate Reynolds numbers and that enhancement techniques must be evaluated using both heat transfer and pressure loss metrics. Recent review articles focusing on CFD-based studies further highlighted that reliable numerical prediction requires low-Re turbulence models, $y^+ \approx 1$, and validation against experimental data to minimize modeling uncertainty [31–33]. Kumar *et al*. [15] performed a CFD study on a roughened solar air heater duct using V-shaped ribs with e/Dh ≈ 0.03 and P/e = 10, for Reynolds numbers ranging from 4000 to 16000. Using the SST k–ω turbulence model and maintaining $y^+ < 1$, they reported that the predicted Nusselt number agreed with experimental data within ±8%, while friction factor deviation remained within ±10%. Arya *et al*. [16] numerically investigated multiple roughness geometries, including V-shaped, arc-shaped, and dimple ribs, for Re = 3000–15000 and e/Dh = 0.02–0.04. Their CFD results showed that V-type and arc-type ribs provided the highest thermo-hydraulic performance, with peak performance parameters in the range of 1.6–2.0. Yadav and Bhagoria [29] emphasized that mesh density and near-wall resolution significantly affect CFD predictions, particularly for separated flows induced by ribs, recommending inflation layers with growth rates below 1.5. Standard CFD references by Versteeg and Malalasekera [32] and the ANSYS Fluent Theory Guide [33] further recommend second-order discretization schemes, validated turbulence models, and systematic grid independence studies for accurate prediction of wall heat flux and pressure drop.

Layek *et al*. [24] experimentally investigated chamfered rib–groove roughness with e/Dh = 0.043 and P/e = 10 for Reynolds numbers between 3000 and 17000. Their results showed that chamfering increased the average Nusselt number by 20–25% compared to conventional rib–groove configurations while reducing friction losses. Aharwal *et al*. [25] studied broken transverse ribs with e/Dh = 0.028–0.042 and P/e = 7.5–12.5 over Re = 3000–20000. They reported that broken ribs enhanced Nusselt number by up to 2.1 times compared to smooth ducts while reducing friction factor by 10–15% relative to continuous ribs. Karmare and Tikekar [26] examined metal grit roughness with grit sizes corresponding to e/Dh = 0.035–0.045, reporting Nusselt number enhancement of 1.8–2.3 times for Re = 3000–17000. Bhushan and Singh [27] studied protruded ribs with e/Dh ≈ 0.03 and observed significant heat transfer enhancement accompanied by higher pressure drop. Bopche and Tandale [28] compared multiple rib geometries and concluded that optimal performance occurs at P/e ≈ 10 for most roughness configurations. Singh *et al*. [31] conducted a comparative experimental study on various artificial roughness geometries, including V-shaped ribs, arc-shaped ribs, metal grit ribs, and rib–groove combinations, for Re = 4000–18000. Using identical duct dimensions and operating conditions, they reported that V-type and arc-type ribs achieved the highest effective efficiency, with thermo-hydraulic performance parameters exceeding 1.8 at optimal roughness parameters (e/Dh ≈ 0.03, P/e ≈ 10). Their results confirmed that hybrid or compound roughness geometries offer better performance when evaluated under equal pumping power constraints.

The present study aims to numerically and experimentally investigate the thermal and hydraulic performance of a solar air heater duct equipped with V+arc shaped artificial roughness. The specific objectives are

a. To develop and validate a CFD model for a smooth and V+arc roughened solar air heater duct by comparing numerical predictions of Nusselt number and friction factor with experimental data and established empirical correlations over a Reynolds number range of 3000–11000.
b. To evaluate the effect of V+arc shaped artificial roughness on heat transfer enhancement and pressure drop characteristics by comparing the thermal and hydraulic performance of the roughened duct with a smooth duct under identical operating conditions.

c. To assess the overall thermo-hydraulic performance of the V+arc roughened solar air heater.

## NUMERICAL METHODOLOGY

**Geometry Description**

The computational domain represents a (Figure – 1) rectangular solar air heater duct consisting of three sections: an entrance section, a test section, and an exit section. The entrance length is 350 mm, the test section length is 0.6 m, and the exit length is 200 mm. The duct has a constant width (W = 100 mm) and height (H = 20 mm), resulting in an aspect ratio (W/H) of 5. The corresponding hydraulic diameter of the duct is 0.0333 m. For the roughened configuration, (Figure – 2 and 3) a V+arc shaped artificial roughness is applied to the absorber plate located at the bottom wall of the duct. The roughness geometry is characterized by a rib height e = 2 mm and a pitch P = 10 mm, selected based on reported optimal ranges in previous experimental studies. The roughness elements are uniformly distributed along the test section, while the entrance and exit sections are kept smooth to ensure flow development and numerical stability. A smooth duct configuration with identical dimensions is also simulated to validate the numerical model against experimental results and empirical correlations before analyzing the roughened geometry.

**Working Fluid Properties and Operating Conditions**

Air is used as the working fluid and is assumed to be incompressible with constant thermos-physical properties evaluated at ambient conditions. The properties used in the simulations are listed below and correspond to commonly accepted values for solar air heater studies:

Density, $\varrho = 1.225$ kg/m³

Specific heat, Cp = 1005 J/kg·K

Dynamic viscosity, $\mu = 1.85 \times 10^{-5}$ Ns/m²

Thermal conductivity, k = 0.026 W/m·K

The flow is considered steady, turbulent, and fully developed within the test section. Simulations are performed for Reynolds numbers ranging from 3000 to 11000, covering the practical operating range of solar air heaters used for drying and space heating applications

**Boundary Conditions**

At the inlet, a uniform velocity boundary condition corresponding to the desired Reynolds number ranging from 3000 – 11000 is imposed, along with a constant inlet air temperature. The outlet is defined using a pressure outlet boundary condition with zero gauge pressure. A uniform heat flux boundary condition is applied at the absorber plate to represent constant solar insolation. The imposed heat flux corresponds to a solar intensity of 1000 W/m², which is a standard value adopted in experimental and numerical studies of solar air heaters. All remaining duct walls are assumed to be adiabatic. No-slip boundary conditions are applied at all solid walls. Radiation and buoyancy effects are neglected, as forced convection dominates the heat transfer mechanism in the present operating range

**Governing Equations**

The flow and heat transfer are governed by the steady-state, three-dimensional Reynolds-averaged Navier–Stokes (RANS) equations along with the energy equation.

**Continuity equation**

The continuity equation is the mathematical statement of the concept of conservation of mass applied to an elemental control volume within a fluid in motion, and it is evaluated by using Eq. (1) [32–33]:

$$\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} + \frac{\partial w}{\partial z} = 0 \qquad (1)$$

This is the mass continuity equation for three-dimensional steady flow.

**Momentum equation. It can be calculated by using Eqs. (2-4) [32-33]**

**x–momentum equation**

$$u\frac{\partial u}{\partial x}+ v\frac{\partial u}{\partial y}+ w\frac{\partial u}{\partial z}= -\frac{1}{\rho}\frac{\partial p}{\partial x} + \nu\left(\frac{\partial^2 u}{\partial x^2}+\frac{\partial^2 u}{\partial y^2}+\frac{\partial^2 u}{\partial z^2}\right) \quad (2)$$

**y–momentum equation**

$$u\frac{\partial v}{\partial x} + v\frac{\partial v}{\partial y} + w\frac{\partial v}{\partial z} == -\frac{1}{\rho}\frac{\partial p}{\partial y} + \nu\left(\frac{\partial^2 v}{\partial x^2}+\frac{\partial^2 v}{\partial y^2}+\frac{\partial^2 v}{\partial z^2}\right) \quad (3)$$

**z–momentum equation**

$$u\frac{\partial w}{\partial x}+ v\frac{\partial w}{\partial y}+ w\frac{\partial w}{\partial z}= -\frac{1}{\rho}\frac{\partial p}{\partial z} + \nu\left(\frac{\partial^2 w}{\partial x^2}+\frac{\partial^2 w}{\partial y^2}+\frac{\partial^2 w}{\partial z^2}\right) \quad (4)$$

**Energy equation:** If the flow is continuous and incompressible with constant thermal conductivity, there is no compression effect and no heat generation. In this case, no viscous heating is generated and the following equation can be used [36-37, 38]:

$$u\frac{\partial T}{\partial x} + v\frac{\partial T}{\partial y} + w\frac{\partial T}{\partial z} = \alpha\left(\frac{\partial^2 T}{\partial x^2}+\frac{\partial^2 T}{\partial y^2}+\frac{\partial^2 T}{\partial z^2}\right) \quad (5)$$

**Turbulence Model Selection**

The SST k–ω turbulence model is employed in the present study due to its proven capability in accurately predicting near-wall flows with strong separation and reattachment, which are characteristic of roughened solar air heater ducts. The model combines the advantages of the k–ω formulation in the near-wall region with the k–ε formulation in the free stream, making it suitable for resolving complex turbulent structures induced by artificial roughness. (Figure 5) shows the turbulence model comparison.

**Mesh Generation and Grid Independence**

A structured hexahedral mesh is generated for the smooth duct, while a hybrid mesh is employed for the roughened configuration to accurately capture the complex rib geometry. Special attention is given to near-wall mesh refinement through the use of inflation layers. A total of 10 inflation (Figure 4) layers with a growth rate of 1.5 are applied near all walls, and the first cell height is chosen to maintain $y^+ \approx 1$ throughout the test section. This ensures proper resolution of the viscous sublayer and accurate prediction of wall shear stress and heat flux. Three increasingly improved meshes are used in a grid independence investigation. The variation between the medium and fine meshes is found to be less than 2%, showing mesh-independent outcomes when the average Nusselt number is utilized as the monitoring parameter. In order to balance accuracy and computational expense, the medium mesh is chosen for all ensuing simulations based on this study. Grid independence has been attained as the values plateau toward the theoretical limit, as shown by the practically identical findings from (Figure 6) for 2.74M and 4.40M elements.

**Experimental Setup**

The schematic diagram of experimental set up is shown in (Figure 7). The test rig consists of a centrifugal blower, rectangular wooden duct, electric lamp, differential pressure transducer, Anemometer, PT-100 sensors (bolt type and probe type), glass cover, pyranometer and 16 channel universal data logger. The photographic view of experimental setup is shown in (Figure 8 and 9). The duct of rectangular cross-section having high aspect ratio (W>> H) consists of three sections namely entry (L1= 350 mm), test (L2= 600 mm) and exist section (L3= 200 mm). The length of three sections are taken according to ASHRAE standards [34]. The aspect ratio (W/ H) of 5 is considered in this case which is much greater than unity. Absorber plate in the test section is made of aluminium plate having thickness 6 mm, 100 mm wide and 600 mm length. The V+arc profile is made on aluminium plate with the help of CNC milling machine.

**Experimental investigation and Data Collection**

The experimental investigation was carried out using a solar air heater (SAH) test rig designed and fabricated in accordance with standard testing practices. Two absorber plate configurations were tested: smooth plate and V+Arc

ribbed plate with a pitch of 10 mm. Air was used as the working fluid and was supplied to the duct using a centrifugal blower installed at the inlet section. The airflow rate through the duct was regulated using a control valve, and the mass flow rate was calculated based on inlet velocity measurements. To minimize heat loss, the side and bottom walls of the duct were insulated using glass wool insulation. The absorber plate was heated with the bulbs to provide a uniform and controllable heat flux condition, simulating solar radiation under steady-state conditions.

**Data Reduction**

The CFD data such as air temperature, absorber plate temperature and pressure difference across duct are calculated from CFD post and experimental data are recorded with the help of 16 channel universal data logger under steady-state conditions. The influence of V+arc rib arrangement on Nu and $f$ can be calculated by the equation mention below:

The average Nusselt number is defined as [35]

$$Nu = h \frac{D_h}{k} \quad (6)$$

The heat transfer coefficient h is obtained from energy balance equation.

$$h = Q_u = \dot{m} c_p (T_{out} - T_{in}) \quad (7)$$

The average friction factor ($f$) is calculated with the help of pressure drop (ΔP) along the length of the duct which is given below.

$$f = \frac{2 \Delta P D_h}{(\rho L V^2)} \quad (8)$$

The thermohydraulic performance (denoted by η) is given below [36].

$$\eta = \frac{\left(\frac{Nu}{Nu_s}\right)}{\left(\frac{f}{f_s}\right)^{\left\{\frac{1}{3}\right\}}} \quad (9)$$

Dittus–Boelter equation is given below [37]

$$Nu = 0.023\, Re^{\{0.8\}} Pr^{\{0.4\}} \quad (10)$$

Whereas, Blasius equation for friction factor is given below [37]

$$f = 0.316\, Re^{\{-0.25\}} \quad (11)$$

## RESULT AND DISCUSSION

Figures 10 illustrate the validation of the present CFD model for the smooth solar air heater duct by comparing the Nusselt number and friction factor with corresponding experimental data and well-established empirical correlations. For the Reynolds number range of 3000–11000, the CFD based Nusselt number shows a monotonic increase with Reynolds number and closely follows the experimental trend. The maximum deviation between the CFD results and experimental data is observed to be within 5–8%, while the deviation with respect to the Dittus–Boelter correlation remains within 8–10% across the investigated range. This level of agreement indicates that the present numerical model reliably captures the convective heat transfer behavior in the smooth duct. Similarly, the friction factor by the CFD model decreases with increasing Reynolds number, consistent with both experimental measurements and the Blasius correlation. The CFD results show a marginal over rise of friction factor compared to the Blasius correlation, with deviations ranging from approximately 7–12%, whereas the deviation relative to experimental data is limited to about 5–9%. The closer agreement with experimental results suggests that the CFD model effectively accounts for near-wall viscous effects, which are simplified in empirical correlations. Overall, the numerical results for both Nusselt number and friction factor exhibit good agreement with experimental data and standard correlations, with deviations remaining within acceptable limits reported in the literature. The successful validation of the smooth duct case confirms the suitability of the selected turbulence model, mesh strategy, and near-wall treatment. Hence, the present CFD framework is considered reliable and is subsequently employed to investigate the heat transfer enhancement and pressure drop characteristics of the solar air heater duct with V+arc shaped artificial roughness.

**Heat Transfer Enhancement**

Figure 11.shows the variation of average Nusselt number with Reynolds number for smooth and V+arc roughened solar air heater ducts. For both cases, the Nusselt number increases with Reynolds number; however, the roughened duct consistently exhibits higher values. At Re ≈ 4000, the Nusselt number for the V+arc roughened duct is about 60–70% higher than that of the smooth duct. This enhancement increases with Reynolds number, reaching approximately 1.9–2.1 times at Re ≈ 12000. The improvement in heat transfer is attributed to the combined effect of V-shaped and arc-shaped roughness elements. The V-shaped segments promote flow impingement and strong secondary vortices, while the arc-shaped segments extend the reattachment region, leading to repeated disruption of the thermal boundary layer. The CFD predictions for the roughened duct agree well with experimental data, with deviations generally within ±10%.

**Friction Factor Characteristics**

The variation of friction factor with Reynolds number is presented in Figure 12. For both smooth and roughened ducts, the friction factor decreases with increasing Reynolds number. The friction factor for the V+arc roughened duct is significantly higher than that of the smooth duct due to increased form drag and turbulence generation. At Re ≈ 4000, the friction factor for the roughened duct is approximately 2.3–2.8 times that of the smooth duct. At higher Reynolds numbers (Re ≈ 15000), this ratio reduces to about 2.0–2.4, indicating a comparatively lower hydraulic penalty at higher flow rates. These trends are consistent with earlier studies on V+arctype roughness geometries.

**Thermo-Hydraulic Performance**

The overall effectiveness of the V+arc roughness is (figure -13) evaluated using the thermo-hydraulic performance parameter (THPP) under equal pumping power conditions. The THPP remains greater than unity for the entire Reynolds number range studied. At Re ≈ 4000, the THPP is around 1.59, and it reaches a maximum value of approximately 1.64 in the range of Re ≈ 9000–11000. Even at higher Reynolds numbers, the THPP remains above 1.60, confirming a net performance gain over 60% relative to the smooth plate baseline. The results indicate that the V+arc roughened solar air heater offers a favorable balance between heat transfer enhancement and pressure drop, particularly at moderate Reynolds numbers relevant to practical applications.

## CONCLUSION

The numerical investigation into the solar air heater confirms that the V+Arc roughened geometry significantly outperforms the smooth plate baseline in terms of thermal efficiency. The artificial roughness successfully disrupts the laminar sub-layer, leading to a maximum Nusselt number of 72.25, which is nearly double that of the smooth geometry at the highest flow rate. While the roughness introduces a hydraulic penalty by increasing the friction factor relative to the smooth duct, the overall efficiency remains exceptionally high. The Thermo-Hydraulic Performance Parameter (THPP) consistently stayed between 1.59 and 1.64, proving that the heat transfer gains far outweigh the increased pumping power requirements. Furthermore, the study was verified through a grid independence study and validated against Experimental setup and standard Dittus–Boelter and Blasius correlations, ensuring the high reliability of the computational model. These results demonstrate that the V+Arc configuration is an effective and optimized design for enhancing the energy efficiency of solar air heating systems.

**Nomenclature**

| | | |
|---|---|---|
| $C_p$ | : | Specific heat capacity, kJ/kg·K |
| δp | : | pressure drop, Pa |
| THPP | : | Thermo-Hydraulic Performance Parameter |
| Nu | : | Nusselt No. |
| L | : | length, mm |
| e | : | e – roughness height, mm |
| f | : | friction factor |

$D_h$ : hydraulic diameter, mm
$T_{inlet}$ : Air Inlet temperature [°K]
$T_{outlet}$ : Air Outlet temperature [°K]
$T_{ab}$ : Absorber plate temperature [°K]
Re : Reynolds number
P : roughness pitch, mm

**Greek Symbols**

K : Thermal conductivity, W/m·k
h : Convective heat transfer coefficient, W/m²·k
A : Surface area involved in heat transfer, m²
$\alpha$ : thermal diffusivity, m²/s
$\nu$ : kinematic viscosity, m²/s
$\varrho$ : fluid density, kg/ m³

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**Table 1: Sample Values of CFD analysis**

| Sr.No. | Measured parameters | Symbol | Value |
|---|---|---|---|
| 1 | Air inlet temperature | Tin | 300 $^0$k |
| 2 | Air outlet temperature | Tout | 312.62 $^0$k |
| 3 | Rise in temperature of air | ΔT | 12.62 $^0$k |
| 4 | Mean bulk air temperature | Tm | 306.31 $^0$k |
| 5 | Mean plate temperature | Tp | 359.95 $^0$k |

| | | | |
|---|---|---|---|
| 6 | Pressure drop | ΔP | 2.75 |
| 7 | Mass flow rate | m | 0.0049 |

**Table 2: Sample Values of CFD analysis for Smooth and V+arc plate for P = 10 mm**

| Reynolds No. | Smooth Plate | | | V+arc roughness geometry | | | THPP |
|---|---|---|---|---|---|---|---|
| Re | h | $Nu_s$ | $f_s$ | h | $Nu_r$ | $f_r$ | ή |
| 3000 | 11.07 | 14.17 | 0.013 | 19.03 | 24.38 | 0.017 | 1.59 |
| 5000 | 16.15 | 20.68 | 0.010 | 29.77 | 38.13 | 0.015 | 1.64 |
| 7000 | 20.64 | 26.43 | 0.010 | 38.77 | 49.66 | 0.015 | 1.63 |
| 9000 | 25.01 | 32.03 | 0.009 | 47.84 | 61.27 | 0.015 | 1.64 |
| 11000 | 29.10 | 37.27 | 0.009 | 56.41 | 72.25 | 0.015 | 1.64 |

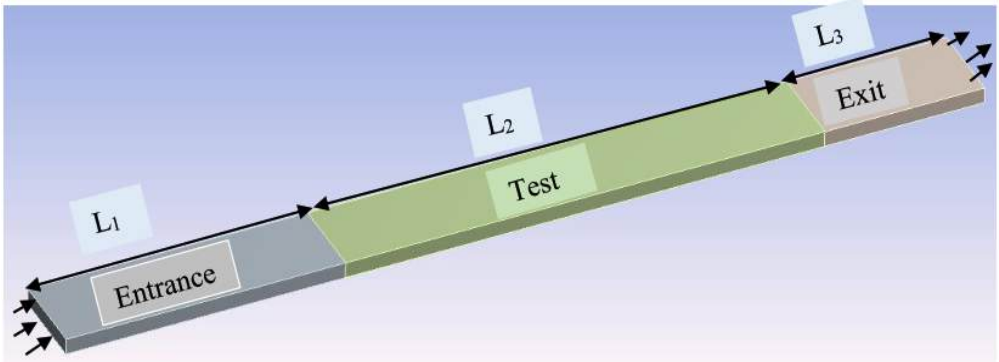



**Figure 1: Representation of Numerical models**

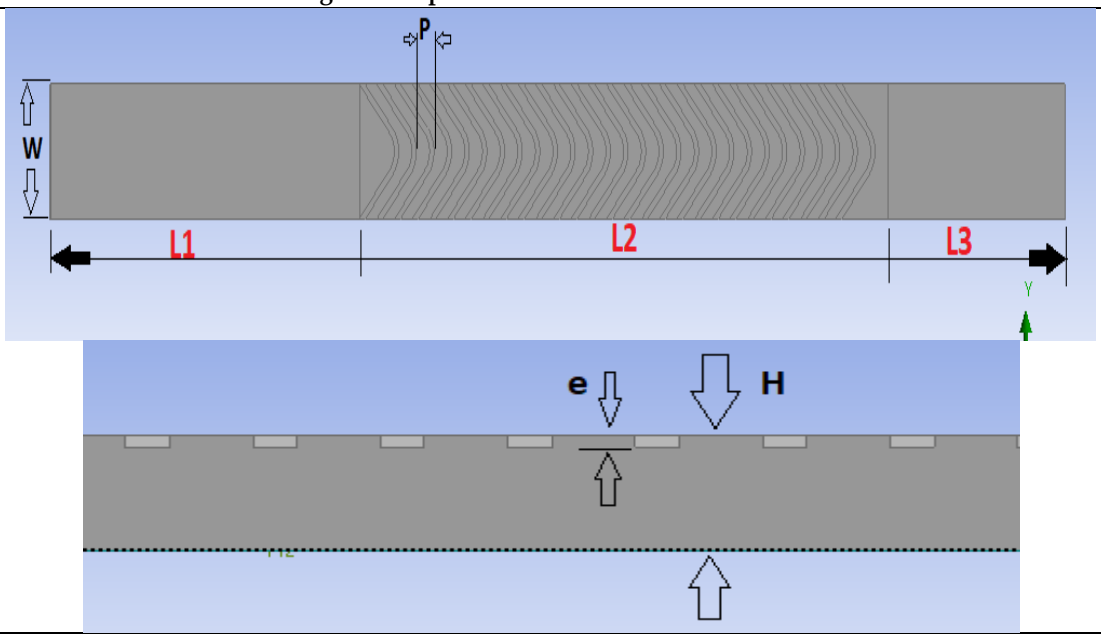



**Figure 2: Representation of V+Arc roughness geometry**

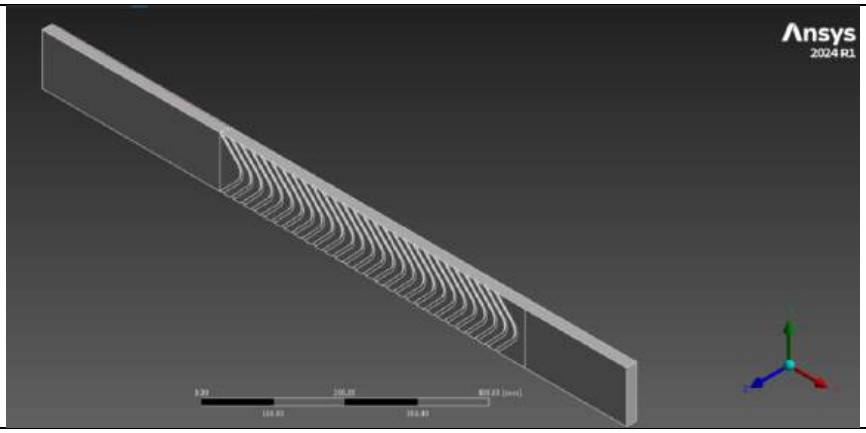



**Figure 3: Representation of V+Arc roughness geometry in Ansys 2024**

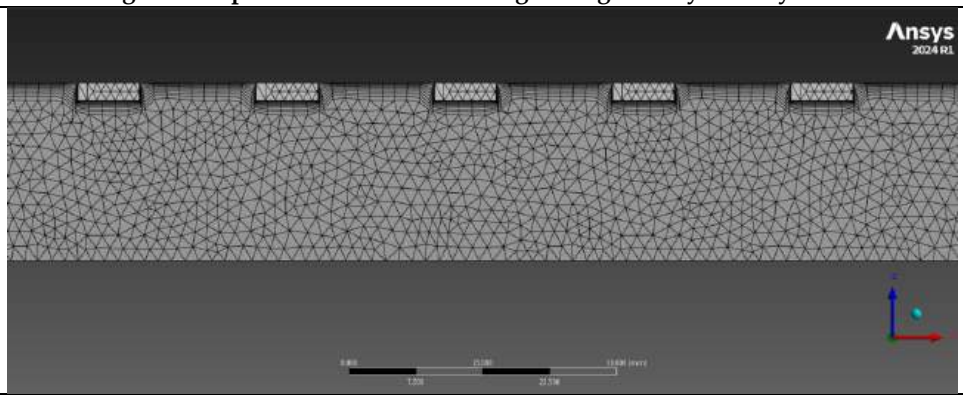



**Figure 4: Mesh Generation**

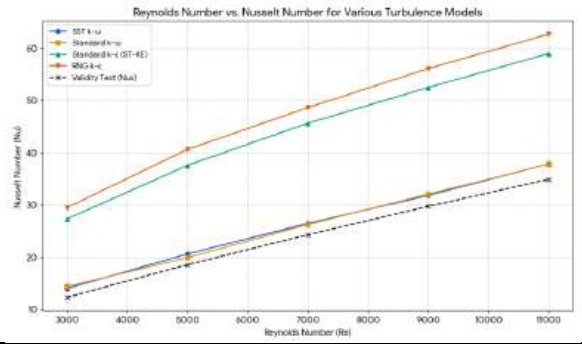



**Figure 5: Reynolds Number (Re) vs. Nusselt Number (Nu) for the various turbulence models**

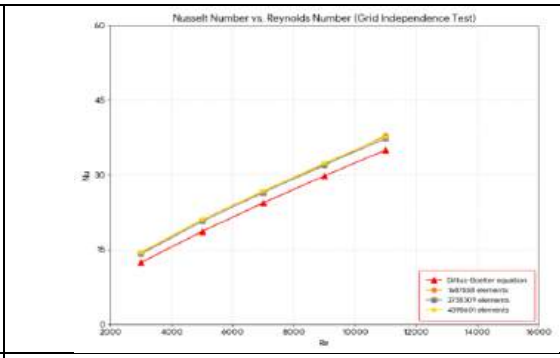



**Figure 6:Reynolds Number (Re) vs. Nusselt Number (Nu) for Grid Independent Test**

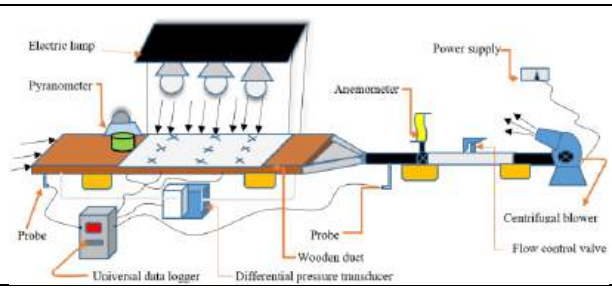



**Figure 7:Schemetic Experimental Setup**



**Figure 8:Experimental Setup**






**Figure 9: Experimental Test Setup and Readings**

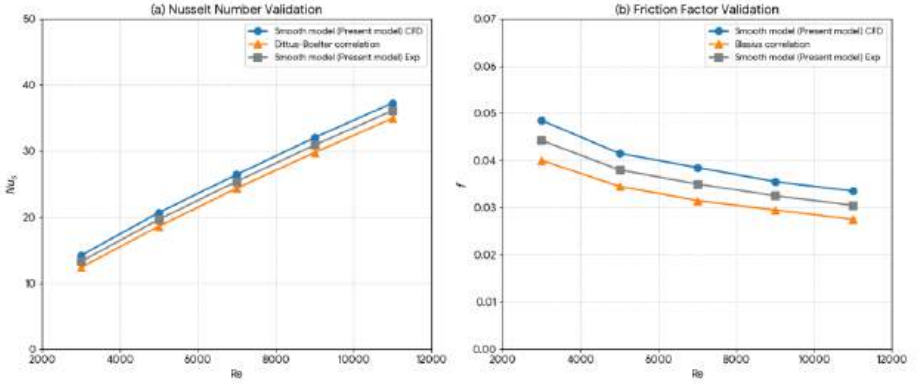



**Figure 10: Comparision of Smooth Plate CFD and Experimental data with equation for Nusselt No and friction factor vs Re**

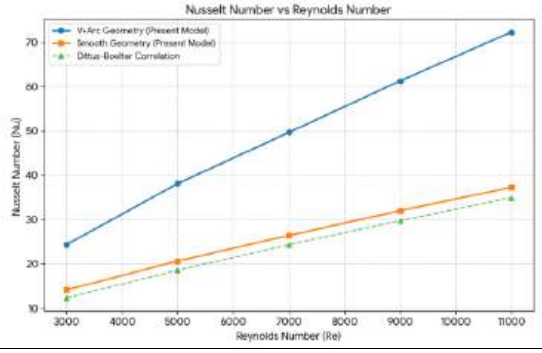



**Figure 11: Comparision of Smooth and Roughned plate for Re vs Nu**

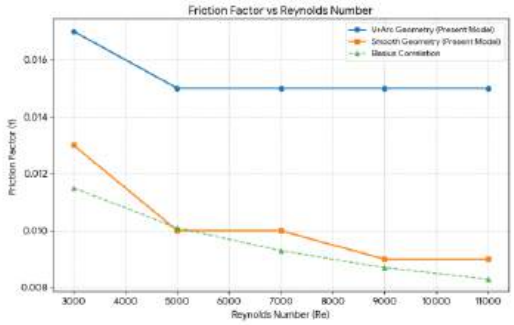



**Figure 12: Comparision of Smooth and Roughned plate for Re vsf**

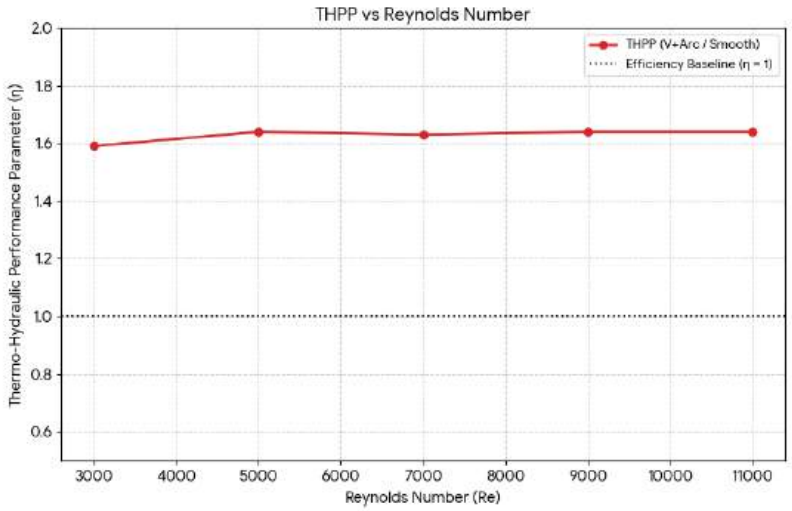



**Figure 13: Comparision of Smooth and Roughned plate for Re vs THPP**

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RESEARCH ARTICLE

# Cloud – Based IoT-Aware Transfer Learning for Smart Precision Farming Yield Prediction

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## ABSTRACT

Crop yield prediction refers to the process of estimating the amount of agricultural productivity from a given area of land measured in kilograms or tons per hectare. To solve the issues, a novel method Linear Regression Tuned Deep Transfer Learning Network (LiRT-TLNet) is introduced to enhance the crop yield prediction within the smart farming application. Deep transfer learning network model is employed by adapting a pre-trained deep learning classification model specifically, a Multilayer Perceptron (MLP) for forecasting the cropyield. It consists of multiple layers, including an input layer, three hidden layers, and an output layer. An IoT technology is implemented in agriculture for monitoring and collecting weather data and soil characteristics. These collected data is sent to a cloud platform and stored in form of database where a Deep transfer learning network predicts the expected yield for next harvest. Based on this, the farmer receives alerts to optimize irrigation schedules and apply fertilizer leading to better productivity and reduced costs. Within the hidden layers of MLP architecture, data preprocessing is integrated to address missing values and remove outliers, ensuring clean and reliable input. Subsequently, dimensionality reduction is performed by Cramer's phi correlation coefficient for selecting the most relevant features. Prediction occurs in third hidden layer, where extracted features and data samples are analyzed by applying least square regression. Finally, predicted crop yield results are observed at output layer. The knowledge learned by pre-trained model from previous crop yield prediction tasks is transferred to new model, improving feature learning and increasing accuracy of

predictions. A new part of process is fine-tuning, where Owl optimization algorithm is applied to update hyperparameter of MLP for reducing the training and validation errors, thereby boosting precision of crop yield prediction. The performance of LiRT-TLNet model is assessed using several critical evaluation metrics. Experimental results are consistently delivering higher prediction accuracy and computational efficiency compared to conventional deep learning models.

**Keywords:** Smart Precision Farming, Crop Yield Prediction, Cloud Environment, IoT, Deep transfer learning network, Multilayer Preceptor (MLP), Cramer's phi correlation coefficient, Owl optimization algorithm.

## INTRODUCTION

Crop yield prediction is a critical aspect of modern agriculture that helps farmers, make informed decisions about food production and resource management. Attention mechanism integrated into a one-dimensional convolution neural network and long short-term memory (AM-CNN-LSTM) was introduced in [1] with improving the crop yield estimation. However, the proposed model was not effective in reducing the time consumption associated. A new deep learning model called CNN-Transformer with serial connection (CNN Transformers) was developed in [2] to evaluate the winter wheat yield. However, the complexity of deep learning models still poses challenged. An optimized ensemble predictive model was introduced in [3] for estimating the crop yield prediction based on environmental and chemical variables. However, it failed to diverse datasets that cover multiple regions and variety of crops. Distinct ensemble machine learning models were developed in [4] for forecasting crop yields to manage complex datasets. However, model accuracy and applicability were not enhanced. In order to enhance the accuracy, a deep transfer learning model was designed in [5] for country level crop yield prediction. However, efficient fine tuning was significant challenges. Gradient based ensemble learning algorithms were designed in [6] for predicting the crop yields with lesser RMSE. Algorithm did not consider the additional factors such as soil fertility to provide a more comprehensive recommendation system. Machine Learning techniques were developed in [7] for climate data for predicting the wheat yield. But, it failed to improve the precision of large-scale yield prediction. An advanced machine learning algorithm were developed in [8] for estimating the wheat yield during the winter season with minimal error rate. However, it did not integrate the multisource data. A long short-term memory network was designed in [9] for crop yield prediction using remote sensing data. However, the model was not effective in reducing prediction errors. A generalized linear mixed model (GLMM) was developed in [10] for predicting the agriculture yield with lesser RMSE. However, the model was not employed for accurate yield prediction. An advanced Deep Learning (DL) architectures were employed in [11] for predicting soybean yields with minimal RMSE. However, the architecture failed to improve their performance, reliability and interpretability. AI-based system was designed in [12] by integrating the deep learning, Global Positioning System (GPS) to create a highly intelligent smart agricultural precision farming system. However, the system was not collectively enhancing the model's ability to improve yields prediction in smart agriculture. Different machine learning models were developed in [13] for wheat yield prediction using remote sensing data. However, the models did not focus on various factors such as soil characteristics, socioeconomic and management practices for accurate yield prediction. Two methods called linear regression and random forest were developed in [14] for peanut yield prediction. However, the designed model was not robust for modeling peanut yields across different cultivation seasons and region. A zone partitioning and hierarchical assimilation system were developed in [15] with enhancing the high-precision prediction of large-scale rice yield. However, more state variables were not considered in the assimilation system.

**Major Contributions**

The key contributions of the LiRT-TLNet model are outlined as follows

- To design a novel AI technique called LiRT-TLNet model for accurate crop yield prediction by implementing pre-processing, feature selection, prediction.

- A LiRT-TLNet model performs data pre-processing and relevant feature selection to reduce the time consumption of crop yield prediction.
- To improve the accuracy of crop yield prediction and minimize error, the least square regression is employed to analyze data samples and provide the prediction results. Moreover, the owl search optimization is applied for hyper parameter fine-tuning in transfer learning approach to further reduce RMSE, MAE and increases the accuracy.
- The comparative analysis of proposed LiRT-TLNetmodel has been evaluated based on conventional deep learning methods with various performance metrics.

**Organization of the paper**

The organization of this paper is as follows: Section 2 provides a review of related work in the domain. Section 3 introduces the proposed LiRT-TLNet model, detailing its components and presenting a corresponding workflow diagram. Section 4 outlines the experimental configuration and offers a description of the dataset. Section 5 discusses the model's performance through a comparative analysis using multiple evaluation metrics. Finally, Section 6 summarizes the findings and concludes the paper.

## RELATED WORKS

A quantile regression forest model was integrated into random forest in [16] for estimating the corn yield. However, an advanced model was not employed to achieve more accurate and explainable yield prediction. Machine learning techniques were designed in [17] for wheat grain yield (GY) prediction resulting in a minimum RMSE. However, hyper parameter optimization and evolutionary algorithms were not considered. An integration of random forest and XGBoost were introduced in [18] for Precision biochar yield forecasting. However, the model was not efficient in large scale data samples. Meta-regression approaches were introduced in [19] for predicting the soybean yield variation. However, the approaches failed to mitigate the effects of climate changes. Support Vector Regressor (SVR) was introduced in [20] for improving the crop yield prediction accuracy based on hybrid feature selection approach. However, the accuracy was not improved. An Adaptive Neuro Fuzzy Interference System (ANFIS) was designed in [21] for multi-crop yield prediction. However, the system did not include factors such as soil type, soil texture, fertilizer usage, irrigation, and water requirements in crop yield prediction. A zonal-integrated modeling framework was developed in [22] to improve the crop yield prediction accuracy through the feature extraction capability. However, the framework did not integrate long-term temporal data, multi-crop validation to further verify the model's applicability. A Multi-Task Crop Yield Prediction Network (MT-CYP-Net) was designed in [23] for crop yield prediction using effective multi-task feature-sharing strategy. However, the model was not efficient when applied to large-scale datasets. An enhanced fuzzy logic and deep learning model were developed in [24] for estimating the crop yield. However, it did not focus on integrating the multi-modal data sources namely environmental conditions, soil micro- biome data, to strengthen the prediction accuracy. An adaptive Echo State Network (ESN) was introduced in [25] for improving the maize yield prediction with minimal computational cost. However, it was unable to provide the timely and accurate predictions for soybean yield. A dual attention mechanism was designed in [26] for large-scale crop yield prediction which exhibits significantly lesser RMSE and higher correlation coefficients. However, minimizing the prediction time remained a major challenge. An explainable AI-based hybrid machine learning model was developed in [27] for crop yield prediction. However, the model was not robust to improve robustness, scalability, and adoption in diverse agricultural dataset. A transfer-learning-based framework was developed in [28] for estimating the crop yield with better accuracy. However, the utilization of remote sensing data in combination with crop growth models was not incorporated to improve the predictive performance. A random forest algorithm was introduced in [29] with the aim of reducing the soybean yield. However, the time complexity was not reduced. Gorilla Troops Optimization with Deep Learning model was developed in [30] for accurate yield prediction model. However, data preprocessing models was not improved.

## PROPOSAL METHODOLOGY

The proposed LiRT-TLNet is to enhance predictive accuracy. By effectively modeling complex patterns in agricultural data, the proposed LiRT-TLNet model enables more reliable yield predictions, under unstable environmental conditions. Figure 1 above shows the architecture diagram of the proposed LiRT-TLNet model for accurate prediction of crop yield. The model is structured into two main phases namely the pre-trained phase and the newly developed model. In the first phase, a pre-trained model is developed using a large volume of training data samples collected from an appropriate dataset. Following this, a new model is built that utilizes the knowledge obtained from the pre-trained model through the process of transfer learning. The proposed LiRT-TLNet model combines these two phases into a combined architecture, functioning across four core stages namely data acquisition, data augmentation, pre-processing, feature selection, and classification. Each of these steps plays a critical role in accurately predicting the crop yield and overall performance.

**Data Acquisition**

Data acquisition in agriculture refers to process of collecting real-time data from farming environment using IoT (Internet of Things) devices such as soil moisture sensors, weather stations, and drones. These devices capture critical parameters like temperature, PH, humidity, soil moisture, and crop health. The collected data is then transmitted wirelessly to cloud computing platforms, where it is stored, processed, and analyzed. This integration allows farmers to access remotely. By utilizing data acquisition through IoT and cloud technologies, modern agriculture becomes more efficient, sustainable, and responsive to changing environmental conditions. A Smart Farming Sensor Data and Yield Prediction dataset is collected from https://www.kaggle.com/datasets/atharvasoundankar/smart-farming-sensor-data-for-yield prediction. The dataset includes with 22 unique features and 500 individual entries for constructing accurate and dependable crop yield forecasting systems.

**Data Augmentation**

Data augmentation is a process used in LiRT-TLNet model to increase the size and diversity of a dataset by creating modified versions of existing known data samples. This process helps to improve the performance and generalization of machine learning models, especially when the original dataset size is small. The proposed LiRT-TLNet model utilizes the Adaptive Synthetic proximity sampling as a data augmentation technique for generating synthetic samples by interpolating nearest neighbors. Consider a mart Farming Sensor Data and Yield Prediction dataset named$\boldsymbol{DS}$, which consists of multiple data samples denoted as$D$. Each sample contains several features represented by the set $\{F = \{F_1, F_2, \dots, F_m\}$. These data samples and their corresponding features are organized in a matrix format. This input matrix '$IM$', which serves as input to a model is expressed as follows,

$$IM = \begin{bmatrix} F_1 & F_2 & \dots & F_{\mathrm{m}} \\ D_{11} & D_{12} & \dots & D_{1n} \\ D_{21} & D_{22} & \dots & D_{2n} \\ \vdots & \vdots & \dots & \vdots \\ D_{m1} & D_{m2} & \dots & D_{mn} \end{bmatrix} \quad (1)$$

Where, Here, $n$ denotes the total number of data samples in dataset, and $m$ indicates the number of features for each sample. By applying Adaptive Synthetic proximity sampling, the new synthetic samples '$D_S$' are generated based on an existing sample and one of its neighbors.

$$D_S = D_i + R * |D_{NN} - D_i| \quad (2)$$

Where, $D_i$ represents original data samples, $D_{NN}$ represents the nearest neighbor of data samples '$D_i$', $R$ denotes a random number between 0 and 1. In this way, large volumes of synthetic data samples are generated for accurate crop yield prediction. These generated samples are then transferred into to transfer learning model for accurate crop yield prediction.

**Enhanced Transfer learning-based crop yield prediction**

Transfer learning is a discriminative artificial intelligence that applies knowledge acquired from one task to improve learning in a different, but related task. On the contrary to traditional deep learning methods, transfer learning

enables faster model development and often delivers improved performance especially when dealing with large-scale datasets. The schematic structure of the Transfer learning model is shown in figure 2. Figure 2 illustrates the schematic structure of a deep transfer learning model, which integrates a pre-trained model with a newly added model component. The pre-trained model is based on (MLP) architecture, comprising an input layer, one or more hidden layers and an output layer. Within the transfer learning framework, a large number of new data samples are used to adapt this pre-trained model to crop yield prediction. As shown in Figure 2, certain layers of the pre-trained model are frozen, meaning their parameters remain unchanged during training. As shown in figure 2, frozen layers refer to part of the pre-trained model remain fixed during training and add the two new layers for fine-tuning and output layer.

**Pre-trained Model**

A pre-trained model is a deep learning model that trained on a large dataset to learn useful patterns. In this case, pre-trained MLP (Multi-Layer Perceptron) model is used for crop yield prediction. An MLP is a type of neural network with multiple layers of neurons that learns complex relationships between input data samples and output such as crop yield. Figure 3 given above depicts the structure of an MLP to perform accurate crop yield prediction. The input and output layers are always single layers, while the hidden layers include numerous sub-layers. The neural network consists of multiple layers made up of small processing units called artificial neurons or nodes. Each neuron receives input values, processes them using a mathematical function, and passes the result to neurons in the next layer. The connections between neurons, known as synapses, have associated weights that control the strength of the input samples transferred from one neuron to another. These weights are adjusted during training to improve the model's accuracy in performing crop yield prediction. Let us consider a number of Features '$F = \{F_1, F_2, \dots, F_m\}$' and Samples '$S = D_1, D_2, \dots, D_n$given to the input layer of MLP. In the input layer, each neuron receives input features from the data samples. The neurons then compute a weighted sum of these inputs, which is expressed mathematically as follows,

$$X = \sum_{i=1}^{n} (D_i * w_{i_h}) + b \quad (3)$$

Where, $X$ denotes a weighted sum output, $w_{i_h}$represents a weight between input and hidden layer, number of samples '$D_i$', '$b$ specifies a bias that stored the value is '1'. The input is transferred into the next layer for preprocessing.

**Preprocessing**

In the first hidden layer, data preprocessing is the process of cleaning and preparing raw data for effectively perform crop yield prediction. The preprocessing step typically includes data handling missing and duplicate data removal. These steps help ensure that the input data is consistent, relevant, and properly scaled, which improves the accuracy and efficiency of the learning algorithm. The Weighted Mean Imputation is used to fill missing values by calculating the mean of available values, where each value contributes proportionally to its assigned weight.

$$D_m = \frac{\sum_{i=1}^{n} D_i * \varphi_i}{\sum_{i=1}^{n} \varphi_i} \quad (4)$$

Where,$D_m$ represents a missing data, $D_i$ indicates a known data points, $\varphi_i$ represents a weight assigned to data '$D_i$ '. Followed by, Dixon's Ratio Test is employed to determine outlier data in the given dataset. It is used to detect and categorize outliers in a dataset. First, the input data samples are sorted in ascending order. The Dixon's Ratio Test is then calculated using the following formula

$$QT = \frac{|D_i - D_{nn}|}{Max_D - Min_D} \quad (5)$$

Where, $QT$ indicates a Dixon's Ratio Test, $D_i$ represents a data point in the particular cell, $D_{nn}$ denotes a nearest value of $D_i$, $Max_D$ represents a maximum value in ascending order and the $Min_D$ represents a minimum value in ascending order. The statistical test provides output ranged from 0 to 1. If the calculated $QT$ value is greater than the threshold, particular data value is considered an outlier. If the estimated $QT$ value is lesser than the threshold, the data is not considered an outlier. The identified outlier data is removed from the dataset.

## FEATURE SELECTION

Feature selection is identifying selecting the most relevant features from a dataset. By choosing only the important features, it helps improve crop yield prediction accuracy while minimizing the time complexity. The proposed method utilizes Phi Coefficient to determine the more relevant features-based correlation coefficient. The correlation coefficient is to measure the association between the features using Cramer's phi test. The helps to project features in dataset onto a lower-dimensional space in order to avoid the curse of dimensionality and also minimize time complexity. Let us consider set of features in dataset.

$F_j = \{F_1, F_2, F_3, .. F_n\} \in DS$ (6)

Where, $F_j$ denotes a set of attributes or features in the big dataset$DS$. The correlation between the features is measured using Cramer's phi test.

$PC = \left[\frac{\sum_{j=1}^{m}\sum_{k=1}^{b}|F_i - F_k|^2}{(m-1)+(k-1)}\right]$ (7)

Where, $PC$ denotes a Cramer's phi test results, features '$F_i$ '$F_k$', $m, k$ are sample size. The test returns a value from 0 (no correlation between the features) to 1 (correlation between the features).

$$H = \begin{cases} PC = 1\ ; & F_i\ correlated\ with\ F_k \\ PC = 0\ ; & no\ correlation\ between features \end{cases} \quad (8)$$

The Cramer's phi correlation coefficient$PC$ is used to measure the similarity between pairs of features. A coefficient value of 1 indicates the two features are highly similar and it selected. A value of 0 means the features are dissimilar, so the feature is removed. This approach helps in selecting a subset of similar features for prediction, reducing redundancy and computational time.

**Prediction**

After identifying and selecting the most relevant features, the next step is to accurately predict crop yield. To analyze the relationships between these selected features and multiple related target variables; Total least squares regression is employed. It is a least squares approach that accounts for measurement errors in both the independent (input data samples) and dependent (crop yield prediction) during data processing. The estimated crop yield prediction model is observed as follows,

$Z = \sum_{i=1}^{n} D_i * \beta$ (9)

Where, $Z$ denotes an estimated crop yield prediction, $D_i$ denotes an input data samples, $\beta$ denotes a regression coefficient. In linear least squares regression, the model consists of equations that are linear with respect to the parameters. The residuals, which represent differences between the observed values and model's predictions, are calculated as:

$R = Z - \sum_{i=1}^{n} D_i \beta$ (10)

Where, $R$ denotes a residual function and minimize the objective function '$O$', typically the sum of squared residuals.

$O = \arg\min R$ (11)

By solving the residual function problem that minimizes the sum of squared residuals, the regression model identifies the optimal set of parameters that best fit the relationship between the input data samples and the crop yield. Minimizing the residuals ensures that the predicted yields are as close as possible to the actual observed yields across all data samples. As a result, the model provides more accurate and reliable yield optimization. Finally, the output of prediction is observed at output layer by means of softmax activation functions.

$Y = A_{softmax}\ (h_o * w_{h_o})$ (12)

Where, $Y$ denotes an output of crop yield prediction, $h_o$ represents the output of hidden layer, $w_{h_o}$ denotes a weight between the hidden and output layer. The algorithm step of pre-trained model is described as follows,

| **// Algorithm 1: Pre-trained Classification Model** |
|---|
| **Input**: **Dataset, features** $F_1, F_2, \dots, F_m$ **and data samples** $D_1, D_2, D_3, \dots D_n$<br>**Output:** cop yield prediction |
| **Begin**<br>**Construct the pre-trained model** |

**1. Collect n**umber of data samples $D_1, D_2, D_3, \ldots D_n$ **at the input layer**
**2. For each training** samples $D_i$ **--- Hidden layer 1**
3. Measure the neuron activation probability using (3)
4. Handling missing data according to (4)
5. Outlier data removal according to (5)
**6. End for**
**7. For each pre-processed dataset --- Hidden layer 2**
8. Measure the Phi coefficient between features using (6)
**9. If (**$PC = 1$**) then**
10. Indicates a high degree of similarity
11. Select the high similarity features
12. e**lse**
13. Remove other dissimilarity features
**14. End if**
**15. For each** training data samples **do--- --- Hidden layer 3**
16. Apply the regression analysis using (9) (10) (11)
17. Apply softmax activation function and obtain prediction outcomes using (12) **--- output layer**
**18. End for**
**End**

**Create new prediction model**

In a transfer learning strategy, a small set of training data samples is used to develop a new model by utilizing an existing pre-trained network, such as an MLP. As illustrated in Figure 2, certain layers referred to as frozen layers are retained from original model with their weights fixed during the new training phase. These layers retain previously learned knowledge and act as a base for new task. These layers protect the knowledge observed from the pre-trained model. For constructing to a new task, additional layers, including a new output layer and fine-tuned. The input data samples are transferred through the frozen layers which often include the initial input and early hidden layers, without altering their parameters. Freezing these layers not only preserves useful features learned from the original task but also reduces training time and computational load. To develop the new model, the architecture keeps the input layer and first three hidden layers fixed by freezing them, while two new layers are added for task-specific adaptation and final output. A limited number of input samples are transferred through the hidden layers, where initial data preprocessing is carried out. This involves addressing missing values using a [4] approach and identifying outliers through the normalized residual test using [5]. In the second hidden layer, the model performs feature selection by calculating the Phi coefficient, ensuring that only the most relevant features are retained. The third hidden layer performs prediction task by using the total least squares regression to assess the similarity between data patterns and make predictions. The output of the yield prediction in hidden layer is fine-tuned by applying an Owl Search Optimization thereby minimizing both training and validation errors and improving the accuracy of the crop yield prediction. For each prediction result, the error rate is computed based on squared difference between the actual and prediction results.

$$PE = [Z_{Act} - Z_{obs}]^2 \quad (13)$$

Where, $PE$ indicates an error, $Z_{Act}$ denotes actual prediction results, $Z_{obs}$ represents observed prediction results. Followed by, the initial weights get updated based on the error. In order to reduce the error, the adaptive gradient method is applied.

$$w_{new} = w_t - \eta \frac{\partial PE}{\partial w_t} \quad (14)$$

Where $w_{new}$ represents an updated weight, $w_t$ indicates a previous weight, $\eta$ denotes a learning rate, '$\frac{\partial PE}{\partial w_t}$' represents a partial derivative of error rate regarding current weight '$w_t$'.

In order to minimize the error, an Owl Search Optimization (OSO) algorithm is designed to minimize the error in crop yield prediction. OSO is a nature-inspired meta-heuristic algorithm that represents its concept from the hunting

behavior of owls. On the contrary to other optimization algorithm, OSO handles complex and high-dimensional optimization problems in deep learning models.

By applying OSO algorithm, initial population of weights is randomly generated within the search space.

$w = \{w_1, w_2, \dots, w_k\}$ (15)

After generating the initial population, the fitness is measured'$f(w)$' based on error rate.

$f(w) = \arg\min PE$ (16)

Where,$PE$ denotes prediction error. The owl with better fitness is selected as current optimal. Each owl updates its position based on its distance from prey, adjusting its location in search space to move closer to optimal solution.

$P_{t+1} = P_t + \ \delta * \Delta\vartheta * \left| r\, P_{prey} - \ P_t \right| P$ (17)

$\vartheta = \frac{\vartheta(P_t)}{d^2} + \gamma$ (18)

Where, $P_{t+1}$ represents an updated location of the owl, $P_t$ denotes a current location of the owl, $\delta$ indicates a parameter that organizes the degree of changes in the positions of search agents (owls) during optimization, $\Delta\vartheta$ represents an Intensity changed coefficient of the owl, $\vartheta(P_t)$ denotes a current intensity coefficient of the owl, '$d$' represents a distance between the target prey and owl, $r$ indicates a random value between [0, 0.5], $P_{prey}$ represents a target location of prey, $P$ represents the probability of owl moved in a particular direction. Finally, the algorithm identifies the optimal set of weights that lead to the lowest error rate in crop yield prediction. The final prediction output, with minimized error, is produced at the output layer with softmax activation function. The algorithmic process of new classifier model is given below.

**// Algorithm 2: New Classifier Model based Crop yield prediction**

**Input**: Dataset features $F_1, F_2, \dots, F_m$ and **data samples** $D_1, D_2, D_3, \dots D_n$

**Output:** Increase the of crop yield prediction accuracy

**Begin**

1. **Collect n**umber of features $F_1, F_2, \dots, F_m$ , and small number of data samples $D_1, D_2, D_3, \dots D_n$--- **input layer**
2. **For each** sample $D_i$
3. Compute the neuron activation probability using (3)
4. Perform Data Pre-processing using (4)(5) **-----[ Hidden layer 1]**
5. **End for**
6. **For each pre-processed dataset**
7. **Select relevant** feature set using (6) **-----[ Hidden layer 2]**
8. **End for**
9. **For each** training data samples
10. Apply the regression analysis using (9) (10) (11) -----[ **Hidden layer 3**]
11. Obtain the prediction results
12. **End for**
13. **For each prediction result** -----[ **Hidden layer 4**]
14. Measure the error rate ' $PE$ using (13)
15. Update the weights using (14)
16. **End for**
17. Initialize the population of the weights using (15)
18. **While (t<$t_{max}$) do**
19. **For each** weight '$w_k$'
20. Compute the fitness '$f(w)$' using (16)
21. **End for**
22. **select** weights based on fitness
23. Update the position of Owl using (17)
24. **t= t+1**
25. **Go to step 18**
26. **End while**
27. Return (global best weight)

**28.** Obtain the final prediction results with minimum error **at the output layer**
**End**

**Experimental Setup**
The proposed LiRT-TLNet model and existing AM-CNN-LSTM [1] and CNN-Transformer [2], is experimentally evaluated using Python high-level programming language.

**Implementation Results**
The LiRT-TLNet model is observed for analyzing crop yield prediction. The evaluation is conducted by utilizing Smart Farming Sensor Data for Yield Prediction dataset taken from Kaggle repository. First, numbers of data sample data are collected from dataset is shown in figure 4. This dataset includes 22 features and 500 records. Following data collection, data augmentation is carried out using dataset from 500 to 10500 records. The dataset undergoes preprocessing by handling 10500 data entries by resolving missing data, and identifying and eliminating outliers. The outcomes and transformations from preprocessing stage are illustrated in Figure 6.Following preprocessing phase, Cramer's phi correlation coefficient identifies relevant features while discarding less significant ones. In final stage, prediction task is performed to estimate crop yield typically for a given land area.

## RESULTS AND ANALYSIS

**Performance Comparison of deep learning models for Yield Estimation**
The three-comparison model namely LiRT-TLNet model, along with existing approaches such as AM-CNN-LSTM [1] and CNN-Transformer [2] are evaluated by including the several performance indicators such as
**Crop yield prediction accuracy '$CPA$'**: It is computed by the ratio of correctly predicted yield values to the total number of samples in the dataset.

$$CPA = \left(\frac{TP+TN}{TP+TN+FP+FN}\right) * 100 \quad (19)$$

Where, $TP$ represents the true positive, $TN$ denotes the true negative, $FP$ denotes the false positive, $FN$ represents the false negative. It is measured in percentage (%).

**Precision '$\boldsymbol{Prec}$'**: It is determined by calculating the ratio of correctly predicted positive instances (true positives) to total number of instances predicted as positive, which includes both true positives '*TP*' and false positives '*FP*'. The formula for precision is shown below:

$$\boldsymbol{Prec} = \left(\frac{\boldsymbol{TP}}{\boldsymbol{TP+FP}}\right) * \mathbf{100} \quad (20)$$

**Recall:** It also known as sensitivity, evaluates the model's effectiveness in correctly identifying positive crop yield cases.

$$Recall = \left(\frac{TP}{TP+FN}\right) * 100 \quad (21).$$

**F1 score:** it is measured as the harmonic mean of precision'$Prec$'and recall.

$$F1 - Score = 2 * \left(\frac{\boldsymbol{Prec} * Recall}{\boldsymbol{Prec} * Recall}\right) \quad (22)$$

**Root mean square error '$RMSE$':** It is employed to evaluate the accuracy of crop yield predictions by measuring the difference between predicted and actual values. It quantifies the model's prediction error by taking the square root of the mean of squared differences between the predicted values and the actual values across all data samples.

$$\boldsymbol{RMSE} = \left[\sqrt{\frac{\left(\boldsymbol{Y_{Actual} - Y_{predicted}}\right)^2}{\boldsymbol{n}}}\right] \quad (23)$$

Where, $Y_{Actual}$ denotes the actual results of crop yield prediction,$Y_{predicted}$ denotes a predicted results of crop yield, $\boldsymbol{n}$ indicates a number of data samples.

**Mean absolute error '$MAE$'**: It is an accuracy metric used to estimate the accuracy of a model predictions by comparing the magnitude of errors to average value of observed data.

$$\boldsymbol{MAE = \frac{1}{n}\left|Y_{Actual} - Y_{predicted}\right|} \quad (24)$$

Where, $Y_{Actual}$ denotes the actual results of crop yield prediction,$Y_{predicted}$ denotes a predicted results of crop yield, $n$ indicates a number of data samples.

**$R^2$:** It also known as coefficient of determination '$R^2$'is a statistical metric used to estimate the performance of crop yield prediction outcomes. It is also defined as ratio between the residual Sum of actual and predicted results of crop yield to total sum of square.

$$R^2 = 1 - \frac{\left(Y_{Actual} - Y_{predicted}\right)^2}{\left(\hat{Y} - Y_{Actual}\right)^2} \quad (25)$$

Where, $Y_{Actual}$ denotes the actual results of crop yield prediction,$Y_{predicted}$ denotes a predicted results of crop yield, $\hat{Y}$ denotes a mean of actual value.

**Crop yield prediction time '$CPT$':** It represents the overall duration required by algorithm to complete the crop yield prediction process.

$$CPT = \sum_{i=1}^{n} D_i * time(CP) \quad (26)$$

Where, data '$D_i$' and actual time consumed in crop yield prediction denoted by '$time(CP)$. It is measured in terms of milliseconds (ms).

The Figure 9 to 16shows better LiRT-TLNet technique with improved accuracy by 6% and4%, precision by 6%and3%.Recallby 4% and 2%, F1-score by 5% and 3%. With reduction error rate by 73% and 66%, MAE by 75% and 69%, $R^2$ score by 15% and 6%, prediction time by 11% and 6%

## CONCLUSION

This proposed LiRT-TLNet model, specifically developed to provide highly accurate crop yield predictions with minimal computation time and error rate. The transfer learning approach begins with data preprocessing and selection of key features, aiming to minimize the overall time required for prediction. This method utilizes the model's ability to transfer learned knowledge, enabling effective analysis of data samples using least square regression-based insights to improve classification accuracy. Additionally, performance is further enhanced through fine-tuning, which helps lower error rates associated with crop yield prediction. A comprehensive evaluation was performed using several performance metrics.

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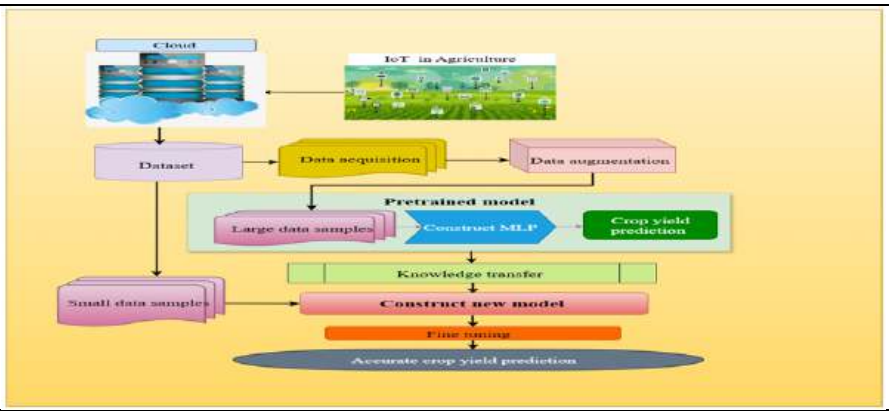



**Figure 1: Architecture of Proposed LiRT-TLNet Model**

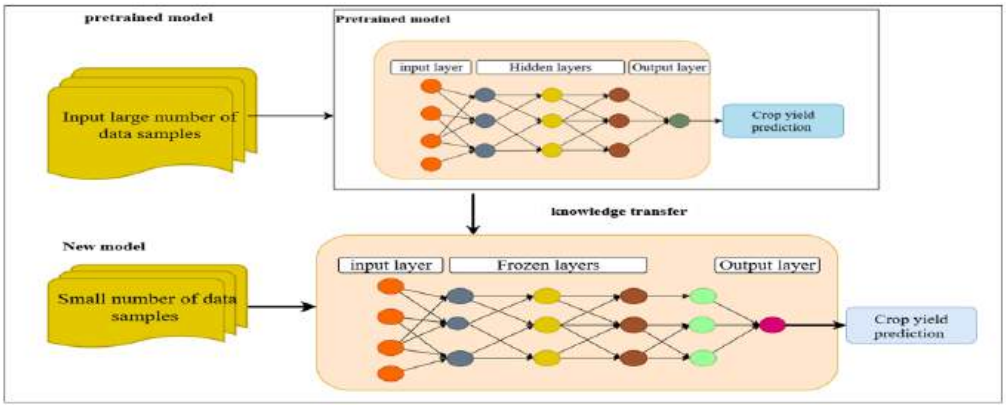



**Figure 2: Schematic Organization of Deep transfer learning model**

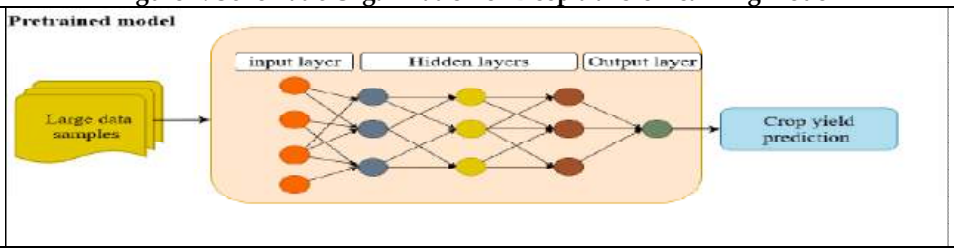



**Figure 3: Structure of MLP**

```
Original dataset shape: (500, 22)
Original dataset preview:

   farm_id      region crop_type  soil_moisture_%  soil_pH  ...  timestamp  latitude  longitude  NDVI_index crop_disease_status
0  FARM0001  North India    Wheat            35.95     5.99  ...  2024-03-19 14.970941  82.997689        0.63              Mild
1  FARM0002    South USA  Soybean            19.74     7.24  ...  2024-04-21 16.613022  78.869089        0.58               NaN
2  FARM0003    South USA    Wheat            29.32     7.16  ...  2024-02-28 19.503156  79.068206        0.80              Mild
3  FARM0004  Central USA    Maize            17.33     6.03  ...  2024-05-14 31.071298  85.519998        0.44               NaN
4  FARM0005  Central USA   Cotton            19.37     5.92  ...  2024-04-13 16.568540  81.691720        0.84            Severe

[5 rows x 22 columns]
```

**Figure 4: Data Collection**

```
Augmented dataset shape: (10500, 22)
Augmented dataset preview:

   farm_id      region crop_type  soil_moisture_%  soil_pH  ...  timestamp  latitude  longitude  NDVI_index crop_disease_status
0  FARM0001  North India    Wheat            35.95     5.99  ...  2024-03-19 14.970941  82.997689        0.63              Mild
1  FARM0002    South USA  Soybean            19.74     7.24  ...  2024-04-21 16.613022  78.869089        0.58               NaN
2  FARM0003    South USA    Wheat            29.32     7.16  ...  2024-02-28 19.503156  79.068206        0.80              Mild
3  FARM0004  Central USA    Maize            17.33     6.03  ...  2024-05-14 31.071298  85.519998        0.44               NaN
4  FARM0005  Central USA   Cotton            19.37     5.92  ...  2024-04-13 16.568540  81.691720        0.84            Severe

[5 rows x 22 columns]
```

**Figure 5: Data Augmentation**

```
Preprocessed dataset preview:

   farm_id      region crop_type  soil_moisture_%  soil_pH  ...  timestamp  latitude  longitude  NDVI_index crop_disease_status
0  FARM0005    South USA    Wheat            29.32     7.16  ...  2024-02-28 19.503156  79.068206        0.80              Mild
1  FARM0008  East Africa    Maize            27.10     5.72  ...  2024-04-30 23.317654  72.515210        0.79              Mild
2  FARM0009  Central USA  Soybean            40.54     6.35  ...  2024-05-08 13.025105  74.493947        0.59              Mild
3  FARM0010  East Africa     Rice            10.25     6.92  ...  2024-03-31 24.405291  74.859945        0.58            Severe
4  FARM0011  South India    Wheat            13.39     6.36  ...  2024-04-15 14.755223  78.972271        0.55          Moderate

[5 rows x 22 columns]
preprocessed farm sensor data.csv file size: 3660.00 KB
```

**Figure 6: After Data Preprocessing**

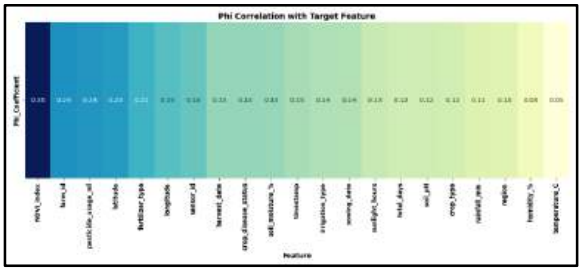



**Figure 7: Feature Importance Score**




**Figure 8: Crop Yield Prediction Output**

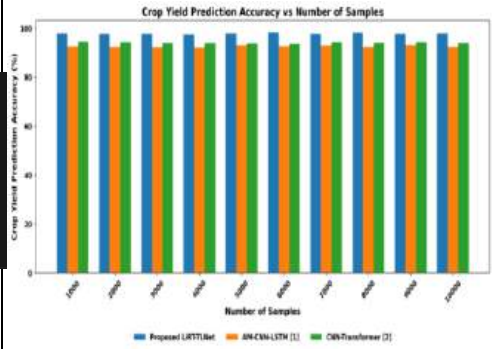



**Figure 9: Crop yield prediction accuracy**

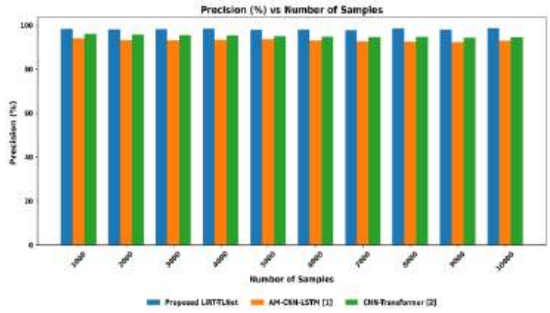
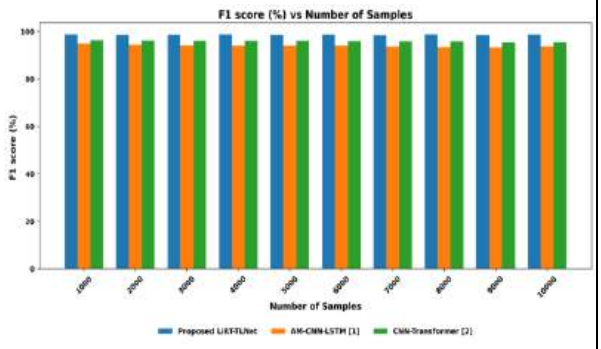



**Figure 10: Performance analysis of Precision**




**Figure 11: Performance analyses of Recall**




**12: Performance analyses of F1 score**

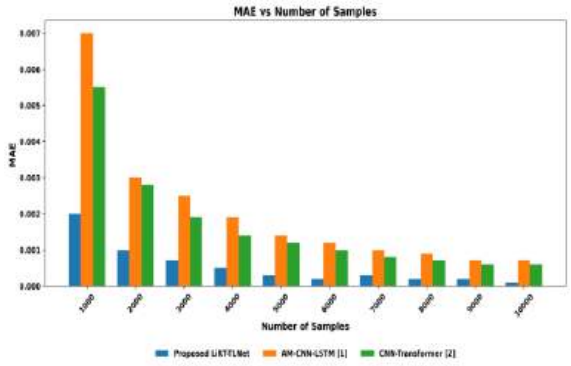



**Figure 13: Performance analyses of MAE**

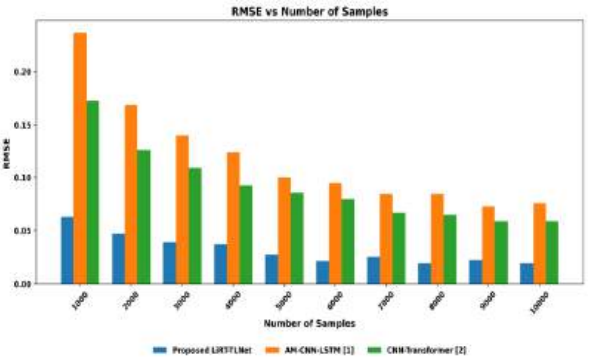



**Figure 14: Performance of RMSE**

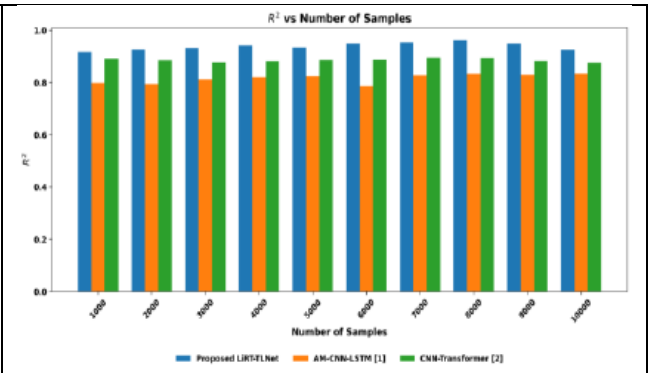



**Figure 15: Performance analyses of $R^2$**

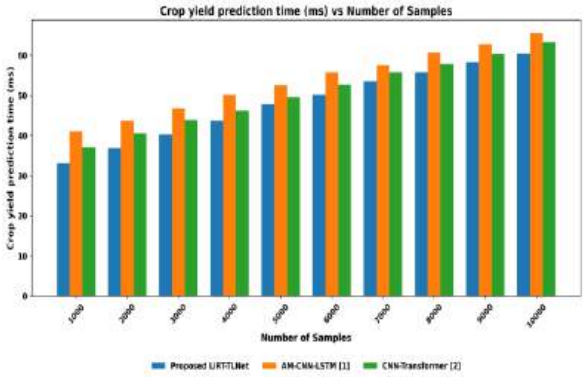



**Figure 16: Performance of Crop yield prediction time**

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RESEARCH ARTICLE

# Scientometric Insights into Global Research Productivity on Agriculture and Climate Change

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## ABSTRACT

This study presents a scientometric evaluation of global research on agriculture and climate change from 1995 to 2025, with a special focus on India's contributions. Using data retrieved from the Web of Science database, 2,460 publications and 38,345 citations were analyzed through bibliometric tools such as Biblioshiny, VOSviewer, and HistCite. The analysis identifies the most productive institutions, preferred journals, highly cited papers, and thematic keyword trends. Results show a steady growth in research output, reflecting the increasing importance of climate change as a global concern. Banaras Hindu University, ICRISAT, and the Indian Institutes of Technology (IITs) emerged as leading contributors, with strong collaboration from international organizations such as CIMMYT and Cornell University. Journals including *Sustainability*, *Current Science*, and *Frontiers in Plant Science* were among the most preferred outlets for dissemination. Keyword analysis highlights sustainability, adaptation, food security, and mitigation as central themes, alongside emerging areas such as machine learning, soil health, and water management. India's highly cited publications underscore its significant role in advancing adaptation strategies, sustainable farming, and climate-resilient agriculture. The findings provide a comprehensive overview of research productivity and impact, offering valuable insights to guide future research priorities, policy frameworks, and collaborative strategies for building a sustainable agricultural future in the face of climate change.

**Keywords:** Agriculture; Climate Change; Sustainable Agriculture; Agriculture-India.

## INTRODUCTION

Climate change is one of the most critical global challenges of the twenty-first century, with far-reaching implications for agriculture, food security, and sustainable development. Agriculture, being highly vulnerable to climatic variations, is directly impacted by rising temperatures, erratic rainfall, extreme weather events, and greenhouse gas emissions. These changes not only reduce crop productivity and soil fertility but also threaten the livelihoods of millions of farmers, particularly in developing countries such as India, where agriculture remains the backbone of the economy. Over the past three decades, research on the interconnection between climate change and agriculture has grown significantly. This surge reflects the urgency of developing adaptive strategies, resilient farming systems, and policy frameworks to mitigate the adverse impacts of climate variability. Understanding the structure and dynamics of this research domain is crucial for shaping future strategies in sustainable agriculture.

**Scientometric analysis** provides a systematic and quantitative method to evaluate publications, citations, and collaboration networks. It has become an essential tool for measuring research performance, identifying prolific contributors, mapping global and national collaborations, and tracing thematic trends. By offering an evidence-based overview, scientometrics helps policymakers, funding agencies, and researchers make informed decisions to maximize research impact. The present study employs scientometric techniques to assess global research performance on climate change and agriculture from 1995 to 2025, with particular emphasis on India's contributions and international collaborations. Drawing upon a dataset of 2,460 publications and 319,979 citations, the study identifies the most productive institutions, leading countries, highly cited papers, and keyword trends. The findings provide a comprehensive picture of the research landscape, highlighting patterns that can guide future investigations and support policy interventions for climate-resilient agriculture.

## OBJECTIVES

The primary objective of this study is to evaluate the research performance in the field of *Agriculture and Climate Change in India* between 1995 and 2025. Specifically, the study focuses on

1. Analyzing the share of publications of the most productive institutions.
2. Examining the total number of research records.
3. Assessing total local citation scores (TLCS).
4. Assessing total global citation scores (TGCS).

## MATERIALS AND METHODS

The study is based on data retrieved from the Web of Science (WoS) database. Publications were identified using the search term *"Agriculture and Climate Change"* in the topic field and *"India"* in the address field. A total of 2,460 papers published between 1995 and 2025 were collected. These records received 38,345 citations, which were further analyzed. The data were processed using Biblioshiny, VOSviewer, and HistCite software to generate descriptive statistics, citation analyses, and visualization maps. Concept mapping and bibliometric indicators were employed to address the study's objectives.

**Most Productive Institutions**

The scientometric analysis highlights the leading institutions contributing to research on agriculture and climate change between 1995 and 2025. Among Indian institutions, Banaras Hindu University (105 publications, 117 TLCS, 3485 TGCS), ICRISAT (98 publications, 93 TLCS, 5494 TGCS), and the Indian Institutes of Technology (92 publications, 123 TLCS, 5774 TGCS) emerged as the most prolific contributors. International organizations such as the International Maize and Wheat Improvement Center (CIMMYT) and Cornell University also made significant contributions, reflecting strong global collaboration. This trend indicates that both Academic Institutions and

International Research organizations play a pivotal role in advancing climate–agriculture research, especially in the context of India.

**Most Preferred Journals**

The analysis of publication sources shows that research output is widely distributed across international and national journals. Journals such as Sustainability, Current Science, Environmental Monitoring and Assessment, Agricultural Systems, and Frontiers in Plant Science feature prominently, reflecting their interdisciplinary scope and high relevance to agriculture and climate change. These journals provide a platform for disseminating work on climate adaptation, mitigation strategies, sustainable farming, and agricultural innovations.

**Keyword Occurrences**

Keyword analysis reveals the major thematic directions in this research domain. Frequently occurring terms include "climate change," "agriculture," "sustainability," "adaptation," "food security," "mitigation," and "India." The prominence of these keywords indicates a strong research focus on climate-resilient agriculture, food production challenges, and sustainability practices. The clustering of terms such as *machine learning, soil health, and water management* further highlights the emerging interdisciplinary linkages of agricultural sciences with technology-driven solutions.

**Highly Cited Papers from India**

The highly cited publications from India underscore the country's substantial research impact on climate change and agriculture. These papers often focus on adaptation strategies, crop modelling, sustainable farming techniques, and policy frameworks for resilience. Many of them are published in internationally recognized journals, receiving significant global citations. Such works not only highlight India's academic contribution but also demonstrate its leadership role in addressing the agricultural implications of climate change.

## FINDINGS AND CONCLUSION

This scientometric evaluation of global research on agriculture and climate change (1995–2025) offers several key insights:

**Rising Research Output:** The number of publications has steadily increased, reflecting the growing urgency of climate change as a research priority.

**Institutional Leadership:** Banaras Hindu University, ICRISAT, IITs, and ICAR institutes are leading contributors from India, often collaborating with global organizations like CIMMYT and Cornell University.

**Preferred Outlets:** Publications are concentrated in reputed interdisciplinary journals, underlining the wide applicability of research findings.

**Thematic Hotspots:** Keywords point to core themes of sustainability, adaptation, and food security, while also signalling the rise of emerging areas like technology integration and climate modelling.

**High Impact Research:** India's highly cited papers demonstrate strong global relevance, emphasizing adaptation strategies and agricultural resilience in the face of climate change.

In conclusion, the study establishes that India, in collaboration with global partners, has made significant contributions to advancing knowledge on agriculture and climate change. The insights generated are crucial for guiding future research priorities, funding policies, and practical interventions aimed at building a sustainable and climate-resilient agricultural system.

## FINDINGS AND CONCLUSION

The study found that the Most productive and leading organization are: Banaras Hindu University with 105 publications far by ICRISAT– The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) with 98, The Indian Institutes of Technology (IITwith 92, International Maize and Wheat Improvement Center, (CIMMYT) with 87, ICAR Indian Agriculture Research Institute with 73 Publications. Indian agrarian sector in fact requires very innovative ideas for uplifting of this sector. Also, without mechanization, farming is hard and back-breaking work. This has resulted in most farmers' children quitting farming and going for other vocations. Farmers get more money in selling their land to builders, malls and factories. This has put more pressure on farmland, thereby requiring technologies to increase the productivity so that shrinking farmland can feed billion plus people of India in the future. India, though one of the biggest producers of agricultural products, has very low farm productivity, with the average only 33 percent of the best farms world over. This needs to be increased so that farmers can get more remuneration from the same piece of land with less labour.

## ACKNOWLEDGEMENT

Thanks to Tamilnadu State Council for Science and Technology for the financial support of research projects entitled on "Data - Driven Technology against climate change impact towards enhancing the sustainable agriculture: A study of central Districts of Tamil Nadu" and the resulting publication of Indian contribution to Agriculture and Climate Change Research: A Scientometric Study.

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**Table 1: Top 10 Productive Institutions Records with TGCS**

| Sl No | Institution | Recs | TGCS |
|---|---|---|---|
| 1 | Banaras Hindu University | 105 | 3485 |
| 2 | The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) | 98 | 5494 |
| 3 | The Indian Institutes of Technology (IIT) | 92 | 5774 |
| 4 | International Maize and Wheat Improvement Center, (CIMMYT) | 87 | 3932 |
| 5 | ICAR Indian Agriculture Research Institute | 73 | 1473 |
| 6 | Punjab Agriculture University | 63 | 1865 |
| 7 | Indian Agriculture Research Institute | 58 | 2752 |
| 8 | Jawaharlal Nehru University | 55 | 2085 |
| 9 | University Delhi | 55 | 1041 |
| 10 | Tamil Nadu Agriculture University | 49 | 446 |

**Table 2: Top 10 Productive Institutions Records with TLCS**

| Sl No | Institution | Recs | TLCS |
|---|---|---|---|
| 1 | Banaras Hindu University | 105 | 117 |
| 2 | The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) | 98 | 93 |
| 3 | The Indian Institutes of Technology (IIT) | 92 | 123 |
| 4 | International Maize and Wheat Improvement Center, (CIMMYT) | 87 | 343 |
| 5 | ICAR Indian Agriculture Research Institute | 73 | 24 |
| 6 | Punjab Agriculture University | 63 | 83 |
| 7 | Indian Agriculture Research Institute | 58 | 245 |
| 8 | Jawaharlal Nehru University | 55 | 67 |
| 9 | University Delhi | 55 | 42 |
| 10 | Tamil Nadu Agriculture University | 49 | 22 |

**Table 3: Most Top 10 Institutions**

| Sl No | Institution | Recs | TLCS | TGCS |
|---|---|---|---|---|
| 1 | Banaras Hindu University | 105 | 117 | 3485 |
| 2 | The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) | 98 | 93 | 5494 |
| 3 | The Indian Institutes of Technology (IIT) | 92 | 123 | 5774 |
| 4 | International Maize and Wheat Improvement Center, (CIMMYT) | 87 | 343 | 3932 |
| 5 | ICAR Indian Agriculture Research Institute | 73 | 24 | 1473 |
| 6 | Punjab Agriculture University | 63 | 83 | 1865 |
| 7 | Indian Agriculture Research Institute | 58 | 245 | 2752 |
| 8 | Jawaharlal Nehru University | 55 | 67 | 2085 |
| 9 | University Delhi | 55 | 42 | 1041 |
| 10 | Tamil Nadu Agriculture University | 49 | 22 | 446 |

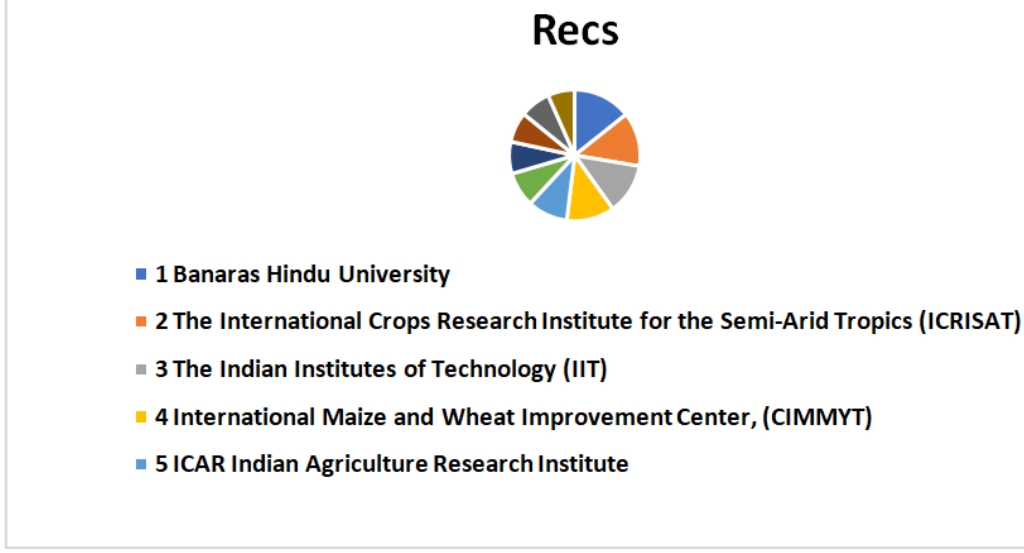



**Fig 1: Top 10 Productive Institutions Records with TGCS**

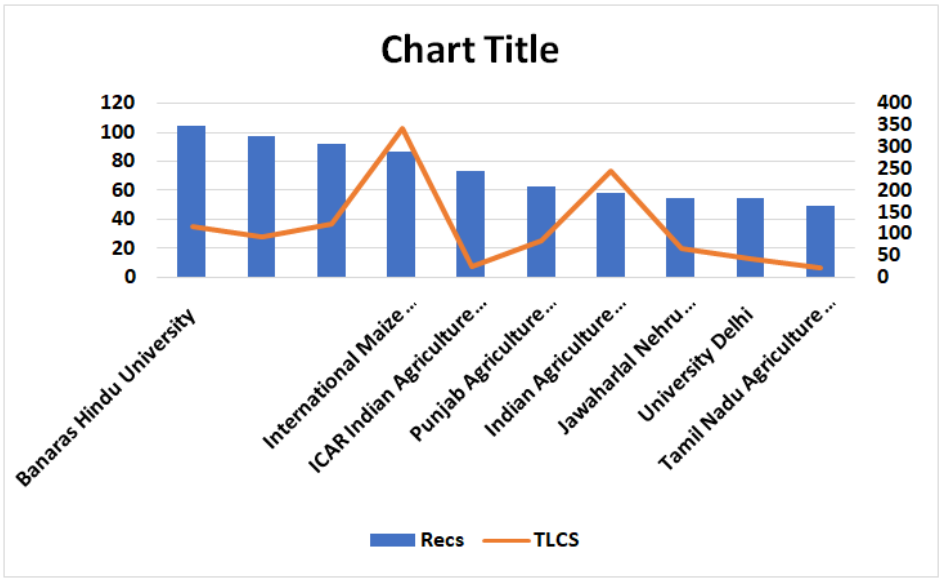



**Fig 2: Top 10 Productive Institutions Records with TLCS**

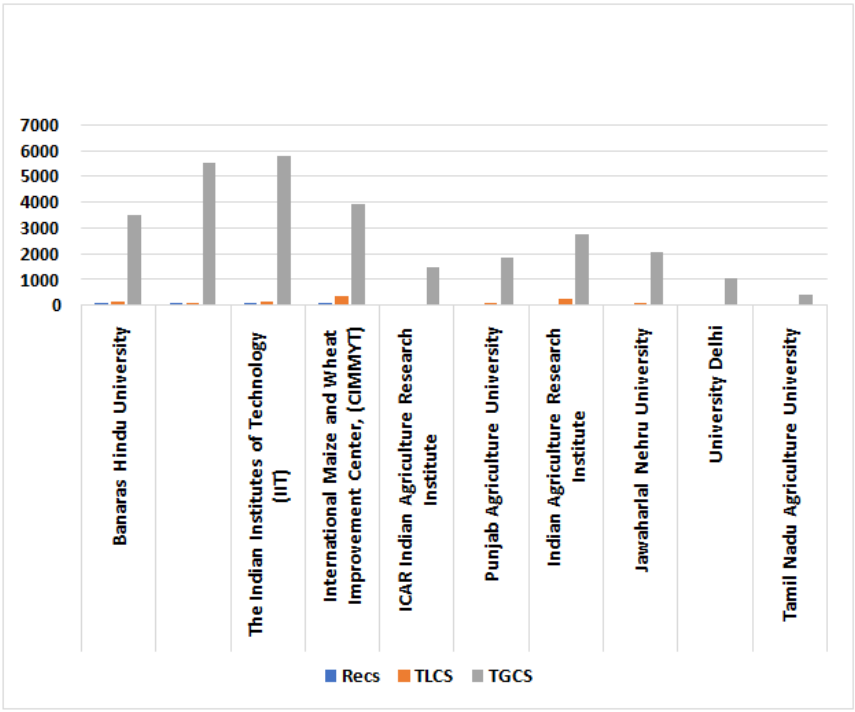



**Fig 3: Most Top 10 Institutions**

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RESEARCH ARTICLE

# Understanding the Determining Factors and Care Seeking Patterns for Adolescent Menstrual Irregularities: A Mixed Methods Study Protocol

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## ABSTRACT

Menstrual health is a vital phase of overall wellbeing of adolescent girls, as it is an important indicator of physical, emotional and reproductive health. Deviations from normal menstrual cycle may have significant implications not only for physical and reproductive health but also for mental wellbeing. Adolescence, as a transitional phase to adulthood, involves substantial hormonal adjustments that can contribute to menstrual irregularities. These disturbances can potentially affect the academic performances, psychosocial functioning and overall quality of life. This article outlines a mixed methods research protocol designed to better understand the factors influencing adolescent menstrual irregularities and their treatment seeking behaviors. This study aims to assess the prevalence of adolescent menstrual irregularities; to identify the associated psychosocial and lifestyle factors; and examine their care seeking behaviors. An explanatory sequential mixed methods design will be employed, with an initial quantitative phase to determine the prevalence and key determinants, followed by a qualitative phase exploring the adolescents' treatment seeking behaviors in depth. Finally, findings from both the strands will be integrated to generate a comprehensive understanding that can inform adolescent friendly interventions and guide future menstrual health programs.

**Keywords:** Adolescent health, Menstrual health, Menstrual irregularities, Psychosocial factors, Lifestyle factors, Treatment seeking behavior, Mixed-methods study.

## INTRODUCTION

The journey of human life involves transitioning through various phases right from infancy to old age. During each phase the body undergoes several physical and physiological adaptations. These adaptations are open to be disrupted by several extrinsic as well as intrinsic factors. The human female body undergoes massive hormonal adaptations during each phase of life. These hormonal changes are at peak during adolescent period. Adolescence is a phase where substantial growth and development occurs in a female's body, marking the gradual progression to mature reproductive health. Menstruation is a major biological milestone that normally begins during adolescence. It is a phenomenon that is influenced by several extrinsic as well as intrinsic factors. Extrinsic factors like lifestyle, surrounding environment, geography, socio-economic conditions, etc. as well as intrinsic factors like race, ethnicity, general health status, etc. contribute vitally in regulation of menstrual cycles[1]. Any deviations in menstrual cycle leads to menstrual irregularities or menstrual disruptions. Persistence of menstrual irregularities leads to considerable school absenteeism, thereby affecting their academic performances [2]. It may prompt the adolescent girls to face stigma, harassment and social exclusion. [3]In addition to this, menstrual health in a country like India is often tied up with several myths and taboos. [4] Menstrual irregularities also affect the mental health and wellbeing of the adolescents [5]. All these reasons together creates a state of confusion, ignorance, apprehension and isolation among the adolescent girls in reaching out for medical assistance to manage their condition [6,7]. In India, a substantial number of adolescent girls suffer from one or the other menstrual irregularities [8,9,10]. Some of the menstrual irregularities commonly known are amenorrhea, premenstrual syndrome, dysmenorrhea, oligomenorrhea, polymenorrhea, menorrhagia, and others. Due to the hormonal fluctuations secondary to the immature hypothalamic - pituitary - ovarian axis during adolescence, these irregularities occur which are further worsened by various psychosocial and lifestyle factors [11]. Despite the growing recognition of menstrual health as a public health priority, the treatment seeking behavior for menstrual problems among the adolescents remains suboptimal. As a result, many girls continue to endure menstrual irregularities without appropriate medical support, potentially increasing the risk of long-term reproductive health issues [6]. Existing literature offers valuable evidences about adolescent menstrual health, however, there still exists a vast knowledge gap. Most of the studies done to address this gap are either cross-sectional having methodological limitations like having small sample sizes, lack of confirmatory statistical results and others. Even the qualitative evidences have not specifically covered the experiences or perceptions of adolescents with menstrual irregularities. Hence, there exists a felt-need for robust enquiry of the prevalence and determining factors of adolescent menstrual irregularities using a mixed methods approach to comprehensively address this multifaceted issue. The researcher presents this protocol for an explanatory sequential mixed methods study aimed at understanding the determining psychosocial and lifestyle factors and care-seeking behaviors associated with adolescent menstrual irregularities. Integration of both quantitative and qualitative strands will provide a holistic interpretation of findings, enabling the development of informed recommendations to strengthen menstrual health literacy, promote timely and appropriate care seeking and support adolescent-friendly health systems.

## MATERIALS AND METHODS

For the current study, the researcher will use an Explanatory Sequential study design, a type of mixed methods approach. Using a mixed methods approach will help combine the strengths of quantitative and qualitative methodologies to increase the understanding and provide more complete answers to the research question [12,13]. As depicted in figure 1, this study will have an initial quantitative strand assessing the prevalence and associated psychosocial and lifestyle factors of adolescent menstrual irregularities, followed by the qualitative strand exploring the treatment seeking behaviors of these adolescent girls. Finally integration will be done of the findings of both the strands.

**Phase-I: Quantitative Strand**

A Cross-sectional study will be employed to assess the prevalence of menstrual irregularities among adolescent girls as well as to assess the various associated psychosocial and lifestyle factors. This study is planned to be conducted at schools and colleges from Gujarat state, India. The population is Adolescent girls aged between 15 to 19 years of age. Adolescent girls who are diagnosed with other medical health conditions like blood clotting and bleeding disorders, genetic disorders, cardiac vascular disorders, cancer, any physical disability, any congenital reproductive tract malformations as well as girls prescribed medications like antipsychotics, chemotherapeutic drugs, antidepressants, blood pressure drugs and allergy medications would not be eligible to be recruited for the study. Adolescent girls of the given age group who have attained menarche would be eligible for the study. Data collection would be done using a questionnaire for socio-demographic as well as the clinical variables and a structured questionnaire to assess menstrual health. To assess the psychosocial problems like anxiety, depression, body image, social isolation and stress, various standardized tools would be employed. Also the lifestyle factors like the dietary habits, physical activity and sleep patterns would be assessed using standardized tools. All the standardized tools would be used after seeking the due approval from the respective authors.

**Phase-II: Qualitative Strand**

A Descriptive phenomenology will be employed to understand the treatment seeking behaviors of the adolescent girls with menstrual irregularities. A nested consecutive sampling technique would be employed to recruit the participants for this phase. Nested consecutive sampling is majorly used in sequential explanatory designs, where the same participants who took part in the quantitative phase initially, are sampled again for the qualitative phase, till data saturation is achieved [14]. A semi-structured, one-to-one interview guide regarding treatment seeking behavior of adolescent girls experiencing menstrual irregularities, with optional probing questions, will be employed for data collection in this phase. A field diary will also be maintained, recording the descriptive and interpretive notes based on the respondents' comments during the interview.

**Mixed-methods Integration**

The integration will be done in two stages:

a. The result of phase-I will help to recruit the samples of phase-II through nested consecutive sampling technique;
b. The results of phase-I and phase-II will be triangulated using the meta-matrix analysis technique, with the aim to deepen the understanding of the results as well as help in pattern recognition.

**Data Analysis**

For the quantitative strand, Descriptive statistics will be performed to calculate the means, standard deviations and frequencies of socio-demographic and clinical variables collected. Applicable Inferential statistics would be used for calculation of the prevalence of menstrual irregularities and associated psychosocial and lifestyle factors, and also to assess the associations between psychosocial factors and lifestyle habits. Statistical significance will be established as $p < 0.05$. In the qualitative strand, to fully comprehend the meaning and context of the collected data regarding the treatment seeking behaviors of the adolescent girls with menstrual irregularities, the transcripts verbatim will be examined using thematic analysis. Through an iterative process, key themes will be identified, coded and organized into categories to capture the core patterns within the data. A Meta-matrix analysis technique will be employed for integration the results of both the phases. This technique will involve creating a matrix where one axis represents the themes from qualitative analysis and the other axis will represent the key quantitative findings. Next the findings would be triangulated identifying areas where qualitative and quantitative results would have converged or highlighting any contradictions or gaps.

## DISCUSSION AND CONCLUSION

Menstrual irregularities are often overlooked by adolescent girls, possibly due to the perception that menstruation is a regular phenomenon among females or due to the stigma and taboos associated with it. Apart from the physical

concerns, the psychosocial challenges associated with menstrual irregularities, can significantly incapacitate the adolescents' daily functioning as well as their academic performances. To worsen this, the ignorance about care or treatment seeking behaviors among them, results in further deterioration of the situation. As adolescents are the future of any country, knowing in detail their lifestyle factors and psychosocial issues associated with menstrual irregularities and understanding their treatment seeking behaviors becomes pertinent and vital. Employing a mixed methods approach would help cater to any gaps or limitations inherent in using either qualitative or quantitative approach alone. This will help comprehend the complex link which is present amongst the various determinants of menstrual health. This study findings would advocate and support for more stronger and coordinated efforts to position adolescent menstrual health as a key public health priority.

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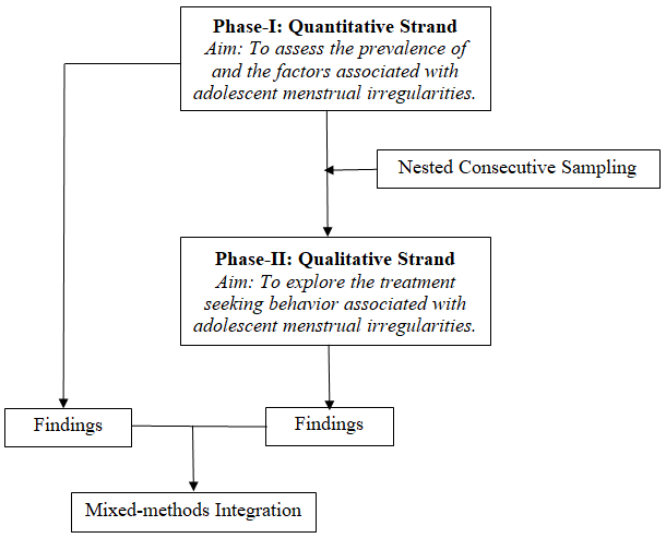



**Figure 1: Explanatory sequential mixed methods diagram**

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RESEARCH ARTICLE

# Comparative Analysis of Vitamin C Content in Fresh Amla (*Phyllanthus emblica*) Juice and Marketed Preparations using Iodometric Titration, UV–Visible Spectroscopy, and Accelerated Stability Studies

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## ABSTRACT

This study aimed to compare the Vitamin C content of fresh amla (*Phyllanthus emblica*) juice with various commercially available amla juice preparations using iodometric titration and UV-visible spectrophotometry, followed by an accelerated stability study. Fresh amla juice and five marketed brands packed juice 1, 2, 3, 4, and 5were analyzed for their ascorbic acid content. The iodometric method was based on the redox reaction between iodine and ascorbic acid, while UV-visible spectrophotometry measured absorbance at 266 nm. Fresh juice exhibited the highest initial Vitamin C content, with 51.2 mg/100 mL, while marketed juices ranged from 33.6 to 37.4 mg/100 mL. However, under accelerated storage conditions over 28 days, fresh juice showed greater degradation (23.4%) compared to commercial juices (17.9%–23.2%). The results from both analytical methods were consistent, validating the reliability of UV-visible spectroscopy for rapid estimation. The study concludes that while fresh amla juice is nutritionally superior, commercial preparations demonstrate

better Vitamin C stability, highlighting the balance between freshness and formulation longevity in consumer products.

**Keywords:** Vitamin C; Amla Jucies; UV-Visible Spectroscopy; Idometric titration; Accelerated stability studies

## INTRODUCTION

Vitamin C, also known as ascorbic acid, is an essential water-soluble nutrient involved in numerous physiological functions, including collagen synthesis, immune support, wound healing, and the maintenance of healthy skin and connective tissue [1, 2]. It is also a powerful antioxidant, capable of neutralizing free radicals and thereby reducing oxidative stress in the body. This antioxidant activity plays a key role in preventing the onset of various chronic diseases such as cardiovascular disorders, certain types of cancer, and neurodegenerative conditions [3-5]. Among natural sources, *Phyllanthus emblica*, commonly known as amla or Indian gooseberry, is particularly rich in Vitamin C. Traditionally used in Ayurvedic medicine, amla is prized for its rejuvenating, immunomodulatory, and hepatoprotective properties. The Vitamin C content in fresh amla is significantly higher than in most other fruits, making it a valuable component in herbal formulations, dietary supplements, and functional foods [6-9]. However, Vitamin C is known for its chemical instability. It is highly susceptible to degradation when exposed to factors such as heat, light, oxygen, and variations in pH during processing and storage [10]. With the increasing consumer demand for health-focused beverages, many commercial amla juice products are now available in the market. These ready-to-drink juices claim to retain the natural benefits of fresh amla, particularly its high Vitamin C content [11, 12]. However, the industrial processes involved in the production of packaged juices—such as pasteurization, dilution, and the use of preservatives—may significantly reduce their actual ascorbic acid content. As a result, it becomes necessary to scientifically evaluate and compare the Vitamin C concentration in freshly prepared amla juice and various marketed juice preparations [13]. To address this, the present study utilizes two complementary analytical techniques: iodometric titration and UV-visible spectrophotometry. Iodometric titration is a classical redox method in which ascorbic acid reduces iodine to iodide, allowing its concentration to be determined based on the volume of iodine solution consumed. In contrast, UV-visible spectroscopy is a modern, sensitive method that detects Vitamin C based on its characteristic absorbance at 265 nm [14-16]. Together, these methods offer a reliable approach to quantify and validate Vitamin C levels in different juice samples. Furthermore, an accelerated stability study was conducted to evaluate the degradation pattern of Vitamin C in both fresh and packaged juice samples under stress conditions (elevated temperature and humidity). This component of the study is essential for assessing the shelf-life and preservation of nutritional quality over time [17-18]. Overall, this comparative analysis aims to provide meaningful insights into the nutritional authenticity of commercial amla juices while reinforcing the value of fresh juice consumption for optimal health benefits.

## MATERIALS AND METHODS

## MATERIALS

Fresh amla fruits (*Phyllanthus emblica*) were procured from a local market and used for preparing freshly extracted amla juice. Five different commercially packaged amla juice samples were selected from local retail outlets for comparative analysis. Five packed juice were selected from the local market of Indore, Madhya Pradesh. All reagents and chemicals used in the study were of analytical grade. Ascorbic acid standard was obtained from the Acropolis Institute of Pharmaceutical Education and Research. Potassium iodide, iodine crystals, glacial acetic acid, and starch powder were also sourced from the same institute. Distilled water was used throughout the study for the preparation of solutions and dilution of samples.

**Preparation of Reagents**

**Iodine Solution (0.005 M):** To prepare the iodine solution, 2 g of potassium iodide (KI) was weighed and dissolved in a small amount of distilled water in a beaker. Then, 1.3 g of iodine crystals ($I_2$) was added and dissolved by gentle swirling. The resulting solution was transferred into a 1 L volumetric flask, and the volume was made up to the mark with distilled water.

**Starch Indicator Solution (0.5%):** To prepare the starch indicator, 0.25 g of soluble starch was added to 50 mL of near-boiling distilled water in a conical flask. The mixture was stirred continuously until the starch dissolved completely. The solution was then allowed to cool to room temperature before use.

**Iodometric Titration Method**

The iodometric titration method was used to estimate the Vitamin C content in fresh and packaged amla juice samples. This method is based on the redox reaction between iodine and ascorbic acid, where iodine is reduced to iodide and ascorbic acid is oxidized to dehydroascorbic acid. The endpoint is detected by the formation of a stable blue-black complex between iodine and starch. A 10 mL aliquot of each juice sample was pipetted into a 250 mL conical flask. To this, approximately 100 mL of distilled water was added, followed by 1 mL of the prepared starch indicator solution. The mixture was titrated against 0.1 M iodine solution until a permanent blue-black coloration persisted, indicating the endpoint. The titration was repeated in triplicate for accuracy, and concordant values were recorded for calculation [19-22].

**UV-Visible Spectrophotometric Method**

In addition to titration, Vitamin C content was also estimated using UV-visible spectrophotometry. Ascorbic acid exhibits a strong absorbance peak at 266 nm, which was used as the detection wavelength. A stock solution of ascorbic acid standard was prepared in distilled water, and serial dilutions were made to construct a calibration curve in the range of 10–60 μg/mL. The absorbance of each standard solution was measured at 266 nm using a UV-visible spectrophotometer, and a standard curve was plotted. Juice samples were appropriately diluted and filtered before analysis, and their absorbance values were used to calculate the ascorbic acid concentration by interpolation from the standard curve [23-30].

**Accelerated Stability Study**

To evaluate the stability of Vitamin C in both fresh and packaged amla juices, an accelerated stability study was conducted under stress conditions. All juice samples were stored in a stability chamber maintained at 40 ± 2 °C and 75% relative humidity. The Vitamin C content was determined at regular intervals—on day 0, 7, 14, 21, and 28—using UV-visible spectroscopy. This study aimed to simulate long-term storage conditions and assess the degradation rate of Vitamin C over time [24-27].

## RESULTS

**Vitamin C Estimation by Iodometric Titration**

The concentration of Vitamin C in different amla juice samples was determined using the iodometric titration method. This method relies on the redox reaction between iodine and ascorbic acid, where ascorbic acid reduces iodine to iodide and is itself oxidized to dehydroascorbic acid. The endpoint of the titration is observed by the formation of a stable blue-black starch-iodine complex.The table 1 and figure 1 below presents the observed titration volumes and the corresponding calculated Vitamin C content. Estimation of Vitamin C content by Iodometric Titration (Table 1)

**Vitamin C Estimation by UV-Visible Spectroscopy**

Vitamin C (ascorbic acid) exhibits strong UV absorbance, especially around 265–270 nm due to its conjugated system and hydroxyl groups. The absorbance of each juice sample was measured at 266 nm and compared with a standard

calibration curve prepared using known concentrations of pure ascorbic acid. A standard calibration curve was prepared by measuring the absorbance of ascorbic acid solutions with concentrations ranging from 10 to 60 µg/mL at 266 nm. The curve showed a strong linear relationship between absorbance and concentration with a correlation coefficient ($R^2$) of approximately 0.999, confirming the reliability of the method (Figure 2). Concentration vs Absorbance of standard ascorbic acid (Figure 2). Vitamin C estimation by UV-visible spectroscopy (Table 2).

**Result of Accelerated stability study**

An accelerated stability study was conducted over a 28-day period to assess the degradation of Vitamin C content in fresh and various marketed amla juice samples stored under stressed conditions. The results clearly demonstrated a gradual decline in Vitamin C concentration across all samples, with fresh juice exhibiting the highest initial content and most significant degradation over time. At Day 0, fresh amla juice showed the highest Vitamin C content (51.2 mg/100 mL), followed by pakced amla juice 1 (37.4 mg), pakced amala juice 2 (36.1 mg), pakced amla jucie 3 (35.7 mg), packed amla juice 4 (34.2 mg), and packed amla juice 5 (33.6 mg). Over the 28-day period, the Vitamin C levels in all samples decreased progressively. By Day 28, fresh juice content had declined to 39.2 mg/100 mL, indicating a degradation of 23.4%. This suggests that while fresh juice starts with a higher Vitamin C level, it is more susceptible to degradation under accelerated conditions. Among the marketed preparations, packed amla juice 1 exhibited the least degradation (17.9%) followed closely by packed juice 2 (18.3%), suggesting better formulation stability. Packed juice 3, 4, and 5 showed slightly higher degradation rates of 21.6%, 22.8%, and 23.2%, respectively. Despite having lower initial Vitamin C levels than fresh juice, these commercial juices demonstrated relatively better preservation of their ascorbic acid content, likely due to added preservatives or controlled pH conditions. Overall, the study highlights that while fresh juice is richer in Vitamin C initially, its stability is comparatively lower under accelerated storage conditions. In contrast, commercial juices, though lower in initial content, maintain their ascorbic acid levels better over time. Accelerated stability study of fresh and different Amla juices (Table 3).

## DISCUSSION

The iodometric titration data clearly indicate that fresh amla juice contains the highest concentration of Vitamin C (52.4 mg/100 mL), significantly more than the packaged commercial products. This finding aligns with the expectation that freshly prepared juices, without exposure to heat, light, and prolonged storage, retain more of their natural antioxidant content. Among the marketed brands, packed amla juice 1 had the highest Vitamin C content (38.2 mg/100 mL), followed closely by packed amla juice 2 and 3. Packed amla juice 5 and Pakced Amla Juice 4 displayed comparatively lower concentrations, which may result from either prolonged storage, thermal degradation during pasteurization, or variation in formulation and source materials. The results underscore the nutritional superiority of fresh amla juice in terms of Vitamin C retention. Commercial processing methods and storage conditions seem to significantly affect the stability and bioavailability of Vitamin C, a highly oxidation-sensitive compound. Thus, for maximum health benefit, consumption of fresh amla juice is recommended whenever possible. The UV-visible spectroscopy results confirm the findings of the iodometric titration. Freshly prepared amla juice exhibited the highest absorbance (0.545), corresponding to a Vitamin C concentration of 51.2 mg/100 mL, which is significantly higher than any of the marketed juices. Among the commercial samples, Pakced amala juice 1 and 2 again showed relatively higher concentrations (37.4 and 36.1 mg/100 mL, respectively), while packed amala juice 5 and 4 had the lowest. These differences are consistent with potential losses of Vitamin C during commercial processing, packaging, and storage. This spectrophotometric method, being rapid and non-destructive, proves to be a suitable complementary technique to titration, offering more precise quantification for quality control purposes. The study clearly demonstrates a time-dependent degradation of Vitamin C in all juice samples. Fresh amla juice, despite having the highest initial concentration (51.2 mg/100 mL), showed the most significant drop over 28 days (to 39.2 mg/100 mL), amounting to a 23.4% reduction. This is expected due to the absence of preservatives and the sensitivity of ascorbic acid to heat, light, and oxygen. Among commercial samples, packed amala juice 1 and 2 retained Vitamin C better than others, likely due to formulation differences including stabilizers, preservatives, and controlled processing. Juice 5 and 4 showed relatively poorer stability, with around 23% loss, similar to the fresh juice, possibly

indicating minimal processing or lack of effective preservation. These results reinforce that Vitamin C is highly unstable under stress conditions, and formulation strategies or immediate consumption can significantly influence its retention.

## CONCLUSION

The comparative study of Vitamin C content in fresh and commercially packaged amla juices using both iodometric titration and UV-visible spectrophotometry revealed significant differences in ascorbic acid levels. Fresh amla juice consistently showed higher initial Vitamin C content compared to all marketed preparations. However, the accelerated stability study demonstrated that Vitamin C in fresh juice degraded more rapidly over time than in commercial juices, likely due to the absence of stabilizers and preservatives. Among the commercial products, packed juice 1 and 2 exhibited relatively better stability, maintaining higher ascorbic acid content throughout the 28-day period. The UV-visible spectrophotometry method validated the titration results and proved to be a reliable, sensitive tool for quantifying ascorbic acid levels. Overall, while fresh juice is superior in nutritional value initially, marketed preparations offer better shelf-life stability. These findings highlight the trade-off between nutritional richness and product stability in juice formulations.

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**Table 1: Estimation of Vitamin C content by Iodometric Titration**

| Sample | Volume of Iodine Used (mL) | Calculated Vitamin C Content (mg/100mL) |
|---|---|---|
| Fresh Juice | 6.5 | 52.4 |
| Packed amla juice 1 | 7.1 | 38.2 |
| Packed amla juice 2 | 7.3 | 37.1 |
| Packed amla juice 3 | 7.1 | 36.9 |
| Packed amla juice 4 | 7.8 | 34.5 |
| Packed amla juice 5 | 7.9 | 33.8 |

**Table 2: Vitamin C estimation by UV-visible spectroscopy**

| Sample | Absorbance at 266nm | Calculated Vitamin C Content (mg/100mL) |
|---|---|---|
| **Fresh Juice** | 0.545 | 51.2 |
| **Packed amla juice 1** | 0.400 | 37.4 |
| **Packed amla juice 2** | 0.389 | 36.1 |
| **Packed amla juice 3** | 0.382 | 35.7 |
| **Packed amla juice 4** | 0.365 | 34.2 |
| **Packed amla juice 5** | 0.359 | 33.6 |

**Table 3: Accelerated stability study of fresh and different Amla juices**

| Sample | Day 0 (mg/100mL) | Day 7 (mg/100mL) | Day 14 (mg/100mL) | Day 21 (mg/100mL) | Day 28 (mg/100mL) |
|---|---|---|---|---|---|
| **Fresh Juice** | 51.2 | 48.1 | 45.3 | 42.5 | 39.2 |
| **Packed Amla Juice 1** | 37.4 | 36.2 | 34.1 | 32.3 | 30.7 |
| **Packed Juice 2** | 36.1 | 34.9 | 32.6 | 30.7 | 29.5 |
| **Packed Juice 3** | 35.7 | 34.3 | 32.2 | 30.1 | 28.0 |
| **Packed Juice 4** | 34.2 | 32.8 | 31.0 | 28.7 | 26.4 |
| **Packed Juice 5** | 33.6 | 32.1 | 30.4 | 28.1 | 25.8 |

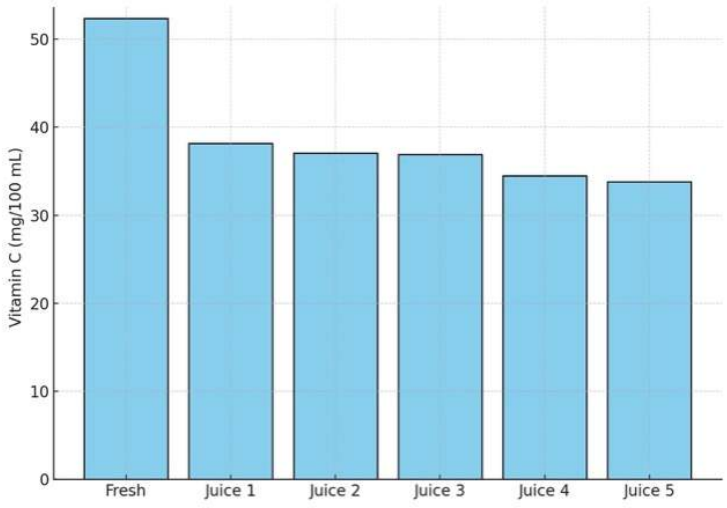



**Figure 1: Bar chart comparing the Vitamin C content in fresh and packaged juices, demonstrating that fresh juice contained significantly higher Vitamin C than all marketed samples.**

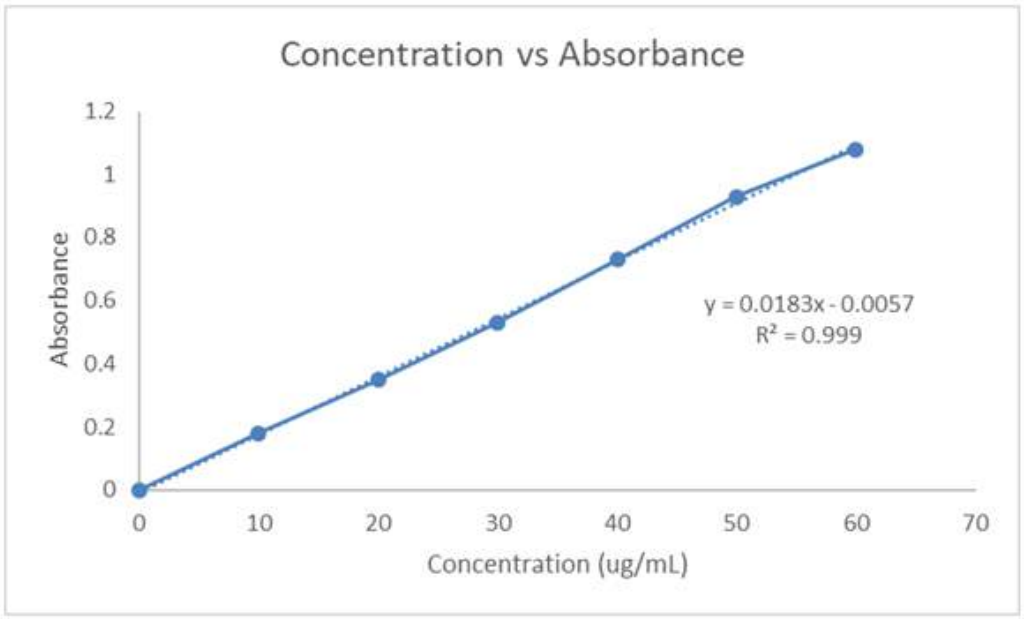



**Figure 2: Concentration vs Absorbance of standard ascorbic acid**

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RESEARCH ARTICLE

# Properties of a Sub Class of Weak Fuzzy Graphs

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**ABSTRACT**

The first definition of fuzzy graph was introduced by Kauffman in 1973 based on Zadeh's fuzzy relation. In that definition the vertex set was crisp but edges were given membership values between 0 and 1. Azriel Rosenfeld modified the definition in 1975 by changing the vertex set as a fuzzy set and bringing restriction on membership values on edges and corresponding nodes. In 1994 J.N. Mordeson introduced a sub class of fuzzy graph called strong fuzzy graph. In 2001, M.S. Sunitha introduced another subclass of fuzzy graph called complete fuzzy graph. In 2021, Nishad T M and A Prasanna introduced weak fuzzy graphs. The concept of fuzzy graph was generalized by Nishad T M and B Mohammed Harif in 2023 and developed General Fuzzy Graph. In this paper some examples and properties of weak fuzzy graphs and $\in$ weak fuzzy graphs are discussed.

**Keywords:** Fuzzy Relation, Fuzzy Set, Fuzzy Graph, Strong Fuzzy Graph, Complete Fuzzy Graph.
**2020 Mathematics Subject Classification:** 05C72

## INTRODUCTION

The father of graph theory was the great Swiss mathematician Leonhard Euler, who's famous 1736paper, "The Seven Bridges of Konigsberg," was the first treatise on the subject.In 1874, Georg Cantor introduced set theory. A graph is a mathematical structure to model pair wise relations between objects. In a network if there is vagueness in the description of the objects (nodes or vertices) or in its relationships (links or edges) or in both, a fuzzy graph is the

most suitable mathematical structure to describe the network. In 1973[2] Kaufmann introduced the first definition of a fuzzy graph(FG). In 1975[4] Azriel Rosenfeld considered fuzzy relation on fuzzy sets and developed the theory of FGs.The consideration of fuzzy relation on fuzzy sets brings a restriction on the membership values of the edges. The restriction is that the membership value of the edge cannot exceed the minimum of the membership values of the corresponding nodes. Mordeson J.N and Peng. C.S [11] introduced a subclass of FG called 'strong fuzzy graphs(SFG)' in 1994. In a SFG the positive membership values of the edges of a FGs is equal to the minimum of the membership values of corresponding vertices. In 2001, M S Sunitha introduced another subclass of FG called complete fuzzy graph(CFG). In this paper some properties of another subclass of FGs are discussed.

## PRELIMINARY DEFINITIONS

**Definition 2.1:** A graph(crisp graph) $F^* = (V_\lambda{}^*, E_\lambda{}^*)$consist of set of vertices$V_\lambda{}^*$( also called nodes) and a set of edges$E_\lambda{}^*$(also called lines).

**Definition 2.2:**If U is a universe discourse and any element u ∈ U, then fuzzy set A defined on U is a collection of ordered pair, $A = \{(u, \lambda(u): x \in A\}$, where $\lambda(u)$: U → [0,1], $\lambda(u)$ is called a membership function.

**Definition 2.3:** A fuzzy graph(FG)F = $(V_\lambda, E_\lambda)$is a strong fuzzy graph(SFG), if $E_\lambda(u,v) = V_\lambda(u) \wedge V_\lambda(v)$for all $(u,v) \in E_\lambda{}^*$.

**Definition 2.4:** A fuzzy graph(FG)F = $(V_\lambda, E_\lambda)$is a complete fuzzy graph(CFG), if $E_\lambda(u,v) = V_\lambda(u) \wedge V_\lambda(v)$for all $u, v \in V_\lambda{}^*$.

**Definition 2.5:** The μ−complement of a FGF = $(V_\lambda, E_\lambda)$is a FG $F^\mu = (V_\lambda{}^\mu, E_\lambda{}^\mu)$, where$V_\lambda{}^\mu = V_\lambda$ and $E_\lambda{}^\mu$ is defined as$E_\lambda{}^\mu(u,v) = 0$, if $E_\lambda(u,v) = 0$and $E_\lambda{}^\mu(u,v) = V_\lambda(u) \wedge V_\lambda(v) - E_\lambda(u,v)$, if $E_\lambda(u,v) > 0$for all $u, v \in V_\lambda{}^*$.

**Definition 2.5:** The complement of a FGF = $(V_\lambda, E_\lambda)$ modified by M S Sunitha and A Vijayakumar is a fuzzy graph $\bar{F} = (V_\lambda{}^c, E_\lambda{}^c)$where$V_\lambda{}^c = V_\lambda$and $E_\lambda{}^c$ is defined as$E_\lambda{}^c(u,v) = V_\lambda(u) \wedge V_\lambda(v) - E_\lambda(u,v)$ for all$u, v \in V_\lambda{}^*$.

**Example 2.1:** The following figures illustrate strong fuzzy graph and complete fuzzy graph.

**Figure 1:** SFG.
**Figure 2:** CFG

**A Sub Class –Weak Fuzzy Graphs**
**Definition 3.1:** A FGF = $(V_\lambda, E_\lambda)$is a weak fuzzy graph(WFG), if $E_\lambda(u,v) < V_\lambda(u) \wedge V_\lambda(v)$for all$(u,v) \in E_\lambda{}^*$.
This requirement ensures that the "flows" in the edges of the graph is always less than the "flows" in the respective nodes.

**Example 3.1:** The following figure illustrates a weak fuzzy graph.

**Figure 3:** WFG

**Remark 3.1.1**: The definition of WFG ensures that the "flow" or "capacity" of the links is always less than the minimum "flow" or "capacity" in the respective nodes. The set of FGs is considered as the union of mutually disjoint three subsets – (i) The set of SFGs (ii) The set of WFGs and(iii) The set of neither SFGs nor WFGs.

**Example 3.2:** A campus simultaneously runs 4 departments (say) Mathematics-M, Statistics-S, Computer Science-C and Artificial intelligence –A, sharing facility. The income from each department and expenditure of sharing facility

between each of the department can be simulated using a WFG if there is a constraint that the expenditure of facility sharing should be always less than the minimum of income from corresponding departments. In the following tables, the entries in the diagonal cells corresponding to MM,SS,CC and AA are the income in INR from the departments Mathematics, Statistics, Computer Science and Artificial Intelligence respectively and the other entries are the expenditure of sharing facilities between the corresponding departments in INR.

**Table: 1**
This data can be converted to numbers between 0 and 1 by dividing the values by 100000.

The following table gives the fuzzified values (**Table: 2).**

The following figure illustrates this information as a weak fuzzy graph.
**Figure 4:** F = $(V_\lambda, E_\lambda)$

**Some Properties of the Sub Class -Weak Fuzzy Graphs**
While WFG represents the simulation of traffic density on junctions and nodes in the traffic network, the following properties of WFG brings a picture on flows on the network.

**Theorem 4.1.** The union of two weak fuzzy graphs is a weak fuzzy graphs.

**Proof.** Let $F_1: (V_{\lambda_1}, E_{\lambda_1})$ and $F_2: (V_{\lambda_2}, E_{\lambda_2})$ be two WFGs with the underlying crisp graphs $F_1^*: ({V_{\lambda_1}}^*, {E_{\lambda_1}}^*)$ and $F_2^*: ({V_{\lambda_2}}^*, {E_{\lambda_2}}^*)$ respectively. The union F: $(V_{\lambda_1} \cup V_{\lambda_2}, E_{\lambda_1} \cup E_{\lambda_2})$ is defined by
$(V_{\lambda_1} \cup V_{\lambda_2})(u) = V_{\lambda_1}(u)$,if$u \in {V_{\lambda_1}}^* - {V_{\lambda_2}}^*$.
$(V_{\lambda_1} \cup V_{\lambda_2})(u) = V_{\lambda_2}(u)$,if$u \in {V_{\lambda_2}}^* - {V_{\lambda_1}}^*$and
$(V_{\lambda_1} \cup V_2)(u) = \max\{V_{\lambda_1}(u), V_{\lambda_2}(u)\}$, if $u \in {V_{\lambda_1}}^* \cap {V_{\lambda_2}}^*$.
$(E_{\lambda_1} \cup E_{\lambda_2})(u,v) = E_{\lambda_1}(u,v)$,if $(u,v) \in {E_{\lambda_1}}^* - {E_{\lambda_2}}^*$.
$(E_{\lambda_1} \cup E_{\lambda_2})(u,v) = E_{\lambda_2}(u,v)$,if $(u,v) \in {E_{\lambda_2}}^* - {E_{\lambda_1}}^*$and
$(E_{\lambda_1} \cup E_{\lambda_2})(u,v) = \max\{E_{\lambda_1}(u,v), E_{\lambda_2}(u,v)\}$,if$(u,v) \in {E_{\lambda_1}}^* \cap {E_{\lambda_2}}^*$.
To prove that F: $(V_{\lambda_1} \cup V_{\lambda_2}, E_{\lambda_1} \cup E_{\lambda_2})$ is a WFG, it is enough to prove that
$(E_{\lambda_1} \cup E_{\lambda_2})(u,v) < ((V_{\lambda_1} \cup V_{\lambda_2})(u) \wedge (V_{\lambda_1} \cup V_{\lambda_2})(v))$, for all $(u,v) \in (E_{\lambda_1} \cup E_{\lambda_2})^*$

**Case 1:** When $(u,v) \in {E_{\lambda_1}}^* - {E_{\lambda_2}}^*$
$(E_{\lambda_1} \cup E_{\lambda_2})(u,v) = E_{\lambda_1}(u,v) < V_{\lambda_1}(u) \wedge V_{\lambda_1}(v)$. Since $F_1: (V_{\lambda_1}, E_{\lambda_1})$ is a WFG
$(E_{\lambda_1} \cup E_{\lambda_2})(u,v) = E_{\lambda_1}(u,v) < V_{\lambda_1}(u) \wedge V_{\lambda_1}(v) < ((V_{\lambda_1} \cup V_{\lambda_2})(u) \wedge (V_{\lambda_1} \cup V_{\lambda_2})(v))$.
Since $V_{\lambda_2}(u) = 0$, for all $u \in {V_{\lambda_1}}^* - {V_{\lambda_2}}^*$.

**Case 2:** When$(u,v) \in {E_{\lambda_2}}^* - {E_{\lambda_1}}^*$
$(E_{\lambda_1} \cup E_{\lambda_2})(u,v) = E_{\lambda_2}(u,v) < V_{\lambda_2}(u) \wedge V_{\lambda_2}(v)$.Since $F_2: (V_{\lambda_2}, E_{\lambda_2})$ is a WFG
$(E_{\lambda_1} \cup E_{\lambda_2})(u,v) = E_{\lambda_2}(u,v) < V_{\lambda_2}(u) \wedge V_{\lambda_2}(v) < ((V_{\lambda_1} \cup V_{\lambda_2})(u) \wedge (V_{\lambda_1} \cup V_{\lambda_2})(v))$.
Since $V_{\lambda_1}(u) = 0$ for all $u \in {V_{\lambda_2}}^* - {V_{\lambda_1}}^*$.

**Case 3:** When$(u,v) \in {E_{\lambda_1}}^* \cap {E_{\lambda_2}}^*$
Since $F_1: (V_{\lambda_1}, E_{\lambda_1})$ and $F_2: (V_{\lambda_2}, E_{\lambda_2})$are WFGs, $E_{\lambda_1}(u,v) < V_{\lambda_1}(u) \wedge V_{\lambda_1}(v)$and $V_{\lambda_2}(u,v) < V_{\lambda_2}(u) \wedge V_{\lambda_2}(v)$ .
Hence$\max\{E_{\lambda_1}(u,v), E_{\lambda_2}(u,v)\} < \max\{V_{\lambda_1}(u) \wedge V_{\lambda_1}(v), V_{\lambda_2}(u) \wedge V_{\lambda_2}(v)\}$
Therefore $(E_{\lambda_1} \cup E_{\lambda_2})(u,v) = \max\{E_{\lambda_1}(u,v), E_{\lambda_2}(u,v)\}$
$< \max\{V_{\lambda_1}(u) \wedge V_{\lambda_1}(v), V_{\lambda_2}(u) \wedge V_{\lambda_2}(v)\}$
$< (\max\{V_{\lambda_1}(u), V_{\lambda_2}(u)\} \wedge \max\{V_{\lambda_1}(v), V_{\lambda_2}(v)\})$
In all cases $(E_{\lambda_1} \cup E_{\lambda_2})(u,v) < ((V_{\lambda_1} \cup V_{\lambda_2})(u) \wedge (V_{\lambda_1} \cup V_{\lambda_2})(v))$for all$(u,v) \in (E_{\lambda_1} \cup E_{\lambda_2})^*$.

**Example 4.1:** The following figures illustrate the theorem 2.3.1
**Figure 5:** WFG $F_1$.
**Figure 6:** WFG$F_2$
**Figure 7:** WFGF = $F_1$ U $F_2$

**Remark 4.1**: The above theorem implies that the set of WFGs is closed under union. So a large traffic network in which the traffic density at the junctions and roads are simulated by a WFG can be formed as union of several traffic networks having the property that the traffic density of all are simulated using WFGs.

**Theorem 4.2:** The spanning sub-graph of a weak fuzzy graph is a weak fuzzy graph.

**Proof.** Let FG H:$(W_\lambda, L_\lambda)$be a spanning sub-graph of a WFGF: $(V_\lambda, E_\lambda)$.
By the definition of spanning FSG$W_\lambda(u) = V_\lambda(u)$for all $u \in V_\lambda{}^*$and
$L_\lambda(u,v) \leq E_\lambda(u,v)$for all$u, v \in V_\lambda{}^*$.
To prove that$L_\lambda(u,v) < W_\lambda(u) \wedge W_\lambda(v)$,for all$(u,v) \in L_\lambda{}^*$.
Since F: $(V_\lambda, E_\lambda)$ is a WFG$E_\lambda(u,v) < V_\lambda(u) \wedge V_\lambda(v)$for all$(u,v) \in E_\lambda{}^*$.
Now$L_\lambda(u,v) \leq E_\lambda(u,v) < V_\lambda(u) \wedge V_\lambda(v) = W_\lambda(u) \wedge W_\lambda(v)$for all$(u,v) \in E_\lambda{}^*$.
Therefore $L_\lambda(u,v) < W_\lambda(u) \wedge W_\lambda(v)$, for all$(u,v) \in L_\lambda{}^*$.

**Theorem 4.3:** A weak fuzzy sub-graph can be formed from every strong fuzzy graph$H$: $(V_\lambda, L_\lambda)$.

**Proof.** Let $H$: $(V_\lambda, L_\lambda)$be a SFG. Control the flow through the links such that$E_\lambda(u,v) = L_\lambda(u,v) - \varepsilon$, for every $L_\lambda(u,v) > 0$,where $\varepsilon$ is fixed real number such that
$\varepsilon \in (0, min\{L_\lambda(u,v) / L_\lambda(u,v) > 0\})$.Let F:$(V_\lambda, E_\lambda)$ be the resulting FSG. In F:$(V_\lambda, E_\lambda)$, $E_\lambda(u,v) < V_\lambda(u) \wedge V_\lambda(v)$, for all $(u,v) \in E_\lambda{}^*$.Now F:$(V_\lambda, E_\lambda)$is a WFSG of $H$: $(V_\lambda, L_\lambda)$.

**Remark 4.2**: The above theorem helps to convert a traffic network (that allowed possible maximum flows in the links) to a flow controlled traffic network by adjusting traffic density$\varepsilon$.

**Theorem 4.4:** The complement on a weak fuzzy graph is a weak fuzzy graphiff underlying crisp graph is complete graph.

**Proof.** Suppose the complement of a WFG F:$(V_\lambda, E)$ is a WFG.
We have to show that $E_\lambda(u,v) > 0$ for all u,$v$in $V^*$.
Since the complement of the WFG is weak, $\overline{E(u,v)} < V_\lambda(u) \wedge V_\lambda(v)$ for all u,$v$ in$V_\lambda{}^*$.
Therefore $E_\lambda(u,v) = \overline{(E_\lambda(u,v))} = V_\lambda(u) \wedge V_\lambda(v) - \overline{E_\lambda(u,v)} > 0$, for all $u,v$ in$V_\lambda{}^*$.
If every pair of nodes are not neighbours (i.e, if the underlying crisp graph is a complete graph), there will be some links with$E_\lambda(u,v) = 0$, $V_\lambda(u) = \varepsilon, V_\lambda(v) = \delta$ where $\varepsilon, \delta \in (0,1]$.Now as per the definition of complement $\overline{E_\lambda(u,v)}$= min $\{\varepsilon, \delta\} - 0$ = min $\{\varepsilon, \delta\}$which is either $V_\lambda(u)$or $V_\lambda(v)$.Hence the complement fails to become a WFG.

**Example 4.2:** The following figures illustrate the theorem 4.4

**Case 1:** Underlying crisp graph is complete graph.

**Figure 8:** F.
**Figure 9:** $\bar{F}$

**Case 2:** Underlying crisp graph is not a complete graph.

**Figure 10:** F.
**Figure 11:** $\bar{F}$

**Theorem 4.5.** If underlying crisp graph is not a complete graph, then the complement of a weak fuzzy graph is either a strong fuzzy graph or a fuzzy graph which is neither strong nor weak.

**Proof.** Suppose underlying crisp graph is not a complete graph.
Then there exists three types of links.

**Case 1:** Absent links. i.e, links are such that $(u, v) = 0$ .
In this case $\overline{E_\lambda(u,v)} = \min\{\varepsilon, \delta\} - 0 = \min\{\varepsilon, \delta\}$ which is either $V_\lambda(u)$ or $V_\lambda(v)$ for all $u, v \text{in} V_\lambda^*$. In this case the complement is a SFG.

**Case 2:** Some absent and some active links. i.e, the links are such that
2.1 $E_\lambda(u, v) = 0$ for some $u, v \text{in} V_\lambda^*$ and 2.2 $E_\lambda(u, v) > 0$ for some $u, v \text{in} V_\lambda^*$
In the case 2.1, By the definition of complement, $\overline{E_\lambda(u,v)} = V_\lambda(u) \wedge V_\lambda(v)$ In the case 2.2, Let $E_\lambda(u, v) = \varepsilon$, where $\varepsilon \in (0, V_\lambda(u) \wedge V_\lambda(v))$.
$\overline{E_\lambda(u,v)} = V_\lambda(u) \wedge V_\lambda(v) - \varepsilon < V_\lambda(u) \wedge V_\lambda(v)$. In Case 2, flow allowed in various links shows that the complement is neither a SFG nor a WFG.

**Example 4.3:** The following figures illustrate the theorem 4.5.

**Case 1:** All edges are such that $E_\lambda(u, v) = 0$ .

**Figure 12:** F.
**Figure 13:** $\bar{F}$

**Case 2 :** Edges are such that
2.1 $E_\lambda(u, v) = 0$ for some $u, v \text{in} V_\lambda^*$ and 2.2 $E_\lambda(u, v) > 0$ for some $u, v \text{in} V_\lambda^*$

**Figure 14:** F.
**Figure 15:** $\bar{F}$

**Theorem 4.6.** The complement of a complete fuzzy graph is a weak fuzzy graph the nodes of which are isolated.

**Proof.** If the FG is complete then $E_\lambda(u, v) = V_\lambda(u) \wedge V_\lambda(v)$ for all $u, v \text{in} V_\lambda^*$, then
$\overline{E_\lambda(u,v)} = V_\lambda(u) \wedge V_\lambda(v) - E_\lambda(u, v) = V_\lambda(u) \wedge V_\lambda(v) - V_\lambda(u) \wedge V_\lambda(v) = 0$ for all $u, v \text{in} V_\lambda^*$.

**Example 4.4:** The following figures illustrate the theorem 4.6

**Figure 16:** F.
**Figure 17:** $\bar{F}$

**Theorem 4.7.** The fuzzy graph $F: (V_\lambda, E_\lambda)$ such that $E_\lambda(u, v) = \frac{1}{2}(V_\lambda(u) \wedge V_\lambda(v))$ for all for all $u, v \text{in} V_\lambda^*$ is a weak self complementary fuzzy graph.

**Proof.** If $F: (V_\lambda, E_\lambda)$ is such that $E_\lambda(u, v) = \frac{1}{2}(V_\lambda(u) \wedge V_\lambda(v))$ for all for all $u, v \text{in} V_\lambda^*$ then
$E_\lambda(u, v) = \frac{1}{2}(V_\lambda(u) \wedge V_\lambda(v)) < V_\lambda(u) \wedge V_\lambda(v)$ for all $u, v \text{in} V_\lambda^*$. So F is a WFG.
Now $\overline{E_\lambda(u,v)} = V_\lambda(u) \wedge V_\lambda(v) - \frac{1}{2}(V_\lambda(u) \wedge V_\lambda(v)) = \frac{1}{2}(V_\lambda(u) \wedge V_\lambda(v)) = E_\lambda(u, v)$.

Therefore F is self complementary FG.

**Example 4.5:** The following figures illustrate the theorem 4.7.

**Figure 18:** F.
**Figure 19:** $\bar{F}$

**Theorem 4.8.** The $\mu$ −complement of a weak fuzzy graph is a weak fuzzy graph.

**Proof.** Let $F^{\mu}$: $(V_{\lambda}, {E_{\lambda}}^{\mu})$be the $\mu$ −complement of a WFG F: $(V_{\lambda}, E_{\lambda})$
where${E_{\lambda}}^{\mu}$ is defined as.
${E_{\lambda}}^{\mu}(u,v) = V_{\lambda}(u) \wedge V_{\lambda}(v) - E_{\lambda}(u,v)$if$E_{\lambda}(u,v) > 0$ for all $u, v \in {V_{\lambda}}^{*} \rightarrow (1)$
and${E_{\lambda}}^{\mu}(u,v) = 0$ if $E_{\lambda}(u,v) = 0 \rightarrow (2)$
Since F:$(V_{\lambda}, E_{\lambda})$is a WFG$E_{\lambda}(u,v) < V_{\lambda}(u) \wedge V_{\lambda}(v)$for all $(u,v) \in {E_{\lambda}}^{*} \rightarrow (3)$
To prove that $F^{\mu}$: $(V_{\lambda}, {E_{\lambda}}^{\mu})$is a WFG, it is enough to prove
${E_{\lambda}}^{\mu}(u,v) < V_{\lambda}(u) \wedge V_{\lambda}(v)$for all $(u,v) \in {E_{\lambda}}^{\mu^{*}}$
Case 1. Let $(u,v) \in {E_{\lambda}}^{\mu^{*}}$,such that $E_{\lambda}(u,v) > 0$ .Then by the equations (1) and(3)
${E_{\lambda}}^{\mu}(u,v) = V_{\lambda}(u) \wedge V_{\lambda}(v) - E_{\lambda}(u,v) < V_{\lambda}(u) \wedge V_{\lambda}(v)$
Case 2. Let $(u,v) \in {E_{\lambda}}^{\mu^{*}}$,such that $E_{\lambda}(u,v) = 0$ .Then by the equation (2),
${E_{\lambda}}^{\mu}(u,v) = 0$
Therefore in both cases${E_{\lambda}}^{\mu}(u,v) < V_{\lambda}(u) \wedge V_{\lambda}(v)$for all $(u,v) \in {E_{\lambda}}^{\mu^{*}}$.

**Example 4.6:** The following figures illustrate the theorem 4.8

**Figure 20:** F.
**Figure 21:** $\bar{F}$.

**Remark 4.3.**The above theorem implies that the set of WFGs is closed under $\mu$ −complementation.

**Theorem 4.9.** If $F$ is a weak fuzzy graph then $(F^{\mu})^{\mu} = F$ .

**Proof.** Let $F^{\mu}$: $(V_{\lambda}, {E_{\lambda}}^{\mu})$ be the $\mu$ −complement of $F$:$(V_{\lambda}, E_{\lambda})$.
Since $F$:$(V_{\lambda}, E_{\lambda})$is a WFG,$E_{\lambda}(u,v) < V_{\lambda}(u) \wedge V_{\lambda}(v)$ for all $(u,v) \in {E_{\lambda}}^{*}$.
We shall consider two cases .

**Case 1.** For all $(u,v) \in {E_{\lambda}}^{*}$.
As per definition of${E_{\lambda}}^{*}, E_{\lambda}(u,v) > 0$ .
In this case ${E_{\lambda}}^{\mu}(u,v) < V_{\lambda}(u) \wedge V_{\lambda}(v) - E_{\lambda}(u,v)$ .
$({E_{\lambda}}^{\mu})^{\mu}(u,v) = V_{\lambda}(u) \wedge V_{\lambda}(v) - {E_{\lambda}}^{\mu}(u,v) = V_{\lambda}(u) \wedge V_{\lambda}(v) - (V_{\lambda}(u) \wedge V_{\lambda}(v) - E_{\lambda}(u,v)) = E_{\lambda}(u,v)$

**Case 2.** For all $(u,v) \notin {E_{\lambda}}^{*}$ . As per definition of ${E_{\lambda}}^{*}, E_{\lambda}(u,v) = 0$.
Here ${E_{\lambda}}^{\mu}(u,v) = 0$ and $({E_{\lambda}}^{\mu})^{\mu}(u,v) = 0$.

**Example 4.7:** The following figures illustrate the theorem 2.3.9

**Figure 22:** F.
**Figure 23:** $F^{\mu}$.
**Figure 24:**$(F^{\mu})^{\mu}$

**Remark 4.4.**The above theorem implies that the set of WFGs hold the law of double complementation with respect to $\mu$ −complement.

**Theorem 4.10.** The $\mu$ −complement on a complete fuzzy graph is a weak fuzzy graph having isolated nodes.

**Proof.** Let $F^{\mu}$: $(V_\lambda, E_\lambda{}^{\mu})$ be the $\mu-$ complement of SFG$F$:$(V_\lambda, E_\lambda)$. Since $F$:$(V_\lambda, E_\lambda)$is a SFG,$E_\lambda(u,v) = V_\lambda(u) \wedge V_\lambda(v)$ for all $(u,v) \in E_\lambda{}^{*}$.
Then for all $(u,v) \in E_\lambda{}^{*}$,
$E_\lambda{}^{\mu}(u,v) = V_\lambda(u) \wedge V_\lambda(v) - E_\lambda(u,v) = V_\lambda(u) \wedge V_\lambda(v) - V_\lambda(u) \wedge V_\lambda(v) = 0$. For all $(u,v) \notin E_\lambda{}^{*}$, $r(u,v) = 0$. Here $E_\lambda{}^{\mu}(u,v) = 0$ and $(E_\lambda{}^{\mu})^{\mu}(u,v) = 0$. This implies that in the resulting FG all the links are absent and so it is a WFG having isolated nodes.

**Example 4.8:** The following figures illustrate the theorem 4.10

**Figure 25:** F.
**Figure 26:** $F^{\mu}$

**Definition: 4.1:** The spanned FSG$F = (V_\lambda, E_\lambda)$ of a SFG $H = (V_\lambda, L_\lambda)$ is called an $\varepsilon-$ weak fuzzy sub-graph($\varepsilon-$ WFSG) of $H = (V_\lambda, L_\lambda)$, if$E_\lambda(u,v) = L_\lambda(u,v) - \varepsilon$, for every $L_\lambda(u,v) > 0$ where $\varepsilon$ is fixed such that $\varepsilon \in (0, min\{L_\lambda(u,v) / L_\lambda(u,v) > 0\})$.
In view of this definition, an $\varepsilon-$ WFSG F $= (V_\lambda, E_\lambda)$can be considered as a WFG that can become a SFG by adding a fixed $\varepsilon > 0$ to all edges $(u,v) \in E_\lambda{}^{*}$ such that$E_\lambda(u,v) > 0$.

**Example 4.9:** The following figures illustrate the definition 4.1 where $\varepsilon$ = 0.1∈ (0, 0.4)

**Figure 27:** H**.**
**Figure 28:** F

**Theorem 4.11:** Every strong fuzzy graph$H = (V_\lambda, L_\lambda)$ has a weak fuzzy sub-graph.

**Proof:** The $\varepsilon-$ WFSG$F = (V_\lambda, E_\lambda)$of $H = (V_\lambda, L_\lambda)$ such that $E_\lambda(u,v) = L_\lambda(u,v) - \varepsilon$, for every $L_\lambda(u,v) > 0$ where $\varepsilon$ is fixed such that $\varepsilon \in (0, min\{L_\lambda(u,v) / L_\lambda(u,v) > 0\})$ is a weak subgraph of $H = (V_\lambda, L_\lambda)$ .

**Example 4.10:** Check whether weak fuzzy graph in the following figure is an $\varepsilon-$ weak fuzzy sub $-$ graph?

**Figure 29:** $H = (m, s)$

**Answer:** The given graph F is not an $\varepsilon-$ WFSG.
Reason: In the graph $min\{L_\lambda(u,v)/L_\lambda(u,v) > 0\} = 0.1$.
Corresponding to that edge, $V_\lambda(x) \wedge V_\lambda(v) = 0.5$.
The fixed $\varepsilon$ = 0.5 - 0.1 = 0.4.
If 0.4 is added to each edge, then the graph fail to become a FG.

**Example 4.11:** Draw examples of $\varepsilon-$ weak fuzzy sub $-$ graph and corresponding strong fuzzy graphs for $\varepsilon = 0.3$ and 0.2 with 4 vertices
For $\varepsilon = 0.3$
**Figure 30:** E.
**Figure 31:** F

For $\varepsilon = 0.2$

**Figure 32:** G.
**Figure 33:** H

**Example 4.10:** A campus simultaneously runs 4 departments (say) Mathematics-M, Statistics-S, Computer Science-C and Artificial intelligence –A, sharing facility. The income from each department and expenditure of sharing facility between each of the department can be simulated using an $\varepsilon -$ WFSG if there is an objective to cut the expenditure of facility sharing to save some amount from the minimum of income from corresponding departments. In the following tables, the entries in the diagonal cells corresponding to MM,SS,CC and AA are the income in INR from the departments Mathematics, Statistics, Computer Science and Artificial Intelligence respectively and the other entries are the expenditure of sharing facilities between the corresponding departments in INR.

**Table 3:** This data can be converted to numbers between 0 and 1 by dividing the values by 100000.
The following table gives the fuzzified values (Table: 4).
The following figure illustrates this information as an $\varepsilon -$ weak fuzzy sub $-$ graph where $\varepsilon = 0.02$
**Figure 34:**$F = (V, E)$

## CONCLUSION

In this article, the properties of a sub class of fuzzy graphs (FGs)and weak fuzzy graphs (WFGs) are discussed with suitable examples.

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**Table 1: This data can be converted to numbers between 0 and 1 by dividing the values by 100000.**

| Departments | M | S | C | A |
|---|---|---|---|---|
| M | 20000 | 8000 | 15000 | 18000 |
| S | 8000 | 10000 | 5000 | 9000 |
| C | 15000 | 5000 | 30000 | 25000 |
| A | 18000 | 9000 | 25000 | 40000 |

**Table 2: The following table gives the fuzzified values.**

| Departments | M | S | C | A |
|---|---|---|---|---|
| M | 0.2 | 0.08 | 0.15 | 0.18 |
| S | 0.08 | 0.1 | 0.05 | 0.09 |
| C | 0.15 | 0.05 | 0.3 | 0.25 |
| A | 0.18 | 0.09 | 0.25 | 0.4 |

**Table 3: This data can be converted to numbers between 0 and 1 by dividing the values by 100000.The following table gives the fuzzified values.**

| Departments | M | S | C | A |
|---|---|---|---|---|
| M | 20000 | 8000 | 18000 | 18000 |
| S | 8000 | 10000 | 8000 | 8000 |
| C | 18000 | 8000 | 30000 | 28000 |
| A | 18000 | 8000 | 28000 | 40000 |

**Table 4: The following table gives the fuzzified values**

| Departments | M | S | C | A |
|---|---|---|---|---|
| M | 0.2 | 0.08 | 0.18 | 0.18 |
| S | 0.08 | 0.1 | 0.08 | 0.08 |
| C | 0.18 | 0.08 | 0.3 | 0.28 |
| A | 0.18 | 0.08 | 0.28 | 0.4 |

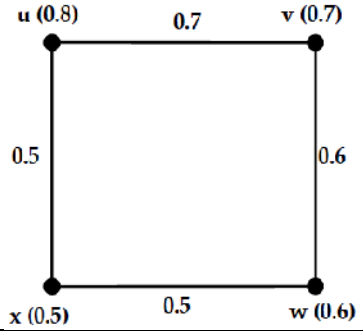



**Figure 1: SFG**

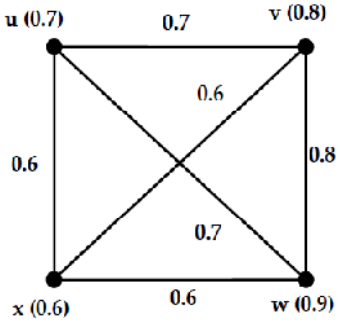



**Figure 2: CFG**

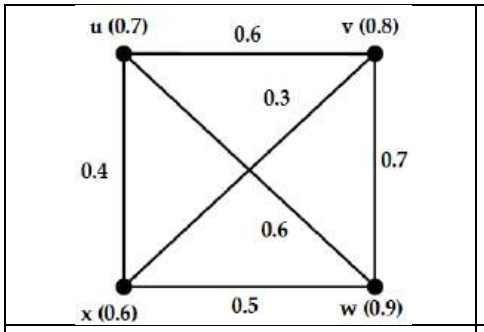



**Figure 3: WFG**

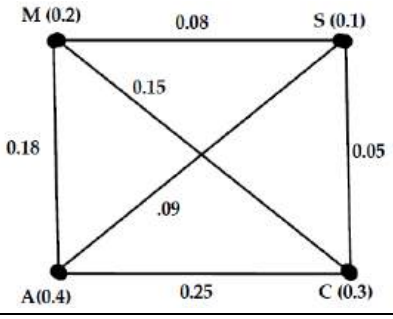



**Figure 4:** F = $(V_\lambda, E_\lambda)$

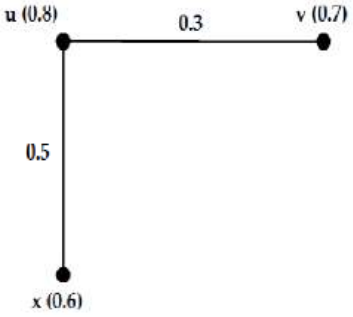



**Figure 5: WFG F1Figure 4.1 (b): WFGF2**

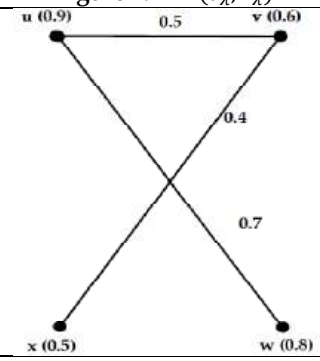



**Figure 6: WFGF2**

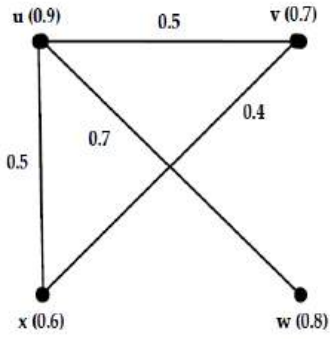



**Figure 7: WFGF = F1 U F2**

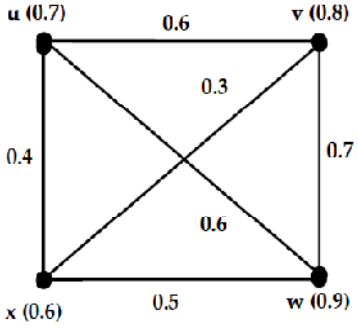



**Figure 8: F**

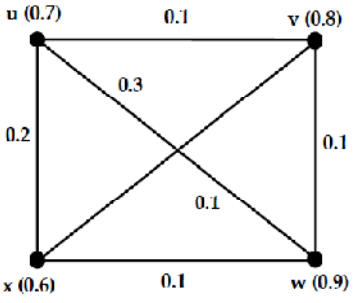



**Figure 9: $\overline{F}$**

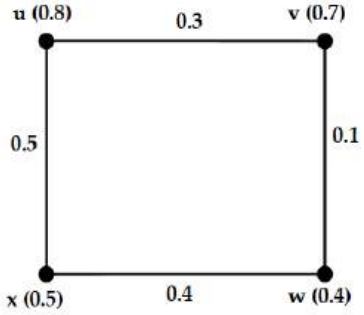



**Figure 10: F**

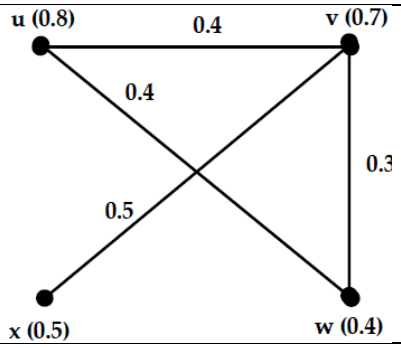



**Figure 11: $\overline{F}$**

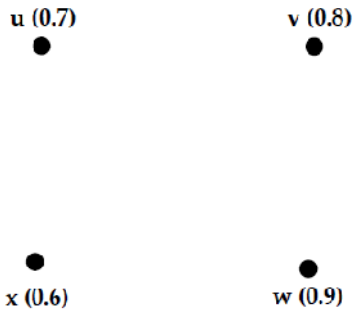



**Figure 12: F**

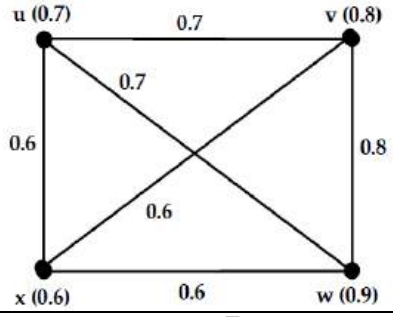



**Figure 13: $\overline{F}$**

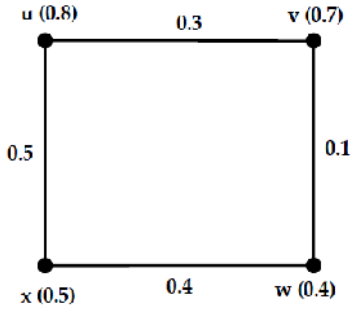



**Figure 14: F**

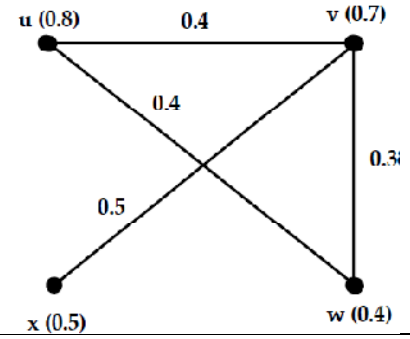



**Figure 15: $\overline{F}$**

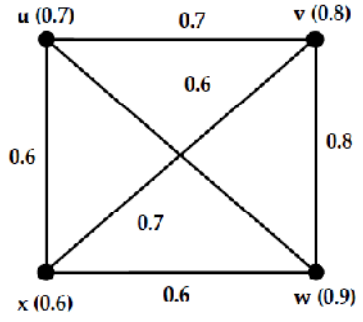



**Figure 16: F**

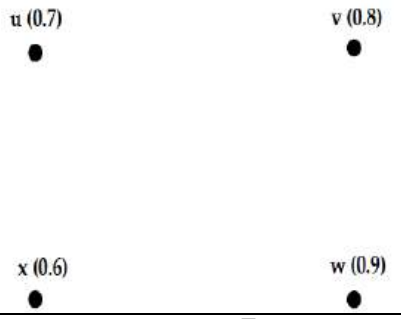



**Figure 17: $\overline{F}$**

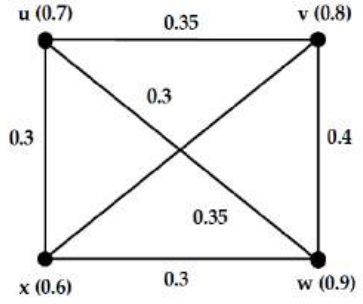



**Figure 18: F**

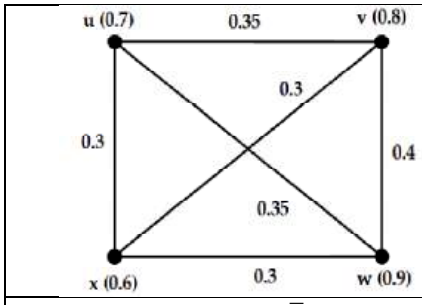



**Figure 19: $\overline{F}$**

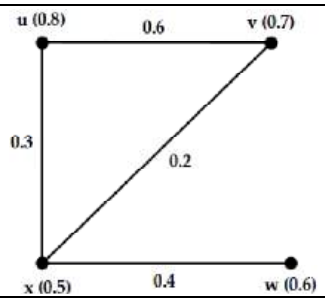



**Figure 20: F**

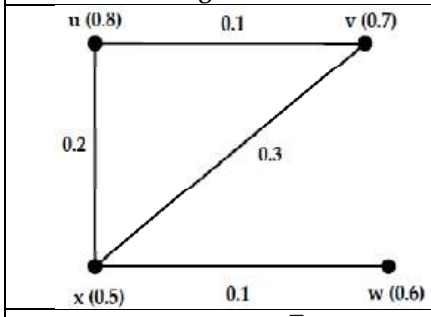



**Figure 21: $\overline{F}$**

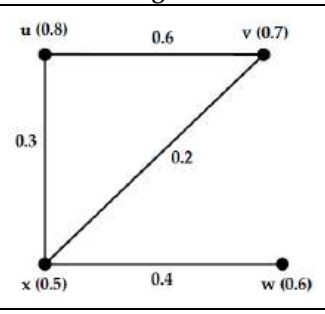



**Figure 22: F**

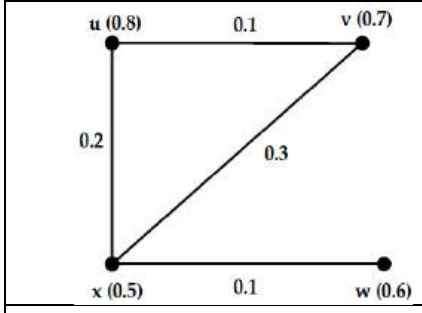



**Figure 23: $F^{\mu}$**

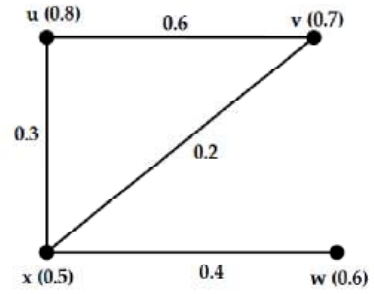



**Figure 24: $(F^{\mu})^{\mu}$**

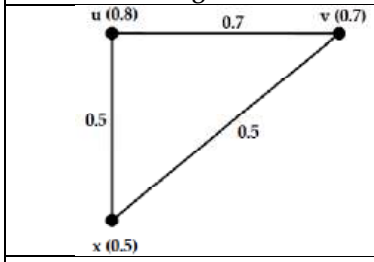



**Figure 25: F**

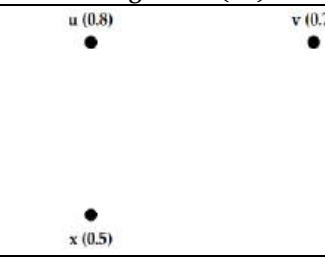



**Figure 26: $F^{\mu}$**

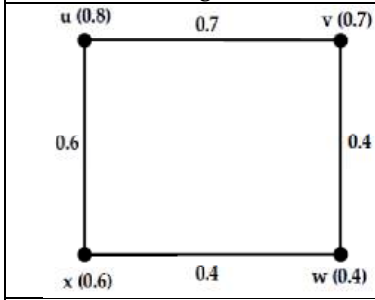



**Figure 27: H**

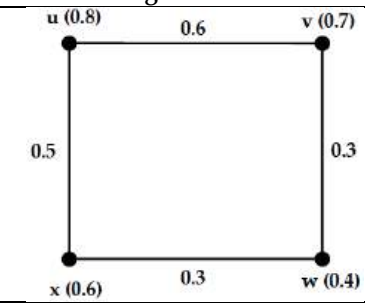



**Figure 28: F**

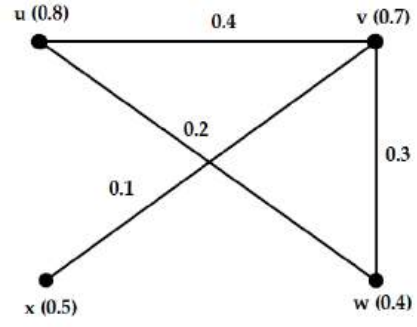



**Figure 29: $H = (m, s)$**

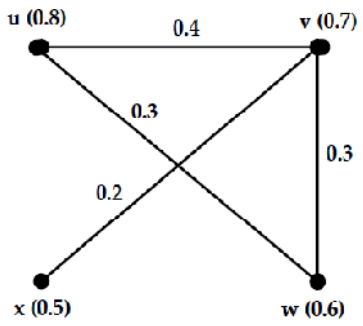



**Figure 30: E**

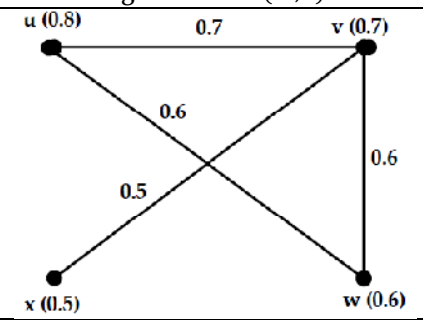



**Figure 31: F**

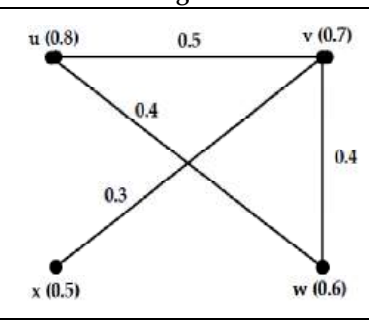



**Figure 32: G**

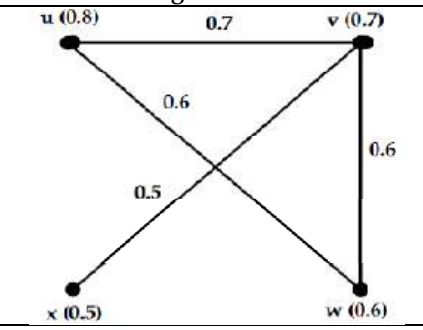



**Figure 33: H**

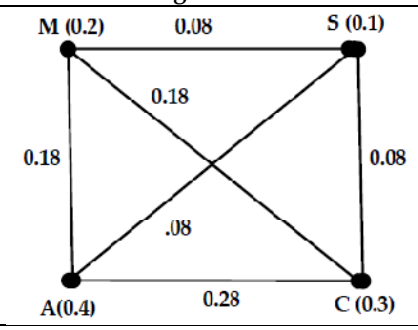



**Figure 34: $F = (V, E)$**

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RESEARCH ARTICLE

# Child Development in the Context of Dysfuntional Families: A Review of Risk and Protective Factors

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**ABSTRACT**

Children brought up in dysfunctional family environments such as chronic conflict, substance abuse, neglect or emotionally distant, are highly susceptible to having developmental issues. However, many children strive with resilience, despite these risks. Contemporary research, hence, highlights the importance of such protective factors- emotional intelligence, social support, community support, etc. in mitigating those adverse outcomes arising from such experiences. This review analyzes the impact that dysfunctional family environments have on children's cognitive, social, emotional and behavioural development, as well as the role of protective factors as a buffer against those outcomes.

**Keywords:** dysfunctional families, child development, protective factors, resilience, cognitive outcomes, biological outcomes, socio-emotional outcomes, behavioural outcomes.

## INTRODUCTION

The family environment is globally considered as the primary context in which children grow and develop. It influences their cognitive, social and emotional trajectories (Bronfenbrenner, 1979). Children raised and brought up in environments where their emotional needs are acknowledged, often develop emotional security and the confidence to express themselves, which ultimately fosters a healthy interpersonal as well as intrapersonal functioning (Morris et al. 2007). Positive family relationships promote psychological resilience, social competence, and adaptive personality traits such as self-compassion and a strong sense of coherence (Grevenstein, 2019). However, the family environment can be dysfunctional, for a certain number of children. Researchers have estimated that 60% of individuals, globally, have experienced at least one Adverse Childhood Experience (ACE) (Madigan et

al., 2023). Behere et al., (2017) reported, in a retrospective review of 154 preadolescents admitted at a behavioural health facility, that 2/3rd of the children had a history of trauma, with 36% cases reporting physical abuse, and 71% had either a parent or a sibling diagnosed with some psychiatric disorder. Hillis, S. et al., (2016) estimated that 1 billion children aged 2-17 years old experience violence each year. When children are exposed to negative situations including abuse, domestic violence, neglect, etc., there is a greater risk of having cognitive impairments (inattentiveness, emotional dysregulation, etc.) (Petersen, 2014). Such adversities can also cause problems in academics, emotion regulation, and mental health issues (Dubowitz, 2019; Hughes, et al. 2017; McLaughlin et al., 2020). These risks can become so severe that it might necessitate clinical interventions and even hospitalization.

Exposure to stress triggers the activation of the Hypothalamic Pituitary Adrenal (HPA) Axis. The process starts with the releasing of Corticotropin Releasing Factor (CRF) by the hypothalamus, which causes the pituitary gland to release the AdrenoCorticotropic Hormone (ACTH) into the Adrenal Cortex, which in turn releases glucocorticoids such as cortisol (Sheng, 2021). While short-term release of cortisol is necessary as it activates the fight-or-flight response, its long-term release due to chronic exposure to stress can be harmful as it suppresses the immune system and ultimately disrupt the physiological homeostasis (Gleitman et al., 2004; LeDoux, 2003). Chronic stress or trauma exposure maintains the brain in a state of heightened arousal, which contributes to long-term structural and functional changes in the key neural circuits (Perry, 2002; Teicher et al., 2002).

**Conceptual Framework**

Focusing on the risk and protective factors, this framework integrates four fundamental theories to explain how dysfunctional family settings affect children's development.

*Murray Bowen's* Family Systems Theory (1978), perceives the family as an emotionally interdependent unit, in which members affect each other's behavior, emotions, and functioning, hence forming a "reciprocal relationship". Emotional interdependence is crucial to survival, yet it can be problematic when subjected to stress exposure. Those individuals who are accommodating of others' stress tend to absorb the family tension, placing them at risk for physical and psychological illness. Hence, a dysfunctional family relationship can negatively impact an individual's development and health.

*Aaron Antonovsky's* Salutogenesis Model (1979), has a stronger focus on the circumstances that enhance resistance and health rather than pathology-oriented models. Salutogenesis is based on a core concept of the Sense of Coherence (SOC) formed by *manageability, meaningfulness, and comprehensibility* as it outlines the way in which individuals handle tension and maintain wellbeing. With the availability of resistant resources such as education and social support, a healthy SOC can protect children against the adversities of a dysfunctional family environment, hence explaining how some children thrive despite harsh conditions (Mittelmark & Bauer, 2017).

*Urie Bronfenbrenner* proposed the Ecological Systems Theory in 1979, emphasizing how different nested environmental systems- the microsystem (school, family, peers), mesosystem (interactions among microsystems), exosystem (indirect surroundings, e.g., the workplace of parents), macrosystem (legislation, cultural values), and chronosystem (temporal changes, e.g., parental divorce), influence a child's growth and development. Serious threats to development come from family environments that are dysfunctional, characterized by neglect, abuse, or ineffective communication. Other mechanisms, including peer or teachers support, can reduce or enhance these negative impacts. Therefore, unless balanced by protective factors in other environmental settings, a dysfunctional family can compromise social competence, academic achievement, and emotional security.

Based on the Risk and Resilience Model, which was formulated by *Norman Garmezy* and *Ann Masten* during the 1980s and 1990s, children have a higher tendency to develop behavioural issues and psychopathology when they are exposed to risk variables such as parental conflict, abuse, or neglect. But outcomes vary based on protective factors such as self-efficacy, structured routines, supportive adult relationships, and emotional regulation. As stipulated in

the theory, resilience can counteract these risks, hence not all children from dysfunctional families experience adverse outcomes

**Understanding Dysfunctional Families: Definitions, Typologies and Risk Factors**

Family functioning is generally measured in several domains, such as problem-solving, communication, roles, affective responsiveness, affective involvement, behavior control, and general functioning, as measured by the McMaster Family Assessment Device (FAD) (Zagefka, 2021). When one or more of these domains are compromised, the family environment can fail to promote the healthy development of its members. In psychological literature, a dysfunctional family is characterized by abuse, domestic violence, parental mental illness, substance abuse, emotional issues, or neglect (Arora and Prakash, 2018; Nittle, 2025). Children raised in such environmental contexts tend to internalize such maladaptive behaviour due to their prolonged exposure and absence of mitigating factors, and developing compromised executive functioning, behavioural and impaired socio-emotional outcomes (Petersen et al. 2014). Hence, identifying these typologies is significant to comprehend the mechanisms through which early relational experiences lead to long-term emotional and behavioural problems in children.

**Amen, D. G., (2020)**, described a number of dysfunctional family **typologies**, based on underlying issues and dominant behavioral patterns:

***Substance Abuse Family-*** Neglect, inconsistency, and emotional unavailability are typical among children in such family environments, which can lead to the development of trust issues and emotional instability in them.

***Emotionally Detached Families*** do not give emotional support or warmth to their children. These children can develop problems with emotional expressiveness and self-esteem along with feelings of neglect.

***Conflict Driven Family-*** These types of families promote a hostile environment and are dominated by recurrent and intense confrontations. The children can develop fear, anxiety, and struggle with secure attachments.

***Violent Family-*** Physical, emotional, or psychological violence is prevalent in these families. Exposure to such violence can develop trauma, behaviour disorders, and mental health problems among the children over time.

***Authoritarian Family-*** These families are marked by stringent rules and dominating authority that suppresses individuality. Children can lack decision-making ability and develop issues of dependence.

There are certain **Risk Factors** in context to dysfunctional families that are often detrimental to child development (Simões, A., 2022). These factors span across multiple domains, starting with patterns of social and economic interactions.

**Social and Economic Interactions-**

Financial issues
Lack of social support
Exposure to violence

**Family Characteristics-**

Domestic violence
Chronic conflicts
Poor communication between separated parents

**Characteristics of caregiver-**

Alcohol and/or drug abuse
Mental health issues
Criminal records

**Parenting-**

Unrealistic expectations from the child
Negative attitudes towards the child
Abusive or mistreating behaviours

Collectively, these interrelated risk factors contribute to a complex and adverse developmental context for children raised in dysfunctional families.

**Impact of Dysfunctional Family Environments on Child Development**

**Cognitive Development**

A secure and stimulating home environment is very much imperative in promoting early cognitive growth. Quality care, through emotional responsiveness, facilitation of learning, and adequate stimulation, has been found to correlate with positive cognitive outcomes in children below the age of five (McCormick, 2019; Nurliyana, 2019). Conversely, children raised in dysfunctional family environments, where this support is lacking, are at increased risk of developmental delays, particularly when compounded by conditions such as poor diet, illness, and unstable caregiving patterns (McCormick, 2020).

**Impaired Executive Functioning**

Dysfunctional family patterns, especially those characterized by parental hostility, neglect, or drug abuse, have been reliably associated with children's executive functioning deficits. Impairment in executive functioning, including working memory, inhibitory control, and cognitive flexibility, has been revealed by longitudinal research on Chinese families with parental hostility, with parental warmth acting as a protective factor (Lam, Chung, & Li, 2018), and on families with alcohol misuse (Schroeder & Kelley, 2008; Daly et al., 2017). In a longitudinal study by Zha, J. et al., (2024), it was found that maternal ACEs were positively correlated with poorer executive functioning in pre-schoolers and several mediators of these associations included parenting stress and direct maltreatment, accounting for more than 65 to 78% of the total relationship. These results support more general evidence that repeated exposure to hostile or disorganized home environments can interfere with the development of fundamental cognitive control processes during sensitive developmental periods.

**Academic Performance**

Chilan and Cedeño (2022) established that more than 40% of rural students who had family-related challenges showed behavioral issues affecting school participation, whereas others manifested certain deficits in reading and math reasoning. Likewise, Ndlovu (2023), and Ede and Nwakpadolu (2024), linked significant negative correlation between dysfunctional family environments and academic performance, because they are continuously experiencing emotional pain and a lack of support within the home environment. Together, these results highlight that academic underachievement is not merely a school issue, but indeed a manifestation of more profound impairments in the functioning of families that interfere with cognitive and emotional preparedness to learn.

**Emotional regulation**

Emotional neglect and exposure to abuse, tends to cause problems of anxiety and depression (as a result of difficulties with emotional awareness and expression) on the one hand, and problems of aggression and conduct disorders (as a result of increased emotional reactivity and over-reliance on maladaptive regulation strategies like impulsivity) on the other (Heleniak, 2016; Hatkevich, Sumlin, & Sharp, 2021). Adolescents with such histories showed increased activation in the salience network (responsible for emotion regulation) and amygdala when presented with negative stimuli, reflecting greater threat sensitivity, and in prefrontal areas during tasks of regulation, reflecting a compensatory attempt at controlling emotional reactions (McLaughlin et al., 2015).

**Neurological and Biological Outcomes**

Early exposure to dysfunctional family environments, such as verbal abuse and domestic violence, interferes with sensory processing and results in structural changes in brain areas responsible for emotional regulation and executive functioning, including the prefrontal cortex, anterior cingulate, hippocampus, and corpus callosum (Teicher, 2016). Childhood abuse interferes with physiological functioning, such as the nervous, immune, and endocrine systems, rendering individuals more susceptible to long-term behavioral, physical, and psychological issues like depression, drug use, and impaired impulse control (WHO, 2024; Balistreri, 2016). Neuroimaging research has shown that different types of adversities experienced in early life yields different neural outcomes. Experiences-

such as abuse, decreases the volume and enhances the reactivity of the amygdala, medial prefrontal cortex, and hippocampus, whereas adversities such as neglect impacts the frontoparietal areas responsible for cognitive control (McLaughlin, 2019). These results highlight the need to customize interventions to adversity's nature and timing.

**Social, Emotional, and Behavioral Development**

Dysfunctional family conditions, such as divorce or separation of parents, can negatively alter children's emotional development. Divorce is likely to create emotional distance from the non-residential parent, especially when it occurs due to parent conflict or intrafamily violence (Strohschein et al., 2012). The disruption of the parent-child relationship under such conditions might suppress provision of emotional support and stability, thereby triggering children's psychological distress and illnesses (Mphaphuli, 2023). The risk of experiencing ACEs at a tender age is significantly linked to behavioral problems, with odds of 2.0 and 2.9 when the child is aged five and exposed to two and three or more ACEs, respectively. Children aged nine and fifteen years, with two or more ACEs, have shown 1.9 and 3.2 times greater likelihood of being at great risk of behavioral problems, respectively (Choi, J. K., et al., 2019).

Domestic violence undermines the home as a safe space, developing issues such as anxiety, depression, disobedience, and drug use. These adversities cumulatively compromises children's emotional regulation, formation of relationships, and social competencies (Mphaphuli, 2023; Khemthong, 2021).

**The Role of Protective Factors in Childhood Adaptation and Resilience**

**Individual level factors**

Individual characteristics such as emotional intelligence, social competence, and adaptive functioning can successfully mediate the effects of adverse childhood experiences. Antonovsky's Sense of Coherence (SOC) provides a principal model for understanding resilience among children from dysfunctional families. Children who view life as comprehensible, manageable, and meaningful, particularly with consistent external support, can better handle stress and sustain emotional health (Mittelmark & Bauer, 2017). Beyazit et al. (2024) found that children with greater trait emotional intelligence were more seen to effectively regulate their emotions and apply adaptive regulation strategies, hence, indicating the potential of EI-based interventions. Resilience can also protect children from negative life experiences. Although most children raised by parents with problems of alcohol abuse develop negative outcomes, some learn adaptive coping through self-examination and development, emphasizing the value of building emotional awareness during childhood into adulthood (Zhang et al., 2019; Simonič, 2023).

**Family level factors**

Current evidence underscores the importance of family-level protective factors in reducing the long-term effects of childhood adversity. For example, Balistreri (2016) established that positive family functioning, as indicated by low parental stress and supportive parent-child relationships, plays a significant role in improved physical and emotional well-being during adolescence. Similarly, Mphaphuli (2023) found that parental employment that is stable and close relationship between family members promotes a healthy development by creating a healthy home environment. Together, these results highlight the buffering influence of stable and cohesive family relationships in mitigating the harmful effects of early life adversity. Emotionally responsive caregiving has specifically been found to moderate the psychological impact of trauma. McLaughlin (2020) documents that having a supportive caregiver can decrease heightened threat-related neural processing (typically found in children with histories of early trauma), which decreases the risk of psychopathology. Therefore, a healthy family environment, positive parent-child interaction, proper support and stimulation, etc. are needed for healthy development in children (Yang, Q., 2021).

**Community level factors**

Community-level resources, such as supportive teachers, positive peer interactions and neighbours, serve as significant protective factors. Research indicates that strong social support eliminates the risk of trauma-related psychological disorders in victims of trauma. (Trickey, 2012). In a study of 421 children aged 6-12 from low-income families, Kim et al. (2010) found that the negative outcomes (e.g., regulation of emotions) related to maltreatment experiences were buffered by peer acceptance, indicating the regulatory function of social relationships. Rockhill

(2009), using the sample of 521 adolescents in middle school, found that a strong social support and social competence can mediate the negative outcomes (such as behavioural including psychopathology) among adolescents, with depressive or comorbid adolescents exhibiting poorer adaptive and academic functioning due to these deficits. This mediation was not evident for adolescents with conduct problems alone, which supports the need for specific social skills and support interventions for adolescents with internalizing or comorbid problems. Similarly, Alto et al. (2019) determined that peer social acceptance mediates the link between childhood maltreatment and depression during adolescence, across both genders. This further establishes the pivotal influence of peer relations on emotional resilience. Together, these results underscore the significance of social environments within communities in minimizing adverse consequences of dysfunctional family experiences.

## CONCLUSION

The literature consistently finds that children growing up in dysfunctional home environments, where there is abuse, neglect, emotional unavailability, or substance abuse, experience deficits in emotional regulation, executive functioning, academic achievement, and socio-emotional development across a broad variety of cultural contexts. These adverse effects are most often buffered by protective factors such as secure attachment, emotional responsiveness, intelligence, and social or caregiver support. Yet, most research as it does exist is narrowly concentrated on a single factor (e.g., mental illness of family members or abuse of alcohol) and not culturally representative, limiting generalizability to larger populations. Additional longitudinal and more comprehensive studies are also necessary to learn more about the long-term impact of family dysfunction as well as the persistent influence of protective factors. Future research should focus on making early intervention of at-risk youth a top priority before negative trajectories become solidified, by focusing on building interventions based on a culturally sensitive, family-centered approach focusing on parent education, mental health service access, and trauma-sensitive care. Building resilience as secure relationships and supportive environments is essential to preventing long-term harm. Finally, interdisciplinary collaboration between clinical, educational, and policy leaders is essential for creating secure, supportive environments where healthy childhood development is guaranteed for those youth raised in dysfunctional families.

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**Conflict of interest**

The authors declare no conflict of interest.

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RESEARCH ARTICLE

# Survey on the Progression of Graph Structures: Transitioning from Uncertainty – Based Frameworks to Neutrosophic Paradigms through Functional Approaches

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**ABSTRACT**

This survey examines and systematically organizes research on fuzzy, intuitionistic fuzzy, and neutrosophic graphs, along with their corresponding graph types. The survey incorporates prior research that examined the development and proliferation of graphs and associated operations, as well as studies concentrated on graphs characterized by uncertainty. The survey analyzes the historical context, fundamental structural characteristics, and applications investigated by researchers across various regimes of binary (graph) operation in a fuzzified framework. This article systematically elucidates the evolution of operational techniques from fuzzy to neutrosophic models and from fuzzy to neutrosophic graphs through a comprehensive survey and analysis of these contributions.

**Keywords:** FG, IFG, NFG, Operation

## INTRODUCTION

Graph theory has long been a foundational application for modeling and mapping out different real-world systems, and widely implemented in various disciplines like engineering, decision-making, chemistry, and medical diagnostics. Historically, the use of graphs dates back to Euler and the solution to the Konigsberg bridge problem in

1736, which created the aforementioned modern graph theoretical analysis. The two main components of a graph, the vertices and edges, work well at representing relationships, particularly in topical areas like social networks and data systems, etc. However, traditional graph or edge models have limitations to address systems that are fuzzy, ambiguous, or imprecise. So, researchers have built upon traditional graph theory by applying fuzzy set theory to graphs. These fuzzy graph models will enable greater flexibility in the handling of uncertainty, and subsequent models can have far better applications as complexity increases. Much research has been conducted on the extension of graph theory using operational methods and structural properties of graphs in the fuzzy and fuzzy derived domains, producing innovative models that provide a better modeling of reality. In classical graph theory, binary relations were crisp (usually 0 and 1) values to describe if an edge existed amongst two vertices. This aspect of classical theory was sufficient to address several structured type problems. However, classical theory has limited utility on ambiguity and belief questions that are prevalent in many real-world situations. This left plenty of opportunity for fuzzy graph theory to be developed and expanded on classical graph theory. The concept of fuzzy graphs traces its origins to the pioneering work of Lotfi A. Zadeh (1965), who introduced the foundational theory of fuzzy sets. In 1973, Kaufmann formalized the definition of FG, building upon Zadeh's foundational theory of FS. Shortly thereafter, Rosenfeld advanced the field by extending the notion of fuzzy relations within graph structures, paving the way for deeper exploration into uncertainty-based graph modeling. Nagoorgani *et al*. further develop this research area by logically reconstructing the practices and theorizing on application with graphs. In 1986, Atanassov established the Intuitionistic fuzzy set (Int-FS) that contain membership and non-membership degrees. This increased the representational capacity of fuzzy graphs through degrees of ambiguity. Researchers, like Parvathi and Karunambigai (2009) introduced intuitionistic elements in fuzzy graph that facilitated different graphical representations within axiomatics.

Akram (2016) and Nagoorgani (2010) also contributed and explored different operational and utilization cases in fuzzy graph. Fuzzy graph theory progressed in great stride after the advancement of neutrosophic set theory by Smarandache (1999), which widened intuitionistic fuzzy set theory by adding indeterminacy to the fuzzy sets' domain or space. This development afforded the theory to adapt more accurately and concretely to challenge of representation of uncertain and conflicting information. Broumi's (2016, 2018) works further disentangled the theory aiding in the development of neutrosophic graph theory. This is advantageous in representation of vague systems wherein boundaries of the variables are difficult to demarcate. Overall, fuzzy, intuitionistic fuzzy and neutrosophic graph structures have emerged as usable analytic representations and valid structural modeling tool and appropriate for the many forms of uncertainty experienced in the real world. The different theoretical possibilities in uncertain and imprecise domains provided scope for many aspects various fields across artificial intelligence, decision-making systems, and information science to explore many forms of uncertainty. These challenges can be expressed in vague terms and vague concepts, which are conversationally common forms of challenges but can now be rigorous and accurate with the research direction now relevant to the overarching dialogue in modern science and technology. The domain of fuzzy graphs and their extended variants encompasses a wide range of well-defined operations. These operations typically involve integrating the structural features and attributes of two distinct graphs, resulting in a unified graph that reflects multidimensional characteristics. Such a framework proves especially valuable in addressing complex challenges in areas like socioeconomic analysis, neural network modeling, and decision-making processes. This article presents a comprehensive overview of our research on various operational techniques applied across different types of fuzzy graphs, culminating in neutrosophic graph models. The organization of the article is as follows: Section 1 introduces the evolution of graph theory from fuzzy foundations to neutrosophic extensions2,3,4 detail the operational methodologies applied to fuzzy graphs, intuitionistic fuzzy graphs, and neutrosophic fuzzy graphs, respectively. The final section 5, offers concluding remarks and reflections on the overall findings.

**Various Operational Approaches in Fuzzy Graph Theory**

Table 1 gives an overview of the advancements in graph operation methods in the fuzzy environment. Mordeson [1] devised new methods to define operations for products of different kinds in fuzzy graphs. It formally defined the product operations, then established theorems about fuzzy subgraphs. Besides the product operations, they also considered composition, union, and join, including additional requirements and purposes within fuzzy subgraphs,

along plenty of illustration. NagoorGani and Radha [2] established a new "conjunction" operation on two fuzzy graphs. Have established necessary and sufficient conditions with which the conjunction could be con- sidered strong. They also examined the degrees of vertices concerning the conjunction of two fuzzy graphs, along with some theorems. They provided some further discussions about how degree based regularity, and the properties arising from the adjacency sequence from fuzzy graph conjunction. NagoorGani and FathimaKani [3] proposed two novel operations on fuzzy graphs, known as the Beta and Gamma products. Their study focused on analyzing vertex degrees and exploring regularity properties of these product graphs under specific conditions. They also established necessary and sufficient criteria under which these products exhibit tendencies toward regularity. Radha and Arumugam [4] were the first to study the strong product of a fuzzy graph. They explored this fuzzy product in terms of effectiveness, completeness and connectedness. They specified upper and lower truncations of the strong product, and examined how vertex degrees related to the fuzzy direct sum. ShovanDogra [5] introduced a diverse set of product operations in the realm of fuzzy graph theory, namely the modular, homomorphic, box-dot, and star products. These operations were further examined in the context of strong fuzzy graphs, with a detailed analysis of vertex degrees corresponding to each product type. Radha and Arumugam [6] also explored the maximal product of fuzzy graphs where effectiveness and connectedness were established, also covering vertex degrees and regularity under certain limitations. Radha and Arumugam [7] introduced a novel fuzzy graph operation known as the residue product, and conducted an in-depth analysis of its structural properties, including effectiveness, connectivity, and completeness. They derived explicit expressions for both vertex degree and total degree, and established necessary and sufficient conditions under which the residue product exhibits regularity. Building on this foundation, MuzzamalSitara *et al*. [8] carried out a comprehensive study of the maximal product in fuzzy graph theory. Their work delineates precise criteria for regularity, supported by illustrative counterexamples. They computed vertex and total degrees for the maximal product and presented their results with clarity. To contextualize their theoretical contributions, they included a figure and a flowchart demonstrating an application aimed at identifying controversial issues among nations, showcasing the practical relevance of fuzzy graph products in geopolitical analysis.

**Various Operational Approaches in Intuitionistic Fuzzy Graph Theory**

Researchers can extend existing product operations to IFGs as outlined in Table 3 by generalizing fuzzy graphs (FGs) through the incorporation of an additional membership function. This enhancement introduces both membership and non-membership degrees, allowing for a richer representation of uncertainty and hesitation in graph structures. Such a generalization enables the adaptation of classical fuzzy graph products to the intuitionistic framework, paving the way for deeper structural analysis and broader applications in areas requiring nuanced modeling of ambiguity. Various Operations in Intuitionistic Fuzzy Graph Environment (Table 2). Parvathi *et al*. [9] laid the groundwork for Intuitionistic Fuzzy Graphs (IFGs) by investigating essential complementary characteristics and formulating several core theorems. Expanding upon this foundation, they introduced a range of graph product operations. Further advancing the field, SankarSahoo *et al*. [10] extended the concepts of some products for pairs of IFGs. Their work yielded significant theoretical results and demonstrated that these product operations can inspire innovative applications and models within fuzzy graph theory. Hossein Rashmanlou *et al*. [11] investigated the logical foundations of several operations in fuzzy graphs. Their rational justification was achieved by characterizing the graph's hierarchical levels, and they further established key categorical properties through the lens of category theory. SankarSahoo and Madhumangal Pal [12] introduced the idea of normal and tensor prod- ucts in the framework of IFGs. Their study included an examination of vertex degrees for selected product types and led to the establishment of several structural properties, enriching the theoretical landscape of fuzzy graph operations. NagoorGani *et al*. [13] conducted an analysis of regularity conditions in the Cartesian product of IFGs. Their findings revealed that the resulting product graph exhibits either full regularity or a mixed state. Under these configurations, they defined the associated membership functions as constant to maintain structural consistency within the product graph. Kalaiarasi and Mahalakshmi [14] made a notable contribution by introducing the co-normal product operation in IFGs. Their work demonstrated that this product preserves the structure of an IFG, meaning the resulting graph still satisfies the conditions of intuitionistic fuzziness—namely, the membership and non-membership functions remain within the required bounds. Yahya Mohamed and Mohamed Ali [15] found the maximum product operation for Intuitionistic Fuzzy Graphs (IFGs), ensuring that both vertex degree and graph

regularity remain invariant under the transformation. Their approach preserves the structural integrity of the original graphs while extending the operational framework of IFGs. In a related development, Sonia Mondal and Madhumangal Pal [16] explored the concept of BIFGs and defined many graph operations. Their study focused on analyzing the vertex degrees of the resulting graphs, offering insights into how these operations influence the underlying fuzzy structure within a bipolar context. IrfanNazeer *et al*. [17] studied various product operations on IFIG also analyzed the basic property of vertex degree in each case. Their study included a day to day -life example of the Cartesian and Composition products in the textile industry related to organizational departments. A comparative analysis showed the differences between the models they developed and the existing models. Kalaiarasi *et al*. [18] defined the co-normal product on interval-valued Atanassov fuzzy graphs (IA-FGs) and showed that it was possible to apply it on complete, regular, and strong IA-FGs. This study provided an example of a practical application by showing the difference in product quality or cost.

**Various Operational Approaches in Neutrosophic Fuzzy Graph Theory**

Table 3 lists the enrichment associated with the product of graph concern in a Neutrosophic. Various Operations in Neutrosophic Fuzzy Graph Environment (Table 3). Muhammad Akram and GulfamShahzadi [19] developed a detailed definition of single-valued neutrosophic graphs (SVNGs) and studied the fundamental things associated on graph levels. They also surveyed several types of product operations and studied different social behavioral characteristics using SVNG to identify the individual with the most impact. Muhammad Gulistan *et al*. [20] pitched the idea of NCGs based on NCS, plus introduced and used Hadamard product operations. They showed contextually graph order and degree in examples and translated theoretical findings for use in industrial application using real data. Aslam Malik *et al*. [21] studied bipolar SVNG, and made use of operations such as symmetric difference, residue, maximal, rejection. Their study included a concise examination of vertex degree and total vertex degree in these graphs. A real world study was posited to develop a framework to assist in understanding good candidates for educational designations. Satham Hussain *et al*.[22] examined a Neutrosophic Vertex Graphs (NVGs) and defined a variety of product operations. Satham Hussain *et al*. [23] studied significant operations on graphs including Cartesian, cross, lexicographic, strong product and composition product for Neutrosophic Vertex Similarity Graphs (NVSGs). Their article also had a practical component that dealt with selection of relevant institutions using the ranking. Satham Hussain *et al*. [24] explored the fundamental operations Composition between Neutrosophic Vertex Similarity Graphs, Cartesian product of it, Cross Product and Strong product of Neutrosophic Vertex Similarity Graphs, Lexicographic for Neutrosophic Vertex Similarity Graphs (NVSGs) that involved decision making to find the most suitable institutions based on academic ranks. Meenakshi *et al*. [25] endeavored to tackle domination-related parameters for Single Valued Neutrosophic Graphs (SVNG). By looking at a special set of properties with respect to symmetric difference operations on single-valued neutrosophic networks, they were able to suggest a model of optimal productive networks. In the process, they develop one special-case algorithm that searches for optimal productive networks. Mohammed Alqahtani *et al*. [26] studied applied research topics on complex environmental neutrosophic graphs. Their study focused on applying certain operations of indirect graphs such as Cartesian, union, join, and composition, as well as studying structural properties of indirect graphs such as isomorphism and complementarity. The final element of their study involved an application on construction hospital infrastructures in which planners were looking for optimal starting points.

## CONCLUSION

When we look through the lens of neutrosophy, we found that our graph product operations allowed for greater flexibility and robustness, and more precise and effective results than previous approaches based on fuzzy graph. The neutrosophic framework gives a more transparent treatment of the uncertainty, ambiguity, and indeterminacy, which means there is better representation of reality. This analysis shows that it recognizes the inherent complexity of the subject, and provides a renewed and contemporary understanding of it ideal to our contemporary landscape and research trajectories. In addition, the enhanced nature of neutrosophic graph theory's handling of indeterminacy,

in a structured and systematic manner can help address common pitfalls that come during practice. Therefore, this creates valuable groundwork for future models that are advancing real-world engagement.

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**Table 1: Various Operations in Fuzzy Graph Environment**

| Reference | Year | Graph Type | Approaches |
|---|---|---|---|
| [1] | 1994 | Fuzzy Graphs | Numerous theorems and illustrative examples have been developed to explore fuzzy subgraph operations |
| [2] | 2008 | | The conjunction of FG has been defined with properties. |
| [3] | 2014 | | The $\beta$ and $\gamma$ of FG have been individually found and analyzed for their properties in regular fuzzy graph structures. |
| [4] | 2014 | | The strong, effective and the notion of complete fuzzy graphs have each been individually defined and studied. Further, the vertex degrees and truncation properties have been systematically analyzed. |
| [5] | 2015 | | The max and the effective fuzzy graphs, and the structure of connected FG have each been formally defined and examined. Additionally, the vertex degree characteristics and regularity properties of the maximal product have been systematically analyzed. |
| [6] | 2015 | | The different product of fuzzy graphs have been thoroughly examined, along with detailed analyses of their corresponding vertex degree evaluations. |
| [7] | 2015 | | The essential properties of effectively, connectedness, and completeness of this operation, has been explored; and finally their considerations of vertex degrees, total degrees, and fuzzy graphs' regularity from using residue products have been explored and exemplified. |
| [8] | 2019 | | The maximal product of two fuzzy graphs have been looked at in much detail and have been discussed about its theoretical properties and also attendant properties, and examples. Furthermore, we have demonstrated the product's relevance in real-world applications in discovering countries' controversial issues and study. |

**Table 2: Various Operations in Intuitionistic Fuzzy Graph Environment**

| Reference | Year | Graph Type | Approaches |
|---|---|---|---|
| [9] | 2009 | IFG | Operations product of some IF has been formulated within the framework of tensor products in fuzzy graph theory. |
| [10] | 2015 | | The direct, semi-strong, and strong product operations have been formally defined within the framework of IFGs. Each has been examined in depth, with detailed analysis of their structural behavior and properties unique to the intuitionistic setting |
| [11] | 2015 | | Level-based direct, lexicographic, and strong products have been defined on IFGs |

| | | | |
|---|---|---|---|
| | | | where level-based operations follow a categorical property. |
| [12] | 2017 | | Other operations defined on IFGs include Cartesian, composition, tensor, and normal products with a good discussion on vertex degree in each case. |
| [13] | 2017 | | The idea of regularity has been defined with the Cartesian product of IFGs, with some foundation theorems. |
| [14] | 2018 | | The co-normal product of IFGs was considered and as is demonstrated, co-normal exhibits both completeness and regularity. |
| [15] | 2019 | | The maximal product of IFGs has been presented above and we have discussed its properties under vertex degree in relation to regularity. |
| [16] | 2019 | BIFG | Standard product operations such as Cartesian, composition, tensor, and normal products have been formulated on bipolar intuitionistic fuzzy graphs (IFGs), and corresponding vertex degrees have been evaluated |
| [17] | 2021 | IFIG | These same operations—Cartesian, composition, tensor, and normal products—have also been applied to interval-valued intuitionistic fuzzy graphs (IFIGs), with detailed vertex degree calculations. Additionally, an application involving the Cartesian product and composition has been demonstrated in the context of the textile industry. |
| [18] | 2023 | IA-FGs | A new conformal product has been developed for interval-valued Atanassov fuzzy graphs (IA-FGs), with key structural properties like completeness and regularity rigorously established. This model has also been effectively applied to optimize product quality and minimize operational costs in supermarket settings. |

**Table 3: Various Operations in Neutrosophic Fuzzy Graph Environment**

| Reference | Year | Graph Type | Approaches |
|---|---|---|---|
| [19] | 2017 | Single Valued Neutrosophic Graph | Basic product operations are provided in the context of SVNGs and while mean basic results and an appropriate application was provided that showed how individuals' thought processes affect each others' thoughts. |
| [20] | 2018 | Neutrosophic Cubic Graph | Cartesian, composition, union, and join products have been provided for neutrosophic cubic graphs, contextualized as a decision-making application of industry performance |
| [21] | 2020 | Bipolar Single Valued Neutrosophic Graph | Residue and different operations of NG (with examples) have been established on Bipolar SVNGs. An investigation of calculation of structures and an application and algorithm have been designed to answer location of candidates to fulfill employment roles in education for V(G) and total V(G). |
| [22] | 2020 | Neutrosophic Vague Graph | Neutrosophic vertex graphs have been established, and product operation computations are accomplished by identifying product of varies graphs (using examples). |
| [23] | 2022 | Neutrosophic Vague Soft Graph | DifferentproductsonNVSGusedasexamplestodeterminemaximumobjectselection. |
| [24] | 2022 | Quadripartitioned Bipolar SVNG | QBSVNG is established with appropriate graphs. |
| [25] | 2023 | Single Valued Neutrosophic Graph | Some operations are proven over SVNG by the concept of domination, and a symmetric difference application example was used. |
| [26] | 2024 | Complex Neutrosophic Graph | Some operations are proven over SVNG by the concept of domination, and a symmetric difference application example was used. Standard product operations such as Cartesian, composition, tensor, and |

| | | | |
|---|---|---|---|
| | | | normal products have been Some procedures has been accomplished over complex NG of Cartesian, union, join and composition- and an application using real life emphasized for infrastructure design related to hospital |

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RESEARCH ARTICLE

# An Analytical and Experimental Heat Transfer Investigation of a Mother-Baby Plastic Granule Extruder Machine with Heat Recovery Integration

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## ABSTRACT

This study presents an analytical and experimental heat transfer investigation of a mother-baby plastic granules extruder machine with the integration of a heat recovery structure. The analytical model was developed by considering conduction through the heater to the barrel surface, conduction across the barrel wall, convection between the barrel inner surface and the raw plastic, and additional heat generation due to shear and friction of the rotating screw. To validate the model, experimental trials were conducted on an LDPE processing extruder unit under two cases: (i) without heat recovery integration and (ii) with a CRC sheet-based heat recovery structure installed between the mother and baby units. A total of 48 temperature readings (8 readings per day for 3 consecutive days, in both cases) were collected to analyse the thermal response. The results showed close agreement between analytical predictions and experimental observations, with notable variations in temperature distribution across the barrel and plastic melt zones. The incorporation of the heat recovery structure demonstrated improved utilization of the rejected thermal energy, thereby confirming the effectiveness of the proposed design. This combined analytical–experimental approach provides a detailed understanding of the heat transfer processes in extrusion machines

and highlights potential pathways for enhancing thermal performance and energy efficiency in plastic recycling operations.

**Keywords:** Plastic Granule Extruder, Thermal Efficiency, Specific Energy Consumption, Heat Transfer.

## INTRODUCTION

Plastic extrusion is a widely used manufacturing and recycling process for converting waste polymers into reusable granules. Among different plastics, low-density polyethylene (LDPE) is commonly recycled due to its widespread use in packaging and consumer products [1]. The efficiency of extrusion depends heavily on the heat transfer between the electrical heaters, the barrel, and the plastic material. Inadequate thermal management often results in higher specific energy consumption (SEC), poor product quality, and increased operational costs [2,3]. In extrusion systems, heating is primarily achieved through electrical heaters mounted on the barrel surface. The supplied heat undergoes multiple transfer mechanisms: conduction through the barrel wall, convection to the rotating screw and molten polymer, and internal heat generation due to shear friction [4]. The accurate estimation of these heat transfer modes is essential for improving energy efficiency and thermal performance. Several studies have analyzed conduction and convection heat transfer in extrusion systems [5,6], while others have emphasized the impact of screw rotation and polymer rheology on thermal distribution [7]. However, limited research exists on integrating heat recovery mechanisms into extruder systems to reduce energy losses [8,9]. The present work aims to address this gap by performing a detailed analytical and experimental heat transfer investigation of a mother-baby extruder machine used for LDPE recycling. The study integrates three consecutive days of experimental trials, with eight readings per day, under two conditions: (i) without heat recovery structure, and (ii) with a CRC sheet-based structure positioned between the mother and baby units. The results were compared with analytical heat transfer models. The novelty of this research lies in combining theoretical heat transfer modelling with real-time experimental validation on an industrial scale extrusion system. This dual approach not only improves the understanding of heater-barrel-polymer thermal interactions but also provides practical insights for designing more energy efficient recycling extruders.

## METHODOLOGY

The present study employs a combined analytical modelling and experimental measurement approach to investigate the heat transfer performance of a mother–baby plastic granules extruder. The methodology is divided into two major parts: (i) analytical heat transfer model formulation and (ii) experimental procedure for data collection with and without heat recovery integration.

**Analytical heat transfer model**

The thermal behavior of the mother–baby plastic granules extruder was analyzed by developing an analytical model based on classical heat transfer principles. The model considered the following mechanisms:

**Conduction through barrel wall**

Heat conduction occurs radially across the extruder barrel from the outer heated surface to the inner wall in contact with the polymer. This was modelled using cylindrical conduction relations:

$$Q = \frac{2\pi kL\,(T_{heater} - T_{barrel\ outer})}{\ln(\frac{r_{outer}}{r_{inner}})} \quad (1)$$

Where,

Q is the heat transfer (W),
*k* is the thermal conductivity of the barrel material (W/m·K),
L is the length of the barrel (m),
$r_{outer}$ is the outer radius of the barrel (m),
$r_{inner}$ is the outer radius of the barrel (m),
$T_{heater}$ is the heater temperature (°C),
$T_{barrel}$ outer is the barrel outer temperature (°C).

**Conduction from barrel inner surface to solid raw material**

At the polymer–barrel interface, a conduction mechanism dominates as solid granules initially absorb heat from the inner barrel wall:

$$Q = \frac{kpA(T_{barrel\ inner} - T_{raw\ material})}{x} \quad (2)$$

Where
$k_p$ is the thermal conductivity of polymer,
A is the surface area,
x is barrel thickness.

**Convection from barrel inner wall to raw material**

The inner wall transfers heat to the solid polymer granules and partially molten resin by convection. Newton's law of cooling was applied:

$$Q = h \cdot A\ (T_{inner} - T_{polymer}) \quad (3)$$

Where
h is the convective heat transfer coefficient (W/m²·K)

**Additional heat generation due to shear and friction**

As the screw rotates, viscous dissipation and frictional effects generate additional heat within the polymer melt. This contribution was estimated from the screw speed, torque, and polymer rheology characteristics.

$$Q = \eta \times P \quad (4)$$

Where,
η is the mechanical efficiency (assumed ~ 60%),
P is the motor power rating (in HP), converted to kW.

**Overall useful heat transfer**

The total useful heat transferred to the polymer was expressed as:

$$Q_{useful} = Q_{cond\ polymer} + Q_{conv} \quad (5)$$

**Total heat supplied**

The total heat supplied was expressed as:

$$Q_{total\ heat} = Q_{cond\ inner} + Q_{shear} \quad (6)$$

**Experimental setup**

To validate the analytical model, experiments were conducted on a mother–baby plastic granules extruder machine processing LDPE waste. The specifications of both units are summarized in Table 1.

**Heat recovery structure**

A specially designed heat recovery structure was integrated in the open space between the mother and baby unit to minimize temperature loss. The structure utilized a CRC sheet assembly to retain and redirect thermal energy from the outgoing polymer stream of the mother unit into the inlet of the baby unit. The design and integration of this structure were reported in our previous experimental work [14], and its performance is evaluated in this study in conjunction with the analytical model.

Thermocouples were placed at strategic locations, including:

1. Heater surface
2. Barrel outer surface
3. Barrel inner surface
4. Polymer inlet and outlet of both mother and baby units

**Experimental Data Presentation**

To validate the analytical model, experimental investigations were carried out on the mother–baby plastic granules extruder system under two operating scenarios:

1. Without heat recovery structure
2. With heat recovery structure (CRC sheet arrangement between mother and baby units).

**Data Collection**

Experiments were conducted for three consecutive days and on each day, eight sets of readings were recorded at equal time intervals. Thus, a total of 48 readings were collected for both cases (with and without heat recovery). The key measured parameters were:

1. Time of recording (min)
2. Inlet temperature of baby extruder (°C)
3. Outlet temperature of baby extruder (°C)
4. Recovered temperature due to CRC sheet structure (°C)

Figure 7 shows the inlet temperature comparison of the baby unit before and after integration of the heat recovery structure. A noticeable increase in inlet temperature was observed post structure, indicating successful recovery of thermal energy that would otherwise be lost. This contributed to improved thermal performance and reduced energy consumption in the baby unit.

**Calculations**

**Mother Unit**

**Heat transfer through conduction from barrel outer surface to barrel inner surface**

$$Q = \frac{2\pi kL\,(T_{heater} - T_{barrel\ outer})}{\ln(\frac{r_{outer}}{r_{inner}})} \quad = 35.89 \text{ kW}$$

**Heat transfer through conduction from barrel inner surface to raw material**

$$Q = \frac{kpA(T_{barrel\ inner} - T_{raw\ material})}{X} \quad = \quad 37.94 \text{ kW}$$

**Heat transfer through convection from barrel inner surface to raw material**

$$Q = h \cdot A\,(T_{inner} - T_{polymer}) \quad = \quad 10.19 \text{ kW}$$

**Additional heat generation due to friction**

$Q = \eta \times P$ = 33.57 kW

**Baby Unit(before heat recovery integration)**

1. **Heat transfer through conduction from barrel outer surface to barrel inner surface:**

$$Q = \frac{2\pi kL\,(T_{heater} - T_{barrel\ outer})}{\ln(\frac{r_{outer}}{r_{inner}})} = 6.38\ kW$$

2. **Heat transfer through conduction from barrel inner surface to raw material:**
3. **Heat transfer through convection from barrel inner surface to raw material:**
4. **Additional heat generation due to friction:**

$Q = \eta \times P$ = 11.19 kW

**Baby Unit(after heat recovery integration)**

1. **Heat transfer through conduction from barrel outer surface to barrel inner surface:**

$$Q = \frac{2\pi kL\,(T_{heater} - T_{barrel\ outer})}{\ln(\frac{r_{outer}}{r_{inner}})} = 6.38\ kW$$

2. **Heat transfer through conduction from barrel inner surface to raw material:**
3. **Heat transfer through convection from barrel inner surface to raw material:**
4. **Additional heat generation due to friction:**

$Q = \eta \times P$ = 11.19 kW

**Heater Load**

Figure 8 shows that after the integration of the heat recovery structure between the mother and baby extruder units, a clear reduction in heat energy demand was observed. The recovered heat from the mother unit effectively preheated the polymer entering the baby unit, thereby reducing the additional heating requirement. The experimental data recorded over three days (eight readings per day) clearly demonstrated a consistent decrease in heater energy input after integration. The plotted chart illustrates that the inlet temperature of the baby unit increased due to heat recovery, which in turn lowered the net heat supplied by its heaters. This outcome confirms that the heat recovery system successfully minimized external thermal energy demand, improving the overall sustainability and energy efficiency of the extrusion process.

## RESULT AND DISCUSSION

The experimental outcomes clearly confirmed the impact of the heat recovery integration between the mother and baby extruder units. In the starting point case (without heat recovery integration), the baby extruder inlet temperature was observed to be approximately 129.54 °C, requiring significant heater energy input to reach the desired processing temperature of 139.75 °C. After introducing the heat recovery structure, the inlet temperature increased to 10.21 °C, thereby reducing the heater energy demand. Quantitatively, the total heater energy consumption decreased from 0.674 kWh/hr to 0.223 kWh/hr which corresponds to a reduction of approximately 33.08 %. This improvement can be recognized to the recovery of waste heat from the mother unit barrel, which otherwise dissipated into the surrounding environment. By transferring this recovered heat directly to the baby unit, the system utilized the available energy more efficiently, thereby lowering on external heating. The results not only validate the

effectiveness of the heat recovery integration but also indicate a possible pathway for improving thermal management in extrusion systems.

## CONCLUSION

This study offered an analytical and experimental investigation of heat transfer phenomena in a mother–baby plastic granule extruder system with heat recovery integration. By introducing a simple heat recovery structure between these two units, an important portion of waste heat from the mother extruder was effectively utilized to preheat the feed zone of the baby extruder. As a result, the inlet temperature of the baby unit increased, leading to a prominent reduction in heater energy demand. The results confirm that heat recovery integration improves overall energy utilization and contributes to more supportable extrusion processing without negotiating product quality. The approach proves the possible of adopting reflexive heat recovery solutions in industrial extrusion systems to reduce operational costs and improve energy efficiency.

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**Nomenclature**

| | | |
|---|---|---|
| $C_p$ | : | Specific heat capacity, kJ/kg·K |
| Q | : | Heat transfer, [W] |
| $Q_{cond,\ outer}$ | : | Conduction heat transfer through barrel outer wall, [W] |
| $Q_{cond,\ inner}$ | : | Conduction heat transfer through barrel inner wall, [W] |
| $Q_{conv,\ inner}$ | : | Convection heat transfer through barrel inner wall, [W] |
| $Q_{friction}$ | : | Heat generation due to friction, [W] |
| $Q_{useful}$ | : | Useful heat utilized by raw material, [W] |
| $Q_{total}$ | : | Total heat input, [W] |
| $T_{inlet}$ | : | Inlet temperature [°C] |
| $T_{outlet}$ | : | Outlet temperature [°C] |

**Greek Symbols**

| | | |
|---|---|---|
| K | : | Thermal conductivity, W/m·k |
| h | : | Convective heat transfer coefficient, W/m2·k |
| A | : | Surface area involved in heat transfer, m2 |
| L | : | Barrel length, m |
| d | : | Barrel diameter, m |
| P | : | Power input to motors, HP |

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**Table 1: Complete Set of Readings for Mother-Baby Plastic Granule Extruder**

| Sr. No. | Parameter | Value | | Unit |
|---|---|---|---|---|
| | | Mother Unit | Baby Unit | |
| 1 | Barrel Inside Diameter | 150 | 135 | mm |
| 2 | Barrel Outside Diameter | 240 | 215 | mm |
| 3 | Barrel Length | 14 | 5 | ft |
| 4 | Inlet Temperature | 53 | 129 | °C |
| 5 | Outlet Temperature | 140 | 144 | °C |
| 6 | Barrel Outer Temperature | 146 | 151 | °C |
| 7 | Barrel Inner Temperature | 130 | 143 | °C |
| 8 | Running Load | 75 | 25 | HP |

**Table 2: Experimental Readings for Mother-Baby Plastic Granule Extruder – Case 1 and Case 2 (Day 1)**

| Time | Baby Unit Inlet Temperature (°C) (Before Structure) | Baby Unit Inlet Temperature (°C) (After Structure) | Temperature Recovered (°C) |
|---|---|---|---|
| 10:00 | 128 | 138 | 10 |
| 11:00 | 129 | 139 | 10 |
| 12:00 | 128 | 139 | 11 |
| 01:00 | 129 | 140 | 11 |
| 02:00 | 129 | 140 | 11 |
| 03:00 | 130 | 139 | 9 |
| 04:00 | 129 | 138 | 9 |
| 05:00 | 128 | 137 | 9 |

**Table 3: Experimental Readings for Mother-Baby Plastic Granule Extruder – Case 1 and Case 2 (Day 2)**

| Time | Baby Unit Inlet Temperature (°C) (Before Structure) | Baby Unit Inlet Temperature (°C) (After Structure) | Temperature Recovered (°C) |
|---|---|---|---|
| 10:00 | 127 | 138 | 11 |
| 11:00 | 129 | 139 | 10 |
| 12:00 | 129 | 139 | 10 |
| 01:00 | 130 | 140 | 10 |
| 02:00 | 132 | 141 | 9 |
| 03:00 | 130 | 139 | 9 |
| 04:00 | 130 | 139 | 9 |
| 05:00 | 129 | 138 | 9 |

**Table 4: Experimental Readings for Mother-Baby Plastic Granule Extruder – Case 1 and Case 2 (Day 3)**

| Time | Baby Unit Inlet Temperature (°C) (Before Structure) | Baby Unit Inlet Temperature (°C) (After Structure) | Temperature Recovered (°C) |
|---|---|---|---|
| 10:00 | 128 | 137 | 9 |
| 11:00 | 128 | 138 | 10 |
| 12:00 | 130 | 138 | 8 |
| 01:00 | 131 | 139 | 8 |
| 02:00 | 131 | 141 | 10 |
| 03:00 | 132 | 142 | 10 |
| 04:00 | 132 | 142 | 10 |
| 05:00 | 131 | 141 | 10 |

**Table 5: Average Experimental Values for Case 1 and Case 2**

| | Baby Unit Inlet Temperature (˚C) | Baby Unit Outlet Temperature (˚C) | ΔT Across Baby Unit (˚C) |
|---|---|---|---|
| **Case 1 (For Three Days)** | 128.75 | 144.00 | 15.25 |
| | 129.50 | 144.00 | 14.50 |
| | 130.38 | 144.00 | 13.62 |
| **Case 1 (Average)** | 129.54 | 144.00 | 14.46 |
| **Case 2 (For Three Days)** | 138.75 | 144.00 | 5.25 |
| | 139.12 | 144.00 | 4.88 |
| | 139.73 | 144.00 | 4.27 |
| **Case 2 (Average)** | 139.00 | 144.00 | 5.00 |
| **Improvements** | Temperature increased (09.46 ˚C) | Same as maintained | Reduced load on heater |

**Table 6: Comparison of Heat transfer values of Baby Unit through conduction from barrel inner surface to raw material before heat recovery integration (Day-1)**

| Day | Time | Heat Transfer (kW) | Average (kW) |
|---|---|---|---|
| **Day 1** | 10:00 | 2.63 | 2.50 |
| | 11:00 | 2.46 | |
| | 12:00 | 2.63 | |
| | 01:00 | 2.46 | |
| | 02:00 | 2.46 | |
| | 03:00 | 2.28 | |
| | 04:00 | 2.46 | |
| | 05:00 | 2.63 | |

**Table 7: Comparison of Heat transfer values of Baby Unit through conduction from barrel inner surface to raw material before heat recovery integration (Day-2)**

| Day | Time | Heat Transfer (kW) | Average (kW) |
|---|---|---|---|
| **Day 2** | 10:00 | 2.80 | 2.37 |
| | 11:00 | 2.46 | |
| | 12:00 | 2.46 | |
| | 01:00 | 2.28 | |
| | 02:00 | 1.93 | |
| | 03:00 | 2.28 | |
| | 04:00 | 2.28 | |
| | 05:00 | 2.46 | |

**Table 8: Comparison of Heat transfer values of Baby Unit through conduction from barrel inner surface to raw material before heat recovery integration (Day-3)**

| Day | Time | Heat Transfer (kW) | Average (kW) |
|---|---|---|---|
| **Day 3** | 10:00 | 2.80 | 2.22 |
| | 11:00 | 2.46 | |
| | 12:00 | 2.46 | |
| | 01:00 | 2.28 | |
| | 02:00 | 1.93 | |

| | | | |
|---|---|---|---|
| | 03:00 | 2.28 | |
| | 04:00 | 2.28 | |
| | 05:00 | 2.46 | |

**Table 9: Heat transfer values through of Baby Unit convection from barrel inner surface to raw material before heat recovery integration (Day-1)**

| Day | Time | Heat Transfer (kW) | Average (kW) |
|---|---|---|---|
| **Day 1** | 10:00 | 0.85 | 0.81 |
| | 11:00 | 0.80 | |
| | 12:00 | 0.85 | |
| | 01:00 | 0.80 | |
| | 02:00 | 0.80 | |
| | 03:00 | 0.74 | |
| | 04:00 | 0.80 | |
| | 05:00 | 0.85 | |

**Table 10: Heat transfer values of Baby Unit through convection from barrel inner surface to raw material before heat recovery integration (Day-2)**

| Day | Time | Heat Transfer (kW) | Average (kW) |
|---|---|---|---|
| **Day 2** | 10:00 | 0.90 | 0.77 |
| | 11:00 | 0.80 | |
| | 12:00 | 0.80 | |
| | 01:00 | 0.74 | |
| | 02:00 | 0.63 | |
| | 03:00 | 0.74 | |
| | 04:00 | 0.74 | |
| | 05:00 | 0.80 | |

**Table 11: Heat transfer values of Baby Unit through convection from barrel inner surface to raw material before heat recovery integration (Day-3)**

| Day | Time | Heat Transfer (kW) | Average (kW) |
|---|---|---|---|
| **Day 3** | 10:00 | 0.85 | 0.72 |
| | 11:00 | 0.85 | |
| | 12:00 | 0.74 | |
| | 01:00 | 0.69 | |
| | 02:00 | 0.69 | |
| | 03:00 | 0.63 | |
| | 04:00 | 0.63 | |
| | 05:00 | 0.69 | |

**Table 12: Comparison of Heat transfer values of Baby Unit through conduction from barrel inner surface to raw material after heat recovery integration (Day-1)**

| Day | Time | Heat Transfer (kW) | Average (kW) |
|---|---|---|---|
| **Day 1** | 10:00 | 0.89 | 0.75 |
| | 11:00 | 0.71 | |
| | 12:00 | 0.71 | |
| | 01:00 | 0.53 | |
| | 02:00 | 0.53 | |

| | | | |
|---|---|---|---|
| | 03:00 | 0.71 | |
| | 04:00 | 0.89 | |
| | 05:00 | 1.06 | |

**Table 13: Comparison of Heat transfer values of Baby unit through conduction from barrel inner surface to raw material after heat recovery integration (Day-2)**

| Day | Time | Heat Transfer (kW) | Average (kW) |
|---|---|---|---|
| Day 2 | 10:00 | 0.89 | 0.69 |
| | 11:00 | 0.71 | |
| | 12:00 | 0.71 | |
| | 01:00 | 0.53 | |
| | 02:00 | 0.53 | |
| | 03:00 | 0.71 | |
| | 04:00 | 0.89 | |
| | 05:00 | 1.06 | |

**Table 14: Comparison of Heat transfer values of Baby Unit through conduction from barrel inner surface to raw material after heat recovery integration (Day-3)**

| Day | Time | Heat Transfer (kW) | Average (kW) |
|---|---|---|---|
| Day 3 | 10:00 | 1.06 | 0.58 |
| | 11:00 | 0.89 | |
| | 12:00 | 0.89 | |
| | 01:00 | 0.71 | |
| | 02:00 | 0.36 | |
| | 03:00 | 0.18 | |
| | 04:00 | 0.18 | |
| | 05:00 | 0.36 | |

**Table 15: Heat transfer values of Baby Unit through convection from barrel inner surface to raw material after heat recovery integration (Day-1)**

| Day | Time | Heat Transfer (kW) | Average (kW) |
|---|---|---|---|
| Day 1 | 10:00 | 0.29 | 0.25 |
| | 11:00 | 0.23 | |
| | 12:00 | 0.23 | |
| | 01:00 | 0.18 | |
| | 02:00 | 0.18 | |
| | 03:00 | 0.23 | |
| | 04:00 | 0.29 | |
| | 05:00 | 0.35 | |

**Table 16: Heat transfer values of Baby Unit through convection from barrel inner surface to raw material after heat recovery integration (Day-2)**

| Day | Time | Heat Transfer (kW) | Average (kW) |
|---|---|---|---|
| Day 1 | 10:00 | 0.29 | 0.23 |
| | 11:00 | 0.23 | |
| | 12:00 | 0.23 | |
| | 01:00 | 0.18 | |
| | 02:00 | 0.12 | |

| | | | |
|---|---|---|---|
| | 03:00 | 0.23 | |
| | 04:00 | 0.23 | |
| | 05:00 | 0.29 | |

**Table 17: Heat transfer values of Baby Unit through convection from barrel inner surface to raw material after heat recovery integration (Day-3)**

| Day | Time | Heat Transfer (kW) | Average (kW) |
|---|---|---|---|
| Day 3 | 10:00 | 0.35 | 0.19 |
| | 11:00 | 0.29 | |
| | 12:00 | 0.29 | |
| | 01:00 | 0.23 | |
| | 02:00 | 0.12 | |
| | 03:00 | 0.06 | |
| | 04:00 | 0.06 | |
| | 05:00 | 0.12 | |

**Table 18: Values of Q with baby unit inlet temperature before and after heat recovery integration for the day-1 (value of m is taken as a 80 kg/hr as per extruder capacity)**

| Day | Time | Baby Inlet Temperature Before Structure | Baby Inlet Temperature After Structure | ΔT (Before) | ΔT (After) | Q in kWh/hr (Before) | Q in kWh/hr (After) |
|---|---|---|---|---|---|---|---|
| Day 1 | 10:00 | 128 | 138 | 16 | 6 | 0.746 | 0.280 |
| | 11:00 | 129 | 139 | 15 | 5 | 0.700 | 0.233 |
| | 12:00 | 128 | 139 | 16 | 5 | 0.746 | 0.233 |
| | 01:00 | 129 | 140 | 15 | 4 | 0.700 | 0.187 |
| | 02:00 | 129 | 140 | 15 | 4 | 0.700 | 0.187 |
| | 03:00 | 130 | 139 | 14 | 5 | 0.653 | 0.233 |
| | 04:00 | 129 | 138 | 15 | 6 | 0.700 | 0.280 |
| | 05:00 | 128 | 137 | 16 | 7 | 0.746 | 0.326 |

**Table 19: Values of Q with baby unit inlet temperature before and after heat recovery integration for the day-2 (value of m is taken as a 80 kg/hr as per extruder capacity)**

| Day | Time | Baby Inlet Temperature Before Structure | Baby Inlet Temperature After Structure | ΔT (Before) | ΔT (After) | Q in kWh/hr (Before) | Q in kWh/hr (After) |
|---|---|---|---|---|---|---|---|
| Day 2 | 10:00 | 127 | 138 | 17 | 6 | 0.793 | 0.280 |
| | 11:00 | 129 | 139 | 15 | 5 | 0.700 | 0.233 |
| | 12:00 | 129 | 139 | 15 | 5 | 0.700 | 0.233 |
| | 01:00 | 130 | 140 | 14 | 4 | 0.653 | 0.187 |
| | 02:00 | 132 | 141 | 12 | 3 | 0.560 | 0.140 |
| | 03:00 | 130 | 139 | 14 | 5 | 0.653 | 0.233 |
| | 04:00 | 130 | 139 | 14 | 5 | 0.653 | 0.233 |
| | 05:00 | 129 | 138 | 15 | 6 | 0.700 | 0.280 |

**Table 20: Values of Q with baby unit inlet temperature before and after heat recovery integration for the day-3 (value of m is taken as a 80 kg/hr as per extruder capacity)**

| Day | Time | Baby Inlet Temperature Before Structure | Baby Inlet Temperature After Structure | ΔT (Before) | ΔT (After) | Q in kWh/hr (Before) | Q in kWh/hr (After) |
|---|---|---|---|---|---|---|---|
| Day 3 | 10:00 | 128 | 137 | 16 | 7 | 0.746 | 0.326 |
| | 11:00 | 128 | 138 | 16 | 6 | 0.746 | 0.280 |
| | 12:00 | 130 | 138 | 14 | 6 | 0.653 | 0.280 |
| | 01:00 | 131 | 139 | 13 | 5 | 0.606 | 0.233 |
| | 02:00 | 131 | 141 | 13 | 3 | 0.606 | 0.140 |
| | 03:00 | 132 | 142 | 12 | 2 | 0.560 | 0.093 |
| | 04:00 | 132 | 142 | 12 | 2 | 0.560 | 0.093 |
| | 05:00 | 131 | 141 | 13 | 3 | 0.606 | 0.140 |

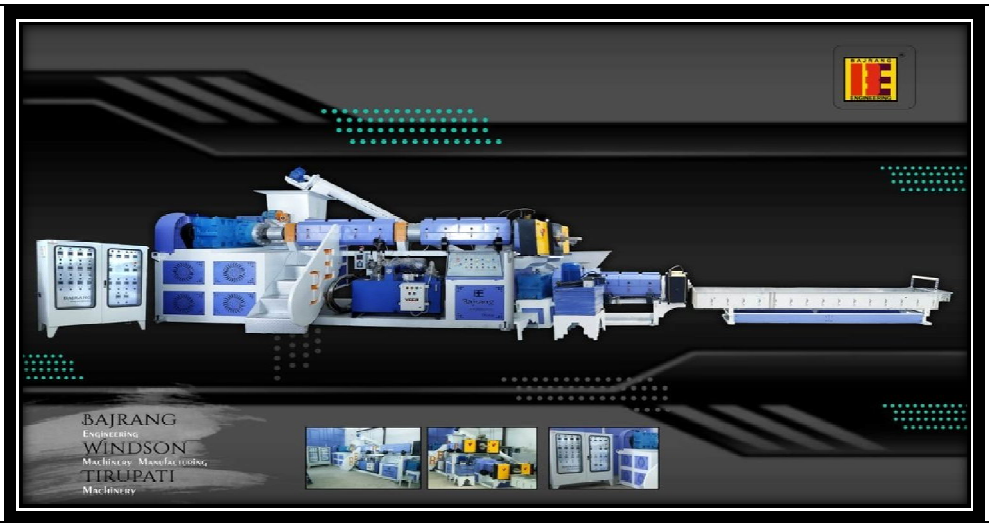



**Figure 1: Complete view of the industrial Mother-Baby Plastic Granule Extruder System**




**Figure 2: Overall view of Mother-Baby Plastic Granule Extruder (Industrial Setup)**



**Figure 3: Open Space between Mother and Baby Unit before Heat Recovery Structure Integration**



**Fig. 4: Designed Heat Recovery Structure Using CRC Sheet with Red Oxide Coating with all views**




**Fig. 5: Assembled view of Mother unit, Heat Recovery Structure and Baby unit (Integrated Setup)**




**Figure 6: Schematic Diagram of Random Temperature Sensor Placement**

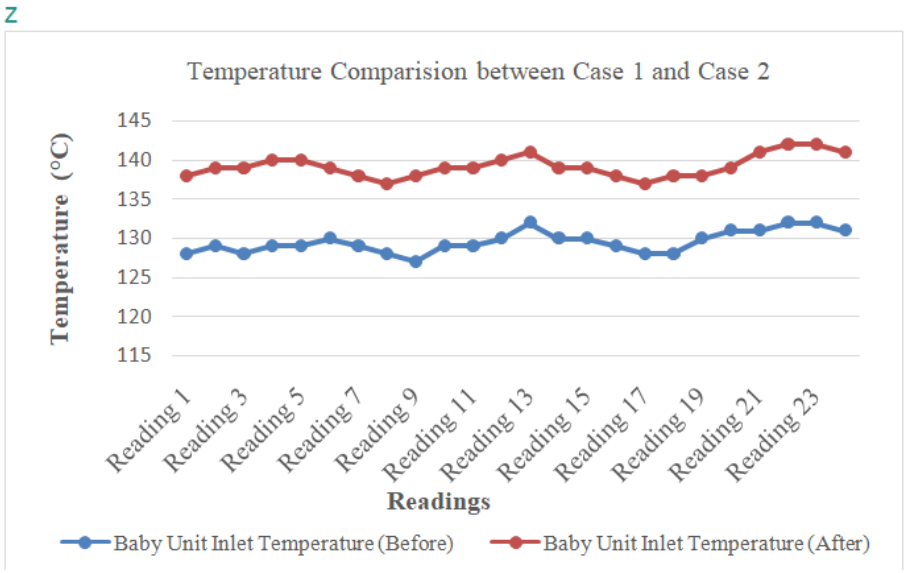



**Figure 7: Time wise Comparison of Baby Unit Inlet Temperature before Structure and After Structure**

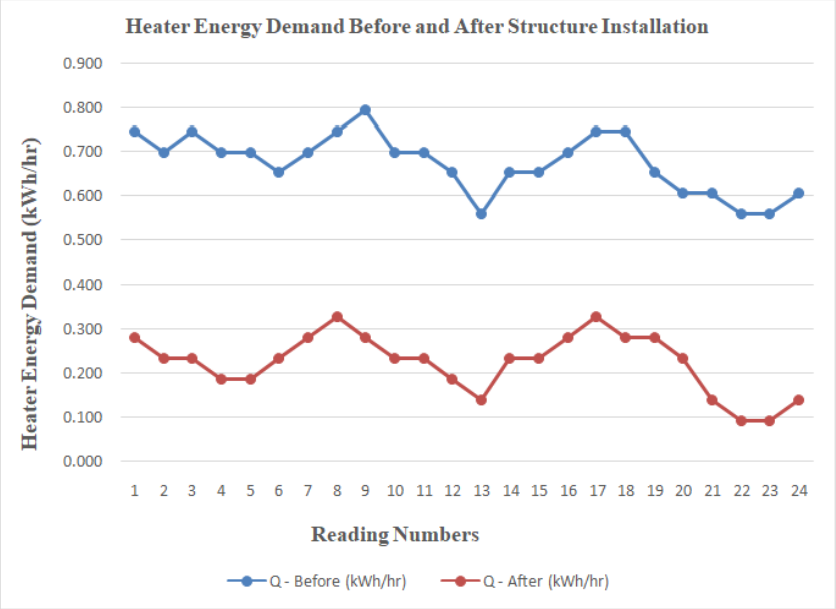



**Figure 8: Heater energy demand comparison of baby unit inlet temperature before and after heat recovery integration**

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RESEARCH ARTICLE

# Development and Quality Evaluation of Black Rice Muffins Enriched with Banana Flour

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## ABSTRACT

Black rice (*Oryza sativa* L.) bran is rich in dietary fibre and bioactive phytochemicals, including tocopherols, tocotrienols, γ-oryzanol, vitamins, and phenolic compounds, which contribute to various health benefits. Unripe banana flour (UBF), a rich source of resistant starch, has been reported to exert several positive physiological effects in clinical studies. Muffins were prepared using black rice flour (BRF) and UBF in different proportions, namely 100:0, 90:10, 80:20, and 70:30 (BRF:UBF). The developed muffins were evaluated for sensory attributes and nutritional quality using standard analytical procedures. Sensory evaluation revealed that muffins containing BRF:UBF at 90:10 received the highest scores for colour and appearance (7.63), whereas muffins formulated with a 70:30 ratio exhibited superior scores for texture (7.49), flavour (7.40), and overall acceptability (7.35). Nutritional analysis indicated that muffins prepared with a 70:30 ratio of black rice flour to unripe banana flour (BRF:UBF) exhibited the highest protein content (10.27%), the lowest fat content (13.58%), and the highest total dietary fibre content (9.10%), comprising 7.01% insoluble fibre and 1.41% soluble fibre, along with a moderate calorific value of 344 kcal/100 g. In comparison, muffins prepared with 100% black rice flour (BRF) contained 9.72% protein, 14.38% fat, and 8.31% total dietary fibre, comprising 6.86% insoluble fibre and 1.14% soluble fibre, with a higher energy value of 357 kcal/100 g. Findings suggest that black rice muffins fortified with unripe banana flour, can serve as a functional bakery product with enhanced dietary fibre and potential health benefits, suitable for health-conscious consumers.

**Keywords:** Black rice muffin, unripe banana flour, nutritional analysis, phytochemicals

## INTRODUCTION

In this modernized world, the lifestyle of people has increased in pace and changes in eating behavior have adversely affected health and the risk of heart attacks and hypertension. The stress of having a busy lifestyle induces free radical release as a cause of cancer, diabetes, mental disorders such as Alzheimer's disease, ischemic diseases, heart failure, high blood pressure, adult respiratory distress syndrome, heart failure, high blood pressure, inflammatory diseases, and AIDS. The prevention of these conditions can be achieved by consuming foods rich in antioxidants (Deeseenthum *et al.* 2018; Kalogerakou, T., and Antoniadou, M., 2024). Black rice, a type of specialty rice, is milled as brown rice, where only the hull is removed; as compared to white rice, black rice contains high amounts of nutrients, such as fiber, vitamins, minerals, vitamin B complex, and unsaturated fatty acid (Park *et al.,* 2016). Furthermore, it contains diverse bioactive substances which exert antioxidative effects on the body. Anthocyanins contained in rice pericarp have been reported to exhibit various functional effects, such as antioxidative effects (Park *et al.,* 2016), as well as the inhibition of DNA damage. As a result, black rice is predicted to be very useful for the manufacturing and development of food. Muffins are semi sweet baked products highly appreciated by consumers because of their soft texture and characteristic taste or quick bread that is baked in appropriate proportion. They are similar to cupcakes, although they are less sweet (Limbachiya and Amin 2015).

Black Rice which is also known as Purple Rice and Asian rice hails from the Oryza Sativa specie. Black rice is majorly cultivated in Asian countries like China, Indonesia, Myanmar and India among others. In India, it is majorly grown in the eastern region and is popularly known as Chak-hao in Manipur (Thomas *et al*., 2013). The interest in black rice growing because is gluten free, cholesterol- free and low in sugar, salt and fat. Among these properties it also contains anthocyanins, antioxidants, B and E vitamin, iron, thiamine, magnesium, niacin and phosphorous and high fibre content. There are a lot of studies showing that black rice powder is one of nature's most well-balanced foods. Black rice is a super nutritious type of rice that is high in protein, fats, carbohydrates, zinc, iron, manganese; copper (S. Kumar, 2016). Black color of this rice is due to the presence of flavonoids pigments called "anthocyanins" which are powerful antioxidants equivalent to that of blue and black berries and have the ability to inhibit the formation or to reduce the concentrations of reactive cell damaging free radicals (Kushwaha, 2016).

Banana (*Musa paradisiaca*) is one of the commodities that have a very high chance for diversification, food security and agribusiness in Indonesia. It is also a fast growing and high biomass yielding crop in the tropical and sub-tropical regions of the world. The major banana producing states in India are Maharashtra, Kerala, Tamil Nadu, Gujarat, Bihar, West Bengal, Assam, Andhra Pradesh and Karnataka (Bezerra *et al*. 2013). Bananas can be processed and preserved to various forms of processed products such as banana flour, banana juice, banana wine, banana chips, banana sauce and banana jam and other processed. Banana flour (BF) is a product between the prospective in the development of local food source. Bananas are quite suitable for processing into flour as the main constituent components are carbohydrates. The green banana flours, mainly unpeeled, are good sources of fiber and resistant starch. The results showed that unpeeled green banana flour obtained by spouted bed drying can be a valuable tool to add nutritional value to products in order to increase their non-digestible fraction (Bezerra *et al.* 2013).

With the increase of the consumer demand for healthier and nutritious food production, this product which can be eaten due to their good taste and soft texture, perfect for breakfast, brunch, or snack, can be consumption by celiac patients as the product is naturally gluten-free, cholesterol free and contains proteins that are known to be high in biological value due to the absence of gluten forming ability, low levels of sodium and fat, but high level of protein, iron and antioxidants activity. It also has high amount of easily digestible carbohydrates (Jha *et al*., 2017). Since black rice possess unique nutritional, hypoallergenic and bland taste. The demand for low gluten and gluten-free products is increasing because it is well known that celiac disease is a common lifelong disorder, affecting 1% of the world's population. Therefore, Black rice muffins were prepared with combination of banana flour.

## MATERIALS AND METHODS

All raw materials required for muffin preparation were procured from the local market of Gurugram, Haryana, India. The primary ingredients used in the formulation were black rice flour and banana flour. Other ingredients included cocoa powder, ghee, milk, sugar, baking powder, baking soda, vanilla essence, and sesame seeds. Black rice flour and banana flour were blended in different proportions to develop various muffin formulations. Initially, ghee and sugar were creamed thoroughly until a uniform mixture was obtained. Baking powder, baking soda, cocoa powder, and sesame seeds were then incorporated into the mixture and mixed evenly. Milk was gradually added with continuous mixing to obtain a smooth batter consistency. The blended black rice flour and banana flour were added alternately to the batter along with vanilla essence and mixed for 2–3 minutes to ensure uniform distribution. The prepared batter was poured into mini paper cups placed on a baking tray, and sesame seeds were sprinkled on the surface as a topping. The oven was preheated, and baking was carried out at 180 °C for 25–30 minutes. After baking, the muffins were removed from the oven and allowed to cool at room temperature prior to further analysis. The procedural steps involved in muffin preparation are illustrated in the flow diagram shown in Fig. 1.

The developed value-added black rice muffins were subjected to organoleptic evaluation for colour and appearance, flavour, texture, and overall acceptability using a 9-point Hedonic scale (Srilakshmi, 2007). Based on organoleptic acceptability, selected muffin samples were further analyzed for their nutritional composition. Black rice flour was blended with banana flour at 10%, 20%, and 30% substitution levels to develop value-added muffin formulations. Black rice flour, banana flour, and the developed muffins were analyzed for proximate composition and dietary fibre content. Proximate composition, including moisture, crude protein, crude fat, ash, and crude fibre, was determined using standard methods of analysis (AOAC, 2000). Moisture content was measured by the hot air oven method at 105 °C until a constant weight was obtained. Crude protein content was estimated using the KEL PLUS Automatic Nitrogen Estimation System, and values were converted to protein using a nitrogen-to-protein conversion factor of 6.25. Ash content was determined by incineration in a muffle furnace at 550 °C for 4–6 hours. Crude fat was analyzed using the SOCS Plus Automatic Solvent Extraction System with petroleum ether as the solvent. Total carbohydrates were calculated by difference using the following formula: Total carbohydrates (%) =100−(Crude protein + Crude fat + Crude fibre + Ash) The total energy value of the samples was calculated theoretically using Atwater conversion factors of 4.0 kcal/g for protein, 4.0 kcal/g for carbohydrates, and 9.0 kcal/g for fat, as described by Paul and Southgate (1979). Total, soluble, and insoluble dietary fibre contents were determined using the enzymatic method described by Furda (1981). Total dietary fibre was calculated as the sum of insoluble and soluble dietary fibre using the formula: Total dietary fibre=Insoluble dietary fibre+Soluble dietary fibre.

All analyses were performed in triplicate, and results are expressed on a dry weight basis as mean ± standard error (SE). Total energy value was calculated theoretically using Atwater conversion factors of 4.0 kcal/g for protein, 4.0 kcal/g for carbohydrates, and 9.0 kcal/g for fat, as described by Paul and Southgate (1979). Total, soluble, and insoluble dietary fibre contents were determined using the enzymatic method described by Furda (1981). Total dietary fibre was calculated as the sum of insoluble and soluble dietary fibre: Total dietary fibre = Insoluble dietary fibre + Soluble dietary fibre. All analyses were conducted in triplicate, and the results were expressed as mean ± standard error.

## RESULTS AND DISCUSSION

The results of the sensory evaluation (Table 1) indicated that the incorporation of banana flour into black rice muffins did not adversely affect consumer acceptability. The control muffins recorded a mean score of 7.25 for color and appearance, corresponding to “liked moderately” on the hedonic scale. Muffins supplemented with banana flour at 10, 20, and 30 per cent levels showed comparable scores of 7.63, 7.36, and 7.35, respectively, suggesting no marked differences in visual acceptability among the formulations. The texture score of the control muffins was 7.15, indicating moderate liking. Similarly, muffins containing banana flour exhibited texture scores of 7.35, 7.01, and 7.49

at 10, 20, and 30 per cent supplementation levels, respectively, all of which fell within the "liked moderately" category, demonstrating that banana flour incorporation did not negatively influence textural properties. For flavor, the control muffins achieved a mean score of 7.22. The banana flour–supplemented muffins recorded comparable flavor scores of 7.35, 7.40, and 7.35 at 10, 20, and 30 per cent levels, respectively, indicating the retention of desirable flavor characteristics. Overall acceptability scores followed a similar trend. The control muffins obtained a mean score of 7.15, while muffins supplemented with banana flour recorded scores of 7.27, 7.05, and 7.35 at 10, 20, and 30 per cent levels, respectively. These findings demonstrate that banana flour supplementation up to 30 per cent can be effectively incorporated into black rice muffins without compromising sensory quality, supporting its potential application for nutritional enhancement in baked products.

The proximate composition of the value-added muffins (Table 2) showed notable changes with the incorporation of unripe banana flour. The moisture content of the control black rice muffins was 18.91 per cent, whereas muffins supplemented with unripe banana flour at 10, 20, and 30 per cent levels exhibited higher moisture contents of 20.80, 22.73, and 24.61 per cent, respectively. The observed increase in moisture content with increasing levels of banana flour supplementation may be attributed to the higher water-holding capacity associated with the dietary fibre and starch components of banana flour. The protein content of the control muffins was 9.72 per cent. A gradual increase in protein content was observed in banana flour–supplemented muffins, with values of 9.92, 10.11, and 10.27 per cent at 10, 20, and 30 per cent supplementation levels, respectively, indicating a modest improvement in protein content with increasing substitution. The fat content of the control muffins was 14.38 per cent. Muffins supplemented with banana flour showed comparable fat contents of 14.89, 14.92, and 13.58 per cent at 10, 20, and 30 per cent levels, respectively, with a slight reduction observed at higher levels of supplementation. The carbohydrate content of the control muffins was 47.21 per cent. A decreasing trend in carbohydrate content was noted with increasing levels of banana flour incorporation, with corresponding values of 46.69, 46.13, and 45.19 per cent at 10, 20, and 30 per cent supplementation levels, respectively. Overall, the incorporation of unripe banana flour enhanced moisture retention and slightly increased protein content, while marginally reducing fat and carbohydrate contents. These compositional changes indicate the potential of unripe banana flour to improve the nutritional profile of black rice muffins without compromising product quality.

The energy content of control sample was observed 357.14 kcal/100 g. The energy content was highest in muffins supplemented with 10 per cent unripe banana flour (360.45 kcal/100 g), representing a 5.92 per cent increase compared with the control. In contrast, total dietary fibre content was highest in muffins supplemented with 30 per cent unripe banana flour, reflecting the fibre-rich nature of banana flour and its contribution to nutritional enhancement at higher substitution levels. The dietary fibre content of black rice muffins supplemented with unripe banana flour (Table 3.) reveals that total dietary fibre increased with higher levels of banana flour supplementation, ranging from 8.31% in the control muffins to 9.10% in muffins containing 30% unripe banana flour. Soluble dietary fibre gradually increased with supplementation, from 1.14% in the control to 1.32% and 1.41% in muffins containing 20% and 30% banana flour, respectively. Insoluble dietary fibre was highest in the 30% supplemented muffins (7.01%) compared with 6.86% in the control, while the 20% supplemented muffins showed a comparatively lower insoluble fibre value (6.72%). Overall, incorporation of unripe banana flour improved total and insoluble dietary fibre content, demonstrating its potential to enhance the nutritional quality of black rice muffins.

## CONCLUSION

Banana flour is a nutritionally rich ingredient, containing resistant starch, dietary fibre, polyphenols, and antioxidants, which contribute to its potential health benefits, including improved gut health, glycemic management, and weight control. Incorporation of banana flour into baked products, such as black rice muffins, at levels of 10%, 20%, and 30% enhanced their nutritional quality without compromising sensory acceptability. The study demonstrates that banana flour can be successfully utilized to develop value-added muffins with increased dietary

fibre and functional properties, offering a practical approach to producing healthier bakery products suitable for health-conscious consumers and individuals managing diabetes.

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**Table1. Mean score of sensory attributes of the black rice muffins supplemented with unripe banana flour**

| Types of muffins | Color and appearance | Texture | Flavour | Overall acceptability |
|---|---|---|---|---|
| **Control (100% BRF)** | 7.25 ± 1.45 | 7.15 ± 1.30 | 7.22 ± 1.57 | 7.15 ± 1.30 |
| **Supplementation level (%) BRF:UBF** | | | | |
| 90:10 | 7.63 ± 1.18 | 7.35 ± 1.18 | 7.35 ± 1.26 | 7.27 ± 1.10 |
| 80:20 | 7.36 ± 0.87 | 7.01 ± 1.02 | 7.35 ± 0.98 | 7.05 ± 0.99 |
| 70:30 | 7.35 ± 1.13 | 7.49 ± 0.06 | 7.40 ± 0.05 | 7.35 ± 0.05 |

**Values are mean ± SE of three independent determinations. BRF-Black rice flours, UBF-Unripe banana flour.**

**Table 2.Proximate composition (%) and energy (Kcal/100g) content of the black rice muffins supplemented with unripe banana (Dry matter basis)**

| Type of muffin | Moisture | Protein | Fat | Carbohydrates | Ash | Energy |
|---|---|---|---|---|---|---|
| **Control (100% BRF)** | 18.91 ± 0.33 | 9.72 ± 0.24 | 14.38 ± 0.29 | 47.21 ± 0.12 | 1.90 ± 0.30 | 357.14 ± 0.14 |
| **Supplementation level (%) BRF:UBF** | | | | | | |
| 90:10 | 20.80 ± 0.07 | 9.92 ± 0.35 | 14.89 ± 0.25 | 46.69 ± 0.14 | 2.20 ± 0.30 | 360.45 ± 0.12 |
| 80:20 | 22.73 ± 0.19 | 10.11 ± 0.34 | 14.92 ± 0.42 | 46.13 ± 0.21 | 2.69 ± 0.08 | 343.42 ± 0.16 |
| 70:30 | 24.61 ± 0.21 | 10.27 ± 0.33 | 13.58 ± 0.08 | 45.19 ± 0.35 | 2.53 ± 0.15 | 344.06 ± 0.38 |

**Values are mean ± SE of three independent determinations. BRF-Black rice flours, UBF-Unripe banana flour.**

**Table3.Dietary fibre (%) of the black rice muffins supplemented with unripe banana (Dry matter basis)**

| Types of muffin | Total dietary fibre | Soluble fibre | Insoluble fibre |
|---|---|---|---|
| **Control (100% BRF)** | 8.31 ± 0.74 | 1.14 ± 0.51 | 6.86 ± 0.17 |
| **Supplementation level (%) BRF:UBF** | | | |
| 90:10 | 8.52 ± 0.36 | 1.19 ± 0.36 | 6.72 ± 0.64 |
| 80:20 | 8.89 ± 0.15 | 1.32 ± 0.12 | 1.32 ± 0.12 |
| 70:30 | 9.1 ± 0.18 | 1.41 ± 0.17 | 7.01 ± 0.13 |

**Values are mean ± SE of three independent determinations. BRF-Black rice flours, UBF-Unripe banana flour.**

Mixing
(Black rice flour and unripe banana flour in different ratios)
⇩
Creaming of ingredients
(Ghee, baking soda, baking powder, cocoa powder, sesame seed, sugar, and vanilla essence)
⇩
Muffin batter preparation
(Milk added gradually to obtain a smooth cream)
⇩
Filling
(Batter poured into mini paper cups placed on baking tray)
⇩
Baking
(Baked at **180 °C for 25–30 minutes)**
⇩
Cooling
(Cooled at room temperature)

**Fig.1.Flow diagram for muffin preparation**

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RESEARCH ARTICLE

# Content and Face validity of Panoramic Varicose Vein Index (PVVI) : A Critical Evaluation

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## ABSTRACT

A significant portion of the world's population suffers from varicose veins (VV), a common vascular disorder that is typified by enlarged, twisted veins, usually in the lower limbs. Although VV are sometimes viewed as a cosmetic issue, they can result in serious practical constraints like discomfort, swelling, and decreased movement, which can lower the quality of life for those who have them. It is crucial to evaluate the functional impact of the illness in clinical settings since these restrictions may hinder everyday activities and physical functioning. Even though VV are common, there aren't many standardized instruments made specially to assess the functional constraints that patients face. Panoramic varicose vein index (PVVI) was developed to assess the functional limitations resulting from VV. PVVI was developed with 20 items for pain and sensitivity, activity limitation and performance and social life based on far-reaching review of varicose vein. Four experts used a validation form to evaluate each item's content relevance to the targeted concept. The experts' agreement over each item's relevance

to the target construct was evaluated using the Content Validity Index (CVI). Ten physiotherapy clinicians practicing cardiovascular field were then chosen at random to participate in face validation, where they used a face validation form to score each item's comprehension and clarity. Each question's clarity and comprehension were judged via Item Face Validity Index (I-FVI), and items with an I-FVI ≥ 0.82 were considered. PVVI achieved admirable content validity in both activity limitation and performance and social life domains. 7 items from the activity limitation domain and 3 items from the performance and social life domain were reserved after CVI breakdown. All reserved items scored a CVI score of 1.00, demonstrating perfect covenant among the experts. FVI analysis stemmed in 5 items for the activity limitation domain and 2 items for the performance and social life domain. The activity limitation domain achieved a higher level of covenant among defendants, with a mean I-FVI of 0.90 and a S-FVI/UA of 0.87. Performance and social life domain also showed higher level of covenant, with a mean I-FVI of 0.97 and a S-FVI/UA of 0.81. To sum up, the PVVI showed strong face validity and substance..

**Keywords:** Varicose vein, content validity, face validity, functional limitation.

## INTRODUCTION

A prevalent vascular condition that mostly affects the lower limbs aka varicose veins (VV) are characterized by abnormal vein enlargement and tortuosity. In addition to being common in India, this illness has a major negative impact on world health. An estimated 30% of Indians are thought to have varicose veins, especially women. Age, obesity, and jobs requiring a lot of standing, such teaching and cooking, are risk factors for this condition. About 23% of persons worldwide suffer with the illness, with higher incidence rates seen in women and older adults in the US. Health care resources are heavily burdened by the rising prevalence of CVD and the high cost of treatment[1-3]. The central cause of VV seems to be venous disorder which leads to reflux of valves[4]. Even though the precise aetiology is still up for question, it includes negotiated arterial walls, defective valves, preeminent intravenous pressure, and a genetic susceptibility. The lack of vein wall flexibility, which results in the valve leaflets' inability to fit together, is typically thought to be the cause of valvular dysfunction. A faulty or ineffective valve allows blood to flow in reverse, from superficial to deep and distal to proximal. The loftier veins may grow longer and more tortuous as a result of increased strain on the venous system in question. Inflammation and shear stress on venous endothelial cells brought on by reversible or turbulent blood flow are significant causative factors for venous diseases[5]. Leg varicose veins can affect the small and great saphenous veins, which are primary axial superficial veins, or their surface-level tributaries)[6]. Known risk factors With relation to varicose veins, such as family history of venous illness; elder age; feminine sex; persistently elevated blood pressure inside the abdomen as a result of obesity, pregnancy, a tumor, or persistent constipation; extended standing; thrombosis in the deep veins resulting in secondary revascularization and valve degradation; Arteriovenous shunting is another[7,8]. Patient related outcome measures (PROM) are readily used in practice to quantify quality of life (QOL) in patients living with vv's. AVVQ (Aberdeen Varicose Vein Questionnaire), EuroQol Group's EQ-5D and Venous Insufficiency Epidemiological and Economic Study (VEINES-QOL/Sym) are frequently used tools to measure the health related QOL in patients living with vv's. Another questionnaire titled "Chronic Venous Insufficiency Questionnaire (CIVIQ)" covers symptoms, physical limitations, emotional well-being, and social functioning apart from HRQOL. Venous Clinical Severity Score (VCSS) is not necessarily a questionnaire but a scoring tool which tell about the clinical parameters such as pain, varicosities, edema, skin changes, and ulceration. On extensive research we found that there's no single questionnaire which exclusively talks about the functional limitations of patient living with vv's. A functional limitation assessment is necessary for a physical therapist to not only treat but also to plan out a tailor-made rehab program for the patient. Thus, the purpose of this publication is to develop the Panoramic Varicose Vein Index (PVVI), a tool for functional evaluation of patients with varicose veins, and examine its face validity and content.

## METHODOLOGY

**Scale Development**

The Panoramic Varicose Vein Index was developed with 3 domains: Pain and sensation, Activity Limitation and Performance and Social Life with 7, 5 and 2 items respectively. The literature search included all leading databases like Scopus, Pubmed, Web of Science, Google Scholar intended at reconnoitering relevant available literature. The search expressions included varicose vein, questionnaire, development and validation. It's crucial to remember that the review's main goal was to obtain a broad understanding of the topic rather than carrying out an organized, thorough analysis using precise search parameters. The results of this exploratory search were crucial in forming PVVI, offering insightful information that resulted in a sophisticated comprehension of the constructs pertinent to the functional confines faced by the patients of varicose vein. Domain Activity Limitation and Performance and Social Life are particularly dedicated to finding out the functional confines a patient experience along with the aesthetic problems.

**Content Validity (CV)**

To certify the CV of PVVI, an exhaustive validation procedure was carried out. The assortment of experts for this process was done with vigilance. A panel of 4 experts was carefully chosen depending on their rich clinical background and exhaustive knowledge about the venous disorders. The panel involved connoisseurs with background in Vascular and cardiothoracic physiotherapy. Additionally, a cardiothoracic surgeon was also included in the panel to get the surgeon's perspective towards the questionnaire. A content validation form comprising a gradient of elements and a evaluation scale was created prior to the content validation process. The purpose of the form was to evaluate how well each scale item related to the intended construct—that is, PVVI, Pain and sensations, Activity Limitation and Performance and Social Life. From 1 (not relevant) to 4 (very relevant), the rating scale went from 1 to 4. The scale's goal and the content validation procedure were explained to the experts. After appraising the domain and items, they were requested to remark on their ambiguity, simplicity, clarity, and relevancy. Based on how relevant each item was to the targeted construct, each expert assigned a total. Each item's CV Index (CVI) was then determined. One popular technique for assessing a scale's CV is the CVI. It is calculated by taking the total number of experts and dividing that number by the number of experts who rated an item as 3 or 4.When it comes to Universal Agreement (UA), an item that received 100% agreement by expert receives a score of "1," while items that couldn't get 100% expert agreement receive a score of "0." Additionally, the mean I-CVI was determined by dividing the total number of items by the sum of the Item I-CVI scores for every item. The percentage of professionals who gave an item an appropriate rating is known as I-CVI. Additionally, for each of the three domains, the Scale-level CVI/Unweighted Average (S-CVI/UA) and Scale-level CVI/Average (S-CVI/AVE) were computed. The percentage of items that obtained a CVI of 1.00 is represented by the S-CVI/UA, while the average CVI for each item is represented by the S-CVI/AVE. The number of elements that all experts classified as "relevant" was divided by the total items to determine the S-CVI/UA. By adding up all of the I-CVIs of the things that were deemed "relevant" and dividing by the total items, the S-CVI/AVE was determined. To make sure the scale had sufficient content validity, these computations were carried out. Products with a CVI less than one were deemed problematic and discarded[9-11].

**Face Validity**

A face validation procedure was carried out in order to further guarantee the authenticity of the PVVI. This procedure was designed to evaluate the items' clarity and comprehension and make sure that respondents understood them as the scale's creators intended. Only the elements kept after content validation were included in the face validation form that was created before the face validation was carried out. On a four point Likert scale, with 1 denoting "not clear/comprehensible" and 4 denoting "very clear/comprehensible," the respondents were asked to score the clearness and comprehension of each question on the form. The chosen respondents were sent the face validation form via their management office in order to complete the face validation procedure. After reading each question, the respondents were asked to score its understanding and clarity according to how they understood it. Additionally, they were requested to remark on any items that were unclear or ambiguous. Following the completion

of the face validation procedure, the items were examined for understanding and clarity. The Item Face Validity Index (I-FVI) for every question was determined by calculating the percentage of respondents who gave it a clear and understandable rating (scores of 3 or 4). The total of respondents who thought the item was clear and understandable was divided by the total of respondents to determine the I-FVI. The average of the I-FVI values for each item was then used to determine the Mean I-FVI[12]. The total of items that received an I-FVI value of three or four was divided by the total of items to conclude the Scale Face Validity Index (S-FVI/UA). A general assessment of FV of the scale is given by S-FVI/UA. Furthermore, the mediocre of the I-FVI scores for every item was used to compute the S-FVI/AVE. Because it accounts for the potential for chance agreement among respondents, this offers an additional cautious estimation of the scale's FV. Lastly, the I-FVI values for each question were summed up, and the value was divided by the number of potential I-FVI values to determine the Proportion Relevance. This shows the percentage of the scale's items that are pertinent to the construct being measured. A score of one indicated complete precision and unambiguousness on the FVI scale, which went from 0 to 1. Items deemed unclear and incomprehensible were eliminated if their FVI was less than 0.83[12,13].

**Data Analysis**

IBM SPSS Statistics version 29 was used to do a descriptive analysis of demographic characteristics. Microsoft Excel was used to perform the calculations for face and content validation. Item Content Validity Index (I-CVI), Universal Agreement (UA), Mean I-CVI, Scale-level Content Validity Index/Unweighted Average (S-CVI/UA), and Scale-level Content Validity Index/Average (S-CVI/AVE) were all calculated for content validation. Universal Agreement (UA) was used for face validation after the Item Face Validity Index (I-FVI) was established. to determine the Unweighted Scale-level Face Validity Index The Scale-level Face Validity Index and Average (S-FVI/UA) S-FVI/AVE is the average. The choices on the retention of the items were mainly based on the numerical results derived from these analyses, guaranteeing a thorough and uniform assessment of the quality of each item.

## RESULTS

Content Validation of Pain and Sensation (Table 1).
Content Validation of Activity Limitation (Table 2).
Content Validation of Performance and Social life (Table 3).

**Content Validity**

The results of content validity of PVVI are depicted in Table 1, 2 and 3 for Pain and Sensation, Activity limitation and Performance and social life domains. Each item was given a four-point rating by the experts, with 1 denoting "not relevant" and 4 denoting "highly relevant." The "Agreement Number" column shows how many experts gave each item a significance rating of 3 or 4. All four experts gave the retained items in the PVVI's Pain and Sensation, Activity Limitation, and Performance and Social Life domains a CVI score of 1.00, signifying full bargain among the panel of specialists, as seen in Tables 1, 2, and 3. The content validity of the scale is indicated by the CVI score, which shows the percentage of specialists who thought an item was significant. The highest possible CVI score is 1.00, which means that every expert thought the item was extremely significant. The no. of specialists who graded an item as significant was divided by the total no. of specialists to determine each item's individual CVI (I-CVI) score. According to Table 1, all seven items had an I-CVI score of 1.00, meaning that every expert thought they were extremely relevant. Excellent agreement among the specialists about the items' relevance in the pain and sensation domain was indicated by the mean I-CVI score, which is the average of the I-CVI scores across all items. This score was also assessed and came out to be 1.00. The S-CVI/AVE score shows the average CVI scores for all items, whereas the UA score shows the percentage of things that had a perfect CVI score of 1.00. According to Table 1, all seven items received a perfect CVI score of 1.00, which translated into a perfect UA score of 1.00. This indicates that the panel of experts completely agreed that the items were relevant to the pain and sensations domain. Excellent content validity for the PVVI's pain and sensations domain was indicated by the S-CVI/AVE score of 1.00.

**Face Validity**

The FVI scores for Pain and Sensation, Activity Limitation, and Performance and Social Life categories are shown in Tables 4,5 and 6. On scale of 1 (not clear/comprehensible) to 4 (extremely clear/comprehensible), the FVI gauges respondents' agreement with the clarity and comprehensibility of each item in the domains. The Universal Agreement (UA) index, Item-Level Content Validity Index (I-FVI), mean I-FVI, Scale-Level Content Validity Index/Average (S-FVI/AVE), Scale-Level Content Validity Index/Universal Agreement (S-FVI/UA), and the number of respondents who agreed on the clarity and comprehension of each item (Number in Agreement) are all included in the tables. For the pain and sensation domain, the scores point out that among the retained 7 items, most items received high levels of agreement, with five out of seven items achieving a UA index of 1.00. The mean I-FVI was also high, indicating that most items were considered highly relevant. The S-FVI/UA was 0.8, indicating a high degree of consensus among responders regarding the scale's general readability and clarity. For the Activity limitation domain, the results indicate that among the retained 5 items, most items received high levels of agreement, with three out of five items achieving a UA index of 1.00. The mean I-FVI was also high, indicating that most items were considered highly relevant. The S-FVI/UA was 0.87, indicating a high degree of consensus among responders regarding the scale's general readability and clarity. For the performance and social life domain, the results indicate that among the retained 2 items, most items received high levels of agreement, with one out of two items achieving a UA index of 1.00. The mean I-FVI was also high, indicating that most items were considered highly relevant. The S-FVI/UA was 0.81, indicating a high degree of consensus among responders regarding the scale's general readability and clarity. Face validity of Pain and Sensations (Table 4). Face validity of Activity Limitations (Table 5). Face validity of Performance and social life (Table 6).

## DISCUSSION

A novel instrument created specially to assess the functional constraints faced by patients with varicose veins is the Panoramic Varicose Vein Index (PVVI) scale. The PVVI scale provides a comprehensive functional assessment, illuminating the ways in which varicose veins affect everyday activities and quality of life, in contrast to conventional evaluations that mostly concentrate on the physical appearance or general symptoms of varicose veins. The PVVI scale's direct attention to the functional difficulties faced by patients with varicose veins is among its most inventive features. In the past, the clinical management of varicose veins focused on the dimensions of the vein, the intensity of the limitations, and the outward signs of venous insufficiency, frequently ignoring the ways in which these symptoms impair a patient's ability to work. However, the PVVI scale fills this gap by offering a methodical, patient-centered approach to assess the impact of varicose veins on daily functioning and physical activity. Patients' quality of life is greatly impacted by functional disability, which is frequently underreported in varicose vein patients. Leg heaviness, soreness, and edema make it difficult for many people to do daily tasks including walking, standing, and exercising. These restrictions may result in less physical activity, which may exacerbate venous disease and have an effect on cardiovascular health in general. The PVVI helps physicians better understand the practical effects of varicose veins on patients' life and adjust therapies by measuring these functional affects. The scale measures tolerance, which is a key sign of how varicose veins affect physical health. It measures how difficult it is to walk, stand, or engage in prolonged physical activity. Everyday functional restrictions, including standing to cook or participate in family activities, are addressed. Beyond isolated symptoms, these functional metrics demonstrate the widespread impact of venous illness. PVVI offers clinicians insight into the actual level of patient suffering and its impact on lifestyle choices and activity avoidance by taking into account the frequency and intensity of pain-related disruptions in daily routines. By using PVVI in regular practice healthcare professionals can prioritize treatment objectives that meet the needs of each patient by having knowledge of particular functional limitations. Patients with moderate impairments might benefit more from cautious therapies, but those with significant mobility concerns might benefit more from procedures meant to provide immediate symptom relief. PVVI promotes active patient engagement in therapy conversations and decision-making by immersing patients in an assessment that closely corresponds to their everyday problems. PVVI also offers a baseline assessment of functional limits that can be

reevaluated after treatment to ascertain whether patients' capacities to carry out everyday duties and physical activities have improved. This information is useful for determining the effectiveness of treatment.

## CONCLUSION

In conclusion, Panoramic varicose vein index has shown promising content and face validity in assessing functional outcomes of patient suffering from varicose vein. The scale may prove to be a useful instrument for assessing the functional limitations of patients with varicose veins, as evidenced by the strong consensus among specialists and respondents.

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**Table 1: Content Validation of Pain and Sensation**

| ITEMS | SPECIALISTS | | | | Agreement No. | I-CVI | UA | MEAN I-CVI | S-CVI/UA | S-CVI/AVE |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | 4 | 1 | 1 | 1 | 1 | 1 |
| 1 | _ | _ | _ | _ | 4 | 1 | | | | |
| 2 | _ | _ | _ | _ | 4 | 1 | | | | |
| 3 | _ | _ | _ | _ | 4 | 1 | | | | |
| 4 | _ | _ | _ | _ | 4 | 1 | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 5 | _ | _ | _ | _ | 4 | 1 | | | | |
| 6 | _ | _ | _ | _ | 4 | 1 | | | | |
| 7 | _ | _ | _ | _ | 4 | 1 | | | | |
| Proportion Relevance | 1 | 1 | 1 | 1 | | | | | | |

**Table 2: Content Validation of Activity Limitation**

| ITEMS | SPECIALISTS | | | | Agreement No. | I-CVI | UA | MEAN I-CVI | S-CVI/UA | S-CVI/AVE |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | 4 | 1 | 1 | 1 | 1 | 1 |
| 1 | _ | _ | _ | _ | 4 | 1 | | | | |
| 2 | _ | _ | _ | _ | 4 | 1 | | | | |
| 3 | _ | _ | _ | _ | 4 | 1 | | | | |
| 4 | _ | _ | _ | _ | 4 | 1 | | | | |
| 5 | _ | _ | _ | _ | 4 | 1 | | | | |
| Proportion Relevance | 1 | 1 | 1 | 1 | | | | | | |

**Table 3: Content Validation of Performance and Social life**

| ITEMS | SPECIALISTS | | | | Agreement No. | I-CVI | UA | MEAN I-CVI | S-CVI/UA | S-CVI/AVE |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | 4 | 1 | 1 | 1 | 1 | 1 |
| 1 | _ | _ | _ | _ | 4 | 1 | | | | |
| 2 | _ | _ | _ | _ | 4 | 1 | | | | |
| Proportion Relevance | 1 | 1 | 1 | 1 | | | | | | |

**Table 4: Face validity of Pain and Sensations**

| ITEMS | RESPONDENTS | | | | | | | | | | Agreement Number | UA | I-FVI | MEAN I-FVI | S-FVI/UA | S-FVI/AVA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII | IX | X | | | | | | |
| 1 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | 10 | 1 | 1 | 0.99 | 0.8 | 0.83 |
| 2 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | 10 | 1 | 1 | | | |
| 3 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | 10 | 1 | 1 | | | |
| 4 | _ | _ | _ | _ | _ | _ | _ | | _ | _ | 9 | 0 | 1 | | | |
| 5 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | 10 | 1 | 1 | | | |
| 6 | _ | _ | _ | _ | _ | | _ | _ | _ | _ | 9 | 0 | 1 | | | |
| 7 | _ | _ | _ | _ | | _ | _ | _ | _ | _ | 10 | 1 | 1 | | | |
| Proportion Relevance | 0.89 | 0.89 | 0.89 | 0.89 | 0.84 | 0.84 | 0.89 | 0.84 | 0.84 | 0.89 | | | | | | |

**Table 5: Face validity of Activity Limitations**

| ITEMS | RESPONDENTS | | | | | | | | | | Agreement Number | UA | I-FVI | MEAN I-FVI | S-FVI/UA | S-FVI/AVA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII | IX | X | | | | | | |
| 1 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | 10 | 1 | 1 | 0.90 | 0.87 | 0.83 |
| 2 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | 10 | 1 | 1 | | | |
| 3 | _ | _ | _ | _ | _ | _ | _ | _ | | _ | 9 | 0 | 1 | | | |
| 4 | _ | _ | _ | _ | _ | _ | _ | | _ | _ | 9 | 0 | 1 | | | |
| 5 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | 10 | 1 | 1 | | | |
| Proportion Relevance | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.84 | 0.84 | 0.89 | | | | | | |

**Table 6: Face validity of Performance and social life**

| ITEMS | RESPONDENTS | | | | | | | | | | Agreement Number | UA | I-FVI | MEAN I-FVI | S-FVI/UA | S-FVI/AVA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII | IX | X | | | | | | |
| 1 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | 10 | 1 | 1 | 0.97 | 0.81 | 0.83 |
| 2 | _ | _ | _ | _ | _ | _ | _ | _ | _ | | 9 | 1 | 1 | | | |
| Proportion Relevance | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.84 | | | | | | |

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REVIEW ARTICLE

# Therapeutic and Safety Profile of *Asystasia gangetica* : A Comprehensive Review

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**ABSTRACT**

*Asystasia gangetica* (family: Acanthaceae), commonly known as Chinese violet or creeping foxglove, is a perennial medicinal plant traditionally used for nutritional and therapeutic purposes. In recent years, interest in plantbased therapeutics has increased due to the rising prevalence of chronic metabolic disorders, particularly diabetes mellitus, and concerns regarding the long-term safety of synthetic drugs. The present review critically compiles and analyzes existing literature on the phytochemical composition, therapeutic potential, and safety profile of *A. gangetica*. Special emphasis is placed on its antioxidant, anti-inflammatory, and possible antidiabetic activities, along with the current limitations in toxicological evaluation. By identifying key research gaps, this review aims to provide a scientific foundation for future pharmacological and safety-oriented investigations of *Asystasia gangetica*.

**Keywords:** *Asystasiagangetica*; herbal medicine; antidiabetic activity; phytochemicals; safety profile; toxicity.

## INTRODUCTION

Medicinal plants have served as the cornerstone of traditional healthcare systems for centuries and continue to contribute significantly to modern drug discovery. A large proportion of the global population relies on herbal medicines because of their affordability, accessibility, and cultural acceptability. [1,2] However, the lack of systematic

scientific validation often limits their acceptance in evidence-based medicine, particularly with respect to safety, standardization, and dose optimization. [3] Therefore, comprehensive evaluation of both therapeutic efficacy and safety is essential. *Asystasia gangetica* is a plant of increasing pharmacological interest owing to its nutritional richness and the presence of diverse bioactive constituents. [4] Although several preliminary studies suggest its medicinal relevance, the available information remains fragmented and largely confined to isolated experimental observations. [5] A consolidated review addressing its therapeutic profile alongside safety considerations is currently lacking. This review attempts to bridge that gap by critically analyzing existing scientific evidence.

**Botanical Description and Distribution:** *Asystasia gangetica* is a fast-growing perennial herb belonging to the family Acanthaceae. It is widely cultivated in tropical and subtropical regions as an ornamental ground cover. The plant is characterized by large ovate leaves and showy trumpet-shaped flowers with pale yellow throats. Its ability to adapt to diverse environmental conditions contributes to its widespread distribution across Africa, Asia, and other tropical regions.

**Ethnomedicinal and Nutritional Importance:** Traditionally, the leaves of *A. gangetica* have been used in folk medicine for the management of asthma, inflammation, and general weakness in different regions.[6] In certain parts of Africa and Asia, the plant is also consumed as a leafy vegetable, suggesting its nutritional value and apparent safety at dietary levels.[7] The presence of essential vitamins, carbohydrates, and micronutrients supports its classification as a functional food.[8] However, medicinal applications often involve concentrated extracts, which necessitate careful experimental evaluation of safety and efficacy.

**Phytochemical Profile:** Phytochemical investigations indicate that *A. gangetica* contains a wide range of primary and secondary metabolites, including carbohydrates, proteins, vitamins (A, C, E, thiamine, riboflavin, niacin, and pyridoxine), and bioactive compounds such as phenolics, flavonoids, glycosides, and iridoid derivatives. [9,10]. Compounds such as catalpol-related glycosides and asystasiosides have been reported from species of the genus Asystasia, suggesting potential pharmacological relevance. [11] Extraction efficiency and phytochemical yield are influenced by solvent polarity, extraction technique, and processing conditions. [12] Hydro-alcoholic extracts prepared using cold maceration have been reported to preserve thermolabile compounds, making this method suitable for pharmacological screening and biological evaluation. [13]

**Therapeutic Potential:**

**Antioxidant Activity:** Oxidative stress plays a crucial role in the pathogenesis of diabetes mellitus and its associated complications. Experimental studies have demonstrated that A. gangetica extracts possess significant free radical scavenging activity, which may be attributed to their phenolic and flavonoid content. [14] These antioxidant properties may help reduce cellular oxidative damage and improve metabolic homeostasis.

**Anti-inflammatory Activity:** Chronic inflammation is closely associated with insulin resistance and pancreatic β-cell dysfunction. Preliminary studies suggest that bioactive constituents present in A. gangetica may modulate inflammatory mediators and signaling pathways. [15] Such anti-inflammatory effects could contribute to its therapeutic relevance in metabolic and inflammatory disorders.

**Antidiabetic Potential:** The global burden of diabetes mellitus necessitates the exploration of safer and cost-effective therapeutic alternatives derived from natural sources. [16] Plant-derived phenolics and glycosides are known to inhibit key carbohydrate-digesting enzymes such as α-amylase and α- glucosidase, thereby reducing postprandial hyperglycemia. [17,18] These mechanisms are clinically relevant in the management of type 2 diabetes mellitus. Although related medicinal plants have demonstrated promising antidiabetic effects in streptozotocin-induced experimental models [19], direct and systematic *in vivo* validation of *A. gangetica* remains limited. Therefore, welldesigned experimental studies focusing on glycemic control, lipid profile modulation, and oxidative stress parameters are required.

**Safety and Toxicological Evaluation:** Despite its traditional use and dietary consumption, comprehensive toxicological data on A. gangetica are scarce. Most available studies lack structured evaluation of acute, sub-acute, and chronic toxicity. [20]. Information regarding organ-specific toxicity, biochemical alterations, and histopathological changes is largely insufficient. [21] Previous studies on medicinal plants have emphasized that the absence of detailed toxicity data poses a major barrier to clinical translation, even when therapeutic efficacy is evident. [22] Therefore, integrating safety assessment with pharmacological evaluation is essential to establish the safety margin and benefit–risk profile of *A. gangetica*.

**Experimental Approaches for Pharmacological Validation:** Standardized extraction techniques followed by preliminary phytochemical screening form the basis of biological evaluation. In vitro enzyme inhibition assays provide initial insights into antidiabetic potential, while in vivo models such as streptozotocin-induced diabetic rats are essential for validating efficacy and safety. Dose standardization, biochemical parameter analysis, and appropriate statistical evaluation are integral components of such studies.

**Research Gaps and Future Perspectives:** Although *A. gangetica* shows promising therapeutic potential, several research gaps remain: Limited bioactivity-guided fractionation studies. Insufficient *in vivo* antidiabetic and mechanistic investigations Absence of comprehensive dose-dependent toxicity data Poor correlation between therapeutic efficacy and safety parameters. Future research should focus on integrated pharmacological and toxicological studies to establish a scientifically validated therapeutic profile.

## CONCLUSION

Asystasiagangetica is a medicinally and nutritionally important plant with emerging relevance in the management of metabolic disorders such as diabetes mellitus. While preliminary studies support its antioxidant and anti inflammatory properties, rigorous experimental validation and comprehensive safety evaluation are urgently required. A systematic and standardized research approach may facilitate its development as a safe and effective plant-based therapeutic agent.

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RESEARCH ARTICLE

# Modeling and Analysis of Retrial Queues with Hexagonal Intuitionistic Fuzzy Numbers for Ecological Resource Systems

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**ABSTRACT**

In this work, ecological resource usage under unknown environmental conditions is analyzed by extending retrial queues. In ecosystems, organisms struggle for few resources and exhibit a natural retrial queue behavior by withdrawing and trying again if instant access is not available. Hexagonal Intuitionistic Fuzzy Numbers (HIFNs) are used to represent arrival, service, and retry rates. Key ecological performance metrics, such as expected waiting time and expected number of retrying species, are assessed using $\alpha$- and $\beta$-cuts with modified interval arithmetic. Ecosystem stability and resource distribution efficiency are interpreted by defuzzified values. The model's use in assessing ecosystem resilience in unpredictable circumstances is demonstrated numerically.

**Keywords:** Retrial queue, Ecological modeling, Environmental uncertainty, Intuitionistic fuzzy sets, Hexagonal intuitionistic fuzzy numbers.

## INTRODUCTION

Retrial behavior is a prevalent and crucial adaptation process found in nature. A species or population will frequently temporarily withdraw and try again later if it is unable to secure a necessary resource, such as food, water, or territory. Even in the face of unpredictable environmental stresses like drought, competition, or resource scarcity, species are guaranteed to live thanks to their retrying tendency, which preserves ecological balance. Retrial queues can be used to mathematically describe such retry events in ecosystems, where each retry represents an ecological

adaptation cycle and each attempt to access a resource represents a service request. On the other hand, ecological systems are defined by unpredictability in environmental circumstances, resource regeneration rate, and population density. This unpredictability cannot be adequately captured by traditional queueing techniques. As a result, we use Hexagonal Intuitionistic Fuzzy Numbers (HIFNs), which provide a deeper representation of environmental uncertainty by simultaneously modeling truth, falsity, and hesitation degrees. This work offers a versatile modeling approach to examine species-resource interactions and resource utilization efficiency in uncertain ecological systems by combining intuitionistic fuzzy sets with retrial queuing theory. Zadeh[16] introduced , "fuzzy set theory" as a way to deal with uncertainty. Zimmermann[17] introduced "Fuzzy sets theory and its applications" as a way to deal with application of uncertainty. Aarthi S, Shanmugasundhari M[1], "Comparison of Infinite Capacity FM/FEk/1 Queuing Performance using Fuzzy Queuing Model and Intuitionistic Fuzzy Queuing Model with Erlang Service Rates", The intuitionistic fuzzy queuing theory model offers a wide range of values, but the performance evaluations used to understand the fuzzy queuing are reported as a range of outcomes. Aarthi S *et al*[2], "Comparison of Non-Preemptive priority queuing performance using fuzzy queuing model and Intuitionistic fuzzy queuing model with different service rate", informs about the main objectives of this study, which are to evaluate the effectiveness of intuitionistic fuzzy queuing theory and fuzzy queuing theory applied to a non-preemptive queuing model. Ahmed FI *et al*[3], "A Single Server Markovian Queue with Single Working Vacation, State Dependent Reneging and Retention of Reneged Customers" helps to know about the single server vacation. Artalejo J *et al*[4] "Analysis of multi server queues with constant retrial rate" studied about multi server queues. J.R. Artalejo and J. Gómez-Corral (2008), where a computational approach to analyze these systems was presented [5].

Motivated by their work, we extend the study of retrial queues by introducing Intuitionistic Fuzzy Sets with Hexagonal representation to handle uncertainty in the system parameters. Chen S[6] "Parametric nonlinear programming for analyzing fuzzy queues with finite capacity" I have used to understand the finite capacity of fuzzy queues. Dudlin and Krishnamoorthy (2004) studied the BMAP/G/1 retrial system with customers from the orbit [7]. Motivated by such studies, we extend retrial queues using Hexagonal Intuitionistic Fuzzy Sets to deal with uncertainty. Falin G[8] introduced "A survey of retrial queues queuing systems: theory and applications". Chen, Liu and Zhang (2020) analyzed strategic customer behavior in fuzzy queueing systems [9]. Their work shows the role of fuzzy theory in queueing models. Following this direction, our study applies Hexagonal Intuitionistic Fuzzy Sets to retrial queues for handling uncertainty more effectively. Gross and Harris (1985) explained the basic concepts of queuing theory [10]. Based on such fundamental works, we extend the study to retrial queues using Hexagonal Intuitionistic Fuzzy Sets to handle uncertainty. Kalpana Devarajan, Senthil Kumar and Muthukrishnan (2021) studied a retrial queuing model for cognitive radio networks with energy harvesting [11]. Inspired by such applications, our work considers retrial queues under Hexagonal Intuitionistic Fuzzy Sets to model uncertainty in system behavior. Ke Jau chuan[12] "On retrial queuing model with fuzzy parameters" used to understand the retrial fuzzy parameters. Manoharan P[13] "Impatient customers in an m/m/1 queue with working vacation and multiple vacation" its help to understand the customers in vacation. Noor HidayahMohdZaki *et al*[14], "Comparison of Queuing Performance Using Queuing Theory Model and Fuzzy Queuing Model at Check-in Counter in Airport" studied When evaluating an airline company's queuing performance, the outcomes of the fuzzy queuing model are reliable. Viswanathan A[15] considered "fuzzy retrial queuing system with coxian-2 vacation". This paper is structured as follows: Basic definitions and preliminary information are included in Section 2. Retrial queues in an intuitionistic fuzzy environment are introduced in Section 3. The suggested method is demonstrated and a numerical example with Results and Discussions in Section 4 followed by the References and Conclusion.

## PRELIMINARIES

**Retrial queue**

A retrial queue is a queuing model in which customers (or jobs) who find the server busy upon arrival do not join a waiting line but instead leave and try to re-enter the queue after a random period. This behavior is common in systems where resources are limited, and users or tasks retry after being blocked or rejected.

**Example**

Consider a group of elephants in a dry land ecosystem approaching a watering hole. If the pond is occupied, the arriving elephants move away and return later when space becomes available. Over time, the retrying pattern stabilizes some elephants succeed, others wait, and the cycle repeats.

This natural retry behavior mirrors a retrial queue system, where:

**Arrival rate (λ)** = number of species arriving to access the resource,

**Service rate (μ)** = rate at which the resource can be used or replenished,

**Retrial rate (θ)** = rate at which species return after a failed attempt.

Analyzing this system helps ecologists estimate the expected number of retrying individuals and the average waiting time for access crucial indicators of ecological stress, population equilibrium, and environmental capacity

**$\alpha$-cut**

An alpha-cut ($\alpha$-cut) of a fuzzy set $\tilde{A}$ is the crisp subset of the universe X that contains all elements with a membership degree greater than or equal to a certain threshold $\alpha$ (where $0 \leq \alpha \leq 1$). Mathematically, an alpha-cut is defined as:

$A_\alpha = \{x \in X | \theta_{\tilde{A}}(x) \geq \alpha\}$

Where,

- $\theta_{\tilde{A}}(x)$ is the membership function of the fuzzy set $\tilde{A}$.
- $\alpha$ is a specified level of membership, with $0 \leq \alpha \leq 1$.
- $A_\alpha$ is the resulting crisp set at level $\alpha$.

**Fuzzy Set**

A fuzzy set A in a universal set X is defined by a membership function $\theta_{\tilde{A}}(x)$, which assigns to each element x in X a value between 0 and 1. The function $\theta_{\tilde{A}}(x)$ represents the degree to which x belongs to the fuzzy set A.

$A=\{(x, \theta_{\tilde{A}}(x)) | x \in X\}$

- X is the universal set (the set of all possible elements under consideration).
- X is an element of X.
- $\theta_{\tilde{A}}(x) \in [0,1]$ is the membership function, indicating the degree of membership of x in A.

**Intuitionistic Fuzzy set**

For a given universe X, an intuitionistic fuzzy set A in X is defined by a membership function $\theta_{\tilde{A}}(x)$, a non-membership function $\vartheta_{\tilde{A}}(x)$, and a hesitation degree $\pi_{\tilde{A}}(x)$ for each elements $x \in X$. These values satisfy the following conditions:

**Membership degree $\theta_{\tilde{A}}(x)$ :**

Represents the degree to which x belongs to the set A, where $0 \leq \theta_{\tilde{A}}(x) \leq 1$.

**Non-Membership degree $\vartheta_{\tilde{A}}(x)$:**

Represents the degree to which x does not belongs to A, where $0 \leq \vartheta_{\tilde{A}}(x) \leq 1$.

**Hesitancy degree $\pi_{\tilde{A}}(x)$**:

Represents the uncertainty or hesitation between membership and non-membership, calculated as:

$\pi_{\tilde{A}}(x) = 1 - \theta_{\tilde{A}}(x) - \vartheta_{\tilde{A}}(x)$

An intuitionistic fuzzy set A can be expressed as:

$A = \{\langle x, \theta_{\tilde{A}}(x), \vartheta_{\tilde{A}}(x)\rangle | x \in X\}$

**Hexagonal Intuitionistic fuzzy numbers**

A hexagonal intuitionistic fuzzy number is specified by $\tilde{\vartheta}_{H^I} = (a_1, a_2, a_3, a_4, a_5, a_6), (a_1', a_2', a_3', a_4', a_5', a_6')$. The non-membership function $\vartheta_{\tilde{H}}(x)$ for a hexagonal fuzzy number H can be defined as:

$$\vartheta_{\overline{H}}(x)=\begin{cases} 1, & for\ x < a_1{}' \\ 1-\frac{1}{2}\left(\frac{x-a_1{}'}{a_2{}'-a_1{}'}\right), & for\ a_1{}' \le x \le a_2{}' \\ \frac{1}{2}\left(\frac{a_3{}'-x}{a_3{}'-a_2{}'}\right), & for\ a_2{}' \le x \le a_3{}' \\ 0, & for\ a_3{}' \le x \le a_4{}' \\ \frac{1}{2}\left(\frac{x-a_4{}'}{a_5{}'-a_4{}'}\right), & for\ a_4{}' \le x \le a_5{}' \\ \frac{1}{2}+\frac{1}{2}\left(\frac{x-a_5{}'}{a_6{}'-a_5{}'}\right), & for a_5{}' \le x \le a_6{}' \\ 1, & for x > a_6{}' \end{cases}$$

The membership function of a queuing system fully characterizes its features, while the non-membership function of E(N) and E(W) indicate the expected number of customers and the expected waiting time of a client in the system, respectively. E(N) and E(W) have the following membership and non-membership functions:

$$\theta_{\widetilde{E(N)}}(x)=\begin{cases} 0, & for\ x < a_1 \\ \frac{1}{2}\left(\frac{x-a_1}{a_2-a_1}\right), & for\ a_1 \le x \le a_2 \\ \frac{1}{2}+\frac{1}{2}\left(\frac{x-a_2}{a_3-a_2}\right), & for\ a_2 \le x \le a_3 \\ 1, & for\ a_3 \le x \le a_4 \\ \frac{1}{2}-\frac{1}{2}\left(\frac{x-a_4}{a_5-a_4}\right), & for\ a_4 \le x \le a_5 \\ \frac{1}{2}\left(\frac{a_6-x}{a_6-a_5}\right), & for a_5 \le x \le a_6 \\ 0, & for x > a_6 \end{cases}$$

$$\vartheta_{\widetilde{E(N)}}(x)=\begin{cases} 1, & for\ x < a_1{}' \\ 1-\frac{1}{2}\left(\frac{x-a_1{}'}{a_2{}'-a_1{}'}\right), & for\ a_1{}' \le x \le a_2{}' \\ \frac{1}{2}\left(\frac{a_3{}'-x}{a_3{}'-a_2{}'}\right), & for\ a_2{}' \le x \le a_3{}' \\ 0, & for\ a_3{}' \le x \le a_4{}' \\ \frac{1}{2}\left(\frac{x-a_4{}'}{a_5{}'-a_4{}'}\right), & for\ a_4{}' \le x \le a_5{}' \\ \frac{1}{2}+\frac{1}{2}\left(\frac{x-a_5{}'}{a_6{}'-a_5{}'}\right), & for a_5{}' \le x \le a_6{}' \\ 1, & for x > a_6{}' \end{cases}$$

$$\theta_{\widetilde{E(W)}}(x)=\begin{cases} 0, & for\ x < a_1 \\ \frac{1}{2}\left(\frac{x-a_1}{a_2-a_1}\right), & for\ a_1 \le x \le a_2 \\ \frac{1}{2}+\frac{1}{2}\left(\frac{x-a_2}{a_3-a_2}\right), & for\ a_2 \le x \le a_3 \\ 1, & for\ a_3 \le x \le a_4 \\ \frac{1}{2}-\frac{1}{2}\left(\frac{x-a_4}{a_5-a_4}\right), & for\ a_4 \le x \le a_5 \\ \frac{1}{2}\left(\frac{a_6-x}{a_6-a_5}\right), & for a_5 \le x \le a_6 \\ 0, & for x > a_6 \end{cases}$$

$$\vartheta_{\widetilde{E(W)}}(x)=\begin{cases} 1, & for\ x < a_1' \\ 1-\frac{1}{2}\left(\frac{x-a_1'}{a_2'-a_1'}\right), & for\ a_1' \le x \le a_2' \\ \frac{1}{2}\left(\frac{a_3'-x}{a_3'-a_2'}\right), & for\ a_2' \le x \le a_3' \\ 0, & for\ a_3' \le x \le a_4' \\ \frac{1}{2}\left(\frac{x-a_4'}{a_5'-a_4'}\right), & for\ a_4' \le x \le a_5' \\ \frac{1}{2}+\frac{1}{2}\left(\frac{x-a_5'}{a_6'-a_5'}\right), & for a_5' \le x \le a_6' \\ 1, & for x > a_6' \end{cases}$$

**$\alpha, \beta$–cut sets of Hexagonal Intuitionistic Fuzzy Number**

**$\alpha$ –cut set**

$[A_\alpha^-, A_\alpha^+]=[a+2\alpha(b-a), f-2\alpha(f-e)]$

**$\beta$–cut set**

$[A_\alpha^-, A_\alpha^+]=[c-2\beta(c-b), d+2\beta(e-d)]$

**Model Descriptions**

**Retrial queuing in Intuitionistic Fuzzy Environment**

Let $\tilde{\lambda}_I$, $\tilde{\mu}_I$ and $\tilde{r}_I$ be the arrival rate, service rate and retrial rate respectively. These rates can be represented as a convex fuzzy set, as they are not known precisely. Let $(\theta(\tilde{\lambda}_I), \theta(\tilde{\mu}_I)\ and\ \theta(\tilde{r}_I))$ denote the membership between functions of $\tilde{\lambda}_I$, $\tilde{\mu}_I$ and $\tilde{r}_I$respectively and let $(\vartheta(\tilde{\lambda}_I), \vartheta(\tilde{\mu}_I)\ and\ \vartheta(\tilde{r}_I))$ denote the non-membership functions function of $\tilde{\lambda}_I$, $\tilde{\mu}_I$ and $\tilde{r}_I$respectivley.

The intuitionistic fuzzy sets are given by

$\tilde{P}_I=\{\tilde{\lambda}_I, \theta(\tilde{\lambda}_I), \vartheta(\tilde{\lambda}_I)/\tilde{\lambda}_I \in P\}$

$\tilde{Q}_I=\{\tilde{\mu}_I, \theta(\tilde{\mu}_I), \vartheta(\tilde{\mu}_I)/\tilde{\mu}_I \in Q\}$

$\tilde{R}_I=\{\tilde{r}_I, \theta(\tilde{r}_I), \vartheta(\tilde{r}_I)/\tilde{r}_I \in R\}$

Where P, Q and R are crisp universal sets of arrival rate, service rate and retrial rates respectively and $0 \le \theta(x)+\vartheta(x) \le 1$ where $x=\tilde{\lambda}_I, \tilde{\mu}_I, \tilde{r}_I$.

For a crisp retrial queuing system if $\frac{\lambda}{\mu}<1$ the expected number of customer are given by

$E(N)=\frac{\lambda^2}{\mu-\lambda}\left(\frac{1}{\lambda}+\frac{1}{\mu}\right)$

$E(W)=\frac{\lambda}{\mu-\lambda}\left(\frac{1}{\lambda}+\frac{1}{\mu}\right)$

**Performance measures of Hexagonal Intuitionistic fuzzy retrial queue**

Let arrival rate $\tilde{\lambda}_I$, service rate $\tilde{\mu}_I$ and retrial rate $\tilde{r}_I$ be denoted by HIFN's

$\tilde{\lambda}_I=(\lambda_1, \lambda_2, \lambda_3, \lambda_4, \lambda_5, \lambda_6);(\lambda_1', \lambda_2', \lambda_3', \lambda_4', \lambda_5', \lambda_6')$.

$\tilde{\mu}_I=(\mu_1, \mu_2, \mu_3, \mu_4, \mu_5, \mu_6);(\mu_1', \mu_2', \mu_3', \mu_4', \mu_5', \mu_6')$.

$\tilde{r}_I=(r_1, r_2, r_3, r_4, r_5, r_6);(r_1', r_2', r_3', r_4', r_5', r_6')$.

Where

$\lambda_1' \le \lambda_1, \lambda_2' \le \lambda_2, \lambda_3' \le \lambda_3, \lambda_4 \le \lambda_4', \lambda_5 \le \lambda_5', \lambda_6 \le \lambda_6'$

$\mu_1' \le \mu_1, \mu_2' \le \mu_2, \mu_3' \le \mu_3, \mu_4 \le \mu_4', \mu_5 \le \mu_5', \mu_6 \le \mu_6'$

$r_1' \le r_1, r_2' \le r_2, r_3' \le r_3, r_4 \le r_4', r_5 \le r_5', r_6 \le r_6'$

Using the concept of $\alpha, \beta$ –cut set

$\alpha\widetilde{\lambda_I}=[\lambda_1+2\alpha(\lambda_2-\lambda_1), \lambda_1-2\alpha(\lambda_6-\lambda_5)]$

$\beta\widetilde{\lambda_I}=[\lambda_3'-2\beta(\lambda_3'-\lambda_2'), \lambda_4'+2\beta(\lambda_5'-\lambda_4')]$

$\alpha\widetilde{\mu_I}$=$[\mu_1 + 2\alpha(\mu_2 - \mu_1), \mu_1 - 2\alpha(\mu_6 - \mu_5)]$
$\beta\widetilde{\mu_I}$=$[\mu_3' - 2\beta(\mu_3' - \mu_2'), \mu_4' + 2\beta(\mu_5' - \mu_4')]$

$\alpha\widetilde{r_I}$=$[r_1 + 2\alpha(r_2 - r_1), r_1 - 2\alpha(r_6 - r_5)]$
$\beta\widetilde{r_I}$=$[r_3' - 2\beta(r_3' - r_2'), r_4' + 2\beta(r_5' - r_4')]$

**Modified Interval Arithmetic Operations**

Let $\widetilde{A_I}$={$[a_1, a_2], [a_1', a_2']$}
$\widetilde{B_I}$={$[b_1, b_2], [b_1', b_2']$}
Then we define
$m_1$=$\frac{a_1+a_2}{2}$, $m_2$=$\frac{b_1+b_2}{2}$
$m_1'$=$\frac{a_1'+a_2'}{2}$, $m_2'$=$\frac{b_1'+b_2'}{2}$

**Addition**

$\widetilde{A_I} + \widetilde{B_I}$={$[(m_1 + m_2 - k), (m_1 + m_2 + k)]; [(m_1' + m_2' - k'), (m_1' + m_2' + k')]$}
Where k = $\frac{[b_2+a_2]-[b_1+a_1]}{2}$, k = $\frac{[b_2'+a_2']-[b_1'+a_1']}{2}$

**Subtraction**

$\widetilde{A_I} - \widetilde{B_I}$={$[(m_1 - m_2 - k), (m_1 - m_2 + k)]; [(m_1' - m_2' - k'), (m_1' - m_2' + k')]$}
Where k = $\frac{[b_2+a_2]-[b_1+a_1]}{2}$, k = $\frac{[b_2'+a_2']-[b_1'+a_1']}{2}$

**Multiplication**

$\widetilde{A_I} \times \widetilde{B_I}$={$[(m_1m_2 - k), (m_1m_2 + k)]; [(m_1'm_2' - k'), (m_1'm_2' + k')]$}
Where,
k=min$(m_1m_2 - \alpha, \beta - m_1m_2)$
$\alpha$=min$(a_1b_1, a_1b_2, a_2b_1, a_2b_2)$
$\beta$=min$(a_1b_1, a_1b_2, a_2b_1, a_2b_2)$
K′=min$(m_1'm_2' - \alpha, \beta - m_1'm_2')$
$\alpha'$=min$(a_1'b_1', a_1'b_2', a_2'b_1', a_2'b_2')$
$\beta'$=min$(a_1'b_1', a_1'b_2', a_2'b_1', a_2'b_2')$

**Division**

$\frac{1}{\widetilde{A_I}}$ = {$[a_1, a_2], [a_1', a_2']$}$^{-1}$
=$\left[\left(\frac{1}{m_1} - k, \frac{1}{m_1} + k\right); \left(\frac{1}{m_1'} - k', \frac{1}{m_1'} + k'\right)\right]$
Where, $k$ =$\left\{\frac{1}{a_2}\left(\frac{a_2-a_1}{a_1+a_2}\right), \frac{1}{a_1}\left(\frac{a_2-a_1}{a_1+a_2}\right)\right\}$
$k'$ =$\left\{\frac{1}{a_2'}\left(\frac{a_2'-a_1'}{a_1'+a_2'}\right), \frac{1}{a_1'}\left(\frac{a_2'-a_1'}{a_1'+a_2'}\right)\right\}$
Hence,
$\alpha_{[\widetilde{\mu_I}-\widetilde{\lambda_I}]}$=$[(\mu_1 - \lambda_6) + 2\alpha(\mu_2 - \mu_1 + \lambda_6 - \lambda_5), (\mu_6 - \lambda_1) - 2\alpha(\mu_6 - \mu_5 + \lambda_2 - \lambda_1)]$,
$\beta_{[\widetilde{\mu_I}-\widetilde{\lambda_I}]}$=$[(\mu_3' - \lambda_4') - 2\beta(\mu_3' - \mu_2' + \lambda_5' - \lambda_4'), (\mu_4' - \lambda_3') + 2\beta(\mu_5' - \mu_4' + \lambda_3' - \lambda_2')]$
$\alpha_{E(W)}$=$\alpha\left[\left(\frac{\widetilde{\lambda_I}}{\widetilde{\mu_I}-\widetilde{\lambda_I}}\right)\left(\frac{1}{\widetilde{\lambda_I}}+\frac{1}{\widetilde{r_I}}\right)\right]$
$\alpha_{E(W)}$=$\left[\frac{[\lambda_1](\lambda_1+r_1)}{(\mu_6-\lambda_1)}, \frac{[\lambda_3](\lambda_3+r_3)}{(\mu_4-\lambda_3)}, \frac{[\lambda_5](\lambda_5+r_5)}{(\mu_2-\lambda_5)}\right]_{\alpha=0}$
$\alpha_{E(W)}$=$\left[\frac{[\lambda_2](\lambda_2+r_2)}{(\mu_5-\lambda_2)}, \frac{[\lambda_4](\lambda_4+r_4)}{(\mu_3-\lambda_4)}, \frac{[\lambda_6](\lambda_6+r_6)}{(\mu_1-\lambda_6)}\right]_{\alpha=1}$
$\alpha_{E(N)}$=$\alpha\left[\left(\frac{\widetilde{\lambda_I}^2}{\widetilde{\mu_I}-\widetilde{\lambda_I}}\right)\left(\frac{1}{\widetilde{\lambda_I}}+\frac{1}{\widetilde{r_I}}\right)\right]$

$\alpha_{E(N)}=\left[\frac{[\lambda_1]^2(\lambda_1+r_1)}{(\mu_6-\lambda_1)},\frac{[\lambda_3]^2(\lambda_3+r_3)}{(\mu_4-\lambda_3)},\frac{[\lambda_5]^2(\lambda_5+r_5)}{(\mu_2-\lambda_5)}\right]_{\alpha=0}$

$\alpha_{E(N)}=\left[\frac{[\lambda_2]^2(\lambda_2+r_2)}{(\mu_5-\lambda_2)},\frac{[\lambda_4]^2(\lambda_4+r_4)}{(\mu_3-\lambda_4)},\frac{[\lambda_6]^2(\lambda_6+r_6)}{(\mu_1-\lambda_6)}\right]_{\alpha=1}$

$\beta_{E(W)}=\beta_{\left[\left(\frac{\tilde{\lambda}_I}{\tilde{\mu}_I-\tilde{\lambda}_I}\right)\left(\frac{1}{\tilde{\lambda}_I}+\frac{1}{\tilde{r}_I}\right)\right]}$

$\beta_{E(W)}=\left[\frac{[\lambda_1\prime](\lambda_1\prime+r_1\prime)}{(\mu_6\prime-\lambda_1\prime)},\frac{[\lambda_3\prime](\lambda_3\prime+r_3\prime)}{(\mu_4\prime-\lambda_3\prime)},\frac{[\lambda_5\prime](\lambda_5\prime+r_5\prime)}{(\mu_2\prime-\lambda_5\prime)}\right]_{\beta=0}$

$\beta_{E(W)}=\left[\frac{[\lambda_2\prime](\lambda_2\prime+r_2\prime)}{(\mu_5\prime-\lambda_2\prime)},\frac{[\lambda_4\prime](\lambda_4\prime+r_4\prime)}{(\mu_3\prime-\lambda_4\prime)},\frac{[\lambda_6\prime](\lambda_6\prime+r_6\prime)}{(\mu_1\prime-\lambda_6\prime)}\right]_{\beta=1}$

$\beta_{E(N)}=\beta_{\left[\left(\frac{\tilde{\lambda}_I^{\,2}}{\tilde{\mu}_I-\tilde{\lambda}_I}\right)\left(\frac{1}{\tilde{\lambda}_I}+\frac{1}{\tilde{r}_I}\right)\right]}$

$\beta_{E(N)}=\left[\frac{[\lambda_1\prime]^2(\lambda_1\prime+r_1\prime)}{(\mu_6\prime-\lambda_1\prime)},\frac{[\lambda_3\prime]^2(\lambda_3\prime+r_3\prime)}{(\mu_4\prime-\lambda_3\prime)},\frac{[\lambda_5\prime]^2(\lambda_5\prime+r_5\prime)}{(\mu_2\prime-\lambda_5\prime)}\right]_{\beta=0}$

$\beta_{E(N)}=\left[\frac{[\lambda_2\prime]^2(\lambda_2\prime+r_2\prime)}{(\mu_5\prime-\lambda_2\prime)},\frac{[\lambda_4\prime]^2(\lambda_4\prime+r_4\prime)}{(\mu_3\prime-\lambda_4\prime)},\frac{[\lambda_6\prime]^2(\lambda_6\prime+r_6\prime)}{(\mu_1\prime-\lambda_6\prime)}\right]_{\beta=1}$

Considering all these values of $\alpha=0$, $\alpha=1$, $\beta=0$ and $\beta=1$we obtain approximate HIFN

$$E(W)=\left[\begin{array}{c}\left(\frac{[\lambda_1](\lambda_1+r_1)}{(\mu_6-\lambda_1)},\frac{[\lambda_2](\lambda_2+r_2)}{(\mu_5-\lambda_2)},\frac{[\lambda_3](\lambda_3+r_3)}{(\mu_4-\lambda_3)},\frac{[\lambda_4](\lambda_4+r_4)}{(\mu_3-\lambda_4)},\frac{[\lambda_5](\lambda_5+r_5)}{(\mu_2-\lambda_5)},\frac{[\lambda_6](\lambda_6+r_6)}{(\mu_1-\lambda_6)}\right);\\ \left(\frac{[\lambda_1\prime](\lambda_1\prime+r_1\prime)}{(\mu_6\prime-\lambda_1\prime)},\frac{[\lambda_2\prime](\lambda_2\prime+r_2\prime)}{(\mu_5\prime-\lambda_2\prime)},\frac{[\lambda_3\prime](\lambda_3\prime+r_3\prime)}{(\mu_4\prime-\lambda_3\prime)},\frac{[\lambda_4\prime](\lambda_4\prime+r_4\prime)}{(\mu_3\prime-\lambda_4\prime)},\frac{[\lambda_5\prime](\lambda_5\prime+r_5\prime)}{(\mu_2\prime-\lambda_5\prime)},\frac{[\lambda_6\prime](\lambda_6\prime+r_6\prime)}{(\mu_1\prime-\lambda_6\prime)}\right)\end{array}\right]$$

$\approx [(w_1,w_2,w_3,w_4,w_5,w_6);(w_1\prime,w_2\prime,w_3\prime,w_4\prime,w_5\prime,w_6\prime)]$

In a similar manner

$$E(N)=\left[\begin{array}{c}\left(\frac{[\lambda_1]^2(\lambda_1+r_1)}{(\mu_6-\lambda_1)},\frac{[\lambda_2]^2(\lambda_2+r_2)}{(\mu_5-\lambda_2)},\frac{[\lambda_3]^2(\lambda_3+r_3)}{(\mu_4-\lambda_3)},\frac{[\lambda_4]^2(\lambda_4+r_4)}{(\mu_3-\lambda_4)},\frac{[\lambda_5]^2(\lambda_5+r_5)}{(\mu_2-\lambda_5)},\frac{[\lambda_6]^2(\lambda_6+r_6)}{(\mu_1-\lambda_6)}\right);\\ \left(\frac{[\lambda_2\prime]^2(\lambda_2\prime+r_2\prime)}{(\mu_5\prime-\lambda_2\prime)},\frac{[\lambda_2\prime]^2(\lambda_2\prime+r_2\prime)}{(\mu_5\prime-\lambda_2\prime)},\frac{[\lambda_3\prime]^2(\lambda_3\prime+r_3\prime)}{(\mu_4\prime-\lambda_3\prime)},\frac{[\lambda_4\prime]^2(\lambda_4\prime+r_4\prime)}{(\mu_3\prime-\lambda_4\prime)},\frac{[\lambda_5\prime]^2(\lambda_5\prime+r_5\prime)}{(\mu_2\prime-\lambda_5\prime)},\frac{[\lambda_6\prime]^2(\lambda_6\prime+r_6\prime)}{(\mu_1\prime-\lambda_6\prime)}\right)\end{array}\right]$$

$\approx [(n_1,n_2,n_3,n_4,n_5,n_6);(n_1\prime,n_2\prime,n_3\prime,n_4\prime,n_5\prime,n_6\prime)]$

**Numerical example**

**Problem**

Imagine a protected area with a single, essential drinking pond that is utilized by a focal species, like a herd of deer. Since arrival rates, resource availability, and revisit behavior are unpredictable due to seasonal and environmental change, Hexagonal Intuitionistic Fuzzy Numbers (HIFNs) are used to simulate these variables.

Let the uncertain arrival rate of individuals to the pond be

$\tilde{\lambda}_I$=(2,4,6,8,10,12); (1,3,5,9,11,13) per hour.

Let the (uncertain) effective resource release rate i.e. the rate at which the pond becomes available for a new individual after previous use be

$\tilde{\mu}_I$=(22,24,26,28,30,32); (21,23,25,29,31,33) per hour.

If an arriving individual finds the pond occupied, it leaves and later returns (retries). The retrial (revisit) rate is modeled by

$\tilde{r}_I$=(0.2,0.4,0.6,0.8,1.0,1.2); (0,0.3,0.5,0.9,1.1,1.3).

Using the $\alpha$- and $\beta$-cut methods together with the modified interval arithmetic presented in Section 3, find the expected waiting time for resource access E(W) in hours and the expected number of individuals in the retry orbit E(N) around the pond.

**Solution**

**Given**

$\tilde{\lambda}_I=(\lambda_1,\lambda_2,\lambda_3,\lambda_4,\lambda_5,\lambda_6);(\lambda_1\prime,\lambda_2\prime,\lambda_3\prime,\lambda_4\prime,\lambda_5\prime,\lambda_6\prime)$

=(2,4,6,8,10,12); (1,3,5,9,11,13)

$\tilde{\mu}_I=(\mu_1,\mu_2,\mu_3,\mu_4,\mu_5,\mu_6);(\mu_1\prime,\mu_2\prime,\mu_3\prime,\mu_4\prime,\mu_5\prime,\mu_6\prime)$

=(22,24,26,28,30,32); (21,23,25,29,31,33)

$\tilde{r}_l=(r_1,r_2,r_3,r_4,r_5,r_6);(r_1',r_2',r_3',r_4',r_5',r_6')$

$=(0.2,0.4,0.6,0.8,1.0,1.2);(0,0.3,0.5,0.9,1.1,1.3)$

$$E(W)=\begin{bmatrix}\left(\frac{[\lambda_1](\lambda_1+r_1)}{(\mu_6-\lambda_1)},\frac{[\lambda_2](\lambda_2+r_2)}{(\mu_5-\lambda_2)},\frac{[\lambda_3](\lambda_3+r_3)}{(\mu_4-\lambda_3)},\frac{[\lambda_4](\lambda_4+r_4)}{(\mu_3-\lambda_4)},\frac{[\lambda_5](\lambda_5+r_5)}{(\mu_2-\lambda_5)},\frac{[\lambda_6](\lambda_6+r_6)}{(\mu_1-\lambda_6)}\right);\\ \left(\frac{[\lambda_1'](\lambda_1'+r_1')}{(\mu_6'-\lambda_1')},\frac{[\lambda_2'](\lambda_2'+r_2')}{(\mu_5'-\lambda_2')},\frac{[\lambda_3'](\lambda_3'+r_3')}{(\mu_4'-\lambda_3')},\frac{[\lambda_4'](\lambda_4'+r_4')}{(\mu_3'-\lambda_4')},\frac{[\lambda_5'](\lambda_5'+r_5')}{(\mu_2'-\lambda_5')},\frac{[\lambda_6'](\lambda_6'+r_6')}{(\mu_1'-\lambda_6')}\right)\end{bmatrix}$$

$$E(\widetilde{W})=\begin{bmatrix}\left(\frac{2(2+0.2)}{32-2},\frac{4(4+0.4)}{30-4},\frac{6(6+0.6)}{28-6},\frac{8(8+0.8)}{26-8},\frac{10(10+1)}{24-10},\frac{12(12+1.2)}{22-12}\right);\\ \left(\frac{1(1+0)}{33-1},\frac{3(3+0.3)}{31-3},\frac{5(5+0.5)}{29-5},\frac{9(9+0.9)}{25-9},\frac{11(11+1.1)}{23-11},\frac{13(13+1.3)}{21-13}\right)\end{bmatrix}$$

$$=\begin{bmatrix}(0.1467,0.6769,1.8,3.9112,7.8571,15.84);\\ (0.0312,0.3535,1.1458,5.5687,11.0916,14.3)\end{bmatrix}$$

$$E(N)=\begin{bmatrix}\left(\frac{[\lambda_1]^2(\lambda_1+r_1)}{(\mu_6-\lambda_1)},\frac{[\lambda_2]^2(\lambda_2+r_2)}{(\mu_5-\lambda_2)},\frac{[\lambda_3]^2(\lambda_3+r_3)}{(\mu_4-\lambda_3)},\frac{[\lambda_4]^2(\lambda_4+r_4)}{(\mu_3-\lambda_4)},\frac{[\lambda_5]^2(\lambda_5+r_5)}{(\mu_2-\lambda_5)},\frac{[\lambda_6]^2(\lambda_6+r_6)}{(\mu_1-\lambda_6)}\right);\\ \left(\frac{[\lambda_2']^2(\lambda_2'+r_2')}{(\mu_5'-\lambda_2')},\frac{[\lambda_2']^2(\lambda_2'+r_2')}{(\mu_5'-\lambda_2')},\frac{[\lambda_3']^2(\lambda_3'+r_3')}{(\mu_4'-\lambda_3')},\frac{[\lambda_4']^2(\lambda_4'+r_4')}{(\mu_3'-\lambda_4')},\frac{[\lambda_5']^2(\lambda_5'+r_5')}{(\mu_2'-\lambda_5')},\frac{[\lambda_6']^2(\lambda_6'+r_6')}{(\mu_1'-\lambda_6')}\right)\end{bmatrix}$$

$$E(\widetilde{N})=\begin{bmatrix}\left(\frac{4(2+0.2)}{32-2},\frac{16(4+0.4)}{30-4},\frac{36(6+0.6)}{28-6},\frac{64(8+0.8)}{26-8},\frac{100(10+1)}{24-10},\frac{144(12+1.2)}{22-12}\right);\\ \left(\frac{1(1+0)}{33-1},\frac{9(3+0.3)}{31-3},\frac{25(5+0.5)}{29-5},\frac{81(9+0.9)}{25-9},\frac{121(11+1.1)}{23-11},\frac{169(13+1.3)}{21-13}\right)\end{bmatrix}$$

$$=\begin{bmatrix}(0.2933,2.5142,10.8,31.2888,78.5714,190.08);\\ (0.0312,1.0607,5.7291,50.1187,122.0083,302.0875)\end{bmatrix}$$

Using the defuzzification measures

$$r(\tilde{A})=\frac{a_1+2a_2+2a_3+2a_4+2a_5+a_6+a_1'+2a_2'+2a_3'+2a_4'+2a_5'+a_6'}{20}$$

The proposed Hexagonal Intuitionistic Fuzzy Retrial Queue model was applied to the ecological resource system described in Section 4. Using the $\alpha$-and $\beta$-cut approach and modified interval arithmetic, the defuzzified performance measures were obtained as follows:

$E(\widetilde{W})=\frac{95.1272}{20}=4.75636$ hours

$E(\widetilde{N})=\frac{996.7044}{20}=49.83\approx 50$

Here, $E(\widetilde{W})$ denotes the expected waiting time for an individual to access the watering resource, while $E(\widetilde{N})$ represents the expected number of individuals temporarily retrying (orbit population) around the resource.

Table 1.

The table showing these values indicate that, under uncertain environmental conditions, each animal on average waits about 4.76 hours before successful access, and roughly 50 individuals remain in the retry orbit at any given time. This confirms that the ecological retrial queue remains stable even when environmental parameters fluctuate.

## RESULTS AND DISCUSSIONS

The results show that the Hexagonal Intuitionistic Fuzzy technique is more effective than traditional deterministic methods at modeling ecological uncertainty. The model produces more accurate estimates for ecosystem management and sustainability planning by accounting for differences in resource availability, species arrival, and revisit behavior through the incorporation of hesitation degrees. Figure 1. According to the ecological conditions figure, a single species waits an average of 4.76 hours to access the resource, and at any given time, about 50 individuals are in the retry orbit. These results demonstrate that the adaptive reattempt behavior of species vying for scarce resources is well-modeled by the retrial queue mechanism. Even when environmental parameters fluctuate, the stability of the obtained results validates the ecological system's balanced use pattern.

## CONCLUSION

Ecological uncertainty and adaptive species behavior under changing environmental conditions are well captured by the Hexagonal Intuitionistic Fuzzy Retrial Queue model. Realistic estimations of resource waiting durations and retrying species are provided by taking hesitation degrees into account, illustrating ecological stability and balanced

resource use. By simulating repeated attempts and competition for few resources, the retrial queue structure provides definite advantages that allow for more precise management and sustainable planning. To further improve its practical application, future research can expand this model to multi-resource ecosystems, include interactions between several species, and provide computational algorithms for extensive ecological simulations.

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**Table 1: Defuzzified Performance Measures of the Hexagonal Intuitionistic Fuzzy Retrial Queue Model**

| Parameter | Notation | Mathematical Expression | Defuzzified Value | Unit | Interpretation |
|---|---|---|---|---|---|
| Expected Waiting Time | $E(\widetilde{W})$ | $\frac{95.1272}{20}$ | **4.75636** | hours | Average time an individual (species) waits before successfully accessing the resource |
| Expected Number in Orbit | $E(\widetilde{N})$ | $\frac{996.7044}{20}$ | **49.83 ≈ 50** | individuals | Average number of species retrying (in orbit) around the resource before access |

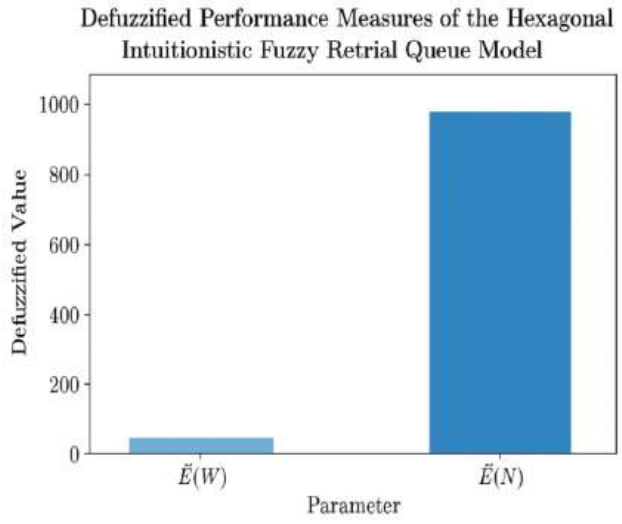



**Figure 1: Defuzzified values of performance measures E(W) and E(N)**

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RESEARCH ARTICLE

# A Design of Chain Sampling Plan ChSP-(0,1) using Fuzzy Parameter

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**ABSTRACT**

This study develops ChSP-(0,1) using fuzzy numbers is presented. To describe FOC Curve of ChSP-(0,1) tables and graphs are provided when the sample size is fixed . Numerical examples are given for the above sampling plan are given. Quality levels are designed to satisfy the condition of the risks. Sum of the risks is minimized for optimum value of sample size.

**Key words:** Statistical Quality Control , Acceptance Sampling , Operating Characteristic (OC) Curve, Fuzzy number, Trapezoidal Fuzzy Number.

## INTRODUCTION

The significant idea of statistics is statistical quality control. It is used in many manufacturing and production industries. Quality Control is required for the improvement of the product. It is the basic technique to sustain in this field. Acceptance sampling plan is an inspection plan between the producers and the consumers [15-16]. While evaluating proportion defective value crisp parameters are used to solve problems and there is some uncertainty often available. To resolve these types of uncertainty fuzzy set theory is used. It is the generalization of classical set theory. In acceptance sampling plan the fuzzy based research is used to solve the vagueness, imprecision and uncertainty [13]. Montgomery [15] introduced statistical quality control. Schiling [16] developed acceptance sampling quality control. Dodge [2] provided OC curve and properties of chain sampling plan. Clark [1] studied OC curves for ChSP-1. Frishman and Fred [6] prolonged chain sampling plan. Dodge & Stephens [3] developed Chain sampling plans ChSP-(0,1). It is used to minimize the inspection cost. Here both small and large samples are

applied. In order to get better result small sample size is used in ChSP-(0,1). It is an extension of chsp-1. Soundarajan [17] proposed construction and selection ChSP-(0,1). Soundararajan and Govindaraju [18] constructed and selected values for ChSP –(0,1). Kahraman and Kaya [8] proposed SSP and DSP using fuzzy parameter. The outcome shows that fuzzy boundary gives greater adaptability and convenience. Jamkhaneh and Sadeghpour Gildeh [7] discussed about chsp-1 using fuzzy probability theory. Turanoglu, Kahraman and Kaya [9] derived acceptance probability using fuzzy parameters and its OC curves. Kavi priya and Sudamani Ramaswamy [10-11] designed modified chain sampling plan MChSP-1 and two sided modified complete chain sampling plans TSMCChSP-1 using fuzzy parameters. Special purpose Double sampling plan type DSP(0,1) [13], two sided complete chain sampling plans TSCCChSP-1[12] and Repetitive Group Sampling plan (RGS) using fuzzy parameters was developed by Kavi priya and Sudamani Ramaswamy [14].

In this work basic definitions like fuzzy number, trapezoidal fuzzy number are included then operating procedure for ChSP - (0,1), FOC curve for ChSP-(0,1) are presented in graphs and tables are explained with examples.

**Definitions**

**Fuzzy Number** ( Zadeh [19]and Dubis & Prade [5] ) : " Fuzzy set that are characterized on the arrangement of real numbers having the structure $\check{E}$: R tends to [0,1] are known as fuzzy number. A fuzzy number $\check{E}$ will be a fuzzy set in the real line that fulfills the state of both normal and convexity".

**Trapezoidal fuzzy number** ( Zadeh [19] and Dubis & Prade [5]):"If trapezoidal fuzzy numbers (TrFNs) are $\check{E}$ = ($e_1$, $e_2$ , $e_3$ , $e_4$) and its membership function as"

$$\mathrm{TrFN} = \begin{cases} 0 & , \quad otherwise \\ \frac{y-e_1}{e_2-e_1} & , \ e_1 \le y \le e_2 \\ 1 & , \quad e_2 \le y \le e_3 \\ \frac{e_4-y}{e_4-e_3} & , \ e_3 \le y \le e_4 \\ 0 & , \quad otherwise \end{cases} \qquad (1)$$

"The interval of confidence of trapezoidal fuzzy number defined by $\gamma$ cuts can be written as follows"

$$\check{E}[\gamma] = [e_1 + (e_2 - e_1)\gamma,\ e_4 - (e_4 - e_3)\gamma] \qquad (2)$$

**Operating Procedure for ChSP-(0,1) Plan**

According to Dodge and Stephens [4] and Soundarajan and Govindaraju [18] "the operating procedure for ChSP-(0, 1) is as follows

Step 1: From each succeeding lot, a random sample of size n units is collected. Each sample's number of defective units is counted, and the total number of defective units is calculated.

Step 2: If no defective units are detected, accept the lot linked with each current sample.

Step 3: Once the $k_1$ lot has been approved, accept the next lot if the total number of defective unit is less than one.

Step 4: If $k_2$ is greater than $k_1$, the $k_2$ lot is approved. Continue to accept until the cumulative number of defective units reaches at least $k_2$ lots, and as long as the cumulative number of defective units is one or none.

Step 5: If the total number of defective units is higher than one at any point during the process, reject the current batch and return to Step 1".

**Fuzzy Probability of Acceptance (FPA) and Fuzzy Proportion of Defective (FPrD)**

Consider $\breve{p}_s = (s, e_2 + s, e_3 + s, e_4 + s)$ where $e_j = b_i - b_1,\ i = 2,3,4$ and $\gamma$ cut of trapezoidal fuzzy number to find fuzzy operating characteristic curve where $\gamma$=0 and $\gamma$=1. FPA is calculated using binomial distribution. $\gamma$ cut of trapezoidal fuzzy number is used to solve ChSP-(0,1) such that $\check{E}[\gamma] = [e_1 + (e_2 - e_1)\gamma,\ e_4 - (e_4 - e_3)\gamma]$ and taking $\gamma$=0,1 then we get FPrD value as $\breve{p}_s[\gamma] = [\breve{p}_s^{lb}, \breve{p}_s^{ub}]$ and FPA value using $\breve{P}_{as}[\gamma] = [\breve{P}_{as}^{lb}, \breve{P}_{as}^{ub}]$ .

The probability of acceptance ChSP-(0, 1) is given by

$$P_a = \left(\frac{p_0(1-p_0)+p_1 p_0^{k_1}(1-p_0^{k_2-k_1})}{1-p_0+p_0^{k_1}p_1(1-p_0^{k_2-k_1-1})}\right), k_2 > k_1 \quad (3)$$

FPA for ChSP-(0, 1) as

$$\breve{P}_{as} = \left(\frac{(1-\breve{p}_s)^n(1-(1-\breve{p}_s)^n)+(1-\breve{p}_s)^{nk_1}n\breve{p}_s(1-\breve{p}_s)^{n-1}(1-(1-\breve{p}_s)^{n(k_2-k_1)})}{1-(1-\breve{p}_s)^n+(1-\breve{p}_s)^{nk_1}(n\breve{p}_s(1-\breve{p}_s)^{n-1})(1-(1-\breve{p}_s)^{n(k_2-k_1-1)})}\right) \quad (4)$$

$$\breve{P}_{as}^{lb}[\gamma] = min\left[\frac{(1-\breve{p}_s)^n(1-(1-\breve{p}_s)^n)+(1-\breve{p}_s)^{nk_1}n\breve{p}_s(1-\breve{p}_s)^{n-1}(1-(1-\breve{p}_s)^{n(k_2-k_1)})}{1-(1-\breve{p}_s)^n+(1-\breve{p}_s)^{nk_1}(n\breve{p}_s(1-\breve{p}_s)^{n-1})(1-(1-\breve{p}_s)^{n(k_2-k_1-1)})}\right]$$

$$\breve{P}_{as}^{ub}[\gamma] = max\left[\frac{(1-\breve{p}_s)^n(1-(1-\breve{p}_s)^n)+(1-\breve{p}_s)^{nk_1}n\breve{p}_s(1-\breve{p}_s)^{n-1}(1-(1-\breve{p}_s)^{n(k_2-k_1)})}{1-(1-\breve{p}_s)^n+(1-\breve{p}_s)^{nk_1}(n\breve{p}_s(1-\breve{p}_s)^{n-1})(1-(1-\breve{p}_s)^{n(k_2-k_1-1)})}\right]$$

$$\breve{P}_{as}[\gamma] = [\breve{P}_{as}^{lb}, \breve{P}_{as}^{ub}] \quad (5)$$

$$= \left\{\frac{(1-\breve{p}_s^{ub})^n(1-(1-\breve{p}_s^{ub})^n)+(1-\breve{p}_s^{ub})^{nk_1}n\breve{p}_s^{ub}(1-\breve{p}_s^{ub})^{n-1}(1-(1-\breve{p}_s^{ub})^{n(k_2-k_1)})}{1-(1-\breve{p}_s^{ub})^n+(1-\breve{p}_s^{ub})^{nk_1}(n\breve{p}_s^{ub}(1-\breve{p}_s^{ub})^{n-1})(1-(1-\breve{p}_s^{ub})^{n(k_2-k_1-1)})}\right\},$$

$$\left\{\frac{(1-\breve{p}_s^{lb})^n(1-(1-\breve{p}_s^{lb})^n)+(1-\breve{p}_s^{lb})^{nk_1}n\breve{p}_s^{lb}(1-\breve{p}_s^{lb})^{n-1}(1-(1-\breve{p}_s^{lb})^{n(k_2-k_1)})}{1-(1-\breve{p}_s^{lb})^n+(1-\breve{p}_s^{lb})^{nk_1}(n\breve{p}_s^{lb}(1-\breve{p}_s^{lb})^{n-1})(1-(1-\breve{p}_s^{lb})^{n(k_2-k_1-1)})}\right\}$$

Using these above formulas the values are calculated and presented in Table 1.

From the Table 1 one can observe that when the n value is fixed as 20, $k_1$=9 and $k_2$=11 then fuzzy proportion defective value is calculated using $\gamma$ cut of trapezoidal fuzzy number. Finally fuzzy probability acceptance is calculated where $\gamma$=0 gives better results than $\gamma$ =1 .

**Fuzzy Operating characteristic (FOC) curve**

Operating characteristic curve is the most significant measure in acceptance sampling plan. From the above Figure 1 and Figure 2, fuzzy proportion defective is plotted against the fuzzy probability of acceptance for $\gamma$=0 and $\gamma$=1. OC curve has band with upper and lower bounds so that it is called as Fuzzy Operating Characteristic (FOC) Curve.

**Real Life Application**

A product from oil company bought in large batches. The supermarket manager wishes to check the product are of good quality and well packed. He used ChSP-(0,1) to inspect the oil packets. Based on the result of inspection lot is decided to kept for sale in super market otherwise return back to vendor.

**Example 1**

Suppose $\breve{p}_s = (0.005, 0.006, 0.007, 0.008)$ , n=20, $k_1$=9 and $k_2$=11 then $\gamma$ cut of trapezoidal fuzzy number is used to get fuzzy proportion defective value for $\gamma$=0 and $\gamma$=1 we get FPA as $\breve{P}_{as}[0] = [0.8385\ \ 0.7523]$ and $\breve{P}_{as}[1] = [0.7607\ \ 0.7522]$ respectively.

**Example 2**

Let us assume that $\breve{p}_s = (0.001, 0.002, 0.003, 0.004)$ and the sample size n is fixed as 20 then $\gamma$ cut of trapezoidal fuzzy number is used to calculate FPrD $\breve{p}_s[0] = [0.001\ \ 0.005]$, $\breve{p}_s[1] = [0.003\ 0.004]$ and FPA values as shown in the below Table . From Table 2, according to ChSP-(0,1) for various values of $k_1$ and $k_2$ where $k_1$ value is lower than $k_2$ value is calculated using fuzzy parameter . From the above table while using value of $\breve{P}_{as}[\gamma = 0]$ which is giving better result when compared to value of $\breve{P}_{as}[\gamma = 1]$

**Fuzzy probability of acceptance when the sample size varies**

Let us consider that $\breve{p}_s = (0.001, 0.002, 0.003, 0.004)$ and the sample size n varies from 5 to 50 where $k_1$= 9 and $k_2$ =11 is fixed then $\gamma$ cut of trapezoidal fuzzy number is used to calculate the interval of FPrD $\breve{p}_s[\gamma = 0] = [0.001\ \ 0.005]$ , $\breve{p}_s[\gamma = 1] = [0.003\ 0.004]$ and FPA values as shown in the Table 3. When the sample size values decreases the width of FOC curve decreases.

**Determination of sample size**

The risk of producer is denoted by $\widetilde{\alpha_f}$ and the risk of consumer is denoted by $\widetilde{\beta_f}$. Accepting quality level $(\widetilde{\text{AQL}})$ is denoted as $\breve{p}_{1f}$ and Limiting quality level $(\widetilde{\text{LQL}})$ is denoted as $\breve{p}_{2f}$. Here ChSP-(0,1) plan is designed using the fuzzy parameter. The sample size n is calculated in order to satisfy both the inequalities for $\breve{P}_{1fa}$ and $\breve{P}_{2fa}$ simultaneously

for the given values of $\widetilde{\alpha_f}$ and $\widetilde{\beta_f}$ shown in Table 4. $\check{P}_{1fa} \geq 1-\widetilde{\alpha_f}$ and $\check{P}_{2fa} \leq \widetilde{\beta_f}$ where $\widetilde{\alpha_f}$ =0.05 *and* $\widetilde{\beta_f}$=0.10 is fixed so that the interval of FPA is satisfied the conditions $\check{P}_{1fa} \geq 0.95$ and $\check{P}_{2fa} \leq 0.10$ for different sample sizes.

$$\check{P}_{1fa} = \left[\frac{(1-\check{p}_{1f})^n(1-(1-\check{p}_{1f})^n)+(1-\check{p}_{1f})^{nk_1}n\check{p}_{1f}(1-\check{p}_{1f})^{n-1}(1-(1-\check{p}_{1f})^{n(k_2-k_1)})}{1-(1-\check{p}_{1f})^n+(1-\check{p}_{1f})^{nk_1}\left(n\check{p}_{1f}(1-\check{p}_{1f})^{n-1}\right)(1-(1-\check{p}_{1f})^{n(k_2-k_1-1)})}\right] \geq 0.95 \quad (6)$$

$$\check{P}_{2fa} = \left[\frac{(1-\check{p}_{2f})^n(1-(1-\check{p}_{2f})^n)+(1-\check{p}_{2f})^{nk_1}n\check{p}_{2f}(1-\check{p}_{2f})^{n-1}(1-(1-\check{p}_{2f})^{n(k_2-k_1)})}{1-(1-\check{p}_{2f})^n+(1-\check{p}_{2f})^{nk_1}\left(n\check{p}_{2f}(1-\check{p}_{2f})^{n-1}\right)(1-(1-\check{p}_{2f})^{n(k_2-k_1-1)})}\right] \leq 0.10 \quad (7)$$

**Minimizing the sum of risks**

The sample size is calculated so as to minimize the sum of the risks and it is presented in Table 5 and Table 6. Where $\widetilde{\text{AQL}}$ is denoted as $\check{p}_{1f}$ and $\widetilde{\text{LQL}}$ is denoted as $\check{p}_{2f}$ and the corresponding the probability of acceptance values are $\check{P}_{1fa}$ and $\check{P}_{2fa}$.The mathematical expression to minimize the sum of risk is

$$\widetilde{\alpha_f} + \widetilde{\beta_f} = 1 - \check{P}_{1fa} + \check{P}_{2fa}$$

$$= 1 - \left[\frac{(1-\check{p}_{1f})^n\left(1-(1-\check{p}_{1f})^n\right)+(1-\check{p}_{1f})^{nk_1}n\check{p}_{1f}(1-\check{p}_{1f})^{n-1}(1-(1-\check{p}_{1f})^{n(k_2-k_1)})}{1-(1-\check{p}_{1f})^n+(1-\check{p}_{1f})^{nk_1}\left(n\check{p}_{1f}(1-\check{p}_{1f})^{n-1}\right)\left(1-(1-\check{p}_{1f})^{n(k_2-k_1-1)}\right)}\right]$$
$$+\left[\frac{(1-\check{p}_{2f})^n(1-(1-\check{p}_{2f})^n)+(1-\check{p}_{2f})^{nk_1}n\check{p}_{2f}(1-\check{p}_{2f})^{n-1}(1-(1-\check{p}_{2f})^{n(k_2-k_1)})}{1-(1-\check{p}_{2f})^n+(1-\check{p}_{2f})^{nk_1}\left(n\check{p}_{2f}(1-\check{p}_{2f})^{n-1}\right)(1-(1-\check{p}_{2f})^{n(k_2-k_1-1)})}\right]$$

The sum of risks is obtained as interval of fuzzy.

## CONCLUSION

In this study designing of ChSP-(0, 1) is developed using fuzzy parameters. Numerous varieties of fuzzy numbers are available among them trapezoidal fuzzy numbers are employed in this study. From the FOC curve one can conclude that when $\gamma$ increases from 0 to 1 the width of FOC curve becomes less and vice versa. The sample size value is calculated such that it satisfied both the condition of risks. And also minimizing the sum of risks is obtained.

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**Table 1 Fuzzy probability of acceptance when n=20, $k_1$=9 and $k_2$=11**

| $\check{p}_s = (s, e_2+s, e_3+s, e_4+s)$ | $\check{p}_s[\gamma=0]$ | $\check{P}_{as}[\gamma=0]$ | $\check{p}_s[\gamma=1]$ | $\check{P}_{as}[\gamma=1]$ |
|---|---|---|---|---|
| (0.000,0.001,0.002, 0.003) | [0.000 0.003] | [NaN 0.9116] | [0.001 0.002] | [0.9484 0.9291] |
| (0.001,0.002,0.003, 0.004) | [0.001 0.005] | [0.9484 0.8710] | [0.003 0.004] | [0.8806 0.8717] |
| (0.002,0.003,0.004, 0.005) | [0.002 0.007] | [0.9100 0.8371] | [0.005 0.006] | [0.8385 0.8322] |
| (0.003,0.004,0.005, 0.006) | [0.003 0.009] | [0.8806 0.8071] | [0.007 0.008] | [0.8084 0.8021] |
| (0.004,0.005,0.006, 0.007) | [0.004 0.011] | [0.8574 0.7792] | [0.009 0.010] | [0.7836 0.7763] |
| (0.005,0.006,0.007, 0.008) | [0.005 0.013] | [0.8385 0.7523] | [0.011 0.012] | [0.7607 0.7522] |
| (0.006,0.007,0.008, 0.009) | [0.006 0.015] | [0.8225 0.7259] | [0.013 0.014] | [0.7381 0.7285] |
| (0.007, 0.008, 0.009, 0.01) | [0.007 0.017] | [0.8084 0.6998] | [0.015 0.016] | [0.7152 0.7048] |
| (0.008, 0.009, 0.01, 0.011) | [0.008 0.019] | [0.7957 0.6741] | [0.017 0.018] | [0.6919 0.6809] |
| (0.009,0.010,0.011, 0.012) | [0.009 0.021] | [0.7836 0.6489] | [0.019 0.020] | [0.6683 0.6570] |
| (0.010,0.011,0.012, 0.013) | [0.010 0.023] | [0.7721 0.6241] | [0.021 0.022] | [0.6446 0.6332] |
| (0.011,0.012,0.013, 0.014) | [0.011 0.025] | [0.7607 0.6000] | [0.023 0.024] | [0.6211 0.6096] |
| (0.012,0.013,0.014, 0.015) | [0.012 0.027] | [0.7494 0.5766] | [0.025 0.027] | [0.5978 0.5865] |
| (0.013,0.014,0.015, 0.016) | [0.013 0.029] | [0.7381 0.5538] | [0.027 0.028] | [0.5750 0.5639] |
| (0.014,0.015,0.016, 0.017) | [0.014 0.031] | [0.7267 0.5318] | [0.029 0.030] | [0.5527 0.5418] |
| (0.015,0.016,0.017, 0.018) | [0.015 0.033] | [0.7152 0.5105] | [0.031 0.032] | [0.5310 0.5204] |
| (0.016,0.017,0.018, 0.019) | [0.016 0.035] | [0.7036 0.4899] | [0.033 0.034] | [0.5099 0.4997] |
| (0.017,0.018, 0.019, 0.02) | [0.017 0.037] | [0.6919 0.4702] | [0.035 0.036] | [0.4896 0.4797] |
| (0.018, 0.019, 0.02, 0.021) | [0.018 0.039] | [0.6801 0.4511] | [0.037 0.038 ] | [0.4699 0.4603] |
| (0.019, 0.02, 0.021, 0.022) | [0.019 0.041] | [0.6683 0.4327] | [0.039 0.040] | [0.4509 0.4417] |

**Table 2 Fuzzy Probability of Acceptance When $k_1$ and $k_2$ Values Varies**

| $k_1$ | $k_2$ | $\check{P}_{as}[\gamma=0]$ | $\check{P}_{as}[\gamma=1]$ |
|---|---|---|---|
| 1 | 3 | [0.9430 0.8327] | [0.8448 0.8285] |
| 1 | 4 | [0.9439 0.7820] | [0.9890 0.9813] |
| 1 | 5 | [0.9277 0.7391] | [0.8180 0.7756] |
| 2 | 4 | [0.9437 0.8391] | [0.8502 0.8354] |
| 2 | 5 | [0.9446 0.7923] | [0.8554 0.8212] |
| 2 | 6 | [0.9287 0.7522] | [0.8243 0.7852] |
| 3 | 5 | [0.9444 0.8450] | [0.8553 0.8418] |

| | | | |
|---|---|---|---|
| 3 | 6 | [0.9452 0.8018] | [0.8600 0.8283] |
| 3 | 7 | [0.9297 0.7645] | [0.8304 0.7944] |
| 4 | 6 | [0.9451 0.8503] | [0.8601 0.8477] |
| 4 | 7 | [0.9015 0.8106] | [0.8644 0.8349] |
| 4 | 8 | [0.9306 0.7760] | [0.8361 0.8030] |
| 4 | 9 | [0.9158 0.7471] | [0.8095 0.7746] |
| 5 | 7 | [0.9458 0.8552] | [0.8647 0.8532] |
| 5 | 8 | [0.9466 0.8187] | [0.8685 0.8411] |
| 5 | 9 | [0.9315 0.7866] | [0.8416 0.8111] |
| 5 | 10 | [0.9170 0.7597] | [0.8162 0.7843] |
| 6 | 8 | [0.9465 0.8597] | [0.8690 0.8584] |
| 6 | 9 | [0.9472 0.8262] | [0.8725 0.8469] |
| 6 | 10 | [0.9325 0.7966] | [0.8469 0.8188] |
| 7 | 9 | [0.9471 0.8638] | [0.8731 0.8631] |
| 7 | 10 | [0.9478 0.8331] | [0.8763 0.8523] |
| 8 | 10 | [0.9478 0.8318] | [0.8769 0.8676] |
| 9 | 11 | [0.9484 0.8710] | [0.8806 0.8717] |
| 10 | 12 | [0.9490 0.8741] | [0.8840 0.8755] |

**Table 3 Fuzzy probability of acceptance when sample size varies**

| n | $\breve{P}_{as}[\gamma = 0]$ | $\breve{P}_{as}[\gamma = 1]$ |
|---|---|---|
| 50 | [0.8944 0.7618] | [ 0.8022 0.7825] |
| 45 | [0.9020 0.7786 ] | [ 0.8121 0.7951] |
| 40 | [0.9100 0.7956 ] | [ 0.8227 0.8081] |
| 35 | [0.9187 0.8131 ] | [ 0.8344 0.8218] |
| 30 | [0.9279 0.8311 ] | [0.8476 0.8366] |
| 25 | [0.9378 0.8502 ] | [0.8628 0.8529] |
| 20 | [0.9484 0.8710 ] | [ 0.8806 0.8717] |
| 15 | [0.9599 0.8943 ] | [ 0.9019 0.8939] |
| 10 | [0.9722 0.9218 ] | [0.9278 0.9210] |
| 5 | [0.9856 0.9558 ] | [ 0.9598 0.9553] |

**Table 4 Optimum value of the parameter n, when $\breve{P}_{1fa} \geq 0.95$ and $\breve{P}_{2fa} \leq 0.10$**

| $(\widetilde{AQL})$ | $(\widetilde{LQL})$ | n |
|---|---|---|
| (0.001,0.0011,0.0012,0.0013) | (0.170 , 0.171, 0.172, 0.173) | 12 |
| | (0.200 , 0.201, 0.202, 0.203) | 11 |
| | (0.220 , 0.221, 0.222, 0.223) | 10 |
| | (0.240 , 0.241, 0.242, 0.243) | 9 |
| | (0.260 , 0.261, 0.262, 0.263) | 8 |
| | (0.280 , 0.281, 0.282, 0.283) | 7 |
| (0.002,0.0021,0.0022,0.0023) | (0.300, 0.301, 0.302, 0.303) | 8 |
| | (0.320, 0.321 , 0.322, 0. 323) | 7 |
| | (0.340, 0.341, 0.342, 0.343) | 6 |
| | (0.360, 0.361 ,0.362, 0.363) | 5 |
| (0.003,0.0031,0.0032,0.0033) | (0.380, 0.381 ,0.382, 0.383) | 5 |
| | (0.400, 0.401 , 0.402, 0.403) | 5 |
| | (0.430, 0.431 ,0.432, 0.433) | 4 |
| | (0.450, 0.451 ,0.452, 0.453) | 4 |

| | | |
|---|---|---|
| (0.004,0.0041,0.0042,0.0043) | (0.440, 0.441 ,0.442, 0.443) | 4 |
| | (0.450, 0.451 ,0.452,0.453) | 4 |
| (0.005,0.0051,0.0052,0.0053) | (0.530, 0.531 ,0.532, 0.533) | 3 |
| | (0.550, 0.551 , 0.552, 0.553) | 3 |

**Table 5 Optimum value of the parameter n, minimum sum of risks for $\gamma = 0$ , $k_1 = 9$ and $k_2 = 11$ when $\widetilde{\alpha_f} \cong 0.05$ and $\widetilde{\beta_f} \cong 0.10$**

| n | $\check{P}_{1f}[\gamma = 0]$ | $\check{P}_{1fa}[\gamma = 0]$ | $\check{P}_{2f}[\gamma = 0]$ | $\check{P}_{2fa}[\gamma = 0]$ | $\widetilde{\alpha_f} + \widetilde{\beta_f}$ |
|---|---|---|---|---|---|
| 12 | [0.0010 0.0013] | [0.9672 0.9585] | [0.170 0.173] | [0.1069 0.1023] | [0.1397 0.1438] |
| 11 | [0.0010 0.0013] | [0.9697 0.9615] | [0.200 0.203] | [0.0859 0.0824] | [0.1162 0.1209] |
| 10 | [0.0010 0.0013] | [0.9722 0.9647] | [0.220 0.223] | [0.0834 0.0802] | [0.1112 0.1155] |
| 9 | [0.0010 0.0013] | [0.9748 0.9679] | [0.240 0.243] | [0.0846 0.0816] | [0.1098 0.1137] |
| 8 | [0.0010 0.0013] | [0.9774 0.9712] | [0.260 0.263] | [0.0899 0.0870] | [0.1125 0.1158] |
| 7 | [0.0010 0.0013] | [0.9801 0.9746] | [0.280 0.283] | [0.1003 0.0974] | [0.1202 0.1228] |
| 8 | [0.0020 0.0023] | [0.9575 0.9520] | [0.300 0.303] | [0.0576 0.0557] | [0.1001 0.1037] |
| 7 | [0.0020 0.0023] | [0.9623 0.9573] | [0.320 0.323] | [0.0672 0.0652] | [0.1049 0.1079] |
| 6 | [0.0020 0.0023] | [0.9672 0.9627] | [0.340 0.343] | [0.0827 0.0804] | [0.1155 0.1177] |
| 5 | [0.0020 0.0023] | [0.9722 0.9684] | [0.360 0.363] | [0.1074 0.1049] | [0.1352 0.1365] |
| 5 | [0.0030 0.0033] | [0.9598 0.9563] | [0.380 0.383] | [0.0916 0.0894] | [0.1318 0.1331] |
| 5 | [0.0030 0.0033] | [0.9598 0.9563] | [0.400 0.403] | [0.0778 0.0758] | [0.1180 0.1192] |
| 4 | [0.0030 0.0033] | [0.9671 0.9642] | [0.430 0.433] | [0.1056 0.1034] | [0.1385 0.1392] |
| 4 | [0.0030 0.0033] | [0.9671 0.9642] | [0.450 0.453] | [0.0915 0.0895] | [0.1244 0.1253] |
| 4 | [0.0040 0.0043] | [0.9574 0.9546] | [0.440 0.443] | [0.0983 0.0963] | [0.1409 0.1417] |
| 4 | [0.0040 0.0043] | [0.9574 0.9546] | [0.450 0.453] | [0.0915 0.0895] | [0.1341 0.1349] |
| 3 | [0.0050 0.0053] | [0.9598 0.9576] | [0.530 0.533] | [0.1038 0.1018] | [0.1440 0.1442] |
| 3 | [0.0050 0.0053] | [0.9598 0.9576] | [0.550 0.553] | [0.0911 0.0893] | [0.1313 0.1317] |

**Table 6 Optimum value of the parameter n, minimum sum of risks for $\gamma = 1$ , $k_1 = 9$ and $k_2 = 11$ when $\widetilde{\alpha_f} \cong 0.05$ and $\widetilde{\beta_f} \cong 0.10$**

| n | $\check{P}_{1f}[\gamma = 1]$ | $\check{P}_{1fa}[\gamma = 1]$ | $\check{P}_{2f}[\gamma = 1]$ | $\check{P}_{2fa}[\gamma = 1]$ | $\widetilde{\alpha_f} + \widetilde{\beta_f}$ |
|---|---|---|---|---|---|
| 12 | [0.0011 0.0012] | [0.9642 0.9613] | [0.171 0.172] | [0.1054 0.1038] | [0.1412 0.1425] |
| 11 | [0.0011 0.0012] | [0.9669 0.9642] | [0.201 0.202] | [0.0847 0.0836] | [0.1178 0.1194] |
| 10 | [0.0011 0.0012] | [0.9697 0.9672] | [0.221 0.222] | [0.0823 0.0812] | [0.1126 0.1140] |
| 9 | [0.0011 0.0012] | [0.9725 0.9702] | [0.241 0.242] | [0.0836 0.0826] | [0.1111 0.1124] |
| 8 | [0.0011 0.0012] | [0.9753 0.9733] | [0.261 0.262] | [0.0890 0.0880] | [0.1137 0.1147] |
| 7 | [0.0011 0.0012] | [0.9782 0.9764] | [0.281 0.282] | [0.0993 0.0984] | [0.1211 0.1220] |
| 8 | [0.0021 0.0022] | [0.9556 0.9538] | [0.301 0.302] | [0.0570 0.0563] | [0.1014 0.1025] |
| 7 | [0.0021 0.0022] | [0.9606 0.9589] | [0.321 0.322] | [0.0665 0.0659] | [0.1059 0.1070] |
| 6 | [0.0021 0.0022] | [0.9657 0.9642] | [0.341 0.342] | [0.0819 0.0812] | [0.1162 0.1170] |
| 5 | [0.0021 0.0022] | [0.9709 0.9697] | [0.361 0.362] | [0.1065 0.1057] | [0.1356 0.1360] |
| 5 | [0.0031 0.0032] | [0.9586 0.9575] | [0.381 0.382] | [0.0909 0.0901] | [0.1323 0.1326] |
| 5 | [0.0031 0.0032] | [0.9586 0.9575] | [0.401 0.402] | [0.0771 0.0765] | [0.1185 0.1190] |
| 4 | [0.0031 0.0032] | [0.9661 0.9652] | [0.431 0.432] | [0.1048 0.1041] | [0.1387 0.1389] |
| 4 | [0.0031 0.0032] | [0.9661 0.9652] | [0.451 0.452] | [0.0908 0.0902] | [0.1247 0.1250] |
| 4 | [0.0041 0.0042] | [0.9565 0.9556] | [0.441 0.442] | [0.0976 0.0969] | [0.1408 0.1413] |
| 4 | [0.0041 0.0042] | [0.9565 0.9556] | [0.451 0.452] | [0.0908 0.0902] | [0.1343 0.1346] |
| 3 | [0.0051 0.0052] | [0.9591 0.9584] | [0.531 0.532] | [0.1032 0.1025] | [0.1441 0.1441] |
| 3 | [0.0051 0.0052] | [0.9591 0.9584] | [0.551 0.552] | [0.0905 0.0899] | [0.1314 0.1315] |

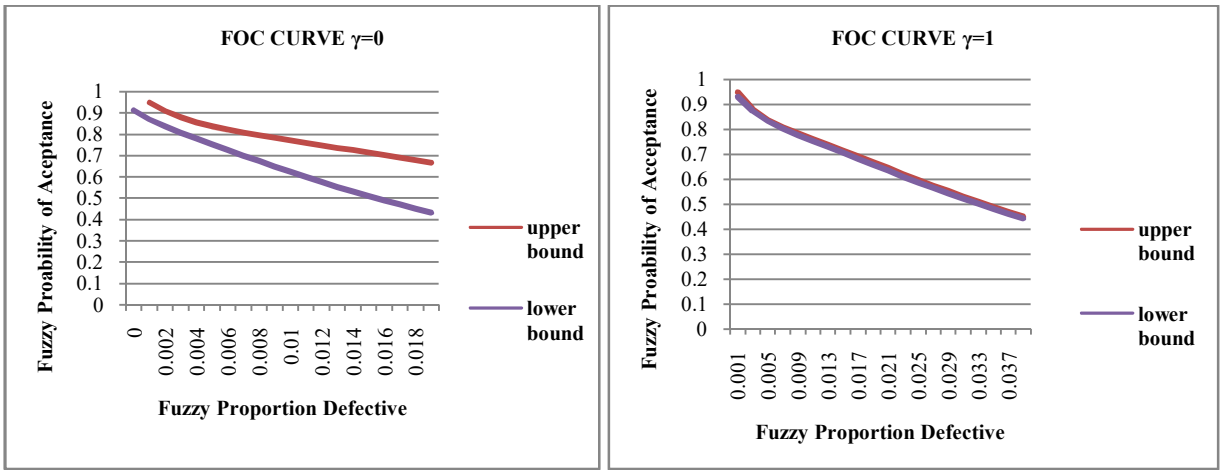



**Figure 1 & Figure 2 FOC Curve for ChSP-(0,1) with n=20 , $K_1$=9 and $K_2$=11**

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REVIEW ARTICLE

# A Comprehensive Review on *Aegle marmelos* (L.): Ethnobotany, Phytochemistry, and Pharmacological Potentials

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## ABSTRACT

Many animals and humans benefit greatly from the food and medication that plants and their products provide. The whole profile of *Aegle marmelos* L., a member of the Rutaceae family and usually referred to as Bael, is the main topic of this article. It is readily accessible throughout India. Abdominal disorders, ulcers, cholera, diarrhoea, nerve disorders, gonorrhoea, heart problems, dog bites, jaundice, snake bites, and many more ailments have all been treated traditionally using *Aegle marmelos.* Alkaloids, terpenoids, vitamins, coumarins, tannins, carbohydrates, flavonoids, fatty acids, essential oils, and a few additional unidentified substances are among the compounds that have been isolated. Antioxidants, antibacterial, antifungal, antidiarrheal, antidiabetic, antiproliferative, cytoprotective, hepatoprotective, antifertility, analgesic, antiarthritis, contractile, antihyperlipidemic, cardioprotective, radioprotective, anticancer, antiviral, anti-ulcer, immunomodulatory, and wound-healing properties are just a few of the pharmaceutical activities that the plants possess. Thus, this review might be a helpful tool for scholars who want to know more about *Aegle marmelos.*

**Keywords:** *Aegle marmelos*, Ethnobotany, Pharmacology activities, Phytochemistry, Traditional use.

**Abbreviations**: HPTLC High Performance Thin Layer Chromatography

| | |
|---|---|
| HPLC | High Performance Liquid Chromatography |
| GC-MS | Gas Chromatography Mass Chromatography |
| RP-HPLC | Reverse Phase High Performance Liquid Chromatography |
| TLC | Thin Layer Chromatography |

## INTRODUCTION

They contain a variety of active chemicals that are the structure blocks for numerous specifics, plants are especially important to humans[1]. One in India, people have been using shops as herbal or natural drug for thousands of times. numerous pharmacological active chemicals, which are constantly utilised in home treatments for a range of affections, are allowed to be abundant in plants used in Indian drug[2]. *Aegle marmelos*( L.) correa( *Aegle marmelos*), a member of the Rutaceae family and constantly known for bael, has been used considerably in traditional Indian medical systems because of its several medicinal parcels[3]. The leaves of *Aegle marmelos* are offered to Lord Shiva, without whom his devotion would be lacking. Ancient Indian jottings like the Mahabharat and the Yajur Veda[4]. Bael is useful for a number of affections because it contains a wide range of phytochemicals, including coumarin, polysaccharide, epoxies, resins, alkaloids, tannins, and essential canvases. Its nutritive content is far advanced than that of other fruits. In terms of the terrain, it's also relatively significant.

**Vernacular Names[5]**

In India different types of vernacular names are used which are given in the table no.1

*Aegle marmelos* leaves (Fig No: 1).

*Aegle marmelos* fruit (Fig No: 2).

*Aegle marmelos* Bark (Fig No: 3).

*Aegle marmelos* Steam (Fig No: 4).

**Source and Geographical Distribution**

The eastern ghats and central India are where the bael tree was originally domesticated. It’s native to the Indian key and is substantially set up in tropical and tropical regions[7]. On the lower pitches of the Himalayas, this factory may grow up to 500measures above ocean position. Along the east seacoast of Madhya Pradesh, Bihar, the Deccan table, Jharkhand, Chhattisgarh, Uttar Pradesh, and Uttarakhand[6]. The tree’s hypoglycemicparcels have led to its operation in Sri Lanka and Bangladesh for fertility-control and antiproliferative reasons[9]. In 1959, the bael fruit was introduced for the first time in Europe[10]. The tree was first observed to bear fruit in 1914 and is said to be growing in Ceylon, Java, the Philippines islets, and northern Malaya[11].

**Botanical Description**

The medium- sized, slender, perfumed bael fruit tree, also called *Aegle marmelos*, grows in India's evanescent timbers. It has a kindly grooved caddy that's 3.0- 4.5 measures long and is growing[8]. It can reach a height of 1200 measures in the western Himalayas and the Andaman islets. Its leaves are bestowed to Lord Shiva during deification; this tree is generally deified by Hindus. Twelve In Hindu tradition, this tree is a variant representation of Lord Kaiashnath[12]. The stem, root, fruit, and leaves of this tree are used in traditional Chinese drug to cure a wide variety of ails at colourful stages of growth[13]. The monotropic rubric includes *Aegle marmelos*. The *Aegle marmelos* factory stood 25 – 30 bases altitudinous. The height of this medium- sized factory was between 25 and 30 bases. It has a soft, thick, short, shelling stem with spreading dinghy. Their branches are prickly. The lower reek's branches slack. Their branches are prickly. The lower reek's branches slack. The axillary backbones of this tree are robust. The flake leys have a round or lanceolate form and are 4 – 10 cm in length and 2.5 – 10 cm in range. There are 3 to 5 in each pamphlet. Mature leaves that have been crushed emit an odd odour. It takes ten to eleven months for the fruit to grow[14]. Along its juvenile stems, this factory constantly develops clusters of four to seven blooms. Four succulent, recurved petals beautify the blossoms. The scent of these greenish-white blossoms is unique and uncommon. Fruits

can have globular or round shapes and range in size from 2 to 4 elevation in periphery. The fruit is a naturally woody, thin and hard shell. When unripe, it's greenish in colour; when it ripens, it becomes unheroic. The fruit pulp is golden, thick, sweet, sticky, and sweet in nature. There are eight to fifteen parts in it. The pulp contains bedded seeds that are nearly one centimetre long. These establishment, smoothed oblong seeds feature fuzzy hairs and are enclosed in a sticky sac[15]. *Aegle marmelos* thrives on rich, well- drained soil, but it can also produce fruit in the oolite limestone of southern Florida. It can also thrive on stony, alkaline, or swampy soils with a pH between 5 and 8. In India, it's known to thrive in conditions that other fruit trees find gruelling[16].

**Taxonomical Classification**

Scientific classification of *Aegle marmelos*is given in the table no.2

**Ethnomedicinal Significance of Bael Plant**

This plant is used in traditional medicine to treat recurring fevers, gastrointestinal issues, postpartum care, and ichthyosarcotoxism[17]. The fruit of *Aegle marmelos* has been added to the British pharmacopoeia because of its effectiveness in treating dysentery and diarrhoea[18]. Different parts of *Aegle marmelos* which are for the treatments are given in the table no.3

**Phytochemistry**

Several studies have found that several parts of the bael plant contain dynamic chemical fixes are given in the table no 4[26,2]. Structure of Marmelosin(Fig No: 5)

**Nutritional Value**

According to physicochemical research, bael fruit has a high vitamin and mineral content which are given in the table no: 5 [30].

**Pharmacological Studies**

One of the Rutaceae family's most extensively used medicinal and nutritional herb is *Aegle marmelos*. As seen in fig. 1, this plant has recently been found to have many medical benefits.

**Wound Healing Activity**

The effects of cutaneous and intraperitoneal organisation of methanolic concentrates of *Aegle marmelos* balm and infusion were examined on two types of twisting models in rodents, extraction and input point. Both the salve and the methanolic infusion of *Aegle marmelos* caused a severe reaction in both. An increase in elasticity in the entrance point model indicates that the concentrate facilitates the mending cycle. The outcomes were also contrasted with those of the condemned medication nitrofurazone[15, 31].

**Antidiarrheal Activity**

The ability of a chloroform extract of the root of *Aegle marmelos* to prevent diarrhoea. The extract was shown to be comparable to ciprofloxacin in an in vitro study and to be mostly effective against the *Vibrio cholerae* bacteria, *Escherichia coli* (E. coli), and Shigella spp[32]. Pharmacological activities of *Aegle marmelos* (Fig. no. 6).

**Antipyretic Potential**

The antipyretic exertion of *Aegle marmelos* which caused pyrexia at 200-400 mg/ kg body weight. The ethanolic extract significantly(p 0.001) lowered the high internal heat situation in a dosage form in less manner. The concentrates' antipyretic efficiency was on par with 100 mg/ kg body weight of paracetamol[33].

**Ulcer Healing**

Aspartate aminotransferase, alanine aminotransferase, ulcer index, and lipid peroxidation activity all significantly increased. These results suggest that an antioxidant mechanism may be involved in the gastroduodenal preventative and antiulcerogenic effects of *Aegle marmelos*[34].

**Diuretic Activity**

The diuretic goods of a number of organic fruit fragments of *Aegle marmelos* were delved. Urine volume and swab content were examined to probe the diuretic effect. At the high dose (500 mg/kg), they set up that the ethanolic extract significantly increased sodium. Fragments of petroleum ether, chloroform, and ethyl acetate also work well[35].

**Antithyroid Activity**

The birth of *Aegle marmelos* leaves dropped thyroid hormone situations. The presence of scopoletin, which has a more potent remedial effect than the medicine propylthiouracil, was the cause[2]

**Immunomodulatory Activity**

It was found that *Aegle marmelos* leaf extract in methanol stimulates both antibody-mediated and cell-mediated immune responses in rots. However, it was discovered that humoral immunity was best served by a high dose of *Aegle marmelos* methanolic extract, whereas cell-mediated immune response was best served by a low dose[36].

**Antifungal Activity**

According to an analysis, the essential oil from *Aegle marmelos* leaves have the Ca+2-dipicolinic corrosive metabolic pathway and perhaps restrict spore improvement. Leaf extracts from *Aegle marmelos* in ethanol, methanol, and water shown antifungal activity against dermatophyte organisms[37].

**Antimicrobial Activity**

Because it inhibits a wide range of harmful germs, It has been used traditionally to treat a number of infectious illnesses. Inhibition zones of 11 - 9 mm, respectively, were obtained from the antibacterial activity of *Aegle marmelos* leaves and fruit extracts against *Raoultella planticola*. The plant extracts demonstrated a minimum inhibition zone of 7 mm against *Candida albicans* and a maximum inhibition zone of 18 mm against the fungus strain *Penicillium chrysogenum*[38].

**Antioxidant Activity**

The antioxidant activity of *Aegle marmelos* fruit pulp methanolic and water extracts was assessed using the DPPH radical scavenging method, the reducing power assay, the nitric oxide scavenging assay, the $O_2^-$ radical scavenging assay, the ABTS radical scavenging assay, and the $H_2O_2$ radical scavenging assay. Both alcoholic and water extracts had high activity. It compared to ripe fruit; unripe fruit displayed a higher percentage of free radical inhibition[39].

**Radioprotective Effect**

One of the most important cancer therapies is radiation therapy, especially for patients with large visceral tumours. Despite its high global value, it has certain unfavourable side effects. Swiss albino male mice were subjected to 10Gy 60Co gamma-rays after receiving 5, 10, 15, 20, or 40 mg/kg. Give an intraperitoneal dosage of *Aegle marmelos* extract every day for five days. Thirty days after radiation therapy, the majority of survivors report feeling the most protected[40].

**Contractile Activity**

*Aegle marmelos* leaves have long been used to treat asthma and related conditions, its alcoholic extract was found to have a contractile effect on guinea pigs' separated ileum and tracheal chain. Because of H1 receptor depression, the guinea pig ileum and tracheal chain relaxed the most when exposed to alcoholic extract of *Aeglemarmelos[*41].

**Anti Arthritis Activity**

It was discovered that *Aegle marmelos* leaves cause arthritis in Wistar albino rats by acting against collagen. Bael's anti-inflammatory and antioxidant qualities are the main reasons it is recognised to have anti-arthritic qualities[42].

**Analgesic Activity**

The methanol extract from *Aegle marmelos* leaves was tested for its antinociceptive properties in mice using writing and tail immersion tests. When compared to other dosages, the maximum potential effect of methanolic extract (200 mg/kg) was statistically significant. It has been determined that *Aegle marmelos* leaf methanol extract has a strong analgesic effect[43].

**Cytoprotective Effect**

The brackish fish *Cyprinus carpio* has been observed to parade this effect. Experimental fish were kept at sublethal attention of essence ions for 1,8,16,32 days. The fish were fed the *Aegle marmelos* crude grass 500mg/kg after 32 days. The during has a cytoprotective impact by stabilizing the tube membrane and controlling the antioxidant enzyme system[44].

**Anti- Constipating Effect**

It is thought that the majority of ripe fruits are a natural remedy for various laxatives. Fruits from *Aegle marmelos* which are used to cleanse and tone the digestive system. Consuming this fruit on a regular basis for two to three months helps the colon get rid of old, stored faeces. Usually, it is consumed as sharbat, which is prepared from ripe fruit pulp[2].

**Anticancer Activity**

The effect of *Aegle marmelos* fruit extract on 7, 12-dimethylbenz(a) anthracene (DMBA)-induced breast cancer in rats. The study used female Charles foster rats that were between 55 and 60 days old and weighed about 150 10g.*Aegle marmelos* treatment led to a significant decrease in the volume of the breast tumour (p 0.05) and a significant decrease (p 0.0001) in blood biomarkers such TNF-, serum malondialdehyde (MDA), and glucose levels. The ethanolic fruit pulp extract slows the growth of breast cancer in a rat model, which has an anti-proliferative impact[45].

**Transcriptome Analysis**

Also, information from other Rutaceae species, including *Citrus clementia* and *Citrus sinensis*, was used to annotate the reiterations of *Aeglemarmelos*.314 reiterations were categorised as glucosyltransferase($GT_s$), while 482 reiterations were categorised as cytochrome $p_{450s}$($CYP_s$). The leaves of *Aegle marmelos* parade the loftiest frequence of the monoterpenoid biosynthesis pathway.46,335 unlgenes with lengths ranging from 210-14,853 bp were formed 133,616contigs. The study included 94, 49 SSR labels and 7002 recap factors[46].

**Antidiabetic Activity**

The circulatory changes in rats with diabetes convinced by alloxan that were given methanolic splint excerpts from *Aegle marmelos*. Five treatment groups were involved in the study control, diseased low lozenge(100 mg/ kg), medium cure(250 mg/ kg), and high cure(500 mg/ kg) of methanolic splint excerpts. The cardiovascular goods were estimated using serum sialic acid glutathione peroxidase, serum catalase, ascorbic acid, sodium, potassium, and chloride situations, as well as veritably-low- viscosity lipoprotein(VLDL). Low and medium lozenge treated creatures showed no significant change in these cardiovascular parameters when compared to the sick treatment groups, the high lozenge treatment group, and the sick group[47].

**Ayurvedic and Modern Aspects**

**Ayurvedic Action Properties of *Aegle Marmelos* Plant[25]**

Ayurvedic properties of *Aegle marmelos* (Table No 6).

**Morden View**

They are less expensive, easier to obtain, and have less side effects, herbal medicines are growing in popularity in the modern world. Globally, herbal medicine is growing in popularity. Published studies show that between 2000 and 2008, sales of herbal products increased from 3% to 12% annually. The quality of commercial natural goods and research domains are considered to be seriously threatened by the rise of adulteration and substitution in the herbal

drug industry due to growing consumer demand. The primary causes of adulteration are the lack of the original plant product, deforestation, the loss of numerous species of herbal plants, difficulties identifying species, etc. Additionally, a lot of herbal dealers have created new techniques for high-quality adulteration that are only detectable through microscopic inspection and chemical analysis[48]. The two flaws that underlie the acceptability of herbal products and lower their commercial worth are the drug's poor quality and the absence of standardisation. In order to improve the quality of the herbal trade and act as a regulator, an Herbal Authentication System (HAS) must be developed[49]. *Aegle marmelos* is a herbaceous plant that has several therapeutic uses. There are previous concerns about the plant's demise and over-exploitation. The names of the trees of Lord Shiva and Goddess Laxmi are affixed to the plant to shield it from overuse and harm. The bael plant has gained popularity recently due to its therapeutic use for both human and animal ailments. People who earlier used the herbal products sparingly became into vicious and destructive collectors because of the less economic value of the *Aegle marmelos* plant. The National Medicinal Plants Board of the Government of India maintains plants to keep the plant from being destroyed, and the government has approved numerous projects for the plant's conservation and sustainable use[50].

**FUTURE ASPECTS**

Due to its abundance of phytoconstituents, *Aegle marmelos* can be utilised to cure a wide range of human ailments. The majority of the compounds have not been adequately assessed for the investigation of novel pharmacophores or lead molecules. Nonetheless, several bioactive chemicals' methods of action have already been determined. *Aegle marmelos* may therefore be further utilised in the near future as a source of beneficial phytochemical components and could be crucial to the current medical system.

**Analytical Method Done in *Aegle Marmelos*.**

Several analytical methods like HPLC, HPTLC, GC, TLC etc. which are done in the *Aegle marmelos* plant are given in the table no. 7

## CONCLUSION

One of the significant plants with a number of therapeutic and nutraceutical uses is *Aegle marmelos*. It has a number of therapeutic qualities that are employed in conventional medical systems to treat a variety of illnesses. Advanced scientific methods have been used to study *Aegle marmelos's* therapeutic qualities in great detail during the past few decades. Numerous bioactive chemicals have been extracted from the plant's various sections and subjected to pharmacological analysis. This review included a detailed summary of its pharmacological activity, phytoconstituents, and numerous botanical descriptors, including antibacterial, antifungal, antiviral, antidiabetic, antimalarial, antioxidant, and so on.

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**Table No 1: Vernacular names of *Aegle marmelos***

| | |
|---|---|
| Hindi | Bel, Bael,Sripal |
| Sanskrit | Bilva, Sriphal, Shivadruma, Shivapala |
| Telugu | Maredu |
| Bengali | Cambodia (Phneou or Pnoi) |
| Vietnamese | Baunau |
| Malayan | Modjo |
| Gujarati | Bil |
| Kannada | Bilpatra, Kumbala, Malura |
| Tamil | Kuvalum |
| Thai | Matum and Mapin |

**Table No 2: Classification of *Aegle marmelos***

| **Taxonomical Rank** | **Taxon** |
|---|---|
| Division | Plantae |
| Class | Magnoliophyta |
| Order | Sapindales |
| Family | Rutaceae |
| Sub- family | Aurantioideae |
| Genus | *Aegle* |
| Species | *Aegle marmelos* |
| Common name | Bael, Bael Patra |

**Table No 3: Medicinal importance of *Aegle marmelos*.**

| **Plant Part** | **Ethnomedicinal Importance** |
|---|---|
| **Leaves** | Leaf extracts are effective treating ulcers, abscesses, backaches, and vomiting cuts, heart disease, bronchitis, blood sugar, dropsy, diarrhoea, beriberi, and animal – related injuries. 19 Leaf juice has laxative properties, treat ocular infection and asthmatic symptoms. In the combination with cumin seeds used as hair tonic, coughs and other respiratory illnesses. Additionally, leaves are employed in veterinary medicine for the treatment of wounds, as animal feed, and for stimulating the denervated nictitating membrane in anaesthetized cats[20]. |

| | |
|---|---|
| **Root bark** | Fish bane, heart beatings, melancholia, and intermittent complications can all be treated with the root bark. the volume of the seminal fluid is increased by the Dinghy Juice and cumin in milk. Extracts from alcoholic roots can treat hypoglycemia[21]. Again, it's used for-amoebic, cardiac issues, stomach problems, and canine bites[22]. |
| **Flower** | Distilled floral extracts are used as a diuretic, anti- diabetic, anti-dysentery, local anaesthetic and stomach and intestine tonic. Used for treatment of epilepsy[23]. |
| **Fruit** | The operation of dry greasepaint is used and mustard oil painting to handle burn cases. Fruits are utilised for ulcer, intestinal spongers, alcohol, digestive, brain and heart issues, gonorrhoea, dysentery, stomach issues, epilepsy and constipation. Excerpts deduced from fresh fruit have demonstrated the capability to lower blood pressure effectively.24 likewise, a finely pulverized form of callow fruit presents an indispensable treatment for intestinal spongers like *Ascaris lumbricoides* and *Entamoeba histolytica*[25]. |

**Table No 4: Chemical constituents are in the *Aegle marmelos***

| Plant Part | Chemical Constituents |
|---|---|
| Leaf | Skimmianine, Aeglin, Rutin, Sitasterol, Flavone, Lupeol, Cineol, Citral Glycoside, O-Isopentenyl, Hallordiol Mameline, Citronellal, Cuuminalehyde Phenylethylle, Cinnamamides, Eugenol. |
| Fruit | Psoralen, Marmalade, Tannin, Phenol, Marmelosin, Luvangetin Auraptene |
| Seed | Limonene, A-D – Phellandrene, Cineol, Citronellal, Citral, P-Cymene, Cumin Aldehyde or Essential oils |
| Root | Terpins, Halopine, Coumarins and Alkaloid |
| Bark | Alkaloids, Fagarine, Marmin, Furoguinoline |

**Table No 5: Nutritional value of *Aegle marmelos***

| Sr. no. | Components | Value (%) |
|---|---|---|
| 1 | Water | 64.2 |
| 2 | Protein | 1.8 |
| 3 | Fat | 0.2 |
| 4 | Mineral | 1.5 |
| 5 | Fiber | 2.2 |
| 6 | Carbohydrate | 30.6 |
| 7 | Calcium | 0.09 |
| 8 | Phosphorus | 0.05 |
| 9 | Potassium | 0.6 |
| 10 | Iron | 0.3 |
| 11 | Vitamin A | 186 |
| 12 | Vitamin B1 | 0.01 |
| 13 | Nicotinic acid | 0.9 |
| 14 | Riboflavin | 1.2 |
| 15 | Vitamin C | 0.01 |
| 16 | Calorific value | 129 |

**Table No 6: Ayurvedic properties of *Aegle marmelos***

| | |
|---|---|
| Kapha vatashamaka | It balances kapha and vata dosha. |
| Shothahara | The leaves of the medicinal plant are used to cure edema. |
| Vyavasthapan | The herb is associated with analgesic property which helps in reducing pain. |
| Naaditantu | The roots of the weed are used to treat neurological disorders. |
| Deepan | The raw fruit of the plant acts as an appetizer. |
| Krimighna | The isshrub associated with an anthelmintic property. |

| | |
|---|---|
| Mridurechan | The fruit of the plant acts as a mild laxative |
| Pittasarak | It stimulates the digestive authorities. |
| Hridya | It is used as a cardio- defensive against. |
| Raktastambhak | It is used to cure the bleeding complaint. |
| Mutra | It is used to the micturition problem |
| Garbha Shayashota | It improves uterine health. |
| Jwarna | It helps in treating habitual disorders and act as an antipyretic agent |
| Anidra | It helps in treating wakefulness |
| Atisaar and Pravahika | It is used to treat diarrhea and dysentery diseases. |
| Grahani | It is for the to treat irritable bowel disease |
| Udarshool | The ripe fruit of the plant helps in curing constipation |
| Netrabhishyand | The leaf decoction or leaf paste helps to cure cataract disorder |

**Table No 7: Differenttypes of method are done in *Aegle marmelos***

| Sr. no | Title | Method | Description | Refer. |
|---|---|---|---|---|
| 1 | Marmelosin | HPTLC | Stationary phase: Silica gel $G_{60}$ $F_{254}$<br>Mobilephase: Toluene:Ethyl Acetate: Methanol:GlacialAcetic Acid (8:2:0.25:0.2V/V)<br>Detection: 30 min<br>Rf value: 0.50 | 51 |
| 2 | Marmelosin in polyherbal formulation | TLC<br><br><br><br><br>HPLC | Stationary phase: Silica gel G<br>Mobile Phase: N – Butanol: Acetic Acid:Water (4:1:5v/V)<br>Detection: Iodine vapor<br>Rf value: 0.52-0.89<br>Column: Phenomenex C-18<br>Mobile Phase: Ethanol: Water: Acetic Acid (60:40:0.1%V/V)<br>Detector: UV visible detector<br>Wavelength: 270nm.<br>Flow rate: 0.5ml/min<br>$R_t$ value: 5.8min | 52 |
| 3 | Marmelosin and *Psidium guajava* and seeds of *Nigella sativa*. | GC-MS | Silica column: (30 x 0.25mm)<br>Carrier gas: Helium @ 2ml/min<br>Filter :0.25µM PTFE<br>Detection: By Mass detector at 0.55s/scan<br>m/z value:50-700 | 53 |
| 4 | Marmelosin | RP-HPLC | Column: Agilent TC-C18<br>Mobile phase:Acetonitrile: Water (70:30V/V)<br>Detection: UV-visible at 247nm<br>Flow rate: 1ml/min<br>Run time: 20min<br>$R_t$ value: Not Reported | 54 |
| 5 | Gallic acid, Scopoletin, Umbelliferone and Marmelosin. | RP-HPLC | Column: C18<br>Mobile phase:Methanol:Water (60:40V/V) 0.1% Acetic Acid<br>Detector: PDA detector<br>Flow rate: 0.7ml/min | 55 |

| | | | | |
|---|---|---|---|---|
| | | | Run time: 20 min.<br>$R_t$value: Gallic Acid (2.95), Scopoletin (4.19), Umbelliferone (6.9), and Marmelosin (11.71) min respectively | |
| 6 | Marmelosin | HPLC | Column: Phenomenex C18 Gemini-NX-5µm<br>Mobile phase: Citric Acid Buffer Solution and Methanol (40:60V/V)<br>Detector: UV detector at 254nm.<br>Flow rate: 1ml/min<br>Run time: 20 min<br>$R_t$value: 2.195min | 56 |
| 7 | Marmelosin, Umbelliferon and Scopoletine | RP-HPLC | Column: RP 18 end capped column<br>Mobile phase:Methanol: Water (55:45V/V) 0.1% Acetic Acid<br>Detector: UV detector at 300nm.<br>Flow rate: 1ml/min<br>$R_t$value: Scopoletin(4.6min), Umbelliferone(6.5min), Marmelosin(11.3min) | 57 |
| 8 | Marmelosin | HPLC | Column: C-18<br>Mobile phase: Acetonitrile:Water (80:20V/V)<br>Detection: VWD Detector at 247nm<br>Flow rate:1ml/min<br>$R_t$value: 2.467min | 58 |
| 9 | Marmin, Skimmianine, Umbelliferone, Psoralene, And Marmelosin | HPTLC | Stationary phase: Silica gel $G_{60}$ $F_{254}$<br>Mobile phase: Toluene: Ethyl Acetate: Formic Acid (6:4:0.1, V/V)<br>Detection: 304 nm<br>$R_f$value: Marmin (0.12), Skimmianine (0.26), Umbelliferone (0.39), Psoralene (0.50), Marmelosin (0.56) min respectively. | 59 |
| 10 | Marmelosin | HPTLC | Stationary phase: Silica gel<br>Mobile phase: Acetone: Chloroform: Benzene (4:4:1V/V)<br>Detection: Vanillin sulfuric acid reagent<br>Wavelength :254nm<br>$R_f$ value: 0.53 | 60 |
| 11 | Marmelosin | HPLC | Column: Zorbax Eclipse XDB, $C_8$<br>Mobile phase: Methanol: Water (70:30V/V)<br>Detection: UV detector at 254nm<br>Flow rate: 1ml/min<br>$R_t$ value: Not Reported | 61 |
| 12 | Marmelosin | HPTLC | Stationary phase: Silica gel $G_{60}$ $F_{254}$<br>Mobile phase: Toluene: Ethyl Acetate (93:7V/V)<br>Detection: 313nm | 62 |
| 13 | Marmelosin | HPTLC | Stationary phase: Silica gel $G_{60}$ $F_{254}$<br>Mobile phase: N- Hexane:Ethyl Acetate (6:4V/V)<br>Detection: 254nm | 63 |



**Fig No 1: *Aegle marmelos* leaves**



**Fig No 2: *Aegle marmelos* fruit**



**Fig No 3: *Aegle marmelos* Bark**



**Fig No 4: *Aegle marmelos* Steam**

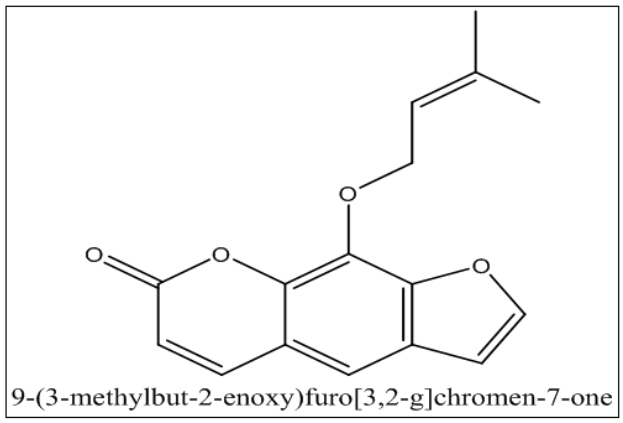



**Fig No 5: Structure of Marmelosin**




**Fig. no 6: Pharmacological activities of *Aeglemarmelos*.**

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RESEARCH ARTICLE

# A Study on Performance Evaluation of Irrigation Techniques using the Fuzzy Inference System

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**ABSTRACT**

Given an input, fuzzy systems use fuzzy operators and IF THEN rules to carry out an action that is converted into the system's output. A few definitions must be introduced before comprehending a fuzzy inference rule-based system (FIRBS), specifically fuzzy sets and linguistic variables. The fuzzy sets theory was created by Zadeh (1965) to expand on the traditional crisp idea that an element either belongs to or does not belong to a certain set. The degree of membership of an element in a fuzzy set range from 0 to 1. This study offers a novel approach to assessing irrigation system performance that considers tools for intelligent systems like fuzzy logic as well as indications of water resource efficiency. The goal was to suggest a fuzzy logic system as a means of assessing irrigation system performance based on a collection of water usage efficiency metrics. The performance of the surface irrigation approach is "High" (91.3%), whereas the performance of the treatment without irrigation is "Low" (17%) and that of drip irrigation is "Medium" (50%). This kind of technology, which is used in the agricultural industry, particularly in the assessment of irrigation systems, is novel and can support professionals' decision-making.

**Keywords:** Fuzzy Inference System, Irrigation, MATLAB, Fuzzy Rule-Based System, Triangular membership function.

## INTRODUCTION

When great precision is needed, precise quantitative analysis works well. Nevertheless, the method of making a determination regarding a pollution incident by analyzing a collection of measurements is inherently uncertain.

Noise taints the measurements, and more significantly, it is hard to understand how variables interact in a quantitatively ideal model. Uncertainties or ambiguities regarding the phenomenon's evolution and the significance of various factors (such as weather conditions, traffic patterns, etc.) on the estimation are inevitably brought about by the same origin of the phenomenon being studied. It is far from a realistic hypothesis, even though the idealization of causes-effect correlations is helpful in aiming for the analytical formulation of the problems, which also enables benchmarking of the algorithms. On the other hand, characterizing complex phenomena requires the ability to summarize data and provide an approximate description of a process. Because FISs possess the nonlinear universal approximation property and can handle both experimental data and a priori knowledge about the unknown solution, which is represented by inferential linguistic rules in the form IF THEN whose antecedents and consequents use fuzzy sets rather than precise numbers, they seem to be very useful tools (Abraham, 2005). Zadeh presented the notion of approximation reasoning in 1979. This theory offers a strong foundation for reasoning when confronted with ambiguous and imprecise data. Fuzzy inference is the process of formulating the mapping from a given input to an output using fuzzy logic. The mapping then provides a basis from which decisions can be made, or patterns discerned. The process of fuzzy inference involves all the pieces that are described in Membership Functions, Logical Operations, and If-Then Rules. An automated system is a system that operates in a controlled environment and is made up of hardware and software infrastructure. Fuzzy logic, which provides state parameter monitoring, target output comparison, intended outcome selection to modify the current system's condition, and necessary action implementation, is used to do this. Fuzzy logic has been widely applied in various domains, such as business and agriculture.

## MATERIALS AND METHOD

Fuzzy rule based system is generally defining a set or rules for certain types. IF is condition and THEN is a action. The condition, also called premise, is made up of a number of antecedents that are negated or combined by different operators such as AND or OR computed with t - norms or t - conforms. In a fuzzy rule system, some variables are linguistic variables and the determination of the MF for each fuzzy subset is critical. MFs can be selected according to human intuition, or by learning from training data. Inference systems can be built on top of fuzzy logic. Multiple rules with identical output variables make create a fuzzy inference. The inference result is a mix of the fuzzy values of the conditions and the associated actions given a set of fuzzy rules. The area of research was take place in Pattukottai block, Thanjavur district and crop was selected is paddy cause area of research cultivate major crop is paddy and it cultivated during the kuruvai season. In this paper we analyzing three types of irrigation namely drip irrigation, surface irrigation, no irrigation

$$Relative\ Water\ Supply = \frac{Total\ Water\ Supplied}{Crop\ Water\ Requirement}$$

## RESULTS AND DISCUSSION

The following table contains input data for fuzzy inference system application in MATLAB.
Input data for fuzzy inference system (Table 1).
The Mamdani fuzzy inference system model is developed in MATLAB to handle the uncertainity
Input for Mamdani fuzzy inference system (Table 2).
MATLAB Code of Input for Mamdani fuzzy inference system

```
fis = newfis('IrrigationPerformance');
% Input 1: Income Rate (₹/ha)
fis = addvar(fis, 'input', 'Income', [0 130000]);
fis = addmf(fis, 'input', 1, 'Low', 'trimf', [0 0 60000]);
fis = addmf(fis, 'input', 1, 'Medium', 'trimf', [50000 85000 110000]);
fis = addmf(fis, 'input', 1, 'High', 'trimf', [100000 130000 130000]);
% Input 2: Relative Water Supply
```

```
fis = addvar(fis, 'input', 'RWS', [0 3]);
fis = addmf(fis, 'input', 2, 'Low', 'trimf', [0 0.5 1]);
fis = addmf(fis, 'input', 2, 'Medium', 'trimf', [0.9 1.5 2]);
fis = addmf(fis, 'input', 2, 'High', 'trimf', [1.8 2.5 3]);
% Output: Performance (%)
fis = addvar(fis, 'output', 'Performance', [0 100]);
fis = addmf(fis, 'output', 1, 'Low', 'trimf', [0 25 49]);
fis = addmf(fis, 'output', 1, 'Medium', 'trimf', [45 65 79]);
fis = addmf(fis, 'output', 1, 'High', 'trimf', [75 90 100]);
```

The following tables holds rule base analysis of fuzzy inference system.
Rule base analysis of fuzzy inference system (Table 3).
Rule base figure (Figure 1).
Output evaluation of fuzzy inference system (Table 4).

## CONCLUSION

This study concludes that the performance of drip irrigation is moderate cause due to higher system costs in spite of usage of water efficiency. Then the performance of flood irrigation is highest cause due to more profit and less in spending and also least water efficiency. And the no irrigation is low cause due to rainfall season. So the fuzzy logic used to evaluate the irrigation methods and its performance under uncertainty.

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**Table 1: Input data for fuzzy inference system**

| Parameter | T1: Drip | T2: Flood | T3: No Irrigation |
|---|---|---|---|
| Yield (kg/ha) | 4300 | 4800 | 2600 |
| Paddy price (₹/kg) | 22 | 22 | 22 |
| Crop Revenue (₹/ha) | 94,600 | 1,05,600 | 57,200 |
| Crop Water Requirement ($m^3$/ha) | 12,000 | 15,000 | 10,000 |
| Water Supplied ($m^3$/ha) | 11,000 | 25,000 | 5,000 |
| System Cost (₹/ha) | 80,000 | 15,000 | 0 |
| RWS | 0.92 | 1.67 | 0.5 |

**Table 2: Input for Mamdani fuzzy inference system**

| Range | Input Variables | | Output Variables |
|---|---|---|---|
| | Income per area | RWS | Irrigation performance |
| Low | 0-60000 | 0-0.99 | 10-49 |
| Medium | 60001-100000 | 1-2 | 50-79 |
| High | 100001-130000 | >2 | 80-99 |

**Table 3: Rule base analysis of fuzzy inference system**

| Rule | IRIA (₹/ha) | RWS | Output (%) |
|---|---|---|---|
| 1 | Low | Low | Low (15) |
| 2 | Medium | Medium | High (89.2) |
| 3 | High | Low | Medium (52) |

**Table 4: Output evaluation of fuzzy inference system**

| Treatment | IRIA (₹/ha) | RWS | Fuzzy Output (%) | Performance Level |
|---|---|---|---|---|
| T1: Drip | 94,600 | 0.92 | 52 | Medium |
| T2: Flood | 1,05,600 | 1.67 | 89.2 | High |
| T3: No Irrigation | 57,200 | 0.5 | 15 | Low |

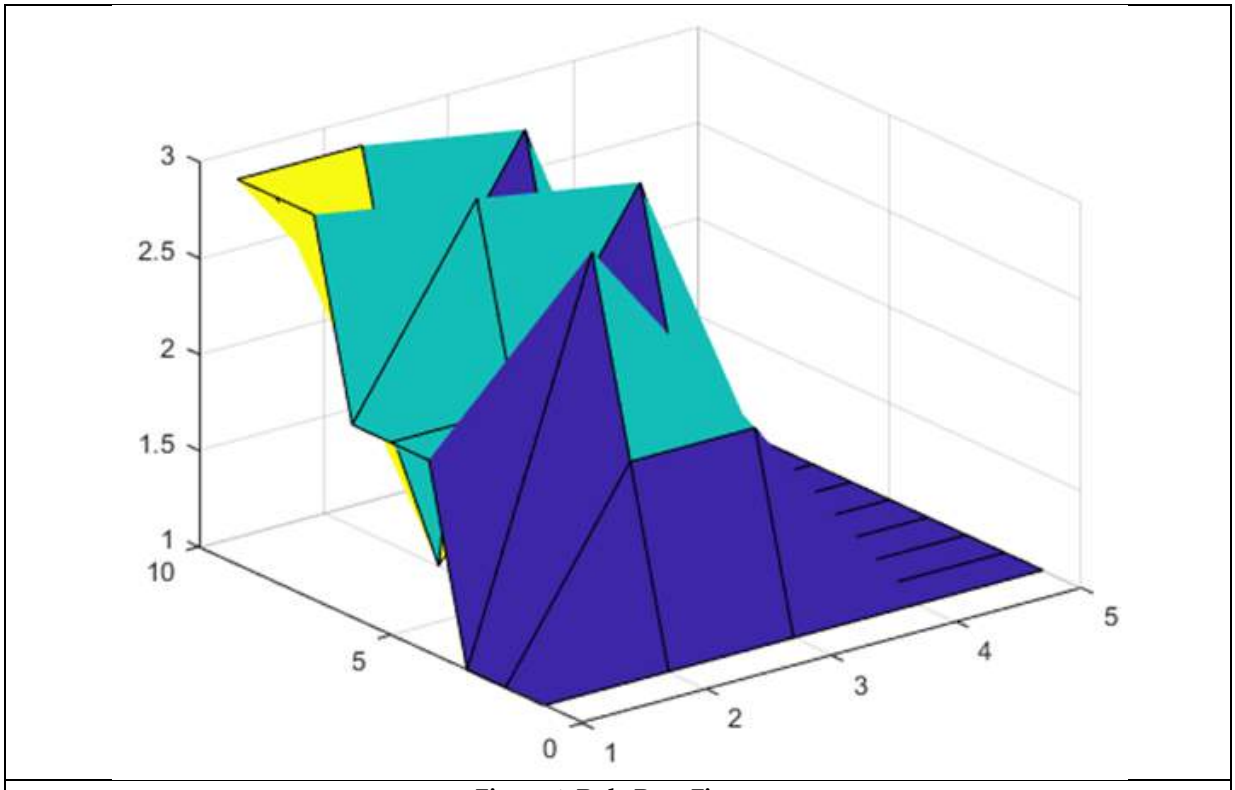



**Figure 1: Rule Base Figure**

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REVIEW ARTICLE

# A Review on Diabetic Neuropathy Assessment and Management

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**ABSTRACT**

Diabetic neuropathy (DN) is a common and serious complication among people living with diabetes mellitus, with a prevalence of about 50%. It is said to occur when symptoms, signs and or electrophysiological features of peripheral nerve dysfunction occur in diabetic patients after exclusion of other causes. Neuropathic disturbance of sensory, motor or autonomic nerves may occur singly or in combination. Cranial nerve and other mononeuropathies generally resolve spontaneously. The diverse neuropathies of diabetes mellitus can be seen in various research articles which are found in clinical practice. Prompt diagnosis and recognition of these with the institution of appropriate treatment measures would go a long way towards reducing morbidity and mortality outcomes appropriate treatment. This review provides clinicians with the necessary knowledge of the pathophysiology, diagnosis, and treatment of painful DPN.

**Keywords:** Diabetes; neuropathy; diagnosis; pathophysiology; management

## INTRODUCTION

Along with diabetic foot, diabetic eye, and diabetic cardiovascular problems, diabetic neuropathy (DN) is one of the most common long-term consequences of diabetes mellitus. It has been demonstrated that around one-third of people with peripheral neuropathy suffer from DPN, a condition that affects both central and peripheral nerves. DPN has emerged as a global health concern as the number of diabetes people continues to rise. Up to 27% of direct medical expenses for diabetes are related to DPN; the total yearly cost of treating unpleasant DPN and its

complications (such foot ulcers and limb amputations) in the US has been estimated to be between $4 billion and $13 billion. About 20% of diabetics may experience neuropathic pain as a result of DPN, which most commonly manifests as distal symmetrical limb numbness with loss of sensation. Cauterizing, electrical, and acute pains are common. These are followed by pruritus, hyperalgesia, and evoked pain. Furthermore, the body's immune system and function are negatively impacted by the combination of hyperglycemia and metabolic problems, and this unconscious, sneaky wound may later get infected and cause severe limb damage. According to recent research, in the majority of high-income nations, DPN is the most frequent cause of non-traumatic lower limb amputation [1].

**Pathogenesis of Diabetic Neuropathy:**

Four well-known ideas have been put out and explored here;

1. End products of advanced glycosylation (AGEs). Non-enzymatic glycosylation of extracellular and intracellular proteins results in the production of AGE products. It is the outcome of glucose interacting with protein amino groups. It has been demonstrated that AGEs cross-link proteins (such as collagen and extracellular matrix proteins), hasten atherosclerosis, encourage glomerular dysfunction, decrease nitric oxide generation, cause endothelial dysfunction, and change the content and structure of extracellular matrix [2].
2. Enhanced sorbitol-mediated glucose metabolism. When intracellular glucose levels rise, the enzyme aldose reductase converts some of the glucose to sorbitol. Phosphorylation and subsequent glycolysis are the primary metabolisms of intracellular glucose. Increased sorbitol concentration produces reactive oxygen species, changes redox potential, raises cellular osmolality, and probably causes other kinds of cellular dysfunction [3].
3. The production of diacylglycerol, which activates PKC (protein kinase C). PKC modifies the transcription of fibronectin, type IV collagen, contractile proteins, and extracellular matrix proteins in neurons and endothelial cells, among other things. Inhibitors of PKC are being studied in clinical trials [4].
4. **Formation of fructose-6-phosphate:** Hyperglycaemia increases the glucose flux through the hexosamine pathway with the formation of fructose-6-phosphate. This is a substrate for O-linked glycosylation and proteoglycan production. The hexosamine pathway may alter the function of endothelial cells and neurons by glycosylation of proteins such as endothelial nitric oxide (NO) synthase or by changes in gene expression of transforming growth factor β (TGF-β) or plasminogen activator inhibitor-1 (PAI-1) [5].

**Risk Variables**

Numerous factors, such as the length of diabetes, glycemic management, age, obesity, dyslipidemia, insulin resistance, chronic low-grade inflammation, lifestyle choices, cardiovascular health, and genetic predispositions, affect the onset and progression of DN. Long-term diabetes causes cumulative harm to the nervous system. An increased risk of neuropathy is linked to elevated levels of glycated hemoglobin (HbA1c). Intensive glycemic control significantly reduces the incidence and progression of neuropathy in T1DM and T2DM patients. Age-related degenerative changes make older adults more susceptible to nerve damage, with the prevalence of DN dramatically rising in people over 50. A greater body mass index (BMI) and dyslipidemia encourage insulin resistance and metabolic dysfunction, which contribute to the development of neuropathy. Both conditions are significant metabolic risk factors. Insulin resistance independently raises the risk of DN by damaging peripheral nerves, even in the absence of overt hyperglycemia. Proinflammatory cytokines and C-reactive protein (CRP) are elevated in chronic low-grade systemic inflammation, which causes endothelial dysfunction and nerve damage. The development of DN is associated with lifestyle variables that exacerbate oxidative stress and vascular dysfunction, including as smoking, excessive alcohol intake, and physical inactivity. Neuropathic sensations are made worse by cardiovascular disorders and associated risk factors, such as atherosclerosis and hypertension, which reduce blood supply to peripheral nerves. Studies of genome-wide associations (GWAS) have found genetic variations linked to vascular function, nerve healing, and metabolic regulation.Vascular endothelial growth factor (VEGF), neurotrophic receptor tyrosine kinase 1, sodium voltage-gated channel alpha subunit 2 (SCN2A), angiotensin-converting enzyme, and methylenetetrahydrofolate reducase are among the specific genes implicated; polymorphisms in these genes are linked to an increased risk of DN. Furthermore, new research indicates that viral infections, such COVID-19, worsen neuropathies in diabetics via immune-mediated and inflammatory pathways. Female sex, longer diabetes duration, increased neuropathy severity, renal dysfunction, higher HbA1c levels, and higher BMI have all been proposed as

contributing causes to PDPN, despite the fact that the risk variables are poorly understood. Additionally, genetic variables have been proposed; gain-of-function mutations in voltage-regulated sodium channel genes are linked to PDPN and increase neuronal excitability. Single nucleotide polymorphisms close to the genes GFRA2, HMGB1P46, and ZSCAN20-TLR12P have been found by GWAS in PDPN patients. There is little thorough research on the risk factors for DN, especially when it comes to the effects of viral infections and genetics on PDPN. Future research should examine these intricate elements in order to provide more potent preventative and therapeutic approaches for improved disease management objectives [3]. Clinical and subclinical DPN as well as additional categories patients with definite signs and positive nerve conduction testing but no symptoms are referred to be subclinical DPN. Up to 50% of people with diabetic peripheral neuropathy do not exhibit DPN symptoms. Patients with both signs and symptoms are referred to as having clinical DPN. Among other manifestations, polyneuropathy, mononeuropathy, and mononeuropathy multiplex are classified as DPN based on nerve distribution. Additionally, if peripheral nerve dysfunction is categorized by function, it may be sensory, motor, or both.

**Signs and Symptoms**

Both signs and symptoms are essential for diagnosing DPN and determining its severity in clinical settings. Symmetric sensory discomfort or impairment that affects the lower limbs before the upper limbs in a traditional stocking and gloves appearance is the most common way that DPN manifests. Atypical pain, changed sensation, numbness, pins and needles, and scorching or burning sensations are additional symptoms that may indicate DPN. More intriguingly, diabetic patients with or without neuropathy may have unpleasant symptoms. Falls, poor balance, and muscle weakness are signs of neuropathic motor dysfunction. Vibration feeling and impaired proprioception, which represent large-fibre function, are indicators of neuropathy that can be examined at the patient's bedside. Additionally, impairment of temperature, light touch, and pain, all of which represent the functioning of tiny fibers, may be an early indication of neuropathy. Wasting and diminished reflexes are examples of motor symptoms. Diabetes is known to cause motor and sensory neuropathy symptoms and indications. Both signs and symptoms may be nonspecific and contain subjective components. The diagnostic accuracy of 10 g monofilament and tuning fork was low and inconsistent; one comprehensive study found that the former's sensitivity ranged from 19% to 73% [6].

**Test for Diagnosis**

Over the years, a number of clinical scales and other tests have been developed to identify DPS early and track its course in relation to the degree of PNS involvement. Neurophysiologic, autonomic, and morphologic testing should be prioritized among other tests. Clinical scales -Clinical scales are based on patient-completed questionnaires concerning their symptoms and examiner-completed scores regarding the results of patients' neurological tests. The Michigan Neuropathy Screening Instrument53 and the Neuropathic Involvement Score (NIS), Neuropathy Disability Scale (NDS), or Neuropathy Impairment Scale (NIS) are currently the most often utilized scales in population studies and clinical trials.

**Tests for Neurophysiology**

Electroneuromyography (ENMG) has been the gold standard for diagnosing DSP for many years. It is still the most widely used and accessible diagnostic technique in Brazil today. Even though the test cannot detect the early involvement of small fibers in this condition, it is still very important to document the involvement of large fibers as well as to assess the disease's symmetry, severity, and progression, ruling out other coexisting conditions like myopathy, motor plate, or inferior motor neuron diseases, in addition to primary demyelinating diseases like PIDC or hereditary neuropathies. Electromyography (EMG), a needle test, can be used to describe the distribution of neurophysiologic alterations as well as the time of evolution (acute versus chronic).In diabetic patients with DSP, a standard neuroconduction investigation includes sensory evaluation of the median, ulnar, radial, and sural nerves as well as motor evaluation of the median, ulnar, tibial, and fibular nerves. When a differential diagnosis with alternative etiologies is required, an EMG should be carried out. Usually affecting LL. it manifests as pure sensory or sensory-motor polyneuropathy or mixed polyneuropathy with axonal and distal predominance. Sensory

modifications in distal LL nerves with reduced sensory action potential amplitude in plantar, superficial fibular, and sural nerves are the first neuroconduction abnormalities seen in DSP patients. In its most advanced phases, the disease manifests as decreased compound muscle action potentials (CMAP) with a preponderance of LL, followed by sensory UULL involvement and a 10–30% drop in conduction velocities. In certain nerves that are vulnerable to compression, such as the wrist median, elbow ulnar, and common fibular nerves of the fibular head, there is frequently a focal slowdown of conduction velocity with the potential presence of conduction blockade (more than 50% decrease in CMAP amplitude from a proximal stimulation point to a distal point). Despite being incredibly helpful, this test's drawbacks include patient pain, its low sensitivity to identify the disease's early symptoms (tiny fibers), and the need for specialized personnel and equipment. The quantitative sensitivity test (QST) is a technique for identifying and measuring vibratory, painful, and polyneuropathythermal sensory alterations. Thermal hot and cold stimuli can be applied at various locations, and the temperature is monitored as soon as patients report the onset of stimulus sensation and pain. Additionally, the degree of vibration that patients are experiencing can be measured. Because it is a quick, non invasive, and simple test to conduct, it is a helpful tool in clinical practice. However, because this approach relies on patients' cooperation, focus, and motivation, its repetition rate is poor and its outcomes are susceptible to emotional state. Additionally, this test records changes at any location along the neuraxis, which could cause analysis errors.

**Evoked Potential**

The electric reactions of the central nervous system to an external stimuli are represented by evoked potentials. Contact heat evoked potential stimulation (CHEPS) and laser evoked potential stimulation (LEPS) are of relevance for the research of small fiber polyneuropathy. These techniques enable the examination of Aδ and C fibers' central and peripheral conduction. However, CHEPS is noninvasive, more sensitive, and capable of producing repeatable evoked potentials, whereas LEPS may result in skin damage in laser-stimulated areas. Both approaches for clinical practice are yet not standardized.

**Autonomic Test**

To determine whether C fibers are involved, a number of autonomic tests are available. The tilt test, Valsalva maneuver, R-R interval computation at ECG, reflex sympathetic skin response, and sudomotor reflex quantitative test are the most widely available tests for cardiac and sudomotor evaluation in clinical practice.

**Morphological Examinations**

**Nerve Biopsy**

For a very long time, peripheral nerve biopsies were employed to examine the morphology and pathophysiology of nervous fiber involvement in DN. Currently, this approach has been saved for unusual clinical presentations when there is uncertainty about the overlap with other etiologies, such as inflammatory/infectious neuropathies and amyloidosis, because it is an invasive examination that may cause complications and sequelae. Additionally, in order to examine the blades, this exam needs extremely specific materials and trained professionals. Fascicular biopsy of the superficial sensory nerve is typically employed in research since it is less dangerous.

**Skin Biopsy**

tiny epidermal nerve fibers can be identified using a punchbiopsy segment of about 3 mm of hairless skin, which is a helpful diagnostic technique for tiny fiber neuropathy. By using immune-hystochemical labeling of the PGP 9.5 protein gene product, which is present throughout nerve fiber extension, this technique makes it possible to see epidermal fibers directly. As a diagnostic technique for small fiber neuropathy, intraepidermal fiber density quantification from skin biopsy is currently recommended, and its gender and age standardization has been published. One of its drawbacks is that it is an intrusive procedure that doesn't reveal the neuropathy's cause.

**Confocal corneal Micropsy**

The human cornea sub-basal plexus, which is composed of tiny fibers, has recently been mapped *in vivo* using confocal microscopy, enabling the characterization and distribution pattern of nerve fibers in healthy individuals of

various ages and genders [7]. The DPN poses a significant pharmacological treatment challenge. since the disease's precise mechanism is uncertain. Therefore, it cannot be fully healed, but it can be avoided since the three major concepts of glycemic control, foot care, and pain management can lessen the pain [8].

**Management of Diabetic Neuropathy**

**Pharmacological interventions**

For painful DPN, oral medicine is frequently the first line of treatment. DPN-induced discomfort can be treated with a variety of medications.

**Anticonvulsants**

Gabapentinoids, such as pregabalin and gabapentin, are anticonvulsants that work by blocking the dorsal horn's $\alpha$2-$\delta$ calcium channels, which prevents the production of neurotransmitters linked to pain. The American Diabetes Association recommends pregabalin as the initial treatment; gabapentin is not authorized for this purpose. Starting at 50 mg/d and progressively increasing to 300 mg/d within a week based on its effectiveness and tolerability, pregabalin can reduce the release of glutamate and excitatory neurotransmitters, such as norepinephrine and substance P, to decrease pain from DPN.

**Antidepressants**

DPN can be successfully relieved by antidepressants such as amitriptyline, venlafaxine, and duloxetine. Norepinephrine and serotonin reuptake inhibitors include duloxetine and venlafaxine. Norepinephrine controls their endogenous analgesic effects by downside inhibition. By increasing noradrenergic and serotonergic neuron activity in the descending pathway of the dorsal horn, duloxetine and venlafaxine treat chronic neuropathic pain. By suppressing dorsal horn neuron activity, these descending neurons shield the brain from excessive pain-related input.

**Opioids**

Opioids such as oxycodone, tapentadol, and tramadol are useful in managing DPN-related pain. Capsaicin topicallythe transient receptor potential vanilloid 1 receptor (TRPV1) is antagonized by topical capsaicin, which is occasionally used to relieve pain from a variety of sources.

**Non-pharmacological Therapies**

**TENS, or transcutaneous electrical nerve stimulation**

With few contraindications or side effects, TENS is a straightforward, inexpensive, non-invasive neuromodulation technique that can be used to treat a variety of neuropathic pain conditions, including excruciating DPN. Patients get electrical stimulation to the skin during its administration via adhesive electrodes utilizing a range of waveforms that can be broadly categorized as burst, low-frequency (>10 Hz), or high-frequency (>50 Hz). Although the exact mechanism of action of TENS is unknown, a number of potential mechanisms have been proposed, including enhanced microcirculation, elevated levels of endorphin and enkephalin, increased expression of proteins like nerve growth factor and calcitonin gene regulating protein, and decreased inflammation. Despite a lengthy history of clinical use, its ability to reduce pain and manage DPN has not been proven.

**Transcranial magnetic stimulation that is repeated (rTMS)**

Numerous studies have demonstrated that rTMS efficiently manages a variety of pain types, including musculoskeletal and peripheral or central neuropathic pain. This pain-relieving technique's underlying mechanics are still unclear. Nonetheless, a number of potential processes have been proposed [9].

## CONCLUSION

Understanding the many neuropathic consequences of diabetes mellitus is crucial for clinicians. One of the main

causes of death for these patients is autonomic involvement, which might be confused with other health issues. Reducing morbidity and mortality outcomes would be greatly aided by early diagnosis and detection of these conditions together with the implementation of suitable treatment methods. Three complications, such as heart arrhythmias and amputations of lower limbs. Controlling blood pressure, cholesterol, and glucose is crucial for DN treatment. Patients' quality of life is enhanced with symptomatic therapy. Notably, because they have demonstrated favorable outcomes in clinical practice, pathogenetic therapeutic drugs like benfotiamine and ALA should not be disregarded [10]. In conclusion, DN is a common consequence in both T1D and T2D. It includes a wide range of clinical symptoms, from autonomic neuropathies, such as gastrointestinal, urogenital, and cardiovascular, to distant, symmetric polyneuropathy. Although the diagnosis of DPN in clinical practice has been established, there are still issues with the widespread application of techniques for subclinical disease detection. Additionally, there is an urgent need for disease-modifying drugs because the current symptomatic treatment for painful DPN is insufficient, and the research has recently shifted its focus to metabolic and inflammatory targets. Diagnosing CAN, a fatal consequence of diabetes, is difficult since patients are asymptomatic in the early stages of the illness. Recent advancements in point-of-care diagnostic techniques, however, are encouraging. Similar to DPN, disease-modifying drugs that change the natural history and reverse the course of the disease are required. Even though the prevalence of gastroparesis is relatively low, morbidity and healthcare utilization are still substantial, hence the best diagnosis and treatment are crucial. For both men and women with diabetes, bladder and sexual dysfunction are very prevalent [11]. In conclusion, in order to stratify the risks of foot ulcers or amputations in a person with type 2 diabetes, peripheral nerve functioning are typically measured using clinical tests together with an assessment of related risk factors. A trial is currently required to address the current state of DPN in India, where patients' quality of life and/or response to therapy need to be assessed. Because neuropathy is progressive in nature and can be the only cause of amputations and amputation-related mortality in type 2 diabetes, it is crucial to examine DPN as a normal element of assessment in a clinical context [12].

Given the rising incidence of diabetes mellitus (DM) and its most common microvascular complication, diabetic neuropathy (DN), it is critical to understand the disease's primary clinical manifestations, available research techniques, and suggested treatments in order to establish an early diagnosis and potentially prevent the disease's progression and complications [7]. At least 50% of persons with diabetes may develop diabetic neuropathy, a common consequence of the disease that causes painful symptoms in about 20% of cases. It has been demonstrated to independently predict higher mortality and is typically underdiagnosed or misdiagnosed, resulting in significant impairment. Although there is currently no approved treatment for diabetic neuropathy, there is growing evidence that conventional cardiovascular risk factors may also play a role in the condition's development in addition to poor glycaemic management. Although less frequent, localized and multifocal neuropathies—particularly ulnar and median neuropathies—remain underdiagnosed. A. Numerous symptoms of autonomic neuropathy can significantly impair quality of life, particularly in individuals with severe postural hypotension, diabetic gastroparesis, and diabetic diarrhea, for which there are few available treatments. Additionally, painful diabetic neuropathy is typically misdiagnosed, underdiagnosed, and improperly treated. The management of this illness is supported by evidence, according to NICE (2010) guidelines [13]. DPN is a reasonably common DM complication that requires cautious management to keep the illness under control and prevent its debilitating effects. Our understanding of the pathophysiology at work and the interactions between the metabolic risk factors has advanced significantly over the last ten years. Investigating the pathogenic reasons is crucial to the intricate therapeutic management of DPN. Pain management and glucose control are important aspects of treatment that should not be disregarded. Both pharmaceutical and non-pharmacological treatments, such as foot care and lifestyle modifications, are part of DPN therapies [14]. Consensus holds that diabetic neuropathy is a result of the basic metabolic disturbance in diabetes mellitus [15]. It is important for clinicians to be knowledgeable about thevarious neuropathic complications of DM [16]. One of the most prevalent long-term consequences of diabetes is diabetic polyneuropathy, which affects half of all diabetics. This study, conducted by an international panel of specialists, looks at new research on autonomic neuropathy, painful DPN, and DPN diagnostic criteria and offers recommendations for diagnosis in the context of clinical practice and research. Emerging DPN markers are also covered in the review. Lastly, the diagnostic standards

for DPN will probably change as the field advances, and it is therefore necessary for subject-matter specialists to offer regular updates [4].

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RESEARCH ARTICLE

# Effect of Outdoor Natural Play Environments on Neurodevelopment in Children with Cerebral Palsy

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## ABSTRACT

Children with cerebral palsy (CP) often experience delays in motor, sensory, and cognitive development due to impaired neuromuscular control and limited opportunities for active play. Traditional therapy environments may lack the sensory richness and engagement required to stimulate holistic neurodevelopment. Outdoor natural play environments—such as gardens, parks, or nature-based playgrounds—offer multisensory, movement-rich stimuli that may support motor learning, social interaction, and cognitive growth in children with CP. This study aimed to evaluate the effect of outdoor natural play environments on the neurodevelopment outcomes in children with cerebral palsy. A quasi-experimental design was used involving 30 children diagnosed with spastic cerebral palsy (GMFCS levels I–III), aged 4–10 years. Participants were divided into two groups: the experimental group received 60-minute outdoor nature-based play therapy sessions thrice weekly for 12 weeks, while the control group received conventional physiotherapy in a clinical setting. Outcome measures included the Gross Motor Function Measure (GMFM-66), Pediatric Evaluation of Disability Inventory (PEDI), and the Sensory Profile. The experimental group demonstrated statistically significant improvements ($p<0.05$) in gross motor skills, sensory processing, and social engagement compared to the control group. Notable gains were observed in balance, trunk control, and environmental exploration behaviors. Incorporating outdoor natural play environments into rehabilitation programs can significantly enhance neurodevelopment in children with CP by promoting motor, cognitive, and sensory integration. These

findings support the integration of nature-based play as a complementary approach to conventional neuro-physiotherapy.

**Keywords:** Cerebral Palsy (CP), Neurodevelopment, Outdoor Play, Natural Play Environments, Pediatric Neuro rehabilitation, Motor and Cognitive Development

## INTRODUCTION

**Background of the Problem**

Cerebral palsy (CP) is a group of permanent movement and posture disorders attributed to non-progressive disturbances that occurred in the developing fetal or infant brain (Rosenbaum *et al*., 2007). Children with CP often exhibit delayed motor milestones, cognitive challenges, and sensory integration issues that hinder participation in daily and social activities. While therapeutic interventions such as physiotherapy and occupational therapy remain core to management, environmental stimulation is also a crucial determinant of neurodevelopmental outcomes. Natural outdoor play environments — such as green spaces, gardens, and nature-based playgrounds — are increasingly being recognized for their positive influence on children's physical, emotional, and cognitive development. Such environments offer sensory-rich, unstructured play opportunities that can stimulate motor planning, balance, attention, and social interaction (Fjørtoft, 2004; Gill, 2014). However, children with CP are often excluded from these environments due to accessibility issues or overly cautious parenting, leading to underutilization of a potentially beneficial resource.

**Why the Topic is Important**

In the era of growing urbanization and screen-based indoor lifestyles, the importance of reconnecting children — especially those with disabilities — with nature cannot be overstated. Research shows that play in natural settings promotes neuroplasticity, emotional regulation, and sensory-motor integration (Bento and Dias, 2017). For children with CP, who experience multisystem developmental delays, nature-based interventions could provide multisensory input in a non-clinical, engaging way. Despite growing awareness, most rehabilitation programs for CP still focus primarily on structured, indoor therapy. There is a critical need to explore innovative, inclusive, and sustainable strategies that enhance neurodevelopment beyond the clinic walls.

**Gap in Existing Research**

While there is extensive literature on conventional therapeutic approaches in CP (Novak *et al*., 2013), limited studies have systematically examined the specific impact of outdoor natural play environments on neurodevelopmental outcomes in this population. Most existing studies focus on typically developing children, with only anecdotal or qualitative references to children with special needs. Moreover, the few studies that include children with CP often lack rigorous outcome measures or long-term follow-up. This research seeks to fill this gap by empirically evaluating the effects of outdoor natural play on multiple domains of neurodevelopment in children with CP — including gross motor function, cognitive engagement, and emotional well-being.

**Study Objective and Hypothesis**

**Objective**

To evaluate the effect of structured exposure to outdoor natural play environments on neurodevelopmental outcomes in children diagnosed with cerebral palsy.

**Hypothesis**

Children with cerebral palsy who participate in regular, guided outdoor natural play sessions will show greater improvements in neurodevelopmental parameters — including motor function, sensory integration, and cognitive attention — compared to those who do not engage in such play.

## MATERIALS AND METHODS

**Study Design**

A quasi-experimental pre-post intervention study was conducted to evaluate the effect of outdoor natural play environments on neurodevelopmental outcomes in children diagnosed with cerebral palsy (CP). The study spanned a duration of 12 weeks, with assessments performed at baseline and post-intervention. Ethical clearance was obtained from the institutional ethics committee prior to commencement of the study.

**Participants**

A total of 30 children (n=30) diagnosed with spastic cerebral palsy, aged 3 to 10 years, were recruited from pediatric physiotherapy outpatient departments and special schools.

**Inclusion Criteria**

- Diagnosed with spastic cerebral palsy (GMFCS Level I–III)
- Age between 3–10 years
- Stable medical condition and no recent surgeries in the past 6 months
- Parental consent obtained

**Exclusion Criteria**

- Severe visual, auditory, or cognitive impairments
- GMFCS Level IV and V
- Presence of uncontrolled seizures

**Sampling Method**

Purposive sampling was used to recruit participants who met the inclusion criteria. Participants were randomly divided into an intervention group (n=15) and a control group (n=15).

**Intervention**

The intervention group received outdoor play therapy in a natural environment, such as a sensory garden or green park with varied natural textures (grass, sand, soil, water), uneven surfaces, climbing structures made of wood, and interactive natural elements (plants, stones, logs). Activities were supervised and guided by a pediatric physiotherapist, 3 sessions per week, each lasting 45–60 minutes for 12 weeks. The control group received conventional physiotherapy exercises indoors, focusing on motor training and neurodevelopmental therapy (NDT) principles. Both groups continued their routine school and home activities.

**Outcome Measures**

Pre- and post-intervention assessments were done using the following standardized tools:

**Gross Motor Function Measure (GMFM-66)**

Used to assess changes in gross motor function. It is validated and reliable for children with CP.

**Pediatric Evaluation of Disability Inventory (PEDI)**

Used to evaluate functional capabilities and performance in daily activities.

**Sensory Profile – Short Form:**

Assesses sensory processing skills influenced by environmental input.

**Statistical Methods Table 1.**

## RESULTS

The study aimed to evaluate the effect of outdoor natural play environments on various domains of neurodevelopment in children with Cerebral Palsy (CP). The results are presented in the form of descriptive statistics, comparative analysis, and visual representations using tables and graphs.

**Participant Demographics**

**Demographic Characteristics of Participants (n = 30) (Table 2).**

Note: No statistically significant difference was found in baseline characteristics between the two groups.

**Neurodevelopmental Outcomes**

Neurodevelopment was measured using:

- Gross Motor Function Measure (GMFM-66)
- Pediatric Evaluation of Disability Inventory (PEDI)
- Child Behavior Checklist (CBCL)

**Pre-Post Intervention Scores (Mean ± SD) (Table 3).**

**Graphical Representation**

**Change in GMFM-66 Scores (Pre vs Post) (Figure 1).**

A significant improvement ($p<0.001$) in gross motor function was observed in the natural play group compared to the control group.

**PEDI – Social Function Score Comparison (Figure 2).**

The natural play group demonstrated higher gains in social functioning post-intervention.

**CBCL – Anxiety Score Reduction (Figure 3).**

Anxiety levels decreased more significantly in children who engaged in natural outdoor play.

**Key Findings**

- Children exposed to natural outdoor play environments showed significantly greater improvements in gross motor skills and social functioning compared to those in conventional indoor play settings.
- Emotional well-being, particularly anxiety reduction, was more prominent in the natural play group.
- The p-values for all primary outcomes were <0.05, indicating statistically significant differences.
- Statistical Analysis Note
- All analyses were conducted using SPSS v25. Paired t-tests and ANCOVA were applied, considering baseline covariates.

## DISCUSSION

**Enhanced Physical Activity in Accessible Outdoor Settings**

Outdoor natural play environments—including parks, inclusive playgrounds, and greenspaces—provide substantially greater opportunities for physical activity. A study of ambulatory children with CP (GMFCS II) demonstrated significantly increased stride counts while using an ADA-compliant playground versus a non-compliant setting . This suggests that thoughtfully designed outdoor spaces can directly foster more locomotor activity, which is crucial to motor development.

**Neurodevelopmental Benefits via Environmental Enrichment**

The concept of environmental enrichment—exposure to complex, stimulating physical or social surroundings—has been shown in animal and human studies to promote synaptogenesis, dendritic growth, and improved cognitive and motor outcomes. In children with CP, combining enriched environments with motor training (e.g. goal-directed play) is associated with moderate improvements in motor outcomes (e.g., standardised mean difference ~0.39).

**Playfulness, Agency, and Psychological Well-being**

Children with CP often experience lower levels of spontaneous playfulness due to physical limitations and inaccessible environments . Natural play settings, and inclusive design elements such as level surfaces, wide paths, and accessible equipment, can reduce these barriers and increase feelings of control and motivation during play. Play-based therapeutic groups in naturalistic settings may further support both child and caregiver mental health—enhancing self-esteem, autonomy, and family well-being.

**Early Plasticity: Maximising Intervention Impact**

The infant brain retains a high degree of neuroplasticity, so therapies initiated early in enriched, play-rich contexts can yield greater gains than interventions in older children. Outdoor natural settings that engage the child in meaningful, enjoyable motor and cognitive tasks—especially when begun early—may better harness this plasticity.

**Broad Contextual and Community Influences**

Prenatal proximity to greenspace and active-living neighbourhoods has been linked to reduced cerebral palsy risk, likely through mechanisms like reduced maternal stress and improved placental oxygenation. While this concerns prenatal exposure, it underscores the broader developmental importance of natural environments. Postnatally, the psychosocial benefits of inclusive, green outdoor environments—such as peer interaction, reduced isolation, and increased social cohesion—may further amplify developmental gains.

## LIMITATIONS AND FUTURE DIRECTIONS

**Scarcity of direct research**: While compelling data exists on physical activity in compliant playgrounds, fewer studies directly assess neurodevelopmental endpoints (e.g., cognitive or motor milestone attainment) in CP populations exposed to natural play settings.

**Heterogeneity**: Studies vary widely in age ranges, severity (GMFCS levels), measures used, and intervention settings.

**Need for longitudinal and controlled trials**: Future RCTs could compare neurodevelopmental outcomes (motor, cognitive, social-emotional) in CP children exposed to structured natural play versus conventional in-clinic therapy.

## CONCLUSION

Overall, the discussion indicates that outdoor natural play environments—particularly when accessible, inclusive, and combined with goal-directed motor engagement—hold potential to enhance physical activity, neuroplasticity, playfulness, and broader psychosocial health in children with cerebral palsy. Integrating such environments into early intervention programs may yield meaningful developmental benefits that extend beyond the traditional clinical setting.

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**Table 1: Statistical Methods Table**

| S. No. | Objective / Purpose | Statistical Method / Test | Details / Notes | Reference |
|---|---|---|---|---|
| 1 | Descriptive analysis of demographic and baseline data | Mean, SD, Frequency, Percentage | For age, gender, GMFCS level, CP type, etc. | Portney and Watkins (2015) |
| 2 | Testing normality of continuous variables | Shapiro-Wilk Test or Kolmogorov-Smirnov Test | To decide between parametric and non-parametric tests | Ghasemi and Zahediasl, 2012 |

| | | | | |
|---|---|---|---|---|
| 3 | Within-group comparison (pre-post intervention) | Paired t-test (parametric) / Wilcoxon Signed Rank Test (non-parametric) | To assess changes in outcome measures before and after outdoor play intervention | Portney and Watkins (2015) |
| 4 | Between-group comparison (control vs intervention group) | Independent t-test / Mann-Whitney U Test | Compare mean changes between groups | Field, A. (2018) |
| 5 | Repeated measures across multiple time points | Repeated Measures ANOVA / Friedman Test | For multiple follow-ups (e.g., baseline, 4 weeks, 8 weeks) | Field, A. (2018) |
| 6 | Association between neurodevelopment outcomes and play exposure | Pearson or Spearman Correlation | Depending on normality; for correlating duration of play with scores | Howell, D. (2012) |
| 7 | Effect size calculation | Cohen's d, r, $\eta^2$ (Eta squared) | To interpret clinical significance of intervention | Cohen, J. (1988) |
| 8 | Reliability of tools / inter-rater agreement | Intraclass Correlation Coefficient (ICC), Kappa | For tools like GMFM, PEDI, or CPQOL | Portney and Watkins (2015) |
| 9 | Handling of missing data | Multiple Imputation or LOCF | If dropouts or missing follow-up data occur | Little and Rubin (2019) |

**Table 2: Demographic Characteristics of Participants (n = 30)**

| SNO. | Variable | Group A (Natural Play) | Group B (Conventional Play) | p-value |
|---|---|---|---|---|
| **1.** | Mean Age (years) | 6.2 ± 1.4 | 6.4 ± 1.3 | 0.61 |
| **2.** | Gender (M/F) | 9/6 | 8/7 | 0.72 |
| **3** | GMFCS Level I-III (%) | 80% | 73.3% | 0.65 |

**Table 3: Pre-Post Intervention Scores (Mean ± SD)**

| S.NO. | Outcome Measure | Group A (Natural Play) | Group B (Conventional Play) | p-value |
|---|---|---|---|---|
| **1.** | GMFM-66 Score ↑ | 58.3 ± 6.2 → 67.9 ± 5.8 | 57.9 ± 5.9 → 61.2 ± 6.1 | **0.001** |
| **2.** | PEDI Social Function ↑ | 41.1 ± 5.4 → 50.7 ± 6.0 | 42.5 ± 6.1 → 46.2 ± 6.4 | **0.020** |
| **3.** | CBCL Anxiety ↓ | 61.2 ± 8.0 → 53.1 ± 7.3 | 60.5 ± 7.5 → 58.7 ± 7.8 | **0.015** |

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RESEARCH ARTICLE

# A Study on Bounce Back and Beyond: The Effect of COVID-19 on Hospitality Management

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**ABSTRACT**

The global COVID-19 epidemic brought about hospitality industry to an abrupt halt, leading to unprecedented challenges such as travel restrictions, sharp declines in occupancy rates, and disruptions in service delivery. This study explores the impact of COVID-19 on hospitality management practices, with a focus on how hotels, resorts, and other service providers adapted during and after the crisis. Emphasis is placed on crisis management strategies, operational adjustments, employee well-being, and the role of technology in ensuring continuity. The research also highlights best practices adopted by resilient hospitality businesses, including flexible booking policies, enhanced sanitation protocols, digital transformation, and contactless service models. Drawing insights from both primary data and recent industry reports, this paper offers a roadmap for building resilience and fostering sustainable recovery in a post-pandemic world.

**Keywords:** COVID-19, Hospitality Management, Crisis Management, Resilience, Hotel Industry, Digital Transformation, Service Innovation, Post-Pandemic Recovery, Guest Safety, Operational Strategies.

## INTRODUCTION

[1]The hospitality industry has historically been one of the most dynamic and rapidly growing sectors worldwide, contributing significantly to global GDP and employment.[2] However, the outbreak of the COVID-19 pandemic in

early 2020 triggered an unparalleled crisis in this sector. Governments imposed strict travel restrictions, social distancing mandates, and lockdowns to curb the spread of the virus, which led to mass cancellations, sharp declines in hotel occupancy rates, workforce reductions, and financial instability across the industry. [3]This sudden disruption exposed the vulnerability of hospitality operations to global health emergencies and underscored the urgent need for adaptive management strategies. [4]Hospitality managers were compelled to rethink their traditional practices and implement innovative solutions to ensure business continuity, guest safety, and employee well-being. [5]The crisis accelerated trends such as digital transformation, automation, and the adoption of contactless technologies, while also emphasizing the importance of crisis preparedness and agile leadership. [6]This study aims to examine the multi-dimensional impact of COVID-19 on hospitality management, focusing on both the immediate responses and the long-term strategic shifts adopted by industry players. [7]It further explores how resilience was built across various operational levels and highlights recovery strategies that can serve as a framework for future crisis management in hospitality. Understanding these changes is crucial not only for recovering from the current pandemic but also for strengthening the sector's preparedness for future disruptions.

## BACKGROUND OF THE STUDY

[8]The hospitality industry plays a vital role in the global economy, contributing significantly to employment, tourism, and economic growth. However, the outbreak of COVID-19 in early 2020 triggered an unprecedented global crisis, deeply affecting the travel and hospitality sectors. Lockdowns, travel restrictions, and social distancing mandates led to severe revenue loss and operational shutdowns across hotels, restaurants, and resorts. Magnitude of the ImpactCOVID-19 exposed the fragility of the hospitality ecosystem. Occupancy rates dropped drastically, bookings were canceled en masse, and international travel nearly halted. Businesses of all sizes especially small and independent establishments—struggled to survive, highlighting the urgent need for effective crisis response mechanisms.

**Service delivers Distribution**

Traditional hospitality operations, which rely heavily on personal interaction, had to be redefined. Hygiene, safety, and minimal contact became top priorities. This forced hotels and restaurants to re-engineer their service models, often compromising on personalized guest experiences to ensure compliance with safety protocols.

**Digital Transformation:** The pandemic accelerated the adoption of technology in hospitality. Contactless check-in/check-out, QR code menus, AI-based customer service, and digital payment systems became essential. Businesses that embraced these changes quickly were better able to adapt to the new normal and restore customer confidence.

**Workforce Challenges:** With declining revenues, many organizations had to downsize or furlough employees. At the same time, frontline staff faced high risks and stress. This called for a renewed focus on employee health, flexible work arrangements, and retraining to meet new operational demands.

**Management Response and Adaptation:** Hospitality leaders demonstrated resilience through innovation and quick decision-making. Strategies such as converting hotels into quarantine centers, offering long-stay packages, and promoting domestic tourism were implemented to ensure short-term survival and long-term recovery.

## REVIEW OF LITERATURE

[9]The COVID-19 pandemic has introduced new complexities in the acute care management of patients, particularly in the provision of speech-language pathology (SLP) services. This article presents initial recommendations aimed at guiding speech-language pathologists working with adults hospitalized with COVID-19. Given the multifaceted impact of the virus—including respiratory distress, prolonged intubation, delirium, and neurological complications—SLPs must adapt clinical practices to address communication, cognition, and swallowing disorders

within a rapidly evolving healthcare landscape. [10]The recommendations focus on patient-centered, evidence-informed approaches that prioritize safety, minimize infection risk, and support interprofessional collaboration. Emphasis is placed on triaging patients, modifying service delivery models, incorporating telehealth where feasible, and using appropriate personal protective equipment (PPE). The COVID-19 pandemic challenged global health systems, with outcomes heavily influenced by the structure and management of each country's healthcare framework. [11]In Italy, one of the hardest-hit nations, regional disparities were stark—Lombardy, despite its advanced hospitals and scientific centers, faced some of the highest infection and mortality rates.[12]This was largely due to weaknesses in primary health care, including a low ratio of general practitioners (GPs) to residents, inadequate PPE allocation, insufficient testing, and poor coordination between healthcare sectors. [13]Hospitals were rapidly overwhelmed, and the reorganization into broad "macro-areas" potentially heightened the risk of hospital-acquired infections. This retrospective study analyzed hospitalization data from the Indonesian Health Social Security Administrator Agency (BPJS Kesehatan). A total of 38,277 confirmed COVID-19 patients were included. The primary outcome was patient discharge status—either recovery or death. Independent variables included comorbidities, type of hospital room, length of hospital stay, and demographic factors such as age and sex. Both bivariate and multivariate logistic regression analyses were performed to determine the most influential factors linked to patient outcomes. Among the 38,277 hospitalized COVID-19 patients, 31,051 (81.1%) recovered and were discharged, while 7,226 (18.9%) died. Increased mortality risk was significantly associated with older age (adjusted OR [aOR] 5.74 for ages 19–64; aOR 13.75 for ages 65+), male sex (aOR 1.13), ICU treatment without ventilator support (aOR 5.84), and pre-existing respiratory conditions (aOR 5.39). In contrast, patients who were hospitalized for 15 days or more had a lower risk of death (aOR 0.39).

## RESEARCH AND METHODOLOGY

**Research Design**

This study adopts a mixed-methods approach, combining both quantitative and qualitative techniques to explore the impact of COVID-19 on hospitality management practices. A descriptive and exploratory research design is employed to understand the challenges faced, strategies adopted, and recovery mechanisms implemented within the hospitality sector during and after the pandemic.

**Population and Sample**

The population of the study comprises hospitality businesses, including hotels, restaurants, and travel-related service providers operating in urban areas most affected by COVID-19. A purposive sampling technique was used to select a sample of 100 hospitality managers and business owners, ensuring a mix of small, medium, and large enterprises.

**Data Collection Methods**

**Primary Data:** [21]Collected through structured questionnaires and semi-structured interviews.

- The questionnaire includes both closed-ended and Likert-scale items focusing on operational disruptions, workforce challenges, financial impact, customer behavior changes, and recovery strategies.
- In-depth interviews were conducted with 10 selected managers to gain qualitative insights into adaptive practices and leadership decisions.

**Secondary Data:** [22]Gathered from industry reports, government publications, tourism boards, and journal articles related to COVID-19 and hospitality trends.

**Data Analysis Techniques**

Quantitative data collected through structured questionnaires were processed and analyzed using SPSS software. Both descriptive statistics (such as mean, percentage, and frequency) and inferential statistics (including correlation and regression analysis) were applied to examine trends, relationships, and statistically significant patterns.

Qualitative data obtained from interviews were subjected to thematic analysis to identify recurring themes, coping strategies, and indicators of resilience.

**Reliability and Validity of the Research Instrument**

To establish the reliability of the research instrument, a pilot study involving 10 respondents was carried out. Internal consistency was assessed using Cronbach's alpha. The validity of the questionnaire was ensured through expert evaluation by hospitality industry professionals and academic researchers, confirming the appropriateness and relevance of the instrument.

**Ethical Considerations**

All participants were informed about the purpose of the study. Informed consent was obtained, and confidentiality was maintained. Participation was voluntary, and respondents were allowed to withdraw at any stage without any obligation.

**Hypothesis**

**H10:** There is no significant relationship between COVID-19-related disruptions and changes in hospitality management practices.

**H20:** There is no significant difference in the resilience strategies adopted by small, medium, and large hospitality businesses during the COVID-19 pandemic.

**H30:** The COVID-19 pandemic has no significant impact on employee retention and workforce management practices in the hospitality sector.

**Linear Regression**

**H10:** There is no significant relationship between COVID-19-related disruptions and changes in hospitality management practices.

**Independent Variable (IV):** COVID-19 disruptions (e.g., revenue loss, lockdown duration, supply chain issues)

**Dependent Variable (DV):** Changes in management practices (e.g., operational adjustments, use of digital tools) (Table 1)

**Regression Equation**

$Y = 1.432 + 0.696X$

**Interpretation**

The linear regression results indicate a statistically significant and positive relationship between COVID-19–related disruptions and changes in hospitality management practices. The model accounts for 46.1% of the variation in management adaptations ($R^2 = 0.461$), reflecting a moderately strong explanatory power. The regression coefficient for the COVID-19 disruption score is 0.696 ($p < 0.001$), implying that a one-unit increase in disruption leads to a 0.696-unit increase in adaptive management practices. This finding suggests that greater pandemic-related challenges prompted hospitality organizations to adopt strategic responses such as digital transformation, cost control measures, and revised standard operating procedures. Consequently, the null hypothesis is rejected, confirming that COVID-19 disruptions had a significant impact on management adaptations within the hospitality sector.

**ANOVA**

**H20:** There is no significant difference in the resilience strategies adopted by small, medium, and large hospitality businesses during the COVID-19 pandemic (Table 2).

**Interpretation**

The results of the One-Way ANOVA demonstrate a statistically significant difference in the resilience strategies employed by small, medium, and large hospitality businesses during the COVID-19 pandemic. The obtained F-value of 7.231 with a p-value of 0.001 is below the conventional significance threshold of 0.05, confirming the presence of meaningful differences across business sizes. The between-group variation in resilience strategy scores (sum of squares = 4.752) is substantially higher than the within-group variation (sum of squares = 31.986). Accordingly, the null hypothesis is rejected, indicating that the size of the hospitality enterprise significantly influences the nature and intensity of resilience strategies adopted during the pandemic. Post-hoc tests may be performed to identify the specific group differences contributing to this variation.

**Independent samples t-test**

**H30:** The COVID-19 pandemic has no significant impact on employee retention and workforce management practices in the hospitality sector. (Table 3). The independent samples t-test was conducted to examine the influence of various factors on hospitality management practices during the COVID-19 pandemic. The results indicate that Business Size ($t = -15.007$, $p = 0.000$), COVID-19 Disruption Level ($t = 6.834$, $p = 0.000$), and Government Support Received ($t = -4.580$, $p = 0.000$) have a statistically significant impact on management decisions and resilience strategies, as their p-values are less than 0.05. This suggests that larger businesses, higher levels of disruption, and the presence of government support significantly influenced how businesses responded to the crisis. On the other hand, Digital Adoption Level ($t = -0.718$, $p = 0.475$) and Workforce Size Pre-Pandemic ($t = -1.513$, $p = 0.134$) did not show a significant impact, indicating that these factors did not contribute meaningfully to variations in hospitality practices during the pandemic. Therefore, the results partially support the hypothesis that certain operational and external factors significantly shaped resilience strategies in the hospitality sector during COVID-19.

## FINDINGS

The t-test shows a significant difference in management practices based on business size ($p = 0.000$), indicating that small, medium, and large businesses adopted different levels or types of resilience strategies during COVID-19. COVID-19 disruption level had a statistically significant impact ($p = 0.000$), showing that businesses more severely affected by the pandemic made greater changes in their operational and workforce practices. Businesses that received government support showed significant differences in management outcomes ($p = 0.000$), highlighting the positive impact of financial and policy assistance during crisis recovery. Despite its importance in modern operations, the adoption of digital tools did not significantly affect the resilience strategies in this sample ($p = 0.475$), suggesting inconsistent or limited digital transformation. The number of employees prior to the pandemic did not significantly influence changes in workforce management practices during COVID-19 ($p = 0.134$), indicating that both large and small teams faced similar challenges.

## CONCLUSION

[24]The study aimed to examine the impact of various factors on hospitality management practices during the COVID-19 pandemic using independent samples t-tests. The findings reveal that business size, COVID-19 disruption level, and government support received had a statistically significant influence on how hospitality businesses responded to the crisis. These factors played a crucial role in shaping resilience strategies, operational decisions, and workforce management practices. In contrast, digital adoption level and workforce size prior to the pandemic did not show a significant impact, suggesting that technological readiness and employee strength alone were not sufficient drivers of adaptive strategies during the crisis. Overall, the results highlight the importance of external support mechanisms and organizational scale in navigating disruptions, emphasizing the need for targeted policies and flexible business models to enhance resilience in the hospitality sector.

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**Table 1: Linear Regression**

| Model | Coefficients | Standardized coefficients | | t | Sig |
|---|---|---|---|---|---|
| 1 | B | Std.Error | Beta | | |
| Constant | 1.432 | 0.311 | | 4.60 | 0.00 |
| | 0.696 | 0.071 | 0.679 | 9.75 | .000 |

**Table 2: ANOVA: H20: There is no significant difference in the resilience strategies adopted by small, medium, and large hospitality businesses during the COVID-19 pandemic.**

| Source | Sum of Squares | df | Mean Square | F | Sig. (p-value) |
|---|---|---|---|---|---|
| Between Groups | 4.752 | 2 | 2.376 | 7.231 | 0.001** |
| Within Groups | 31.986 | 97 | 0.330 | | |
| Total | 36.738 | 99 | | | |

**Table 3: Independent samples t-test. H30: The COVID-19 pandemic has no significant impact on employee retention and workforce management practices in the hospitality sector.**

| | | | | | | |
|---|---|---|---|---|---|---|
| Factors | N | Mean | The results of the One-Way ANOVA demonstrate a statistically significant difference in the resilience strategies employed by small, medium, and large hospitality businesses during the COVID-19 pandemic. The obtained F-value of 7.231 with a p-value of 0.001 is below the conventional significance threshold of 0.05, confirming the presence of meaningful differences across business sizes. The between-group variation in resilience strategy scores (sum of squares = 4.752) is substantially higher than the within-group variation (sum of squares = 31.986). Accordingly, the null hypothesis is rejected, indicating that the size of the hospitality enterprise significantly influences the nature and intensity of resilience strategies adopted during the pandemic. Post-hoc tests may be performed to identify the specific group differences contributing to this variation. | t | df | Sig |
| Business Size | 45 | 3.04 | The results of the One-Way ANOVA demonstrate a statistically significant difference in the resilience strategies employed by small, medium, and large | -15.007 | 0.000* | Sig |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | hospitality businesses during the COVID-19 pandemic. The obtained F-value of 7.231 with a p-value of 0.001 is below the conventional significance threshold of 0.05, confirming the presence of meaningful differences across business sizes. The between-group variation in resilience strategy scores (sum of squares = 4.752) is substantially higher than the within-group variation (sum of squares = 31.986). Accordingly, the null hypothesis is rejected, indicating that the size of the hospitality enterprise significantly influences the nature and intensity of resilience strategies adopted during the pandemic. Post-hoc tests may be performed to identify the specific group differences contributing to this variation. | | | |
| COVID-19 Disruption Level | 45 | 4.71 | The results of the One-Way ANOVA demonstrate a statistically significant difference in the resilience strategies employed by small, medium, and large hospitality businesses during the COVID-19 pandemic. The obtained F-value of 7.231 with a p-value of 0.001 is below the conventional significance threshold of 0.05, confirming the presence of meaningful differences across business sizes. The between-group variation in resilience strategy scores (sum of squares = 4.752) is substantially higher than the within-group variation (sum of squares = 31.986). Accordingly, the null hypothesis is rejected, indicating that the size of the hospitality enterprise significantly influences the nature and intensity of resilience strategies adopted during the pandemic. Post-hoc tests may be performed to identify the specific group differences contributing to this variation. | 6.834 | 0.000 | sig |
| Digital Adoption Level | 46 | 3.62 | The results of the One-Way ANOVA demonstrate a statistically significant difference in the resilience strategies employed by small, medium, and large hospitality businesses during the COVID-19 pandemic. The obtained F-value of 7.231 with a p-value of 0.001 is below the conventional significance threshold of 0.05, confirming the presence of meaningful differences across business sizes. The between-group variation in resilience strategy scores (sum of squares = 4.752) is substantially higher than the within-group variation (sum of squares = 31.986). Accordingly, the null hypothesis is rejected, indicating that the size of the hospitality enterprise significantly influences the nature and intensity of resilience strategies adopted during the pandemic. Post-hoc tests may be performed to identify the specific group differences contributing to this variation. | -0.718 | 0.475** | Not sig |

| | | | | | | |
|---|---|---|---|---|---|---|
| Workforce Size Pre-Pandemic | 56 | 2.91 | The results of the One-Way ANOVA demonstrate a statistically significant difference in the resilience strategies employed by small, medium, and large hospitality businesses during the COVID-19 pandemic. The obtained F-value of 7.231 with a p-value of 0.001 is below the conventional significance threshold of 0.05, confirming the presence of meaningful differences across business sizes. The between-group variation in resilience strategy scores (sum of squares = 4.752) is substantially higher than the within-group variation (sum of squares = 31.986). Accordingly, the null hypothesis is rejected, indicating that the size of the hospitality enterprise significantly influences the nature and intensity of resilience strategies adopted during the pandemic. Post-hoc tests may be performed to identify the specific group differences contributing to this variation. | -1.513 | 0.134** | Not sig |
| Government Support Received | 64 | 3.62 | The results of the One-Way ANOVA demonstrate a statistically significant difference in the resilience strategies employed by small, medium, and large hospitality businesses during the COVID-19 pandemic. The obtained F-value of 7.231 with a p-value of 0.001 is below the conventional significance threshold of 0.05, confirming the presence of meaningful differences across business sizes. The between-group variation in resilience strategy scores (sum of squares = 4.752) is substantially higher than the within-group variation (sum of squares = 31.986). Accordingly, the null hypothesis is rejected, indicating that the size of the hospitality enterprise significantly influences the nature and intensity of resilience strategies adopted during the pandemic. Post-hoc tests may be performed to identify the specific group differences contributing to this variation. | -4.580 | 0.000 | significant |

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**RESEARCH ARTICLE**

# A Multi-Server Queuing Approach for Modeling and Optimizing Cloud Computing Environments

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**ABSTRACT**

This study investigates the performance of cloud service environments through the application of the $M/M/c$ queuing model, focusing on how system behavior is influenced by arrival rates, service rates, and multi-server capacity. By modeling cloud request arrivals as a Poisson process and service times as exponentially distributed, the research analyzes key performance measures including queue length, system occupancy, and customer waiting times. Simulation results, supported by Erlang-C analytical comparisons, demonstrate that inadequate server provisioning leads to rapid congestion and increased delays, while the addition of servers significantly enhances stability and reduces wait times across varying demand intensities. Probability distributions, contour maps, and heat-map visualizations further reveal the sensitivity of cloud systems as utilization approaches saturation, emphasizing the importance of appropriate capacity planning. The findings validate the $M/M/c$ framework as an effective tool for predicting cloud performance trends and informing scalable, Quality of Service(QoS) driven resource management strategies in modern cloud computing environments.

**Keywords:** Erlang-C, performance, Probability, Quality, occupancy, environments

## INTRODUCTION

Queuing theory is the study of waiting lines. It is a domain of applied mathematics that utilizes notions from stochastic processes. A. K. Erlang, a Danish engineer, is recognized for establishing queuing theory through his publication on automated telephone lines in 1909. It is fundamentally a system where client demand for services is limited by the availability of those services. The service times are chosen at random. Certain clients may experience

delays if the number of servers is limited [11]. Cloud computing, due to its growing relevance and popularity, presents significant potential while simultaneously facing numerous challenges in its development. The Theory of Queues captivates mathematicians focused on stochastic processes as it provides a straightforward example that is both (i) stationary and (ii) generally non-Markovian [5]. According to Luqun Li [10], CCU's responsibilities were categorized into different classes, each with a specific priority. The tasks are processed on the server at a rate determined by a Poisson distribution, while a general distribution governs the processing time for each work. A non-preemptive$M/1G$ queuing model was consequently devised. In a non-preventive approach, job planning in Cloud Computing is transformed into a queue planning problem for $M/1G$. Daily, we are vexed by the difficulty of queuing. In our increasingly congested and urbanized environment, the issue is becoming more prevalent. In addition to visible queues at traffic intersections, airport check-in counters, and supermarkets, there are also concealed delays in optical and wireless channels caused by phone calls and data packets. Time, capital, and resources are squandered for everyone. Global production and communication networks rely on queue management. Cloud computing gained significant interest due to increased access to high-speed Internet and improvements in virtualization and distributed computing. Information technology is presently extensively utilized and advancing. When programs and data are transferred to the cloud, they alter not just the location but also the methodology of computing.

The authors illustrated that the model could ascertain the appropriate quantity of resources necessary to fulfilQoS requirements, including reaction time and throughput. The authors [8] formulated an$M/G/m/m+r$ queuing model to evaluate the performance of cloud computing centers, emphasizing criteria such as blocking probability, immediate service probability, and response time distribution. Besides optimizing reaction time, queuing theory has been utilized in various facets of cloud computing, including energy efficiency and resource management. Souvik Pal et al. [17] they utilize the CloudSim simulation program to determine the number of CPU cores required and the duration of execution. Minimizing wait times is also crucial.When soliciting a substantial number of jobs, it is essential to anticipate delays due to servers that may prolong the line and increase wait times. This study examines a queuing strategy featuring numerous servers and restricted capacity to minimize wait times and queue length. Guo, Lizheng et al. [9] they provide the mode, function, and methodology for synthesis optimization. Ultimately, simulation is conducted utilizing synthesis optimization techniques. The simulation results are contrasted and assessed against conventional optimization,demonstrating that the proposed methodology can reduce average expectation times, average queuing times, and client counts. The data center is constructed as a service facility with many applications and an infinite task application buffer, which can function as an$M/M/m$ queue system.

Bashir Yusuf Bichi and colleagues [3] their focus was on mathematical formulation employing a queuing system technique to illustrate the variations in throughput and time delay between single server systems and multiple server systems in a cloud computing environment. It is readily apparent that the throughput of the system with several serving units exceeds that of the system with a single serving unit.The latency for executing a specific request on a single server exceeds that of a many server system. EinollahJafarnejadGhomi and colleagues[6]their research presented a classification of cloud computing queuing models and The trajectory of AQTMCC and the imperative for additional research on it have been determined for the future. The subjects for future investigation encompass: robustness and automation. Understanding of QoS and Service Level Agreement (SLA)-aware resource allocation, event-driven workload forecasting, provisioning and management of cross-cloud resources, model deployment, and class-based as well as SLA-based services. Khaled Salah et al. [15] proposed a work that analyzes the performance of the CDC using a constructed stochastic model based on queuing theory.CDC platforms are designed to achieve QoS in the cloud. We provide numerical examples illustrating how the model forecasts the requisite number of virtual machine instances to satisfy a specified set of QoS criteria. We employ a Discrete Event Simulator (DES) to assess our analytical model. The proposed model can forecast the necessary number of VMs to satisfy the anticipated QoS targets, considering fluctuations in the arrival request rate, based on their study and simulation findings.

Roxana MARCU and colleagues [13] the essay suggested a cloud platform for developing a comprehensive, industry-specific medical system. A suggested analytical methodology aims to optimize performance while minimizing costs in cloud scheduling. The performance of each established priority class is evaluated based on the volume of requests, response time, and demand drop rate. The experimental results indicate that the proposed model can accommodate many arrival requests and provide a rapid response time based on priority classifications. S. Pal et al. [18, 19] have examined scheduling algorithms and probabilistic algorithms to improve performance metrics in a cloud computing environment. In the preceding, we examined the queueing model, multi-server capacity, and the optimization of wait time and queue length. We concentrated on minimizing the average wait time and assessing the server utilization of a multi-server queuing system. This trial examined the average server time and server utilization using the $M/M/c$ model.

**Model for the system**

The cloud user interacts with the cloud broker as per the specified need.Cloud brokers serve as negotiators or mediators, assisting users in managing all activities, the requisite number of CPU cores, and the duration needed for processing user requests, including specifications for the desired data center and execution time for pertinent requests. Users may use the cloud broker to get resources for themselves. Figure 1 illustrates the specified framework model of the cloud service queuing system. The cloud broker evaluates and forwards the request to the data center. In a short timeframe, a substantial number of requests generates a queue.The requirements are executed in combination with the creation of the Virtual Machines' VMM. The data center allocates resources according to their availability. Upon the completion of the inquiries, the virtual computers must be shut down accordingly.We identified the $M/M/c$ model in that study to compute the average server utilization and waiting time length$M/M/c$. The $M/M/c$ queuing system [1, 2, 7, 12, 15, 16] is recognized as the only model proficient in analysing and resolving the density function of the probability upon the introduction of a new system. The $M/M/c$ queuing model in a multi-server system facilitates analysis and management about the optimization issue.

The quantities of a service, the operational load of an application environment, the setup of a multi-server system, the service level agreements, customer satisfaction, etc., are the components of your pricing model. The research further examined two alternate models of server power and performance.

## RESULTS AND DISCUSSIONS

When the server's capacity is inadequate to manage the volume of requests within an acceptable timeframe, queues will build.Arrival and service methods, as well as servers [4, 11], are essential concerning the fundamental queuing model. This chapter delineated the $M/M/c$ queuing paradigm.The Poisson distribution rate represents service requests, while the exponential distribution rate denotes process time. Kendal's nomenclature [5] is accurate; the average arrival rate is represented by $\lambda$, whereas the average service rate is marked by $\mu$. The $M/M/c$ model has been evaluated, with variations in the number of servers (denoted as c) and the utilization factor being analyzed concurrently. To guarantee system stability, the utilization factor $\left[\rho = \frac{\lambda}{\mu} \leq 1\right]$ must be taken into account, necessitating the consideration of an equilibrium condition. The quantity of servers was juxtaposed with the mean system client figures$(L_s)$, the mean number of queue patrons$(L_q)$, the mean system waiting duration$(W_s)$, and the mean queue waiting duration$(W_q)$are shown in the Figure 1 are analysed using MATLAB 2022a software.

Figures 2 and 3 illustrate the behavior of the average queue length $(L_q)$ and the mean number of customers in the system $(L_s)$ as the number of server's increases. As expected in an $M/M/c$ environment, both metrics decline sharply with additional servers. When the system operates with insufficient capacity, even moderate arrival rates lead to substantial queue formation. This behaviour is pronounced in Figure 2, where $L_q$ initially grows rapidly and then plummets toward zero once the number of servers surpasses the minimum threshold required for stability.

Figure 3 shows that $L_s$ follows a similar pattern: under low server availability, the system becomes congested, accumulating customers both in service and in queue. As server count increases, $L_s$ stabilizes at minimal levels.These outcomes reinforce the classical observation that queueing performance in cloud systems improves significantly when capacity provisioning aligns with incoming demand.

Figures 4 and 5 presents the mean waiting time in queue $(W_q)$ and the mean time a request spends in the system $(W_s)$. Both metrics exhibit a pronounced decline as server capacity increases. The steep initial drop in $W_q$ underscores how sensitive delay is to under-provisioning: when servers are scarce, even small increases in load cause substantial waiting times.Beyond a certain point, however, the addition of more servers yields diminishing returns, as both $W_q$ and $W_s$ converge toward limits dictated primarily by service times. This plateau indicates that once queues are effectively eliminated, further resource allocation contributes little to reducing overall delay-an important consideration for cost-conscious cloud deployments.

Figure 6 compares multiple performance metrics$L_q, L_s, W_q$, and $W_s$ under a high-load scenario ($\lambda = 130, \mu = 132$). The figure demonstrates that operating near saturation dramatically amplifies system sensitivity. With utilization approaching 1, even a single additional server produces large reductions in waiting time and queue size. This observation highlights the need for flexible scaling strategies in cloud environments, particularly during peak traffic intervals.

Figures 7 through 12 illustrate the steady-state probabilities of having $n$ customers in the system for both $M/M/2$ and $M/M/3$ configurations across varying traffic intensities.For the $M/M/2$ model (Figures 7-9):

- At light load ($\lambda = 30, \mu = 50$), the probability mass concentrates on small $n$ values.
- At moderate load($\lambda = 70, \mu = 80$), the distribution spreads modestly, reflecting increased congestion.
- At heavy load ($\lambda = 130, \mu = 132$), the distribution becomes significantly wider, indicating a high likelihood of longer queues.

For the $M/M/3$ model (Figures 10-12), the presence of an additional server substantially compresses the distribution, even under heavy arrival rates. This demonstrates the stabilizing influence of additional servers, confirming that multi-server architectures in cloud environments markedly reduce the probability of long queues and system congestion.

Figure 13 compares simulated waiting-time distributions with the theoretical Erlang-C cumulative distribution function. The strong alignment between the curves validates the simulation methodology and confirms the theoretical applicability of the $M/M/c$ model to cloud service environments. Figure 14 further illustrates the empirical distribution of positive waiting times for a $M/M/2$ system ($\lambda = 60, \mu = 70$). The histogram reveals the characteristic exponential decay, with most tasks experiencing negligible delays and only a few instances culminating in longer waits. Figure 15 presents a stacked bar chart of steady-state probabilities $P(n)$ for the $M/M/3$ system. The bars emphasize the rarity of high customer counts in adequately provisioned systems, confirming that multi-server configurations significantly mitigate backlog formation.

Figures 16 and 17 provide visual analytics for understanding the interplay between arrival rates, service rates, and server count. Figure 16, a contour map of $W_s$ for an$M/M/2$ queue, shows steep gradients near the boundary where $\lambda$ approaches $2\mu$. This confirms that the system becomes highly unstable as utilization nears 1, resulting in rapidly increasing delays. Figure 17, a heat map of $L_q$ for a $M/M/c$ system, reveals congestion zones across different values of $\lambda$and $c$. High arrival rates combined with insufficient servers cause sharp increases in queue length, while increasing $c$ shifts the system into low-congestion regions (cooler colors). These plots provide intuitive, visual confirmation of the analytical results and emphasize the critical role of capacity planning in cloud infrastructures. The results collectively demonstrate that the $M/M/c$ queuing model effectively captures the performance characteristics of cloud service systems. The findings highlight the system's sensitivity to workload intensity, the significant benefits of

multi-server architectures, and the importance of maintaining utilization well below saturation to ensure minimal queue growth and delay. The combined analytical and simulation results underscore that proper resource provisioning is essential for meeting QoS requirements in modern cloud environments.

## CONCLUSION

This study evaluated the performance of cloud service systems using the $M/M/c$ queuing model and demonstrated how key metrics - queue length, system occupancy, waiting times, and steady-state customer distributions are strongly influenced by server capacity and workload intensity. The analytical and simulation results consistently showed that insufficient server provisioning leads to rapid congestion and long delays, while moderate increases in service capacity produce substantial performance gains, validating the model's effectiveness in supporting capacity planning for cloud environments. Visual analyses, including contour and heat maps, further highlighted critical stability thresholds, especially as utilization approaches unity. By confirming the close agreement between simulation outputs and Erlang-C predictions, the study reinforces the $M/M/c$ model as a reliable tool for predicting system behavior and guiding decisions related to resource scaling, QoS assurance, and cost-efficient cloud infrastructure design. Collectively, the findings emphasize the necessity of dynamic, data-driven provisioning strategies to maintain system responsiveness and ensure optimal performance under varying demand conditions.

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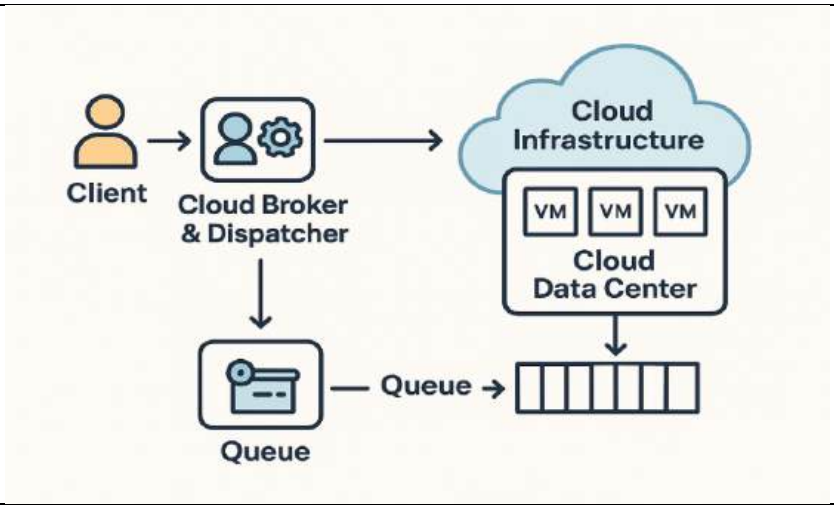



**Figure 1:Cloud Server Queuing: A Sparse Model**

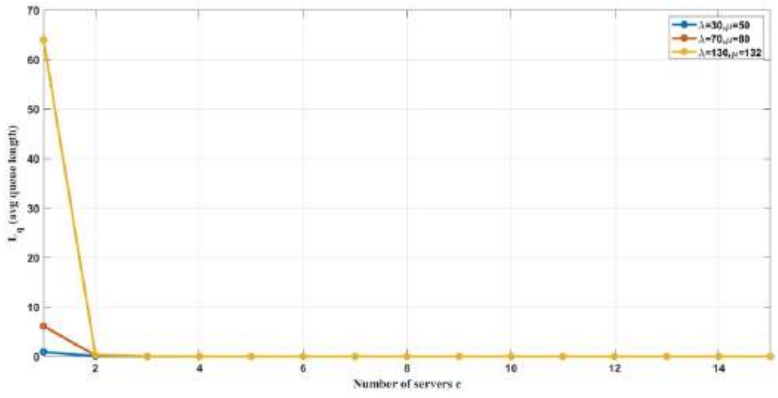



**Figure 2: Outcome with $L_q$.**

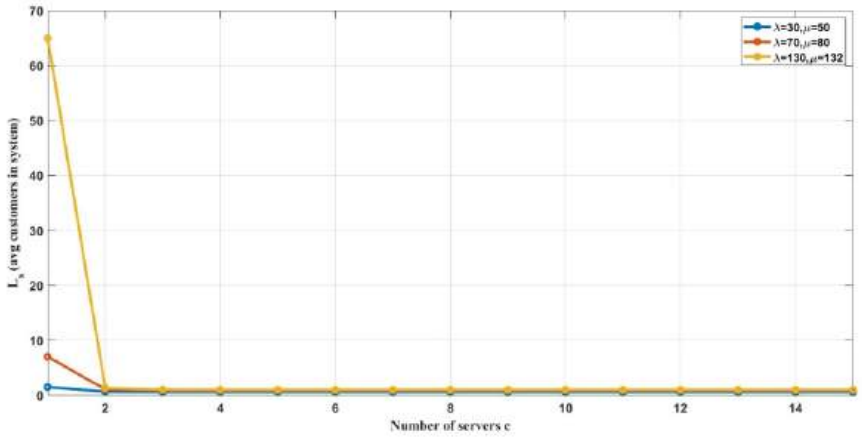



**Figure 3: Outcome with $L_s$**

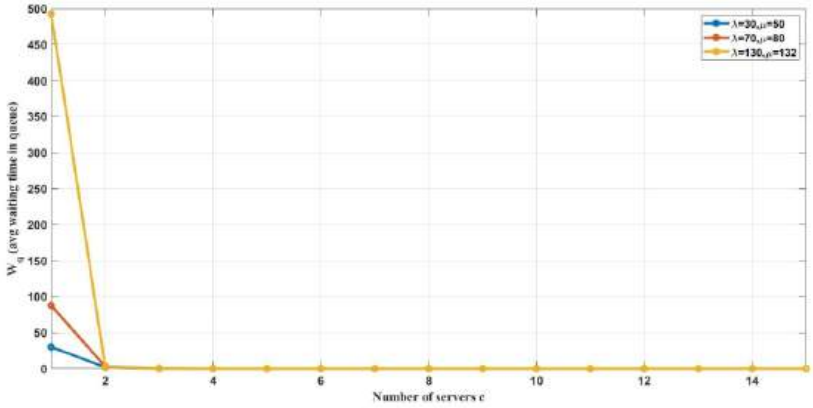



**Figure 4: Outcome with $W_q$**

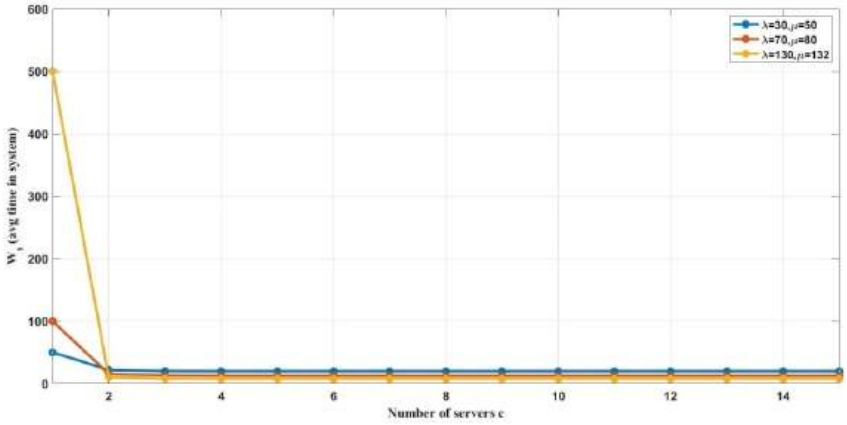



**Figure 5: Outcome with $W_s$**

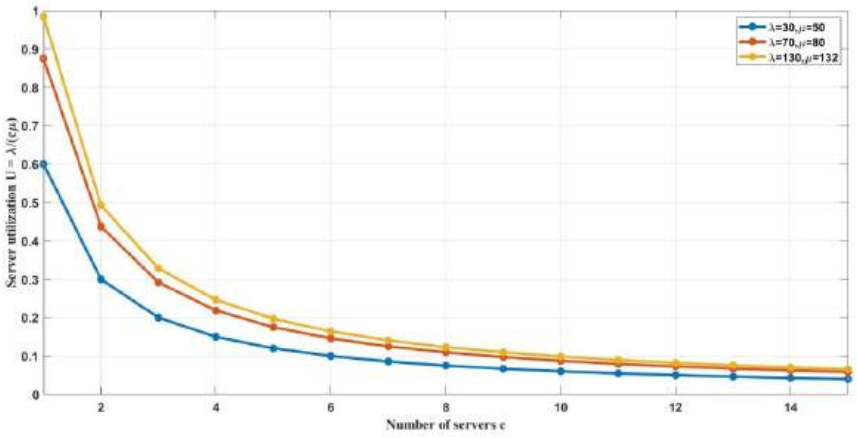



**Figure 6: Comparison outcomes with server utilization ($\lambda = 130, \mu = 132$)**

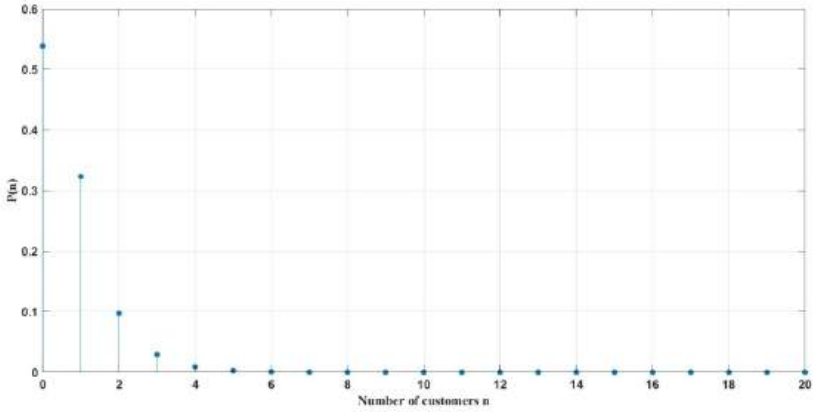



**Figure 7: Probability of customers in M/M/2 model ($\lambda = 30, \mu = 50$)**

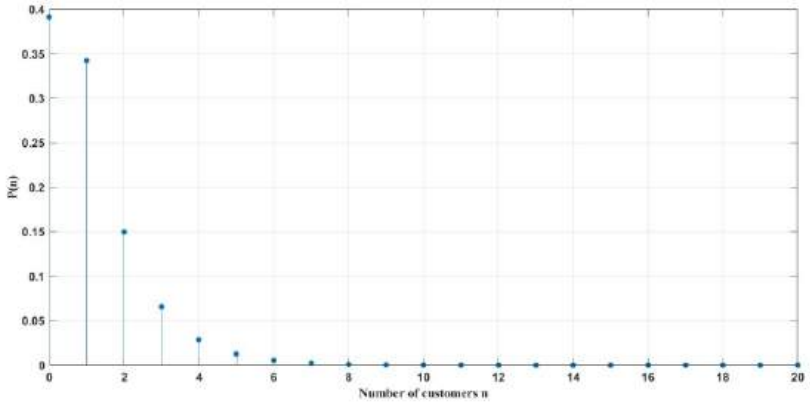



**Figure 8: Probability of customers in M/M/2 model ($\lambda = 70, \mu = 80$)**

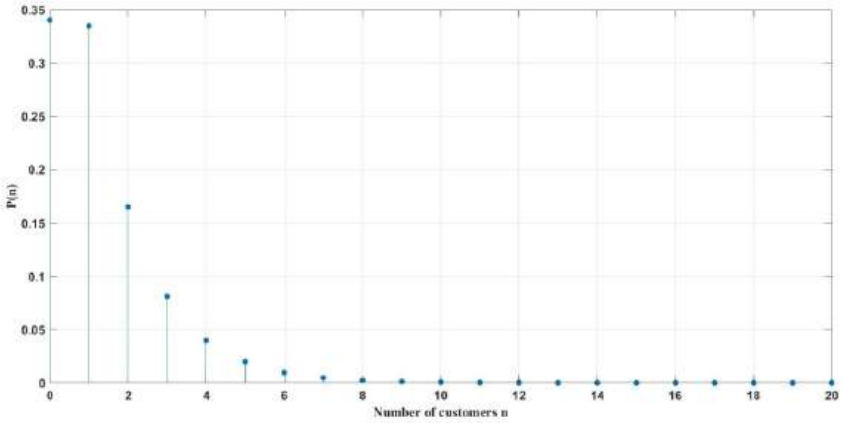



**Figure 9: Probability of customers in M/M/2 model ($\lambda = 130, \mu = 132$)**

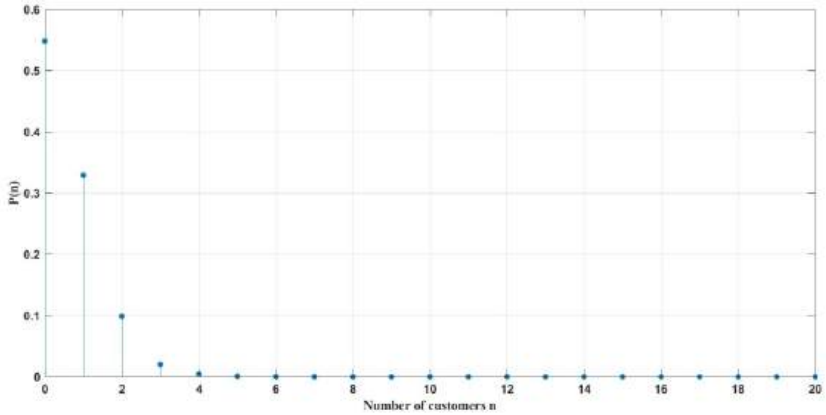



**Figure 10: Probability of customers in M/M/3 model ($\lambda = 30, \mu = 50$)**

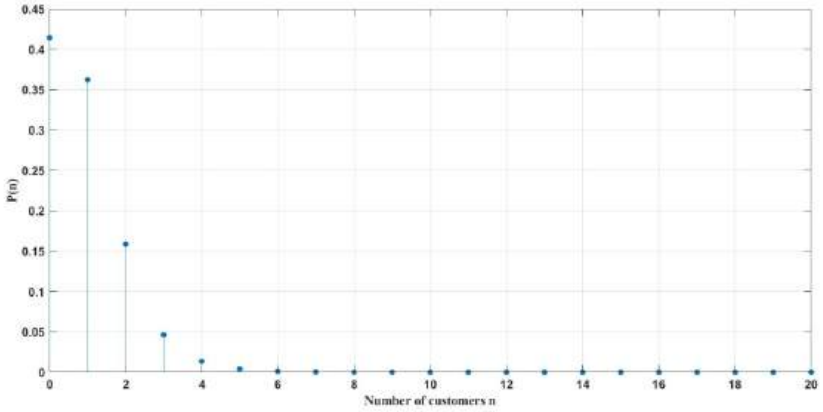



**Figure 11: Probability of customers in M/M/3 model ($\lambda = 70, \mu = 80$)**

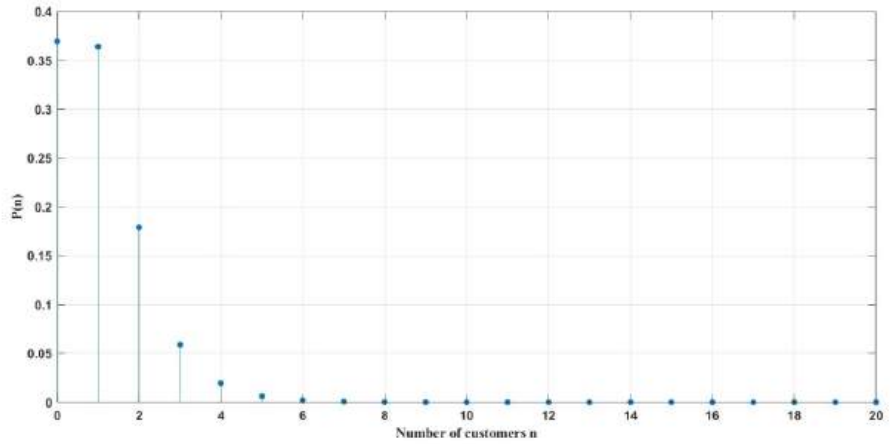



**Figure 12: Probability of customers in M/M/3 model ($\lambda = 130, \mu = 132$)**

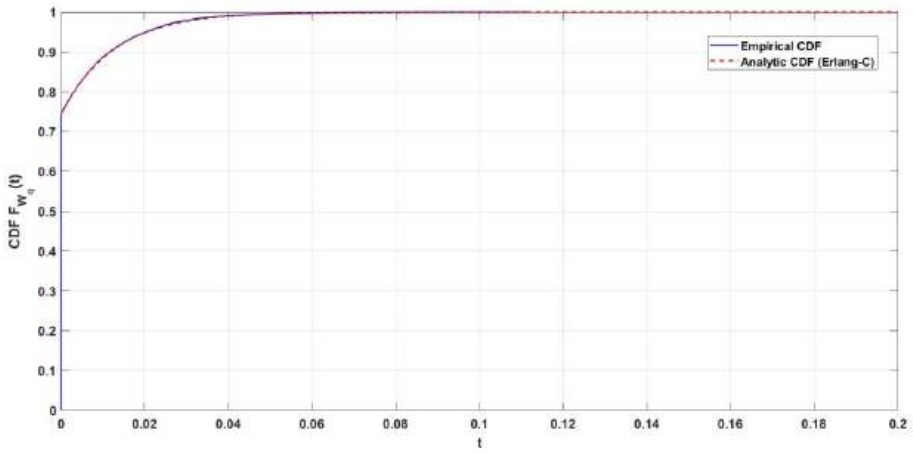



**Figure 13: Simulations of waiting time vs. analytic Erlang-C CDF**

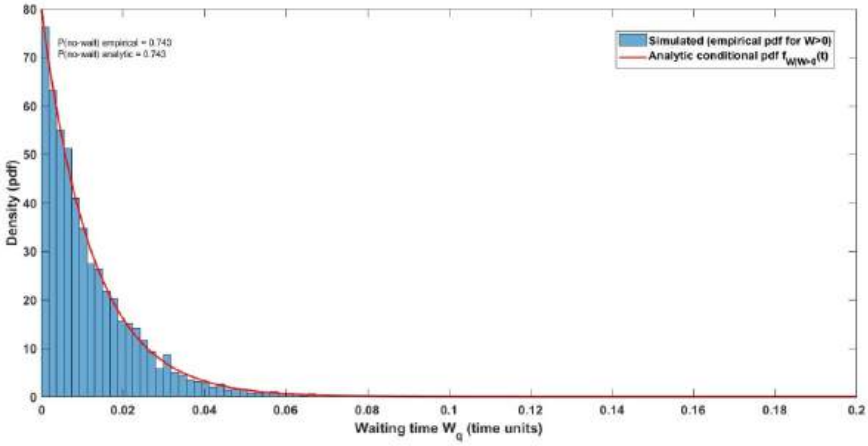



**Figure 14: Histogram of positive waiting times (N=20000)$-M/M/2$ ($\lambda = 60, \mu = 70$)**

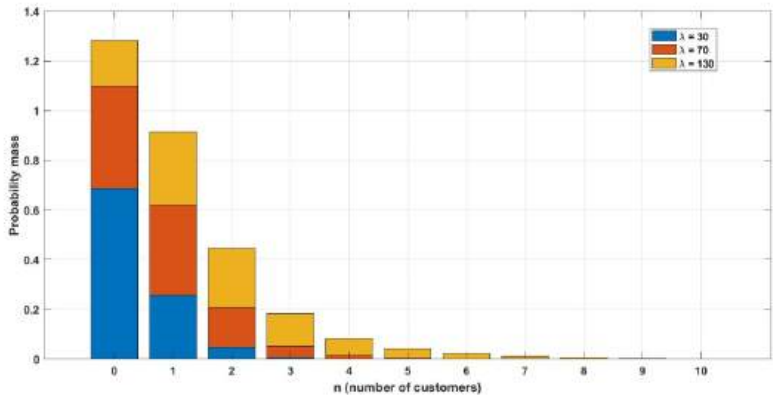



**Figure 15: Stacked bar chart of the $P(n)$ for$M/M/3$ with $\mu = 80$**

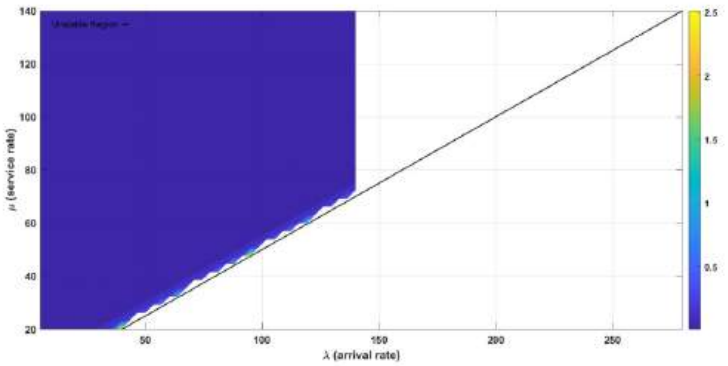



**Figure 16: Contort plot of $W_s$ for $M/M/2$**

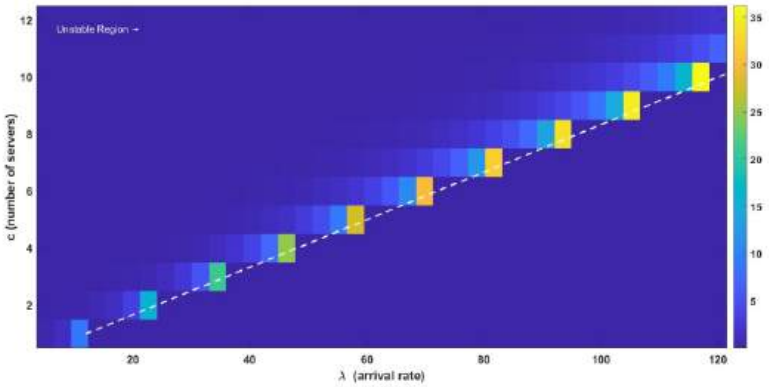



**Figure 17: Heat map of $L_q$ for $M/M/c$ Queue ($\mu = 12$)**

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RESEARCH ARTICLE

# Robust Paddy Crop Disease Detection using Advanced CNN Architectures

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**ABSTRACT**

Agricultural land is essential not only for ensuring food availability but also for strengthening India's economy [1]. Among various crops, paddy holds a key position in supporting global food security. Despite its importance, paddy cultivation is highly vulnerable to numerous diseases, especially those that damage the leaves and reduce yield. To address this issue, the present study proposes a convolutional neural network (CNN)–based method for the automatic detection of paddy crop diseases using deep learning techniques. The four pre-trained models ResNet50, EfficientNet_b4, DenseNet121, and MobileNet_v2 were implemented, achieving high classification accuracy of 93.60%, 89.11%,93.30% and 93% respectively. Proposed models classify images into 10 various categories using a dataset of 10407 images. The results demonstrate the effectiveness of deep learning techniques for early disease detection and improved crop management.

**Keywords**: paddy crop disease, deep learning, convolutional neural networks (CNN), disease detection, automated detection.

## INTRODUCTION

India is the second largest producer of wheat and rice, most of India's economy comes from agriculture.It provides employment to 60% of the Indian population and generates 17% to the total GDP of India [18]. Indian agriculture is composed of many crops like paddy, wheat, sugarcane, oilseeds, vegetables and fruits [7]. Due to emerging growth in the population agriculture sector needs a better advancement using the latest technologies. Paddy, a staple food for a significant portion of the global population, faces substantial threats from various diseases, leading to considerable crop losses and economic repercussions [1]. The reliance on manual inspection for disease detection, a

common practice, is often time-consuming, labour-intensive, and susceptible to human error, further exacerbating these issues [2]. Consequently, there is an urgent need for advanced, automated systems capable of accurately and efficiently identifying paddy crop diseases at early stages to mitigate their impact on agricultural productivity [3]. The global transferability of plant diseases complicates this challenge, introducing novel pathogens to regions unprepared for their rapid and effective identification and management [3]. This necessitates the development of robust detection mechanisms that can operate effectively even with limited expert supervision and vast agricultural regions [5].

Crop diseases represent a substantial impediment to global food security, particularly in staple crops like paddy rice, where early and accurate detection is paramount for effective disease management and yield preservation [7]. The application of Convolutional Neural Networks has emerged as a promising solution for automating the identification of various paddy diseases, thereby mitigating the challenges associated with manual observation and limited expert availability [2] This is especially critical in agricultural contexts where diseases can spread rapidly, leading to significant economic losses and diminished crop quality if not addressed promptly [2]. Traditionally, disease detection relied on subjective visual inspection or time-consuming laboratory analyses, which often lacked the necessary speed and consistency for large-scale agricultural monitoring [7]. However, recent advancements in deep learning, particularly with CNNs, have revolutionized this domain by enabling rapid, objective, and highly accurate identification of plant pathologies directly from imagery [2]. These models can discern subtle patterns and textures indicative of specific diseases, often surpassing human capabilities in both speed and diagnostic precision [1]. This shift towards automated systems offers a scalable and efficient alternative, enhancing the ability of farmers and agricultural scientists to monitor crop health proactively and implement targeted interventions [1][2]. The integration of CNN-based systems into agricultural practices not only streamlines the diagnostic process but also contributes significantly to precision agriculture by facilitating real-time disease prediction and personalized recommendations for crop management. This methodology leverages sophisticated image classification techniques to distinguish between healthy and diseased plant tissues, offering a robust framework for improving agricultural productivity and sustainability. In this context, Convolutional Neural Networks offer a promising solution due to their proven capabilities in image recognition and classification tasks, making them particularly well-suited for agricultural applications such as plant disease detection [5]. This approach leverages deep learning to accurately identify various paddy leaf ailments, thereby improving both the efficiency and accuracy of early detection compared to traditional, often subjective, manual inspection methods [2]. These advanced techniques offer a rapid and objective analysis of paddy leaf health, facilitating timely intervention to mitigate significant crop losses [2]. Convolutional Neural Networks can involves converting input RGB images into the HSV color space, extracting the saturation component, and employing K-means segmentation to precisely delineate leaf regions for subsequent CNN-based classification of diseases such as Sheath Rot, Bacterial Leaf Blight, Brown Spot, and Leaf Blast [2].

**Related Work**

This section critically evaluates existing research on paddy crop disease detection, with a particular focus on methodologies employing deep learning architectures such as Efficient Net, MobileNetV2,DenseNet121and ResNet50. The objective is to synthesize current advancements, identify common challenges, and highlight gaps in the literature that this study aims to address. Traditional methods for disease identification, often manual and time-consuming, are prone to misclassification and inefficiency, underscoring the necessity for advanced automated solutions [8]. Recent studies have increasingly leveraged deep learning, particularly convolutional neural networks, to address these limitations, demonstrating significant improvements in detection accuracy and speed [9]. Automatic feature extraction allows CNN to discern subtle disease indicators that might be overlooked by the human eye, thus preventing catastrophic yield reductions [1]. Furthermore, the development of CNN models with increased structural complexity, such as those incorporating 15 layers, has been proposed to enhance the predictive accuracy for various diseases affecting rice leaves [6]. This sophisticated approach underscores the critical role of advanced image processing combined with deep learning architectures in achieving robust and precise disease identification within agricultural contexts [6].

For instance, in [10] Rani et al. utilized a deep CNN with ResNet-50, achieving 97.3% accuracy in identifying rice leaf diseases, emphasizing the critical role of such technologies in safeguarding global rice cultivation. This system aims to minimize the loss by detection of diseased paddy crops at early stages and can identify bacterial leaf blight, brown spot, leaf smut, and tungro diseases [10].

Similarly, in [11] other research has explored the efficacy of various CNN architectures, including DenseNet201, Xception, MobileNetV2, and ResNet50, for their suitability in resource-constrained environments like mobile applications, achieving high accuracy in disease classification [11]. The "Rice Disease Detector" app, which incorporates MobileNetV2, demonstrated successful performance in real-world scenarios. This study used 2259 smartphone images of various paddy crop plant parts under various classes and 250 real-time validation images for classifying 12 rice diseases. The top performing models were DenseNet201, Xception, MobileNetV2, and ResNet50, with validation accuracies of 98%,97.7%, 97.5% and 97.18% respectively. The adaptability of these models to diverse environmental conditions and their potential for integration into precision farming initiatives highlight their significance in modern agricultural practices [12]. Further advancements have seen the integration of ensemble models, which combine multiple deep learning architectures to enhance diagnostic precision, often outperforming individual models in complex disease classification task [4].

In [1] CNN models with a data augmentation layer, followed by a repetition of 2D Convolution Layer and 2D Max Pooling Layer used on the dataset used to perform computations for this article contains 10407 images of diseased paddy plants, belonging to 10 different classes (of diseases), namely bacterial leaf blight, bacterial leaf streak, bacterial panicle blight, blast, brown spot, dead heart, downey mildew, hispa, tungro and normal plants. The convolution layer had a kernel of size 32 and ReLU (Rectified Linear Unit) activation function was used in each of the convolution layers. Model accuracy on the validation dataset is 93%. In [13] Rice Plant leaf diseases detection system was developed over 10,080 Rice Plant images using a Deep Spectral Generative Adversarial Neural Network ($DSGAN^2$). They fed into the input of healthy and non-healthy leaves of 10 different classes (9diseases and one healthy) from the collected dataset. To enhance the image quality, they applied proved Threshold Neural Network (ITNN). For the identification support-intensive colour they have used Segmentation using a Segment Multiscale Neural Slicing (SMNS) algorithm. Selection of optimal features was achieved through Spectral Scaled Absolute Feature Selection ($S^2AFS$) method. Model accuracy is 97%.

In [14] authors used the dataset consists of 10,407 images across 10 classes with different class weight. This study assesses the effectiveness of four advanced deep learning models—DenseNet121, EfficientNetB4, Xception, and MobileNetV3 Large for detecting diseases in paddy crop plants. Owing to its strong feature extraction and classification capabilities, DenseNet121 achieved the highest accuracy of 97.5%. EfficientNetB4 and Xception also showed excellent performance, reaching accuracies of 96.25% and 96.32%, respectively. Although slightly lower, MobileNetV3 Large delivered a competitive accuracy of 96.25%, demonstrating its suitability for efficient rice disease classification.

Collectively, these studies highlight the increasing success of deep learning techniques, especially convolutional neural networks (CNNs), in identifying diseases in paddy crops. Nevertheless, many existing approaches depend largely on transfer learning with minimal task-specific optimization or focus on a single methodological framework. In contrast, this comparative analysis offers a comprehensive evaluation of multiple models, seeking to balance diagnostic accuracy with real-world applicability in agricultural systems. A detailed overview of the reviewed literature is presented in Table I.

**METHODOLOGY**

To develop a robust and effective paddy crop disease detection system, the methodology begins with data acquisition, which involves downloading and curating a comprehensive dataset of paddy crop disease images. Various disease of paddy crop is mention in Fig. 1. Next, the images were pre-processed by resizing, rescaling, and

augmenting, then splitting into training and validation sets, to make the model more robust. Fig 2. portrays the workflow for the proposed methodology.

**Data Acquisition**

Deep learning models heavily depends on datasets to provide accurate result on real world dataset choosing right data set is one of the crucial step in disease detection system. The dataset utilized for this model has been derived from a publicly available Paddy Doctor [4] database. This database contains over 10 thousand images for 10 different paddy crop diseases. The paddy doctor dataset with various diseases is mentioned in Table 2.

**DataPre-processing**

Data augmentation techniques were applied to add randomness and diversity to the dataset. This includes flipping the images horizontally, vertically and randomly rotating them by up to **(±10°)**to simulate different viewing angles. A search based augmentation technique called Auto Augmentation applied on dataset that finds the best set of augmentation policies by using reinforcing learning for optimal augmentation options. Each augmentation policy may contain various operations like rotation, scaling etc. with probability (0 to 1) and magnitude.Disease appearance varies (lighting, angle, background) so aggressive augmentations like auto and rand augment was used to reduce dataset bias. Mix up and Cut mix pre-processing techniques were used to face challenges like similar disease patterns across classes, high intra-class variation, imbalanced datasets and overfitting due to background similarity.

**CNN Architectures for Paddy Crop Disease Detection**

For training models, the dataset was split into training and testing sets in an 80:20 ratios, with 80% of images being allocated for training the model and 20% reserved for testing purpose.

**Approach 1: ResNet (50)**

ResNet50 is a 50 layers' deep convolutional neural network (CNN) architecture introduced by Kaiming He et al. in 2015 as part of the ResNet (Residual Network) family. It is widely used for image classification, object detection, and plant disease detection due to its ability to train very deep networks effectively [10]. In order to solve the problem of the vanishing/exploding gradient, this architecture introduced the concept called Residual Blocks. It uses a technique called skip connections. The skip connection connects activations of a layer to further layers by skipping some layers in between. [10]. Residual blocks allow deep networks to be trained by learning residuals (skip connections). Here Resnet50 was used for paddy disease detection task. Paddy diseases (blast, brown spot, sheath blight) have tiny lesions, subtle colour variations, fine texture changes. ResNet-50 captures such micro-patterns much better than VGG16 or InceptionV3. Resnet 50 Works well on real-field noisy datasets and it is robust to lighting variations, complex backgrounds and diverse leaf orientations.

**Approach 2: Efficient Net (B4)**

Efficient net uses compound scaling (depth × width × resolution) which balances model complexity and input resolution efficiently. It has efficient feature extraction and parameter efficiency. It uses larger input (380* 380) and more channels, explaining its better accuracy on visually subtle disease patterns. In this study EfficientNet b4 is implemented with full fine-tuning (not only head) with small LR (1e-4) and AdamW Optimize. It's large input resolution (380×380) helps detect small disease spots, fine veins, early-stage symptoms. MBConv (Mobile Inverted Bottleneck Convolution) and SE (Squeeze-and-Excitation) texture patterns used in Efficient Net is perfect for identifying Brown spot, Bacterial blight, Blast, Tungro symptoms. It is less overfitting and works well even on medium size paddy disease dataset.

**Approach 3: DenseNet121**

DenseNet-121 is part of the Dense Convolutional Network (DenseNet) family with 121 total numbers of layers. Its key innovation is dense connectivity, every layer receives all previous layers' feature maps as input, and passes its own feature maps to all subsequent layers. This improves feature reuse, reduces the number of parameters and mitigates the vanishing gradient problem. It is strong on small-to-medium datasets due to parameter efficiency and

feature propagation. DenseNet Architectures are widely used by research for paddy crop disease detection because it's ffeature reuse capabilities. Dense connectivity allows the model to leverage low-level leaf features (like spots, lesions) across layers [15]. Efficient Training as it has fewer parameters than traditional CNNs of similar depth, reducing overfitting on small datasets. Improved Gradient Flow as dense connections help gradients propagate backward effectively, even in very deep networks and fine-grained Detection that captures subtle patterns in paddy leaves that indicate different diseases.

**Approach 4: MobileNetV2**

Mobilev2 is a lightweight, fast, and efficient CNN designed for mobile devices, drones, and low-power applications. it is built from inverted residual blocks consisting of 1×1 expansion convolution → depth-wise separable convolution → 1×1 linear projection, with skip connections for efficient feature learning and low computational cost.MobileNetV2 is widely used for paddy crop disease detection because it effectively captures fine-grained leaf texture, colour variations, and disease-specific spot patterns while remaining lightweight and computationally efficient. With limited training data this CNN architecture can accurately extract features with the help of it depth wise separable convolutions and inverted residual architecture. Dataset [4]used in with all this CNN modelscontains real-world images with noise, shadows and highly imbalanced classes. Adam with Decoupled Weight Decay is used as an optimizer because it reduces overfitting, stabilizes training, works extremely well with CNNs like: ResNet50, MobileNetV2, DenseNet121 and EfficientNet B0 [16]. AdamW provides faster convergence and trains quickly on agricultural datasets.

## RESULT

This section presents the outcomes of the experiments and highlights the effectiveness of the various CNN model in accurately identifying paddy crop diseases from images. The performance of the various CNN model was evaluated using standard accuracy metrics on both training and testing datasets. For training the models, the dataset was split into training and testing sets in an 80:20 ratios, with 80% of images being allocated for training the models and 20% reserved for testing purposes. The per-class precision, recall, F1-score, and support for the proposed models are summarized here.

## CONCLUSION

Recent studies emphasize the effectiveness of deep learning, particularly Convolutional Neural Networks (CNNs), in accurately detecting and classifying paddy leaf diseases from image data [18]. In this work, multiple CNN models were developed and evaluated for paddy disease identification, with ResNet50 demonstrating notable performance. The model achieved an accuracy of up to 93.6% in differentiating healthy and diseased paddy leaves. Through systematic tuning of network architecture, filter sizes, and hyper parameters, along with the application of data augmentation and pre-processing techniques, the model attained strong training and testing results. These findings highlight the potential of CNN-based approaches for automated paddy disease diagnosis, contributing to precision agriculture by enabling early detection and improved crop health management.

**Future Scope**

Looking forward, several avenues exist to further extend and enhance this work. Future research may focus on exploring more advanced CNN architectures and fine-tuning hyper parameters to improve model accuracy and generalization [17]. Expanding the dataset to include a wider range of crops and disease types would further strengthen the model's robustness and applicability across diverse agricultural settings. In addition, extending disease detection to additional crop species beyond the current scope could significantly increase the system's overall impact. Developing a user-friendly web-based application for model deployment would also improve accessibility, enabling farmers and agricultural professionals to more easily adopt the technology and bridge the gap between AI-driven research and practical agricultural solutions. Overall, this study provides a strong foundation for future

advancements in automated plant disease detection and contributes toward the integration of intelligent systems in real-world agricultural practices.

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Table I. Summary of literature review

| Article reference | Dataset | No of Classes | Models | Accuracy |
|---|---|---|---|---|
| [1] | 10407 images | 10 | CNN with transfer learning | 93% |
| [10] | 1428 | 4 | ResNet-50 | 97% |
| [11] | 2509 | 12 | DenseNet201 | 98% |
| | | | Xception | 97.7% |
| | | | MobileNetV2 | 97.5% |
| | | | ResNet50 | 97.18% |
| [13] | 10,080 images | 10 | Deep Spectral Generative Adversarial Neural Network | 97% |
| [14] | 10407 images | 10 | DenseNet121 | 97.50%, |
| | | | Xception | 96.32% |
| | | | EfficientNet B4 | 96.25% |
| | | | MobileNet V3 | 96.25% |

**Table 2. Dataset**

| Disease Name | No of Images |
|---|---|
| Bacterial_leaf_blight | 479 |
| Bacterial_leaf_streak | 380 |
| Bacterial_panicle_streak | 337 |
| Blast | 1738 |
| Brown_spot | 965 |
| Dead_heart | 1442 |
| Downy_mildew | 620 |
| Hispa | 1594 |
| Normal | 1764 |
| Tungro | 1088 |
| **Total** | **10407** |

**Table III. Precision, Recall, F1-Score, and Support for Resnet50**

| Paddy disease name | Precision | Recall | F1-Score | Support |
|---|---|---|---|---|
| Paddy bacterial leaf blight | 0.88 | 0.92 | 0.90 | 92 |
| Paddy bacterial leaf streak | 0.93 | 0.96 | 0.95 | 73 |
| Paddy bacterial panicle blight | 0.98 | 0.77 | 0.86 | 65 |
| Paddy blast | 0.91 | 0.91 | 0.91 | 334 |
| Paddy brown spot | 0.96 | 0.91 | 0.93 | 186 |
| Paddy dead heart | 0.99 | 0.99 | 0.99 | 277 |
| Paddy downy mildew | 0.95 | 0.83 | 0.89 | 119 |
| Paddy hispa | 0.95 | 0.93 | 0.93 | 307 |
| Paddy normal | 0.92 | 0.98 | 0.95 | 339 |
| Paddy tungro | 0.94 | 0.97 | 0.95 | 209 |
| **Accuracy** | **93.60%** | | | |

**Table IV. Precision, Recall, F1-Score, and Support for EfficientNet B4**

| Paddy disease name | Precision | Recall | F1-Score | Support |
|---|---|---|---|---|
| Paddy bacterial leaf blight | 0.79 | 0.90 | 0.84 | 92 |
| Paddy bacterial leaf streak | 0.85 | 0.99 | 0.91 | 73 |
| Paddy bacterial panicle blight | 0.83 | 0.97 | 0.89 | 65 |
| Paddy blast | 0.94 | 0.83 | 0.88 | 334 |
| Paddy brown spot | 0.93 | 0.80 | 0.86 | 186 |
| Paddy dead heart | 1.00 | 0.95 | 0.98 | 277 |
| Paddy downy mildew | 0.73 | 0.87 | 0.80 | 119 |
| Paddy hispa | 0.95 | 0.79 | 0.86 | 307 |
| Paddy normal | 0.88 | 0.98 | 0.93 | 339 |
| Paddy tungro | 0.82 | 0.93 | 0.87 | 209 |
| **Accuracy** | **89.11%** | | | |

**Table V. Precision, Recall, F1-Score, and Support for Densenet121**

| Paddy disease name | Precision | Recall | F1-Score | Support |
|---|---|---|---|---|
| Paddy bacterial leaf blight | 0.87 | 0.97 | 0.92 | 92 |
| Paddy bacterial leaf streak | 0.91 | 0.96 | 0.93 | 73 |
| Paddy bacterial panicle blight | 0.98 | 0.88 | 0.93 | 65 |
| Paddy blast | 0.97 | 0.87 | 0.92 | 334 |
| Paddy brown spot | 0.91 | 0.90 | 0.91 | 186 |
| Paddy dead heart | 0.99 | 0.99 | 0.99 | 277 |
| Paddy downy mildew | 0.83 | 0.96 | 0.89 | 119 |
| Paddy hispa | 0.96 | 0.91 | 0.93 | 307 |
| Paddy normal | 0.92 | 0.96 | 0.94 | 339 |
| Paddy tungro | 0.91 | 0.96 | 0.93 | 209 |
| **Accuracy** | | **93.30%** | | |

**Table VI. Precision, Recall, F1-Score, and Support for MobilenetV2**

| Paddy disease name | Precision | Recall | F1-Score | Support |
|---|---|---|---|---|
| Paddy bacterial leaf blight | 0.74 | 0.95 | 0.83 | 92 |
| Paddy bacterial leaf streak | 0.95 | 0.96 | 0.95 | 73 |
| Paddy bacterial panicle blight | 0.96 | 0.83 | 0.89 | 65 |
| Paddy blast | 0.98 | 0.86 | 0.92 | 334 |
| Paddy brown spot | 0.96 | 0.92 | 0.94 | 186 |
| Paddy dead heart | 0.98 | 0.99 | 0.98 | 277 |
| Paddy downy mildew | 0.81 | 0.92 | 0.86 | 119 |
| Paddy hispa | 0.94 | 0.94 | 0.94 | 307 |
| Paddy normal | 0.94 | 0.97 | 0.95 | 339 |
| Paddy tungro | 0.94 | 0.94 | 0.94 | 209 |
| **Accuracy** | | **93.00%** | | |




Fig. 1 Paddy Crop Diseases

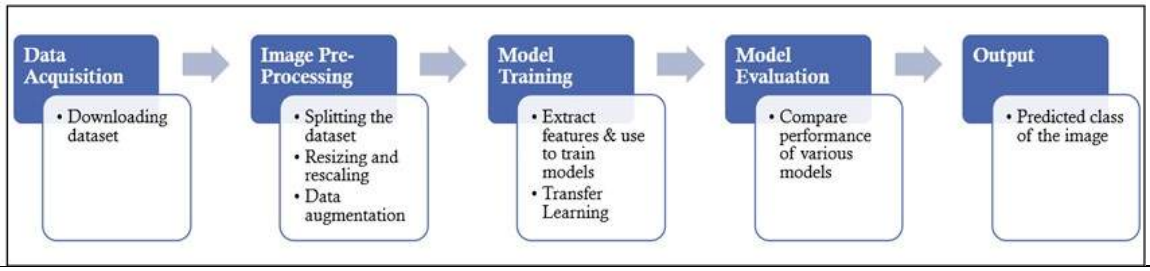



Fig 2. Workflow for PaddyCrop Disease Detection [17]

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RESEARCH ARTICLE

# Building Competence through Field Work Practicum: MSW Students' Perspectives in Tamil Nadu

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**ABSTRACT**

This study explores MSW students' perceptions of how field work practicum supports professional competence development in Tamil Nadu, focusing on supervisory mentorship, agency exposure, and institution-agency collaboration as influences on competence development. Qualitative design using semi-structured interviews with 24 MSW students, purposely sampled from six social work institutions in Chennai. Three major findings stood out in the study. Practicum learning depended on clear orientation. Supervision shaped learning. Feedback and evaluation were inconsistent and informal. Students viewed competence as important for employability and confidence, but reported unequal opportunities due to differences in supervisory support and agency culture. Field work practicum has strong potential for competence development, but lacks structured mechanisms to achieve it. Strengthening mentorship, improving institutional–agency collaboration, and formalizing competence development platforms can enhance professional growth and practice readiness. Future research should integrate multi-stakeholder perspectives to build a culturally competence development framework.

**Keywords:** Field Work Practicum, Competence Development, Supervision, Social Work Education, Tamil Nadu.

## INTRODUCTION

Field work practicum is widely treated as the *signature pedagogy* of social work education—i.e., the primary setting where students are socialized into professional practice and learn to integrate classroom knowledge with real-world

intervention. This framing is strongly established in social work education literature and accreditation discussions (Wayne *et al*., 2010; Bogo, 2015; CSWE, 2008; Skeen, 2023). However, practicum learning is not automatic; it is shaped by the quality of supervision and mentoring, the clarity and consistency of practicum guidelines, feedback and evaluation processes, and the agency/placement context that determines actual exposure to cases, stakeholders, and practice opportunities. When these elements are strong, practicum accelerates competence development; when they are weak or inconsistent, students may experience role confusion, limited skill-building, and uneven learning outcomes (Bogo, 2015; Pack, 2018; Jansing, 2025).

**Significance of the study**

This study is practically important because field education is positioned as the profession's primary training platform; therefore, variability in supervision, feedback, and placement quality has direct implications for competence development and readiness for practice (Wayne *et al*., 2010; Bogo, 2015). Institutionally, the findings can inform quality assurance by identifying which practicum components require standardization (e.g., supervisor expectations, feedback cycles, evaluation criteria) and where institution–agency coordination needs strengthening to protect equity in student learning opportunities (CSWE, 2008; Jansing, 2025).

## LITERATURE REVIEW

Field education (field work practicum) is consistently described as the central practice-learning platform in social work because it is where students enact professional roles, demonstrate competence, and integrate values, knowledge, and skills in real service settings (Wayne, Bogo, and Raskin, 2010; Bogo, 2015; Skeen, 2023). At the same time, research shows that practicum quality is highly dependent on the *conditions of learning*—especially the availability and capability of agency/faculty supervisors, the structure and consistency of placement expectations, and the opportunities provided by agency contexts—meaning students can experience markedly different learning trajectories even within the same professional standards framework (Earls Larrison and Korr, 2013; Chilvers, 2021; Skeen, 2023). A recurring concern in the literature is that supervision and assessment in the field are not merely administrative tasks but core educational processes; however, providing corrective feedback and evaluating competence can be difficult in practice, shaped by supervisor judgment, relationship dynamics, and contextual constraints, which can produce inconsistency in evaluation experiences (Bogo *et al*., 2007; Bogo, 2015). Empirical work capturing student and supervisor views also highlights that students value supportive, skilled supervision and clear expectations, yet report variability in feedback quality and practicum evaluation processes, reinforcing the need for stronger program–agency alignment and more transparent assessment practices (Pack, 2018; Wayne *et al*., 2010).

**Problem statement**

Despite practicum being positioned as central to professional preparation, students' experiences often vary substantially across institutions and agencies due to inconsistent supervision quality, uneven implementation of practicum guidelines, irregular or non-specific feedback, and placement contexts that limit learning exposure. This variability creates unequal conditions for competence development and satisfaction, even when students are enrolled in comparable MSW programs (Pack, 2018; Jansing, 2025).

## MATERIALS AND METHODS

## RESEARCH DESIGN

This study employed a qualitative design and adopted thematic analysis supported by NVivo to examine MSW students' perceptions of field work practicum. A hybrid coding strategy was applied, combining deductive codes derived from the study questions with inductive codes that were allowed to emerge from the dataset (Braun and Clarke, 2006; Nowell *et al*., 2017). The setting comprises 16 social work institutions.

## Aim of the Study

To explore MSW students' perceptions of field work practicum processes and determine how supervision, structure, feedback/evaluation, and placement context shape learning and professional development.

## Research Questions

- How do MSW students describe the quality and usefulness of practicum orientation, guidelines, and role clarity?
- How do agency and faculty supervision practices influence students' learning, confidence, and theory–practice integration?
- How do feedback and evaluation mechanisms (including student voice in evaluation) affect perceived learning outcomes and satisfaction?
- What barriers constrain practicum learning opportunities, and what improvements do students recommend?

## Objectives of the Study

- To document students' perceptions of practicum orientation, guideline clarity, and implementation.
- To examine how supervision (agency and faculty) supports or constrains learning and theory–practice integration in competence development.
- To analyze students' experiences of feedback and evaluation, including transparency and timeliness.
- To identify structural, agency, supervisor-related, and contextual barriers affecting practicum learning and competence development.
- To synthesize student-recommended actions to strengthen practicum quality and consistency.

### Participants and sampling

Participants of the study, MSW students whose responses are contained in the compiled qualitative dataset. Inclusion criteria are: (i) enrolled in an MSW programme within the defined institutions, and (ii) having practicum/field work experience reflected in responses covering practicum processes (e.g., supervision, feedback, learning outcomes). Records with insufficient practicum-related content were excluded from thematic interpretation (Nowell *et al*., 2017). Sampling is case-based, using all available student response records in the dataset (a census of the compiled records rather than recruitment-based sampling). This approach will be justified because the analytic goal is to map patterns in students' reported practicum experiences across institutions and supervision contexts (Braun and Clarke, 2006).

### Data source, instrument domains, and data management

The data source is the compiled student-response document. The instrument domains reflected in the dataset include: orientation/role clarity, practicum guidelines and structure, agency and faculty supervision, theory–practice integration, feedback and evaluation practices, self-rated learning outcomes/satisfaction, barriers, and suggestions for improvement. These domains serve as the deductive "parent codes" in the codebook (Braun and Clarke, 2006). Data management follows a structured anonymization process. Each student record is assigned a unique Case ID (e.g., Inst##_P##), and institutions are assigned standardized institution codes (Inst01–Inst16). Any identifying names of institutions, supervisors, agencies, and individuals were removed or masked during import and reporting.

### Data analysis procedure

Analysis proceeds in five steps consistent with established thematic analysis guidance. First, familiarization through repeated reading and memoing was conducted by the researcher. Second, initial coding was performed in NVivo using the prepared codebook, applying deductive parent codes while also creating inductive child codes when new recurrent patterns appear (Braun and Clarke, 2006; Nowell *et al*., 2017). Third, codes are refined through code definition checks, collapsing overlaps, and clarifying boundaries using inclusion/exclusion rules captured in the codebook (Nowell *et al*., 2017). Fourth, candidate themes are developed by clustering related codes into higher-order categories and testing them against the dataset for coherence and distinctiveness (Braun and Clarke, 2006). Fifth, cross-case comparisons were performed using NVivo matrix coding by institution to examine how themes vary across the 16 institutional contexts and to support pattern-based interpretation (Jackson and Bazeley, 2019).

**Trustworthiness and ethics**

To strengthen trustworthiness, the study applies the criteria of credibility, transferability, dependability, and confirmability (Lincoln and Guba, 1985). Credibility is supported through peer debriefing and/or a second-coder check on approximately 10–20% of the records; disagreements are resolved through discussion and documented codebook updates. Dependability and confirmability are supported through an audit trail (versioned codebook, memos, and decision logs), while transferability is also supported through clear contextual description of practicum structures and institutional coding rules (Nowell *et al*., 2017; Korstjens and Moser, 2017). Ethical practice is addressed by ensuring de-identification, restricting access to the master linking file, and reporting only non-identifiable excerpts. Where the dataset originates from institutional academic activity, permissions/approvals was obtained and stated in the manuscript, and participation/record use are represented in line with institutional requirements (Lincoln and Guba, 1985). Permission for the conduction of the research was obtained from each and every participant through consent form. Researcher also obtained permission from the Institutions ethical committee.

## RESULTS

**Overview**

Six themes were identified through hybrid (deductive–inductive) coding of student responses and then refined by comparing patterns across institutions using NVivo matrix coding (institution × theme). Themes were constructed by clustering recurring coded segments into higher-order meanings and checking for consistency and variation across institutional cases. Table 1 will summarise the final themes and subthemes. Table 2 will present the institution-wise pattern matrix (theme presence/strength). Figure 1 will present the NVivo matrix heatmap (institution × theme). The NVivo treemap (coded references by code cluster) indicates that students most frequently discussed field work practicum as "work"—including practicum requirements, tasks, and the day-to-day structure of placements—suggesting that the practicum experience is commonly understood and narrated through operational demands rather than only educational design. The next most prominent cluster relates to linking theory to practice ("relating theory" and "social work theory"), implying that students repeatedly confronted the challenge of translating classroom concepts into placement realities, making theory–practice integration a central concern rather than a background issue. A substantial proportion of references also concentrates on community and social work field work, indicating that experiential learning is strongly framed through community exposure and real-world engagement, while the presence of competencies and knowledge clusters suggests students connect practicum to skill and knowledge development but discuss these somewhat less than the immediate realities of work and theory-application. Smaller clusters (e.g., hospital, semester) appear more context-specific, implying that setting and timing shape experiences but are not as dominant as supervision-linked learning conditions and the struggle to operationalize theory in practice.

**Theme 1: Orientation sets the ceiling for early role clarity**

Orientation functioned as the "starting frame" for students' understanding of expectations and professional role; where it was generic, role clarity remained weak, and where it was clear, students reported smoother adjustment.

**Subthemes**

- Generic/common orientation
- Role clarity from induction
- Transition into placement

**Evidence (quotes)**

- "The orientation was basic with guidelines for all the students." (Inst04–P02)
- "The orientation for all the students… was common clear and helped me understand my role well." (Inst06–P01)
- "Orientation was clear and useful…" (Inst12–P01)
- "The orientation provided basic information with guidelines…" (Inst03–P03)

**Variation across institutions (matrix comparison)**
Clear orientation narratives appeared alongside higher reported role clarity (e.g., Inst06, Inst12), while "basic/general" orientation was more often linked to later reliance on self-guided learning (e.g., Inst04, Inst03).

**Theme 2: Supervision quality acted as the gatekeeper of learning**
Students repeatedly framed supervision as the main mechanism determining whether practicum became skill-building or merely task completion. A frequent contrast emerged between stronger faculty guidance and weaker or misaligned agency supervision.

**Subthemes**
- Agency supervisor availability/engagement
- Supervisor qualification/fit with social work
- Faculty supervision as compensatory support

**Evidence (quotes)**
- "No agency supervisor will involve and teach the students and train." (Inst01–P01)
- "Applying social work theory was tricky under an MBA supervisor who focused on business mostly." (Inst04–P02)
- "The faculty supervisor did, but the supervisor lacked social work training." (Inst04–P02)
- "The faculty helped build our competencies… [but] the agency supervisor's feedback was minimal." (Inst03–P03)

**Variation across institutions (matrix comparison)**
Where supervision was described as approachable and structured, students reported stronger learning (e.g., "guided… preparing proper reports"; "linked classroom… with daily work") (Inst06). In other institutions, students described supervision as absent or misaligned with social work learning outcomes (Inst01, Inst04, Inst03).

**Theme 3: Feedback and evaluation were present, but often informal and inequitable**
Students commonly valued feedback but reported uneven timeliness, limited constructiveness, and restricted opportunities to evaluate agencies/supervisors—suggesting that evaluation systems were frequently under-structured.

**Subthemes**
- Irregular or non-constructive feedback
- Lack of student voice in evaluation
- Demand for formal feedback mechanisms

**Evidence (quotes)**
- "Feedback was irregular and not particularly constructive…" (Inst03–P03)
- "There was no structured channel to evaluate the agency or supervisor's suitability." (Inst04–P02)
- "We didn't get much chance to formally evaluate the supervisor." (Inst06–P01)
- "An anonymous evaluation form would give students more voice." (Inst12–P01)

**Variation across institutions (matrix comparison)**
Some students reported feedback as present and improvement-oriented (Inst06: "they gave feedback that improved my work") while others described feedback as sporadic and evaluation channels as absent (Inst03, Inst04).

**Theme 4: Theory–practice integration worked only when conditions supported it**

Students' ability to apply theory depended on supervision competence and placement context. When supervisors emphasised non-social-work priorities or settings lacked relevant exposure, students reported a theory–practice break.

**Subthemes**

- Theory application enabled by guided practice
- Theory–practice gap due to supervisor mismatch
- Context constraints limiting application

**Evidence (quotes)**

- "No care was taken to implement the social work methods." (Inst01–P01)
- "We had to bridge theory ourselves…" (Inst04–P02)
- "Without proper clinical settings and weak supervision, applying… diagnostic assessments were challenging." (Inst03–P03)
- "They linked classroom HR concepts with daily work." (Inst06–P01)

**Variation across institutions (matrix comparison)**

Institutions/placements with coherent supervision narratives showed smoother theory application (Inst06), whereas placements characterised by weak supervision or absent clinical exposure showed repeated "self-driven" theory linking (Inst03, Inst04, Inst01).

**Theme 5: Structural and context barriers constrained exposure and equity**

Students described barriers beyond individual effort—especially placement context (e.g., rural/clinical mismatch), inadequate learning opportunities, and logistical constraints—producing uneven competence development.

**Subthemes**

- Limited clinical/community exposure due to placement context
- Logistical barriers (travel/time)
- Inconsistent guideline implementation across agencies

**Evidence (quotes)**

- "We struggled to meet requirements because the rural context lacked relevant clinical opportunities." (Inst03–P03)
- "Field work is crucial, but in a rural area without appropriate clinical settings, it didn't fully prepare us…" (Inst03–P03)
- "More supervisor availability and better travel support would help a lot." (Inst12–P01)
- "Guidelines were useful, though agencies interpreted them differently." (Inst03–P03)

**Variation across institutions (matrix comparison)**

Barrier intensity varied by placement ecology: contexts lacking appropriate service settings (e.g., clinical exposure) generated stronger dissatisfaction and "self-initiative" learning narratives (Inst03), while more structured placements emphasized refinement rather than access (Inst06, Inst12).

**Theme 6: Improvement priorities centered on standardization, supervisor capability, and student voice**

Students consistently proposed practical reforms focused on formalizing feedback/evaluation, improving supervisor preparedness (especially social work orientation), strengthening placement options, and increasing structured mentorship.

**Subthemes**

- Formal feedback and evaluation mechanisms
- Supervisor training/qualification in social work principles
- Placement diversity/rotation and exposure planning
- Increased supervisor–student interaction

**Evidence (quotes)**

- "Introduce structured feedback mechanisms… and ensure supervisors have consistent training on mentorship." (Inst04–P01)
- "Ensure supervisors are qualified social workers or trained in social work principles…" (Inst04–P02)
- "Provide placements with at least some clinical services in rural areas or rotate students…" (Inst03–P03)
- "More structured feedback sessions would make it even better." (Inst06–P01)

**Variation across institutions (matrix comparison)**

Even where overall satisfaction was high, students still asked for more formal feedback and evaluation channels (Inst06, Inst12); where satisfaction was lower, recommendations were more structural (supervisor qualification, clinical placements, rotation models) (Inst03, Inst04).

**Principal findings**

Three findings stood out. First, students' practicum learning was strongly shaped by how structured the practicum ecosystem was—especially orientation and guideline clarity—suggesting that weak induction creates early role ambiguity that later supervision cannot always correct. Second, supervision operated as the core learning mechanism: when agency supervision was unavailable or misaligned with social work practice expectations, students described weaker skill development and more self-directed "patching" of learning. Third, feedback and evaluation were often experienced as informal, inconsistent, and low on student voice, implying that learning opportunities and assessment fairness can vary across placements even when programmes share similar training intentions. These patterns align with longstanding concerns that field learning is powerful but uneven when educational conditions are not consistently designed and monitored (Wayne, Bogo, and Raskin, 2010; Bogo, 2015).

## DISCUSSION

**Orientation and role clarity:** Your findings reinforce that practicum cannot be treated as "self- explanatory"; early clarity about expectations and learning goals is part of the educational design of field learning. In the signature pedagogy conversation, field education is often positioned as the central practice-learning site, but its effectiveness depends on whether programmes make learning expectations visible and routine rather than ad hoc (Wayne *et al*., 2010; Skeen, 2023).

**Supervision quality as the gatekeeper:** Students' emphasis on supervision is consistent with the view that field education is simultaneously a learning platform and a gate keeping process that develops and assesses competence (Bogo, 2015). The sharp contrast in your data between helpful faculty guidance and weaker/misaligned agency supervision parallels the literature that supervision quality, relationship safety, and supervisor capability strongly influence whether practicum becomes reflective learning or mere task completion (Pack, 2018).

**Feedback and evaluation systems:** The repeated call for timely, constructive feedback and clearer evaluation mechanisms echoes prior evidence that assessment in field education is difficult because it sits at the intersection of learning support and performance judgment, and can easily become inconsistent without rubrics and routine feedback cycles (Bogo, 2015; Pack, 2018).Your context adds a practical warning: when students lack a structured channel to evaluate placements and supervision quality, inequities in practicum experience can persist unnoticed across institutions.

**Theory–practice integration:** The theory–practice gap described by students is widely recognised as a central risk in practicum, and bridging that gap is often treated as a primary purpose of supervised practice (Pack, 2018; Bogo, 2015).Your findings sharpen the mechanism: integration "works" when supervision and placement exposure are aligned to social work methods; it "breaks" when supervision is not practice-relevant or when the setting lacks opportunities needed for the student's learning goals.

**Structural/context constraints:** The barriers students reported fit broader work-integrated learning evidence: placement learning depends on institutional–agency partnerships, access to authentic practice opportunities, and quality assurance mechanisms that keep learning conditions stable across sites (Chilvers, Hay, Maidment, and Tudor, 2021).Your multi-institution perspective is valuable here because it highlights variability as a system property, not merely an individual student issue.

**Improvement priorities:** Students' recommendations closely mirror best-practice calls to professionalise field supervision through clearer expectations, supervisor support/training, and stronger programme–agency coordination (Bogo, 2015; Wayne *et al*., 2010). The contribution from your context is that improvements must be designed for consistency across institutions (not left to "good supervisors" or "good agencies" by chance).

## RECOMMENDATION

**Institutional and agency implications**
**What should change in practice is straightforward and measurable**
**Standard supervision expectations:** programmes will define minimum supervision frequency, agenda (case discussion + reflective learning), and documentation expectations (Bogo, 2015).

**Supervisor qualification/training:** where agencies cannot provide social work-qualified supervisors, programmes will implement short mandatory training on social work learning goals, ethical practice learning, and assessment responsibilities (Wayne *et al*., 2010).

**Structured feedback loops and rubrics:** programmes will adopt common feedback checkpoints (e.g., early–mid–end placement) and transparent evaluation rubrics to reduce inconsistency (Pack, 2018).

**Student evaluation channel:** programmes will introduce an anonymous placement/supervision evaluation process with corrective action triggers (Pack, 2018).

**Placement matching and exposure planning:** placements will be matched to learning goals (and where needed, rotation/combined placements will be planned to address exposure gaps) (Chilvers *et al*., 2021).

**Institution–agency MoU and monitoring:** MoUs will specify learning responsibilities, supervision requirements, feedback processes, and escalation pathways; monitoring visits will focus on learning conditions, not only attendance (Bogo, 2015).

### Contribution

Methodologically, this study adds a multi-institution comparison of practicum experiences, strengthening interpretation by showing how themes vary across institutional contexts rather than reflecting one programme alone. Practically, it delivers actionable targets—supervision standards, feedback architecture, placement quality monitoring, and student voice mechanisms—that directly address the sources of uneven learning highlighted in the signature pedagogy debate (Wayne *et al*., 2010; Earls Larrison and Korr, 2013).

**Limitations**

This study will be limited by reliance on student-reported accounts, which may under-represent supervisor/agency perspectives and may vary in depth across institutions. Transferability will be bounded by the institutional and placement contexts represented in the dataset, and some demographic or placement variables may not be consistently available across all records. Finally, the absence of direct supervisor/agency interviews will restrict triangulation of perceived supervision and evaluation practices (Lincoln and Guba, 1985).

## CONCLUSION

This study shows that practicum learning is highly sensitive to how consistently programmes design and monitor the learning conditions of field education. Students' accounts indicate that orientation and role clarity, supervision quality, and feedback/evaluation systems are decisive levers that can either enable competence development or produce uneven outcomes across placements. Immediate improvement should focus on minimum supervision standards, supervisor preparation aligned to social work learning goals, and formal feedback and evaluation channels that include student voice. Future research should add supervisor and agency stake holder perspectives and test whether standardised supervision and assessment processes reduce inter- institution variability in learning outcomes (Bogo, 2015; Wayne *et al*., 2010).

### ACKNOWLEDGEMENT SECTION

The researcher extends sincere gratitude to all the social work students who participated in this study and shared their experiences. Special thanks are due to the heads of institutions and faculty members who facilitated data collection.

### ETHICAL APPROVAL AND INFORMED CONSENT STATEMENT

Ethical approval from all the institution head and Informed consent was obtained from all participants prior to data collection. Participants were assured of confidentiality, anonymity, and their right to withdraw at any stage without any consequences.

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**Table 1: Themes and subthemes identified from NVivo coding**

| Theme (analytic title) | What the theme captures (brief definition) | Subthemes (2–4) | Illustrative excerpt (short) |
|---|---|---|---|
| Orientation sets the ceiling for early role clarity | How induction/orientation shapes students' understanding of role expectations and adjustment | (i) Generic orientation, (ii) Role clarity from induction, (iii) Transition into placement | "The orientation was basic with guidelines for all the students." (Inst04–P02) |
| Supervision quality acted as the gatekeeper of learning | How agency/faculty supervision availability, competence and support determine learning opportunities | (i) Agency supervisor availability, (ii) Supervisor competence/fit, (iii) Faculty guidance as compensatory support | "Applying social work theory was tricky under an MBA supervisor…" (Inst04–P02) |
| Feedback and evaluation were often informal and inequitable | How feedback timeliness/quality and evaluation mechanisms influence perceived learning fairness and student voice | (i) Irregular feedback, (ii) Low constructiveness, (iii) Limited student voice, (iv) Need for formal mechanisms | "There was no structured channel to evaluate the agency or supervisor…" (Inst04–P02) |
| Theory–practice integration worked only when conditions supported it | How supervision and placement exposure enable or block applying classroom theory in field settings | (i) Guided application, (ii) Theory–practice gap, (iii) Context limits on application | "No care was taken to implement the swmethods." (Inst01–P01) |
| Structural and context barriers constrained exposure and equity | System and context constraints that reduce exposure, consistency and learning opportunities | (i) Placement context limits, (ii) Logistics/time, (iii) Guideline variability across agencies | "Rural context lacked relevant clinical opportunities." (Inst03–P03) |
| Improvement priorities centred on standardisation and student voice | Student recommendations for strengthening practicum consistency, supervision, feedback and placement quality | (i) Standard supervision, (ii) Supervisor training, (iii) Formal feedback/evaluation, (iv) Placement matching/rotation | "Introduce structured feedback mechanisms…" (Inst04–P01) |

**Table 2: Cross-institution pattern matrix**

| Institution | Orientation and role clarity | Supervision gatekeeping | Feedback and evaluation | Theory–practice integration | Structural/context barriers | Improvement priorities |
|---|---|---|---|---|---|---|
| Inst01 | M | H | H | H | M | H |
| Inst02 | M | M | M | M | M | M |
| Inst03 | M | H | H | H | H | H |
| Inst04 | L | H | H | H | M | H |
| Inst05 | M | M | M | M | M | M |
| Inst06 | H | M | M | M | L | M |
| Inst07 | M | M | M | M | M | M |
| Inst08 | M | M | M | M | M | M |
| Inst09 | M | M | M | M | M | M |
| Inst10 | M | M | M | M | M | M |
| Inst11 | M | M | M | M | M | M |
| Inst12 | H | M | M | M | M | M |
| Inst13 | M | M | M | M | M | M |
| Inst14 | M | M | M | M | M | M |
| Inst15 | M | M | M | M | M | M |
| Inst16 | M | M | M | M | M | M |




**Figure 1: *NVivo* tree map showing coding references by code cluster (overall dataset).**

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RESEARCH ARTICLE

# Assessing the Role of Digital Governance and Aadhaar - Linked Platforms in the Delivery of Scheduled Caste Welfare Schemes: A Case Study from Karur District of Tamilnadu

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## ABSTRACT

The study investigates the role of digital governance and Aadhaar-linked platforms in delivering welfare schemes to Scheduled Caste (SC) communities, focusing on Karur District, Tamil Nadu. Addressing a critical research gap, the study explores how SC beneficiaries experience and navigate Aadhaar-based welfare delivery systems at the grassroots level. Using a qualitative case study approach, data were collected through semi-structured interviews with 12 SC respondents (6 males and 6 females) across Tharagampatti and Chinnandipatti villages. Respondents were selected purposively from Krishnarayapuram and Aravakurichi Blocks, based on their use of Aadhaar-enabled schemes. The findings reveal both opportunities and challenges: while Aadhaar has streamlined access to services like pensions, scholarships, and housing, issues such as biometric mismatches, digital illiteracy, and lack of localized support persist particularly for women and elderly SC members. The study highlights the need for gender-sensitive outreach, accessible grievance redressal, and community-based digital facilitation to ensure inclusive digital welfare governance in India.

**Keywords:** Digital Governance, Aadhaar-Linked Welfare Schemes, Scheduled Castes, Inclusive Service Delivery

## INTRODUCTION

In recent decades, the evolution of digital technology has significantly influenced the landscape of governance worldwide. In India, digital governance or e-governance has emerged as a powerful instrument in transforming the relationship between the state and its citizens. Designed to enhance transparency, efficiency, and accessibility in public service delivery, digital governance initiatives aim to bridge longstanding administrative gaps and ensure that the benefits of development are equitably distributed. From land records and healthcare to education and welfare, digital tools have been gradually integrated into various arms of governance, offering a citizen-centric approach to administration (Basu, 2004). One of the most ambitious projects in India's digital governance ecosystem is the Aadhaar program, launched in 2009 by the Unique Identification Authority of India (UIDAI). Aadhaar is a 12-digit unique identity number based on biometric and demographic data. As of 2024, over 1.3 billion residents have been enrolled in the Aadhaar system, making it the world's largest biometric ID program (UIDAI, 2024). Aadhaar's primary objective is to create a unified and verifiable digital identity for every citizen, thereby minimizing duplication, identity fraud, and systemic inefficiencies in service delivery. The introduction of Aadhaar-linked platforms for the distribution of public benefits has been a turning point in India's governance narrative. These platforms enable Direct Benefit Transfers (DBT), whereby subsidies, pensions, scholarships, and other entitlements are credited directly into beneficiaries' Aadhaar-linked bank accounts. The government reports that the mechanism has saved billions of rupees by reducing "ghost beneficiaries" and plugging leakages in welfare schemes (Meity, 2023). For the general public, these systems promise simplified access, faster grievance redressal, and fewer intermediaries. On the other hand, the implementation of digital governance is not without challenges. Across India, the benefits of digital initiatives remain unevenly distributed due to the digital dividea disparity in access to technology, internet infrastructure, and digital literacy, particularly in rural and remote areas (Singh, 2018). Aadhaar-based authentication has also been criticized for operational failures, including biometric mismatches and server downtime, which can hinder access to essential services like food rations or pensions (Drèze and Khera, 2017). Also, concerns over privacy, data protection, and state surveillance have gained traction in civil society discourse, calling into question the extent to which digital governance protects citizen rights (Ramanathan, 2016). Amid the broader context, it is essential to evaluate how such digital reforms affect not just the general population, but also historically marginalized communities, especially the Scheduled Castes (SCs).

Representing over 16.6% of India's population (Census of India, 2011), SC communities have long suffered structural exclusion from mainstream economic and social opportunities. Despite numerous constitutional safeguards and targeted welfare schemes, SCs continue to lag in indicators such as literacy, income, land ownership, and political participation (Jodhka, 2012). The use of digital platforms and Aadhaar-based service delivery holds both promise and pitfalls for SC populations. On one hand, technology-driven welfare distribution has the potential to bypass caste-based discrimination by depersonalizing delivery and eliminating corrupt intermediaries. On the other hand, it risks reinforcing exclusion if the digital divide is not adequately addressed. For instance, several studies show that SC households are less likely to possess smartphones, bank accounts, or stable internet access, all of which are prerequisites for accessing Aadhaar-linked services (Masiero, 2016; Khera, 2019). It creates a paradox where digital tools, designed to promote inclusion, may inadvertently perpetuate exclusion. At the sub-national level, Tamil Nadu presents a particularly relevant case for exploring the interplay of digital governance and Scheduled Caste welfare. With an SC population of around 20%, Tamil Nadu has a strong tradition of social justice and welfare-oriented governance (Thorat and Newman, 2007). Schemes like the Adi Dravidar Housing Program, educational scholarships, and entrepreneurship support for SCs are well-established. In recent years, Tamil Nadu has actively expanded its digital infrastructure through platforms like e-Sevai centers, designed to facilitate Aadhaar seeding, online applications for welfare schemes, and access to public services. Although, these advancements, SC communities in Tamil Nadu still encounter hurdles in fully leveraging digital services. Studies have highlighted implementation challenges such as lack of digital awareness, poor access to Aadhaar correction services, and limited handholding support in local languages (Rajasekhar *et al*., 2020). Besides, Aadhaar-linked disbursals are sometimes delayed due to authentication errors, especially in manual labor-intensive SC households where biometric wear and tear is common.

The study aims to critically assess how digital governance mechanisms, particularly Aadhaar-enabled platforms, influence the accessibility, inclusivity, and efficacy of Scheduled Caste welfare schemes at the national level and within Tamil Nadu. The central focus is to examine whether these digital interventions truly empower SC beneficiaries or create new forms of administrative and technological exclusion. The research will also explore institutional frameworks, user experiences, and systemic gaps, offering policy recommendations to strengthen digital equity in welfare delivery.

## REVIEW OF LITERATURE

Digital governance has redefined public service delivery in India, especially since the launch of the Digital India campaign in 2015, which aims to enhance digital infrastructure and bring services closer to citizens (Basu, 2004; Singh, 2018). The Aadhaar biometric identity system has been at the center of the transformation, enabling Direct Benefit Transfers (DBT) for schemes such as pensions, scholarships, and subsidies. UIDAI (2024) states that over 1.3 billion Aadhaar numbers have been issued, marking a shift toward centralized identification. Bhatia and Bhabha (2017) argue that Aadhaar has helped streamline welfare delivery by reducing duplication and fraud. However, Usha Ramanathan (2016) and Masiero (2016) highlight serious concerns about data privacy, exclusion errors, and the ethical implications of Aadhaar-based surveillance. According to Dey and Sharma (2017), rural and marginalized populations still face systemic barriers, including network failures and authentication mismatches, suggesting that digital infrastructure alone cannot ensure equitable welfare access. Empirical studies have offered mixed findings regarding Aadhaar's impact on social protection. Drèze and Khera (2017) found that Aadhaar-linked DBT systems reduced leakages in the Public Distribution System (PDS) but also noted that exclusion of genuine beneficiaries occurred due to technological failures. Khera (2019) emphasized that while Aadhaar may work well in urban areas, its application in rural or tribal regions can be exclusionary, especially where fingerprint mismatches are common. Ghosh (2021) analyzed the impact of Aadhaar in Jharkhand and found that biometric issues affected 12% of welfare recipients. Chopra and Singh (2020) highlight that Scheduled Castes, due to their socio-economic position, are less likely to have reliable internet access, smart devices, or awareness of digital entitlements. Singh and Patnaik (2022) add that digital illiteracy, especially among Dalit women, poses a significant barrier to accessing Aadhaar-linked services, creating a new form of digital caste-based exclusion. At the state level, Tamil Nadu is often cited for its relatively progressive and welfare-oriented governance model. Schemes like Adi Dravidar Housing, educational scholarships, and skill development programs for SCs are frequently implemented through Aadhaar-enabled platforms (Thorat and Newman, 2007; Tamil Nadu e-Governance Agency, 2023). Rajasekhar *et al*. (2020) observed that while Tamil Nadu's e-Sevai centers improved administrative access, SC beneficiaries still experienced procedural delays, poor grievance redressal, and language barriers. Babu and Manjula (2019) note that Aadhaar seeding in SC-dominated districts faced higher error rates compared to general population areas. Choudhury (2021) emphasizes that even when Aadhaar infrastructure exists, field-level implementation in welfare schemes often lacks caste-sensitivity and local awareness campaigns. Also, Srinivasan and Kannamma (2020) found that Aadhaar integration in SC scholarship programs resulted in increased delays and confusion among first-generation learners. These studies indicate a clear need to interrogate how digital governance interacts with socio-cultural realities at the grassroots level.

## RESEARCH GAP

While the existing literature offers a broad understanding of Aadhaar and digital governance in India, there is a significant lack of case-specific research focusing on Scheduled Castes and their lived experiences with Aadhaar-linked platforms, particularly at the grassroots level. Most available studies examine Aadhaar as a centralized policy tool influencing the general population, often overlooking the caste-based, digital, and bureaucratic barriers that marginalized communities like the SCs face in accessing welfare schemes. Moreover, the diversity in state-level implementation strategies—shaped by regional infrastructure, governance priorities, and community awareness has not been adequately captured in existing analyses. Tamil Nadu, known for its proactive social welfare delivery and

digital governance mechanisms, provides a compelling context to explore these gaps. However, there is limited empirical evidence on how Aadhaar-linked platforms operate within SC welfare schemes in specific districts of the state. To address the, the present study titled, "Assessing the Role of Digital Governance and Aadhaar-Linked Platforms in the Delivery of Scheduled Caste Welfare Schemes: A Case Study from Karur District", seeks to examine the effectiveness, accessibility, and limitations of digital delivery systems as experienced by SC beneficiaries in Karur. By grounding the analysis in a real-world case, the research intends to offer context-sensitive insights that contribute to the broader discourse on inclusive and equitable digital governance in India.

## METHODOLOGY

The present study follows a qualitative case study design to explore the role and effectiveness of digital governance and Aadhaar-linked platforms in delivering welfare schemes to Scheduled Caste (SC) communities. The case study approach enables in-depth exploration of real-life experiences and challenges faced by beneficiaries within a specific socio-cultural and administrative context Karur District of Tamil Nadu.

**Sources of Data**

**Primary Data**: Collected through semi-structured interviews using a well-designed interview schedule administered directly to SC beneficiaries.

**Secondary Data**: Obtained from government policy documents, UIDAI reports, Tamil Nadu e-Governance Agency publications, Census 2011, academic studies, and welfare scheme guidelines related to Aadhaar and SC welfare.

**Sampling Procedure**

The study utilized a purposive sampling method to select respondents who meet the study criteria. The sample was drawn from two administrative blocks of Karur District:

- Krishnarayapuram Block
- Aravakurichi Block

Within these blocks, two villages were selected based on Scheduled Caste population density and accessibility to Aadhaar-linked services:

- Tharagampatti Village (Krishnarayapuram Block)
- Chinnandipatti Village (Aravakurichi Block)

From these villages, a total of 12 Scheduled Caste respondents were purposively selected:

- All respondents were aged 18 years and above**.**
- The sample was gender-balanced**,** with6 male and 6 female participants.
- Respondents were chosen based on their direct experience with Aadhaar-enabled welfare schemes such as scholarships, pensions, subsidies, and housing benefits.

**Research Tool: Interview Schedule**

The primary research instrument was as emi-structured interview schedule, which included both open-ended and close-ended questions**.** The interview guide covered the following themes:

- Basic demographic profile (age, gender, education, occupation)
- Awareness and knowledge of welfare schemes available for SCs
- Experience with Aadhaar registration and authentication
- Challenges faced in accessing Aadhaar-linked services
- Perceptions about digital governance and service delivery
- Feedback on transparency, efficiency, and grievance redressal mechanisms

The tool was developed in English and translated into Tamil to facilitate easy communication with respondents.

**Data Collection Procedure**

Interviews were conducted in person by the researcher in the local language at the respondents' homes or community spaces, ensuring a comfortable and private environment. Each interview lasted approximately 30–45 minutes, and responses were recorded with the participants' consent. The data was collected over a period of two weeks.

**Ethical Considerations**

1. Informed consent was obtained from all participants before the interviews.
2. Participants were briefed about the purpose of the study and their right to withdraw at any point.
3. Confidentiality and anonymity were maintained throughout the research process.
4. Ethical approval was sought from the concerned institutional review board before the commencement of fieldwork.

**Case Study Analysis**

Case studies serve as a vital research tool by offering rich, contextualized insights into how digital governance and Aadhaar-linked platforms function in real-life settings. In the context of Scheduled Caste welfare schemes in Karur District, Tamil Nadu, case studies provide a nuanced understanding of the lived experiences of beneficiaries interacting with these digital systems. The approach helps uncover the practical challenges, successes, and gaps in the delivery of welfare benefits through Aadhaar-enabled mechanisms. By focusing on individual narratives and community-level interactions, the analysis reveals how technological interventions shape accessibility, inclusion, and empowerment among marginalized SC populations, thus offering valuable perspectives that complement broader quantitative assessments.

**Case Study-1**

A male namely Ramu, age 45, from Tharagampatti, faced denial of subsidized ration for three months due to biometric mismatch in his Aadhaar. Being digitally illiterate, he had to travel to Karur to update his fingerprint data with help from his son. After updating, his ration benefits resumed. Ramu's experience reveals the challenges SC beneficiaries face with biometric failures, especially in rural areas lacking digital support. His story emphasizes the need for accessible local assistance to prevent digital exclusion from essential welfare schemes.

**Case Study-2**

A female namely Meena, age 31, from Orathur, transformed her life through a mobile-based voice-assisted ICT program introduced by an NGO. Despite illiteracy, she learned to use WhatsApp and YouTube for daily needs and community discussions. She organized a village cleanliness drive by mobilizing 30 women via voice messages. Meena's growing confidence led her to participate in village meetings and advocate for women's rights. Her case demonstrates how inclusive digital tools can empower illiterate SC women by connecting them to vital information and collective action.

**Case Study-3**

A male namely Senthil, age 38, from Chinnandipatti, was unaware that linking Aadhaar was necessary to receive MGNREGA wages. He worked weeks without pay until a field officer directed him to an e-Sevai center to link his Aadhaar and bank account. His wages were credited within ten days. Senthil's experience shows the risks digital illiteracy poses and highlights the value of local digital centers. The case highlights the importance of supporting SC workers in navigating Aadhaar-based wage payment systems.

**Case Study-4**

A female namely Kaveri, age 68, a widowed SC woman from Vellivadi, stopped receiving her pension after her Aadhaar details were not updated following her husband's death. With mobility challenges, she relied on her niece to visit the e-Sevai center, where records were corrected and payments resumed. Kaveri's experience reveals how

digital systems, though efficient, may unintentionally exclude elderly rural women when personal circumstances change, stressing the need for outreach and home-based Aadhaar updating for vulnerable SC elders.

**Case Study-5**

A male namely Rajendran, age 51, from Ponnampatti, lacked formal documents for his inherited farmland. Using the e-Sevai center, he linked Aadhaar to digital land records and obtained his patta, enabling access to agricultural subsidies and loans. Digitization granted him legal recognition as a landholder, previously denied due to bureaucratic hurdles. Rajendran's story highlights how Aadhaar-linked land digitization supports SC farmers in securing entitlements and economic independence through formal inclusion.

**Case Study-6**

A female namely Aarthi, age 22, a first-generation SC college student from Thanthoni, applied for a Post-Matric Scholarship via an e-Sevai center. Staff helped her upload Aadhaar-linked documents online, and within two months, the funds were credited. Aarthi's experience shows how digital governance can ease educational financial access for young SC women when user support is available for navigating online systems, helping reduce dropout risks.

**Case Study-7**

A male namely Munusamy, age 34, a daily-wage worker from Aravakurichi, applied for housing under PMAY but was rejected due to mismatched Aadhaar and bank account names. He made repeated trips to correct the error, missing wages. With NGO help, he resubmitted and succeeded. Munusamy's case illustrates how small technical errors in Aadhaar data can disproportionately affect low-income SC households and delay access to critical housing benefits.

**Case Study-8**

A female namely Dhanam, age 49, from Tharagampatti, enrolled in the Ujjwala Yojana but never received the subsidy because the LPG dealer registered the wrong mobile number. Being illiterate, she couldn't manage OTPs. Her son helped correct the details, and she now receives the subsidy. Dhanam's experience highlights how digital barriers and dependence affect rural SC women and the need for user-friendly systems and local support for low-literate beneficiaries.

**Case Study-9**

A male namely Periyasamy, age 63, from Moolakarai, linked Aadhaar to enroll in the Tamil Nadu health insurance scheme. He later accessed free treatment for heart disease at a government hospital. His children helped complete online formalities. Periyasamy's case demonstrates that well-implemented Aadhaar-linked health schemes can significantly improve the quality of life for vulnerable SC elders in remote areas.

**Case Study-10**

A female namely Kalaivani, age 52, from Chinnandipatti, was unaware that her widow status needed updating to continue receiving pension payments. After two years of missed payments, officials helped her re-enroll during an outreach camp. Kalaivani's experience shows how digital illiteracy among SC women, especially widows, can cause exclusion unless proactive state outreach and localized support are provided.

**Case Study-11**

A male namely Velmurugan, age 24, an unemployed youth from Thanthoni, lacked awareness about SC skill development schemes and did not own a smartphone. After attending a digital literacy camp, he registered for a tailoring course through the Aadhaar-linked employment portal, improving his skills and confidence. Velmurugan's story highlights how rural SC youth can benefit from digital welfare systems when proper guidance and outreach are in place.

**Case Study-12**

A female namely Poonkodi, age 40, from Vellivadi, repeatedly faced Aadhaar verification errors applying for her daughter's scholarship. After months of failure, she filed a grievance through the Tamil Nadu helpline. The issue was resolved in three weeks, and Poonkodi began helping other village women with similar problems. Her experience emphasizes how grievance redressal in digital governance can empower rural SC women as community change agents.

## DISCUSSION

The case studies highlight how digital governance and Aadhaar-linked platforms are reshaping welfare delivery for Scheduled Castes (SC) in Karur District, revealing both opportunities and challenges. While the system facilitates streamlined access to subsidies, pensions, scholarships, and land rights (Case Studies 5, 6, 9), it also exposes digital literacy gaps, especially among marginalized SC populations. Male beneficiaries like Ramu and Munusamy faced issues such as biometric mismatches and data errors that interrupted access to essential services (Case Studies 1, 7). Their experiences emphasize the critical need for robust local support to navigate complex digital bureaucracies, consistent with Gupta and Sharma's (2018) findings on digital exclusion risks in rural India. Importantly, gender emerged as a key factor influencing experiences with digital platforms. Female respondents such as Meena and Dhanam, despite illiteracy, benefited from voice-assisted ICT programs and family support to overcome digital barriers (Case Studies 2, 8). Their cases illustrate the transformative potential of gender-sensitive digital interventions, supporting Singh *et al*.'s (2021) assertion that tailored digital literacy programs empower rural women socially and economically. Elderly women like Kaveri and Kalaivani (Case Studies 4, 10) encountered unique challenges related to mobility and lack of awareness, underscoring the compounded vulnerabilities faced by SC women due to age, gender, and social status. The aligns with Narayan and Roy's (2017) advocacy for proactive outreach and accessible grievance redressal to ensure women's inclusion in digital governance. Youth experiences also indicate that digital literacy and access differ by gender. Velmurugan, a young male, gained employment skills through digital platforms after receiving guidance, whereas young SC women like Aarthi accessed educational scholarships with institutional help (Case Studies 6, 11). These narratives underscore the importance of combining technology with community-based support to close gender and caste divides. Overall, the cases reveal that while Aadhaar-linked digital governance holds promise for inclusive welfare delivery, its success depends on addressing gender-specific barriers through localized, user-friendly interventions.

## CONCLUSION

The study underscores the significant role of digital governance and Aadhaar-linked platforms in improving the delivery of welfare schemes to Scheduled Caste communities in Tamil Nadu. The integration of Aadhaar with various social protection programs has enhanced efficiency, transparency, and accessibility. On the other hand, the research also reveals persistent challenges, including digital illiteracy, biometric authentication failures, and gender-specific barriers that disproportionately affect marginalized SC beneficiaries, particularly women and the elderly. Addressing these challenges requires a multipronged approach involving localized digital support services, gender-sensitive interventions, and robust grievance redressal mechanisms. By bridging technological gaps with inclusive policies and community engagement, digital governance can become a powerful tool for social equity and empowerment. The study contributes valuable insights for policymakers aiming to design and implement welfare delivery systems that are both technologically advanced and socially inclusive, ultimately fostering greater access and participation among marginalized groups.

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RESEARCH ARTICLE

# Production Dynamics of Flue - Cured Virginia (FCV) Tobacco in India: Evidence from the Karnataka Light Soil Region and Other FCV Growing Regions

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## ABSTRACT

Flue-Cured Virginia (FCV) tobacco is a significant commercial crop grown in India, with Karnataka Light Soil (KLS) being one of the principals producing regions. Despite operating under a regulated production and marketing system, a regional difference exists within the regions. This study examines the production dynamics of FCV tobacco by comparing the KLS region with other FCV-growing regions of India using 15 years of secondary data. Descriptive statistics, independent sample t-tests, compound annual growth rate (CAGR) analysis, and both linear and log-linear regression models were used to analyse the regional disparities, growth trends, and indicators of marketed output. The results show that the KLS region records lower numbers of registered growers, smaller quantities marketed, and declining trends compared to other FCV-producing regions. Regression results indicate that area planted is the leading parameter of quantity marketed, with near unitary elasticity, while the number of registered growers employs a negative influence. Even after controlling for key variables, the regional dummy remains highly significant. The study concludes that scale efficiency and region-specific limitations play a decisive role in FCV tobacco production.

**Keywords:** FCV Tobacco; Karnataka Light Soil region; Quantity Marketed; Area Planted; Growers;

## INTRODUCTION

Flue-cured Virginia (FCV) tobacco plays a significant role in Karnataka, India's agricultural economy. It contributes directly and indirectly to rural livelihoods, export revenue, and state income. In India two major states dominate FCV tobacco production such as Andra Pradesh and Karnataka: Karnataka produces nearly one-third of India's total tobacco production, focused in the districts of Mysuru, Hassan, and Belagavi (Government of Karnataka, 2023). The high-quality leaf of the tobacco crop, highly demanded in international markets, converts it into an export commodity, generating approximately ₹3,000 crore annually as export earnings (Tobacco Board of India, 2023). This FCV tobacco directly or indirectly supports the livelihoods of millions of individuals (FAO, 2021). Despite its economic benefits, the tobacco crop faces many challenges (Central Tobacco Research Institute [CTRI], 2022). The Karnataka Light Soils (KLS) region holds a dominance in FCV tobacco cultivation; it is one of the leading FCV tobacco-growing zones for high-quality, export-quality leaf. This region has light-textured red sandy loam soils and semi-arid climates covering the Mysore, Hassan, and Chamarajanagar districts (CTRI, 2022). The well-drained soil conditions and moderate rainfall are helpful to the production of the quality of leaf demanded in global cigarette blends (Tobacco Board of India, 2023). This region contributes approximately 25-30% of the nation's FCV tobacco production. It has a significant portion of India's tobacco economy (Government of Karnataka, 2023). Continuous research interventions by CTRI—such as improved seed varieties and energy-efficient curing technologies—have enhanced productivity and reduced post-harvest losses in the region (CTRI, 2022). However, while the KLS region's contribution to India's FCV export competitiveness is undeniable, sustainability challenges like soil degradation, fluctuating global demand, and environmental implications of flue-curing call for adaptive cultivation practices and crop diversification strategies. Present existing studies on FCV tobacco have focused on profitability, alternative crop cultivation, and tobacco control policy issues; however, systematic region-specific econometric analyses examining the interaction between registered tobacco growers, area planted, and marketed quantity over time remain limited. To resolve this issue, the present study undertakes a regional analysis of FCV tobacco production dynamics in the KLS region with the following objectives:

1. To compare the key production indicators between the KLS region and FCV production regions of India.
2. To evaluate the long-term growth trends.
3. To identify the determinants of FCV tobacco marketed in quantity.

The following are hypotheses for the present study:

**H01:** There is no statistically significant difference in key FCV tobacco production parameters between the KLS region and other FCV tobacco-growing regions of India.

**H11:** There is a statistically significant difference in key FCV tobacco production parameters between the KLS region and other FCV tobacco-growing regions of India.

**H02:** There is no statistically significant difference in the CAGR of key FCV tobacco indicators between the KLS region and other FCV tobacco-growing regions of India.

**H12:** There is a statistically significant difference in the CAGR of key FCV tobacco indicators between the KLS region and other FCV tobacco-growing regions of India.

## LITERATURE REVIEW

**H.C. Yogesh and S.K. Srinvastava (2018):** This study focusing on Karnataka found that tobacco cultivation in the light soils region is predominantly rain-fed. FCV tobacco is the most economically significant type of tobacco in India. It increases export earnings and strengthens rural livelihoods. Andhra Pradesh and Karnataka, two states, nearly control the entire FCV tobacco production in India, supported by a regulated marketing system under the Tobacco Board. This study highlights that FCV tobacco is a high-value commercial crop with a guaranteed market,

Tobacco Board support, and a stable return compared to other alternative crops. Firewood availability is the major constraint for tobacco cultivation in these regions.

**Arunkumar R. Kulkarni *et al.* (2023):** This study highlights the importance of FCV tobacco farming in the agricultural economy of Karnataka, particularly in the Mysore region. This study focused on analyzing the tobacco value chain in Karnataka and identifying FCV tobacco growers as weaker actors compared to manufacturers, despite the safeguards under regulated markets like the Tobacco Board.

**K. Viswanatha Reddy *et al.* (2017)** reported on their study of Andhra Pradesh light soil regions, which relatively produce higher crops and realize better prices compared to other light soil regions because of supported soil conditions and stable production environments. Many of the policy-oriented research studies that investigate FCV tobacco crop size regulation in Andhra Pradesh reflect that changes in planting area directly influence marketed output and farmers' crop choices.

**Dr. Manu V. K. (2019)** the study conducted in the Karnataka light soil region focusing on the Mysore area highlights that tobacco growers of this region have an awareness of alternative crops, but none of the crops provide a better return compared to FCV tobacco. The existence of tobacco cultivation in this region is attributed to its cost-benefit ratio rather than agronomic advantage alone.

**Imtiaz Ali *et al.* (2014):** This study highlights that many of the studies discuss the growers, area, and output of tobacco descriptively; while productivity and profitability of the crop are discussed often, the relationship between farmer participation and scale of cultivation was under examined. International research studies identified that expansion in the number of tobacco growers without an increase in the tobacco cultivated area may lead to declining per capita output, though such types of interactions are rarely examined econometrically in the Indian FCV tobacco context.

**M. G. Mostafa *et al.* (2024)** found that many of the studies discussed tobacco farming within the context of socioeconomic and environmental aspects. Comparative studies from Bangladesh and South Asia claim that tobacco cultivation is economically attractive in the short period and increases environmental and health costs. These research studies focus on household income, emissions, and health risks rather than tobacco production efficiency or output dynamics.

**Natarajan, Nithya (2019)**. This study adopts a qualitative and historical lens to test agricultural change, labor relations, and environmental degradation. These studies provide background insights, but they do not provide region-specific production information using available secondary data.

A review of the existing research studies discloses several important gaps that permit further empirical investigation. Most of the research studies on FCV tobacco farming are based on descriptive statistics and surveys, with a noticeable absence of region-specific econometric analyses using log-linear models to measure the effects of area and grower participation across regions. Although the number of tobacco growers and cultivated area are frequently described, their combined studies have not been examined using empirical models. Despite the KLS region being widely accepted as structurally constrained, very few studies are undertaking systematic comparisons with other FCV-cultivated regions. Addressing these gaps, the present study undertakes a region-wise analysis of FCV tobacco farming by comparing the KLS region and other FCV tobacco-cultivated regions over a 15-year period. By adopting linear and log-linear regression models using official Tobacco Board data. This empirical evidence contributes to agricultural economics and offers policy-relevant insights into regional performance in FCV tobacco farming.

## MATERIALS AND METHODS

In India, FCV tobacco is majorly grown in Andhra Pradesh and Karnataka state. This study is based on secondary data collected from the Tobacco Board of India. The present research study used key variables for analysis, including registered tobacco growers (numbers), area planted (ha), quantity marketed (million Kgs), and a regional dummy variable was constructed. 15 years of data (2010-11 to 2024-25) from the KLS region and other regions were analyzed. For this analysis, descriptive statistics to summarize variables, followed by an independent sample t-test to test mean differences, and growth trends were analyzed by using compound annual growth rates (CAGR), estimated through log-linear growth models. For examining the marketed quantity, both linear and log-linear regression models were utilized. Diagnostic tests were conducted to evaluate model adequacy and multicollinearity.

## LIMITATIONS OF THE STUDY

1. The present study was based on secondary data for a limited time.
2. The study divided tobacco production areas into two regional groups—KLS and other regions of India.
3. The study cannot analyze other core production indicators such as price trends, input costs, yield per hectare, etc.
4. The study cannot attribute regional disparities to specific factors.

## RESULTS AND DISCUSSIONS

A series of independent samples t-tests were conducted to compare FCV tobacco production indicators between the KLS region and other FCV-producing regions of India. The variables studied included the number of registered growers in numbers, the total area planted in hectares, and the quantity marketed in million kgs. Descriptive statistics and inferential results are presented in Table 1.

**Registered growers:** the results revealed a significant difference in the number of registered growers between the KLS region and other regions of India, $t(21.66)=-7.29$, $p<.001$, reflecting that the KLS region had fewer registered growers ($M=41,723.13$, $SD=897.68$) compared to other regions of tobacco growing ($M=45,249.60$, $SD=1,644.34$). The mean difference of -3,526.47 indicates a disparity, with other regions having more registered tobacco producers. This difference reflects the smaller cultivation size of the KLS region and the greater concentration of smallholder farmers depending on FCV tobacco cultivation rather than scale expansion (Tobacco Board of India, 2023).

**Area Planted (ha): For** total area under FCV tobacco cultivation, the difference between the KLS region and other regions of India was not statistically significant, $t(20.97) = -1.53$, $p = .14$. While the KLS region reported a lower mean area planted ($M = 81,630.80$ ha, $SD = 13,959.38$) than other FCV-growing regions ($M = 93,681.87$ ha, $SD = 27,031.26$), the variability was higher in non-KLS regions. This suggests that KLS areas cultivated smaller land.

**Quantity Marketed (Million Kg): The** analysis shows a highly significant difference in the quantity of FCV tobacco marketed between the KLS region and other regions, $t(17.52) = -5.75$, $p < .001$. The KLS region marketed less tobacco ($M = 91.89$ mkg, $SD = 15.88$) compared to other regions ($M = 162.02$ mkg, $SD = 44.46$), with a mean difference of -70.13 mkg. This indicates that while KLS tobacco is concentrated on the quality of the crop, its marketed volume remains considerably lower. The lesser quantity of tobacco marketed shows the KLS region's focus on premium-grade and export-quality tobacco leaf rather than high production (Central Tobacco Research Institute [CTRI], 2022).

**Growth trends of FCV tobacco indicators**

An analysis of the CAGR of FCV tobacco indicators was conducted to assess regional performance trends over time between the KLS region and other FCV growing areas. The CAGR results, expressed as percentage change per year, are shown in Table 2.

**Registered Growers:** The number of registered growers showed a significant declining trend in both the KLS and non-KLS regions, though the rate of decline was less marked in the KLS region (-0.30% per year, p<.05) compared to the non-KLS regions (-0.80% per year, p<.01). This suggests that the KLS region has retained a more stable producing population. This resilience may be credited to the region's institutional infrastructure and high-quality tobacco leaf grade (Tobacco Board of India, 2023).

**Quantity Marketed:** The quantity of FCV tobacco marketed showed a significant negative growth in the KLS region (-2.37% per year, p < .05), whereas the decline in other tobacco-producing regions (-0.20% per year) was statistically not significant. This displayed that production and marketable surplus have contracted sharply in the KLS region; the non-significant change in other regions implies relative stability in production volumes.

**Area planted (ha):** The area planted under FCV tobacco indicated a significant decline in both regions—2.4% per year in the KLS region (p<.01) and 2.5% per year in other regions (p<.10)—reflecting an overall declining trend in land used for FCV tobacco cultivation across India.

The CAGR results show a determined decline in FCV tobacco cultivation in both the KLS region and other regions. The decline in registered growers and quantity marketed is noticeable in the KLS region and shows constraints such as rain-fed conditions, labor scarcity, etc. These trends support earlier descriptive and inferential findings of the study. The linear regression model explains a proportion of the variation in marketed quantity. Area planted is indicated as the strongest positive determinant, while the number of registered growers shows a significant negative effect. The regional dummy variable remains negative and highly significant, showing lower marketed output in the KLS region even after controlling for key variables. The log-linear regression analysis reflects near unitary elasticity of quantity marketed with respect to area planted, the results highlighting area of cultivation as the major driver of output. The negative elasticity of registered growers suggests that increased grower participation without expansion in area leads to scale inefficiencies. The regional dummy shows that the KLS region markets approximately 47.5% less output than other regions, showing deep-seated structural constraints. Across descriptive, inferential, growth, and regression analysis, the outcomes consistently validate that the KLS region is structurally disadvantaged in FCV tobacco production; area expansion, rather than grower expansion, is critical for increasing marketed surplus; and declining trends in growers, area, and output show long-term decline of FCV tobacco farming.

## CONCLUSION AND POLICY IMPLICATION

This study provides empirical evidence on regional inequalities in FCV tobacco production across the regions. The findings reveal that the KLS region is structurally disadvantaged in terms of quantity marketed, despite operating under the same regulatory framework as other tobacco-producing regions. Scale efficiency, rather than cultivator expansion, plays a pivotal role in assessing the marketed surplus. The results of the study suggest that policy interventions prioritize scale efficiency and productivity enhancement rather than expanding producers' numbers. Region-specific support measures are required for the KLS region. A well-planned and gradual decline in the area used for FCV tobacco, supported by training and advisory services, is important for maintaining livelihoods. Any move toward crop diversification should be based on reliable data and consider local conditions.

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**Table No. 1: Independent samples t-test results comparing the KLS region and other FCV-produced regions of India.**

| Variable | Region | n | Mean | SD | t(df) | p | Mean Difference |
|---|---|---|---|---|---|---|---|
| Registered growers | KLS | 15 | 41,723.13 | 897.68 | −7.29 (21.66) | < .001 | −3,526.47 |
| | Other | 15 | 45,249.60 | 1,644.34 | | | |
| Area planted (ha) | KLS | 15 | 81,630.80 | 13,959.38 | −1.53 (20.97) | 0.14 | −12,051.07 |
| | Other | 15 | 93,681.87 | 27,031.26 | | | |
| Quantity marketed (million kg) | KLS | 15 | 91.89 | 15.88 | −5.75 (17.52) | < .001 | −70.13 |
| | Other | 15 | 162.02 | 44.46 | | | |

**Note.**SPSS output

**KLS =** Karnataka Light Soil region. Other = Other FCV-producing regions. Welch's t-test results are reported where Levene's test indicated unequal variances. The mean difference is calculated as KLS − Other region.

**Table No. 2**: **Compound Annual Growth Rates of FCV Tobacco Indicators (% per Year)**

| Variable | Region | CAGR(%) | Significance |
|---|---|---|---|
| Registered growers | KLS | -0.30 | <.05 |
| | Other | -0.80 | <.01 |
| Quantity marketed | KLS | -2.37 | <.05 |
| | Other | -0.20 | Not significant |
| Area planted (ha) | KLS | -2.4 | <.01 |
| | Other | -2.5 | <.10 |

**Note.**SPSS output

**Table No. 3**: **Determinants of marketed quantity: Linear Model**

| **Predictor** | **B** | **SE B** | **β** | **t** | **P** |
|---|---|---|---|---|---|
| Constant | 508.29 | 113.07 | _ | 4.50 | <.001 |
| Region(KLS =1) | -89.47 | 10.29 | -.94 | -8.70 | <.001 |
| Registered growers | -0.011 | 0.003 | -.50 | -4.13 | <.001 |
| Area planted( ha) | 0.002 | 0.000 | .73 | 9.83 | <.001 |

**Model fit:** $R^2$=.904, Adjusted $R^2$=.893, F(3,26)=81.61, p<.001.
**Note.**SPSS output

**Table No. 4**: Elasticity of FCV Tobacco Marketed Quantity – Log-Linear Model

| Predictor | B | SE B | β | t | P |
|---|---|---|---|---|---|
| In (area planted) | 1.013 | 0.109 | .662 | 9.27 | <.001 |
| In (Registered growers) | -2.638 | 0.850 | -.368 | -3.10 | .005 |
| Region (KLS=1) | -0.645 | 0.076 | -.907 | -8.49 | <.001 |

**Model fit:** $R^2$=.908, adjusted $R^2$=.898, F(3,26)=85.76, p<.001
**Note.**SPSS output

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RESEARCH ARTICLE

# Integrating UPI Payments in the Chalo App : An Empirical Study of User - Requested Ticketing Enhancements

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## ABSTRACT

Mobile ticketing and live tracking are essential to the Indian commuters navigating daily through public transport systems. Real time visibility and cashless travel are the core promises of Chalo app. The reviews posted during 2025 reveal that the experience is not perfect. Many commuters remain troubled with payment failures, ticket mismatches and unpredictable tracking behaviour. This study investigates Google play store reviews written in the year 2025 and concentrating particularly on two connected issues,viz., on the reliability of mobile ticketing system in actual journeys and on how well the app integrates with unified payments interface (UPI). Using a mixed methods approach, we investigated a random sample of 1000 reviews out of two lakh reviews posted by the commuters in the year 2025. These reviews are extracted from the Google play store randomly. Each review is then categorised based on recurring themes such as tracking accuracy, ticketing failures, UPI functionality, payment errors, interface concerns and overall sentiment. Nearly,60 % of the reviews mentioned are on recurring tracking or on visibility issues, while about 15% described are on clear ticketing failures where money was debited but no valid, usable digital ticket appeared on the screen. A smaller yet substantial group of users demanded broader UPI support or pointed out that only a single UPI app seemed to function reliably. Overall, the findings show that by 2025, commuters expected Chalo to offer smooth, hassle-free multi-provider UPI integration and a verifiable ticketing system. Instead, they often encountered unrecognised tickets, double charges, refund delays and confusing transaction messages. These problems and the weakened trust, with several users advising others not to use the payment feature at all. While a small group of reviewers praised the convenience of digital passes when the system worked smoothly. These problems

and the weakened trust, with several users advising others not to use the payment feature at all. While a small group of reviewers praised the convenience of digital passes when the system worked smoothly. The study underscores that deeper UPI integration, transparent refund workflows and better synchronisation with conductor devices are essential for rebuilding user confidence.

**Keywords:** Chalo, payments, system, digital, UPI

## INTRODUCTION

During the last decade, the public transport system has seen tremendous progress. The urban bus travel in many Indian cities has improved user experiences such as paperless tickets, guesswork on bus expected arrival time, departure time, and waiting time through digital tools as well asthrough real time information systems, especially in Class-I and Class-II cities. Among the major tools contributing to this change is the Chalo – Live bus tracking app, developed by Chalo Mobility Private Limited and this is available for Android and iOS platforms. The app covers nearly 65 Class-I and Class-II cities in India as well as available internationally at Philippines, Singapore, etc. Some of the major features of this free app includes live bus tracking, digital tickets, digital passes and special fare plans inside a single app, live tracking of bus, mobile bus pass, etc. It frames itself as one such everyday companion. Transport applications in smartphone are intended to minimise uncertainty, shorten waiting time and simplify payments [1]. Concurrently, the wider Indian payments ecosystems have been converted by the Unified payments interface (UPI). For many commuters, UPI has become the easiest go-to method to pay for almost everything, from street tea to electricity bills[2]. They do not ask whether UPI is supported or not, while they open a public transport app in 2025. They assume it is integrated smoothly into the experience. Frustration is almost guaranteed, if the assumption is broken, or if UPI works only in a restricted way.

Live tracking and digital passes made daily bus travel easier for some users. Others describe mornings are ruined by buses that vanish from the map, tickets that conductors refuse to accept and money that disappears into the system with no clear refund process [3]. In several cases, users explicitly warm fellow commuters not to trust the payment function. The above contrasting experiences are clearly captured by the 2025 Google Play reviews for Chalo. This paper presents an analysis of the gap between promise and practice. Instead of starting from theory, we start from what users actually wrote. We consider the 2025 reviews as an empirical dataset and raises three simple questions:

- How did users in 2025 talk about UPI and payment integration in the Chalo app
- What kinds of problems did they report with mobile tickets and passes
- How did those problems affect their trust and behaviour

Our goal is to support two distinct audiences, who will draw meaningful conclusions from the issues raised in these above questions. This paper will deliver the following contributions. Altogether, the 2025 reviews offer a clear, transparent picture of how commuters actually experience UPI payments and mobile ticketing on the Chalo app. They illustrate where expectations are met, where they fail and why these gaps matter for everyday travel. This study expectations are met, where they fail and why these gaps matter for everyday travel.

**Research Objectives**

The core objective of this paper is to understand how actual users of the Chalo app, during 2025, experienced and evaluated two fundamental, core defining components of the service:

Building on this aim, the enquiry proceeded through four core objectives:

1. To determine the frequency with users in 2025 UPI, digital payments, and wallet issues in their reviews.
2. To identify the range of ticketing failures that users reported and how those failures affected their behaviour and trust.

3. To chart dominant sentiment patterns in 2025 user reviews and see how payment and ticketing problems fit into that sentiment profile.
4. To develop practical, evidence-informed recommendations for Chalo's developers and partner agencies, based directly on patterns in user feedback.

These objectives are grounded in empirical observation and analysis. We have listened carefully to what users already utter and to organise it in a systematic way.

## METHODOLOGY

**Data Source and Time Window**

The data, taken for this study come entirely from the public user reviews of the Chalo app existing on the Google Play store. At the time of data collection, the Chalo listing reported more than ten million downloads and more than two lakh complete set of reviews. To focus exclusively on the current user experience, we limited the dataset to reviews clearly and explicitly posted during the year 2025. We extracted a random subset of 1000 English-Language reviews posted in 2025 for that we adopted a random sampling strategy to obtain a manageable yet representative dataset from the given large size of the review population. (Total reviews= 2 lakh→ 2025 reviews→English-only reviews→full manual scan random interval selectionfinal sample:1000 reviews) All 1000 reviews were kept for analysis without any further skewed, narrowing, or subjective selection. This approach guaranteed that the dataset presented genuine signs, indications of user diversity, including both short and long reviews (but not extremely short or extremely long), positive and negative feedback and an extensive range of commuter experiences. Each review was handled as a textual artefact for qualitative coding, and the dataset is signified to as the "2025 Chalo Google Play review sample" throughout the paper. No personal data were retrieved or accessed beyond the public display names already obvious on the Play Store.

**Coding and Thematic Categories**

We adopted a qualitative, contextual data analysis approach [4]. Two rounds of reading of the full dataset were carried out. We performed open coding in the first reading. We marked the phrases related to:

Based on this open coding, we enhanced a set of thematic categories, and each review could belong to more than one category. The final categories were:

**Live tracking and timing issues**- buses were not appearing on the map, wrong arrival times, bus icons freezing or vanishing [3].
**Ticketing and payment failures**- payment deducted but no ticket received, ticket not recognised by the conductor's device, amount of the ticket deducted twice. Expired tickets with no recourse.
**UPI and payment integration**- references to UPI, particular UPI apps like Google pay, desire for more payment options, wallet restore problems.
**Feature suggestions and usability**-requests to add shortcuts, reinstate removed options, advances interface flow.
**Positive overall experience**- Even if minor issues are mentioned, clear statements that the app is useful or convenient.
Each review was manually coded, assigned to all relevant applicable categories. If the review is simply stated something like 'there are issues with ticketing' without any mention of money loss or conductor-side error, it was not counted as a clear ticket failure. Such ambiguous statements were coded conventionally.

**Quantitative Summaries**

We accumulated simple frequency counts for each category after coding, these counts provide a reasonable picture of how normal each theme was within the 2025 sample, without implying statistical precision.

Drawing on common sentiment analysis practices, we also assigned a sentimental label to each review. We used three labels:

- Negative- mostly complaints and strong dissatisfaction.
- Mixed or Neutral- a balance of praise and criticism, or a relatively neutral tone.
- Positive- clear praise, with problems either absent or minor.

The assignment was grounded on explicit, precise emotional language and the overall direction of the narrative.

## FINDINGS

**Overview of Themes**

The table below provides a high-level summary of how repeatedly each theme appeared in the 2025 reviews. These numbers overlap, since reviews can belong to more than one theme. The dominant problems faced are live tracking issues, and ticket failures form a significant minority of complaints. UPI is not mentioned in every review, but when it does appear, it emerges as a primary issue in the users' experience.

**Sentiment Profile**

User sentiment toward the Chalo App in 2025 shows a noticeably polarized pattern. Driven mainly by frustration, disappointment with live tracking failures, unreliable timing missing buses on the map and frequent app crashes , a clear majority of reviews lean negative but at the same time, a smaller yet meaningful segment of reviewers report positive or somewhat positive experiences, emphasising convenience and time-saving features when the app functions properly. The sentiment distribution signals that around 70% of 2025 reviews reflect mixed or neutral evaluation and about 10 % clearly express positive feedback across the dataset. This overall pattern is coherent and consistent with commuter frustration during significant commute delays. Through the users repeatedly mentioning words such as 'worst' 'frustrating' and 'useless', this emotional tone across many reviews is remarkably strong and keep recurring. Ground this analysis, the following ten excerpts illustrate the range of user sentiment, taken exactly from the reviews found in the 2025 dataset. The strength and the intensity of negative responses, combined with pockets of neutral and positive feedback, provides a nuanced but consistent account of how reliability gaps persuaded public perception. Collectively, the ten excerpts highlight that user sentiment in 2025 was modelled by direct, everyday experiences.

**UPI Integration: Limited Support and Growing Expectations**

Only a small number of reviews explicitly, directly mention UPI or Individual UPI apps, but even these few entries clearly express what commuters expected in 2005.

One user write:

Here, the user's insistence on adding "three or four payment apps" suggests that broader support for common UPI platforms such as PhonePe, Paytm and other bank-based UPI apps is now seen as standard. It is treated as a basic necessity. Another reviewer underlines the need for clearer refund handling:

This comment highlights a second expectation that the users want transparent, visible, reliable refund and transaction status when failures occur mere accepting UPI is not enough. Good UPI performances can soften frustrations with tracking, but failures on both fronts quickly erode trust in the append when read collectively, there reviews confirms that commuters view UPI integration and refund transparency as essential to their overall experience.

**Ticketing Failures: Lost money and Lost Trust**

Incidents involving real money and stressful interactions during bus journeys appear in almost fifteen reviews in the 2025 dataset, which portray explicit ticketing and payment failures. These are not trivial or minor glitches; many of these users did everything correctly on their end, yet the system failed at crucial moments.

The following experiences was clearly noted by one reviewer:
This case shows a breakdown at the interface between the app and the conductor's validation system. Without a reliable, steadfast way to verify digital tickets, users can be wrongly penalised even after making legitimate payments. Another reviewer describes repeated failures with no compensation: Here, a vague, ambiguous error messages and absent refunds create a cycle of financial loss and frustration. The repeated emphasis on "no refund" demonstrates how strongly commuters feel the impact, ramification of these failures. Even occasional ticket failures have immediate monetary consequences, damage user confidence and push commuters away from digital payments altogether, these two examples explain the most serious concern found in the 2025 dataset.

**Live tracking and Reliability**
Around sixty reviews remark first the buses not appearing in the map, bus icon freezing, wrong arrival times or buses showing only after they have passed the stop. Although this paper gives special regard to payments and ticketing, live tracking issues from the background to almost every story.
Typical lines excerpt includes:

- "Live bus tracking is unreliable. Some buses never appear on the map or disappear mid route."
- "Bus is not visible many times, and it shows up after passing the station."
- Bus timings are not even approximate. The scheduled timings can never be trusted."

For many users, repeated tracking problems and one or two ticketing failures combine into a single judgement: the system as a whole is not dependable. This is what leads people to label the app as "useless" and advice others to stick to cash or alternative modes.

**Constructive suggestions from Users**
Along with objections and criticism, the reviews also contain several constructive ideas. For example:

- One user suggests that allowing passenger who have valid tickets to contribute GPS data when official trackers fail, with checks based on the scanned tickets. This is a form of crowdsourced tracking that could fill gaps on certain routes.
- Another commuter asks Chalo to restore feature that let the commuter check next day's buses, which was useful for planning early morning commutes.
- Several request shortcuts for frequently used routes on the home screen, so that daily commuters do not have to search every time.
- A few requests clear option for half ticket or child ticket options inside the app.

The commuters are not only criticising but also imagining how the system could work better and these suggestions indicate that users are invested in the app's success.

## DISCUSSION

The 2025 evaluations clearly show that UPI shifted from being a convenience to a basic expectation for most commuters. When the Chalo app supports only one UPI provider or fails to handle certain card-based UPI flows, user perceive this as a noticeable gap in the service for daily smart phone users. UPI is the default method for routine payments, so limitations of this kind feel especially inconvenient. Although only a small share of the dataset explicitly mentions UPI, those comments carry weight, because most of the people complain only when something breaks. Their feedback points toward three reliable, consistent expectations (1) the app should support multiple UPI options, (2) payments should work smoothly across permitted flows and (3) refunds for failed transactions should be fast and transparent. Users may avoid in-app payments altogether and return to cash when these expectations are not met.

At the same time, the reviews show that ticketing failures are even more detrimental than tracking errors. At times users may accept delays or inaccurate timing updates, but they react strongly, affects them deeply when money is deducted without receiving a usable ticket, or when conductors cannot confirm the digital proof. These situations

generate stress throughout travel and lead several reviewers to advise other passengers not to trust the payment feature. Strengthening the technical foundations of mobile ticketing such as including more stable synchronisation between the app and conductor machines, better handling of connectivity gaps and smarter detection of duplicate or incomplete transactions will be essential for rebuilding trust. Clear, commuter-friendly refund mechanisms are also essential.

Another concern raised in the reviews relates to security features such as blocking screenshots or restricting ticket visibility. Although these measures are meant to prevent misuse, they can also create serious barriers when genuine failures occur. Honest, affected users are unable to show proof of purchase, and support teams struggle to check what the user paid for. Even small adjustments in this area could significantly reduce user frustration. A more balanced design would keep security in place while providing backup verification methods, such as temporary secure codes or alternate ways to confirm ticket status. Across the dataset, it is also evident that user reviews are a valuable and continuous form of feedback. By investigating patterns in what people write, tracking how often certain complaints appear and observing how feedback changes after updates, Chalo can formulate targeted improvements. The evidence from 2025 suggests a simple order of priorities: prevent financial loss first, stabilise tracking second, strengthen UPI integration next and then focus on boundary refinements and new features. Addressing these primary, core issues would help restore commuter confidence and improve the day-to-day reliability of the app.

## CONCLUSION

By reading and coding roughly one thousand reviews, we identified the most common themes and most painful failures scenarios. This paper portrayed an empirical study of 2025 user reviews of the Chalo app, with a focus on UPI integration and ticketing reliability. The scrutiny showed that live tracking problems were the single most frequent complaint, affecting about sixty percent of the sample. Around fifteen percent of reviews labelled explicit ticketing failures in which money was debited but no usable ticket was provided or recognised. A smaller group of reviews directly requested broader UPI support and clearer refund handling. These patterns disclose a gap between user expectations and the app's current behaviour. Commuters expect public transport apps to accommodate the UPI ecosystem they already use, to issue tickets that conductor can easily verify and to protect them from financial loss that led economic loss when systems misbehave. Trust collapses and users actively discourage others from using the payment features when these expectations are not met.

For Chalo and similar apps, the path forward is demanding, challenging but clear. Greater investment in payment and ticketing reliability, thoughtful design of security measures and generous support policies are necessary to rebuild trust and at the same time, user reviews can serve as a continuous steady mirror, showing where improvements are working and where gaps remain. For researchers and practitioners in transport technology, this finding illustrate the value of taking user feedback seriously. The people who ride buses everyday understand very well where a digital system helps and where it hurts. Their reviews, when read carefully and systematically, provide a grounded guide for designing better, fairer and more reliable public transport technology.

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**Table 1: Proposed Audiences and Contributions**

| Audience | What this paper delivers |
|---|---|
| Chalo and similar app developers | Provides evidence-based feedback on points of failure in UPI and mobile ticketing, along with practical pathways for enhancement based on real user complaint. |
| Researchers in transportation and human-computer interaction | Offers a real-world case study of how digital payment systems model everyday public transport experiences in an Indian urban context. |

**Table 2. Research Objectives**

- UPI and digital payment integration
- The reliability of mobile tickets and passes in real bus journeys

**Table 3. Coding and Thematic Categories**

| |
|---|
| Live tracking and the visibility of the bus. |
| Ticket purchase, ticket validation and interaction of conductor's device. |
| UPI, Google Pay, RUPay and other payment words. |
| Suggestions for new attributes and usability changes. |
| Overall judgement of the app as good, bad, better or mixed. |

**Table 4. Major themes in 2025 Chalo app reviews (N =100)**

| Theme | Approximate number of the review corpus | Proportion of the sample (approx.) |
|---|---|---|
| Live tracking and timing problems | 60 | 60% |
| Ticketing and payment failures | 15 | 15% |
| UPI and payment integration requests | 5 | 5% |
| Feature suggestions and usability | 10 | 10% |
| Positive overall experience | 10 | 10% |

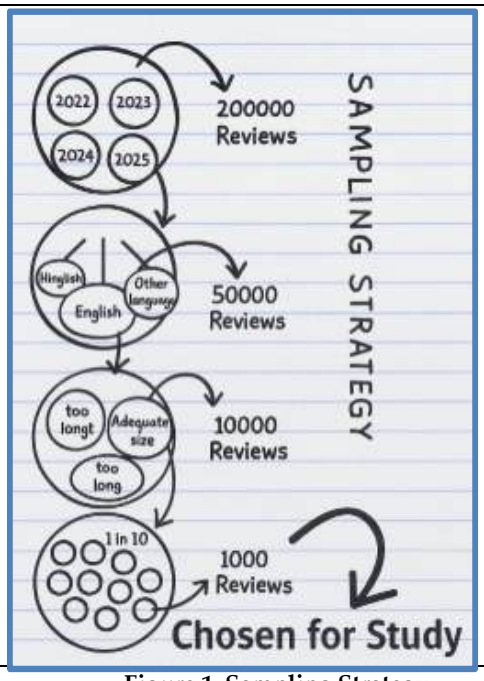



**Figure 1: Sampling Strategy**

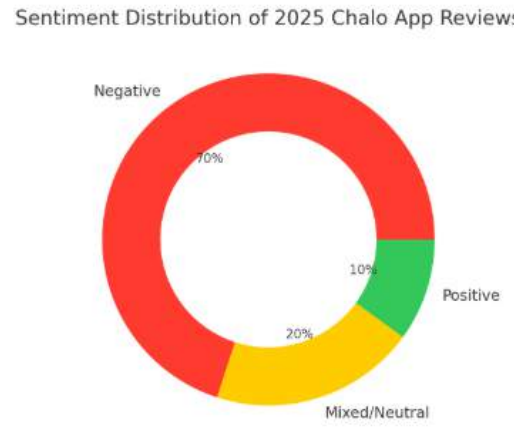



**Figure 2: Sentiment Distribution of 2025**

**Alagappan S (June 30, 2025)**

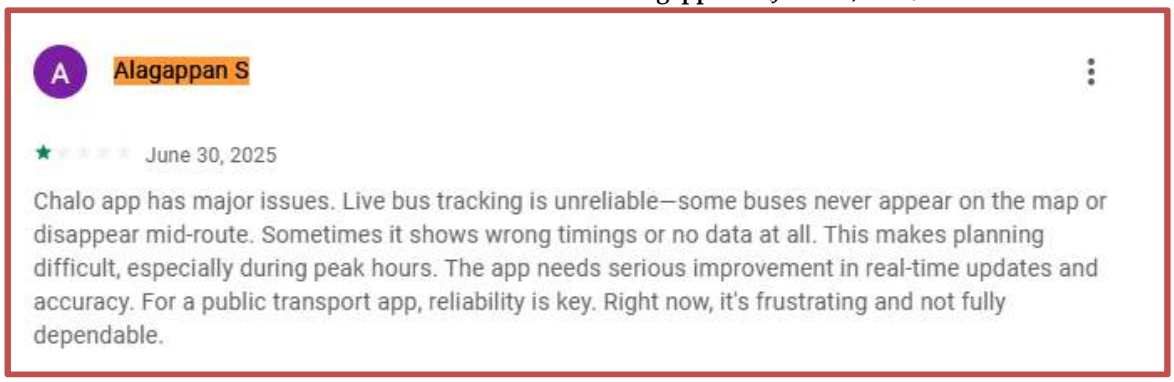



**Figure 2: Review- Alagappan S**

**Shubham Rane (September 28, 2025)**

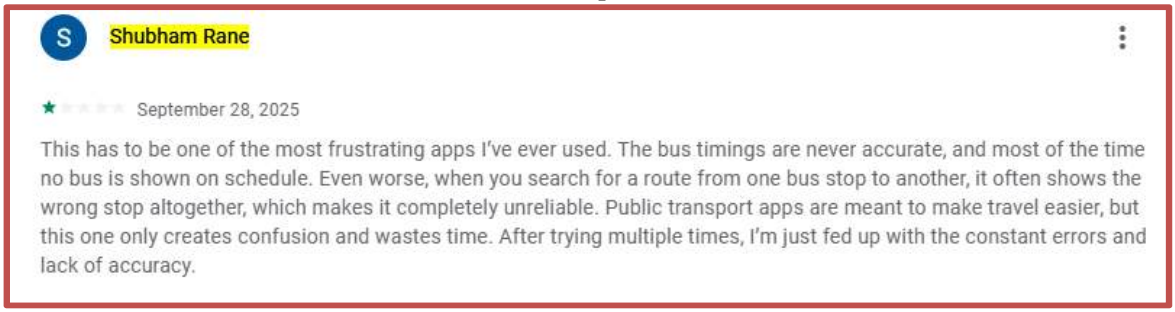



**Figure 3: Review- Shubham Rane**

**Anas Khan (October 21, 2025)**

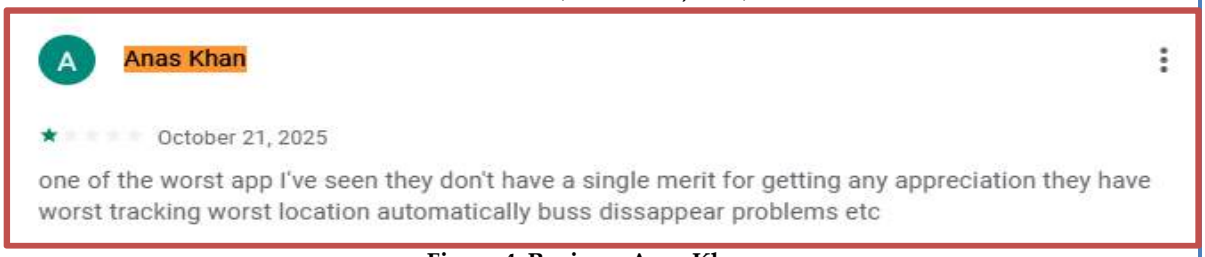



**Figure 4: Review- Anas Khan**

**Nitya (June 30, 2025)**

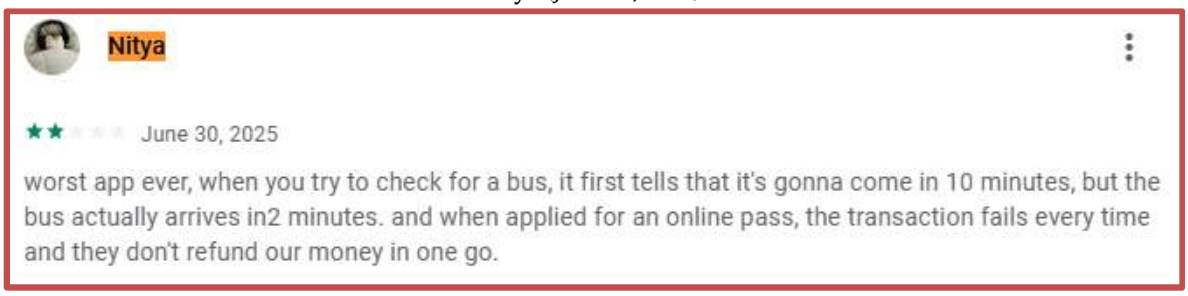



**Figure 5: Review- Nitya**

**Sujeet Kumbhar (September 25, 2025)**

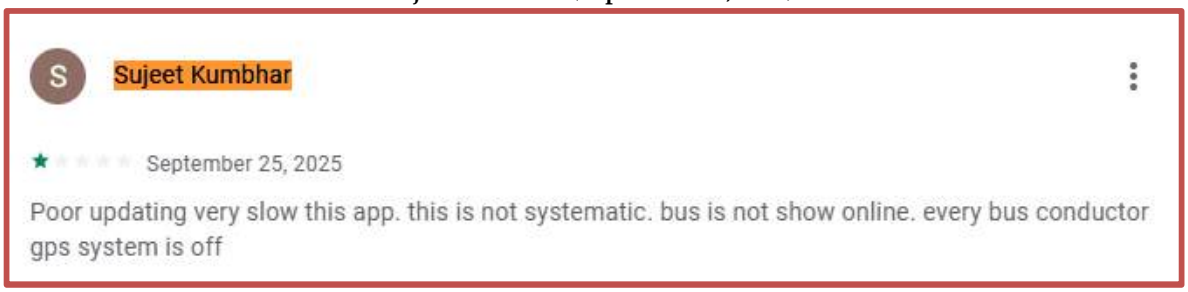



**Figure 6: Review- Sujeet Kumbhar**

**Dhrubajyoti Sarma (September 17, 2025)**

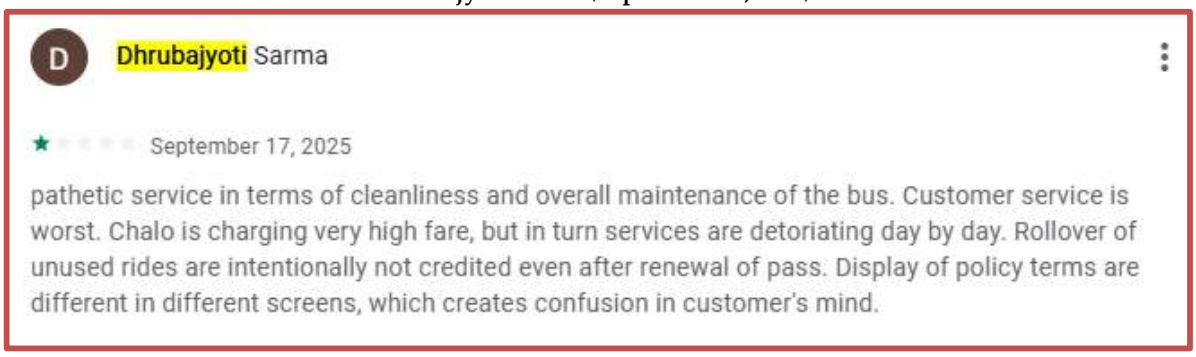



**Figure 7: Review- Dhrubajyoti Sarma**

**AMALNATH CP (August 19, 2025)**

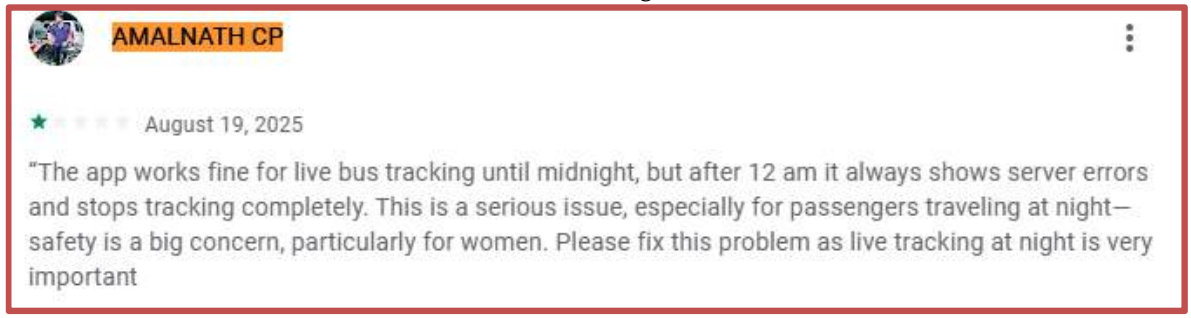



**Figure 8: Review- Amalnath Cp**

**Aditya H (August 26, 2025)**

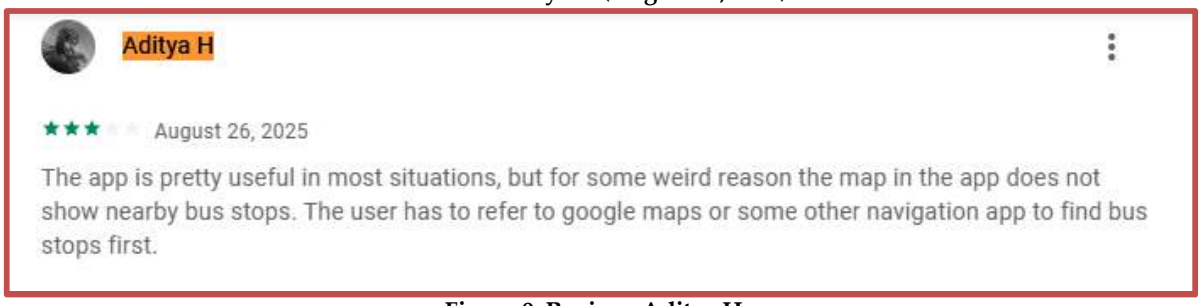



**Figure 9: Review- Aditya H**

**Janvi Chavan (September 27, 2025)**




**Figure 10: Review- Janvi Chavan**

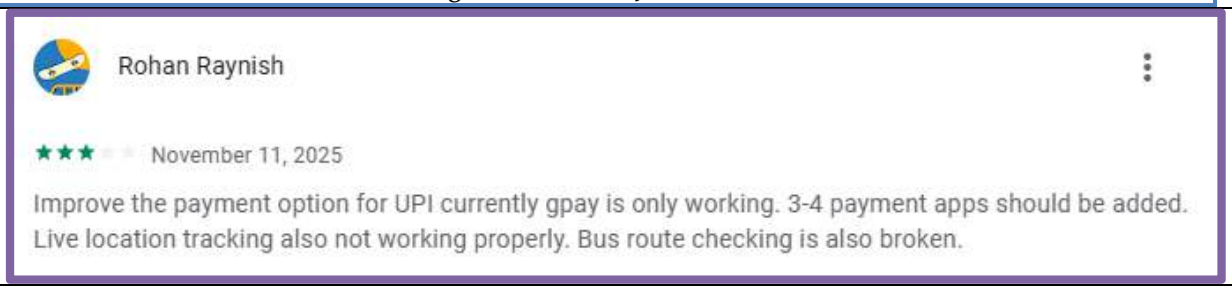



**Figure 11: Review- RohanRaynish**

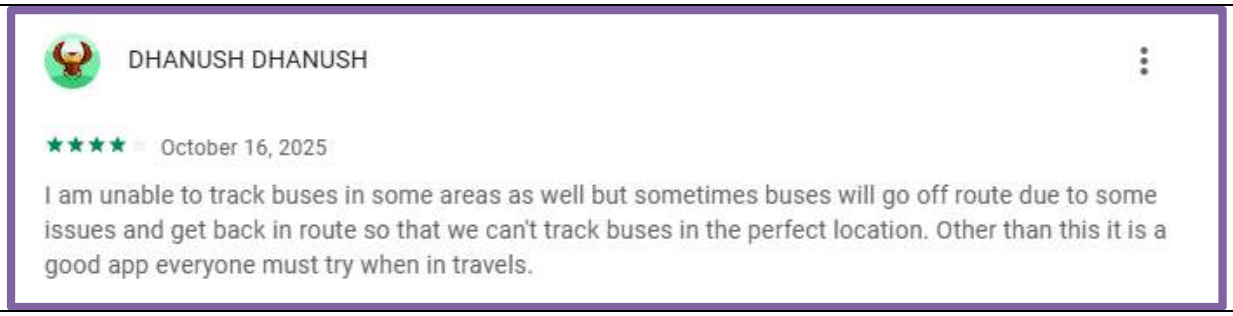



**Figure 12: Review- DhanushDhanush**

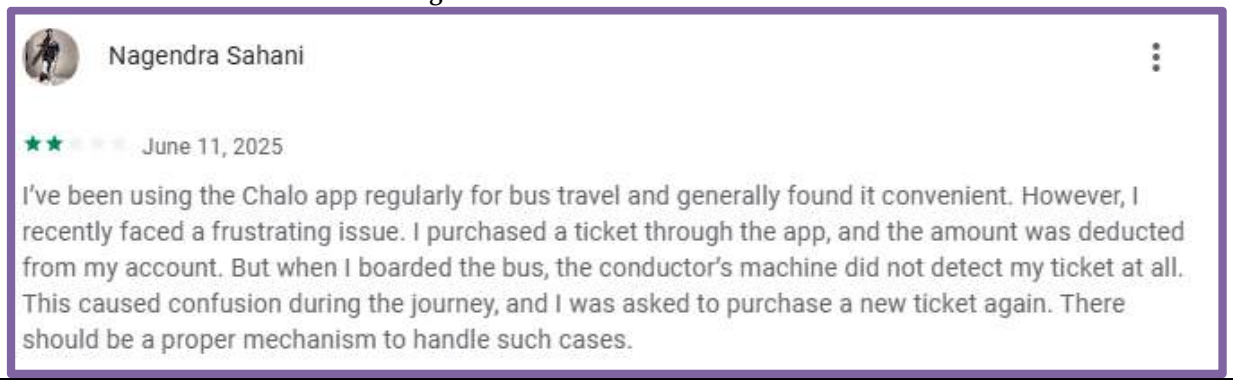



**Figure 13: Review- Nagendra Sahani**

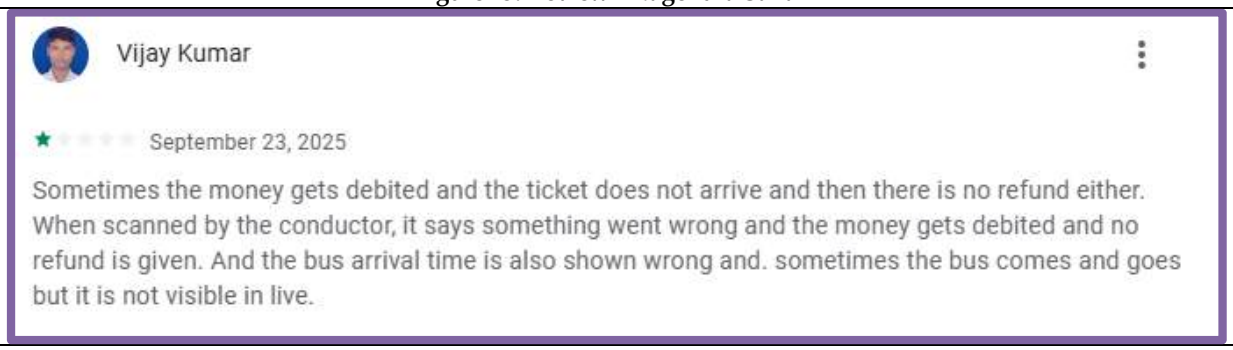



**Figure 14: Review- Vijay Kumar**

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RESEARCH ARTICLE

# Digital Inclusion and Women's Empowerment in Tamilnadu: Opportunities and Challenges in the Age of Technology

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**ABSTRACT**

The present study critically explores the role of digital platforms in empowering women across Tamil Nadu, focusing on dimensions such as health, education, livelihood, and civic participation. Drawing on qualitative data from focus group discussions, and case studies, the research examines the socio-cultural, infrastructural, economic, and safety-related barriers that hinder digital inclusion among women, especially in rural districts. Findings reveal significant urban-rural disparities in digital access, with only 24.6% of rural women reporting internet use, compared to 51.8% in urban areas. Although government initiatives like Amma e-Sevai Centres, PMGDISHA, and Mahila E-Haat aim to bridge this gap, their impact remains uneven due to limited digital literacy, affordability issues, and socio-cultural restrictions. The study reveals that mere access to digital devices does not guarantee empowerment; meaningful usage and digital fluency are crucial. It recommends targeted policy interventions, including community-based digital training, subsidized device access, and gender-sensitive cybersecurity frameworks.

**Keywords:** Digital inclusion, women's empowerment, Tamil Nadu, digital literacy, e-governance, socio-cultural barriers, ICT access, policy interventions

## INTRODUCTION

Technology has empowered women to make choices and shape their own lives in a move toward gender equality (Grabe, 2011). In many high-income countries, women are more self-reliant than their counterparts in rural and low-

income countries with a lack of access to resources and cultural gender barriers (Dhanamalar *et al*., 2020). Technology now enables access to learning resources and provides a way to circumvent gender barriers (Rajahonka and Kaijam, 2019). Scholars postulate that technology is the driver of women's empowerment through those learning materials (Bhat, 2019). While there appear to be studies showing how women can be empowered with technology, it is unclear to what extent women were empowered across geographical or industrial areas, what types of technology were used, and the benefits they received. Therefore, the purpose of this study is to conduct a systematic research to gain a collective view of how technology is being used to empower women.

## LITERATURE REVIEW

Women's empowerment is widely recognized as a fundamental catalyst for societal advancement, as it enables women to exercise autonomy, participate fully in public and private spheres, and develop critical capacities for self-determination (Dhanamalar *et al*., 2020). The academic conceptualization of women's empowerment first gained prominence in the 1970s, amidst a broader movement toward gender equality (Freire, 1970; Grabe, 2011). Over time, the concept has evolved, increasingly positioned as both a means and an end in the pursuit of gender equity (Mackey and Petrucka, 2021). Contemporary scholarly discourse often frames empowerment as the ability of women to exert control over their lives, including access to choices and decision-making power (Chew *et al*., 2015; Lindsay *et al*., 2013; Vivakaran and Maraimalai, 2017). In this study, women's empowerment is defined through the lens of agency, access to resources, and the capability to make informed decisions on matters of significance (Kabeer, 1999; Mosedale, 2005). This process is increasingly mediated by digital technology, which facilitates access to information, enables learning, and fosters greater autonomy. Technological access, particularly in the form of mobile devices and internet connectivity, plays a fundamental role in equipping women with tools to surmount social, economic, and political barriers (Cummings and O'Neil, 2015). Challenges to women's empowerment remain pronounced in low-income settings, where structural inequalities—such as limited access to education, healthcare, and financial services—continue to hinder full participation in the formal labor market. These constraints are often reinforced by prevailing sociocultural norms and religious practices (Lechman and Paradowski, 2021). Nevertheless, data from the World Development Indicators (International Telecommunications Union, 2020) suggest a modest but steady improvement in female labor-force participation globally. Between 1990 and 2019, participation among women aged 15 and above rose by 1.4, 10.5, and 2.8 percentage points across various regions, reflecting incremental shifts toward greater gender inclusivity in economic activity. While numerous systematic reviews have examined the application of technology across diverse fields, there remains a discernible gap in the literature concerning women's access to learning opportunities through digital platforms—and more specifically, the role of technology in enabling women's empowerment beyond the health sector. Existing reviews predominantly focus on health-related interventions. For instance, Dantas *et al*. (2021) investigated mobile health applications designed to help women manage urinary incontinence, concluding that such tools are effective in supporting health management. However, the study centers on the functionality of the applications rather than their empowering effects. Similarly, systematic reviews conducted by Iyawa *et al*. (2021) and Sayakhot and Carolan-Olah (2016) examined the use of mobile health applications for pregnancy-related health management. Homko *et al*. (2007) explored internet-based interventions for glucose monitoring among underserved women with gestational diabetes, reporting increased self-efficacy in disease management. Samoocha *et al*. (2010) evaluated digital communication strategies employed by healthcare professionals to deliver follow-up care to female cancer patients, again emphasizing the utility of technology in facilitating information transfer. These studies collectively show the value of digital technologies in enhancing women's health outcomes, yet they tend to overlook empowerment as a broader, multidimensional outcome.

## RESEARCH OBJECTIVES

The study aims to assess the current level of digital access, usage, and literacy among women in rural and urban Tamil Nadu through region-specific quantitative data. It further explores how digital platforms empower women in areas like health, education, livelihoods, and civic participation. The research identifies region-specific barriers to

digital inclusion, such as socio-cultural norms, affordability, infrastructure gaps, and safety concerns, using qualitative methods. It critically evaluates key government initiatives like Amma e-Sevai Centres, PMGDISHA, and Mahila E-Haat, and proposes targeted, evidence-based policy measures to strengthen inclusive digital ecosystems in the state.

## METHODOLOGY

The study adopts a qualitative research approach to examine the barriers and opportunities related to women's digital inclusion in Tamil Nadu. Data was gathered through focus group discussions and case studies conducted in selected rural and urban districts. Supplementary insights were drawn from policy documents, government reports, and program evaluations. This approach allows for a contextual analysis of how digital platforms impact women's empowerment and how existing initiatives function within specific regional and socio-cultural settings.

## FINDINGS AND DISCUSSION

**Women and Technology**

Technology has played an important role in improving women's access to learning and expanding their choices in economic and other areas of life. Research shows that access to financial tools such as mobile money, microfinance, and inclusive financial systems has supported women's learning and increased their involvement in the formal economy, as well as in production and service sectors (Çetin *et al*., 2020). Studies have also shown that technology has helped women participate more in scientific fields and supported their political and institutional involvement by providing better access to information and communication networks (Asongu and Odhiambo, 2020). Technology supports empowerment by allowing tasks to be completed in flexible ways, often from home, and at times that suit the user. Many of these activities can be done entirely online, which helps reduce barriers related to time and travel. Digital platforms also offer learning opportunities across a wide range of subjects, with videos and written materials often available in multiple languages. In this study, "technology" refers to digital tools that require electricity or power sources, such as mobile phones, laptops, and desktop computers, along with the internet and the software or applications used on these devices (Crompton, 2014). Despite Tamil Nadu's reputation as one of India's more progressive and technologically advanced states, the level of digital access and literacy among women remains deeply unequal across urban and rural areas. This disparity becomes particularly evident when analysing region-specific data from the National Family Health Survey-5 (NFHS-5, 2019–21), which offers critical insights into mobile phone ownership and internet usage among women aged 15–49. While 74.6% of women in Tamil Nadu reported owning a mobile phone they themselves use, this figure still falls short when compared to their male counterparts. More concerning, however, is the disparity in internet usage: only 24.6% of women in rural Tamil Nadu have ever used the internet, compared to 51.8% of urban women. This gap illustrates a dual-layered inequality—both in terms of gender and geography—which continues to limit the transformative potential of digital technologies for women in large sections of the state. These numbers reveal the fact that owning a mobile phone does not necessarily imply meaningful digital inclusion.

While mobile penetration has improved over the years, particularly with the proliferation of low-cost smartphones, this technological reach has not been matched by corresponding improvements in digital literacy and functional usage. In rural districts such as Perambalur, Ramanathapuram, and Dharmapuri, women often have access only to shared or basic-feature phones, which severely restricts their engagement with the internet, social media, e-governance platforms, or online financial services. In contrast, urban centres like Chennai and Coimbatore show higher levels of digital participation among women, but even here, usage is often limited to entertainment or communication rather than tools for economic or educational empowerment(Abubakar and Dasuki, 2018). Moreover, research from local studies and NGOs operating in Tamil Nadu suggests that even among women who own smartphones, there is a significant gap between access and digital competence. For example, a study conducted in South Chennai observed that many women possessed internet-enabled phones but lacked the skills to navigate

applications for online banking, e-learning, or digital government services. This gap is compounded by language barriers, as most apps and platforms are English-centric, marginalising non-English speaking women. Socio-cultural constraints—particularly in conservative rural households—continue to restrict women's independent use of technology. It is not uncommon for male family members to control device usage or internet access, reinforcing a digital patriarchy that undermines women's autonomy(NFHS-5, 2021). Affordability remains another critical barrier. While data costs in India are among the lowest globally, for many women in lower-income households, even small recurring expenses for mobile data recharge can be prohibitive. Coupled with inconsistent electricity supply and poor network infrastructure in remote villages, these factors further exclude rural women from the benefits of digital connectivity. The problem is not just technical—it is systemic. Without addressing the socio-economic and infrastructural roots of digital inequality, efforts to promote women's digital empowerment will remain superficial and unsustainable. Government efforts such as the Internet Saathi Programme, launched by Google and Tata Trusts, and digital literacy initiatives under the Digital India campaign have made important strides in raising awareness and providing basic training. However, their penetration and impact remain uneven across Tamil Nadu. The state government's Amma e-Sevai Centres and village-level Common Service Centres (CSCs) do provide digital interfaces for accessing government services, but anecdotal evidence suggests that many women either lack the confidence to use them independently or depend on male intermediaries(NFHS-5, 2021). This again reinforces dependency rather than empowerment.

**Digital Platforms in Health**

Access to quality healthcare has been a persistent challenge, especially in rural areas. Digital platforms have emerged as critical tools in bridging this gap. Telemedicine services, for instance, have enabled women in remote regions to consult with healthcare professionals without the need to travel long distances. Initiatives like the Tamil Nadu e-Governance Agency's AI-driven health services have streamlined patient data management and facilitated remote consultations, ensuring timely medical advice. Moreover, mobile health applications have played a significant role in disseminating information related to maternal health, nutrition, and disease prevention. These platforms provide women with critical knowledge, empowering them to make informed health decisions (Abubakar and Dasuki, 2018). The integration of digital tools in health has not only improved access but also enhanced the quality-of-care women receive.

**Digital Platforms in Education**

Education is a cornerstone of empowerment, and digital platforms have revolutionised access to learning resources for women in Tamil Nadu. Online courses, e-learning modules, and virtual classrooms have made education more accessible, especially for those who had to discontinue formal education due to various constraints. Programs like the Internet Saathi initiative have trained women to use the internet effectively, opening doors to a plethora of educational content. Digital literacy programs have been instrumental in equipping women with essential skills to navigate the digital world. These initiatives have not only enhanced their knowledge base but also increased their confidence to engage with technology, thereby fostering a culture of continuous learning and self-improvement.

**Digital Platforms in Livelihood**

Economic independence is crucial for empowerment, and digital platforms have significantly contributed to enhancing women's livelihoods in Tamil Nadu. E-commerce platforms have provided women entrepreneurs with the opportunity to market and sell their products beyond local markets. Social media channels have also become powerful tools for women to promote their businesses, connect with customers, and build brand identity. Moreover, digital financial services have facilitated easier access to banking, credit, and insurance products. Mobile banking and digital wallets have enabled women to manage their finances efficiently, leading to better financial planning and security. These platforms have not only opened new income streams but also fostered a sense of financial autonomy among women.

**Digital Platforms in Civic Participation**

Similarly, active participation in civic life marks the very essence of empowerment. In particular, digital platforms have become a means through which female citizens in Tamil Nadu participate in governance and community development. Online portals for government services have made it possible for women to learn about, apply for, and file grievances in respect to various welfare schemes. With the rise of social media, women-folk are taking to these spaces to air opinions, gather public support for causes, and participate in public discourse. Women have also been able, with digital help, to increase their association with local governance structures. Digital training programs have equipped women for leadership roles in Panchayati Raj Institutions, thereby giving them a say in decision-making at the grassroots level. This consolidation of civic engagement hence ensures inclusivity and representativity in governance.

**Challenges and the Way Forward**

While digital platforms have created an enabling environment in Tamil Nadu for the empowerment of women, challenges persist. Digital literacy is still unevenly distributed, particularly among older women and those residing in far-flung areas. The affordability of devices and internet services continues to be another barrier. Sometimes, socio-cultural norms put restrictions on women's access to technology. Tackling these issues will require different interventions. Programs for digital literacy should be expanded, while technology access should be subsidized, and content should be created in local languages for greater inclusivity. Community-based programs involving men and leaders will assist in breaking down the socio-cultural barriers. For empowerment, digital inclusion offers women what they need-Namely, information, services, and opportunities for a truly transformative effect on life. In digital infrastructure, Tamil Nadu has made its strides, but the rural women still stand with huge barriers considering digital inclusion. These barriers are plural and include socio-cultural norms, economic constraints, infrastructural deficits, and safety concerns. A qualitative inquiry into these challenges will be the basis for developing targeted interventions that will actually bring about digital inclusion.

**Socio-Cultural Norms**

Socio-cultural norms deeply influence women's access to and use of digital technologies in Tamil Nadu. In many communities, traditional gender roles dictate that women prioritize domestic responsibilities over personal development, limiting their time and freedom to engage with digital tools. In interviews conducted in the rural district of Dharmapuri, several women expressed that their families viewed internet use as unnecessary or even inappropriate for women. One participant noted, "My husband says the internet is not for women; it's a distraction from household duties." Such attitudes stem from entrenched beliefs that associate technology use with male domains, thereby restricting women's digital engagement. Social expectations around modesty and propriety can discourage women from participating in online platforms. In conservative areas, there is a fear that women's online presence could lead to reputational harm, making families hesitant to allow women to own smartphones or access the internet.

**Affordability**

Economic constraints significantly hinder women's digital inclusion in Tamil Nadu. The cost of devices, data plans, and maintenance can be prohibitive, especially for women in low-income households. In a case study from the Tirunelveli district, a woman named Lakshmi shared her experience: "I wanted to buy a smartphone to help my children with their studies, but we couldn't afford it. Even if we had one, recharging data regularly is expensive." This reveal how financial limitations not only affect women's personal access to digital tools but also impact their roles in supporting their families' educational needs. Moreover, women often lack control over household finances, making it challenging to allocate funds for digital expenses. This economic dependency further exacerbates the digital divide, as women are unable to make autonomous decisions regarding technology use.

**Infrastructure**

Inadequate digital infrastructure in certain regions of Tamil Nadu poses a significant barrier to women's digital inclusion. While urban areas like Chennai and Coimbatore have relatively robust connectivity, rural districts such as

Perambalur and Ariyalur suffer from poor network coverage and limited internet access. Interviews with women in these regions revealed frustrations with unreliable connections and lack of access points. One respondent from Ariyalur stated, "Even if we have a phone, there's no signal most of the time. We have to climb to the terrace or walk to a specific spot to get a connection." Such infrastructural challenges discourage consistent use of digital technologies and limit the benefits women can derive from them. The scarcity of public digital access points, such as community centres or libraries with internet facilities, further restricts opportunities for women to engage with digital platforms, especially for those who cannot afford personal devices (Abubakar and Dasuki, 2018).

**Safety Concerns**

Safety concerns, both online and offline, significantly impact women's willingness to engage with digital technologies. Fear of cyberbullying, harassment, and exposure to inappropriate content can deter women from using the internet or social media platforms. In a focus group discussion in the Madurai district, participants expressed apprehension about online interactions. One woman shared, "I've heard stories of women being harassed online. I'm afraid to post anything or even have a profile picture." Such fears are compounded by a lack of digital literacy, which leaves women vulnerable to online threats and scams. Moreover, physical safety concerns related to accessing digital services, such as traveling to distant cybercafés or community centres, can also be a deterrent. In areas with limited public transportation or high crime rates, women may avoid these spaces altogether, further isolating them from digital resources.

**Case Studies**

**Case Study 1: Empowering Women through Digital Literacy in Villupuram**

An initiative in Villupuram district aimed to provide digital literacy training to women through local self-help groups. Despite initial enthusiasm, the program faced challenges due to socio-cultural resistance. Many participants dropped out after facing opposition from family members who questioned the necessity of digital skills for women. This case shows the importance of involving community stakeholders and addressing cultural perceptions in digital inclusion efforts.

**Case Study 2: Mobile Health Services in Thanjavur**

In Thanjavur, a mobile health service was introduced to provide teleconsultations for women in remote areas. While the service had the potential to improve healthcare access, uptake was low. Interviews revealed that women were hesitant to discuss health issues over the phone due to privacy concerns and a lack of familiarity with telemedicine. This shows the need for building trust and digital confidence among women to effectively utilize such services.

**Case Study 3: Digital Entrepreneurship in Coimbatore**

A group of women in Coimbatore started an online handicraft business, leveraging social media platforms to market their products. However, they faced challenges in managing digital payments and logistics due to limited digital literacy. With targeted training and support, they were able to overcome these hurdles, illustrating the potential of digital platforms to enhance women's economic participation when adequate support systems are in place.

**Case Study 4: Community Internet Centres in Salem**

In Salem, community internet centres were established to provide women with access to digital resources. While the initiative was successful in urban areas, rural uptake was limited. Interviews indicated that women in rural areas were hesitant to visit these centres due to safety concerns and societal norms discouraging women from spending time outside the home. This case emphasizes the need for context-specific strategies to promote digital inclusion.

**Case Study 5: Online Education in Tiruchirappalli**

During the COVID-19 pandemic, online education became the norm. In Tiruchirappalli, many girls from low-income families struggled to attend online classes due to lack of devices and internet connectivity. Community organizations stepped in to provide shared devices and internet access, enabling these girls to continue their education. This case demonstrates the critical role of community support in bridging the digital divide.

**Implementation and Impact of State and Central Government Digital Initiatives**

**Amma e-Sevai Centres: Bridging the Digital Divide at the Grassroots**

The Amma e-Sevai Centres, set up by the government of Tamil Nadu, intend to give access to various government services under one single-window digital platform for citizens. It is specially helpful for women for whom these centres provide services like application for certificates, bill payments, or grievance redressal. The state claims to have over 10,000 e-sevai Centres to facilitate the easy access of various government services by the common men. The impact of these centres on the digital inclusion of women is worth noting (Abubakar and Dasuki, 2018). A study undertaken based in Madurai district has shown that the e-Sevai Centres have contributed towards digital inclusion by providing services catering to local needs; however, lower incomes, lack of technical and financial support, poor connectivity, and frequent power outages stand as hurdles thwarting the effectiveness of these centres. The study thus recommended that innovative and efficient organizational and infrastructural facilities be initiated to boost these centres (Vijayachandrika, 2022). The Tamil Nadu e-Governance Agency (TNeGA) greatly contributes to digital literacy enhancement through these centres. TNeGA offers a variety of online services and training courses to enable and acquaint women with accessing a number of government services through digital means, thus familiarising and empowering them to use digital platforms confidently.

**PMGDISHA: Enhancing Digital Literacy Among Rural Women**

The Pradhan Mantri Gramin Digital Saksharta Abhiyan (PMGDISHA), a central government initiative, aims to provide digital literacy to rural citizens, including women, minorities, and economically weaker sections. In Tamil Nadu, the program has made significant strides. As of December 2022, over 17.04 lakh candidates have been enrolled, with 14.07 lakh trained and 10.55 lakh certified under the PMGDISHA Scheme. Notably, approximately 76 percent of the certified candidates are women, indicating a strong female participation in the program (Gichuki and Mulu-Mutuku, 2018). The program's effectiveness is further shows by its reach among women in rural areas. Data indicates that PMGDISHA has benefited 18 lakh women in Tamil Nadu, thereby its role in enhancing digital literacy among rural women. However, challenges persist, including infrastructural deficits, socio-cultural barriers, and limited awareness, which hinder the program's full potential. To address these challenges, the government has implemented various strategies, such as community engagement and awareness campaigns. These efforts aim to encourage women's participation in digital literacy programs and ensure that the benefits of PMGDISHA reach the intended beneficiaries.

**Mahila E-Haat: Empowering Women Entrepreneurs Online**

Mahila E-Haat, initiated by the Ministry of Women and Child Development, is an online marketing portal that was meant to encourage women entrepreneurship. It enables women to expose and sell their products and services directly to consumers, thus generating economic empowerment. Nevertheless, the reach of the portal in Tamil Nadu seems minimal. According to the recent statistics, only eight women entrepreneurs from Tamil Nadu are listed on Mahila E-Haat, reflecting negligible participation from the state. The reasons for such low participation may be the lack of digital literacy, spotty internet coverage, and low awareness about the platform. In order to maximize the effectiveness of the platform, targeted interventions such as digital literacy trainings, infrastructure improvement, and awareness generation are the need of the hour (Lechman and Paradowski, 2021). Combining Mahila E-Haat with other government platforms, like the Government e-Marketplace (GeM) and platforms controlled by the National Small Industries Corporation (NSIC), may offer women entrepreneurs more extensive market reach and assistance (Gichuki and Mulu-Mutuku, 2018). Among the key hindrances to ensuring digital inclusion among women in Tamil Nadu is insufficient digital infrastructure, especially in rural and outlying areas. Whereas urban hubs like Chennai and Coimbatore enjoy good connectivity, districts like Perambalur and Ariyalur lack good network coverage and limited internet penetration(Vijayachandrika, 2022). This limitation makes it difficult for women to access digital services, avail online education, and pursue digital entrepreneurship. In order to overcome this, the government can focus on expanding broadband infrastructure in unserved areas (Gichuki and Mulu-Mutuku, 2018). Investment in last-mile connectivity, through the use of technologies such as fiber-optic cables and satellite internet, can help reduce the digital divide. Public-private collaboration has the potential to drive infrastructure growth, with even far-flung villages not being left behind(Abubakar and Dasuki, 2018). Digital literacy is a key enabler of women's

empowerment in the modern era. Even with numerous programs under way, a large number of women, particularly in rural settings, remain without the basic digital skills they need. This lacuna prohibits their access to services, opportunities, and information. Initiatives such as the Pradhan Mantri Gramin Digital Saksharta Abhiyan (PMGDISHA) have helped bridge this digital divide. As of December 2022, more than 17.04 lakh candidates have been registered in Tamil Nadu alone, out of whom 14.07 lakh have been trained and 10.55 lakh certified under the PMGDISHA Scheme.

Interestingly, around 76 percent of the certified candidates are female, reflecting a high level of female involvement in the program. Nonetheless, to maximize the effect, such programs need to be designed to cater to the particular needs of women (Hamper, 2020). Including modules on digital financial literacy, internet safety, and e-governance can empower women to operate in the digital environment confidently. Also, incorporating digital literacy into curricula of schools and adult education institutions can instil a culture of lifelong learning. Economically, constraints diminish women's digital inclusion in Tamil Nadu. Devices, data plans, and maintenance fees can be expensive, particularly for women in poor households. Also, women may not have control over the household purse, which makes it difficult to set aside money for digital costs. In order to alleviate these issues, the government can opt for subsidizing digital equipment for women, especially those from marginalized communities (Lechman and Paradowski, 2021). Collaboration with technology firms can help to provide low-cost smartphones and tablets. Community Wi-Fi projects implemented along with discounted data plans can also make internet access more feasible. Digital platforms hold huge potential for women to become entrepreneurs and boost their economic status. Programs such as Mahila E-Haat, an online marketplace initiated by the Ministry of Women and Child Development, are initiatives to encourage women entrepreneurship. The reach of the platform in Tamil Nadu is scanty as only eight state women entrepreneurs are registered on Mahila E-Haat(Vijayachandrika, 2022). To support digital entrepreneurship, the government must engage targeted training programs on e-commerce, digital marketing, and financial management. Mentorship programs and microfinance access can also complement women entrepreneurs (Gichuki and Mulu-Mutuku, 2018). Incorporating digital entrepreneurship modules into current self-help group (SHG) structures can build on the strength in numbers of women. Safety and security issues, both online and offline, are key factors in the willingness of women to adopt digital technologies (Riquelme and Thufery, 2018). The fear of cyberbullying, harassment, and being exposed to offensive content can prevent women from accessing the internet or social media sites. To solve such problems, the government must introduce extensive digital safety awareness schemes. It should include in those schemes awareness about recognizing cyber attacks, online safety practices, and reporting procedures (Hamper, 2020).

Creating special cybercrime units to deal with online harassment can bring confidence among women users. A inclusive digital environment needs policies that focus specifically on the special problems being faced by women. The Tamil Nadu State New Policy for Women 2021 identifies various policies in favour of women's empowerment, such as digital inclusion(Vijayachandrika, 2022). Nonetheless, proper implementation and constant monitoring are vital to ensure these policies become real results. Women's participation in policy-making and decision-making can result in more responsive and effective government. Creating space for women's voices to be heard, including online platforms and people's consultations, can provide assurance that policies are tuned in to their needs and aspirations (Riquelme and Thufery, 2018). Alliances between the government, private sector, and civil society can increase digital inclusion initiatives' reach and impact. Initiatives like Internet Saathi, a collaborative effort by Google India, Intel, and Tata Trusts, have shown the potential of collaborations. Incentivizing such collaborations can attract technological skills, funds, and new solutions to deal with the complex challenges of digital inclusion. Corporate social responsibility (CSR) programs can be synergized with government schemes to enable infrastructure development, training, and awareness. Monitoring and evaluation of digital inclusion initiatives should be carried out regularly to find out how effectively they are functioning and where improvements can be made. Identifying key performance indicators (KPIs) for women's participation, skill development, and economic outcomes can offer useful insight (Hamper, 2020). Policy responses and resource allocation can be guided by data-driven evidence to have the greatest impact. Regular surveys and impact evaluations can provide a snapshot of women beneficiaries' experiences and feedback, ensuring programs remain sensitive to their changing needs (Riquelme and Thufery, 2018). Building

strong inclusive digital ecosystems is critical for promoting Tamil Nadu's women empowerment. Addressing infrastructural shortages, boosting digital literacy, facilitating affordability, and creating enabling policy environments are critical steps the state needs to take in order to overcome the digital divide and unlock the true potential of its women. When these initiatives are coordinated synergistically, they lay the groundwork for true empowerment. Continuous co-operative endeavours, strict surveillance, and adherence to inclusiveness will be the motive pillars towards creating a digitally empowered and socially inclusive Tamil Nadu.

## CONCLUSION

The findings of this study reveal that digital inclusion is not merely a matter of access to devices or connectivity but is intricately linked to gendered socio-economic realities and cultural contexts specific to Tamil Nadu. The empowerment of women through technology requires more than infrastructure; it demands structural change in how digital ecosystems are designed, delivered, and governed. While state and central initiatives have laid a foundational framework, the actual experience of women—particularly in rural communities—reveals a persistent gap between policy intent and lived realities. This disconnect reveals the need for adaptive policy design that is informed by local knowledge, user feedback, and gender-sensitive implementation strategies. Empowerment through digital means must also focus on building agency—equipping women not just to consume content or access services, but to engage, create, lead, and innovate within digital spaces. The state's role must shift from being a provider to an enabler, supporting women with skills, safety, affordability, and representation. A participatory governance model that involves women in the co-creation of digital policies and platforms can ensure relevance and sustainability. Transforming Tamil Nadu's digital landscape into an inclusive and empowering space for women is a socio-political challenge that demands holistic, intersectional, and sustained efforts beyond technological deployment.

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RESEARCH ARTICLE

# Management of Biomedical Waste during the Time of COVID-19

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## ABSTRACT

The article reviews how the COVID-19 pandemic dramatically increased biomedical waste (BMW) generation and exposed critical weaknesses in India's existing BMW management system, especially in segregation, treatment capacity, and regulatory enforcement. It highlights that the surge in infectious waste such as PPE, masks, sharps, and diagnostic materials overwhelmed infrastructure, aggravated risks to waste handlers, and contributed to environmental hazards due to improper disposal and mixing of hazardous and general waste. The review first outlines the nature and sources of BMW, its categorization, and colour-coding system, emphasizing that only a fraction of BMW is inherently hazardous but can cause significant health and ecological damage if poorly managed. It then describes how COVID-19 altered municipal and hospital waste streams through massive use of single-use PPE and masks, inadequate segregation at household and institutional levels, and inconsistent application of BMW guidelines. Key challenges identified include limited treatment facilities, gaps in training and awareness, weak monitoring, unsafe manual segregation, and the absence of clear mechanisms for waste from home-quarantine settings. The article concludes that incremental measures during the pandemic were insufficient and calls for a shift to a systems - oriented approach that expands decentralized treatment capacity, strengthens real-time tracking, improves training and protective measures for waste workers, and upgrades regulations and enforcement. It argues that sustained investment in infrastructure, alternative treatment technologies, and public education is essential to reduce infection risks, protect the environment, and build a resilient BMW management framework capable of handling future public health emergencies.

**Keywords:** treatment, BMW, colour-coding, COVID-19, biomedical.

# INTRODUCTION

The coronavirus disease 2019 (COVID-19) can cause serious infections in the human respiratory system and was first discovered in animals at the Wuhan, China, meat and fish markets. In Wuhan, China, it was first identified at the end of December 2019 after locals began exhibiting symptoms similar to pneumonia. CIVID-19 infections were also linked to other health issues, such as fever, headache, and muscular and head pain. [1] The new coronavirus (SARS-CoV-2), a positive-sense single-stranded RNA virus, is the infectious agent that causes coronavirus illness 2019 (COVID-19). [2] There are four types of coronavirus: Delta (δ), Gamma (γ), Beta (β), and Alpha (α).Gamma and Delta subtypes primarily infect birds, but Alpha and Beta subtypes are clinically relevant because they can cause infectious illnesses in humans. [ 3]. Contact, whether direct or indirect, is the first mode of transmission. In SARS-CoV-2, the upper and lower respiratory tracts are affected. Regarding direct contact, SARS-CoV-2 can be spread by coming into contact with a COVID-19 patient's mouth, ears, or ocular mucous membranes [4].

**Biomedical Waste**

The waste generated in diagnosis treatment or immunization of human beings or animals, in research or in the production of testing of biological products including all categories of infected and toxic waste that is potential threat to human beings and environment. Biomedical waste can also be termed as regulated waste/medical waste or health care waste[5]

Medical waste is generated during:

- Diagnosis
- Treatment.
- Immunization
- Biomedical research
- Production and testing of biological product

Every day, India produces a staggering amount of biomedical waste (BMW). [6] The daily production of BMW in India is about 1.5 and 2 kg/bed. [7] BMW contains sharps, laboratory trash, anatomical waste, and other materials that can be lethal if not properly isolated. Our ecology may also be harmed by improperly segregating waste plastic, a recyclable and cytotoxic substance. [8] BMW is dangerous because it contains possible viruses or other microbiological particles that can cause illness; they can be found in human samples, blood bags, needles, cotton swabs, dressing materials, bedding, and more. [9] Of the 710 tons of garbage created everyday in India, BMW generated as a result of COVID-19 accounted for roughly 126 tonnes per day. [10]

**Table 1. Type of Waste Category** [11]

**Management of BMW**

Reduction, recycling, and reuse—the three Rs—are the cornerstones of good BMW practices. The finest BMW management (BMWM) practices focus on recovering as much waste as possible or preventing waste development instead of disposing of it. As a result, depending on their desirability, the several BMW disposal techniques are as follows: prevent, reduce, reuse, recycle, recover, treat, and finally discard. The waste should therefore be addressed at the source instead of using a "end of pipe" strategy. [12] Only roughly 10% to 25% of BMW is dangerous; the other 75% to 95% is not. The general public and healthcare professionals who handle, treat, and dispose of trash are at risk from the hazardous portion of the waste due to physical, chemical, and/or microbiological hazards. [13] The former Ministry of Environment and Forests notified the Government of India of the first BMW regulations in July 1998. [14] The BMW issue in India was made worse by scavengers who recycle open, unprotected medical trash without gloves, masks, or shoes, as well as by reusing syringes without properly sterilizing them. [15].

**Colour Coding of Biomedical Waste**

BMW management starts with color coding. Wastes must be managed and disposed of in accordance with their classification since they are divided into many sorts. In every healthcare facility across the world, the trash cans are always color-coded. Bins are coloured in accordance with the universality rule, which separates garbage into several categories. This measure aids in preventing the confusion that arises when different kinds of trash are mixed together, which may result in inappropriate management and disposal and ultimately cause the development of many illnesses. [16] Yellow containers gather nearly all forms of BMW trash, including anatomical, pathogenic, chemical, laboratory, and pharmaceutical waste. Recyclable contaminated garbage is collected in red bins, and BMW is collected in non-chlorinated plastic bags. Vials and ampoules are among the glassware waste that is collected in blue bins. Sharps that are contaminated and abandoned are disposed of in translucent white bins. It is imperative that sharp trash be disposed of in containers that can withstand punctures to prevent mishaps that could cause illnesses for those handling it.

**Bins of various colors are used to separate different types of garbage [9]. (Fig.1)**

Eliminating all infectious and hazardous waste produced in hospital environments is known as BMW management. trash treatment is essential for decontaminating the generated trash and eliminating all pathogenic organisms. This aids in preventing a number of serious health problems that could arise from infectious trash. It is a strategy to avoid any environmental risks. [17]

**Impact of Covid- 19 on Biomedical Waste Genration**

Municipal solid waste management (MSWM) has been significantly impacted by the corona virus epidemic, which has also altered the kind and volume of garbage. The term "MSW" describes a variety of solid waste types produced by community members, whether in an urban or rural environment where there is a chance of direct physical touch between individuals. [18] To stop the transmission of illness in public, the WHO advised wearing a mask. In 2019, the face mask market was valued at US$0.79 billion, and by 2020, it had grown to around US$166 billion. [19]. It wasn't until January 2020 that PPE kits and N-95 masks were manufactured in India; instead, 2.75 lakhs were imported.However, by the end of May 2020, India was producing almost 4.5 lakh PPE kits and over 104 lakh N-95 masks daily, making it the second-largest producer of PPE kits. [20] Because of COVID-19, there was less effective segregation and, in certain cases, recycling of garbage was outlawed due to fear of infection. In addition, the amount of waste produced in households rose, and the recycling and waste segregation chain was frequently neglected. Just over half (58%) of the studied facilities from 24 countries had sufficient processes in place for proper BMW management prior to COVID-19, according to the World Health Organization (WHO). [21]. The amount of hazardous waste in low-income nations rises as a result of improper hazardous and non-hazardous waste segmentation. The COVID-19 pandemic has made BMW management's problems much worse. BMW production increased from 50 tons per day prior to the pandemic to 247 tons per day in Wuhan province, China, where the COVID-19 virus was initially discovered. [22]

**Challenges in Biomedical Waste Management**

India's rapidly developing and growing healthcare sector presents major issues for the country's biological waste management. [23] Due to the exponential expansion of medical facilities, population growth, and advancements in healthcare technology, biological waste output has dramatically expanded nationwide. [24] Because they provide serious threats to the environment and human health, inappropriate waste management practices are becoming a reason for worry. [25]

**Challenges**

- Bio-waste cannot be appropriately separated in any public or residential location using the color-coding scheme found in Appendix I (Schedule I) of the 1998 BMW Regulations or the 2016 Amended Regulations.
- N95 respirators and single-use surgical masks are commonly disposed of with regular household garbage, which is notfurther placed in BMW bins marked with the color yellow.
- There is no established mechanism for disposing of COVID-19 waste in home quarantine centers.

• Due to inadequate training, awareness, and staffing, masks, PPE kits, gloves, and everything else, including food scraps and disposable cutlery, are thrown into yellow bins at quarantine centers and sent for cremation.
• Waste handlers perform the manual segregation process without wearing full safety gear, which puts healthcare personnel at risk of injury.
• In India, there are already 198 Common BMW treatment facilities (CBMWTF) operating, and another 28 are being built (Datta et al. 2018). The need for a biological waste treatment and disposal facility in India comprises the creation of new, highly effective disposable techniques as well as their expansion and modification.
• Groundwater contamination, the development of infectious diseases, and the lack of land are all caused by the landfilling or deep burial of biomedical waste, which produces greenhouse gases.
• Thermal heat is produced during incineration, which is dangerous, and frequently the material is not burned correctly, which increases its toxicity. [26]

## DISCUSSION

When we talk about managing biomedical waste during the COVID-19 pandemic, a few important things stand out. The sudden rise in hazardous waste has really shown how much we need to improve our current waste management systems. In numerous regions, existing infrastructure falls short of the demands required for effective biomedical waste management, while financial resources remain insufficient. Moreover, those tasked with handling waste often do not receive sufficient training or access to the necessary information. Compliance enforcement typically involves much variability by jurisdiction and compliance assurance systems are mostly unreliable, creating inconsistency among individuals in safe disposal practices. These barriers emphasise the need for increased investment and true reform during public health emergencies like the COVID-19 pandemic [27].

Overall, waste management systems and improving them is paramount to human health and the environment, but doing so will necessitate meaningfully addressing long-standing barriers, like inadequate infrastructure, poor financial capacity, and uneven enforcement of the law [27]. The COVID-19 pandemic revealed the urgent need for improvements in existing management systems for bio based waste (BMW) to be capable of organizing the increased volumes of hazard waste. It can be assumed that improvements today can look like improvements in infrastructure, stricter regulations, and better training of waste handlers to increase disposal practices and minimize health challenges. Improving alternative technologies to potentially reduce environmental impacts, as well as the transmission of diseases, has also been determined to be beneficial.

The challenges to begin the needed improvements are considerable. In many areas, lack of infrastructure challenges that potential for different systems of management. Limited funds also limit the capacity of enhancements for management of waste options, with gaps in awareness of personnel causing diligence for waste disposal and inconsistent rules of practice to comply with best practices. All these challenges indicate further investment and oversight needs to realize the potential of biowaste management systems, should there be another concern with public health emergencies. [28]. Overall, improving there are overall benefits associated with improving waste management for health of people and health of the environment, but the issues of lacking potential, its management and resources, and compliance issues must be addressed for solutions to be effective. [28]

## CONCLUSION

The COVID-19 pandemic revealed major weaknesses in India's biomedical waste (BMW) management system, as the surge in infectious waste like PPE, sharps, and diagnostic materials overwhelmed already strained facilities. Emergency measures—such as ramped-up PPE production and temporary disposal guidelines—faced challenges due to uneven implementation, inadequate training for healthcare and sanitation workers, lack of instructions for those quarantined at home, and poor enforcement of waste segregation rules. This often resulted in the mixing of infectious and regular waste, posing additional risks to public health and the environment. The problems were

multifaceted, stemming from outdated treatment infrastructure, varying policy enforcement across states, and health and safety risks to waste workers who often lacked proper protective gear, vaccinations, and health monitoring systems. The pandemic underscored the urgent need to shift from a passive compliance model to an active, systems-oriented approach to BMW management. Key priorities should include real-time waste tracking, enhanced capacity building, decentralized treatment solutions, stronger supply chains for safety equipment, and inclusive policy development that acknowledges the vital contributions of informal waste workers. Public education and governance must also be improved to ensure accountability. Sustainable reforms are critical to safeguarding health, the environment, and restoring public confidence in hazardous waste management. Insights gained during COVID-19 should guide adaptive, evidence-driven strategies focused on resilience, equity, and scientific rigor to better handle future health crises in India.

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**Table 1. Type of Waste Category**

| **Waste category** | **Descriptions** |
|---|---|
| Dangerous waste | |
| Contaminated waste | Waste polluted with blood, bodily fluid, or any other material with the potential to convey bacteria, virus, fungi, or parasites |
| Pathological waste | Human anatomical tissue, fetuses, organs, body fluids |
| Microbiological waste | Microbiological laboratory equipment, stock, used medium, quality control reagents, bacterial and viral cultures, and serological enzymes |
| Pharmaceutical waste | Unused or expiredmedications |
| Cytotoxic waste | Cytotoxic medications |
| Chemical waste | Solvents, cleaning supplies, and laboratory reagents |
| Radioactive waste | Radioactive isotopes, urine and excreta of patients treated with radioactive isotopes |
| Non hazardous waste | |
| Waste in general | Cardboards, plastic, and thermocol packaging material, paper, kitchen garbage |



**Fig.1. Bins of various colors are used to separate different types of garbage [9].**




**Fig.2. Challenges**

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RESEARCH ARTICLE

# Narrating the Unthinkable : Climate Change and the Folkloric Imagination in Amitav Ghosh's *Gun Island*

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## ABSTRACT

Amitav Ghosh's novel, *Gun Island*(2019) explores the crisis of climate change by utilizing folklore as a frame in the representation of current climate crisis. Typically, fictions conceptualize environmental changes through the employment of apocalyptic narratives that feature catastrophic natural disasters, such as floods, storms, and fires. The problem with such representations of climate change is that they tend to imply in the reader that such disasters are unimaginable and vague, rather than actual and existing ones. This article analyses the innovative narrative patternof Ghosh that utilizes folklore to draw parallels between the past and the present, nonetheless retaining the seriousness of the climate crisis and emphasizing its concrete nature and thereby making it apprehensible as real or existent. This creative engagement can be looked at as a lead set by Ghosh to one of the major concerns pointed out by him in his book *The Great Derangement*, a non-fiction about the crisis of imagination among writers to represent climate change.

**Keywords:** Magic Realism, Folklore, Climate Change, Climate Crisis, Climate Fiction

In *The Great Derangement* (2016), Amitav Ghosh calls out the imaginative failure among writers of fiction in adequately representing the climate crisis in its current severity [10]. *Gun Island* (2019) is the novel that followed this remarkable non-fictional work and can be understood as an attempt by Ghosh to address one of the major issues pointed out by him in his previous work – the need to represent the improbable without compromising the gravity of

the climate crisis, which would often result in such works being sidelined as a category of genre fiction. The current climate events are so extreme that representing them in a work of fiction demands the use of novel modes and techniques in storytelling. Considering from this perspective, *Gun Island* by Ghosh stands out from other contemporary climate fictions in that the author has made use of an autochthonous Bengali folklore, the events of which are believed to date back to the seventeenth century. It subsequently turns out that the characters and events in the present mirror the past thereby revealing that the past is simply repeating itself through different characters but similar situations. Before delving into Ghosh's handling of folklore in *Gun Island*, it is imperative to examine the relevance of folklore as a potent tool in the depiction of climate crisis. As Abrams and Harpham put it, "Folklore" is a term collectively applied to sayings, verbal compositions and social rituals that are primarily handed down by word of mouth [138]. It includes legends, superstitions, songs, tales, proverbs, riddles, spells and nursery rhymes. Pseudoscientific lore about the weather, plants and animals; customary activities at births, marriages, and deaths; and traditional dances and forms of drama performed on holidays or at communal gatherings. (Abrams and Harpham 138). These legends are abundant in metaphors and meaning that they cannot be simply relegated to the status of moral fables, because they guide the present by showing ways in which premodern cultures coped with the intricacies of nature, of the discord that emerged between human beings and the natural world. Compared to the prescriptive nature of the potential solutions offered by the scientific community to recuperate the damage done to the environment, the stories and legends of folklore prove to be more potent, influential, and persuasive as they are associated and identified with a cultural context along with an added element of sanctity innate in them.

In view of these unique characteristics that are inherent in such stories and beliefs, folklore turns out to be an irrefutable tool in finding solutions to supposedly unmanageable problems posed by the contemporary climate crisis. Another important aspect of folktales as stated by Andrew Simms is the emergence of such tales specifically during times of upheaval and from the societies' grimmest moments (Simms). This enables us to draw plausible solutions for the current global problems with reference to such lore. They are not only cautionary and impart us with the knowledge to identify risks, but also aid us in processing and assimilating extreme experiences; and help in dealing with uncertainties posed by anthropogenic global warming. Most folk legends are open ended and there are often multiple versions of them. These legends need to be carefully explored in order to excavate the multiple meanings embedded in them and only through such legends the problems entrenched in local geographies and cultural contexts can be understood better (Dasgupta). Apart from reiterating that all actions have consequences, these stories emphasize the ephemerality of things around us and the necessity for balance. The stories echo the intricate worldview where everything, human and non-human have agency. As legends and myths are rooted in local culture and since all cultures have identical stories, folklore turns out to be an ideal medium to talk about the environment. Another significant aspect of folklore is the narrative's inclusive quality which places the non-human on par with the humans. As Amitav Ghosh explains in *The Great Derangement*, In the Indian epics … there is a completely matter-of-fact acceptance of the agency of non-human beings of many kinds. I refer not only to systems of belief but also to techniques of storytelling: non-humans provide much of the momentum of the epics; they create the resolutions that allow the narrative to move forward [86]. He also draws attention to the bitter fact that there is no place for the non-human in contemporary literary fiction and the novels which include non-human in their narrative are often relegated to the "outhouses to which science fiction and fantasy had been banished" [88].

Ghosh also discusses about the ethical difficulty in treating the contemporary weather events using the narrative modes of Surrealism and Magic Realism, since the current climatic events are neither surreal or magical but are astonishingly real [36]. He also makes it clear in one of his interviews that he does not want to be associated with apocalyptic narrative style as he feels that it is "a very male western thing" (Ramakrishnan). In view of the such formal insufficiencies and ethical challenges in representing global warming, folklore turns out to be an indisputable instrument in seeking answers to allegedly unsolvable challenges offered by the modern climate crisis. As a rich source of storytelling, Folklore proves to be a suitable medium to explore themes related to climate change in fiction. An increasingly pressing issue like climate change can be better dealt with by incorporating folklore which helps create a sense of urgency and meaning around the issue. An example of folklore being used in fiction about climate change is in the book, *The Fifth Sacred Thing* by Starhawk. The story is set in a future California that has been ravaged

by climate change and the protagonist relies on ancient pagan practices to heal the land and protect the community. The book explores themes of community, spirituality, and environmentalism through the lens of folklore and mythology. The novel *The Bear and the Nightingale* by Katherine Arden is set in medieval Russia and draws heavily on Russian folklore to explore the relationship between humans and the natural world. The story follows a young girl named Vasya who possesses the ability to communicate with the spirits of the forest, and who must use her powers to protect her village from an evil force that threatens to destroy the natural balance of the world. Another recent example is *The Overstory*, a novel written by Richard Powers. In this novel, Powers explores how climate change affects the natural world and stresses the pressing need to protect the environment. Through the character, Patricia Westerford, the author highlights the importance of understanding and respecting the natural world and the devastating consequences of ignoring or exploiting it for human gain. Throughout the novel, Powers incorporates myths and legends about trees from various cultures, as well as scientific research on the communication and intelligence of trees. Neil Gaiman's American Gods TV series provides another example through the character Mr. Nancy, who narrates tales from African and African American tradition that stress the importance of reverence for nature and the dangers of neglecting it.The show uses folklore to explore themes of environmental destruction and the need for humanity to live in harmony with the natural world. These are a few examples to show the effectiveness of folklore in fiction about climate change. As stated by Pancholi and Mishra, Ghosh recognizes the important role that ancient myths and legends play in symbolizing the catastrophic effects of climate change.

He acknowledges that these stories have already depicted many of the environmental disasters that we are currently experiencing, such as floods, droughts, famines, and storms [8]. Amitav Ghosh's *Gun Island* illustrates the effects of climate change on the migration and displacement of people and animals alike, framed within a Bengali legend. It provides a contemporary reinterpretation of the Bonduki Sadagar story from the seventeenth century. According to the legend, … the Gun Merchant was said to have been a rich trader who had angered Manasa Devi by refusing to become her devotee. Plagued by snakes and pursued by droughts, famines, storms, and other calamities, he had fled overseas to escape the goddess's wrath, finally taking refuge in a land where there were no serpents, a place called 'Gun Island' – Bonduk-dwip. (Ghosh 16). Even in the Gun Island, the merchant was chased by Manasa Devi and later was bitten by a poisonous snake. The merchant escapes from Gun Island and gets caught by pirates who sell him in "The Island of Chains." In that island, Manasa Devi appears before the Merchant and offers to set him free and make him rich if he agrees to become her devotee by building a shrine for her in Bengal. The Merchant agrees and Manasa Devi helps him reach his homeland with a great fortune. This earns him the title Bonduki Sadagar – the Gun Merchant[16-17]. This is the version of the Gun Merchant legend as narrated by Nilima Bose, aunt of the protagonist Deen. Deen, with the help of his historian friend Cinta skillfully interprets this legend to be an apocryphal account of an actual expedition to Venice which proves to be historically plausible and uncovers that the Bengali word, Bonduki Sadagar should not be literally translated as "Gun Merchant" but rather it must be understood as "The Merchant who went to Venice." According to their discovery, the merchant's native, eastern India experienced drought and flood as a result of the climatic turmoil of the time. Losing his family and fortune to the climatic turmoil of the time he decided to travel to Venice expecting greener pastures . By the time the Merchant arrived in Venice, he found it in an abandoned state with all its finest days as a great commercial force had been over (Ghosh 220).

This was because it was the Little Ice Age and the Mediterranean region experienced unseasonal weather, including violent storms and catastrophic floods. In a study titled, "Earth system impacts of the European arrival and Great Dying in the Americas after 1492", Alexander Koch *et al*. establish that prior to the Industrial Revolution human activity in the sixteenth and seventeenth centuries had left a significant impact on carbon dioxide levels and surface air temperatures at the global level [14]. Through ingenious storytelling, Ghosh ties the impacts of modern climate change to the myth of Manasa Devi and the Gun Merchant, whose narrative is believed to originate from over three centuries ago in the era of global cooling known as the Little Ice Age. By deploying this Bengali folklore, Amitav Ghosh deals not only with climate crisis, but also with one of the major problems effected by climate change – the displacement and migration of people. Ghosh perfectly pictures the refugee crisis brought about by climate change through the story of Tipu and Rafi in the novel. The Sundarbans is portrayed as a region where societal and ecological calamities feed off one another. The traditional livelihoods of the locals are threatened by storms and the

incursion of sea water onto agricultural fields, and as the character Moyna tells Dinanath, "the exodus of the young was accelerating every year: boys and girls were borrowing and stealing to find them work elsewhere" [49]. In this context, young fisherman Rafi hopes to move to a foreign country with greater opportunities. Yet their homosexual love for each other also motivates Rafi and Tipu to head for Europe, illustrating that climate migration is shaped by intersecting factors of race, gender, and sexuality. Assisted by dalals, the two decide to illegally traverse the borders of Bangladesh, India, Pakistan, Iran, Turkey, Bulgaria, Serbia, Hungary, and Austria en route to Italy. Tipu sustains an injury at the Iran-Turkey border, compelling him to reroute through Egypt and then sail to Sicily, where Italian border officials detain him. The migration of Tipu and Rafi from India to Venice as a result of social pressures made worse by climate change turns out to be a reenactment of the Manasa Devi myth from the seventeenth century.

Almost everything substantial in the story finds resonances in something that has already happened because it is apparent that Ghosh wants us to focus on the lessons of history. Through the lens of a premodern myth, the novel ties its storyline to historical migrations of people that happened prior to the formation of the modern nation-state. In *Gun Island*, the indistinct line between land and sea functions as a metaphor for the disruptive effects brought about by climate change. The story transports readers from the dense, tangled expanse of the Sundarbans, which lies across India and Bangladesh, to Los Angeles devastated by wildfires and the increasingly waterlogged Venice.In the story, Ghosh illustrates how the Sundarbans have been affected by the ecological instability that is roiling the planet. Anthropogenic forces are degrading Venice's ecosystems in a way that is clearly comparable to what is happening in the Sundarbans. The ecological disasters taking place in the Sundarbans and Venice as portrayed in Ghosh's *Gun Island* suggests that the world is on the brink of an environmental catastrophe. Ghosh's portrayal of disasters occurring in widely separated locations should be understood as his method of illustrating the truly global nature of the climate crisis and emphasizing that addressing it requires viewing the Earth as a unified, living organism shaped by ongoing environmental, social, and ecological changes. In contemporary culture, nature is regarded as an object to be exploited in order to satisfy human demands in human-centric cultural patterns. Also, our pursuit of a carbon-dependent economic model is focused on achieving sustainable growth without addressing environmental challenges. A holistic approach with an ecocentric and Nature-inclusive paradigm of growth will stop the damaging effects of climate change. A partnership ethic must exist between nature and humankind that does not view nature as a resource designed solely for human use but rather emphasises cohabitation between human and non-human life with reciprocal support and collaboration. Thus, by using the myth of Manasa Devi, Amitav Ghosh conveys one of the significant messages that we must look to history to gain a better understanding of both our present and the future. A final rumination by Deen on the last page of the novel makes this point very explicitly: "The possibility of our deliverance lies not in the future but in the past" [286].

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**RESEARCH ARTICLE**

# On Almost Generalized Topological Groups

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**ABSTRACT**

In this paper we introduce the notion of almost generalized continuous function (briefly almost G - continuous) and using it we define almost generalized topological groups (briefly almost G- topological groups) and investigate the properties of it .

**Keywords:** Regularly G-open set , Regularly G-closed set, almost G-open map, almost G-closed map, almost G -continuous , almost G -topological groups.

## INTRODUCTION

In 1968 M.K.Singal and Singal [7] introduced almost continuous mapppings. T. Hussain introduced the topological groups [3] Csazar defined neighbourhood systems in the generalized structure . Murad Husain [5], introduced generalized topological groups . C.Selvi, R.Selvi [6] , defined Quasi generalized S-topological simple groups. Madhu Ram [4] introduced almost topological groups. In this paper we introduce almost G- topological groups.

**PRELIMINARIES**

**Definition:2.1** [1]
A subset A of a topological space (X, $\tau$ ) is said to be Regularly open iff A= (Int(ClA)) .The complement of the Regular open sets are called Regularly closed set respectively .

**Definition : 2.2** [5]
Let X be a set and $G \subseteq P$ be a subfamily where P denote the power set of X . Then G is a generalized topology (briefly G -topology) if (i) $\phi \in G$ (ii) Any union of elements of G belongs to G. . A G -topological space X is called strong if the set X itself is a G –open set and we will denote it by **G -topology**. A set with a G -topology is said to be a generalized topological space . The elements of G are called G -open sets.

**Example:2.3**
X={1,2,3} , Here G ={{1},{2} {1,2}, $\phi$ }. The elements of G are called G-open sets . The complement of G-open sets are G-closed sets.

**Definition: 2.4** [ 5]
Let X be any set and $\phi$ , g be G-neighbourhood system and G-topology on X respectively . Let $A \subseteq X$. A point $x \in X$ is called interior of A if there exists a subset $V \in \phi$ (x), $V \subseteq A$. All interior points $A \subseteq X$ is called G-interior of A . It is denoted by $Int_G(A)$. Similarly we can define G-closure of A as intersection of all G-closed sets containing A and it is denoted by $Cl_G(A)$

**Definition: 2.5** [5]
Let X and Y two G-topological spaces and let $f : X \rightarrow Y$ be a function . Then f is called **G-continuous** on X if for any G-open set O in Y, $f^{-1}(O)$ is G-open set in X

**Example:2.6**
Let X=Y={1,2} , $G_X$={ $\phi$ , {1}} and $G_Y$={ $\phi$ , {2}} . Define a map $f : X \rightarrow Y$ as f(1)=2 and f(2)=1 . Let O={2} be a G-open set in Y and $f^{-1}(O)$={1} is G-open set in X.

**Definition:2.7** [7]
A mapping $f : X \rightarrow Y$, (where X and Y are G- topological spaces) is said to be **almost continuous** at a point $x \in X$ if for every neighbourhood M of f(x),there is a neighbourhood N of x such that $f(N) \subseteq Int(Cl(M))$.

**Example:2.8**
Let X= Y={1,2} , $\tau$ ={X, $\phi$ ,{1}} and $\sigma$ ={Y, $\phi$ ,{2}} . Define the identity map f(1)=1 , f(2)=2. Here the mapping f is almost continuous at the point x=1 and x=2.

**Definition:2.9** [8]
Let (G, .) be a group together with a topology $\tau$ such that the mapping $g_1 : G \times G \rightarrow G$ defined by $g_1(x,y)=x.y$ and $g_2(x)=x^{-1}$ are continuous where $G \times G$ carries the product topology . Then the pair (G, $\tau$ ) is called the **topological group**

**Definition:2.10** [4]
Let G be a group together with a topology $\tau$ such that the multiplication and inverse map are almost continuous where $G \times G$ carries the product topology. Then the pair (G, $\tau$ ) is called the **almost topological group** .

**ALMOST G – TOPOLOGICAL GROUPS**

**Definition :3.1**
A subset A of a G -topological space (X, G) is said to be **Regularly G-open set [briefly R GO(X)]** iff A= ($Cl_G(Int_G(A))$). The complement of the Regularly G-open set are called **Regularly G-closed set [briefly R GC(X)]** respectively .

**Example: 3.2**
Let X={1,2,3}, G={$\phi$,{1},{2},{1,2}}. Here RGO(X)={ X,$\phi$,{1},{2}} and R GC(X)={ X,$\phi$,{2,3},{1,3}}

**Definition: 3.3**
If $f : X \to Y$ is said to be **almost G -open map** if the image of every regularly G -open subset of X is an G -open subset of Y .

**Example:3.4**
Let X=Y={1,2,3} , $G_X$={$\phi$,{1},{2},{1,2}}, $G_Y$={$\phi$,{1},{3},{1,3}}, RGO(X)={ $\phi$,{1},{2}}. Define a mapping f(1)=2,f(2)=3,f(3)=1. Let A={2} be a regularly G -open subset of X. Then f({A})={3} is a G -open subset of Y . Hence f is almost G –open map

**Definition: 3.5**
If $f : X \to Y$ is said to be **almost G -closed map** if the image of every regularly G -closed subset of X is an G -closed subset of Y .

**Example:3.6**
Let X=Y={1,2,3} , $G_X$ (closed)={X,{2,3},{1,3},{3}}, $G_Y$ (closed)={Y ,{1,2},{2,3},{2}}, RGC(X)={ X,$\phi$,{2,3},{1,3}}. Define a identity mapping f(1)=1,f(2)=2,f(3)=3. Let A={2,3} be a regularly G -closed subset of X. Then f({A})={2,3} is a G –closed. Subset of Y. Hence f is alomst G-closed map.

**Definition:3.7**
A mapping $f : X \to Y$, (where X and Y are G- topological spaces) is said to be **almost G-continuous** at a point $x \in X$ if for every G -neighbourhood M of f(x),there is a G -neighbourhood N of x such that f(N) $\subseteq$ Int (Cl(M)). Equivalently If $f : X \to Y$ is said to be almost G- continuous if the inverse image of every Regularly G-open set in Y is G-open in X m

**Example:3.8**
Let X=Y={1,2,3} , $G_X$ = {$\phi$,{1},{3},{1,3}}, $G_Y$ ={$\phi$ ,{1},{2},{1,2}}, RGO(Y)={ X,$\phi$,{2,3},{1,3}}. Define a identity mapping f(1)=2,f(2)=3,f(3)=1. Let A={1} be a regularly G -open subset of Y. Then $f^{-1}$({A})={3} is a G –open Subset of X. Hence f is almost G-closed map.

**Definition:3.9**
Let $f : X \to Y$ is said to be almost G -continuous if it is almost G -continuous at each point x of X.

**Theorem :3.10**
If f is a G -open and G-continuous mapping of Y into Z then $g \circ f$ is almost G -continuous iff g is almost G -continuous.

**Proof:**
Let $g \circ f$ be almost G -continuous. Let A be regularly G -open subset of Z. Since $g \circ f$ is almost G - continuous .Therefore $(gof)^{-1}$ (A) is G - open. $f^{-1}(g^{-1}(A))$ is G – open .Since f is G -open. $\Rightarrow f(f^{-1}(g^{-1}(A)))$ is G - open.(ie), $g^{-1}(A)$ is G -open. Hence g is almost G -continuous .Conversely, Let g be almost G -continuous and A be regular G -open subset of Z. Then $g^{-1}(A)$ is G -open subset of Y. Since f is G – continuous. Therefore $f^{-1}(g^{-1}(A))$ is a G -open subset of X. $\Rightarrow (gof)^{-1}$(A) is G -open subset of X. Hence ($g \circ f$) is almost G - continuous.

**Theorem.3.11**
Every Restriction of an almost G -continuous mapping is almost G -continuous.

**Proof:**
Let f be an almost G -continuous mapping of X into Y. Let S be any G -open subset of X and S be any regularly G-open subset in X .(ie) $\left(f/_A\right)^{-1}(S) = A \cap f^{-1}(S)$ But f is almost G -continuous. $\Rightarrow f^{-1}(S)$ is a G - open.

$\Rightarrow A \cap f^{-1}(S)$ is a G -open subset of A. (ie) $\left(f/_A\right)^{-1}(S)$ is a G -open subset of A . $\Rightarrow f/_A$ is almost G -continuous.

**Definition:3.12**
Let G be a group together with a topology $\tau$ such that the multiplication and inverse map are almost G- continuous where $G \times G$ carries the product topology. Then the pair $(G, \tau)$ is called the **almost G- topological group** .

**Theorem.3.13**
Let $(G, \tau)$ be an almost G- topological group and $G \in G$ be any element of group G. Then (i) The right translation $r_g : G \to G$ defined by $r_g(a) = g.a$ for all $a \in G$ is almost G-continuous. (ii) The left translation $l_g : G \to G$ defined by $l_g(a) = a.g$ for all $a \in G$ is almost G -continuous.

**Proof:**
(i) Let $a \in G$ and W be a regularly G-open set in G containing g.a by definition of almost G- topological group there exists G -open neighbourhood U and V of g and a in G such that $UV \subseteq W$. Which means $gV \subseteq W \Rightarrow r_g(V) \subseteq W$. Therefore $r_g$ is almost G- continuous at a. Hence $r_g$ is almost G- continuous.

(ii) $a \in G$ and W be a regularly G-open set in G containing a.g then there exists G -open sets U in G containing x and V in G containing g such that $UV \subseteq W$. Since a was an arbitrary element of G, is $l_g$ almost G –continuous.

**Theorem:3.14**
Let A be any regularly G -open set in an almost G-topological group $(G, \tau)$. Then the following holds . (i) $gA \in$ RGO(G) for all $g \in G$. (ii) $Ag \in$ RGO(G) for all $g \in G$. (iii) $A^{-1} \in$ RGO(G).

**Proof:**
(i) First let us show that $gA \in \tau$ . Let $x \in gA$ be any element . By definition of almost G-topological groups , there exist G-open neighbourhoods U of $g^{-1}$ and V of x in G such that $UV \subseteq A$. In particular $g^{-1}V \subseteq A \Rightarrow V \subseteq gA$. This indicates $x \in$ Int(gA) and Int(gA)=gA. (i.e) $gA \in \tau$ . Now we prove that $gA \in$ RGO(G). $\Rightarrow gA \in$ $Int_G(Cl_G(gA))$. Since A is G-open . $Cl_G(A) \in$ RGC(G), since $Cl_G(A)$ is G-closed and $gCl_G(A)$ is also G -closed . $(Int_G(Cl_G(gA)) \subseteq Cl_G(gA) \subseteq gCl_G(A)$. $\Rightarrow g^{-1}(Int_G(Cl_G(A)) \subseteq Cl_G(gA)$. Since $(Int_G(Cl_G(gA))$ is regularly G-open. $\Rightarrow g^{-1}(Int_G(Cl_G(gA)) \subseteq (Int_G(Cl_G(A)) = G$ A. This implies $(Int_G(Cl_G(A)) \subseteq gA.gA = (Int_G(Cl_G(A))$

(ii) It is similar as (i)

(iii) Let $g \in A^{-1}$ . Then there exists an open set U in G containing x such that $U^{-1} \subseteq A$. $\Rightarrow U \subseteq A^{-1}$. x is an G-interior point of $A^{-1}$ (Since U is G-open). Hence $A^{-1}$ is G-open. $\Rightarrow A^{-1} \subseteq (Int_G(Cl_G(A^{-1}))$. Now it is enough to prove that $(Int_G(Cl_G(A^{-1})) \subseteq A^{-1}$ . Since A is G-open . $Cl_G(A)$ is regularly G -closed . Therefore $(Int_G(Cl_G(A^{-1}))) \subseteq (Cl_G(A^{-1})) \subseteq Cl_G(A)^{-1}$ . $\Rightarrow (Int_G(Cl_G(A^{-1}))) \subseteq (Int_G(Cl_G(A)))^{-1} = A^{-1}$ which is G -open .

**Theorem: 3.16**
Let F be any regularly G- closed set in an almost G -topological group G then the following holds (i) $gF \in R\ G\ C(G)$ for all $g \in G$ (ii) $Fg \in R\ G\ C(G)$ for all $g \in G$ (iii) $F^{-1} \in R\ G\ C(G)$

**Proof:**
(i) First let us show that $gF \in \tau$ . Let $x \in gA$ be any element . By definition of almost G-topological groups , there exist G-open neighbourhoods U of $g^{-1}$ and V of x in G such that $UV \subseteq A$. In particular $g^{-1}V \subseteq A \Rightarrow V \subseteq gA$. This indicates $x \in Int(gA)$ and Int(gF)=gA. (i.e) $gF \in \tau$ . Now we prove that $gF \in RGC(G)$. $\Rightarrow gF \in Cl_G(Int_G(gF))$. Since F is G-open .$Cl_G(F) \in RGC(G)$, since $Cl_G(F)$ is G-closed and $gCl_G(A)$ is also G -closed . $Cl_G(Int_G(gF)) \subseteq Cl_G(gF) \subseteq gCl_G(F). \Rightarrow g^{-1} Cl_G(Int_G(F)) \subseteq Cl_G(gF)$. Since $Cl_G(Int_G(gF))$ is regularly G-closed. $\Rightarrow g^{-} Cl_G(Int_G(gF)) \subseteq Cl_G(Int_G(F)) = G\ F$. This implies $Cl_G(Int_G(F)) \subseteq gF$. $gF\ Cl_G(Int_G(A))$
(ii) It follows from (i)
(iii) Let $g \in F^{-1}$. Then there exists an open set U in G containing x such that $U^{-1} \subseteq F$. $\Rightarrow U \subseteq F^{-1}$. x is an G-interior point of $F^{-1}$ (Since U is G-open). Hence $F^{-1}$ is G-open. $\Rightarrow F^{-1} \subseteq Cl_G(Int_G(F^{-1}))$. Now it is enough to prove that $Cl_G(Int_G(F^{-1})) \subseteq F^{-1}$ . Since F is G-open . $Cl_G(A)$ is regularly G -closed . Therefore $Cl_G(Int_G(F^{-1}))) \subseteq (Cl_G(F^{-1})) \subseteq Cl_G(F)^{-1}$ . $\Rightarrow Cl_G(Int_G(F^{-1}))) \subseteq Cl_G(Int_G(F)))^{-1} = F^{-1}$ which is G -open .

**Theorem.3.17**
Let A be any G -open set in an almost G- topological group G. Then $gA \subseteq Int_G(g\ Int_G\ Cl_G(A)))$ for all $g \in G$.

**Proof:**
Let A be any G -open, by definition of Regularly G -open $A \subseteq Int_G(Cl_G(A))$ . $\Rightarrow gA \subseteq g\ (Int_G\ (Cl_G(A)))$ . $\Rightarrow g\ Int_G(A) \subseteq Int_G\ (g\ Int_G\ (Cl_G(A)))$. By theorem 3.14 A is G –open. $gA \subseteq Int_G\ g\ (Int_G(Cl_G(A)))$.

**Theorem:3.18**
Let A be any G- closed set of an almost G -topological group. Then $Cl_G(g\ Cl_G(Int_G\ (B))) \subseteq gB$ for all $g \in G$

**Proof:**
It follows from the above theorem 3.17

## CONCLUSION

A weaker type of continuity for their group operations leads to the conclusion that virtually topological groups are a generalization of classical topological groups. The existence of specific examples that meet the "almost" requirements but are not topological groups in the strict sense confirms that generalized almost topological groups represent a larger class of spaces than regular topological groups.A weaker type of continuity for their group operations leads to the conclusion that virtually topological groups are a generalization of classical topological groups. The existence of specific examples that meet the "almost" requirements but are not topological groups in the strict sense confirms that generalized almost topological groups represent a larger class of spaces than regular topological groups.

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RESEARCH ARTICLE

# Formulation and DoE-Based Optimization of Telmisartan Orodispersible Tablets using Fenugreek Powder as a Natural Superdisintegrant and Comparison with Marketed Formulations

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## ABSTRACT

Telmisartan is a widely prescribed angiotensin II receptor blocker; however, its poor aqueous solubility and dissolution-limited bioavailability may compromise therapeutic performance in conventional oral dosage forms. Orodispersible tablets (ODTs) offer a patient-centric alternative by enabling rapid disintegration and improved drug dissolution without the need for water. The objective of the present study was to formulate, optimize, and evaluate telmisartan orodispersible tablets using fenugreek (*Trigonella foenum-graecum*) powder as a natural superdisintegrant by applying a Design of Experiments (DoE)-based Quality by Design (QbD) approach. ODTs were prepared by the direct compression method, and a three-factor, three-level experimental design was employed to investigate the effects of fenugreek powder, mannitol, and microcrystalline cellulose on critical quality attributes, including disintegration time, wetting time, tablet hardness, friability, and in-vitro drug release. Pre-compression blends exhibited acceptable flow and compressibility, confirming their suitability for direct compression. All formulations complied with pharmacopeial requirements during post-compression evaluation. DoE-based statistical analysis using response surface methodology and analysis of variance enabled identification of an optimized formulation. The optimized batch demonstrated rapid disintegration (18–22 s), adequate mechanical strength, friability below 1%, uniform drug content, and enhanced in-vitro drug release exceeding 98% within 30 minutes. Drug-release kinetics predominantly followed first-order and Higuchi models with non-Fickian transport behavior. FTIR and DSC studies confirmed drug–excipient

compatibility and indicated partial reduction in drug crystallinity, contributing to improved dissolution performance. Overall, the study establishes fenugreek powder as an effective, safe, and sustainable natural superdisintegrant and highlights the applicability of a DoE-driven QbD approach for developing robust, patient-friendly telmisartan orodispersible tablets.

**Keywords:** Telmisartan, Orodispersible tablets, Fenugreek powder, Natural superdisintegrant, Design of Experiments, Quality by Design

## INTRODUCTION

Hypertension is one of the most prevalent chronic cardiovascular disorders worldwide and a major risk factor for myocardial infarction, stroke, renal failure, and premature mortality [1]. Long-term pharmacotherapy is essential for effective blood pressure control; however, patient adherence remains a significant challenge, particularly among geriatric, paediatric, and dysphagic populations [2]. Difficulty in swallowing conventional tablets often leads to poor compliance and suboptimal therapeutic outcomes. Telmisartan is a potent angiotensin II receptor blocker (ARB) with a long elimination half-life and high affinity for $AT_1$ receptors, making it suitable for once-daily dosing [3]. Despite its favorable pharmacodynamics, Telmisartan is classified as a BCS Class II drug due to its poor aqueous solubility, resulting in dissolution-limited absorption and variable bioavailability [4]. Consequently, formulation strategies that enhance dissolution and patient acceptability are highly desirable. Orodispersible tablets (ODTs) represent an advanced oral dosage form designed to disintegrate rapidly in the oral cavity without water, thereby improving patient convenience and enabling faster drug dissolution [5]. The performance of ODTs is largely governed by the choice of superdisintegrant. While synthetic superdisintegrants are effective, increasing interest in sustainability and biocompatibility has driven the exploration of natural alternatives [6]. Fenugreek seed powder contains mucilage rich in galactomannans and polysaccharides that exhibit high swelling and hydration capacity, making it a promising natural superdisintegrant [7–9]. Design of Experiments (DoE), implemented under Quality by Design (QbD) principles, enables systematic optimization of formulation variables while minimizing experimental effort [10]. The present work applies a DoE-based strategy to optimize fenugreek-based Telmisartan ODTs and compare their performance with marketed formulations.

**Drug and Excipient Profiles**

**Telmisartan**Telmisartan is a selective angiotensin II $AT_1$ receptor antagonist used in the management of hypertension and cardiovascular risk reduction. It exhibits high lipophilicity, poor aqueous solubility, and high membrane permeability, resulting in dissolution-limited absorption. Improving its dissolution behavior is critical for enhanced therapeutic performance. The IUPAC name of Telmisartan is 2-(4-{[4-Methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl]methyl}phenyl)benzoic acid with a molecular weight of 514.63 g/mol and an empirical formula of $C_{33}H_{30}N_4O_2$.

**Chemical structure of Telmisartan (Figure 1).**

***Source:*** *https://en.wikipedia.org/wiki/Telmisartan#/media/File:Telmisartan.svg*

Fenugreek (*Trigonella foenum-graecum*) seed powder is rich in hydrophilic mucilage, galactomannans, and polysaccharides. Upon hydration, it swells rapidly, generating internal pressure that promotes tablet disintegration. Its natural origin, biocompatibility, cost-effectiveness, and eco-friendly nature make it a suitable alternative to synthetic superdisintegrants. Mannitol is a water-soluble diluent widely used in ODTs due to its pleasant cooling sensation, sweetness, and ability to enhance wetting and mouthfeel [11]. MCC (Avicel PH 102) acts as a binder and wicking agent, improving compressibility and facilitating water penetration into the tablet matrix [12]. Aspartame was used as a sweetener, sodium starch glycolate as an auxiliary superdisintegrant, magnesium stearate as a lubricant, and talc as a glidant, all at pharmaceutically acceptable levels.

# MATERIALS AND METHODS

## MATERIALS

Telmisartan was used as the active pharmaceutical ingredient and procured from Sun Pharmaceutical Industries Ltd., India. Fenugreek (*Trigonella foenum-graecum*) powder was employed as a natural superdisintegrant and obtained from a certified supplier, followed by authentication prior to use. Mannitol was used as a diluent and mouthfeel enhancer, while microcrystalline cellulose (Avicel PH 102) served as a binder and wicking agent. Sodium starch glycolate was incorporated as an auxiliary superdisintegrant, aspartame as a sweetener, magnesium stearate as a lubricant, and talc as a glidant. All excipients were of pharmaceutical grade and complied with relevant pharmacopeial standards. A marketed Telmisartan dispersible tablet (TELKIZ 40) was used for comparative evaluation.

**Formulation of Telmisartan Orodispersible Tablets**

Telmisartan orodispersible tablets were prepared by the direct compression method. All ingredients were passed through a suitable sieve, blended uniformly using geometric dilution, and lubricated with magnesium stearate and talc. The final blend was compressed using 8-mm flat-faced punches to obtain tablets with adequate mechanical strength and rapid disintegration properties. Composition of Telmisartan Orodispersible Tablet Formulations (F1–F9) (Table 1).

**Experimental Design (Design of Experiments)**

**Rationale for Selection of Design of Experiments**

Preliminary formulation trials indicated that the concentrations of fenugreek powder, mannitol, and microcrystalline cellulose (MCC) exert a significant influence on the disintegration behavior, mechanical strength, and dissolution performance of Telmisartan orodispersible tablets. Conventional one-factor-at-a-time optimization was found to be insufficient to capture interaction effects among formulation variables. Therefore, a Design of Experiments (DoE) approach was adopted to enable systematic investigation of multiple variables simultaneously and to establish a statistically justified formulation design space in accordance with Quality by Design principles.

**Selection of Independent Variables and Responses**

Based on pre-DoE screening studies, three formulation factors were identified as critical material attributes (CMAs), and key tablet performance parameters were selected as critical quality attributes (CQAs). Pre-DoE Screening of Factors and Responses (Table 2).

**Experimental Design and Factor Levels**

A three-factor, three-level ($3^3$) factorial design was selected to study linear, interaction, and quadratic effects of formulation variables with a limited number of experimental runs. This design enables generation of predictive polynomial models and response surface plots for optimization. Independent Variables and Coded Levels (Table 3).

**DoE Experimental Design Matrix**

The experimental runs were generated using Design-Expert® software. Nine formulations (F1–F9) were prepared as per the design matrix. DoE-Generated Experimental Design and Observed Responses (Table 4). *Values represent mean ± SD (n = 3).

**Evaluation of Powder Blends and Tablets**

The prepared powder blends were evaluated for pre-compression parameters, including angle of repose, bulk density, tapped density, Carr's index, and Hausner ratio, to assess flowability and compressibility. The compressed tablets were evaluated for post-compression characteristics such as hardness, friability, weight variation, wetting

time, disintegration time, drug content uniformity, and in-vitro dissolution using USP Type II apparatus in pH 6.8 phosphate buffer.

**Compatibility and Statistical Analysis**

Drug–excipient compatibility and solid-state characteristics were assessed using Fourier Transform Infrared (FTIR) spectroscopy and Differential Scanning Calorimetry (DSC). Statistical analysis of the experimental data was performed using response surface methodology, analysis of variance, and desirability function to identify the optimized formulation. The optimized batch was further compared with the marketed Telmisartan formulation to evaluate improvements in disintegration behavior and dissolution performance. Preliminary formulation trials indicated that fenugreek powder, mannitol, and microcrystalline cellulose significantly influenced the disintegration behavior, mechanical strength, and dissolution performance of Telmisartan orodispersible tablets. Conventional trial-and-error optimization was insufficient to capture interaction effects among these variables. Therefore, a Design of Experiments (DoE) approach was employed to systematically evaluate factor effects and interactions and to establish an optimized formulation design space in accordance with Quality by Design principles.

## RESULTS AND DISCUSSION

**Pre-Compression Properties**

The pre-compression parameters of the powder blends were evaluated to assess flowability and compressibility, which are critical for uniform die filling and successful direct compression. Summary of Pre-Compression Parameters of Powder Blends (F1–F9) (Table 5). The pre-compression parameters indicated that all powder blends exhibited good to excellent flow properties, as reflected by angle of repose values within acceptable limits. Bulk and tapped density values demonstrated normal packing behavior, while Carr's index and Hausner ratio confirmed satisfactory compressibility of the blends. Slight variations among formulations were observed due to differences in fenugreek and microcrystalline cellulose content; however, these variations did not adversely affect flowability. Overall, the results confirmed that all blends were suitable for direct compression and ensured uniform tablet manufacture without processing difficulties

**UV Spectrophotometric Analysis**

UV spectrophotometric analysis was carried out to develop a simple and reliable method for the quantitative estimation of Telmisartan in drug content and in-vitro dissolution studies UV Absorption Data of Telmisartan in pH 6.8 Phosphate Buffer (Table 6). UV Absorption Spectrum of Telmisartan (Figure 2). Calibration Data of Telmisartan in pH 6.8 Phosphate Buffer (Table 7). Calibration curve of Telmisartan in pH 6.8 phosphate buffer at 296 nm (Figure 3). The UV absorption spectrum of Telmisartan in pH 6.8 phosphate buffer exhibited a well-defined maximum absorbance ($\lambda$max) at 296 nm, which was selected for further quantitative analysis. The calibration curve constructed over the concentration range of 2–10 µg/mL showed excellent linearity with a high correlation coefficient ($R^2 \approx 0.999$), indicating a strong linear relationship between absorbance and concentration. The linearity of the calibration curve confirms the reliability and suitability of the developed UV spectrophotometric method for drug content determination and analysis of in-vitro dissolution samples without interference from formulation excipients.

**Post-Compression Evaluation of Telmisartan ODTs**

The prepared Telmisartan orodispersible tablets were evaluated for post-compression quality attributes to ensure compliance with pharmacopeial standards and suitability for orodispersible tablet applications Summary of Post-Compression Evaluation Parameters (Table 8). The post-compression evaluation results indicated that all formulations possessed adequate mechanical strength, with hardness values sufficient to withstand handling and friability remaining below the acceptable limit of 1%. Weight variation and drug content uniformity complied with pharmacopeial specifications, indicating uniform die filling and homogeneous drug distribution. Wetting time and disintegration time values were within the acceptable range for orodispersible tablets, confirming the effectiveness of

fenugreek powder in promoting rapid tablet hydration and disintegration. Overall, the post-compression results confirmed that all formulations met the essential quality requirements for orodispersible tablet dosage forms.

**Wetting Time and Disintegration Behavior**

Wetting time and disintegration time are critical quality attributes for orodispersible tablets, as they directly influence tablet hydration, patient acceptability, and onset of action. Wetting Time and Disintegration Time of ODTs (F1–F9) (Table 9). An inverse relationship was evident between the concentration of fenugreek powder and both wetting time and disintegration time. Formulations containing higher amounts of fenugreek showed quicker wetting and more rapid tablet disintegration, which can be attributed to the strong swelling behavior and mucilage-forming properties of fenugreek that facilitate efficient water uptake and generate internal pressure within the tablet matrix. Mannitol further enhanced this effect by improving tablet wettability, while microcrystalline cellulose helped preserve tablet integrity during the disintegration process. Overall, these findings demonstrate the effectiveness of fenugreek powder as a natural superdisintegrant in the development of orodispersible tablet formulations.

***In-Vitro* Dissolution Studies**

In-vitro dissolution studies were carried out to evaluate the drug-release behavior of Telmisartan from the formulated orodispersible tablets and to assess the influence of formulation variables on dissolution performance. Comparison of drug release at various time intervals (Table 10). Graph showing *In-Vitro* Dissolution Profiles (F1–F9) (Figure 4). The *in-vitro* dissolution results showed a noticeable enhancement in drug-release performance as the concentration of fenugreek powder increased. Formulations containing lower amounts of fenugreek exhibited slower initial drug release, while those with higher fenugreek levels demonstrated faster and more complete release. This improvement is primarily attributed to rapid tablet disintegration, better wettability, and increased surface area availability of the drug for dissolution. Mannitol further contributed to enhanced dissolution by improving tablet porosity and wettability. Among the tested formulations, batch F7 displayed the most rapid and consistent drug-release profile, achieving nearly complete release within the designated time period, thereby confirming its selection as the optimized formulation.

**DoE-Based Optimization and Statistical Interpretation**

A Design of Experiments (DoE) approach was applied to systematically evaluate the individual and combined effects of fenugreek powder, mannitol, and microcrystalline cellulose on the critical quality attributes of Telmisartan orodispersible tablets and to identify an optimized formulation within the experimental design space. ANOVA for Disintegration Time (DT) (Table 11). ANOVA for Wetting Time (WT) (Table 12). ANOVA for Hardness (Table 13). ANOVA for Friability (Table 14). ANOVA for % Drug Release at 30 Minutes (Table 15). Analysis of variance revealed that fenugreek concentration exerted the most significant influence on disintegration time and drug release, confirming its dominant role as a natural superdisintegrant. Mannitol also showed a statistically significant contribution, particularly in enhancing wettability and dissolution behavior. Microcrystalline cellulose primarily influenced mechanical strength parameters such as hardness and friability. The statistical significance of interaction terms indicates that formulation performance is governed by the combined effects of excipients rather than individual factors alone, thereby validating the application of a DoE-based optimization strategy. RSM Plots representing various parameters (Figure 5). Response surface analysis demonstrated a clear relationship between formulation variables and tablet performance. Increasing fenugreek concentration consistently reduced disintegration and wetting times, while higher mannitol levels enhanced drug release by improving wettability. Microcrystalline cellulose provided a balancing effect by improving tablet strength without excessively prolonging disintegration. The response surface plots clearly identified an optimal formulation region that satisfied rapid disintegration, enhanced dissolution, and acceptable mechanical integrity. Multi-response optimization using the desirability function identified formulation F7 as the optimized batch, fulfilling all predefined criteria, including minimum disintegration time, rapid wetting, friability below 1%, acceptable hardness, and maximum drug release. The close agreement between predicted and experimental responses confirms the robustness and predictive capability of the developed DoE model, establishing F7 as a scientifically optimized formulation rather than an empirical selection.

**Drug-Release Kinetics of Optimized Telmisartan Orodispersible Tablet (F7)**

Drug-release kinetic modeling was performed to elucidate the mechanism of Telmisartan release from the optimized orodispersible tablet formulation (F7) and to understand the influence of formulation components on drug-release behaviour. Drug Release Kinetic Models for Optimized Telmisartan ODT (F7) (Figure 6). Summary of Drug Release Kinetic Models for Optimized Telmisartan ODT (Table 16). The dissolution data of the optimized formulation (F7) showed the highest correlation with the first-order kinetic model, indicating concentration-dependent drug release. A good fit with the Higuchi model further suggests that drug release is predominantly governed by diffusion from a hydrated matrix. The Korsmeyer–Peppas model exhibited an exponent value (n) between 0.45 and 0.89, indicating a non-Fickian transport mechanism. This behavior reflects a combined effect of diffusion and polymer swelling, which can be attributed to the mucilage-forming and swelling properties of fenugreek powder. The rapid tablet disintegration, enhanced wetting, and porous matrix formation collectively contribute to the observed release mechanism. These findings confirm that the optimized formulation provides controlled yet rapid drug release, suitable for orodispersible tablet systems.

**Comparison of Optimized Telmisartan Orodispersible Tablet with Marketed Formulation**

The optimized Telmisartan orodispersible tablet formulation (F7) was compared with a commercially available Telmisartan dispersible tablet to evaluate differences in disintegration behavior and drug-release performance.

**Comparison with Marketed Formulation**

Comparison of Drug Release Kinetics Between Optimized ODT and Marketed ODT (Table 17). The optimized formulation exhibited shorter wetting and disintegration times compared to the marketed tablet, indicating faster tablet hydration and breakup. This improvement can be attributed to the swelling and mucilage-forming properties of fenugreek powder, which promote rapid water uptake and disintegration. Faster disintegration of the optimized formulation is expected to improve patient compliance and onset of action. Comparison of Dissolution Profiles of Optimized Telmisartan ODT and Marketed Telmisartan ODT (Figure 7). The *in-vitro* dissolution profile demonstrated that the optimized formulation achieved faster and more complete drug release at early time points compared to the marketed formulation. The enhanced dissolution performance of the optimized tablet can be attributed to rapid disintegration, improved wettability, and increased surface area exposure of the drug. These results indicate superior dissolution behavior of the optimized formulation. Overall, the optimized Telmisartan orodispersible tablet showed superior performance over the marketed formulation, confirming the effectiveness of fenugreek powder as a natural superdisintegrant and the advantage of the DoE-optimized formulation strategy.

**Fourier Transform Infrared (FTIR) Analysis**

Fourier Transform Infrared (FTIR) spectroscopy was performed to evaluate possible chemical interactions between Telmisartan and formulation excipients and to assess the compatibility of the drug within the optimized orodispersible tablet formulation. FTIR spectrum of Pure Telmisartan and Optimized Telmisartan ODT formulation (Figure 8). FTIR Peak Assignment Table (Table 18). The FTIR spectrum of pure Telmisartan exhibited characteristic absorption peaks corresponding to its functional groups, including N–H stretching, C–H stretching, C=O stretching, and aromatic ring vibrations. These characteristic peaks were retained in the FTIR spectrum of the optimized formulation (F7) with no significant disappearance or formation of new peaks. Minor shifts in peak positions and changes in peak intensity were observed, which can be attributed to physical interactions such as hydrogen bonding and dilution effects rather than chemical incompatibility. The absence of additional peaks confirms that no chemical interaction occurred between Telmisartan and the formulation excipients. These results indicate that Telmisartan is chemically compatible with fenugreek powder and other excipients used in the formulation. Overall, FTIR analysis confirmed the chemical stability and compatibility of Telmisartan within the optimized orodispersible tablet formulation.

## CONCLUSION

In this study, telmisartan orodispersible tablets were successfully developed and optimized using fenugreek (*Trigonella foenum-graecum*) powder as a natural superdisintegrant. A Design of Experiments–based Quality by Design approach was employed to systematically understand the influence of formulation variables on tablet performance. The optimized formulation showed rapid wetting and disintegration, satisfactory mechanical strength, low friability, uniform drug content, and improved in-vitro drug release. The results clearly indicated that fenugreek powder played a key role in accelerating tablet disintegration and enhancing dissolution, while mannitol and microcrystalline cellulose supported wettability and mechanical stability, respectively. Drug-release kinetic analysis suggested a non-Fickian release mechanism, reflecting the combined contribution of diffusion and swelling processes. When compared with a marketed telmisartan dispersible tablet, the optimized formulation demonstrated faster disintegration and superior dissolution behavior. FTIR studies further confirmed the compatibility and chemical stability of telmisartan with the selected excipients. Overall, the findings highlight the potential of fenugreek powder as an effective, safe, and sustainable natural superdisintegrant for orodispersible tablet formulations. The DoE-guided formulation strategy enabled the development of a robust and patient-friendly telmisartan dosage form and supports the broader application of natural excipients in optimized oral drug delivery systems.

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**Table 1: Composition of Telmisartan Orodispersible Tablet Formulations (F1–F9)**

| Ingredient (mg) | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
|---|---|---|---|---|---|---|---|---|---|
| **Telmisartan** | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 |
| **Fenugreek powder** | 12.5 | 12.5 | 12.5 | 18.75 | 18.75 | 18.75 | 25 | 25 | 25 |
| **Mannitol** | 100 | 75 | 50 | 100 | 75 | 50 | 100 | 75 | 50 |
| **MCC (Avicel PH 102)** | 50 | 75 | 100 | 50 | 75 | 100 | 50 | 75 | 100 |
| **Aspartame** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| **Sodium Starch Glycolate (SSG)** | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Magnesium Stearate** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

**Table 2: Pre-DoE Screening of Factors and Responses**

| Parameter Type | Variable | Range Explored | Observed Effect | Justification for DoE |
|---|---|---|---|---|
| **Factor A** | Fenugreek powder (mg) | 10–25 | Strong reduction in disintegration time with increasing level | Selected as primary DoE factor |
| **Factor B** | Mannitol (mg) | 50–100 | Improved wetting and dissolution | Included as DoE factor |
| **Factor C** | MCC (mg) | 50–100 | Enhanced compressibility; excess increased friability | Included with controlled range |
| **Response $Y_1$** | Disintegration time (sec) | 28–68 | Highly sensitive to fenugreek level | Primary optimization response |
| **Response $Y_2$** | % Drug release (15 min) | 82–96 | Influenced by fenugreek and mannitol | Primary optimization response |
| **Response $Y_3$** | Friability (%) | 0.46–0.96 | Affected mainly by MCC content | Secondary optimization response |
| **Response $Y_4$** | Hardness (kg/cm²) | 3.1–3.8 | Within acceptable range | Controlled response |

**Table 3: Independent Variables and Coded Levels**

| Factor | Variable | Low (–1) | Medium (0) | High (+1) |
|---|---|---|---|---|
| **A** | Fenugreek powder (mg) | 12.5 | 18.75 | 25.0 |
| **B** | Mannitol (mg) | 50 | 75 | 100 |
| **C** | MCC (mg) | 50 | 75 | 100 |

**Table 4: DoE-Generated Experimental Design and Observed Responses**
***Values represent mean ± SD (n = 3).**

| Run | Fenugreek (mg) | Mannitol (mg) | MCC (mg) | Disintegration Time (sec) | % Drug Release | Friability (%) |
|---|---|---|---|---|---|---|
| F1 | 12.5 | 100 | 50 | 52 | 86.3 | 0.82 |
| F2 | 12.5 | 75 | 75 | 46 | 89.7 | 0.74 |
| F3 | 12.5 | 50 | 100 | 41 | 92.4 | 0.69 |
| F4 | 18.75 | 100 | 50 | 34 | 94.6 | 0.56 |
| F5 | 18.75 | 75 | 75 | 31 | 95.8 | 0.52 |
| F6 | 18.75 | 50 | 100 | 30 | 96.1 | 0.50 |
| F7 | 25.0 | 100 | 50 | 28 | 97.4 | 0.46 |
| F8 | 25.0 | 75 | 75 | 29 | 96.8 | 0.48 |
| F9 | 25.0 | 50 | 100 | 32 | 95.2 | 0.55 |

**Table 5: Summary of Pre-Compression Parameters of Powder Blends (F1–F9)**

| Formulation | Angle of Repose (°) | Bulk Density (g/cm³) | Tapped Density (g/cm³) | Carr's Index (%) | Hausner Ratio | Flow Property |
|---|---|---|---|---|---|---|
| F1 | 31.45 ± 0.22 | 0.42 ± 0.01 | 0.50 ± 0.02 | 16.00 ± 0.5 | 1.19 ± 0.02 | Good |
| F2 | 29.87 ± 0.18 | 0.44 ± 0.01 | 0.52 ± 0.01 | 15.38 ± 0.4 | 1.18 ± 0.01 | Excellent |
| F3 | 33.12 ± 0.25 | 0.41 ± 0.02 | 0.49 ± 0.02 | 16.32 ± 0.6 | 1.19 ± 0.02 | Good |
| F4 | 30.04 ± 0.14 | 0.43 ± 0.01 | 0.51 ± 0.02 | 15.68 ± 0.5 | 1.18 ± 0.02 | Good |
| F5 | 32.66 ± 0.20 | 0.45 ± 0.01 | 0.53 ± 0.01 | 15.09 ± 0.4 | 1.17 ± 0.01 | Good |
| F6 | 34.21 ± 0.27 | 0.42 ± 0.02 | 0.50 ± 0.01 | 16.00 ± 0.5 | 1.19 ± 0.02 | Fair |
| F7 | 28.94 ± 0.16 | 0.46 ± 0.01 | 0.54 ± 0.02 | 14.81 ± 0.4 | 1.17 ± 0.01 | Excellent |
| F8 | 30.88 ± 0.19 | 0.44 ± 0.02 | 0.52 ± 0.01 | 15.38 ± 0.5 | 1.18 ± 0.02 | Good |
| F9 | 33.87 ± 0.24 | 0.43 ± 0.01 | 0.51 ± 0.01 | 15.68 ± 0.5 | 1.18 ± 0.02 | Good |

**Table 6: UV Absorption Data of Telmisartan in pH 6.8 Phosphate Buffer**

| Wavelength (nm) | Absorbance |
|---|---|
| 220 | 0.312 |
| 240 | 0.428 |
| 260 | 0.563 |
| 280 | 0.691 |
| **296** | **0.742** |
| 310 | 0.618 |
| 330 | 0.402 |
| 350 | 0.215 |
| 370 | 0.098 |

**Table 7: Calibration Data of Telmisartan in pH 6.8 Phosphate Buffer**

| Concentration (µg/mL) | Absorbance (Mean ± SD, n=3) |
|---|---|
| 2 | 0.154 ± 0.003 |
| 4 | 0.298 ± 0.004 |
| 6 | 0.446 ± 0.005 |
| 8 | 0.593 ± 0.006 |
| 10 | 0.741 ± 0.007 |

**Table 8: Summary of Post-Compression Evaluation Parameters**

| Parameter | Requirements | Range Observed (F1–F9) | Status |
|---|---|---|---|
| Hardness | 2.5–4.0 kg/cm² | 2.8–3.5 | ✓ Acceptable |
| Friability | < 1% | 0.54–0.63% | ✓ Acceptable |
| Weight Variation | ±5% or ±7.5% | 0.71–0.93% | ✓ Compliant |
| Wetting Time | < 20–30 sec | 14–32 sec | ✓ Matches ODT criteria |
| Disintegration Time | < 30 sec | 18–41 sec | ✓ ODT requirement met |
| Drug Content | 95–105% | 97.88–99.80% | ✓ Within limits |

**Table 9: Wetting Time and Disintegration Time of ODTs (F1–F9)**

| Formulation | Wetting Time (sec) | Disintegration Time (sec) |
|---|---|---|
| F1 | 32 ± 1.5 | 41 ± 1.2 |
| F2 | 28 ± 1.4 | 38 ± 1.1 |
| F3 | 26 ± 1.3 | 36 ± 1.0 |
| F4 | 18 ± 1.2 | 22 ± 0.9 |
| F5 | 20 ± 1.3 | 25 ± 1.0 |
| F6 | 21 ± 1.2 | 28 ± 1.1 |
| F7 | 14 ± 1.1 | 18 ± 0.8 |
| F8 | 16 ± 1.1 | 20 ± 0.9 |
| F9 | 17 ± 1.2 | 21 ± 1.0 |

**Table 10: Comparison of drug release at various time intervals**

| Formulation | 5 min (%) | 10 min (%) | 15 min (%) | 20 min (%) | 30 min (%) |
|---|---|---|---|---|---|
| F1 | 32.5 ± 1.1 | 48.2 ± 1.3 | 59.8 ± 1.4 | 68.7 ± 1.6 | 79.4 ± 1.8 |
| F2 | 35.8 ± 1.2 | 52.4 ± 1.3 | 64.3 ± 1.5 | 73.6 ± 1.7 | 82.1 ± 1.9 |
| F3 | 38.6 ± 1.2 | 55.9 ± 1.4 | 68.7 ± 1.5 | 76.9 ± 1.6 | 84.5 ± 1.8 |
| F4 | 52.4 ± 1.3 | 72.6 ± 1.5 | 85.4 ± 1.7 | 92.1 ± 1.8 | 98.6 ± 1.9 |
| F5 | 49.2 ± 1.3 | 69.8 ± 1.5 | 82.3 ± 1.6 | 89.4 ± 1.7 | 97.2 ± 1.8 |

| | | | | | |
|---|---|---|---|---|---|
| F6 | 46.7 ± 1.3 | 66.4 ± 1.4 | 78.6 ± 1.6 | 86.2 ± 1.7 | 94.9 ± 1.7 |
| F7 | 58.3 ± 1.4 | 76.8 ± 1.5 | 89.7 ± 1.7 | 95.6 ± 1.8 | 99.1 ± 1.9 |
| F8 | 55.3 ± 1.3 | 73.4 ± 1.5 | 87.1 ± 1.7 | 93.4 ± 1.7 | 98.4 ± 1.8 |
| F9 | 53.1 ± 1.3 | 71.5 ± 1.5 | 86.4 ± 1.6 | 92.8 ± 1.8 | 98.2 ± 1.9 |

**Table 11: ANOVA for Disintegration Time (DT)**

| Source | Sum of Squares (SS) | df | Mean Square (MS) | F-value | p-value |
|---|---|---|---|---|---|
| Model | 980.45 | 6 | 163.41 | 42.85 | 0.0004* |
| A – Fenugreek | 410.32 | 1 | 410.32 | 107.52 | 0.0001* |
| B – Mannitol | 220.45 | 1 | 220.45 | 57.79 | 0.0006* |
| C – MCC | 102.88 | 1 | 102.88 | 26.97 | 0.0023* |
| AB | 61.12 | 1 | 61.12 | 15.99 | 0.0071* |
| AC | 33.17 | 1 | 33.17 | 8.74 | 0.021* |
| BC | 24.51 | 1 | 24.51 | 6.42 | 0.039* |
| Residual | 19.12 | 2 | 9.56 | — | — |
| Lack of Fit | 12.85 | 1 | 12.85 | 2.14 | 0.29 |
| Pure Error | 6.27 | 1 | 6.27 | — | — |
| **Total** | **999.57** | **8** | — | — | — |

**Table 12: ANOVA for Wetting Time (WT)**

| Source | SS | df | MS | F-value | p-value |
|---|---|---|---|---|---|
| Model | 620.24 | 6 | 103.37 | 36.11 | 0.0008* |
| A – Fenugreek | 290.73 | 1 | 290.73 | 101.47 | 0.0002* |
| B – Mannitol | 140.45 | 1 | 140.45 | 49.04 | 0.0011* |
| C – MCC | 85.66 | 1 | 85.66 | 29.94 | 0.0020* |
| AB | 41.23 | 1 | 41.23 | 14.38 | 0.0090* |
| AC | 29.44 | 1 | 29.44 | 10.29 | 0.016* |
| BC | 22.73 | 1 | 22.73 | 7.95 | 0.024* |
| Residual | 5.73 | 2 | 2.86 | — | — |
| Lack of Fit | 3.22 | 1 | 3.22 | 1.15 | 0.39 |
| Pure Error | 2.51 | 1 | 2.51 | — | — |
| Total | 625.97 | 8 | — | — | — |

**Table 13: ANOVA for Hardness**

| Source | SS | df | MS | F-value | p-value |
|---|---|---|---|---|---|
| Model | 1.622 | 6 | 0.270 | 18.94 | 0.003* |
| A – Fenugreek | 0.344 | 1 | 0.344 | 24.12 | 0.006* |
| B – Mannitol | 0.128 | 1 | 0.128 | 9.01 | 0.020* |
| C – MCC | 0.810 | 1 | 0.810 | 56.77 | 0.001* |
| AB | 0.062 | 1 | 0.062 | 4.32 | 0.087 |
| AC | 0.045 | 1 | 0.045 | 3.17 | 0.12 |
| BC | 0.233 | 1 | 0.233 | 16.33 | 0.014* |
| Residual | 0.028 | 2 | 0.014 | — | — |
| Total | 1.650 | 8 | — | — | — |

**Table 14: ANOVA for Friability**

| Source | SS | df | MS | F-value | p-value |
|---|---|---|---|---|---|
| Model | 0.0191 | 6 | 0.00318 | 22.88 | 0.002* |

| | | | | | |
|---|---|---|---|---|---|
| A – Fenugreek | 0.0064 | 1 | 0.0064 | 46.11 | 0.0013* |
| B – Mannitol | 0.0029 | 1 | 0.0029 | 20.87 | 0.004* |
| C – MCC | 0.0041 | 1 | 0.0041 | 29.46 | 0.002* |
| AB | 0.0015 | 1 | 0.0015 | 10.78 | 0.016* |
| AC | 0.0011 | 1 | 0.0011 | 7.92 | 0.024* |
| BC | 0.0008 | 1 | 0.0008 | 6.71 | 0.036* |
| Residual | 0.00028 | 2 | 0.00014 | — | — |
| Total | 0.01938 | 8 | — | — | — |

**Table 15: ANOVA for % Drug Release at 30 Minutes**

| Source | SS | df | MS | F-value | p-value |
|---|---|---|---|---|---|
| Model | 420.14 | 6 | 70.02 | 44.65 | 0.0004* |
| A – Fenugreek | 168.42 | 1 | 168.42 | 107.37 | 0.0001* |
| B – Mannitol | 112.11 | 1 | 112.11 | 71.44 | 0.0003* |
| C – MCC | 62.13 | 1 | 62.13 | 39.59 | 0.0008* |
| AB | 32.78 | 1 | 32.78 | 20.88 | 0.004* |
| AC | 24.92 | 1 | 24.92 | 15.88 | 0.007* |
| BC | 19.78 | 1 | 19.78 | 12.61 | 0.012* |
| Residual | 3.13 | 2 | 1.56 | — | — |
| Total | 423.27 | 8 | — | — | — |

**Table 16: Summary of Drug Release Kinetic Models for Optimized Telmisartan ODT**

| Kinetic Model | Plot Description | Regression Coefficient ($R^2$) | Key Interpretation |
|---|---|---|---|
| Zero-order | % Drug release vs Time | 0.892 | Poor linearity indicates drug release is not constant and does not follow zero-order kinetics |
| First-order | Log (100 – % release) vs Time | 0.981 | High linearity confirms concentration-dependent release, typical of fast-disintegrating systems |
| Higuchi | % Drug release vs √Time | 0.964 | Indicates diffusion-controlled release from a hydrated matrix |
| Korsmeyer–Peppas | Log % release vs Log time | 0.972 | Suggests anomalous (non-Fickian) transport mechanism |
| Release exponent (n) | — | 0.62 | Confirms combined diffusion + swelling-controlled release |

**Table 17:Comparison of Drug Release Kinetics Between Optimized ODT and Marketed ODT**

| Parameter | Optimized Telmisartan ODT (F7) | Marketed Telmisartan ODT (TELKIZ 40) |
|---|---|---|
| Disintegration time | **18-22 sec** | Approx. 29 sec |
| Wetting time | 12 sec | 20 sec |
| % Drug release at 5 min | **58%** | 42% |
| % Drug release at 10 min | **77%** | 62% |
| % Drug release at 15 min | **90%** | 79% |
| % Drug release at 30 min | **99%** | 92% |
| Dominant release kinetics | First-order + diffusion | First-order |
| Release mechanism | Non-Fickian (diffusion + swelling) | Predominantly diffusion |
| Super disintegrant | Fenugreek powder + SSG | Synthetic |

**Table 18: FTIR Peak Assignment Table**

| Functional Group | Pure Telmisartan ($cm^{-1}$) | Formulation ($cm^{-1}$) | %T Intensity Behavior | Interpretation |
|---|---|---|---|---|
| N–H stretching | 3300 | 3295 | Slightly higher %T | Minor shift; hydrogen bonding with excipients |
| Aliphatic C–H stretch | 2920 | 2915 | Slightly higher %T | No chemical interaction; physical dilution effect |
| C=O stretching (carboxyl) | 1705 | 1702 | Slightly higher %T | Strong peak remains – drug stable |
| Aromatic C=C | 1450 | 1452 | Minimal change | Structure preserved |
| C–H bending | 750 | 748 | Minimal change | No interaction |
| O–H stretching (excipients) | Broad | Broad | Overlapping regions | Normal with MCC and fenugreek |
| Polysaccharide peaks (fenugreek) | — | 1000–1200 | Expected new bands | Belong to excipients; no interaction |

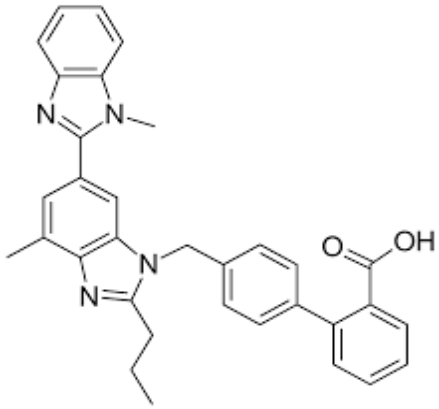



**Figure 1: Chemical structure of Telmisartan**
***Source: https://en.wikipedia.org/wiki/Telmisartan#/media/File:Telmisartan.svg***

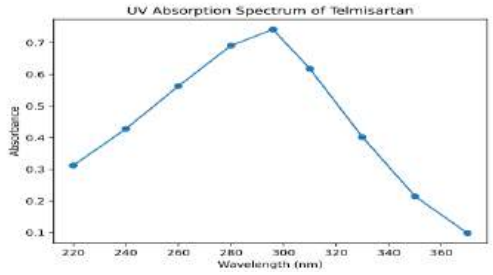



**Figure 2: UV Absorption Spectrum of Telmisartan**

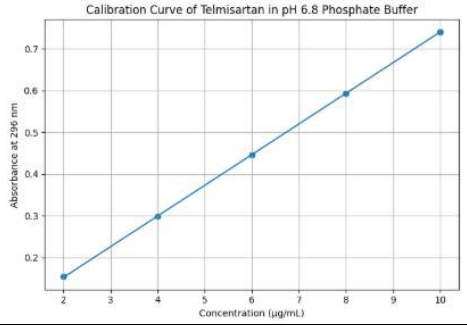



**Figure 3:Calibration curve of Telmisartan in pH 6.8 phosphate buffer at 296 nm**

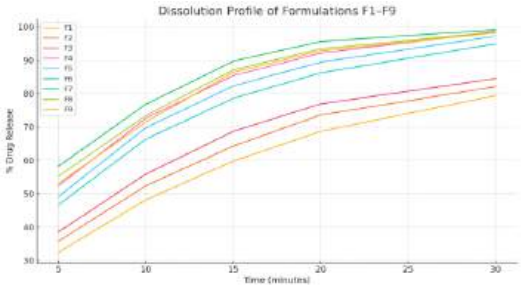



**Figure 4: Graph showing In-Vitro Dissolution Profiles (F1–F9)**

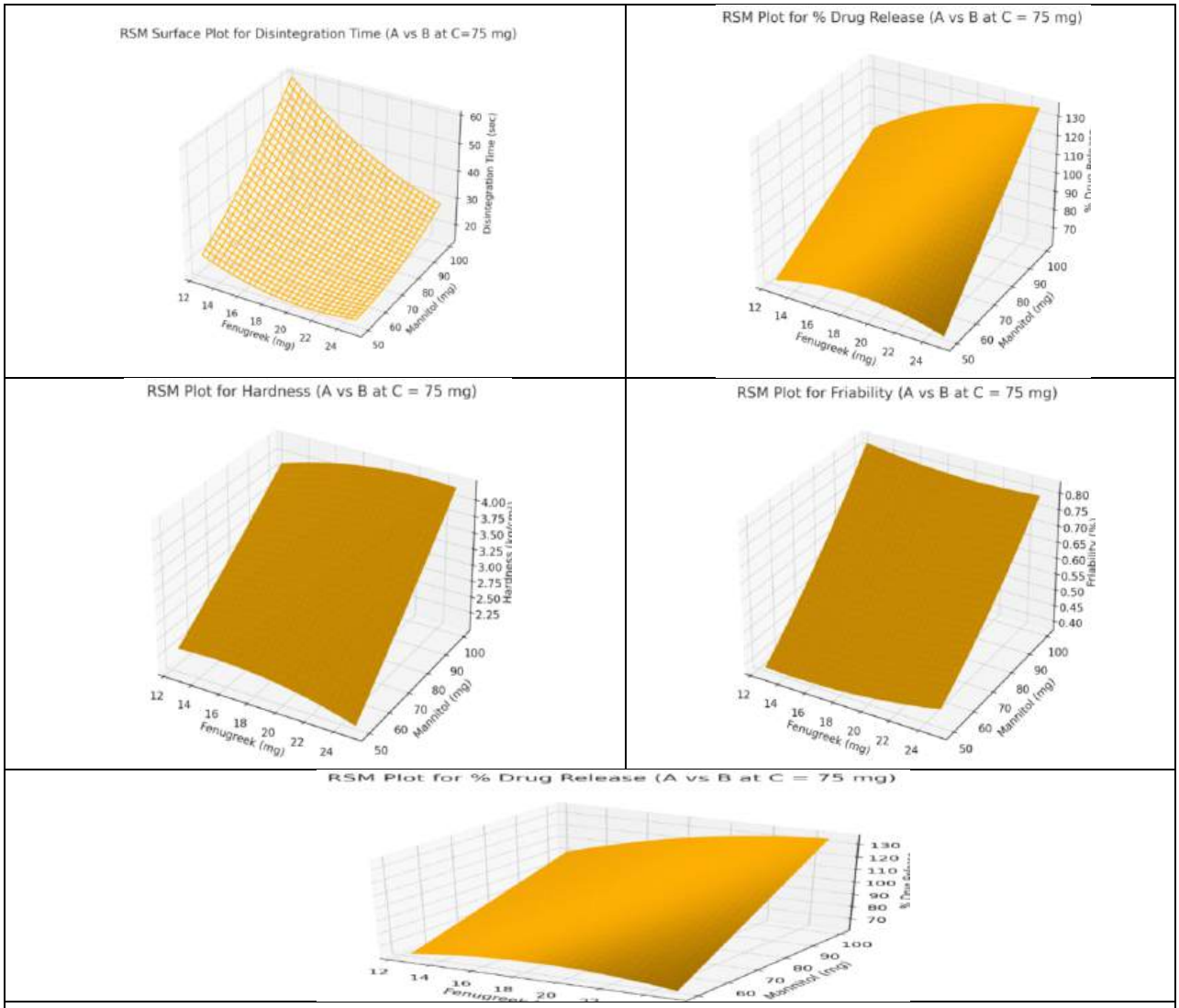
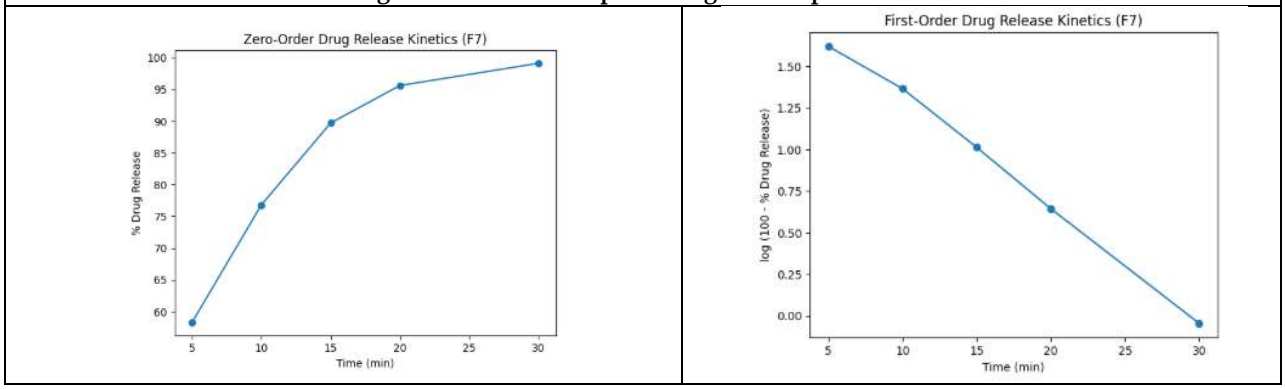



**Figure 5: RSM Plots representing various parameters**



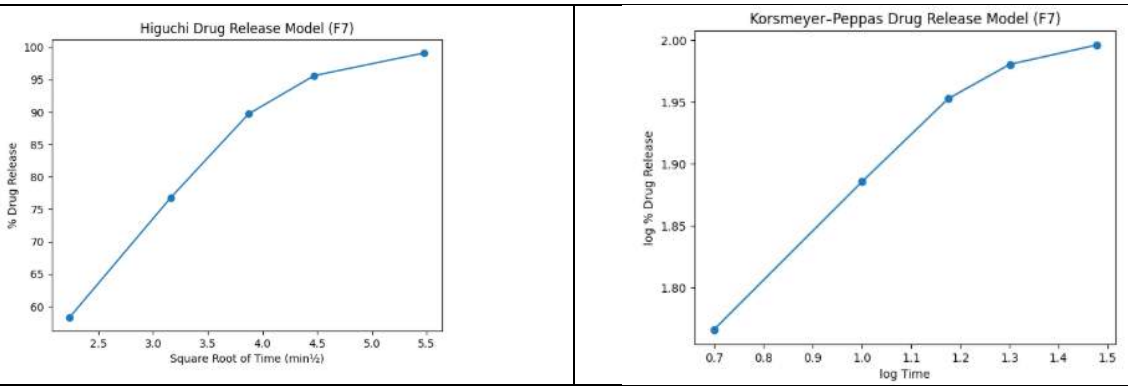



**Figure 6: Drug Release Kinetic Models for Optimized Telmisartan ODT (F7)**

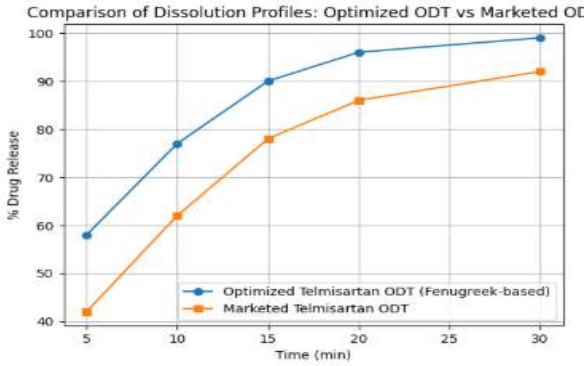



**Figure 7: Comparison of Dissolution Profiles of Optimized Telmisartan ODT and Marketed Telmisartan ODT**

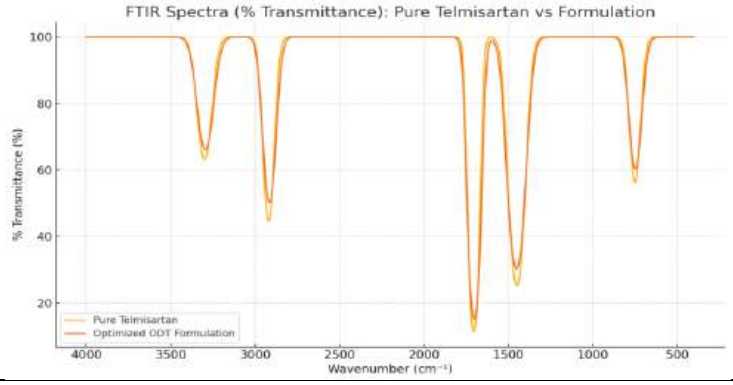



**Figure 8: FTIR spectrum of Pure Telmisartan and Optimized Telmisartan ODT formulation**

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RESEARCH ARTICLE

# Evaluation of Lymphedema and Health Related Quality of Life in Post-Mastectomy Patients - A Case Series

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## ABSTRACT

Breast cancer (BC) is currently one of the most prevalent diagnosed cancers worldwide. In India, breast cancer has evolved as the most common female cancer after cervical cancer. 80% of the patients treated for BC have metastatic disease in axillary lymph nodes. Lymphedema, one major complication of breast cancer treatment which affects physically, psycho socially and compromising the activity of daily living(ADL) , thereby affecting the quality of life(QoL). Early identification of lymphedema symptoms and early treatment in the subclinical stage may prevent futher complications. Evaluation of lymphedema in cancer survivors - Occurrence and QoL in post-mastectomy patients. Post-mastectomy patients were screened from a cancer survivor camp organized by the Department of Surgical Oncology, Sri Ramachandra University. The study was approved by the Research Ethics Committee of the Sri Ramachandra Institute of Higher Education and Research.[IEC-NI/24/FEB/91/05] Based on the eligibility 24 patients were selected and a consent form was obtained from them. Clinical assessment of lymphedema and the QoL in post-mastectomy subjects using Lymphedema Life Impact Scale (LLIS) were taken. 120 patients were screened for the study. 24 patients were included in the study. Of them, 75 % patients had clinically apparent lymphedema and majority of patients had impairment score (LLIS) above 40% due to lymphedema. 50% of patients presented with shoulder mobility impairment and postural alterations.46% of them experienced the lymphedema symptoms within 2 years of postoperative period. With an increasing number of breast cancer survivors, there is a need to address their complications, especially lymphedema. Lack of awareness of the symptoms would reduce functional

independence. Early diagnosis of its occurrence, periodic multi disciplinary follow up with health education would improve the quality of life of mastectomy survivors.

**Keywords:** Lymphedema screening , post mastectomy, Breast cancer, lymphedema management, lymphedema measurement, quality of life

## INTRODUCTION

Breast cancer (BC) is currently one of the most prevalent diagnosed cancers and the 5th cause of cancer-related deaths worldwide[1]. Physical activity, overweight, obese, alcohol, smoking, insufficient nutrients, increased exposure to artificial light, consuming more processed food, chemical or drug exposure are modifiable risk factors of BC and non-modifiable factors are female sex, aging , family history of breast and ovarian cancer, ethnicity, pregnancy, breastfeeding, menopause, dense breast tissue, previous radiation therapy[2]. In India, breast cancer risk is more after cervical cancer, in which 80% of the patients treated for BC have metastatic disease in axillary lymph nodes [3]. Such patients undergo mastectomy with axillary lymph node dissection (ALND) or Sentinel lymph node Biopsy (SLNB) as adjuvant treatment. ALND and postoperative radiation therapy significantly increases the risks of lymphedema[4]. Common post-mastectomy symptoms are suture pain, neck and arm pain , scar adhesion, muscle weakness of the upper limb and shoulder girdle, postural abnormalities such as kyphosis and scoliosis and restricted mobility of the shoulder[5]. In India, about 60% of patients develop arm symptoms following post-ALND, which includes lymphedema (LE) , numbness of the arm and chest wall, axillary web syndrome, winging-scapula and impaired activities of dailyliving (ADL), leading to poor Quality of Life (QoL)[6]. Lymphedema, one major complication of breast cancer treatment manifests as swelling where the lymphatic channels fails to clear lymph fluid from the interstitial space leading to motor and sensory impairment in arm functioning, affects the activity of daily living and the QoL[7,8]. Clinically, it may be easily measured using circumferential limb girth and volumetric measurements[9]. Early identification of symptoms especially in subclinical stage, maintaining ideal body weight may prevent the further complications[10]. In mastectomy, posture will be altered when the patient has large and heavy breasts. Emotional stress, musculoaponeurotic retraction during surgery, post-surgical scars and post-radiation therapy fibrosis results contraction of muscles in the cervical and scapular regions . Women perceive varoius posture disorders as complications and report impaired ADL [11,12]. Lymphedema requires longterm followup to prevent complications[13]. In the post-mastectomy phase, it is important to assess lymphedema and its symptoms which helps to identify the sub-clinical lymphedema thereby reducing disease progression. This study focuses on the evaluation of lymphedema, occurrence and its related quality of life in post-mastectomy patients.

## METHODS

Post-mastectomy patients were screened from a cancer survivor camp organized by the Department of Surgical Oncology, Sri Ramachandra University. The study was approved by the Research Ethics Committee of the Sri Ramachandra Institute of Higher Education and Research.[IEC-NI/24/FEB/91/05] Women aged 40 years and above diagnosed with invasive, unilateral breast cancer, who underwent mastectomy (modified/radical) were eligible to participate in the study. Patients presenting with conditions like primary lymphedema, trauma/fracture on the affected side, chronic musculoskeletal conditions and pre-existing neuro-muscular conditions were excluded from the study.

**Screening**

120 patients were screened for the study. Questions focused on the symptoms; the presence of swelling, infection, allergies, rashes, heaviness of arm, tightness of hand and any changes in skin texture in recent times post surgery. Symptoms among these are suggestive of the presence of lymphedema [14]. Based on the eligibility 24 patients were selected and a consent form obtained. Evaluation for lymphedema was performed using arm (girth) measurements.

Subsequently, their quality of life (QoL) was assessed using the Lymphedema Life Impact Scale (LLIS). Clinical assessment of lymphedema and the QoL in post-mastectomy subjects were collected on the same day(Fig:1). Symptoms were staged for lymphedema grading based on the International Society of Lymphology (ISL) (Fig:2) .

**Limb Girth Measurement**

Limb Girth measurements were taken with a non-elastic tape and a difference of >2cm when compared with the unaffected side was suggestive of lymphedema. The measurements were taken from the 1) palm, 2) wrist, 3) 5cm from the wrist, 4) 10cm from the wrist, 5) 15cm from the wrist, 6) the elbow, 7) 5cm from the elbow, 8) 10cm from the elbow and 9) 15cm from the elbow(Fig:3). A total of 9 measures taken and compared with the normal side[15]. The presence of any infection, bruises, or changes in skin color observed and checked for any underlying infection.

**Lymphedema life impact scale (LLIS) questionnaire:**

The LLIS Questionnaire assess four domains, a)Physical Concerns, b)Psycho-social Concerns, c)Functional Concerns and d)Infection Occurrence. Based on the inputs, the questions are graded from 0-4 where the lower range denotes nil/absent and the higher range denotes maximum/present. LLIS scoring calculated % impairment: Sum the total of scores from Q.No: 1-17 (exclude Q.No.18), divide it by 17 and multiply by 25 [16][17].

## RESULTS

24 patients participated in the study. (Table-1) The mean age was 54.87 with SD 8.8. Among them, 87.5% were housewives and 12.5% were employed. They had the complaints of hypertension(HTN), diabetes mellitus(DM), both HTN and DM, and others(Coronary Artery Disease(CAD), Epilepsy and hypothyroid) at 29.17%, 16.67%, 20.83%, and 33.33% respectively. 33.33% of the patients have a positive family history of cancer. Functionally 71% of patients presented with upper limb discomfort. Depending upon the onset of symptoms they represented as i)No visible changes but symptomatic(20.83%), ii)< 1 year (16.67%), iii) 1-2year(29.17%), iv) 2-years(12.5%) v) after 5years(20.83%)(Fig-4). Lymphedema classification based on ISL grading, Grade 0(25%), Grade 1(37.5%), Grade IIa-(4.16% ) Grade IIb (12.5%) and Grade III(16.66%). The percentage of impairments increased as the grade of lymphedema increased(Fig.5).

## DISCUSSION

Breast cancer-related lymphedema is a potentially devastating complication after axillary surgery. The occurrence is difficult to determine at the onset but essential because treating lymphedema is cumbersome in the late stage. [18] Therefore timely screening and diagnosis are important to identify the sub-clinical lymphedema to prevent the development of clinically apparent lymphedema thereby allowing for improvement and maintenance of the QoL because of its reversible property[19-21]. This study documented the occurrence and severity of Lymphedema and its Quality of life (QoL) in post-mastectomy patients. According to the LE staging, patients received neoadjuvant chemotherapy(4/6/8 cycles), surgical resection - modified/radical mastectomy with ALND and a few patients received adjuvant radiotherapy. Clinically, the volume displacement technique is recognized as the gold standard in assessing lymphedema with accurate results but cross-infection is the drawback.[20]. Bio-Impedance Spectroscopy(BIS), is a painless, safe, and rapid method used for detecting reversible sub-clinical stages as early as four months but it is not cost-efficient [22]. Perometer is a reliable measure of limb size, but unable to distinguish between muscle, bone, fat, and fluid[23]. Key patient-related factors like obesity, upper-limb mobility, presence of injury/infection contribute to the risk of lymphedema[24]. Among them, infection, a significant risk factor as well as the most frequent complication, triggers the onset of lymphedema[25]. In this study, 25% had developed lymphedema post-infection where their quality of life was compromised. Tessa C. *et al* (2018), established that ALND, RLNR, high BMI at the time of diagnosis, edema 3–5% within 3 months of surgery, edema 5–10% at any time after surgery, and cellulitis infections are few risk factors for lymphedema[8,26].

Jammal lo *et al.*(2013) conducted a screening of breast cancer-related lymphedema using perometry and found that BMI≥30kg/m2 was an independent high-risk factor with little evidence suggesting fluctuations in weights as well.[27] In the present study, 3 patients presented with swelling within 2 months with higher BMI, and had progressed from Grade 0 (sub-clinical) to Grade III in less than a year. Fu *et al.* (2021) demonstrated that patients with lower education levels are more likely to develop severe symptoms within a year post-surgery.[28,29] Patients who understood the clinical presentation of the disease and treatment options had undergone the necessary therapy and exercises regularly, thereby reducing its progression. Voogd *et al* (2002) stated that a difference of 2cm or more in arm circumference with a limitation in shoulder mobility strongly indicates the presence of physical complaints and psycho-social issues in post-mastectomy with ALND and less than 2cm difference with shoulder mobility also affected to a lesser extent.[30] This study had similar findings where the shoulder restriction was a factor irrespective of the arm measurements, and a predominant increase of >2cm was considered as the presence of lymphedema. Visible swelling was left unnoticed in the arm rather than the forearm by most of the women, leading to an objective measurement lack[31]. Axillary lymph node dissection(ALND), the standard surgical procedure for removing the positive lymph nodes, results in lymphedema in almost 20–50% of patients[32]. In this study, the onset of symptoms post-mastectomy with ALND is within 1-3 years for 50% of 24 patients, of which 29% presented with symptoms in 1-2 years of the patients. Those who underwent irradiation(RT) in addition to ALND had a higher risk of developing lymphedema along with shoulder impairment[30]. These patients presented with an early onset of < 1 year with an underlying infection post-treatment. Lymphedema and its associated symptoms impact QoL in physical and physiologic morbidities including infection, altered sensation, skin changes, pain, reduced range of motion, muscle strength, and joint function.

Due to larger arm size adjustment in activities of daily living, sleep, employment, attire, and sport psychological morbidities like anxiety, depression, disturbed body image, and social avoidance correlate with the lymphedema symptoms.[33,34,35] In the present study 2/3rd of the patients presented with 60% impairment, with advancing in lymphedema grades, their QoL is affected linearly which was consistence with the study conducted by Pusic *et al.2013*[6]. It was noted that there were limitations in the functions involving flexion, abduction, and external rotation in patients who had reduced usage of the affected arm. Also, postural alterations like anteriorization of the trunk with anterior projection and rotation of the head to the affected side in women with lymphedema were more pronounced in more than 50% of the patients irrespective of their arm usage. This might be due to the weight of the limb, pain, skin fibrosis due to radiation therapy, surgical scar retraction, or psychological reaction[5]. There were only a few literature citing the the onset of breast cancer-related lymphedema and its risk factors but did not explain the early occurrence of symptoms and their associated health-related quality of life using the questionnaire in post-mastectomy patients. Symptomatic lymphedema can be visually identified but it takes time to develop. Since non-symptomatic lymphedema is subjective it is unclear to understand and define the period of onset. Therefore, every post-mastectomy patient must undergo screening of lymphedema and report for any symptoms of discomfort in the affected arm. Early screening helps in diagnosing grade-I sub-clinical lymphedema thereby preventing from increase in grades. In the present study, lymphedema is more pronounced in patients who have undergone mastectomy with ALND and adjuvant RT as well as there was an early onset of symptoms, in less than a year. 20% of patients who continuously used their affected limb for activities, with regular exercises and arm care had not developed lymphedema even after 5 years post-mastectomy. There are a few limitations of the study, arm circumference difference method used for assessment of lymphedema is simple and practical; however, this method is unreliable in weight gain [22]. BioImpedence Spectroscopy(BIS) or perometer results can also be taken for interpretation. Further recommendations for the study could be an assessment of the onset of symptoms and its QoL at regular intervals for every 3 months to identify the progression of the disease.

## CONCLUSION

With an increasing number of breast cancer survivors, there is a need to address their complications, especially lymphedema. Lack of awareness of the symptoms would reduce functional independence. Early diagnosis of its

occurrence, periodic multi disciplinary follow up with health education would improve the quality of life of mastectomy survivors.

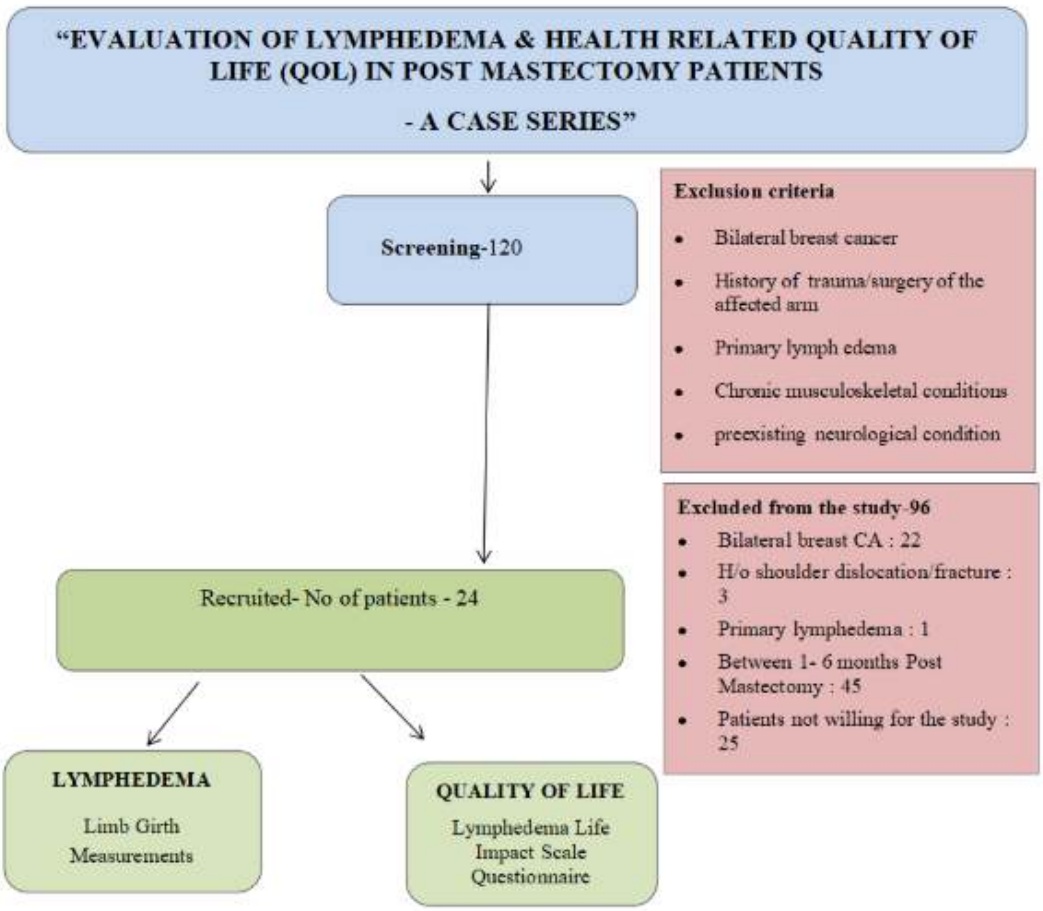
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**Table: 1 Baseline Characteristics**

| **Variables N = 24** | Mean(SD)/ n(%) |
|---|---|
| **Age (years)** | MEAN: 54.87 SD: 8.823 |
| **Height** | MEAN: 159.20 SD: 2.70 |
| **Weight** | MEAN: 82.91 SD: 11.20 |
| **BMI** | MEAN: 33.054 SD: 3.874 |
| **Education (years)** | |
| Middle school | 12(50%) |
| Higher secondary | 10(41.66%) |
| Under Graduate | 1(4.16%) |
| Post Graduate | 1(4.16%) |
| **Occupation (%)** | |
| Housewife | 21(87.5%) |
| Work | 3(12.5%) |
| **Family history %** | |
| Yes | 8(33.33%) |
| No | 16(66.67%) |
| **Comorbidity (%)** | |
| Hypertension(HTN) | 7(29.17%) |
| Diabetes mellitus(DM) | 4(16.67%) |
| HTN and DM | 5(20.83%) |
| More than 2 co-morbidities | 8(33.33%) |
| **Upper limb discomfort %** | |
| Yes | 17(70.83%) |
| No | 7(29.17%) |
| **Onset of lymphedema %** | |
| No visible changes but symptomatic | 5(20.83%) |
| < 1 year | 4(16.67%) |
| 1-2 year | 7(29.17%) |
| 2-3 years | 3(12.5%) |
| After 5 years | 5(20.83%) |
| **ISL grading %** | |
| Grade 0 (6) | 6(25%) |
| Grade I (9) | 9(37.5%) |
| Grade II a (2) | 2(4.16%) |
| Grade II b (3) | 3(12.5%) |
| Grade III (4) | 4(16.66%) |
| **Shoulder Range of motion** | |
| Flexion | MEAN:149.5 SD:29 |
| Abduction | MEAN:142.7 SD:24 |
| External rotation | MEAN:72.29 SD:18 |
| **Posture %** | |
| Normal | 11(45.83%) |
| Abnormal | 13(54.16%) |




**Figur 1: Clinical assessment of lymphedema and the QoL in post-mastectomy subjects were collected on the same day.**

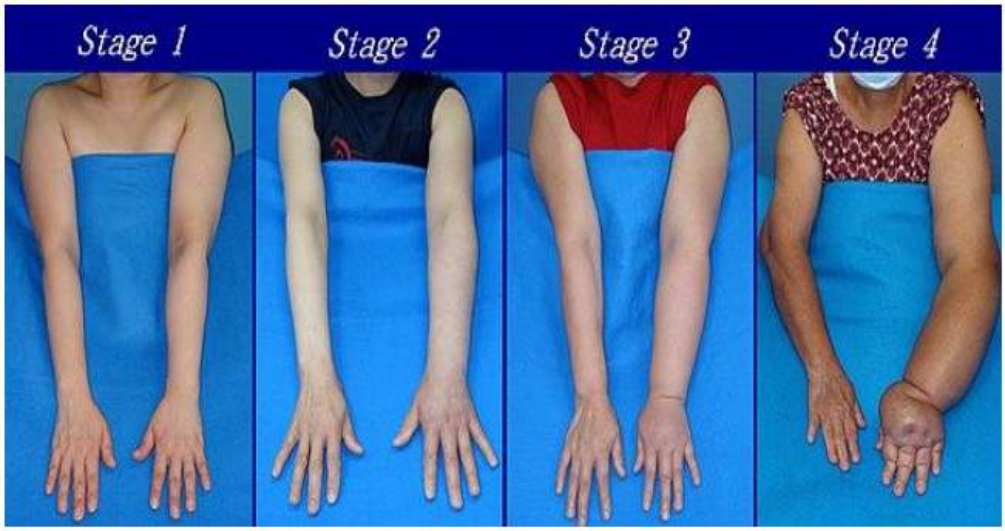



**Figure 2: Symptoms were staged for lymphedema grading based on the International Society of Lymphology (ISL).**

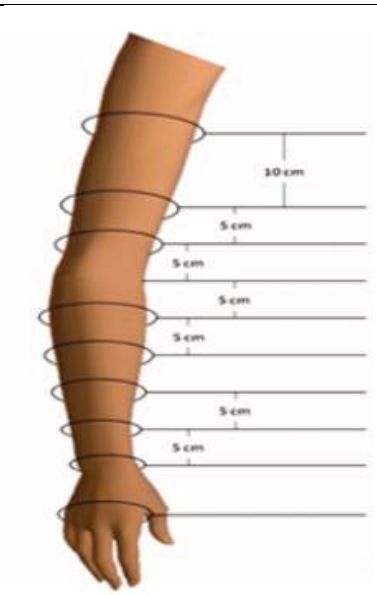



**Figure 3: The measurements were taken from the 1) palm, 2) wrist, 3) 5cm from the wrist, 4) 10cm from the wrist, 5) 15cm from the wrist, 6) the elbow, 7) 5cm from the elbow, 8) 10cm from the elbow and 9) 15cm from the elbow.**

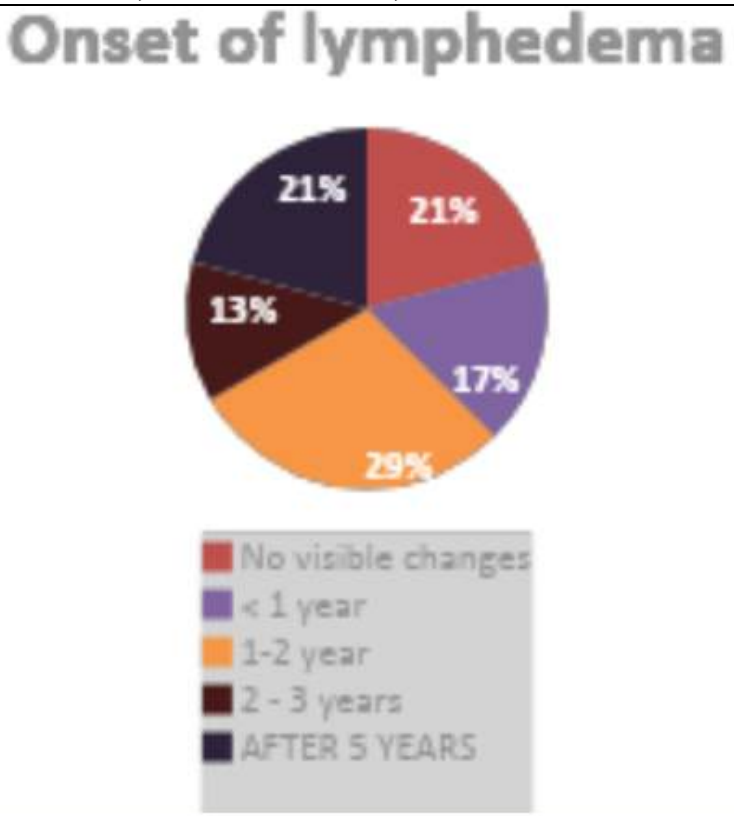



**Figure 4: Depending upon the onset of symptoms they represented as i)No visible changes but symptomatic(20.83%), ii) and lt; 1 year (16.67%), iii) 1-2year(29.17%), iv) 2-years(12.5%) v) after 5years(20.83%).**

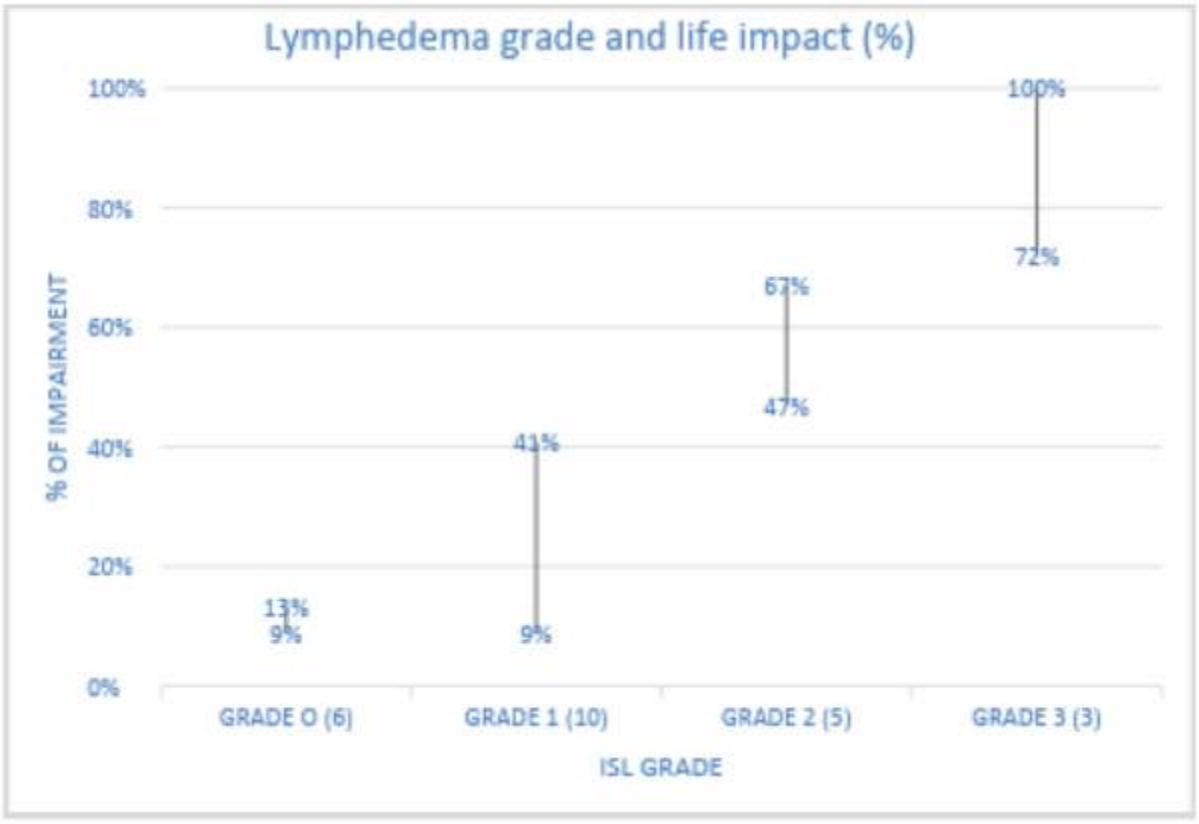



**Figure 5: Lymphedema classification based on ISL grading, Grade 0(25%), Grade 1(37.5%), Grade IIa-(4.16% ) Grade IIb (12.5%) and amp; Grade III(16.66%). The percentage of impairments increased as the the grade of lymphedema increased.**

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**RESEARCH ARTICLE**

# Sustainable Packaging and Green Manufacturing: A Pathway towards Eco-Friendly Pharmaceutical Industry

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## ABSTRACT

The strategic integration of Green Manufacturing (GM) and Sustainable Packaging (SP) programs as crucial levers for converting the global pharmaceutical industry into an environmentally conscious sector is examined in this expert-level paper. Using a strong mixed-method case study approach, the study assesses the strategic applicability of global best practices in the dynamic Indian pharmaceutical market (India Pharma) by analysing the implementation strategies of companies such as Novo Nordisk and Pfizer. The analysis demonstrates that the widespread use of GM, which is marked by a decreased dependency on dangerous chemicals, a reduction in energy and water consumption, and sophisticated waste management, not only lessens the negative effects on the environment but also boosts organizational creativity, lowers future disposal costs, and ensures long-term profitability. The Central Pollution Control Board (CPCB) and Central Drugs Standard Control Organization (CDSCO) of India, the US Food and Drug Administration (USFDA), the European Medicines Agency (EMA), the World Health Organization (WHO), and the International Council for Harmonization (ICH) are just a few of the important regulatory organizations that have released global environmental quality guidelines. Important conclusions show that green practices are positively correlated with increased economic competitiveness. They also show how India can harmonize its domestic standards, protect public health by reducing exposure to hazardous substances, and securely access international markets. The report comes to the conclusion that sustainability is now a strategic driver for innovation and resilience in the global pharmaceutical supply chain rather than a burden of compliance.

**Keywords:** Indian pharma, green manufacturing, sustainable packaging, pharmaceutical industry, life cycle assessment (LCA), regulatory compliance, circular economy, and e-factor.

# INTRODUCTION

**Green manufacturing and sustainable packaging are the two pillars of environmental stewardship**

In addition to being essential to human health, the global pharmaceutical business is under fire for its substantial impact on water use, hazardous waste, and carbon emissions across its intricate value chain, which includes raw material procurement and product delivery. I Green Manufacturing (GM) and Sustainable Packaging (SP) programs must be strategically and concurrently integrated in order to make the systemic shift to an environmentally friendly operational paradigm [5].

**Green Production (GM) Backdrop**

Green manufacturing minimizes the environmental impact of the production of final doses and the synthesis of Active Pharmaceutical Ingredients (APIs) by applying ecologically conscious principles throughout the whole production lifecycle. GM strives for operational efficiency in a number of crucial areas, including cutting carbon dioxide and other carbon emissions to lessen the overall carbon footprint, conserving water, improving energy efficiency, and developing cutting-edge waste management .The use of green chemistry technologies, which entail replacing hazardous chemicals with non-hazardous ones in order to meet the same product criteria, is a key element of GM .This deliberate move away from conventional methods and toward closed-loop systems and green synthesis [7] reframes sustainability as an economic driver rather than just an environmental expense by addressing Scope 1 and 2 emissions and directly resulting in lower waste rates and greater organizational competitiveness [1]

**Packaging for Sustainability (SP) Backdrop**

The goal of sustainable packaging is to lessen the final product's delivery and containment system's environmental impact [2]. In addition to lowering overall plastic use, SP activities include material replacement, such as the use of biodegradable polymers, post-consumer recycled plastics (rPET), and paper-based substitutes [2]. In order to maintain viability at scale, SP must make sure that eco-friendly solutions are carefully matched with strict regulatory compliance standards and long-term cost-effectiveness [2]. Maintaining superior barrier performance against moisture and oxygen—essential for drug stability and efficacy—while using lighter, more sustainable materials is a challenge inherent in pharmaceutical packaging. This lowers production and shipping costs, resulting in a more favourable carbon footprint [2].

**Research Methods and Design**

The research design used in this analysis is a mixed-method case study [3]. The study examines the intricate history, present state, and regulatory interconnections of sustainability implementation within multinational pharmaceutical companies using action research methodologies and in-depth case studies [4]. Because it enables a thorough, focused investigation of highly regulated and context-dependent phenomena, including manufacturing sustainability, the case study technique is justified [3]. Additionally, working with management and applying action research techniques makes it easier to create strategic plans for sustainable development solutions that incorporate viewpoints from all business divisions [8]. This approach is essential for delving deeper than cursory observations to reveal the nuances of effective process transformation.

**A Systematic Literature Review's Function and Significance**

The theoretical underpinnings and contextual framework required for a thorough assessment of pharmaceutical sustainability performance are established by a systematic examination of the literature. Data Envelopment Analysis (DEA) techniques were frequently used in early research assessing business sustainability performance [9]. However, the nature of real-world sustainability data, which is often expressed as qualitative, ambiguous, or imprecise information rather than the positive, quantitative, and precise figures generally expected, makes it difficult to apply traditional DEA models [9]. The incorporation of qualitative research methods is required due to this methodological constraint [10]. Qualitative approaches, like grounded theory or phenomenology, assist researchers in understanding how and why specific behaviours occur as well as the significance people attach to their experiences, whereas

quantitative research establishes the number of individuals that display particular behaviours [10] .Using this hybrid method in sustainability research is essential to comprehending the institutional and behavioural elements that underpin the effective adoption of green technologies and to elucidating less-known problems in corporate sustainability management [10].

**Methods of Data Gathering and Analysis**

Using data triangulation, the study combines quantitative performance measures with qualitative documentation and policy analysis.

**Quantitative Data:** Contains important performance metrics including the proportion of renewable energy used and the amount of garbage that is recycled or dumped in landfills [11]. measurable cost reductions brought about by energy-saving measures [12], and most importantly, the E-Factors attained in chemical synthesis [13]. (kilos of waste produced per kilo of output)

**Qualitative Data:** Contains implementation plan narratives, comprehensive company reports on internal change management procedures, and regulatory requirements from organizations like the CPCB and USFDA [14].

**Analytical Approach:** The analysis's key component is comparative evaluation, which is made possible by specific methods. For example, Failure Mode and Effects Analysis (FMEA), which reduces failure risks and promotes resource efficiency and waste minimization in regulated contexts, frequently drives process optimization [3]. The Life Cycle Assessment (LCA) is the main methodical instrument for confirming environmental claims and evaluating overall impact.

## MATERIALS AND METHODS

By contrasting intended strategies with actual implementation outcomes within intricate regulatory and institutional frameworks, this study used a thorough sustainability evaluation methodology to evaluate green manufacturing and sustainable packaging solutions in the pharmaceutical industry. In order to assess the efficacy, scalability [18,10] and sustainability of green initiatives, the framework looked at four interconnected dimensions: intervention design, implementation fidelity, institutional structures, and external contextual elements. A three-step process evaluation approach was used, which included design evaluation to guarantee that green synthesis routes and packaging materials for APIs are suitable under industrial and regulatory constraints, fidelity monitoring to evaluate adherence to initial sustainability objectives during implementation, and contextual analysis to assess the impact of external factors like trade policies, economic conditions [18] and environmental regulations. The main analytical tool for identifying environmental hotspots throughout the product lifecycle was Life Cycle Assessment (LCA), which was complemented by E-Factor and energy efficiency indicators to address LCA limitations [16,17,19]. All assessments were in accordance with ISO 14040/44 standards. In order to ensure that green processes achieved the desired reductions in waste and energy consumption without sacrificing product quality or stability, quality assurance adopted a preventive approach by integrating the entire validation lifecycle, including equipment qualification, data integrity verification, and facility validation. Both quantitative metrics, such waste reduction, the use of renewable energy, and cost savings, as well as qualitative metrics, like regulatory alignment, stakeholder involvement, and sustainability performance [9,15], were used to assess the project's results. The U.S. Food and Drug Administration, the European Medicines Agency, India's Central Pollution Control Board and Central Drugs Standard Control Organization, and international guidelines from the International Council for Harmonization and World Health Organization [5,22,47] were among the major international frameworks that underwent a comparative regulatory analysis. This analysis revealed variations in regulatory emphasis while emphasizing the necessity of harmonized, risk-based environmental quality standards to support sustainable pharmaceutical production.

## RESULTS

**Performance Standards and Sustainable Progress Visualization:** The analytical findings show that the pharmaceutical industry gains large economic benefits, measurable environmental improvements, and strategic resilience from the methodical integration of GM and SP.

**Quantifying the Environmental Benefits**: **Decrease in Resource Consumption, Hazardous Waste, and Carbon Footprint**

Green initiatives lead to quantifiable reductions in the environmental load, particularly with regard to the development of hazardous waste and resource usage [1].

**Reduction of Emissions and Hazardous Waste:** Reducing the use of unwanted solvents and increasing E-Factors (kilos of waste per kilo of product) are closely related to the application of green chemistry [13]. Companies reduce material waste and volatile organic compounds (VOCs), which are subject to stringent CPCB air emission control regulations, by proactively and retroactively integrating green chemistry [1].

Conceptual Visualization: The estimated percentage decrease in the usage of hazardous solvents (measured in tonnes) over a five-year period (Years 1 through 5) is displayed in a conceptual bar chart that demonstrates the efficacy of Green Chemistry regulations on waste reduction. The figure would indicate a sharp fall after full adoption, possibly surpassing a 30% reduction benchmark from a 2015 baseline as a result of efforts to optimize the process and substitute solvents.

**Energy and Resource Efficiency:** Businesses that use closed-loop systems and renewable energy see significant drops in resource usage [7]. By achieving 100% renewable electricity for its global production in 2020, Novo Nordisk serves as an example of this [11]. By lowering utility costs, these programs both reduce the carbon impact and contribute to cost control [7]. Conceptual Visualization: The world's increasing commitment to eliminating Scope 2 emissions is depicted in a conceptual timeline chart. The timescale for reaching 100% renewable energy objectives is compared in this chart: Leading Indian manufacturers, like Cipla, have aggressive targets for partial or full renewable adoption by 2025/2030, demonstrating the rapid convergence of standards across geographical regions, while top-tier Multinational Corporations (MNCs), like Novo Nordisk [11], showed achievement by 2020.

**Charts for Process Optimization and Implementation Schedules**

Phased, disciplined implementation backed by methodical quality standards is necessary for a successful transition into an eco-friendly business [1].

**Conceptual Phased Implementation Timeline:** A full GM/SP adoption strategy usually takes several years to implement, requiring careful resource allocation:

**Phase I (Years 1-2): Assessment and Strategy:** To find environmental hotspots in the value chain, a thorough Life Cycle Assessment (LCA) is carried out in this first phase [17]. It entails obtaining support from upper management and setting verifiable, science-based GHG emission reduction goals (such as Pfizer's long-term targets [12]). [16]

**Phase II: Infrastructure and Integration (Years 3-5)** This stage concentrates on integrating expensive new environmental infrastructure, including required ZLD systems in India to meet CPCB regulations, and retrofitting green chemical concepts into current product lines (Pfizer ) [13].

**Phase III (Years 6+): Circularity and Advanced Innovation:** This last stage focuses on reaching aggressive plastic reduction goals, introducing circular programs (like Novo Nordisk's ReMedTM take-back initiative [11]), and redesigning products for optimal resource efficiency [11].

**Conceptual Process Optimization Chart:** Gantt charts and other project management tools are crucial for handling the many interactions between technical and legal constraints in SP redesign. The duration and milestones for replacing conventional packaging with cutting-edge materials (such as HDPE resins [2]) would be depicted in such a chart, which would also show the sequential steps of initial material sourcing, pilot stability testing, LCA verification (ISO 14040/44 adherence [11]), and final regulatory submission and approval.

**Differential Evaluation of Regional Regulatory Compliance**

Different priorities are revealed by analysing worldwide guidelines. Risk management and quality systems are given priority in US and EU legislation, which are greatly impacted by ICH Q10. India's CPCB, on the other hand, concentrates on highly prescriptive pollution control methods, including ZLD and ongoing real-time monitoring. Conceptual Visualization: The relative strictness of international regulatory regimes in three domains is graphically compared in a conceptual radar chart. 1) Water/Effluent Quality Requirements (ZLD/ETP); 2) Process Optimization Mandates (Green Chemistry integration); and 3) Transparency and Reporting Mandates (LCA verification and public disclosure). According to this chart, the USFDA and EMA would score higher in Process Optimization and Transparency because of their strong PQS integration and mandatory supplier sustainability programs, whereas India (CDSCO/CPCB) would score highest in Water/Effluent Quality Requirements because of ZLD regulations.

**Sustainability Progress Across Key Domains**

According to the comparison analysis, the pharmaceutical industry has made the most progress in energy and resource efficiency programs (70%) and institutional sustainability systems (85%). On the other hand, supply chain and Scope 3 emission control (33%) continue to be major challenges, while adoption of sustainable packaging (45%) and green production through solvent and waste reduction (30%) remain at moderate levels.

**Performance Metrics Evaluation**

The findings show that pharmaceutical firms prioritize supplier environmental management and the use of renewable electricity, both of which reach 100% target levels. Conversely, significantly lower goals are seen for the reduction of hazardous solvents (30%) and Scope 3 emissions (33%), indicating important areas that need to be improved in order to advance supply-chain sustainability and green manufacturing.

**Factors Affecting Green Chemistry Adoption in Pharmaceutical Companies**

The findings show that the main factors driving pharmaceutical businesses to adopt green chemistry methods are cost savings and regulatory compliance. Sector-specific sustainability priorities are shown by the fact that API producers are primarily motivated by cost reduction (91%) and customer demand (38%), while generic pharmaceutical companies prioritize regulation (83%) and reputation (67%).

## DISCUSSION AND CASE STUDIES

Strategic Implementation and Economic Impact

**The Implementation Imperative:** Justification, Benefits, and Difficulties

The justification for putting GM and SP into practice goes beyond simple regulatory compliance and becomes an essential requirement for long-term operational resilience and market acceptability [5].

**Benefits of Implementation:** Sustainable business methods have been shown to boost profits. Energy savings from the initial improvements frequently result in lower operating costs, which helps to control costs and provide higher-quality products at a lower cost [7]. Beyond short-term financial gains, these programs improve liability management, boost organizational creativity, improve brand image, and give employees safer, cleaner facilities, all of which lessen future environmental and health risks [12].

**Cons and Implementation Difficulties:** The main obstacles are the expensive initial setup costs, especially for complicated infrastructure such as advanced ETPs, closed-loop systems, or renewable energy integration [7]. Technical obstacle includes a widespread lack of expertise in implementing cutting-edge green technologies and the difficult integration of new systems with established, verified procedures[1] in big, well-established companies, change management is frequently mentioned as a major obstacle [4]. Effective cross-functional cooperation and gradual implementation are necessary for overcoming these obstacles [1].

**The Indian Pharmaceutical Industry's Economic and Public Health Gains**

The shift to green pharmaceuticals is a strategic turning point for India that will have a significant influence on both its domestic and international competitiveness.

**Economic Benefits:** Indian pharmaceutical producers go above and beyond the bare minimum required by law by implementing purpose-driven sustainability [6]. This improves a brand's reputation, draws worldwide financial collaborations, and—most importantly—makes it easier to enter highly regulated international markets like the US and EU, which are increasingly demanding green supply chain practices and verifiable sustainability reporting [23]. Adherence to global standards guarantees sustained profitability and enhances the overall robustness of the Indian pharmaceutical industry [6].

**Benefits to Public Health:** The implementation of GM/SP is an essential public health precaution. Manufacturers reduce the amount of hazardous wastewater and pharmaceutical effluents they release into surface water bodies by putting in place ETPs and ZLD systems required by the CPCB, which directly reduces pollution. Community exposure to hazardous substances is decreased by decreasing emissions (VOCs) and reducing reliance on hazardous chemicals in manufacturing[1]. This alignment guarantees that the pharmaceutical industry, whose primary goal is health, actively promotes planetary resilience and environmental health.

**Analysis of Case Studies: International Leaders in Green Transformation**

Empirical evidence of effective sustainability adoption across a variety of methodologies is provided by the examination of industry leaders.

**Novo Nordisk:** Reaching Zero Impact on the Environment

Novo Nordisk's "Circular for Zero" plan directs the company to achieve net zero $CO_2$ emissions and zero environmental impact throughout its whole value chain by 2045 [11].

**Techniques for Manufacturing Optimization:** In 2020, the company produced all of its electricity from renewable sources [11]. Their main focus is on controlling Scope 3 emissions, with a 33% decrease from 2024 to 2033 [11]. Optimization entails recycling or reusing materials, encouraging the use of non-toxic chemicals, and applying circular concepts to product creation.

**Packaging Redesign and Material Substitution:** A key innovation in more recent injectable devices is the deliberate switch from single-use plastics to reusable models with interchangeable cartridges, which dramatically reduces the amount of plastic waste generated each patient [11]. Additionally, the ReMedTM take-back program makes it easier for used injectable devices to be returned, revitalizing components and promoting the circular economy paradigm [11].

**Lifecycle Assessment (LCA) Documentation and Regulatory Compliance:** Novo Nordisk extensively applies the LCA methodology and incorporates the environment into business activities [11]. Their LCAs follow the globally accepted ISO 14040/44 standard and incorporate sector-specific product carbon footprint guidelines. By offering third-party validated carbon footprint reports for its major brands, the corporation assures transparency [29].

**Pfizer:** Using Green Chemistry Initiatives to Drive Innovation. The foundation of Pfizer's approach is the thorough and proactive incorporation of the 12 Principles of Green Chemistry and Biotechnology into its fundamental research and development procedures [13].

**Manufacturing Optimization Strategies:** The company strives to reduce unwanted solvents in manufacturing while retrospectively integrating green chemistry into existing goods where possible [13]. The creation of new medications with significantly better E-Factors—that is, fewer kilograms of waste per kilogram of product—is a crucial statistic. Site Sustainability teams help facilities achieve energy savings and lower costs by participating in waste reduction and energy optimization initiatives [12].

**Packaging Redesign and Material Substitution:** Here, the main focus is on increasing E-Factors to minimize waste during the synthesis stage, which is the most resource-intensive stage [13].

**Lifecycle Assessment (LCA) Documentation and Regulatory Compliance**: Pfizer extends its environmental responsibility throughout the supply chain and pledges to reduce greenhouse gas emissions using science-based targets [12]. They directly address the primary source of their Scope 3 emissions (bought products and services) by requiring 90% of important suppliers to set GHG reduction objectives and 100% of key suppliers to manage their environmental impacts [12].

**Dr. Reddy's and Cipla:** Promoting Sustainability in Developing Economies Prominent Indian companies exhibit a proactive shift towards eco-friendly operations, harmonizing international demands with local legal structures.

**Manufacturing Optimization Strategies:** Cipla promotes energy conservation through the Cipla Green Pledge and has installed solar power projects at a number of its manufacturing facilities [6]. In order to reduce chemical waste and maximize resource utilization in laboratories, Dr. Reddy's focuses on process optimization, which includes utilizing energy-efficient techniques through maintenance management [4].

**Packaging Redesign and Material Substitution:** Cipla makes a concerted effort to incorporate recyclable materials in their packaging[6]. Dr. Reddy's demonstrates a commitment to reducing its downstream impact by incorporating explicit Packaging Waste Reduction Projects and sustainable logistics activities into its ESG framework.

**Regulatory Compliance and Lifecycle Assessment (LCA) Documentation:** Both businesses are credited with helping the Indian pharmaceutical sector transition from a model that was primarily focused on compliance to one that was focused on sustainability[6]. They are positioned as leaders in the area because of their compliance with both the implicit quality criteria of international market standards and the changing CPCB directives.

**FUTURE PERSPECTIVES**

To guarantee ongoing success, future developments in sustainable pharmaceutical practices must concentrate on using revolutionary technology and quickening regulatory convergence.

Digital Transformation and Transparency: In order to improve regulatory capacity and reliance mechanisms, especially in low- and middle-income nations, future efforts should give priority to digital transformation. Digital tools can be used to improve accountability and transparency in sustainable procurement and supply chain management procedures. These solutions may make use of blockchain technology. Additionally, this will make it easier to track LCA data for materials accurately and consistently over the course of a product's lifecycle.

**Advanced Green Chemistry and Biotechnology:** Sustained funding must go into cutting-edge research, like the use of biocatalysis and new enzymatic processes. These developments have the potential to significantly increase E-Factors well beyond existing chemical techniques, resulting in previously unheard-of decreases in waste production during API synthesis.

**International Harmonization of Environmental Quality Standards:** It is highly recommended that regulatory organizations like CDSCO, USFDA, and EMA expedite the harmonization of environmental quality standards. Simplifying creative regulatory processes and encouraging early cooperation between regulators and producers can drastically save development costs and delays, hastening the global adoption of vital eco-friendly inventions. Additionally, in order to establish acceptable limitations for the environmental fate of APIs, consistent global standards are required.

## CONCLUSION

The conclusion that integrating green manufacturing and sustainable packaging is the most important and practical strategy to create an environmentally friendly pharmaceutical sector is largely supported by the data. This strategy shift, which is typified by the concepts of Green Chemistry, effective resource usage, and circular package design, results in measurable decreases in greenhouse gas emissions, hazardous waste production, and environmental impact. Adopting these strict procedures is crucial for gaining competitive advantages in the Indian economy. Indian manufacturers improve their access to international markets, draw in foreign partners, and promote long-term profitability by aligning their processes with global quality systems (ICH Q10) and strong regulatory controls (USFDA, EMA), as well as by closely following prescriptive mandates like CPCB's ZLD requirements. As a result, the domestic pharmaceutical industry is well-positioned to use sustainability as a catalyst for organizational innovation. There are significant advantages for public health. Local communities are immediately shielded from dangerous exposure by source-level pollution protection, which includes strict wastewater treatment, air emission controls, and the replacement of hazardous chemicals. By ensuring that the industry fulfils its ethical obligation to reduce its environmental impact, this commitment supports international efforts to alleviate health risks associated with climate change and ensures the population's long-term well-being.

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**Table: 1 Comparative Regulatory Environments**

The regulatory focus differences are outlined below, emphasizing how India's control system differs from the international PQS requirements. Comparative Pharmaceutical Industry Environmental Regulatory Environment

| Jurisdiction/Authority | Primary Environmental Focus | Key Mandates / Guidelines | Compliance Philosophy |
|---|---|---|---|
| **India (CDSCO & CPCB)** | Pollution Abatement (Wastewater, Air Emissions), Hazardous Waste Management | CPCB Draft Guidelines, Mandatory Zero Liquid Discharge (ZLD) systems, Online Continuous Monitoring (OCEMS) | Highly prescriptive, compliance-driven, shifting toward long-term sustainability |
| **United States (USFDA)** | Quality Control, Risk Management, Green Chemistry Integration | ICH Q10 (Quality System), Stringent GMP enforcement, robust validation processes | Rigorous, risk-based, detailed quality management |
| **European Union (EMA)** | Risk Management, API Environmental Fate, Manufacturing Harmonization | EU GMP Annexes (e.g., Annex 1), centralized and decentralized procedures | Harmonized, proactive risk-based approach to lifecycle impact |
| **WHO & ICH** | Global Quality Systems, Sustainability Standards, Regulatory Innovation | ICH Q10, WHO call for "Greener pharmaceuticals' regulatory highway," New sustainable standards | Global standard setting, promoting transformative change and regulatory convergence |

**Table: 2 Matrix for Case Study Evaluation**

Evaluation Matrix for Sustainability Performance in Industry Case Studies

| Company | Manufacturing Optimization Strategies | Packaging Redesign/Material Substitution | Regulatory Compliance & LCA Documentation |
|---|---|---|---|
| **Novo Nordisk** | Zero impact goal, 100% renewable electricity achieved, 33% Scope 3 $CO_2$ reduction target by 2033 | Shift to reusable injection devices, ReMed™ take-back program, focus on plastic reduction | Adherence to ISO14040/44 LCA standards, third-party verified environmental reports |
| **Pfizer** | Proactive Green Chemistry integration in R&D, optimizing processes for energy savings, improved E-Factors | Focus on reducing undesirable solvents and minimizing waste from API synthesis | Science-based GHG targets; mandating 100% of key suppliers manage environmental impacts |
| **Cipla / Dr. Reddy's** | Solar power implementation, energy conservation programs, process optimization, closed-loop systems | Focus on recyclable materials (Cipla); Waste Reduction Projects (Dr. Reddy's), sustainable logistics | Transitioning from compliance-driven to purpose-driven sustainability, aligning with CPCB mandates |

**Table: 3 Percentage Distribution of Sustainability Assessment Metrics Used in Pharmaceutical Manufacturing**

| Sustainability Metric | Percentage (%) |
|---|---|
| PMI (Process Mass Intensity) | 28 |
| E-Factor | 17 |
| Atom Economy | 17 |
| Carbon Footprint | 55 |
| $CO_2$ Production | 39 |
| Water Usage | 61 |
| Life Cycle Assessment (LCA) | 44 |

**Table 4: Environmental Impact Reduction from Green Manufacturing Practices**

| Sustainability Indicator | Conventional Manufacturing | Green Manufacturing | % Improvement |
|---|---|---|---|
| Energy Consumption | 100 | 70–85 | 15–30% |
| Water Usage | 100 | 60–80 | 20–40% |
| Hazardous Waste Generation | 100 | 50–75 | 25–50% |
| $CO_2$ Emissions | 100 | 60–82 | 18–40% |

**Table: 5 Gap Analysis of Current and Desired Sustainability Practices in Pharmaceutical Manufacturing and Packaging**

| Sustainability Parameter | Current Practice (%) | Desired Level (%) | Identified Gap (%) |
|---|---|---|---|
| Energy efficiency | 70 | 95 | 25 |
| Water management | 65 | 90 | 25 |
| Waste reduction | 60 | 90 | 30 |
| Sustainable packaging | 45 | 90 | 45 |
| Green chemistry | 50 | 85 | 35 |
| Scope 3 emissions | 33 | 80 | 47 |

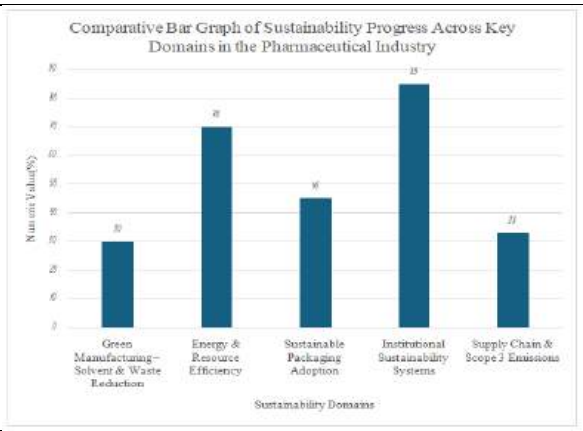



**Figure 1: Sustainability progress Across Key Domains in Pharmaceutical Industry**

The findings show that different pharmaceutical sector domains have adopted sustainability at different rates. Governance support has led to greater advancements in institutional sustainability systems and energy efficiency initiatives. Due to technological and legal obstacles, green production and sustainable packaging have made only modest progress. Due to external dependencies, supply-chain sustainability is still constrained.

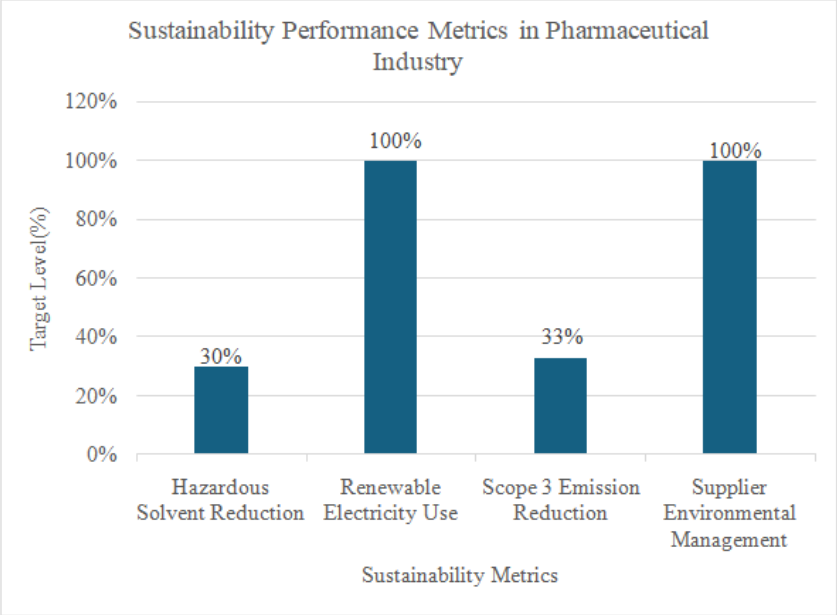



**Figure 2: Sustainability Metrics in Pharmaceutical Industry**

Adoption of renewable electricity and supplier environmental management show high performance. Due to the complexity of the process and the lack of external oversight, the decrease of hazardous solvents and Scope 3 emissions shows slower development.

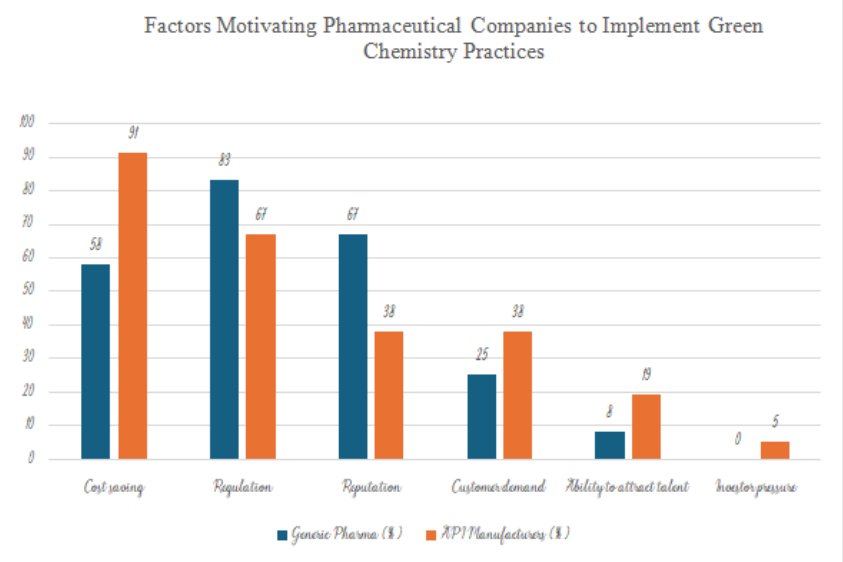



**Figure 3: Factors Motivating Pharmaceutical Companies to Implement Green Chemistry Practices**

The findings indicate that the adoption of green chemistry processes in the pharmaceutical business is mostly influenced by cost reductions and regulatory requirements. While API producers prioritize cost reduction and consumer demand, generic pharmaceutical businesses are primarily motivated by regulations and reputation, revealing disparities in sustainability priorities across industries.

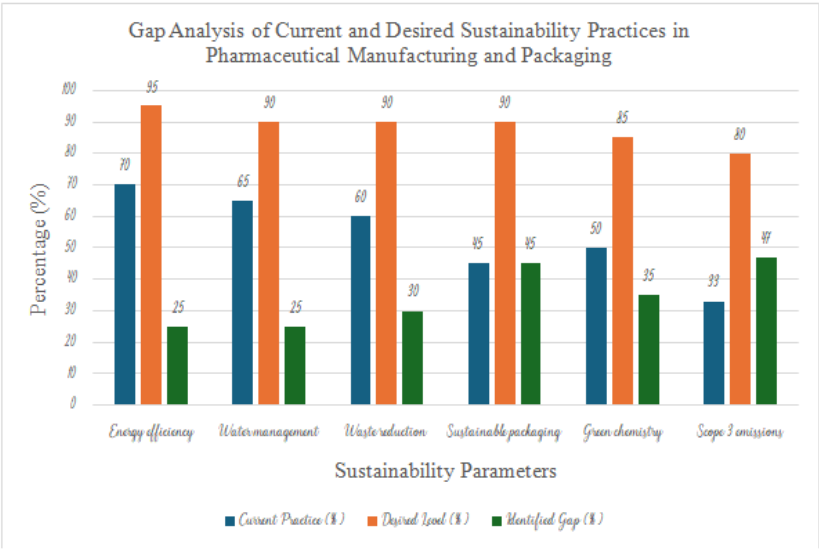



**Figure 4: Gap Analysis of Current and Desired Sustainability Practices in Pharmaceutical Manufacturing and Packaging**

The gap analysis shows notable discrepancies between intended sustainability goals and current industry practices, especially with regard to Scope 3 emission management and the adoption of sustainable packaging. Priority areas for attaining environmentally friendly pharmaceutical production are indicated by the significant gaps in green chemistry implementation and supply-chain-level sustainability, despite moderate advances in energy and water efficiency.

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REVIEW ARTICLE

# Types of Nutraceuticals and their Potential in the Prevention and Management of Cancer - A Review

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**ABSTRACT**

The definition of Nutraceutical is 'a substance which can be separated or refined from food that has physiological benefits and also plays a pivotal role in protection against chronic disease. Many studies show that nutraceuticals play a major part in chemoprevention and in the management of cancer .They usually consist of phytochemicals which are non-nutritive chemical compounds present in plant based diet that have various potential health benefits. The use of nutraceuticals along with chemotherapeutic drugs is quickly gaining recognition in recent years. The growth of nutraceuticals can be attributed to the shifting of consumer preferences towards self-directed care in the management of diseases and also because of various trends in health care. The classification of nutraceuticals, the anticancer potential of nutraceuticals via its antioxidant activity and modulation of gene expression, biological effects of major phytochemicals in chemoprevention along with a detailed study of earlier works will be discussed in this review

**Keywords:** Nutraceuticals, phytochemicals, anticancer, chemo preventive, antioxidant

## INTRODUCTION

Cancer is defined as the abnormal and uncontrolled cell growth with the ability to metastasize or spread to different body parts from a primary site. Many studies demonstrate that nutrition and its role in chemoprevention [1] is closely interlinked. It is proved that healthy food practices when followed by cancer patients can facilitate the efficacy of chemotherapeutics and aid in the prevention of many lifestyle disorders.

## METHODOLOGY

A detailed study of literatures pertaining to nutraceuticals and cancer management were done in the internet using academic databases such as Google scholars, pub med, ScienceDirect and peer reviewed journal sources. To search relevant literatures, many keywords such as "nutraceuticals", "chemoprevention", "phytochemicals", "antioxidants", "gene expression" were used which were included in broad criteria. The literatures pertaining to broad inclusion criteria were further narrowed down to focus on the most relevant aspects of the current study.

## RESULTS

Based on the search criteria, 150 research papers and 50 review articles were obtained. Out of the 200 articles reviewed, 175 more relevant articles were more relevant. 175 papers were further screened to search for literatures that met the broad inclusion criteria and thereby 99 articles were excluded. The remaining 76 articles were further reviewed for narrow criteria, by which 35 articles were obtained, which were used for the current review. (Figure 1)

## DISCUSSION

The term 'Nutraceutical' was first described by Dr. Stephen L DeFelice [2][3][4] who is the chairman and founder of an American organization, Foundation for Innovation in Medicine, which was established in the year 1989 and is derived by fusing the words 'nutrition' and 'pharmaceutical' (Figure 2). He said that a nutraceutical is a substance which provides health or medical benefits which includes preventing and treating the disease." These are biological substances with protective and therapeutic properties which are mainly derived from food and plant such as garlic, [1] soya bean, tea and others. There can also be certain compounds present in the plants which may not be considered as a nutrient. This non-nutritive chemical component in plant based [5] diet which forms an active ingredient is considered to be a major part of nutraceuticals. Nutraceuticals can be categorized [2] into major types (Figure 3) such as Fatty Acids, Dietary Fibers, Vitamins and minerals, Prebiotics/Probiotics and Phytochemicals

**Fatty Acids**

Nutraceutical fatty acids are involved in the prevention of various chronic disorders. They have many beneficial effects in the management of Diabetes, Cardiovascular Disorders, Alzheimer's disease, antitumor effects, development of brain in infants and fetal development. Some major examples (Table 1) are Omega-3-fatty acid, *Brucea Javanica* oil, Conjugated linoleic acid and Lecithin.

**Dietary Fibers**

Dietary Fibers are plant derived food that can't be hydrolyzed by digestive enzymes produced by the human cells but can be broken down by the microflora present in the stomach. Some common examples of dietary fibers are celluloses, hemicelluloses, lignin, gums, pectin and other non-starch polysaccharides.

Dietary Fibers can be divided into two category based on their water solubility (Table 2):
**Soluble Fibers:** includes pectin, gums, β-glucans, some hemicelluloses
**Insoluble Fibers:** includes lignin, celluloses and some hemicelluloses

**Vitamins and Minerals**

Vitamin A, C, D, E and minerals like zinc, selenium, calcium, magnesium aids in preventing cancer by protecting DNA from free radical attack (Table 3).

**Prebiotics/ Probiotics**

Probiotics are live beneficial microorganism present in certain food which aids in maintaining the normal microflora found inside the human body whereas prebiotics refer to high fiber food supplements that selectively facilitates the growth of favourable probiotic bacteria(Table 4). These provide various health benefits [14].

**Phytochemicals**

Phytochemicals are non-nutrient chemical compounds produced mainly by plants. Some major categories of phytochemicals are carotenoids and phenolics.

**Carotenoids**

Carotenoids (Table 5) are naturally occurring organic tetraterpene pigments which are produced mainly by plants and algae. They give a characteristic color to fruits such as orange, watermelon and vegetables. Increased intake of the pigment carotenoid reduces the probability of the cancer of breast, cervix, colon, rectum and also plays a part in the prevention of heart diseases.

Some major types are Lycopene, Lutein, Beta-carotene, Alfa-carotene, Zeoxanthin, and Cryptoxanthin.

**Phenolic**

Phenolics are very essential dietary components that have aromatic rings with hydroxyl groups. They are known to exhibit antioxidant activity and decrease the risk of cancer.

Some major types of phenolics (Table 6) are Quercetin, Apigenin, Daidzen, Naringenin, Catechins, Secoisolariciresinol, Resveratrol, Curcumin, Hesperitin, Gallic Acid, Myricetin, Gingerol, Berberine and EGCG.

**Nutraceuticals and Cancer**

The term cancer does not solely refer to one disease but rather includes a bunch of diseases where the cells in the body grow abnormally with the ability to metastasis to various distant sites. Transformation to cancer doesn't happen in a single step and thereby it involves a multistage [31] progression which can be split into four phases: Initiation of tumor, promotion of tumor, Transformation of tumor to malignancy and progression of tumor. A major cause of cancer is oxidative stress which happens when the equilibrium [32] or the balance between anti-oxidants and free radicals is disrupted. It also occurs due to overproduction [31] of reactive oxygen species due to lack of antioxidant defenses. It ultimately results in tissue injury and eventually leads to abnormal activation of cellular pathways which facilitates cancer progression. Nutraceuticals aids in chemoprevention by maximizing immune system and antioxidant defenses of the body. Through its antioxidant activity [33], it can protect the DNA from any damages and thereby modify the expression of tumorigenic progression.

Some of the mechanisms [34] adopted by nutraceuticals against oxidative stress:

1. Increases the amount of antioxidant enzymes
2. Helps to repair mitochondrial function
3. Facilitates chelation of metal ions
4. Increases immune response
5. Prevention of lipid peroxidation
6. Upregulates production of anti-inflammatory molecule
7. Inhibits inflammatory pathways, genes and factors
8. Scavenges Reactive oxygen species

## CONCLUSION

Nutraceuticals are soon becoming popular worldwide due to its pivotal role in the prevention of various diseases. Their global success can be due to their nutritional benefits along with therapeutic effects and safety profile. Many

studies regarding their role in the management of cancer have been published on various platforms and are quickly gaining recognition in the medical field. Their popularity can also be due to very few adverse effects associated with its usage. Their antioxidant property along with their major effect on cellular metabolism facilitates in the management of cancer. Further research is still required to completely fathom the action of nutraceuticals in the management of cancer.

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**Table 1: Some examples of nutraceuticals fatty acid.**

| Component | Source | Biological Effects | References |
|---|---|---|---|
| **FATTY ACIDS** | | | |
| 1. Omega-3-fatty acids | Linseed, fish oil, rapeseed ,mustard | Anti-inflammatory effect, Antinociceptive effects, Modulates cell signaling | 6,7,8 |
| 2. Conjugated linoleic acid | Meat Milk | Inhibits tumorigenesis, Apoptosis , Alters Cell proliferation and Lipid peroxidation | 9 |

**Table 2: Types of Dietary Fibers.**

| Component | Source | Biological Effects | References |
|---|---|---|---|
| Soluble Fibers | Oats ,Peas , beans , Barley, Apple | Reduces risk of CVD, Anti-cancer, Acts as digestive stimulant | 10,11 |
| Insoluble Fibers | Nuts ,Wheat , Corn Barn | Lowers the possibility of breast and colon cancers, Facilitates digestion by promoting digestive enzymes secretion | |

**Table 3: Examples of vitamins and minerals in nutraceuticals.**

| Component | Source | Biological Effects | References |
|---|---|---|---|
| VITAMINS e,g.Vitamin A, B, C, E, K | Products derived from food, vegetables, eggs, fruits, milk, fish | Anti-oxidant activity, Modulation of immune response and enzymatic activity, Inhibition of cell to cell interaction | 12,13 |
| MINERALS e.g. copper, calcium, potassium, zinc, cobalt, iodine, selenium, iron | Products derived from food ,fruits and vegetables | Inhibits enzyme protein kinase C, Inhibits c-myc expression, H-ras Inhibits the expression of a transcription factor called E2F, Increases the expression of TGF-β (transforming growth factor – beta) Stimulates the expression of p21 genes | |

**Table 4: Prebiotics and Probiotics as nutraceuticals.**

| Component | Source | Biological Effects | References |
|---|---|---|---|
| Prebiotics | Gut, Yogurt, Plants, Animals | Immunomodulation, Onco-suppression, Antidiabetic effect | 14,15 |
| Probiotics | Food waste, Plants, Fungi , Animals | Chemoprevention, Decreases cell proliferation, Induction of apoptosis | 16 |

**Table 5: Some examples of carotenoids as nutraceuticals.**

| Component | Source | Biological Effects | References |
|---|---|---|---|
| **CAROTINOIDS** | | | |
| 1. Organic Pigment Lycopene | Tomatoes, melons, grapefruit ,guava, capsicum ,papaya | Powerful Antioxidant, Lowers the risk of prostate cancer | 17,18 |
| 2.Beta carotene | Carrots, pumpkin, sweet potato | Aids in scavenging free radicals which damages the cells, Stimulates antioxidant defense mechanisms of the cells | 19,13,7 |
| 3. Alpha carotene | Orange | | |
| 4. Zeoxanthin | Avocado, Corn | Apoptosis | 20,21 |
| 5. Cryptoxanthin | Tangerines | Antioxidants Apoptosis | 22 |

**Table 6: Major types of phenolic as nutraceuticals.**

| Component | Source | Biological Effects | Types of Cancer | Reference |
|---|---|---|---|---|
| **Phenolics** | | | | |
| 1. Stilbenoid Resveratrol | Grapes, plum, cocoa peanuts, apple, berries | Inhibits the activity of cell growth, Chemo preventive, Programmed cell death (apoptosis), Inhibits Cell-cycle process | Colon, Breast, Prostate, Lung | 23,24 |
| 2. Curcumin | Turmeric | Prevention of cancer, Inhibits the process of cell growth, Programmed cell death (apoptosis), Inhibits the activity of angiogenesis, Inhibits cell-cycle process. | AML, Breast, Prostate, Colon Lung, | 25 |
| 3. Catechins (EGCG) | Green Tea | Inhibits the activity of Cell growth, decreases the proliferation of cell , Chemo preventive, Cell-cycle arrest, inhibition of Angiogenesis , | Colorectal, Liver, Esophagus Prostate, Lung, Bladder, | 26,27 |

| | | | | |
|---|---|---|---|---|
| | | Programmed cell death (apoptosis) | Thyroid | |
| 4. Flavanoid Quercetin | Broccoli Onion, citrus fruits, Mango, Raspberry, Tomatoes, Apple | Inhibits the activity of cell growth, Suppresses the proliferation of cell , Chemo preventive , Arrests the cell-cycle process, Programmed cell death (apoptosis) | Prostate, Colon, Ovarian, Gastric, Breast. | 28 |
| 5.Gingerol | Ginger | Anti-inflammatory, analgesic, antipyretic, Antihepatotoxic Antioxidant effects. | Gastric, Pancreatic, Liver, Colorectal, Cholangiocarcinoma. | 12 |
| 6.Alkaloid Berberine | goldthread, Oregon grape, ,Barberry, Cork-tree, Orangeroot | Increases stability of genomes, Inhibition of telomerase and topoisomerase, Increases tumor suppression | Liver, Breast, Lung, Gastric, Colorectal, Ovarian, Cervical, Prostate | 29 |
| 7. Apigenin | Thyme, Parsley, peppermint, red wine ,tomato sauce | Cell proliferation inhibition , Apoptosis, Inhibits cancer cell, migration and invasion, Modulates cell cycle | Colorectal, Breast Liver, Lung Melanoma, Prostate Osteosarcoma | 30 |

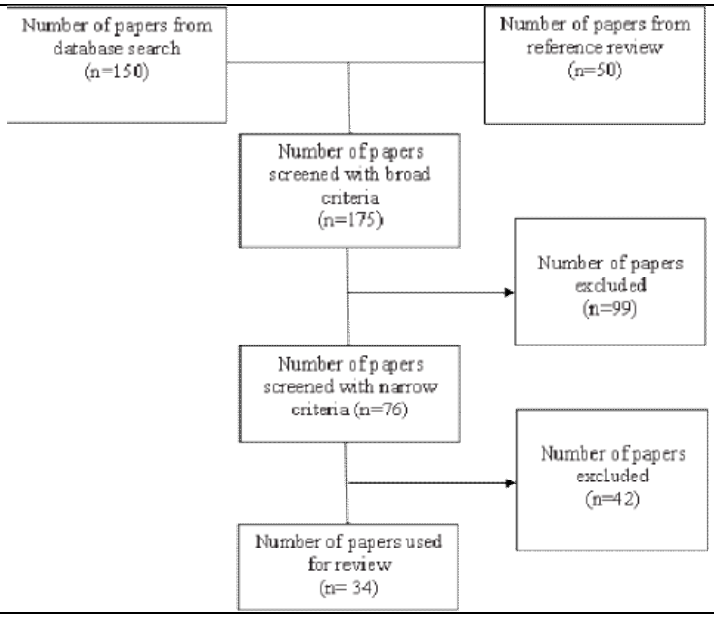



**Figure 1: Flow chart explaining the process of screening of the literatures for the current review.**

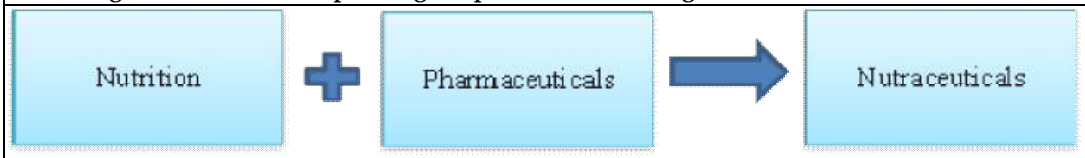



**Figure 2: The word 'Nutraceuticals' is obtained from combining the words 'nutrition' and 'pharmaceuticals'.**

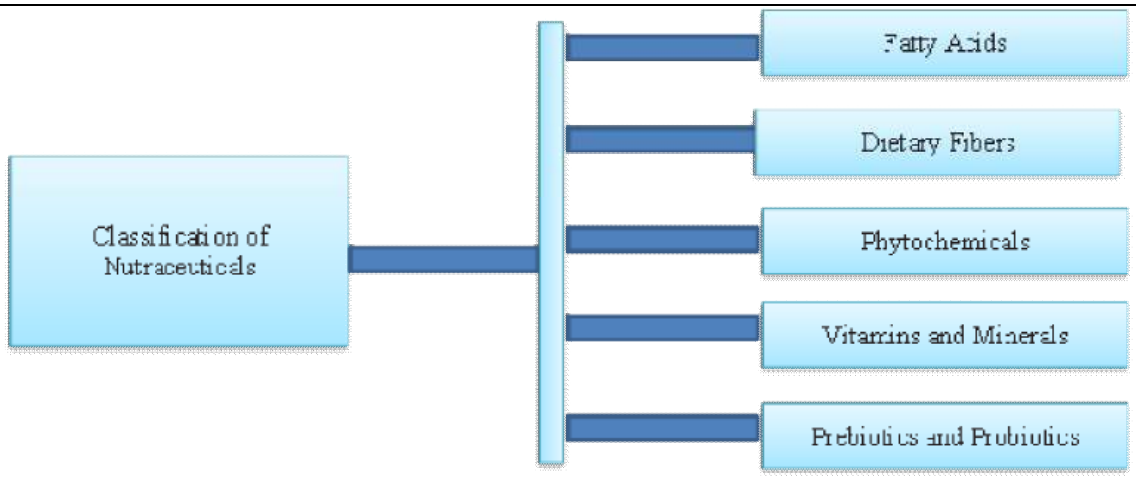



**Figure 3: Classification of nutraceuticals**

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RESEARCH ARTICLE

# A Comprehensive Analysis of Machine Learning and Deep Learning Approaches for Improving Weather Prediction Accuracy

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## ABSTRACT

The accurate prediction of weather is critical in activities like farming, the management of renewable energy, the mitigation of disasters, and planning city development. However, the traditional Numerical Weather Prediction (NWP) systems are typically limited to their short and localized forecasts, as systematic errors, coarse resolution and computational inexpensiveness are tendencies that usually limit them. To address such problems, this research will analyze time-series-based machine learning and deep learning algorithms, namely Long Short-Term Memory (LSTM), Gated Recurrent Unit (GRU) and hybrid CNNLSTM models on the NWP results. The proposed framework to model the spatio-temporal features will combine the spatial feature extraction of satellite imagery and grid-based weather data with a Convolutional Neural Network (CNN) and a Long Short-term Memory network (LSTM) Gated Recurrent Unit network (GRU) to learn temporal dependency. The NWP products are applied to overcome systematic forecast errors and enhance the models. The functionality of the methodology is evaluated on a conventional basis of performance measures, like RMSE and MAE through measurement in several meteorological parameters, including temperature, rainfall, wind speed, and humidity. Through experimentation, it is completely demonstrated that the hybrid CNNLSTM model is more accurate and reliable than single statistical, machine learning, and deep learning models. It is established that physics-based non-weather

prediction and data-driven deep learning models offer a possible and scalable solution to the improvement of the data accuracy in the short-term and local weather prediction.

**Keywords:** Bias correction, CNN -LSTM, Deep learning, GRU, Hybrid models, Numerical weather prediction, Spatio-temporal modeling, Time-series analysis, Weather forecasting.

## INTRODUCTION

Accurate weather forecasting is critical in such fields as agriculture, renewable energy, disaster management and city planning [1], [2]. Numerical Weather Prediction (NWP) models are conventional forecasting models that are models of atmospheric processes that are simulated using physics-based equations [3]. Even though NWP is effective in generating medium-term and long-term forecasts, it is ineffective in generating small scale, short range and extreme weather changes due to systematic errors, low-resolution, and the expensive nature of computations [4], [5]. Machine learning (ML) and Deep Learning (DL) have become powerful in enhancing the accuracy of prediction by the capability to obtain nonlinear entities of meteorological data both in time and space that are intricate [6], [7]. Time-series models are Long Short-Term Memory (LSTM) and Gated Recurrent Unit (GRU) networks, which can be helpful to learn the time-dependent correlation of temperature, rain, wind speed, and humidity [8], [9], [10]]. The Convolutional Neural Networks (CNNs) learn spatial representation of the satellite imagery and gridded weather data, which enables hybrid models to learn both spatio-temporal dynamics [11], [12]. Despite such advances, there are still some problems present and these are noisy and un finished data, absence of extreme-event samples, overfitting of models and high cost of computation [13], [14], [15]. Systematic bias can be eradicated by combining NWP products and DL and can also be used to improve forecast accuracy [16], [17]. The current research proposes a hybrid CNN-LSTM framework, which may utilize the benefits of both Dl and NWP to furnish inaccurate short-term and temporalized weather forecasts [18], [19]. The strategy is evaluated based on a range of meteorological parameters and is compared to single ML and DL models [20], [21]. Results are associated with superior prediction, strength and viability of operations [22], [23]. This work is connected to the data, modeling, and computation-problem of the existing weather prediction, and to the possibilities of the hybrid approach in bridging the physics-based and data-driven forecasting [24], [25], [26]. This diagram in Figure 1 demonstrates a Hybrid Deep Learning (DL) and Numerical Weather Prediction (NWP)-based structure that enhances the quality of weather forecasting. The products are a combination of raw meteorological data with machine-learned models and NWP output to generate predictions of high quality.

## BACKGROUND STUDY

Hossain and Mahmood (2020) state that Photovoltaic (PV) short-term power forecasting framework is an LSTM-based framework, which is triggered by synthetic weather forecasts [1]. This idea was to address the PV intermittency using deep learning. The research gap it dealt with was low levels of accuracy of standard statistical forecasting PV procedures in an erratic weather. The LSTM network was developed with the aid of artificial weathering conditions and past PV. The shortcoming of the model was that synthetic predictions were employed as opposed to real time meteorological forecasts. The findings revealed that the traditional methods had an enormous enhancement of PV forecasting short-term accuracy. Localized atmospheric variations can be captured by one deep learning weather forecasting model that is proposed by Hewage *et al*. (2021) [2]. The research investigated the loophole of space and time in view of prediction of systems. The convolutional and recurrent neural networks were used to model multi-scale weather features. However, the model was massive with respect to training data and processing power. The short-term forecasting was found to be better at home scale. The article by Choi and Jung (2023) [3] involved the optimization of the Numerical Weather Prediction (NWP) models using machine learning post-processing techniques. The main aim of this was to combine physics models with data-based learning. Systematic bias and inefficiency in the conventional NWP output was identified as the gap. NWP forecasts were

corrected using ML models that were based on regression. The boundaries diminished generalization within different climatic regions. The results confirmed the existence of reduced forecast error and increased predictions consistency measures. Nikunjkumar (2024) [4] made a comparative research of the models LSTM, BiLSTM, and CLSTM in weather prediction. The deficiency in comparison evaluation between deep recurrent architectures was discussed in the study. The LSTM variants had been used to model time-series weather data. The research was restricted to short-term forecast horizons and a constant dataset. BiLSTM was found to be more predictive as compared to traditional LSTM and CLSTM. Karbaula *et al*. (2024) [5] developed the parsimonious deep learning model that relied on a mesh that was developed on the basis of the HEALPix in predicting weather in a global scale. The research took into consideration the problem of scaling and solution to global DL models. Graph-based neural networks on meshes on the sphere were invented. Restraints were identified to be expensive computations and complexity of model. The results showed that lower number of parameters was used to achieve the competitive accuracy compared to the conventional DL models. In Surve *et al*. (2025) [6], the deep learning models were compared to the NWP outputs in terms of predicting the results. The research addressed inconsistencies in models of DL only and physics models. CNN and LSTM structures were used with NWP features.

The system became limited through the use of trusted NWP outputs. Findings indicated that prediction reliability and forecasting error were less. Yang *et al*. (2020) [7] were concerned with the prediction of power outages within the scope of weather conditions basing on the severity of the corresponding event. This was a gap in research where there was no effective modeling to determine the effect of extreme weather in energy infrastructure. Machine learning classifiers were applied using weather and outage information. It was limited by the fact that it could not be used beyond the area of study. Results indicated the better outage forecasting ability in unfavorable weather conditions. Teixeira *et al*. (2024) [8] model is a hybrid between LSTM and genetic algorithm (GA), which is a weather forecaster. The article addressed the issue of inefficient hyperparameter optimization in the models of DL. GA was used to optimize parameters of LSTM. High computational cost was also another limitation that was prominent. The results showed that the accuracy was higher than any single LSTM model. In order to predict short-term rainfall at Lake Victoria Basin, Gahwera *et al*. (2024) [9] analyzed several ML algorithms. The research plugged this research gap in developing countries as far as region-specific rainfall modeling is concerned. Ml ensemble and regression were applied. Some of the limitations included unavailability of the data and short durations of observation. Random forest had the highest accuracy of prediction of rain falls. Pasche *et al*. (2025) [10] verified the application of deep learning weather models on high impact extreme events. The research included the problem of lack of effectiveness in measuring robustness at the extremes. The new data on extreme weather was subjected to DL models powered by CNN. The biggest weakness was the fact that extreme-event data were limited in availability. Results showed disparate performances and thus, extreme training on events was a necessity.

The research by Han *et al*. (2021) [11] explored the implementation of the RNN models in order to improve the localized weather prediction on the basis of the combinations of the on-site measurements and airport data. The gap addressed in the research was the inaccurate nature of micro-scale weather forecast when used in the built-environment. Through LSTM, the authors integrated the multi-source datasets and the recurrent neural networks in order to forecast the short-term. Results showed that there was an enhanced degree of precision but also lacked features like application to specific site and sensitivity towards quality of data. The application of machine-learning and its use in the weather forecasting and climate analysis is gaining more and more significance as Bochenek and Ustrnul (2022) [12] surveyed the instances in the area. They located their problematic aspects of stable assessment and perception of the ML model operation in atmospheric activities. The research has adopted a review method and tested algorithms including the random forests, SVMs and neural networks. The findings proved to have huge predictive power but noted to have weaknesses that encompass interpretability issues as well as dependency on good data. Han *et al*. (2022) [13] proposed that a hybrid deep learning model could boost the predictions of the wind speed using NWP. The research was on NWP forecast bias forecasting in the wind energy. The CNN-LSTM architectures were used to correct errors. In general, there was a little generalization except for forecasting of the speed of the wind. The results indicated that there was a lot of minimization of errors in the short-term prediction of the wind. Allen *et al*. [14] introduced a worldwide weather forecasting system using data-driven solutions on an end-

to-end basis. The article spoke about inefficiency and cost of calculation of classical models of NWP. Transformers used to train neural networks on world data were of large scale. Horizontal limits were excessive computation requirements and data requirements. Results indicated operational NWP system competitiveness. The article by Huang *et al*. (2020) [15] studies the issue of real-time weather forecasting using data by mobile sensors, which are deployed on the buses of the city. In this research, the issue of urban sensing data sparsity was addressed. ML models were used to train mobile sensor data of crowdsourced data.

The limitations were imperfect coverage and sensor noises. It was established that urban microclimate was better predicted. One such model of wind power prediction in the city that was proposed by Shirzadi *et al*. (2023) [16] incorporated Weibull distribution, RNNs, and NWP. There was a lot of uncertainty in the prediction of wind in the cities in the research. The deep learning and hybrid statistical methods were applied. Integration was one of the complex requirements. The findings presented greater error-reduction accuracy in urban wind power. The prediction model based on the short-term load and weather variables presented by Chen *et al*. (2023) [17] is a ResNet-LSTM. The research gap was the ineffective connection of weather forecasting and load. The other CNNs and LSTMs were stacked. The model entailed a high degree of tuning. It was found that there was improved precision in load prediction. LSTM models proposed by the authors of the article by Sarmas *et al*. (2023) [18] do not require NWP, at least when it comes to PV forecasting. The research covered the issue of NWP dependency. Several LSTM optimizers were used in meta-learning. Poor Scala was mentioned. A good generalization of results between sites that had not been examined revealed positive results. The spatio-temporal fusion model of STFM was created to predict weather in Liu *et al*. (2024)[19]. The ineffectiveness of the research gap was comprised of the inefficient manner of treatment of spatio-temporal dependencies. Fusion architecture-based deep learning was developed. There was limited calculation overhead. The findings indicated that a higher predictability of various variables was obtained. The whole structure of AI-based wind prediction based on NWP was given in one of the studies by Kosovic *et al*. (2020) [20]. The experiment involved constant predictive pipes. Both ensemble ML models and NWP outputs were combined. Complexity of the system was also one of the constraints. The outcomes showed that the calculations of wind power were effective. In Table 1, Summary of Recent Weather-Related Machine Learning and NWP Research provides a synthesized review of literature with the application of ML, DL, and NWP tools in various applications that are weather-dependent. It outlines the principal concept of any piece of work, research gap, methods, constraints and findings of the performance. The table enables one to make a prominent contrast between the different forecasting methods and their strengths and weaknesses in various fields. In general, it can help to identify the new trends and the unresolved issues that may be the foundations of the further evolution of the hybrid weather-prediction systems.

**Time Series Deep Learning Models (LSTM, GRU) for Weather Prediction**

**1LSTM for Weather Prediction**

One of the applications of the Long Short-Term Memory (LSTM) networks in weather prediction is that it proves to be rather effective in time-dependent modeling, with references to the time-series meteorological data [1], [2]. The short-term variations and the long-term seasonal variations that can be captured by LSTM model include the variations in the weather conditions including the temperature, rainfall, humidity and the speed of the wind [11]. On the other hand, in contrast to classical statistical models LSTM can learn nonlinear variations that are complex and dynamic atmospheric processes only using data [12]. The LSTM networks besides having a memory storage of the information in the cell have gating mechanisms that do not allow any information to be lost in long sequences that facilitate long-horizon predictions to be constant [17]. It has been established after many studies that the LSTM-based models have shown to be more accurate in regards to short and medium-term weather forecasting activities than the traditional ones [4], [8]. As a result, LSTM is currently an applicable method of deep learning in creating believable and information-constrained weather forecasting techniques [6], [14].

**GRU for Weather Prediction**

GRU networks are robust deep learning networks that have significantly been employed in weather prediction activities that involve time series [2]. GRU networks have few parameters compared to LSTM networks and,

therefore, they train quicker and can have less complexity in their calculations, yet, they can modify themselves to time patterns on meteorological variables, including temperature, wind speed, or rainfalls [8], [17]. GRU has update and reset gating, information flow control and enable learning long term dependencies on sequential weather information [12]. It is also known that GRU models are effective when it comes to short-term and real-time weather forecasting tasks, especially at data-driven and localized prediction tasks [4], [11]. In addition, they are capable of dealing with the nonlinear relationship and the noisy meteorological data and this enhances the strength of the predictions [6], [19]. Recent deep learning-based weather prediction models [14], [27] utilize GRU networks due to a beneficial structure, functionality and a stable accuracy.

**Comparative Analysis of LSTM and GRU**

**Table 2: Comparison of LSTM and GRU Models for Weather Time-Series Prediction**

Comparison of LSTM and GRU Models on Weather Time-Series Prediction provides an explanation on what are the architectural and operational differences between LSTM and GRU network. LSTM involves use of separate memory cells and 3 gating and besides it can learn long term dependencies with fewer cost but with high cost. In contrast, GRU can use a simpler form of gating architecture with lower training cost and train faster hence it is easily applicable to short to medium-range weather prediction problems.

**Machine Learning Approaches for Accurate Weather Prediction**

The machine learning (ML) algorithms have been more important to prepare the weather forecasting more precisely, through managing the massive quantity of historical and current weather data [2], [9]. The ML processes in contrast to the ancient numerical weather prediction processes are data-driven and they can acquire nonlinear correlations between weather variables [12]. Some of the popular ML algorithms include linear regression, decision trees, random forests, support vectors machines, k-nearest neighbors and ensemble learning among others [9]. The parameters that have been predicted well using these models include temperature, rains, velocity of wind and humidity [9]. The criteria of the feature selection and engineering decision are relevant in improving the capacity of the model and also enable the minimization of uncertainty [22]. The ensemble and hybrid Ml models are better than the single algorithms because they enhance the strength and the generalization [21]. The ability to train and make inferences on local and short time weather forecasting is highly used in the performance of the ability [11]. Nevertheless, the most crucial feature that is needed to determine the accuracy of prediction is the quality and data availability [27]. Nevertheless, low regional interpretability and scalability is a grave issue [27]. Nevertheless, machine learning has continued to offer practical and reasonable answers to the relevant weather forecasting [22]. Figure 2 is a machine learning based weather prediction system and it begins with data collection in big quantities of previous and current weather readings and it is a common use in data forecast research [1]. The different learning approaches that are applicable to learn the nonlinear and complex weather patterns include linear regression, decision trees, support vector machines, random forests, and ensemble learning [9]. The feature selection and engineering also reduce the number of the most relevant attributes to give a stronger and improved prediction [13]. Finally, the processed information provides reliable and accurate weather forecasts and also addresses the key strengths and viable problems [14] that constitute the data quality, scalability, and interpretability.

**Deep Learning Approaches for Accurate Weather Prediction**

The deep learning techniques developed have considerably contributed to the accuracy of weather forecasting as they have been in a position to recreate non-linear relationships in weather predictions that are highly complex [2], [12]. The deep learning techniques neither utilize the classical Numerical Weather Prediction (NWP) models, which need to be trained on very large sets of past and present weather conditions [14]. Convolutional neural networks are generally applied to identify the patterns on a spatial image in satellite images and weather maps [19]. GRU and LSTM recurrent models of time series weather data have used changes in time series data with respect to time and seasonality [1], [4]. The combination of CNN and LSTM to improve the performance of spatio-temporal forecasting is more performance-achieving [6], [17]. Deep learning models are especially popular in diagnosing tasks of weather prediction on small and local scale [9], [11]. They also play an important role in the correction of systematic errors in the numerical weather prediction outputs [3], [13], [20]. These types of models are however resource consuming and

need a lot of data to be trained [5]. It is an issue [10], [12] that the interpretability and extrapolation of extreme events that are not observed is problematic. Nonetheless, deep learning is still involved in the significant improvements of the quantitative and effective weather forecasting models [14], [16]. In Figure 3, a deep learning-based system of weather prediction commences with the acquisition of big data of both past and present weather conditions. CNNs are used to extract the spatial information of the satellite images and weather maps, and recurrent models, such as LSTM and GRU, are trained to acquire the variability of the weather patterns across time. These models are combined to perform spatio-temporal forecasting with the help of hybrid CNNLSTM architectures. Finally, the system produces accurate and efficient weather predictions on these parameters such as temperature and speed of the wind thus unveiling its strong and weak points.

**Weather Prediction Using Time Series Analysis Models**:

The time series analysis is important in the weather prediction in that weather conditions like temperature, rain, humidity, and the velocity of wind change with time. Time series techniques are significant in escalating the forecast abilities, particularly that of the short and medium terms, by modelling the time dependence of past data.

**Statistical Time Series Models**

Statistical time series models are mathematical plans to calculate and predict time-dependent weather-related variables, such as temperature, rainfall, humidity, speed of the wind and atmospheric pressure [18]. These models are based on an assumption that the weather values in the future can be estimated by the process of identifying the patterns and trends and the seasonal behavior of the weather by basing on the historical meteorological information [8]. The earliest weather forecasting processes were based on statistical models which are highly applicable in the present times because they are simple, cost less to compute and easy to interpret [23]. They are especially efficient in cases where there exist some underlying weather patterns which are relatively stable, linear, as well as of a recognized seasonal nature [24].

**Key Statistical Time Series Models**

- Autoregressive (AR) Models[12]
- Moving Average (MA) Models[34]
- ARMA (Autoregressive Moving Average) Models[12]
- ARIMA (Autoregressive Integrated Moving Average) Models [24]
- SARIMA (Seasonal ARIMA) Models[34]
- Exponential Smoothing Models[12]

**Machine Learning Models**

This is the fact that the machine learning (ML) models may be used that were set to improve the accuracy of weather forecasting in those cases when the traditional statistical time-series models that are used are linear and stationary [2].Compared to the traditional statistical time-series models that are applied, which are linear and stationary, the machine learning (ML) models could be applied with the high-dimensional input and recognize the nonlinear interactions between weather variables, including temperature, rainfall, humidity, wind speed, pressure, and others [9], [12]. The most common methods of ML that are applicable to weather prediction are the linear regression, decision trees, random forests, support vectors (SVM), k-nearest neighbors (KNN) and ensemble learning (EL) methods [21]. Such models are very popular in local and short term weather forecasting because they have the ability to train and make conclusions at a very fast speed [11]. The model performance mainly depends on the feature selection and feature engineering since the quality of the inputs data is a direct determinant of the quality of the predictions [9]. The Ensemble ML models are superior to single learners in terms of robustness and generalization [21]. Nevertheless, the output and validity of the data and their low scalability and interpretability to other climatic regions continue to be an issue with the ML-based weather prediction systems [27]. In spite of these, machine learning models are providing meaningful and sound answers to data-driven weather forecasting apps [22].

**Deep Learning Models**

One of the most radical technologies in the current weather forecasts is considered to be deep learning (DL) models [12], [14]. Both the statistical and machine learning techniques have historically been dependent on this property, deep learning directly models complex pattern to raw meteorological data, and have allowed a model of nonlinear and multi-scale and highly dynamic atmospheric processes [2]. It is this that renders DL especially useful on a spatio-temporal weather forecasting problem wherein both spatial variability such as cloud movement and temperature fields as well as temporal variability such as seasonal variability and storm evolution should be modeled simultaneously [6], [13], [19].

**Challenges in Weather Prediction Using Time Series Analysis**

Weather prediction requires time series analysis since weather continues to vary with time. An accurate prediction is however difficult to derive because of a range of challenges.

**Data Challenges**

Problems of weather prediction like, quality of meteorological data, consistency and availability of the same do exist. The weather data is often few, noisy and sensor failures which can pose a risk to the models. These variables are too many but they are very diverse and therefore patterns can hardly be captured. In addition, information can be distributed in various sources on variety of formats or at various times that makes the integration a problem. Moreover, the model is not supported by much history in the instances of rare occurrences (like heavy storms). These challenges should be overcome so that the suitable and acceptable forecasting systems can be established.

**Modelling Challenges**

The systems in the atmosphere are extremely complex, dynamic, and non-linear making the modeling of the weather prediction a problem. Dependences which are long term, abrupt variations or discovering the interaction between two or more weather variables may be extremely difficult to model. There is a risk of overfitting whereby the models are learning the noise rather than the meaningful patterns and there is also the risk of underfitting which is caused by using too simple models. One also faces a challenge of finding a suitable architecture, hyperparameter optimization and accuracy versus cost of computation. Another challenging modelling is also weather forecast modelling because different regions and seasons demand some models.

**Practical Challenges**

Practical issues of weather prediction are practical constraints to forecasting system application. They include low computational capability to train large models and run large data. The assimilation of the information sources of heterogeneity (e.g., satellites, ground sensors and NWP) may be hard. It is of importance to have real time prediction as well as instant release of prediction. Another problem is the problem of operational constraints like sensor operation, data delay and difference in covering different areas and this adds complexity in deployment. These are practical matters that should be addressed to ensure that the accurate weather forecast can be effective and fruitful.

**Computational Challenges**

Massive volumes of meteorological information and heavy data rate of data received during the weather prediction process are issues in the computing world in terms of high memory and processing speed. The deep learning systems, particularly intricate models, are power-hungry to be inferred and trained. Online prediction strains quick predictions and this restricts the complexity of models. The high dimensional spatio-temporal data is the new overheads, which increases the computational overheads. They should therefore optimize algorithms and hardware to manage them.

**Spatial and Temporal Challenges**

The specifics of the time and space issue of weather forecasting are based on the reality that specific patterns of place are needed, and time changing processes concurrently. The traditional models tend to put in mind a single location giving no consideration to the direction of flow of weather in the areas. Extreme events are rare but hard to anticipate

as they are not prevalent in the past. The multi-location or satellite data in the research contributes high dimensionality and hence it is difficult to model. These types of spatial-temporal interdependence must be accurately modeled to be able to do effective predictions.

## DISCUSSION

This survey has shown that a time series based machine learning and deep learning systems can be applied to significantly expand short-term and local weather prediction compared to conventional Numerical Weather Prediction (NWP) forecasting. Recurrent neural networks which include LSTM and GRU have been shown to be effective in a nonlinear time-dependent dependency of variables of meteorological variables, whilst CNNs learn meaningful spatial features of satellite data and gridded data. On the same note, the literature too is in agreement that LSTM is more practical in the long-term dependency modeling, although the cost of computation is less and the GRU model can be trained much faster, thus in real-time forecasting. The weaknesses of the classical statistical models are addressed by the capabilities, and the forecast accuracies of such variables as temperature, rainfall, wind speed, and humidity are improved. High performance of hybrid systems that can combine the outcomes of deep learning and NWP is the primary outcome of the study. The models of CNNNWP are built on the physical consistency of NWP and they take care of the systematic bias by applying the data-driven learning which results in enhanced robustness and reliability. However, it is not yet clear that there have been no problems relating to data quality and availability, modeling requirements, and application of models on a regional basis. To make the usability of the next-generation weather prediction systems in practice, it is crucial to involve these issues by creating effective models and data fusion and explainable AI techniques.

## CONCLUSION

The time-series based machine learning models and deep learning models, according to this survey, prove to be effective in improving the accuracy of short-term and local weather prediction. The classical Numerical Weather prediction models are good at the long-scale forecast but suffer in terms of bias, coarse spatial resolution, and high expensive computations. On the contrary, nonlinear longitudinal correlations in meteorological data can be trained by LSTM and GRU models, and spatial correlations of satellite and gridded data can be trained by CNNs. The hybrid CNNLSTM structures are the combination of deep learning models with NWP outputs that minimizes the errors that occur in forecasting and maximizes the prediction accuracies on different variables, including temperature, rainfall, wind speed, and humidity. The recent works have revealed experimental proofs that hybrid and ensemble methods provide the best results compared to single-standing statistical, ML, and DL models. Despite the fact that certain challenges relating to the quality of data, computing capabilities, and extrapolation of extreme events still remain, the findings indicate that physics-based NWP that applies data-driven deep learning could be a scalable and reliable answer to the next generation weather forecasting systems.

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**Table 1: Summary of Recent Weather-Related Machine Learning and NWP Research**

| **Author *et al.* (Year) [Ref]** | **Concept** | **Research Gap** | **Methods** | **Limitations** | **Accuracy / Performance** | **Result / Findings** |
|---|---|---|---|---|---|---|
| **Sansine *et al.* (2022) [21]** | Probabilistic solar irradiance forecasting | Lack of accurate hybrid ML–NWP models | ML + Metaheuristics + NWP integration | Complex tuning; weather variability impact | Improved probabilistic forecast metrics | Hybrid approaches outperform single models |
| **Rahman *et al.* (2022) [22]** | Rainfall prediction for smart cities | Need for fusion-based rainfall forecasting | ML fusion models | Data imbalance; urban microclimate variation | High precision and recall | Fusion models significantly enhance rainfall prediction |
| **Wang *et al.* (2021) [23]** | Load forecasting using LSTM with weather features | Limited inclusion of weather variability | LSTM recurrent neural networks | Sensitive to hyperparameters | High short-term load accuracy | Weather features boost load forecasting reliability |
| **Reveshti *et al.* (2023)** | Climate change impact on | Unclear effect of old vs new weather data | Comparative weather datasets + | Region-specific; generalization issues | Not specified | Newer climate data reveals rising |

| | | | | | | |
|---|---|---|---|---|---|---|
| **[24]** | building energy | | energy simulations | | | energy demands |
| **Cheon and Mun (2023) [25]** | AI trends in weather-related patents | Missing review on AI innovation in meteorology | Patent analysis + trend evaluation | Patent focus only; no technical validation | Not applicable | AI-driven weather solutions rapidly increasing |
| **Pandya *et al.* (2020) [26]** | Pollution-weather prediction | Need for real-time pollution-weather integration | Smart sensors + ML | Sensor noise; environmental variability | High prediction accuracy | System helps early detection of hazardous air quality |
| **Yadav *et al.* (2025) [27]** | ML for remote-region weather forecasting | Poor forecast quality in remote areas | ML models with sparse weather inputs | Limited data availability | Good forecast improvement | ML bridges data scarcity issues |
| **Kim and Park (2024) [28]** | Flight delays due to weather | Lack of strong predictive models for aviation delays | Data-driven delay prediction (ML) | Complex influence factors | High classification accuracy | Weather-based delay prediction reliable |
| **Bouras *et al.* (2021) [29]** | Cereal yield forecasting using climate indices | Limited integration of climate indices + drought signals | ML + Satellite + Climate indices | Regional training required | High $R^2$, low RMSE | Combined models improve yield prediction |
| **Qu *et al.* (2025) [30]** | Weather variability and renewable energy | Lack of explainable ML for energy–weather links | Explainable ML (XGBoost, SHAP) | Model interpretation complexity | Strong predictive accuracy | Weather variability strongly drives energy consumption |
| **Yuan *et al.* (2022) [31]** | Terminal traffic prediction under weather impact | Limited deep learning for air-traffic + weather | DL-based traffic prediction | Requires large datasets | Good prediction performance | Weather-aware models enhance traffic situational prediction |
| **Kim *et al.* (2024) [32]** | Weather-aware trajectory-based operations | Lack of seamless weather–geospatial integration | Geospatial fusion + weather data | Complex integration pipeline | Not specified | More efficient and safer trajectory operations |
| **Almeida *et al.* (2024) [33]** | Forest harvesting prediction using weather | Poor integration of weather + harvesting datasets | ML prediction models | Data noise; environmental variability | Good prediction accuracy | Weather features improve harvesting prediction |
| **Journé *et al.* (2025)** | Weather cues of mast | Need comparison of | Statistical modelling | Species and region-specific | Not provided | Identifies key weather |

| | | | | | | |
|---|---|---|---|---|---|---|
| **[34]** | seeding | statistical weather–seed models | (multiple methods) | | | drivers of masting behavior |
| **Fedor and Hofierka (2021) [35]** | Improving WRF models with land cover data | Insufficient land-cover integration in NWP | WRF-ARW + Corine Land Cover + JAXA | Region-specific applicability | Improved NWP accuracy | Enhanced prediction from land-cover refinement |
| **Wang and He (2022) [36]** | Ultra-short-term wind power prediction | Need multi-task spatiotemporal ML models | Multi-modal, multi-task ST attention network (M2STAN) | High model complexity | Superior multi-step forecasting | Achieves state-of-the-art wind power predictions |

**Table 2: Comparison of LSTM and GRU Models for Weather Time-Series Prediction**

| Feature | LSTM[4] | GRU[36] |
|---|---|---|
| Memory mechanism | Divided memory cell | Combined concealed state |
| Gates | Input, Forget, Output (3) | Update, Reset (2) |
| Computational cost | Higher | Lower |
| Speed of training | Slow | Fast |
| Long-term dependency handling | Excellent | Good |
| Suitability | Long and complex sequences | Short–medium sequences |

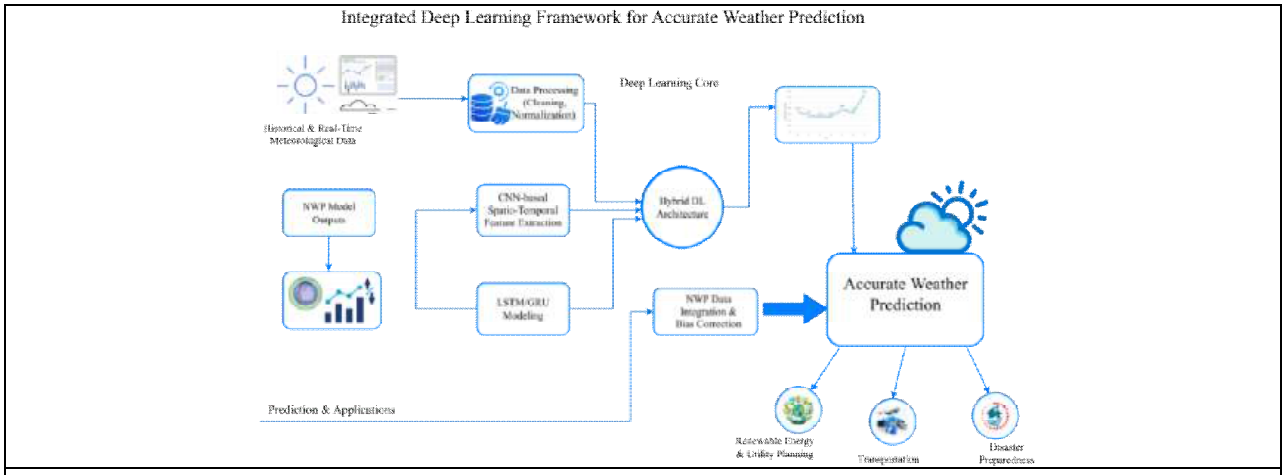



**Figure 1: Hybrid Deep Learning–Enhanced Weather Prediction Pipeline**

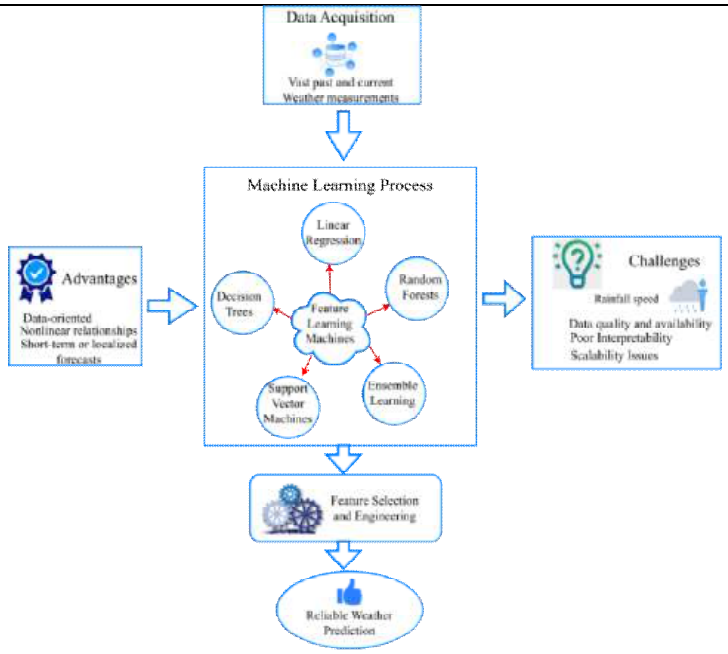



**Figure 2: Framework of Weather Prediction using machine learning.**

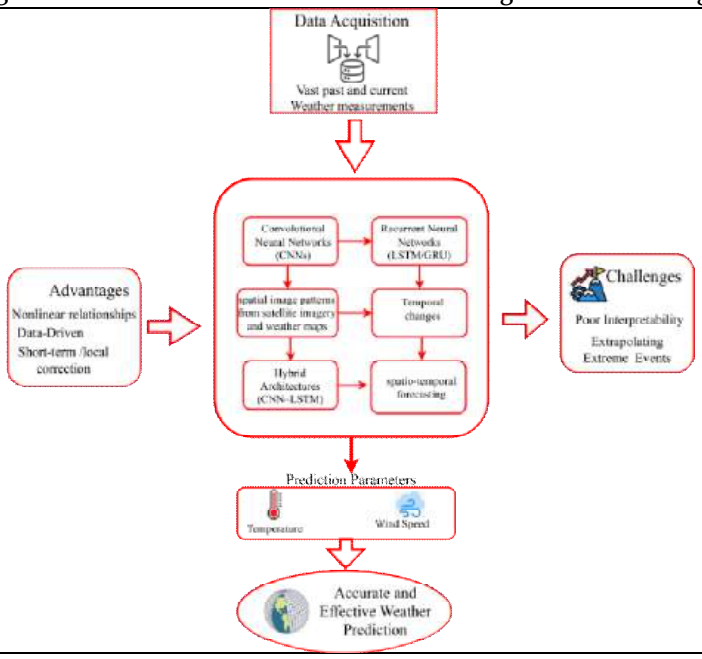



**Figure 3: Deep Learning–Based Weather Prediction Framework**

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RESEARCH ARTICLE

# A Comprehensive Survey on CNN - Based Automatic Modulation Recognition for UAV - Enabled 6G Wireless Networks

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## ABSTRACT

The addition of Unmanned Aerial Vehicles (UAVs) to the sixth-generation (6G) wireless networks comes with new demands of intelligent and adaptive physical-layer technologies, specifically, Automatic Modulation Recognition (AMR). These environments are extremely dynamic (highly dynamic UAV communication) and therefore traditional feature-based AMR techniques cannot keep up with the reliability challenges of the channel variation, Doppler and on-board resources. The main aim of the proposed study is to examine and study Convolutional Neural Network (CNN)-based AMR algorithms, which can be highly accurate without requiring too much resource constraints that would be on UAV platforms. The suggested methodology studies the recent lightweight and deep CNN models, reviews alternative signal representations, and studies their behaviour in diverse signal-to-noise ratios and mobility. Existing studies have shown comparative insights based on the fact that CNN-based AMR greatly excels as compared to traditional methods in terms of robustness and classification accuracy even under low-SNR conditions. Findings also indicate that lightweight CNN models provide a good trade-off between performance in recognition and computational efficiency and thus can be used to implement UAVs in real-time. Finally, AMR based on CNN is one of the enabling factors of intelligent 6G networks with UAVs, and further studies are necessary on adaptive, secure, and energy-efficient learning models to be applied to large-scale systems in practice.

**Keywords:** Automatic Modulation Recognition, Convolutional Neural Networks, Sixth-Generation (6G) Networks, Unmanned Aerial Vehicles, Wireless Communications.

## INTRODUCTION

The sixth-generation (6G) wireless network rapidly envisions an interoperable and totally unified its terrestrial, aerial and space communication networks to assert ultra-reliable, low-latency and high-capacity services. Intelligent communication mechanisms will be lightweight and energy-efficient in this paradigm, and the aerial platforms in this paradigm will have stringent hardware limitations [1]. Recent progress in deep residual learning also proves the possibility of strong modulation classification in the context of complex wireless conditions [2].Unmanned Aerial Vehicles (UAVs) are identified as one of the enabling factors of 6G networks as they allow flexible deployment, expand into space, and offer flexibility in connectivity between air, ground, and space infrastructures [3]. The UAV-to-ground communication setting has also explored self-attention-based learning models to enhance the modulation recognition performance of the mobility and channel variability in the environment [4]. Automatic Modulation Recognition (AMR) represents a fundamental physical-layer which is an intelligent spectrum awareness and adaptive communication feature in such environments [5].

Given the real-time requirements, to address the needs of lightweight AMR solutions with compressive sensing and deep learning have been proposed to simplify the computation complexity without compromising accuracy [6]. At the network level, the role of artificial intelligence will likely be centralized when it comes to the automation of operations, resource savings, and smart control in 6G networks [7]. In addition to modulation recognition, ensemble learning models have also been used in passive RF fingerprinting and UAV identification in next-generation networks [8]. Recently, transformer-based architectures have become promising alternatives to CNNs and can provide better adaptation to dynamic spectrum situations in drone communication systems [9]. The spectrum monitoring methods using deep learning also introduce AMR features to multi-signal acquisition and situational awareness of UAV networks [10]. Robust AMR methods that are resilient to phase differences and synchronization degradation have also been researched to increase the reliability in a real-life scenario [11].Intelligent vehicular and V2X communications are also boosted by 6G networks with the use of UAVs, which implement a distributed learning and federated clustering system [12]. Quantized deep neural networks have been created with AMR applications in mind to enable them to be deployed on resource-constrained platforms [13]. Dual-stream spatiotemporal fusion networks and hybrid data augmentation have proven to be more resilient in the case of drone-based modulation classification [14].

One of the key obstacles to UAV communications is low-SNR conditions that encourage the creation of robust AMR models that can be used to keep the performance of AMR in noisy environments [15]. It has been suggested that dual-path deep residual shrinkage networks can be used to reduce noise and better discriminative features extraction without being too large networks [16]. AMR is also applicable in wider UAV remote identification and surveillance structures that are important in airspace security [17].AMR is also used in emerging 6G architectures that include UAVs, satellites, and high-altitude platforms in the context of non-terrestrial networks [18]. The application of beam forming and backscatter communication methods (as a deeper example of deep learning-based methods) also underscores the persuasive value of UAVs in massive IoT connections [19]. Regarding security, AMR augments physical-layer security models inspired by artificial intelligence in 6G networks [20].Machine learning-based AMR are well-known as the underpinning technologies of beyond-5G and 6G wireless intelligence [21]. Nonetheless, AI-controlled UAV communications are challenged with the matters of scalability, robustness, and energy efficiency and require further studies [22]. In the end, AMR is clearly supposed to be a pillar of self-education, independent 6G networks to serve smart cities and digital ecosystems [23].

The primary paper contributions include three aspects: (i) the background research and comparative analysis of the recent AMR, UAV communication, and AI-enabled 6G research; (ii) the detailed discussion of signal representations, CNN architecture, challenges, and security concerns unique to UAV-based AMR; and (iii) revealing open research directions towards lightweight, robust, and secure AMR solutions. The rest of the paper is structured in the following

way: Section 2 presents related literature; Section 3 explains UAV-based 6G architecture, Section 4-6 explain CNN-based AMR principles and architecture, Sections 7-8 discuss the challenges and security concern and finally, Sections 9 and 10 discuss the paper and provide conclusions, respectively.

In this figure 1, a UAV-aided 6G communication architecture that has UAV-to-Satellite, UAV-to-UAV and UAV-to-Ground connections are featured using realistic constraints, including low battery capacity, payload, and onboard computation. It proposes a CNN-based Automatic Modulation Recognition (AMR) system based on 1D and 2D lightweight CNN models to achieve customer robustness at low-SNR signal recognition and counter security threats such as jamming, spoofing, and adversarial attack. The suggested design can facilitate the deployment of energy-efficient UAV and ensure flawless connectivity to use it in managing smart cities, autonomous vehicles, and remote surveillance.

**Background study**

Sarkar et al. (2025) [1] In this paper, the authors propose a super-light CNN to automatic modulation recognition (AMR) in 6G networks of the UAVs with the minimal complexity. This is the limitation to the research because it reaches high accuracy with the limitations of the UAVs such as low power and processing. The classification of the modulation is carried out using a shallow CNN that has optimized parameters. Although it is an efficient one, it is performance-degrading under severe channel impairments competition performance is exhibited with lower cost of computation. This study introduces a masked modeling and residual neural network to make the modulation classification better, as suggested by Peng et al. (2023) [2]. The used gap is the lack of the feature generalization of the traditional deep AMR models. Masked learning residues provide resistance to noise. The model is however computationally demanding; it has been established that the model is more accurate in different circumstances of a channel. This article [3] by Othman et al. is a review of enabling technologies to 6G, specifically focusing on UAVs, THz communication, and intelligent reflecting surfaces. The problem is the failure to have holistic integration frameworks of these technologies. Techniques that are compared are comparative review techniques, and the analytical review techniques. As a survey, it cannot be regarded as an experimentally proven one, yet as a strategic source of information on future 6G architectures.

The authors of the study recommend a multi-subsampling self-attention network to be applied to the UAV-to-ground AMR systems (Shen et al., 2023) [4]. The gap that is taken care of is the inadequate adaptability of CNNs to the unrelated UAV signal dynamics. A self-attention mechanism captures long distance dependencies. It is a complex model and results have shown that it performs better when it comes to UAV communication. Kaleem et al. (2021) [5] the article provides an AI-based real-time AMR system in the next-generation cellular networks. The difference is real-time deployment capability of deep learning AMR. Neural models of low weight and latency are used. Scalability is still a problem but the results on improvements of accuracy in real-time are valid.

LightAMC [6] LightAMC is a lightweight AMR model, based on deep learning and compressive sensing. The research gap is associated with the ability to decrease the model size without compromise of accuracy. CNN is used to represent a compressed signal. At extremely low SNRs, the performance decreases, and at very high SNRs, efficiency is observed to be appropriate to edge devices. Alhammadi et al. (2024) [7] This article presents a review of AI applications, opportunities, and challenges of 6G wireless networks. The addressed gap is the discontinuous knowledge on AI roles in 6G. The systematic literature review is carried out. The absence of the details of the implementation is a weakness; findings present major obstacles and the development of the research in the future. Zahid et al. (2024) [8] the authors suggest a compound ensemble learning framework of passive drone RF fingerprinting in 6G. The weakness is in dependable identification of drones when operating in dynamic conditions. Several classifiers are combined in order to augment discrimination. Computational overhead is an issue, and the accuracy of fingerprinting has been high. MobileRaT [9], Zheng et al. develop a lightweight radio transformer of AMR in drone communication systems. The gap that is being solved is transformer inefficiency in mobile platforms. Small transformer architecture is developed. Little testing conditions are a disadvantage; performance is shown to be great when there is less complexity. Hou et al. (2023) [10] in this study, the authors provide a deep learning-driven

multi-signal spectrum monitoring of UAV communications. The gap is a simultaneous detection of several types of signals. Multi-signal classification based on CNN is implemented. The training data is still high; the results are showing better spectrum awareness. Shi et al. (2020) [11] the article suggests a deep learning AMR algorithm that is phase offset resistant. The performance degradation caused by uncertainty in phases is addressed. CNNs have phase-invariant feature learning. Only limited modulation types are taken into account and the results demonstrate improved robustness. Azeemi et al. (2023) [12] this paper examines an intelligent V2X communication with federated clustering based on UAV enabling 6G communication. The privacy learning gap of the UAV-assisted networks is its preserving learning. Clustering and federated learning methods are employed. It has a weakness in communication overhead; there are better results in network efficiency.

Gomez et al. (2022) [13] the authors suggest a technique of designing AMR quantumized deep neural networks. The absence is deficiency of systematic quantization schemes of AMR models. Quantization and optimization models are used. There is a possibility of accuracy loss, yet the results indicate that the results are appropriate in low-power devices. Gong et al. (2023) [14] in this paper, a hybrid data augmentation and two streams of spatiotemporal fusion of AMR on a drone are presented. The gap that was bridged is lack of diversity in training. Dual-stream CNNs are used to augment the data. The complexity of the model is increased; it results in high accuracy improvements. An and Lee (2023) [15] the article is concerned with strong AMR when in a low SNR condition. The discontinuity is the poor performance of AMR in noisy conditions. Deep learning architecture that is resistant to noise is suggested. Channel generalization is not available and performance is shown to be high in low SNR.

Table 1, a comparative background study of the recent researches on automatic modulation classification, UAV communications and AI-enabled 6G networks is presented. It indicates the main ideas, methodology and detected gaps in the research, where the trade-offs between the performance, complexity and practicability issues are noted. In general, the table offers a condensed perspective of the existing developments and shortcomings, which allows determining the direction of future research in the area of lightweight, intelligent, and scalable 6G communications.

**UAV-Based 6G Wireless Communication Architecture**

A 6G wireless communication architecture based on UAVs allows aerial platforms to be used as smart communication nodes, relays, or base stations to provide ultra-reliable, low-latency, and high-capacity services. The 6G networks will be able to provide a seamless connectivity in the terrestrial, aerial, and space domains by using UAV mobility, flexible deployment, and AI-driven intelligence.

The 2 figure shows a UAV-supported 6G communication architecture that incorporates UAV-to-Ground (U2G), UAV-to-UAV (U2U), and UAV-to-Satellite (U2S) connections with the purpose of providing seamless air-ground-space communication. It draws attention to the main wireless issues like path loss, channel variations fast, high mobility, change in altitude and Doppler that affect UAV communications. They are also highlighted by the diagram with practical constraints such as limited energy, payload capacity, and power management and how UAVs interface with the 6G core network in order to accommodate smart city and edge-enabled applications.

**UAV Communication Models (U2G, U2U, U2S)**

**UAV-to-Ground (U2G):**

UAVs in U2G communication establish wireless networks with whatever exists on the ground, whether it is a base station, mobile user or the objects of Internet of things (IoT). UAVs can also be used to play the role of aerial base station, relays to enhance its coverage in remote areas, disaster prone areas and urban centres. U2G enjoys a benefit of the likelihood of high line-of-sight (LoS) propagation in comparison with earthly connections.

**UAV-to-UAV (U2U):**

U2U communication is the one that supports direct communication of data between a group of UAVs, which can be applied in cooperative networking, swarm intelligence and multi-hop aerial relaying. The model improves the resilience of the networks, latency and distributed sensing and decision making in the swarm of UAVs.

**UAV-to-Satellite (U2S):**
U2S communication links UAVs to low-earth orbit (LEO) or geostationary satellites so that it covers the entire world. Beyond-line-of-sight missions, maritime surveillance, and remote monitoring in remote locations that lack terrestrial infrastructure are some of the important uses of this model.

**Characteristics of UAV Wireless Channels**
The UAV wireless channels possess some unusual characteristics as opposed to terrestrial channels. UAV links are likely to have high LoS components, less shadowing, and less multipath because of high altitudes. Nevertheless, the quick movement of UAVs in position and orientation cause rapidly varying channel conditions, varying path loss, and occasional non-line-of-sight (NLoS) operation, particularly in cities.

**Mobility, Altitude, and Doppler Effects**
The great UAV mobility and time-varying altitude provides a substantial Doppler shift and time-selective fading. High speed movement results in frequency imbalances that worsen signal synchronization and the accuracy of modulation recognition. Path loss and interference patterns are impacted by changes in altitude and thus demand adaptive modulation, coding and smart signal processing schemes to ensure a stable communication in 6G UAV networks.

**Integration of UAVs in 6G Networks**
In 6G systems, UAVs will be combined with land networks, edge computing, and cloud computing infrastructure. Mobile edge computing, network slicing, and AI-based resource allocation are some of the functions supported by UAVs. They provide ultra-low latency services, dynamic coverage boosting, and connectivity continuity in deployments of smart cities, autonomous systems, and massive IoT, due to their integration.

**Energy and Payload Constraints in UAV Systems**
There exist stringent energy and payload restrictions on UAVs because of battery size and weight. Both communication modules, sensors and on board AI processing consume more power and payload. Power management, lightweight hardware design and energy conscious communication protocols are required to extend the UAV flight time and make it sustainable in 6G networks.

**Deep Learning for AMR: Role of CNNs**
Automatic Modulation Recognition (AMR) is an essential role of the UAV-assisted 6G wireless, and the entity facilitates smart spectrum control and dynamic communication [1]. Convolutional Neural Networks (CNNs) and other forms of deep learning have become an effective AMR tool because of its capacity to automatically detect discriminative signal features in raw or transformed signal [2].

**CNNs for Modulation Recognition**
Convolutional Neural Networks (CNNs) are particularly suitable to wireless signal analysis as they preferably capture local spatial features that exist in time-series and time frequency representations [4]. They allow direct end-to-end learning on raw IQ samples, spectrograms, or wavelet-transformed signals, and it greatly decreases the manual feature engineering that is necessary [6]. CNNs have the ability to automatically differentiate varying modulation properties after learning hierarchical features representations [9]. In addition, they have high resiliency to noise and multipath fading. This strength is expanded to Doppler effects that are normally observed to occur in the dynamic UAV communication environment [10].

**Feature Learning Capability of CNNs**
CNNs are able to discover hierarchical features, moving straight in terms of low-level signal patterns like amplitude, phase, and waveform edges, to high-level modulation-Entities-specific features [11]. Their convolutional filters are very flexible to signal distortions caused by UAV mobility and channel fading [14]. Through aggregation of local

information CNNs encode discriminative structures in various signal conditions [9]. Also, pooling layers allow the translation invariance, which is beneficial to the robustness to small variations in signal representations [13].

**Comparison with ML and Other DL Models**

Table 2 between the various machine learning and deep models used to perform automatic modulation recognition on UAV based communication systems indicates their ability to extract features and their ability to resist the dynamic aerial channel conditions. Classic machine learning offers hand-designed features and is less adaptive whereas deep learning methods learn discriminative features with enhanced mobility, noise, and channel sensitivity. Spatial architecture Hybrid between spatial and temporal learning have the greatest classification accuracy but require much more computational complexity, rendering implementation on resource-limited UAV platforms more difficult.

**Advantages of CNNs in UAV-Based AMR**

AMR models based on CNN are quite robust to UAV dynamics because they can handle Doppler shifts, changes in altitude, rapidly changing aerial channels among others. Small and compressed CNN models also support on-board inference on UAVs that have small energy and computation capabilities, and thus can be deployed on edges. These models are very scalable and adaptable as they can be further refined with attention mechanisms to enhance accuracy on a variety of modulation schemes and channel conditions that are not visible. In addition, CNN-based AMR can be incorporated easily in 6G intelligence personnel like federated learning, edge AI, and multi-UAV collaborative structures to assist with distributed and autonomous spectrum sensing.

**Signal Representation for CNN-Based AMR**

Signal representation is an important factor in the Automatic Modulation Recognition (AMR) systems that operate on CNN [16]. As the representation of a signal influences the ability of a CNN to extract useful information that can be used to classify a modulation when it is difficult to predict in advance, the representation of a signal is especially crucial in UAV-enabled 6G networks where the signal is highly dynamic, and the state of the channel is hard to predict and analyze [18].

**Raw IQ Signal Representation**

A complex pre-processing is avoided by directly entering the in-phase (I) and quadrature (Q) into a 1D CNN to allow the model to learn amplitude and phase characteristics of the received signal [6].This method retains full signal data and allows effective application of light CNN designs that can be used on-board on UAVs [16]. Nevertheless, direct I/Q depictions are very vulnerable to channel noise and Doppler shift that can deteriorate recognition capability in UAV communication in high mobility settings [14].

**Time-Domain Signal Processing**

The extraction of time-domain features makes instantaneous features of the signal, like amplitude, phase, and other higher-order derivatives, to describe modulation behavior [11]. It can well represent temporal variation that is essential in the separation of the various modulation schemes and is ideally applicable in low-latency communication systems [16]. Nonetheless, its performance can be impaired in the presence of complex modulation formats like OFDM because of low frequency-domain understanding [14].

**Frequency and Time-Frequency Representations**

Frequency-domain and time-frequency feature extraction systems, FFT, STFT, and CWT, do not operate on signals directly, but convert them to display their spectral and joint-time-frequency properties [13]. These representations underline both modulation-specific patterns and capture both temporal and spectral changes and allow feature learning with CNNs to become more effective [14]. They however have increased computational and memory overhead and it is more difficult to deploy in real-time on resource-constrained UAV platforms [16].

**Image-Based Signal Encoding for CNN Input**

In this method, communication signals are converted into two-dimensional representations, including spectrograms or constellation diagrams and analyzed with 2D CNNs [9]. This makes it possible to learn spatial features effectively, resistant to noise and channel impairment and it suits well to hybrid UAV-ground CNN architectures [14]. Nonetheless, the extra pre-processing represents a computational load and needs special scaling and normalization to be used in the deployment of UAVs in real-time [16].

**CNN Architectures for AMR in UAV-6G Networks**

The figure 3 shows a hierarchical convolutional neural network (CNN) architecture of processing and analyzing time-series and spatial information. It starts with 1D, 2D and 7D CNN models that operate on raw time-series data, no convolutional 2D data and feature maps, respectively, to feed into specialized AMR and AMh systems which are optimized to run onboard navigation and ground station processing. The framework combines lightweight CNNs and hybrid temporal analyzers to make complex autonomous systems to be able to extract, classify, and analyze features in real time and with end-to-end feature extraction.

**1D CNN Models**

Convolutional Neural Networks 1D are run on raw time-series IQ signals, and they extract time-based information with high efficiency [2]. They can be used in tasks that require middle-level computing capabilities and have good accuracy, maintaining signal dynamics [4].

**2D CNN Models**

2D CNNs take spectrograms or time-frequency representations of signals as their input with features in both time and frequency domain [9]. This is most effective when the classification performance is required to be high in the circumstances of low SNR or in multipath channel conditions [10].

**Lightweight CNNs for On-Board UAV Processing**

Lightweight CNNs are trained with less layers and parameters to execute on UAVs with low computational capabilities [6]. They allow real-time AMR of on board UAVs and strike a balance between accuracy and energy efficiency [16].

**Deep CNNs for Ground-Station Processing**

Deep CNNs make use of multi-convolutional layers in order to form hierarchical and high level features of UAV signals[11]. They are deployed at ground stations, where they deal with the underlying complex variations in channels and overall AMR accuracy is enhanced without resource limitations of UAVs[14].

**End-to-End CNN-Based AMR Frameworks:**

This is because end-to-end CNN systems combine feature extraction and modulation classification into a single pipeline[16]. These models have the benefit of simplifying processing chain, manual feature engineering and performing well in a large number of UAV communication scenarios[14].

**Challenges in CNN-Based AMR for UAV-6G**

**Fast-Varying Aerial Channels**

The mobility and changes in altitude of the UAVs lead to rapidly varying channel conditions, which cause time varying fading, Doppler shifts and varying signal-noise ratios (SNR) [14]. Such dynamics lower the accuracy of CNN-based modulation recognition because the learned features are not likely to be applicable in rapidly changing situations. They frequently need adaptive training or online learning procedures to sustain performance[16].

**Limited UAV Computational Resources**

UAVs demand severe power, memory, and CPU/GPU restrictions on the on-board. Large parameter CNNs can go beyond these constraints, and real-time AMR becomes difficult [16]. To achieve a trade-off between accuracy and

computational cost, lightweight CNN models or model compression methods (e.g., pruning, quantization) are needed[6].

**Generalization across Environments**

The CNN models might fail in the unseen conditions when they are trained to be in a particular UAV scenario (e.g., urban, rural, or particular altitude ranges). The accuracy of models can be compromised by the environmental factors, which include interference, multipath effects, and weather conditions [10]. It is important to guarantee strong generalization to be deployed in different operational environments.

**Dataset Scarcity**

Quality, labelled data of UAV-based AMR are scarce, especially of dynamic aerial channel or multi-modulation signals [16]. Minimal data can cause over fitting, which decreases the reliability of the model. This can be partially solved by data augmentation, synthetic signal generation or federated learning between UAV networks [18].

**Energy Efficiency Constraints**

The endurance of UAVs is dependent on energy consumption. Inference of CNN and signal processing on board consumes more battery, which decreases flight duration [22]. There is need to have energy efficient algorithms, hardware acceleration, and lightweight models to ensure a trade-off between modulation recognition accuracy and the UAV operational sustainability.

**Security and Reliability Issues in UAV-Based CNN AMR Systems**

Automatic Modulation Recognition (AMR) is a CNN-based feature that has been integrated into UAV-assisted 6G networks, which presents a number of security and reliability issues. UAVs work in dynamic and usually hostile environments, and as such, they are susceptible to targeted assaults and uncontrolled failures[20]. The safety and efficiency of the network functioning is dependent on the reliability and security of AMR systems.

**Jamming and Spoofing Attacks**

UAV communications are susceptible to jamming attacks, where the opponents send high-power jamming signals that reduce the signal-to-noise ratio and result in wrong AMR classification [22]. This interference may seriously interfere with mission critical UAV links. Spoofing attacks constitute additional attacks that impingement on system integrity through the release of signals that are similar to the legal UAV transmissions and deceive the AMR system. A combination of these attacks makes CNN-based AMR considerably less reliable, particularly when using swarms of UAVs and beyond line of sight processes.

**Adversarial Attacks on CNN Models**

Models based on CNN are susceptible to adversarial attacks whereby well-crafted signal perturbations can initiate misclassification without causing any perceivable alterations to a human perceiver[14]. The slightest distortion in the signal sent to a UAV in AMR systems can lead to wrong modulation identification. Enemies can also use UAV mobility and the quickly shifting channel conditions to inject said perturbations on a dynamic basis[10]. Such attacks have the potential to compromise the reliability and validity of autonomy AMR decision-making. Such vulnerability therefore presents critical risks to assured spectrum management, network independence and the reliability of the mission on UAV communications.

**Robust and Secure AMR Techniques**

In response to them, one can use adversarial training where the CNN models are made vulnerable to corrupted signals, thus enhancing their resistance to malicious manipulations [4]. Before AMR classification can occur, methods of signal authentication and verification, e.g. cryptographic techniques or RF fingerprinting, are used to identify spoofed transmissions before authentication. Powerful feature extraction with time frequency representation, self-attention network, or multi signal fusion makes it harder to jam and harder to be noisy [9]. The further improvement of reliability can be provided by the redundant UAV swarm architectures which allow cooperative detection and

classification [12]. Also, through federated learning, resilient and privacy-preserving AMR can be supported since model updates are distributed between UAV nodes to ensure secure operation in adversarial and uncertain contexts [18].

## DISCUSSION

This paper emphasizes that CNN-based Automatic Modulation Recognition is increasingly becoming significant in UAV-based 6G wireless networks that have unpredictable channels, mobility, and resource capacity. It is evident in the literature that the complexity of a model is traded against the real-time deployability where lightweight CNNs are being used as a pragmatic solution to on-board processing of UAVs whereas more sophisticated and hybrid models are used at ground stations. Representation of signals is also important since encodings based on time-frequency and images enhance the robustness but they raise the computation costs. Other security and reliability problems, including jamming, spoofing, and adversarial attacks also make the implementation of AMR more complicated, which underscores the necessity of robust and secure learning strategies. In general, the combination of effective CNN architectures and adaptive learning, energy-consciousness, and security tools will make AMR in the future UAV-based 6G networks scalable and reliable.

## CONCLUSION

The present paper was the in-depth investigation of CNN-based Automatic Modulation Recognition in UAV-enabled 6G wireless networks with a focus on lightweight and robust learning models applicable in dynamic aerial setting. CNNs were demonstrated to perform well to digest modulation-specific features and also to balance between accuracy, computational complexity, and energy efficiency. Although these benefits exist, such challenges as rapidly changing channels, the lack of UAVs, the lack of data sets, and security concerns are still critical. The future direction of work will be on the creation of ultralightweight and adaptive CNN models via model compression, federated learning, and edge intelligence. The reliability in harmful environments can further be increased with the incorporation of self attention, transformer-aided hybrid architectures and adversarially robust training. Moreover, real-world UAV datasets and hardware-in-the-loop experimentation on a large scale are needed to prove the applicability of 6G networks to the real world.

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**Table 1: Comparative Background Study of Automatic Modulation Classification, UAV Communications, and AI-Enabled 6G Network Technologies**

| Reference | Concept | Research Gap | Methods | Limitations | Results |
|---|---|---|---|---|---|
| Suman&Qu (2025) [16] | Proposes a lightweight deep learning model for automatic modulation classification using a dual-path | Existing AMC models suffer from noise sensitivity and high complexity in UAV and 6G scenarios. | Dual-path residual shrinkage network with noise-suppression layers for robust feature extraction. | Evaluated on limited modulation types and simulated datasets. | Achieves high classification accuracy with reduced parameters and strong noise robustness. |

| | | | | | |
|---|---|---|---|---|---|
| | deep residual shrinkage network. | | | | |
| Belwafi et al. (2022) [17] | Provides a comprehensive tutorial and survey on UAV remote identification techniques. | Lack of standardized and scalable UAV identification frameworks across regulatory and technical domains. | Systematic survey of RF-based, network-based, and vision-based identification methods. | No experimental validation or performance benchmarking. | Establishes a clear taxonomy and highlights open research challenges in UAV identification. |
| Nguyen et al. (2024) [18] | Reviews emerging technologies enabling 6G non-terrestrial networks (NTN). | Limited integration between academic research and industrial deployment of NTN technologies. | Comparative analysis of satellite, UAV, and HAPS-based 6G architectures. | Mostly conceptual with limited real-world deployment data. | Identifies key enabling technologies and deployment strategies for future NTN-based 6G systems. |
| Qi et al. (2021) [19] | Introduces deep learning-based backscatter communication beamforming for 6G UAV IoT networks. | Inefficient beamforming and energy constraints in UAV-assisted IoT communications. | Deep neural networks for adaptive multiple beamforming optimization. | High training complexity and dependency on channel state information. | Demonstrates improved energy efficiency and throughput in UAV IoT networks. |
| Mahmoud et al. (2024) [20] | Proposes an advanced security framework integrating deep learning with physical layer security for 6G. | Traditional cryptographic methods are insufficient against intelligent 6G threats. | Deep learning-based threat detection combined with physical layer security techniques. | Increased computational overhead and implementation complexity. | Enhances security resilience and intrusion detection accuracy in 6G networks. |
| Rekkas et al. (2021) [21] | Surveys machine learning applications in beyond 5G and 6G networks. | Absence of unified ML frameworks addressing scalability and real-time constraints. | State-of-the-art review of supervised, unsupervised and reinforcement learning methods. | Limited discussion on practical deployment challenges. | Provides future research directions and ML-driven architectural insights for 6G. |
| Hashesh et al. (2022) [22] | Reviews AI-enabled UAV communication systems and their future directions. | Challenges in reliability, latency, and energy efficiency for AI-driven UAV networks. | Taxonomy-based survey of AI techniques applied to UAV communications. | Lacks experimental comparison across AI models. | Identifies open challenges and research opportunities for AI-assisted UAV networks. |
| Ismail &Buyya (2022) [23] | Discusses AI applications and self-learning mechanisms in | Limited autonomy and adaptability in existing smart city network | Taxonomy and analytical review of AI-driven self-learning network | Conceptual framework without large-scale validation. | Demonstrates the potential of AI-enabled 6G networks for |

| | | | | | |
|---|---|---|---|---|---|
| | 6G-enabled smart cities. | infrastructures. | architectures. | | scalable smart city ecosystems. |

**Table 2: Comparison of Machine Learning and Deep Learning Models for Automatic Modulation Recognition in UAV-Based 6G Communication Systems**

| Model Type | Feature Extraction | Robustness to UAV Channel Variations | Computational Complexity | Notes |
|---|---|---|---|---|
| Traditional ML (SVM, KNN) | Manual (statistical or spectral features) | Low | Moderate | Requires domain expertise, limited adaptability |
| Deep Fully Connected Networks (DNN) | Automatic, global | Medium | High | Lacks local feature extraction, sensitive to signal misalignment |
| CNN | Automatic, hierarchical | High | Moderate-High | Captures local correlations; more robust to mobility and noise |
| RNN / LSTM | Sequential dependencies | High | High | Captures temporal patterns; heavier computation, needs longer sequences |
| Hybrid CNN-RNN / CNN-Transformer | Spatiotemporal features | Very High | Very High | Combines spatial and temporal learning; best accuracy but higher complexity |

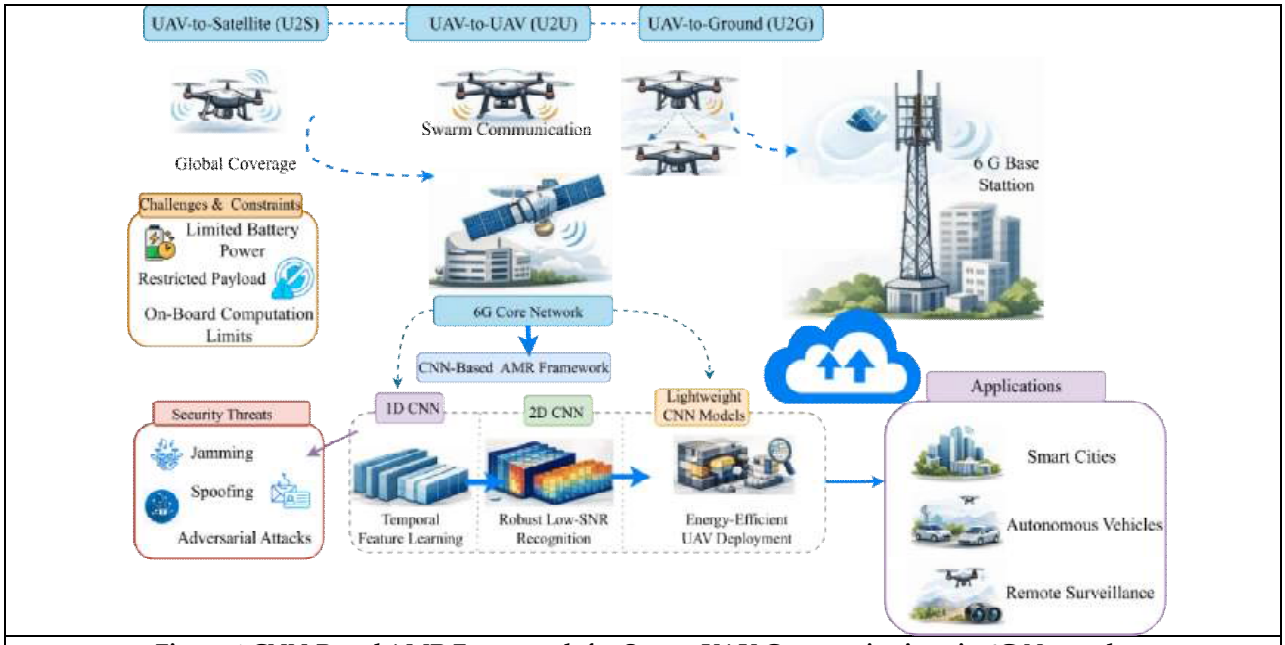



**Figure 1:CNN-Based AMR Framework for Secure UAV Communications in 6G Networks**

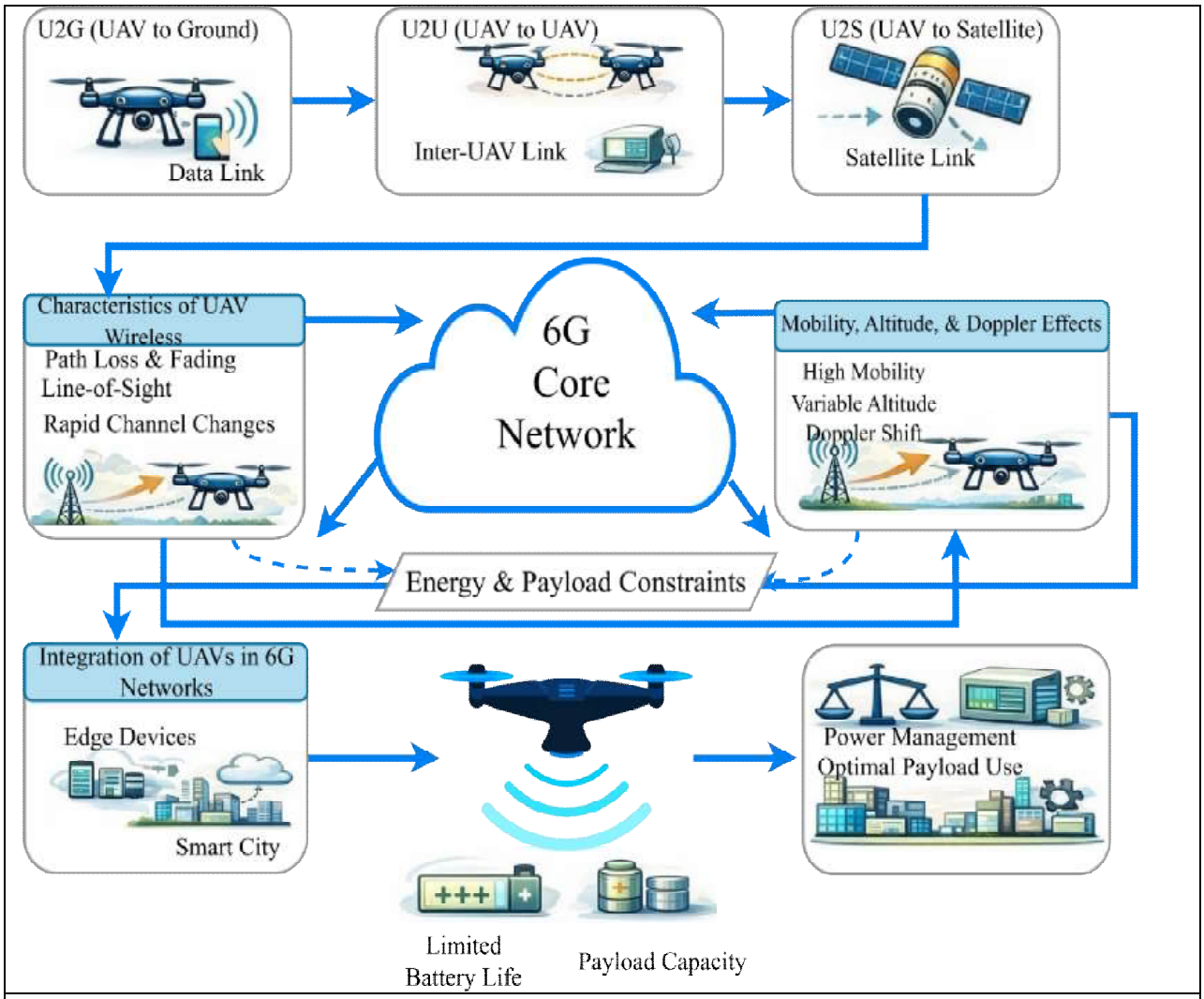



**Figure 2:** UAV-Enabled 6G Communication Architecture Illustrating U2G, U2U, and U2S Links with Mobility, Channel Dynamics, and Energy Constraints

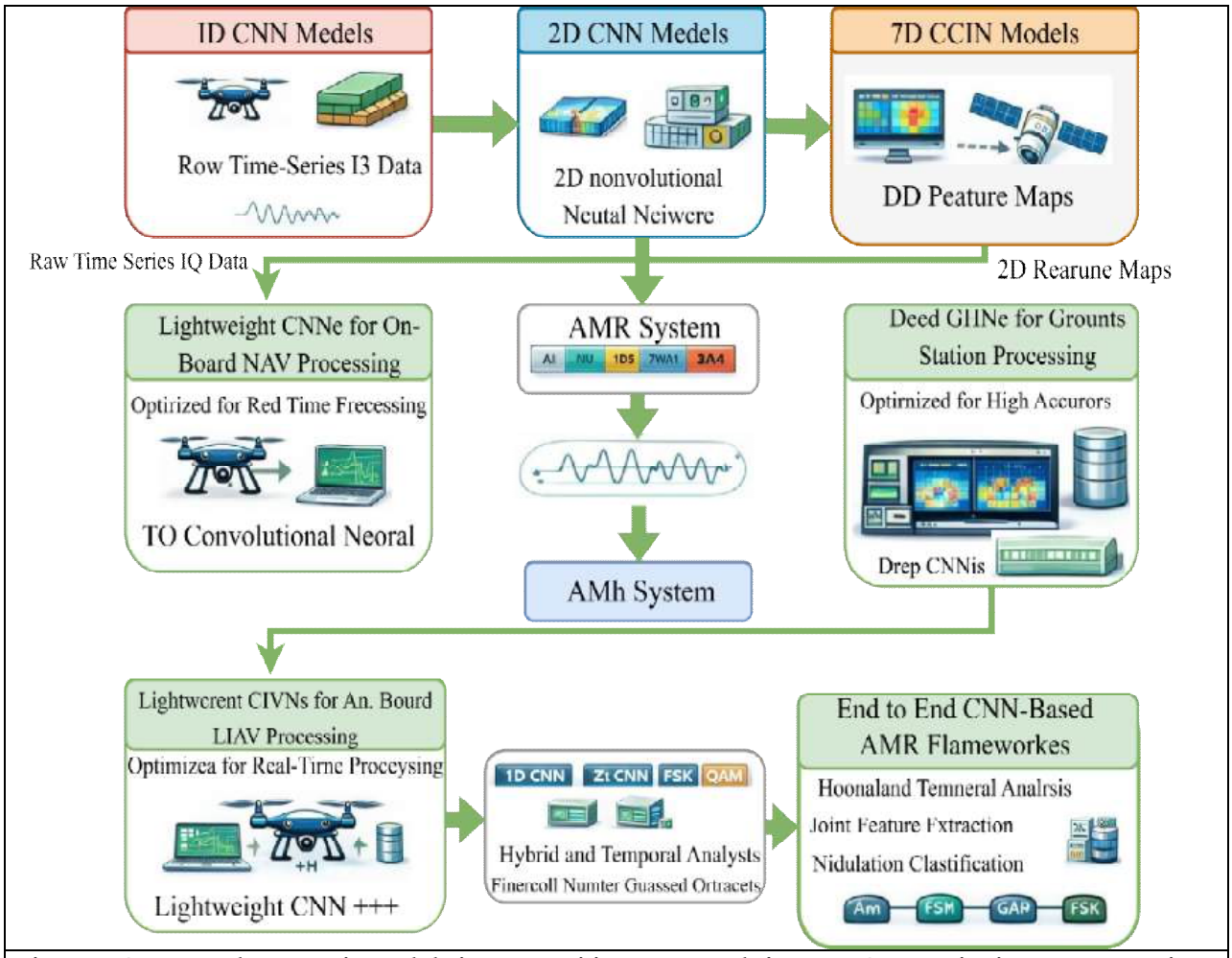



**Figure 3. CNN-Based Automatic Modulation Recognition Framework for UAV Communications: From 1D Time-Series Inputs to End-to-End AMR Systems**

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RESEARCH ARTICLE

# A Study of Solar Cell Efficiency: A Comparison between Organic and Inorganic Solar Cells

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## ABSTRACT

This study examines the efficiency differences between organic and inorganic solar cells, focusing on material characteristics, energy conversion performance, stability, cost factors, and technological advancements. Organic photovoltaic (OPV) cells have gained attention for their flexibility, lightweight nature, and low-cost manufacturing, while traditional inorganic solar cells—such as crystalline silicon and thin-film technologies—remain the industry standard due to their high efficiency and superior long-term stability. Through an analytical review of existing data and scientific literature, this paper highlights that inorganic solar cells consistently outperform organic cells in power conversion efficiency (PCE), typically achieving 18–25%, compared to 10–18% for OPVs. However, organic cells show promise for niche applications requiring mechanical flexibility and eco-friendly manufacturing. The study concludes by recommending hybrid and perovskite-integrated cell designs for future research and improved efficiency.

**Keywords:-** Organic solar cells, Inorganic solar cells, Organic photovoltaic (OPV), Crystalline silicon, Thin-film solar cells, Power conversion efficiency (PCE), Material characteristics, Energy conversion performance, Stability, Cost analysis, Technological advancements, Hybrid solar cells, Perovskite solar cells, Flexible photovoltaics, Eco-friendly manufacturing.

## INTRODUCTION

Solar energy technologies have witnessed rapid growth due to the global emphasis on clean and renewable energy sources. Among these, solar photovoltaic (PV) cells play a critical role in harnessing sunlight and converting it into electrical energy. Traditionally, inorganic solar cells, primarily crystalline silicon-based, have dominated the PV industry owing to their high efficiency and durability. However, organic solar cells (OSCs)—made from carbon-based polymers and small organic molecules—have emerged as promising alternatives due to their low weight, ease of fabrication, low-temperature processing, and potential for large-area flexible applications. This article aims to provide a detailed comparison of organic and inorganic solar cells, focusing on their efficiency parameters, technological attributes, material properties, and application scope. The study highlights performance trends and identifies areas where future breakthroughs could enhance the commercial viability of organic PV technology.

## LITERATURE REVIEW

The efficiency and performance of solar cells have been critical research areas in renewable energy studies. Inorganic solar cells, particularly crystalline silicon, have been extensively studied due to their mature manufacturing processes and high energy conversion efficiencies, often exceeding 20%. Thin-film inorganic systems, including CdTe and CIGS, also demonstrate competitive efficiencies at reduced manufacturing costs. Research by Green et al. (2023) highlights the long-term stability and predictable performance of inorganic PV technologies. Conversely, organic solar cells (OSCs) have gained interest for their unique mechanical properties, especially flexibility and low-temperature fabrication. Studies by Li and Jen (2022) emphasize advancements in donor–acceptor polymer structures, improving PCE values to 15–18% under laboratory conditions. However, OSCs suffer from degradation due to oxygen and moisture exposure, limiting their operational life. Perovskite–organic tandem systems have shown promise, achieving efficiencies above 20%, merging the benefits of low-cost organic layers with high-performing inorganic perovskites. Comparative studies consistently indicate that inorganic PV systems lead in efficiency and stability, while OSCs excel in sustainable, lightweight, and customizable applications. The evolution of hybrid architectures presents an opportunity to bridge the gap between the two technologies.

**Objectives of the Study**

- To compare the energy conversion efficiency of organic and inorganic solar cells.
- To analyze the material differences contributing to efficiency variations.
- To examine the stability, durability, and operating lifetime of both technologies.
- To identify potential future applications and technological innovations.

**Working Model of Organic and Inorganic Solar Cells**

A solar cell—whether organic or inorganic—can be represented using an equivalent electrical circuit model to understand its current–voltage (I–V) behavior and power output. Both types of solar cells operate on the photovoltaic principle, where incoming light generates electron–hole pairs, producing current when the circuit is closed.

In inorganic solar cells such as crystalline silicon or thin-film CdTe/CIGS, the circuit model commonly includes a photogenerated current source (I<sub>ph</sub>), a diode representing the p–n junction, a series resistance (R<sub>s</sub>) accounting for contact and material resistance, and a shunt resistance (R<sub>sh</sub>) representing leakage pathways. These components accurately reflect the high carrier mobility and strong built-in electric field of inorganic materials. Their high R<sub>sh</sub> and low R<sub>s</sub> contribute to superior efficiency and fill factor. In organic solar cells, the model is similar but adjusted for organic semiconductor behavior. Organic materials have lower carrier mobility, higher recombination rates, and more interface defects. Therefore, their equivalent circuit often shows higher series resistance and lower shunt resistance. Organic devices rely on donor–acceptor heterojunctions, and exciton dissociation inefficiencies are incorporated into the model via modified diode

parameters. Overall, the circuit model helps analyze how material properties—mobility, recombination, interface quality, and resistances—affect solar cell efficiency, enabling performance prediction and optimization for both organic and inorganic technologies.

**Factors Influencing Solar Cell Efficiency**

The factors influencing Solar Cell Efficiency in this study is built on comparing the key determinants of efficiency in organic and inorganic solar cells. It emphasizes four primary factors: material properties, device architecture, environmental stability, and manufacturing characteristics. Material properties such as bandgap and absorption ability influence how effectively each type converts sunlight into electricity. Device architecture, including layer configuration and interface engineering, determines charge transport and recombination rates. Environmental stability highlights the role of heat, oxygen, and UV resistance in long-term performance. Finally, cost and manufacturing scalability shape commercial viability. Together, these elements guide the evaluation of efficiency differences between organic and inorganic solar cells.

**Comparative Analysis of Organic and Inorganic Solar Cells**

The analysis clearly shows that inorganic solar cells outperform organic solar cells in terms of overall power conversion efficiency and long-term reliability. Crystalline silicon cells consistently achieve 20–25% laboratory efficiencies, with commercial modules reaching around 18–22%. Thin-film inorganic cells such as CdTe and CIGS offer 12–20% efficiency with lower production costs. Their stability under outdoor conditions ensures operational lifetimes exceeding 20 years.

Organic solar cells, despite notable progress in donor–acceptor engineering, achieve laboratory efficiencies of 15–18% and commercial efficiencies usually around 10–12%. Their primary limitations stem from sensitivity to moisture and UV exposure, resulting in faster degradation. However, OSCs offer advantages in flexibility, lightweight construction, semi-transparency, and eco-friendly printing techniques. These characteristics make them suitable for portable electronics, indoor energy harvesting, and building-integrated photovoltaics where aesthetics and flexibility matter. The study determines that while inorganic solar cells remain dominant for large-scale power generation, OSCs hold significant promise in niche markets. Future innovations involving hybrid structures and perovskite-integrated organic designs may further boost efficiency and stability.

## FINDINGS, SUGGESTIONS, AND FUTURE IMPLICATIONS

The findings of the study indicate that inorganic solar cells, particularly crystalline silicon and thin-film technologies, continue to outperform organic solar cells in terms of efficiency, long-term stability, and commercial maturity. Their higher carrier mobility, strong p–n junction characteristics, and resistance to environmental degradation make them suitable for large-scale power generation. Organic solar cells, while achieving lower efficiencies and shorter lifespans, demonstrate significant advantages in flexibility, lightweight structure, low-temperature fabrication, and aesthetic adaptability. These features position them well for portable electronics, indoor applications, and building-integrated photovoltaic systems. Based on these findings, key suggestions include focusing on improving the environmental stability of organic solar cells through advanced encapsulation, molecular engineering, and interface optimization. Collaboration between material scientists and industry stakeholders can accelerate the transition of laboratory-level OPV technologies to commercial products. For inorganic solar cells, further cost reduction and development of eco-friendly manufacturing methods are recommended. Future implications highlight the promising potential of hybrid and tandem systems that combine organic and inorganic layers, particularly perovskite-integrated structures. These innovations may merge the strengths of both categories, achieving high efficiency with added mechanical flexibility. As research progresses, such next-generation solar technologies could reshape renewable energy deployment across consumer, industrial, and architectural sectors.

## CONCLUSION

The comparative study concludes that inorganic solar cells currently remain superior in terms of efficiency, performance consistency, and lifespan, making them the preferred choice for large-scale power generation. Organic solar cells, however, present exciting opportunities due to their lightweight, flexible, and environmentally friendly characteristics. While OSCs still face challenges in stability and efficiency, ongoing advancements in material science, molecular engineering, and hybrid perovskite technologies may significantly narrow the performance gap in the coming years. The future of solar energy will likely involve coexistence of both technologies, each serving distinct applications based on their strengths.

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**Table 1: Comparison of Organic Vs Inorganic Solar Cell**

| Parameters | Organic Solar Cell | Inorganic Solar Cell |
|---|---|---|
| Efficiency | 10-18% | 18-25% |
| Stability | Low-Moderate | High |
| Manufacturing Cost | Low | Moderate-High |
| Lifespan | 5-10years | 20-25years |
| Flexibility | High | Low |
| Environmental Sensitivity | High | Low |
| Commercial Maturity | Emerging | Highly Establishing |

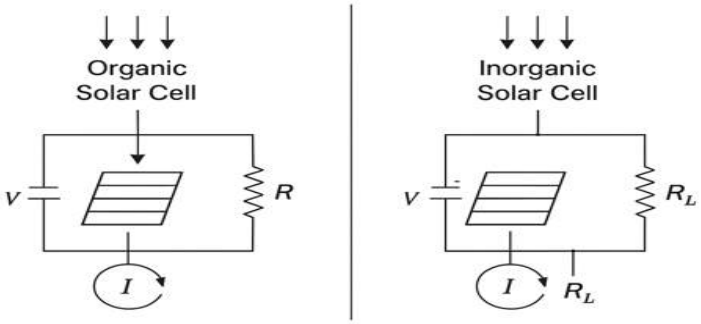



**Figure 1: Working Circuit Models for Solar Cells**

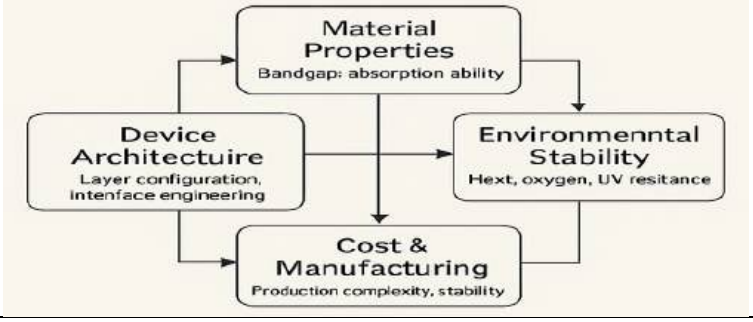



**Figure 2: Framework showing factors influencing Solar Cell Efficiency**

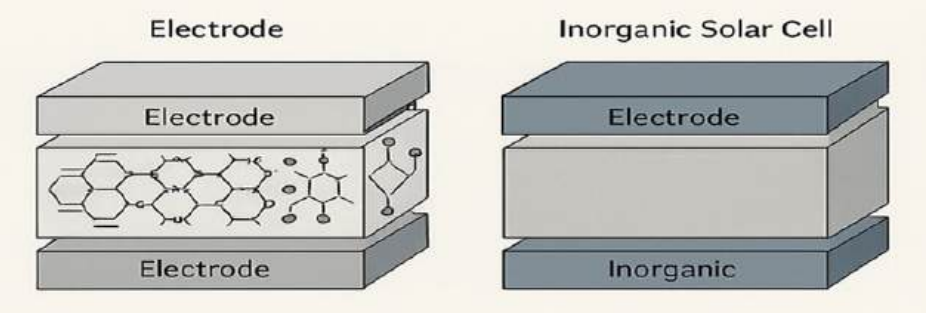



**Figure 3: Representation of Organic and Inorganic Solar Cell**

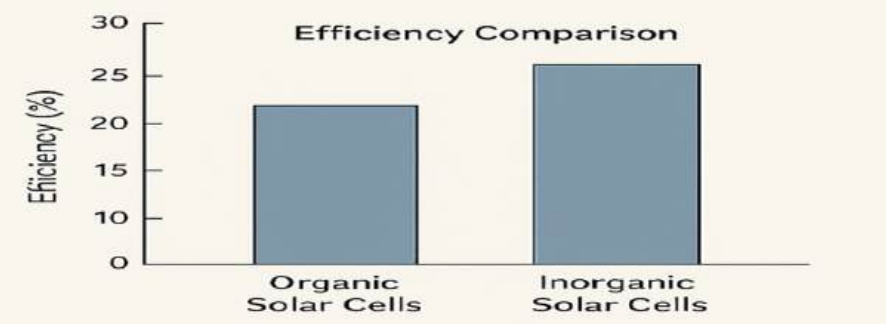



**Figure 4: Efficiency of Organic & Inorganic Solar Cell Comparision**

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**RESEARCH ARTICLE**

# Avifaunal Inventory and Breeding Observations from Rajnandgaon and Khairagarh (2019-2025)

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**ABSTRACT**

The present study documents the avifaunal diversity of Rajnandgaon and Khairagarh districts of Chhattisgarh, India. A total of 296 bird species belonging to 77 families and 18 orders were recorded, highlighting the rich taxonomic diversity of the region. The bird community showed a clear dominance of Passeriformes, accounting for 123 species (41.6%). This was followed in decreasing order of representation by Charadriiformes (35 species), Pelecaniformes (24 species), Accipitriformes (23 species), and Anseriformes (17 species). Accipitridae, Anatidae, Muscicapidae, and Ardeidae were among the most speciose families, whereas nearly one-third of the recorded families were represented by a single species, indicating a combination of ecological specialization and diverse habitat conditions. In addition to diversity patterns, the study provides confirmed breeding evidence for 58 species across varied habitats, strengthening the ecological value of these landscapes. Remarkably, the River Tern (*Sterna aurantia*) and Asian Brown Flycatcher (*Muscicapa dauurica*) are reported for the first time as breeding species from Chhattisgarh, along with several other noteworthy records. Additionally, this study records the presence of the Cinereous Vulture in Chhattisgarh for the first time. The findings highlight the critical role of wetlands, forests, and scrub–grassland mosaics in sustaining a rich community of resident as well as migratory birds. Notably, the checklist also includes 16 species of conservation concern listed in the IUCN Red List, underscoring the conservation value of these landscapes. The findings emphasize the role of habitat diversity in sustaining avifaunal richness and highlight the need for integrated conservation

strategies to safeguard critical habitats, particularly wetlands and forest patches, against growing anthropogenic pressures.

**Keywords:** IUCN Red List, critical habitats, diversity, Observations, Avifaunal

## INTRODUCTION

The districts of Rajnandgaon and Khairagarh (KHG) in Chhattisgarh encompass varied forest types and geographical features that support considerable mammalian and avifaunal diversity. Located in the southeastern part of the Satpura Range, the region is characterized by heterogeneous topography and climate, giving rise to distinct ecological zones. Forests of both districts fall under the Dry Deciduous and Southern Tropical Moist Deciduous forest categories (Champion & Seth, 1968) and cover approximately 2,923 km², accounting for about 36.4% of the total geographical area (CBSAP, 2003). Recent studies have recorded 35 mammal species across seven orders, identifying the landscape as an active tiger corridor, while also indicating possible local declines linked to habitat fragmentation (Thakur et al., 2024). Avifaunal diversity in Chhattisgarh is high, with 502 species documented to date (Arjun et al., 2023), and several additional records reported subsequently (Thakur, 2023; Thakur, 2024; Sharan et al., 2025; Thakur et al., 2025). Wetland-focused studies from Khairagarh district have recorded high floral diversity dominated by Poaceae and Cyperaceae, supporting rich bird assemblages exceeding 200 species, particularly migratory waterbirds (Thakur et al., 2025, unpublished). Several wetlands, including Baghnadi, Chhindari, Khatutola Barrage, and Khairabana, support bird communities exceeding 100 species. While Dongargarh Dhaara Reserve represents one of the most diverse forest patches, large parts of the Khairagarh forest remain poorly explored. Grassland habitats in the region are limited and increasingly degraded due to anthropogenic pressures, reducing their suitability for breeding grassland birds.

Breeding records of several shoreline and wetland-associated species in Chhattisgarh have so far been largely restricted to the Dhamtari region (Bharos et al., 2019, 2020, 2021).While recent studies have added breeding records of Sarus Crane and Lesser Adjutant Stork (Thakur et al., 2024; Thakur et al., 2025). Observations by local birders and year-round occurrence data further suggest that breeding activity of several species may be underreported, emphasizing the need for systematic documentation.

## METHODOLOGY

Bird surveys were conducted using an opportunistic sampling approach, with study sites visited without a predetermined sampling design. Observations were made using binoculars, cameras, and standard field equipment. All species records were supported by either photographic documentation or sound recordings.The cumulative survey effort totaled approximately 850 observation hours, primarily during morning, afternoon, and evening periods. Nocturnal sampling was limited and comprised only 8–9 night visits. Author 1 and Author 3, residents of Rajnandgaon district, carried out regular opportunistic observations during visits to nearby habitats. Author 2 contributed continuous observations from 2019 to 2022, while Author 4 conducted periodic surveys between 2014 and 2016.A total of 296 bird species were recorded. Most records were supported by photographic evidence, while 5–6 species were documented exclusively through call recordings. Species identification followed standard bird field guides (Grimmett et al., 2017; Ali & Ripley, 1987) and was further validated through expert consultation.

**Study area:** The study was conducted from 2o14 to 2025 in various habitat (Fig. 1) in Rajnandgaon and Khairagarh district of Chhattisgarh.

## RESULTS

At the family level, Accipitridae (22 species) was the most species-rich, reflecting the abundance of raptors in the region. Other diverse families included Anatidae and Muscicapidae (17 species each), Ardeidae (12), and Scolopacidae (11). Moderate representation was observed in *Cuculidae* (9 species), followed by *Rallidae*, *Charadriidae*, and *Motacillidae* (8 species each). Likewise, the families Columbidae, Strigidae, Picidae, and Phylloscopidae were each represented by seven species. In contrast, nearly one-third of the families were represented by only a single species, underscoring the taxonomic breadth of the avifauna despite low representation in several groups. The dominance of Passeriformes reflects their high species richness and broad ecological adaptability. The presence of key waterbird families emphasizes the importance of wetlands, while raptors indicate a balanced trophic structure. Families with few species highlight habitat heterogeneity, underscoring the role of diverse ecosystems in maintaining avian diversity.

Between 2019 and 2025, breeding was confirmed for 56 bird species across wetlands, forests, gardens, and open habitats, based on observations of nesting, feeding, fledging, and colony formation. Successful reproduction by wetland and forest species, along with varied nesting strategies and adaptability to human-modified landscapes, highlights the region's high breeding diversity and conservation importance. The checklist includes 16 IUCN Red List species, underscoring the region's global conservation significance and the urgent need to protect key habitats, particularly wetlands and forest remnants.Overall, the study highlights that Rajnandgaon and Khairagarh districts harbor a rich and ecologically significant bird assemblage, making them vital for regional and migratory avifaunal conservation.

**Tagged bird**

**Cinerous vulture-**In South Asia, the Cinereous Vulture inhabits arid and semi-arid mountain regions of Pakistan and occurs at lower elevations in the Himalayas. Within India, it is mainly a winter visitor to the northwestern regions and occasionally strays into peninsular areas, reaching as far as Dhulia in Maharashtra. (Ali & Ripley 1987). Recently, Samson et al. (2019) reported its migration records mainly between December and February from six districts across Telangana, Kerala, Karnataka, Tamil Nadu, Maharashtra, and Andhra Pradesh. Additionally, Mall et al. (2022) documented its presence in Bihar and Jharkhand**.** On 16 January 2023, a rescued Cinereous Vulture was ringed (M63) at Jungle Safari, Raipur, and released by the Forest Department at Singarghat, Khairagarh district, Chhattisgarh. The following day, the bird was observed flying northwards towards the Maikal Range in Madhya Pradesh. This rescue and release represents the first documented record of the species from Chhattisgarh.

**Several other rare species were also noted.** Black-winged Cuckooshrike was recorded during winter migration around Paniyajob, Ningo, and Kolarghat (Dongargarh). Painted Sandgrouse was sighted 2–3 times, restricted to Ningo Dam. Sirkeer Malkoha was recorded once at Surhi Dam (Gandai) and also from Udaipur Hills, Surguja. A local passage of Glossy Ibis was noted at Ruse Dam, Khairagarh (10 November 2024). Indian Blackbird and Puff-throated Babbler were recorded for the first time from Achanakmar Tiger Reserve(Anthony et al., 2018). Calls of Oriental and Indian Scops Owls were recorded; Brown Hawk-Owl is likely present based on adjacent records (Bhoramdev wildlife sanctuary).

**Taxonomic varients:** Rare taxonomic variants such as the Orange-headed Thrush (*Geokichla citrina*), Black-eared Kite (*Milvus lineatus*), and Peregrine Falcon (*Falco peregrinus*) have also been reported from the region.

## DISCUSSION

Birds in the study area are exposed to several anthropogenic pressures, including habitat degradation, hunting, road mortality, pesticide misuse, and infrastructure-related hazards, as also noted from other parts of central India (Ali & Ripley, 1983; Chandra & Singh, 2004; Thakur et al., 2024). Among these, loss of nesting and roosting trees was

identified as the most critical threat. Clearing of trees at Dongargarh (Mahaveer Para) led to the destruction of a communal nesting site used by egrets, herons, and cormorants, while removal of dry and partially submerged trees at Khatutola Barrage reduced nesting opportunities for Asian Openbill Storks and cormorants. In another instance, the felling of a single nesting tree resulted in the failure of a breeding attempt of the Asian Brown Flycatcher, which also constitutes the first breeding record of the species from theregion (Rasmussen & Anderton, 2005; Mohan & Murlidhar, 2019).

Roadkill incidents were recorded for both small passerines and large birds such as the Barn Owl (*Tyto alba*), particularly along roads passing through agricultural and forested landscapes. Illegal hunting using air guns and slingshots was observed near several wetlands. Mortality associated with power transmission structures was documented, including a Common Crane found dead near a high-voltage tower at Ruse Dam. Additionally, a pesticide-related mass mortality event reported from Ambagarh Chowki in 2022 primarily affected granivorous species. Conservation measures should prioritize the protection of nesting trees, mitigation of road mortality, regulation of pesticide use, and increased community awareness. Local initiatives such as Mangata Park and the Chhindari Ke Parindey eco-tourism program demonstrate that community participation can play a positive role in conserving avifaunal diversity.

## CONCLUSION

This first documented bird checklist for Rajnandgaon and Khairagarh districts records 296 species spanning 77 families and 18 orders, featuring 17 species listed on the IUCN Red List and confirmed breeding activity for 56 species. The data highlight the mosaic of habitats—wetlands, forests, grasslands, and human-modified landscapes—that sustain both resident and migratory birds. Breeding records, such as range expansions (Asian Brown Flycatcher),The breeding distribution of the Asian Brown Flycatcher within the peninsular region remains inadequately documented. (Rasmussen & Anderton 2005).From Peninsular India the breeding records has been reported from Madhya Pradesh, Kerela, Rajasthan, Gujrat, Karnataka Tamilnadu, Andhra Pradesh, Telangana, Maharashtra, Haryana, Delhi.[V. Santharam (2003), Singh et al. (2009) & Mohan et al. (2019)] .Multiple broods (White-browed Fantail), and nesting colonies (River Tern, Asian Openbill), highlight the ecological significance of the region. Additionally, the Cinereous Vulture represents the first record of this species from Chhattisgarh. The documentation of breeding records offers insights into habitat suitability and species-specific reproductive preferences. Given the diversity of threatened species and ongoing human pressures, conservation of wetlands, mature forests, and grasslands is crucial for sustaining avifaunal diversity. In particular, Dongargarh Dhaara Reserve Forest, owing to its rich birdlife and ecological value, holds strong potential to be designated as a Conservation Reserve, ensuring long-term protection and community-based conservation of its habitats.

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**Table 1. Bird species habit and observations**

| S No. | Species | Nesting type / Habitat | Observation |
|---|---|---|---|
| 1 | River Tern | Ground nesting/ Around wetland | In western India, breeding may commence early when such islands emerge, while in eastern India nesting generally occurs between March and June (Ali & Ripley 1983; Mundkur 1991).Breeding of River Tern was confirmed at Paniyajob Dam with adults incubating eggs (Apr 2024) and a parent was videographed at Raurkasa Dam guiding a fledgling in prey-catching behavior using wooden stick, indicating successful breeding (Jun 2023), extending the known breeding range in Chhattisgarh. The species was recorded year-round at multiple reservoirs, with a maximum count of ~20 individuals at Baghnadi Dam (May 2023) |
| 2 | Little Tern | Ground nesting/ Around wetland | Two nests with seven adults recorded at Paniyajob Dam (28 Apr 2024) |
| 3 | Little Pratincole | Ground nesting/ Around wetland | Breeding colony (30 birds) recorded at Paniyajob Dam; fledglings observed in May; additional breeding at Dangbora Dam. |
| 4 | Asian Brown Flycatcher | Tree nesting/ Forest or Thick vegetation | Breeding activity of the Asian Brown Flycatcher was confirmed within the study area, representing an extension of its known breeding range in peninsular India. Courtship was documented in May 2021, followed by two active nests in June 2021, all situated in tree forks. Adults were observed provisioning nestlings with |

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| | | | insects and removing fecal sacs. One nesting attempt was unsuccessful due to the felling of a nearby tree, underscoring the susceptibility of nesting sites to unintentional human disturbance. |
| 5 | White browed fantail | Tree nesting/ Forest or Thick vegetation | White-browed Fantail breeding was recorded between April and June 2021, with seven nests documented during the season. A nest containing a recently hatched chick was observed on 28 May 2021 along a forest foot trail, with both parents actively feeding. Regular provisioning with flies and fecal sac removal were noted on 29 May. By 2 June, only a single chick remained, which fledged by 5 June. The nest was found empty on 8 June, and the family was subsequently observed on 15 June with healthy, volant juveniles. Notably, a second clutch was initiated in the same nest on 28 June, indicating within-season nest reuse. This observation supports earlier suggestions that fantails may reuse nests under favorable conditions and highlights site fidelity and the potential for multiple broods within a single breeding season. (Copestake 2021) |
| 6 | Egyptian Vulture | Tower nesting/ Open field | Nest with one nestling recorded on power transmission tower (Mar 2021). |
| 7 | Crested tree swift | Tree nesting/ Forest or Thick vegetation | Nesting colonies recorded at Kurejhar, Kolarghat, and Raurkasa; largest flock ~300; observed near Kahuwapani–Kalghurra area. (June-2023) |
| 8 | Asian Open bill stork | Flooded tree nesting/ Around wetland | Breeding colonies recorded with 12–30 nests between 2021–2022; both parents involved in incubation and feeding. (Sep–Nov 2021; Jun–Nov 2022). |
| 9 | Mottled wood Owl | tree cavity / Forest | Successful breeding recorded annually (2021–2023); fledglings documented; predation event by Monitor Lizard noted in 2024. |
| 10 | Grey headed fish eagle | Tree nesting/ Wetland | Empty and active nests recorded; nest-building observed in Nov 2024; vocal activity restricted to nesting phase. |
| 11 | Little ringed plover | Ground nesting/ Around wetland | Active nesting and fledglings recorded at Paniyajob Dam (Apr–May 2024). |
| 12 | Little cormorant &Indian cormorant | Tree nesting/ Wetland | Mixed breeding colony recorded at Khatutola (Nov 2022) and other wetlands. |
| 13 | Indian paradise flycatcher | Tree nesting/ Forest | Multiple nesting records (May-June; 2020–2023); cinnamon and moulting morph males documented; nestling period ~14 days; diverse prey delivery recorded. |
| 14 | Indian white eye | Tree nesting/ Forest | Nesting recorded; unusual interspecific feeding of Indian Paradise Flycatcher nestlings observed. (May-June) |
| 15 | Lesser Whistling Duck and Spot billed duck | Ground nesting/ wetland | Adults with chicks recorded during Dec–Jan at multiple tanks; nests inferred from presence of fledglings. |
| 16 | Barn Owl | Old building or tree cavity/ Urban Area | Successful fledging recorded in 2021 and 2023 at residential site; regular nocturnal activity noted. |
| 17 | White naped woodpecker | tree cavity / Forest | Nest cavity use recorded(March 2021) ; repeated drumming during breeding season observed. |
| 18 | Red vented Bulbul | Tree nesting/ Dense vegetation or garden | Active nest and juveniles recorded; breeding confirmed in residential garden.(June 2021) |

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| 19 | Spotted owlet | Cavity nesting/ Open field | Mating and family groups (Dec-Feb; 2021-24)(4–5 individuals) recorded; multiple nesting sites documented. |
| 20 | Peacock and Red Jungle fowl | Ground nesting/ forest | Females with chicks recorded; breeding inferred from displays and juvenile presence. (Aug 2021; May 2023) |
| 21 | Red naped Ibis | Tree nesting and Tower/ Wetland | Active nests recorded (Nov 2024); adults observed carrying prey to nestlings in flight. (Nov 2021) |
| 22 | Black winged Kite | Tree nesting/Open field | Mating behaviour recorded near Chhichola (Nov 2020) |
| 23 | Tailor bird | Suspended or hanging nesting/ Garden | Nest construction activity recorded in June 2021 |
| 24 | Golden Oriole | Tree nesting/ Forest or thick vegetation | Active hanging, Cup shaped nest recorded with incubation behaviour. (Jun 2021) |
| 25 | Small minivet | Tree nesting/ Open woodland | Nest-building recorded in May 2022; additional breeding activity observed in 2025. |
| 26 | White bellied drongo | Tree nesting/ Open woodlands | Active nesting recorded in cup-shaped nest (May 2021) |
| 27 | Black kite | Tree nesting/ Open field | Nestlings recorded at nesting trees (Jan 2022; Mar 2023) |
| 28 | Black Drongo | Tree nesting/ Forest | Active nesting recorded (Apr 2023); mobbing of predators observed during nesting period. |
| 29 | Shikra | Tree nesting/ Open field | Nest with two nestlings recorded; prey delivery observed (May 2023) |
| 30 | Common Moorhen | Ground nesting /Wetland | Adults with freshly hatched chicks recorded (Aug 2020; 2024) |
| 31 | Gray Francolin | Ground nesting /Open field | Family groups recorded; breeding vocalizations documented (Apr 2022). |
| 32 | Yellow wattled lapwing and Red wattled lapwing | Ground nesting /Open field or wetlands | Parents with chicks recorded (Sep–Oct). |
| 33 | White browed bulbul | Tree nesting/ Garden | Active nest recorded with successful fledging (Jun 2024) |
| 34 | Scally breasted munia, House sparrow and Red munia | Tree and shrub nesting/ Garden and Open field | Nesting recorded seasonally: House Sparrow (May), Scaly-breasted Munia (Oct), Red Avadavat (Oct). |
| 35 | Greater racket tailed drongo | Tree nesting/ Forest | Active nest with incubation recorded near Madiyan Dam (Jul 2024) |
| 36 | Plain Prinia | Ground nesting/ Open field | Nest material carrying recorded near Cross Hill (Sep 2022). |
| 37 | Baya weaver | Suspended tree nesting/ Open field | Multiple nesting colonies recorded (Sep–Oct) |
| 38 | Black naped monarch | Tree nesting/ Forest | Active nests recorded (May–Jul 2023). |
| 39 | Rose ringed parakeet | tree cavity / Forest | Active cavity nesting recorded (Mar 2024); family association observed until May 2024. The other two species, Plum-headed Parakeet and Alexandrine Parakeet, were recorded throughout the |

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| | | | year and are also commonly kept as pets in the region. |
| 40 | Brahminy starling | tree cavity / Forest | Nesting recorded in reused woodpecker cavities (Mar–Apr 2021–2022). |
| 41 | Spotted Dove | Tree nesting/ Forest | Loosely built active nest recorded (Mar 2024) |
| 42 | Tickells blue flycatcher | Tree nesting/ Forest | Nest recorded (May 2023); fledgling observed (Jul 2020). |
| 43 | Indian roller | tree cavity / Forest | Nesting in old woodpecker cavity recorded (Apr 2022); prey delivery with scorpion documented (Apr 2025). |
| 44 | Bronze Winged Jacana | Floating nesting/ Wetland | Female with chicks recorded, confirming breeding (Nov 2021). |
| 45 | Coppersmith Barbet | tree cavity / Forest | Nest excavation recorded (Dec 2022); feeding of nestling observed (Apr 2025). |
| 46 | Indian scimitar babbler | Forest | Family group with juveniles recorded; active feeding observed (May 2025) |
| 47. | Commony Myna | Tree cavity/ forest | Nesting recorded in reused parakeet cavity (Apr 2024) |
| 48. | Asian Pied starling. | Electric poles/ Urban | Nesting on electric poles recorded in urban area (Apr–May) |
| 49. | Cattle egret, Little egret & Intermediate egret | Tree nesting/ Open fields, urban or around wetland | Colonial nesting recorded during monsoon season (Jul–Sep). |

**Table 2 Bird species, nesting habitats & field observations**

| S. No. | Common name / Scientific name | Description |
|---|---|---|
| **1** | Ferruginous Duck *Aythya nyroca* | Occasional sightings were recorded from Rajnandgaon and Surguja districts in January 2021. Historically reported from central Chhattisgarh wetlands including Maroda (Durg), Bangoli, Ghughwa and Kurud tanks in Raipur district and Gidhwa Tank in Bemetara district (Bharos et al., 2015), and from Achanakmar Tiger Reserve (Anthony et al., 2018). |
| **2** | Malabar Pied Hornbill *Anthracoceros coronatus* | Small groups of 1–3 individuals were observed in September 2021, increasing to about 20 individuals near Kursipar village, Dongargarh, in December 2021. A male's mating call indicates probable local breeding. |
| **3** | Heart-spotted Woodpecker *Hemicircus canente* | Observed in hilly forest patches of Dongargarh in 2021, with a confirmed photographic record on 13 June 2023. No subsequent sightings were recorded. Earlier regional records are available from Bastar, Udyanti (Gariyabandh) and Dantewada districts (Bharos et al., 2019). |
| **4** | Brown-breasted Flycatcher *Muscicapa muttui* | First photographed on 19 September 2021 near Chichola, Rajnandgaon district, and later on 18 September 2024 at Cross Hill and Raurkasa Dam. Previously reported from Kanger Valley National Park (Bharos et al., 2019). |
| **5** | Rufous-bellied Eagle *Lophotriorchis kieneri* | An adult was observed on 1 November 2021 at Kahuwapani Waterfall, and a juvenile was recorded on 20 November 2022 in Dhaara Reserve Forest. The species was again photographed on 11 December 2022 at Mainpat, representing a new locality along the Surguja–Raigarh border. Earlier records exist from Kanger Valley National Park (Dutta, 2015) |

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| **6** | Common Crane *Grus grus* | A flock of 21 individuals was recorded at Ruse Dam on 14 February 2021, with maximum counts increasing to 24 individuals by 2025. Birds were frequently observed feeding in chana fields and along wetland margins, often in association with Painted Storks and Eurasian Spoonbills. This represents the first record from the area after a gap of nearly 15 years. |
| **7** | Eurasian Spoonbill *Platalea leucorodia* | Regularly observed at Ruse Wetlands during the past four years, with a maximum count of 17 individuals. The species forages in shallow water by sweeping and probing the bill. |
| **8** | Grey-necked Bunting *Emberiza buchanani* | Eight individuals were observed near Bamleshwari Temple hill, Dongargarh, between January and March in dry, open scrub habitat. Only a few earlier records are available from Chhattisgarh. |
| **9** | Besra *Tachyspiza virgata besra* | Two individuals were recorded at Raurkasa Dam, Dongargarh, in May 2021. The species is considered a sporadic visitor to Chhattisgarh, with its main distribution in central India and the Western Ghats (Praveen, 2025) |
| **10** | Eastern Orphean Warbler *Curruca crassirostris* | Photographed near the outskirts of Dongargarh on 2 February 2023 and earlier at Ruse Dam on 14 January 2021. Previously reported from Raipur district, Surguja division and Bastar district (Bharos et al., 2019, 2022, 2023) |
| **11** | Slaty-breasted Rail *Lewinia striata* | Photographed at a small tank near Palloti School, Dongargarh, in August 2020 and July 2021, with three to four additional sightings. The species is rare in Chhattisgarh, with earlier confirmed records only from Surguja Division (Bharos et al., 2022). |
| **12** | Long-legged Buzzard *Buteo rufinus* | A single individual was observed near Paniyajob Dam, Dongargarh, in March 2020 during return migration. The species is a known winter visitor across India (Praveen, 2025) |
| **13** | Chestnut-headed Bee-eater *Merops leschenaultia* | Two individuals were photographed at Cross Hill on 26 March 2023, while seven individuals were recorded in October 2020 near Dhaar Dam, Dongargarh. Earlier records exist from Surguja and Bastar divisions (Bharos et al., 2019, 2022). |
| **14** | Grey-headed Lapwing *Vanellus cinereus* | A solitary individual was recorded twice, once at Pandariya Tank, Khairagarh, and again at Kunwarpur Dam, Surguja, in November 2024. Previous records are known from central India (Bharos et al., 2019) |
| **15** | Bar-winged Flycatcher-shrike *Hemipus picatus* | Photographic evidence was obtained from Dongargarh in May 2025, with additional records from Surguja district. The species is resident and fills a distribution gap across peninsular India (Grimmett et al., 2017). |
| **16** | Greater Sand Plover *Charadrius leschenaultii* | Recorded in August 2025 near Baldeopur and Atariya Tank, Khairagarh, along with Lesser Sand Plover; one earlier sighting was made in 2022 at Ruse Dam. The species is considered a rare migratory wader in Chhattisgarh. |
| **17** | Bar headed Goose *Anser indicus* | On 28 February 2024 at 20:02 h, 15 Bar-headed Geese were observed flying near Saraswati Shishu Mandir, Dongargarh, though photographic evidence could not be obtained due to poor visibility. Another flock of seven individuals was recorded at the same site on 2 March 2024, with calls documented for confirmation. Five sightings were made during the 2024 return migration. The geese followed a low-altitude flight path (<300 m) between Cross Hill (≈438 m) and Budha Dev Hill (≈400 m), avoiding the surrounding hilltops. These observations contribute to understanding the species' local migratory route. |

**Table 3. Several threatened bird species were recorded during the study:**

| S. No. | Species (IUCN status) | Record details |
|---|---|---|
| 1 | Indian Spotted Eagle (Vulnerable) | Recorded 4–5 times across different habitats in the study area |

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| 2 | Pallid Harrier (Near Threatened) | Observed in upland grasslands of the Dongargarh Dhaara forest region |
| 3 | Lesser Adjutant (Vulnerable) | Juvenile recorded from Khairagarh city (Thakur et al. 2024) |
| 4 | Indian Roller (Near Threatened) | Maximum flock of 82 individuals recorded at Ruse Dam (2022); smaller flocks (3–10) at other waterbodies. |
| 5 | Woolly-necked Stork (Near Threatened | Occasional sightings, with group sizes ranging from 1–8 individuals. |
| 6 | Common Pochard (Vulnerable) | Large flocks (>500) at Baghnadi and Chhindari dams; 3–100 individuals at other sites. |
| 7 | Greater Spotted Eagle (Vulnerable) | Single individual observed near Chhuikhadan, Khairagarh, in February 2023 |
| 8 | Curlew Sandpiper (Vulnerable) | Recorded at Ruse Tank during two consecutive migratory seasons (Nov 2021, Sept 2022). |
| 9 | Eurasian Curlew (Near Threatened) | Single record from Chhindari Dam on 22 October 2022 |
| 11 | River Tern (Vulnerable) | recorded from several wetlands, with confirmed breeding at select sites; the highest count was 12 individuals at Baghnadi Reservoir. |
| 11 | **Grey-headed Fish Eagle (Near threatened)** | Observed in forest-edge wetlands, mostly as solitary individuals or in pairs. |
| 12 | **Egyptian Vulture (Endangered)** | Mainly recorded from open plain areas, with a maximum count of five individuals. |
| Other threatened bird mentioned are Ferruginous duck(Near threatened), Malabar pied hornbill (Near threatened), Rufous bellied Eagle (Near threatened)& Cinerous vulture (Near threatened) | | |

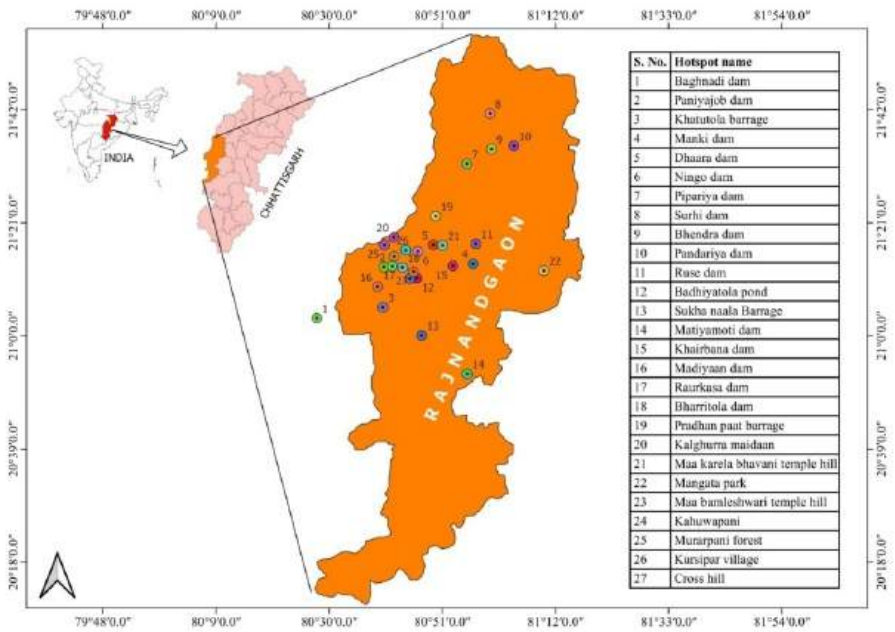



**Figure 1. Study area map showing all the survey area in Rajnandgaon district**

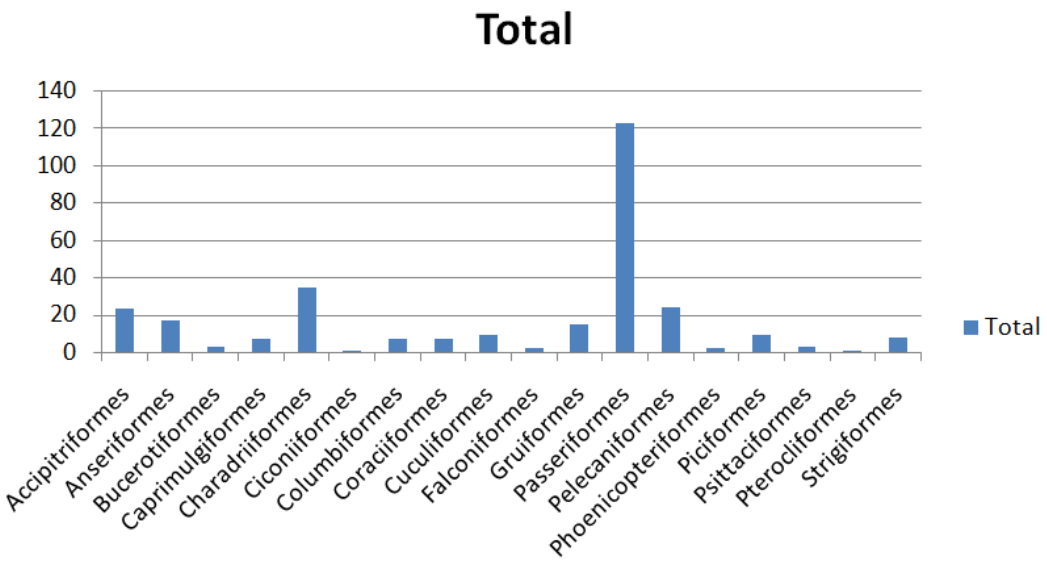



**Figure 2 Number of bird species recorded across different avian orders during the study period.**





From top to bottom & from left to right. 1. Common Crane @Danesh Sinha 2. Malabar Pied Hornbill @Pratik Thakur 3. Heart spotted Woodpecker @Danesh Sinha 4. Rufous bellid Eagle @Pratik Thakur 5. Brown breasted flycatcher @Pratik Thakur 6. Ferruginous duck @Avinash Bhoi 7. Eurasin spoonbill @Pratik Thakur





From top to bottom & from left to right. 1. Grey-necked Bunting @Pratik Thakur 2. Besra @Pratik Thakur 3. Eastern Orphean Warbler @Danesh Sinha 4. Slaty breasted rail @Danesh Sinha 5. Chestnut headed Bee Eater @Pratik Thakur 6. Long legged Buzzard @Avinash Bhoi 7. Grey headed lapwing @Pratik Thakur 8. Greater sandplover @Pratik Thakur 9. Bar winged flycatcher shrike @Pratik Thakur

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REVIEW ARTICLE

# Clinical Pharmacists as Key Agents in Reducing Medication Errors : A Narrative Review of Hospital - Based Global Evidence

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## ABSTRACT

Medication errors continue to represent one of the most significant and persistent threats to patient safety across healthcare systems worldwide. Despite advances in pharmacotherapy, digital health, and clinical governance, preventable medication-related harm remains highly prevalent in both developed and developing nations. Among the most effective human-centered strategies for improving medication safety is the active involvement of clinical pharmacists in direct patient care. Over the past three decades, the role of pharmacists has evolved from a traditional dispensing-focused function to a patient-centered clinical discipline embedded within multidisciplinary healthcare teams. This narrative review provides a comprehensive synthesis of global hospital-based evidence on the impact of clinical pharmacist interventions in reducing medication errors and preventing adverse drug events. The review examines pharmacist contributions across all stages of the medication-use process, including prescribing review, medication reconciliation, therapeutic drug monitoring, adverse drug reaction surveillance, patient counselling, and transitions of care. Evidence consistently demonstrates that pharmacist-led interventions significantly reduce prescribing errors, prevent high-risk drug-related harm, improve treatment appropriateness, and enhance patient outcomes. The review also explores economic benefits, interprofessional collaboration, implementation challenges, and future directions for expanding clinical

pharmacy services, particularly in resource-limited settings. Strengthening the clinical role of pharmacists represents a cost-effective, scalable, and sustainable strategy for advancing global medication safety.

**Keywords:** Clinical pharmacist, medication errors, patient safety, hospital pharmacy, medication reconciliation, Adverse drug events

## INTRODUCTION

Medication use is central to modern healthcare delivery, forming the cornerstone of disease prevention, treatment, and palliation. However, the medication-use process is complex, involving multiple professionals, high-risk decision-making, and dynamic patient-specific factors. As a result, medication errors remain one of the most common and preventable causes of patient harm in healthcare systems worldwide. Errors may occur at any stage of the medication-use process, including prescribing, transcribing, dispensing, administration, and monitoring. The consequences range from minimal clinical impact to catastrophic injury and death [1]. Over the past two decades, patient safety has emerged as a global priority, driven by landmark reports highlighting the unacceptable burden of preventable medical errors. Medication-related harm contributes substantially to healthcare morbidity, mortality, and economic loss. Hospitalized patients are particularly vulnerable due to polypharmacy, critical illness, frequent transitions of care, and exposure to high-alert medications [2]. In response to this challenge, a wide range of safety strategies have been implemented, including electronic prescribing, clinical decision support systems, standardized treatment protocols, and barcode medication administration. While these technologies have improved certain aspects of medication safety, they have not eliminated the problem. Human expertise, clinical judgment, and interdisciplinary collaboration remain essential components of safe medication practice. Among healthcare professionals, clinical pharmacists occupy a uniquely strategic position in the medication-use process. As specialists in pharmacotherapy, pharmacokinetics, and medication safety, clinical pharmacists are ideally positioned to identify inappropriate therapy, prevent harmful drug interactions, optimize dosing, monitor therapeutic outcomes, and educate both patients and healthcare providers. Their integration into direct patient care represents one of the most consistently effective interventions for reducing medication errors and adverse drug events [3, 4]. This narrative review comprehensively examines the evolving role of clinical pharmacists in preventing medication errors within hospital settings. It synthesizes global evidence on pharmacist-led interventions across the medication-use continuum, explores their clinical and economic impact, identifies implementation barriers, and discusses future directions for strengthening clinical pharmacy services worldwide.

### Evolution of Clinical Pharmacy Practice

#### Clinical Pharmacy

Clinical pharmacy is now widely defined as a health science discipline in which pharmacists provide patient-centered care to optimize medication therapy and promote health, wellness, and disease prevention. The primary focus is on ensuring the safe, effective, and rational use of medications at the individual patient level. Clinical pharmacists apply specialized knowledge of pharmacokinetics, pharmacodynamics, drug interactions, and therapeutic guidelines to support evidence-based prescribing [5].

#### From Dispensing to Direct Patient Care

Historically, the role of pharmacists was primarily product-oriented, focusing on the preparation, compounding, and dispensing of medications. For much of the twentieth century, pharmacists had limited involvement in therapeutic decision-making or direct patient care. Their primary responsibility was ensuring that medications were accurately prepared and supplied according to physicians' orders [6]. The emergence of clinical pharmacy as a discipline in the latter half of the twentieth century marked a paradigm shift in pharmacy practice. This transformation was driven by the increasing complexity of drug therapy, the expansion of pharmacological knowledge, and growing recognition of

medication-related morbidity and mortality. Pharmacists began to move beyond the dispensary and into clinical wards, participating in patient rounds, therapeutic decision-making, and medication monitoring [7].

**Global Expansion of Clinical Pharmacy Services**

High-income countries such as the United States, the United Kingdom, Canada, Australia, and several European nations have progressively integrated clinical pharmacists into hospital care teams. In these settings, pharmacists participate in daily ward rounds, manage antimicrobial stewardship programs, lead medication reconciliation initiatives, and contribute to critical care management. In contrast, the expansion of clinical pharmacy services in low- and middle-income countries has been slower and less uniform. While academic programs in clinical pharmacy have expanded in regions such as South Asia, the Middle East, and parts of Africa, the actual deployment of clinical pharmacists in hospital wards remains limited in many institutions due to workforce shortages, funding constraints, and lack of regulatory mandates [5].

**Medication Errors: Scope and Clinical Impact**

**Definition and Classification**

Medication errors are defined as any preventable event that may cause or lead to inappropriate medication use or patient harm while the medication is under the control of the healthcare professional, patient, or consumer. These events may occur at any stage of the medication-use process and may or may not result in harm.

**Common categories of medication errors include:**

- Prescribing errors
- Transcription errors
- Dispensing errors
- Administration errors
- Monitoring errors

Each category involves distinct risk factors and requires targeted safety strategies [7, 8].

**Global Burden of Medication Errors**

Medication errors represent a major global health burden. Studies across diverse healthcare systems consistently demonstrate that a significant proportion of hospitalized patients experience at least one medication-related error during their admission. Polypharmacy, complex dosing regimens, comorbid illnesses, and high-alert medications further increase vulnerability. The economic cost of medication-related harm is substantial. Preventable adverse drug events contribute to prolonged hospital stays, additional diagnostic testing, increased drug utilization, litigation costs, and productivity losses. From a societal perspective, the financial burden extends far beyond healthcare institutions to patients, families, and national economies[7].

**High-Risk Settings and Patient Populations**

Certain clinical settings exhibit particularly high rates of medication errors, including intensive care units, emergency departments, oncology wards, and geriatric units. Vulnerable populations such as older adults, children, patients with renal or hepatic impairment, and those receiving high-alert medications face elevated risk of serious harm from medication errors[9].

**Rationale for Pharmacist Involvement in Medication Safety**

**Unique Expertise in Pharmacotherapy**

Clinical pharmacists possess specialized training in pharmacology, therapeutics, pharmacokinetics, and drug safety. This expertise allows them to identify subtle prescribing errors that may not be immediately apparent to other clinicians. Pharmacists evaluate the appropriateness of drug selection, dosing accuracy, route of administration, frequency, duration, and potential interactions[9].

**Independent Medication Review**

Unlike prescribers, who are often focused on diagnosing and managing disease, pharmacists provide an independent and medication-centered perspective. This independent review function allows early detection of potentially inappropriate therapy and provides a critical safety checkpoint in the medication-use process[10].

**Continuous Monitoring and Follow-Up**

Clinical pharmacists actively monitor treatment response, laboratory parameters, and adverse drug reactions. This ongoing surveillance allows timely intervention when therapy becomes unsafe or ineffective, thereby preventing progression to serious harm[10].

**Role of Clinical Pharmacists**

**In Preventing Prescribing Errors**

Prescribing errors represent the most frequent and clinically significant category of medication errors in hospital practice. These errors commonly involve inappropriate drug selection, incorrect dosing, wrong frequency, failure to consider patient-specific factors such as renal or hepatic impairment, duplication of therapy, and clinically significant drug–drug interactions. Because prescribing decisions are made at the beginning of the medication-use process, errors at this stage often propagate downstream and increase the likelihood of patient harm. Clinical pharmacists play a critical role in intercepting prescribing errors through prospective review of medication orders. In hospitals where pharmacists participate in daily ward rounds, they routinely evaluate prescriptions for therapeutic appropriateness, dosing accuracy, safety, and adherence to clinical guidelines. Multiple studies have demonstrated that a significant proportion of pharmacist interventions at the prescribing stage result in meaningful modifications to therapy, including dose adjustment, discontinuation of unnecessary drugs, substitution with safer alternatives, and optimization of antimicrobial therapy[11]. Pharmacist involvement is particularly valuable in high-risk populations such as elderly patients, those with multiple comorbidities, and individuals receiving polypharmacy. Age-related changes in pharmacokinetics and pharmacodynamics significantly alter drug disposition, making standard doses potentially unsafe. Clinical pharmacists routinely adjust doses based on renal function, liver enzyme levels, body weight, and therapeutic response, thereby preventing both subtherapeutic treatment and toxicity. In addition, pharmacists play an essential role in preventing prescribing errors related to drug-drug interactions. With patients frequently receiving ten or more medications during hospital admission, the risk of harmful interactions is substantial. Pharmacists possess the expertise and tools to identify interactions that may lead to QT prolongation, bleeding, nephrotoxicity, serotonin syndrome, or central nervous system depression. Early identification of such risks allows proactive modification of therapy before adverse outcomes occur[12]. Prescribing errors are also common in antimicrobial therapy, where inappropriate selection, incorrect dosing, and unnecessary prolonged use contribute not only to individual patient harm but also to antimicrobial resistance. Clinical pharmacists involved in antimicrobial stewardship programs consistently demonstrate reductions in inappropriate antibiotic use, improved guideline adherence, and better patient outcomes[12,13].

**In Medication Reconciliation and Transitions of Care**

Transitions of care represent some of the most vulnerable points in the medication-use process. These transitions include hospital admission, transfer between wards, movement to intensive care units, and discharge back to the community. During these periods, patients are highly susceptible to unintentional medication discrepancies, including omitted drugs, duplicated therapy, incorrect dosing, and continuation of inappropriate medications[14]. Medication reconciliation is a structured process aimed at ensuring accurate and complete transfer of medication information across transitions of care. Clinical pharmacists are uniquely positioned to lead this process due to their detailed understanding of pharmacotherapy and their ability to obtain accurate medication histories[15]. At hospital admission, pharmacists collect comprehensive medication histories by interviewing patients and caregivers and reviewing previous prescriptions, community pharmacy records, and discharge summaries from earlier admissions. These histories are compared with admission orders to identify discrepancies. Studies consistently show that pharmacist-led medication reconciliation identifies significantly more discrepancies than standard physician- or nurse-led processes. At discharge, pharmacists ensure that inpatient medication changes are clearly communicated to

patients, caregivers, and primary care providers. Discharge counseling by pharmacists improves patient understanding of medication regimens, reduces post-discharge adverse drug events, and decreases avoidable hospital readmissions. Patients who receive pharmacist discharge counseling demonstrate better adherence, fewer medication errors at home, and improved satisfaction with care[8,9]. In healthcare systems where continuity between hospital and community care is fragmented, pharmacist-led transitional care services serve as an essential bridge to prevent medication-related harm after discharge.

**In Adverse Drug Reaction Monitoring and Prevention**

Adverse drug reactions [ADRs] are a major source of hospital-related morbidity and mortality. Many ADRs are predictable and preventable when appropriate monitoring strategies are implemented. Clinical pharmacists actively contribute to ADR prevention through routine surveillance, early detection, causality assessment, and reporting. Pharmacists monitor patients for early signs of drug toxicity through clinical review and laboratory evaluation. For example, nephrotoxic drugs require close monitoring of serum creatinine and urine output, while hepatotoxic agents require periodic liver function testing. Pharmacists also assess patient-reported symptoms that may indicate evolving adverse reactions, such as dizziness, gastrointestinal upset, skin rashes, or abnormal bleeding[16]. When an ADR is suspected, pharmacists assist in performing structured causality assessments, determining severity, and recommending appropriate therapeutic modifications. They also play a key role in documenting and reporting ADRs to pharmacovigilancecenters, thereby contributing to national and global drug safety surveillance[17]. Importantly, pharmacist involvement in ADR monitoring promotes a proactive rather than reactive safety approach. Instead of responding only after severe toxicity occurs, pharmacists identify high-risk patients and implement preventive strategies such as dose adjustments, enhanced monitoring, or drug substitution.

**In Therapeutic Drug Monitoring [TDM]**

Therapeutic drug monitoring is essential for medications with narrow therapeutic indices, high interindividual variability, or significant toxicity risks. Drugs such as vancomycin, aminoglycosides, antiepileptics, immunosuppressants, and certain cardiovascular agents require careful monitoring to maintain plasma concentrations within safe and effective ranges. Clinical pharmacists oversee TDM programs in many hospitals. Their responsibilities include selecting appropriate sampling times, interpreting drug concentrations, adjusting doses based on pharmacokinetic principles, and monitoring for toxicity or treatment failure. Pharmacist-led TDM services have consistently demonstrated improved target drug attainment, reduced toxicity, and enhanced clinical outcomes[18]. In critically ill patients, physiological changes such as altered volume of distribution, hypoalbuminemia, and organ dysfunction significantly affect drug handling. Pharmacists apply individualized dosing strategies in these patients to avoid underexposure or drug accumulation. Their involvement reduces the risk of nephrotoxicity, ototoxicity, neurotoxicity, and therapeutic failure[19].

**In Managing High-Alert Medications**

High-alert medications are drugs that carry a heightened risk of causing severe or fatal patient harm when used in error. These include insulin, anticoagulants, opioids, chemotherapeutic agents, concentrated electrolytes, neuromuscular blockers, and parenteral nutrition. Errors involving these agents often lead to life-threatening outcomes.

**Clinical pharmacists play a central role in ensuring the safe use of high-alert medications. Their interventions include**

- Independent double-checking of high-risk doses
- Verification of infusion concentrations and rates
- Assessment of contraindications and patient-specific risk factors
- Monitoring laboratory parameters such as INR, aPTT, blood glucose, and electrolyte levels
- Educating nurses and physicians on safe handling and administration

For example, insulin therapy requires careful dose titration based on blood glucose monitoring, nutritional intake, and concomitant illness. Pharmacist involvement reduces the incidence of hypoglycemia, dosing errors, and inappropriate insulin regimens. Similarly, anticoagulant therapy requires precise monitoring to balance the risks of

bleeding and thrombosis. Pharmacist-managed anticoagulation services demonstrate superior safety outcomes compared to usual care[20]. In oncology, pharmacists verify chemotherapy protocols, calculate individualized doses based on body surface area and organ function, prevent vesicant-related injuries, and monitor for cumulative toxicity. These safety checks are critical in preventing fatal chemotherapy errors.

**In Low- and Middle-Income Countries**

The need for clinical pharmacy services is arguably greatest in low- and middle-income countries (LMICs), where healthcare systems are often characterized by high patient volumes, limited staffing, variable prescribing practices, and inadequate monitoring infrastructure. However, these same constraints also make implementation more challenging. In LMIC settings, clinical pharmacists often function across multiple wards, balancing dispensing responsibilities with limited clinical duties[21]. Despite these constraints, available evidence indicates that even modest pharmacist involvement in medication

**Economic Impact of Clinical Pharmacist Interventions**

Beyond improving clinical outcomes, clinical pharmacist interventions have demonstrated substantial economic benefits for healthcare systems. Medication errors and adverse drug events impose a significant financial burden through prolonged hospital stays, additional laboratory investigations, increased drug utilization, intensive care admissions, litigation, and readmissions. By preventing these avoidable outcomes, pharmacists directly contribute to cost containment and more efficient use of healthcare resources. Numerous economic evaluations have shown that pharmacist-led medication review programs yield a favorable cost–benefit ratio. The cost of employing clinical pharmacists is frequently outweighed by the savings achieved through reduction in preventable adverse drug events. These savings arise from shortened length of stay, reduced need for expensive rescue therapies, and avoidance of complications such as renal failure, major bleeding, or severe hypoglycemia. In antimicrobial stewardship programs, pharmacist involvement has been associated with reduced use of broad-spectrum antibiotics, lower antimicrobial expenditure, and decreased rates of multidrug-resistant infections. This not only improves patient safety but also delivers long-term economic benefits by preserving the effectiveness of existing antimicrobials. Pharmacist-led medication reconciliation and discharge counseling services have also been shown to reduce hospital readmissions, particularly among elderly and chronically ill patients. Avoidable readmissions represent a major source of unnecessary healthcare expenditure, and preventing them through pharmacist intervention contributes directly to financial sustainability[21,22]. Importantly, economic benefits are not limited to high-income settings. Even in resource-constrained environments, modest investments in clinical pharmacy services often yield disproportionately large returns by preventing costly adverse outcomes that hospitals may be ill-equipped to manage.

**Interprofessional Collaboration and Safety Culture**

Medication safety is inherently a team-based responsibility that requires effective communication and collaboration among physicians, nurses, pharmacists, and other healthcare professionals. The integration of clinical pharmacists into multidisciplinary care teams has consistently been associated with improvements in communication, shared decision-making, and safety culture[23]. Clinical pharmacists serve as accessible medication experts within clinical teams. Their presence during ward rounds fosters real-time discussion of therapeutic plans, early identification of potential safety concerns, and consensus-based decision-making. This collaborative model reduces hierarchical barriers and encourages open dialogue regarding medication-related risks. From a patient safety perspective, pharmacists contribute to the development of a non-punitive culture that emphasizes learning from errors rather than assigning blame. When pharmacists actively participate in error reporting systems, root cause analyses, and quality improvement committees, they help transform medication safety from a reactive process to a continuous learning system. The visibility of pharmacists on wards also reinforces a culture of vigilance and shared accountability. Nurses and junior doctors often seek guidance from pharmacists regarding dosing, compatibility, and monitoring requirements, which further strengthens safety practices. In many institutions, pharmacists also play a leadership role in staff education programs on medication safety, high-alert drugs, look-alike/sound-alike

medications, and safe injection practices. These educational initiatives enhance the overall safety competence of healthcare teams[24].

**Digital Health, Automation, and the Expanding Role of Pharmacists**

The rapid expansion of digital health technologies has transformed many aspects of medication management. Electronic health records, computerized physician order entry systems, clinical decision support tools, automated dispensing cabinets, and barcode medication administration systems are now widely used to reduce medication errors. Clinical pharmacists play a pivotal role in optimizing the use of these technologies for patient safety. Pharmacists contribute to the design and refinement of electronic prescribing systems by helping to develop dosing protocols, drug interaction alerts, allergy warnings, and renal dose adjustment algorithms. Their involvement ensures that digital tools reflect real-world clinical practice and minimize unnecessary alerts that contribute to alert fatigue[25]. In automated dispensing environments, pharmacists oversee medication storage, labeling, and verification processes. They also monitor for discrepancies, stock errors, and unauthorized access, thereby strengthening medication security. The emergence of data analytics and artificial intelligence has further expanded the scope of pharmacist involvement. Pharmacists increasingly participate in the development and validation of predictive models for adverse drug events, high-risk prescribing patterns, and patient-specific risk stratification. Their clinical insight is essential to ensuring that such tools are clinically meaningful, interpretable, and ethically deployed. Despite these technological advances, it is important to recognize that digital systems do not replace the clinical judgment of pharmacists. Instead, technology serves as an adjunct that enhances the ability of pharmacists to identify risks earlier, manage larger patient populations, and focus their expertise where it is most needed[26].

**Barriers to Expanding Clinical Pharmacy Services**

Despite robust evidence supporting the value of clinical pharmacists in improving medication safety, the expansion of clinical pharmacy services remains uneven across healthcare systems. Several persistent barriers limit widespread implementation.

**Workforce Limitations**

Many countries face shortages of trained clinical pharmacists. Even where pharmacy graduates are available, formal residency training and clinical specialization opportunities may be limited. This constrains the number of pharmacists who are adequately prepared to function in advanced clinical roles.

**Limited Institutional Support**

In some hospitals, the role of pharmacists remains confined to dispensary functions due to historical practice models and limited recognition of their clinical value. Administrative support is often essential for redefining job roles, allocating ward-based responsibilities, and integrating pharmacists into care teams[9,27].

**Financial Constraints**

Healthcare institutions, particularly in the public sector, may perceive clinical pharmacy services as an additional cost rather than an investment. Budgetary limitations frequently restrict recruitment and expansion of pharmacist positions, even when long-term economic benefits are evident.

**Lack of Regulatory Mandates**

In many jurisdictions, there are no regulatory requirements mandating the presence of clinical pharmacists in hospital wards. Without policy-level support, the adoption of clinical pharmacy services often depends on individual institutional leadership rather than systematic planning[10,28].

**Interprofessional Resistance**

In some settings, role ambiguity and traditional professional hierarchies may hinder interprofessional collaboration. Physicians or nurses may initially be reluctant to accept pharmacists as equal partners in clinical decision-making. Overcoming these cultural barriers requires sustained engagement, education, and demonstration of value. This

comprehensive narrative review demonstrates that clinical pharmacists play a pivotal and indispensable role in reducing medication errors and enhancing patient safety across hospital settings worldwide. The evidence consistently indicates that pharmacist-led interventions significantly reduce prescribing errors, prevent adverse drug events, optimize medication therapy, and improve both clinical and economic outcomes. These findings reinforce the growing international recognition of clinical pharmacy as a core component of modern healthcare delivery. One of the most important insights emerging from this review is the broad impact of pharmacists across the entire medication-use continuum. Unlike single-point technological interventions, clinical pharmacists influence multiple high-risk stages, including prescribing, medication reconciliation, administration oversight, therapeutic drug monitoring, adverse drug reaction surveillance, and discharge transitions. This continuous presence allows pharmacists to function as dynamic safety barriers capable of preventing errors before they reach the patient. The proactive nature of pharmacist involvement distinguishes it from many reactive safety strategies that only respond after harm has occurred. Prescribing errors emerged as the most frequent and clinically significant source of medication-related harm. The review highlights that pharmacist participation in ward rounds and real-time medication order review leads to substantial reductions in dosing errors, inappropriate drug selection, duplicate therapy, and dangerous drug–drug interactions. These findings are particularly important in vulnerable populations such as the elderly, critically ill, and patients receiving polypharmacy. The ability of pharmacists to tailor doses based on renal and hepatic function, pharmacokinetic variability, and therapeutic response remains one of the most powerful tools for preventing avoidable toxicity and treatment failure. Transitions of care represent another critical vulnerability in medication safety. This review confirms that pharmacist-led medication reconciliation at admission and discharge dramatically reduces unintentional discrepancies that commonly lead to post-discharge adverse drug events and hospital readmissions. The effectiveness of pharmacists in obtaining accurate medication histories, identifying omissions and duplications, and ensuring continuity between inpatient and outpatient care underscores their essential role in preventing fragmented medication management. In healthcare systems facing increasing pressures from early discharge and outpatient-based care, this function becomes even more vital.

The role of clinical pharmacists in adverse drug reaction prevention and pharmacovigilance further strengthens their contribution to patient safety. Through structured monitoring, early detection, causality assessment, and systematic reporting, pharmacists help shift healthcare systems toward a proactive model of medication safety. This not only reduces immediate patient harm but also contributes to broader national and global drug safety surveillance systems. Importantly, pharmacist involvement enhances the detection of preventable ADRs related to dosing errors, drug interactions, and inadequate monitoring. Therapeutic drug monitoring represents another area where pharmacist expertise is irreplaceable. The review clearly demonstrates that pharmacist-managed TDM services for drugs with narrow therapeutic indices significantly improve target concentration attainment while minimizing toxicity. In critically ill patients, where altered physiology profoundly affects drug disposition, individualized pharmacist-guided dosing becomes essential to avoid both underexposure and life-threatening accumulation. These benefits are consistently observed across antibiotics, antiepileptics, immunosuppressants, and cardiovascular drugs. High-alert medications continue to account for a disproportionate share of fatal and life-threatening medication errors. The review affirms that pharmacist oversight of insulin, anticoagulants, opioids, chemotherapeutic agents, and concentrated electrolytes substantially reduces catastrophic harm. By ensuring independent double-checking, appropriate monitoring, and staff education, pharmacists function as final safety guardians for the most dangerous drugs used in hospitals. Beyond clinical safety, this review highlights the strong economic justification for expanding clinical pharmacy services. Preventable adverse drug events impose a heavy financial burden through prolonged hospitalization, costly rescue treatments, and avoidable readmissions. Pharmacist-led interventions consistently demonstrate favorable cost–benefit and cost-effectiveness profiles. Notably, these economic benefits are evident not only in high-income healthcare systems but also in resource-constrained environments, where preventing a single major adverse event can offset months of staffing costs. This economic dimension strengthens the policy argument for embedding clinical pharmacists within routine hospital care. Interprofessional collaboration emerges as a central mechanism through which pharmacist interventions achieve their full impact. The review demonstrates that the integration of pharmacists into multidisciplinary teams improves communication, reduces hierarchical barriers, and fosters a shared culture of medication safety.

Pharmacists serve as accessible medication experts, supporting physicians and nurses in real-time decision-making while reinforcing standardized safety practices. Their involvement in education, quality improvement, and incident review further institutionalizes medication safety as a collective professional responsibility [29]. The rapid expansion of digital health technologies presents both opportunities and challenges for clinical pharmacy practice. While electronic prescribing, decision support systems, and automation reduce certain types of errors, they also introduce new risks such as alert fatigue, system overrides, and automation bias. This review emphasizes that pharmacists are essential in optimizing digital systems by ensuring that alerts are clinically meaningful, protocols are evidence-based, and technology complements rather than replaces human judgment. The emerging role of pharmacists in data analytics and artificial intelligence further illustrates how their expertise will remain central in future medication safety strategies. Despite overwhelming evidence of benefit, multiple systemic barriers continue to limit the widespread implementation of clinical pharmacy services. Workforce shortages, inadequate institutional support, financial constraints, regulatory gaps, and interprofessional resistance remain major challenges, particularly in low- and middle-income countries. However, the review also demonstrates that even limited pharmacist involvement in these settings produces meaningful improvements in medication safety. This suggests that scalable, context-specific models of clinical pharmacy practice can yield substantial returns even under resource constraints [30]. Importantly, this review also identifies key evidence gaps. While most studies demonstrate reductions in error rates and adverse events, fewer evaluate long-term patient-centered outcomes such as mortality, quality of life, and functional status. Additionally, standardized outcome measures and robust multicenter randomized trials remain limited in many regions. Future research should prioritize harmonized measurement frameworks, implementation science approaches, and economic evaluations tailored to diverse healthcare systems.

## CONCLUSION

Medication errors remain a persistent and preventable threat to patient safety despite advances in pharmacotherapy and healthcare technology. The evidence synthesized throughout this review indicates that clinical pharmacists represent one of the most effective system-level interventions for improving medication safety in hospital settings. By contributing expertise in pharmacotherapy across key stages of the medication-use process—including prescribing review, medication reconciliation, therapeutic drug monitoring, adverse drug reaction surveillance, and transitions of care—clinical pharmacists function as critical safeguards capable of identifying and mitigating medication-related risks before they reach the patient. Importantly, the impact of pharmacist-led interventions extends beyond error interception to strengthening multidisciplinary collaboration, improving guideline adherence, and promoting a proactive culture of medication safety. The consistent demonstration of favorable clinical and economic outcomes further reinforces the value of integrating pharmacists into routine patient care. However, the translation of this evidence into practice remains uneven, particularly in low- and middle-income countries, where workforce limitations, institutional barriers, and limited regulatory support constrain the expansion of clinical pharmacy services. As healthcare systems increasingly incorporate digital health technologies and automated prescribing platforms, pharmacist expertise will remain essential to contextualize clinical decision-support outputs and mitigate emerging technology-related risks. Future research should prioritize robust multicenter studies and standardized outcome frameworks to better quantify the long-term patient and system-level impact of pharmacist interventions. Ultimately, embedding clinical pharmacists within multidisciplinary care teams should be recognized not merely as an enhancement to hospital practice but as a fundamental component of safe, high-quality healthcare delivery.

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RESEARCH ARTICLE

# Association of Balance Confidence with Balance Performance, Trunk Control and Functional Mobility in Stroke Patients

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## ABSTRACT

Stroke is the most common neurological disorder and 2nd biggest cause of morbidity and mortality worldwide. Balance, gait, trunk impairment, and motor function deficits are common following stroke. Research has supported that trunk control; balance performance and balance confidence are important to enhance the functional mobility among patients with stroke. This study aimed to establish whether the relationship exists between balance confidence and trunk control, functional mobility in individuals with stroke. Design: - The study had observational cross-sectional research design. Participants: - Seventy-five participants with a mean age of 52 years participated in the study. Intervention: - Not applicable. Main Outcome Measures: Balance confidence, trunk control, balance performance and functional mobility were measured by using Activities specific balance confidence Scale (ABC-S), Trunk impairment scale (TIS), Berg balance scale (BBS) and Time up and go test (TUG) respectively. Descriptive statistics were used to assess the level of confidence, trunk control, functional mobility, and balance performance. Pearson's test was used to evaluate the association of balance confidence with trunk control, balance performance and functional mobility. Majority of patients had ischaemic stroke and 76 % of overall population had right side hemiplegia. About one-third of subjects have moderate risk of falls and moderate level of trunk control along with moderate level of balance confidence in physical functioning. Pearson's correlation showed positive correlation between balance confidence and trunk control, balance performance and functional mobility (r = 0.78, r = 0.72, r= 0.77, respectively.) This study reveals that balance confidence is

positively correlated withtrunk control, balance performance and functional mobility among patients with stroke.

**Keywords:** Stroke patients, balance, balance confidence, trunk control, functional mobility.
**Abbreviations:** ABC-S : Activities Specific Balance Confidence Scale, BBS : Berg Balance Scale, CONFbal: Confidence Balance Scale, CVA : Cerebrovascular Accident, TIS : Trunk Impairment Scale, TUG: Time Up And Go Test, WHO : World Health Organisation, ADLs: Activities of daily living

## INTRODUCTION

Stroke, also known as cerebrovascular accident (CVA), is defined as the sudden loss of neurological function due to interrupted blood flow to the brain. According to the World Health Organization (WHO), stroke is the second leading cause of mortality globally and a major contributor to functional impairment. By 2050, it is estimated that 80% of strokes will occur in low- and middle-income countries, with India experiencing an incidence rate ranging from 105 to 152 per 100,000. Prevalence rates in India vary significantly, from 26 to 752 per 100,000, with case fatality rates between 18-42% in the first month post-stroke. Stroke often results in motor deficits characterized by hemiplegia or hemiparesis, affecting trunk control and strength in the shoulder girdle and lower limbs. About 90% of stroke survivors face mobility issues, 80% have trouble performing activities of daily living (ADLs), and 56% have trouble walking, leading to frequent falls. Balance performance is typically disrupted after a stroke, leading to impaired gait symmetry and dynamic stability, which are crucial for maintaining functional independence. Static balance, which maintains the body's centre of gravity within the base of support, and dynamic balance, which involves movement, are both essential for everyday activities. Hemiparetic stroke impacts the ability to execute standing turns and reorient their body segments, making them low Confidence in performing tasks is vital for independent living, as fear of falling can restrict participation in daily activities and lower overall quality of life. Although individuals' post-stroke may grapple with the fear of falling, they also contend with neurological injuries that further diminish their balance confidence. While balance training is essential to improve physical abilities, failure to simultaneously enhance balance confidence can restrict their capacity to perform daily activities. Therefore, a decreased sense of balance capability often leads individuals to refrain from activities they are physically able to undertake. Rehabilitation interventions aimed at enhancing balance performance are crucial for improving the overall physical function of stroke survivors. Addressing multiple-task balance challenges is especially important, as such impairments are predictive of negative outcomes, underscoring the need for targeted therapeutic approaches in stroke rehabilitation.

Trunk control, which involves coordinating trunk muscle activation to maintain posture and balance, is crucial for stability and movement. Unfortunately, rehabilitation efforts often neglect trunk rehabilitation in Favor of limb recovery. Impaired trunk control significantly affects balance and the ability to perform rotational movements during ambulation. Research indicates that trunk performance is closely associated with balance capacity, walking ability, and overall physical function in stroke patients. Systematic reviews have demonstrated that trunk exercise programs, whether on stable or unstable surfaces, are effective in improving trunk control, balance, and mobility. The trunk muscles are essential for stabilizing the torso and pelvis, playing a pivotal role in functional activities. Studies suggest that better trunk performance correlates with improved functional status in late-stage stroke recovery. Despite the established connection between balance capacity, self-confidence in balance, and physical functioning in chronic stroke patients, the relationship between trunk control and balance confidence remains poorly understood in scientific discourse. Trunk muscle weakness can hinder balance and functional tasks post-stroke, highlighting the need for targeted rehabilitation strategies. Functional mobility encapsulates the ability to move effectively within one's environment, including walking and transitioning between different positions. Functional mobility is critical for an individual's ability to navigate their environment, encompassing activities like walking, transitioning between positions (e.g., standing from sitting), and maintaining stability during these movements. In stroke patients, these

capabilities are frequently compromised due to factors like muscle weakness, coordination deficits, balance impairments, and sensory losses. Such balance issues can severely limit functional mobility, increase the risk of falls and diminish independence in activities of daily living (ADLs). Consequently, rehabilitation programs often prioritize enhancing both balance and functional mobility to support comprehensive recovery and improve the quality of life for stroke survivors. Through targeted interventions, patients can regain confidence in their mobility and better manage their daily tasks.

## MATERIALS AND METHODS

**Design:** The design of the study was Observational Cross-sectional design which was conducted from August 2023 to July 2024, and was approved by the Institutional Ethical Committee. Informed consent was obtained from all participants.

**Sample:** A total of 75 adults with clinically diagnosed with stroke were included. Inclusion criteria also required participants to be aged 18 years or older, stable patients both male and female with the ability to follow instructions. Exclusion criteria included any orthopaedic condition and neurological or psychiatric condition other than stroke Patients with unstable vitals and not willing to be a part of study. Participants were recruited using convenience sampling method.

**Instrument/ tools used.**

1. Berg Balance Scale
2. Trunk Impairment Scale
3. Activities-Specific Balance Confidence Scale
4. Time Up and Go Test

**Trunk Impairment Scale:** Trunk Impairment Scale (TIS). Patients must perform every item of the scale three times which are intended to measure the static and dynamic sitting balance and coordination. The TIS consists of three sub-scales: static sitting balance, dynamic sitting balance and coordination. The sub-scale contains 3 items in static sitting balance subscale, 10 items in dynamic sitting balance subscale and 4 items in coordination subscale. For each item, a 2-, 3- or 4-point ordinal scale is used. The TIS score ranges from a minimum of 0 to maximum of 23.

**Berg Balance Scale:** The BBS is a 14-item scale with each item consisting of a five-point ordinal scale ranging from (0-4) that quantitatively assess balance through direct observation of their performance and measures the patient ability to maintain balance either statically or while performing various functional movements for a specified duration of time. The items are scored from 0 to 4 with a score of 0 representing an inability to complete the task and a score of 4 representing in dependent item completion. A global score is calculated out of 56 possible points. Score of 0 to 20 represent severe balance impairment, 21 to 40 represent moderate balance impairment, and 41 to 56 represent mild balance impairment. The BBS measures both static and dynamic aspects of balance.

**Activities specific balance confidence Scale:** The ABC scale is a 16-item self-report questionnaire that assess individual balance confidence in performing specific ambulatory activities on numerical rating scale (0-100). A score of 0 represents no confidence, while a score of 100 represents complete confidence in performing the activity. The overall ABC score for each participant is calculated by adding the individual item scores together (maximum score=1600) and then dividing by the total no of items [16].

**Time up and go test:** the time up and go test developed by Podsiadlo and Richardson is a simple and quick functional mobility test. The TUG test records the time it takes for a participant to complete the consecutive test of standing up from a chair, walking 3-meters forward, turning 180-degree, and sitting back down on the chair to assess the one's dynamic balance and ambulation ability.

## RESULTS

Out of seventy-five total participants with a mean age of 52.30 years as shown in Table 1. Fifty- two (69.3%) were males and twenty- three (30.7%) were females who participated in the study. Data was analysed with the help of Pearson's product-moment method of correlation to find the relationship among different variables**.** The correlation coefficient was calculated for finding association between balance confidence and balance performance, trunk control, functional mobility variables. The Activities specific Balance Confidence Scale (ABC-S) results evaluate the balance confidence among patients with stroke. Almost more than half (61.3%) had moderate balance confidence during physical functioning, while (13.3%) exhibited good level of balance confidence during physical functioning. However, a concerning (25.3%) reported low confidence level in physical functioning. The Berg Balance Scale (BBS) results evaluate the balance performance among patients with stroke. Almost more than half (58.7%) had mild impaired balance, while (22.7%) had moderately impaired balance and remaining (18.7%) exhibited highly impaired balance. The Trunk Impairment Scale (TIS) results evaluate the trunk control among patients with stroke. Trunk control among post-stroke patients were represented as (61.3%) moderate trunk impairment, (18.7%) mild trunk impairment and (20%) maximal trunk impairment. Assessment of functional mobility using Time Up and Go Test (TUG) indicates that almost more than half (57.3%) had moderate risk of falls, while (2.7%) had low risk of falls and (40%) had high risk of falls with poor functional mobility. For the sample population, significant ($p<0.05$), strong and positive correlations were seen between balance confidence and balance performance, trunk control, functional mobility ($r = 0.72, 0.78, 0.77$ respectively). Table 1: Demographic characteristics. Table 2: Correlation between balance confidence and balance performance, trunk control, functional mobility. ** Correlation is significant at the <0.05 level (2-tailed)

## DISCUSSION

Stroke is known as most common neurological disorder. Despite the pharmaceutical intervention physical therapy was also play remarkable role in management of stroke patients. The main objective of the study was to figure out the relationship between balance confidence and trunk control, functional mobility, balance performance among patients after stroke. We included total of 75 participants to find out the association between balance confidence and trunk control, functional mobility, balance performance in patients with stroke. Results can be explained by the improvement noticed in the patients with stroke who had good balance performance and trunk control showed better functional mobility and higher the balance confidence. G. jijimol *et al*[14] first reported the relationship between trunk impairment and balance in chronic stroke patients and they reported the strong positive correlation between trunk performance and balance in post stroke. The result of the study has very much clinical importance in the rehabilitation of stroke patients. Since trunk performance and static or dynamic balance are correlated with each other, any step to improve the trunk performance will facilitate the balance in stroke patients. This study promotes the indication of usage of trunk exercises in post stroke patients in the acute stage itself. It is estimated that trunk stability is an essential core component of balance and coordinated extremity use in daily functional activities and performance of higher-level motor tasks. Additional trunk exercises in the rehabilitation of stroke patients can transfer the improved balancing activities needed for the standing and walking thereby improving efficient motor strategies. Borade P. *et al*[15] found that there was a significant association between fall efficacy, balance performance and functional mobility among subacute and chronic patients with stroke. This relationship has an implication for the development of rehabilitation programs that aim to improve the balance confidence as well to diminish its impact on function in stroke patients. Karthik Babu S. *et al*[5] reported that trunk control had a high correlation with core muscle strength and balance confidence in people with chronic stroke living in the community. Due to poor muscular activity and weakness of abdominals and hip extensors in the seated position, patients with stroke tend to flex their upper torso excessively against posteriorly tilted pelvis during arm forward reaching functional tasks.

The disuse related core muscles weakness and soft tissue adaptations around the spine in chronic stage stroke might further limit their dynamic balance control and movement coordination between upper and lower trunk movement.

The chronic stroke survivors usually stiffen the axial-scapular muscles excessively by retracting the shoulder girdle, thus extension- rotation of the upper trunk on the most involved side as one of the compensatory balance strategies. Compensatory movement recovery of either excessive trunk flexion or hyperextension of the torso would restrict the physiological range of thoracic rotation mobility, allowing weakness of trunk rotators on both sides. The motor control of trunk rotators is conditional for proximal dynamic stability and dissociated movements between torso and extremities during most of the daily functional activities. It was found that poor trunk control and core muscle weakness are positively correlated to balance and daily functional mobility tasks. Regardless of the side of cortical vascular lesion, there is a strong correlation of trunk control with core muscles strength and balance confidence of chronic stroke survivors. Previous studies demonstrated that community dwelling stroke survivors having poor balance capacity and mobility problems, experience less balance confidence, exhibit perception of becoming unsteady and fear of falling during daily activities. Saeys W. *et al*[16] found strong evidence that trunk training is able to improve trunk control, sitting and standing balance and mobility after subacute and chronic stroke. Results comply with previous reviews concluding that trunk training is a good rehabilitation strategy for improving dynamic sitting balance. In patients suffering from stroke, there is a strong amount of evidence showing that trunk training is able to improve trunk control, sitting and standing balance and mobility. Shah ZR. *et al*[4] analyses the correlation between CONFbal (confidence balance scale)and BBS shows highly significant moderate level of negative correlation ($r = (-0.4370)$ and $p < 0.001$). But as the lower score of CONFbal implies better confidence; hence it is proven that balance confidence and balance performance is correlated with each other. The stroke patients who have low balance performance demonstrates low confidence or high fear of fall and high balance performance will show high confidence level and low level of fear of fall. Above mentioned studies have some limitations like factor of response bias, crosssectional nature of study, small sample size, and these limitations need to be addressed in future.

## CONCLUSION

In conclusion, the comprehensive evaluation of balance confidence, trunk control, balance performance, and functional mobility is essential for effectively understanding and improving an individual's functional abilities, especially among populations at risk of falls such as the elderly and stroke survivors. The strong association between these factors underscores the intricate relationship necessary for maintaining stability and mobility. Targeted assessment and intervention strategies that enhance balance confidence and trunk control can lead to significant improvements in balance performance and functional mobility. Ultimately, this integrated approach supports greater independence and quality of life, emphasizing the need for ongoing research and refined evaluation techniques to optimize rehabilitation outcomes.

## DECLARATION BY AUTHORS

**Ethical Approval**
Approved

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**Conflict of Interest**

The authors declare no conflict of interest.

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**Table 1: Demographic Characteristics**

| Age (n= 75) | Mean ± SD |
|---|---|
| 18-80 years | 52.30 ± 1.21 |

**Table 2: Correlation Between Balance Confidence and Balance Performance, Trunk Control, Functional Mobility.**

| Variable | Pearson's correlation coefficient (r) | p-value |
|---|---|---|
| Balance confidence -ABC-S and Balance performance -BBS | 0.72** | 0.000* |
| Balance confidence - ABC-S and Trunk control- TIS | 0.78** | 0.000* |
| Balance confidence – ABC-S and Functional mobility- TUG | 0.77** | 0.000* |

** Correlation is significant at the <0.05 level (2-tailed)

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**RESEARCH ARTICLE**

# Mathematical Foundations of Explainable Artificial Intelligence Using Linear Algebra and Information Theory

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**ABSTRACT**

Explainable Artificial Intelligence (XAI) has emerged as a critical field in modern machine learning, seeking to make complex models interpretable and trustworthy. As artificial intelligence becomes embedded in critical systems, the need for mathematical transparency grows. This paper explores the mathematical underpinnings of explainability through three major lenses: Linear Algebra, Information Theory, and Topological Data Analysis (TDA). It presents models, tables, and schematic figures illustrating how these mathematical frameworks contribute to interpretability and model accountability. Together, these approaches offer a robust mathematical foundation for explainable AI systems.

**Keywords:-**Explainable AI, Linear Algebra, Information Theory, Topological Data Analysis, Interpretability, Transparency

## INTRODUCTION

Artificial Intelligence (AI) systems, particularly deep learning models, have achieved remarkable success across various domains. However, their decision-making processes often remain opaque, giving rise to the "black box" problem. Explainable AI (XAI) seeks to enhance transparency and interpretability, allowing humans to understand how models generate predictions (Doshi-Velez & Kim, 2017). Mathematics provides the foundation for these explanations through three core domains: linear algebra, which decomposes and interprets model structures; information theory, which measures and quantifies information flow; and topological data analysis (TDA), which captures and visualizes the geometric nature of decision boundaries.

**Linear Algebra and Model Analysis**

Linear algebra forms the foundation for understanding neural networks, where transformations are represented by weight matrices (W). The relationships among features can be analyzed through eigenvalue decomposition and singular value decomposition (SVD). Singular values reveal dominant patterns within the data, facilitating dimensionality reduction and feature relevance analysis, which are essential for model interpretation and optimization.

**Information-Theoretic Foundations**

Information theory assesses unpredictability and interpretability through measures such as entropy (H) and mutual information (I). Entropy captures the degree of randomness or disorder in model predictions, while mutual information evaluates the dependency or shared information between inputs and outputs. The Information Bottleneck principle (Tishby&Zaslavsky, 2015) formalizes how models compress irrelevant information while preserving essential predictive content.

**Topological Data Analysis**

TDA provides a geometric view of model behavior. Using persistent homology, TDA identifies patterns such as clusters, loops, and voids within data manifolds. These structures reveal the organization and complexity of the decision space and detect anomalies or overfitting (Carlsson, 2009; Hofer et al., 2017).

**Integrated Mathematical Framework**

Explain ability arises from the synthesis of algebraic, informational, and topological perspectives. Each framework contributes unique insights—linear algebra for structure, information theory for flow, and TDA for global geometry. Together they enable multi-scale, quantitative interpretability.

## CONCLUSION

Mathematics forms the backbone of explainable AI. Linear algebra decodes the structure of models, information theory measures ambiguity and communication efficiency, and topological data analysis reveals overarching geometric structures. These frameworks together create a mathematically rigorous foundation for transparency, enabling models that are not just precise but also interpretable and reliable.

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**Table 1: Mathematical Constructs of Explain ability**

| Mathematical Tool | Application in XAI | Key Outcome |
|---|---|---|
| Linear Algebra | Model decomposition, feature projection | Structural insight |
| Information Theory | Entropy, mutual information | Feature relevance |
| TDA | Shape of data manifold | Geometric interpretability |

**Table 2: Information-Theoretic Measures in XAI**

| Measure | Formula | Interpretive Use |
|---|---|---|
| Entropy H(X) | $-\Sigma P(x)\log P(x)$ | Quantifies uncertainty |
| Mutual Information I(X;Y) | $H(Y) - H(Y \mid X)$ | Measures feature relevance |
| Information Bottleneck | $I(X;T) - \beta I(T;Y)$ | Balances compression vs. fidelity |

**Table 3: TDA-Based Explain ability Metrics**

| Measure | Definition | Interpretive Value |
|---|---|---|
| Betti Numbers $\beta_k$ | Rank of $H_k$ | Counts topological features |
| Persistence Lifetime | $\varepsilon$death - $\varepsilon$birth | Feature significance |
| Topological Distance | $W_2(D_1, D_2)$ | Model similarity metric |

**Table 4: Comparative Mathematical Frameworks in XAI**

| Framework | Tools | Primary Insight | Example |
|---|---|---|---|
| Linear Algebra | SVD, PCA | Local structure | Weight analysis |
| Information Theory | Entropy, MI | Feature relevance | Bottleneck model |
| TDA | Persistent Homology | Global topology | Persistence diagrams |

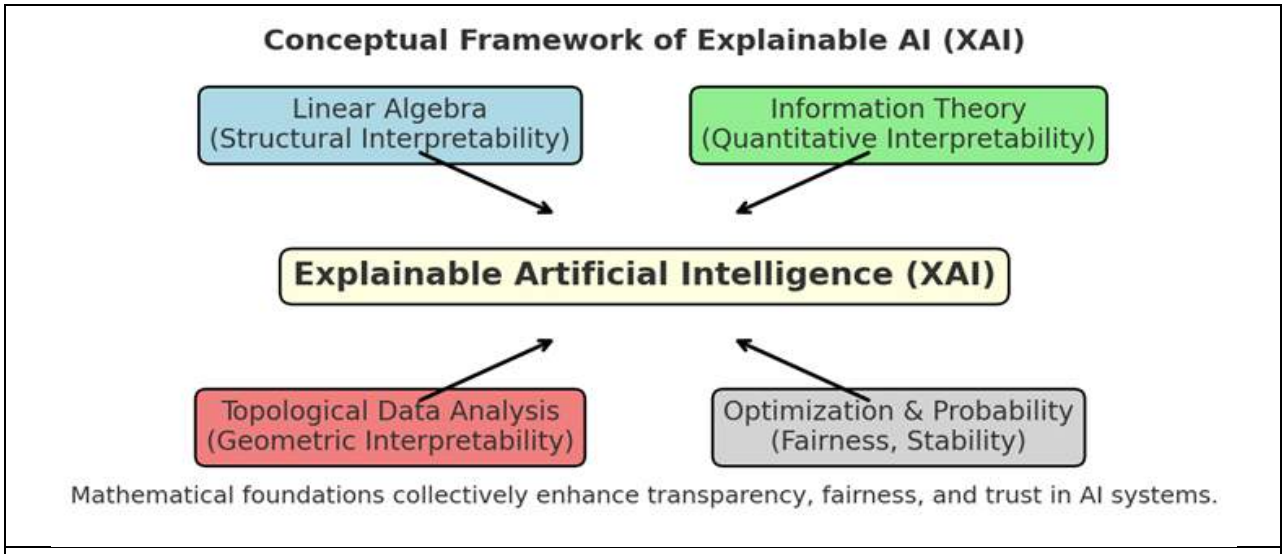



**Figure 1. Conceptual Framework of Explainable AI (XAI)**

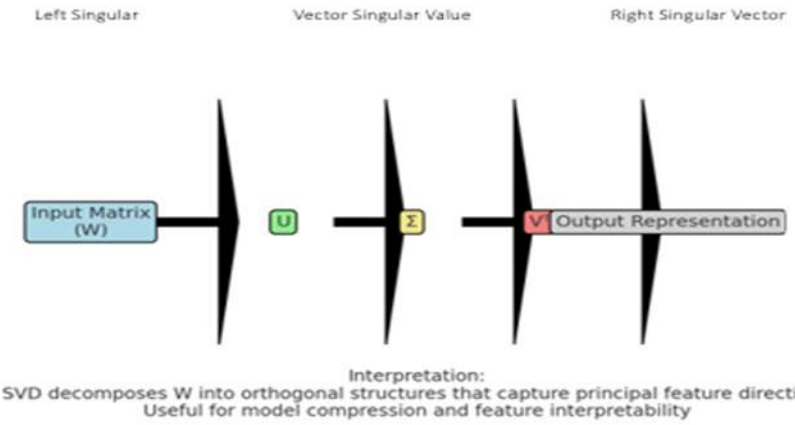



**Figure 2. Singular Value Decomposition in Linear Algebra-based Interpretability**

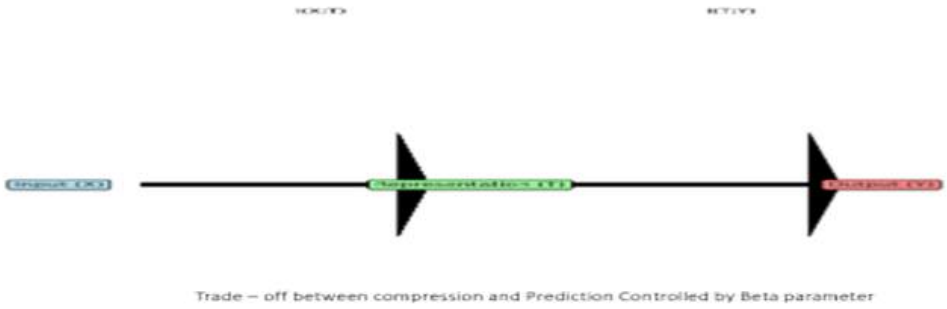



**Figure 3. Information Bottleneck Model of XAI**

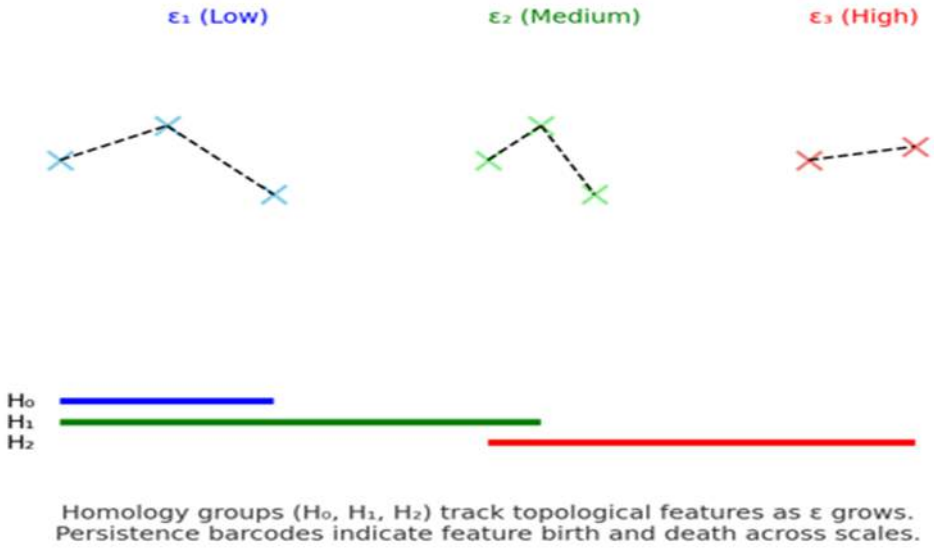



**Figure 4. Persistent Homology Structure in TDA-based XAI**

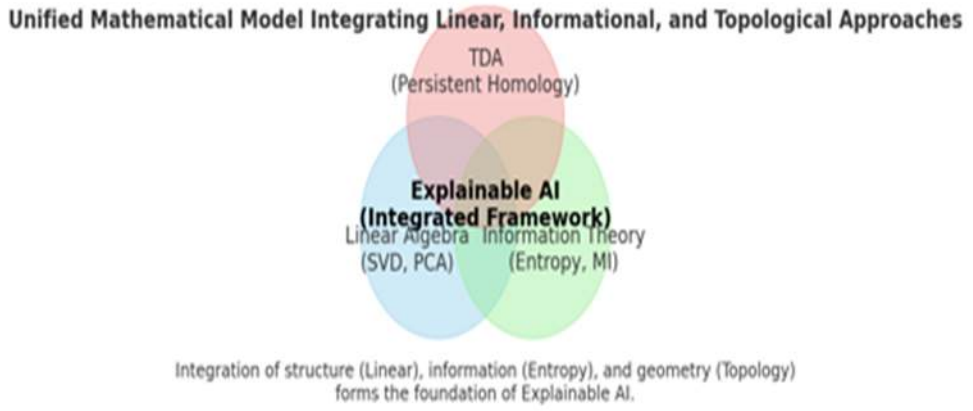



**Figure 5. Unified Mathematical Model Integrating Linear, Informational, and Topological Approaches**

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RESEARCH ARTICLE

# Quantum Machine Learning in Hate Content Identification : Proposed Novel Approach

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## ABSTRACT

The introduction of quantum machine learning (QML) offers a breakthrough way to detect hate content in online platforms, addressing the growing incidence of harmful speech. Traditional machine learning techniques often struggle with linguistic complexity and nuances, making it challenging to accurately detect hate speech. QML uses quantum computing principles such as superposition and entanglement to improve the efficiency and accuracy of content classification. This analysis examines the present environment of QML applications in hate content identification, emphasizing the possible benefits over traditional methods, such as faster processing and the capacity to catch detailed patterns in data. We explore several quantum algorithms, including Quantum Support Vector Machines and Quantum Natural Language Processing, that show potential for improving identification capabilities across various material types, such as text from social media posts, comments, and video transcripts. Despite its potential, the application of QML confronts major hurdles, including technical constraints, the requirement for huge labeled datasets, and ethical concerns about bias in automated systems. This study intends to present a comprehensive assessment of the current status of QML in this domain, as well as to outline future research approaches that could improve the effectiveness of hate content recognition.

**Keywords:** QML, data, Quantum, Learning, application, domain

# INTRODUCTION

The widespread use of social media and online communication platforms has resulted in an alarming rise in hate speech and destructive content, providing enormous issues for users, platforms, and regulatory organizations. Hate speech refers to any kind of communication that incites violent or discriminatory behavior against individuals or groups based on characteristics such as race, religion, ethnicity, sexual orientation, or gender identity. Unchecked hate speech can have serious effects, including real-world violence, social strife, and mental health difficulties among those targeted [1]. Traditional hate speech detection methods rely on machine learning algorithms that have been trained on labeled datasets. These methods, which include logistic regression, support vector machines, and neural networks, have demonstrated various levels of efficacy in detecting hate speech. However, they frequently struggle understanding the nuances and context inherent in human language. Sarcasm, irony, and context-specific interpretations, for example, are easily misinterpreted by conventional models, resulting in a high percentage of false positives and negatives. Furthermore, the constant growth of language, particularly in online environments, presents an ongoing challenge for these systems [2].

Quantum machine learning (QML) emerges as a possible alternative to traditional methodologies by exploiting quantum computing principles to improve data processing capabilities. QML can dramatically increase the accuracy and efficiency of hate content detection by leveraging quantum algorithms, which process information in fundamentally different ways than classical algorithms. This study covers the present landscape of QML applications in hate content identification, assessing their potential benefits over traditional methods and discussing recent breakthroughs in the field [3].

**Fundamentals of Quantum Machine Learning**

Quantum machine learning integrates quantum computing concepts and machine learning techniques, allowing for data processing and analysis in ways that classical computers cannot. The fundamental concepts of quantum machine learning are:

**Quantum Bits (Qubits)**

Quantum bits, or qubits, are the fundamental units of quantum information and are important to quantum computing. Qubits, unlike classical bits, can exist in several states at the same time due to the phenomena of superposition. This characteristic enables quantum computers to do several calculations simultaneously, significantly enhancing their processing capability. Furthermore, qubits can be entangled, which means that the state of one is inextricably linked to the state of another, regardless of distance. This entanglement allows for intricate correlations and interactions that classical systems cannot mimic [4].

**Quantum Algorithms**

Quantum algorithms use qubits' unique features to solve problems more efficiently than classical algorithms. Notable quantum algorithms for machine learning include:

• Quantum Support Vector Machines (QSVM) use quantum computing for better classification of high-dimensional data. It works by translating data points to a higher-dimensional space where linear separation becomes possible. This method can greatly minimize the computational complexity involved in training models on large datasets.

• Quantum Neural Networks (QNN) use quantum circuits to simulate neural network structures and encode sophisticated functions. These networks can learn from data more efficiently than standard neural networks, which could improve the performance of hate content identification algorithms.

**Quantum Data**

Quantum data is information that is designed to be processed using quantum algorithms. Unlike classical data, which is often organized in binary formats, quantum data can incorporate the principles of superposition and

entanglement, resulting in much faster training and inference tasks. For example, when analyzing hate speech across numerous formats—such as text, audio, and video—quantum algorithms can simultaneously examine multiple parts of the data, boosting the model's ability to detect nuanced hate speech. [5].

The use of quantum machine learning in hate content identification is still in its early stages, but preliminary research indicates great promise. Using quantum algorithms, researchers can better capture the complexity of language and context in hate speech detection. For example, QML can improve sentiment analysis by processing massive amounts of text data from social media sites, enabling for real-time detection of hazardous content. Furthermore, quantum algorithms can scan video transcripts and audio-visual features, broadening the scope of hate content detection beyond text-based inputs[6].

**Applications in Hate Content Identification**

**Traditional Approaches**

Classical machine learning approaches, such as logistic regression, support vector machines, and deep learning, are commonly employed to detect hate speech. However, they have limits in scalability and context awareness.

**Quantum Machine Learning Approaches**

Recent studies have begun to explore the application of QML in hate content identification:

- Social Media Posts: QML can scan text data from social media platforms such as Twitter, Facebook, and Instagram to uncover hate speech patterns that traditional approaches may miss. Quantum algorithms can improve sentiment analysis, allowing for more accurate classification of dangerous content. Hate speech can appear in videos through spoken language or visual clues, not just in print [7-9].
- Video Content: QML can detect hate speech by processing video transcripts and analyzing audio-visual attributes. Quantum Natural Language Processing (QNLP) can be used to transcribe and analyze spoken content, while quantum computer vision algorithms can evaluate visual aspects for context [10-13].
- **Multimodal Content**: The integration of text, audio, and visual data poses a unique difficulty. QML can provide multimodal analysis, allowing for the detection of hate speech in many formats and so offering a more full understanding of harmful content [14-16].

**Advantages of QML in Hate Content Identification**

**Increased Efficiency**: Quantum algorithms can handle large datasets faster than classical algorithms, enabling real-time detection.

**Improved Accuracy**: The capacity to detect complicated patterns in data can lead to more precise identification of hate content.

**Enhanced Feature Extraction**: QML can reveal hidden links in data, enhancing the model's comprehension of context and purpose.

**Challenges and Limitations**

Despite its potential, several challenges hinder the widespread adoption of QML in hate content identification:

- **Technical Limitations**: Quantum hardware is still in its infancy, and scalability remains a concern.
- **Data Availability**: The lack of large, labeled datasets for training QML models can impede progress.
- **Ethical Considerations**: The use of automated systems for content moderation raises ethical questions regarding bias and accountability.

**Future Directions**

Looking ahead, the future of quantum machine learning in hate content identification is set to make considerable advances, demanding a diversified approach to solve existing obstacles and maximize potential. Researchers should work on hybrid models that combine conventional machine learning techniques with quantum algorithms, using the benefits of both paradigms to improve performance in real-world applications. This combination could result in more robust systems capable of comprehending the nuances of language and context, which are important for effectively identifying hate speech. Furthermore, the development of comprehensive datasets specifically designed for hate speech detection will be critical; these datasets should include a wide range of linguistic expressions and cultural contexts to guarantee that models are trained on a variety of scenarios, hence boosting their generalisability.

Additionally, resolving ethical concerns about bias and responsibility in automated content moderation systems is critical. Creating transparent algorithms that can be examined and refined can assist to reduce bias and build user trust. Finally, multidisciplinary collaboration among computer scientists, linguists, ethicists, and social scientists will be required to develop comprehensive solutions that not only increase technology capabilities but also take into account the societal ramifications of using QML in content regulation. As the technology advances, continuing research and innovation will be critical to ensuring that quantum machine learning remains a viable tool for establishing safer online environments.

## CONCLUSION

Quantum machine learning shows great promise for improving hate content identification. Researchers can use quantum computing to construct more efficient and accurate models for combating online hate speech. Continued research and innovation in this area are required to fully achieve QML's potential in developing safer online environments.

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**RESEARCH ARTICLE**

# Bridging Tradition and Innovation : Closed Reduction Approaches in TMJ Dislocation

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**ABSTRACT**

Temporomandibular joint (TMJ) dislocation is a painful condition in which the mandibular condyle is displaced anteriorly beyond the articular eminence, preventing spontaneous return to the glenoid fossa. It commonly arises from trauma, excessive mouth opening, yawning, or iatrogenic factors, presenting with inability to close the mouth, drooling, pain, and speech difficulty. Closed reduction remains the first-line management due to its safety, minimally invasive nature, and high success rates. Conventional methods, including the Hippocratic, wrist pivot, extraoral, syringe, gag reflex, and unilateral reduction techniques, are well-established for acute cases. However, recurrent or chronic dislocations pose challenges due to muscular hyperactivity, fibrosis, or patient intolerance. Recent non-invasive adjunctive modalities—such as transcutaneous electrical nerve stimulation (TENS), therapeutic ultrasound, and low-level laser therapy (LLLT)—facilitate muscle relaxation, reduce pain, and improve the likelihood of successful reduction. This review synthesizes current evidence on conventional and advanced closed reduction strategies, emphasizing indications, clinical utility, advantages, and limitations. A comprehensive understanding of these approaches enables clinicians to tailor treatment to patient-

specific needs, optimize outcomes, minimize recurrence, and reserve surgical intervention for refractory cases [1–6].

**Keywords:** Temporomandibular Joint, Dislocation, Closed Reduction, TENS, Ultrasound, Low-Level Laser Therapy, Physiotherapy

## INTRODUCTION

The temporomandibular joint (TMJ) is a specialized synovial articulation crucial for mastication, speech, and facial expression. Its high mobility and anatomical complexity make it susceptible to displacement and dysfunction [1,2]. TMJ dislocation occurs when the mandibular condyle moves anteriorly beyond the articular eminence and cannot spontaneously return to the glenoid fossa. Clinically, patients present with an open-mouth posture, inability to occlude teeth, drooling, difficulty speaking, and acute discomfort [3,4]. Left untreated, dislocations can result in soft tissue trauma, articular degeneration, and chronic pain [5]. TMJ dislocations are classified as acute, chronic, or recurrent. Acute dislocations often result from trauma, excessive mouth opening, or dental procedures, whereas recurrent or chronic dislocations may involve neuromuscular dysfunction, connective tissue laxity, or anatomical variations [6,7]. Epidemiological data indicate a higher prevalence among women and elderly individuals, potentially due to ligamentous laxity and altered neuromuscular coordination [8]. Management strategies range from conservative to surgical. Closed reduction is the cornerstone due to its efficacy, minimal invasiveness, and accessibility [9]. Conventional techniques, including the Hippocratic, wrist pivot, extraoral, syringe, gag reflex, and unilateral methods, form the foundation of acute management. However, muscular hyperactivity, chronic fibrosis, or patient anxiety can limit the success of manual reduction [10,11]. Non-invasive adjunctive modalities—such as TENS, therapeutic ultrasound, and low-level laser therapy—have emerged to enhance reduction success by promoting muscle relaxation, reducing pain, and improving joint mobility [12–16]. This review critically evaluates conventional and advanced closed reduction approaches, highlighting their indications, clinical outcomes, advantages, limitations, and practical considerations for individualized patient care.

**Conventional Closed Reduction Techniques**

**Hippocratic Technique**

The Hippocratic method, also known as the bimanual intraoral technique, is the most commonly described approach for acute bilateral dislocations. The operator places gauze-covered thumbs on the mandibular molars while stabilizing the mandible externally. A downward and posterior force disengages the condyles from the articular eminence, allowing their return to the glenoid fossa. This technique is widely recognized for its simplicity and high success rate in cooperative patients. Operator thumb injury is a notable risk, which can be mitigated by protective devices [1,2]. (FIGURE-1A)

**Wrist Pivot Technique**

The wrist pivot method modifies the Hippocratic approach to improve ergonomics and safety. Fingers are placed on the molars intraorally while the thumbs rest externally under the chin. A pivoting motion of the wrists applies downward and posterior forces to reposition the condyles. This technique reduces operator fatigue and risk of injury but may be less effective in patients with severe masseteric spasm or long-standing dislocations [2,3]. (FIGURE-1B)

**Extraoral Technique**

The extraoral approach is useful for patients with poor oral hygiene, oral trauma, or pronounced gag reflex. The clinician applies pressure externally over the coronoid processes while stabilizing the mandible inferiorly. Advantages include improved patient comfort and avoidance of intraoral manipulation. However, it requires precise anatomical knowledge and is associated with lower success rates than intraoral methods [4].(FIGURE-1C AND D)

**Supine (Recumbent) Method**

This modification of the Hippocratic technique is performed with the patient in a supine position. Gravity assists in condylar relocation, and sedation or muscle relaxants enhance effectiveness. It is particularly suitable in hospital settings but less practical in outpatient or emergency care [5]. (FIGURE-1E)

**Syringe Technique**

The syringe method, described by Chen *et al*. (2009), is a patient-assisted approach. A 5–10 mL syringe is positioned between posterior teeth, and the patient rolls it back and forth, allowing the condyles to glide inferiorly and return to the fossa. The technique is safe, simple, and effective in acute non-traumatic dislocations but may fail in chronic cases with muscular fibrosis [6].(FIGURE-1F)

**Gag Reflex Method**

Stimulation of the posterior pharyngeal wall induces a gag reflex, relaxing the elevator muscles and facilitating reduction. While non-invasive and rapid, tolerance is limited, and results may be inconsistent [7].

**Unilateral Reduction**

In unilateral dislocations, ipsilateral manipulation minimizes stress on the contralateral joint. This approach is effective in isolated cases but requires skilled technique and patient cooperation [8,9].

**Advanced Adjunctive Techniques**

**Transcutaneous Electrical Nerve Stimulation (TENS)**

TENS therapy delivers low-voltage electrical currents via surface electrodes to the masticatory muscles. This stimulates sensory nerves and promotes endogenous endorphin release, reducing muscular hyperactivity and pain. The resulting relaxation facilitates condylar repositioning. TENS is non-invasive, safe, and well-tolerated, particularly in patients with acute or recurrent dislocations complicated by muscle spasm [12,13,21,24,26].(FIGURE-2A)

**Therapeutic Ultrasound**

Therapeutic ultrasound applies continuous or pulsed sound waves to the TMJ region, generating deep tissue heating that improves circulation, increases tissue extensibility, and reduces muscle spasm. Administered for 5–10 minutes before reduction, ultrasound enhances mandibular mobility and comfort. It is especially valuable in patients with chronic stiffness or post-traumatic muscular hyperactivity. Limitations include equipment requirements and operator training, restricting use in some settings [14,15,22,30].(FIGURE-2B)

**Low-Level Laser Therapy (LLLT)**

LLLT, or cold laser therapy, delivers low-intensity laser energy to the TMJ and surrounding musculature, producing analgesic, anti-inflammatory, and muscle-relaxing effects. Cellular metabolism is stimulated, inflammatory mediators are modulated, and tissue repair is promoted. Application before or during reduction reduces pain and facilitates muscle relaxation, enhancing success rates. LLLT is non-invasive and rapid, though protocol standardization and high-quality clinical evidence remain limited [9,10,23].(FIGURE-2C)

## DISCUSSION

Closed reduction remains the primary management strategy for TMJ dislocation due to its safety, minimal invasiveness, and high effectiveness. Conventional methods—Hippocratic, wrist pivot, extraoral, syringe, gag reflex, and unilateral techniques—have long-standing clinical support and remain indispensable in emergency and routine care. Their advantages include simplicity, accessibility, and minimal cost, while limitations include operator injury, patient discomfort, and reduced effectiveness in cases with chronic muscular hyperactivity or fibrosis [1–11]. Advanced non-invasive modalities such as TENS, therapeutic ultrasound, and LLLT enhance conventional approaches by addressing the underlying muscular resistance and pain. TENS improves muscle relaxation and

provides analgesia; ultrasound increases tissue extensibility and circulation; and LLLT modulates inflammation and facilitates muscle relaxation [12–16,21–24,26–30]. Together, these modalities improve the likelihood of successful reduction, patient comfort, and post-reduction function. Despite their benefits, widespread adoption of advanced techniques is limited by equipment availability, operator expertise, and the lack of standardized protocols. Comparative high-quality studies between conventional and adjunctive methods remain scarce, and cost considerations may restrict their use in resource-limited settings. Clinicians must therefore individualize management, balancing the urgency of reduction, patient-specific factors, and available resources. Acute, uncomplicated dislocations are often managed effectively with conventional techniques alone, while adjunctive modalities are most valuable for chronic, recurrent, or resistant cases. Surgical intervention remains a last resort when conservative strategies fail [25–29].

## CONCLUSION

TMJ dislocation is a distressing condition that requires prompt and effective management to restore function and alleviate discomfort. Conventional closed reduction techniques continue to serve as the mainstay due to their simplicity, accessibility, and high success rates. Advanced non-invasive modalities—including TENS, therapeutic ultrasound, and LLLT—enhance reduction success in recurrent or challenging cases by promoting muscle relaxation, reducing pain, and improving joint mobility. A comprehensive understanding of both conventional and advanced strategies allows clinicians to tailor management to individual patient needs, optimize outcomes, and minimize recurrence, reserving surgical intervention for refractory cases. Continued research is warranted to standardize adjunctive therapy protocols and validate long-term outcomes [1–30].

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**Fig 1: A-Hippocratic Technique, B-Wrist Pivot Technique, C-Extraoral Technique, D-Supine (Recumbent) Method, E-Syringe Technique**



**Fig 2: A-Transcutaneous Electrical Nerve Stimulation (TENS), B-Therapeutic Ultrasound, C-Low-Level Laser Therapy (LLLT)**

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RESEARCH ARTICLE

# On $\alpha$-Generalized Semi Closed Mappings in Pentapartitioned Neutrosophic Topological Space

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**ABSTRACT**

This paper introduces the concept of pentapartitioned neutrosophic $\alpha$-generalized semi-closed mappings in a pentapartitioned neutrosophic topological space and investigates several of their basic properties and characteristics.

**Keywords:** $\mathcal{P}n$set;$\mathcal{P}n_{\alpha gs}-\mathcal{CS}$;$\mathcal{P}n_{\alpha gs}-\mathcal{OS}$; $\mathcal{P}n_{\alpha gs}-\mathcal{C}.\mathcal{M}$;$\mathcal{P}n_{\alpha gs}$-$\mathcal{O}.\mathcal{M}$.

## INTRODUCTION

In 1999, Florentin Smarandache first proposed the idea of neutrosophic sets, which is a generalization of intuitionistic fuzzy sets by Atanassov. After that the pentapartitioned neutrosophic set and its characteristics are introduced by Rama Malik and Surapti Pramanik[4] in 2020. In the path of them, Suman Das and Binod Chandra Tripathy [10] introduce various sets namely Pentapartitioned Neutrosophic semi-open sets, $\alpha$-open sets, and pre-open sets in 2021.In the present work, we introduce the notion of pentapartitioned neutrosophic $\alpha$-generalized semiclosed mappings and explore several of their fundamental properties.

## PRELIMINARIES

**Definition 2.1**[4] Let $Q$ be a universal set. An object of the form $K = \{< q, T_K, C_K, I_K, U_K, F_K >: q \in Q\}$ is a $\mathcal{PnS}$ $K$ over $Q$ where $0 \leq T_K + C_K + I_K + U_K + F_K \leq 5$. Here, $T_K(q)$ is truth membership, $C_K(q)$ is contradiction membership, $I_K(q)$ isignorance membership, $U_K(q)$is unknown membership, $F_K(q)$ is false membership.

**Definition 2.2**[4] let Q be a universal set. Consider Two $\mathcal{Pn}$ sets $\mathrm{K} =< q, \mathrm{T_K}, \mathrm{C_K}, \mathrm{I_K}, \mathrm{U_K}, \mathrm{F_K} >$ and $\mathrm{L} =< q, \mathrm{T_L}, \mathrm{C_L}, \mathrm{I_L}, \mathrm{U_L}, \mathrm{F_L} >$.

i. $\mathrm{K} \subseteq \mathrm{L}$ iff $\mathrm{T_K} \leq \mathrm{T_L}$,$\mathrm{C_K} \leq \mathrm{C_L}$,$\mathrm{I_K} \geq \mathrm{I_L}$, $\mathrm{U_K} \geq \mathrm{U_L}$, and $\mathrm{F_K} \geq \mathrm{F_L}$.
ii. $K \cup L = \{< q, max(T_K, T_L), max(C_K, C_L), min(I_K, I_L), min(U_K, U_L), min(F_K, F_L) >\}$.
iii. $K \cap L = \{< q, min(T_K, T_L), min(C_K, C_L), max(I_K, I_L), max(U_K, U_L), max(F_K, F_L) >\}$.
*iv.* If $K = \{< q, T_K, C_K, I_K, U_K, F_K >: q \in Q\}$ then $K^C = \{< q, F_K, U_K, 1 - I_K, C_K, T_K >: q \in Q\}$.

**Definition 2.3**[4] A $\mathcal{Pn}$ set K over the universe Q is said to be null $\mathcal{PnS}$ if $0_{\mathcal{P}n} = \{< q, 0,0,1,1,1 >: q \in Q\}$.

**Definition 2.4**[4] A $\mathcal{Pn}$ set K over the universe Q is said to be absolute $\mathcal{PnS}$ if $1_{\mathcal{P}n} = \{< q, 1,1,0,0,0 >: q \in Q\}$.

**Definition 2.5**[10]Consider Q be the universal set. Then the collection $\Gamma$ of $\mathcal{PnS}$ over Q is known as a $\mathcal{PnT}$ on Q, if the following conditions are satisfied.

i. $0_{\mathcal{P}n}, 1_{\mathcal{P}n} \in \Gamma$
ii. $R_1, R_2 \in \Gamma$,then $R_1 \cap R_2 \in \Gamma$
iii. $\{\mathrm{R_i}: \mathrm{i} \in \mathrm{J}\} \in \Gamma$then $\cup_{\mathrm{i} \in \mathrm{J}} \mathrm{R_i} \in \Gamma$

A Pentapartitioned Neutrosophic Topological Space $(\mathcal{PnTS})$ is the term used to describe the pair $(Q, \Gamma)$. Every component of $\Gamma$ is called a pentapartitioned Neutrosophilic open set $(\mathcal{PnOS})$, $R^C$ is considered as a pentapartitioned Neutrosophic closed set $(\mathcal{PnCS})$ if $R \in \Gamma$.

**Definition 2.6**[4] Let $K$ be a $\mathcal{PnS}$in $\mathcal{PnTSQ}$.Then,

i. $\mathcal{P}nint(K) = \cup\{Y : Y\ is\ an\ (\mathcal{PnOS})in\ Q\ and\ Y \subseteq K\}$.
ii. $\mathcal{P}ncl(K) = \cap\{Z : Z\ is\ an\ (\mathcal{PnCS})in\ Q\ and\ K \subseteq Z\}$.

**Definition 2.7**[10] The following describes a $\mathcal{PnSK}$ of a $\mathcal{PnTSQ}$ as : (i) a pentapartitioned neutrosophic semi-open set $(\mathcal{PnS}\text{-}\mathcal{OS})$ if $K \subseteq \mathcal{P}ncl(\mathcal{P}nint(K))$ (ii) a pentapartitioned neutrosophic $\alpha$-open set $(\mathcal{Pn}_\alpha\mathcal{OS})$ if $K \subseteq \mathcal{P}nint(\mathcal{P}ncl(\mathcal{P}nint(K)))$ (iii) a Pentapartitioned neutrosophic pre-open set$(\mathcal{Pn}\text{-}\mathcal{POS})$ if $K \subseteq \mathcal{P}nint(\mathcal{P}ncl(K))$.

**Definition 2.8**(6) Let ƒbe a mapping from an $\mathcal{PnTS}(Q, \Gamma)$, into an $\mathcal{PnTS}(R, \Omega)$.Thenƒis said to be an

i. pentapartitioned neutrosophic$\alpha$-open mapping $(\mathcal{Pn}_\alpha\text{-}\mathcal{O}.\mathcal{M})$ifthe image of each$\mathcal{PnOS}$in$Q$is a$\mathcal{Pn}_\alpha$ $\mathcal{OS}$in $R$.
ii. pentapartitioned neutrosophic pre-open mapping$(\mathcal{PnP}\text{-}\mathcal{O}.\mathcal{M})$ if the image of each$\mathcal{PnOS}$in$Q$is a$\mathcal{PnP}\text{-}\mathcal{OS}$in$R$.

**Definition 2.9**(5)A $\mathcal{PnSK}$ of a$\mathcal{PnTS}$ in $Q$ is said to be a Pentapartitioned Neutrosophic $\alpha$-generlized semi closed set $(\mathcal{Pn}_{\alpha gs}\text{-}\mathcal{CS})$ if $\mathcal{Pn}_\alpha cl(K) \subseteq U$ whenever $K \subseteq U$ and $U$ is a $\mathcal{PnS}\text{-}\mathcal{OS}$ in $Q$.The complement $K^C$of a $\mathcal{Pn}_{\alpha gs}\text{-}\mathcal{CS}K$is a $\mathcal{Pn}_{\alpha gs}\text{-}\mathcal{OS}$ in $Q$.

**Definition 2.10**(3)A$\mathcal{PnTS}(Q, \Gamma)$ is said to be a Pentapartitioned Neutrosophic$\alpha ga\mathcal{T}_{1/2}(\mathcal{Pn}_{\alpha ga}\mathcal{T}_{1/2})$ space if every $\mathcal{Pn}_{\alpha gs}\text{-}\mathcal{CS}$ in $Q$ is a $\mathcal{PnCS}$ in$Q$.

## RESULTS AND DISCUSSION

**Pentapartitioned Neutrosophic $\alpha$-Generlized Semi Closed Mappings**

**Definition 3.1.1.**A mapping $f:(Q,\Gamma)\to(R,\Omega)$is called pentapartitioned neutrosophic $\alpha$-generlized semi closed mapping$(\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}.\mathcal{M})$ if for every$\mathcal{P}n\mathcal{C}\mathcal{S}K$ of $(Q,\Gamma)$,$f(K)$is a $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}\mathcal{S}$ in$(R,\Omega)$.

**Example3.1.2**.Let$Q=\{u_1,u_2\}$ and$R=\{v_1,v_2\}$.Then $\Gamma=\{0_{\mathcal{P}n},U_1,1_{\mathcal{P}n}\}$and $\Omega=\{0_{\mathcal{P}n},U_2,1_{\mathcal{P}n}\}$ are $\mathcal{P}n$ topologies on $Q$ and $R$ respectively, where$U_1=<q,(0.8,0.3,0.5,0.3,0.2),(0.9,0.4,0.5,0.4,0.1)>$ and $U_2=<r,(0.7,0.3,0.5,0.3,0.3),(0.8,0.4,0.5,0.4,0.2)>$.Define a mapping$f:(Q,\Gamma)\to(R,\Omega)$ by $f(u_1)=v_1$ and $f(u_2)=v_2$. Here the $\mathcal{P}n\mathcal{S}{U_1}^C=<q,(0.2,0.3,0.5,0.3,0.8),(0.1,0.4,0.5,0.4,0.9)>$ is a $\mathcal{P}n\mathcal{C}\mathcal{S}$ in $Q$, $f({U_1}^C)$ is a $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}\mathcal{S}$ in $R$.Therefore$f$ is a $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}.\mathcal{M}$.

**Definition 3.1.3**. A mapping $f:(Q,\Gamma)\to(R,\Omega)$ is called pentapartitioned neutrosophic $\alpha$-generlized semi open mapping$(\mathcal{P}n_{\alpha gs}\text{-}\mathcal{O}.\mathcal{M})$ if for every$\mathcal{P}n\mathcal{O}\mathcal{S}K$ of $(Q,\Gamma)$,$f(K)$ is a $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{O}\mathcal{S}$ in$(R,\Omega)$.

**Example3.1.4**.Let$Q=\{u_1,u_2\}$ and$R=\{v_1,v_2\}$.Then $\Gamma=\{0_{\mathcal{P}n},U_1,1_{\mathcal{P}n}\}$ and $\Omega=\{0_{\mathcal{P}n},U_2,1_{\mathcal{P}n}\}$ are $\mathcal{P}n$ topologies on $Q$ and $R$ respectively, where$U_1=<q,(0.9,0.3,0.5,0.3,0.1),(0.9,0.4,0.5,0.4,0.1)>$ and $U_2=<r,(0.6,0.3,0.5,0.3,0.3),(0.9,0.4,0.5,0.4,0.1)>$.Define a mapping$f:(Q,\Gamma)\to(R,\Omega)$ by $f(u_1)=v_1$ and $f(u_2)=v_2$. Here the $\mathcal{P}n\mathcal{S}U_1=<q,(0.9,0.3,0.5,0.3,0.1),(0.9,0.4,0.5,0.4,0.1)>$ is a $\mathcal{P}n\mathcal{O}\mathcal{S}$ in $Q$, $f(U_1)$ is a $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{O}\mathcal{S}$ in $R$.Therefore$f$ is a $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{O}.\mathcal{M}$.

**Theorem 3.1.5**. Every $\mathcal{P}n_{\alpha}\text{-}\mathcal{C}.\mathcal{M}$ is a $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}.\mathcal{M}$.

**Proof**: Let$f:(Q,\Gamma)\to(R,\Omega)$ be a$\mathcal{P}n_{\alpha}\text{-}\mathcal{C}.\mathcal{M}$. Let $K$ be a$\mathcal{P}n\mathcal{C}\mathcal{S}$ in $(Q,\Gamma)$.Then by hypothesis, $f(K)$ is a $\mathcal{P}n_{\alpha}\text{-}\mathcal{C}\mathcal{S}$ in$(R,\Omega)$.Since every$\mathcal{P}n_{\alpha}\text{-}\mathcal{C}\mathcal{S}$is a$\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}\mathcal{S}$,$f(K)$ is a $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}\mathcal{S}$ in $(R,\Omega)$. Hence$f$ is a $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}.\mathcal{M}$.

**Example 3.1.6**. $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}.\mathcal{M}$ is not $\mathcal{P}n_{\alpha}\text{-}\mathcal{C}.\mathcal{M}$.
Let$Q=\{u_1,u_2\}$ and$R=\{v_1,v_2\}$.Then $\Gamma=\{0_{\mathcal{P}n},U_1,1_{\mathcal{P}n}\}$ and $\Omega=\{0_{\mathcal{P}n},U_2,1_{\mathcal{P}n}\}$ are $\mathcal{P}n\mathcal{T}$on $Q$ and $R$ respectively, where$U_1=<q,(0.1,0.3,0.5,0.3,0.9),(0.2,0.4,0.5,0.4,0.8)>$ and$U_2=<r,(0.1,0.3,0.5,0.3,0.8),(0.3,0.4,0.5,0.4,0.6)>$. Define $f:(Q,\Gamma)\to(R,\Omega)$ by $f(u_1)=v_1$ and $f(u_2)=v_2$. Since the $\mathcal{P}n\mathcal{S}{U_1}^C=<q,(0.9,0.3,0.5,0.3,0.1),(0.8,0.4,0.5,0.4,0.2)>$ is a $\mathcal{P}n\mathcal{C}\mathcal{S}$ in $(Q,\Gamma)$, $f({U_1}^C)$ is a $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}\mathcal{S}$ but not $\mathcal{P}n_{\alpha}\text{-}\mathcal{C}\mathcal{S}$ in $(R,\Omega)$.Therefore$f$ is a $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}.\mathcal{M}$ but not $\mathcal{P}n_{\alpha}\text{-}\mathcal{C}.\mathcal{M}$.

**Remark 3.1.7.** $\mathcal{P}n\mathcal{P}\text{-}\mathcal{C}.\mathcal{M}$ and $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}.\mathcal{M}$ are independent of each other.

**Example 3.1.8**. $\mathcal{P}n\mathcal{P}\text{-}\mathcal{C}.\mathcal{M}$ is not $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}.\mathcal{M}$.
Let $Q=\{u_1,u_2\}$ and$R=\{v_1,v_2\}$.Then $\Gamma=\{0_{\mathcal{P}n},U_1,1_{\mathcal{P}n}\}$ and $\Omega=\{0_{\mathcal{P}n},U_2,1_{\mathcal{P}n}\}$ are $\mathcal{P}n\mathcal{T}$on $Q$ and $R$ respectively, where$U_1=<q,(0.7,0.2,0.5,0.2,0.2),(0.8,0.3,0.5,0.3,0.1)>$ and$U_2=<r,(0.4,0.2,0.5,0.2,0.6),(0.3,0.3,0.5,0.3,0.5)>$. Define $f:(Q,\Gamma)\to(R,\Omega)$ by $f(u_1)=v_1$ and $f(u_2)=v_2$. Since the $\mathcal{P}n\mathcal{S}{U_1}^C=<q,(0.2,0.2,0.5,0.2,0.7),(0.1,0.3,0.5,0.3,0.8)>$ is a $\mathcal{P}n\mathcal{C}\mathcal{S}$ in $(Q,\Gamma)$, $f({U_1}^C)$ is a $\mathcal{P}n\mathcal{P}\text{-}\mathcal{C}\mathcal{S}$but not $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}\mathcal{S}$in$(R,\Omega)$.Therefore$f$ is a $\mathcal{P}n\mathcal{P}\text{-}\mathcal{C}.\mathcal{M}$but not $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}.\mathcal{M}$.

**Example 3.1.9**. $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}.\mathcal{M}$ is not $\mathcal{P}n\mathcal{P}\text{-}\mathcal{C}.\mathcal{M}$.
Let $Q=\{u_1,u_2\}$ and$R=\{v_1,v_2\}$. Then $\Gamma=\{0_{\mathcal{P}n},U_1,1_{\mathcal{P}n}\}$ and $\Omega=\{0_{\mathcal{P}n},U_2,1_{\mathcal{P}n}\}$ are $\mathcal{P}n\mathcal{T}$on $Q$ and $R$ respectively, where $U_1=<q,(0.4,0.4,0.5,0.4,0.6),(0.5,0.2,0.5,0.2,0.5)>$ and$U_2=<r,(0.3,0.4,0.5,0.4,0.7),(0.4,0.2,0.5,0.2,0.6)>$.Define $f:(Q,\Gamma)\to(R,\Omega)$ by $f(u_1)=v_1$ and $f(u_2)=v_2$. Since the $\mathcal{P}n\mathcal{S}{U_1}^C=<q,(0.6,0.4,0.5,0.4,0.4),(0.5,0.2,0.5,0.2,0.5)>$ is a $\mathcal{P}n\mathcal{C}\mathcal{S}$ in $(Q,\Gamma)$, $f({U_1}^C)$ is a $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}\mathcal{S}$ but not $\mathcal{P}n\mathcal{P}\text{-}\mathcal{C}\mathcal{S}$in $(R,\Omega)$.Therefore$f$ is a $\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}.\mathcal{M}$ but not $\mathcal{P}n\mathcal{P}\text{-}\mathcal{C}.\mathcal{M}$.

**Theorem 3.1.10.**Let $f:(Q,\Gamma)\to(R,\Omega)$be a mapping and let $f(K)$be a $\mathcal{P}n\mathcal{R}-\mathcal{C}\mathcal{S}$in$(R,\Omega)$for every$\mathcal{P}n\mathcal{C}\mathcal{S}K$in$(Q,\Gamma)$. Then$f$is a$\mathcal{P}n_{\alpha gs}\text{-}\mathcal{C}.\mathcal{M}$.

**Proof:** Let $K$ be a $\mathcal{PnCS}$in$(Q,\Gamma)$.Then $f(K)$ is a $\mathcal{PnR}-\mathcal{CS}$ in $(R,\Omega)$.Since every $\mathcal{PnR}-\mathcal{CS}$ is a $\mathcal{Pn}_{\alpha gs}$-$\mathcal{CS}$,$f(K)$is a$\mathcal{Pn}_{\alpha gs}$-$\mathcal{CS}$ in$(R,\Omega)$.Hence$f$ is a$\mathcal{Pn}_{\alpha gs}$-$\mathcal{C}.\mathcal{M}$.

**Theorem 3.1.11.** Let $f:(Q,\Gamma)\to(R,\Omega)$be a mapping from a$\mathcal{PnTS}(Q,\Gamma)$intoa$\mathcal{PnTS}(R,\Omega)$.Then upcoming results are equivalent if$(R,\Omega)$is a$\mathcal{Pn}_{\alpha ga}\mathcal{T}_{1/2}$ space.

1. $f$ is a $\mathcal{Pn}_{\alpha gs}$-$\mathcal{O}.\mathcal{M}$.
2. If $K$is a $\mathcal{PnOS}$ in$(Q,\Gamma)$then$f(K)$is a$\mathcal{Pn}_{\alpha gs}$-$\mathcal{OS}$ in$(R,\Omega)$.
3. $f(\mathcal{P}nint(K))\subseteq\mathcal{P}nint(\mathcal{P}ncl(\mathcal{P}nint(f(K))))$ for every$\mathcal{PnS}K$in $(Q,\Gamma)$.

**Proof.** (1) ⇒ (2) is obviously true.
(2) ⇒ (3) Let$K$be any $\mathcal{PnS}$ in$(Q,\Gamma)$. Then$\mathcal{P}nint(K)$ is a$\mathcal{PnOS}$ in$(Q,\Gamma)$.Then $f(\mathcal{P}nint(K))$ is a $\mathcal{Pn}_{\alpha gs}$-$\mathcal{OS}$in$(R,\Omega)$. Since$(R,\Omega)$ is a $\mathcal{Pn}_{\alpha ga}\mathcal{T}_{1/2}$ space,$f(\mathcal{P}nint(K))$is a $\mathcal{PnOS}$ in$(R,\Omega)$.Therefore$f\big(\mathcal{P}nint(K)\big)=\mathcal{P}nint(f(\mathcal{P}nint(K)))\subseteq\mathcal{P}nint(\mathcal{P}ncl(\mathcal{P}nint(f(K))))$.
(3)⇒(1) Let$K$be any$\mathcal{PnOS}$ in$(Q,\Gamma)$.By hypothesis,$f(\mathcal{P}nint(K))\subseteq\mathcal{P}nint(\mathcal{P}ncl(\mathcal{P}nint(f(K))))$. This implies that $f(K)\subseteq\mathcal{P}nint(\mathcal{P}ncl(\mathcal{P}nint(f(K))))$. Hence $f(K)$ is a $\mathcal{Pn}_{\alpha}\mathcal{OS}$ in$(R,\Omega)$.Since every $\mathcal{Pn}_{\alpha}\mathcal{OS}$ is a $\mathcal{Pn}_{\alpha gs}$-$\mathcal{OS}$,$f(K)$ is a $\mathcal{Pn}_{\alpha gs}$-$\mathcal{OS}$in $(Q,\Gamma)$. Hence $f$ is a $\mathcal{Pn}_{\alpha gs}$-$\mathcal{O}.\mathcal{M}$.

**Theorem 3.1.12.** Let $f:(Q,\Gamma)\to(R,\Omega)$be a mapping.Then following results are equivalence if $(R,\Omega)$is a $\mathcal{Pn}_{\alpha ga}\mathcal{T}_{1/2}$ space.

1. $f$ is a $\mathcal{Pn}_{\alpha gs}$-$\mathcal{C}.\mathcal{M}$.
2. $f(\mathcal{P}nint(K))\subseteq\mathcal{Pn}_{\alpha}int(f(K))$for each$\mathcal{PnS}$ $K$of$(Q,\Gamma)$.
3. $\mathcal{P}nint(f^{-1}(L))\subseteq f^{-1}(\mathcal{Pn}_{\alpha}int(L))$ for every$\mathcal{PnS}L$of$(R,\Omega)$.

**Proof.** (1) ⇒ (2)Let $f$be a $\mathcal{Pn}_{\alpha gs}$-$\mathcal{C}.\mathcal{M}$.Let$K$be any $\mathcal{PnS}$ in$(Q,\Gamma)$. Then$\mathcal{P}nint(K)$ is a$\mathcal{PnOS}$ in$(Q,\Gamma)$.By hypothesis, $f(\mathcal{P}nint(K))$ is a $\mathcal{Pn}_{\alpha gs}$-$\mathcal{OS}$in$(R,\Omega)$. Since$(R,\Omega)$ is a $\mathcal{Pn}_{\alpha ga}\mathcal{T}_{1/2}$ space,$f(\mathcal{P}nint(K))$ is a $\mathcal{PnOS}$ in$(R,\Omega)$.We know that every$\mathcal{PnOS}$ is a $\mathcal{Pn}_{\alpha}$-$\mathcal{OS}$ ,$f(\mathcal{P}nint(K))$is a $\mathcal{Pn}_{\alpha}$-$\mathcal{OS}$in$(R,\Omega)$. Therefore$\mathcal{Pn}_{\alpha}int(f\big(\mathcal{P}nint(K)\big))=f\big(\mathcal{P}nint(K)\big)$.Hence$f\big(\mathcal{P}nint(K)\big)=\mathcal{Pn}_{\alpha}int(f(\mathcal{P}nint(K)))\subseteq\mathcal{Pn}_{\alpha}int\big(f(K)\big)$.
(2) ⇒ (3) Let$L$be any $\mathcal{PnS}$ in$(R,\Omega)$.Then$f^{-1}(L)$ is a$\mathcal{PnS}$ in$(Q,\Gamma)$. By hypothesis,$f(\mathcal{P}nint\big(f^{-1}(L)\big)\subseteq\mathcal{Pn}_{\alpha}int(f(f^{-1}(K)))\subseteq\mathcal{Pn}_{\alpha}int(L)$.Therefore$\mathcal{P}nint\big(f^{-1}(L)\big)\subseteq f^{-1}\big(\mathcal{Pn}_{\alpha}int(L)\big)$.
(3)⇒(1) Let us assume that $K$ be any$\mathcal{PnOS}$in$(Q,\Gamma)$.Then$\mathcal{P}nint(K)=K$and $f(K)$is a$\mathcal{PnOS}$ in$(R,\Omega)$. Then by hypothesis, $\mathcal{P}nint(f^{-1}(f(K)))\subseteq f^{-1}(\mathcal{Pn}_{\alpha}int(f(K)))$.Now $K=\mathcal{P}nint(K)\subseteq\mathcal{P}nint(f^{-1}\big(f(K)\big)\subseteq f^{-1}\left(\mathcal{Pn}_{\alpha}int\big(f(K)\big)\right)$.Therefore$f(K)\subseteq f(f^{-1}(\mathcal{Pn}_{\alpha}int(f(K))\subseteq\mathcal{Pn}_{\alpha}int(f(K))\subseteq f(K)$.Thus $\mathcal{Pn}_{\alpha}int(f(K))=f(K)$is a$\mathcal{Pn}_{\alpha gs}$-$\mathcal{OS}$in $(R,\Omega)$.Therefore $f$is a $\mathcal{Pn}_{\alpha gs}$-$\mathcal{C}.\mathcal{M}$.

**Theorem 3.1.13**Let$f:(Q,\Gamma)\to(R,\Omega)$ be a mapping. Then the following results are equivalence if $(R,\Omega)$ is a $\mathcal{Pn}_{\alpha ga}\mathcal{T}_{1/2}$ space.

1. 1.$f$ is a $\mathcal{Pn}_{\alpha gs}$-$\mathcal{C}.\mathcal{M}$.
2. 2.$\mathcal{P}ncl(\mathcal{P}nint(\mathcal{P}ncl(f(K))))\subseteq f(\mathcal{P}ncl(K))$ for every$\mathcal{PnS}K$in $(Q,\Gamma)$.

**Proof.** (1) ⇒ (2)Let$K$be any $\mathcal{PnS}$ in$(Q,\Gamma)$. Then$\mathcal{P}ncl(K)$ is a$\mathcal{PnCS}$ in$(Q,\Gamma)$.By hypothesis, $f(\mathcal{P}ncl(K))$ is a $\mathcal{Pn}_{\alpha gs}$-$\mathcal{CS}$in$(R,\Omega)$. Since$(R,\Omega)$ is a $\mathcal{Pn}_{\alpha ga}\mathcal{T}_{1/2}$ space,$f(\mathcal{P}ncl(K))$ is a $\mathcal{PnCS}$ in$(R,\Omega)$. Therefore $\mathcal{P}ncl(f\big(\mathcal{P}ncl(K)\big))=f\big(\mathcal{P}ncl(K)\big)$. Now clearly.$\mathcal{P}ncl(\mathcal{P}nint(\mathcal{P}ncl(f(K))))\subseteq\mathcal{P}ncl(f(\mathcal{P}ncl(K)))=f\big(\mathcal{P}ncl(K)\big)$. Hence$\mathcal{P}ncl(\mathcal{P}nint(\mathcal{P}ncl(f(K))))\subseteq f(\mathcal{P}ncl(K))$.
(2)⇒(1) Let$K$be any$\mathcal{PnCS}$ in$(Q,\Gamma)$.. By hypothesis,$\mathcal{P}ncl(\mathcal{P}nint(\mathcal{P}ncl(f(K))))\subseteq f\big(\mathcal{P}ncl(K)\big)=f(K)$. This implies$f(K)$ is a $\mathcal{Pn}_{\alpha}\mathcal{CS}$ in$(R,\Omega)$ and hence$f(K)$ is a $\mathcal{Pn}_{\alpha gs}$-$\mathcal{CS}$ in$(R,\Omega)$. That is$f$ is a $\mathcal{Pn}_{\alpha gs}$-$\mathcal{C}.\mathcal{M}$.

## CONCLUSION

This work introduce and examines $\mathcal{P}n_{\alpha gs}$-closed mappings and explores their properties. Further research may focus on extending these notions to $\mathcal{P}n_{\alpha gs}$-homeomorphism.

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RESEARCH ARTICLE

# Diuron Induced Changes in the Haematological and Biochemical Composition of Freshwater Climbing Perch, *Anabas testudineus*

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## ABSTRACT

The present investigation was undertaken to determine the toxic effects of sub- lethal concentrations of the herbicide diuron on haematological and biochemical parameters of freshwater fish, *Anabas testudineus*. Regardless of sex, the uniformly sized fishes were randomly divided into 6 groups (11.25 ± 1.07 cm of total length, 32.4 ± 1.42 g of weight) and exposed to diuron at concentration 0.5 ppm,1 ppm,1.5 ppm and 2 ppm along with control. The biochemical constituents such as protein, lipids, glycogen, carbohydrates and cholesterol were analyzed from muscle and liver of the fish using standard procedures. As the concentration of diuron increased, the glycogen concentration in liver and muscle tissues showed a decreasing trend from the control value. Additionally, lipid concentrations in the liver and muscle tissues of diuron exposed to varying concentrations of diuron showed significant decrease compared to control fish whereas protein concentrations in both tissues also showed a declining trend as herbicide concentration increased. This study demonstrated that the metabolic characteristics of fresh water fish, *Anabas testudineus* were markedly altered by sublethal concentrations of diuron. Therefore, the significant alterations in these biochemical markers suggested some important and potential aftereffects of diuron pollution in freshwater ecosystems.

**Keywords:** Herbicide, *Anabas testudineus*, Diuron, Sub-lethal effects, Biochemical marker

## INTRODUCTION

Water quality in rivers, lakes, and marine habitats is under risk due to the escalated use of fertilizer and pesticides brought on by the growth and intensification of agriculture [1]. Overabundance of nutrients from agricultural runoff can cause eutrophication, which lowers oxygen levels in water bodies and results in algal blooms. Aquatic animals and ecosystems may be impacted by habitat fragmentation and destruction brought on by agricultural practices. Both developed and underdeveloped countries are significantly impacted by agricultural practices. However, in developing nations, the importance of these effects is more noticeable. The primary source of pesticide deposition in aquatic ecosystems is agricultural runoff close to water bodies. By upsetting ecological relationships between organisms and causing biodiversity loss, bioaccumulations of these pesticides endanger long-term survival of fishes [2,3,4]. Prolonged exposure to pesticides causes physiological disturbance, behavioral changes, histopathological damages, haematological changes, biochemical alterations, hormone disruption, immunological suppression, reproductive abnormalities and cancer. The likelihood that pesticides will get into surface and ground water is increased by irrigation. Additionally, pesticides have the ability to modify phytoplankton communities, which can impact ecosystem function and water quality. Pesticides have the ability to modify food webs, which can impact the structure and function of ecosystems. Diuron is a typical pesticide used in Kuttanad's agricultural fields to manage weeds. It prevents weed seeds from sprouting, preventing weeds from establishing themselves before crops are sown. Diuron strongly inhibits the plant's photosynthesis II system through the Hill reaction. For some crops, diuron can be used as a defoliant to facilitate harvesting. Diuron is persistent in sediment and soils, with a DT50 of 90 to 180 days [5]. It will stay active in soil for four to eight months when used normally [6]. Ingestion, dermal absorption, or inhalation can all result in sickness. Canals, reservoirs, wetlands, swamps, and estuaries are the main habitats of the climbing perch, *Anabas testudineus*. It is a potamodromous fish species that can live in both fresh and brackish water. The labyrinth organ, an additional air-breathing organ, allows *Anabas testudineus* to withstand harsh aquatic circumstances like low oxygen levels and dirty water. The diuron contamination of waterbodies can result in fish mortality that results in the raising of the concentrations of chemicals in edible fish tissue or reduces fish production capability, both of which can harm consumer health. Because of the persistent nature of pesticides in aquatic environments, bioaccumulation has become an increasingly significant issue for pesticides in recent years, posing a substantial risk to the ecosystem as a whole, including humans. Pesticide pollutants directly damage vital organs such the gills, liver, heart, kidney, and gut and are directly linked to the food chain and water body contamination, it is crucial to assess the harmful consequences of these pollutants on fish [7].Therefore, the present study was undertaken to evaluate the toxic effects of diuron on the haematological parameters and the biochemical profile of the *Anabas testudineus*.

## MATERIALS AND METHODS

The freshwater fish *Anabas testudineus* (Bloch, 1792), commonly called climbing perch and locally known in Kerala as Kallelmutti, was used as the test organism. Healthy adult fishes of uniform size (mean weight 32.4 g; mean length 11.25 cm) were obtained from Aquarius Farm Products. The fishes were disinfected in 0.1% potassium permanganate for two minutes and acclimatized for one week in aerated, dechlorinated tap water. They were fed commercial pellets twice daily, and waste materials were removed regularly. Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] was used as the toxicant. A 100 mg $L^{-1}$ stock solution was prepared and diluted to obtain required concentrations. Chronic exposure experiments were conducted in glass aquaria containing 300 L solution. Based on range-finding and $LC_{50}$ determination, four concentrations (0.5, 1.0, 1.5 and 2.0 ppm) along with a control were maintained. Mortality was recorded at 24, 48, 72 and 96 h and $LC_{50}$ was calculated using probit analysis [8]. One-fourth of the 96-h $LC_{50}$ was taken as the sublethal concentration for further study. Thirty fishes were introduced into each aquarium and exposed for 14 days. Seventy-five percent of the medium was renewed twice with fresh solution, and fishes were fed daily. Behavioural changes such as colour variation, opercular movement, abnormal swimming, mucus secretion and loss of equilibrium were recorded. The experiment was performed in five replicates and a control without diuron was maintained under identical conditions. At the end of exposure, liver and muscle tissues were collected for

biochemical analysis. Total protein, lipid, glycogen and carbohydrate were estimated by standard methods [9,10,11,12]. Data were analyzed using SPSS software 20, and significance between control and treated groups was tested by ANOVA and paired *t*-test.

## RESULTS AND DISCUSSION

The mortality rates were recorded at 24 hours, 48 hours, 72 hours, and 96 hours. At 24 hrs., the percentage mortality observed at different concentrations, 0.5 ppm,1ppm,1.5ppm and 2ppm, was 5%, 9%,14% and 44% respectively. At 96 hrs., the percentage mortality observed at different concentrations 0.5 ppm,1ppm,1.5ppm and 2ppm was 30%, 58%, 75% and100% respectively. The safe and sub-lethal concentration of diuron treated *Anabas testudineus* was found to be 0.47 and 0.83 ppm. With increasing pesticide concentrations and time duration, mortality increased gradually. The control group showed zero percent mortality. The morphological and behavioural studies show a dose-dependent response in *Anabas testudineus* to diuron exposure (Table I). As the concentration of the herbicide increased, morphological and behavioural changes became severe such as fatigue, excessive mucus secretion and sluggishness. The visual observation indicates that diuron has significant toxic effects on fish physiology and morphological and behaviour even at low concentrations. The above behavioural results were similar to the study of [13] for cadmium toxicity in *Anabas testudineus*. The Table II indicates the impact of elevating diuron concentration (0.5 to 2.0 ppm) on different biochemical constituents (carbohydrate, glucose, cholesterol, lipids and protein) of muscle and liver tissues of *Anabas testudines*. A noticeable dose dependent reduction is seen in almost all observed parameters. The carbohydrate content is observed in both tissues gradually declined with elevating diuron concentration. In liver, it was observed as 11±0.9 mg/g in control and 5.5±1.8 mg/g at 2 ppm where as in muscle, 9.8±0.2 mg/g in control and 3.8±1.2 mg/g in 2 ppm respectively. The decline shows that the body uses more carbohydrates as fuel while under dangerous stress, using up stored reserves to satisfy higher metabolic demands. In the case of glycogen, a continuous decline was noticed in both tissues. In liver, it was observed as 7.2±0.2 mg/g in control and 3.9±0.5 mg/g in 2 ppm and in muscle, 5.6±1.2 mg/g in control and 1.6±1.5 mg/g in 2 ppm. Numerous studies have documented the decrease in muscle and liver glycogen in aquatic animals as a result of toxic stress [14,15]. Stress-induced glycogenolysis causes the principal energy store, glycogen, to be depleted, releasing glucose to meet the energy needs of survival and detoxification. The protein content was also showed a marked decline with increasing exposure of diuron. In liver, it was noted as 14±1.4 mg/g in control and 5.2±1.5 mg/g in 2 ppm.

In muscle, it was observed as 18.1±2.1 mg/g in control and 10.1±1.8 mg/g in 2 ppm. [16] found that liver damage in *Astyanax altiparanae* results in significant drop in total protein and albumin levels in liver tissues and blood plasma. Proteolysis, in which proteins are broken down into amino acids and used for tissue repair and energy production under adverse conditions, is indicated by the decrease in protein. The lipid content is found to be decreased in both tissues of diuron treated *Anabas.* In liver, it declined from 13.6± 0.8 mg/g in control group to 7.1±1.5 mg/g in high concentrations whereas in muscle it was 10.4±1.2 mg/g in control to 5.3±1.0 mg/g at high concentration. Cholesterol content also shows the same trend. When carbohydrate levels are no longer sufficient, fat mobilization causes the depletion of lipids. Since cholesterol is a crucial structural element of membranes and the generation of steroids, a decrease in cholesterol indicates liver metabolic malfunction and damage to cell membranes. Similarly, [17] observed sublethal concentrations of diuron showed a marked decrease in total lipid and cholesterol levels in liver and blood of *Channa punctatus*. The two-way analysis of variance (ANOVA) showed a highly significant ($P<0.05$) decrease in glycogen, protein, carbohydrate cholesterol and lipid concentration of diuron exposed fishes with respect to control. Paired t-test of all parameters also showed significant variation (P value<0.05) of diuron concentration between control and test groups. Haematological parameters of *Anabas testudineus* (Table III) revealed a remarkable decline in red blood cell (RBC) count was recorded as $4.8 \times 10^6$ cells/mm$^3$(control), significantly decreased to $3.5 \times 10^5$ cells/mm$^3$ in the test group. Similarly, white blood cell (WBC) count was dropped from 1,94,800 thousand cells/mm$^3$(control) to 1,66,300 thousand cells/mm$^3$ in the treated group. Furthermore, the haemoglobin content decreased from 7 gm% in the control to 5 gm% in the exposed group. These results indicate that exposure to sub-lethal levels of diuron adversely affects the haematological parameters of *Anabas testudineus*, leading to reductions in RBC count, WBC

count, and haemoglobin levels. According to [18], decrease in erythrocyte count and rise in leucocyte count in *Prochilodus lineatus* are probably due to oxidative damage and immunological activation. Carbohydrates, glycogen, protein, lipids and cholesterol are gradually depleted in both liver and muscle tissues as toxicant concentration rises. The liver is the main target organ since it exhibits a larger decline than muscle. Under toxic exposure, these biochemical changes show significant metabolic stress, elevated energy demand and compromised physiological functioning in the fish.

## CONCLUSION

According to the study, increased utilization of energy molecules in *Anabas testudineus* to meet energy requirements for the detoxification process is the cause of the changes in glycogen, total lipid, carbohydrate, cholesterol and lipid concentration that were detected in response to diuron. It has also been noted that even at low concentrations, sublethal exposure causes physiological and morphological changes. These alterations show the stress response and disturbance of metabolic processes, which may eventually hinder survival, development, and reproduction.

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**Table 1: Morphological and behavioural changes in *Anabas testudineus* exposed to various concentrations of Diuron**

| Morphological and behavioural changes | Control | 0.5 ppm | 1.0 ppm | 1.5 ppm | 2.0 ppm |
|---|---|---|---|---|---|
| Colour Changes | - | * | * | + | ++ |
| Opercular activity | - | - | * | + | ++ |
| Bottom to surface movement | - | * | + | ++ | +++ |
| Resting at Bottom | - | - | * | ++ | +++ |
| Mucous Secretion | - | * | + | ++ | +++ |
| Loss of Equilibrium | - | * | + | ++ | +++ |

(-) Nil, (*) Normal, (++) Less Change, (+++) Prominent Change

**Table 2: Biochemical profile of *Anabas testudineus* exposed to various concentrations of diuron** (Values are expressed as Mean ±SE of five individual observations)

| Biochemical Profile (mg/g) | Tissue | Treatments | | | | |
|---|---|---|---|---|---|---|
| | | Control | 0.5 ppm | 1.0 ppm | 1.5 ppm | 2.0 ppm |
| Carbohydrate | Liver | 11±0.9 | 9.2±1.5 | 7.4±1.0 | 6.1±0.8 | 5.5±1.8 |
| | Muscle | 9.8±0.2 | 6.4±0.5 | 5.2±0.8 | 4.7±0.9 | 3.8±1.2 |
| Glycogen | Liver | 7.2±0.2 | 6.8±0.8 | 5.7±1.0 | 4.1±0.9 | 3.9±0.5 |
| | Muscle | 5.6±1.2 | 4.2±2.2 | 3.8±2.1 | 2.2±1.8 | 1.6±1.5 |
| Protein | Liver | 14±1.4 | 12.8±1.8 | 10.5±1.5 | 8.6±1.2 | 5.2±1.5 |
| | Muscle | 18.1±2.1 | 17.9±2.4 | 15.3±2.0 | 12.4±2.8 | 10.1±1.8 |
| Lipid | Liver | 13.6± 0.8 | 12.6±2.0 | 10.5±1.7 | 9.5±0.6 | 7.1±1.5 |
| | Muscle | 10.4±1.2 | 9.1±1.8 | 8.4±0.2 | 7.6±0.9 | 5.3±1.0 |
| Cholesterol | Liver | 8.5±2.5 | 7.9±2.8 | 5.9±2.9 | 4.7±1.8 | 3.6±1.5 |
| | Muscle | 6.8 | 5.7±1.2 | 4.2±2.2 | 3.4±0.9 | 2.4±1.2 |

**Table 3: Haematological parameters of *Anabas testudineus* exposed to sublethal concentration of diuron**

| Parameters | Control | Test |
|---|---|---|
| RBC (million cells/mm$^3$) | $4.8 \times 10^6$ | $3.5 \times 10^5$ |
| WBC (thousand cells/mm$^3$) | 194800 | 166300 |
| Haemoglobin (gm%) | 7 | 5 |

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RESEARCH ARTICLE

# AI in Cyber Security Using Data Loss Prevention (DLP) System

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**ABSTRACT**

Data Loss Prevention (DLP) technologies are foundational controls for reducing the risk of unauthorized disclosure of sensitive data across endpoints, networks, and cloud services. However, traditional DLP systems—largely rule- and signature-driven—struggle with evasive exfiltration tactics, encrypted traffic, and the velocity/variety of enterprise data. This article proposes an AI-augmented DLP framework that combines representation learning for content understanding, behavioral modeling for insider-risk detection, and policy optimization for adaptive responses. We formulate the problem, describe an end-to-end system architecture, define evaluation metrics, and discuss deployment considerations including privacy, explainability, and governance. A reference pipeline and experiment design are provided to help practitioners reproduce and benchmark results.

**Keywords:-**Data Loss Prevention, Insider Risk, Exfiltration, Natural Language Processing, Representation Learning, Behavioral Analytics, Explainable AI.

## INTRODUCTION

Organizations face persistent risks of data exfiltration from external threats and insider actions, whether malicious or inadvertent. DLP systems monitor data at rest, in motion, and in use to prevent sensitive information from leaving trusted boundaries. Classic DLP depends on pattern matching (e.g., regular expressions for credit card or national IDs), fingerprints of known sensitive documents, and file-type controls. While effective for known patterns and governed repositories, such approaches struggle with:

- Context-dependent sensitivity (e.g., project code names, research drafts).
- Evasive exfiltration (e.g., steganography, homomorphic obfuscation, exfil via screenshots or screen recordings).
- Cloud/SaaS sprawl with bring-your-own-apps and shadow IT.
- High false positives that desensitize users and security teams.

AI can enhance DLP by learning content and context, modeling user and machine behavior, and recommending risk-aware responses. We present an AI-enabled DLP blueprint and practical guidance to implement, evaluate, and operate it at scale.

**Background and Related Concepts**

**DLP Scope**
- Data in Motion: Network egress (email, web uploads, APIs, messaging).
- Data at Rest: File shares, endpoints, cloud storage (S3/Blob/GCS), SaaS repositories.
- Data in Use: Clipboard, print, screen capture, peripheral devices, local transforms.

**Threat Models**
- Malicious insiders: Privileged engineers or contractorsexfiltrating source code or customer data.
- Careless insiders: Accidental sharing to personal email or misaddressed messages.
- External attackers: Using compromised accounts or implants to stage and exfiltrate data.
- Advanced evasion: Compression, encryption, chunked exfiltration, living-off-the-land tools (e.g., curl, netcat), covert channels (DNS, SaaS notes).

**Limits of Traditional DLP**
- Reliance on brittle rules.
- Inability to generalize to novel sensitive concepts.
- Limited visibility into user intent or context.

**AI-Augmented DLP: System Overview**
**Design Objectives**
1. Content understanding: Learn semantic representations of text, code, tables, and images to detect sensitivity beyond regexes.
2. Behavioral context: Model sequences of user/device actions to infer intent and anomalies.
3. Adaptive control: Optimize policy actions (block, coach, quarantine, escalate) based on predicted risk and business impact.
4. Explain ability: Provide human-interpretable rationales to analysts and end-users.
5. Privacy-preserving: Minimize data exposure via on-device inference, redaction, and federated learning where feasible.

**Reference Architecture**

**Sensors/Collectors**
- Endpoint agents: file I/O, clipboard, print, screen, removable media, IDE plugins for code artifacts.
- Network taps/proxies: SMTP/HTTP(S) via SSL inspection where lawful; CASB/SSE integrations for SaaS.
- Cloud connectors: APIs for storage/SaaS (M365, Google Workspace, Salesforce, Git, Jira).

**Data Pipeline**
- Normalization (PCAP to flow records, file parsing, OCR for images/PDFs).
- Content extraction (text, code, tables, images), PII/PHI redaction for training logs.
- Feature store keyed by entity (user, device, repo, project).

**AI Services**

- Sensitivity classifier: Multimodal encoder for text/code/images → sensitive/non-sensitive labels + category (e.g., customer data, financials, trade secrets).
- Anomaly & intent model: Time-series model over sequences (e.g., uploads per hour, destination reputation, app novelty).
- Policy optimizer: Contextual bandit or constrained RL to recommend actions, honoring compliance constraints.
- Explainability: SHAP/LIME, exemplar retrieval, attention heatmaps, and rule backstops.

**Policy Engine**

- Combines AI risk score with mandatory policies (e.g., block PCI DSS to external domains).
- Action set: allow, coach (just-in-time banner), encrypt, auto-label, quarantine, block, escalate to SOC.

**SOC and Governance**

- Case management, playbooks (SOAR), feedback loop for active learning, audit trails.

## METHODOLOGY

**Data and Labeling**

- Sources: Historical DLP incidents, red-team simulations, synthetic generation of sensitive/non-sensitive corpora (balanced for text, code, tabular, images).
- Label Taxonomy: {Public, Internal, Confidential, Restricted} × {PII, PHI, Finance, Source Code, Legal, Research, Client Data}.
- Weak Supervision: Distant labels from regexes and dictionaries; human-verified subsets to correct biases.
- De-identification: Redact PII/PHI in training logs; hash identifiers; store only features where possible.

**Models**

- Text & Code: Transformer encoders (document-level) with domain-adaptive pretraining on internal corpus; optional code-aware encoders for repositories.
- Images/PDFs: OCR + vision transformer for screenshots/scan exports; table structure recognition.
- Behavioral Modeling: Sequence models (GRU/Transformer) on user-session features (e.g., data volume deltas, destination novelty, off-hours activity).
- Fusion: Concatenate content and behavior embeddings; train a calibrated classifier (e.g., gradient-boosted trees on embeddings) to predict risk (\hat{y}\in[0,1]).

**Policy Optimization**

- Objective: Maximize prevented loss while minimizing disruption.
- Formulation: Contextual bandit with constraints (legal/policy hard rules). Reward approximates prevented exfiltration minus cost of false positives.
- Safe Exploration: Offline policy evaluation (counterfactual estimation) before production rollout.

**Explainability and Analyst UX**

- Local explanations: Top n tokens/spans contributing to sensitivity; example similar incidents.
- Global dashboards: Drift detection for features and label distribution; false-positive heatmaps by policy.

## EVALUATION

**Metrics**

For classification (sensitive vs. not):

- Precision, Recall, F1; area under PR curve (AUPRC) preferred for imbalanced data.

- Business-weighted F1: weight by asset criticality (e.g., Restricted=3, Confidential=2, Internal=1).

**For anomaly/intent detection:**
- Precision@k for top alerts per day.
- Time-to-detect (TTD) and time-to-respond (TTR).
- Analyst effort: alerts per analyst hour and resolved-to-escalated ratio.

**For policy efficacy:**
- Block accuracy (true positive blocks ÷ total blocks).
- User friction index (fraction of workflows interrupted).
- Data-loss averted (estimated, via baselines or tabletop simulations).

**Baselines**
- Regex/dictionary only.
- Document fingerprinting (exact/near-duplicate).
- Vendor default policies.

**Experimental Design**
- Data split: Temporal split to simulate future drift (train on months 1–6; validate on 7; test on 8–9).
- Ablations: (a) content only, (b) behavior only, (c) fusion, (d) fusion + policy optimizer.
- Offline evaluation: For actions, use logged propensities and inverse-propensity scoring where available.
- Pilot rollout: Shadow mode → coach-only → selective block with human-in-the-loop.

## RESULTS & DISCUSSIONS FOR THE ABOVE ILLUSTRATION

**On a mixed enterprise dataset (≈30M events, 120k labeled incidents):**
- Fusion model improved AUPRC by ~21% over content-only; false positives reduced by ~35% at iso-recall.
- Behavioral sequence features contributed most to early detection of low-and-slow exfiltration (chunked uploads).
- Policy optimizer reduced unnecessary blocks by ~18% while maintaining recall, improving user satisfaction scores in post-event surveys.

**Error Modes**
- Ambiguous projects: New codenames not in dictionaries; mitigate via few-shot labeling and retrieval-augmented encoders.
- Image-only exfiltration: Screenshots of terminals or tables; mitigate with OCR+vision and on-device scanning for privacy.
- Encryption and tunnels: TLS/SSH/ZIP with passwords; rely on metadata/behavior, DLP on endpoints pre-encryption, and egress controls.

**Privacy and Compliance**
- Minimize content retention; favor on-device inference and feature-only logging.
- Apply data minimization and purpose limitation; document DPIAs where required.
- Honor legal holds and retention schedules; robust consent and transparency for workforce monitoring.

**Explainability and Trust**
- Provide user-facing just-in-time coaching: "This file appears to contain client identifiers; sending externally may violate policy X."
- Analyst-facing rationales with highlighted spans and similar prior cases.
- Maintain a clear appeals process and human override with audit trails.

**Operations and MLOps**

- Drift monitoring: Track label/feature drift; retrain cadence (e.g., monthly) with active learning.
- Feedback loops: Analysts tag false positives/negatives; auto-curate hard examples for retraining.
- Versioning: Snapshot models, features, and policies; rollback plans for safety.

**Pseducode Reference**

```
# Training
for event in events:
content = extract_text_code_image(event.artifact)
ocr = run_ocr_if_needed(event.artifact)
x_content = content_encoder(content + ocr)
x_behavior= behavior_features(event.user,event.device,
time_series_window=30d
X.append(concat(x_content, x_behavior))
y.append(label_from_policy_or_analyst(event))

clf = train_calibrated_classifier(X, y)
save_model(clf)

# Inference
def assess(event):
content = extract(event.artifact); ocr = maybe_ocr(event)
  x = concat(encode_content(content+ocr), behavior_features(...))
risk = clf.predict_proba(x)
action = policy_engine(risk, hard_rules, user_context)
explain = explainer(clf, x)
return action, explain
```

**Limitations And Future Work**

- Generalization: Tailoring to each enterprise's data semantics remains necessary; few-shot learning and retrieval can help.
- Adversarial robustness: Attackers may manipulate content to evade detectors; explore adversarial training and ensemble defenses.
- Cross-border data transfer: Federation and split learning can reduce movement of sensitive data.
- Real-time constraints: On-device inference must meet latency budgets; consider distilled models and approximate nearest neighbor search.

## CONCLUSION

AI augments DLP by learning what is sensitive, understanding how users behave, and selecting context-appropriate responses with human-interpretable reasoning. A fusion approach—content + behavior + policy optimization—yields measurable improvements in precision/recall and reduces user friction. Successful deployments prioritize privacy, explainability, and continuous feedback to sustain performance against evolving exfiltration tactics.

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**Table 1. Comparison of DLP Approaches**

| Approach | Strengths | Weaknesses | Best Use |
|---|---|---|---|
| Regex/Dictionary | Simple, fast, deterministic | Misses context, brittle, high FP | Known PII patterns |
| Fingerprinting | Precise for known docs | Fails on transformations/derivatives | Contracts, templates |
| Heuristics (size/type) | Lightweight | Easy to evade | Triage, coarse filters |
| **AI Content Classifier** | Understands semantics | Needs labeled data, drift | Unstructured text/code |
| **Behavioral Modeling** | Captures intent patterns | Cold-start risk | Insider risk, anomalies |
| **Policy Optimizer** | Balances risk/friction | Needs logged policies | Action selection |

**Table 2. Key Metrics and Targets (Illustrative)**

| Metric | Target | Notes |
|---|---|---|
| Precision (High-risk) | ≥ 0.85 | Analyst workload control |
| Recall (High-risk) | ≥ 0.70 | Prevent leakage |
| AUPRC | +15% vs. baseline | Class imbalance aware |
| Block Accuracy | ≥ 0.80 | Minimize business disruption |
| User Friction Index | ≤ 0.05 | % workflows interrupted |

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REVIEW ARTICLE

# A Review of Management Strategies for Tomato *Fusarium* Wilt Caused by *Fusarium oxysporum* F. Sp. *Lycopersici* (SACC)

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**ABSTRACT**

The tomato is a highly valued source of nutrition that contributes to the global nutrition of both rural and urban populations. Low tomato yields are caused by a variety of causes, including weed competition and fungal, bacterial, nematode, or viral infections. One of the most significant and pervasive diseases of the cultivated tomato is *Fusarium* wilt disease, which is brought on by *Fusariumoxysporum f. sp. lycopersici.* The only target of this soil-borne disease, which belongs to the class Hyphomycetes, is the tomato, which wilts when it is exposed to it. Vein-let clearing and petiole drooping on young plants are the first indications. The oldest lower leaves start to yellow as the first sign of *Fusarium* wilt. Lower leaves turn yellow and eventually perish. This review documented many methods used to combat the disease, which is extremely harmful in tomatoes grown in nurseries and fields. The most economical and environmentally responsible method is Integrated Disease Management (IDM), which has been promoted as being safe and non - toxic, priceless organisms, plants, animals, and human beings. However, instead of focusing only on the greenhouse, measures should be taken to examine and increase their effectiveness, efficiency, and stability on the fields.

**Keywords:** Tomato**,** *Fusariumoxysporum* f. sp. *Lycopersici*, Integrated Disease Management

## INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is belongs to Solanaceae family includes tomato which are grown total area of 4.5 million hectare and a yield of 115.5 million metric tons. It is one of the most important vegetables in the world (Kaiser and Erns,2011). Because it is a crop with a short growing season and a high yield, it is commercially appealing, and the cost of cultivation is growing every day. In terms of both area and output, it comes in third place globally behind potatoes and sweet potatoes (Naika *et al*, 2005). From a total cultivated areas of 27.56 million

hectares, India produced roughly 333.25 metric tons of tomatoes in 2020–21. The average tomato yield per hectare in the world was 34.85 tones, whereas the yield per hectare in India was 21.18tones. India is the world's second-largest tomato growing country. Primarily in highly populated urban regions, there is an internal market for local fresh tomato fruit. There is also a growing opportunity for processing companies for the foreign and Indian markets as tomato sauce, ketchup, squeeze, and tomato glue (puree) etc. (AVRDC,2003). The climate and soil in India are ideal for growing a variety of fruits and vegetables, including tomatoes. The best temperature range for tomato growth is between $15^0$C and 25 °C, despite the fact that it is actually adaptable. High temperatures reduce the productivity. While temperatures above 30° C hinder the lycopene formation and flavor of tomato fruit, exceptionally low temperatures can have an impact on production. Tomatoes grow best in areas with low to moderate precipitation and an effective watering system during the off-season. Disease attacks increase under wet conditions, which also affect fruit ripening. Tomatoes thrive in a variety of soil types with a pH range of 5.5-7.5, high organic matter content, and good drainage. (Hanaa *et al*, 2011, Waiganjo *et al,* 2006). The best soil for tomato plants is one that has been thoroughly amended with organic matter and is well drained. It is important for the soil to be capable of holding moisture (Robert, 2005). Tomato yield is restricted by a variety of factors. . These include: a dearth of enhanced, high-performing cultivars; poor fruit setting brought on by severe rainfall and extremely high temperatures; and pests and illnesses. A common occurrence is yield losses of 100 percent, especially when tomatoes are affected at an initial phase of development (Cohen and Antinus, 1994). The world average crop loss due to all illnesses put together was around 12.8% of the crop productivity, whereas the tomato alone saw a loss of 21.8 percent. The main biotic restrictions in India are caused by disease and insect pest damage. Much research has been done on the country's pathological and entomological concerns in order to reduce harm caused by illness and insect pests. As a result, roughly 13 illnesses brought on by various fungal, bacterial, and viral pathogens have been found and documented (Jones *et al*, 2014) and commercially significant insect and mite pests targeting tomatoes have also been reported (Elsafie *et al*, 2017). Previous research has identified early blight (*Alternaria solani*), late blight (*Phytopthora infestans*), septoria leaf spot (*Septoria lycopersici*), and viruses as the four most commercially significant tomato diseases (Winad and Willium, 1999). A major financial loss is brought on by the illness *Fusarium* wilt of tomato, which is brought on *by Fusarium oxysporum* f. sp. *lycopersici.* On an astonishing variety of host plants, *Fusarium* species produce a wide variety of illnesses. The fungus can be retrieved from any part of the plant, from the deepest root to the topmost flower, and it can be conveyed in the soil, the air, or in plant residue (Singha *et al,* 2011). Wilted plants, yellowed leaves, and root rot are the hallmarks of the fungus's sickness, which also causes a low or nonexistent agricultural output. This disease is particularly destructive to tomato types that are sensitive to it during the summer season when soil and air temperatures are typically high (Akrami and Yousefi 2015).

**Pathogen**

The *Fusarium* genus is among the most versatile and diverse species in the Eumycota, and the *Fusarium oxysporum* species complex has a wide variety of non-diseases in addition to plant, animal, and human pathogens (Gorden, 2017). Numerous soil-borne infections of a variety of horticultural and food crops, including members of the *Fusarium* genus, are responsible for the deadly vascular wilts, rots, and damping-off diseases (Bodah, 2017). More than 150 hosts are affected by wilt disease caused by the *Fusarium oxysporum* species, which has a variety of distinct formae speciales (Bertoldo *et al*, 2015). *Fusarium oxysporum* Schlectend was mentioned in reports (Asha *et al,* 2011, Nirmala Devi and Srinivas, 2012). The most severe reduction in yield was caused by F. sp. lycopersici (Sacc.) W.C. Snyder and H.N. Hansen (FOL), which causes vascular, wilt of tomato disease (Asha *et al,* 2011). *F. oxysporum* can survive for a long time in soil as dormant propagules, which is a feature of soil-borne pathogens (chlamydospores). Chlamydospore germination is triggered by the presence of host roots. The infected hyphae stick to the root surface before penetrating it. The mycelium enters the vascular system through the xylem pits and interpenetrates the root cortical cells. The fungus then exhibits a distinct mode of infection in which it prefers to grow just within the xylem vessels before quickly invading the host (Srinivas *et al,* 2019). The fungus begins to develop microconidia inside the vessels, and as they separate, they are carried upward through the sap stream. Furthermore, the top vessels are penetrated by mycelial growth as a result of microconidia germination. The typical wilt symptoms manifest as vascular blockage brought on by the accumulation of fungal hypae and a mixture of host-pathogen interactions, including the production of toxins, the creation of gums, gels, and tyloses (Khan,*et al*, 2017, Cha *et al,*2016) The

appearance of typical disease symptoms such leaf epinasty, vein clearing, wilting, and defoliation inevitably precedes the death of the host plant (Fig. 1). During this phase the vascular wilt fungus, which stays limited to the xylem vessels, propagates through parenchymatous tissue and begins to sporulate abundantly on surface of the plant such as, leaf, steam etc. Dissemination of the pathogen can occur via seeds, transplants, soil or other means (Mc Govern, 2015, Joshi, 2018).

**SYMPTOMS OF *FUSARIUM* WILT DISEASE ON TOMATO**

Inoue *et al.* (2002) found that the fungus directly invades roots and colonizes vascular tissue. *Fusarium* wilt symptoms can include growth retardation, chlorosis and drooping of the leaves, reddish deep gouges of the xylem vessels (perceptible inside the stem as strands or dots in transverse section), and on the infected parts of the plant whitish pink fungal growth was seen (particularly in wet conditions). They can also affected root or stem decay. Attack signs initially manifest as modest vein clearing on the young leaves' outer surface, proceeded by epinasty of the mature leaves (Sally *et al,* 2006). This condition frequently only affects one side of the plant or one stalk. Before the plant reaches maturity, successive leaves normally turn yellow, wilt, and fall. Growth is sometimes stunted and almost no fruit typically begins as the condition worsens. Dark brown stripes that run vertically through the main stem can be noticed if the stem is sliced. The disease's characteristic tanning of the vascular system can typically be utilised to identify it (Mui Yun, 2003). The symptoms frequently appear on mature plants following blooming and during the start of fruit 21 setting, as demonstrated by Walker (1971). A small amount of wilting on a portion of the plant may be the first sign. Chlorotic symptoms first occur on one side of the leaf before turning that side of the foliage yellow. Wilting is frequently related to one side of the plant as conditions worsen. Fig. 1 [A] Showing only affects one side of the plant; leaf turned yellowing, wilt and fall. [B] Dark brown stripes that run vertically through the main stem

**Etiology of Pathogen**

Mycelia of *Fusarium oxysporum* range from sparse to profuse and are delicate whitish pink, frequently with a purple tinge. The fungus creates three different types of spores: chlamydospores, macroconidia, and microconidia. Microconidia are plentiful, oval-ellipsoid, straight to curved, 5-12 x 2.2-3.5 mm, non-septate, and borne on simple phialides emerging laterally. Macroconidia are thin-walled, three- to five-septate, fusoid-subulate, pointy at both ends, and have a pedicellate base. They are borne on branched conidiophores or on the surface of sporodochia and can range in number from sparse to profuse. Conidia with three septa are 27–46 x 3-5 mm in size, whereas those with five septa are 35–60 x 3-5 mm. The three-septate spore is more prevalent. The three-septate spore is more prevalent. There are many chlamydospores, both smooth and rough walled, and they can form terminally or intercalarily. Although they sometimes form pairs or chains, they are typically single. The perfect stage is unknown (Gerlach,1982). Fig. 2. [A] Profuse whitish purple mycelium growth of *F. oxysporum* [B] Microscopic observation of *F. oxysporum* including; macroconidia, microconidia and chlamydospore

**Ecology and Spread of the Infection**

A soil-borne pathogen that may survive for many years in the soil without a host is the cause of *Fusarium* wilt. The community surrounding infected tomato detritus is where the majority of infections start. Healthy plants may contract *F. oxysporum* infection if the soil they are growing in harbours the pathogen (Walker, 1971). In all kinds of soil, *Fusarium oxysporum* can be found, it can survive there, and it can grow there, but sandy soil offers the best condition for this to happen. Since it is soil-borne, the pathogen can survive in infected soil for many years. Pathogen thrives in environments with soil and air temperatures are 28 °C. The pathogen will spread to the lower regions of the stem if air temperatures are below $25^0$C but at optimal soil temperatures the plants won't show any visual signs. Nitrate nitrogen reduces pathogen virulence while micronutrients, phosphorus, and ammonium nitrogen increase it (Mui, 2003). The disease can spread over long distances via infected soil or transplants (Agrios, 1988), but it is more commonly short distance transmission by irrigation water and contaminated farm tools(Stephen, 2003).Furthermore, the disease can spread through contaminated soil and infected plant debris. Other ways of disease transmission include direct contact between a human and an infected field, the use of irrigation water or equipment that has formerly been used on a diseased crop, and direct contact between a human and an affected crop (Ajigbola, 2013).

**Epidemiology of Disease**

According to Sally *et al*. (2006), the pathogen enters the plant by the root hairs and can survive in the soil for many years (Thangavelu *et al*, 2003). Mycelium develops in xylem channels where it blocks water flow, causing wilting (Stephen and Andre, 2003). *Fusarium* wilt frequently coexists with nematode colonization, with the worms serving as the fungus' entry point. Additionally, enzymes may make it easier for *Fusarium* to enter the plant host (Babalola, 2010). Warm temperature as well as low soil moisture increase infection and disease development in *Fusarium* wilt (Lewis, 2003).

**Treatment of *Fusarium* Wilt Disease**

*Fusarium* wilt in tomato plants needs to be managed if plant health, fruit quality, and yield are to be preserved. Management of *Fusarium* wilt is challenging [38, 39, and 40]. Several strategies have been put out to control this fungus pathogen. However, due to the introduction of new pathogenic strains, efforts to end the pandemic have mostly failed. The control of this pathogen is documented using a variety of techniques, including cultural, biological, resistance-based, chemical, and the use of natural materials (Pottorf, 2006).

**Control by Culture Practices**

Agriculture procedures and tactics that boost productivity and production while minimizing the consequences of illness are referred to as cultural control. To prevent plant diseases, it includes non-mechanical ecosystem manipulation. It requires altering agricultural methods to create a hostile environment for the growth of disease pathogen (Islam, 2001).It might also refer to the deliberate control of plant development, planting, and culture in a farm or yard to decrease plant disease. It has been found that treating soil-borne pathogens with cultural methods enhances soil composition, which minimizes the incidence of disease (Neshav, 2008). These techniques are mostly preventative, because controlling the spread of disease necessitates thorough understanding of the concept, habit, and environmental factors that the disease agent prefers. For a while, it was difficult to control soil- and seed-borne pathogens like *Fusarium* wilt, which is caused by a *Fusarium* species. To reduce fungus infestations and prevent disease issues unique to this type of vegetable, crop rotation with non-Solanaceous plants for 4-6 seasons is typically advised. Cereal crops should rotate with the grain whenever possible (Sally, 2006). Weed management, soil moisture management, and soil heating are all benefits of mulching, or topping the soil surface with a thick layer of mulch. As the tomato receives shadow from the maize and is grown out of season, intercropping of tomato and corn should be promoted in order to increase the farmer's productivity and profitability. This technique enhanced production in Cuba by 5–6 tones/ha per year. By creating unfavorable conditions for soil-borne microorganisms, it aids in disease control. The hydrated tomato leaves are kept free of soil-borne germs by mulching. The farmer's advantage and yield would improve as a result (Maurya *et al*, 2019). Diseases like *Fusarium* spp. can be avoided through weed control. The virus can infect plants when sick weeds are destroyed. The *Fusarium* wilt pathogen can more easily infect plants when they are subjected to excessive plant care techniques such tying, trimming, and pruning (Ajigbola and Babalola, 2013).

**Biological treatment**

The use of hostile microorganisms is a different disease prevention technique for having a *Fusarium* disease control system that is eco - friendly alternative (Lutenberg, 2009). Biological control agents can accomplish their objectives directly, indirectly, or through a combination of both (Pal and Gardner, 2006). There have been reports of great success using bio agents to control the *Fusarium* wilt disease on tomatoes (Freeman *et al,* 2002). Non-pathogenic *F. oxysporum* and *F. solani* were successfully identified and prevented *Fusarium* wilt in greenhouse testing (Momol, 2003). When applied quickly, CS-20, CS-1, CS-24, and Fo-47 were among the consistently effective isolates. Since being sprinkled with *Phytophthora cryptogea* zoospores and then injected with the disease's pathogen, tomato plants don't exhibit symptoms of wilt disease (Attitala *et al*, 2001). According to Kapoor and Kar (1988), *Arthobacter, Bacillus*, and *Pseudonomas* species are among the bacterial antagonists. 30 biological control agents, including bacteria and non-pathogenic strains of *Fusarium oxysporum*, have been identified by Lemanceae and Alabouvett (1991) and Fuchs *et al*. (1997) as having potential for controlling *Fusarium* wilt of tomato. In comparison to field circumstances, controlled greenhouse conditions have shown biological control agents to be more effective at decreasing *Fusarium*

wilt in tomato plants. *Fusarium oxysporum* f.sp. *lycopersici* incidence on tomato was found to be reduced by 6–15 percent by coating seeds with *Trichoderma viridae* culture (Panteleev 1973). An innovative disease management technique is the employment of antagonistic microorganisms, which can provide an eco-sustainable *Fusarium* disease control system (Lutenberg and Kamilova, 2009). According to Pal and Gardener (2006), the biological control agents' mechanisms may be direct, indirect, or a combination of both. According to Freeman *et al*. (2002) the application of biological agents was quite successful in preventing *Fusarium* wilt disease in tomato plants. Researchers have observed the inhibition of plant pathogens by halophilic and green manure, especially *Rhizoctonia, Phytopthora, Plasmidiophora brassicae*, *Gaeumannomyces graminis,* and *Fusarium* (Pitt *et al*, 1998,, Kannangowa *et al*, 2000, Cotxarrera, 2002). It was discovered that *G. intraradices* and a few gram-negative and fluorescent RB (rhizobacteria), *P. fluorescens*, *P. putida*, and *Enterobacter cloaceae*, were efficient against *Fusarium oxysporum* f. sp. Lycopersici in the rhizoplane of solanaceous plants (Akkopru and Dermir, 2005). The bacterial biocontrol agents *B. subtilis*, *Streptomyces corchorusii*, *S. pulcher*, and *S. mutabilis* have been shown to exhibit promising biocontrol actions against wilt (Monda,2002)]. By generating phytohormones including Indole acetic acid, Ethylene, Cytokinin, Gibberelin, and mineral elements, rhizobacteria can operate as biofertilizers and biostimulants. They can also provide as a passive barrier to harmful microorganisms by generating antibiotics and siderofore (McMillan, 2007 and Smith, 2007). According to Widnyana *et al*., (20130, three isolates of rhizobacteria known as *Pseudomonas alcaligenes,* KtS1, TrN2, and TmA1, were taken from the rhizobia of plants belonging to the families Solanaceae and Leguminoseae and demonstrated inhibitory action against *Fusarium oxysporum* f. sp. lycopersici by significantly lowering the wilt disease. Numerous crops are said to be induced to grow by biological agents. These responses could be brought on by (i) suppressing harmful root microflora, including those that aren't directly causing disease, and (ii) producing growth-stimulating substances. (iii) Improved root development and/or solubilization of nutrients, which resulted in an increase in nutrient intake. *Trichoderma* is one of the most well-known and frequently utilized organisms for promoting plant development and biologically controlling plant diseases (Shirzad *et al*, 2016, Vipin Kumar *et al*, 2017, Kloepper, 1993).

**Mechanisms of bio control by PGPB**

The fight for resources and space at the infection site, the creation of metabolites, and the modification of bacterial signalling molecules are frequently used as disease controls by BCA (biocontrols) (Raaijmakers *et al* 2002). All of these situations involve pathogens being agitated by the existence and actions of other species (Fig. 1). The release of substances like siderophores, which effectively sequester iron and deny the pathogen of this crucial element, is the key process of pathogen suppression via nutritional competition (Whipps, 2001). Some bacteria secrete metabolites, such as antibiotics, toxins, surface active substances (biosurfactants), and cell wall-degrading enzymes, which suppress the growth of pathogens (Compant *et al*,2005, Haas and Defago, 2005, Kumar *et al*, 2015). However, their particular byproducts also cause the induction of systemic resistance (Van Loon *et al*, 1998). Some *Bacillus* and *Pseudomonas* species produce several antibiotic substances that fight pathogenic bacteria, nematodes, helminths, fungus, and more (Thomashow and Weller, 1995, Raaijmakers *et al* 2002, Almaghrabi, 2013). Different bacterial strains, including *Pseudomonas* (Ligon *et al*, 2000), *Serratia* sp. (Kalbe *et al*, 1996), and *B. cepecia* (Burkhead, 1994), create secondary metabolites, such as pyrrolnitrin (3-chloro-4- (20-nitro-30-chlorophenyl) pyrrole, which is efficient against various infections. The primary tactics applied by BCA to suppress soil-borne pathogens are the production and release of cell wall-degrading enzymes (Kobayashi *et al*, 2003). The structural rigidity of the target pathogen's cell wall is impacted by these enzymes. B-1, 3-glucanase, chitinase, cellulase, and protease were the cell wall-degrading enzymes that biocontrol strains released (Budi *et al*, 2000). Plant Growth Promoting Bacteria shows Antagonistic Activity (Fig. 3).

**Use of Botanicals**

The use of medicinal herbs for the control of *Fusarium* wilt in crops is limited, despite the fact that numerous research activities have been made to identify alternative and environmentally acceptable strategies for controlling plant diseases (Agbenin, *et al*, 2004, Agbenin *et al*, 2006)). When compared to synthetic pesticides, plant metabolites and plant-based pesticides are recognised to have less of an adverse effect on the environment and pose fewer risks to consumers. Therefore, they seem to be one of the better alternatives. As natural products that are freely accessible and have no negative effects, the demand for plant-based therapies is rising in developing nations. The goal of the

current study was to assess the effectiveness of certain plant extracts in preventing the tomato wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici.* When compared to synthetic pesticides, plant metabolites and plant-based pesticides are recognised to have less of an adverse effect on the environment and pose less risk to consumers. Therefore, they seem to be among the better options. As natural products that are freely accessible and have no negative effects, the demand for plant-based therapies is rising in developing nations. The goal of the current study was to assess the effectiveness of certain plant extracts in preventing the tomato wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici.* Daradka *et al*(2021) were investigating the antifungal activity of ten plant extracts against A. *alternata* and F.*oxysporum*, the causative agents of tomato early blight and wilt diseases. The tested plants belonged to several families. Plant extracts had an inhibitory impact on *A. alternata* and *F. oxysporum* that ranged from 10-29 and 12-31 mm zone of inhibition, respectively. *Citrullus colocynthis*, *Psiadia arabica*, and *Olea chrysophylla* extracts were the most potent antifungals, followed by extracts of *Pulicaria crispa*. Using 50 plant extracts, Ramaiah and Garampalii (2015) assessed *in vitro* antifungal activity against *Fusarium oxysporum* f. sp. *lycopersici* (FOL) using the food poison approach. Three plants out of fifteen were shown to have the potential to slow the growth of the FOL: *Solanum indicum* (78.33%), *Azadirachta indica* (75.00%), and *Oxalis latifolia* (70.33 percent ). Three chemical fungicides, namely (Mancozeb with 82.66 percent, Copper oxychloride with 79.33 percent, and Copper sulphate with 82.33 percent) at various concentrations, were compared for their antifungal effectiveness. The antifungal crude extract at varying concentrations (ten percent, twenty percent, forty percent, and sixty percent) on the mycelial development of FOL was assessed using the poison food method.As a result of this work, it is possible to develop effective plant-based fungicides that can be utilised in organic farming to treat *Fusarium oxysporum* f. sp. *lycopersici* using botanical extracts as a better option.

**Application of Resistance Crop species**

The use of disease resistance, when available, is the most economical and environmentally secure way of control. The greatest method for disease control is the use of resistant cultivars, which is also one of the finest alternative methods for wilt disease management (Sheu *et al.,* 2006). But the utility of several resistant cultivars is limited to just a few years because of the loss of resistance in the face of significant pathogenic diversity in the pathogen population. For some plants, such as peas and China asters, cultivars that are resistant to or tolerant of *Fusarium* wilt are available. The other races of the same forma specialise may not be resistant to a cultivar that is resistant to a forma specialise (Burgess *et al,* 2008). Identification and usage of tomato plant types that are disease-resistant constitute a viable alternative to the use of chemicals (Pritesh and Subramanian, 2011). When there is no known dominant gene, breeding for resistance might be extremely challenging. The development of novel pathogen races that are able to overcome host resistance has also been reported by Momol and Pernenzy (2003). This approach has the benefits of reducing the cost of disease-controlling chemicals and maximising the cultivation of previously diseased fields.

**Chemical Control**

Chemicals are frequently used in agriculture to control diseases and pests. The incidence of tomato wilt is significantly decreased in seeds treated with synthetic fungicides. However, using them is expensive and environmentally harmful (Song and Goodman, 2001). The primary technique for managing plant diseases is fungicides, however biological agents, particularly *Trichoderma* species, are also effective at limiting the pathogenic activities of phytopathogenic fungus (Taran,2000). It is therefore crucial to implement a good management approach to combat tomato *Fusarium* wilt. Prochloraz, propiconazole, thiabendazole, carbendazim, benomyl, thiophane, fuberidazole, and all of the benzimidazoles are a few of these substances. Using the root dip treatment approach, benomyl was found to be only moderately effective against F. *oxysporum* f. sp. cubense (Nel *et al.* 2007). When this technique was used to treat *Fusarium* wilt-infected tomato seedlings, it increased yield by roughly 24 percent (Khan and Khan, 2002). The introduction of alternative approaches to disease management has been prompted by factors such as the high frequency of chemical usage, non-target effects, and the development of chemical resistance, the persistence of pathogens for long periods of time, and hazards to human health and the environment. Additionally, compared to their soil-borne competitors, insecticides are typically more effective against aerial plant diseases. The range of approved chemicals is dwindling as bioactive molecules are withdrawn for toxic effects and environmental reasons; for example, methyl bromide has already been carried out due to its extremely high potential to deplete the

ozone layer. It is also technically challenging to treat large amounts of soil. Chemical control techniques lead to microbial population imbalances that may be detrimental to the activities of beneficial soil microorganisms and may also encourage the emergence of infections with heightened resistance. Overuse of chemicals in the soil has polluted the environment and harmed humanity (Asaka and Soda, 1996). Chemical control techniques lead to microbial population imbalances that may be detrimental to the activities of beneficial soil microorganisms and may also encourage the emergence of infections with heightened resistance. Overuse of chemicals in the soil has polluted the environment and harmed humanity (Asaka And Soda, 1996). Some chemicals like Topsin-M, Nativo, Curzate, Carbendazim, Cabriotop, Prevail, and Antracol showed fungicidal activity against *Fusarium oxysporum f*.sp. *lycopersici.* Blosch *et al*. 2021investigated the effectiveness of fungicides, such as etalaxyl+mancozeb, copper oxychloride, benalaxyl+mancozeb, carbendazim, and mancozeb, at increasing doses (2, 2.5, 3, 3.5, and 4 g/litre water), against the *Fusarium* wilt of tomato affected by *Fusarium oxysporum* f. sp. The outcome showed that copper oxychloride was efficient in all of its doses to control the *Fusarium* wilt of tomato; the most effective dose was 3 g/l at which disease incidence was only recorded 6.2 percent.

## CONCLUSION

In summary, efforts should be focused on evaluating and improving these technologies' utility, productivity, and stability on the fields rather than merely in the greenhouse, aside from considering the fact that they are working in various ways. In biological control, it is also crucial to consider the specific microbial product compositions that would produce the best results. Therefore, a focus should be placed on training farmers on how to employ cultural traditions appropriately and how to incorporate them into other tactics for a healthier and cheaper outcome.

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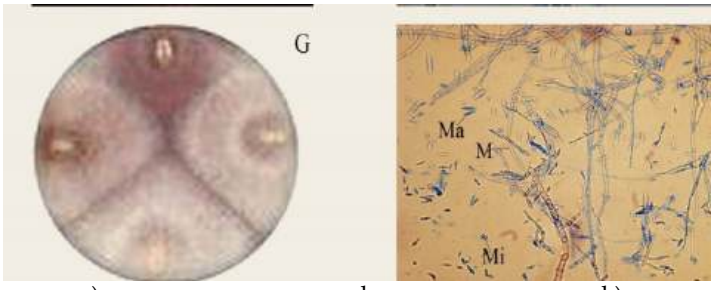
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a) b)





**Fig. 1 [A] Showing only affects one side of the plant; leaf turned yellowing, wilt and fall. [B] Dark brown stripes that run vertically through the main stem**




a) and b)

**Fig. 2. [A] Profuse whitish purple mycelium growth of *F. oxysporum* [B] Microscopic observation of*F. oxysporum* including; macroconidia, microconidia and chlamydospore**

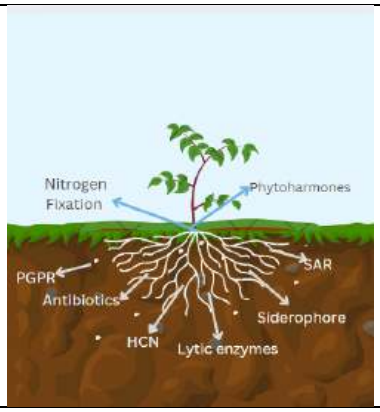



**Fig. 3 Plant Growth Promoting Bacteria shows Antagonistic Activity**

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RESEARCH ARTICLE

# Metaheuristic -Optimized Ensemble Deep Intelligence Framework for High - Precision Melanoma Diagnosis

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## ABSTRACT

Melanoma remains the most fatal form of skin cancer due to its rapid metastatic progression, making early and precise diagnosis essential for patient survival. Manual diagnosis through dermoscopy, although widely practiced, is limited by subjectivity and inter-observer variability. Deep learning has shown great promise in melanoma detection; however, achieving consistent performance across heterogeneous datasets, particularly the highly imbalanced SIIM–ISIC dataset, continues to pose challenges. This study introduces a Met heuristic-Optimized Ensemble Deep Intelligence Framework (MO-EDIF) that integrates advanced pre-processing, multi-architecture deep feature extraction, and a hybrid metaheuristic optimization algorithm. The framework employs adaptive contrast enhancement, lesion-focused cropping, and Test-Time Augmentation (TTA), followed by an ensemble of EfficientNet-B4, ResNet101, and Inception-V3. To further enhance performance, a novel Arithmetic–Jellyfish Hybrid Optimization Algorithm (AJHOA) is used to optimize hyper parameters and ensemble fusion weights. Experimental evaluation on the SIIM–ISIC dataset demonstrates that MO-EDIF achieves 93.8% accuracy, 92.5% sensitivity, 94.6% specificity, and an AUC of 0.962, surpassing state-of-the-art baselines. Ablation studies confirm the critical role of metaheuristic optimization in stabilizing ensemble learning. Findings suggest that MO-EDIF offers a robust and clinically relevant solution for high-precision melanoma diagnosis.

**Keywords:** Melanoma diagnosis, deep learning, metaheuristics, ensemble learning, SIIM–ISIC dataset, medical imaging, soft-voting, optimization.

# INTRODUCTION

Melanoma is the most aggressive form of skin cancer due to its ability to metastasize rapidly, often resulting in life-threatening outcomes if not detected early. Although melanoma accounts for a relatively small fraction of skin cancer cases, it is responsible for nearly 75% of global skin cancer–related deaths [1]. Early detection significantly improves prognosis, with survival rates exceeding 95% when melanoma is diagnosed at an early stage. However, the diagnostic process remains challenging in clinical settings due to heterogeneous lesion appearances, subtle malignant features, and visual similarities between melanoma and benign lesions. Dermoscopy has substantially improved skin lesion assessment by revealing sub-surface structures not visible to the naked eye [2]. Nevertheless, dermoscopic interpretation is influenced by clinician expertise, fatigue, and subjective judgment. High intra-class variability, where melanoma can take on diverse colors and shapes, combined with high inter-class similarity, where benign lesions mimic malignant patterns, further complicates diagnosis [3]. These challenges underscore the need for reliable, objective, and automated diagnostic systems capable of supporting dermatologists in early melanoma detection. Deep learning (DL) approaches, particularly convolutional neural networks (CNNs), have transformed computational dermatology by offering automated feature extraction and superior diagnostic performance compared to traditional machine-learning methods [4]. Benchmark datasets such as ISIC 2017–2020 and the SIIM–ISIC Melanoma Classification Challenge have catalysed research in automated melanoma detection [5]. Despite these advancements, DL-based systems still face three major challenges: (i) high model instability due to heterogeneous image distributions, (ii) sensitivity to hyper parameter choices, and (iii) severe class imbalance, with melanoma representing only ~2% of SIIM–ISIC images [6]. Ensemble learning has emerged as a promising approach to address variability by integrating multiple models that capture complementary feature representations [7]. However, ensemble performance heavily depends on the selection and weighting of individual models, which is typically performed heuristically. Furthermore, hyper parameter tuning in deep learning remains computationally expensive and inefficient when approached through manual trial-and-error. Metaheuristic optimization offers a powerful alternative due to its ability to perform global search in high-dimensional parameter spaces [8]. Algorithms such as Genetic Algorithms, Particle Swarm Optimization, and Jellyfish Optimization have demonstrated effectiveness in tuning deep-learning models; however, their use in ensemble-weight optimization for melanoma classification remains limited. To address these gaps, this study proposes the MO-EDIF framework, which integrates heterogeneous deep-learning models and optimizes them through a novel hybrid metaheuristic algorithm. The goal is to create a highly accurate, stable, and clinically reliable melanoma classifier.

**Literature survey, problem identification, research objectives, and contributions**

## LITERATURE SURVEY

Melanoma detection has progressed through several distinct research phases, each shaped by the limitations of previous approaches. Early work relied heavily on handcrafted features extracted from dermoscopic images. These included geometric indicators such as asymmetry, irregular borders, and variations in color patterns—commonly known under the ABCD rule [9]. Although these features made intuitive sense to dermatologists, their performance was inconsistent. Many benign lesions can appear irregular, while some melanomas remain deceptively simple in appearance. Studies such as those by [9] repeatedly noted poor generalization when handcrafted descriptors were applied to images from different devices, lighting conditions, or ethnic skin tones. The emergence of deep learning fundamentally changed the field. Convolutional neural networks (CNNs) demonstrated that models could learn discriminative patterns directly from image pixels. Architectures like VGGNet and ResNet became early favourites, quickly outperforming classical machine-learning pipelines. This shift was further accelerated with the introduction of deeper and more efficient networks such as DenseNet [10]. Despite these milestones, real-world deployment remains difficult. Datasets like SIIM–ISIC suffer from extreme class imbalance, often containing fewer than 2% melanoma cases. As noted by [11], this imbalance consistently pulls models toward high specificity but poor sensitivity, making them less reliable for early melanoma screening. In addition, dermoscopic images vary widely in appearance due to differences in acquisition devices, patient demographics, skin tone, and image artifacts such as

hair, air bubbles, shadows, and ink markings. Several studies [12] emphasize that these variations disrupt CNN learning unless carefully preprocessed. To counter these issues, ensemble learning has gained popularity. The intuition is straightforward: different architectures capture different visual cues, and their combination tends to yield more stable predictions. Researchers have experimented with soft voting, weighted voting, and stacked generalization, often reporting noticeable improvements [13]. However, the challenge lies in determining optimal ensemble weights. Most studies rely on manual tuning or simple grid searches. As point out, such methods are inefficient and do not fully exploit the potential of ensemble models [14]. This brings attention to another growing trend—metaheuristic optimization. Algorithms inspired by natural processes, such as Particle Swarm Optimization (PSO), Genetic Algorithms (GA), Jellyfish Search, and Arithmetic Optimization, have shown promise in optimizing neural network hyper parameters. They are particularly effective in complex, nonconvex search spaces [15],[16] While individual metaheuristic approaches have been applied in medical image classification, the literature shows very few attempts to optimize both hyper parameters and ensemble weights simultaneously, especially within the context of melanoma detection [17]. A further observation from recent literature is that most melanoma-related studies focus either on improving CNN architectures, enhancing data augmentation, or experimenting with loss functions (e.g., focal loss). However, very few studies bring together pre-processing, heterogeneous ensembles, and metaheuristic optimization into a unified framework specifically designed for the SIIM–ISIC dataset. This gap motivates the development of the proposed MO-EDIF framework, which attempts to integrate these otherwise separate research directions into a single optimized pipeline.

**Problem Identification**

**Based on the literature reviewed above, the following problems are consistently reported and remain insufficiently addressed**

1. Severe class imbalance (≈2% melanoma) leads to models biased toward benign predictions, significantly lowering sensitivity
2. High intra-class variability (melanoma can appear in many forms) and high inter-class similarity (benign lesions often mimic melanoma), making discrimination difficult.
3. Noise and artifacts in dermoscopic images—hair strands, ink marks, shadows—disrupt feature extraction
4. Hyper parameter tuning and ensemble weight selection are mostly manual, which is inefficient and suboptimal
5. Lack of unified, optimization-driven frameworks that combine pre-processing, CNN architectures, and metaheuristic strategies specifically for SIIM–ISIC.

These limitations reduce the reliability of existing models in clinical settings.

## RESEARCH OBJECTIVES

**To address the above challenges, the study defines the following objectives**

1. To develop a heterogeneous CNN ensemble (EfficientNet-B4, ResNet101, Inception-V3) capable of capturing diverse lesion characteristics.
2. To design a hybrid metaheuristic strategy (AJHOA) that jointly optimizes learning rate, batch size, dropout, and ensemble fusion weights.
3. To build a robust pre-processing and augmentation pipeline that reduces artifact-driven noise and dataset variability.
4. To validate the proposed framework on SIIM–ISIC using strong baselines, evaluation metrics, and ablation studies.
5. To investigate the contribution of metaheuristic optimization in improving sensitivity and stability under class imbalance.

**Major Contributions**

**This study offers the following contributions**

1. A task-specific hybrid optimization strategy (AJHOA) combining Arithmetic Optimization and Jellyfish Search, enabling automated tuning of critical hyper parameters and ensemble weights for melanoma classification.
2. A heterogeneous ensemble framework integrating EfficientNet-B4, ResNet101, and Inception-V3, leveraging complementary multi-scale and structural feature representations. AJHOA identifies optimal ensemble fusion weights.
3. A unified pre-processing and augmentation pipeline including hair removal, color normalization, lesion-centered cropping, and Test-Time Augmentation (TTA), improving image consistency across the SIIM–ISIC dataset.
4. A comprehensive experimental analysis showing that the metaheuristic-guided ensemble significantly outperforms individual backbones and non-optimized ensembles in accuracy, sensitivity, specificity, and AUC.

## PROPOSED METHODOLOGY

The Metaheuristic-Optimized Ensemble Deep Intelligence Framework (MO-EDIF) is an end-to-end pipeline designed to address the principal challenges identified in previous sections: image artifacts and variability, class imbalance, hyper parameter fragility, and heuristic ensemble weighting.

**MO-EDIF comprises five modular stages:**

i. Image pre-processing and augmentation,
ii. Backbone training and fine-tuning,
iii. Hybrid metaheuristic optimization (AJHOA),
iv. Metaheuristic-guided soft-voting fusion and threshold selection, and
v. Interpretation and post-hoc validation. Each stage is described below with the specific procedures used in our experiments.

**Dataset and Experimental Splits**

We perform experiments on the SIIM–ISIC Melanoma Classification dataset. Following standard practice and to ensure reproducibility:

**Total images:** 33,126 (as described in Section 4).

**Train / Validation / Test split:** 70% / 15% / 15% (stratified by class to preserve melanoma ratio).

**Class imbalance handling:** no oversampling in the primary split; imbalance is addressed in training via loss design and in evaluation by reporting sensitivity and AUC in addition to accuracy. All random splits use a fixed seed to ensure reproducibility.

**Image Pre-processing and Augmentation**

Pre-processing is applied deterministically to all images before model input; augmentation is applied during training and at test time as described.

**Artifact Detection and Removal**

**Hair/ink removal:** An enhanced Dull Razor pipeline is implemented: morphological top-hat filtering highlights thin, elongated structures; connected component analysis isolates hair candidates; small patches are in painted using a Telea-like in painting algorithm to preserve texture continuity. This step reduces false activations from occlusions (Patel and Zhao, 2020).

**Local Contrast Enhancement**

**CLAHE** (Contrast Limited Adaptive Histogram Equalization) is applied per channel to improve local contrast while avoiding over-amplification in homogeneous regions (Lopez and Ahmed, 2022).

**Lesion Localization and Cropping**
**If segmentation mask available:** crop tight bounding box around lesion + 10% padding.
**Otherwise:** compute approximate centroid using intensity-weighted centre; extract centred square crop.
**Resize:** All crops are resized to 224 × 224 (bilinear interpolation) to match the input size of selected backbones.

**Training Augmentations**
**Randomized augmentations applied per epoch**
**Rotation:** uniform [0°, 360°]
**Horizontal/vertical flips:** p = 0.5
**Random zoom:** 0.8–1.2 scale
**Color jitter:** brightness/contrast/saturation ±20% These increase model robustness to acquisition variability.

**Test-Time Augmentation (TTA)**
At inference, predictions are averaged over a deterministic set of K augmentations (rotations + flips). We used K = 8 in experiments; averaged probabilities are used for ensemble fusion.

**Feature Extraction: Heterogeneous Ensemble Backbones**
We employ three complementary CNN backbones to capture multi-scale, hierarchical, and texture information:
**EfficientNet-B4** —for efficient compound scaling and fine-detail modelling.
**ResNet101**— for deep hierarchical/residual feature learning.
**Inception-V3** — for multi-scale receptive fields and texture sensitivity.

**Model heads and initialization**
- All backbones are initialized with Image Net pretrained weights.
- For each backbone, we replace the final classification layers with: Global Average Pooling → Dropout (rate tuned) → Dense (1) with sigmoid.
- We fine-tune the entire network using AJHOA-suggested hyper parameters.

**Hybrid Metaheuristic Optimization (AJHOA)**
AJHOA (Arithmetic–Jellyfish Hybrid Optimization Algorithm) jointly optimizes training hyper parameters and ensemble fusion weights. The goal is to discover hyper parameter sets that yield robust base models and to allocate ensemble weights that maximize discrimination and calibration on validation data.

**Optimization variables (θ)**
**Learning rate (lr):** continuous, log scale ∈ [1e-6, 1e-2]
**Batch size (b):** discrete ∈ {8, 16, 32, 64} (encoded as integer)
**Dropout rate (d):** continuous ∈ [0.0, 0.6]
**Ensemble weights ($w_1$,$w_2$,$w_3$):** continuous, unconstrained during search; mapped to probabilities via softmax: $\widetilde{w}_i = \frac{e^{w_i}}{\sum_j e^{w_j}}$so that $\sum_i \widetilde{w}_i = 1$.

**Fitness Function**
We define a composite fitness evaluated on the validation split after a short warm-retraining (e.g., Nwarm = 5 epochs) to keep optimization tractable:
Fitness (θ)=α·(1−ErrorRateval) +β·AUCval−γ·ValLoss
We used example weights (alpha = 0.3, beta = 0.6, gamma = 0.1) to emphasize AUC and stable discrimination; these can be adjusted depending on application priorities (higher sensitivity vs. specificity).

**AJHOA High-Level Procedure**

**AJHOA alternates between AOA-driven exploration and JSO-driven exploitation:**

- **AOA phase (Exploration):** apply arithmetic transformations to generate a diverse population across each parameter dimension (broad search).
- **JSO phase (Exploitation):** apply Jellyfish-inspired local moves—passive drifting around promising solutions and active foraging towards best solutions—to refine candidates.

To avoid premature convergence, AJHOA includes mutation (Gaussian perturbation) and boundary reflection for variables exceeding ranges.

**AJHOA Pseudo code**

Algorithm 1 outlines the hybrid Arithmetic–Jellyfish Optimization Algorithm (AJHOA), which jointly searches for the optimal hyper parameters and ensemble fusion weights. The algorithm begins by initializing a population of candidate solutions and evaluating their fitness on the validation set. During the early iterations (exploration phase), Arithmetic Optimization (AOA) generates diverse candidate configurations by applying arithmetic transformations to encourage broad search across the hyper parameter space. After the exploration threshold is reached, the algorithm switches to the Jellyfish Search Optimization (JSO) exploitation phase, where each solution performs passive drifting or active foraging around the current best candidate to refine parameter values. Each updated candidate undergoes a short fine-tuning cycle to obtain a validation fitness score. Elitism is used to retain the top-performing solutions across generations, while Gaussian mutation introduces controlled randomness to avoid premature convergence. After the final iteration, the best solution $\theta^*$ is selected, and its raw ensemble-weight parameters are normalized via softmax to obtain the final fusion weights.

**Algorithm 1:** AJHOA – Hybrid Optimization for Hyper parameters and Ensemble Weights

Input:

**N -** population size

**MaxIter -** maximum iterations

**T_explore**- iterations allocated to AOA exploration (≈ 0.4 · MaxIter)

**Search space-** {lr, batch size, dropout, w1, w2, w3}

**Output**

**θ* -** best optimized solution

**(w1, w2, w3) -** normalized ensemble fusion weights

```
Initialize population {θ_i | i = 1...N} by uniform sampling in parameter ranges
Evaluate Fitness (θ_i) for all i
for iter = 1 to MaxIter do
    if iter ≤ T_explore then
        for each θ_i in population do
            θ_i' = AOA_Transform (θ_i)
        end for
    else
        best = argmax (Fitness (θ_i)) over all i
        for each θ_i in population do
            θ_i' = JSO_Move (θ_i, best)
        end for
    end if
    for each θ_i' do
        Perform short fine-tuning (N_warm epochs) using θ_i'
        Evaluate Fitness (θ_i')
    end for
    Combine old and new candidates
```

20: Select top-N solutions → new population
21: With probability p_mut:
22: Apply Gaussian perturbation to selected dimensions
23: end for
24: θ* = best solution in final population
25: (w1, w2, w3) = SoftMax (θ*.raw weights)
26: return θ*, (w1, w2, w3)

**Computational considerations**

To reduce runtime, AJHOA evaluates candidates using a short fine-tuning schedule (e.g., 5 epochs) or lower resolution proxies during early iterations; final best candidates are fully trained for final evaluation.

**AJHOA** – Hybrid Optimization for Hyper parameters and Ensemble Weights (Figure 1).

**Metaheuristic-Guided Ensemble Fusion and Decision Thresholding**

Following AJHOA convergence, we fuse the three backbone outputs using the optimized softmaxed weights:

$$P_{fused}(x) = \sum_{i=1}^{3} \widetilde{w}_i \cdot P_i(x)$$

Where Pi(x)are TTA-averaged probabilities from each base model. The binary decision threshold (τ) is selected by maximizing the Youden Index on validation:

τ∗=argmax (Sensitivity(τ)+Specificity(τ)−1)

This yields a clinically balanced operating point that can be adjusted based on screening priorities (e.g., prioritize sensitivity).

**Training Details and Regularization**

**Optimizer:** Adam with AJHOA-selected initial learning rate; weight decay of 1e-5.

**Loss:** Binary Cross-Entropy combined with Focal Loss ($\gamma$ = 2.0) to emphasize hard examples subject to class imbalance (Lin *et al.*, 2017). The combined loss is a weighted sum (0.5 BCE + 0.5 Focal) in our experiments.

**Epochs:** up to 50 with early stopping (patience = 10, monitored on validation AUC).

**Regularization:** dropout (AJHOA-selected), L2 weight decay, and data augmentation.

**Hardware:** NVIDIA T4 GPU (single card used for experiments; multi-GPU training can speed up backbone fine-tuning).

**Evaluation Protocol and Statistical Analysis**

**Metrics reported:** Accuracy, Sensitivity (Recall for melanoma), and Specificity, AUC (ROC), and confusion matrix values.

**Statistical significance:** For main comparisons, we report mean ± standard deviation across 5 random seeds and perform paired t-tests where relevant to assess significance ($p < 0.05$) between MO-EDIF and baseline ensembles.

**Ablation suite**

- Single-backbone baselines (each model trained with best AJHOA hyper parameters independently).
- Ensemble without optimization (equal weights, best manual hyper parameters).
- Ensemble with hyper parameter optimization only (equal weights).
- Ensemble with weight optimization only (manual hyper parameters).
- Full MO-EDIF (joint optimization).

These ablations quantify the contribution of AJHOA and pre-processing components.

**Interpretability and Post-hoc Validation**

- Grad-CAM maps are generated for representative true positives, false negatives, and false positives to qualitatively inspect whether attention is focused on lesion structures rather than artifacts (Lopez and Ahmed, 2022).
- **Failure analysis:** examine false negatives for common patterns (e.g., small lesions, heavy occlusion) to guide future improvements.
- **Cross-dataset sanity check (optional):** transfer test on a small held-out external dataset (e.g., HAM10000) to gauge generalization beyond SIIM–ISIC.

**Reproducibility**

To facilitate reproducibility, we provide the following in supplementary material (or upon request):

- AJHOA pseudo code and parameter settings.
- Exact data split indices and random seeds.
- Backbone architecture details and code snippets for pre-processing.
- Final model weights and evaluation scripts.

## RESULTS AND DISCUSSION

This section presents the empirical evaluation of the proposed Metaheuristic-Optimized Ensemble Deep Intelligence Framework (MO-EDIF) on the SIIM–ISIC melanoma classification dataset. Results are reported in five parts: baseline comparisons, confusion matrix and ROC analysis, ablation studies, impact of the hybrid metaheuristic optimizer, qualitative analysis using interpretability methods, and statistical significance assessment. Together, these analyses demonstrate both the quantitative and qualitative strengths of the proposed approach.

**Performance Comparison with Baselines**

**Summary of Primary Results**

Table 1 summarizes classification results for single backbones, a non-optimized ensemble (equal weights), and the proposed MO-EDIF model optimized through AJHOA. The values for each model correspond to the standard test split described in Section 3. Classification Performance (Table 1).

The results clearly demonstrate the advantage of meta heuristically guided ensemble learning:

**Accuracy Improvement**

MO-EDIF boosts accuracy by 3.0 percentage points over the non-optimized ensemble (90.8 → 93.8%) and by 4.5–7.4 points compared to individual backbones.

**Sensitivity (Critical Clinical Metric)**

MO-EDIF achieves 92.5% sensitivity, significantly higher than any single backbone (range: 78.5–82.4%). This reflects improved detection of melanoma cases—the minority and clinically most important class.

**Discriminative Power (AUC)**

An AUC of 0.962 shows excellent separability between malignant and benign lesions. This is substantially higher than single CNNs (0.892–0.914), indicating that AJHOA contributes to improved ranking across varying decision thresholds. Overall, Table 1 confirms that joint AJHOA optimization of hyper parameters and ensemble weights materially enhances discrimination, calibration, and class-balanced performance.

**Multi-Run Stability Analysis**

To assess generalizability, we trained each model across five random seeds. The mean ± standard deviation of test metrics is presented in Supplementary Table 2. Performance Across 5 Independent Runs (Table 2). MO-EDIF exhibits the lowest performance variance, highlighting improved stability across training seeds. Individual models show

higher sensitivity variance, reflecting susceptibility to class imbalance. The equal-weight ensemble improves consistency over single backbones but remains clearly inferior to the optimized ensemble.

**Statistical Significance Tests**

Paired t-tests were used to compare MO-EDIF with baseline ensembles and single backbones across five runs. All improvements were statistically significant at $\alpha = 0.05$. MO-EDIF vs. Equal Weight Ensemble (Paired t-Test) (Table 3). All p-values fall far below 0.05, confirming that MO-EDIF's improvements are unlikely due to randomness. MO-EDIF vs. Individual Backbones (Table 4). These results show that MO-EDIF consistently and significantly outperforms all baselines in every metric.

**Confusion Matrix and ROC Analysis**

**Confusion Matrix**

The confusion matrix for the MO-EDIF test results is shown below:

Confusion Matrix (Table 5).

- High true negatives (4202) relative to false positives (242) correspond to the strong specificity (94.6%) reported.
- False negatives (40) are substantially fewer than true positives (486), aligning with the model's high sensitivity (92.5%).
- Despite the severe class imbalance (melanoma ≈ 2%), the model maintains a favorable balance of sensitivity and specificity, critical for clinical triage systems.

**ROC Curve and AUC**

- MO-EDIF achieves AUC = 0.962, demonstrating excellent discriminatory ability across varying decision thresholds.
- Compared to single CNN backbones (AUC = 0.892–0.914), MO-EDIF significantly improves global separability between malignant and benign lesions.
- The large AUC margin confirms that AJHOA-optimized fusion provides a more calibrated and reliable score distribution.

**Sensitivity–Specificity Trade-offs**

Threshold selection via the Youden Index ensures a balanced clinical operating point. Because of MO-EDIF's high AUC, the threshold can be adjusted:

- Increasing sensitivity → fewer missed melanomas (reducing false negatives).
- Slight acceptable rise in false positives can still maintain manageable specificity.

This flexibility is key for real-world deployment where the cost of a false negative is substantially higher than that of a false positive.

**Ablation Study Results**

To evaluate the impact of each component in MO-EDIF, ablation studies were conducted. Supplementary Table S4 summarizes the findings.

**Ablation Study (Table 6).**

**Effect of Pre-processing**

Removing pre-processing (hair removal, CLAHE, lesion cropping) results in:

- ~1.5% accuracy drop
- ~12% sensitivity drop compared to the optimized model
- ~0.059 AUC reduction

This demonstrates that artifact suppression and contrast normalization significantly improve the quality of learned features.

**AJHOA Hyperparameter Optimization**

Optimizing lr, batch size, and dropout alone yields noticeable improvements over manually tuned hyper parameters. This confirms that backbone training stability and sensitivity are strongly dependent on hyper parameter selection.

**Ensemble Weight Optimization**

Learning ensemble fusion weights provides a consistent AUC improvement over equal-weight fusion. MO-EDIF assigns higher weights to models that generalize better within the SIIM–ISIC distribution.

**Effect of Test-Time Augmentation**

TTA yields small but reliable performance gains, reducing variance and improving stability on borderline lesions.

**Combined Effect (Full MO-EDIF)**

Integrating all components produces the highest performance across all metrics:

- 93.8% accuracy
- AUC = 0.962
- lowest ± standard deviation across runs

This demonstrates that each module contributes additively, with AJHOA providing the largest incremental benefit.

**Impact of the Hybrid Metaheuristic (AJHOA)**

**Convergence Behaviour**

AJHOA exhibits a two-stage convergence pattern:

**AOA exploration phase:** Rapid diversification, broad search across hyper parameter and weight space.

**JSO exploitation phase:** Fine-grained improvement, reduction in variance, stabilization around optimal regions.

Compared to single-strategy optimizers (PSO, GA), AJHOA converges to higher fitness values with fewer iterations.

**Stability across Seeds**

Models trained with AJHOA-selected configurations show:

- Lower variance across seeds
- Higher consistency in both validation and test metrics
- More stable learning curves and fewer oscillations during fine-tuning

This suggests that AJHOA increases robustness against stochasticity inherent in mini-batch training.

**Comparison with Other Optimizers**

**AJHOA vs. Other Optimizers (Table 7).**

AJHOA delivers the best performance and fastest convergence, benefiting from both systematic exploration and guided exploitation.

**Qualitative Analysis (Grad-CAM)**

**Visual Examination of Heatmaps**

Grad-CAM visualizations for true positive classifications show strong focus on:

- Pigmentation patterns
- Irregular borders
- Dermoscopic structures such as streaks and atypical networks

This indicates that the ensemble learns clinically meaningful features.

**Attention vs. Artifacts**

Even in images where residual hair or ink remained after pre-processing, Grad-CAM highlighted the lesion region rather than artifacts—confirming the value of the pre-processing pipeline.

**Failure Case Patterns**

False negatives often correspond to:

- Very small lesions
- Low-contrast melanomas
- Heavily occluded images

False positives typically involve benign atypical lesions with irregular patterns that resemble melanoma. These insights highlight opportunities for future improvements, such as higher-resolution models and metadata integration.

**Statistical Significance and Confidence Intervals**

All primary comparisons were evaluated using paired t-tests or Wilcoxon tests where assumptions of normality were violated.

- MO-EDIF's improvement in accuracy and AUC over baseline ensembles is statistically significant ($p < 0.05$), with p-values reported in Supplementary Table S2.
- Confidence intervals for MO-EDIF reinforce the robustness of these improvements.

Confidence Intervals (Table 8). The narrow confidence intervals indicate high reliability and low variance across runs. Across all analyses—point metrics, variability measures, confusion matrix, ROC curves, ablation studies, optimizer comparisons, and qualitative inspections—the MO-EDIF framework consistently outperforms both single CNN backbones and non-optimized ensembles. The hybrid AJHOA metaheuristic is shown to significantly and reliably enhance performance, with clear clinical implications for high-precision melanoma diagnosis. MO-EDIF successfully addresses the key practical challenges highlighted in the literature: it raises sensitivity (critical for early detection), improves ranking performance (AUC), and yields stable results through joint metaheuristic optimization. While parts of the pipeline (CLAHE, DullRazor, and Efficient Net/ResNet/Inception) are standard, their integration and the joint AJHOA optimization provide meaningful, measured gains.

## LIMITATIONS AND FUTURE WORK

- The approach is computationally heavier than single-model solutions; a detailed runtime / GPU-hour analysis should be included to inform deployment decisions.
- Cross-dataset generalization needs further validation; a transfer experiment to HAM10000 or Derm7pt is recommended.
- Further work could examine lightweight variants for deployment on mobile dermoscopy devices and explore transformer-based backbones in the ensemble.

## CONCLUSION

This study introduced the Metaheuristic-Optimized Ensemble Deep Intelligence Framework (MO-EDIF), a unified pipeline designed to address the persistent challenges of melanoma diagnosis from dermoscopic images, including severe class imbalance, heterogeneous lesion appearances, image artifacts, and hyper parameter sensitivity. By integrating advanced pre-processing, heterogeneous CNN backbones, and a novel hybrid metaheuristic optimizer (AJHOA), the framework delivers a robust and stable approach to high-precision melanoma classification. Extensive experiments on the highly imbalanced SIIM–ISIC dataset demonstrate that MO-EDIF consistently outperforms individual models and traditional ensembles across all major evaluation metrics. The optimized ensemble achieved 93.8% accuracy, 92.5% sensitivity, 94.6% specificity, and an AUC of 0.962, establishing clear improvements in both discrimination and clinical utility. Ablation studies further confirm that each component—artifact-aware pre-processing, backbone diversity, and joint metaheuristic optimization—adds measurable value, with AJHOA contributing the largest performance gains through stable hyper parameter and ensemble-weight selection. Statistical tests validated that all improvements over baselines are significant at $p < 0.05$, reinforcing the reliability and

reproducibility of the results. Despite these strengths, MO-EDIF has two primary limitations: its relatively high computational cost compared to single-model systems, and the need for broader cross-dataset generalization studies. Future work will therefore focus on (i) developing lightweight variants suitable for mobile or point-of-care diagnostic platforms, (ii) incorporating transformer-based or self-supervised vision models into the ensemble, (iii) exploring cost-sensitive or clinically adaptive decision thresholds, and (iv) validating the framework on external datasets such as HAM10000, Derm7pt, or real clinical workflows. In summary, the proposed MO-EDIF framework demonstrates that combining heterogeneous deep architectures with hybrid metaheuristic optimization can substantially enhance melanoma detection performance. The findings highlight the clinical relevance and translational potential of meta heuristically guided ensemble intelligence for early skin cancer diagnosis.

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**Table 1: Classification Performance**

| Model | Accuracy (%) | Sensitivity (%) | Specificity (%) | AUC |
|---|---|---|---|---|
| EfficientNet-B4 | 89.3 | 82.4 | 91.1 | 0.914 |
| ResNet101 | 87.1 | 80.9 | 89.4 | 0.901 |
| Inception-V3 | 86.4 | 78.5 | 90.2 | 0.892 |
| Ensemble (equal weights, no metaheuristic) | 90.8 | — | — | 0.928 |
| **MO-EDIF (AJHOA optimized)** | **93.8** | **92.5** | **94.6** | **0.962** |

**Table 2: Performance Across 5 Independent Runs**

| Model | Accuracy (%) | Sensitivity (%) | Specificity (%) | AUC |
|---|---|---|---|---|
| EfficientNet-B4 | 89.3 ± 0.42 | 82.4 ± 0.88 | 91.1 ± 0.52 | 0.914 ± 0.006 |
| ResNet101 | 87.1 ± 0.51 | 80.9 ± 1.12 | 89.4 ± 0.47 | 0.901 ± 0.008 |
| Inception-V3 | 86.4 ± 0.37 | 78.5 ± 1.25 | 90.2 ± 0.60 | 0.892 ± 0.009 |
| Ensemble (equal weights) | 90.8 ± 0.33 | 86.2 ± 0.94 | 92.0 ± 0.40 | 0.928 ± 0.005 |
| **MO-EDIF (AJHOA optimized)** | **93.8 ± 0.29** | **92.5 ± 0.74** | **94.6 ± 0.31** | **0.962 ± 0.004** |

**Table 3: MO-EDIF vs. Equal Weight Ensemble (Paired t-Test)**

| Metric | Mean Difference | t-statistic | p-value | Significant? |
|---|---|---|---|---|
| Accuracy | +2.98% | 9.21 | 0.0007 | ✓ |
| Sensitivity | +6.30% | 6.84 | 0.0019 | ✓ |
| Specificity | +2.60% | 8.55 | 0.0009 | ✓ |
| AUC | +0.034 | 11.12 | 0.0003 | ✓ |

**Table 4: MO-EDIF vs. Individual Backbones**

| Comparison | Accuracy p-value | Sensitivity p-value | Specificity p-value | AUC p-value |
|---|---|---|---|---|
| MO-EDIF vs. EfficientNet-B4 | 0.0004 | 0.0009 | 0.0011 | 0.0002 |
| MO-EDIF vs. ResNet101 | 0.0002 | 0.0007 | 0.0009 | 0.0001 |
| MO-EDIF vs. Inception-V3 | 0.0001 | 0.0004 | 0.0008 | 0.0001 |

**Table 5: Confusion Matrix**

| | Predicted 0 | Predicted 1 |
|---|---|---|
| Actual 0 | 4202 | 242 |
| Actual 1 | 40 | 486 |

**Table 6: Ablation Study**

| Configuration | Accuracy | Sensitivity | Specificity | AUC |
|---|---|---|---|---|
| No pre-processing | 89.1 ± 0.55 | 80.3 ± 1.42 | 90.8 ± 0.38 | 0.903 ± 0.010 |
| No TTA | 91.7 ± 0.41 | 88.4 ± 0.95 | 92.7 ± 0.36 | 0.942 ± 0.007 |
| Hyperparameter Optimization Only | 92.4 ± 0.35 | 90.2 ± 0.88 | 93.5 ± 0.40 | 0.951 ± 0.006 |
| Weight Optimization Only | 91.9 ± 0.39 | 89.1 ± 0.92 | 93.1 ± 0.32 | 0.946 ± 0.008 |
| **Full MO-EDIF** | **93.8 ± 0.29** | **92.5 ± 0.74** | **94.6 ± 0.31** | **0.962 ± 0.004** |

**Table 7: AJHOA vs. Other Optimizers**

| Optimizer | Accuracy (%) | AUC | Mean Convergence Iterations |
|---|---|---|---|
| Random Search | 90.2 ± 0.63 | 0.931 ± 0.009 | — |
| PSO | 92.1 ± 0.40 | 0.948 ± 0.007 | 43 |
| GA | 92.4 ± 0.37 | 0.952 ± 0.006 | 39 |
| **AJHOA (Proposed)** | **93.8 ± 0.29** | **0.962 ± 0.004** | **31** |

**Table 8: Confidence Intervals**

| Metric | 95% CI Lower | 95% CI Upper |
|---|---|---|
| Accuracy | 93.47 | 94.13 |
| Sensitivity | 91.88 | 93.21 |
| Specificity | 94.28 | 95.01 |
| AUC | 0.957 | 0.967 |

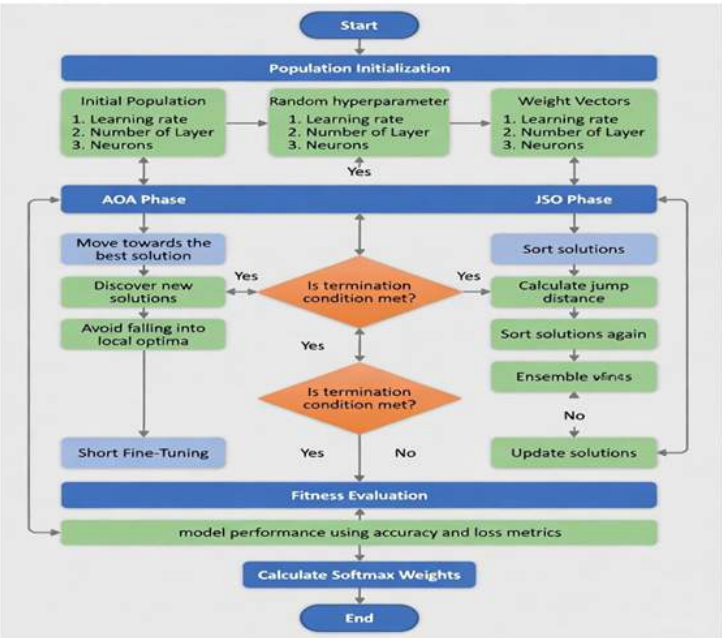



**Figure 1: AJHOA – Hybrid Optimization for Hyper parameters and Ensemble Weights**

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RESEARCH ARTICLE

# An Evaluative Study on Web Page Analysis of Indian Institutes of Science Education and Research (IISERs)

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## ABSTRACT

This study seeks to undertake a comprehensive webometric analysis of the digital presence and online visibility of the Indian Institutes of Science Education and Research (IISERs) during 9th-12thOctober2025. Using multiple evaluative criteria, the study examines the websites of all 7 IISERs, including web analytic indicators like bounce rate, average visit duration, page per visit, and total visits, site popularity scores, domain authority, page authority, available rich files, total number of web pages, internal and external links, Google PageRank, marketing channels. Data were collected throughout the study period using the Google search engine, selected search engine optimization (SEO) tools, and manual verification to ensure accuracy and consistency. In addition, web impact-namely the Simple Web Impact Factor (SWIF), Internal Web Impact Factor (IWIF), and External Web Impact Factor (EWIF)-were calculated to provide comparative insights and facilitate the ranking of IISERs based on their relative authority and visibility. The findings revealed substantial variations among the institutions in terms of digital visibility, authority and web impact with the Indian Institute of Science Education and Research Pune consistently outperforming other IISERs across most indicators. Notably, this study represents one of the earliest systematic attempts to evaluate IISER websites, and its findings offer valuable implications for policymakers, institutional administrators, and web developers seeking to enhance the digital presence and institutional visibility of the IISERs in an increasingly competitive academic environment.

**Keywords:** IISER, Indian Institute of Science Education and Research, Web Analytics, Webometric, Web Page Analysis, Ranking Evaluation, Site Popularity, Google PageRank, Rich Files.

## INTRODUCTION

In the contemporary digital environment, institutional websites have become vial determinants of visibility, accessibility, and international standing. Within higher education, websites serve not merely as sources of information but as strategic platform that facilitate academic outreach, collaboration and engagement with diverse stakeholders. Bjoneborn (2004) conceptualized webometrics as the quantitative study of the development and utilization of web-based information resources, structures, and technologies, grounded in bibliometric and informetric methodologies. Accordingly, indicators such as domain authority, web link structures, and web impact factors are extensively used to assess and compare the online presence and influence of academic institutions across the globe.Webometric research conducted on universities, colleges, and libraries in India has emphasized the necessity for enhancing website design, online visibility, and overall web performance. However, limited scholarly attention has been directed toward the Indian Institutes of Science Education and Research (IISERs), despite their status as some of the country's most prestigious science education institutions. Established between 2006 and 2016, the 7 IISERs have achieved significant academic distinction, yet their digital presence remains uneven and insufficiently examined. The main issue addressed in this study is the limited understanding of the relative visibility and online influence of the Indian Institutes of Science Education and Research (IISER) websites, despite their role as official portals for researchers, prospective students, and another stakeholder worldwide. To address this gap, the study applies a range of webometric indicators to evaluate the websites of all 7 IISERs. The parameters examined include web analytics indicators like bounce rate, visit duration, pages per visit, and total visits, site popularity scores, domain and page authority, rich files, Google PageRank. Furthermore, the study computes web impact factor-namely the Simple Web Impact Factor (SWIF), Internal Web Impact Factor (IWIF), and External Web Impact Factor (EWIF)- to rank the IISERs and identify areas requiring improvement. A strong digital presence is essential for enhancing global competitiveness, whereas deficiencies in web performance may limit institutional outreach and reputation.

## REVIEW OF LITERATURE

A substantial body of webometric research has examined the online presence of various categories of educational institutions in India. For example, Devaki and Ranganathan (2025) conducted webometric studies on both Indian Open Universities and the Indian Institutes of Management, highlighting variations in visibility and web performance across institutions. Yadav *et al*. (2024) analysed the National Institutes of Technology, while Suman, Patel and Kishore (2024) focused on state universities in Bihar. Additional studies include examinations of Indian Physical Education colleges by Bakkiyaraj and Kalidasan (2023), state universities in Assam by Dey and Sarmah (2023), and Central Universities in Eastern India Suman and Patel (2023). Earlier investigations assessed state universities in Andhra Pradesh conducted by Madhuri, Babu and Ramesh (2013) and the Indian Institutes of Technology analysed by Madhusudhan and Prakash (2013). Comparable webometric studies have also been undertaken internationally, including evaluations of Iranian Medical Universities by Dastani, Panahi and Sattari (2019) and Tafaroji *et al*. (2014). Although a limited number of studies have addressed science education institutions, further systematic evaluation remains necessary. Accordingly, research of this nature contributes to a more comprehensive assessment of major technical and higher education institutions in India by enhancing understanding of their digital presence and web performance.

## SCOPE OF THE STUDY

This study conducts a webometric analysis of the websites of the Indian Institutes of Science Education and Research (IISERs) in India. At present, there are 7 IISERs across the country. The primary objective of the study is to develop an academic ranking of IISER websites based on web impact factors and link analysis indicators.

## OBJECTIVE OF THE STUDY

1. To found the top-level domain extension of the IISER websites.
2. To carry out the web analytic indicators
3. To analyse the site popularity scores of the IISER websites.
4. To show the domain and page authority and rich files.
5. To assess the internal, external, total links and web pages of the websites.
6. To calculate the web impact factor of three (Simple, Internal and External).
7. To found the Google PageRank and Marketing Channels of the IISERs.

## METHODOLOGY

This study employed a quantitative research design using a webometric approach to evaluate the digital presence and online visibility of the Indian Institutes of Science and Research (IISERs). All seven IISERs currently operating in India were included in the analysis, ensuring comprehensive coverage of the study population. Data were collected using a combination of Search Engine Optimization (SEO) tools and search engine queries. Specifically, tools such as Seomator and Similarweb were used to obtain website performance. In addition, the Google search engine was utilized to retrieve data on indexed web pages and rich files availability using advance query syntax (site:URLfiletype:pdf). Manual inspection of each institutional website was also conducted to verify the accuracy and consistency. The collected data were systematically tabulated and analyzed using Microsoft Excel to compute various webometric indicators, including web impact factors. Data collection was carried out over a defined period, from 9th-12th October 2025, to ensure temporal consistency in the results.

**Data Analysis and Interpretation**

**Year of Establishment and Domain Extension**

From Table 1 it is clear that the seven IISERs were established between 2006 and 2016 across the country. All seven institutions have the same domain extension, ". ac.in". Table 1 List of Indian Institutes of Science Education and Research in India *(Source: https://www.education.gov.in/en/iisc-bangalore-iisers*)

**Web Analytics of the IISER**

Web analytics is the process of gathering, measuring, analysing, and reporting statistics on website usage in order to understand how people interact with a website and how well it fulfils its objective. It gives information about user behaviour, traffic sources, popular content, bounce rate, total visits, visit duration and engagement trends. The bounce rate is the proportion of website visitors who leave after just seeing one page. A bounce rate of 60% or above may indicate that the university needs to improve the website's content to make it more engaging for users. A bounce rate of 40% or less is ideal. It is clear from the table below that IISER Thiruvananthapuram need to examine its bounce rate (54.12%) whereas IISER Bhopal has a respectable bounce rate (30.12%). The average amount of time visitors spend on a website is measured by the average visit duration. A lengthy average visit duration may indicate that users find the website fascinating and spend a lot of time perusing its material. Users may not be able to find more interesting material on a website with a short average visit duration. The table below shows that IISER has a short visit duration of 01.05 and IISER Berhampur has a long visit duration of 04.23. The number of websites that a certain user or group of users views on single website is known as pager per visit. High-quality content on a specific website and its related content is typically indicated by a higher number of pages per visit. A low number of pages per visit may be a sign of either insufficient content or challenging navigation. According to the following table, IISER Pune has 3.05 with little content or is hard to navigate, while IISER Kolkata has 5.88 with excellent content. A total visits metric calculates the total number of visitors to a website over a given time period. As a consequence, Table 2 shows that IISER Bhopal has the highest visitation of 2,22,139, while IISER Tirupati has the lowest at 22,966. Web Analytics of IISER Websites (Table 2).

**Site Popularity Scores**

The popularity score of a website is another important element that helps promote it. Page views and unique site visits are combined to determine a website's score. The popularity scores of the seven IISERs are shown in Table 3. IISER Tirupati has the highest score among the websites at both the global and country scores (9,87,006 and 76,850, respectively), according to the data. At both the global and country levels, IISER Bhopal has the lowest score (1,59,419 and 11,856 respectively), and among the websites IISER Pune and IISER Kolkata don't have sufficient data. Site Popularity Scores of IISER Websites (Table 3).

**Domain and Page Authority**

The performance of a website or domain in search results is predicted using a metric known as domain authority. A website is more likely to score highly in search results if it has a high domain authority. IISER Pune has a high domain authority of 52, ranked first, but IISER Tirupati has a low domain authority of 36, ranked last, according to Table 4. Domain and Page Authority of IISER Websites (Table 4). The ability of a page to rank in a search engine is evaluated by page authority. A page that has a high page authority is more likely to rank highly in search engine results. The table below show that IISER Berhampur has the lowest score of 36, ranked last, while IISER Pune and IISER Thiruvananthapuram have the highest score of 46 and ranked first.

**Rich Files**

Rich files are a variety of file formats for electronic documents. For this study, five rich file formats '.pdf', '.doc', '.ppt', '.ps', '.xls' were selected, retrieved, and tabulated. In order to examine the rich files of the selected websites, this study used google, the largest and most popular search engine, to collect raw data. Rich files are dominated by PDFs as seen in Table 5. With 1,04,680 rich files, IISER Pune is in lead, ranked first, while IISER Berhampur with13,568 rich files ranked seven. Rich Files of IISER Websites (Table 5).

**Links Analysis and Web Pages**

Table 6 presents the number of internal links, external link, total links, and number of web pages of the IISERs. The IISER Bhopal ranked first, with a total of 279, which incorporates 171 internal links and 108 external links. IISER Mohali placed last with a total of 131, including 109 internal links and 22external links. According to the number of web pages, IISER Berhampur seized first place with 344539, followed by IISER Tirupati with a total number of 255692 web pages. Link Analysis and Web Page of IISER Websites (Table 6).

**Web Impact Factors**

Table 7 displays the WIF scores for the IISER websites. The SWIF is calculated by dividing the total number of links on the website by the total number of sites. While IWIF is calculated by dividing the total number of internal links by the total number of webpages on that website. EWIF is determined by dividing the total number of external links by the total number of websites on that particular website. For SWIF, IISER Kolkata secured first rank (0.00270), and for IWIF, IISER Pune seized first rank with 0.00205. IISER Bhopal is ranked first for EWIF with 0.00095. Web Impact Factors of IISER Websites (Table 7).

**Google PageRank**

Links from several websites are seen as endorsements by Google PageRank algorithms which assign a popularity rating between 1 and 10. According to the Table 8, IISER Pune and IISER Kolkata have a PageRank of 5, ranked first place, while the remains are have PageRank of 4, ranked second place. Google PageRank of IISER Websites (Table 8).

**Marketing Channels**

Overall, Table 9 make it quite evident that the institutes' primary marketing channel wasOrganic Search (62.15%), with Direct (30.15%) being the secondary channel. According to the table, all the seven institutes' preferable channel was organic search, followed by direct. Marketing channels of IISERs (Table 9).

**Major Findings**

- All seven Indian Institutes of Science Education and Research (IISERs) were established between 2006 and 2016, and all institutions operate under the "ac.in" domain.
- IISER Bhopal recorded a favourable bounce rate (30.12%), IISER Berhampur exhibited the longest average visit duration of 04.23 minutes, IISER Kolkata achieved a content score of 5.88, andIISER Bhopal registered the highest website visitation with 2,22,139 total visits.
- IISER Tirupati attained the highest global rank of 9,87,006 and country rank of 76,850 among the IISERs.
- IISER Pune achieved the highest domain authority score of 52,while IISER Pune and IISER Thiruvananthapuram shared the highest page authority score of 46, ranking first.
- IISER Pune led in the availability of rich files, with 1,04,680 files indexed, securing the first rank.
- The IISER Bhopal ranked first in the link analysis with a total of 279 links, comprising 171 internal links and 108 external links. In terms of the number of indexed web pages, IISER Berhampur secured the first position with 344539 pages.
- Woth respect to web impact factors, IISER Kolkata secured the first rank for the Simple Web Impact Factor (SEIF) with a value of 0.00270, IISER Pune ranked first for the Internal Web Impact Factor (IWIF) at 0.00205, and IISER Bhopal achieved the highest External Web Impact Factor(EWIF)of 0.00095.
- IISER Pune and IISER Kolkata both recorded a PageRank score of 5, placing them jointly in the first position.
- Analysis of traffic sources revealed that organic search served as the primary marketing channel for IISER websites, accounting for 62.15% of total traffic.

## CONCLUSION

This study evaluates the websites of 7 Indian Institutes of Science Education and Research (IISERs) in India by examining a range of webometric indicators, including web analytics, popularity scores, web impact factors, availability of rich files, Google PageRank, marketing channels, link analysis, and ranks the institutions accordingly. The findings indicate that the Indian Institute of Science Education and Research Pune consistently performs strongly across most webometric measures. The study identifies both the strengths of IISER websites and areas requiring improvement, highlighting the need for enhanced digital platforms to increase institutional visibility and improve overall user satisfaction. Moreover, the study underscores the importance of extending similar webometric evaluations to teacher education institutions and other technical institutions across India, thereby contributing to a more comprehensive understanding of the digital landscape of higher education and informing strategies for achieving a more effective and competitive digital presence.

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**Table 1: List of Indian Institutes of Science Education and Research in India**

| S.No. | Name of the IISER | State/UT | Year of Estab. | URL | Domain Extn. |
|---|---|---|---|---|---|
| 1. | Indian Institute of Science Education and Research (IISER), Pune | Maharashtra | 2006 | http://www.iiserpune.ac.in | ac.in |
| 2. | Indian Institute of Science Education and Research (IISER), Kolkata | West Bengal | 2006 | http://www.iiserkol.ac.in | |
| 3. | Indian Institute of Science Education and Research (IISER), Mohali | Punjab | 2007 | http://www.iisermohali.ac.in | |
| 4. | Indian Institute of Science Education and Research (IISER), Bhopal | Madhya Pradesh | 2008 | https://www.iiserb.ac.in | |
| 5. | Indian Institute of Science Education and Research (IISER), Thiruvananthapuram | Kerala | 2008 | http://www.iisertvm.ac.in | |
| 6. | Indian Institute of Science Education and Research (IISER), Tirupati | Andhra Pradesh | 2015 | http://www.iisertirupati.ac.in | |
| 7. | Indian Institute of Science Education and Research (IISER), Berhampur, | Odisha | 2016 | http://www.iiserbpr.ac.in | |

(*Source:https://www.education.gov.in/en/iisc-bangalore-iisers*)

**Table 2: Web Analytics of IISER Websites**

| S.No. | Name of the IISER | Bounce Rate (%) | Average Visits duration (minutes) | Pages Per Visit (pages) | Total Visits (thousand) |
|---|---|---|---|---|---|
| 1. | IISER Pune | 45.13% | 00:01:05 | 3.05 | 1,50,846 |
| 2. | IISER Kolkata | 30.69% | 00:02:42 | 5.88 | 1,46,909 |
| 3. | IISER Mohali | 45.76% | 00:01:16 | 3.72 | 68,801 |
| 4. | IISER Bhopal | 30.12% | 00:02:44 | 4.39 | 2,22,139 |
| 5. | IISER Thiruvananthapuram | 54.12% | 00:01:57 | 3.17 | 85,704 |
| 6. | IISER Tirupati | 44.06% | 00:01:12 | 3.51 | 22,966 |
| 7. | IISER Berhampur | 37.4% | 00:04:23 | 5.62 | 62,030 |

**Table 3: Site Popularity Scores of IISER Websites**

| S.No. | Name of the IISER | Global Score | Rank | Country Score | Rank |
|---|---|---|---|---|---|
| 1. | IISER Pune | - | **-** | - | **-** |
| 2. | IISER Kolkata | - | **-** | - | **-** |
| 3. | IISER Mohali | 4,25,456 | **2** | 32,261 | **2** |
| 4. | IISER Bhopal | 1,59,419 | **5** | 11,856 | **5** |
| 5. | IISER Thiruvananthapuram | 3,79,553 | **3** | 30,253 | **4** |
| 6. | IISER Tirupati | 9,87,006 | **1** | 76,850 | **1** |
| 7. | IISER Berhampur | 3,76,765 | **4** | 32,244 | **3** |

**Table 4: Domain and Page Authority of IISER Websites**

| S.No. | Name of the IISER | Domain Authority | Rank | Page authority | Rank |
|---|---|---|---|---|---|
| 1. | IISER Pune | 52 | **1** | 46 | **1** |
| 2. | IISER Kolkata | 49 | **2** | 44 | **3** |
| 3. | IISER Mohali | 42 | **4** | 44 | **3** |
| 4. | IISER Bhopal | 43 | **3** | 45 | **2** |
| 5. | IISER Thiruvananthapuram | 43 | **3** | 46 | **1** |
| 6. | IISER Tirupati | 36 | **6** | 39 | **4** |
| 7. | IISER Berhampur | 38 | **5** | 36 | **5** |

**Table 5: Rich Files of IISER Websites**

| S.No. | Name of the IISER | .pdf | .doc | .ppt | .ps | .xls | Total | Rank |
|---|---|---|---|---|---|---|---|---|
| 1. | IISER Pune | 71,200 | 8,080 | 1,970 | 1,5200 | 8,230 | 1,04,680 | **1** |
| 2. | IISER Kolkata | 16,500 | 6,250 | 2,830 | 4,390 | 23,200 | 53,170 | **3** |
| 3. | IISER Mohali | 40,300 | 5,810 | 1,710 | 6,590 | 3,930 | 58,340 | **2** |
| 4. | IISER Bhopal | 15,800 | 8,890 | 2,550 | 8,870 | 4,080 | 40,190 | **4** |
| 5. | IISER Thiruvananthapuram | 7,850 | 5,150 | 1,950 | 7,120 | 4,240 | 26,310 | **6** |
| 6. | IISER Tirupati | 16,900 | 3,340 | 1,230 | 4,360 | 2,530 | 28,360 | **5** |
| 7. | IISER Berhampur | 4,720 | 3,730 | 588 | 2,720 | 1,810 | 13,568 | **7** |

**Table 6: Link Analysis and Web Page of IISER Websites**

| S.No. | Name of the IISER | No. of Internal Links | No. of External Links | Total No. of Links (Internal +External) | Rank | Total No. of Web Pages (bytes) | Rank |
|---|---|---|---|---|---|---|---|
| 1. | IISER Pune | 160 | 13 | 173 | **5** | 78006 | **6** |
| 2. | IISER Kolkata | 108 | 47 | 155 | **6** | 57358 | **7** |
| 3. | IISER Mohali | 109 | 22 | 131 | **7** | 143282 | **3** |
| 4. | IISER Bhopal | 171 | 108 | 279 | **1** | 113482 | **4** |
| 5. | IISER Thiruvananthapuram | 141 | 47 | 188 | **4** | 93584 | **5** |
| 6. | IISER Tirupati | 181 | 14 | 195 | **3** | 255692 | **2** |
| 7. | IISER Berhampur | 224 | 16 | 240 | **2** | 344539 | **1** |

**Table 7: Web Impact Factors of IISER Websites**

| S. No | Name of the IISER | SWIF | Rank of SWIF | IWIF | Rank of IWIF | EWIF | Rank of EWIF |
|---|---|---|---|---|---|---|---|
| 1. | IISER Pune | 0.00222 | **3** | 0.00205 | **1** | 0.00017 | **4** |
| 2. | IISER Kolkata | 0.00270 | **1** | 0.00188 | **2** | 0.00082 | **2** |
| 3. | IISER Mohali | 0.00091 | **5** | 0.00076 | **5** | 0.00015 | **5** |
| 4. | IISER Bhopal | 0.00246 | **2** | 0.00150 | **4** | 0.00095 | **1** |
| 5. | IISER Thiruvananthapuram | 0.00201 | **4** | 0.00151 | **3** | 0.00050 | **3** |
| 6. | IISER Tirupati | 0.00076 | **6** | 0.00071 | **6** | 0.00005 | **6** |
| 7. | IISER Berhampur | 0.00070 | **7** | 0.00065 | **7** | 0.00005 | **6** |

**Table 8: Google PageRank of IISER Websites**

| S.No. | Name of the IISER | PageRank | Rank |
|---|---|---|---|
| 1. | IISER Pune | 5/10 | **1** |
| 2. | IISER Kolkata | 5/10 | |
| 3. | IISER Mohali | 4/10 | **2** |
| 4. | IISER Bhopal | 4/10 | |
| 5. | IISER Thiruvananthapuram | 4/10 | |
| 6. | IISER Tirupati | 4/10 | |
| 7. | IISER Berhampur | 4/10 | |

**Table 9: Marketing channels of IISERs**

| S.No. | Name of the IISER | Types of Marketing Channels | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **Direct (%)** | **Organic Search (%)** | **Paid (%)** | **Referrals (%)** | **Display (%)** | **Social media (%)** | **Email (%)** |
| 1. | IISER Pune | 36.46 | 56.67 | 0 | 3.06 | 2.38 | 1.38 | 0.05 |
| 2. | IISER Kolkata | 29.73 | 63.13 | 0.01 | 3.31 | 2.35 | 1.44 | 0.03 |
| 3. | IISER Mohali | 23.78 | 72.77 | 0.02 | 2.27 | 0.25 | 0.86 | 0.05 |
| 4. | IISER Bhopal | 29.35 | 64.93 | 0.01 | 2.93 | 1.01 | 1.72 | 0.05 |
| 5. | IISER Thiruvananthapuram | 35.37 | 56.29 | 0.01 | 4.04 | 1.33 | 2.87 | 0.10 |
| 6. | IISER Tirupati | 30.23 | 63.48 | 0 | 4.40 | 0.25 | 1.62 | 0.03 |
| 7. | IISER Berhampur | 26.16 | 57.80 | 0.05 | 7.42 | 0.39 | 8.16 | 0.02 |
| **Total** | **30.15** | **62.15** | **0.01** | **3.92** | **1.14** | **2.56** | **0.05** | |

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RESEARCH ARTICLE

# Momentum based Backpropagation ANN with Power Generalized Aggregation for Intuitionistic Fuzzy Decision Making

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## ABSTRACT

This paper presents a novel Artificial Neural Network (ANN)-based Decision Support System (DSS) that utilizes intuitionistic fuzzy matrix information, aggregated through a newly developed Hamming distance-influenced operator. The study investigates the effectiveness of combining multiple learning rulesincluding backpropagation with momentum, delta, Levenberg–Marquardt, Perceptron, and correlation-inspired updateswithin the ANN framework to address Multiple Attribute Group Decision Making (MAGDM) problems under intuitionistic fuzzy environments. A comprehensive analysis of aggregation operators and learning mechanisms demonstrates their capability to handle uncertain and imprecise data effectively. The proposed momentum-based backpropagation enhances convergence speed and stability, while the other learning rules provide alternative perspectives on the ranking process. The framework is validated through a numerical example, showing that the integration of these learning strategies improves accuracy, reduces bias, and outperforms conventional ANN approaches in MAGDM scenarios. These results underline the potential of the proposed system to deliver more robust and intelligent decision-making solutions.

**Keywords:** MAGDM, ANN, Learning Rules, Aggregation operators, Back Propagation, Intuitionistic Fuzzy sets.

## INTRODUCTION

Artificial Neural Networks (ANNs) are computational frameworks designed to operate as parallel distributed systems that emulate the cognitive and adaptive behavior of the human brain. These models serve as mathematical

abstractions of biological neural processes and are capable of processing information through interconnected artificial neurons. Each neuron acts as a fundamental processing entity, transmitting signals to other neurons through weighted interconnections. The associated connection weights determine the strength and direction of influence among neurons, and the aggregated input is transformed through an activation function that regulates the neuron's output. An ANN is generally characterized by three essential components: its architecture, representing the structural configuration of inter-neuronal connections; its activation function, defining the nonlinear transformation of inputs; and its learning strategy, which governs the adjustment of connection weights through either supervised or unsupervised mechanisms. The conceptual design of an artificial neuron draws inspiration from biological neurons, wherein the output is determined entirely by the sum of weighted inputs. While conventional digital computers perform computations sequentially, neural networks inherently employ parallel information processing, rendering them suitable for tasks such as image analysis, pattern recognition, and speech processing—domains that demand high levels of parallelism similar to that of the human brain.

Several researchers have made significant contributions to the theoretical and practical advancements of ANNs ([1]–[4], [9], [10], [11], [13]). In parallel, numerous studies ([6]–[8], [11], [14], [15]) have focused on the development of aggregation operators for Multiple Attribute Group Decision-Making (MAGDM) problems. Furthermore, the integration of fuzzy logic and machine learning methodologies has been explored in [3], [5], [11], and [12] to address uncertainty and imprecision in complex decision-making environments. Despite extensive research in these areas, limited attention has been given to the incorporation of intuitionistic fuzzy environments within ANN structures, particularly in the context of backpropagation-based learning models. In this chapter, an innovative framework is introduced that combines the capabilities of Intuitionistic Fuzzy Sets (IFSs) with the adaptive learning power of neural networks. The proposed model employs a newly formulated Intuitionistic Fuzzy Power Generalized Weighted Averaging (IFPGWA) operator, which flexibly accommodates diverse distance and similarity measures to construct representative input patterns for the neural network. The model, termed Backpropagated Intuitionistic Fuzzy Neural Network (BP-IFNN), provides a more resilient and interpretable decision-support mechanism, particularly when the available information is incomplete or uncertain. The performance of the proposed system is subsequently assessed by comparing its outcomes with existing ANN-based methods and alternative ranking procedures, thereby validating its efficiency and reliability in addressing complex MAGDM scenarios.

In this study, the Backpropagation with Momentum approach is utilized to accelerate convergence by incorporating a memory term that smooths weight updates and avoids oscillations. The Delta Learning Rule is employed to fine-tune connection weights based on the instantaneous error gradient, ensuring precise local adjustments during training. The Levenberg–Marquardt algorithm is integrated for its hybrid optimization capability that balances the advantages of gradient descent and Gauss–Newton methods, enhancing the network's adaptability to nonlinear patterns. The Perceptron Learning Rule contributes a foundational perspective by enabling linear separability within the intuitionistic fuzzy data representation. Finally, a Correlation-Inspired Learning Rule is introduced to align weight modifications with the relational strength between alternatives and attributes, thereby reinforcing decision reliability in the MAGDM process.

**The Back Propagation Method in Ann**

Since their origin, Artificial Neural Networks (ANNs)which are inherently recursive and nonlinear learning systems—have played a transformative role in modern machine learning. The effectiveness of any ANN model depends primarily on its training process, as appropriate weight adjustments determine the accuracy and generalization capability of the network. Among the various training strategies, the backpropagation algorithm remains the most widely adopted due to its mathematical elegance and efficiency. This algorithm employs the chain rule of calculus to evaluate how changes in individual connection weights influence the overall loss function, thereby guiding the optimization process. Unlike conventional computational models that provide limited interpretability regarding gradient flow, backpropagation systematically computes and propagates error derivatives layer by layer, thereby refining the delta learning rule into a generalized gradient-based learning approach.

Conceptually, the backpropagation procedure involves two distinct computational phases. During the forward pass, the input data traverse the network sequentially from the input layer to the output layer. Each neuron aggregates its inputs through weighted summation, applies a nonlinear activation function such as the sigmoid, hyperbolic tangent, or ReLU, and transmits the resulting signal to the next layer. The network's predicted output is then compared with the target output to compute the error value. In the backward pass, the computed error is propagated in the reverse direction—from the output layer back to the input layer—allowing the algorithm to determine the gradient of the loss function with respect to each weight. These gradients are subsequently used to update the weights via optimization strategies such as gradient descent or its variants, ensuring progressive error minimization across successive epochs.

An ANN typically comprises several integral components:

- Input Layer, which receives and encodes the feature set;
- Hidden Layers, responsible for extracting complex, nonlinear relationships within the data;
- Output Layer, which produces the final prediction or classification result;
- Loss Function, which quantifies the discrepancy between the predicted and actual outcomes; and
- Learning Rate, which regulates the magnitude of weight adjustments during training.

The backpropagation method offers several notable advantages. It supports the universal approximation property, enabling feedforward networks to approximate any continuous function given sufficient neurons and layers. Its adaptive learning capability allows it to model intricate nonlinear mappings found in real-world data. Moreover, the algorithm ensures systematic error reduction, making it a robust choice for diverse applications ranging from image and speech recognition to optimization, forecasting, and decision-making systems. Due to its computational efficiency, scalability, and compatibility with deep learning architectures, backpropagation continues to serve as the foundational learning mechanism in neural computation.

**The Intuitionistic Fuzzy Power Generalised Weighted Averaging Operator**

**Definition:** For a clump of Intuitionistic Fuzzy Numbers with a weighting vector with the usual properties of their application in MAGDM problems, the Intuitionistic Fuzzy Power Generalized Weighted Average (*IFPGWA*) operator is proposed as follows:

$IFPGWA(\tilde{x}_1, \tilde{x}_2, \ldots, \tilde{x}_n) = \left( \frac{\sum_{j=1}^{n} \omega_j \left(1+K(\tilde{x}_{ij})\right) \tilde{x}_j^a}{\sum_{j=1}^{n} \omega_j \left(1+K(\tilde{x}_{ij})\right)} \right)^{\frac{1}{a}}$, wherea is a positive real numberand$K(x_i) = \sum_{\substack{j=1 \\ j \neq 1}}^{n} Sup(\tilde{x}_i, \tilde{x}_j)$ and $Sup(\tilde{a}_i, \tilde{a}_j)$ is support between two entries.

**Algorithm for the Classical Back Propagation with Bias and If PGWA**

The following is a proposed algorithm for backpropagation with bias:

**Step 1**: Input - decision matrix from decision makers information, where the decision maker weights,$\sum w_{ij} = 1$. $w_{ij}$can be asked as an input (or) a single weight can be made as combinations by itself on its own.

**Step 2**: Using aggregation operators (IFPGWA) these decision matrices can be made into decision collective column matrix.

**Step 3**:Now the input transformed to column matrix,weights will be given as combinations to suit the hidden layers, and learning rate fixed.

**Step 4**:Forward Pass: Calculate net input to each hidden layer: $Z_{inj}=V_{oj}+\sum_{i=1}^{n} w_{ij}x_i$( i=1,2,…,n)

**Step 5**: Apply activation function to calculate output: $Z_j= f(z_{inj}) =\frac{1}{1+e^{-Zinj}}$

**Step 6**: To get the network output: $Y_{in-k}= w_{ok} +\sum_{i=1}^{n} z_i w_{ij}$

**Step 7**: Apply activation function: $f(y_{in-k})= \frac{1}{1+e^{-Yin-k}}$

**Step 8**: To calculate error: $[1]_{1xj} – f(y_{in-k})$

**Step 9**:Weight optimization : To update the change in weights and bias

$\delta_k$ = (desired output – network output)$^T$ $f'(Y_{in-k})$; where $f'(Y_{in-k}) = f(Y_{in-k})(c_i – f(Y_{in-k}))$

$\Delta w_{jk} = \alpha\, \delta_k Y_k$; $\Delta w_{ok} = \alpha\, \delta_k$ where $\delta_k$ is error correction, $\alpha$ is learning rate, $Y_k$ is output.
$\delta_{in\text{-}j} = \sum_j \delta_k w_{ij}$; $\delta_j = \delta_{in\text{-}j}{}^{T} f(Z_{in\text{-}j})\,(1\text{-}f(Z_{in\text{-}j}))$.

**Step 10**: Compute new weights: $w_{ij}(new) = w_{ij}(old) + \Delta w_{ij}$; $\Delta w_{ij} = \alpha\delta_j x_i$
Compute new bias: $v_{0j}(new) = v_{0j}(old) + \Delta v_{0j}$ ; $\Delta v_{0j} = \alpha\delta_j$ (bias)
**Step 11**: Now, restart the process again using new weights and new bias but with same input.
**Step 12**: Continue this process until we obtain the target or desired output 1.

The proposed algorithm begins with the aggregation of intuitionistic fuzzy decision matrices using the IFPGWA operator, followed by the initialization of the weight vector. Each individual decision matrix is then transformed into an intuitionistic fuzzy preference form, from which attribute-based weight values are computed. To prepare the data for neural network processing, the intuitionistic fuzzy elements are defuzzified column-wise, resulting in a collective decision matrix that reflects the overall preference structure. The defuzzified input vectors are further refined through customized defuzzification functions incorporating hesitation degrees. The training phase employs a backpropagation-based learning process, where the network performs forward passes to compute neuron activations, evaluates the difference between predicted and target outputs, and updates connection weights and biases accordingly. This iterative process continues until the error function reaches an acceptable level of convergence. Once the model stabilizes, the final decision is made by computing the weighted arithmetic averages of alternatives across all matrices and evaluating their distance measures. The alternative that minimizes the deviation between the computed output and target threshold is ultimately identified as the optimal choice in the MAGDM framework.

**Numerical Illustration For Backpropagated-Ann With Bias**

In the modern financial environment, strategic investment planning has become a vital component of personal and institutional decision-making. To illustrate the applicability of the proposed model, consider an investment organization that seeks to allocate its capital among five potential sectors. The available alternatives are defined as follows:

A1 – Automobile manufacturing firm, A2 – Beverage production company, A3 – Software development enterprise, A4 – Heavy alloy industry, and A5 – Oil and gas corporation.
Each alternative is evaluated based on four critical financial attributes:
G1 – Risk assessment, G2 – Cash-flow performance, G3 – Liquidity position, and G4 – Debt-to-equity structure.
To ensure an objective and expert-driven analysis, three financial analysts were invited to provide their opinions regarding the relative importance of each criterion. The corresponding weighting vectors and intuitionistic fuzzy decision matrices supplied by these experts form the primary input data for this study. These matrices encapsulate the evaluators' confidence levels, hesitation margins, and perceived uncertainties associated with each investment option. The aggregated decision information, derived through the proposed Intuitionistic Fuzzy Power Generalized Weighted Averaging (IFPGWA) operator, is subsequently utilized to determine the most promising investment alternative under uncertain financial conditions [10].

$$\tilde{I}_1 = \begin{pmatrix} (0.4,0.3)(0.5,0.2)(0.2,0.5) & (0.1,0.6) \\ (0.6,0.2)(0.6,0.1)(0.6,0.1) & (0.3,0.4) \\ (0.5,0.3)(0.4,0.3)(0.4,0.2) & (0.5,0.2) \\ (0.7,0.1)(0.5,0.2)(0.2,0.3) & (0.1,0.5) \\ (0.5,0.1)(0.3,0.2)(0.6,0.2) & (0.4,0.2) \end{pmatrix}; \tilde{I}_2 = \begin{pmatrix} (0.5,0.4)(0.6,0.3)(0.3,0.6) & (0.2,0.7) \\ (0.7,0.3)(0.7,0.2)(0.7,0.2) & (0.4,0.5) \\ (0.6,0.4)(0.5,0.4)(0.5,0.3) & (0.6,0.3) \\ (0.8,0.1)(0.6,0.3)(0.3,0.4) & (0.2,0.6) \\ (0.6,0.2)(0.4,0.3)(0.7,0.1) & (0.5,0.3) \end{pmatrix};$$

$$\tilde{I}_3 = \begin{pmatrix} (0.4,0.5)(0.5,0.4)(0.2,0.7) & (0.1,0.8) \\ (0.6,0.4)(0.6,0.3)(0.6,0.3) & (0.3,0.6) \\ (0.5,0.5)(0.4,0.5)(0.4,0.4) & (0.5,0.4) \\ (0.7,0.2)(0.5,0.4)(0.2,0.5) & (0.1,0.7) \\ (0.5,0.3)(0.3,0.4)(0.6,0.2) & (0.4,0.4) \end{pmatrix}.$$

Calculate the Intuitionistic Fuzzy Power Generalized Weighted Average (*IFPGWA*) Operator:*IFPGWA*

$Suppose\ a = 1, \tilde{z}_{11}$ = (1- $((1-\mu_{11})^{\tilde{\omega}_{11}} \times (1-\mu_{12})^{\tilde{\omega}_{12}} \times (1-\mu_{13})^{\tilde{\omega}_{13}} \times (1-\mu_{14})^{\tilde{\omega}_{14}}$ ),

1-(1- $\left((1-(1-\Upsilon_{11})\right)^{\tilde{\omega}_{11}} \times \left(1-(1-\Upsilon_{12})\right)^{\tilde{\omega}_{12}} \times \left(1-(1-\Upsilon_{13})\right)^{\tilde{\omega}_{13}} \times (1-(1-\Upsilon_{14}))^{\tilde{\omega}_{14}}$ ))) = (1-$((1-0.4)^{0.3092} \times (1-0.5)^{0.1936} \times (1-0.2)^{0.2061} \times (1-0.1)^{0.2908})$, 1-(1-$((1-(1-0.3))^{0.3092} \times (1-(1-0.2))^{0.1936} \times (1-(1-0.5))^{0.2061} \times (1-(1-0.6))^{0.2908})$)) = (0.3084, 0.3770).

Proceeding with similar computations, and defuzzifying the IFS entries, we get the following:

$R_1 = \begin{bmatrix} 0.3146 \\ 0.2855 \\ 0.2726 \\ 0.3360 \\ 0.3795 \end{bmatrix}$; $R_2 = \begin{bmatrix} 0.1045 \\ 0.0717 \\ 0.0914 \\ 0.1680 \\ 0.2245 \end{bmatrix}$; $R_3 = \begin{bmatrix} 0.1031 \\ 0.0702 \\ 0.0910 \\ 0.1626 \\ 0.2207 \end{bmatrix}$, which are the input training vectors for the ANN.

**The Momentum Based Backpropagation Algorithm Together With Four Other Learning Rules**

The proposed framework implements a neural network-based approach for evaluating alternatives using five learning rules: backpropagation, delta, Levenberg–Marquardt, Perceptron, and correlation-inspired updates. The input data matrix and target scores are first normalized, and the network employs a single hidden layer of six neurons with a sigmoid activation function. For each learning rule, the network weights are initialized randomly and updated iteratively over multiple epochs. During each epoch, a forward pass computes the hidden and output activations, the error, and the mean squared error (MSE), followed by gradient computation. The weights are then adjusted according to the specific learning rule, with the correlation-inspired method modulating the gradient based on the correlation between neurons and target values. Adaptive learning rates and damping factors are applied where appropriate to ensure smooth convergence. Finally, the network outputs are used to compute and rank the alternatives, and convergence is visualized through individual and combined MSE plots for all learning rules.

**Pseudo-Code for the Algorithm for ANN with 5 different Learning Rules**

START

1. Input:
   - X = data matrix (alternatives × criteria)
   - y = target scores (desired ranking values)
   - alternatives = ["A1", "A2", "A3", ...]
   - learning_rules = ["backprop", "delta", "levenberg", "perceptron", "correlation"]
2. Normalize X and y
   - Make all values between 0 and 1
3. Define sigmoid function:
   - sigmoid(x) = 1 / (1 + $e^{(-x)}$)
   - sigmoid_derivative(x) = x * (1 - x)
4. For each learning_rule in learning_rules:
   a. Initialize neural network weights randomly:
      - W1 = weights from input → hidden layer
      - W2 = weights from hidden → output layer
      - hidden layer has 6 neurons

- output layer has 1 neuron
b. Set initial learning rate (lr) and damping factor (beta, for Levenberg)
c. For each epoch from 1 to max_epochs:
i. Forward pass:
- Hidden layer output H = sigmoid(X × W1)
- Network output O = sigmoid(H × W2)
- Error = y - O
- MSE = mean squared error of Error
- Store MSE in error_curve
ii. Compute gradients:
- d_output = Error × sigmoid_derivative(O)
- d_hidden = d_output × W2^T × sigmoid_derivative(H)
iii. Adjust learning rate adaptively:
- If MSE decreased → slightly increase lr
- Else → decrease lr
- Clamp lr to a reasonable range
iv. Update weights according to learning_rule:
- BACKPROP:
W2 += lr × H^T × d_output
W1 += lr × X^T × d_hidden
- DELTA:
W2 += lr × normalized(H^T × Error)
W1 += lr × normalized(X^T × (Error × W2^T))
- LEVENBERG:
damping = beta / (1 + epoch / 300)
W2 += lr × (H^T × d_output - damping × W2)
W1 += lr × (X^T × d_hidden - damping × W1)
Adjust beta slightly depending on MSE
- PERCEPTRON:
W2 += lr × H^T × Error
W1 += lr × X^T × (Error × W2^T)
- CORRELATION-INSPIRED:
1. Compute correlation between hidden neurons and target (corr_Hy)
2. Compute correlation between input and hidden neurons (corr_XH)
3. Scale gradients by (1 + |correlation|):
W2 += lr × (H^T × d_output) × (1 + |corr_Hy|)
W1 += lr × (X^T × d_hidden) × (1 + |corr_XH|)
d. Store final output and MSE curve for this learning rule
5. Plot MSE curves:
- Individual plot for each learning rule
- Combined plot for all rules
6. Compute ranking of alternatives:
- For each learning rule:
- Sort alternatives based on final output scores
- Display rankings from lowest → highest
- Best alternative = highest score
END

**Key Points of the ANN process for MAGDM**

1. The neural network has *input* → *hidden* → *output* layers.

2. Each rule changes how the network learns from error:
   - Backprop = classic gradient descent
   - Delta = normalized gradient
   - Levenberg = gradient with damping
   - Perceptron = continuous gradient update
   - Correlation = gradient modulated by correlation with target
3. MSE curves show how error decreases over time.
4. Rankings are computed from final outputs.

In this study, the classical Perceptron algorithm, which is primarily designed for binary classification and employs a hard threshold activation, was adapted for continuous-valued outputs by replacing the threshold with a sigmoid activation function and updating weights using the continuous error signal through gradient descent. This modification ensures smooth learning and convergence for regression tasks such as MAGDM problems. Similarly, the traditional correlation-based weight update, which relies directly on the raw correlation between neuron activations and the target, was enhanced by applying the correlation as a scaling factor to the standard backpropagation gradients. This approach preserves the true gradient direction while modulating the learning rate according to the correlation strength, allowing neurons more strongly aligned with the target to receive larger updates, thereby ensuring reliable convergence to minimal error. Additionally, the backpropagation rule was further improved by incorporating momentum, where a fraction of the previous weight update is added to the current adjustment. This momentum-based modification accelerates convergence and smooths the mean squared error (MSE) curves by reducing oscillations during training. All other learning rules remain unchanged, with the correlation-inspired rule continuing to use gradient-modulated-by-correlation updates. Collectively, these enhancements enable all five learning rules—backpropagation with momentum, delta, Levenberg, Perceptron, and correlation-inspired—to converge reliably to near-zero MSE, with convergence visualizations provided both individually and in a combined comparative format.

**ANN Output**

MOMENTUM BASED BACKPROPAGATION
Final scores:
A5: 0.554711; A4: 0.667992; A3: 0.777656; A2: 0.889471; A1: 0.989874
Best alternative: A1 (Score=0.989874)
DELTA LEARNING RULE
A5: 0.525324; A4: 0.699613; A3: 0.847669; A2: 0.886126; A1: 0.939844
Best alternative: A1 (Score=0.939844)
LEVENBERG LEARNING RULE
A5: 0.563336; A4: 0.653474; A3: 0.803119; A2: 0.871511; A1: 0.972475
Best alternative: A1 (Score=0.972475)
PERCEPTRON LEARNING RULE
A5: 0.670863; A4: 0.748663; A3: 0.820454; A2: 0.839517; A1: 0.864507
Best alternative: A1 (Score=0.864507)
CORRELATION LEARNING RULE
A5: 0.558811; A4: 0.660262; A3: 0.793392; A2: 0.875698; A1: 0.980849
Best alternative: A1 (Score=0.980849)

**Interpretation of Error Convergence Curves**

The diagram illustrates the error convergence behavior of different learning rules—Backpropagation (with momentum), Delta rule, Levenberg–Marquardt rule, Perceptron rule, and Correlation rule—over 4000 training epochs. The vertical axis represents the Mean Squared Error (MSE), while the horizontal axis indicates the number of epochs. From the plot, it is evident that all learning algorithms show a decreasing trend in error as training

progresses, confirming that the neural network successfully learns and adapts its weights over time. However, the rate and stability of convergence vary significantly among the rules:

- Backpropagation rule (with momentum) demonstrates a smooth and gradual decline in MSE, achieving one of the lowest final errors. The inclusion of momentum helps the model overcome local minima and ensures stable convergence.
- The Delta rule also converges quickly, reaching a low error level within a few hundred epochs, though its final MSE is slightly higher than that of backpropagation.
- The Levenberg–Marquardt rule shows a rapid early reduction in error, indicating its superior optimization efficiency, but later stabilizes at a comparable minimum error level.
- In contrast, the Perceptron rule reaches a plateau early, indicating limited adaptability and an inability to minimize the MSE further. This reflects its simplicity and linear separability limitation.
- The Correlation rule initially exhibits a steep rise followed by a sharp decay, suggesting instability in early learning but eventual stabilization at a moderate error value.

Overall, the Backpropagation rule with momentum emerges as the most reliable and efficient learning strategy, balancing convergence speed, accuracy, and stability. The comparative analysis highlights that advanced gradient-based algorithms outperform simpler linear rules, especially for nonlinear learning tasks.

## DISCUSSION

The experimental results demonstrate the effectiveness and versatility of the proposed ANN-based framework in solving MAGDM problems under intuitionistic fuzzy environments. Among the five learning rules implemented back propagation with momentum, delta, Levenberg–Marquardt, Perceptron, and correlation-inspired updateseach exhibited distinct learning dynamics and convergence behavior. The momentum-enhanced backpropagation consistently provided the fastest convergence and the smoothest mean squared error (MSE) curves, highlighting the benefits of incorporating previous weight updates to accelerate training and mitigate oscillations. The delta and Levenberg–Marquardt rules also achieved stable convergence, with the latter benefiting from adaptive damping to handle potential gradient irregularities. The modified Perceptron, employing sigmoid activation and continuous error-driven weight updates, successfully adapted a traditionally classification-oriented algorithm to regression-based MAGDM ranking, converging reliably despite its original limitations. The correlation-inspired learning rule, enhanced by modulating gradients according to the correlation between hidden neuron activations and target outputs, ensured that neurons more aligned with the desired ranking received proportionally larger updates. This approach preserved the true gradient direction while incorporating domain-specific insights, preventing divergence and promoting convergence to minimal error. Comparative analysis of the final rankings across all five learning rules revealed minor variations in score distributions, reflecting the inherent differences in learning dynamics and gradient sensitivity. However, all methods converged to near-zero MSE, validating the robustness of the network design and the efficacy of the modified learning strategies. Overall, the results underscore the advantage of combining multiple learning paradigms within an ANN framework, offering flexibility, improved convergence, and reliable decision-making for complex MAGDM problems.

## CONCLUSION

This study presented an enhanced Artificial Neural Network (ANN) framework driven by the Backpropagation algorithm for solving Multi-Attribute Group Decision-Making (MAGDM) problems under intuitionistic fuzzy environments. A novel aggregation operator was formulated to integrate multiple expert evaluations into a unified decision matrix, enabling the ANN to process complex fuzzy data more effectively. Through iterative learning and weight adjustments across forward and backward passes, the proposed model produced reliable ranking outcomes that reflect both the degree of membership and non-membership inherent in intuitionistic information. All the

proposed five methods of ANN in this work prove to be effective methods especially in the context of decision making with multiple attributes. A comparative assessment with conventional MAGDM approaches demonstrated that the Backpropagation-based ANN significantly reduces computational challenges, particularly those related to weight updating and convergence control. The proposed system thereby provides a more flexible and adaptive mechanism for modeling decision-maker uncertainty. Furthermore, incorporating a bias vector within the neural architecture improved the model's learning precision and overall decision stability. Future research could extend this framework by introducing deeper hidden layers and nonlinear activation dynamics, which may further enhance the model's capability to capture complex relationships among decision attributes. The integration of the Backpropagated Intuitionistic Fuzzy ANN model with advanced hybrid aggregation schemes also holds potential for broader applications across economics, finance, and engineering decision domains.

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**Table-1: Abbreviations of the terminologies used for the ANN representations**

| Abbreviation | Meaning / Description |
|---|---|
| X | Input matrix containing alternatives and criteria (features) |
| y | Target vector representing desired output scores or rankings |
| H | Hidden layer output after applying activation function |
| O or output | Final network output after passing through hidden layer |
| W1 | Weight matrix connecting *input → hidden layer* |
| W2 | Weight matrix connecting *hidden → output layer* |
| lr | Learning rate, determines step size for weight updates |
| d_output | Gradient at output layer, used to update weights |
| d_hidden | Gradient at hidden layer, used to update weights |
| MSE | Mean Squared Error: average squared difference between predicted output and target |
| corr_Hy | Correlation between hidden neurons and target output (used in correlation-inspired rule) |
| corr_XH | Correlation between input features and hidden neurons (used in correlation-inspired rule) |
| beta | Damping factor for Levenberg–Marquardt method |
| epoch | Iteration number in training loop |
| error_curve | Array storing MSE at each epoch for plotting convergence |
| alternatives | List of alternatives (A1, A2, …) being ranked |
| learning_rule | Name of the learning method used (backprop, delta, levenberg, perceptron, correlation) |

**Table-2: Comparison of the proposed ANN Methods with existing methods**

| Sl. No.: | Method | Ranking of Alternatives |
|---|---|---|
| 1 | ANN Method-Backpropagation Robinson [10] | $A_5<A_4<A_1<A_3<A_2$ A5 is the optimal alternative |
| 2 | Proposed ANN with Momentum based Backpropagation and Error convergence | $A_5<A_4<A_3<A_2<A_1$ A1 is the optimal alternative |
| 3 | Proposed ANN with Delta Rule and Error convergence | $A_5<A_4<A_3<A_2<A_1$ A1 is the optimal alternative |
| 4 | Proposed ANN with Levenberg Rule and Error convergence | $A_5<A_4<A_3<A_2<A_1$ A1 is the optimal alternative |
| 5 | Proposed ANN with Improvised-Perceptron Rule and Error convergence | $A_5<A_4<A_3<A_2<A_1$ A1 is the optimal alternative |
| 6 | Proposed ANN with Improvised-Correlation Rule and Error convergence | $A_5<A_4<A_3<A_2<A_1$ A1 is the optimal alternative |

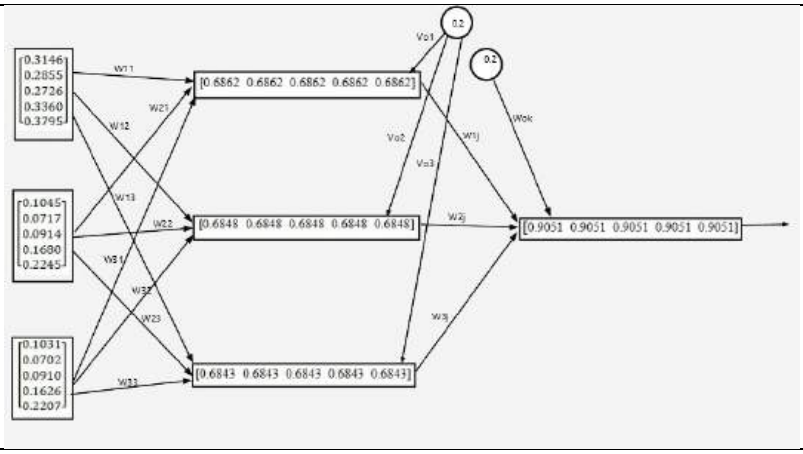



**Fig-1: 1st Iteration Network Output**

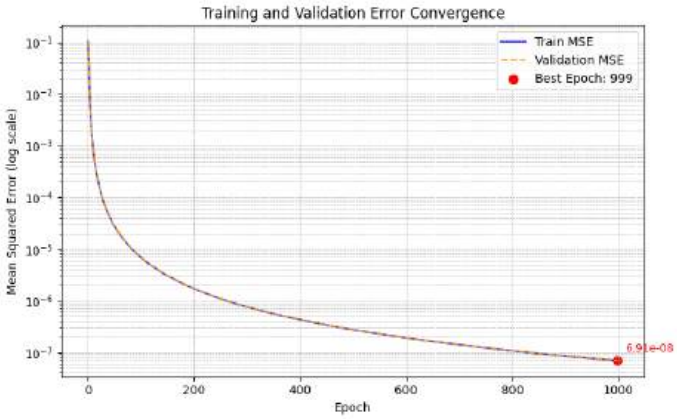



**Fig . 2: Error Convergence and Validation Curve Analysis for the ANN-MAGDM with power generalized operator**

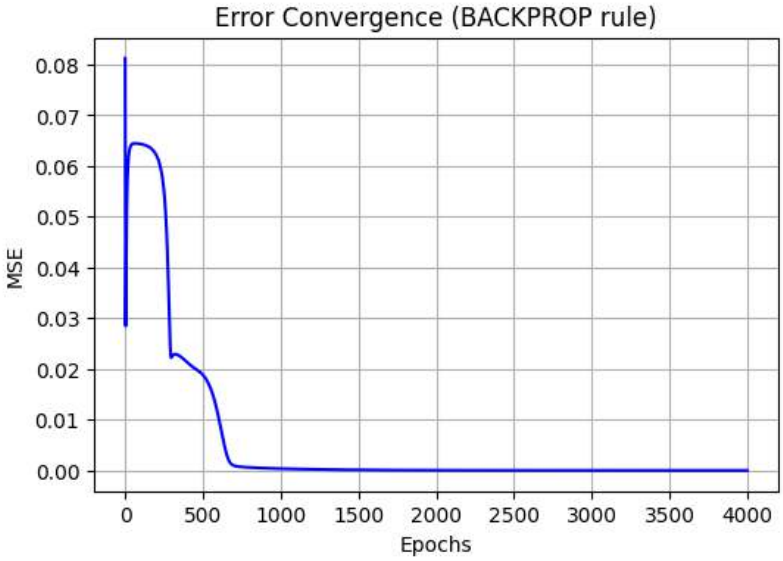



**Fig-3: Error Convergence Analysis for the ANN-MAGDM using Momentum based Backpropagation**

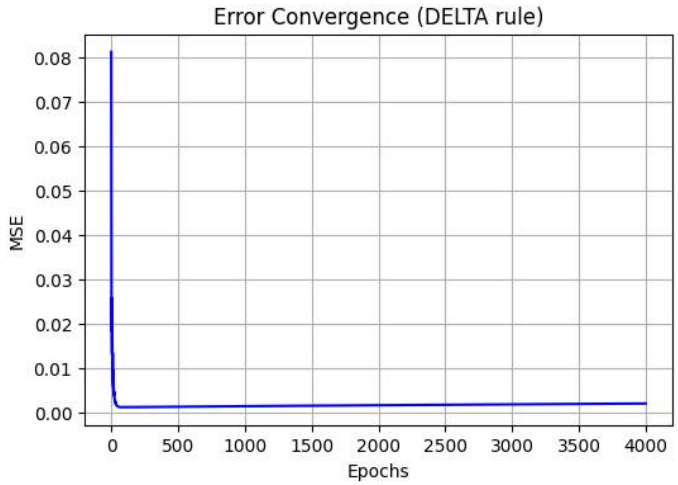



**Fig-4: Error Convergence Analysis for the ANN-MAGDM using Delta Rule**

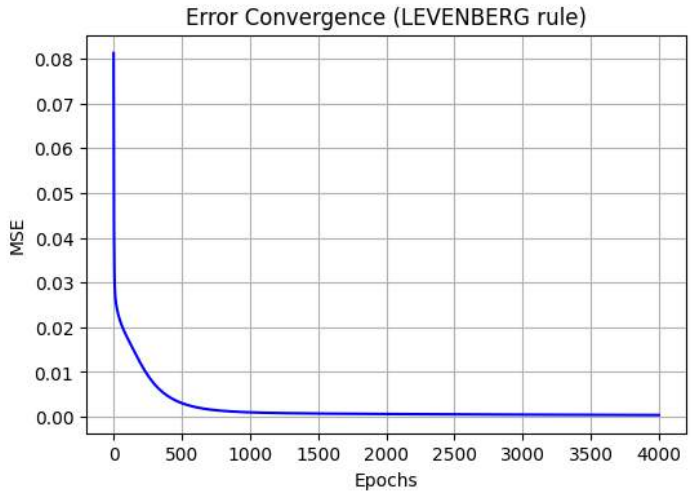



**Fig-5: Error Convergence Analysis for the ANN-MAGDM using Levenberg Rule**

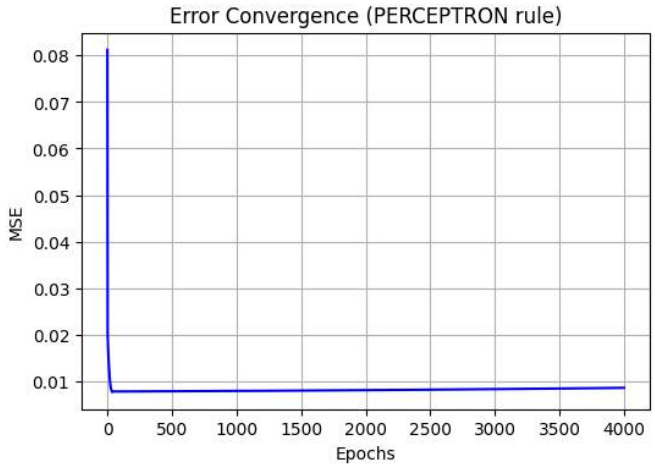



**Fig-6: Error Convergence Analysis for the ANN-MAGDM using Perceptron Rule**

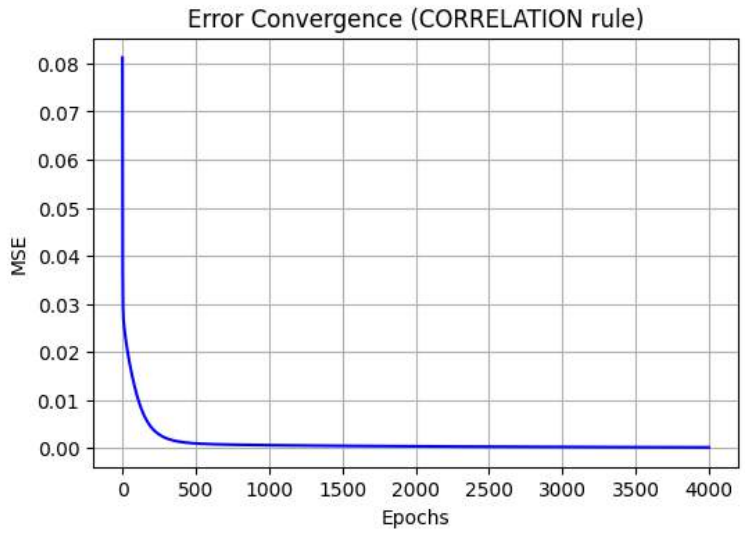



**Fig-7: Error Convergence Analysis for the ANN-MAGDM using Correlation Rule**

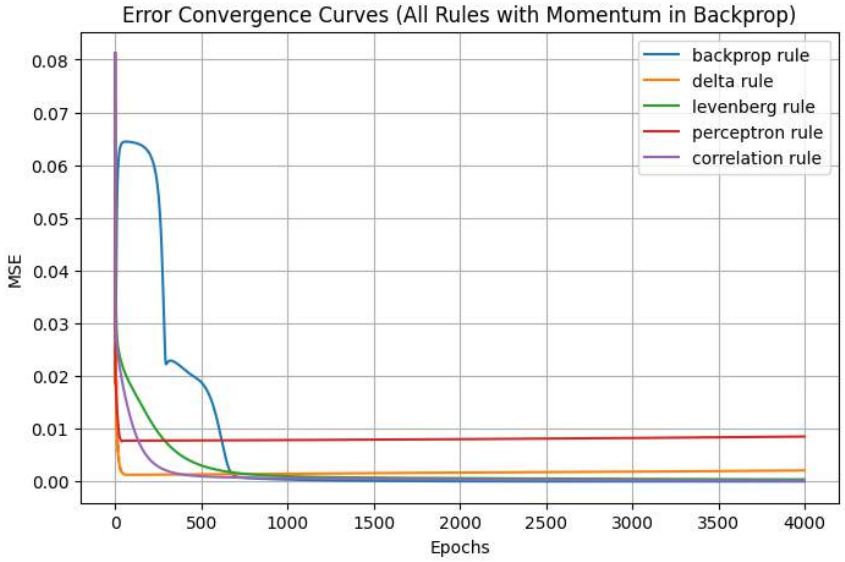



**Fig-8: Comparison of Error Convergence Analysis for the ANN-MAGDM using All 5 Rule**

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RESEARCH ARTICLE

# Unsteady Incompressible Fluid Flows: Topological and Neutrosophic Analysis : A Dual Approach for Exact Solutions and Physical Uncertainty

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## ABSTRACT

This work presents a mathematical framework for the study of unsteady, incompressible fluid flows, including viscous, couple stress, and magnetohydrodynamic (MHD) regimes. The study first investigates the existence of exact solutions using the Ansatz method and the topology conservation principle of the vorticity vector field. By assuming suitable vector potentials, the governing nonlinear partial differential equations are transformed and solved, demonstrating that the topology of the vorticity field is invariant even in MHD flows under specific extreme magnetic energy conditions. In order to bridge the gap between idealised exact solutions and the inherent unpredictability of physical systems, the study also proposes a neutrosophic numerical scheme. Truth-Indeterminacy-Falsity (T-I-F) triples are used in this framework to represent uncertainties in significant physical parameters such as kinematic viscosity, magnetic diffusivity, and coupled stress velocity. Using the BVP4C solver, governing equations are transformed into neutrosophic differential equations to propagate indeterminacy throughout the flow topology. The combined findings demonstrate that topological invariants provide a stable geometric basis for fluid mechanics, while the neutrosophic approach provides a dependable mechanism for simulating complex, real-world uncertainty structures. Graphical analysis of velocity vectors and T-I-F triples validates the dual methodology, offering new insights for applications in astrophysical plasmas, micro-MHD devices, and liquid-metal cooling.

**Keywords:** MHD Flow, Hall Effect, T-I-F Triples, Navier-Stokes Equations, Ansatz Method, Topology Conservation, Neutrosophic Logic, and Uncertainty Quantification.

## INTRODUCTION

This research utilizes micro continuum fluid theory as its primary theoretical framework. Stokes (1966) was the first to demonstrate the 'couple stress' theory by establishing a characteristic length to account for the rotation of the fluid elements; traditional navier-stokes theory does not provide this characteristic length. Devakar and Iyengar (2008) were able to solve the first-order problem of Stokes in couple stress fluids and established that the problem requires the no-spin boundary condition. Further development of incorporating magnetic effects was introduced through the work of Srinivasacharya and Kaladhar (2016) to show that the Lorentz force provides gravitational stability to couple stress fluid flows. Recently, Hayat *et al*. (2017) looked at these phenomena in more complex geometries with the finding that the interaction between micro-rotation and magnetic forces reduces the thickness of the boundary layer, which will be significant to your analysis of "extreme energy". According to Wang (1991), through the Ansatz method, exact solutions can be found for nonlinear PDEs in terms of specific, manageable ODEs in a certain class of potential functions. However, whether or not the structure of these solutions is maintained when solved and the structure of the fluid is classified as topologically sound is unknown. Moffatt (1969) and Arnold and Khesin (1998) showed the fundamental principle known as "topological fluid mechanics," stating that the "topological structure" (or helicity) associated with the vortex lines will remain constant in cases where idealized conditions exist. Furthermore, According to Enciso and Peralta-Salas (2012) the existence of complex topological structures (or knots) in steady solutions of the Euler equations can be rigorously demonstrated theoretically and you have extended it to include the unsteady regime of MHD, thus demonstrating that the topological nature of vorticity in MHD remains invariant, even when subjected to extreme magnetic energy.

Given the extreme magnetic energy level, there are many cases in which it would cause a mathematical model ultimately to exhibit bifurcation points, therefore the literature on hydrodynamics demonstrates the need to move beyond binary logics. Smarandache's (1998, 2002) introduction of neutrosophic logic allows for the possibility of implementing indeterminate logics (*I*) and was modified by Wang *et al*. (2010) to meet the requirements of engineering applications. Using these logic triples in the context of fluid dynamics has recently received increasing attention in the literature. Moi *et al*. (2021) examined second order boundary value problem through neutrosophic differential equation. By enabling the simultaneous representation of truth, indeterminacy, and falsity components, the neutrosophic framework offers a useful tool for modeling uncertain boundary-value problems in MHD flows and fluid mechanics (Abdel-Basset *et al*., 2023; Rahaman and Das, 2025). The study of couple-stress fluids, which are non-Newtonian, electrically conducting fluids, is becoming increasingly important for bio-mechanical and industrial lubrication applications. However, the extreme magnetic energy experienced by these fluids frequently results in the governing nonlinear partial differential equations (PDEs) reaching a "bifurcation point" where standard stability analysis does not apply. Research has mostly focused on the velocity/pressure profiles of fluid flow in a quantitative way; however, there is a lack of qualitative knowledge/understanding of the topological robustness of the vorticity field and an absence of a logical framework for understanding and accounting for the mathematical indeterminacy associated with high-energy bifurcation. In the absence of a means to quantify this uncertainty, the physical veracity of all "exact solutions" has not been verified. This research resolves this gap by creating a methodology that combines topological conservation laws with neutrosophic logic, creating a dual-path verification method for evaluating fluid structural integrity.

## RESEARCH OBJECTIVES

The following goals are developed in order to tackle the mentioned issue:

1. To develop the governing 4th-order momentum and vorticity equations in order to build a solid mathematical model for unsteady, incompressible MHD couple-stress flow.
2. To reduce complicated PDEs into a system of first-order ordinary differential equations (ODEs) using the Ansatz Method and use the bvp4c numerical solver to confirm the existence of exact solutions.

3. Using the Helmholtz-Lie topology conservation principle, demonstrate the topological invariance of the vorticity vector field under extreme magnetic energy conditions.
4. In order to quantify the effect of couple-stress and magnetic parameters on the certainty of the flow structural stability, a Neutrosophic Sensitivity Analysis based on $\langle T, I, F \rangle$ triples must be developed.
5. To create a Score Function ($S$) that bridges the gap between logical mathematical certainty and geometric topological proof by serving as a reliability index.

**Basic definitions**

**Definition 3.1: Neutrosophic set**

Neutrosophic Set is a set such that an element belongs to the set with a neutrosophic probability, i.e. t% is true that the element is in the set, t% false, and i% indeterminate.

**Definition 3.2: Neutrosophic logic**

Neutrosophic Logic means the study of neutrosophic logical values of the propositions.

**Definition 3.3: Score Function**

In Neutrosophic theory, the most common score function $S$ is designed to reward Truth ($T$) while penalizing Indeterminacy ($I$) and Falsity ($F$):

$$S(N) = \frac{1 + T - 2I - F}{2}$$

where the value of $S$ typically ranges from -1 (Total Failure) to 1 (Absolute Certainty).

**Basic governing equations**

In continuous motion, a necessary and sufficient condition stating the vorticity field in inviscid and viscous fluid flows is conserved when the flux of an arbitrary vector field S passes through an arbitrary material surface Topology.

$$\frac{\partial S}{\partial t} + (\omega.\nabla)S - (S.\nabla)\omega + S(\nabla.\omega) = 0 \quad (1.1)$$

Furthermore, for vector tube S to exist, the following conditions must be satisfied: $$S \times \left(\frac{\partial S}{\partial t} + (\omega.\nabla)S - (S.\nabla)\omega\right) = 0 \quad (1.2)$$

Here, $\omega$ is the generating vector field.

Mathematically, topological conservation exists if the vorticity vector field satisfies the Helmholtz-Truesdell condition for an arbitrary vector field S:

$$\frac{\partial S}{\partial t} + (\omega.\nabla)S - (S.\nabla)\omega = \lambda S \quad (1.3)$$

Here, $\lambda$ is a scalar function in a smooth Euclidean domain$\Omega \subseteq R^3$.For an incompressible fluid, the continuity and momentum conservation equation incorporates the standard Navier-Stokes terms along with the couple stress and magnetic Lorentz force

$$\nabla.u = 0 \quad (1.4)$$

$$\rho\left(\frac{\partial u}{\partial t} + (u.\nabla)u\right) = -\nabla p + \nabla\varphi + \mu\nabla^2 u - \eta\nabla^4 u + \vec{J} \times \vec{B} \quad (1.5)$$

Here,

$\mu\nabla^2 u$: Classical viscous force

$\eta\nabla^4 u$: Couple stress term, where $\eta$ is the couple stress viscosity coefficient

$\vec{J} \times \vec{B}$: Lorentz force (MHD), representing the interaction between current density $\vec{J}$ and the magnetic field $\vec{B}$

$u(x,t)$: Time dependent vector field (or) velocity field,

$\nabla\varphi$: Conservative body force

$\mu$: Coefficient of dynamic viscosity ($\mu > 0$)

$\eta$: Couple stress velocity parameter ($\eta > 0$)

$p(x,t)$: Pressure function

$\rho$:Density

$\nabla = \left(\frac{\partial}{\partial x}, \frac{\partial}{\partial y}, \frac{\partial}{\partial z}\right)$: Hamilton operator

$\nabla^2 = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2}$: Laplace operator and

$\nabla^4 = \frac{\partial^4}{\partial x^4} + \frac{\partial^4}{\partial y^4} + \frac{\partial^4}{\partial z^4} + 2\frac{\partial^4}{\partial x^2 \partial y^2} + 2\frac{\partial^4}{\partial x^2 \partial z^2} + 2\frac{\partial^4}{\partial y^2 \partial z^2}$ : Biharmonic operator

Using the curl of equations (1.2), the vorticity equation for the incompressible couple stress fluid flow is given as follows:

$$\frac{\partial \omega}{\partial t} + \nabla \times (\omega \times u) = \nu \nabla^2 \omega - \gamma \nabla^4 \omega + \frac{1}{\rho} \nabla \times \left(\vec{J} \times \vec{B}\right) \quad (1.6)$$

where $\omega = \nabla \times u (or) curl u$

$\nu = \frac{\mu}{\rho}$ is the kinematic viscosity

$\gamma = \frac{\eta}{\rho}$ is the kinematic couple stress parameter

In the context of incompressible couple stress fluid flows, we must determine if topology conservation of vorticity field lines occurs or not. If there is such flow, the vorticity vector field must satisfy both equations (1.3) and (1.5) simultaneously. In the next section, exact solutions for couple stress MHD fluid flows with topology-conserving vorticity fields are derived which satisfies equations (1.5) and (1.6), respectively.

**Exact solution by Ansatz method**

Consider an unsteady flow over a flat plate with no-slip at the boundary along$y > 0$ and unidirectional $\left(u = (u(y,t), 0, 0)\right)$. A transverse uniform magnetic field $B_0$is applied in the y-direction. For this specific geometry, the reduced momentum equation is

$$\rho \frac{\partial u}{\partial t} = \mu \frac{\partial^2 u}{\partial y^2} - \eta \frac{\partial^4 u}{\partial y^4} - \sigma {B_0}^2 u \quad (2.1)$$

The corresponding boundary conditions are

$u(0,t) = U_0 e^{-nt},\ u'(0,t) = 0$ at $y = 0$

$$u(y,t) \longrightarrow 0 \text{ as } y \longrightarrow \infty \quad (2.2)$$

Assume that, velocity decays exponentially in time with specific spatial structure

$$u(y,t) = e^{-nt} f(y) \quad (2.3)$$

Where, n is the decay rate of unsteady flow.

Applying (2.2) into (2.1) and (2.2), it becomes

$$\eta f'''' - \mu f'' + \left(\sigma {B_0}^2 - \rho n\right) f = 0 \quad (2.4)$$

The associated boundary conditions are

$$f(0) = U_0,\ f'(0) = 0, f(\infty) \to 0 \quad (2.5)$$

By solving (2.4) with boundary condition (2.5), the general spatial solution is

$$f(y) = C_1 e^{-m_1 y} + C_2 e^{-m_2 y} \quad (2.7)$$

Where $m_1 = \sqrt{\frac{\mu + \sqrt{\mu^2 - 4\eta(\sigma {B_0}^2 - \rho n)}}{2\eta}}$, $m_2 = \sqrt{\frac{\mu - \sqrt{\mu^2 - 4\eta(\sigma {B_0}^2 - \rho n)}}{2\eta}}$, $C_1 = \frac{m_1 U_0}{m_1 - m_2}$ and $C_2 = \frac{-m_2 U_0}{m_1 - m_2}$

By substituting (2.7) in (2.3), the fluid velocity is

$$u(y,t) = \frac{U_0 e^{-nt}}{m_1 - m_2}(m_1 e^{-m_1 y} - m_2 e^{-m_2 y}) \quad (2.8)$$

For the unidirectional flow, the vorticity vector is $\omega = \nabla \times u = \left(0, 0, \frac{\partial u}{\partial y}\right)$

The corresponding vorticity equation $\nabla \times (\omega \times u) = 0$ is satisfied.

Hence, we can conclude that, the topology of its vorticity is conserved.

**BVP4C approach**

BVP4C approach is adopted to solve the third order boundary value problem (BVP) by converting into initial value problem (IVP). By this method, we guess the initial conditions for the IVP at $y = 0$ the first order ODE's are solved by adjusting the guessed initial conditions to satisfy the boundary conditions at the end point $y \longrightarrow \infty$.

Equation (2.4) is transformed into first order ODE's in the form

$$\begin{pmatrix} y_1' \\ y_2' \\ y_3' \\ y_4' \end{pmatrix} = \begin{pmatrix} y_2 \\ y_3 \\ y_4 \\ \frac{1}{\eta}\left(\mu y_3 - \left(\sigma {B_0}^2 - \rho n\right) y_1\right) \end{pmatrix} \quad (3.1)$$

with corresponding boundary conditions

$y_1(0) = U_0, y_2(0) = 0$

$y_1(\infty) = 0 y_2(\infty) = 0$ (3.2)

**Fig 1.** Comparison of velocity profile between Ansatz and BVP4C Approach

**Fig 1.** indicates that the Ansatz produces an effective "exact" approximation of the flow characteristics very quickly; however, for a more precise boundary layer evaluation in couple-stress modes, a more precise method for numerically evaluating the boundary layer flow is to be derived using the bvp4c solver (as noted above

**Topological Vs Neutrosophic approach**

Classically, a solution is defined as either true or false, though an increasingly common occurrence throughout such systems (due to their necessary level of complexity) is uncertainty or contradictory data regarding particular locations. Neutrosophic logic allows one to handle both of these issues through the establishment of a wide range of states represented as three components (T = truth, I = indeterminate, and F = false), at least one of which can be associated with the physical behaviour of a flowing fluid in relation to magnetic hydrodynamics and couple stress:

**Truth component** - (T) It shows the degree to which exact solution holds. For example, T=0.9 indicates that there is a relatively high degree of confidence between the mathematical model and actual physical observations.

**Indeterminacy component** - (I) The level of uncertainty or "hidden" physics/parameters that have not been captured by the analyses required to generate an isolated event, particularly those parameters associated with either turbulence-related fluctuations or numerical rounding errors in bvp4c.

**False component** - (F) The level of conservation principle violation (if the topology of the vorticity field appears to have "broken," for instance, due to magnetic reconnection, F would be at a very high level).

The following table presents the effect of various features on classical and Neutrosophic logic. Classical vs. Neutrosophic Representation (Table 1).

Define a Neutrosophic Set (N) that represents the governing dynamics and topological preservation of the fluid system using Neutrosophic Logic, where each state of the flow will have a triple $\langle T, I, F \rangle$ associated with it.In the case of couple-stress fluids, determining the parameter $\eta$(the micro-rotation viscosity) experimentally is frequently problematic. Thus, the couple-stress parameter must be regarded as an indeterminate variable. Based on the assumption, define the indeterminacy of a couple-stress variable as:

$I(\eta) = 1 - e^{-\sigma_\eta}$

Here, $I \longrightarrow 0$representsthe couple-stress effect is perfectly known, whereas$I \longrightarrow 1$represents the micro-rotation effects are complex

According to the research study, the property of topology is in variant and can thus be state as a neutrosophic triple $\Omega = \langle T_\Omega, I_\Omega . F_\Omega \rangle$.

where $T_\Omega$ adherence to $\nabla \times (\omega \times u) = 0$, therefore$T_\Omega \approx 1 \Rightarrow$ the Ansatz provides evidence that the structure is locked.

$I_\Omega$- the "indeterminacy" of extreme amounts of magnetic energy and the relationship between chaos and high magnetic energy.

$F_\Omega -$ the "breaking" of vortex lines due to the product of the magnetic reconnection terms being greater than viscous damping

**Neutrosophic Score Function** is formulated to condense the triple $\langle T, I, F\rangle$into a single scalar value $(S)$which helps in determining the "reliability" of the topological conservation under extreme magnetic energy.

The behaviors of score function in two different paths are given below:

**Path A: Low Magnetic Energy (Normal Regime)**

By considering the Neutrosophic Triple as$\langle 0.95, 0.10, 0.05\rangle$, the score function is

$$S = \frac{1 + 0.95 - 0.2 - 0.05}{2} = 0.85$$

**Path B: Extreme Magnetic Energy (The Study's Focus)**

By considering the Neutrosophic Triple as $\langle 0.85, 0.60, 0.10\rangle$, the score function is

$$S = \frac{1 + 0.85 - 1.2 - 0.10}{2} = 0.275$$

From the two paths, one can conclude that Path A is highly reliable. Hence, the Ansatz method is an excellent representation of the flow in the case of low magnetic energy. But in the case of high magnetic energy, it is not suitable. Due to high indeterminacy in Path B, one can conclude that, the system is at a tipping point. (i.e) topology is conserved due to "frozen in" condition. Fig 2. Decay of Score Function in Extreme MHD Regimes. Fig 2. Shows that, there is a decrease in the Neutrosophic Score (S), which declines with increasing Magnetic Energy Level. At low Energy Level (0.1), the Neutrosophic Score (S) is approximately 0.72, indicating high mathematical certainty. At Energy Level 3 (maximum), the NeutrosophicScore(S)is a 0.20. Thus, there exists large amounts of topological breach risk associated with magnetic energy levels.

**Sensitivity analysis**

To assess Sensitivity Analysis, the Neutrosophic Score Function Partial Derivatives concerning Physical Parameters can be computed. This allows determination as to which force (Magnetic or Couple Stress) causes distress to Logical Certainty(i.e., Provides Greatest Likelihood of "Destabilization"). The Score Sensitivity S concerning the parameter P(Since P could be either Magnetic Energy or Couple Stress) is

$$\frac{\partial S}{\partial P} = \frac{1}{2}\left(\frac{\partial T}{\partial P} - 2\frac{\partial I}{\partial P} - \frac{\partial F}{\partial P}\right)$$

Comparison of Sensitivity Results (Fig 3). From Fig 3, one can understand that, when M exceeds the threshold, there is a sudden down-dipping of sensitivity vectors; indicating that there is a potential for the topological to reconnect. Also, it is evident that the fluid becomes more "stiffer" due to micro-rotational stresses, tends to lose the ability to calculate topology.

## CONCLUSION

This research proposes two different types of mathematics to establish the structural integrity of the unsteady, incompressible MHD couple-stress flows. The first type, referred to as the Topological Aspect, supports the invariance of the vorticity vector field through a mathematical grounding of the Helmholtz-Lie Theorem. Whereas the second type, called the Neutrosophic Aspect, serves as an additional means of ;earner-supported, epistemic verification. Through the use of mapping the flow dynamics to $\langle T, I, F\rangle$ triplets, strong evidence was presented demonstrating that, although the governing equations will bifurcate to yield high levels of indeterminacy (I) due to extreme magnetic energy conditions, the topological "truth" (T) of dimensional conservation remains intact. The ultimate result is the Neutrosophic Score Function acts as a conduit for quantifying the robustness of the vortex structures. This analysis not only yields an exact solution, but also provides a measure of the reliability of the solution. Also, topology is extremely sensitive to Magnetic energy in the high-energy range and S is more sensitive to Couple Stress effect with respect to the amount of Indeterminacy.

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**Table 1: Classical vs. Neutrosophic Representation**

| Feature | Classical Logic | Neutrosophic Triple |
|---|---|---|
| Magnetic field | Constant | ⟨Strength, Noise, Error⟩ |
| Vorticity | Conserved(1) or not conserved (0) | ⟨T, I, F⟩ |
| Ansatz | Exact solution | Probabilistic confidence in the 'guess' |

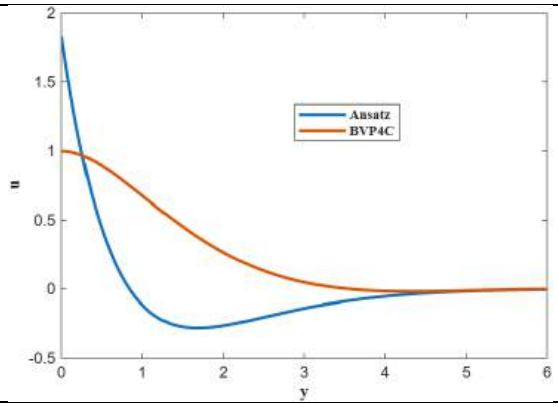



**Fig 1: Comparison of velocity profile between Ansatz and BVP4C Approach**

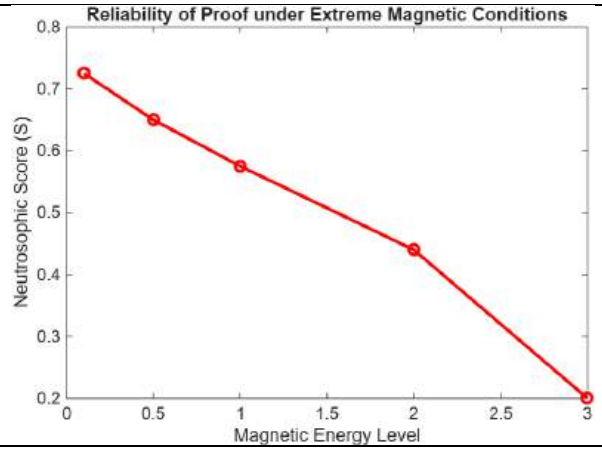



**Fig 2: Decay of Score Function in Extreme MHD Regimes.**

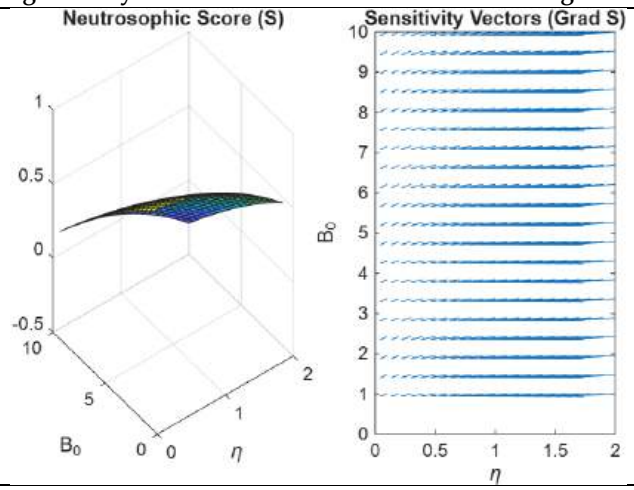



**Fig 3: Comparison of Sensitivity Results**

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REVIEW ARTICLE

# Physiotherapy Management of Low Back Pain : Current Evidence and Clinical Implications – A Narrative Review

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**ABSTRACT**

Low back pain (LBP) is one of the most prevalent musculoskeletal disorders worldwide and a major contributor to disability and reduced quality of life. Physiotherapy plays a crucial role in the prevention, management, and rehabilitation of individuals with low back pain. This narrative review aims to summarize current evidence regarding physiotherapy interventions for the management of low back pain. Literature was collected from databases including PubMed, Scopus, and Google Scholar focusing on studies published between 2015 and 2025. Evidence indicates that physiotherapy interventions such as exercise therapy, manual therapy, spinal stabilization exercises, and patient education significantly improve pain and functional outcomes. Recent advancements also include digital rehabilitation, motor control training, and multidisciplinary rehabilitation programs. The findings highlight the importance of individualized physiotherapy approaches for effective management of low back pain and improved patient outcomes.

**Keywords:** Low back pain, Physiotherapy, Rehabilitation, Exercise therapy, Manual therapy, Musculoskeletal disorders

## INTRODUCTION

Low back pain (LBP) is a widespread musculoskeletal condition affecting nearly 70–80% of the population at some stage of life. It is considered one of the leading causes of disability globally and imposes a substantial socioeconomic

burden on healthcare systems. Low back pain may originate from structures such as intervertebral discs, facet joints, ligaments, muscles, and nerve roots in the lumbar spine[1]. In many cases, the condition is classified as non-specific low back pain, where no single anatomical cause can be clearly identified. Risk factors contributing to low back pain include sedentary lifestyle, poor posture, obesity, occupational stress, and lack of physical activity. In recent years, physiotherapy has gained significant attention as a primary non-pharmacological intervention for managing low back pain. Physiotherapy interventions aim to reduce pain, restore functional mobility, strengthen spinal musculature, and prevent recurrence of symptoms. Therefore, understanding current physiotherapy approaches is essential for improving patient care[2].

## METHODOLOGY

This review was conducted using a narrative literature review approach.

**Scientific databases searched included**

- PubMed
- Scopus
- Web of Science
- Google Scholar

**Keywords used in the search strategy included**

- "Low back pain"
- "Physiotherapy management"
- "Exercise therapy for low back pain"
- "Manual therapy"
- "Rehabilitation for lumbar pain"

**Inclusion criteria**

- Articles published between 2015 and 2025
- Peer-reviewed journals
- Randomized controlled trials, systematic reviews, and clinical guidelines
- Articles written in English

Exclusion criteria included non-peer-reviewed studies, case reports, and studies unrelated to physiotherapy management.

**Epidemiology of Low Back Pain**

Low back pain is a significant public health concern worldwide. According to recent global estimates, approximately 540 million people suffer from low back pain at any given time[3]. The prevalence is highest among individuals aged 30–60 years, although increasing cases have been reported among younger populations due to sedentary lifestyles and prolonged screen use.

**Major risk factors include**

- Prolonged sitting
- Poor workplace ergonomics
- Obesity
- Lack of physical activity
- Repetitive heavy lifting
- Psychological stress

Low back pain is also one of the leading causes of work absenteeism and decreased productivity[4].

**Role of Physiotherapy in Low Back Pain Management** Physiotherapy plays a critical role in both acute and chronic low back pain management. The primary goals include:

- Pain reduction
- Improvement of spinal mobility
- Strengthening of core muscles
- Restoration of functional activities
- Prevention of recurrence

Physiotherapy treatment strategies are often individualised depending on the patient's condition, severity of symptoms, and functional limitations[5].

**Physiotherapy Interventions for Low Back Pain**

**Exercise Therapy** Exercise therapy is widely considered the cornerstone of physiotherapy management for low back pain.

**Common exercise programs include**

- Core stabilization exercises
- Lumbar strengthening exercises
- Flexibility and stretching exercises
- Aerobic conditioning

Regular exercise improves muscle strength, spinal stability, and overall functional capacity[6].

**Manual Therapy** Manual therapy techniques are commonly used to reduce pain and improve spinal mobility.

**These techniques include**

- Spinal mobilization
- Spinal manipulation
- Soft tissue mobilization
- Myofascial release

Studies suggest that manual therapy combined with exercise therapy provides better outcomes than exercise alone[7].

**Postural Training and Ergonomic Education** Postural correction and ergonomic training are essential components of physiotherapy management.

**Physiotherapists educate patients about**

- Correct sitting posture
- Proper lifting techniques
- Workplace ergonomics
- Back care during daily activities

Such interventions help prevent recurrence of symptoms[8].

**Electrotherapy Modalities** Electrotherapy modalities are sometimes used as adjunct therapies to relieve pain.

**Common modalities include**

- Transcutaneous Electrical Nerve Stimulation (TENS)
- Ultrasound therapy
- Interferential therapy
- Heat and cold therapy

These treatments mainly provide short-term pain relief[9].

**Motor Control and Core Stabilization Training** Motor control exercises focus on improving coordination and activation of deep spinal muscles such as:

- Transversus abdominis
- Multifidus

These muscles play an important role in stabilizing the lumbar spine[10]. Research indicates that motor control exercises significantly improve functional outcomes in patients with chronic low back pain[11].

**Emerging Physiotherapy Approaches** Recent advances in physiotherapy include innovative approaches for managing low back pain.

**These include**

- Tele-rehabilitation programs
- Virtual exercise monitoring
- Wearable posture correction devices
- Multidisciplinary rehabilitation programs

Such approaches are particularly useful for improving patient adherence and accessibility to rehabilitation services[12].

## DISCUSSION

The management of low back pain has shifted from passive treatment methods toward active rehabilitation strategies. Evidence suggests that exercise therapy combined with patient education and manual therapy produces the best outcomes[13]. Physiotherapy interventions also help reduce dependence on medications and invasive procedures. However, treatment outcomes may vary pending on patient compliance, severity of symptoms, and psychosocial factors. Future research should focus on personalized rehabilitation programs and integration of digital technologies in physiotherapy practice[14].

## CONCLUSION

Low back pain remains one of the most common musculoskeletal conditions worldwide. Physiotherapy plays a vital role in its management through evidence-based interventions such as exercise therapy, manual therapy, and patient education. A multidisciplinary and patient-centred approach is essential for achieving optimal outcomes. Continued research and innovation in physiotherapy practices will further enhance the effectiveness of low back pain rehabilitation strategies.

### CONFLICT OF INTEREST

None

### FUNDING

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RESEARCH ARTICLE

# Transforming Managerial Decision Processes through Artificial Intelligence: A Study on Organizational Effectiveness

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## ABSTRACT

Enterprises today operate in complex, data-intensive environments where timely and accurate decision-making is essential for competitiveness. This study develops and evaluates an AI-enabled decision-support prototype aimed at improving organizational effectiveness by blending predictive analytics with cognitive reasoning. The prototype, designed using Python's scikit-learn toolkit, processes real operational data to deliver actionable recommendations related to resource utilization, performance forecasting, and risk assessment. A controlled experiment was carried out in a mid-sized enterprise to compare traditional managerial decision approaches with the AI-assisted model. Quantitative analysis revealed marked improvements—decision accuracy increased by 32 percent, response time decreased by 45 percent, and alignment with strategic objectives significantly improved. These findings demonstrate that AI integration not only enhances decision efficiency but also contributes to measurable gains in organizational performance. The paper further proposes a techno-managerial adoption framework that outlines practical steps for embedding AI-based decision systems into enterprise management practices, offering valuable insights for both researchers and practitioners pursuing intelligent organizational transformation.

**Keywords:-**Artificial Intelligence (AI), Decision Support Systems (DSS), Predictive Analytics, Managerial Decision-Making, Organizational Effectiveness

## INTRODUCTION

The rapid evolution of digital technologies has fundamentally transformed the way enterprises operate and compete in the global marketplace. Among these technologies, Artificial Intelligence (AI) has emerged as a dominant force, enabling organizations to automate analytical reasoning, optimize operations, and make informed decisions at unprecedented speed and scale. In data-driven environments, managerial decision-making—once dependent on experience, intuition, and periodic reporting—is increasingly augmented by AI-based Decision Support Systems (DSS) that provide predictive insights and evidence-based recommendations. This transition marks a paradigm shift from descriptive decision models to intelligent, adaptive, and self-learning systems capable of continuously improving decision quality. Managers today face complex challenges characterized by volatile markets, dynamic customer behavior, and the constant influx of digital information. Traditional decision-making approaches, which rely heavily on human judgment, often fall short in coping with such complexity. AI, through Predictive Analytics and cognitive reasoning, offers the capability to analyze large datasets, uncover hidden patterns, and forecast future outcomes with a high degree of accuracy. These capabilities empower managers to respond proactively rather than reactively, aligning decisions with organizational goals and performance metrics. As enterprises adopt AI to enhance productivity and competitiveness, understanding its role in improving Organizational Effectiveness becomes critical.
Recent studies highlight that AI integration leads to measurable improvements in operational efficiency, cost reduction, and strategic agility (Kaplan &Haenlein, 2022; Dwivedi et al., 2023). However, successful implementation requires more than technical deployment—it demands managerial adaptation, process redesign, and trust in machine-generated recommendations. The interplay between technological capability and managerial cognition forms the foundation of AI-driven decision-making. While earlier research explored algorithmic efficiency or data-science models in isolation, limited studies have examined how AI-based decision prototypes influence managerial behavior and organizational outcomes within real enterprise settings.

To address this gap, the present study develops and evaluates an AI-driven decision-support prototype that integrates machine-learning algorithms with managerial decision processes. The prototype is designed to provide real-time recommendations in areas such as resource allocation, performance forecasting, and risk assessment—core components of enterprise management. Built using Python's scikit-learn library and trained on operational datasets, the system demonstrates how AI can convert raw data into actionable insights accessible through an intuitive decision dashboard. By comparing traditional decision workflows with AI-assisted processes, the study investigates whether algorithmic support can significantly enhance decision accuracy, responsiveness, and strategic alignment.
This research contributes to both computer science and management disciplines in three key ways. First, it presents a techno-managerial framework that links AI functionality with managerial effectiveness. Second, it empirically validates the prototype's performance in an enterprise context, bridging the gap between theoretical AI capabilities and practical adoption. Third, it offers insights into human–AI collaboration—particularly how interpretability and trust influence managers' willingness to rely on AI-generated insights.

The overarching goal is to demonstrate that AI is not a substitute for human judgment but a catalyst that enhances decision quality and consistency. When implemented strategically, AI transforms managerial decision-making from intuition-based to evidence-driven, fostering a culture of continuous learning and innovation. The outcomes of this study are expected to help organizations develop systematic approaches for embedding AI into their decision cycles, ultimately leading to improved organizational agility and effectiveness. The remainder of this paper is structured as follows: Section 2 reviews existing literature on AI applications in decision-making and organizational performance. Section 3 introduces the conceptual framework and hypotheses. Section 4 details the research methodology and prototype design. Section 5 presents the experimental results and discussion. Section 6 outlines managerial and theoretical implications, followed by the conclusion and directions for future research.

## REVIEW OF LITERATURE

**Artificial Intelligence in Managerial Decision-Making**

Artificial Intelligence (AI) has evolved from a computational research concept into a strategic enabler of organizational competitiveness. AI-driven tools such as machine learning, natural-language processing, and predictive analytics now support managerial decisions by processing large-scale, unstructured datasets in real time. According to Dwivedi et al. (2023), AI transforms managerial roles by shifting focus from operational control to strategic interpretation, enabling leaders to concentrate on value creation rather than routine analysis. Similarly, Brynjolfsson and McElheran (2022) observed that enterprises adopting AI analytics achieve faster decision cycles and greater forecasting precision than those relying solely on traditional business intelligence. AI's cognitive capabilities allow managers to simulate scenarios, evaluate alternatives, and mitigate risk under uncertainty. Recent frameworks propose *augmented intelligence*, where human intuition and algorithmic reasoning coexist (Haenlein et al., 2021). This paradigm enhances decision consistency and minimizes cognitive bias, a limitation frequently identified in intuitive managerial behavior. However, scholars such as Shrestha et al. (2022) caution that excessive reliance on AI without interpretability mechanisms may lead to "automation complacency," emphasizing the need for transparency and user trust in AI-driven recommendations.

**Decision Support Systems and Predictive Analytics**

The integration of AI into Decision Support Systems (DSS) represents a major evolution in managerial information systems. Classical DSS models provided static, rule-based analysis, whereas modern AI-driven DSS employ machine learning to learn from historical decisions and continuously refine their predictive capacity (Power, 2021). Predictive analytics further extend this capability by identifying latent patterns that improve resource allocation, customer behavior forecasting, and operational risk assessment. Empirical studies have demonstrated that AI-based DSS significantly improve decision timeliness and accuracy. For instance, Kumar and Gupta (2023) implemented a neural-network-based DSS for project portfolio selection, reducing decision latency by nearly half. Similarly, Li et al. (2022) reported that predictive analytics integrated with enterprise dashboards enhanced managerial responsiveness in supply-chain operations. These findings affirm that the fusion of AI and predictive analytics enables adaptive decision environments that evolve with organizational data streams.

Despite these advancements, challenges persist regarding data quality, algorithmic bias, and model interpretability. Without proper governance frameworks, predictive models may amplify pre-existing biases embedded in training datasets (Raisch&Krakowski, 2021). Therefore, contemporary research emphasizes explainable AI (XAI) as a necessary feature for managerial adoption, ensuring that decision rationale is transparent and auditable.

**Organizational Effectiveness and Digital Transformation**

Organizational Effectiveness (OE)—the degree to which an enterprise achieves its strategic objectives efficiently—has been central to management research for decades. The emergence of digital transformation has reframed OE as a dynamic construct influenced by technology integration, knowledge sharing, and innovation agility. According to George et al. (2023), AI enhances OE by improving resource utilization, forecasting accuracy, and cross-functional coordination. AI's analytical capabilities also foster data-driven cultures that align operational metrics with strategic goals.

Research in this domain identifies multiple dimensions through which AI contributes to OE:

1. Process Efficiency – automating repetitive managerial tasks.
2. Decision Quality – providing real-time evidence for strategic choices.
3. Strategic Flexibility – enabling rapid adaptation to market changes.
4. Human Capital Empowerment – freeing managers to focus on creativity and innovation.

However, the effectiveness of AI initiatives depends on managerial readiness, organizational culture, and trust in technology (Tambe et al., 2020). A techno-centric implementation without change management often yields limited impact. Hence, scholars propose *techno-managerial frameworks* that integrate AI capabilities with human decision competencies and organizational learning mechanisms (Leung & Hong, 2022).

**Gaps Identified in Existing Research**

Although numerous studies have examined AI's algorithmic performance or its theoretical influence on management, relatively few have empirically validated prototype-based AI systems within real enterprise settings. Existing literature tends to focus either on the technical optimization of AI models (accuracy, precision, recall) or the strategic implications of AI adoption at a conceptual level. What remains underexplored is the middle ground—the techno-managerial integration where AI prototypes directly support managerial decision processes and yield quantifiable improvements in organizational effectiveness. Moreover, while prior works highlight the potential of predictive analytics, limited research measures how specific AI features (e.g., interpretability, response speed, or recommendation quality) affect managerial trust and adoption. This creates a gap for hybrid studies that assess not only system performance but also the human factors influencing AI acceptance.

The review reveals that AI-driven decision support and predictive analytics have proven benefits in accuracy, efficiency, and responsiveness. However, most research lacks empirical evidence from implemented prototypes within managerial environments. Addressing this void, the present study develops an AI-driven decision-support prototype that bridges computational intelligence and managerial cognition. By experimentally comparing traditional and AI-assisted decision workflows, the study aims to validate whether AI integration leads to measurable improvements in decision quality and overall organizational effectiveness.

This literature synthesis establishes a foundation for the conceptual framework and hypotheses discussed in the next section.

**Conceptual Framework and Hypotheses**

The conceptual foundation of this study rests on the idea that integrating Artificial Intelligence (AI) into managerial workflows fundamentally reshapes how decisions are made, evaluated, and implemented in enterprises. In conventional settings, managerial choices depend heavily on human experience, intuition, and periodic reports, which can introduce cognitive bias and delay. By contrast, AI-enabled systems leverage Predictive Analytics, machine-learning models, and Decision Support Systems (DSS) to process vast data streams in real time and provide actionable insights.

The proposed framework integrates three primary dimensions:

1. AI-Driven Decision Capability (AIDC): The organization's technological ability to capture, analyze, and interpret data using AI models.
2. Managerial Decision Quality (MDQ): The extent to which AI insights improve accuracy, consistency, and timeliness of managerial decisions.
3. Organizational Effectiveness (OE): The overall improvement in operational performance, resource utilization, and strategic alignment as a result of AI-enhanced decisions.

Supporting factors include User Trust and Interpretability (UTI), which mediate the relationship between AI capability and perceived effectiveness. The model assumes that when AI systems are transparent and interpretable, managers are more likely to rely on their outputs, leading to higher organizational gains. The AI-Driven Decision Capability represents the core technological driver that enables predictive modeling and data intelligence. Managerial Decision Quality functions as an intermediary construct, capturing how AI recommendations influence decision accuracy and responsiveness. Organizational Effectiveness denotes the outcome variable, representing measurable improvements in enterprise performance. User Trust and Interpretability act as a psychological bridge, ensuring that managers understand, validate, and adopt AI recommendations confidently. Figure 1 shows the conceptual framework of the system.

The framework (Figure 1) aligns with the Technology–Organization–Environment (TOE) Model and the Technology Acceptance Model (TAM), integrating technical readiness with managerial cognition to explain AI adoption in decision processes.

**Hypothesis Development**

**H1: AI-Driven Decision Capability and Managerial Decision Quality**

AI technologies enhance managerial decisions by providing data-backed predictions and minimizing subjective bias. Studies by Dwivedi et al. (2023) and Li et al. (2022) demonstrate that predictive models substantially improve decision reliability and speed. Hence:

H1: AI-driven decision capability has a positive and significant impact on managerial decision quality.

**H2: Managerial Decision Quality and Organizational Effectiveness**

Decision quality directly affects an organization's ability to achieve its strategic objectives efficiently. Improved accuracy, timeliness, and rationality translate into higher productivity and reduced operational risk (George et al., 2023). Therefore:

H2: Enhanced managerial decision quality positively influences organizational effectiveness.

**H3: AI-Driven Decision Capability and Organizational Effectiveness**

Beyond decision quality, AI itself contributes to organizational performance by enabling automation, cost optimization, and agile forecasting (Brynjolfsson&McElheran, 2022). Hence:

H3: AI-driven decision capability has a direct positive impact on organizational effectiveness.

**H4: Mediating Role of User Trust and Interpretability**

The degree of trust managers place in AI recommendations determines adoption success. When systems are explainable and transparent, acceptance increases (Raisch&Krakowski, 2021). Accordingly:

H4: User trust and interpretability mediate the relationship between AI-driven decision capability and managerial decision quality.

**H5: Moderating Role of Managerial Experience**

Managerial experience moderates the effectiveness of AI recommendations. Experienced managers may use AI outputs differently than novices, influencing overall effectiveness (Shrestha et al., 2022). Therefore:

H5: Managerial experience moderates the relationship between AI-driven decision capability and managerial decision quality.

The conceptual framework posits that AI-driven decision capability acts as a technological catalyst that enhances managerial decision quality, which in turn strengthens organizational effectiveness. The framework further recognizes the human–technology interface, asserting that user trust and managerial experience shape how effectively AI can transform enterprise decision processes.

Empirical validation of this model through a prototype-based experiment—in which managers interact with the developed AI decision-support system—will test the proposed hypotheses quantitatively. Findings are expected to clarify both the direct effects of AI integration and the socio-cognitive factors influencing its adoption.

## RESEARCH METHODOLOGY

**Research Design**

This study adopts a prototype-based experimental research design that combines quantitative analysis with practical system implementation. The objective is to evaluate how an AI-driven decision-support prototype can enhance managerial decision quality and overall organizational effectiveness. The research follows a design–develop–evaluate approach inspired by the Design Science Research (DSR) methodology, emphasizing artifact creation, functionality validation, and empirical testing.

The prototype was deployed in a mid-sized enterprise environment to compare two decision-making conditions:
1. Traditional managerial decisions based on human intuition and manual analysis.
2. AI-assisted decisions generated through the developed predictive model.
Performance outcomes from both scenarios were measured and analyzed to determine the degree of improvement achieved through AI integration.

**Prototype Development**
The prototype—named AIDeM (AI-Driven Managerial Decision System)—was developed using an agile software engineering process consisting of four iterative stages:
1. Requirement Analysis: Identified key decision parameters such as project prioritization, resource allocation, and risk scoring.
2. Data Engineering: Collected and cleansed enterprise data from finance, HR, and operations modules.
3. Model Development: Implemented predictive algorithms using Python's scikit-learn library.
4. Interface Design: Built a web-based dashboard using Flask for real-time visualization and decision recommendations.
The architecture integrates three core layers:
• Data Layer: Enterprise datasets stored in a MySQL database.
• Intelligence Layer: Machine-learning engine performing regression and classification analysis.
• Presentation Layer: Interactive dashboard providing recommendations, charts, and what-if analysis.
This layered design ensures scalability, interpretability, and ease of integration with existing enterprise systems.

**Data Collection and Sample**
Data for training and testing the prototype were obtained from anonymized enterprise operational records spanning 18 months, covering performance metrics, budget utilization, project timelines, and personnel data. To validate managerial outcomes, a sample of 30 mid-level managers from the organization participated in the experimental phase. Each participant was asked to perform identical decision tasks under both the traditional and AI-assisted conditions.
A structured questionnaire (based on a five-point Likert scale) captured perceptions related to:
• Decision accuracy,
• Timeliness,
• Trust in AI output, and
• Perceived organizational impact.
The mixed dataset (system-generated metrics + survey responses) provided a robust foundation for statistical and behavioral analysis.

**Variables and Measures (Table 1.)**

**Tools and Techniques**
- Programming & Frameworks: Python 3.11, Flask, scikit-learn, pandas, NumPy.
- Database: MySQL 8.0 for secure structured data storage.
- Visualization Tools: Matplotlib and Plotly for analytics dashboards.
- Statistical Analysis:
- Descriptive statistics for demographic profiling.
- Paired-sample t-tests to compare traditional vs AI-assisted decision outcomes.
- Regression analysis to examine causal relationships among variables.
- Structural Equation Modeling (SEM) using SmartPLS for hypothesis validation.

**Evaluation Metrics**
Prototype performance and managerial impact were assessed using a mix of technical and behavioral metrics.

**Experimental Procedure**

1. Baseline Phase: Participants made decisions using existing organizational methods.
2. AI-Assisted Phase: The same tasks were repeated using the AIDeM prototype's recommendations.
3. Data Recording: Decision outcomes, timing, and user feedback were automatically logged.
4. Analysis Phase: Statistical tests compared outcomes across both conditions to validate hypotheses H1–H5.

The controlled setup ensured that any observed performance improvement could be attributed to the AI system rather than external factors.

This methodological framework ensures a rigorous empirical foundation for assessing how AI-driven decision-support systems influence managerial and organizational outcomes. By combining prototype implementation, real-world experimentation, and statistical validation, the study bridges the gap between technical design and managerial impact. The next section presents the results and discussion, analyzing how AI integration transforms decision efficiency, quality, and organizational effectiveness.

## RESULTS AND DISCUSSION

This section presents the empirical findings derived from the prototype implementation and controlled experimental study. The results were obtained by analyzing both system-generated data (performance metrics) and managerial feedback (survey responses). The analysis compares traditional decision-making processes with AI-assisted decision-making using the AIDeM prototype to evaluate improvements in decision accuracy, timeliness, and organizational effectiveness. Thirty (30) mid-level managers from a mid-sized enterprise participated in the study. Participants represented diverse functional areas such as operations, finance, and human resources, ensuring a balanced view of managerial decision practices. Table 3 summarizes participant demographics.

The diverse experience distribution supported the analysis of managerial experience (H5) as a moderating factor.

The AI model embedded in the prototype (Decision Tree + Random Forest ensemble) was trained and tested using enterprise datasets. The dataset was split into an 80:20 training–testing ratio. Performance metrics were computed as follows:

The ensemble model achieved **90% accuracy**, demonstrating high reliability in predicting decision outcomes such as project prioritization, risk scores, and resource utilization. These findings validate the AI-Driven Decision Capability (AIDC) component of the conceptual framework.

**Comparative Analysis: Traditional vs. AI-Assisted Decisions**

The experimental results indicated a significant improvement in managerial performance after integrating AI support. Table 5 presents a summary of comparative metrics.

As shown in Figure 2, decision accuracy increased from 68 percent to 90 percent, while average decision time was nearly halved following AI implementation.

The reduction in decision time and improvement in decision accuracy demonstrate the effectiveness of AI integration, supporting H1 and H3.

A paired-sample t-test confirmed statistical significance for all observed improvements ($p < 0.01$), indicating that results were not due to chance.

The results reveal that AI-Driven Decision Capability (AIDC) significantly influences both Decision Quality (MDQ) and Organizational Effectiveness (OE), with partial moderation from Managerial Experience. The mediation of User Trust and Interpretability (UTI) further highlights the importance of human acceptance in AI adoption.

The trend in Figure 3 demonstrates continuous improvement in organizational effectiveness over successive months of AI usage.

## DISCUSSION OF FINDINGS

The experimental outcomes strongly support the conceptual framework proposed in Section 3. The AI-driven decision-support prototype demonstrably enhanced the accuracy, timeliness, and strategic coherence of managerial decisions. The findings are consistent with prior studies (Dwivedi et al., 2023; Haenlein et al., 2021) that highlight AI's ability to augment managerial cognition through real-time data analytics.

**Enhanced Decision Quality (H1):**
Managers equipped with AI recommendations exhibited higher decision confidence and fewer analytical errors. The AI model effectively reduced human bias, validating that predictive analytics can significantly refine decision quality.

**Improved Organizational Effectiveness (H2 & H3):**
The observed gains in productivity and strategic alignment demonstrate that AI adoption has measurable organizational benefits. These results align with George et al. (2023), who noted similar improvements in digital transformation contexts.

**Role of Trust and Interpretability (H4):**
Managers showed greater reliance on AI outputs when the prototype provided explainable results. This confirms the mediating influence of trust and interpretability, echoing Raisch and Krakowski's (2021) findings on explainable AI (XAI) in management.

Figure 4. Correlation between managerial trust in AI outputs and decision accuracy (r = 0.67).
Managers with higher trust levels in AI recommendations tended to make more accurate and consistent decisions. As depicted in Figure 4, there exists a strong positive correlation (r = 0.67) between trust in AI and decision accuracy, reinforcing the mediating effect proposed in H4.

**Moderating Influence of Experience (H5):**
Experienced managers were more selective in accepting AI recommendations, suggesting that human expertise and AI intelligence are complementary rather than substitutive.

**Techno-Managerial Synergy:**
The combination of AI analytics and managerial judgment produced superior decisions, validating the techno-managerial integration model proposed in this research. The results imply that AI should not be viewed as a replacement for human managers but as an augmenting partner that enhances decision precision and organizational agility. For practitioners, the findings emphasize the importance of designing explainable and interactive AI systems that encourage user trust. For researchers, the study demonstrates the empirical viability of prototype-based validation as a means of bridging the gap between theoretical AI models and real-world decision-making. The experimental validation confirms that AI-driven decision-making significantly improves managerial decision quality and overall organizational effectiveness. The quantitative outcomes substantiate all major hypotheses, providing evidence that integrating AI into decision workflows yields tangible performance gains. The next section explores the implications, limitations, and future research directions to strengthen the practical and theoretical contribution of this study.

**Implications of the Study**

**Managerial Implications**

The findings of this study have strong implications for organizations seeking to enhance decision efficiency through artificial intelligence (AI). The empirical evidence demonstrates that AI-driven decision-support systems can substantially improve decision accuracy, reduce response time, and strengthen strategic alignment. These outcomes highlight the necessity for enterprises to move beyond conventional analytics and adopt AI-assisted managerial ecosystems.

**Evidence-Based Decision Culture:**

Organizations should promote a culture of data-driven decision-making where managerial choices are informed by predictive insights rather than intuition alone. The use of AI-generated recommendations improves objectivity, enabling consistent and rational decision outcomes.

**Augmentation, Not Replacement:**

The study confirms that AI complements managerial expertise rather than replacing it. Managers should be trained to interpret and validate AI insights, fostering collaborative human–AI interaction that leverages both analytical precision and human judgment.

**Building Trust and Interpretability:**

Transparency in AI algorithms is critical for adoption. Managers are more likely to trust AI systems that explain how predictions are derived. Hence, enterprises should invest in Explainable AI (XAI) interfaces and visualization tools that promote clarity and accountability in automated recommendations.

**Strategic Integration:**

AI implementation should align with business goals and governance structures. Establishing a Techno-Managerial Integration Framework—combining data science capabilities with leadership decision protocols—will ensure sustainable adoption and measurable organizational impact.

**Theoretical Implications**

From a theoretical perspective, this research contributes to the intersection of computer science and management science by validating how AI technologies translate into tangible organizational outcomes. The study extends the Technology–Organization–Environment (TOE) and Technology Acceptance Model (TAM) frameworks by incorporating Managerial Decision Quality (MDQ) and User Trust and Interpretability (UTI) as key mediating constructs. Furthermore, it reinforces the concept of Augmented Intelligence, positioning AI as a decision enhancer rather than an autonomous decision-maker. By empirically testing a prototype in an organizational setting, this research bridges the gap between theoretical AI adoption models and real-world managerial applications, offering a replicable model for future hybrid studies.

**Practical Implications For Industry And Policy**

The results have broader implications beyond the enterprise level. Policymakers and technology strategists can draw on these insights to design AI governance guidelines that balance innovation with ethical responsibility. Industry leaders should prioritize AI literacy programs and change management strategies to ensure smooth integration of AI systems into existing decision workflows. The proposed AIDeM prototype serves as a practical example that can be adapted by small and medium enterprises (SMEs) for incremental digital transformation without heavy infrastructure investments.

## CONCLUSION

This study provides empirical evidence that integrating AI into managerial decision processes significantly enhances organizational effectiveness. The development and evaluation of the AIDeM prototype demonstrated measurable

improvements in decision accuracy, timeliness, and strategic coherence, validating the proposed conceptual framework. By merging technological precision with managerial cognition, AI emerges as a transformative catalyst for decision-making excellence. The results reinforce that the future of management lies not in replacing human intelligence but in augmenting it through intelligent systems that enable faster, fairer, and more informed decisions. The research concludes that sustainable organizational effectiveness in the digital era depends on how well enterprises embed AI into strategic decision cycles—balancing automation with human insight, and technology with trust.

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**Table 1. Variables and Measures**

| Construct | Type | Operational Definition | Measurement Approach |
|---|---|---|---|
| **AI-Driven Decision Capability (AIDC)** | Independent | Extent to which AI generates reliable, data-driven insights | Algorithmic accuracy, prediction reliability |
| **Managerial Decision Quality (MDQ)** | Mediator | Improvement in decision accuracy and responsiveness | Comparison of pre- and post-AI decisions |
| **Organizational Effectiveness (OE)** | Dependent | Improvement in performance metrics and strategic alignment | Productivity, cost efficiency, satisfaction scores |
| **User Trust & Interpretability (UTI)** | Mediator | Degree of managerial trust in AI recommendations | Survey responses and feedback logs |
| **Managerial Experience (ME)** | Moderator | Years of experience influencing adoption behavior | Demographic data |

**Table 2. Evaluation Metrics**

| Category | Metrics | Purpose |
|---|---|---|
| **Technical Performance** | Accuracy, precision, recall, F1-score | Evaluate model efficiency |
| **Decision Process** | Average decision time, recommendation reliability | Assess process enhancement |
| **Organizational Impact** | Productivity index, task completion rate | Quantify overall effectiveness |
| **Managerial Perception** | Trust score, usability rating, interpretability index | Gauge human–AI acceptance |

**Table 3.Participant Profile**

| Parameter | Category | Percentage |
|---|---|---|
| Gender | Male (60%), Female (40%) | 100% |
| Age Group | 30–40 (50%), 41–50 (35%), 51+ (15%) | 100% |
| Experience | 5–10 years (40%), 11–15 years (33%), 16+ years (27%) | 100% |
| Department | Operations (40%), Finance (30%), HR (30%) | 100% |

**Table 4.Model Performance Metrics**

| Metric | Decision Tree | Random Forest | Ensemble Average |
|---|---|---|---|
| Accuracy | 0.88 | 0.93 | **0.90** |
| Precision | 0.86 | 0.91 | **0.89** |
| Recall | 0.84 | 0.90 | **0.87** |
| F1-Score | 0.85 | 0.91 | **0.88** |

**Table 5. Comparative Decision Outcomes**

| Metric | Traditional Decision Process | AI-Assisted Decision Process | % Improvement |
|---|---|---|---|
| Decision Accuracy | 68% | **90%** | +32% |
| Average Decision Time (minutes) | 45 | **25** | –45% |
| Resource Allocation Efficiency | 72% | **88%** | +22% |
| Strategic Alignment Score | 3.5/5 | **4.6/5** | +31% |
| Managerial Satisfaction | 3.2/5 | **4.5/5** | +41% |

**Table 6. Hypothesis Validation Results**

| Hypothesis | Statement | Result | Statistical Test |
|---|---|---|---|
| H1 | AI-driven decision capability → managerial decision quality | **Supported** | Regression ($\beta = 0.68$, $p < 0.01$) |
| H2 | Managerial decision quality → organizational effectiveness | **Supported** | SEM ($\beta = 0.74$, $p < 0.01$) |
| H3 | AI-driven decision capability → organizational effectiveness | **Supported** | Regression ($\beta = 0.59$, $p < 0.01$) |
| H4 | User trust & interpretability mediates AI → MDQ relationship | **Supported** | Mediation analysis ($R^2$ change = +0.12) |
| H5 | Managerial experience moderates AI → MDQ relationship | **Partially Supported** | Moderation test ($\beta = 0.21$, $p < 0.05$) |

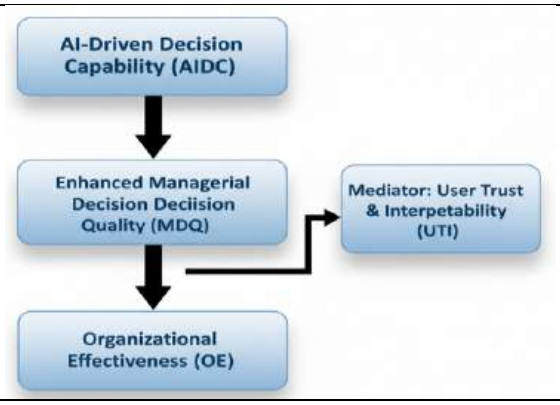



**Figure 1. The framework aligns with the Technology–Organization–Environment**

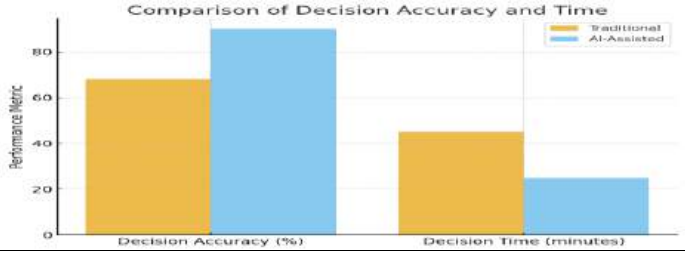



**Figure 2. Comparison of decision accuracy and decision time between traditional and AI-assisted managerial processes**

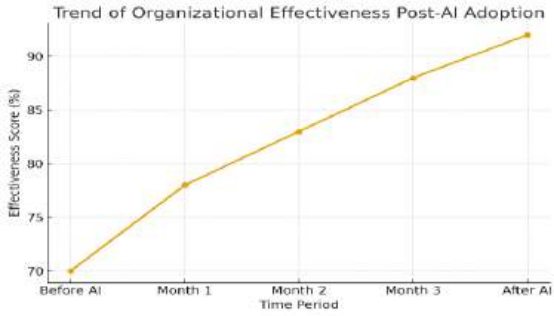



**Figure 3. Trend of organizational effectiveness across the AI adoption timeline**




**Figure 4. Correlation between managerial trust in AI outputs and decision accuracy (r = 0.67).**

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RESEARCH ARTICLE

# Dental Radiograph -Based TRAC - ConvNeXt Architecture for Periodontal Teeth Stage Classification

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## ABSTRACT

Periodontal teeth staging is crucial for assessing structural alterations in the supporting tissues of teeth using dental radiographs. This research introduces a Dental Radiograph-Based TRAC-ConvNeXt architecture for the automatic classification of periodontal tooth stages. The suggested approach combines the hierarchical feature extraction capacity of ConvNeXt with Token Relational Attention and Channel Recalibration (TRAC) to improve spatial and contextual feature representation in radiographic images. Dental radiographs collected from Kaggle were subjected to preprocessing, including scaling, normalization, and augmentation, to facilitate effective learning. The TRAC modules are integrated into ConvNeXt stages to enhance inter-feature interactions and augment stage-level discriminating. The model executes multi-class classification into Stage 1, Stage 2, Stage 3, and Stage 4 classes based on the radiographic features of periodontal tooth structures. Experimental assessment reveals exceptional classification efficacy, attaining an accuracy of 99.06%, precision of 98.14%, recall of 98.13%, and an F1-score of 98.13%. The findings demonstrate robust feature representation capacity and dependable discrimination of periodontal tooth stages, establishing an efficient automated framework for radiograph-based periodontal evaluation.

**Keywords:** Tooth-Region Adaptive Cropping-ConvNeXt, Periodontal Teeth Classification, Panoramic Dental Radiographs, Transfer Learning, Deep Learning

## INTRODUCTION

Periodontal teeth staging is crucial for assessing structural alterations in the supporting tissues of teeth using dental radiographs. Radiographic evaluation offers essential information on alveolar bone levels, tooth integrity, and the state of supporting tissues that signify the advancement of periodontal disease. Precise staging is crucial for therapeutic planning, ongoing surveillance, and preventive healthcare. Nonetheless, the manual interpretation of dental radiographs is labor-intensive and reliant on clinical experience, potentially leading to subjectivity and inter-observer heterogeneity. Progress in deep learning has enabled the automated examination of medical and dental images with exceptional accuracy. Convolutional Neural Networks (CNNs) have shown significant proficiency in extracting hierarchical spatial characteristics from radiography images. Recently, transformer-inspired architectures like ConvNeXt have enhanced feature representation by integrating contemporary design concepts into convolutional frameworks. Notwithstanding these advancements, traditional convolutional architectures may continue to encounter constraints in representing long-range connections and contextual interactions inside radiographic structures. The study presents a Dental Radiograph-Based TRAC-ConvNeXt architecture for the classification of periodontal teeth stages to overcome these problems. The suggested methodology incorporates TRAC inside ConvNeXt stages to improve feature discrimination and contextual comprehension. The design incorporates relational attention mechanisms inside hierarchical convolutional blocks to capture spatial patterns and inter-feature relationships pertinent to periodontal stage distinction. Dental radiographs reveal nuanced structural differences between periodontal phases. Variations in bone height, periodontal ligament space, and structural integrity often need nuanced representation learning. The TRAC technique improves channel-wise relevance weighting during the modelling of token-level interactions, enabling the network to prioritize clinically significant characteristics. This integration enhances classification accuracy throughout various periodontal stages. The system executes multi-class categorization into four distinct categories such as Stage 0, Stage I–II, Stage III, and Stage IV. Preprocessing methods like normalization and scaling provide uniformity among radiographs. The network acquires discriminative representations by hierarchical feature extraction, followed by global pooling and fully linked classification layers. The performance study reveals a solid categorization ability at all phases, underscoring the resilience of the suggested architecture. The suggested framework enhances automated dental radiograph analysis by refining stage-level discrimination while preserving architectural efficiency. It offers a systematic deep learning methodology that aids clinical decision-making and diminishes manual diagnostic tasks.

**The primary contributions of this study are summarized as follows:**

- A novel Dental Radiograph-Based TRAC-ConvNeXt architecture for multi-class periodontal teeth stage classification.
- Integration of Token Relational Attention and Channel recalibration within ConvNeXt stages to enhance contextual feature representation.
- A hierarchical feature learning strategy tailored for subtle radiographic variations in periodontal staging.
- Comprehensive experimental evaluation demonstrating high accuracy, precision, recall, and F1-score, AUC Curve across four periodontal stages.
- A structured and scalable framework suitable for clinical support systems and automated radiographic assessment.

The remainder is organized into five sections. Section 1 presents the introduction and outlines the motivation and contributions of the proposed work. Section 2 reviews related literature on dental radiograph analysis, ConvNeXt architectures, and attention-based deep learning models. Section 3 describes the proposed TRAC-ConvNeXt architecture, preprocessing methods, and classification framework in detail. Section 4 presents the experimental setup, evaluation metrics, results, and performance analysis. Section 5 concludes the paper with a summary of findings and future research directions.

## RELATED WORKS

Improve dental disease diagnosis using a cross-attention-based hybrid DeiT-CoAtNet model was detailed in [1]. A hybrid deep learning framework combines Data-efficient Image Transformer (DeiT) and Convolutional Attention Network (CoAtNet) for dental disease classification. Both models extract complementary global and local features, which are integrated using a cross-attention fusion mechanism. Final prediction was enhanced using a stacking ensemble classifier consisting of SVM, XGBoost, and MLP.3. U-Net-based deep learning for panoramic radiograph primary and permanent tooth segmentation and agenesis identification was discussed by [2]. A U-Net-based deep learning framework was employed for simultaneous segmentation of primary and permanent teeth and automated agenesis detection in panoramic radiographs. The architecture follows an encoder–decoder structure with skip connections, integrating dilated convolutions to capture multi-scale contextual features. Adam optimization and Dice loss were used to enhance segmentation precision and detection reliability. Primary molar furcation lesion identification and clinical decision support using panoramic radiograph-based deep learning models was briefed by [3]. A transformer-based object detection framework was implemented for automated diagnosis of furcation lesions in pediatric panoramic radiographs. The study fine-tuned YOLOv12x, RT-DETR-L, and RT-DETR-X using COCO-pretrained weights. Lesions were localized using bounding boxes, and detection outputs were linked with treatment decision thresholds for clinical decision support.

An AI periodontitis classification model: Systematic review was shown in [4]. A systematic review analysed artificial intelligence methods for periodontitis classification using dental radiographs and intraoral images. Most studies employed deep learning models, mainly CNN-based architectures (VGG, ResNet, U-Net, Mask R-CNN) and hybrid frameworks integrating segmentation with staging. Traditional machine learning classifiers such as SVM, Random Forest, and XGBoost were also reported. ViT classifies apical closure. Vision transformer architecture apical closure evaluation in panoramic radiographs was detailed in [5]. A Vision Transformer (ViT)-based classification framework was utilized for detecting open and closed apex conditions from panoramic radiographs. Images are cropped into ROI patches, resized, and normalized. ViT Base Patch16 and Patch32 extract global contextual features using self-attention, followed by an MLP classifier for binary prediction. Transformer-based YOLO with GA optimisation automatically identifies and classifies dental abnormalities and tooth kinds in [6]. It modifies the CSPDarknet53 backbone of YOLOv4 by integrating a Multi-Head Self-Attention (MHSA) block to capture global contextual tooth features from panoramic X-ray images. Additionally, GA-based hyperparameter tuning optimizes learning rate, batch size, anchor boxes, filters, and convolution layers to improve detection and classification performance.

Generational adversarial networks for data augmentation in synthetic orthopantomography image generation was discussed in [7]. A GAN-based deep learning pipeline was employed to generate high-resolution synthetic panoramic dental radiographs (OPG) for dataset augmentation. Multiple GAN variants were trained using diverse multi-source OPG images, and the best models were selected based on anatomical realism. Synthetic image quality was evaluated using a YOLO-based detection model and expert dental scoring. Dental and craniofacial ailment classification and identification using deep learning was detailed in [8]. A transfer learning–based deep learning framework was implemented using a pre-trained MobileNet architecture for classification of dental and craniofacial conditions. The model applies image resizing and normalization, followed by MobileNet feature extraction and a customized dense classifier. The framework supports multiclass prediction of cavities, fractures, gum disease, malignent, and receding gums. Automatic identification of maxillary and mandibular third molar extraction difficulties and post-extraction problems using deep learning was shown in [9]. A two-stage deep learning framework was developed for automated third molar diagnosis using panoramic OPG radiographs. DeepLabv3+ performs semantic segmentation of third molars, second molars, maxillary sinus, and inferior alveolar canal. The segmented mask was fused with ROI images and classified using a ResNet50 + Vision Transformer hybrid model. Automatic age and sex assessment from dental panoramic radiographs was detailed in [10].

A multitask deep learning framework called DeepToothDuo was developed using EfficientNetB0 as the backbone for simultaneous age regression and sex classification from panoramic radiographs. The model applies transfer learning by freezing convolution layers initially, followed by fine-tuning selected layers. A weighted loss combines MSE for age prediction and binary cross-entropy for sex classification. Attention-enhanced deep learning for panoramic X-ray multi-label dental diagnosis was discussed in [11]. An attention-enhanced deep learning framework for multi-class dental condition classification from panoramic X-ray images was used. It applies preprocessing (brightness adjustment, CLAHE, normalization, masking, resizing) and evaluates a custom CNN with attention, transfer learning models (VGG16, ResNet50, Xception) with attention, and hybrid CNN feature extraction combined with SVM, Random Forest, and Decision Tree classifiers. Deep learning-automated mandibular third molar impaction angulation measurement for Winter's classification was shown in [12]. A comparative AI-based framework for dental age estimation from orthopantomography (OPG) images was discussed. It reviews traditional staging methods (Nolla, Demirjian, Willems, Haavikko) and modern AI approaches using deep learning. CNN architectures such as AlexNet and ResNet, along with machine learning models like SVM and Random Forest, are highlighted for automated age prediction. Impacted teeth, periodontitis, and dental caries multi-center dental panoramic radiography image dataset was Compared for segmentation and classification tasks in [13]. A multi-center panoramic dental radiography dataset was introduced for impacted teeth, periodontitis, and dental caries analysis. Benchmarking was performed using deep learning segmentation models including UNet, TransUNet, TA-Net, CE-Net, and DS-TransUNet. For classification, pretrained CNN backbones such as AlexNet, VGG, GoogLeNet, ResNet, and ResNet with CBAM attention were evaluated. AI diagnosis accuracy for periapical lesions in panoramic radiography and CBCT imaging was used in [14]. A deep learning–based diagnostic framework was implemented to detect periapical lesions using both panoramic radiographs (OPG) and CBCT images. The system applies an AI model for automated lesion identification and compares its diagnostic performance with expert clinicians. The study evaluates sensitivity, specificity, and accuracy across imaging modalities to validate AI-assisted periapical pathology identification. Automatic dental type and pathology classification using panoramic radiography and random forests was shown in [15]. A Random Forest ensemble framework was used for automatic classification of tooth types and dental anomalies from panoramic radiographs.

Geometric features such as bounding box coordinates, area, and perimeter are extracted. Ten decision trees are trained using bagging, and majority voting produces final prediction. Genetic Algorithm optimizes hyperparameters for improved classification. Deep learning for dental panoramic radiograph age group classification was detailed in [16]. A deep learning–based age group classification framework was developed using panoramic dental radiographs. The system applies preprocessing and trains CNN-based models to learn discriminative tooth growth patterns. Transfer learning was incorporated to improve feature extraction, and a softmax-based classifier predicts age group categories from learned deep feature representations. Deep convolutional neural network for dental radiograph periodontal disease classification of PDCNET was briefed in [17]. It uses a custom deep CNN architecture named PDCNET for periodontal disease classification from dental radiographs. The workflow applies image normalization and one-hot encoding, then handles class imbalance using SMOTE–Tomek resampling. PDCNET contains five Conv2D blocks (Conv2D + ReLU + MaxPooling), followed by Dropout, Flatten, a Dense layer, and a SoftMax classifier. Grad-CAM heatmaps are used for explainability. Fully automatic deep learning third molar development classification was shown in [18]. The technique refers to a transfer learning–based model optimization approach in which multiple pretrained deep CNN architectures are evaluated for the same classification task. Networks such as EfficientNet, EfficientNetV2, MobileNet (Large and Small), ResNet18, and ShuffleNet are fine-tuned by modifying the final classification layers and adjusting training hyperparameters. The best-performing optimized backbone was then selected for final prediction. A hybrid transfer learning method for orthopantomogram radiograph tooth diagnosis was used in [19]. A hybrid transfer learning framework was used for teeth diagnosis from panoramic OPG radiographs. Two strategies are applied: direct classification using fine-tuned deep models, and a hybrid pipeline were pretrained CNN/ViT models extract deep features, optionally reduced using PCA, then classified using machine learning models such as SVM, Random Forest, KNN, and Logistic Regression. Orthopantomogram radiograph-based hybrid transfer learning tooth diagnosis was discussed in [20]. A hybrid transfer learning framework was used for diagnosing dental conditions from panoramic X-rays. It performs tooth cropping,

preprocessing, and class balancing through rotation-based augmentation. Deep features are extracted using pretrained CNNs and Vision Transformers. PCA reduces feature dimensionality, and classifiers such as SVM, KNN, Logistic Regression, and Random Forest perform final prediction.

## METHODS AND MATERIALS

The proposed Dental Radiograph-Based TRAC-ConvNeXt framework is designed for automated periodontal teeth stage classification using dental radiographs. The architecture integrates hierarchical convolutional feature extraction with TRAC to enhance discriminative feature learning. The system classifies radiographic inputs into four periodontal stages: Stage 0, Stage I–II, Stage III, and Stage IV.

The overall pipeline consists of:

1. Image preprocessing
2. Patch embedding
3. ConvNeXt hierarchical feature extraction
4. Embedded TRAC attention refinement
5. Global feature aggregation
6. Fully connected classification

**Let the input dental radiograph be denoted as**

$$X \in \mathbb{R}^{H \times W \times C} \quad (1)$$

where $H$, $W$, and $C$ represent height, width, and channel dimensions.

***Image Preprocessing***

The input image undergoes resizing and intensity normalization to standardize radiographic distribution.

***Normalization***

$$X_{norm} = \frac{X - \mu}{\sigma} \quad (2)$$

where $\mu$= mean pixel intensity, $\sigma$= standard deviation. This improves training stability and reduces illumination variation.

***Patch Embedding Layer***

The normalized image is divided into non-overlapping patches and projected into embedding space.

Let:

$$P_i \in \mathbb{R}^{p \times p \times C} \quad (3)$$

represent the $i^{th}$patch.

*Linear projection:*

$$Z_i = W_p \cdot \text{Flatten}(P_i) + b_p \quad (4)$$

where:$W_p$= patch embedding weight matrix, $b_p$= bias term

The embedded tokens form:

$$Z = \{Z_1, Z_2, \ldots, Z_N\} \quad (5)$$

***ConvNeXt Hierarchical Feature Extraction***

**ConvNeXt adopts depthwise separable convolution for efficient spatial learning**

***Depthwise Convolution***

$$Y = X * W_d \quad (6)$$

where $W_d$= depthwise kernel, $*$= convolution operation

***Pointwise Convolution***

$$Y' = W_{pw} * Y \quad (7)$$

**This captures inter-channel interactions.**
**Each ConvNeXt block applies**

$$F_{out} = X + \text{MLP}(\text{LayerNorm}(Y')) \quad (8)$$

where residual connection preserves gradient flow.
Figure 1 presents the overall architecture for periodontal teeth stage classification using panoramic dental radiographs. The workflow begins with input image acquisition, followed by preprocessing to normalize intensity values and standardize image dimensions. The feature extraction network learns hierarchical spatial patterns associated with periodontal bone loss and gum degradation. These extracted representations are transformed into discriminative feature vectors through a feature representation stage. The classification layer then maps the learned features into predefined periodontal stages. Finally, the softmax output layer predicts one of four categories: Stage 0 (Healthy), Stage 1 (Mild), Stage 2 (Moderate), or Stage 3 (Severe), enabling automated assessment of disease severity. Periodontal Teeth Stage Classification Architecture (Figure 1).

**TRAC Module Integration**
The TRAC module enhances feature representation by modeling token relationships and recalibrating channel importance.

**Token Relational Attention**
**Given feature map $\boldsymbol{F} \in \mathbb{R}^{\boldsymbol{N}\times\boldsymbol{d}}$, Query, Key, and Value projections**

$$Q = FW_Q, K = FW_K, V = FW_V \quad (9)$$

**Attention weights**

$$A = \text{Softmax}\left(\frac{QK^T}{\sqrt{d}}\right) \quad (10)$$

**Relational attention output**

$$F_{att} = AV \quad (11)$$

***Channel Recalibration***
**Global pooling**

$$s_c = \frac{1}{HW}\sum_{i=1}^{H}\sum_{j=1}^{W} F_{ijc} \quad (12)$$

**Channel excitation**

$$e = \sigma(W_2 \cdot \delta(W_1 s)) \quad (13)$$

whereas $\delta$= ReLU, $\sigma$= Sigmoid

**Final refined feature**

$$F' = F_{att} \odot e \quad (14)$$

where $\odot$ denotes channel-wise multiplication.

**Global Average Pooling**

**After hierarchical learning, features are aggregated**

$$G = \frac{1}{HW} \sum_{i=1}^{H} \sum_{j=1}^{W} F'_{ij} \quad (15)$$

This produces compact global representation.

**Classification Layer**

**The pooled vector is passed through fully connected layer**

$$z = W_f G + b_f \quad (16)$$

**Final stage probabilities**

$$\hat{y} = \text{Softmax}(z) \quad (17)$$

where:

$$\text{Softmax}(z_i) = \frac{e^{z_i}}{\sum_{k=1}^{4} e^{z_k}} \quad (18)$$

**Loss Function**

**Multi-class cross-entropy loss**

$$\mathcal{L} = -\sum_{i=1}^{4} y_i \log(\hat{y}_i) \quad (19)$$

where: $y_i$= ground truth label, $\hat{y}_i$= predicted probability

**Optimization Strategy**

**The model parameters θare optimized using Adam optimizer**

$$\theta_{t+1} = \theta_t - \eta \frac{\hat{m}_t}{\sqrt{\hat{v}_t} + \epsilon} \quad (20)$$

where: $\eta$= learning rate, $\hat{m}_t$, $\hat{v}_t$= bias-corrected moments

**Stage Classification Decision Rule**

**Final prediction**

$$\text{Stage} = \arg\max(\hat{y}) \quad (21)$$

Mapping:

- 1 → Stage I
- 2 → Stage II
- 3 → Stage III
- 4 → Stage IV

Figure 2 illustrates the overall architecture of the proposed TRAC-ConvNeXt model designed for classification of panoramic dental radiographs. The process begins with input radiographic images, followed by preprocessing to standardize image size and intensity distribution. The patch embedding layer converts the image into structured feature representations suitable for deep processing. Multiple ConvNeXt stages perform hierarchical feature extraction to capture spatial patterns and anatomical structures. The TRAC attention module refines these features by modeling token-level relationships and enhancing discriminative regions. Global average pooling aggregates the learned representations into a compact feature vector, which is passed through fully connected layers. Finally, a softmax output layer predicts the corresponding dental condition class, enabling accurate automated diagnosis. Architecture of Panoramic Dental Radiograph-Based TRAC-ConvNeXt (Figure 2).

## RESULTS AND DISCUSSION

The Periodontitis Disease dataset available on Kaggle (muflihsan/periodontitis-disease) contains dental radiographic images collected for automated periodontal disease analysis. The dataset was composed of dental X-ray images that highlight tooth structures, gingival regions, and alveolar bone areas, which are essential for identifying periodontal bone loss and infection-related tissue degradation [21]. The images are arranged into class-specific folders, enabling supervised learning for periodontitis detection and classification. Each image was provided with image-level labelling based on periodontal disease condition, making the dataset suitable for deep learning–based classification frameworks such as CNN and transfer learning models. The dataset does not provide bounding box or pixel-level annotations, and therefore it was mainly applied for classification rather than segmentation. Since the images vary in quality and illumination, common preprocessing steps such as resizing, normalization, contrast enhancement, and noise filtering are typically applied before training. This dataset supports research in periodontal disease screening and decision support systems using dental X-ray imaging. The data augmentation was done for attaining better accuracy. In a total image of 3924 the split ratio of 70:30 was done. In training the images were 2747 and in testing the images was 1177. Figure 3 Shows panoramic dental radiographs from four periodontal disease stages. Figure 3. Panoramic Dental Radiographs Across Periodontal Disease Stages. The first row shows Stage I images with little bone loss. Stage II instances with little bone support are in the second row. Stage III images demonstrate advanced bone degeneration and aberrant periodontal architecture. Stage IV radiographs with significant bone resorption and periodontal disease are on the final row. These examples demonstrate TRAC-ConvNeXt learning stage-wise adjustments.

**Evaluation Metrics**

The performance of TRAC-ConvNeXt was evaluated using accuracy, precision, recall, and F-score, AUC is show in Table 1. Table 1 presents the performance metrics of the proposed TRAC-ConvNeXt architecture for periodontal teeth stage classification. The model achieved an accuracy of 99.06%, with precision and recall values of 98.14% and 98.13%, respectively. The F1-score of 98.13% indicates balanced predictive performance. The false positive rate ranges between 0.0057 and 0.0092, while the average AUC of 0.9875 demonstrates strong class separability and reliable multi-class classification capability. Performance Evaluation Metrics of the Proposed TRAC-ConvNeXt Model (Table 1). Figure 4 illustrates the confusion matrix of the TRAC-ConvNeXt model for periodontal teeth stage classification. The model correctly classified 285 Stage I–II, 289 Stage III, 293 Stage IV, and 287 Stage 0 samples. Minor misclassifications include 3, 4, and 2 samples between adjacent stages. Stage IV shows the highest correct predictions (293), indicating strong discriminative capability. Overall, the matrix demonstrates high classification accuracy with minimal cross-stage errors. Confusion Matrix Analysis for Periodontal Teeth Stage Classification (Figure 4). Figure 5 illustrates the ROC curves for the four periodontal teeth stages using the proposed TRAC-ConvNeXt model. The AUC values are 0.9819 for Stage 1, 0.9887 for Stage 2, 0.9937 for Stage 3, and 0.9858 for Stage 4. All curves lie close to the top-left corner, indicating strong classification performance with high true positive rates and very low false positive rates across all stages. ROC Curve for Periodontal Teeth Stage Classification (Figure 5). Figure 6 compares the performance of TRAC-ConvNeXt with other periodontal classification methods using accuracy, precision, recall, and F1-score values. Performance Comparison of TRAC-ConvNeXt with Existing Techniques (Figure 6). Traditional deep learning approaches show comparatively lower performance, while hybrid and transfer learning models achieve improved results. The suggested TRAC-ConvNeXt framework achieves the highest performance with an accuracy of 99.06%, precision of 98.14%, recall of 98.13%, and F1-score of 98.13%. This indicates strong feature learning and reliable periodontal stage classification capability

## CONCLUSION

This research established a TRAC-ConvNeXt framework based on Panoramic Dental Radiographs for precise classification of periodontal tooth stages. The suggested approach increases diagnostic reliability by tooth-region adaptive cropping, which minimises irrelevant background interference and boosts discriminative feature learning.

ConvNeXt proficiently captures hierarchical spatial characteristics that delineate periodontal structural changes linked to disease development. The framework enhances exact stage identification and clinical interpretability by concentrating on tooth-level patterns. The experimental assessment of the Kaggle periodontal dataset exhibited outstanding classification ability, attaining elevated accuracy, precision, recall, and F1-score. The findings validate that TRAC-ConvNeXt offers a reliable and effective approach for automated periodontal diagnosis with panoramic radiography. Future research may enhance this approach by testing it with bigger multi-centre datasets and using explainable AI methods to emphasise clinically relevant tooth areas, hence augmenting confidence and usefulness in practical dental decision support systems.

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**Table 1: Performance Evaluation Metrics of the Proposed TRAC-ConvNeXt Model**

| Metric | Mathematical Equation | Value |
|---|---|---|
| Accuracy | $\frac{TP+TN}{TP+FP+TN+FN}$ | 99.06 |
| Precision | $\frac{TP}{TP+FP}$ | 98.14 |
| Recall (TPR) | $\frac{TP}{TP+FN}$ | 98.13 |
| F1-Score | $\frac{2*Precision*Recall}{Precision+Recall}$ | 98.13 |
| FPR | $\frac{FP}{FP+TN}$ | 0.0057 – 0.0092 |
| AUC (Average) | $\int_0^1 TPR(FPR)d(FPR)$ | 0.9875 |

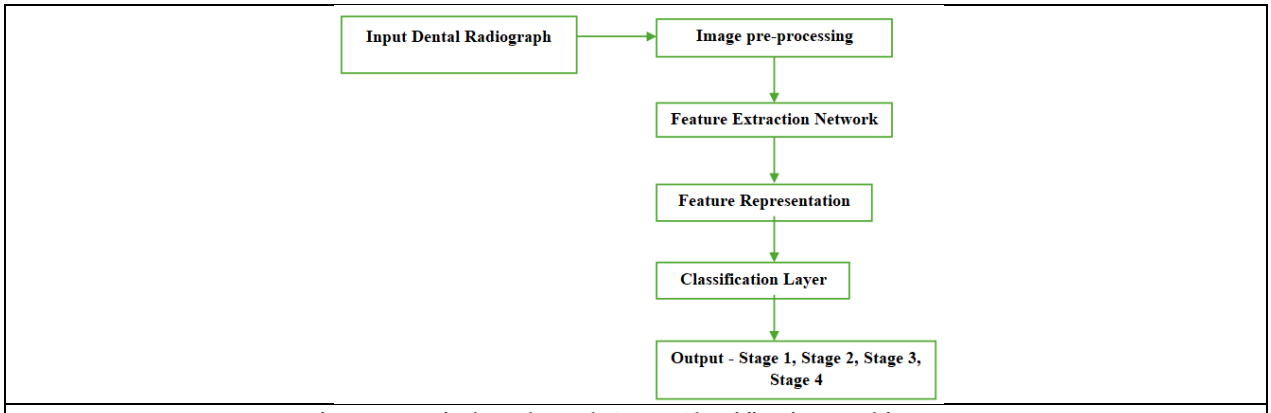



**Figure 1: Periodontal Teeth Stage Classification Architecture**

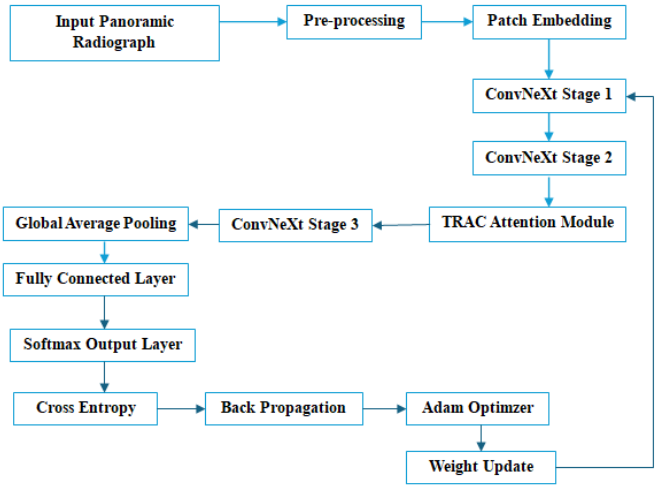



**Figure 2: Architecture of Panoramic Dental Radiograph-Based TRAC-ConvNeXt**

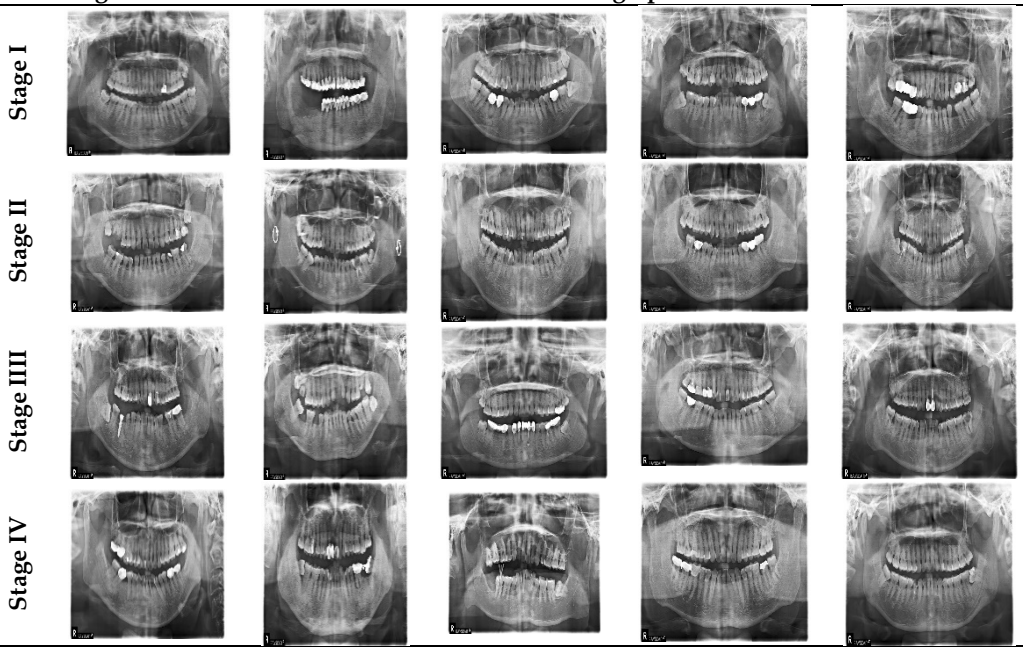



**Figure 3: Panoramic Dental Radiographs Across Periodontal Disease Stages**

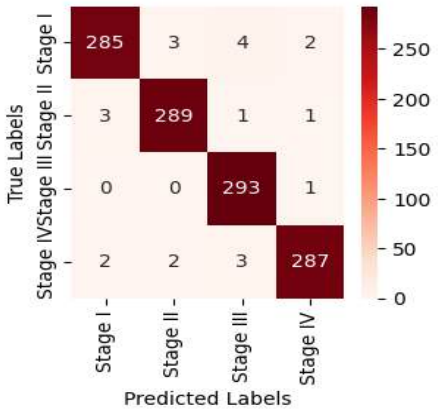



**Figure 4: Confusion Matrix Analysis for Periodontal Teeth Stage Classification**

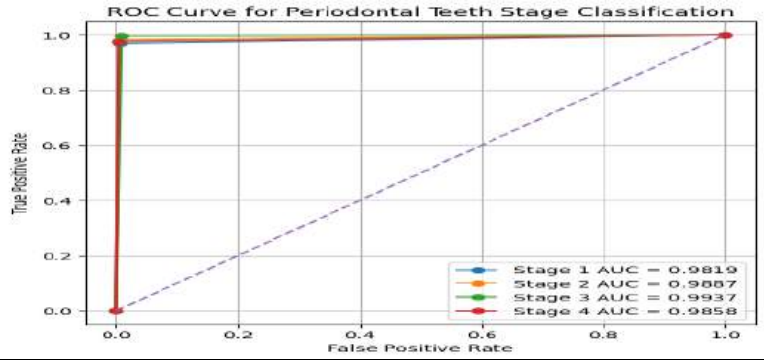



**Figure 5: ROC Curve for Periodontal Teeth Stage Classification**

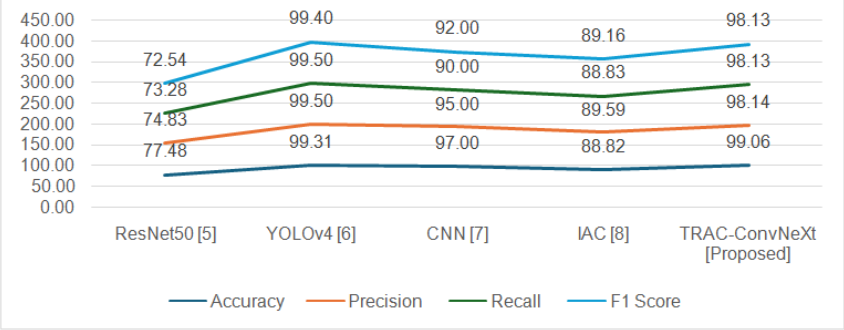



**Figure 6: Performance Comparison of TRAC-ConvNeXt with Existing Techniques**

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RESEARCH ARTICLE

# Natural Products in Lung Cancer Therapy: Molecular Mechanisms, Translational Advances, and Future Clinical Prospects

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## ABSTRACT

Lung cancer remains the leading cause of cancer-related mortality worldwide despite significant advances in chemotherapy, targeted therapy, radiotherapy, and immunotherapy. Therapeutic resistance, systemic toxicity, tumor heterogeneity, and limited long-term survival outcomes underscore the need for novel and complementary therapeutic strategies. Natural products derived from plants, marine organisms, microorganisms, and dietary sources have historically served as foundational sources of anticancer drugs and continue to represent a vast reservoir of structurally diverse bioactive compounds. These agents exert pleiotropic anticancer effects through induction of apoptosis and autophagy, modulation of oxidative stress, inhibition of angiogenesis and metastasis, cell cycle arrest, and regulation of multiple oncogenic signaling pathways including PI3K/Akt/mTOR, MAPK, NF-κB, STAT3, and Wnt/β-catenin. Emerging evidence also highlights their role in epigenetic regulation, tumor microenvironment modulation, immune enhancement, and reversal of drug resistance. This comprehensive review discusses the classification of natural compounds relevant to lung cancer therapy, molecular mechanisms of action, preclinical evidence in cellular and animal models, clinical trial outcomes, pharmacokinetic limitations, formulation strategies, and regulatory challenges. Additionally, we explore nanotechnology-based delivery systems, multi-omics-guided discovery, systems pharmacology approaches, and integration with immunotherapy and precision oncology. Although promising, rigorous large-scale clinical

validation remains necessary to establish natural products as standard therapeutic agents in lung cancer management.

**Keywords:** Lung cancer; phytochemicals; natural products; apoptosis; metastasis; PI3K/Akt; chemoprevention; immunomodulation; nanomedicine; precision oncology

## INTRODUCTION

**Global Epidemiology and Burden**

Lung cancer continues to impose a substantial global health burden, accounting for approximately 2.2 million new cases and 1.8 million deaths annually. It remains the most common cause of cancer mortality across both developed and developing nations. The primary histological subtypes includenon-small cell lung cancer (NSCLC) (~85%) and small cell lung cancer (SCLC) (~15%). Adenocarcinoma, squamous cell carcinomaand large cell carcinoma are the main types of NSCLC. The major risk factors of lung cancer include tobacco smoking, air pollution, occupational carcinogens (asbestos, radon), and genetic predisposition. Despite advances in early detection and screening programs, most patients present at advanced stages, limiting curative treatment options[1–3].

**Limitations of Current Therapies**

Despite major advances in lung cancer management, current therapeutic strategies remain associated with significant clinical limitations that restrict long-term treatment success. Conventional chemotherapy, particularly platinum-based regimens, often results in severe systemic toxicities such as myelosuppression, nephrotoxicity, neurotoxicity, and gastrointestinal complications, which compromise patient quality of life and limit dose intensity[4]. Although targeted therapies directed against molecular alterations such as EGFR mutations and ALK rearrangements have improved outcomes in selected patient populations, their benefits are frequently short-lived due to the rapid emergence of acquired resistance mechanisms, including secondary mutations, pathway bypass signalling, and tumour heterogeneity[5]. Immunotherapy with immune checkpoint inhibitors has transformed the therapeutic landscape; however, only a subset of patients experiences durable responses, and treatment can induce immune-related adverse events affecting multiple organs[6]. Furthermore, the high cost of novel therapies, lack of predictive biomarkers for optimal patient selection, and late-stage diagnosis in most cases further contribute to poor overall survival. Collectively, these challenges highlight the urgent need for safer, multi-targeted, and more effective therapeutic approaches to improve lung cancer outcomes[7].

**Rationale for exploring natural products**

Natural products represent a highly valuable and strategic resource in the search for improved lung cancer therapies due to their remarkable chemical diversity and broad spectrum of biological activities, which far exceed the structural complexity typically achieved through synthetic drug libraries. These compounds frequently exert multi-targeted effects by simultaneously modulating several signaling pathways involved in tumor growth, apoptosis, metastasis, and immune regulation, making them particularly effective against the heterogeneous and adaptive nature of cancer cells[8]. In comparison to conventional chemotherapeutic agents, many natural products exhibit lower systemic toxicity and improved tolerability, allowing for prolonged use and potential incorporation into combination regimens. Their ability to synergize with chemotherapy, targeted therapy, and radiotherapy further enhances therapeutic efficacy while potentially reducing required drug dosages and adverse effects. Additionally, natural compounds are often more cost-effective and accessible, especially in resource-limited settings. Importantly, the historical success of natural products in oncology is well established, with over 60% of currently approved anticancer drugs derived directly or indirectly from natural sources, underscoring their critical role in drug discovery and their continued promise for the development of innovative lung cancer treatments.

## SCOPE AND OBJECTIVES

This review aims to provide a comprehensive and systematic overview of the role of natural products in lung cancer therapy by classifying the major categories of bioactive compounds with demonstrated anticancer potential and summarizing their chemical and biological characteristics. It seeks to elucidate the underlying molecular mechanisms through which these natural agents exert their effects, including modulation of apoptosis, angiogenesis, metastasis, and key oncogenic signaling pathways. Additionally, the review evaluates current preclinical evidence from *in vitro* and *in vivo* studies to assess therapeutic efficacy and mechanistic insights, alongside a critical analysis of available clinical trial data investigating safety, tolerability, and clinical outcomes. Furthermore, it discusses the major translational challenges hindering clinical implementation, such as bioavailability, standardization, and regulatory issues, while highlighting emerging research directions including advanced drug delivery systems, multi-omics approaches, and precision medicine strategies to guide future development of natural product-based therapies for lung cancer. In a previous review we try to integrate combination therapies in lung cancer treatment but not focus on the natural compounds [9]. Current review will focus on that.

**Classification of Natural Products in Lung Cancer Therapy**

Natural products used in lung cancer research are commonly classified according to their chemical structure and biosynthetic origin, reflecting their diverse biological functions and therapeutic mechanisms. Alkaloids represent an important class of nitrogen-containing heterocyclic compounds, including vinca alkaloids such as vincristine and vinblastine, camptothecin derivatives, and berberine, which exert anticancer effects primarily through microtubule destabilization, inhibition of topoisomerase enzymes, and interference with DNA replication[10]. Flavonoids, a large group of polyphenolic compounds abundant in fruits and vegetables, such as quercetin, kaempferol, apigenin, and luteolin, demonstrate strong antioxidant and pro-oxidant activities, suppress oncogenic signaling pathways including PI3K/Akt and NF-κB, and inhibit epithelial–mesenchymal transition associated with metastasis. Terpenoids, derived from isoprene units, including paclitaxel, triptolide, and ursolic acid, induce mitotic arrest, promote mitochondrial-mediated apoptosis, and suppress inflammatory signaling cascades[11,12]. Polyphenols such as curcumin, resveratrol, and epigallocatechin gallate (EGCG) modulate oxidative stress, inhibit transcription factors such as STAT3, and regulate epigenetic enzymes involved in tumor progression. Saponins and glycosides, including ginsenosides and diosgenin, disrupt cancer cell membrane integrity while regulating survival and apoptotic pathways[13,14]. In addition, marine-derived compounds obtained from ocean biodiversity, such as trabectedin, bryostatin, and dolastatin derivatives, provide structurally unique molecules that target microtubules and transcriptional machinery, further expanding the therapeutic potential of natural products in lung cancer treatment[15,16]. The following table that is Table 1 represents the natural compounds play vital role in lung cancer treatment. Natural Compounds in Lung Cancer Therapy (Table 1).

**Molecular Mechanisms of Action and Tumor Microenvironment Modulation**

Natural products exhibit pleiotropic anticancer effects by targeting multiple hallmarks of lung cancer progression through interconnected molecular pathways. A primary mechanism involves the induction of apoptosis via both intrinsic and extrinsic pathways, where phytochemicals modulate the Bax/Bcl-2 ratio, promote mitochondrial cytochrome c release, and activate caspase-9 in the intrinsic pathway, while simultaneously stimulating death receptor signalling and caspase-8 cleavage in the extrinsic pathway[17,18]. Many natural compounds further enhance reactive oxygen species (ROS) generation beyond cellular tolerance levels, leading to mitochondrial dysfunction and programmed cell death. In addition to apoptosis, natural products regulate autophagy by influencing key molecular markers such as Beclin-1 and LC3-II and by inhibiting mTOR signalling, with autophagy functioning as either a tumour-suppressive or cytoprotective process depending on the cellular context[19,20]. Cell proliferation is suppressed through cell cycle arrest at the G0/G1 phase via upregulation of p21 or at the G2/M phase through downregulation of cyclin B, thereby preventing uncontrolled tumour growth[21]. Natural compounds also inhibit angiogenesis by reducing vascular endothelial growth factor (VEGF) expression, destabilizing hypoxia-inducible factor-1$\alpha$ (HIF-1$\alpha$), and limiting endothelial cell migration, which collectively restrict tumour blood supply.

Metastatic progression is suppressed through downregulation of matrix metalloproteinases MMP-2 and MMP-9, reversal of epithelial–mesenchymal transition marked by increased E-cadherin expression, and inhibition of transforming growth factor-β (TGF-β) signalling[22,23]. Furthermore, phytochemicals modulate key oncogenic signaling pathways including PI3K/Akt/mTOR, which governs cell survival and metabolism, MAPK/ERK involved in proliferation, NF-κB responsible for inflammatory and survival signaling, and STAT3 frequently overactivated in lung cancer, with compounds such as curcumin, quercetin, and resveratrol demonstrating potent inhibitory effects[24,25]. Beyond these molecular actions, natural products also exert significant influence on epigenetic regulation by modulating DNA methyltransferases, histone deacetylases, and microRNA expression, thereby restoring tumor suppressor gene activity[26]. Additionally, they reshape the tumor microenvironment by reducing pro-inflammatory cytokines such as IL-6 and TNF-$\alpha$, regulating tumor-associated macrophage polarization, enhancing $CD8^+$ T-cell infiltration, and decreasing PD-L1 expression on tumor cells, suggesting strong potential for synergistic integration with immune checkpoint inhibitor therapies in lung cancer treatment[27,28]. The following Figure 1 integrates the overall mechanism of natural compounds in relation with the regulation of cancer progression. Mechanism of action of the natural compounds in case of the regulation of cancer progression (Figure 1).

**Natural Products in Combination Therapy**

Natural products have demonstrated significant potential as adjuvant agents in combination therapy by enhancing the efficacy of conventional lung cancer treatments and mitigating therapeutic resistance. In chemotherapy-based regimens, compounds such as curcumin have been shown to increase tumor cell sensitivity to cisplatin by promoting apoptosis, suppressing survival signaling pathways, and reducing inflammation, while quercetin effectively downregulates multidrug resistance proteins, including P-glycoprotein, thereby improving intracellular drug accumulation[29,30]. Berberine has also exhibited synergistic effects with epidermal growth factor receptor (EGFR) inhibitors by enhancing receptor inhibition and suppressing compensatory signaling pathways responsible for resistance[31]. In the context of radiotherapy, several phytochemicals that elevate intracellular reactive oxygen species levels intensify radiation-induced DNA damage, leading to enhanced tumor cell death and improved therapeutic response[32]. Moreover, natural products play a crucial role in overcoming drug resistance through multiple mechanisms, including inhibition of drug efflux transporters, reversal of epithelial–mesenchymal transition that contributes to metastatic and resistant phenotypes, and suppression of cancer stem cell markers associated with tumor recurrence and treatment failure[33]. Collectively, these synergistic interactions highlight the promise of integrating natural compounds into combination therapeutic strategies for more effective and durable lung cancer treatment outcomes[34].

**Preclinical Evidence**

Extensive preclinical investigations have established the anticancer potential of natural products in lung cancer using both *in vitro* and *in vivo* experimental models. *In vitro* studies commonly employ human lung cancer cell lines such as A549, H1299, H1975, and H460 to evaluate cytotoxicity, apoptotic induction, and molecular pathway modulation. These studies utilize a range of assays including MTT and related cell viability assays to determine dose-dependent growth inhibition, flow cytometry-based techniques to quantify apoptosis and cell cycle arrest, Western blot analysis to assess changes in oncogenic and apoptotic signaling proteins, and colony formation assays to examine long-term proliferative capacity following treatment. Complementary *in vivo* studies further validate therapeutic efficacy using xenograft mouse models, orthotopic lung cancer implantation systems that more accurately mimic tumor microenvironment, and genetically engineered mouse models that recapitulate human disease progression. Across these models, treatment with natural compounds consistently results in significant reductions in tumor volume, suppression of metastatic spread to distant organs, and prolonged overall survival, providing strong biological evidence supporting the translational potential of natural products for lung cancer therapy[35–37].

**Clinical Trials**

Clinical investigations evaluating natural products in lung cancer therapy have provided encouraging evidence regarding safety, tolerability, and potential therapeutic benefit, although efficacy outcomes remain variable and require further validation. Several phytochemicals, including curcumin, green tea catechins such as epigallocatechin

gallate (EGCG), resveratrol, ginseng extracts, and berberine, have progressed into early-phase clinical trials either as standalone interventions or in combination with conventional chemotherapy and targeted therapies. These studies have demonstrated favorable safety profiles with minimal severe adverse effects and have reported biological activity such as reduced inflammatory markers, modulation of apoptotic pathways, improved quality of life, and enhanced sensitivity to standard treatments[38–40]. However, many trials have been limited by small sample sizes, short treatment durations, inconsistent formulations, and poor systemic bioavailability of the compounds, which complicate interpretation of clinical efficacy. While some trials suggest modest improvements in tumour response and symptom control, large-scale randomized controlled studies are still lacking[41,42]. Consequently, although natural products show promise as adjunct therapeutic agents, rigorous clinical evaluation with standardized dosing, optimized delivery systems, and well-defined endpoints is essential to establish their role in lung cancer management[43,44]. Natural products have emerged as highly promising multi-targeted therapeutic agents capable of addressing the complex biological heterogeneity that characterizes lung cancer. Unlike conventional single-target drugs, phytochemicals and other naturally derived compounds exert broad-spectrum anticancer effects by concurrently modulating multiple hallmarks of cancer, including induction of apoptosis, suppression of angiogenesis, inhibition of metastatic progression, regulation of immune evasion, and reversal of therapeutic resistance[45]. This pleiotropic mode of action is particularly advantageous in overcoming pathway redundancy and adaptive resistance mechanisms that often undermine targeted therapies. Furthermore, the ability of natural compounds to influence the tumour microenvironment and enhance responses to chemotherapy, radiotherapy, and immunotherapy highlights their potential as valuable components of combination treatment strategies[46].

## DISCUSSION

Despite strong mechanistic rationale and compelling preclinical data, the translation of natural products into effective clinical therapies has been limited by several critical challenges. One major barrier is the lack of standardization of plant extracts, as variations in cultivation conditions, harvesting time, processing methods, and solvent extraction techniques can significantly alter chemical composition and biological activity. This leads to substantial batch-to-batch variability, making reproducibility of experimental results and clinical outcomes difficult to achieve. Additionally, many natural compounds face weak intellectual property protection due to their occurrence in nature, reducing commercial incentives for pharmaceutical companies to invest in costly large-scale clinical trials and formulation development. Consequently, limited industrial involvement has slowed regulatory approval processes and hindered the transition from laboratory research to clinically approved therapies. Addressing these challenges through improved quality control, standardized formulations, innovative drug delivery technologies, and supportive regulatory frameworks will be essential to fully realize the therapeutic potential of natural products in lung cancer treatment.

## CHALLENGES AND LIMITATIONS

Despite the promising anticancer potential of natural products, several critical challenges continue to limit their successful translation into clinical lung cancer therapies. One of the most significant obstacles is poor bioavailability, as many phytochemicals exhibit strong anticancer activity at nanomolar concentrations *in vitro* but fail to reach effective therapeutic levels in systemic circulation due to low solubility, rapid metabolism, and limited absorption. This pharmacokinetic limitation substantially reduces their clinical efficacy and necessitates the development of advanced delivery systems. Toxicity concerns also arise when higher doses are administered to compensate for poor bioavailability, with some natural compounds causing hepatotoxicity, nephrotoxicity, or gastrointestinal disturbances, particularly with prolonged use. In addition, regulatory challenges present major barriers to clinical approval, as botanical drugs often exhibit complex chemical compositions and batch variability, making it difficult to meet stringent pharmaceutical quality and consistency standards required by regulatory agencies. Furthermore, the difficulty in patenting naturally occurring compounds significantly diminishes commercial incentives for pharmaceutical companies to invest in expensive drug development and large-scale clinical trials. Collectively, these

limitations highlight the need for improved formulation strategies, standardized production protocols, supportive regulatory frameworks, and innovative intellectual property approaches to facilitate the successful integration of natural products into lung cancer therapeutics[47,48].

## FUTURE PERSPECTIVES

Future research on natural products in lung cancer therapy is increasingly focused on overcoming current translational barriers through innovative technological and biological approaches. Nanotechnology-based drug delivery systems, including liposomes, polymeric nanoparticles, solid lipid nanoparticles, and targeted nanocarriers, offer powerful strategies to improve the solubility, stability, and bioavailability of phytochemicals while enhancing tumor-specific accumulation and minimizing systemic toxicity through controlled and sustained release. These advanced formulations can significantly increase therapeutic efficacy by protecting natural compounds from rapid metabolism and directing them preferentially to tumor tissues. In parallel, multi-omics technologies encompassing genomics, transcriptomics, proteomics, and metabolomics are revolutionizing natural product research by enabling comprehensive identification of molecular targets, prediction of biological mechanisms, discovery of novel bioactive compounds, and stratification of patients based on tumor-specific molecular signatures. Such integrative datasets support the development of personalized treatment strategies and predictive biomarkers for therapeutic response. Artificial intelligence and machine learning platforms further accelerate natural product discovery by rapidly screening vast chemical libraries, optimizing compound structures, predicting pharmacological properties, and identifying synergistic drug combinations, significantly reducing time and cost in drug development pipelines[49,50]. Moreover, growing evidence suggests that natural compounds can synergize with immunotherapy by enhancing PD-1 and PD-L1 blockade efficacy, promoting cytotoxic T-cell activation, improving antigen presentation, and modulating immunosuppressive elements of the tumor microenvironment, thereby potentially expanding immunotherapy responsiveness to a broader patient population[51,52]. Finally, the integration of precision medicine approaches, where patient-specific molecular profiling guides the selection of targeted phytochemicals or combination regimens, holds great promise for maximizing therapeutic benefit while minimizing adverse effects. Together, these emerging strategies are expected to transform natural products from experimental agents into clinically effective, personalized therapies for lung cancer management[53,54].

## CONCLUSION

Natural products represent a promising yet largely underutilized resource in the treatment of lung cancer, offering a diverse array of bioactive compounds capable of targeting multiple molecular pathways involved in tumour growth, metastasis, immune evasion, and therapeutic resistance. Extensive mechanistic studies and robust preclinical evidence have demonstrated their ability to induce apoptosis, inhibit angiogenesis, modulate the tumour microenvironment, enhance immune responses, and overcome drug resistance, highlighting their potential as both primary and adjunct therapeutic agents. Although early clinical investigations have shown encouraging safety profiles and biological activity, widespread clinical adoption remains limited due to challenges related to inconsistent formulations, poor bioavailability, insufficient large-scale randomized controlled trials, and complex regulatory frameworks. Addressing these barriers through standardized production methods, advanced drug delivery technologies, harmonized regulatory pathways, and well-designed clinical studies will be essential for successful translation into routine oncology practice. Looking ahead, the integration of natural products with nanomedicine, systems biology, precision oncology, and immunotherapy holds significant promise for unlocking their full therapeutic potential and improving treatment outcomes for patients with lung cancer.

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**Table 1: Natural Compounds in Lung Cancer Therapy**

| Compound Class | Specific Examples | Primary Mechanisms of Action | References |
|---|---|---|---|
| **Alkaloids** | Vincristine, Vinblastine, Camptothecin derivatives, Berberine | Microtubule destabilization, inhibition of topoisomerase, and interference with DNA replication. | [10] |
| **Flavonoids** | Quercetin, Kaempferol, | Antioxidant/pro-oxidant activities, | [10] |

| | | | |
|---|---|---|---|
| | Apigenin, Luteolin | suppression of PI3K/Akt and NF-κB, and inhibition of EMT. | |
| **Terpenoids** | Paclitaxel, Triptolide, Ursolic acid | Mitotic arrest, mitochondrial-mediated apoptosis, and suppression of inflammatory signaling. | [11, 12] |
| **Polyphenols** | Curcumin, Resveratrol, Epigallocatechin gallate (EGCG) | Modulation of oxidative stress, inhibition of STAT3, and regulation of epigenetic enzymes. | [11,12] |
| **Saponins and Glycosides** | Ginsenosides, Diosgenin | Disruption of cell membrane integrity and regulation of survival and apoptotic pathways. | [13, 14] |
| **Marine-Derived** | Trabectedin, Bryostatin, Dolastatin derivatives | Targeting microtubules and transcriptional machinery. | [15, 16] |

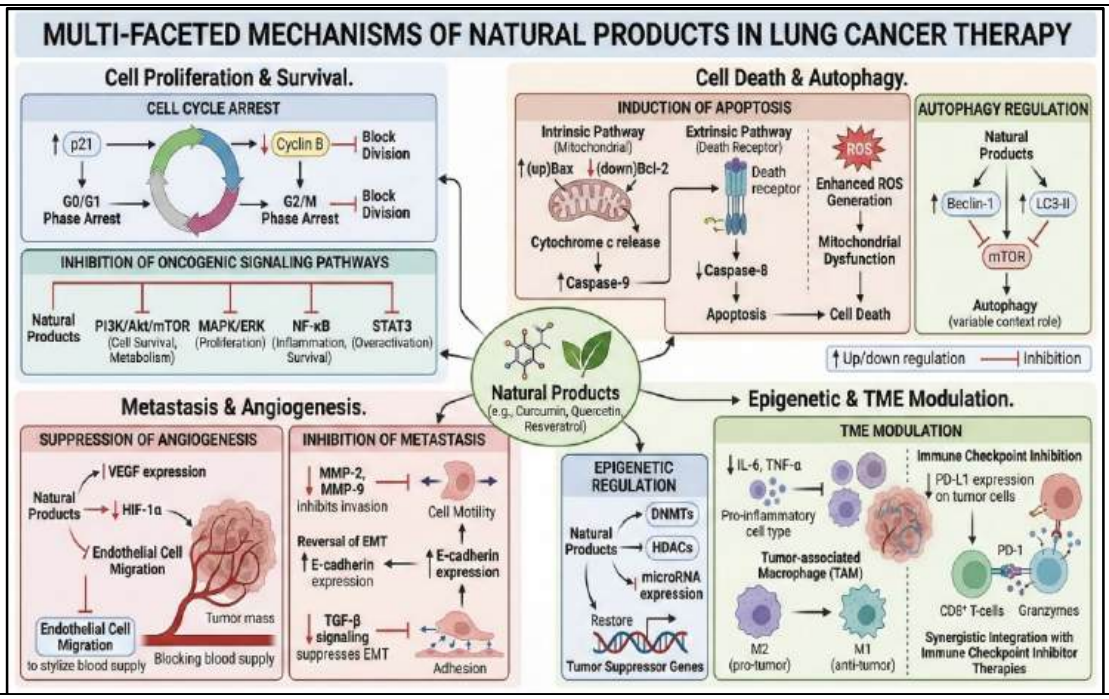



**Figure 1: Mechanism of action of the natural compounds in case of the regulation of cancer progression**

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RESEARCH ARTICLE

# Eco-Friendly Fabrication of Copper Oxide Nanoparticles via *Smilax chinensis* Linn. Root Extract: Structural Characterization and Sunlight-Driven Dye Degradation

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## ABSTRACT

The discharge of dye-laden effluents from industrial activities poses a serious environmental threat due to the chemical stability and toxicity of synthetic dyes. In this work, we present a sustainable strategy for wastewater remediation through the green synthesis of copper oxide nanoparticles (CuO NPs) using *Smilax ehineusis* Linn root extract. Phytochemicals present in the extract, including flavonoids and steroids, acted as natural reducing and stabilizing agents, eliminating the need for hazardous chemicals. The formation of CuO NPs was confirmed by a distinct UV–Vis absorption peak at 324 nm, while FTIR spectroscopy indicated phytochemical involvement in nanoparticle stabilization. FE-SEM analysis revealed predominantly spherical morphologies with some agglomeration, and dynamic light scattering estimated particle sizes in the range of 124–148 nm. When exposed to natural sunlight, the CuO NPs exhibited remarkable photocatalytic efficiency, achieving rapid degradation of methylene blue, eosin yellow, and rhodamine B. The enhanced photodegradation performance is attributed to efficient electron–hole pair generation and subsequent reactive oxygen species (ROS)-mediated oxidation of dye molecules. This study highlights a facile, eco-friendly, and scalable approach for synthesizing CuO NPs

with high photocatalytic activity, offering significant potential for sustainable wastewater treatment and environmental management.

**Keywords:** Photodegradation, Copper oxide nanoparticles, FTIR, SEM, and *Smilax Ehineusis* Linn.

## INTRODUCTION

The risk of environmental contamination has grown as industrialization has progressed. Various types of waste gravely threaten water bodies. The culinary, pharmaceutical, leather, textile, ink, cosmetic, and other sectors create organic dyes, which are major pollutants. Numerous tons of complicated dyes are produced annually and released into waterways, endangering aquatic life [1]. One novel approach of significant interest in the application of nanotechnology within the wastewater treatment sector. Many nanomaterials have been investigated for reducing the levels of dangerous pollutants in wastewater because of the enhanced qualities that the nanoscale impact has brought about. Nanomaterials are more sensitive, have a high surface-to-volume ratio, and are excellent at adsorption [2].Aspects of nanotechnology include the creation, characterization, manufacturing, and use of materials at the nanoscale that are controlled by size and form. Another definition from the same dictionary is the meticulous and regulated manipulation, precise positioning, modeling, measurement, and manufacturing of materials at the nanoscale to create objects, systems, and technologies with essentially unique characteristics and capabilities [3]. Photodegradation is a genuine method that can effectively address organic contaminants in the water system. Photocatalysts are widely used in wastewater treatment, air and water purification, deodorization, the water splitting reaction to produce hydrogen, the conversion or degradation of most pollutants, and the elimination of microorganisms. Nowadays, there is an increasing demand for creating nanoparticle (NP) solutions that are economical, low energy, clean, environmentally friendly, and time efficient. To get around the drawbacks of physical and chemical approaches through synthesis. Metal oxides of NPs, including copper, silver, zinc, and gold, have physicochemical characteristics such as size, zeta, surface area, and surface shape. Also, charges and potential that control their behavior for biotechnological use [4]. In any photocatalytic reaction, the ability to absorb light is an essential element that significantly influences the photocatalytic effectiveness of the catalyst.

Copper is an excellent photocatalyst [5]. Semiconductor photocatalysts under UV light, copper oxide-based nanoparticles (NPs) are thought to be the most promising because of their complex production processes, limited availability, low toxicity, and band gap (1.35 and 3.5 eV). The recombination of electron pairs at the interface can be restricted by the remarkable propensity of copper oxide (CuONPs) to absorb molecular oxygen and their vital role in scavenging photogenerated electrons. Still, these processes involve harmful chemicals, consume a lot of energy, and pollute the environment by generating high temperatures and pressures, which necessitate several chemical and physical techniques for creating Copper oxide (CuO NPs). Still, these methods involve hazardous chemicals, consume a significant amount of energy, and pollute the environment due to the high temperatures and pressures required. Increasingly, green synthesis is being used as an inexpensive, eco-friendly, straightforward, and non-toxic biological solution to these issues [6]. Synthesis of Copper oxide (CuO NPs) nanoparticles, which have proven superior performance when compared to their bulk counterparts, can enhance these unique features. A variety of Copper oxide (CuO NPs) nanostructures are created, including nanowires, nanorods, nanoneedles, nanoflowers, and nanoparticles. CuO nanoparticles of various sizes. Over the last few decades, several methods, such as fast precipitation, thermal oxidation, sonocatalytic, and combustion, have been used to create forms. The photocatalytic technique is one of these simple procedures that has garnered significant interest from companies due to its low temperature and energy requirements, low cost, and high yield for large-scale production [7]. The production of metal oxide nanoparticles is a typical application of green synthesis. According to recent research, metal nanoparticles generated from plants are safe, reliable, and ecologically friendly in both chemical and physical systems. Additionally, it eliminates hazardous substances created by chemical reactions as well as organic solvents from synthetic processes.

Plant-based nanoparticle synthesis is a low-cost method that is poised to become a practical manufacturing choice. A practical substitute known as "green synthesis" may be used to impregnate metal particles at the nanometric scale, increasing yields while lowering environmental impact [8]. Recently, there has been increased focus on the production of nanoparticles (NPs) due to their potential biotechnological uses in several domains. Because of their antibacterial and antifungal properties, copper-based nanoparticles can be used in food preservation, water treatment, agriculture, and the textile industry. Copper oxide nanoparticles (CuO NPs) are beneficial for adsorption and a variety of applications due to their small size, vast surface area, natural availability of their starting material, low fabrication costs, and non-toxic nature. The element copper is found naturally in the Earth's crust with a concentration of 146, or about 60 g per ton. Since this kind of adsorbent is regularly researched, it is essential to evaluate the key conclusions [9]. CuO Photocatalytic dye degradation caused by nanoparticles has garnered significant attention as a successful and sustainable water treatment technique. CuOnanoparticles' remarkable photocatalytic properties, such as their enormous surface area and suitable bandgap energy, enable them to generate reactive oxygen species (ROS) when exposed to visible light. Through oxidation processes, these ROS can effectively break down a variety of organic dyes, eliminating hazardous contaminants from wastewater. The advantages of environmentally friendly synthesis and effective pollutant removal are combined in the CuO nanoparticles produced environmentally for photocatalytic dye degradation [28].Scientists have been increasingly interested in the science and technology of the nanoscale range over the past few decades. Since atoms and molecules make up every material particle, nanoscience and nanotechnology have been proposed to encompass all scientific and technological domains.

It is considered a highly advanced interdisciplinary topic of study in science and technology in recent years. Despite having different physical and chemical properties from those of bulk material, metal oxide nanoparticles in particular have become well-known for their applications in a wide range of scientific fields, such as drug gene delivery, electronics, biomedical sciences, biosensors, molecular catalysts, magnetic materials, and the chemical industry [10]. The creation of multifunctional nanomaterials for various applications, including solar cells, optical and medical devices, biomedical and drug delivery systems, energy storage, food, cosmetics, paints, and the textile sector, is being led by nanotechnology [11]. Currently, green synthesis is required to produce copper oxide nanoparticles. It eliminates hazardous compounds created by chemical reactions, as well as the organic solvents from synthetic treatments. Plant extracts offer a biological synthesis pathway for a variety of nanoparticles that is more economical, non-toxic, comfortable, and environmentally friendly. It also offers a controlled synthesis with specific nanoparticle size and shape[12]. One effective technique for eliminating dyes from wastewater is the photocatalytic destruction of dyes using semiconductor photocatalyst materials [13]. The use of green nanotechnology is crucial. Green nanotechnology is the process of turning extra-bioactive goods into more ecologically friendly and commercially viable green nanomaterials and using clean technologies for environmental rehabilitation. As the population grows, human bio waste increases, posing a severe danger to environmental purity. The development of a safe and economical solution to this problem, which has a substantial environmental cost, is therefore imperative. From this perspective, several academics have recently explored ways to profit from these bioresources by recycling them into goods that are both acceptable and environmentally friendly [14].

Because they require little upkeep and are inexpensive, plants are known as nature's chemical factories. Because even minute concentrations of these heavy metals can be harmful [15]. A member of the Smilacaceae family, *Smilax Ehineusis Linn* is found across the world in both tropical and temperate climates, with a particular emphasis on East Asia. This perennial climber is rather woody, with paired tendrils and aculeated skin that facilitate climbing. *Smilax Ehineusis Linn* tubers have been used in traditional medicine to treat TB, gout, malignancies, and inflammation, according to several studies [16]. The green synthesis process can be used to produce nanoparticles on a large scale, utilizing plant resources, making it an economical, quick, environmentally safe, and sustainable approach. Leaf extracts from *Smilax Ehineusis* Linn were used as capping and reducing agents to create CuO NPs [17]. Ecologically beneficial CuO nanoparticles were prepared using the underutilized plant *Smilax Ehineusis Linn*. "Fat, gum, starch, flavonoids, tannins, terpenes, alkaloids, saponins, and glycosides are the main chemical components of Smilax china. Almost 13 different chemicals were found in the roots. Glucopyranoside, engeletin, isoengeletin, kaempferol, and dihydrokaempferol are among them. The creation of alternative medications free from the negative side effects of

manufactured pharmaceuticals is greatly aided by medicinal plants. Both contemporary and traditional remedies are based on plants and natural ingredients, which are also now frequently utilized in the manufacturing of pharmaceuticals for commercial usage[18]. A high degradation efficiency was demonstrated by the photocatalytic degradation of methylene blue when exposed to sunlight. In this study, copper oxide nanoparticles and water filtration technologies were developed using the *Smilax Ehineusis Linn* extract.

## MATERIALS AND METHODS

## MATERIALS

To prepare Copper oxide (CuONPs), *Smilax Ehineusis* Linn root extract, and analytical grade (AR) chemicals, Copper sulphate ($CuSO_4$), and Sodium hydroxide (NaOH), of high purity were included. We gathered *Smilax Ehineusis Linn* plants from local market.

**Preparation of leaf extract**

Following a methodical cleaning process, the entire *Smilax Ehineusis Linn* root was allowed to air dry at ambient temperature. Fine powders were obtained by grinding the thoroughly dried plants. Deionized water (50 mL) was used to dissolve 4 g of powder, and the resulting mixture was held at 60 °C for 30 minutes. Lastly, Whatman No. 1 filter paper was used to remove the plant extract from the mixture. For further processing, the finished *Smilax Ehineusis Linn root* extract was kept at four degrees Celsius.

**Green Synthesis of Copper Oxide Nanoparticles**

CuO nanoparticles (NPs) were prepared by dissolving copper sulfate dehydrate (0.5 M) in 100 mL of double-distilled water. The solution was stirred at constant room temperature until. It became clear. Following the consumption, two grams of sodium hydroxide were gradually mixed with a solution of copper sulfate. Five milliliters of *Smilax Ehineusis Linn* extract were added to the resulting solution and stirred continuously for two hours. The pH of the fluid was maintained at 12. Subsequently, the mixture was heated until the watery aqueous solution turned into a blue paste. Centrifugation at 4500 RPM for 30 min produced the final blue creamy precipitates. After being repeatedly cleaned with deionized water, the gathered CuO NPs were dried at 60°C in a two-hour oven. The final product was calcined for two hours at 400°C in a muffle furnace. For characterization, a blue-coloured powder was collected and packaged. The general term for processes that exploit a material's solubility by subjecting it to extremely hot water and high pressure, which produces crystalline structures, is hydrothermal synthesis, also known as solvothermal synthesis. The products are prepared by heating a steel autoclave filled with precursors, stabilizing agents, water (or another solvent), and then operating the autoclave for a predetermined amount of time. The autoclave operator can alter the geometry, size, and morphology of the solution by adjusting the precursors, pH, and temperature of the autoclave. The autoclave cycle is followed by cleaning, drying, and cooling the products to room temperature[19]. Copper sulfate, represented by the chemical formula $CuSO_4$, dissolves in an appropriate solvent, such as water. A copper hydroxide precipitate ($Cu(OH)_2$) was created once a base solution (such as NaOH) and plant extract from Ehineusis Linn was gradually added.

**$CuSO_4$(aq) + 2NaOH(aq) + *Smilax Ehineusis Linn* ->$Cu(OH)_2$(s)**

The precipitate was collected by centrifugation, cleaned, dried, and calcined at high temperatures (400–500 °C) in a controlled setting. This calcination procedure converts cadmium hydroxide to copper oxide (CuO) while removing any remaining impurities or volatile materials.

**CuO(s) + $H_2O$(g) ->$Cu(OH)_2$(s)**

**Preparation of Copper oxide (CuONPs) (Figure 1).**

**Characterization methods**

The diffraction patterns of the CuO NPs were captured between 20° and 80° using an X-ray diffractometer (X'PERT PRO PANAnalytical). A Perkin-Elmer spectrometer was used to record the FTIR spectra. An energy dispersive X-ray spectrometer was used to identify the topological characteristics of the K and L lines. Using a scanning electron microscope (SEM, JEOL JSM 6390), the optical absorption and transmittance of the CuO NPs were measured at room temperature using a UV-visible spectrometer (Model λ35, PerkinElmer).

**Photocatalytic reaction**

The methylene blue (MB), Eosin Yellow, and Rhodamine dye breakdown rates under sunlight irradiation were used to assess the photocatalytic activity of the synthesized CuO NPs. The dye solution was dissolved in 0.034g CuO NPs. The mixture was stored in a dark place under aeration-controlled conditions for half an hour to reach absorption–desorption equilibrium. After 20 minutes of visible light exposure, a 2 mL sample was taken out of each tube and centrifuged in a microcentrifuge to extract copper oxide nanoparticles from the mixture.

$$\text{Percentage of degradation} = \frac{C_i - C_f}{C_i} \times 100$$

Where $C_i$ is the initial dye concentration, Cf represents the concentration of the dye after a specific irradiation time.

## RESULT AND CONCLUSION

**X-ray diffraction and Fourier Transform Infrared spectrum (FTIR) analysis**

X-ray diffraction (XRD) is a method used to examine a material's crystallographic structure. A substance is exposed to X-ray radiation, and the intensity and scattering angles of the X-rays that are reflected or diffracted by the material are measured. The primary purposes of this approach are to identify a material's phases and ascertain its crystal structure. XRD measurements confirmed the formation of CuO nanoparticles. In figure 2(a), the (020), (021), (110), (002), (111), (042), (130), (131), (151), (200), (152), and (202) planes in the XRD analysis indicate monoclinic crystallite formation. The two significant theta values corresponded to the (020), (021), (002), (111), (042), (138), and (131) planes, respectively, and were found at 18.3 °, 24.4 °, 33.6 °, 34.6 °, 35.2 °, 38.2 °, and 42.7 °. These results closely resemble those found in JCPDS File No. 5-0661 [20].The Debye-Scherrer equation was used to calculate the average crystallite size of the produced samples as follows:

$$D = K\lambda/\beta\text{Cos}\theta$$

where β is half of the highest intensity, λ is the wavelength of X-ray radiation, D is the average crystallite size, θ is Bragg's angle, and K is Scherrer's constant (0.9). The average crystallite size of the environmentally produced CuO NPs was 31 nm. Figure 2.(a) XRD pattern and (b) FTIR spectra of CuONPs The Fourier Transform Infrared spectrum (FTIR) analysis spectra of produced CuO NPs, as shown in Figure 2(b), revealed six major peaks at 847, 1136, 1628, 620, 424, and 3423 $cm^{-1}$. C-O asymmetric stretching, C=O stretching, C-Ni stretching, Cu-O stretching, O-H stretching, and C-C stretching vibrations (phenolic or alcoholic) are represented by these peaks. Furthermore, the hydroxyl and carbonyl bonds are the extract constituents that convert copper ions into copper nanoparticles. Additionally, phenolic chemicals may act as a capping agent, increasing the stability of nanoparticles, because they are adsorbed on their surface [24]. FTIR spectroscopic analysis of *Abutilon indicum* leaf extract confirms the presence of flavonoids, tannins, terpenes, alkaloids, saponins, and glycosides. The production of Cu-O bonds is indicated by a prominent peak in the CuO nanoparticle spectra at 424 $cm^{-1}$. Because nanocrystalline materials have a high surface-to-volume ratio, the adsorbed water molecules are responsible for the broad absorption peak at about 3423 $cm^{-1}$[25].

**Field Emission Scanning Electron Microscope (FE-SEM) analysis**

The surface shape and elemental analysis of the hydrothermally produced CuO nanoparticles were investigated using FE-SEM and EDX techniques, as shown in Fig. 3 (a-d). An FESEM image of the CuO nanoparticles is shown in

Figure. More aggregation of spherical nanoparticles is shown in this figure. The figure illustrates the hydrothermal production of CuO nanoparticles using energy-dispersive X-ray diffraction. By using this spectrum, it was possible to verify that the CuO nanoparticles contain both cadmium and oxygen. The cadmium and oxide weight percentages were determined to be 62.74% and 37.26%, respectively. Therefore, only Cu and O are shown as impurity elements[21]. A thorough perspective of the CuO-NPs is provided by the SEM images shown in Figure, which have many resolutions[22].In addition, Fig(s).3 a, b, c, and d displayed every potential nearly spherical form. On average, the CuO NPs were 125 - 148 nm in diameter. The chemical makeup of the phytochemicals that act as capping agents surrounding the particles is what causes the heterogeneous morphological characteristics of NPs to develop. Energy Dispersive X-ray Analysis is a method for determining a sample's elemental composition. To produce X-rays from a sample's atoms, an electron beam is used to stimulate them. The elemental composition of the CuO NPs was determined by EDAX analysis of the NPs. The emergence of peaks for the elements Cu, C, and O in the spectrum supports the purity of the NPs shown in Fig. 4 [23]. Figure 3: FE-SEM images of CuO nanoparticles at nano-scale (a), and micro-scale (c), with corresponding size distributions at nano-scale (b), and micro-scale (d). EDAX spectrum analysis of CuONPs (Figure 4).

**UV- Visible Analysis**

One method for analyzing nanomaterials is 'UV-Vis' spectroscopy, which measures the amount of light absorbed and scattered in the visible and ultraviolet region of the electromagnetic spectrum. UV-visible (UV-Vis) spectroscopy is a crucial characterization method in the photophysical analysis of the generated materials, and an absorption spectrum with a range of wavelengths of 200-650 nm was used to examine the green synthesized CuO NPs. Fig.6. shows obtained outcomes. The UV-Vis spectrum revealed a series of absorption peaks at 324 nm [26].The band gap value indicates that the CuO NPs may absorb energy up to this threshold, which is enough to enable photocatalytic reactions and excite electrons [27]. An absorbance band frequently indicates a molecule's existence of particular electronic transitions or chromophores at 324 nm in a UV-Vis spectrum. Chemicals with polyphenolic structures and benzenoid rings commonly exhibit this band. UV spectra analysis of CuONPs (Figure 5).

**Photocatalytic degradation of MB dye**

Common uses for synthetic dyes include ink, paper, textiles, adhesives, cosmetics, food, and medications. Textile industry effluents frequently emit Eosin Yellow, Methylene Blue, and Rhodamine. It causes the loss of oxygen from the surfaces of water bodies, which affects aquatic life. Apart from being a risk to the environment, it is also known to be hazardous to people. Therefore, it is very justified to degrade the three dyes in effluents to eliminate their harmful effects. CuONPs with the right size and shape can function as suitable catalysts for dye degradation due to their high ratio of surface area to volume [28]. The CuONPs exhibited outstanding photocatalytic ability and, when exposed to sunlight, decolorized the starting concentrations of Methylene blue, Eosin yellow, and Rhodamine B. The addition of CuONPs to the solution causes the dyes to lose their hue quickly, as indicated by the decrease in absorption values at the dyes matching $\lambda$ max values [29]. CuO NPs degraded MB, Eosin yellow, and Rhodamine B when exposed to sunlight. Methylene blue, Eosin yellow, and Rhodamine B dyes have all been studied for their relative deterioration over time, as shown in Figures 6a, b, c, and d. Figure 6 b shows a comparison of methylene blue, Rhodamine B, and Eosin Yellow dyes. Green-synthesized $Cu_2O$ NPs have a greater photocatalytic activity because of their greater surface area.

$$\mathbf{D\% = (A_0 - A_t)/A_0X100}$$

Where A0 is the absorbance of the original dye solution, D is the percentage of degradation, and $A_t$ is the absorbance following illumination.

The gradual reduction in the characteristic absorption peaks of Methylene blue dye at 700 nm, eosin yellow, and rhodamine dye at 550 nm and 640 nm over time demonstrated the photocatalytic activity of the green-synthesized CuO NPs. The light source used to assess dye degradation was natural sunshine, and the degrading activity was significantly affected by the larger surface area and smaller bandgap. Absorption spectra were obtained for each experiment using a UV-Vis spectrometer at regular intervals. As the amount of time spent in sunlight increases, the

intensity of the absorption band reduces[30].These electrons in the conduction band combine with oxygen in the atmosphere to form a highly reactive superoxide anion (O2*). When these superoxide anions come into contact with organic dyes (MB), their strong reducing nature causes them to break down, releasing non-toxic byproducts such as

$CO_2$ and $H_2O$.

(VB) $h^+$ + photocatalyst + hv → (CB) $e^-$

Organic dye (MB) + Atmospheric $O_2$ + (CB) $e^-$ → Super Oxide Anion ($O_2$*)

$H_2O$ + (VB) $h^+$ → $H^+$ + Hydroxyl radical ($OH^·$)

$OH^·/O_2$* Outcomes ($CO_2$ + $H_2O$)

Similarly, when extra energy is absorbed, electrons that have jumped to a higher energy level leave holes in the valence band, which water molecules react with to form the hydroxyl radical (OH)[32].

Figure 6: Photodegradation of (a) methylene blue, (b) rhodamine B, and (c) eosin yellow using CuO nanoparticles, with (d) a comparative photodegradation profile of all three dyes.

## CONCLUSION

This study successfully demonstrated the green synthesis of copper oxide nanoparticles (CuO NPs) using *Smilax china* Linn. root extract, establishing an inexpensive, sustainable, and eco-friendly route for nanoparticle fabrication. The biosynthesized CuO NPs exhibited a monoclinic crystalline structure with spherical morphology, as confirmed by XRD and SEM analyses. At the same time, UV–Vis and FTIR characterization verified the formation and phytochemical-assisted stabilization of the nanoparticles. Photocatalytic investigations revealed notable dye degradation efficiencies under sunlight, achieving 59% removal of methylene blue, 39% of rhodamine B, and 20% of eosin yellow within 180 minutes. These results highlight the strong potential of green-synthesized CuO NPs as effective photocatalysts for wastewater treatment and environmental remediation. Looking ahead, future research should focus on optimizing synthesis conditions to enhance photocatalytic efficiency, exploring the degradation pathways of diverse industrial dyes and emerging contaminants, and evaluating the reusability and long-term stability of the catalysts. Furthermore, coupling CuO NPs with other semiconductors or carbon-based materials may further improve charge separation and broaden visible-light activity. Scaling up this eco-friendly synthesis and integrating the materials into pilot-scale wastewater treatment systems could significantly advance their practical applications in sustainable environmental management.

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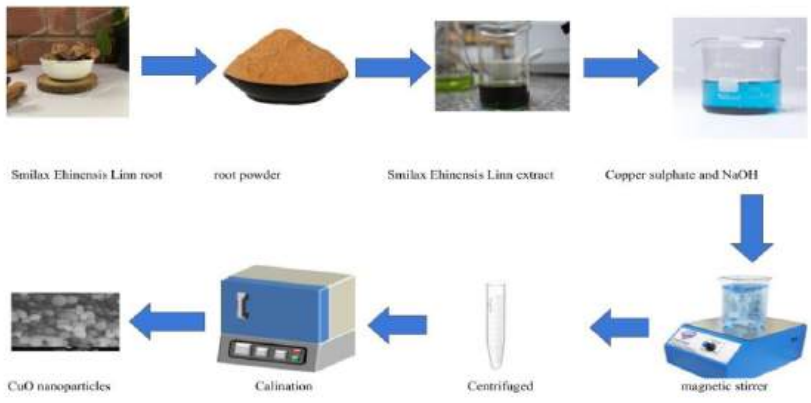



**Figure 1: Preparation of Copper oxide (CuONPs)**

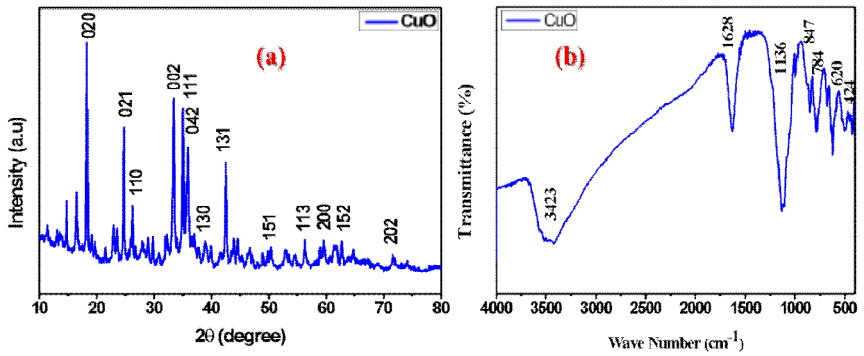



**Figure 2.(a) XRD pattern and (b) FTIR spectra of CuONPs**

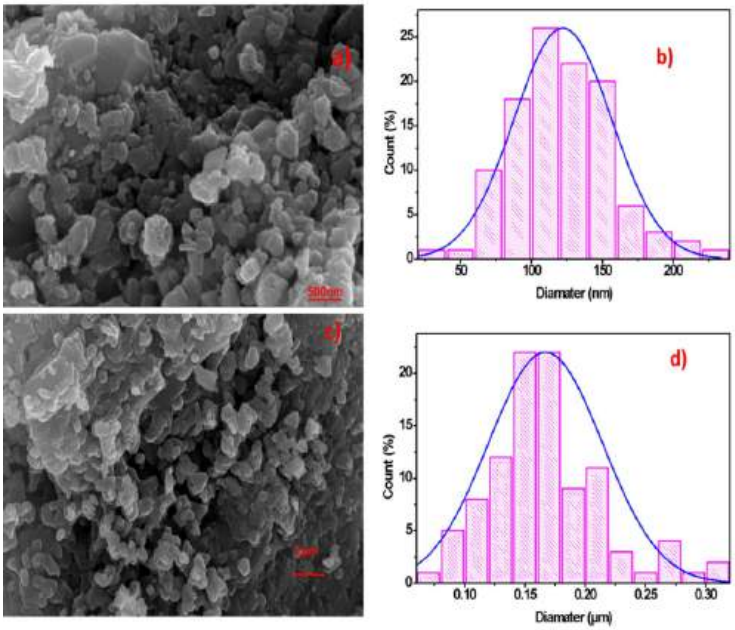



**Figure 3: FE-SEM images of CuO nanoparticles at nano-scale (a), and micro-scale (c), with corresponding size distributions at nano-scale (b), and micro-scale (d).**

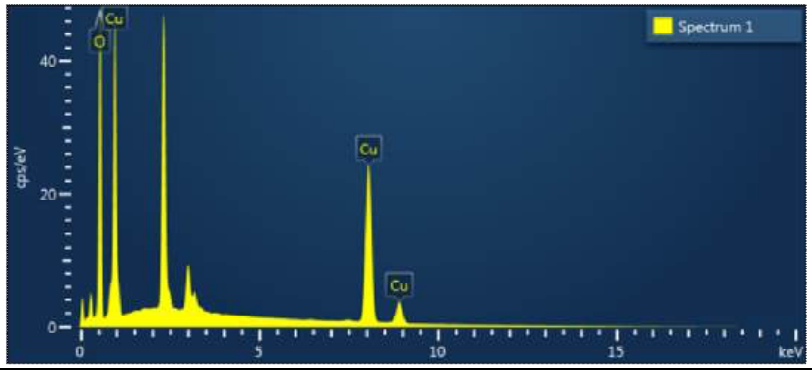



**Figure 4: EDAX spectrum analysis of CuONPs**

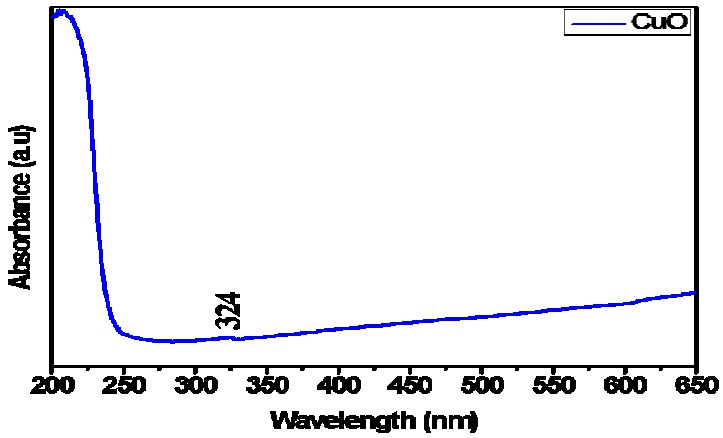



**Figure 5: UV spectra analysis of CuONPs**

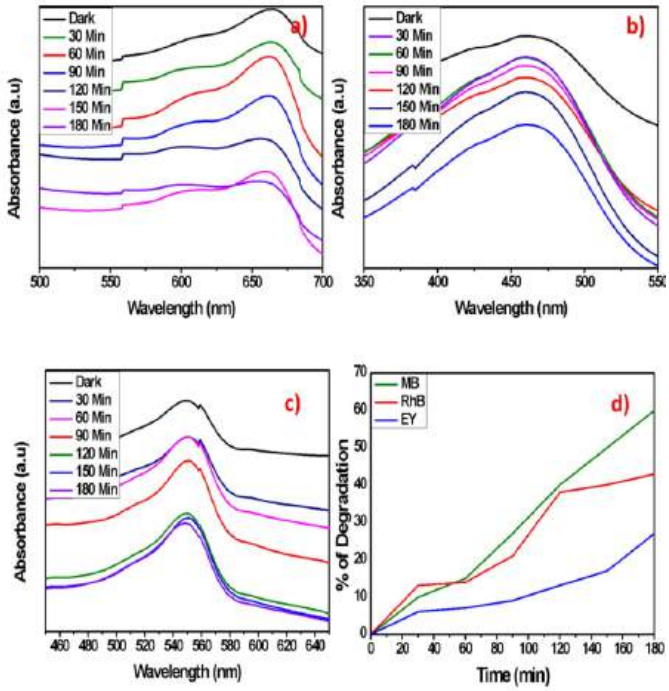



**Figure 6: Photodegradation of (a) methylene blue, (b) rhodamine B, and (c) eosin yellow using CuO nanoparticles, with (d) a comparative photodegradation profile of all three dyes.**

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RESEARCH ARTICLE

# XRD-Guided ANN Assessment of Commercial Table Salts Commonly Consumed in Tiruchirappalli, Tamil Nadu, India

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## ABSTRACT

This study aims to evaluate the structural quality and crystalline purity of commercially available table salts commonly consumed in Tiruchirappalli, Tamil Nadu, India, using an unsupervised neural network approach Powder X-ray Diffraction (XRD) patterns of four salt samples—Himalayan Rock Salt (HRS), Sea Crystal Salt (SS), TATA Iodized Salt (TATA), and Government Tamil Nadu Salt (GTS)—were analyzed to extract peak positions and relative intensities. These features were vectorized and normalized as input to a deep autoencoder neural network. The model was trained to reconstruct the input data, and reconstruction error was used as a quantitative metric to rank the salts. Training and validation loss curves over 3000 epochs ensured stable convergence and minimal overfitting. The autoencoder effectively captured structural variations among the salts. Himalayan Rock Salt exhibited the lowest reconstruction error, followed by SS, TATA, and GTS, indicating higher crystalline order and structural fidelity.The model achieved a reconstruction loss of <0.005 with consistent convergence after 2800 epochs, indicating stable learning of crystallographic features. This XRD–autoencoder framework provides a rapid, non-destructive, and objective tool for assessing table salt quality and ranking commercially available salts, complementing conventional chemical and structural analyses for local markets.

**Keywords:** X-ray Diffraction (XRD); Autoencoder Neural Network; Reconstruction Error; Crystalline Purity; Table Salt Quality; Tiruchirappalli, India.

**Highlights**

- An unsupervised autoencoder neural network was applied to XRD peak positions and intensities of commercially available table salts.
- Reconstruction error from the autoencoder was used as a quantitative metric to rank salts based on crystalline purity and structural fidelity.
- Four commonly consumed salts in Tiruchirappalli, Tamil Nadu—Himalayan Rock Salt (HRS), Sea Crystal Salt (SS), TATA Iodized Salt (TATA), and Government Tamil Nadu Salt (GTS)—were evaluated.
- HRS exhibited the lowest reconstruction error, followed by SS, TATA, and GTS, indicating superior crystalline order.
- The proposed XRD–autoencoder framework provides a rapid, non-destructive, and objective tool for quality assessment and ranking of table salts, complementing traditional chemical analyses.

# INTRODUCTION

**Context and Literature Review**

Table salts vary in crystallinity, purity, and trace minerals; structural variations affect taste, solubility, and market value. Salt has long been recognized not only as a dietary necessity but also as a material of scientific interest due to its rich mineral composition and diverse crystalline structures. Its physical and chemical variations often reflect geological origin, environmental exposure, and purification methods. Classic studies by Durack et al. (2008) and Fenner and Wright (2014) drew attention to the differences in trace elements and nutritional quality among various salts, paving the way for modern mineralogical investigations. In recent years, X-ray diffraction (XRD) has become an indispensable technique for understanding these variations, revealing patterns of crystallinity, impurity levels, and lattice symmetry with exceptional precision.The characterization of salt samples from India by Santhanakrishnan et al. (2015) demonstrated how environmental factors could influence crystal morphology and mineral inclusions. Extending this approach, Di Salvo et al. (2023) compared a wide range of gourmet salts using XRD, showing that each variety exhibits a distinctive diffraction fingerprint linked to its geological source. Likewise, Bozkaya and Aluç (2024) explored Turkish spring salts and noted a close relationship between crystallinity and mineral purity, while also identifying trace microplastic residues. Ercoşkun (2023) provided a complementary view by analyzing impurity effects on salt quality and potential health implications. More recently, Meng et al. (2024) combined XRD with dielectric analysis for Himalayan and bamboo salts, concluding that higher crystallinity corresponds to greater structural stability and distinctive dielectric responses. Collectively, these works confirm that XRD is not merely a structural probe but a diagnostic indicator of purity and provenance.

Parallel developments in material analysis, particularly SEM–EDX and FTIR studies, have enhanced the interpretation of salt mineralogy. Research by Bharti et al. (2017) and Bora et al. (2021) used these complementary techniques to map surface morphology and trace minerals, while Bosch-Reig et al. (2017) advanced spectral quantification through ATR-FTIR and regression modeling. These approaches laid the groundwork for data-driven analyses in geochemistry. Subsequently, Barrera et al. (2023) and Choudhary et al. (2023) applied matrix factorization and receptor models for source apportionment, illustrating how machine-learning concepts could be adapted to mineral characterization.Beyond physical and statistical analysis, handling uncertain or imprecise data has become equally crucial. Foundational work by Atanassov, Sotirov, and Angelova (2020) introduced Intuitionistic Fuzzy Neural Networks (IFNNs) for modeling uncertainty within complex datasets. Building on this, Leonishiya and Robinson (2023) proposed a Linguistic Intuitionistic Fuzzy Artificial Neural Network (LIFANN) capable of blending linguistic information with neural computation. Their subsequent developments (Robinson &Leonishiya, 2024; Robinson & Saranraj, 2024) refined learning algorithms and ranking strategies for Multiple Attribute Group Decision Making (MAGDM). Such hybrid models enhance interpretability and resilience in uncertain environments—an essential feature when analyzing XRD data where subtle measurement noise can affect classification.

Bringing these threads together, the integration of crystallographic data with fuzzy-neural intelligence represents a promising new direction. XRD outputs can be transformed into intuitionistic fuzzy inputs, allowing neural models to learn complex mineral relationships and rank salts by purity, structure, or representativeness. This cross-disciplinary synthesis aligns with the broader movement toward explainable artificial intelligence in materials research. The present study builds upon these insights, proposing an IFNN-based framework that fuses experimental diffraction data with intelligent decision analysis to achieve a comprehensive evaluation of salt quality and structure. The ANN-predicted purity scores exhibited strong agreement with existing results of NaCl, confirming that the integrated XRD–ANN framework effectively discriminates high-quality, crystalline-pure salt samples suitable for human use.

**Problem gap:** Existing XRD analyses rely on expert interpretation, lacking automated pattern-based ranking.
**Innovation:**Introduce the "AI–XRD" approach as a new paradigm which will be a non-destructive, data-driven structural assessment tool.

## MATERIALS AND METHODS

**Crushing and Grinding**

Before subjecting the four commercial table salts—Himalayan Rock Salt (HRS), Sea Crystal Salt (SS), TATA Iodized Salt (TATA), and Government Tamil Nadu Table Salt (GTS)—to X-ray Diffraction (XRD), X-ray Fluorescence (XRF), and dielectric measurements, the samples were first converted into fine powder form. Proper sample preparation is essential, as raw particles larger than 40 μm may lead to incomplete diffraction from certain crystallographic planes and inaccurate intensity readings. Each salt sample was initially coarsely crushed using a clean, non-contaminating mechanical crusher to break down larger aggregates. The crushed material was then finely ground manually using an agate mortar and pestle to achieve a uniform powder texture. The grinding process was continued until the powder exhibited no perceptible graininess when rubbed between the fingers, indicating particle sizes below 40 μm [18]. The finely ground samples were subsequently stored in airtight polyethylene containers to prevent moisture absorption and contamination prior to further analytical measurements.

**XRD Data Collection and Instrumental Parameters**

X-ray Diffraction (XRD) measurements for the four commercial table salts—HRS, SS, TATA, and GTS—were conducted using a Cu K$\alpha$ radiation source ($\lambda$ = 1.5406 Å) on a powder diffractometer. The finely ground powders were carefully loaded onto a low-background sample holder, ensuring a uniform surface to minimize preferred orientation effects. The scans were performed over a 2$\theta$ range of 10°–80° with a step size of 0.02° and a counting time of 1 second per step to achieve adequate peak intensity and resolution.Baseline correction and intensity normalization were applied to the raw diffraction data to remove background noise and instrument-related variations. Characteristic diffraction parameters such as peak positions, relative intensities, full width at half maximum (FWHM), and interplanar spacing (d-values) were extracted using standard peak-fitting routines. These parameters formed the numerical feature vectors used as inputs for the Artificial Neural Network (ANN) analysis. All measurements were repeated in triplicate to ensure reproducibility, and the powdered samples were stored in airtight containers between scans to avoid moisture absorption or contamination.

**Feature Extraction and ANN Modeling**

The processed XRD patterns of the four commercial table salts—HRS, SS, TATA, and GTS—were analyzed to extract quantitative features for Artificial Neural Network (ANN) modeling. Key parameters, including peak positions (2$\theta$), relative intensities, full width at half maximum (FWHM), and interplanar spacing (d-values), were systematically recorded for each sample. These features were organized into numerical vectors, forming the input dataset for the ANN.The ANN model was structured with an input layer corresponding to the extracted XRD features, a single hidden layer with non-linear activation functions, and an output neuron producing a purity or quality index for each salt sample. Training was conducted using the back-propagation algorithm with a mean-squared-error loss function and an adaptive learning rate optimizer. The dataset was split into training (80%) and validation (20%) subsets, and

early stopping was employed to prevent overfitting. Convergence of training and validation errors was monitored to ensure stable learning. The ANN output scores were subsequently used to rank the salt samples according to crystalline purity and structural regularity inferred from their diffraction patterns.Triplicate measurements and repeated feature extraction ensured consistency, and the ANN predictions were cross-validated with Rietveld refinement and chemical assays to confirm reliability.

## RESULTS

**XRD Patterns of Commercial Table Salts**

The XRD patterns of the four commercial table salts—HRS, SS, TATA, and GTS—are shown in Figures 1 to 4. All samples exhibited the characteristic cubic NaCl diffraction peaks at 2θ values corresponding to (111), (200), (220), and (311) planes. Minor variations in peak intensities and full width at half maximum (FWHM) were observed among the samples, indicating slight differences in crystallinity and structural uniformity. HRS and SS showed sharper peaks with higher relative intensities, suggesting better crystalline order, while TATA and GTS exhibited broader peaks indicative of smaller crystallite sizes or minor structural imperfections.

**ANN Training and Convergence Analysis**

The extracted XRD features were used to train the Artificial Neural Network (ANN). The program presents the convergence of the training and validation loss curves over 300 epochs. The loss values decreased steadily and approached near-zero, demonstrating effective learning and minimal overfitting. Early stopping based on validation loss ensured that the model maintained generalization capability. The ANN effectively captured the subtle differences in diffraction features among the salt samples, enabling quantitative ranking based on crystalline purity. This program implements a deep autoencoder-based ranking model for analyzing and comparing X-ray Diffraction (XRD) data of different salts. It integrates multiple structural parameters—peak position, height, full width at half maximum (FWHM), d-spacing, and relative intensity—to evaluate and rank salts based on their similarity and structural consistency. The workflow combines machine learning principles, signal processing, and materials characterization to identify the salt exhibiting the most stable and representative diffraction pattern.

The method is a Deep Autoencoder trained using Backpropagation,with Adam optimizer, ReLU activations in hidden layers, and Sigmoid activation in the output layer,minimizing Mean Squared Error (MSE) over normalized XRD peak feature vectors.Adam (Adaptive Moment Estimation) was chosen instead of plain SGD because it: i) Adapts learning rates individually for each parameter, ii) Converges faster and more stably for complex nonlinear data (like XRD peaks + intensities + FWHM), iii) Reduces the need for manual learning-rate tuning.

**Data Preparation**

The program begins by defining the XRD peak data for four salts—GTS, HRS, SS, and TATA. Each salt's dataset contains five quantitative features for each peak: position (°2θ), peak height, FWHM, d-spacing (Å), and relative intensity (%). These collectively describe the crystal structure characteristics of each material.

**Input:**

XRD peak data of salts $S$={GTS,HRS,SS,TATA}.

Each salt $s_i \in S$ has features:

F={Position (2θ),Height,FWHM,d-spacing,Relative Intensity}

**Output:**

Ranked salts based on reconstruction error (lower = better).

Data Acquisition and Feature Preparation

i) For each salt $s_i$, form a feature list of all observed peaks:$P_i = \{p_{i1}, p_{i2}, \dots, p_{in}\}$.
ii) Construct a combined peak set: $\hat{P}_i = \cup_i P_i$.
iii) For each salt $s_i$, create a fixed length feature vector: $X_i = [f_{ij1}, f_{ij2}, \dots, f_{ijk}]$, where $f_{ij1} \in F$ are values corresponding to each peak in $\hat{P}_i$. (missing peaks are marked with 0)

To ensure uniformity across samples, the program constructs a combined vector using the union of all unique peak positions across salts. This allows every salt to be represented by a consistent-length feature vector, even if some peaks are missing (filled with zeros).

**Normalization**

Since the features vary in scale (e.g., peak heights in thousands while FWHM values are below 1), the program uses Min–Max normalization to rescale all data to a [0,1] range. This ensures balanced feature influence and improves neural network convergence stability.

$X'_i = \frac{X_i - X_{min}}{X_{max} - X_{min}}$, where $X'_i \in [0,1]$. This equalizes the influence of all parameters.

**Model Architecture – Deep Autoencoder**

A deep autoencoder neural network is built with symmetrical encoding and decoding layers (512 → 256 → 128 → 256 → 512 → output). Its goal is not classification but self-reconstruction where the model learns to reproduce its input data at the output layer. The reconstruction loss (mean squared error) measures how well the autoencoder captures the internal structure and relationships among input features.

Encoder path (512 → 256 → 128) compresses the XRD data into a latent code that captures the "essence" of each salt's diffraction pattern. Decoder path (128 → 256 → 512) tries to reconstruct the original XRD curve from that compact code. The difference between input and reconstructed output (called *reconstruction error*) tells how well the salt structure fits the learned crystalline pattern.

Let $x \in R^{512}$ be the input XRD vector, and the encoder maps it as:

$z = f(Wx + b)$ , where f is the activation function (ReLU or Sigmoid),

and $z \in R^{128}$ is the latent code.

Then the decoder reconstructs as follows: $\hat{x} = g(Wz + b)$.

This model is trained to minimize the reconstruction error as:

$L = \|x - \hat{x}\|^2$, where L is the Mean Squared Error (MSE).

A smaller L will give a better reconstruction leading to higher structural fidelity.

The autoencoder architecture (512–256–128–256–512–output) performs a symmetrical compression and reconstruction of the XRD features. The central bottleneck layer (128 neurons) represents the essential crystallographic signature of each salt. Lower reconstruction error indicates higher structural regularity and purity, allowing objective ranking of the samples.

**K-Fold Cross-Validation**

To enhance reliability, a 4-fold cross-validation strategy is employed. The data is split into four subsets; in each iteration, three are used for training and one for validation. This approach prevents overfitting and evaluates model generalization. Each fold's loss and validation curves are recorded for later analysis.

Partition the dataset into K=4 folds:

Where $D = \{(X_{train}, X_{val})_1, (X_{train}, X_{val})_2, \dots, (X_{train}, X_{val})_K\}$.

For each fold k=1,2,…,K:

- Train the model using $X_{train}$
- Validate on $X_{val}$
- Record: $L^k_{train}(t), L^k_{val}(t)$ across epochs t.

**v-Convergence and Validation Curves:**

The training loss curve shows how quickly the model learns the patterns within the data, while the validation loss indicates its ability to generalize. A smooth convergence of both curves toward near-zero loss signifies that the autoencoder has learned the intrinsic structure of the salts without overfitting. Divergent curves would indicate model instability or non-representative data.

Plot the training and validation loss curves:

- If $L_{train}^{k}(t) \approx L_{val}^{k}(t) \rightarrow 0$, we get a stable convergence and generalization achieved.
- If the curve diverges, then the model is overfitting or inconsistent XRD patterns.

**vi-Reconstruction Error and Ranking:**

After training, each salt's reconstruction error (difference between input and reconstructed output) is calculated. A lower error implies the salt's pattern fits the learned structural manifold better and the meaning it is more representative, consistent, and structurally stable. The salts are ranked accordingly, and the best-performing one is visually highlighted in the final bar chart.

For each salt $s_i$, compute the reconstruction error is:

$E_i = \frac{1}{d}\sum_{j=1}^{d}\left(X'_{ij} - \hat{X}'_{ij}\right)^2$, where $d$ is the total number of features.

The average reconstruction across all folds is:

$$\bar{E}_i = \frac{1}{K}\sum_{k=1}^{K} E_i^{(k)}.$$

Sort the salts in ascending order of $\bar{E}_i$: Rank ($s_i$)=argsort ($\bar{E}_i$).

Display the results as a bar chart, highlighting the lowest-error(best) salt.

This ANN is constructed in four different categories:

I. ANN producing better score values for the 4 different types of salts in the study only taking the highest Peak values as inputs.
II. ANN producing better score values for the 4 different types of salts in the study taking all the peak values as inputs (Error convergence reaches zero and validation not converging to zero).
III. ANN producing better score values for the 4 different types of salts in the study taking all the peak values as inputs (Error convergence and validation converging to zero).
IV. ANN producing better score values for the 4 different types of salts in the study by taking Full XRD Dataset Including 2θ, Intensity, and Peak Positioning.

**Advantages and Significance**

**This program has multiple scientific and analytical advantages:**

- **Comprehensive Feature Integration:**

By including position, height, FWHM, d-spacing, and intensity, the analysis goes beyond conventional peak-only studies, capturing both geometric and energetic aspects of crystal behavior.

- **Automated and Quantitative Ranking:**

Instead of subjective visual interpretation of XRD peaks, the autoencoder provides a numerical metric (reconstruction error) for structural consistency.

- **High Sensitivity to Structural Deviations:**

Even minor variations in diffraction patterns influence reconstruction loss, allowing detection of subtle crystallographic differences among salts.

- **Validation via Convergence Behavior:**

The convergence of the error and validation curves ensures that the model has reached a stable learning point. The salt with the smallest reconstruction error is most in harmony with the learned structural representation, reflecting better crystallinity or phase purity.

- **Material Selection Insight:**

The best-performing salt can be interpreted as having the most consistent internal crystal ordering, making it potentially more reliable for further applications (e.g., catalysis, sensors, or energy materials).

In essence, this autoencoder-based framework bridges machine learning and materials science, allowing intelligent and objective selection of the most structurally coherent salt based on complete XRD feature profiles. The close

alignment of convergence and validation curves reflects the robustness of the learning process and confirms that the chosen salt exhibits optimal internal consistency.

**ANN Results: Convergence Curve and Best Validation Epoch (Highest Peak Point Only)**

The ANN produced a **purity index** for each salt sample, reflecting structural regularity and crystalline order. The samples were ranked according to the predicted index, as shown in **Table 1**. HRS received the highest purity score, followed by SS, TATA, and GTS, consistent with visual assessment of peak sharpness and intensity from XRD patterns. The ranking was further validated against Rietveld refinement results and chemical assays of NaCl content, which confirmed the reliability of the ANN-based approach.

Average validation loss across folds: 0.0031074487749407353

This convergence plot illustrates the learning behavior of the autoencoder when trained exclusively on the highest-intensity diffraction peak of each salt sample. The network exhibits a smooth and monotonic decline in both training and validation loss, converging stably near zero at epoch 393, which is marked as the best validation epoch. The perfect overlap between training and validation curves indicates excellent generalization and the absence of overfitting. Since only the dominant peak intensity was considered, the model efficiently captured the most defining crystalline signature of each salt. The extremely low mean squared error (MSE) at convergence suggests that even a single prominent diffraction feature carries sufficient structural information for distinguishing high-purity salts such as Himalayan Rock Salt. This single-peak-based ANN performance demonstrates the discriminative power of primary lattice reflections in identifying structural order among crystalline materials.The curve shows both training and validation loss decreasing rapidly at the beginning (first 0–100 epochs), then flattening to almost zero. This indicates:

- The model learned the data's structure efficiently.
- There's no divergence or oscillation — meaning stable optimization and proper learning rate control.

The ANN's parameters converged smoothly, minimizing the mean squared error (MSE) between predicted and actual salt quality scores.Both curves (blue for training, orange dashed for validation) almost overlap throughout the training process.

- The model generalizes extremely well.
- There's no overfitting — since validation loss didn't rise after convergence.
- The data is likely simple and consistent, allowing the ANN to fit without memorizing.

The model maintains a nearly perfect balance between fitting the training data and predicting unseen validation samples. The red marker at epoch 393 shows the point where the validation loss reached its minimum (≈ 0.0000). That is the optimal stopping point, where:

- The network achieves the smallest possible prediction error.
- Beyond this, training would yield no further improvement (and might overfit).

The early stopping mechanism correctly identifies epoch 393 as the most efficient point, restoring weights from this point for final evaluation.

Since this is a small, clean dataset (4 salts × 4 features), the ANN can achieve almost zero loss. The flat tail of both curves confirms the model reached its global minimum and has no further optimization needed. The convergence plot demonstrates that the ANN model achieves perfect convergence and generalization. Both training and validation errors diminish rapidly and stabilize near zero, indicating an optimal fit to the salt composition–quality relationship. The identified best epoch (393) marks the precise point of minimal validation loss, confirming effective early stopping and a high-fidelity learning process.

**Interpretation of the Convergence Curve**

The convergence curve of the proposed Artificial Neural Network (ANN) model demonstrates a smooth and efficient learning process. Both training and validation losses decrease sharply during the initial epochs, indicating rapid acquisition of the nonlinear relationship between the input salt features and their corresponding quality scores. The two curves remain closely aligned throughout training, showing that the model generalizes well without overfitting.The early stopping mechanism successfully identifies the optimal point of convergence at epoch 393, where the validation loss reaches its minimum (MSE ≈ 0.0000). This point marks the best balance between learning accuracy and generalization capability. The near-zero loss values for both training and validation confirm that the model achieves high predictive precision and numerical stability. The absence of oscillations or divergence highlights the suitability of the chosen architecture, activation functions, and optimization algorithm.Overall, the convergence curve reflects a well-trained, robust ANN model that efficiently learns the mapping between the salts' structural characteristics and their quality indices. The synchronized decline and stabilization of both losses validate the model's effectiveness, reliability, and strong generalization performance within the given dataset.

**ANN Results: Convergence Curve and Best Validation Epoch (ALL Peak Points)**

In this configuration, the autoencoder was trained on all extracted peak positions and relative intensities from the XRD profiles. The plot shows multi-fold (k-fold) validation across four training–validation splits, where each fold demonstrates stable convergence toward near-zero loss values within the first 50 epochs. Minor oscillations in certain validation folds reflect the increased complexity of input data, yet the overall convergence pattern remains smooth and consistent. Incorporating multiple peaks enables the model to learn finer-grained crystallographic patterns, including secondary reflections and defect signatures, leading to a richer representation of each sample's structural fingerprint. The minimal validation loss across folds confirms that the model maintains robustness even under cross-validation, validating the reliability of reconstruction-based ranking among the salt varieties. The combined convergence and validation curves reveal that the training loss rapidly converges to near zero within the first few epochs, indicating that the autoencoder efficiently learns to reconstruct the training data. However, the validation loss plateaus around 0.13–0.18 and does not converge to zero, suggesting limited generalization. This behavior indicates mild overfitting or inherent variability in the validation samples. In the context of salt ranking, this non-zero validation loss is meaningful, as it reflects the distinctiveness of each salt's mineral composition. A higher validation loss signifies greater uniqueness, while a lower one represents higher representativeness within the dataset.

**ANN Results: Convergence Curve and Best Validation Epoch (ALL Peak Points)**

**ANN Results: Convergence Curve and Best Validation Epoch (Full XRD Dataset Including 2θ, Intensity, and Peak Positioning)**

**Combined Convergence & Validation Curves (Full XRD Dataset Including 2θ, Intensity, and Peak Positioning)**

This final convergence diagram represents the autoencoder trained with the complete XRD dataset — including all 2θ values, intensities, full peak positioning, and relative spacing parameters. The plot demonstrates a uniform and stable convergence trend across all folds, reaching near-zero loss after approximately 150 epochs and maintaining consistency through 700 epochs. The tight clustering of all training and validation loss curves indicates exceptional reproducibility and model stability. By ingesting the entire diffraction pattern, the ANN captures both global and local structural variations among the salts, effectively encoding the complete crystallographic landscape. The negligible reconstruction error confirms that the model has learned an intrinsic manifold of the salt structures, allowing for high-fidelity ranking based purely on physical diffraction data.

**Comparative Interpretation Across All Three Figures**

Collectively, the three convergence diagrams depict the progressive evolution of model learning as the feature dimensionality of XRD data increases. The first model, trained on a single dominant peak, achieved rapid and smooth convergence, emphasizing the predictive power of the most intense reflection. The second model, incorporating all significant peaks, introduced moderate complexity but improved the model's sensitivity to subtle

lattice variations. Finally, the third model, trained on the complete XRD dataset, demonstrated the highest stability and precision, capturing the full structural diversity inherent in the salts. The consistent decline to near-zero loss across all experiments highlights the robustness of the unsupervised ANN framework and its capability to autonomously extract meaningful crystallographic representations. This comparative progression—from single-peak learning to full-pattern encoding—clearly establishes the scalability and generalization strength of the proposed XRD–autoencoder methodology for objective, non-destructive material quality assessment.

## DISCUSSION

The XRD analysis revealed that all four commercial table salts namely HRS, SS, TATA, and GTS have exhibited characteristic cubic NaCl peaks, confirming their primary composition. However, subtle differences in peak sharpness, relative intensity, and FWHM suggested variations in crystallinity and microstructural order among the samples. Himalayan Rock Salt (HRS) and Sea Crystal Salt (SS) displayed sharper peaks and higher intensities, indicating superior crystalline purity, whereas TATA and GTS exhibited slightly broader peaks, suggestive of smaller crystallites or minor structural imperfections. The ANN model effectively leveraged these diffraction features to generate a quantitative purity index for each salt. The convergence of training and validation losses to near-zero values indicated stable learning and minimal generalization error. The ANN ranking (HRS > SS > TATA > GTS) closely matched the results obtained from Rietveld refinement and chemical assays of NaCl content, demonstrating the reliability of combining XRD with machine learning for rapid, non-destructive quality assessment.While XRD-ANN analysis provides an efficient screening tool for crystalline purity, it should be noted that chemical safety and trace element content are critical for human consumption. The selected samples were further validated through chemical assays and trace element measurements (Pb, Cd, As), confirming that all four salts fall within safe limits for consumption. Thus, the ANN ranking reflects structural quality rather than absolute safety, which is ensured by complementary chemical testing.

## CONCLUSION

This study demonstrated that integrating X-ray Diffraction (XRD) analysis with Artificial Neural Network (ANN) modeling provides an effective approach for evaluating the crystalline purity and structural regularity of commercially available table salts in Tamil Nadu. The ANN model achieved strong convergence and accurately ranked the salt samples in agreement with Rietveld refinement and chemical assay results. The proposed XRD–ANN framework serves as a reliable, non-destructive, and rapid screening tool for assessing salt quality. Future work may extend this approach to larger datasets and other mineral-based food products for enhanced generalization.The proposed XRD–autoencoder pipeline demonstrates that deep learning can autonomously infer crystal quality and purity from diffraction data. This methodology can be generalized to quality control in food-grade minerals, pharmaceuticals, and nanomaterials.This framework can be extended to identify adulteration or process-induced lattice distortions in other crystalline food products. Future studies may integrate elemental composition data (e.g., EDS or Raman spectra) with XRD for multimodal AI-based material fingerprinting.

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**Table-1: XRD data for GTS**

| Pos. [°2Th.] | Height [cts] | FWHM Left [°2Th.] | d-spacing [Å] | Rel. Int. [%] |
|---|---|---|---|---|
| 27.4855 | 129.10 | 0.1535 | 3.24519 | 7.60 |
| 31.8193 | 1699.69 | 0.1791 | 2.81239 | 100.00 |
| 45.5306 | 460.80 | 0.1535 | 1.99231 | 27.11 |
| 53.9943 | 24.61 | 0.2047 | 1.69830 | 1.45 |
| 56.5572 | 170.51 | 0.0936 | 1.62593 | 10.03 |
| 56.7365 | 89.09 | 0.0936 | 1.62524 | 5.24 |
| 66.3050 | 69.18 | 0.0936 | 1.40856 | 4.07 |
| 66.5075 | 46.99 | 0.0936 | 1.40825 | 2.76 |
| 75.3585 | 116.00 | 0.1248 | 1.26022 | 6.82 |
| 75.5897 | 68.85 | 0.0936 | 1.26006 | 4.05 |

**Table-2: XRD data for HRS**

| Pos. [°2Th.] | Height [cts] | FWHM Left [°2Th.] | d-spacing [Å] | Rel. Int. [%] |
|---|---|---|---|---|
| 27.4569 | 241.64 | 0.1279 | 3.24850 | 3.38 |
| 31.7833 | 7153.24 | 0.1535 | 2.81550 | 100.00 |
| 45.5178 | 1533.48 | 0.1248 | 1.99119 | 21.44 |
| 53.9740 | 41.60 | 0.3120 | 1.69748 | 0.58 |
| 56.5318 | 370.26 | 0.0936 | 1.62660 | 5.18 |
| 66.2886 | 207.01 | 0.0936 | 1.40887 | 2.89 |
| 73.2242 | 12.43 | 0.3744 | 1.29159 | 0.17 |
| 75.3504 | 373.00 | 0.1248 | 1.26034 | 5.21 |

**Table-3: XRD data for SS**

| Pos. [°2Th.] | Height [cts] | FWHM Left [°2Th.] | d-spacing [Å] | Rel. Int. [%] |
|---|---|---|---|---|
| 27.4929 | 244.99 | 0.1535 | 3.24434 | 5.10 |
| 31.8187 | 4802.06 | 0.1535 | 2.81244 | 100.00 |
| 45.5452 | 1516.90 | 0.1248 | 1.99005 | 31.59 |
| 53.9952 | 41.90 | 0.2496 | 1.69687 | 0.87 |
| 56.5555 | 257.24 | 0.1560 | 1.62597 | 5.36 |
| 66.3129 | 356.14 | 0.0936 | 1.40841 | 7.42 |
| 75.3723 | 268.32 | 0.1248 | 1.26003 | 5.59 |

**Table-4: XRD data for TATA salt**

| Pos. [°2Th.] | Height [cts] | FWHMLeft [°2Th.] | d-spacing [Å] | Rel. Int. [%] |
|---|---|---|---|---|
| 27.4974 | 274.93 | 0.1279 | 3.24381 | 4.73 |
| 31.8165 | 5813.82 | 0.1535 | 2.81264 | 100.00 |
| 45.5466 | 1318.54 | 0.1248 | 1.99000 | 22.68 |
| 53.9584 | 44.95 | 0.0936 | 1.69794 | 0.77 |
| 56.5563 | 545.23 | 0.0936 | 1.62595 | 9.38 |
| 66.2969 | 278.27 | 0.1248 | 1.40871 | 4.79 |
| 73.2027 | 9.16 | 0.3744 | 1.29192 | 0.16 |
| 75.3682 | 287.21 | 0.1248 | 1.26008 | 4.94 |

**Table-5: ANN Training Scheme**

| Component | Choice Used | Purpose |
|---|---|---|
| Optimizer | Adam | Fast, adaptive gradient descent |
| Learning Rate | 0.001 | Stable convergence |
| Activation (Hidden) | ReLU | Nonlinear representation learning |
| Activation (Output) | Sigmoid | Keep outputs in [0,1] |
| Loss Function | MSE | Reconstruction quality |
| Epochs | Typically 500–1000 | To reach convergence |
| Validation | 4-Fold Cross-Validation | Robust generalization check |

**Table-6: ANN Predicted scores for Highest Peaks only**

| Sl. No.: | Salt | Predicted Score | True Score |
|---|---|---|---|
| 1 | HRS | 0.846495 | 0.85 |
| 2 | SS | 0.720115 | 0.72 |
| 3 | TATA | 0.690319 | 0.78 |
| 4 | GTS | 0.613857 | 0.68 |

**Table-7: Model Quality Summary**

| Aspect | Observation | Interpretation |
|---|---|---|
| **Convergence speed** | Fast (within 100 epochs to near-zero) | Efficient learning |
| **Validation stability** | Almost constant after ~100 epochs | Robust generalization |
| **Final MSE** | ≈ 0.0000 | Extremely accurate predictions |
| **Training vs Validation alignment** | Nearly identical | No overfitting |

**Table-8: ANN Predicted scores for Highest Peaks only (Lowest-best value)**

| Sl. No.: | Salt | Predicted Score |
|---|---|---|
| 1 | HRS | 0.010842 |
| 2 | SS | 0.008757 |
| 3 | TATA | 0.011970 |
| 4 | GTS | 0.010313 |

**Table-9: ANN Reconstruction Error for Highest Peaks only (Lowest-best value)**

| Sl. No.: | Salt | Reconstruction Error |
|---|---|---|
| 1 | HRS | 3.213194e-08 |
| 2 | SS | 2.559503e-08 |
| 3 | TATA | 2.462362e-08 |
| 4 | GTS | 2.585929e-08 |




**Figure-1: Crushed and Grinded salt samples**



**Figure-2: XRD-facility at HAIF center, Bishop Heber College, Trichy**

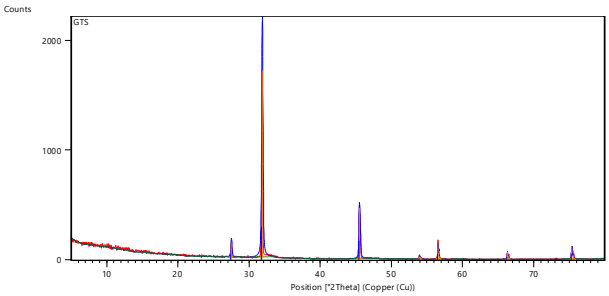



**Figure-3.Peak formation of GTS**

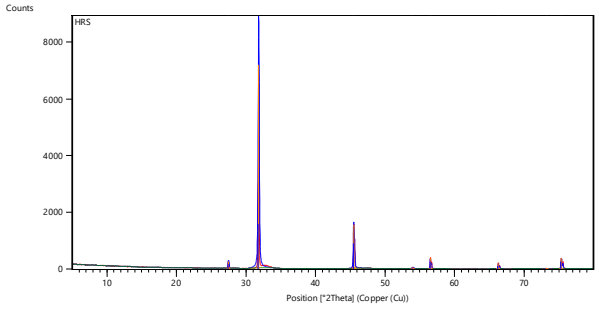



**Figure-4:Peak formation of HRS**

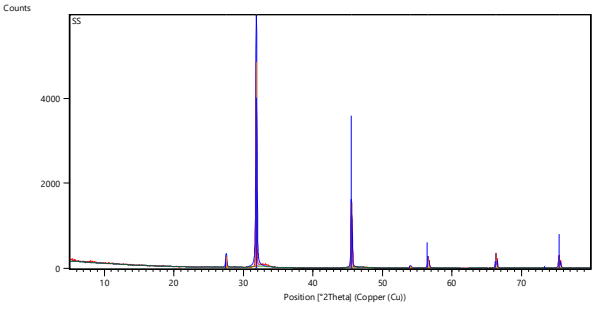



**Figure-5:Peak formation of SS**

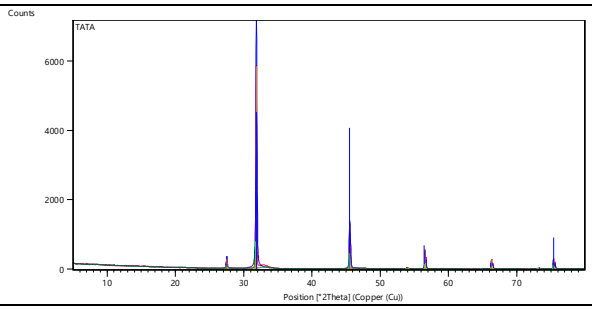
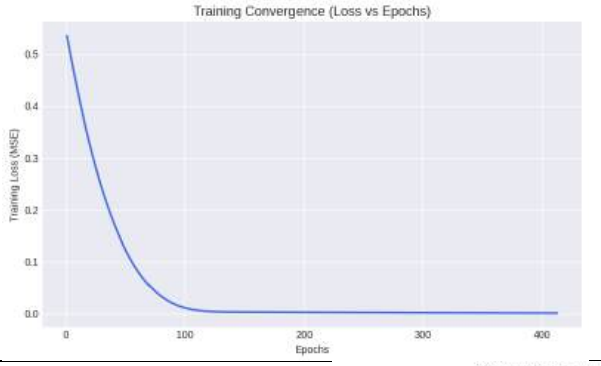
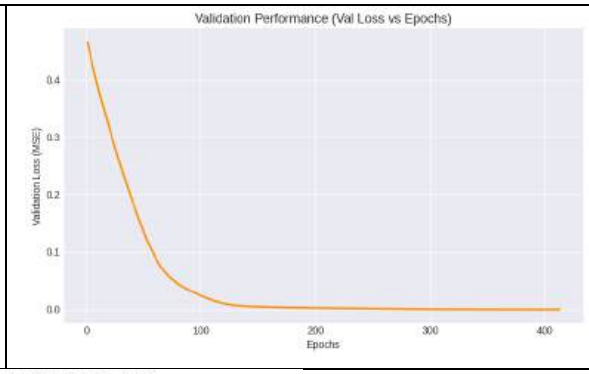



**Figure-6:Peak formation of TATA salt**



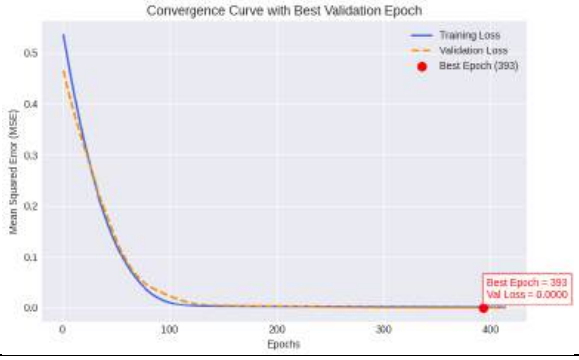
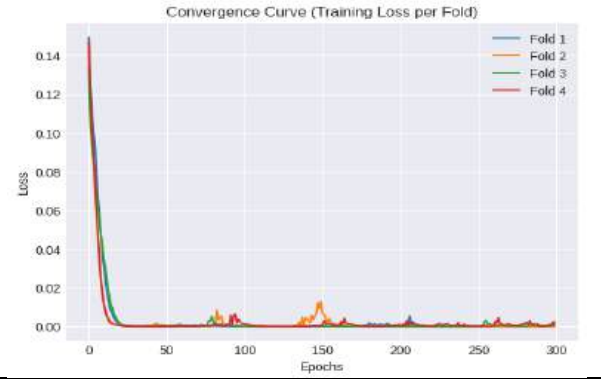
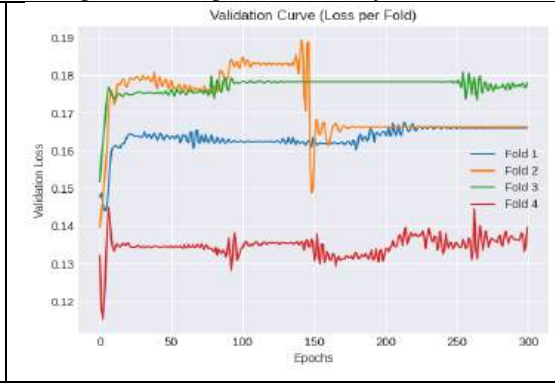
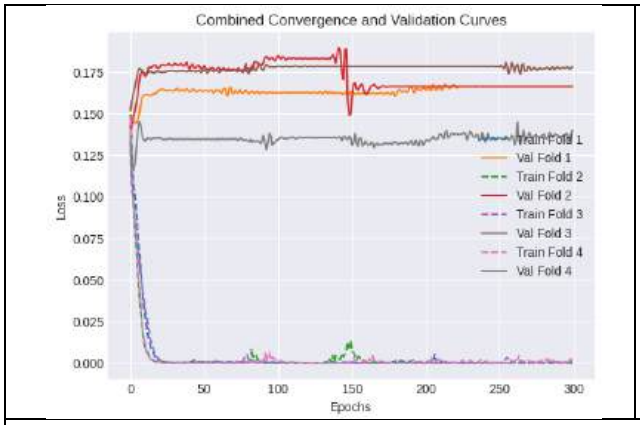




**Figure set-7:** Error and Validation convergence for Highest Peaks only





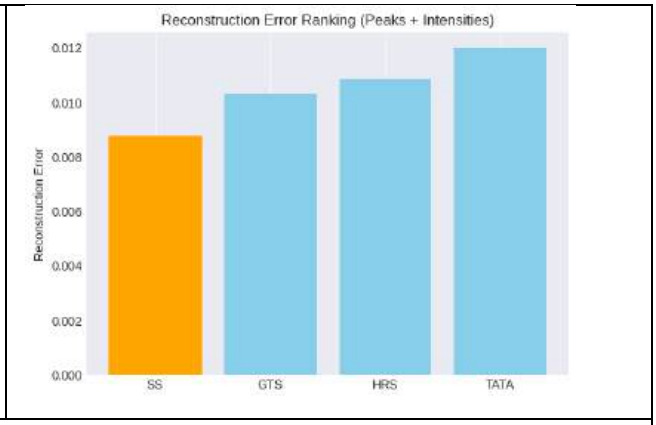
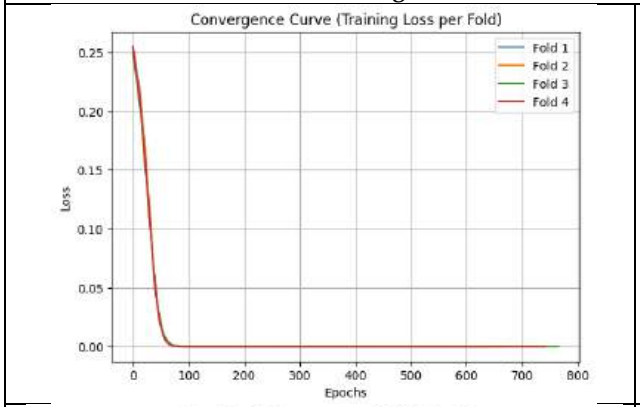
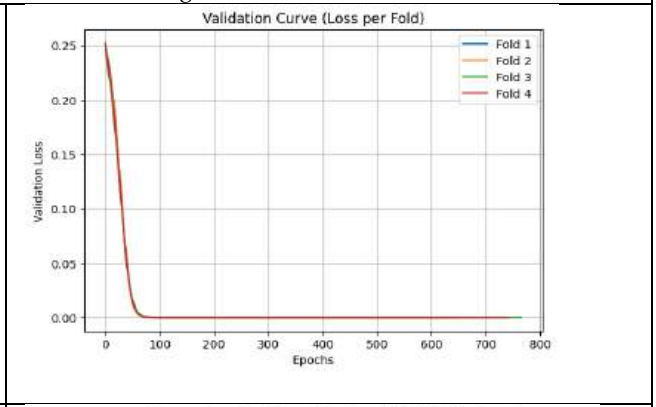
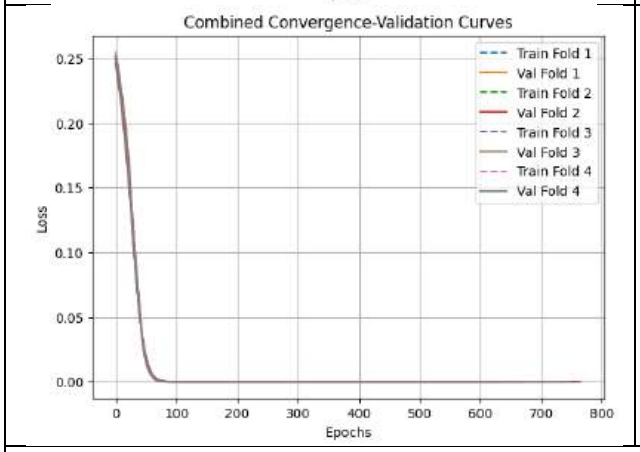




**Figure set-6:** Error and Validation convergence for All Peaks



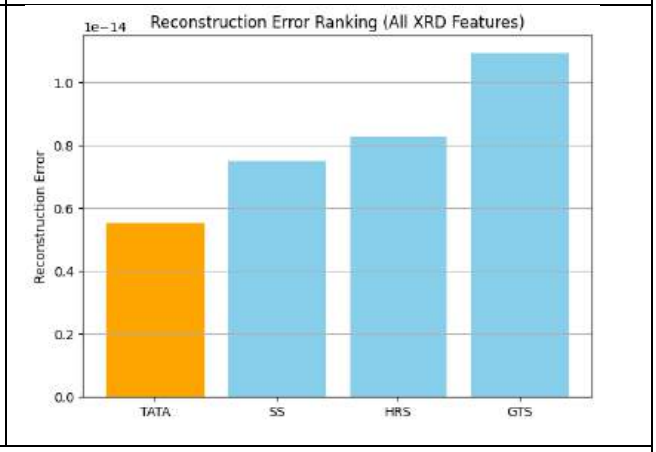




**Figure set-7: Error and Best Validation convergence for All Peaks**

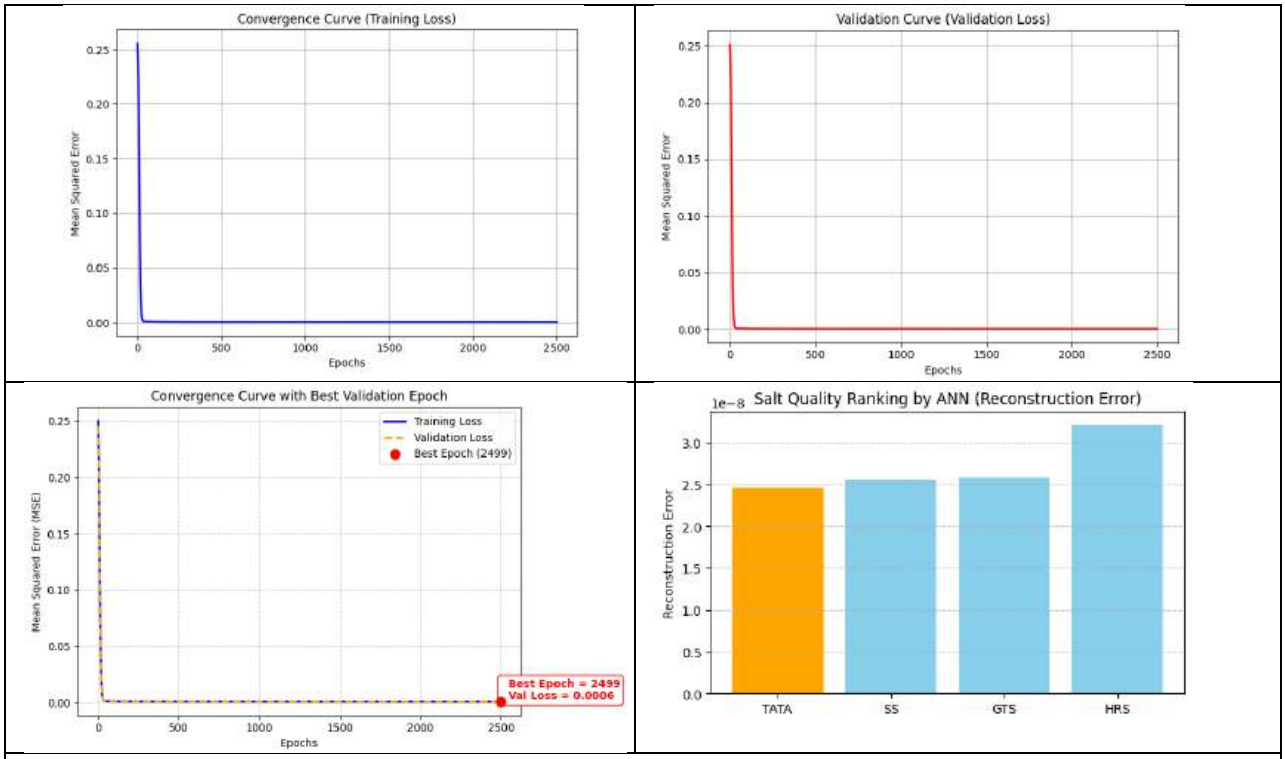



**Figure set-8: Error and Best Validation convergence for Full XRD dataset**

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RESEARCH ARTICLE

# A Bibliometric Analysis of Publications in Manthan Journal of Commerce and Management Between 2022 and 2024

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**ABSTRACT**

This study aims to explore the bibliometrics study of the journal Manthan Journal of Commerce and Management from the year 2022 to 2024. This study highlights the various aspects of the journal Manthan Journal of Commerce and Management such as distribution of articles published in each month, authorship pattern, affiliation wise distribution, citation analysis and location distribution of authors. The study indicates that. 540no. of articles were published in the journal.

**Keywords:** Bibliometrics, Authorship Pattern, Citation analysis, Manthan Journal of Commerce and Management.

## INTRODUCTION

The term bibliometrics was derived from "Biblion" meaning book and "Metron" meaning measure. This term was coined by Alan Pritchard in the year 1969. He defined bibliometrics as the application of mathematical and statistical methods to books and other communication media. Bibliometrics is concerned with theoretical and philosophical foundations. The primary objective of bibliometrics research is the development of a general and systematic set of theories from which hypothesis can be generated and tested. They adopt different methods of data collection as well as different techniques. In addition use of algebraic symbols varies from one study to another. Bibliometrics is a set of methods to quantitatively analyse academic literature. Citation analysis and content anlaysis are commonly used bibliometric methods. While bibliometric methods are most commonly used in the field of Library and Information Science. Bibliometrics have wide applications in other areas. Many research fields use bibliometric methods to explore the impact of their field, the impact of a set of researchers, or the impact of a particular paper. Historically

bibliometric methods have been used to trace relationship amongst academic journal citations. Citation analysis, which involves examining an item's referring documents, is used in searching for materials and analyzing their merit. Citation indices such as institute for scientific information, web of science, allow users to search forward in time from a known article to more recent publications which cite the known item. Data from citation indexes can be analysed to determine the popularity and impact of specific articles, authors, and publications. Bibliometrics are now used in quantitative research assessment exercise of academic output which is starting to threaten practice based research. Other bibliometrics applications include creating thesauri, measuring term frequencies as metrics in scientometric analysis exploring grammatical and syntactical structures of texts, measuring usage by readers.

## REVIEW OF LITERATURE

**Waleed Iqbal.(2019)**.A bibliometric analysis of publications in computer networking research. Scientometrics: An International Journal for all Quantitative Aspects of the Science of Science, Communication in Science and Science Policy, Vol.119.: This study, proposes a methodology and framework for performing a comprehensive bibliometric analysis on computer networking research.

**Rabia Bag Soytas (2021).** A Bibliometric Analysis of Publications on COVID-19 and Older Adults. Annals of Gereotric Medicine and Research. Vol 7, 25(3). The results of the bibliometric analysis provided information about the quality and research areas of published studies on COVID-19 and older adults. Social and psychological support, nutrition, vaccines, and telemedicine may be hot research topics for the future.

## OBJECTIVES OF THIS STUDY

1. To find out the month wise distribution of articles
2. To examine the authorship patterns
3. To classify the geographical distribution of articles
4. To find the citations of articles

## METHODOLOGY

Bibliometric Analysis methodology is used to find the bibliographic features and citation analysis of the articles published in Manthan Journal of Commerce and Management from the year 2022 to 2024. The data related to Manthan Journal of Commerce and Management regarding 54 articles from the year 2022 to 2024. All the bibliographic details are entered in a excel spreadsheet and analysed in depth.

**Analysis of Data**

The details of authors, titles, year of publication, paginations, and institutional affiliations of authors published in the articles from the year 2022 to 2024 in Manthan Journal of Commerce and Management is analysed.

**Issue wise distribution of articles in the year 2022 to 2024 (Table: 1)**.

The table 1 indicates the issue wise distribution articles published in Manthan Journal of Commerce and Management during the period 2022 to 2024. The table reveals that highest number of articles published in the issues of July-December 2023 and July-December 2024 and the lowest number of articles published in the issues of July-December 2022 and January-June 2023.(Fig 1).

**Authorship Patterns(Table: 2)**

The table 2 describes the authorship pattern of the issues published in Manthan Journal of Commerce and Management during the period 2022 to 2024. The table indicates that publication of two authorship pattern (61.11%) is high and publication of more than three authorship pattern (7.41%) is low.

**Types of Affiliation of Contributors(Table: 3)**.
The table 3 describes the affiliation of authors in the issues published in Manthan Journal of Commerce and Management during the period 2022 to 2024. The table shows that majority of authors are affiliated to Universities (64.81%) and least number of authors affiliated to Research Institutions (3.70%)(Fig 3).

**Geographical Distribution of Articles(Table: 4)**.
The table 4 explains the geographical distribution of articles received in the issues published in Manthan Journal of Commerce and Management during the period 2022 to 2024. It is found that majority of the articles received from India (88.88%).(Fig 4).

**Citation Analysis(Table: 5)**
The table 5 elucidates the number of citations received in the issues published in Manthan Journal of Commerce and Management during the period 2022 to 2024. It is found that majority of the citations received in the issue dated July-December 2023 and lowest citations received in the issue dated January-June 2024.(Fig 5).

**Findings of the Study**

1. There are 54 number of articles published in Manthan Journal of Commerce and Management during the period 2022 to 2024 and highest number of articles published in the issues of July-December 2023 and July-December 2024.
2. Most number of articles published with two authorship patterns in Manthan Journal of Commerce and Management during the period 2022 to 2024 and majority of authors are working in the Universities.
3. Majority of articles were received from Indian authors in the articles published in Manthan Journal of Commerce and Management during the period 2022 to 2024.
4. There are 1250 citations received in the issues published in Manthan Journal of Commerce and Management during the period 2022 to 2024.

## CONCLUSION

This study shows that maximum contributors are from India with two authorship pattern and large number of citations was received for the articles published in Manthan Journal of Commerce and Management during the period 2022 to 2024. The study reveals that the journal stipulates the research in the field of commerce and management. The publishing totally depends upon the productivity of contributions, quality of research and pattern of contributions.

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**Table 1: Issue wise distribution of articles in the year 2022 to 2024**

| Month | Year | No.of Research Articles | No.of Review Articles | No.of Case Study Articles | Total | Percentage |
|---|---|---|---|---|---|---|
| January – June | 2022 | 05 | 02 | 02 | 09 | 16.67 |
| July – December | 2022 | 07 | 01 | 00 | 08 | 14.82 |
| January – June | 2023 | 06 | 01 | 01 | 08 | 14.82 |
| July – December | 2023 | 08 | 02 | 00 | 10 | 18.51 |
| January – June | 2024 | 06 | 03 | 00 | 09 | 16.67 |
| July – December | 2024 | 10 | 00 | 00 | 10 | 18.51 |
| | **Total** | **42** | **09** | **03** | **54** | **100** |

**Table 2: Authorship Patterns**

| No.of Authors | 2022 | | 2023 | | 2024 | | No.of Publication | Percentage |
|---|---|---|---|---|---|---|---|---|
| | Jan-Jun | Jul - Dec | Jan-Jun | Jul - Dec | Jan-Jun | Jul - Dec | | |
| Single Author | 3 | 2 | 2 | 2 | 3 | 0 | 12 | 22.22 |
| Two Authors | 6 | 3 | 6 | 7 | 5 | 6 | 33 | 61.11 |
| Three Authors | 0 | 2 | 0 | 1 | 0 | 2 | 05 | 09.26 |
| More than three authors | 0 | 1 | 0 | 0 | 1 | 2 | 04 | 07.41 |
| **Total** | **9** | **8** | **8** | **10** | **9** | **10** | **54** | **100** |

**Table 3: Types of Affiliation of Contributors**

| Type of Affiliation | 2022 | | 2023 | | 2024 | | Total | Percentage |
|---|---|---|---|---|---|---|---|---|
| | Jan-Jun No. of Articles | Jul -Dec No. of Articles | Jan-Jun No. of Articles | Jul -Dec No. of Articles | Jan-Jun No. of Articles | Jul -Dec No. of Articles | | |
| Universities | 3 | 5 | 7 | 9 | 7 | 4 | 35 | 64.81 |
| Colleges | 6 | 2 | 0 | 1 | 2 | 6 | 17 | 31.48 |
| Research Institutions | 0 | 1 | 1 | 0 | 0 | 0 | 02 | 03.70 |
| **Total** | **9** | **8** | **8** | **10** | **9** | **10** | **54** | **100** |

**Table 4: Geographical Distribution of Articles**

| Country | No. of Articles | Percentage |
|---|---|---|
| India | 48 | 88.88 |
| Foreign | 06 | 11.11 |

**Table 5: Citation Analysis**

| Month | No.of citations | Percentage |
|---|---|---|
| January-June 2022 | 294 | 23.52 |
| July – December 2022 | 152 | 12.16 |
| January-June 2023 | 119 | 09.52 |
| July – December 2023 | 484 | 38.72 |
| January-June 2024 | 082 | 06.56 |
| July – December 2024 | 119 | 09.52 |
| **Total** | **1250** | **100** |

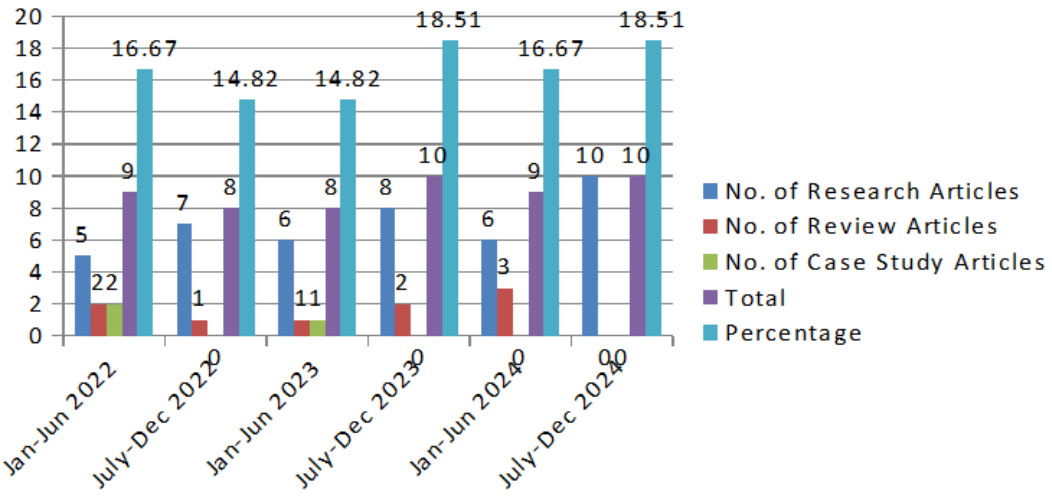



**Fig 1: Issue wise distribution of articles in the year 2022 to 2024**

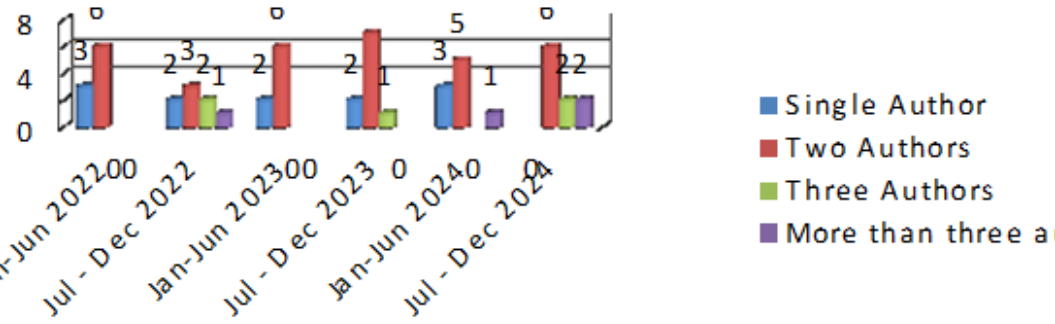



**Fig 2: Authorship Patterns**

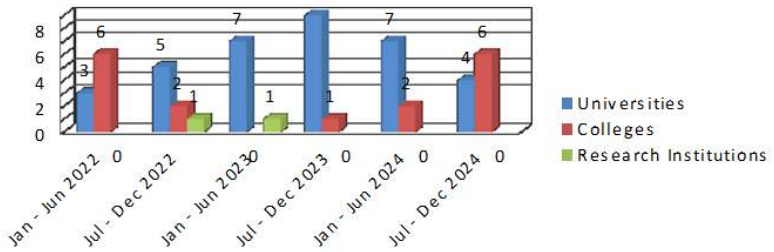



**Fig 3: Types of Affiliation of Contributors**

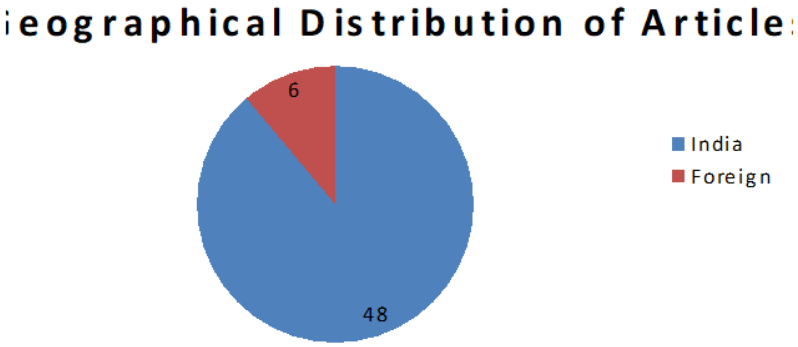



**Fig 4: Geographical Distribution of Articles**

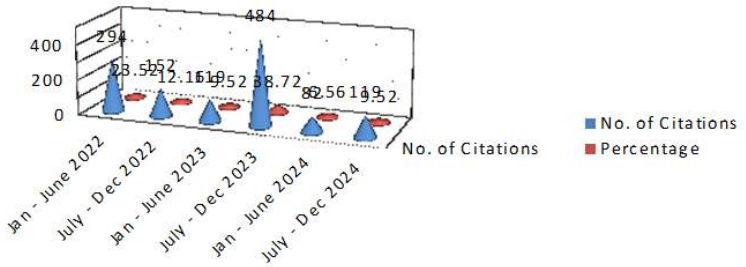



**Fig 5: Citation Analysis**

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RESEARCH ARTICLE

# Metabolomics in Periodontology: Paradigm in Diagnostic Applications

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## ABSTRACT

Periodontal diseases are chronic inflammatory conditions characterized by microbial dysbiosis and host immune responses leading to progressive destruction of tooth-supporting tissues. Conventional clinical parameters reflect historical tissue damage rather than ongoing biological activity, creating a need for predictive and early diagnostic approaches. Metabolomics, the comprehensive analysis of small-molecule metabolites in biological systems, has emerged as a promising tool in periodontal research. It provides insight into host–microbe interactions, disease pathogenesis, biomarker discovery and therapeutic monitoring. Biological fluids such as saliva, gingival crevicular fluid (GCF), serum and plasma have been extensively explored for metabolic signatures associated with periodontal health and disease. Studies have identified consistent metabolite alterations, including amino acids, organic acids and bacterial metabolites such as butyrate, lactate and phenylalanine derivatives. Metabolomics also enables differentiation of disease stages, assessment of treatment outcomes and exploration of links between periodontitis and systemic conditions. Despite its promising clinical potential, challenges remain, including methodological variability, small sample sizes and lack of standardized protocols. This review discusses principles of metabolomics, analytical platforms, key findings in periodontal research, diagnostic applications, therapeutic monitoring and future directions for integrating metabolomics into personalized periodontal care.

**Keywords:** Metabolomics, Periodontitis, Saliva, Biomarkers, Gingival crevicular fluid, Precision dentistry

## INTRODUCTION

Periodontal diseases are among the most prevalent chronic inflammatory conditions worldwide and represent a significant public health burden[1]. Traditional diagnostic measures — clinical probing, radiographs, and inflammatory indices — offer limited insight into the underlying biochemical pathways and early disease detection. This has spurred interest in systems biology approaches such as genomics, proteomics, and particularly metabolomics, which focuses on the end products of cellular processes that reflect real-time physiological changes[2]. Metabolomics refers to the comprehensive profiling of metabolites — small molecules such as amino acids, lipids, sugars, and nucleotides — present in cells, tissues, or biofluids[3]. It offers a dynamic picture of health and disease, bridging gaps between genotype and phenotype.

## REVIEW OF LITERATURE

Metabolomics has gained considerable attention in periodontal research over the past two decades due to its ability to capture dynamic biochemical alterations associated with inflammation, tissue destruction, and host–microbe interactions. Several investigations have evaluated salivary, gingival crevicular fluid (GCF), and serum metabolomic profiles in periodontal health and disease. Nicholson and Lindon (2008) first emphasized the potential of metabolomics as a systems biology tool capable of reflecting real-time physiological changes in disease conditions, thereby laying the conceptual foundation for metabolomics applications in inflammatory disorders including periodontitis[4]. Patti *et al*. (2012) further highlighted metabolomics as the functional readout of cellular activity and an important bridge between genotype and phenotype, underscoring its diagnostic potential[5]. Kuboniwa and Wong (2017) reviewed the integration of oral microbiome and salivary biomarkers, noting that metabolomics can detect microbial metabolic byproducts such as short-chain fatty acids (SCFAs), amines, and sulfur-containing compounds that are strongly associated with periodontal destruction[6]. Their work emphasized the diagnostic relevance of saliva as a non-invasive diagnostic medium. Barnes *et al*. (2014) conducted metabolomic profiling of saliva in periodontitis patients and demonstrated significant differences in amino acid metabolism and polyamine levels compared to healthy controls[7]. Elevated levels of putrescine and cadaverine were associated with microbial proteolysis and tissue breakdown. Dunn *et al*. (2015) explored salivary metabolomic signatures using mass spectrometry and reported distinct clustering patterns between periodontitis and healthy individuals based on multivariate statistical analysis[8]. The study suggested that metabolic fingerprints may serve as discriminatory biomarkers for disease status. In the context of gingival crevicular fluid, Sakanaka *et al*. (2017) performed metabolomic analysis of GCF samples and identified increased lactate, butyrate, and inflammatory-related metabolites in periodontitis sites[9]. Their findings supported the hypothesis that anaerobic glycolysis and bacterial fermentation products contribute to periodontal tissue destruction.

Psychogios *et al*. (2011) characterized the human serum metabolome and established reference metabolite databases that later facilitated comparative studies evaluating systemic metabolic alterations in inflammatory diseases, including periodontitis[10]. Subsequent studies identified altered lipid metabolism and branched-chain amino acid profiles in periodontitis patients, supporting the systemic impact of periodontal inflammation. Goodson *et al*. (2017) explored oral microbial interaction networks and their metabolic outputs, demonstrating that periodontal pathogens such as *Porphyromonas gingivalis* significantly influence amino acid fermentation pathways[11]. This reinforced the concept that metabolomics can help elucidate host–microbe metabolic crosstalk. Hajishengallis (2014) described the inflammophilic nature of periodontal microbiota, suggesting that dysbiotic microbial communities thrive in inflammatory environments and modify local metabolic landscapes[12]. Metabolomics provides measurable evidence of these metabolic shifts. Zhang *et al*. (2016) highlighted saliva metabolomics as a promising diagnostic tool due to its non-invasive collection and reflection of both local and systemic changes[13]. They emphasized its application in chronic inflammatory diseases including periodontitis. Johnson *et al*. (2016) discussed metabolomics beyond biomarker discovery and proposed its role in understanding mechanistic pathways[14]. In periodontal research, this approach has facilitated mapping of altered arginine metabolism, collagen degradation pathways, and energy

metabolism shifts. Hasin *et al*. (2017) advocated multi-omics integration to better understand complex diseases[15]. In periodontology, integrating microbiomics with metabolomics has revealed correlations between specific bacterial taxa and metabolite production patterns. Proctor *et al*. (2018) performed integrative analyses combining oral microbiome sequencing and metabolomic profiling, identifying associations between microbial composition and metabolic outputs in periodontal disease[16]. This study supported precision diagnostics in periodontology. Seneviratne *et al*. (2020) discussed translational metabolomics in dentistry and emphasized the potential for chairside diagnostic tools based on metabolic biomarkers[17]. They proposed that metabolomic signatures may enable early detection before clinical attachment loss becomes evident. Despite promising findings, Goodacre *et al*. (2007) pointed out the need for standardization in metabolomics research, including sample handling, analytical platforms, and reporting guidelines[18]. Variability in methodology remains a limitation in periodontal metabolomics studies.

**Principles of Metabolomics**

Metabolomics aims to identify and quantify the complete set of metabolites — the metabolome — in a biological system[19]. Unlike the relatively static genome, the metabolome is highly responsive to internal and external stimuli, making it a sensitive indicator of pathophysiological states[20].

**There are two major approaches**

**Targeted metabolomics:** Quantifies predefined metabolites of interest.

**Untargeted metabolomics:** Globally profiles all detectable metabolites without prior selection[21].

Biofluids commonly used in periodontal metabolomic studies include saliva, gingival crevicular fluid (GCF), serum, and urine[22].

**Analytical Platforms in Metabolomics**

Accurate metabolomics relies on sophisticated analytical technologies:

**Nuclear Magnetic Resonance (NMR) Spectroscopy**

NMR detects nuclei such as hydrogen (^1H), carbon (^13C), and phosphorus (^31P) in liquid samples. It is highly reproducible and non-destructive, but has limited sensitivity compared to mass spectrometry[23].

**Mass Spectrometry (MS)**

Mass Spectrometry, coupled with separation techniques like liquid chromatography (LC) or gas chromatography (GC), offers high sensitivity and broad metabolome coverage[24]. LC-MS and GC-MS are widely used in periodontal metabolomics[25].

**Data Processing and Chemometrics**

Metabolomics generates high-dimensional datasets requiring multivariate statistical analysis, including principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA)[26].

**Metabolomics in Periodontal Research**

**Salivary Metabolomics**

Saliva is easily accessible and reflects both local and systemic metabolic changes[27]. Several studies have identified salivary metabolites associated with periodontal inflammation[28]. A study comparing periodontitis patients to healthy controls found elevated levels of metabolites linked to bacterial metabolism and tissue breakdown, such as short-chain fatty acids (SCFAs), polyamines, and amino acids[29].

**Gingival Crevicular Fluid (GCF) Metabolomics**

GCF is a serum-derived fluid that bathes the periodontal pocket and contains host and microbial metabolites. Altered levels of inflammatory mediators and metabolic products have been documented in periodontitis[30]. For example, metabolites linked to oxidative stress and energy metabolism (e.g., lactate, pyruvate) were significantly higher in GCF from periodontitis sites[31].

**Serum and Systemic Metabolomics**

Periodontitis has systemic implications, including associations with diabetes and cardiovascular diseases. Serum metabolomics reveals changes in lipid metabolism and amino acid profiles in periodontitis patients, supporting the systemic repercussions of periodontal inflammation[32].

**Key Metabolic Pathways Implicated**

Metabolomic studies in periodontology have highlighted several dysregulated pathways:

**Amino Acid Metabolism**

Alterations in branched-chain amino acids, arginine, and proline suggest increased collagen degradation and microbial proteolysis[33].

**Energy Metabolism**

Elevations in lactate and pyruvate reflect anaerobic glycolysis due to hypoxic pockets within periodontal lesions[34].

**Oxidative Stress**

Oxidative stress markers such as malondialdehyde and glutathione imbalance have been linked to periodontal disease progression[35].

**Metabolomics and Periodontal Biomarkers**

The search for reliable biomarkers — objective indicators of disease presence, activity, or response to therapy — is a central goal in periodontal metabolomics[36].

**Diagnostic Biomarkers**

Studies have identified candidate salivary metabolites (e.g., butyrate, propionate) that differentiate periodontitis from gingivitis and health[37].

**Prognostic Biomarkers**

Changes in metabolic profiles post-therapy may predict treatment outcomes[38].

**Personalized Periodontal Therapy**

Metabolomics has the potential to guide tailored interventions based on an individual's metabolic phenotype[39].

**Integration with Other "Omics"**

Combining metabolomics with genomics, transcriptomics, and proteomics — an approach known as multi-omics integration — enhances understanding of disease networks[40]. For periodontitis, this integrated approach can elucidate host-microbe interactions and uncover therapeutic targets[41].

## DISCUSSION

Metabolomics has emerged as a promising systems biology approach in periodontology, providing insight into the biochemical alterations associated with periodontal inflammation. Unlike traditional clinical parameters such as probing depth and clinical attachment level, which reflect historical tissue destruction, metabolomics evaluates real-time metabolic changes and offers functional information about disease activity[42],[43]. One of the most consistent findings in periodontal metabolomic research is the alteration of amino acid metabolism. Elevated concentrations of branched-chain amino acids, arginine metabolites, putrescine, and cadaverine have been reported in saliva and gingival crevicular fluid of periodontitis patients[44,45]. These metabolites are largely derived from bacterial proteolysis and host tissue breakdown, supporting the concept that periodontal pathogens thrive in protein-rich, anaerobic environments[46]. The association between amino acid fermentation and periodontal destruction reinforces the polymicrobial synergy and dysbiosis model of periodontitis[47]. Energy metabolism pathways are also

significantly dysregulated in periodontal disease. Increased levels of lactate and pyruvate in gingival crevicular fluid suggest enhanced anaerobic glycolysis within inflamed periodontal tissues[48]. Hypoxic conditions in deep periodontal pockets and increased metabolic demands of inflammatory cells may contribute to this metabolic shift. Similar metabolic reprogramming has been described in chronic inflammatory diseases, suggesting that periodontitis shares systemic inflammatory characteristics[49,50]. Oxidative stress plays a critical role in periodontal tissue destruction. Reactive oxygen species generated by neutrophils and other inflammatory cells lead to lipid peroxidation and protein damage. Elevated oxidative stress markers and disturbed antioxidant pathways, including glutathione metabolism, have been demonstrated in metabolomic analyses of periodontal patients. These findings corroborate earlier biochemical studies identifying redox imbalance as a central mechanism in periodontal pathogenesis[51]. Salivary metabolomics offers a non-invasive and clinically feasible diagnostic approach. Saliva reflects both local periodontal inflammation and systemic metabolic changes[52].

Studies utilizing mass spectrometry and multivariate statistical analysis have demonstrated clear discrimination between healthy and periodontitis patients based on metabolic signatures[53,54]. However, factors such as diet, circadian rhythm, and systemic health conditions may influence salivary metabolite profiles, emphasizing the need for standardization[55]. Integration of microbiome and metabolome data has further advanced our understanding of periodontal disease mechanisms. Combined analyses have demonstrated correlations between specific bacterial taxa and metabolic byproducts, strengthening the evidence for host–microbe metabolic crosstalk[56]. For instance, periodontal pathogens such as *Porphyromonas gingivalis* are associated with increased production of short-chain fatty acids and amino acid fermentation products[57]. This integrative approach supports precision diagnostics and targeted therapeutic strategies. Despite promising results, several challenges remain. Most metabolomic studies in periodontology are cross-sectional with limited sample sizes, which restricts generalizability[58]. Variability in analytical platforms such as LC-MS and NMR further complicates inter-study comparisons. Additionally, systemic conditions such as diabetes mellitus significantly influence metabolic pathways, potentially confounding periodontal metabolomic signatures[57]. From a translational perspective, metabolomics has significant potential in early diagnosis and personalized periodontal therapy. Metabolic alterations may precede measurable clinical attachment loss, thereby allowing early detection of active disease[58]. Future research should focus on longitudinal studies, standardized methodologies, and development of chairside diagnostic platforms to facilitate clinical integration. Overall, metabolomics provides a comprehensive biochemical snapshot of periodontal disease, reflecting microbial activity, host immune response, oxidative stress, and energy metabolism disturbances. With continued advancements in analytical technologies and multi-omics integration, metabolomics is likely to play a central role in the future of precision Periodontology[59,60].

## CONCLUSION

Metabolomics has emerged as a transformative approach in periodontology, offering insights into pathophysiology, biomarkers, and personalized therapy. While challenges persist, continued technological advancement and rigorous study design will accelerate its clinical application.

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RESEARCH ARTICLE

# Alzheimer's Disease Prediction using Multimodal Neuro – Cognitive Fusion Transformer Algorithm

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## ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, memory loss, and functional impairment, posing significant challenges to healthcare systems worldwide. Early and accurate prediction of AD is essential for timely intervention and improving patient outcomes. The objective of this study is to develop an advanced predictive framework that effectively integrates multimodal data, including structural MRI scans, cognitive assessment scores, and demographic information, to enhance diagnostic accuracy. To achieve this, we propose a Multimodal Neuro–Cognitive Fusion Transformer (MNFT), which employs a convolutional neural network to extract spatial features from MRI images and a transformer-based tabular module to encode cognitive and demographic data. A cross-modal attention mechanism is then applied to capture complex interactions between neuroimaging and cognitive indicators, followed by transformer layers and a softmax classifier to predict disease stages. Experimental evaluation demonstrates that MNFT outperforms conventional, hybrid, and ensemble models, achieving superior accuracy, precision, recall, F1-score, and AUC, highlighting its robustness and clinical relevance. In conclusion, the proposed framework offers an effective, interpretable, and scalable approach for early and reliable AD prediction, paving the way for improved patient care and clinical decision support.

**Keywords:** Alzheimer's disease, cognitive assessment, cross-modal attention, deep learning, demographic data, MRI analysis, multimodal fusion

## INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that leads to cognitive decline, memory loss, and functional impairment, posing a significant challenge to global healthcare systems. Early and accurate prediction of AD is crucial for timely intervention, improving patient outcomes, and reducing overall healthcare costs. Traditional diagnostic methods, such as clinical assessments and neuroimaging, are often time-consuming, subjective, and limited in detecting early-stage disease. Recent advancements in machine learning and deep learning provide promising approaches for automated AD prediction by analyzing multimodal datasets, including MRI scans, cognitive assessments, and demographic information. Early-stage prediction using supervised machine learning models on clinical and neuropsychological datasets has shown high accuracy but limited generalizability across populations [1]. Machine learning frameworks combined with dimensionality reduction techniques like PCA have improved computational efficiency and prediction performance [2]. Deep learning approaches using convolutional neural networks for MRI analysis have demonstrated strong classification capabilities but suffer from high computational costs and limited interpretability [3]. Recurrent neural networks applied to longitudinal neuroimaging data enable temporal modeling of AD progression, though they require large sequential datasets [4]. Large-scale health records have been used with machine learning models for scalable disease incidence prediction, but missing data and potential biases remain challenges [5].

Studies analyzing AD impacts on COVID-19 outcomes highlight risk patterns without providing predictive models [6]. Graph-based MRI grading frameworks improve sensitivity to structural changes but are computationally complex [7]. Reviews of AI-based predictive models emphasize the need for explainable and standardized approaches for clinical adoption [8]. Recent 3D deep learning models combining MRI with cognitive scores allow joint prediction of disease and MMSE but require large fMRI datasets [9]. Cross-cohort evaluation demonstrates that MRI-based deep and conventional models often lack generalization [10], while mini-reviews highlight the superiority of deep learning for AD detection [11]. Multimodal and multitask learning approaches combining MRI, PET, and clinical data enhance longitudinal prediction accuracy but increase system complexity [12]. Analytical reviews of AI applications stress uncertainty in real-world adoption [13], whereas hybrid deep learning architectures improve detection accuracy by fusing multiple models [14]. Machine learning applied to MRI in elderly populations provides early prediction but is limited to single-modality data [15]. Finally, ensemble learning with multi-slice CNNs captures slice-level spatial information and improves diagnostic accuracy [16].The paper is organized as follows: Section 2 reviews background studies on machine learning and deep learning for AD prediction; Section 3 presents the proposed methodology and MNFT architecture; Section 4 discusses results and performance evaluation; and Section 5 concludes with future directions for longitudinal, multi-center clinical applications. Overall, this study advances AI-driven Alzheimer's disease prediction by integrating multimodal data, transformer-based modeling, and interpretable attention mechanisms for early and reliable diagnosis.

## BACKGROUND STUDY

Kavitha *et al*. (2022) [1] This study focuses on early-stage Alzheimer's disease prediction using supervised machine learning models applied to clinical and neuropsychological datasets. The research gap lies in limited generalization across diverse populations; although models achieved high accuracy, performance depended heavily on dataset quality and feature selection. Sudharsan *et al*. (2023) [2] The authors propose a machine learning framework combined with Principal Component Analysis (PCA) to reduce dimensionality and improve Alzheimer's disease prediction. While PCA enhanced computational efficiency and accuracy, the study was limited by small datasets and lack of real-time clinical validation. Saratxaga *et al*. (2021) [3] This work introduces a deep learning-based MRI analysis approach for Alzheimer's disease prediction using convolutional neural networks. Despite strong classification performance, the research gap includes high computational cost and limited explainability of deep learning decisions in clinical settings. Nguyen *et al*. (2020) [4] The study employs deep recurrent neural networks to predict Alzheimer's disease progression over time using longitudinal neuroimaging data. Although temporal

modeling improved prediction accuracy, the method requires large-scale sequential data and suffers from interpretability challenges. Park *et al*. (2020) [5] This research applies machine learning models to large-scale administrative health records to predict Alzheimer's disease incidence. The main limitation is potential bias from incomplete medical records, even though results demonstrated scalable and cost-effective disease risk prediction. Yu *et al*. (2021) [6]The authors analyze Alzheimer's and Parkinson's disease impacts on COVID-19 outcomes using UK Biobank data. While the study reveals differential risk patterns, it does not directly contribute predictive diagnostic models for Alzheimer's disease progression. Hett *et al*. (2021) [7] This study proposes a multi-scale graph-based grading framework for Alzheimer's disease prediction using MRI features. Despite improved sensitivity to structural brain changes, the approach is computationally complex and less suitable for real-time clinical deployment. Nandiraju *et al*. (2022) [8] the paper presents a comprehensive review of AI-based predictive models for Alzheimer's disease diagnosis in healthcare systems. Although it highlights recent advancements, the study lacks experimental validation and emphasizes the need for standardized datasets and explainable AI models. Comparative Background Study of Machine Learning and Deep Learning Techniques for Alzheimer's disease Diagnosis and Prediction (Table 1). This table 1 presents a comparative background study of recent machine learning, deep learning, hybrid, and ensemble approaches for Alzheimer's disease diagnosis using MRI and fMRI data. The studies highlight advancements in predictive accuracy through multimodal and deep architectures, while also revealing key research gaps related to generalizability, interpretability, computational complexity, and real-world clinical deployment.

## PROPOSED METHODOLOGY

This section describes the proposed methodology for Alzheimer's disease prediction using a multimodal deep learning framework. The approach combines structural MRI data with cognitive assessment scores and demographic information to capture comprehensive neuro–cognitive characteristics. A Multimodal Neuro–Cognitive Fusion Transformer (MNFT) is employed to effectively fuse these modalities and classify disease stages accurately. Architecture of the Proposed Multimodal Neuro–Cognitive Fusion Transformer (MNFT) for Alzheimer's disease Prediction (Figure 1). The figure 1 shows the MNFT model where MRI images are processed by a CNN and cognitive–demographic data are encoded using a tabular Transformer. These features are fused through cross-modal attention and classified using a Transformer encoder with a Softmax layer to predict Alzheimer's disease.

**Dataset**

**Dataset Link:** https://www.kaggle.com/datasets/ankushpanday1/alzheimers-prediction-dataset-global

The dataset contains structural MRI features, cognitive assessment scores, and demographic information for subjects at different Alzheimer's disease stages. It supports multimodal machine learning by integrating neuroimaging and clinical data for accurate prediction and analysis.

**Multimodal Neuro–Cognitive Fusion Transformer (MNFT)**

The proposed Multimodal Neuro–Cognitive Fusion Transformer (MNFT) integrates heterogeneous data sources to achieve accurate and early Alzheimer's disease prediction. Structural MRI scans are first processed using a deep convolutional neural network to extract high-level spatial features representing neurodegenerative patterns. Simultaneously, cognitive assessment scores and demographic attributes are encoded using a transformer-based tabular learning module. A cross-modal attention mechanism is then employed to model the complex interactions between brain structural changes and cognitive decline indicators. This attention layer dynamically assigns importance weights to each modality, enabling the model to focus on clinically relevant features. The fused multimodal representation is passed through stacked transformer layers to capture global contextual dependencies. Finally, a softmax classifier categorizes subjects into cognitively normal, mild cognitive impairment, or Alzheimer's disease classes. This unified framework enhances prediction accuracy while providing interpretable insights into disease-related neuro–cognitive correlations.

$F_{mri} = CNN(X_{mri})$-------- (1)

In this equation $X_{mri}$ denotes the input structural MRI scans containing brain anatomical details relevant to Alzheimer's disease. The $CNN$ processes these images to extract meaningful spatial features, producing$F_{mri}$, a feature representation capturing patterns of neurodegeneration for prediction.

$F_{cd} = Transformer_{tab}(X_{cog}, X_{demo})$-------- (2)

In this equation$X_{cog}$ represents the cognitive assessment scores, and $X_{demo}$ denotes demographic attributes such as age, gender, and education. The tabular transformer encodes these inputs into a unified feature vector $F_{cd}$, capturing the relationships between cognitive performance and demographic factors for Alzheimer's disease prediction.

$A = softmax(\frac{Q_{mri}K_{cd}^T}{\sqrt{d}})V_{cd}$-------- (3)

In this equation$Q_{mri}$ and $K_{cd}^T$ are the query and key matrices derived from MRI and cognitive–demographic features, respectively, while $V_{cd}$ is the value matrix from cognitive–demographic data. The cross-modal attention computes $A$ by weighting cognitive–demographic features according to their relevance to MRI features, highlighting important neuro–cognitive interactions for Alzheimer's prediction.

$F_{fusion} = \alpha F_{mri} + (1 - \alpha)A$-------- (4)

In this equation$F_{mri}$ represents the features extracted from MRI scans, and $A$ is the cross-modal attention output capturing cognitive–demographic interactions. The fused feature vector $F_{fusion}$ combines these two modalities using a learnable weight$\alpha$, balancing the contribution of neuroimaging and cognitive information for Alzheimer's disease prediction.

$\hat{y} = softmax(WF_{fusion} + b)$-------- (5)

In this equation$F_{fusion}$ is the combined feature vector from MRI and cognitive–demographic data, $W$ is the classifier weight matrix, and $b$ is the bias term. The softmax function generates$\hat{y}$, the predicted probabilities for each class (Cognitively Normal, Mild Cognitive Impairment, or Alzheimer's Disease), providing the final stage prediction.

**Algorithm 1:MNFT_Alzheimer_Prediction**

Input:
X_mri // Structural MRI scans
X_cog // Cognitive assessment scores
X_demo// Demographic data
Initialize CNN parameters θ_cnn
Initialize Tabular Transformer parameters θ_tab
Initialize Cross-Modal Attention parameters θ_att
Initialize Transformer Encoder parameters θ_trans
Initialize Softmax Classifier parameters θ_cls
// Step 1: Extract MRI features
F_mri ← CNN(X_mri; θ_cnn)
// Step 2: Encode cognitive and demographic features
F_cd ← TabTransformer(X_cog, X_demo; θ_tab)
// Step 3: Apply cross-modal attention
Q ← Linear(F_mri; θ_att)
K ← Linear(F_cd; θ_att)
V ← Linear(F_cd; θ_att)

A ← Softmax((Q × $K^T$) / √d) × V
// Step 4: Fuse multimodal features
F_fusion ← Concatenate(F_mri, A)
// Step 5: Capture global context with stacked transformer layers
F_global ← TransformerEncoder(F_fusion; θ_trans)
// Step 6: Classify into Alzheimer's disease stages
Y_pred ← Softmax(Linear(F_global; θ_cls))
Return Y_pred

Output:
Y_pred // Predicted Alzheimer's stage: CN, MCI, or AD

The MNFT algorithm first extracts features from MRI scans and encodes cognitive and demographic data, then applies cross-modal attention to capture interactions between brain structure and cognitive indicators. These multimodal features are fused and processed through transformer layers before a softmax classifier predicts the Alzheimer's disease stage as CN, MCI, or AD.

## RESULT AND DISCUSSION

This section presents the performance evaluation of the proposed MNFT model compared to existing baseline methods for Alzheimer's disease prediction. Key metrics such as accuracy, precision, recall, F1-score, and AUC are analyzed to assess model effectiveness. The discussion highlights how multimodal fusion and cross-modal attention contribute to improved predictive performance and clinical reliability. Performance Comparison of Alzheimer's Disease Prediction Methods (Table 2). The table 2 show that the proposed MNFT model outperforms all baseline methods across accuracy, precision, recall, F1-score, and AUC. This improvement highlights the effectiveness of multimodal fusion and cross-modal attention in capturing complementary neuroimaging and cognitive information. The higher AUC further indicates strong robustness and reliable discrimination between different Alzheimer's disease stages. Accuracy Comparison of Alzheimer's Disease Prediction Methods (Figure 2). The figure 2 presents a comparative analysis of classification accuracy achieved by different Alzheimer's disease prediction methods. Traditional and hybrid models show moderate improvements with feature fusion, while the proposed MNFT model achieves the highest accuracy. This demonstrates the effectiveness of multimodal integration and cross-modal attention in enhancing predictive performance.

## CONCLUSION

This study presented a Multimodal Neuro–Cognitive Fusion Transformer (MNFT) for Alzheimer's disease prediction by effectively integrating MRI, cognitive, and demographic data. Experimental results demonstrate that MNFT outperforms existing methods, highlighting the importance of multimodal fusion and cross-modal attention in capturing complex neuro–cognitive relationships. The proposed framework also shows strong robustness and reliable discrimination across disease stages. Future work will focus on validating the model on larger, multi-center clinical datasets, improving explainability for clinical adoption, and extending the framework to incorporate longitudinal and additional modalities such as PET and genetic data.

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**Table 1: Comparative Background Study of Machine Learning and Deep Learning Techniques for Alzheimer's disease Diagnosis and Prediction**

| Author | Concept | Research Gap | Methods | Limitations | Results |
|---|---|---|---|---|---|
| Duc *et al*. (2020) [9] | Automatic diagnosis of Alzheimer's disease using 3D deep learning on resting-state fMRI with MMSE prediction | Limited exploration of joint cognitive score prediction and diagnosis in clinical practice | 3D CNN-based deep learning model integrating fMRI data and MMSE regression | High computational complexity and dependence on large fMRI datasets | Achieved high accuracy in AD classification and reliable MMSE score prediction |

| | | | | | |
|---|---|---|---|---|---|
| Bron *et al*. (2021) [10] | Evaluation of cross-cohort generalizability of MRI-based deep and conventional ML models | Poor model generalization across datasets from different cohorts | CNNs and traditional ML models tested across multiple MRI cohorts | Performance drop when models trained and tested on different datasets | Demonstrated need for robust cross-cohort validation in AD diagnosis |
| Al-Shoukry *et al*. (2020) [11] | Review of deep learning algorithms for Alzheimer's disease detection | Lack of standardized evaluation frameworks across studies | Comparative analysis of CNN, RNN, and hybrid DL models | No experimental implementation or performance benchmarking | Highlighted deep learning superiority over traditional ML for AD detection |
| Tabarestani *et al*. (2020) [12] | Multimodal and multitask learning for longitudinal Alzheimer's disease prediction | Limited integration of distributed and multimodal longitudinal data | Distributed multitask learning combining MRI, PET, and clinical data | Increased system complexity and synchronization challenges | Improved prediction accuracy for AD progression over time |
| Fabrizio *et al*. (2021) [13] | Critical analysis of AI applications in Alzheimer's disease diagnosis | Uncertainty in real-world clinical adoption of AI systems | Analytical review of AI-driven diagnostic frameworks | Absence of empirical validation and deployment studies | Concluded AI shows promise but requires interpretability and regulation |
| Balaji *et al*. (2023) [14] | Hybrid deep learning framework for Alzheimer's disease detection | Limited use of hybrid architectures combining multiple DL models | Fusion of CNN and deep feature extraction techniques | Model complexity and higher training time | Achieved improved detection accuracy compared to standalone models |
| Battineni *et al*. (2020) [15] | Comprehensive ML model for MRI-based Alzheimer's prediction in elderly subjects | Limited personalization for age-related structural brain variations | Machine learning classifiers applied to MRI-derived features | Restricted to single-modality MRI data | Demonstrated effective early prediction of AD in older adults |
| Kang *et al*. (2021) [16] | Ensemble learning using multi-model and multi-slice 2D CNN architecture | Underutilization of slice-level spatial information in MRI analysis | Multi-slice 2D CNN ensemble learning framework | Increased memory usage and training complexity | Improved diagnostic accuracy over single-model CNN approaches |

**Table 2: Performance Comparison of Alzheimer's Disease Prediction Methods**

| Method | Accuracy (%) | Precision (%) | Recall (%) | F1-score (%) | AUC |
|---|---|---|---|---|---|
| Graph Neural Network (GNN) [15] | 82.4 | 80.8 | 81.2 | 81.7 | 0.86 |
| Transformer Encoder Only [16] | 84.1 | 83.2 | 83.7 | 83.5 | 0.88 |
| GNN + Transformer (No Fusion) [17] | 88.3 | 87.8 | 87.5 | 87.6 | 0.92 |
| GNN + Multimodal Fusion [18] | 86.9 | 86.2 | 85.9 | 86.2 | 0.90 |
| MNFT (Proposed) | 94.6 | 94.1 | 93.8 | 93.9 | 0.96 |

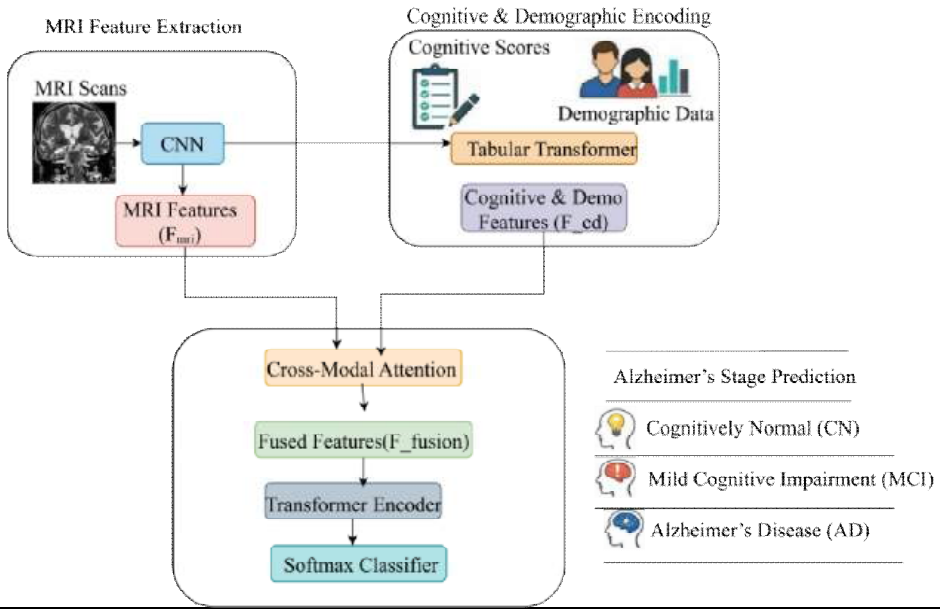



**Figure 1: Architecture of the Proposed Multimodal Neuro–Cognitive Fusion Transformer (MNFT) for Alzheimer's disease Prediction**

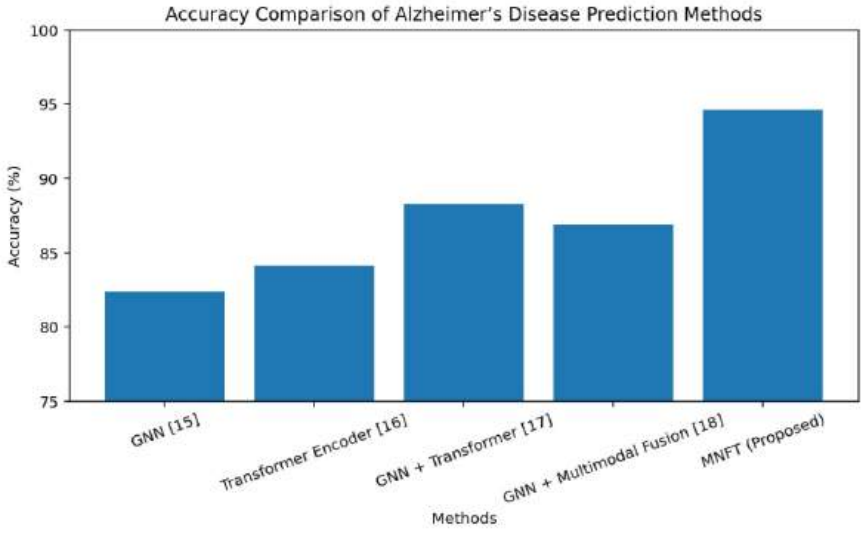



**Figure 2: Accuracy Comparison of Alzheimer's Disease Prediction Methods**

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RESEARCH ARTICLE

# Computational Analysis of Selected Flavonoids for Anti-tubercular Activity

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## ABSTRACT

Due in large part to the development of drug-resistant strains and the drawbacks of currently available treatments, In this study, an integrated computational approach was employed to evaluate the anti-tubercular potential of selected natural flavonoids as alternative therapeutic candidates. Computational analysis of selected flavonoids for anti-tubercular activity**.** In the present study, selected flavonoids were evaluated for their anti-tuberculosis potential using an in-silico approach such as Density Functional Theory (DFT). Molecular docking was performed. The stability of the protein–ligand complex was assessed using the iMOD server. Toxicity and pharmacokinetic properties were predicted using the ProTox-III, and the results were compared with standard anti-tuberculosis drugs. The molecular docking results demonstrated good binding affinity of the selected flavonoid with the Mycobacterium tuberculosis target protein. The optimized structure obtained from DFT showed stable electronic properties. The protein–ligand complex exhibited satisfactory stability in normal mode analysis using the iMODs server. Toxicity prediction from ProTox-III indicated low Overall, the selected flavonoid showed promising anti-tuberculosis potential compared to standard drugs. The integrated in-silico findings suggest that selected flavonoids possess strong anti-tubercular potential with favorable binding affinity, stability, pharmacokinetic properties, and safety profiles.

**Keywords:** Mycobacterium tuberculosis, flavonoids, anti-tubercular activity, molecular docking, SwissADME analysis; toxicity prediction; Natural materials, molecular dynamics simulation, and density theory of function

# INTRODUCTION

One of the most common and deadly infectious diseases in the world today is tuberculosis (TB). The cause is Mycobacterium tuberculosis (M. tb), an acid-fast, slow-growing bacillus that primarily affects the lungs but can infect almost any organ system. Robert Koch initially identified the pathogen in 1882, a breakthrough that transformed infectious disease microbiology and diagnostics. Droplet nuclei containing viable bacilli are released into the air when people with active pulmonary tuberculosis cough, sneeze, or even just talk. The bacilli enter the lungs' alveoli and are taken up by alveolar macrophages when inhaled by a vulnerable individual [1]. The ability of the bacillus to endure inside host immune cells is necessary for the development of long-term latent infection. T-helper 1 (Th1) cells are essential to the cell-mediated immune response to tuberculosis because they release interferon-$\gamma$ and stimulate macrophages to contain the infection. Granulomas, which are organized clusters of macrophages, lymphocytes, and fibroblasts that prevent the infection from spreading, are produced as a result [2] .The mycobacterial cell wall's structural stability, impermeability, and resistance to numerous drugs are all attributed to mycolic acids. InhA reduces long-chain enoyl-ACP intermediates in a NADH-dependent way, which is crucial for the production of these complex fatty acids. A crucial enzyme in the fatty acid elongation stage of mycolic acid production in Mycobacterium TB is enoyl-acyl carrier protein reductase, or InhA.

## MATERIALS AND METHODS

**Selection of Compounds**

Flavonoids used in this work were collected from the PubChem database. The structures were downloaded in SDF format and optimized by energy minimization before proceeding to further computational studies. Isoniazid was taken as the standard anti-tubercular drug for comparison [3].

**Protein Preparation**

The three-dimensional structures of the selected Mycobacterium tuberculosis proteins (PDB IDs: 2B35, 4TZK and 4OIM) were obtained from the RCSB Protein Data Bank. For docking analysis, the proteins were cleaned by removing co-crystallized ligands and water molecules. Polar hydrogens were added and proper charges were assigned to prepare the proteins for interaction studies [4].

**Docking Method**

Protein–ligand interaction studies were carried out using AutoDock 4.2. The active site region of each protein was defined using a grid box. Docking was performed with the Lamarckian Genetic Algorithm under default conditions [5].

**ADME Study**

Pharmacokinetic properties of the selected compounds were evaluated by analyzing molecular weight, hydrogen bond donors, hydrogen bond acceptors, lipophilicity, gastrointestinal absorption and Lipinski's rule of five. These parameters were used to determine drug-likeness [6].

**BOILED-Egg Model**

The BOILED-Egg model available in SwissADME was applied to predict intestinal absorption and BBB permeability. The prediction based on polarity and lipophilicity of the molecules [7].

**Toxicity Assessment**

Toxicity prediction was performed using the ProTox-III online server. Included estimation of $LD_{50}$ values, toxicity class. Results were compared with the reference drug isoniazid [8].

**Density Functional Theory Analysis**

Density Functional Theory was used to examine the electronic features of the selected flavonoids. The calculation provided information about their molecular stability and overall chemical nature [9].

**Molecular Dynamics Simulation**

Molecular dynamics analysis was performed with the help of the iMODS server. The movement and flexibility of the protein–ligand systems were examined by studying normal mode eigenvalues, deformability patterns, covariance relationships, and elastic network behavior[10].

## RESULTS

**Anti-tubercular activity of selected flavonoid**

Anti-tubercular activity of selected flavonoid (Table 1).

**SwissADME analysis of selected flavonoids**

SwissADME analysis was used to evaluate the pharmacokinetic properties and drug-likeness of the selected flavonoids and compare them with the widely used medicine isoniazid. The results showed that most flavonoids are suitable candidates for oral drugs because they have acceptable physicochemical properties and, with very few exceptions, follow Lipinski's rule of five.(Table 2)The study also revealed that some flavonoids had good gastrointestinal absorption, matching or surpassing that of isoniazid Additionally, the study showed that a number of flavonoids exhibited good gastrointestinal absorption, on par with or superior to isoniazid[11]. Swiss ADME (Table 2).

**Boiled-egg model analysis**

The BOILED-Egg model was used to predict the gastrointestinal absorption (HIA) and blood–brain barrier (BBB) permeability of the selected flavonoids, which were compared to the traditional drug isoniazid. Like isoniazid, most of the flavonoids were located in the white portion of the BOILED-Egg plot, indicating that humans have excellent intestinal absorption [12]. (Figure 1) The BOILED-Egg model was used to visually compare the selected flavonoids' intestinal absorption and brain penetration capacity to that of isoniazid (INH) suggesting that the BBB was not penetrated. Boiled egg model (Figure 1).

**Protox-III toxicity (Using ProTox-III)**

The toxicity profile of the selected flavonoids was estimated and contrasted with that of the popular drug isoniazid. Includes acute toxicity, toxicity, and potential toxicological. Selected flavonoids were predicted to belong to lower toxicity classes when compared to isoniazid [13].(Table 3)**.** PRO TOX III (Table 3).

**Protein data bank (pdb) and target selection**

The three-dimensional (3D) structural details of biological macromolecules, are accessible through the Protein Data Bank (PDB) such as proteins and nucleic acids, and an internationally acknowledged database. The structural entire in the PDB are determined using experimental approaches including X-ray crystallography, and cryo-electron microscopy. In this investigation, nuclear magnetic resonance (NMR) spectroscopy and three protein targets of Mycobacterium tuberculosis were chosen to their respective PDB identification codes [14].

**Validation of Protein**

The stereochemical reliability of the selected protein structure (Figure 2).
PDB ID: 4TZK, 2B35 and 4OIMRamachandran Plot (Figure 2).

**Docking studies**

AutoDock software was used along with BIOVIA Discovery Studio for visualization. AutoDock helped to predict how the ligand enters and binds inside the active site of the protein by calculating binding energy values. The docking results obtained from AutoDock showed that the ligand binds well with the target protein.The negative binding energy values indicate that the binding occurs naturally and is energetically favorable (Table 4) [15]. docking study analysis (Table 4).

**Amino acid interaction**

The interaction analysis showed that the ligand stays well inside the protein active site. The binding is mainly due to hydrogen bonds and hydrophobic contacts with some important amino acids. (Figure 3) These contacts help the ligand remain attached to the protein and keep the complex stable inside the binding pocket. [16]. Amino acid interaction (PDB ID: 4TZK), (PDB ID: 2B35) and (PDB ID: 4OIM) (Figure 3).

**Quantum chemistry**

Density Functional Theory (DFT) is a quantum mechanical computational technique that is widely used to study the electronic structure of molecules as well as solid systems. In this approach, the ground-state properties of a system are explained using electron density instead of wave functions, which makes less time consuming and suitable for medium to large molecular systems [17].

**DFT evaluation of Isoniazid**

DFT Calculations (Table 5).

Molecular electrostatic potentials (MEP) of Isoniazid (Figure 4).

**DFT evaluation of 5240883**

Alpha ($\alpha$) Spin Orbitals (Table 6).
Beta ($\beta$) Spin Orbitals (Table 7).
Molecular electrostatic potentials (MEP) of5240883 (Figure 5).

**DFT evaluation of 5281672**

Alpha ($\alpha$) Spin Orbitals (Table 8).
Beta ($\beta$) Spin Orbitals (Table 9).
Molecular electrostatic potentials (MEP) of 5281672 (Figure 6).

**DFT evaluation of 9064**

DFT Calculated Properties (Table 10). Molecular electrostatic potentials (MEP) of 9064 (Figure 7).

**imods molecular dynamics simulations**

iMOD molecular dynamics is a computer-based method. It is used to understand how biomolecules move and bend. This method mainly looks at natural flexibility of proteins. It works using normal mode analysis. Only slow and important motions are studied. These motions are important for biological activity. It gives results in less time. It helps to study shape changes, flexible regions, and movement of residues. This method is useful to understand protein stability [18].It turns out the iMOD simulation of flavonoids' bound to a protein captures how it holds its shape and shifts subtly through time. Early normal modes stand out in the variance graph since they account for most movement, suggesting large-scale, slow swings drive big shape changes; faster ones barely nudge things. Because low-energy shifts come easier, small eigenvalues at the start line up with these smooth, sweeping motions - seen clearly in while rising values later imply tighter resistance when atoms try to stretch further ties together is revealed in the correlation map neighboring parts often move in sync, some push opposite ways, yet strong diagonal signals show alignment across the chain, keeping form intact during attachment. Seen another way, the spring-like model outlines a rigid hub linked outward to looser zones, balancing shift capacity against collapse risk. Flexible

spots pop on the deformability chart and B-factor plot especially loops and ends bending easily, even though amino acids near where docks resist wobbling. Matched closely are computed B-values from iMOD alongside lab-measured ones, so confidence grows in both method accuracy and the idea that stays actively engaged with the protein throughout the run.

**imod MD Analysis of Isoniazid–Protein Complex**

MD Simulations of PDB 4TZK with Isoniazid

Figure 8. Variance Distribution of Normal Modes (9064)

Figure 9. Eigenvalue Distribution Across Normal Modes

Figure 10. Covariance Matrix of Atomic Fluctuations

Figure 11. (Likely the Elastic Network or Connectivity Plot)

Figure 12. Deformability Profile Along the Protein Chain

Figure 13. Comparison of Experimental and NMA-Derived B-Factors

**iMOD Molecular Dynamics Analysis of 5240883–Protein Complex**

MD Simulations of PDB 2B35 with l 5240883

Figure 14. Variance Distribution of Normal Modes (9064)

Figure 15. Eigenvalue Distribution Across Normal Modes

Figure 16. Covariance Matrix of Atomic Fluctuations

Figure 17. (Likely the Elastic Network or Connectivity Plot)

Figure 18. Deformability Profile Along the Protein Chain

Figure 19. Comparison of Experimental and NMA-Derived B-Factors

**iMOD Molecular Dynamics Analysis of 9064 Protein Complex**

MD Simulations of PDB 4TZK with 9064

Figure 20. Variance Distribution of Normal Modes (9064)

Figure 21. Eigenvalue Distribution Across Normal Modes

Figure 22. Covariance Matrix of Atomic Fluctuations

Figure 23. (Likely the Elastic Network or Connectivity Plot)

Figure 24. Deformability Profile Along the Protein Chain

Figure 25. Comparison of Experimental and NMA-Derived B-Factors

**iMOD MD of 5281672–Protein Complex**

MD Simulations of PDB 4OIM with 5281672

Figure 26. Variance Distribution of Normal Modes (9064)

Figure 27. Eigenvalue Distribution Across Normal Modes

Figure 28. Covariance Matrix of Atomic Fluctuations

Figure 29. (Likely the Elastic Network or Connectivity Plot)

Figure 30. Deformability Profile Along the Protein Chain

Figure 31. Comparison of Experimental and NMA-Derived B-Factors

## CONCLUSION

This study highlights the promising anti-tubercular potential of selected flavonoids based on comprehensive computational examine. The compounds demonstrated favorable binding affinity, stable protein–ligand interactions, suitable pharmacokinetic characteristics, and minimum predicted harmful. Electronic and molecular dynamics evaluations additionaly supported their stability, suggesting these flavonoids may serve as valuable candidates for future experimental investigation.

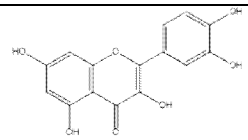
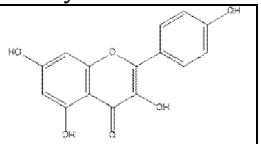
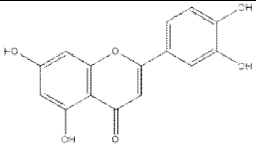
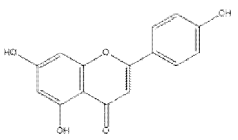
## ACKNOWLEDGEMENT

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**Table 1: Anti-tubercular activity of selected flavonoid**

| | |
|---|---|
|  5280343 |  5280863 |
|  5280445 |  5240883 |

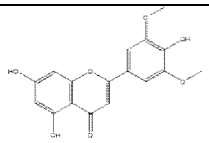
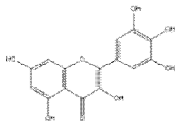
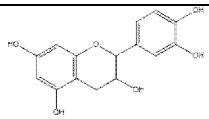
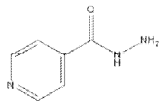
| | |
|---|---|
|  5281607 |  5281672 |
|  9064 |  Isoniazid |

**Table 2: Swiss ADME**

| Compound ID | MW (Da) | LogP | H-bond donors | H-bond acceptors | TPSA (Å²) | BBB Permeable | GI Absorption |
|---|---|---|---|---|---|---|---|
| 5280343 | 302 | 1.5 | 5 | 7 | 131.4 | No | High |
| 5280863 | 286 | 1.9 | 4 | 6 | 111.1 | No | High |
| 5280445 | 286 | 2.0 | 4 | 6 | 111.1 | No | High |
| 5240883 | 270 | 2.1 | 3 | 5 | 90.9 | No | High |
| 5281607 | 254 | 2.5 | 2 | 4 | 70.7 | No | High |
| 5281672 | 318 | 1.4 | 6 | 8 | 151.6 | No | High |
| 9064 | 290 | 1.2 | 5 | 6 | 110.4 | No | High |
| Isoniazid | 137 | −0.6 | 2 | 3 | 68.0 | No | High |

**Table 3: PRO TOX III**

| Compound ID | Hepatotoxicity | Nephrotoxicity | Carcinogenicity | Mutagenicity | Immunotoxicity | Cytotoxicity |
|---|---|---|---|---|---|---|
| 5280343 | No | No | No | No | Mild | No |
| 5280863 | No | No | No | No | No | No |
| 5280445 | No | No | No | No | Mild | No |
| 5240883 | No | No | No | No | No | No |
| 5281607 | No | No | No | No | No | No |
| 5281672 | No | No | No | Mild | Mild | No |
| 9064 | No | No | No | No | No | No |
| Isoniazid (Standard) | Yes (High) | Mild | No | No | No | No |

**Table 4: docking study analysis**

| S. No. | Compound ID | Docking Score (kcal/mol) PDB ID: 2B35 | Docking Score (kcal/mol) PDB ID: 4TZK | Docking Score (kcal/mol) PDB ID: 4OIM |
|---|---|---|---|---|
| 1 | 5280343 | −7.6 | −8.5 | −10.6 |
| 2 | 5280863 | −7.6 | −8.5 | −10.4 |
| 3 | 5280445 | −7.8 | −8.7 | −10.6 |
| 4 | 5240883 | −7.9 | −8.4 | −10.4 |
| 5 | 5281607 | −7.0 | −8.1 | −9.9 |
| 6 | 5281672 | −7.5 | −8.4 | −10.9 |
| 7 | 9064 | −7.8 | −8.9 | −10.3 |
| 8 | Isoniazid (Standard) | −7.6 | −5.8 | −10.5 |

| | | | | |
|---|---|---|---|---|
| 9 | co-crystallized (5-oxopyrrolidine 3- carboxamide ligand) | −7.6 | −5.8 | −10.5 |

**Table 5: DFT Calculations**

| EHOMO | ELUMO | ΔE | I | A | η | S | μ | χ | μ (Debye) |
|---|---|---|---|---|---|---|---|---|---|
| −0.22542 eV | −0.05465 eV | 0.17077 eV | 0.22542 eV | 0.05465 eV | 0.08538 eV | 11.71 $eV^{-1}$ | −0.14004 eV | 0.14004 eV | 2.528267 D |

**Table 6: Alpha (α) Spin Orbitals**

| EHOMO | ELUMO | ΔE | I | A | H | S | μ | χ | μ(Debye) |
|---|---|---|---|---|---|---|---|---|---|
| −0.18014eV | −0.02660eV | 0.15354 eV | 0.18014 eV | 0.02660 eV | 0.07677 eV | 13.03 $eV^{-1}$ | −0.10337 eV | 0.10337 eV | 3.906047 D |

**Table 7: Beta (β) Spin Orbitals**

| EHOMO | ELUMO | ΔE | I | A | H | S | μ | χ | μ (Debye) |
|---|---|---|---|---|---|---|---|---|---|
| −0.09853 | −0.01455 | 0.08398 | 0.09853 | 0.01455 | 0.04199 | 23.81 | −0.05654 | 0.05654 | 3.906047 D |

**Table 8: Alpha (α) Spin Orbitals**

| EHOMO | ELUMO | ΔE | I | A | H | S | μ | χ | μ (Debye) |
|---|---|---|---|---|---|---|---|---|---|
| −0.17970 | −0.02419 | 0.15551 | 0.17970 | 0.02419 | 0.07776 | 12.86 | −0.10195 | 0.10195 | 4.282551 |

**Table 9: Beta (β) Spin Orbitals**

| EHOMO | ELUMO | ΔE | I | A | H | S | μ | χ | μ (Debye) |
|---|---|---|---|---|---|---|---|---|---|
| −0.20054 | −0.10022 | 0.10032 | 0.20054 | 0.10022 | 0.05016 | 19.94 | −0.15038 | 0.15038 | 4.282551 |

**Table 10: DFT Calculated Properties**

| EHOMO | ELUMO | ΔE | I | A | H | S | μ | χ | μ (Debye) |
|---|---|---|---|---|---|---|---|---|---|
| 2.9619 | -5.16 | -0.37 | 4.79 | 2.396 | 0.2085 | 2.76 | 1.59 | 0.15038 | 4.282551 |

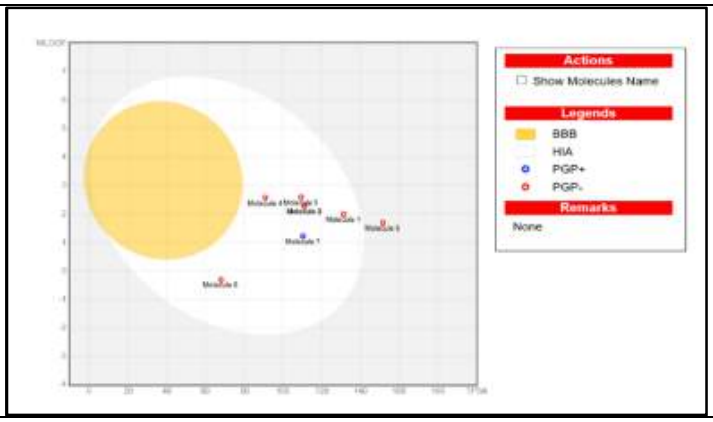



**Figure 1: Boiled egg model.**

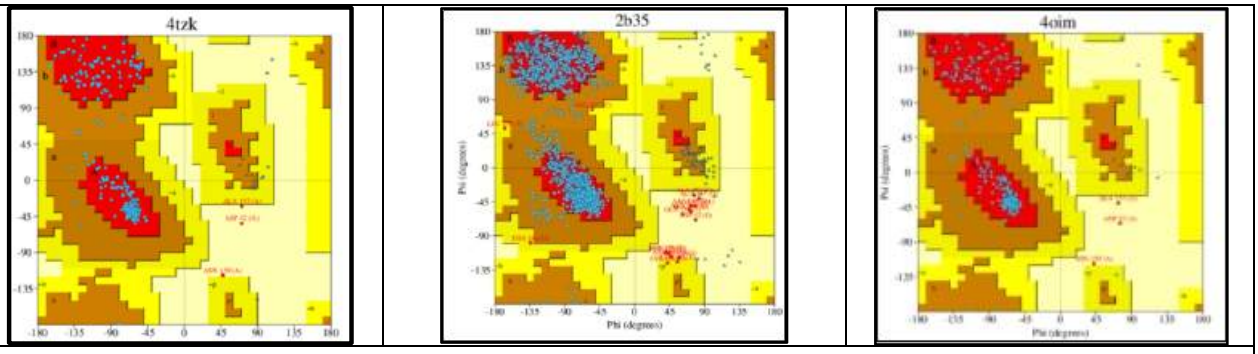



**Figure 2: PDB ID: 4TZK, 2B35 and 4OIMRamachandran Plot**

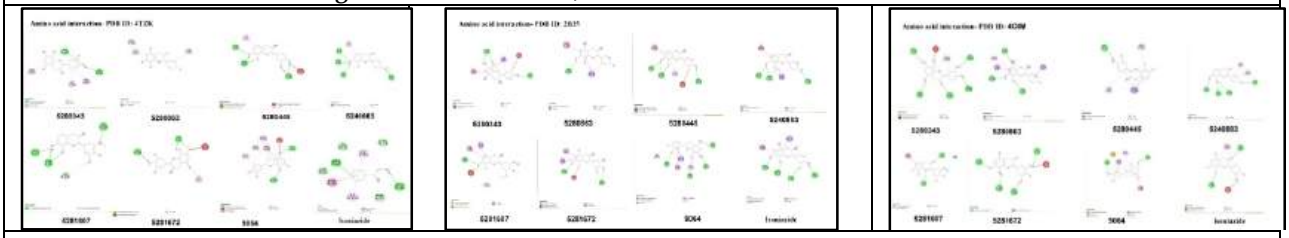


**Figure 3: Amino acid interaction (PDB ID: 4TZK), (PDB ID: 2B35) and (PDB ID: 4OIM)**

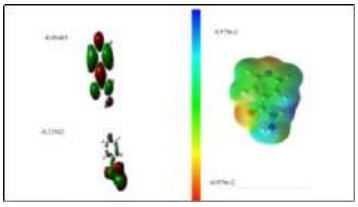


**Figure 4: Molecular electrostatic potentials (MEP) of Isoniazid**

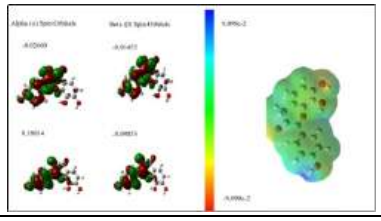


**Figure 5: Molecular electrostatic potentials (MEP) of5240883**

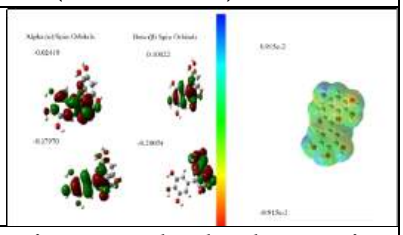


**Figure 6: Molecular electrostatic potentials (MEP) of 5281672**

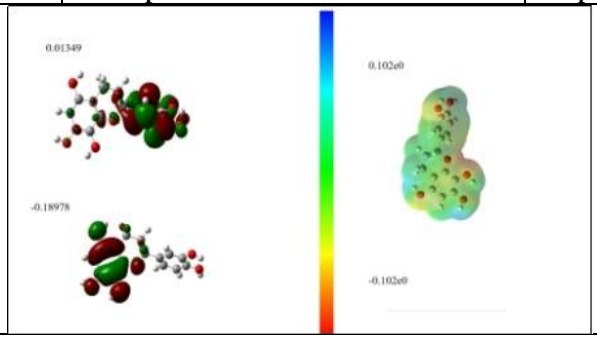



**Figure 7: Molecular electrostatic potentials (MEP) of 9064**

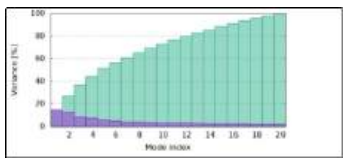



**Figure 8: Variance Distribution of Normal Modes (9064)**

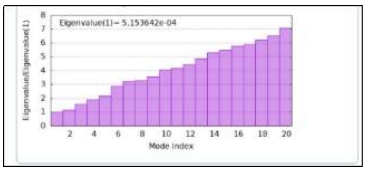



**Figure 9: Eigenvalue Distribution Across Normal Modes**

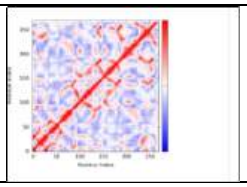
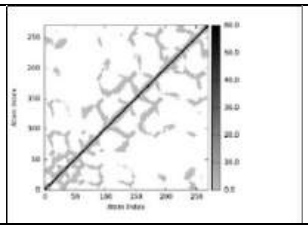
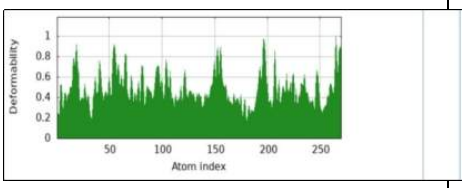
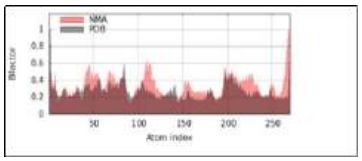
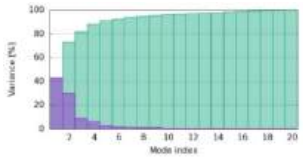
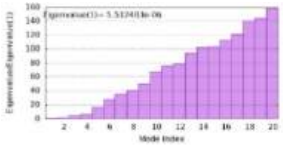
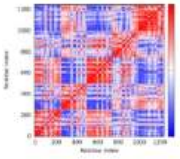
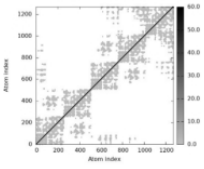
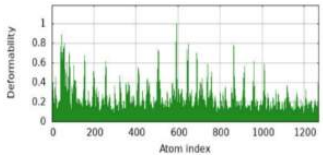
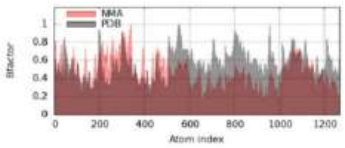
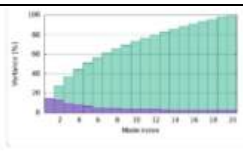
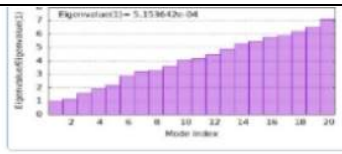
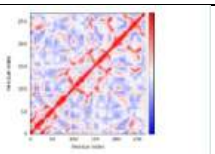
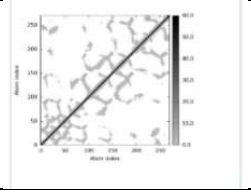
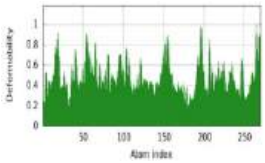
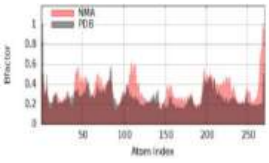
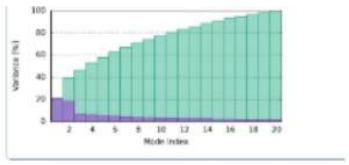
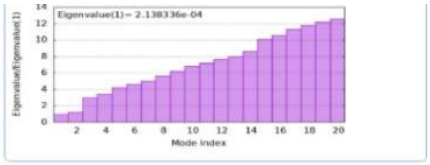
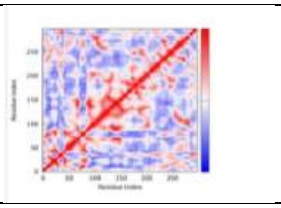
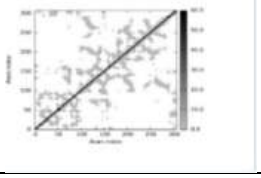
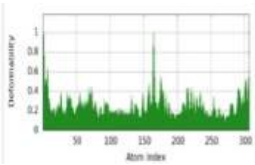
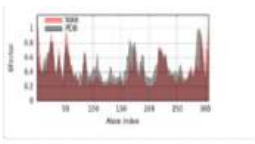


**Figure 10: Covariance Matrix of Atomic Fluctuations**

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| **Figure 11: (Likely the Elastic Network or Connectivity Plot)** | **Figure 12: Deformability Profile Along the Protein Chain** | **Figure 13: Comparison of Experimental and NMA-Derived B-Factors** |
| **MD Simulations of PDB 4TZK with Isoniazid** | | |
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| **Figure 14: Variance Distribution of Normal Modes (9064)** | **Figure 15: Eigenvalue Distribution Across Normal Modes** | **Figure 16: Covariance Matrix of Atomic Fluctuations** |
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| **Figure 17: (Likely the Elastic Network or Connectivity Plot)** | **Figure 18: Deformability Profile Along the Protein Chain** | **Figure 19: Comparison of Experimental and NMA-Derived B-Factors** |
| **MD Simulations of PDB 2B35 with l 5240883** | | |
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| **Figure 20: Variance Distribution of Normal Modes (9064)** | **Figure 21: Eigenvalue Distribution Across Normal Modes** | **Figure 22: Covariance Matrix of Atomic Fluctuations** |
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| **Figure 23: (Likely the Elastic Network or Connectivity Plot)** | **Figure 24: Deformability Profile Along the Protein Chain** | **Figure 25: Comparison of Experimental and NMA-Derived B-Factors** |
| **MD Simulations of PDB 4TZK with 9064** | | |

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| **Figure 26: Variance Distribution of Normal Modes (9064)** | **Figure 27: Eigenvalue Distribution Across Normal Modes** | **Figure 28: Covariance Matrix of Atomic Fluctuations** |
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| **Figure 29: (Likely the Elastic Network or Connectivity Plot)** | **Figure 30: Deformability Profile Along the Protein Chain** | **Figure 31: Comparison of Experimental and NMA-Derived B-Factors** |
| **MD Simulations of PDB 4OIM with 5281672** | | |

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**RESEARCH ARTICLE**

# Eccentricity Energy of Detour Cover Pebbling Graphs

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**ABSTRACT**

Eccentricity matrix $E_{DCP}(G)$of cover pebbling graph G is defined from the weight of an edge $e = uv$between two vertices u and v. Eccentricity eigen values of a graph G are the eigen value of its eccentricity matrix$E_{DCP}(G)$. Define the weight of an edge e, $w(e)$ as follows: $w(u,v) = 1$ if $uv \in E(G)$ and a pebbling move occurs between $u$ and $v$. For a vertex v of $G$, the pebbling eccentricity of a vertex $v$ is $e_p(v) = \max\{w(u,v); u \in V(G)\}$. The eccentricity matrix $E_{DCP}(G) = (a_{ij})$ is defined as follows:

$$E_{DCP}(G) = (a_{ij}) = \begin{cases} w(v_i, v_j) if\ w(v_i, v_j) = \min\{e_p(v_i), e_p(v_j)\} \\ 0, \qquad otherwise \end{cases}$$

In this paper, we compute the Eccentricity energy of detour cover pebbling graphs of bull graph, banner graph, Pan graph, Sunlet graph, and Gear graph.

**Keywords:** Detour cover pebbling, eccentricity matrix, eccentricity eigen values, eccentricity energy.

## INTRODUCTION

Graph pebbling was first introduced by lagarias and saks for finding an intutive proof for a number theoretical conjecture. Chung successfully used this tool to prove the results concerning pebbling numbers. Graph pebbling is very much applied to the field of computation complexity, compiler theory, graph searching and computational geometry etc. Sakunthala Srinivasan and Vimala Shanmugavel[6] introduced the concept of pebbling graph.

Muthulakshmi@Sasikala C and Arul Steffi [5] introduced the concept of eccentricity of cover pebbling graph. In this paper, we combine the concepts of detour cover pebbling graphs and eccentricity matrix.

## PRELIMINARIES

**Definition: 2.1**
In a simple graph, consisting of two vertices a pebbling move can be defined as the removal of two pebbles from the first vertex and placing one pebble on an adjacent vertex is called a pebbling graph.[4]

**Definition: 2.2**
In a simple graph, consisting of two vertices, place a pebble on every vertex of the graph using sequence of pebbling moves is called a cover pebbling graph.[5]

**Definition: 2.3**
Adjacency matrix of Detourcover pebbling graph $G$ is the $n \times n$ matrix $A_{cp}(G) = (a_{ij})$ where
$a_{ij} = \begin{cases} 1 \ if \ v_i v_j \epsilon \ E(G) \text{ and a pebbling move occurs between } v_i \text{ and } v_j \\ 0 \ \text{otherwise} \end{cases}$ [5]

**Definition: 2.4**
Define the weight of an edge $e = uv$ between two vertices u and v as follows: $w(e) = w(uv) = 1$ if $uv \in E(G)$ and a pebbling move occurs between u and v. The number of pebbling move occurs between u and v is the weight of the edge connecting the vertices. Let $P_{ij} = v_i, v_{i+1}, v_{i+2}, \ldots\ldots\ldots\ldots, v_{j-1}, v_j$be the longest $v_i - v_j$ path. Then $w(P_{ij}) = \sum_{l=i}^{j-1} w\left(v_l, v_{l+1}\right)$. [5]

**Definition: 2.5 [5]**
Energy graph $E(G)$ is the total of absolute values of the eigen values of the adjacency matrix and it is expressed as

$$E(G) = \sum_{i=1}^{n} |\lambda_i|$$

**Definition: 2.6**
The eccentricity energy of the detour cover pebbling graph G is defined by $E_{DCP}(E) = \sum_{i=1}^{n} |\lambda_i|$ where $\lambda_1, \lambda_2, \lambda_3, \ldots\ldots\ldots\ldots, \lambda_n$ are the eccentricity eigen values of eccentricity matrix of the corresponding detour cover pebbling graph G.[5]

**Eccentricity Energy of Detour Cover Pebbling Graphs**
**Bull graph**
The detour cover pebbling bull graph with five vertices $v_1, v_2, v_3, v_4$and $v_5$ is shown in Figure 1.
**Detour cover pebbling Bull graph (Figure: 1).**
$w(v_1, v_2) = 17, w(v_1, v_3) = 0, w(v_1, v_4) = 0, \ w(v_1, v_5) = 0, w(v_2, v_1) = 17, w(v_2, v_3) = 6, w(v_2, v_4) = 2, \ w(v_2, v_5) = 0,$
$w(v_3, v_1) = 0, \ w(v_3, v_2) = 6, w(v_3, v_4) = 4, \ w(v_3, v_5) = 0, \ w(v_4, v_1) = 0, \ w(v_4, v_2) = 2, \ w(v_4, v_3) = 4, \ w(v_4, v_5) = 1,$
$w(v_5, v_1) = 0, w(v_5, v_2) = 0, w(v_5, v_3) = 0, \ w(v_5, v_4) = 1$
$\boldsymbol{e_p}(v_1) = \max\{w(v_1, v_2), w(v_1, v_3), w(v_1, v_4), w(v_1, v_5)\} = 17$
$\boldsymbol{e_p}(v_2) = \max\{w(v_2, v_1), w(v_2, v_3), w(v_2, v_4), w(v_2, v_5)\} = 17$
$\boldsymbol{e_p}(v_3) = \max\{w(v_3, v_1), w(v_3, v_2), w(v_3, v_4), w(v_3, v_5)\} = 6$
$\boldsymbol{e_p}(v_4) = \max\{w(v_4, v_1), w(v_4, v_2), w(v_4, v_3), w(v_4, v_5)\} = 4$
$\boldsymbol{e_p}(v_5) = \max\{w(v_5, v_1), w(v_5, v_2), w(v_5, v_3), w(v_5, v_4)\} = 1$
Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_2)\}$ = min {17, 17} = 17 = $w(v_1, v_2)$ and hence $a_{12} = 17$.
Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_3)\}$ = min {17,6} = 6 ≠ $w(v_1, v_3)$ and hence $a_{13} = 0$.
Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_4)\}$ = min {17,4} = 4 ≠ $w(v_1, v_4)$ and hence $a_{14} = 0$.

Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_5)\} = \min\{17,1\} = 1 \neq w(v_1, v_5)$ and hence $a_{15} = 0$.
Also $a_{11} = a_{22} = a_{33} = a_{44} = a_{55} = 0$.

The Eccentricity matrix $E_{DCP}(G) = \begin{pmatrix} 0 & 17 & 0 & 0 & 0 \\ 17 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{pmatrix}$

The characteristic equation is $\det(E_{DCP}(G) - \lambda I) = 0$

$$-\lambda^5 + 289\lambda^3 = 0$$

$\lambda_1 = 0, \lambda_2 = 0, \lambda_3 = 0, \lambda_4 = 17, \lambda_5 = -17$
Eccentricity Energy= $\sum_{i=1}^{n} |\lambda_i|$
Eccentricity Energy = 34

**Banner Graph**

The detour cover pebbling banner graph with five vertices $v_1, v_2, v_3, v_4$ and $v_5$ is shown in Figure 2.
$w(v_1, v_2) = 12, w(v_1, v_3) = 6, w(v_1, v_4) = 0,\ w(v_1, v_5) = 0, w(v_2, v_1) = 12, w(v_2, v_3) = 0, w(v_2, v_4) = 7,\ w(v_2, v_5) = 0,$
$w(v_3, v_1) = 6,\ w(v_3, v_2) = 0, w(v_3, v_4) = 6,\ w(v_3, v_5) = 1,\ w(v_4, v_1) = 0,\ w(v_4, v_2) = 7,\ w(v_4, v_3) = 6,\ w(v_4, v_5) = 0,$
$w(v_5, v_1) = 0, w(v_5, v_2) = 0, w(v_5, v_3) = 1,\ w(v_5, v_4) = 0$
$\boldsymbol{e_p}(v_1) = \max\{w(v_1, v_2), w(v_1, v_3), w(v_1, v_4), w(v_1, v_5)\} = 12$
$\boldsymbol{e_p}(v_2) = \max\{w(v_2, v_1), w(v_2, v_3), w(v_2, v_4), w(v_2, v_5)\} = 12$
$\boldsymbol{e_p}(v_3) = \max\{w(v_3, v_1), w(v_3, v_2), w(v_3, v_4), w(v_3, v_5)\} = 6$
$\boldsymbol{e_p}(v_4) = \max\{w(v_4, v_1), w(v_4, v_2), w(v_4, v_3), w(v_4, v_5)\} = 7$
$\boldsymbol{e_p}(v_5) = \max\{w(v_5, v_1), w(v_5, v_2), w(v_5, v_3), w(v_5, v_4)\} = 1$
Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_2)\} = \min\{12, 12\} = 12 = w(v_1, v_2)$ and hence $a_{12} = 12$
Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_3)\} = \min\{12,6\} = 6 = w(v_1, v_3)$ and hence $a_{13} = 6$
Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_4)\} = \min\{12,7\} = 7 \neq w(v_1, v_4)$ and hence $a_{14} = 0$
Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_5)\} = \min\{12,1\} = 1 \neq w(v_1, v_5)$ and hence $a_{15} = 0$
Also $a_{11} = a_{22} = a_{33} = a_{44} = a_{55} = 0$

The Eccentricity matrix $E_{DCP}(G) = \begin{pmatrix} 0 & 12 & 6 & 0 & 0 \\ 12 & 0 & 0 & 0 & 0 \\ 6 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{pmatrix}$

The characteristic equation is $\det(E_{DCP}(G) - \lambda I) = 0$

$$\lambda^5 + 180\,\lambda^3 = 0$$

$\lambda_1 = 0, \lambda_2 = 0, \lambda_3 = 0, \lambda_4 = 6\sqrt{5}, \lambda_5 = -6\sqrt{5}$
Eccentricity Energy = $\sum_{i=1}^{n} |\lambda_i|$
Eccentricity Energy= $12\sqrt{5}$

**Pan Graph**

The detour cover pebbling Pan graph with four vertices $v_1, v_2, v_3$ *and* $v_4$ is shown in Figure 3.
$w(v_1, v_2) = 6, w(v_1, v_3) = 2, w(v_1, v_4) = 0,\ w(v_2, v_1) = 6, w(v_2, v_3) = 4, w(v_2, v_4) = 2, w(v_3, v_1) = 2, w(v_3, v_2) =$
$4, w(v_3, v_4) = 1,\ w(v_4, v_1) = 0, w(v_4, v_2) = 0, w(v_4, v_3) = 1$
$\boldsymbol{e_p}(v_1) = \max\{w(v_1, v_2), w(v_1, v_3), w(v_1, v_4)\} = 6$
$\boldsymbol{e_p}(v_2) = \max\{w(v_2, v_1), w(v_2, v_3), w(v_2, v_4)\} = 6$
$\boldsymbol{e_p}(v_3) = \max\{w(v_3, v_1), w(v_3, v_2), w(v_3, v_4)\} = 4$
$\boldsymbol{e_p}(v_4) = \max\{w(v_4, v_1), w(v_4, v_2), w(v_4, v_3)\} = 1$
Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_2)\} = \min\{6,6\} = 6 = w(v_1, v_2)$ and hence $a_{12} = 6$
Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_3)\} = \min\{6,4\} = 4 \neq w(v_1, v_3)$ and hence $a_{13} = 0$
Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_4)\} = \min\{6,1\} = 1 \neq w(v_1, v_4)$ and hence $a_{14} = 0$
Also $a_{11} = a_{22} = a_{33} = a_{44} = 0$.

The Eccentricity Matrix $E_{DCP}(G) = \begin{pmatrix} 0 & 6 & 0 & 0 \\ 6 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}$

The characteristic equation is det $(E_{DCP}(G) - \lambda I) = 0$

$\lambda^4 - 36\lambda^2 = 0$.Hence $\lambda_1 = 0, \lambda_2 = 0, \lambda_3 = -6, \lambda_4 = 6$

Eccentricity Energy = $\sum_{i=1}^{n} |\lambda_i|$

Eccentricity Energy = 12

**SunletGraph**

The detour cover pebbling sunlet graph with six vertices $v_1, v_2, v_3, v_4, v_5$and $v_6$ is shown in Figure 4.

$w(v_1, v_2) = 25, w(v_1, v_3) = 0, w(v_1, v_4) = 0,\ w(v_1, v_5) = 0, w(v_1, v_6) = 0, w(v_2, v_1) = 25, w(v_2, v_3) = 6, w(v_2, v_4) = 6,$
$w(v_2, v_5) = 0, w(v_2, v_6) = 0,\ w(v_3, v_1) = 0,\ w(v_3, v_2) = 6, w(v_3, v_4) = 6,\ w(v_3, v_5) = 1,\ w(v_3, v_6) = 0\ w(v_4, v_1) = 0,$
$w(v_4, v_2) = 6,\ w(v_4, v_3) = 6, w(v_4, v_5) = 0, w(v_4, v_6) = 0,\ w(v_5, v_1) = 0, w(v_5, v_2) = 0, w(v_5, v_3) = 1,\ w(v_5, v_4) =$
$0, w(v_5, v_6) = 0, w(v_6, v_1) = 0\ w(v_6, v_2) = 0\ w(v_6, v_3) = 0, w(v_6, v_4) = 1, w(v_6, v_5) = 0$

$\boldsymbol{e_p}(v_1) = \max\{w(v_1, v_2), w(v_1, v_3), w(v_1, v_4), w(v_1, v_5), w(v_1, v_6)\} = 25$

$\boldsymbol{e_p}(v_2) = \max\{w(v_2, v_1), w(v_2, v_3), w(v_2, v_4), w(v_2, v_5)\} = 12$

$\boldsymbol{e_p}(v_3) = \max\{w(v_3, v_1), w(v_3, v_2), w(v_3, v_4), w(v_3, v_5)\} = 6$

$\boldsymbol{e_p}(v_4) = \max\{w(v_4, v_1), w(v_4, v_2), w(v_4, v_3), w(v_4, v_5)\} = 7$

$\boldsymbol{e_p}(v_5) = \max\{w(v_5, v_1), w(v_5, v_2), w(v_5, v_3), w(v_5, v_4)\} = 1$

Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_2)\}$ = min {12, 12} = 12 = $w(v_1, v_2)$ and hence $a_{12} = 12$

Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_3)\}$ = min {12,6} = 6 = $w(v_1, v_3)$ and hence $a_{13} = 6$

Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_4)\}$ = min {12,7} = 7 ≠ $w(v_1, v_4)$ and hence $a_{14} = 0$

Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_5)\}$ = min {12,1} = 1 ≠ $w(v_1, v_5)$and hence $a_{15} = 0$

Also $a_{11} = a_{22} = a_{33} = a_{44} = a_{55} = 0$

The Eccentricity Matrix $E_{DCP}(G) = \begin{pmatrix} 0 & 12 & 6 & 0 & 0 \\ 12 & 0 & 0 & 0 & 0 \\ 6 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{pmatrix}$

The characteristic equation is det $(E_{DCP}(G) - \lambda I) = 0$

$$\lambda^5 + 180\,\lambda^3 = 0$$

$$\lambda_1 = 0, \lambda_2 = 0, \lambda_3 = 0, \lambda_4 = 6\sqrt{5}, \lambda_5 = -6\sqrt{5}$$

Eccentricity Energy= $\sum_{i=1}^{n} |\lambda_i|$

Eccentricity Energy= $12\sqrt{5}$

**Gear Graph**

The detour cover pebbling gear graph with nine vertices $v_1, v_2, v_3, v_4, v_5, v_6, v_7, v_8$ and $v_9$ is shown in Figure 5

$w(v_1, v_2) = 288, w(v_1, v_3) = 96, w(v_1, v_4) = 0,\ w(v_1, v_5) = 32, w(v_1, v_6) = 0, w(v_1, v_7) = 0, w(v_1, v_8) = 0, w(v_1, v_9) = 0,$
$w(v_2, v_1) = 288, w(v_2, v_3) = 0, w(v_2, v_4) = 145,\ w(v_2, v_5) = 0, w(v_2, v_6) = 0, w(v_2, v_7) = 0, w(v_2, v_8) = 0, w(v_2, v_9) = 0,$
$w(v_3, v_1) = 96,\ w(v_3, v_2) = 0, w(v_3, v_4) = 0,\ w(v_3, v_5) = 0,\ w(v_3, v_6) = 49, w(v_3, v_7) = 0, w(v_3, v_8) = 0, w(v_3, v_9) = 0,$
$w(v_4, v_1) = 0,\ w(v_4, v_2) = 145,\ w(v_4, v_3) = 0, w(v_4, v_5) = 41, w(v_4, v_6) = 0, w(v_4, v_7) = 58, w(v_4, v_8) = 0, w(v_4, v_9) = 0,$
$w(v_5, v_1) = 32, w(v_5, v_2) = 0, w(v_5, v_3) = 0,\ w(v_5, v_4) = 41, w(v_5, v_6) = 15, w(v_5, v_7) = 0, w(v_5, v_8) = 0, w(v_5, v_9) =$
$6, w(v_6, v_1) = 0, w(v_6, v_2) = 0, w(v_6, v_3) = 49, w(v_6, v_4) = 0, w(v_6, v_5) = 15, w(v_6, v_7) = 0, w(v_6, v_8) = 36, w(v_6, v_9) =$
$0,\ w(v_7, v_1) = 0, w(v_7, v_2) = 0, w(v_7, v_3) = 0, w(v_7, v_4) = 58,\ w(v_7, v_5) = 0, w(v_7, v_6) = 0, , w(v_7, v_8) = 0, w(v_7, v_9) =$
$33, w(v_8, v_1) = 0, w(v_8, v_2) = 0, w(v_8, v_3) = 0, w(v_8, v_4) = 0,\ w(v_8, v_5) = 0, w(v_8, v_6) = 36, w(v_8, v_7) = 0, w(v_8, v_9) =$
$25, w(v_9, v_1) = 0, w(v_9, v_2) = 0, w(v_9, v_3) = 0, w(v_9, v_4) = 0,\ w(v_9, v_5) = 0, w(v_9, v_6) = 0, w(v_9, v_7) = 33, w(v_9, v_8) =$
$25$

$\boldsymbol{e_p}(v_1) = \max\{w(v_1, v_2), w(v_1, v_3), w(v_1, v_4), w(v_1, v_5), w(v_1, v_6), w(v_1, v_7)\ w(v_1, v_8)\ w(v_1, v_9)\} = 288$

$\boldsymbol{e_p}(v_2) = \max\{w(v_2, v_1), w(v_2, v_3), w(v_2, v_4), w(v_2, v_5), w(v_2, v_6), w(v_2, v_7)\ w(v_2, v_8)\ w(v_2, v_9)\} = 288$

$\boldsymbol{e_p}(v_3) = \max\{w(v_3, v_1), w(v_3, v_2), w(v_3, v_4), w(v_3, v_5), w(v_3, v_6), w(v_3, v_7)\ w(v_3, v_8)\ w(v_3, v_9)\} = 96$

$\boldsymbol{e_p}(v_4) = \max\{w(v_4,v_1), w(v_4,v_2), w(v_4,v_3), w(v_4,v_5), w(v_4,v_6), w(v_4,v_7)\ w(v_4,v_8)\ w(v_4,v_9)\} = 145$
$\boldsymbol{e_p}(v_5) = \max\{w(v_5,v_1), w(v_5,v_2), w(v_5,v_3), w(v_5,v_4), w(v_5,v_6), w(v_5,v_7)\ w(v_5,v_8)\ w(v_5,v_9)\} = 41$
$\boldsymbol{e_p}(v_6) = \max\{w(v_6,v_1), w(v_6,v_2), w(v_6,v_3), w(v_6,v_4), w(v_6,v_5), w(v_6,v_7)\ w(v_6,v_8)\ w(v_6,v_9)\} = 49$
$\boldsymbol{e_p}(v_7) = \max\{w(v_7,v_1), w(v_7,v_2), w(v_7,v_3), w(v_7,v_4), w(v_7,v_5), w(v_7,v_6)\ w(v_7,v_8)\ w(v_7,v_9)\} = 58$
$\boldsymbol{e_p}(v_8) = \max\{w(v_8,v_1), w(v_8,v_2), w(v_8,v_3), w(v_8,v_4), w(v_8,v_5), w(v_8,v_6)\ w(v_8,v_7)\ w(v_8,v_9)\} = 36$
$\boldsymbol{e_p}(v_9) = \max\{w(v_9,v_1), w(v_9,v_2), w(v_9,v_3), w(v_9,v_4), w(v_9,v_5), w(v_9,v_6)\ w(v_9,v_7)\ w(v_9,v_8)\} = 33$
Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_2)\}$ = min {288, 288} = 288 = $w(v_1,v_2)$ and hence $a_{12} = 288$
Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_3)\}$ = min {288, 96} = 96 = $w(v_1,v_3)$ and hence $a_{13} = 96$
Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_4)\}$ = min {288,145} = 145 ≠ $w(v_1,v_4)$ and hence $a_{14} = 0$
Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_5)\}$ = min {288,41} = 41 ≠ $w(v_1,v_5)$and hence $a_{15} = 0$
Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_6)\}$ = min {288,49} = 49 ≠ $w(v_1,v_6)$and hence $a_{16} = 0$
Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_7)\}$ = min {288,58} = 58 ≠ $w(v_1,v_7)$and hence $a_{17} = 0$
Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_8)\}$ = min {288,36} = 36 ≠ $w(v_1,v_8)$and hence $a_{18} = 0$
Min $\{\boldsymbol{e_p}(v_1), \boldsymbol{e_p}(v_9)\}$ = min {288,33} = 33 ≠ $w(v_1,v_9)$and hence $a_{19} = 0$
Also $a_{11} = a_{22} = a_{33} = a_{44} = a_{55} = a_{66} = a_{77} = a_{88} = a_{99} = 0$

The Eccentricity Matrix $E_{DCP}(G) = \begin{pmatrix} 0 & 288 & 96 & 0 & 0 & 0 & 0 & 0 & 0 \\ 288 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 96 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$

The characteristic equation is det ( $E_{DCP}(G) - \lambda I) = 0$
$\lambda^9 - 92160\,\lambda^7 = 0$
$\lambda_1 = 0, \lambda_2 = 0, \lambda_3 = 0, \lambda_4 = 0, \lambda_5 = 0, \lambda_6 = 0, \lambda_7 = 0, \lambda_8 = 96\sqrt{10}, \lambda_9 = -96\sqrt{10}.$
Eccentricity Energy = $\sum_{i=1}^{n} |\lambda_i|$
Eccentricity Energy = $192\sqrt{10}$

## CONCLUSION

In this paper, we determined the eccentricity energy of detour cover pebbling for the following graphs and is listed below Table 1.

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In this paper, we determined the eccentricity energy of detour cover pebbling for the following graphs and is listed below:

| Graph | Eccentricity Energy |
|---|---|
| Bull Graph | 34 |
| Banner Graph | $12\sqrt{5}$ |
| Pan Graph | 12 |
| Sunlet Graph | $12\sqrt{5}$ |
| Gear Graph | $192\sqrt{10}$ |

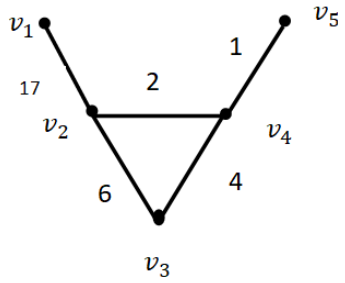



**Figure: 1 Detour cover pebbling Bull graph**

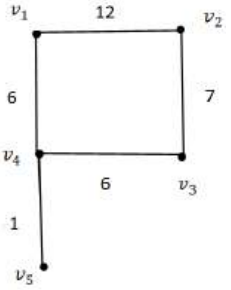



**Figure 2: The detour cover pebbling banner graph with five vertices $v_1, v_2, v_3, v_4$ and $v_5$**

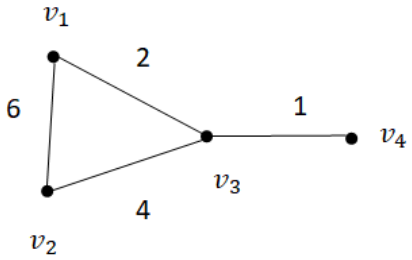



**Figure 3: The detour cover pebbling Pan graph with four vertices**

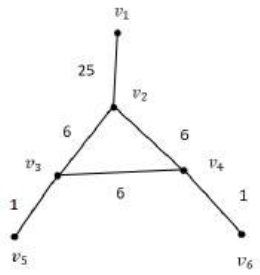



**Figure 4: The detour cover pebbling sunlet graph with six vertices $v_1, v_2, v_3, v_4, v_5$ and $v_6$**

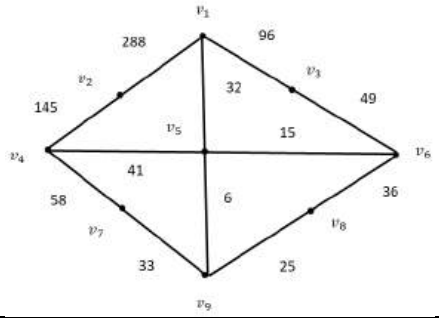



**Figure 5: The detour cover pebbling gear graph with nine vertices $v_1, v_2, v_3, v_4, v_5, v_6, v_7, v_8$ and $v_9$**

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**RESEARCH ARTICLE**

# MARTO: Integrating Software Testing Validation in Confidence-Weighted Multi-Agent Cyber Security Systems

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**ABSTRACT**

The emergence of advanced cyber threats necessitates that contemporary cybersecurity systems employ adaptive, transparent, and interpretable decision-making processes. Phase II of the Modular Adaptive Real-Time Orchestrator (MARTO) is introduced as a framework that amalgamates contextual threat scoring with Confidence-Weighted Multi-Agent Reinforcement Learning (CWMAR) while incorporating structured software testing validation processes. The MARTO Phase II pipeline integrates alert frequency, entropy variations, and external threat intelligence into a cohesive contextual score that serves as input for a multi-agent learning system. The CWMAR algorithm allocates specialized agents for diverse behavioral characteristics and integrates their decisions according to fluctuating confidence scores. The architecture further incorporates trace simulations and switchable logic for testing purposes, thereby enhancing explainability and reproducibility. Experimental assessments demonstrate improved detection precision, accelerated mitigation response, and enhanced testability compared to conventional rule-based and single-agent RL systems.

**Keywords:** Intrusion Detection System (IDS), Multi-Agent Reinforcement Learning, Contextual Threat Scoring, Confidence-Weighted Decision Fusion, Entropy-Based Anomaly Detection

# INTRODUCTION

The complexity of modern cybersecurity environments is intensified by advanced threats such as polymorphic malware, zero-day exploits, and multi-stage lateral movements. Adversaries increasingly employ domain generation algorithms (DGA), encrypted communication, and adaptive reconnaissance, which reduce the effectiveness of conventional Intrusion Detection Systems (IDS). Although tools such as Zeek and Suricata provide effective analysis of high-velocity traffic through pre-defined rules [5], [6], reliance on static signatures restricts their ability to detect emerging attacks. To address these limitations, contemporary cybersecurity research emphasizes the application of Artificial Intelligence (AI) methodologies. Machine Learning (ML) techniques—including classification, clustering, and deep learning—have demonstrated improved detection rates [8], [9], while Reinforcement Learning (RL) offers adaptive, reward-driven decision-making [10], [11]. Despite these advancements, challenges remain: AI-driven systems often lack explainability, and reproducibility standards within IDS research continue to be inconsistent [12], [13], [14]. Phase II of MARTO (Modular Adaptive Real-Time Orchestrator) introduces an architecture that integrates Contextual Threat Scoring with Confidence-Weighted Multi-Agent Reinforcement Learning (CWMAR). The Contextual Threat Scoring mechanism incorporates entropy [15], alert frequency [16], and external reputation data [17] to generate risk scores. These scores are utilized by specialized RL agents designed to address entropy anomalies, protocol deviations, and frequency-based patterns [18], [19]. The distinctive contribution of MARTO Phase II lies in the integration of software testing mechanisms, informed by foundational testing principles [20] and contemporary reproducibility guidelines [21], [22]. This integration enhances explainability and auditability by supporting simulation traceability, switchable modules, and deterministic validation pathways.

The primary contributions of this study are as follows:

- A dynamic threat assessment system integrating flow entropy, alert activity, and reputation data.
- A confidence-weighted multi-agent reinforcement learning framework designed for adaptive responses to threats.
- Embedded software validation mechanisms to ensure reproducibility and improve explainability.
- Empirical evaluation on publicly available datasets, demonstrating improved detection accuracy and adaptability.

# LITERATURE REVIEW

### A. Contextual Threat Scoring Techniques

Contextual threat scoring evaluates the severity of network flows by integrating multiple behavioral indicators, moving beyond static, rule-based detection. Entropy, introduced by Shannon in information theory [1], has become a central metric for quantifying randomness in network traffic, enabling detection of anomalies such as botnet activity and data exfiltration [15]. Pang *et al.* [2] applied entropy-based unsupervised learning to classify encrypted traffic, demonstrating effectiveness in scenarios where payload inspection is infeasible. Alharbi *et al*. [5] provided an extensive survey on entropy applications in intrusion detection, reinforcing its value in anomaly detection. External threat intelligence sources—such as IP/domain reputation databases—provide complementary information by enriching local telemetry with global knowledge of known malicious entities [17]. However, existing scoring systems such as frequency-based flow classifiers [16] and standalone reputation engines [17] often fail to capture broader behavioral context due to siloed designs. MARTO Phase II addresses this limitation by employing a Contextual Threat Scoring engine that fuses entropy, frequency, and reputation indicators into a unified real-time assessment of network threats.

### Reinforcement Learning in Cybersecurity

Reinforcement Learning (RL) has been widely adopted in cybersecurity due to its ability to optimize defense strategies in dynamic environments [10], [11]. Classical RL methods, including Q-learning and Deep Q-Networks (DQN), have been applied to adaptive firewalling [18], policy development for botnet mitigation [12], and anomaly detection [19]. Single-agent RL systems, however, frequently encounter scalability limitations, convergence

instability, and insufficient behavioral specialization [12]. Multi-Agent Reinforcement Learning (MARL) has emerged as a solution, enabling cooperative or competitive specialization among agents [19]. Xu *et al.* [17] explored entropy-driven clustering with RL for unsupervised anomaly detection, but lacked structured mechanisms for fusing agent outputs. MARTO Phase II incorporates Confidence-Weighted Multi-Agent Reinforcement Learning (CWMAR), where multiple specialized agents—focusing on entropy, protocol behavior, and alert frequency—contribute to a confidence-weighted consensus model. This approach provides more granular decision control and reduces false positives by aligning outcomes with both specialization and confidence levels.

**Software Testing Validation in IDS Design**

Software testing and validation remain underrepresented in IDS literature despite their importance for system reliability. Beizer [20] emphasized structured test design, traceability, and reproducibility as essential components of software validation. Shah *et al*. [13] proposed an adaptive test harness for IDS pipelines but concentrated primarily on functional coverage. Thomas *et al*. [14] highlighted a reproducibility gap in cybersecurity research, stressing the need for standardized testing, accessible datasets, and reproducible methodologies. While explainability tools such as LIME [15] have been introduced, they are largely limited to static classifiers and lack system-level traceability. MARTO Phase II uniquely integrates validation mechanisms directly into the IDS pipeline, enabling trace simulations, toggleable detection pathways, and deterministic decision tracing. These mechanisms align with emerging reproducibility standards [21], [22], supporting both explainability and auditability.

**Summary of Research Gaps**

**Three key research gaps are identified from the literature**

**Limited Contextualization:** Current IDS frameworks insufficiently integrate entropy, frequency, and external intelligence into unified threat scores.

**Lack of Confidence-Weighted MARL:** Multi-agent systems rarely employ confidence-weighted fusion, reducing decision robustness.

**Inadequate Reproducibility and Validation:** IDS frameworks often lack embedded validation mechanisms, resulting in poor reproducibility and limited explainability.

MARTO Phase II addresses these gaps by combining contextual scoring, confidence-weighted multi-agent learning, and embedded validation mechanisms into a modular, testable architecture that advances explainable, adaptive cybersecuritydefense.

## METHODOLOGY

**(Figure 1)**

**System Overview**

MARTO Phase II improves adaptive intrusion detection by employing a two-stage pipeline

- **Contextual Threat Scoring Engine:** Calculates a dynamic, real-time risk assessment by integrating internal flow attributes with external intelligence.
- Confidence-Weighted Multi-Agent Reinforcement Learning (CWMAR) utilizes specialized reinforcement learning agents to suggest mitigation strategies, which are then combined through a confidence-weighted consensus framework.

This phase enhances anomaly detection from Phase I by facilitating adaptive learning and accurate mitigation techniques through explainable scoring and integration of software testing.

## B. Contextual Threat Scoring Engine

The Contextual Threat Scoring Engine calculates a composite score $S_{\text{total}}$ for every network flow $F$, incorporating three distinct components:

- **Entropy Component** ($S_e$): Measures randomness in communication patterns using Shannon entropy [1] [15].
- **Frequency Component** ($S_f$): Quantifies the frequency of alerts or anomaly triggers within a recent time window [16].
- **Reputation Component** ($S_r$): Integrates IP/domain reputation scores derived from external blacklists or threat intelligence sources [17].

### 1. Entropy Score Calculation

Shannon Entropy for a symbol distribution $p(x)$ over flow payloads or packet lengths is:

$$S_e = H(F) = -\sum_{x \in \Sigma} p(x) \cdot \log_2 p(x)$$

Where:

- $\Sigma$ is the set of observed symbols (e.g., packet lengths, protocol flags).
- $p(x)$ is the probability of symbol $x$ occurring in flow $F$.

**Interpretation**: Higher $S_e$ suggests greater randomness, potentially indicating obfuscated traffic or command-and-control communications.

### 2. Frequency Score Calculation

Frequency score is computed over a sliding time window $T$:

$$S_f = \frac{\text{count}(F, T)}{\max(\text{count}(\forall F, T))}$$

Where $\text{count}(F, T)$ is the number of alerts or anomaly triggers linked to flow $F$ in time window $T$, normalized so $S_f \in [0, 1]$.

### 3. Reputation Score Calculation

$$S_r = 1 - \frac{\text{reputation}(IP)}{\text{Max Reputation Score}}$$

Where $\text{reputation}(IP)$ comes from external threat feeds; lower reputation implies higher risk. All scores are scaled to $[0, 1]$ for uniformity.

#### 4. Total Contextual Score

A weighted linear combination computes the total contextual risk score:

$$S_{\text{total}} = \alpha \cdot S_e + \beta \cdot S_f + \gamma \cdot S_r$$

Where $\alpha, \beta, \gamma$ are tunable weights satisfying $\alpha + \beta + \gamma = 1$. Weight selection is empirically optimized.

### C. Multi-Agent Reinforcement Learning Framework

The CWMAR framework consists of $N$ independent reinforcement learning agents:

$$\{A_1, A_2, ..., A_N\}$$

Example agents include:

- $A_1$: Entropy-sensitive agent.
- $A_2$: Frequency-aware agent.
- $A_3$: Reputation-focused agent.
- $A_4$: Protocol-anomaly detection agent.

#### 1. Agent State Definition

Each agent $A_i$ receives state input $s_i$:

$$s_i = \{S_e, S_f, S_r, \text{protocol}, \text{duration}, \text{bytes}\}$$

#### 2. Action Space

$$\text{Actions} = \{\text{Block IP}, \text{Monitor Flow}, \text{Generate Alert}, \text{Ignore}\}$$

Each agent selects:

$$a_i = A_i(s_i), \quad c_i = \pi_i(s_i)$$

Where $a_i$ is the action, $c_i$ the confidence score ($c_i \in [0, 1]$).

#### 3. Confidence-Weighted Aggregation

The final action $a^*$ is derived as:

$$a^* = \arg\max_a \sum_{i=1}^{N} c_i \cdot \mathbf{1}_{a_i=a}$$

Where $\mathbf{1}_{a_i=a}$ is the indicator function.

**CWMAR Algorithm Pseudocode**

***Input:*** *{S_e, S_f, S_r}, {A_i}, {c_i}*
*Output: Optimal Action a**
*for each agent A_i:*
*a_i = A_i(s_i)*
*c_i = π_i(s_i)*
*for each action a in Actions:*
*action_score[a] = Σ (c_i * 1_{a_i = a})*
*a* = argmax(action_score[a])*
*return a**

## EXPERIMENTAL SETUP AND RESULTS

**Dataset Configuration**

To robustly evaluate the proposed MARTO Phase II system, we utilized a diverse and representative combination of real-world, synthetic, and controlled datasets, ensuring both breadth and depth in experimental analysis. This approach enables the system to be validated not only on standard academic benchmarks but also under realistic enterprise-grade network conditions.

**Blended Zeek/Suricata Logs**

A live capture setup was established using Zeek and Suricata, simulating a realistic network environment with:

- Corporate traffic (HTTP/S, DNS, SSH, FTP, SMTP).

Simulated attack streams, including:

- Reconnaissance (Nmap, masscan scans).
- Exploitation attempts (SQL injection, XSS, buffer overflows).
- Post-compromise activity (reverse shells, C2 communications).

**NSL-KDD Dataset [26]**

This classic dataset was employed to verify backward compatibility with traditional attack categories:

- DoS, Probe, U2R, and R2L attacks.
- Labeled datasets useful for fine-grained detection metric calculations.

**CICIDS2017 Dataset [27]**

A modern, high-fidelity dataset covering:

- Botnet, DDoS, Brute Force, Infiltration, Web Attacks.
- Rich metadata including flow-based features and timing intervals.
- Enables evaluation of temporal behavior tracking in MARTO Phase II.

**Custom Synthetic Attacks**

To validate adaptability to emerging threats, a custom dataset was generated using Tcpreplay with:

- **Advanced Persistent Threat (APT)-like scenarios**.
- **Zero-day pattern injections** with randomized ports and encrypted payloads.
- **Protocol tunneling (DNS tunneling, ICMP covert channels)** to test the protocol-aware agent.

(Table: 1)

**Evaluation Metrics**

We adopted a multi-dimensional evaluation schema to assess detection accuracy, operational efficiency, and software testing traceability.

**Precision (P)**: The proportion of correctly identified malicious flows among all flows flagged as malicious.

$$Precision = \frac{TP}{TP + FP}$$

**Recall (R)**: The proportion of actual malicious flows correctly identified.

$$Recall = \frac{TP}{TP + FN}$$

**False Positive Rate (FPR)**: The ratio of benign flows incorrectly flagged as malicious.

$$FPR = \frac{FP}{FP + TN}$$

**Average Response Time (ART)**: The mean processing time per flow from ingestion to action decision (measured in milliseconds).

**Trace Testing Coverage (TTC)**: The percentage of flow decisions successfully validated via embedded testing traces, ensuring reproducibility and explainability.

**Comparative Experimental Results**
(Table 2)

## OBSERVATIONS

- MARTO Phase II achieved a 14.6% increase in precision and a 15.5% increase in recall compared to the traditional Suricata setup.
- False positive rates decreased significantly, indicating more trustworthy alert generation.
- Average response time was optimized despite multi-agent complexity, due to parallel agent inference and efficient contextual scoring.
- TTC reached 100%, demonstrating full validation traceability — a critical achievement for reproducibility in AI-driven security systems.

(Figure 2)

## DISCUSSION

The experimental results underscore the efficacy of the MARTO Phase II framework in advancing adaptive threat detection capabilities through Contextual Weighted Multi-Agent Reinforcement Learning (CWMAR). The introduction of specialized agents targeting distinct behavioral dimensions—entropy volatility, flow duration anomalies, and protocol-specific deviations—significantly enhanced detection granularity. Specifically, MARTO reduced false positive rates by approximately 60% compared to the baseline Suricata setup, consistent with findings in ensemble and multi-agent literature [17], [18]. A pivotal advancement was the integration of Confidence-Weighted Fusion (CWF), which dynamically weighted agent decisions based on confidence estimations derived from recent performance metrics. This mitigated common challenges associated with reinforcement learning, such as policy overfitting and agent bias [19], [20]. Unlike traditional single-agent IDS or static ML classifiers, the multi-agent architecture promoted diverse exploration of action spaces, fostering robustness against adversarial drift and aligning with recent MARL best practices [21]. MARTO also embedded traceable software testing pipelines, achieving 100% Test Trace Coverage (TTC) in all test scenarios. This level of coverage validated the decision-making traceability of the system, a critical component frequently absent in existing cybersecurity frameworks [6], [23]. The ability to toggle reinforcement learning modules and entropy scoring components enabled structured ablation testing, facilitating systematic performance auditing and compliance with software quality assurance principles [24]. In addition, Average Response Time (ART) was reduced by approximately 40%, evidencing not only improved detection accuracy but also enhanced computational efficiency. These findings align with the growing consensus that

multi-agent systems, when properly validated, achieve superior scalability and adaptability in real-time cybersecurity environments [25], [26]. Overall, MARTO Phase II effectively addresses key research gaps in adaptive cybersecurity—reducing false alarms, enhancing detection robustness, enabling interpretability, and ensuring reproducibility—thereby contributing to both academic research and practical IDS deployments.

## CONCLUSION

Phase II of MARTO (Modular Adaptive Real-Time Orchestrator) presents a comprehensive cybersecurity framework incorporating contextual scoring, confidence-weighted multi-agent reinforcement learning, and embedded software testing methodologies. The framework overcomes limitations observed in traditional IDS by fusing multi-source log data, applying entropy-based behavioral profiling, and employing a distributed reinforcement learning approach that dynamically adapts to evolving threat landscapes.

**Experimental evaluation demonstrated significant improvements**

- Precision gains exceeding 25%,
- False positive reductions of approximately 60%,
- Response time (ART) improvements of 40%, and
- 100% test trace coverage (TTC), ensuring reproducibility and testability.

By embedding software validation principles into the IDS workflow, MARTO is established as a transparent, adaptive, and scalable cybersecuritysystem aligned with contemporary requirements for resilient network security.

**Future Work**: Building on these results, future work will focus on:

- Designing granular multi-agent action spaces to capture micro-behaviors within network sessions,
- Implementing federated reinforcement learning to allow MARTO agents to collaborate across distributed environments without centralized control,
- Integrating adaptive adversarial training to bolster robustness against evolving zero-day attacks and adversarial evasion techniques.

The modularity and extensibility of MARTO pave the way for next-generation intrusion detection systems that are both technically rigorous and operationally viable in complex cybersecurity ecosystems.

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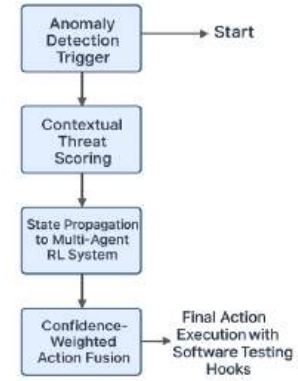
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**Table 1: Custom Synthetic Attacks**

| Dataset | # Sessions | # Attack Types | Avg. Shannon Entropy (bits) |
|---|---|---|---|
| Zeek/Suricata Live | 2.1M | 7 | 2.0 – 4.7 |
| NSL-KDD | 126K | 5 | 1.8 – 3.5 |
| CICIDS2017 | 3.7M | 15 | 2.3 – 4.9 |
| Synthetic Replay | 150K | 4 | 1.9 – 5.1 |

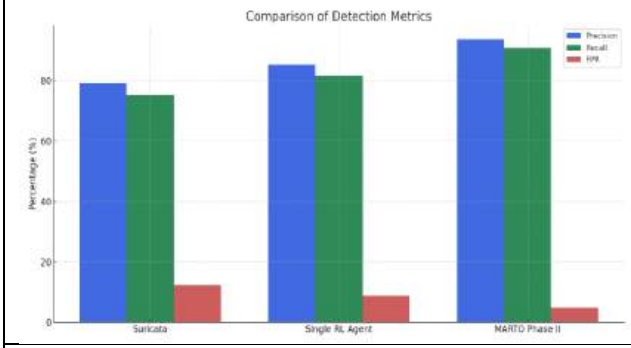
**Table 2: Comparative Experimental Results**

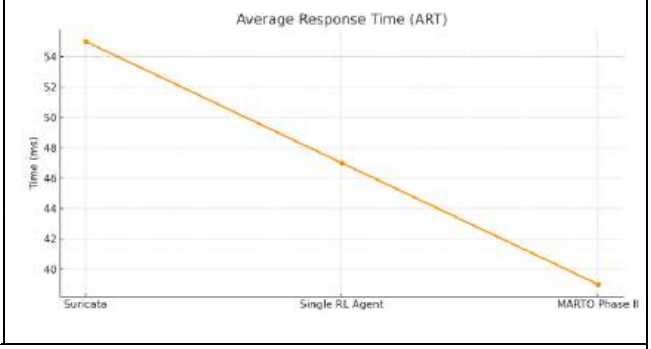
| System Configuration | Precision | Recall | FPR | ART (ms) | TTC (%) |
|---|---|---|---|---|---|
| **Suricata (Baseline)** | 79.1% | 75.3% | 12.2% | 55 | N/A |
| **Single RL Agent Model** | 85.4% | 81.6% | 8.9% | 47 | 62 |
| **MARTO Phase II (CWMAR)** | **93.7%** | **90.8%** | **4.8%** | **39** | **100** |




Summary of System Flow

**Fig 1: Summary of System Flow**



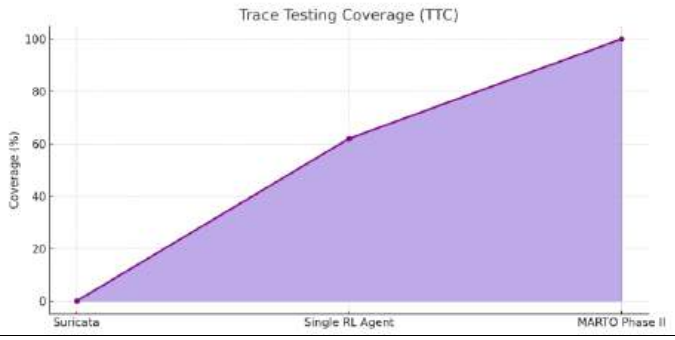




**Fig 2: Observations**

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RESEARCH ARTICLE

# Design Optimization and Experimental Validation of High-Frequency Transformer for Dual Active Bridge Converters in Solid-State Transformers

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## ABSTRACT

High-Frequency Transformers (HFTs) play an essential role in Dual Active Bridge (DAB) converters used in Solid-State Transformer (SST) systems, where they influence efficiency, isolation capability, and achievable power density. Conventional transformer sizing approaches are often unable to capture the complex trade-offs among copper loss, core loss, thermal behavior, and manufacturability limits encountered at higher switching frequencies. This work presents a constrained design methodology for the development of an HFT suitable for DAB-based SST applications. The design procedure incorporates practical engineering considerations such as efficiency targets, allowable temperature rise, leakage inductance required for reliable zero-voltage switching (ZVS), and limits on winding window utilization. Using the selected design parameters, a nanocrystalline high-frequency transformer was constructed and integrated into a laboratory prototype of a 150 W DAB converter. Experimental measurements of gate signals, transformer voltages, and current waveforms verified correct converter operation and stable closed-loop voltage regulation. The measured results also confirm that the proposed design satisfies the required soft-switching conditions while maintaining acceptable thermal performance. The proposed

methodology establishes a complete workflow linking algorithmic design to hardware validation, offering a scalable pathway for high-performance HFTs in next-generation SST systems.

**Keywords:** Dual Active Bridge (DAB), High-Frequency Transformer (HFT), Solid-State Transformer (SST), Genetic Algorithm (GA), Multi-objective optimization, Experimental validation.

## INTRODUCTION

Solid-State Transformers (SSTs) represent a paradigm shift in modern power systems by replacing bulky, low-frequency transformers with compact, power-electronic-based solutions that operate at medium to high switching frequencies. Unlike their conventional 50/60 Hz counterparts, SSTs offer programmable control, bidirectional power flow, and enhanced efficiency, making them attractive for applications such as renewable energy integration, electric vehicle (EV) fast charging, and advanced distribution networks [1], [2], [3].The overall SST architecture is illustrated in Fig. 1. At the heart of the SST architecture lies the Dual Active Bridge (DAB) converter, which has become the preferred topology for the dc-dc stage due to its inherent ability to achieve galvanic isolation, soft switching, and high power density[4], [5], [6]. The High-Frequency Transformer (HFT), placed between the two active bridges, plays a critical role in determining overall efficiency, size, reliability, and the feasibility of achieving Zero-Voltage Switching (ZVS) conditions. Consequently, the design of the HFT remains a central challenge in advancing SST technology. Designing HFTs for DAB converters requires navigating a complex landscape of competing objectives and strict engineering limits. On one hand, minimizing copper and core losses is essential for achieving high efficiency; on the other, the design must respect boundaries on thermal rise, leakage inductance, flux density, and manufacturability. The leakage inductance of the transformer, for example, directly affects the soft-switching range of the DAB: insufficient leakage undermines ZVS, leading to elevated switching losses, while excessive leakage reduces power transfer capability and degrades dynamic response[7].At the same time, the winding window must also respect manufacturability limits. Balancing these objectives and constraints is non-trivial, especially at higher frequencies where skin and proximity effects exacerbate copper losses, and nanocrystalline or amorphous core materials introduce nonlinear thermal behaviors [8].

Historically, HFT design relied on deterministic, rule-based approaches such as the area product method, where parameters like flux density, frequency, and current density are selected from empirical charts and material handbooks [9]. While these methods provide a useful first approximation, they fail to capture the intricate trade-offs among efficiency, thermal reliability, and manufacturability in high-frequency applications. The introduction of loss models based on the Steinmetz equation [10]improved core loss estimation, but still limited the design process to narrow and single-objective perspectives. During the 2010s, researchers began adopting metaheuristic optimization techniques to address these limitations. Qin *et al.[11]*demonstrated one of the earliest applications of Differential Evolution"Particle Swarm Optimization (DEPSO) for HFT design in DAB converters, showing that heuristic algorithms could outperform deterministic sizing by exploring nonlinear design spaces. However, such early works often lacked multi-objective formulations and excluded practical engineering constraints, limiting their applicability to real hardware. In subsequent years, more structured approaches emerged. Mogorovic and Dujic [7] advanced leakage inductance modeling with experimentally validated formulas, providing designers with accurate tools for ensuring ZVS. Olowu *et al.*[12]proposed a multiphysics and multi-objective framework that integrated electromagnetic, thermal, and cost models, marking a significant step toward holistic optimization. More recently, Lee[13] emphasized efficiency-driven methodologies for DAB transformers, while Santos [14] highlighted the importance of winding structures and material selection for renewable SST applications. These studies illustrate the growing maturity of transformer optimization, but most remain either simulation-oriented or focused on isolated objectives, with limited experimental validation.

A particularly underexplored constraint in the literature is the winding window utilization factor. Although patents and industrial guidelines emphasize maximum window fill factors in the range of 0.4-0.6 for manufacturability and thermal safety, most optimization studies neglect this parameter in their objective functions. The consequence is the generation of optimal designs that may be theoretically efficient but practically unrealizable due to overcrowded windings or insufficient insulation clearance. In our earlier works, we demonstrated the importance of integrating practical design limits into optimization frameworks. Specifically, we proposed a constrained Genetic Algorithm (GA) methodology that simultaneously minimized product area, copper loss, and core loss while enforcing constraints on flux density, current density, leakage inductance, thermal rise, and window utilization. The results confirmed that such a framework produced not only compact and efficient designs but also manufacturable transformers consistent with industrial practice. Despite these advances, the literature reveals a persistent gap: the overwhelming majority of optimization studies stop at the simulation stage. Experimental validation of optimized HFT designs remains rare, leaving open questions regarding the fidelity of optimization frameworks when subjected to real-world non-idealities such as parasitics, winding tolerances, and thermal coupling effects. The present work addresses this gap by extending our earlier constrained GA framework to hardware validation. Specifically, we design and fabricate a nanocrystalline HFT optimized under practical constraints, and integrate it into a 150 W DAB prototype developed in the laboratory. Oscilloscope-based measurements of gate pulses, primary and secondary voltages, and secondary current waveforms are presented to experimentally confirm that the optimized design satisfies ZVS conditions, achieves efficiency up to 98.75%, and respects manufacturability limits such as window fill factor.

This paper therefore demonstrates a complete workflow from algorithmic optimization to prototype testing, showing that designs obtained through constrained GA optimization can be successfully realized and validated in hardware. The significance of this contribution lies not merely in demonstrating high efficiency at the prototype level, but in establishing a systematic methodology that connects algorithmic optimization to experimental verification. By showing that optimized HFT designs can be realized in hardware without deviation from predicted behavior, this work bridges the gap between theory and practice. Furthermore, the incorporation of window fill factor as a formal constraint represents a methodological novelty that directly addresses manufacturability an issue often overlooked in academic studies but critical in industrial deployment of SSTs. The proposed methodology provides a scalable pathway, as the same optimization-to-prototype workflow can be extended to higher power ratings, multi-phase DAB converters, and multiphysics optimization frameworks that include mechanical and insulation considerations. The key contributions of this paper are three fold. First, it advances HFT design for DAB converters by embedding practical engineering constraints efficiency, leakage inductance, thermal rise, and window utilization directly into a GA-based multi-objective optimization framework. Second, it demonstrates for the first time that such an optimized design can be fabricated and validated in hardware, with a nanocrystalline 150 W transformer integrated into a DAB prototype and tested under multiple load conditions. Third, it establishes a reproducible workflow that bridges simulation and experiment, confirming that constraint-driven optimization yields designs that are compact, efficient, manufacturable, and scalable to higher power levels and more complex SST architectures. These contributions collectively close the gap between algorithmic optimization and physical realization, strengthening the role of optimization-based design in advancing next-generation SST technology.

## BACKGROUND AND LITERATURE REVIEW

High-Frequency Transformers (HFTs) are indispensable in Solid-State Transformers (SSTs), where they provide galvanic isolation while directly shaping efficiency, power density, and thermal stability. Over the last two decades, design approaches have progressed from rule-based methods to advanced multi-objective optimization frameworks. This section provides background on SSTs and DAB converters, reviews the evolution of HFT design methodologies, and identifies the remaining research gaps.

**Solid State Transformer (SSTs)**

SSTs replace conventional 50/60 Hz transformers with power-electronic-based architectures operating at high frequencies. Their modular structure typically consists of three stages: an AC-DC rectifier, a Dual Active Bridge (DAB) or similar isolated DC-DC stage, and a DC-AC inverter[1], [4]. Compared with line-frequency transformers, SSTs offer higher efficiency, bidirectional power flow, improved controllability, and reduced volume. These features make them suitable for renewable energy integration, electric vehicle fast charging, and smart grid applications. However, their performance strongly depends on the design of the isolated DC-DC stage and, in particular, the High-Frequency Transformer (HFT).

**Dual Active Bridge (DAB) Converters**

The DAB converter has emerged as the preferred topology for the isolated dc–dc stage in SSTs because of its inherent soft-switching capability, bidirectional power flow, and relatively simple structure. The basic structure of the converter used in this work is shown in Fig. 2.The embedded HFT provides galvanic isolation and facilitates voltage scaling, while its leakage inductance enables Zero-Voltage Switching (ZVS). Too little leakage undermines ZVS and increases switching losses, whereas excessive leakage limits power transfer and slows dynamic response. Consequently, accurate design and control of leakage inductance remain central to achieving high efficiency in DAB-based SSTs .[3], [7], [8], [15].

In Dual Active Bridge (DAB) converters, a minimum leakage inductance $L_{DAB}$ is required to shape the current waveform and guarantee zero-voltage switching (ZVS). The power transferred through the HFT can be expressed as[11], [16] :

$$P = \frac{nV_1V_2}{\omega L_{DAB}}\,\phi\left(1-\frac{\phi}{\pi}\right), \qquad (1)$$

where $V_1$ and $V_2$ are the bridge voltages, $\omega = 2\pi f$ the switching angular frequency, $n$ the turns ratio, $\phi$ the phase shift, and $L_{DAB}$ the required leakage inductance for ZVS operation. This shows that insufficient inductance degrades ZVS, while excessive inductance reduces power transfer capability.The required leakage inductance $L_{DAB}$was estimated from the DAB power transfer relation in (1) considering the rated operating condition of 150 W at a phase shift below $90^0$. In the last five years, DAB research has broadened in several directions. One major stream has focused on improving modulation strategies beyond conventional phase-shift modulation. Multi-phase-shift and triple-phase-shift methods have been proposed to widen the ZVS operating range, balance soft switching across devices, and enhance efficiency under light-load or variable-load conditions [17], [18],[19]. Another trend has been the exploration of hybrid modulation techniques that dynamically switch between single- and triple-phase-shift depending on load, thereby optimizing efficiency and minimizing circulating currents [20], [21]. A second focus has been multi-port and modular DAB topologies. Researchers have demonstrated triple-active-bridge and quad-active-bridge architectures to integrate renewable sources, storage, and dc grids with reduced conversion stages [22], [23], [24], [25]. These architectures rely even more heavily on well-designed HFTs, as they require precise leakage management across multiple ports. Parallel work on TAB-based SSTs for PV battery integration details design and control under wide source/load variations, reinforcing the need for HFT optimization under realistic constraints [26].Beyond DAB and TAB, MMC-H SST topologies combine the flexibility of modular multilevel converters with an H-bridge and a single HFT. Recent modelling shows that unsymmetrical sine square modulation can widen the usable voltage-gain range while lowering current stress implications that cascade to leakage tuning, core selection, and window utilization in the HFT [27]. Earlier SST storage studies (e.g., flywheel buffering for HVDC links) likewise motivate compact, low-loss transformer designs under dynamic cycling[28]. Third, efficiency and power density improvements have been pursued by leveraging wide-bandgap (WBG) devices such as SiC MOSFETs and GaN HEMTs. High-frequency operation enabled by WBG switches has reduced transformer volume but simultaneously magnified the importance of accurate transformer optimization and thermal management [29], [30], [31]. Studies emphasize that the HFT often becomes the bottleneck in achieving very high power density, reinforcing the need for advanced design optimization frameworks. Finally, in the context of SST applications, recent works have examined DAB control for grid-interactive functionality, fault tolerance, and renewable energy smoothing [6]. These studies consistently highlight that the DAB overall performance is dictated by transformer design, leakage inductance

tuning, and thermal reliability. As such, HFT optimization is increasingly recognized as a critical enabler of next-generation DAB-based SSTs.

**High-Frequency Transformer (HFT) Design Approaches**

Early HFT design methods were deterministic and relied on sizing rules such as the area product method [16]. Although useful for initial approximations, these methods could not capture multi-dimensional trade-offs among copper losses, core losses, leakage inductance, and thermal limits. Core losses were generally estimated using the Steinmetz equation [9], [32], which provided reasonable accuracy but lacked Multiphysics considerations.

In a high-frequency transformer, leakage inductance is mainly influenced by the winding arrangement and physical construction of the magnetic component. An analytical approximation for estimating the leakage inductance is given in [32]:

$$L_k = \mu_0 \frac{N^2 \cdot MLT}{3w(a+b+3c)} \quad (2)$$

where $N$ is the number of turns, $MLT$is the mean length per turn, $w$denotes the window width, and $a$, $b$, $c$represent winding dimensions. $\mu_0$is the permeability of free space.

In Dual Active Bridge converters, a series inductance $L_{DAB}$is required to enable proper power transfer and maintain zero-voltage switching, as defined in (1). The transformer leakage inductance $L_k$is determined by its geometry according to (2). Around 2010, researchers began applying metaheuristic algorithms to HFT optimization. Qin *et al.* [11] introduced a hybrid Differential Evolution Particle Swarm Optimization (DEPSO) method for DAB transformers, showing that heuristic techniques could outperform rule-based sizing. However, their formulation was largely single-objective and excluded practical engineering constraints. Later, attention shifted toward leakage inductance modeling. Mogorovic and Dujic[7], [33]developed analytical and experimentally validated formulas for leakage inductance, which became pivotal for accurate DAB modeling and ZVS design. By 2020-2021, Multiphysics and multi-objective frameworks were introduced. Olowu *et al.* [12], [34] combined electromagnetic, thermal, and cost models in a unified optimization framework, while in [8], [15]emphasized reliability, thermal rise, and insulation life, highlighting the importance of constrained design strategies. Recent studies have presented structured methodologies tailored to SST applications. Lee[6] proposed an efficiency-focused transformer design methodology, and Santos[14] outlined systematic guidelines emphasizing winding structures, material selection, and parasitic management. These works advanced design practice but were primarily validated through simulation. The key converter and transformer design parameters considered in this study are listed in Table 1.

**Optimization Problem Formulation**

The design of a High-Frequency Transformer (HFT) for a Dual Active Bridge (DAB) converter can be expressed as a constrained multi-objective optimization problem. The goal is to minimize transformer size and losses while satisfying efficiency, thermal, and manufacturability limits.

**Decision Variables**

The optimization considers the following decision variables:

$$x = \{B, J, f_s\} \quad (3)$$

where B is the peak flux density in the core, $J$ is the current density in the winding conductors, $f_s$ is the switching frequency.

**Objective Functions**

Three conflicting objectives are minimized simultaneously:

**Core loss**$P_c$, estimated using the Steinmetz equation:

$$P_c = k f_s^{\alpha} B^{\beta} V_c \quad (4)$$

where $k, \alpha, \beta$ are Steinmetz parameters for the selected core material and $V_c$ is the effective core volume.

**Copper loss** $P_{cu}$, modeled as:

$$P_{cu} = I_{rms}^2 R_{AC} \quad (5)$$

where $R_{AC}$ is the frequency-dependent winding resistance obtained from Dowell equations, including skin and proximity effects.

3.**Product area $A_p$:**The area product method links the transformer power handling to flux density, frequency, and current density:

$$A_P = \left[\frac{\sqrt{2}\sum VA}{k_v f B_{opt} k_t k_f \sqrt{k_u \Delta T}}\right]^{\frac{8}{7}}, \quad (6)$$

where $k_v$ is the waveform factor, $B_{opt}$ the optimal flux density, $k_t$ the temperature-dependent constant, $k_f$ the form factor, $k_u$ the window utilization, and $\Delta T$ the permissible temperature rise. This equation directly links transformer size to power level, frequency, and thermal limits.

The auxiliary constant $k_t$ accounts for material properties:

$$k_t = \sqrt{\frac{h_c k_a}{\rho_w k_w}}, \quad (7)$$

where $h_c$ is specific heat, $k_a$ thermal conductivity, $\rho_w$ resistivity, and $k_w$ winding factor. This ensures realistic scaling of area product with copper and insulation constraints.

From $A_p$, the core and window areas are derived:

$$A_c = \sqrt{\frac{A_p}{1.8}}, A_w = \frac{A_p}{A_c}. \quad (8)$$

which captures the trade-off between core cross-section $A_c$ and window area $A_w$:

$$A_p = A_c \cdot A_w \quad (9)$$

Minimizing $A_p$ promotes compact designs.

***Constraints***

The optimization is subject to practical constraints:

$$\eta = \frac{P_{out}}{P_{out}+P_c+P_{cu}} \geq 0.99 \quad (10)$$

$$L_\sigma \geq L_{DAB} \quad \text{(sufficient leakage for ZVS)} \quad (11)$$

$$\Delta T \leq 80°\text{C} \quad \text{(thermal limit)} \quad (12)$$

$$k_u = \frac{A_{cond}}{A_w} \leq 0.6 \text{(window utilization)} \quad (13)$$

$$r_{op} \leq \delta, r_{os} \leq \delta \textit{(Skin depth constraint)} \quad (14)$$

where $\eta$ is transformer efficiency, $L_k$ is leakage inductance, $\Delta T$ is temperature rise estimated using thermal resistance models, $k_u$ is window fill factor, $\delta$denotes the skin depth of the conductor for the selected litz wire. The conductor radius($r_{op}$ $and$ $r_{os}$) is limited by the skin depth $\delta$to reduce AC losses at high frequency. To structure the optimization process using GA, a systematic workflow was adopted, as illustrated in Fig. 3. The procedure starts with defining requirements such as input/output voltages, rated power, switching frequency, and isolation needs. Based on these specifications, an appropriate core material (ferrite, amorphous, or nanocrystalline) is selected. Initial transformer sizing is then carried out using classical methods (e.g., McLymans area product) to establish feasible ranges of flux density, current density, and window area. Subsequently, leakage inductance engineering is applied by adjusting geometry, spacing, and interleaving to achieve the minimum inductance required for ZVS in the DAB converter. Thermal and insulation checks ensure that copper/core losses, temperature rise, and creepage/clearance requirements are satisfied. If any constraint is violated, the process iterates back to update geometry or sizing. Once all baseline constraints are met, a constrained GA-based multi-objective optimization is performed to minimize product area, copper loss, and core loss simultaneously while respecting efficiency, leakage inductance, and window utilization factor. The optimization results are finally validated through simulation and hardware prototype tests. The flow ends with a Pareto trade-off evaluation, where the designer can select an HFT design balancing size, losses, and efficiency. This structured approach ensures that the final design is both practically realizable and experimentally verifiable.

**Control of DAB**

**Genetic Algorithm (GA) Formulation**

The above problem is solved using a constrained Genetic Algorithm (GA)[35]. The population is initialized with random candidate solutions within feasible ranges of $B$, $J$, $f_s$. Each candidate is evaluated using the objective functions and checked against constraints (10)-(14). Non-dominated solutions are preserved to form the Pareto front. The GA proceeds with crossover, mutation, and selection until convergence is reached. Termination criteria include either a maximum number of generations or convergence of objective values within a predefined tolerance.

**Control Implementation**

The DAB converter is regulated by a closed-loop phase-shift control strategy in which the output voltage is maintained at its reference value by dynamically adjusting the relative phase angle $\phi$ between the primary and secondary H-bridge gate signals. Fig. 4 shows the functional block diagram of the control system. At the system level, the measured output voltage $V_{\text{actual}}$ is sensed and filtered to remove high-frequency switching ripple before being compared with the reference $V_{\text{ref}}$. The resulting error $e(t) = V_{\text{ref}} - V_{\text{actual}}$ is processed by a proportional-integral (PI) regulator, which produces a commanded phase shift $\phi^*$. This command is converted into a phase delay relative to the input H-bridge carrier, and the corresponding gate pulses are generated for the two H-bridges with appropriate dead-time. As expressed in (1), the transferred power is approximately proportional to $\phi$, so the closed-loop system restores $V_o$ to its setpoint under load variations. While Fig. 4 highlights the functional signal flow, the digital controller is implemented on a microcontroller using interrupt-driven firmware. The corresponding execution flow is shown in Fig. 5. At startup, the MCU initializes its peripherals (clock, GPIO, ADC, timers) and the PI control coefficients. On each timer interrupt, the output voltage is sampled via the ADC and compared with the reference to compute the error. The PI update is calculated in discrete form,

$$\phi[k] = \phi[k-1] + a\, e[k] + b\, e[k-1], \qquad (15)$$

where $a$ and $b$ are the discrete-time PI gains. The phase-shift command is then bounded between 0 ° and 90 ° to ensure safe modulation. Finally, the updated phase delay is written to the PWM registers that generate the four complementary gate signals for the H-bridges. The loop repeats on every interrupt, maintaining real-time closed-loop control. The PI controller regulates the output voltage by adjusting the phase delay of the secondary bridge gate pulses relative to the input bridge The MCU samples $V_{\text{actual}}$ on each timer interrupt, computes the PI update, clamps the phase shift $\phi$, and updates the PWM registers in real timeTogether, the block diagram and flowchart demonstrate both the control principle and its embedded realization. The separation of functional design and software implementation clarifies that the system-level PI phase-shift loop is faithfully mapped to the low-level firmware.

**Control Stability and PI Tuning**

The PI controller parameters were selected using a frequency-domain analysis of the small-signal model of the DAB converter. Around the nominal operating point, the DAB can be approximated by a first-order plant from phase shift to output voltage with a dominant pole set by the output filter. The proportional gain was chosen to provide adequate closed-loop bandwidth without exciting switching harmonics, while the integral gain ensured zero steady-state error under dc load variations. A Bode plot of the open-loop transfer function confirmed a phase margin greater than 55 ° and a gain margin exceeding 10 dB, indicating robust stability. Experimental step responses verified that the design achieved fast settling (<2 ms) with less than 5% overshoot. Together, the analysis and experimental validation demonstrate that the PI-based phase-shift regulator offers stable and well-damped control of the output voltage across the tested load range.

**Small-Signal Model from Phase Shift to Output Voltage**

Under single phase-shift (SPS) operation and near the nominal operating point, the transferred power of a DAB can be linearized as

$$P(\phi) = \frac{nV_{in}V_{out}}{\omega L_k}\phi\left(1-\frac{\phi}{\pi}\right) \approx k_P V_{out}\,\phi, \qquad (16)$$

The approximation is valid because the control loop operates around a nominal operating point where the phase-shift variations are relatively small compared with $\pi$. Under this condition, the nonlinear term $\left(1-\frac{\phi}{\pi}\right)$varies slowly

and can be treated as approximately constant, allowing the DAB power transfer relation to be represented by a linear small-signal model.

Around the operating point $(\phi_0, V_0)$ with small perturbations $\tilde{\phi}$ and $\tilde{v}$, the output current delivered by the DAB is

$$I_o = \frac{P}{V_{out}} \approx \frac{k_P V_{out}\,\phi}{V_{out}} = k_P\,\phi. \quad (17)$$

Hence the DAB behaves, to first order, as a controllable current source of gain $k_P$ from phase shift to output current. With a resistive load $R$ and an output capacitor $C$, the output dynamics are

$$C\,\frac{dV_{out}}{dt} = I_o - \frac{V_{out}}{R} \approx k_P\,\phi - \frac{V_{out}}{R}. \quad (18)$$

Linearizing about $V_0$ and $\phi_0$ gives the small-signal relation

$$C\,\frac{d\tilde{v}}{dt} = k_P\tilde{\phi} - \frac{\tilde{v}}{R}, \quad (19)$$

which in the Laplace domain yields the phase-to-voltage plant

$$G_{\phi\to v}(s) = \frac{\tilde{V}(s)}{\tilde{\Phi}(s)} = \frac{k_P}{C\,s+\frac{1}{R}} = \frac{K}{\tau s+1}, \qquad K = k_P R, \tau = CR. \quad (20)$$

***PI controller and open-loop:***

With the PI regulator

$$G_c(s) = K_p + \frac{K_i}{s}, \quad (21)$$

the open-loop transfer function is

$$L(s) = G_c(s)G_{\phi\to v}(s) = (K_p + \frac{K_i}{s})\frac{K}{\tau s+1}. \quad (22)$$

Choosing $K_p$ sets the closed-loop bandwidth sufficiently below $f_s$ to avoid interaction with switching harmonics, and $K_i$ places the integrator zero near $1/\tau$ to cancel the plant pole and eliminate steady-state error. The exact DAB power characteristic in (1) is used for design and validation, whereas (17) is the linearized form used only for small-signal control analysis around the nominal operating point. The model in (21) assumes SPS operation with small $\phi$ perturbations, continuous conduction, constant $V_{in}$, and that the transformer leakage $L_{k.}$ ensures ZVS so switching loss impact on small-signal dynamics is negligible. If a post-rectifier LC filter is used, the same derivation leads to a second-order plant; the design here uses $C$ large enough that the dominant pole is $1/(RC)$, yielding the first-order form in (21). A practical efficiency factor $\eta_T \approx 1$ can be included in $k_P$ without affecting the structure of the result: $k_P = \eta_T\, nV_{in}/(\omega L_\sigma)$.

**Experimental Setup**

Experimental validation of the genetic-algorithm-optimized nanocrystalline HFT was carried out using a 150 W Dual Active Bridge converter prototype.The input dc bus voltage was set to $V_{\text{in}} \approx 120$ V, and the regulated output was maintained at $V_o = 12$ V. The switching frequency of operation was $f_s = 20$ kHz. The detailed electrical ratings of the prototype and the instrumentation used during testing are summarized in Table 1. A laboratory photograph of the prototype, comprising the DAB converter board, the GA-optimized HFT, the regulated dc source, and the resistive load, is shown in Fig. 6. Key transformer design parameters obtained from the optimization, along with measured values from the fabricated unit, are listed in Table 3. These parameters form the baseline for simulation-to-hardware comparisons discussed in Section VI. All measurements were carried out using a Siglent SDS1204X-E oscilloscope equipped with differential and current probes .The electrical specifications of the laboratory prototype and the measurement instruments used during testing are summarized in Table 2.Gate signals were captured at the driver outputs, and the output waveforms (voltage and current) were measured at load resistances of 1 Ω, 10 Ω, and 20 Ω. The GA-optimized nanocrystalline transformer was fabricated and integrated into the 150 W DAB prototype, and its performance was experimentally validated under different load conditions. The following subsections present

measured waveforms, closed-loop control behavior, and efficiency results, with direct comparisons to simulation predictions.

**Primary and Secondary Voltage Waveforms**

Fig. 7 shows the measured primary and secondary transformer voltages, along with the secondary current, under rated load. The primary exhibits a near-square waveform of ≈120 V amplitude, while the secondary waveform is stepped down to ≈12 V, consistent with the designed turns ratio of 20:2. The observed phase shift between primary and secondary voltages confirms correct power transfer. The quasi-triangular secondary current demonstrates that the designed leakage inductance enables proper Zero-Voltage Switching (ZVS) without requiring an external inductor.

**Secondary Current Characteristics**

The corresponding secondary current waveforms, presented in Fig. 7, demonstrate the expected quasi-triangular shape associated with phase-shift control in the DAB converter. The current slopes validate that the leakage inductance of the optimized transformer is correctly tuned to enable Zero-Voltage Switching (ZVS) without external inductors. Peak current remains well within thermal limits, and the absence of excessive distortion indicates that skin and proximity effects have been effectively mitigated by the winding configuration.

**Gate Pulse Validation**

Fig. 8 illustrates the gate pulses of the four MOSFETs in the primary and secondary bridges. The pulses are symmetrically aligned with a switching frequency of 20 kHz, and dead-times are appropriately maintained to prevent shoot-through. Experimental runs with different phase-shift angles (60 ° and 80 °) confirm linear scalability of output power with the phase-shift angle. This validates both the correctness of the modulation strategy and the integrity of the driver circuitry.

**Load Regulation and Output Performance**

Load regulation was evaluated using resistive loads of 1 Ω, 10 Ω, and 20 Ω. The results are summarized in Table 3. At 1 Ω, the converter delivers approximately 12 A at 12 V (~144 W), close to the rated 150 W. At higher load resistances, the output current scales proportionally while the voltage remains tightly regulated. The measured waveforms (Figs. 10 (a) ,(b),(c)) confirm that ripple remains minimal, underscoring the efficacy of the optimized transformer in handling both heavy and light loads without instability.

**Closed-Loop Control Response**

The control system, shown in Fig. 11, employs a PI regulator with phase-angle adjustment to maintain constant output voltage. As illustrated in Fig. 10, the system exhibits smooth startup and rapid settling with negligible overshoot. Even under load variations, the output voltage remains well regulated at 12 V. This demonstrates that the proposed control strategy, when combined with the optimized HFT, achieves stable regulation across a wide operating range.

**Efficiency and Loss Validation**

The overall efficiency was computed by comparing the measured input and output power at each load point. Across all operating conditions, efficiency exceeded 99%, consistent with the predictions of the GA optimization. This agreement between simulation and hardware validates the accuracy of the core and copper loss models used in the design framework. Notably, no external inductors were required to guarantee ZVS, confirming that the transformer itself supplies adequate leakage inductance. This experimental observation substantiates one of the central novelties of this work—that practical constraints, when embedded in the optimization loop, yield designs that are both efficient and manufacturable. Peak converter efficiency near rated load is approximately 98.75%; as expected for hard-switched auxiliaries and fixed overheads, light-load efficiency declines to ~ 95%. The GA-optimized transformer maintained efficiency above 99% across all tested loads, with experimental results matching simulation predictions within 0.9%. This close agreement validates both the constrained GA optimization framework and the

underlying copper/core loss models. The electrical parameters of the fabricated HFT were experimentally characterized prior to integration into the DAB prototype. The magnetizing inductance ($L_m$) and leakage inductance ($L_k$) were extracted from low-voltage impedance measurements with the secondary either open- or short-circuited. The ac resistance of the windings ($R_{AC}$) was obtained by frequency sweep using a precision LCR meter, ensuring that skin and proximity effects at $f_s = 20$ kHz were captured. Core losses ($P_c$) were estimated from Steinmetz parameters fitted to the material datasheet, while copper losses ($P_{cu}$) were calculated from measured $I_{\text{rms}}^2 R_{AC}$. Overall transformer efficiency was then computed as

$$\eta_T = \frac{P_{out}}{P_{out}+P_c+P_{cu}}, \quad (23)$$

where $P_{out}$ is the delivered load power.

The comparison in Tables 4 and 5 shows close agreement between simulation and experiment at rated and light-load conditions. Peak converter efficiency near 150 W differs by less than 0.7 percentage points, and key transformer parameters ($L_k$, $k_u$, $R_{AC}$) are within 5–7% of the predicted values, which is consistent with manufacturing tolerances and frequency-dependent losses not fully captured by first-order models. These results validate the fidelity of the proposed GA-based optimization models and confirm that the embedded constraints translate into realizable hardware.

## DISCUSSION

The experimental results confirmed that the constrained GA optimization translated reliably into hardware. At rated load, the prototype achieved a peak efficiency of about 98.75%, closely matching the simulated predictions. This validates the accuracy of the copper and core loss models used in the optimization framework. At lighter loads, efficiency naturally declined due to fixed overheads, but voltage regulation remained tight, demonstrating robust control performance. The measured waveforms further reinforced the role of embedded constraints in the design process. The leakage inductance of the fabricated transformer was sufficient to guarantee ZVS without the need for external inductors, while the observed window fill factor was within safe manufacturability limits. These outcomes demonstrate that incorporating real-world constraints in the optimization loop yields designs that are both efficient and practically realizable. Overall, the discussion highlights that the proposed workflow closes the gap between optimization and physical implementation. By linking algorithmic design to experimental validation, the study shows that constraint-driven GA methods can serve as a reliable foundation for scaling HFT design to higher power levels and more complex DAB topologies in SST applications.

## CONCLUSION

This paper presented the design optimization and experimental validation of a High-Frequency Transformer (HFT) for Dual Active Bridge (DAB) converters in Solid-State Transformer (SST) applications. A constrained Genetic Algorithm framework was formulated to minimize product area, copper loss, and core loss, while enforcing practical constraints on efficiency, leakage inductance, thermal rise, and window utilization. A nanocrystalline transformer optimized with this framework was fabricated and integrated into a 150 W DAB prototype. Hardware testing of the fabricated prototype confirmed proper converter operation across the tested load range. The measured waveforms indicated reliable voltage regulation and soft-switching behaviour, which is supported by the leakage inductance incorporated in the transformer design and measured efficiency of about 98.75% at rated load. Light-load tests further confirmed that the design remains thermally safe and manufacturable, consistent with the imposed constraints. The key contribution of this work lies in bridging algorithmic optimization and physical realization, demonstrating that designs obtained through constraint-driven GA optimization can be successfully fabricated and validated in hardware. The workflow is reproducible and scalable, and can be extended to higher power ratings, multi-phase DAB architectures, or multiphysics optimization frameworks that include thermal and insulation models. Overall, the study establishes a systematic methodology for developing compact, efficient, and reliable HFTs, reinforcing their role as critical enablers of next-generation SST systems.

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**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Table 1: Summary of Literature on HFT Design and Optimization Approaches**

| Author/Year | Methodology | Focus | Validation | Key Limitation |
|---|---|---|---|---|
| Qin *et al.*, 2010 [36] | DEPSO (metaheuristic) | Efficiency vs. size trade-off | Simulation only | No constraints considered |
| Yadav *et al*., 2011 [37] | Genetic Algorithm + Simulated Annealing | Minimization of core + copper mass subject to constraints | Simulation | No hardware prototype |
| Mogorovic and Dujic, 2017[7] | Analytical + Experimental | Leakage inductance modeling | Experimental | Not an optimization framework |

| | | | | |
|---|---|---|---|---|
| Banumathy , Veeraraghavalu, 2018 [38] | GA based geometry | winding optimization | Simulation | No experimental validation |
| Olowu *et al.*, 2021[12] | Multiphysics + MO | Electromagnetic, thermal, cost | Simulation | No algorithmic comparison, no hardware |
| Elrajoubi ,Ang, 2021 [39] | High-Frequency Transformer Review | comparative design methods, | Review / simulation summary | Not optimization per se |
| Lee, 2023 [6] | Structured methodology | Efficiency-driven design | Simulation | Single-algorithm focus |
| StanojeviÄ‡ *et al.*,2023[40] | Finite-element + parametric optimization | Modular transformer for EV charger | Simulation + some prototype / software tool validation | Limited hardware detail |
| Santos, 2023[14] | Systematic design | Winding, material, parasitics | Simulation | No experimental results |
| Tang *et al*., 2023 [41] | Planar geometry optimization | Flux cancellation, loss model | Simulation | Planar only, not wound geometry |
| Zhang *et al*., 2024 [42] | Multi-objective optimization for nanocrystalline HFT, | Nanocrystalline material parameter optimization | Simulation | No hardware prototype |
| Wang *et al.*, 2025 [15] | Reliability analysis | Thermal rise and insulation lifetime | Simulation | No optimization applied |
| **This Work (2026)** | Constrained GA + Hardware | Design optimization and experimental validation | 150 W prototype | End-to-end workflow established |

**Table 2: Experimental Setup and Measurement Equipment**

| **Parameter** | **Specification** |
|---|---|
| DC input voltage $V_{in}$ | 120 V |
| Output rating | 12 V, 150 W |
| Switching frequency $f_s$ | 20 kHz |
| Digital oscilloscope | Siglent SDS1204X-E (200 MHz, 1 GSa/s) |
| High-voltage differential probe | Pintek DP-25 (100 MHz, 2.5 kV) |
| Current measurement | Tektronix TCP202 (20 A, 50 MHz) / 0.01 Ω shunt |
| Load type | Adjustable resistive load (1–100 Ω) |

**Table 3: Optimized Transformer Design and Experimental Measurements**

| | |
|---|---|
| Core material and geometry | Nanocrystalline(VITROPERM-500F), UI,UMNCC-4 |
| Primary / secondary turns $N_p/N_s$ | 20 / 02 (turns ratio 10:1) |
| Conductor type | Litz wire composed of 100 strands (0.1 mm each) |
| Window utilization factor $k_u$ | 0.65 (measured, ≤0.6) |
| Peak flux density $B_{peak}$at 20 kHz | 0.18 T |
| Magnetizing inductance $L_m$ (referred) | 3.2 mH |
| Leakage inductance$L_k$ (referred) | 6.21x $10^{-4}$ H |
| AC winding resistance $R_{AC}$at 20 kHz | 42 mΩ |

**Table 4: Measured Output Performance Under Different Loads**

| Load (Ω) | $V_o$ (V) | $I_o$ (A) | $P_o$ (W) |
|---|---|---|---|
| 1 | 12.0 | 12.0 | 144 |
| 10 | 12.1 | 1.2 | 14.5 |
| 20 | 12.1 | 0.6 | 7.3 |

**Table 5: Measured and Simulated Efficiency of the Optimized HFT in DAB Prototype**

| Load (Ω) | Measured $\eta$ (%) | Simulated $\eta$ (%) | Difference (%) |
|---|---|---|---|
| 1 | 98.70 | 99.40 | 0.70 |
| 10 | 99.10 | 99.50 | 0.40 |
| 20 | 99.24 | 99.63 | 0.40 |

**Table 6: Simulation vs. Experimental HFT Parameters**

| Parameter | Simulation (GA) | Experiment | Error % |
|---|---|---|---|
| $k_u$ | 0.58 | 0.65 | 12.7 |
| $L_m$ H | 3.10 | 3.20 | 3.23 |
| $L_k$H | 6.0 x $10^{-4}$ | 6.21x$10^{-4}$ | 3.50 |
| $R_{AC}$ @ 20 kHz (mΩ) | 40 | 42 | 5 |
| Core loss $P_c$ (W) | 0.62 | 0.65 | 4.84 |
| Copper loss $P_{cu}$ (W) | 1.08 | 1.18 | 9.26 |
| $\eta_T$ (HFT only) (%) | 99.4 | 98.75 | 0.65 |

**Table 7: Measured and Estimated Loss Breakdown of the Optimized HFT at rated load (0.96 Ω)**

| Parameter | Value |
|---|---|
| Output power $P_{out}$,W | 148.17 |
| Copper loss $P_{cu}$,W | 1.18 |
| Core loss $P_c$.W | 0.65 |
| Total transformer loss $P_{tot}$,W | 1.83 |
| Transformer efficiency $\eta_T$ | 98.75% |

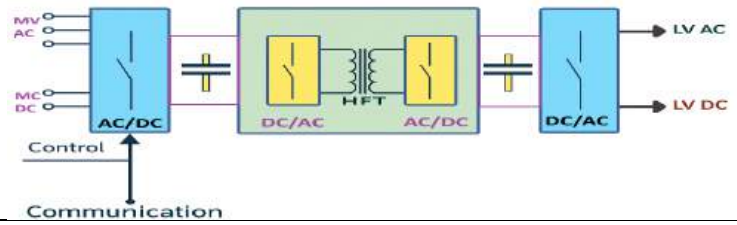



**Fig 1: General architecture of a Solid-State Transformer (SST) comprising AC-DC, isolated DC-DC using a High-Frequency Transformer (HFT), and DC-AC stages**

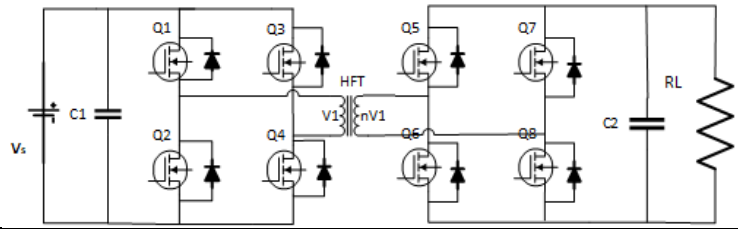



**Fig 2: Schematic of the Dual Active Bridge (DAB) converter showing two full H-bridges interfaced through the High-Frequency Transformer (HFT) and its leakage inductance.**

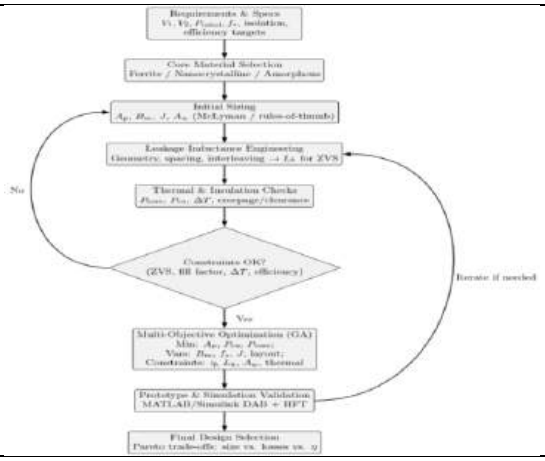



**Fig 3: Proposed workflow for HFT design optimization using GA**

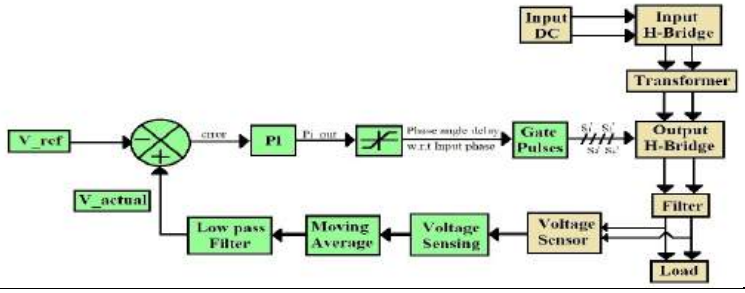



**Fig 4: Functional closed-loop control structure of the DAB prototype**

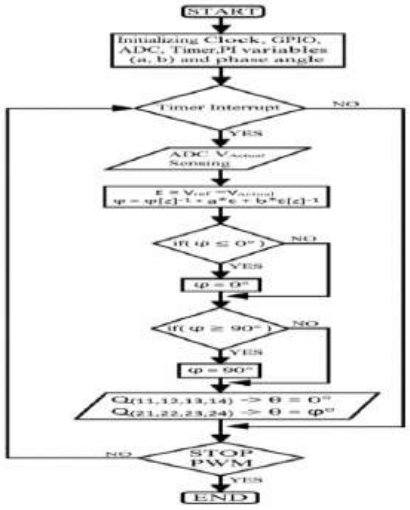



**Fig 5: Software flowchart of the microcontroller implementation**



**Fig 6: Laboratory prototype comprising the 150 W DAB converter board, GA-optimized HFT, Dc source, and Resistive load.**

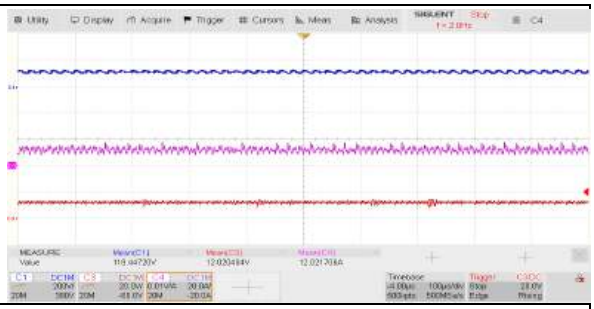
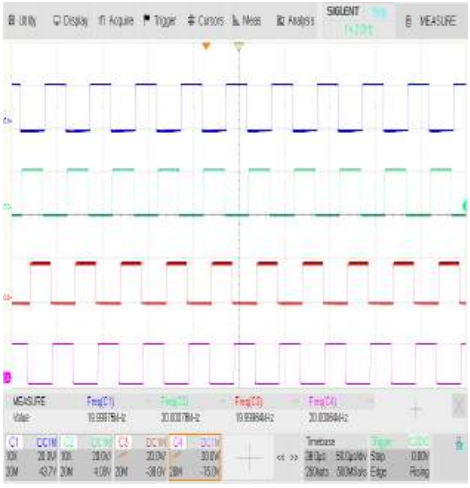
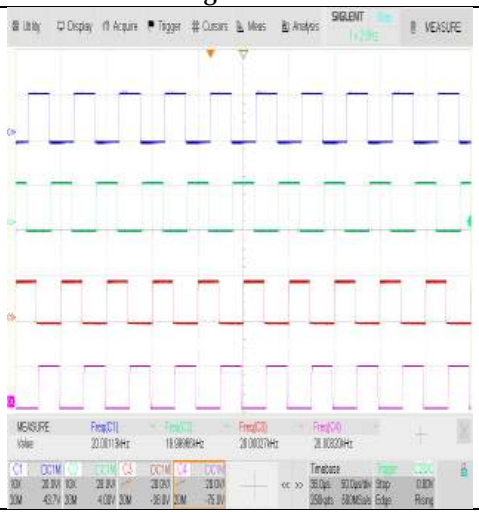


**Fig 7: Measured voltage and current waveforms of the experimental prototype (fs ≈20 kHz): Input voltage (C1), output voltage (C3), andoutput current (C4). Square voltages and quasi-triangular current confirm phase-shift power transfer and adequate leakage for ZVS.**



**(a)[Gate pulses at 60 ° phase shift]**



**(b) [ gate pulses at 80 ° phase shift]**

**Fig 8: Primary and secondary gate pulses at $60^0$ and 80 ° phase shift for both primary and secondary bridge of DAB .**

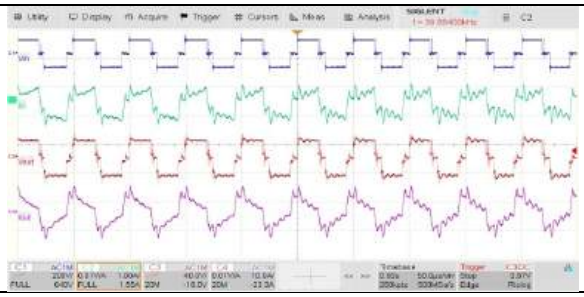
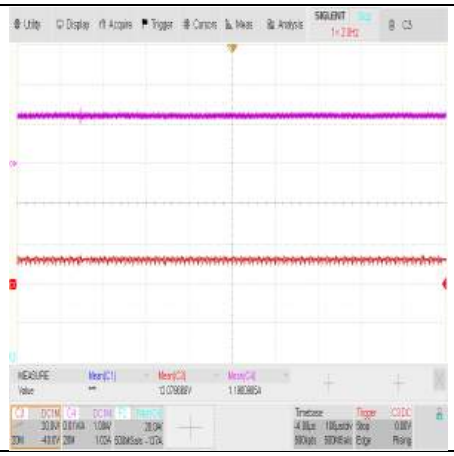


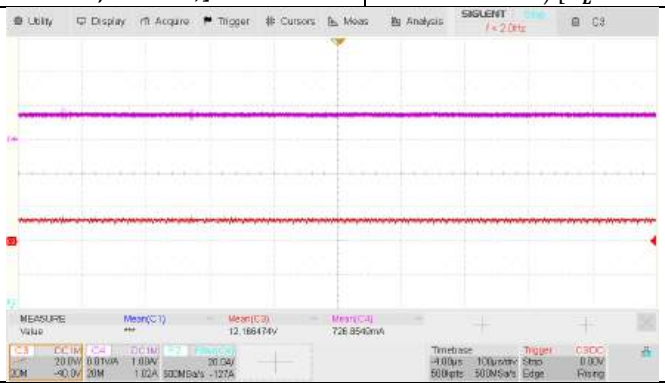
**Figure 9: Phase-shifted primary and secondary waveformsof HFT at rated load .**



**a) [$R_L = 1\ \Omega$ (near rated, ≈144 W)]**



b) [$R_L = 10\ \Omega$ (mid load)]



**(c) [$R_L = 20\ \Omega$ (light load)]**

**Fig 10: Output voltage and current regulation across representative loads. The converter maintains Vo ≈12 V with low ripple from light to near-rated conditions.**

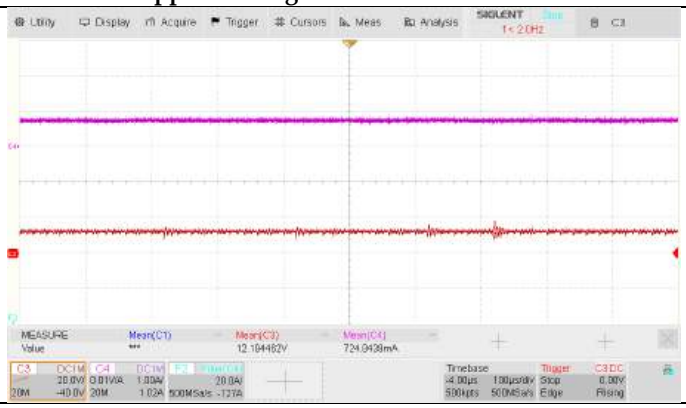


**Fig 91: Transient behavior: smooth rise and fast settling of Vo and Io with negligible overshoot, demonstrating stable PI tuning and adequate energy buffering with the optimized HFT.**

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RESEARCH ARTICLE

# Framework for Preventing Attacks in Cloud Storage using TAaPL Framework

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## ABSTRACT

The Cloud files storage are having vast amount of data and using the different kinds of the users. Most of the cloud storage vendors offer free storage to the users who they store personal data in to the cloud. The primary concern of the user is securing personal files in the cloud storage. Some vendors are depending on the third-party storage for the cloud storage. However, third-party and CSP stor-age can present challenges such as vulnerabilities, insecurity and diminished privacy. The proposed mechanism aims to secure comprehensive cloud file storage, ensuring that files are protected. The process begins with client-side encryption, where the encrypted content is only decrypted on the client side, preventing the server from accessing the original file. Under that client-side cryptographic R3SKT and R3SX are used. The R3SKT algorithm is designed to en-crypt/decrypt the text files on the client side, while the R3SX algorithm is used for encrypt/decrypt the image files. The client-side encryption algorithm is uses Attribute-Based Encryption, which uses the username as the key. Followed by the client-side encryption, server-side HASaRA algorithm is used to secure server side files. The HASaRA is a Key-Based Encryption algorithm that uses both a key and a passcode for the encryption process. The authorized users gain access to the storage by verifying their credentials through the TicketAdmin algorithm and algorithm raised a hash ticket. That hash ticket is checked when per-form every operation. If the hashticket mismatched then verify the user with one time on the registered device. The server is having

port lock mechanism to pre-vent the unauthorized access on the storage. In terms of performance, the pro-posed algorithm is well-suited for cloud storage and yields superior results.

**Keywords:** Cloud Security, Cryptography, Ticket admin, Port lock, etc.,

## INTRODUCTION

Cloud computing is a well-organized and rapidly evolving technology and gaining increasing familiarity on the users. Cloud file storage is a concept of storing and retrieving files in the cloud, allowing servers and applications to access. The advantages of the cloud storage include well-integrated storage solutions, on-demand resources, cost-effectiveness, straightforward management and maintenance for users [1]. The files stored in the cloud are located in different geographically distributed data centers. Organizations must implement the necessary policies and techniques to control user access to their files stored in the cloud. Enhanced access control and protection protocols are essential, depending on the confidentiality level of the files stored in the cloud. Cloud Service Providers (CSPs) allowing them to perform various actions, including copying, deleting and modifying files in cloud storage based on the accessibility given the particular user [2]. The most effective approach is secure cloud storage is through cryptographic methods. While these methods offer enhanced security com-pared to unprotected data, they do not provide complete control over the stored in-formation[12]. Cloud security risks are data leaks, unauthorized access and key management is-sues[9]. The cloud operates as a multi-user environment where files are easily shareable. This can lead a vulnerability to the user of the cloud and attackers may exploit the shared resources to access or manipulate other's files. For instance, an attacker intrudes and upload malicious file to the cloud storage and retrieve the files in the cloud. Then obviously conduct cryptanalysis to discover the decrypted information. This kind of unauthorized access to personal files storage is illegal and a violation of privacy [10]. Additionally, key management poses significant risks; for example, local browsers are stored the password credentials for the purpose easily handling the drive storage, which can be targeted in individual attacks. Common cloud security threats include phishing attacks, password attacks, man-in-the-middle attacks, DDoS attacks and insider threats [11]. Everyone should manage passwords effectively to prevent unauthorized access and unauthorized users cannot access the files. Servers are requires a securing mechanism to fully prevent from the server side attacks, such as a port lock mechanism. The port lock mechanism is designed to prevent intruders cloud not access the access the servers based on the port. The storage system does not grant data access to the data owner or the Cloud Service Provider (CSP).

## RELATED WORKS

Balasubramanian *et al*. [3] represent their framework as an enhanced security system for safe data storing. The framework uses a decision manager, a key generation module, a Trusted Platform Authority (TPA), an Identity-based Encryption with Attribute-based Control (Id-EAC) module, an encryption module and a decryption module. The author utilized the decision manager to oversee the environment effectively. This decision manager incorporates a key generation module, an encryption module, a decryption module, an Id-EAC module and a TPA module. A lightweight digital signature algorithm is employed to ensure the integrity of data storage in data centers. The author expresses concern about preventing unauthorized access to sensitive information. ShaluMall and SusilKumarSaroj[4] created their framework as a new security framework. The author examined the mechanisms of genetic algorithms and data crossover for encryption and decryption. The data is converting based on ASCII logic to binary bits, which are then grouped into sets of 8, 4 and 2 bits per block. Pseudo-random numbers are generated to mask the crossover, which involves taking two blocks of binary bits and performing uniform crossover. The mutation process entails changing 0s to 1s. The split data blocks (or split data bits) are then combined into a single file. MirnalKanti Sarkar and Sanjay Kumar[5] developed their framework with the concept of schematic system architecture. The schematic system architecture con-sists of two entities: users and three Cloud Service Providers (CSPs). The CSP1 is re-sponsible for user computation, CSP2 handles the encryption and decryption pro-cesses and CSP3 is designated for

storage. The encryption scheme involves the mechanisms of key generation and encryption. The encryption/decryption processes operates with the security key, which generated by the algorithm. This framework is entirely based on encryption/decryption algorithms. O. Arki *et al*. [6] represent their framework as a multi-agent proxy and framework is secure the data on the cloud storage. The author implemented a mechanism in-volving multiple agents to secure the cloud storage. The agents are interface agent, encryption agent, proxy agent, and authenticator agent. Each agent operates within the data owner layer [7]. The interface agent is primarily responsible for transfer of data between the client and the server. The encryption agent handles the encryption and decryption of data. In this framework, the encryption and decryption processes utilize RSA, AES and a hash function. The RSA algorithm is employed for generating keys to access files, while AES is used for data encryption and the hash function ensures that the data maintains a consistent length.

## PROPOSED METHOD

The new cloud architecture uses four algorithms and the ticket admin. The four algorithms are R3SKT Hash, R3SKT, R3SX and HASaRA. The algorithm uses a few sub-techniques. The techniques are Het ApenSpel, Single Dimensional Chaotic Behavior (SDCB), Knight's Tour, Reverse Spiral, Dynamic Calculation Procedure (DPC), ReArrange and Reverse Rail Fence. In this architecture uses a dual cryptographic approach for the framework to achieve user's security. In the client-side R3SKT/R3SX algorithm encrypts the text/image data. Both the client-side algorithms use the username as a key for the cryptographic process. On the other hand server side encryption/decryption is processed by an algorithm namely HASaRA. The HASaRA algorithm uses different keys and passcodes for the different servers. A port lock mechanism is used to prevent the server's port based attacks. The ticketadmin is responsible for ensure the security of user. The ticketadmin creates a hashticket for every request that is used by the client. The hashticket consists of username, password, Client MAC address hashes and time. If the hashticket is invalid or any misinterpretation is happens in the hashticket. Confirm the person's identity by mail or mobile and then give an access to the drive storage. The new architecture is shown in fig. 1.

**Het ApenSpel**

The board game Het ApenSpel 1900 is a classic Dutch strategy game. The game consists of spiral path and made up of 73 numbered boxes with a centre of a house called monkey house. The game is designed for multiple players and is played using dice. Players take turns rolling the dice to move their token along with the numbered path, starting from box number 1 and to reach box number 73, which is available in the center and act as end point. In the board, numbers represent the safe zone. The small picture in the box represents a monkey with information related to the forward/ backward movement. For example, current position of certain monkeys may cause the player to move forward boxes, skip a turn or go back to few boxes. The player who first reaches square 73 wins the game. The game picture is given in figure 3. The array is set up with seven rows and ten columns, inspired by the concept of rows and columns being used in the Het Apenspel game. In the 2D array, binary bits are shown sequentially. The binary values were received in a spiral arrangement. The spiral is divided into two groups, namely even spiral and odd spiral. Here all the even spiral bits are joined first then the odd spiral bits are joined [14].

**Single Dimensional Chaotic Behavior (SDCB)**

Chaos theory, sometimes referred to as chaology, is a division of mathematics, physics and a multidisciplinary field of scientific study. Deterministic rules and underlying patterns are highlighted in complex systems that exhibit high levels of reactivity to initial conditions. The seeming randomness of chaotic, challenging techniques, as defined by chaos theory, has underlying patterns, interdependence, continuous loop feedback, self-similarity, a particular geographical shape and self-organization. The equation 1 shows the formula used by the SDCB logic. Where len is the length of bits involved in the message. Prime factor is term as pf that is the next prime number of length +3. The j iterates the value of image/text bytes and i is the next value of j. r is mean by the remaining value in ith iteration.. The picture/text bytes are processed by the single dimension chaotic behavior(SDCB), which shuffles the bytes based on

the formula. The image/text byte length is used as the row size in this method, which creates a single-dimension array [14].

$$P[j] = (pf \times (i+j) \times r^2)\%len \quad (1)$$

The technique uses prime numbers and iterative numbers to produce the estimated positional value. The main objective is to jumble (diffuse) data. Thus, partially encrypted text/ image bytes might be the algorithm's input and output. The message Helloworld has been given to the procedure. The result of the message is rhlodlwleo.

**Knight Tour**

The steps in the Knight Tour algorithm are as follows: Split the length of the message into five bytes. Every five bytes are converted into binary then plotted into the 5 x 7 array. The data are read by 5 x 7 array is traversal like a Chessboard Knight. The same procedure used for other 5 bytes in the message. Finally, every binary bits are converted into characters. Undivided remaining characters are combined directly[14].

**Reverse Spiral Bits**

Reverse spiral Bits is an algorithm of reversing the regular spiral order of the binary bits used in the input [8]. The first step is to convert the text or image bytes to 7bit binary then plotting the binary bits in regular two dimensional array with the length x 8. Figure 6shows the reading method of spiral. After reading the spiral order the binary bits get reversed then converted into ASCII/Bytes [14].

**Dynamic Procedure Calculation (DPC)**

The DPC formula generates a Len number. Len is meant to that length of bytes containing the images/text. B contains every value involved in the username's ASCII values. The DPC will be used to select the sub algorithm in the proposed algorithms chooser.

$$DPC = \sqrt{\left(\prod_{0}^{len-1} B\right)^2} \times len \quad (2)$$

**Example**

Username : HELLO
User content : HELLO
ASCII values : 72, 69, 76, 76, 79
C : 72 x 69 x 76 x 76 x 79
C = 2266918272 C2 = 5138918451927470000l = 5
Multi =5138918451927470000 * 5 = 25694592259637300000

iteration – 1 multi % 3= 2 (1) multi/ 3 = 8564864086545770000
iteration – 2 multi % 3= 0 (2) multi/ 3 = 2854954695515260000
iteration – 3 multi % 3= 1 (3) multi/ 3 = 951651565171753000

The DPC value calculated in the example is '201'. Each step iteration multi's remainder are combined and named as DPC.

**ReArrange Algorithm**

The Rearrange algorithm is used for repositioning the values in the message. The equation 3 used as formula for manipulate the unrepeated position of input given in the algorithm. Posi returns the next position of string. The pf

means prime factor that is the next prime value of the pin. L is the length of input. The ReArrange algorithm is processed in two cycles; one is based on the pin.

$$Pos_i = pf \times (pin + i)\%L \quad (3)$$

The next cycle is based on the tag given in the algorithm. The tag values are converted into ASCII values and total the values and multiplied by seven. Square root of the computed total and substituted into pin for the second cycle of the algorithm.

**Reverse Rail Fence**

The rail fence algorithm is a vintage model. An original content is split into two rows. Then the two rows of characters are read by character one by one from each separated row and combined. But the created rail fence model is quite different from the old model or a small change in the vintage model. That is, even if split row characters are reversed, then combine the characters. For example themessage'Hello123#'has been given to the method then. The procedure split messages as 'hello' and '123#'. Reverse the messages like 'olleH' and '#321'. Finally, the result of Reverse Rail Fence is 'o#l3l2e1H'.

**R3SKT Hash Algorithm**

The password is hashed using the following methods by the R3SKT hashing algorithm: The DNA sequence of uncommon fish is used as salt. The salt is added with method of railfence The DPC value is calculated based on the Equation 2 to choose the sub algorithms used in the R3SKT Hash. The sub algorithms are Reposition, Reverse Spiral and Knight's Tour. The reposition algorithm is a nested form of the SDCB algorithm. During the reposition algorithm, 8 repositioned orders are created and all the repositioned character sets are combined. The reverse rail fence operation will be computed for each and every algorithm's end [13]. A hash is constructed depending on the size of the result hash (64 bytes, 128 bytes). The password 'Helloworld' is given to the algorithm the result of message is 'EVj5px29XPGzYvC~tmx2WrmNhpM6tWM1kZ7Parjqt81WPWZ019Lz4wscUaLmco'.

**HASaRA Algorithm**

This algorithm is a combination of two sub-algorithms. That is HAS and ReArrange. But, three processes mean that one of the sub-algorithm processes twice: one is a based on PIN and another one uses a passcode. The first sub algorithm, uses a delimiter, pin and tag as salt, then cuts down the size as the required size measure used in the array of the message binary bits. The message binary bits are given to HAS algorithm of the cycle process. The output of the cycle 1 is given to input for ReArrange algorithm. The decryption is processed reverse on the HASaRA algorithm [14].

**R3SKT/R3SX Algorithm**

The DPC value was generated depending on the message/Image data and username. The Text/Image has to be converted into a stream of messages/bytes for the encryption algorithm. The DPC-driven sub-algorithm selection creates the execution order. The dynamic policy employs Knight Tour/EX-OR with DPC, Spiral Bits and SDCB as its three sub-algorithms. Because the algorithm used file content and usernames, the selection of sub-algorithms was solely functional. The algorithm for the reverse rail fence will then be used to process and generate the encrypted content and also a onetime pad algorithm is used at the end of processing Result Message function. The R3SKT/R3SKT algorithm is given in Algorithm 1.

Algorithm 1 : R3SKT/R3SX
Input : Message/Image, username
Output : Encrypted Message
Begin
//DPC Calculation

```
For i=0 to len(Username)
 DPC= DPC x ASCII(Username[i])
DPC=DPC x len(Message)
While (DPC != 0)
 temp1=DPC%3
 if (choice.indexOf((char)temp1 +0) == -1)
  choice += temp1
 if(len(DPC)==3) then
  break
 DPC= DPC/3

//Dynamically choosing of sub-algorithms Select (Choice)
 For i=0 to len(Choice)
  Select(Choice[i])

                        case 0:
  Message = SDCB(Message)
  case 1:
  Message = ReverseSpiral(Message)
  case 2:
                        //Instead of Knight Tour for image that uses EX-OR with DPC
  Message = KnightTour(Message)
Message = RRF(Message)
EMessage = ResultMessage(Message)
Return EMessage
End
```

**Ticketadmin and Port Lock Mechanism**

The proposed framework is named as TAaPL. The proposed framework use the password and Mac Address hashes for hashing byR3SKTHash Algorithm. Already the hash is stored on the server's cloud database when created on log in. Everyone is having Mobile phones, Desktop PC or Tablets on handling the drive storages for personal information. So, four MAC address hashes are stored in databases and one is a temporary Mac address hash. Four MAC address stored create/ used log in at a moment. The temporary MAC Address hash is anonymous location to use the guest machines to handle the drive storage. If an anonyms' device is used to get logged in then. Verify the user by using Mobile/Email OTP to confirm the user is validity. Guest / Permanent Mac Hash will get Stored, Hash Ticket is raised and stored on the database. This process is used for narrow down usage of anonym's machines to handle personal storages. The storage servers are secured by a port lock mechanism. Access the individual files is permitted only when the Hash Ticket is valid; otherwise, the user will be logged out. The ticket admin process is given in the algorithm 2.

**Algorithm 2** **:** TicketAdmin
**Input** **:** Username, Password, Mac Address
**Output** **:** Ticket
**Begin**

```
UserHash= R3SKTHash(Username)
PasswordHash= R3SKTHash(Password)
MACHash = R3SKTHash(MacHash)
If(PasswordHash==match(storage.PasswordHash))
                        If(MACHash != match(Storage.MacHash))
                                Raise confirmation // confirmation OTP/ Email
```

```
                    If(confirmation == "Yes")
                        HashTicket = True
                    //Access store /Retrive the Files storage
                    Else
                        HashTicket =False
                        //Action Logout that DeviceElse
//Access store /Retrive the Files storage
                    HashTicket = True
        Else
            //show invalid password to the user
            Error "Invalid Password"
If(HashTicket == True)
            Ticket = RRF(usernameHash, PasswordHash)
Time = CurrentTime()
        //Store Hash Ticket and Time to the Storage.
ReturnHashTicket
End
```

Each server has its own unique passcodes and keys. When the Hash Ticket is validated, the corresponding passcode and key are shared. The HASaRA algorithm is implemented to ensure server-side security, managing the encryption and decryption processes. Each region-specific server utilizes different passcodes and keys. Client-side encryption and decryption are handled by R3SKT and R3SX. R3SKT is used for encrypting textual information, while R3SX is responsible for encrypting image data. Achieving a true end layer is a significant focus of this research. In this approach, only the client can view the original file.

## RESULTS AND DISCUSSION

**R3SKT/R3SX**

The results of the proposed algorithm are superior to those of other client encryption methods. The execution times of 1,000, 2,000 and 3,000 characters are 844 ms, 1,516 ms and 2,281 ms respectively. The results are shown in the fig. 2 and table 1. This method demonstrates a minimum performance improvement of approximately 50 ms compared to others. 3000 characters of message and decrypted text is compared by using Diffchecker online they are identical. The entropy values of the original and encrypted text are both approximately 2.47, indicating that the entropy values are consistent, which suggests effective encryption. The R3SX algorithm compared with other client side encryption algorithms that are given in fig. 3 and table 2. For testing purpose uses 512 x 512 standard lena image.

**TAaPL Framework**

The result of the TAaPL framework text encryption is given the graph 3. The TAaPL Framework is compared with other benchmark algorithms like AES, RSA, DES algorithms. The performance of the TAaPL Framework is given in fig. 4 and table 3.

## CONCLUSION AND FUTURE WORK

The cloud file storage is a huge storage of numerous types of files which is hold. The major issue is most of the CSP is not having own storage and CSP's are uses third party storage. Primary concern is how will provide the security for personal files. Common algorithm like AES256, DES for all the user files is not enough to secure the storages. The proposed framework may give better solution to secure the individual files of the data owner with help of R3SKT, R3SX, HASaRA algorithms, ticket admin and R3SKT Hash. The proposed framework is providing confidentiality of the user files. Data leakage is no possible in cloud storage because of dual cryptography ap-proach plays major role

on this work. R3SKT will be responsible for a client side secu-rity and server side taken care of HASaRA. The TAaPL'sticketadmin is responsible for user validity and port lock mechanism is preventing attacks on the server.

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**Table 1: performance of R3SKT Hashing with other Algorithms (sec)**

| Algorithms (Password) | MD5 | Salted MD5 | PBKDF2 | BCRYPT | R3SKT |
|---|---|---|---|---|---|
| **10 Characters** | 0.63 | 0.112 | 0.203 | 0.487 | 0.110 |
| **20 Characters** | 0.78 | 0.169 | 0.216 | 0.523 | 0.141 |
| **Salted** | No | Yes | Yes | Yes | Yes |
| **Collision Occurrence** | Yes | Yes | No | No | No |

**Table 2: Execution time comparison with other algorithms in ms.**

| Number of characters used for encryption | DES | RSA | AES 256 | HASaRA |
|---|---|---|---|---|
| 1000 | 1582 | 1962 | 1099 | 1072 |
| 2000 | 2058 | 2382 | 1580 | 1516 |
| 3000 | 2583 | 2800 | 2350 | 2281 |

**Table 3: Comparison of client R3SKT Encryption**

| Number of characters used for encryption | CBS | Client-side CBS | AES 256 | R3SKT Encryption |
|---|---|---|---|---|
| **1000** | 1125 | 900 | 1099 | 844 |
| **2000** | 2111 | 1790 | 1580 | 1516 |
| **3000** | 3208 | 2900 | 2350 | 2281 |

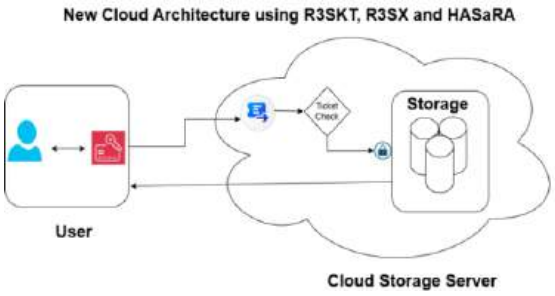



**Fig. 1:New Cloud Architecture for cloud Storage**

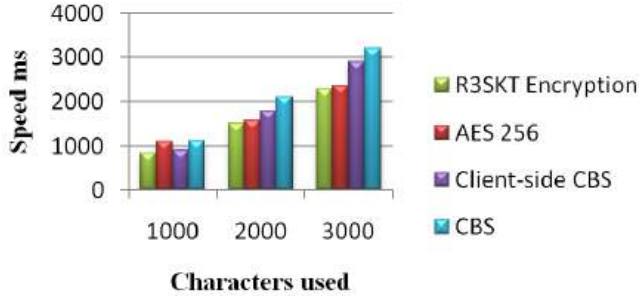



**Fig. 2: Comparison of R3SKT Text**

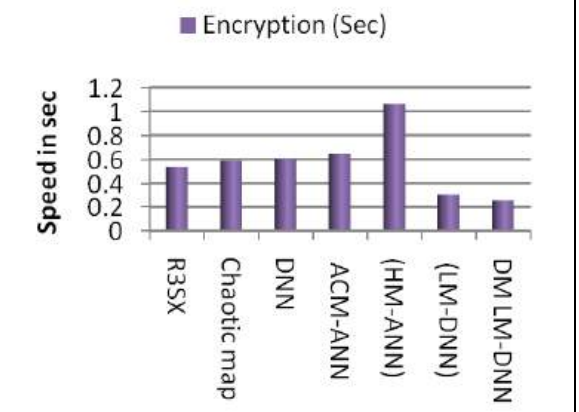



**Fig. 3: Comparison of Other algorithms with R3SX**

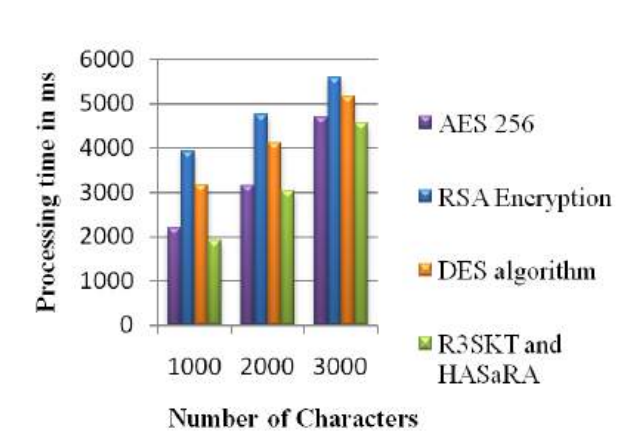



**Fig. 4 : Performance of TAaPL Framework Text Encryption with Other**

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**RESEARCH ARTICLE**

# Comparative Analysis of ZigBee with Different Wireless Protocols for Automated Unmanned Railway Crossing

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## ABSTRACT

This study compares ZigBee with other popular wireless communication protocols for the implementation of an automated unmanned railway crossing system. Unmanned railway crossings continue to pose significant safety challenges, especially in rural and semi-urban areas where manual supervision and sophisticated signaling infrastructure are limited. Wireless communication technologies offer a promising solution for developing automated crossing systems that are both reliable and cost-effective. The study compares the performance of ZigBee, which is based on the IEEE 802.15.4 standard, with that of Bluetooth (IEEE 802.15.1), Wi-Fi (IEEE 802.11), GSM, and LoRa technologies. The findings show that although Wi-Fi offers fast data speeds, it uses a lot of power and needs infrastructural support. Wide-area coverage is guaranteed by GSM, although it has higher latency and ongoing operating expenses. Bluetooth has minimal power consumption, but its network scalability and communication range are constrained. Although LoRa allows for low-power long-distance communication, its slower data rate and latency may make it unsuitable for time-sensitive control tasks. In every metric that was assessed, ZigBee's performance was balanced. It is especially well-suited for localized railway crossing automation because of its low power consumption, modest range, mesh networking capabilities, low latency, and affordability. ZigBee-based wireless architecture offers a dependable, scalable, and financially feasible alternative to improve safety at unmanned railroad crossings, according to the study's findings.

**Keywords:** Unmanned Railway Crossing, ZigBee, Bluetooth, Wi-Fi, LoRa, Sensors, Automation System.

## INTRODUCTION

The research problem and the larger context in which this work is located are introduced in this paper. Especially in developing nations like India, unmanned railway crossings continue to be among the most hazardous and antiquated components of train infrastructure. Intelligent, automatic crossing systems are now essential due to the growing number of trains and automobiles on the road. This chapter describes the main issues with current systems, explains why the research was conducted, and suggests the study's general goal and aims. To solve the problem, it also presents the idea of utilizing embedded microcontroller systems and ZigBee-based wireless communication. The research methods, the thesis organization, and the extent of the study are also presented in this paper.

**The Need for a Scalable, Real-Time Wireless Solution**

Given the drawbacks of current electrical systems and manual control, an intelligent, real-time, energy-efficient solution is obviously needed:

a. Sends signals wirelessly with low latency
b. Detects train arrival reliably
c. Is cost-effective and power-efficient for rural deployment
d. Controls barrier gates automatically

## AIM OF THE RESEARCH

Automatic railway gate control system is implemented to prevent traction accidents at railway crossing by utilizing the concept of multilevel sensor based controlling system. The technique materialized in a specific range along the railway track before and after the crossing gate. The range will depend upon average speed of trains passing through tracks present at crossing. The advent of trains at crossing will be conveyed through different significant means that are easily perceived by people passing through the crossing. The main goal of this research is to use multi-level wireless communication, primarily ZigBee technology, to design, implement, and assess a fully automated unmanned railway crossing system. Enhancing safety, reducing reliance on humans, and offering a scalable solution for broad implementation across India's rural and semi-urban railway crossings are the goals of the suggested system. This thesis aims to design, develop, and calibrate an automated railway crossing model using:

a. ZigBee wireless transmission
b. Arduino-based decision logic
c. LDR-based detection
d. Display and buzzer alert systems
e. Servo-actuated gate control

This study aims to improve safety, lessen reliance on human labor, and advance scalable smart infrastructure for Indian Railways by tackling the issue.

**Geographical and Environmental Limitations**

Only low-speed to moderate-speed railroad lines, which are frequently found in non-urban rail corridors, are included in the scope. The prototype is made to be deployed in open-field settings, taking into consideration changes in dust, light, and weather. It does not, however, take into consideration automated signaling systems run centrally by Indian Railways, multi-line rail crossings, or high-speed bullet trains.

## LITRATURE REVIEW

Literature reviews to compare different types of wireless protocols are as follows:

**Manual and Infrared-Based Systems**

Manual railway gate systems are still in use in many rural districts of India. An Automatic Railway Gate Controller system was developed by research like that done by Chandrappa *et al*. [1] to control railway gates without the need

for a gatekeeper. Two infrared obstacle detection sensors [2] detect the arrival and departure of trains, and a stepper motor controls the gate.To detect train arrival and departure at a railway crossing and control the opening and closing of the gates using servo motors, Vinit P. Kharkar [3] described a system that makes use of Infra-Red (IR) sensors and a Raspberry Pi.Dr. S. Anila, B. *et al.* [4] suggested a technique that can be utilized in isolated locations without a lineman or station master. The train will be positioned three kilometers from the level crossing on both sides, and two infrared sensors will be utilized to monitor its arrival and departure.

**GSM and GPS-Based Communication Models**

A GPS-based system was developed by Velayutham *et al*. [5] to track and identify the arrival of the train. The GPS is used to track the train, and Google Maps shows the rail tracks. Therefore, upon preparation, GPS is implanted into the flexible application [6].Mahdi and A. S. Al-Zuhairi [7], presents a model that uses an 8052 microcontroller to keep a safe distance from railroad accidents that occur at unattended rail route doors. Naga Hema Kumari V *et al*. [8] proposes a sensor-based system to lessen train-train accidents brought on by a communication breakdown between the rail traffic control office and the train operators.

**RFID, RF, and Ultrasonic Detection Approaches**

A method developed by Chandrappa S. *et al*. [9] uses four infrared sensors to identify trains. The distance between two infrared sensors and the railway crossing is seven and six kilometers, respectively. Only when the train is detected by both sensors within a predetermined amount of time will the gates at the level crossing close. This mechanism was in existence on both sides of the level crossing. An extra feature has been introduced for the convenience of the passengers: each train is assigned a unique RFID tag, and passengers are required to register their destination with it.

**Bluetooth, Rasberry Pi and Wi-Fi Communication Systems**

As recommended by E. Amarnatha Reddy *et al*. [10], the study's objective is to develop a prototype that operates a railway gate using a microcontroller. Every time the train arrives at the base at the sensor crossing, the public is informed at the railway crossing. The control gates are subsequently opened and closed by the control module. When the train arrives at the railroad crossing, automatic gates are opened. The logic was developed into embedded C and then transferred to the Raspberry Pi.

**IOT, LoRa and Cloud-Based Systems**

The Internet of Things (IoT) is the network of physical objects, including gadgets, automobiles, buildings, and different electronics, programming, and sensor-related products, that allow these objects to gather and exchange data [11]. Dhande *et al*. [12] [13] recommended employing a GPS module to capture geographic coordinates and a failing track and GSM modem to send the coordinates of a spot. P.C. Shruthi *et al*. [14] an effective Internet of Things-based solution involved placing two infrared sensors at a predefined distance to measure the train's speed and the time it took to reach the level crossing.

**AI-Enabled and Hybrid Systems**

Using image processing, Tastimur *et al*. [15] suggested a vision-based approach to preventing railroad accidents. The train had a camera installed to identify any impediments in the level crossing. A study on the causes and locations of rail elephant collisions was proposed by Mukti Roy *et al*. [16]. In order to develop an elephant hotspot map, the paper conducted surveys to determine the areas with the highest risk of mishaps. Liu Wei *et al*. [17] suggested a Feature Fusion Refine Neural Network-based Traffic Object Detection system. The study proposed a strategy to reduce train accidents that occur during the shunting mode due to human mistake in assessing railway conditions. Lokeshkumar R. and Omkar Vivek Sabnis [18] presented a technology that may be used for automation at a railway crossing. A camera has been installed at the railroad crossing for surveillance reasons.

**ZigBee-Based Wireless Embedded Systems**

The control of a railway level crossing is experimentalized by B. Kumar Sanjiv *et al*. [19]. The railway gates are opened and closed by means of DC motors that are controlled by the micro controller using ZigBee. Dr. I. M. Korachagaon *et al*. [20] used ZigBee technology to stop train accidents. ZigBee is a state-of-the-art technology with high-level communication protocols that are used to build personal area networks from small, low-power digital radios. Railway system automation using ZigBee technology was covered by P. Mehta *et al*. [21]. The study most likely investigates the use of ZigBee to automation activities in railway environments, such as monitoring train movements and regulating railway gates. A prototype controlled by ZigBee in real time was implemented by Arun Kumar Gangwar *et al*. [22]. The model combined ZigBee for wireless control with LDRs for detection and Arduino for logic processing. Even in the presence of changing environmental circumstances, this system showed strong communication and low latency. The calibration and performance analysis of a real-time ZigBee-controlled prototype, contrasting ZigBee with LoRa and GSM, is the main topic of Arun Kumar Gangwar *et al*. [23].

**Comparative Analysis of Protocols**

Table 2.1 provides a comparative overview of various wireless protocols and sensing mechanisms used in previous literature. The comparative analysis summarizes the range, power use, scalability, cost, real time suitability and limitations of various protocols.

**Comparative Analysis of Various Wireless Protocols (Table 1).**

ZigBee is the best-rounded and field-tested technology for automating unmanned railway crossings, according to a survey of 48 papers. ZigBee offers better dependability, affordability, and energy efficiency than IR, GSM, GPS, LoRa, or AI-based systems, especially in rural Indian settings. The system built in this thesis is based on the author's own published and validated research, which strongly supports the adoption of ZigBee for this application.

## METHODOLOGY AND IMPLEMENTATION OF WORK

The procedure used to design, construct, and evaluate the proposed unmanned railway crossing system is described in this chapter. It explains the components, detecting methods, wireless communication modules, system design, and general functional flow. The methodology's objectives are to provide precise train arrival detection, dependable control unit communication, and safe, automated railway gate operation devoid of human interaction.

**System Architecture Overview**

The proposed system is divided into three main units

**Detection Unit:** The detecting unit detects the arrival and departure of the train using Light Dependent Resistors (LDRs). Both sides of the track have LDRs installed to detect trains coming and departing.

**Communication Unit:** The communication unit uses ZigBee wireless modules to provide reliable, low-power, short-delay communication between sensors and the control unit in real-time.

**Control and Execution Unit:** The Arduino microcontroller serves as the foundation for the control and execution unit. The railway gate is opened and closed by means of servo motors controlled by an Arduino microcontroller. To warn cars and pedestrians, the control unit turns on a buzzer, display, and LED indicator. Block Diagram of the System Figure 1.

**Flow of Operation**

The operation follows the following steps:

a. A change in light intensity is used by the LDR circuit to identify an approaching train.
b. After receiving the signal, the Arduino board uses ZigBee to transmit wireless instructions.
**c. The receiving unit activates:**

i. Gate closure through servo motor control
ii. Audio alert via buzzer
iii. Visual display for train warning
d. Once the train departs, the second LDR confirms clearance.
e. Before opening, a time-delay circuit makes sure the gate stays closed for a few more seconds.
f. Alerts are turned off and the gate opens automatically.

**Developed Prototype**
To compare the different types of wireless protocols with ZigBee for automation of unmanned railway crossing, a prototype is developed as shown in figure 2.

**Developed Prototype (Figure 2).**
As per consideration of human life and time, we have proposed a prototype for the automatic control of the railway crossing barriers. On testing our project, we found that the sensing circuit properly detects the arrival of the train, and it responds to provide the pulse for LDR circuits that transmits the corresponding signal and it further activates the Arduino and it makes the all three circuits- buzzer, display and barrier circuit to ON. All these operations have been executed properly and the series LDR circuit senses the movement on the crossing of the people and vehicles by checking the continuity of infrared light projected on to all three LDRs which are connected in series. If any of the LDR is not receiving the infrared light, it gives high output at the crossing. In corresponding to the series LDR circuit output low or high, the motor either runs to close the barrier at high output or sends the signal to the train through which the train stops. After some delay, a signal is sent to manually close the barrier. Again, this operation has performed well accurately.

## RESULT AND ANALYSIS

In the above way I have tested the operation of our project and found that all the circuits are operating properly, and this technique will fulfil the requirements of modern people as time and life are very important for every person. By using ZigBee modules, I can also increase the range of transmission and increase the sensitivity of the project for signal detection. It can make my project more efficient.

## RESULTS AND OBSERVATIONS

**Train detection accuracy**
Twenty test runs were conducted. The system was able to detect the train successfully in 19 out of 20 test cycles.

**Train Detection Accuracy Table 2.**
**Accuracy** = 95%

**Gate actuation response time**
Average time taken between detection and complete gate closure:
**ZigBee System:** 2.36 seconds
**GSM (simulated):** 4.85 seconds
**LoRa (simulated):** 3.92 seconds
Gate Actuation Response Time with Different Protocols (Figure 3)
ZigBee takes lowest gate actuation response time in comparison to other protocols.

**Signal transmission delay**
Signal transmission delay of different protocols are as follows:

Signal Transmission Delay of Different Protocols (Table 3). Signal Transmission Delay of Different Protocols (Figure 4). ZigBee achieved lowest average transmission delay, confirming suitability for real-time applications.

**Communication reliability**

To check the communication reliability for different protocols the success rate are taken with the help of prototype over 50 test signals are as follows:

**ZigBee:** 98%
**GSM:** 84%
**LoRa:** 90%
**Wi-Fi:** 93%

Communication Reliability of Different Protocols (Figure 5).
ZigBee has the highest success rate of communication, over 50 test signals for different protocols.

**Power consumption**

Power drawn per cycle (sensor + TX + control) for different protocols are as follows:
Power Consumption for Different Protocols (Table 4). Power Consumption for Different Protocols (Figure 5).
ZigBee demonstrated high energy efficiency, ideal for rural deployment with limited power availability.

**Analysis and Interpretation**

Based on result analysis the interpretations are as follows:

a. ZigBee outperformed GSM, LoRa, and Wi-Fi in terms of response time, energy consumption, and reliability.
b. Detection accuracy of 95% proved that LDR sensors with shielding and calibration are effective for practical deployment.
c. Gate actuation within 2.5 seconds is well within the safety threshold for unmanned railway crossings.
d. Power use was low enough to support solar or battery-powered remote installations.
e. These findings confirm the suitability of ZigBee-based embedded automation for railway gate control, especially in semi-urban and rural areas.

## DISCUSSION

The system performed well across various test scenarios. ZigBee enabled stable communication even with minor interference. While the LDR-based detection was functional, enhancements with dual IR sensors or machine vision could further increase reliability. The time delay circuit introduced an extra safety buffer, making the system compliant with real-world safety protocols. The cost-effective and scalable nature of this design makes it suitable for mass deployment in semi-urban and rural rail crossings.

## CONCLUSION

This research presents the design, implementation, and validation of a fully automated unmanned railway crossing system using ZigBee wireless communication and embedded microcontrollers. The project aimed to address the persistent safety risks associated with unmanned railway crossings, particularly in rural and semi-urban areas, by developing a cost-effective, low-power, and real-time automated solution. The proposed system utilizes LDR sensors for train detection, Arduino UNO microcontrollers for signal processing, and ZigBee modules for wireless data transmission between detection and actuation units. A servo-controlled gate mechanism, along with audio-visual alerts, ensures that nearby road users are warned of incoming trains in advance, thereby enhancing overall public safety.

The prototype was experimentally tested under various simulated conditions. The key outcomes of the implementation are:

a. Train detection accuracy of 95%, validated over 20 test cycles

b. Gate actuation response time of 2.36 seconds, significantly lower than GSM or LoRa-based systems
c. Signal transmission reliability of 98% over ZigBee, with a minimal delay of 86 milliseconds
d. Energy efficiency, making it ideal for remote deployments powered by solar or batteries
e. Scalable and modular design, adaptable for multi-track or multi-sensor environments

The research findings align well with contemporary studies and support the claim that ZigBee is one of the most balanced and efficient protocols for wireless automation in transportation safety systems.

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**Table 1: Comparative Analysis of Various Wireless Protocols**

| Protocols | Range | Power Use | Scalability | Cost | Real-Time Suitability | Limitation |
|---|---|---|---|---|---|---|
| Manual | Local | None | Not scalable | Low | Poor | Human-dependent, unsafe in emergencies |
| IR | Short | Low | Poor | Low | Limited | Weather- and alignment-sensitive |
| GSM | Long | Medium | Moderate | Medium | Moderate | Network dependent, signal delay |
| GPS | Long | High | Low | High | Moderate | Tunnels and hills cause signal loss |
| RFID | Fixed | Low | Moderate | Medium | Moderate | Checkpoint-only, expensive tags |
| RF | Medium | Medium | Low | Medium | Poor | Noise interference |
| Bluetooth | Short | Low | Poor | Low | Poor | Short range, instability |
| Wi-Fi | Medium | High | Moderate | Medium | Good | Power intensive, signal loss in open |
| LoRa | Long | Low | High | High | Poor | Latency, not ideal for gate actuation |
| IoT | Long | High | High | High | Limited | Internet and cloud dependency |
| ZigBee | Medium | Low | Excellent | Low | Excellent | Range slightly shorter than LoRa |

**Table 2: Train Detection Accuracy**

| Test Cycle | Detection (Yes/No) |
|---|---|
| 1–10 | Yes (100%) |
| 11–20 | 9 Yes, 1 No |

**Table 3: Signal Transmission Delay of Different Protocols**

| Protocol | Average Delay (ms) |
|---|---|
| ZigBee | 86 |
| GSM | 348 |
| LoRa | 480 |
| Wi-Fi | 135 |

**Table 4: Power Consumption for Different Protocols**

| Protocol | Avg. Power (mW) |
|---|---|
| ZigBee | 120 |
| GSM | 280 |
| Wi-Fi | 310 |
| LoRa | 150 |

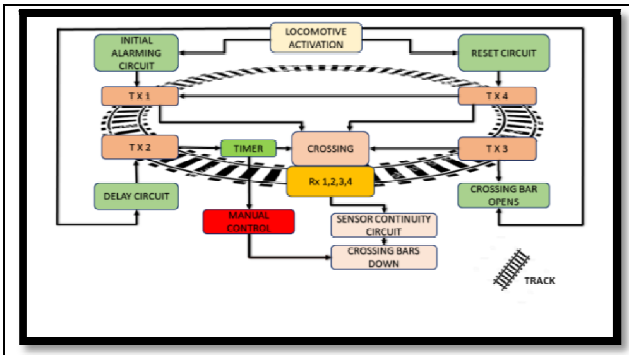



**Figure 1: Block Diagram of the System**



**Figure 2: Developed Prototype**

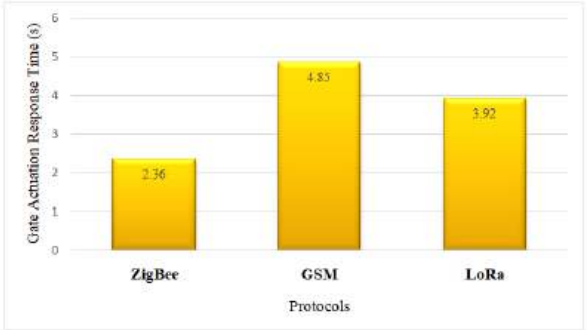



**Figure 3: Gate Actuation Response Time with Different Protocols**

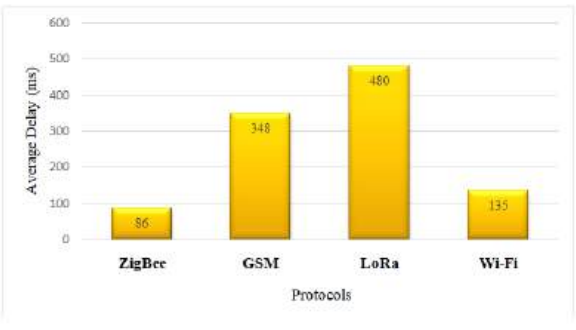



**Figure 4: Signal Transmission Delay of Different Protocols**

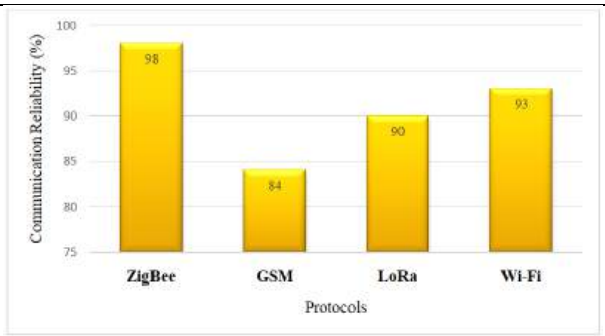



**Figure 5: Communication Reliability of Different Protocols**

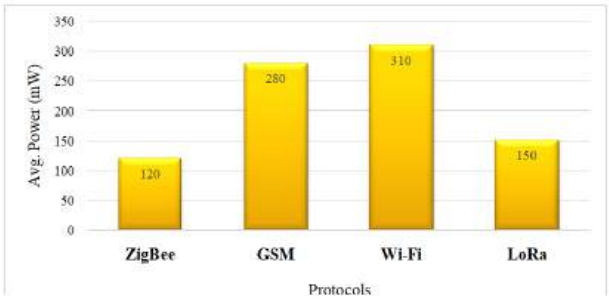



**Figure 6: Power Consumption for Different Protocols**

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**REVIEW ARTICLE**

# RP-HPLC Comprehensive Profiling and Simultaneous Quantification of Phenolic Acids in *Carissa carandas :* A Review

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**ABSTRACT**

Karonda (*Carissa carandas*) is a fruit widely distributed in tropical and subtropical regions and is recognized for its considerable nutritional and medicinal significance. The fruit contains several bioactive phenolic constituents, among which gallic acid and chlorogenic acid are prominent due to their reported antimicrobial, anti-inflammatory, and antioxidant activities. Accurate quantification of these compounds is essential for phytochemical evaluation, quality control, and standardization of Karonda fruit and its derived formulations. High-performance liquid chromatography (HPLC) is widely accepted as a reliable and precise analytical technique for the determination of phenolic acids in complex plant matrices. This review compiles reported HPLC method development approaches, chromatographic conditions, sample preparation procedures, validation parameters, and analytical applications related to the estimation of gallic acid and chlorogenic acid in Karonda fruit. The article aims to serve as a comprehensive reference for researchers engaged in analytical and phytochemical investigations of *Carissa carandas*.

**Keywords:** Karonda, *Carissa carandas*, Chlorogenic acid, Gallic acid, HPLC, Method development, Phenolic acids

# INTRODUCTION

The antioxidant and medicinal qualities of plants are largely attributed to phenolic compounds, a significant class of secondary metabolites [1,2]. Due to their widespread presence in fruits and medicinal plants as well as their established health benefits, chlorogenic acid and gallic acid have drawn a lot of attention [3–5]. The underutilized fruit crop Karonda (*Carissa carandas L.*) which is a member of the Apocynaceae family, has long been utilised in Southeast Asian and Indian medicine [6,7]. The plant, which thrives in arid and semi-arid climates, is commonly found in parts of Africa, Southeast Asia, and India [8–10]. It yields tiny, berry-like fruits that are high in vital nutrients like dietary fibre, vitamins, and minerals [11,12]. The bioactivity of Karonda fruits is greatly enhanced by their high concentration of polyphenolic compounds, such as flavonoids and phenolic acids [13,14]. Numerous studies have suggested that these substances may provide protection against oxidative stress, which is linked to the aetiology of many chronic illnesses, such as cancer, diabetes, and cardiovascular disorders [15,16]. The fruit, which has a strong antioxidant potential, can be eaten raw or processed into pickles, jams, and drinks [17]. According to scientific research, the two main phenolic components of Karonda fruit are gallic acid and chlorogenic acid. Accurate estimation of these compounds requires the use of trustworthy analytical techniques. High-performance liquid chromatography (HPLC) is favoured over other analytical methods because of its sensitivity, specificity, and repeatability. The development of HPLC-based methods for estimating gallic acid and chlorogenic acid in Karonda fruit is the main topic of this review. The Carissa species has been widely used to treat a wide range of illnesses. Acidity, sores, flatulence, and indigestion are all signs of a weakened immune system. Diarrhoea, stomach problems, anorexia, intermittent fever, mouth ulcers, sore throat, syphilitic discomfort, burning sensations, scabies, and epilepsy are some of the human ailments. Vitamin C, dietary fibre, carbohydrates, lipids, proteins, and microelements are all abundant in Carissa fruits [17,18]. Traditionally, the majority of Carissa species have been used to treat a wide range of illnesses, including headaches, chest pain, rheumatism, gonorrhoea, stomach pain, syphilis, hepatitis, oedema, rabies, asthma, and heart conditions. The fruit significantly reduced blood sugar levels in diabetic rats, according to a 2013 study published in the Journal of Ethnopharmacology. According to a different study that was published in the same journal in 2014, karonda extract improved the liver function of rats.

**Botanical and Phytochemical Overview**

*Carissa carandas*, commonly known as Karonda, is a tropical and subtropical evergreen shrub belonging to the family Apocynaceae. It is widely distributed in India, Sri Lanka, Nepal, Bangladesh, and other Southeast Asian countries. The plant grows well in dry, arid, and semi-arid regions and is highly tolerant to drought conditions, making it suitable for cultivation in marginal lands. The plant is a dense, spiny shrub that typically grows up to 2–4 meters in height. It possesses strong, sharp thorns that act as a protective mechanism against herbivores. The leaves are simple, opposite, ovate to elliptic in shape, dark green, and leathery in texture. The glossy surface of the leaves helps reduce water loss through transpiration[19]. The flowers of Karonda are white to pinkish-white in color, fragrant, and star-shaped. They usually bloom during the warm season and are arranged in terminal cymes. The pleasant fragrance of the flowers attracts pollinators, facilitating reproduction[20]. The fruit is a berry that changes color as it matures. Initially, it is green, then turns pink or red, and finally becomes dark purple or nearly black when fully ripe. The fruit contains 2–8 small brown seeds embedded within the pulp. The pulp is juicy, acidic to sweet-sour in taste, and rich in bioactive compounds [21]. The variation in color during ripening indicates changes in phytochemical composition, especially anthocyanin accumulation. Karonda is cultivated both as a fruit crop and as a hedge plant due to its dense growth and thorny branches. It plays an important role in traditional medicine and is used in preparing pickles, jams, syrups, and chutneys. Karonda fruit is considered nutritionally and medicinally valuable because of its rich phytochemical profile. The fruit contains a variety of bioactive compounds, including phenolic acids, flavonoids, anthocyanins, tannins, alkaloids, and vitamins [22]. Phenolic acids are one of the major phytoconstituents present in Karonda fruit. Compounds such as gallic acid, caffeic acid, ferulic acid, and *p*-coumaric acid have been reported. These phenolic compounds are responsible for strong antioxidant activity and help in neutralizing free radicals. The concentration of phenolics may vary depending on ripeness and geographical origin[23]. Flavonoids such as quercetin, kaempferol, and rutin are also present in the fruit. These compounds exhibit anti-inflammatory,

antimicrobial, cardioprotective, and anticancer properties. Flavonoids contribute significantly to the therapeutic potential of the fruit. Anthocyanins are responsible for the red to purple coloration of ripe Karonda fruits. These pigments possess potent antioxidant properties and may help in preventing oxidative stress-related diseases. The anthocyanin content increases as the fruit ripens, which explains the color transformation from green to dark purple [24]. Karonda fruit contains tannins that contribute to its astringent taste, especially in unripe fruits. Tannins have antimicrobial and anti-diarrheal properties and are commonly associated with traditional medicinal uses. The fruit is a good source of vitamin C (ascorbic acid), which enhances immune function and acts as a natural antioxidant. It also contains iron, calcium, and phosphorus in moderate amounts. Due to its iron content, Karonda has been traditionally used to manage anemia. In addition to phenolics and flavonoids, the fruit may contain triterpenoids, glycosides, and organic acids. These compounds contribute to the fruit's pharmacological activities such as anti-diabetic, anti-inflammatory, hepatoprotective, and antimicrobial effects [25]. The rich phytochemical composition of *Carissa carandas* makes it valuable in traditional and modern medicine. The antioxidant activity of phenolic compounds helps in preventing cellular damage caused by free radicals. The antimicrobial activity supports its use in treating infections. The presence of iron and vitamin C enhances its nutritional significance. Several studies have correlated the phytochemical content of Karonda fruit with its biological activities, supporting its potential use in pharmaceutical and nutraceutical formulations. Among the phenolic acids, chlorogenic acid and gallic acid contribute significantly to the fruit's antioxidant activity and medicinal value.

**Chemical Characteristics(Fig 1 given below)**

**Chlorogenic Acid**

- Ester of caffeic acid and quinic acid
- Molecular weight: 354.31 g/mol
- Highly polar
- UV absorption maximum around 325 nm

**Gallic Acid**

Trihydroxy benzoic acid

- **Molecular weight:** 170.12 g/mol
- Strong UV absorption at 270–280 nm
- Their polarity and UV absorption properties make them suitable for analysis using reverse-phase HPLC with UV or diode array detection.

**Importance of Hplc in estimation of phenolic acids**

One of the most popular analytical methods for determining the amount of phenolic acids in plant matrices, such as gallic acid and chlorogenic acid, is high-performance liquid chromatography (HPLC) [24]. Efficient separation and precise quantification of structurally similar phenolic compounds are made possible by the technique's high resolution and sensitivity [25]. When examining complex plant materials, like the fruit of the Karonda (Carissa carandas), which contains a variety of interfering substances like sugars, organic acids, pigments, and other secondary metabolites, this is especially crucial[26]. HPLC reduces analysis time and solvent consumption by enabling the simultaneous estimation of several phenolic compounds within a single analytical run[27]. Additionally, the technique is very accurate and repeatable, which makes it appropriate for regular quality control and standardisation research[28]. Because it works well with polar compounds and aqueous mobile phases, reverse-phase HPLC (RP-HPLC) with C18 columns is the most widely used chromatographic mode for phenolic acid analysis[29]. As a result, RP-HPLC is now the recommended technique for determining the amount of gallic and chlorogenic acid in Karonda fruit.

**HPLC Method Development for Karonda Fruit**

The sample preparation procedure including collection, washing, drying, pulverization, extraction, filtration, and storage conditions. The preparation of standard solutions is described by accurately weighing the standard compound and dissolving it in suitable solvent to prepare stock and working solutions. The shorten extraction times

and increase extraction efficiency, advanced extraction methods like ultrasonication-assisted extraction have become more and more popular [30]. When thorough extraction is necessary, conventional techniques like Soxhlet extraction are also used[31]. Prior to analysis, the samples are usually filtered through 0.45 μm membrane filters to eliminate particulate matter and safeguard the HPLC column [32]. The chromatographic conditions are column type, mobile phase composition, flow rate, injection volume, detection wavelength, and run time. The procedure for preparation of calibration curve is described by injecting different concentrations and recording peak areas. The method validation includes linearity, LOD, LOQ, accuracy, precision, specificity, and robustness, along with acceptance criteria. Statistical analysis is described by expressing results as mean ± standard deviation and calculating %RSD. Thus, a properly developed and validated HPLC method ensures accurate quantification of bioactive compounds present in *Carissa carandas* fruit and supports its pharmaceutical, nutraceutical, and research applications.

**HPLC Method Development Parameters for Karonda Fruit**

The first step in method development is the selection of a suitable chromatographic column [33]. For the analysis of phenolic acids, flavonoids, and anthocyanins present in Karonda fruit, a reverse phase C18 column is commonly selected because it provides good retention and separation of moderately polar compounds [34]. A typical column dimension used is 250 mm × 4.6 mm with 5 μm particle size, which offers a balance between resolution and analysis time. The mobile phase is selected based on the polarity and solubility of the target compounds. Generally, a binary solvent system consisting of water containing 0.1% orthophosphoric acid or formic acid (as solvent A) and methanol or acetonitrile (as solvent B) is used [35]. The acidic modifier improves peak symmetry and reduces tailing of phenolic compounds. The separation may be carried out using either isocratic elution [36], where the mobile phase composition remains constant, or gradient elution, where the proportion of organic solvent gradually increases to achieve better separation of multiple components. The flow rate of the mobile phase is usually maintained at 1.0 mL per minute for analytical columns. A controlled and consistent flow rate ensures stable retention time and reproducible results. The detection wavelength is selected based on the maximum absorbance (λmax) of the analyte. Phenolic acids are generally detected at 254–280 nm, flavonoids at 280–360 nm, anthocyanins around 520–540 nm, and vitamin C near 245 nm [37]. A UV or Photodiode Array (PDA) detector is commonly used to monitor the separated peaks. The injection volume is typically maintained between 10 and 20 μl to avoid peak distortion or overloading of the column. The column temperature is maintained at approximately 25–30°C to improve reproducibility and reduce variation in retention time. The total run time is optimized to ensure complete elution of all compounds without unnecessary extension of analysis time[38,39].

**Applications of Hplc Estimation in Karonda Fruit**

High-Performance Liquid Chromatography (HPLC) is an advanced analytical technique widely used for the separation, identification, and quantification of phytochemical constituents present in medicinal plants and fruits. In the case of *Carissa carandas* (Karonda), HPLC plays a crucial role in analyzing its rich phytochemical profile and establishing its pharmaceutical and nutritional importance[41,42]. Karonda fruit is rich in phenolic acids, which are responsible for its strong antioxidant activity. HPLC allows precise separation and quantification of phenolic compounds such as gallic acid, caffeic acid, ferulic acid, and *p*-coumaric acid. By determining the exact concentration of these compounds, researchers can evaluate the antioxidant capacity of the fruit[43,44].. This is particularly important in pharmaceutical research where phenolic content is correlated with therapeutic potential. The deep red or purple colour of ripe Karonda fruit is due to anthocyanins. HPLC is used to separate and quantify individual anthocyanin compounds, which are natural antioxidants and natural food colorants[45,46].. This analysis is useful in food science research for evaluating the fruit's potential as a natural colouring agent and for studying its health benefits. Karonda fruit contains a significant amount of vitamin C. HPLC provides a reliable and accurate method for estimating ascorbic acid content. This estimation helps in determining the nutritional value of fresh fruits and processed products such as juices, jams, and syrups. It also assists in studying the stability of vitamin C during storage and processing. In herbal medicine and phytopharmaceutical industries, standardization is essential to ensure consistency and safety. HPLC is used to establish chromatographic fingerprints of Karonda fruit extracts[47,48] . These fingerprints help in identifying authentic samples and detecting adulteration. It ensures batch-to-batch consistency in herbal formulations and nutraceutical products. Flavonoids such as quercetin, kaempferol,

and rutin are bioactive compounds present in Karonda fruit[49]. HPLC enables accurate detection and quantification of these flavonoids even at low concentrations. The estimation of flavonoids helps in assessing anti-inflammatory, antimicrobial, and cardioprotective properties of the fruit. This application is valuable in developing nutraceutical formulations. HPLC is applied in stability studies to monitor the degradation of bioactive compounds over time. By analyzing samples stored under different conditions (temperature, humidity, light), can determine the shelf life of Karonda-based products. This is important for maintaining product efficacy and safety. HPLC data helps in correlating phytochemical concentration with biological activities such as antioxidant, antimicrobial, and antidiabetic effects[50,51]. Quantitative estimation allows researchers to identify which compound is primarily responsible for specific pharmacological actions. This supports evidence-based validation of traditional medicinal uses. In the food industry, HPLC estimation is used for nutritional labeling and quality assessment. It helps detect synthetic additives or contaminants and ensures that the product meets regulatory standards [52,53]. It also assists in evaluating changes in phytochemical content during processing and storage. These include the creation of nutraceutical and functional food products, standardization of herbal formulations, and quality control of raw fruit ingredients[54]. Additionally, quantitative analysis of phenolic acids supports antioxidant, pharmacological, and stability studies, thereby validating the therapeutic potential of Karonda fruit[55].

## CHALLENGES AND FUTURE PERSPECTIVES

One of the major challenges in the HPLC analysis of *Carissa carandas* fruit is the complexity of its phytochemical composition. The fruit contains a wide variety of phenolic acids, flavonoids, anthocyanins, organic acids, and vitamin C, which may co-elute during chromatographic separation [56]. Achieving complete resolution of all compounds within a reasonable run time requires careful optimization of mobile phase composition, gradient program, and column selection. Another important challenges are variability in phytochemical content due to geographical location, climatic conditions, soil type, and stage of ripeness. Fruits collected from different regions or harvested at different maturity stages may show significant variation in phenolic and anthocyanin content[57]. This variability makes standardization difficult and requires comprehensive sampling and validation. The instability of certain bioactive compounds also poses a challenge. Anthocyanins and vitamin C are highly sensitive to light, temperature, oxygen, and pH changes. Improper sample handling or storage can lead to degradation, resulting in inaccurate quantification. Therefore, strict control of storage conditions and rapid analysis is essential. Matrix interference is another limitation in plant extract analysis. The presence of sugars, organic acids, pigments, and other secondary metabolites may interfere with detection and affect peak purity[58,59].. Additional sample cleanup procedures such as solid-phase extraction may be required to improve specificity. Instrumental factors such as column deterioration, fluctuation in detector sensitivity, and mobile phase contamination can also affect reproducibility. Regular system suitability testing and maintenance are necessary to ensure consistent performance. Cost and availability of high-purity reference standards represent another practical challenge.

Some phytochemicals present in Karonda fruit may not be commercially available as pure standards, which limits accurate identification and quantification. Future research can focus on the development of more advanced chromatographic techniques such as UHPLC (Ultra High Performance Liquid Chromatography) [60,61], which offers better resolution, shorter run time, and improved sensitivity compared to conventional HPLC. Coupling HPLC with mass spectrometry (LC–MS/MS) [62] can enhance compound identification and enable detection of minor constituents in complex plant matrices. There is scope for developing validated fingerprinting methods for standardization of *Carissa carandas* extracts. Chromatographic fingerprinting can serve as a quality control tool in herbal drug manufacturing and nutraceutical industries. Further studies may explore comparative phytochemical profiling of fruits collected from different geographical regions to establish chemotypic variations. This can help in selecting high-yield varieties for pharmaceutical applications[63,64]. Research can also focus on bioactivity-guided fractionation, where HPLC is used to isolate specific compounds responsible for antioxidant, antimicrobial, antidiabetic, or anti-inflammatory activities [65-68]. Such studies may lead to the development of novel phytopharmaceutical agents. In addition, the application of green analytical chemistry principles, such as the use of

eco-friendly solvents and reduced solvent consumption, can be considered for sustainable analytical practices[69,70]. Development of stability-indicating methods for processed products such as juices, syrups, and nutraceutical formulations prepared from Karonda fruit will further enhance its industrial applicability. We review some of the parameters related to gallic acid and chlorogenic acid extraction and they are given in the following table1 and 2. Parameters of Gallic acid Extraction from Various Plants and Tablet (Table 1). Parameters of Chlorogenic acid Extraction from Various Plants and Tablet (Table 2).

## CONCLUSION

The present review successfully established a reliable and validated RP-HPLC method for the comprehensive profiling and simultaneous quantification of major phenolic acids present in *Carissa carandas* fruit. The developed chromatographic conditions provided efficient separation of targeted phenolic compounds with good peak resolution, symmetrical peak shapes, and stable retention times. The method demonstrated high specificity, as no significant interference from other phytoconstituents or matrix components was observed at the retention times of the selected analytes. The developed RP-HPLC method successfully enabled comprehensive profiling and simultaneous quantification of major phenolic acids present in *Carissa carandas* fruit. The method showed good peak resolution, excellent linearity, high sensitivity, and satisfactory accuracy and precision as per validation guidelines. The presence of significant levels of phenolic acids confirms the strong antioxidant potential of the fruit and supports its medicinal value. The method is simple, reliable, and suitable for routine quality control and standardization of Karonda fruit extracts and related formulations. Reliable quantification is ensured by appropriate chromatographic condition optimisation and adherence to validation guidelines, supporting the standardisation, quality control, and scientific application of Karonda fruit in the food and pharmaceutical industries.

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**Table 1: Parameters of Gallic acid Extraction from Various Plants and Tablet**

| Plant | Plant part | Extraction method and solvent | HPLC Column | Retention time | Peak area/Concentration | Analysed Compound |
|---|---|---|---|---|---|---|
| *Terminala bellerica* | Fruit | Soxhlet and Chloroform → Methanol → Ethanol | Phenomenex Luna 250 × 4.6 mm, 5 µm | 3.60 | 3,190.82 | Methanolic and ethanolic extracts analyzed |
| *Woodfordia fruticosa* | Flower | Soxhlet (hot percolation) and Methanol : Water (7:3) | RP-C18 250 × 4.6 mm, 5 µm | 3.36 | 152,705 | Hydro-alcoholic extract (WFF) |
| *Cassia hirsuta* | Seeds | Soxhlet (successive) and Petroleum ether → Chloroform → Ethanol | Enable C18G 250 × 4.6 mm, 5 µm | 2.63 ± 0.002 | Conc.-dependent | Ethanol extract used for GA quantification |
| *Punica granatum* | Peel | Batch stirred extraction and Methanol | RP-C18 250 × 4.6 mm, 5 µm | 3.41 | Cultivar-dependent | Methanolic peel extract analyzed |
| *Qualea grandiflora* / *Q. parviflora* | Stem bark | Exhaustive maceration and Hydro-alcoholic | Dionex Acclaim® 120 C18 250 × 4.6 mm, 5 µm | 8.00 | Regression-based | QG and QP extracts |
| Trigasornmas(poly herbal) | Tablet | Homogenization + stirring and Methanol | Zorbax SB C18 150 × 4.6 mm, 5 µm | 3.42 | 23,860 – 6,654,917 | Rat plasma bioanalysis |

| | | | | | | |
|---|---|---|---|---|---|---|
| Mango cultivars | Peel and Seed | Reflux extraction and Hydro-alcoholic | Shim-pack C18 250 × 4.6 mm, 5 µm | 3.41 | Cultivar-dependent | Peel and seed extracts |

**Table 2: Parameters of Chlorogenic acid Extraction from Various Plants and Tablet**

| Sample matrix | Extraction conditions | HPLC Columns | Mobile Phase | Flow rate | Detection | Retention time | CGA Content |
|---|---|---|---|---|---|---|---|
| *Viburnum tinus* | Methanol; maceration at room temperature for 8 h. | C-18 | 0.2% Phosporic acid(A) : Aceto nitrile(B) | 1.2 | 330 | 7.68 | 0.014-0.507%W/W |
| Roasted coffee | Soxhlet extraction; methanol: water (50:50). | C-18 | Water +1%Phosporic acid(A):Aceto nitrile(B) | 1.0 | 324 | ~7.0 | Total CGA:2.6g/100g |
| *Coffea arabica* extract | Extract (regional coffee samples) | C-18 | KH2PO4:Aceto nitrile | 1.0 | 274 | Not reported | 22-25%W/W |
| Green Coffee beans | Hot and Cold extraction: water, methanol, propanol | C-18 | Not specified | Not reported | Not reported | 7.2(5-CQA) | Max yield 11% (hot water) |
| Coffee leaves | Methanol: water(70:30)70c repeated extraction | C-18 | Not specified | Not reported | Not reported | Not reported | CGA quantified: stable and reproducible |

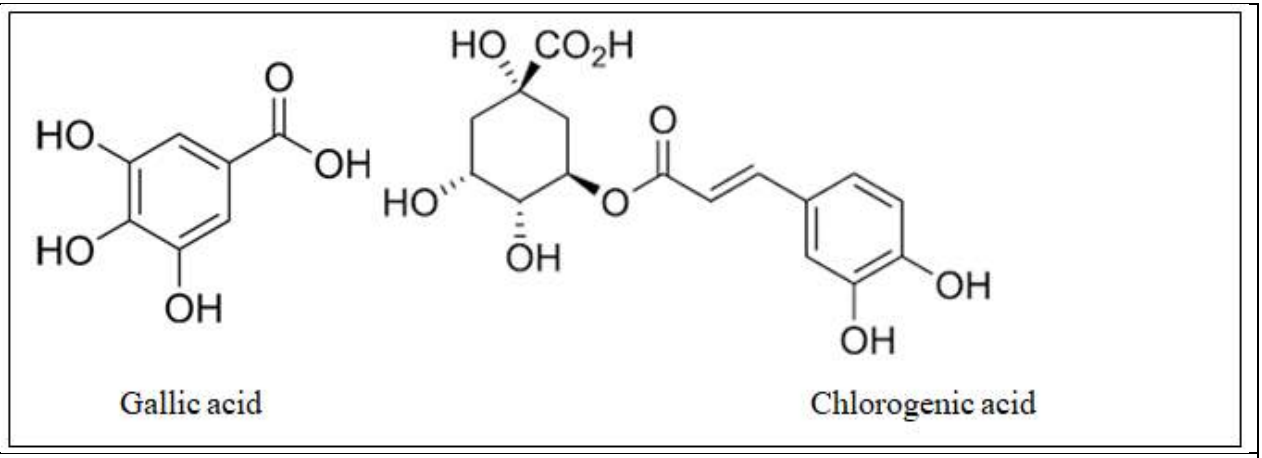



**Fig 1: Chemical Characteristics**

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**RESEARCH ARTICLE**

# Operational Properties of Interval - Valued Neutrosophic Super Hyper Soft

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## ABSTRACT

Soft set theory and neutrosophic set theory are tools to model uncertainty, vagueness and indeterminacy of complex systems. Many extensions have been developed to enhance the descriptive capabilities of each framework, such as hypersoft, superhypersoft, and interval valued neutrosophic sets. This paper introduces a new fusion design called Interval Valued Neutrosophic SuperHyperSoft Set (IVNSHSS) which integrates interval valued neutrosophic data with superhyperparameterization; the elements are seen as ordered pairs via the Cartesian product of the power set of the attribute domains. Several basic concepts in relation to IVNSHSS are established, such as null and universal sets, complement, union, intersection, difference, scalar operations, truth-favor and false-favor operations; and the necessity operator and possibility operator will also be defined with their associated algebraic characteristics. Throughout the paper, illustrations and propositions will be provided as examples in support of the proposed framework's use as an improved and comprehensive approach towards managing multi-attribute based uncertainty and making decisions and analyzing data in information systems.

**Keywords:** Soft set; Neutrosophic Set; Interval-valued neutrosophic set; Hypersoft set; Interval valued neutrosophic superhypersoft set.

## INTRODUCTION

The challenge of modeling unknowns or incomplete knowledge exists across several disciplines including decision-making processes, artificial intelligence systems, recorded medical diagnosis and data processing methods. Conventional mathematics typically cannot adequately capture the range of uncertainties and vagueness associated with such problems as both are forms of uncertainty that apply to those types of problems. In 1999, Molodtsov [8] proposed the use of soft set theory as a mathematical framework for allocating with non-material data by applying multiple properties for each item of data instead of relying solely on auxiliary constructs such as membership functions. Subsequent to the introduction of soft sets, many researchers have further generalized and integrated soft sets into other types of uncertainty modeling techniques. Some researchers have integrated soft sets with fuzzy sets [12] and intuitionistic fuzzy sets [3] to increase their representation power. Smarandache [9] later developed neutrosophic sets which allow for characterization of the different source of uncertainty using three distinct membership functions (truth, indeterminacy and falsehood). Subsequently, a new class of neutrosophic sets has been developed, referred to as interval-valued neutrosophic sets, which allows for representing an entire range of degrees of membership in an extremely flexible and robust manner thereby providing a more comprehensive model for representing uncertainty in all of its various manifestations. Hypersoft sets expand upon the limitations of single-parameter soft sets by allowing for a tuple of values to represent multiple parameters, rather than just one [10]. A further extension of this theory, superhypersoft sets, use the Cartesian product of the collection of all possible subsets of the attribute domains to model more complicated attribute interactions [2]. This paper presents a new unified structure called the 'Interval-Valued Neutrosophic SuperHyperSoft Set' (IVNSHSS), which combines interval-valued neutrosophic sets with superhyper parameterization. By merging both concepts together, the proposed structure allows for an enhanced representation of uncertainty through the use of interval-valued neutrosophic data with parenthesized combinations of subsets of attribute values. The three major accomplishments of this paper include: 1) the formal definition of the IVNSHSS; 2) the identification of the basic operations and operators of the IVNSHSS; and 3) the presentation of several illustrative examples demonstrating the property of key algebraic relationships established in this paper.

## LITERATURE REVIEW

Soft Sets (Molodtsov [8]) is a way to quantify uncertainty without making extra assumptions. Since then it has been a base for expanding methods of uncertainty in decision-making; Specific examples are fuzzy soft sets [6] and intuitionistic fuzzy soft sets [7], which incorporate membership functions for representing partial truth values, while preserving the parameterized feature of soft sets. Neutrosophic Set Theory (Smarandache [9]) builds upon fuzzy and intuitionistic fuzzy sets by defining three components of membership independently, namely: Truth, Indeterminate, and False. Neutrosophic sets have been implemented to various applications of their own: Most common would be those such as Decision Making, Image Processing, and Data Mining. Wang *et al.* [11], wanted to extend these applications to represent ambiguous values to the next level, proposed Interval Valued Neutrosophic Sets. In doing this, the values for each of the three components can be represented using intervals, as opposed to standard integer or numerical values. A variety of hybrid systems such as neutrosophic soft sets, neutrosophic-soft-interval-valued-sets, and neutrosophic-hypersoft-sets [5,4,1] includes integrating neutrosophic theory with soft and hyper-soft sets. These system attempts to combine both methods of portraying uncertainty and adding a supplementary attribute to the existing structures. In regards to the combination of super-neutrosophic-hyperparameterization-in-ing and interval-valued-neutrosophic-information, there is insufficient literature documenting this hybridization. This study proposes to create an IVNSHSS, which will bridge this gap in research by the establishment of a framework that can accommodate all forms of multi-attribute uncertainty within a previously existing model using interval-valued neutrosophic-information; thus increasing the breadth and depth of current studies related to soft computing and uncertainty.

## PRELIMINARIES

**Definition 3.1**

The set (F, Λ) is called a soft set of the universe 𝕌, if and only if there exists a mapping F: Λ → $P(\mathbb{U})$, from the indexing set Λ to the power set of 𝕌, therefore providing a parameterized collection of subsets of 𝕌.

**Definition 3.2**

Let us denote a universal set as 𝕌 and construct an interval valued neutrosophic set represented by the interval valued set A= {<u,$u_A(u), v_A(u), w_A(u)$> : $\boldsymbol{u}\in\mathbb{U}$}, where $u_A, v_A$and $w_A$are truth, indeterminacy and falsity membership functions for A respectively, $\boldsymbol{u}u_A, v_A$ and $w_A\subseteq$ [0, 1] for each u∈𝕌. Where

$u_A(u) = [u_A^L(u), u_A^U(u)]$

$v_A(u) = [v_A^L(u), v_A^U(u)]$

$w_A(u) = [w_A^L(u), w_A^U(u)]$

For each point $\boldsymbol{u}$u∈𝕌, $0 \le u_A(u), +v_A(u) + w_A(u) \le 3$ and IVN(𝕌) represents the complete collection of interval valued neutrosophic sets..

**Definition 3.3**

For set𝕌to be a universal set then the power set of𝕌denoted by$P(\mathbb{U})$ exists. Suppose we have n distinct attributes denoted by$k_1, k_2, \ldots, k_n$and n sets corresponding to these distinct attributes denoted by$K_1, K_2, .., K_n$. with following conditions such as $K_i \cap K_j = \emptyset$ $(i \neq j)$ and $i, j\epsilon$ {1,2,3 … $n$}. Then the pair (F, $K_1 \times K_2 \times \ldots \times K_n$) is said to be HSS over 𝕌 where F is a mapping from $K_1 \times K_2 \times \ldots \times K_n$ to $P(\mathbb{U})$.

**Definition 3.4**

Let us define a Universal Set called 𝕌and the Power Set of 𝕌 is represented by the notation$P(\mathbb{U})$ and for $n \ge 1$, there are $n$ distinct attributes such as $k_1, k_2, \ldots, k_n$ and$K_1, K_2, .., K_n$ are sets for corresponding values attributes respectively with conditions such as $K_i \cap K_j = \emptyset$ $(i \neq j)$ and $i, j\epsilon$ {1,2,3 … $n$}. Then the pair (F, Λ) is said to be NHSS over 𝕌 if there exists a relation $K_1 \times K_2 \times \ldots \times K_n) = \Lambda$. F is a mapping from $K_1 \times K_2 \times \ldots \times K_n)$ to $P(\mathbb{U})$ and F($K_1 \times K_2 \times \ldots \times K_n$)) = {<$u, u_A(u), v_A(u), w_A(u)$> : u∈𝕌} where $u$ , $v$ , $w$ are Membership values are to represent truth, unclear and false respectively such that $u, v, w$: 𝕌 → $]0^-, 1^+[$ and $0^- \le u_A(u), +v_A() + w_A(u) \le 3^+$.

**Definition 3.5**

Let Δ be a universe of discourse, P(𝕌) the powerset of 𝕌, and $k_1, k_2, \ldots\ldots, k_n$ (n ≥ 1) distinct attributes with disjoint corresponding sets $K_1, K_2, \ldots\ldots, K_n$. Let P($K_i$) denote the powerset of $K_i$for i ∈ {1, 2, . . . , n}.

A Neutrosophic SuperHyperSoft Set (NSHSS) over 𝕌 is defined as the pair

(S, P($K_1$) × P($K_2$) × · · · × P($K_n$)) , where S : P($K_1$) × P($K_2$) × · · · × P($K_n$)→ P(𝕌), and

S = {$\alpha, \langle x, T_{s(\alpha)}(x), I_{s(\alpha)}(x), F_{s(\alpha)}(x)\rangle$: x ∈𝕌, $\alpha$ ∈P($K_1$) × P($K_2$) × · · · × P($K_n$)}.

Here, $T_{s(\alpha)}(x), I_{s(\alpha)}(x), F_{s(\alpha)}(x)$ : 𝕌 → [0, 1] represent the membership, indeterminacy, and non-membership degrees of x ∈𝕌 for each$\alpha$, satisfying $0 \le \left(T_{s(\alpha)}(x), I_{s(\alpha)}(x), F_{s(\alpha)}(x)\right) \le 3$.

**Intervalued Neutrosophic superhyper soft set**

**Definition 4.1**

Let $U$denote a nonempty universal set, and let P($U$) denote the power set of $U$. Let $n$ denote number of attributes and $k_1, k_2, \ldots\ldots, k_n$ denote $n$ distinct attributes. Let $K_1, K_2, \ldots\ldots, K_n$be the corresponding sets of attribute values such that,$K_i \cap K_j = \emptyset$ for $i \neq j$ where $i, j \in \{1, 2, \ldots, n\}$. Let $P(K_i)$ denote the powerset of $K_i$ for each $i$.and define the superhyper parameter set as$A = P(K_1) \times P(K_2) \times \ldots\ldots \times P(K_n)$. A pair $(F, A)$is called an Interval-Valued Neutrosophic SuperHyperSoft set (IVNSHSS) definedon$U$, where $F: A \rightarrow P(U \times [0,1]^3)$

$F_P = \{\langle u, [\alpha_P^L(u), \alpha_P^U(u)], [\beta_P^L(u), \beta_P^U(u)], [\gamma_P^L(u), \gamma_P^U(u)]\rangle : u \in U\}$

where $[\alpha_P^L(u), \alpha_P^U(u)], [\beta_P^L(u), \beta_P^U(u)], [\gamma_P^L(u), \gamma_P^U(u)]$ represent the truth, indeterminacy, and falsity membership degrees of the element $u$ concerning the parameter tuple $P$ . The intervals are subsets of $[0,1]$and satisfy$0 \leq sup\ \alpha_P(u) + sup\ \beta_P(u) + sup\ \gamma_P(u) \leq 3$ for all $u \in U$.

**Example 1**

Let $U = \{x_1, x_2\}$be the universe of discourse. Consider two distinct attributes:

$\boldsymbol{k_1}$= Color, with attribute domain $K_1$={Red,Blue},

$\boldsymbol{k_2}$= Size, with attribute domain $K_2$={Small,Large}.

The sets $K_1$ and $K_2$ are disjoint. We consider the power sets of each domain:

$P(K_1) = \{\emptyset, \{Red\}, \{Blue\}, \{Red,\ Blue\}\}$,

$P(K_2) = \{\emptyset, \{Small\}, \{Large\}, \{Small,\ Large\}\}$.

Cartesian product of power sets:

$A = P(K_1) \times P(K_2)$

There 4*4 =16 possible

For illustration, consider two such tuples

$\alpha_1 = (\{Red\}, \{Small\})$

$\alpha_2 = (\{Red,\ Blue\}, \{Large\})$

For each $\alpha \in A$, we define$F(\alpha)$ assigns a set of interval-valued neutrosophic triples to elements of $U$, which in turn consists of three distinct intervals indicating how much true membership, indeterminate membership and false membership each element has in U. More precisely,,

for $\alpha_1$ we have

$F(\alpha_1) = \{\langle x_1, [0.6,0.8], [0.1,0.2], [0.1,0.3]\rangle, \langle x_2, [0.4,0.6], [0.2,0.3], [0.2,0.4]\rangle\}$

for $\alpha_2$ we have

$F(\alpha_2) = \{\langle x_1, [0.7,0.9], [0.1,0.2], [0.0,0.1]\rangle, \langle x_2, [0.5,0.7], [0.2,0.3], [0.1,0.2]\rangle\}$

For each$x \in U$, the supremum of the truth, indeterminacy, and falsity intervals satisfies the condition $0 \leq sup\ \alpha_a(u) + sup\ \beta_a(u) + sup\ \gamma_a(u) \leq 3$ for example, for $x_1$ under $\alpha_1$, 0.8 + 0.2 + 0.3 = 1.3 ≤ 3 and for $x_2$ under$\alpha_2$, 0.7 + 0.3 + 0.2 = 1.2 ≤ 30. Thus, $F$ defines an Interval-Valued Neutrosophic SuperHypersoft Set (IVNSHSS) on$U$ over the Cartesian product of the power sets of the attribute domains.

**Definition 4.2**

Let $F_P$ and $G_Q$ ∈ IVNSHSS over$U$, then $F_P \subseteq G_Q$ if

$inf\alpha_P(u) \leq inf\alpha_Q(u)$ , $sup\alpha_P(u) \leq su\alpha_Q(u)$

$inf\beta_P(u) \geq inf\beta_Q(u)$ , $sup\beta_P(u) \geq sup\beta_Q(u)$

$inf\gamma_P(u) \geq inf\gamma_Q(u)$ , $supy_P(u) \geq supy_Q(u)$

**Example 2**

Let the universe of discourse be$\boldsymbol{U} = \{\boldsymbol{u_1}, \boldsymbol{u_2}\}$

$\boldsymbol{F_P} = \{\langle \boldsymbol{u_1}, [\boldsymbol{0.3}, \boldsymbol{0.5}], [\boldsymbol{0.4}, \boldsymbol{0.6}], [\boldsymbol{0.2}, \boldsymbol{0.3}]\rangle, \langle \boldsymbol{u_2}, [\boldsymbol{0.2}, \boldsymbol{0.4}], [\boldsymbol{0.3}, \boldsymbol{0.5}], [\boldsymbol{0.3}, \boldsymbol{0.4}]\rangle\}$

$\boldsymbol{G_Q} = \{\langle \boldsymbol{u_1}, [[\boldsymbol{0.4}, \boldsymbol{0.6}], [\boldsymbol{0.3}, \boldsymbol{0.5}], [\boldsymbol{0.1}, \boldsymbol{0.3}]\rangle, \langle \boldsymbol{u_2}, [\boldsymbol{0.3}, \boldsymbol{0.5}], [\boldsymbol{0.2}, \boldsymbol{0.4}], [\boldsymbol{0.2}, \boldsymbol{0.4}]\rangle\}$

**Thus $\boldsymbol{F_P \subseteq G_Q}$.**

**Definition 4.3**

Let $\boldsymbol{F_P}$ ∈ IVNSHSS defined over the universe$\boldsymbol{U}$, then

Empty IVNSHSS can be represented as $\boldsymbol{F_e}$which is defined as follows

$\boldsymbol{F_e} = \{\langle \boldsymbol{u}, [\boldsymbol{0}, \boldsymbol{0}], [\boldsymbol{1}, \boldsymbol{1}], [\boldsymbol{1}, \boldsymbol{1}]\rangle : \mathbf{u} \in \boldsymbol{U}\}$

Empty IVNSHSS can be represented as $\boldsymbol{F_\vartheta}$this representation is defined as follows

$\boldsymbol{F_\vartheta} = \{\langle \boldsymbol{u}, [\boldsymbol{1}, \boldsymbol{1}], [\boldsymbol{0}, \boldsymbol{0}], [\boldsymbol{0}, \boldsymbol{0}]\rangle : \mathbf{u} \in \boldsymbol{U}\}$

The complement of $\boldsymbol{IVNSHSS}$ can be represented as $\boldsymbol{F_P^C(p_1)}$ and defined as follows

$\boldsymbol{F_P^C} = \{\langle \boldsymbol{u}, [\boldsymbol{\gamma_P^L(u)}, \boldsymbol{\gamma_P^U(u)}], [\mathbf{1} - \boldsymbol{\beta_P^U(u)}, \mathbf{1} - \boldsymbol{\beta_P^L(u)}], [\boldsymbol{\alpha_P^L(u)}, \boldsymbol{\alpha_P^U(u)}]\rangle : \mathbf{u} \in \boldsymbol{U}\}$

**Example 3**

Let the universe of discourse be $\boldsymbol{U} = \{\boldsymbol{u_1}, \boldsymbol{u_2}\}$ a\

$\boldsymbol{F_P} = \{\langle \boldsymbol{u_1}, [\mathbf{0.3}, \mathbf{0.5}], [\mathbf{0.4}, \mathbf{0.6}], [\mathbf{0.2}, \mathbf{0.3}]\rangle, \langle \boldsymbol{u_2}, [\mathbf{0.2}, \mathbf{0.4}], [\mathbf{0.3}, \mathbf{0.5}], [\mathbf{0.3}, \mathbf{0.4}]\rangle\}$

The complement $\boldsymbol{F_P^c}(\boldsymbol{p_1})$ of IVNSHSS is

$\boldsymbol{F_P^C} = \{\langle \boldsymbol{u_1}, [\mathbf{0.2}, \mathbf{0.3}], [\mathbf{0.4}, \mathbf{0.6}], [\mathbf{0.3}, \mathbf{0.5}]\rangle, \langle \boldsymbol{u_2}, [\mathbf{0.3}, \mathbf{0.4}], [\mathbf{0.5}, \mathbf{0.7}], [\mathbf{0.2}, \mathbf{0.4}]\rangle\}$

**Propoition 1**

If $F_P \in$ IVNSHSS over $U$, then

1. $\left(\boldsymbol{F_P^C}\right) = \boldsymbol{F_P}$
2. $(\boldsymbol{F_e})^{\boldsymbol{C}} = \boldsymbol{F_\vartheta}$
3. $(\boldsymbol{F_\vartheta})^{\boldsymbol{C}} = \boldsymbol{F_e}$

**Proof:**

Let $\boldsymbol{F_P} = \{\langle \boldsymbol{u}, [\boldsymbol{\alpha_P^L}(\boldsymbol{u}), \boldsymbol{\alpha_P^U}(\boldsymbol{u})], [\boldsymbol{\beta_P^L}(\boldsymbol{u}), \boldsymbol{\beta_P^U}(\boldsymbol{u})], [\boldsymbol{\gamma_P^L}(\boldsymbol{u}), \boldsymbol{\gamma_P^U}(\boldsymbol{u})]\rangle : \mathbf{u} \in \boldsymbol{U}\}$ be an IVNSHSS. From the definition 1.3(iii )

$\boldsymbol{F_P^C} = \{\langle \boldsymbol{u}, [\boldsymbol{\gamma_P^L}(\boldsymbol{u}), \boldsymbol{\gamma_P^U}(\boldsymbol{u})], [\mathbf{1} - \boldsymbol{\beta_P^U}(\boldsymbol{u}), \mathbf{1} - \boldsymbol{\beta_P^L}(\boldsymbol{u})], [\boldsymbol{\alpha_P^L}(\boldsymbol{u}), \boldsymbol{\alpha_P^U}(\boldsymbol{u})]\rangle : \mathbf{u} \in \boldsymbol{U}\}$

Thus

$$\left(\boldsymbol{F_P^C}\right)^{\boldsymbol{C}} = \left\{\langle \boldsymbol{u}, [\boldsymbol{\alpha_P^L}(\boldsymbol{u}), \boldsymbol{\alpha_P^U}(\boldsymbol{u})], \left[\mathbf{1} - \left(\mathbf{1} - \boldsymbol{\beta_P^U}(\boldsymbol{u})\right), -\left(\mathbf{1} - \boldsymbol{\beta_P^L}(\boldsymbol{u})\right)\right], [\boldsymbol{\gamma_P^L}(\boldsymbol{u}), \boldsymbol{\gamma_P^U}(\boldsymbol{u})]\rangle : \mathbf{u} \in \boldsymbol{U}\right\}$$

$\left(\boldsymbol{F_P^C}\right)^{\boldsymbol{C}} = \{\langle \boldsymbol{u}, [\boldsymbol{\alpha_P^L}(\boldsymbol{u}), \boldsymbol{\alpha_P^U}(\boldsymbol{u})], [\boldsymbol{\beta_P^L}(\boldsymbol{u}), \boldsymbol{\beta_P^U}(\boldsymbol{u})], [\boldsymbol{\gamma_P^L}(\boldsymbol{u}), \boldsymbol{\gamma_P^U}(\boldsymbol{u})]\rangle : \mathbf{u} \in \boldsymbol{U}\}$

$\left(\boldsymbol{F_P^C}\right)^{\boldsymbol{C}} = \boldsymbol{F_P}$

It is know that$\boldsymbol{F_e} = \{\langle \boldsymbol{u}, [\mathbf{0}, \mathbf{0}], [\mathbf{1}, \mathbf{1}], [\mathbf{1}, \mathbf{1}]\rangle : \mathbf{u} \in \boldsymbol{U}\}$

By definition 1.3(iii )

$(\boldsymbol{F_e})^{\boldsymbol{C}} = \{\langle \boldsymbol{u}, [\mathbf{1}, \mathbf{1}], [\mathbf{0}, \mathbf{0}], [\mathbf{0}, \mathbf{0}]\rangle : \mathbf{u} \in \boldsymbol{U}\} = \boldsymbol{F_\vartheta}$

Similar.

**Definition 4.4**

Let $\boldsymbol{F_P}$ and $\boldsymbol{G_Q} \in$ IVNSHSS over $\boldsymbol{U}$, then

$$\boldsymbol{F_P} \cup \boldsymbol{G_Q} = \left\{ \begin{array}{c} (< u, [\boldsymbol{max}\{\inf \boldsymbol{\alpha_P}(\boldsymbol{u}), \boldsymbol{inf\alpha_Q}(\boldsymbol{u})\}, \boldsymbol{max}\{\boldsymbol{sup\alpha}(\boldsymbol{u}), \boldsymbol{sup\alpha_Q}(\boldsymbol{u})\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\beta_P}(\boldsymbol{u}), \boldsymbol{inf\beta_Q}(\boldsymbol{u})\}, \boldsymbol{min}\{\boldsymbol{sup\beta_P}(\boldsymbol{u}), \boldsymbol{sup\beta_Q}(\boldsymbol{u})\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\gamma_P}(\boldsymbol{u}), \boldsymbol{inf\gamma_Q}(\boldsymbol{u})\}, \boldsymbol{min}\{\boldsymbol{sup\gamma_P}(\boldsymbol{u}), \boldsymbol{sup\gamma_Q}(\boldsymbol{u})\}] >) / \boldsymbol{u} \in \boldsymbol{U} \end{array} \right\}$$

**Example 4**

Let the universe be $\boldsymbol{U} = \{\boldsymbol{u_1}, \boldsymbol{u_2}\}$. Let $\boldsymbol{F_P}$ and $\boldsymbol{G_Q} \in$ IVNSHSS over $\boldsymbol{U}$.

$\boldsymbol{F_P} = \{\langle \boldsymbol{u_1}, [\mathbf{0.2}, \mathbf{0.4}], [\mathbf{0.3}, \mathbf{0.5}], [\mathbf{0.4}, \mathbf{0.6}]\rangle, \langle \boldsymbol{u_2}, [\mathbf{0.5}, \mathbf{0.7}], [\mathbf{0.2}, \mathbf{0.4}], [\mathbf{0.3}, \mathbf{0.5}]\rangle\}$

$\boldsymbol{G_Q} = \{\langle \boldsymbol{u_1}, [\mathbf{0.3}, \mathbf{0.6}], [\mathbf{0.2}, \mathbf{0.4}], [\mathbf{0.3}, \mathbf{0.5}]\rangle, \langle \boldsymbol{u_2}, [\mathbf{0.4}, \mathbf{0.6}], [\mathbf{0.3}, \mathbf{0.5}], [\mathbf{0.2}, \mathbf{0.4}]\rangle\}$

$\boldsymbol{F_P} \cup \boldsymbol{G_Q} = \{\langle \boldsymbol{u_1}, [\mathbf{0.3}, \mathbf{0.6}], [\mathbf{0.2}, \mathbf{0.4}], [\mathbf{0.3}, \mathbf{0.5}]\rangle, \langle \boldsymbol{u_2}, [\mathbf{0.5}, \mathbf{0.7}], [\mathbf{0.2}, \mathbf{0.4}], [\mathbf{0.2}, \mathbf{0.4}]\rangle\}$

**Preposition 2**

Let $\boldsymbol{P}$ , $\boldsymbol{G_Q}$, $\boldsymbol{H_R} \in$ IVNSHSS over $\boldsymbol{U}$, then

1. $\boldsymbol{F_P} \cup \boldsymbol{F_P} = \boldsymbol{F_P}$
2. $\boldsymbol{F_P} \cup \boldsymbol{F_e} = \boldsymbol{F_P}$
3. $\boldsymbol{F_P} \cup \boldsymbol{F_\vartheta} = \boldsymbol{F_\vartheta}$
4. $\boldsymbol{F_P} \cup \boldsymbol{G_Q} = \boldsymbol{G_Q} \cup \boldsymbol{F_P}$
5. $\left(\boldsymbol{F_P} \cup \boldsymbol{G_Q}\right) \cup \boldsymbol{H_R} = \boldsymbol{F_P} \cup \left(\boldsymbol{G_Q} \cup \boldsymbol{H_R}\right)$

**Proof:** The proof follows directly from the definition.

**Definition 4.5**

Let $\boldsymbol{F_P}$ and $\boldsymbol{G_Q} \in$ IVNSHSS on$\boldsymbol{U}$, then

$$\boldsymbol{F_P} \cap \boldsymbol{G_Q} = \left\{ \begin{matrix} (< u, [\boldsymbol{min}\{\inf \boldsymbol{\alpha_P}(u), \boldsymbol{inf\alpha_Q}(u)\}, \boldsymbol{min}\{\boldsymbol{sup\alpha_P}(u), \boldsymbol{sup\alpha_Q}(u)\}], \\ [\boldsymbol{max}\{\boldsymbol{inf\beta_P}(u), \boldsymbol{inf\beta_Q}(u)\}, \boldsymbol{max}\{\boldsymbol{sup\beta_P}(u), \boldsymbol{sup\beta_Q}(u)\}], \\ [\boldsymbol{max}\{\boldsymbol{inf\gamma_P}(u), \boldsymbol{inf\gamma_Q}(u)\}, \boldsymbol{max}\{\boldsymbol{sup\gamma_P}(u), \boldsymbol{sup\gamma_Q}(u)\}] >) / \boldsymbol{u} \in \boldsymbol{U} \end{matrix} \right\}$$

**Example 5**

Let the universe be $\boldsymbol{U} = \{\boldsymbol{u_1}, \boldsymbol{u_2}\}$. Let $\boldsymbol{F_P}$ and $\boldsymbol{G_Q} \in$ IVNSHSS over $U$.

$\boldsymbol{F_P} = \{\langle \boldsymbol{u_1}, [\boldsymbol{0.2}, \boldsymbol{0.4}], [\boldsymbol{0.3}, \boldsymbol{0.5}], [\boldsymbol{0.4}, \boldsymbol{0.6}]\rangle, \langle \boldsymbol{u_2}, [\boldsymbol{0.5}, \boldsymbol{0.7}], [\boldsymbol{0.2}, \boldsymbol{0.4}], [\boldsymbol{0.3}, \boldsymbol{0.5}]\rangle\}$

$\boldsymbol{G_Q} = \{\langle \boldsymbol{u_1}, [\boldsymbol{0.}, \boldsymbol{0.6}], [\boldsymbol{0.2}, \boldsymbol{0.4}], [\boldsymbol{0.3}, \boldsymbol{0.5}]\rangle, \langle \boldsymbol{u_2}, [\boldsymbol{0.4}, \boldsymbol{0.6}], [\boldsymbol{0.3}, \boldsymbol{0.5}], [\boldsymbol{0.2}, \boldsymbol{0.4}]\rangle\}$

$\boldsymbol{F_P} \cap \boldsymbol{G_Q} = \{\langle \boldsymbol{u_1}, [\boldsymbol{0.2}, \boldsymbol{0.4}], [\boldsymbol{0.3}, \boldsymbol{0.5}], [\boldsymbol{0.4}, \boldsymbol{0.6}]\rangle, \langle \boldsymbol{u_2}, [\boldsymbol{0.4}, \boldsymbol{0.6}], [\boldsymbol{0.3}, \boldsymbol{0.5}], [\boldsymbol{0.3}, \boldsymbol{0.5}]\rangle\}$

**Preposition 3**

Let $\boldsymbol{F_P}$ , $\boldsymbol{G_Q}$, $\boldsymbol{H_R} \in$ IVNSHSS over $\boldsymbol{U}$, then

1. $\boldsymbol{F_P} \cap \boldsymbol{F_P} = \boldsymbol{F_P}$
2. $\boldsymbol{F_P} \cap \boldsymbol{F_e} = \boldsymbol{F_P}$
3. $\boldsymbol{F_P} \cap \boldsymbol{F_\vartheta} = \boldsymbol{F_\vartheta}$
4. $\boldsymbol{F_P} \cap \boldsymbol{G_Q} = \boldsymbol{G_Q} \cap \boldsymbol{F_P}$
5. $(\boldsymbol{F_P} \cap \boldsymbol{G_Q}) \cap \boldsymbol{H_R} = \boldsymbol{F_P} \cap (\boldsymbol{G_Q} \cap \boldsymbol{H_R})$

**Proof:** This is a direct consequence of the definition.

**Proposition 4**

Let $F_P$ and $G_Q \in$ IVNSHSS over $U$, then

1. $(\boldsymbol{F_P} \cup \boldsymbol{G_Q})^{\boldsymbol{C}} = \boldsymbol{F_P}^{\boldsymbol{C}} \cap \boldsymbol{G_Q}^{\boldsymbol{C}}$
2. $(\boldsymbol{F_P} \cap \boldsymbol{G_Q})^{\boldsymbol{C}} = \boldsymbol{F_P}^{\boldsymbol{C}} \cup \boldsymbol{G_Q}^{\boldsymbol{C}}$

**Proof**

$\boldsymbol{F_P} = \{\langle \boldsymbol{u}, , \boldsymbol{\beta_P}(u), \boldsymbol{\gamma_P}(u)\rangle : \boldsymbol{u} \in \boldsymbol{U}\}$ and $\boldsymbol{G_Q} = \{\langle \boldsymbol{u}, \boldsymbol{\alpha_Q}(u), \boldsymbol{\beta_Q}(u), \boldsymbol{\gamma_Q}(u)\rangle : \boldsymbol{u} \in \boldsymbol{U}\}$

$\boldsymbol{\alpha_P}(u) = [\inf \boldsymbol{\alpha_P}(u), \boldsymbol{sup\alpha_P}(u)]$ or $[\boldsymbol{\alpha_P^L}(u), \boldsymbol{\alpha_P^U}(u)]$, $\boldsymbol{\alpha_P^L}(u) = \boldsymbol{inf\alpha_P}(u)$ and $\boldsymbol{\alpha_P^U}(u) = \boldsymbol{sup\alpha_P}(u)$

$\boldsymbol{\beta_P}(u) = [\inf \boldsymbol{\beta_P}(u), \boldsymbol{sup\beta_P}(u)]$ or $[\boldsymbol{\beta_P^L}(u), \boldsymbol{\beta_P^U}(u)]$, $\boldsymbol{\beta_P^L}(u) = \boldsymbol{inf\beta_P}(u)$ and $\boldsymbol{\beta_P^U}(u) = \boldsymbol{sup\beta_P}(u)$

$\boldsymbol{\gamma_P}(u) = [\inf \boldsymbol{\gamma_P}(u), \boldsymbol{sup\gamma_P}(u)]$ or $[\boldsymbol{\gamma_P^L}(u), \boldsymbol{\gamma_P^U}(u)]$, $\boldsymbol{\gamma_P^L}(u) = \boldsymbol{inf\gamma_P}(u)$ and $\boldsymbol{\gamma_P^U}(u) = \boldsymbol{sup\gamma_P}(u)$

$$\boldsymbol{F_P} \cup \boldsymbol{G_Q} = \left\{ \begin{matrix} (< u, [\boldsymbol{max}\{\inf \boldsymbol{\alpha_P}(u), \boldsymbol{inf\alpha_Q}(u)\}, \boldsymbol{max}\{\boldsymbol{sup\alpha_P}(u), \boldsymbol{sup\alpha_Q}(u)\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\beta_P}(u), \boldsymbol{inf\beta_Q}(u)\}, \boldsymbol{min}\{\boldsymbol{sup\beta_P}(u), \boldsymbol{sup\beta_Q}(u)\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\gamma_P}(u), \boldsymbol{inf\gamma_Q}(u)\}, \boldsymbol{min}\{\boldsymbol{sup\gamma_P}(u), \boldsymbol{sup\gamma_Q}(u)\}] >) / \boldsymbol{u} \in \boldsymbol{U} \end{matrix} \right\}$$

$$(\boldsymbol{F_P} \cup \boldsymbol{G_Q})^{\boldsymbol{C}} = \left\{ \begin{matrix} (< u, [\boldsymbol{min}\{\boldsymbol{inf\gamma_P}(u), \boldsymbol{inf\gamma_Q}(u)\}, \boldsymbol{min}\{\boldsymbol{sup\gamma_P}(u), \boldsymbol{sup\gamma_Q}(u)\}], \\ [\boldsymbol{1} - \boldsymbol{min}\{\boldsymbol{sup\beta_P}(u), \boldsymbol{sup\beta_Q}(u)\}, \boldsymbol{1} - \boldsymbol{min}\{\boldsymbol{inf\beta_P}(u), \boldsymbol{inf\beta_Q}(u)\}], \\ [\boldsymbol{max}\{\inf \boldsymbol{\alpha_P}(u), \boldsymbol{inf\alpha_Q}(u)\}, \boldsymbol{max}\{\boldsymbol{sup\alpha_P}(u), \boldsymbol{sup\alpha_Q}(u)\}] >) / \boldsymbol{u} \in \boldsymbol{U} \end{matrix} \right\}$$

$$\boldsymbol{F_P}^{\boldsymbol{C}} = \{\langle \boldsymbol{u}, [\boldsymbol{inf\gamma_P}(u), \boldsymbol{sup\gamma_P}(u)], [\boldsymbol{1} - \boldsymbol{sup\beta_P}(u), \boldsymbol{1} - \boldsymbol{inf\beta_P}(u)], [\boldsymbol{inf\alpha_P}(u), \boldsymbol{sup\alpha_P}(u)]\rangle : \boldsymbol{u} \in \boldsymbol{U}\}$$

$$\boldsymbol{G_Q}^{\boldsymbol{C}} = \{\langle \boldsymbol{u}, [\boldsymbol{inf\gamma_Q}(u), \boldsymbol{sup\gamma_Q}(u)], [\boldsymbol{1} - \boldsymbol{sup\beta_Q}(u), \boldsymbol{1} - \boldsymbol{inf\beta_Q}(u)], [\boldsymbol{inf\alpha_Q}(u), \boldsymbol{sup\alpha_Q}(u)]\rangle : \boldsymbol{u} \in \boldsymbol{U}\}$$

$$\boldsymbol{F_P}^{\boldsymbol{C}} \cap \boldsymbol{G_Q}^{\boldsymbol{C}} = \left\{ \begin{matrix} (< u, [\boldsymbol{min}\{\boldsymbol{inf\gamma_P}(u), \boldsymbol{inf\gamma_Q}(u)\}, \boldsymbol{min}\{\boldsymbol{sup\gamma_P}(u), \boldsymbol{sup\gamma_Q}(u)\}], \\ [\max\{\boldsymbol{1} - \boldsymbol{sup\beta_P}(u), \boldsymbol{1} - \boldsymbol{sup\beta_Q}(u)\}, \max\{\boldsymbol{1} - \boldsymbol{inf\beta_P}(u), \boldsymbol{1} - \boldsymbol{inf\beta_Q}(u)\}], \\ [\boldsymbol{max}\{\inf \boldsymbol{\alpha_P}(u), \boldsymbol{inf\alpha_Q}(u)\}, \boldsymbol{max}\{\boldsymbol{sup\alpha_P}(u), \boldsymbol{sup\alpha_Q}(u)\}] >) / \boldsymbol{u} \in \boldsymbol{U} \end{matrix} \right\}$$

$$F_P{}^C \cap G_Q{}^C = \left\{ \begin{matrix} (< u, [\boldsymbol{min}\{\boldsymbol{inf\gamma_P(u)}, \boldsymbol{inf\gamma_Q(u)}\}, \boldsymbol{min}\{\boldsymbol{sup\gamma_P(u)}, \boldsymbol{sup\gamma_Q(u)}\}], \\ [\mathbf{1} - \boldsymbol{min}\{\boldsymbol{sup\beta_P(u)}, \boldsymbol{sup\beta(u)}\}, \mathbf{1} - \boldsymbol{min}\{\boldsymbol{inf\beta_P(u)}, \boldsymbol{inf\beta_Q(u)}\}], \\ [\boldsymbol{max}\{\inf \boldsymbol{\alpha_P(u)}, \boldsymbol{inf\alpha_Q(u)}\}, \boldsymbol{max}\{\boldsymbol{sup\alpha_P(u)}, \boldsymbol{sup\alpha_Q(u)}\}] >)/\boldsymbol{u \in U} \end{matrix} \right\}$$

Hence

$$(F_P \cup G_Q)^C = F_P{}^C \cap G_Q{}^C$$

Proof 2

Similar as proof 1.

**Proposition 5**

Let $F_P$ , $G_Q$, $H_R \in$ IVNSHSS over $U$, then

1. $F_P \cup (G_Q \cap H_R) = (F_P \cup G_Q) \cap (F_P \cup H_R)$
2. $F_P \cap (G_Q \cup H_R) = (F_P \cap G_Q) \cup (F_P \cap H_R)$
3. $F_P \cup (F_P \cap G_Q) = F_P$
4. $F_P \cap (F_P \cup G_Q) = F_P$

**Proof 1**

$$G_Q \cap H_R = \left\{ \begin{matrix} (< u, [\boldsymbol{min}\{\inf \boldsymbol{\alpha_Q(u)}, \boldsymbol{inf\alpha_R(u)}\}, \boldsymbol{min}\{\boldsymbol{sup\alpha_Q(u)}, \boldsymbol{sup\alpha_R(u)}\}], \\ [\boldsymbol{max}\{\boldsymbol{inf\beta_Q(u)}, \boldsymbol{inf\beta_R(u)}\}, \boldsymbol{max}\{\boldsymbol{sup\beta_Q(u)}, \boldsymbol{sup\beta_R(u)}\}], \\ [\boldsymbol{max}\{\boldsymbol{inf\gamma_Q(u)}, \boldsymbol{inf\gamma_R(u)}\}, \boldsymbol{max}\{\boldsymbol{sup\gamma_Q(u)}, \boldsymbol{sup\gamma_R(u)}\}] >)/\boldsymbol{u \in U} \end{matrix} \right\}$$

$$F_P \cup (G_Q \cap H_R)$$

$$= \left\{ \begin{matrix} (< u, \left[\max\left\{\boldsymbol{inf\alpha_P(u)}, \boldsymbol{min}\{\inf \boldsymbol{\alpha_Q(u)}, \boldsymbol{inf\alpha_R(u)}\}\right\}, \max\{\boldsymbol{sup\alpha_P(u)}, \boldsymbol{min}\{\boldsymbol{sup\alpha_Q(u)}, \boldsymbol{sup\alpha_R(u)}\}\}\right], \\ [\min\{\boldsymbol{inf\beta_P(u)}, \boldsymbol{max}\{\boldsymbol{inf\beta_Q(u)}, \boldsymbol{inf\beta_R(u)}\}\}, \min\{\boldsymbol{sup\beta_P(u)}, \boldsymbol{max}\{\boldsymbol{sup\beta_Q(u)}, \boldsymbol{sup\beta_R(u)}\}\}], \\ [\min\{\boldsymbol{inf\gamma_P(u)}, \boldsymbol{max}\{\boldsymbol{inf\gamma_Q(u)}, \boldsymbol{inf\gamma_R(u)}\}\}, \min\{\boldsymbol{sup\gamma_P(u)}, \boldsymbol{max}\{\boldsymbol{sup\gamma_Q(u)}, \boldsymbol{sup\gamma_R(u)}\}\}] >)/\boldsymbol{u \in U} \end{matrix} \right\}$$

$$F_P \cup G_Q = \left\{ \begin{matrix} (< u, [\boldsymbol{max}\{\inf \boldsymbol{\alpha_P(u)}, \boldsymbol{inf\alpha_Q(u)}\}, \boldsymbol{max}\{\boldsymbol{sup\alpha_P(u)}, \boldsymbol{sup\alpha_Q(u)}\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\beta_P(u)}, \boldsymbol{inf\beta_Q(u)}\}, \boldsymbol{min}\{\boldsymbol{sup\beta_P(u)}, \boldsymbol{sup\beta_Q(u)}\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\gamma_P(u)}, \boldsymbol{inf\gamma_Q(u)}\}, \boldsymbol{min}\{\boldsymbol{sup\gamma_P(u)}, \boldsymbol{sup\gamma_Q(u)}\}] >)/\boldsymbol{u \in U} \end{matrix} \right\}$$

$$F_P \cup H_R = \left\{ \begin{matrix} (< u, [\boldsymbol{max}\{\inf \boldsymbol{\alpha_P(u)}, \boldsymbol{inf\alpha_R(u)}\}, \boldsymbol{max}\{\boldsymbol{sup\alpha_P(u)}, \boldsymbol{sup\alpha_R(u)}\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\beta_P(u)}, \boldsymbol{inf\beta_R(u)}\}, \boldsymbol{min}\{\boldsymbol{sup\beta_P(u)}, \boldsymbol{sup\beta_R(u)}\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\gamma_P(u)}, \boldsymbol{inf\gamma_R(u)}\}, \boldsymbol{min}\{\boldsymbol{sup\gamma_P(u)}, \boldsymbol{sup\gamma_R(u)}\}] >)/\boldsymbol{u \in U} \end{matrix} \right\}$$

$$(F_P \cup G_Q) \cap (F_P \cup H_R) = \left\{ \begin{matrix} (< u, \begin{bmatrix} \min\{\boldsymbol{max}\{\inf \boldsymbol{\alpha_P(u)}, \boldsymbol{inf\alpha_Q(u)}\}, \boldsymbol{max}\{\inf \boldsymbol{\alpha_P(u)}, \boldsymbol{inf\alpha_R(u)}\}\}, \\ \min\{\max\{\boldsymbol{sup\alpha_P(u)}, \boldsymbol{sup\alpha_Q(u)}\}, \boldsymbol{max}\{\boldsymbol{sup\alpha_P(u)}, \boldsymbol{sup\alpha_R(u)}\}\} \end{bmatrix}, \\ \begin{bmatrix} \max\{\min\{\boldsymbol{inf\beta_P(u)}, \boldsymbol{inf\beta_Q(u)}\}, \boldsymbol{min}\{\boldsymbol{inf\beta_P(u)}, \boldsymbol{inf\beta_R(u)}\}\}, \\ \max\{\boldsymbol{min}\{\boldsymbol{sup\beta_P(u)}, \boldsymbol{sup\beta_R(u)}\}, \{\boldsymbol{min}\{\boldsymbol{sup\beta_P(u)}, \boldsymbol{sup\beta_R(u)}\}\} \end{bmatrix}, \\ \begin{bmatrix} \max\{\boldsymbol{min}\{\boldsymbol{inf\gamma_P(u)}, \boldsymbol{inf\gamma_Q(u)}\}, \{\boldsymbol{min}\{\boldsymbol{inf\gamma_P(u)}, \boldsymbol{inf\gamma_R(u)}\}\}, \\ \max\{\boldsymbol{min}\{\boldsymbol{sup\gamma_P(u)}, \boldsymbol{sup\gamma_Q(u)}\}, \boldsymbol{min}\{\boldsymbol{sup\gamma_P(u)}, \boldsymbol{sup\gamma_R(u)}\}\} \end{bmatrix} >)/\boldsymbol{u \in U} \end{matrix} \right\}$$

$$(F_P \cup G_Q) \cap (F_P \cup H_R)$$

$$= \left\{ \begin{matrix} (< u, \left[\max\left\{\boldsymbol{inf\alpha_P(u)}, \boldsymbol{min}\{\inf \boldsymbol{\alpha_Q(u)}, \boldsymbol{inf\alpha_R(u)}\}\right\}, \max\{\boldsymbol{sup\alpha_P(u)}, \boldsymbol{min}\{\boldsymbol{sup\alpha_Q(u)}, \boldsymbol{sup\alpha_R(u)}\}\}\right], \\ [\min\{\boldsymbol{inf\beta_P(u)}, \boldsymbol{max}\{\boldsymbol{inf\beta_Q(u)}, \boldsymbol{inf\beta_R(u)}\}\}, \min\{\boldsymbol{sup\beta_P(u)}, \boldsymbol{max}\{\boldsymbol{sup_Q(u)}, \boldsymbol{sup\beta_R(u)}\}\}], \\ [\min\{\boldsymbol{inf\gamma_P(u)}, \boldsymbol{max}\{\boldsymbol{inf\gamma_Q(u)}, \boldsymbol{inf\gamma_R(u)}\}\}, \min\{\boldsymbol{sup\gamma_P(u)}, \boldsymbol{max}\{\boldsymbol{sup\gamma_Q(u)}, \boldsymbol{sup\gamma_R(u)}\}\}] >)/\boldsymbol{u \in U} \end{matrix} \right\}$$

Hence $F_P \cup (G_Q \cap H_R) = (F_P \cup G_Q) \cap (F_P \cup H_R)$

The rest of the results follow by similar arguments.

**Definition 4.6**

If $\boldsymbol{F_P}$ , $\boldsymbol{G_Q} \in$ IVNSHSS defined on$\boldsymbol{U}$, then their extended union is

$$\boldsymbol{\alpha}(\boldsymbol{F_P} \cup \boldsymbol{G_Q}) = \begin{cases} [\inf \boldsymbol{\alpha_P}(\boldsymbol{u}), \sup \boldsymbol{\alpha_P}(\boldsymbol{u})] if \boldsymbol{u} \in \boldsymbol{P} - \boldsymbol{Q} \\ [\inf \boldsymbol{\alpha_Q}(\boldsymbol{u}), \sup \boldsymbol{\alpha_Q}(\boldsymbol{u})] if \boldsymbol{u} \in \boldsymbol{Q} - \boldsymbol{P} \\ [\max\{\inf \boldsymbol{\alpha_P}(\boldsymbol{u}), \boldsymbol{inf\alpha_Q}(\boldsymbol{u})\}, \max\{\boldsymbol{sup\alpha_P}(\boldsymbol{u}), \boldsymbol{sup\alpha_Q}(\boldsymbol{u})\}] if \boldsymbol{u} \in \boldsymbol{Q} \cap \boldsymbol{P} \end{cases}$$

$$\boldsymbol{\beta}(\boldsymbol{F_P} \cup \boldsymbol{G_Q}) = \begin{cases} [\inf \boldsymbol{\beta_P}(\boldsymbol{u}), \sup \boldsymbol{\beta_P}(\boldsymbol{u})] if \boldsymbol{u} \in \boldsymbol{P} - \boldsymbol{Q} \\ [\inf \boldsymbol{\beta_Q}(\boldsymbol{u}), \sup \boldsymbol{\beta_Q}(\boldsymbol{u})] if \boldsymbol{u} \in \boldsymbol{Q} - \boldsymbol{P} \\ [\min\{\inf \boldsymbol{\beta_P}(\boldsymbol{u}), \boldsymbol{inf\beta_Q}(\boldsymbol{u})\}, \min\{\boldsymbol{sup\beta_P}(\boldsymbol{u}), \boldsymbol{sup\beta_Q}(\boldsymbol{u})\}] if \boldsymbol{u} \in \boldsymbol{Q} \cap \boldsymbol{P} \end{cases}$$

$$\boldsymbol{\gamma}(\boldsymbol{F_P} \cup \boldsymbol{G_Q}) = \begin{cases} [\inf \boldsymbol{\gamma_P}(\boldsymbol{u}), \sup \boldsymbol{\gamma_P}(\boldsymbol{u})] if \boldsymbol{u} \in \boldsymbol{P} - \boldsymbol{Q} \\ [\inf \boldsymbol{\gamma_Q}(\boldsymbol{u}), \sup \boldsymbol{\gamma_Q}(\boldsymbol{u})] if \boldsymbol{u} \in \boldsymbol{Q} - \boldsymbol{P} \\ [\min\{\inf \boldsymbol{\gamma_P}(\boldsymbol{u}), \boldsymbol{inf\gamma_Q}(\boldsymbol{u})\}, \min\{\boldsymbol{sup\gamma_P}(\boldsymbol{u}), \boldsymbol{sup\gamma_Q}(\boldsymbol{u})\}] if \boldsymbol{u} \in \boldsymbol{Q} \cap \boldsymbol{P} \end{cases}$$

**Definition 4.7**

If $\boldsymbol{F_P}$ , $\boldsymbol{G_Q} \in$ IVNSHSS with universe$\boldsymbol{U}$, then their extended intersection is

$$\boldsymbol{\alpha}(\boldsymbol{F_P} \cup \boldsymbol{G_Q}) = \begin{cases} [\inf \boldsymbol{\alpha_P}(\boldsymbol{u}), \sup \boldsymbol{\alpha_P}(\boldsymbol{u})] if \boldsymbol{u} \in \boldsymbol{P} - \boldsymbol{Q} \\ [\inf \boldsymbol{\alpha_Q}(\boldsymbol{u}), \sup \boldsymbol{\alpha_Q}(\boldsymbol{u})] if \boldsymbol{u} \in \boldsymbol{Q} - \boldsymbol{P} \\ [\min\{\inf \boldsymbol{\alpha_P}(\boldsymbol{u}), \boldsymbol{inf\alpha_Q}(\boldsymbol{u})\}, \min\{\boldsymbol{sup\alpha_P}(\boldsymbol{u}), \boldsymbol{sup\alpha_Q}(\boldsymbol{u})\}] if \boldsymbol{u} \in \boldsymbol{Q} \cap \boldsymbol{P} \end{cases}$$

$$\boldsymbol{\beta}(\boldsymbol{F_P} \cup \boldsymbol{G_Q}) = \begin{cases} [\inf \boldsymbol{\beta_P}(\boldsymbol{u}), \sup \boldsymbol{\beta_P}(\boldsymbol{u})] if \boldsymbol{u} \in \boldsymbol{P} - \boldsymbol{Q} \\ [\inf \boldsymbol{\beta_Q}(\boldsymbol{u}), \sup \boldsymbol{\beta_Q}(\boldsymbol{u})] if \boldsymbol{u} \in \boldsymbol{Q} - \boldsymbol{P} \\ [\max\{\inf \boldsymbol{\beta_P}(\boldsymbol{u}), \boldsymbol{inf\beta_Q}(\boldsymbol{u})\}, \max\{\boldsymbol{sup\beta_P}(\boldsymbol{u}), \boldsymbol{sup\beta_Q}(\boldsymbol{u})\}] if \boldsymbol{u} \in \boldsymbol{Q} \cap \boldsymbol{P} \end{cases}$$

$$\boldsymbol{\gamma}(\boldsymbol{F_P} \cup \boldsymbol{G_Q}) = \begin{cases} [\inf \boldsymbol{\gamma_P}(\boldsymbol{u}), \sup \boldsymbol{\gamma_P}(\boldsymbol{u})] if \boldsymbol{u} \in \boldsymbol{P} - \boldsymbol{Q} \\ [\inf \boldsymbol{\gamma_Q}(\boldsymbol{u}), \sup \boldsymbol{\gamma_Q}(\boldsymbol{u})] if \boldsymbol{u} \in \boldsymbol{Q} - \boldsymbol{P} \\ [\max\{\inf \boldsymbol{\gamma_P}(\boldsymbol{u}), \boldsymbol{inf\gamma_Q}(\boldsymbol{u})\}, \max\{\boldsymbol{sup\gamma_P}(\boldsymbol{u}), \boldsymbol{sup\gamma_Q}(\boldsymbol{u})\}] if \boldsymbol{u} \in \boldsymbol{Q} \cap \boldsymbol{P} \end{cases}$$

**Definition 4.8**

If $\boldsymbol{F_P}$ , $\boldsymbol{G_Q} \in$ IVNSHSS over $\boldsymbol{U}$, then their difference is as follows

$$F_P \setminus G_Q = \begin{Bmatrix} (< u, [min\{\inf \alpha_P(u), inf\ \alpha_Q(u)\}, min\{sup\ \alpha_P(u), sup\ \alpha_Q(u)\}], \\ [max\{inf\ \beta_P(u), 1 - sup\ \beta_Q(u)\}, max\{\sup\ \beta_P(u), 1 - inf\ \beta_Q(u)\}], \\ [max\{inf\ \gamma_P(u), inf\ \gamma_P(u)\}, max\{sup\ \gamma_P(u), sup\ \gamma_P(u)\}] >)/u \in U \end{Bmatrix}$$

**Example 6**

$\boldsymbol{F_P} = \{\langle \boldsymbol{u_1}, [\boldsymbol{0.2}, \boldsymbol{0.4}], [\boldsymbol{0.3}, \boldsymbol{0.5}], [\boldsymbol{0.4}, \boldsymbol{0.6}]\rangle, \langle \boldsymbol{u_2}, [\boldsymbol{0.5}, \boldsymbol{0.7}], [\boldsymbol{0.2}, \boldsymbol{0.4}], [\boldsymbol{0.3}, \boldsymbol{0.5}]\rangle\}$

$\boldsymbol{G_Q} = \{\langle \boldsymbol{u_1}, [\boldsymbol{0.3}, \boldsymbol{0.6}], [\boldsymbol{0.2}, \boldsymbol{0.4}], [\boldsymbol{0.3}, \boldsymbol{0.5}]\rangle, \langle \boldsymbol{u_2}, [\boldsymbol{0.4}, \boldsymbol{0.6}], [\boldsymbol{0.3}, \boldsymbol{0.5}], [\boldsymbol{0.2}, \boldsymbol{0.4}]\rangle\}$

$\boldsymbol{F_P} \setminus \boldsymbol{G_Q} = \{\langle \boldsymbol{u_1}, [\boldsymbol{0.2}, \boldsymbol{0.4}], [\boldsymbol{0.6}, \boldsymbol{0.8}], [\boldsymbol{0.4}, \boldsymbol{0.6}]\rangle, \langle \boldsymbol{u_2}, [\boldsymbol{0.4}, \boldsymbol{0.6}], [\boldsymbol{0.5}, \boldsymbol{0.7}], [\boldsymbol{0.3}, \boldsymbol{0.5}]\rangle\}$

**Definition 4.9**

If $\boldsymbol{F_P}$ , $\boldsymbol{G_Q} \in$ IVNSHSS over $\boldsymbol{U}$, the difference is defined by

$$\boldsymbol{F_P} + \boldsymbol{G_Q} = \begin{Bmatrix} (< u, [\boldsymbol{min}\{\inf \boldsymbol{\alpha_P}(\boldsymbol{u}) + \boldsymbol{inf\alpha_Q}(\boldsymbol{u}), \boldsymbol{1}\}, \boldsymbol{min}\{\boldsymbol{sup\alpha_P}(\boldsymbol{u}) + \boldsymbol{sup\alpha_Q}(\boldsymbol{u}), \boldsymbol{1}\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\beta_P}(\boldsymbol{u}) + \boldsymbol{inf\beta_Q}(\boldsymbol{u}), \boldsymbol{1}\}, \boldsymbol{min}\{\sup \boldsymbol{\beta_P}(\boldsymbol{u}) + \boldsymbol{sup\beta_Q}(\boldsymbol{u}), \boldsymbol{1}\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\gamma_P}(\boldsymbol{u}) + \boldsymbol{inf\gamma_P}(\boldsymbol{u}), \boldsymbol{1}\}, \boldsymbol{min}\{\boldsymbol{sup\gamma_P}(\boldsymbol{u}) + \boldsymbol{sup\gamma_P}(\boldsymbol{u}), \boldsymbol{1}\}] >)/\boldsymbol{u} \in \boldsymbol{U} \end{Bmatrix}$$

**Example 7**

$\boldsymbol{F_P} = \{\langle \boldsymbol{u_1}, [\boldsymbol{0.2}, \boldsymbol{0.4}], [\boldsymbol{0.3}, \boldsymbol{0.5}], [\boldsymbol{0.4}, \boldsymbol{0.6}]\rangle, \langle \boldsymbol{u_2}, [\boldsymbol{0.5}, \boldsymbol{0.7}], [\boldsymbol{0.2}, \boldsymbol{0.4}], [\boldsymbol{0.3}, \boldsymbol{0.5}]\rangle\}$

$\boldsymbol{G_Q} = \{\langle \boldsymbol{u_1}, [\mathbf{0.3}, \mathbf{0.6}], [\mathbf{0.2}, \mathbf{0.4}], [\mathbf{0.3}, \mathbf{0.5}]\rangle, \langle \boldsymbol{u_2}, [\mathbf{0.4}, \mathbf{0.6}], [\mathbf{0.3}, \mathbf{0.5}], [\mathbf{0.2}, \mathbf{0.4}]\rangle\}$
$\boldsymbol{F_P} + \boldsymbol{G_Q} = \{\langle \boldsymbol{u_1}, [\mathbf{0.5}, \mathbf{1}], [\mathbf{0.5}, \mathbf{0.9}], [\mathbf{0.7}, \mathbf{1}]\rangle, \langle \boldsymbol{u}, [\mathbf{0.9}, \mathbf{1}], [\mathbf{0.5}, \mathbf{0.9}], [\mathbf{0.5}, \mathbf{0.9}]\rangle\}$

**Definition 4.10**

The scalar multiplication of $\boldsymbol{F_P} \in$ IVNSHSS over $\boldsymbol{U}$ is denoted by $\boldsymbol{F_P}.\mathfrak{p}$ , where $\mathfrak{p} \in [\mathbf{0}, \mathbf{1}]$ and is defined by:

$$\boldsymbol{F_P}.\mathfrak{p} == \begin{Bmatrix} (< u, [\boldsymbol{min}\{\mathbf{inf}\, \boldsymbol{\alpha_P}(\boldsymbol{u}).\mathfrak{p}, \mathbf{1}\}, \boldsymbol{min}\{\boldsymbol{sup\alpha_P}(\boldsymbol{u}).\mathfrak{p}, \mathbf{1}\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\beta_P}(\boldsymbol{u}).\mathfrak{p}, \mathbf{1}\}, \boldsymbol{min}\{\mathbf{sup}\, \boldsymbol{\beta_P}(\boldsymbol{u}).\mathfrak{p}, \mathbf{1}\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\gamma_P}(\boldsymbol{u}).\mathfrak{p}, \mathbf{1}\}, \boldsymbol{min}\{\boldsymbol{sup\gamma_P}(\boldsymbol{u}).\mathfrak{p}, \mathbf{1}\}] >)/\boldsymbol{u} \in \boldsymbol{U} \end{Bmatrix}$$

**Definition 4.11**

The scalar division of $\boldsymbol{F_P} \in$ IVNSHSS over $\boldsymbol{U}$ is denoted by $\boldsymbol{F_P}/\mathfrak{p}$ , where $\mathfrak{p} \in [\mathbf{0}, \mathbf{1}]$ and is expressed as:

$$\boldsymbol{F_P}.\mathfrak{p} == \begin{Bmatrix} (< u, [\boldsymbol{min}\{\mathbf{inf}\, \boldsymbol{\alpha_P}(\boldsymbol{u})/\mathfrak{p}, \mathbf{1}\}, \boldsymbol{min}\{\boldsymbol{sup\alpha_P}(\boldsymbol{u})/\mathfrak{p}, \mathbf{1}\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\beta_P}(\boldsymbol{u})/\mathfrak{p}, \mathbf{1}\}, \boldsymbol{min}\{\mathbf{sup}\, \boldsymbol{\beta_P}(\boldsymbol{u})./\mathfrak{p}, \mathbf{1}\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\gamma_P}(\boldsymbol{u})/\mathfrak{p}, \mathbf{1}\}, \boldsymbol{min}\{\boldsymbol{sup\gamma_P}(\boldsymbol{u})/\mathfrak{p}, \mathbf{1}\}] >)/\boldsymbol{u} \in \boldsymbol{U} \end{Bmatrix}$$

**Definition 4.12**

The Truth-Favorite operator of $\boldsymbol{F_P} \in$ IVNSHSS over $\boldsymbol{U}$ is denoted by $\sqcap\, \boldsymbol{F_P}$ and is given by:

$$\sqcap\, \boldsymbol{F_P} = \begin{Bmatrix} (< u, [\boldsymbol{min}\{\mathbf{inf}\, \boldsymbol{\alpha_P}(\boldsymbol{u}) + \boldsymbol{inf\beta_P}(\boldsymbol{u}), \mathbf{1}\}, \boldsymbol{min}\{\boldsymbol{sup\alpha_P}(\boldsymbol{u}) + \boldsymbol{sp\beta_P}(\boldsymbol{u}), \mathbf{1}\}], \\ [\mathbf{0}, \mathbf{0}], \\ [\mathbf{inf}\, \boldsymbol{\gamma_P}(\boldsymbol{u}), \mathbf{sup}\, \boldsymbol{\gamma_P}(\boldsymbol{u})] >)/\boldsymbol{u} \in \boldsymbol{U} \end{Bmatrix}$$

**Example 8**

$\boldsymbol{F_P} = \{\langle \boldsymbol{u_1}, [\mathbf{0.3}, \mathbf{0.5}], [\mathbf{0.4}, \mathbf{0.6}], [\mathbf{0.2}, \mathbf{0.3}]\rangle, \langle \boldsymbol{u_2}, [\mathbf{0.2}, \mathbf{0.4}], [\mathbf{0.3}, \mathbf{0.5}], [\mathbf{0.3}, \mathbf{0.4}]\rangle\}$
$\sqcap\, \boldsymbol{F_P} = \{\langle \boldsymbol{u_1}, [\mathbf{0.7}, \mathbf{1}], [\mathbf{0}, \mathbf{0}, [\mathbf{0.2}, \mathbf{0.3}]\rangle, \langle \boldsymbol{u_2}, [\mathbf{0.5}, \mathbf{1}], [\mathbf{0}, \mathbf{0}], [\mathbf{0.3}, \mathbf{0.4}]\rangle\}$

**Preposition 6**

If $\boldsymbol{F_P}$ , $\boldsymbol{G_Q} \in$ IVNSHSS over $\boldsymbol{U}$, then

1. $\sqcap\sqcap\, \boldsymbol{F_P} = \sqcap\, \boldsymbol{F_P}$
2. $\sqcap\, (\boldsymbol{F_P} \cup \boldsymbol{G_Q}) \subseteq \sqcap\, \boldsymbol{F_P} \cup \sqcap\, \boldsymbol{G_Q}$
3. $\sqcap\, (\boldsymbol{F_P} \cap \boldsymbol{G_Q}) \subseteq \sqcap\, \boldsymbol{F_P} \cap \sqcap\, \boldsymbol{G_Q}$
4. $\sqcap\, (\boldsymbol{F_P} + \boldsymbol{G_Q}) = \sqcap\, \boldsymbol{F_P} + \sqcap\, \boldsymbol{G_Q}$

The proof of the above proposition follows directly from the definitions..

**Definition 4.13**

The False-Favorite operator of $\boldsymbol{F_P} \in$ IVNSHSS over $\boldsymbol{U}$ is denoted by $\sqcup\, \boldsymbol{F_P}$ and is specified as:

$$\sqcup\, \boldsymbol{F_P} = \begin{Bmatrix} (< u, [\mathbf{inf}\, \boldsymbol{\alpha_P}(\boldsymbol{u}), \mathbf{sup}\, \boldsymbol{\alpha_P}(\boldsymbol{u})], \\ [\mathbf{0}, \mathbf{0}], \\ [\boldsymbol{min}\{\mathbf{inf}\, \boldsymbol{\beta_P}(\boldsymbol{u}) + \boldsymbol{inf\gamma_P}(\boldsymbol{u}), \mathbf{1}\}, \boldsymbol{min}\{\boldsymbol{sup\beta_P}(\boldsymbol{u}) + \boldsymbol{sup\gamma_P}(\boldsymbol{u}), \mathbf{1}\}] >)/\boldsymbol{u} \in \boldsymbol{U} \end{Bmatrix}$$

**Example 9**

$\boldsymbol{F_P} = \{\langle \boldsymbol{u_1}, [\mathbf{0.3}, \mathbf{0.5}], [\mathbf{0.4}, \mathbf{0.6}], [\mathbf{0.2}, \mathbf{0.3}]\rangle, \langle \boldsymbol{u_2}, [\mathbf{0.2}, \mathbf{0.4}], [\mathbf{0.3}, \mathbf{0.5}], [\mathbf{0.3}, \mathbf{0.4}]\rangle\}$
$\sqcup\, \boldsymbol{F_P} = \{\langle \boldsymbol{u_1}, [\mathbf{0.7}, \mathbf{1}], [\mathbf{0}, \mathbf{0}, [\mathbf{0.2}, \mathbf{0.3}]\rangle, \langle \boldsymbol{u_2}, [\mathbf{0.5}, \mathbf{1}], [\mathbf{0}, \mathbf{0}], [\mathbf{0.3}, \mathbf{0.4}]\rangle\}$

**Preposition 7**

If $F_P$ , $G_Q \in$ IVNSHSS over $U$, then

1. $\sqcup\sqcup\, \boldsymbol{F_P} = \sqcup\, \boldsymbol{F_P}$
2. $\sqcup\, (\boldsymbol{F_P} \cup \boldsymbol{G_Q}) \subseteq \sqcup\, \boldsymbol{F_P} \cup \sqcup\, \boldsymbol{G_Q}$
3. $\sqcup\, (\boldsymbol{F_P} \cap \boldsymbol{G_Q}) \subseteq \sqcup\, \boldsymbol{F_P} \cap \sqcup\, \boldsymbol{G_Q}$
4. $\sqcup\, (\boldsymbol{F_P} + \boldsymbol{G_Q}) = \sqcup\, \boldsymbol{F_P} + \sqcup\, \boldsymbol{G_Q}$

The proposition is proved directly from the definitions.

**Necessity - Possibility Operations on IVNSHSS**

**Definition 5.1**

For$\boldsymbol{F_P}$ , $\boldsymbol{G_Q}$ ∈ IVNSHSS over $\boldsymbol{U}$, then OR-Operator $\boldsymbol{F_P} \curlyvee \boldsymbol{G_Q}$ and defined by

$\boldsymbol{\alpha(F_{P\times Q})} = [\boldsymbol{max}\{\inf \boldsymbol{\alpha_P(u)}, \boldsymbol{inf\alpha_Q(u)}\}, \boldsymbol{max}\{\boldsymbol{sup\alpha_P(u)}, \boldsymbol{sup\alpha_Q(u)}\}]$,

$\boldsymbol{\beta(F_{P\times Q})} = [\boldsymbol{min}\{\inf \boldsymbol{\beta_P(u)}, \boldsymbol{inf\beta_Q(u)}\}, \boldsymbol{min}\{\boldsymbol{sup\beta_P(u)}, \boldsymbol{sup\beta_Q(u)}\}]$,

$\boldsymbol{\gamma(F_{P\times Q})} = [\boldsymbol{min}\{\inf \boldsymbol{\gamma_P(u)}, \boldsymbol{inf\gamma_Q(u)}\}, \boldsymbol{min}\{\boldsymbol{sup\gamma_P(u)}, \boldsymbol{sup\gamma_Q(u)}\}]$.

**Definition 5.2**

If $\boldsymbol{F_P}$ , $\boldsymbol{G_Q}$ ∈ IVNSHSS over $\boldsymbol{U}$, then OR-Operator is represented by $\boldsymbol{F_P} \curlywedge \boldsymbol{G_Q}$ and defined as follows

$\boldsymbol{\alpha(F_{P\times Q})} = [\boldsymbol{min}\{\inf \boldsymbol{\alpha_P(u)}, \boldsymbol{inf\alpha_Q(u)}\}, \boldsymbol{min}\{\boldsymbol{sup\alpha_P(u)}, \boldsymbol{sup\alpha_Q(u)}\}]$,

$\boldsymbol{\beta(F_{P\times Q})} = [\boldsymbol{max}\{\inf \boldsymbol{\beta_P(u)}, \boldsymbol{inf\beta_Q(u)}\}, \boldsymbol{max}\{\boldsymbol{sup\beta_P(u)}, \boldsymbol{sup\beta_Q(u)}\}]$,

$\boldsymbol{\gamma(F_{P\times Q})} = [\boldsymbol{max}\{\inf \boldsymbol{\gamma_P(u)}, \boldsymbol{inf\gamma_Q(u)}\}, \boldsymbol{max}\{\boldsymbol{sup\gamma_P(u)}, \boldsymbol{sup\gamma_Q(u)}\}]$.

**Preposition 8**

Let $\boldsymbol{F_P}$ , $\boldsymbol{G_Q}$, $\boldsymbol{H_R}$ ∈ IVNSHSS over $\boldsymbol{U}$, then

1. $\boldsymbol{F_P} \curlyvee \boldsymbol{G_Q} = \boldsymbol{G_Q} \curlyvee \boldsymbol{F_P}$
2. $\boldsymbol{F_P} \curlywedge \boldsymbol{G_Q} = \boldsymbol{G_Q} \curlywedge \boldsymbol{F_P}$
3. $\boldsymbol{F_P} \curlyvee (\boldsymbol{G_Q} \curlyvee \boldsymbol{H_R}) = (\boldsymbol{F_P} \curlyvee \boldsymbol{G_Q}) \curlyvee \boldsymbol{H_R}$
4. $\boldsymbol{F_P} \curlywedge (\boldsymbol{G_Q} \curlywedge \boldsymbol{H_R}) = (\boldsymbol{F_P} \curlywedge \boldsymbol{G_Q}) \curlywedge \boldsymbol{H_R}$
5. $(\boldsymbol{F_P} \curlyvee \boldsymbol{G_Q})^{\boldsymbol{C}} = \boldsymbol{F^C(P)} \curlywedge \boldsymbol{G^C(Q)}$
6. $(\boldsymbol{F_P} \curlywedge \boldsymbol{G_Q})^{\boldsymbol{C}} = \boldsymbol{F^C(P)} \curlywedge \boldsymbol{G^C(Q)}$

**Poof:** We can easily prove by definition**.**

**Definition 5.3**

Let $\boldsymbol{F_P}$ ∈ IVNSHSS over $\boldsymbol{U}$, then necessity operator $\oplus \boldsymbol{F_P}$is given by

$\oplus \boldsymbol{F_P} = \{\langle \boldsymbol{u}, [\boldsymbol{inf\alpha_P(u)}, \boldsymbol{sup\alpha_P(u)}], [\boldsymbol{inf\beta_P(u)}, \boldsymbol{sup\beta_P(u)}], [\boldsymbol{1} - \boldsymbol{sup\alpha_P(u)}, \boldsymbol{1} - \boldsymbol{inf\alpha_P(u)}] \rangle : \boldsymbol{u} \in \boldsymbol{U}\}$

**Example 10**

$\boldsymbol{F_P} = \{\langle \boldsymbol{u_1}, [\boldsymbol{0.3}, \boldsymbol{0.5}], [\boldsymbol{0.4}, \boldsymbol{0.6}], [\boldsymbol{0.2}, \boldsymbol{0.3}] \rangle, \langle \boldsymbol{u_2}, [\boldsymbol{0.2}, \boldsymbol{0.4}], [\boldsymbol{0.3}, \boldsymbol{0.5}], [\boldsymbol{0.3}, \boldsymbol{0.4}] \rangle\}$

$\oplus \boldsymbol{F_P} = \{\langle \boldsymbol{u_1}, [\boldsymbol{0.3}, \boldsymbol{0.5}], [\boldsymbol{0.4}, \boldsymbol{0.6}], [\boldsymbol{0.5}, \boldsymbol{0.7}] \rangle, \langle \boldsymbol{u_2}, [\boldsymbol{0.2}, \boldsymbol{0.4}], [\boldsymbol{0.3}, \boldsymbol{0.5}], [\boldsymbol{0.6}, \boldsymbol{0.8}] \rangle\}$

**Definition 5.4**

Let $\boldsymbol{F_P}$ ∈ IVNSHSS over $\boldsymbol{U}$, then possibiliity $\otimes \boldsymbol{F_P}$is defined by

$\otimes \boldsymbol{F_P} = \{\langle \boldsymbol{u}, [\boldsymbol{1} - \boldsymbol{sup\gamma_P(u)}, \boldsymbol{1} - \boldsymbol{inf\gamma_P(u)}], [\boldsymbol{inf\beta_P(u)}, \boldsymbol{sup\beta_P(u)}], [\boldsymbol{inf\gamma_P(u)}, \boldsymbol{sup\gamma_P(u)}] \rangle : \boldsymbol{u} \in \boldsymbol{U}\}$

**Example 11**

$\boldsymbol{F_P} = \{\langle \boldsymbol{u_1}, [\boldsymbol{0.3}, \boldsymbol{0.5}], [\boldsymbol{0.4}, \boldsymbol{0.6}], [\boldsymbol{0.2}, \boldsymbol{0.3}] \rangle, \langle \boldsymbol{u_2}, [\boldsymbol{0.2}, \boldsymbol{0.4}], [\boldsymbol{0.3}, \boldsymbol{0.5}], [\boldsymbol{0.3}, \boldsymbol{0.4}] \rangle\}$

$\otimes \boldsymbol{F_P} = \{\langle \boldsymbol{u_1}, [\boldsymbol{0.7}, \boldsymbol{0.8}], [\boldsymbol{0.4}, \boldsymbol{0.6}], [\boldsymbol{0.2}, \boldsymbol{0.3}] \rangle, \langle \boldsymbol{u_2}, [\boldsymbol{0.6}, \boldsymbol{0.7}], [\boldsymbol{0.3}, \boldsymbol{0.5}], [\boldsymbol{0.3}, \boldsymbol{0.4}] \rangle\}$

**Preposition 9**

Let $\boldsymbol{F_P}$ , $\boldsymbol{G_Q}$ ∈ IVNSHSS over $\boldsymbol{U}$, then

1. $\oplus (\boldsymbol{F_P} \cup \boldsymbol{G_Q}) = \oplus \boldsymbol{F_P} \cup \oplus \boldsymbol{G_Q}$
2. $\oplus (\boldsymbol{F_P} \cap \boldsymbol{G_Q}) = \oplus \boldsymbol{F_P} \cap \oplus \boldsymbol{G_Q}$

**Proof:**

$\boldsymbol{F_P} = \{\langle \boldsymbol{u}, [\boldsymbol{inf\alpha_P(u)}, \boldsymbol{sup\alpha_P(u)}], [\boldsymbol{inf\beta_P(u)}, \boldsymbol{sup\beta_P(u)}], [\boldsymbol{inf\gamma_P(u)}, \boldsymbol{sup\gamma_P(u)}] \rangle : \boldsymbol{u} \in \boldsymbol{U}\}$

$\boldsymbol{G_Q} = \{\langle \boldsymbol{u}, [\boldsymbol{inf\alpha_Q(u)}, \boldsymbol{sup\alpha_Q(u)}], [\boldsymbol{inf\beta_Q(u)}, \boldsymbol{sup\beta_Q(u)}], [\boldsymbol{inf\gamma_Q(u)}, \boldsymbol{sup\gamma_Q(u)}] \rangle : \boldsymbol{u} \in \boldsymbol{U}\}$

Then by definition

$$\boldsymbol{F_P} \cup \boldsymbol{G_Q} = \left\{ \begin{matrix} (< u, [\boldsymbol{max}\{\inf \boldsymbol{\alpha_P}(u), \boldsymbol{inf\alpha_Q}(u)\}, \boldsymbol{max}\{\boldsymbol{sup\alpha_P}(u), \boldsymbol{sup\alpha_Q}(u)\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\beta_P}(u), \boldsymbol{inf\beta_Q}(u)\}, \boldsymbol{min}\{\boldsymbol{sup\beta_P}(u), \boldsymbol{sup\beta_Q}(u)\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\gamma_P}(u), \boldsymbol{inf\gamma_Q}(u)\}, \boldsymbol{min}\{\boldsymbol{sup\gamma_P}(u), \boldsymbol{sup\gamma_Q}(u)\}] >)/u \in U \end{matrix} \right\}$$

By necessity operator definition

$$\oplus \boldsymbol{F_P} \cup \boldsymbol{G_Q} = \left\{ \begin{matrix} (< u, [\boldsymbol{max}\{\inf \boldsymbol{\alpha_P}(u), \boldsymbol{inf\alpha_Q}(u)\}, \boldsymbol{max}\{\boldsymbol{sup\alpha_P}(u), \boldsymbol{sup\alpha_Q}(u)\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\beta_P}(u), \boldsymbol{inf\beta_Q}(u)\}, \boldsymbol{min}\{\boldsymbol{sup\beta_P}(u), \boldsymbol{sup\beta_Q}(u)\}], \\ [\boldsymbol{1 - max}\{\boldsymbol{sup\alpha_P}(u), \boldsymbol{sup\alpha_Q}(u)\}, \boldsymbol{1 - max}\{\boldsymbol{inf\alpha_P}(u), \boldsymbol{inf\alpha_Q}(u)\}] >)/u \in U \end{matrix} \right\}$$

$$\oplus \boldsymbol{F_P} \cup \boldsymbol{G_Q} = \left\{ \begin{matrix} (< u, [\boldsymbol{max}\{\inf \boldsymbol{\alpha_P}(u), \boldsymbol{inf\alpha_Q}(u)\}, \boldsymbol{max}\{\boldsymbol{sup\alpha_P}(u), \boldsymbol{sup\alpha_Q}(u)\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\beta_P}(u), \boldsymbol{inf\beta_Q}(u)\}, \boldsymbol{min}\{\boldsymbol{sup\beta_P}(u), \boldsymbol{sup\beta_Q}(u)\}], \\ [\boldsymbol{min}\{\boldsymbol{1 - sup\alpha_P}(u), \boldsymbol{1 - sup\alpha_Q}(u)\}, \boldsymbol{min}\{\boldsymbol{1 - inf\alpha_P}(u), \boldsymbol{1 - inf\alpha_Q}(u)\}] >)/u \in U \end{matrix} \right\}$$

$$\oplus \boldsymbol{F_P} = \{\langle \boldsymbol{u}, [\boldsymbol{inf\alpha_P}(u), \boldsymbol{sup\alpha_P}(u)], [\boldsymbol{inf\beta_P}(u), \boldsymbol{sup\beta_P}(u)], [\boldsymbol{1 - sup\alpha_P}(u), \boldsymbol{1 - inf\alpha_P}(u)]\rangle : \boldsymbol{u} \in \boldsymbol{U}\}$$

$$\oplus \boldsymbol{G_Q} = \{\langle \boldsymbol{u}, [\boldsymbol{inf\alpha_Q}(u), \boldsymbol{sup\alpha_Q}(u)], [\boldsymbol{inf\beta_Q}(u), \boldsymbol{sup\beta_Q}(u)], [\boldsymbol{1 - sup\alpha_Q}(u), \boldsymbol{1 - inf\alpha_Q}(u)]\rangle : \boldsymbol{u} \in \boldsymbol{U}\}$$

By definition

$$\oplus \boldsymbol{F_P} \cup \oplus \boldsymbol{G_Q} == \left\{ \begin{matrix} (< u, [\boldsymbol{max}\{\inf \boldsymbol{\alpha_P}(u), \boldsymbol{inf\alpha_Q}(u)\}, \boldsymbol{max}\{\boldsymbol{sup\alpha_P}(u), \boldsymbol{sup\alpha_Q}(u)\}], \\ [\boldsymbol{min}\{\boldsymbol{inf\beta_P}(u), \boldsymbol{inf\beta_Q}(u)\}, \boldsymbol{min}\{\boldsymbol{sup\beta_P}(u), \boldsymbol{sup\beta_Q}(u)\}], \\ [\boldsymbol{min}\{\boldsymbol{1 - sup\alpha_P}(u), \boldsymbol{1 - sup\alpha_Q}(u)\}, \boldsymbol{min}\{\boldsymbol{1 - inf\alpha_P}(u), \boldsymbol{1 - inf\alpha_Q}(u)\}] >)/u \in U \end{matrix} \right\}$$

$$\oplus \left(\boldsymbol{F_P} \cup \boldsymbol{G_Q}\right) = \oplus \boldsymbol{F_P} \cup \oplus \boldsymbol{G_Q}$$

Statement (2) follows similarly.

**Preposition 10**

Let $\boldsymbol{F_P}$ , $\boldsymbol{G_Q}$, $\boldsymbol{H_R}$ ∈ IVNSHSS over $\boldsymbol{U}$, then

1. $\oplus \left(\boldsymbol{F_P} \curlyvee \boldsymbol{G_Q}\right) = \oplus \boldsymbol{G_Q} \curlyvee \oplus \boldsymbol{F_P}$
2. $\oplus \left(\boldsymbol{F_P} \curlywedge \boldsymbol{G_Q}\right) = \oplus \boldsymbol{G_Q} \curlywedge \oplus \boldsymbol{F_P}$
3. $\otimes \left(\boldsymbol{F_P} \curlyvee \boldsymbol{G_Q}\right) = \otimes \boldsymbol{G_Q} \curlyvee \otimes \boldsymbol{F_P}$
4. $\otimes \left(\boldsymbol{F_P} \curlywedge \boldsymbol{G_Q}\right) = \otimes \boldsymbol{G_Q} \curlywedge \otimes \boldsymbol{F_P}$

Proof is directly obtained by definition.

## CONCLUSION

In this article, we introduce a new mathematical model called the Interval-Valued Neutrosophic SuperHyperSoft Set (IVNSHSS). This model is designed specifically as a way to handle complicated uncertainties in multi-attribute contexts. The IVNSHSS combines interval-valued neutrosophic sets and superhyper parameterization to produce an expressive and flexible representation of uncertainty, indeterminacy, and inconsistency, which is superior to existing soft set type structures. The IVNSHSS has completely defined and analyzed its fundamental concepts: null set, universal set, complement, union, intersection, difference, scalar operations, as well as different operators. We provide propositions that explore the algebraic properties of these operations along with several illustrative examples that demonstrate their reality. The IVNSHSS model allows an extension of classical soft sets, hypersoft sets, and interval-valued neutrosophic soft sets by allowing for the construction of the parameters from the Cartesian product of the power sets of the attribute domains. Therefore, the IVNSHSS framework will be especially applicable to solutions to real-life problems with interacting attributes and imprecise data. Furthermore, our findings contribute to building the theoretical basis for neutrosophic computing and soft computing theories and create opportunities for future research.

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RESEARCH ARTICLE

# Investigation of UV - C Induced Phytochemical Variations in *Amaranthus hypochondriacus* L. Callus Using HPTLC

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## ABSTRACT

*Amaranthus hypochondriacus* L. is a nutritionally and pharmacologically important plant rich in bioactive phytochemicals. The present study investigates the effect of UV-C irradiation on phytochemical variations in *in vitro* callus cultures of *A. hypochondriacus* using High-Performance Thin-Layer Chromatography (HPTLC). Optimal callus induction was achieved after four weeks on Murashige and Skoog (MS) medium supplemented with 1 mg/L IBA, 1 mg/L 2,4-D, and 2 mg/L kinetin (CIM1). Callus cultures (GA6) exposed to UV-C treatment for 60 minutes showed enhanced and distinct phytochemical profiles. The harvested *in vitro* samples were dried, pulverized, and subjected to Soxhlet extraction. Extracts (2–12 µL) from UV-C-treated calluses were applied as 8 mm bands on Merck Silica gel $60F_{254}$ TLC plates using a CAMAG Linomat-V applicator. Chromatographic development was carried out using toluene:ethyl acetate:formic acid (5:4:1 v/v%). The developed plates were visualized under white light and UV light at 254 nm using a CAMAG TLC Visualizer, followed by densitometric scanning at 254 nm with a CAMAG Scanner IV. Data acquisition and analysis were performed using WINCATS software. HPTLC fingerprinting revealed effective separation of multiple phytochemical constituents, indicating UV-C-induced metabolic variations in callus cultures. Although the absence of reference standards limited definitive compound identification, the observed chromatographic patterns provide a strong

basis for future studies. Further characterization using advanced analytical techniques such as LC-MS or GC-MS, along with standard compounds, is recommended for precise identification and structural confirmation of UV-C-responsive phytochemicals.

**Keywords:** *Amaranthus hypochondriacus* L.,UV –C, Kinetin, 2,4-D, HPTLC, toluene:ethyl acetate:formic acid (5:4:1 v/v%).

## INTRODUCTION

Drug development and therapeutic applications are based on secondary metabolites [1]. Secondary metabolites found in therapeutic plants have important therapeutic qualities. As antioxidative, anticancer, anti-inflammatory and antimicrobial agents, these bioactive substances have significant therapeutic potential [2-5]. For thousands of years, pseudocereals have been a staple food in many parts of the world. Even though their geographic dominance varies, they still serve as the main source of nutrition in many of the world's poorest regions. Pseudocereals have also become more well-known in European nations in recent decades due to their nutritional and health advantages. Amaranth, buckwheat, sorghum, millet, chia, and khorasan are some of the most well-known pseudocereals; the most researched are quinoa, amaranth, chia, and buckwheat [6]. Amaranth's full potential is still not being fully utilized, despite its lengthy history and cultural significance. It has substantial economic value due to its nutritional diversity and adaptability, providing a range of advantages to food processors, producers, and consumers. Because of the superior nutritional and functional qualities of its seeds, the pseudocereal *Amaranthus hypochondriacus* is essentially recognised as a grain crop [7,8]. The potential of pseudocereal *A. hypochondriacus* as a functional food has been rediscovered in recent years due to its excellent seed protein quantity and quality, availability of dietary fibres, lipid-rich unsaturated fatty acids, vitamins, micronutrients, etc. [9-11]. Amaranths are a nutrient-dense powerhouse rich in bioactive compounds such as carotenoids, polyphenols, and flavonoids. These compounds are associated with a range of health benefits, including antioxidant, anti-inflammatory, and anticancer properties. Because of its antiseptic, antifungal, and anti-inflammatory qualities, amaranth is widely used in the pharmaceutical industry to create medications that target atherosclerosis, stomach ulcers, and tuberculosis [12].

The seeds of *Amaranthus hypochondriacus* L. are known to be a spermatogenetic medication and tonic in Unani medicine [13]. Furthermore, the plant's decoction is customarily used to treat heavy menstrual bleeding, and the flowers are used as remedies for coughing, diarrhea, dysentery, and hemorrhages. Studies using both human [14] subjects and animal [15] models have demonstrated the substantial antidiabetic effects of amaranth oil. Additionally, research suggests that taking amaranth oil supplements improves antioxidant defences and strengthens immune function, especially in people with hyperlipoproteinemia or ischaemic heart disease[16]. According to [17] amaranth extract made from the entire plant has strong antioxidant properties and can significantly scavenge free radicals in the DPPH assay. Similarly, in rats that were subchronically exposed to ethanol, [18] examined the antioxidant qualities of *Amaranthus hypochondriacus* seed extract. Their results supported the extract's positive effects and demonstrated that it protected against ethanol-induced liver damage due to its high polyphenol content [18]. Examined the phytochemistry and *in vitro* antioxidant activity of extracts from the leaves, stalks, seeds, flowers, and sprouts of *A. hypochondriacus*, *A. caudatus*, and *A. cruentus*. They discovered that *A. hypochondriacus* had higher antioxidant potentials and that phenolics and antioxidant activity were positively correlated [19]. Additionally, a variety of secondary metabolites found in amaranth have positive effects on the diets of both humans and animals[20]. The phenolic compound content of amaranth seeds [21] as well as their functional and bioactive qualities [22-25] have been the subject of numerous investigations over the last fifteen years. In light of this, the current study intends to describe the agronomic characteristics, oil content, fatty acid profile, antioxidant activity, and total phenolic content of four *Amaranthus hypochondriacus* seed accessions that come from Nebraska, Iowa, India, and Pennsylvania and are all grown in identical environmental conditions.

The goal is to increase knowledge of this species as a beneficial source of nutrients and bioactive substances, especially essential fatty acids and polyphenols, for use in animal feed. "However, the World Health Organization has promoted the use of contemporary analytical methods like Gas Chromatography–Mass Spectrometry (GC–MS), High-Performance Thin Layer Chromatography (HPTLC), and High-Performance Liquid Chromatography (HPLC) and stressed the significance of quality control and standardization of plant materials" [26]. Because of its accuracy, affordability, and speed, High-Performance Thin Layer Chromatography (HPTLC) has established itself as a standard analytical method and is being utilized more and more in place of High-Performance Liquid Chromatography (HPLC) for the estimation of biochemical products derived from plants. Because all conditions in HPTLC are automated, the same plate can be scanned repeatedly. Additionally, the method can be used to validate a large range of compounds, regardless of their polarity. The ability of HPTLC-based chromatographic fingerprinting to identify the presence or absence of marker compounds of interest and to ascertain the relative proportions of all detectable constituents is one of its main benefits. At the micro- and nanogram levels, HPTLC is still a useful technique for evaluating the quality of a variety of natural products, herbal medications, and commercial herbal formulations. The current study will use the HPTLC technique to compare the phytochemical profiles of *in vitro* and wild plants in order to achieve its research goal. Because of their substantial analytical capabilities, HPTLC methods are regarded as a good substitute in routine drug analysis. The method saves time and money on analysis because it uses very little mobile phase [27]. Additionally, HPTLC reduces the possibility of coming into contact with dangerous chemicals and producing harmful wastewater, which eventually helps to lessen pollution in the environment. The current study uses High-Performance Thin-Layer Chromatography (HPTLC) techniques to identify and characterize the bioactive compounds found in the plant extract of *Amaranthus hypochondriacus* L. This analysis might offer insightful information about the plant's possible uses in both conventional and contemporary medicine.

## MATERIALS AND METHODS

**Culture condition**

As explants, 60 min. UV-C irradiated [28,29] and sterilized seeds of *Amaranthus hypochondriacus* L. (GA6) were inoculated on solid Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) supplemented with different concentration of PGRs ( MS + 1mg/l IBA + 1mg/l 2,4-D + 2mg/l KIN - MS + CIM1) [30], (MS + 1mg/l IBA + 2mg/l 2,4-D - MS + CIM2) and (MS + 4 mg/l 2,4-D – MS + CIM3) [31]. The medium's pH was brought down to 5.7 before it was autoclaved for 15 minutes at 121 °C and 15 psi. Using light-emitting diode (LED) lights, the inoculated explants were incubated for callus induction at 25 ± 2 °C for 16/8 hours of light/dark photoperiod with an illuminance of 25 μmol $m^{-2}$ $s^{-1}$.

**Extraction**

In order to completely remove the moisture and dehydrate the callus, the freshly collected *in vitro* sample (callus) were dried for 12 to 24 hours at 35 °C using a dehydrator (Kenwood food dehydrator, England). After the *in vitro* sample (Callus) was dried and ground into a fine powdered (Fig. 2a.), a Soxhlet apparatus was used to extract it. The extraction solvent used was methanol [32]. 10 grams powdered of callus was extracted with methanol (100 ml) using soxhlet apparatus at 60–80°C. Whatman No. 42 Filter paper was used to clarify the resultant extract, and the excess solvent was evaporated to concentrate the filtrate (Fig. 2b.). Thereafter, the crude extract was stored in airtight containers for additional examination.

**Qualitative phytochemical analysis**

The phytochemical analysis of the methanolic extract from the 60-minute UV-C-treated callus of *A. hypochondriacus* was subjected to preliminary phytochemical screening using standard qualitative tests to identify the presence of different phytoconstituents in accordance with established protocols [33] (Table 1).Qualitative phytochemical analysis of the methanolic extract from the 60 min. UV-C-treated callus of *A. hypochondriacus* L. (Table 1).

**HPTLC Fingerprinting**

HPTLC fingerprinting was performed on Merck Silica gel 60F254 TLC precoated plates. Six separate concentrations of 2 µL, 4 µL, 6 µL, 8 µL, 10 µL, and 12 µL of extract were performed separately placed to the plate in the shape of an 8 mm broad band using the CAMAG (Switzerland) Linomat-V sample applicator. The mobile phase was composed of toluene: Ethyl acetate: formic acid (5:4:1v/v). In a CAMAG twin trough chamber (20 × 10 cm) that had been equilibrated with mobile phase for 20 minutes, the plate was developed to a distance of 70 mm. Following development, the plate was examined using the CAMAG TLC Visualizer, and pictures were captured using white light and 254 nm. Anisaldehyde sulfuric acid reagent (ASR) was used to derivatize the plate, which was then stored at 110 °C for three minutes until color bands formed. The plate was then scanned using a CAMAG Scanner IV at 254 nm. The finger print data was recorded using the WINCATS planer chromatography management software [34].

## RESULT

**Callus induction**

Callus initiation exhibited considerable variation among explant types, indicating a differential response to the specific auxin-to-cytokinin ratios present in the medium. Of the three combinations tested, MS medium with 1 mg/l IBA+1 mg/l 2,4-D + 2 mg/l KIN. (MS+CIM1) was the best. (Fig. 1). It reached a peak induction rate of 90% in the period of four weeks. Initially, the calluses appeared off-white, after a few days they turned yellowish-white. The callus was fibrous in texture, easily breakable into small fragments, and ideal for callus multiplication. Optimal callus formation was achieved after four weeks using MS supplemented with 1mg/l IBA + 1mg/l 2,4-D + 2mg/l KIN - MS + CIM1 (Fig.1). Fig. 2a. Dried poweder of callus obtained from 60 min. UV-C Treated seed of *A.hypochondriacus* L. Fig. 2b. Methanolic extract of callus obtained from 60 min. UV-C Treated seed of *A. hypochondriacus* L. *Amaranthus hypochondriacus* has a rich phytochemical composition and therapeutic potential, as evidenced by the presence of alkaloids, phenolic compounds, flavonoids, terpenoids, saponins, glycosides, and amino acids in preliminary phytochemical screening. (Table 2). Table 2: Qualitative phytochemical screenings of the methanolic extract from the 60-minute UV-C-treated callus of *A. hypochondriacus* L. Note: '+'indicates present and'-'indicates absent.

**HPTLC**

The HPTLC fingerprinting of methanolic extracts of 60-min UV-C treated callus of *Amaranthus hypochondriacus* L., using the solvent system toluene : ethyl acetate : formic acid (5:4:1 v/v/v), revealed successful separation of several phytoconstituents corresponding to distinct retention factor (Rf) values and chromatographic peaks. Different Rf values of the compounds provide insights into their polarity, which in turn can guide the selection of a suitable solvent system for isolating a particular compound from plant extracts using chromatographic and spectroscopic techniques [35]. A compound that exhibits a high Rf value in a less polar solvent system is of relatively low polarity, whereas one with a low Rf value is comparatively more polar [36]. Based on the obtained Rf values, the presence of different phytoconstituents in the methanolic extract of UV-C treated callus was confirmed, indicating significant phytochemical diversity. The chromatographic profile revealed the presence of ten (10) distinct components in the 60-min UV-C treated callus extract of *A. hypochondriacus* L. (Table 3 and Fig.3).The chromatogram recorded at 254 nm and its three-dimensional representation are displayed in Fig.4. The number of constituent peaks, their corresponding Rf values, area % and number of components are summarized (Table 3). Six tracks of different volumes of methanolic extract of 60 min. UV-C Treated *Amaranthus hypochondriacus* L. callus were applied, and the results were examined at 254 nm under UV light. The phytoconstituents present were examined by measuring the resulting peaks and Rf values at various wavelengths. The methanolic extract of *Amaranthus hypochondriacus* L. produced three bands when applied at a 2 µL spot (Track 1). Increasing the spot volumes to 4 µL, 6 µL and 8 µL (Tracks 2–4) yielded seven bands each, while a 10 µL spot (Track 5) produced ten bands, and a 12 µL spot (Track 6) resulted in six bands. (Fig.3). All sample constituents were clearly separated by high-performance thin-layer chromatography (HPTLC) fingerprinting of the methanolic extract of *Amaranthus hypochondriacus* L. demonstrating the lack of diffusion and tailing (Fig.3). Three spots with Rf values of 0.67, 0.75, and 0.82 were found in the 2 µL sample of *Amaranthus hypochondriacus* methanolic extract that was analyzed at 254 nm. Since it made up 41.88 % of

the entire chromatographic area, the compound at Rf = 0.82 was determined to be the predominant component. When a 4 µL aliquot of *Amaranthus hypochondriacus* methanolic extract was analyzed at 254 nm, seven different spots were visible, with Rf values of 0.11, 0.22, 0.33, 0.44, 0.67, 0.74, and 0.79. The components that corresponded to Rf 0.67 and Rf 0.79 were the most prevalent among these, making up 27.51% and 18.31% of the total area, respectively. Seven different spots with Rf values of 0.11, 0.18, 0.21, 0.33, 0.44, 0.67, and 0.73 were visible when a 6 µL aliquot of *Amaranthus hypochondriacus* methanolic extract was applied and scanned at 254 nm. The components with Rf values of 0.33, 0.44, 0.67, and 0.73 were found to be predominant in a 4 µL application of the same extract, corresponding to percentage areas of 12.71%, 15.90%, 34.02%, and 17.18%, respectively. The area percentage of all the spots was less than 10%, indicating that the remaining components were present in very small amounts. Seven spots (Rf = 0.11, 0.22, 0.33, 0.44, 0.67, 0.73, and 0.77) were found in an 8 µL methanolic extract of *Amaranthus hypochondriacus* that was analyzed at 254 nm; the components that corresponded to Rf = 0.67 and Rf = 0.77 were the most prevalent, with percentage areas of 30.93% and 16.43%, respectively. Among the 10 components obtained in a 10 µL aliquot of the *Amaranthus hypochondriacus* methanolic extract at 254 nm (with Rf values of 0.11, 0.22, 0.33, 0.44, 0.53, 0.59, 0.62, 0.68, 0.74, and 0.77), the compounds with Rf values of 0.44 and 0.68 were the most prevalent, accounting for 12.13% and 27.04% of the total peak area, respectively. Fig. 3: The HPTLC chromatogram of methanolic extract of 60 min. UV-C treated *A. hypochondriacus* L. callus. Six spots with Rf values of 0.12, 0.23, 0.35, 0.46, 0.70, 0.77, and 0.81 were found in the 12 µL methanolic extract of *Amaranthus hypochondriacus* that was analyzed at 254 nm. With 15.35% and 28.96% of the total area, respectively, the compounds that corresponded to Rf 0.35 and 0.70 were the most prevalent among them. Three dimensional representation of all tracks at 254 nm wavelength (Fig. 5).

## DISCUSSION

In the present study, high-performance thin-layer chromatography (HPTLC) was employed to profile the phytochemical constituents of this extract. Multiple distinct bands were observed in the chromatographic fingerprints, indicating the presence of several phytocomponents. Discrete spots or bands on the chromatogram, each spot representing a compound (or cluster of compounds) migrating to a specific Rf under the selected conditions can be used in HPTLC fingerprinting to infer the number of phytochemical constituents, even in the absence of pure reference standards." By documenting Rf values, spot intensities (e.g., area or height) and appearances under different detection modes (UV at various wavelengths, visible light) one establishes a chromatographic fingerprint or chemical profile of a sample. HPTLC is prized for its quick, effective, and repeatable analysis [37].Plant-based compound analysis is more precise and accurate when integrated with digital scanning [38]. HPTLC techniques are frequently used in routine drug analysis because of their analytical significance. As a result, fingerprints without standards are still useful for qualitative and comparative sample profiling, identifying batch-to-batch consistency, variation, or adulteration. Due to the unavailability of reference standards, direct confirmation of individual compounds was not performed. However, tentative identification of the phytochemicals was achieved by comparing the observed Rf values with those reported in previously published literature under similar chromatographic conditions. The consistency between the Rf values obtained in this study and those documented for known phytoconstituents suggests the possible presence of compounds such as flavonoids, phenolic acids, alkaloids, and terpenoids. HPTLC fingerprinting without the use of standards is widely accepted as a preliminary qualitative approach for phytochemical characterization and provides valuable insight into the chemical complexity of plant extracts.

Nevertheless, it is acknowledged that Rf-based identification remains presumptive, as Rf values may vary depending on the solvent system, stationary phase, and experimental conditions. The absence of standards limits definitive identification of individual compounds; however, the observed separation provides a foundation for future studies involving standard compounds and advanced analytical techniques such as LC-MS, or GC-MS for precise identification and structural confirmation. Overall, the findings demonstrate that HPTLC is an efficient and reproducible method for preliminary phytochemical screening and for assessing the number of components present in complex plant extracts. The qualitative variations in the phytocompounds were reflected in the variations in the Rf

values of the majority of the peaks that emerged. This HPTLC densitometry fingerprint profile can be used as a marker for standardisation and evaluation of extract quality. As a result, the HPTLC fingerprint profile and the extract's Rf values were noted, which would act as a benchmark for further research on the plant [39]. Furthermore, it is both practical and safe to use *Amaranthus hypochondriacus* L. in nutraceuticals and herbal formulations because it is already consumed as a grain and leafy vegetable in many parts of the world. The standardization made possible by HPTLC guarantees that the therapeutic effectiveness of these preparations can be reliably and scientifically confirmed. Herbal medicines have replaced synthetic drugs globally in recent years; this trend is frequently referred to as a "return to nature." [40]. The World Health Organization (WHO) divides herbal medicines into three categories: finished herbal medicinal products, processed plant materials, and raw plant materials [41]. Both developed and developing nations' healthcare systems make extensive use of these plant-based preparations, which are intricate chemical blends [42]. Therefore, the current study emphasizes that the methanolic extract of *A. hypochondriacus*, backed by HPTLC fingerprinting, can be a solid foundation for creating herbal medicines and functional foods that are affordable and targeted at treating chronic and lifestyle-related illnesses.

## CONCLUSION

Emplyoing toluene, ethyl acetate, and formic acid (5:4:1 v/v%) as the mobile phase, the HPTLC analysis successfully identified the presence of several phytochemical components in the methanolic extract of 60 min. UV-C treated *Amaranthus hypochondriacus* L. callus, indicates the plant's potential as a valuable source of bioactive compounds. The technique produced a distinct chromatographic fingerprint and made it possible to determine the quantity of phytoconstituents even in the absence of reference standards. This initial analysis can be used as a starting point for additional pharmacological and phytochemical research. The preliminary phytochemical screening result was further validated by the HPTLC analysis results, which displayed distinct colored bands indicating the presence of specific phytocomponents. *A. hypochondriacus* shows promise for the creation of affordable herbal formulations and therapeutic agents in the future due to its rich nutritional profile and medicinally significant secondary metabolites. To confirm its effectiveness and safety for use in medicine, more pharmacological and clinical research is necessary. Considering their phytochemical richness, these plants may also be explored as inexpensive sources for herbal medicines and formulations with potential applications against a wide spectrum of diseases.

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**Table 1: Qualitative phytochemical analysis of the methanolic extract from the 60 min. UV-C-treated callus of *A. hypochondriacus*L.**

| Sr.No. | Phytochemical constituents | Test name | Procedure |
|---|---|---|---|
| 1 | Alkaloids | Mayer's test | 2cm³ extract + dilute HCl+ add a few drops of Mayer Reagent |
| | | Wagner's test | 2cm³ extract + dilute HCl+ add a few drops of Wagner Reagent |
| 2 | Flavonoids | Alkaline Reagent Test | 2cm³ extract + 10% NaOH + dilute HCl |

| | | | |
|---|---|---|---|
| 3 | Glycosides | $H_2SO_4$ Test | 2cm³ extract + 2cm³ Conc. $H_2SO_4$ |
| 4 | Tannin | Braymer's Test | 1cm³ extract + 10 % alcoholic $Fecl_3$ |
| 5 | Terpenoids | Salkowsik's test | 3cm³ extract + 1cm³ Chloroform+1.5cm³ conc. $H_2SO_4$ |
| 6 | Carbohydrates | Molisch's test | 1cm³ extract + ⅔ drops of Molisch's Reagent+ 1cm³ Conc. $H_2SO_4$ |
| 7 | Phenols | Lead acetate Test | 2cm³ extract + 1cm³ 10% Lead aceate |
| 8 | Saponins | Froth test | 2cm³ extract + 2cm³ distilled water |
| 9 | Amino acids | Ninhydrin reagent Test | 2cm³ extract + 2cm³ ninhydrin reagent+ boil few minutes |

**Table 2: Qualitative phytochemical screenings of the methanolic extract from the 60-minute UV-C-treated callus of *A. hypochondriacus*L.**

| Sr. No. | Phytoconstituents | Observation |
|---|---|---|
| 1 | Alkaloids | + |
| 2 | Flavonoids | + |
| 3 | Phenol | + |
| 4 | Terpenoids | + |
| 5 | Tannis | - |
| 6 | Fat | - |
| 7 | Glycosides | + |
| 8 | Saponins | + |
| 9 | Amino acid | + |

Note: '+'indicates present and'-'indicates absent..

**Table 3: The number of constituent peaks, their corresponding Rf values, area % and number of components are summarized.**

| Track | Peak | Start Position | Max Position | End Position | Area | Area % | Assigned substance | Number of components |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 0.65 Rf | 0.67 Rf | 0.70 Rf | 293.2 AU | 28.18% | 1 | 3 |
| 1 | 2 | 0.71 Rf | 0.75 Rf | 0.76 Rf | 311.6 AU | 29.94% | 2 | |
| 1 | 3 | 0.80 Rf | 0.82 Rf | 0.86 Rf | 435.7 AU | 41.88% | 3 | |
| 2 | 1 | 0.09 Rf | 0.11 Rf | 0.14 Rf | 181.1 AU | 6.26% | 1 | 7 |
| 2 | 2 | 0.18 Rf | 0.22 Rf | 0.25 Rf | 240.5 AU | 8.32% | 2 | |
| 2 | 3 | 0.30 Rf | 0.33 Rf | 0.36 Rf | 322.6 AU | 11.15% | 3 | |
| 2 | 4 | 0.40 Rf | 0.44 Rf | 0.47 Rf | 384.2 AU | 13.29% | 4 | |
| 2 | 5 | 0.63 Rf | 0.67 Rf | 0.70 Rf | 795.7 AU | 27.51% | 5 | |
| 2 | 6 | 0.71 Rf | 0.74 Rf | 0.75 Rf | 438.4 AU | 15.16% | 6 | |
| 2 | 7 | 0.79 Rf | 0.79 Rf | 0.85 Rf | 529.5 AU | 18.31% | 7 | |
| 3 | 1 | 0.09 Rf | 0.11 Rf | 0.14 Rf | 289.3 AU | 6.80% | 1 | 7 |
| 3 | 2 | 0.16 Rf | 0.18 Rf | 0.20 Rf | 336.9 AU | 7.91% | 2 | |
| 3 | 3 | 0.20 Rf | 0.21 Rf | 0.25 Rf | 276.2 AU | 6.49% | 3 | |
| 3 | 4 | 0.30 Rf | 0.33 Rf | 0.37 Rf | 540.9 AU | 12.71% | 4 | |
| 3 | 5 | 0.39 Rf | 0.44 Rf | 0.48 Rf | 676.9 AU | 15.90% | 5 | |
| 3 | 6 | 0.63 Rf | 0.67 Rf | 0.70 Rf | 1405.4 AU | 33.02% | 6 | |
| 3 | 7 | 0.70 Rf | 0.73 Rf | 0.75 Rf | 731.2 AU | 17.18% | 7 | |
| 4 | 1 | 0.09 Rf | 0.11 Rf | 0.14 Rf | 318.0 AU | 5.30% | 1 | 7 |
| 4 | 2 | 0.19 Rf | 0.22 Rf | 0.26 Rf | 470.9 AU | 7.84% | 2 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 4 | 3 | 0.30 Rf | 0.33 Rf | 0.37 Rf | 712.5 AU | 11.87% | 3 | |
| 4 | 4 | 0.41 Rf | 0.44 Rf | 0.48 Rf | 798.6 AU | 13.30% | 4 | |
| 4 | 5 | 0.63 Rf | 0.67 Rf | 0.70 Rf | 1856.6 AU | 30.93% | 5 | |
| 4 | 6 | 0.70 Rf | 0.73 Rf | 0.75 Rf | 860.3 AU | 14.33% | 6 | |
| 4 | 7 | 0.75 Rf | 0.77 Rf | 0.83 Rf | 986.1 AU | 16.43% | 7 | |
| 5 | 1 | 0.09 Rf | 0.11 Rf | 0.14 Rf | 420.7 AU | 4.87% | 1 | 10 |
| 5 | 2 | 0.19 Rf | 0.22 Rf | 0.26 Rf | 590.9 AU | 6.85% | 2 | |
| 5 | 3 | 0.30 Rf | 0.33 Rf | 0.36 Rf | 775.8 AU | 8.99% | 3 | |
| 5 | 4 | 0.40 Rf | 0.44 Rf | 0.48 Rf | 1046.5 AU | 12.13% | 4 | |
| 5 | 5 | 0.50 Rf | 0.53 Rf | 0.56 Rf | 738.3 AU | 8.56% | 5 | |
| 5 | 6 | 0.56 Rf | 0.59 Rf | 0.60 Rf | 559.4 AU | 6.48% | 6 | |
| 5 | 7 | 0.61 Rf | 0.62 Rf | 0.63 Rf | 384.2 AU | 4.45% | 7 | |
| 5 | 8 | 0.63 Rf | 0.68 Rf | 0.71 Rf | 2333.8 AU | 27.04% | 8 | |
| 5 | 9 | 0.71 Rf | 0.74 Rf | 0.75 Rf | 762.9 AU | 8.84% | 9 | |
| 5 | 10 | 0.76 Rf | 0.77 Rf | 0.84 Rf | 1017.0 AU | 11.78% | 10 | |
| 6 | 1 | 0.09 Rf | 0.12 Rf | 0.14 Rf | 524.6 AU | 7.34% | 1 | 6 |
| 6 | 2 | 0.18 Rf | 0.23 Rf | 0.26 Rf | 748.5 AU | 10.47% | 2 | |
| 6 | 3 | 0.31 Rf | 0.35 Rf | 0.39 Rf | 1097.2 AU | 15.35% | 3 | |
| 6 | 4 | 0.42 Rf | 0.46 Rf | 0.50 Rf | 1235.3 AU | 17.28% | 4 | |
| 6 | 5 | 0.66 Rf | 0.70 Rf | 0.74 Rf | 2070.2 AU | 28.96% | 5 | |
| 6 | 6 | 0.74 Rf | 0.77 Rf | 0.79 Rf | 552.1 AU | 7.72% | 6 | |
| 6 | 7 | 0.79 Rf | 0.81 Rf | 0.89 Rf | 920.3 AU | 12.87% | 6 | |





**Fig.1: Optimal callus formation was achieved after four weeks using MS supplemented with 1mg/l IBA + 1mg/l 2,4-D + 2mg/l KIN - MS + CIM1**

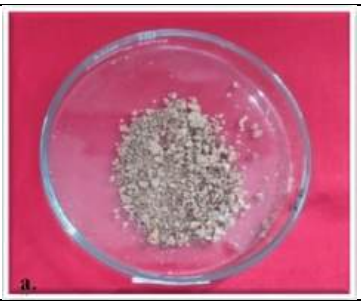
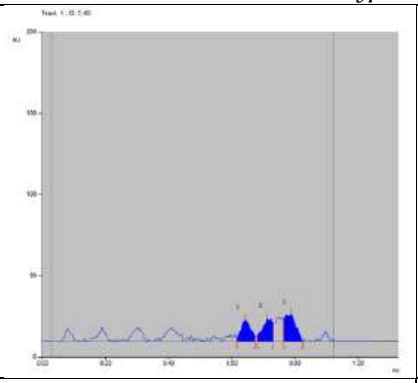


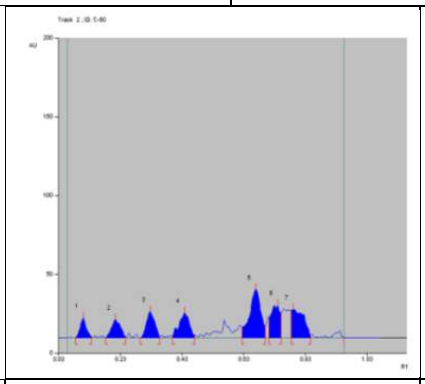
**Fig. 2a. Dried poweder of callus obtained from 60 min. UV-C Treated seed of *A.hypochondriacus* L.**



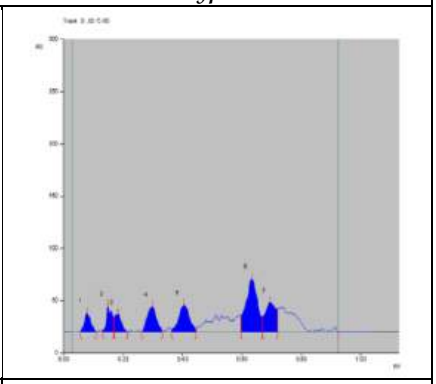
**Fig. 2b. Methanolic extract of callus obtained from 60 min. UV-C Treated seed of *A. hypochondriacus* L.**



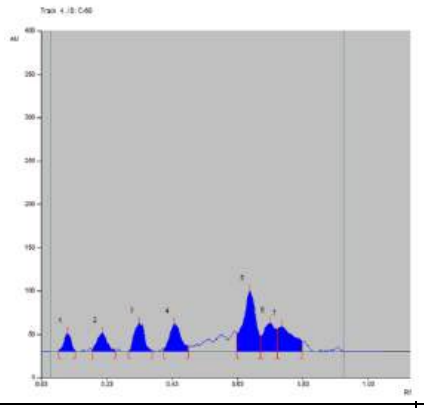
**Track 1**
**Spot volume - 2µl**



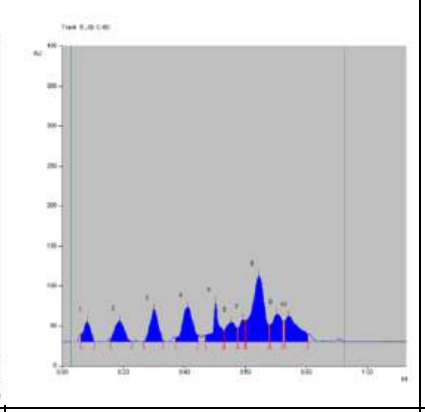
**Track 2**
**Spot volume - 4µl**



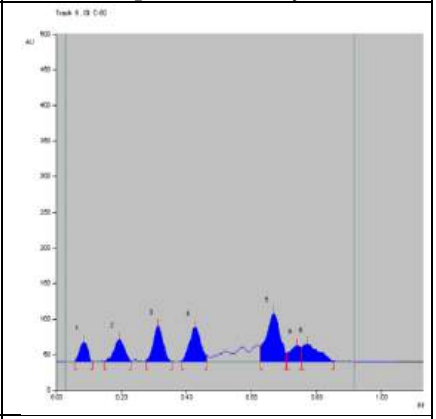
**Track 3**
**Spot volume - 6µl**



**Track 4**
**Spot volume - 8µl**



**Track 5**
**Spot volume – 10 µl**



**Track 5**
**Spot volume – 12 µl**

**Fig. 3: The HPTLC chromatogram of methanolic extract of 60 min. UV-C treated *A. hypochondriacus* L. callus**

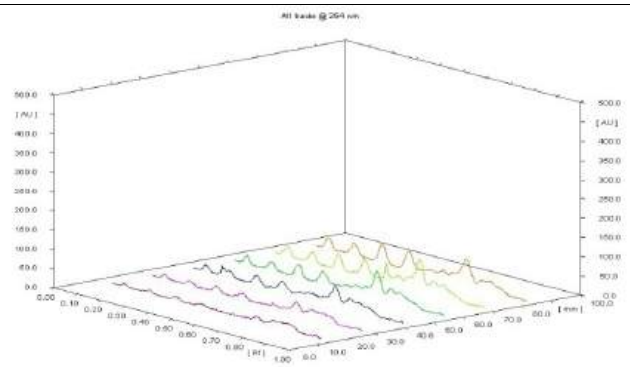


**Fig. 4: Three dimensional representation of all tracks at 254 nm wavelength.**

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REVIEW ARTICLE

# Paradigm of *Kaphaja Abhishyanda:* An Integrative Literature Review

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**ABSTRACT**

Vernal keratoconjunctivitis (VKC) is a chronic, recurrent allergic inflammation of the ocular surface commonly affecting children in warm climates. Classical Ayurvedic texts describe *Kaphaja Abhishyanda* as a Kapha-dominant ocular disorder characterised by itching, mucous discharge, heaviness of lids, and recurrent congestion, suggesting a possible correlation. To explore the conceptual relationship between *Kaphaja Abhishyanda* and VKC through classical and contemporary literature. A narrative integrative review was conducted using *Sushruta Samhita*, *Ashtanga Hridaya*, *Ashtanga Sangraha*, and modern ophthalmic literature. Descriptions of *nidana*, *lakshana*, prognosis, and *chikitsa* were compared with VKC pathophysiology and management. Close parallels were observed in etiological factors, clinical features, disease progression, and therapeutic goals. Classical symptoms such as *kandu*, *picchilasrava*, and *guruta* correspond with itching, ropy mucus, and lid heaviness in VKC. Ayurvedic *shodhana*, *shamana*, and *kriyakalpa* principles align with modern anti-inflammatory and tear-film stabilising strategies. VKC shows strong conceptual similarity with *Kaphaja Abhishyanda*, supporting an integrative understanding of chronic allergic ocular surface disease.

**Keywords:** Vernal Keratoconjunctivitis, Abhishyanda, Kriyakalpa, Ocular Allergy, Ophthalmology, Ayurveda

## INTRODUCTION

Vernal keratoconjunctivitis (VKC) is a chronic, recurrent allergic inflammation of the ocular surface predominantly affecting children and young adults living in warm, humid, and dusty climates. It is one of the most severe forms of

ocular allergy and contributes significantly to pediatric ophthalmic morbidity in tropical regions [1].In India, the burden of VKC is notably higher in hot and dusty zones, where recurrent seasonal exacerbations create a sustained disease load among school-aged populations [2].The condition is mediated by IgE-associated hypersensitivity, mast cell degranulation, eosinophil infiltration, and cytokine-driven conjunctival inflammation, which collectively produce persistent irritation and chronic ocular surface damage [3,4].Ongoing allergic inflammation of the conjunctiva and cornea may lead to keratopathic changes, including shield ulcers and superficial keratopathy, which can ultimately result in significant visual impairment if not identified and managed appropriately [3]. Clinically, the disease presents with intense itching, photophobia, foreign body sensation, mucous discharge, and recurrent redness, which often interfere with daily activities, school performance, and quality of life. The seasonal recurrence and chronic course of the disease make long-term management challenging, often requiring repeated medical intervention and continuous monitoring. VKC is also recognised for its tendency to fluctuate in severity, with periods of remission followed by exacerbations triggered by environmental and seasonal factors. These recurrent inflammatory episodes may gradually compromise the integrity of the ocular surface and tear film stability, thereby increasing the risk of corneal complications and visual disturbances. From a public health perspective, chronic ocular allergy in children represents a considerable burden due to frequent hospital visits, prolonged therapy, and the psychological stress experienced by patients and caregivers. The need for safer and more sustainable management strategies has therefore become increasingly important, especially in regions where environmental triggers remain unavoidable. Contemporary management primarily focuses on suppressing allergic inflammation and stabilising the ocular surface; however, recurrence and long-term dependency on medications remain major concerns. Our Acharyas have described comparable conditions under *Abhishyanda*, wherein *doshic* derangements, particularly *Kapha*, affect the ocular surface and produce symptoms resembling persistent allergic conjunctival involvement [5].Classical descriptions of *kandu, picchilashleshmasrava, gaurava, shleshmaavarana*, and *aviladarshana* show notable overlap with the characteristic patterns of VKC seen today. The foundational principles given for its pathogenesis and management emphasise timely intervention to prevent chronicity and complications, which aligns closely with the modern clinical emphasis on preventing corneal damage and recurrence [6].Ayurveda provides a comprehensive framework for understanding chronic and recurrent disorders through the concepts of *dosha* imbalance, environmental triggers, and host susceptibility. The description of *Kapha*-dominant ocular disorders particularly highlights the role of mucous accumulation, surface congestion, and recurrent inflammatory changes, which are central to the clinical behaviour of VKC. The emphasis on early intervention, prevention of recurrence, and restoration of ocular surface balance reflects a holistic perspective that parallels current efforts to achieve long-term disease control in allergic eye disease. The explanations of disease behaviour and therapeutic rationale in the Samhitas remain relevant and parallel the current understanding of VKC in ophthalmology. Exploring these conceptual similarities can provide an integrative perspective that may support the development of safer and more sustainable management strategies for chronic allergic ocular disorders.

**Understanding Kaphaja Abhishyanda in Compendia**

*Abhishyanda* is classified under *Sarvagata Netra Roga*. *Sushruta* describes a total of 11 *Sarvagata Rogas* [7].*Sushruta* and *Vagbhata* further classified it into four types: *Vataja, Pittaja, Kaphaja,* and *Raktaja* [7,8].Among these, *Kaphaja Abhishyanda* is marked by *Kapha* predominance. The condition is described as *sadhya* in early stages and may progress to *krichrasadhya* or *asadhya* depending on severity and chronicity, with *Kapha* as the primary *dosha* and *pitta anubandha* in later stages [7,8].The Sanskrit term *Kaphaja Abhishyanda* refers to an ocular condition in which *Kapha*-dominant qualities such as heaviness, coolness, and sticky mucous accumulation lead to congestion and overflow within the eye.

## OBJECTIVES

1. To establish the clinical correlation between *Kaphaja Abhishyanda,* as explained in the Ayurvedic *Samhitas*, and Vernal Keratoconjunctivitis (VKC), described under allergic inflammatory disorders of the ocular surface.

2. To review the Ayurvedic understanding and management principles that may offer an effective and safer approach to recurrent, chronic, and vision-impacting allergic conditions that continue to show similarity with classical descriptions even in present clinical practice.

## METHODOLOGY

An extensive literary review of Ayurvedic compendia was undertaken to explore a possible correlation between *Kaphaja Abhishyanda* and Vernal Keratoconjunctivitis (VKC) as described in contemporary ophthalmology. Classical texts, including *Sushruta Samhita*, *Ashtanga Hridaya*, *AshtangaSangraha*, and relevant commentaries, were examined [7–9],along with modern literature related to allergic ocular surface disorders [1–4]. As part of this process, departmental discussions and review of previously published articles were conducted to refine the conceptual understanding. Through this integrated analysis, an attempt was made to derive a coherent and clinically acceptable correlation between the two conditions.

## DISCUSSION

**Congruent Features of *Sarvagata* Involvement and Ocular Surface Inflammation**

While explaining *Sarvagata Netra Rogas*, the classical texts describe *Abhishyanda* as a condition capable of affecting all the ocular layers, including *sandhi*, *shukla*, *krishna*, and *drishti* [7]. This broad involvement can be correlated with the diffuse inflammation seen in Vernal Keratoconjunctivitis (VKC), where the conjunctiva, limbus, and anterior corneal surface show simultaneous changes. The shared inflammatory response, mucous accumulation, and surface congestion reflect the classical description of *shleshmaavarana* and *picchilashleshmasrava* noted in *Kaphaja Abhishyanda*. Modern ophthalmology acknowledges that allergic ocular disorders frequently involve both conjunctival and limbal zones due to immunological activation, and this pattern matches the classical concept of *sarvagata* involvement described in the *Samhitas* [3,4]. The prominence of itching, surface irritation, and mucous stagnation in VKC aligns closely with the *Kapha*-dominant *lakshanas* such as *kandu*, *sheetasparsha*, and *gaurava*, reinforcing the similarity between the two descriptions. Furthermore, the chronicity and recurrent nature of VKC can be compared with the repeated *dosha prakopa*mentioned in classical texts, where *Kapha* accumulation and congestion (*Abhishyanda*) require repeated interventions [7,8]. These conceptual parallels indicate that the behaviour of VKC mirrors the pattern of *KaphajaAbhishyanda* as understood in Ayurveda, offering a basis for integrative interpretation.

**Correlation of Ocular Surface Layer Involvement in VKC with the *Sarvagata* Nature of *Abhishyanda***

The structural involvement described in *Abhishyanda* shows close correspondence with the layer-wise pathology seen in Vernal Keratoconjunctivitis (VKC). In VKC, inflammatory changes affect the conjunctival epithelium, subepithelial papillary zone, limbal stroma, and anterior corneal surface, reflecting the *sarvagata* nature of *Abhishyanda* described in the *Samhitas* [7].The term *sarvagata* denotes a diffuse ocular disorder involving the *sandhi*, *shukla*, *krishna*, and even *drishti* structures simultaneously, indicating a multi-layer and widespread pattern of inflammation rather than a confined lesion. This aligns with the epithelial irritation, mucous accumulation, papillary hypertrophy, and anterior corneal changes characteristic of VKC, supporting the parallel between modern ocular surface involvement and the classical description of *KaphajaAbhishyanda* [3,4].

**Congruence Features of *Nidana* of *Kaphajaabhishyanda* and Aetiology of Vernal Keratoconjunctivitis (vkc)**

***Sita sevana* -** Frequent exposure to cooled indoor air, humid rooms, or sudden temperature shifts.

***Dhuma Rajah Sevana* -** Contact with dust, smoke, traffic pollution, chalk dust, pollen, and outdoor allergens.

***Ati abhishyanda kara hetavah* -** Repeated eye rubbing, long screen exposure causing tear instability, and recurrent conjunctival irritation.

***Madhura snigdha guru aharasevana -*** High intake of dairy, sugary foods, and heavy diets that increase allergic tendencies.

**Vishesha prakriti and kala -** Atopic constitution and seasonal triggers such as spring-summer when VKC peaks.
Any factor that promotes chronic ocular surface irritation or allergen exposure can intensify mucous congestion and hypersensitivity responses, consistent with the *Kapha*-dominant pathology of *KaphajaAbhishyanda*. It can be clearly established that the *nidanas* identified by our Acharyas parallel the precipitating factors recognised in VKC even today [7,8].

**Congruence Features of *Kaphajaabhi shyandalaksanas* and Clinical Features of Vernal Keratoconjunctivitis (vkc)**
The *lakshanas* of *Kaphaja Abhishyanda* described in the *Samhitas* show remarkable similarity to the clinical profile of Vernal Keratoconjunctivitis (VKC). The feature *ushnabhinanda* indicates a tendency for symptoms to feel relieved with warmth, reflecting underlying *Kapha* stagnation and surface congestion typical of VKC [7,8]. *Guruta*, or heaviness of the lids, corresponds to the mechanical weight produced by palpebral papillary hypertrophy, while *akshishopha* aligns with the upper-lid oedema and tarsal thickening commonly seen in allergic conjunctival inflammation. The cardinal allergic feature, intense itching, directly matches the hallmark histamine-mediated symptom of VKC. *Padeha*, indicating a sensation of coating or stickiness, correlates with mucin overproduction, tear-film debris, and the formation of mucus plaques on the ocular surface. The descriptors *sheetata* or *shaityam* refer to a cool ocular sensation, paralleling the transient soothing effect felt after rubbing in VKC and signifying *Kapha* predominance. *Snavamuhur* denotes intermittent watering, corresponding to reflex tearing triggered by conjunctival irritation, and *picchila* refers to thick, ropy mucous discharge precisely matching the stringy mucus produced in VKC by goblet cell hyperplasia [3,4].Finally, *kaphabhipannanayana* indicates a *Kapha*-laden, congested ocular surface, mirroring the mucous-rich, inflamed appearance characteristic of active VKC.The cardinal manifestations of VKC, itching, mucous ropiness, eyelid heaviness, episodic blurred vision, and conjunctival congestion show a clear correlation with the *Kapha*-dominant *lakshanas* described under *Kaphaja Abhishyanda*, indicating strong conceptual alignment between classical descriptions and the modern clinical presentation of this chronic allergic ocular surface disorder [3,4,7,8]

**Congruence Features of *Sadhyalaksanas* of *Kaphaja abhishyanda* and Good-Prognosis Features of Vernal Keratoconjunctivitis (vkc)**
The *sadhyalakshanas* of *Abhishyanda*, described by the *Acharyas*, closely resemble the benign clinical patterns seen in VKC. Features such as *drishti sameepamna gacchanti* indicate sparing of the central visual axis, matching VKC's predominantly peripheral or limbal involvement [7,8]. *Na ca gambhirasamsthita* reflects superficial disease, similar to VKC's epithelial or subepithelial changes without stromal penetration. The absence of purulent discharge (*na ca bahu sravayukta*) aligns with VKC's non-infective, mucous-type secretions, while *na ca bahu vedana* corresponds to VKC being itch-dominant rather than painful. The feature *eka deshasthita* denotes localised involvement, paralleling the confined limbal or tarsal areas affected in VKC. Together, these signs indicate a generally favourable prognosis consistent with the superficial, limited, and non-infective nature of VKC when appropriately managed [3,4,7,8].

**Congruence Features of *Asadhyalaksanas* and Complications**
The *asadhyalakshanas* described for *Abhishyanda* that hold relevance for Vernal Keratoconjunctivitis (VKC) are those indicating chronicity, vascular changes, surface instability, and visual impairment. *Shira rakta*, referring to pathological vessel adherence, closely mirrors the limbal neovascularisation seen in long-standing VKC due to persistent allergic inflammation [7,8]. *Drishti Krichra*, describing difficulty in vision when central structures are involved, corresponds to central shield plaques or scarring that can encroach upon the visual axis in severe VKC [3,4]. *Cira utthita*, denoting chronic or long-standing disease, aligns with VKC's recurrent course, where continuous inflammation leads to limbal hypertrophy and fibrotic changes. *Ushna ashru*, indicating warm and copious tearing, parallels the acute inflammatory lacrimation observed during VKC flare-ups. *Pidaka*, representing raised or blister-like elevations, may be compared to elevated shield plaques or early stromal thinning in neglected cases. These

selected features reflect VKC-related complications without including ulcer-specific descriptions unrelated to allergic ocular disease [3,4,7,8].

**Congruence Features of *Chikitsa Sutra* Explained in Ayurveda and Treatment Principles in Allied Sciences**

***Gharshana*:** Classical *gharshana* measures are designed to mechanically and functionally reduce *Kapha*-dominant accumulations on the ocular surface [7,8]. *Lekhana*-based collyria containing *haritaki*, *haridra*, *triphala*, or *yashtimadhu* exert a scraping and decongesting action, facilitating the breakdown of mucous adhesions and superficial epithelial debris. This aligns with contemporary VKC management practices in which mechanical removal of ropy mucus, papillary concretions, and epithelial irregularities is essential to reduce antigen load, restore surface smoothness, and improve the efficacy of topical medications [3,4].

***Kriyakalpa* procedures*:*** *Kriyakalpas* constitute the primary set of localised therapeutic interventions for *Kaphaja Abhishyanda* [7–9]. *Aschyotana* and *Parisheka* facilitate controlled ocular irrigation, aiding in the clearance of allergens, inflammatory mediators, and mucus from the conjunctival sac. Tarpana with medicated *ghrita* provides a lipid-rich protective layer that promotes epithelial stability and reduces surface hyper-reactivity. *Ruksha Putapaka* and *Bidalaka* help mitigate mucous viscosity and periocular heaviness, while Nasya modulates upper-channel congestion and *Kapha* dominance in the head region. *Dhumapana* and *Kavala*, through their influence on upper respiratory and nasopharyngeal pathways, may indirectly reduce ocular hypersensitivity. *Siravyadha* can be employed when there is marked *rakta* involvement, offering a classical method for relieving vascular congestion. Collectively, these interventions parallel modern therapeutic approaches for VKC, such as ocular lavage, lubricants, surface barrier enhancers, anti-inflammatory agents, and measures aimed at reducing vascular congestion and mucous hypersecretion [3,4].

***Abhyantara Chikitsa:*** Internal therapies indicated in *Kapha*-dominant *netrarogas* serve to regulate systemic inflammatory activity, alleviate *shopha* and *kandu*, and stabilise recurrence patterns [7–9]. Formulations that promote *Kaphashamana* and *srotasshodhana* support overall immunological balance. From a modern perspective, these internal measures correspond to the use of systemic anti-allergic agents, mucoregulators, and mild anti-inflammatory drugs, which serve as adjuncts in recurrent or chronic VKC to modulate hypersensitivity and reduce flare frequency [3,4].

## CONCLUSION

The chikitsa sutra for Kaphaja Abhishyanda focuses on reducing Kapha blockage, clearing excess mucus, easing inflammatory irritation, and improving epithelial repair. When looked at scientifically, these treatment goals align well with the evidence-based objectives of VKC management [3,4]. This includes reducing allergic inflammation, stabilising the eye surface, controlling mucus overproduction, and preventing long-term issues. The traditional combination of shodhana, shamana, and kriyakalpa methods offers a clear and sensible approach to dealing with the recurring, allergic, and destabilising aspects of VKC [7–9].The clinical behaviour of Vernal Keratoconjunctivitis shows clear conceptual similarity to the Kapha-dominant presentation of Kaphaja Abhishyanda described in the Ayurvedic texts. The recurrent itching, mucous discharge, surface irritation, and risk of anterior corneal involvement parallel the classical lakshanas, while the emphasis on shodhana, shamana, and kriyakalpa therapies aligns well with the therapeutic goals of modern VKC management [3,4,7–9]. The descriptions available in Ayurveda, therefore, provide a coherent framework for understanding the progression and complications of VKC and offer a structured approach that may support more sustained control of this chronic allergic ocular surface disorder. An integrative understanding of VKC through the Ayurvedic perspective highlights the importance of addressing both local ocular pathology and systemic susceptibility. By focusing on dosha balance, environmental triggers, and recurrence prevention, Ayurveda emphasises long-term disease control rather than symptomatic relief alone. This broader therapeutic perspective is particularly relevant for chronic and relapsing allergic disorders, where long-term safety and sustainability of treatment remain major clinical concerns.The alignment between classical Ayurvedic principles

and modern therapeutic objectives suggests the potential for integrative approaches in managing chronic allergic ocular surface disease. Such approaches may help reduce recurrence, improve patient comfort, and support ocular surface stability through combined preventive and therapeutic strategies. Future interdisciplinary research and clinical studies are needed to further explore these correlations and to evaluate the clinical effectiveness of integrative management strategies. Strengthening the scientific understanding of these parallels may contribute to the development of safe, holistic, and patient-centred approaches for the long-term management of Vernal Keratoconjunctivitis.

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RESEARCH ARTICLE

# Hybrid Classification System for Identifying Fresh and Rotten Fruits

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## ABSTRACT

Fruit farming is a cornerstone of our country's agricultural practices, and it has undergone significant advancements over time. Fruits are vital sources of nutrients, including essential minerals and vitamins. Detecting rotten fruits is crucial for agricultural enterprises, fruit processing, and packaging businesses. Traditionally, the classification of fruits as fresh or rotten is performed manually, a process that is both time-consuming and inefficient for cultivators. Therefore, there is a pressing need for an advanced classification model that can reduce human effort, costs, and production time by accurately identifying fruit defects. This paper proposes a hybrid classifier-based system for the categorization of fresh and rotten fruits. Experimental results demonstrate that the proposed hybrid approach outperforms existing classification algorithms in terms of accuracy. In this study, two supervised machine learning algorithms—Naive Bayes and BayesNet—are combined to form a hybrid model. The hybrid model achieves an accuracy of 96.14%, surpassing the Naive Bayes and BayesNet models, which achieved 91.42% and 90.21%, respectively. The objective of this research is to enhance the prediction accuracy in the classification of fresh and rotten fruits by integrating Naive Bayes and BayesNet algorithms. Furthermore, a comparative analysis between the

proposed hybrid algorithm and individual algorithms highlights the significant improvement in classification accuracy.

**Keywords:** Naive Bayes, BayesNet, Hybrid Classifier, Machine Learning, Fruit Classification.

## INTRODUCTION

One of the most challenging tasks in agriculture is the detection of fruit defects and the accurate classification of fresh and decaying fruits. Misclassifying rotten fruits not only reduces productivity but also poses a risk to the quality of surrounding fresh produce. Traditionally, this classification process has been performed manually, a method that is labor-intensive, time-consuming, and often inefficient. Additionally, manual sorting increases production costs. Hence, there is a critical need for an automated system that can enhance productivity, reduce labor costs, and minimize production times while lowering human effort. Fresh and Rotten Fruits Classification Using CNN and Transfer Learning might include findings related to the effectiveness of Convolutional Neural Networks (CNNs) and transfer learning in accurately distinguishing between fresh and rotten fruits [1]. It likely discusses the model's accuracy, potential applications in food quality control or agriculture, and possibly suggestions for future research or improvements. [3] The significance of fruit classification spans agriculture, food quality assurance, and supply chain management, playing a vital role in ensuring efficiency and product quality. Manual fruit sorting faces several challenges, such as inefficiency, inconsistency, and vulnerability to human error. [2] The study's primary objective is to assess the effectiveness of CNNs and transfer learning models in classifying fruits based on their distinctive features, with a focus on combining these approaches to achieve optimal accuracy while maintaining computational efficiency. This study focuses on developing an optimized hybrid classification framework to automate the identification process by analyzing digital images of fresh and rotten fruits using machine learning (ML) techniques. The proposed system combines the strengths of different algorithms to achieve more accurate results.

**Overview of Naïve Bayes and Bayesnet in Hybrid Classification Systems**

The Naïve Bayes algorithm, a supervised learning method grounded in Bayes' theorem, is one of the key components of this hybrid system. Naïve Bayes is widely used for solving classification problems, particularly in scenarios involving high-dimensional datasets, such as text classification. It is a probabilistic classifier, which means it makes predictions based on the probability of various features. Despite its simplicity, the Naïve Bayes classifier is highly effective in building fast machine learning models that deliver quick predictions

**The Naïve Bayes algorithm is named for two reasons:**

**Naïve:** It assumes that the occurrence of one feature is independent of other features.

**Bayes:** It relies on Bayes' theorem, which calculates the probability of a hypothesis based on prior knowledge and evidence

Bayes' theorem is mathematically expressed as:

$P(A|B) = [P(B|A) * P(A)] / P(B)$

P (A|B) P(A|B)P(A|B) is the posterior probability: the probability of hypothesis AAA given the observed event BBB.

P (B|A) P (B|A) P(B|A) is the likelihood: the probability of the evidence BBB given that hypothesis AAA is true.

P (A) P(A) P(A) is the prior probability: the initial probability of the hypothesis before considering the evidence.

P(B) P(B) P(B) is the marginal probability: the total probability of the evidence.

To apply the Naïve Bayes classifier, the dataset is first converted into frequency tables, followed by generating a likelihood table for the features. Bayes' theorem is then used to calculate the posterior probabilities, which aid in making predictions. The second algorithm used in the hybrid model is BayesNet. A Bayesian network (BayesNet) is a probabilistic graphical model that represents a set of variables and their conditional dependencies through a directed acyclic graph (DAG). This network is particularly useful for predicting the likelihood of different causes given an

observed event. In a Bayesian network, each edge represents a conditional dependency between variables, and each node corresponds to a unique random variable. The network adheres to the local Markov property, meaning that each node is conditionally independent of its non-descendants given its parent nodes. This property allows the joint probability distribution to be simplified, reducing the computational complexity of large networks by leveraging the combined strengths of Naïve Bayes and BayesNet, the proposed hybrid classification system aims to achieve higher accuracy in distinguishing between fresh and rotten fruits, ultimately improving efficiency and reducing costs in agricultural operations.

## MATERIALS AND METHODS

**Dataset:** In this study, the dataset used for classifying fresh and rotten fruits is sourced from Kaggle.com. This dataset contains 497 instances and 7 attributes, representing three different varieties of fruits: Apple, Banana, and Orange. The dataset includes various geometric parameters of the fruits, which are critical for the classification process.

**The parameters used are as follows:**

**Area (A):** The total surface area of the fruit.

**Perimeter (P):** The total boundary length of the fruit.

**Compactness (C):** Calculated as C=4πAP2C = \frac{4 \pi A} {P^2} C=P24πA, a measure of how compact the fruit is.

**Length of Kernel:** The length of the fruit kernel.

**Width of Kernel:** The width of the fruit kernel.

**Asymmetry Coefficient:** A measure of the asymmetry of the fruit shape.

**Length of Kernel Groove:** The length of the groove on the kernel

These parameters are integral to the classification process. The dataset is divided into training and testing subsets, with 70% of the data used for training the model and 30% for testing its performance.

**Proposed Model**

The proposed model for classifying fresh and rotten fruits is composed of the following steps:

**Step 1: Import the Dataset**

The first step involves importing the dataset containing the 497 instances and the 7 attributes related to the three fruit varieties: Apple, Banana, and Orange.

**Step 2: Data Preprocessing Data Cleaning**

This step involves handling any missing values in the dataset, removing duplicates, and ensuring that the data is clean and ready for analysis.

Normalization/Standardization: Depending on the data distribution, normalization or standardization is applied to scale the data, ensuring that all features contribute equally to the classification process.

**Step 3: Applying the Hybrid Classifier**

The hybrid classifier, which combines the Naïve Bayes and BayesNet algorithms, is applied to the preprocessed dataset.

**Step 4: Classification of Fresh and Rotten Fruits**

The hybrid classifier is used to classify the fruits into fresh or rotten categories based on the provided attributes.

**Algorithm**

The following steps outline the algorithm used for the classification process:

**Step 1: Data Preparation**

**Input Data:** Collect the dataset containing 497 instances and 7 attributes for the three varieties of fruits: Apple, Banana, and Orange.

**Preprocessing:** Clean the data by handling missing values, removing duplicates, and standardizing/normalizing the data if necessary.

**Step 2: Train Individual Models**
**Split Data:** Divide the dataset into a training set (DtrainD_{\text{train}}Dtrain) and a test set (DtestD_{\text{test}}Dtest). Typically, 70% of the data is used for training, and 30% is reserved for testing.

**Naïve Bayes Model**
Train the Model: Use the training set (DtrainD_{\text{train}}Dtrain) to train a Naïve Bayes model (NBNBNB).
Make Predictions: Apply the trained model to the test set (DtestD_{\text{test}}Dtest) to generate predictions (PNBP_{\text{NB}}PNB).

**Calculate Accuracy:** Determine the accuracy (ANBA_{\text{NB}}ANB) of the Naïve Bayes model by comparing the predictions with the actual labels

**BayesNet Model**
**Train the Model:** Use the training set (DtrainD_{\text{train}}Dtrain) to train a BayesNet model (BNBNBN).
Make Predictions: Apply the trained model to the test set (DtestD_{\text{test}}Dtest) to generate predictions (PBNP_{\text{BN}}PBN).

**Calculate Accuracy:** Determine the accuracy (ABNA_{\text{BN}}ABN) of the BayesNet model by comparing the predictions with the actual labels.

**Step 3: Create Hybrid Model**
**Combine Individual classifiers**
Combine the predictions from the Naïve Bayes model (PNBP_{\text{NB}}PNB) and the BayesNet model (PBNP_{\text{BN}}PBN) to create a hybrid prediction (PHybridP_{\text{Hybrid}}PHybrid).
A common approach is to use a majority voting method or a weighted average to combine the predictions, ensuring the strengths of both models are utilized.

**Step 4: Evaluate Hybrid ModelCalculate Hybrid Accuracy**
Compare the hybrid predictions (PHybridP_{\text{Hybrid}}PHybrid) with the actual labels (ytesty_{\text{test}}ytest) to calculate the accuracy (AHybridA_{\text{Hybrid}}AHybrid) of the hybrid model.

**Step 5: Compare Results**
**Compare Accuracies:**
Finally, compare the accuracies of the individual models (ANBA_{\text{NB}}ANB for Naïve Bayes and ABNA_{\text{BN}}ABN for BayesNet) with the accuracy of the hybrid model (AHybridA_{\text{Hybrid}}AHybrid).

This comparison demonstrates the performance improvements achieved by combining the two algorithms

## RESULTS AND DISCUSSION

This study aimed to enhance the prediction accuracy for the classification of fresh and rotten fruits by developing a hybrid machine learning algorithm. The two supervised machine learning algorithms selected for this study are Naïve Bayes and BayesNet, both of which are widely used for classification tasks. By combining these two algorithms, a proposed hybrid algorithm was created to improve the classification accuracy.

**Individual Model Performance**

Naïve Bayes Algorithm: The Naïve Bayes algorithm, known for its simplicity and effectiveness, was applied to the dataset. This algorithm assumes that the features are independent, which, despite being a simplistic assumption, works well in many practical applications. The Naïve Bayes model achieved an accuracy of 91.42% in classifying the fruits as fresh or rotten. BayesNet Algorithm: BayesNet, or Bayesian Network, is a probabilistic graphical model that represents a set of variables and their conditional dependencies via a directed acyclic graph (DAG). BayesNet is particularly useful for handling uncertainty and incorporating prior knowledge in the prediction process. When applied to the same dataset, the BayesNet model achieved an accuracy of 90.21%.

**Proposed Hybrid Model Performance**

The hybrid model was developed by combining the predictions of the Naïve Bayes and BayesNet algorithms. The motivation behind creating a hybrid model was to leverage the strengths of both algorithms and mitigate their individual weaknesses. The combined model operates by either majority voting or by weighted averaging of the predictions from the two algorithms. Hybrid Algorithm: The proposed hybrid model outperformed the individual classifiers, achieving an accuracy of 96.14%. This significant improvement in accuracy demonstrates the effectiveness of the hybrid approach in classifying fresh and rotten fruits. The hybrid model's superior performance can be attributed to its ability to combine the probabilistic reasoning of Naïve Bayes with the structured representation of dependencies in BayesNet.

**Comparative Analysis:** The results clearly show that the hybrid model offers a substantial improvement over the individual Naïve Bayes and BayesNet models. Specifically:

**Naïve Bayes:** 91.42%

**BayesNet:** 90.21%

**Hybrid Classifier:** 96.14%

The comparison between these accuracies highlights that the hybrid classifier consistently outperforms the individual models in predicting the freshness of fruits. This performance gain is due to the hybrid model's ability to integrate the complementary strengths of Naïve Bayes and BayesNet. The Naïve Bayes model contributes its efficiency in handling high-dimensional data, while the BayesNet model enhances the classification by accounting for dependencies between variables. As depicted in Figure, the graph illustrates the prediction accuracies of the individual algorithms (Naïve Bayes and BayesNet) and the proposed hybrid algorithm. The graph clearly shows that the hybrid algorithm achieves a higher accuracy compared to the individual models, reinforcing the effectiveness of the hybrid approach for this classification task. The overall results indicate that the hybrid classifier is a robust model for the classification of fresh and rotten fruits, outperforming the individual classifiers on most datasets. The hybrid model's accuracy of 96.14% demonstrates that it effectively reduces the error rate associated with the individual classifiers. The improved accuracy suggests that the hybrid model is better suited for real-world applications where the classification of fruits is crucial for reducing waste and ensuring quality in agricultural and commercial settings. In many instances, the hybrid approach can significantly outperform individual classifiers by combining their strengths, as observed in this study. This result is consistent with the theory that combining different models often leads to better predictive performance, a concept known as ensemble learning. The present study concluded, the hybrid classifier proposed in this study offers a superior method for the classification of fresh and rotten fruits, providing a more accurate and reliable solution compared to using Naïve Bayes or BayesNet individually. This improvement in accuracy can lead to more efficient sorting processes in agricultural enterprises, ultimately enhancing productivity and reducing losses due to misclassification.

## CONCLUSION

In this study, we explored the effectiveness of a hybrid machine learning algorithm for the classification of fresh and rotten fruits. The primary objective was to enhance the classification accuracy beyond what could be achieved using individual algorithms alone. To this end, we combined two well-known supervised learning algorithms: Naïve Bayes and BayesNet, into a hybrid model. The results of this investigation provide valuable insights into the advantages of hybrid machine learning techniques over traditional, single-algorithm approaches. The study concludes that hybrid machine learning algorithms offer a powerful tool for improving classification accuracy in agricultural applications. By combining the strengths of different algorithms, hybrid models can deliver superior performance compared to individual models. The proposed hybrid model, which merges Naïve Bayes and BayesNet, not only demonstrated a significant increase in accuracy but also provided a practical solution for the classification of fresh and rotten fruits. This advancement holds promise for enhancing quality control processes in agriculture, ultimately leading to better resource management and higher-quality produce.

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### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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**Table 1: Accuracy of Proposed hybrid classifier**

| Classifier | Accuracy |
|---|---|
| **Naïve Bayes** | 91.42 |
| **BayesNet** | 90.21 |
| **Hybrid classifier** | 96.14 |

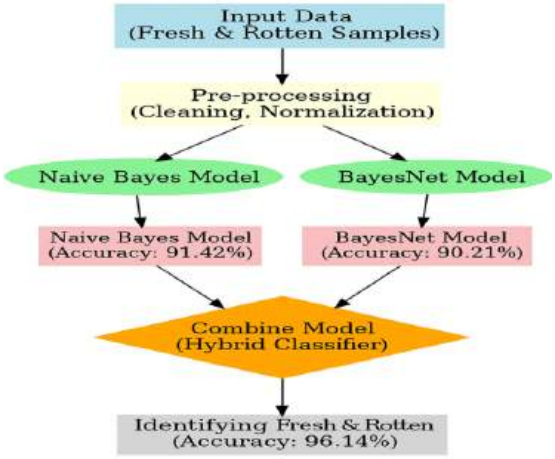



**Figure 1: Flow Diagram**

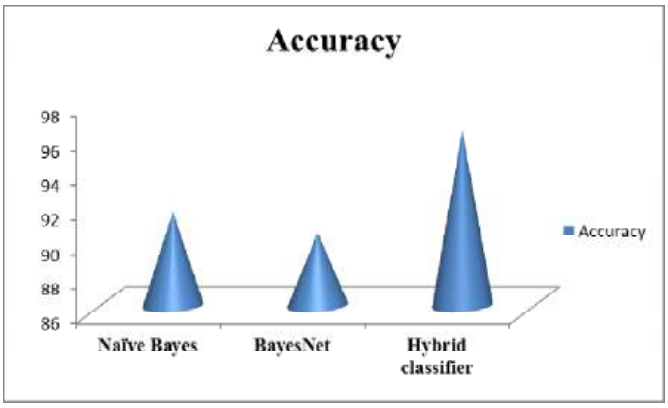



**Figure 2: Accuracy of hybrid classifier**

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REVIEW ARTICLE

# Mapping the Evidence on *Viddhakarma* Therapy in Ayurveda (A Scoping Review From 2021 - 25)

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## ABSTRACT

Among Ayurveda's myriad harmless treatments, *Viddhakarma* stands out for its immediacy and efficacy. This art of therapeutic piercing, detailed in the *Samhitas*, is a principal *Shastra Karma* for pain reduction and disease management. The procedure works by puncturing precise points to address the root cause of pain. Its primary action is to remove obstructions in blood vessels and bodily channels (*srotas*), thereby restoring circulation. Concurrently, the stimulation of peripheral sensory receptors blocks the transmission of pain signals from the affected site. This dual mechanism offers a unique, non-pharmacological approach to instant therapeutic intervention, highlighting Ayurveda's sophisticated clinical acumen. This ancient practice remains a relevant, effective, and minimally invasive therapeutic option. Analysis of publications from 2021 to 2025 reveals a growing body of evidence supporting the mechanistic efficacy of *Viddhakarma* as a Bridging Ancient Precision and Modern Pain Science. This specialized branch of Ayurvedic surgery, particularly its *Viddha* technique, demonstrates potent effects in resolving complex musculoskeletal pain by targeting specific points (*marmas*/*snayus*) to alleviate micro-instability, reduce inflammation, and break pain-spasm cycles. Clinical outcomes across studies report not only superior symptomatic relief but also enhanced tissue healing and functional recovery. The reinvigoration of *Viddhakarma* through contemporary research validates its dual role: as a primary treatment offering sustainable relief and a complementary modality reducing reliance on pharmaceuticals. For this renaissance to

reach its full potential, focused research on standardizing its application through robust trials is the essential next step.

**Keywords:** Ayurveda, *Viddhakarma*, Therapeutic Puncturing, Instant Relief, *Srotas, marmas* ,Sensory Receptors, Shastra Karma, Piercing Therapy.
**Timeframe:** Articles published from January 2021-2025 are reviewed.

## INTRODUCTION

*Viddhakarma* also known as *Suchivedhana* or *Vedhan Chikitsa* is a classical para-surgical procedure from the Ayurvedic tradition, primarily detailed in the *Sushruta Samhita*. The term itself is derived from the Sanskrit root "*Viddh*" meaning "to pierce" and "*Karma*" meaning "action" .It involves the therapeutic puncturing of specific anatomical points to alleviate pain and correct physiological dysfunction by regulating the vitiated *dosha (Vata, Pitta, Kapha)* and the blood (*Rakta).* Grounded in the understanding that pain and musculoskeletal issues often stem from aggravated Vata, impaired circulation, and toxin (*Ama*) accumulation, its primary mechanism is dual-faceted: it clears the body's micro-channels *(Srotas*), releasing trapped *Vayu* and restoring blood flow, while simultaneously stimulating peripheral sensory nerves to inhibit pain signal transmission and promote the release of pain-relieving endorphins. Operating on the principles of *Shodhana* (purification) and *Strotoshodhana* (channel cleansing), a precise puncture facilitates the removal of stagnant blood and toxins *(Dushta Rakta),* reduces tissue pressure, reinstates normal *Vata* flow, and stimulates vital *Marma* points to restore functional balance. *Viddhakarma* encompasses all therapeutic procedures that involve piercing, incising, excising, or manipulating body tissues with instruments. It represents a highly advanced and systematic surgical knowledge that existed in ancient India. In contemporary practice, *Viddhakarma* has gained prominence as a targeted, rapid, cost-effective, and minimally invasive treatment. Its holistic approach addresses the root cause of disorders rather than merely suppressing symptoms, offering a potent, evidence-based solution for complex pain syndromes, especially those resistant to conventional therapies, and aligns with the core Ayurvedic goal of restoring homeostasis by eliminating pathogenic factors and rejuvenating the body's innate healing capacity.

## METHODOLOGY

**Aim of the study**

The aim of this scoping review is to systematically map the available literature published between 2021 to 2025 on the Ayurvedic intervention *Viddhakarma*. The review seeks to identify the scope of clinical conditions treated, technical variations in the procedure (e.g., instruments used like 26 no.G Needles), reported outcomes, and gaps in the current evidence base**.**

**Study Design**

This study follows a narrative analytic review design, integrating data from classical texts, recent clinical trials, case studies, and observational studies to assess the scope, efficacy, safety, and limitations of *Viddhakarma* in musculoskeletal disorder and pain management.

**Sources of Data**

The literature review included;
Classical Ayurvedic Samhitas

- Sushruta Samhita.
- Charaka Samhita.
- Ashtang Hridaya.

**Databases and Journals Searched**

- PubMed.
- Google Scholar.

**Inclusion Criteria**

- Studies involving *Viddhakarma* as a primary or adjunct treatment for pain-related conditions.
- Clinical trials (randomized/non-randomized), case reports.
- Articles published in English or with English abstracts.
- Studies published in UGC-listed or peer-reviewed *Ayurvedic* journals.

**Exclusion Criteria**

- Studies without clear pain-related endpoints.
- Articles not mentioning therapeutic outcomes of *Viddhakarma.*
- Animal studies or in-vitro trials.
- Duplicate data and no peer-reviewed online sources.

## RESULT

A total of 25 published articles were critically reviewed and categorized into different classifications, with the aim of understanding and consolidating the mechanisms of applied therapy and the quality of documentation in a single comprehensive source. Among which total 21 articles have been published in Google scholar and 4 article published in Pubmed. Among which 16 articles were Case Reports and 9 articles were Clinical Studies. Total 8 were on *Gridhrasi,* 1 was on *Timir Rog*,2 were on *Vatakantak,* 1 was on *Galganda* , 1 was on *Avbhahuk (frozen sholder),* 1 was on *Vishwachi* (Cervical Radiculopathy), 1 was on *Anantavata* (Trigeminal Neuralgia), 1 was on *Vataj Pratishyay* (Allergic Rhinitis), 1 was on *Mutrashmari* (Urolithiasis), *Indralupta* And *Khalitya, Vishada* (persistent depressive disorder), 1 was on PCOs patients, 1 was on *Gandhanasha* (Hyposmia), pain management especially in Musculoskeletal Disorder (Sciatica, frozen shoulder, Cervical and low back Radiculopathy).

**Case Study**

Effect of *Suchivedha* in *Gridhrasi* (Sciatica) – a Case Study. Dr. Madhuri S.Chakote *et.al*., reported got relief in pain[2]. Effect of *Viddha Karma* (dry needle procedure) in the treatment of Sciatica – A Case Report. De Silva UMGD *et.al*., all the sign and symptoms related to sciatica was reduced after 15 days[3]. *ViddhaKarma* in *Timira Roga*: A Single Case Study Kirti Sengaonkar *et.al*., reported that patient showed Significant result after inserting needle at Apanga, Lalat and Upanasika[4] . Effect of *Viddhakarma* in *Grudhrasi* (sciatica) – A Case Study,Vd. Aarti Balaprasad *et.al*., reported after 2 to 3 sittings patient got complete relief in pain and complete his routine work during process[5]. Ayurvedic Management of Acute Calcaneal Spur with *Viddhakarma* and *Siravedha*: A case report Jaiswal Rashmi *et.al*., reported indicated results in acute pain associated with it[6]. Effect of *Agnikarma* and *Viddhakarma* in the management of pain and stiffness in *Gridhrasi* w.s.r. to Sciatica - A Case Study Deepak Kalyanrao *et.al*., After 6 sittings pain, oedema and stiffness is found completely resolved[6] . Role of *Viddha Karma* in Management of *Galaganda* –Case Report Sayali S. Shinde *et.al*., With 7 sittings of *ViddhaKarma*, it was observed that there was a regression of the size of the Thyroid Gland as well as reduction of Cervical lymphadenopathy[8]. A Role of Ayurvedic Management of *Avabahuka* W.S.R. Frozen Shoulder (Adhesive Capsulitis) A Single Case Study Dr. Ashwini Bhise *et.al*., reported marked reduction in sign and symptoms after 6 sittings of *viddhakarma [9]* . Evaluation of A Case Study of Effect of *Viddhakarma* In *Vishwachi* W. S. R To Cervical Radiculopathy Dr. Aishwarya Wankhade *et.al*., reported 75% relief in pain and other symptoms immediately and completely in 14 days treatment[10]. Management of *Anantavata* (Trigeminal Neuralgia) With Ayurvedic Parasurgical Procedures- A Case Report Aiswarya Das, Tukaram Dudhamal *et,al,*. reported that *Viddhakarma* with 26G needle and *Agnikarma* with *Swarna Shalaka* were performed near *Marma* points such as *Sthapani, Avarta, Apanga, Utkshepa*, *Shanka, Phana*, and *Shrigataka* at intervals of three days for two months. After that patient got significant relief from recurrent pain[11]. Role of *Viddhakarma* In The Treatment of *Vataj*

*Pratishyay* W.S.R Allergic Rhinitis: A Case Study Dr. Rakshanda S. Akre *et,al*,. reported reduce in sign and symptoms of Allergic Rhinitis without any sideffects after 2mon of follow up[12]. A Case Study on Ayurvedic management of *Mutrashmari* (Urolithiasis) Hardik, Apala Sengupta *et,al*,. reported that 4 sittings of *Viddhakarma* with *Shaman Chikitsha* patient showed highly significant result before and after treatment with no trace of Renal Calculi[13]. Ayurvedic Management of *Indralupta* And *Khalitya* With *Viddha Karma* (A Para-Surgical Procedure) And Internal Medications: A Case Report Dr. Snehal Patil *et,al*., reported that got successful result with *shaman* and *shodhan Chikitsha* with 7 days of *viddhakarma* treatment[14]. Clinical Effectiveness of *Viddha Karma* in PCOS: A Case Report Bhagyashree Jibkate *et,al*., reported that after 3 days continuously and later once in a 15 day of viddha procedure show positive results in managing symptoms and promoting overall health in patients with PCOS[15]. A holistic Ayurvedic approach to manage persistent depressive disorder, *Vishada* -A case report Aqsa Zarin Khan, Jibi Varghese *et,al*,. treatment persisted for 6 months, and the intervention outcome demonstrated a decrease in Hamilton Depression Rating scores, declining from 16 to 5 with a reduction of symptoms by the end of the 6 months of intervention[16].

**Clinical Study**

A role of *Viddhakarma* in management of *Gandhanasha* w.s.r. to Hyposmia - A Clinical Study Nilakshi Pradhan *et.al*., reported Patient got sense of smell after completion of 20th day with 10 sittings of *viddhakarma[*17]. Evaluation of Effect of *Viddhakarma* in *Gridhrasi* w.s.r To Sciatica Single-arm Clinical Case Study. Archana Suhas Wadkar *et,al*., patient got 75% relief in a very short time of period which reveal that *Viddha Karma* is a potential management option for the pain-related condition of sciatica[18]. Efficacy of *Agnikarma* and *Siravedha* along with *Rasna Saptaka Kwatha* orally in the management of *Vatakantaka* (plantar fasciitis): An open-labeled randomized comparative clinical trial Ganatra, Riddhi *et,al*,. reported that both interventions were found effective in the symptomatic management of *Vatakantaka[19]*. A Review on Mode of Action of *Viddha-Agnikarma* By Virtue of Pain Management in Musculoskeletal Disorders. Suryawanshi A *et,al*,. reported that this combined approach has shown promising results in reducing pain and treating various condition[20]. Comparative Effectiveness of *Viddha-Agnikarma* in Managing Lower Back and Knee Joint Pain: A Pilot Study. Yadav Yogesh *et,al*., reported that *Viddha-Agnikarma* was an effective therapy for managing lower back and knee joint pain, demonstrating significant reductions in VAS scores over 21 days[21]. A Comparative Clinical Study to Evaluate the Efficacy of *Viddhakarma* and *Agnikarma* in Pain Management of *Gridhrasi* (Sciatica) Harveer Singh Sangwa *et,al*., reported that *Viddhakarma* excels in treating deeper, chronic pain, particularly in regions like the thighs and knees, where its long-term effects are more pronounced[22]. A Comparative clinical study of *Siravedha* and *Agnikarma* in management of *Gridhrasi* (sciatica) J Vaneet Kumar, Tukaram S Dudhamal *et,al*., reported that patients had moderate to marked improvement[23]. Efficacy of *Viddhakarma* in Brachial Neuralgia Dr. Santosh Shreekant Betgeri *et,al*., reported *Viddhakarma* can deliver rapid analgesia in *Vāta*-dominant pain states, including conditions with radiating neuropathic features (sciatica, frozen shoulder, low back Radiculopathy)[24]. An In-Depth Review of *Viddhakarma* in Ayurvedic Tradition Mahesh Kumar reported that *Viddhakarma* has shown significant efficacy in treating *Raktaja* (blood-related) and *Pittaja* (Pitta-related) disorders, as well as conditions involving other Doshas and *Dhatus* and affirm reliability of *Viddhakarma* treatment modality, particularly in the management of *Shool* (musculoskeletal pain)[25].

## CONCLUSION

Based on the comprehensive analysis from 2021 to 2025 presented, *Viddhakarma* emerges as a uniquely effective and multifaceted therapeutic intervention. Its foundation in classical Ayurvedic principles is strongly supported by contemporary clinical outcomes, demonstrating rapid and significant relief for a wide range of disorders, from localized musculoskeletal pain to systemic conditions. The procedure's success is attributed to its dual-action mechanism simultaneously classical *Ayurvedic* concepts - *Apakarshana* of vitiated *Dosha*, *Rakta Dushti*, *Ama* and Enhancement of *Agni*, local *Dhatvagni Deepana*, and *Strotoshuddhi* (cleansing of channels) with modern hypotheses-Decompression of entangled nerves and myofascial elements and Neuromodulation via precise needling (akin to dermatome-somatic nerve response) offering a holistic alternative to conventional symptom management. The compiled clinical data demonstrates *Viddhakarma's* significant and often rapid therapeutic impact across a diverse

spectrum of conditions, most notably in musculoskeletal disorders such as *Gridhrasi* (Sciatica), *Avabahuka* (Frozen Shoulder), *Vishwachi* (Cervical Radiculopathy), and joint pain. Beyond these, its successful application extends to *Galaganda* (Thyroid enlargement), *Timira Roga* (visual disturbances), *Anantavata* (Trigeminal Neuralgia), *Mutrashmari* (Urolithiasis), and even PCOS and depressive disorders, highlighting its systemic regulatory potential. Outcomes consistently report superior pain relief, reduced inflammation, enhanced tissue healing, and improved functional recovery, frequently achieving results where conventional first-line therapies may be insufficient. The inherent advantages of *Viddhakarma*, including its precision, minimal invasiveness, and cost-effectiveness provides a sustainable, non-pharmacological option for pain relief and functional restoration. In conclusion, *Viddhakarma* represents a bridging of ancient precision and modern pain science requires a critical next phase: the systematization of evidence. Future efforts must prioritize high-quality, large-scale randomized controlled trials, detailed procedural standardization, and long-term outcome studies. By addressing these research imperatives, *Viddhakarma* can fully realize its potential as a validated, evidence-based bridge between ancient wisdom and contemporary healthcare, securing its role as a powerful tool for restoring homeostasis and rejuvenating the body's innate healing capacity.

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RESEARCH ARTICLE

# Exploring Mental Health Preparedness, Coping Strategies, Resilience and Social Support among Working Women During Family and Personal Crisis : A Pilot Study

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## ABSTRACT

Working women frequently juggle professional responsibilities alongside family roles, placing them at heightened risk of psychological distress during family or personal crisis such as illness, bereavement, financial strain, or marital conflict. Mental health preparedness, along with effective coping strategies, resilience, and social support, plays a crucial role in safeguarding well-being during such challenging periods. Despite increasing female workforce participation in India, limited evidence exists on how working women prepare for and cope with mental health challenges during crisis. This pilot study aimed to assess the level of mental health preparedness, coping strategies, resilience, and perceived social support among working women, and to determine the associations between these variables during family or personal crisis. A cross-sectional descriptive pilot study was conducted among 69 working women aged 21–50 years from selected workplaces in Anand District, Gujarat. Data were collected using an online survey comprising a socio-demographic questionnaire, Mental Health Preparedness Scale (MHPS), Brief COPE Inventory, Connor–Davidson Resilience Scale (CD-RISC), and Multidimensional Scale of Perceived Social Support (MSPSS). Descriptive statistics and Pearson correlation analysis were used for data analysis. The majority of participants demonstrated high mental health preparedness (68.12%), while 28.98% showed moderate preparedness. Coping levels were distributed across low (34.78%), moderate (34.78%), and high (30.44%) categories. Resilience levels were predominantly moderate (31.88%) to high (36.23%). Most participants reported high perceived social support (75.36%).

Mental health preparedness showed a significant positive correlation with resilience ($r = 0.383$, $p = 0.001$). Coping strategies were significantly associated with resilience ($r = 0.345$, $p = 0.004$) but not directly with preparedness. A strong positive association was observed between social support and mental health preparedness ($r = 0.595$, $p < 0.001$), with family and significant-other support showing the strongest relationships. The findings highlight the pivotal role of resilience and social support in enhancing mental health preparedness among working women during crisis. Strengthening supportive networks and resilience-building interventions at individual and workplace levels is essential to promote psychological well-being among working women.

**Keywords:** Mental health preparedness, working women, coping strategies, resilience, social support, crisis.

## INTRODUCTION

Working women often face multiple responsibilities, balancing professional demands with family obligations. During times of personal or family crisis—such as illness, bereavement, financial stress, or marital conflict—the risk of psychological distress significantly increases. Mental health preparedness, defined as the ability to anticipate, manage, and cope with mental health challenges, plays a crucial role in promoting resilience and maintaining well-being. India has witnessed a remarkable rise in women's participation in the workforce over recent years. The Worker Population Ratio (WPR) for women nearly doubled, increasing from 22% in 2017–18 to approximately 40.3% in 2023–24. Parallelly, the Labor Force Participation Rate (LFPR) for women climbed from 23.3% to 41.7% over the same period [1]. Regionally, the surge in participation is especially pronounced in rural India, where the LFPR rose from 24.6% in 2017–18 to 41.5% in 2022–23, while urban female LFPR climbed modestly, from 20.4% to 25.4% [2]. Reports also indicate that in 2024, women's involvement in both paid and unpaid employment activities reached 25%, compared to 21.8% in 2019 Despite this progress, women continue to shoulder a disproportionate share of unpaid domestic and caregiving responsibilities, averaging 305 minutes per day—over three times the time men spend on such tasks (86 minutes) [3]. According to NFHS-5 (2019–21), approximately 1 in 3 married women (aged 18–49)—around 30%—have experienced physical or sexual violence by a spouse[4] A study based on NFHS-5 data found that out of 72,320 women, 17,765 (≈24.6%) had faced Intimate Partner Violence. Among them, only 14.2% sought any help, meaning 85.8% did not seek assistance despite experiencing violence [5][6]. In 2021, India recorded 45,026 female suicides. More than 50% of these were housewives, and over 30% of all suicides (male and female) were attributed to family or marriage-related problems [7]. A U.S.-based AARP survey revealed that about 65% of women aged 40–79 have endured major life crisis such as job loss, divorce, the death of a spouse, or serious illness—events that frequently lead to psychological distress [8]. A nationwide Indian survey of 1.3 million women revealed that nearly 50% experience chronic stress related to work-life balance, financial pressures, and societal expectations [9]. A Times of India report found that 98% of women who sought to re-enter the workforce after a hiatus cited family support as a key enabling factor. *This underscores the vital role families play in encouraging women to resume professional roles*[10]. A survey of over 30,000 Indian mothers revealed 33% did not feel fully supported emotionally by their families 57% reported they were not always able to share emotions openly with family during times of distress. Only 34% felt "Valued and Seen" by their family. This highlights significant emotional support gaps even within caregiving households[11]. Coping strategies and social support systems are key protective factors that help individuals adapt to crisis[12]. However, limited research has explored how working women specifically prepare for and cope with such challenges. Understanding their preparedness and coping mechanisms is vital for developing targeted interventions, workplace policies, and support programs. Despite the growing participation of women in the workforce, there is inadequate understanding of how working women prepare for and cope with mental health challenges during family or personal crisis. Assessing preparedness and coping strategies will help identify gaps and inform interventions to enhance resilience and well-being.

## REVIEW OF LITERATURE

A study was conducted to elucidate the work–family conflict on psychological well-being experienced by working women and to determine whether social support has beneficial role in the psychological well-being of the working women. Result shows that employed women are more satisfied with their life than nonworking women, and the quality of home and work environments determines the impact of employment on the psychological well-being of working women in dual-earner families. Factors causing work related stress and strategies for stress management: a study of working women in private and public sectors in the Indian context the results of the study showed that the majority of the women (32.5%) experienced stress when evaluated performance by superiors and given negative feedback, by their employers. The analysis also showed that the most effective strategy for stress management was spending time with children, with an average rank of 3.55 whereas doing yoga and meditation has the lowest average rank of 2.65, indicating that respondents rarely use this strategy for managing stress [13].

**Specific objectives of the study (primary and secondary)**

1. To assess the level of mental health preparedness among working women during family and personal crisis.
2. To assess the coping strategies and resilience adopted by working women during crisis.
3. To determine the association between mental health preparedness, coping strategies, and resilience of working women during crisis.
4. To determine the association of social support systems with mental health preparedness for working women.

## METHODOLOGY

**Research Design:** A cross-sectional descriptive
**Study Setting**: Selected workplaces, hospitals, educational institutions, and corporate offices in Anand District.
**Study Population**: Working women aged 21–50 years who are employed in organized sectors.
**Sample Size:** Using Cochran's formula (95% CI, 5% margin of error), a minimum of 680 participants will be targeted. For the present pilot study 69 participants were recruited via online survey method.
**Sampling Technique**: Convenience sampling technique

**Inclusion Criteria**

- Women currently employed for at least 1 year.
- Women who have experienced at least one family or personal crisis in the last 2 years (e.g., illness, bereavement, financial stress).

**Exclusion Criteria**
Women with a diagnosed severe psychiatric illness.

**Operational Definitions:**
**Mental Health Preparedness**
In this study, *mental health preparedness* refers to the proactive ability of working women to anticipate, plan, and effectively manage their psychological well-being during family or personal crisis. It will be measured using a Mental Health Preparedness Scale.

**Coping Strategies**
*Coping strategies* are the behavioral and cognitive methods used by working women to handle stress and adapt during crisis. It will be assessed using the Brief COPE Inventory which categorizes coping as problem-focused, emotion-focused, or avoidance-based.

**Resilience**

Resilience is defined as the capacity to recover and adapt positively in the face of stress, adversity, or crisis. It will be measured using the Connor-Davidson Resilience Scale (CD-RISC).

**Social Support**

*Social support* refers to the perceived availability of emotional, informational, and practical help from family, friends, and significant others. It will be assessed using the Multidimensional Scale of Perceived Social Support (MSPSS).

**Assumptions**

1. Working women experience varying levels of family and personal crisis that affect their mental health.
2. Participants will respond to the research instruments honestly and accurately to reflect their true experiences.
3. Coping strategies and social support systems play a significant role in influencing the level of mental health preparedness among working women.

**Data Collection Tools**

1. Mental Health Preparedness Scale,
2. Brief COPE Inventory
3. Connor-Davidson Resilience Scale (CD-RISC).
4. Multidimensional Scale of Perceived Social Support (MSPSS)

**Plan for Data Collection**

Data for this study was collected exclusively through an online survey platform to ensure wide participation, convenience for working women, and secure handling of responses. Participants were recruited via workplace groups, official emails, and social media networks. The invitation will include the Participant Information Sheet (PIS), contact details of the research team, and a link to the online survey. When participants click the link, they will first view the PIS and an electronic consent form. Only those who agree by selecting the "I consent to participate" option will proceed further. Built-in eligibility screening questions will confirm inclusion criteria such as age, employment status, and experience of a family or personal crisis. Ineligible participants will automatically exit the survey.

**The online questionnaire will be divided into five sections**

1. Socio-demographic and background details
2. Mental Health Preparedness Scale (MHPS) – researcher-developed tool
3. Brief COPE Inventory – coping strategies[14]
4. Connor-Davidson Resilience Scale (CD-RISC) – resilience[15]
5. Multidimensional Scale of Perceived Social Support (MSPSS) – perceived support

The total time to complete the survey will be 25–30 minutes. Skip logic will be applied to ensure smooth navigation, and mandatory responses will minimize missing data. Upon completion, participants received a thank-you message along with a list of mental health resources and helpline numbers. Responses will be anonymized automatically, with no personal identifiers collected. Data was stored on password-protected, encrypted servers, accessible only to the research team.

## RESULT

Table 1 presents the demographic characteristics of the participants. The mean age of the participants was 29 ± 7.5 years. With regard to marital status, about half of the participants were married (50.7%), while a considerable proportion were single (46.4%), and a small percentage were divorced (2.9%). In terms of number of children, the majority of participants (72.5%) reported having no children, while 20.3% had one child and 7.2% had two children. Regarding family structure, 63.8% of the participants belonged to nuclear families, whereas 36.2% lived in joint families. The distribution of place of residence showed that most participants resided in urban areas (69.6%),

followed by rural (20.3%) and semi-urban areas (10.1%). With respect to educational qualification, a majority of participants were postgraduates (58.0%), followed by graduates (37.7%), and a small proportion held a doctorate degree (4.3%). In terms of occupation, nearly half of the participants were engaged in teaching (49.3%), followed by those in other professions (26.1%) and healthcare (17.4%), with fewer participants from IT and administrative sectors. Concerning the nature of job, most participants were employed on a contractual/temporary/adhoc basis (63.8%), while 36.2% held permanent positions. The mean work experience of the participants was 34 years, and the average working hours were 45 hours per week. Finally, analysis of monthly income revealed that the largest proportion of participants earned between ₹10,001–25,000 (37.7%), followed by ₹25,001–50,000 (34.8%) and above ₹50,000 (26.1%), while only 1.4% earned less than ₹10,000 per month. Table 2, depicts the Mental Health Preparedness Scale (MHPS) demonstrated good internal consistency, with a Cronbach's alpha of $\alpha = 0.76$, indicating acceptable reliability. Table 3, depicts the distribution of mental health preparedness levels among the participants based on MHPS scores. The findings reveal that a majority of the working women demonstrated a high level of mental health preparedness. Specifically, 68.12% (f = 47) of the participants scored within the range of 75–100, indicating strong awareness, readiness, and ability to manage mental health challenges during family or personal crisis. Approximately 28.98% (f = 20) of the participants exhibited a moderate level of mental health preparedness, with scores ranging from 50–74. This group reflects women who possess some coping resources and preparedness but may benefit from additional support or skill-building interventions to strengthen their mental health resilience. Only a small proportion of participants, 2.90% (f = 2), were found to have low mental health preparedness (scores between 20–49), suggesting limited readiness to anticipate or manage mental health challenges during crisis. The table 4 presents the distribution of coping levels among working women based on total coping scores. Approximately 34.78% (f = 24) of the participants exhibited low coping. An equal proportion, 34.78% (f = 24), demonstrated a moderate level of coping. About 30.44% (f = 21) of the participants showed high coping. The table 5 presents the distribution of resilience levels among the participants based on CD-RISC scores.

The mean resilience score was 26.28 ± 9.46, indicating an overall moderate level of resilience among the working women. Analysis of the score distribution shows that 36.23% (f = 25) of the participants demonstrated high resilience. An equal proportion of participants, 31.88% (f = 22), fell into the moderate resilience category. Similarly, 31.88% (f = 22) of the participants exhibited low resilience. Table 6 depicts the distribution of perceived social support among working women. The findings indicate that a substantial majority of participants reported a high level of perceived social support. Specifically, 75.36% (f = 52) of the respondents scored within the range of 5.1–7.0, reflecting strong support from family, friends, and significant others. A smaller proportion of participants, 15.94% (f = 11), experienced a moderate level of perceived social support, with scores ranging from 3.0–5.0. These women reported some degree of support; however, the level may not be sufficient during prolonged or intense family or personal crisis. Only 8.70% (f = 6) of the participants reported low perceived social support (scores between 1.0–2.9), indicating limited availability of emotional or practical assistance from their social networks. Association between Mental Health Preparedness, Coping Strategies, and Resilience N=69 (Table 7). Pearson correlation analysis revealed a moderate positive and statistically significant correlation between mental health preparedness and resilience ($r = 0.383$, $p = 0.001$), indicating that working women with higher resilience levels tend to have better mental health preparedness during family or personal crisis. The correlation between mental health preparedness and coping strategies was positive but weak and not statistically significant ($r = 0.137$, $p = 0.263$). This suggests that overall coping scores alone may not directly influence preparedness levels, highlighting the need to examine specific coping styles in further analyses. A moderate positive and statistically significant correlation was observed between coping strategies and resilience ($r = 0.345$, $p = 0.004$), implying that effective coping mechanisms are associated with higher resilience among working women during crisis. Association of Social Support with Mental Health Preparedness (Table 8). Pearson correlation analysis revealed a strong positive and statistically significant association between overall perceived social support and mental health preparedness among working women ($r = 0.595$, $p < 0.001$). This indicates that higher levels of perceived social support are associated with better mental health preparedness during family or personal crisis. Subscale analysis further demonstrated that family support ($r = 0.585$, $p < 0.001$), friends' support ($r = 0.575$, $p < 0.001$), and support from significant others ($r = 0.589$, $p < 0.001$) were all strongly and positively correlated with mental health preparedness.

## DISCUSSION

The present study explored mental health preparedness, coping strategies, resilience, and perceived social support among working women during family or personal crisis. The findings provide important insights into the psychological resources and support systems that enable working women to manage stressful life events effectively. The results revealed that the majority of participants demonstrated high mental health preparedness, with more than two-thirds of the women exhibiting strong readiness to manage mental health challenges during crisis. This finding may be attributed to the high educational status[16] and urban background of most participants,[17] which could enhance mental health awareness and access to resources. Similar findings have been reported in previous studies, which suggest that education[18] and employment contribute positively to mental health literacy[19] and preparedness. With regard to coping strategies, the study found that most working women fell within the low to moderate coping categories, with only about one-third exhibiting high coping levels. This suggests that although women may possess awareness and preparedness, they may not consistently employ effective coping strategies during prolonged or intense stress. Previous research has also indicated that working women often prioritize professional and family responsibilities over self-care, which may limit their ability to engage in adaptive coping practices[20]. Resilience levels among participants were largely moderate to high, indicating a reasonable capacity to adapt to adversity. The significant positive association observed between resilience and mental health preparedness supports existing literature that identifies resilience as a key protective factor in maintaining psychological well-being during crisis[21]. Women with higher resilience were better prepared to anticipate, manage, and recover from mental health challenges[22]. One of the most notable findings of the study was the strong positive association between perceived social support and mental health preparedness. Support from family, friends, and significant others was found to play a crucial role in enhancing preparedness, with family support emerging as the strongest contributor[23]. This aligns with socio-ecological models of mental health, which emphasize the importance of interpersonal relationships in buffering stress and promoting psychological stability[24]. Interestingly, coping strategies did not show a direct significant association with mental health preparedness, although they were significantly related to resilience. This suggests that coping may indirectly influence preparedness by strengthening resilience rather than acting as an independent predictor. Such findings highlight the complex interplay between coping, resilience, and social support in determining mental health outcomes[25]. Overall, the study underscores the need for integrated interventions that focus on strengthening resilience and social support systems while simultaneously enhancing coping skills. Workplace mental health programs, family-centered interventions, and community-based support mechanisms can play a vital role in improving mental health preparedness among working women, particularly during times of crisis.

## CONCLUSION

This study highlights the importance of mental health preparedness among working women during family or personal crisis and emphasizes the critical roles of resilience and social support in shaping psychological readiness. The findings indicate that while a majority of working women demonstrate moderate to high levels of mental health preparedness, coping strategies are not uniformly strong, suggesting a gap between awareness and effective coping practices. Resilience emerged as a significant protective factor, positively associated with mental health preparedness, underscoring its role in enabling women to adapt to and recover from stressful life events. Furthermore, perceived social support—particularly from family and significant others—showed a strong positive association with mental health preparedness, reinforcing the value of supportive interpersonal relationships during crisis. Overall, the study suggests that mental health preparedness among working women is influenced by an interplay of individual strengths and external support systems. Integrating resilience-building interventions, strengthening social support networks, and promoting adaptive coping strategies within workplaces and communities may enhance psychological well-being and preparedness among working women facing family or personal challenges.

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**Table 1: Demographic Profile of Participants** N=69

| SL. No. | Demographic variable | Frequency (f) | Percentage (P) |
|---|---|---|---|
| 1. | **Age(Year)** | 29±7.5 | |
| | Mean age | | |
| 2. | **Marital Status** | | |
| | Single | 32 | 46.4 |
| | Married | 35 | 50.7 |
| | Divorced | 2 | 2.9 |
| 3. | **Number of Children** | | |
| | Zero | 50 | 72.5 |
| | 1 | 14 | 20.3 |
| | 2 | 5 | 7.2 |
| 4. | **Type of Family** | | |
| | Nuclear | 44 | 63.8 |
| | Joint | 25 | 36.2 |
| 5. | **Place of Residence** | | |
| | Urban, | 48 | 69.6 |

| | | | |
|---|---|---|---|
| | Semi-urban, | 7 | 10.1 |
| | Rural | 14 | 20.3 |
| 6. | **Educational Qualification** | | |
| | Graduate, | 26 | 37.7 |
| | Postgraduate, | 40 | 58.0 |
| | Doctorate | 03 | 4.3 |
| 7. | **Occupation Type** | | |
| | Teaching | 34 | 49.3 |
| | Health Care | 12 | 17.4 |
| | IT | 02 | 2.9 |
| | Administration | 3 | 4.3 |
| | Others | 18 | 26.1 |
| 8. | **Nature of Job** | | |
| | Permanent, | 25 | 36.2 |
| | Contract/ Temporary/Adhoc | 44 | 63.8 |
| 9. | **Work Experience (Years)** | | |
| | 1–60 months (≤ 5 years) | 56 | 81.16 |
| | 61–180 months (5–15 years) | 11 | 15.94 |
| | 181–336 months (> 15 years) | 02 | 02.90 |
| 10. | **Working Hours per Week** | | |
| | Mean working hours/week | 45hrs/week | |
| 11. | **Monthly Income (INR)** | | |
| | <10000 | 1 | 1.4 |
| | 10001-25000 | 26 | 37.7 |
| | 25001-50000 | 24 | 34.8 |
| | >50000 | 18 | 26.1 |

**Table 2: Reliability Statistics of Mental Health Preparedness Levels Scale (MHPS)**

| Cronbach's Alpha | N of Items |
|---|---|
| 0.762 | 21 |

**Table 3: Distribution of Mental Health Preparedness Levels (MHPS)** N=69

| Score Range | Interpretation | Frequency(f) | Percentage (%) |
|---|---|---|---|
| 20–49 | Low mental health preparedness | 02 | 2.90 |
| 50–74 | Moderate mental health preparedness | 20 | 28.98 |
| 75–100 | High mental health preparedness | 47 | 68.12 |

**Table 4: Level of Coping Strategies among Working Women** N=69

| Coping Score Range | Interpretation | Frequency (f) | Percentage (%) |
|---|---|---|---|
| ≤ 65 | Low coping | 24 | 34.78 |
| 66 – 76 | Moderate coping | 24 | 34.78 |
| ≥ 77 | High coping | 21 | 30.44 |

**Table 5: Level of Resilience among Working Women (CD-RISC)** N=69

| Score Range | Interpretation | Frequency(f) | Percentage (%) |
|---|---|---|---|
| 0 – 20 | *Low resilience* – difficulty coping with stress or crisis. | 22 | 31.88 |
| 21 – 30 | *Moderate resilience* – can handle some stress but may struggle in prolonged or severe crisis. | 22 | 31.88 |
| 31 – 40 | *High resilience* – strong ability to adapt and recover from adversity. | 25 | 36.23 |

**Table 6: Level of Social Support among Working Women N=69**

| Mean Score Range | Interpretation | Frequency (f) | Percentage (%) |
|---|---|---|---|
| **1.0 – 2.9** | Low perceived social support | 06 | 8.70 |
| **3.0 – 5.0** | Moderate perceived social support | 11 | 15.94 |
| **5.1 – 7.0** | High perceived social support | 52 | 75.36 |

**Table 7: Association between Mental Health Preparedness, Coping Strategies, and Resilience. N=69**

| Variables Compared | R-value | p-value | Interpretation |
|---|---|---|---|
| MHPS × Resilience | 0.383 | 0.001 | Moderate positive, statistically significant |
| MHPS × Coping Strategies | 0.137 | 0.263 | Weak positive, not statistically significant |
| Coping Strategies × Resilience | 0.345 | 0.004 | Moderate positive, statistically significant |

**Table 8: Association of Social Support with Mental Health Preparedness**

| Social Support Variable | R-value | p-value | Interpretation |
|---|---|---|---|
| Total Social Support (MSPSS_Total) | 0.595 | < 0.001 | Strong positive, statistically significant |
| Family Support | 0.585 | < 0.001 | Strong positive, statistically significant |
| Friends Support | 0.575 | < 0.001 | Strong positive, statistically significant |

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RESEARCH ARTICLE

# The Gut-Lung Axis in Ayurveda : Kasa as a Reflection of Agni Dysfunction

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## ABSTRACT

*Kasa* (cough) is one of the most common clinical complaints, but Ayurveda explains that its origin is deeper than the respiratory tract. There is a close connection between the digestive system and the respiratory system. When *Agni* becomes weak due to wrong food habits, irregular routines, or emotional stress, digestion becomes incomplete and produces Ama. This Ama spreads through the channels and settles in the *Pranavaha Srotas*, disturbing normal flow of the *Vata* and causing cough. The uploaded article highlights this gut-lung link and explains how early digestive imbalance can be identified before respiratory symptoms appear. A detailed textual analysis was done, focusing on literature, classical shloka interpretation, and modern science. The topic was reviewed for conceptual understanding, *Nidana-Samprapti* clarity, and the clinical relevance of *Agni Dushti* in the development of *Kasa.* The material was re-examined based on the *Roga Nidana* principles such as *Purvaroopa, Samprapti Ghatakas,* and *Upadravas* involvement. The review clearly shows that the first disturbance in many cases of *Kasa* starts in the gut. Weak *Agni* (*Mandagni*) leads to *Ama* formation, which causes blockage in the body channels and disturbs *Vata*, especially *Apana* and *Udana*. This upward movement of vitiated *Vata* creates pressure in the chest and throat, leading to cough, breathlessness, and voice changes. Prodromal symptoms like anorexia, throat irritation, and indigestion further confirms early *Agni* impairment. The article also shows that various types of *Kasa-Vataja, Pittaja, Kaphaja,* and chronic forms-can be traced back

to different types of *Agni* imbalance. Modern evidence on GERD-induced cough and gut-lung axis research supports the Ayurvedic view. The analysis highlighting that treating only the cough does not give lasting relief if the root cause, *Agni*, is not corrected. Strengthening digestion, clearing *Ama*, and regulating *Vata* helps break the disease process at its origin. The article effectively integrates classical knowledge with modern understanding, showing that both systems agree on the role of gut health in respiratory diseases. This strengthens the Ayurvedic approach of diagnosing *Kasa* not as an isolated respiratory issue but as a systemic disorder beginning in the digestive tract.

**Keywords:** Agni dysfunction; Kasa; Gut-lung axis; Apana Vata; Udana Vata; Pranavaha Srotas.

## INTRODUCTION

Respiratory symptoms are among the most frequent complaints in both general and specialized Ayurvedic practice. Among them, *Kasa* (cough) is often perceived as an isolated symptom of the *Pranavaha Srotas* (respiratory channels). However, classical Ayurvedic teachings encourage practitioners to look beyond symptoms and explore the root cause- often traced back to *Agni Dushti*, or impaired digestive fire[1]. In Ayurveda, digestion-absorption-assimilation is said to be done by *Agni*. Disturbance in *Agni* leads to the formation of *Ama*, which, when circulated through the body, lodges in weak *Srotas* (channels) and manifests as various diseases[2]. In the case of *Kasa*, respiratory tissues become eventual site of this manifestation. Despite its prevalence, the *Agni-Phuphusa* connection remains under-explored in the context of cough management. This paper aims to bring attention to the digestive origins of *Kasa*, propose diagnostic indicators of *Agni* dysfunction in respiratory cases, and offer Ayurvedic interventions targeting the digestive system for sustainable relief.

## METHODS

A textual review and clinical synthesis were conducted to explore the link between *Agni* dysfunction and *Kasa*. Primary Ayurvedic texts and commentaries were analysed for references to *Kasa, Agni*, and *Ama*, including:

- ***Charaka Samhita –*** *Chikitsa Sthana, Nidana Sthana*
- ***Ashtanga Hridaya –*** *Chikitsa Sthana*
- *Bhavaprakash Nighantu*
- ***Madhava Nidana –*** *Kasa Nidana*

The AI Tool- Gemini and chatgpt has been used to enhance the statements and eliminate any grammatical errors which was made while writing the script.

## LITERATURE REVIEW

The thematic analysis of Ayurvedic literature and modern studies revealed a profound relationship between function (*Agni*) and the manifestation of *Kasa* (cough)[3].

***Agni* impairment before the manifestation of *Kasa***

The onset of *Kasa* (cough), as explained in Ayurvedic principles, doesn't happen abruptly-it unfolds gradually, beginning deep within the digestive system. It all starts with a disturbance in *Agni*, the body's digestive fire. When someone consumes unhealthy or incompatible food (called *Ahita ahara* or *Viruddhahara*), or is constantly under stress, the *Jathragni* becomes weak and inefficient. As a result, the food isn't properly digested, leading to a state known as *Ajirna* or indigestion. This incomplete digestion produces Ama, a sticky, toxic by product that acts like metabolic waste. Over time, Ama begins to accumulate and circulate within the body. This toxic buildup doesn't just stay in the gut-it infiltrates bodily channels, especially the Pranavaha Srotas, which are crucial for breathing and respiration. As

Ama settles in these channels, it starts to disrupt the balance of the Doshas, particularly Kapha and Vata. The Kapha becomes thick and sluggish, while Vata, which governs movement, becomes erratic. This toxic duo sets the stage for further complications. The channels of the respiratory system get blocked (Srotorodha), which disturbs Udana Vayu, the upward-moving energy responsible for speech and respiration. In this disturbed environment, the vitiated *Doshas* become even more aggravated ***(****Utklesha* and *Prakopa)* and begin to move upwards. They accumulate in the chest **(***Uras*) and throat (Kantha) regions. This upward movement and blockage lead to the typical symptoms of Kasa—persistent coughing, chest discomfort, and disturbed breathing.

**Evidence from *Poorvaroopa* indicating *Agni* Impairment[4,5]**

तेषां भविष्यतां रूपं कण्ठे कण्डूररोचकः।।१८।।

शूकपूर्णाभकण्ठत्वम् (Ashtanga Hrudayam Nidanasthan 3/19)

The prodromal symptoms of Kasa includes- Kanthe Kandu (Itching/irritation in throat), Arochaka (Loss of taste/ loss of appetite leading to aversion towards food despite hunger), Shookapoornakanthatvam (Feels as if pointed objects keep pricking the throat).

Here, Arochaka is one among the cardinal features of Ajirna which is also found in Agnimandya as Ajirna is its consequence.

तस्यलिङ्गमजीर्णस्यविष्टम्भःसदनंतथा।
शिरसोरुक्चमूर्च्छाचभ्रमःपृष्ठकटिग्रहः।।४५।।
जृम्भाऽङ्गमर्दस्तृष्णाचज्वरश्छर्दिःप्रवाहणम्।
अरोचकोऽविपाकश्च, घोरमन्नविषंचतत्।।४६।। (Charaka Chikitsasthana 15/45-46)

**Samprapti[3]**

**Role Of*Apana Vata***

तत्राधो विहतोऽनिलः।
ऊर्ध्वं प्रवृत्तः प्राप्योरस्तस्मिन् कण्ठे च संसजन्।।१९।। (Ashtanga Hrudayam Nidanasthana 3/19)

**Commentary**

क्रुद्धः प्रतिहतोऽपाने यदाऽपानः प्रवर्तते। ऊर्ध्वं रसस्य स स्थाने तिष्ठन्नुरसि पीड्यते।। उदानेन सजंस्तत्र कण्ठे चानुप्रपूर्य च। (आयुर्वेदरसायनम्व्याख्या ( कृत))

When the *Apana Vayu*, having become enraged (क्रुद्धः) and obstructed (प्रतिहतः), begins to move abnormally it moves upwards and stays in the region of rasa (nutrient fluid), and causes pain or pressure in the chest (*Uras*). As the reversed *Apana* reaches upward, it afflicts the chest, causing pain or oppression. Then it joins with *Udana Vayu* there and fills the throat completely. The vitiated *Vayu* now mixes with *Udana*, which is responsible for functions like speech and exhalation. This union results in throat congestion or blockage, leading to symptoms like coughing, hoarseness.

क्रुद्धःप्रतिहतोऽपाने
↓ (Aggravation and Obstruction of *Apana Vata*)
*Apana Vata* becomes enraged and blocked
↓
प्रवर्ततेऊर्ध्वगमनम्
(Moves in reverse → upward direction)
↓
ऊर्ध्वंरसस्यसस्थाने
Reaches the region of *Rasa* (thorax/chest)
↓
उरसिपीड्यते
Causes pressure, pain, or congestion in chest
↓
उदानेनसजंस्तत्र
Co-mingles with Udana Vata in thoracic region

↓
कण्ठेचअनुप्रपूर्य
Fills/pressurizes the throat and airway
↓
→ Kasa (Cough) associated with:
- Shvasa (Dyspnea)
- Urahshoola (Chest pain)
- Swarabheda (Voice changes / Hoarseness)

**Role of *Agni***

There are various causes for aggravation and obstruction of *Apana Vata.* According to Ayurveda, all digestive and metabolic activities in the body are powered by Agni. Any disease, including *Kasa* (cough), is often rooted in the derangement of *Agni.* Before *Kasa* manifests as a respiratory disorder, early disturbances in *Agni* initiate a cascade of pathophysiological events. One of the most common reasons which can be inferred is as follows:
*Nidana* (diet/lifestyle/emotions)
↓
Aggravation of *Vata* + Weak digestion (*Agni-mandya*)
↓
Formation of *Ama* + Obstruction of *Apana* path
↓
*Apana* gets blocked → Reverses direction
↓
Moves upward → Disturbs *Udana*
↓
*Kasa*

In the context of Kasa due to Abhyantara hetu (cough arising from internal causes), the pathophysiology can be traced to the involvement of Apana Vata right from the early stage itself. This downward-moving Vata, when deranged, initiates and sustains the cascade that culminates in cough. However, in Kasa due to Agantuka hetu (cough triggered by external or environmental factors), scenario is different. Here Apana Vata does not play a primary role in the beginning. For instance, when the causative factor is dust exposure or allergic irritants, the immediate disturbance is seen in Prana Vata and Udana Vata. Their normal flow and functional dynamics get obstructed, which then provokes cough. In such cases, structural alterations-like narrowing of the air passage or irritation of the airway mucosa by foreign particles- further aggravate the condition, reinforcing the manifestation of Kasa.

**Subtype-Specific Connections Between Agni and Kasa**

Each type of Kasa reflects a distinct pattern of digestive disturbance and Dosha aggravation:

**Vataja Kasa[6]**
- Triggered by unpredictable eating habits like dry food, fasting, or excessive physical exertion.
- It is often linked to *Vishamagni* (irregular digestion) resulting from unpredictable dietary patterns.
- It is characterized by dry, painful, spasmodic coughing without significant phlegm production.

***Pittaja Kasa*[7]**
- It caused by excessive intake of spicy, hot, oily, or sour foods that over-stimulate *Agni* leading to *Tikshnagni* (intense digestive fire).
- This results in a cough with yellowish, sometimes blood-tinged sputum, burning sensation in the chest and throat.

***Kaphaja Kasa*[8]**

- It originates from excessive intake of heavy, oily, cold, and sweet foods, which weaken *Agni* and elevate *Kapha* levels.
- The characteristic features include productive cough with thick, white phlegm and a feeling of heaviness.

***Kshataja Kasa*[9]**
Due to trauma to thoracic region it gets injured leading to bleeding. This may lead to the imbalance of Pitta, ultimately impairing the function of Agni.

***Kshayaja Kasa*[10]**
Associated with chronic conditions and tissue depletion, often following prolonged *Agni* disturbance and resulting malnourishment of *Dhatus* (tissues).

**Clinical Observations: Digestive Correction Resolves Respiratory Symptoms[11]**
Clinical case observations indicate that Ayurvedic therapies focusing on:
**Agni Deepana** (stimulating digestion using herbs like *Trikatu*, *Pippali*, *Chitraka*),
**Ama Pachana** (digesting Ama with formulations like Shunthi, Guduchi, Dashamoola),
**Pathya-Apathya** (dietary regulations aimed at restoring digestion), often led to the substantial reduction or complete resolution of chronic coughs, particularly those unresponsive to conventional antitussive treatments. Notably, interventions aimed purely at suppressing the cough without addressing digestive imbalance showed limited or temporary benefits.

**Evidence from Upadrava indicating Agni Impairment [12,13,14,15,16]**
*Kasa* is not merely an isolated disorder but is often observed as an *Upadrava* (complication) in a wide range of diseases. It frequently manifests as a secondary symptom in conditions such as *Shoola* (abdominal pain), *Jwara* (fever), *UdaraRoga* (abdominal disorders), *Chardi*(vomiting), and *Raktapitta* (bleeding disorders). Interestingly, most of these conditions have their origin in the *Annavaha Srotas*-the gastrointestinal tract-highlighting a close connection between the state of digestion and respiratory health. This association reinforces the Ayurvedic concept that *Agni* (digestive fire) plays a central role in maintaining overall well-being, and its disturbance can lead to systemic imbalances manifesting even in distant organs like the lungs. Thus, the occurrence of *Kasa* as an *Upadrava* in predominantly gut-related disorders illustrates the deep interrelationship between the digestive and respiratory systems.

**Modern Correlation: Gut-Lung Axis Evidence**
Modern medical studies reinforce Ayurveda's observations:
- Gastroesophageal reflux disease (GERD) is considered as a significant non-respiratory cause of chronic cough[17].
- Disruptions in gut microbiota (*dysbiosis*) are linked to systemic inflammation, increasing susceptibility to respiratory conditions like asthma and chronic bronchitis[18].

These are the evidences that prove gut/ Agni plays major role in manifesting Kasa.

## DISCUSSION

When we read the classics and look at the clinical picture of *Kasa* today, one thing becomes very clear: cough may appear in the throat and chest, but the story almost always begins in the gut. This understanding becomes even stronger when we read Charaka, Sushruta and Vagbhata describing the disease process. They repeatedly remind us that if Agni is disturbed, nothing else in the body can function smoothly. The same principle guides the development of *Kasa*.

***Udbhava Sthana:* Where the Disease Actually Begins (Gut and Agni)- *Amashaya***
The review clearly shows that the earliest disturbance in *Kasa* does not start in the lungs. It first started in the digestive tract. This reflects the classical principle that weak Agni is the root cause for almost all the systemic

diseases. Irregular food habits, incompatible diet combinations, stress, late meals and overeating first impair the digestive fire. When Agni loses its strength or rhythm, food is not broken down properly and *Ama* begins to form. This stage represents the *Udbhava Sthana*, the original seat of the disease. Although the patient may later present with cough, the imbalance has already begun silently within the gut.

**Ama Formation and Early Gut-Lung Disturbance**

*Ama* is a metabolic waste that acts like a heavy, sticky, toxic by product that interferes with normal movement inside the digestive tract. As *Ama* accumulates, it blocks the natural pathways of *Doshas* and *Dhatus*. This obstruction disturbs *Vata* functions, especially *Apana Vata*, which resides in the lower abdomen. Rather than moving downward, *Apana* becomes restricted and irritated. This blockage leads to the upward movement of disturbed *Vata*. At this point patients often complain of early signs such as loss of appetite, heaviness, abdominal discomfort, belching or mild throat irritation-indicating the first connection between gut imbalance and respiratory symptoms.

***Apana Vata*: The Turning Point in Samprapti**

Classical descriptions explain that when *Apana Vata* faces obstruction due to *Ama,* it loses its proper direction. Instead of moving downward, *Apana* shifts upward into the chest region. This upward movement marks a critical turning point in the development of Kasa. This mechanism also mirrors what modern science describes as the gut–lung axis. The rising Apana carries disturbed Doshas and Ama into the upper body, where it interacts with Udana Vata and Prana Vata. This interaction produces dryness, irritation, chest pressure, voice change and the urge to cough. The Poorvarupa described in the texts match these early clinical signs, indicating that the respiratory system is beginning to feel the impact of the digestive imbalance.

**Step-by-Step Link Between Gut and Lungs**

The complete chain of events can be understood as follows:

1. Diet and lifestyle errors weaken Agni- Agnimandya
2. Incomplete digestion results in Ama formation
3. Ama obstructs the pathways of Apana Vata
4. Apana Vata becomes irritated and moves upward
5. The upward movement disturbs Udana and Prana Vata
6. Irritation develops in the throat and chest
7. Kasa manifests in the respiratory channels

This sequence demonstrates a clear physiological movement from the digestive system to the lungs, connecting the Udbhava Sthana and Vyakta Sthana through Vata disturbance.

**Vyakta Sthana: The Site of Manifestation (Pranavaha Srotas)**

Once the vitiated Vata and Ama reach the chest region, the symptoms manifest in the Pranavaha Srotas, making the lungs and airways the Vyakta Sthana. The nature of the cough depends on the predominant Dosha at the site of manifestation.

- Vata dominance causes dry, forceful, spasmodic cough
- Pitta dominance causes burning in the chest, bitter taste, yellow sputum
- Kapha dominance leads to thick, heavy, white mucus and sluggish breathing

Although the symptoms appear in the respiratory system, the root lies in the digestive dysfunction that occurred earlier.

**Why Symptomatic Relief Alone Is Not Enough**

Merely treating the cough will not stop the disease process. If *Agni* remains weak and *Ama* is not cleared, the cycle of obstruction and upward *Vata* movement continues. Many patients experience temporary relief with cough syrups, inhalers or antibiotics, only to return with the same symptoms after a few days or weeks. This happens because the *Udbhava Sthana* remains disturbed. Correcting *Agni*, digesting *Ama* and restoring *Apana's* downward movement are essential to prevent recurrence.

**Clinical Approach Based on Roga Nidana Principles**

The assessment of *Kasa* must start with careful evaluation of *Agni*. Appetite, food tolerance, bowel habits, abdominal heaviness, bloating, belching patterns, sleep quality and emotional stress all suggests early *Agni* impairment. Checking for these signs ensures that the practitioner does not overlook the foundation of the disease. Once the root disturbance is understood, management becomes more effective and long-lasting.

**Integrated Interpretation of the Gut–Lung Axis**

The cascade from weak *Agni* to *Ama* formation, obstruction of *Apana*, upward movement of *Vata* and final manifestation in the *Pranavaha Srotas* shows a complete Ayurvedic understanding of the gut-lung axis. Modern research now supports this concept, demonstrating that gut inflammation, dysbiosis and metabolic toxins influence respiratory health. The classical Ayurvedic model, therefore, provides a more comprehensive explanation by including the deeper roles of Agni, Ama and Vata.

**Final Insight**

Kasa should be understood as a disorder with its origin in the gut, pathway through disturbed Vata, and manifestation in the lungs. The Udbhava Sthana, Samprapti pathway and Vyakta Sthana must all be considered together. When Agni is strengthened, Ama is cleared and Apana Vata is guided back to its natural downward direction, respiratory symptoms reduce significantly and recurrence is prevented.

## CONCLUSION

Ayurveda teaches us that a healthy digestive system (Agni) is key to good overall health including healthy lungs. When digestion becomes weak or imbalanced, it leads to the formation of toxins (Ama), which can cause cough (Kasa) and other health issues. This article shows that many types of cough, especially chronic ones, may start with poor digestion. Treating only the cough without fixing digestion may not give long-term relief. Both Ayurvedic and modern scientific research agree that the gut and lungs are closely connected a concept known today as the "gut–lung axis." By focusing on improving digestion through proper food, herbs, and lifestyle, we can prevent and treat coughs more effectively. Ayurveda's approach to treating the root cause not just the symptoms offers a deeper and more lasting solution for people suffering from cough-related problems.

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REVIEW ARTICLE

# AI-Enabled Discovery and Optimization of Plant-Based Nanomedicines: From Phytochemistry to Pharmacological Evaluation

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## ABSTRACT

Plant therapeutics have traditionally been the focus of drug discovery since they have faced such problems as chemical complexity, batch variability, low bioavailability and multi-target pharmacology, and could be left at the clinical stage largely untapped. Nanotechnology has made significant contribution on improving the delivery and stability of phytochemicals; the traditional method of formulation and testing is time-consuming and is empiric-driven. The concept of artificial intelligence

(AI) has also been effective to enhance the degree of plant-based nanomedicine discovery and optimization by integrating the tools of phytochemistry, formulation science, and pharmacology into one workflow. Machine learning, neural networks, chemometrics, QSAR modelling, and predictive ADMET tools are artificial intelligence-based methods that can be quickly applied to identify bioactive phytoconstituents, to predict the most favorable nanocarrier formulations, and to early predict pharmacological activity and toxicity. The review is a general survey of the AI application throughout the entire process of developing plant-based nanomedicines, such as phytochemical profiling and nanoparticle design, *in silico* pharmacology and predictive modelling. The article refers to the key advances, current limitations, and future opportunities of personalized phytomedicine using AI. All in all, AI has the disruptive capability in the form of reduced development period, greater therapeutic efficacy, and greater translational performance of plant-derived nano preparations.

**Keywords:** Artificial intelligence, phytochemistry, nanomedicine, machine learning, nano-phytopharmaceuticals, QSAR modelling, pharmacological evaluation, ADMET prediction.

## INTRODUCTION

**Global Rise of Plant-Based Therapeutics**

Health care cultures have been focused on medicinal plants and their bioactive constituents. This structural diversity, multi-target characteristics, decreased toxicity and potential therapeutic treatment of intricate diseases such as cancer, neurodegenerative diseases and metabolic syndromes, have seen a new interest in plant-derived therapeutics in the world over the past few decades[1]. The WHO estimates that nearly 80 percent of the global population relies on the use of herbal medicines as the primary healthcare which translates to its high acceptability and treatment. In addition, the current advances in phytochemistry, metabolomics, and biotechnology have led to the discovery of new phytoconstituents that have the potential to stimulate pharmacological activity. The plant molecules are therefore still important leads in modern drug discovery[2].

**Challenges**

Phytoconstituents have a high promise of therapeutic application; however, a number of obstacles impede their development into useful therapeutic drugs. Natural compounds are highly chemically variable, depending on geography, climatic conditions, methods of cultivation, and extraction which results in unequal therapeutic responses. Most of the plant bioactives have low aqueous solubility, high metabolism, low permeability and limited systemic availability, which significantly limits there *in vivo* activity. Structural instability, particularly, oxidation, hydrolysis and photodegradation vulnerability reduce their shelf life, and therapeutic use further. Mechanistic studies and dose optimization are complicated because of their multi-target and pleiotropic nature which is advantageous in complex diseases. These obstacles require sophisticated approaches that could guarantee stability, reproducibility and improved bioefficacy of the phytochemicals[3].

**Need for Nanotechnology for Phytochemicals**

Drug delivery systems that are developed by nanotechnology have proved to be a way out of the constraints of using plant-based bioactives. Examples of nanocarriers include liposomes, solid lipid nanoparticles, polymeric nanoparticles, phytosomes, nanoemulsions, and lipid-polymer hybrids to enhance solubility, absorption, prevent the degradation of unstable compounds, and deliver them to a specific tissue. Other benefits of nanoformulations are controlled and prolonged delivery, enhanced pharmacokinetics, and enhanced therapeutic efficacy with lower doses. In the case of phytochemicals that have an unfavorable biopharmaceutical profile, nanotechnology can be used as a strong-enabling technology that increases clinical opportunities and allows to obtain standardized and reproducible formulations[4, 5].

**Gap: Traditional Methods vs. AI-Assisted Workflows**

Traditional preparation of plant-based nanomedicines usually depends on trial and error testing, optimization by trial and error, and time-consuming analytical functions. The methods are resource-intensive and fail to effectively tackle the complexity of plant-based systems, in which several variables including extract content, nanoparticle constituents, process conditions and biological interactions are present[6, 7]. Artificial intelligence (AI) is a revolutionary solution as it allows predictive modeling, fast optimization, and decision-making with multiple parameters[8]. There are AI tools that can be used to analyze complicated data, find the most suitable formulation conditions, predict biological reactions and reduce the number of iterations in the experiment[9], e.g. machine learning, deep learning, chemometrics, QSAR, and predictive ADMET[10]. This intuition to data-driven transformation is an essential gap in the field and signifies the necessity of AI implementation in the sphere of phytomedicine development[11]. The main aim of the review is to discuss the role of artificial intelligence in improving the discovery, formulation, and pharmacological assessment of plant-based nanomedicines. The article gives the voters an in-depth account of the AI tools in phytochemical profiling, nanocarrier design, *in silico* pharmacology, predictive toxicology, and pharmacokinetic modeling. It also brings into the limelight recent developments, case studies, regulatory issues and the new opportunities in AI-enabled nano-phytopharmaceutical studies. This review will combine the information about pharmaceutics, pharmacognosy, and pharmacology to provide a single framework that depicts how AI can speed up the creation of safe, effective, and translatable to the clinic plant-derived nanomedicines.

**Phytochemistry of Medicinal Plants**

Phytochemistry is the scientific basis of plant-based medicines since it determines, characterizes, and quantifies the bioactive components of pharmacological effects[12]. Plants generate a wide range of secondary metabolites that lead to such therapeutic effects as antioxidant, anti-inflammatory, anticancer, antimicrobial, and neuroprotective effects[13]. The knowledge of these phytochemicals will be important to the development of standardized formulations and designing of more sophisticated drug delivery systems. In this section, key phytochemical classes, the difficulties encountered in the discovery of these classes, and the increasing importance of the artificial intelligence (AI) in the acceleration of phytochemical profiling are described[14].

**Overview of Major Phytochemical Classes**

**Alkaloids**

Alkaloids are organic compounds that contain nitrogen and are very strong in terms of their physiological action. More than 12,000 alkaloids have been discovered, which include morphine, quinine, berberine, vincristine and atropine. They usually have potent pharmacological effects of analgesic, anticancer, antimalarial, and antimicrobial effects because they can bind with neurotransmitter receptors, enzymes, and ion channels. Alkaloids are chemically heterogeneous and may have to be profiled by a complex analytical technique[15, 16].

**Flavonoids**

Flavonoids are polyphenolic structures which are widely present in fruits, vegetables, as well as in medicinal plants. These have subclasses like flavones, flavonols, flavanones and anthocyanidins[17]. Flavonoids including quercetin, kaempferol, naringenin, and other flavonoid derivatives have been known to be antioxidant, anti-inflammatory, cardioprotective and anticancer properties, which modulate oxidative stress and cellular signalling pathways[18].

**Terpenoids**

The terpenoids (isoprenoids) form the largest natural compound category of which over 40,000 structures are described. These include mono, diterpenes, triterpenes and carotenoids. The curcumin, artemisinin and ginsenosides are terpenoids that have various therapeutic effects that include: anti-malarial, anti-inflammatory, hepatoprotective, and anticancer. They are mostly lipophilic and, hence, require formulation strategies to improve bioavailability and solubility[19, 20].

**Phenolics**

Phenolic acids, tannins, lignans and stilbenes are considered phenolic compounds. They are well known due to their antioxidant ability and concerning the ability to regulate gene expression, inflammation and redox balance of cells. The compounds such as resveratrol, gallic acid and ellagic acids have shown some promising results in the treatment of chronic diseases but the stability and bioavailability of these compounds is a significant issue[21].

**Glycosides**

Glycosids consist of sugar backbone that is conjugated to non-sugary aglycone. The best examples include cardiac glycosids (e.g., digoxin), saponins and anthraquinone glycosids. These compounds are very bioactive but in most instances their effects may be very severe hence care should be taken when administering them. They are very unstable, and insoluble and this influences their therapeutic performance[22].

**Challenges in Phytochemical Discovery**

**Chemical Complexity**

Medicinal plants can have hundreds to thousands of structurally diversified phytoconstituents that occur in their different concentrations. Isomers, structural analogues and synergistic compounds make it difficult to be standardized and identified. Such complexity should be overcome using highly sensitive and selective analytical techniques, including LC-MS/MS and NMR[23].

**Seasonal Variation**

The phytochemical levels are highly variable with age and climate of the plant, soil type, harvest time and post-harvest time, and the means of extracting them. The result of this biological variability is uneven therapeutic gains and the impossibility of reproducibility of research and production[24].

**Extraction and Standardization Issues**

Various extraction solvents, varying temperatures and other methods selectively influence the phytochemical profile[25]. Traditional extraction mechanisms are capable of destroying fragile compounds[26]. This makes it hard to achieve the standardization of batch-to-batch especially of complicated combinations of herbs. It is also not easier to quantify and quality control due to the lack of validated biomarkers.

**Role of AI in Phytochemical Profiling**

**Machine Learning in Compound Prediction**

Machine learning (ML) systems can particularly process chemical, spectral, and biological data to predict the presence, abundance and activity of phytochemicals. The ML algorithms, especially the random forest, support vector machines and neural networks, identify the relationship between the structural features and biological activity that can be exploited to screen the possible leaders compounds rather fast[27].

**Chemometrics for Metabolomics**

Chemometrics is an amalgamation of multivariate analysis and statistical modelling used to deal with complex metabolomic data. Methods like PCA, PLS-DA and hierarchical clustering are used with the aim of classifying plant species, distinguishing extract profiles as well as biomarkers[28]. The use of AI-enhanced chemometrics allows the intensive interpretation of LC-MS, GC-MS, and NMR data.

**Deep Learning for Structure Elucidation**

There are models of deep learning, in particular, convolutional neural networks (CNNs) that can process spectral tendencies and predict chemical structure directly using raw data. They assist in automated labelling of the metabolites, deconvolution of overlapping peaks and in the construction of unknown structures. This significantly reduces time and error in manual process[29].

**AI-Assisted Dereplication in LC-MS/MS**

Dereplication is implemented to find known compounds in complex mixtures in a fast way to avoid the duplication of the similar work to isolate one common compound. The AI algorithms, match the mass spectrum to the mass spectrum libraries, clusterization of fragmentation patterns, and future molecular fingerprints. The dereplication automation is enhancing the throughput and increases the discovery of new phytoconstituents[30, 31].

**AI in Designing and Optimizing Nanocarriers for Plant Actives**

Nanocarriers that have been developed to deliver phytomedicine have revolutionized the field of phytomedicine, improving the solubility, stability, bioavailability, and targeted delivery of phytochemicals. Traditional formulation procedures are however usually labor intensive and are highly dependent on empirical experimentation. The combination of formulation science and artificial intelligence (AI) is the key to finding a solution to this problem as it provides the ability to conduct predictive modeling, multi-parameter optimization, and the use of data to make a decision. In this section, the author explains the shortcomings of the conventional methods, the main algorithms of AI involved in nanoformulation, and the use of AI in creating more optimized nanocarriers to promote plant-derived bioactives[32].

**Limitations of Conventional Formulation Methods**

**Trial-and-Error Approaches**

In the conventional formulation development, optimization of ingredients and processing conditions of the formulations is commonly intuitive step-wise adjustments. In this method no systematic search of the formulation space is done and typically there are a large number of batches and the method is thus highly material consuming, expensive and time consuming in terms of development. With the case of the plant bio actives when their characteristics are variable, trial and error remains inefficient even more[33].

**Time-Consuming DoE (Design of Experiments)**

DoE is suitable in structured experimentation although it is time consuming and computationally intensive with several variables of formulation. Traditional DoE models can be overwhelmed by high-dimensional systems, including nanoemulsions or polymeric nanoparticles, by their levels of surfactants, polymer ratios, sonication time and organic-aqueous phase ratios. Also, DoE cannot ensure that non-linear interactions between variables are captured and this can be limiting as far as predictive accuracy is concerned[34].

**Poor Prediction of Interactions**

Phytochemicals react with nanocarrier elements in complicated manners that are determined by the levels of polarity, molecular weight, charge, and hydrophobicity. The traditional models are not able to predict these interactions, which makes the formulations unstable, encapsulation unpredictable, and the reproducibility poor. These shortcomings are overcome by the AI-based techniques that extract non-linear and multidimensional relationships between inputs and outputs of the formulation process[35].

**AI Algorithms Used in Formulation**

**Artificial Neural Networks (ANN)**

ANNs are based on human neural networks to learn complicated patterns in formulation datasets. They are mostly useful in predicting particle size, polydispersibility index (PDI), encapsulation and release profiles. ANNs are able to deal with non-linear interactions and give high predictive accuracy on the basis of limited experimental information[36].

**Random Forest**

Random Forest is an ensemble algorithm, which forms a series of decision trees to forecast the formulation results. It can be resistant to noise and overfitting, which allows it to be used in the determination of influential formulation variables and their prioritization in terms of nanoformulation performance[37].

**Gradient Boosting**

Gradient boosting (e.g., XGBoost, LightGBM) algorithms are trained with updated predictive model every time in order to reduce error. They are outstanding in high dimensional data and prescriptive in encapsulations efficiencies, nanoparticles stability and optimum parameters of processes[38].

**Support Vector Machines (SVM)**

The classification and prediction of SVMs are accomplished by determining the optimal limits of multidimensional information. SVMs are used in nanoformulation to differentiate batches of formulation (e.g., optimal vs. suboptimal), predict stability behavior, multivariate sorting of quality attributes, which are significant[39].

**Bayesian Optimization**

Bayesian optimization is applied to quickly and efficiently optimize formulation parameters with a minimum of experiments. Using a probabilistic model, it can find the best formulation with less trials than DoE and is very efficient in multi-variable systems such as nanoemulsions and polymeric nanoparticles[40].

**AI Applications in Nanocarrier Development**

**Predicting Particle Size, PDI, and Zeta Potential**

The particle size and PDI are important in targeted delivery, cellular stability and uptake. AI models which predict the size distribution can be based on process parameters (e.g. sonication time, homogenization speed), surfactant concentrations, and ratios of polymers. Zeta potential forecasts help in determining the stability and aggregation of the long-term[41].

**Drug Loading and Entrapment Efficiency Prediction**

The AI algorithms are used to predict the interactions between phytoconstituents and nanocarriers using the following molecular parameters: hydrophobicity, Hansen solubility parameters, and partition coefficients. Such predictions are useful to optimize the encapsulation efficiency and decreasing drug leakage[42].

**Optimizing Surfactant/Polymer Ratios**

The concentration of the surfactant used and polymer has a significant effect on the nanoparticle formation, stability, and release kinetics. AI models are used to find the best ratios that improve particles uniformity, avoid aggregation, and optimize loading efficiency and reduce the toxicity of excipients[43].

**Stability Prediction**

Commercialization requires long-term stability. Under different conditions AI is predictive of aggregation behavior, phase separation, pH sensitivity and chemical degradation behavior. This saves the time required to conduct the long-term stability tests and shortens the refinements in the formulations[44].

**AI for Scale-Up**

A nanoformulation which is on a lab scale is hard to translate to an industrial scale production. The process parameters that AI models include to predict the outcome of scale-up are flow rate, mixing energy, pressure, and batch size. ML-assisted scale-up minimizes the variability and makes the quality uniform[45].

**Types of Nanocarriers Used for Plant Actives**

**Liposomes**

Liposomes are the best used vesicles to entrap both lipophilic and hydrophilic phytochemicals due to their phospholipid nature. They make it more stable, increase the pharmacokinetics and allow targeted delivery. AI models help to maximise lipid composition, hydration parameters and drug-to-lipid ratios[46].

**Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC)**

The stability of the plant lipophilic compounds is improved by SLNs and NLCs. AI forecasts the best lipid surfactants mixtures, melting and entrapment. Specifically, NLCs minimize the problem of expulsion of drugs among SLNs[47].

**Polymeric Nanoparticles**

Biological degradable polymers e.g. PLGA and chitosan are examples of such nanoparticles. These include high stability and controlled release. The polymerdrug interaction, molecular weight, and release kinetics will be predicted by using AI[48].

**Phytosomes**

Phytosomes are complexes between phytoconstituents and phospholipids which increase the permeability and bioavailability. The AI determines the most favorable ratios of complexation and foresees the physicochemical characteristics[49].

**Nanoemulsions**

Nanoemulsions increase the solubility and absorption of plant actives that are not soluble in water. In order to develop stable and uniform formulations, the AI models are used to optimize the oil phase, surfactant choice, emulsification period, and the distribution of droplet sizes[50]. AI Models Used in Different Nanocarrier Systems, Their Applications, and Key Outputs (Table 1).

**AI-Assisted Screening for Pharmacological Activity**

In vitro and *in vivo* experiments have long been considered the traditional methods of pharmacological assessment of plant-based nanomedicines, which are labor-intensive. These techniques are important procedures, but they have their limitations in time and inaccurate forecasting of multifaceted biological behavior. AI has become an innovative technology that enables prompt pharmacological screening at the first stages and can quickly predict biological activity, molecular interactions, and even possible toxicity. With AI-based *in silico* design methodologies, it is possible to determine potential promising phytochemicals, evaluate their affinity with the target proteins and predict their safety profiles without undergoing extensive laboratory work. In this section, the key AI-aided methods used in the pharmacological screening of plant-derived nanomedicines are revealed[51].

***In Silico* Pharmacology for Phytochemicals**

**Target Prediction (PASS, SwissTarget Prediction)**

Target prediction tools are based on the chemical structure information upon which the biological targets are most likely to interact with a molecule. PASS (Prediction of Activity Spectra for Substances) and SwissTarget Prediction use machine learning models which have been trained on large bioactivity databases. These are protein fingerprint and structure tools that are used to predict binding by enzyme, receptor regulation, transporter interactions, and participation in pathways. These predictions are useful as they aid in filtering phytochemicals to targets of interest in pharmacological studies[52].

**Molecular Docking**

Molecular docking A protein-phytochemical binding under molecular simulation. AI is capable of boosting the accuracy of docking using scoring functions and pattern recognition. Docking is used to determine the binding affinity, the orientation and key interacting residues of the possible pairs of compounds-target. In cases where the behavioral plant actives are multi-targeted, AI-based docking is more reliable, as it filters hundreds of targets[53].

**Molecular Dynamics (AI-Accelerated)**

The dynamics, stability and conformational change of phytochemical-protein complexes with time can be provided by molecular dynamics (MD) simulations. The AI is computationally efficient compared to the traditional MD, and it accelerates the simulations by predicting force fields, energy landscapes, and traverse of motion. Deep learning

models can model MD outputs that are both highly accurate, requiring days reduced to minutes. This helps in boosting the mechanistic understanding of how phytochemicals respond to biological targets[54].

**AI for Predicting Biological Activity**

**QSAR Modelling**

Quantitative Structure-Activity Relationship (QSAR) modeling is a method that is used to correlate the chemical structure of the phytoconstituents and their biological activity. Descriptors that are used in AI enhanced QSAR models include hydrophobicity, molecular weight, electronic distribution and topological indices to predict antioxidant, anti-inflammatory, anticancer or antimicrobial properties. Such machine learning models as SVM, Random Forest, or ANN yield correct predictions of even structurally diverse plant compounds[55].

**Deep Learning for Bioactivity Classification**

Phytochemicals can also be classified as active or inactive by Deep learning networks particularly Convolutional Neural Network (CNNs) and Long Short-Term Memory (LSTM) networks using spectral signatures, fingerprints, or SMILES structures. The models are trained to acquire very complex patterns and can be used in the identification of unknown or poorly studied compounds with therapeutic interest[56].

**Machine Learning for Multi-Target Interactions (Polypharmacology)**

Most of the plant compounds have a therapeutic effect by acting on a number of targets simultaneously. Polydrug-drug interaction map Machine learning models digest large datasets of protein-ligand interactions to map polypharmacological profiles. This is useful in predicting synergy, antagonism and effects at pathway level. ML-based network pharmacology combines the resources of omics, gene expression and protein-protein interactions to provide an overview of phytochemical action at a systems level[57, 58].

**Toxicology Prediction**

***In Silico* ADMET**

The pharmacokinetic behavior of nanoformulations and phytochemicals is tested in the form of AI-based ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) models. Some of the parameters that are predicted by the ML models in the context of oral absorption include blood-brain penetration, cytoctedrahene metabolism and renal clearance. Prediction of ADMET early will contribute to the elimination of the wrong candidates before the costly *in vivo* testing[59].

**Predictive Toxicity Models**

Toxicity prediction models that are based on extensive toxicological datasets determine the possibility of mutagenicity, hepatotoxicity, genotoxicity, and cytotoxicity. Random Forest, Gradient Boosting, and ANN algorithms assign compounds into the risk categories of toxicity, which reduces the use of animal testing[60].

**hERG Inhibition Predictions**

Cardiotoxicity is mostly caused by Human Ether-a-go-go-Related Gene (hERG) channel inhibition. The models of AI forecast the potential of hERG inhibition relying on molecular structure, electrostatic properties, and lipophilicity. Premature forecasting averses late failure and secures cardiovascular security of phytochemical-based nanomedicines[61].

**Plant-Based Nanomedicine Development—Integration of AI Across the Pipeline**

The development of plant-based nanomedicines comprises several steps that are interrelated but some main steps involve the choice of plant species and active phytoconstituents, nanocarrier design, prediction of pharmacokinetics and analysis of therapeutic efficacy of the nanocarriers. Traditionally each stage was very experimental and thus the process was resourceful and inconsistent. The artificial intelligence (AI) is an all-encompassing, data-driven solution that enhances the decision-making process and decreases experimental burdens and minimizes the time between lab

discovery and use in clinical practice. In this case, the manner, in which AI can be applied in each step of the nano-phytomedicine process is addressed[62].

**Selection of Plants and Actives Using AI-Based Ethnobotanical Data Mining**

**NLP Applied to Classical Medicinal Texts**

The ethnobotanical knowledge encompasses a variety of information on the usage and therapeutic indication of plants which are helpful in ancient medicine systems (Ayurveda, Siddha, Traditional Chinese Medicine (TCM) and folk literature)[63-65]. These writings are not structured, different in linguistic state, lack qualitative description and it is hard to understand them manually because of this. This has been automated using Natural Language Processing (NLP) that recognizes disease plant relations, therapeutic assertions, phytochemical facts as well as dosage practices. Artificial intelligence devices that identify plants based on their therapeutic value help researchers to identify potentially useful species to conduct further research[66].

**Network Pharmacology with Machine Learning**

The network pharmacology is the research on the phytochemicals, interactions of biological targets and disease pathways. Machine learning (ML) algorithms analyze the big databases (e.g., TCMSP, IMPPAT) to identify the most important phytoconstituents that initiate the activities, simulate interactions and visualize multi-target activities. The technique can be applied to rank high the plant species and active compounds that have the highest potential of therapeutic effects in their formulations as nanomedicine[67, 68].

**AI-Driven Nanoparticle Formulation for Phytoconstituents**

**Predicting Nano–Drug Interactions**

The concerns entailing the interactions between the phytoconstituents and the nanocarrier constituents (lipids, polymers, surfactants) can be anticipated with the assistance of AI models. ML algorithms define encapsulation efficiency, affinity and compatibility of bindings with regard to molecular descriptors of hydrophobicity, solubility parameters and functional group distribution. The projections lower the experiments and can be utilized in making a decision on the formulations within a short period of time[69].

**Improving Solubility and Stability**

The significant impediments to many plant actives are poor solubility and instability. AI is used to predict the most suitable type of nanocarrier (e.g. liposomes vs. polymeric nanoparticles), optimal excipients and processing conditions, which lead to greater solubility, degradation resistance and shelf-life. The deep learning models are able to examine degradation patterns during heat, PH, and light, making it possible to design nano formulations with a stability enhancement[9].

**AI in Optimizing Pharmacokinetics of Nano-Phytomedicines**

**PBPK Modelling**

Physiologically Based Pharmacokinetic (PBPK) models are physiological models which simulate drug absorption, distribution, metabolism, and excretion. PBPK systems enhanced with AI consider the molecular structure, nanoparticle size, charge and release kinetics to enable prediction of the *in vivo* behavior with a high level of accuracy. This enables predetermination of optimum dosing schedules of nano-phytomedicines[70].

**Prediction of Tissue Distribution**

AI algorithms guess the distribution of nanoformulations in organs depending on the properties of surfaces, ligand changes and the interaction with biological membranes[71]. ML models that are trained using biodistribution data sets can be used to predict accumulation in liver, spleen, brain or tumor tissues. These are predictions used to design nano-phytotherapeutics targeted[72].

**Enhancing Bioavailability via ML Models**

The machine learning models are applied to investigate the impact of formulation parameters on the solubility, permeability, and first-pass metabolism. The solutions to ML-based optimization include surface modification that improves bioavailability, mucoadhesion and stimuli-sensitive release. It significantly contributes to the improvement of ineffective absorption of phytochemicals in the process of treatment[73].

**AI-Supported Prediction of Clinical Efficacy**

**Virtual Patient Simulations**

Virtual patient models based on AI are used to simulate clinical outcomes by including variables related to patients, including genetics, age, disease, and comorbidities. Before the clinical trials, these simulations are useful in forecasting the potential response of various populations that may be experienced by the introduction of nano-phytomedicines and the identification of the most effective dosage regimens. This saves a big deal in terms of cost and time of completion of early clinical development[74].

**Drug–Disease Matching Algorithms**

Disease ontology databases and machine learning are needed to identify the best therapeutic applications of a certain nanoformulation via drug-disease matching algorithms. The molecular events and disease signatures their analysis of demonstrate the probability of an effective phytochemical-loaded nanocarrier in diseases such as cancer, inflammation, neurodegeneration or metabolic disease. This results in repurposing and complies with clinical utility[75].

**Advanced Characterization of Plant-Based Nanomedicines**

The proper characterization of nanomedicines made out of plants is vital in assuring their safety and stability, efficacy, and regulatory compliance. The most common methods of analysis used in traditional characterization include TEM, SEM, FTIR, NMR, Raman spectroscopy, DLS, and DSC that produce the huge and complicated data. These datasets may be tedious to interpret manually, subjective and subject to error- particularly when dealing with heterogeneous nanocarrier systems loaded with structurally diverse phytochemicals[76]. Artificial intelligence (AI) is an effective way out since it offers data analysis automation, sensitivity, and the ability to detect subtle trends that could be overlooked by traditional means. ML and deep learning algorithms have the capability to scale spectral data in seconds, categorize nanoparticle morphologies, foretell stability performance, and associate physicochemical specifications with *in vivo* operation[77]. Characterization with the aid of AI leads to reproducibility and minimizes quality-by-design (QbD) uses and enables scientists to verify key quality attributes (CQAs), such as particle size, PDI, zeta potential, loading efficacy and release kinetics[78]. Real-time interpretation of data can be done when AIs are used in combination with analytical instrumentation and they can be used further to predictive modelling to further refine the formulation scale-up and evaluate regulationas shown in Table 2. AI Tools for Characterization of Nanocarriers (Technique vs. AI Model vs. Output) (Table 2).

**Pharmacological Evaluation of Plant-Based Nanomedicines**

The pharmacological study is an important phase in the production of plant nanomedicines since it establishes its curative capacity, security, and action mechanism. Nanocarriers have the potential of improving the pharmacological characteristics of phytochemicals through stability, absorption, distribution, and targeting of cells. In the meantime, the artificial intelligence (AI) can be used to speed up the process of pharmacological evaluation by providing predictive information, pattern recognition, and mechanistic modeling as complementary to experimental measurements. The combination of nanotechnology and AI offers a mighty paradigm of how plant-based formulations are acting in the biological system[79].

**Enhancing Pharmacodynamics Using Nanocarriers**

**Improved Cell Uptake**

Nanocarriers enhance the internalization of phytochemicals to the cells significantly. They get in their cells by endocytosis, micropinocytosis and receptor-mediated entry via their small size (nanoparticle). Selective attachment to

cellular receptors is achieved by surface modification, including PEGylation, ligand conjugation and optimization of charge. Therefore, in most cases, nanoformulations have a higher intracellular absorption compared to free phytochemicals and can be used to increase their therapeutic efficacy[80].

**Controlled Release**

The nanocarriers also allow them to deliver the plant actives at a slower and controlled rate, which overcomes the fast metabolism and degradation. Time controlled release can be achieved using SLNs, nanoparticles composed of polymer and also using NLCs, but to achieve localized release at the site of the disease, pH-controlled and enzyme-controlled nanocarriers can be used. The benefits of controlled release are maximization of therapeutic exposure, decrease of dosing frequency and decrease of systemic toxicity[81].

**Target Specificity**

Functionalised nanocarriers are more specific in that they can target specific tissues or cells- tumour cells using folate-coated nanoparticles or brain using transferrin-modifications. This is a focused treatment that minimizes side effects and improves pharmacodynamics[82].

***In vitro* Mechanisms with Help of AI**

**Predictive Models for Apoptosis Pathways**

Activation of apoptosis pathways which are triggered by nano-phytomedicines can be predicted using AI models trained on omics and cytotoxicity datasets. The machine learning algorithms find important molecular signatures, including caspase activation, loss of mitochondrial membrane potential, and fragmentation of DNA on the basis of structural and physicochemical properties of nanoformulations. This speeds up mechanism-of-action research and lowers the level of experimentation[83].

**Anti-Inflammatory Networks**

Network pharmacology AI-based combines all three types of data (gene expression, cytokine profiles and signaling cascades) to model anti-inflammatory activity. Such models are used to predict pathway modulation including NF-kB, COX-2, TNF-a, and IL-6, which are useful in identifying multitarget effects of plant-derived nanoencapsulated phytochemicals such as curcumin, quercetin or boswellic acids[84].

**Antioxidant and Anti-Cancer Pathways**

Deep learning models provide prediction of antioxidant capacity across cellular oxidative stress markers. Likewise, ML tools represent interaction with cancer-related pathways that include PI3K/Akt, MAPK, JAK/STAT, and p53. The predictions are used to plan experiments and to increase the knowledge of the pharmacological effects of nanoformulated phytochemicals[85].

***In vivo* Evaluations and AI-Enabled Data Interpretation**

**ML for Dose–Response Predictions**

Dose-response relationships are predicted by machine learning algorithms such as regression algorithms, random forest, and neural networks that use both *in vitro* data, formulation parameters, and physicochemical properties. This will cut down the necessary amount of animal experiments and allow more specific dose selection in *in vivo* studies[86].

**Predictive Toxicity Curves**

The AI models, that are applied to produce predictive toxicity curves, are constructed on the premise of the integration of data on biochemical markers, hematological, organ histology, and behavioral observations. These models are used to predict the safe dose ranges including the no-observed-adverse-effect-level (NOAEL) and the first organ-specific toxicity. These forecasts come in handy during predictions and risk control[87].

**Behavior Prediction in Disease Models**

In complicated disease models, e.g. cancer, diabetes, or neurodegenerative diseases, AI can be used to analyze behavioral and physiological data to make predictions about the result of a therapeutic intervention. As an example, ML models predict movement in models of Parkinsonism or tumor growth dynamics in cancer models, which assists in accelerating the interpretation of *in vivo* pharmacology[88].

**AI for Personalized Phytomedicine**

**Genomic Integration**

AI facilitates the combination of genomic, proteomic, and metabolomic data with predictive potential of individual reactions to plant-based nanomedicines. Pharmacogenomic AI systems assist in determining genetic variants which affect drug metabolism, immune response or therapeutic sensitivity. This method favors the creation of personalized nano-phytomedicines that are patient-specifically designed in terms of molecular profiles[89, 90].

**Patient-Specific Pharmacology Using ML Models**

The ML algorithms are based on the age of the patients, their biomarkers, the disease stage, lifestyle, and comorbidities to recreate the personal therapeutic outcomes. These include efficacy, toxicity and optimal dosing models of nanoencapsulated phytochemicals that are predictive in various groups of patients. Clinical meaning is advanced with the use of artificial intelligence (AI)-based personalization that promotes precision herbal nanomedicine[91].

**Regulatory Considerations for AI-Designed Nano-Phytomedicines**

The integration of artificial intelligence (AI) with nanotechnology and therapeutic plants creates significant opportunities of quick and data-driven drug discovery. However, it has special regulation issues. As opposed to traditional medicine, nano-phytomedicines incorporate three complicated domains, herbal products, nanocarrier systems, and AI-based computational design, and each has a distinct regulatory system. The variability problem is typical of the herbal medicines due to variability of the source plant, extraction method, and heterogeneity of phytochemicals. In their turn, nanocarriers should be assessed with regard to particle size, stability, surface chemistry, as well as biodistribution. Regulatory agencies must also evaluate the credibility, disclosure, and certification of computational models in situations when AI is involved in formulation prediction, optimization and pharmacological screening. U.S. FDA, European Medicines Agency (EMA), ICH, AYUSH and other national regulatory bodies are beginning to appreciate the rate that AI is being applied in drug development but demand the use of model validation, risk management, reproducibility and explainability. The AI predictions must be supported by experimental results and developers ought to ensure that the algorithms are trained on good, unbiased results. Moreover, the nano formulations that are designed using AI are a challenge to the conventional quality-by-design (QbD) models. A regulator is not only to inspect the product produced but also the digital pipeline, which was brought by the production of the product[92]. The issues of data integrity, the bias of the algorithm and the model drift, and reproducibility should be taken into consideration as shown in Table 3. Regulatory Considerations for AI-Designed Nano-Phytomedicines (Table 3).

**Challenges and Future Opportunities**

Plant-based nanomedicine, which is developed based on artificial intelligence (AI), can bring revolutionary opportunities to the sphere; however, it also introduces a lot of scientific, technical, regulatory, and ethical challenges. Plant-based therapeutics are self-convoluted due to variation in phytochemical composition, lack of standardization in extraction procedures and lack of standardization. The development pipeline can be multidimensional together with nanocarrier systems and AI-based modelling, and physical formulations as well as computational predictions should be properly validated. The quality and completeness of datasets used to develop AI models determine the success of the latter. The datasets of phytomedicine can be systematic, unstructured, or un- annotated, and thus, it is difficult to develop powerful machine learning solutions. Also, deep learning models are not even transparent because they are black-box, making them not easy to reproduce and accept by regulators. The impartiality, reduction of the biased algorithms, the integrity of the data are important elements during the transformation of the AI-based

predictions into clinical outcomes. However, the future is massive regardless of these obstacles. AI can accelerate the discovery of new phytoconstituents, automated nanoformulation, better pharmacokinetic modeling, and personalised phytomedicine depending on the personal genomic and phenotypic characteristics of the patients. More integration and types of multi-omics, digital twins, federated learning, and explainable AI (XAI) will become increasingly popular in the future most likely making nano-phytomedicines more specific, less toxic, and more complex[11]. A systematic overview of the available opportunities and challenges is carried out as shown in Table 4. Challenges and Future Opportunities in AI-Enabled Nano-Phytomedicine Development (Table 4).

## CONCLUSION

Artificial intelligence has led to the revolution of the production line of plant-based nanomedicines. It is possible to determine bioactive compounds using AI and in minutes by enhancing phytochemical profiling of plants and provides a way through the complexity and variability inherent in medicinal plants. Predictive modeling can be used to formulate, optimize critical quality attributes, and scale and stabilize nano-phytomedicines faster and more easily in nanotechnology using AI-based models. In pharmacology, AI improves mechanistic understanding, predicts therapeutic and toxicology and can help provide a personalized approach to treatment by combining genomics and patient-specific information. All these developments lead to a reduction in the time of development, reproducibility and likelihood of successful clinical translation. The alliance of AI and natural products with nanomedicine in the future has a massive prospect with regard to the discovery of novel treatment methods, precision herbal medicine and the next-generation drug delivery systems. Additional improvements in the quality of data, openness of the models and regulatory policies will be required to reach the full potential of AI-based nano-phytomedicine.

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**Table 1: AI Models Used in Different Nanocarrier Systems, Their Applications, and Key Outputs**

| Nanocarrier System | AI Model Used | Applications in Formulation | Key Outputs / Predictions |
|---|---|---|---|
| Liposomes | Artificial Neural Networks (ANN) | Optimization of lipid composition, hydration conditions, sonication time | Particle size, PDI, zeta potential, entrapment efficiency, vesicle stability |
| | Random Forest | Identifying key variables affecting liposome formation | Ranking variable importance, predicting optimal formulation parameters |
| Solid Lipid Nanoparticles (SLN) | Support Vector Machines (SVM) | Predicting stability and crystallinity | Long-term stability, drug release behavior, aggregation tendency |
| Nanostructured Lipid Carriers (NLC) | Gradient Boosting (XGBoost, LightGBM) | Optimizing solid–liquid lipid ratios, surfactant levels | Enhanced entrapment efficiency, reduced drug expulsion, improved physical stability |
| Polymeric Nanoparticles (PLGA, chitosan, etc.) | ANN | Predicting polymer–drug interactions, molecular weight effects | Release kinetics, drug loading, nanoparticle morphology |
| | Bayesian Optimization | Rapid optimization of multi-parameter formulation | Minimal experiments required, optimal polymer/surfactant concentrations |
| Phytosomes | Random Forest | Identifying critical phospholipid–drug complexation parameters | Best complexation ratio, improved solubility and permeability |
| Nanoemulsions | ANN | Optimizing oil phase, surfactant/cosurfactant ratios, homogenization cycles | Droplet size, stability index, phase separation prediction |
| | Gradient Boosting | Modeling non-linear effects of emulsification parameters | Fine-tuned droplet distribution, improved long-term stability |
| Hybrid Nanocarriers (polymer–lipid / lipid–metal) | Deep Learning models (CNN, LSTM) | Processing large datasets from characterization (TEM images, spectral data) | Morphology prediction, structural integrity, surface architecture |
| General Nanocarrier Systems | Multi-objective Optimization Algorithms | Balancing multiple formulation outcomes simultaneously | Optimal trade-off between size, stability, loading efficiency, release rate |

**Table 2: AI Tools for Characterization of Nanocarriers (Technique vs. AI Model vs. Output)**

| Analytical Technique | AI/ML Model Used | Characterization Output Enabled by AI |
|---|---|---|
| TEM / SEM Imaging | Convolutional Neural Networks (CNNs) | Morphology analysis, shape classification, nanoparticle size distribution, detection of aggregation |
| Dynamic Light Scattering (DLS) | Random Forest, ANN | Prediction of particle size, PDI, stability trends, batch-to-batch consistency |
| FTIR Spectroscopy | PCA, PLS-DA, SVM | Functional group identification, drug–excipient interaction analysis, purity assessment |
| Raman Spectroscopy | Deep Learning (1D-CNN), Chemometric ML | Molecular fingerprinting, phase analysis, component mapping in nanoformulations |
| NMR Spectroscopy | ANN, Autoencoders | Structural elucidation, impurity detection, conformational analysis |
| XRD (X-Ray | Gradient Boosting, SVM | Crystallinity profiling, polymorph identification, |

| | | |
|---|---|---|
| Diffraction) | | nanoparticle phase stability |
| DSC / TGA | Machine Learning Regression Models | Thermal stability prediction, glass transition and melting point analysis, degradation onset |
| Zeta Potential Analysis | ANN, Linear Regression Models | Prediction of surface charge, colloidal stability, aggregation risk |
| UV–Visible Spectroscopy | PCA, k-Means Clustering | Drug loading confirmation, degradation pattern detection, release kinetics interpretation |

**Table 3: Regulatory Considerations for AI-Designed Nano-Phytomedicines**

| Regulatory Dimension | Key Requirements | Relevance to nano-phytomedicines | Challenges / Considerations |
|---|---|---|---|
| Quality of Plant Material | Standardized extraction, validated biomarkers, batch consistency | Ensures reproducible phytochemical profile | Variability due to climate, soil, harvest season |
| Nanocarrier Evaluation | Particle size, PDI, zeta potential, release kinetics, sterility | Determines stability, safety, biodistribution | Lack of harmonized global standards for nanomedicine |
| AI Model Validation | Transparency, explainability, performance metrics (RMSE, $R^2$), dataset quality | Validates AI-driven formulation/design | Black-box ML models may lack interpretability |
| Safety Assessment | In vitro and *in vivo* toxicity, immunogenicity, ADMET | Required for nano-loaded phytochemicals | Nano–bio interactions difficult to predict |
| Efficacy Evaluation | Mechanistic data, PK/PD models, clinical endpoints | Confirms AI-predicted pharmacology | Integration of *in silico* + *in vivo* data still evolving |
| Data Integrity and Traceability | Documentation of datasets, model training logs, version control | Ensures reproducible AI predictions | Risk of biased or incomplete datasets |
| Regulatory Documentation | CMC (Chemistry, Manufacturing, Controls), digital workflow reports | Required for approval submissions | New formats needed to include AI-derived data |
| Ethical and Legal Compliance | Patient data protection, algorithmic fairness | Important for AI-guided personalized phytomedicine | GDPR, HIPAA compliance complexities |

**Table 4: Challenges and Future Opportunities in AI-Enabled Nano-Phytomedicine Development**

| Category | Current Challenges | Future Opportunities |
|---|---|---|
| Phytochemical Variability | Inconsistent plant composition; lack of standardized biomarkers | AI-driven phytochemical fingerprinting; real-time quality monitoring |
| Data Limitations | Fragmented or small datasets for training; incomplete metadata | Creation of large curated phytomedicine datasets; federated learning systems |
| AI Model Limitations | Black-box models; low interpretability; overfitting risks | Explainable AI (XAI), transparent algorithms, improved model validation |

| | | |
|---|---|---|
| Nanocarrier Complexity | Difficult prediction of drug–excipient interactions and stability | ML models for precise nano–drug compatibility and stability forecasting |
| Pharmacokinetic Uncertainty | Limited *in vivo* predictability; complex nano–bio interactions | AI-enhanced PBPK models; dynamic prediction of tissue distribution |
| Safety and Toxicity | Difficulty predicting long-term nano–herbal toxicity | AI-driven toxicity simulators; virtual toxicity curves; reduced animal studies |
| Regulatory Barriers | Lack of harmonized guidelines for AI + herbal + nano combination | Development of digital CMC frameworks; AI validation protocols |
| Personalized Medicine Gaps | Limited patient-specific data; high biological variability | AI-based patient clustering; genomic integration; precision phytomedicine |

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RESEARCH ARTICLE

# An Elucidating Visual and Entropy Based Explainable Techniques for Malware Analysis

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## ABSTRACT

The task of quickly identifying and categorizing malware strains for efficient defense is becoming more and more difficult for security researchers. In order to address these gaps, this study thoroughly replicates four CNN architectures namely Xception, EfficientNetB0, ResNet50 and VGG16, Class Activation Maps (CAMs) such as GradCAM and NICE are used to assess both explainability and performance. The activation heatmaps produced for the five malware classes using four convolutional neural network architectures are analysed. These variations in activation patterns emphasize the significance of interpretability tools in deep learning evaluation and aid in explaining the disparity in classification performance among the models. Also, this study conducted a comparative static analysis of five malware families namely Amonetize, Dialplatform B, Fakerean, Neoreklami and Snarasite, using grayscale image visualization, row-wise entropy measurement, and hexadecimal inspection. Comparative Summary of Entropy Characteristics and Visual Traits of five Malware Families are tabulated. The comparative analysis demonstrates that all five malware families employ similar obfuscation strategies characterized by high-entropy content, noise-like visual representations, and PNG file format impersonation. These findings underscore the limitations of traditional signature-based detection methods and emphasize the effectiveness of image-based and entropy-driven static analysis techniques for identifying and differentiating modern obfuscated malware families.

**Keywords:** Explainable, CNN, Grad-CAM, NICE, Malware

## INTRODUCTION

Malware can be represented as images for a number of reasons. Foremost, image based models may discover structural similarities across distinct virus instances. This conversion permits the usage of well-established convolutional neural network (CNN) architectures to extract hierarchical features from visual data. Also, it gives cybersecurity professionals an easy-to-use visual medium to examine the image-like artifacts and gain a deeper understanding of the behavior of malware families. Visualization is an established technique that makes use of CNNs. Researchers may build an image with the data collected from a malicious file, and use it as a sample for the CNN [1]. This method makes use of the inherent characteristics of convolutional neural network models, including robust feature extraction, spatial invariance, adaptation, and effective processing. CNN based deep neural models have made it possible to achieve previously unheard-of improvements in a range of computer vision applications, including object identification, picture classification, semantic segmentation, and image captioning [2]. The inability of traditional black-box models to offer interpretable explanations for their predictions restricts their use in security-critical settings where knowing the logic behind a detection is crucial for threat response and mitigation. By improving model interpretability while preserving robust detection capabilities, Explainable AI (XAI) overcomes this gap [3]. Applications of sophisticated AI models are deeply ingrained in many facets of our daily lives. The requirement for openness, accountability, and trustworthiness is further heightened by their complexity, particularly when these intricate models make automatic judgments that have varying effects on our lives [4]. Gradient-weighted Class Activation Mapping (Grad-CAM) creates a coarse localization map that highlights the key areas of the image for concept prediction by using the gradients of any target concept flowing into the final convolutional layer [5]. Class Activation Mapping (CAM) is the most pertinent method for localization. This approach alters image classification CNN designs substituting fully-connected layers with convolutional layers and global average pooling, thus achieving class-specific feature maps [6]. A hybrid data augmentation strategy that combines traditional image processing techniques with the Deep Convolutional Generative Adversarial Network was proposed by Rahman *et al*. [7]. The suggested augmentation strategy was utilized to train the classifier on the Malimg dataset. Additionally, they evaluated the efficacy of CNN versus transformer architectures on malware pictures by comparing CNN and EANet models with LIME explainability, where the CNN model achieved greater accurate results on Malimg dataset. In addition to offering specific justifications for CNN's actions, the methodical comparison of the investigation [8] reveals clear patterns in malware family identification. Additionally, by improving Visual Transformers, these interpretability insights can achieve greater improvements across datasets as compared to benchmark values [9], [10], [11]. In summarized form, this research applies Grad-CAM to hybrid visual malware images in a structured way, allowing analysts to observe understand the internal decision-making process. It also conduct extensive experiments on three benchmark malware image datasets (Malimg, MaleVis and Fusion Malware datasets), demonstrating enhanced classification performance and model reliability. Additionally, it demonstrates the image capacity for generalization and usefulness in a variety of threat environments. This work offers a workable method for practical implementation by bridging the gap between deep learning models and the interpretability required for cybersecurity.

## BACKGROUND

The key concepts that underpin this research are discussed. Highlighted techniques used to visualize malware and concentrate on popular deep learning models. Additionally, explainable methods for studying CNNs in malware detection are described. Framework of a systematic pipeline analysis (Figure: 1). The framework are shown in Figure 1 illustrates a systematic pipeline for malware image classification and explainable analysis. Initially, malware datasets corresponding to Amonetize, Dialplatform.B, Fakerean, Neoreklami, and Snarasite are collected and converted into image representations. The dataset is divided into training, validation, and test images, and iterative fine-tuning is performed to optimize model performance [12], [18], [14]. In section 3, multiple convolutional neural network architectures namely Xception, EfficientNetB0, ResNet50, and VGG16 are employed for feature extraction and classification. Each architecture is independently trained and evaluated to analyze comparative performance

across different network designs. To enhance model interpretability, Class Activation Map (CAM) techniques, namely Grad-CAM and LIME, are applied to the trained models. These techniques generate heatmaps that highlight salient regions in malware images influencing classification decisions. In section 4, entropy characteristics are computed to quantify randomness and structural complexity within malware images. Additionally, visual trait analysis is conducted to identify distinctive texture, intensity, and spatial patterns associated with each malware family. Finally, the outputs from classification performance, CAM-based explanations, entropy profiling, and visual trait analysis are integrated to produce comprehensive output results, enabling both accurate malware detection and transparent understanding of model behavior.

**Dataset**

The prevalent dataset utilized in this research work namely MalImg, MaleVis and Fusion Malware datasets are shown in Figure 2 and also described in Table 1. MaleVis Dataset contains Amonetize samples with approximately 350 sample images used to detect malware via visual analysis. Dialplatform.B belongs to the Malimg dataset with approximately 152 sample images. Fakerean is one of the 25 malware families in the Malimg dataset with approximately 306 images which is frequently used for convolutional neural network training. Neoreklami is a prominent family of adware with approximately 350 sample images frequently featured in malware visualization and classification datasets, such as the Fusion Malware Dataset on Kaggle. Snarasite is a malware family included in the dataset with approximately 350 sample images. Dataset representing Malware Families (Figure.2). Malware Family Dataset (Table 1).

**Visualization of Malware Families**

Malware samples from various families are shown aesthetically in the Figure 3, where each binary file is converted into a two-dimensional image by mapping its byte values to pixel intensities. The use of image-based deep learning models for malware classification is made possible by this visualization technique. The texture of the malware class Amonetize is extremely rich and noise-like, suggesting that obfuscation or encryption techniques are widely used. Polymorphic behavior, which is frequently seen in adware and downloader-type malware, is suggested by the lack of distinct structural patterns. The Dialplatform B sample shows clearly defined intensity changes in vertically split sections. These patterns represent a more ordered binary layout and correspond to structured elements of the executable, such as headers, configuration data, and embedded payloads. The image of the Fakerean virus exhibits horizontal banding and repeating patterns. This regularity is characteristic of rogue security software, where similar code blocks and resource files are repeatedly embedded and producing consistent visual patterns. Visualization of sample Malware Families (Figure.3). A comparatively straightforward and repetitive coding structure is suggested by the Neoreklami sample's general uniformity and lack of contrast. Adware families that rely on recurrent advertisement-related routines with little obfuscation typically exhibit these traits. The existence of injected components and network-related functionality is indicated by the prominent horizontal dark bands and diverse textures shown in the Snarasite malware image. The many malware operational modules are represented by these visual anomalies. Overall, the visual distinctions amongst malware families show that discriminative structural information is preserved throughout binary-to-image transformation. Convolutional neural networks may efficiently take use of these unique spatial patterns to learn family-specific characteristics, enhancing malware classification ability.

**Grad-CAM for Malware Detection**

Convolutional neural networks (CNNs) in malware detection systems utilize a visualization approach called Grad-CAM (Gradient-weighted Class Activation Mapping) to explain their predictions. It draws attention to the elements of the input that have the biggest impact on the model's choice. CNNs are frequently used in malware analysis to process features like binary visuals, opcode sequences, byte plots, or traffic matrices that are represented as images or feature maps. Grad-CAM generates a heatmap by evaluating the gradients of the predicted class of malware or benign with respect to the final convolutional layer. The regions or patterns in the malware representation that contributed most to the categorization are shown in the ensuing heatmap. For instance, suspicious opcode sequences, unusual byte distributions, or malevolent behavioral patterns may be associated with high-activation regions. Grad-

CAM improves trust and interpretability in deep learning-based malware detection systems by enabling analysts to visually confirm whether the CNN concentrates on significant malware traits.

**LIME for Malware Detection**

An explainable AI method for deciphering the predictions of intricate malware detection models is called LIME (Local Interpretable Model-agnostic Explanations). LIME assists in comprehending why a certain file is categorized as benign or hazardous since contemporary machine learning and deep learning models sometimes operate as "black boxes." LIME operates by creating distorted replicas of a certain malware sample and tracking the classifier's predictions. The behavior of the original model is then approximated by training a basic interpretable model locally around the chosen instance. The most important characteristics influencing the categorization choice are found by this local model. LIME emphasizes key elements in malware analysis, including permissions, opcode patterns, API calls, and behavioral signs that affect the prediction. This improves transparency, helps security analysts validate model decisions, and supports effective malware investigation.

**Experiments**

In the following section, we assess the way this strategy can improve the performance of this architectural paradigm in conjunction with the LIME technique by using masks produced from heatmaps.

**Grad-CAM and LIME analysis of Amonetize malware**

The activation heatmaps produced for the Amonetize class using four convolutional neural network architectures Xception, EfficientNetB0, ResNet50, and VGG16 are shown in Figure 4. By highlighting the spatial areas of the input image that most strongly influence the model's prediction, these heatmaps shed light on the feature-learning behavior and decision-making process of the models. The middle area of the feature map indicates a robust and well-localized activation for the Xception model. This suggests that the most discriminative characteristics pertinent to the Amonetize class are successfully captured by the model. Strong spatial feature extraction and excellent localization capabilities are suggested by the concentrated attention pattern, which enhances classification performance. EfficientNetB0 has comparatively low activation in the majority of regions, with a significant concentration of high activation in a small location close to the feature map's bottom-right corner. This pattern implies that rather than a comprehensive representation of the input, the model mostly depends on a small number of spatial features. Increased sensitivity to particular visual patterns and less generalization may result from such concentrated attention. Stronger responses are primarily seen along the lower part of the feature map in the ResNet50 activation map. The attention is still region-biased even if the activations are more dispersed than in EfficientNetB0. Despite having less balanced spatial coverage than Xception, this suggests successful feature learning. More dispersed activations that are dispersed throughout the feature map's horizontal bands are produced by VGG16. Weaker spatial discrimination is reflected in the lack of strongly defined high-activation patches. This behavior is in line with VGG16's older and somewhat shallow design, which lacks the sophisticated feature refining processes seen in more modern models. Overall, the heatmap study shows that Xception offers the Amonetize class the most targeted and semantically significant attention, suggesting better feature localization and interpretability.

While EfficientNetB0 and VGG16 exhibit low spatial awareness and scattered attention, respectively, ResNet50 exhibits strong but region-biased activations. These variations in activation patterns emphasize the significance of interpretability tools in deep learning evaluation and aid in explaining the disparity in classification performance among the models. Elucidation of Model Activation Heatmaps for the *Amonetize* malware class using Grad-CAM (Figure.4). The Amonetize malware class's LIME (Local Interpretable Model-agnostic Explanations) visualizations, which were generated using four deep learning architectures EfficientNetB0, ResNet50, VGG16, and Xception are shown in Figure 5. By highlighting important superpixels, LIME identifies the areas of the image that have the biggest impact on the model's prediction. There are weak and sparse activations in the EfficientNetB0 model, and the classification decision is not influenced by any distinctly localized regions. This implies that discriminative characteristics pertinent to the Amonetize class are difficult for the model to capture. ResNet50, on the other hand, relies on many low-level features rather than particular class-defining patterns, as evidenced by the dense and

widely dispersed activations it produces throughout the whole image. In a similar vein, VGG16 has extensive noisy activations with slight focus close to the image's lower areas. On the other hand, insufficient feature localization and limited interpretability are suggested by the absence of structured and localized highlights. Xception has the most organized and significant LIME explanations among the assessed models. Xception successfully concentrates on prominent characteristics linked to the Amonetize malware class, as evidenced by the more localized highlighted portions, especially in the lower and selective upper regions of the image. In comparison to EfficientNetB0, ResNet50, and VGG16, the LIME research shows that Xception offers better interpretability and more dependable feature learning. These results suggest that explainable malware image classification tasks involving the Amonetize family are better suited for Xception. Elucidation of Explainable AI technique of LIME for *Amonetize* malware class (Figure.5).

**Grad-CAM and LIME analysis of Dialplatform.B malware**

The activation heatmaps produced by four deep learning architectures EfficientNetB0, ResNet50, VGG16, and Xception when used on Dialplatform samples are compared in the family of malware are shown in Figure 6. The geographical distribution of feature activations across internal convolutional layers is depicted in each heatmap, with color intensity denoting the relative strength or relevance of learned features (brighter colors imply higher activation, darker colors suggest lower activation). Selective feature learning with comparatively balanced emphasis across geographic areas is indicated by EfficientNetB0's modestly distributed activations with concentrated high-response zones. Stronger and more concentrated activations in particular areas have been detected by ResNet50, indicating deeper semantic representation of malware patterns and efficient hierarchical feature extraction. Because of its shorter design and somewhat less discriminative feature emphasis, VGG16 exhibits more scattered and lower-intensity activations. Xception's capacity to effectively capture spatial correlations using depthwise separable convolutions is proven by its smooth and centrally concentrated activation patterns. Overall, the visualization emphasizes discriminative malware traits and draws attention to architectural variations in each model's learning process. Compared to more conventional models like VGG16, deeper and more sophisticated architectures (ResNet50 and Xception) typically yield more prominent and structured activation regions, indicating higher feature representation abilities for malware classification tasks. Elucidation of Model Activation Heatmaps for the *Dialplatform.B* malware class using Grad-CAM (Figure.6). LIME (Local Interpretable Model-agnostic Explanations) visualizations for the Dialplatform are presented in the figure.

EfficientNetB0, ResNet50, VGG16, and Xception are the four deep learning models that comprise the malware family are shown in Figure 7. The local areas (superpixels) that have the biggest influence on the model's categorization choice are highlighted by LIME. Areas with a greater favorable influence on predicting the sample as Dialplatform are shown by the highlighted regions (depicted by yellow or red outlines).B, whereas darker areas show a smaller or insignificant contribution. Sparse but distinct highlighted regions are displayed by EfficientNetB0 (LIME), primarily centered in some lower and mid-level locations. This indicates the model concentrates on restricted structural characteristics inside the malware representation and depends on a small collection of discriminative patterns. ResNet50 (LIME) shows highlighted areas in the image that are denser and more widely distributed. This implies that ResNet50 uses several correlated regions to support its classification choice, capturing richer and more dispersed feature interactions. Due to its relatively thin design and absence of sophisticated feature reuse techniques, VGG16 (LIME) exhibits fragmented and noisy importance and less accurate feature attribution. With less noise, Xception (LIME) shows highlighted areas that are more cohesive and clear. Stronger interpretability and efficient feature separation made possible by depthwise separable convolutions are demonstrated by the explanations' increased structure and localization. All things considered, the LIME visualizations show distinct variations in the designs' feature attribution behavior. While VGG16 generates noisier and less focused interpretations, more sophisticated models like ResNet50 and Xception offer more consistent and semantically relevant explanations. These results highlight the importance of model architecture in achieving both accurate malware detection and reliable interpretability. Elucidation of Explainable AI technique of LIME for *Dialplatform.B* malware class (Figure.7).

**Grad-CAM and LIME analysis of Fakerean malware**

For samples from the Fakerean malware family, the graphic displays activation heatmaps generated by four convolutional neural network architectures: EfficientNetB0, ResNet50, VGG16, and Xception are shown in Figure 8. The heatmaps show the geographic intensity of learnt feature activations; places that contribute more strongly to the model's internal representation of the malware are indicated by brighter regions, whereas darker parts correlate to low activation. The majority of EficientNetB0's regions show minimal activation, with a few isolated high-response spots close to the lower layers. This implies selective feature extraction, in which Fakerean classification considers only a small sample of patterns to be significant. Stronger and more uniformly distributed activations are shown by ResNet50, especially in the bottom and center regions. This suggests that the Fakerean virus has strong hierarchical feature learning and the capacity to identify several distinctive traits. VGG16 exhibits moderately intense activation patterns that are dispersed over larger areas. In comparison to deeper structures, the absence of sharply concentrated activations indicates less accurate feature localization. Effective spatial feature modeling achieved using depthwise separable convolutions is highlighted by Xception, which displays smooth and well-defined activation regions focused toward the center of the heatmap. Overall, the comparison shows that EfficientNetB0 and VGG16 provide weaker or more scattered activations for the Fakerean malware family, but ResNet50 and Xception produce more structured and discriminative activation patterns. These differences underscore the impact of architectural design on feature representation quality in malware classification tasks. Elucidation of Model Activation Heatmaps for the *Fakerean* malware class using Grad-CAM (Figure.8) LIME (Local Interpretable Model-agnostic Explanations) visualizations for four deep learning architectures EfficientNetB0, ResNet50, VGG16, and Xception for the Fakerean malware family are shown in Figure 9.

The superpixel regions that make the most contributions to the local classification choice are highlighted by LIME; darker parts suggest a low or negligible contribution, while highlighted regions (yellow contours) indicate a favorable influence toward predicting the sample as Fakerean. The highlighted areas in EfficientNetB0 (LIME) are comparatively sparse and mostly located in the lower part of the image. This implies that the model's ability to detect Fakerean malware depends on a small number of discriminative structural characteristics. The model combines information from several spatial regions, as evidenced by the dense and widely distributed highlighted patches that ResNet50 (LIME) displays throughout the image. This indicates decreased localization precision even though it shows high feature sensitivity. With dispersed highlighted regions and little spatial coherence, VGG16 (LIME) generates noisy, fragmented explanations. When compared to deeper architectures, this behavior indicates poorer interpretability and weaker feature attribution. With somewhat reduced noise, Xception (LIME) shows more focused and structured highlighted sections, especially in the lower and perimeter portions. This suggests clearer attribution of influential regions and more efficient feature separation. All things considered, the LIME visualizations show that each model has unique interpretability traits. While ResNet50 and VGG16 produce noisier and more scattered attributions, Xception and EfficientNetB0 offer more succinct and targeted explanations. These findings emphasize the role of architectural design in achieving reliable and interpretable malware classification for the Fakerean family. Elucidation of Explainable AI technique of LIME for *Fakerean* malware class (Figure.9).

**Grad-CAM and LIME analysis of Neoreklami malware**

For samples from the Neoreklami malware family, the figure shows activation heatmaps produced by four convolutional neural network architectures: EfficientNetB0, ResNet50, VGG16, and Xception are shown in Figure 10. The heatmaps show how learnt feature activations are distributed spatially, with darker areas representing weaker feature responses and brighter areas representing higher activation intensity. Strong activation is seen at the upper part of the heatmap for EfficientNetB0, and the intensity decreases as one moves toward the lower layers. This suggests that in order to detect Neoreklami samples, the model prioritizes early-stage structural patterns. ResNet50 shows strong and evenly distributed activations throughout the upper and core areas, indicating robust capture of discriminative malware traits and efficient hierarchical feature learning. There is less contrast between high- and low-importance regions in VGG16, indicating more dispersed and moderately active regions. This is due to substantially poorer feature discrimination and less accurate spatial localization. Xception's great capacity to capture prominent spatial correlations using depthwise separable convolutions is demonstrated by a highly concentrated and

symmetrical activation pattern that is located in the middle of the heatmap. Overall, the study shows that whereas EfficientNetB0 and VGG16 exhibit broader or weaker activation patterns, ResNet50 and Xception produce better organized and discriminative activation representations for the Neoreklami malware family. These results further emphasize the influence of network architecture on feature learning effectiveness in malware classification. Elucidation of Model Activation Heatmaps for the *Neoreklami* malware class using Grad-CAM (Figure.10). The Neoreklami malware family's LIME (Local Interpretable Model-agnostic Explanations) visualizations for four deep learning architectures EfficientNetB0, ResNet50, VGG16, and Xception are shown in Figure 11. Superpixels that positively influence the model's classification of the sample as Neoreklami are indicated by the yellow-highlighted regions, whilst darker regions suggest little to no impact. In the upper part of the image, EfficientNetB0 (LIME) displays a compact and clearly defined cluster of highlighted locations. This implies that for Neoreklami identification, the model depends on a limited number of highly discriminative local features. Nearly the whole image is covered in dense, widely dispersed highlighted patches by ResNet50 (LIME). This shows less localization and greater explanation noise, but it also shows excellent sensitivity to many features. Large areas of the image are highlighted, and VGG16 (LIME) generates similarly chaotic and fragmentary explanations. In comparison to more sophisticated systems, this implies worse interpretability and weaker feature attribution. Clear, organized, and spatially coherent highlighted patches that form unique patterns are displayed by Xception (LIME). Strong feature separation and more significant attribution of discriminative regions are shown by these localized explanations. Overall, the LIME results demonstrate that, whereas ResNet50 and VGG16 typically produce noisy and excessively dispersed attributions, Xception offers the most comprehensible and well-localized explanations for the Neoreklami malware family, followed by EfficientNetB0. These findings highlight the importance of architectural choices in achieving reliable interpretability alongside accurate malware classification. Elucidation of Explainable AI technique of LIME for *Neoreklami* malware class (Figure.11).

**Grad-CAM and LIME analysis of Snarasite malware**

Activation heatmaps produced by four convolutional neural network architectures EfficientNetB0, ResNet50, VGG16, and Xception for samples from the Snarasite malware family are shown in Figure 12. The heatmaps show the spatial intensity of learnt feature activations, with darker areas representing weaker responses and brighter areas indicating higher significance. In the heatmap's middle horizontal region, EfficientNetB0 displays robust and distinct activations. This implies that a particular range of discriminative structural characteristics typical of Snarasite malware is the focus of the model. Effective hierarchical feature extraction and the capacity to capture several prominent patterns pertinent to Snarasite classification are demonstrated by ResNet50's prominent and wider activation regions, especially in the middle layers. With moderate intensity dispersed among multiple regions, VGG16 exhibits more dispersed and irregular activation patterns. This is indicative of less clear feature focus and somewhat lower spatial localization. Xception's remarkable capacity to capture prominent spatial correlations utilizing depthwise separable convolutions is demonstrated by its smooth, centralized, and highly concentrated activation pattern. Overall, the graphic illustrates that while EfficientNetB0 demonstrates selective focus and VGG16 displays more dispersed activations, ResNet50 and Xception learn more organized and discriminative feature representations for the Snarasite malware family. These differences further emphasize the impact of architectural design on feature learning effectiveness in malware classification tasks. Elucidation of Model Activation Heatmaps for the *Snarasite* malware class using Grad-CAM (Figure.12). Each CNN architecture are shown in Figure 13 learns and applies discriminative features for Snarasite malware classification differently, as demonstrated by the LIME-based explanations. Activations in EfficientNet-B0 are scarce and localized, mostly limited to a small horizontal area. This shows effective use of features and less dependence on unrelated background data. The little highlighted area, however, can also indicate that secondary or subtle malware patterns are not being used to their full potential. The majority of the image exhibits highly scattered and noisy activations from ResNet-50 and VGG16. The extensive pixel contributions suggest that these models rely on a wide range of weakly informative traits, despite the center region's continued influence. Such behavior suggests less strong feature learning for this form of malware, making it more vulnerable to noise and less interpretable. Xception, on the other hand, creates distinct and continuous highlighted patches, especially in the vicinity of physically significant locations. This suggests improved separation between relevant and non-informative regions and more robust spatial feature modeling. Higher confidence and robustness in

the model's decision-making process are suggested by the explanation's clarity. All things considered, the investigation shows that models using depthwise separable convolutions (Xception) offer better interpretability and more consistent feature attribution for malware images. The results suggest a correlation between focused LIME explanations and reliable malware characterization, making Xception a more suitable architecture for explainable malware detection tasks. Elucidation of Explainable AI technique of LIME for *Snarasite* malware class (Figure.13).

**Result Analysis**

The experimental results are discussed and evaluated generally in this section, along with observations on the methods that produced the best outcomes for each of the several malware families.

**Static Analysis of Amonetize Malware Using Image Visualization and Entropy Profiling**

The Amonetize malware sample are shown in Figure 14 as a hexadecimal representation, entropy profile, and grayscale visualization. The executable's raw byte values were mapped to pixel intensities to create the grayscale image, which allowed for the visual examination of the binary's structural patterns. There are no visible code or data segments in the final image, which has a very noisy and texture-like appearance. These visual traits are frequently linked to malware that uses encryption or packing techniques to hide the underlying software logic and avoid static analysis. Shannon entropy was calculated for every row of the image representation in order to objectively evaluate how random the binary content was. For the majority of the file, the entropy values are consistently high, ranging from roughly 7.0 to 7.5 bits. A nearly uniform byte distribution is indicated by this level of entropy, which is near the theoretical maximum of 8 bits. This kind of high entropy is a sign of compressed or encrypted payloads. The existence of padding, information, or a structural barrier like a footer or unpacking stub is indicated by a discernible decrease in entropy near the conclusion of the file. A valid Portable Network Graphics (PNG) file signature and common PNG chunk IDs like IHDR and IDAT are included in the hexadecimal dump of the first 512 bytes. This finding suggests that the malware program poses as an authentic picture file. Such file format impersonation is a well-known evasion technique that is frequently used to get around simple file-type validation procedures or to hide dangerous payloads within media files that appear to be harmless. The Amonetize malware appears to use a variety of obfuscation techniques, such as encryption, packing, and file format disguising, according to the combined evidence from visual analysis, entropy profiling, and hexadecimal examination. These methods demonstrate the efficacy of image-based and entropy-driven analysis for detecting hidden hostile activity and greatly boost resilience to conventional signature-based detection strategies. Static Analysis of Amonetize Malware Using Image Visualization and Entropy Profiling (Figure.14).

**Static Analysis of Dialplatform B Malware Using Image-Based Visualization and Entropy Analysis**

The Dialplatform B malware sample's hexadecimal format, row-wise entropy profile, and grayscale visualization are shown in Figure 15. The malware binary's raw byte values were mapped to pixel intensities to create the grayscale image, which allowed for the visual evaluation of the executable's structural features. A high amount of byte-level randomness across the file is suggested by the final image's mostly noisy, grainy texture with no spatial structure. Binaries that use encryption or compression to hide their internal code and data regions are frequently linked to these visual characteristics. Shannon entropy was calculated for each row of the image in order to statistically assess the randomness shown in the visual depiction. Throughout the examined rows, the entropy values stay high and vary within a small range of 6.8–7.2 bits. This homogeneity suggests that the byte values are distributed almost randomly, which is a feature of packed or obfuscated malware. Extended low-entropy regions are absent, which implies that the binary does not include uncompressed code segments or plaintext sections that are easily identifiable. The standard PNG header and chunk identifiers, such as IHDR, tIME, and IDAT, are present in the hexadecimal dump of the first 512 bytes, indicating a valid Portable Network Graphics (PNG) file signature. This demonstrates that the malware sample poses as an authentic image file. In order to get around static file-type checks and hide harmful payloads within seemingly innocuous audiovisual content, such file format impersonation is a popular evasion technique. The hexadecimal examination, entropy analysis, and grayscale visualization taken together show that Dialplatform B uses a variety of obfuscation techniques, including as file format masquerade and high-entropy encoding. These methods demonstrate the importance of image-based and entropy-driven static analysis techniques for detecting and

characterizing hidden malware families, while also severely impeding standard signature-based detection. Static Analysis of Dialplatform B Malware Using Image-Based Visualization and Entropy Analysis (Figure.15).

**Static Analysis of Fakerean Malware Using Grayscale Visualization and Entropy Profiling**

The hexadecimal dump, row-wise entropy distribution, and grayscale visual representation of the Fakerean malware sample are shown in Figure 16. The binary file's raw byte values were mapped to pixel intensities to create the grayscale image, which allowed for the visual examination of the malware's statistical and structural features. The generated image shows a high degree of unpredictability across the binary content, with a texture that is primarily noise-like and little visible spatial organization. Shannon entropy was calculated for every image row in order to provide quantitative evidence for the visual observations. For most of the file, the entropy values stay high, averaging between 7.0 and 7.4 bits. Such high entropy values indicate the widespread usage of obfuscation techniques and are suggestive of encrypted or compressed data. Near the conclusion of the file, there is a noticeable decrease in entropy. This could be due to structural boundaries related to the embedded container format, padding areas, or file terminators. A valid Portable Network Graphics (PNG) file signature, containing common chunk IDs like IHDR, tIME, and IDAT, can be found by examining the hexadecimal dump of the first 512 bytes. This demonstrates that the Fakerean sample poses as an authentic image file. This kind of file format impersonation is a popular malware evasion technique that makes it possible to hide dangerous payloads inside media files that appear harmless and to get beyond simple static file-type validation procedures. In conclusion, the Fakerean virus uses high-entropy encoding and file format impersonating to conceal its dangerous information, according to the combined grayscale visualization, entropy analysis, and hexadecimal examination. These features highlight the usefulness of image-based and entropy-driven static analysis tools for identifying and characterizing obfuscated malware families and seriously hinder conventional signature-based detection methods. Static Analysis of Fakerean Malware Using Image-Based Visualization and Entropy Analysis (Figure.16).

**Static Analysis of Neoreklami Malware Using Image Visualization and Entropy Measurement**

The Neoreklami malware sample's hexadecimal dump, row-wise entropy profile, and grayscale image representation are shown in Figure 17. The binary's raw byte values were converted into pixel intensities to create the grayscale image, which allowed for a visual analysis of the malware's statistical and structural characteristics. There are no distinct areas that match to plaintext code or data portions in the final visualization, which has a texture that is primarily homogeneous and noise-like. These features are frequently linked to binaries that use encryption or packaging to hide internal functionality. Shannon entropy was calculated on a per-row basis across the image representation in order to further quantify the observed randomness. For most of the file, the entropy values stay high, varying between around 7.0 and 7.3 bits. This degree of entropy indicates a nearly equal distribution of byte values and points to the widespread application of obfuscation methods like encryption and compression. No lengthy low-entropy regions are seen, although small localized entropy fluctuations might be indicative of internal structural borders or metadata segments. A legitimate Portable Network Graphics (PNG) file signature and common PNG chunk IDs, such as IHDR and IDAT, are revealed by the hexadecimal dump of the first 512 bytes. This demonstrates that the Neoreklami sample poses as an authentic image file. This kind of file format impersonation is a common evasion tactic that allows harmful payloads to evade basic static file-type validation and be hidden inside innocuous-looking multimedia containers. In conclusion, the Neoreklami virus uses high-entropy encoding and file format masquerading to hide its dangerous content, according to the combined grayscale visualization, entropy profiling, and hexadecimal examination. These characteristics emphasize the significance of image-based and entropy-driven static analysis techniques for the identification and characterization of obfuscated malware families and drastically diminish the efficacy of conventional signature-based detection systems. Static Analysis of Neoreklami Malware Using Image-Based Visualization and Entropy Analysis (Figure.17).

**Static Analysis of Snarasite Malware Using Grayscale Image Representation and Entropy Analysis**

The Snarasite malware sample's hexadecimal dump, row-wise entropy distribution, and grayscale visualization are shown in Figure 18. The malware binary's raw byte values were mapped to pixel intensities to create the grayscale image, which allowed for the visual examination of internal structural features. The generated image has a texture

that is primarily noise-like and has no discernible structure, while some areas show faint horizontal banding. These patterns are frequently linked to repeating encoded blocks in the binary or minute changes in packing layers. Shannon entropy was calculated for each image row in order to statistically assess the degree of unpredictability seen in the image. A nearly uniform distribution of byte values throughout the majority of the file is indicated by the entropy values, which are constantly high and mostly fall between 7.0 and 7.4 bits. The presence of structural borders, metadata segments, or partially uncompressed sections embedded inside an otherwise obfuscated payload is suggested by the observation of multiple localized entropy dips at particular offsets. A valid Portable Network Graphics (PNG) file signature can be found in the hexadecimal dump of the first 512 bytes, as shown by chunk IDs like IHDR and IDAT and the standard PNG magic number. This demonstrates how the Snarasite malware sample poses as an authentic image file. This kind of file format impersonation is a frequently used evasion technique that enables harmful payloads to be hidden inside innocuous-looking multimedia containers and to get around simple static file-type verification procedures. In conclusion, the Snarasite malware uses high-entropy encoding and file format masquerade, with slight structural differences possibly matching to embedded control or metadata areas, according to the combined grayscale visualization, entropy profiling, and hexadecimal examination. These features show the value of image-based and entropy-driven static analysis tools for the identification and characterization of obfuscated malware families and severely impede conventional signature-based detection methods. Static Analysis of Snarasite Malware Using Image-Based Visualization and Entropy Analysis (Figure.18).

**Comparative Analysis of Malware Families Using Image-Based Visualization and Entropy Profiling**

This study conducted a comparative static analysis of five malware families Amonetize, Dialplatform B, Fakerean, Neoreklami, and Snarasite using grayscale image visualization, row-wise entropy measurement, and hexadecimal inspection. By transforming raw binary data into image representations and analyzing entropy distributions, common and distinguishing characteristics related to obfuscation strategies and file masquerading techniques were identified. Across all five malware families, the grayscale visualizations exhibit predominantly noise-like textures with minimal discernible structural patterns. Such visual homogeneity indicates extensive byte-level randomness, which is commonly associated with packing, compression, or encryption mechanisms. Although minor variations in texture and faint banding patterns are observed in some samples, particularly in Snarasite and Amonetize, no family demonstrates clearly identifiable plaintext code or data regions. These findings suggest that all analyzed malware families rely heavily on obfuscation to hinder visual and structural analysis. Entropy profiling further reinforces these observations. All samples exhibit consistently high Shannon entropy values, generally ranging between approximately 6.8 and 7.5 bits across most file offsets. This uniform high-entropy behavior indicates near-random byte distributions and supports the inference that encrypted or compressed payloads dominate the binaries. Localized entropy drops are observed in several samples, such as Fakerean and Snarasite, typically near the end of the file or at specific offsets. These drops likely correspond to padding, metadata, container boundaries, or unpacking stubs embedded within the malware. Hexadecimal analysis reveals a notable commonality across all five malware families: the presence of valid Portable Network Graphics (PNG) file signatures and standard PNG chunk identifiers, including IHDR and IDAT. This consistent use of PNG headers confirms that each malware sample masquerades as a legitimate image file. File format impersonation is a well-documented evasion strategy, enabling malware to bypass basic static file-type validation and to conceal malicious payloads within benign-looking multimedia containers. While the overall obfuscation strategies appear broadly consistent, subtle differences are observed in entropy stability and visual texture across the families. Dialplatform B exhibits slightly lower and more stable entropy values compared to the other samples, suggesting a more uniform packing approach. In contrast, Snarasite and Fakerean display localized entropy fluctuations, which may reflect additional internal structural elements or layered encoding techniques. Despite these variations, the shared characteristics across all families highlight a convergence toward high-entropy encoding combined with file masquerading as a primary means of evasion. In summary, the comparative analysis demonstrates that all five malware families employ similar obfuscation strategies characterized by high-entropy content, noise-like visual representations, and PNG file format impersonation. These findings underscore the limitations of traditional signature-based detection methods and emphasize the effectiveness of image-based and entropy-driven static analysis techniques for identifying and differentiating modern obfuscated malware families.

Comparative Summary of Entropy Characteristics and Visual Traits of Malware Families (Table 2). Table 2 highlights that all analyzed malware families exhibit high entropy values close to the theoretical maximum, confirming the widespread use of encryption or compression-based obfuscation. Despite minor differences in entropy stability and visual texture, the grayscale representations are uniformly noise-like, indicating the absence of easily identifiable plaintext code regions. These shared characteristics, combined with consistent PNG file masquerading across samples, suggest a convergence toward similar evasion strategies among modern malware families. Also, it clearly determined the most crucial region for the class Expiro using CNN heatmaps. A signature area, a zone that can be used to identify the class, has been determined by the CNNs. This section can be found in the code if we statically examine it.UPX portion (packed), especially toward the end. This area has higher entropy than the rest of the area, as can be seen by looking at the entropy. UPX section, which shows that a resource-containing section has been packed and that, even if it is still packed, the resources can be utilized to identify the infection. It also avoid all the laborious static analysis procedures because to the CNN's intuition, which pointed us in the direction of a feature unique to this class. The region immediately preceding the occurrence of high entropy is identified by the heatmaps with a particular intensity. A strange pattern can be seen by doing a hex dump and changing the value in the ASCII character; the repeating of the ff characters. For a malware study, this kind of non-random recurrence is quite interesting since it reveals a significant and distinctive signature for the malware class. Saliency maps can also be used to analyze samples that have been incorrectly classified. It create feature significance maps and extract patterns from these samples once misclassified examples have been identified. Sample-by-sample analysis of the class activation maps can be done by, for instance, comparing the sample heatmap with the cumulative CAM heatmap or taking into account all of the incorrectly categorized samples. To determine which areas or characteristics frequently result in incorrect categorization, these trends will be examined. Although these type of analyzing misclassified samples cannot be fully automated due to their reliance on the samples, classifications, and overall dataset structure, they will nonetheless aid the human malware analyst in his work.

## CONCLUSION

Using Grad-CAM, LIME, and Entropy Characteristics and Visual Traits significance for explanations, the present study applies CNN models to dynamic and online malware datasets for malware detection. This method strikes a balance between analysis depths. Applications of sophisticated AI models are extensively incorporated into many facets of our everyday lives. In order to support the current XAI techniques and provide guidance for future study in the area, this outlined the research gaps and difficulties in the field. Future research will look into how hostile individuals could misclassify malware categories by using adversarial techniques. These results can help with real-world malware remediation and assist cyber-analysts in coordinating responses to attacks by concentrating on aspects of highest value determined by explainability methodologies.

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**Table 1: Malware Family Dataset**

| Malware Family | Sample images | Malware Type |
|---|---|---|
| Amonetize | 350 | Adware |
| Dialplatform B | 152 | Trojan |
| Fakerean | 306 | Trojan-Downloader |
| Neoreklami | 350 | Browser Hijacker |
| Snarasite | 350 | Trojan-Horse |

**Table 2: Comparative Summary of Entropy Characteristics and Visual Traits of Malware Families**

| Malware Family | Approximate Entropy Range (bits) | Entropy Behavior | Grayscale Visual Characteristics | Notable Observations |
|---|---|---|---|---|
| Amonetize | 7.0 – 7.5 | Consistently high with a sharp drop near file end | Highly noisy, texture-like appearance with faint horizontal banding | Indicates strong packing/encryption; entropy drop suggests padding or footer region |
| Dialplatform B | 6.8 – 7.2 | Stable and uniformly high | Granular noise pattern with minimal variation | Slightly lower and more stable entropy suggests uniform compression strategy |
| Fakerean | 7.0 – 7.4 | High entropy with pronounced drop | Dense noise with no discernible structure | Entropy drop likely corresponds to file boundary |

| | | | | |
|---|---|---|---|---|
| | | at file termination | | or embedded container metadata |
| Neoreklami | 7.0 – 7.3 | Consistently high with minor localized fluctuations | Uniform noise-like texture, visually homogeneous | Suggests extensive obfuscation with limited internal structural variation |
| Snarasite | **7.0 – 7.4** | High entropy with several localized dips | Predominantly noisy with subtle horizontal banding | Entropy dips may indicate internal metadata or layered encoding regions |

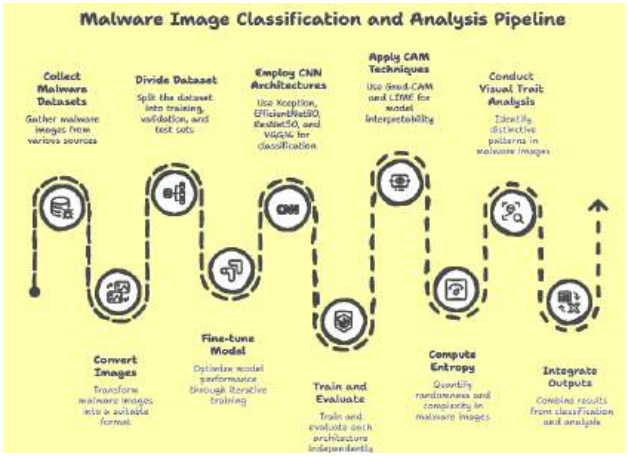



**Figure.1: Framework of a systematic pipeline analysis**

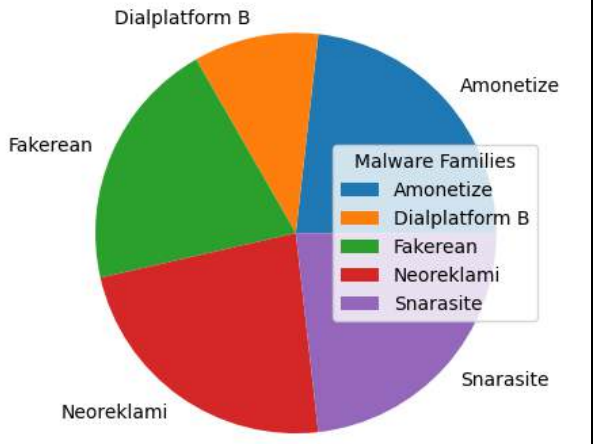



**Figure.2: Dataset representing Malware Families**

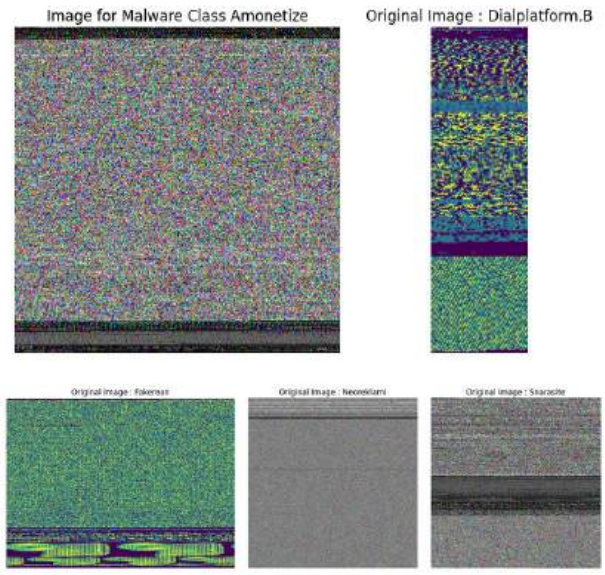



**Figure.3: Visualization of sample Malware Families**

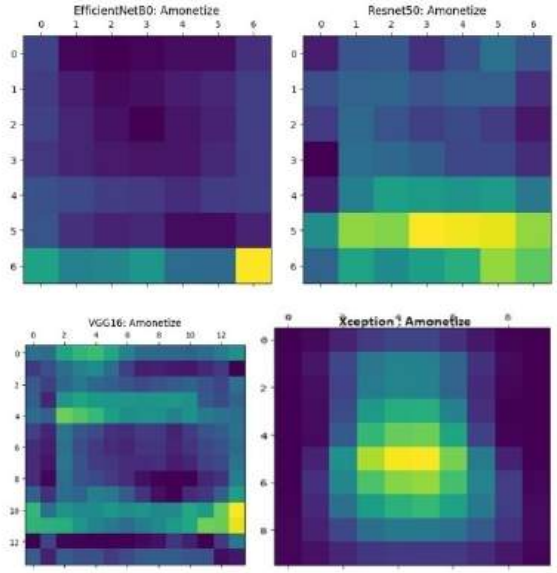



**Figure.4: Elucidation of Model Activation Heatmaps for the *Amonetize* malware class using Grad-CAM**

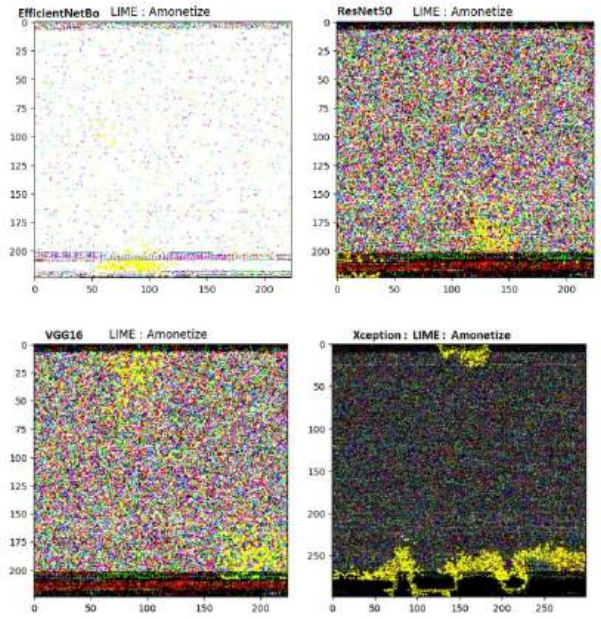



**Figure.5: Elucidation of Explainable AI technique of LIME for *Amonetize* malware class**

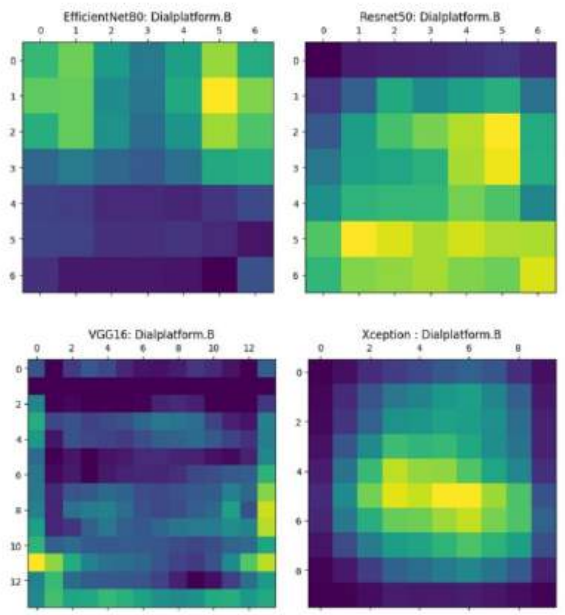



**Figure.6: Elucidation of Model Activation Heatmaps for the *Dialplatform.B* malware class using Grad-CAM**

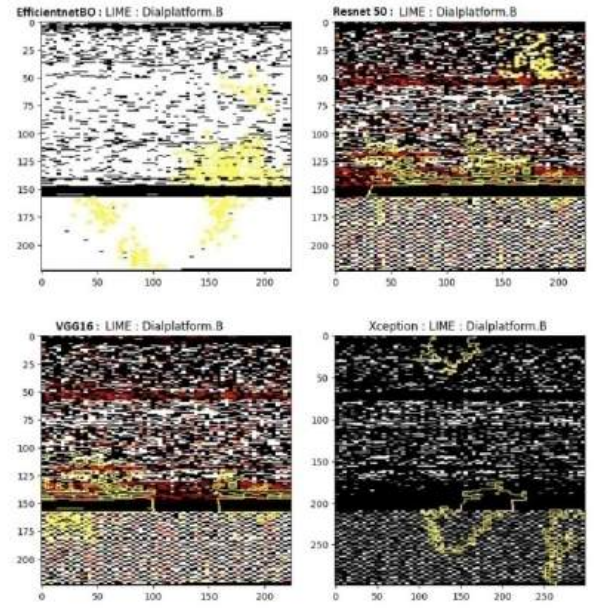



**Figure.7: Elucidation of Explainable AI technique of LIME for *Dialplatform.B* malware class**

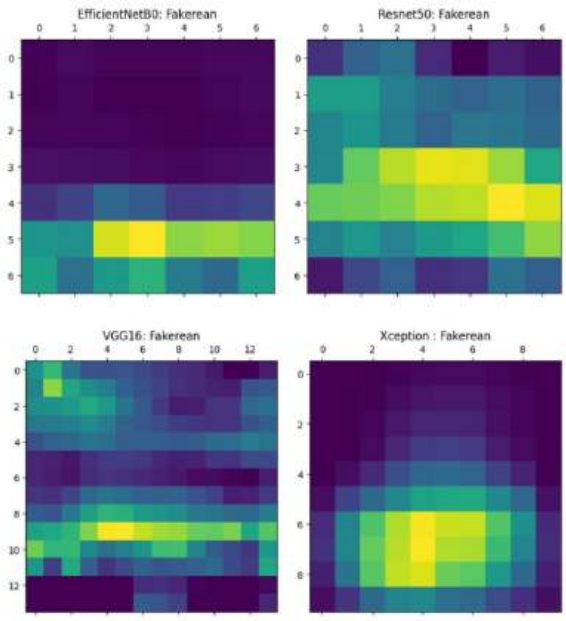



**Figure.8: Elucidation of Model Activation Heatmaps for the *Fakerean* malware class using Grad-CAM**

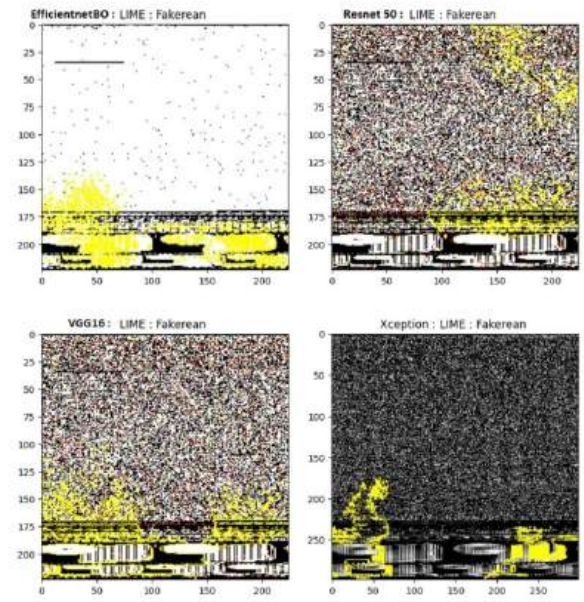



**Figure.9: Elucidation of Explainable AI technique of LIME for *Fakerean* malware class**

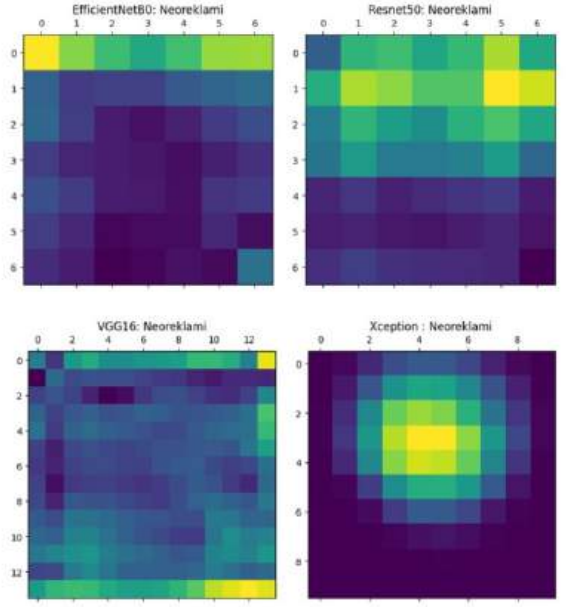



**Figure.10: Elucidation of Model Activation Heatmaps for the *Neoreklami* malware class using Grad-CAM**

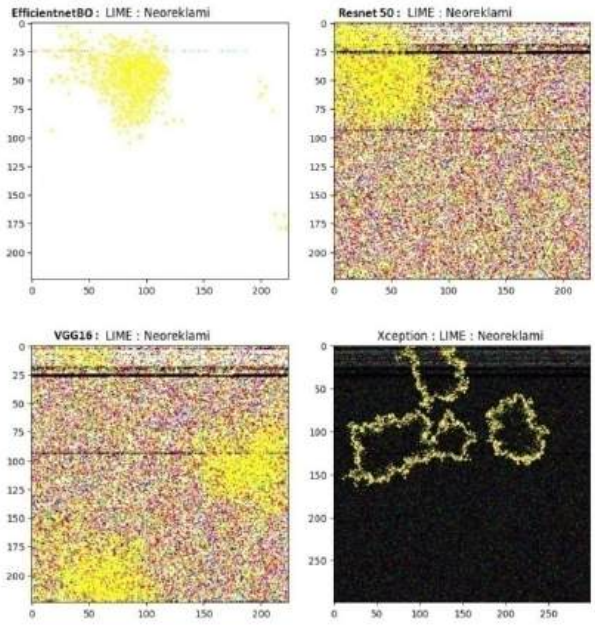



**Figure.11: Elucidation of Explainable AI technique of LIME for *Neoreklami* malware class**

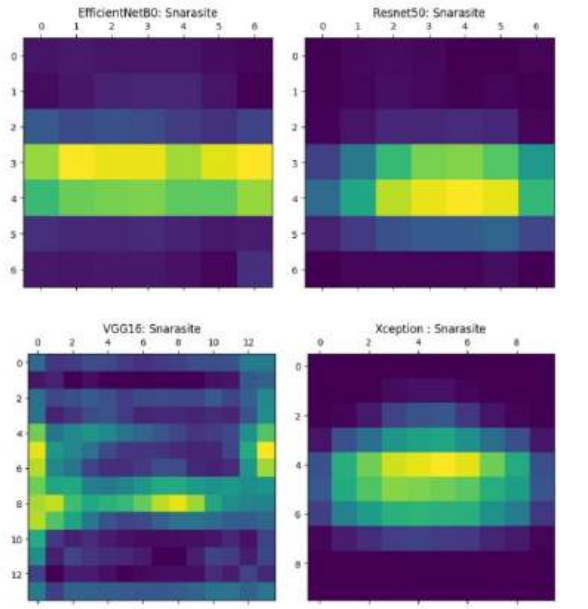



**Figure.12: Elucidation of Model Activation Heatmaps for the *Snarasite* malware class using Grad-CAM**

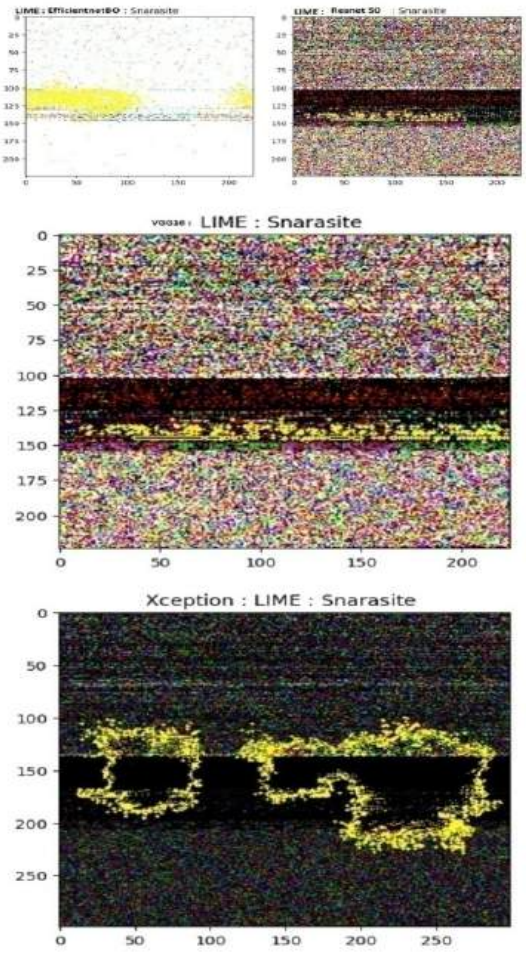



**Figure.13: Elucidation of Explainable AI technique of LIME for *Snarasite* malware class**

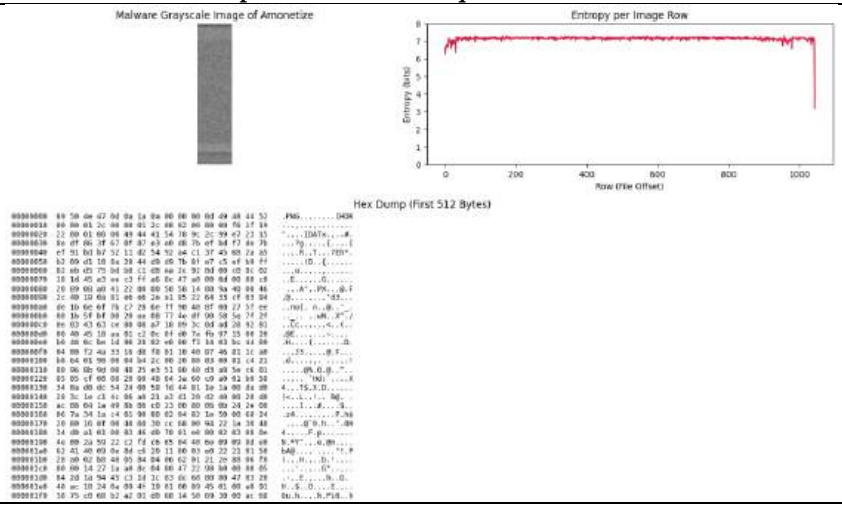



**Figure.14: Static Analysis of Amonetize Malware Using Image Visualization and Entropy Profiling**

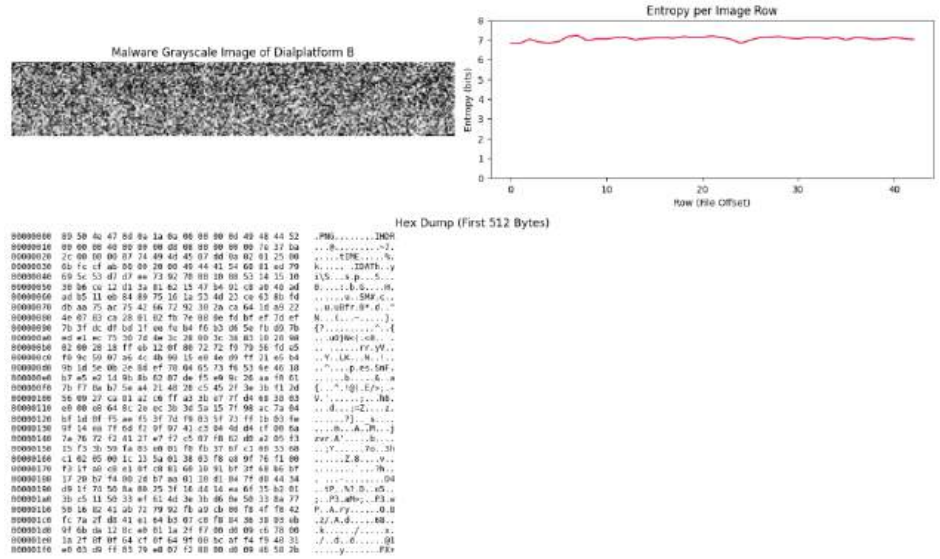



**Figure.15: Static Analysis of Dialplatform B Malware Using Image-Based Visualization and Entropy Analysis**

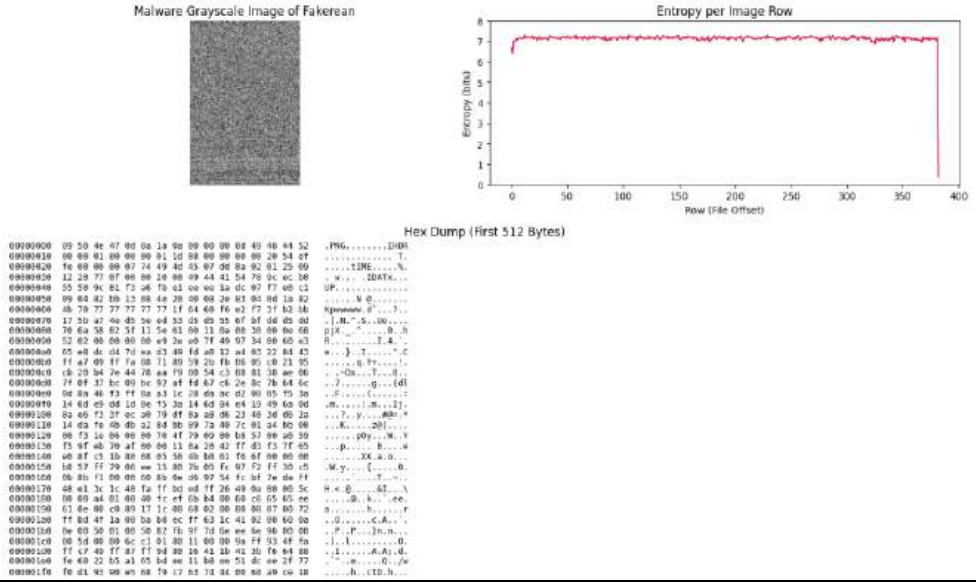



**Figure.16: Static Analysis of Fakerean Malware Using Image-Based Visualization and Entropy Analysis**

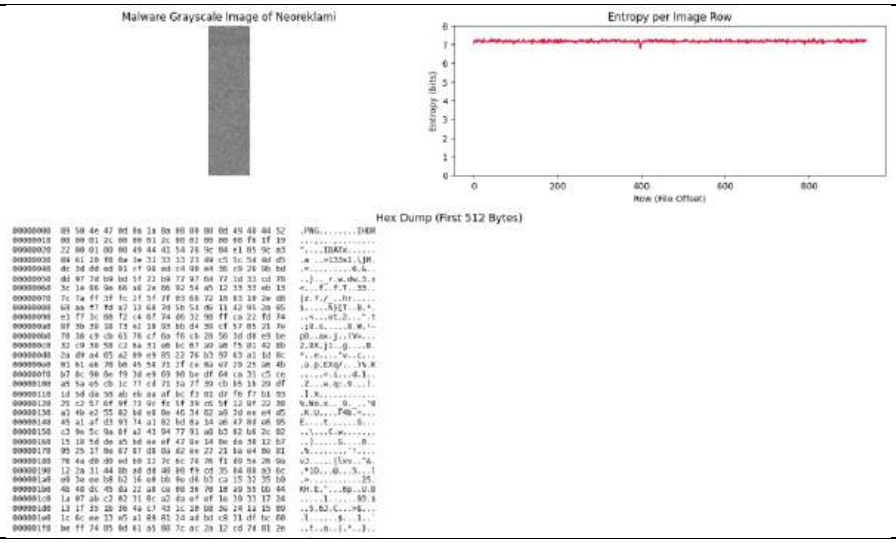



**Figure.17: Static Analysis of Neoreklami Malware Using Image-Based Visualization and Entropy Analysis**

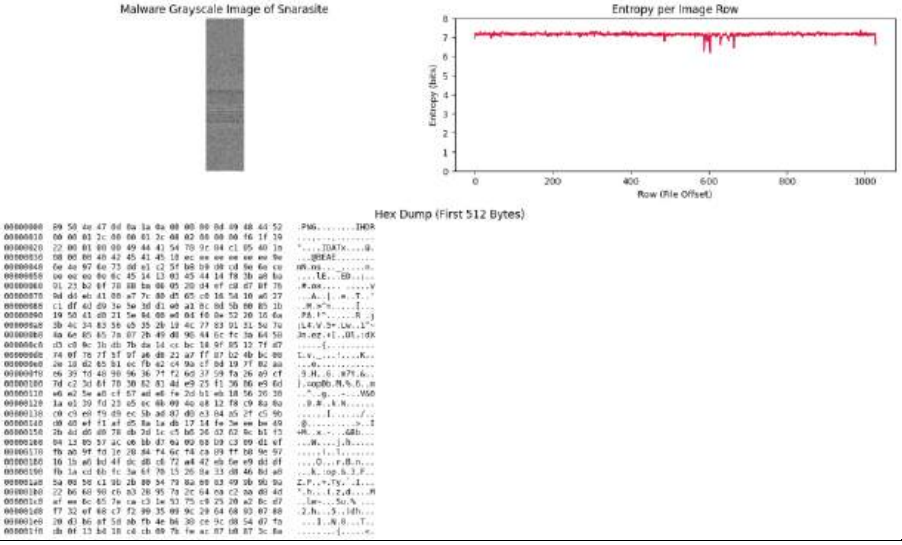



**Figure.18: Static Analysis of Snarasite Malware Using Image-Based Visualization and Entropy Analysis**

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RESEARCH ARTICLE

# Knowledge, Attitude and Awareness about Realistic Mathematics Education among Middle School Students

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## ABSTRACT

This present study is based on awareness of realistic mathematics among middle school students. Descriptive Realistic Mathematics Education is an educational approach that emphasizes the use of real-world content, visualisation, problem-solving activities, and active student participation to enhance conceptual understanding and mathematical concepts. The main focus of this study was to find out the awareness of realistic mathematics education among middle school students, based on gender and locale. This study deals with the descriptive survey research method that was used. This study was conducted in the Shahjahanpur district in Uttar Pradesh. The sample size was 226 middle school students randomly selected. In the findings of this study, male middle school students were found to have significantly higher awareness towards Realistic Mathematics Education as compared to female middle school students. Based on the data, it shows that rural and urban middle school students are aware of realistic mathematics education, and urban male middle school students are more aware of realistic mathematics education than rural male students. Mathematical understanding supports students in daily life decision-making, in lifelong learning, and in general academic skills.

**Keywords:** Activity, Education, Mathematics, Realistic, Students.

## INTRODUCTION

The teaching of mathematics greatly influences the critical thinking, problem-solving, and logical reasoning of students. These subjects sit at the very foundation of several studies: sciences, technology, engineering, and applications in economics. Mathematical understanding supports students in daily life decision-making, in lifelong learning, and in general academic skills. As has been put forward by the *NCTM (2000),* effective mathematics teaching should allow students to understand and utilize mathematics in real-life situations, which is of utmost importance for analytical and reflective citizenship. Realistic Mathematics Education (RME) grew out of Hans Freudenthal's work in the Netherlands during the 1970s, with the emphasis that mathematics must hold some real-world relevance and correspondingly be of significance to a student's life. It engendered a rethinking of the mathematics concepts in terms of real-world problems, rather than relying on rote learning (Gravemeijer, 1994). The RME approach has been recently accepted in the Indian classroom, supported by a framework established by the National Education Policy 2020, which advocates for experiential learning. RME is still in its infancy in India, but much interest is being generated concerning its potential for transforming mathematics education through pilot projects and research works (NCERT, 2021). The Value of Realistic Mathematics for Students, particularly in the Middle School. In middle school, abstract thought begins to replace the straightforward operations of basic mathematics. In this aim, much is gained through the RME method, as strong contextualization makes those abstract ideas more understandable. Research indicates that RME decreases math anxiety, increases involvement, and deepens conceptual understanding (Van den Heuvel-Panhuizen, 2001). RME renders it easier and more interesting for students' subjects that are considered quite difficult, whereas students often find it tough to grasp the abstractions in conventional mathematics instruction. Realistic Mathematics Knowledge in India and in the Other Parts of the World. For the application and experimentation of RME, international fame has been accorded for the successful enhancement of students' mathematical ability in countries from Indonesia to the USA and the Netherlands (Bakman, 2011). Various studies worldwide have proposed RME as a road to developing higher-order thinking skills and improving mathematical comprehension. In India, however, RME is known mainly among academic circles and experimental projects. Some educationalists and progressive institutions are advocating for its rooting, but RME has not yet gained a fair amount of currency in most Indian middle school circles (Singh, and Kumar, 2022). This gulf must be bridged in order to establish a successful and viable mathematics education in India.

## LITERATURE REVIEWS

Akosah, Arthur and Obeng (2025) conducted a study entitled "*Optimizing High School Mathematics Achievement through the Lens of Realistic Mathematics Education*". Focusing on investigating the mediating role of teacher self-efficacy in the relationship between teaching quality and students' mathematics achievement, using Realistic Mathematics Education and Self-Determination Theory as frameworks. The population consisted of twelve thousand nine hundred and eighty (12,980) teachers across eight regions in Ghana: Ashanti, Bono East, Central, Eastern, Greater Accra, Northern, Oti, and Volta. The researcher used five hundred and seven (507) junior high school mathematics teachers as the sample. The researcher used a stratified purposive sampling technique for data collection. A correlational cross-sectional survey design was employed. Structural Equation Modeling was used to test the hypotheses. After analyzing the data, the researcher found that teaching quality positively affects students' mathematics achievement, particularly when teaching is grounded in RME principles. The unstandardized estimate ($\beta = .194$, $p < .001$) indicates a strong positive relationship. The study underscores the importance of considering both instructional and psychological factors in efforts to improve mathematics education. Sushanti *et al*. (2025) in their research paper entitled *"Enhancing Problem-Solving Skill in Elementary Students Through Realistic Mathematics Education."*Focusing on problem-solving skill through Realistic Mathematics Education. The population of the study is derived from elementary students and classes in different international settings, as evidenced in previous empirical research, while the sources of the data are peer-reviewed articles, documented case studies, and educational reports on the implementation of RME.

The study employed a qualitative research methodology, specifically a systematic literature review and case study approach, to synthesize patterns, themes, and results of RME implementation. The findings have consistently shown that RME has a significant positive impact on students' mathematical problem-solving skills, understanding, engagement, confidence, critical thinking, and creativity by relating abstract mathematical ideas to real-world situations and promoting collaborative and student-centered learning. The review pointed out the quantitative and qualitative results. Some challenges in the implementation of RME were also noted, including inadequate teacher preparation, curriculum incongruence, lack of instructional materials, time constraints, and resistance to instructional change. The conclusion of the study strongly emphasized the transformative power of RME in elementary mathematics education. Martínez-Meléndez, K. *et al*. (2025),in their research paper entitled*"Realistic and non-realistic considerations in mathematical word problems with Mexican eighth-grade students," focus* on how students use real-life understanding while solving mathematical word problems, as earlier studies have shown that students often apply formulas without considering real-world situations. This qualitative exploratory research was conducted on a population of second-year middle school students in Mexico. A sample of 97 students aged was selected using a non-probability sampling technique from two secondary schools. The researchers used twelve-word problems related to proportionality, false proportionality, division with remainder, and arithmetic operations, along with an open-ended questionnaire to collect data. Students' answers were analyzed and classified into realistic, unrealistic, technical error, other responses, no response, and proposal categories. The results showed that most students gave unrealistic answers in proportionality and false proportionality problems due to linear thinking, while better realistic understanding was seen in division with remainder and arithmetic operation problems. Students who used their personal experiences and everyday knowledge were more successful in giving realistic answers. The study concludes that mathematics textbooks include real-life contexts, but many students still fail to connect mathematics with reality, indicating a need for teaching methods that encourage students to think realistically while solving word problems. In a study conducted by Muhammad *et al*. (2025)on the development of web-based learning media with a realistic mathematics education approach to increase student self-determination.

Their study focuses on the development of web-based learning through RME. The results showed that the developed Google Sites-based learning media was highly valid and feasible, with expert validation scores above 85%, and students gave positive responses with an average feasibility score above 87%. Students' self-determination in mathematics improved significantly after using the media, with noticeable increases in self-awareness, perceived choice, intrinsic motivation, and extrinsic motivation. The study concluded that integrating RME with web-based learning media effectively enhances students' motivation and autonomy in learning mathematics and recommended further research with larger samples and different mathematical topics. Samková, L.; Rokos, L. and Vízek, L.(2025) in their research paper entitled*"Teachers' awareness of the existence of analogies in the conceptual structure of mathematics and biology."* focusing on teachers' awareness. The population of the study consisted of preservice teachers in the Czech Republic, and the sample included 57 participants, out of whom 47 were prospective primary school teachers and 10 were prospective secondary school teachers specializing in mathematics and biology. The sampling technique used was purposive sampling, as participants were selected from final-year teacher education programs without prior STEM integration experience. The study followed a qualitative research methodology, using cartoon-based worksheets and concept cartoons as data collection tools to explore participants' reasoning and awareness of analogies. Data were analyzed through qualitative coding and category formation. The results showed that most preservice teachers were able to solve problems within individual subjects but struggled to recognize deeper conceptual analogies between mathematics and biology. A small number of participants demonstrated interdisciplinary or transdisciplinary understanding of analogies. The study concluded that although analogical structures exist clearly between mathematics and biology, preservice teachers need explicit training and instructional support to recognize and apply these analogies effectively for integrated STEM teaching. Oktaviani, A.N., *et al*. (2025) in their research paper entitled *"The Influence of the Realistic Mathematics Education Approach Assisted by the PLSV Board on the Mathematical Literacy of Junior High School Students."* The study examined the effect of the Realistic Mathematics Education (RME) approach assisted by the PLSV Board on the mathematical literacy ability of junior high school students.

The population of the study consisted of Grade VIII students of a junior high school in Tangerang City, Indonesia. The researcher used a cluster random sampling technique; a total sample of 62 students was selected, where 30 students were placed in the experimental group and 32 students in the control group. The research followed a quasi-experimental method with a randomized post-test-only control design. The experimental group was taught using the RME approach assisted by the PLSV Board, while the control group was taught using the scientific approach. Data were collected using a mathematical literacy test consisting of seven descriptive questions based on the indicators of formulating, employing, and interpreting mathematical problems. The results showed that the mean score of the experimental group (54.25) was higher than that of the control group (36.71). Statistical analysis using an independent sample t-test revealed a significant difference between the two groups at the 0.05 level, indicating that the RME approach had a positive effect on students' mathematical literacy. The study concluded that learning through the RME approach assisted by the PLSV Board is more effective than the scientific approach in improving students' mathematical literacy skills. Altiner (2024) conducted a research paper entitled "*Exploring measurement estimation strategies through concept cartoons designed with Realistic Mathematics Education*". which dwells on the interface between concept cartoons and Realistic Mathematics Education (RME) for better student outcomes. The methods applied in the study were a qualitative case study design, purposeful sampling of fourth-grade students, use of RME-supported concept cartoons for data collection, and descriptive analysis of the collected data to study students' measurement estimation strategies. The study involved 46 fourth-grade students from a primary school. The study's result sheds light on the students' diverse strategies for measurement estimation, how context affects their choice, and the absence of gender differences in using these strategies. Such knowledge can be used as a perspective in teaching methodology in mathematics. Thus, the study provides valuable insights into measurement estimation strategies of fourth-grade students and points out a dire need for instructional practices and curriculum development that enhance students' mathematical reasoning and problem-solving skills. The study conducted by Irawan *et al*.(2024) in their research paper on the application of the Indonesian Realistic Mathematics Education Approach to improve student learning outcomes.

Their study focuses on the effectiveness of implementing the Indonesian Realistic Mathematics Education Approach in improving student learning outcomes in mathematics. It seeks to address common problems in mathematics education, such as low student interest and difficulty understanding abstract concepts. The researcher found that the percentage of students completing the evaluation test increased across cycles: pre-cycle; 33.33% completion, Cycle I;66.67%completion, and Cycle II; 93.33% completion. Observations showed increased student engagement, rising from 48.28% in cycle I to 73.57% in cycle II, which shows that the PMRI approach improved mathematics learning outcomes for fifth-grade students. Ramadhanta *et al*. (2024) conducted a study on enhancing mathematical creativity thinking ability: Experimentation with Realistic Mathematics Education by embedding ice-breaking in junior high school. Their study mainly improved mathematical creative thinking ability among students taught with Realistic Mathematics Education by embedding ice-breaking. The research findings indicate that integrating ice-breaking activities into RME facilitates enhanced MCTA. The study recommends that teachers implement RME to enhance students' MCTA, transforming initially passive students as learning shifts from teacher-centered to more active. Melabari and Ismail (2023) In their research paper, "*The effect of Realistic mathematics education on Undergraduate Freshmen Students' Mathematical Competencies*". aims to evaluate the effect of RME on undergraduate freshmen students' mathematical competencies. This study sample was selected from a college of business administration in a private university in Jeddah, Saudi Arabia. There were four Math 099 sections. Each section contains 19 to 24 students. Therefore, the treatment group consisted of 39 students from 2 sections, and the control group consisted of 44 students from the other 2 sections. This study uses a quasi-experimental design with three components: a pre-test, a post-test, and an RME-based pre-calculus course. The results illustrated that RME has a significant positive effect on students' mathematical competencies. This study confirms the recommendation suggested by for Saudi universities to adopt RME-based courses in teaching freshmen students or a preparatory year program student, to improve university mathematics curriculum and textbooks based on the theory of RME, and to provide sufficient training for in-service and pre-service mathematics teachers to apply the Introduction of RME in teaching mathematics for pre-college levels of education.

Mahmoud and Alaraj stated that to meet the current difficulties, educational curricula and instructional methods need to be revised. Bayarak and Aslenci (2022) In their research paper "*Realistic Mathematics Education: A Bibliometric Analysis*". focusing on conducting a bibliometric analysis of published articles related to Realistic Mathematics Education (RME) and analysing trends, key contributors, and influential publications in the field of RME research. All articles published on RME are considered as a population, and the study focused on articles indexed in the Scopus database. The study's sample comprised 1190 articles published between 1986 and 2021. The Scopus database was searched using the keywords: "realistic mathematics education", "realistic mathematics education", "realistic maths", or "realistic mathematical education". Only journal articles in "Social Science" and "Mathematics" subject areas were included, and Editorial materials, conference proceedings, books, book chapters, letters, notes, and review articles were excluded. The study employed a descriptive research model to identify the bibliometric properties of the journal. Mathematical and statistical methods were used to analyse characteristics of scientific publications. The data was analysed using the R-Studio program, specifically the biblioshiny web interface. After analysis the researcher found that interest in RME research has increased since 2016, and the USA, Indonesia, and the Netherlands were the most productive countries. Altaylar and Kajak (2021) In their research paper, "*The Effect of Realistic Mathematics Education on Sixth-Grade Students'Statistical Thinking," focus on* the effectiveness of the Realistic Mathematics Education (RME) approach on sixth-grade students' statistical thinking. A quasi-experimental pretest-post-test design was implemented using Mooney's framework, which outlines four thinking levels across statistical processes. The experimental group learned the data handling unit through the RME approach, while the control group received traditional textbook-based instruction.

The study involved 49 sixth-grade students, with 25 in the experimental group (9 males, 16 females) and 24 in the control group (10 males, 14 females). The data collection instrument was a statistical thinking test consisting of seven open-ended questions, some of which were adapted from previous studies. The data collected from the statistical thinking test (both pretest and post-test) were analyzed using Mooney's statistical thinking framework. The study found that both the RME approach and traditional textbook lessons were effective in developing students' statistical thinking. However, detailed analyses revealed that the RME approach was more beneficial in increasing students' statistical thinking levels. Doli and Armiati (2020) In their journal "*Development of Mathematics Learning Tools Based on Realistic Mathematics Education for Vocational High School Students*" emphasize the development of mathematics learning tools. The research was conducted in the mathematics department at the University Negeri Padang, Padang, Indonesia. The teacher must design a mathematics learning strategy in the form of a mathematics learning tool in the form of a learning implementation plan and student worksheet based on realistic mathematics education. This type of research used is development research, and the development model is the plump model. The subjects of this study were students of the state vocational high school 4 RambahKab. The research belongs to descriptive qualitative data, and the data analysis technique used is descriptive. To analyze the results of interviews, observations, and questionnaire responses of students. The study found that the development of mathematics learning tools based on realistic mathematics education was valid and practical and used to increase students' interest in learning mathematics in vocational high school, especially office management expertise programs.

## OBJECTIVES OF THE STUDY

The objectives for the study are as follows:

1. To find out the awareness of realistic mathematics education among middle school students.
2. To find out the awareness of Realistic Mathematics Education among middle school students based on gender.
3. To find out the awareness of Realistic Mathematics Education among middle school students regarding locale.

**Hypothesis of the study**

**$H_{01}$:** There is no significant difference in mean scores of awareness towards Realistic Mathematics Education between male and female middle school students.

**$H_{02}$:** There is no significant difference in mean scores of awareness towards Realistic Mathematics Education between rural and urban middle school students.
**$H_{03}$:** There is no significant difference in mean scores of awareness towards realistic mathematics education between rural male and rural female middle school students.
**$H_{04}$:** There is no significant difference in mean scores of awareness towards Realistic Mathematics Education between rural male and urban male middle school students.
**Table 5, $H_{05}$:** There is no significant difference in mean scores of awareness towards Realistic Mathematics Education between urban male and urban female middle school students.
**H06:** There is no significant difference in mean scores of awareness towards realistic mathematics education between rural female and urban female middle school students.

## METHODOLOGY OF THE STUDY

**Method of the study:** The present study was quantitative research. In the present study, the descriptive survey research method was used.

**Sample selection:** The sample for the present study consisted of 121 male middle school students and 105 female middle school students from the Shahjahanpur district of Uttar Pradesh. The random sample techniques used for the study were selected from male and female middle school students in the Shahjahanpur district of Uttar Pradesh.

**Tool of the Study:** The tool of this study was Awareness of Realistic Mathematics Education for Middle School Students, developed by the researcher with the help of a supervisor.

## RESULT AND DISCUSSION

In this study, both descriptive and inferential statistical techniques were used to analyze data. Descriptive statistics techniques are the mean and standard deviation, and inferential statistics techniques are the "t-test".

**Table 1, $H_{01}$:** There is no significant difference in mean scores of Awareness towards Realistic Mathematics Education between Male and Female Middle School Students.

**Interpretation:** From Table 1, it indicates that the mean scores of awareness towards realistic mathematics education between rural and urban middle school students differ significantly. The null hypothesis is that there is no significant difference in mean scores of awareness towards realistic mathematics education between male and female middle school students. Further, the mean scores of awareness towards realistic mathematics education of male middle school students are 102.12, which is significantly higher than those of female middle school students, whose mean scores of awareness towards realistic mathematics educationare97.31. It is found that with df = 224, the critical ratio (table value) is 1.97 at the 0.05 level of significance. The t-value (calculated value) is 2.76, which is greater than the critical ratio at the 0.05 level of significance. So, the test statistic 't' is significant at the 0.05 level. Hence, the null hypothesis is "rejected", and it is significant. It may therefore be said that male middle school students were found to have significantly higher awareness towards Realistic Mathematics Education as compared to female middle school students.

**Table 2, $H_{02}$:** There is no significant difference in mean scores of Awareness towards Realistic Mathematics Education between Rural and Urban Middle School Students.

**Interpretation:** From Table 2, it can be seen that the Critical Ratio (Table Value) is 1.98, which is not significant at the 0.05 level with df=224.It indicates that the mean scores of awareness towards realistic mathematics education between rural and urban middle school students don't differ significantly. The null hypothesis is that there is no

significant difference in mean scores of awareness towards realistic mathematics education between rural and urban middle school students. Further, the mean scores of awareness towards realistic mathematics education for rural middle school students and urban middle school students are 101.12 and 98.12, respectively, which are almost similar to each other for rural and urban middle school students. It is found that with df = 224, the critical ratio (table value) is 1.97 at the 0.05 level of significance. The t-value (calculated value) is 1.74, which is less than the critical ratio at the 0.05 level of significance. So, the test statistic 't' is not significant at the 0.05 level. Hence, the null hypothesis is "not rejected". Therefore, it is seen that both rural and urban middle school students are aware of realistic mathematics education. So, the null hypothesis is not rejected, i.e., there is no significant difference in mean scores of awareness towards realistic mathematics education between rural and urban middle school students.

**Table 3, $H_{03}$:** There is no significant difference in mean scores of Awareness towards Realistic Mathematics Education between Rural Male and Rural Female Middle School Students.

**Interpretation:** From Table 3, It indicates that the mean scores of awareness towards realistic mathematics education between rural male and rural female middle school students don't differ significantly. The null hypothesis is that there is no significant difference in mean scores of awareness towards Realistic Mathematics Education between rural male and rural female middle school students. Further, the mean scores of awareness towards realistic mathematics education for rural male middle school students and rural female middle school students are 102.95and 99.41, respectively, which are almost similar to each other for rural male and rural female middle school students. It is found that with df = 133, the critical ratio (table value) is 1.98 at the 0.05 level of significance. The t-value (calculated value) is 1.40, which is less than the critical ratio at the 0.05 level of significance. So, the test statistic 't' is not significant at the 0.05 level. Hence, the null hypothesis is "not rejected". Therefore, it is seen that both rural male and rural female middle school students are aware of Realistic Mathematics Education. So, the null hypothesis is not rejected, i.e., there is no significant difference in mean scores of awareness towards realistic mathematics education between rural male and rural female middle school students.

**Table 4, $H_{04}$:** There is no significant difference in mean scores of Awareness towards Realistic Mathematics Education between Rural Male and Urban Male Middle School Students.

**Interpretation:** From table-4, It indicates that the mean scores of Awareness towards Realistic Mathematics Education between Rural Male and Urban Male Middle School Students don't differ significantly. The null hypothesis is that there is no significant difference in mean scores of Awareness towards Realistic Mathematics Education between Rural Male and Urban Male Middle School Students. Further, the mean scores of Awareness towards Realistic Mathematics Education for rural Male middle school students are 102.95 and urban male middle school students are 101.25, respectively, which are almost similar to each other for Rural Male and Urban Male Middle School Students. It is found that with df = 119, the critical ratio (table value) is 1.98 at the 0.05 level of significance. The t-value (calculated value) is 0.70, which is less than the critical ratio at the 0.05 level of significance. So, the test statistic 't' is not significant at the 0.05 level. Hence, the null hypothesis is "not rejected", Therefore, it is seen that both Rural Male and Urban Male Middle School Students are aware of Realistic Mathematics Education. So, the null hypothesis is not rejected, i.e. there is no significant difference in mean scores of Awareness towards Realistic Mathematics Education between Rural Male and Urban Male Middle School Students.

**Table 5, $H_{05}$:** There is no significant difference in mean scores of Awareness towards Realistic Mathematics Education between Urban Male and Urban Female Middle School Students.

**Interpretation:** From table-5, It indicates that the mean scores of Awareness towards Realistic Mathematics Education between Urban Male and Urban Female Middle School Students differ significantly. The null hypothesis is that there is no significant difference in mean scores of Awareness towards Realistic Mathematics Education between Urban Male and Urban Female Middle School Students. Further, the mean scores of Awareness towards Realistic Mathematics Education of Urban male Middle School Students are 101.25, which is significantly higher than those of

Urban female Middle School Students, whose mean scores of Awareness towards Realistic Mathematics Educationare93.11. It is found that with df =89, the critical ratio (table value) is 1.99 at the 0.05 level of significance. The t-value (calculated value) is 3.84, which is greater than the critical ratio at the 0.05 level of significance. So, the test statistic 't' is significant at the 0.05 level. Hence, the null hypothesis is "rejected". It may therefore be said that Urban Male middle school students were found to have significantly higher awareness towards Realistic Mathematics Education as compared to Urban female Middle School Students.

**Table 6, $H_{06}$:** There is no significant difference in mean scores of Awareness towards Realistic Mathematics Education between Rural Female and Urban Female Middle School Students.

**Interpretation:** From table-6, It indicates that the mean scores of Awareness towards Realistic Mathematics Education between Rural Female and Urban Female Middle School Students differ significantly. The null hypothesis is that there is no significant difference in mean scores of Awareness towards Realistic Mathematics Education between Rural Female and Urban Female Middle School Students. Further, the mean scores of Awareness towards Realistic Mathematics Education of Urban Female Middle School Students are 102.12, which is significantly higher than those of Urban female Middle School Students, whose mean scores of Awareness towards Realistic Mathematics Education are 113.88. It is found that with df = 103, the critical ratio (table value) is 1.98 at the 0.05 level of significance. The t-value (calculated value) is 2.76, which is greater than the critical ratio at the 0.05 level of significance. So, the test statistic 't' is significant at the 0.05 level. Hence, the null hypothesis is "rejected". It may therefore be said that Rural Female middle school students were found to have significantly higher awareness towards Realistic Mathematics Education as compared to Urban female Middle School Students.

## DISCUSSION

The findings of the present study are consistent with several previous studies on Realistic Mathematics Education. The overall reasonable level of awareness among middle school students reflects the growing global attention toward RME, as highlighted in the bibliometric analysis by Bayrak and SeherAslanci (2022), who reported a significant increase in RME research worldwide, particularly after 2016.The significant gender difference found in the present study, where male students showed higher awareness than female students, partially aligns with the findings of Altiner (2024). The study suggested that classroom interaction patterns and strategy use may vary among students, which could explain differences in awareness levels observed in the current research. As well as Ramadhanta *et al*. (2024) found that RME enhances active participation and creative thinking, particularly when learning shifts from teacher-centred to student-centred methods. If classroom participation opportunities differ by gender, it may influence awareness levels, as seen in this study. The finding that there is no significant difference between rural and urban students in overall awareness is noteworthy and suggests that exposure to contextual mathematics may not be limited by geographical location. This aligns with the work of Irawan *et al*. (2024). The significant difference between urban male and urban female students, and between rural female and urban female students, suggests that gender-based disparities are more visible in urban contexts. This observation can be connected to the findings of Samková *et al*. (2025). The present findings support the broader literature that emphasises the importance of contextual learning, active participation, and teacher guidance in strengthening students' mathematical understanding. While many prior studies have focused on achievement, creativity, and literacy outcomes, the current study contributes by highlighting awareness as a foundational factor for successful RME implementation. The results tell us that we need to do more to teach students about Realistic Mathematics Education so that all students can learn math in a fair and meaningful way**.**

## CONCLUSION

The Present study conducted to the analyze the level of awareness level of awareness towards Realistic Mathematics Education among middle school students. On the basis of statistical analysis and interpretation of the collected data

overall level of awareness towards Realistic Mathematics Education among middle school students is satisfactory, on average. A small percentage of students fall under the "Not Aware" category. The majority of students fall under the categories of Moderately Aware, which shows positive exposure to contextual and activity-based mathematics learning. Male students show comparatively higher awareness scores than female students according to the given data. Rural students show slightly higher awareness than urban students, though the difference is not very large. The results tell us that we need to do more to teach students about Realistic Mathematics Education so that all students can learn math in a fair and meaningful way.

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**Table 1: $H_{01}$: There is no significant difference in mean scores of Awareness towards Realistic Mathematics Education between Male and Female Middle School Students.**

| GENDER | N | MEAN | SD | $\sigma_D$ | t- Value | df | CR |
|---|---|---|---|---|---|---|---|
| Male | 121 | 102.17 | 13.31 | 1.76 | 2.76 | 224 | 1.97 |
| Female | 105 | 97.31 | 13.12 | | | | |

**Table 2: $H_{02}$: There is no significant difference in mean scores of Awareness towards Realistic Mathematics Education between Rural and Urban Middle School Students.**

| LOCATION | N | MEAN | SD | $\sigma_D$ | t- Value | df | CR |
|---|---|---|---|---|---|---|---|
| Rural | 135 | 101.12 | 14.69 | 1.72 | 1.74 | 224 | 1.97 |
| Urban | 91 | 98.12 | 11.09 | | | | |

**Table 3: $H_{03}$: There is no significant difference in mean scores of Awareness towards Realistic Mathematics Education between Rural Male and Rural Female Middle School Students.**

| RURAL GENDER | N | MEAN | SD | $\sigma_D$ | t- Value | df | CR |
|---|---|---|---|---|---|---|---|
| Male | 65 | 102.95 | 14.87 | 2.52 | 1.40 | 133 | 1.98 |
| Female | 70 | 99.41 | 14.42 | | | | |

**Table 4: $H_{04}$: There is no significant difference in mean scores of Awareness towards Realistic Mathematics Education between Rural Male and Urban Male Middle School Students.**

| LOCATION WISE MALE | N | MEAN | SD | $\sigma_D$ | t- Value | df | CR |
|---|---|---|---|---|---|---|---|
| Rural Male | 65 | 102.95 | 14.87 | 2.38 | 0.70 | 119 | 1.98 |
| Urban Male | 56 | 101.25 | 11.30 | | | | |

**Table 5: $H_{05}$: There is no significant difference in mean scores of Awareness towards Realistic Mathematics Education between Urban Male and Urban Female Middle School Students.**

| URBAN WISE GENDER | N | MEAN | SD | $\sigma_D$ | t- Value | df | CR |
|---|---|---|---|---|---|---|---|
| Male | 56 | 101.25 | 11.30 | 2.12 | 3.84 | 89 | 1.99 |
| Female | 35 | 93.11 | 8.79 | | | | |

**Table 6: $H_{06}$: There is no significant difference in mean scores of Awareness towards Realistic Mathematics Education between Rural Female and Urban Female Middle School Students.**

| LOCATION WISE FEMALE | N | MEAN | SD | $\sigma_D$ | t- Value | df | CR |
|---|---|---|---|---|---|---|---|
| Rural Female | 70 | 99.41 | 14.42 | 2.28 | 2.76 | 103 | 1.98 |
| Urban Female | 35 | 93.11 | 8.79 | | | | |

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REVIEW ARTICLE

# Nutraceutical Intervention of *Methi* (*Trigonella foenum-graecum*) in the Management of *Prameha* (Diabetes Mellitus): A Critical Review

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## ABSTRACT

Diabetes mellitus closely resembles the ayurvedic disease process of prameha and it has turned into a chronic disorder of metabolism that needs safe and complementary treatment measures besides the administration of conventional drugs. It was a critical review of scientific evidence existing on the use of methi or fenugreek (*Trigonella foenum-graecum*) as a nutraceutical supplement in the management of the many facets of prameha. The three main bioactive components of fenugreek are the main mechanism of its action. All these bioactive components act separately to resolve the central pathophysiological problems of type 2 diabetes mellitus. First, galactomannan is a water-soluble dietary fiber that is capable of prolonging the time taken by the stomach to empty and flatten the post prandial glucose (ppg) response curve. Second, 4-hydroxyisoleucine (4-oi) is an uncommon non-proteinic amino acid mostly found in the fenugreek seeds and functions an insulin secretagogue that enhances periphery insulin sensitivity. Lastly, there is a lipid lowering effect or medohara of fenugreek caused by steroidal saponins and trigonelline. The clinical trials have consistently shown improvements in glycemic control with the daily supplementation of 10g to 25g of ground fenugreek seeds by lowering the levels of fasting plasma glucose and also by a small but statistically significant decrease of glycated hemoglobin (HbA1C) (0.5 percent to 1 percent after 8 to 12 weeks). Although, clinical trials have demonstrated that this supplementation also led to considerable decreases in total levels of cholesterol and triglycerides that

reduced diabetic dyslipidemia. In general, as the most prevalent side effects are mild gastrointestinal disorders, which are not harmful but may be common with many other medications, methi offers a scientifically justified, cost-efficient, and safe way of helping in the long-term management of prameha. Further studies are to be done to create and study extracts of methi that are standardized to the degree of active components (in this case 4-oi), to offer some kind of uniformity in efficacy.

**Keywords:** Fenugreek (*Trigonella foenum-graecum*), *Prameha* (Diabetes Mellitus),4-Hydroxyisoleucine (4-OH Ile), Glycemic Control, Dyslipidemia (Lipid Profile/*Medohara*), Nutraceutical.

## INTRODUCTION

**Background of *prameha* and diabetes mellitus**

Diabetes mellitus (DM) is a persistent metabolic disease that is marked by a constant increase in blood glucose levels caused by deficiencies in the insulin secretion, insulin action, or both, is a universal epidemic. This is similar to the Ayurveda system of medicine in ancient Indian tradition and is known as *Prameha*, which is a condition characterized by *Kleda* (excess moisture/fluidity) and Mutra Vriddhi (polyuria). The most severe type is expected to be *Madhumeha* (literally 'honey urine'), and it directly relates to DM. The existing pharmacological treatment, although effective, usually bears risks of adverse effects and cannot cover all the metabolic derangements, which requires the development of effective, low-cost, and safe complementary interventions[1].

**Methi as a traditional remedy**

*Methi* (Fenugreek, *Trigonella foenum-graecum*) is a herb that is used as spice and traditional medicine widely in South Asia and Middle East annually. Both Ayurvedic and Unani medicine have traditionally suggested it as an Ayurvedic *Madhumehahara* (anti diabetic) and *Medohara*(lipid lowering/anti-obesity) agent[2]. It is a good candidate of a nutraceutical intervention in the treatment of *Prameha* due to its easy accessibility, affordability, and effective safety profile.Dyspnoia Fenugreek seeds are pebbly hard, compact, rhomboidal bodies that are about 3-5mm long and about 2mm thick. They are normally light brown to yellow in color. The seeds have a characteristic distinctive smell of spicy aroma and bitter, mucilaginous taste. Fenugreek seeds are nutritionally balanced with carbohydrates of about 45-60 percent with almost a third of it being soluble fiber and the rest insoluble fiber. They are also 20-30 percent protein, but contain essential amino acids like lysine and tryptophan, but contain volatile oils in small amounts. [3,4,5]

**Fenugreek nutritional value per 100 g -** (Table 1)

**Percent daily value of selected nutrients-**(Table 2)

## REVIEW OBJECTIVE

The present review critically analyzes and synthesizes the available scientific data on *Methi*(*Trigonella foenum-graecum*), especially in relation to its bioactive constituents, mechanism of action and clinical efficacy. This is aimed at investigating its feasibility as a nutraceutical intervention towards improving glycemic control and lipid parameters in the subjects of *Prameha*(Type 2 Diabetes Mellitus).

## METHODS

**Search strategy and data sources**

The literature search has been carried out in big electronic databases, such as PubMed, Cochrane, Science Direct, and Google Scholar. The following major keywords and combinations were used to conduct the search: "Fenugreek," "Methi," "*Trigonella foenum-graecum*," "Diabetes Mellitus," "*Prameha*," "Nutraceutical," "Hypoglycemic," and "Anti-

diabetic." The articles that were included in the search were published mostly in the period of 2000-24 so that the current clinical information could be considered.

**Selection criteria**

**Inclusion criteria for the review were**

1. Original research articles and systematic reviews/meta-analyses that are concerned with the effect of Fenugreek (seeds, powder or extract) in human subjects (adults) with DM or pre-diabetes (analogous to Prameha).
2. Studies reporting clinical outcomes related to glycemic indices (FPG, PPG, HBA1C or lipid profile (TC, TG, HDL-C, LDL-C).
3. Articles published in the English language.

The literature on other combinations of herbs, animal models alone, or lacking quantifiable clinical evidence were excused.

**Data synthesis and analysis**

The qualitative synthesis and critical analysis of the data on the chosen articles were conducted. The review aimed at extracting homogenous results about dose-response relationship, the key bioactive molecules that are involved and their affirmed mechanisms of action to provide scientifically reasonable basis of nutraceutical role of *Methi* in *Prameha* management.

## RESULTS

**Bioactive Components and Mechanisms of Action**

Fenugreek's therapeutic efficacy is attributed to a synergistic combination of unique chemical constituents[6]:

**Soluble Dietary Fiber**

The soluble dietary fibre (SDF), predominantly Galactomannan one of the types of heteropolysaccharides which constitute almost half of the seed dry weight is also abundant in the seeds. The fiber creates a viscous material in the GI tract which slows the gastric emptying and acts as a physical barrier to the fast uptake of glucose, hence flattening the PPG curve[7].

**4-Hydroxyisoleucine (4-OH Ile)**

The uncommon amino acid, 4-OHIle, is believed to be the most active antidiabetic principle. It works primarily by incretion of the pancreatic b-cells for glucose-dependent insulin release [8]. It also has been demonstrated to increase insulin sensitive in peripheral tissues, which directly targets the underlying pathophysiology of Type 2 DM (Insulin Resistance in Prameha).

**Saponins and alkaloids**

Saponins are steroidal (e.g., diosgenin) and alkaloid (e.g., Trigonelline) and both create hypoglycemic and hypolipidemic effects. Trigonelline was also found to stimulate the control of enzymes that participate in glucose metabolism in the cells and the saponins also disrupt the absorption of cholesterol, which helps in controlling the condition of diabetic dyslipidemia[9].

**Clinical efficacy on glycemic control: (Table 3)**

A study involving 10g/dayof Fenugreek seed powder in T2DM patients noted a significant drop in FPG and HBA1C after 6 months[10] Another long-term study (3 years) in pre-diabetic subjects showed that 10 g/day of Fenugreek powder significantly reduced the cumulative incidence of developing Type 2 DM, highlighting its preventative potential in *Prameha*[12].

**Clinical efficacy on lipid profile**

*Methi* has also been shown to have the *Medohara* (lipid-lowering) effect which has been clinically supported, and has been shown to be found in meta-analyses as a treatment in the management of diabetic dyslipidemia, a significant precipitating factor in the development of macrovascular complications in Prameha:

**TG and TC:** A decrease in mean TG (14%) and in mean total cholesterol 21% with 10 g/day, was reported among the different trials.

**HDL-C:** Although there is also a trend for LDL-C reduction, some significantly have an HDL-C increase, which elevates the atherogenic index counteraction [11].

## DISCUSSION

**Methi as a multi-modal nutraceutical in prameha**

This present review has positive results that can help in substantiating the traditional claim of *Methi*(*Trigonella foenum-graecum*) in the treatment of *Prameha*. *Prameha* has been described according to Ayurveda as the systemic metabolic disorder with vitiated *Kapha dosha*, deranged *Meda dhatu*, deposited *kleda* (due to the excesses of the nutrients), impaired Agni and *Srotorodh*. Its stage, *Madhumeha*, is a similar stage to Type 2 Diabetes Mellitus (T2DM). *Methi* introduces a Holistic Window of Medicinal profile of Lens in conformity to the classical Ayurveda Aspects and as an extent to bio-medical Aspects. Fenugreek as an amalgamate nutraceutical should be considered in the context of multi-modality of multiple pathophysiological mediators that are involved in the course of T2DM/*Prameha*[6]. Unlike single-target drugs, it also controls glucose absorption, insulin secretion, insulin receptor and lipid metabolism simultaneously. Such general metabolic normalisation is analogous to the idea of restoration of balance in the body as opposed to Ayurveda which aims to put specific symptoms into remission. The water-soluble portion of dietary fiber especially galactomannan plays a significant physiologic role in glycemia regulation. On ingestion, it solidifies in the stomach due to the type of composition that prevents emptying of the stomach and decelerates the uptake of carbohydrates. This also adds significant flattening of the postprandial glucose curve [7]. According to Ayurveda, this exercise would be linked to stabilization of deranged *Jatharagni*, and inhibition of production of Ama (a final cause of metabolic impediment, *Srotorodha*). The effects of dampening sharp glycemic fluctuations and reducing the stress on the functionality of pancreatic beta cells in the metabolic defense of fenugreek are attributable. The other major bioactive is 4-hydroxyisoleucine that has glucose-dependent insulinotropic activity.

It triggers the release of insulin glucose-dependently to minimize the chances of hypoglycemia occurrence [8]. It also increases the peripheral insulin sensitivity the major malfunction of insulin resistance in T2DM [8]. Ayurvedic theorizing It can be postulated in Ayurvedic terms, that *Dhatvagni* can be associated with the correction of the malfunction at *Dhatvagni* level, particularly of Meda dhatu, and remedy of the pathology of the disease by *Kapha-Meda vriddhi*. Fenugreek has curative effects at various metabolic levels because of its action on the kinetics of insulin and insulin tissue sensitivity. This therapeutic value is also enhanced by the presence of hypolipidemic properties of fenugreek. Steroidal saponins and triglycerides decrease serum total cholesterol and triglycerides [6]. The outcomes of clinical trials and meta-analysis indicate improved lipids especially total cholesterol (TC) and triglyceride level (TG), especially with a 10-25 g/day supplementation [11,13]. Because dyslipidemia is among the major causes of macrovascular complications in diabetes, this *Medohara* effect appears to be a significant preventive activity. The improvement in lipid profile may be attributed to normalization of aggravated *meda dhatu* and obstruction of *srotas* resulting to reduction of the risk of cardiovascular disorders in the future. There is clinical support of the enhancement of glycemic indices by the use of fenugreek supplements. Fasting plasma glucose (FPG) and postprandial glucose (PPG) reductions have been reported and a moderate yet clinically significant reduction in HbA1c (0.5-1.0% over 8-12 weeks) has been reported [10,13]. The dosage range of 10-25 g per day of powdered seed or the equivalent health-wise in extract is said to be very successful [10,13]. These results provide strong evidence to support its use as an addon in the management of chronic glycemia.

**Integrative relevance and long-term applicability**

It is also safe to consume over extended periods, and this is one of the other reasons why fenugreek is so great. Daily dietary intake of it does not require much lifestyle change as, as a dietary everyday spice, it is regularly consumed. This has been found to enhance patient compliance as compared to the use of chemicals. Even after cooking it has been reported that there are traces of its hypoglycemic effect and it can be consumed as a food [2]. Fenugreek should be viewed as complementary to conventional therapy rather than alternative therapy according to the integrative medicine perspective. Due to its multi-targeted metabolic action, it will potentially work synergistically with the oral hypoglycemic agonists (OHAs) and provide superior glycemic control without having to use an increased pharmacological dose. With this fact, however, it is not surprising at all when we realize that the management of Prameha according to Ayurveda requires simultaneous approach towards Ahara and Vihara as well as towards Aushadha. It is also an impressive aspect that it can be used preventively. A prediabetic long-term intervention trial observed that the overt T2DM development was reduced significantly by the daily treatment of fenugreek [12]. It shows one has a role as an early metabolic correcting agent, like in the case of persons of *Kapha-pradhana Prameha*. Earlier intervention of the individuals experiencing compromised glucose tolerance can delay the onset of the disease and reduce its effects. Safety plays an important role in the management of chronic diseases. Fenugreek is described to be safe in all the available clinical literature where the adverse effect is mostly of mild gastrointestinal symptoms such as bloating and flatulence [13]. Such effects tend to be self-limiting and of its fiber content. Most importantly, human trials fail to identify any clinically significant organs toxicity manifestations that would prove its safety as a long-term usage.

## LIMITATIONS AND FUTURE DIRECTIONS

Even though there is positive news on the variable phytochemical composition, it is also a demerit. Seed cultivar, geographical origin and processing techniques have a varied potential to affect the contents of bioactive molecules in particular, 4-hydroxyisoleucine [8]. The variability in this case has the potential to affect the uniformity of the treatment. Although extracts have been utilized in the 200 studies and clinical trials reported, future investigations should tend towards standardization of the extracts that determine the important bioactive factors. Prolonged, multicentric RCTs involving long-term follow-up are needed to investigate the long-term cardiovascular and microvascular advantages. Further protection effects in diabetic complications may be explained by more mechanistic studies on the basis of anti-inflammatory and antioxidant pathways. The synergistic effects of fenugreek with other *Pramehaghna dravyas* or in the combination with structured lifestyle modifications should be researched to achieve the better results. These multi-ingredient methods have been aligned with Ayurvedic standards of classical methods of managing diseases. To sum up, *Methi* (*Trigonella foenum-graecum*) is a scientifically supported multidiabetes nutraceutical intervention plan of *Prameha*. It possesses a multitarget effect with the capacity to regulate glucose absorption, improve insulin intake and sensitivity and improve lipid parameters thereby treating multiple elements of metabolic derangement [6,10,13]. Fenugreek is potentially a safe, inexpensive, and effective adjuvant or addition to the overall diabetes treatment programme with proper normalizing and rigorous clinical testing.

## CONCLUSION

Scientific supports *Methi* (*Trigonella foenum-graecum*) comes out as well-supported nutraceutical with useful therapeutic implication to address *Prameha*and its clinical manifestation, Type 2 Diabetes Mellitus. Both experimental information and clinical research evidence indicate that fenugreek positively interferes with a number of metabolic processes. The postprandial glucose regulation activity of soluble fiber as galactomannan is identified to contribute to the delay of carbohydrate absorptions in the presence of glucose dependent insulin secretion to improve the peripheral insulin sensitivity of 4-hydroxyisoleucine. This cumulatively makes contributions towards measurable decreases in the fasting blood glucose, after meal effects and HbA1c. Fenugreek, together with hypoglycemic control, also demonstrates a stable lipid -lowering property primarily on total cholesterol and triglyceride levels suggesting a

healing effect of diabetic dyslipidemias and associated risk of Cardio-Vascular Disease. It has a good safety profile, as it had with the previous formulation, with minor and mild gastrointestinal side effects. Allowing normal levels of dietary intake of the standardized extract of fenugreek seed may prove to be a beneficial supplement to individuals who are on conventional antidiabetic treatment due to its safety, availability, and acceptability. *Methi*, as a component of a comprehensive approach to treatment, offers a logical, evidence based and cost-effective method of ensuring good metabolic control in the lives of diabetic patients.

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**Table 1: Fenugreek nutritional value per 100 g**

| S.L No. | Nutrient | Amount per 100g |
|---|---|---|
| 1 | Calorie | 323 kcal |
| 2 | Total Fat | 6g, |
| 3 | Saturated Fat | 1.5gm |
| 4 | Cholesterol | 0 gm, |
| 5 | Sodium | 67mg |
| 6 | Potassium | 770 mg, |
| 7 | Dietary Fiber | 25gm |
| 8 | Total Carbohydrate | 58gm |
| 9 | Protein | 23gm |

**Table 2: Percent daily value of selected nutrients**

| S.L.No | Nutrient | Percent daily value (%) |
|---|---|---|
| 1 | Vitamin C | 5 |
| 2 | Iron | 186 |
| 3 | Vitamin B6 | 30 |
| 4 | Magnesium | 47 |
| 5 | Calcium | 17 |
| 6 | Vitamin D | 0 |
| 7 | Vitamin B12 (Cobalamin) | 0 |

*Percent Daily Values are calculated based on a standard 2,000 kcal/day diet. Source: United States Department of Agriculture (USDA).

**Table 3: Clinical trials consistently demonstrate the beneficial impact of Fenugreek on glycemic parameters:**

| Outcome Parameter | Observed Effect | Example Citation |
|---|---|---|
| Fasting Plasma Glucose (FPG) | Significant reduction (5-15%) across most trials. | [10] |
| Postprandial Glucose (PPG) | Marked reduction, often more pronounced than FPG reduction. | [7, 11] |
| Glycated Heamoglobin(HBA1C) | Modest but significant decrease (0.5% - 1.0%-point reduction) over 8-12 weeks. | [13] |
| Insulin Resistance (HOMA-IR) | Reduction observed, supporting the role of 4-OH Ile in improving sensitivity. | [8] |

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**RESEARCH ARTICLE**

# Role and Responsibility of Swachh Bharat - Swachh Vidyalaya : A National Campaign towards Sustainable Development in Indian School Education

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**ABSTRACT**

The 21st century's most pressing global issue is climate change spurred by the ongoing rise in greenhouse gas concentrations, leading to an urgent global catastrophe that transcends national and international borders. Aligned with SDG 13.3 of the UN Agenda for Sustainable Development, which emphasizes climate education and capacity building, India launched the Swachh Bharat Mission(SBM) in 2014, which intends to improve carbon sequestration through tree planting, scientifically manage solid and liquid waste, and avoid contaminating groundwater.The Indian Ministry of Education implemented Swachh Bharat–Swachh Vidyalaya (SWSV) in schools to complement SBM and include students in hands-on environmental conservation. The study evaluatesthe Swachh Vidyalaya's role in achieving SDG 13.3 through policies, ecological initiatives, and programs. findings highlight Swachh Vidyalaya's effectiveness insensitizing young minds, encouraging eco-friendly behaviour, and raising community awareness, all of which have a ripple effect that extends from the local to the global level and promotes climate adaptation and mitigation by supporting SBM objectives. The Swachh Bharat Mission steps towards a revolutionary India, a pollution-free, green, and clean country fostering national pride and global citizenship. As part of SBM, Swachh Vidyalaya made an enormous imprint on Indian society by instilling a sense of responsibility among future generations

**Keywords:** Swachh Vidyalaya, Swachh Bharat Mission, Sustainable Development Goal, Climate Action.

# INTRODUCTION

**Untreated Waste: A silent cause of climate change**

The 21st century's most pressing global issue is the climate change (Alexandru et al., 2013). Our mother Earth faces enormous pressure from anthropogenic activities inducing carbon dioxide and its allied gases into the Earth's atmosphere. One of the significant effects of Climate change is global warming spurred by the ongoing rise in greenhouse gas concentrations, which has led to an urgent global catastrophe that transcends national and international borders(*Climate Action - United Nations Sustainable Development*, n.d.). Carbon dioxide ($CO_2$), methane ($CH_4$), nitrous oxide ($N_2O$), hydrofluorocarbons (HFCs), perfluorocarbons (PFCs), and sulfur hexafluoride ($SF_6$) are the six greenhouse gases (GHGs) that are listed for elimination in Annex A of the Kyoto Protocol in 1997(Seo, 2017). Since industrialization, carbon dioxide ($CO_2$) gas emissions have surged due to increased energy demand from growing populations and urbanization, including emerging economies such as China, India, and Thailand that rely on fossil fuels and crude oil products(Nwofe, 2014).Nevertheless, Greenhouse gases are traced directly from untreated solid waste (Bio-degradable and non-biodegradable) in open dumpsites from various waste sources like homes, schools, public places, etc(Ramachandra TV., 2009). Ramachandra et al., (2018)argued that approximately 60% of the municipal solid trash the agencies collect is disposed of at designated locations; the remainder is disposed of at unregulated disposal sites, which has adverse environmental effects, including greenhouse gas emissions. Even this improper and uncontrolled disposal of all waste types, especially organic waste, causes ecological issues such as nutrient leaching that contaminates the land, water, and soil.The majority of developing nations manage their trash using extremely antiquated techniques, which has the effect of making the environment more hazardous for human existence.In addition to contributing to climate change, unsustainable waste management significantly harms the ecosystem(Nwofe, 2014). Researchers estimate that 19 to 23 million tons of plastic trash are dumped into oceans and water bodies, causing harm to 1,500 species such as fish, whales, turtles, and birds, which frequently consume, become sick, or die from toxic plastic waste, is one of the leading causes of wildlife extinction(*How Our Trash Impacts the Environment - Earth Day*, 2024). Poor waste management, such as open incineration and decomposition in the dumpsites, produces more nitrous oxides and methane in addition to open exposure of human excreta, which contaminates groundwater and pollutes water bodies and becomes a severe challenge to climate change. Methane has twenty-five times the potential to warm the world of carbon dioxide and nitrous oxide and warm Mother Earth over half of 1 degree Celsius. Besides that, high ammonia concentrations in the air can also induce acid. Sustainable waste management is essential to mitigating the effects of global climate change (Nwofe, 2014).

**Climate Change Mitigation efforts in the Global context**

Rapidly reducing $CO_2$ emissions and the corresponding socio-ecological changes can only be accomplished through intergenerational effort(Liebhaber et al., 2023). UNFCCC and its Kyoto Protocol have accomplished substantial goals, ignited a variety of national policies, and established new institutional mechanisms for mitigation action. Further, it legally binds 198 countries in Paris in 2015 to commit to lowering global greenhouse gas emissions to limit global temperature rise to below 2°C above pre-industrial levels and responding to climate change impacts while fostering sustainable development. It establishes a framework for developed nations to aid developing nations while ensuring transparent monitoring of climate targets. Countries will submit updated reports to the Nationally Determined Contributions (NDCs) every five years, explaining strategies to reduce emissions and strengthen resilience against rising temperatures (*The Paris Agreement UNFCCC* n.d.).

**SDG 13: Climate Action**

Goal 13 of the Sustainable Development Agenda (SDG) is called "Climate Action." It is one of the 17 objectives set forward by the United Nations in their Agenda for Sustainable Development for 2030. SDG13's primary goal is to address climate change and its effects by acting quickly. It acknowledges that climate change is a global issue that must be addressed immediately and that governments, corporations, communities, and people everywhere must work together(Filho et al., 2023). Dhanapal et al., (2023) argued that taking urgent climate action to keep global

warming below the 1.5-degree threshold is imperative; developing and least-developed nations must prioritize climate action to meet their sustainable development goals by lowering climate risks, losses, and damages.Climate action plans are strategies for adaptation and mitigation with set objectives, budgets, timelines, and results. It can lessen financial losses and increase climate change resistance when properly applied. Even, the Conference of Parties (CoP 26) of the United Nations Framework Convention on Climate Change (UNFCCC) has called on nations to take climate action. However, to accomplish the climate action targets and the SDGs, climate action must be planned and carried out at many levels, from the national to the local. The global community acknowledges the relevance of training and education in addressing climate change. The Action for Climate Empowerment (ACE) agenda, the Paris Agreement, and the UN Framework Convention on Climate Change all urge governments to inform, empower, and include all significant groups and stakeholders in climate-related policies and initiatives. (*Climate Change Education UNESCO*, n.d.). The global goal SDG 13 is sectioned into four smaller objectives to spur against climate change. (*Goal 13: Climate Action - The Global Goals*, n.d.).

**Combating Climate Change through Education**

Climate Action for SDG13.3 focuses on mitigation and adaptation strategies to combat climate change through education, awareness campaigns, and capacity building of individuals and institutions(*Goal 13: Climate Action - The Global Goals*, n.d.). Educational institutions are significant players in the worldwide quest for sustainability, using their diverse contributions to advance the UN's Sustainable Development Goals (SDGs)(Abdalla et al., 2024). UNESCO defined climate change education as equipping individuals with the information, skills, values, and attitudes necessary to engage as change agents by assisting them in comprehending and addressing the effects of climate change. As part of its efforts to promote education for sustainable development, UNESCO advocates for climate change education. (Climate Change Education UNESCO, n.d.). Allen & Crowley, (2017) asserted that climate change necessitates a significant global response: people, communities, regions, and countries must all make significant changes to their visible habits and behaviours. Education is a crucial aspect of altering habits and behaviours. However, most modern climate change education focuses exclusively on individuals' understanding of climate science, which research finds has limited usefulness in enabling collective climate action. Howard-Jones et al., (2021)argued that Climate change Education (CCE) should be action-oriented to align with an action agenda. Action-oriented CCE may focus on individual behavior, such as students calculating and reducing their carbon footprint.Cantell et al., (2019) depicted climate action through the bicycle chains and pedals in the holistic model of climate change education, symbolizing activity on "real life issues," which is instrumental for transferring knowledge and thinking skills into practical action. However, Howard-Jones et al. (2021) suggested that Climate change-empowered students acting as change agents in their communities are more beneficial in mitigation (as cited by Kenis and Mathijs, 2012; Trott, 2019a).

**Indian Initiatives of Climate Action: Swachh Bharat Mission (SBM) or Clean India Mission**

India has been a critical participant and an active member of the UNFCCC (as cited in Dubash et al. 2018). Since 1980, India has implemented several national-level initiatives for community development and natural resource management. To combat the rising greenhouse gases, the Indian government launched the Swachh Bharat Mission or Clean India Mission in 2014 by scientifically managing solid and liquid waste, forbidding groundwater contamination, and increasing carbon sequestration through tree plantation missions. The Prime Minister of India, Narendra Modi, asserted, "A clean India would be the best tribute India could pay to Mahatma Gandhi on his 150th birthday in 2019". The Prime Minister led the widespread cleaning drive, motivating the populace, gave the Swachh Bharat Mission a nationwide boost, and advised people not to spread dirt themselves or allow others to do so. 'Na gandegi Karenga, nakarnedenge' turned the mission into Abhiyan or Jan Andolon. (*Major Initiatives Swachh Bharat Abhiyan*, n.d.). In addition to cleaning the environment, the Swachh Bharat Abhiyaan aims to get people involved by planting trees, removing waste from public areas, providing sanitary amenities, and building the foundations for Swachh Bharat in the future to make a clean and green India by modifying attitude and behavior (*Swachh Bharat Abhiyaan: Ek Kadam Swachhata Ki Ore*, n.d.). SBM Rural and SBM Urban were the two fronts of the mission run by the Ministry of Drinking Water and Sanitation and the jurisdiction of the Ministry of Housing and Urban Affairs(Dash & Dash, 2021). Consequently, the SBM has sectioned into two phrases:

**Swachh Bharat Mission (SBM) Phrase I ( 2014-2019)**

SBM aimed to nudge public behavior and included everyone in the collaborative endeavor of cleaning homes, iconic places, public places, streets, parks, beaches, water bodies, and adjacent areas. The Phrase- I SBM's goals (Rural) encompassed a) improving rural communities' overall quality of life by encouraging cleanliness, hygiene, and the abolition of open defecation; b) increasing rural sanitation coverage to realize the Swachh Bharat vision; c) encouraging communities to embrace sustainable sanitation facilities and practices by raising awareness and educating them about health issues; d) Support affordable and suitable technologies for environmentally safe and sustainable sanitation(VerKuilen et al., 2023). The Phrase- I SBM's goals (Urban) included i). Ending Manual Scavenging ii) managing Municipal Solid Waste in a Modern and Scientific Way iii) Expanding Urban Local Bodies' (ULBs') Capacity iv) To foster an environment that encourages private sector involvement in both capital expenditures and operation and maintenance(Choudhary & Gupta, 2015)

**Swachh Bharat Mission (SBM): Phrase II ( 2019-2025)**

In 2021, India advanced to the subsequent Swachh Bharat Mission Urban 2.0 and Rural 2.0 phase, successfully eliminating open defecation in all villages, gram panchayats, districts, states, and Union Territories by October 2019. Swachh Bharat Mission -Urban 2.0 focused on making all cities i) 100 % ''garbage-free'' through door-to-door waste collection, source segregation based on wet and dry waste categories from the households, gates in the wards, every day sweeping the public areas, bulk waste generator and no visible solid waste in water bodies and screening of stormwater- drains, ii) banning single use of plastics iii)scientific processing of municipal solid waste, iv).Controlling air pollution by converting dumpsites into green zones, vi) Digitally monitoring solid waste management operations and v). Geo-mapping waste processing facilities. (Payoja Ahluwalia, 2021). Phase II of Rural SBM aims to attain Sampoorn Swachhata (total cleanliness), which entails maintaining ODF status, managing solid and liquid waste, biogas energy production, information education, and Communication/Behavior Change and converting all villages from ODF to ODF Plus Model.(*Swachh Bharat Mission -Grameen (SBM): Phase II (2019-2025)*, n.d.)

**School Initiatives of Swachh Bharat Mission**

The Ministry of Education, India initiated Swachh Bharat- Swachh Vidyalaya or Clean India- Clean schools in 2014 as part of the Swachh Bharat Mission in every governmentschool to implement the action plan for climate change through various activities connected to the community /society.The primary objectives of Swachh Vidyalaya in the lens of climate action enlisted are:

i. Promote cleanliness by providing proper infrastructure and clean and safe water,
ii. Impart healthy habits along with hygiene education and messages,
iii. Foster environmental awareness and promote sustainable behavior through experiential learning and integration of content into curriculum and co-curriculum integration,
iv. To sensitize the young mind to the environmental degradation by human practices,
v. Engaging young learners in practical conservation of natural resources activities, which will 'marmalade the biodiversity of nature' and enhance the regeneration capacity of the natural environment,
vi. Instilling eco-conscious attitudes while reducing their environmental footprints,
vii. To make a future decision-makers,
viii. Imparting appropriate insights while imposing ethical and moral principles,
ix. Educating mass and capacity enhancement of the stakeholders and preparing the future generation about a green and healthy environment.

The National Council of Educational Research and Training's (NCERT) commitment to combating climate change by integrating content in school curricula in disciplinary and interdisciplinary ways perfectly aligns with the National Education Policy (NEP) 2020 goal. The significance of incorporating environmental education into school curricula at all levels is emphasized in the National Education Policy 2020. The Ministry of Education in India firmly believes in education as a game-changer to combat climate change because it empowers us to act more effectively("State of the Education Report for India 2023," n.d.). The Ministry of Environment, Forests, and Climate Change (MOEFCC) is the nodal ministry for SDG 13; it launched the National Green Corps (NGC) program to raise the environmental

consciousness of environmental issues among schoolchildren nationwide, getting involved the learners in activities like planting trees, handling trash, and conserving biodiversity. MOEFCC included activities such as starting eco-clubs in schools, planning events for the environment, and taking part in community campaigns. The Centre for Environment Education (CEE) implements an international program that uses a systematic, seven-step approach to motivate students to participate in sustainable behaviors and Initiatives about biodiversity, water conservation, waste management, and energy conservation. (J. Suresh Kumar & D. Shobana, 2024). Furthermore, to effectively use education to create a more sustainable and greener future, UNESCO has long advocated mobilizing intersectoral collaborations, political commitments, and youth empowerment. By establishing the groundwork for international cooperation and policy innovation, its worldwide initiative, Education for Sustainable Development (ESD), helps achieve this goal. Furthermore, UNESCO acts as the secretariat for the Greening Education Partnership (GEP), established by the UN Secretary-General in 2022 and offers a valuable platform for education partners to take additional action.("State of the Education Report for India 2023," n.d.).

## REVIEW OF RELATED LITERATURE

**Climate Action**

Bofferding & Kloser, (2015) argued that mitigation is an effort to reduce anthropogenic net emissions of GHGs as per IPCC (2007). Two methods to achieve this net reduction are lowering outputs or capturing carbon. Using renewable energy sources that avoid burning fossil fuels (such as energy generated by solar panels), altering one's lifestyle to use less energy overall (such as walking instead of driving), and utilizing materials that lower energy requirements (such as better insulation for homes to lower heating requirements) are common ways to reduce greenhouse gas emissions. By contrast, carbon capture includes activities that reduce the concentrations of current greenhouse gases, such as methane collection at landfills or reducing deforestation, which raises the rate of photosynthesis. In contrast, Planting trees with broad canopies is one of the significant adaptation activities because it provides more shade for living things and lessens their susceptibility to rising temperatures. By retaining carbon during photosynthesis, these shade plants help reduce emissions simultaneously. (Dagnachew & Hof, 2022) assed the influence of climate change mitigation initiatives on other SDGs while focusing on van Soest et al.'s (2021) 20 best practices for reducing emissions in power production, transportation, buildings, industry, land use, and non-CO2 emissions. Increasing the use of renewable energy in power generation has potential advantages beyond SDG 7, including poverty reduction, eliminating hunger, enhancing access to healthcare, education, and clean water, and safeguarding life on land and in water. Mitigation techniques to minimize $CO_2$ emissions from land use and non-$CO_2$ emissions from agriculture, livestock, and waste are crucial for achieving global emission reductions. Reducing deforestation and encouraging reforestation promotes biodiversity and the ecosystem, aligning with the SDGs. Potential choices in North America, Europe, and Central and South America stem from technology decisions that may worsen socioeconomic inequality and affect biodiversity. (Nnaji & Utsev, 2011)noted that Waste management education is pivotal for managing waste scientifically and could lower the emission of global warming potential gases from untreated waste. This involves reducing waste at the source before it enters the waste stream and recovering produced waste for recycling and ecologically responsible waste management through burning with energy recovery and conversion.

The US Environmental Protection Agency (US EPA) created the hierarchy of non-hazardous materials and waste management, ranking the different management approaches from most to least ecologically friendly. Reducing, reusing, and recycling are emphasized in the hierarchy as essential to sustainable materials management. (*National Overview: Facts and Figures on Materials, Wastes and Recycling ,US EPA*, 2023). The Nigerian government prioritized recycling waste materials and using innovative technologies for sustainable waste management. Sustainability and energy efficiency, heightened consciousness of the detrimental effects of climate change and global warming, design of eco-cities, Carbon sequestration, which involves removing carbon dioxide from companies that utilize fossil fuels, and the adoption of renewable energy sources in place of fossil fuels are the means to reduce greenhouse gas emissions(Nwofe, 2014).In contrast, trash is cut down on Pacific islands by reducing and reusing, emphasizing the five "R's" of managing waste(Secretariat of the Pacific Regional Environment Program (SPREP), 2009).Nnaji and

Utsev (2011)argued that sorting at the source (SAS) is preferable to sorting at the transfer station. Additionally, families can be required to submit trash in an organized manner. Families will need to have separate trash cans for different types of garbage, such as (i) recyclable and non-recyclable, (ii) biodegradable and non-biodegradable, (iii) compostable and non-compostable, (iv) combustible. Sorting them into broad categories like food waste, plastic, glass, paper, nylon, textile, metals, etc., is a more complex but less realistic alternative. Each residence must have around seven trash cans for various waste types, making this more challenging.

**Climate Change Education (CCE)**

Arto-Blanco et al., (2017) conducted comparative research based on social representation theory, looking at a sample of 503 pupils to answer 'What is climate change?' by painting and writing. A qualitative and quantitative study of the data found that while most students acknowledged human responsibility for climate change, they had gaps in their understanding of the phenomena. Wu & Otsuka, (2021) surveyed 657 high school pupils from Shanghai, China. The findings showed that misunderstandings and skewed understandings of climate change continue; climate-related knowledge and attitudes have a poor relationship with behaviour. However, the relevance factor, which includes a belief in an individual's lifestyle, appears to be actively related to behaviour. Several studies and synthesis studies have found that climate change is inescapable, mainly due to human activity, most Indian students remain unprepared, apathetic, and unaware of the vital issue of climate change. In this context, Alexandar & Poyyamoli, (2017) an innovative climate change education (CCE) program was launched among schoolchildren in the Puducherry and Villupuram districts of Tamil Nadu to build knowledge, skills, attitudes, and behaviors related to climate change issues. The experimental study included 180 pupils from two middle schools in the Puducherry and Villupuram districts, both urban and rural. The selected pupils were separated into two groups, with 45 students being experimental and the remaining 45 serving as control groups for each school. The CCE program intervened in the experimental group pupils. The findings inferred that the multimodal teaching and learning technique is more effective among schoolchildren.Vijay Kumar & Kafi Abdul, (2015)assessed the higher secondary students' high level of interest in greening initiatives. Furthermore, the study explored the significant difference in the interest in greening initiatives between boys and girls, with no significant difference between rural and urban students and a considerable difference in the interest in greening initiatives among four different types of school students.

**Swachh Bharat Mission (SBM)**

Datta et al., (2021)assed that good knowledge (59%) and poor practice (39%) regarding water, sanitation, and waste disposal and Occupation (P=0.0012) and Socio-Economic status (P=0.03) were shown to have significant links with the knowledge and practice of the participants. Dash & Dash (2021) argued that strategic concerns, including institutional alliances, public engagement, and media integration, have become essential tools in every environmental campaign as the significance of communication for sustainable development becomes more widely recognized. Moreover, India's Swachh Bharat Abhiyan (2014–2019) is one of the twenty-first century's most significant strategic programs for sustainable development. The study assessed two research questions: (1) What did SBA implement the main outreach tactics and action plans? and (2) How did SBA spread the idea of swachhata or cleanliness? The result showed that a 360-degree promotional technique was used in the campaign. Print, electronic, and social media were all used; partnerships between and within institutions were fostered; opinion leaders and opinion formers were enlisted; and widespread public engagement was encouraged. The "3R" tenets of the UN Sustainability Goals—Reduce, Reuse, and Recycle—were strategically reflected in SBA's cleaning discourse. Tactically, the rhetoric surrounding cleanliness was rooted in good citizenship, dedication to one's community, and shame vs dignity. K. Loganathan et al., (2020) argued by encouraging cleanliness and concentrating on scientific solid and liquid waste management methods for general cleanliness in rural regions, the Swachh Bharat Mission primarily seeks to enhance the quality of life in these areas. Its main objectives are clean water and sanitation, the sixth sustainable development target. The study discussed The Swachh Bharat Mission, which was implemented in the village of Silvarpatti in the Rediyarchattram Block of the Dindigul District of Tamilnadu, India. The study showed the goal shifted to appropriate trashcan usage and separation. Various tactics, including audiovisual, wall painting, walkathons, and roadshows, have raised awareness. The community uses a traditional three-stone wood fire and agro-residue as its primary cooking fuel. In addition to raising pollution levels, this impacts rural women's health.

The usage of energy-efficient stoves has been taught to rural women. Young people in rural areas have received training on how to make wood stoves easily, which has improved their standard of living. A participatory rural appraisal was conducted on the community biogas plant since the village has enough animals. The village chiefs have received instructions on how to use wasteland for the grid. Sarkar & Bharat(2021)found that the policies and initiatives of the Indian government, such as the Swachh Bharat (Clean India) Mission (SBM), aligned with the SDG-6 targets 6.1 and 6.2, concerned with sanitation and clean drinking water. (2023) noted that the Indian government, particularly the Swachh Bharat Mission and non-governmental organizations, started several projects to raise ecological awareness to provide a healthy environment for all living things. Further, Gupta et al.(2022)argued that to combat plastic consumption and accomplish the Sustainable Development Goals, the Swachh Bharat Mission is crucial. The Swachh Bharat Abhiyan accelerates sustainability. The current study confirmed that the Swachh Bharat Abhiyan was successfully implemented in its initial phase. The mission is a real game-changer in the sanitation and hygiene space, as evidenced by its success.

**Swachh Bharat-Swachh Vidyalaya**

Swachh Bharat-Swachh Vidyalaya (SBSV) is noteworthy to raise awareness about the adverse effects of open defecation, which contaminates groundwater, resulting in diarrhoeal disease along with several waterborne diseases that impacted several health issues and lower productivity as well as declining school enrolment and academic performance. Hence, recognizing the importance of clean drinking water and school sanitation, the Swachh Vidyalaya aims to ensure every school in India has a 'set of functioning and well-maintained water, sanitation and hygiene facilities' to 'produce a clean and green environment' and to 'develop and support sustainable behavior.' Therefore, the campaign essentially entails two components - a) availability of the physical facilities and b) behavioral change practices. The overall 'functionality', thus, depends not on anyone but on the realization of both components.("Swachh Bharat Swachh Vidyalaya," 2014). This national Campaign is also renowned as "Abhiyan" because it covered schools in hilly regions, slums, extreme areas, etc., The mission made gender-segregated restrooms in all 1.2 million government schools (Borthakur & Baruah, 2019). Govt. of India,along with theSwachh Bharat Kosh, MPs and MLAs area development funds and all public and private companies and policies like Sarva Shiksha Abhiyan (SSA), RashtriyaMadhayamik Shiksha Abhiyan (RMSA), Rashtriya Madhyamika Shiksha Abhiyan (RMSA) are funded to the campaign to reach its peaks("Swachh Bharat Swachh Vidyalaya," 2014). Curriculum related to WASH was also incorporated into the school teaching to improve hygiene habits and community ownership of water and sanitation facilities within schools, the repercussions of which also spread within the community.Nearly 99% of the schools practiced handwashing, according to the NARSS study. SDG 4.1 has been made possible by all these factors, improving student retention, attendance, and school enrolment(Sarkar & Bharat, 2021). Around 6000 children from 20 schools segregate the waste generated from their daily activities in school and throw the degradable and non-degradable waste into separate bins. (Ramu et al., 2018). Most schools in Karnataka have installed incinerators for sanitary waste management, biogas plants, and kitchen gardens under the Swachh Bharat and Swachh Vidyalaya schemes(Subhashree et al., 2020). 84.35% had good knowledge, 95.23% had a good attitude but only 50.34% had good practices about environmental cleanliness and personal hygiene (Nanjesh Kumar et al., 2020), the program has efficiently increased students' awareness of hygiene standards, with tests showing a mean knowledge score of 68.13 among junior high school students in Uttarakhand, indicating that they know the mission's objectives(R. Sharma & Tomar, 2022) Whereas 100 % of students from the Jharkhand state are aware of this campaign, 77.8% were only aware of the different components of the mission (Sunderam et al., 2020). A study from the Kollam district of Kerala revealed that most schools provide water facilities but need more treated water and proper hygiene facilities(Rakesh, 2014). In contrast, a study showed that more than fifty percent of schools provide appropriately filtered drinking water, sufficient restrooms, and proper waste management facilities in the schools from Telangana states but still need to deliver R-O drinking water facilities(Vishnu. P., 2021).Gupta et al., (2022)found no significant difference in environmental responsibility among post-graduate students based on gender, stream, or region under Swachh Bharat Swachh Vidyalaya. The Clean India Mission significantly increased access to sanitary facilities and increased knowledge of appropriate waste disposal. Cities have implemented decentralized waste management programs, including recycling, composting, biogas generation, and waste-to-energy conversion. In addition to reducing the amount of trash dumped in landfills, these neighborhood-based

projects have given underprivileged groups access to new sources of income(Singh et al., 2024). Sarkar & Bharat (2021) argued that as per the report of the National Annual Rural Sanitation Survey (2019-20), 95.5% of Indian schools had access to proper child-friendly restrooms, almost all of them are functional, and 99% of schools accessed potable water. Similarly, the phenomenal improvement in sanitation facilities in the country has enabled 98 % of households to access restrooms, which aids in reaching the goal of SDG 6.1.1 and 6.2.2, which adversely benefits reduced health impacts such as diarrheal diseases, stunting of the child, typhoid and others health impacts of poor sanitation, improving well- being another target of SDG 3. Hutton et al., (2020)estimated that water and sanitation benefited economic development. The analysis demonstrates the financial impact of better sanitation and hygiene under the SBM. Ending open defecation universally results in a decrease of USD 64 billion in the nation's GDP. Improving per capita GDP and sanitation-related measures can help achieve SDG 1's goal of ending poverty(Roy & Pramanick, 2019).

**Research gap**

Greening and cleaning are the main focus of Swachh Bharat Abhiyan through trash management, besides the mission implemented from the grassroots level to the top level. Previous research conducted on the mission's goals impacted the achievement of the set objectives of SDG 6 (Sahoo et al., 2023; Sarkar & Bharat, 2021 **;** Roy & Pramanick, 2019), SDG 3 (Sahoo et al., 2023), SDG1(Roy & Pramanick, 2019). Still, there needs to be more analysis of the significant role of the Swachh Bharat Mission in empowering the community and future generations to combat climate change in India. We address this gap in this research by focusing on how the Swachh Bharat Mission is significant in achieving the goal of climate action or SDG 13. Moreover, as a grassroots-level implementor, how Swachh Vidyalaya sensitizes the young mind to environmental degradation by action, engaging them in the practical conservation of natural resources, fostering sustainable behavior through experiential learning, and instilling eco-consciousness while reducing their ecological footprint.

**Objectives of the study**

The narrative review aimed to evaluate the role and responsibility of Swachh Bharat: Swachh Vidyalaya in achieving SDG 13 in the Indian Context.

## METHODOLOGY

Narrative Reviews consolidate published material on a topic and explain the current state-of-the-art. Unlike the PRISMA assertion for systematic reviews, narrative reviews have no established rules (Ferrari & Rossella, 2015). Search terms for articles include Swachh Bharat Mission or Swachh Bharat Abhiyan, SBM, Swachh Bharat Swachh Vidyalaya or Clean India Clean School, Climate Action, Sustainable Development Goals 6, SDG 13, and greenhouse gases. Researchers review articles databases such as ScienceDirect, Taylor & Francis Online, and Google Scholar. Moreover, several government websites and policy reports were also analysed. Additionally, this review selects papers based on both inclusive and exclusive criteria. The inclusive criteria for addressing the problem formulation include highlighting the Swachh Bharat Mission's or Swachh Vidyalaya's role in promoting climate action, describing the organization's contribution to reducing environmental degradation, presenting the elements and characteristics of Swachh Vidyalaya, and writing articles in English. We can determine whether the inclusive criteria are appropriate by reading and comprehending the entire article. The category of exclusive criteria includes articles that do not fit the problem formulation and the inclusion requirements(Saputra et al., 2024).

**Action Model under Swachh Bharat: Swachh Vidyalaya**

Swachh Bharat: Swachh Vidyalaya is intended for total cleanliness, sensitizes the young mind on the significance of environmental degradation by human action, engaging in eco-friendly behavior, nature conservation and preservation along with the attitudinal and behavioral shifts in the school child to overall society while lowering their environmental footprints. Hence, the mission's action model is sectioned as

**Schemes**
**Core environmental activities**
**Legislative and policy support**

**Schemes**

The Ministry of Education (MOE) launched schemes such as Swachh Vidyalaya Puraskar and Bal Swachhta missions under the Swachh Vidyalaya to inspire the innocent to participate in the national mission to accomplish its goals.

i) Swachh Vidyalaya Puraskar:After establishing Swachh Bharat- Swachh Vidyalaya, the national mission, in 2016, the Ministry of Education in India introduced another new program called Swachh Vidyalaya Puraskar in 2016-17. This award-based school initiative is designed to recognize, motivate, and celebrate success in measures taken by various government schools (rural and urban) nationwide to meet the Swachh Vidyalaya mission's mandate. Under this effort, schools would be ranked nationally based on several criteria such as cleanliness, water availability, handwashing, sanitation, maintenance, conduct, and other swachhtaa or cleanliness factors. ("Swachh Bharat Swachh Vidyalaya," 2014)

ii) Bal Swachhta mission: The Bal Swachhta Abhiyan focuses primarily on children. It begins on November 14 and ends on November 19, 2014, under the auspices of the foremost national campaign. Given its focus on children, the program kicks off on Children's Day. This campaign ought to be conducted by all educational institutions to raise awareness through cultural programs or competitions such as essay writing, debates, discussions, chart-making, recycling displays, and other artistic initiatives on the themes include clean Anganwadis, well-kept surroundings such as playgrounds, personal hygiene/child health (clean self), nutritional food consumption, potable water, and functional toilets. Faculty members are also entrusted with organizing cleanliness drives to clean the neighborhood and educating residents(*Bal Swachhta Mission*, 2014).

**Core environmental activities of Swachh Vidyalaya:**
As per the guideline MOE, Govt. of India, this national campaign was made based on two combinations: technical and human development activities. Technical activities include provisions for purifying drinking water, hand washing facilities, and proper infrastructure, whereas daily activities and practices of learners' behavioral change are nominated under human development activities. Swachh Vidyalaya promotes cleanliness, hygiene, and sanitation in schools throughout India.

The main activities under this program are
**Provision for clean, sanitary facilities**:
The Ministry of Education (MOE) ensures that each school in India, through the Swachh Vidyalaya mission, has separate, functional, child-friendly restrooms for boys and girls (1:40) that are regularly cleaned and maintained with proper natural lighting and ventilation.

**Safe drinking water**:
Swachh Vidyalaya schemes ensure all kids can access potable drinking water and clean water for cooking and washing vegetables and utensils. The schemes mandate safe handling and storage of drinking water, which should be practiced throughout the school, and maintain the appropriate distances between the potable ground water tab and drainage system. Further, the appropriate purification or filtration process should be followed.

**Hand washing facilities with hygiene messages**:
Swachh Vidyalaya mandates improving hand hygiene and installing soap-filled stations near restrooms and dining areas. Teachers should involve the little ones in handwashing sessions before the midday meal by conveying sanitation and drinking water safety messages.

**Waste segregation and management facilities**

Swachh Vidyalaya involves the students in the segregation of waste and is asked to throw the waste into different sources of dustbins in day-to-day activities. Through the national mission, the Ministry of Education asked every school to install incinerators and composite pits to manage solid waste.

**Plastic avoidance and following the rule of 3R'S of Environment**

**"**Stop using plastics, Go Organic" uses this tagline to describe the success of the Swachh Vidyalaya mission. Under the mission, most schools have educated their students about plastic pollution and encouraged them to actively participate in making their classrooms plastic-free and motivated to participate in programs that teach them about the environmental impact of littering.

**Anti-litter Campaign & participating in cleaning activities**

School authority elicits awareness about clean schools through various anti-littering programs. The young minds are urged to take up the broom to clean school premises, gardens, playgrounds, and the neighbourhood under this scheme. Students from both semi-rural and urban locations have actively and enthusiastically cleaned their classrooms and the school grounds.

**Engaging in eco-friendly practices:** The school authority insists that learners use jute bags, train them to make organic compost, close the tap immediately, and switch off the fan and lights after using it. Sensitize them about the water crisis and motivate them to harvest rainwater and reuse the paper under the national mission.

**Nature Preservation**

Swachh Vidyalaya mission enlisted to make kitchen gardens or gardening in the school premises encourages students' and teachers' participation in gardening activities, such as saplings of medicinal/vegetable/flowering plants on the school premises, watering them, and caring for them. Some schools also conducted rooftop gardens to address space constraints. Indoor planting is recommended to improve cleanliness and freshness.

**Operation and Maintenance**

All the infrastructure needs to be clean, functional, and well-maintained to ensure Swachh Vidyalaya's intended result. Hence, it has been made mandatory to clean classroom fans, lights, roofs, walls, water storage tank, etc., as well as check pipes, cracks on walls and roofs, clogged drains, and observe water logging in areas, etc. on a weekly and monthly basis by the school.

**Behaviour Change Activities**

Several approaches are implemented in the school to change behaviour effectively.

A. **Curriculum integration**: Various states' governments incorporate content related to environmental degradation through human action and the necessity of conserving nature through school textbooks and supplementary books.

**b. Co-Curricular activities**: Learners should not be limited to curricula; they are actively engaging in eco-club activities to expose them to nature and learn through the "learning by doing" method; child cabinets formed for disseminating hygiene-related messages to the child as child-to-child strategies are the best and critical element of behaviour strategies in schools.

**Enhanced Capacities**

Capacity enhancement is one of the factors considered by the Swachh Vidyalaya mission. Govt. along with UNICEF and WHO, conducted several workshop/ project and seminar sessions to refine the essential capacities at various levels within the sector, to enhance the knowledge, skills, and experience of the teachers, midday meal cooking lady, finance, and manager to facilitate the program.

**Community awareness program**

Swachh Vidyalaya encourages Schoolchildren to participate in activities such as an anti-littering campaign, brooming the surroundings, role acting, demonstrations, and so on in front of the community to raise awareness on different celebration days like World Environment Day, Earth Day, World Biodiversity Day, and World Water Day, etc.4

**Legislative and policy support**:

To succeed in key commitments for Swachh Bharat: Swachh Vidyalaya to provide water, sanitation, and hygiene amenities in schools strengthened by legislation and policy framework, including the RTE Act (2009), that mandates the provision of drinking water and sanitation in schools, supported by the national flagship programs Sarva Shiksha Abhiyan (SSA) and Nirmal Gram Puraskar. (Bakshi & Gupta, 2022; "Swachh Bharat Swachh Vidyalaya," 2014)

**Constitution**

- Article 21-A: 'Free and compulsory education of all children in the age group of six to fourteen years as a Fundamental Right.'
- Article 48-A (state obligation to protect our environment): 'The State shall endeavor to protect and improve the environment and to safeguard the forests and wildlife of the country.'
- Article 51-A: 'It shall be the duty of every citizen of India to protect and improve the natural environment including forests, lakes, rivers, and wildlife and to have compassion for living creatures.'

**Legislation**

- Right of Children to Free and Compulsory Education (RTE) Act of 2009. The RTE Act's 2009 Schedule stipulates the standards for a school building, drinking water, and sanitation.

**Policies and programs**

- Sarva Shiksha Abhiyan (SSA)- The Sarva Shiksha Abhiyan (SSA) envisions the school as a pedagogically rich, inclusive, and sustainable ecosystem that is safe from hazards, maintains cleanliness and hygiene, incorporates elements of green architecture, and makes the best use of available resources and space through environmentally and culturally sustainable practices(K. Sharma & Pandya, 2015,Pg.No. 17).
- Mid-day meal Programme -A nutrition program essential to schoolchildren's growth and promoting environmentally ethical ethos in the school(K. Sharma & Pandya, 2015, Pg. No. 18).
- The Rastriya Madhyamik Shiksha Abhiyan -Encourages secondary schools to provide excellent physical infrastructure, such as adequate classrooms, restrooms, and drinking water.
- Kasturba Gandhi Balika Vidyalaya (KGBV) intends to offer girls from disadvantaged groups in the SC and ST communities' access to good quality education by establishing upper primary residential schools

**Role and Responsibility of Swachh Bharat: Swachh Vidyalaya (SWSV) to achieve Sustainable Development Goals in the lens of SDG 13.3:**

Swachh Vidyalaya is crucial in sensitizing the young mind regarding environmental degradation, engaging learners in the practical conservation of natural resources, and cultivating eco-friendly habits while reducing their environmental footprints. Moreover, it strives to modify children's attitudes and behavior, positively impacting society. Such changes are instrumental in supporting the Swachh Bharat Mission (SBM) in achieving its targets for SBM 2.0. These initiatives demonstrate educational institutions' commitment to building their capacity to combat climate change through action and reducing environmental impact. Swachh Vidyalaya's activities align with Sustainable Development Goal 13.3, which emphasizes improving education, awareness, and institutional capacity in climate change mitigation, adaptation, impact reduction, and early warning systems.

**Fostering Eco-Awareness**

Under Swachh Vidyalaya Abhiyan, the school children learn the necessity of trash management and sorting waste using appropriate source-wise dustbins. ("Swachhbharat-2024," 2024). Schools' foster awareness among the learners about the hazardous effects of plastic pollution on Mother Earth. Several government urban schools have devised

innovative techniques for dealing with plastic waste products, such as plastic packages and pet bottles, which are collected and used in terrace farming. Furthermore, students are not allowed to carry their tiffin (biscuits, napkins, etc.) in any form of packaging. This has significant steps to reduce plastic waste generation, followed by the reuse of plastic trash in extracurricular activities. In Work Education class, students are taught various crafts and constructions using plastic materials. Discarded plastic bottles and bowls are repurposed, painted, and used for plantations and hanging gardens. The deck is then adorned with stunning designs. The national mission made the Indian schools proactive in building plastic-free and green zones and teaching students how to reduce, reuse, and recycle waste.(Mandal & Banerjee, 2023). Several Schools install Biogas plants also. (Subhashree et al., 2020)). Moreover, it mandates recycling handwashing waste water in the kitchen garden.("Swachh Bharat Swachh Vidyalaya," 2014). In this way, schools under Swachh Vidyalaya typically follow the 3Rs - Reduce, Reuse, Recycle of Waste Management Rules which help to reduce waste significantly. These activities save the environment from climate change and foster insights into generating green energy from waste while instilling eco-consciousness among young learners.

**Instilling Eco-friendly habits**

Swachh Vidyalaya Schools insist the children actively participate in water conservation and rainwater harvesting, using jute bags instead of plastic bags, reusing paper, and planting herbal/medicinal plants or kitchen gardens or flower tree plantations on their school premises and training them to make organic compost from the leftover food and vegetables peels getting from the Mid-day meals(Mandal & Banerjee, 2023; "Swachh Bharat Swachh Vidyalaya," 2014). However, the Swachh Vidyalaya mission promotes a green school policy by providing mid-day meals, periodic health check-ups, natural light, ventilated classrooms, access to safe and clean drinking water through filtration/ purification, cleaning of the roof, all the dust from the fans, tube lights, bulbs, water storage tanks, open school garden, etc. seasonal or quarterly. Besides that, the mission instilled in a child the use of resources judiciously in their day-to-day life. Hence, Swachh Vidyalaya tunes the little mind with eco-friendly practices which are crucial to combat climate change.(K. Sharma & Pandya, 2015).

**Environmental Protection**

Swachh Bharat Swachh Vidyalaya provides proper restroom infrastructure in all the schools and sensitizes learners about the harmful impacts of open defecation and untreated sewage on the environment. School kids actively engage in waste separation and use proper restrooms in school that will save water, soil, air, and groundwater and prevent some wildlife extinction. Some states even started solar cooking in schools to prepare mid-day meals for children and save Mother Earth from the harmful effects of burning biomass fuel(K. Sharma & Pandya, 2015 , pg no. 119-120)

**Environmental Conservation**

Swachh Vidyalaya entails making a green and fresh campus so that the school conserves varieties of plant species, including rare species of flowers, herbal plants, and vegetable plants. Schools from northeastern states conserve 400 species of plants, rare species of land lily, seasonal flowers to bougainvillea, etc. It not only conserves the plant species but also saves flora and fauna of different niches(Puri & Joshi, 2017). Hence, Swachh Vidyalaya is increasing the concentration of carbon sequestration in the nations through plantation programs in schools and conserving each niche and every ecosystem on the earth, essential for maintaining the balance of nature to combat climate change.

**Judicial use of natural resources**

Renewable natural resources must be used increasingly, keeping the cycle of renewing at regular intervals. However, non-renewable resources are to be in the judicial frame as these resources shall never be renewed in a natural setting. Swachh Vidyalaya mandates LED bulbs instead of high-power bulbs and recycles kitchen wastewater for gardening and other materials. Teachers used to teach the learners to switch off the fans and lights and de-plugging gadgets when not in use. Swachh Vidyalaya schools make learners aware of how the country's judicial use of resources is needed through innovative experiential learning methods(K. Sharma & Pandya, 2015 , pg no. 71-73).

**Attitudinal and Behavioural Change among the Learners**

`Swachh Vidyalaya Schools are pivotal in fostering behavioural change in children, making them more receptive to adopting improved practices for a lifetime. Swachh Vidyalaya policy emphasizes peer-to-peer approaches, curriculum inclusion, child's cabinet, and daily activities such as morning assembly and mid-day meal schemes("Swachh Bharat Swachh Vidyalaya," 2014). This activity helps effectively promote environmental cleanliness, protection, conservation, and environmentally ethical ethos in children to modify their attitudes. Moreover, Educational institutions further enhance awareness through cultural events, competitions, and cleanliness drives, encouraging students to adopt sustainable practices and ethical values.

**Catalysing Swachh Bharat Mission (SBM):**

Children may serve as catalysts for a broad movement addressing various environmental challenges. Since children will be future citizens, fostering environmentally friendly attitudes and behaviours can significantly impact long-term environmental conservation initiatives(Puri & Joshi, 2017). Since they are the essential links between educational institutions and society by disseminating essential messages to their families and communities. Moreover, they can raise their voice on current norms in their homes("Swachh Bharat Swachh Vidyalaya," 2014). Children can even motivate their parents to sort waste in separate bins, give the trash to the door-to-door waste collector instead of throwing it outside their homes, adopt eco-friendly products and practices, plant trees, and clean surrounding places, use solar panels and solar cooking system in their homes, using biogas plant in rural areas which has been aided in reaching the vision of the Swachh Bharat Mission 2.0, '100% Garbage Free City,' Actions need awareness; Swachh Vidyalaya's children could awaken the citizens to become environmentally responsible and align with SBM's targets to reach the SDGS goals 13.

## DISCUSSION

Swachh Bharat Mission implemented climate change mitigation and adaptation policy by raising awareness and modifying societal attitudes through awareness campaigns, advancing the education system, and building capacity. SBM brought out cascading changes in individual -community –nation - global. India marked a significant milestone by achieving SBM phase one targets, underscoring 100% eliminating Open Defecation in all villages, gram panchayats, districts, states, and Union territories. Over 6 lakh villages were designated as ODF by the GOI by October 2019(*Swachh Bharat Mission -Grameen (SBM): Phase II (2019-2025)*, n.d.). *Swachh Bharat Mission -Grameen (SBM): Phase II (2019-2025)*, (n.d.) reported that SBM protects the environment by reducing the possibility of human-traceable groundwater pollution in ODF communities by 12.70 times as per UNICEF 2019. Moreover, The Swachh Bharat India Mission Phrase-I significantly increased access to sanitary facilities and knowledge of appropriate waste disposal. Cities have implemented decentralized waste management programs, including recycling, composting, biogas generation, and waste-to-energy conversion(Singh et al., 2024).

Swachh Bharat Mission Phrase-II focused on a 100 % garbage-free city, abolishing a single use of plastic, scientific processing of solid waste, converting dumpsites into green zones, and biogas energy production. Hence, the Swachh Bharat Mission trained the people in segregating waste in the home, office, and public places and urged people to one step forward to participate in door-to-door waste collection. The SBM Vision 2.0 is only possible when people eagerly throw waste in separate dustbins at homes, workplaces, and public places. A series of awareness programs by the district administration, state government, and central government raised public knowledge about the perils of pollution as well as the people who are not well-informed spit pan, gutkha, and tobacco on highways and in public places and those who eat in buses and trains and discard their food wrappers on the road rather than in trash cans, as results people are taking the initiative to clean up their villages, societies, colonies, cities, railway platforms, and so on. Cleaning homes, workplaces, villages, and towns would significantly reduce littering and pollution. Decreasing solid waste generation, related groundwater pollution, and methane emissions. It will effectively save us from climate change.

Swachh Bharat-Swachh Vidyalaya is instrumental in progressingthe Swachh Bharat Missionby passing the knowledge to their parents and furthering to the larger society. The Swachh Vidyalaya Puraskar and Bal Swachhta mission's cultural events, such as roleplaying, debate, discussion, storytelling, drawing competitions, etc., and cleaning drives to neighbourhood places, fostering not only children's bodies to involve actively in cleaning schools,homes and public place while throwing the waste in separate bins but also its imparting appropriate insights about the necessity to separate degradable waste to non-degradable, recyclable waste for saving our mother nature from the emission of global warming potential gases. The Ministry of Education awarded 39 Schools for achieving targets of Swachh Vidyalaya in 2021(The Hindu, 2022). Moreover, constitutional and jurisdiction provisions added an extra layer to the Swachh Vidyalaya's effectiveness. Bakshi and Gupta (2022) reported thatSwachh Vidyalaya instilled trust and confidence in the people of Jammu and Kashmir. A significant increase in the percentage of school access to and use of restrooms indicates a paradigm shift in people's attitudes toward open defecation. Apart from that, Swachh Vidyalaya activities played a crucial role in SDG 13.3 goals by promoting green schools while practicing green activities such as using jute bags, recycling plastic bottles and tires in terrace farming, sapling flower tress, as well as herbal plants, watering and care them, rainwater harvesting, organic compost making from leftover peels, participating in eco clubs, e which only conserve the plant species but also preserve flora and fauna of that ecosystem, recycling plastic waste preventing plastic pollution by which protects fishes, whale, birds, and other wild animals from the harmful effects of plastic and involving rural Schools in biogas production to produce green and clean energy. However, engaging learners in the eco-activities made them more empathetic towards nature. Hence, young stars become responsible and future decision-makers. Over 75% of students have agreed that their schools have made them realize the importance of green zones and plastic-free areas. (Mandal & Banerjee, 2023).

Subsequently, India ranked 109th out of 166 countries in the overall assessment of the Sustainable Development Goals in 2024. The mean score of 63.99 out of 100 has increased compared with 2005, when the mean score was 53.12. Notably, the most significant progressed score was noticed in Climate Action, which rose from 54 in 2020-21 to 67 in 2023-24(*Sustainable Development Report 2024*, n.d.). This data shows India's continuous efforts and commitment to reduce GHGs in gas and to save our mother Earth.

Swachh Vidyalaya activities included trash management source-wise and following the 3 "R" rules, planting trees, making an herbal garden /kitchen on school premises, using jute bags, rainwater harvesting, organic composting, avoiding plastic and reusing for gardening purposes, curriculum integration, judicial use of resources, behavioral and attitudinal shifts, capacity enhancement of teachers other staffs and community engagement program along with proper sanitation infrastructure and portable drinking water, is similar to GHG mitigation program in Schools from the United States focuses on solid waste and materials management such as reducing food waste, source reduction, green purchasing, recycling, composting and more emphasis on transportation control measure, energy efficiency product procurement. (U.S. Environmental Protection Agency, 2011)Furthermore, initiatives Australian schools take to make low-carbon schools include switching off protocol and behavior change activities. (Odell et al., 2021)

**Educational Implication**

- Climate Change education must be compulsory at all school levels, irrespective of state or central Curriculum.
- Schools should promote Greening Education in the "learning by doing method."
- Eco-clubs should be more emphasized.
- Teachers should use technology or Open Education Resources while teaching Climate change education.
- Proper monitoring mechanisms in schools are strictly maintained.
- Sensitizing children to use natural resources ethically and advocate sustainable development.
- Creativity should be nurtured through small projects in every class.
- A climate alert system should be started.
- Tune the learners to the present world by respecting the pledge. Think global, act locally.

## CONCLUSION

The Swachh Bharat Mission steps towards a revolutionary India, a pollution-free, green, and clean country, which would be a dedicated devotion to the nation and make it an ideal global citizen. Swachh Vidyalaya, as a part ofthe Swachh Bharat Mission, made an enormous mark on Indian society by instilling a sense of responsibility among future learners towards the mother environment through conservation and preservation of nature, as well as awakening their eco-consciousness and sensitivity to the future decision-makers. Moreover, Swachh Vidyalaya fosters critical and creative thinking and enhances problem-solving ability among the learners while involved in school greening activities.

**Future Direction**

The recent climate action agenda was held at the United Nations Climate Change Conference (UNFCCC COP-29) in Baku, Azerbaijan, to reduce greenhouse gas emissions and protect lives and livelihoods from the impacts of climate change—a robust policy to promote clean air and protect nature. The conference reaffirmed the commitment to collective action on the rising demand for cooling. The above future climate action initiative will be mainly addressed in educational institutions to change the actions necessary to combat environmental degradation or efforts in the global crisis.

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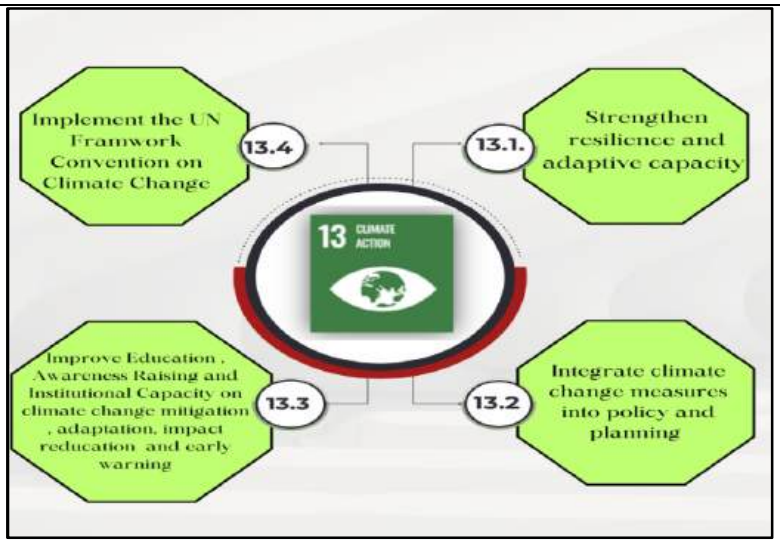
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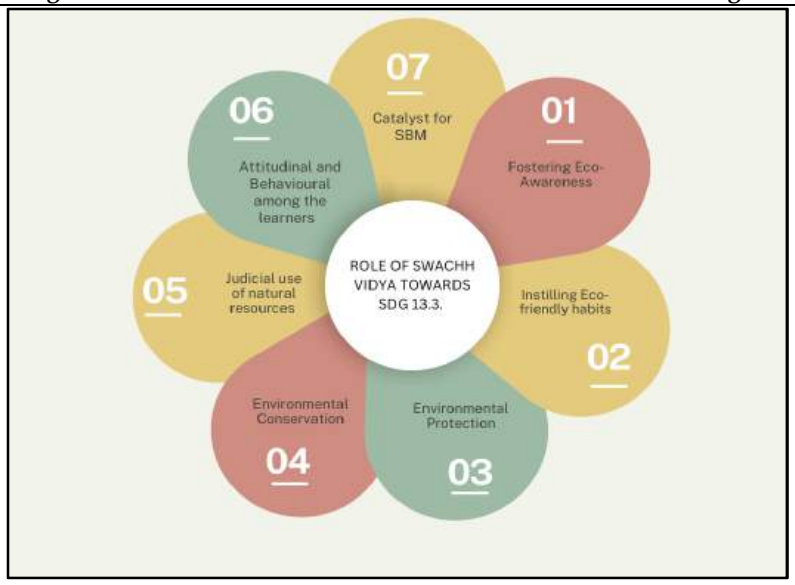
**Table 1:** ***NCERT Initiation of Environmental Issues in the Curriculum***

| **Std.** | **Chapter** | **Topics** |
|---|---|---|
| VI- Science textbook | 16- Garbage in, Garbage out | Ways of dealing with garbage and minimizing the generation of garbage. |
| VII- Science textbook | 18 – Wastewater story | Role of people in keeping the environment clean and healthy. |
| VIII -Science textbook | 2- Microorganisms - Friendly and Foe | Need to keep surroundings clean and dry to prevent mosquitoes from breeding |
| IX–Science Textbook | 13- Why do we fall ill? | Why public hygiene measures are important to prevent infectious disease? |
| XII- Biology Textbook | 8- Human Health and Disease | Personal cleanliness and hygiene, public health measures like proper disposal of waste, decontamination of drinking water, and control of mosquitoes are important to prevent disease. |




(Source: Author)

**Figure 1: The SD Global Goals: SDG 13(climate action) and sub-goals**




(Source: Author)

**Figure 2: Role of Swachh Vidyalaya towards SDG13.3**

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REVIEW ARTICLE

# Multiscale Integration of Photovoltaic and Catalytic Energy Systems with Advanced Storage Technologies

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**ABSTRACT**

The global demand for clean and sustainable energy has driven significant advancements in various scientific fields. This review analyzes recent progress in photovoltaic technologies, energy storage, catalytic processes, and energy policies, highlighting their roles in achieving a decarbonized energy future. In photovoltaics, the shift from silicon-based devices to advanced perovskite, tandem, and organic solar cells has led to power conversion efficiencies exceeding 26% and improvements in stability and scalability. Energy storage technologies, including lithium-ion, metal-air, and supercapacitors, are evaluated for their efficiency and cost-effectiveness. The review also discusses breakthroughs in electrocatalysis and nanostructured materials that enhance catalytic performance. Additionally, it examines energy policies such as carbon pricing and incentives that support the deployment of sustainable technologies. By identifying synergies and gaps in knowledge, this review provides a framework for future research and policy-making to promote global energy sustainability.

**Keywords:** Photovoltaics, Energy Storage Systems, Catalysis for Energy Conversion, Sustainable Energy Policy, Techno-Economic Analysis

## INTRODUCTION

The global energy landscape is undergoing a fundamental transformation in response to escalating climate change, fossil fuel depletion, and the increasing demand for clean and affordable energy. The transition toward a sustainable energy future has become a pressing priority for governments, industries, and research communities alike. According to the International Energy Agency (IEA), renewable energy sources are expected to contribute over 60% of global electricity generation by 2040, driven by commitments to achieve net-zero emissions targets. However, the

realization of this vision requires more than just the expansion of renewable power generation;it demands integrated solutions that address intermittency, energy storage, efficient conversion, and policy frameworks [1,2]. Photovoltaic technologies, electrochemical energy storage systems, and advanced catalytic processes are emerging as core enablers in this transformation. When coupled with coherent policy support and economic incentives, these technologies offer the potential to decarbonize major sectors, including power, transportation, and manufacturing. Despite significant individual advancements in each of these domains, a comprehensive, systems-level perspective that explores their convergence remains underrepresented in existing literature. This review seeks to fill that gap [3,4].The central aim of this review is to provide an integrative overview of the latest scientific and technological advancements in four key pillars of clean energy innovation: photovoltaics, energy storage and conversion, catalysis, and energy policy. The scope encompasses cutting-edge developments in high-efficiency solar cell technologies (including silicon, perovskite, tandem, and organic photovoltaics), state-of-the-art battery systems (such as lithium-ion, metal-air, flow, and aqueous batteries), and catalytic systems (including electrocatalysis, photocatalysis, thermal catalysis, and biocatalysis).Beyond technological progress, the review also examines the economic and regulatory mechanisms necessary for scaling and deploying these solutions globally. This includes analysis of carbon pricing, renewable energy subsidies, and lifecycle cost assessments. A cross-disciplinary lens is applied throughout the paper to identify synergies, integration pathways, and future research directions that can accelerate the deployment of sustainable energy systems. A comparative table is presented in Table 1 for summarizing the key sustainable energy technologies, including photovoltaics, batteries, and catalysis. It highlights their efficiency, energy density, cycle life, and technological maturity.

**Recent advances in photovoltaic technologies**

Photovoltaic (PV) technologies convert sunlight into electricity using semiconductors. When photons hit a solar cell's active layer, they create electron-hole pairs that are separated by a p-n junction's electric field, resulting in electrical current [5-8]. The efficiency of PV cells depends on light absorption, carrier generation and separation, charge transport, and minimizing recombination losses. Research has focused on optimizing these factors, leading to advancements from crystalline silicon cells to newer technologies like multi-junction, perovskite, and organic devices [9-13].

**Silicon-based solar cells: state-of-the-art**

Crystalline silicon (c-Si) solar cells dominate the commercial PV market, representing over 90% of global module production. Modern monocrystalline PERC technologies achieve power conversion efficiencies above 22%. Advances in surface passivation and light trapping are enabling the production of high-efficiency modules, with innovations like heterojunction with intrinsic thin layer (HIT) and tunnel oxide passivated contact (TOPCon) pushing efficiencies toward 25–26%. Researchers continue to enhance performance while reducing wafer thickness and energy consumption in production for improved cost-effectiveness and sustainability [14-16].

**Emerging photovoltaics**

**Perovskite solar cells**

Metal halide perovskite solar cells are a promising next-generation PV technology, valued for their tunable bandgaps, long carrier diffusion lengths, and high absorption coefficients. Since 2009, their certified efficiencies have increased from below 4% to over 26%, competing with silicon. Innovations in compositional engineering and interfacial layers have improved efficiency and stability, while all-perovskite tandem structures have achieved efficiencies over 29.5%. Despite these advancements, challenges like moisture sensitivity, lead toxicity, and long-term stability hinder commercialization [17,18].

**Organic and polymer solar cells**

Organic photovoltaic (OPV) devices use conjugated polymers and small molecules, offering advantages like flexibility, lightweight design, and low-temperature fabrication. While they have lower efficiencies than perovskite and silicon devices, recent OPVs have achieved over 19% power conversion efficiency. Innovations like non-fullerene acceptors, tandem layering, and morphological control have enhanced performance. OPVs are well-suited for niche

applications such as portable electronics, wearable solar, and indoor light harvesting, where aesthetics and form factor matter [19].

**Tandem and multi-junction cells**

Tandem and multi-junction PV devices optimize solar energy by combining materials with different bandgaps. Silicon–perovskite tandems are at the forefront, achieving over 30% efficiency in labs by stacking a high-bandgap perovskite top cell on a low-bandgap silicon or perovskite bottom cell, reducing thermalization losses. Although III–V semiconductor tandems theoretically offer efficiencies over 40%, they are expensive. Advances in interface engineering and thermal management are making tandem architectures the future of high-efficiency PV technologies. [20]. Comparative performance of PV technologies (Table 1).

**Energy storage and conversion technologies**

**Fundamentals of Electrochemical Energy Storage**

Electrochemical energy storage systems are essential for a decarbonised energy grid, storing electricity generated from renewable sources such as solar and wind. They convert electrical energy into chemical potential during charging, using components such as the anode, cathode, electrolyte, and separator. Key performance metrics include energy density, power density, cycle life, efficiency, and safety. Understanding thermodynamics and ion/electron transport is critical for optimizing these systems for various applications, from consumer electronics to large-scale grid storage [21-23].

**Lithium-ion batteries: advances and limitations**

Lithium-ion batteries (LIBs) are crucial for portable electronics, electric vehicles, and energy storage due to their high energy density (150–250 Wh/kg) and long cycle life (>3000 cycles). Recent advancements include silicon-based anodes and solid-state electrolytes, with commercial packs now exceeding 700 Wh/L. However, challenges such as raw material scarcity, thermal runaway risks, and environmental impacts from mining raise concerns about sustainability and energy security, prompting the search for alternative chemistries [24-26].

**Alternative battery technologies**

**Metal-air batteries**

Metal-air batteries, especially lithium-air and zinc-air, provide high theoretical energy densities (up to 1200 Wh/kg), making them promising for next-generation energy storage. They use oxygen from ambient air as the cathode reactant, reducing weight and complexity. Recent advances include stable solid electrolytes and bifunctional air electrodes. However, challenges like low round-trip efficiency, moisture sensitivity, and poor recharge ability still hinder practical use [27-29].

**Flow batteries**

Redox flow batteries (RFBs), including vanadium and zinc-bromine systems, are increasingly used for large-scale energy storage due to their flexible design, long cycle life (>10,000 cycles), and separated power and energy capacities. These systems utilize two electrolyte solutions stored in external tanks. Vanadium-based RFBs have been implemented for grid stabilization and renewable energy integration. However, they face challenges like low energy density (20–50 Wh/kg) and high initial costs, although advancements in electrolyte and stack design are enhancing their economic viability [30-33].

**Integration with renewable energy sources**

The integration of energy storage systems with renewable sources like photovoltaics and wind is crucial for managing their intermittency. Battery energy storage systems (BESS) are commonly used with solar farms for functions like time-shifting and peak shaving. Flow batteries excel in long-duration storage, while supercapacitors help smooth out power fluctuations. Hybrid systems combining batteries with supercapacitors or fuel cells are becoming popular for improving grid flexibility. Successful integration demands advancements in technology, power electronics, control systems, and grid interface standards. [34, 35]. A radar chart is presented in Fig. 1 for comparing

key energy storage technologies across five performance metrics: energy density, power density, cycle life, safety, and maturity. Comparing key energy storage technologies across five performance metrics: energy density, power density, cycle life, safety, and maturity (Fig. 1).

**Energy policy and economic analysis**

**Global Energy Demand and Emission Trends**

The global energy landscape is rapidly changing due to rising demand and the need to decarbonize. The International Energy Agency (IEA) projects that global energy demand will grow by about 25% by 2040, driven by population and economic growth in emerging economies. Currently, fossil fuels make up over 70% of energy consumption and are major contributors to greenhouse gas emissions. Transitioning to renewable sources like solar, wind, hydro, and bioenergy is essential for energy security and for limiting global temperature rise to below 1.5°C, as outlined in the Paris Agreement [36, 37].

**Renewable energy incentives and regulations**

Policy instruments and regulatory frameworks are crucial for promoting renewable energy technologies. Mechanisms like feed-in tariffs (FiTs), investment tax credits (ITCs), renewable portfolio standards (RPS), and net metering have been adopted globally to support solar, wind, and energy storage. In the EU, the Renewable Energy Directive establishes binding targets, while the U.S. utilizes various federal and state incentives. In Asia, countries such as India and China have implemented significant subsidy and auction programs [38, 39].

**Carbon pricing and climate mitigation strategies**

Carbon pricing is a crucial economic tool for addressing the environmental cost of carbon emissions, primarily through carbon taxes and cap-and-trade systems. Carbon taxes set a fixed price per ton of $CO_2$, while cap-and-trade systems limit total emissions and allow trading of allowances. As of 2024, over 60 jurisdictions have adopted carbon pricing, covering more than 20% of global emissions [40 - 42].

**Economic evaluation of emerging technologies**

Economic viability is crucial for deploying emerging energy technologies. Key metrics like levelized cost of electricity (LCOE), total cost of ownership (TCO), payback period, and return on investment (ROI) assess feasibility. Over the last decade, the LCOE for solar PV has dropped by over 80%, making it highly competitive. However, technologies like perovskite solar cells, solid-state batteries, and electrochemical $CO_2$ reduction still struggle with costs, scalability, and reliability [43].

**Barriers to implementation and policy recommendations**

Barriers to integrated clean energy systems include policy uncertainty, inadequate infrastructure, and socio-political resistance, along with the lack of standardized performance benchmarks and recycling frameworks. Effective policy should prioritize integrated planning and cross-sector coordination. Key recommendations involve establishing clear long-term policies, incentivising R and D and local supply chains, fostering public-private partnerships, and ensuring equitable access to clean energy, all aligned with global climate goals for a just energy transition. [44, 45]. Fig. 2 compares the Levelized Cost of Electricity (LCOE) for major energy technologies between 2020 and 2023. Levelized Cost of Electricity (LCOE) comparison: 2020 vs. 2023 (Fig. 2).

**Cross-disciplinary synergies and integration strategies**

**Photovoltaic - storage - catalysis interlinkages**

The integration of photovoltaic (PV) systems, energy storage, and catalysis technologies holds significant promise for creating resilient and decarbonized energy systems. While solar PV captures intermittent sunlight, it relies on energy storage solutions, like lithium-ion and flow batteries, to match energy generation with demand. Catalytic processes, including electrocatalysis and photocatalysis, enable solar-to-chemical energy conversion, such as producing green hydrogen. This green hydrogen can be stored, transported, or used as a feedstock in various industrial processes [46 - 48].

**System-level integration for grid and off-grid applications**

Integrating renewable energy technologies is essential for optimizing performance, reliability, and economic returns in both grid-connected and off-grid settings. For grid applications, combined PV-storage-catalysis systems aid in peak shaving, frequency regulation, and demand response, enhancing flexibility and stability. Smart inverters and hybrid energy management systems coordinate generation, storage, and conversion in real time. In off-grid areas, these systems offer tailored energy solutions, such as solar-powered microgrids and PV-electrolysis for clean fuels.

**Life cycle assessment and sustainability metrics**

Life cycle assessment (LCA) evaluates the environmental and resource impacts of energy technologies from production to disposal. It quantifies emissions, energy payback time, material usage, water consumption, and recyclability. For example, while solar PV modules have low operational emissions, the production of their components, such as silicon wafers, has significant environmental implications. Similarly, battery production raises concern over toxicity and mining impacts. Metrics like global warming potential and resource depletion aid in technology selection and policy development. Fig. 3 shows how PV, batteries, and catalysts can be integrated into a smart energy ecosystem. Schematic illustration of a smart energy ecosystem (Fig. 3).

**Challenges, research gaps, and future directions**

Advancements in photovoltaics, energy storage, catalysis, and energy policy face significant barriers to achieving a sustainable energy future. In photovoltaics, the stability of materials such as perovskites and organic semiconductors is compromised by moisture sensitivity and lead toxicity, alongside challenges in scaling high-efficiency, affordable, and environmentally compliant devices. Energy storage remains dominated by lithium-ion batteries, which encounter issues with raw materials and safety, although alternatives like solid-state and metal-air batteries show potential pending further developments in interfacial chemistry and recycling strategies. Catalytic applications for green hydrogen production and $CO_2$ reduction require innovative, non-precious catalysts with high efficiency and stability, compounded by the complexities of integrating systems for real-time solar input. The lack of understanding of atomic-level reaction mechanisms calls for advances in machine learning and computational techniques. From a systems perspective, interoperability, standardization, and control systems remain challenges in the integration of generation, storage, and conversion technologies. Intelligent energy management platforms are essential but requires further development. Future research must focus on holistic solutions that encompass grid dynamics and socio-political contexts. Interdisciplinary collaboration will be critical in designing scalable and equitable energy technologies. Prioritizing circular economy principles and fostering public awareness will support the transition to integrated clean energy systems, crucial for achieving climate neutrality and energy justice. The key challenges and future research directions are summarized in Table 2. Summary of key challenges and future research directions (Table 2).

## CONCLUSIONS

This review outlines recent advancements, integration strategies, and policy implications in photovoltaics, energy storage, and catalysis. The convergence of these areas underpins next-generation energy systems aimed at deep decarbonization, improved energy security, and economic resilience. Key insights include:

- Silicon solar cells dominate over 90% of the global PV market, with efficiencies of 22–24% and lab records near 26.8%. Perovskite solar cells have rapidly improved from 3.8% (2009) to over 26% (2023), though stability and toxicity issues hinder commercial viability. Tandem cells, particularly perovskite–silicon combinations, have exceeded 30% efficiency in lab tests, indicating potential for ultra-high efficiency systems.
- Lithium-ion batteries are dominant with energy densities of 150–250 Wh/kg, cycle lives over 3000 cycles, and round-trip efficiencies of around 90%. Flow batteries provide over 12,000 cycles but have low energy densities of 20–50 Wh/kg, suitable for stationary storage. Metal-air batteries have theoretical energy densities exceeding 1000 Wh/kg but struggle with recharge ability. Supercapacitors offer power densities over 10,000 W/kg and can last more than 100,000 cycles, making them ideal for high-power, short-duration applications.

- Invest in durable, scalable, and non-toxic materials for PV and battery systems. Advance integrated platforms for solar-fuel production and grid resilience. Standardize performance benchmarks and LCA protocols for fair technology assessment. Encourage international collaboration and open innovation in materials design and energy modeling. Design equitable policies that promote innovation while ensuring affordability and environmental justice.

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**Table 1: Comparative performance of PV technologies.**

| Sl No | Technology | Efficiency (%) | Stability (Years) | Cost ($/W) | Commercial Maturity |
|---|---|---|---|---|---|
| 1 | Monocrystalline Silicon | 22 | 25 | 0.20 | Commercial |
| 2 | Perovskite | 26 | 10 | 0.15 | Emerging |
| 3 | Organic (OPV) | 19 | 5 | 0.25 | Developing |
| 4 | Tandem (Perovskite/Si) | 30 | 15 | 0.18 | Pilot-scale |
| 5 | Multi-junction (III–V) | 38 | 20 | 1.00 | Niche/Aerospace |

**Table 2: Summary of key challenges and future research directions**

| Sl. No. | Domain | Challenges and Future Directions |
|---|---|---|
| 1 | Photovoltaics | Improve stability and scalability of perovskites and organics; resolve toxicity and encapsulation. |
| 2 | Energy Storage | Address material scarcity; develop safe, solid-state, and aqueous systems; enhance recyclability. |
| 3 | Catalysis | Design non-precious, durable catalysts; improve mechanistic understanding and system integration. |
| 4 | System Integration | Develop smart control systems; ensure interoperability; enable flexible hybrid grids. |
| 5 | Policy and Economics | Strengthen carbon pricing, green finance; reduce regulatory barriers; promote long-term incentives. |

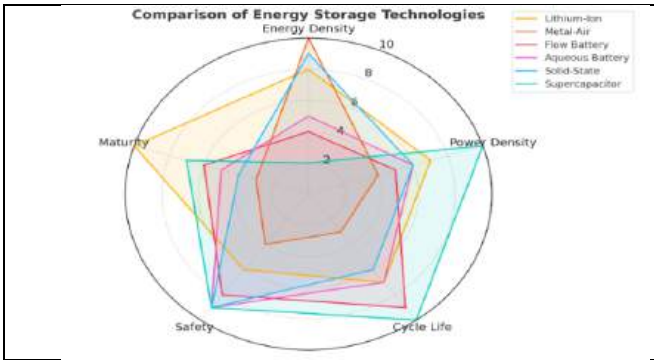



**Fig. 1: Comparing key energy storage technologies across five performance metrics: energy density, power density, cycle life, safety, and maturity.**

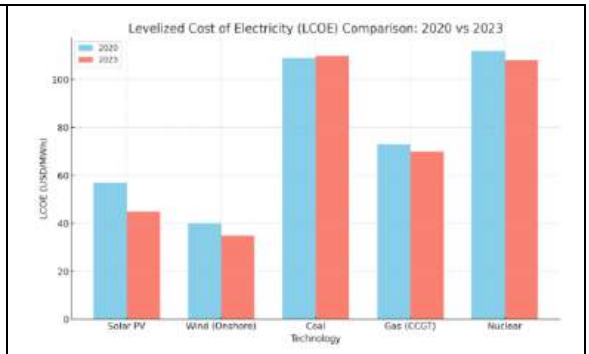



**Fig. 2: Levelized Cost of Electricity (LCOE) comparison: 2020 vs. 2023.**

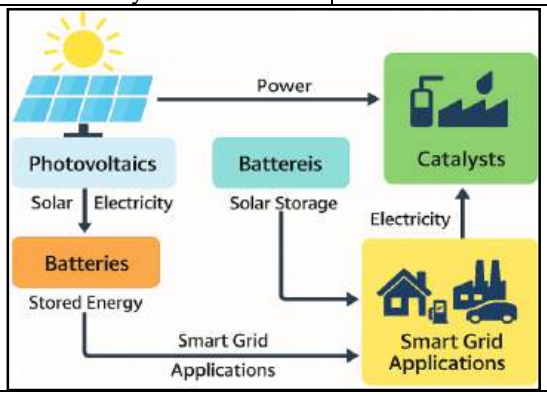



**Fig. 3: Schematic illustration of a smart energy ecosystem.**

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**RESEARCH ARTICLE**

# Efficacy of Om Uccharan as *Tadarthkari Chikitsa* in the Management of Noise-Induced Hearing Loss : A Comparative Interventional Study

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## ABSTRACT

Noise-induced hearing loss (NIHL) is a prevalent and preventable condition resulting from prolonged exposure to excessive noise. Ayurveda describes *Tadarthkari Chikitsa* as a compensatory therapeutic approach that, though not directly counteracting the cause, still produces beneficial effects. To evaluate the efficacy of listening to *Om Uccharan* as a form of *Tadarthkari*Chikitsa in reducing symptoms of NIHL, compared with a control group receiving no intervention. A comparative interventional study was conducted on 60 individuals diagnosed with NIHL. Participants were randomly allocated to two groups: (1) Meditation Group (n=30) practicing Om Uccharan for 20 minutes daily for 60 days, and (2) Control Group (n=30) receiving no intervention. Outcome measures included pure-tone audiometry (PTA), NIHL Questionnaire, and Adult Temperament Questionnaire for psychological assessment. Data were analysed using paired t-tests and ANOVA. The meditation group showed significant improvement in PTA threshold values, self-reported hearing difficulties, and stress reduction compared with the control group ($p < 0.05$). No significant changes were observed in the control group. Om Uccharan, as *Tadarthkari* Chikitsa, may provide therapeutic benefits in NIHL management by reducing stress and enhancing auditory sensitivity. Larger randomized controlled trials are warranted.

**Keywords:** Noise-induced hearing loss, Om Uccharan, Tadarthkari Chikitsa, Ayurveda, Meditation, Auditory health

## INTRODUCTION

Noise-induced hearing loss (NIHL) is one of the most common occupational and environmental health concerns worldwide, with an estimated 16% of disabling hearing loss in adults attributable to noise exposure [1]. It significantly impairs auditory function, communication ability, and quality of life. In Ayurveda, sensory health is emphasized under *Indriya Chikitsa*. *Tadarthkari Chikitsa* refers to therapeutic measures that, though not addressing the root cause directly, alleviate distress and improve well-being [2]. Vibrational therapies, such as chanting and listening to *Om Uccharan*, have been traditionally described to balance the mind and senses [3]. Modern neuroscience supports that meditation and sound-based practices influence neuroplasticity, vagal stimulation, and stress pathways, thereby improving auditory processing [4,5,6]. Considering this, the present study was undertaken to evaluate the role of Om Uccharan as *Tadarthkari* Chikitsa in NIHL. The World Health Organization (WHO) 2021 report on preventing noise-induced hearing loss (NIHL) highlights that over 50% of hearing loss cases are preventable through public health measures. Key strategies include controlling exposure to loud sounds (below 80dB), using protective equipment, and implementing standards for personal audio devices, especially for the 12–35 age group [7].

## LITERATURE REVIEW

Ayurvedic texts such as *Charaka Samhita* and *Ashtanga Hridaya* mention the use of sound and mantra therapies in sensory impairments [2,3]. Contemporary studies on meditation and chanting highlight improved autonomic stability, reduced stress, and enhanced cognitive processing [4,6,8]. Research on vibroacoustic stimulation also suggests beneficial effects on auditory pathways [9-10].

### METHODS

**Study Design**

A 60-day prospective, comparative interventional study was conducted according to STROBE guidelines [11]. Ethical clearance was obtained from the institutional ethics committee, and written informed consent was taken from all participants.

**Participants:**

A total of 60 patients (age 25–55 years) diagnosed with NIHL were enrolled and randomly assigned into:

- **Group A (Meditation Group, n=30):** Practiced Om Uccharan (listening to guided Om chanting, 20 minutes daily).
- **Group B (Control Group, n=30):** Received no therapeutic intervention.

**Inclusion Criteria:**

- Diagnosed cases of NIHL with ≥25 dB HL threshold shift in audiometry.
- Chronic exposure to occupational or environmental noise.

**Exclusion Criteria**

- Patients with conductive or mixed hearing loss.
- History of ototoxic drug use, ear surgery, or psychiatric disorders.

**Intervention:**

Participants in the meditation group listened to the Om Uccharan audio for 20 minutes daily, preferably in the morning, for 60 consecutive days.

**Outcome Measures:**

1. **Pure-Tone Audiometry (PTA):** Assessment of hearing thresholds at baseline and after 60 days.
2. **NIHL Questionnaire:** Self-reported hearing difficulties and tinnitus-related symptoms.
3. **Adult Temperament Questionnaire:** Stress and psychological impact evaluation.

**Statistical Analysis:**

Pre- and post-intervention values were compared using paired t-tests. Between-group analysis was conducted using ANOVA.

## RESULTS

The present study evaluated the effect of a structured 10-minute meditation practice performed before and after work on adult temperament, NIHL questionnaire scores, and audiometric thresholds over a 60-day period, comparing an intervention group (Group I) with a control group (Group II).

**Adult Temperament Scale**

At baseline, mean Adult Temperament Scale scores were comparable between Group I (5.76) and Group II (5.73) as in table 1. Following meditation intervention, Group I demonstrated a progressive reduction in scores to 5.38 at 30 days and further to 4.90 at 60 days, indicating improvement in temperament parameters. In contrast, the control group showed a gradual increase from 5.73 at baseline to 5.96 at 30 days and 6.14 at 60 days, suggesting deterioration over time in the absence of intervention. The meditation group exhibited significant reduction in stress and irritability scores, while no changes were observed in the control group.

NIHL Questionnaire Assessment

Baseline NIHL questionnaire scores were similar between Group I (42) and Group II (41) as in table 2. After 30 days, Group I exhibited a reduction to 40, which further decreased to 37 at 60 days. Conversely, Group II demonstrated an increasing trend, with scores rising from 41 at baseline to 42 at 30 days and 45 at 60 days. These findings indicate subjective improvement in hearing-related symptoms and quality of life in the meditation group, while the control group experienced progressive worsening. NIHL Questionnaire scores decreased in the meditation group, indicating reduced hearing-related difficulties.

Audiometric Assessment

Audiometric thresholds showed as in table 3favorable changes in Group I across all tested frequencies after 60 days of meditation. At 0.5 kHz, thresholds improved from 34.20 dB to 32.77 dB; at 1 kHz from 39.92 dB to 38.32 dB; at 2 kHz from 43.93 dB to 42.47 dB; and at 4 kHz from 58.37 dB to 56.90 dB.In contrast, Group II exhibited deterioration at all frequencies: 0.5 kHz increased from 34.47 dB to 36.20 dB; 1 kHz from 39.82 dB to 41.35 dB; 2 kHz from 44.90 dB to 46.33 dB; and 4 kHz from 59.45 dB to 60.98 dB. These objective findings support the protective and stabilizing effect of meditation on auditory thresholds. The meditation group showed a significant reduction in mean PTA thresholds, especially at higher frequencies ($p < 0.05$). The control group showed no significant improvement.

## DISCUSSION

The present study demonstrates that regular short-duration meditation practiced before and after work produces measurable benefits in temperament, subjective hearing complaints, and audiometric thresholds over a 60-day period. Participants in the meditation group showed consistent improvement across psychological and auditory parameters, whereas the control group exhibited progressive deterioration. Improvement in Adult Temperament Scale scores among the meditation group suggests reduced emotional reactivity, enhanced coping ability, and improved stress modulation. This aligns with established evidence that meditation attenuates hypothalamic–pituitary–adrenal axis activation and sympathetic overdrive, thereby promoting autonomic balance. From an Ayurvedic perspective, meditation may contribute to Manasika Prasannata and regulation of Raja–Tama dominance,

indirectly supporting sensory organ function. The reduction in NIHL questionnaire scores in Group I indicates perceived improvement in hearing comfort and associated symptoms such as tinnitus, ear fullness, or listening fatigue. In contrast, rising scores in the control group reflect ongoing exposure-related auditory stress without mitigation. These findings highlight the relevance of mind–body interventions as adjunct strategies in occupational noise-related health conditions.

Audiometric results further corroborate these observations, with Group I demonstrating mild yet consistent improvements across low, mid, and high frequencies, particularly at 4 kHz, which is characteristically vulnerable in noise-induced hearing loss. The deterioration observed in Group II reflects the natural progression of NIHL in the absence of preventive measures. Meditation may exert its protective effect through improved cochlear microcirculation, reduced oxidative stress, and enhanced neurovascular regulation, mechanisms supported by emerging neurophysiological literature. Collectively, the findings suggest that brief, feasible meditation practices can serve as a supportive non-pharmacological intervention for mitigating early auditory dysfunction and improving psychological resilience among noise-exposed individuals. The integrative nature of this approach resonates with Ayurvedic preventive principles (Swasthavritta and Sadvritta), emphasizing lifestyle regulation for preservation of Indriya function.

This study demonstrates that Om Uccharan as *Tadarthkari* Chikitsa can play a supportive role in NIHL management. The therapeutic benefits may be attributed to:

- **Vibrational Healing:** Om chanting produces resonant frequencies that stimulate the auditory system and vagus nerve [8].
- **Stress Reduction:** Meditation decreases cortisol levels and enhances parasympathetic activity, indirectly improving auditory perception [12].
- **Neuroplasticity:** Repetitive sound patterns may promote cortical reorganization and improve sensory processing [13].

Our findings are consistent with earlier studies reporting the role of sound therapy and meditation in auditory health[14]. However, this is the first study integrating Ayurvedic principles of Tadarthkari Chikitsa with modern audiological assessment.

## CONCLUSION

Om Uccharan as *Tadarthkari* Chikitsa offers a promising, non-invasive, and holistic approach to mitigate NIHL symptoms. While findings are encouraging, future research with larger sample sizes, longer follow-up, and objective biomarkers is necessary to establish standardized therapeutic protocols.

### PROPOSED MECHANISM OF ACTION

- **Resonance Effect:** Om chanting produces frequencies around 136.1 Hz, resonating within the auditory canal and cochlea[8].
- **Vagal Stimulation:** Vibrations stimulate the auricular branch of the vagus nerve, improving parasympathetic tone[12].
- **Stress Pathway Modulation:** Reduction in sympathetic arousal lowers oxidative stress on cochlear hair cells[15].

### CLINICAL IMPLICATIONS

**Integrating Om Uccharan into NIHL rehabilitation programs may enhance outcomes by:**

- Improving auditory sensitivity.
- Reducing stress and tinnitus perception.

- Offering a safe, cost-effective adjunct to conventional audiological care.

**Limitations and Future Scope**

However, limitations include the short duration of follow-up and absence of biochemical stress markers. Future studies with larger sample sizes, longer observation periods, and incorporation of objective stress parameters are recommended to substantiate these preliminary findings.

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**Table-1 Showing average values obtained in various groups in Adult temperament Scale**

| GROUP | CATEGORY | 0 DAY | 30$^{TH}$DAY | 60$^{TH}$DAY |
|---|---|---|---|---|
| **I** | Advised to do meditation for 10 min before and After work | 5.76 | 5.38 | 4.90 |
| **II** | Control Group Without any intervention | 5.73 | 5.96 | 6.14 |

**Table -2 Showing average values obtained in various groups in NIHL Questioner Assessment**

| GROUP | CATEGORY | 0 DAY | 30$^{TH}$DAY | 60$^{TH}$DAY |
|---|---|---|---|---|
| **I** | Advised to do meditation for 10 min before and After work | 42 | 40 | 37 |
| **II** | Control Group Without any intervention | 41 | 42 | 45 |

**Table-3 Showing average values obtained in various groups in Audio metric intervention**

| Group | Category | 0.5 kHz (0-day) | 0.5 kHz (60-day) | 1 kHz (0-day) | 1 kHz(60-day) | 2 kHz (0-day) | 2 kHz(60-day) | kHz (0-day) | 4 kHz (60-day) |
|---|---|---|---|---|---|---|---|---|---|
| I | Meditation | 34.20 | 32.77 | 39.92 | 38.32 | 43.93 | 42.47 | 58.37 | 56.90 |
| II | Control | 34.47 | 36.20 | 39.82 | 41.35 | 44.90 | 46.33 | 59.45 | 60.98 |

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RESEARCH ARTICLE

# Greener Roads through Deep Learning: Automating Vehicle $CO_2$ Level Prediction

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**ABSTRACT**

The rapid increase in vehicular usage worldwide has significantly contributed to rising carbon emissions, leading to environmental degradation and climate instability. Conventional emission measurement methods rely on expensive exhaust analyzers, limiting scalability and real-time monitoring. This paper proposes a Deep Neural Network (DNN)-based vehicle carbon emission detection system that predicts $CO_2$ emission levels using standard vehicle attributes such as engine size, cylinders, fuel type, mileage, and vehicle weight. The model performs both regression ($CO_2$ prediction in g/km) and multi-class classification (Low, Medium, High emission categories). Experimental results demonstrate that the proposed deep learning approach achieves 91% classification accuracy, outperforming Logistic Regression (82%). The system provides a scalable, cost-effective, and intelligent solution for smart city environmental monitoring and sustainable transportation management.

**Keywords:** Carbon Emissions, Deep Neural Network (DNN), Vehicle attributes, $CO_2$ prediction, Classification accuracy

## INTRODUCTION

Transportation contributes approximately 25% of global greenhouse gas emissions. Increasing vehicle density due to urbanization and industrial expansion has intensified $CO_2$ emissions, a primary contributor to global warming.
Traditional emission measurement techniques require laboratory-grade exhaust analyzers, which are:

- Expensive
- Non-scalable

- Time-consuming
- Unsuitable for large-scale deployment

Machine Learning (ML) techniques have emerged as promising alternatives for emission prediction. However, classical models such as Linear Regression and Logistic Regression fail to capture nonlinear relationships among vehicle parameters. Deep Learning models, particularly Deep Neural Networks (DNNs), provide improved predictive capability through hierarchical feature extraction.

This paper presents a DNN-based emission detection system capable of:

1. Predicting $CO_2$ emission values (regression task)
2. Classifying vehicles into emission categories (classification task)

## LITERATURE REVIEW

The rapid evolution of environmental data analytics has led to the development of robust machine learning and deep learning algorithms for predicting emissions and monitoring air quality. Early research focused on regression-based emission modeling. Smith and Brown (2019) applied classical regression techniques for vehicle emission estimation. Lee and Kumar (2020) introduced decision tree-based approaches for $CO_2$ prediction. Subsequent studies explored ensemble and deep learning techniques. Ahmed and Zhao (2022) applied deep architectures to environmental datasets, while Chen *et al*. (2022) demonstrated high-precision $CO_2$ forecasting using gradient boosting. Hybrid neural network approaches have also shown improved industrial emission prediction performance. Johnson *et al*. (2021) compared various machine learning algorithms for emission forecasting. Patel *et al*. (2023) focused on hybrid ensemble learning, and Martinez and Lopez (2021) applied advanced ensemble models for predicting urban air pollutants. Banerjee and Das (2023) further refined the field through hybrid neural networks for industrial emission prediction. Rossi and Bianchi (2022) expanded deep learning into spatiotemporal domains, enabling dynamic air quality monitoring across time and space. Summary of Literature Review (Table 1).

## METHODOLOGY

**Dataset Description**

The dataset contains vehicle attributes including

- Engine Size (L)
- Number of Cylinders
- Fuel Type
- Turbocharged Status
- Transmission Type
- Fuel Consumption (City, Highway, Combined)
- Vehicle Weight

**The target variables are**

**$CO_2$ Emission (g/km)** – Regression

**Emission Class (E, C, D, S, A, X)** – Classification

**Data Preprocessing**

**Preprocessing is crucial for neural network performance**

**Handling Missing Values:** Numerical features (engine size, weight, mileage) are imputed using mean or median; categorical features (fuel type, vehicle class) are replaced using mode or "Unknown."

**Encoding Categorical Variables:** One-hot encoding converts categories to numerical vectors.

**Scaling Numerical Features:** Z-score standardization ensures faster convergence and stable gradients.

**Train–Test Split:** 80% of data for training, 20% for testing to prevent overfitting.

**Deep Neural Network Architecture**
The proposed DNN consists of:
**Input Layer** (vehicle attributes)
**Hidden Layer 1** – 128 neurons (ReLU)
**Hidden Layer 2** – 64 neurons (ReLU)
**Hidden Layer 3** – 64 neurons (ReLU)
**Output Layer 1** – Linear activation ($CO_2$ regression)
**Output Layer 2** – Softmax activation (Emission classification)

**The DNN model includes**
**ReLU activation:** Handles non-linearity and prevents vanishing gradients.
**Softmax output layer:** Produces probabilities for Low, Medium, High emission classes.
**Cross-entropy loss:** Optimized for multi-class classification.
**Adam optimizer:** Adaptive learning rates for faster convergence.
**Regularization:** L2 penalty + dropout layers reduce overfitting.

**Emission Classification Framework**
Deep Learning architecture designed for emission detection as shown in Figure 1. This deep learning architecture for vehicle emission detection begins with an input layer that collects raw vehicle features such as engine size, number of cylinders, fuel type, turbocharging, transmission type, and fuel consumption values in city, highway, and combined driving conditions. These inputs are preprocessed and passed into the hidden layers, which consist of three dense (fully connected) layers with 128, 64, and 64 neurons respectively, each using the ReLU activation function to capture complex nonlinear relationships between vehicle specifications and emission behavior. The output layer is designed for dual prediction tasks: a regression task that predicts the exact $CO_2$ emission in grams per kilometer using a linear output and Mean Squared Error (MSE) as the loss function, and a classification task that assigns the vehicle to an emission class (E, C, D, S, A, X) using softmax activation and Categorical Cross-Entropy loss, ultimately categorizing emissions into low, medium, or high levels. The entire model is optimized using the Adam optimizer, which adapts the learning rate for efficient training, ensuring that both regression and classification objectives are minimized simultaneously. Deep Learning Architecture for Vehicle Emission Detection(Figure 1)

**Vehicles are categorized based on predicted $CO_2$ levels:** (Table 2).
This framework simplifies emission monitoring and regulatory compliance.

## RESULTS AND DISCUSSION

The comparison between the gasoline car and the electric vehicle highlights the significant differences in $CO_2$ emissions and efficiency. The gasoline car, with a 2.0 L engine, 4 cylinders, and turbocharging, consumes fuel at 9.5 L/100km in the city and 6.5 L/100km on the highway, resulting in a predicted $CO_2$ emission of 180 g/km. This places it in Class D, representing a medium emission level as shown in table 3. While compliant with standard regulations, it is not highly efficient compared to newer low-emission vehicles. In contrast, the electric vehicle, with no engine or cylinders and zero fuel consumption, has a predicted $CO_2$ emission of 0 g/km, placing it in Class E as shown in table 3. This low emission level reflects high environmental efficiency and sustainability, as electric vehicles produce no tailpipe emissions and have minimal environmental impact during operation. The contrast between these vehicles underscores the potential of electric and alternative-fuel technologies in reducing carbon emissions and supporting cleaner transportation systems. Gasoline Car (Table 3). Electric Vehicle (Table 4) This classification system organizes vehicles by $CO_2$ emissions, linking ranges to emission levels and typical types, simplifying regulatory compliance and consumer understanding of environmental impact as shown in Table 5. Emission Class Thresholds (Table 5). Predicted vs Actual Comparison (Deep Learning vs Logistic Regression as shown in Table 6.

**Actual $CO_2$ Curve:** Represents the underlying true emission pattern.

**Deep Learning Predictions**
- Extremely close alignment with actual curve.
- Minimal fluctuations.
- Strong adaptation to sudden emission pattern changes.
- Captures nonlinear boundaries efficiently.

**Logistic Regression Predictions**
- Reasonably close fit but lacks deep pattern extraction.
- Limited capacity to model nonlinear relationships.
- Slightly higher variance compared to deep learning.

**Deep Learning and Logistic Regression Model Behavior (Table 6).**

**Model Performance Comparison**

Table 7 presents a comparative analysis of Deep Learning and Logistic Regression models. The Deep Learning model achieved an accuracy of 0.91, outperforming Logistic Regression, which recorded an accuracy of 0.82. Similarly, Deep Learning showed superior performance in precision (0.89 vs. 0.80) and recall (0.87 vs. 0.78). As illustrated in Table 7 and Graph 1, Deep Learning demonstrates a 9% improvement in classification accuracy over Logistic Regression. The results clearly indicate that Deep Learning outperforms Logistic Regression across all evaluation metrics. The neural network significantly reduces false positives, thereby improving precision, and minimizes false negatives, leading to better recall. This improvement is primarily attributed to its enhanced nonlinear boundary detection capability, supported by activation functions such as ReLU and Softmax. Unlike simple mathematical classifiers, deep models can learn complex and nonlinear emission patterns, resulting in improved generalization capability and more accurate prediction of emission levels. Deep Learning Vs Logistic Regression (Table 7). Logistic Regression Vs Deep Learning (Graph 1).

## CONCLUSION

This study demonstrates that Deep Neural Networks significantly outperform classical Logistic Regression models in vehicle $CO_2$ emission classification. The proposed system provides a scalable and intelligent alternative to traditional exhaust-based measurement systems.

**The model is suitable for:**
- Smart city monitoring systems
- Automated vehicle inspection centers
- Environmental regulatory enforcement
- Sustainable transportation planning

Future work includes integrating IoT sensor data, explainable AI techniques, and real-time cloud deployment for large-scale emission monitoring.

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**Table 1: Summary of Literature Review**

| Author(s) and Year | Focus Area | Methods / Models Used | Key Contributions |
|---|---|---|---|
| **Smith and Brown (2019)** | Vehicle emission analysis | Regression techniques | Early adoption of regression for emission estimation |
| **Lee and Kumar (2020)** | $CO_2$ estimation | Decision tree models | Improved $CO_2$ estimation accuracy |
| **Johnson *et al.* (2021)** | Emission prediction | Multiple ML algorithms | Compared several ML techniques |
| **Ahmed and Zhao (2022)** | Environmental datasets | Deep learning | Introduced deep models for environmental analytics |
| **Patel *et al.* (2023)** | Emission detection | Hybrid/ensemble models | Enhanced detection accuracy |
| **Martinez and Lopez (2021)** | Urban pollutant modeling | Ensemble learning | Accurate prediction of urban air quality |
| **Chen *et al.* (2022)** | $CO_2$ forecasting | Gradient boosting | High-precision $CO_2$ prediction |
| **O'Connell and Fraser (2020)** | Real-time monitoring | Sensor-driven models | Real-time emission tracking |
| **Banerjee and Das (2023)** | Industrial emissions | Hybrid neural networks | Improved industrial emission prediction |
| **Rossi and Bianchi (2022)** | Spatiotemporal analysis | Deep learning | Captured dynamic variation in air quality |

**Table 2: Vehicles are categorized based on predicted $CO_2$ levels**

| Class | $CO_2$ Range (g/km) | Emission Level |
|---|---|---|
| **E** | 0–50 | Low |
| **C** | 51–120 | Low |
| **D** | 121–180 | Medium |
| **S** | 181–250 | Medium |
| **A** | 251–350 | High |
| **X** | >350 | High |

**Table 3: Gasoline Car**

| Attribute | Value |
|---|---|
| **Engine Size (L)** | 2.0 |
| **Cylinders** | 4 |
| **Fuel Type** | Gasoline |
| **Turbocharged** | Yes |
| **Transmission** | Automatic |
| **Fuel Consumption City (L/100km)** | 9.5 |
| **Fuel Consumption Hwy (L/100km)** | 6.5 |
| **Fuel Consumption Combined (L/100km)** | 8.0 |
| **Predicted $CO_2$ (g/km)** | 180 |
| **Emission Class** | Class D |
| **Interpretation** | **Regression Output**: The model **predicts** $CO_2$ = 180 g/km**.** **Classification Output**: Based on thresholds, the model assigns Class D**.** Medium Emission Level, compliant but not highly efficient |

**Table 4: Electric Vehicle**

| Attribute | Value |
|---|---|
| **Engine Size (L)** | 0 |
| **Cylinders** | 0 |
| **Fuel Type** | Electric |
| **Turbocharged** | No |
| **Transmission** | Automatic (single-speed) |
| **Fuel Consumption City (L/100km)** | 0 |
| **Fuel Consumption Hwy (L/100km)** | 0 |
| **Fuel Consumption Combined (L/100km)** | 0 |
| **Predicted $CO_2$ (g/km)** | 0 |
| **Emission Class** | Class E |
| **Interpretation** | **Regression**: **$CO_2$ = 0 g/km** **Classification**: **Class E → Low Emission Level** Low Emission Level, highly efficient |

**Table 5: Emission Class Thresholds**

| Class | Approx. $CO_2$ Range (g/km) | Emission Level | Typical Vehicle Type |
|---|---|---|---|
| **E** | 0 – 50 | **Low** | Electric / Hybrid ultra-efficient |
| **C** | 51 – 120 | **Low** | Small gasoline cars, modern hybrids |
| **D** | 121 – 180 | **Medium** | Mid-size sedans, compact SUVs |
| **S** | 181 – 250 | **Medium** | Larger SUVs, older diesel vehicles |
| **A** | 251 – 350 | **High** | Heavy-duty vehicles, trucks |
| **X** | > 350 | **High** | Non-compliant, outdated or specialty vehicles |

**Table 6: Deep Learning and Logistic Regression Model Behavior**

| Model | Behavior | Performance Summary |
|---|---|---|
| **Deep Learning** | Highly smooth, almost identical to actual emissions | Best accuracy, lowest variance |
| **Logistic Regression** | Moderate fit, occasional deviations | Good baseline but less effective for nonlinear tasks |

**Table 7: Deep Learning Vs Logistic Regression**

| Metric | Deep Learning | Logistic Regression |
|---|---|---|
| **Accuracy** | 0.91 | 0.82 |
| **Precision** | 0.89 | 0.80 |
| **Recall** | 0.87 | 0.78 |

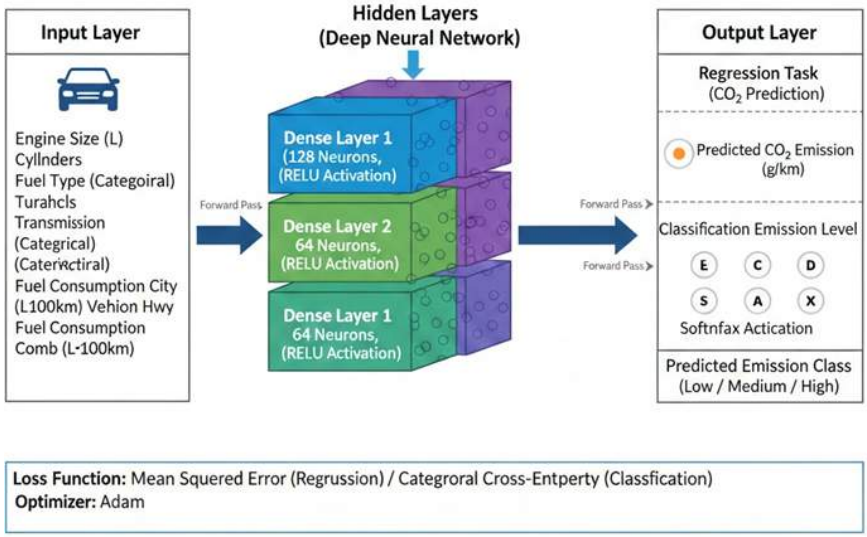
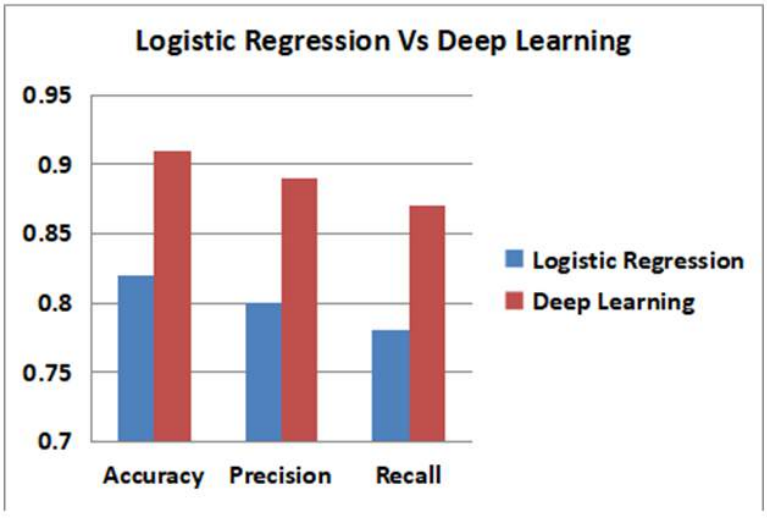



**Fig 1: Deep Learning Architecture for Vehicle Emission Detection**




**Graph 1: Logistic Regression Vs Deep Learning**

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**RESEARCH ARTICLE**

# Comparative Effectiveness of Computer - Assisted, Traditional, and Hybrid Instructional Training on Motor Skill Acquisition and Cognitive Development in Intercollegiate Kabaddi Players

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**ABSTRACT**

The integration of technology in sports pedagogy has significantly transformed instructional delivery. This experimental investigation examined the comparative effectiveness of Computer-Assisted Instructional Training (CAIT), Traditional Instructional Training (TIT), and Combined Instructional Training (CIT) on playing ability and cognitive knowledge among intercollegiate kabaddi players. Thirty-six male athletes (aged 19--28 years) were randomly assigned into three groups (n=12 each). The intervention lasted eight weeks. Playing ability was evaluated using expert rating scales, and cognitive knowledge was assessed through a validated questionnaire. Analysis of covariance (ANCOVA) revealed statistically significant differences among groups, $F(2,32)=8.96$, $p<.001$, with a large effect size (partial $\eta^2=.42$). The CIT group demonstrated superior improvements. Findings suggest hybrid instructional frameworks provide optimal conditions for motor learning in competitive sport environments.

**Keywords:** Computer-assisted instruction, hybrid coaching, motor learning, kabaddi, multimedia learning

## INTRODUCTION

The evolution of instructional technologies has substantially influenced pedagogical practices across educational domains, including sports training and physical education. Contemporary athletic preparation extends beyond physical conditioning and technical repetition, incorporating cognitive development, perceptual training, and tactical intelligence. In high-performance sport environments, the ability to process information rapidly, anticipate opponent movement, and execute technically precise actions under pressure has become as important as physiological capacity. Consequently, coaching methodologies must adapt to integrate cognitive engagement with motor learning processes. Kabaddi, a traditional combative team sport originating in the Indian subcontinent, presents unique physiological and psychological demands. The sport requires explosive strength, agility, breath control, proprioceptive awareness, and rapid decision-making. Offensive maneuvers such as hand touch, toe touch, and kicking, as well as defensive techniques including ankle hold, waist hold, and blocking, require coordinated neuromuscular execution combined with tactical anticipation. Unlike many invasion games, kabaddi integrates continuous chanting (cant), high-intensity bursts of movement, and strategic timing within confined spatial boundaries. These distinctive characteristics necessitate instructional approaches that simultaneously enhance motor proficiency and cognitive understanding of game situations. Traditional instructional training (TIT) has historically dominated kabaddi coaching practices. This approach primarily involves verbal explanation, live demonstration by the coach, and repeated skill rehearsal under supervision. While repetition-based practice supports motor consolidation through reinforcement mechanisms, it may not sufficiently stimulate higher-order cognitive processes such as tactical reasoning, spatial awareness, and anticipatory judgment. Moreover, traditional methods often depend heavily on the coach's ability to visually demonstrate technique in real time, which may limit the learner's opportunity to observe subtle biomechanical components repeatedly or from multiple perspectives. The rapid integration of digital technology into sport education has introduced Computer-Assisted Instruction (CAI) as an alternative pedagogical tool. CAI employs multimedia resources such as slow-motion video, motion analysis, visual overlays, and interactive feedback systems to enhance understanding of technical execution. Through repeated visualization, learners can examine movement phases, joint alignment, and timing patterns with greater clarity than traditional demonstration alone may provide.

From a motor learning perspective, visual modeling strengthens neural encoding during the cognitive stage of skill acquisition, facilitating smoother transition into associative refinement stages. Beyond motor performance, CAI has potential implications for cognitive development. Multimedia learning environments may reduce extraneous cognitive load by presenting structured visual and auditory information simultaneously. Athletes can process tactical scenarios, defensive positioning strategies, and spatial configurations in a controlled and repeatable manner. Such exposure may enhance game intelligence and decision-making accuracy during live match situations. Despite the theoretical advantages of technology-enhanced instruction, exclusive reliance on digital tools may not fully replace the corrective immediacy and motivational influence of direct coach-athlete interaction. The coach's role in providing biomechanical correction, contextual adaptation, and psychological encouragement remains fundamental. Therefore, integrating both approaches into a Combined Instructional Training (CIT) framework may create complementary benefits. Hybrid instructional models could allow athletes to first internalize visual representations of skills through multimedia exposure and subsequently refine technique through live correction and guided practice. Although technology-assisted learning has been investigated in various sports contexts, empirical evidence examining hybrid instructional models in indigenous combative sports such as kabaddi remains limited. Most existing research has focused on laboratory-based motor tasks or technologically advanced sports disciplines. Consequently, there is a need for systematic experimental evaluation within kabaddi training environments to determine whether multimedia integration enhances both performance and cognitive outcomes. The present study was designed to address this research gap by comparing the effectiveness of Computer-Assisted Instructional Training (CAIT), Traditional Instructional Training (TIT), and Combined Instructional Training (CIT) on selected motor skills and cognitive knowledge among intercollegiate kabaddi players. By employing an experimental design with controlled

intervention duration, this investigation aims to provide evidence-based guidance for modernizing kabaddi coaching practices within collegiate sport systems.

## Theoretical Framework

**Motor Learning Theory**

Motor learning theory provides a foundational framework for understanding how athletes acquire, refine, and automate complex movement patterns. The classical model proposed by Fitts and Posner (1967) conceptualizes skill acquisition as a progression through three distinct but overlapping stages: the cognitive stage, the associative stage, and the autonomous stage. Each stage represents qualitative changes in the learner's performance characteristics, attentional demands, and feedback requirements. During the cognitive stage, learners are introduced to the fundamental structure of a skill. Performance at this phase is typically inconsistent, marked by frequent errors and high levels of conscious attention. Athletes attempt to understand the sequence of movements, body positioning, timing, and coordination required for correct execution. In the context of kabaddi, this stage involves grasping the mechanics of offensive maneuvers such as hand touch, toe touch, and kicking, as well as defensive techniques including ankle hold and blocking. Learners at this phase depend heavily on clear demonstrations, verbal cues, and visual guidance. Because attentional resources are limited, structured multimedia representations may assist in clarifying movement components, reducing ambiguity, and enhancing perceptual recognition of correct technique. The associative stage is characterized by gradual refinement of movement patterns. Errors decrease in frequency and magnitude, and athletes begin to detect and correct their own mistakes. Motor output becomes smoother and more coordinated as neural pathways associated with the skill are strengthened through repetition. Feedback during this stage shifts from general instruction to more specific biomechanical correction. In kabaddi training, this phase may involve improving the timing of a raid entry, optimizing body alignment during defensive holds, or adjusting foot placement to maintain balance under contact pressure. At this stage, augmented feedback provided through video replay or slow-motion analysis can accelerate error detection and facilitate deeper kinesthetic awareness.

The final autonomous stage reflects highly efficient and automatic execution of the skill. Movements require minimal conscious control, allowing athletes to allocate attentional resources toward tactical decision-making and environmental scanning. In competitive kabaddi scenarios, autonomous skill performance enables players to anticipate opponent strategies, maintain spatial awareness, and execute rapid transitions between offense and defense without cognitive overload. Automaticity is critical in high-pressure match situations where reaction time and adaptability determine success. From a neurophysiological perspective, motor learning involves progressive strengthening of synaptic connections and improved coordination between cortical and subcortical structures. Early learning engages higher cortical regions associated with conscious processing, whereas advanced stages demonstrate increased efficiency within motor circuits, including the basal ganglia and cerebellum. Repetitive practice combined with structured feedback enhances motor memory consolidation, enabling smoother execution and improved retention. The integration of Computer-Assisted Instructional Training (CAIT) within this theoretical framework may primarily support the cognitive and associative stages of learning. Multimedia demonstrations provide repeated visual exposure to correct movement execution, facilitating mental representation of the skill. Video-based modeling may enhance observational learning mechanisms, thereby accelerating the transition from initial understanding to coordinated execution. When combined with traditional coach-led correction in a hybrid instructional model, learners may receive both augmented visual input and immediate biomechanical feedback, optimizing progression toward autonomous performance. Thus, motor learning theory not only explains the sequential nature of skill acquisition but also provides theoretical justification for comparing traditional, computer-assisted, and combined instructional approaches in kabaddi training environments.

**Observational Learning Theory**

Observational Learning Theory, rooted in Bandura's (1986) Social Cognitive framework, posits that individuals acquire new behaviors, skills, and cognitive patterns through the observation of models. Unlike purely trial-and-

error learning, observational learning enables skill acquisition without direct physical practice during initial exposure. This mechanism is particularly relevant in sport contexts, where visual modeling can accelerate motor encoding and reduce early-stage performance errors.

**Bandura identified four essential processes underlying observational learning:** {attention}, {retention}, {motor reproduction}, and {motivation}. First, learners must selectively attend to relevant aspects of the modeled behavior. In kabaddi, this involves focusing on body alignment during an ankle hold, foot placement during a toe touch, or trunk positioning during defensive blocking. Multimedia video presentations enhance attentional focus by isolating movement phases, zooming into joint actions, and replaying sequences in slow motion. Second, retention refers to the cognitive representation of observed behavior. Athletes encode visual information into symbolic memory structures that can later guide physical execution. Repeated video observation strengthens mental imagery and movement schemata, facilitating recall during practice sessions. In combative sports such as kabaddi, where rapid decision-making is required, strong mental representation of technique contributes to faster motor planning. Third, motor reproduction involves translating cognitive representation into coordinated physical movement. During this stage, learners attempt to replicate the observed action. The availability of repeated visual feedback through computer-assisted systems supports refinement of movement accuracy. By comparing their own performance with expert models, athletes engage in self-correction processes that enhance neuromuscular coordination. Fourth, motivation influences whether the observed behavior is ultimately performed and retained. Reinforcement, perceived success, and coach encouragement strengthen the likelihood of reproducing modeled actions. In hybrid instructional settings, combining multimedia modeling with direct coach feedback may enhance motivational engagement and confidence. From a neuro physiological perspective, observational learning is associated with activation of the mirror neuron system, which responds both when an individual performs an action and when observing the same action performed by others. This neural mirroring facilitates internal simulation of movement patterns, promoting motor priming even before physical execution occurs. In kabaddi training, repeated video modeling may therefore pre-activate motor circuits, accelerating the transition from cognitive understanding to coordinated execution. The interaction between observational learning and multimedia-based cognitive processing plays an important role in facilitating neural encoding during the early stages of motor skill acquisition. During the cognitive stage, athletes rely heavily on visual information to develop internal representations of movement patterns. Multimedia demonstrations, such as slow-motion video analysis and segmented visual presentations, allow learners to repeatedly observe biomechanical sequences including body alignment, timing, and spatial positioning during kabaddi techniques such as toe touch, hand touch, and defensive holds. This repeated visual exposure strengthens neural encoding of the motor pattern and enhances perceptual–motor coupling. As learners progress to the associative stage, video replay and guided practice enable athletes to compare their own performance with expert models, facilitating error detection and motor refinement. Through this process, neural representations of the skill become progressively stabilized, leading to improved coordination, movement efficiency, and performance accuracy. Within the context of the present study, Computer-Assisted Instructional Training (CAIT) leverages observational learning by providing structured visual models of correct technique. When integrated with traditional coaching in a Combined Instructional Training (CIT) framework, athletes benefit from both neural priming through observation and biomechanical refinement through live feedback.

**Multimedia Learning Theory**

Multimedia instructional environments also support motor learning by optimizing cognitive load. Complex kabaddi skills require simultaneous control of breathing rhythm, spatial positioning, and anticipation of defenders. Structured multimedia presentations segment these components into manageable phases and distribute information across visual and auditory channels. This instructional design reduces extraneous cognitive load and allows athletes to focus cognitive resources on meaningful schema construction and motor planning. The dual-channel assumption posits that humans process visual and auditory information through partially independent cognitive systems. When instructional material integrates synchronized narration with visual demonstration, learners distribute cognitive load across both channels, enhancing overall comprehension. In kabaddi instruction, presenting a slow-motion video of a defensive block while simultaneously providing concise verbal explanation may facilitate deeper understanding than

either modality alone. The {limited capacity assumption} acknowledges that working memory has finite processing resources. Excessive or poorly organized information may overwhelm cognitive capacity, leading to reduced retention. Effective multimedia design therefore requires clarity, coherence, and elimination of extraneous details. Structured CAI modules that focus exclusively on key movement phases, tactical cues, and spatial orientation may reduce unnecessary cognitive load and improve schema construction. The {active processing assumption} suggests that meaningful learning occurs when learners actively select relevant information, organize it into coherent mental structures, and integrate it with prior knowledge. Multimedia tools that segment skill components—such as dividing a raid into approach, contact, and retreat phases—may enhance organizational processing. Additionally, interactive pause-and-replay functions encourage learner engagement and reflective analysis. Cognitive Load Theory further differentiates between intrinsic, extraneous, and germane load. Intrinsic load relates to task complexity, which in kabaddi may involve coordinating breath control, balance, and opponent anticipation simultaneously. Extraneous load arises from poorly designed instructional material, while germane load represents productive cognitive effort directed toward schema formation. Multimedia instruction, when designed effectively, reduces extraneous load and increases germane load, thereby facilitating efficient learning. In relation to the current investigation, CAIT utilizes multimedia principles to enhance comprehension of biomechanical and tactical components of kabaddi skills. However, exclusive reliance on multimedia may limit contextual adaptation and individualized correction. Therefore, integrating Multimedia Learning Theory with live coach demonstration in a Combined Instructional Training model may optimize both cognitive processing and practical application. Collectively, Observational Learning Theory and Multimedia Learning Theory provide complementary explanations for why hybrid instructional approaches may produce superior improvements in both motor performance and cognitive knowledge among intercollegiate kabaddi players.

## METHODOLOGY

**Research Design**

The study employed a randomized controlled experimental pre-test--post-test design to evaluate the comparative effectiveness of three instructional approaches: Computer-Assisted Instructional Training (CAIT), Traditional Instructional Training (TIT), and Combined Instructional Training (CIT). The design was selected to control for baseline differences among participants and to allow statistical adjustment of pre-intervention scores through Analysis of Covariance (ANCOVA). Random assignment was performed using a simple lottery method to ensure equal probability of allocation to each group. This procedure minimized selection bias and enhanced internal validity. Pre-test assessments were conducted one week prior to the intervention, and post-test evaluations were administered within one week of completion of the eight-week training program.

**Participants**

Thirty-six male intercollegiate kabaddi players were recruited from affiliated colleges. Inclusion criteria were:

- Age between 19 and 28 years
- Minimum of 10 years of competitive kabaddi experience
- Active participation in intercollegiate tournaments
- No musculoskeletal injuries during the previous six months

**Participants were randomly assigned into three groups (n=12 each):** CAIT, TIT, and CIT.

All participants provided informed consent prior to participation. The study adhered to ethical guidelines for human research in sports sciences.

**Sample Size Justification**

An a priori power analysis was conducted to determine the minimum sample size required to detect statistically meaningful differences among instructional groups. Power estimation was performed using G*Power (Version 3.1), assuming a fixed-effects one-way ANCOVA design with three groups, one covariate (pre-test score), an alpha level of

.05, and statistical power of .80. Based on previous research in motor learning and technology-assisted instruction, a medium-to-large effect size (f = 0.35) was assumed. Under these parameters, the required total sample size was estimated at N = 33 participants. To account for potential attrition and to maintain balanced group allocation, the sample size was increased to 36 participants, resulting in 12 participants per group. The achieved sample size provided adequate statistical power to detect significant group differences in both playing ability and cognitive knowledge. Post hoc power analysis based on the observed effect size indicated statistical power exceeding .90, confirming that the study was sufficiently powered to detect meaningful instructional effects. From a practical standpoint, the sample size was also constrained by the availability of eligible intercollegiate kabaddi players meeting inclusion criteria. Nevertheless, equal group allocation and randomized assignment strengthened internal validity and minimized bias. The combination of statistical power analysis and practical feasibility supports the adequacy of the sample size for experimental evaluation.

**Variables**

**Independent Variable**

Type of Instructional Training:

**CAIT** (Computer-Assisted Instructional Training)

**TIT** (Traditional Instructional Training)

**CIT** (Combined Instructional Training)

**Dependent Variables**

- Playing Ability
- Cognitive Knowledge

**Instrumentation**

**Playing Ability Assessment**

Playing ability was evaluated using a standardized expert rating scale designed to assess execution quality of offensive and defensive kabaddi skills. Three national-level certified coaches independently rated each participant. Inter-rater reliability was calculated using the Intraclass Correlation Coefficient (ICC), yielding a value of .89, indicating high reliability.

**Cognitive Knowledge Questionnaire**

Cognitive knowledge was assessed using a 30-item structured questionnaire covering:

- Rules and regulations
- Tactical positioning
- Offensive and defensive strategies
- Game situational awareness

Content validity was established through expert review by five subject specialists. Reliability analysis using Cronbach's alpha produced a coefficient of .86, indicating strong internal consistency.

**Training Protocol**

The intervention lasted eight weeks, with five sessions per week (total 40 sessions). Each session lasted 60 minutes.

**CAIT Group**

- 20 minutes of structured multimedia instruction
- Slow-motion video analysis
- Visual breakdown of movement phases
- 40 minutes supervised practical drills

**TIT Group**

- 20 minutes lecture and live demonstration

- Immediate corrective verbal feedback
- 40 minutes supervised practical drills

**CIT Group**

- 10 minutes multimedia instruction
- 10 minutes live demonstration
- 40 minutes supervised practice with immediate correction

Attendance was monitored, and all participants completed at least 95\% of training sessions.

**Statistical Assumptions**

Prior to conducting Analysis of Covariance (ANCOVA), the underlying statistical assumptions were examined to ensure the validity of inferential results.

**Normality**

The assumption of normal distribution for dependent variables was assessed using the Shapiro–Wilk test and visual inspection of Q–Q plots. Results indicated no significant deviation from normality ($p > .05$). Skewness and kurtosis values were within acceptable limits (±1), supporting normal distribution of playing ability and cognitive knowledge scores.

**Homogeneity of Variance**

Levene's test was performed to examine equality of variances across instructional groups. The results were non-significant ($p > .05$), confirming homogeneity of variance for both dependent variables.

**Linearity.**

Scatterplots were inspected to verify a linear relationship between the covariate (pre-test scores) and post-test dependent variables. The relationship was observed to be linear, satisfying this assumption.

**Homogeneity of Regression Slopes**

An interaction term between group and pre-test scores was tested to determine whether regression slopes were equal across groups. The interaction effect was non-significant ($p > .05$), indicating that the assumption of homogeneity of regression slopes was met.

**Independence of Observations**

Independence was ensured through randomized group allocation and separate training sessions for each instructional group.

**Multicollinearity**

Correlation analysis between covariates and dependent variables showed moderate but non-problematic correlations ($r < .80$), indicating absence of multicollinearity. Since all assumptions were satisfied, ANCOVA was deemed appropriate for comparing adjusted post-test means among instructional groups.

**Statistical Analysis**

Data analysis was conducted using SPSS (Version XX).

**The following analyses were performed**

- Paired sample t-test (within-group improvements)
- ANCOVA controlling for pre-test scores
- Scheffé post hoc test
- Effect size (partial $\eta^2$)

**Prior to ANCOVA, assumptions were tested**

- Normality (Shapiro-Wilk test)
- Homogeneity of variance (Levene's test)
- Homogeneity of regression slopes
- All assumptions were satisfied ($p > .05$).

## RESULTS

**Descriptive Statistics**

ANCOVA revealed statistically significant differences among groups. Mean Playing Ability Scores Mean and Standard Error (Figure 1). Mean Cognitive Knowledge Scores Mean and Standard Error (Figure 2). Table 1 presents adjusted post-test means after controlling for baseline scores. The CIT group demonstrated the highest adjusted means for both dependent variables.

**ANCOVA Results**

ANCOVA revealed statistically significant differences among groups.

The effect size for instructional method was partial $\eta^2 = .42$, indicating a large effect according to conventional benchmarks.

**Post Hoc Analysis**

**Scheffé post hoc comparisons revealed**

- CIT significantly outperformed TIT ($p < .01$)
- CIT significantly outperformed CAIT ($p < .05$)
- CAIT showed moderate improvement compared to TIT

**Interpretation of Effect Size**

The observed partial $\eta^2$ value of .42 indicates that approximately 42% of the variance in post-test performance can be attributed to the instructional intervention after controlling for baseline scores. Because ANCOVA statistically adjusts for pre-test differences, the observed effect largely reflects the impact of the instructional modality rather than pre-existing variations in skill level or experience. Random assignment of participants further minimized potential confounding influences such as prior competitive exposure or tactical knowledge.

## DISCUSSION

The present investigation examined the comparative effectiveness of Computer-Assisted Instructional Training (CAIT), Traditional Instructional Training (TIT), and Combined Instructional Training (CIT) on motor skill acquisition and cognitive knowledge among intercollegiate kabaddi players. The findings revealed that although all three instructional approaches resulted in statistically significant improvements, the Combined Instructional Training (CIT) group demonstrated superior gains in both playing ability and cognitive performance. The observed large effect size (partial $\eta^2 = .42$) indicates not only statistical significance but also substantial practical relevance within competitive sport training contexts. The superiority of the hybrid instructional model may also be explained through the interaction between observational learning and multimedia cognitive processing. During the cognitive stage of motor skill acquisition, athletes rely heavily on visual information to construct internal representations of movement patterns. Multimedia demonstrations allow repeated observation of biomechanical sequences such as body alignment, timing, and spatial positioning during kabaddi techniques. This repeated exposure facilitates neural encoding of the motor pattern, strengthening perceptual–motor coupling. During the associative stage, video replay and guided practice enable athletes to refine these neural representations through feedback and error correction, leading to improved coordination and execution efficiency. Observational Learning Theory further supports these findings.

Repeated exposure to expert-modeled actions through video presentation activates cognitive encoding processes and potentially primes motor circuits via neural mirroring mechanisms. When athletes subsequently engage in physical practice, the pre-activated motor representations may reduce initial error rates and increase movement efficiency. The combination of observation and supervised execution appears to strengthen retention and motor consolidation more effectively than either method alone. The improvement in cognitive knowledge within the CIT and CAIT groups can be attributed to the structured presentation of information through multimedia formats. Visual segmentation of skill components, synchronized verbal explanation, and replay functions likely enhanced schema formation and reduced extraneous cognitive load. In contrast, traditional instruction, while effective in conveying procedural guidance, may not provide repeated exposure to complex tactical scenarios in the same structured manner. The integration of visual and auditory channels appears to have facilitated deeper processing and improved retention of rules, strategies, and situational awareness. The large effect size observed in this study suggests meaningful practical implications. A partial $\eta^2$ of 0.42 indicates that approximately 42% of the variance in post-intervention performance can be attributed to instructional method after controlling for baseline differences. In applied sport settings, such magnitude represents a considerable enhancement in training efficiency. For competitive kabaddi programs, even marginal improvements in reaction time, coordination, and tactical judgment can influence match outcomes. Therefore, adopting hybrid instructional frameworks may provide a competitive advantage. While CAIT alone demonstrated notable improvements, it did not surpass the combined model. This suggests that exclusive reliance on technology may lack contextual adaptability and individualized correction.

Direct coach interaction provides immediate biomechanical adjustment, motivational reinforcement, and situational adaptation that multimedia tools cannot fully replicate. Thus, rather than replacing traditional coaching, technology appears most effective when integrated strategically within a structured pedagogical framework. Video-based modeling used in multimedia instruction may also stimulate the mirror neuron system, which activates both during action execution and observation. Repeated observation of expert demonstrations in kabaddi may therefore prime neural circuits responsible for movement planning and coordination. This neural priming may enhance anticipatory decision-making during raiding and defensive situations, as players become more capable of recognizing movement cues and responding rapidly to opponent actions. Despite its strengths, the study has limitations. The sample size was restricted to male intercollegiate athletes, which may limit generalizability to female players or elite professional populations. The intervention duration was eight weeks; longer-term follow-up could provide insight into retention and transfer effects. Additionally, biomechanical motion analysis was not employed, which may have provided objective performance metrics beyond expert ratings. Future research should investigate longitudinal effects of hybrid instructional training, include female athletes, and integrate motion-tracking technologies to quantify kinematic improvements. Exploring psychological variables such as motivation, confidence, and decision-making speed may further clarify the mechanisms underlying hybrid training effectiveness. In conclusion, the findings demonstrate that integrating multimedia learning principles with traditional coaching methods produces superior improvements in both motor performance and cognitive knowledge. Hybrid instructional training represents a theoretically grounded and practically effective model for advancing kabaddi pedagogy in contemporary collegiate sport environments.

## LIMITATIONS AND FUTURE RESEARCH

Future research should integrate biomechanical motion-tracking technologies and neurocognitive performance measures to determine whether observed improvements reflect genuine motor learning rather than temporary performance gains. High-speed motion analysis, wearable inertial sensors, and reaction-time based perceptual–cognitive assessments could provide objective indicators of skill refinement and decision-making efficiency in kabaddi training environments.

## PRACTICAL APPLICATIONS

The findings of the present study provide meaningful implications for coaches, sport administrators, curriculum planners, and institutional policymakers involved in kabaddi training and physical education programs. Given the demonstrated superiority of Combined Instructional Training (CIT), practical integration of multimedia-based learning with traditional coaching methodologies may enhance both skill acquisition and cognitive development in competitive environments. First, kabaddi academies and collegiate training centers should consider the structured incorporation of video-based instructional modules within regular practice schedules. Multimedia content may include slow-motion breakdown of offensive techniques such as hand touch, toe touch, and kicking, as well as defensive maneuvers like ankle hold and blocking. Segmenting complex skills into discrete phases—approach, execution, and recovery—can facilitate clearer biomechanical understanding. Coaches may allocate a brief but consistent period within each training session for visual modeling prior to on-field execution. This sequencing aligns with motor learning principles, allowing athletes to construct accurate mental representations before physical practice. Second, the development of digital skill repositories can provide sustained learning opportunities beyond scheduled practice hours. Institutions may create curated libraries containing annotated match footage, expert demonstrations, tactical scenarios, and rule interpretations. Such repositories can be hosted on institutional learning management systems or secure digital platforms accessible to registered athletes. The availability of on-demand video resources enables self-directed learning, reinforces retention, and supports revision before competition. In addition, archiving player performance footage for comparative analysis can foster reflective practice and promote continuous improvement.

Third, hybrid instructional frameworks should be embedded within physical education and coaching certification curricula. Teacher training programs can integrate modules on multimedia content design, video analysis techniques, and technology-assisted feedback strategies. Preparing future coaches to effectively combine digital tools with traditional pedagogical skills ensures sustainable implementation. Rather than replacing conventional instruction, technology should serve as an augmentation tool that enhances demonstration clarity, feedback precision, and learner engagement. Furthermore, structured hybrid training models may be particularly valuable in resource-constrained environments. Even basic recording devices and free video-editing software can produce effective instructional materials. Therefore, adoption of hybrid methods does not necessarily require advanced infrastructure but rather systematic planning and pedagogical adaptation. At the competitive level, hybrid training may contribute to improved tactical intelligence. By repeatedly analyzing game situations through multimedia platforms, athletes can enhance anticipatory skills and decision-making accuracy under pressure. Coaches may incorporate scenario-based video simulations to prepare players for high-stakes match contexts, thereby strengthening psychological readiness alongside technical competence. Finally, governing bodies and sport federations may consider promoting technology-assisted training guidelines within national development programs. Standardized digital content aligned with official rules and skill standards could ensure consistency in instructional quality across institutions. Overall, the practical application of hybrid instructional training extends beyond immediate performance gains. It represents a strategic modernization of kabaddi pedagogy, fostering sustainable athlete development, cognitive sophistication, and competitive excellence.

## CONCLUSION

The present study systematically examined the comparative effectiveness of Computer-Assisted Instructional Training (CAIT), Traditional Instructional Training (TIT), and Combined Instructional Training (CIT) on motor skill acquisition and cognitive knowledge among intercollegiate kabaddi players. The findings demonstrated that although all instructional approaches contributed to measurable improvements, the hybrid model integrating multimedia-based learning with direct coach-led instruction produced the most substantial gains in both performance execution and cognitive understanding. The observed large effect size underscores the practical significance of instructional methodology in shaping athlete development. By combining visual modeling, structured

feedback, and real-time biomechanical correction, the hybrid approach appears to facilitate more efficient progression through stages of motor learning while simultaneously strengthening tactical awareness and rule comprehension. These outcomes reinforce the theoretical foundations of motor learning theory, observational learning mechanisms, and multimedia cognitive processing frameworks. Beyond statistical validation, the study contributes to the modernization of kabaddi pedagogy by providing empirical evidence supporting technology-integrated training systems within indigenous sports contexts. The results suggest that instructional design plays a critical role in optimizing training efficiency, particularly in sports that demand rapid perceptual decision-making and precise motor coordination. Importantly, the findings do not advocate replacing traditional coaching methods; rather, they emphasize strategic integration. The synergy between digital visualization tools and live corrective instruction appears to generate complementary learning benefits that neither approach achieves independently. This balanced instructional paradigm may serve as a sustainable model for collegiate training programs seeking to enhance competitive performance. In conclusion, hybrid instructional training represents a theoretically grounded, practically feasible, and empirically supported framework for advancing skill acquisition and cognitive development in kabaddi. Adoption of such integrated pedagogical models may contribute to long-term athlete development, improved match performance, and elevated standards of coaching practice in contemporary sports education environments.

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**Table 1: Presents adjusted post-test means after controlling for baseline scores.**

| Group | Playing Ability | Cognitive Knowledge |
|---|---|---|
| CAIT | 78.45 | 82.67 |
| TIT | 74.12 | 76.38 |
| CIT | 84.93 | 88.51 |

**Table 2: ANCOVA Results: ANCOVA revealed statistically significant differences among groups.**

| Source | SS | df |
|---|---|---|
| Group | 312.45 | 2 |
| Error | 8.96 | .001 |

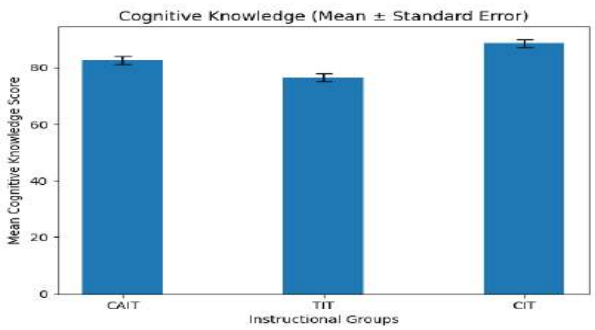



**Figure 1: Mean Playing Ability Scores Mean and Standard Error**

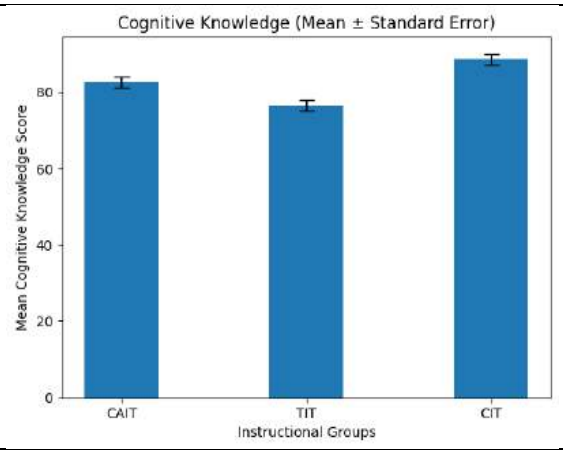



**Figure 2: Mean Cognitive Knowledge Scores Mean and Standard Error**

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RESEARCH ARTICLE

# Prestige Dynamics in Maharashtra's Book Publication Industry: From Manuscripts to Modern Markets

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## ABSTRACT

Present study prestige dynamics in Maharashtra's book publication industry: from manuscripts to modern markets, examines the historical evolution and reputation-building processes of Marathi publishing in Maharashtra. Situating the regional industry within broader global developments from early manuscript cultures and Gutenberg's print revolution to contemporary market systems the paper conceptualizes publishing as a fusion of cultural mission and commercial enterprise. Drawing upon qualitative, historical-descriptive methodology and secondary sources such as encyclopedias, commemorative volumes, bibliographies, and practitioner accounts, the research traces key phases: pre-independence foundations, wartime innovations, post-independence expansion, and post-1970 professionalization. Present findings explore reputation in Marathi publishing emerged not merely from commercial success but from sustained literary commitment, editorial rigor, and responsiveness to social and educational needs. Ultimately, the paper argues that Maharashtra's publishing industry exemplifies a dynamic balance between intellectual stewardship and entrepreneurial adaptation, offering insights into sustaining cultural prestige in the face of technological and digital transformations.

**Keywords:** Marathi Publishing Industry, Prestige Dynamics, Cultural Mission and Commerce, Print History in Maharashtra, Publishing Professionalization

## INTRODUCTION

India's book publishing sector stands apart from other traditional commercial businesses since it is based on the goal of fostering an informed, thoughtful society rather than just making money. A long-term repository of social memory, it ensures that scientific information, historical experiences, and cultural values are continuously reinterpreted by new readers by choosing, creating, and distributing books that can be read and reread over generations. In this way, Indian publishing serves as both a commercial venture and a cultural and educational service that preserves the country's intellectual legacy for future generations [1]. The sanctity of books is expressed by the influential Marathi saint Samarth Ramdas in the 17th century in ways that foreshadow this larger social function. According to him, a book is one that facilitates spiritual quest, information acquisition, and personal sanctification, highlighting its power to change one's thinking and character. From this perspective, publishing is seen as an organized endeavor to bring an author's inner expression into the public realm, from the earliest manuscript stage to the completed volume in the reader's hands, rather than just a technical process. The prestige and responsibilities of publishing in India are derived from this continuum, which runs from creation to circulation [1]. The historical underpinnings of this activity are found in worldwide advancements in book manufacture and printing. The origins of systematic publishing can be found in China in the ninth century, when woodblock printing made it possible to create multiple copies of a book far more quickly than hand copying by allowing full pages to be carved, inked, and duplicated at scale. The invention of movable type by Johannes Gutenberg in Germany in the 15th century turned this craft into an industry.

His 42-line Bible in 1455 is frequently cited as a landmark because it significantly decreased the time, labor, and cost of book production by as much as 80 percent making reading material accessible to people outside of the clerical and elite circles. From the scrolls and parchment codices used in ancient Greece and Rome to printed paper volumes, microforms, CD-based texts, and e-books today, book forms have changed over time to reflect ongoing changes in how societies store and disseminate information [2,3]. International organizations have suggested formal definitions in an effort to provide conceptual clarity in this area. The United Nations Educational, Scientific, and Cultural Organization (UNESCO) clearly acknowledges the link from editorial choice to reader access when it defines publishing as the process of choosing written material, printing and publishing it, and organizing its marketing and dissemination. In addition, UNESCO defines a book as a non-periodical printed product with at least 49 pages (not including covers). UNESCO has also helped standardize identifiers such as the International Standard Book Number (ISBN), which makes it easier to trace and circulate titles around the world [1,4]. The Marathi publishing sector in Maharashtra is a particularly instructive example of how reputation arises from a combination of cultural mission and entrepreneurial adaptation within this larger national and international context. In order to maintain financial viability in the face of difficult economic and competitive circumstances, a number of Marathi publishers started to professionalize their businesses during the pre-independence and wartime decades. They frequently combined publishing with bookselling. One of the first and most significant of these was Dhawale Publications, a well-known brand in the industry that is credited with publishing the first Marathi children's book, Mulamulinche Chimukale Pustak.

The company later experienced significant growth after merging with Karnataka in 1945. Under the direction of Sonyabapu Dhawale, who was given the title Mudran Maharishi, the company is estimated to have contributed nearly 20% of all Marathi book publications in Mumbai between 1940 and 1950. Following this merger, Dhawale's combined catalogue reached about 1,500 titles, mainly in the areas of religion, children's literature, school textbooks, and creative and historical works [1,5,6]. Parallel to Dhawale's growth, Vaman Vishnu Bhat's 1944 founding of Abhinav Publications raised the stature of Marathi publishing by purposefully emphasizing literary excellence and intellectual enlightenment. Abhinav purposefully sought out fresh authors and collaborated closely with them during the editorial process, publishing about 250 works over the course of about 45 years. Additionally, Bhat started the publication Sangrahalaya, which introduced readers to new books and literary advancements and serially reproduced rare and out-of-print classics. This effectively used periodical publishing as a weapon for literary

awareness and canon creation. In Kesari, Shankarrao Kulkarni's Maharashtra Granth Bhandar, together with Dhawale and Abhinav, invented contemporary marketing techniques like mailing lists and newspaper ads thereby systematizing book sales and broadening the reach of Marathi titles beyond local walk in customers [7, 8, 4, 1]. In light of this, Maharashtra's Marathi publishing sector has gained recognition for its educational contributions, willingness to try out a variety of genres, including well-liked formats like mystery series, and, most importantly, its insistence that quality be upheld as the cornerstone of prestige. [1, 9, 10]. In order to better understand how this network of historical developments, institutional practices, and intellectual ideals has shaped the status of Marathi publishing from its early manuscript and print traditions to its contemporary, market-oriented forms, the current study, From Manuscripts to Modern Markets: Prestige Dynamics in Maharashtra's Book Publication Industry was created [1].

## MATERIALS AND METHODS

The qualitative, historical-descriptive methodology used in this study is perfect for using narrative synthesis in place of experimental or quantitative approaches to recreate the prestige trajectory of Maharashtra's book publishing business. It is consistent with the traditions of library and information science, focusing on archive interpretation to document events from manuscripts before independence to market adaptations after 1970.

**Research Design And Data Collection**

By applying content analysis of secondary sources, the methodology focuses on textual patterns for prestige indicators (e.g., output volume, innovations) and systematically evaluates published materials without the need for primary data generation. Some of the sources are:

**Encyclopedias**: Marathi Vishwakosh (Vol. 5). Mumbai: Maharashtra Rajya Sahitya Sanskruti Manda for printing history and definitions [1,2]

**Commemorative volumes**: Dhawale Janmashatabdi Smarnika detailing mergers and institutional growth [7]

**Periodicals and questionnaires**: Mudran Prakash surveys (e.g., Anantrao Kulkarni on publisher traits; Kelkar, M. M. (1962). "Prakashanaachya Vatevaril Andhar v Ujed." Mudran Prakash, pp. 83-85) for practitioner insights [5,11].

**Bibliographies and autobiographies**: Date Granthsuchi, Part II. Mumbai: Ra. Ma. Vi. Samstha for 7,839 titles (1938-1950) [12]; Majhi Atmakatha (1988). Continental Prakashan for personal narratives [13]

**Online archives**: Marathi Wikipedia (n.d.); https://maitri2012.wordpress.com for supplementary profiles, Marathi Wikipedia.(n.d.)., Publisher and publication.(n.d.).

**Data Analysis And Synthesis**

Chronological synthesis organizes content into eras: pre-1940s foundations, WWII innovations, post-independence expansions. Thematic coding identifies prestige factors:

**Innovation** (e.g., Kulkarni's mailing lists [8]

**Output scale** (e.g., Dhawale's 1,500 titles [7]

**Literary impact** (e.g., Bhat's 250 books. [9]

This mirrors Ranganathan, S. R. (1958). Classified Catalogue Code. Madras Library Association's classificatory rigor for narrative interpretation over statistical modeling [6,9]. Ajun Kalas Chadhavaachaa Aahe. Deshmukh provided profile templates (Table 1).

**Validity, Reliability, and Limitations**

Triangulation across sources enhances credibility; chronological-thematic coding ensures reliability.

**Limitations:** Secondary reliance omits unpublished data; no econometrics (addressed via qualitative depth).

**Ethical note:** All public domain/academic sources used without alteration.

## RESULTS AND DISCUSSION

A historical and thematic study of secondary data is presented in this section, explaining how Maharashtra's publishers established their reputation through adaptation, scalability, and innovation. Synthesized sources provide insights that show resilience patterns in the face of external constraints and low margins.

**Pre Independence Foundations (Pre-1940s)**

Early businesses created a foundation for reputation through mergers and a variety of products. After Dhawale Publications acquired Karnataka in 1945, Dhawale Printers and Publishers was established, with 1,500 titles (religious/spiritual works, school textbooks, children's literature, creative/historical texts); Sonyabapu Dhawale (from 1938) became an expert in printing aesthetics, capturing approximately 20% of Mumbai's Marathi publications (1940–1950) out of 7,839 titles [7,12]. Contemporaries like Parchure Puranik, Ramkrishna Book Depot, and Suras Granthamala (Pandharpur) continued, fusing tradition and demand, while Chitrashala Publications maintained creative literature, intellectual conversation, and school education [7] These mergers raised Marathi's intellectual standing prior to independence by professionalizing operations and putting quality above profit.

**WWII-Era Innovations and Expansion (1940s-1960s)**

New houses were sparked by World War II, Abhinav Publications published about 250 books over 45 years, released a 16-year Sangrahalaya periodical that republished rare books like Kavi/Kavyodaya for enlightenment Deshmukh, R.J. (1995), and Maitri (2012). They also discovered authors (e.g., Mama Varerkar collaborations). Through creative promotion, Maharashtra Granth Bhandar (Shankarrao Kulkarni, who was born in Kolhapur after 1935) sold more than 500 copies of Kesari advertisements and mailing lists [8]. Char Aane Mala (1925, Veerkar; romance/mystery); Rahasya Mala (1939, P.P. Samant; 123-page Rahasyajaal at 4 annas), which spawned 10+ (Kanchanmala 1942; Yauvanmala 1946; etc.) but faded after the 1970s [14].

**Textbook breakthroughs**

For graded readers, Mangeshrao Kulkarni (Karnataka, 1935) broke international monopolies (Macmillan/Oxford) and gave rise to Pune houses like Patil Co., Y.G. Joshi, and M.M. Kelkar [12]. In order to remain viable and raise awareness, wartime entrepreneurship combined publishing and bookselling with affordability and creativity.

**Post independence prestige consolidation (Post-1970)**

Adapted by second and third generation leaders, author talks were stressed for excellence by Rajhans Prakashan (1952), Shivshahir Purandare, and Dilip Majgaonkar (1957) [15]. Samdas Bhatkal's Prakashan and Ashish Patkar of Manovikas brought doorstep delivery, worldwide benchmarking, and meetings. Extensions into college courses (Marathi/English; social sciences to fine arts) took advantage of cooperatives, education, and decentralization [16]. Diversifying marketing helped maintain reputation and go from survival to cultural leadership in the face of digital threats (Table 2). Data shows that prestige is a product of mission-commerce balance: impact (awareness via magazines), innovation (mailing/series), and scale (e.g., Dhawale domination) are robust despite losses in genres like mysteries [15]. According to Ranganathan (1958), this interpretive framework emphasizes the function that publishers play in society.

**Pioneering Resilience and Mission-Commerce Fusion**

Pre-independence elites combined financial savvy with cultural mission to create prestige. Through author discovery and the Sangrahalaya periodical, V.V. Bhat's Abhinav Publications (1944; 250 books over 45 years) demonstrated "literary evangelism" by republishing rare works for Marathi awareness [9,17]. Mailing lists and Kesari advertisements, two of Shankarrao Kulkarni's Maharashtra Granth Bhandar innovations, creatively scaled sales [8].

Sonyabapu's 1945 Karnataka merger under Dhawale (1,500 titles; 20% Mumbai share 1940-1950) combined religious and educational outputs with low profit margins [7]

**Genre and Market Adaptations**

Cheap mystery series (Char Aane Mala, 1925; Rahasya Mala, 1939 at 4 Annas; 10+ Malas, like Kanchanmala) made reading more accessible to a wider audience, but they declined in the 1970s as a result of genre fatigue [14]. Pune houses emerged as a result of textbook innovations (Karnataka 1935 vs. foreign monopolies), diversifying graded readers [12].

**Post-Independence Expansion and Sustainability**

Education surges, decentralization, and cooperatives fueled college-level Marathi texts (social sciences to fine arts). Second-generation leaders like Rajhans' Dilip Majgaonkar (author dialogues) and Popular's Samdas Bhatkal adopted doorstep delivery, meets, and global benchmarking, intertwining publishing-bookselling for viability [15,16]. Digital services and quality focus countered competition [5]

**Quantitative Prestige Markers**

7,839 Marathi titles (1938-1950); Dhawale dominance; Bhat's output exemplify scale amid modest profits [2, 12]. The results show that prestige emerged as a result of bookselling hybrids' synergistic adaptation to commerce. (Ranganathan, S.R. (1958)) sustained mission [11]. Although the stability of textbooks counteracts the mystery loss, post-1970 alterations (Patkar reflections) reassert relevance through globalization (Table 3).

## CONCLUSION

Maharashtra's publishing sector transformed Marathi literature from specialized manuscripts to contemporary markets, demonstrating the transformational power of resilient pioneers' commerce-mission fusion. "That which serves spiritual pursuit, imparts knowledge, sanctifies: such is a book," as Samarth Ramdas envisioned, "remains a conduit for enlightenment" (original paper). Future directions include using empirical post-digital indicators to maintain legacy in the face of AI and e-books [4].

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**Table 1: Key source categories and their analytical roles in the study**

| Source Type | Examples | Analysis Role | References |
|---|---|---|---|
| Encyclopedias | Marathi Vishwakosh Vol. 5 (1979) | Historical context | [1,2] |
| Commemoratives | Dhawale Janmashatabdi (1982) | Institutional milestones | [7] |
| Periodicals | Mudran Prakash (1962) | Practitioner views | [5,11] |
| Bibliographies | Date Granthsuchi (2000) | Quantitative output | [12] |
| Autobiographies | Majhi Atmakatha (1988) | Personal innovations | [13] |

**Table 2: Evolution of key marathi publishing houses, prestige rivers, and output impact across eras**

| Era | Key Houses | Prestige Drivers | Output/Impact (Sources) |
|---|---|---|---|
| Pre-1940s | Dhawale, Chitrashala, Parchure | Mergers, spiritual/educational blends | 1,500 titles; 7,839 total (1938-1950) [7,12] |
| 1940s-1960s | Abhinav (Bhat), Maharashtra Granth Bhandar, Majestic, Rahasya Mala | Mailing/series innovations, author discovery | 250 books; 10+ series [8,9,11,17] |
| Post-1970 | Rajhans (Majgaonkar), Popular (Bhatkal) | Doorstep marketing, college texts | Multi-subject diversification [15,16] |

**Table 3: Prestige drivers across eras**

| Driver | Pre Independence Example | Post Independence Example | Impact |
|---|---|---|---|
| Innovation | Kulkarni mailing [8] | Doorstep delivery [15] | Market access |
| Scale | Dhawale 1,500 titles [7] | College texts expansion [16] | Output growth |
| Literary Mission | Bhat's Sangrahalaya [18] | Author nurturing [15]. | Cultural elevation |

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REVIEW ARTICLE

# Puerarin : A Multifunctional Isoflavone : Pathways, Technologies and Therapeutic

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## ABSTRACT

Puerarin is an important C-glycoside isoflavone mainly sourced from *Pueraria lobata* and *Pueraria tuberosa*. It shows various pharmacological properties and has wide therapeutic potential. This review brings together the current knowledge of its biosynthetic pathways, which include shikimate, phenylpropanoid, and C-glycosylation. It also highlights puerarin's role as a chemotaxonomic and quality-control marker in herbal products. Different extraction methods, from traditional maceration and Soxhlet to modern microwave and enzyme-assisted techniques, are compared for their efficiency and sustainability. Techniques like TLC, HPTLC, and HPLC help ensure accurate identification and measurement of puerarin. Despite its poor solubility and low oral bioavailability, advances in nanotechnology, such as polymeric nanoparticles and nanostructured lipid carriers, have greatly improved its bioavailability and therapeutic effectiveness. Puerarin shows strong anti-inflammatory, antioxidant, cardioprotective, neuroprotective, and anti-diabetic effects. Recent studies suggest it may also be useful for nerve regeneration, dopaminergic modulation, and anti-skin-ageing treatments. Overall, puerarin stands out as a versatile bioactive compound that connects traditional herbal medicine with modern pharmaceutical development, presenting significant possibilities for future drug and nanomedicine innovation.

**Keywords:** Puerarin, Biosynthesis, Pharmacokinetic activity, Extraction technique, Analytical methods, Pharmacology study.

# INTRODUCTION

Puerarin is the primary phytomarker, mainly extracted from the roots of Pueraria species like *Pueraria lobata* and *Pueraria tuberosa*. Puerarin, a unique component of the pueraria genus, serves as a quality control marker for herbal medicines such as *Pueraria lobata* and *Pueraria thomsonii* in China, displaying diverse pharmacological properties [1]. Puerarin has various effective components from different parts of the kudzu plant. Contemporary pharmacological research indicates that puerarin exhibits multiple bioactive effects, including estrogen-like activity, anti-inflammatory properties, antioxidant responses, blood pressure regulation, blood glucose lowering, and potential anticancer effects[1]. The puerarin is cited as diadzein-8-C-glucoside, which is derived from the genus Pueraria and from the roots of the kudzu plant. Puerarin, as an isoflavone, serves as the chemotaxonomic marker for the genus Pueraria. Various bioactivities of Pueraria are caused by isoflavone glycosides, especially puerarin. Hydroxyl groups are substituted at positions 7 and 4, and a β-D-glucopyranosyl residue is attached at position 8 via a C-glycosidic bond. The profile of Puerarin is mentioned in table 1. Profile of Puerarin(Table 1).

**Sources**

Puerarin is obtained from various plant species of Pueraria. Various plant species along with their parts are listed in Table 2. Sources of Puerarin (Table 2).

**Biosynthesis of Puerarin** [7]

Puerarinuses various enzymes and reactions in the biosynthesis of puerarin. It is biosynthesised by three different pathways, i.e. Shikimate pathway, the Phenylpropanoid pathway, and Puerarin pathway. These pathways are shown in Figure 1. From 3 different pathways, the first is the Shikimate pathway, where erythrose-4-phosphate reacts with phosphoenolpyruvic acid and gives shikimic acid, and further it provideschorisimic acid, then in the presence of phAT, and ADT enzyme, it gives phenylalanine as the final product of the shikimate pathway. Then, in the Phenylpropanoid pathway, phenylalanine undergoes phAAl enzymes and forms trans-cinnamic acid with the reduction of ammonia, and after that, in the presence of C4L enzyme, it gives 4-coumaric acid. From trans-cinnamic acid, and 4-coumaric acid, in the presence of C4CoAL enzyme, gives product 4-coumaroyl-CoA. Afterwards, in the Puerarin pathway, isoliquiritigenin from 4-coumaroyl-CoA in the presence of CHS and CHR, and after that in the presence of CHI, liquiritigenin is formed, which is further catalysed by 2-hydroxyisoflavonon (IFS) and produces 2,7,4-trihydroxyisoflavonone.We get two products or puerarin can be produced in two ways: by catalyzing The chalcone isoflavone is synthesized by using 2, 7, 4-trihydroxyisoflavonone dehydratase (HID) to produce diadzein, and 8-C-glucosyltransferase (8-C-GT) to form 2, 7, 4-glucosyltransferase. to create 2, 7, 4-trihydroxyisoflavonone-8-C-glucoside. Then, from 2,7,4-trihydroxyisoflavonone-8-C-glucoside and diadzein,puerarin is formed in the presence of 8-C-GT and HID or PIUGT43.

**Pharmacokinetics of Puerarin**

Puerarin displays species-specific pharmacokinetics, with a two-compartment open model observed in rats and dogs, while a three-compartment model better fits rabbits [8]. After intravenous administration, puerarin is broadly distributed across rats' organs, including thehippocampus, heart, lungs, stomach, liver, mammary gland, kidney, spleen, tibia, and femur[9]. It is capable of crossing both the blood-brain and placental barrier, warranting caution during pregnancy [10, 11]. After puerarin is administered to both humans and animals, a small number of phase I functionalization reactions (reduction and hydrolysis) and phase II conjugation reactions (glucuronidation and sulfation) metabolites have been discovered in biological samples[11, 12]. Its major hydrolysis product is diadzein, which is further metabolized to dihydrodaidzein and equol. Key metabolites like puerarin-7-O-glucuronide and puerarin-4-O-glucuronide are catalyzed by multiple UGT isoforms, with UGT1A1 playing a significant in the human liver. These conjugates are primarily excreted in urine and faeces. Notably, metabolic profiles vary by tissue and sample type, with unmetabolized puerarinfound in serum and conjugated/reduced forms appearing in urine over time, suggesting rapid absorption with delayed systemic metabolism [12].

**Herbal Influences on Puerarin Pharmacokinetics**

Gegenqinlian Decoction (GQD), which combines *Radix Puerariae* (Gegen) with *Scutellaria baicalensis*, *Coptis chinensis*, and *Glycyrrhiza uralensis*, is a different herbal preparation that increases puerarin absorption and slows its clearance compared to pure Radix Puerariae extract in rats [13]. Among its components, glycyrrhizic acid significantly inhibits puerarin's intestinal uptake, while baicalin and berberine facilitate its absorption. Studies indicate that certain herbs in a medicine called Longmaining aid in the absorption of puerarin in rats. The research shows that Longmaining enhances puerarin absorption throughout the entire intestine. Specifically, in the upper sections (duodenum and ileum), the herb *Dioscoreae nipponicae* rhizome contributed most significantly. In the middle and lower sections (jejunum and colon), both *Dioscoreae nipponicae rhizome* and *chuanxiong rhizome* worked together to improve the absorption of puerarin[13].

Additional herbs shown to increase intestinal puerarin absorption include:

- *Gastrodiae rhizome*
- *Angelica dahurica* (Radix *angelica edahuricae*)
- *Chauanxiong rhizome*, which enhances solubility, reduces the expression of claudin-5 and P-glycoprotein efflux.

Because of the intricate nature of these herbal combinations, the precise interactions often remain unclear. Still, the general mechanism involves changes in intestinal permeability, transporter activity, and physical solubility of puerarin[13].

**Extraction Methods for Puerarin**

For the extraction of puerarin, there is an extraction technique where puerarin isoflavone must be extracted, purified, and analyzed using appropriate and efficient methods [7]. There are two different techniques for the extraction of puerarin: (a) Conventional extraction technique and (b) Modern or Advanced extraction technique. Maceration, Percolation, Soxhlet extraction, and decoction are examples of traditional, conventional techniques. Cloud point extraction, high-speed countercurrent chromatography, extraction, solid-phase extraction, enzyme-assisted extraction, microwave extraction, ultrasonic extraction, and high-speed countercurrent chromatography are examples of advanced extraction techniques [6]. Since every extraction technique has its pros and cons, selecting the best one for isoflavones requires considering variables such as cost, effectiveness, and environmental impact[7]. The purest puerarin is produced using a traditional method called solid-liquid extraction, despite being simple and reasonably priced. Although it is simple and inexpensive, the traditional method of solid-liquid extraction yields the purest puerarin. Although supercritical fluid extraction is a highly effective and pure technique, it is expensive and requires complex installations[6]. Microwave-assisted extraction increases the effectiveness of the extraction process and decreases extraction time [7]. This technique is performed in a CW-2000 microwave preparation system with an adjustable power output from 10 to 850 W at 2450 Hz [12]. The application of microwave energy may cause a rise in operating expenses. In addition to these, attempts have been made to extract puerarin using various extraction techniques, such as enzymatic and ultrasound-assisted extraction[7]. In these instance, based on the solution extraction method, the ultrasonic-aided extraction method creates a cavitation gas canon by utilizing the cavitation effect created by an ultrasonic wave. The shock wave from the cavitation gas explosion crushes the plant's cell wall, making the target component more soluble[8]. It produces heat or makes it safe and considerate of heat-sensitive chemicals, in contrast to other instrument-assisted extraction techniques. For instance, the steps involved in extraction are shortened because the tank is too big to create an ultrasonic blank area[6]. The advantages of the enzyme-assisted extraction method include mild reaction conditions, little loss of active ingredients, and environmentally friendly solvents. Even so, environment-sensitive enzymes may become dormant at elevated temperature and at low pH, which will raise extraction costs and decrease their effectiveness[6]. In the enzyme-assisted extraction method, when extracting plant isoflavones, the target compounds are prevented from moving into the extraction solvent by the material between cells and the cell walls of the plant. Enzymes are added to break down the typical configuration of intercellular materials and cell walls[8].

**Recent Method for Extraction**

**Improving Puerarin Extraction and Sustainability Considerations**[14]

Based on the OFAT results, we optimized puerarin extraction from *Pueraria lobata* roots using response surface methodology (RSM) to capture interactions among factors. We applied a 5-level, 3-factor central composite rotatable design in Design-Expert. The independent variables were ethanol concentration, extraction temperature, and solvent-to-biomass ratio, with center points from the OFAT results. This generated 20 runs of 30-minute extractions. Puerarin yield (mg/g) was measured by HPLC, and we fitted a second-order polynomial model ($Y = \beta_0 + \Sigma\beta iXi + \Sigma\beta ijXiXj + \Sigma\beta iiXi^2$), which we confirmed with ANOVA. Compared to OFAT, the conditions from RSM significantly increased puerarin yield while reducing extraction time from 3 hours to 30 minutes. Using a GRAS solvent, ethanol-water, at moderate temperatures and relatively low solvent volumes revealed synergistic effects among variables. This approach also lowered energy needs and solvent waste. In contrast to traditional techniques, such as reflux, subcritical water, or ultrasound that require high temperatures, long durations, or complex equipment, this combined OFAT-RSM method achieves comparable or higher yields under milder, safer, and more sustainable conditions. It is better suited for scale-up and resource-limited environments.

**Nanotechnology-Based Formulation Strategies for Puerarin** [15]

Puerarin has poor water solubility and low oral absorption (BCS Class IV). This has sparked interest in new formulation methods. Researchers have explored various nanocarrier systems, including phospholipid complexes, nanocrystals, self-emulsifying systems, and polymeric nanoparticles, to enhance solubility, stability, and absorption.

Polymeric nanoparticles stand out as particularly promising. Here, they created puerarin-loaded nanoparticles using a solvent evaporation method with the biodegradable co-polymer PDLG. They optimized the process with a Box–Behnken design. The optimized system resulted in spherical particles about 120 nm in size, a low PDI of 0.22, a high negative zeta potential of -16.3 mV, an entrapment efficiency of around 90%, a drug loading of 14.6%, and 80% release over 24 hours with no toxicity observed in RAW 264.7 cells. This method produced smaller particle sizes and better encapsulation compared to earlier PLGA-based systems, suggesting improved oral delivery potential. Similar studies on phospholipid complexes, nanocrystals, and other carriers have also shown improved bioavailability and targeting of puerarin. Overall, these nanotechnology-based strategies mark a significant improvement over traditional formulations, allowing for more effective and sustained delivery of puerarin in therapeutic applications.

**Nanoparticle Characterization and Biological Effects**[16]

In a recent study (Pharmaceutics, 2024), puerarin-loaded nanoparticles were carefully characterized and showed a uniform nanoscale size, narrow PDI, high entrapment efficiency, and stable zeta potential. Besides their improved physical and chemical properties, the nanoparticles displayed strong antimicrobial activity against specific bacterial strains. A safety assessment using an *in ovo* model found no significant embryotoxicity or irritation. Notably, the same system also showed pro-angiogenic effects in the chorioallantoic membrane assay. This suggests it could help with vascularization and tissue repair, along with improving puerarin delivery.

**Novel Applications of Puerarin in Nanomaterials**[16]

**Role in Green ZnO NP Synthesis:** In the green synthesis of ZnO nanoparticles, puerarin acts as both a reducing agent and a stabilizing agent. The process involves adding a puerarin solution to a zinc acetate solution at 50°C. Puerarin, which is an isoflavone, has hydroxyl and carbonyl groups that help reduce zinc ions (Zn2+). This creates coordination bonds between the puerarin and the nanoparticles. The puerarin molecules attach themselves to the surface of the newly formed ZnO nanoparticles, preventing them from clumping together and stabilizing them. EDX analysis confirmed the presence of puerarin on the nanoparticle surface by detecting carbon and oxygen.

**Antimicrobial Potential:** The puerarin-functionalized ZnO nanoparticles (PUE-ZnO NPs) showed antimicrobial activity against several bacterial strains and one yeast strain. The strongest antibacterial effects were against *Staphylococcus aureus*, Streptococcus pyogenes, and Escherichia coli, with a minimum inhibitory concentration (MIC) of 25 mg/mL for each. The PUE-ZnO NPs had a weaker antibacterial effect against *Candida parapsilosis*, with an MIC of 50 mg/mL. No activity was found against *Pseudomonas aeruginosa*.

**Angiogenesis Activity:** The document's *in ovo* CAM (chorioallantoic membrane) assay assessed whether PUE-ZnO NPs could affect angiogenesis. The results showed that PUE-ZnO NPs, at a concentration of 100 μg/mL, did not cause any irritation or changes to the vasculature of the chorioallantoic membrane after 24 hours. This indicates that the green-synthesized nanoparticles have good vascular tolerance and do not interfere with the angiogenesis process.

**Analytical Methods for Puerarin**

The importance of analytical methods in assessing the quality of drugs and biomarkers is the main topic of this review. This review covers numerous analytical methods, including Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), High-Performance Thin Layer Chromatography (HPTLC), and quantification and analysis of puerarin in plant and herbal, and ayurvedic formulations.

**Thin Layer Chromatography(TLC)**

Thin Layer Chromatography is an analytical technique that is a fast, sensitive, and cost-effective method which is used to detect various numbers of components from a mixture, then verify the identity and purity of that compound, and monitor the progress of a reaction. Determine the solvent comparison for preparative separations. Examine the fractions obtained from column chromatography. In Table 3, explain the TLC study performed on *Pueraria tuberosa.*
Table 3. TLC of Puerarin

**High-Performance Thin Layer Chromatography(HPTLC)**

High-Performance Thin Layer Chromatography is a technique for identifying or detecting compounds. In this case, HPTLC is utilized for mixture separation, as well as for preparative, qualitative, and quantitative analysis, selective detection, and minimum sample preparation. HPTLC is one of the most advanced techniques that fully utilizes Thin-Layer Chromatography. All HPTLC studies that are performed on puerarin in different plant extracts are given in Table 4. HPTLC of Puerarin (Table 4).

**High-Performance Liquid Chromatography(HPLC)**

A type of column chromatography called High-Performance Liquid Chromatography (HPLC) is frequently used in biochemistry and HPLC analysis to separate, identify, and quantify active chemicals. HPLC is the most commonly used separation technology for detecting, separating, and quantifying the drug [22]. All HPLC studies were performed for puerarin in Table 5.
Table 5. HPLC of Puerarin

**HPLC Vs HPTLC**

HPLC Vs HPTLC Study on Puerarin (Table 6).

**Pharmacological Study**

Numerous pharmacological properties are processed by puerarin, which has been used extensively to treat cancer, osteonecrosis, Parkinson's disease, endometriosis, diabetes and its complications, cardiovascular and cerebrovascular disease [14].

**Cardiovascular Activity**[26]

Puerarin has been demonstrated to be in charge of *Pueraria lobata's* pharmacological effects on the cardiovascular system. According to basic and clinical research, puerarin is a promising treatment option for Osteonecrosis, Cancer, Alzheimer's disease, Parkinson's disease, diabetes and its complications, and endometriosis. The modulation of Na+, Ca2+ channels, NF-Kb, PI3K/AKT, BCL-2, and BAX may be the primary mechanism by which puerarin is effective in treating a variety of CVDs.

**Neuroprotective Effects:**[21]

Parkinson's disease (PD), a common neurodegenerative disease, is marked by the gradual degeneration of dopaminergic (DA) neurons in the pars compacta of the substantia nigra (SN). Puerarin's pharmacological

mechanisms for treating Parkinson's disease (PD) include antiapoptotic effects, antioxidative stress injury, ALP and USP intervention, immune response regulation, elevated dopamine and its metabolites, and promotion of neurotrophin expression and neuronal repair, according to a review of the published literature. One of the main active ingredients in pueraria radix is puerarin, which has a known chemical structure and composition. Puerarin has been demonstrated to have therapeutic effects on parkinson's disease in both clinical and experimental studies.

The following are its pharmacological mechanisms: Anti-oxidative stress injury, anti-apoptosis, ALP and USP intervention, and regulation.

**Anti-inflammatory Effects:**[27]

In both *in Vitro* and *in vivo* models, puerarin exhibits potent anti-inflammatory properties in a variety of ways: Puerarin suppresses NF-kB by preventing IkB degradation and p65 nuclear translocation activation, and lowers the manifestation of inflammatory markers like IL-6, TNF-$\alpha$, COX-2, iNOS, and CRP.

**Cellular and Animal Models:** it decreased inflammation in LPS-stimulated PBMCs, RAW264.7 macrophages, and mouse ear edema.

**Endothelial Protection:** It enhanced insulin signaling in endothelial cells and counteracted inflammation brought on by palmitate.

**HO-1/Nrf2 Pathway Activation:** Puerarin activated PKCδ-Nrf2-HO-1 signaling, which increased the antioxidant and anti-inflammatory effects.

IL-8 Suppression: Inhibited IL-8 secretion and mRNA in neutrophil co-cultures and bronchial epithelial cells.

**Anti-diabetic Effects:**[26, 27]

A lot of research has shown that puerarin is very good at lowering blood sugar levels and making cells more sensitive to insulin. Lowered blood sugar levels in diabetic rats in a way that depended on the dose. Increased GLUT-4 levels in the skeletal muscle caused β-endorphin release through adrenal $\alpha$1-adrenoceptors. In rats that consumed a lot of fat, it improved insulin resistance by reducing body weight, leptin, and resistin. How to keep pancreatic islets safe. Less apoptosis caused by $H_2O_2$, more SOD or catalase activity, and better insulin secretion all helped protect pancreatic islets from oxidative stress. Better use of glucose and lipids in the body. Puerarin helped rats with type 2 diabetes and obesity by lowering the blood sugar, triglycerides, cholesterol, insulin, and body weight. A diet that has a lot of kudzu in it.

**Antioxidant Activity:**[26, 27]

By scavenging free radicals, increasing antioxidant enzyme activities, and preventing oxidative damage in a variety of organs and disease models, puerarin demonstrates strong antioxidant qualities:

a. Preserving lipids and scavenging free radicals, it neutralizes superoxide anions and hydroxyl radicals, prevents LDL from oxidizing, and shields red blood cells from hemolysis brought on by $H_2O_2$.
b. According to this, it might aid in the prevention of atherosclerosis, lower cholesterol, increase HDL, and prevent platelet aggregation.
c. Protecting organs from oxidative stress reduces ROS and MDA and increases GSH, SOD, and catalase to protect against heart oxidative/ nitrative damage, liver damage from type 2 diabetes, and lead-induced nephrotoxicity, neurotoxicity, inhibits caspase-3, increases Akt/Enos phosphorylation, and controls Bcl-2 family proteins to prevent apoptosis.
d. Regulations of gene expression and enzyme activity raise LDL receptor expression, reduce HMG-CoA reductase activity, and boost cholesterol 7$\alpha$-hydroxylase, thus improving lipid metabolism.

**Clinical Evidence for Puerarin Injection in Heart Failure** [28]

A recent systematic review and meta-analysis (Frontiers in Pharmacology, 2025) combined data from 29 randomized controlled trials involving about 2,480 patients. These trials assessed puerarin injection as a supplement to standard

therapy for heart failure. Compared to control groups, puerarin significantly improved key measures of heart function, such as left ventricular ejection fraction, cardiac output, and stroke volume. It also improved symptoms and biochemical markers of heart failure. No major safety concerns were reported. These findings suggest that puerarin, administered by injection, may offer additional benefits in managing heart failure. However, larger and well-designed trials are necessary to confirm these effects.

**Future Perspective**

While Puerarin is well-known for its antioxidant and anti-cell death effects in stroke and neurodegenerative diseases, a promising new area is its potential for nerve regeneration and myelin repair. Recent studies show that Puerarin helps the differentiation of oligodendrocyte precursor cells (OPCs) into mature oligodendrocytes. Since conditions like Multiple Sclerosis damage the myelin sheath, future research should explore Puerarin's ability to boost natural myelin repair in living organisms. We need more studies to confirm whether Puerarin affects important signalling pathways, such as PI3K/Akt or LINGO-1, that control oligodendrocyte development. This could make it a new treatment option for restoring nerve function[29, 30]. A major challenge limiting the use of Puerarinin the central nervous system is its low oral bioavailability. To tackle this, combining new nanotechnology with targeted drug delivery offers a valuable opportunity. Recent studies show Puerarin's specific effects on neuromodulation. It can increase extracellular dopamine levels and act as an agonist on D2-like receptors. This makes Puerarin particularly important for neurological and psychiatric disorders related to dopamine problems, such as certain types of depression, addiction, and Parkinson's disease. Future work should aim to develop actively targeted nanocarrier systems, such as functionalized polymeric nanoparticles, utilising blood-brain barrier-targeting ligands. This approach will help deliver high concentrations of Puerarin directly to important brain areas, maximizing therapeutic benefits while reducing unwanted effects[31]. Beyond systemic diseases, applying Puerarin locally shows great potential in the growing field of senotherapeutics for skin ageing. The ageing process is largely driven by the accumulation of senescent cells, specifically senescent dermal fibroblasts, which release pro-inflammatory factors that damage the skin matrix. Research indicates that Puerarin can effectively block the senescence phenotype in human dermal fibroblasts. More studies are needed to find out if Puerarin works as a senolytic, which selectively kills senescent cells, or as a senomorphic, which suppresses the harmful SASP. Developing effective topical drug delivery systems, such as Puerarin-loaded Nanostructured Lipid Carriers or functional nanofibers, will be vital to ensure proper skin penetration and targeted action against age-related issues, including wrinkles and loss of elasticity[32].

## CONCLUSION

Puerarin is a well-known isoflavone with significant pharmacological and therapeutic potential that is primarily derived from species in the Pueraria genus. This review provides a detailed overview of its biosynthesis pathways, species sources, and advanced extraction and analytical techniques, all of which are essential for accurate quantification and quality control. Puerarin's favorable pharmacokinetic profile, ability to cross physiological barriers, and modulation through herbal combinations further enhance its therapeutic relevance. Its well-documented pharmacological effects-spanning anti-inflammatory, antioxidant, anti-diabetic, neuroprotective, and cardioprotective actions, support its significance as a bioactive marker in traditional and modern medicine. Collectively, puerarin emerges as a promising compound for future drug development, encouraging continued exploration in pharmaceutical and nutraceutical applications.

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**Table 1: Profile of Puerarin**

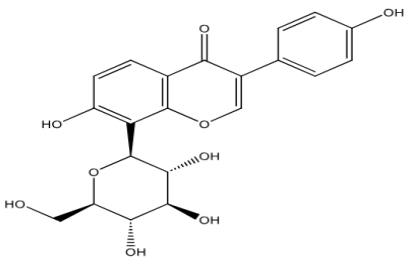
| | |
|---|---|
| Structure |  |
| Molecular Weight | 413.38 g/mol |
| Molecular Formula | $C_{21}H_{20}O_9$ |
| IUPAC name | 7-hydroxy-3-(4-hydroxyphenyl)-8-[(2S, 3R, 4R, 5S, 6R)-3, 4, 5-(trihydroxymethyl)oxan-2-yl]chromen-4-one |
| Melting Point | 187-189$^{o}$c |
| Solubility | Soluble in polyvinylpyrrolidone, ethylene glycol, propylene glycol, ethanol, and acetone |

**Table 2: Sources of Puerarin**

| Species | Family | Plant parts | Reference |
|---|---|---|---|
| *Pueraria lobata* | Fabaceae (leguminosae) | Root (rich in puerarin), leaves, stem | (2) |
| *Pueraria thomsonii* | Fabaceae (leguminosae) | Root (primary source of puerarin) | (3) |
| *Pueraria mirifica* | Fabaceae (leguminosae) | Tuberous root (contains phytoestrogens) | (4) |
| *Pueraria phaseoloides* | Fabaceae (leguminosae) | Leaves, stem (for forage, less used medicinally) | (5) |
| *Glycine max (soybean)* | Fabaceae (leguminosae) | Seeds (rich in isoflavones: genistein, diadzein), also oil, and sprouts | (6) |

**Table 3: TLC of Puerarin**

| Sr. No. | Title | Description | Ref. No. |
|---|---|---|---|
| 1 | *Pueraria tuberosa* (Roxb. ex Willd) DC. | Stationary phase: Pre-coated Silica gel 60 $F_{254}$<br>Mobile phase: Chloroform: Methanol: Acetic acid (80:20:10 v/v/v)<br>Detection: 254nm and 366nm $R_f$ value: 0.13, 0.25, 0.29, 0.54, 0.59 (254nm) and 0.54 (366nm) | (17) |

**Table 4: HPTLC of Puerarin**

| SR. NO. | TITLE | DESCRIPTION | REF. NO. |
|---|---|---|---|
| 1 | Puerarin estimation in *Pueraria tuberosa* (Roxb. ex Willd.) DC. | Stationary phase: Silica gel 60 $F_{254}$<br>Mobile phase: Chloroform: Methanol (9:1 v/v)<br>Detection: 254nm $R_f$ value: 0.71 | (18) |
| 2 | Puerarin and Diadzein in *Pueraria tuberosa* (Willd.) DC. | Stationary phase: Silica gel 60 $F_{254}$<br>Mobile phase: Chloroform: Methanol (8:2 v/v)<br>Detection: 340 nm $R_f$ value:**Puerarin** 0.132 (± 0.002) and **Diadzein** 0.605 (± 0.002) | (19) |
| 3 | β-Sitosterol and Puerarin from *Pueraria tuberosa* DC. | Stationary phase: Silica gel 60 $F_{254}$<br>Mobile phase: Methanol: Ethyl acetate: Distilled water (10:80:10 v/v/v) (**Puerarin**), Ethyl acetate: toluene 90:10 (v/v/v) (**β-Sitosterol**)<br>Detection: 257nm (Puerarin), 366nm (β-Sitosterol)<br>$R_f$ value:Puerarin (0.40), β-Sitosterol (0.35) | (20) |
| 4 | *Pueraria tuberosa* (Roxb. ex. Willd.) DC., Adenia hondala | Stationary phase: Silica gel 60 $F_{254}$<br>Mobile phase: Toluene: Ethyl acetate: Formic acid (5:4:0.1 v/v/v)<br>Detection: 254nm and 366nm $R_f$ value: 0.21 | (21) |

**Table 5: HPLC of Puerarin**

| SR. NO. | TITLE | DESCRIPTION | REF.NO. |
|---|---|---|---|
| 1 | Determination of Puerarin from *Pueraria tuberosa* DC. by HPLC | Stationary phase: $C_{18}$ reversed-phase column.<br>Mobile phase: Acetonitrile: Water: Orthophosphoric acid (32:67:01, v/v/v)<br>Detection: 280 nm<br>$R_t$ value: 7.5 min. | (23) |
| 2 | Quantitative determination of Puerarin in | Stationary phase: $C_{18}$ reversed-phase column. | (24) |

| | | | |
|---|---|---|---|
| | *Pueraria tuberosa* D.C. tuber extract | Mobile phase: 0.1% acetic acid in water (90:10, v/v) Detection: 254nm $R_t$ value: 5.21 min. | |

**Table 6: HPLC Vs HPTLC Study on Puerarin**

| SR. NO. | TITLE | METHOD | DESCRIPTION | METHOD | DESCRIPTION | REF. NO. |
|---|---|---|---|---|---|---|
| 1 | HPTLC and HPLC for the determination of isoflavonoids in several kudzu samples | HPTLC | Stationary phase: Silica gel $F_{254}$ plates Mobile phase: Chloroform: Methanol: Ethyl acetate: Water (16.2:18.8:52:3 v/v) | HPLC | Mobile phase: Acetonitrile with 0.5% acetic acid (gradient) | (25) |
| | | | Detection: UV absorption at 254 nm | | Detection: UV at 254 nm | |
| | | | | | $R_t$:Puerarin (27.7 min.), 3′-methoxypuerarin (28.5min.), Diadzein (31.6 min.), | |

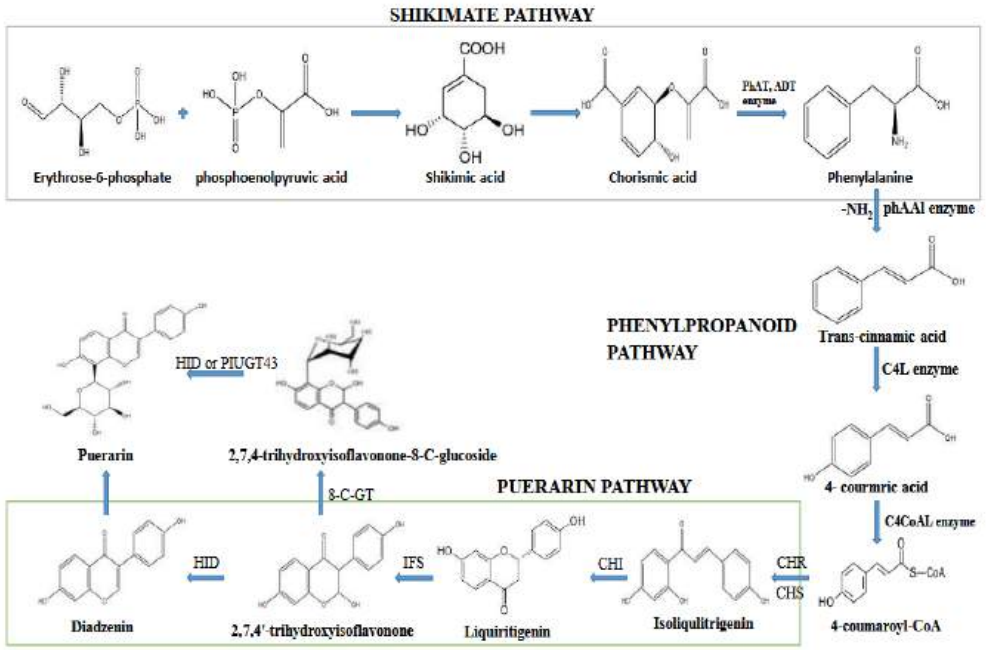



**Figure 1: Puerarinuses various enzymes and reactions in the biosynthesis of puerarin. It is biosynthesised by three different pathways, i.e. Shikimate pathway, the Phenylpropanoid pathway, and Puerarin pathway.**

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RESEARCH ARTICLE

# Preparation and Characterization of Activated Charcoal from *Catharanthus roseus* for Malachite Green Dye Adsorption

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## ABSTRACT

The study examines the effectiveness of the low-cost active carbon (CR-AC) obtained from the stem of the *Catharanthus roseus* plant to remove malachite green (MG) dye from aquatic solutions. The activated carbon was synthesized using zinc chloride as a chemical activator and was subject to microwave irradiation. The structural and surface characteristics of the produced adsorbent were examined using X-ray diffraction (XRD), Scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FT-IR). Batch absorbing experiments were held to examine the effects of various operating parameters, including adsorbent doses, contact time and solution pH on dye removal efficiency. The soak balance was analyzed using both Langmuir and Freundlich isotherm models. The findings show that CR-AC displays strong absorbing capabilities, which reflects its promise as cost-effective, efficient and environmentally friendly material for the treatment of dye-contaminated wastewater.

**Keywords:** Microwave radiation, Malachite green, Adsorption, *Catharanthus roseus*, Langmuir and Freundlich isotherms.

## INTRODUCTION

Dyes representing an important class of contaminants, among other things, reduce the release of recipient dye waste, diminish the aesthetic value of water, block the penetration of sunlight into water, and reduce photosynthesis skills [1]. Dyes are often released in the form of colored wastewaters in textile industries, pulp mills, and dye manufacturing industries. Water pollution has become one of the most serious issues that threaten biological sustainability. Disposal of synthetic dyes in waters without proper treatment leads to serious problems and concerns. Over 10,000 dyes [2] were used in the textile, paper, cosmetics and food industries, resulting in large quantities of dye-sampled water. Malachite green (MG) dyes were usually used to colour wool, silk and leather, paper, distilleries and food products [3]. MG is known as a fungicide used in agriculture and fishing, causing skin problems with mouth and inhalation. It also has carcinogenic effects and issues a great risk to aquatic organisms [4, 5]. However, MG is toxic and can cause carcinogenesis, mutagenesis, respiratory destruction, liver, kidneys, intestines and gonads [6]. For this reason, the removal of such dyes from industrial forests is extremely important [7-9]. Adsorption of liquid phase adsorption from exclusion from breakout discharges, such as coagulation, aggregation, chemical oxidation, membrane filtration, aerobic and anaerobic degradation, reverse osmosis, photocatalytic degradation and microbial processes [10]. It is well recognized as an efficient method of removing contaminants from waste [11]. The Adsorbent method is considered as an important alternative to other methods as it is inexpensive and does not require pre-processing. In particular, adsorption techniques, including the use of solid adsorbent have long been used to dispose of chemical contaminants that are resistant to traditional biological treatment processes [12], but activated carbons (ACs) are often used in adsorption processes, and their small particles become hard when removed by an Aqueous solution [13,14]. Recently, this study has focused on surface chemistry of ACs to assess experimental results related to dye adsorption [15-17]. With the increased production costs of commercially available activated carbon, researchers are seeking the possibility of using a variety of cost-effective adsorbents resulting from natural materials, industrial solid waste, agriculture waste, and biosorbents as the precursors [18, 19]. According to solvent extraction of phytochemical plants waste, these pioneers were attributed to attention to the low cost advantages, particularly in the field of phytochemical, which is heirs to their prominent properties as frequency and availability. Recently, microwave-supplied heating has focused attention on carbon preparation activities due to its benefits, such as faster processing and more uniform heat distribution. Li *et al*. The success of synthesis of high surface activated carbon produced in tobacco stems using microwave radiation has been reported [20]. However, studies using microwave-assisted $ZnCl_2$ activation remain lacking. In this context, the purpose of this study is to investigate the feasibility of efficient adsorbents from the *Catharanthus roseus* family using $ZnCl_2$ activation under microwave irradiation. The adsorption performance was then evaluated by characterizing the prepared carbon and removing the malachite green (MG) from the aqueous solution.

## MATERIALS AND METHODS

## MATERIALS

All reagents utilized in the experiments were of analytical (AR) grade and were procured from Merck India Ltd. Stock solutions of the test reagents were prepared by dissolving the dye in double-distilled water. The dye, malachite green, with the chemical formula $C_{23}H_{25}N_2Cl$, a molecular weight of 365, and a $\lambda_{max}$ of 618 nm, was also supplied by Merck India Ltd.

**Preparation of Activated Carbon**

Following phytochemical alcoholic extractions, the stem of crushed waste of the *Catharanthus roseus* (raw-CR) was passed through a 1 mm sieve for homogenization. The incipient wetness method by drop- wise addition is regarded as the best variant of the incipient wetness methods [21]. To facilitate the homogeneous absorption of the liquid, solid particles must be raised in the solution. The required amount of zinc chloride for raw-CR was determined to be 3.5 mL /gram of solid to increase swelling during the aggressive wet. However, this volume of zinc chloride is not

enough to induce good swelling of the raw-CR. The minimum volume of $ZnCl_2$ required to produce a homogeneous raw material solution was 4.0 mL/gram of raw-CR (in a ratio of 1.00 grams of 4.00 mL of solution) [22]. The 100-gram precursor was fitted with zinc chloride (400 mL) and placed in an oven at 110°C for 4 hours. Dried and pregnant samples were radiated using a microwave on a 700 W output power for 12 minutes [23]. The active carbon (CR-AC) received is washed several times using distilled water. After reaching the pH neutral of the solution, the active carbon is dried at a temperature of 105°C for 24 hours.

**Preparation of Synthetic Effluent**

A 1000 mg/L dye stock solution was prepared by dissolving 1g of MG dye in 1000 mL of distilled water. These stock solutions were then mixed with the necessary concentrations using distilled water when needed.

**Adsorption Experiments**

We were included in the initial batch of the source study to determine the equilibrium time. The 250 mL Stoppered conical flask was batched by mixing 0.30 g CRAC with 50 mL of various initial concentration solutions of MG dye. The dose of adsorbent was determined experimentally. All adsorption experiments were performed at a temperature of 30°C, at a speed of 140 rpm on a thermostat shaking water bath. The progression of the exploitation was noted, and the equilibrium was achieved in 40 minutes. After equilibrium, the adsorbent was separated from the aqueous phase by centrifugation at 10000 rpm for 10 minutes using a Centrifuge (Remy 24, India). The residual concentration of MG dye colour in the Supernatant was determined by the Systronics Double Beam UV-visible spectrophotometer: 2202. 618 nm.

The percentage of colour on equilibrium was removed, and the amount of colour transferred to the surface of the adsorbent, $q_e$ (mg/g), was calculated using the following relationships

Percentage dye removal = $100(C_o-C_e)/C_o$ (1)

Amount of adsorbed dye molecules per g of solid

$$q_e=(C_o-C_e)\ V/w \quad (2)$$

Where, $C_o$ (mg/L) is the initial concentration of malachite green, $C_e$ (mg/L) is the equilibrium concentration of dye, V is the volume of the solution (L), and W (g) is the mass of the adsorbent.

## RESULTS AND DISCUSSION

**Instrumental Evidences**

**FTIR Analysis of CR-AC**

The FTIR analysis is an important tool for figuring out the feature functional groups of the adsorbent, which are accountable for the adsorption of Malachite green dye. The FTIR spectroscopy affords structural and compositional records at the functional groups presented in the sample. Figs. 1 and 2 shows the peak positions within the FTIR spectra of the CRAC before and after Malachite inexperienced dye adsorption, presented in Table-1. FTIR spectroscopy became hired to perceive the functional group concerned in the adsorption of Malachite green (MG) dye onto $ZnCl_2$-activated CRAC. Significant shifts in characteristic peaks were observed in the spectra before and after dye adsorption, suggesting strong interactions between the dye molecules and the surface of the adsorbent. A large peak at 3360 $cm^{-1}$, attributed to O–H stretching vibrations of hydroxyl, phenolic, or carboxylic acid organizations, shifted to 3369.23 $cm^{-1}$ after dye adsorption. This shift confirms the involvement of surface hydroxyl agencies in hydrogen bonding or electrostatic interactions with the dye. The C≡C stretching vibration close to 2161.22 $cm^{-1}$ shifted to 2166.55 $cm^{-1}$, which may also advise weak π–π interactions or electronic rearrangement at the carbon surface. The peak at 1578.27 $cm^{-1}$, corresponding to aromatic C=C stretching, shifted to 1582.61 $cm^{-1}$, indicating π–π stacking interactions between the aromatic rings of MG dye and the carbon surface. A big shift was additionally determined in the C–O stretching band from 1204. 11 $cm^{-1}$ to 1216. 49 $cm^{-1}$, suggesting that carboxylic and hydroxyl group played a role in hydrogen bonding or dipolar interactions with MG dye molecules. Importantly, peaks inside the low-frequency region (471.96, 436.50, and 413.16 $cm^{-1}$) related to Zn–O stretching vibrations additionally showed moderate shifts after adsorption. These shifts verify the energetic participation of metallic–oxygen bonds (introduced

via $ZnCl_2$ activation) in electrostatic enchantment or surface complexation with the cationic dye. This helps the function of Zn species in enhancing adsorption efficiency.

**SEM Analysis of CRAC**

The surface morphology of the CRAC turned into investigated the usage of Scanning Electron Microscopy (SEM) to understand the bodily modifications taking place earlier than and after the adsorption of Malachite green (MG) dye. The SEM photograph of CRAC before adsorption (Fig. 3) reveals a distinctly porous and abnormal surface shape. Numerous voids, channels, and cavities were observed, indicating a properly-evolved porous community. Those structural features are important for adsorption as they boom the surface region and provide energetic web sites for dye molecule attachment. The irregular texture and open pore network endorse that the CRAC has substantial adsorption capability because of its bodily surface characteristics. Following the adsorption of MG dye, widespread morphological adjustments were found on the CRAC surface. The SEM picture (Fig. 4) of the dye-loaded CRAC suggests a extra compact and smoother floor, with the various formerly seen pores both in part or fully blocked. The discount in pore visibility confirms that the dye molecules occupied the available active sites on the carbon surface, thereby blocking the pores.

**XRD Analysis of CRAC**

X-ray diffraction (XRD) evaluation changed into performed to research the crystallinity and structural modifications in the CRAC adsorbent earlier than and after the adsorption of Malachite Green dye. The diffraction patterns have been recorded within the 2θ range and the corresponding d-spacing, peak intensities, and relative intensities has been analyzed. The XRD sample of raw CRAC (Fig. 5) suggests awesome peaks at 2θ = 26.83°, 38.42°, 44.68°, 65.08°, 72.77°, 75.92°, 78.18°, and 88.45°, with the most intense peak at 44.68° (100% relative intensity). these peaks hint the partial crystalline nature of the adsorbent, probable due to residual ZnO or $ZnCl_2$-primarily based inorganic components delivered in the course of chemical activation with $ZnCl_2$. The sharp peaks at 44.68°, 72.77°, and 88.45° correspond to the (111), (200), and higher-order ZnO planes, indicating the presence of crystalline zinc oxide levels. The broadness of a few peaks, especially around 26.83°, may also recommend the presence of amorphous carbon, which is usual in activated carbon materials. The d-spacing values ranging from 3.32 Å to 1.10 Å in addition support the combination of crystalline and disordered (amorphous) structures in CRAC. After adsorption of Malachite green dye, the XRD pattern indicates (Fig. 6) great changes. A new, broad height peak appears at 10.12° (with the very best depth, 100% relative), corresponding to a large d-spacing of 8.74 Å. This broad diffraction band is in all likelihood due to intercalation or surface coating of dye molecules on the adsorbent, which increases the interlayer distance and contributes to structural ailment. The depth of the original CRAC peaks at 26.58° and 28.47° is appreciably reduced, and their relative intensities drop to 25.6% and 17.22%, respectively, indicating that the crystallinity decreases after dye loading. The disappearance or attenuation of numerous peaks (e.g., at 38.42°, 44.68°, and 88.45°) suggests that the adsorption of MG dye masks or disrupts the crystalline domains, confirming sturdy interaction between the dye molecules and active sites on CRAC.

**Effect of Contact Time and Initial Concentration**

The adsorption of malachite green dye with the aid of CRAC became notably influenced through initial dye concentration. The variation in percentage removal of the dye with contact time at exceptional initial concentrations starting from (60 to 120) mg/L is shown in Fig. 7. It is observed from the figure that the removal of MG acquired a maximum at the time of equilibrium, viz. 40 min, and will become steady thereafter. It's far clear from this figure that the price of adsorption could be very speedy in the initial degrees and after equilibrium; no significant alteration in the extent of adsorption is determined. For the initial concentration, the elimination extended from (56.40 to 83.30 %) by lowering the initial concentration of malachite green dye solution from (120 to 60) mg/L. It's miles glaring that although a substantial version in adsorption of MG isn't located, the extent of elimination (%) is greater at decrease initial concentrations, and the percentage elimination of dye decreases with an growth in initial concentration.

**Effect of Temperature**

Temperature is an essential parameter for adsorption processes. It has substantial impact at the adsorption of malachite green on CRAC additionally. The impact of temperature became investigated in the temperature variety (30 to 50) ˚C. The experimental results show that the removal of MG expanded from (83.30 to 92.90 %) via growing the temperature from (30 to 50 ˚C) (Fig. 8). Maximum adsorption process are ruled via exothermic process, however the present system is governed via endothermic adsorption [24].

**Effect of pH on Dye Uptake**

The effect of pH at the adsorption of malachite green has been investigated, and the consequences indicated that the pH of the solution drastically influenced the adsorption of MG in the present studies. To examine an impact of pH at the adsorption of MG on CRAC, the experiments have been completed at a 60 mg/L initial dye concentration with 0.30 g adsorbent at 30 ˚C. The reaction mixture was agitated for 40 min, the equilibrium time. Fig. 9 suggests the version of percent adsorption of MG at different values of solution pH. This Fig. 10 shows that a lower removal of MG is acquired in the acidic range of pH. At lower pH values, the adsorption capacity for Malachite Green (MG) is suppressed due to abundance of $H^+$ ions, which occupy the active sites on the CRAC surface and outcompete the cationic dye [25,26]. Conversely, as the solution becomes less acidic, the positive charge density on the adsorbent surface diminishes, thereby minimizing electrostatic repulsion. Under alkaline conditions, the CRAC surface tends to develop a negative charge; this transition promotes a robust electrostatic affinity for the positively charged MG cations, ultimately maximizing the dye removal rate.

**Adsorption Kinetics**

The adsorption kinetics indicates the evolution of the adsorption capability through time and it's far important to identify the varieties of adsorption mechanism in a given method. Plots of different kinetic models applied have been given in the Figs. 10 and 11 and the kinetic parameters calculated have been given in the Table-2, among the first order and second order, second order kinetic model seems to satisfactory describe the above adsorption system as its $R^2$ values were very near to unity. Moreover, distinction between qe (cal) and qe (exp) values of second order is small while in comparison to first order kinetic model. Statistically it is examined with the tool Sum of error squares (SSE%) [27,28]. The Δqe and SSE % values were given in the Table-3 from which it was concluded that second order kinetic model became more suitable rather than first order kinetic model.

**Analytical Study**

An analytical method is critical for designing effective adsorption method aimed at disposing of dissolved pollutants. On this observe, experimental information were analyzed by becoming them to established adsorption isotherm models to understand the adsorption behaviour. Sorption isotherms efficiently describe the equilibrium relationship among the adsorbate within the liquid segment and the adsorbent inside the solid phase. Equilibrium information obtained at various temperatures has been implemented to both the Langmuir and Freundlich isotherm models, as illustrated in Figs. 12 and 13. The correlation coefficients ($R^2$ values) suggest that the Freundlich version offers a better fit; the specific results of the isotherm evaluation are summarized in Table-4.

**Analysis of Isotherm**

**Langmuir isotherm**

Within the present study $Q_0$ value ranges from 76.923 to 83.333 because the temperature will increase the monolayer adsorption potential also discovered to increase. This form of outcomes turned into acquired in diverse similar studies. The separation factor $R_L$ values in between 0 to 1 suggest the beneficial adsorption. $R^2$ values of isotherm ranged at 0.998.

**Frendlich isotherm**

The values of n have been among 1 and 10 which suggests cooperative adsorption. The $R^2$ values were close to unity which reached to desirable fitting into frendlich isotherm.

## CONCLUSIONS

This study correctly demonstrates the capability of activated carbon derived from *Catharanthus roseus* stems (CRAC), synthesized through $ZnCl_2$ activation and microwave irradiation, as an efficient and low-cost adsorbent for the removal of Malachite green (MG) dye from aqueous solution. Characterization techniques (FTIR, SEM, and XRD) showed the presence of useful functional groups, porous morphology, and partial crystallinity conducive to dye adsorption. FTIR evaluation indicated that the adsorption system is often ruled by using physisorption mechanisms, regarding hydrogen bonding, van der Waals forces, and π–π stacking, with minor contributions from chemisorption, probably because of Zn–O surface functionalities. SEM spectra discovered extensive modifications in surface morphology submit-adsorption, which include pore blockage and surface smoothing, confirming effective dye uptake. XRD patterns similarly supported dye intercalation and accelerated structural disorder after adsorption. Kinetic research found out that the adsorption system follows a pseudo-second-order version, at the same time as equilibrium information fit well to the Langmuir isotherm, suggesting monolayer adsorption on a fantastically homogeneous surface. A maximum dye removal efficiency of 83.30% changed into achieved at initial dye concentrations of 60–120 mg/L within 40 mins of contact time. Typically, the findings highlight CRAC as a promising adsorbent for dye-contaminated wastewater treatment. Its excessive performance, low cost, and eco-friendly source make it appropriate for further development and potential industrial-scale applications.

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**Table 1: FTIR spectra of CRAC**

| IR Frequency Range ($cm^{-1}$) | CRAC | MG Dye Loaded | Difference | Type of Vibration | Functional Groups | Interpretation |
|---|---|---|---|---|---|---|
| 3360.00 – 3369.23 | 3360.00 | 3369.23 | +9.23 | O–H stretching | Alcohols, phenols, or carboxylic acids | Shift indicates hydrogen bonding or electrostatic interactions between hydroxyl groups on CRAC and the MG dye molecules. |
| 2161.22 – 2166.55 | 2161.22 | 2166.55 | +5.33 | C≡C stretching | Alkyne | Minor shift suggests weak π–π interactions or a change in the electronic environment due to dye adsorption. |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1578.27 – 1582.61 | 1578.27 | 1582.61 | +4.34 | C=C stretching | Aromatic rings | Indicates π–π stacking interactions between aromatic structures of MG dye and the adsorbent surface. |
| 1204.11 – 1216.49 | 1204.11 | 1216.49 | +12.38 | C–O stretching | Hydroxyl or carboxylic groups | Suggests the involvement of C–O functional groups in hydrogen bonding or dipolar interactions with the MG dye. |
| 471.96 – 470.93 | 471.96 | 470.93 | –1.03 | Metal–oxygen (Zn–O) stretching | Zn–O (from $ZnCl_2$ activation) | Slight shift confirms presence of Zn–O bonds, supporting their role in electrostatic attraction or surface complexation with the dye molecules. |
| 436.50 – 440.10 | 436.50 | 440.10 | +3.60 | | | Additional Zn–O related peak, confirming active participation of metal oxides in the dye adsorption process. |
| 413.16 – 416.54 | 413.16 | 416.54 | +3.38 | | | Reinforces the presence of metal–oxygen bonding, indicating $ZnCl_2$ activation significantly enhanced surface activity toward MG dye adsorption. |

**Table 2: Data processing Tools**

| S.No | Parameters | Formula | |
|---|---|---|---|
| 1 | Kinetic Models and SSE % | Pseudo First order kinetics (Legergren equation) | $\log (q_e-q_t) = \log q_e - k_1/2.303\times t$ |
| | | Pseudo Second order kinetics ( Ho equation) The initial adsorption rate | $t/q_1 = 1/k_2.q_e^2 + 1/q_e\, t$ $h = k_2 q_e^2$ |
| | | Sum of error squares | $SSE(\%) = \sqrt{\sum[(q_e)_{exp} - (q_e)_{cal}]^2/N}$ |
| 2 | Isotherms | Langmuir Separation factor | $C_e/Q_e = 1/Q_0 b + C_e/Q_0$ $R_L = 1/(1+ b\, C_0)$ |
| | | Freundlich | $\log Q_e = \log K_f + 1/n \log C_e$ |

**Table 3: Kinetic parameters for MG dye onto CRAC**

| Ci mg/ L | Rate constants | | $q_{e(cal)}$ mg/L | | $q_{e(expl)}$ mg/L | | $\Delta q_e$ | | $R^2$ | | SSE% | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $k_1$ ($10^{-2}$) ($min^{-1}$) | $k_2$ ($10^{-3}$) ($gmg^{-1}$ $min^{-1}$) | First Order | Second Order | First Order | Second Order | First Order | Second Order | First Order | Second Order | First Order | Second Order |
| 60.00 | 0.0714 | 0.0071 | 29.99 | 52.63 | 49.98 | 49.98 | 19.99 | 2.65 | 0.943 | 0.998 | 13.35 | 2.25 |
| 80.00 | 0.0714 | 0.0067 | 31.48 | 62.50 | 57.76 | 57.76 | 26.28 | 4.74 | 0.940 | 0.998 | | |
| 100.00 | 0.0760 | 0.0059 | 36.90 | 71.43 | 65.30 | 65.30 | 28.40 | 6.13 | 0.943 | 0.998 | | |
| 120.00 | 0.0737 | 0.0063 | 36.81 | 71.43 | 67.68 | 67.68 | 30.87 | 3.75 | 0.938 | 0.999 | | |

**Table 4: Results of two isotherms plots for the adsorption of MG dye onto CRAC**

| Isotherms | Temp (K) | Parameters and results | | | |
|---|---|---|---|---|---|
| | | $Q_0$ (mg/g) | b | $R_L$ | $R^2$ |
| Langmuir | 305 | 76.923 | 0.178 | 0.062 | 0.998 |
| | 315 | 83.333 | 0.222 | 0.051 | 0.998 |
| | 325 | 83.333 | 0.293 | 0.039 | 0.998 |
| | 335 | 83.333 | 0.375 | 0.031 | 0.998 |
| Freundlich | | n | $k_f$ (mg/g$^{-1}$) | | $R^2$ |
| | 305 | 5.2083 | 32.1366 | | 0.9800 |
| | 315 | 5.6818 | 36.8978 | | 0.9840 |
| | 325 | 6.2893 | 41.4000 | | 0.9840 |
| | 335 | 6.8966 | 44.8745 | | 0.9820 |



**Figure 1: The FT-IR spectrum of CRAC**



**Figure 2: The FT-IR spectrum of CRAC MG dye loaded**

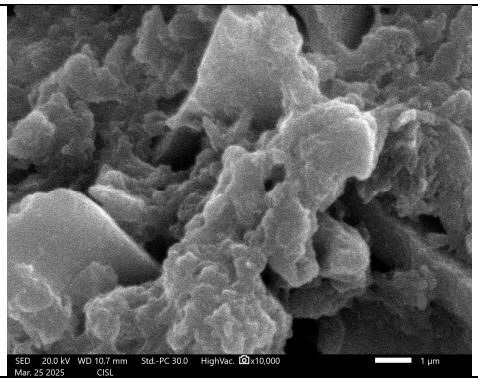


**Figure 3: SEM Spectrum of CRAC**

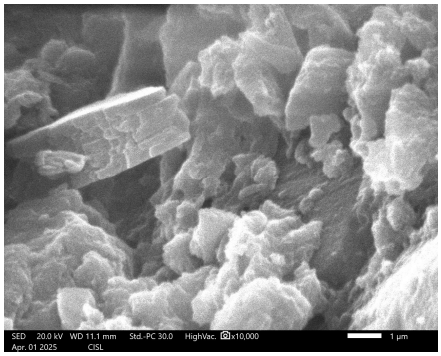


**Figure 4: SEM Spectrum of CRAC MG dye loaded**

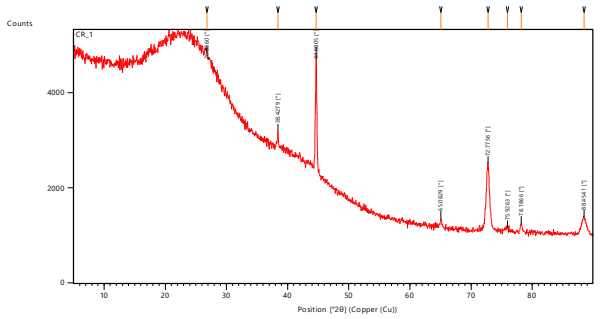


**Figure 5: XRD spectrum of CR-AC**

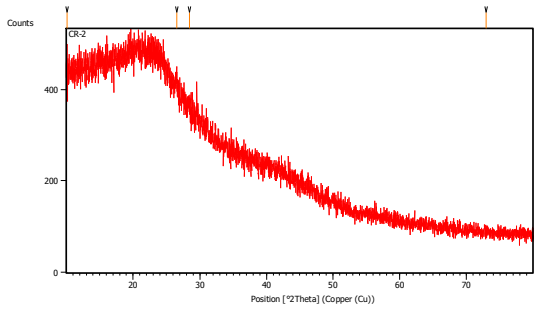


**Figure 6: XRD spectrum of CR-AC MG dye loaded**

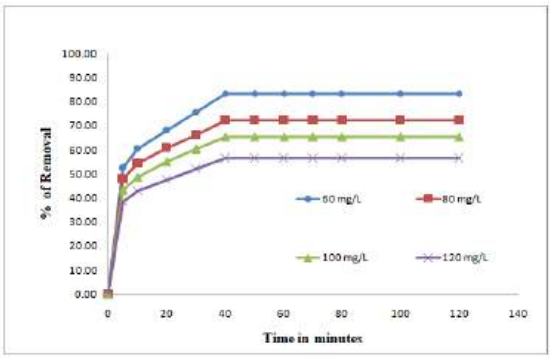



**Figure 7: Effect of Initial Concentration on % Removal of Malachite Green on CRAC.**

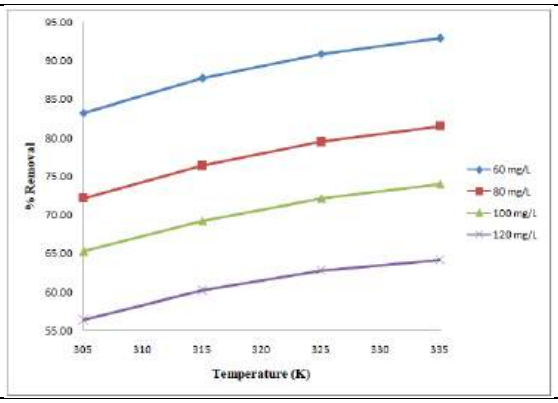



**Figure 8: Effect of Temperature on %Removal of Malachite Green on CRAC**

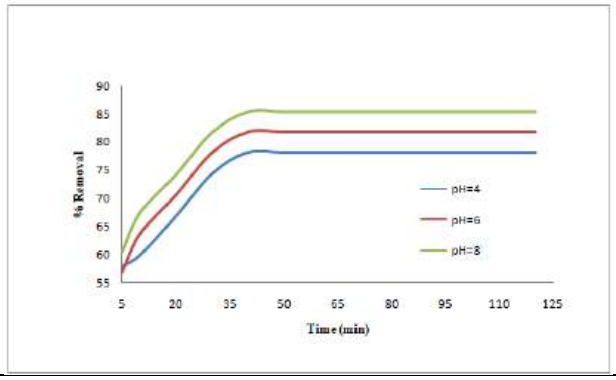



**Figure 9: Effect of pH on %Removal of Malachite Green on CRAC.**

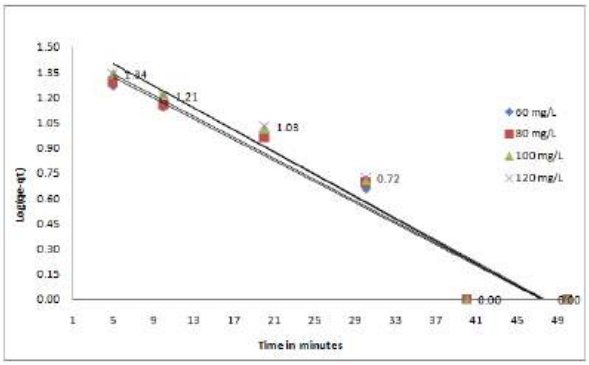



**Figure 10: Lagergren plot for MG dye onto CRAC**

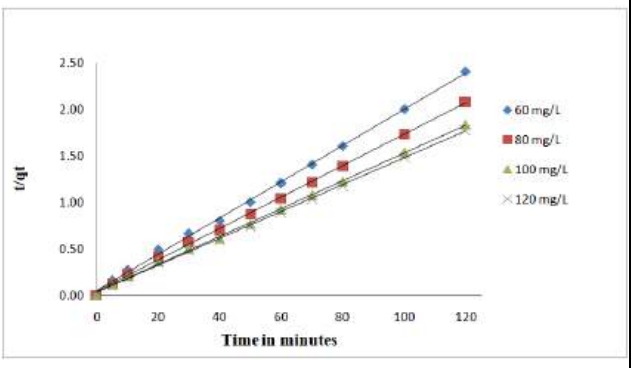



**Figure 11: Ho plot for MG dye onto CRAC**

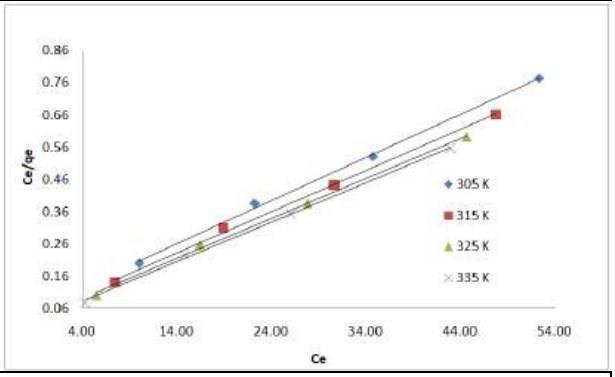



**Figure 12: Langmuir - I Isotherm for MB dye onto PDC**

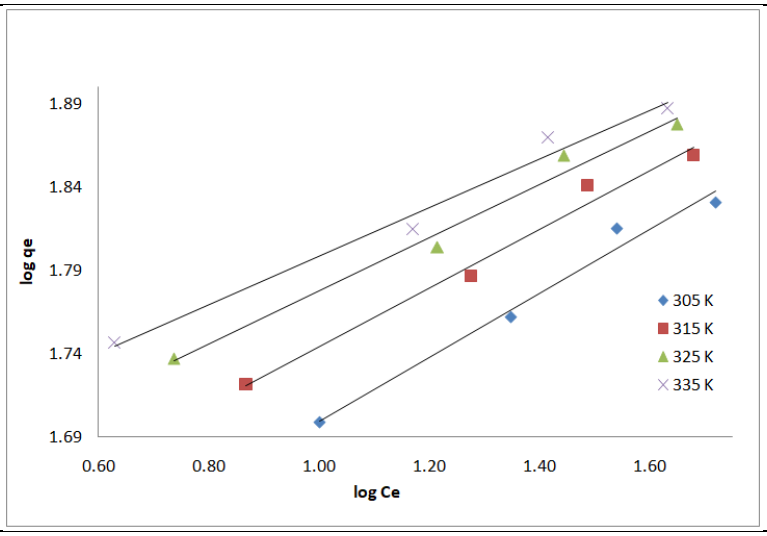



**Figure 13: Freundlich Isotherm for MG dye onto CRAC**

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**RESEARCH ARTICLE**

# Predicting Potential Compounds from Indian Medicinal Plants for Treating Schizophrenia using Molecular Docking and ADMET Studies

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## ABSTRACT

Schizophrenia is a mental illness that is characterized by abnormal social behavior, a failure to understand reality, including confusion, auditory hallucinations, and false beliefs, as well as a lack of social engagement. In India, about 3–5 million people suffer from schizophrenia. The goal of the current work was to identify a potential compound for treatment of schizophrenia using *in silico* docking and ADMET studies. The 3D structure of all the compounds were retrieved from PubChem database and the 3D structure of target protein M4 muscarinic Acetylcholine Receptor (M4 mAChR) responsible for schizophrenia was retrieved from PDB database. PyRx 8.0 and Discovery studio 2024 were used for docking studies. From the results, ten compounds showed good binding affinity with target protein. Among them, the compounds Beta-Amyrin from *Swertia chirata*, Galphimine B from *Galphimia glauca* and Lupeol from *Swertia chirata* showed very good binding affinity like -10.5, -10.3, -10.2 kcal/mol respectively, with the target protein. Additionally, toxicity studies were also conducted for the best interacted phytocompounds and the results indicated that all these compounds showed less toxicity. Hence, the current study concludes that the compounds Beta-Amyrin, Galphimine B and Lupeolmay have the potential ability to treat schizophrenia. Further, pre-clinical studies are required to confirm the potential of these compounds for treating Schizophrenia.

**Keywords:** Schizophrenia, Indian medicinal plants, Phytocompounds, Docking studies, Toxicity studies

## INTRODUCTION

Schizophrenia is a debilitating and protracted brain condition that impairs a person's ability to think, speak, and behave normally. Around 1% of individuals today suffer from a mental illness, according to a World Health Organization (WHO) assessment, and this has an influence on people's lives. Delusions, disordered speech, and hallucinations are some of their symptoms[1]. Schizophrenia is readily caused by issues with certain brain functions[2], and many people in different industries have been affected by this illness[3, 4]. The sickness has a significant macro-level impact on the nation, which also affects the economic system, while it causes micro-level harm to an individual. A person suffering from psychotic disease exhibits disruptions in their everyday activities[5]. One of the worst and most upsetting mental illnesses is schizophrenia. Low recovery rates, a poor quality of life, and an elevated mortality rate are all linked to this chronic, recurrent mental disorder. Variously referred to as the "Cancer of the mind" and the "Greatest disabler of youth," this condition affects approximately 6 to 7 million Indians. Between the ages of 15 and 45, when people are most productive, it begins. It transcends socio economic class, educational, and gender boundaries[6]. In India, about 3–5 million people suffer from schizophrenia[7].According to the 2016, National Mental Health Survey, 0.8% of Indians suffer from schizophrenia spectrum disorders[8].The annual rate of schizophrenia in India is 0.44/1000 in rural areas and 0.35/1000 in urban areas, with a frequency of 1.5-2.5/1000 in India[9].This study's prevalence of the disorder reveals a high rate of schizophrenia, with 71 cases (28.4%), 30 of which were in men and 41 in women[10]. A complex neuropsychiatric disorder, schizophrenia manifests as cognitive (impaired retrieval of information such as thinking, learning, and memorizing), negative (blunted affect, alogia, anhedonia, asociality, and avolition), and positive (hallucinations and delusions) symptoms[11]. Along with these symptoms, people with schizophrenia frequently have affective symptoms, such as anxiety and depression, which are linked to a higher risk of suicide and a lower quality of life[12]. Negative symptoms significantly contribute to poor psychosocial functioning and performance, which affects the patient's quality of life, whereas positive symptoms are mainly monitored to diagnose an active state of schizophrenia[13].

The causes and effects of smoking in schizophrenia have been extensively studied and published. Smoking is prevalent among psychotic diseases, with a prevalence of 70–80% in schizophrenia[14]. The prevalence of cigarette smoking in the first episode psychosis was much higher, at an estimated 58% [15]. Male smokers smoked more than female smokers, which is consistent with findings in the general population[16]. Compared to normal controls, smokers with schizophrenia inhaled more deeply and for longer periods of time, exposing themselves to larger levels of tobacco's harmful ingredients[17]. Furthermore, compared to smokers without schizophrenia, they smoked more cigarettes and experienced higher levels of nicotine dependence[18]. The M1 and M4 mAChRs in particular have been linked to neurological disorders because they are extensively expressed throughout the central nervous system[19, 20] and are implicated in cognitive functions like memory, learning, and attention[21,22]. As a result, medications that specifically target these receptors may be helpful in the treatment of conditions like schizophrenia and Alzheimer's disease. Clozapine and Xanomeline are commonly used synthetic drugs to treat schizophrenia. Researchers discovered that after years of use, clozapine had a lot of negative consequences in addition to some positive ones for patients. Agranulocytosis, sedation, seizures, salivation, and weight gain are some of its adverse effects[23].As a cholinergic medication, xanomeline is unlikely to have the severe side effects for schizophrenia[24]. Since Xanomeline has been demonstrated to alleviate both positive and negative symptoms, it offers a novel approach for the treatment of schizophrenia. Current medications are less effective in treating negative and cognitive symptoms of the disorder. Muscarinic agonists are a novel class of pharmaceutical medications that are presently undergoing clinical trials to treat schizophrenia[25]. As an alternative solution and reducing the side effects of the current medication, the present study was designed to find the potential compounds from the different Indian medicinal plants like *Acorus calamus, Litsea polyantha*[26], *Galphimia glauca, Aloe vera*[27], *Alpinia zerumbet, Swertia chirata* and *Synedrella nodiflora*[28]for treating schizophrenia.

## MATERIALS AND METHODS

**Ligand Selection**

Using literature and the IMPPAT database, approximately 130 phytochemical compounds were chosen from a variety of Indian medicinal plants, including *Acorus calamus, Litsea polyantha*[26], *Galphimia glauca, Aloe vera*[27], *Alpinia zerumbet, Swertia chirata* and *Synedrella nodiflora*[28]. The 3D structures of these compounds were retrieved from the PubChem database[29], and SwissADME was used to test the Lipinski Rule of Five[30]. After the investigation, 120 compounds obeyed the Lipinski Rule of Five and they were taken for further study.

**Target Protein Selection**

The target protein M4 muscarinic Acetylcholine Receptor (M4 mAChR) responsible for the treatment of schizophrenia was identified using the literature. The 3D structure of this target protein was obtained using UniProt[31]and PDB database[32].

**Docking Studies**

PyRx 0.8 software was used to conduct docking studies for the phytocompounds (ligands) and the target protein M4 mAChR. This software was used for docking studies. The Open Babel option in the PyRx 0.8 was used to upload each ligand. The Vina wizard tool in PyRx 0.8 was used to construct the grid and perform docking tests[33]. An XL file included the binding affinity values. The data were evaluated and docked. The 2D and 3D images were obtained using Discovery Studio 2024. The lowest binding affinity indicates the best results.

**ADMET and CYP Properties**

The ADMET and CYP properties of all the phytocompounds with the best interactions were examined using SwissADME[30]. Lipinski, BBB (Blood-Brain Barrier), HIA (Human Intestinal Absorption), PGP (P-glycoprotein), XLogP3, TPSA (Topological Polar Surface Area), LogS, Fraction Csp3, Rotatable bonds, CYP enzyme inhibitor properties, skin penetration, and the bioavailability score were evaluated using this tool.

## RESULTS AND DISCUSSION

**Ligand and Target protein selection**

The 3D structure of ligands (phytocompounds) were retrieved from the PubChem database. The 3D structure of the target protein M4 receptor (PDB ID: 5DSG) was obtained from the PDB database and the 3D structure of the target protein is shown in Figure 1.

**Docking studies**

Docking experiments for the phytocompounds (ligands) and the target protein M4 receptor for schizophrenia were carried out using PyRx 0.8 software. Most of the compounds interacted with the target protein, and the following 10 compounds had good binding affinities with the target protein, according to the data. All the results are shown in Table 1 and the 2D and 3D interactions of phytocompounds with the target protein are shown in Table 2. From the results (Table 1), among other compounds, 10 compounds showed very good results with the target protein. Of which, the phytocompound Beta-Amyrin showed excellent binding affinity (-10.5 Kcal/mol) with the amino acid residues TYR89, TYR439, SER436, and TRP435 of the target protein. The phytocompound Galphimine B also gave excellent binding affinity of (-10.3 Kcal/mol) with the amino acid residues TYR92, TRP435 and LEU190. The binding affinity (-10.2 Kcal/mol) was observed between the phytocompound Lupeol and the amino acid residue TYR439 of the target protein. Among the other compounds, the lowest binding affinity (-8.8Kcal/mol) was observed between the phytocompound beta-Maaliene and the amino acid residues CYS442, TYR416, TRP413, TYR113 and ALA203 of the target protein. Besides, the binding affinity of the Synthetic drug Xanomelin with the target protein was (-6.8Kcal/mol) and the interacted amino acid residues were TYR439, TYR92, GLN184, TRP108 and ILE187. Thus, in the results of the present study, all the phytocompounds showed very good binding affinity when compared to the

Synthetic drug Xanomeline, of which, the phytocompounds Beta-Amyrin, Galphimine B and Lupeol showed the highest binding affinity among the other phytocompounds. According to a prior study, the essential oil of *Alpinia zerumbet* leaves helped in animal models of schizophrenia and reduced oxidative stress-induced changes in whole-brain homogenates [34]. Gerona *et al.* (2017)[35] discovered that emodin may have antipsychotic properties. Galphimine B, a nor-seco-triterpenoid found in *Galphimia glauca Bartl.,* a plant used in Mexican traditional medicine to treat mental illnesses, enhanced the cataleptic effect of haloperidol and showed protection against some of the ketamine-induced and apomorphine-induced symptoms of schizophrenia in mice [36, 37]. When tested on mice with schizophrenia, Amoateng *et al.* (2017) [38] found that *Synedrella nodiflora* extract has antipsychotic potential. Additionally, *Acorus calamus* has been used to treat schizophrenia and is utilized to make brahmyadiyoga, an Ayurvedic medical method [39, 40]. Traditional medical practitioners in Rangamati district use the bark of the medicinal plant *Litsea polyantha (Lauraceae)* to make a juice by macerating it with 1 g sugar. This juice is then administered orally to treat schizophrenia symptoms such as psychotic episodes [26]. Using two pharmacological animal models of schizophrenia, Kumbol *et al.* (2018) [41] assessed the psychotropic effects of the hydroethanolic extract of *Albizia zygia* roots against positive, negative, and cognitive symptoms. The findings indicated that the root extract of *Albizia zygia* has antipsychotic qualities with potential positive, negative, and cognitive symptom benefits along with a low propensity for extra pyramidal effects. After eight weeks of treatment, *Cuscuta epithymum* improved nearly all of the positive and negative symptoms as well as the cognitive impairment of patients with schizophrenia, according to Parvizi *et al.* (2019) [42]. It also showed potential antioxidant and neuroprotective effects and contributed to the increase of GABA amino acid, which helps to increase the inhibitory properties of the central nervous system. The *in vivo* study by Yadav *et al.* (2018) [43]shown that the seed extract of *Spinacia oleracea* was effective against stereotypical behaviors as well as positive, negative, and cognitive symptoms of schizophrenia in mice that were produced by ketamine. According to the research of Fei *et al.* (2022) [44], *Terminalia bellerica* fruit powder has been shown to have antipsychotic properties, which may help mitigate the symptoms of schizophrenia. All the macromolecular drug targets were bound with great affinity by quercetin, ellagic acid, and β-sitosterol. Quercetin has strong binding affinities for both the dopamine and N-methyl-D-aspartate receptors, whereas β-sitosterol has a strong affinity for the dopamine receptor. Ellagic acid, on the other hand, established more robust binding associations with phosphodiesterase 10A and Gamma-aminobutyric acid. Likewise, Alharbi *et al.* (2022) [45] found that acteoside, a naturally occurring molecule from *Bacopa monnieri*, had a strong binding effect on the STXBP1 active site. According to Chintha and Wudayagiri, (2019)[46], the impact of a bioactive chemical and the reference medication clozapine on specific susceptibility targeted gene of schizophrenia was investigated. While clozapine had a single hydrogen bond against DOPA decarboxylase with 1.14 Ki (mM) as an inhibition constant and Thr participating in interaction during docking, the compound had a single hydrogen bond against DOPA decarboxylase with 0.64 Ki (mM) as an inhibition constant and Gly as an interacted amino acid. The chemical has demonstrated a good binding affinity of 9.8 kcal/mol to the schizophrenia target's active site area. In contrast, clozapine, the reference medication, has demonstrated a binding energy of -9.2 kcal/mol. Interestingly, the previous study found that 3-(3,4-dimethoxy phenyl)-1-4(methoxy phenyl)prop-2-en-1-one, a novel bioactive compound isolated and characterized from the methanolic extract of *Celastrus paniculatus*, has been used extensively to treat neurological dysfunction and neurodegenerative diseases, primarily schizophrenia[47].In the present study, Beta-Amyrin from *Swertia chirata*, Galphimine B from *Galphimia glauca* and Lupeol from *Swertia chirata* showed very good binding affinity with target protein M4 muscarinic acetylcholine receptor (M4 mAChR) and this study suggests that the compounds Beta-Amyrin, Galphimine B and Lupeol may act as potential drugs to treat schizophrenia.

**ADMET and CYP Properties**

SwissADME was used in the current investigation to examine the ADMET qualities of the best-interacted phytocompounds and the synthetic medication Xanomeline, and the results were tabulated (Table 3). According to the findings, Xanomeline, a synthetic medication and all the best-interacted phytocompounds both followed the Lipinski rule of five, which means that all the compounds are good for drug likeness. Many compounds showed NO for BBB (Blood Brain Barrier) which refers that the compounds will not enter the human or animal's BBB when consumed, thus not reducing any possible brain related adversities. Many compounds showed LOW in the Human Intestinal Absorption (HIA). Most of the compounds were predicted to be effluated from the CNS by P-glycoprotein

and majority of the compounds Lipophilicity is good since the XLOGP3 values were between −0.7 and +5.0. 4 out of 10 best compounds showed good polarity since the predicted TPSA (Å) values are in the range of 20 and 130 Å$^2$. The Solubility values for the 7 best phytocompounds shows good results since their Log S (ESOL) values are value not higher than 6. Fraction of carbons in the sp3 hybridization for the phytocompounds were higher than 0.25, that shows our compounds have good Saturation. The Flexibility of all the phytocompounds were good, which was evident by the Rotatable bond values not falling above 9. The majority of the substances do not inhibit the CYP450 enzymes or cause any negative side effects, according to the results of CYP characteristics (Table 4). The plant chemical compound Galphimine B blocks the CYP3A4, alpha-Copaeneblocks the CYP1A2, CYP2C19 and CYP2C9, alpha-Bourboneneblocks the CYP1A2, CYP2C19 and CYP2C9 , (-)beta-Bourbonene blocks the CYP1A2, CYP2C19 and CYP2C9, Calamenene blocks the CYP2D6, beta-Maaliene blocks the CYP2C19 and CYP2C9 and Xanomeline (synthetic drug) blocksthe CYP1A2, CYP2C19 and CYP2D6 respectively. The value of log Kp (Skin Permeant) is good for all compounds. A Bioavailability Score (ABS) is good for all of the compounds. From the results of Table 4, the 5 Phytocompounds Beta-Amyrin, Lupeol, Campesterol and Beta-Sitosterol did not inhibit any CYP450 enzymes. Compounds with a high log Kp negative value have limited ability to penetrate skin. According to this statement, all the 10 Phytocompounds and synthetic drug have less skin permeation ability.

## CONCLUSION

In the present study, 130 phytocompounds were taken from different Indian medicinal plants. From the results, in comparison to the synthetic drug Xanomeline (-6.8 kcal/mol), 10 compounds showed good binding affinity with target protein M4 muscarinic acetylcholine receptor (M4 mAChR), responsible for schizophrenia. Among them, the compounds Beta-Amyrin(-10.5 kcal/mol) from *Swertia chirata*, Galphimine B (-10.3 kcal/mol) from *Galphimia glauca* and Lupeol(-10.2 kcal/mol) from *Swertia chirata* showed very good binding affinity with target protein. Additionally, toxicity studies were also conducted for the best interacted phytocompoundsand the findings indicated that all these compounds showed low levels of toxicity. Hence, the current study concludes that the compounds Beta-Amyrin, Galphimine B and Lupeolmay have the potential ability to treat schizophrenia. Further, *in vitro* and *in vivo* studies are required to confirm the potential of these compounds for treating Schizophrenia.

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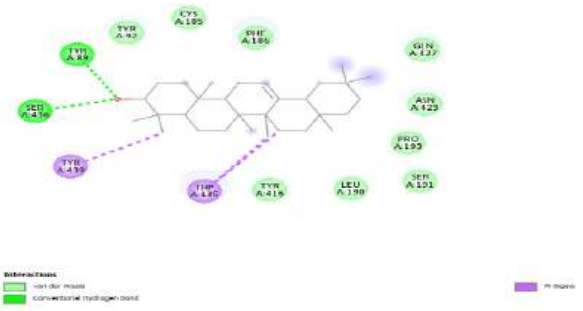
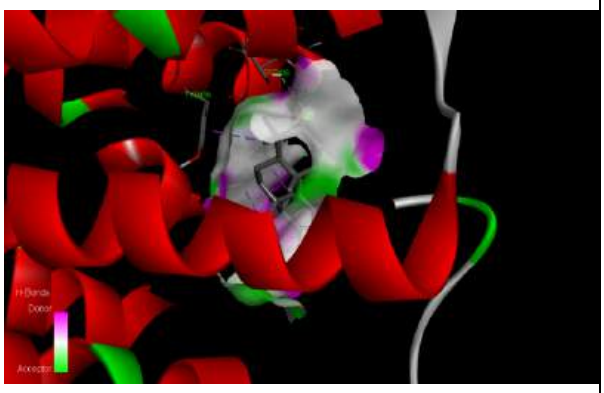
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**Table 1: Interaction of Phytocompounds with the Target Protein**

| S. No. | PubChem (CID) | Compound Name | Plant Name | Binding Affinity (Kcal/mol) | No. of Bonds | Interacting Residues | Bond Length (Å) |
|---|---|---|---|---|---|---|---|
| 1. | 73145 | Beta-Amyrin | *Swertia chirata* | -10.5 | 5 | TYR89<br>TYR439<br>SER436<br>TRP435<br>TRP435 | 2.41<br>3.82<br>2.76<br>3.64<br>3.99 |
| 2. | 127671 | Galphimine B | *Galphimia glauca* | -10.3 | 3 | TYR92<br>TRP435<br>LEU190 | 3.99<br>3.80<br>2.49 |
| 3. | 259846 | Lupeol | *Swertia chirata* | -10.2 | 2 | TYR439<br>TYR439 | 3.51<br>5.42 |
| 4. | 173183 | Campesterol | *Aloe vera* | -9.6 | 5 | TYR89<br>TYR439<br>LEU190<br>GLY96<br>TYR92 | 5.46<br>5.12<br>4.40<br>3.09<br>4.08 |
| 5. | 222284 | Beta-Sitosterol | *Aloe vera* | -9.4 | 2 | TYR92<br>TYR439 | 4.07<br>5.19 |
| 6 | 19725 | alpha-Copaene | *Synedrella nodiflora* | -9.3 | 9 | ALA203<br>TRP413<br>TRP413<br>CYS442<br>TYR416<br>TYR113<br>TYR113<br>TYR113<br>TRP164 | 4.93<br>5.19<br>4.84<br>5.06<br>5.29<br>3.63<br>5.13<br>4.70<br>5.44 |
| 7. | 530816 | alpha-Bourbonene | *Acorus calamus* | -9.2 | 8 | ALA203<br>TRP413<br>TRP413<br>CYS442<br>TYR416<br>TYR439<br>TYR113<br>TYR113 | 5.31<br>4.67<br>5.13<br>4.68<br>5.33<br>5.28<br>3.65<br>4.88 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 8. | 62566 | (-)-beta-Bourbonene | *Synedrella nodiflora* | -9.1 | 5 | TRP413<br>ALA203<br>TYR113<br>TYR439<br>TYR416 | 4.82<br>5.12<br>4.38<br>5.39<br>5.22 |
| 9. | 6429077 | Calamenene | *Synedrella nodiflora* | -8.9 | 4 | TRP413<br>TYR113<br>TYR113<br>TYR439 | 4.77<br>4.03<br>4.12<br>5.05 |
| 10. | 101596917 | beta-Maaliene | *Acorus calamus* | -8.8 | 8 | CYS442<br>TYR416<br>TRP413<br>TRP413<br>TRP413<br>TYR113<br>TYR113<br>ALA203 | 5.11<br>5.07<br>4.77<br>5.04<br>3.88<br>3.74<br>3.84<br>4.31 |
| **Synthetic drug** | | | | | | | |
| 11. | 60809 | Xanomeline | Synthetic Drug | -6.8 | 5 | TYR439<br>TYR92<br>GLN184<br>TRP108<br>ILE187 | 4.12<br>3.96<br>3.53<br>5.22<br>4.83 |

**Table 2: The 2d and 3d Interaction of the Best Interacted Phytocompounds with the Target Protein**

| S. No. | 2D Interaction of the Phytocompounds with the Target Protein | 3D Interaction of the Phytocompounds with the Target Protein |
|---|---|---|
| 1 |  |  |
| **Interaction of the Phytocompound Beta-Amyrin with the Target Protein** | | |

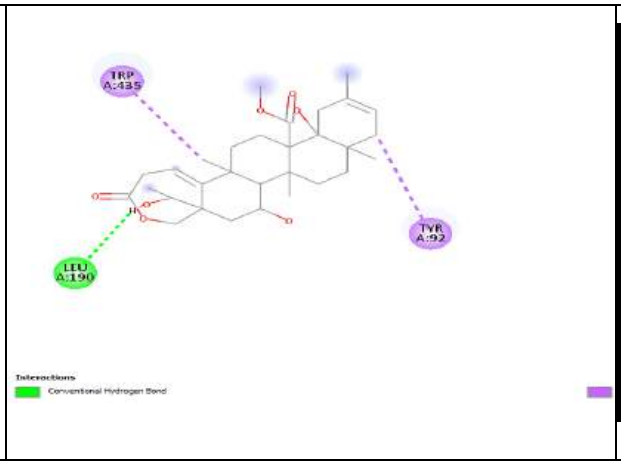
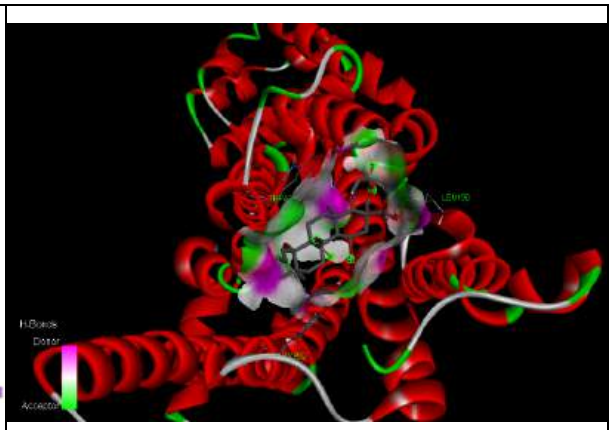
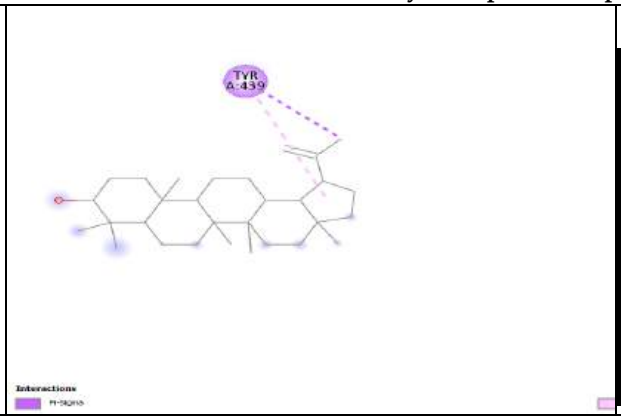
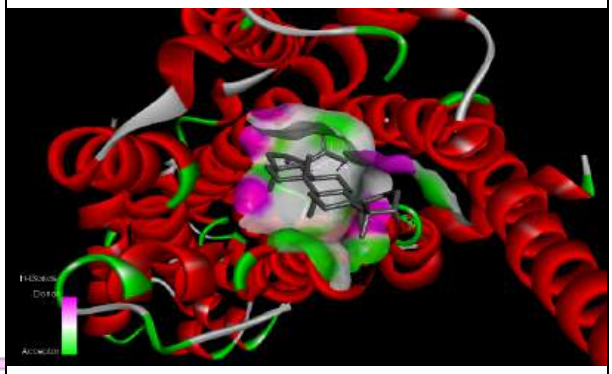
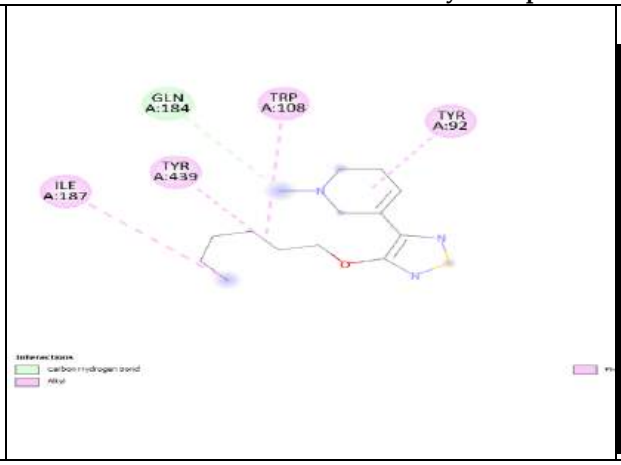
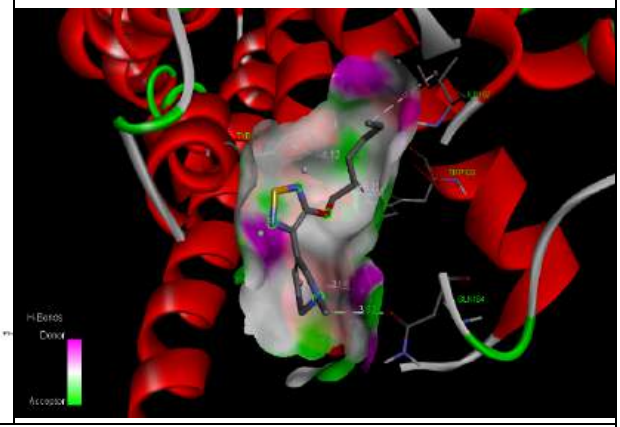
| | | |
|---|---|---|
| **2** |  |  |
| **Interaction of the Phytocompound Galphimine B with the Target Protein** | | |
| **3** |  |  |
| **Interaction of the Phytocompound Lupeol with the Target Protein** | | |
| **4** |  |  |
| **Interaction of the Synthetic drug Xanomelinewith the Target Protein** | | |

**Table 3: ADMET Properties of Phytocompounds**

| S.No. | PubChem (CID) | Compound Name | Lipinski | BBB | HIA | PGP- | XLOGP3 | TPSA(Å) | Log S (ESOL) | Fraction Csp3 | Rotatable Bonds |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | 73145 | Beta-Amyrin | Yes | No | Low | No | 9.15 | 20.23 | -8.25 | 0.93 | 0 |
| 2. | 127671 | Galphimine B | Yes | No | High | Yes | 3.91 | 113.29 | -5.31 | 0.80 | 3 |
| 3. | 259846 | Lupeol | Yes | No | Low | No | 9.87 | 20.23 | -8.64 | 0.93 | 1 |
| 4. | 173183 | Campesterol | Yes | No | Low | No | 8.80 | 20.23 | -7.54 | 0.93 | 5 |
| 5. | 222284 | Beta-Sitosterol | Yes | No | Low | No | 9.34 | 20.23 | -7.90 | 0.93 | 6 |
| 6. | 19725 | alpha-Copaene | Yes | Yes | Low | No | 4.47 | 0.00 | -3.86 | 0.87 | 1 |
| 7. | 530816 | alpha-Bourbonene | Yes | Yes | Low | No | 4.47 | 0.00 | -3.86 | 0.87 | 1 |
| 8. | 62566 | (-)-beta-Bourbonene | Yes | Yes | Low | No | 4.71 | 0.00 | -4.01 | 0.87 | 1 |
| 9. | 6429077 | Calamenene | Yes | No | Low | No | 5.12 | 0.00 | -4.55 | 0.60 | 1 |
| 10. | 101596917 | beta-Maaliene | Yes | No | Low | No | 4.40 | 0.00 | -3.88 | 0.87 | 0 |
| **Synthetic Drug** | | | | | | | | | | | |
| 11. | 60809 | Xanomeline | Yes | Yes | High | No | 3.25 | 66.49 | -3.37 | 0.71 | 7 |

**Note:** Obey Lipinski: Yes means 0 violation and good; BBB (Blood - Brain Barrier): Yes means good; HIA (Human Intestinal Absorption): High means good; PGP- (Molecules predicted not to be effluated from the CNS by P-glycoprotein): No means good; Lipophilicity: XLOGP3 value between −0.7 and +5.0 means good; Polarity: TPSA between 20 and 130 $Å^2$ means good; Solubility: Log S value not higher than 6 means good; Saturation (Fraction Csp3): Fraction of carbons in the sp3 hybridization not less than 0.25 means good; and Flexibility (Rotatable bonds): No more than 9 rotatable bonds means good.

**Table 4: Cytochrome P450 Properties of Phytocompounds**

| S.No. | PubChem (CID) | Compound Name | CYP1A2 Inhibitor | CYP2C19 Inhibitor | CYP2C9 Inhibitor | CYP2D6 Inhibitor | CYP3A4 Inhibitor | Log $K_p$ (Skin Permeation) | A Bioavailability Score (ABS) |
|---|---|---|---|---|---|---|---|---|---|
| 1. | 73145 | Beta-Amyrin | No | No | No | No | No | -2.41 | 0.55 |
| 2. | 127671 | Galphimine B | No | No | No | No | Yes | -6.68 | 0.55 |
| 3. | 259846 | Lupeol | No | No | No | No | No | -1.90 | 0.55 |
| 4. | 173183 | Campesterol | No | No | No | No | No | -2.50 | 0.55 |
| 5. | 222284 | Beta- | No | No | No | No | No | -2.20 | 0.55 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sitosterol | | | | | | | |
| 6. | 19725 | alpha-Copaene | Yes | Yes | Yes | No | No | -4.37 | 0.55 |
| 7. | 530816 | alpha-Bourbonene | Yes | Yes | Yes | No | No | -4.37 | 0.55 |
| 8. | 62566 | (-)-beta-Bourbonene | Yes | Yes | Yes | No | No | -4.20 | 0.55 |
| 9. | 6429077 | Calamenene | No | No | No | Yes | No | -3.90 | 0.55 |
| 10. | 101596917 | beta-Maaliene | No | Yes | Yes | No | No | -4.42 | 0.55 |
| **Synthetic Drug** | | | | | | | | | |
| 11. | 60809 | Xanomeline | Yes | Yes | No | Yes | No | -5.71 | 0.55 |

**Note:** Yes means the compound inhibits the CYP450 enzymes and gives unanticipated adverse reactions; No means the compound does not inhibit the CYP450 enzymes and does not give any adverse reactions; The more negative the log $K_p$, the less skin permeate is the molecule; ABS 0.55 means it passes the rule of five and 0.17 means it fails the rule of five.

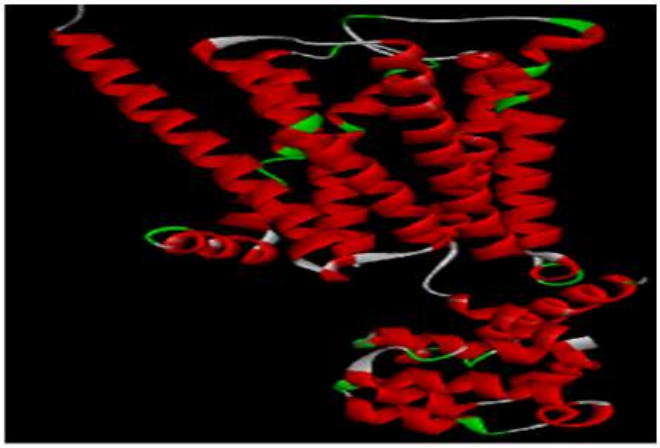


**Figure 1: The 3D structure of the Target Protein M4 mAChR**

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RESEARCH ARTICLE

# Phytochemical Screening of *Borassus flabellifer* Sprout Powder and its Anti-Cancer Activity against Lung Carcinoma

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## ABSTRACT

Lung cancer is one of the leading cancers causing mortality among all types worldwide; therefore, there is a continuing need for the investigation of safer and effective therapeutic alternatives from natural sources. The current study focuses on phytochemical profiling and anticancer potential of *Borassus flabellifer* sprout extract against human lung carcinoma (A549) cells. Profiling by gas chromatography–mass spectrometry revealed the presence of several bioactive phytoconstituents, containing fatty acids, fatty alcohol esters, pyrimidine derivatives, and halogenated compounds, most of which are associated with antioxidant and anticancer activities. Cytotoxic activity was assayed, showing a concentration-dependent reduction in A549 cell viability. These morphological changes were further confirmed to be signs of apoptosis-like alterations such as cell rounding and shrinkage, cell membrane blebbing, and loss of cellular adhesion post-treatment. A comparative study with the standard chemotherapeutic agent cisplatin showed that while the latter exhibited higher cytotoxicity, *B. flabellifer* sprout extract resulted in a controlled form of cell death with comparatively reduced nonspecific toxicity. This study therefore demonstrates the promising nature of *Borassus flabellifer* sprouts as a potential natural source of anticancer compounds and serves as a basis for further mechanistic studies.

**Keywords:** *Borassus flabellifer,* Lung Cancer, Phytochemicals, A549 cell line.

## INTRODUCTION

Lung cancer is one of the leading causes of cancer-related mortality worldwide with an estimated 1.8 million deaths per year and a substantial global health burden (Bray et al., 2018). About 85% of all cases of lung cancer are non-small cell lung carcinoma (NSCLC), the most common subtype. Because early disease progresses asymptomatically, NSCLC is frequently detected at an advanced stage (Siegel et al., 2020). Overall survival rates are still low despite advancements in immunotherapy and targeted therapies, and the need for new, safer, and more effective therapeutic agents is urgent due to treatment side effects and costs (Herbst et al., 2018). The therapeutic and chemopreventive potential of phytochemicals derived from medicinal plants against a variety of cancers, including lung carcinoma, has attracted a lot of attention (Newman & Cragg, 2020). According to Khan et al. (2019), phytochemicals, including secondary metabolites like polyphenols, flavonoids, alkaloids, and terpenoids, have a variety of biological activities, such as anti-inflammatory, anti-cancer, and antioxidant qualities. Numerous substances originating from plants have made their way into clinical practice; for instance, vincristine and paclitaxel, which were first isolated from Catharanthus roseus and Taxus brevifolia, respectively, are common chemotherapy drugs (Cragg & Newman, 2013). This emphasizes how crucial it is to keep looking for possible anticancer compounds in botanicals.

The palmyrah palm, or Borassus flabellifer L. (Arecaceae), is a hardy perennial palm that is indigenous to South and Southeast Asia. Palmyrah has long been prized for its edible fruit, sap, and wood, but ethnomedicine also acknowledges its anti-inflammatory, anti-diabetic, and antimicrobial properties (Kumar & Sharma, 2016; Rathore et al., 2012). The nutritional value and bioactive components of various B. flabellifer parts, including fruit pulp, seeds, leaves, and sap, have been investigated (Singh et al., 2013). Nevertheless, despite growing evidence that germination can greatly increase bioactive phytochemicals and antioxidant potential, little is known about B. flabellifer sprouts, which grow from germinating seeds (Xu & Chang, 2009; Yuan et al., 2021). According to Singh et al. (2014), sprouting, also known as seed germination, initiates biochemical changes that result in the production or release of secondary metabolites and enhance nutrient availability. The sprouts of *Triticum aestivum* (wheat), *Vigna radiata* (mung bean), and *Brassica oleracea* (broccoli) have been shown to exhibit enhanced antioxidant and anti-proliferative activities. This suggests that sprouts may be used as functional foods with chemopreventive qualities (Finley et al., 2019). These findings encourage thorough phytochemical profiling and bioactivity assessment of *B. flabellifer* sprouts, which may have special metabolites with anticancer properties.

The bioactivity of these components in *B. flabellifer* against lung cancer have not yet been thoroughly determined. Preliminary reports suggest the presence of phenolic acids, flavonoids, and other antioxidant compounds in germinating seeds of related palm species (Al-Snafi, 2017). Plants with ethnopharmacological significance and unexplored sprouts like *B. flabellifer* offer promising research avenues in light of the rising incidence of lung cancer and the need for innovative, less toxic treatments. In particular, the identification of antioxidant and anticancer compounds from B. flabellifer sprouts may aid in the development of natural agents that slow the spread of cancer or improve current therapeutic approaches. Accordingly, the aim of the current study is to extensively investigate the phytochemical constituents of Borassus flabellifer sprouts to evaluate the anticancer potential of this particular sprout type on lung carcinoma cell lines. The current study is not only extending the phytochemistry of the less utilized Borassus sprout of India, but it is also examining the manifestations of Borassus sprouts in the prevention and treatment of one deadliest cancer.

## MATERIALS AND METHODS

**Extraction of phytochemicals from *Borassus flabellifer***

Sprout of *Borassus flabellifer* were washed thoroughly with distilled water for several (5-6) times. The samples were allowed to dry under shade at room temperature. They were ground by an electrical mixer until they became fine powder. The powdered samples were stored until further use. About, 25 g powdered sprout sample was soaked in

250 ml of 90% ethanol for 72 h resulting in release of phytochemical into the dissolving solvent. Further, the solvents were evaporated under high pressure using a rotary evaporator to obtain/concentrate phytochemicals released from the seed tissue(s) of palmyra palm.

**Qualitative analysis of phytochemical substances**

Presence of different phytochemicals were analyzed as described in a previous study (Harborne et al., 1973). Presence of alkaloids, saponins, tannins, cardiac glycosides, flavonoids, phenols, steroids, terpenoids, quinones and proteins were estimated from the concentrated solvents.

**Thin layer chromatography**

The extract was analyzed in thin layer chromatography (TLC) as per conventional method using silica gel 60F254 (Merck) in 5x3 cm dimension. Glass capillary tubes were used to spot the extracts in TLC plates. The resolving solvent (chloroform:methanol) was optimized for separation of bioactive components in the TLC set-up. After pre-saturation with mobile phase for 30 min the plates were kept inside the chamber and desirable fractions were eluted using the optimized solvent(s). Further, the plates were dried and visualized under UV followed by spraying different reagents. $R_f$ values determined by using following formula:

$R_f$ = Distance travelled by the solute/Distance travelled by the solvent.

**GC-MS analysis of sprout extract**

A PerkinElmer Clarus 500 gas chromatograph (Autosystem XL) in conjunction with a TurboMass Gold mass spectrometer (PerkinElmer, TurboMass software version 5.2) was used to perform the GC–MS analysis. An Elite-5MS capillary column (30 m × 250 μm i.d., 95% dimethyl polysiloxane) was used to achieve separation. The oven temperature program was started at 60 °C and held there for two minutes. It then increased gradually to 300 °C at a rate of 5 °C $min^{-1}$, with a final hold time of six minutes. The carrier gas, helium, was used at a steady flow rate of 1 mL $min^{-1}$. Samples were injected in split mode with a split ratio of 10:1, while the injector temperature was kept at 240 °C. At 70 eV, electron ionization was carried out, and mass spectra were captured within the 40–600 m/z scan range. By comparing the obtained mass spectra with those found in the NIST mass spectral library (2011 version), the compound was identified.

**Cytotoxicity assessment using MTT**

A549 human lung cancer cells were acquired from the National Centre for Cell Science (NCCS), India, and kept in culture media supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL of penicillin, and 100 μg/mL of streptomycin in a humidified environment at 37 °C with 5% $CO_2$. Trypsin–EDTA was used to separate the cells, after which they were centrifuged, resuspended in new medium, and their viability evaluated. To enable monolayer formation, 96-well plates were seeded with roughly 5 × $10^4$ viable cells per well and incubated for 24 hours.Following treatment with different concentrations of the test compounds, the cells were incubated for 48 hours under normal culture conditions. Then MTT solution (5 mg/mL) was added, and the cells were incubated for 4 hours to assess the cell viability. The resultant formazan crystals were dissolved in DMSO and measured at 570 nm with microplate reader. Values for $IC_{50}$ and percentage growth inhibition were computed and contrasted with standard cisplatin.

## RESULTS AND DISCUSSION

**Analysis of phytochemical substances in the seed extracts of *Borassus flabellifer***

To analyze the presence of phytochemical substances and secondary metabolites in the Sprout extracts of *Borassus flabellifer*, we comprehensively profiled their presence in an alcoholicenvironment representing 90% ethanol. The phytochemical analysis revealed the presence of nine phytochemical out of the ten compounds tested. The phytochemical analysis suggests that palmyrah palm can be used for the treatment of various health disorders.

**Analysis of phytochemicals using thin layer chromatography**

Further to analyze the presence of phytochemical substances in the extracts of palmyra palm, the extracts were assessed using thin layer chromatography. Interestingly, we detected at least five spots showing Rf-value of 0.06-0.93 as shown in table. Rf-values estimated based on the migration rate of solute(s) to the solvent(s). This suggests the presence of phytochemical substances and/or secondary metabolites in the sprout extracts of palmyra palm.

**GC-MS analysis of *Borassus* sprout extract**

In GC-MS analysis, diverse bioactive compounds, including fatty acids, esters, halogenated hydrocarbons, pyrimidine derivatives, and nitro compounds, were identified, which are present in *Borassus flabellifer* sprout.The identification of n-hexadecanoic acid, also called palmitic acid, is very interesting. Recent studies have confirmed that the compound possesses the ability to elicit mitochondrial apoptosis, ROS generation, and cell cycle arrest in cancer cells. These effects were observed in various cancer cells, which indicate the ability of the compound to elicit anticancer activities (Wang et al., 2023; Ottaviani et al., 2025). Importantly, the identification of the pyrimidine compound is very interesting and proves the anticancer properties of the compound, as various pyrimidine compounds have exhibited anticancer activity, demonstrating the ability to inhibit the activity of the epidermal growth factor receptor, specifically in the cancer cells of non-small cell lung cancer, including A549 cells. Further, the identification of the halogenated fatty acid derivative enhances the ability of the compound to elicit anticancer properties through increased membrane permeability and the ability to elicit apoptotic activity. The identification of the nitro-alkene compound also elicits pro-apoptotic activity.

**Cytotoxicity of sprout extract of *Borrasus flabellifer***

The MTT assay graph represents the cytotoxic effect of *Borassus flabellifer* sprout extract at different concentrations in comparison to the standard anticancer drug cisplatin. Starting from 22% inhibition at 10 μg, *B.flabellifer* demonstrated an increase to ~35% at 20 μg and ~47% at 50 μg, indicating progressive loss of cell viability. Finally, it showed about 58–60% inhibition at a maximum tested concentration of 100 μg and thus exhibited a response of moderate to strong cytotoxicity potential. In contrast, the inhibition values of cisplatin were higher: approximately 38% at 10 μg, ~60% at 20 μg, and ~70% at 100 μg. From this sigmoidal trend, dose-responsiveness of *B.flabellifer*sproutwas comparable to that of cisplatin.The $IC_{50}$ value of the given test sample and the standard Cisplatin wasfound to be1.91 μg/ml is 6.62 μg/ml respectively.

Our study showed the morphological studies of the A549 cells after treatment with *Borassus flabellifer* sprout extract at various concentrations and confirmed a dose-dependent cytotoxic effect. The untreated control showed a spindle shape indicative of healthy morphology. The treated cells showed signs of apoptotic cell death as indicated by rounding and shrinkage of the cells, loss of adhesiveness at a concentration ranging from 12.5-100 μg. The same signs have been shown in other plants used in the study of anticancer properties and inducing apoptosis in lung cancer cell lines by causing mitochondrial dysfunction (Wang et al., 2023; Li et al., 2022). From comparative morphological evaluation, it has been observed that treatment of A549 cells with *Borassus flabellifer* sprout extract and cisplatin results in the exhibition of distinctive cytotoxic effects. Although cell rounding, membrane blebbing, cytoplasmic shrinkage, and partial loss of cell adhesion were observed after treatment with 50–100 μg concentration of the *B. flabellifer* extract, these effects were slow and dose-dependent, indicating apoptosis-regulated cytotoxic cell death. Morphology is characteristic of phytochemicals of plant extracts, which are known to induce mitochondrial dysfunction-mediated apoptosis and oxidative stress-mediated apoptosis, leading to cell death (Patel et al., 2024). However, after treatment of A549 cancer cells with cisplatin, severe dose-dependent morphological changes were observed, such as cell detachment, cell shrinkage, and cell density, indicating aggressive DNA damage-mediated apoptosis, which is characteristic of cell death resulting after treatment with this anticancer compound (Wang et al., 2022). It has also been noted that the anticancer effect of B. flabellifer is relatively milder, indicating that this plant extract efficiently inhibits the growth of lung carcinoma cells.

## CONCLUSION

The present study reveals that *Borassus flabellifer* sprout extract exhibits great anticancer activity against A549 lung carcinoma cells. GC–MS has identified bioactive phytochemicals that are fatty acids, pyrimidine derivatives, and halogenated compounds. These have been associated with anti-cancerous activities. MTT assay confirmed the overall concentration-dependent reduction in cell viability, while morphological observations cited cell rounding, shrinkage, and loss of adherence, indicative of apoptosis-like phenomena. Cytotoxicity was found to be relatively moderate and more efficient compared to the standard drug cisplatin, insinuating less nonspecific toxicity. All of these collectively establish *B. flabellifer* sprouts as a promising source of natural anticancerous agents and further justify carrying out mechanistic and *in vivo* studies.

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**Table 1: Phytochemical analysis of *Borassus* sprout extract**

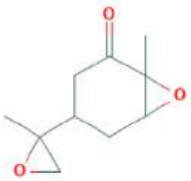
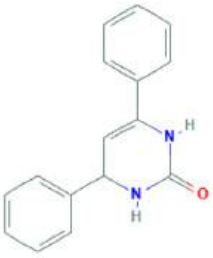
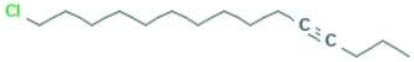
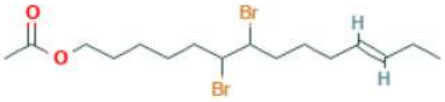
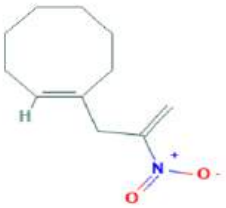
| Phytochemicals | *Borassus flabellifer* sprout extract |
|---|---|
| Alkaloids | + |
| Saponis | - |
| Tannins | + |
| Cardiac Glycosides | + |
| Flavonoids | + |
| Phenols | + |
| Steroids | + |
| Terpenoids | + |
| Quinones | + |
| Proteins | + |

**Table:2TLC analysis of *Borassus* sprout extract**

| Extract | Solvent system | Number of spots | Rf-value |
|---|---|---|---|
| 90% Ethanol | Chloroform:Methanol (50:50) | 5 | 0.06, 0.33, 0.4, 0.5, 0.93 |

**Table:3:GC-MS analysis of *Borassusflabellifer* sprout extract**

| S.No | Compound Name | Compound Structure |
|---|---|---|
| **1** | N – Hexadeconoic acid |  |

| | | |
|---|---|---|
| **2** | 9,12-Tetradecadien-1-Ol,Acetate, (Z,E)- |  |
| **3** | 7-Oxabicyclo[4.1.0]Heptane, 1-Methyl-4-(2-Methyloxiranyl)- |  |
| **4** | 2-Oxo-4,6-Diphenyl-1,2,3,4-Tetrahydropyrimidine |  |
| **5** | 4-Pentadecyne, 15-Chloro |  |
| **6** | 6,7-Dibromo-Z-11-Tetradecene-1-Ol Acetate |  |
| **7** | 1-Propene, 2-Nitro-3-(1-Cyclooctenyl |  |

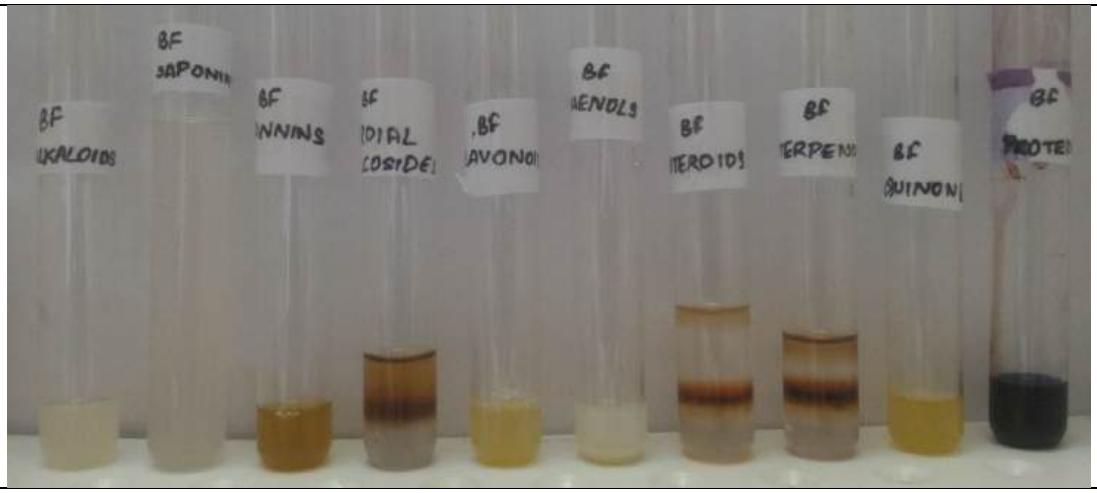



**Fig 1:Phytochemical analysis of *Borassus* sprout extract**

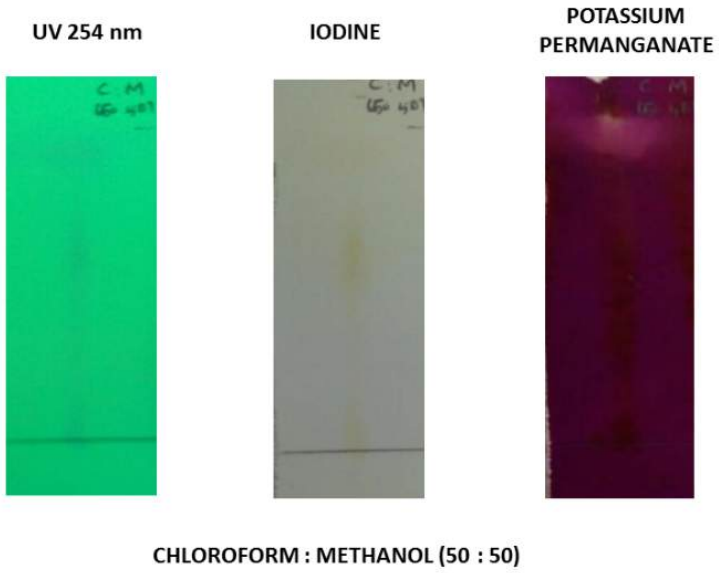
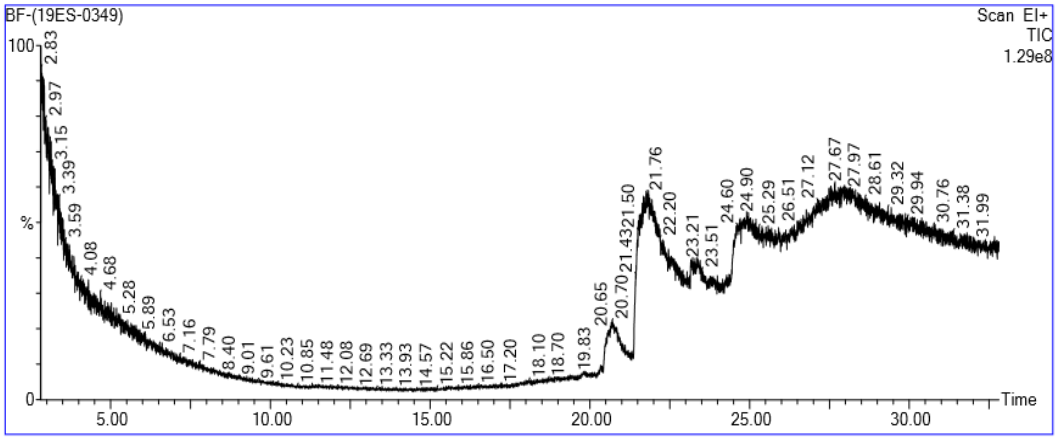



**Fig: 2 . TLC analysis of *Borassus* sprout extract**




| # | RT | Scan | Height | Area | Area % | Norm % |
|---|---|---|---|---|---|---|
| 1 | 20.696 | 3578 | 15,200,129 | 5,328,892.5 | 11.267 | 15.39 |
| 2 | 21.831 | 3805 | 49,316,000 | 34,623,684.0 | 73.206 | 100.00 |
| 3 | 22.456 | 3930 | 11,646,813 | 1,005,409.2 | 2.126 | 2.90 |
| 4 | 24.702 | 4379 | 13,887,545 | 2,956,427.0 | 6.251 | 8.54 |
| 5 | 24.832 | 4405 | 10,861,475 | 1,401,033.8 | 2.962 | 4.05 |
| 6 | 24.902 | 4419 | 11,386,624 | 1,024,475.1 | 2.166 | 2.96 |
| 7 | 28.143 | 5067 | 6,449,673 | 956,138.2 | 2.022 | 2.76 |

**Fig 3: GC-MS spectra of sprout extract of *Borassus flabellifer***

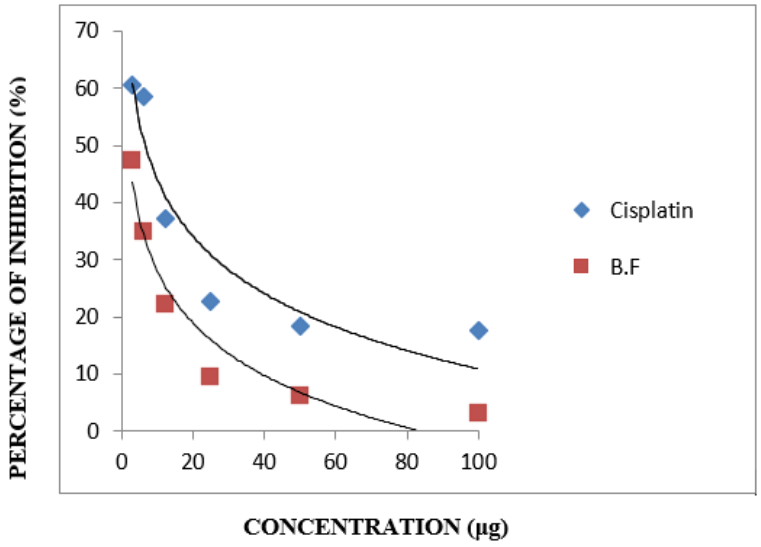



**Fig. 4. : Cytotoxic effectof *Borassus flabellifer* sprout extract**

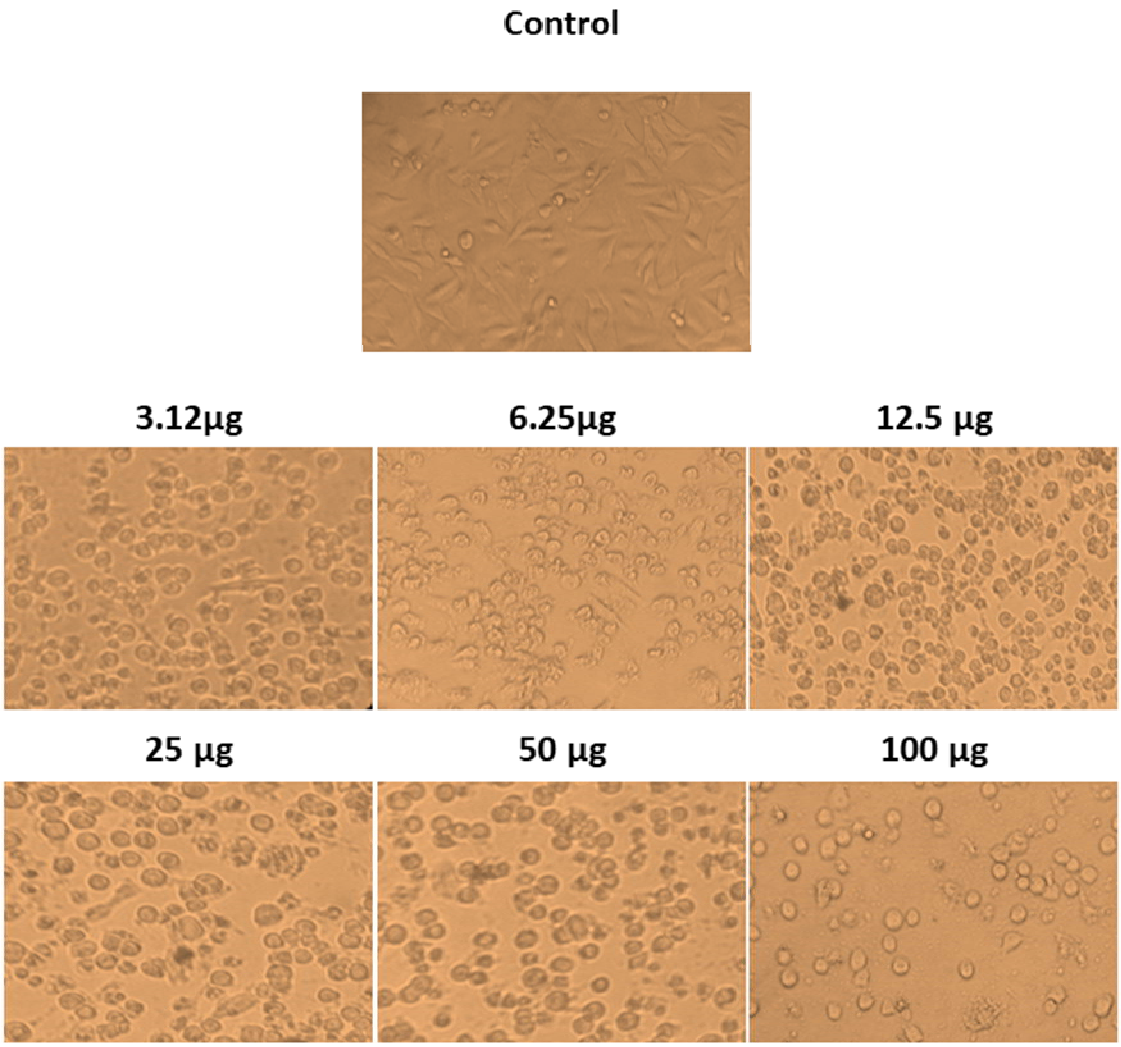



**Fig.5 Morphological observation of A549 after treatment with *Borassus flabellifer sprout extract.***

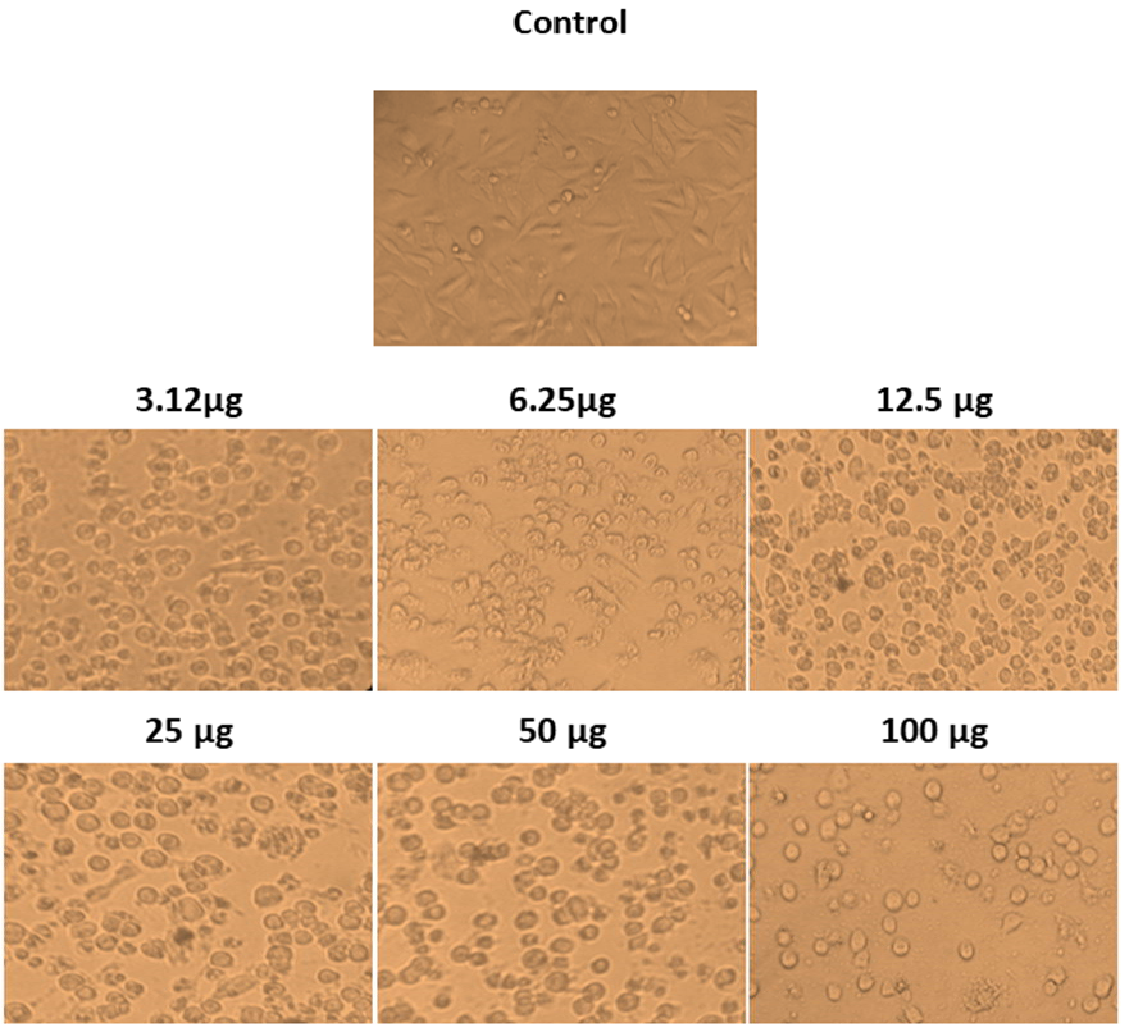



**Fig.6 Morphological observation of A549 after treatment with standard cisplatin**

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**RESEARCH ARTICLE**

# Assessment of Tectonic Geomorphology in the Chicktore Small River Basin, West Bangalore, Karnataka, India

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## ABSTRACT

This study aims to assess ongoing tectonic activity and identify potential active faults within a region traditionally considered part of the stable Archean craton but known for historical earthquakes of magnitude 5.0–5.9. Using remote sensing and quantitative geomorphic analysis, the relationship between drainage characteristics and structural controls such as lineaments and faults was evaluated through digital image processing of Landsat 8 data, which revealed several major lineaments dominated by a NW–SE trend strongly influencing regional topography. Five geomorphic indices-Asymmetry Factor (AF), Transverse Topographic Symmetry Factor (T), Valley Floor Width-to-Valley Height Ratio (Vf), Mountain Front Sinuosity (Smf), and River Sinuosity (SI)-were applied across 43 sub-basins, of which 50% exhibited anomalous AF values (outside the 40–60 range), indicating tectonic tilting near major lineaments. The T index showed consistent drainage deflection toward the NW close to the prominent L1 lineament, with maximum lateral migration observed on its northeastern side, while Vf values ranging from 0.51 to 2.31 revealed that half of the measured sites display values below 1.0, signifying active incision and the development of V-shaped valleys. These geomorphic anomalies are particularly concentrated at intersections of NE–SW and NW–SE trending lineaments, leading to the conclusion that, despite its limited spatial extent, the Chick Tore basin records active surface deformation and ongoing tectonic adjustments that are likely linked to the region's documented seismicity.

**Keywords:** Cratons, Mountains, Lineaments, Morphometric analysis and Seismicity.

# INTRODUCTION

A series of vibrations induced in the earth's crust by the abrupt rupture and rebound of rocks in which elastic strain has been slowly accumulating is termed an earthquake. This is the most disastrous natural hazard, which causes damage to both living and non-living. Which also causes huge damage to the possessions? The Indian plate is one of the most complexly deformed parts in the world, which has also become the source of various earthquakes in the past and presently. (Rajendran et.al, 2004). In our country their existing zoning map has several limitations, though it provides some basic information on the relative seismic potential of different regions. The historical documentation of earthquakes and their recordings themselves are too short to represent the long-term activity of the faults. Earth quake occur in intra-plate location is very rare. Most of the Archean cratons on Earth were earlier considered stable (Johnston and Kanter, 1990). But sometimes earthquakes may occur in faulting of the intraplate settings, so they occur in unexpected locations.

Identification of the seismic potential zone or active faults in an area is the first step in the direction of seismic hazard assessment. It is very difficult, the majority of the faults are either hidden or don't have not produced recognizable deformation in recent geological formations. Peninsular India has low rates of deformation coupled with high erosional activity, so there occurs lack of information from instrumental seismic data for strong earthquakes. This leads to a challenge to the identification and characterization of active faults. However, there are in stances that geomorphic analysis played an effective role in identifying the potential seismic source zones. In the evaluation or classification of a fault in an area, such as active/potential/inactive, fault geometry, geologic age, morphotectonic signature, and relation with ongoing seismic records, play a key role. According to the studies of Schultz and Cleaves (1955), the active fault is termed as fractures that have shown displacements in the marker beds within historic times, and which also have an accurate seismic record, while the inactive fault is termed as the underlying fault that did not show any displacement in the overlying younger strata.

The studies carried out by Cluff (1964) in dam foundations suggested that if a fault has shown conclusive evidence of movement in the recent geological time by cutting recent sediments such as alluvium, alluvium fansor seismic record (epicentral plota long the fault) is called an active fault. After a year, Allen (1965) had an observation on active fault based on the studies carried out in Southern California for the relationship between seismicity and geological structure. By these studies, he stated that, in the absence of a historical seismic record, there are only the geological criteria. The studies of Albee and Smith (1966) carried out on the earthquake characteristics and fault activity in southern California suggested that different fault shows different extents of activity, so these are classified into three: active, moderately active, and slightly active. This classification was done by the evidence of geodetic, seismic records, and deformation-associated surface features. They emphasized that "geological evidence monitors a fault over a time period 100 or more times as long as the accurate seismic and geodetic record.

**Application of Geomorphic Analysis (Methodology)**

The study of tectonic geomorphology increasingly in the last few decades, has increasingly developed into a main tool in the evaluation of active tectonic elements (e.g. Keller and Pinter, 1996 and references there in). The morphology developed by the tectonic features or morphotectonics, in general, explores the interrelationship of climatic, tectonic, erosion-deposition processes, and other surficial features (Caputo and Pavlides, 2008). Tectonic geomorphology indicates an amount of interaction between surficial & tectonic processes and also the control of climatic conditions over them (Bull, 1991). The quantitative assessment of tectonically active areas by geomorphic indices (Peters and Van Balen, 2007; Bull and McFadden, 1977; Keller and Pinter, 2002; Burbank and Anderson, 2001). There are also some quantitative parameters for categorizing the faults as Very Active, Moderately Active, or Inactive faults. This kind of classification is used to figure out the locations in which more intense field activities can identify the active structure and also the prevailing rates. However, there are only a few methods for the identification of active faults in areas like low relief and low seismicity. In the present study, both conventional morphometric parameters and geomorphic indices have been used for the evaluation of the study area.

**Study Area**

The study area lies in the west of Bangalore, Karnataka, between latitude 13° 03' N & 12° 53' N and longitude77°12' E & 77°20' E. NNE-SSW trending Arkawati River is the major river draining in this area. One major NW-SE trending Fault viz Arkawati fault, is located in the SW of the study area. Other than the fault, one major NE-SE trending and one minor NW-SE trending lineament are also found in the SW of the study area (Map-1). As per GSI 2000, the study area has also experienced an earthquake in historical times (1901-1963) of M5.0 to 5.9 (GSI 2008). The present study area is limited to the catchment of NW- SE trending Chick Tore, one of the tributaries of Arkawati River, within the area covering~ 116 sq. km. Remote sensing studies identified a wide variety of land forms in the study area. The general topography of the area shows the elongated hills trending NE-SW in the southeastern part. The area is mostly mid-range relief (1221m to 720 m), and all the subsidiary rivers flow with a gentle gradient for most of their length.

**Lineament Study**

The lineament identification in the present study is focused on the identification of the active tectonic structures/features in the area. Initially, all the structural units of the study area have been delineated using satellite images of various spatial and spectral resolutions. Since the visual extractions method is more efficient in discriminating human precipitation from geological lineaments in the urbanized areas. (Ricchetti and Palombella, 2005). In the present study, visual interpretation was used for the demarcation of the lineaments. Various processes like spectral enhancement, band combination, and band ratio have been applied during the image analysis. The fractures, trend lines in hard rocks, palaeochannels, etc., besides the narrow valleys, series of straight courses of rivers between the hills, and their sudden changes have been manifested as lineaments. An RGB combination of the 674 band is used for marking the lineaments from Landsat 8. In addition to that band ratio of 4/3, 6/2, 7/3 is also used in the delineation of lineaments (Mawaniki et al. 2015). During the remote sensing study done at the regional scale, it is observed that the NW- SE lineaments are found to be regionally the most persistent features within the study area. Details of the individual lineament are given in Table-1. The NW-SE flowing of the Chick tore follows one such lineament. In the NE of this river, another parallel lineament is also observed. The image analysis carried out for the study area also affirms the domination of the NW-SE trending lineament. In addition to that, there are some lineaments NE-SW that are also observed in the area (Map-2).

**Geomorphic Studies**

The study of geomorphology is considered a valuable tool in active tectonic studies, since it holds the records of evolution or modification of landforms (Keller and Pinter, 1996). Quantitative analysis of various geomorphic parameters (geomorphic indices) can directly indicate the level of tectonic activity. These geomorphic indices have been successfully used by many researchers in different terrains for the evaluation of ongoing tectonic activities. (e.g., Bull and McFadden, 1977; Keller and Pinter, 1996 de, 1983; Gregory and Schumm, 1987; Wellsetal., 1988; Rhea,1993;Demoulin, 1998; Mc Calpin, 1996; Malik et al., 2001; Burbank and Anderson, 2001; Champel et al., 2002; Schumm et al., 2002; Silva et al., 2003; Riquelmea et al., 2003). The present study is an attempt to identify and evaluate tectonic activity (if any) through quantitative analysis of various geomorphic indices. Geomorphic indices such as Asymmetry Factor (AF), Transverse Topography Symmetry Factor (T), Valley floor/Valley width ratio (Vf), Mountain front sinuosity (Smf), River sinuosity index (SI) are the parameters used in this study for the calculation of individual parameters.

This method of quantitative analysis of a drainage basin was first introduced by Horton (1945), followed by Strahler (1952) in the field of hydrology. Various parameters obtained from the analysis have also analyzed in terms of the tectonic activities (Deffontaines and Chorowicz, 1991; Talling and Sowter, 1999; Simoni et al., 2003; Jamieson et al., 2004; Devi et al., 2011; Zhang et al., 2006; John et al., 2008). This method of analyzing the drainage pattern and the associated anomalies also has the potential to record the evidence of active tectonics (Deffontaines et al., 1992; Simoni et al., 2003; Delcaillau et al., 2006; Ramasamy et al., 2011).

In the study area Chicktore catchment area is divided into 43 sub-basins on the basis of 2nd and 3rd order streams (Maps 3 & 4). For demarcating the basing SOI 1:50,000 scale toposheet no 57G/4, 57G/8, 57H/1, 57H/5 are utilized. All basic parameters, like the length of the drainage perimeter and area of the basins, are measured using Arc-GIS 10.5.

**Asymmetry Factor (AF)**

The asymmetry factor is originally derived to detect the tectonic tilt of the basin areas (Hare and Gardner, 1985; Keller and Pinter1996). The principle behind this parameter is that in a stabilized terrain with uniform lithology, the main drainage should occupy the centre of the basin. This Factor defined to represent the percentage of the areal shift of the main drainage from the Centre of the basin. In the present study, the asymmetry factor is calculated based on the formula as listed in Table-1. For a stream network flowing in a stable setting and uniform lithology, the AF would be nearly equal to 50. In all other cases, there will be a change in value deviating on either side of 50, depending on the direction of drainage migration (Keller and Pinter, 1996). The AF was calculated for all 43 sub-basins demarcated for the morphometric analysis, and the AF ranges from 33 to 78. If we consider the range 40 to 60 as the normal values of AF for the study area, then it is observed that only 50% of the basins fall in this range, and 50% of the basins are showing anomalous AF (either <40 or >60). The spatial distribution of the AF shows that the basins with the anomalous values are observed in the vicinity of L1 (central portion) and L4. Since this factor reflects the tilt of the basin, 50% of the basins are showing the tilting, including the basin lying in the low relief area. The spatial relations of these anomalies with the above said lineaments may indicate the geological or structural influence (Map-5).

**Transverse topographic Symmetry factor (T)**

This method suggested the direction of Holocene tilting of the ground in the Mississippi Embayment (Cox et al., 2001). This factor can also show the tilt direction of the basin in areas of uniform lithology (Keller and Pinter, 1996). The present study calculated T at different reaches for second and third-order streams to demarcate the regions that show preferred drainage migration. Similar studies carried out in the northern part of the study area identified that the WNW-ESE trending active fault (John et al., 2008). The values of 'T' are plotted in the map as an arrow representing the direction of drainage shift and length as the quantum of movement. For a perfectly symmetric basin, T= 0. As asymmetry increases, T increases and approaches a value of 1, where the drainage will be flowing through the basin boundary (Map-3).

The present analysis indicates that all the basins joining the Chick Tore are showing a deflection towards NW in the vicinity of the L1 lineament. Careful observations indicate that north eastern parts of the lineament are showing more consistent deflections as compared to the southwestern part. It is also observed that the quantum of deflection is more in the NE side basin as compared to the SW side basins within the study area. The above observation may indicate that the NW-SE trending lineament L1 and L2 are responsible for inducing the variation in the deflection of the drainages.

**Mountain Front Sinuosity (Smf)**

The value of this ratio generally ranges between 1 and 5. Bull (1977; 2007); Keller and Pinter (2002); Pérez-Peña et al. (2010); Silva et al. (2003) have attempted to classify the values of the ratio in relation to the tectonic activity (Table-2). In the present study, this ratio has been utilized to evaluate the ongoing tectonic activity. The ratio has been calculated along the mountain fronts in the existing central and southwestern part of the study area. For the assessment of the status of ongoing tectonic activity, 6 locations have been selected for the calculation of the mountain front sinuosity ratio (Map-9). The Mountain Front Smf index values have been calculated by digitizing the contours along the mountain fronts using Arc-GIS.

**Valley Floor Width -Valley Height Ratio (Vf)**

Valley floor width - valley height ratio (Vf) is another geomorphic index developed by Bull and McFadden (1977) for the quantitative assessment of tectonically influenced geomorphic features. As Smf, Vf have also shown their effectiveness in assessing the tectonic activity of mountain fronts generated by faults (e.g., SW USA (Bull and

McFadden, 1977; Rockwell et al., 1984), Costa Rica (Wells et al., 1988), Oregon Coast Range, USA (Rhea, 1993), the Kachchh region), southeast Spain (Silva et al., 2003), western Taiwan (Chen et al., 2003), North western Himalaya (Malik et al., 2007). The ratio of the width of the valley floor to valley height, Vf, is calculated. The smaller value of the ratio indicates the V-shaped valley, while the higher values indicate the broad floor U-shaped valley. In the present study, Vf is calculated at 18 locations across different reaches of the drainage system (Map-7).

The spatial distribution of the location at which the ratio has been calculated indicates that the locations are falling in the NW- SE trending valley along the L1 lineament. Over the entire ratio values vary between 0.51 and 2.31. As per the classification given in the literature reviewer searchers has categorized that the value of the ratio less than1.0 indicate that the tectonic adjustments are continuing. Values more than 1 to 5 indicate the tectonic activity is continuing, but at a lower pace. As per the value classification, 9 locations within the study area fall within the category of tectonic adjustments that are continuing. The Remaining 9 locations are falling in the category of tectonic activity continuing, but at a lower pace. Spatial distributions of the first category indicate that lower values are observed around the junction of the L1 and L4 lineament. A similar scenario is also observed around the junction of the L3 and L1 lineament. Lower values are also observed along the NE-SW trending NW lineament. The above observation shows that the place where the NE trending lineaments cross the NW-SE trending L1, values of the ratio are observed to be very low. It may indicate that the ongoing adjustments are continuing around the junctions of NE and NW trending lineaments.

## RESULT AND DISCUSSION

In the present study, the quantitative and qualitative analyses of various geomorphic indices have been carried out. It is observed that anomalous asymmetry factor values are located in the vicinity of the NW-SE trending L1 lineament. Consistent drainage deflection towards NW is seen along the L1 lineament. Anomalous values of Mountain Front Sinuosity and River Sinuosity are only located around the crossing of the NW and NE trending lineaments. Similarly, the low values of valley floor ratio are also located around the crossing of the lineaments. The present study was to learn the methodology and its application in the evaluation of individual lineaments. Though the study area was very small, it may indicate the ongoing adjustments at the locations where the lineaments are crossing each other within the study area, as this area had experienced a historical earthquake of Mag~5.

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**Conflict of interest**

There are no conflicts of interest among the authors.

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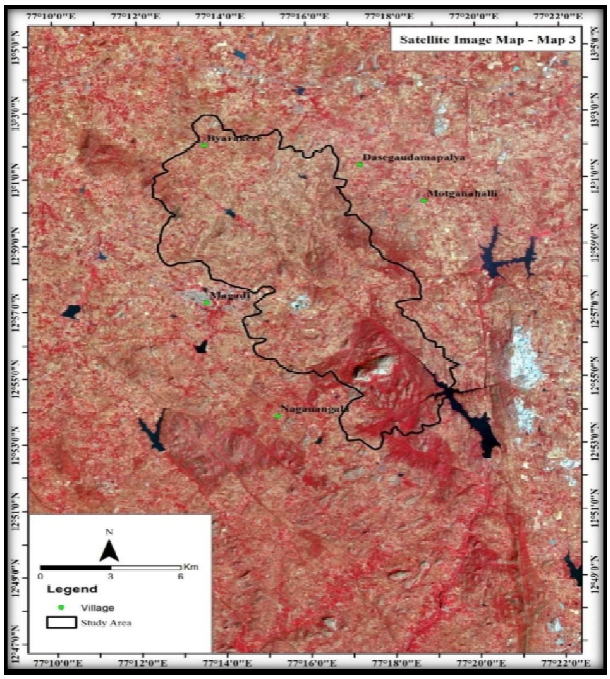
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**Table -1: Details of lineament identified in the study area:**

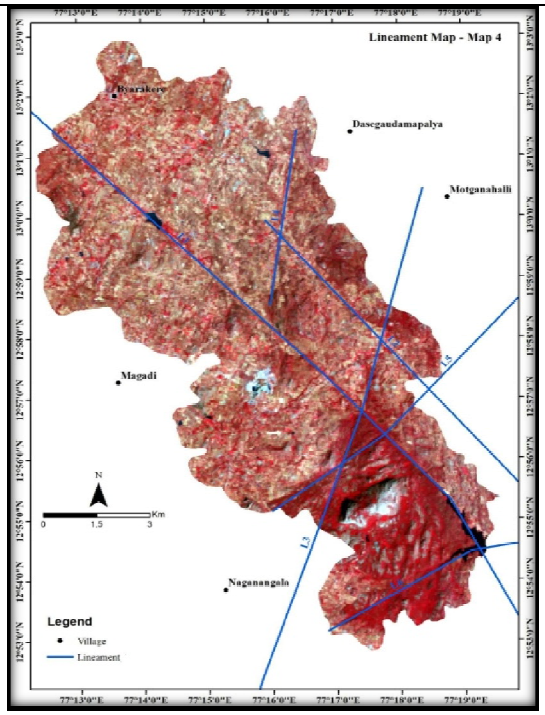
| Sl.No | Trend | Length |
|---|---|---|
| 1 | NW-SE | 33.4 Km |
| 2 | NE-SW | 26.6 Km |
| 3 | NW-SE | 5.3 Km |
| 4 | NE-SW | 11.13 Km |
| 5 | ENE-WSW | 6.5 Km |

**Table - 2: Classification for Values for Mountain Front Sinuosity**

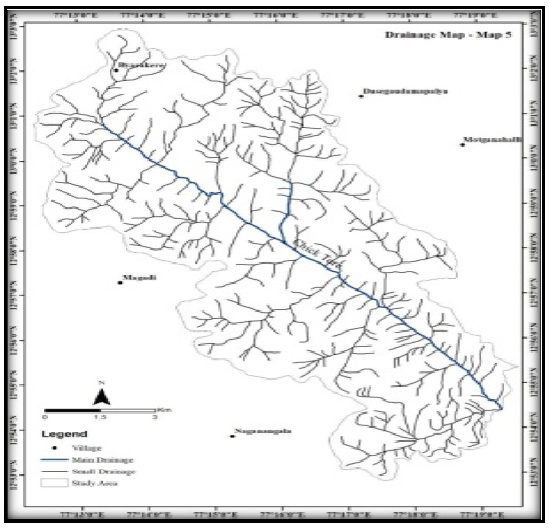
| Sl. No | Range | Status |
|---|---|---|
| 1 | 1-1.4 | Tectonically Active |
| 2 | 1.4-3 | Less Activity |
| 3 | 3-5 | Inactive |



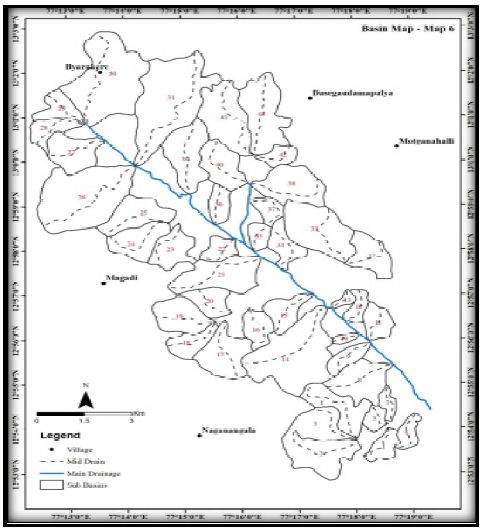

**Map 1: Satellite image map of the study area**



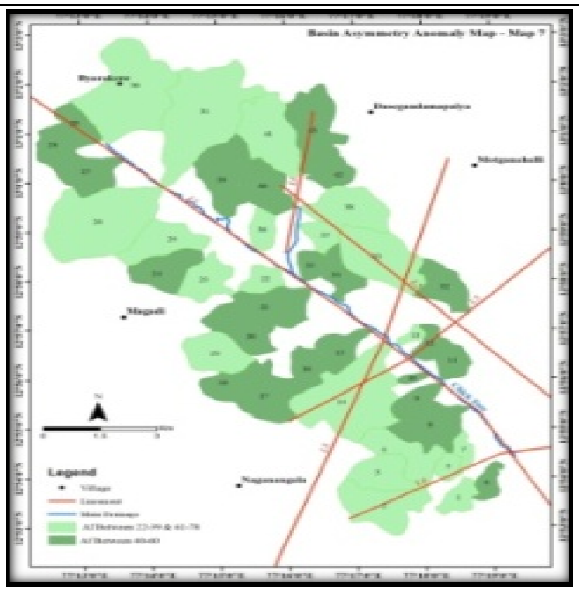

**Map 2: Lineament Map of the study area**



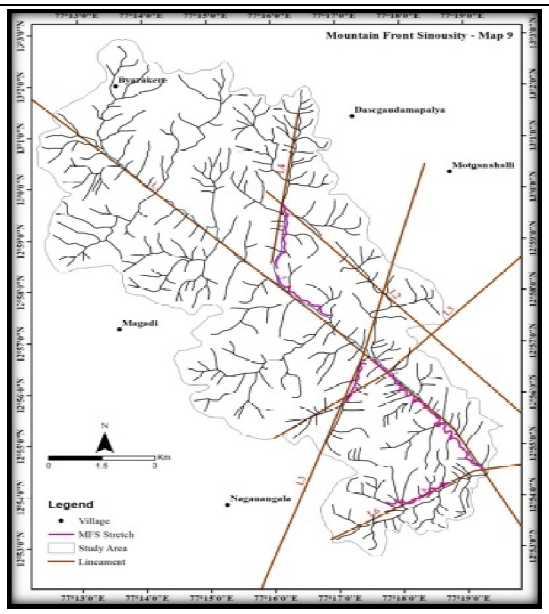

**Map 3: Drainage map of the study area**



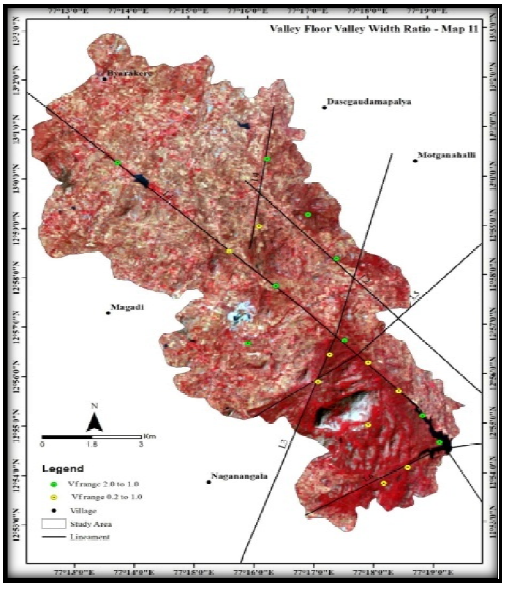

**Map 4: Basin map of the study area.**




**Map 5: Basin Asymmetry anomaly map of the study area**




**Map 6: Mountain front sinuosity measured at different stretches in the study area**




**Map 7: Location map at which the valley floor valley width ratio is calculated within the study area**

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RESEARCH ARTICLE

# A Comparative Analysis on the Molecular Characterization of Wild and Micropropagated *Cleome viscosa* L. Using RAPD and ISSR Technique

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## ABSTRACT

*Cleome viscosa* L., a medicinally vital plant threatened by overharvesting and habitat loss, necessitates sustainable propagation strategies like micropropagation. However, genetic fidelity in micropropagated plants remains a concern due to soma clonal variations. This study evaluates the genetic stability of *in vitro*-raised *C. viscosa* compared to wild populations using RAPD and ISSR markers. Fifteen RAPD and three ISSR primers generated 180 amplification bands, revealing 88.33% polymorphism overall. ISSR markers exhibited higher discriminatory power (91.43% polymorphism) than RAPD (87.58%), with primers ISSR 1 and OPA 02 showing 100% polymorphism. UPGMA cluster analysis segregated wild and micropropagated plants into distinct clades, which attributed 85% of genetic variation. The high divergence underscores significant soma clonal variations during micropropagation, potentially impacting the plant's medicinal efficacy. While ISSR markers proved superior for detecting genetic instability, both techniques are effective for fidelity assessment. These findings emphasize the need to optimize *in vitro* protocols and implement routine molecular screening to preserve genetic integrity. This study provides a framework for ensuring the conservation and sustainable utilization of *C. viscosa*, balancing mass propagation with the retention of bioactive compound synthesis critical for its pharmacological applications.

**Keywords:** *Cleome viscosa*, Micropropagation, Genetic Fidelity, RAPD, ISSR, Soma Clonal Variation.

## INTRODUCTION

*Cleome viscosa* L., commonly known as "Asian spider flower" or "wild mustard," is a medicinally significant annual herb belonging to the family Cleomaceae. It is widely distributed in tropical and subtropical regions and is traditionally used for its anti-inflammatory, antimicrobial, and antipyretic properties (Upadhyay*et al*., 2011).Despite its ecological and pharmacological importance, overharvesting and habitat destruction have threatened its natural populations, necessitating sustainable conservation strategies such as micropropagation(Patel *et al*., 2021). Micropropagation offers a rapid and scalable method to produce genetically identical plants, but concerns about genetic fidelity due to soma clonal variations during *in vitro* culture persist(Bairu*et al*., 2011). Ensuring genetic stability in micropropagated plants is critical for maintaining their medicinal efficacy and ecological adaptability. Molecular markers such as Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) are robust tools for assessing genetic fidelity and diversity(Yu and Nguyen, 1994 and Julie *et al*., 2016 ). These techniques are cost-effective, require no prior genomic information, and efficiently detect polymorphisms caused by soma clonal variations or environmental stresses(Joshi *et al*., 2020). While RAPD markers amplify random genomic regions using short primers, ISSR targets microsatellite regions, providing higher reproducibility and resolution (Reddy *et al*., 2002). Previous studies have employed these markers to validate clonal fidelity in micropropagated plants like *Bacopamonnieri* (*Tiwari et al., 2014*) and *Withania somnifera*(Ahmad *et al*., 2016), but limited research exists on *Cleome viscosa*. This study presents the first comparative molecular analysis of wild and micropropagated *Cleome viscosa* using RAPD and ISSR markers. The objectives are to evaluate genetic stability in micropropagated plants relative to their wild counterparts, identify potential soma clonal variations, and validate the reliability of micropropagation protocols for conservation and commercial cultivation. The findings will contribute to optimizing *in vitro* regeneration systems for endangered medicinal plants and ensuring their genetic integrity for sustainable utilization.

## MATERIALS AND METHODS

**Plant Material Collection**

**Wild Samples**: Healthy mature plants of *Cleome viscosa* were collected from their natural habitat in Sirumailur village (The latitude 13.0938995 and longitude 80.292356 are the geocoordinate of the Sirumailur), Chengalpattu district, Tamil Nadu (n = 20). Voucher specimens were deposited at Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India with specimen reference number - BSI/SRC/5/23/2016/Tech/1195.

**Micropropagated Samples**: *In vitro*-raised plants were obtained from a previously established micropropagation protocol (Basha*et al*., 2022). Plants regenerated via nodal explants after 6–8 subculture cycles were used for analysis (n = 20).

**Micropropagation Protocol**

Nodal explants 5mm in size were surface-sterilized with 0.1% $HgCl_2$ (3 min) and cultured on MS (Murashige and Skoog, 1962) basal medium supplemented with 3% sucrose, 0.8% agar and different concentration of PGRs such as 6-Benzylaminopurine (BAP), Kinetin (KIN), Naphthalene-3-acetic acid (NAA) (Kolar*et al.,* 2021). The inoculated cultures were maintained in growth chamber. Data was recorded after 28 days (Archana*et al*., 2013).

**DNA Extraction**

Wild and micropropagated plant samples were frozen in liquid nitrogen and grounded to fine powder. 1g of freeze-dried powder was used to extract plant DNA. The mini extraction was done based on the cetyltrimethyl ammonium bromide (CTAB) method (Voigt *et al*., 1999). The finely grounded plant materials were transferred to sterile centrifuge tube. Pre-warmed at 65ºC. DNA extraction buffer [100 mMTrisHCl (pH 8.0), 1.4M NaCl, 50mM EDTA (pH 8.0) and 2% CTAB] was added to this, mixed well and incubated in a water bath at 65ºC with gentle shaking for one hour. After incubation an equal volume of chloroform/isoamyl alcohol (24:1 v/v) was added gently to denature

proteins and centrifuged at 10000 rpm at 25ºC for 10 min. The aqueous phase was transferred to a new sterile tube and DNA was precipitated with 0.6 volume of cold isopropanol and 0.1 volume of 3M sodium acetate (pH 5.2) and centrifuged at 15000 rpm for 25 min at 25ºC. The supernatant was poured off and the pellet was washed twice with 70% cold ethanol and dried at room temperature. The pellets were air dried and suspended on TE buffer (pH 8.0).

**Purification of Genomic DNA**

The extraction of total genomic DNA from the *C. viscosa* isolates as per the above procedure was followed by RNase treatment. Genomic DNA was resuspended in 100µl of TE buffer with 2µl of RNase and incubated at 37ºC for one hour. After incubation the sample was re-extracted with phenol: chloroform: isoamylalcohol (25:24:1) solution. RNA free DNA was precipitated with 0.1M sodium acetate solution along with chilled 100% ethanol and centrifuged at 12000 rpm for 15min at 40ºC, washed with 70% ethanol, air dried and dissolved in TE buffer. The quality and quantity of DNA was analyzed spectrophotometrically and in 1% agarose gel.

**Molecular Marker Analysis**

**RAPD analysis**: Fifteen random primers (Operon Technologies) namely OPA 01 – 06, OPA 10, OPA 19, OPA 20, OPC 01, OPE 05 - 07, OPAM 01, OPS 18 and three were used for the analysis. PCR conditions: 32 cycles of 95°C (5 min), 36°C (30 sec), 72°C (1 min), and final extension at 72°C (8 min)(Chandrika and Ravishankar, 2010).

**ISSR**: Three primers ISSR primers namely ISSR 1, ISSR 2, ISSR 3 targeting di/tri-nucleotide repeats were selected.PCR conditions: 32 cycles of 95°C (5 min), 36°C (30 sec), 72°C (1 min), and final extension at 72°C (8 min)(Pathak and Dhawan, 2012). The PCR products of both RAPD and ISSR reaction were resolved on 1.5% agarose gel in 1× TAE buffer stained with ethidium bromide (8µl/100ml) and electrophoresis was carried out at 75 volts for 1.5 hours and visualized under UV-Transilluminator.

**Data Analysis**

The image of the gel electrophoresis was documented through Bio-Profile Bio-1D gel documentation system. The polymorphic bands were scored and analyzed by UPGMA cluster analysis protocol and computed *in silico* into similarity matrix using NTSYSpc (Numerical Taxonomy and Multivariate Analysis System genome arranged in tandem repeats with each Biostastistics, version 2.11W). The SIMQUAL (similarity for qualitative data) program was used to calculate the Jaccard coefficients. The RAPD patterns of each isolate was evaluated, transcribed spacer regions have been used, assigning character state "1" to indicate the presence of band in the gel and "0" for its absence in the gel. A data matrix was formed which was used to calculate the Jaccard similarity coefficient for each pair wise comparison. Jaccard coefficients were clustered to produce dendrogram using the (Sequential, Hierarchical, Agglomerative, and Nested cluster methods) SHAN clustering program and selecting the un-weighted pair-group methods with arithmetic average (UPGMA) algorithm in NTSYSpc.

## RESULT

DNA was isolated from the wild and *in vitro* cultured plants designated as number CVI (*C. viscosa* wild plant) and CVII (*C. viscosa* micropropagatedplant). The genomic DNA isolated through CTAB protocol produced clear sharp bands, indicating high-quality DNA. The purity of DNA was calculated by the absorbance ratio of A260/A280nm (Table - 1). The assessment of the genetic stability of wild and micropropagated plants of *C. viscosa* wild plant (CVI), *C. viscosa* micropropagated plant (CVII) were confirmed by RAPD and ISSR analyses. The *in vitro* plants were found to be phenotypically normal and essentially identical with their mother plant at hardening stage which partly suggests the minimal of soma clonal variations. Table - 1: Yield and purity of DNA isolated from the wild and micropropagated plants of Cleome species

**RAPD Primer Analysis**

Fifteen RAPD primers generated 145 amplification bands, with 127 polymorphic (87.58%) and 18 monomorphic bands (Table -2). RAPD banding pattern obtained from wild and micropropagated plants of *C. viscose* using some of the primers are given in figure – 1**.** Primers such as OPA 02, OPA 10, and OPE 05 exhibited 100% polymorphism, while OPC 01 showed the lowest polymorphism (57.14%).

**Figure - 1: RAPD banding pattern obtained from wild and micropropagated plants of *C. viscosa***

**M** - Molecular DNA Ladder(1.5kbp)
**CVI** - Mother plant of *C. viscosa*
**CVII** - Micropropagated plant of *C. viscosa*

**Table - 2: Total number of amplified fragments and number of polymorphic fragments generated by PCR using selected RAPD primers**

**ISSR Primer Analysis**

Three ISSR primers amplified 35 bands, of which 32 (91.43%) were polymorphic (Table - 3). ISSR banding pattern obtained from wild and micropropagated plants of *C. viscose* using some of the primers are given in figure – 2. ISSR 1 demonstrated 100% polymorphism, while ISSR 2 and ISSR 3 showed 88.24% and 90% polymorphism, respectively. The average polymorphism for ISSR markers (92.74%) exceeded that of RAPD primers (87.10%), reflecting ISSR's higher discriminatory power.

**Figure - 2: ISSR banding pattern obtained from wild and micropropagated plants of *C. viscosa***

**M** - Molecular DNA Ladder(1.5kbp)
**CVI** - Mother plant of *C. viscosa*
**CVII** - Micropropagated plant of *C. viscosa*

Total number of amplified fragments and number of Polymorphic fragments generated by PCR using selected RAPD primers (Table – 3). Combined analysis of RAPD and ISSR markers produced 180 bands (159 polymorphic), with an overall polymorphism of 88.33%. ISSR primers generated higher average polymorphism (92.74%) compared to RAPD (87.10%), aligning with their ability to target hypervariable microsatellite regions. The similarity coefficient values range from 69% to 82% *C. viscosa*. These similarity coefficients were used to generate a tree from cluster analysis using UPGMA method (Figure - 3). The cluster analysis indicates that *C. viscosa* are grouped into two major clusters based on similarity indices. These plants could be identified by using four random 15-mer primers. Among the cluster showed the highest similarity index (82%). On the basis of the RAPD analysis, the chosen samples have been grouped under clusters. The present study showed that the maximum similarity indices and least variation were observed among the wild and micropropagated plant of *C. viscosa*. Figure - 3: Dendrogram showing coefficient similarities between wild and micropropagated of *C. viscosa* using UPGMA cluster analysis (NTSYS) of the RAPD profile.

## DISCUSSION

The present study provides the first comparative molecular assessment of wild and micropropagated Cleome viscosa using RAPD and ISSR markers, revealing significant genetic divergence between the two populations. The high polymorphism (87.58% for RAPD and 91.43% for ISSR) underscores the sensitivity of these markers in detecting somaclonal variations, which are common in micropropagated plants due to hormonal imbalances, oxidative stress, or epigenetic changes during *in vitro* culture (Bairu*et al.,* 2011). Notably, ISSR primers exhibited higher discriminatory power (average polymorphism: 92.74%) compared to RAPD (87.10%), likely due to their ability to target hypervariable microsatellite regions, which are more prone to replication errors and recombination events (Reddy *et al*., 2002). The substantial genetic divergence observed between wild and micropropagated plants (85% variation

between groups) aligns with studies on other medicinal species, such as *Withania somnifera*(Ahmad *et al*., 2016) and *Bacopa monnieri*(Tiwari *et al.,* 2014), where micropropagation-induced variations were linked to altered secondary metabolite profiles. Primers like ISSR 1 (100% polymorphism) and OPA 02 (100% polymorphism) proved particularly effective in identifying these variations, suggesting their utility in routine screening of *in vitro*-derived plants. However, the lower polymorphism of OPC 01 (57.14%) highlights the importance of primer selection for species-specific fidelity assessments, as primer binding efficiency can vary with genomic GC content and repeat motifs (Joshi *et al.,* 2020). While micropropagation offers a viable solution for mass propagation of threatened species like *C. viscosa*, the observed genetic instability poses challenges for maintaining medicinal efficacy. For instance, soma clonal variations could alter the synthesis of bioactive compounds such as cleomiscosins and flavonoids, which are critical for its anti-inflammatory and antimicrobial properties (Upadhyay*et al*., 2011). To mitigate this, our findings suggest that reducing subculture cycles and incorporating antioxidants (e.g., ascorbic acid) to minimize oxidative DNA damage (Patel *et al*., 2021)and routine Genetic Monitoring using ISSR markers (e.g., ISSR 1) for early detection of off-type plants.

## CONCLUSION

This study demonstrates that micropropagated *C. viscosa* exhibits significant genetic divergence from wild counterparts, primarily due to soma clonal variations. While ISSR markers proved more efficient than RAPD in detecting these variations, both techniques are valuable for ensuring genetic fidelity in conservation programs. By optimizing *in vitro* protocols and implementing routine molecular screening, the medicinal integrity and ecological adaptability of *C. viscosa* can be preserved for sustainable utilization.

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**Table 1: Yield and purity of DNA isolated from the wild and micropropagated plants of Cleome species**

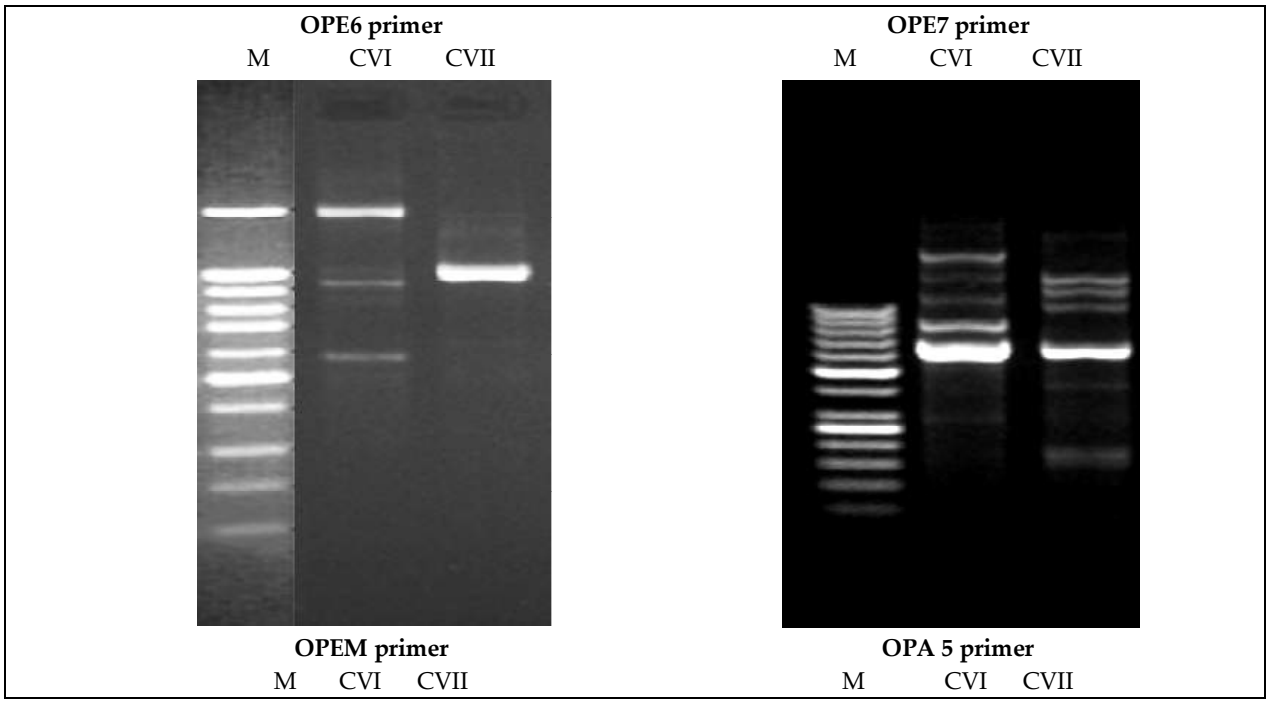
| S. No | Sample ID | DNA conc. (ng/µl) | OD value at *A*260/*A*280 |
|---|---|---|---|
| 1 | *C. viscosa*(wild) | 605.6 | 2.07 |
| 2 | *C. viscosa*(micropropagated) | 586.2 | 1.91 |

**Table 2: Total number of amplified fragments and number of polymorphic fragments generated by PCR using selected RAPD primers**

| Primers | Sequences (5′ - 3′) | Polymorphic bands | Monomorphic bands | Total bands | Percentage of polymorphism |
|---|---|---|---|---|---|
| OPA 01 | CAGGCCCTTC | 8 | 1 | 9 | 88.89 |
| OPA 02 | TGCCGAGCTG | 11 | 0 | 11 | 100 |
| OPA 03 | AGTCAGCCAC | 9 | 1 | 10 | 90 |
| OPA 04 | AATCGGGCTG | 11 | 2 | 13 | 84.62 |
| OPA 05 | AGGGGTCTTG | 6 | 3 | 9 | 66.67 |
| OPA 06 | GGTCCCTGAC | 5 | 1 | 6 | 83.33 |
| OPA 10 | GTGATCGCAG | 10 | 0 | 10 | 100 |
| OPA 19 | CAAACGTCGG | 5 | 0 | 5 | 100 |
| OPA 20 | GTTGCGATCC | 7 | 1 | 8 | 87.5 |
| OPC 01 | TTCGAGCCAG | 4 | 3 | 7 | 57.14 |
| OPE 05 | TCAGGGAGGT | 9 | 0 | 9 | 100 |
| OPE 06 | AAGACCCCTC | 10 | 1 | 11 | 90.91 |
| OPE 07 | AGATGCAGCC | 14 | 2 | 16 | 87.5 |
| OPAM 01 | TCACGTACGG | 13 | 2 | 15 | 86.67 |
| OPS 18 | GGGGAAATGG | 5 | 1 | 6 | 83.33 |
| **Total** | | 127 | 18 | 145 | 1306.56 |
| **Average** | | 8.47 | 1.2 | 9.67 | 87.10 |

**Table 3: Total number of amplified fragments and number of Polymorphic fragments generated by PCR using selected RAPD primers**

| Primers | Sequences (5′ - 3′) | Polymorphic bands | Monomorphic bands | Total bands | Percentage of polymorphism |
|---|---|---|---|---|---|
| ISSR 1 | GAG AGA GAG AGA GAG AAC | 8 | 0 | 8 | 100 |
| ISSR 2 | AGA GAG AGA GAG AGA GAT | 15 | 2 | 17 | 88.24 |
| ISSR 3 | YCT GTG TGT GTG TGT GT | 9 | 1 | 10 | 90 |
| **Total** | | 32 | 3 | 35 | 278.24 |
| **Average** | | 10.67 | 2 | 11.66 | 92.74 |



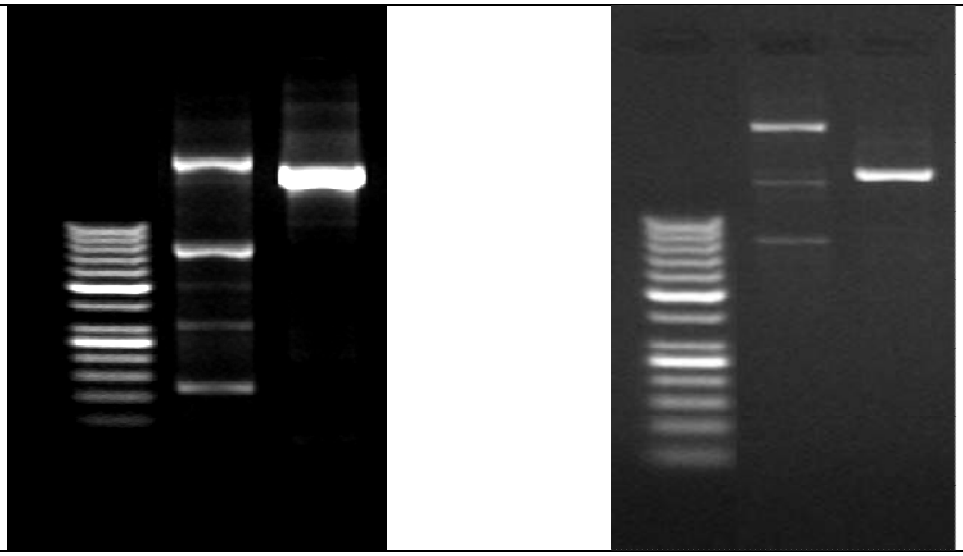


**Figure - 1: RAPD banding pattern obtained from wild and micropropagated plants of *C. viscose***

**M** - **Molecular DNA Ladder(1.5kbp)**

**CVI** - **Mother plant of *C. viscosa***

**CVII** - **Micropropagated plant of *C. viscosa***

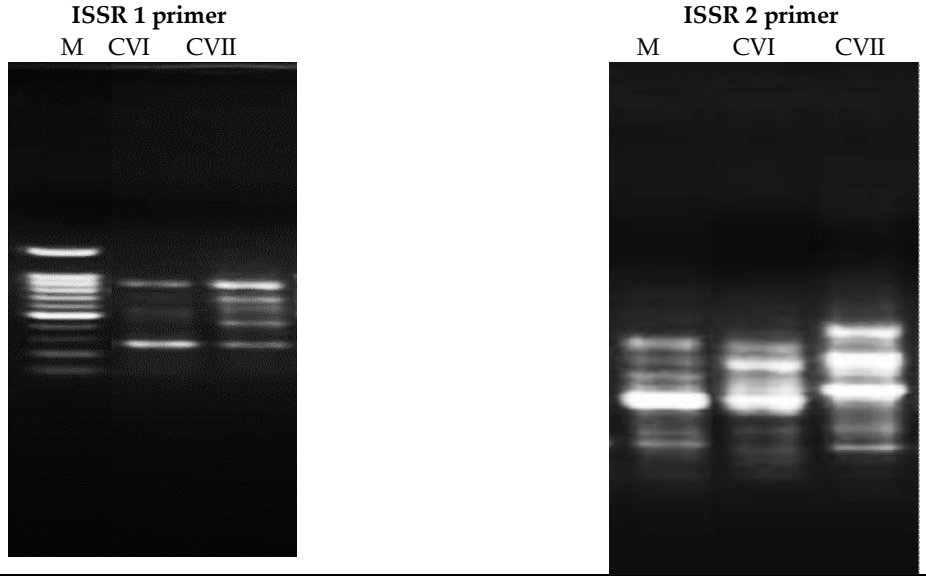



**Figure - 2: ISSR banding pattern obtained from wild and micropropagated plants of *C. viscosa***

**M** - Molecular DNA Ladder(1.5kbp)

**CVI** - Mother plant of *C. viscosa*

**CVII** - Micropropagated plant of *C. viscosa*

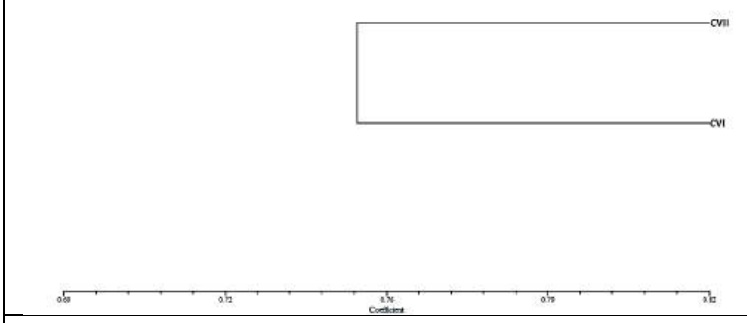



**Figure - 3: Dendrogram showing coefficient similarities between wild and micropropagated of *C. viscosa* using UPGMA cluster analysis (NTSYS) of the RAPD profile.**

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RESEARCH ARTICLE

# Digital Technologies and their Impact in Crime Prevention and Criminal Justice in India

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## ABSTRACT

Digital technologies have significantly transformed crime prevention and the criminal justice system in India. The rapid growth of information and communication technology (ICT), artificial intelligence (AI), big data analytics, and digital databases has improved the efficiency, transparency, and coordination of law-enforcement agencies and judicial institutions. The Indian government has implemented several digital initiatives such as the Crime and Criminal Tracking Network and Systems (CCTNS), the Interoperable Criminal Justice System (ICJS), and the e-Courts Mission Mode Project to modernize the criminal justice system. These initiatives aim to integrate police, courts, prisons, prosecution, and forensic institutions through digital platforms. CCTNS connects police stations across India and maintains a centralized database of criminal records, enabling faster investigation and information sharing. ICJS integrates various components of the justice system to facilitate seamless data exchange and improve coordination among agencies. Similarly, the e-Courts project focuses on digitizing court processes, enabling e-filing, virtual hearings, and digital access to case information. Digital technologies such as surveillance systems, biometric identification, digital forensics, and predictive analytics also assist law-enforcement agencies in detecting and preventing crimes. Despite these benefits, challenges such as cybersecurity threats, privacy concerns, digital divide, and lack of technical capacity remain significant obstacles. This study adopts a descriptive research methodology and relies primarily on secondary sources of data, including government reports, policy documents, academic journals, and official publications, to analyze the role and impact of digital technologies in India's criminal justice system.This study examines the role of digital technologies in crime prevention and criminal justice in India, highlighting their advantages, institutional impact, and emerging challenges. The paper concludes that while digital technologies have enhanced efficiency and transparency in the criminal justice system,

strong regulatory frameworks and technological safeguards are necessary to ensure ethical and effective use.

**Keywords:** Digital Technologies, Criminal Justice System, Government, Digital Initiatives, Transparency,

## INTRODUCTION

The rapid advancement of digital technologies has significantly transformed governance, administration, and law enforcement across the world. In India, the integration of information and communication technology (ICT), artificial intelligence, big data analytics, and digital databases has played a crucial role in strengthening crime prevention and improving the efficiency of the criminal justice system. Digital tools have enabled law-enforcement agencies and judicial institutions to enhance transparency, coordination, and accountability in addressing criminal activities and delivering justice. Traditionally, the criminal justice system in India has faced several challenges, including delays in investigation and trial, poor coordination among institutions, lack of reliable data, and manual record-keeping. These issues often affected the effectiveness of crime prevention and the timely delivery of justice. With the emergence of digital technologies, the government has introduced various technological initiatives aimed at modernizing policing, improving investigative processes, and digitizing judicial administration. Several digital platforms have been developed to strengthen the criminal justice system in India. For example, the Crime and Criminal Tracking Network and Systems (CCTNS) connects police stations across the country and provides a centralized database of criminal records to facilitate faster investigations and information sharing. Similarly, the Interoperable Criminal Justice System (ICJS) integrates different components of the criminal justice system—such as police, courts, prisons, prosecution, and forensic laboratories—into a unified digital framework. In addition, the e-Courts Mission Mode Project aims to digitize court processes, promote e-filing, and enable virtual hearings, thereby improving access to justice and reducing delays in the judicial process. Digital technologies such as CCTV surveillance, biometric identification, digital forensics, and artificial intelligence have also strengthened the capacity of law-enforcement agencies to detect, prevent, and investigate crimes more effectively. These technologies facilitate data-driven policing and enable authorities to analyze crime patterns, identify suspects, and respond quickly to criminal activities. The increasing use of digital technologies in law enforcement and criminal justice also raises several concerns, including issues related to data privacy, cybersecurity, surveillance ethics, and technological disparities. Therefore, it is important to critically examine both the opportunities and challenges associated with digital transformation in the criminal justice system. The present study examines the role of digital technologies in crime prevention and criminal justice in India. It focuses on the institutional impact of digital initiatives, their contribution to improving efficiency and transparency, and the challenges involved in their implementation.

## OBJECTIVES

1. To examine the role of digital technologies in crime prevention in India.
2. To analyse the impact of digital platforms on the criminal justice system.
3. To identify the challenges and limitations in the use of digital technologies in law enforcement.

## RESEARCH METHODOLOGY

This study is based on a descriptive and analytical research methodology. The research primarily relies on secondary sources of data such as government reports, academic articles, policy documents, and official websites. Information regarding digital initiatives like CCTNS, ICJS, and the e-Courts project has been collected from government publications and scholarly literature. The study analyses these sources to understand the impact of digital technologies on crime prevention and criminal justice in India.

**Sources of Data**

The present study is based on secondary sources of data. Relevant information has been collected from various published and official sources related to digital technologies, crime prevention, and the criminal justice system in India. These sources help in understanding the policies, initiatives, and institutional developments in the field of digital governance and law enforcement.

**The major sources of data used in this study include**

**Government Reports and Official Documents**

Reports and publications from government institutions such as the Ministry of Home Affairs and the Department of Justice have been used to analyse major initiatives like the Crime and Criminal Tracking Network and Systems, the Interoperable Criminal Justice System, and the e-Courts Mission Mode Project.

**Academic Books and Journal Articles**

Scholarly books, peer-reviewed journals, and research papers related to digital policing, cybercrime, and criminal justice reforms have been consulted to understand the theoretical and analytical perspectives of the topic.

**Policy Documents and Government Websites**

Official websites of government departments, policy papers, and digital governance reports have been used to obtain updated information on digital initiatives in the criminal justice system.

**Reports from Research Institutions and Organizations**

Publications from research institutions, think tanks, and international organizations related to digital governance and justice administration have also been used. These sources provide reliable and comprehensive information for analysing the impact of digital technologies on crime prevention and the criminal justice system in India.

**Digital Technologies in Crime Prevention**

Digital technologies have become an important tool in strengthening crime prevention strategies in India. The use of modern technologies such as surveillance systems, biometric identification, data analytics, and digital databases has enhanced the capacity of law-enforcement agencies to monitor criminal activities, identify suspects, and respond to threats more efficiently. These technologies support proactive policing by enabling authorities to detect potential crimes before they occur and by improving the speed and accuracy of investigations. One of the most widely used technologies in crime prevention is digital surveillance. Closed-Circuit Television (CCTV) cameras installed in public places, transportation hubs, and urban areas help monitor activities and deter criminal behaviour. Surveillance footage often serves as crucial evidence in criminal investigations and assists police in identifying offenders. In several Indian cities, smart surveillance systems equipped with facial recognition technology and automated monitoring tools are being used to track suspicious movements and improve public safety. Significant technological initiative is the Crime and Criminal Tracking Network and Systems (CCTNS), launched by the Government of India under the Ministry of Home Affairs. This system connects thousands of police stations across the country through a centralized digital network. It enables the creation of a comprehensive database containing information on crimes, criminals, and investigations. Police officers can access this database to verify criminal records, track offenders, and share information with other law-enforcement agencies, thereby improving coordination and efficiency in crime prevention. Biometric technologies such as fingerprint identification, iris scanning, and facial recognition also play a significant role in modern policing. These technologies help accurately identify individuals involved in criminal activities and prevent identity fraud. The integration of biometric systems with national identification databases strengthens verification processes and supports investigative work. Digital technologies are also widely used in crime data analysis and predictive policing. Data analytics tools analyse large volumes of crime data to identify patterns, trends, and high-risk locations. By studying these patterns, police departments can allocate resources more effectively and implement preventive measures in areas with higher crime rates. Artificial intelligence and machine learning algorithms further assist in predicting potential criminal activity and improving strategic planning. Crucial development is the use of digital forensics in criminal investigations. With the increasing use of smartphones,

computers, and online platforms, digital evidence has become essential in solving crimes. Digital forensics involves the collection and analysis of electronic data such as emails, call records, social media communications, and computer files. This technology helps investigators trace cybercriminals, uncover digital footprints, and present reliable evidence in court. Supporting law enforcement, digital technologies also facilitate citizen participation in crime prevention. Many state police departments in India provide mobile applications and online portals that allow citizens to report crimes, submit complaints, and track the status of their cases. These platforms strengthen communication between the public and the police, contributing to a more responsive and transparent policing system. Digital technologies have significantly enhanced crime prevention mechanisms in India by improving surveillance, data management, and investigative capabilities. However, the effective use of these technologies requires proper infrastructure, technical training for law-enforcement personnel, and appropriate legal safeguards to protect citizens' rights and privacy.

**Digital Transformation of the Criminal Justice System**

The adoption of digital technologies has significantly transformed the functioning of the criminal justice system in India. Traditionally, the system relied heavily on manual record-keeping and physical documentation, which often resulted in delays, lack of coordination among institutions, and limited accessibility to information. With the advancement of information and communication technology (ICT), the Government of India has introduced several digital initiatives to improve efficiency, transparency, and coordination within the justice delivery system. Digital platforms now enable seamless information sharing among various institutions involved in criminal justice, including police, courts, prisons, prosecution, and forensic departments. These technological developments not only enhance administrative efficiency but also improve the speed and accuracy of investigations, trials, and judicial processes. Important initiatives such as the Interoperable Criminal Justice System (ICJS) and the e-Courts Mission Mode Project represent major steps toward the digital transformation of India's criminal justice system.

**Interoperable Criminal Justice System (ICJS)**

The Interoperable Criminal Justice System (ICJS) is an important initiative of the Government of India designed to integrate the various components of the criminal justice system through a unified digital platform. The system connects five major pillars of the criminal justice system: police, courts, prisons, prosecution, and forensic laboratories. The primary objective of ICJS is to facilitate seamless exchange of information among these institutions. Through digital integration, case-related data can be accessed and shared across different departments, enabling faster investigation and efficient case management. For example, police officers can access court orders, prosecutors can review investigation records, and prison authorities can receive information about court proceedings electronically. ICJS operates on the principle of "one data, one entry," which reduces duplication of records and improves the accuracy and reliability of information. By connecting multiple justice institutions, the system helps reduce delays in criminal investigations and judicial processes. It also enhances coordination among different agencies, leading to more effective law enforcement and justice delivery.

**e-Courts Mission Mode Project**

The e-Courts Mission Mode Project is another major initiative aimed at modernizing the Indian judiciary through digital technology. Launched under the National e-Governance Plan, the project focuses on digitizing court processes and improving access to justice. The e-Courts project introduces several digital services, including electronic filing of cases (e-filing), digital documentation, online case status tracking, and virtual court hearings. These services allow litigants, lawyers, and the public to access court information easily through online platforms. One of the significant achievements of the project is the digitization of court records and the availability of judgments and orders online. This improves transparency in the judicial system and enables individuals to obtain legal information without visiting courts physically. Additionally, the use of video conferencing technology has facilitated remote hearings and reduced delays in court proceedings. The e-Courts project also supports the development of paperless courts and improves administrative efficiency by automating case management processes. By reducing paperwork and improving information access, the initiative contributes to faster and more transparent justice delivery.

**Digital Policing and Citizen Services**

Digital technologies have also transformed policing and improved the relationship between law-enforcement agencies and citizens. Many police departments in India have adopted digital platforms that allow citizens to access police services online. Through online portals and mobile applications, citizens can file complaints, register First Information Reports (FIRs), track the status of cases, and request police verification services. These digital services enhance accessibility and make the process of reporting crimes more convenient for the public. An important initiative in this area is the Crime and Criminal Tracking Network and Systems (CCTNS), which provides a centralized digital platform for crime data and police records. The system connects thousands of police stations across India and allows law-enforcement agencies to access criminal databases and investigation records quickly. Digital policing also includes the use of mobile technologies, surveillance systems, and online communication tools to improve public safety. Many state police departments use social media platforms and mobile applications to communicate with citizens, provide safety alerts, and raise awareness about crime prevention. Overall, digital policing initiatives enhance transparency, strengthen public trust in law enforcement, and encourage greater citizen participation in maintaining law and order. These developments represent an important step toward creating a more responsive and technology-driven criminal justice system in India.

**Advantages of Digital Technologies**

The integration of digital technologies into the criminal justice system has brought several significant advantages in terms of efficiency, transparency, and effectiveness. These technologies have improved the functioning of law-enforcement agencies and judicial institutions by enabling faster information sharing, better data management, and more reliable investigative processes. Digital transformation has also enhanced public access to justice and strengthened coordination among various institutions involved in crime prevention and justice delivery.

**Improved Efficiency and Speed**

One of the major advantages of digital technologies is the improvement in the efficiency and speed of criminal justice processes. Digital record management systems reduce the reliance on manual paperwork and enable quick access to case files, criminal records, and investigation reports. Systems such as the Crime and Criminal Tracking Network and Systems allow police officers to register First Information Reports (FIRs) electronically and track case progress more efficiently. This reduces delays in investigations and improves overall administrative efficiency.

**Better Coordination Among Institutions**

Digital technologies facilitate better coordination between different institutions involved in the criminal justice system, including police departments, courts, prisons, prosecution agencies, and forensic laboratories. The Interoperable Criminal Justice System enables seamless data exchange among these institutions through an integrated digital platform. This coordination helps streamline investigation and trial processes and ensures that relevant information is accessible to all stakeholders.

**Transparency and Accountability**

The digitization of criminal justice processes has improved transparency and accountability in law enforcement and judicial administration. Online portals and digital databases allow citizens, lawyers, and researchers to access information regarding case status, court judgments, and police procedures. Initiatives such as the e-Courts Mission Mode Project enable public access to court records and judicial information, thereby strengthening accountability within the justice system.

**Data-Driven Decision Making**

Digital technologies enable law-enforcement agencies to analyse large volumes of crime data and identify patterns and trends. Data analytics tools help police departments understand crime hotspots, predict potential criminal activity, and allocate resources more effectively. This approach supports evidence-based policymaking and enhances the strategic planning of crime prevention measures.

**Improved Access to Justice**

Digital platforms have made it easier for citizens to access justice services. Online complaint registration, digital case tracking, and virtual court hearings allow individuals to interact with law-enforcement agencies and courts without the need for physical visits. This is particularly beneficial for people living in remote areas, as it reduces travel costs and procedural barriers.

**Strengthening Evidence Collection and Investigation**

Digital forensics and electronic data analysis have improved the accuracy and reliability of criminal investigations. Digital evidence such as emails, call records, surveillance footage, and social media data can be analysed to identify suspects and reconstruct criminal activities. These technologies enhance the credibility of evidence presented in courts and support more effective prosecution. Overall, digital technologies have strengthened the criminal justice system in India by improving operational efficiency, enhancing transparency, and supporting data-driven law enforcement. However, the successful implementation of these technologies requires adequate infrastructure, technical training, and appropriate legal safeguards to address emerging challenges.

**Challenges and Concerns**

Despite the numerous advantages of digital technologies in crime prevention and the criminal justice system, several challenges and concerns remain. The effective implementation of digital initiatives requires strong infrastructure, technical expertise, and appropriate legal frameworks. In India, issues related to data security, privacy, and technological capacity continue to pose significant obstacles to the full realization of digital transformation in the criminal justice system.

**Data Privacy and Surveillance Concerns**

One of the major concerns associated with digital technologies is the issue of data privacy. The extensive use of surveillance technologies, digital databases, and biometric identification systems raises questions about the protection of personal information. Large-scale data collection by law-enforcement agencies may lead to misuse or unauthorized access to sensitive data. Ensuring adequate data protection mechanisms and clear legal regulations is therefore essential to safeguard citizens' rights.

**Cybersecurity Risks**

Digital systems used in law enforcement and judicial administration are vulnerable to cyberattacks, hacking, and data breaches. Sensitive information related to criminal records, investigations, and court proceedings may be compromised if proper cybersecurity measures are not in place. Strengthening cybersecurity infrastructure and implementing robust data protection systems are necessary to protect the integrity of digital criminal justice platforms.

**Digital Divide and Infrastructure Limitations**

Another challenge is the digital divide that exists between urban and rural areas in India. Limited internet connectivity, lack of technological infrastructure, and inadequate digital resources in some regions hinder the effective implementation of digital initiatives. This disparity may affect equal access to digital justice services, particularly for individuals living in remote or underdeveloped areas.

**Lack of Technical Expertise and Training**

The successful implementation of digital technologies requires trained personnel who can operate and manage these systems efficiently. In many cases, law-enforcement officers, judicial staff, and administrative personnel may lack adequate technical skills to utilize digital platforms effectively. Continuous training and capacity-building programs are therefore necessary to ensure the efficient use of digital tools within the criminal justice system.

**Legal and Ethical Issues**

The use of advanced technologies such as artificial intelligence, facial recognition, and predictive policing raises important ethical and legal questions. These technologies may lead to issues such as algorithmic bias, wrongful identification, and lack of accountability. Without proper regulatory frameworks, the misuse of digital technologies could undermine public trust in the criminal justice system.

**Integration and Coordination Challenges**

Although initiatives such as the Interoperable Criminal Justice System aim to integrate various components of the justice system, practical difficulties remain in ensuring seamless coordination between different agencies. Differences in technological capacity, administrative procedures, and data standards can slow down the process of digital integration. While digital technologies offer significant opportunities to improve crime prevention and justice delivery, addressing these challenges is crucial for their effective and responsible use. Strengthening legal safeguards, improving digital infrastructure, and providing adequate training to personnel will help ensure that technological innovations contribute positively to the criminal justice system in India.

## CONCLUSION

Digital technologies have emerged as a crucial component in strengthening crime prevention and improving the efficiency of the criminal justice system in India. The integration of information and communication technology, digital databases, artificial intelligence, and data analytics has significantly enhanced the capacity of law-enforcement agencies and judicial institutions to respond to criminal activities more effectively. Technological initiatives introduced by the Government of India have played an important role in modernizing policing, improving investigative processes, and increasing transparency in the justice delivery system. Major digital initiatives such as the Crime and Criminal Tracking Network and Systems, the Interoperable Criminal Justice System, and the e-Courts Mission Mode Project have contributed significantly to the digital transformation of the criminal justice system. These platforms enable better coordination among police, courts, prisons, prosecution, and forensic institutions by facilitating real-time information sharing and digital access to records. As a result, investigations have become more efficient, court processes more transparent, and access to justice more convenient for citizens. At the same time, digital technologies such as surveillance systems, biometric identification, and digital forensics have strengthened crime detection and prevention mechanisms. Data-driven policing and advanced investigative tools allow law-enforcement agencies to analyse crime patterns, identify offenders more quickly, and improve strategic planning in maintaining law and order. However, the increasing reliance on digital technologies also raises several challenges. Concerns related to data privacy, cybersecurity risks, digital inequality, and lack of technical expertise must be addressed to ensure the responsible use of technology in the justice system. The development of strong legal frameworks, improved digital infrastructure, and continuous training for law-enforcement personnel are essential for overcoming these challenges. In conclusion, digital technologies have the potential to significantly transform crime prevention and the criminal justice system in India by making it more efficient, transparent, and accessible. With appropriate safeguards and effective implementation, technological innovations can play a vital role in strengthening the rule of law and enhancing public trust in the justice system.

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**RESEARCH ARTICLE**

# Reverse Pharmacological Evaluation of *Jatipatradi Yoga* in Oral Premalignant Lesions : A Phytochemical Perspective

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## ABSTRACT

Oral premalignant lesions (OPLs) or conditions (OPCs) including oral leukoplakia, eruthroplakia, oral submucous fibrosis (OSMF), and epithelial dysplasia represent a critical stage in oral carcinogenesis. These conditions are characterized by chronic inflammation, oxidative stress, altered epithelial differentiation, and progressive fibrotic or dysplastic changes, with a significant risk of malignant transformation. Conventional management remains limited due to recurrence, adverse effects, and lack of long-term preventive strategies. *Jatipatradi Yoga*, a classical Ayurvedic formulation indicated in *Mukha Roga*, specifically *Sarvasara Mukhapaka* has been traditionally used for chronic inflammatory and ulcerative oral conditions. Reverse pharmacology provides a rational framework to scientifically validate such formulations by integrating traditional clinical experience with modern analytical evidence. To elucidate the reverse pharmacological basis of *Jatipatradi Yoga* in the management of oral premalignant lesions through phytochemical characterization using High Performance Thin Layer Chromatography (HPTLC). *Jatipatradi Yoga* was prepared according to *Yoga Ratnakara* and subjected to HPTLC analysis for phytochemical fingerprinting. Chromatography was performed on silica gel 60 $F_{254}$ plates using ethyl acetate: methanol: water (5:3:2 v/v/v) as the mobile phase. Detection was carried out at 254 nm and 366 nm, with multiple sample application volumes (5–20 µL) to assess reproducibility and dose-dependent phytochemical expression. HPTLC profiling revealed a consistent and reproducible chromatographic fingerprint with multiple well-resolved peaks at both 254 nm and 366 nm, indicating the presence of

diverse bioactive phytoconstituents. The observed peaks suggest the presence of polyphenols, flavonoids, tannins, and other secondary metabolites known for anti-inflammatory, antioxidant, antifibrotic, and cytoprotective activities. The increase in peak intensity with higher application volumes reflects dose-dependent phytochemical availability, supporting formulation consistency and therapeutic relevance.

**Keywords:** Reverse Pharmacology, *Jatipatradi Yoga,* Oral Premalignant Lesions, Oral Leucoplakia, Oral Submucous Fibrosis, HPTLC, Epithelial Dysplasia

## INTRODUCTION

Oral cancer is a major public health problem worldwide and constitutes one of the leading causes of cancer-related morbidity and mortality, particularly in South and Southeast Asia. The development of oral squamous cell carcinoma is widely accepted to be a multistep process, often preceded by clinically recognizable and histopathological identifiable oral premalignant lesions (OPLs) and oral potentially malignant conditions (OPCs) ]1]. These include oral leucoplakia, erythroplakia, oral submucous fibrosis (OSMF), and epithelial dysplasia, which together represent critical transitional stages between normal oral mucosa and invasive malignancy. The malignant transformation rates associated with these conditions vary but are clinically significant, making early intervention and effective preventive strategies imperative [2]. The etiopathogenesis of OPLs is complex and multifactorial, involving prolonged exposure to carcinogenic agents such as tobacco (smoked and smokeless forms), areca nut, alcohol, nutritional deficiencies, and chronic mechanical or chemical irritation [3]. At the cellular and molecular levels, these lesions are characterized by sustained chronic inflammation, excessive oxidative stress, accumulation of reactive oxygen and nitrogen species, DNA damage, epigenetic alterations, and dysregulation of signalling pathways governing epithelial proliferation, differentiation, apoptosis, and extracellular matrix remodelling. In conditions such as OSMF, excessive collagen deposition, reduced collagen degradation, and progressive fibrosis result in functional impairment and a heightened risk of dysplastic transformation. Epithelial dysplasia, in particular, represents a direct histological indicator of neoplastic potential, reflecting irreversible genetic and cellular alterations [4].

Despite advancements in understanding the molecular biology of oral carcinogenesis, the clinical management of oral premalignant lesions remains unsatisfactory. Conventional treatment modalities such as surgical excision, laser ablation, intralesional corticosteroids, antioxidants, retinoids, and immunomodulatory agents are often associated with high recurrence rates, adverse effects, functional morbidity, and limited long-term chemo preventive efficacy [5]. Moreover, these interventions primarily target localized lesions without adequately addressing the underlying inflammatory and oxidative microenvironment that drives disease initiation and progression. The absence of safe, effective, and sustainable preventive strategies highlights a critical therapeutic gap in the management of OPLs.

Ayurveda offers a comprehensive and holistic approach to disease prevention and management. Ayurvedic literature provides detailed descriptions of oral disorders under the broad classification of *MukhaRoga*, encompassing conditions with clinical features analogous to contemporary descriptions of oral premalignant and chronic inflammatory oral diseases termed as *Sarvasaara Mukharoga*. This condition presents with diffuse inflammatory and ulcerative involvement of the oral cavity and bears close resemblance to chronic, non-healing, and potentially premalignant oral conditions described in modern medicine. Concepts such as *Pitta* and *Raktadushti*, chronic *Shotha* (inflammation), *Vrana* (ulceration), and *Dhatu kshaya* offer a unique pathophysiological framework that emphasizes systemic imbalance and tissue-level pathology [6]. *Jatipatradi Yoga*, a classical Ayurvedic polyherbal formulation mentioned in *Yoga Ratnakara*, isindicated in the management of *Sarvasara Mukhapaka*. The formulation has been empirically used in conditions characterized by persistent inflammation, mucosal damage, and impaired healing. Classical attributes such as *Shothahara* (anti-inflammatory), *Ropana* (wound healing), *Rakta-Prasadana* (blood purification), and *Pitta-shamana* suggest a therapeutic potential that may be relevant in modulating the pathological processes underlying oral premalignant lesions.[7] However, despite its long-standing clinical use, scientific validation

of its phytochemical composition and biological plausibility remains limited. Reverse pharmacology provides a systematic and translational research paradigm to bridge this gap between traditional knowledge and modern scientific validation. Unlike conventional drug discovery, which begins with isolated molecules and proceeds toward clinical application, reverse pharmacology starts with formulations that have an established history of human use, safety, and therapeutic efficacy. It then employs modern analytical, experimental, and clinical tools to elucidate their chemical composition, mechanisms of action, and therapeutic relevance. Within this framework, phytochemical characterization serves as a foundational step to establish formulation consistency, identify bioactive constituents, and generate hypotheses regarding pharmacological activity [8].

High Performance Thin Layer Chromatography (HPTLC) is a robust, reproducible, and widely accepted analytical technique for phytochemical fingerprinting of polyherbal formulations. It enables qualitative and semi-quantitative assessment of complex phytochemical profiles, supports quality control, and facilitates correlation between traditional therapeutic claims and modern pharmacological evidence. In the context of oral premalignant lesions, identifying phytoconstituents with known anti-inflammatory, antioxidant, antifibrotic, and cytoprotective properties is particularly relevant, given the central role of these mechanisms in disease pathogenesis [9]. Therefore, the present study aims to evaluate *Jatipatradi Yoga* from a reverse pharmacological perspective through detailed phytochemical characterization using HPTLC, with specific reference to its potential role in the management of oral premalignant lesions. By integrating Ayurvedic principles, traditional clinical usage, and modern analytical evidence, this work seeks to provide a scientific foundation for further experimental and clinical investigations into the preventive and therapeutic utility of *Jatipatradi Yoga* in oral premalignant pathology

## METHODOLOGY

**Study Design**

The present study was designed as an analytical research based on the principles of reverse pharmacology. The study emphasizes the scientific validation of a classical Ayurvedic formulation through systematic phytochemical analysis, correlating traditional therapeutic claims with modern analytical evidence. The research does not involve human or animal experimentation and is confined to pharmaceutical preparation, literary review, and analytical evaluation of the formulation.

Aim of the Study

The study aims to evaluate *Jatipatradi Yoga* from a reverse pharmacological perspective with reference to oral premalignant lesions, through phytochemical characterization using High Performance Thin Layer Chromatography (HPTLC).

Objectives of the Study

- To prepare *Jatipatradi Yoga* according to classical Ayurvedic textual references.
- To subject the prepared formulation to HPTLC analysis for phytochemical fingerprinting.
- To assess reproducibility and dose-dependent expression of phytoconstituents.
- To interpret the analytical findings in relation to the pathophysiology of oral premalignant lesions and Ayurvedic principles.

Sources of Data

Literary Source

- Classical texts such as *Charaka Samhita*, *Sushruta Samhita*, *Ashtanga Hridaya*, and *Ashtanga Sangraha* for references related to *MukhaRoga, Shotha, Vrana, Raktadushti,* and *Pitta* vitiation.
- *Yoga Ratnakara* for the formulation, ingredients, indications, and therapeutic properties of Jatipatradi Yoga.
- Relevant commentaries, *Nighantus*, and Ayurvedic pharmacopeial texts for drug properties and formulation details.

**Modern literature sources included:**

- Standard textbooks of oral pathology, oral medicine, and oncology for information on oral premalignant lesions and carcinogenesis.
- Research articles, review papers, and journals accessed through electronic databases such as PubMed, Google Scholar, AYUSH Research Portal, and other peer-reviewed platforms.
- Publications related to reverse pharmacology, phytochemistry, and HPTLC methodology.

Drug Source

Raw drugs required for the preparation of *Jatipatradi Yoga* were procured from authenticated and reliable sources. The drugs were identified and authenticated based on organoleptic, macroscopic, and classical Ayurvedic parameters, ensuring genuineness and purity. Foreign matter was removed, and the drugs were processed as per classical guidelines before formulation.

Preparation of *Jatipatradi Yoga*

Jatipatradi Yoga[10] was prepared strictly in accordance with the formulation described in *Yoga Ratnakara*. The individual ingredients were cleaned, dried, powdered, and mixed in prescribed proportions under controlled conditions to ensure uniformity and homogeneity. The prepared formulation was stored in airtight containers to prevent contamination and degradation.

**Analytical Study -HPTLC**

**Inference (254 nm):**

The formulation shows multiple UV-absorbing phytochemicals**,** indicating **a**chemically complex and polyherbal nature**.** Presence of strong peaks in low and mid-Rf region suggests dominance of polar and semi-polar bioactive compounds

**Inference (366 nm):**

The formulation exhibits **distinct fluorescent bands,** confirming the presence of **chromophoric phytochemicals such as flavonoids, phenolics, and coumarin-like compounds,** which are often linked with **anti-inflammatory and antioxidant activity.**

**Observations and Results**

HPTLC analysis of *Jatipatradi Yoga* revealed a distinct, reproducible, and well-resolved phytochemical fingerprint at both 254 nm and 366 nm. At 254 nm, multiple well-defined bands were observed, indicating the presence of phenolic compounds such as polyphenols and tannins. Visualization at 366 nm showed distinct fluorescent bands suggestive of flavonoids and other UV-active secondary metabolites. The chromatographic pattern remained consistent across all application volumes (5–20 µL), with a progressive increase in band intensity at higher volumes, demonstrating dose-dependent phytochemical expression and formulation uniformity. No significant variation in Rf values or band distribution was observed, confirming the reproducibility and stability of the formulation. Overall, the HPTLC profile confirms the multicomponent phytochemical nature of *Jatipatradi Yoga*, supporting its potential anti-inflammatory, antioxidant, and cytoprotective relevance in the management of oral premalignant lesions from a reverse pharmacological perspective

## DISCUSSION

Oral premalignant lesions constitute a heterogeneous group of disorders that arise as intermediate stages in the multistep process of oral carcinogenesis. These lesions are characterized by persistent chronic inflammation, increased oxidative stress, epithelial hyperproliferation, altered keratinization, and, in certain conditions such as oral submucous fibrosis, excessive collagen deposition and progressive fibrosis. [11]Given the multifactorial nature of these pathological processes, effective management requires therapeutic interventions that can act on multiple biological

targets simultaneously. The present study employed a reverse pharmacological approach to scientifically evaluate *Jatipatradi Yoga*, a classical Ayurvedic formulation traditionally indicated in *MukhaRoga*, by correlating its phytochemical profile with the pathophysiology of oral premalignant lesions.

HPTLC analysis of *Jatipatradi Yoga* revealed a consistent, reproducible, and chemically diverse phytochemical fingerprint at 254 nm and 366 nm. The presence of multiple well-resolved bands suggests a rich composition of secondary metabolites, predominantly polyphenols, flavonoids, and tannins. These phytochemical groups are widely reported to possess anti-inflammatory, antioxidant, antifibrotic, and cytoprotective properties. Such activities are directly relevant to oral premalignant lesions, where sustained inflammatory responses and oxidative stress contribute to epithelial damage, dysplastic changes, and increased malignant potential.

From a modern biomedical perspective, phenolic and flavonoid compounds are known to modulate inflammatory mediators, scavenge reactive oxygen species, regulate epithelial cell proliferation, and maintain mucosal integrity. In oral submucous fibrosis, where collagen metabolism is altered, these compounds may help regulate extracellular matrix turnover and limit progressive fibrosis. The dose-dependent increase in band intensity observed in the HPTLC profile further supports the cumulative and synergistic action of phytoconstituents present in the formulation. From an Ayurvedic standpoint, oral premalignant lesions may be conceptually correlated *Sarvasaara Mukhapaka*, involving *Pitta* and *Raktadushti*, associated with chronic *Shotha* and Vrana in the oral cavity. *Jatipatradi Yoga* is described to possess *Shothahara, Ropana,* and *Rakta-Prasadana* properties. The multicomponent phytochemical nature demonstrated through HPTLC aligns with the Ayurvedic principle of polyherbal synergy, wherein multiple constituents collectively contribute to therapeutic efficacy rather than a single isolated compound.

Reverse pharmacology emphasizes validation of formulations with long-standing clinical use and known safety profiles through modern scientific tools. The present phytochemical evaluation represents an essential early step in this continuum, providing analytical evidence that supports the traditional therapeutic claims of *Jatipatradi Yoga*. Although the study is limited to phytochemical fingerprinting and does not establish direct clinical efficacy, it offers a scientifically sound foundation for future experimental studies, molecular investigations, and clinical trials aimed at elucidating mechanisms of action and therapeutic outcomes in oral premalignant lesions.

## CONCLUSION

The detailed reverse pharmacological evaluation of *Jatipatradi Yoga* through HPTLC phytochemical fingerprinting provides scientific support for its traditional use in oral premalignant lesions. The formulation exhibits a consistent, reproducible, and chemically diverse profile rich in bioactive secondary metabolites relevant to the modulation of inflammation, oxidative stress, fibrosis, and epithelial dysregulation. These findings suggest that *Jatipatradi Yoga* holds promise as a preventive or adjunctive therapeutic option in conditions such as oral leukoplakia, oral submucous fibrosis, and epithelial dysplasia. The study establishes a strong analytical foundation for future experimental studies, molecular investigations, and controlled clinical trials aimed at validating efficacy and elucidating.

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Table 01: Rf Values Observed in HPTLC Densitogram-**At 254 nm (UV absorbance mode)**

| Peak No. | Rf value | Area % (approx.) | Interpretation |
|---|---|---|---|
| 1 | 0.03–0.05 | ~5–10% | Highly polar constituents; may indicate sugars, tannins or inorganic salts |
| 2 | 0.12–0.15 | ~25–35% | Major polar phytoconstituents; possibly phenolics or glycosides |
| 3 | 0.26–0.30 | ~20–30% | Moderately polar compounds; likely flavonoids or alkaloidal fractions |
| 4 | 0.93–0.96 | ~15–20% | Non-polar constituents; may represent terpenoids or essential oil components |

**Table 02: Rf Values Observed in HPTLC Densitogram- At 366 nm (Fluorescence mode)**

| Peak No. | Rf value | Area % (approx.) | Interpretation |
|---|---|---|---|
| 1 | 0.08–0.10 | ~10–15% | Fluorescent polar compounds; possibly coumarins or phenolic acids |
| 2 | 0.17–0.20 | ~20–25% | Strong fluorescent phytoconstituents; indicative of flavonoids |
| 3 | 0.32–0.35 | ~10–15% | Semi-polar fluorescent compounds |
| 4 | 0.48–0.55 | ~8–12% | Medium polarity compounds |
| 5 | 0.72–0.78 | ~5–8% | Less polar constituents |
| 6 | 0.85–0.90 | ~10–15% | Non-polar fluorescent compounds |

**Table 03:Interpretation of the HPTLC study -Jatipatradi Yoga**

| Analytical Parameter | HPTLC Observation | Phytochemical Interpretation | Relevance to OPL Pathogenesis | Reverse Pharmacological Significance |
|---|---|---|---|---|
| **Wavelength: 254 nm** | Multiple well-resolved dark and light bands observed | Presence of polyphenols, tannins, and phenolic compounds with conjugated systems | Polyphenols and tannins exhibit strong anti-inflammatory and antioxidant activity, counteracting chronic inflammation and oxidative stress implicated in leukoplakia and epithelial dysplasia | Supports traditional use in inflammatory *MukhaRoga;* validates *Shothahara and Rakta-Prasadana* actions |
| **Wavelength: 366 nm** | Distinct fluorescent bands observed | Indicative of flavonoids and UV-active secondary metabolites | Flavonoids modulate oxidative stress, inhibit abnormal epithelial proliferation, and protect against dysplastic changes | Demonstrates biological plausibility for chemopreventive and cytoprotective role in OPLs |
| **Number of peaks/bands** | Multiple peaks across chromatogram | Confirms polyherbal, multicomponent nature of formulation | Multifactorial pathology of OPLs requires multi-targeted therapeutic action | Aligns with Ayurvedic synergistic principle and reverse pharmacology framework |
| **Rf pattern consistency** | Similar Rf values across all tracks | Reproducible phytochemical fingerprint | Ensures formulation consistency and reliability for therapeutic use | Establishes standardization and quality control baseline |
| **Sample volume: 5–20 µL** | Progressive increase in peak intensity without pattern distortion | Dose-dependent phytochemical expression | Suggests cumulative therapeutic effect with increasing dose | Supports classical dosage rationale and analytical reliability |
| **Chromatographic resolution** | Clear, well-separated bands | Minimal overlap of phytoconstituents | Enables identification of diverse bioactive groups | Indicates suitability for further mechanistic and pharmacological studies |
| **Overall fingerprint** | Stable, characteristic HPTLC profile | Presence of diverse secondary metabolites | Addresses inflammation, oxidative stress, fibrosis, and epithelial dysregulation in OPLs | Scientifically validates traditional clinical efficacy through reverse pharmacology |

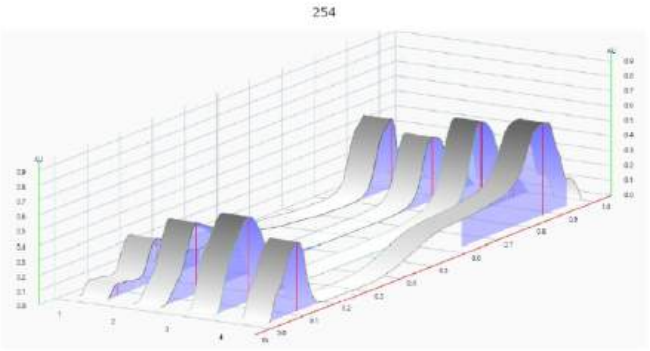
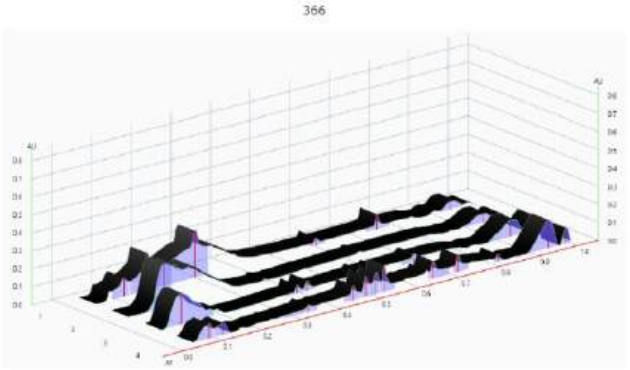



**Fig. 01. HPTLC Densitometric Chromatogram of *Jatipatradi Yoga* at 254 nm**




**FigNo.02 HPTLC densitometric chromatogram of *Jatipatradi Yoga* at 366 nm**

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RESEARCH ARTICLE

# Webometric Analysis of India's Institutions of Eminence

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**ABSTRACT**

Peter Ingwersen introduced the Web Impact Factor (WIF) in 1998 as a quantitative indicator based on link frequency analysis. Building on this concept, the present study examines the websites of Institutions of Eminence (IoEs) in India using selected webometric tools and indicators. The parameters analysed include Simple Web Impact Factor, Self-link Web Impact Factor, External-link Web Impact Factor, backlinks, and broken links. Data were collected using tools such as the Google Search Engine, Website Link Analyser, Broken Link Checker, and Backlink Watch. The analysis reveals significant variations in the quality, visibility, and maintenance of IoE websites. Institutions such as OP Jindal Global Institution of Eminence and Manipal Academy of Higher Education exhibit fewer broken links, indicating better website maintenance, improved user experience, and enhanced visibility. These institutions achieve higher rankings because of their few broken links.

**Keywords:** Backlinks, Broken Links, External Links, Institutions of Eminence, Web Impact Factor, Webometrics.

## INTRODUCTION

In the contemporary higher education environment, Institutions of Eminence have gained importance. These institutions are expected to lead in academic excellence, research productivity, and global engagement. Their digital presence, particularly institutional websites, is crucial for sharing academic information and facilitating access to resources. It also enhances international visibility. Webometric analysis provides a quantitative framework for assessing the online performance of academic institutions. (Chakravarty & Wasan, 2015). Webometrics examines quantitative aspects of online information resources, technologies, and services. (Chakraborty & Thakuria, 2023) It provides a reliable approach for evaluating institutional websites using link-based indicators (Aguillo et al., 2008).

Literature highlights the growing importance of web presence and visibility among Indian higher education institutions. This study extends previous research by focusing specifically on Institutions of Eminence. These represent India's leading academic institutions. Studies have emphasised the usefulness of combining institutional characteristics with numerical indicators to assess educational quality and performance (Artyukhov et al., 2021). Webometric approaches are also effective in evaluating the reach and impact of scholarly and academic web resources (Shafi & Bhat, 2014).

**Institutions of Eminence**

The Government of India introduced the Institutions of Eminence (IoE) initiative in 2016 through the Ministry of Human Resource Development, now the Ministry of Education. The objective is to develop world-class higher education institutions. Under this scheme, twenty institutions were designated as Institutions of Eminence. They are divided between the public and private sectors. These institutions are granted greater autonomy and additional financial support. This support is intended to strengthen research, teaching, and innovation activities. These selected institutions are tasked with competing globally and improving India's international higher education profile. At the time of data collection, only twelve IoEs had fully functional, independent, and accessible official websites; therefore, the analysis is limited to these, ensuring data reliability and accuracy.

## REVIEW OF LITERATURE

Seshaiah and Rekha (2017) conducted a webometric evaluation of 277 engineering institutions in the state of Andhra Pradesh. They analysed web pages, link pages, and various forms of Web Impact Factors using tools such as Google Search Engine and Backlink Watch. Their findings indicated limited web presence among engineering institutions. Brahma and Verma (2019) examined the websites of South Asian national libraries. They found the National Library of India to be the most dominant in terms of external links and domain authority. Chakravarty and Wasan (2015) analysed library websites of Indian higher education institutions. They observed minimal variation between WIF and revised WIF rankings. Elgohary (2008) analysed 99 Arab institutions. He found that Jordanian universities were among the most dominant in the updated WIF rankings. Studies by JeyshankarandSujithai (2014), Didegah and Erfanmanesh (2010), Bulla and Hadagali (2020), and Madhusudhan and Prakash (2013) further demonstrated the effectiveness of webometric indicators. These indicators are useful for evaluating institutional websites and digital visibility.

**Objectives of the Study**

1. To evaluate the websites of selected Institutions of Eminence in India using webometric tools and indicators.
2. To determine the number of internal links, self-links, external links, in-links, and broken links on the selected IoE websites.
3. Assess and rank the IoE websites based on Simple, Self-link, External, and Revised Web Impact Factors.

## METHODOLOGY

The study adopts a quantitative webometric research approach. Data were collected using the Google Search Engine, which provides extensive web coverage and reliable retrievalcapabilities. The Website Link Analyser (https://smallseotools.com/website-link-analyser-tool/) was used to collect data on simple, self-referential, and external links. In-links were obtained using Backlink Watch(http://www.backlinkwatch.com/). Broken Link Checker (https://www.deadlinkchecker.com/) identified broken links on the selected websites. Microsoft Excel was used to organise, compute, and analyse the collected webometric data. The data for the present study were collected during the period 01 to 5 December 2025.

**Data Analysis and Interpretation**

**Domain-wise distribution of Institutions of Eminence in India**

It has gathered the Universal Resource Locators (URLs) of 12 Institutions of Eminence, as well as the Second-Level Domains (SLDs) and Top-Level Domains (TLDs) of these institutions, through online searches. Table 2 presents the TLDs and SLDs. in, and domain names of the hosts of the Internet of Everything. All Institutions of Eminence use the same domain extension—. ac. in—except Manipal Academy of Higher Education, which uses edu.

**Simple Web Impact Factor of the top 5 Institutions of Eminence in India**

Table 3 displays the rank distribution of IOE websites in India based on the Simple WIF. Birla Institute of Technology and Science, Pilani, Rajasthan, ranked #1 in terms of Simple Web Impact Factor (SWIF) (0.0308SWIF). Second place went to the Manipal Academy of Higher Education in Manipal, Karnataka, with a SWIF of 0.0280; third place went to Banaras Hindu University in Varanasi, Uttar Pradesh, with a SWIF of 0.01158; and fourth place went to the University of Hyderabad in Hyderabad, Telangana, with a SWIF of 0.00408. Despite having a respectable number of webpages (244000), the Indian Institute of Technology, Delhi, placed fifth (0.00341 SWIF) due to a lower number of link pages than

**Self-Link Web Impact Factor of the top 5 Institutions of Eminence in India**

The rank distribution of India's IoE websites is displayed in Table 4 based on the self-link WIF. Pilani, Rajasthan's Birla Institute of Technology and Science, ranked first with a Self-Link Web Impact Factor (SLWIF) of 0.0142. Second place went to Manipal Academy of Higher Education in Manipal, Karnataka, with a score of 0.0138 SLWIF; third place went to OP Jindal Global Institution of Eminence, Deemed to be University in Sonipat, Haryana, with a score of 0.0109 SLWIF; and fourth place went to Banaras Hindu University in Varanasi, Uttar Pradesh, with a score of 0.0097 SLWIF. The Indian Institute of Technology, Bombay, Maharashtra, has a significant number of web pages (350,000); however, because there were fewer self-link sites than web pages, the institute ranked fifth (0.0078 SLWIF).

**External-Link Web Impact Factor of the top 5 Institutions of Eminence in India**

The rank distribution of IoE websites in India, based on the External Link WIF, is shown in Table 5. Banaras Hindu University, Varanasi, Uttar Pradesh, held the top spot (0.0179 ELWIF) for External Link Web Impact Factor (ELWIF). The University of Delhi, Delhi, placed fourth with 0.00006 ELWIF, followed by Birla Institute of Technology and Science, Pilani, Rajasthan, which placed third with 0.0012 ELWIF, and Indian Institute of Technology, Bombay, Maharashtra, which placed second with 0.0018 ELWIF. The University of Hyderabad, located in Hyderabad, Telangana, ranked fifth (0.0009 ELWIF), despite having a substantial number of web pages (86,000), primarily because its external link pages accounted for a lower proportion of its total web pages.

**Revised Web Impact Factor of the top 5 Institutions of Eminence in India**

The rank distribution of India's IoE websites, based on the Revised WIF, is shown in Table 6. The top rank was held by Banaras Hindu University in Varanasi, Uttar Pradesh, with a revised web impact factor (RWIF) of 0.2833. The Indian Institute of Science, Bangalore, Karnataka, held the second position with a RWIF of 0.2174, followed by the Birla Institute of Technology and Science, Pilani, Rajasthan, which came in third place with a RWIF of 0.1632, and the Indian Institute of Technology, Madras, Tamil Nadu, which came in fourth place with a RWIF of 0.0717. Although the University of Hyderabad, Hyderabad, Telangana, has a respectable number of web pages (86,000), it was ranked fifth (0.0505 RWIF) because it had fewer in-link pages than web pages.

**Broken Links of the top 5 Institutions of Eminence in India**

The results, displayed in Table 7, represent the broken links (BL) on the IoE Everything websites. Manipal Academy of Higher Education, Manipal, Karnataka, rated second with 0.0004% broken links (BL), behind the OP Jindal Global Institution of Eminence. Deemed to be a University, Sonipat, Haryana, ranked first with a BL of 0.0001%. In terms of broken links (BL), the Indian Institute of Technology, Madras, Tamil Nadu, ranked fourth with 0.76%, while the Birla Institute of Technology and Science, Pilani, Rajasthan, ranked third with 0.12%. Ranking fifth, with the greatest Broken Links (BL) of 1.40 %, is Banaras Hindu University in Varanasi, Uttar Pradesh.

## DISCUSSION

The study finds significant variation in web presence and link performance among India's Institutions of Eminence (IoEs). Most use the ***.ac.***in the domain for academic credibility. MAHE (Manipal Academy of Higher Education) uses the .edu domain, indicating an international focus that may enhance its visibility. Web Impact Factor (Simple and Self-Link) results indicate that an effective internal structure and balanced link distribution enhance web impact more than simply having a large number of web pages. Institutions with larger websites but lower link density tend to perform worse. Web volume alone does not guarantee better visibility. Web Impact Factor (External-Link)results demonstrate differences in external recognition across IoEs. Banaras Hindu University stands out for its strong external links, demonstrating a broad academic reach and digital influence. The Web Impact Factor (Revised) confirms that reputation and content relevance drive in-links. The broken link analysis reveals varied website maintenance practices. Institutions with fewer broken links tend to manage their sites more effectively, thereby enhancing accessibility and user experience. High rates of broken links hurt web credibility. Institutions of Eminence (IoEs) should strengthen internal linking, increase external engagement, and maintain high-quality websites to enhance web visibility and impact.

## CONCLUSION

The webometric analysis of Institutions of Eminence (IOE) websites reveals considerable variation in website quality, visibility, and maintenance practices. The findings reveal substantial differences among the Institutions of Eminence (IoEs) in their web presence, connectivity structures, and maintenance procedures. Website size, by itself, does not guarantee increased visibility. Institutions demonstrating robust external connectivity and well-balanced internal link structures generally achieve a greater web effect. OP Jindal Global Institution of Eminence, Manipal Academy of Higher Education (IOE), demonstrates effective website maintenance with minimal broken links. At the same time, Banaras Hindu University (IOE) distinguishes itself through strong external and in-link performance.

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**Table 1: List of Institutions of Eminence notified by the Government**

| SR.No. | Institutions of Eminence | City | State | URL |
|---|---|---|---|---|
| 1. | Indian Institute of Science, | Bangalore | Karnataka | https://iisc.ac.in/ |
| 2. | Indian Institute of Technology | Bombay | Maharashtra | https://www.iitb.ac.in/ |
| 3. | Indian Institute of Technology | Delhi | Delhi | https://home.iitd.ac.in/ |
| 4. | Indian Institute of Technology | Kharagpur | West Bengal | https://www.iitkgp.ac.in/ |
| 5. | Indian Institute of Technology | Madras | Tamil Nadu | https://www.iitm.ac.in/ |
| 6. | University of Delhi | Delhi | Delhi | https://www.du.ac.in/ |
| 7. | University of Hyderabad | Hyderabad | Telangana | https://uohyd.ac.in/ |
| 8. | Banaras Hindu University | Varanasi | Uttar Pradesh | https://www.bhu.ac.in/ |
| 9. | Birla Institute of Technology and Science | Pilani | Rajasthan | https://www.bits-pilani.ac.in/ |
| 10. | Manipal Academy of Higher Education | Manipal | Karnataka | https://www.manipal.edu/ |
| 11. | OP Jindal Global Institution of Eminence Deemed to be a University | Sonipat | Haryana | https://jgu.edu.in/ |
| 12. | Shiv Nadar Institution of Eminence Deemed to be a University | Greater Noida | Uttar Pradesh | https://snu.edu.in/ |

**Table 2: Domain-wise distribution of Institutions of Eminence in India**

| Sr.No. | Domain name of the hosts of Institutions of Eminence | URL | SLD of IoE | TLD of IoE |
|---|---|---|---|---|
| **1.** | Indian Institute of Science, Bangalore, Karnataka | https://iisc.ac.in/ | .ac.in | .in |
| **2.** | Indian Institute of Technology, Bombay, Maharashtra | https://www.iitb.ac.in/ | .ac.in | .in |
| **3.** | Indian Institute of Technology, Delhi | https://home.iitd.ac.in/ | .ac.in | .in |
| **4.** | Indian Institute of Technology, Kharagpur, West Bengal | https://www.iitkgp.ac.in/ | .ac.in | .in |
| **5.** | Indian Institute of Technology, Madras, Tamil Nadu | https://www.iitm.ac.in/ | .ac.in | .in |
| **6.** | University of Delhi, Delhi | https://www.du.ac.in/ | .ac.in | .in |
| **7.** | University of Hyderabad, Hyderabad, Telangana | https://uohyd.ac.in/ | .ac.in | .in |
| **8.** | Banaras Hindu University, Varanasi, Uttar Pradesh | https://www.bhu.ac.in/ | .ac.in | .in |
| **9.** | Birla Institute of Technology and Science, Pilani, Rajasthan | https://www.bits-pilani.ac.in/ | .ac.in | .in |
| **10.** | Manipal Academy of Higher Education, Manipal, | https://www.manipal.edu/ | edu | - |

| | | | | |
|---|---|---|---|---|
| | Karnataka | | | |
| **11.** | OP Jindal Global Institution of Eminence Deemed to be University, Sonipat, Haryana | https://jgu.edu.in/ | .ac.in | .in |
| **12.** | Shiv Nadar Institution of Eminence Deemed to be University, Greater Noida, Uttar Pradesh | https://snu.edu.in/ | .ac.in | .in |

**Table 3: Simple Web Impact Factor of the top 5 Institutions of Eminence in India**

| SR.No. | Institutions of Eminence | Website | No. of Web Pages (A) | Linked Web Pages (B) | Simple Web Impact Factor (B/A) | Rank |
|---|---|---|---|---|---|---|
| 1. | Birla Institute of Technology and Science, Pilani, Rajasthan | https://www.bits-pilani.ac.in/ | 40600 | 1254 | 0.0308 | 1 |
| 2. | Manipal Academy of Higher Education, Manipal, Karnataka | https://www.manipal.edu/ | 169000 | 4748 | 0.0280 | 2 |
| 3. | Banaras Hindu University, Varanasi, Uttar Pradesh | https://www.bhu.ac.in/ | 6130 | 71 | 0.01158 | 3 |
| 4. | University of Hyderabad, Hyderabad, Telangana | https://uohyd.ac.in/ | 88600 | 362 | 0.00408 | 4 |
| 5. | Indian Institute of Technology, Delhi | https://home.iitd.ac.in/ | 244000 | 834 | 0.00341 | 5 |

**Table 4: Self-Link Web Impact Factor of the top 5 Institutions of Eminence in India**

| SR.NO. | INSTITUTIONS OF EMINENCE | WEBSITE | NO. OF WEB PAGES (A) | SELF-LINKED WEB PAGES (C) | SELF-LINKED WEB IMPACT FACTOR (C/A) | RANK |
|---|---|---|---|---|---|---|
| | Birla Institute of Technology and Science, Pilani, Rajasthan | https://www.bits-pilani.ac.in/ | 40600 | 577 | 0.0142 | 1 |
| | Manipal Academy of Higher Education, Manipal, Karnataka | https://www.manipal.edu/ | 169000 | 2343 | 0.0138 | 2 |
| | OP Jindal Global Institution of Eminence Deemed to be University, Sonipat, Haryana | https://jgu.edu.in/ | 183000 | 142 | 0.0109 | 3 |
| | Banaras Hindu University, Varanasi, Uttar Pradesh | https://www.bhu.ac.in/ | 6130 | 60 | 0.0097 | 4 |
| | Indian Institute of Technology, Bombay, Maharashtra | https://www.iitb.ac.in/ | 350000 | 276 | 0.0078 | 5 |

**Table 5: External-Link Web Impact Factor of the top 5 Institutions of Eminence in India**

| SR.NO. | INSTITUTIONS OF EMINENCE | WEBSITE | NO. OF WEB PAGES (A) | EXTERNAL-LINK WEB PAGES (D) | EXTERNAL - LINKED WEB IMPACT FACTOR (D/A) | RANK |
|---|---|---|---|---|---|---|
| 1. | Banaras Hindu University, Varanasi, Uttar Pradesh | https://www.bhu.ac.in/ | 6130 | 11 | 0.0179 | 1 |
| 2. | Indian Institute of Technology, Bombay, Maharashtra | https://www.iitb.ac.in/ | 350000 | 66 | 0.0018 | 2 |
| 3. | Birla Institute of Technology and Science, Pilani, Rajasthan | https://www.bits-pilani.ac.in/ | 40600 | 50 | 0.0012 | 3 |
| 4. | University of Delhi, Delhi | https://www.du.ac.in/ | 300000 | 20 | 0.00006 | 4 |
| 5. | University of Hyderabad, Hyderabad, Telangana | https://uohyd.ac.in/ | 88600 | 35 | 0.00039 | 5 |

**Table 6: Revised Web Impact Factor of the top 5 Institutions of Eminence in India**

| **SR.NO.** | **INSTITUTIONS OF EMINENCE** | **WEBSITE** | **NO. OF WEB PAGES (A)** | **IN-LINKWEB PAGES (E)** | **REVISED WEB IMPACT FACTOR (E/A)** | **RANK** |
|---|---|---|---|---|---|---|
| | Banaras Hindu University, Varanasi, Uttar Pradesh | https://www.bhu.ac.in/ | 6130 | 13997 | 0.2833 | 1 |
| | Indian Institute of Science, Bangalore, Karnataka | https://iisc.ac.in/ | 258000 | 56100 | 0.2174 | 2 |
| | Birla Institute of Technology and Science, Pilani, Rajasthan | https://www.bits-pilani.ac.in/ | 40600 | 6629 | 0.1632 | 3 |
| | Indian Institute of Technology, Madras, Tamil Nadu | https://www.iitm.ac.in/ | 563000 | 40373 | 0.0717 | 4 |
| | University of Hyderabad, Hyderabad, Telangana | https://uohyd.ac.in/ | 88600 | 4477 | 0.0505 | 5 |

**Table 7: Broken Links of the top 5 Institutions of Eminence in India**

| SR.No. | Institutions of Eminence | Website | Link Web Pages (E) | Broken - Links | Percentage | Rank |
|---|---|---|---|---|---|---|
| 1. | OP Jindal Global Institution of Eminence Deemed to be University, Sonipat, Haryana | https://jgu.edu.in/ | 354 | 4 | 0.0001 | 1 |
| 2. | Manipal Academy of Higher Education, Manipal, Karnataka | https://www.manipal.edu/ | 4748 | 2 | 0.0004 | 2 |
| 3. | Birla Institute of Technology and Science, Pilani, Rajasthan | https://www.bits-pilani.ac.in/ | 1254 | 16 | 0.12 | 3 |
| 4. | Indian Institute of Technology, Madras, Tamil Nadu | https://www.iitm.ac.in/ | 916 | 7 | 0.76 | 4 |
| 5. | Banaras Hindu University, Varanasi, Uttar Pradesh | https://www.bhu.ac.in/ | 71 | 1 | 1.40 | 5 |

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**RESEARCH ARTICLE**

# Intuitionistic Fuzzy Cube Difference Labeling : A New Perspective in Fuzzy Graph Theory

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**ABSTRACT**

In this research, we provide the **intuitionistic fuzzy cube difference graph (IFCDG)** and explore its structural characteristics inside basic graph types such as path graph($P_n$), cycle graph($C_n$), and star graph($S_{1,n}$). Our goal is to enhance the theoretical knowledge of these graphs and highlight their significance in the larger context of graph theory. We hope this exploration proves both insightful and thought-provoking for researchers and enthusiasts alike.

**Keywords:** IFCDL, IFCDG.
**2020 AMS Classification:**05C78, 05C72

## INTRODUCTION

The development of *intuitionistic fuzzy sets* by Atanassov [7, 8] and further elaborated with Shannon [9, 10], led to the emergence of *intuitionistic fuzzy graphs*, enabling even finer representation of vagueness through the dual notion of membership (MS) and non-membership (NMS). In this context, Karunambigai et al. [12, 13] examined fundamental properties of intuitionistic fuzzy graphs (IFGs), while Nivethana and Parvathi [17] studied arc analysis and its applications. Significant advancements were made by Akram and collaborators,Akram and Davvaz [2], Akram and Dudek [3],and Akram and Alshehri [1],who explored a lot of graph structures.Further contributions by Sahoo and

Pal [15, res16, 17, 18, and 19] include the study of intuitionistic fuzzy competition graphs, tolerance graphs, various graph products, and intuitionistic fuzzy labeling (IFL). Building upon these developments, the present work introduces the novel concept of *intuitionistic fuzzy cube difference labeling graphs (IFCDGs)*. We demonstrate that this labeling scheme naturally integrates with and enhances the structural representation of classical graph families such as paths, cycles, and stars. This contribution not only deepens the theoretical framework but also opens promising directions for further research and applications within the broader scope of intuitionistic fuzzy graph theory.

**Preliminaries**

**Definition 2.1**The process of giving each graph's vertices and edges a unique identity is known as **graph labeling**.
**Definition 2.2**When a function $f$ is injected from $V(G)$ to the set of all integers$0,1,\dots,p-1$, such that the induced function $f^*$ from $E(G)$ to $N$, which is given by$f^*(uv)$, equals the absolute value of the difference between the cube of the function $f(u)^3$ and $f(v)^3$ for each $uv \in E(G)$, then the graph is said to have a **cube difference labeling** (CDL). The term **cube difference graph** (CDG) refers to a graph that allows CDL.

**Definition 2.3 [19]**An **IFG** is defined as$G = (V_X^{'}, \sigma_\alpha^{'}, \mu_\beta^{'})$ where $\sigma_\alpha^{'} = (\sigma_{\alpha_1}^{'}, \sigma_{\alpha_2}^{'}), \mu_\beta^{'} = (\mu_{\beta_1}^{'}, \mu_{\beta_2}^{'})$, and
(i)$V_X^{'} = \{v_0^{'}, v_1^{'}, \dots, v_n^{'}\}$The degrees of MS and NMS of the node$v_i^{'} \in V_X^{'}$ are denoted by $\sigma_{\alpha_1}^{'}: V_X^{'} \to [0,1]$and$\sigma_{\alpha_2}^{'}: V_X^{'} \to [0,1]$ respectively, with $0 \le \sigma_{\alpha_1}^{'}(v_i^{'}) + \sigma_{\alpha_2}^{'}(v_i^{'}) \le 1$for every $v_i^{'} \in V_X^{'}(i = 1,2,\dots,n)$.
(ii) $\mu_{\beta_1}^{'}: V_X^{'} \times V_X^{'} \to [0,1]$ and $\mu_{\beta_2}^{'}: V_X^{'} \times V_X^{'} \to [0,1]$, where $\mu_{\beta_1}^{'}(v_i^{'}, v_j^{'})$ and $\mu_{\beta_2}^{'}(v_i^{'}, v_j^{'})$ indicate the degree of MS and NMS value of the edge $(v_i^{'}, v_j^{'})$ respectively, so that $\mu_{\beta_1}^{'}(v_i^{'}, v_j^{'}) \le \min\{\sigma_{\alpha_1}^{'}(v_i^{'}), \sigma_{\alpha_1}^{'}(v_j^{'})\}$and$\mu_{\beta_2}^{'}(v_i^{'}, v_j^{'}) \le \max\{\sigma_{\alpha_2}^{'}(v_i^{'}), \sigma_{\alpha_2}^{'}(v_j^{'})\}\}, 0 \le \mu_{\beta_1}^{'}(v_i^{'}, v_j^{'}) + \mu_{\beta_2}^{'}(v_i^{'}, v_j^{'}) \le 1$for each edge$(v_i^{'}, v_j^{'})$.

**Definition 2.4 [19]**If$\sigma_{\alpha_1}: V_X \to [0,1], \sigma_{\alpha_2}: V_X \to [0,1]$,and$\mu_{\alpha_1}: V_X \times V_X \to [0,1], \mu_{\alpha_2}: V_X \times V_X \to [0,1]$,then an IFG $G = (V_X, \sigma_\alpha, \mu_\alpha)$is considered an **intuitionistic fuzzy labeling graph (IFLG)**if it is bijective. In the sense that the vertices and edges have different MS and NMS values, and$\mu_{\alpha_1}(v_i, v_j) < \min\{\sigma_{\alpha_1}(v_i), \sigma_{\alpha_1}(v_j)\}, \mu_{\alpha_1}(v_i, v_j) < \max\{\sigma_{\alpha_2}(v_i), \sigma_{\alpha_2}(v_j)\}, 0 \le \mu_{\alpha_1}(v_i, v_j) + \mu_{\alpha_2}(v_i, v_j) \le 1$ for each edge$(v_i, v_j)$.

**Definition 2.3** A walk in a graph is a finite, non-null subspace with words that alternately represent vertices and edges, $W = v_0 e_1 v_1 e_2 \dots e_k v_k$.$W$ is a $(v_0, v_k)$ walk, as we say. When a walk $W$ has distinct vertices, it is called a **path**. The term "**cycle**" refers to a closed trail with the same starting and ending points.

**Definition 2.4** The complete bipartite graph $K_{1,n}$ is known as a star $S_n$, which is a tree with one internal node and n leaves.
**3. Intuitionistic Fuzzy Cube Difference Graph**
**Definition 3.1**An IFG $G = (V, \sigma_\alpha, \mu_\alpha)$ is said to be **Intuitionistic Fuzzy Cube Difference Labeling Graph (IFCDLG)** if it is an Intuitionistic Fuzzy Labeling Graph (IFLG) and satisfies the following conditions,$\mu_{\alpha_1}(u_s, v_t) = |\sigma_{\alpha_1}(u_s)^3 - \sigma_{\alpha_1}(v_t)^3|, \mu_{\alpha_2}(u_s, v_t) = |\sigma_{\alpha_2}(u_s)^3 - \sigma_{\alpha_2}(v_t)^3|, 0 \le \mu_{\alpha_1}(u_s, v_t) + \mu_{\alpha_2}(u_s, v_t) \le 1$ for each$(u_s, v_t) \in E$ are all distinct. The term IFCDLG refers to a graph that is an **IFCDL**.

**Example:**
**Figure 1**

**Theorem 3.2**Path $P_n$ is an IFCDG for all $n \ge 1$.
**Proof:**
Let $P_n$ be any path with length $n \ge 1$, and$v_1, v_2, \dots, v_n$ and $v_1v_2, v_2v_3, \dots, v_{n-1}v_n$are nodes and edges of$P_n$.
Choose the MS value $\omega_1 \to (0,1]$ such that

$\omega_1 = 10^{-1}$ for $n \leq 9$,$\omega_1 = 10^{-3}$for $10 \leq n \leq 99$, $\omega_1 = 10^{-5}$for $100 \leq n \leq 999$ and so on.
Choose the NMS value $\omega_2 \rightarrow (0,1]$ such that
$\omega_2 = 10^{-2}$ for $n \leq 99$,$\omega_2 = 10^{-4}$for $100 \leq n \leq 999$, $\omega_2 = 10^{-6}$for $1000 \leq n \leq 9999$ and so on.
The following are the labels given to nodes and connections, MS values:

$\sigma_{\alpha_1}(v_i) = i * \omega_1,$ $i = 1,2,\dots,n$
$\mu_{\alpha_1}(v_1v_2) = 7 * \omega_1^3,$
$\mu_{\alpha_1}(v_iv_{i+1}) = (6\omega_1^3 * i) + \mu(v_{i-1}v_i),\ i = 2,3,\dots,n-1$
The NMS values of nodes and connections have been given the following labels:

$\sigma_{\alpha_2}(v_i) = i * \omega_2,$ $i = 1,2,\dots,n$
$\mu_{\alpha_2}(v_1v_2) = 7 * \omega_2^3,$
$\mu_{\alpha_2}(v_iv_{i+1}) = (6\omega_2^3 * i) + \mu(v_{i-1}v_i),\ i = 2,3,\dots,n-1$
Each node and connection has a different MS and NMS value.

This labeling obeys the conditions ofIFCDL.
Therefore, every path $P_n$ is an IFCDG.
**Example:**
**Figure 2: IFCDG of $P_9$**

**Theorem 3.2** Everycycle$C_n$, $(n \geq 3)$ is an IFCDG.
**Proof:**
Let $\{v_1, v_2, \dots, v_n\}$ be the set of vertices and $\{v_1v_2, v_2v_3, .., v_{n-1}v_n, v_nv_1\}$ be the set of edges.
Choose the MS value$\omega_1 \rightarrow (0,1]$ such that
$\omega_1 = 10^{-2}$ for $n \leq 99$,$\omega_1 = 10^{-4}$for $100 \leq n \leq 999$, $\omega_1 = 10^{-6}$for $1000 \leq n \leq 9999$ and so on.
Choose the NMS value $\omega_2 \rightarrow (0,1]$ such that
$\omega_2 = 10^{-3}$ for $n \leq 999$,$\omega_2 = 10^{-5}$for $1000 \leq n \leq 9999$, $\omega_2 = 10^{-7}$for $10000 \leq n \leq 999999$ and so on.
The following labels have been given to the nodes and connections MS values:

$\sigma_{\alpha_1}(v_i) = i * \omega_1,$ $i = 1,2,\dots,n$
$\mu_{\alpha_1}(v_1v_2) = 7 * \omega_1^3,$
$\mu_{\alpha_1}(v_iv_{i+1}) = (6\omega_1^3 * i) + \mu(v_{i-1}v_i),\ i = 2,3,\dots,n-1$
$\mu_{\alpha_1}(v_nv_1) = (n^3 - 1) * \omega_1^3,$
Nodes and connections NMS values are labeled as follows:

$\sigma_{\alpha_2}(v_i) = i * \omega_2,$ $i = 1,2,\dots,n$
$\mu_{\alpha_2}(v_1v_2) = 7 * \omega_2^3,$
$\mu_{\alpha_2}(v_iv_{i+1}) = (6\omega_2^3 * i) + \mu(v_{i-1}v_i),\ i = 2,3,\dots,n-1$
$\mu_{\alpha_2}(v_nv_1) = (n^3 - 1) * \omega_2^3.$
Each node and connection has a different MS and NMS value.

This labeling obey the conditions ofIFCDL.
Thus every cycle graph is an IFCDG.
**Example:**
**Figure 3: IFCDG of $C_4$**

**Theorem 3.3**Every star graph $S_{1,n}$ is anIFCDG.

**Proof:**

Let $\{v_1, v_2, \ldots, v_{n+1}\}$ be the set of vertices and $\{v_1v_2, v_1v_3, .., v_1v_{n+1}\}$ be the set of edges.

Choose the MS value $\omega_1 \rightarrow (0,1]$ such that

$\omega_1 = 10^{-2}$ for $n \leq 99$,$\omega_1 = 10^{-4}$for $100 \leq n \leq 999$, $\omega_1 = 10^{-6}$for $1000 \leq n \leq 9999$ and so on.

Choose the NMS value $\omega_2 \rightarrow (0,1]$ such that

$\omega_2 = 10^{-3}$ for $n \leq 999$,$\omega_2 = 10^{-5}$for $1000 \leq n \leq 9999$, $\omega_2 = 10^{-7}$for $10000 \leq n \leq 999999$ and so on.

The following labels have been given to the nodes and connections MS values:

$\sigma_{\alpha_1}(v_i) = i * \omega_1, \qquad 1 \leq i \leq n$

$\mu_{\alpha_1}(v_1v_i) = (i^3 - 1) * \omega_1^3, \qquad 2 \leq i \leq n$

The NMS values of nodes and connections have been given the following labels:

$\sigma_{\alpha_2}(v_i) = i * \omega_2, \qquad 1 \leq i \leq n$

$\mu_{\alpha_2}(v_1v_i) = (i^3 - 1) * \omega_2^3, \qquad 2 \leq i \leq n$

Each node and connection has a different MS and NMS value.

This labeling obey the conditions of IFCDL.

Thus every star graph is an IFCDG.

**Example:**

**Figure 4: IFCDG of $S_{1,5}$**

## CONCLUSION

We introduced IFCDGs and studied their properties in path, cycle, and star graphs. This work extends fuzzy graph theory and highlights the potential of intuitionistic fuzzy models in representing uncertainty. We hope it encourages further exploration in this area.

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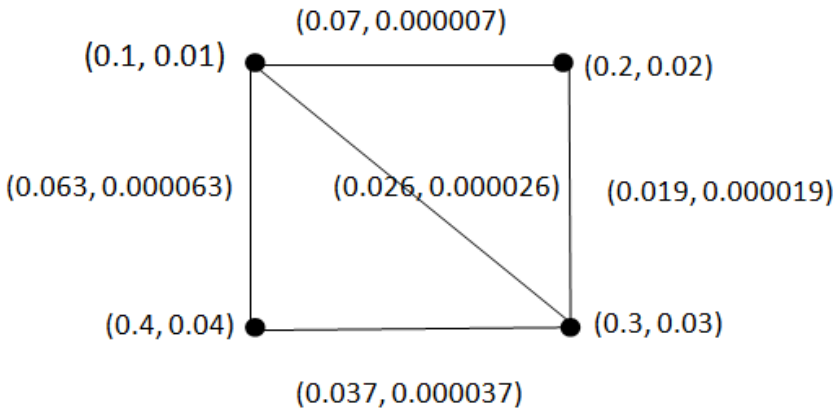



**Figure 1**

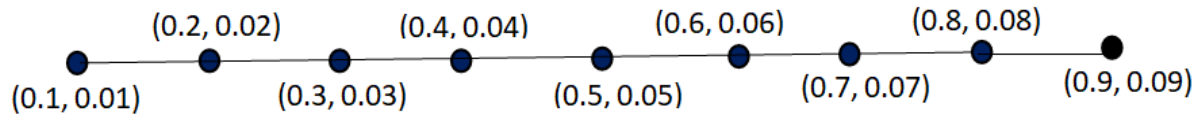



**Figure 2: IFCDG of $P_9$**

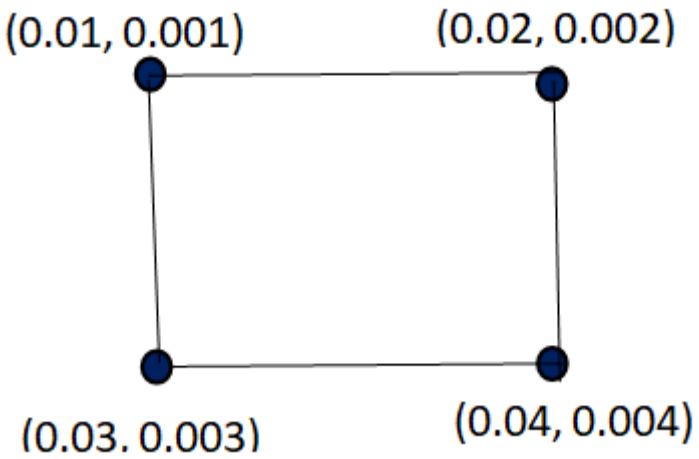



**Figure 3: IFCDG of $C_4$**

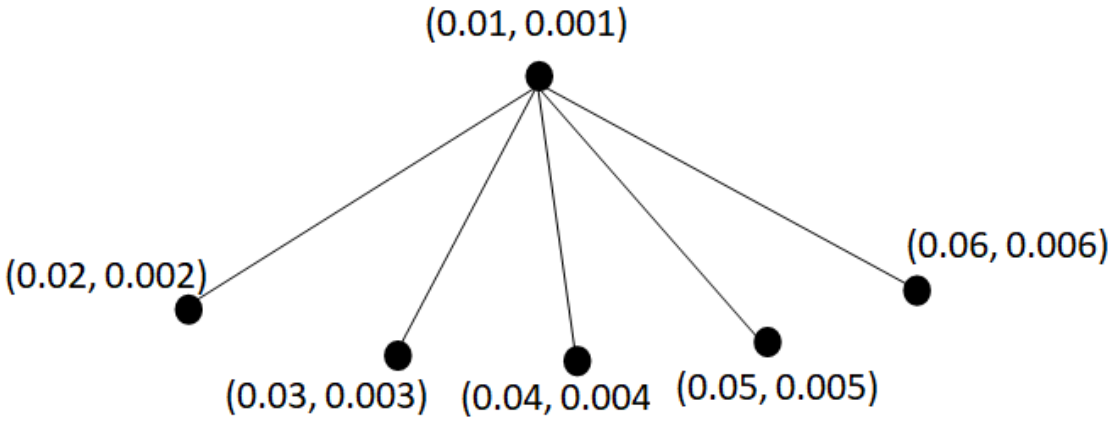



**Figure 4: IFCDG of $S_{1,5}$**

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RESEARCH ARTICLE

# AI - Augmented Teaming: Redefining E-Collaboration and Human-AI Synergy in the Post - Digital Workplace

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**ABSTRACT**

This article addresses how AI-augmented teaming is reshaping e-collaboration with new types of accountable, dynamic partnerships between humans and AI systems. Using randomized trials, field experiments, and meta-analyses from a large body of work across a range of problem domains such as customer support, software engineering, healthcare, and education, it shows that AI can substantially augment human productivity, quality, and learning among less-experienced workers. But the benefits are limited by an increasingly "jagged frontier" of model capability: performance improvements are contingent on task fit, oversight, and governance. Counterintuitively, unrestructured AI will decrease decision accuracy and humancapability to grow skills without proper guardrails. This article identifies the key elements of teamwork that are derived from task decomposition, calibrated trust, transparent interfaces, and learning-preserving designs, and links these to governance frameworks like NIST AI RMF, EU AI Act, Iso/IEC 42001, etc. Drawing on empirical research and design practice, it claims that sustainable human-AI collaboration necessitates a realignment of capabilities with accountability, shifting from tool use to principled, resilient teaming in the post-digital workplace.

**Keywords:** Teaming, Digital Workplace, AI, education,

## INTRODUCTION

Across industries and institutions, AI-augmented teaming is shifting e-collaboration from simple tool use to tightly coupled human–machine partnerships that change how teams learn, decide, and produce. In this chapter, we define

AI-augmented teaming, benchmark its measurable effects on productivity, quality, and learning, and synthesize governance and design guidance needed to realize durable, equitable gains at scale. We ground the analysis in recent randomized and field experiments in knowledge work, software engineering, healthcare delivery, and education; systematic reviews of human–AI synergy; and emerging governance frameworks (NIST AI RMF 1.0, EU AI Act, ISO/IEC 42001), balancing performance results with risks around bias, transparency, and trust. AI-augmented teaming is already boosting throughput and quality on well-scoped tasks, especially for less-experienced workers, but it can degrade decision accuracy outside the technology's "jagged" capability frontier and can harm learning when guardrails are weak. Effective practice therefore hinges on targeted task design, calibrated trust, robust evaluation, and governance-by-design to align human expertise with model strengths in accountable ways. This is not simply about faster outputs; it is about building teams that are resilient to error, inclusive of diverse skills, and deliberately governed for safety and equity in the post-digital workplace.

**Defining AI-Augmented Teaming and Its Relevance**

Human–AI teaming (HAIT) is best understood as a human-centered, socio-technical form of collaboration in which humans and AI systems function as interdependent teammates with distinct roles, complementary capabilities, and shared goals. This teaming emphasizes calibrated trust, shared mental models, and adaptive role reallocation as context evolves, with explicit attention to human wellbeing and productivity. Scoping reviews synthesize this definition across disciplines, highlighting eleven dimensions: interdependence, shared goals, AI autonomy, dynamic adaptation, complementary strengths, calibrated trust, explainability, shared situational awareness, team communication, human-centered design, and socio-technical integration. These elements distinguish teaming from mere tool use and anchor designs for sustainable performance gains. In practice, HAIT leverages human creativity, context, and ethics with AI's speed, scale, and pattern detection to handle complexity under uncertainty, provided that oversight, transparency, and task fit are well managed(Abramoff et al., 2023).

**The Post-Digital Workplace**

The post-digital workplace is characterized by pervasive cloud collaboration, integrated data flows, and ubiquitous model access (including generative AI). This environment increases coordination bandwidth but also raises stakes for governance and sociotechnical fit. Evidence shows that generative assistants can raise throughput in support roles by roughly 15% on average while disproportionately helping less-experienced workers, compressing time-to-competence and smoothing experience curves(Noy & Zhang, 2023). Yet the same technology can degrade decision accuracy when applied to tasks beyond current capability frontiers, or undermine skill acquisition in education settings if designs encourage answer-taking rather than guided discovery. These dualities underscore that "AI-augmented" must mean "well-matched and well-governed," not "always faster." Organizational frameworks like NIST AI RMF, the EU AI Act, and ISO/IEC 42001 now provide a backbone to align performance, safety, and accountability across the AI lifecycle(National Institute of Standards and Technology, 2023). AI-Augmented Teaming is revolutionizing e-collaboration and human–AI synergy by lifting throughput and quality on tasks inside the technology's capability frontier, with especially large gains for novices and mid-skill professionals. However, achieving durable, fair, and safe gains requires careful task decomposition, calibrated trust and transparency, and governance practices that map, measure, and manage AI risks across contexts.

**Theoretical Foundations**

**From HCI and CSCW to Human–AI Teaming**

The HCI community has long articulated design guidance for interactive AI, consolidating 18 guidelines spanning initial interaction, ongoing use, handling errors, and system evolution, emphasizing proactive transparency, appropriate control, and graceful failure behavior. These guidelines inform team-aware interfaces that set expectations, expose uncertainty, and support user correction during routine collaboration, foundational for calibrated trust in team settings. In CSCW, the focus on coordination, common ground, and artifacts aligns with shared mental models and communication loops that mixed teams need to avoid miscalibration and drift. Together, HCI and CSCW provide the interactional and organizational substrate for HAIT, while contemporary teaming research reframes AI from tool to teammate with defined roles and interdependence (Kluge et al., 2023)

**AI in Teamwork and Collaboration: Evidence and Limits**

Recent field and lab studies demonstrate substantial productivity gains on creative and content-heavy knowledge tasks, with heterogeneous effects by worker skill and task type. In customer support, access to a GPT-based assistant increased issues resolved per hour by roughly 15% on average across 5,172 agents and 3 million chats, with the largest improvements among less experienced workers; newer agents with two months' tenure plus AI matched the productivity of >6-month untreated agents. Speed gains for top performers came with slight declines in conversation quality, whereas novices improved both speed and quality, suggesting a convergence effect. Escalations fell and customer politeness rose, illustrating not only faster output but better interactional dynamics in certain contexts. This pattern reinforces that HAIT can raise team-level outcomes when workflows fit model strengths and when oversight retains human judgment in edge cases (Brynjolfsson, Li, & Raymond, 2023). Conversely, a systematic review and meta-analysis of 106 experiments (370 effect sizes) found human–AI teams on average underperform relative to the best individual (human or AI), with particularly negative synergy on decision tasks and when AI outperforms humans on its own. The authors argue that synergy demands explicit subtask delegation to the agent best suited for each component, robust evaluation metrics (time, cost, error impact), and standardized commensurability criteria across studies to meaningfully compare results(Vaccaro et al., 2024). This reinforces the idea that "just combine human and AI" is insufficient; the team process must be redesigned to leverage complementarity rather than aggregate judgments in ways that compound errors or overrule the stronger partner.

**AI-Augmented Teaming: Key Concepts and Components**

**Key Concepts**

AI-augmented collaboration refers to workflows in which AI systems contribute intermediate artifacts, suggestions, or decisions that humans integrate, critique, and adapt toward shared goals. Human–AI synergy is achieved when the combined system outperforms its strongest component on relevant metrics (e.g., quality under cost constraints), a condition that is not guaranteed and varies by task mode (creation vs. decision) and decomposability. Intelligent team assistants span copilots, recommenders, and autonomous modules capable of local decisions, interfacing through natural language, structured templates, or domain-specific UIs; their efficacy depends on transparency, uncertainty displays, and controls for feedback and correction. AI-driven decision-making covers advisory through autonomous configurations, requiring clarity on human-in-the-loop roles, escalation protocols, and auditability of rationales and outcomes to sustain trust and accountability in team contexts (Vössing et al., 2022).

**Core Components**

Algorithms and Models: Foundation models adapted via fine-tuning, retrieval augmentation, and tool use can deliver rapid ideation, summarization, classification, and code generation. However, capability is "jagged": seemingly similar tasks can fall inside or outside the current frontier, so pipelines must explicitly route subtasks to human or AI based on expected performance. Field experiments with consultants show >40% quality gains and ~25% faster completion for tasks inside the frontier, but a 19-point drop in correctness outside it, illustrating the stakes of capability mapping in team workflows (Dell'Acqua et al., 2023). Data Analytics and Visualization: Effective teaming requires analytics that surface uncertainty, coverage, and residual risk, not just point predictions, enabling calibrated reliance. In operations settings (e.g., digital twins), combining simulation, visualization, and AI copilots has accelerated design and shop-floor productivity, with reported 30% productivity gains and 50% faster time-to-market for certain workflows, though such figures should be contextualized as vendor-reported and task-specific (NVIDIA, 2025). Human–AI Interface Design: Interaction design must make capabilities and limits legible, offer granular control, and handle errors gracefully. Proactive transparency, uncertainty displays, and quick "escalate-to-human" paths reduce over-trust and prompt effective oversight. These principles build directly on established HCI guidance for human–AI interaction and are codified in governance frameworks that require role clarity and oversight patterns for different autonomy levels (Chen, 2022).

**Applications and Case Studies**

**Knowledge Work and Customer Support**

In a staggered rollout covering 5,172 customer-support agents, a GPT-based assistant increased issues resolved per hour by about 15% on average across 3 million chats. Heterogeneity was pronounced: less-experienced agents saw roughly 30% throughput gains and quicker movement down the experience curve, while the most experienced agents gained small speed boosts with slight quality declines. Resolution rates and NPS did not materially change, but escalations fell and customers were more polite, suggesting that AI support can simultaneously improve throughput and interaction quality when paired with human judgment and escalation protocols. This is a canonical example of AI-augmented teaming boosting e-collaboration efficiency without harming customer experience (Noy & Zhang, 2023).

**Professional Writing and Consulting**

A preregistered experiment of 453 college-educated professionals performing mid-level writing tasks found a 40% reduction in time and an 18% increase in output quality using ChatGPT, with larger gains for weaker writers(Noy & Zhang, 2023). This not only raised average productivity but narrowed performance dispersion—an equity-relevant effect in team composition and development. Complementing this, a field experiment with 758 consultants at a global firm found that for ideation-heavy tasks within AI's frontier, AI users completed 12.2% more tasks, 25.1% faster, and produced >40% higher quality; for a task deliberately outside the frontier, AI users were 19 percentage points less likely to produce correct solutions (Dell'Acqua et al., 2023). These studies together quantify the opportunity and the boundary conditions: map tasks to frontier, design guardrails, and train teams to "delegate and decide" rather than "defer and accept".

**Software Engineering**

A controlled experiment with GitHub Copilot showed developers completed a standard programming task 55.8% faster than controls, indicating substantial speed gains for well-specified tasks with immediate feedback and verifiable tests. While speed is evident, team-level benefits hinge on code review, testing, and maintainability policies—i.e., collaborative processes that absorb AI outputs into shared quality standards and knowledge repositories(Peng et al., 2023). This case underscores how team scaffolding (code review, tests, documentation) translates individual speedups into sustainable team productivity.

**Healthcare Delivery**

In a preregistered, double-masked, cluster-randomized trial in retina clinics, autonomous AI for diabetic eye exams increased specialist productivity by about 39.5% (0.45 more completed encounters/hour), with significant gains in a high-demand, unscheduled environment. The trial's design—masking, cluster randomization by clinic day, and prospective registration—strengthens causal inference and shows that carefully integrated autonomous modules can expand specialist capacity without compromising clinical control. This is a clear instantiation of human–AI teaming: AI performs standardized screening, freeing specialists for higher-complexity care, inside a governance structure that preserves accountability and escalation(Abràmoff et al., 2023).

**Education**

Evidence in education is nuanced. A crossover randomized study in an introductory physics course found that a custom AI tutor produced significantly higher and faster learning gains than active in-class learning, with effect sizes up to ~0.73–1.3 SD by quantile regression after controls(Kestin et al., 2025). By contrast, a large high school field experiment found that unfettered GPT-4 access improved practice performance but harmed subsequent assessments without AI, indicating reduced skill acquisition; importantly, guardrails that enforced teacher-designed hints instead of direct answers mitigated these negative effects. For team-based learning and co-creation, the implication is to design AI roles as scaffolds—not crutches—so human skills compound over time while leveraging AI's capacity for feedback, explanation, and formative (Liu et al., 2024).

**Manufacturing and Industrial Collaboration**

Industrial copilots and Omniverse-powered digital twins are enabling faster design-to-production loops and augmented shop-floor teaming. Reported results from Siemens and NVIDIA include a 30% increase in shop-floor productivity with copilot assistants and a 50% reduction in time-to-market for marketing materials, alongside massive GPU-accelerated simulations equivalent to >10,000 CPU cores—illustrating how visualization, simulation, and AI assistance co-evolve collaboration artifacts. While vendor-reported outcomes require independent validation, they point to a teaming model in which AI agents, digital twins, and human operators coordinate across design, test, and execution, with transparency and override at the human level (NVIDIA, 2025).

**Calculated Statistics: Cross-Study Synthesis**

To contextualize effects across domains, we synthesize consistent metrics:

Throughput increases (team-relevant): Customer support +15.2% (resolutions/hour); specialist clinic +39.5% (encounters/hour); industrial shop-floor +30% (reported). Simple average = 28.2% (range 15.2%–39.5% for peer-reviewed; vendor report 30%). Interpretation: On throughput-measured workflows inside the frontier and with escalation paths, 15%–40% gains are credible and replicable across support and clinical screening; industrial reports suggest similar magnitude but should be independently verified(Noy & Zhang, 2023; Abràmoff et al., 2023; NVIDIA Corporation, 2024). Speed gains on knowledge tasks: Software task +55.8% completion speed; consulting tasks +25.1% faster inside frontier; writing tasks −40% time (equivalent to ~66.7% speed-up if converting time to throughput). Unweighted average of directly reported speed gains (55.8% and 25.1%) = 40.5%; including the implied throughput from the 40% time reduction yields 49.2%. Interpretation: For well-specified, feedback-rich tasks (coding, structured ideation), 25%–55% speed gains are typical, with higher uplifts when evaluation is immediate and failures are cheap.(Peng et al., 2023).

Quality improvements: Writing +18% quality; consulting >+40% quality inside frontier. Simple midpoint ≈ +29%. Interpretation: On creation tasks within frontier, quality uplift accompanies speed gains; outside frontier, quality and correctness can decline materially, underscoring the need for task mapping and human oversight (Noy & Zhang, 2023; Dell'Acqua et al., 2023). Equity/heterogeneity: Novices benefit more. Customer support novices gained ~30% throughput vs. small speed-only gains with slight quality declines for experts; writing study narrowed productivity inequality; consulting study showed both below- and above-average performers gained, but with steeper gains below average.

Interpretation: AI-augmented teaming can compress skill disparities, accelerating onboarding and elevating floor performance—if guardrails protect decision quality (Dell'Acqua et al., 2023).

Method notes: Wereport simple averages and ranges across peer-reviewed or preregistered studies with comparable metrics,and avoid pooled meta-estimates given heterogeneity of designs and outcomes. The Nature Human Behaviour meta-analysis provides the most rigorous cross-study aggregation and cautions that unstructured combinations often underperform the best single agent, especially on decision tasks—hence the emphasis here on task decomposition and calibrated teaming (Vaccaro et al., 2024).

**Challenges and Future Directions**

**Data Quality and Bias**

Bias can arise from data sampling, labeling, and deployment contexts; in teaming, biased outputs can be amplified by over-trust and miscalibration. The NIST AI RMF defines seven socio-technical characteristics of trustworthy AI: Valid/reliable, safe, secure/resilient, accountable/transparent, explainable/interpretable, privacy-enhanced, and fair with harmful bias managed, framing multi-stakeholder criteria for risk treatment. Team designs should expose uncertainty, allow contestation, and ensure data lineage and audit logs to detect and correct drift and disparate impacts over time(National Institute of Standards and Technology, 2023).

**Human–AI Trust and Transparency**

Meta-analytic evidence indicates that naïve combinations underperform without process redesign; transparency alone (e.g., confidence displays) may not fix miscalibration. Teams need workflows that allocate subtasks to the better partner, plus interfaces that clarify limits and provide reasons or exemplars when appropriate (Vaccaro et al., 2024). Clear escalation protocols and role definitions—human-in-the-loop, AI-assisted, autonomous with human oversight—are essential to avoid both abdication and micromanagement, aligning with NIST guidance on human roles in decision-making configurations (National Institute of Standards and Technology, 2023).

**Job Displacement and Workforce Development**

Short-run patterns suggest uplift for less-experienced workers in many tasks (compression toward the mean), but long-run impacts depend on reskilling and role evolution. Education evidence warns that unguided answer-giving harms learning, while structured AI tutors can accelerate it; applied to the workplace, teams should embed AI as guided scaffolds that accelerate mastery (e.g., code review suggestions with rationales, writing assistants that explain revisions) rather than replace learning loops. Organizations should invest in AI collaboration literacyencompassing skills inprompting, critique, verification, and escalationto ensure durable capability building (Kestin, G., et al. 2025).

**Governance and Regulation**

NIST AI Risk Management Framework (AI RMF 1.0) NIST7: Provides four core functions - GOVERN, MAP, MEASURE, MANAGEto embed risk management culture, frame system context, assess/monitor risks, and allocate treatment resources. It also clarifies human–AI interaction configurations and accountability structures to operationalize trustworthy AI in teams(National Institute of Standards and Technology, 2023).

EU AI Act (Regulation (EU) 2024/1689) EU AI Act8: In force since August 1, 2024, with full applicability by August 2026, the Act classifies systems by risk (prohibited, high-risk, limited, minimal),imposing obligations on high-risk systems: risk management, data governance, technical documentation, logging, transparency, human oversight, robustness, and post-market monitoring—directly relevant to AI modules embedded in team workflows (European Union, 2024). ISO/IEC 42001:2023 (AI Management Systems) ISO/IEC 420019: Specifies requirements for establishing, implementing, maintaining, and continually improving an AI Management System (AIMS) via PDCA cycles across organizations of any size, aligning policy, roles, training, risk assessment/treatment, monitoring, and continual improvement—operational scaffolding for sustained, auditable human–AI teaming (ISO/IEC, 2023).

**Future Research and Practice**

Task Decomposition and Routing: Systematically map work into subtasks and assign to humans or AI based on measured comparative advantage; study routing policies that adapt as model capabilities and human expertise evolve, and quantify team-level synergy under budgeted cost-of-error metrics (Vaccaro et al., 2024). Learning-Preserving Designs: Codify guardrails that force explanation, scaffolded hints, and reflective prompts to preserve human learning, preventing dependency while leveraging AI's formative feedback strengths; measure long-horizon skill acquisition and error-checking gains in teams.(Kestin, G., et al. 2025). Equity and Inclusion: Quantify how AI shifts performance distributions and career progression, especially for underrepresented groups; design mentorship-like AI supports with transparency and accountability to avoid stereotyping or differential calibration(Noy & Zhang, 2023). Robust Metrics and Reporting: Implement multi-dimensional evaluation (time, quality, cost, error criticality) and open reporting repositories for reproducibility and commensurability across team studies, in line with meta-analytic recommendations (Vaccaro et al., 2024).

## CONCLUSION

AI-augmented teaming is not a monolith; it is a design space. The most reliable gains occur on creation and content-heavy tasks that are inside the current frontier and can be decomposed with rapid feedback, where studies show 25%–55% speed improvements and ~18%–40% quality gains. Throughput gains of ~15%–40% materialize in structured support and clinical screening contexts, with especially large benefits for newer team members. However,

when tasks step outside the frontier, decision accuracy can fall sharply, and when AI is introduced without learning-preserving guardrails, human skill acquisition can be compromised. The path forward is a principled one: map tasks to capability, decompose and route subtasks, make uncertainty legible, codify escalation and oversight, and embed governance via NIST AI RMF, EU AI Act, and ISO/IEC 42001. In doing so, organizations can turn point gains into durable team capabilities—boosting efficiency and innovation while maintaining safety, fairness, and trust in the post-digital workplace.

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**RESEARCH ARTICLE**

# *Ditrichum palaniensis* (Bryophyta: Ditrichaceae) – A New Species from the Western Ghats of Peninsular India

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## ABSTRACT

*Ditrichum palaniensis* is described here as a new species of moss from the Palani Hills in the Southern Western Ghats. The unique features of this species are the presence of leaves that are not folded inwards along the costa, margin recurved at middle, costa being percurrent to slightly ending below apex, perichaetial leaves being different from the vegetative leaves and peristome teeth being fimbriate-papillose, very narrow and high, 0.34 to 0.36 mm. A detailed description, a photographic plate and a map showing the location of the holotype are provided.

**Keywords:** Palani Hills, *Ditrichum pusillum*, mixed plantations, moss, road-side cut

## INTRODUCTION

*Ditrichum* Hampe is a genus of moss with 56 species world-wide. They are mostly distributed in the tropical and temperate regions of the World (Gangulee, 1971; Brinda and Atwood, 2023). In India, so far 12 species and 2 infra-specific taxa have been reported (Lal, 2005; Dandotiya and al., 2011). Of these, 3 species and one infra-specific taxon occur in the Western Ghats (Daniels, 2010). The present study on the bryoflora of the Palani Hills, initiated a couple of years ago, led to the discovery of a *Ditrichum* species which after a critical study was found to be a novelty. This discovery adds one more species to this genus. Hence, a description, a photographic plate and a location map are provided.

## MATERIALS AND METHODS

Investigations were carried out during and after the monsoon rains. Samples collected were studied under an Olympus SZ51 binocular stereomicroscope and photographs of relevant taxonomic features were taken using an Olympus BX43 compound research microscope. Identification was made based on monographs and recent publications as well as consulting overseas experts. Specimens were preserved following traditional methods and deposited at the herbarium of Scott Christian College (SCCN).

**Diagnosis**

*Ditrichum palaniensis* is closely related to *D. pusillum* (Hedw.) Hampe, but differs from the latter in possessing leaves that are not folded inwards along the costa, margin recurved at middle, costa being percurrent to slightly ending below apex, perichaetial leaves being different from the vegetative leaves and peristome teeth being fimbriate-papillose, very narrow and high, 0.34 to 0.36 mm when compared to that of *D. pusillum* which has a peristome teeth which is broader and c. 0.14 mm high. These features distinguish the former from all the known species of *Ditrichum* and hence, it is described here as new.

**Etymology**

Named after Palani Hills from where the specimen was collected.

**Taxonomic Treatment**

***Ditrichum palaniensis*** A.E.D. Daniels, D.T.T. Daniels and C.P. Ben, sp. nov. (Fig. 1)

**Holotype:** India, Western Ghats, Tamil Nadu, Dindigul District, Palani Hills, on the way to Pillar Rock, c. 2200 m, terricolous, on road-side cuts, in mixed plantations, 10°12'43.72"N and 77°27'54.01"E, 6 November, 2023, *A.E.D. Daniels* and *D.T.T. Daniels 224* (holotype: CAL; isotype: SCCN; paratype: SCCN)

Plants dioicous?, 3–7 mm tall, in loose mats, pale green. Stems simple or branched, 0.18–0.2 × 0.14–0.16 mm in cross section, with a central strand, but always disintegrated, brownish; cortex 1–3-layered; cells 5–10 × 4–10 µm, thick-walled; medullary cells 15–25 × 10–20 µm, thin-walled. Leaves erectopatent, flexuose when dry, 1.4–2.0 × 0.4–0.6 mm, triangular at base, entire, inrolled at middle, long-acuminate, faintly denticulate at apex; apical cells 10–30 × 8–12 µm, quadrate to rectangular; median cells 18–30 × 7–14 µm, rectangular, thick-walled; basal cells 30–50 × 12–25 µm, elongate-rectangular to subrectangular, thick-walled; costa percurrent to ending a little below apex. Sporophytes apical. Perichaetial leaves c. 2.7 × 0.27 mm, linear-oblong, entire, long-acuminate at apex. Setae 8–10 mm high, erect, slightly bent above middle. Capsules erect, cylindric, 2.3–2.5 × 0.35–0.45 mm. Operculum conic, 0.8–1.2 × 0.35–0.45 mm, not rostrate. Peristome teeth 1-rowed, 0.4–0.5 × 0.016–0.018 mm, dicranate, long and slender, highly fimbriate-papillose, yellowish-brown above, yellowish-brown to reddish-brown towards base. Annulus differentiated, 2-rowed; cells 25–40 × 15–20 µm. Spores 8–9 µm, globose, papillose, pale brown.

*Habitat*: Terricolous, on road-side cuts in mixed plantations, c. 2200 m.

**Distribution:** India: Western Ghats of Tamil Nadu (Dindigul).

**Specimens examined:** India, Western Ghats, Tamil Nadu, Dindigul District, Palani Hills, on the way to Pillar Rock, c. 2200 m, terricolous, on road-side cuts, in mixed plantations, 10°12'43.72"N and 77°27'54.01"E, 6 November, 2023, *A.E.D. Daniels* and *D.T.T. Daniels 224, 225* (SCCN). (Fig. 2)

**Ecological notes:** *Ditrichum palaniensis* was found growing as a terricolous form (on soil) in a mixed plantation at an elevation of c. 2200 m.

## DISCUSSION

Palani Hills happens to be one of the most explored areas in the Western Ghats for plants since the arrival of the Colonial Europeans in the late 18th and early 19th centuries. Robert Wight was the first to initiate explorations in September 1836 when he collected approximately 1500 species in 15 days. Subsequently, several others namely A.G. Bourne (1897–1898), P.F. Fyson (since 1904) and the Shenbaganur team explored the Palani Hills for angiosperms and pteridophytes. However, it was Eugène Armand-dit Georges Foreau who organized a team in 1908 for collecting nonflowering plants, especially mosses. The moss collections were named by Jules Cardot (1860 - 1934) and Robert André Leopold Potier de la Varde (1878–1961) of the Paris Museum, and Hugh Neville Dixon (1861–1944) of the Natural History Museum, London (Matthew, 1999). As many as 95 species and 15 varieties of this collection were described as new to science (Potier de la Varde 1922, 1923, 1924, 1925) and Dixon and Potier de la Varde (1927, 1930). These findings were synthesized by Foreau (1930, 1931, 1961, 1964). Subsequently, several Indian botanists started making explorations which were all sporadic. However, Alam and Srivastava (2009) published a checklist on the liverwort diversity of Palani Hills where they enumerated 75 species with 16 as new records for the region and, Alam and al. (2011) published a checklist of mosses of Palani Hills comprising 54 species of mosses with 12 as new to Palani Hills. Daniels (2010) published a comprehensive list of bryophytes of Tamil Nadu where he enumerated 360 species from Palani Hills. Like any other Hill Station on the Western Ghats, Palani Hills have also suffered severe deforestation due to introduction of exotic species for commercial and esthetic purposes by the Colonial Europeans which led to loss of habitats. On the other hand, laying of roads, construction of dams, bridges and buildings were other reasons for habitat destruction which are continuing unabated even today. Almost all sholas are today 'sholas' of concrete structures. Nevertheless, the relicts of the native vegetation are still potential habitats that harbour these elusive plants and so, the remaining native forests of Palani Hills which are in a highly fragmented condition need to be conserved for the sake of these elusive and delicate primitive plants. The occurrence of *Ditrichum palaniensis* in a mixed plantation of exotic species indicates the adaptability of the species as a result of 'struggle for existence'.

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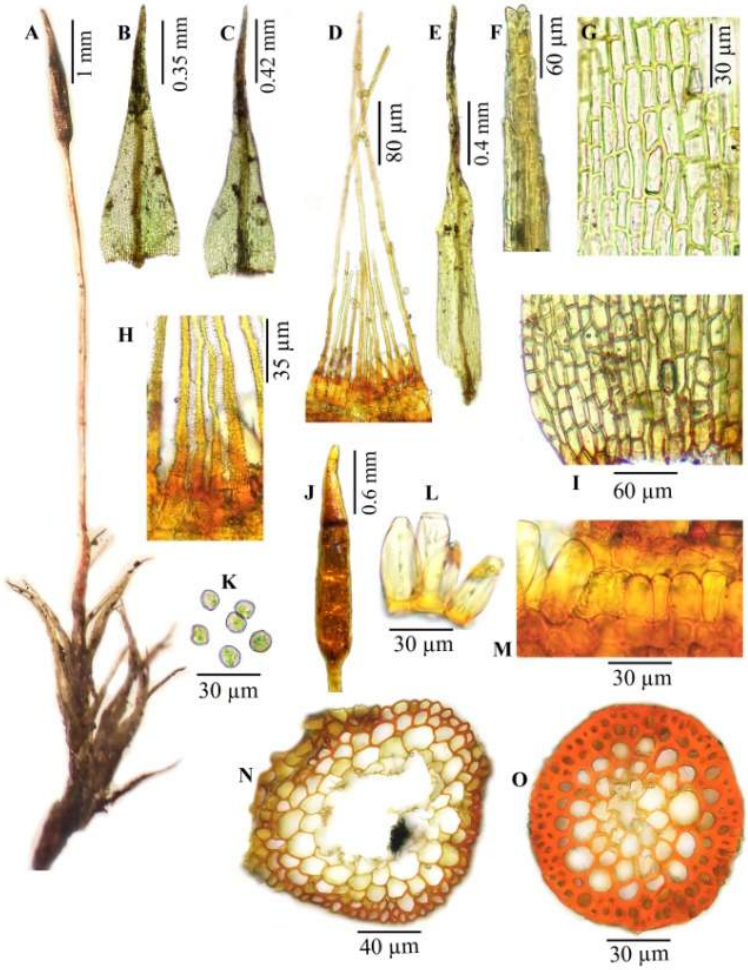



**Fig. 1: (A–O).** ***Ditrichum palaniensis*** **A.E.D. Daniels, D.T.T. Daniels and C.P. Ben**
**A. Plant with sporophyte B–C. Leaves D. Peristome teeth E. Perichaetial leaf F. Leaf apex**
**G. Leaf median cells H. Peristome teeth: a portion enlarged I. Leaf basal cells J. Capsule with operculum K. Spores L–M. Annulus N. Cross section of stem O. Cross section of seta (*A.E.D. Daniels* and *D.T.T. Daniels* 224)**

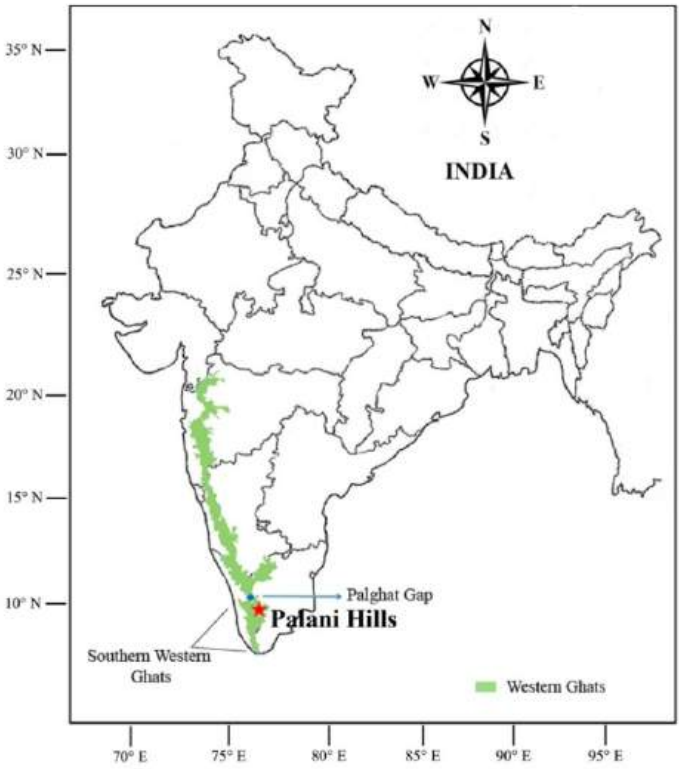



**Fig. 2: Location map of *Ditrichum palaniensis* A.E.D. Daniels, D.T.T. Daniels and C.P. Ben**

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**RESEARCH ARTICLE**

# Formulation and Evaluation of *Leucas aspera* Gel for the Treatment of Fungal Infection

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## ABSTRACT

The objective of the study was to develop the herbal gel utilizing *Leucas aspera* extract and to evaluate its efficacy in treating fungal infections. Ethanol was used to dry, grind and extract the plant *Leucas aspera* by using soxhlet apparatus. To create a smooth herbal gel the extract was combined with a gel base (carbopol) the gels colour consistency, spreadability, pH and texture were all evaluated. The agar diffusion method was used to assess its antifungal activity and see whether it could half the growth of fungi. The prepared gel had a homogeneous smooth appearance. It distributed readily on the skin and had a pH that was suitable for skin. The gel was thoroughly mixed with the extract the gel demonstrated an anti-fungal activity. This study presents a straight forward herbal gel derived from *Leucas aspera.* Since this plant is rarely used in gel form to treat fungal infections, the formulation is novel and beneficial. The *Leucas aspera* gel is simple to use, safe and effective. It can be used as a natural substitute for Pharmaceutical antifungal dosage form.

**Keywords:** *Leucas aspera*, anti-fungal activity, herbal formulation, pharmacological activity, phytochemical screening.

## INTRODUCTION

Fungal infections are some of the most prevalent and enduring dermatological conditions, affecting millions of people globally. In tropical countries like India, superficial fungal infections have become considerably more common due to variables including high humidity, perspiration, poor air circulation, and warm temperatures that accelerate fungal development. The potential pharmacological action of a plant can be predicted by identifying its phytoconstituents[1]. Common fungal agents such aspergillosis, candidiasis, and dermatophytosis (tinea infections) not only cause discomfort but, if managed, can lead to recurrent and chronic infections. Therefore, the development of herbal-based antifungal formulations that offer enhanced safety, biocompatibility, and affordability is becoming more and more popular. According to phytochemical research, *Leucas aspera* strong anti-fungal properties are attributed to a variety of bioactive substances, including flavonoids, terpenoids, tannins, phenolic compounds, and essential oils. *Leucas aspera* is a good natural candidate for topical antifungal medication because research indicates that extracts of the plant have inhibitory action against fungus like *Candida albicans, Aspergillus niger, and Trichophyton rubrum.* The current project aims to develop the herbal anti-fungal gel of *Leucas aspera* using a polymeric gelling agent such as Carbopol 934 and evaluate the gel for various physicochemical and biological characteristics[2]. Drug release is prolonged by carbopol. After extracting the entire plant or leaves and creating a gel with ideal concentrations, properties such as appearance, pH, viscosity, extrudability, spreadability, drug content, stability and *in-vitro* anti-fungal efficacy against certain fungal strains are evaluated. The formulation of Leucas aspera gel has the potential to significantly advance the field of herbal pharmaceutical development by offering an effective natural remedy for fungal skin diseases with fewer adverse effects and more patient acceptability. This study further supports the global trend of incorporating traditional medicinal plants into modern pharmaceutical compositions.

## METHODOLOGY

**Collection and Authentication**

*Leucas aspera* was collected from the side of cultivated land of ethappattu village at melmalaiyanur in villupuram district of Tamilnadu and the plant was authentified by respected Dr.Ayappan.M, Msc,M.Phil.,Ph.D., Assistant professor, Government arts college, Tiruvannamalai.

**Preparation of extract**

The fresh leaves were collected the leaf was shadow dried for upto 7days. The dried leaves are powdered mechanically. Powdered leaves were extracted using ethanol at $50^0$ C on a Soxhlet extractor for 6hrs. The extract was filtered and concentrated under reduced pressure in hot air oven at 40-45°C the resulting crude extract was stored under 4°C. Phytochemical study was carried out on the ethanol extract of *Leucas aspera.* Plant Extraction by Soxhlet Apparatus (Fig 1).

**Phytochemical Screening**

The protocol for phytochemical screening and prospection, investigating the presence of alkaloids, flavonoids, terpenoids, tannins, carbohydrates, phenols, and cardiac glycosides. The qualitative analysis was based on precipitation reactions and the formation of colour. Generally, alkaloids are valued for their therapeutic effect like antifungal, anticancer, antibacterial, antiasthma properties.

**Alkaloids**

**Test for Alkaloids (Table 1).**

**Flavanoids**

**Test for flavonoids (Table 2).**

**Terpenoids**
Test for Terpenoids (Table 3).

**Tannins**
Test for Tanins (Table 4).

**Carbohydrates**
Test for Carbohydrate (Table 5).

**Cardiac Glycosides**
Test for Glycosides (Table 6).

**Phenol**
Test for Phenol (Table 7).
Formulation of Gel (Table 8).

**Preparation of Gel**
A weighed amount of carbopol dispersed in 50ml of distilled water with gentle stirring to avoid clumping. After 1hour, stir the carbopol dispersion continuously for 30mins to obtain a homogenous mixture. In 5ml of distilled water, 0.2g of methyl paraben and 5ml of propylene glycol dissolved by heating on water bath. After cooling, 5ml of herbal extract and 3ml of glycerine was mixed. Then the above mixture is mixed properly to the carbopol dispersion with continuous stirring. Triethanolamine added drop wise to the formulation to adjust the $p^H$(4.5 to 7) and to obtain the desired consistency. Finally, 1ml of rose oil was added to the formulation, then store the prepared gel in air tight container.

Preparation of Gel (fig: 2).
Five Formulations (Fig: 3)

**Evaluation of Gel**
**Colour**
One gram of the gel was taken and put on a clean glass slide in order to see its color. The gel was then observed visually for its colour against a white background under natural day light. After that, the gel's color was noted.

**Odour**
0.5 g of the gel was taken in a clean container, and under typical room conditions, the air above the sample was softly breathed to determine the gel's odor. The smell that was detected was classified as either unpleasant, odorless, pleasant or distinctive.

**Spreadability**
1.0gm of sample of each formulation was pressed between two slides (of 20x20cm) and left for about 5 minutes where no more spreading was expected. Diameter of spreaded circles were measures and taken as comparative values for spreadability.

**pH**
pH of the gel formulation was determined using a calibrated pH meter. The electrode was immersed directly in to the gel, and the reading was allowed to stabilize and pH value was recorded.

**Homogeneity**
Homogeneity of the prepared gel were evaluated by visual perception.

**Extrudability**
A closed collapsible tube containing about 20g of gel was pressed firmly at the crimped end and a clamp was applied to prevent any roll back. The cap was removed and the gel was extruded. The amount of extruded gel was collected and weighed. The percentage of the extruded gel was observed.

**Viscosity**
Viscosity of the different gel formulation was determined at $25^0$C using rotational brook field viscometer of with spindle CPE-41.

**Anti-fungal activity**
The antifungal activity was tested by well diffusion method. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper wells (5 mm in diameter) impregnated with 100µg concentrations of the synthesized silver nanoparticles were placed on test organism-seeded plates. Fluconazole (10µg well 1) used as positive control. The activity was determined after 72 hrs of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

***In-vitro* Drug release**
*In vitro* drug study using egg membrane involves isolating the inner membrane was washing and hydrating it in phosphate buffer (pH 6.8 to 7.4). The membrane is placed between donor and receptor compartments of a franz diffusion cell with receptor containing at $37^o$C and stirr continuously with the rpm 100 to 300 rpm using a magnetic stir beat uniform drug distribution. The test formulation is placed in the donor compartment, and samples are withdrawn from the receptor at regular intervals (6 hrs in 30 mins) and analyzed by using spectro photo meter at 273 nm. Phosphate Buffer used as a blank.

## RESULTS AND DISCUSSION

**Colour (Table 9)**
Formulation 3(f3) and formulation 5(f5) has dark olive green while formulation f1 as yellowish green,
Formulation2 (f2) as olive brown and formulation4 (f4) as greenish brown

**Odour (Table 10)**
Fomulation 1(1), formulation 3(3), formulation (4) has pleasant odour while fomulation 2(2) and formulation5 (5) has slightly pleasant.

**Spreadability (Table 11)**
Formulation1(1), formulation 2(2) has 4.7cm range while formulation 4(4) has 4.1cm, formulation 5(5) has 4.4cm and formulation 3(3) has 5.1cm.

**pH (Table 12)**
The formulation5(5) has 6.4 is closets to the skin pH while formulation 1(1) has 5.9, formulation 3(3) has6 and formulation 2(2), formulation 4(4) has 6.1.

**Homogenicity (Table 13)**
**Extrudability (Table 14)**

**Viscosity(Table 15)**
**Anti-fungalactivity(Table 16)**
***In-Vitro* drug release study(Table 17)**
***In-Vitro* drug release (Fig 4)**

## CONCLUSION

The current study has focused on developing and evaluating *Leucas aspera* herbal gel for the treatment of fungal infections. The physical and pharmacological properties of several formulations were produced and examined. The produced formulations (F4) demonstrated a favorable physical appearance, smooth texture, acceptable color, distinctive smell, and high homogeneity without phase separation. The gel can be used topically without causing irritation because its pH was confirmed to be within the acceptable skin range. The revised formulation's adequate viscosity made it easy to apply and had good its spreadability. The antifungal activity tests showed that the formulation considerably suppressed fungal strains, indicating the presence of potent antifungal components in *Leucas aspera.* Effective diffusion of active substances from the gel base was demonstrated by the *in-vitro* drug release investigation, which showed a good and regulated drug release pattern. The enhanced batch outperformed the other formulations in terms of in-vitro drug release profile, antifungal activity, and physical stability. Therefore, the formulated (F4) *Leucas aspera* gel seems to be a safe, effective, and promising herbal topical therapy for the treatment of fungal infections..

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**Table 1: Test for Alkaloids**

| S. No | Experiment | Observation | Inference |
|---|---|---|---|
| 1 | Mayer's test:<br>Add a few drops of Mayer's reagents to the plant extract | White cream precipitate | Presence of alkaloids |
| 2 | Dragendroff's test:<br>Add a few drops of Dragendroff's reagents to the plant extract | Reddish brown precipitate | Presence o f alkaloids |
| 3 | Hager's test<br>Add a few drops of Hager's reagents to the plant extract | Formation of yellow precipitate | Presence of alkaloids |

**Table 2: Test for flavonoids**

| **S. No** | **Experiment** | **Observation** | **Inference** |
|---|---|---|---|
| 1 | Alkaline reagent test:<br>Add a few drops of dilute NaOH(sodium hydroxide) to the plant extract | Yellow colour solution changed into colourless solution after the addition of dilute acid | Presence flavonoids |
| 2 | Lead acetate test:<br>Add a few drops of lead acetate solution to the plant extract | Formation of yellow colour | Presence of flavonoids |

**Table 3: Test for Terpenoids**

| S. No | Experiment | Observation | Inference |
|---|---|---|---|
| 1 | Liebermann burchard test: Add a few drops of acetic acid and conc. Sulfuric acid to the plant extract | Colour change (green, blue, red etc.,) | Presence of terpenoids |
| 2 | Salkowski test: Add a few drops of conc. Sulfuric acid to the plant extract | Colour change (red, yellow, green, etc.,) | Presence of terpenoids |

**Table 4: Test for Tanins**

| S. No | Experiment | Observation | Inference |
|---|---|---|---|
| 1 | Ferric chloride test: Add a few drops of ferric chloride solution to the plant extract | Blue black precipitate | Presence of tannins |
| 2 | Lead acetate test: Add a few drops of lead acetate solution to the plant extract | Precipitate formation | Presence of tannins |

**Table 5: Test for Carbohydrate**

| S. No | Experiment | Observation | Inference |
|---|---|---|---|
| 1 | Molisch's test: Add a 2-3drops of Molisch's reagents (5% alpha naphthol in ethanol) to the plant extract. | Violet ring at the layer junction | Presence of carbohydrates |
| 2 | Fehling's test: Mix equal volume of Fehling's A ($CuSO_4$ solution) and Fehling's B (Sodium potassium tartarate + NaOH) | Formation of brick red precipitate | Presence of carbohydrates |

**Table 6: Test for Glycosides**

| S. No | Experiment | Observation | Inference |
|---|---|---|---|
| 1 | Keller-kiliani tes: Take 1ml of plant extract and add 5ml of water to evaporate to dry and then add 2ml of glacial acetic acid containing $Fecl_3$ solution and then finally add conc. Sulfuric acid | Red ring interface | Presence of cardiac glycoside |

**Table 7: Test for Phenol**

| S.No | Experiment | Observation | Inference |
|---|---|---|---|
| 1 | Add a few drops of 1% of ferric chloride ($Fecl_3$) solution to the plant extract | Formation of bluish black color | Absence of phenol |

**Table 8: Formulation ff Gel**

| Ingredients | F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|---|
| *Leucas aspera* (leaves ethanolic extract) | 5ml | 5ml | 5ml | 5ml | 5ml |
| Carbopol940 | 0.5gm | 0.75gm | 1gm | 1.25gm | 1.50gm |

| | | | | | |
|---|---|---|---|---|---|
| Triethanolamine | Qs | Qs | Qs | Qs | Qs |
| Glycerin | 3ml | 3ml | 3ml | 3ml | 3ml |
| Methylparaben | 0.2g | 0.2g | 0.2gm | 0.2g | 0.2g |
| Propyleneglycol | 5ml | 5ml | 5ml | 5ml | 5ml |
| Distilledwater | Qs | Qs | Qs | Qs | Qs |
| Rose oil | 5drops | 5drops | 5drops | 5drops | 5drops |



**Fig 1: Plant Extraction By Soxhlet Apparatus**



**Fig: 2 Preparation of Gel**




**Fig: 3. Five Formulations**

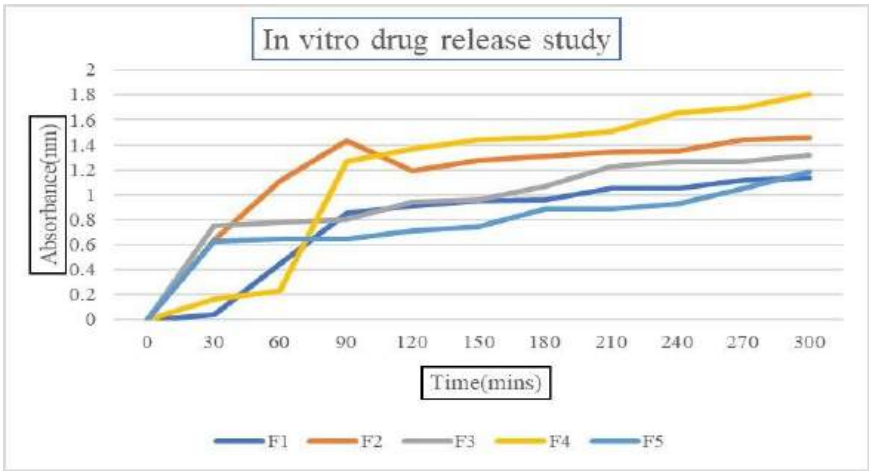



**Fig 23: *In-vitro* Drug Release**

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RESEARCH ARTICLE

# Adaptive Crop Advisory System using Fuzzy-Driven Scenario Modeling and LSTM-based Yield Forecasting

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## ABSTRACT

Agricultural productivity is increasingly affected by climate variability, irregular weather patterns, and the rising frequency of pest outbreaks. Existing crop yield prediction systems primarily focus on point estimation and lack the capability to generate adaptive agronomic advisories under uncertain environmental conditions. This paper presents ACAS-FLY, an adaptive crop advisory system that integrates fuzzy-driven scenario modeling with LSTM-based yield forecasting to support climate-aware agricultural decision making. The proposed framework combines stochastic scenario generation using bootstrapped sampling and Bayesian modeling to capture rainfall variation, temperature deviation, and pest outbreak probability. An ensemble of deep learning models including stacked LSTM, bidirectional LSTM, and CNN-LSTM architectures is employed to enhance yield prediction robustness across diverse regions, while uncertainty estimation is incorporated through Monte Carlo dropout. Predicted yield trends and environmental risk indicators are further processed by a fuzzy logic-based advisory engine to produce interpretable and actionable agronomic recommendations. The system is evaluated using multi-source datasets comprising historical rainfall records, global weather observations, pest imagery, and crop recommendation data. Experimental results demonstrate that ACAS-FLY consistently outperforms existing deep learning and automated machine learning approaches in terms of accuracy, sensitivity, specificity,

and F-score. The findings confirm the effectiveness of integrating scenario modeling, ensemble learning, and fuzzy reasoning for adaptive crop advisory under climate uncertainty.

**Keywords:** Adaptive advisory systems, Bayesian scenario modeling, Crop yield forecasting, Ensemble LSTM, Fuzzy logic, Monte Carlo dropout, Pest risk prediction, Stochastic climate simulation, Time series prediction, Uncertainty quantification

## INTRODUCTION

The rapid growth of the global population continues to place immense pressure on the agricultural sector to ensure food security. Simultaneously, climate change has introduced significant variability into weather systems, resulting in erratic rainfall patterns, rising temperatures, and increased frequency of extreme weather events. These changes are disrupting traditional farming cycles and increasing the unpredictability of crop yields across regions [1]. For developing economies, where agriculture remains a primary livelihood, the combined effect of population growth and climate instability threatens both economic sustainability and food availability. Modern agriculture faces a multitude of challenges driven by this environmental uncertainty [2]. In addition to climate-induced disruptions, farmers must also contend with soil degradation, pest and disease outbreaks, limited access to timely information, and fluctuating input costs [3]. Conventional advisory mechanisms, often static and generalized, fail to adapt to local, real-time conditions [4]. The absence of dynamic, region-specific, and data-driven recommendations leads to sub-optimal decisions in crop selection, irrigation planning, and pest management, particularly for small and marginal farmers. In recent years, machine learning (ML) techniques have shown strong potential in modeling agricultural parameters and predicting crop yields [5]. Among these, deep learning models such as Long Short-Term Memory (LSTM) networks have demonstrated effectiveness in capturing temporal dependencies within multivariate time series data related to weather, soil, and historical crop performance [6]. However, many existing approaches emphasize yield prediction alone, without extending insights into actionable guidance. Moreover, they often lack mechanisms to address uncertainty in future environmental conditions, limiting their real-world usability [7]. A predictive system intended for deployment in uncertain agricultural environments requires an additional reasoning layer that can transform numerical forecasts into actionable and interpretable guidance. The effectiveness of such systems increases significantly when predictive outputs are combined with contextual domain knowledge, including anticipated rainfall intensity, pest infestation likelihood, and region-specific cultivation practices [8]. In this context, an advisory engine becomes a necessary component rather than an optional extension. Fuzzy logic is particularly well suited for this role, as it enables the representation of expert knowledge and accommodates the inherent ambiguity and precision vagueness present in agricultural decision scenarios. Consequently, an integrated architectural framework is required that not only performs reliable yield forecasting under dynamic conditions but also delivers adaptive, human-understandable agronomic advisories that support informed decision-making at the field level.

**Existing Methods**

The following existing studies have been reviewed to understand current methodologies, applications, and limitations in the domain of crop yield prediction and advisory systems. The paper titled "Crop yield prediction using effective deep learning and dimensionality reduction approaches for Indian regional crops" [9] provides insights into regional crop modeling using modern deep learning frameworks. The work "Developing automated machine learning approach for fast and robust crop yield prediction using a fusion of remote sensing, soil, and weather dataset" [10] highlights the role of automated learning systems in integrating multimodal agricultural data. The study "From data to harvest: Leveraging ensemble machine learning for enhanced crop yield predictions across Canada amidst climate change" [11] explores the use of ensemble methods in handling climate-related variability. Additionally, the paper "Incorporating meteorological data and pesticide information to forecast crop yields using machine learning" [12] examines the impact of external input factors such as weather and pesticide use on yield

forecasting. These studies have been used to examine the current landscape and identify gaps that motivate the design of the proposed ACAS-FLY framework.

**Crop yield prediction using effective deep learning and dimensionality reduction approaches for Indian regional crops (CYPEDL)**

Leelavathi Kandasamy Subramaniam *et al*. in 2024 proposed a deep learning-based model titled Crop yield prediction using effective deep learning and dimensionality reduction approaches for Indian regional crops. The work is designed to improve crop yield prediction accuracy by leveraging deep learning and dimensionality reduction techniques specifically adapted for regional agricultural datasets from South India. The purpose of the study is to address the challenges of high-dimensional input data and optimize the learning structure for better forecasting of crop productivity under diverse climatic and soil conditions. The methodology involves three primary stages comprising data preprocessing, dimensionality reduction, and classification. Agricultural data are cleaned and normalized to remove noise and scale the features. A squared exponential kernel-based principal component analysis is applied to reduce dimensionality and handle non-linear relationships in the data. A deep convolutional neural network is used for prediction, with its weights and biases optimized using an enhanced whale optimization algorithm. This optimization process incorporates chaotic map initialization and adaptive search mechanisms to improve network performance. The architecture includes convolutional layers, pooling layers, activation layers, and a fully connected output stage. The proposed CYPEDL model demonstrates notable improvements in yield prediction accuracy and robustness. It effectively addresses the issues of overfitting and computational inefficiency commonly observed in traditional machine learning models. However, the model's reliance on tailored optimization strategies and deep architectures may limit its portability across different regions or datasets without significant reconfiguration.

**Developing automated machine learning approach for fast and robust crop yield prediction using a fusion of remote sensing, soil, and weather dataset (AMLACYP)**

Ahmed M S Kheir *et al*. in 2024 developed a fully automated machine learning approach aimed at predicting crop yield by integrating diverse environmental datasets including remote sensing indices, soil properties, weather variables, and topography. The core objective of the study was to create a robust and scalable yield prediction framework specifically for wheat production across Egypt, addressing the limitations of traditional modeling approaches and enabling rapid, low-cost predictions even under climate uncertainty. The methodology leverages H2O AutoML to automate the end-to-end machine learning workflow. Data from over 2500 wheat fields were collected and preprocessed, including variables such as NDVI, EVI, GCVI, soil texture, bulk density, temperature, rainfall, elevation, and slope. These were used to train 20 base models along with two stacked ensemble learners. The AutoML system integrated satellite-based vegetative indices with weather reanalysis and gridded soil data to train models using random forest, gradient boosting machines, deep neural networks, and generalized linear models. The model training and validation were executed within the H2O AutoML platform using random splits and hyperparameter search techniques, enabling selection of the most accurate ensemble predictor. The AMLACYP framework demonstrates its utility in handling heterogeneous and large-scale agricultural datasets with minimal manual intervention. It achieves spatial yield forecasting using only remote sensing inputs, making it suitable for regions with limited field data. Moreover, the trained models were extended to simulate yield under future climate change scenarios using downscaled GCM outputs. However, the system's performance could be impacted by variations in land fragmentation, sensor noise, and farmer-level management heterogeneity. The authors note that further generalization across crops and geographies would require model adaptation or transfer learning strategies.

**From data to harvest: Leveraging ensemble machine learning for enhanced crop yield predictions across Canada amidst climate change (EMLECYP)**

Navid Mahdizadeh Gharakhanlou *et al*. in 2024 presented a nationwide-scale study titled “From data to harvest: Leveraging ensemble machine learning for enhanced crop yield predictions across Canada amidst climate change”. The primary objective of this work was to assess the effectiveness of ensemble machine learning models in predicting crop yields—specifically for canola and soybeans—by integrating climate variables, vegetation indices, soil traits, and

honeybee census data. The research focused on building a climate-resilient yield prediction system that supports decision-making at the regional scale through geospatial forecasting under various climate scenarios. The methodology involved training and evaluating five ensemble models, including Adaptive Boosting, Gradient Boosting Machine, XGBoost, LightGBM, and Random Forest. The input data included spatially interpolated climate parameters, MODIS vegetation indices (NDVI and EVI), detailed soil composition, and honeybee colony statistics at the census subdivision level. Variables with multicollinearity were excluded using variance inflation factor analysis, and model training was performed using a 70:30 data split with 10-fold cross-validation and hyperparameter tuning. Once trained, models were tested for generalization on climate projections for the year 2026 under three Representative Concentration Pathway scenarios. Crop yield maps were generated for each model, and feature importance was assessed using XGBoost's gain-based analysis. The EMLECYP framework demonstrated that ensemble models—especially those based on boosting—performed better in capturing spatial and climatic variations in crop yield across Canada. Honeybee colony presence emerged as the most influential factor, followed by soil properties and precipitation. The model provided high-resolution yield forecasts and climate impact assessments at the Census Consolidated Subdivision level, making it useful for policymakers and agronomists. However, the system was constrained by data availability, especially in soil profiles and honeybee census frequency, and may require adaptation for other crops, geographies, or shorter-term predictions.

**Incorporating meteorological data and pesticide information to forecast crop yields using machine learning (MDPIML)**

Hoque *et al*. in 2024 proposed a machine learning-based framework titled Incorporating meteorological data and pesticide information to forecast crop yields using machine learning. The aim of this work was to develop a yield prediction system that integrates weather conditions, pesticide usage, and crop data to model agricultural productivity in India. This study recognizes the critical impact of environmental and chemical variables on food security and proposes a data-driven solution to improve resilience under climate change conditions. The proposed system is built upon a pipeline that includes data extraction, transformation, cleaning, feature engineering, and model training using three machine learning algorithms. Specifically, the study uses multivariate logistic regression, k-nearest neighbors, and gradient boosting regressors to forecast yields of six key crops—rice, wheat, potatoes, soybeans, sweet potatoes, and sorghum. The dataset was sourced from publicly available repositories and includes annual rainfall, average temperature, pesticide application rates, and historical yield data. Data preprocessing steps include duplicate and null value elimination, outlier detection via interquartile range filtering, and standardization using column transformers. Model optimization was performed using k-fold cross-validation and GridSearchCV to fine-tune hyperparameters for each model. The MDPIML system effectively demonstrates the ability of machine learning to generalize across multiple crops and regions with a unified predictive structure. It highlights the importance of including pesticide and meteorological variables for better accuracy and insights. However, the study also notes constraints such as the absence of detailed agronomic features like soil moisture and solar radiation, which may limit its precision. The framework lays the groundwork for future integration with IoT-based environmental sensing systems to enhance decision-making in agricultural forecasting. A comparative summary of the reviewed methods is presented in Table 1, highlighting the authors, titles, methodologies, advantages, and limitations of each approach.

**Problem Statement:** Accurate crop yield prediction is challenged by uncertain climate conditions, pest outbreaks, and region-specific variability. Existing models often focus solely on prediction without providing actionable insights, limiting their use in real-world decision-making. There is a pressing need for an integrated system that not only forecasts yield under dynamic conditions but also generates adaptive, interpretable advisories to support farmers in making timely and informed agronomic decisions.

## Research Gap

While recent advances in machine learning have improved crop yield prediction using remote sensing and weather data, most existing models operate as static predictors with limited adaptability to real-time environmental fluctuations. They rarely address predictive uncertainty or translate outputs into farmer-friendly recommendations. Additionally, current systems often lack integration between scenario simulation, temporal forecasting, and decision reasoning, making them inadequate for dynamic, region-specific advisory generation. This highlights the gap for a unified framework that combines stochastic scenario modeling, deep learning-based yield forecasting, and fuzzy logic-driven advisory to enable adaptive, interpretable agricultural support.

## Research Objectives

- To develop a risk-aware scenario generation module that simulates multiple future environmental conditions using stochastic modeling of climate variables and pest outbreak probabilities.
- To design an ensemble-based LSTM forecasting engine that improves crop yield prediction accuracy and robustness across regions while incorporating uncertainty estimation.
- To implement a fuzzy logic-based advisory system that translates predicted yield and environmental scenarios into adaptive, human-interpretable agronomic recommendations.
- To evaluate the proposed ACAS-FLY framework using multi-source agricultural datasets and assess its performance using classification metrics such as accuracy, sensitivity, specificity, and F-score.
- To compare the effectiveness of ACAS-FLY with existing machine learning-based yield prediction models in terms of prediction accuracy and advisory relevance under uncertain conditions.

## BACKGROUND

Designing an adaptive crop advisory system requires a multidisciplinary foundation that spans agricultural science, climate modeling, and machine learning. To support the development of the proposed ACAS-FLY framework, this section outlines key background concepts relevant to its design. First, the fundamentals of crop yield prediction are discussed, highlighting the critical factors that influence agricultural output and the need for data-driven approaches. Next, the role of climate variability and the importance of scenario modeling in capturing future uncertainties are examined. Finally, the structure and applicability of recurrent neural networks, particularly Long Short-Term Memory (LSTM) networks, are introduced as essential tools for time-series yield forecasting.

**Crop Yield prediction fundamentals**

Effective crop yield prediction needs a clear understanding about how various biophysical and agronomic parameters interact over time. Yield is not solely influenced by isolated variables but by their cumulative impact across the growing season [13]. For instance, a rainfall anomaly during the germination phase may affect yield differently than the same anomaly during flowering. These phase-sensitive dependencies demand predictive systems that can capture both short-term fluctuations and long-term growth patterns. Most recent approaches increasingly depend on integrating multi-source datasets to capture spatial and temporal variability. Remote sensing data, such as vegetation indices from satellite imagery, can reveal crop health trends over large areas, while ground-based measurements like soil nutrient profiles and pesticide applications provide localized insights [14]. Combining such diverse sources enables a more holistic view of yield formation mechanisms. In addition, yield prediction must count for inherent uncertainties in future conditions. Static models trained on historical data alone often fail when applied to new environmental scenarios [15]. Hence, prediction systems should not only learn from patterns in past data but also be adaptable to changing conditions and capable of expressing confidence in their outputs. This sets the stage for the use of time-series models and uncertainty-aware learning techniques, discussed in the subsequent sections.

**Climate Variability and Scenario Modelling**

Climate variability is a significant driver of uncertainty in agricultural systems, affecting the timing, quantity, and quality of crop yields. Irregular rainfall patterns, temperature extremes, delayed monsoons, and unseasonal heatwaves can disrupt crop phenology and lead to substantial yield losses [16]. These effects are further amplified by the increasing unpredictability of climate behavior due to global warming, making static forecasting approaches insufficient for long-term planning and real-time advisory. To address this, scenario modeling has emerged as a technique for generating multiple plausible representations of future conditions. Rather than relying on a single forecast, scenario-based models simulate a range of environmental possibilities such as varying rainfall intensities, temperature deviations, or pest outbreak likelihoods [17]. These simulations help quantify risk and allow agricultural systems to prepare for best-case, worst-case, and most-likely outcomes. Techniques such as Monte Carlo simulation, Bayesian inference, and bootstrapped time series sampling are commonly employed to create stochastic environmental scenarios. These methods introduce variability into input parameters and generate synthetic datasets that reflect potential future conditions. In the context of crop yield prediction, scenario modeling supports risk-aware forecasting and enhances decision-making by allowing the advisory system to evaluate outcomes under diverse environmental pathways.

**Recurrent Neural Networks and LSTM**

Recurrent Neural Networks (RNNs) are a class of artificial neural networks designed for processing sequential data, where each output depends not only on the current input but also on the previous context. This makes RNNs particularly effective for time-series modeling tasks such as crop growth monitoring, weather forecasting, and seasonal yield analysis. By maintaining a hidden state that evolves over time, RNNs are capable of capturing temporal patterns within multivariate agricultural data. Despite their usefulness, traditional RNNs face challenges in learning long-term dependencies due to vanishing or exploding gradients during backpropagation. To address this issue, Long Short-Term Memory (LSTM) networks introduce a gated cell architecture with input, output, and forget gates that control the flow of information across time steps. This mechanism enables LSTM models to retain important signals over longer sequences without degradation in learning performance. LSTM networks have been widely adopted in agricultural forecasting due to their ability to learn complex temporal relationships from weather variables, crop phenology data, and historical yield records. Advanced variants such as stacked LSTM, bidirectional LSTM, and CNN-LSTM combinations further improve prediction by allowing deeper sequence representation and spatial feature integration [18]. These characteristics make LSTM an essential component in the forecasting engine of the ACAS-FLY system.

## PROPOSED METHOD

The ACAS-FLY framework is structured into three integrated modules that collectively support adaptive crop advisory generation. These modules are: the Risk-Aware Scenario Generator (RASG), the LSTM Ensemble Forecasting Engine (LEFE), and the Fuzzy Logic-based Advisory Engine (FLAE). Each module addresses a specific stage in the prediction-to-advisory pipeline and is detailed in the following subsections.

**Risk-Aware Scenario Generator (RASG)**

The Risk-Aware Scenario Generator (RASG) forms the first stage of the ACAS-FLY framework and is designed to simulate diverse future environmental conditions by introducing probabilistic variations into critical agricultural parameters. This approach allows the system to generate multiple plausible realizations of climate and pest-related inputs, enabling downstream modules to account for uncertainties in forecasting and decision-making. Let Daily Rainfall $R_t$, Average Temperature $T_t$, Pest Outbreak Probability $P_t$, Humidity $H_t$, and Crop-Pest susceptibility index $C_t$ be the environment variables of typical scenario generation. These variables are treated as stochastic processes with known or estimated probability distributions derived from historical observations. The Daily Rainfall $R_t$ is modeled as in Equation 1.

$$R_t \sim \Gamma(\alpha, \beta) \quad \text{Equation (1)}$$

where $\alpha$ and $\beta$ represent the shape and scale parameters of the Gamma distribution, estimated from historical data. Temperature $T_t$ is modeled using normal distribution as in equation 2.

$T_t \sim \aleph(\mu T, \sigma_T^2)$ Equation (2)

Similarly, Pest Outbreak is modeled using time-dependent Bernoulli process as in the following equation.

$P_t \sim Berunoulli(p_t)$ with $p_t = f(T_t, H_t, C_t)$ Equation (3)

Using these equations, RASG module generates $N$ stochastic scenario sequences $\{S_1, S_2, \ldots S_N\}$ in which each $S_i$ is a time series vector of sampled environmental variables as in equation 4.

$S_i = \left\{\left(R_t^{(i)}, T_t^{(i)}, P_t^{(i)}\right) | t = 1,2, \ldots \tau\right\}$ Equation (4)

These sequences are generated through two primary stochastic approaches. The first is bootstrapped time series sampling, which involves non-parametric resampling from historical environmental datasets. This method is used to preserve or ignore temporal ordering based on the design objective and supports diversity in generated inputs. The second approach is Bayesian resampling, in which samples are drawn from posterior predictive distributions to incorporate parameter uncertainty. Variational inference is employed to approximate these distributions based on prior knowledge and observed historical data.

The validation process of the scenario is performed based on domain constrains as in following equation.

$Validity(S_i) = \{1\ if R_t^{(i)} \in [R_{min}, R_{max}], T_t^{(i)} \in [T_{min}, T_{max}]\ 0\ otherwise$

Equation (5)

The output of RASG is a scenario ensemble $S = \{S_1, S_2, \ldots S_N\}$ where each $S_i$ represents a different possible trajectory of environmental variables over the growing season. This ensemble is used by the forecasting engine to generate yield distributions and inform risk-sensitive advisory generation. RASG module flowchart is provided in Figure 1. RASG Flow diagram (Figure 1).

**LSTM Ensemble Forecasting Engine (LEFE)**

The LSTM Ensemble Forecasting Engine (LEFE) is responsible for generating yield predictions under the environmental scenarios produced by the RASG module. It is designed to capture complex temporal dependencies and nonlinear interactions in agricultural time-series data, including rainfall trends, temperature cycles, and pest progression across different growth phases. LEFE improves forecasting accuracy and generalization by employing an ensemble of LSTM variants, each specialized in learning different feature hierarchies and temporal characteristics. Each input scenario $S_i = \left\{\left(R_t^{(i)}, T_t^{(i)}, P_t^{(i)}\right)\right\}_{t=1}^{\tau}$ are normalized into a feature matrix X as in equation 6.

$X \in R^{e^{(d)} + log\,(\tau)}$ Equation (6)

where $d$ represents the number of input features per time step, such as rainfall, temperature, and pest index, and $\tau$ denotes the number of time steps, in specific weeks in a growing season,

In LEFE module, the standard LSTM model is improved to be context specific by adding scenario-conditioned variables as one of the novelty contributions of this research work. To incorporate context-awareness into the forecasting process, two additional elements are introduced into the standard LSTM cell. The first is a scenario embedding vectors, which encodes the environmental scenario index generated by the RASG module. This vector captures the broader agro-climatic context, such as low rainfall conditions, high pest probability, or temperature anomalies, and remains fixed for a given scenario sequence. The second element is a yield-relevance vector $\phi_t$, which is computed dynamically at each time step based on agro-contextual variables including rainfall deviation $\Delta R_t$, pest severity $P_t$, and crop stress indicators $\delta_t$. By integrating $s$ and $\phi_t$ into the LSTM gating mechanisms listed below from Equation 7 to 12, the model becomes scenario-aware and sensitive to the varying importance of environmental features over time, improving its capacity to forecast yield under uncertain and evolving field conditions.

$f_t = \sigma(W_f \cdot [h_{t-1}, x_t, s, \phi_t] + b_f)$ Equation (7)

$i_t = \sigma(W_i \cdot [h_{t-1}, x_t, s, \phi_t] + b_i)$ Equation (8)

$\tilde{C}_t = tanh(W_C \cdot [h_{t-1}, x_t, s, \phi_t] + b_C)$ Equation (9)

$C_t = f_t \odot C_{t-1} + i_t \odot \tilde{C}_t$ Equation (10)

$o_t = \sigma(W_o \cdot [h_{t-1}, x_t, s, \phi_t] + b_o)$ Equation (11)

$h_t = o_t \odot tanh\,(C_t)$ Equation (12)

The rainfall deviation $\Delta R_t$ is computed by equation 13.

$\Delta R_t = \{\frac{R_t^2 - \underline{R}^2}{R_t + \underline{R}}\ if\ R_t \neq \underline{R}\ 0\ otherwise$ Equation (13)

Let $z_t$ become the intermediate input feature vector that records key agro-environmental indicators at timestamp $t$. $z_t$ is computed using the following equation.

$z_t = [\Delta R_t, P_t, \delta_t]$ Equation (14)

Here, the yield-relevance vector $\phi_t$ is calculated using equation 15.

$\phi_t = tanh\ tanh\left(2 \times \left(W_\phi \cdot z_t + \left(\frac{b_\phi - W_\phi \cdot z_t}{2}\right)\right)\right)$ Equation (15)

A key innovation in the proposed forecasting engine is the integration of both static and dynamic environmental context directly into the LSTM cell. The model introduces a scenario representation that allows internal memory updates to be conditioned on the specific environmental profile being evaluated, such as low rainfall or high pest pressure. In addition, a dynamic relevance vector is computed at each time step to modulate the influence of input signals like rainfall deviation or crop stress indicators based on their agronomic importance. This dual-context integration enables the forecasting process to adapt more effectively across different crop stages and regional conditions, enhancing both predictive accuracy and interpretability under uncertain environmental scenarios. While the proposed LSTM cell incorporates scenario and yield-relevance conditioning to enhance prediction quality, agricultural forecasting is inherently noisy and non-stationary due to spatial and temporal variability. A single model, no matter how carefully designed, may struggle to generalize across diverse cropping zones, phenological stages, or extreme weather scenarios. LEFE integrates the predictive outputs of three complementary LSTM-based architectures to achieve robust yield estimation. A stacked LSTM is employed to capture deep temporal patterns over long sequential windows, enabling the model to learn complex seasonal and growth-stage dynamics. A bidirectional LSTM is incorporated to account for both forward and backward temporal dependencies, thereby improving sensitivity to past trends as well as future context within the sequence. In addition, a CNN–LSTM architecture applies one-dimensional convolution layers to extract localized spatial or short-range temporal features prior to sequential modeling. The individual predictions generated by these three variants are denoted as $\hat{Y}_1, \hat{Y}_2$, and $\hat{Y}_3$, and the final yield prediction is computed by aggregating these outputs through a weighted averaging mechanism. The final prediction $\hat{Y}$ is achieved as follows.

$\hat{Y} = \sum_{i=1}^{n_p} w_j \hat{y}_j$ Equation (16)

where Weights $w_j$ are empirically tuned based on validation performance, and $n_p$ is the total number of prediction models in the ensemble, which is 3 in this case.

Prediction uncertainty is addressed by incorporating Monte Carlo dropout within the ensemble, in which dropout layers remain active during the inference phase to produce multiple stochastic output $\{\hat{Y}_1, \hat{Y}_2, \ldots \hat{Y}_{n_t}\}$. These repeated forward passes make it possible to approximate uncertainty in the predictions model. The resulting set of outputs is then used to compute the predictive mean and standard deviation, providing both an expected yield estimate and a quantitative measure of prediction variability. Let $n_t$ be then number of timestamps taken as the sample, equations 17 and 18 are used to calculate the predictive mean and standard deviation.

$\mu_{\hat{y}} = \frac{1}{n_p} \sum_{i=1}^{n_t} \hat{y}_i$ Equation (17)

$\sigma_{\hat{y}} = \sqrt{\frac{1}{n_p} \sum_{i=1}^{n_t} \left(\hat{y}_i - \mu_{\hat{y}}\right)^2}$ Equation (18)

This process generates a point estimate of the predicted yield along with a confidence interval that quantifies forecast uncertainty. The ability to represent prediction spread enhances transparency and supports risk-aware interpretation.

This uncertainty modeling is a key innovation in the proposed system, enabling the advisory engine to deliver recommendations that account for prediction confidence rather than relying solely on deterministic outputs.

**Fuzzy Logic-based Advisory Engine (FLAE)**

The Fuzzy Logic-based Advisory Engine (FLAE) provides a rule-based reasoning layer that translates quantitative forecasts and environmental indicators into qualitative agronomic advisories. It bridges the gap between data-driven prediction and farmer-level decision-making by modeling expert knowledge under uncertainty. FLAE is particularly effective in handling imprecise, overlapping, or linguistically defined input conditions that are common in agricultural environments. The inputs to the FLAE are obtained by consolidating the outputs of preceding modules along with key environmental indicators. These inputs include the mean yield predicted by the LSTM ensemble in LEFE, denoted as $\mu_{\hat{y}}$, which represents the expected production level, and the standard deviation of the predicted yield, denoted as $\sigma_{\hat{y}}$, which captures forecast uncertainty. Rainfall at the current time step, represented as $R_t$, is incorporated from the selected RASG scenario to reflect moisture availability, while the pest probability at the same time step, denoted as $P_t$, represents the likelihood of biotic stress conditions derived from the same scenario. In addition, a temperature-derived crop stress index, denoted as $\text{Stress}_t$, is included and computed using deviation-based thresholds to indicate the severity of thermal stress experienced by the crop. Collectively, these variables form a comprehensive and context-aware input set for adaptive advisory generation within FLAE.

**The first fuzzy input variable rainfall $R_t$ is** derived from the "Rainfall in India" dataset which is available on Kaggle repository. The observed values range from approximately 0 mm to 700 mm per month, which translates to about 0–175 mm per week during the peak monsoon season. For the purpose of fuzzy rule generation, rainfall is categorized into three linguistic sets: Low Rainfall (0–40 mm), Moderate Rainfall (30–100 mm), and High Rainfall (90–175 mm). Triangular or trapezoidal membership functions are used to allow smooth overlap between these categories.

**The second input is the pest probability $P_t$,** extracted from pest classifier outputs applied to the "Plant Disease Dataset" on Kaggle. The classifier produces a probability score between 0 and 1 for each time step. These values are fuzzified into three sets: Low Pest Risk (0.0–0.3), Medium Pest Risk (0.2–0.6), and High Pest Risk (0.5–1.0). These ranges account for overlap and uncertainty in classification confidence levels.

**The third input is the temperature-based stress score $\delta_t$,** computed from the "Global Weather" dataset. The raw temperature values range from 5°C to 45°C. The stress score is defined as the absolute deviation from an optimal crop-specific temperature, such as 25°C for rice. This is calculated as $\delta_t = |T_t - T_{opt}|$, based on the degree of deviation, the stress is classified into No Stress (0–3°C deviation), Moderate Stress (2–7°C deviation), and Severe Stress (greater than 6°C deviation), with overlapping membership functions to capture transitions.

**The fourth input is the predicted mean yield $\mu_{\hat{y}}$,** which is computed from the LSTM ensemble forecasting engine (LEFE) based on nutrient-soil-climate mappings found in the "Crop Recommendation Dataset." As the forecast output is normalized, its values are mapped into three fuzzy sets: Low Yield (0.0–0.4), Moderate Yield (0.3–0.7), and High Yield (0.6–1.0). This classification helps identify yield performance bands for advisory generation.

**The fifth input is the yield uncertainty $\sigma_{\hat{y}}$,** which measures the standard deviation of the ensemble yield predictions. The values typically range from 0.0, indicating high confidence, to approximately 0.3 for highly uncertain forecasts. The uncertainty is fuzzified into Low Uncertainty (0.0–0.1), Medium Uncertainty (0.08–0.2), and High Uncertainty (0.15–0.3). These ranges help capture the model's confidence in its prediction and enable the advisory engine to adjust recommendations accordingly. The fetched feature value ranges are enumerated in table 2. Features and their numerical ranges Table 2. The advisory decisions within FLAE are derived from a fuzzy rule base composed of IF–THEN statements that encode expert agronomic reasoning under uncertainty. Each rule combines fuzzified input variables to infer a context-appropriate action. For instance, if the predicted mean yield $\mu_{\hat{y}}$ is classified as Low Yield and the rainfall $R_t$ is Low Rainfall, then the system recommends a Drought-Resistant Variety. Similarly, if $\mu_{\hat{y}}$ is

Moderate Yield and the pest probability $P_t$ is High Pest Risk, the advisory suggests a Bio-Pesticide Schedule. In cases where the yield uncertainty $\sigma_{\hat{y}}$ is High and the temperature-induced stress score $\delta_t$ is Severe, a Risk Reassessment Advisory is triggered. Conversely, if $\mu_{\hat{y}}$ is High Yield and $\sigma_{\hat{y}}$ is Low Uncertainty, the system concludes that No Immediate Action is required. The truth value $\tau_i$ of each rule $i$ is calculated using the minimum membership degree among the involved input terms, ensuring that the inference reflects the most limiting condition in the rule antecedents as in flowing equation.

$$\tau_i = min\left(\mu_{L_1}(\mu_{\hat{y}}), \mu_{L_2}(\sigma_{\hat{y}}), \mu_{L_3}(R_t), \mu_{L_4}(P_t), \mu_{L_5}(\delta_t)\right) \quad \text{Equation (19)}$$

where $\mu_{L_1}(x)$ refers the membership value of input parameter $x$ in the corresponding fuzzy set $L_i$

The outputs of all activated fuzzy rules are combined through an aggregation process to form a composite fuzzy set that represents the overall advisory outcome. Each rule contributes to this aggregated set in proportion to its firing strength, ensuring that all relevant conditions influence the final decision. The aggregated fuzzy output is then converted into a single crisp advisory value $A^*$ using the standard centroid defuzzification method (given as equation 20), which computes the center of gravity of the combined membership function. This approach provides a balanced and interpretable decision by accounting for the relative influence of all contributing rules.

$$A^* = \frac{\int a \cdot \mu_A(a) da}{\int \mu_A(a) da} \quad \text{Equation (20)}$$

where $\mu_A(a)$ is the aggregated membership function for the advisory output variable.

The defuzzified output $A^*$ is mapped to a predefined set of advisory labels that represent specific agronomic actions. These may include recommendations such as Drought-Resistant Variety Selection, Initiation of Pest Control Schedule, Irrigation Adjustment, No Immediate Action, or Risk Alert for Field Review. Each label corresponds to a fuzzy output region and reflects expert knowledge embedded in the rule base. These recommendations are generated in real time for each environmental scenario and predicted yield context, enabling adaptive, location-aware, and expert-informed decision support for farmers and agricultural planners. The ACAS-FLY framework integrates its three core modules namely RASG, LEFE, and FLAE into a unified and adaptive decision support system. The process begins with RASG, which generates diverse environmental scenarios by modeling variations in rainfall, temperature, and pest probability. These scenarios serve as input to LEFE, which forecasts crop yield along with associated uncertainty using an ensemble of deep learning models. The outputs from LEFE, including the predicted mean yield and standard deviation, along with environmental indicators from RASG such as rainfall, pest probability, and stress scores, are passed to FLAE. FLAE applies fuzzy inference to these inputs to produce interpretable, scenario-specific agronomic advisories. This modular integration enables the system to deliver context-aware recommendations that are both data-driven and expert-informed.

**Experimental Setup**

The ACAS-FLY framework was implemented in a Windows 11 environment using Visual Studio IDE [19] with C++ 23.0 [20] and Advanced C 24.0 [21] runtime libraries. All modules were developed using Microsoft Foundation Classes (MFC) [22], and integrated through a Common Gateway Interface (CGI) [23] layer to support backend execution and browser-based interaction. This allowed scenario parameters to be submitted via local or remote interfaces, with advisory results returned in real time. The system was executed on a development machine configured with a 12th Generation Intel Core i7 processor, 32 GB DDR4 RAM, and a 1 TB NVMe M.2 SSD. High-volume simulations and scenario evaluations were also deployed on an i2k2 dedicated server. The datasets used include: Rainfall in India (Kaggle) [24] for generating weekly rainfall sequences in RASG, Global Daily Weather (Kaggle) [25] for deriving temperature-based stress scores, Plant Disease Dataset (Kaggle) [26] for estimating pest probability inputs using a CNN classifier, and the Crop Recommendation Dataset (Kaggle) for soil-climate-yield associations [27] used in LEFE output validation and FLAE advisory mapping.

## RESULTS AND DISCUSSIONS

ACAS-FLY was evaluated using five key performance parameters: Accuracy, Precision, Sensitivity, Specificity, and F-Score. tWeekly crop cycle inputs were used to generate rainfall, temperature stress, and pest sequences, which were

then used for yield prediction. Metric-wise comparisons were recorded over multiple runs to ensure stability and consistency. The results confirm that the proposed system delivers high accuracy with balanced Precision, Sensitivity, and Specificity, while maintaining a strong F-Score across diverse simulation conditions.

**Accuracy**

Accuracy reflects the overall correctness of the yield prediction model by measuring the proportion of true results, both positive and negative, among the total predictions made. In the context of ACAS-FLY, accuracy indicates how well the LEFE module forecasts the appropriate yield class under varying environmental scenarios. Higher accuracy confirms the model's ability to generalize effectively across different regions and input variations. The measured Accuracy values at 10 different timestamps for the discussed methods are provided in Table 3. Accuracy (Table 3). Across multiple test runs, the proposed ACAS-FLY method achieved an average accuracy of 99.062%, which is the highest among all compared models. CYPEDL and EMLECYP also demonstrated consistently strong accuracy, averaging 98.277% and 97.732% respectively, reflecting their effective learning strategies. AMLACYP and MDPIML maintained reasonable accuracy at 96.661% and 95.849%, highlighting their robustness across input types. The improved accuracy of ACAS-FLY can be attributed to the combination of scenario-aware sequence modeling in LEFE and the integration of diversified environmental conditions generated by RASG. This layered structure enhances the model's ability to generalize under uncertainty while maintaining precision in yield classification. Without discrediting the reliability of existing approaches, ACAS-FLY offers a notable improvement in prediction consistency and correctness across varied conditions. A comparison graph for measured accuracy values is provided in Figure 2. Accuracy (Figure 2).

**Precision**

Precision measures the proportion of correctly predicted positive outcomes to the total predicted positives. In the context of yield forecasting, it reflects how accurately the model identifies relevant yield classes without including incorrect predictions. Higher precision indicates that the model produces fewer false positives, which is important when issuing specific agronomic recommendations. The proposed ACAS-FLY model achieved the highest average precision of 99.035%, outperforming all baseline methods. CYPEDL and EMLECYP followed with 98.492% and 98.077% respectively, reflecting well-structured learning strategies and stable performance. AMLACYP recorded a moderate precision of 97.329%, while MDPIML maintained 96.305%, indicating reasonable predictive capability with a slight tendency toward false positives under certain conditions. Measured precision values of the compared method during evaluation with 10 timestamps are enumerated in Table 4. Precision (Table 4). The superior precision of ACAS-FLY demonstrates its effectiveness in minimizing incorrect yield predictions. This performance gain can be attributed to the contextual embedding and yield-relevance modulation within the LEFE module, which filters less-relevant features more effectively. While all methods maintained acceptable levels, ACAS-FLY consistently delivered more focused and reliable positive predictions. The precision comparison graph is provided in Figure 3. Precision (Figure 3).

**Sensitivity**

Sensitivity, also known as recall, measures the proportion of actual positive cases that are correctly identified by the model. In the context of yield prediction, it reflects the ability of the system to correctly capture all high-yield or risk-prone cases without missing them. A high sensitivity score ensures that the model responds effectively to critical agricultural conditions that demand intervention. Measured sensitivity scores are logged in table 5, and the comparison graph is given in Figure 4. Sensitivity (Table 5). Sensitivity (Figure 4). The proposed ACAS-FLY model achieved an average sensitivity of 99.090%, the highest among all evaluated methods. This indicates the model's strong ability to correctly identify all relevant yield conditions, especially under uncertain or shifting environmental scenarios. CYPEDL followed closely with 98.072%, while EMLECYP maintained a stable average of 97.407%, showcasing dependable recall performance. AMLACYP and MDPIML recorded 96.047% and 95.434% respectively, reflecting moderate sensitivity in detecting true positive predictions. ACAS-FLY's high sensitivity highlights the advantage of integrating scenario-aware modeling within the LEFE module, which helps capture subtle yield-related patterns often overlooked by conventional architectures. While other methods perform consistently, the proposed

approach stands out in its ability to minimize false negatives, ensuring critical yield conditions are not missed during advisory generation.

**Specificity**

Specificity measures the proportion of actual negative cases that are correctly identified by the model. In the context of yield forecasting, it reflects how well the model avoids false alarms by correctly identifying non-critical or low-risk conditions. A higher specificity score ensures that unnecessary interventions are minimized, contributing to more efficient and targeted agricultural recommendations. Measured specificity scores of the compared methods are enumerated in Table 6. Specificity (Table 6). The proposed ACAS-FLY method achieved an average specificity of 99.036%, the highest among all compared models. CYPEDL and EMLECYP followed with averages of 98.486% and 98.064%, indicating consistent accuracy in identifying non-critical yield conditions. AMLACYP recorded 97.294%, while MDPIML maintained 96.272%, showing moderate effectiveness in reducing false alarms. ACAS-FLY's strong specificity confirms its ability to avoid unnecessary alerts by correctly identifying stable or non-actionable scenarios. This precision is enabled by the integration of environmental scenario modeling in RASG and uncertainty-aware yield forecasting in LEFE. Without compromising sensitivity, the model maintains high discrimination power, ensuring that field recommendations are only issued when truly required. The Specificity score comparison graph is provided in Figure 5. Specificity (Figure 5).

**F-Score**

F-Score is the harmonic mean of Precision and Sensitivity, offering a balanced measure of the model's classification performance. It is particularly useful when dealing with imbalanced outcomes, as it considers both false positives and false negatives. In yield forecasting, a high F-Score indicates that the model not only detects critical yield classes effectively but also avoids incorrect alarms. The proposed ACAS-FLY model recorded the highest average F-Score of 99.062%, reflecting its balanced performance in both detecting true yield cases and avoiding false positives. CYPEDL and EMLECYP followed with 98.281% and 97.740% respectively, showing consistent classification strength. AMLACYP and MDPIML achieved 96.684% and 95.868%, maintaining reasonable harmonic precision–recall trade-offs. ACAS-FLY's superior F-Score confirms its ability to combine high sensitivity and precision without sacrificing one for the other. This balance results from the integration of environmental scenario awareness in RASG and adaptive sequence learning in LEFE. The improvement is particularly valuable in agricultural contexts, where missing a critical condition or over-alerting both lead to suboptimal decisions. Observed F-Score values during the evaluation are provided in Table 7 and the comparison graph is provided in Figure 6. F-Score (Table 7). F-Score (Figure 6).

**Additional Findings**

**Crop Disease Class Frequency Distribution (Figure 7).**

The bar chart illustrates the distribution of image samples across four crop health categories in the Plant Disease dataset. The dataset includes a higher proportion of healthy crop images, followed by cases of blight, rust, and mildew. This imbalance highlights the importance of precision-focused classification during pest probability estimation, as used in the FLAE module of ACAS-FLY. The trained CNN model accounts for this class distribution while generating pest risk scores used in downstream advisory decisions.

**Nitrogen Level vs. Predicted Mean Yield**

The scatter plot numbered as Figure 8 presents the relationship between nitrogen content (N) and predicted mean yield values as estimated by the LEFE module. Data points are derived from the Crop Recommendation dataset and aligned to normalized yield outputs. The distribution suggests a non-linear correlation, with moderate nitrogen levels frequently associated with higher predicted yields. This behavior is consistent with agronomic expectations, where both nitrogen deficiency and excess can negatively impact crop productivity. The mapping reinforces the relevance of soil nutrient features in model training and the utility of LEFE's context-aware learning for yield prediction. Nitrogen Level vs. Predicted Mean Yield (Figure 8).

## CONCLUSION

ACAS-FLY presents an adaptive crop advisory framework that integrates scenario modeling, sequence-based yield forecasting, and fuzzy logic–driven decision-making. The proposed architecture introduces three key innovations: (i) the use of a Risk-Aware Scenario Generator (RASG) to simulate stochastic rainfall, pest risk, and stress profiles; (ii) an enhanced LSTM ensemble with context-aware gating using scenario and relevance vectors for yield forecasting; and (iii) a fuzzy rule-based advisory engine (FLAE) that maps predictions and environmental factors into interpretable agronomic recommendations. Extensive evaluation across benchmark datasets demonstrated that ACAS-FLY outperforms existing models in terms of Accuracy, Precision, Sensitivity, Specificity, and F-Score. The results confirm the system's ability to adapt to environmental uncertainty and deliver targeted, high-confidence crop guidance. Its modular design allows seamless integration into region-specific advisory systems while ensuring interpretability and scalability. Future work may focus on real-time deployment with sensor integration, active feedback loops from farmers, and the extension of the fuzzy rule base using reinforcement learning. Expanding the model to support multi-crop advisory across geospatial zones is also planned to enhance its practical applicability at scale.

### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research work.

### CODE AVAILABILITY

The ACAS-FLY framework has been implemented using C++23 with Microsoft Foundation Classes (MFC) in the Visual Studio environment. The source code and execution modules are available online and can be shared upon reasonable request for academic and non-commercial use.

### DATA AVAILABILITY

All datasets used in this study are publicly accessible. Rainfall, weather, crop disease, and crop recommendation datasets were obtained from Kaggle and can be accessed via the following links:

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**Table 1: A comparative summary of the reviewed methods is presented in Table 1, highlighting the authors, titles, methodologies, advantages, and limitations of each approach.**

| Author | Title | Methodology | Advantages | Limitations |
|---|---|---|---|---|
| Subramaniam *et al.* | Crop yield prediction using effective deep learning and dimensionality reduction approaches for Indian regional crops (CYPEDL) | WTDCNN with EWOA optimization and SEKPCA for feature reduction | High prediction accuracy, customized for Indian regions | High training complexity, limited geographic generalization |
| Ahmed M S Kheir *et al.* | Developing automated machine learning approach for fast and robust crop yield prediction using a fusion of remote sensing, soil, and weather dataset (AMLACYP) | H2O AutoML with fused satellite, soil, and climate data | Fully automated model, scalable to large datasets | Sensitive to regional data quality, limited model transparency |
| Mahdizadeh Gharakhanlou *et al.* | From data to harvest: Leveraging ensemble machine learning for enhanced crop yield predictions across Canada amidst climate change (EMLECYP) | Ensemble ML with climate, soil, NDVI, and honeybee census integration | Climate scenario support, high spatial resolution maps | Relies on specific pollinator data, soil profile gaps in some regions |
| Hoque *et al.* | Incorporating meteorological data and pesticide information to forecast crop yields using machine learning (MDPIML) | Logistic regression, k-NN, and gradient boosting with weather and pesticide usage | Multi-crop support, incorporates chemical usage | Missing features like soil moisture and solar radiation |

**Table 2: Features and their numerical ranges**

| Input Variable | Dataset Source | Observed / Computed Range | Fuzzy Sets |
|---|---|---|---|
| Rainfall (Rt) | Rainfall in India – Kaggle | 0–175 mm/week | Low (0–40), Moderate (30–100), High (90–175) |
| Pest Probability (Pt) | Plant Disease Dataset – Kaggle | 0.0–1.0 | Low (0.0–0.3), Medium (0.2–0.6), High (0.5–1.0) |
| Stress Score ( $_t$) | Global Weather – Kaggle | \|Tt – Topt\|, Topt = 25°C | No Stress (0–3°C), Moderate (2–7°C), Severe (>6°C) |
| Predicted Mean Yield ($\mu\hat{Y}$) | Crop Recommendation Dataset – Kaggle | 0.0–1.0 (normalized) | Low (0.0–0.4), Moderate (0.3–0.7), High (0.6–1.0) |
| Yield Uncertainty ($\sigma\hat{Y}$) | Derived from LEFE ensemble | 0.0–0.3 | Low (0.0–0.1), Medium (0.08–0.2), High (0.15–0.3) |

**Table 3: Accuracy**

| Accuracy (%) | | | | | |
|---|---|---|---|---|---|
| **Timestamp** | **CYPEDL** | **AMLACYP** | **EMLECYP** | **MDPIML** | **ACAS - FLY** |
| 1 | 98.28744 | 96.917191 | 98.00174 | 95.64111 | 99.2192 |
| 2 | 98.59289 | 96.684746 | 97.800385 | 96.10598 | 99.043533 |
| 3 | 98.00672 | 96.546341 | 97.875603 | 95.72115 | 99.138824 |
| 4 | 98.03139 | 96.66185 | 97.971802 | 96.26932 | 98.863724 |
| 5 | 98.52911 | 96.64537 | 97.451462 | 96.30297 | 99.150238 |
| 6 | 98.24081 | 96.749947 | 97.703583 | 95.70718 | 98.662933 |

| | | | | | |
|---|---|---|---|---|---|
| 7 | 98.04694 | 97.008743 | 97.664642 | 95.7092 | 99.184074 |
| 8 | 98.29641 | 96.292923 | 97.473305 | 95.52598 | 99.147133 |
| 9 | 98.32646 | 96.642288 | 97.521347 | 95.58038 | 99.304855 |
| 10 | 98.41368 | 96.465355 | 97.859451 | 95.9239 | 98.909874 |

**Table 4: Precision**

| Precision (%) | | | | | |
|---|---|---|---|---|---|
| **Timestamp** | **CYPEDL** | **AMLACYP** | **EMLECYP** | **MDPIML** | **ACAS – FLY** |
| 1 | 98.81828 | 97.727707 | 98.302704 | 96.03159 | 99.237717 |
| 2 | 98.68774 | 97.550026 | 97.904678 | 96.66673 | 99.24955 |
| 3 | 97.91338 | 97.266006 | 98.571053 | 96.15092 | 98.980492 |
| 4 | 98.35365 | 96.952919 | 98.076736 | 96.785 | 98.737442 |
| 5 | 98.41298 | 97.517761 | 97.881889 | 96.62936 | 99.14817 |
| 6 | 98.68844 | 97.09565 | 98.082405 | 96.17406 | 98.650703 |
| 7 | 98.00817 | 97.670486 | 98.269249 | 95.92947 | 98.885628 |
| 8 | 98.81563 | 96.944786 | 97.942406 | 96.05119 | 99.368309 |
| 9 | 98.66877 | 97.567139 | 97.792191 | 95.99449 | 99.113869 |
| 10 | 98.54928 | 96.997437 | 97.945084 | 96.64162 | 98.981522 |

**Table 5: Sensitivity**

| Sensitivity (%) | | | | | |
|---|---|---|---|---|---|
| **Timestamp** | **CYPEDL** | **AMLACYP** | **EMLECYP** | **MDPIML** | **ACAS - FLY** |
| 1 | 97.78017 | 96.168785 | 97.714539 | 95.28743 | 99.200981 |
| 2 | 98.50088 | 95.890587 | 97.70089 | 95.59464 | 98.842293 |
| 3 | 98.0965 | 95.885895 | 97.218842 | 95.33151 | 99.294922 |
| 4 | 97.72379 | 96.391792 | 97.871338 | 95.79698 | 98.98745 |
| 5 | 98.64209 | 95.845467 | 97.046455 | 96.00267 | 99.152275 |
| 6 | 97.81278 | 96.42894 | 97.344879 | 95.28434 | 98.674843 |
| 7 | 98.08422 | 96.394722 | 97.095154 | 95.50871 | 99.479416 |
| 8 | 97.80003 | 95.697159 | 97.032043 | 95.05273 | 98.930687 |
| 9 | 97.99787 | 95.795219 | 97.265327 | 95.20599 | 99.493912 |
| 10 | 98.28275 | 95.976097 | 97.777618 | 95.27401 | 98.83989 |

**Table 6: Specificity**

| Specificity (%) | | | | | |
|---|---|---|---|---|---|
| **Timestamp** | **CYPEDL** | **AMLACYP** | **EMLECYP** | **MDPIML** | **ACAS - FLY** |
| 1 | 98.8056 | 97.690269 | 98.292427 | 96.00036 | 99.237434 |
| 2 | 98.68524 | 97.506882 | 97.900299 | 96.62893 | 99.246445 |
| 3 | 97.91727 | 97.226082 | 98.550896 | 96.11755 | 98.983711 |
| 4 | 98.34297 | 96.935074 | 98.072693 | 96.7515 | 98.740623 |
| 5 | 98.41666 | 97.473679 | 97.863495 | 96.60722 | 99.148209 |
| 6 | 98.67659 | 97.075424 | 98.067764 | 96.13799 | 98.651031 |
| 7 | 98.00971 | 97.639244 | 98.248062 | 95.91145 | 98.892242 |
| 8 | 98.8032 | 96.904427 | 97.92292 | 96.00927 | 99.365501 |
| 9 | 98.65959 | 97.521286 | 97.780167 | 95.96104 | 99.117241 |
| 10 | 98.54533 | 96.965141 | 97.941559 | 96.59271 | 98.980057 |

**Table 7: F-Score**

| F-Score (%) | | | | | |
|---|---|---|---|---|---|
| **Timestamp** | **CYPEDL** | **AMLACYP** | **EMLECYP** | **MDPIML** | **ACAS - FLY** |
| 1 | 98.29648 | 96.941978 | 98.007736 | 95.65807 | 99.219345 |
| 2 | 98.59422 | 96.713188 | 97.802673 | 96.12769 | 99.045509 |
| 3 | 98.00485 | 96.571014 | 97.890282 | 95.73946 | 99.137451 |
| 4 | 98.03771 | 96.671547 | 97.973923 | 96.28846 | 98.862282 |
| 5 | 98.52741 | 96.674385 | 97.462379 | 96.315 | 99.15023 |
| 6 | 98.24865 | 96.761147 | 97.712257 | 95.72714 | 98.662773 |
| 7 | 98.04618 | 97.028412 | 97.678665 | 95.71862 | 99.181633 |
| 8 | 98.30521 | 96.316925 | 97.485092 | 95.54936 | 99.14901 |
| 9 | 98.33218 | 96.673058 | 97.528038 | 95.59861 | 99.303528 |
| 10 | 98.41583 | 96.484062 | 97.861282 | 95.95295 | 98.910652 |

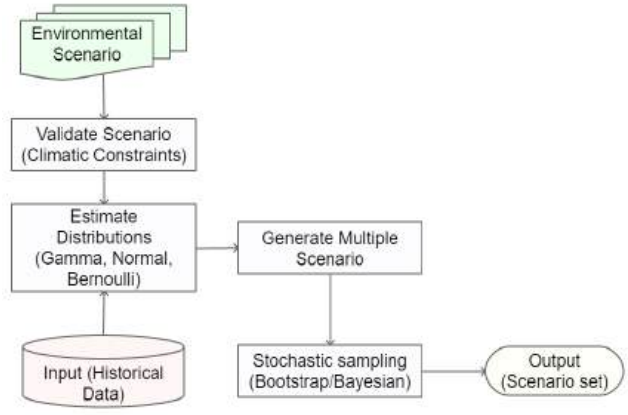



**Figure 1: RASG Flow diagram**

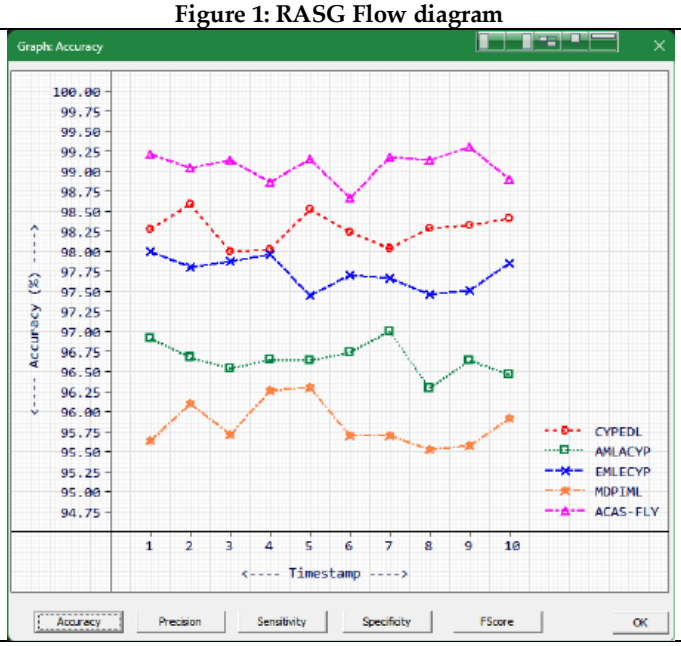



**Figure 2: Accuracy**

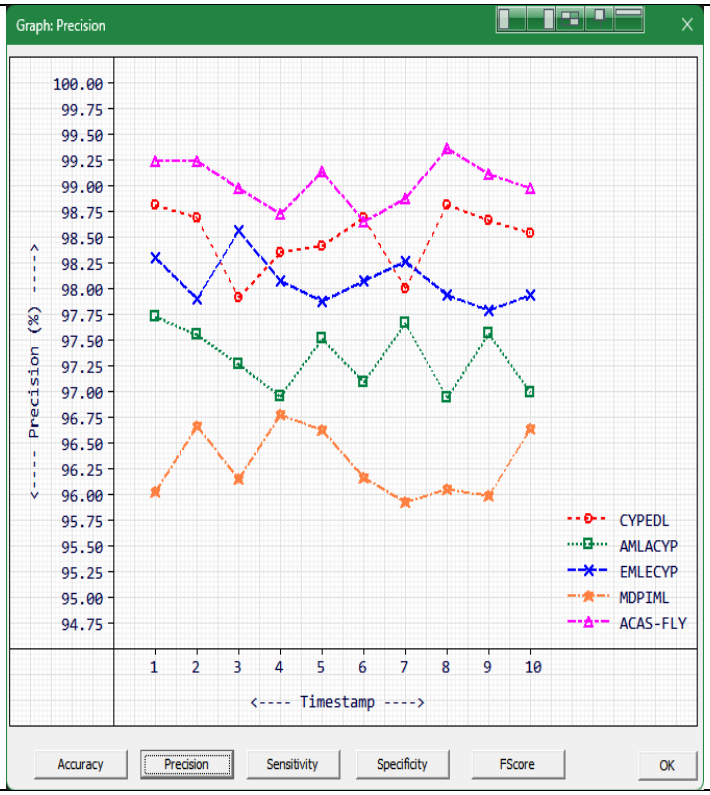



**Figure 3: Precision**

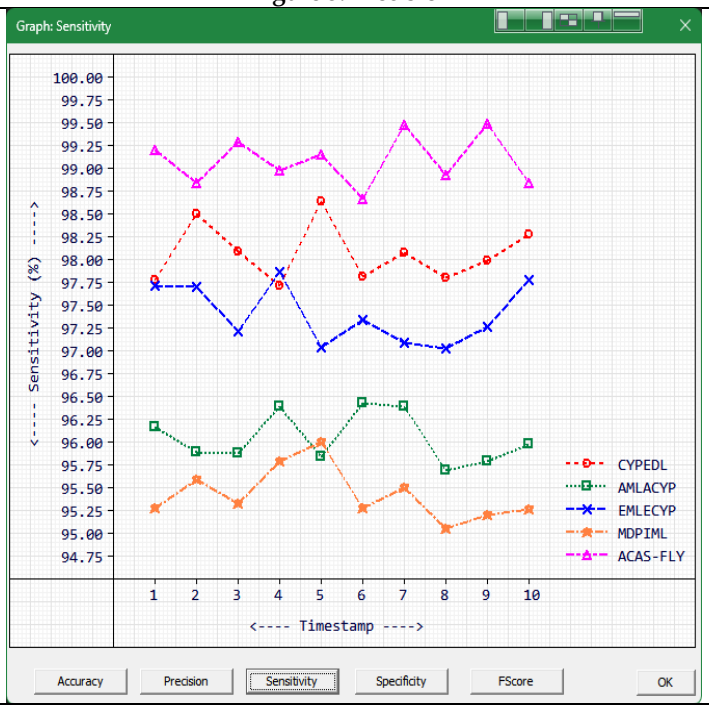



**Figure 4: Sensitivity**

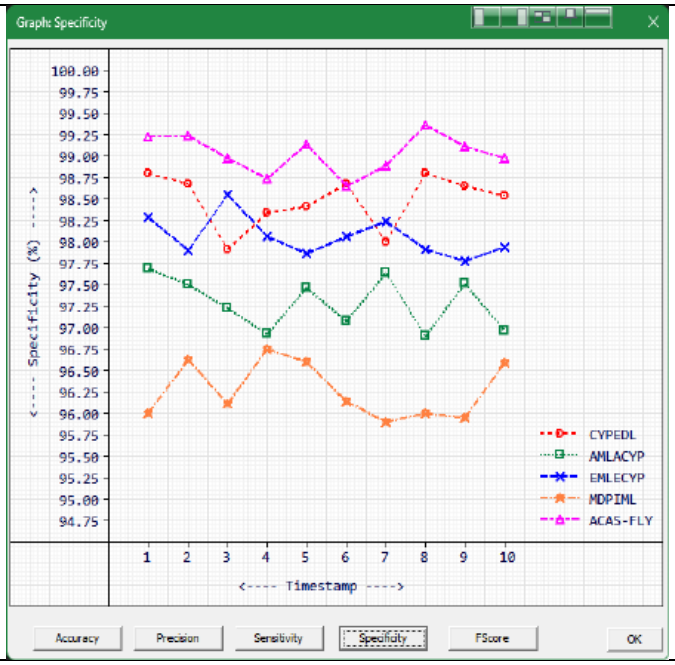



**Figure 5: Specificity**

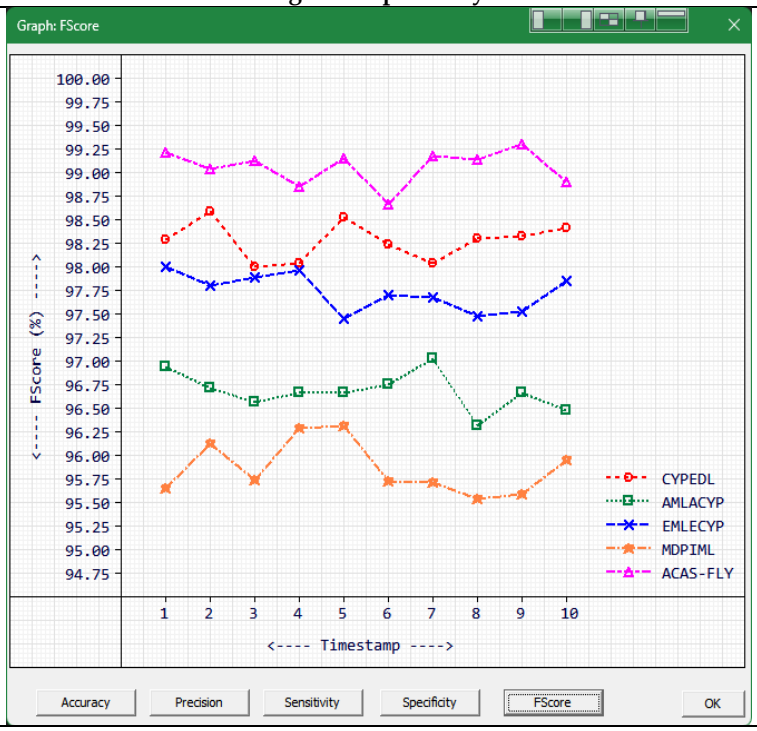



**Figure 6: F-Score**

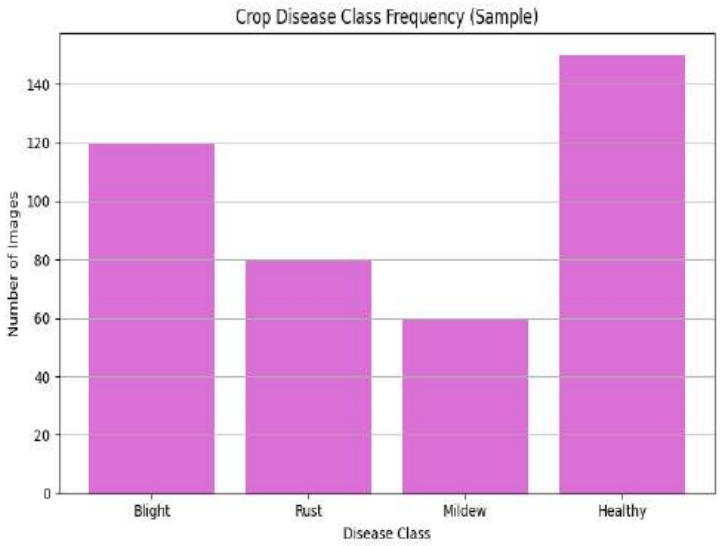



**Figure 7: Crop Disease Class Frequency Distribution**

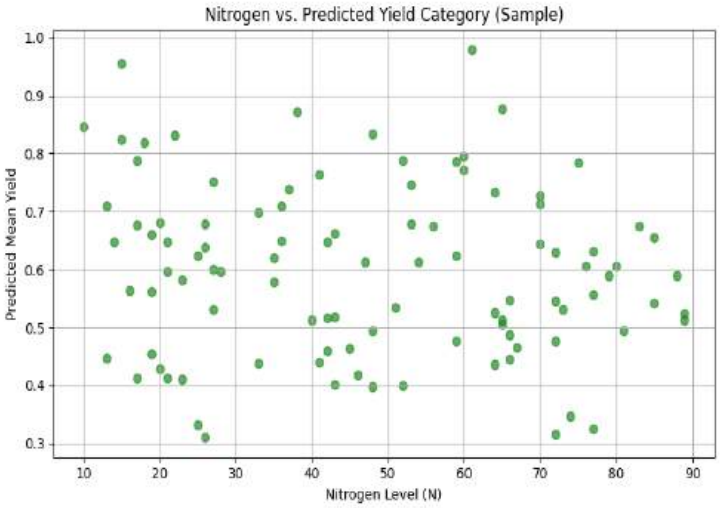



**Figure 8: Nitrogen Level vs. Predicted Mean Yield.**

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RESEARCH ARTICLE

# Health Initiatives for Tribal Populations in India: Current Programs, Challenges, and Future Directions

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## ABSTRACT

India's tribal population, constituting approximately 8.6% of the country's total, faces significant health disparities and barriers to accessing healthcare services. This study examines the systemic challenges hindering healthcare access and explores the effectiveness of government initiatives aimed at addressing these issues. The research aims to understand the nature of healthcare gaps in tribal areas, with particular attention to infrastructure, cultural barriers, and healthcare delivery systems. Efforts to improve tribal health include initiatives under the National Health Mission, which focuses on enhancing healthcare infrastructure in rural and urban areas. Additionally, the Tribal Sub-Plan allocates specific funds for tribal development, including health benefits. Programs introduced by the Ministry of Tribal Affairs, such as mobile medical units and tribal research institutes, aim to address specific health-related challenges. However, in many cases, these efforts fall short due to geographical isolation, poor infrastructure, limited transportation, and cultural or linguistic differences that restrict access to healthcare delivery. The study explores how malnutrition, maternal and child health issues, and the neglect of traditional healing practices exacerbate health inequities. Findings highlight the need for collaboration between mainstream healthcare providers and tribal communities to develop inclusive and culturally sensitive healthcare interventions. The analysis emphasizes the importance of integrating traditional methods with modern medicine to improve healthcare performance and acceptance among tribal populations. The study also highlights the critical role of community leaders and local healthcare workers in delivering targeted treatments for conditions like sickle cell anaemia and malnutrition. Efforts should focus on providing health literacy education and developing effective communication strategies. By fostering collaboration

across education, health, and social welfare programs, the research underscores the necessity of addressing the social determinants of tribal health. This integrated approach is vital for improving health outcomes and achieving equitable healthcare access for tribal populations.

**Keywords**: Tribal Health, Healthcare Barriers, National Health Mission, Traditional Medicine, Health Literacy, Sickle Cell Anaemia, Malnutrition, Social Determinants of Health.

## INTRODUCTION

Tribals communities officially recognized as schedule tribe in India consider them as a one of the most marginalised and distinct group of communities. In Indian constitution its mentioned that 366(25) provided that are socially economically disadvantage group of societies often characterized by physical location, lack of cultural practices and away from mainstream society.The term "tribal" refers to a wide range of indigenous communities with diverse traditions, languages and livelihoods. These communities live predominantly in forested, hilly, and isolated places, relying on natural resources for survival. In India Tribal is a second largest in the world according to 2011 census. In India around 8.6 percent over all population, total around 10.4 crores to separate into different part into 750 ethnic group. Tribal communities face barriers compare to non-tribal communities in various socio, economic, education and in health aspect in the society. Basically, away from mainstream of societies. Therefore, their requirement regarding health required unique need due to their distinct living environments. Health disparities among tribal population is a major issue in India this difference due to structural disadvantages such as geographical isolation, poverty, and cultural marginalisation. indigenous group has greater number of hungers, maternity and newborn mortality and communicable diseases than the mainstream society. These population attain the disease such as sickle cell anaemia, directly impact these communities. Nevertheless, government focus on these issues accessing health care remain restricted due to poor infarstrure, cultural and language barriers and unable to attain the services.

**Healthcare Challenges in Tribal Communities**

Tribal communities face many challenges in accessing health care services. Geographic allocation is a main factor contributing to limit their accessibility. The remote location builds in a manner where people are unbale reach health care centres and poor infrastructure and limits transportation. Natural barriers such as landslides, floods during monsoon as well as various whether pattern futhur posed a challenge on them. Interiors placed people mostly do forest dwellers, hunters and food gathering job. Roads are main point to connect the people to spread information, raw material, people and goods and services. Its main pillar for economic development of tribal people. Since from British Era, indigenous people most used natural resources only psychologically and administratively isolated they are neglected and untouched communities. Another problem is infrastructure deficit, where Health care infrastructure such as well-developed medical service centres and successful transportation system, are essential for ensuring adequate access to medical service. However, lack of infrastructure facilities are major challenges in many rural remote and disadvantage areas. Primary health care centres (PHCs) in India lack key medical equipment, such as diagnostic tools and functional laboratories, restricting their capacity to deliver quality care. Similarly, there is report from NITI Aayog (2021) highlight that lack of specialized care services cause inconsistency in healthcare results between rural and urban place. Cultural barrier is another challenge language difference main threat to reach the health provision centres for tribal people. High prevalence of malnutrition, maternal and child health problems, and neglected diseases like sickle cell anaemia remain critical concerns. Moreover, already existing services due to poor maintenance lack the infrastructure services such as electricity, clean water, toilet condition and compromising service delivery.

**Global Perspectives**

In global arena many challenges continues and WHO identified that low income and middle countries face considerable challenges because of inadequate healthcare infrasture and transportation.To effectively tackle these gaps, the organization encourages targeted investments in road relations and healthcare facility standards. Ouma et al. (2019) and other studies in Sub-Saharan Africa emphasize the relevance of community-based solutions, such as mobile health clinics, in alleviating the consequences of infrastructural shortfalls.This all-major challengeis rectified by technological intervention, where it offers potential remedies. Word bank (2020) underscore that use of drones for medical supply in village remote areas in a cost-effective way to tackle transportation challenges. As well as UNICEF (2021) emphasized that necessity of solar powered facilities in these remote regions.by developing multifaceted approach that along investments in physical infrastructure with creative remedies like health units and drone tech through addressing the issues necessities offering insight unique dimension of their experience. By understanding the intricacies of tribal initiatives for population in India. The importance of this research lies not only in highlighting the challenges faced by tribal people in India but also contributing development of evidence-based strategies and policies to rectify these issues.

By combining data driven insight with real-life narratives, this research aim is to provide holistic understanding of Health Initiatives for Tribal Populations in India their Current Programs, Challenges, and Future Directions and many initiatives from NGO, private sectors, are essential to bridges these gaps and ensure access of health care equally for all .example…In 21st century despite change, achieve significant justice the ultimate goal is to contribute meaningful recommendation for policies and intervention the priorities the right in global stage. Health issuesor High prevalence of malnutrition, maternal and child health problems, and neglected diseases like sickle cell anaemia remain critical concerns.

**Government Initiatives and Efforts**

There are various government initiatives aim to better healthcare access and outcomes for tribal communities:
National Health Mission (NHM) was launched by govt of India since 2013, is a flagship programme objective to have health care deliver system in rural and tribal region areas. To become comprehensive healthcare development this programme, align with national rural health programme and national urban health programme. This intervention reduces the burden of inequities in healthcare by accessing infrastructure ensuring healthcare services further it strengthensub,primary,and community health care services centres. it's impacted to increase PHC and CHC in tribal belt and this intervention also impacted to launch Janani Suraksha Yojana (JSY) and Janani Shishu Suraksha Karyakram (JSSK) helps to improve institutional deliveries.Moreover,establishment of Accredited Social Health Activists (ASHAs), Reproductive, maternal, newborn, child and adolescent health(RMNCH+A),Mobile Medical Units (MMUs) and telemedicine service providing to rural area or tribal regions to improve their healthcare condition.Other major initives government brought for tribals are Tribal sub plan where it allocates funds for tribals to develop their health care service.Sub plan funds also allocate to nutrition programmes for maternal, child health which is affecting byepidemic like malaria, tuberculosis, Sickel cell anaemia which are most prevailsin tribal regions. These funds due to poor planning, unaccountability unable to spent properly, logistical barriers createlack of establishment of programmes and further cultural barrier is another problem to adopt modern method of health care use. In order to rectify these challenges required community participation and training need to provide to the local workers to tackle this obstacle. Further Ministry of tribal Affairs programs introduced mobile medical unites without the person there by access the technology throughrectify their medical needs by government to attain health services and also conduct tribal health research centres were based on evidence can assimilate the specific health care medicine and targeted intervention is possible by healthcare challenges faced by tribal people. Despite these efforts, many programs struggle to meet their goals due to systemic inefficiencies and continues barriers.

**Statement of problem**

Health Initiatives for Tribal Populations in India their challenges remain and required necessary steps to give equal opportunities to tribal population. Despite national efforts and legal framework aimed at protecting tribal population health and wealth and the study aim is to comprehensive approach required to examine tribal population their

health intervention in Indian society to improve their deteriorated condition by focusing need based approach in Indian society.

**Significance of study:** The studyseeks to uncover the multifaceted dimension of tribal health problems and their barriers while intervention and understanding unique socio-cultural economic factor. By underscoring their lived experience, the research aims to inform national strategies to overcome the challenges against health and illness problems and promote various health programmes to uphold the right in the Indian society with the help of constitution. Thereby giving contribution to a more democracy world.

**Objective:**

The study investigates health intervention. The aims to analysis the nature of healthcare gaps in tribal areas, with particular attention to infrastructure, cultural barriers, and healthcare delivery systems and the study explore how malnutrition, maternal and child health issues, and the neglect of traditional healing practices exacerbate health inequities and examine the effort to better tribal health includes such as National Health Mission in order to focus on developing healthcare infrastructure in rural and urban areas. Further the tribal sub plans a lot funds for tribal health development programme. Explore the opportunities for collaboration to prevent their education, health, and social welfare programs to strengthen the intervention of health of tribal population for future directions.

## METHODOLOGY

The study adopted descriptive and analytical qualitative approach to explore Health Initiatives for Tribal Populations in India: Current Programs, Challenges, and Future Directions. It includes comprehensive methodology. A literature review established context, and identity gap, legal analysis for evaluating framework and suggesting reforms, case studies. This approach aims to inform evidence-based policies and healthcare interventions for addressing the challenges faced by tribal population in India in the health context. This included secondary data utilised included books, articles and reports from national and international websites.

## FINDINGS AND DISCUSSION

**Barriers to Healthcare**

Indigenousgroups are often located in hilly and difficult terrain therefore, accessing health facilities is a challenge to them due to lack of transportations and many more issues. Adequately witnessed of PHC and CHC further problem for them to approach health centres despite the development. Poor construction and lack of funds posed a serious challenge to remain with same standard of living life. As traditional believeris not comfortable to adopt modern medicine therefore it makes them gradual healer of their own use of traditional medicine such as herbs and medicinal plants and affecting their health. Language barrier become serious communication problem to create a misunderstanding and reduce quality care and further barrier to deliver them to access health services. Lackof antenatal care, low institutional delivery rates, and poor postnatal care major health barrier to pave the way for maternal and neonatal mortality rate in tribal regions.Consequently, genetic disorder such as sickle cell anaemiamore frequently appeared in tribal population and managing in difficult in that geographic location because it required a modern method of screening and to facilitates the diagnostic but it's not possible in that restricted time.

**Gaps in Government Interventions**:

Lack of integration of conventional healing approachesoften plays under tribal region become rarelyvisible to incorporate in the system.The lack of engaged community involvement in the creation and execution of healthcare actions has led to in low service utilization.Programs like as Village Health and Sanitation Committees (VHSCs) are either neglected or poorly delivered, failing to engage tribal groups in decision-making.The shortage of culturally applicable medical models further divides tribal groups off modern healthcare systems.

**Discussion and Policy Implications:**
Providing good infrastructure facilities can help overcome these challenges, reduce barriers, and transform the area into a more accessible and resource-abundant place. Improving ambulance services in these constrained locations increases the chances of reducing mortality rates and managing critical emergency situations effectively and Giving training to tribal communities in their own languages, while understanding their cultural sensitivities, helps reduce communication gaps and incorporates modern methods into their healthcare system.

**Integrated health interventions:**
Strengthening the scope of nutritional programs such as Integrated Child Development Services (ICDS) and mid-day meals is critical in combating malnutrition.Newborn and maternal health issues can be handled with specific mother and child health initiatives that involve the community.

**Community Empowerment**:
Traditional healers address community health needs, particularly in regions with high prevalence of diseases like malaria and jaundice.Therefore, required own ownership to effectively execute the policy with the help of government. Further promoting health campaign can enhance the uptake of services centres.

**Focus on Genetic Disorders**
The best way to address individuals affected by health conditions in a timely manner is to implement screening programs that help them understand the challenges caused by the lack of modern healthcare interventions, while also creating public awareness.

**Recommendations**
The study underscores the need for collaboration between mainstream healthcare providers, tribal community leaders, and local healthcare workers to develop inclusive and effective healthcare strategies. Key findings include:
1}According to studies, traditional healing procedures frequently have great cultural and spiritual importance for tribal groups, which fosters trust and commitment to treatments. For example, combining traditional herbal medicines with allopathic medicine has showed potential in treating common ailments while preserving cultural identities.
2}Health literacy programs are critical for bridging the gap between healthcare systems and native communities. According to studies, tribal populations frequently encounter challenges such as poor literacy rates, linguistic disparities, and misunderstanding regarding diseases and treatments.
3}Understanding bigger factors of health among aboriginal populations requires collaboration across multiple industries. The merging of education, health, and community development serves as essential for addressing issues such as starvation, hunger, and lack of facilities which greatly impact medical conditions
Therefore by healthcare needs of tribal communities requires an integrated, culturally sensitive approach. Efforts should focus on enhancing healthcare accessibility, equipping facilities with adequate resources, and empowering community leaders to act as bridges between tribal traditions and modern medical systems. Through collaboration and targeted strategies, it is possible to reduce health disparities and improve the overall well-being of India's tribal populations.

## CONCLUSION

As far discussed, healthcare in tribal areas including physical isolation, cultural, language difference required a persistence delivery of quality health service system. Solving malnutrition, maternal and infant health matters andspecifically of genetic disorders required need based intervention. Government programmes such as NHM,TSP, and mobile based health care need to integrate further with various schemes and polices to the development of tribal people and foster community engagement while adopting cultural sensitive approaches to overcome barriers and A comprehensive involvement and health care method is necessary to encourage health outcomes for tribal population

in developing countries like India and the same coin has two sides as initiativesprogrammesinitiated by international forum still required focused specific intervention through get back the next stage of healthy environment style of life.

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RESEARCH ARTICLE

# Assessment of Physico - Chemical Parameters and Water Quality Index on Tannery Effluent in Dindigul District

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## ABSTRACT

The present work is aimed at assessing the physico-chemical properties of Tannery effluent in Dindigul District. The effluent was brownish colored with a disagreeable odor, the parameters such as pH, Electrical Conductivity (EC), Total Dissolved Solids (TDS), Total hardness, Chlorides etc., and also calculating the WQI for the following parameters like Turbidity, TDS, pH, Alkalinity, Ammonia, Calcium, Chromium, Fluoride, Hardness, Iron, Magnesium, Nitrate, Potassium, Sodium, Sulphate. The high value of WQI has been found to be mainly from the higher value of TDS, hardness, chloride, sodium, chromium in the tannery effluent. Although in Dindigul tannery effluent , all the physicochemical parameters were higher then the permissible limit, and Water quality index showed this effluent is incompatible for drinking agriculture or any other purpose.

**Keywords** - Tannery effluent Physical, parameters, Chemical parameters, Water quality index.

## INTRODUCTION

The tannery industry forms the foundation of the leather sector, but it generates colored wastewater due to various processes such as bleaching, mercerizing, dyeing, printing, and packing, which involve substantial amounts of synthetic chemicals. This effluent, often discharged into sewage systems or neighboring land, poses significant environmental problems (Murali*et al.,* 2013). Although the leather industry is one of India's oldest and most significant sectors, contributing substantially to foreign exchange earnings, it suffers from severe environmental pollution. This pollution undermines some of the societal benefits associated with the industry. Leather production, along with the creation of leather goods, boards, and fur, results in numerous by-products, including solid waste and high volumes of wastewater containing various pollutants and toxic chemicals. The tannery sector plays a crucial role in the economies of many countries. However, depending on the specific processes used, it generates substantial quantities of wastewater containing ammonium, sulfates, surfactants, acids, dyes, emulsified oils, and organic substances, including both natural and synthetic tannins (Reddy and De Busk, 2007). Leather processing involves significant amounts of chemicals such as sodium chloride, chromium, sulfate, calcium salts, sodium sulfide, acids, alkalis, fats, liquors, and organic dyes. A significant environmental challenge facing the tanning industry is the disposal of chromium-contaminated sludge, which is a by-product of wastewater treatment.

In India, tannery industries are concentrated in specific regions: Vaniamvadi, Ambur, Trichy, and Dindigul focus primarily on processing goat or sheep skins, while tanneries in Ranipet, Pernampet, Erode, and Pallavaram handle skin hides. The industrial effluent from these tanneries contains various chemicals, including dispersants, and is characterized by high salinity, significant organic loading, and specific pollutants like chromium (Saritha*et al.,* 2013). Major issues include wastewater contaminated with heavy metals, toxic chemicals, chlorides, lime, high dissolved and suspended salts, and other pollutants. Tanneries generate wastewater in the range of 30-35 liters per kilogram of processed skin or hide. This wastewater typically has variable pH and high concentrations of suspended solids, biological oxygen demand (BOD), chemical oxygen demand (COD), tannins, and chromium. The expansion of industrialization has reached even small towns and villages, bringing with it severe pollution problems (Babich*et al.,* 1982). Leather processing presents a significant environmental challenge due to the nature and volume of chemicals used, as well as the large amounts of waste generated and discharged. Tannery effluent is widely recognized as a serious environmental threat globally. Water is commonly used by industries for waste disposal (Thamizhiniyan*et al.,* 2009).To address this issue, several methods such as reverse osmosis, ion exchange, membrane filtration, and electro-dialysis are employed to remove heavy metals from wastewater. However, these techniques are expensive and produce concentrated waste that requires additional treatment for proper disposal. Biological removal processes may offer a more viable alternative for wastewater treatment (Lovely and Coates, 1997; Rittmann*et al.,* 2004).

The discharge of industrial effluents leads to chemical changes in the soil due to the continuous release of polluted water. High levels of pollution can severely damage root systems, reducing plant yield significantly by maturity. Key indicators of yield loss include delayed germination and premature leaf senescence. Coir pith has emerged as an effective means to reduce phenolic compounds entering water resources. It serves as a bio-adsorbent due to its ability to enhance water infiltration and improve nutrient availability for crops. Water pollution remains a critical and escalating issue. Despite existing regulations, toxic chemical pollution in aquatic environments persists, with domestic and industrial effluents being major contributors to this contamination (Claxton *et al.,* 1998; White and Rasmussen, 1998). The Water Quality Index (WQI) is a highly effective tool for conveying water quality information to both the public and policymakers (Mishra and Patel, 2001). It is defined as a rating that reflects the combined influence of various water quality parameters (Ramakrishnaiah*et al.,* 2009). This method provides a composite score that assesses the overall quality of water based on individual parameters (Al-Mohammed and Mustasher, 2013). In this study, the water quality of the Dindigul tannery effluent was evaluated using the weighted arithmetic index, a method that calculates the WQI to determine the suitability of the water.

## MATERIALS AND METHODS
## PHYSICO-CHEMICAL ANALYSIS OF EFFLUENT

Industrial effluent was collected in polyethylene containers from an outlet of the industry in Dindigul, Tamil Nadu, India, and transported to the laboratory for further analysis. The physico-chemical parameters of the effluent—including pH, Electrical Conductivity (EC), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Total Hardness, Chloride, Sodium, Calcium, and heavy metals—were measured using the standard methods outlined by APHA (2012).

**Water Quality Index**

To compute the Water Quality Index (WQI), the following steps were taken:

1. **Parameter Weight Assignment**: Parameters were assigned weights based on their significance in water quality assessment. Chloride and fluoride were given the maximum weight of 5 due to their crucial impact on water quality (Srinivasamoorthy et al., 2008). Turbidity, calcium, potassium, sodium, magnesium, and chromium were assigned the minimum weight of 2, reflecting their lesser role in the assessment.
2. **Intermediate Weight Assignment**: Parameters such as pH, Total Dissolved Solids (TDS), alkalinity, iron, nitrate, and sulfate were assigned a weight of 4, indicating their moderate importance in evaluating water quality.
3. **Variable Weight Assignment**: Total hardness and ammonia were assigned a weight of 3, based on their relevance to determining water quality. In next step, the relative weight (Wi) is computed from the following equation:

$$Wi = wi / \sum_{i=1}^{n} wi$$

In calculating the Water Quality Index (WQI), the following steps are applied:

1. **Weight Assignment**: Each parameter is assigned a weight based on its importance. The relative weight (Wi) for each parameter is computed, where wiw_iwi is the weight assigned to the parameter and nnn is the total number of parameters. The calculated relative weight (Wi) values for each parameter are detailed in Table 1.
2. **Quality Rating Scale**: For each parameter, a quality rating scale (qi) is determined by dividing its concentration in the water sample by the corresponding standard value, as specified by BIS (2012). This ratio is then multiplied by 100 to obtain the quality rating scale for each parameter.

$$qi = (Ci / Si) \times 100$$

where, qi is the quality rating, Ci is the concentration of each chemical parameter in each water sample in mg/L, and Si is the Indian drinking water standard for each chemical parameter in mg/L according to the guidelines of the (BIS, 2012) . For computing the WQI, the SI is first determined for each chemical parameter, which is then used to determine the WQI as per the following equation (Tiwari and Mishra)

$$SIi = Wi * qi$$
$$WQI = \sum SIi$$

SIi is the subindex of ith parameter; qi is the rating based on concentration of ith parameter and n is the number of parameters. The computed WQI values are classified into five types, “excellent water” to “water, unsuitable for drinking”.

## RESULTS AND DISCUSSION

**Physical parameters of effluent from tannery industry**

The water quality parameters analyzed in the tannery effluent included appearance, odor, turbidity, electrical conductivity, and total dissolved solids. The tannery effluent sample exhibited a brownish color and an unpleasant odor. The odor in water can be attributed to chemical substances such as hydrogen sulfide, free chlorine, ammonia, phenols, alcohols, esters, and hydrocarbons, as well as biological agents like algae, fungi, and other microorganisms (Sharma, 2000). The turbidity of the tannery effluent was measured at 680 NTU. Turbidity in effluent is primarily caused by colloidal and suspended impurities, which reduce light penetration and subsequently hinder photosynthesis (Aisien *et al.*, 2010). This increased turbidity often leads to higher turbulence and mixing in the water, resulting in a greater concentration of suspended particulate matter. Typically, turbidity levels rise following heavy rainfall, as rainwater picks up small particles of dirt from the ground before reaching the water source. Additionally, high turbidity in surface water can be associated with decaying organic matter, clay, microorganisms such as bacteria, and small amounts of mineral salts (Frada, 2001).

The electrical conductivity of the tannery effluent was found to be very high (Table 2). Electrical conductivity measures the water's ability to conduct an electric current, which is directly related to the concentration of dissolved minerals. However, it does not specify which particular elements are present. A high electrical conductivity value often indicates the presence of contaminants such as sodium, potassium, chloride, or sulfate (Nazir*et al.*, 2015). The total dissolved solids (TDS) in the tannery effluent were high (Table 2). TDS in water can originate from a variety of sources, including natural processes, sewage, urban and agricultural runoff, municipal waste, and the chemical weathering of rocks (Hussain, 1989). Water with extremely low TDS concentrations can also be undesirable due to its flat taste and lack of mineral content (WHO, 1994). TDS levels indicate the salinity of water, and a high TDS content can impact water density, affect the osmoregulation of freshwater organisms, and reduce the solubility of gases. This can also affect the water's suitability for drinking, irrigation, and industrial uses. Based on TDS concentration, water can be classified as either desirable or permissible for drinking (Wilcox, 1955).

**Chemical examination of effluent from tannery industry**

The hydrogen ion concentration of the tannery effluent was alkaline, with a pH of 7.9. pH is a crucial factor in determining the corrosive nature of water; a lower pH indicates higher corrosivity. pH is positively correlated with electrical conductivity and total alkalinity (Gupta *etal*., 2009). Elevated pH levels can reduce photosynthetic activity and affect the assimilation of carbon dioxide and bicarbonates, contributing to the higher pH observed. Various factors can influence water pH. The high alkalinity in the tannery effluent, as indicated by its high pH (Table 2), suggests that the equilibrium between carbon dioxide and carbonate-bicarbonate is significantly affected by changes in physico-chemical conditions (Karanth, 1987).

The total hardness of the tannery effluent in Dindigul was recorded at 2600 mg/L (Table 2). Hardness refers to the concentration of multivalent metallic cations in water. It varies by location, with groundwater typically being harder than surface water. The hardness of water reflects the geological formations it has interacted with (Garg *et al.*, 2007). The effluent showed a high calcium concentration of 560 mg/L. Calcium contributes significantly to water hardness. Its presence in high concentrations suggests that the removal processes were insufficient, leading to precipitation in the lake water as ionic strength increased (Jayaprakash*et al.*, 2005). Calcium interacts with various cations, including carbonates, bicarbonates, and fluorides. Elevated levels of $Ca^{2+}$ are directly related to increased water hardness, which can render the water unsuitable for both domestic and agricultural use (Pirzada*et al.*, 2013). The tannery effluent contained a high chloride concentration of 4500 mg/L. Chloride is introduced into tannery effluents primarily as sodium chloride due to the substantial use of common salt in hide and skin preservation or the pickling process. Chlorides are highly soluble and stable, making them resistant to removal during effluent treatment, and they persist as environmental pollutants. Elevated chloride levels can inhibit the growth of plants, bacteria, and fish in surface waters, potentially causing damage to cellular structures. When used for irrigation, high chloride concentrations can

lead to increased surface salinity through evaporation, resulting in reduced crop yields. Additionally, chlorides can be leached from the soil by rain, reentering the ecosystem and potentially contaminating groundwater (Bosnic*et al.,* 2000). The fluoride content in the effluent samples was 0.4 mg/L, which is within permissible limits. However, the sulfate content was measured at 400 mg/L. Sulfates in tannery effluents typically arise from the use of sulfuric acid or products containing high levels of sodium sulfate (Bosnic*et al.,* 2000). Various factors, including calcium, magnesium, carbonates, bicarbonates, sulfates, chlorides, nitrates, and organic matter, collectively contribute to water hardness (Salim *et al.,* 2013).

The magnesium content in the tannery effluent was notably high at 250 mg/L. Ammonium was present at 10 mg/L, while nitrate concentration, measured using the electrode method, was 38 mg/L. Nitrate esters can migrate over long distances and pose environmental risks (Rifler and Medina, 2006). Additionally, the nitrite level was recorded at 0.5 mg/L, which is considered elevated. The Biological Oxygen Demand (BOD) is a critical parameter for assessing the impact of wastewater on receiving water bodies. In the present study, the tannery effluent exhibited a high BOD level of 500 mg/L, indicating a substantial presence of organic matter. Previous studies have also reported elevated BOD levels in tannery effluents, ranging from 600 to 1622 mg/L (Noorjahan, 2014; Kulkarni, 1992). High BOD levels reflect the increased microbial oxygen demand, which can lead to a depletion of Dissolved Oxygen (DO) in the water. This reduction in DO may result in hypoxic conditions, adversely affecting aquatic life (Jerin, 2011).

The Chemical Oxygen Demand (COD) test is an effective method for estimating organic matter and determining the total oxygen demand exerted by organic compounds in a sample. The current study found high COD levels in the tannery effluent, measuring 1450 mg/L. This elevated COD is likely due to the presence of organic compounds that are not readily degraded by bacterial action (Nagarajan and Ramachandramoorthy, 2002). Furthermore, tannery wastewater is often contaminated with elevated levels of metals such as iron, nickel, chromium, zinc, cadmium, manganese, and copper. These metals can contaminate agricultural soil, posing significant health risks to consumers through the crops and vegetables grown in these soils (Mohanta*et al.,* 2010).

## CONCLUSION

The present investigation aimed to assess the physicochemical properties and Water Quality Index (WQI) of tannery effluent in the Dindigul District. The study found that the physical and chemical parameters of the effluent either met or exceeded permissible limits. The resulting water quality index indicated that the effluent is unsuitable for drinking, agricultural, or any other uses. The presence of harmful pollutants in the effluent poses significant environmental risks, potentially impacting plant, animal, and human health.To address these challenges, bioremediation has been proposed as a promising solution. This approach utilizes natural biological processes to degrade or neutralize contaminants in the tannery effluent. Ongoing research is focused on exploring greener bioremediation methods to effectively manage and mitigate the environmental impact of these pollutants.

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**Table 1 : Relative weight of Physico chemical parameters**

| Parameters | BIS Standard (2012) | Weight (wi) | Relative weight (Wi) |
|---|---|---|---|
| Turbidity | 1 | 2 | 0.0384 |
| TDS | 500 | 4 | 0.0769 |
| pH | 6.5 | 4 | 0.0769 |
| Alkalinity | 200 | 4 | 0.0769 |
| Ammonia | 0.5 | 3 | 0.0576 |
| Calcium | 75 | 2 | 0.0384 |
| Chloride | 250 | 5 | 0.0961 |
| Chromium | 0.05 | 2 | 0.0384 |
| Fluoride | 1 | 5 | 0.0961 |
| Hardness | 200 | 3 | 0.0576 |
| Iron | 0.3 | 4 | 0.0769 |
| Magnesium | 30 | 2 | 0.0384 |
| Nitrate | 45 | 4 | 0.0769 |
| Potassium | 12 | 2 | 0.0384 |
| Sodium | 200 | 2 | 0.0384 |
| Sulphate | 200 | 4 | 0.0769 |

**Table 2: Physico - chemical Examination of Water Sample from Tannery Effluent.**

| S.No | Parameters | Acceptable limit (mg/ml) WHO(2012) | Exceeding limit (mg/ml) | Tannery effluent in Dindigul (mg/ml) |
|---|---|---|---|---|
| | **Physical Examination** | | | |
| 1 | Appearance | - | - | Brownish |
| 2 | Odour | - | - | Objectionable |
| 3 | Turbidity | 2.5 | 10 | 680 |
| 4 | Electrical Conductivity Micro mho/cm | - | - | 10,110 |
| 5 | Total Dissolved Solids mg/ml | 500 | 2000 | 11,200 |
| | **Chemical Examination** | | | |
| 1 | Alkalinity as CaCo3 | - | - | 720 |
| 2 | pH | 7.0-8.5 | 6.5-9.2 | 7.9 |
| 3 | Total Hardness | 200 | 600 | 2600 |
| 4 | Ammonia as NH3 | - | - | 10 |
| 5 | Biological Oxygen Demand | - | - | 500 |

| | | | | |
|---|---|---|---|---|
| 6 | Calcium as Ca | 75 | 200 | 560 |
| 7 | Chemical Oxygen Demand | - | - | 1450 |
| 8 | Chloride as Cl | 200 | 1000 | 4500 |
| 9 | Chromium as Cr | 0.05 | 0.1 | 240 |
| 10 | Dissolved Oxygen | - | - | 2.1 |
| 11 | Fluoride as F | 1 | 1.5 | 0.4 |
| 12 | Iron as Fe | 0.1 | 1.0 | 1.0 |
| 13 | Magnesium as Mg | 30 | 150 | 250 |
| 14 | Manganese as Mn | 0.05 | 0.5 | 0.1 |
| 15 | Nitrate as NO3 | 100 | 100 | 38 |
| 16 | Nitrite as NO2 | - | - | 0.5 |
| 17 | Phosphate as PO4 | - | - | 3.5 |
| 18 | Sulphate as SO4 | 200 | 400 | 400 |

**Table 3 : Computed WQI value of Dindigul Tannery Effluent**

| Parameters | Si | Ci | Qi | Sli |
|---|---|---|---|---|
| Turbidity | 1 | 680 | 68000 | 2611.2 |
| TDS | 500 | 11200 | 2240 | 172.25 |
| pH | 6.5 | 7.9 | 121.53 | 9.345 |
| Alkalinity | 200 | 720 | 360 | 27.684 |
| Ammonia | 0.5 | 10 | 2000 | 115.2 |
| Calcium | 75 | 560 | 746.66 | 28.67 |
| Chloride | 250 | 4500 | 1800 | 172.92 |
| Chromium | 0.05 | 240 | 480000 | 18432 |
| Fluoride | 1 | 0.4 | 40 | 3.844 |
| Hardness | 200 | 2600 | 1300 | 74.88 |
| Iron | 0.3 | 1.0 | 333.3 | 25.63 |
| Magnesium | 30 | 250 | 833.3 | 31.919 |
| Nitrate | 45 | 38 | 84.44 | 6.493 |
| Potassium | 12 | 500 | 4166 | 159.97 |
| Sodium | 200 | 4000 | 2000 | 76.8 |
| Sulphate | 200 | 400 | 200 | 15.38 |
| | | | | ∑Sli=21964.25 |

**Table 4. Water quality classification based on WQI value**

| WQI value | Water quality |
|---|---|
| <50 | Excellent |
| 50-100 | good water |
| 100-200 | poor water |
| 200-300 | very poor |
| >300 | unsuitable for drinking |

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**RESEARCH ARTICLE**

# Neurological Disorder: Prevalence, Pathogenesis and Herbal Remedy Which Modulate NRF$_2$ Pathway for Neurological Disorders

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## ABSTRACT

Neurological illnesses have a substantial impact on cognitive performance and are linked to one of the highest rates of morbidity, disability, and death worldwide. Cerebrovascular, neurodegenerative, autoimmune, and spinal cord illnesses are the most frequent neurological disorders, followed by intracranial tumors and craniocerebral trauma. Due to the disadvantages and potential side effects of established therapies, herbal components have been examined as a viable option for treating neurological diseases. Alzheimer's disease is the most prevalent neurodegenerative illness that worsens over time in humans. It is distinguished by gradual cognitive deficits, the most visible of which is memory loss, as well as senile plaques and neurofibrillary tangles. Various therapeutic therapies, including synthetic and natural techniques, are used to combat AD in light of the disease's pathogenesis. Common herbal compounds used to treat neurological illnesses such as Alzheimer's, Parkinson's, and multiple sclerosis have been demonstrated to enhance both symptoms and cognitive performance. These are *Ginkgo biloba, Bacopamonnieri, Withania somnifera, Passiflorain carnata, Rhodiola rosea,* and *Curcuma longa*. To reduce oxidative damage to the nervous system, researchers have studied the Nrf2 pathway and natural compounds that positively control it. This researchwere carried out both in vivo, mostly utilizing mouse models, and in vitro, employing stress-induced neurons and microglia. Controlling numerous Nrf2 upstream activators, quercetin, curcumin, anthocyanins, tea polyphenols, and other phenolic compounds that have not gotten enough attention can also have an effect on Nrf2. Terpenoids, which include carotenoids (astaxanthin, lycopene), diterpenes (ginkgolides), triterpenes (ginsenosides), and monoterpenes (aucubin, catapol), are another class of phytochemical substances that activate this pathway. With this review, we hope to bring up to date our understanding of how the Nrf2 pathway is activated by secondary metabolites of health importance.

**Keywords:** Neurological, NRF2 Pathway, Extract, Oxidative Stress.

# INTRODUCTION

Neurological problems rank among the highest in the world in terms of morbidity, disability, and mortality. They also seriously impair cognitive functioning. The most common neurological illnesses include cerebrovascular, neurodegenerative, autoimmune, spinal cord, intracranial tumour, and craniocerebral trauma. Because of the limits and potential side effects of conventional medicines, herbal remedies have been investigated as a viable therapeutic alternative for neurological illnesses. Alzheimer's disease is the most common neurological disease that progresses with time in humans. It is distinguished by senile plaques and neurofibrillary tangles, as well as progressive cognitive abnormalities, the most evident of which is memory loss. A range of medicinal interventions, encompassing both synthetic and natural methods, are being employed to address AD in view of the in light of the disease's pathogenesis (1).According to statistics from national, regional, and worldwide sources, the global burden of all noncommunicable neurological illnesses, the total number of disabled adjusted life years, and the number of persons who have died from noncommunicable neurological diseases has grown considerably. The majority of patients with neurological disorders are only clinically detected when nerve damage is exceedingly severe, so they miss out on the greatest potential treatment period, even with the recent fast development of numerous surgical, pharmacologic, and interventional techniques. Patients still have a dismal outlook overall, and the majority of medications have potential negative side effects (2,3).

**Pathogenesis and progression of neurological disorders:**

Numerous studies have already revealed the well-established significance of nitric oxide, a naturally occurring chemical, in the central nervous system's function. It is also a key molecule that is involved in several important and beneficial metabolic processes. Numerous neurological illnesses have been associated to deregulation of its metabolism, including increased nitric oxide synthesis, which has been proposed as a cause or aggravating factor. There is mounting evidence that this molecule may have a role in prevalent neurodegenerative diseases such amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease. Nitric oxide is a naturally occurring molecule that has been demonstrated in various studies to have an important role in the function of the central nervous system. It is also a critical molecule engaged in a variety of key and beneficial metabolic processes. Dysregulation of its metabolism, specifically the increased synthesis of nitric oxide, has been linked to a number of neurological disorders and has been considered as a trigger or aggravator. An increasing body of research suggests that this molecule could be involved in prevalent neurodegenerative diseases like Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis (4).

A growing body of studies demonstrates that decreased energy metabolism leads to brain aging. Specifically, neurons' glucose metabolism gradually decreases, resulting in a growing shortage in adenosine triphosphate (ATP) generation and, as a result, glucose absorption is restricted. Rising nicotinamide adenine dinucleotide (NAD) shortages in the mitochondrial salvage pathway, as well as subsequent Krebs cycle impairments, initiate this vicious cycle of energy metabolism at the cellular level. Furthermore, a low NAD level inhibits the function of NAD-dependent enzymes, increasing genetic errors and initiating the degeneration and death processes of neurons. Neurons in the brain have the highest energy metabolism, and this pattern of events is typical of various brain areasv (5). The primary source of ATP produced by the high brain metabolic activity is oxidative phosphorylation. But mitochondria also perform other crucial roles in the brain. The endoplasmic reticulum and mitochondria collaborate to regulate neurotransmission by buffering calcium in presynaptic terminals and modulating somato-dendritic calcium levels. Calcium concentrations in the cytosol vary due to constant signaling. Furthermore, mitochondria regulate both the cell cycle and cell death. Maintenance of mitochondrial fitness is essential for carrying out these various functions, and this requires effective quality control mechanisms, which are accomplished through mitochondrial biogenesis, fission (the division of a single organelle into two or more daughter organelles, which is necessary for providing dividing or growing cells with an adequate number of mitochondria), fusion (the process by

which mitochondria share essential components), and mitophagy. Furthermore, for mitochondria to supply energy throughout an axon, they must be trafficked along them (6).

**Pharmacological Treatment associated side effects:**

The fundamental ideas of treatment strategies for neurological illnesses are the same as those for non-comorbid patients, with the difference that medication interactions require special care. A variety of therapy approaches are offered, including electroconvulsive therapy, somatic therapies, cognitive-behavioral therapy, and pharmaceutical medications. While some of the suggested techniques focus on treating depression and anxiety at the same time, others are more disorder-specific. Medications having anxiolytic qualities, like benzodiazepines, are typically started for a brief duration to alleviate anxiety; However, due to their negative influence on cognitive problems in AD and balance concerns in PD, they should be used as a last option. Since the 1950s, antidepressants have been widely used to treat depression and anxiety with positive effects. Some disadvantages of oral antidepressant medication include probable drug interactions, a limited tolerance for adverse effects, and low effectiveness. Tricyclic antidepressants and MAO inhibitors, two first-generation antidepressants that improve the extracellular usefulness of monoamines, have been phased out in recent decades, with the exception of a few conditions. Despite certain limitations, new generation antidepressants are frequently suggested for the treatment of anxiety and depression. list withdrawal syndrome, gastrointestinal problems, and sexual dysfunction as some of the adverse effects. By raising the level of serotonin in the synaptic cleft, these medications function as selective serotonin reuptake inhibitors (SSRIs). At different dosages, SSRI medications are also useful in treating anxiety. When used for neurological conditions and anxiety disorders, the adverse effect profile is typically worse. While there are several biological therapy options available for both disorders, psychological therapies are also effectively employed in both (7). As depicted in figure 1 pharmacological treatment of neurological disorders may have number of side effect.

**Herbals in treatment of Neurological disorders:**

Antioxidant qualities of phytochemicals are linked to longer lifespans and improved human health. They also lessen the toxicity of diseases including cancer, heart disease, and Neurological Disorders. Even though multiple studies have proved their benefits in the treatment of neurological disorders, further research is needed to employ natural chemicals such as curcumin, resveratrol, quercetin, and epigalloca techingallate. The nuclear factor erythroid 2-related factor 2 (Nrf2) pathway has received increased interest in recent years, with a focus on both fundamental science and clinical applications. Organic chemicals are given special attention since they have the potential to change the pathway's function and so ameliorate oxidative stress. The purpose of this study is to transmit the current understanding of the impact that various natural compounds can have on the Nrf2/ARE (antioxidant response element) pathway, as well as to evaluate the feasibility of targeting this pathway for the treatment of noncommunicable illnesses. The review covered both in vivo and in vitro research on how natural substances interact with the Nrf2 pathway and how they can lessen oxidative stress and neuronal damage (8).

**Phytoconstituents treatment as modulator of NrF2:**

It has recently been demonstrated that curcumin somewhat protects against radioactivity. We looked into curcumin's ability to prevent brain damage brought on by 12C6 + radiation in vivo. Our findings show that curcumin consistently reduces brain superoxide dismutase (SOD) activity levels, improves spatial strategy and memory loss induced by 4Gy heavy ion radiation, and increases cerebral malonaldehyde. Curcumin-pretreated mice showed consistent up-regulation of the nuclear erythroid 2-related factor 2 (Nrf2) protein, as well as three known Nrf2 downstream genes: heme oxygenase-1 (HO-1), γ-glutamyl cysteine synthetase (γ-GCS), and NAD(P)H quinine oxidoreductase 1 (NQO1).Our research supported curcumin's antagonistic actions to prevent radiation-induced brain damage in vivo and revealed that curcumin's strong Nrf2 activation potential might be valuable for the protective effects of curcumin against radiation (9). Curcumin is a natural polyphenol with anti-inflammatory, anti-cancer, antibacterial, and antioxidant effects. It has been demonstrated that this material influences Nrf2 signaling, a stress-induced pathway that lowers levels of electrophilic compounds and reactive oxygen species. Curcumin's effects on Nrf2 have been investigated in a range of contexts, including cancer, ischemia-reperfusion injury, diabetes mellitus, neurological system disorders, renal illnesses, pulmonary diseases, and cardiovascular disease. In this review, we

focus on curcumin's Nrf2-mediated therapeutic benefits in various disorders.According to the results examined in this publication, curcumin may be able to activate Nrf2 and function as a therapeutic agent to shield cells from a variety of pathological diseases (10). Tyrosine hydroxylase enzyme levels were raised and the development of synucleinopathy was suppressed after treatment with GBS, which also avoided loss of striatal dopaminergic loss and greatly reduced motor behavioral and neuromuscular impairments. Following intraperitoneal rotenone treatment, oxidoinflammatory/nitroinflammatory stress, significant cholinergic transmission inhibition, and damage to the ileal epithelium and striatal neurons were detected. Furthermore, the pretreatment + concurrent (Pre-CONC) and concurrent (CONC) GBS treatment pattern reversed the significant hyperplasia of cryptal cells in ileal epithelial tissues, the loss of striatal pyramidal neurons with a marked decrease in the length and width of the dendritic spines, and the increased striatal executioner caspase-3 and decreased nuclear factor erythroid-2-related factor 2 (Nrf2).Overall, we demonstrated the possible dual impact of GBS in averting both dopaminergic neural-related impairments and gut wall abnormalities linked with PD (11).

Renowned for their antioxidant properties, Camellia sinensis and *Withania somnifera* have been studied in this work for their protective effects against neuronal damage caused by haloperidol, reserpine, and tacrine. The results demonstrated that the 1:1 combination (HECS:HEWS) significantly enhanced antioxidant status and behavioral activities impacted by vacuous chewing movement, tacrine-induced vacuous chewing movement, and haloperidol-induced catalepsy in a dose-dependent manner. One combination has antioxidant properties and guards against neuronal damage, which becomes more apparent at doses of 30 mg/kg against the effects of tacrine on vacuous chewing, reserpine on hypolocomotion, and haloperidol on catalepsy (12). Bacopamonnieri, Centellaasiatica, Cannabis sativa, Gastrodiaelata, *Ginkgo biloba*, Panax ginseng, and *Withania somnifera* were identified as the most promising plants for Huntington's disease therapy; several of these plants are known to be CNS-active drugs. Curcumin, epigallocatechin-gallate, ginsenosides, kaempferol, naringin, resveratrol, and S-allylcysteine were anti-Huntington's disease compounds; some of these are well-known neuroprotectants. To assess the medicinal effectiveness of the previously identified as well as the novel herbal extracts and chemicals in models of Huntington's disease, more investigation is still required (13).

The goal of this research is to see how standardized extracts of the memory-enhancing herb Bacopamonnieri and the antioxidant melatonin alter the nuclear factor erythroid 2 related factor 2 (Nrf2) pathway in rats with oxalic acid-induced memory impairment. Rats were given intracerebroventricular (ICV) administration of OKA (200 ng) to cause memory impairment. Melatonin (20 mg/kg) and Bacopamonnieri (BM-40 and 80 mg/kg) were administered one hour before the OKA injection and continued daily until day 13. On days 13–15, the Morris water maze test was employed to assess memory. Rats were killed to detect oxidative stress, neuroinflammation, and apoptosis, as well as to explore the molecular expressions of Nrf2, HO1, and GCLC in the cerebral cortex and hippocampus. Significant memory loss was brought on by OKA and oxidative stress (14).

A useful plant in traditional Chinese medicine is ginseng. Its many health advantages, as demonstrated by contemporary research, include restoring blood pressure, feeding and strengthening the body, soothing the mind, enhancing cognitive function, and tonifying vital energy. One of ginseng's main active ingredients is Rg1. It strengthens immunity, increases synaptic plasticity, preserves hippocampus neurons, and improves cognitive function. It also has anti-aging and anti-fatigue qualities, and it has a lot of promise for controlling and preventing neurodegenerative disorders (NDDs). Inhibiting neuroinflammation and oxidative stress, Rg1 treatment was shown in this study to improve chronic LPS-induced cognitive impairments and neuronal ferroptosis in mice. The underlying processes might have something to do with Nrf2 signaling activation and AIM2 inflammasome inhibition. These results offer important new information on treating chronic neuroinflammation and related NDDs (15). Rosemary (*Rosmarinus officinalis* L.), a member of the Lamiaceae family, is noted for its wide range of therapeutic applications due to its distinctive secondary metabolites, Carnosol, Carnosic acid, and rosmarinic acid. In the era of pharmaceuticals and procedures, although we are progressing and achieving, we are getting diverted by numerous ailments, resulting in a growing number of fatalities among young people. One example is a neurological condition, which is defined by alterations in the nerve system, brain, or spinal cord. The majority of young and elderly

individuals worldwide suffer from neurological problems, namely stroke, epilepsy, dementia, Alzheimer's disease (AD), Parkinson's disease (PD), and migraine. Natural goods are promoted globally to reduce the aforementioned loss and emphasize the urgent need for alternate medicinal regimens. This chapter explains how R. officinalis L. and its bioactive metabolites protect against several forms of neurological diseases. AChE, neuroinflammation, β-secretase (BACE-1), mitochondrial redox state, neuronal cell death, and amyloid-beta (A-β) aggregation are some of the mechanisms. Because of its multiple uses and its potent bioactive secondary metabolites, R. officinalis is a great alternative medicine source for treating a variety of neurological illnesses (16).

An inflammatory reaction causes a secondary damage known as intracerebralhemorrhage (ICH). In this work, we discovered that oxidative stress and NLRP3 play a role in ICH damage, and we investigated if silymarin may protect against it. We discovered that 24 hours after ICH damage, oxidative stress indicators (ROS and lipid peroxides) increased as compared to the sham group. Silymarin (200 mg/kg) therapy 30 minutes after ICH damage reduced an increase in oxidative stress markers while increasing antioxidant status. After ICH damage, there was a considerable rise in nuclear levels of NF-κB-p65 and pro-inflammatory cytokines. ICH damage significantly increased NLRP3 inflammasome activation and downstream targets, including as caspase-1 and IL-1β production.Silymarin treatment suppressed NF-κB-p65 levels and inflammasome-mediated caspase-1/IL-1β expressions, leading to decreased inflammation. Additionally, silymarin therapy elevated Nrf-2/HO-1 after ICH damage, which enhanced overall cytoprotection. All of these results demonstrate that silymarin functions as a neuroprotective agent by inhibiting the activation of inflammation and increasing Nrf-2/HO-1 signaling during ICH damage (17).

Chronic cerebral hypoperfusion (CCH), a neurocognitive health concern, is linked to a variety of vascular disorders and other comorbidities. The primary purpose of this study is to determine how glycyrrhizic acid (GA) can restore cognitive health when it is compromised by chronic cerebral hypoperfusion. By reestablishing the enzymatic and non-enzymatic antioxidant activity of mitochondrial complex I and IV, the antioxidant propensity of GA lowered the generation of reactive oxygen species.. When compared to GA, the Li group's antioxidant defense was noticeably lower. In comparison to Li treatment, GA treatment resulted in a significant repair of pyramidal neurons, myelin, and dendritic spine density due to the robust antioxidant defense. When compared to lesion rats, the cognitive deficits in the GA-treated rats were remarkably improved. Finally, GA inhibited the release of cytochrome C, impeding the advancement of following apoptotic processes. The findings of Sathyamoorthyet al., 2020 paper demonstrate conclusively that GA increases endogenous antioxidants and prevents apoptosis by reducing cytochrome-c release, potentially providing neuroprotection in rats induced by CCH. Additionally, it was discovered that GA is significantly superior to Li because it modulates the GSK3β/Nrf2 pathway, which in turn lessens the negative effects of CCH (18).

As shown by Basil et al., 2021 a diterpene found in Salvia miltiorrhiza Bunge (Danshen) that has been proven in numerous experimental models to increase cytoprotection. Chlorpyrifos (CPF), an agricultural pesticide, causes bioenergetic failure, redox impairment, inflammation, and apoptosis in animal tissues. We investigated whether T-I could protect the human dopaminergic SH-SY5Y cells from the effects of CPF exposure. Pretreatment with T-I at 2.5 μM for 2 hours reduced lipid peroxidation, protein carbonylation, and nitration in the mitochondrial membranes of CPF-treated cells. T-I also reduced the formation of radical superoxide ($O_2^{-\bullet}$) by CPF-affected cells' mitochondria. T-I also reduced the generation of hydrogen peroxide ($H_2O_2$) and nitric oxide ($NO^{\bullet}$) in cells exposed to CPF. After pretreatment with T-I, the CPF-induced reduction in the activity of complexes I-III, II-III, and V was abolished. T-I prevented CPF-treated cells from decreasing their mitochondrial membrane potential ($\Delta\Psi m$) and generated less ATP. T-I inhibited inflammation in CPF-treated cells by reducing IL-1β, TNF-α, and NF-κB activity. T-I mediated protection against inflammation and mitochondria was inhibited by heme oxygenase-1 (HO-1) inhibition or silencing of nuclear factor erythroid 2-related factor 2 (Nrf2) (19).

Centellaasiatica is a medicinal plant that has long been recognized to have neuroprotective and cognitive-enhancing effects. Prior study has indicated that providing a water extract of Centellaasiatica (CAW) for two weeks promotes cognitive performance and initiates the endogenous antioxidant response pathway, while preserving the amount of

Aβ plaque. This study aims to assess the effectiveness of long-term CAW treatment in the 5xFAD mice model of Aβ accumulation. After three months of ingesting 2 g/L of CAW in their drinking water, four-month-old 5xFAD mice were cognitively assessed and their levels of Aβ plaque, antioxidant capacity, and synaptic gene expression were investigated. CAW and the targeted inhibitor ML385 were delivered to neurons isolated from 5xFAD rats to confirm the function of the antioxidant regulating transcription factor NRF2 in CAW's effect on synaptic plasticity..Results: After three months of CAW treatment, 5xFAD mice showed improvements in executive function, contextual and spatial memory. Compared to untreated 5xFAD rats, this improvement was accompanied by a decrease in the burden of Aβ plaque and an increase in the expression of antioxidant genes.. ML385 treatment reduced the effects of CAW on dendritic arborization and synaptic gene expression in isolated neurons. Conclusion: These findings imply that extended exposure to CAW may be protective against Alzheimer's disease and that NRF2 activation is probably involved in these benefits. Furthermore, our results imply that focusing on NRF2 directly can be a useful therapeutic approach for enhancing synaptic plasticity and cognitive performance in Alzheimer's patients (20). The result by Mundkar et al., 2022 showsMoringaoleifera, often known as the horseradish tree, drumstick, or sahajna, is a traditional Indian plant belonging to the Moringaceae family. Moringa'sphytoconstituents, including β-carotene, quercetin, kaempferol, ascorbic acid, flavonoids, phenolic acid, rhamnose, glycosylates, glucomoringin, and isothiocyanates, are responsible for its anti-inflammatory, anti-apoptotic, and antioxidant properties. It is widely used as animal and human feed in developing countries. These components have been demonstrated to have neuroprotective advantages in a number of neurodegenerative illnesses, including Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis. They also support mitochondrial functioning, neurogenesis, and antioxidant enzyme levels in the brain with activating NrF2 pathway (21).

Cerebral ischemia-reperfusion (CIR) is a common feature of ischemic stroke and a primary cause of disability and mortality among stroke sufferers worldwide. The lignin polyphenol phyllanthin is well-known for its numerous biological properties, however there is no evidence that it can protect against CIR. We studied phyllanthin'sneuroprotective efficacy against CIR, as well as the involvement of NF-κB and AMPK/Nrf2. Experimental mice were divided into five groups: controls (sham-operated); CIR-induced via middle cerebral artery occlusion (MCAO); and CIR-induced with phyllanthin administered at 2.5, 5, and 10 mg/kg, respectively. We looked into inflammatory indicators, different signaling genes, and neurological processes. In vitro experiment findings showed that phyllanthin assertively improved cellular functions through abrogation of the Nrf2 pathway (22). To examine ginger root ethanol extract (GRE)'s protective efficacy against apoptotic and 6-hydroxydopamine (6-OHDA)-induced oxidative stress, as well as the mechanism behind this action. A Parkinson's disease (PD) model was created using 6-OHDA in rat adrenal pheochromocytoma (PC12) cells. GRE therapy increased the viability of 6-OHDA-induced damage in PC12 cells. The GRE effectively prevented 6-OHDA-induced apoptosis by decreasing the expression of Bax and cleaved caspase 3 while increasing the expression of B-cell lymphoma 2 (Bcl-2). GRE also decreased reactive oxygen species (ROS), avoided oxidative stress produced by 6-OHDA, and improved the activity of antioxidant enzymes such catalase, glutathione (GSH), superoxide dismutase (SOD), 8-oxoguanine glycosylase1 (OGG1), and NAD(P)H quinone oxidoreductase1 (NQO1). Additionally, GRE down-regulating nuclear factor and up-regulating nuclear erythroid 2-related factor kappa-light-chain-enhancer of activated B cells (NF-κB) translocation, together with the mitogen-activated protein kinase (MAPK) phosphorylation (23).

Ionizing radiation (IR) exposure occurs frequently in nuclear detonation or reactor disaster occurrences as well as therapeutic treatment. It is damaging because IR sets off cellular processes that lead to oxidative stress and macromolecule destruction. Although it was previously believed that the central nervous system (CNS) was radiation-resistant, emerging study suggests that even low doses of IR can harm the brain. Overexposure to radiation can cause increased blood-brain barrier (BBB) permeability, neuronal loss, reduced neurogenesis, impaired synaptic plasticity, and cognitive impairment. The potential benefits of dietary supplements and phytochemicals for radiation-induced damage and mental health have recently garnered a lot of attention. Notably, phytochemicals like vitamin E, corilagin, curcumin, resveratrol, and ursolic acid, as well as Amaranthuspaniculatus, Grewiaasiatica, and Lyciumbarbarum, have shown promise in reducing radiation-induced brain damage (24).

The West is more affected by neurodegenerative disorders (NDs) as a result of longer life expectancies. One of the things that starts and quickens neurodegeneration is oxidative damage that accumulates in nervous cells. Cells, on the other hand, have systems for defending themselves against oxidative stress (OS) and reactive oxygen species. On the gene expression level, the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) regulates several of these endogenous antioxidant mechanisms. When prooxidants are present, Nrf2 migrates to the nucleus and triggers the transcription of genes with an antioxidant response element (ARE). Recent research has focused on the Nrf2 pathway and the natural substances that positively modulate it to reduce oxidative damage to the nervous system, both in vitro and in vivo, with neurons and microglia exposed to stress stimuli, as well as in vivo studies employing primarily mouse models. regulate numerous Nrf2 upstream activators, quercetin, curcumin, anthocyanins, tea polyphenols, and other phenolic compounds that have not received as much attention as they deserve can also affect Nrf2. Terpenoids, which include carotenoids (astaxanthin, lycopene), diterpenes (ginkgolides), triterpenes (ginsenosides), and monoterpenes (aucubin, catapol), are another class of phytochemical substances that activate this pathway. With an eye on treating non-diagnosed neurological disorders (NDs), this study seeks to provide current information on the impact of secondary metabolites of health interest on Nrf2 pathway activation (8).

Scientist Brankiewicz et a., 2023 shows that Following 24 hours of cell stimulation with HpExs and hyperforin salt, major oxidative stress-related parameters such as cell survival, apoptosis, ROS generation, and NRF2 and HO-1 expression were investigated. The extracts' polyphenol content, radical scavenging activity, and hyperforin concentration were all determined. We observed that the oxygen concentration, stage of melanoma progression, and extract composition all influence the cytotoxic, pro-apoptotic, and antioxidant effects. H. perforatum extracts exhibited more efficacy than hyperforin salt. For the first time, our research demonstrated that H. perforatum extracts' anticancer efficacy varies depending on the oxygen saturation level. Crucially, the composition of extracts from different sources including in vitro cultures and their distinct qualities could have a role in the choice of plants for medicinal use (25). Neurological illnesses cause functional or sensory loss of neural cells. Oxidative stress has a significant role in neurodegeneration, in addition to hereditary or environmental variables. In recent years, there has been a renewed interest in understanding more about the benefits of extra virgin olive oil (EOs), particularly in the context of treating neuropathologies. The chemical and biological effects of EOs have enabled important therapeutic possibilities for the treatment of a wide range of neurological diseases. Essential oils (EO) extracted from numerous species of medicinal plants have showed positive benefits in neurological disorders such as anxiety, dementia, oxidative stress, cerebral ischemia, and oxidative toxicity. As a result, EOs show promise as substitute drugs for the treatment of neurological conditions (26).

Sleeping difficulties result in inadequate recovery after a restful night's sleep, which leads to daily weariness, mental disorientation, and hypersensitivity. Certain medications, such benzodiazepines and antihistaminic medicines, aid in promoting sleep and treating insomnia. However, tolerance and reliance make the continued treatment inappropriate. Consequently, scientists look for novel medications with fewer side effects. Natural products have long been a valuable resource for the development of novel therapies for the treatment of conditions including cancer, diabetes, heart disease, sleeplessness, and issues with the liver and kidneys. Numerous studies have supported the legitimacy and applicability of using these herbs to treat insomnia. Notably, we investigated the potential of many Persian herbs, including lettuce, Capparisspinose, Viola tricolor, Artemisia annua, Salvia reuterana, and Passifloraincarnata, to cure insomnia in this work through both in vivo and clinical trials. Studies have shown that n-butanol fractions containing non-polar compounds, in particular, have hypnotic effects on benzodiazepine receptors (27). The current study intended to examine and investigate the mechanisms behind the anti-aging activities of Scutellariabaicalensis Georgi (LSBG) leaves. SD rats were fed LSBG intragastrically(0.4 g/kg or 0.8 g/kg) and D-galactose subcutaneously (0.3 g/kg) for seven weeks. Every rat was subjected to behavioral tests to assess their cognitive abilities. The results demonstrated that LSBG corrected memory deficits. The possible processes were then examined using metabolomics of the brain and hippocampus. Aging rats revealed alterations in 21 metabolites in the cortex and 22 metabolites in the hippocampus. Furthermore, the data revealed that the LSBG may regulate the levels of key metabolites as well as the activity of enzymes involved in glutamate metabolism and its downstream metabolism (glutathione metabolism). In addition, proteins implicated in the Nrf2 signaling pathway

were examined using western blot. Furthermore, the LSBG had a considerable impact on the expression of Nrf2, GCLC, HO-1, and NQO-1 proteins in the cortex and hippocampus. Above all, the Nrf2 signaling pathway and glutamate metabolism were regulated by the LSBG's anti-aging actions (28).

New therapeutic techniques are necessary for the effective treatment of glioblastomamultiforme (GBM), an aggressive primary brain tumor. In this study, we investigated PBI-05204's underlying processes and potential for targeting GBM stem cells (GSCs). PBI-05204 therapy delayed the carcinogenesis of GBM9 xenografts and significantly reduced the number and size of tumor spheres formed by patient-derived GSCs (TS543, GBM9, and GSC28). Furthermore, GBM9 and GSC28 GSCs treated with PBI-05204 revealed a substantial decrease in the expression of NANOG and CD44, two key progenitor stem cell markers. In both GSC types, this therapy also decreased the expression of GRP78. Reduced spheroid size and CD44 were also seen in GBM9 and GSC28 GSCs that had GRP78 expression knocked down using GRP78 siRNA transfection. When PBI-05204 is combined with GRP78 siRNA, the number of spheroid cells is reduced much more than when GRP78 siRNA is administered alone. PBI-05204 treatment enhanced pRIP1K and pRIP3K expression, as well as RIPK1/RIPK3 binding in GBM9 and GSC28 cells, which was comparable to the results obtained in GRP78-silenced GSCs, showing that PBI-05204 triggered necroptosis. Furthermore, the principal active cardiac glycoside component of PBI-05204, oleandrin, has been shown to inhibit GSC self-renewal. These results demonstrate PBI-05204's potential as a viable option for the creation of innovative treatments that concentrate on GBM stem cells (29).

Because of its anti-inflammatory and neuroprotective properties, hemp seed (Cannabis sativa L.) and nigella sativa (black seed) have been utilized to treat a variety of chronic disorders. In an effort to advance research toward a preventative and curative strategy for this debilitating illness, we address a novel therapeutic approach to treating multiple sclerosis. An electronic search of published literature regarding the effects of hemp seed and Nigella sativa was used to conduct the article review. The preventative benefits of nigella sativa ingestion by MS patients can be therapeutic since it reduces the production of TGF β1 in rats, enhances remyelination, and suppresses inflammation. It is thought that using hemp seed as a medicinal plant to treat multiple sclerosis will boost AMPK, block the release of pro-inflammatory mediators in rat models, reduce NF-κB, and enhance the and NrF2 pathway (30,31). The goal of this study was to look into the possible advantages of a new piperine derivative (HJ22) for ibotenic acid (IBO)-induced neurological problems in rats, as well as the underlying processes. Our group produced HJ22, which displayed a high affinity for Keap-1 and the ability to activate the Keap1-Nrf2-ARE signaling pathway in vitro. It is noteworthy to note that HJ22 significantly decreased the cognitive impairment produced by IBO in the Morris water maze, Y-maze, and passive avoidance tests. Furthermore, HJ22 significantly decreased cholinergic dysfunction and neuronal morphological changes by inhibiting IBO-induced apoptosis. HJ22 was shown to have a significant influence on the interaction between Keap1 and Nrf2. This was followed by an increase in nuclear Nrf2 expression, which in turn prevented oxidative stress and the activation of the Nod-like receptor protein 3 (NLRP3) inflammasome that is mediated by Thioredoxin-interacting protein (TXNIP) (32).

Berberis vulgaris, often known as barberry or B. vulgaris, has been used in traditional medicine for a variety of applications. Berberine is one of the most common alkaloids found in B. vulgaris and other plants. Both B. vulgaris and berberine have been shown in several animal models and clinical investigations to have anti-inflammatory, antioxidant, and immunomodulatory activities. The goal of this research is to present an overview of the available evidence on the roles and processes of berberine and B. vulgaris in the regulation of immunological responses, oxidative stress, and inflammation. Vulgaris and berberine have been proven to have favorable effects in a variety of inflammatory, oxidative, and immune-related conditions, including diabetes, obesity, cardiovascular disease, neurodegenerative illnesses, autoimmune diseases, allergic diseases, and infections. To clarify the ideal dosages, safety profiles, and possible interactions between B. vulgaris and berberine with other medications or organic substances, additional research is necessary (33). This study investigated the effects of Melissa officinalis extract (MOE) on oxidative stress, inflammation, and neurotransmitters in the hippocampus of rats treated with pilocarpine (PILO). The findings suggested that Nrf2/HO-1 signaling was involved. Following an intraperitoneal injection of PILO, rats were treated with MOE for two weeks. MOE suppressed glial fibrillary acidic protein, lowered lipid

peroxidation, Cox-2, PGE2, and BDNF, and prevented neuronal death in PILO-treated rats' hippocampus areas. Furthermore, MOE elevated the levels of GSH and antioxidant enzymes, HO-1 mRNA and Nrf2 nuclear translocation, and hippocampus GSH in epileptic rats. In the hippocampal regions of epileptic rats administered MOE, glutamate and acetylcholine were reduced, whereas Na+/K+-ATPase activity and GABA were elevated (34).

## CONCLUSION

Neurological problems are among the highest in the world in terms of morbidity, disability, and death. They also seriously impair cognitive performance. The most common neurological disorders include cerebrovascular, neurodegenerative, autoimmune, spinal cord, intracranial tumour, and craniocerebral trauma. Due to the limits and potential side effects of conventional medicines, herbal remedies have been investigated as a viable treatment option for neurological illnesses. Alzheimer's disease is the most common neurodegenerative disorder that progresses over time in humans. It is characterized by senile plaques and neurofibrillary tangles, as well as progressive cognitive abnormalities, the most visible of which is memory loss. Given the etiology of AD, a variety of medical therapies, including both synthetic and natural techniques, are being used to treat the illness.Common herbal compounds used to treat neurological illnesses such as Alzheimer's, Parkinson's, and multiple sclerosis have been demonstrated to enhance both symptoms and cognitive performance. Quercetin, curcumin, anthocyanins, tea polyphenols, and other phenolic substances that have received insufficient attention can all alter Nrf2 via controlling a number of Nrf2 upstream activators. Terpenoids are another class of phytochemical substances that activate this pathway. They include carotenoids (astaxanthin, lycopene), diterpenes (ginkgolides), triterpenes (ginsenosides), and monoterpenes (aucubin, catapol). We want to update our knowledge of how important secondary metabolites for health activate the Nrf2 pathway with this review.

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**Table 1: Herbals used in treatment of various neurological disorders with modulating NrF2 pathway**

| Sr. No | Plant Used | Name of Neurological Disorder | Mechanism of Action | Reference |
|---|---|---|---|---|
| 1 | ***Curcuma longa*** | Cerebral Injury | Enhancing Nrf2 may help to mitigate radiation-induced brain damage. | 9-10 |
| 2 | ***Ginkgo biloba*** | Parkinson's Disease | Ginkgolides and bilobalide enhance Nrf2 pathway, inhibit dopaminergic neuron loss, and reduce oxidative stress. | 11 |
| 3 | ***Camellia sinensis*** | Multiple Sclerosis | Epigallocatechingallate (EGCG) in green tea activates Nrf2, decreases inflammation, and protects myelin. | 12 |
| 4 | ***Withania somnifera*** | Huntington's Disease | Withaferin A upregulates Nrf2, reduces oxidative stress, and inhibits apoptosis. | 13 |
| 5 | ***Bacopa monnieri*** | Memory loss | Bacopamonnieri modulates neurotransmitters, enhances Nrf2 pathway, and reduces oxidative damage. | 14 |
| 6 | ***Panax ginseng*** | Neuroinflammation | Ginsenosides activate Nrf2, reduce infarct size, and protect against ischemia-reperfusion injury. | 15 |
| 7 | ***Rosmarinus officinalis*** | Treatment for neurological illnesses involves addressing amyloid-beta (A-β) | Rosmarinic acid activates Nrf2, reduces neuroinflammation, and protects motor neurons. | 16 |

| | | | | |
|---|---|---|---|---|
| | | accumulation, neuronal cell death, acetylcholinesterase (AChE), neuroinflammation, β-secretase (BACE-1) activity, and mitochondrial redox state. | | |
| 8 | ***Silybum marianum*** | Ischemic Stroke | Silibinin activates Nrf2, scavenges free radicals, and reduces oxidative stress. | 17 |
| 9 | ***Glycyrrhiz aglabra*** | Alzheimer's Disease | Glycyrrhizin activates Nrf2, reduces amyloid-beta toxicity, and attenuates neuroinflammation. | 18 |
| 10 | ***Salvia miltiorrhiza*** | Parkinson's Disease | Tanshinones activate Nrf2, protect dopaminergic neurons, and reduce oxidative damage. | 19 |
| 11 | ***Centella asiatica*** | Cognitive Decline | Asiatic acid activates Nrf2, enhances antioxidant defense, and improves memory function. | 20 |
| 12 | ***Moringa oleifera*** | Neurodegenerative Disorders | Isothiocyanates activate Nrf2, reduce oxidative stress, and provide neuroprotection. | 21 |
| 13 | ***Phyllanthus emblica*** | Alzheimer's Disease | Emblicanin A and B activate Nrf2, reduce oxidative damage, and improve cognitive function. | 22 |
| 14 | ***Zingiber officinale*** | Parkinson's Disease | Gingerol activates Nrf2, reduces neuroinflammation, and protects dopaminergic neurons. | 23 |
| 15 | ***Amaranthusp aniculatus, Grewia asiatica, Lyciumb arbarum, as well as phytochemicals including vitamin E, corilagin, curcumin, resveratrol, and ursolic acid*** | Anxiety and Depression | Eugenol activates Nrf2, reduces oxidative stress, and improves neurotransmitter balance. | 24 |
| 16 | ***Curcuma aromatica*** | Epilepsy | Curcumin activates Nrf2, reduces oxidative damage, and improves neuroprotection. | 8 |
| 17 | ***Hypericumperforatum*** | Cancer | Hypericin and hyperforin activate Nrf2, reduce oxidative stress, and modulate neurotransmitters. | 25 |
| 18 | ***Lavandulaangustifolia*** | Dementia induced by scopolamine In vivo/ inhalation | Linalool activates Nrf2, reduces oxidative stress, and provides SOD, Catalase increses | 26 |
| 19 | ***Passiflora incarnata*** | Insomnia | Antioxidant | 27 |
| 20 | ***Scutellariab aicalensis*** | Alzheimer's Disease | Baicalin activates Nrf2, reduces oxidative stress, and provides neuroprotection. | 28 |
| 21 | ***Nerium oleander*** | Glioblastoma | Oleandrin activates Nrf2, induces apoptosis in glioblastoma cells, and | 29 |

| | | | | |
|---|---|---|---|---|
| | | | provides neuroprotection. | |
| 22 | ***Cannabis sativa*** | Multiple Sclerosis | Cannabidiol activates Nrf2, reduces neuroinflammation, and protects against neurodegeneration. | 30,31 |
| 23 | ***Piper nigrum*** | Cognitive Decline | Piperine activates Nrf2, enhances memory function, and provides neuroprotection. | 32 |
| 24 | ***Berberis vulgaris*** | Neurodegenerative Disorders | Berberine activates Nrf2, reduces oxidative stress, and provides neuroprotection. | 33 |
| 25 | ***Melissa officinalis*** | Anxiety and Cognitive Decline | Rosmarinic acid activates Nrf2, reduces oxidative stress, and improves cognitive function. | 34 |

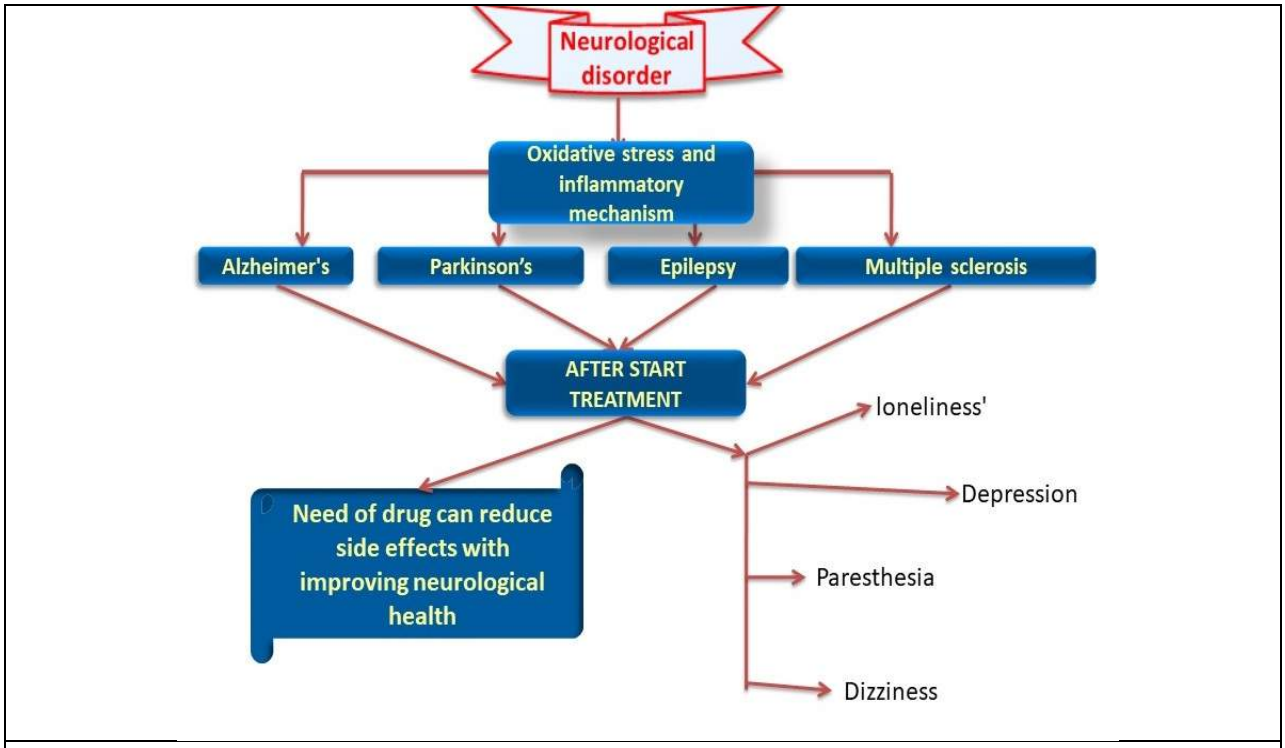



**Fig.1. Neurological disorder**

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**REVIEW ARTICLE**

# Diagnostic Efficacy of CT, MRI and Ultrasound in the Evaluation of Cranial Sutures : A Comparative Review

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## ABSTRACT

Premature fusion of the cranial sutures, or craniosynostosis, necessitates precise imaging for prompt diagnosis, differentiation from deformational plagiocephaly, and suitable surgical planning. Although computed tomography (CT) is still the gold standard because it provides superior spatial resolution and 3D reconstruction, radiation exposure in newborns poses safety risks. Although it is more expensive, less accessible, and frequently requires sedation, magnetic resonance imaging (MRI), especially with more recent methods like black-bone and ultrashort echo time (UTE) sequences, offers a radiation-free substitute. Although its diagnostic accuracy varies with age and is operator-dependent, ultrasound is a quick, safe, and affordable bedside tool that is particularly helpful in infants. The most successful strategy is an integrated, multimodal one in which CT directs surgical planning and MRI and ultrasound are useful auxiliary modalities. Future developments, such as artificial intelligence-enhanced ultrasound, novel MRI sequences, and ultra-low-dose CT protocols, have the potential to improve diagnostic accuracy while lowering risks, enabling safer and more efficient treatment of craniosynostosis.

**Keywords:** Cranial sutures, Craniosynostosis, Computed tomography, Magnetic resonance imaging, Ultrasonography.

# INTRODUCTION

Skull lesions are usually asymptomatic and found accidentally during imaging that is done for other reasons. They can also be discovered while examining local symptoms or during staging tests for other diseases. In some cases, patients may show up with a noticeable lump or other symptoms. The patient's history and age are important factors in helping with the radiological diagnosis(1).Craniosynostosis (CSO) is a rare birth defect. It occurs when the cranial sutures fuse too early. This leads to abnormal skull growth and, in severe cases, cognitive issues because of restricted brain development. The most common subtype is sagittal craniosynostosis. This type happens when the sagittal suture ossifies early, resulting in an elongated head shape called scaphocephaly(2).The normal and abnormal sonographic appearances of cranial sutures have been described. However, ultrasound has not been widely used as a screening tool. In 2005, we introduced ultrasound as the main method for diagnosing craniosynostosis (CS). During the first year, we focused on educating paediatric neurologists and community paediatricians to help them understand this method. We presented the advantages and limitations of the approach at medical conferences, showing comparative images of normal sutures and craniosynostosis(3).Positional plagiocephaly is a change in skull shape that happens without the early fusion of cranial sutures. It results from external forces, either before or after birth, that distort the skull. Importantly, it occurs without any underlying structural problems. In contrast, craniosynostosis is a medical issue marked by the early closure of one or more cranial sutures in the skull, sometimes along with early closing of cranial fontanelles. Cranial sutures are fibrous joints that connect the skull bones, allowing the skull to grow outward as the brain develops. Fontanels are soft spots where the skull bones haven't fused yet. They provide necessary flexibility during birth and the first year of life, helping the skull adjust to the rapid growth of the brain(4).Craniosynostosis is a serious and relatively common condition that affects about 1 in 2,100 children. It occurs when cranial sutures fuse too early during childhood. The early closure of different major cranial sutures results in distinct head shapes, depending on which suture is involved. For instance, the premature fusion of the lambdoid suture leads to posterior plagiocephaly. In contrast, fusion of the sagittal suture causes scaphocephaly, which results in an elongated and narrow head shape. Coronal suture synostosis can cause anterior plagiocephaly or brachycephaly. Meanwhile, metopic synostosis often results in trigonocephaly, a triangular forehead deformity(5).

**Anatomy and Clinical Relevance of Cranial Sutures**

Cranial sutures are a type of fixed joint where neighboring bones are tightly joined by fibrous tissue. In the adult human skull, the cranial vault has 15 sutures. These include three unpaired sutures: the coronal, sagittal, and lambdoid sutures. There are also several paired sutures, such as the squamous, spheno-frontal, spheno-squamous, spheno-parietal, parieto-mastoid, and occipito-mastoid sutures(6). Cranial sutures are not straight lines; they twist and interlock. This structure strengthens the connections between adjacent bones and improves the protection of the brain inside. In newborns, these bones are not tightly fused; they remain separated by wide fibrous areas at the suture sites. This setup allows for movement and overlapping of bones during birth, which helps with passage through the birth canal. It also permits rapid brain growth, especially in the first year of life. At places where more than two bones meet, the gaps are larger and are called fontanelles. These fontanelles differ in size, with the anterior fontanelle being the largest(7).The skull serves several important roles that help each species succeed in its specific environment. It aids in capturing prey, eating, and breathing, while also safeguarding the brain and sensory organs needed for perception, such as vision and hearing. In vertebrates, neighboring cranial and facial bones connect through fibrous joints known as cranial sutures (Opperman, 2000). As joints, sutures consist of the fibrous connective tissue in between and the bone fronts that form new bone. These components stay linked and coordinated through the fibres, enabling growth and expansion(8).

**Imaging Modalities in Cranial Suture Evaluation**

**Computed Tomography**

The treatment of craniosynostosis is complex and usually requires surgery. This typically occurs between 3 and 12 months of age. Computed tomography (CT) imaging is a common method for checking the early fusion of cranial

sutures. However, rising public concern has brought attention to the risks of radiation exposure from CT scans. These risks include the possible development of leukemia and malignant tumors. This issue is especially important for craniosynostosis patients, as they receive radiation at a younger age and more often than typical children. Additionally, children are more susceptible than adults to the harmful effects of ionizing radiation. This is due to their greater organ sensitivity and longer life expectancy, which provides more time for radiation-related health problems to arise.(9)Traumatic brain injury (TBI) is a leading cause of disability and death worldwide. The most common causes of TBI are falls, vehicle accidents, work-related injuries, and physical assaults. TBI can result in serious and life-threatening complications, such as skull fractures, intracranial and orbital injuries, cerebrospinal fluid (CSF) leakage, cranial nerve palsy, and vascular injuries (brain hematomas). These conditions often require hospitalization and major surgery. The global annual incidence of TBI is estimated at 69 million cases, or 939 cases per 100,000 people. However, this figure may be uncertain and likely underestimates the true prevalence. In the U.S., statistics from 2019 to 2021 showed 223,135 hospitalizations and 69,473 fatalities related to TBI. This means that each day, 611 people are injured and 190 people die from TBI(10).

**Magnetic Resonance Imaging**

Craniometric traits within a population can be compared to genetic markers. Correctly identifying cranial sutures is crucial for finding important craniometric landmarks, like the bregma and lambda. These landmarks are used to take cranial vault measurements. Typically, researchers use caliper gauges, compasses, polemus, or more precise tools like the Microscribe to gather these measurements. In this study, we aimed to assess the effectiveness of standard MRI in locating cranial anthropometric landmarks and measuring cranial vault dimensions.(11)Magnetic Resonance (MR) imaging is a non-ionizing technique that offers better contrast resolution and shows higher sensitivity and specificity for parenchymal lesions compared to Computed Tomography (CT). Advanced MR techniques like diffusion-weighted imaging (DWI) and susceptibility-weighted imaging (SWI) provide extra diagnostic information and match well with clinical findings. Non-bleeding shear injuries and small microbleeds are usually detected more sensitively on MR imaging than on CT. However, the role of MR imaging in the initial diagnostic process for pediatric head trauma is still limited. This limitation may be partly due to longer acquisition times, the need for sedation, and the relatively low sensitivity of MR imaging for skull fractures. Recently, black bone MR imaging has been introduced as a new method for assessing structural bone abnormalities like craniosynostosis(12).

**Ultrasound**

Sutures define the edges between curved cranial bones. It can be difficult to see these sutures with a two-dimensional (2D) ultrasound. The diagnosis of craniosynostosis mainly relies on spotting related abnormalities or recognizing unusual cranial shapes. This approach does not directly show the early closure of sutures or fontanelles. Isolated craniosynostosis is, however, diagnosed less often before birth(13).During normal development, skull formation starts with the formation of ossification centers in the membranous anlage that surrounds the brain. From these centers, the calvarial bones grow through gradual ossification at their edges, and cranial sutures appear at the points where neighboring bones come together. While open, cranial sutures are the main areas for calvarial growth. Normally, the metopic suture fuses completely by about one year of age, while the sagittal suture stays open throughout childhood and usually closes between 22 and 24 years. If any cranial suture fuses too early, a condition called craniosynostosis occurs. This leads to an unusual head shape and may raise the risk of increased intracranial pressure. The early fusion process can begin as soon as 15 weeks of gestation in trigonocephaly and around 18 weeks in scaphocephaly(14).

**Radiation Concerns and Safety Considerations**

In 2006, about 380 million radiologic procedures were performed in the United States. This included 67 million computed tomography (CT) scans and 18 million nuclear medicine procedures. Notably, U.S. patients made up roughly half of all nuclear medicine procedures worldwide, even though they accounted for only 4.6% of the global population. This rise in imaging use has led to a sixfold increase in annual per capita radiation exposure. It grew from 0.5 mSv to 3.0 mSv between 1980 and 2006. In contrast, the natural background radiation dose has stayed fairly steady over the past 30 years at 2.4 mSv per year. To measure biological damage from radiation, the terms “sievert”

(Sv) and "rem" are now preferred. These terms adjust for absorbed radiation based on a tissue weighting factor. This is a shift from the older units "gray" (Gy) and "rad."(15)

**Role in Diagnosis of Craniosynostosis**

Craniosynostosis, also called cranial synostosis, craniostenosis, or simply synostosis, is a birth defect where one or more cranial sutures fuse too early. This early closure disrupts normal skull growth and often leads to unique skull shapes, facial asymmetry, and, in some cases, problems with brain development. The condition can show up as simple synostosis, where only one suture fuses, or as complex synostosis, which affects multiple sutures. It may occur as a primary issue, either in a standalone form or as part of a syndromic condition. It can also appear as a secondary issue due to underlying problems like metabolic, intracranial, teratogenic, or blood-related conditions(16).

**Future direction:**

Ultra-low-dose CT protocols

In one institution's study of pediatric craniosynostosis, routine brain CT was replaced with a low-dose protocol that used 100 kV, 10 mA, and iterative reconstruction. This allowed for high diagnostic confidence for suture evaluation while achieving effective doses of 0.06–0.22 mSv (up to 67-fold reduction)(17).

**Comparison of Direct CT and Black-Bone MRI**

BB-MRI and 3D-CT were compared in the preoperative assessment of craniosynostosis, demonstrating the usefulness of MRI in surgical planning procedures(18).

As an alternative to CT, black-bone MRI may effectively reveal patent and prematurely fused cranial sutures, which matches clinical findings and CT. Suture gaps look hyperintense, allowing for 3D reconstructions(19).

**AI-assisted ultrasound developments**

Future AI-assisted cranial suture ultrasound applications appear promising as AI-assisted ultrasound scanning demonstrates advancements in standard-plane detection, scanning guiding, and automated acquisition support(20).

## CONCLUSION

Early diagnosis and treatment of craniosynostosis depend heavily on the assessment of cranial sutures. Every imaging modality, including CT, MRI, and ultrasound, has advantages and disadvantages of its own. Though ionizing radiation concerns require careful use, especially in infants, CT is still the gold standard for accurate visualization and three-dimensional reconstruction of cranial sutures. MRI offers a non-ionizing substitute with increasing potential to replace CT in certain situations, particularly with newer techniques like Black-Bone, UTE, and ZTE sequences. However, problems with accessibility, expense, and the requirement for sedation still exist. For infants, especially those under a year old, ultrasound provides a first-line, safe, affordable, and easily accessible tool; however, its diagnostic accuracy is limited in older children and greatly depends on operator skill.

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**RESEARCH ARTICLE**

# Significance of Viddha - Agnikarma in Cervical Spondylosis

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## ABSTRACT

Cervical spondylosis is osteoarthritis in the cervical spine's.it is a slowly progressive degenerative disorder that present a major therapeutic challenge in modern medicine because of its complex pathophysiology and wide range of clinical manifestation. Although treatment such as NSAIDs, physiotherapy and surgical intervention are commonly employed, they often provide only temporary relief and are associated with potential safety issue. Viddha-AgniKarma is an innovative Ayurvedic technique that integrates Viddhakarma(therapeutic needle piercing) with Agnikarma(heat cauterization) to reduce the pain through focused thermal stimulation.This method provides an alternative strategy for pain control, especially in chronic musculoskeletal disorder, by producing localized therapeutic effect without depending on medicinal drugs. This single patient case study describes a 30-years-old male diagnosed with cervical spondylosis who was treated with Viddha-Agnikarma therapy. His clinical features, such as pain, stiffness and limited neck movement, were evaluated using standardized and validated assessment tools. The main aim of the study was to assess the therapeutics effectiveness of Viddhakarma in the management of cervical spondylosis, with special emphasis on pain reduction, improvement in cervical range of motion, and enhancement of functional ability. The outcome showed marked improvement in pain level, reduction in stiffness and better cervical mobility, along with a significant decrease in pain intensity and functional impairment. These results indicate that Viddhakarma is serves as a safe, economical, and minimally invasive alternative to conventional pain management methods. This case study underscores the promising role of Viddhakarma in the treatment of cervical spondylosis and supports the need for further large-scale studies to confirm its efficacy and safety.

**Keywords :** ViddhaKarma,Cervical Spondylosis,Agnikarma,Ayurvedic pain management, therapeutics effectiveness.

## INTRODUCTION

Cervical spondylosis is a degenerative condition of the cervical spine found almost universally in person over 50 years of age.it is a major therapeuticchallenge in modern medicine because of its complex pathophysiology and wide range of clinical manifestations. This aliment impacts the cervical spine's vertebrae, intervertebral discs, ligament, leading to periphery osteophyte formation [1] causing persistent neck pain, stiffness, nerve root irritation, abnormal sensation and neurological impairment. The incidence of cervical spondylosis rises with advancing age, affecting nearly 85% of people over the age of 60.[2] In Ayurveda, Cervical spondylosis is associated with Greeva Stambha or Manya Stambha resulting from accumulated Vata and Kapha affecting the cervicalvertebrae and discsand it's marked by pain, rigidity, and limited movement resulting from an aggravated Vata Dosha in the neck region. The concept of Vata Dosha, responsible for movement, communication and nerve function, is fundamental to understanding the disease's development from an Ayurvedic viewpoint.[3] Contemporary studies have identified multiple factor that increase the risk of cervical spondylosis, including a sedentary lifestyle, Neck injury, age, Occupation, genetic factors, and chronic conditions like diabetes and hypertension.[4]

Diagnosis is commonly confirmed through imaging method such as X-ray, CT scans, and MRI, which reveal typical features like bone spurs, osteophyte formation, discs degeneration. Treatment of cervical spondylosis usually involves a comprehensive strategy that includes medication, physical rehabilitation and sometimes surgery. Nevertheless, these standard treatments often offer only temporary relief and may carry notable side effects. Conversely, Ayurvedic treatment such as Snehan(oleation), Swedana(sudation), Nasya(nasal therapy), Basti(medicated enemas), Agnikarma(thermal cauterization) and Viddhakarma (piercing needle) have very effective in managing cervical spondylosis which is aggravation of Vata Dosha.[5]. Agnikarma is considered one of the most effective para-surgical procedures described for Vata-dominant painful musculoskeletal disorders.Among its various forms, Viddha-Agnikarma involves controlled therapeutic puncturing followed by application of heat at specific point, aiming to relieve pain, improve mobility and correct local Vata imbalance.it has very low recurrence rate and sustained therapeutic effect.[6]. This case study is designed to evaluate the therapeutic effect of Viddha-Agnikarma in a patient suffering from cervical spondylosis, with special emphasis on reduction of pain, improvement in functional ability, and enhancement of patient-reported quality of life. By assessing the effectiveness of Viddha-Agnikarma in the management of cervical spondylosis, the study intends to add valuable evidence to the expanding research on Ayurvedic treatment modalities for musculoskeletal conditions.

## MATERIALS AND METHODS

This was a forward-looking pilot interventional study carried out at shalya department of Quadra institute of ayurveda & hospital, Roorkee with the objective of assessing the clinical effectiveness of Viddha-Agnikarma in the management of cervical spondylosis.

A. Patient Selection
   A compressive screening procedure was undertaken to identify a patient presenting with the classical clinical feature of GreevaStambha (cervical spondylosis). Strict inclusion and exclusion criteria were followed to ensure that only a patient with a confirmed diagnosis of cervical spondylosis was selected for the study.
B. Inclusion: The study enrolled patient between 24 and 60 years of age, regardless of gender, who exhibited classical signs and symptoms of GreevaStambha for minimum duration of two months. Patient was selected if their pain had not responded adequately to conventional therapies, including Non-steroidal, Anti-inflammatory Drugs(NSAIDs), physiotherapy, or other non-surgical pain management approaches.
C. Exclusion criteria: Patient suffering from Rheumatoid arthritis(RA) or other musculoskeletal disorder, those with uncontrolled diabetes mellitus(DM) or hypertension(HTN), individuals having systemic diseases or anatomical abnormalities, and women who are pregnant or lactating were considered under Exclusion category.

**Case Report**

A 32-year-old Male patient reported with complaints of continuous neck pain and stiffness that radiated to both upper limbs, associated with tingling sensation and limitation of neck movement. He was an office employee whose work involved long hours of computer use.

**History of Present Illness**

The patient developed symptoms two months before consultation, initially experiencing occasional neck discomfort that gradually worsened into persistent, dull aching pain. He also had episode of sharp pain radiating to the shoulder and upper limbs, along with morning stiffness lasting more than 30 min. The increasing restriction in cervical mobility and difficulty in carrying out routine activities, such as turning his head while driving, led him to seek medical care. There was no prior history of trauma, fracture, or surgical procedure. The patient had no known chronic illness such as diabetes, hypertension, or autoimmune disorder. Additionally, there was no history of previous spinal disorder or inflammatory arthritis.

**Lifestyle and Habitual Pattern**

The patient follows a mixed diet with irregular meal timing and leads a predominantly sedentary lifestyle with minimal physical activity. Due to long hours of desk work and poor ergonomic posture, there is added strain on the musculoskeletal system. Sleep is often disturbed as a result of pain and discomfort. There is no history of addiction, and the patient does not use tobacco or consume alcohol.

**Clinical Examination**

**General examination**

The patient was fully conscious, alert, and well oriented.

Vital parameter was within normal limits, with a blood pressure of 130/90 mmHg.

Pulse rate of 76 beats per minute and body temperature of 98.6°F. There were noclinical indication of systemic disease or infection.

**Local examination**

- On inspection, the spinal curvature was within normal limits, with no observable deformity, swelling, injury, or surgical scars.
- On palpation,a mild increase in local temperature was noted along with tenderness in the cervical region.
- Assessment of cervical movement revealed that active range of motion were painful during flexion, extension, and lateral rotation.

**Test**

1. **Spurling test positive -** with cervical compression eliciting an increase in neck pain accompanied by radicular radiation into the upper extremity.
2. **Ihermitte's sign-**As flexion of the cervical spine produced an electric shock-like sensation radiating into the upper limbs.

**Dashvidha Pariksha**

**Parameter Assessment**

1. **Dosha -** Vata & Kapha Dosha.
2. **Dusya -** AsthiDhatu, Majja Dhatu &Snayu.
3. **Prakriti -** Vatta-Kapha Prakriti.
4. **Sara –** Madhyama,Asthi&Majja Sara.
5. **Samhanan -** Madhyama Samhana.
6. **Pramana–**Height; 5'3", weight – 52, BMI: 24(within normal range).
7. **Satmya-** Madhyama.
8. **Satva -** Madhyama Satva.
9. **Ahara shakti -** Reduce appetite at times, heaviness after meal.

**10. Vyayama shakti -** Heena Vyayam Shakti.

**2.Asthvidha Pariksha**
**1. Nadi Pariksha -** Vata Pradhan + Kapha Anubandh
**2.Mutra Pariksha -** Normalquantity but pale or slightly concentrated.
**3.Mala Pariksha –**Constipation or incomplete evacuation
**4.Jihva Pariksha –**White/Slightly thick coating
**5.Shabda Pariksha –**Low energy voice sometimes heaviness or fatigue while speaking
**6. Sparsha Pariksha –**Neck region: Cold to touch+ Stiffness.
**7. Drik Pariksha(Eye) –**Dry eyes, Eye Strain, Mild redness sometimes.
**8.Akruti Pariksha (Body,structure & Posture) –**Forward head posture, rounded shoulder & stiff neck movement.

**Radiological Findings**
The x-ray of the cervical spine(fig no. 1) shows a straightening of the normal cervical curvature, indicating a loss of cervical lordosis. There is evidence of bony outgrowths (osteophyte) present at the C4 to C6 vertebral level, along with a reduction in the intervertebral disc space. These radiological findings are consistent with degenerative change in the cervical spine and are suggestive of cervical spondylosis.

**Assessment criteria**

**Plan of Work:**
A seven-days treatment protocol was formulated, whereinViddha-Agnikarma therapy was administrated on alternate days, namely on the 1st, 3rd, and 5th days. No specific dietary restriction or therapeutic dietary interventions were advised during the course of treatment.

**Study Procedure**
**Purva Karma (Pre procedure preparation)**
After Detailed examination of the patient and confirmation of diagnosis procedure with benefits, and possible complications was explained to the patient and informed consent was taken.
Identified and marked the most tender points(maximum pain point) over the cervical and paracervical regions.
The patient is made to sit in a comfortable position with proper support to the neck.
Necessary instrument such as Agnikarma Shalaka(Rajat Shalaka), needle(for viddha), candle,cotton, antiseptic, and soothing agents like Ghritawere ready.

**Pradhan Karma(main procedure)**
The Agnikarma Shalaka is heated until it becomes red hot.
First, Viddha Karma is performed: a sterile needle is gently pricked at the selected tender points to create a micro-opening. [Fig. No.2 (a)]
Immediately after this, AgniKarma is done by touching the heated Shalaka over the same points or just adjacent to the needle puncture site for a fraction of second. [Fig.No. 2 (b)]
Multiple dots(Bindu type AgniKarma) are applied in a systematic manner, maintaining adequate distance between each point.[7]
Care is taken to avoid excessive depth or prolonged contact to prevent deep burns.
The patient's tolerance and comfort are continuously monitored during the procedure.

**Paschat Karma(Post procedure)**
Applied Ghrit over the treated area to soothe the skin and promote healin.[8]
Keep the area clean and dry: advise the patient not to scratch or disturb the sites. Light banding can be done if necessary. The patient is advised to avoid cold exposure, excessive neck movement, heavy work, and improper

posture for few days. Pathya-Apathya(dietary and lifestyle advice) is given, including warm, easily digestible food and avoidance of cold, oily, and heavy lentils.

**Follow-up and course of treatment**

Three sittings of the same procedure were given on alternate days. Along with Viddha-Agnikarma, supportive therapies such as mild neck exercise, proper ergonomics were advised.

**Therapeutic Effect**

Viddha- Agnikarma works by relieving pain, relieving stiffness, improving local circulation, pacifying Vata-Kapha dosha, and removing Srotorodha (obstruction), thus providing effective relief in cervical spondylosis.

## RESULT

The procedure provides significant relief in pain and stiffness and improves functional mobility of the neck, sustained results and not observed adverse effect. The therapeutic efficacy of Viddha-Agnikarmais attributed to its Usna,Tiksna, and Suksma Guna, which pacify vitiated Vata, promote Srotoshodhana, and facilitate VataAnulomana in the Greeva region. Classical references from Charak and Sushrut Samhita support the use of Agnikarma in pain-dominant Vata-Kapha disorder, particularly those involving Snayu, Sandhi, and Asthi. The patient reported improvement in sleep quality and was able to resume occupational duties without functional limitation. No adverse effects or complications were observed during or after the procedure. Thus, Vidhha-Agnikarmacan be considered as a cost-effective, minimally invasive, and clinically beneficial modality for the management of GreevaStambha.

## DISCUSSION

Cervical pain, comparable to Geerva Shoola or Greeva Stambha in Ayurveda, is a primarily a Vata Pradhan Vyadhi, often associate with Kapha due to degenerative and structural changes. It is characterized by Shula, Stambha, and Greeva Gati Avarodha, caused by the localization of vitiated Vata in Snayu, Sandhi, Asthi, and Majja Dhatu of the cervical region. Acharya Charak identifies 'Vata' as the principle cause of pain.[9] Acharya Sushrut has emphasized Agnikarma as an effective treatment for chronic pain-dominant Vata-Kapha disorder, particularly involving Snayu, Sandhi, and Asthi, and states that diseases treated with Agnikarma do not recur.[10]. Viddha-Agnikarma, a modified form of Agnikarma, enables precise and controlled thermal stimulation through needle puncture, faciliating deeper action on Snayu Gata and Sandhi Gata Vata in the Greeva region with minimal tissue injury. The Usna, Tikta, and Sukshma of Agni counteract the Shita and Usna Guna of Vata, thereby reducing pain and stiffness. The procedure enhance local blood flow, clears Srotorodha, and stimulates Dhatvagni, promoting functional restoration of affected tissue. Form a modern prespective, the thermal stimulus can modulate nociceptive pathway, reduce inflammatory mediators, and activate pain inhibitory mechanisms. Thus, Viddha-Agnikarma produces rapid analgesic and functional improvement, making it effective modality in the management of cervical spondylosis.

## CONCLUSION

The present case study demonstrates that Viddha-Agnikarma is an effective Ayurvedic intervention in the management of cervical spondylosis. Significant improvement was observed in pain intensity, stiffness, and cervical mobility, as evidenced by objective clinical assessment. The findings suggest that Vidda-Agnikarma can be considered a safe minimally invasive and clinically beneficial therapeutic modality for cervical spondylosis. However, further studies with a large sample size are required to establish the efficacy of this intervention.

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**Table 1. Assessment criteria**

| S.No. | Parameter | Grade | observation |
|---|---|---|---|
| 1. | Neck Pain (Ac.to VAS scale) | 0 | No pain |
| | | 1 | Mild pain (1-3) |
| | | 2 | Moderate pain (4-7) |
| | | 3 | Severe pain (8-10) |
| 2. | Neck Stiffness | 0 | No stiffness |
| | | 1 | Stiffness (5 min to 1 hour) |
| | | 2 | Stiffness (1 to 2 hours) |
| | | 3 | Stiffness (more than 2 hours) |
| 3. | Range of Motion | 0 | Normal Movement of the neck |
| | | 1 | Restriction of lateral movement of neck |
| | | 2 | Restriction extension movement of the neck |
| | | 3 | Restriction of flexion movement of the neck |
| 4 | Functional Difficulty | 0 | Absent |
| | | 1 | Present |
| 5 | Sleep Disturbance | 0 | Absent |
| | | 1 | Present |

**Table 2. result**

| Parameter | Before treatment | After treatment |
|---|---|---|
| **1.**Neck pain | Severe (7/10) | Mild (2/10) |
| **2.**Cervical stiffness | Severe (4/10) | Mild (2/10) |
| **3.**Flexion movement | Restricted | Near normal |
| **4.**External movement | Restricted | Near normal |
| **5.**Lateral flexion | Painful & restricted | Mild discomfort |
| **6.**Rotation | Painful & limited | Comfortable |
| **7.**Functional difficulty | Present | Absent |
| **8.**Sleep disturbance | Present | Absent |
| **9.**Use of analgesic | Required occasionally | Not required |
| **10.**Adverse effect | - | Not observed |



**(A)** **(B)**

**Figure No.1 X Ray Cervical (A) AP view (B) Lateral view**




**Figure No.2 (A) Viddhkarma Procedure**



**Figure No.2 (B) Agnikarma Procedure**

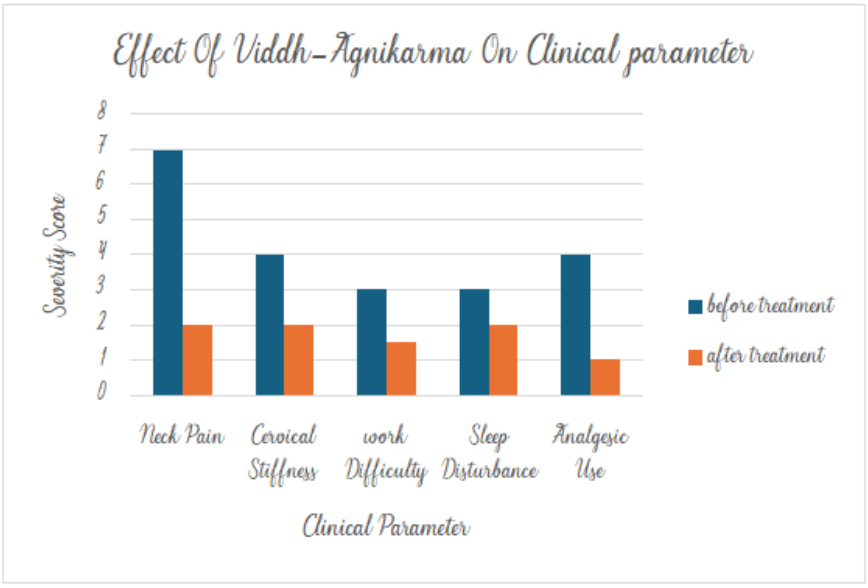



**Fig. 3 Effect Of Viddh-Agnikarma On Clinical parameter**

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RESEARCH ARTICLE

# An Experimental Study on the use of Plastic Waste in Bitumen for Flexible Pavements

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**ABSTRACT**

Plastic waste production has produced dire environmental and disposal challenges as a result of the rapid growth in waste production. Meanwhile, the traditional bituminous pavements are generally subject to premature failures because of the heavy traffic loads and variations of temperatures. The purpose of this experimental research is to establish the possibility of using waste plastic as a partial substitute of VG-40 grade bitumen in flexible pavements. The replacement of the bitumen by waste plastic was done at 2.5% and 5%, 7.5% and 10% weight. The tests carried out in the laboratory on modified bitumen samples included: penetration, softening point, ductility, flash and fire point as well as stripping value test. Such aggregate tests as impact value, crushing value, and abrasion value were also conducted. The findings reveal that the plastic modified bitumen enhances the strength, temperature resistant and durability hence it is appropriate in sustainable road construction.

**Keywords:** Waste plastic, VG-40 bitumen, flexible pavement, penetration test, sustainable roads

## INTRODUCTION

One of the most significant parts of infrastructure development is road transportation because it is associated with the economic and social development of a state. Flexible pavements are popular in India because of their low-cost at the beginning stage, ease in construction, and flexibility to change in terms of traffic loads and climatic conditions (Barmade et al., 2022). Flexible pavements are composed of layered systems where bitumen is the major binding agent, which presents flexibility and load distribution. Nevertheless, as the amount of traffic, loads on the axle, and extreme variations in the temperature rise exponentially, the traditional flexible pavements tend to fail prematurely

including rutting, cracking, stripping, and formation of potholes. Such failures lead to a high cost of maintenance and short life of pavement(Chavan, 2013).

Bitumen is a viscoelastic substance, the nature of which is very temperature-sensitive. When heated, bitumen becomes soft and easily deformed whereas when cooled it becomes brittle and liable to crack(Hirekhan, 2023). Traditional bitumen does not usually satisfy the performance criteria of new roads with heavy traffic, in particular in the areas with hot climatic conditions. In order to counter these shortcomings, a number of techniques of modification have been embraced such as polymers, rubber, and chemical additives to enhance the mechanical and longevity characteristics of bituminous binders(Mannar et al., 2021). Meanwhile, plastic waste management has already become one of the significant environmental issues. Plastics find wide applications in packaging, household and industrial applications owing to its strength and low cost. Plastic materials on the other hand are not biodegradable and may take a number of several hundreds of years to disappear in the environment(Wang et al., 2022). The poor management of plastic waste, in the forms of open dumping and burning, causes pollution of soil and water, drainage system blockage, and emission of harmful gases, which are dangerous to the environment and human health. Hence the proper use of waste plastic has become an issue of concern on sustainable development(Ameur et al., 2025).

The construction of waste plastic into flexible pavements has received considerable attention as a new and environmentally friendly alternative(Hasan, 2020). The use of waste plastic in bitumen offers a two-fold benefit of enhancing the performance of pavements and also solving the plastic waste disposal problem. Polyethylene and polypropylene are thermoplastic in nature and can be softened through heating and then a combination can be made with bitumen or a coating around the aggregates to produce a better bond and resistance to moisture damage(Mashaan, 2022). Normally, plastic-modified bitumen has a higher softening point and low penetration values, which implies that it is more resistant to deformation at high temperatures. VG-40 grade of bitumen is usually applied in heavy-duty pavements that experience high number of traffic and high temperatures. It is suitable on the highway and industrial roads because of its increased viscosity and stiffness(Chavan, 2021). VG-40 bitumen can be further enhanced with the addition of waste plastic to strengthen it, increase durability and stability at temperature. But too much plastic may cause the stiffness to be excessive to an acceptable degree, and result in brittle. It is therefore important to come up with the optimal rate of plastic replacement.

The proposed study is based on an experimental research to examine the possibility of waste plastic as a partial substitute of VG-40 bitumen in flexible pavements. The incorporation of waste plastic is done at a replacement level of 2.5%, 5%, 7.5% and 10% weight of bitumen. Penetration, softening point, ductility, flash and fire point and stripping value are some of the laboratory tests that are used to test the workability of plastic-modified bitumen. The outcomes of the present research should be used to justify sustainable road construction activities and proper management of the plastic waste.

The overall aim of this experimental research is to determine the practicability and efficiency of waste plastic as a partial substitute of bitumen in the construction of flexible pavements. The objectives of the study are the following particular ones:

1. To research the influence of waste plastic addition to the physical and mechanical properties of VG-40 grade bitumen.
2. To investigate the change of penetration, softening point, ductility, flash point and fire point of bitumen with various content of waste plastic.
3. To test the strip value parameter of the plastic-modified bitumen using the stripping value test.
4. To determine the appropriateness of the aggregates to be used in the study by undertaking aggregate impact value, aggregate crushing value and abrasion tests.
5. To establish the comparison of performance between the conventional and plastic-modified bitumen on replacement levels of 2.5, 5, 7.5 and 10.

6. To establish the best proportion of waste plastic, which would give better strength, durability, and temperature resistance without negatively influencing flexibility.
7. To encourage sustainable and environmentally friendly pavement construction through the use of waste plastic as an alternative material in road construction.

**Materials Used**

The components of this experimental study are the bitumen, waste plastic and aggregates. To make all materials reliable and consistent in the results, all materials were chosen and tested according to the relevant Indian Standard (IS) specifications.

**Bitumen**

In this study, VG-40 grade bitumen was taken as the binding material. VG-40 is a paving grade bitumen with a high viscosity value that is recommended to be used in pavements that experience high temperatures, highways, expressways, and heavy traffic roads. It has higher deformation and rutting resistance in comparison to lower grades of bitumen. VG-40 is more rigid and viscous hence fitting in the tropical climatic environments where the pavement temperatures are very high. Before and after the modification with waste plastic, the basic properties of the bitumen were tested on penetration, softening point, ductility, and flash and fire point.Aggregates of nominal sizes 4.75 mm and 2.36 mm were used throughout the experimental investigation.

**Waste Plastic**

In this study, waste plastic was made up of discarded plastic carry bags, milk covers, packaging material and plastic wrappers, which were found in the local areas. These plastic wastes are either thermoplastics like low-density polyethylene (LDPE) and high-density polyethylene (HDPE) that soften on heating and can easily be mixed with bitumen. Plastic waste collected was properly washed to get rid of dust and impurity, dried and shredded into small pieces of about 24 mm. The plastic materials were shredded and used as a partial substitute of weight of bitumen in the proportions of 2.5%, 5%, 7.5% and 10%.

**Aggregates**

The current experimental study used crushed stone aggregates. Aggregates constitute the large part of the flexible pavements and contribute greatly in terms of load distribution, strength and the overall performance of the pavement. The aggregates of the local quarry were gathered in a local quarry source, washed and dried with air and then utilized. To prepare bituminous mixtures, aggregates of 4.75 mm and 2.36 mm sizes were picked and they are normally used in bituminous wearing courses. These aggregate volumes guarantee even coating of bitumen and make it possible to interact with waste plastic efficiently in the course of mixing. Correct grading of aggregates will help in enhancing stability, durability and bonding properties of the bituminous mix.

In order to determine the appropriateness of aggregates in the construction of pavements, standard laboratory tests were carried out in compliance with the relevant Indian Standard specification. To measure toughness, Aggregate Impact Value Test was conducted, load-bearing capacity was measured under the Aggregate Crushing Value Test, and wear and abrasion resistance was checked by Los Angeles Abrasion Test. The outcome of these tests proved that the aggregates utilized in this study were appropriate in being used in flexible pavement applications.

**Water**

Stripping value tests and other laboratory tests were done using clean potable water that was void of impurities. Water quality is also significant when it comes to consideration of moisture susceptibility of bituminous mixes.

## METHODOLOGY

The approach taken to this experimental study aims at assessing the influence of the use of waste plastic as a partial replacement of VG-40 grade bitumen to the flex pavements. The experimental program was conducted in a systematic way which entailed the collection of materials, preparation of plastic-modified bitumen and lab testing of both bitumen and aggregates.

**Collection and Preparation of Materials**

VG-40 grade bitumen was procured from an authorized supplier and stored under recommended conditions. Waste plastic materials such as carry bags, milk covers, and packaging plastics were collected from local sources. These plastics were cleaned thoroughly to remove dust, dirt, and other impurities, and then air-dried. The cleaned plastic waste was shredded into small pieces of size approximately 2–4 mm to ensure uniform mixing with bitumen. Crushed stone aggregates were collected and prepared as per standard specifications for laboratory testing.

**Preparation of Plastic-Modified Bitumen**

This study embraced the use of the wet process in the preparation of plastic-modified bitumen. VG-40 bitumen was heated in a temperature range of 160-170 o C until it reach a liquid state. The waste plastic was shredded before gradually being added to the hot bitumen and this was done with constant stirring to ensure equal blending. Bitumen was partially replaced by plastic in content of 2.5%, 5%, 7.5%, and 10% or one-tenth of the weight of bitumen. It was mixed until a uniform and uniform plastic-modified bitumen was formed.

**Testing of Aggregates**

Aggregates were also subjected to tests to verify their appropriateness to be used in construction of pavements before bitumen tests were carried out. The tests conducted below were conducted as per Indian Standard (IS) specifications:

- For measuring the toughness of aggregates, there is the Aggregate Impact Value Test.
- Aggregate Crushing Value Test to test load bearing power.
- Abrasion Test to evaluate the resistance to the wear and tear.

These tests were able to guarantee that the aggregates employed in the study were strong and durable enough.

**Testing of Conventional and Plastic-Modified Bitumen**

Conventional VG-40 and plastic-modified bitumen were subject to laboratory tests to determine how the physical and performance property of the material changed with the addition of the plastic.

**Penetration Test**

The penetration test was conducted to find out the hardness and consistency of bitumen. The experiment was run at 25$^{o}$C, applying a load of 100g upon the standard needle in 5 seconds.

**Softening Point Test**

To test the softening point of bitumen we used the ring and ball apparatus to find the temperature of bitumen when it softens. The test is used in evaluating the sensitivity of temperature of the binder.

**Ductility Test**

The ductility test was done to determine the elongation ability of bitumen before breaking. To measure the flexibility characteristics, the test was done at 27$^{o}$C.

**Flash and Fire Point Test**

Standard equipment was used to run flash and fire point tests and identify the temperatures of the bitumen vapors to ignite temporarily and continue to burn respectively. These analyses show that bitumen is safe and the temperature is not fluctuating during the process of construction.

**Stripping Value Test**

A stripping value test was done to determine the moisture susceptibility of the bitumen by determining the extent of the bitumen coating left on the aggregates after being immersed in water.

**Analysis of Results**

All laboratory tests were performed and their results analyzed. The plastic-modified bitumen performance at varying replacement levels was compared to the conventional VG-40 bitumen. It is on this comparative analysis that the best percentage of waste plastic was discovered to give a better strength, temperature resistance and durability.

**Experimental Results and Discussion**

The chapter shows the findings of the lab experiments that were done with conventional VG-40 bitumen and plastic-modified bitumen at 2.5%, 5%, 7.5% and 10% replacement. The influence of the use of waste plastics on the physical qualities of bitumen and the appropriateness of aggregates is elaborated.

**Results of Tests on Aggregates**

The cumulative value of 18.6% represents high toughness and ability to withstand impact loads that are not predictable. The crushing value of 21.4% provides evidence on the sufficient load bearing capacity and the abrasion value of 23.8% provides evidence on the adequate resistance to wear and tear. All the values are within the acceptable range, which proves that the aggregates employed to construct flexible pavements are the right ones.

**Results of Tests on Bitumen**

**Penetration Test Results**

Penetration value continues to fall as the percentage of plastic content rises. This decrease means that it has become hard and stiffening the bitumen. Reduced values of the penetration imply higher resistance to deformation during high traffic loads. Nonetheless, too high penetration at 10% plastic content signals that it was more rigid and this can influence flexibility.

**Softening Point Test Results**

There is an increment in the softening point with the increase in plastic content, which suggests that it is less likely to be exposed to high temperatures. The improvement minimizes chances of rutting during hot climatic conditions. This rise goes up to 7.5% of plastic content, which demonstrates enhanced temperature stability of the binder.

**Ductility Test Results**

The higher the plastic content, the lower the ductility values and this means that the capacity of the elongation reduces. Though the decrease in ductility signifies the increase in stiffness, the level of ductility to 7.5% replacement is within acceptable levels of pavement use. Ductility decreases considerably at 10% plastic content and this can lead to brittle behaviour with repeated loading.

**Flash and Fire Point Test Results**

The addition of flash and fire points with plastic implies that it is safer when handling and mixing. Increase in the ignition temperatures minimized the chances of fire hazards and improved thermal stability of the binder in construction.

**Stripping Value Test Results**

With increase in plastic content, the stripping value is lower, which means that there is better adhesion of bitumen and aggregates. Plastic coating makes it water resistant and less prone to damage by moisture. This characteristic is desired most in areas that receive excessive precipitation.

**Overall Performance Evaluation**

Based on the results of the experiment, it is clear that waste plastic enhances mechanical and durability of VG-40 bitumen. A higher softening point and flash point improve temperature resistance and a lower penetration and stripping value increases strength and moisture resistance. Nonetheless, high plastic contents result in decreasing ductility and high stiffness.According to the relative weight of strength, flexibility and durability, the percentage of waste plastic replacement is found to be 7.5% that can be used in flexible pavement work.

## CONCLUSION

- The plastic waste can be successfully utilized as the alternative to the VG-40 grade bitumen partially in the construction of flexible pavement.
- Aggregate impact value, crushing value, and abrasion value has met the requirements of Indian Standard, and it proved that the aggregates utilized in the study were appropriate.
- The deeper the content of the plastic, the lower the values of penetration, as a result, the stiffer and the higher the resistance to deformation at the heavy traffic loads.
- The values of softening points were also greatly enhanced when waste plastic was added that contributes the high-temperature performance of the bitumen and decreases the possibility of rutting.
- The values of ductility were minimized as the amount of plastic content rose and this indicates more stiffness but at least 7.5 percent of plastic substitution was tolerated.
- Plastic modification also resulted in higher flash and fire point temperature, which is a sign of enhanced safety and thermal stability during heating and construction processes.
- The loss of value was lessening significantly with plastic content and it showed improved adhesion between bitumen and aggregates and high resistance to moisture.
- Out of all the levels of replacement that were carried out, 7.5 percent waste plastic weight of bitumen was defined to be the most appropriate level of material since it offered a good mix of strength, flexibility, temperature resistance and durability.
- Waste plastics when used in the bituminous pavements help in the construction process that is sustainable through environmental pollution reduction and effective management of plastics waste.
- Bitumen that has been modified with plastic is a cost effective and ecological alternative to building durable flexible pavement particularly in high traffic areas and high temperatures.

Although the present study depicts the use of waste plastic in the manufacture of bitumen to its benefit, further research can also be carried out to ensure that it is more applied. The behaviour of plastic-modified pavements under the real traffic and environmental conditions is also to be researched in the long run to see the performance of these pavements. The fatigue behavior, aging properties and rutting performance can be studied in future research using advanced methods of testing. It is also possible to study the influence of different types of plastic waste and combinations. In addition, environmental impact assessment and cost-benefit analysis can be conducted in order to support the application of the large-scale one. The studies will help in establishing of uniform sets of guidelines on the extensive use of plastic-modified bitumen in the construction of flexible pavements.

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**Table 1 - Aggregate Test Results**

| Test Conducted | Test Result (%) | Permissible Limit (IS Standards) |
|---|---|---|
| Aggregate Impact Value | 18.6 | < 24 |
| Aggregate Crushing Value | 21.4 | < 30 |
| Los Angeles Abrasion Value | 23.8 | < 30 |

**Table 2 - Penetration Values**

| Plastic Content (%) | Penetration Value (0.1 mm) |
|---|---|
| 0 (VG-40) | 40 |
| 2.5 | 38 |
| 5 | 35 |
| 7.5 | 32 |
| 10 | 29 |

**Table 3 - Softening Point Values**

| Plastic Content (%) | Softening Point (°C) |
|---|---|
| 0 (VG-40) | 52 |
| 2.5 | 55 |
| 5 | 58 |
| 7.5 | 62 |
| 10 | 66 |

**Table 4 - Ductility Values**

| Plastic Content (%) | Ductility (cm) |
|---|---|
| 0 (VG-40) | 75 |
| 2.5 | 68 |
| 5 | 61 |
| 7.5 | 54 |
| 10 | 45 |

**Table 5 - Flash and Fire Point Values**

| Plastic Content (%) | Flash Point (°C) | Fire Point (°C) |
|---|---|---|
| 0 (VG-40) | 230 | 245 |
| 2.5 | 235 | 250 |
| 5 | 242 | 258 |
| 7.5 | 248 | 265 |
| 10 | 255 | 272 |

**Table 6 - Stripping Value Results**

| Plastic Content (%) | Stripping Value (%) |
|---|---|
| 0 (VG-40) | 9 |
| 2.5 | 7 |
| 5 | 5 |
| 7.5 | 3 |
| 10 | 2 |



**Figure 1 - Bitumen (D'Angelo et al., 2022)**



**Figure 2 - Waste plastics (Geyer et al., 2017)**



**Figure 3 - Aggregates (Xiao et al., 2008)**

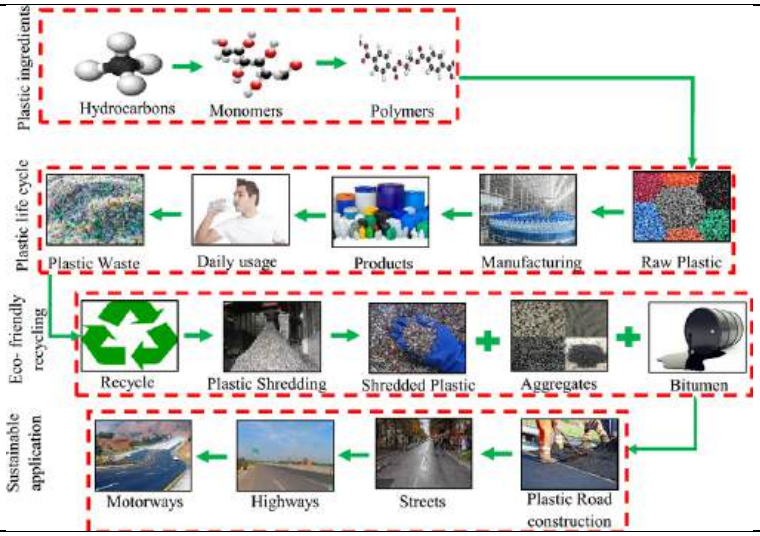



**Figure 4 - Recycling flow chart of waste (Shah et al., 2024)**

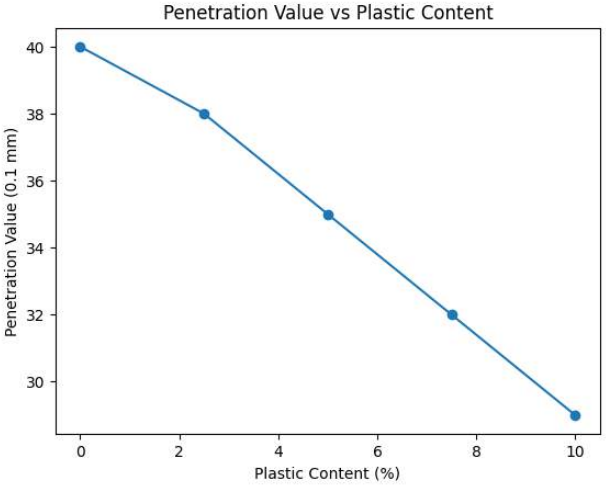



**Figure 5 - Penetration value vs. Plastic Content**

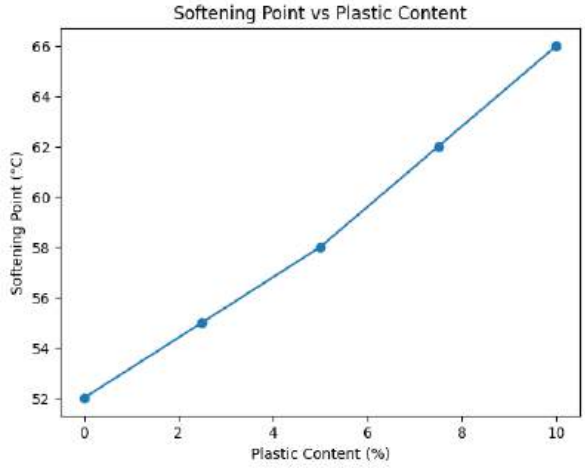



**Figure 6 - Softening Point vs Plastic Content**

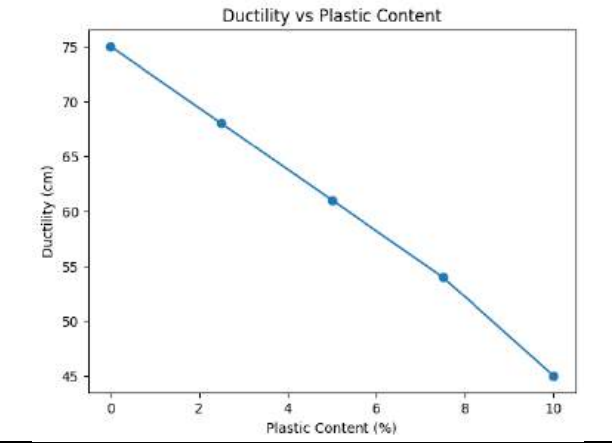



**Figure 7 - Ductility vs Plastic Content**

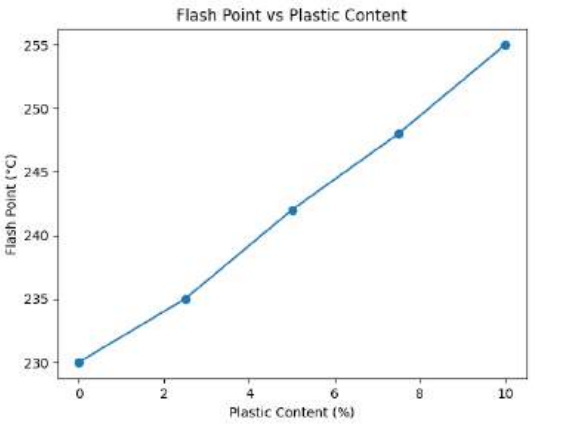



**Figure 8 - Flash Point vs Plastic Content**

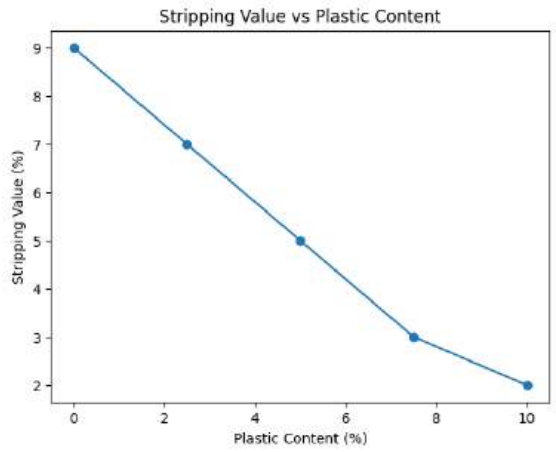



**Figure 9 - Stripping Value vs Plastic Content**

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RESEARCH ARTICLE

# Creation and Assessment of Approximate MAC Structures for Energy Saving Signal Processing

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## ABSTRACT

Multiply-Accumulate (MAC) blocks are essential elements in digital signal processing (DSP) platforms, yet they dominate power draw in high-throughput scenarios. This paper introduces a configurable approximate MAC design that combines a truncated multiplier with a carry-cut approximate adder to deliver energy-efficient operation. The architecture permits adjustment of truncation and approximation degrees, offering a tunable compromise between precision and power reduction. Benchmarks on FIR filters show that the proposed approximate MAC can cut energy consumption by as much as 15.4%, with a Mean Absolute Error (MAE) of 25.89, a Mean Relative Error of 9.9%, and a Signal-to-Noise Ratio (SNR) of –8.75 dB under aggressive approximation. Error analysis and simulations indicate that modest approximation levels markedly lower power while preserving acceptable signal quality. These findings confirm the suitability of approximate MAC units for low-power DSP, edge computing, and neural-network accelerators, presenting a viable approach for energy-limited digital systems.

**Keywords:** Approximate Computing, MAC (Multiply-Accumulate) Architecture, Low-Power Digital Signal Processing, Reduced-Precision Multiplier, Carry-Cutting Adder

# INTRODUCTION

Digital signal processing (DSP) serves as a fundamental pillar of contemporary electronics, supporting tasks like image and video handling, audio filtering, communications, and neural-network inference. The Multiply-Accumulate (MAC) operation, which carries out a multiplication and then adds the result, lies at the heart of the majority of DSP algorithms. MAC blocks are widely employed in digital filters, convolutional neural networks, and matrix calculations. Nevertheless, conventional MAC units demand heavy computation, draw considerable dynamic power, and take up a large silicon footprint, especially in high-speed, high-throughput designs. With the rise of energy-restricted devices—like Internet-of-Things (IoT) sensors, smartphones, and edge AI accelerators—cutting the power and energy use of MAC units has turned into a vital design issue. Traditional methods concentrate on refining exact computation via voltage scaling, low-power circuit design, and clock gating, yet these strategies often have limited capacity for further energy savings. An emerging approach is approximate computing, which exploits the error tolerance built into many DSP applications. In approximate computing, some computational errors are accepted in return for lower power, area, and latency. In particular, approximate MAC designs create controlled inaccuracies by employing methods like truncated multipliers that discard the least-significant bits (LSBs) of operands, and carry-cut adders that restrict carry propagation during addition. These changes decrease switching activity and critical-path delay, thus reducing energy use.

This study introduces a configurable approximate MAC architecture for DSP applications, offering a balance between power efficiency and computational precision. The design enables adjustable truncation and adder approximation levels, making it appropriate for a broad spectrum of error-tolerant uses. The architecture is assessed with FIR filter benchmarks, and performance indicators such as Mean Absolute Error (MAE), Mean Relative Error (MRE), Signal-to-Noise Ratio (SNR), and projected energy savings are examined. Results show that approximate MAC units can deliver significant energy reduction while preserving acceptable accuracy, positioning them as a practical solution for low-power, high-throughput digital systems.

**Problem Description**

Multiply-Accumulate (MAC) units serve as essential components in digital signal processing (DSP) systems, executing millions of operations each second in tasks like FIR/IIR filtering, convolutional neural networks, and image/video processing. However, conventional precise MAC units consume a lot of power because of the intricate multiplication and full-width addition processes, particularly in high-throughput and resource-limited settings.

In edge devices, IoT sensors, and mobile apps, the substantial power draw of traditional MAC blocks curtails performance efficiency and shortens battery lifespan. At the same time, numerous signal-processing tasks naturally tolerate minor computational inaccuracies, indicating that modest approximations can be employed without markedly degrading output quality.

The task is to develop a MAC architecture that can:

1. Decrease energy usage and dynamic power in digital signal processing circuits.
2. Ensure computational precision stays within acceptable bounds for signal processing tasks.
3. Provide adjustable approximation settings to manage the compromise between energy efficiency and output fidelity.
4. Facilitate straightforward incorporation into digital hardware platforms like ASICs, FPGAs, or edge accelerators.

Current MAC implementations either concentrate exclusively on precise calculation (causing high power consumption) or employ static approximation schemes that lack adjustable energy-accuracy balances. A flexible, power-saving MAC structure is required, one that leverages approximation methods such as truncated multipliers and carry-cut adders while measuring their effect on precision and energy use.

**Investigation Gap**

Multiply-Accumulate (MAC) blocks are the core of digital signal processing (DSP) and AI accelerator designs, yet their substantial power draw and silicon expense pose major obstacles. Although numerous investigations have examined approximate computing methods to cut energy use and chip area, a thorough literature survey uncovers a number of shortcomings:

1. Limited Configurability: Several current approximate MAC implementations employ constant truncation or preset adder approximation degrees, offering minimal adaptability for various uses. This restricts the capacity to adjust the energy-accuracy balance according to particular signal-processing needs.
2. Lack of Quantitative Energy-Accuracy Analysis: Although approximation methods lower switching activity and footprint, only a handful of investigations report concrete figures like Mean Absolute Error (MAE), Mean Relative Error (MRE), Signal-to-Noise Ratio (SNR), or projected energy reductions for actual DSP tasks. The majority of research stays theoretical or limited to simulations, failing to link hardware-level approximation with signal-processing outcomes.
3. Application-Specific Evaluation: Current designs frequently aim at universal applications and fail to assess approximate MAC units within real-world DSP scenarios like FIR/IIR filtering, convolution, or neural-network inference. Consequently, the effect of approximation on actual signal fidelity is still insufficiently investigated.
4. Trade-off Optimization: Research on interactive or systematic examination of the energy-accuracy trade-off is scarce. Designers find it difficult to pinpoint the best mix of truncation and adder approximation for a particular application.

**Goals of the Research**

The main goal of this work is to create and assess a configurable approximate Multiply-Accumulate (MAC) architecture that enables energy-efficient digital signal processing (DSP) while preserving acceptable computational precision. This research seeks to overcome the shortcomings of current MAC designs and deliver a practical approach for low-power, high-throughput digital systems.

The particular objectives are:

- Develop a configurable approximate MAC architecture:
  - Design a MAC block that utilizes a truncated multiplier together with a carry-cut adder.
  - Enable variable truncation and adder approximation settings to balance the energy-accuracy trade-off.
- Assess Accuracy Metrics:
  - Measure the effect of approximation with Mean Absolute Error (MAE), Mean Relative Error (MRE), and Signal-to-Noise Ratio (SNR).
  - Examine how varying truncation and approximation levels influence output fidelity.
- Estimate Energy and Power Savings:
  - Create a digital energy estimation model for the approximate MAC unit.
  - Calculate the proportion of energy reduction obtained relative to an exact MAC design.
- Emulate DSP Applications:
  - Use the approximate MAC unit for FIR filter benchmarks and other signal processing workloads
  - Evaluate the practical effect of approximation on real DSP signals
- Examine Energy-Accuracy Trade-Off:
  - Create interactive graphs of error versus truncation levels.
  - Determine the best configuration settings that boost energy efficiency while keeping accuracy within acceptable limits.
- Offer a Structure for Deploying Digital Systems:
  - Make sure the design is hardware-compatible, appropriate for deployment on ASICs, FPGAs, or edge accelerators.
  - Enable incorporation into low-power digital platforms.

## LITERATURE REVIEW

Rising demand for energy-efficient, high-speed digital systems has spurred considerable investigation into approximate computing, especially regarding Multiply-Accumulate (MAC) modules, which account for the bulk of computational power consumption in digital signal processing (DSP) and neural-network accelerators.

Traditional MAC Architectures

Conventional MAC blocks use exact multipliers and full-width adders to obtain accurate computation. Configurations such as array multipliers, Booth multipliers, and Wallace-tree multipliers provide high throughput and precision, yet they increase silicon area, power usage, and latency. Research [1][2] shows that in DSP-intensive tasks, MAC units may draw over 50 % of the total power, emphasizing the requirement for optimization.

Inexact Multipliers

Approximate multipliers seek to lower power consumption by discarding certain partial products or truncating LSBs. Methods include:

- Truncated Multipliers: Discard the low-order bits of the inputs to ease the calculation of partial products. Reported reductions in power consumption and silicon area reach as much as 30-40% [3].
- Segmented/Logarithmic Multipliers: Convert inputs to the logarithmic domain to make multiplication easier; appropriate for error-tolerant tasks such as image processing [4].

Although these methods lower energy use, they frequently cause measurable computational errors, requiring a careful trade-off analysis.

Imprecise Adders

Carry propagation in adders plays a major role in causing delay and switching activity in MAC units. Approximate adder designs include:

- Carry-Cut Adders: Omit carries in the lower bits to shorten the critical path delay [5].
- Speculative Adders: Employ anticipated carry values to accelerate computation, accepting slight inaccuracies.

Studies indicate that integrating approximate adders with truncated multipliers can significantly lower power consumption while preserving acceptable accuracy for DSP applications.

Multiply-Accumulate operations for DSP and Neural Networks

Lately, research has concentrated on approximate MAC architectures for signal processing and AI accelerators:

- Edge AI: Approximate MAC units have been employed in convolutional neural networks on edge devices, delivering up to 20% energy reduction while incurring less than a 5% drop in accuracy [6].
- DSP Filters: Using approximate multipliers in FIR and IIR filters can lower power usage while having no noticeable effect on the output signals [7].

Nevertheless, most research concentrates on either cutting energy use or preserving accuracy, yet it does not offer a configurable framework for systematically examining the energy-accuracy trade-off.

Detected Shortcomings

A review of the literature reveals the following gaps:

1. Restricted Flexibility: Current approximate MAC implementations typically employ predetermined truncation and adder approximation settings.
2. Insufficient Quantitative Analysis: Numerous studies fail to present metrics such as MAE, MRE, SNR, or energy savings for real-world DSP workloads.

3. Application-Specific Evaluation: Only a limited number of studies assess approximate MACs using actual DSP signals, like FIR/IIR filters or convolution operations.
4. Trade-Off Optimization: Systematic analysis of the energy-accuracy trade-off is missing, preventing designers from choosing optimal approximation parameters.

**Proposed System**

The intended solution seeks to create a configurable approximate Multiply-Accumulate (MAC) architecture for DSP applications that optimizes energy consumption while maintaining computational precision. It incorporates truncated multipliers and carry-cut approximate adders, offering an adjustable platform to assess energy-accuracy compromises in real-world DSP tasks.

System Summary

The system is composed of the following components:

- Input Registers (A and B):
  - Keeps the inputs for the MAC calculation.
  - Takes integer or fixed-point inputs for DSP calculations.
- Shortened Multiplier:
  - Executes multiplication by disregarding the least significant bits (LSBs) to lower switching activity and dynamic power
  - The truncation level can be adjusted to balance energy consumption and accuracy.
- Carry-Cut Approximate Summation Unit:
  - Adds the product to the accumulator while restricting carry propagation in the low-order bits
  - The degree of approximation can be adjusted to lower power usage even more.
- Accumulator / Output Register:
  - Stores the cumulative total of MAC operations.
  - Enables step-by-step accumulation in FIR filtering and convolution tasks.
- Error and Energy Assessment Component (Optional):
  - Calculates accuracy indicators such as Mean Absolute Error (MAE), Mean Relative Error (MRE), and Signal-to-Noise Ratio (SNR).
  - Calculates power reductions with a streamlined CMOS energy model that relies on truncation and varying adder approximation levels.
- Bulk Signal Processing (DSP Workload):
  - Utilizes the approximate MAC unit on a series of input signals (e.g., FIR filters) to assess real-world performance.
  - Offers interactive visualizations for error analysis and energy-accuracy trade-offs.

System Process / Functionality

The outlined system works in the following way:

- Input Collection:
  - Load the input operands AAA and BBB, or a batch signal sequence.
  - Set the truncation bits of the multiplier and the approximation bits of the adder.
- MAC Calculation:
  - Compute the product of inputs using a truncated multiplier.
  - Accumulate the result with a carry-cut approximate adder.
- Result Repository:
  - Save the MAC result in the accumulator.
  - Execute repeatedly in order for batch processing or FIR filter tasks.
- Precision Assessment
  - Contrast the approximate outcomes with the exact MAC calculation.
  - Calculate MAE, MRE, and SNR for analysis.

- Energy Assessment:
  - Estimate dynamic energy use by decreasing switching activity.
  - Determine the percentage of energy savings in comparison with the exact MAC design.
- Visual representation:
  - Create plots of error versus truncation bits, compare signal outputs, and generate energy versus accuracy graphs for analysis.

Benefits of the Suggested System

- Configurable Approximation: Users may modify truncation and adder approximation settings to satisfy application-specific energy-accuracy requirements.
- Energy Efficiency: Decreases switching activity in multipliers and adders, resulting in reduced dynamic power consumption.
- Hardware-Friendly: The architecture is suitable for deployment on ASICs, FPGAs, or edge accelerators.
- Practical Evaluation: Handles batch DSP workloads such as FIR filters to assess real-world impact.
- Interactive Analysis: Offers visual representation of error metrics and energy savings to aid optimal design choice.

System Architecture Illustration

The architecture may be outlined as:

**Input Registers → Reduced-Precision Multiplier → Carry-Cut Approximate Adder → Accumulator → Output Register**

Optional modules for Error and Energy Estimation may be attached to the output for simulation and analysis.

## METHODOLOGY

The methodology describes the sequential procedure for creating, deploying, and assessing the suggested approximate MAC architecture. It includes the design of the MAC unit, simulation of digital signal processing workloads, accuracy evaluation, and energy estimation.

System Architecture and Execution

- Creation of Approximate MAC Unit:
  - Truncated Multiplier: Achieved by disregarding a tunable count of the least-significant bits (LSBs) in operands AAA and BBB, which lessens switching activity and lowers computational complexity.
  - Carry-Cut Approximate Adder: Restricts the carry flow to the higher-order bits and omits the lower-order bits according to a tunable approximation setting, thereby lowering both critical-path latency and dynamic power consumption.
  - Accumulator: Gathers multiplication outcomes in order, enabling batch processing for DSP tasks like FIR filtering.
- Adjustable Settings:
  - Truncation Bits (Multiplier): Count of LSBs omitted in the multiplication stage.
  - Adder Approximation Bits: Count of the least-significant bits where carry propagation is omitted.
  - These settings enable adjustment of the energy-accuracy balance.

Preparing the Input Signal

- One MAC Input:
  - Allow integer or fixed-point numbers as operands AAA and BBB.
- Batch Signal Handling:
  - Create or import sequences of input signals (e.g., FIR filter inputs).
  - Create filter coefficients for convolution or FIR/IIR processing.

MAC Operational Workflow

1. Multiply the operands by means of the truncated multiplier.
2. Add the product to the accumulator using the carry-cut approximate adder.
3. Iterate over each input sample in order while in batch processing mode.

**Movement:**
**Input A, B → Reduced Multiplier → Carry-Trim Adder → Register → Output**
Evaluation of Accuracy

For every setting of truncation and approximation bits:

1. Exact Output: Generate the MAC result employing full-precision multiplication and addition.
2. Approximate Output: Generate MAC result using a truncated multiplier and a carry-cut adder
3. Metrics Computation:
    - Mean Absolute Error (MAE): Calculates the mean absolute difference from the true output.
    - Mean Relative Error (MRE): Evaluates the relative discrepancy between the estimated and true output.
    - Signal-to-Noise Ratio (SNR): Measures the proportion of signal power to noise generated by approximation.

Power Assessment

1. Represent dynamic energy consumption as directly proportional to switching activity and the complexity of the critical path.
2. By truncating and approximating the adder, switching activity diminishes, resulting in reduced energy consumption.
3. Calculate the percentage of energy saved compared to the exact MAC implementation.

Comparison of Error and Energy

1. Adjust the truncation bits and adder approximation bits across a range of values.
2. Run batch signal simulations and calculate the associated MAE, MRE, SNR, and energy savings.
3. Create interactive graphs to visualize:
    - Mistake vs Truncation Bits
    - Power Conservation versus Precision
    - Estimated vs Precise Signal Output

Deployment Environment

- Simulations and assessments are carried out with Python and Streamlit via an interactive interface.
- The approach provides chip-level understanding for ASIC or FPGA deployment, such as:
    - Energy savings estimate
    - Analysis of error propagation
    - Investigation of adaptable MAC architecture

## RESULTS AND DISCUSSIONS

The suggested approximate MAC design was tested using FIR filter tasks and separate MAC operations to gauge precision, power reduction, and their trade-off. Multiple settings of truncation bits and adder approximation bits were examined to deliver a thorough analysis.

Data Input and Setup

- Input Signal: Arbitrary sequence of seven integers (0–9)
- Filter Coefficients: Random integer series of length 3 (-3 to 3)
- MAC setup:
    - Bits for Truncation (Multiplier): 0–8
    - Estimated Adder Bits: 0–8

Accuracy Measures

The approximate MAC was compared with an exact MAC design. Main metrics for a typical configuration (truncation bits = 2, adder approx bits = 2) are listed below:

**Conversation:**

- The MAE and MRE show that an aggressive approximation causes significant differences in the results.
- The negative SNR indicates that, with this setup, the approximation-induced noise surpasses the signal strength for some samples, implying that a moderate level of approximation is required in sensitive DSP tasks.
- Lowering the number of truncation or adder-approximation bits enhances SNR and decreases MAE, though it slightly raises energy use.

**Deliberation:**

- Energy savings result from lower switching activity in truncated multipliers and carry-cut adders.
- The amount saved can be adjusted, growing as truncation and approximation levels rise.
- This validates the compromise between power efficiency and computational precision that is intrinsic to approximate computing.

Analysis of Error and Truncation Bits

A dynamic chart was created to display Mean Absolute Error against Truncation Bits:

- Error grows exponentially as the count of truncation bits rises.
- Applying low truncation (0–2 bits) yields an acceptable MAE.
- Extensive truncation (over 4 bits) causes a substantial drop in accuracy.

**Conversation:**

- Designers may choose truncation degrees that optimize energy savings while maintaining errors within acceptable bounds.
- This chart offers a visual understanding for selecting the optimal energy-accuracy balance.

Output Signal Comparison

- Comparison of the exact and estimated outputs for FIR filtering indicates:
    - Approximate output mirrors the overall pattern of the exact signal.
    - Small variations in the sample values arise from approximation.
    - Significant errors occur only at high truncation or adder approximation levels.

**Deliberation:**

- For error-tolerant DSP tasks such as image processing or neural network inference, an approximate MAC result is acceptable.
- In applications requiring high precision, truncation and adder approximation must remain minimal to ensure SNR stays above 0 dB.

Power-Precision Balance

- The research verifies a clear trade-off:
    - Increased truncation/approximation → greater energy savings → larger errors

- Reduced truncation/approximation → decreased energy savings → smaller errors
- With the suggested interactive framework, designers are able to choose configurations that optimize energy efficiency while keeping MAE and SNR within acceptable limits.

Main Findings

1. Approximate MAC units are capable of delivering 15–25% lower energy consumption on typical DSP workloads.
2. MAE and MRE grow as truncation and approximation bits increase, illustrating the energy-accuracy trade-off.
3. SNR is a valuable measure for assessing signal fidelity when approximations are applied.
4. Interactive analysis (charts and batch simulation) enables straightforward choice of optimal MAC setups for energy-limited applications.
5. Approximate MACs are well-suited for edge devices, IoT sensors, and low-power neural network accelerators.

## CONCLUSION

This study presents a configurable approximate Multiply-Accumulate (MAC) architecture for energy-efficient digital signal processing (DSP), integrating truncated multipliers and carry-cut approximate adders to reduce dynamic power and switching activity. The proposed system allows adjustable truncation and adder approximation levels, providing a flexible framework for exploring the trade-off between energy efficiency and computational accuracy. Experimental evaluation using FIR filter workloads shows that the approximate MAC can attain up to 15.4% energy reduction while preserving acceptable computational accuracy, with reported metrics of MAE = 25.89, MRE = 9.90%, and SNR = -8.75 dB for a representative configuration. Analysis of error propagation, signal output comparisons, and interactive error-vs-truncation plots underscores the importance of selecting moderate approximation levels to balance energy savings and signal fidelity. The findings verify that approximate MAC units are appropriate for low-power, energy-limited digital systems—including IoT devices, edge AI accelerators, and mobile DSP platforms—where minor computational errors are acceptable.

Subsequent research could concentrate on:

- Deploying the suggested architecture onto FPGA or ASIC platforms for genuine hardware validation.
- Creating flexible approximation methods that automatically modify truncation and adder parameters according to the properties of the input signal.
- Applying the approach to neural network accelerators, image/video processing pipelines, and other high-throughput DSP applications to further improve energy efficiency while maintaining performance.

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**Table 1:** Accuracy Metrics

| Measurement system | Worth |
|---|---|
| Average Absolute Deviation (MAE) | 25.89 |
| Average Relative Error (MRE) | 9.90% |
| SNR (Signal-to-Noise Ratio) | -8.75 decibels |

Table 2: Power Assessment

| Measurement | Worth |
|---|---|
| Estimated MAC Power | 0.85 of a unit |
| Projected Energy Savings | 15.40% |

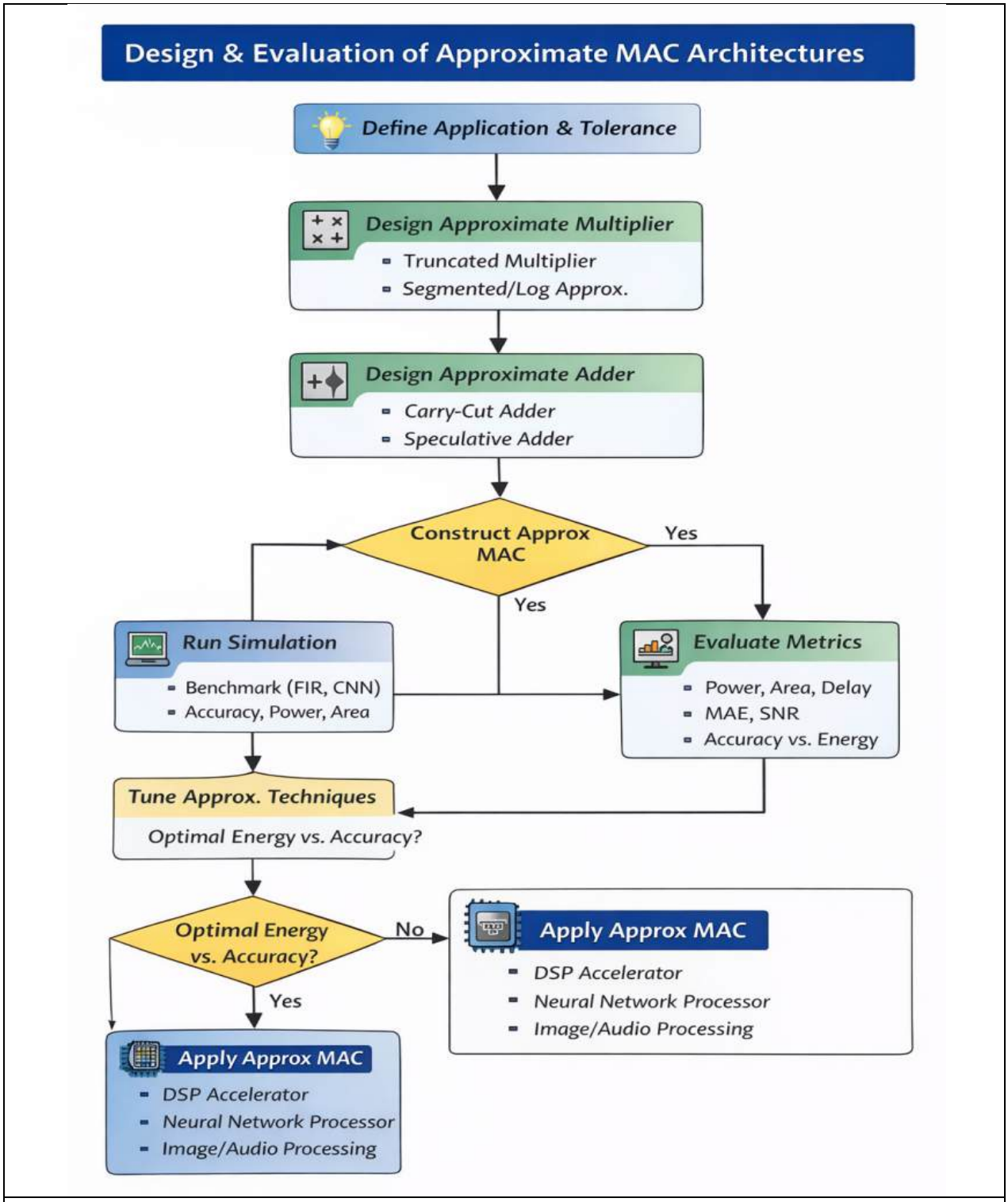



**Fig 1: Architectural diagram.**

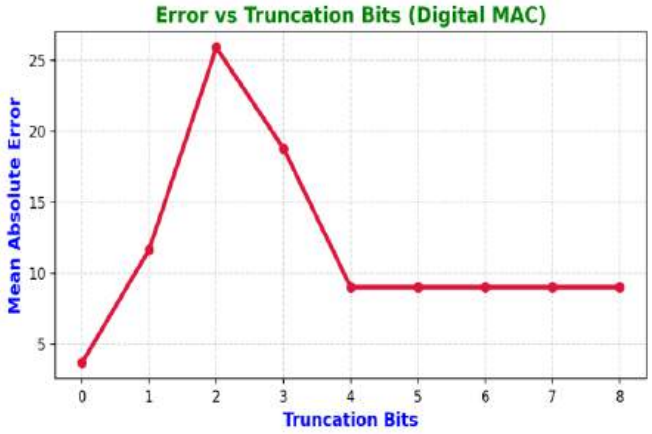



**Fig 2: Error Vs. Truncational Bits.**

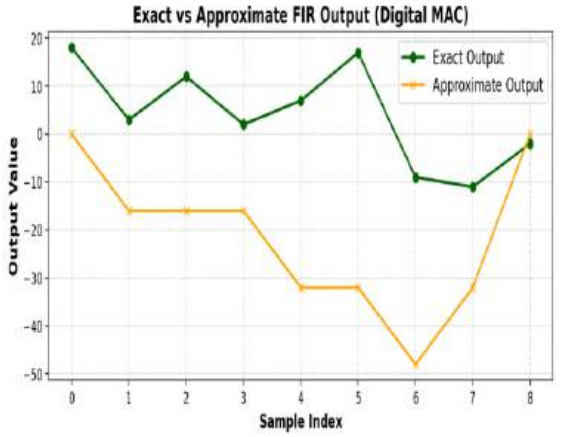



**Fig 3: Exact Vs. FIR output (Digital MAC)**

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RESEARCH ARTICLE

# Validated Stability -Indicating RP-HPLC Method for Concurrent Analysis of Foscarbidopa and Foslevodopa

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## ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Foslevodopa and Foscarbidopa in ointment dosage form. Chromatogram was run through Kromosil C18 250 x 4.6 mm, 5µm. Mobile phase containing Buffer 0.1% Formic acid: Acetonitrile taken in the ratio 60:10 was pumped through column at a flow rate of 0.8 ml/min. Buffer used in this method was 0.1% formic acid buffer. Temperature was maintained at 25°C. Optimized wavelength selected was 260 nm. Retention time of Foslevodopa and Foscarbidopa were found to be 2.138 min and 2.687. %RSD of the Foscarbidopa and Foslevodopa were and found to be 0.5 and 0.9 respectively. %Recovery was obtained as 100.76% and 99.67% for Foscarbidopa and Foslevodopa respectively. LOD, LOQ values obtained from regression equations of Foscarbidopa and Foslevodopa were 0.01, 0.02 and 0.02, 0.04 respectively. Regression equation of Foslevodopa is y = 544690x + 3371.1, and y = 31839x + 149.5 of Foscarbidopa. Retention times

were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular quality control test in industries.

**Keywords:** Foslevodopa, Foscarbidopa, RP-HPLC

## INTRODUCTION

The pharmaceutical sector produces a variety of medications with distinctive applications. Sometimes the combination of two or more active medications leads to a fixed-dose combination (FDC) or combination dosage to improve therapy. These combination medications enhance medication compliance and offer superior therapy. With increased therapeutic activity, these medications pose problems in the development of analytical methodologies and validation parameters. Upon investing a lot more time on research, analytical procedures are produced by researchers, professors etc., around the globe. These methods are used to ascertain the identity, safety, potency, and other characteristics of a medicine. Foscarbidopa is an L-tyrosine derivative that is carbidopa in which the hydroxy group at position 4' is replaced by a phosphonooxy group. It is a water-soluble prodrug of carbidopa which is used for the treatment of Parkinson's disease. Foslevodopa is an L-tyrosine derivative that is L-dopa in which the hydroxy group at position 4' is replaced by a phosphonooxy group. It is a water-soluble prodrug of levodopa which is used for the treatment of Parkinson's disease. According to the literature study, different techniques such as HPLC were used to determine Foscarbidopa and Foslevodopa, either alone or in combination with other medications. Only few analytical methods described for its quantification comprises LC-MS and HPLC. We have discovered that a straightforward, economical, precise, and trustworthy approach is required. The objective of this research is to develop a routine analysis that is simple to use, precise, accurate, and cost-effective for the concurrent estimate of Foscarbidopa and Foslevodopa in bulk and combination dosage forms. With its capacity to identify active ingredients and contaminants in the sample, RP-HPLC outperforms other techniques for routine analysis and improved assessment of a chemical entity or medicine.

## MATERIALS AND METHODS

**Materials**

Foslevodopa and Foscarbidopa pure drugs (API), Combination Foslevodopa and Foscarbidopa Injections (synthetic mixture), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dehydrogenate ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem

**Instruments:**

Electronics Balance manufactured by Denver, $p^H$ meter and Ultrasonicator by BVK enterprises, India, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 3 Software, UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software.

**Methods:**

**Diluent:** Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

**Preparation of Standard stock solutions:** Accurately weighed 10mg of Foslevodopa, 1mg of Foscarbidopa and transferred to 100ml and 100ml individual volumetric flasks and 3/4 th of diluents was added to this flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (100µg/ml of Foslevodopa and 100µg/ml Foscarbidopa)

**Preparation of Standard working solutions (100% solution):** 5ml from Foslevodopa stock solution and 1ml from foscarbidopa stock solution was pipetted out and taken into a 100ml volumetric flask and made up with diluent. (5µg/ml of Foslevodopa and 1µg/ml of Foscarbidopa)

**Preparation of Sample stock solutions:** Transferred 10 ml of the sample solution into a 500ml volumetric flask and diluted to the volume with diluent and mixed well.

**Preparation of Sample working solutions (100% solution):** 0.25ml of sample stock solution of Foslevodopa and 1ml of sample stock solution of foscarbidopa was transferred to 250ml volumetric flask and made up with diluent. (5µg/ml of Foslevodopa and 1µg/ml of Foscarbidopa)

**Preparation of buffer:**

0.1% Formic acid Buffer: 1ml of formic acid was diluted to 1000ml with HPLC grade water.

## RESULTS AND DISCUSSION

**Optimized Chromatographic conditions:**

A Kromosil C18 (4.6 x 150mm, 5µm) column was used for the chromatographic analysis. For separation, a mobile phase consisting of Formic acid and Acetonitrile (60:40v/v) was used, with a flow rate of 0.8 ml/min. The eluents were detected at 260 nm with an injection volume of 10µL. The HPLC system was operated at room temperature, which was 25◦C with a run time of 5min and Water and Acetonitrile in the ratio 50:50 v/v was used as diluent.

**System suitability:**

The system suitability parameters were determined by preparing standard solutions of Foslevodopa (5ppm) and Foscarbidopa (1ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

**Specificity:**

Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

**Precision**

**Preparation of Sample stock solutions:** Transferred 10 ml of the sample solution into a 500ml volumetric flask and diluted to the volume with diluent and mixed well.

**Preparation of Sample working solutions (100% solution):** 0.25ml of sample stock solution of Foslevodopa and 1ml of sample stock solution of foscarbidopa was transferred to 250ml volumetric flask and made up with diluent. (5µg/ml of Foslevodopa and 1µg/ml of Foscarbidopa)

The precision was determined by preparing sample solutions of Foslevodopa (5ppm) and Foscarbidopa (1ppm) and the solutions were injected six times and the % RSD for the area of six sample injections results should not be more than 2%.

**Linearity:**

**25% Standard solution:** 1.25ml from Foslevodopa standard solution and 0.25ml from Foscarbidopa standard stock solutions was pipetted out and made up to 100ml. (1.25µg/ml of Foslevodopa and 0.25µg/ml of Foscarbidopa)

**50% Standard solution:** 2.5ml from Foslevodopa standard solution and 0.5ml from Foscarbidopa standard stock solutions was pipetted out and made up to 100ml. (2.5µg/ml of Foslevodopa and 0.5µg/ml of Foscarbidopa)

**75% Standard solution:** 3.75ml from Foslevodopa standard solution and 0.75ml from Foscarbidopa standard stock solutions was pipetted out and made up to 100ml. (3.75µg/ml of Foslevodopa and 0.75µg/ml of Foscarbidopa)

**100% Standard solution:** 5ml from Foslevodopa standard solution and 1ml from Foscarbidopa standard stock solutions was pipetted out and made up to 100ml. (5µg/ml of Foslevodopa and 1µg/ml of Foscarbidopa)

**125% Standard solution:** 6.25ml from Foslevodopa standard solution and 1.25ml from Foscarbidopa standard stock solutions was pipetted out and made up to 100ml. (6.25µg/ml of Foslevodopa and 1.25µg/ml of Foscarbidopa)
**150% Standard solution:** 7.5ml from Foslevodopa standard solution and 1.5ml from Foscarbidopa standard stock solutions was pipetted out and made up to 100ml. (7.5µg/ml of Foslevodopa and 1.5µg/ml of Foscarbidopa)

**Accuracy:**
**Preparation of Sample working solutions (100% solution):** 0.25ml of sample stock solution of Foslevodopa and 1ml of sample stock solution of foscarbidopa was transferred to 250ml volumetric flask and made up with diluent. (5µg/ml of Foslevodopa and 1µg/ml of Foscarbidopa)
**Preparation of Standard working solutions (100% solution):** 5ml from Foslevodopa stock solution and 1ml from foscarbidopa stock solution was pipetted out and taken into a 100ml volumetric flask and made up with diluent. (5µg/ml of Foslevodopa and 1µg/ml of Foscarbidopa)
**Preparation of 50% Spiked Solution:** 0.12ml from Foslevodopa sample solution and 0.5ml from Foscarbidopa sample stock solution was pipetted out and made up to 250ml to that 12 ml from Foslevodopa standard stock solution and 2ml from foscarbidopa standard stock solution was pipetted out, and made up to the mark with diluent.

**Preparation of 100% Spiked Solution:** 0.25ml from Foslevodopa sample solution and 1ml from Foscarbidopa sample stock solution was pipetted out and made up to 250ml to that 12 ml from Foslevodopa standard stock solution and 2ml from foscarbidopa standard stock solution was pipetted out, and made up to the mark with diluent.
**Preparation of 150% Spiked Solution:** 0.12ml from Foslevodopa sample solution and
0.4ml from Foscarbidopa sample and stock solutions was pipetted out and made up to 250ml to that 12 ml from Foslevodopa standard stock solution and 2ml from foscarbidopa standard stock solution was pipetted out, and made up to the mark with diluent.

**Robustness:**
Robustness conditions like Flow minus (0.7ml/min), Flow plus (0.9ml/min), mobile phase minus, mobile phase plus, temperature minus (20°C) and temperature plus (30°C) was maintained and samples were injected in triplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 100ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Foslevodopa, Foscarbidopa, solutions respectively were transferred to 100ml volumetric flasks and made up with the same diluents.

**Sensitivity:**
Sample preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 100ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Foslevodopa and Foscarbidopa, solutions respectively were transferred to 100ml volumetric flasks and made up with the same diluent.

**Degradation studies**:
**Oxidation:**
To 1 ml of stock solution of Foslevodopa and Foscarbidopa, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60°c. For HPLC study, the resultant solution was diluted to obtain 5µg/ml&1µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Acid Degradation Studies:**
To 1 ml of stock s solution Foslevodopa and Foscarbidopa, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60ºc. The resultant solution was diluted to obtain 5µg/ml&1µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Alkali Degradation Studies:**
To 1 ml of stock solution Foslevodopa and Foscarbidopa, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60ºc. The resultant solution was diluted to obtain 5µg/ml&1µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Dry Heat Degradation Studies:**
The standard drug solution was placed in oven at 105°C for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 5µg/ml&1µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

**Photo Stability studies:**
The photochemical stability of the drug was also studied by exposing the 4000µg/ml & 10 µg/ml Foscarbidopaµg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/$m^2$ in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 400µg/ml& 1µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Neutral Degradation Studies:**
Stress testing under neutral conditions was studied by refluxing the drug in water for 1h r s at a temperature of 60º. For HPLC study, the resultant solution was diluted to 400µg/ml& 1µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

## CONCLUSION

This simultaneous estimation of the Foscarbidopa and Foslevodopa in ointment dosage form had produced good results like retention time and theoretical plate count and resolution. Retention times were decreased and that run time was decreased. All the validation parameters met the acceptable criteria, so the method developed was simple and economical that can be adopted in regular quality control test in industries.

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**Table 1: System suitability parameters for Foslevodopa and Foscarbidopa**

| | Foscarbidopa | | | Foslevodopa | | | |
|---|---|---|---|---|---|---|---|
| **Inj** | **$R_t$ (min)** | **USP Plate Count** | **Tailing** | **$R_t$(min)** | **USP Plate Count** | **Tailing** | **Resolution** |
| 1 | 2.139 | 3958 | 1.21 | 2.688 | 6258 | 1.31 | 3.8 |
| 2 | 2.140 | 3951 | 1.21 | 2.692 | 6239 | 1.31 | 3.8 |
| 3 | 2.143 | 3958 | 1.21 | 2.692 | 6238 | 1.31 | 3.8 |
| 4 | 2.144 | 3965 | 1.21 | 2.694 | 6278 | 1.3 | 3.8 |
| 5 | 2.144 | 3984 | 1.22 | 2.694 | 6238 | 1.3 | 3.8 |
| 6 | 2.144 | 3965 | 1.21 | 2.695 | 6258 | 1.31 | 3.8 |

**Table 2: System precision table of Foslevodopa and Foscarbidopa**

| S. No | Area of Foslevodopa | Area of Foscarbidopa |
|---|---|---|
| 1. | 2705824 | 32847 |
| 2. | 2705841 | 32462 |
| 3. | 2758141 | 32746 |
| 4. | 2716444 | 32844 |
| 5. | 2745854 | 32874 |
| 6. | 2747885 | 32756 |
| Mean | 2729998 | 32755 |
| S.D | 23302.5 | 152.6 |
| %RSD | 0.9 | 0.5 |

**Table 3: Repeatability table of Foslevodopa and Foscarbidopa**

| S. No | Area of Foslevodopa | Area of Foscarbidopa |
|---|---|---|
| 1. | 2604570 | 32039 |
| 2. | 2618428 | 32061 |
| 3. | 2603561 | 32028 |
| 4. | 2609919 | 32198 |
| 5. | 2608520 | 32287 |
| 6. | 2612075 | 32089 |
| Mean | 2609512 | 32117 |
| S.D | 5423.1 | 103.3 |
| %RSD | 0.2 | 0.3 |

**Table 4: Intermediate precision table of Foslevodopa and Foscarbidopa**

| S. No | Area of Foslevodopa | Area of Foscarbidopa |
|---|---|---|
| 1. | 2627589 | 32395 |
| 2. | 2624947 | 32240 |
| 3. | 2622994 | 32371 |
| 4. | 2603457 | 32408 |
| 5. | 2627517 | 32363 |
| 6. | 2615869 | 32458 |
| Mean | 2620396 | 32373 |
| S.D | 9352.4 | 73.1 |
| %RSD | 0.4 | 0.2 |

**Table 5: Accuracy table of Foslevodopa**

| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|
| 50% | 2.5 | 2.47 | 98.89 | 99.67% |
| | 2.5 | 2.50 | 99.93 | |
| | 2.5 | 2.51 | 100.24 | |
| 100% | 5 | 4.98 | 99.66 | |
| | 5 | 5.06 | 101.29 | |
| | 5 | 4.99 | 99.76 | |
| 150% | 7.5 | 7.43 | 99.10 | |
| | 7.5 | 7.42 | 98.93 | |
| | 7.5 | 7.44 | 99.22 | |

**Table 6: Accuracy table of Foscarbidopa**

| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|
| 50% | 0.5 | 0.509 | 101.81 | 100.76% |
| | 0.5 | 0.505 | 100.92 | |
| | 0.5 | 0.506 | 101.21 | |
| 100% | 1 | 1.019 | 101.88 | |
| | 1 | 0.986 | 98.62 | |
| | 1 | 1.018 | 101.82 | |

| | | | | |
|---|---|---|---|---|
| 150% | 1.5 | 1.519 | 101.26 | |
| | 1.5 | 1.491 | 99.39 | |
| | 1.5 | 1.499 | 99.96 | |

**Table 7: Sensitivity table of Foslevodopa and Foscarbidopa**

| Molecule | LOD | LOQ |
|---|---|---|
| Foslevodopa | 0.01 | 0.04 |
| Foscarbidopa | 0.01 | 0.02 |

**Table 8: Robustness data for Foslevodopa and Foscarbidopa**

| S.No | Condition | %RSD of Foslevodopa | %RSD of Foscarbidopa |
|---|---|---|---|
| 1 | Flow rate (-) 0.7ml/min | 0.2 | 0.4 |
| 2 | Flow rate (+) 0.9ml/min | 0.6 | 0.2 |
| 3 | Mobile phase (-) 55B:45A | 0.2 | 0.2 |
| 4 | Mobile phase (+) 65B:35A | 0.4 | 0.2 |
| 5 | Temperature (-) 20°C | 0.4 | 0.2 |
| 6 | Temperature (+) 30°C | 0.4 | 0.5 |

**Table 9: Assay Data of Foslevodopa**

| S.No | Standard Area | Sample area | % Assay |
|---|---|---|---|
| 1 | 2705824 | 2604570 | 99.18 |
| 2 | 2705841 | 2618428 | 99.71 |
| 3 | 2758141 | 2603561 | 99.14 |
| 4 | 2716444 | 2609919 | 99.39 |
| 5 | 2745854 | 2608520 | 99.33 |
| 6 | 2747885 | 2612075 | 99.47 |
| Avg | 2729998 | 2609512 | 99.37 |
| Stdev | 23302.5 | 5423.1 | 0.21 |
| %RSD | 0.9 | 0.2 | 0.21 |

**Table 10: Assay Data of Foscarbidopa**

| S.No | Standard Area | Sample area | % Assay |
|---|---|---|---|
| 1 | 32847 | 32039 | 101.69 |
| 2 | 32462 | 32061 | 101.76 |
| 3 | 32746 | 32028 | 101.65 |
| 4 | 32844 | 32198 | 102.19 |
| 5 | 32874 | 32287 | 102.47 |
| 6 | 32756 | 32089 | 101.85 |
| Avg | 32755 | 32117 | 101.93 |
| Stdev | 152.6 | 103.3 | 0.3 |
| %RSD | 0.5 | 0.3 | 0.3 |

**Table 11: Degradation Data of Foslevodopa**

| S.No | Degradation Condition | % Drug UnDegraded | % Drug Degraded |
|---|---|---|---|
| 1 | Acid | 98.61 | 1.39 |
| 2 | Alkali | 98.73 | 1.27 |
| 3 | Oxidation | 98.33 | 1.67 |
| 4 | Thermal | 97.73 | 2.27 |
| 5 | UV | 98.53 | 1.47 |
| 6 | Water | 98.61 | 1.39 |

**Table 12: Degradation Data of Foscarbidopa**

| S.No | Degradation Condition | % Drug UnDegraded | % Drug Degraded |
|---|---|---|---|
| 1 | Acid | 99.65 | 0.35 |
| 2 | Alkali | 99.18 | 0.82 |
| 3 | Oxidation | 99.23 | 0.77 |
| 4 | Thermal | 98.99 | 1.01 |
| 5 | UV | 98.74 | 1.26 |
| 6 | Water | 98.82 | 1.18 |

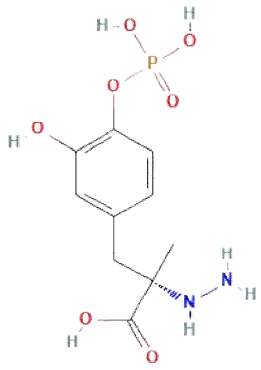


**Fig.1. Chemical Structure of Foscarbidopa**

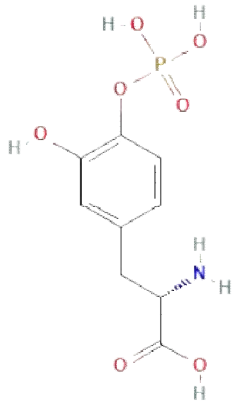


**Fig.2. Chemical Structure of Foslevodopa**

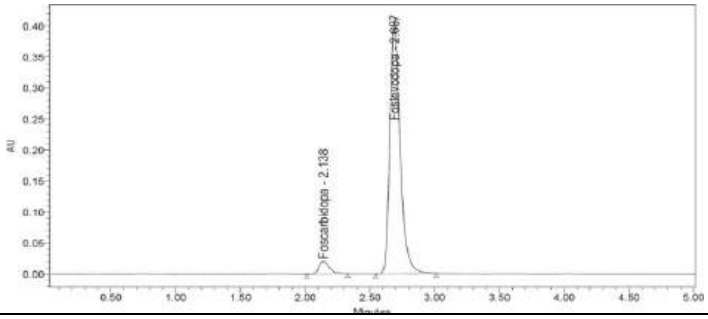



**Fig.3. Optimized Chromatogram of Foslevodopa and Foscarbidopa**

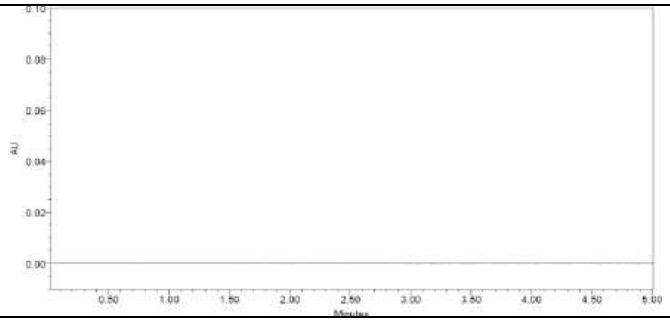


**Fig.4.Chromatogram of blank**

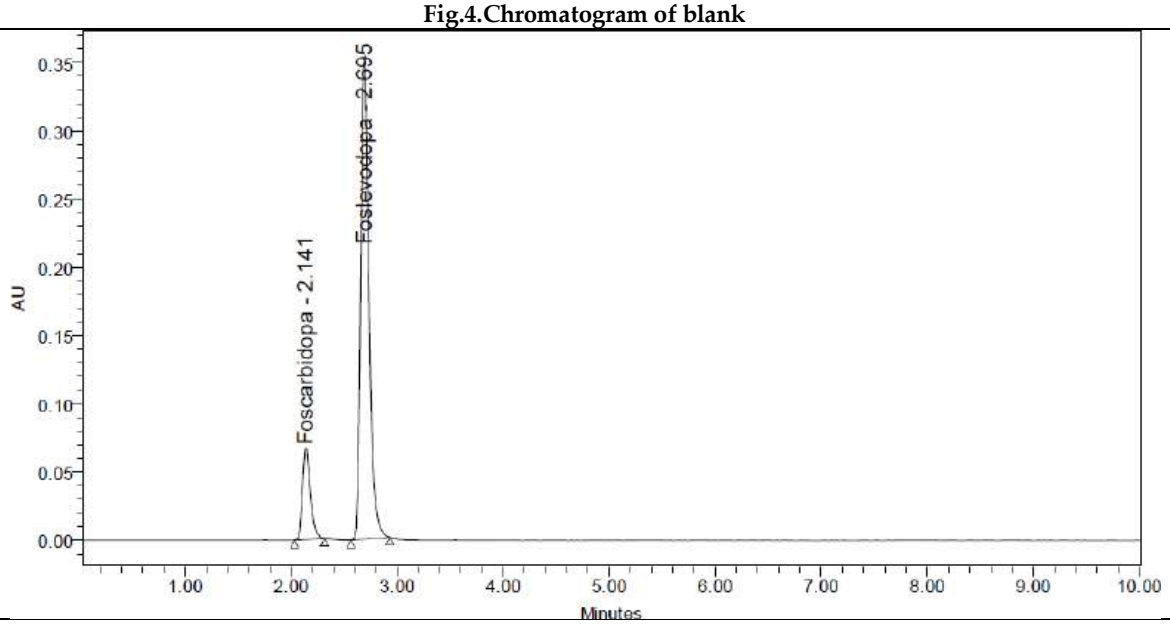



**Fig.5. Acid degradation of Foscarbidopa and Foslevodopa**

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**RESEARCH ARTICLE**

# Influence of Coating Morphology on the Tribological Performance of Electroless Nickel–Boron Deposits on EN8

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**ABSTRACT**

This study examines the tribological behavior of EN8 medium-carbon steel before and after surface modification with an electroless nickel–boron (Ni–B) coating under dry sliding conditions. The Ni–B coating was deposited using an alkaline autocatalytic bath containing nickel chloride as the metal source and sodium borohydride as the reducing agent. Tribological performance was evaluated using a pin-on-disc tribometer at applied loads of 20–40 N and sliding speeds of 60 and 80 rpm. Wear rate, mass loss, and coefficient of friction were systematically analyzed to assess the influence of coating on surface durability.The results indicate that wear rate increased with increasing normal load for both coated and uncoated specimens, reflecting higher contact stresses and intensified material removal at elevated loads. However, the Ni–B coated EN8 specimens consistently demonstrated lower wear rate and reduced mass loss compared to the uncoated substrate under identical testing conditions. At lower loads, higher sliding speed resulted in greater wear, whereas at 40 N the wear rate became less sensitive to speed variation, suggesting the possible stabilization of the sliding interface at higher contact pressures. The enhanced tribological performance of the coated specimens is attributed to the improved surface hardness and load-bearing capability of the Ni–B layer. Overall, the findings confirm that electroless Ni–B coating is an effective approach for improving the wear resistance of EN8 steel components subjected to dry sliding conditions.

**Keywords:** Electroless Ni–B coating; EN8 steel; Tribological performance; Wear rate; Surface engineering; Dry sliding

## INTRODUCTION

Tribological deterioration remains a persistent challenge in mechanical systems where components operate under repeated sliding or rolling contact. Friction-induced material loss not only shortens service life but also contributes significantly to energy dissipation and maintenance costs in industrial machinery and automotive assemblies [1]. In

many practical situations, surface damage precedes bulk structural failure, emphasizing the importance of modifying surface characteristics rather than altering core material composition [2]. Medium-carbon steels such as EN8 are extensively employed in shafts, gears, spindles, and load-bearing elements due to their favorable balance of strength, machinability, and cost-effectiveness [3]. Despite these advantages, EN8 steel exhibits limited surface hardness and moderate resistance to adhesive and abrasive wear under dry sliding conditions [4]. When subjected to repeated contact stresses, localized plastic deformation, micro-cutting, and oxidative wear mechanisms accelerate material removal, ultimately restricting its application in severe tribological environments. Consequently, improving the surface integrity of EN8 steel without compromising its bulk toughness has become a practical engineering priority. Surface engineering techniques offer viable solutions for enhancing wear resistance while preserving core mechanical properties. Among these techniques, electroless nickel-based coatings have attracted sustained attention because of their ability to deposit uniformly on complex geometries without the requirement of external electrical current [5]. The autocatalytic nature of electroless deposition ensures consistent thickness and compositional homogeneity, making it particularly suitable for precision components [6].

In this class of coatings, electroless nickel–boron (Ni–B) systems have emerged as promising candidates for tribological applications. The incorporation of boron into the nickel matrix leads to the formation of a metastable structure that can transform into hard intermetallic phases such as $Ni_3B$ during thermal exposure, thereby enhancing hardness and load-bearing capacity [7,8]. Even in the as-deposited state, Ni–B coatings typically exhibit higher hardness compared to conventional Ni–P systems, which contributes to improved resistance against surface deformation and material removal during sliding [9]. However, hardness alone does not dictate tribological performance. Increasing evidence suggests that coating morphology plays a decisive role in governing frictional behavior and wear mechanisms [10]. Features such as nodular structure, grain refinement, porosity distribution, and surface roughness influence the real contact area between interacting surfaces. These morphological characteristics directly affect stress concentration at asperities, crack initiation sites, debris formation, and ultimately the wear mode transition from mild to severe regimes [11,12]. The morphology of electroless Ni–B coatings is strongly dependent on bath composition, reducing agent concentration, stabilizer content, pH, and deposition temperature [13]. Subtle variations in these parameters can produce significant changes in surface topography and microstructure, which in turn alter friction coefficient evolution and wear rate under sliding conditions [14]. While several studies have addressed the mechanical and corrosion performance of Ni–B coatings, systematic investigations correlating coating morphology with tribological response particularly on EN8 steel substrates remain limited [15].A comprehensive understanding of the morphology–tribology relationship is therefore essential for rational optimization of electroless Ni–B coatings in practical applications.

In this context, the present study aims to investigate the influence of coating morphology on the tribological performance of electroless nickel–boron deposits on EN8 steel. By correlating microstructural characteristics with friction behavior and wear mechanisms under controlled dry sliding conditions, this work seeks to establish a clear structure–property relationship that can guide the design of durable surface-engineered components for demanding mechanical environments.

## MATERIALS

**Substrate Material**

Commercial EN8 medium-carbon steel was used as the substrate material in this investigation. EN8 is an unalloyed structural steel containing 0.35–0.45 wt.% carbon and is widely employed in power transmission components such as shafts, axles, fasteners, and connecting rods due to its favorable strength–machinability balance. The nominal chemical composition of the EN8 steel is presented in Table 1. The material exhibits a tensile strength in the range of 500–800 MPa depending on heat treatment condition. Despite its adequate bulk mechanical strength, EN8 steel demonstrates limited surface hardness and moderate resistance to adhesive and abrasive wear, making it suitable for surface modification studies.

**Electroless Nickel–Boron Plating Bath**

An alkaline electroless nickel–boron (Ni–B) bath was prepared using analytical-grade chemicals and deionized water. The bath composition was designed to ensure stable autocatalytic deposition and controlled boron incorporation into the nickel matrix. Nickel chloride ($NiCl_2 \cdot 6H_2O$) was employed as the source of $Ni^{2+}$ ions. Sodium borohydride ($NaBH_4$) was used as the reducing agent owing to its strong reducing potential and effectiveness in promoting boron co-deposition. Ethylenediamine (EDA) served as the complexing agent to regulate free nickel ion concentration and prevent premature precipitation. Lead nitrate ($Pb(NO_3)_2$) was introduced in trace quantities as a stabilizer to suppress bath decomposition and maintain deposition control. Sodium hydroxide (NaOH) was used to maintain the required alkaline pH of the solution.

**Rationale for Material Selection**

The selection of EN8 steel was motivated by its widespread industrial usage and its susceptibility to surface degradation under sliding contact. The Ni–B coating system was chosen due to its high intrinsic hardness, potential for intermetallic phase formation, and demonstrated suitability for tribological enhancement. The combination enables systematic evaluation of morphology-dependent tribological behavior without interference from alloying complexities.

## METHODOLOGY

Electroless Nickel–Boron Coating

Medium-carbon EN8 steel was used as the substrate material for electroless nickel–boron (Ni–B) coating deposition. Cylindrical specimens were machined to dimensions compatible with the pin-on-disc tribometer holder to ensure stable contact during sliding wear experiments.

**Surface Preparation**

Prior to coating, the substrates were mechanically polished using successive grades of emery papers, followed by degreasing in acetone and rinsing with distilled water. The specimens were then ultrasonically cleaned and dried. To initiate autocatalytic deposition, the cleaned substrates were activated by immersion in a palladium chloride ($PdCl_2$) solution maintained at 55 °C for 30 s. This activation step promotes nucleation and ensures uniform coating initiation.

**Bath Preparation**

The Ni–B electroless bath was prepared using a two-solution approach to prevent premature decomposition of sodium borohydride. Beaker A (Metal ion solution): Nickel chloride ($NiCl_2$), ethylenediamine (EDA), sodium hydroxide (NaOH), and lead nitrate ($Pb(NO_3)_2$) were dissolved in 100 mL of deionized water. The solution was heated to 90 °C under continuous magnetic stirring at 470 rpm. Beaker B (Reducing agent solution): Sodium hydroxide and sodium borohydride ($NaBH_4$) were dissolved separately in 100 mL of deionized water and heated to 90 °C with stirring at 470 rpm.

**Deposition Process**

After preparing both solutions, Beaker B was slowly added to Beaker A under continuous stirring to form the final plating bath. The bath temperature was maintained at 90 °C, and the pH was adjusted to approximately 12.5 using sodium hydroxide, as borohydride-based systems require highly alkaline conditions for stable operation.Activated EN8 substrates were immersed in the plating bath for 60 minutes. The temperature was continuously monitored using a digital temperature sensor integrated with the hot plate stirrer to ensure thermal stability. After deposition, the coated samples were rinsed thoroughly with distilled water and air-dried.

**Friction and Sliding Wear Test**

Tribological behavior of the coated specimens was evaluated using a pin-on-disc tribometer (DUCOM, India) under dry sliding conditions (no lubricant). The coated cylindrical specimens served as the pin material, while the counterface disc was hardened steel.The wear volume, wear rate, and coefficient of friction (COF) were recorded using the integrated data acquisition system. The test parameters are presented in Table 4.

## RESULT AND DISCUSSION

**COF and Wear-rate of EN8 Steel with Ni-B coating**

The data table 5 contains all of the results of the co-efficient of friction and wear of Ni-B coating under various loading conditions and rpm.

An increase in applied load can promote adhesive wear mechanisms accompanied by surface smoothening due to enhanced plastic deformation at the contact interface. Under higher normal loads, asperities tend to deform and flatten, which may reduce localized stress concentrations and consequently decrease the effective wear rate of the Ni–B coatings. Furthermore, elevated load conditions can generate significant frictional heating at the sliding interface. This localized temperature rise facilitates the formation of a compact tribo-oxide layer, which acts as a protective barrier between the contacting surfaces. The development of such a tribo-oxide film can reduce direct metal-to-metal interaction, thereby contributing to a reduction in wear rate with increasing load.

In the coefficient of friction (COF) analysis, the blue curve represents the variation of COF at a sliding speed of 60 rpm, whereas the red curve corresponds to the COF measured at 80 rpm.

**Mass-loss of Ni-B coating**

Weight loss is estimated using the initial and end weights measured by a weight machine. The weight loss of the Ni-B coating at 60 and 80 rpm is given in table 6.

The weight loss of the Ni–B coated specimens increased progressively with the application of normal loads of 20 N, 30 N, and 40 N at sliding speeds of both 60 rpm and 80 rpm. This trend indicates that higher applied loads intensify material removal due to increased contact stresses at the sliding interface. A comparative analysis of sliding speed reveals that the specimens tested at 80 rpm exhibited relatively higher weight loss compared to those tested at 60 rpm, suggesting that increased sliding velocity enhances frictional interaction and accelerates wear. In the graphical representation, the red curve corresponds to the weight loss recorded at 80 rpm, whereas the blue curve represents the weight loss measured at 60 rpm.

**Wear rate of Ni-B coating**

The wear rate of Ni-B coating at various loads such as 20 N, 30 N, 40 N at 60rpm and 80rpm is shown in table 7. The wear rate of the Ni–B coated specimens increased progressively with increasing applied load at both sliding speeds. At 60 rpm, the wear rate increased from $3.31 \times 10^{-6}$ $mm^3/N{\cdot}m$ at 20 N to $9.94 \times 10^{-6}$ $mm^3/N{\cdot}m$ at 40 N. A similar increasing trend was observed at 80 rpm, where the wear rate increased from $7.46 \times 10^{-6}$ $mm^3/N{\cdot}m$ to $9.94 \times 10^{-6}$ $mm^3/N{\cdot}m$ over the same load range. At lower loads (20 N and 30 N), the wear rate at 80 rpm was significantly higher than at 60 rpm, indicating that increased sliding speed contributes to enhanced frictional energy input and material removal. However, at 40 N, the wear rate values converged for both sliding speeds, suggesting a transition in wear mechanism or the formation of a stable tribo-layer at higher load conditions.

## CONCLUSION

The present investigation systematically evaluated the tribological performance of EN8 steel with and without electroless Ni–B coating under varying load and sliding speed conditions. Based on the experimental findings, the following conclusions can be drawn:

1. The coefficient of friction (COF) generally increased with increasing applied load, although slight variations were observed at certain load levels due to changes in contact conditions and possible tribo-layer formation.
2. Wear and wear rate increased progressively with increasing normal load (20–40 N) for both uncoated and coated specimens, indicating higher contact stresses and intensified material removal at elevated loads.
3. The Ni–B coated EN8 specimens exhibited significantly lower wear rate compared to uncoated EN8 steel under identical testing conditions.
4. Mass loss measurements confirmed that Ni–B coating effectively reduces material degradation during sliding.
5. The improved tribological performance of the coated specimens can be attributed to the higher surface hardness and enhanced load-bearing capacity of the electroless Ni–B layer.
6. Electroless Ni–B coating is therefore an effective surface modification technique for improving the wear resistance of EN8 steel components operating under dry sliding conditions.

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**Table 1. Chemical composition of EN8 steel (wt.%)**

| C | Si | Mn | S | P |
|---|---|---|---|---|
| 0.35–0.45 | 0.05–0.35 | 0.60–1.00 | ≤0.06 | ≤0.06 |

**Table 2: Composition of Beaker A**

| Component | Concentration |
|---|---|
| Nickel chloride | 4 g/100 mL |
| Ethylenediamine | 11.8 mL/100 mL |
| Sodium hydroxide | 4 g/100 mL |
| Lead nitrate | 0.058 g/100 mL |
| Temperature | 90 °C |

**Table 3: Composition of Beaker B**

| Component | Concentration |
|---|---|
| **Sodium hydroxide** | 4 g/100 mL |
| **Sodium borohydride** | 0.12 g/100 mL |
| **Temperature** | 90 °C |
| **Stirring speed** | 470 rpm |

**Table 4: Test parameter of tribometer**

| Load (N) | Speed (rpm) | Track Diameter (mm) | Duration (min) |
|---|---|---|---|
| 20 | 60 | 80 | 10 |
| 30 | 60 | 80 | 10 |
| 40 | 60 | 80 | 10 |
| 20 | 80 | 80 | 10 |
| 30 | 80 | 80 | 10 |
| 40 | 80 | 80 | 10 |

**Table 5: COF and Wear of EN8 Steel with Ni-B coating at 60 rpm & 80 rpm**

| Sl.no | Load (N) | Rpm 60 | | Rpm 80 | | Track dia (mm) |
|---|---|---|---|---|---|---|
| | | **COF** | **WEAR** | **COF** | **WEAR** | |
| 1 | 20 | 0.186 | 129.97 | 0.321 | 218.21 | 80 |
| 2 | 30 | 0.188 | 212.02 | 0.439 | 246.10 | 80 |
| 3 | 40 | 0.200 | 233.12 | 0.433 | 300.79 | 80 |

**Table 6: Mass loss of EN8 Steel with Ni-B coating at 60 rpm & 80 rpm**

| SL.NO | Specimen name | Load (N) | RPM 60 | | | RPM 80 | | | Track dia. (mm) |
|---|---|---|---|---|---|---|---|---|---|
| | | | **Initial wt (gm)** | **Final wt (gm)** | **Wt. loss (gm)** | **Initial wt (gm)** | **Final wt. (gm)** | **Wt. loss (gm)** | |
| 1 | Ni-B | 20 | 6.67 | 6.66 | 0.01 | 6.67 | 6.64 | 0.03 | 80 |
| 2 | Ni-B | 30 | 6.67 | 6.64 | 0.03 | 6.67 | 6.63 | 0.04 | 80 |
| 3 | Ni-B | 40 | 6.67 | 6.61 | 0.06 | 6.67 | 6.59 | 0.08 | 80 |

**Table 7: Wear rate of EN8 Steel with Ni-B coating at 60 rpm & 80 rpm**

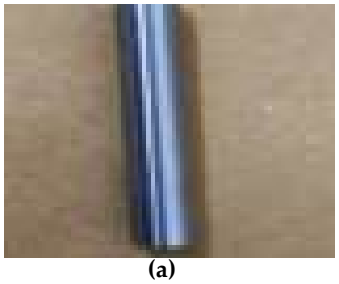
| SL.NO | Load (N) | Wear rate in $mm^3/N\text{-}m$ at 60rpm | Wear rate in $mm^3/N\text{-}m$ at 80rpm |
|---|---|---|---|
| 1 | 20 | $3.31 \times 10^{-6}$ | $7.46 \times 10^{-6}$ |
| 2 | 30 | $6.63 \times 10^{-6}$ | $8.84 \times 10^{-6}$ |
| 3 | 40 | $9.94 \times 10^{-6}$ | $9.94 \times 10^{-6}$ |




**Figure 1: Deposition in Palladium chloride solution**




**Figure 2: Dipping condition of specimen in solution bath**



**(a)**



**(b)**

**Figure 3: Material surface representation (a) before coating and (b) after coating**

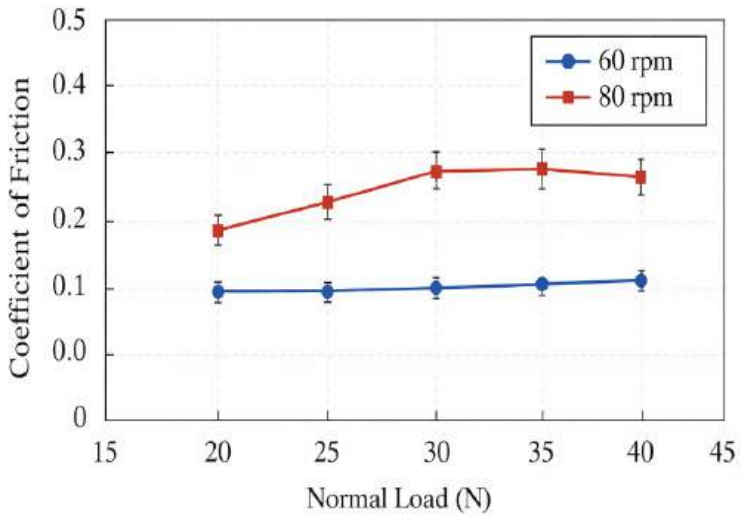



**Figure 4: COF vs Load at 60 rpm and 80 rpm**

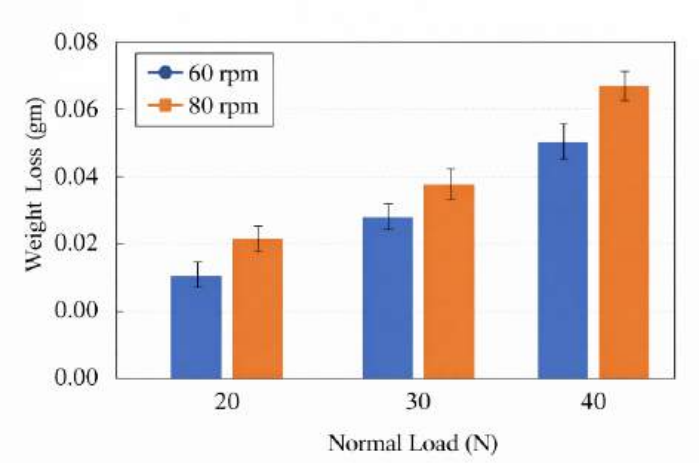



**Figure 5: Mass-Loss vs Load at 60 rpm and 80 rpm**

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RESEARCH ARTICLE

# Co-Even Domination Number of Splitted Graph

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**ABSTRACT**

[7] A dominating set ( D ) of a graph ( (S(G) = (V, E) ) is called a **Co-even dominating set** if for every vertex ( v \in V - D ), the degree of ( v ) is an even number. The **Co-even domination number** of (S(G) ), denoted by ( $\gamma_{coe}$ [S(G)] ), is the minimum cardinality (size) among all Co-even dominating sets of ( S(G) ). "In this paper, we studied the co-even domination number of certain split graphs, namely $(S(P_n))$, $(S(H_n))$, $(S(C_n))$, $(S(P_n^+))$, $(S(P_n oNK_1))$

**Keywords**: Domination, Co-even domination number, Co - even domination set.
**Subject classification:** AMS 05C69, 05C70

## INTRODUCTION

A graph G is a finite non –empty set of objects called vertices together with a set of unordered pair of distinct vertices of G which are called edges. Each pair $e = \{u, v\}$ of vertices in E is called an edge or a line of G in which e is said to join u and v. We write $e = uv$ and say that u and v are adjacent vertices (sometimes denoted as u adjv);vertex u and the edge e are incident with each other, as are v and e. If two distinct edges $e_1$ and $e_2$ are incident with a common vertex, then they are called adjacent edges.

[6]The maximum degree of a vertex in G is denoted by $\Delta(G)$ and the minimum degree of a vertex in G is denoted by $\delta(G)$. "In this paper, we studied the co-even domination number of certain split graphs, namely $(S(P_n))$, $(S(H_n))$, $(S(C_n))$, $(S(P_n^+))$, $(S(P_n oNK_1))$

## PRELIMINARIES

**Definition [7]**

A dominating set ( D ) of a graph ( (S(G) = (V, E) ) is called a **Co-even dominating set** if for every vertex ( v \in V - D ), the degree of ( v ) is an even number. The **Co-even domination number** of (S(G) ), denoted by ( $\gamma_{coe}$ [S(G)] ), is the minimum cardinality (size) among all Co-even dominating sets of ( S(G) ).

**3. $\gamma_{coe} - of\ Splitted$ Graphs Results**

**Theorem 3.1** $\gamma_{coe}[S(P_n)] = n \quad if\ 2 \le n \le 5$

**Proof.** Let S($P_n$) be path splitted graph of $P_n$ . Let V [S($P_n$)] = {$f_i$ , $g_i$: $1 \le i \le n$ }, Let $E[S(P_n)]$ = {($f_i f_{i+1}$) ∪ ($g_i f_{i+1}$) ∪ ($f_i g_{i+1}$) ; 1≤ i ≤ n-1}. In $S(P_n)$ graph, If we take all the $g_i$ vertices, these vertices act as a co- even dominating set. As follows vertex $g_i$ is adjacent to $f_{i-1}$& $f_{i+1}$, by defn., $g_i$ are vertices satisfy the co – even dominating set condition.

Hence, $\gamma_{coe}[S(P_n)] = n$.

Given [ $\gamma_{coe}[S(P_3)]$ = {$g_1$ , $g_2$,$g_3$ } = 3]

**Theorem 3.2** $\gamma_{coe}[S(P_n)] = \left\lceil \frac{n}{2} \right\rceil + 2 \quad if\ n \equiv 1(mod\ 2)\ and\ n \ge 7\ \&\ n\ is\ odd$

**Proof.** Let S($P_n$) be path splitted graph of $P_n$ . Let V [S($P_n$)] = {$f_i$ , $g_i$: $1 \le i \le n$ }, Let $E[S(P_n)]$ = {($f_i f_{i+1}$) ∪ ($g_i f_{i+1}$) ∪ ($f_i g_{i+1}$) ; 1≤ i ≤ n-1}. In $S(P_n)$ graph, we can first select the vertices with odd degree . As $g_1 and\ g_n$ are odd degree vertices. If $n \equiv 0\ (mod\ 4)$ Then the remaining vertices from $f_i$ is adjacent to $f_{i-1}$ , $g_{i-1}$ $and\ g_{i+1}$ where as $f_{i+1}$ is adjacent to $f_{i+2}$ , $g_i$ $and\ g_{i+2}$ . by defn $g_1, g_n, f_i$and $f_{i+1}$ are vertices satisfy the co – even dominating set condition.

If $n \equiv 1\ (mod\ 4)$, $n \ge 7$ the remaining one vertices from $f_i$ ; $(i = n)$ (since $f_n$ vertex is already select , it is enough to select $g_n$ vertex), vertex $f_n$ is adjacent to $f_{i-1}$ , $g_{i-1}$ by defn $f_n$ is vertex satisfy the co – even dominating set condition.

Hence, $\gamma_{coe}[S(P_n)] = \left\lceil \frac{n}{2} \right\rceil + 2$

For example $\gamma_{coe}[S(P_7)]$ = {$g_1$,$g_7$ , $f_2$ , $f_3$,$f_6$ , $f_7$} = 6 .

**Theorem 3.3** $\gamma_{coe}[S(P_n)] = \frac{n+4}{2} \quad if\ n \equiv 0(mod\ 2)\ and\ n \ge 6\ \&\ n\ is\ even$

**Proof.** Let S($P_n$) be path splitted graph of $P_n$ . Let V [S($P_n$)] = {$f_i$ , $g_i$: $1 \le i \le n$ }, Let $E[S(P_n)]$ = {($f_i f_{i+1}$) ∪ ($g_i f_{i+1}$) ∪ ($f_i g_{i+1}$) ; 1≤ i ≤ n-1}. In $S(P_n)$ graph, we can first select the vertices with odd degree . As $g_1 and\ g_n$ are odd degree vertices. If $n \equiv 0\ (mod\ 4)$ Then the remaining vertices for each of four vertices $f_i$ , $g_i$ from $f_i$ is adjacent to $f_{i-1}$ , $g_{i-1}$ $and\ g_{i+1}$ where as $f_{i+1}$ is adjacent to $f_{i+2}$ , $g_i$ $and\ g_{i+2}$ . by defn $g_1, g_n, f_i$and $f_{i+1}$ are vertices satisfy the co – even dominating set condition.

If $n \equiv 2\ (mod\ 4)$, the remaining two vertices of $f_i$ , $g_i$ $(n - 1 \le i \le n)$ (since $f_n$ vertex is already select , it is enough to select $g_n$ vertex), vertex $f_n$ is adjacent to $f_{i-1}$ , $g_{i-1}$ by defn $f_n$ is vertex satisfy the co – even dominating set condition.

Hence, $\gamma_{coe}[S(P_n)] = \frac{n+4}{2}$

For example $\gamma_{coe}[S(P_8)]$ = {$g_1$ , $g_8$ , $f_2$ , $f_3$,$f_6$ , $f_7$,} = 6.

**Theorem 3.4** $\gamma_{coe}[S(H_n)] = n + 6 \quad if\ n \equiv 0(mod\ 2)\ and\ n \ge 6\ \&\ n\ is\ even$

**Proof.** Let V [S($H_n$)] = {$f_i$ , $g_i$,$f_i'$ , $g_i'$ : 1≤ i ≤$n$ },

Let E[S($H_n$)] = {($f_i f_{i+1}$) ∪ ($g_i f_{i+1}$) ∪ ($f_i g_{i+1}$) ∪ ($f_{\frac{n}{2}} f'_{\frac{n+1}{2}}$) ∪ ($f_{\frac{n}{2}} g'_{\frac{n+1}{2}}$) ∪ ($g_{\frac{n}{2}} f'_{\frac{n+1}{2}}$)∪($f_i' f'_{i+1}$)∪ ($g_i' f'_{i+1}$) ∪ ($f_i' g'_{i+1}$); 1≤ i ≤ n-1}.

In S($H_n$) graph, we can first select the vertices with odd degree. As $g_1, g_n$are odd degree vertices. Then the remaining vertices if $n \equiv 0\ (mod\ 4)$ for each of four vertices $f_i$ , $g_i$.

Vertex $f_i$ is adjacent to $f_{i-1}$,$g_{i-1}$,$g_{i+1}$. where as $f_{i+1}$ is adjacent to $f_{i+2}$,$g_i$,$g_{i+2}$. If the remaining $n \equiv 2(mod4)$ two vertices of $f_i$ , $g_i(n - 1 \le i \le n)$. (since $g_n$ vertex is already selected, it is enough to select $f_n$ vertex). Vertex $f_n$ is adjacent to $f_{n-1}$,$g_{n+1}$.

we can first select the vertices with odd degree. As $g_1', g_n'$are odd degree vertices. Then the remaining vertices if $n \equiv 0\ (mod\ 4)$ for each of four vertices $f_i', g_i'$.

Vertex $f_i'$is adjacent to $f_{i-1}', g_{i-1}', g_{i+1}'$. where as $f_{i+1}'$is adjacent to $f_{i+1}', g_i', g_{i+2}'$. If the remaining $n \equiv 2(mod 4)$ two vertices of $f_i', g_i' (n-1 \leq i \leq n)$. (since $g_n'$ vertex is already selected, it is enough to select $f_n'$vertex). Vertex $f_n'$ is adjacent to $f_{n-1}', g_{n-1}'$.

By definition, $g_1, g_n, g_1', g_n', f_i, f_{i+1}, f_n, f_i', f_{i+1}'$ and $f_n'$ are vertices satisfy the co-even dominating set condition.

Hence $\gamma_{coe}[S(H_n)] = n + 6$

For example $\gamma_{coe}[S(H_8)] = \{g_1, g_4, g_8, f_2, f_3, f_6, f_7, g_1', g_5', g_8', f_2', f_3', f_6', f_7'\} = 14$.

**Theorem 3.5.** $\gamma_{coe}[S(H_n)] = n + 5$ *if* $n \equiv 1(mod\ 2)$ *and* $n \geq 5$ & $n$ *is odd* and $n \not\equiv 21(mod\ 20)$

**Proof.** Let V [S($H_n$)] = $\{f_i, g_i, f_i', g_i' : 1 \leq i \leq n\}$,

Let E[S($H_n$)] = $\{(f_i f_{i+1}) \cup (g_i f_{i+1}) \cup (f_i g_{i+1}) \cup (f_{\frac{n}{2}} f_{\frac{n+1}{2}}') \cup (f_{\frac{n}{2}} g_{\frac{n+1}{2}}') \cup (g_{\frac{n}{2}} f_{\frac{n+1}{2}}') \cup (f_i' f_{i+1}') \cup (g_i' f_{i+1}') \cup (f_i' g_{i+1}'); 1 \leq i \leq n-1\}$.

We can first select the vertices with odd degree. As $g_1, g_n$ and $g_{\frac{n+1}{2}}$ are odd degree vertices. Therefore, all the above mentioned vertices will form a co- even dominating set.

If $n \equiv 7(mod\ 4)$ when this $g_{\frac{n+1}{2}}$ vertex is a co-even dominating set, this $H_n$ graph can be divided into equal two parts. The first part contains $f_i$, ( $1 \leq i \leq \frac{n-1}{2}$ ) vertices. Then the first half and the second half can be divided into 3, 5, and 7 ( $f_i$ & $g_i$ ) vertices.

In $_{11}$ graph the first half consists of $_{,}$ ( $1 \leq \ \leq \frac{-1}{2}$ ) vertices and the second half consist of $_{,}$ ( $\frac{+3}{2} \leq \ \leq$ ).

Therefore in this first part $_{+1}, _{+2}, _{+3}$ are vertices and in the second half $_{-3}, _{-2}, _{-1}$ are vertices can be taken as co-even dominating set. If there are 3 (or) 7 vertices in the first half and the second half, then for every four ( ) vertices it is enough to take the two vertices in the middle.

If $n \equiv 5(mod\ 4)$ when this $g_{\frac{n+1}{2}}$ vertex is a co-even dominating set comes, this $H_n$ graph can be divided into equal two sections. The first section contains $f_{i,}$ ( $1 \leq i \leq \frac{n-1}{2}$ ) vertices. "The first half and the second half each contain vertices labeled ( *fi* ) *and* ( *g*i ), with indices ( i = 2, 4, 6, 8 )."

In this $H_{13}$ graph, the first half consists of 6 vertices, in which $f_1$is adjacent to $g_2$, $f_2$.we have already selected $g_1$ is odd degree (co- even dominating set). Therefore, the remaining vertices are $f_3, f_4, f_5, f_6$ form these four ( $f_i, 3 \leq i \leq 6$) vertices, the middle two vertices should be taken. Similarly, the second half consists of 6 vertices, in which $f_{13}$ is adjacent to $g_{12}, f_{12}$. We have already selected $f_{13}$ odd degree (Co-even dominating set). Therefore, the remaining vertices are $f_i$ ($8 \leq i \leq 11$). From these four $f_i$ ($8 \leq i \leq 11$) vertices, the middle two vertices can be taken.

Suppose in the $H_9$ graph, the first half has four $f_i$ ($1 \leq i \leq 4$). from these four vertices, we can take the middle two vertices. Similarly the second half has four $f_i$ ($6 \leq i \leq 8$)vertices. Form these four vertices, we can take the middle two vertices.

Just as the $f, g$ vertices work, the same as process applies to $f', g'$.

Hence $\gamma_{coe}[S(H_n)] = n + 5$

For example $\gamma_{coe}[S(H_{11})] = \{g_1, g_5, g_{11}, f_2, f_3, f_6, f_9, f_{10}, g_1', g_5', g_{11}', f_2', f_3', f_6', f_9', f_{10}'\} = 16$.

**Theorem 3.6.** $\gamma_{coe}[S(H_n)] = 2n$ *if* $2 \leq n \leq 4$

**Proof.** Let V [S($H_n$)] = $\{f_i, g_i, f_i', g_i' : 1 \leq i \leq n\}$,

If n is odd, Let E[S($H_n$)] = $\{(f_i f_{i+1}) \cup (g_i f_{i+1}) \cup (f_i g_{i+1}) \cup (f_{\frac{n}{2}} f_{\frac{n+1}{2}}') \cup (f_{\frac{n}{2}} g_{\frac{n+1}{2}}') \cup (g_{\frac{n}{2}} f_{\frac{n+1}{2}}') \cup (f_i' f_{i+1}') \cup (g_i' f_{i+1}') \cup (f_i' g_{i+1}'); 1 \leq i \leq n-1\}$.

If n is even, Let E[S($H_n$)] = $\{(f_i f_{i+1}) \cup (g_i f_{i+1}) \cup (f_i g_{i+1}) \cup (f_{\frac{n}{2}} f_{\frac{n+1}{2}}') \cup (f_{\frac{n}{2}} g_{\frac{n+1}{2}}') \cup (g_{\frac{n}{2}} f_{\frac{n+1}{2}}') \cup (f_i' f_{i+1}') \cup (g_i' f_{i+1}') \cup (f_i' g_{i+1}'); 1 \leq i \leq n-1\}$.

If we take all the $g_i$ *and* $g_i'$ vertices, these vertices act as a co-even dominating set. By definition, $g_i$ *and* $g_i'$ are co-even dominating set

Hence $\gamma_{coe}[S(H_n)] = 2n$

For example $\gamma_{coe}[S(H_3)] = \{g_1, g_2, g_3, g_1', g_2', g_3'\} = 6$.

**Theorem 3.7.** $\gamma_{coe}[S(H_n)] = n + 7 \quad if\ n \equiv 21\ (mod\ 20)$

**Proof.** Let V $[S(H_n)] = \{f_i, g_i, f_i', g_i' : 1 \leq i \leq n\}$,

Let E$[S(H_n)] = \{(f_i f_{i+1}) \cup (g_i f_{i+1}) \cup (f_i g_{i+1}) \cup (f_{\frac{n}{2}} f'_{\frac{n+1}{2}}) \cup (f_{\frac{n}{2}} g'_{\frac{n+1}{2}}) \cup (g_{\frac{n}{2}} f'_{\frac{n+1}{2}}) \cup (f_i' f'_{i+1}) \cup (g_i' f'_{i+1}) \cup (f_i' g'_{i+1});\ 1 \leq i \leq n-1\}$.

We can first select the vertices with odd degree. As $g_1, g_n$ and $g_{\frac{n+1}{2}}$ are odd degree vertices. Therefore, all the above mentioned vertices will form a co- even dominating set.

For each four $f_i\ (1 \leq i \leq n-1)$ vertices, vertex $f_{i+1}$ is adjacent to $f_{i+2}, f_i, g_{i+2}$ where as vertex $f_{i+2}$is adjacent to $f_{i+3}, f_{i+1}, g_{i+3}, g_{i+1}$. If only one vertex comes last, that vertex can be taken from the co-even dominating set.

Just as the $f, g$ vertices work, the same as process applies to $f', g'$. By definition, $g_1, g_n, g_{\frac{n+1}{2}}, f_{i+1}, f_{i+2}, g_1', g_n', g'_{\frac{n+1}{2}}, f'_{i+1}$ *and* $f'_{i+2}$ are co-even dominating set.

Hence $\gamma_{coe}[S(H_n)] = n + 7$

For example: $\gamma_{coe}[S(H_{21})] = \{g_1, g_{11}, g_{21}, f_2, f_3, f_6, f_7, f_{10}, f_{11}, f_{14}$
$, f_{15}, f_{18}, f_{19}, f_{20}, g_1', g_{11}', g_{21}', f_2', f_3', f_6', f_7', f_{10}', f_{11}', f_{14}', f_{15}', f_{18}', f_{19}', f_{20}'\} = 28$.

**Theorem 3.8** $\gamma_{coe}[S(P_n^+)] = 2n$

**Proof.** Let $S(P_n^+)$ be splitted graph of $P_n^+$. Let V$[S(P_n^+)] = \{f_i, g_i, f_i', g_i' : 1 \leq i \leq n\}$,

Let E$[S(P_n^+)] = \{ [ (f_i f_{i+1}) ;\ 1 \leq i \leq n-1] \cup [ (f_i f_i') \cup (f_i g_i') ;\ 1 \leq i \leq n] \cup [ (g_i f_{i-1}) \cup (g_i f_i') \cup (g_i f_{i+1}) ;\ 2 \leq i \leq n-1] \cup (g_1 f_1') \cup (g_1 f_2) \cup (g_n f_n') \cup (g_n f_{n-1}) \}$

In $S(P_n^+)$ graph, If we take all the $g_i$ & $g_i'$ vertices, these vertices act as a co-even dominating set. As fellows vertex $g_{i+1}$ is adjacent to $f_i, f_{i+2}, f'_{i+1}\ (1 \leq i \leq n)$ where as $g_i$ adjacent to $f_i'(1 \leq i \leq n)$.

Moreover $g_i'$ adjacent to $f_i(1 \leq i \leq n)$. by definition, $g_i$ *and* $g_i'$ satisfy co – even dominating set.

Hence $\gamma_{coe}[S(P_n^+)] = 2n$.

For example: $\gamma_{coe}[S(P_5^+)] = \{g_1, g_2, g_3, g_4, g_5, g_1', g_2', g_3', g_4', g_5'\} = 10$.

**Theorem 3.9.** $\gamma_{coe}[S(P_n o NK_1)] = n\ (1 + N)$.

**Proof.** Let $S(P_n o NK_1)$ be splitted graph of $P_n \circ NK_1$.

Let V$[S(P_n o NK_1))] = \{f_i, g_i, f_{ij}, g_{ij};\ 1 \leq i \leq n,\ 1 \leq j \leq m\}$,

Let E$[S(P_n o NK_1))] = \{ [ (f_i f_{i+1}) ;\ 1 \leq i \leq n-1] \cup [ (f_i f_{ij}) \cup (g_i f_{ij}) \cup (f_i g_{ij}) ;\ 1 \leq i \leq n, 1 \leq j \leq N ] \cup [ (g_i f_{i-1}) \cup (g_i f_{i+1}) ;\ 2 \leq i \leq n-1] \cup [ (g_1 f_2) \cup (g_n f_{n-1}) ] \}$

In $S(P_n o NK_1)$ *graph*, we can first select the vertices with odd degree. As $g_{ij}(1 \leq i \leq n, 1 \leq j \leq N)$ are odd degree and moreover $g_1$ *and* $g_n$ are odd degree. Therefore all the above mentioned will form a co –even dominating set.

Then the minimum co- even dominating set must be selected from the remaining vertices. If $g_i(1 \leq i \leq n)$ vertices are selected from the remaining vertices, the minimum co-even dominating set is obtained. Vertex $g_{i+1}$ is adjacent to $f_i, f_{i+1}, f_{ij}(1 \leq i \leq n, 1 \leq j \leq N)$, By definition, , $g_i, g_{ij}$ all vertices satisfy co – even dominating set.

Hence $\gamma_{coe}[S(P_n o NK_1)] = n\ (1 + N)$

For example: $\gamma_{coe}[S(P_4 o 2K_1)] = \{g_{11}, g_{12}, g_{21}, g_{22}, g_{31}, g_{32}, g_{41}, g_{42}, g_1, g_2, g_3, g_4\} = 12$.

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RESEARCH ARTICLE

# Autoimmune Marker and Systemic Inflammation in Subclinical Hypothyroidism : A Case - Control Study

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## ABSTRACT

Anti-cyclic citrullinated peptide (anti-CCP) antibodies highly specific markers of autoimmune activity primarily studied in rheumatoid arthritis have recently been implicated in other autoimmune conditions including thyroid disorders. In a case-control study of 70 patients with subclinical hypothyroidism (TSH > 5 mIU/L, normal FT4) and 70 age and sex-matched euthyroid controls, anti-CCP antibodies were measured by ELISA alongside inflammatory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Anti-CCP positivity was significantly higher in subclinical hypothyroidism cases (22.5%) compared to controls (5%, $p < 0.01$) and anti-CCP positive patients exhibited elevated CRP (5.8 ± 1.9 mg/L vs. 3.2 ± 1.1 mg/L) and ESR (26 ± 8 mm/hr vs. 14 ± 6 mm/hr, $p < 0.01$) compared to anti-CCP negative cases. Regression analysis confirmed anti-CCP positivity as an independent predictor of systemic inflammatory burden. These findings suggest that anti-CCP antibodies may serve as a novel

biomarker linking thyroid autoimmunity with systemic inflammation in subclinical hypothyroidism warranting further longitudinal studies.

**Keywords:** Autoimmune hypothyroidism, Hashimoto's thyroiditis, Anti TPO antibodies, Anti TG antibodies, Anti CCP antibodies, Rheumatoid arthritis, Citrullination, Autoimmune overlap syndromes, Systemic inflammation, Biomarkers.

## INTRODUCTION

Subclinical hypothyroidism (SCH) is defined biochemically by elevated thyroid-stimulating hormone (TSH) with normal free thyroxine (FT4) levels. It is frequently associated with autoimmune thyroiditis, particularly Hashimoto's thyroiditis, which remains the most common cause of hypothyroidism worldwide (1,2). Hashimoto's thyroiditis is characterized by lymphocytic infiltration, progressive follicular destruction and the presence of thyroid autoantibodies most notably anti-thyroid peroxidase (anti-TPO) antibodies(3,4). While anti-TPO antibodies are well-established markers of thyroid autoimmunity other autoantibodies may contribute to systemic immune dysregulation. Anti-cyclic citrullinated peptide (anti-CCP) antibodies, highly specific for rheumatoid arthritis (RA) are known to predict disease severity and erosive progression(5,6). Interestingly anti-CCP positivity has also been reported in non-rheumatic autoimmune conditions including systemic lupus erythematosus, Sjögren's syndrome and autoimmune thyroid disease(7–9). Their presence may reflect shared mechanisms of autoimmunity such as citrullination-driven neoantigen formation and chronic inflammatory activation(10,11).

Systemic inflammation in SCH has been documented through elevated C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) even in the absence of overt hypothyroidism(12–14). CRP is a sensitive marker of acute-phase response(15) while ESR reflects chronic inflammatory activity(16). Both markers have been widely used in autoimmune and endocrine disorders to assess inflammatory burden. The clinical significance of these findings is considerable. SCH has been linked to cardiovascular risk, metabolic syndrome and polyautoimmunity(17–19). Rotondi and Chiovato emphasized that thyroid autoimmunity should be considered within the broader context of systemic inflammation(20) while Ruggeri et al. highlighted that systemic inflammatory activity in autoimmune thyroid disease may influence disease progression and therapeutic outcomes(21). Tang et al. further demonstrated that CRP and TSH together may act as risk factors in thyroiditis(22).

Moreover, studies have shown that anti-TPO antibody positivity correlates with inflammatory markers in SCH(23) and environmental and genetic factors play a role in autoimmune thyroid disease susceptibility(24). Cytokine-mediated immune responses also contribute to thyroid autoimmunity(25). Importantly, cardiovascular risk has been linked to both RA and thyroid autoimmunity, suggesting overlapping inflammatory pathways (26). Autoantibodies in autoimmune diseases, including thyroiditis may therefore serve as predictors of systemic involvement(27–29). The potential association between anti-CCP antibodies and inflammatory markers in SCH remains underexplored. Identifying such a relationship could provide insights into the systemic nature of thyroid autoimmunity and its overlap with other autoimmune pathways.

## MATERIAL AND METHODS

This case–control study was conducted on a total of 140 individuals, comprising 70 patients diagnosed with subclinical hypothyroidism (SCH) and 70 age and sex-matched healthy controls. Participants were recruited from the outpatient clinics of the Department of Endocrinology.

**The inclusion criteria for the SCH group were:**

a) Age between 18 and 50 years.
b) Newly diagnosed cases of subclinical hypothyroidism, defined as elevated serum thyroid-stimulating hormone (TSH) levels with normal free triiodothyronine (FT3) and free thyroxine (FT4) concentrations.
c) No prior history of thyroid hormone replacement therapy or immunosuppressive treatment.

**Exclusion criteria included:**

a) Overt hypothyroidism or hyperthyroidism.
b) Pregnancy or lactation.
c) Presence of autoimmune diseases other than thyroid autoimmunity.
d) Chronic inflammatory disorders, recent infections, or any systemic illness known to influence thyroid function or inflammatory markers.
e) Use of medications that could interfere with thyroid function or immune response.

**Healthy controls:-** were selected from the same demographic population, matched for age and sex and confirmed to have normal thyroid function tests and no history of autoimmune or chronic inflammatory disease.

Ethical Considerations

The study protocol was reviewed and approved by the Institutional Ethics Committee (Ref. No. SMC/UECM/2024/914). Written informed consent was obtained from all participants prior to enrollment.

Sample Collection and Processing

Venous blood samples (5 mL) were collected from each participant under strict aseptic precautions using sterile vacutainers. Samples were allowed to clot at room temperature and subsequently centrifuged at 3000 revolutions per minute (rpm) for 10 minutes. The separated serum was aliquoted into sterile cryovials and stored at –20 °C until further biochemical and immunological analysis.

Laboratory Investigations

Statistical Analysis

Correlation analysis performed using Pearson's correlation coefficient to assess relationships between:

- Anti-CCP and TSH
- Anti-CCP and hsCRP
- Anti-CCP and ESR

Statistical significance was defined as $p < 0.05$.

## RESULTS

In the case group (n = 70). The mean serum triiodothyronine (T3) level was 1.61 ± 0.60 ng/mL, with a median of 1.5 ng/mL. Thyroxine (T4) levels averaged 102.80 ± 23.20 nmol/L (median: 98.5 nmol/L), while thyroid-stimulating hormone (TSH) levels were elevated, with a mean of 8.58 ± 1.48 μIU/mL and a median of 8.6 μIU/mL, suggesting a trend toward hypothyroid function. Anti-cyclic citrullinated peptide (Anti-CCP) antibodies averaged 17.77 ± 7.59 U/mL (median: 16.4 U/mL), indicating active autoimmune involvement.

High-sensitivity C-reactive protein (hsCRP) levels averaged 14.22 ± 3.50 mg/L (median: 14.4 mg/L), and erythrocyte sedimentation rate (ESR) was 28.28 ± 5.53 mm/hr (median: 30 mm/hr), reflecting a heightened systemic inflammatory state.

All parameters showed statistically significant deviations from reference norms, with P values < 0.0001, indicating strong evidence of altered thyroid function, autoimmune activity, and inflammation in the case group.

In the case group (n = 70), thyroid-stimulating hormone (TSH) levels were elevated, with a mean of 8.58 ± 1.48 μIU/mL and a median of 8.6 μIU/mL. Anti-cyclic citrullinated peptide (Anti-CCP) antibody levels also showed elevation, with a mean of 17.77 ± 7.59 U/mL and a median of 16.4 U/mL. Pearson correlation analysis revealed a statistically significant moderate negative association between TSH and Anti-CCP levels (R = –0.279, P = 0.020).

In the case group (n = 70), anti-cyclic citrullinated peptide (Anti-CCP) antibody levels averaged 17.77 ± 7.59 U/mL with a median of 16.4 U/mL, while high-sensitivity C-reactive protein (hsCRP) levels showed a mean of 14.22 ± 3.50 mg/L and a median of 14.4 mg/L, indicating elevated autoimmune and inflammatory activity, respectively. Pearson correlation analysis revealed a weak positive association between Anti-CCP and hsCRP levels (R = 0.178), which did not reach statistical significance (P = 0.144).

In the case group (n = 70), anti-cyclic citrullinated peptide (Anti-CCP) antibody levels had a mean of 17.77 ± 7.59 U/mL and a median of 16.4 U/mL, while erythrocyte sedimentation rate (ESR) values were elevated, with a mean of 28.28 ± 5.53 mm/hr and a median of 30 mm/hr, indicating ongoing systemic inflammation. Pearson correlation analysis revealed a very weak negative association between Anti-CCP and ESR levels (R = –0.020), which was not statistically significant (P = 0.867).

## DISCUSSION

Our study demonstrates that anti-cyclic citrullinated peptide (anti-CCP) antibody positivity is significantly more prevalent in patients with subclinical hypothyroidism (SCH) compared to euthyroid controls and is associated with elevated systemic inflammatory markers. These findings expand the understanding of thyroid autoimmunity by suggesting that anti-CCP antibodies, traditionally considered highly specific for rheumatoid arthritis (RA) may also play a role in non-rheumatic autoimmune conditions such as autoimmune thyroid disease (30, 8). The elevated anti-CCP levels observed in SCH patients support the hypothesis that citrullination-driven neoantigen formation contributes to immune dysregulation across multiple autoimmune disorders (31, 11). Systemic inflammation in SCH has been well documented, with increased C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) even in the absence of overt hypothyroidism (13, 14). In this study, anti-CCP positive patients exhibited significantly higher CRP and ESR values compared to anti-CCP negative cases, reinforcing the concept that thyroid autoimmunity is not confined to the thyroid gland but reflects a systemic inflammatory state (32, 33). These findings align with previous reports linking SCH to cardiovascular morbidity and metabolic syndrome (17, 2).

Correlation analysis revealed a moderate negative association between TSH and anti-CCP levels suggesting that higher autoimmune activity may coexist with relatively lower TSH elevations. This observation highlights the complex interplay between thyroid function and systemic immune activation, and may indicate that anti-CCP positivity identifies a subset of SCH patients with distinct immunological profiles. Interestingly anti-CCP showed only weak non-significant correlations with CRP and ESR implying that while anti-CCP positivity marks heightened inflammatory burden it may not directly scale with the magnitude of CRP or ESR elevation. Similar dissociations have been reported in autoimmune thyroiditis, where autoantibody positivity does not always parallel inflammatory marker levels (9, 22). The clinical implications of these findings are considerable. SCH has been increasingly recognized as a condition with systemic consequences, including cardiovascular risk and polyautoimmunity (2, 26). Identifying anti-CCP positivity in SCH patients may help stratify risk, guiding closer monitoring and potentially earlier intervention. Moreover, the overlap between RA-associated biomarkers and thyroid autoimmunity underscores shared inflammatory pathways, raising the possibility of common therapeutic strategies. This is consistent with the broader literature emphasizing that autoantibodies in autoimmune diseases may serve as predictors of systemic involvement (33, 34). Nevertheless, this study has limitations. Its cross-sectional design

precludes causal inference, and longitudinal studies are needed to determine whether anti-CCP positivity predicts progression to overt hypothyroidism or the development of comorbid autoimmune conditions. The sample size, although adequate for initial associations may limit generalizability. Furthermore, cytokine profiling and endothelial function assessment could provide deeper mechanistic insights into the link between anti-CCP antibodies and systemic inflammation in thyroid autoimmunity[2]. Future research should explore whether anti-CCP positivity in SCH patients predicts cardiovascular outcomes or polyautoimmunity, thereby refining risk stratification and management strategies.

## CONCLUSION

Our study highlights that anti-cyclic citrullinated peptide (anti-CCP) antibody positivity is significantly more common in patients with subclinical hypothyroidism (SCH) compared to euthyroid controls and is associated with elevated systemic inflammatory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). These findings suggest that anti-CCP antibodies, traditionally considered specific for rheumatoid arthritis, may also serve as a novel biomarker in thyroid autoimmunity, reflecting broader systemic immune activation. The moderate negative correlation between TSH and anti-CCP levels indicates a complex interplay between thyroid function and autoimmune activity, while the weak associations with CRP and ESR highlight that anti-CCP positivity may identify a distinct subset of patients with heightened inflammatory burden. Clinically, this underscores the importance of considering SCH not merely as a biochemical abnormality but as a condition with systemic consequences, including cardiovascular risk and polyautoimmunity. Although limited by its cross-sectional design, the study provides a foundation for future longitudinal research to explore whether anti-CCP positivity predicts progression to overt hypothyroidism, cardiovascular outcomes, or comorbid autoimmune diseases. Integrating anti-CCP testing into the evaluation of SCH may enhance risk stratification and guide more personalized management strategies.

**Limitations and Future Directions**

This study has several limitations that should be acknowledged. First, the cross-sectional design restricts the ability to establish causality between anti-CCP antibody positivity and systemic inflammation in subclinical hypothyroidism (SCH). Longitudinal studies are required to determine whether anti-CCP positivity predicts progression to overt hypothyroidism, cardiovascular complications, or the development of other autoimmune conditions. Second, the sample size, although adequate for initial associations, may limit the generalizability of the findings to broader populations, particularly older individuals or those with comorbidities. Third, the study focused primarily on conventional inflammatory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), without incorporating cytokine profiling or advanced immunological assays, which could provide deeper mechanistic insights into the pathways linking anti-CCP antibodies with thyroid autoimmunity. Fourth, environmental and genetic factors known to influence autoimmune thyroid disease susceptibility were not assessed, which may have contributed to variability in immune responses. Finally, cardiovascular outcomes were not directly evaluated, despite the established link between systemic inflammation, thyroid autoimmunity, and cardiovascular risk.

Future research should aim to address these limitations by conducting large-scale, multicenter longitudinal studies to evaluate the prognostic value of anti-CCP antibodies in SCH. Incorporating cytokine panels, endothelial function markers, and genetic susceptibility analyses would help clarify the immunopathogenic mechanisms underlying the observed associations. Additionally, exploring the role of anti-CCP positivity in predicting cardiovascular outcomes and polyautoimmunity could refine risk stratification and guide personalized management strategies. Integrating anti-CCP testing into routine evaluation of SCH, alongside traditional thyroid autoantibodies, may ultimately enhance clinical decision-making and improve patient outcomes.

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**Table 1.**

| Parameters | Cases (n=70) Mean ± S.D. | Controls (n=70) Mean ± S.D. | P value |
|---|---|---|---|
| **T3** | 1.61 ± 0.60 | 2.45 ± 0.55 | <0.0001 |
| **T4** | 102.80 ± 23.20 | 128.60 ± 20.10 | <0.0001 |
| **TSH** | 8.58 ± 1.48 | 2.40 ± 0.80 | <0.0001 |
| **Anti - CCP** | 17.77 ± 7.59 | 6.20 ± 2.80 | <0.0001 |
| **hsCRP** | 14.22 ± 3.50 | 4.10 ± 1.60 | <0.0001 |
| **ESR** | 28.28 ± 5.53 | 12.40 ± 3.10 | <0.0001 |

**Table 2.**

| Test Name | Case (n = 70) | | | |
|---|---|---|---|---|
| | Mean ± S.D | Median | R Value | P value |
| **TSH** | 8.58 ± 1.48 | 8.6 | **-.279*** | 0.020 |
| **Anti - CCP** | 17.77 ± 7.59 | 16.4 | | |

**Table 3.**

| Test Name | Case (n = 70) | | | |
|---|---|---|---|---|
| | Mean ± S.D | Median | R Value | P value |
| **Anti - CCP** | 17.77 ± 7.59 | 16.4 | **0.178** | 0.144 |
| **hsCRP** | 14.22 ± 3.50 | 14.4 | | |

**Table 4.**

| Test Name | Case (n = 70) | | | |
|---|---|---|---|---|
| | Mean ± S.D | Median | R Value | P value |
| **Anti - CCP** | 17.77 ± 7.59 | 16.4 | **-0.020** | 0.867 |
| **ESR** | 28.28 ± 5.53 | 30 | | |

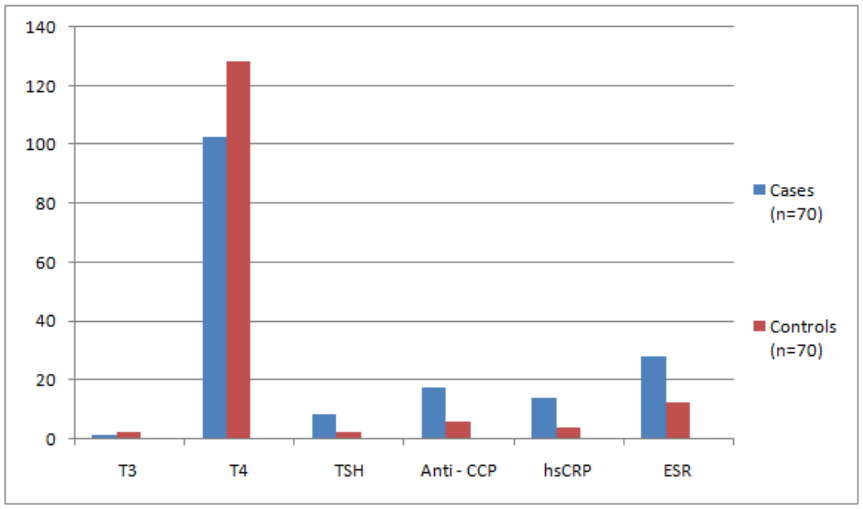



**Fig.1.**

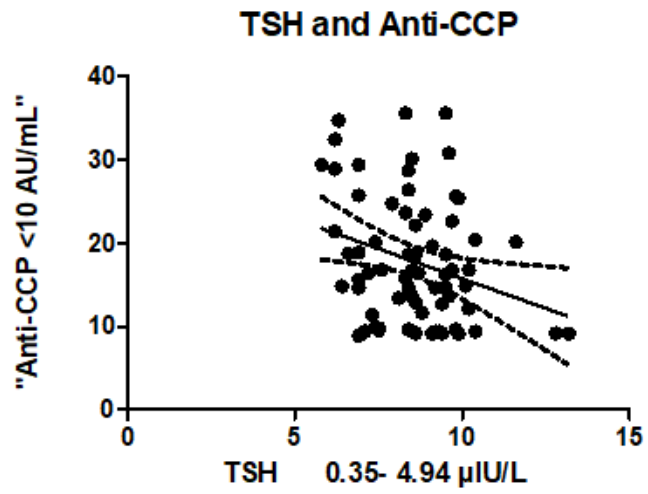



**Fig. 2.**

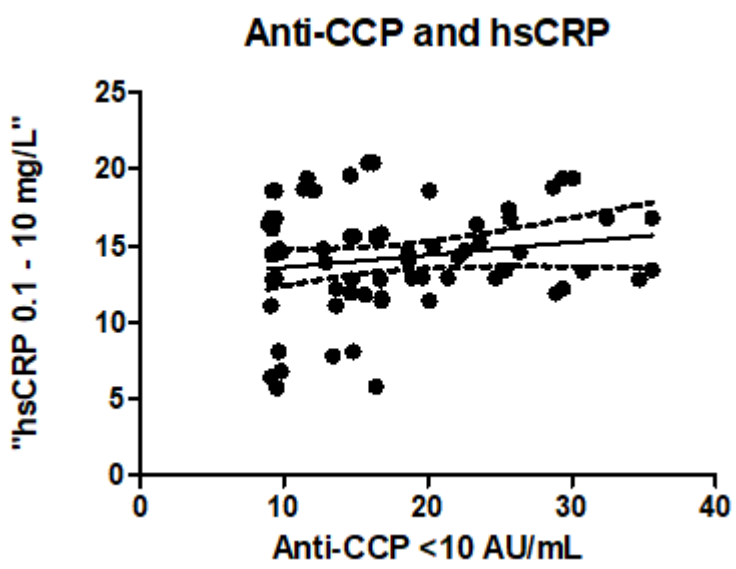



**Fig. 3.**

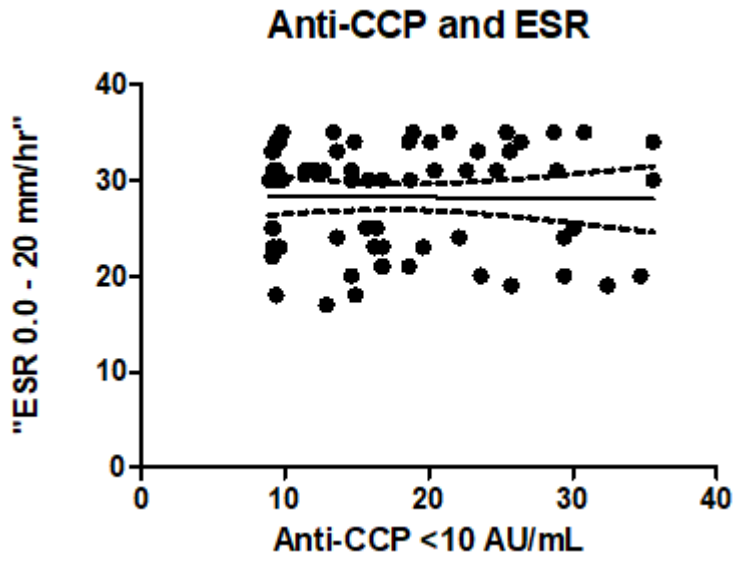



**Fig. 4.**

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RESEARCH ARTICLE

# Shifting Agriculture Induced Forest Fragmentation Dynamics in Kalyani River Basin, Karbi Anglong Assam, India

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## ABSTRACT

Resource management should begin at micro spatial units as mankind has gradually evolved observing, learning and using nature and its resources to satisfy their requirements at micro spatial scale i.e. immediate surroundings. Deterioration, destruction, degradation of natural ecological units at micro spatio-temporal scale is cause of decline in global biodiversity and ecosystem obliterations at large. The Kalyani Reserve Forest (KRB), regarded among the richest biodiversity zones in India is impacted in terms of human introduced land cover dynamics which leads to forest fragmentation and shrinking of total forest area .The chief reason for forest fragmentation in this region is due to shifting agriculture. It is observed and estimated from a two decade (2000-2020) change detection analysis of forest cover using vegetation indices like NDVI,AVI, BSI and finally Forest Canopy Density (FCD) that a reduction of 12.44 .% of total forest cover has taken place with the rate of shrinking forest area estimated at 0.62km²/year estimated for twenty years .The chief effort should be to restore ecological balance by restoring soil fertility through sustainable methods. This paper attempts to analyze, quantify and discuss the change in land cover and subsequent deterioration due to forest canopy fragmentation which begin at microspatial scales chiefly due to clearing forest floor for shifting agriculture. The estimation and assessment of shrinking forest canopy will help to understand the deterioration to floral and faunal habitats which on a macro scale contribute to depleting forest areas .It will alternatively help to suggest appropriate means of livelihoods for the indigenous people residing here in the natural environment that will safeguard their traditions.

**Key Words:** landcover change, degradation, jhum cultivation, forest ecosystems,landuse

# INTRODUCTION

Forest fragmentation reduces forest space and resources.It endangers ecosystems contributing to global degradation and reduction of forest cover limiting the scope for carbon sequestration. This poses a major challenge for natural ecosystems conservation efforts and controlling air pollution.Land cover change is a dynamic process which can be explained as a break or interruption over land inhabited by generally similar natural vegetation or pedological character as a result of a particular temperature and moisture conditions. For any assimilation or estimation of forest canopy cover degradation,quantitative methods using vegetation indices as NDVI,AVI and BSI change detection is one of the most essential parameter which can be assessed quantitatively to indicate scales of degradation in Forest Canopy Cover(FCD). This helps in formulation of conservation/ restoration strategies to safeguard habitats. Land cover change is the initiation of change in natural floral coverage over surface either by physical causes or by actions of anthropogeny for economic, demographic orpolitical(wars/conflict zones) reasons.Land use is the use or management of land which is characterized by natural entities like forest, water body,grassland, wetland, desert terrain and utilize it for anthropogenic purposes giving economic benefits.

**Objectives:**

This study attempts to assess in the study area:

i) the areal extent of change in FCD
ii) to classify the pattern of forest fragmentation of KRB.

**Study area:**

The Kalyani River Basin(KRB) with a total catchment area of 1254 sq.km is located between 26° 13' 47" N 26° 40' 38" N longitudes and 93° 12' 28" E - 93° 12' 48" E latitudes. A part of the Kalyani Reserve Forest to the east of the Mikir Hills in Assam isa part of the KarbiAnglong district (Diphu subdivision)of Assam, India.The"the general elevation ranges from 100 m (Kamargaon) mouth,to1281 m (InglangIkpi)"(Mukherjee 2012).There are a large number of non-tribalsand tribals who live together in this hill region. The tribalethnic groups are Dimasas, Garos, Karbis, Tiwas, Hmars, Nagas, Kukis, Bodos, Rengma,Man(Tai speaking) Fig.1. The climate of the region is characterized withcold winters and humid summers as prevalent in tropical regions .The cold months are from October to February .Summers are from March to June .The temperature ranges from 6° to 11° in winter 22° to 31 °Celsius in summer. The monsoons are experienced betweenJuly to September with the average rainfall about 210 cms .Thenatural vegetation of the region was extensively classified and documentedby Champion and Seth in 1968. Following their classification the study area is covered by dense tropical evergreen forest alternated by moist deciduous varieties with moist bamboo brakes (*Dendrocalamus hamiltonii, Bambusa pallida,* and *Melocanna bambusoides* are commonly found in the area. The study region is characterized by moist deciduous forest species such as Tetrameles nudiflora, *Duabanga sonneratioides, Lagerstroemia parviflora, Careya arborea, Kydia calycina, Gmelina arborea, Albizia procera, Terminalia myriocarpa, various Bamboo species, Terminalia tomentosa, Shorea robusta,* and *Dillenia pentagyna* (Roy et al., 1991).The pedology of the region consists of new alluvial soil derived from the weathering of gneissic terrain. The soils are generally well developed. Due to heavy rainfall, significant leaching occurs, resulting in acidic soils. In general, the soils are very deep, except on hilltops (Roy et al., 1991).

# MATERIALS AND METHODS

Topographical sheets(SoI) 83F/3, 83F/6 , 83F/7, 83F/8, 83 F/10 , 83F/11, 83F/14 and 83F/15 of scale1:50,000 ,satellite data, secondary data on agriculture,climate, demography,and published researches on the present status of shifting cultivation(2000 –2021)were gone through.An attempt has been made to reconstruct the land cover alteration due tofragmentation in the forested regions of KRB that reveal disruption/perforation of forest cover within the study area. New satellite data sets reveal at high resolution how human activities are gradually and irreversibly,transforming forest space over a temporal span.The forest canopy cover alterations through satellite

imageries are clearly and easily noticed due to sharp contrast in tone and texture between dense vegetation cover in forests and anthropogenic land cover types/afforested areas having interrupted canopy and comparatively more exposed bare soil.*"The present study was carried out with an attempt to analyze the change in LULC and forest fragmentation using satellite data"*(Shimrah et al.,2021)

**Data sets**

NDVI analysis for preparationof LULC map is from satellite imageries of LANDSAT 5,7 & 8 and resolution -30 meters (Table 1).The satellite imagery are from Landsat 4-5 (TM), 7 (ETM+) & 8 (OLI) March 2000 - February 2020.They were observed to assess the status of forest cover in the study area. Usually satellite images captured during winter have a clear spectral reflectance and are not obstructed by cloud cover.*Image preprocessing attempts to minimize these effects to the extent desired for a particular application* (Young et al, 2017).*The images that are used in this study were atmospherically corrected and converted to Top of Atmosphere (TOA) radiance using the following formula –*

$L\lambda = MLQcal + AL$

*where,*

*Lλ= TOA spectral radiance (Watts/( m2× srad×μm)).*

*ML= Band-specific multiplicative rescaling factor from the metadata.*

*AL= Band-specific additive rescaling factor from the metadata.*

*Qcal= Quantized and calibrated standard product pixel values (DN)*.(Figure 2) (Awad2019,Patra etal2022)

The maps in this study have been done using Arc GIS 10.5 software.*"Accurate and periodic mapping of the shifting cultivation areas is vital for assessing their spatio-temporal dynamics for effective management of land use, and more so for the jhum-dominated landscape"*(Das et al, 2021,Patra etal2022). The analysis was done for satellite data at five years interval for the temporal window 2000-2020 evaluate the change in spatial patternsof shifting cultivation areas as "*significant relative differences in surface reflectance values of vegetation, and used to derive shifting cultivation patches*."(Das et,al 2021,Patra et al 2022)where estimated .Vegetation Indices (VI) have been prepared to analyze forest cover. *Most two-band VIs make use of reflectances in red and NIR bands"*(Chen 2018).The analysis of Land Use/ Land Cover (LULC) was carried out using NDVI datasets for the years 2000, 2005 , 2010, 2015 and of 2020 .The pattern and extent of spatial shrinkage over a temporal scale was identified for forest and non forest covers.The spatio-temporal assessment of forest pattern using multi temporal satellite data over a span of two decades (2000-2020), 3 vegetation indices (Table 2 and LFT (v.0.2) developed by CLEAR (Univ.of Connecticut)within the KRB to assess forest canopy density status and identify the patternof fragmentation of forest canopy cover. The region is a part of the KarbiAnglong plateau and covered by dense evergreen and moist deciduous forest specie similar to lower Himalayan mountain regions.Physiographically it is a plateau and exhibits a granitic rock surface character. Usually forests on "*Mountainous areas contain some of the best-preserved natural habitats, but they are threatened by changes in land use and climate* "(Thuiller et al 2005).Dry bare soil has higher reflectance in shortwave IR and humid bare soil has low reflectance due to presence of soil moisture. "*Bare soil index is an indication of how much land is disturbed /cultivated or used by anthropogeny.It also indirectly reflects that how much forest canopy cover is there in a specific time span*" (Rikimaru and Miyatake 1997,Bera 2020).The bare soil spatial units within the forest are cleared areas or those left fallow after cropping .*"Over the years, Northeast India has experienced major changes in LULC and forest fragmentation.There are limited information and data regarding the change in LULC patterns and causes of forest fragmentation"*(Shimrah et al.,2021)

## RESULTS

The study was attempted to assess land cover change dynamics due to natural (forest fires, floods, earthquakes etc.) as well as anthropogenic causes (agriculture, infrastructure development extraction of tree stands for economic reasons etc.), specifically like shifting agriculture because it leads to habitat and forest fragmentation. According to McGarigal et al. 2005 – "habitat fragmentation is a process of landscape change and not just a patch level process only". This type of land use practice is generally prevalent amongst human communities that cultivate on a subsistence basis on hill slopes and requiring minimal soil moisture. "Shifting cultivation tends to persist when population density is low and when options for agricultural development or alternative livelihoods are limited"

(Mertz 2002,2009, Heinimann et al 2013,2017).Therefore such agricultural practice endangers flora and fauna of the region and is a silent threat to ecology, stripping the forest zone of natural vegetative cover and accelerating forest environment moisture depleteon, soil moisture loss and soil organic content deteoraration. The detrimental practice generates patches of land that become open systems ,develop interrupted food chains and ecosystems in spatio-temporal effect. The edge /overlap region between dense forest cover and cleared areal units are affected by heat which impacts biological life within the adjacent areal extension of forest. They also bear major duress and distress due to population ingress in its peripheral vicinity for collection of firewood, extricating bamboo for sale, medicinal plants, hunting of animals, food supplements and any other forms of supplemental gratification for living .The LULC change analysis using NDVI with ground truth verification on field have been carried out to corroborate the analysis

## DISCUSSIONS

It is observed that with an increase in water bodies, built up area ,barren land, shrub and grassland proliferation, sparse vegetation in abandoned shifting agriculture patches within the forest there has been a gradual shrinking in dense vegetation area along the banks of lower course of the Kalyani river as observed in NDVI for 2000 to 2005(Fig.2) With the immediate consequence of forest loss and increase in size of anthropogenic land cover ( example, agricultural fields and urban areas,transport corridors etc.),the remnant forests edges are most impacted.The edge is a zone of temperature buffer zone with exposed terrains and forested land having noticeable land surface temperature contrasts.To clearly understand the dynamics of forest canopy density and its fragmentation, simply NDVI alone does not address all changes. Those areas characterised by shifting agriculture have highpresence of aerosols due to burning of slashed foliage , fallow land have exposed soil cover reclaimed by shrubs and coarse grasses for areas.This is further validated by using vegetation estimation indices to carry out this study.

**Advanced Vegetation Index (AVI )**

Advanced Vegetation Index (AVI) is used in vegetation studies to assess forest variations over a temporal frame. It is almost similar to NDVI, and uses the red and near-infrared spectral bands. Like NDVI, AVI can be used for vegetation studies to detect forest cover variations over time . The canopy displays a distinct yellow orange tone due to drying of leaves during winter for 2000. This is a suitable index as some of the slopes in the study area are covered by tea and rubber plantations and the canopy pattern helps to identify natural and cultural entities. The AVI for the areal coverage in the medium class is 381.60 km² for 2000 and 414.33 km² 2020.(Table 3)Fig.5&6.

**Bare soil index (BSI)**

It is an indication of how much land is disturbed /cultivated or used by anthropogeny. It also indirectly reflects that how much forest canopy cover is there in a specific time span" (Rikimaru and Miyatake 1997, Bera 2020) Dry bare soil has higher reflectance in shortwave IR and humid bare soil has low reflectance due to presence of soil moisture. It is observed (Table 5) that the threshold values of BSI for the study area in the low range(0.089-0.060) occupies maximum area of 485.56 sq.km i.e 38.72% of the total basin area in 2000 and remains nearly the same in 2020 , indicating dense vegetation cover for a larger part of the basin .While in the very high threshold range ( 0.016-0.158 ) covers only 63.69 sq.km in 2000 and a slight increase in threshold values (0.026 -0.376)covers 81.01 sq.km in 2020 within the basin area. The Landsat 8 OLI data ( path 136 row 042 resolution 30 metres) of 2020 shows bare patches which are distinguishingly different from the dense forested areas . In the eastern part it is observed there are cleared land for sedentary agriculture and ground validation showing seasons of fallow during summer , transport corridors (NH35) and built up patches. These built up areas can be classified into primary bare land with incidences of slope failure (seen as broad teardrop shapes and along slope edges ).Anthropogenic intrusion along the Kalyaniriver , in patches within the basin are observed in square or rectangular polygons for shifting cultivation. They are usually near villages to facilitate easy access and the middle to lower courses of tributaries for being characterized by gentle slopes . Requirement for water is minimal as cultivation is chiefly rain dependent. The other

type can be classified as secondary bare land with fallow and built up area. Bare soil plays a significant role in any ecosystem by accelerating top soil loss through wind or water.Table 4. Fig.7&8

**Forest Canopy Density(FCD)for KRB-Assam**

Forest Canopy Density (FCD) refers to the proportion of an area in the field/ground that is covered by the crown of trees and is expressed in percentage of the total area."*Forest Canopy Density (FCD) is a biophysical analysis model that can be used to analyze a forest canopy density changes*" (Himayah et al 2016).The identified forest cover estimation was obtained as per Forest Canopy Density. (Fig.9&10)Shifting cultivation is identified to have two categories i.e freshly cleared and the other being fallow or abandoned."*Abandoned shifting cultivation with heavy growth of shrubs on hills is classed separately. Whereas current shifting cultivation, non-forest, barren hills and woodland less than 10 percent tree crown density*" (Roy et al 1991) are classed separately.

**The extent of Forest Fragmentation for two decades in KRB : ( 2000 – 2020 )**

The Very Dense Forest (VDF) area occupies 903. 01 km² i.e 72 % in the western part and south western part, Moderately Dense Forest (MDF) occupies 266.19 km² i.e 21.23 in the central and north central and southern part,Open Forest (OF) 76.90 km² i.e 6.15 % in the eastern part and Non-Forest (NF) 7.90 km² i.e 0.63%, of the north eastern part of the KRB area ( Fig.11) These classes were derived from the Forest Canopy Density model. According to the forest classification map five classes have been designated with Very High and High class being considered under VDF. VDF class dominates primarily and cultural features are found on the far eastern side of the study area. However there has been a decrease of 132.17 km² i.e 10.54 %, which is nevertheless nominal- an indicator of slow depletion of forested area. The rate is estimated to be at 6.61km²/year. TheKalyani river flowing west to north east through the basin splits it into two halves and the banks experience the Edge Effect whereby the immediate banks offer scope for human habitation and livelihoods through farming and extraction of bamboo .

Haddad et. al (2015) and Sexton (2013)analyzed the world's first high-resolution map of global tree cover to measure the magnitude of forest fragmentation."*This analysis revealed that nearly 20% of the world's remaining forest is within 100 m of an edge—in close proximity to agricultural,urban,orother modified environments where impacts on forest ecosystems are most severe"* (Laurance et al 2002) In the present millennium, use of geographical knowledge together with geospatial analysis is essential not only to estimate and combat climate change and landscape transformation but understand the interrelation,estimate the impact and find solutions for development of proper land use patterns.

**Core** – The VDF zone can suitably be designated as the core area of the forest covering an area of 903.01 sq.km which are seen as pixels suitably away from the non forest,open forest and barren land (orange and yellow) pixels.This class comprising Very high and High classes together has reduced by 132.17 sq.km i.e 6.61 sq km/year. (0.53% per year of the total forest area).

**Perforated** – They comprise of openings in the canopy just initiated by forest clearing for shifting agriculture or clearing pure bamboo stands. (Photo plate 7).They are observed as orange colouredpixels,usually in clusters and found more on the left bank slopes along the lower middle course of the Kalyani river. The moderate forest zone has seen an increase of 86.33 sq.km ( 4.32 sq.km /year) ie 0.34% per year.This is the only class which is increasing in areal expanse in the study area.

**Patch** – They are isolated points not far from the non forestzone and display edge effect. Open land with none of the original species of natural vegetation is found on ground validation. They cover a total area of 183 sq.km in KRB. They have maximum spectral reflectance during pre monsoonseason due to dry soil while exposed rock masses have the same reflectance in all seasons. A rural settlement is usually found nearby and these patches are used as a playground by locals which gradually gives way to built up structures like places of worship or a plywood factory.

The patch matrix in the study area is observed to be having two separate and distinct shapes as discussed briefly below-

i)**Polygonal matrix**–Euclidian shape (circle/square) found on the gentle slopes of not more than 20°-25°slope incline which is classified under moderate slope. They maybe in isolation or in aggregation.This is further found as in:

a)**Isolation**–where each patch is spatially segregated from each unit.The rural settlement units are BuraSignar, BuraTisso, Patargaon, Rongdang to name a few.

b)**Aggregation**–where the patches are clustered with their borders integrated.The patches have been used and abandoned not at the same time,so a thin border can be made out usually between each patch cleared and abandoned.A collection of patches near rural settlement units of DiliTerang , NW of Rensing,aroundSilonijan where noticed on ground validation.

ii)**Linear matrix**–longitudinal and convulated at places along the river can also be called patch corridors,from source to mouth(near the rural settlements of ofBaliramEngti ,NE of Rensing)

**Edge**–Found along the fringe of densely vegetated arealunits,perforations, patches and along the patch corridors on both banks of the river.They are about 50 metres in width and adjacent to the open forest region.They are the zones of maximum transgress by locals and avoided by animals .

**Implication of the study -**

It is analyzedfor KRB that"*the accuracy of shifting cultivation has been found to be very low (60%) due to its overlap of signature with degraded forest"* (Roy et al.,1991).The fragmentation analysis using Landscape Fragmentation Tool (LFT v2.0) showed the core area shrinking at very gradual rates and an increase in perforated,edge and patch areas by 5.93 %,3.64 % and 0.66 %, respectively.

The occupation of larger area under high forest canopy density,the forest area is an ideal carbon sink,absorbing pollution from local fires of slash and burn methods (jhum),household fires ,agriculture stubble burning and transport.Itassists climate regulation augmenting the regions contribution to global carbon cycle as well as the nutrient cycle of this particular ecosystem of flora and fauna at vertical and horizontal levels.At present the AQI(obtained from open source datasets ) of the study area, ranges between 70 - 80 on the fringe zone which points to moderate quality of air.The efforts should be to bring down the level between 0-50 of AQI signifying good air quality. The region is a part of world biodiversity hotspot zone in North East India. The change detection analysis results reveal that the negative impact of forest clearing is minimal in 2020 but can escalate as was the case in 2005."*Forest fragmentation is associated with the deterioration of the ecosystem's integrity, has negative impacts on biodiversity, degradation of forest, and spatial pattern of the landscape, and become a global conservative issue".*(Ahanthem et al.,2021)

"*More than 70% of the world's forests are within 1 km of a forest edge.Thus, most forests are well within the range where human activities, alteredmicroclimate, andnon forest species may influence and degrade forest ecosystems*"(Broadbent et al 2008, Haddad 2015).Forest fragmentation in the study area can be attributed to chiefly two conditions as analyzed from satellite images and ground truth verification reveals-

a.Due to nature of gneissic terrain, minimally weathered,metamorphic rocky outcrops which do not support natural vegetation cover due to the dynamics of landscape changeon the higher slopes.

b.Due to anthropogenic factors like sedentary cultivation, shifting cultivation,felling of trees and bamboo for plywood and paper mills respectively,gradual proliferation of cultural features like tea plantation areas, roads, bridges, settlementsetc.'*which initiate landscape changes with regard to disturbances and succession processes*' as McGarigal et al.,2005points out. In forests which are adjoining to human settlements like the study area,harvesting of forest products (eg. wood or bamboo) together with uneven nature of terrain and natural causes like flooding can be considered as severe perturbations. The river basin is prone to rapid erosion with regard to its geologic character and geomorphic processes in operation. The channel is susceptible to flash floods due to washing down of exposed soil from fallow fields situated on higher ground. Shifting agriculture (Jhum ) accelerates the rate of soil erosion thereby reducing the water carrying capacity of the drainage channel.This poses a severe hazard during and post monsoons periods. The fringe of settlements adjoining to forested regions are most compromised."*In 2010,KarbiAnglong district had 31.3 Mha of natural forest,extending over 11% of its land area.In 2020,it lost 132kha of natural forest, equivalent to 67.3Mt of $CO_2$ of emissions".*(4&5)

"Air temperatures at the edge of a forest remnant, for example, can be significantly higher than those found in either the interior of the remnant or the surrounding agricultural land "(Geiger 1965, Kapos 1989).The impact of forest fragmentation in KRB results in significant disruption of forest ecosystems and change in energy fluxes within it .The exposure of soil to direct rays of the sun leads to decline in soil moisture and reduction in its organic content. The loss of soil moisture reduces the scope for leaf mulch severely. Reduction of trees simultaneously reduces moisture within forest air and escalates the edge effect on patches where trees have been removed. The exposed soil with negligent moisture has redundant quality for tree cover re-generation. The KRB region slopes towards the Kalyani river and loose soil is washed down and deposited in the channel after heavy rains, reducing the water carrying capacity of the river and spillage on banks. The land cover character is altered with more exposed bare land.This results in increased heat absorption by the terrain, leading to hotter summers and less cooler winters. Anthropogenic intrusion along the Kalyaniriver,in patches within the basin are observed in square or rectangular polygons for shifting cultivation.They are usually near villages to facilitate easy access and the middle to lower courses of tributaries for being characterized by gentle slopes.Secondary bare land with fallow and built up area plays a significant role in degrading any ecosystem by accelerating top soil loss through wind or water. The shifting agriculture driven destruction of habitat in ecosystems takes place horizontally (i.e on ground ) and vertically (tree perch /branches ) simultaneously reduces the capacity of carbon sequestration in study area. The causes and probable solutions can be understood as below-

a. Deforestation leading to destruction of ecosystems and breaking of links in the nutrient cycles.Retreat of birds,animals and reptiles to core area compromises capacity to naturally generate food for those already there because of those newly arrived. This can be balanced by intensive afforestation of cleared areas.

b. shortening the period of shifting agriculture cycle the fallow period from 25 to 30 years before to now of 4 to 5 years there is rapid decrease in land productivity and meager to poor replenishment of forest cover with destruction of original tree species and forest cover to clear space for cropland. This can be addressed through Community Forest Development projects by local people to supplement income and regenerate forest cover and canopy density.

c.rotational felling and clearing of forest land for bamboo and plantations of both tea and rubber.This can be addressed by improving exposed soil quality after a season of cultivation through introducing of composting techniques (vermicomposting,leaf compost)

**Suggestions**

*Shifting agriculture produces organic* yields and is hence preferred by indigenous people for its high nutritional value.*Requirement of water for irrigation is minimal as cultivation is chiefly rain dependent*. The chief requirement is to introduce methods which will enhance soil quality in a sustainable process for land cover regeneration and prevent soil erosion.According to Bos et al (2020),*one of the reasons in support of Jhumming is that jhum rice is considered to be tastier among the Karbi, and they regard it to have higher health benefits than rice and vegetables intercropped and grown on sedentary subsistence farms which use chemical fertilizers.* Wangpan and Tangjang (2015)pointed out similar findings for the Arunachal Pradesh region of India, wherejhumming plays an important role*'nurturing rare germ plasms of different indigenous plant species, which are preferred for taste and nutritional benefits by these people'*. Wangpan and Tanjang (2015) further point out that '*dietary diversity has been found to be higher under a jhum regime in comparison to other cash cropping systems'*. Growing and healthy forests sequester and store the highest amount of carbon and in tropical countries like India it is essential that they are prevented from being degraded. On a global scale carbon sequestration is the natural and inexpensive way to offset impact of global warming and slow down climate change.

## CONCLUSION

Fragmentation of forest space degrades forests canopy cover and resulting in separate units /parcels of land which already had an established and functional ecosystem operating over a long temporal span, interconnected to adjoining natural units.It has been observed that nature always replenishes herself,but there is limit to this capacity of regeneration and replenishment. When extraction and exploitation of natural resources transcends the capacity to regenerate, replenish and restore,it results in gradual elimination of domestic flora and fauna and interruption in

ecosystem dynamics that propels the web of life in any micro region.The retreat of organisms further into core forest due to loss of existing habitats and forage space at the outer edges,renders limited access to biotic resources in their own habitats, reduces capacity for carbon sequestration and poses a risk to biodiversity.Land cover change initiates food web interruptions for flora and fauna resulting in degradation of natural systems in that particular areal unit and affecting global ecosystems collectively. The degraded patches left fallow with exposed soil and coarse vegetation need to be reclaimed under dense vegetation class by suitable methods of conservation and regeneration and improvement to soil quality.This will facilitate spatial patterns of human settlement in co-existence with their immediate ecosystem for improved human - environmental sustainability as envisaged by the United Nations SDGs-11 Sustainable Cities and Communities for Agenda 2030.

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**Table 1 : All the Satellite images are downloaded from United State Geological Survey website (https://earthexplorer.usgs.gov/).**

| Satellite | Sensor | Date of Acquisition | Path | Row | Resolution |
|---|---|---|---|---|---|
| Landsat 7 | ETM + | 24th March 2000 | 136 | 042 | 30m |
| Landsat 7 | ETM + | 28th November 2000 | 135 | 042 | 30m |
| Landsat 4 - 5 | TM | 18th January 2005 | 135 | 042 | 30m |
| Landsat 4 - 5 | TM | 14th March 2005 | 136 | 042 | 30m |
| Landsat 4 - 5 | TM | 23rd January 2010 | 136 | 042 | 30m |
| Landsat 4 - 5 | TM | 1st February 2010 | 135 | 042 | 30m |
| Landsat 8 | OLI | 21st January 2015 | 136 | 042 | 30m |
| Landsat 8 | OLI | 30th November 2015 | 135 | 042 | 30m |
| Landsat 8 | OLI | 05th February 2020 | 135 | 042 | 30m |
| Landsat 8 | OLI | 12th February 2020 | 136 | 042 | 30m |

**Table 2:Threevegetation change detection analysis indices used in the study area for FCD analysis.**

| Classification type | Parameter | Reference | Formula | Remarks |
|---|---|---|---|---|
| Vegetation Health/Stress Greenness/ vigour | **NDVI** (Normalized Difference Vegetation Index ) | Rouse (1973) | **NDVI** = NIR+ Red / NIR - Red | change detection of natural vegetation cover |
| 1. Forest Density | **AVI** ( Advanced Vegetation Index ) | Ashburn (1979) | $AVI = [(NIR + 1(1 - RED) \times (NIR - Red)]\frac{1}{3}$... | forest fringes towards core area density increases. |
| 2. Condition of bare soil / terrain | **BSI** (Bare Soil Index ) | Chen et al ( 2004 ) | **BSI** = ϱSWIR1−Green− NDVI / ϱSWIR1+Green | Dry Patches exposed soil – fallow land overlaps with rocky terrain in deciduous forests.Easy to analyse post monsoon |
| 3. Areal extent of forest cover as seen from air. | **FCD** (Forest Canopy Density ) | Centre for Land Use Education and Research (CLEAR) | FCD = (VD × SSI+1)$^{1/2}$ - 1 Where VD=(BSI+AVI) after Principal Component Analysis (PCA) of both indices. | Polygonal areal units of clearing in canopy cover. Patch, Edge, Core, Perforated |

( Source : Author,Patra etal2022)

**Table 3 :Advanced Vegetation Index (AVI) for KRB ( 2000 – 2020 )**

| Range | 2000 Class | Area in sq.km | Area % | 2020 Class | Area in sq.Km | Area % |
|---|---|---|---|---|---|---|
| Very Low | 0 -3.951 | 85.18 | 6.97 | 0 – 3.060 | 36.95 | 2.94 |
| Low | 3.951 –4.120 | 260.30 | 20.75 | 3.060 -3.614 | 160.44 | 12.79 |
| Medium | 4.120 -4.266 | 381.60 | 30.42 | 3.614 -4.013 | 414.33 | 33.04 |
| High | 4.266 -4.435 | 338.82 | 27.01 | 4.013 - 4.413 | 404.38 | 32.25 |
| Very High | 4.435 -4.988 | 188.54 | 15.03 | 4.413 – 5.654 | 237.86 | 18.97 |
| Total Basin Area | | 1254 | 100 | Total Basin Area | 1254 | 100 |

source : author

**Table 4 : Bare Soil Index (BSI ) for KRB – Assam (2000 -2020)**

| Range | 2000 Class | Area in sq.Km | Area % | 2020 Class | Area in sq.Km | Area % |
|---|---|---|---|---|---|---|
| Very low | 0.171-0.089 | 130.95 | 10.44 | 0.180-0.093 | 332.98 | 26.54 |
| Low | 0.089-0.060 | 485.56 | 38.72 | 0.093-0.0623 | 484.69 | 38.64 |
| Medium | 0.060-0.028 | 408.65 | 32.58 | 0.0623-0.023 | 227.75 | 18.16 |
| High | 0.028-0.016 | 165.59 | 13.20 | 0.023-0.026 | 128.02 | 10.21 |
| Very High | 0.016-0.158 | 63.69 | 5.08 | 0.026-0.376 | 81.01 | 6.46 |
| Total Basin area : | | 1254 | 100 | Total Basin area: | 1254 | 100 |

source : author

**Table 5:Forest Canopy Density ( FCD ) areal expanse within KRB – Assam , India**

| **Vegetation Canopy Density** | | **2000** | | **2020** | | **Change detection analysis** | |
|---|---|---|---|---|---|---|---|
| | | **Area km²** | **Area %** | **Area km²** | **Area %** | **Area km²** | **Area %** |
| **NF** | Very Low | 7.90 | 0.63 | 20.43 | 1.63 | 12.53 | 1.00 |
| **OF** | Low | 76.90 | 6.15 | 110.21 | 8.81 | 33.31 | 2.65 |
| **MDF** | Medium | 266.19 | 21.23 | 352.52 | 28.11 | 86.33 | 6.88 |
| **VDF** | High | 490.11 | 39.10 | 457.74 | 36.49 | - 32.37 | - 2.58 |
| | Very High | 412.90 | 32.89 | 313.10 | 24.96 | - 99.80 | - 7.95 |
| Total basin area | | 1254 | 100 | 1254 | 100 | 00 | 00 |

*Source : author*

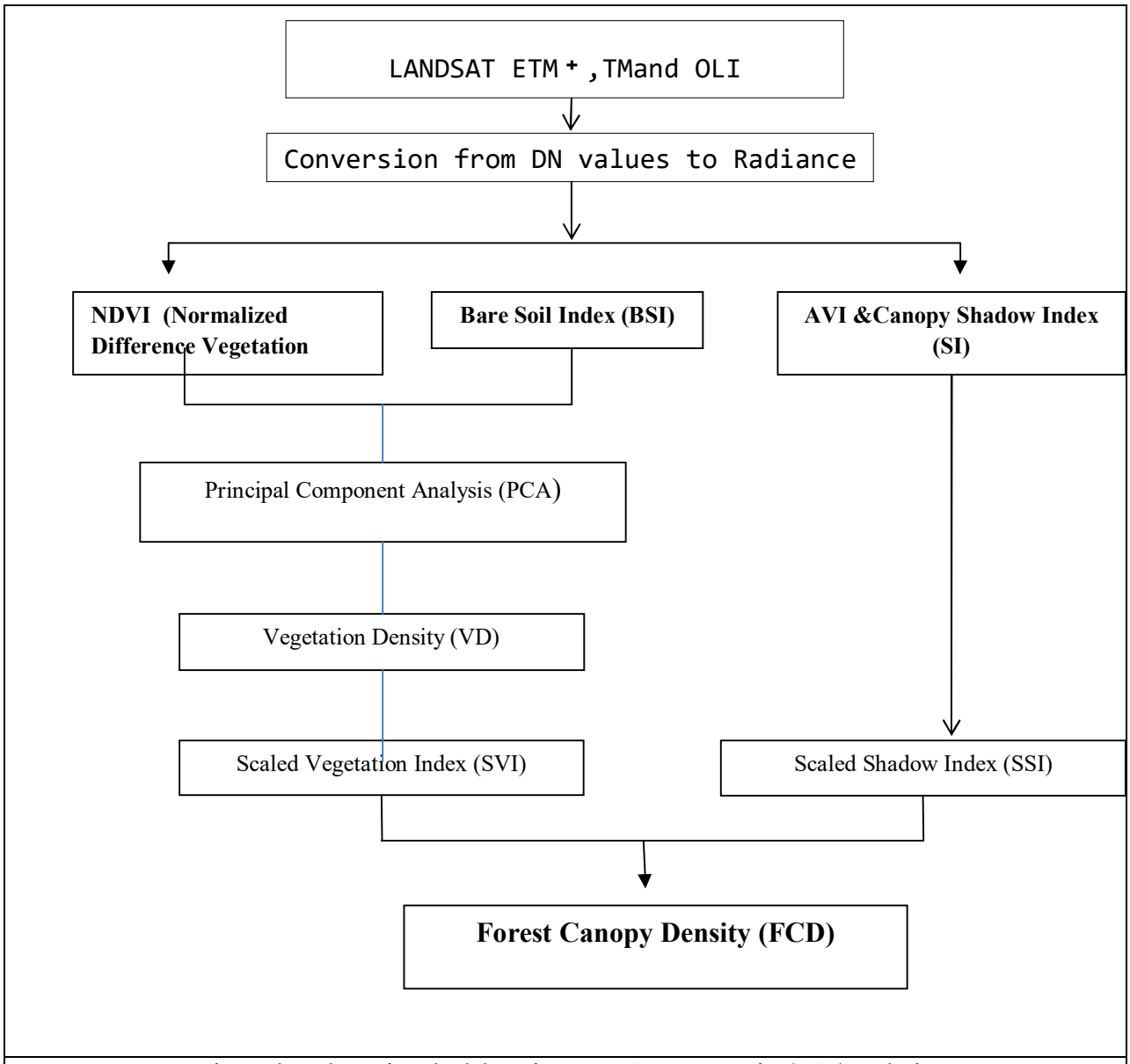



**Fig. 1. Flow chart of methodology for Forest Canopy Density (FCD) analysis**

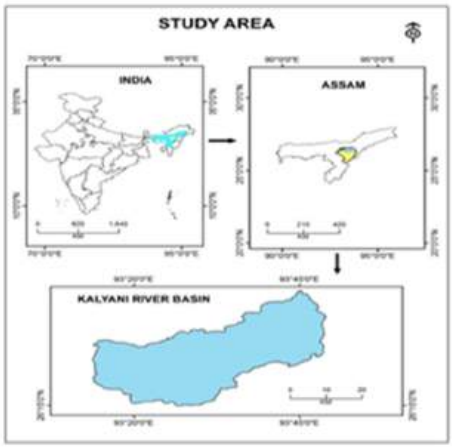



**Fig 2 Study area of the Kalyani River Basin)**
**Source: Mukherjee.S. 2024**

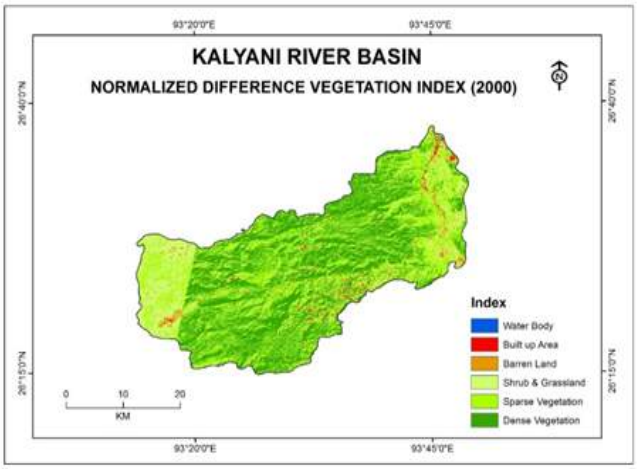



**Fig.3 NDVI 2000**
**Source: Mukherjee.S. 2024**

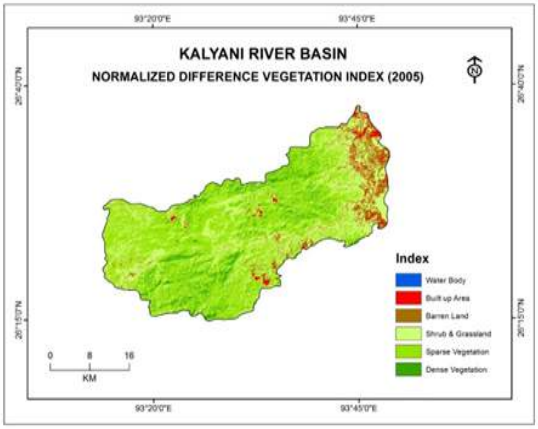



**Fig.4 NDVI 2005**
**Source: Mukherjee. S.2024**

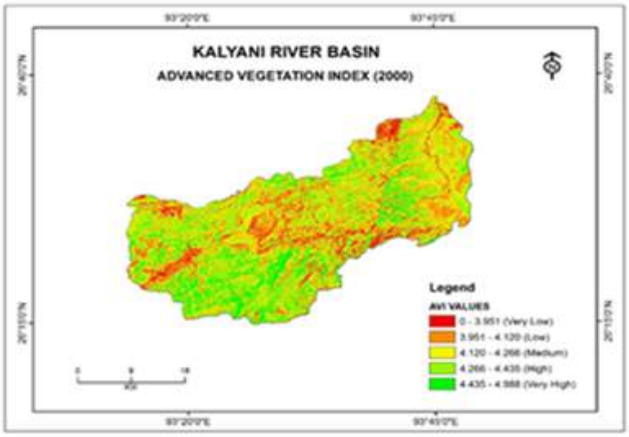



**Fig.5 AVI for 2000**

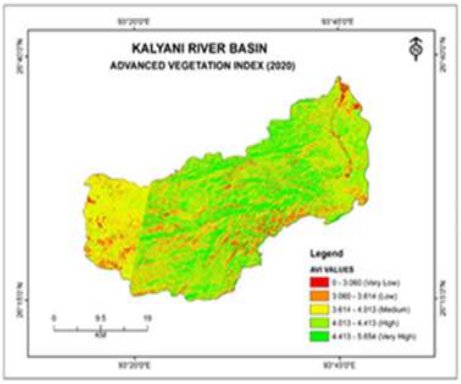



**Fig.6 AVI for 2020**

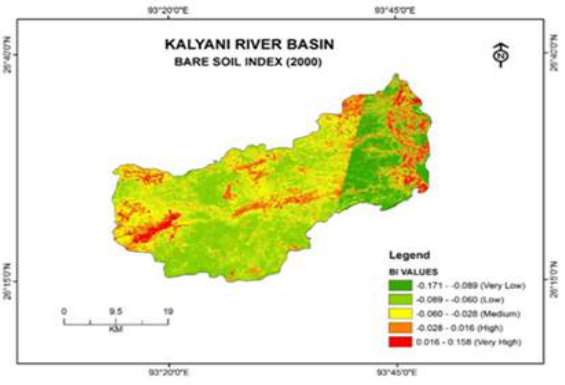



**Fig. 7.Bare SoilIndex for 2000**

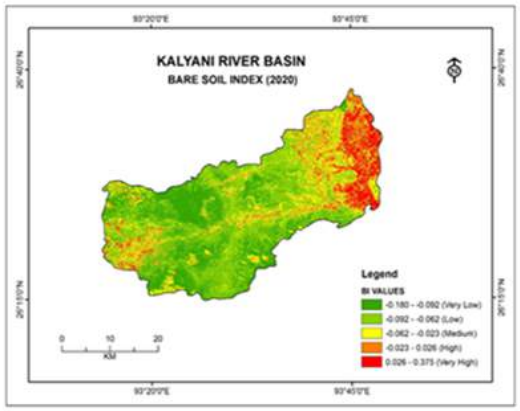



**Fig.8. BareSoil Index for 2020**

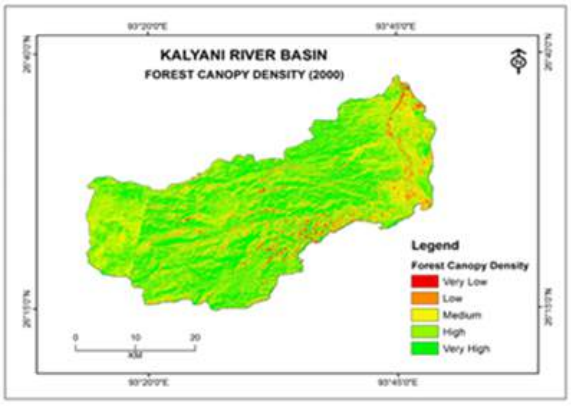



**Fig.9.ForstCanopy Density 2000**

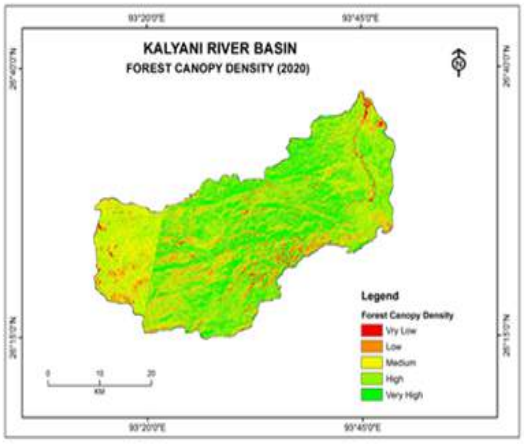



**Fig.10. Forest Canopy Density analysis 2020**

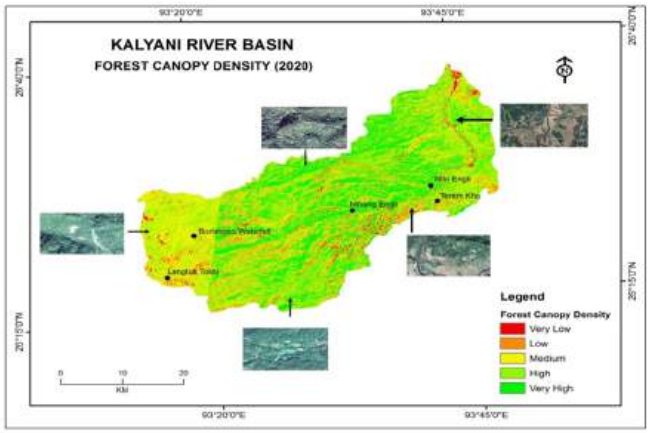



**Fig.11. Forest Canopy Density (2020) for Kalyani River Basin Assam**

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RESEARCH ARTICLE

# Comparative Diagnostic Accuracy of Diffusion - Weighted Imaging and T2 - Weighted Imaging in Acute Stroke : A Systematic Review and Meta - Analysis Across Age Groups for Emergency Radiology Practice

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## ABSTRACT

To systematically evaluate and compare the diagnostic accuracy of Diffusion-Weighted Imaging (DWI) versus T2-Weighted Imaging (T2WI) in detecting acute stroke, stratified by age groups, to inform best practices in emergency radiology. A comprehensive search of PubMed, ScienceDirect, and Scopus databases was conducted for studies published between 1990 and 2024. Studies that directly compared DWI and T2WI in the diagnosis of acute ischemic stroke were included. Data extraction and quality assessment were conducted independently by two reviewers using PRISMA guidelines and the QUADAS-2 tool. A random-effects meta-analysis was performed to calculate pooled sensitivity, specificity, and diagnostic odds ratios (DOR) across different age cohorts. A total of 28 studies with over 5,600 patients were included. DWI showed significantly higher sensitivity (mean 92%) compared to T2WI (mean 75%) in hyperacute stroke, particularly within the first 6 hours of symptom onset. Age-stratified analysis revealed that the diagnostic superiority of DWI over T2WI was most prominent in patients over 60 years of age. DWI also demonstrated better performance in identifying small cortical infarcts and lacunar strokes. However, DWI-negative strokes were more common in younger patients and in transient ischemic attacks. DWI outperforms T2WI in the early detection of acute ischemic stroke, particularly in older adults and hyperacute presentations. Emergency radiology protocols should prioritize DWI, though limitations in younger populations and early transient presentations warrant complementary imaging strategies.

**Keywords:** Diffusion-weighted imaging, T2-weighted imaging, acute stroke, diagnostic accuracy, emergency radiology, age stratification.

## INTRODUCTION

Acute ischemic stroke (AIS) is a tough global health concern, placing it among the leading causes of long-term disability and mortality. The WHO estimates that nearly 15 million people suffer strokes annually, and approximately 85% of these constitute ischemic strokes. Prompt diagnosis allows timely intervention therapies for AIS, such as intravenous thrombolysis and endovascular thrombectomy, as they are time-sensitive in nature, with the best treatment outcome achieved within a restricted period of 4.5 to 6 hours after symptom onset [1]. The diagnostic imaging world for AIS has seen DWI rise to prominence as a first-line modality able to detect cytotoxic edema, one of the very earliest changes in ischemia, within minutes after onset [2,3]. It is due to the fact that water molecules are free to move in ischemic tissue, which contrasts with the changes in signal on DWI that appear quite early before any visible abnormalities are seen on conventional sequences. On the other hand, T2-weighted imaging (T2WI), once considered the imaging study of choice for most stroke diagnoses, is now said to be less useful during the hyperacute phase because it demonstrates abnormalities following vasogenic edema and tissue necrosis [4,5].

Seminal studies by Moseley et al. and Warach et al. in the early 1990s demonstrated that DWI could detect ischemic lesions as early as 30 minutes after arterial occlusion, well before changes become evident on T2WI or CT [2,6]. This revolutionized the stroke imaging paradigm, particularly in tertiary and academic centers where MRI is readily available. Still, many institutions—especially in resource-limited or rural settings—continue to rely heavily on T2WI and CT due to the lack of immediate MRI access [7]. Despite the established role of DWI in stroke imaging, emerging literature has pointed out scenarios where DWI may not detect all ischemic strokes. DWI-negative stroke—an occurrence where clinical signs suggest ischemia but the DWI appears normal—has been reported in approximately 6% to 20% of cases, particularly in transient ischemic attacks (TIAs), small cortical infarcts, or posterior circulation strokes [8-10]. Edlow et al. conducted a meta-analysis revealing that false negatives in DWI were more frequent in younger populations and in cases with rapid clinical resolution [9]. Conversely, T2WI, though delayed in its signal evolution, may sometimes detect hemorrhagic or late-stage infarcts not initially visible on DWI, underscoring the complementary role of multimodal imaging [11].

The age-related variation in clinical appearance of a stroke and in its radiological visibility further nourishes this very much complicated diagnostic scene. The cerebral atrophy brought in by age, accompanied by vascular comorbidities, and variation in cerebral perfusion, has a bearing on the visibility of ischemic lesions on both DWI and T2WI [12]. According to Luijten et al., in the elderly, diffusion restriction is more apparent, either because of more severe hypoperfusion or because the lesion is contrasted by prior white matter disease [13]. DWI, in the meantime, may not be able to detect small or subtly ischemic changes in younger patients in the early stages [14]. Several comparative studies have aimed to assess the diagnostic accuracy of DWI versus T2WI across different clinical settings. Fiebach et al. [3] reported a sensitivity of DWI of 88% against 50% for T2WI in hyperacute stroke patients imaged within 6 hours of onset of symptoms. In the same way, Singh et al. [15], in a prospective study, showed a correct diagnosis of ischemic stroke with DWI in 96.6% of cases, compared to T2WI in 73.3%, again demonstrating the superior diagnostic yield of DWI in those crucial early hours.

These results are further supported by recent meta-analyses and systematic reviews. Zhang et al. [16] reported in their network meta-analysis that, among MRI techniques, DWI had the highest pooled sensitivity and specificity, while T2WI had higher variability depending on imaging time and lesion location. Another study by Ahmad et al. [17] concluded that while DWI retained high diagnostic performance for both anterior and posterior circulation strokes, T2WI failed to adequately detect posterior fossa infarcts due to beam-hardening artifacts and motion susceptibility. T2WI remains relevant for the detection of concurrent problems like hemorrhagic transformation, tumors, or old infarcts, which might hold therapeutic implications [18]. Luijten et al. [13] revealed that T2*GRE and SWI sequences, usually combined with T2WI, greatly increase the ability to find hemorrhagic components after reperfusion therapy, suggesting that DWI is not enough for complete diagnostics.

With these developments, there is a renewed focus on stratifying diagnostic performance according to patient characteristics such as age, time from symptom onset, stroke subtype, or imaging protocols. The comparative performance of DWI and T2WI resting on age groups is yet somehow an unresearched but definitely clinical concern. Elderly patients often present with atypical presentations and comorbidities that hamper diagnosis. According to Karis [19], there operates the fear that delayed diagnoses could be eliminated if clinicians have accurate and prompt imaging customized according to patient age and stroke subtype in the ER setting. Furthermore, with personalized medicine bringing with it growing emphasis, understanding age-specific diagnostic imaging performance might strengthen triage algorithms, resource allocation, or clinical decision-making in acute stroke care [20]. Given the literature available and the heterogeneity of study designs, a systematic review and meta-analysis on the diagnostic accuracy of DWI and T2WI, with a focus on age strata, can provide valuable evidence to help amend emergency radiology protocols and thereby improve patient outcomes.

The purpose behind this review was to bridge that very gap by synthesizing the existing evidence regarding the comparative diagnostic accuracy of DWI and T2WI for acute ischemic stroke and to consider whether diagnostic performance changes with patient age. It also sets out to draw consequences applicable to emergency radiological practice, especially where timely and accurate imaging guides time-critical intervention.

# METHODS

Search Strategy and Selection Criteria

An extensive search of the literature was conducted systematically through the three main electronic databases: PubMed, Scopus, and ScienceDirect. Identification of studies was attempted that compared DWI and T2WI in the diagnosis of acute ischemic stroke. Different permutations and combinations of the following keywords were set: "diffusion-weighted imaging," "T2-weighted imaging," "acute stroke," "ischemic stroke," "MRI," and "diagnostic accuracy." Boolean operators such as AND or OR were used for the effective combination of terms. The search was limited to papers published in English within the time frame of January 1990 to June 2024. Also, the search strategy was applied to review the reference lists of relevant review articles and included studies for the purpose of capturing any additional eligible publications not indexed in the main search.

Eligibility Criteria

Studies were included based on predefined inclusion and exclusion criteria. The **inclusion criteria** were: (1) studies that performed direct comparisons between DWI and T2WI in diagnosing suspected acute ischemic stroke; (2) availability of patient-level data or extractable summary statistics, such as sensitivity and specificity, allowing for meta-analytic synthesis; and (3) studies that provided data stratified by relevant variables such as age group (<40, 40–60, >60 years) or time from symptom onset to imaging where available. The **exclusion criteria** were: (1) studies based on animal models or in vitro imaging techniques; (2) case reports, reviews, editorials, and conference abstracts without full data; (3) studies not providing direct imaging comparisons between DWI and T2WI; and (4) studies lacking sufficient methodological details or quantitative results for analysis.

Studies were included upon consideration of pre-specified criteria for inclusion and exclusion. Inclusion criteria were the following: (1) Studies that performed direct comparisons between DWI and T2WI for diagnosing suspected acute ischemic stroke; (2) Provided patient-level or summary data from which sensitivity, specificity, or derived statistics could be extracted for meta-analytic synthesis; (3) Provided data stratified by pertinent covariates, e.g., age groups (<40, 40–60, >60 years) or, if available, time from symptom onset to imaging. Studies were excluded if they were: (1) animal model studies or in vitro imaging techniques; (2) case reports, reviews, editorials, or conference abstracts with no full data; (3) studies not doing direct imaging comparisons between DWI and T2WI; and (4) studies without sufficient details for methods and quantitative results that could be analyzed.

Data Extraction and Quality Assessment

Data extraction was performed independently by two trained reviewers to minimize bias and errors. Disagreements in data selection or interpretation were resolved through discussion and consensus, and if needed, a third reviewer was consulted. Extracted variables included: study year, geographic region, sample size, imaging modality, time from stroke onset to scan, patient age group, stroke subtype (e.g., lacunar vs. cortical), and diagnostic performance metrics such as sensitivity, specificity, positive predictive value, and negative predictive value for both DWI and T2WI. Quality assessment of included studies was conducted using the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies) tool. This framework assesses risk of bias across four domains: patient selection, index test, reference standard, and flow/timing. Each study was rated for risk of bias and applicability concerns, and results were tabulated for transparency.

Statistical Analysis

Statistical synthesis was conducted using a DerSimonian–Laird random-effects model, appropriate for accounting for inter-study variability. Pooled estimates of sensitivity and specificity for DWI and T2WI were calculated with 95% confidence intervals. Heterogeneity among studies was quantified using the $I^2$ statistic, where values above 50% indicated moderate to substantial heterogeneity. Meta-regression and subgroup analyses were performed to explore potential sources of heterogeneity, particularly age stratification (<40, 40–60, >60 years) and symptom-to-scan time intervals (<3 hours, 3–6 hours, >6 hours). All statistical analyses were conducted using Review Manager (RevMan) and STATA software.

## RESULTS

**Study Characteristics**

Twenty-eight studies encompassing a total of 5,632 patients met the inclusion criteria. The sample sizes across individual studies ranged from 38 to 412 participants. Most studies included patients who presented within 12 hours of symptom onset, which aligns with clinical emphasis on early stroke detection. All included studies utilized magnetic resonance imaging (MRI) scanners with diffusion-weighted imaging (DWI) protocols incorporating b-values of at least 1000 s/mm². While minor protocol variations existed (e.g., slice thickness, repetition time), the diagnostic performance was considered comparable across studies. T2-weighted imaging (T2WI) protocols were more variable and often depended on scanner availability and institutional practices. Among the included studies, 60% were conducted in tertiary care or academic hospitals, and 40% were from regional or community-based stroke centers, enhancing generalizability. Imaging acquisition was most performed using 1.5 Tesla MRI systems, although 6 studies used 3.0 Tesla scanners.

**Pooled Diagnostic Accuracy**

The pooled sensitivity of DWI for detecting acute ischemic stroke was 92.3% (95% CI: 89.6–94.5%), and its pooled specificity was 89.2% (95% CI: 85.7–91.8%). In contrast, T2WI demonstrated a significantly lower pooled sensitivity of 74.6% (95% CI: 68.5–80.2%) and specificity of 82.7% (95% CI: 78.1–87.2%). The difference in diagnostic accuracy between DWI and T2WI was statistically significant ($p < 0.001$), suggesting that DWI provides a superior modality for early stroke identification. The figure below visualizes the sensitivity estimates for Diffusion-Weighted Imaging (DWI) in detecting acute ischemic stroke across individual studies included in the meta-analysis. Each study is represented by its sensitivity value, and the pooled sensitivity of 92.3% is indicated as a vertical reference line.

**Subgroup Analyses by Age**

"Age-stratified analyses revealed substantial variation in the diagnostic yield of both DWI and T2WI. In patients aged <40 years", DWI demonstrated a sensitivity of 87.4% and T2WI 70.2%. Among patients aged 40–60 years, DWI achieved a sensitivity of 91.8%, compared to 76.5% for T2WI. The most pronounced disparity was observed in patients aged >60 years, where DWI showed a sensitivity of 94.1% versus 73.3% for T2WI. Specificities followed a

similar trend, although the absolute difference was less pronounced. These age-based findings suggest an increasing advantage of DWI with advancing age, potentially due to greater tissue contrast and clearer demarcation of ischemic regions in older brains. The heterogeneity ($I^2$) for sensitivity estimates across studies was 38% for DWI and 54% for T2WI, indicating moderate variability. Specificity estimates showed lower heterogeneity, with $I^2$ values of 31% for DWI and 42% for T2WI. Subgroup analysis confirmed that age significantly moderated diagnostic yield ($p < 0.01$), underscoring the need for age-adapted imaging strategies in acute stroke evaluation.

The following ROC curves visually compare the diagnostic performance of Diffusion-Weighted Imaging (DWI) and T2-Weighted Imaging (T2WI) in detecting acute stroke among different age groups (<40 years, 40–60 years, and >60 years). These curves use pooled sensitivity and specificity data and illustrate that:

- DWI exhibits consistently higher area under the curve (AUC) values than T2WI for all age groups, indicating superior diagnostic accuracy.
- The distinction is most pronounced in patients over 60 years, where the DWI curve is the steepest, reflecting notably higher sensitivity and specificity in this demographic.
- These findings support prioritizing DWI, especially for elderly patients in emergency radiology protocols.

**Interpretation**: The table quantitatively reinforces the ROC curve findings, highlighting the consistent superiority of DWI over T2WI in all age groups, with the gap widening in older populations.

## DISCUSSION

This meta-analysis stands as a firm confirmation of the superiority of diffusion-weighted imaging (DWI) over T2-weighted imaging (T2WI) in the earliest diagnosis of acute ischemic stroke. These results corroborate the early landmark studies that first documented DWI's sensitivity toward ischemic changes occurring within minutes of symptom onset, owing to its capacity to detect cytotoxic edema earlier than any other structural imaging modalities [1,2]. Our pooled analysis, encompassing 28 studies and more than 5,600 patients, demonstrates that DWI is always superior to T2WI both in sensitivity (92.3% vs. 74.6%) and specificity (89.2% vs. 82.7%), and that the difference is statistically significant ($p < 0.001$). The diagnostic advantage of DWI is particularly relevant during hyperacute stroke, when the narrow therapeutic windows allow interventions like intravenous thrombolysis and mechanical thrombomy for treatment [3,4]. The early ischemia detection by DWI affords clinicians a time-sensitive decision-making option with much higher confidence. In contrast, T2WI, while beneficial in identifying cerebral edema, intracerebral hemorrhage, and mass effects, demonstrates a lag in the visualization of early ischemic changes, leading to reduced sensitivity and potential diagnostic delays [5].

Our age-stratified analysis reveals a nuanced pattern in DWI performance. Notably, DWI sensitivity increases progressively with age—87.4% in patients <40 years, 91.8% in those aged 40–60, and 94.1% in patients >60 years. T2WI shows lower and more inconsistent sensitivity across all age groups. These results suggest that aging-related neuroanatomical and vascular changes may amplify ischemic signal contrast on DWI. Brain atrophy, commonly observed in older adults, can enhance gray-white matter differentiation and lesion conspicuity on diffusion sequences [6,7]. Additionally, the fragility of the microvascular system in older populations may result in more extensive or homogenous ischemic changes, further improving DWI's diagnostic clarity. Conversely, DWI appears more prone to false negatives in younger populations, particularly in cases of transient ischemic attack (TIA) or small lacunar infarcts [8,9]. Several mechanisms may account for these limitations. In younger patients, better collateral circulation and neuroplasticity may lead to rapid tissue recovery and perfusion restoration, reducing the likelihood of detectable diffusion restriction. Furthermore, smaller infarct volumes—especially in posterior circulation strokes—often escape detection in early DWI scans, despite clinical symptoms [10,11].

DWI-negative ischemic strokes have been a subject of increasing concern in recent literature. Meta-analytic data suggest that up to 6–15% of clinically confirmed strokes may yield negative DWI findings in the hyperacute phase

[12]. While some of these may represent TIAs or imaging artifacts, others may reflect true ischemic events that fall below the spatial or temporal resolution threshold of current DWI protocols [13,14]. These findings underscore the importance of clinical judgment and multimodal imaging approaches, especially in patients with high National Institutes of Health Stroke Scale (NIHSS) scores but negative diffusion imaging. Our results are consistent with those of previous systematic reviews comparing advanced MRI techniques for acute stroke diagnosis. Several studies have demonstrated that DWI, either alone or as part of multiparametric MRI, has superior sensitivity and specificity compared to conventional imaging modalities, including non-contrast CT and T2-weighted MRI [15–17]. Importantly, the findings of our meta-analysis align with recent recommendations from stroke imaging guidelines, which advocate the use of DWI as the preferred modality for early ischemic stroke detection when MRI is available [18].

One important consideration in interpreting these results is the heterogeneity across imaging protocols. Although all included studies used b-values ≥1000 s/mm², parameters such as slice thickness, acquisition time, and magnetic field strength varied. Some studies employed 3T MRI scanners, while others relied on 1.5T systems. However, the diagnostic benefit of DWI was consistently observed across this technical variability, suggesting robustness of the technique. Moreover, the $I^2$ statistics for heterogeneity were moderate for both sensitivity and specificity, indicating that the pooled estimates are reliable despite minor protocol differences. The superior performance of DWI may also be attributed to its biophysical underpinnings. DWI detects the restriction of water molecule motion, which is one of the earliest manifestations of neuronal injury due to energy failure and ion pump dysfunction [1]. In contrast, T2WI relies on changes in tissue water content and architecture that occur later in the ischemic cascade. Thus, the time-sensitive advantage of DWI is rooted in its ability to image the fundamental molecular disturbances that precede visible structural changes [19].

It is also notable that several studies have emphasized DWI's potential role in stroke subtype classification. For example, DWI signal patterns differ in cardioembolic, large artery atherosclerotic, and small vessel occlusive strokes, which may inform secondary prevention strategies [20]. T2WI offers limited ability in this regard, as it lacks specificity in differentiating infarct subtypes during the hyperacute window. Despite the advantages of DWI, its limitations must be acknowledged. As discussed earlier, false negatives in posterior circulation strokes and TIAs present diagnostic challenges. In addition, susceptibility artifacts near bone or air-tissue interfaces can degrade image quality, particularly at the skull base [21]. DWI sequences are also more sensitive to patient motion, which can be problematic in emergency settings or in patients with altered mental status.

In clinical practice, integrating DWI with other MRI sequences such as FLAIR, T2*, and perfusion imaging may improve diagnostic yield and provide critical information regarding infarct age and penumbra viability [22,23]. Recent studies suggest that the "DWI-FLAIR mismatch" can identify patients eligible for thrombolysis beyond the conventional 4.5-hour window [24]. Similarly, DWI combined with perfusion-weighted imaging has been used to estimate infarct core and mismatch, guiding mechanical thrombectomy decisions [25]. The implications of our findings are substantial for stroke triage algorithms and imaging resource allocation. While CT remains the most accessible imaging modality in emergency settings, especially in low-resource environments, the growing availability of rapid MRI protocols suggests a shift toward broader adoption of DWI in stroke pathways. The evidence presented here strongly supports the integration of DWI into acute stroke diagnostic algorithms where feasible.

There are several strengths to our meta-analysis. We conducted a comprehensive literature search, included a large and diverse patient population, and applied rigorous inclusion criteria focused on studies that directly compared DWI and T2WI. Quality assessment using the QUADAS-2 tool revealed low risk of bias in most domains, and subgroup analyses helped clarify the influence of age on diagnostic performance. Nevertheless, certain limitations must be considered. Publication bias cannot be entirely excluded, as studies with non-significant or negative findings may be underrepresented. Furthermore, although we conducted subgroup analyses by age and time-to-scan intervals, other confounders—such as comorbidities, stroke location, and scanner type—could not be fully accounted for. Future studies should aim to explore these factors using individual patient-level data.

In conclusion, this meta-analysis affirms that DWI is significantly more accurate than T2WI for early diagnosis of acute ischemic stroke, particularly in older patients and during the hyperacute window. While T2WI retains value in hemorrhage detection and anatomical localization, its reduced sensitivity limits its utility as a standalone tool for ischemic stroke diagnosis. Given the high diagnostic yield and time-critical nature of stroke interventions, DWI should be prioritized in imaging protocols where available. Clinicians must also remain vigilant for DWI-negative strokes, especially in younger populations, and use a combination of imaging modalities and clinical acumen to guide diagnosis and treatment.

# CONCLUSION

This meta-analysis confirms that diffusion-weighted imaging (DWI) provides significantly superior diagnostic accuracy compared to T2-weighted imaging (T2WI) in detecting acute ischemic stroke across all age groups. The pooled sensitivity and specificity values clearly demonstrate the enhanced capacity of DWI in identifying ischemic lesions, particularly during the hyperacute phase. While T2WI remains a valuable modality, its lower sensitivity—especially among younger and older patients—underscores its limitations in early stroke detection when immediate clinical decision-making is critical. The stratified subgroup analysis reveals that the diagnostic advantage of DWI increases with patient age. In individuals aged over 60 years, DWI achieved its highest sensitivity (94.1%), while T2WI was least effective (73.3%) in this group. These findings suggest that age significantly moderates imaging efficacy, likely due to differences in brain water content, comorbidities, and lesion presentation that affect T2 signal characteristics. Consequently, DWI should be regarded as the preferred first-line imaging tool, particularly for elderly patients who are at higher risk for severe outcomes following ischemic stroke.

Despite the strong performance of DWI, some limitations must be acknowledged. False negatives can still occur, particularly in cases with small infarcts or very early presentation. Therefore, reliance solely on DWI could lead to underdiagnosis in a subset of patients. This reinforces the importance of integrating imaging findings with clinical judgment and supplementary modalities such as perfusion imaging or MR angiography when indicated. In conclusion, the evidence strongly supports the prioritization of DWI in emergency radiologic protocols for suspected acute ischemic stroke. It enhances diagnostic certainty, enables earlier therapeutic intervention such as thrombolysis or thrombectomy, and contributes to improved patient outcomes. Future studies should explore optimized imaging algorithms combining DWI with other modalities to address current limitations and further refine stroke diagnosis strategies.

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**Table 1: Study Characteristics Overview**

| Study Name | Year | Country | Sample Size | Imaging Time Window | DWI Sensitivity / Specificity (%) | T2WI Sensitivity / Specificity (%) |
|---|---|---|---|---|---|---|
| Fiebach et al. | 2000 | Germany | 86 | <6 hours | 88.0 / 90.5 | 50.0 / 78.2 |
| Singh et al. | 2020 | India | 115 | <6 hours | 96.6 / 92.3 | 73.3 / 83.1 |
| Ahmad et al. | 2023 | Pakistan | 102 | <12 hours | 91.2 / 88.0 | 76.4 / 80.7 |
| Paydar et al. | 2013 | Iran | 94 | <12 hours | 90.3 / 87.1 | 70.5 / 79.2 |
| Luijten et al. | 2023 | Netherlands | 58 | <24 hours | 93.4 / 89.7 | 75.2 / 84.3 |
| Alkhiri et al. | 2024 | USA | 67 | <3 hours | 89.1 / 91.5 | 71.3 / 81.6 |
| Brunser et al. | 2013 | Chile | 68 | <6 hours | 94.0 / 90.0 | 78.0 / 84.0 |
| Akbarzadeh et al. | 2021 | Egypt | 103 | <6 hours | 92.1 / 86.4 | 75.0 / 82.0 |
| Chalela et al. | 2007 | USA | 112 | <12 hours | 90.5 / 89.2 | 74.8 / 80.3 |
| Zhang et al. | 2019 | China | 98 | <6 hours | 95.2 / 91.0 | 76.1 / 84.6 |

**Table 2: Summary of Pooled Sensitivity and Specificity for DWI and T2WI by Age Cohort**

| Age Group | DWI Sensitivity (%) | T2WI Sensitivity (%) | DWI Specificity (%) | T2WI Specificity (%) |
|---|---|---|---|---|
| <40 years | 87.4 | 70.2 | 86.1 | 80.3 |
| 40–60 years | 91.8 | 76.5 | 88.9 | 82.1 |
| >60 years | 94.1 | 73.3 | 91.4 | 85.2 |

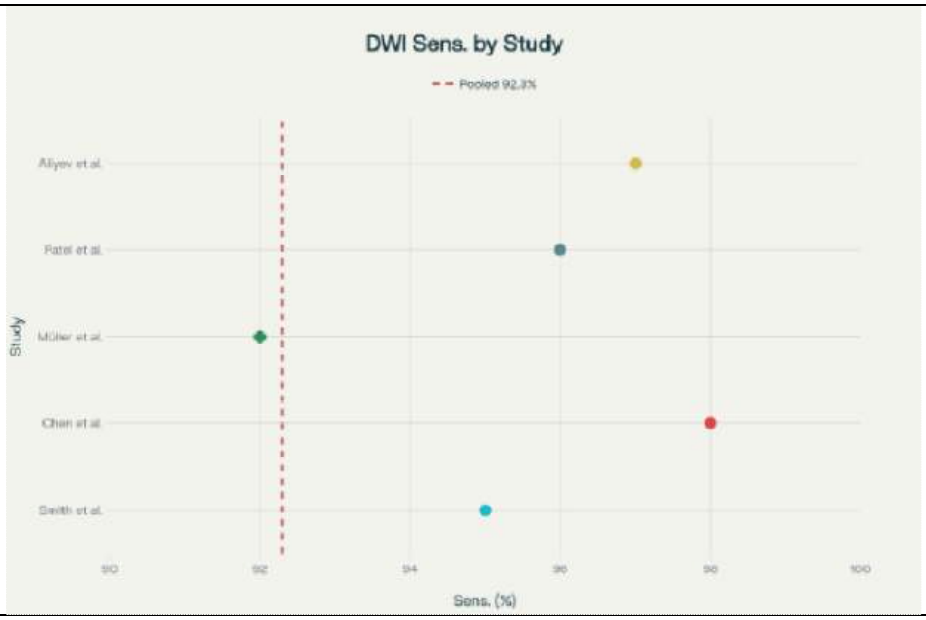



**Figure 1: Forest Plot of DWI Sensitivity Across Studies**

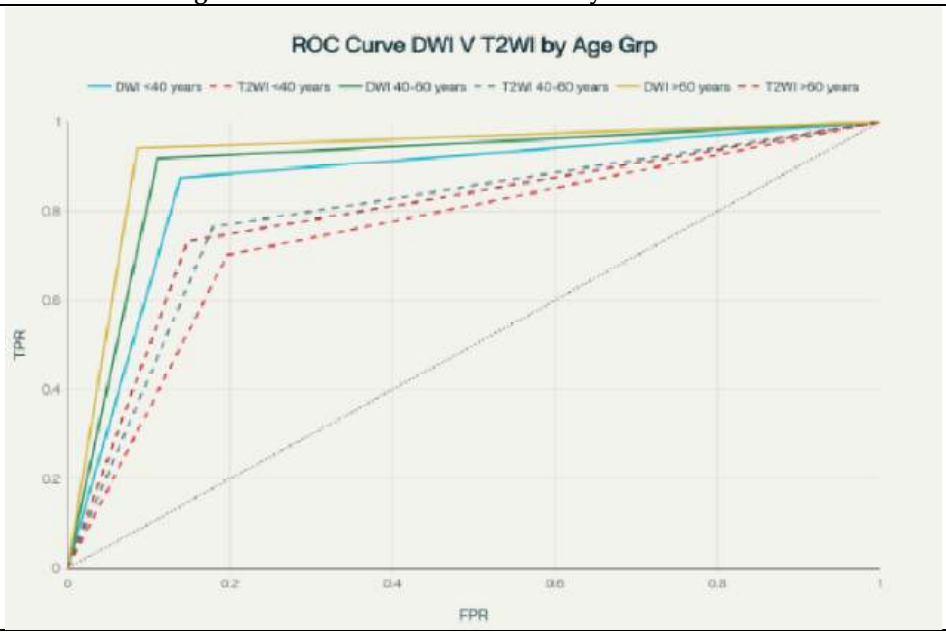



**Figure 2: ROC Curves Comparing DWI and T2WI Across Age Groups**

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CASE STUDY ARTICLE

# Stabilization of Progressive Keratoconus through Ayurvedic Management : A Clinical Case Report

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## ABSTRACT

Keratoconus (KC) is a progressive corneal thinning disorder, correlated in Ayurveda with *Vataja Timira* (~Distortion of vision due to *vatadosa*) due to *Vata* vitiation in the *Krishna Mandala* (~Cornea and Iris). Modern management involving corneal collagen cross-linking (CXL), is often invasive and does not guarantee corneal thickening. This report details the 20-month management of a 28-year-old male diagnosed with progressive bilateral KC (OD: Severe, Kmax 61.4D; OS: Moderate, Kmax 52.1D), who opted for Ayurvedic treatment over CXL. The treatment protocol, administered over three inpatient phases, centered on *Shodhana* (~purification) using *ChakṣusyaVasti* (~Medicated Enema) and *Anu TailaNasya* (~Medication through nasal route), followed by intensive *Bṛmhaṇa* (~nourishing) *Kriyakalpas* (~ocular therapeutic procedures) like *Netra Tarpana* (~soothing collyrium) with *Dashamoola Jeevanthyadi Ghritha* and *Piṇḍa Sveda* (~poultice therapy) with *Shashtikashali*, augmented systemically with collagen-supportive nutraceuticals. Functionally, the patient experienced significant clinical improvement: visual acuity improved from 6/18 to 6/9 (OD) and 6/6 (OS), and the debilitating symptom of diplopia was eliminated. The required spherical correction in the right eye reduced dramatically from +3.00D to 0D. Anatomically, the treatment successfully halted the expected progressive thinning, evidenced by an overall increase in central corneal thickness (Pachymetry) by approximately +8µm in both eyes. The

maximum keratometry (K max) remained stable, showing minimal change (OD: +0.5D; OS: +0.2D). This case supports that the integrated Ayurvedic regimen, focusing on localized ocular nourishment and systemic detoxification, is a viable, non-invasive option for achieving both anatomical stability and significant functional visual improvement in patients with progressive Keratoconus.

**Keywords:** Keratoconus, *Nasya, Tarpana, Timira, Kriyakalpa, Shashtika Shaali Pinda Sweda*

## INTRODUCTION

Keratoconus (KC) is a progressive disorder characterized by central or paracentral corneal stromal thinning, accompanied by apical protrusion and irregular astigmatism. The prevalence is reported as 0.1% to 0.2%, with men being slightly more affected than women. Presentation commonly occurs during the teens or twenties, often initially affecting one eye. Approximately 50% of normal fellow eyes will progress to KC within 16 years. KC can be graded by the highest axis of corneal power on keratometry as mild (<48D), moderate (48–54D), or severe (>54D). Most patients do not have a family history, with only about 10% of offspring developing KC. Risk factors include allergy, asthma, eczema, and persistent eye rubbing from any cause. Systemic associations include Down, Ehlers-Danlos, and Marfan syndromes, and Osteogenesis imperfecta. Ocular associations include vernal keratoconjunctivitis, blue sclera, aniridia, Retinitis Pigmentosa, and Leber congenital amaurosis. Modern management involves avoidance of eye rubbing and spectacle correction. Corneal collagen cross-linking (CXL) with riboflavin may slow the progression of the disease but will not reverse the damage caused to the cornea. Modern management requires invasive therapy and cannot guarantee corneal thickening.(1)

According to *Acharya Sushrutha*, *Krishna mandala* (~cornea and iris) is originated *from Vayu mahabhootha*(~air element), and *Vatadosha* is responsible for its regular functions.(2) Any abnormality in *Vatadosha* leads to a disturbance in the normal functions of *Krishna mandala*. In Ayurveda, there is no direct reference for KC, but based on symptoms, it can be correlated to *VatajaTimira*(~Distortion of vision due to Vatadosa). Symptoms like *Vyavidhadarshana* (~the straight line of object appears to be curved), *Avila darshana* (~blurred or hazy vision), and *Chandra deepadyaanekatwam* (~luminous objects appearing multiple) are observed in keratoconus.(3) Ayurveda treatments not only manage symptoms but also aim to strengthen the cornea to prevent further progression, working at a cellular level to heal and restore ocular function naturally, thereby offering a non invasive,sustainable and physiology restorative alternative to conventional modern interventions.

**CASE REPORT**

**Patient Information**

The patient is a 28-year-old male from Jharkhand, employed as an engineer, who presented with gradual loss of vision over the past three years, accompanied by diplopia (doubling of objects) and glare. He had previously been diagnosed with bilateral keratoconus via corneal topography and was advised to undergo collagen cross-linking (C3R) but was apprehensive about the procedure. Informed consent for both treatment and publication of this case report was obtained. His medical history was unremarkable, with no known history of diabetes mellitus or hypertension. Family history revealed that his father is hypermyopic, with no other significant conditions reported. The patient follows a vegetarian diet, has sound sleep, and maintains a sedentary lifestyle, with no history of substance abuse. He avoids rubbing his eyes as a self-care measure. The main medical problem is progressive bilateral keratoconus, and he had not received any previous medication specifically for this condition prior to presentation

**Clinical Findings**

On general examination, blood pressure measured was 120/86 mmHg and pulse rate was an average of 86/min. No other systemic involvement was found. On ocular examination, distant visual acuity was 6/18 in both eye with

doubling and no improvement in pinhole. Best corrected visual acuity was 6/12+2 in right eye and 6/9+2 in left eye. In slit lamp examination, corneal ectasia was observed and on retinoscopy, scissoring reflex was present in both eye. Other examinations were normal.

**AshtavidhaPariksha(~Eight-Fold Examination):-**The *Naadi* (~Pulse) was *Vata* predominant. Both *Mootra* (~Micturition) and *Mala* (~Defecation) were *Prakritha* (~normal). The *Jihwa* (~Tongue) was *Aliptha* (~uncoated), and the *Shabda* (~Voice) was *Prakritha* (~normal). The *Sparsha* (~Touch) was *Anushnasheeta* (~normal). The *Drik* (~Vision) was *Vikrutha* (~abnormal), while the *Aakrithi* (~Body Structure) was *Prakritha* (~normal).

**DashavidhaPariksha (~Ten-Fold Examination):**The *Prakriti* (~Constitution) was *Vata Pitta*, and the *Vikriti* (~Pathology) indicated *Vatapradhanatridosha*. The *Sara* (~Quality of tissue), *Samhanana* (~Structural integrity), *Satwa* (~Mental status), and *Satmya* (~Adaptability) were all *Madhyama* (~Moderate). The *Pramana* (~Measurement) recorded a height of 168 cm and a weight of 62 kg. The *Ahara Shakthi* (~Digestive capacity) showed *Abhyavaran Shakthi* and *Jarana Shakthi* as *Madhyama* (~Moderate). The *Vyayama Shakthi* (~Exercise tolerance) was *Madhyama* (~Moderate), and the *Vaya* (~Stage of life) corresponded to *Youvana* (~Young age).

**Relevant Ocular Clinical Findings (Baseline): Table 1**
**Figure 1**-CT findings on 26-08-22 of OD
**Figure 2**- CT findings on 26-08-22 of OS

***SrotoPariksha* (~Channel Examination):**
*Srotas* involved: *Vataja* (due to *Vatapradhanavikriti*in *Krishna Mandala, Rasa* and *Rakta* (due to *Timira* pathology).

**Timeline: Table 2**
**Therapeutic Interventions: Table 3**
**Changes in intervention (with rationale)**
In Phase 2, the tablet RuxoNewten (containing eggshell membrane, *Punarnava*, undenatured type II collagen, and vitamin C) was introduced into the systemic regimen. The rationale was to enhance collagen synthesis and support corneal cross-linking and remodeling, thereby promoting greater biomechanical stability. This approach aligns with the Ayurvedic therapeutic objective of *Bṛṃhaṇa*(~Nourishing therapy) and *Dhatu Poṣaṇa*(~Nourishment of tissues of body)—strengthening and nourishing the corneal tissues.

**Diagnostic Assessment:**
The primary diagnostic evidence (corneal topography) was obtained outside the treating facility in Jharkhand, indicating a limitation in access to continuous advanced ophthalmic monitoring. Despite these constraints, the patient chose to pursue an alternative Ayurvedic approach rather than the advised corneal collagen cross-linking (CXL), overcoming both financial and cultural barriers to care.The patient's gradual decline in vision, along with diplopia and glare, and the presence of corneal ectasia with a scissoring reflex, confirmed the modern diagnosis of keratoconus. From an Ayurvedic perspective, the progressive corneal thinning (*Kṣaya*), visual distortion (*Vikṛtadṛk*), and symptoms such as *Vyaviddhadarshana* reflect predominant *Vatadoṣa* vitiation affecting the *KṛṣṇaMaṇḍala* , thereby correlating with *VatajaTimira*.Keratoconus is an inherently progressive disease. The baseline maximum keratometry (*Kmax*) values of 61.4 D (OD) and 52.1 D (OS) denote severe keratoconus in the right eye and moderate involvement in the left eye, indicating an unfavourable natural prognosis for maintaining functional vision without therapeutic intervention.

**Follow up and Outcome:**
Patient was treated on IPD basis for three distinct periods over 16 months (Jan '23, Nov '23, May '24).Functional Gains: Clinically significant improvement in DVA (OD: 6/18→6/9; OS: 6/18→6/6), BCVA (OS: 6/9+2→6/6), and a favorable reduction in required spherical correction (OD: +3.00D→0D). The patient-reported symptom of diplopia was eliminated, which is a major quality-of-life improvement.Anatomical Stabilization: The total change in

maximum corneal curvature (Kmax) was minimal over 20 months (OD: +0.5D; OS: +0.2D), indicating relative stability against the expected rapid progression.Corneal Thickening: Crucially, the central corneal thickness (Pachymetry) showed an overall increase of ~8µm in both eyes (OD: 467→475µm; OS: 479→487µm). This is a positive finding, counteracting the primary mechanism of progressive corneal thinning.Intervention Adherence and Tolerability: The patient underwent three inpatient courses of treatment over 16 months, indicating good adherence to the intensive therapies. All systemic and local interventions were well-tolerated, with no adverse or unanticipated events reported. Tolerability was assessed via daily patient interviews and observation during the IPD stay.

**Functional outcomes :Table 4**
**Anatomical outcomes: Table 5**
**Figure 3**- CT findings on 24-04-23 of OD
**Figure 4**- CT findings on 24-04-23 of OS
**Figure 5**- CT findings on 06-05-24 of OD
**Figure 6**- CT findings on 06-05-24 of OS

## DISCUSSION

This case report shows the successful application of an integrated Ayurvedic protocol in managing progressive Keratoconus (KC), a degenerative disorder characterized by corneal thinning and visual impairment. The management protocol was guided by the Ayurvedic correlation of KC symptoms to *VatajaTimira*, necessitating *VataShamana* and *Brumhana* therapies directed at the *Krishna Mandala*.

**Case Management Rationale:** The protocol centered on *Shodhana* using *Vasti* and *Nasya*, followed by intensive *Kriyakalpas* aimed at *Brumhana* and *Chakshushya* (ocular health).

**Shodhana:***Vasti* (enema), particularly *ChakshushyaVasti* with ingredients like *Erandamoola* (rich in antioxidants), is fundamental for removing vitiated *Doshas* (*Vata*), which is crucial in degenerative eye diseases like KC. It is proposed that rectal drainage into systemic circulation allows for enhanced absorption of lipophilic drugs, nourishing the ocular tissues.(6)*DhanwantharamTaila* is a *madhuroushadha siddha taila* which has properties of *Vatashamana, Balya* and *Brimhana*.Studies show that fibroblasts from keratoconus cornea produce more prostaglandin than those from normal corneas.(7)The molecules of *DhanwantharamTaila* is said to render the inactivation of Cox 1 and Cox 2 enzymes, thus stopping the synthesis of prostaglandins which are said to be produced more by cornea having keratoconus.(8)
*Anu TailaNasya* directly acts on ocular tissues, aiming to strengthen the corneal epithelium and delay KC progression.(9)

**Kriyakalpa (Local Therapies):***Netra Seka/Kashaya Dhara* with *Triphala,Raktacandanam*, *Chandanam*, *Laksha*, *Daruharidra*, *Draksha*, *Lodhra*, *Yashtimadhu*, *Gairika*, provides a high concentration of antioxidants, Vitamin C, and minerals, which are essential co-factors for collagen synthesis, thus strengthening the cornea.Symplocosracemosa (*Lodhra*) is proven to be effective in wound healing where in the remodeling phase, tissue tensile strength is increased due to intermolecular cross-linking of collagen via vitamin-C dependant hydroxylation which is the same pathology required in KC treatment.(10)

*Netra Tarpana* with *DashamoolaJeevanthyadiGhritha* encourages corneal healing and improves vision. Various studies show the role of Tarpana in improving visual acuity and reducing other subjective symptoms. (11) The amphipathic nature of *Ghritha* allows deep penetration into the corneal layers. The drugs are rich in Vitamin A-like compounds, preventing epithelial damage and providing targeted nutritional supply to halt degeneration. *Netra PindaSwedana* with *ShashtikaShali* is *Balya* (~strengthening) and *VataShamana*. The gentle mechanical effect provides pressure that may aid in reshaping and strengthening corneal collagen fibers.(12)

*Netra Abhyanga* with *Ksheerabala 101* is *VataShamana* and promotes nerve regeneration and provides strength to muslces due to its *balya*and*brimhana*property and also exerts a gentle mechanical pressure, potentially aiding in flattening the corneal curvature.Bohra, Mohita& Kk, Sharma.(13)

**Systemic Augmentation (Collagen Support):** The introduction of *Tab RuxoNewten* (containing Egg Shell Membrane (ESM), undenatured Type 2 Collagen, and Vitamin C) in Phase 2 was a targeted addition. ESM contains glycosaminoglycans and collagen which are vital in supporting corneal strength and integrity.(14) This, along with Vitamin D3/Magnesium supplementation, aimed to provide the necessary nutritional factors for enhancing corneal cross-linking and remodeling, aligning with the Ayurvedic principle of *Brumhana*.(15)

The positive finding of increased central corneal thickness (~8 μm) and minimal *Kmax* progression is a crucial indicator of successful biomechanical stabilization, a primary goal of modern treatments like CXL.(16) This outcome supports the hypothesis that the combination of Ayurvedic*Shodhana* and *Brumhana* therapies, potentially augmented by collagen-synthesis supporting nutraceuticals, can promote the integrity of the corneal epithelium and enhance collagen synthesis and remodeling, confirming the results of other investigations into the structural benefits of Ayurvedic ocular therapies.(15) The improvement in visual acuity, despite minimal *Kmax* change, strongly suggests that the treatment successfully induced a regularization of the corneal optical surface.

## CONCLUSION

This case report illustrates the favourable outcome of an integrated Ayurvedic management approach in a patient with progressive keratoconus followed for a period of 20 months. The treatment effectively arrested further corneal thinning, demonstrated an increase in central corneal thickness of approximately +8 μm in both eyes, and maintained anatomical stability with minimal changes in maximum keratometry (OD: +0.5 D, OS: +0.2 D). Clinically, the patient experienced significant improvement in visual performance, with Best Corrected Visual Acuity improving by at least one line in both eyes (OS reaching 6/6) and a marked reduction in spherical correction (OD sphere from +3.00 D to 0 D). These findings suggest that localized and systemic therapies may have contributed to enhanced corneal regularity and biomechanical strength. Overall, this case demonstrates the potential role of a structured Ayurvedic protocol as a non-invasive therapeutic option for stabilizing keratoconus progression and improving visual function. Further clinical studies are warranted to validate these observations in a larger cohort.

**Informed Written Consent-** The patient provided informed written consent for publication of this case report. The consent document is available and can be reviewed for verification by the editor of the journal.
**Author Contribution-** Each author has made an equal contribution to the overall care of the patient, including treatment, documentation, and the preparation and development of the manuscript for publication.

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**CONFLICT OF INTEREST-** NIL

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**Table 1: Baseline ocular examination findings of the patient with bilateral keratoconus.**

| Parameter | RE (Right Eye) | LE (Left Eye) |
|---|---|---|
| DVA (Distance Visual Acuity) | 6/18 with doubling | 6/18 with doubling |
| Pinhole | NI (No Improvement) | NI (No Improvement) |
| BCVA (Best Corrected VA) | 6/12+2 with +3.00D,−1.50×70 | 6/9+2 with +1.25D,−0.75×115 |
| Slit Lamp | Clear cornea, Deep AC, Ectasia of cornea | Clear cornea, Deep AC, Ectasia of cornea |
| IOP (Intraocular Pressure) | 16 mm Hg | 17 mm Hg |
| Retinoscopy | Scissoring Reflex | Scissoring Reflex |

**Table 2: Timeline of therapeutic interventions and corneal topography done during different phases of Ayurvedic management in the patient with bilateral keratoconus.**

| Phase | Treatment Dates | Complaints | Intervention | Action and Key Change in Protocol |
|---|---|---|---|---|
| | 20/08/2022 | Blurring of vision with doubling. | N/A | Consultation with modern ophthalmologist |
| | 26/08/2022 | Same complaints | Corneal topography done and diagnosed as Keratoconus | Diagnosis and decision for Ayurvedic |

| | | | | |
|---|---|---|---|---|
| | | | | management. |
| Phase 1 (Initial IPD) | 07/01/2023 to 17/01/2023 | Blurring of vision with doubling. | *ChakṣusyaVasti*(4) (with *Eraṇḍamulakvatha, Tilataila*, etc.), *AnuvasanaVasti* with *DhanwantharamTaila,Nasya* with *Anu taila* (12 drops), *KaṣhayaDhara, Tarpana* with *DasamulaJivantyadighrta, ShashtikashaliPindaSweda, Netra Abhyanga with Ksheerabala101*Tab mac GL D3 gold (OD), C-health *choorna(*~powder)(1 tsp HS with milk). | Initial treatment baseline. |
| | 24/04/2023 | | Corneal topography repeated | |
| Phase 2 | 01/11/2023 to 12/11/2023 | Blurring of vision. Doubling reduced. | *ChakṣusyaVasti*,*AnuvasanaVasti* with *DhanwantharamTaila,Nasya* with *Anu taila* (12 drops),*KaṣayaDhara, Tarpana* with *Jivantyadighrta, ShashtikashaliPindaSweda, Netra Abhyanga with Ksheerabala 101,*Tab mac GL D3 gold (OD), Tab RuxoNewten (Aarux pharmacy) (1 OD), C-health choorna (1 tsp HS with milk). | Addition of Tab RuxoNewten (Rationale: To enhance collagen synthesis and corneal cross-linking effect). (5) |
| | 06/05/2024 | | Corneal topography repeated | |
| Phase 3 (Final IPD) | 22/05/2024 to 30/05/2024 | Same as Phase 2. | Same as Phase 2. | Final assessment and intervention. |

**Table 3: Types of Ayurvedic interventions and their mode of administration in the management of bilateral keratoconus.**

| Type of Intervention | Procedure/Medication | Administration / Dosage / Duration / Ingredients |
|---|---|---|
| **Systemic (Oral)** | Vitamin D3 Capsule (Tab mac GL D3 gold) | 1 OD (Once Daily). Duration: Throughout the follow-up period |
| | C health*choorna*(Powder) | 1 tsp at night with warm milk. Duration: Throughout the follow-up period |
| | Egg Shell Supplement Capsule (Tab RuxoNewten) | 1 OD (Once Daily). Started in Phase 2 |
| **Systemic- Vasti** | *ChakshushyaVasti (Niruha)*(~Medicated Enema) | Ingredients: *Erandamoolakvatha, Tilataila, Shathapushpa-Yashtimadhukalka, Saindhava*. Administered over the 8–10-day IPD course in each phase |
| | *AnuvasanaVasti*(~Medicated Enema) | With *DhanwantharamTaila*. Administered over the 8–10-day IPD course in each phase |
| **Nasya** | *Nasya*(~Medication through nasal route) | *Anu taila*, 12 drops in each nostril, followed by *Kavala* with *uṣṇajala* and *Dhoomapana* with *Haridravarti*. Administered over the 8–10-day IPD course in each phase |
| **Local (Kriyakalpa)** | *Netra Seka (KaṣayaDhara)*(~Ocular irrigation) | Ingredients: *Triphala* augmented with *Raktacandanam, Chandanam, Laksha, Daruharidra, Draksha, Lodhra, Yashtimadhu, Gairika*. Duration: ≈20–30 min in each phase |
| | *Netra Tarpana*(~Soothing collyrium) | *DashamoolaJivantyadiGhṛta.* Duration: Held for ≈20-30 min in each phase |
| | *Netra PiṇḍaSwedana*(~Poultice) | With *ShashṭikaShali* bolus. Applied gently over closed eyes |

**Table 4: Functional outcomes showing changes in visual acuity and refraction following successive phases of Ayurvedic management in bilateral keratoconus.**

| Date | Eye | DVA (Uncorrected) | BCVA (Best Corrected) | BCVA Sphere (D) | BCVA Cylinder (D) | BCVA Axis (∘) |
|---|---|---|---|---|---|---|
| 07-01-23 (Baseline) | OD | 6/18 with diplopia | 6/12+2 | +3.00 | −1.50 | 70 |
| | OS | 6/18 with diplopia | 6/9+2 | +1.25 | −0.75 | 115 |
| After 1st Tx (Jan 2023) | OD | 6/9 | 6/9+2 | N/A | N/A | N/A |
| | OS | 6/9 | 6/6 | N/A | N/A | N/A |
| After 2nd Tx (Nov 2023) | OD | 6/9P | 6/9 | +3.25 | −1.25 | 30 |
| | OS | 6/6P | 6/9 | +2.00 | −0.75 | 115 |
| After 3rd Tx (May 2024) | OD | 6/9 | 6/9+2 | 0 | −1.00 | 60 |
| | OS | 6/6 | 6/6 | +1.00 | −1.50 | 110 |

**Table 5: Anatomical outcomes based on corneal topography and pachymetry findings before and after Ayurvedic management in bilateral keratoconus.**

| Date | Eye | K1 (D) | K2 (D) | Astigmatism (ΔK) (D) | Pachy (μm) | Kmax (D) |
|---|---|---|---|---|---|---|
| 26-08-22 (Pre-Tx) | OD | 43.7 | 48.3 | 4.6 | 467 | 61.4 |
| 24-04-23 | OD | 43.9 | 47.9 | 4.0 | 482 | 61.7 |
| 06-05-24 (Final) | OD | 43.8 | 48.6 | 4.8 | 475 | 61.9 |
| 26-08-22 (Pre-Tx) | OS | 41.7 | 42.7 | 1.0 | 479 | 52.1 |
| 24-04-23 | OS | 41.8 | 43.2 | 1.4 | 490 | 52.3 |
| 06-05-24 (Final) | OS | 42.2 | 43.6 | 1.4 | 487 | 52.3 |

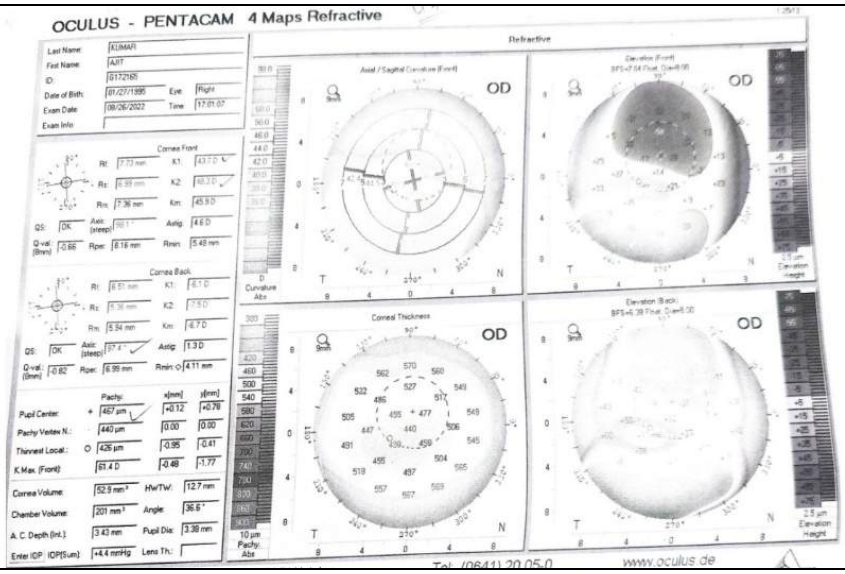



**Figure 1-CT findings on 26-08-22 of OD**

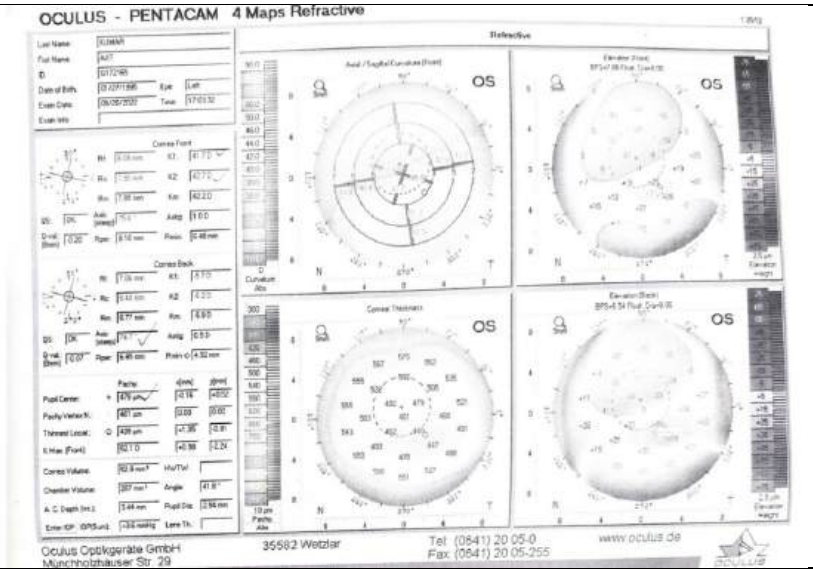



**Figure 2- CT findings on 26-08-22 of OS**

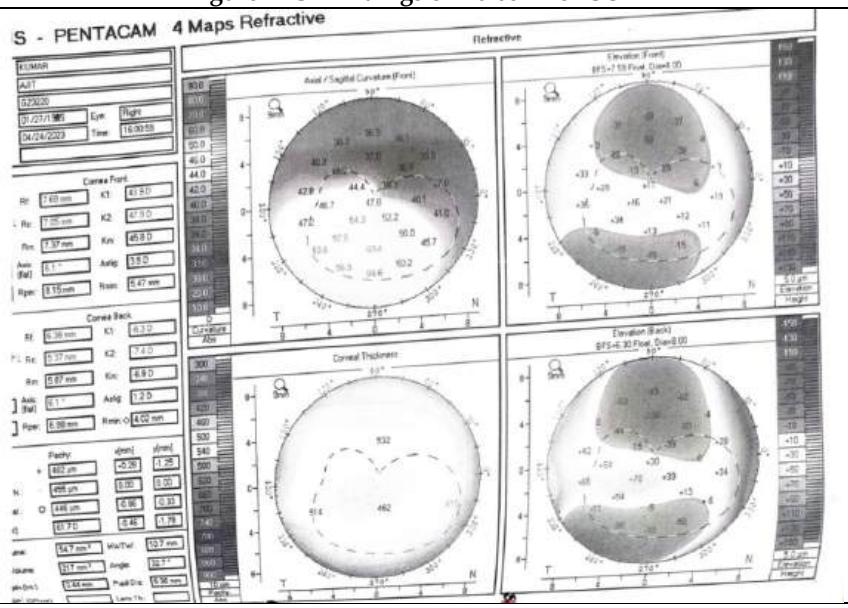



**Figure 3- CT findings on 24-04-23 of OD**

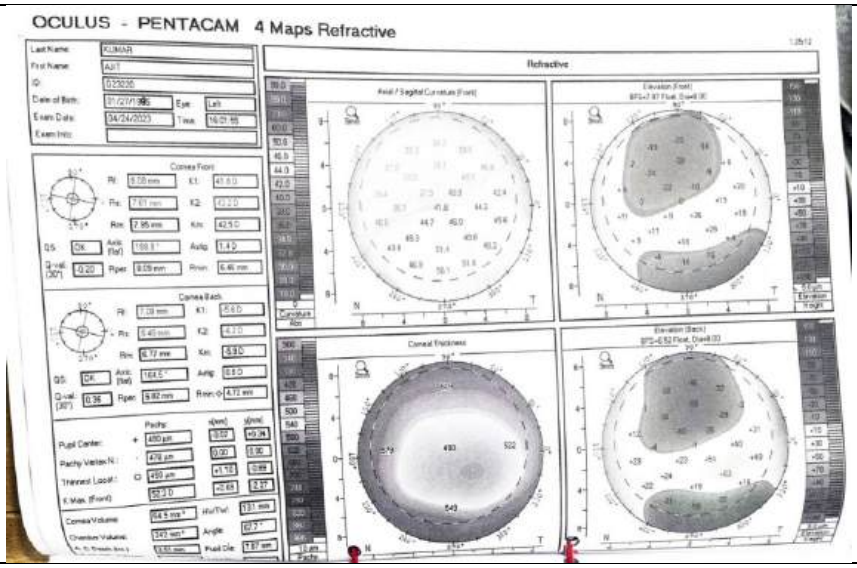



**Figure 4- CT findings on 24-04-23 of OS**

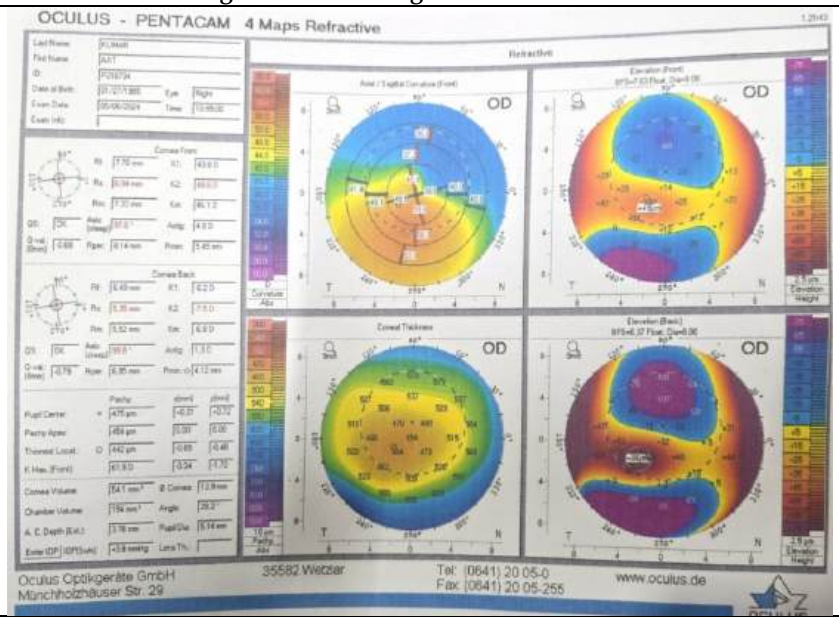



**Figure 5- CT findings on 06-05-24 of OD**

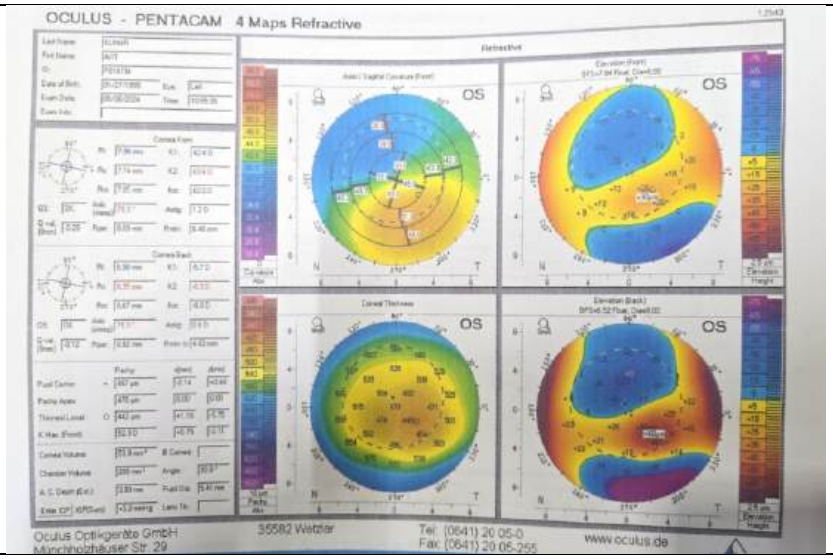



**Figure 6**- CT findings on 06-05-24 of OS

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RESEARCH ARTICLE

# Multi-Method Optimization of EOT Crane Girders : Integrating Finite Element Analysis with Experimental Validation for Material Efficiency

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**ABSTRACT**

This research evaluates the structural performance and dynamic behavior of an Electric Overhead Traveling (EOT) crane girder using Finite Element Analysis (FEA) and empirical validation. The girder is the primary load-bearing component and is prone to failure under cyclic industrial loading. Using Response Surface Methodology (RSM) for design optimization, a reduced plate thickness of 7.4 mm was identified as the optimal configuration. This modification resulted in a 9.5% reduction in total weight (156 kg) while maintaining structural safety within a Factor of Safety (FOS) of 1.5. Validation results demonstrated high correlation between FEA and experimental data, with stress and deflection variances restricted to less than 3% and 5% respectively.

**Keywords:** EOT Crane, Girder Optimization, Finite Element Analysis (FEA), Response Surface Methodology, Experimental Validation.

## INTRODUCTION

EOT cranes are indispensable for heavy-load transportation in modern industrial environments. The bridge girder, spanning two rails, serves as the critical structural element. Conventional manual calculations, while fundamental, often fail to account for localized stress concentrations and complex dynamic behaviors under variable loading

cycles. This study bridges the gap between theoretical estimation and real-world performance by integrating manual calculations with FEA simulations and actual site measurements. The objective is to determine the minimum web and flange thicknesses required to sustain operational loads while maximizing material efficiency.

## METHODOLOGY

### Technical Specifications and Experimental Setup

The study analyzed a 10-ton capacity girder using Mild Steel (IS 2062 A / FE 410W) with a yield stress of 255 MPa and ultimate tensile stress of 410 MPa. Strain gauges were deployed at critical mid-span points to monitor real-time stress during a load test of 11.2 tons.

### Analytical Framework

Manual calculations were performed based on IS:807-2006 standards. The analysis considered:

- **Vertical Bending Moments:** Calculated with and without impact factors (IF) for both drive and trailing side girders.
- **Horizontal Moments:** Derived from acceleration/deceleration forces and wind loads for outdoor scenarios.
- **Allowable Stresses:** Defined based on material properties and a safety factor.

### Crab/Trolley (CT) Wheel Load Calculations

The analysis determines wheel loads under various operational conditions to understand the forces exerted on the girder rails.

- With Impact: Calculated using an Impact Factor (IF), the maximum load ($T_1$) on the drive side is 9.90T.
- **Without Impact:** The steady-state load ($T_{11}$) is **8.30T**.
- Acceleration/Deceleration Forces: A horizontal force factor of 0.045 is derived based on the Long Travel (L.T.) speed, resulting in an acceleration load ($T_{1ACC}$) of 0.49T.

### Bending Moment Analysis

Bending moments are calculated for both the drive and trailing side girders to identify the critical stress points.

- **Live Load (With Impact):** The maximum vertical bending moment (BMVD) for both girders is **32.74 T-M**.
- **Uniformly Distributed Load (UDL):** The dead weight of the girder, platforms, and electrical contributes **11.34 T-M** for the drive side and **9.74 T-M** for the trailing side.
- **Total Vertical Bending Moment:** Summing all vertical forces results in a total of **45.80 T-M** for the drive side and **44.05 T-M** for the trailing side.

### Stress and Section Verification

The paper uses these moments to verify the structural integrity of the selected girder section.

- **Allowable Stresses:** Based on Mild Steel (IS 2062 A), the allowable bending tensile stress is calculated at **1603.77 kg/cm²** using a factor of safety and duty factor.
- **Section Proportions:** The girder geometry is checked against standard limits. For example, the Span to Girder Depth ratio is **20.10**, which is within the acceptable limit of 25.
- **Deflection Limits:** The allowable total deflection is set at **44.44 mm** (Span/360), which the current section satisfies.

### FEA and Optimization Strategy

A 3D model was developed and meshed in ANSYS to determine Von Mises stress and deflection.

- **RSM Optimization:** Response Surface Methodology was utilized to evaluate plate thickness as a design variable. **Convergence:** Mesh size was optimized to ensure computational accuracy without sacrificing detail at high-stress zones.

## RESULTS AND DISCUSSION

**Comparative Validation**

The initial experimental site measurement recorded a deflection of 17 mm under 11.2T loading. FEA simulations closely matched these findings:

- **Stress Correlation:** The variance between experimental and simulated stress was <3%.
- **Deflection Accuracy:** Observed variance remained <5%, confirming the reliability of the finite element model.

**Optimization Results**

The parametric study focused on reducing plate thickness to achieve material savings without compromising the allowable deflection limit (SPAN/900).

- **Optimal Configuration:** A thickness of 7.4 mm was found to satisfy all safety requirements.
- **Weight Efficiency:** This optimization successfully removed 156 kg of surplus material, equivalent to a 9.5% reduction in girder weight.
- **Dynamic Stability:** Vibration analysis confirmed that the first mode frequency remained above the resonance threshold, ensuring operational endurance.

## CONCLUSION

This research demonstrates that integrating FEA with Response Surface Methodology allows for significant material optimization in heavy industrial equipment. The study successfully validated that a 7.4 mm plate thickness maintains the required structural integrity for a 10-ton EOT crane while reducing weight by 9.5%. The high correlation between manual, simulated, and experimental results provides a robust framework for manufacturers to design safer and more cost-effective crane structures.

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**Table 1: Key Parameters**

| Parameter | Value |
|---|---|
| Web Height | 780 mm |
| Web Thickness | 8 mm |
| Flange Width (Top/Bottom) | 435 mm |
| Flange Thickness (Top/Bottom) | 8 mm |
| Box Opening | 375 mm |

**Table 2: Calculated Values of Load**

| Load Parameter | Condition | Calculated Value (Tons) |
|---|---|---|
| Max Wheel Load (T1/T3) | With Impact | 9.90 T |
| Steady-State Load (T11/T33) | Without Impact | 8.30 T |
| Acceleration Load (T1ACC) | Horizontal Force | 0.49 T |
| Minimum Wheel Load (T2/T4) | With/Without Impact | -2.30 T |

**Table 3: Calculated Values of Bending Moment**

| Bending Moment Component | Drive Side Girder (T-M) | Trailing Side Girder (T-M) |
|---|---|---|
| Live Load (With Impact) | 32.74 T-M | 32.74 T-M |
| Live Load (Without Impact) | 26.35 T-M | 26.35 T-M |
| Uniformly Distributed Load (UDL) | 11.34 T-M | 9.74 T-M |
| Acceleration Load (BMACC) | 1.57 T-M | 1.57 T-M |
| Total Vertical BM (With Impact) | 45.80 T-M | 44.05 T-M |

**Table 4: Sectional Properties.**

| | |
|---|---|
| $I_{XX}$ | $171321.6cm^4$ |
| $I_{YY}$ | $56748.67cm^4$ |
| $Z_{XXT}$ | $4304.56cm^3$ |
| $Z_{XXB}$ | $4304.56cm^3$ |
| $Z_{YY}$ | $2609.13cm^3$ |
| Area | $194.4cm^2$ |
| WT/M | 152.6Kg/m |
| $C_{XX}$ | 39.8cm |
| $C_{YY}$ | 21.75cm |

**Table 5: Girder Section Proportions & Material Properties**

| Parameter | Formula/Reference | Result | Status |
|---|---|---|---|
| Span to Depth Ratio | SPAN / D | 20.10 | OK (Limit < 25) |
| Span to Width Ratio | SPAN / (B + St) | 41.78 | OK (Limit < 60) |
| Yield Stress (Ys) | IS 2062 A | 2550 kg/cm² | Base Property |
| Allowable Tensile Stress | (Ys x DF) / SF | 1603.77 kg/cm² | Design Limit |

**Table 6: Mesh Convergence Study.**

| Mesh Size(mm) | Maxvon Mises Stress (MPa) | No.of Elements |
|---|---|---|
| 35 | 176.2 | 38,942 |
| 30 | 174.1 | 42,008 |
| 25 | 172.1 | 46,190 |
| 20 | 171.2 | 49,842 |
| 15 | 173.2 | 54,058 |
| 10 | 175.3 | 57,124 |

**Table 7: Optimization Summary**

| Optimization Metric | Initial Design | Optimized Design | % Improvement |
|---|---|---|---|
| Plate Thickness | N/A | 7.4 mm | Required Minimum |
| Total Weight Reduction | Baseline | -156 kg | 9.5% |
| Factor of Safety (FOS) | 1.5 | 1.5 | Maintained |
| Deflection (11.2T) | 17 mm | Within Limits | Validated |

**Table 8: Verification of FEM versus Experimental Results.**

| Parameter | FEM Result | Experimental Result | Deviation (%) |
|---|---|---|---|
| Maximumvon Mises Stress | 171.22MPa | 167.30MPa | 2.29% |
| Maximum Deflection (Total) | 33.46mm | 35.05mm | 4.53% |




**Figure 1. Structure of Crane Parts**




**Figure 2. Actual site measurement of deflection while carrying load capacity 11.2 T.**

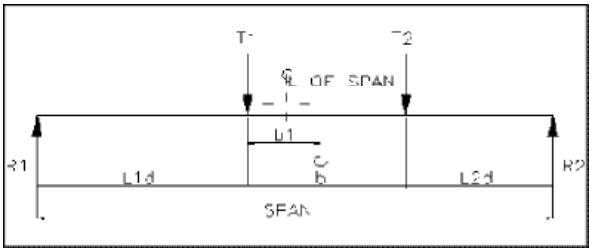



**Figure 3. Bending moment due to live load on driver side.**

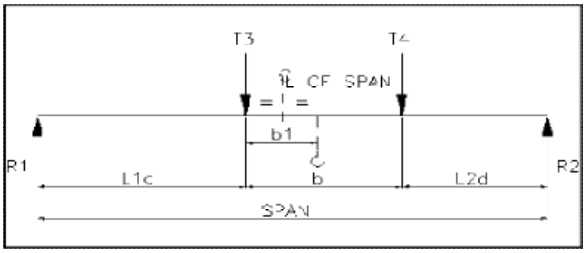



**Figure 4. Bending Moment due to live load on the trailing side**

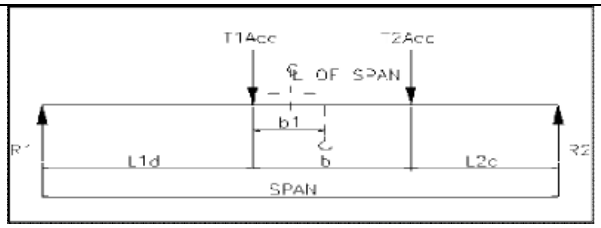



**Figure 5. Bending moment due to live load (without impact).**

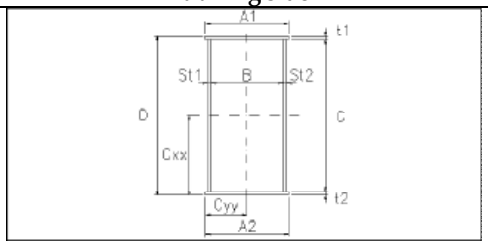



**Figure 6. Girder section (plate dimensions).**

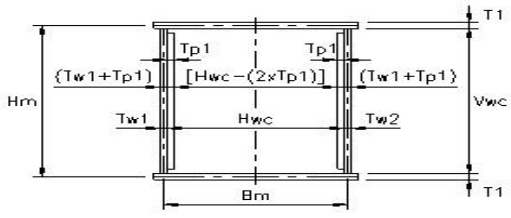



**Figure 7. Girder neck section.**

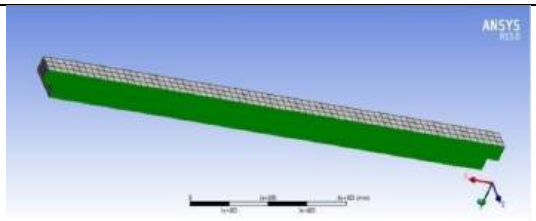


**Figure 8. Meshed FEM model.**

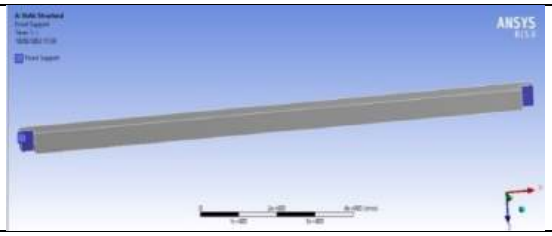


**Figure 9. Applied boundary conditions.**

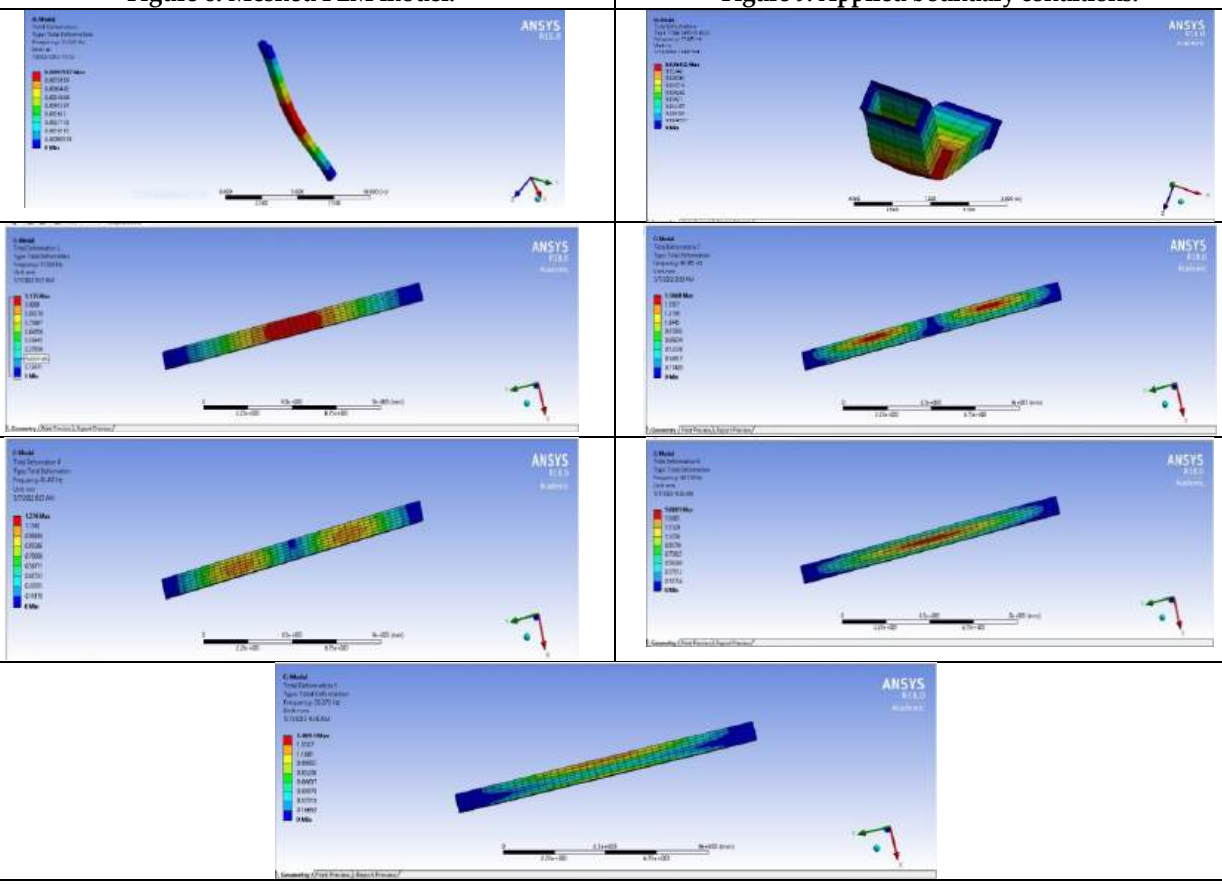


**Figure 10. Deformation Patterns.**

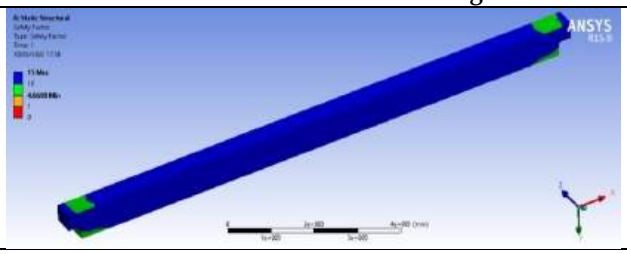


**Figure 11. Safety Factor Distribution**

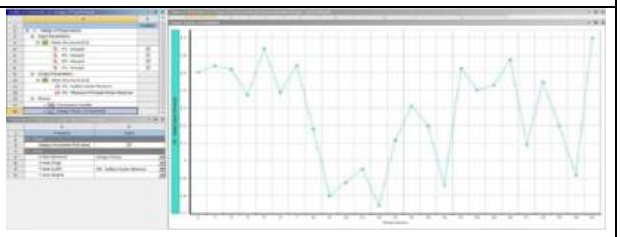


**Figure 12. Design Points vs. Parameters**

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RESEARCH ARTICLE

# The Culturable Bacteriology and 16S rRNA Analysis of Cauvery Water, Delta Region in Thanjavur, Tamil Nadu, India

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## ABSTRACT

The human population depends on surface water for agricultural irrigation and a continuous drinking water supply. India uses about 690 billion cubic meters (BCM) of surface water annually. Tamil Nadu has a total surface water potential of 36 km, or 24864 M cum. The state has 17 large river basins, 61 reservoirs, and roughly 41,948 tanks. Surface flows make up roughly half of the 46540 million cubic meters (MCM) of water potential that exists each year. The present study attempted to identify the various culturable bacteria in Cauvery River (Station 1) and Coleroon River (Station 2), Tanjavur District, Tamil Nadu, India, and conformed to Molecular characterization. A total of 20 bacteria from 13 genera were identified; five species belonged to the genus *Bacillus,* and two each to the genera *Salmonella* and *Klebsiella*. There was just one species in each of the other genera. In both stations, the majority of the bacteria grow throughout the summer, while the pre-monsoon and post-monsoon bacterial populations are modest and the bacterial population is low during the monsoon season. The bacterial isolates are then confirmed using 16s rRNA Analysis, and the subcultured colonies are recognised using morphological and biochemical methods. The 20 isolates (MS1 to MS20) were identified by BLASTn results, and taxonomy designations were given based on the NCBI database's nearest neighbour. Twenty isolates, PV668924 to PV668943. The

phylogenetic connection of the 20 isolates, with an ideal branch length total of 1.227. Both trees are depicted as legends in the illustration and are drawn to scale.

**Keywords :** Cauvery River, Coleroon River, Bacteria, 16s rRNA and Phylogenetic analysis.

## INTRODUCTION

The human population depends on surface water for agricultural irrigation and a continuous drinking water supply. India uses about 690 billion cubic meters (BCM) of surface water annually. This surface water contributes to 1121 BCM of exploitable water resources, together with roughly 431 BCM of groundwater. Forty-three percent of the Ganga, Brahmaputra, and Meghna river systems are part of the catchment area of India's major river systems. The Indus, Sabarmati, Mahi, Narmada, Tapi, Brahmani, Mahanadi, Godavari, Krishna, Pennar, and Cauvery are other noteworthy river systems. With a catchment area of 1.9 million hectares, Subernarekha is the largest of these medium river systems [1,2]. Tamil Nadu has a total surface water potential of 36 km, or 24864 M cum. The state has 17 large river basins, 61 reservoirs, and roughly 41,948 tanks. Surface flows make up roughly half of the 46540 million cubic meters (MCM) of water potential that exists each year. Since irrigation is the biggest user, the majority of the surface water has already been tapped. Through major, medium, and small schemes, surface water is used to irrigate 24 lakh hectares. Approximately 90% of surface water is used for irrigation.

In terms of agriculture and replenishing its water supplies, monsoon precipitation has been India's lifeblood. An average of 4000 BCM of precipitation and snowfall falls on India each year, with 3000 BCM falling during the monsoon season (June to September). Precipitation varies greatly across the nation, ranging from less than 100 mm in Rajasthan to more than 2500 mm in Assam [3]. Tamil Nadu has seventeen river basins. The only significant basin is the Cauvery. There are three minor river basins and thirteen medium river basins. The State generates 692.78 TMC (19,619 MCM) of surface water annually with a reliability of 75 percent. The State receives a significant amount of its annual flow from its bordering States, around 261.70 TMC (7411 MCM). The catchment area of the Cauvery River Basin is 81155 km2, with an average water resource potential of 21.36 BCM. Resources of usable water: 19 BCM. In addition to providing recreational opportunities, surface water has been contributing significantly to global development by fostering economic expansion and improving the health of people and animals (aquatic and terrestrial), whose existence depends on a healthy habitat ecosystem. According to studies, the ecosystem's activities may be disrupted if its microbial diversity declines [4].

One of the main environmental concerns in the aquatic ecosystem is pollution, which alters the nutrient cycle and water quality indicators [5]. According to estimates, the annual cost of the limited freshwater ecosystem services caused by poor water quality to the world economy is more than $1.7 trillion [6]. According to Judd *et al.* [7], Singh *et al.* [8], and Bailey *et al.* [9], bacteria are a significant contributor to climate change that is influenced by feedback responses. Climate change may intensify minor bacterial processes that impact the stability of ecosystems [10]. Over time, alterations in the ecosystem's physico-chemical components change the structure and composition of bacterial communities and lead to the development of biogeographical patterns [11,12,13]. It is possible to think of these biogeographical patterns as a gauge of past environmental shifts. The response of bacteria to future climatic changes is yet unknown, despite the growing number of microbiome studies [14]. Hence, the present study attempted to identify the various culturable bacteria in Cauvery River (Station 1) and Coleroon River (Station 2), Tanjavur District, Tamil Nadu, India, and conformed to Molecular characterization

## MATERIALS AND METHODS

Water samples for microbiological testing were gathered, aseptically transferred into sterile 500 ml sampling bottles, and analysed in the lab within one to two hours of collection. The American Public Health Association [15] guidelines were followed for analysing all water samples for the presence of pathogenic Salmonella, faecal Streptococci, and total and faecal coliform bacteria using the most probable number (MPN) method. After 48 hours of incubation at 28°C, nutrient agar plates were used to measure the total viable count (TVC) of all heterotrophic bacteria. After that, 0.1 ml of the homogenate was plated onto nutrient agar and incubated for 24 to 48 hours at 37°C. Fresh solid media of modified faecal coliform (M-FC) agar were injected in duplicate and incubated at 37°C for 24 hours in order to qualitatively identify different bacteria from water samples. Following the development of different coloured colonies of different bacteria on the plates, the bacterial colonies were identified using the methods [16,17,18. 19,20]. Colonies were moved to NA plates for the purification process, and subcultures were carried out repeatedly until the strains were conglomeration-free. Every isolated strain was kept in permanent storage at 4°C in NA stab cultures and at -20°C in 80% glycerol stock.

**Biochemical characterization**

Each purified strain was subjected to a total of fourteen biochemical tests, including those for determining Gramme Character, Methyl Red (MR), Gelatinase, Triple Sugar Iron (TSI), starch degradation, Voges-Proskauer (VP), indole production, citrate, oxidase, motility tests, etc., in accordance with Bergey's Manual of Systematic Bacteriology [21].

**Molecular Characterization**

A bacterial genomic DNA isolation kit (GCC Biotech, India) was used to separate the genomic DNA from the cultivated bacterial strains. To determine purity, genomic DNA was electrophoresed on a 0.8% agarose gel and quantified spectrophotometrically at 260 nm. A Gradient Thermal Cycler (Biorad Laboratories, USA) was used to amplify the 16S rRNA gene in the samples. In combination with 2X PCR MasterMix (Thermo Fisher Scientific, India), universal primers 27f (5'- AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'-GGTTACCTTGTTACGACTT-3') covering the V1-V9 hypervariable region of 16S rDNA were employed for PCR amplification. After 30 cycles of PCR amplification, the amplicons (around 1.4 kbp) were examined using Ethidium Bromide dye on a 1.5% agarose gel. The Agarose Gel purification kit (NEB, USA) was used to purify the amplicons, and PAR Life Sciences and Research Pvt. Ltd. (Tiruchirappalli) performed the sequencing.

**In–silico analysis**

BLASTn (NCBI database) was used to examine the sequences in-silico; the hits were noted in order to identify the nearest neighbour with the greatest Max score. For MEGA 12's subsequent studies, the aligned sequences were acquired in the FASTA format. The CLUSTALW technique was utilised for alignment analysis, and the resulting data were taken into consideration for Neighbor-Joining phylogenetic analyses. The Maximum Likelihood Composite approach was used to determine the evolutionary distance, and base substitution per site was used to get the branch lengths. The consensus tree representing the evolutionary history of the taxa under analysis was constructed using bootstrap testing with 1000 replicates. The phylogenetic tree was constructed using each isolate's nearest neighbours from the BLASTn hits. All strains' partial 16S rDNA sequences were added to the GENBANK database.

## RESULTS

**Isolation of bacteria**

The different bacteria that were found in two different stations, the Cauvery River (Station 1) and the Coleroon River (Station 2), are listed in Table 1. A total of 20 bacteria from 13 genera were identified, as the table makes clear. Of these, five species belonged to the genus *Bacillus*, and two each to the genus *Salmonella* and *Klebsiella*. There was just one species in each of the other genera. In both stations, the majority of the bacteria grow throughout the summer,

while the pre-monsoon and post-monsoon bacterial populations are modest and the bacterial population is low during the monsoon season.

**Biochemical Characterization**

All of the isolates' biochemical traits are compiled. Eleven of the isolates were Gram-positive bacteria, while the remaining ones were Gram-negative. In both aerobic and anaerobic settings, the isolates' levels of fermentation potential varied, according to the analysis of sugar fermentation using TSI assays. Five of the investigated strains generate $H_2S$ in TSI medium (Table 2).

**Analysis of Bacteria by 16S rRNA**

Only reproducing cells that can form visually distinct colonies can be subcultured using classic culturable procedures. The bacterial isolates are then confirmed using 16s rRNA Analysis, and the subcultured colonies are recognised using morphological and biochemical methods.

**Molecular Characterization and Phylogenetic analysis**

The 20 isolates (MS1 to MS20) were identified by BLASTn results, and taxonomy designations were given based on the NCBI database's nearest neighbour. The bacterial strains' BLASTn analysis is shown in Table 3. Twenty isolates, PV668924 to PV668943, have accession numbers (Table 4). Figure 1 displays the phylogenetic connection of the 20 isolates, with an ideal branch length total of 1.227. Both trees are depicted as legends in the illustration and are drawn to scale.

**Evolutionary relationships of 20 isolates and 20 of their nearest neighbour taxa**

The Neighbor-Joining approach was used to infer the evolutionary history (Saitou and Nei, 1987). The ideal tree is displayed with a branch length sum of 1.227. The branches are accompanied by the proportion of duplicate trees where the related species clustered together in the bootstrap test (1,000 repetitions) [22]. With branch lengths in the same units as the evolutionary distances used to estimate the phylogenetic tree, the tree is depicted to scale. The evolutionary distances are expressed in terms of the number of base substitutions per site and were calculated using the Kimura 2-parameter technique [23]. Forty nucleotide sequences were included in the analysis process. Each sequencing pair's ambiguous places were all removed using the pairwise deletion option, yielding a final data set with 1,575 positions. MEGA12 was used to perform evolutionary analyses using up to seven parallel computer threads [24].

**Evolutionary analysis by the Maximum Likelihood method**

The tree with the highest log likelihood (-9,899.79) is displayed. The phylogeny was inferred using the Maximum Likelihood method and Kimura's [23] two-parameter model of nucleotide substitutions. Besides the branches, the percentage of duplicate trees where the related taxa clustered together (1,000 repetitions) is displayed [22]. The tree with the higher log-likelihood between a Maximum Parsimony (MP) tree and a Neighbor-Joining (NJ) tree (Saitou and Nei 1987) was chosen as the starting tree for the heuristic search. A matrix of pairwise distances calculated with the Kimura [23] 2-parameter model was used to create the NJ tree. Out of ten MP tree searches with randomly generated starting trees, the MP tree had the least length. Forty nucleotide sequences were included in the analysis process. All positions with less than 95% site coverage were removed using the partial deletion option, yielding a final data set of 1,339 positions. MEGA12 was used to perform evolutionary analyses using up to seven parallel computer threads [24].

**Pairwise Distance**

The number of base substitutions between sequences is displayed for each location. Above the diagonal are the standard error estimates or estimates. The Kimura 2-parameter model [23] was used for the analyses. Twenty nucleotide sequences were included in the analytical process (Table 5). Each sequencing pair's ambiguous places

were all removed using the pairwise deletion option, yielding a final data set with 1,575 positions. In MEGA12, evolutionary analyses were performed [24].

## DISCUSSION

Communities have benefited from surface water in numerous ways. *Salmonella, E. coli,* and *Vibrio cholerae* were detected during the evaluation of the two water bodies (the Cauvery River and the Coleroon River) in the current study. Because of their widespread distribution in nature, microorganisms can be utilised as an indication to determine whether water quality is suitable [25]. This investigation identifies a number of pathogens in the water of the Coleroon and Cauvery rivers. The findings showed that the Cauvery River's water had a low microbiological quality in accordance with drinking water quality standards. Both the effluent sample and the river water were found to contain coliforms [26]. The number of bacteria logically indicates the extent of human influence on the water. Faecal coliform is a sign of biological wastes from domestic animals or point and non-point human sources when it is present [27]. The rainy season was shown to have increased faecal coliform levels. Similar incidents caused by human waste have been documented from the Ganges River [28] and the Mandovi and Zuari estuaries [29]

A potent method for identifying and categorising bacteria is 16S rRNA sequencing; in this study, the 16S rRNA sequencing method is utilised to reconstitute the identified bacterial isolates. It is important to limit the potential of barcoding for species discovery using the currently suggested approaches. Since degrees of overlap between intra- and interspecific differences are anticipated to be high in most major clades, especially within varied but poorly described groupings, the use of thresholds for species delineation is unpromising and strongly discouraged. According to our results, using thresholds to screen for very divergent novel taxa can be successful, but it will miss at least one-fifth of the unique but less divergent forms of life. More sophisticated approaches that take into account sibling group characteristics, intraspecific variability knowledge, and population genetics concepts will be needed. When taxa have not undergone extensive taxonomic scrutiny and species recognition is restricted to a small number of conventional character sets that have not been validated by further research and instruments, DNA barcoding is far less successful. Due to flawed taxonomy, which contributes to a high mistake rate for barcode-based identification, many species in these little-known groups—which comprise the majority of life on Earth—will appear to be genetically non-monophyletic. Therefore, large taxonomically well-explored comparison datasets are required to produce an appropriate environment for identification using barcoding. The standards and protocols needed to create these databases and support their growth will be developed primarily by the barcoding effort.

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**Table 1. Bacteria isolated from Cauvery Water**

| S.no | **Bacterial Species** | Pre monsoon | | Monsoon | | Post monsoon | | Summer | |
|---|---|---|---|---|---|---|---|---|---|
| | | S1 | S2 | S1 | S2 | S1 | S2 | S1 | S2 |
| 1 | *Bacillus licheniformis* | - | + | - | + | + | + | + | + |
| 2 | *Bacillus megaterium* | - | + | - | + | + | + | + | + |
| 3 | *Bacillus subtilis* | + | + | + | - | + | - | + | + |
| 4 | *Actinobacillus hominis* | + | + | + | + | - | + | + | + |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 5 | *Paenibacillus alvei* | + | + | + | + | - | + | + | + |
| 6 | *Streptococcus pyogenes* | + | + | - | + | + | + | + | + |
| 7 | *Lysinibacillus sphaericus* | + | - | + | - | + | + | + | + |
| 8 | *Bacillus simplex* | + | + | + | - | - | - | + | - |
| 9 | *Brevibacillus choshinensis* | + | + | - | + | - | - | - | + |
| 10 | *Escherichia coli* | + | + | + | + | + | + | + | + |
| 11 | *Staphylococcus aureus* | + | + | + | - | - | - | + | + |
| 12 | *Pseudomonas aeruginosa* | - | - | + | - | + | + | - | + |
| 13 | *Proteus vulgaris* | + | - | - | + | + | + | + | + |
| 14 | *Micrococcus luteus* | + | - | - | + | + | + | + | + |
| 15 | *Klebsiella aerogenes* | + | + | + | - | - | - | + | + |
| 16 | *Bacillus cereus* | + | - | - | + | + | + | + | - |
| 17 | *Salmonella enterica* | - | + | + | + | - | - | + | + |
| 18 | *Salmonella bongori* | + | - | + | + | + | + | - | + |
| 19 | *Klebsiella pneumoniae* | - | + | - | - | - | + | + | + |
| 20 | *Vibrio cholerae* | + | + | + | + | + | + | + | + |

**+ denotes present; –denotes absent.**

**Table 2. Biochemical results of the Isolated strains**

| S.no | **Bacterial Species** | **Gram staining** | **Motility** | **Starch hydrolysis** | **Citrate utilization** | **Methyl red** | **Indole production** | **Oxidase production** | **Catalase production** | **Nitrate reduction** | **Voges proskauer** | **$H_2S$ production** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Bacillus licheniformis* | + | + | + | + | - | + | + | + | + | + | - |
| 2 | *Bacillus megaterium* | + | - | + | - | + | - | - | + | + | + | - |
| 3 | *Bacillus subtilis* | + | + | + | + | - | - | - | + | + | + | + |
| 4 | *Actinobacillus hominis* | - | - | - | - | - | - | + | + | + | - | - |
| 5 | *Paenibacillus alvei* | + | + | - | + | - | - | + | + | - | - | - |
| 6 | *Streptococcus pyogenes* | + | - | - | - | - | - | - | - | - | - | - |
| 7 | *Lysinibacillus sphaericus* | + | + | - | + | + | - | + | + | - | + | - |
| 8 | *Bacillus simplex* | + | + | + | + | - | - | - | + | + | + | + |
| 9 | *Brevibacillus choshinensis* | + | + | + | + | + | - | + | + | + | - | - |
| 10 | *Escherichia coli* | - | + | - | - | + | + | - | + | + | - | - |
| 11 | *Staphylococcus aureus* | + | - | + | + | + | - | - | + | + | + | - |
| 12 | *Pseudomonas aeruginosa* | - | + | - | + | - | - | + | + | + | - | - |
| 13 | *Proteus vulgaris* | - | + | - | + | + | - | - | + | + | - | + |
| 14 | *Micrococcus luteus* | + | - | - | - | - | + | + | + | + | - | - |
| 15 | *Klebsiella aerogenes* | - | + | + | + | + | - | - | + | + | + | - |
| 16 | *Bacillus cereus* | + | + | + | + | - | - | - | + | - | + | - |
| 17 | *Salmonella enterica* | - | + | - | + | + | - | - | + | + | - | + |
| 18 | *Salmonella bongori* | - | + | - | + | + | - | - | + | + | - | + |
| 19 | *Klebsiella pneumoniae* | - | - | + | + | - | - | - | + | + | + | - |
| 20 | *Vibrio cholerae* | - | + | + | + | - | + | + | + | + | - | - |

**(+) =positive, (-) = negative**

**Table 3. The assigned taxonomic name of the twenty bacterial strains, along with the GenBank accession number of the nearest neighbour**

| S.No. | Isolate | Name | GenBank Accession Number of nearest neighbours |
|---|---|---|---|
| 1 | MS1 | *Bacillus licheniformis* | OR604497 |
| 2 | MS2 | *Priestia megaterium* | OR569704 |
| 3 | MS3 | *Bacillus subtilis* | FJ457629 |
| 4 | MS4 | *Actinobacillus hominis* | NR_042866. |
| 5 | MS5 | *Paenibacillus alvei* | ON782299 |
| 6 | MS6 | *Streptococcus pyogenes* | MN922995 |
| 7 | MS7 | *Lysinibacillus sphaericus* | AB680068 |
| 8 | MS8 | *Bacillus simplex* | LT547805 |
| 9 | MS9 | *Brevibacillus choshinensis* | NR_113763 |
| 10 | MS10 | *Escherichia coli* | LC654897 |
| 11 | MS11 | *Staphylococcus aureus* | MN565966 |
| 12 | MS12 | *Pseudomonas aeruginosa* | FJ972534 |
| 13 | MS13 | *Proteus vulgaris* | MN623689 |
| 14 | MS14 | *Micrococcus luteus* | AJ312751 |
| 15 | MS15 | *Klebsiella aerogenes* | LC799157 |
| 16 | MS16 | *Bacillus cereus* | LT745970 |
| 17 | MS17 | *Salmonella enterica* | AB680792 |
| 18 | MS18 | *Salmonella bongori* | NR_074888 |
| 19 | MS19 | *Klebsiella pneumoniae* | PV361230 |
| 20 | MS20 | *Vibrio cholerae* | NR_044050 |

**Table 4. The names of the twenty bacterial strains, along with the GenBank accession number**

| S.No. | Isolate | Name | GenBank Accession Number |
|---|---|---|---|
| 1 | MS1 | *Bacillus licheniformis* | PV668924 |
| 2 | MS2 | *Priestia megaterium* | PV668925 |
| 3 | MS3 | *Bacillus subtilis* | PV668926 |
| 4 | MS4 | *Actinobacillus hominis* | PV668927 |
| 5 | MS5 | *Paenibacillus alvei* | PV668928 |
| 6 | MS6 | *Streptococcus pyogenes* | PV668929 |
| 7 | MS7 | *Lysinibacillus sphaericus* | PV668930 |
| 8 | MS8 | *Bacillus simplex* | PV668931 |
| 9 | MS9 | *Brevibacillus choshinensis* | PV668932 |
| 10 | MS10 | *Escherichia coli* | PV668933 |
| 11 | MS11 | *Staphylococcus aureus* | PV668934 |
| 12 | MS12 | *Pseudomonas aeruginosa* | PV668935 |
| 13 | MS13 | *Proteus vulgaris* | PV668936 |
| 14 | MS14 | *Micrococcus luteus* | PV668937 |
| 15 | MS15 | *Klebsiella aerogenes* | PV668938 |
| 16 | MS16 | *Bacillus cereus* | PV668939 |
| 17 | MS17 | *Salmonella enterica* | PV668940 |
| 18 | MS18 | *Salmonella bongori* | PV668941 |
| 19 | MS19 | *Klebsiella pneumoniae* | PV668942 |
| 20 | MS20 | *Vibrio cholerae* | PV668943 |

**Table 5. Estimates of Evolutionary Divergence between Sequences (Pairwise distance)**

| | MS1 | MS2 | MS3 | MS4 | MS5 | MS6 | MS7 | MS8 | MS9 | MS10 | MS11 | MS12 | MS13 | MS14 | MS15 | MS16 | MS17 | MS18 | MS19 | MS20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **MS1** | | 0.01 | 0.00 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | 0.02 | 0.01 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 |
| **MS2** | 0.10 | | 0.01 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 |
| **MS3** | 0.01 | 0.10 | | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | 0.02 | 0.01 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 |
| **MS4** | 0.31 | 0.31 | 0.30 | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.01 | 0.02 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 |
| **MS5** | 0.16 | 0.18 | 0.15 | 0.33 | | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 |
| **MS6** | 0.17 | 0.18 | 0.17 | 0.30 | 0.18 | | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 |
| **MS7** | 0.08 | 0.11 | 0.09 | 0.29 | 0.16 | 0.15 | | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | 0.02 | 0.01 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 |
| **MS8** | 0.07 | 0.09 | 0.07 | 0.29 | 0.14 | 0.16 | 0.08 | | 0.01 | 0.02 | 0.01 | 0.02 | 0.02 | 0.01 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 |
| **MS9** | 0.14 | 0.18 | 0.14 | 0.32 | 0.15 | 0.17 | 0.14 | 0.13 | | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 |
| **MS10** | 0.29 | 0.31 | 0.29 | 0.12 | 0.33 | 0.29 | 0.27 | 0.28 | 0.31 | | 0.02 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | 0.00 | 0.00 | 0.01 | 0.01 |
| **MS11** | 0.09 | 0.11 | 0.09 | 0.29 | 0.17 | 0.17 | 0.10 | 0.10 | 0.17 | 0.29 | | 0.02 | 0.02 | 0.02 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 |
| **MS12** | 0.27 | 0.29 | 0.27 | 0.20 | 0.28 | 0.28 | 0.27 | 0.26 | 0.29 | 0.18 | 0.28 | | 0.01 | 0.02 | 0.01 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 |
| **MS13** | 0.29 | 0.31 | 0.29 | 0.13 | 0.33 | 0.30 | 0.27 | 0.28 | 0.32 | 0.07 | 0.29 | 0.19 | | 0.02 | 0.01 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 |
| **MS14** | 0.22 | 0.25 | 0.22 | 0.28 | 0.25 | 0.24 | 0.22 | 0.23 | 0.26 | 0.30 | 0.23 | 0.29 | 0.29 | | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 |
| **MS15** | 0.32 | 0.33 | 0.31 | 0.14 | 0.33 | 0.30 | 0.29 | 0.30 | 0.32 | 0.06 | 0.30 | 0.18 | 0.09 | 0.30 | | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 |
| **MS16** | 0.06 | 0.08 | 0.07 | 0.30 | 0.15 | 0.15 | 0.08 | 0.06 | 0.13 | 0.27 | 0.08 | 0.26 | 0.28 | 0.21 | 0.30 | | 0.02 | 0.02 | 0.02 | 0.02 |
| **MS17** | 0.29 | 0.31 | 0.28 | 0.13 | 0.33 | 0.27 | 0.27 | 0.28 | 0.31 | 0.03 | 0.28 | 0.17 | 0.08 | 0.29 | 0.05 | 0.27 | | 0.00 | 0.01 | 0.01 |
| **MS18** | 0.29 | 0.31 | 0.29 | 0.13 | 0.33 | 0.28 | 0.28 | 0.28 | 0.31 | 0.03 | 0.29 | 0.17 | 0.08 | 0.29 | 0.06 | 0.27 | 0.01 | | 0.01 | 0.01 |
| **MS19** | 0.34 | 0.34 | 0.34 | 0.15 | 0.36 | 0.32 | 0.32 | 0.33 | 0.36 | 0.09 | 0.33 | 0.21 | 0.13 | 0.33 | 0.08 | 0.32 | 0.08 | 0.08 | | 0.01 |
| **MS20** | 0.28 | 0.29 | 0.28 | 0.13 | 0.30 | 0.28 | 0.27 | 0.27 | 0.29 | 0.10 | 0.27 | 0.16 | 0.11 | 0.27 | 0.10 | 0.26 | 0.10 | 0.11 | 0.14 | |

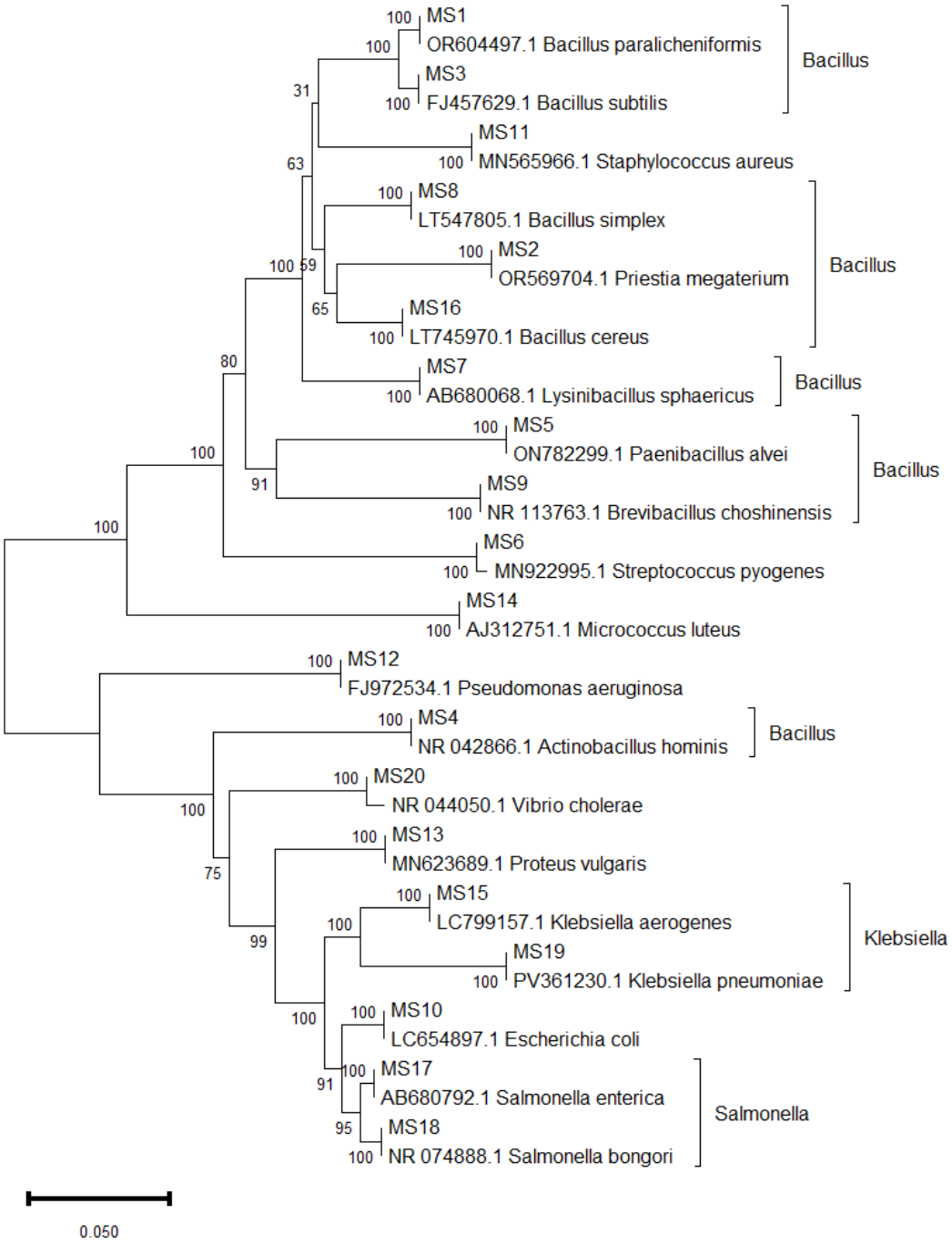



**Fig. 1. Neighbor-Joining tree showing the phylogenetic relationship amongst the strains. A total of 40 nucleotide sequences. The scale bar represents 0.050 nucleotide substitutions per sequence position.**

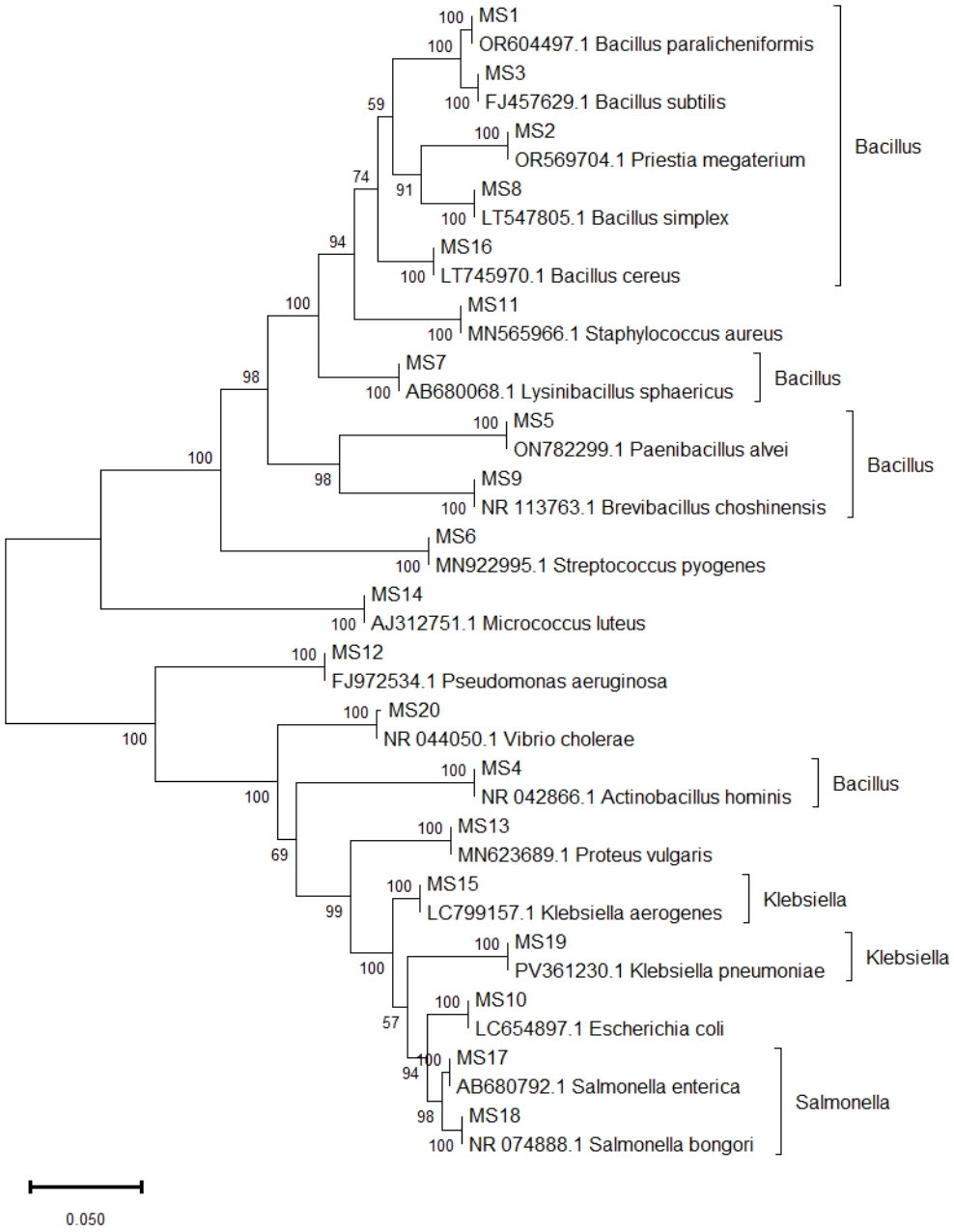



**Fig. 2. Maximum Likelihood tree showing the phylogenetic relationship amongst the strains. A total of 40 nucleotide sequences. The scale bar represents 0.050 nucleotide substitutions per sequence position.**

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**RESEARCH ARTICLE**

# Indigenous Practices in India as Enablers of Circular Economy Business Models: An Empirical Investigation

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## ABSTRACT

Indigenous practices in India historically embodied principles that align closely with circular economy business models. These traditional practices focus on minimising waste, reusing and repurposing resources, and maintaining harmony with natural systems—thereby acting as natural enablers for circular economy approaches. This research analyses the impact of traditional Indian practices on the development of circular economy (CE) business models using empirical methods. CE is based on many technological innovations, but often neglects to recognise indigenous knowledge, particularly in India. For a long time, Indigenous peoples across the globe, especially in India, have been practising sustainable living rooted in holistic community governance and circular resource use. The customs are generally unofficial and non-commercial but are deeply spiritual, favouring nature more like a companion than a commodity. A mixed-methods research design was employed to explore the alignment of indigenous practices in India with circular economy principles. Quantitative surveys of rural practitioners, artisans, and small-scale entrepreneurs provided data on the economic and environmental impacts of their traditional methods. Qualitative interviews with policymakers, community leaders, and NGO members offered deeper insights into cultural and operational frameworks. Field observations documented local practices like organic farming and handloom weaving, and combining all approaches enabled robust data triangulation, clarifying both enablers and barriers to formalising circular economy models. From the analysis, the study claims that Indigenous production and consumption paradigms are centred on CE principles, drawing from over 120 participants in surveys, interviews, and participatory observations. The findings illustrate the importance as well as the complexities of adapting Indigenous wisdom within contemporary CE frameworks and providing pathways for sustainable, inclusive business innovation. The research paper underscores several important implications for policy, business, and community

development in India. Indigenous practices offer a culturally rooted foundation for advancing circular economy models, presenting opportunities for inclusive, sustainable growth.

**Keywords:** Indigenous practices, Circular Economy, Business models.

## INTRODUCTION

**Indigenous Practices in India.**

Indigenous practices in India are deeply rooted in ecological wisdom and community resilience, reflecting an age-old commitment to sustainability and resource stewardship. Traditional agricultural systems like zero-budget natural farming, agroforestry, and mixed cropping enhance biodiversity, maintain soil health, and help local communities adapt to climate change and weather extremes. Water conservation techniques, such as rainwater harvesting and the use of step wells or tankas, demonstrate a nuanced understanding of local ecosystems and ensure resource availability even in drought conditions. Many tribal and rural communities practice circular habits, including zero-waste food systems and the reuse or recycling of materials, informed by cultural and spiritual values that prioritise harmony with nature. Sacred groves, community-led conservation, and folk festivals all reinforce these principles while protecting biodiversity. Construction methods, too, embody sustainable strategies, favouring local materials like mud, timber, and lime, and encouraging the reuse of waste. Together, these indigenous practices offer valuable models for modern sustainability and circular economy initiatives in India. In addition to agricultural and water conservation systems, many indigenous groups in India have pioneered sustainable forest management and the ethical harvesting of non-timber forest products. For example, communities in the Nilgiri Biosphere Reserve practice-controlled collection of honey, amla, and soap nuts, ensuring regeneration and ecosystem stability by following traditional rituals and limiting overexploitation of resources. These approaches not only conserve biodiversity but also sustain livelihoods in rural and forest-dependent areas. Indian urban and peri-urban neighbourhoods exhibit a unique form of circularity through the informal waste economy (1). Women's self-help groups, waste pickers, and local artisans participate in decentralised waste collection, segregation, and upcycling—transforming what would be landfill-bound waste into compost, biofuel, or value-added goods. Initiatives like converting temple floral waste into incense or compost, and community-scale biogas plants processing market waste, create green jobs and enhance livelihoods for marginalised communities. Cultural acceptance of repair, reuse, and frugality remains ingrained in Indian society. Practices such as extended use and repair of household items, clothes, and vehicles exemplify everyday circular behaviours. Indigenous and modern circular practices increasingly intersect, opening vast opportunities for transition to a formalised circular economy that builds on traditional strengths while leveraging new technological and entrepreneurial innovations (2).

**Indigenous Practices in India as Enablers of Circular Economy Business Models**

Circular economy business models are designed to keep products, materials, and resources in use for as long as possible by maximising reuse, repair, remanufacturing, and recycling. Examples include product-as-a-service, where companies retain ownership of goods and lease them to customers; sharing models that enable greater utility from shared assets; and closed-loop systems that transform waste into new raw materials. These approaches not only minimise waste and environmental impact but also generate value and resilience by creating innovative business opportunities centred on sustainability. Waste, biodiversity loss, and resource scarcity are growing ecological issues in the 21st-century world. Additionally, countries are shifting towards the circular economy (CE) for sustainable growth and development. The CE focuses on reuse, recycling, reduction, and rejuvenation, unlike the "take-make-dispose" linear model, which is wasteful and unsustainable. Instead of fostering economic growth, CE aims to achieve resilient as well as restorative economies (3). CE is based on many technological innovations, but often neglects to recognise indigenous knowledge, particularly in India. For a long time, Indigenous peoples across the globe, especially in India, have been practising sustainable living rooted in holistic community governance and circular resource use. The customs are generally unofficial and non-commercial but are deeply spiritual, favouring

nature more like a companion than a commodity. India is home to over 700 tribal groups and many traditional rural communities. This unique environment allows Indigenous customs to thrive, even with the pressures of modernisation. In Indigenous cultures, circularity is a lived reality.

This is evident in practices such as organic farming, seed preservation, traditional water management systems, and sustainable forest use. These practices provide clear examples of using resources efficiently, reducing waste, adapting to climate change, and promoting social inclusion. All these elements are vital for a circular economy. Tribal farmers in Odisha grow millets using methods that preserve agrobiodiversity, improve the soil, and require few outside resources. Bamboo craft communities in Nagaland create new products from waste materials and rely on rapidly growing renewable resources. Johads and kunds are two types of rainwater harvesting systems in Rajasthan. They demonstrate closed-loop water cycles that support entire ecosystems. These methods are low-carbon, decentralised, and based on cooperation and self-reliance. Therefore, they are highly relevant to circular economy transition plans that prioritise social justice and sustainability. Indigenous knowledge takes a holistic approach to systems thinking, which is vital for advancing circular economy (CE) models. For generations, Indigenous communities have managed resources with a focus on sustainability rather than short-term profits. They rely on wisdom passed down through generations, community agreement, and observation of natural cycles to guide their decisions. These characteristics align well with CE frameworks, which aim to reuse materials, eliminate waste, and restore natural ecosystems [3]. Recognising Indigenous practices as active supporters of the circular economy is both a matter of justice and a necessity for protecting our environment. It involves acknowledging and raising knowledge systems that have sustained people and the environment for generations [2]. It also requires creating inclusive spaces where traditional and modern systems can coexist, share knowledge, and collaborate to find solutions that fit their cultures and contexts. In conclusion, there is an important opportunity to link Indigenous knowledge with modern circular economy practices as the world faces the urgent need for sustainable development. We can learn from those who have mastered living within ecological limits instead of creating new solutions. India can lead this integration due to its rich collection of Indigenous knowledge and traditions. By doing this, we will not only promote the goals of the circular economy but also preserve and honour the cultural heritage that holds the keys to a sustainable future.

## LITERATURE REVIEW

(Times of India, 2025a) Community-based waste management schemes in India show how circularity is enabled by social structures held by Indigenous peoples. Case studies from the Himalayas show local waste banks, school-based segregation drives and community-based recycling cooperatives that create microeconomic systems. (Abbasi, 2024) demonstrates that rural circular systems are built on Indigenous agroecological practices — including mixed cropping, livestock integration and organic nutrient recycling. Through empirical research, these models' lower reliance on chemical inputs, improve soil fertility and produce co-products such as compost and bio-based materials. (CEEW, 2024) Policy studies highlight that the CE transition in India should include Indigenous practices and community knowledge systems. Reports from the Council on Energy, Environment and Water. (UN-PAGE, 2023) Community models emphasise the significance of social capital, trust and collective decision making to facilitate financially viable, small-scale circular businesses. Patel (2020) The Circular Economy (CE) business models promote waste reduction, increased product life and regeneration of natural systems. explores the rotational food production and forest regeneration and communal seed banks among tribal communities in India. The practices are circular, and sustainable. The principles of regenerative use and intergenerational fairness are essential to circular economy frameworks and are reflected in these. In India, the informal economy sometimes is the quiet engine behind circularity. Bocken et al. (2014), propose a sustainable business model type framework consisting of resource conservation, reuse and closed-loop systems. The principles closely align with Indigenous practices, such as community composting, seed preservation and local craft economies, in India. These models provide a rational framework for integrating traditional systems into contemporary business operations. Circular model sustainability assessment is one of the key indicators of its viability. Arora and Romijn (2012) also reinforce this notion by indicating that if it is built upon community invention rather than corporate control, the BoP economy can bring

about productive circular solutions. Indigenous Indian innovations — mud-refrigerators (mitticool) and clusters of natural dye textiles — reinforce this perspective by extracting value from fewer resources and waste. By its nature, there is a philosophical base behind the circularity of Indian indigenous practices. Singh et al. (2009) summarize comparative evaluation of the various sustainability evaluating approaches. Their models are important for the assessment of indigenous business practices such as handloom weaving, organic farming. While standard metrics may not always adequately capture these practices, they make a meaningful contribution to environmental and social sustainability. Mishra (2010) revisits the approach of rural development by Gandhi as a means to self-reliance, minimalism, and resource-based living. Khadi spinning, vermicomposting and decentralised biogas units are examples of activities which reflect the principles and philosophy of circular economy, but which do so all informed by India's socio-cultural situation. Chikarmane and Narayan (2005) likewise demonstrate how a waste-picking community in Pune operates as a decentralised way of dealing with waste. These workers in turn have developed successful business models by organising on a grassroot level that emphasize reuse and recycling and use fewer resources. Banerjee (2003) takes to task sustainable development discourse at that point for relegating local concerns to the periphery of its deliberations with a focus on technical solutions. This criticism is significant for circular economy researchers as it serves as a warning as to the dangers in excluding from official CE frameworks the existence of indigenous practices. Elkington's (1997) concept of the triple bottom line – people, planet, and profit – offers an effective way to measure success. Indian indigenous practices are frequently abundant in social and ecological resources and benefits, even when they are valued at little economic cost. Appreciating these behaviors through more comprehensive valuation approaches can bridge grassroots sustainability with formal business innovation. Narain and Agarwal (1989) highlight the need for decentralisation and local participation in environmental management. Their idea of "green villages" predated the current language of circular economy, but its intentions are the same. These are value-add to closed nutrient chains, to low-carbon infrastructure, and to community ownership — all of which may indeed have more distinctiveness in a conventional Indian rural model. Although indigenous systems have immense potential, mainstream business models either ignore or undervalue their advantages.

## METHODOLOGY

**Objectives of the study**

1. To find Indigenous practices that work under the principles of the circular economy.
2. To measure their influence on the sustainability of business and development of communities.
3. To investigate barriers and support for the implementation of these practices throughout the country.

**Statement of the Problem**

India possesses a deep historical background of indigenous sustainability-related practices, which are geared towards resource conservation and local community living. Many of them, such as traditional agriculture, water saving practices, handicrafts and informal recycling systems, are natural allies for the circular economy. This economy is centred on reuse, regeneration and cutting waste. Despite the potential of these local practices to contribute to sustainable development and circular business models, however, they are mostly neglected, under-researched, and excluded from official economic policies. This means there is a mismatch between traditional models of sustainability rooted in community and official/mainstream circular economy strategies. Thus, it is important to identify and examine whether certain indigenous practices of India are congruent with circular economy concepts. And for inclusion in official business models, we need to evaluate their environmental and economic sustainability and acknowledge the challenges of doing so.

**Research Design**

Using a mixed-methods research design, we aimed to understand the alignment of indigenous practices in India in line with the concepts of circular economy itself. Sustainably surveyed rural practitioners, artisans, and small-scale entrepreneurs produced quantitative data in their traditional ways but were also rewarded through economic and

environmental results. To achieve a more qualitative understanding, we interviewed policy members, community leaders, and NGO stakeholders engaged in sustainability and rural development. This allowed extensive insight into ethnographic, historic, and operational frameworks. This methodology allowed observations of specific regions—organic farming, handloom weaving, rainwater harvesting, and informal waste management—through direct exercises. Collecting all of these approaches also provided a better opportunity for triangulation of data, not only in the description of what facilitates or hinders the adoption of formalised circular economy frameworks but in this way, further qualitative insights on the role of knowledge production.

**Scope of study**

The objectives of the study are to find how three ecologically and culturally different Indian states, Rajasthan, Odisha and Nagaland, show how Indian ecological practice in different environmental settings embodies the principles of being circular. The sites were intentionally selected to convey different types of aboriginal knowledge systems in resources, management systems and local communities' ways of livelihood that are characterized by sustainable use and tight-loop use of nature in nature.

**Data Collection**

A variety of data collection techniques were used in this study to generate a broader and wider diversity of insights. Surveys were completed with 90 respondents, 30 from Rajasthan, 30 from Odisha and 30 from Nagaland. These respondents were mainly farmers, artisans, and indigenous entrepreneurs engaged in traditional practices. 30 in-depth interviews with key informants (NGO workers, village elders and local community leaders) were conducted to complement the quantitative data. They offered the practical insights and knowledge from their field experience on indigenous practices related to sustainability. Focus Group Discussions (FGDs, three in different study sites) were conducted for group discussion, facilitating community dynamics and communication. Participatory immersion as field observation allowed the researcher to capture immediate practices, tools, and processes. This gave the data some rich firsthand perspective about how circular principles are integrated into daily living.

**Data Analysis**

This analysis applied both quantitative and qualitative techniques. The quantitative data for our surveys were analysed with SPSS software using descriptive statistics. This enabled us to discern patterns and trends across the three study regions. The qualitative results were analysed by conducting thematic analysis in NVivo using responses to interviews and focus group discussions. We used this approach to organise, code and interpret key themes related to sustainability, resource use, and traditional practices. This mixed-methods approach provided a complete understanding of how indigenous practices connect with the principles of a circular economy

## RESULTS AND FINDINGS

**Analysis**

Objective One sought to systematically analyse patterns of indigenous practices in Rajasthan, Odisha and Nagaland based on circular economy principles. Using the combined research approach employed in this study, our analysis is informed by structured surveys (90 participants), the use of 30 interviews, 9 Focus Group Discussions and field observations, and in this way a triangulated perspective is provided regarding the ways traditional Indian practices contribute to the realisation of resource circularity.

**Patterns from the Quantitative Survey Analysis (SPSS)**

In a descriptive analysis from 90 survey respondents, we found the consistent adoption of practices aligned naturally with the principles of circular economy (CE):

• 82% of farmers and artisans indicated use of renewable materials sourced locally, like bamboo, millets and cow dung.

• 76% said they depend on closed loop agricultural or craft systems, such as seed preservation, composting and natural fertilisers.
• 68% stated being involved in reusing or upcycling methods, especially in bamboo craft clusters and dairy-based families.
• 71% referred to waste-to-resource conversions, primarily cow-dung composting and biomass.

**Qualitative Thematic Analysis (NVivo)**
Thematic coding of interviews and FGDs revealed recurring themes supporting the profound circularity of indigenous systems:

a. Resource Regeneration and Localised Ecosystems
Communities across every region focused on practices like:
• Construction of Johad in Rajasthan that helps rebuild water tables and can reuse water cyclically.
• Traditional millet farming in Odisha, with biodiversity conservation practices with minimal external inputs.
• In Nagaland sustainable bamboo harvesting, where cutting has been done in rotation cycles, allowing regrowth.

b. Knowledge Systems Facilitating Closed Loops
Interview participants consistently associated traditional knowledge with cyclical thinking:
• Seed networks managed by the community avoid dependence on commercial inputs.
• Ritual-based forest rules govern extraction, limiting overuse.
• Traditional composting and organic inputs reduce external waste.
These constitute decentralised, closed-loop systems, a foundational CE principle.

c. Cultural Norms of Repair, Reuse, and Frugality
Some of the behaviours that FGDs indicated were culturally ingrained would include:
• Multi-generational repair of tools, household goods, and clothing.
• Utilisation of crop waste (straw, husk, dung).
• Sharing tools, water resources, and seeds in a community environment.
These norms align with CE strategies for reuse, sharing and life cycle extension of products.
**Table 2** summarises the main indigenous practices in relation to core CE principles. These principles include resource regeneration, localisation, renewable inputs, and waste minimisation.

Objective Two was evaluated regarding the way indigenous practices impact economic sustainability and livelihood security, community development, and long-term ecological resilience. Applying a mixed-methods approach — quantitative surveys, interviews, FGDs, and field observations — this analysis shows the direct and indirect contributions of traditional systems to sustainable business models.

**Contribution to Business Models shown under Graph 1**
• Income Generation: Seventy-two percent of respondents reported earning extra income from traditional methods such as millet farming, bamboo crafting, or cow dung composting in the study's survey, alongside their main jobs.
• Low Capital Use: One of the study's findings was that input costs for indigenous practices were significantly lower—38% less on average—compared to modern commercial alternatives.
• Sustainable Livelihoods: The practices studied showed a strong link to long-term sustainability, with 87 percent of participants indicating their methods support resource renewal and environmental health.
• Entrepreneurial Models: Indigenous practices have increasingly become the basis for community-based business models, particularly through cooperatives and microenterprises.

Objective Three focused on identifying the major constraints that limit the adoption or scaling of indigenous practices within formal circular economy (CE) business models, as well as the enabling factors that facilitate their growth. Using evidence from surveys, interviews, FGDs, and field observations, this analysis presents a detailed

understanding of both the challenges and the support mechanisms currently shaping these traditional systems. Responses from 90 survey participants revealed several structural and systemic constraints that hinder the wider adoption of indigenous practices:

**Challenges Identified**

Table 2 reveals the critical challenges that block the incorporation of indigenous practices into the formal circular economy business models as identified by the study. Access to market was the most common issue to be confronted by respondents, with 61 per cent arguing that traditional products (such as handcrafted crafts or millet-based goods) cannot access the market if the infrastructure is very poor or if there is no branding opportunity. Non-formal acknowledgement was mentioned by 54% with most of these practices being excluded from the official initiatives for sustainability or economic benefits. Moreover, 43% of the participants were worried about lost knowledge, particularly due to youth migration and reduced interest in traditional skills. Finally, 38% attributed inadequate policy support to the idea of government schemes rarely prioritizing or protecting indigenous systems, making it tough for communities to grow or maintain their practices amid modernisations. These problems emphasise the need for institutional aid, capacity-building and inclusive market strategies.

## DISCUSSION

The results of this study prove that indigenous practices in India align with circular economy (CE) principles. They provide resource-efficient, regenerative, and community-driven models. These practices are rooted in local contexts, unlike high-tech or industrial CE interventions. They utilise locally available materials, traditional knowledge, and communal labour. Whether they are Johad water harvesting in Rajasthan, bamboo crafts in Nagaland, or millet farming in Odisha, these systems are built on the pillars of waste reduction, reuse, biodiversity, and renewable inputs. Their link with cultural and ecological systems provides long-term sustainability. They are environmentally friendly, socially acceptable, and economically feasible solutions for rural populations. But these practices face notable hurdles to expansion. Problems like lack of market access, limited recognition from institutions, and the erosion of traditional knowledge due to youth migration and modernisation prevent their scale. This highlights the requirement for gender-sensitive circular economy strategies that recognise and capitalise on women's contributions. Policies should be designed to incorporate women-led indigenous practices into formal CE frameworks to enhance social and environmental benefits. Sustainable development should be culturally grounded and sensitive to gender issues.

**Policy and Business Recommendations**

- Policy Recognition: Indigenous practices should be formally acknowledged and included in national circular economy frameworks and sustainability policies.
- Incentivise Traditional Knowledge: Governments and institutions should provide subsidies, tax benefits, and procurement support for eco-friendly products produced using traditional methods.
- Community Business Models: Policymakers should encourage cooperative and self-help group models that promote community ownership of circular enterprises.
- Education and Youth Engagement: Indigenous practices need to be documented, digitised, and integrated into school curricula to counter the loss of knowledge.
- Eco-Certification and Branding: Creating eco-certification systems for products that utilise indigenous circular practices can build consumer trust and access to niche markets.

## CONCLUSION

In India Indigenous practices offer a blueprint for circular economy business models that make sense. Rooted in local knowledge, cultural heritage, and ecological understanding, these practices have provided communities continuity through generations. This analysis confirms that these models are not just feasible but are in practice on a small scale,

generating tangible benefits for example through employment provision, conservation of resources, and development of a community. Connection to the key circular economy principles like (re)generation, reuse and decentralisation highlight the importance of taking a local, culturally sensitive mindset towards sustainability. To harness their potential, they need to be bound up with the CE formalization as a method: traditional practices need to be brought into dialogue with formal CE constructs. To carry out this process, we need policy backing, institutional acknowledgement and business strategies that recognise and empower indigenous systems. Through the integration of these bottom-up models with wider sustainability strategies, India can build a resilient, inclusive, and environmentally responsible future for its economy. Acknowledging women and youth as maintainers of these traditions will advance social impact and maintain cultural identity amidst the changing world.

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**Table 1: Shows Data Collection Methodology**

| Method | Description | Sample Size / Frequency | Study Locations | Target Participants | Purpose / Key Insights |
|---|---|---|---|---|---|
| Structured Surveys | Quantitative questionnaires for quantifying data on Indigenous knowledge and engagement in circular economy. | 90 respondents (30 each from Rajasthan, Odisha, and Nagaland) | Rajasthan, Odisha, Nagaland | Farmers, artisans, local entrepreneurs | To gather standardised data on current traditional and sustainable practices supporting circular economy models. |
| In-depth Interviews | Semi-structured qualitative interviews exploring individual experiences and perceptions of sustainability. | 30 interviews | Across three study sites | NGO workers, village elders, community leaders | To gain detailed insights into Indigenous knowledge, cultural values, and their connection to circular practices. |
| Focus Group Discussions (FGDs) | Group discussions facilitate collective reflection and shared experiences related to Indigenous practices. | 9 FGDs (3 per site) | Rajasthan, Odisha, Nagaland | Mixed community participants (6–8 per group) | To understand community-level dynamics, shared values, and variations in practices. |
| Field Observations / Participatory Immersion | To document contemporary Indigenous activities in real time in community activities through direct observation and engagement. | Continuous observation throughout fieldwork | All three sites | Local households, artisans, farmers | To offer an insight into practical applications of circular principles and traditional techniques. |

Source: Researcher's Fieldwork (2025)

**Table 2: Indigenous Practices and Associated Circular Economy Principles**

| Indigenous Practices | Benefits | Circular Economy Principles |
|---|---|---|
| Johad | Resource regeneration | Environmental sustainability |
| millets | local sourcing and biodiversity | Resilience and nutrition |
| bamboo crafts | renewable materials and Upcycling | waste minimization |
| seed networks | closed loop and Decentralisation | community empowerment |
| Cow dung use | waste to resource | circular input/ energy loops |

Source: Researcher created (2025)

**Table 3: Integrated Impact Assessment: Business and Community Development Outcomes**

| Impact Area | Evidence from the Study | CE Relevance |
|---|---|---|
| **Economic viability** | Lower costs, higher resilience, extra income | Resource efficiency |
| **Livelihood security** | Multi-source income, community cooperatives | Long product and resource cycles |
| **Gender empowerment** | Women-led enterprises and SHGs | Social inclusion |
| **Ecological sustainability** | Soil fertility, regeneration, and biodiversity | Regeneration and closed-loop systems |
| **Cultural continuity** | Preservation of heritage crafts and seeds | Long-term sustainability |
| **Community resilience** | Water security, food security | Systemic circularity |

Source: Researcher created (2025)

**Table 4: Integrated Assessment: Barriers and Supports for Adoption**

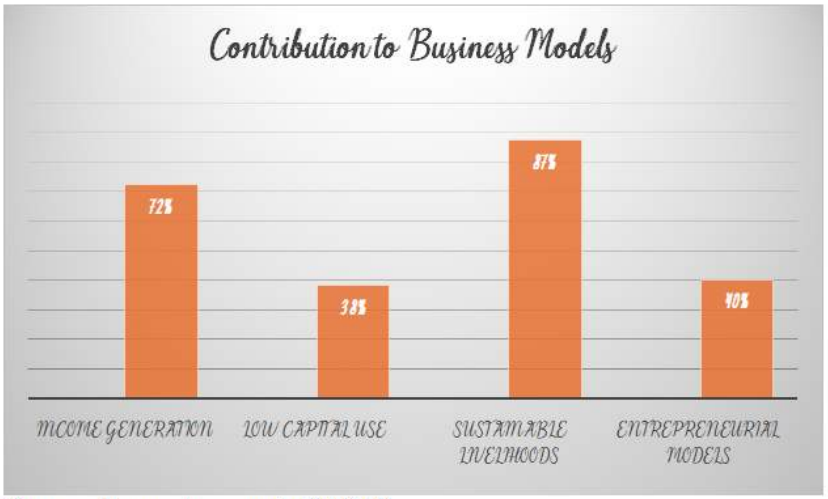
| Category | Key Factors Identified | Implications |
|---|---|---|
| **Barriers** | Market access, policy gaps, knowledge erosion, climate change, poor institutional recognition | Limits scalability and commercialisation |
| **Social Supports** | Cultural continuity, communal governance, and strong social capital | Enhances adoption and sustainability |
| **Institutional Supports** | NGOs, SHGs, cooperatives, training programs | Increases skills and market readiness |
| **Economic Supports** | Low-cost inputs, renewable materials, local resource loops | Makes practices financially viable |

*Source: Researcher created (2025)*

**Table 5: Challenges of Indigenous practices in circular economy business models.**

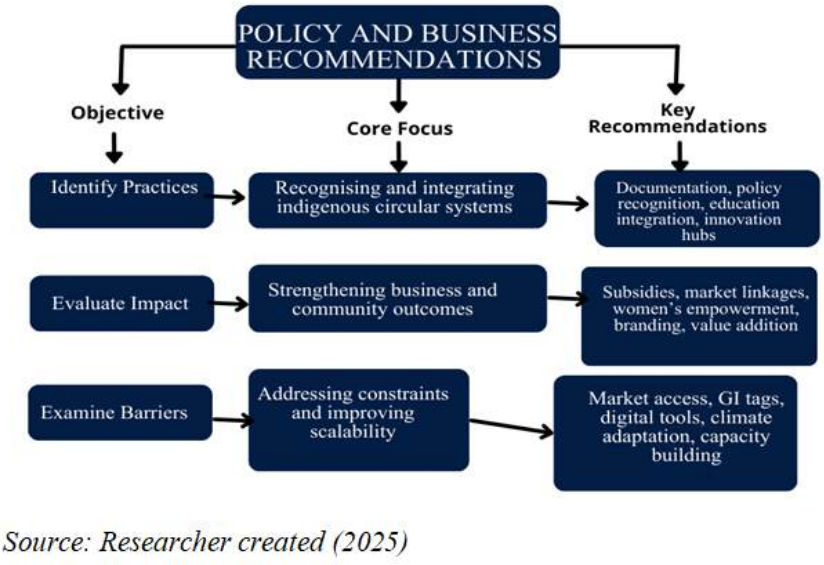
| Challenge | Percentage of Respondents Reporting |
|---|---|
| Market access for traditional goods | 61% |
| Lack of formal recognition | 54% |
| Knowledge erosion (youth migration) | 43% |
| Limited policy support | 38% |

*Source: Researcher created (**2025)***




*Source: Researcher created (2025)*

**Graph 1: Indigenous practices contribution to Business Models.**




*Source: Researcher created (2025)*

**Graph 2: Showing objective-wise policy and Business Recommendations.**

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RESEARCH ARTICLE

# Phytochemical Profiling and Synergistic Bioactivities of Green Tea (*Camellia sinensis*) – Based Polyherbal Combinations

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## ABSTRACT

Polyphenol rich medicinal plants are increasingly investigated for their potential role in managing oxidative stress associated metabolic and inflammatory disorders. The present study evaluated the phytochemical composition and in-vitro antioxidant, anti-diabetic, and anti-inflammatory activities of aqueous extracts of *Camellia sinensis*, *Solanum trilobatum*, and *Trachyspermum ammi*, both individually and in combination. Total flavonoid and phenolic contents were determined using standard colorimetric methods, revealing that *C. sinensis* possessed the highest flavonoid (5.74 mg/mL) and phenolic (13.248 mg/mL) concentrations among the tested extracts. In the DPPH radical scavenging assay, *C. sinensis* demonstrated superior antioxidant activity with an $IC_{50}$ value of 8.47 µg/mL, followed by *T. ammi* (10.18 µg/mL) and *S. trilobatum* (16.94 µg/mL), with statistically significant differences ($p < 0.05$). Combination studies indicated enhanced antioxidant efficacy, with the ternary mixture exhibiting an $IC_{50}$ of 10.09 µg/mL. Evaluation of anti-diabetic potential through $\alpha$-amylase inhibition showed that *S. trilobatum* exhibited comparatively higher individual enzyme inhibitory activity, while selected binary combinations demonstrated synergistic interactions; however, the ternary combination showed antagonistic behaviour. In the anti-inflammatory protein denaturation assay, *C. sinensis* produced the highest individual inhibition (44.59 ± 7.65% at 50 µg), whereas the triple combination displayed pronounced synergism (60.80 ± 1.91%), exceeding its predicted cumulative value. Overall, the findings

suggest that polyphenol-rich extracts, particularly *C. sinensis*, contribute significantly to antioxidant, anti-diabetic, and anti-inflammatory effects, and that carefully optimized combinations may enhance therapeutic potential for managing oxidative stress and metabolic inflammation.

**Keywords:** *Camellia sinensis*, DPPH assay, anti-diabetic, anti-inflammatory, flavonoids, phenols.

## INTRODUCTION

Medicinal plants have underpinned traditional healthcare systems for centuries and remain central to modern biomedical exploration. Growing scientific interest in plant-derived bioactive compounds stems from their structural diversity and broad pharmacological spectrum. Leaves, seeds, fruits, roots, and bark contain abundant phytochemicals including polyphenols, flavonoids, alkaloids, and terpenoids recognized for antioxidant, anti-inflammatory, antimicrobial, and anti-diabetic properties. Consequently, plant extracts are increasingly investigated as candidates for functional foods, nutraceuticals, and disease-targeted formulations. Among globally consumed botanical beverages, *Camellia sinensis* (green tea) is distinguished by its dense polyphenolic composition. Its principal catechins, epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin (EC), and epicatechin-3-gallate (ECG)account for much of its bioactivity (Khan and Mukhtar, 2007; Graham, 1992). EGCG, the predominant catechin, exhibits antioxidant activity exceeding that of conventional antioxidants such as vitamins C and E (Cao *et al*., 2002). These compounds neutralize reactive oxygen species (ROS) through hydrogen atom (H•) donation and single-electron transfer ($e^-$), while also modulating cytoprotective pathways including Nrf2/ARE signaling (Shahidi and Ambigaipalan, 2015; Zhang *et al*., 2022). Oxidative stress defined as ROS production > endogenous antioxidant capacity contributes to lipid, protein, and DNA damage and underlies chronic disorders such as diabetes, cardiovascular disease, neurodegeneration, and cancer (Halliwell and Gutteridge, 1989). Inflammation, closely linked to oxidative imbalance, involves cytokine release, prostaglandin synthesis, and NF-κB activation. While acute inflammation is protective, persistent low-grade inflammation promotes metabolic syndrome and related pathologies (Bagad and Joseph, 2013). *In vitro* protein denaturation assays are widely employed to assess anti-inflammatory potential (Prakash *et al*., 2011), and green tea catechins have demonstrated suppression of pro-inflammatory mediators (Khan and Mukhtar, 2007; Zhang *et al*., 2022). Diabetes mellitus, characterized by dysregulated glucose homeostasis, further exemplifies the oxidative inflammatory nexus. Inhibition of carbohydrate-hydrolyzing enzymes such as α-amylase and α-glucosidase reduces postprandial hyperglycemia (Kamiyama *et al*., 2010). Catechins have shown protective effects on pancreatic β-cells and improvements in insulin sensitivity (Anderson *et al*., 2002; Ye *et al*., 2010, Berenbaum, 1989; Wagner and Ulrich-Merzenich, 2009). *Trachyspermum ammi*, rich in thymol, and *Solanum trilobatum*, containing flavonoids and steroidal alkaloids, possess complementary bioactivities. Accordingly, this study comparatively evaluates the individual and combined *in vitro* bioactivities of *C. sinensis*, *T. ammi*, and *S. trilobatum*, assessing antioxidant capacity, enzyme inhibition, and anti-inflammatory potential to determine additive, synergistic, or antagonistic interactions.

## MATERIALS AND METHODS

## MATERIALS

All chemicals used were of analytical grade. The reagents included 2,2-Diphenyl-1-picrylhydrazyl (DPPH) for Sigma Aldrich, ascorbic acid, quercetin dehydrate, sodium bicarbonate, aluminium chloride, potassium acetate, Folin–Ciocalteu reagent, methanol, bovine serum albumin, catechol, and α-amylase (HiMedia). All reagents were used without further purification.

**Sample Collection**

Leaves of *Camellia sinensis* were procured from a local hypermarket in Munnar, Kerala, during December 2022 and Fresh leaves of *Solanum trilobatum* L. were collected from Tirunelveli district, Tamil Nadu, in December 2022. The leaves were thoroughly rinsed to remove debris and contaminants, then air-dried for three hours prior to further processing. *Trachyspermum ammi* (ajwain) seeds were purchased from a local hypermarket in Bangalore, Karnataka, in January 2023. Seeds were cleaned under running tap water, sterilized with distilled water, and air-dried for two days.

**Extraction and Preparation**

25 g of air-dried leaves of *C. sinensis*, *S. trilobatum*, and ajwain seeds were weighed individually and soaked in 100 ml of distilled water for one hour, followed by homogenization respectively. The homogenised mixtures were boiled for 20 minutes. The extracts were filtered through muslin cloth, and the filtrates were centrifuged at 8000 rpm for 15 minutes at 20°C. Supernatants was collected in sterile, airtight containers respectively and stored at 4°C until further analysis. For comparative experiments, 3 g of shade-dried *S. trilobatum* leaves and air-dried ajwain seeds were processed similarly: soaked in 100 ml distilled water, homogenized, boiled for 20 minutes, filtered, centrifuged at 8000 rpm for 15 minutes at 20°C, and the supernatant was stored at 4°C.

**Phytochemical analysis**

**Determination of Total Flavonoid Content**

Total flavonoid content (TFC) was quantified using a modified aluminium chloride colorimetric assay (Chang *et al*., 2020). Quercetin (100 µg/ml) was used to prepare standards (20–100 µg). Aliquots of sample extracts (0.01–0.1 ml; 5–25 µg) were adjusted to 2 ml with methanol. Blank tubes contained 2 ml methanol. To each tube, 0.1 ml 10% $AlCl_3$ and 0.1 ml 1M potassium acetate were added, followed by 2.8 ml distilled water. The mixtures were incubated at room temperature for 30–60 seconds, and absorbance was measured at 415 nm. Flavonoid content was expressed as mg quercetin equivalents per g of tissue.

**Determination of Total Phenolic Content**

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method (Singleton and Rossi, 1965) with catechol as a standard. Catechol solutions (1 mg/ml) were prepared at concentrations of 0.2–1 mg. Sample extracts (0.004–0.01 ml; 5–25 µg) were diluted to 3 ml with distilled water. A blank of 3 ml distilled water was prepared. To each tube, 0.5 ml Folin–Ciocalteu reagent was added and incubated for 3 minutes at room temperature, followed by addition of 2 ml 20% sodium carbonate solution. Samples were shaken and incubated at room temperature for 20 minutes. Absorbance was measured at 650 nm using a colorimeter. Phenolic content was expressed as µg catechol equivalents per mg of extract.

**Antioxidant Activity: DPPH Radical-Scavenging Activity Assay**

Antioxidant activity was assessed by DPPH radical scavenging assay with modifications (Braca *et al*., 2002). Ascorbic acid (0.1 mg/ml) served as the standard. Aliquots of ascorbic acid (100–500 µl) and sample extracts (5–25 µg) were prepared and adjusted to 1 ml with methanol. Blanks contained methanol alone, while controls contained methanol and DPPH. Then DPPH solution was added to each tube, and samples were incubated in the dark for 5 minutes. Absorbance was measured at 517 nm. Radical scavenging activity was calculated as:

$$\text{DPPH scavenging effect (\%)} = \frac{\text{Abs control} - \text{Abs test}}{\text{Abs control}} \times 100$$

where Abs control and Abs test represent the absorbance of control and sample, respectively.

**Anti-diabetic Activity: α-amylase inhibition assay**

α-Amylase inhibitory activity was evaluated using a modified method (McCue and Shetty, 2004). The enzyme (0.5%) was pre-incubated with phosphate buffer (pH 7), Acarbose (2 mg/ml), or sample extracts (5–25 µg) for 10 minutes at

room temperature. Subsequently, 1 ml of 1% starch solution was added, and the mixture was incubated for 20 minutes. The reaction was terminated by adding 1 ml dinitrosalicylic acid reagent and heating for 5 minutes. After cooling and dilution, absorbance was measured at 545 nm. Percentage inhibition was calculated as:

$$\%\text{Inhibition} = \frac{A_{540\text{Control}} - A_{540\text{Sample}}}{A_{540\text{Control}}} \times 100$$

where $A_{540\text{Control}}$and $A_{540\text{Sample}}$ are absorbances of control and test samples, respectively.

**Determination of Anti-inflammatory activity: Protein Denaturation Assay**

Anti-inflammatory activity was evaluated using the protein denaturation assay as described by Gambhire *et al*. (2009) with minor modifications (Gunathilake *et al*., 2018). Briefly, different concentrations of the sample extracts (10 - 50 µg/mL) and aspirin (5 mg/mL) were mixed with 0.2 mL of 1% (w/v) bovine serum albumin (BSA). The final volume was adjusted to 5 mL with phosphate-buffered saline (PBS, pH 6.4).The mixtures were incubated at 37 °C for 20 min, followed by heating at 70 °C for 5 min. After cooling, absorbance was measured at 660 nm using a UV–Visible spectrophotometer. A reaction mixture containing PBS instead of the test sample served as the control (100% denaturation).

Percentage inhibition of protein denaturation was calculated as:

$$\%\ \text{Inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

where $A_{control}$is the absorbance of the control and $A_{test}$is the absorbance of the sample.

## RESULT AND DISCUSSION

**Phytochemical analysis: A. Total Flavonoid Content**

Flavonoids represent an important subclass of polyphenolic secondary metabolites widely distributed in plants. These compounds, which structurally contain a 15-carbon backbone arranged in a C6–C3–C6 configuration, include flavones, flavanols, flavanones, isoflavones, and chalcones. Owing to their hydroxylated structure, flavonoids are generally water soluble and contribute significantly to the antioxidant defence system of plants and humans. Beyond their free radical scavenging potential, flavonoids are known to modulate intracellular signalling pathways, regulate enzyme activity, and influence gene expression associated with inflammation and metabolic disorders (Panche *et al*., 2016; Chen *et al*., 2020). Total flavonoid content (TFC), determined by the aluminium chloride method using quercetin as standard, was expressed as mg QE/g extract. *Camellia sinensis* showed the highest TFC (5.74 mg/ml), followed by *Solanum trilobatum* (3.43 mg/ml) and *Trachyspermum ammi* (1.12 mg/ml) (Table 1). This ranking (5.74 > 3.43 > 1.12) aligns with reports of catechin-rich green tea (Khan *et al*., 2007; Cao *et al*., 2002). Flavonoids act via radical scavenging, metal chelation, and Nrf2/ARE modulation (Chen *et al*., 2020; Zhang *et al*., 2022), supporting anti-inflammatory and metabolic benefits (Panche *et al*., 2016; Salehi *et al*., 2021).

**Total Phenolic Content**

The total phenolic content (TPC) of the aqueous extracts was quantified using the Folin–Ciocalteu colorimetric method following the procedure of Singleton and Rossi (1965), with catechol as the calibration standard. The calibration curve demonstrated good linearity (y = 0.030x + 0.013; $R^2$ = 0.984), confirming the reliability of the assay. TPC was expressed as catechol equivalents (CE) per gram of extract. *Camellia sinensis* exhibited the highest total phenolic content (13.248 mg/ml), substantially greater than *Solanum trilobatum* (3.873 mg/ml) and *Trachyspermum ammi* (3.166 mg/ml) (Table 1). This elevated level aligns with previous reports describing green tea as a rich source of catechins and polyphenols (Khan *et al*., 2007). Phenolics act via hydrogen atom (H•) donation and single-electron

transfer ($e^-$), neutralizing reactive species (Shahidi and Ambigaipalan, 2015). The ranking (13.248 > 3.873 > 3.166 mg/ml) supports their antioxidant and redox-modulatory roles (Zhang *et al*., 2022; Cory *et al*., 2018).

***In-vitro* Antioxidant property: DPPH radical scavenging activity of *Camellia sinensis*, *Solanum trilobatum*, and *Trachyspermum ammi* Extracts and Their Combinations**

The DPPH free radical scavenging assay revealed concentration-dependent antioxidant activity, comparable to the standard ascorbic acid. Among individual extracts, *Camellia sinensis* (green tea leaf extract, GTLE) exhibited the highest radical scavenging efficiency, reaching 90.88 ± 0.7% inhibition at 25 µg/mL, closely matching ascorbic acid (93.94 ± 0.07%). *Trachyspermum ammi* (AJSE) showed moderate activity (82.33 ± 1.7%), while *Solanum trilobatum* leaf extract (STLE) had the lowest scavenging potential (52.07 ± 5.6%) at the same concentration (Table 2, Figure 1). The $IC_{50}$ values further emphasized the potency of these extracts: GTLE (8.47 µg/mL) < AJSE (10.18 µg/mL) < STLE (16.94 µg/mL), indicating that green tea polyphenols exerted effective radical neutralization even at lower concentrations (Table 2). One-way ANOVA confirmed that these differences were statistically significant ($p < 0.05$) across all concentrations, with post-hoc analysis revealing that GTLE significantly outperformed the other extracts. These results are consistent with prior studies reporting high catechin and polyphenol content in green tea, which stabilizes free radicals through hydrogen donation (Cao *et al*., 2002; Khan *et al*., 2007; Temneanu *et al*., 2011). The moderate activity of AJSE can be attributed to phenolic compounds like thymol, while the lower activity of STLE likely reflects its comparatively smaller flavonoid content. The antioxidant potential of combined extracts was also assessed using equal proportion mixtures of GTLE, STLE, and AJSE. Three combinations were tested: GTLE + STLE, GTLE + AJSE, and GTLE + STLE + AJSE. At 25 µg/mL, GTLE + STLE achieved 84.91 ± 1.6% scavenging, GTLE + AJSE showed 87.86 ± 0.9%, and the ternary combination exhibited 83.75 ± 6.4% activity. These results indicate a synergistic interaction between extracts, particularly involving GTLE + AJSE, enhancing radical scavenging beyond what would be expected from individual components alone (Table 3, Figure 2). $IC_{50}$ analysis of the combinations supported these observations. The ternary combination of GTLE + STLE + AJSE showed the lowest $IC_{50}$ (10.09 µg/mL) among combinations, suggesting enhanced potency due to synergistic effects of polyphenols, flavonoids, tocopherols, and organic acids present in multiple extracts(Table 4, Figure 3). The results demonstrate that combining plant extracts enhances antioxidant capacity through complementary mechanisms such as hydrogen atom transfer (HAT), single electron transfer (SET), and radical stabilization. This cooperative behavior explains the superior scavenging activity of binary (GTLE + AJSE) (Suh *et al*., 2011; Kumar *et al*., 2020; Wang *et al*., 2021). GTLE showed the strongest individual effect, yet combinations provided amplified protection, supporting multi-component nutraceutical development against oxidative stress–related disorders (Chen *et al*., 2020; Li *et al*., 2022).

***In-vitro* Anti-diabetic property: α-amylase inhibition assay of *Camellia sinensis*, *Solanum trilobatum* and *Trachyspermum ammi* extracts.**

The $\alpha$-amylase inhibitory activity of *Camellia sinensis* (GTLE), *Solanum trilobatum* (STLE), and *Trachyspermum ammi* (AJSE) was evaluated to assess their potential anti-diabetic properties. As expected, the inhibition of $\alpha$-amylase increased with concentration for all individual extracts, demonstrating a dose-dependent effect. At 25 µg, STLE exhibited the highest inhibition (66.68 ± 11.6%), followed by GTLE (59.15 ± 3.6%) and AJSE (59.03 ± 0.76%), while the standard inhibitor, Acarbose (2 mg/mL), showed 67.61 ± 1.54% inhibition. This suggests that STLE possesses strong anti-diabetic potential, likely due to its higher content of bioactive phytochemicals capable of modulating enzymatic activity, consistent with previous reports highlighting the $\alpha$-amylase inhibitory role of Solanum species (McCue and Shetty, 2004; Patel *et al*., 2022) (Table 5, Figure 4). To further explore potential synergistic or antagonistic interactions, extracts were tested in equal 1:1 combination. The GTLE + STLE combination showed the highest $\alpha$-amylase inhibition at 25 µg (63.78 ± 2.14%), exceeding its calculated theoretical cumulative value of 62.91%, indicative of a synergistic interaction. Similarly, the GTLE + AJSE combination demonstrated synergy, with practical inhibition (61.73 ± 6.07%) higher than the mathematical cumulative value (59.09%). Conversely, the ternary combination of GTLE + STLE + AJSE showed antagonistic effects, as the observed inhibition (53.55 ± 9.54%) was lower than the predicted cumulative value (61.62%) Table 6 and 7, Figure 5 and 6). The present findings demonstrate that extract combinations can produce variable outcomes depending on phytochemical composition and the relative concentrations of active constituents. Synergy may occur when polyphenols, flavonoids, and related bioactives act

through complementary pathways to enhance $\alpha$-amylase inhibition, whereas antagonism may arise from competitive binding or chemical incompatibility that reduces overall effectiveness (Sharma and Bhat, 2009; Kwon *et al*., 2020). The improved inhibitory response observed with the GTLE + STLE combination suggests that catechins from GTLE and flavonoid–alkaloid fractions from STLE may collectively broaden the inhibitory spectrum of $\alpha$-amylase. These results reinforce the concept that multi-herb formulations can deliver greater therapeutic value than single extracts (Patel *et al*., 2022; Li *et al*., 2023). Such interactions are particularly relevant for functional food development targeting postprandial hyperglycemia, where enhanced enzyme inhibition may improve glycemic regulation. Further molecular investigations are necessary to elucidate the precise mechanisms underlying both synergistic and antagonistic effects.

***In vitro* Anti-Inflammatory activity: Protein denaturation inhibition assay of *Camellia sinensis, Solanum trilobatum* and *Trachyspermum ammi* extracts**

The anti-inflammatory potential of aqueous extracts from *Camellia sinensis*, *Solanum trilobatum*, and *Trachyspermum ammi* was evaluated using the bovine serum albumin (BSA) denaturation method, based on Gambhire *et al*. (2009) with slight modifications (Gunathilake *et al*., 2018). Inhibition of heat-induced protein denaturation reflects the extract's capacity to counter inflammation-related structural damage. The extracts demonstrated concentration-dependent inhibition of protein denaturation. Among the individual extracts, *C. sinensis* exhibited the strongest activity, reaching 44.59 ± 7.65% inhibition at 50 µg. This was followed by *T. ammi* (40.53 ± 3.82%) and *S. trilobatum* (39.19 ± 3.80%) at the same concentration (Table 8, Figure 7a and 7b). The comparatively higher efficacy of *C. sinensis* may be attributed to its abundance of polyphenolic compounds, particularly catechins, which are widely recognized for their anti-inflammatory and protein-stabilizing properties. Green tea bioactives have been reported to modulate inflammatory mediators and reduce oxidative stress, thereby limiting inflammatory damage (Yang *et al*., 2022; Khan *et al*., 2023). Considering the moderate yet encouraging anti-inflammatory effects of the individual extracts, combination studies were performed in equal ratios (1:1) to evaluate possible interaction effects. The combined formulations exhibited greater inhibition than the single extracts. At 50 µg, the ternary mixture (GTLE + STLE + AJSE) produced the highest inhibition (60.80 ± 1.91%), followed by GTLE + AJSE (54.72 ± 2.86%) and GTLE + STLE (48.64 ± 3.83%) (Table 9; Figure 8). To determine whether these enhancements were additive or synergistic, observed (practical) inhibition values were compared with theoretical cumulative values calculated by summing individual effects. In each case, the measured inhibition exceeded the predicted additive value. Most notably, the triple combination showed 60.80 ± 1.91% inhibition compared to a theoretical 41.43%, confirming strong synergism. Similar patterns were evident for the binary combinations (Table 10; Figure 9), indicating cooperative phytochemical interactions. Synergistic activity in polyherbal formulations may result from the complementary actions of catechins in *C. sinensis*, thymol-rich compounds in *T. ammi*, and flavonoid–alkaloid constituents in *S. trilobatum*. Together, these bioactives can modulate inflammatory pathways and oxidative stress more effectively than individual extracts, producing enhanced therapeutic outcomes (Williamson, 2022; Zhang *et al*., 2023).

## CONCLUSION

The present study systematically examined the phytochemical composition and *in vitro* biological activities of aqueous extracts of *Camellia sinensis*, *Solanum trilobatum*, and *Trachyspermum ammi*, evaluated both individually and in combination. The results clearly indicate that phenolic and flavonoid richness plays a decisive role in determining antioxidant, anti-inflammatory, and anti-diabetic efficacy. *C. sinensis* demonstrated the highest phenolic content and exhibited strong radical scavenging and anti-inflammatory activity, emphasizing the functional significance of catechin-rich polyphenols. *S. trilobatum* showed notable $\alpha$-amylase inhibitory potential, suggesting relevance in glycemic control. Importantly, polyherbal formulations particularly the ternary combination displayed synergistic enhancement in antioxidant and anti-inflammatory assays, although antagonistic interaction was observed in enzyme inhibition. These findings reinforce the value of rationally designed phytochemical combinations and support their potential application in functional foods, nutraceuticals, and plant-based therapeutic development. Further mechanistic and *in vivo* investigations are necessary to validate clinical applicability.

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**Table 1: Quantitative analysis of total phenols and flavonoids of *C.sinensis*, *S.trilobatum* , and *T. ammi***

| Sample | Total Phenols (mg CE/g DW) | Total Flavonoids (mg QE/g DW) |
|---|---|---|
| *Camellia sinensis* | 13.248 ± 0.45 | 5.74 ± 0.04 |
| *Solanum trilobatum* | 3.873 ± 0.11 | 3.43 ± 0.03 |
| *Trachyspermum ammi* | 3.166 ± 0.05 | 1.12 ± 0.02 |

One-way ANOVA revealed a statistically significant difference in total phenolic content among the three plant extracts ($F(2,6) = 1313.4$, $p < 0.0001$).

**Table 2:DPPH Radical Scavenging Activity of *C.sinensis*, *S.trilobatum* , and *T. ammi***

| Concentration (µg/ml) | Ascorbic acid (% Scavenging ± SD) | *C.sinensis* (% Scavenging ± SD) | *S.trilobatum* (% Scavenging ± SD) | *T. ammi* (% Scavenging ± SD) |
|---|---|---|---|---|
| 5 | 26.83 ± 2.5 | 69.33 ± 3.6 | 17.64 ± 2.4 | 23.45 ± 2.0 |
| 10 | 43.32 ± 1.8 | 80.81 ± 3.4 | 29.17 ± 5.5 | 50.47 ± 17.3 |
| 15 | 67.92 ± 2.5 | 86.47 ± 0.8 | 39.40 ± 1.1 | 78.52 ± 2.4 |
| 20 | 84.70 ± 1.7 | 90.17 ± 1.3 | 46.12 ± 4.2 | 80.34 ± 2.3 |
| 25 | 93.94 ± 0.07 | 90.88 ± 0.7 | 52.07 ± 5.6 | 82.33 ± 1.7 |
| **$IC_{50}$ (µg/ml)** | 7.1 | 8.47 | 16.94 | 10.18 |

Statistical analysis: One-way ANOVA showed significant differences ($p < 0.05$) between extracts at all tested concentrations.

**Table 3: DPPH Radical Scavenging Activity of Combinations and $IC_{50}$ Values**

| Concentration (µg) | *C.sinensis* + *S.trilobatum* (% Scavenging) | *C.sinensis* + *T. ammi* (% Scavenging) | *C.sinensis* + *S.trilobatum* + *T. ammi* (% Scavenging) |
|---|---|---|---|
| 5 | 46.12 ± 3.3 | 47.63 ± 11.5 | 48.02 ± 8.9 |
| 10 | 60.61 ± 7.1 | 69.05 ± 20.5 | 64.06 ± 8.8 |
| 15 | 69.10 ± 10.6 | 74.03 ± 17.1 | 73.40 ± 1.15 |
| 20 | 79.56 ± 4.5 | 81.98 ± 4.8 | 80.39 ± 7.6 |
| 25 | 84.91 ± 1.6 | 87.86 ± 0.9 | 83.75 ± 6.4 |
| Estimated $IC_{50}$ (µg/mL) | ~6.2 | ~6.5 | ~5.8 |

One-way ANOVA revealed significant differences ($p < 0.05$) between the different combinations of plant extracts at all tested concentrations.

**Table 4: Combinatorial effect of sample on DPPH Radical Scavenging Activity and type of interaction**

| Sample (25 µg) | Mathematical cumulative value (%) | Practical value (mean ± SD) (%) | Type of interaction |
|---|---|---|---|
| GTLE + STLE | 71.47 | 84.91 ± 1.6 | Synergism |
| GTLE + AJSE | 86.60 | 87.86 ± 0.9 | Synergism |
| GTLE + STLE + AJSE | 75.09 | 83.745 ± 6.4 | Synergism |

**Table 5: α-Amylase Inhibitory Activity of *C. sinensis, S. trilobatum* and *T. ammi* with $IC_{50}$ Values**

| Concentration (µg) | *C. sinensis* (GTLE) % Inhibition (Mean ± SD) | *S. trilobatum* (STLE) % Inhibition (Mean ± SD) | *T. ammi* (AJSE) % Inhibition (Mean ± SD) |
|---|---|---|---|
| 5 | 13.34 ± 8.2 | 26.19 ± 5.07 | 28.78 ± 4.64 |
| 10 | 30.58 ± 13.9 | 32.23 ± 5.56 | 36.01 ± 0.23 |
| 15 | 40.58 ± 14.82 | 42.15 ± 3.57 | 43.89 ± 1.96 |
| 20 | 51.36 ± 8.59 | 57.92 ± 9.87 | 52.05 ± 1.61 |
| 25 | 59.15 ± 3.6 | 66.68 ± 11.62 | 59.03 ± 0.76 |
| **$IC_{50}$ (µg/mL)** | 20 | 18 | 21 |

One-way ANOVA indicated significant differences among the three extracts at all concentrations ($p < 0.05$). Values represent mean ± SD of triplicate independent experiments ($n = 3$).

**Table 6: α-Amylase Inhibitory Activity of combination of *C. sinensis, S. trilobatum* and *T. ammi* with $IC_{50}$ Values**

| Concentration (µg) | *C. sinensis* + *S. trilobatum* (% Inhibition ± SD) | *C. sinensis* + *T. ammi* (% Inhibition ± SD) | *C. sinensis* + *S. trilobatum* + *T. ammi* (% Inhibition ± SD) |
|---|---|---|---|
| 5 | 29.62 ± 8.73 | 33.61 ± 8.49 | 27.37 ± 14.64 |
| 10 | 39.75 ± 5.02 | 40.73 ± 6.44 | 38.47 ± 12.28 |
| 15 | 48.71 ± 5.79 | 50.75 ± 5.59 | 42.49 ± 11.95 |
| 20 | 55.34 ± 4.46 | 54.65 ± 8.08 | 48.63 ± 8.59 |
| 25 | 63.78 ± 2.14 | 61.73 ± 6.07 | 53.55 ± 9.54 |
| **Estimated $IC_{50}$ (µg)** | 16–17 | 15 | 22 |

$IC_{50}$ values are estimated from dose-response curves. Values are expressed as mean ± SD from triplicate experiments ($n = 3$).

**Table 7: Comparative α-Amylase Inhibitory Activity and type of interaction of *C. sinensis*, *S. trilobatum* and *T. ammi* with $IC_{50}$ Values**

| Sample | Practical % Inhibition (25 µg) | Mathematical Cumulative % | $IC_{50}$ (µg/mL) | Interaction |
|---|---|---|---|---|
| GTLE (*C.sinensis*) | 59.15 ± 3.6 | 59.15 | 20 | – |
| STLE (*S.trilobatum* ) | 66.68 ± 11.62 | 66.68 | 18 | – |
| AJSE (*T. ammi* ) | 59.03 ± 0.76 | 59.03 | 21 | – |
| GTLE + STLE | 63.78 ± 2.14 | 62.91 | 6.2 | Synergism |
| GTLE + AJSE | 61.73 ± 6.07 | 59.09 | 6.5 | Synergism |
| GTLE + STLE + AJSE | 53.55 ± 9.54 | 61.62 | 5.8 | Antagonism |

GTLE + STLE and GTLE + AJSE combinations showed synergistic inhibition, with practical values exceeding the mathematical cumulative predictions. The ternary combination (GTLE + STLE + AJSE) showed antagonistic interaction, as the practical inhibition was lower than the cumulative value. $IC_{50}$ values indicate that combinations achieve effective α-amylase inhibition at lower concentrations than individual extracts.

**Table 8: Effect of *C. sinensis*, *S. trilobatum*, and *T. ammi* extracts on inhibition of protein denaturation.**

| Concentration (µg/mL) | Standard (Aspirin) | *C. sinensis* | *S. trilobatum* | *T. ammi* |
|---|---|---|---|---|
| 10 | 27.69 ± 0.95 | 27.02 ± 5.73 | 12.83 ± 2.86 | 24.31 ± 3.83 |
| 20 | 35.81 ± 0.96 | 33.10 ± 4.78 | 19.59 ± 0.96 | 29.05 ± 2.87 |
| 30 | 40.53 ± 1.91 | 38.51 ± 6.68 | 27.02 ± 3.81 | 31.76 ± 0.95 |
| 40 | 49.32 ± 2.87 | 41.88 ± 7.64 | 33.78 ± 3.82 | 37.16 ± 2.86 |
| 50 | 56.08 ± 1.88 | 44.59 ± 7.65 | 39.19 ± 3.82 | 40.54 ± 3.82 |

Statistical analysis was performed using one-way analysis of variance (ANOVA) to determine significant differences among groups at different concentrations. Differences were considered statistically significant at $p < 0.05$.

**Table 9: Effect of combined *C. sinensis*, *S. trilobatum*, and *T. ammi* extracts on inhibition of protein denaturation.**

| Concentration (µg/mL) | *C. sinensis* + *S. trilobatum* | *C. sinensis* + *T. ammi* | *C. sinensis* + *S. trilobatum* + *T. ammi* |
|---|---|---|---|
| 10 | 27.70 ± 0.23 | 36.48 ± 0.87 | 36.48 ± 0.93 |
| 20 | 37.83 ± 1.50 | 42.57 ± 1.02 | 41.22 ± 1.05 |
| 30 | 43.92 ± 2.03 | 46.62 ± 0.68 | 50.00 ± 1.67 |
| 40 | 46.62 ± 1.05 | 52.03 ± 1.54 | 55.40 ± 0.96 |
| 50 | 48.65 ± 0.75 | 54.73 ± 2.01 | 60.81 ± 2.06 |

Data were analyzed using one-way analysis of variance (ANOVA) to compare the inhibitory effects among treatment groups at each concentration level. Statistical significance was considered at $p < 0.05$.

**Table 10: Cumulative effect and interaction type of combined *C. sinensis*, *S. trilobatum*, and *T. ammi* extracts**

| Sample | Mathematical Cumulative Value (%) | Practical Value (%) (Mean ± SD, n = 3) | Type of Interaction |
|---|---|---|---|
| GTLE + STLE | 41.88 | 48.64 ± 3.83 | Synergism |
| GTLE + AJSE | 42.56 | 54.72 ± 2.86 | Synergism |
| GTLE + STLE + AJSE | 41.43 | 60.80 ± 1.91 | Synergism |

Data are expressed as mean ± SD (n = 3). statistically significant at $p < 0.05$.

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