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Research Article

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Evaluation Potential of *Strobilanthes Auriculata* var. *Dyeriana* (Mast.) J.R.I. Wood as an Antioxidant and Antimicrobial Agent

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ABSTRACT

Strobilanthes belongs to the Acanthaceae family and possesses various medicinal qualities, including wound healing, antioxidant, antibacterial, antidiabetic, anticancer, and anti-inflammatory effects. One species in this genus, *Strobilanthes auriculata* var. *dyeriana*, is a popular ornamental plant that has long been used as a diuretic to alleviate rheumatism. While few are recognised, this species likely has many more benefits. This study examined the phytochemical composition, phenolic content, anthocyanin content, and antibacterial and antioxidant qualities of *S. auriculata* var. *dyeriana* leaf extract. Finding no anthocyanin, we discovered that the leaves of this species contained phenolic compounds (82.9±0.86 µg/100µg). Furthermore, vigorous antioxidant activity (IC_{50} 96.17 ppm) and growth-inhibiting action against *Salmonella typhimurium* are provided by high phenolic content. According to this study, the leaf of *S. auriculata* var. *dyeriana* may act as a potential antibacterial and antioxidant agent.

Keywords: Antibacterial; Anthocyanin; DPPH; Phytochemical screening; Total phenol content

NOMENCLATURE

ε	: Molar absorptivity of cyanidin-3-glucoside (269000 L/(mol.cm))
DF	: Solubility factor
DPPH	: 1,1-diphenyl-2picylhydrazyl
L	: Cuvette width (1cm)
MW	: The molecular weight of cyanidin-3-glucoside (449.2 g/mol)
TPC	: Total Phenol Content

1. INTRODUCTION

Strobilanthes is a genus from the Acanthaceae family that Blume first described in 1826 from samples taken in West Java. It is the most species-rich genus throughout tropical Asia and Australia. *Strobilanthes* is a well-known genus in Sri Lanka due to its diverse behaviours, gregarious distribution, and gorgeous blooms. Many *Strobilanthes* species have medicinal qualities. In several parts of the world, *Strobilanthes* extract has been used to treat illnesses caused by respiratory viruses, spider poisoning, snake bites, and cerebrospinal meningitis. Furthermore, the leaves of this plant are often used to make indigo dyes[1].

Traditionally, several *Strobilanthes* species are also used as medicinal plants in several parts of the world, particularly in the Ayurvedic medicinal system[2]. *S. ciliatus* and *S. heynianus* are used as medicinal herbs to treat several conditions such as epilepsy, paraplegia, back pain, hemiplegia, and paralysis[2,3]. *S. heynianus* also exhibits potent antioxidant potential[4].

Figure 1. ***Strobilanthes auriculata* var. *dyeriana* is widely recognized as an attractive plant in indonesia because of its magnificent learf color.**

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Strobilanthes auriculata var. *dyeriana*, known as *sembar lilin* in Indonesia, is distinguished by its dark green leaves with metallic-purple stripes radiating from the vein's center[5] (Fig. 1). Despite the popular use of foliar ornamentation plants, this species is also used as an herbal treatment for rheumatism in Indonesia. However, more information is needed on this plant. Most information is primarily about its propagation, but studying its potential as a therapeutic herb still needs to be explored. As a result, several facets of this plant, spanning from botanical features to its uses, still need to be explored. *S. auriculata* var. *dyeriana* is thought to have additional potential benefits that are not commonly known, particularly in herbal medicine. This study aimed to examine the efficacy of *S. auriculata* var. *dyeriana* extract as a natural antioxidant and assess its antibacterial activities.

2. MATERIAL AND METHODS

2.1 Leaves Extraction

The leaves of *S. auriculata* var. *dyeriana* used in this study were obtained from a non-collection ornamental plant in the Eka Karya Bali Botanic Garden area. The leaves were cleaned, thinly cut, and air-dried for five days. The leaf extraction was carried out using the maceration method, slightly modified from the procedure used by Baehaki[6], *et al.* and Andila & Hartanto[7]. In 1000 mL of methanol, 100 g of dried leaves were macerated and then incubated in the dark at 26 °C. The mixture was filtered with filter paper after three days. The crude extract was separated from the solvent using a vacuum rotary evaporator. The concentrated crude extract was then used for further analysis.

2.2 Phytochemical Screening, Total Phenol, and Anthocyanin Content

Phytochemical contents, including saponin, phenols, and steroids, were screened qualitatively according to the methods carried out by Yuniati[8] *et al.,* Putri[9] *et al.,* and Hossain[10] *et al.,* respectively.

TPC was calculated using the linear regression equation of gallic acid standards. The determined content was expressed as equivalents of gallic acid. Total phenolic analysis was performed using the modified method by Ghafoor[11] *et al.,* The sample's TPC was estimated using the gallic acid linear regression equation.

The total anthocyanin content test was carried out using the pH differential method. Sample solutions were prepared from each filtrate, and each sample was measured for its absorbance at its maximum absorption wavelength and λ700 nm (as an absorbance correction) with pH 1.0 and pH 4.5 solutions. The total anthocyanin content (%) was calculated using the following formula:

$$\% \text{ inhibition} = \frac{((A510 \pm A700)pH\ 1{,}0 - (A510 \pm A700)pH\ 4{,}5) \times MW \times DF}{\varepsilon} \times L$$

2.3 Antioxidant Activity Assay

The antioxidant activity of *S. auriculata* var. *dyeriana* leaves was performed according to the modified method by Wibawa[12], *et al.* The stock solution of plant crude extract was diluted into numerous concentration series: 50, 100, 150, 200, 250, 300, and 350 ppm. One ml of each extract concentration was mixed with 4 ml of DPPH (40 ppm). The mixture was mixed and incubated in a dark room for 30 minutes. Ascorbic acid antioxidant activity was tested in various concentrations (2, 4, 6, 8, 10, and 12 ppm) as a comparison. Following that, the absorbance of each mixture was measured at λ 517 nm with a UV-Vis spectrophotometer. The quantitative calculation was performed by determining the free radical inhibitory power of the sample, which was calculated using the following formula:

$$\% \text{ inhibition} = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} \times 100$$

2.4 Antimicrobial Activity Assay

Antimicrobial activity was assessed using the modified Kirby-Bauer disc diffusion method[13] on nutrient agar. The microorganisms tested were those that cause human diseases, including *Candida albicans*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Streptococcus mutans*.

The tested microorganisms were regenerated before the antimicrobial activity using the following protocol: one ose of the microbes tested was transferred aseptically onto a sterile slant nutrient agar, then the bacteria were incubated for 24 h at 37 °C.

For the antimicrobial activity assay, the fresh colonies were transferred into a sterile saline solution, and the turbidity was adjusted to 0.5 McFarland standards before streaking onto the surface of nutrient agar and allowed for 15 minutes before the tested discs were placed on the surface of the agar. The formation of the clear inhibition zone was observed at one to three days of incubation (37 °C).

Table 1. Chemical properties contained in *strobilanthes auriculata* var. *dyeriana* leaces extract

Chemical compounds	Presence
Phenols	√
Steroid	–
Triterpenoid	–
Saponin	–
TPC (equivalent with gallic acid) (μg/100μg extract) ± S.D.	82.9 ± 0.86
Anthocyanin (%)	–

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening, Total Phenol, and Anthocyanin Content

The results of phytochemical screening showed that the leaf extract of *S. auriculata* var. *dyeriana* only contained chemical compounds belonging to the phenol group. No detectable levels of saponin, steroid, and triterpenoid

compounds were found through qualitative testing (Table 1). According to this result, the TPC of this extract was further assayed. As a result, *S. auriculata* var. *dyeriana* leaves extract showed 82.9±0.86 µg/100µg extract of TPC (equivalent to gallic acid), with no anthocyanin detected.

Several studies reported that dark rind contains more polyphenols (flavonoids, anthocyanins, and tannins)[14, 15]. Anthocyanins are plant pigments that produce red, blue, and purple colours in several plant parts, including leaves[16, 17]. Although *S. auriculata* var. dyeriana is commonly known as a purple plant due to its leaf colour, our study showed no anthocyanin compounds in the sample, suggesting that the biosynthesis process of anthocyanin compounds does not occur in the leaf parts of this plant.

3.2 Antioxidant Activity

The present study showed that *S. auriculata* var. *dyeriana* had an IC_{50} value of 96.17 ppm, while ascorbic acid had an IC_{50} value of 5.02 ppm. Compared to the IC_{50} value of ascorbic acid, the *S. auriculata* var. *dyeriana* extract had lower activity but still had strong antioxidant ability.

Because of its high phenolic content, *S. auriculata* var. *dyeriana* was assumed to have high antioxidant potential. According to various reports, phenolic compounds exhibit several biological functions, such as antioxidant, antimutagenic, modified gene expression, cardiovascular protection, antidiabetics, vision improvement, and carcinogenesis suppression[18-20]. The number of -OH groups in the phenolic compound framework can influence antioxidant activity. Their ability as a hydrogen donor atom can neutralize free radicals and prevent oxidation[21].

The high antioxidant activity in *S. auriculata* var. *dyeriana* is also thought to be related to its anti-inflammatory properties. Previous research has linked *S. heyneanus*' high amount of antioxidant activity to its action as an anti-inflammatory medication[22]. Closely related plants, both in family and genus, tend to produce comparable metabolites, implying that their efficiency as therapeutic components is also likely to be similar. Previously, antiviral, anticancer, anti-inflammatory, and anticoagulant activities have been demonstrated from *S. tonkinensis* extract[23], while *S. barbatus* and *S. tonkinensis* have also been found to be high in antioxidants[3,4]. *S. crispus* leaves are frequently employed in traditional medicine for their blood pressure-lowering, antidiabetic, anticancer, and diuretic qualities. Scientifically, it has been proven to have potent antioxidant activity, anti-AIDS, and anticancer properties[4,24]. Furthermore, a study discovered that the aqueous extract of *S. crispus* leaves contains a high level of antioxidants and anticancer potential[25].

3.3 Antimicrobial Activity

The plant extracts' antimicrobial activities were demonstrated by establishing a clear zone in the growth media, indicating the presence of antagonistic metabolites produced by the extracts, hence inhibiting microbial development[28]. We discovered that the extract of *S. auriculata* var. *dyeriana* could only suppress the growth of *S. typhimurium* in this investigation (Fig. 2).

Figure 2. Methanolic leaf extract of Strobilanthes auriculata *var. dyeriana* formed a clear zone when challenged with Salmonella typhimurium. The clear zone emerged on the first day after treatment. the diameter of the clear zone increased with each passing day. this figure was taken on the thrid-day post -treatment.

The ability of the *S. auriculata* var. *dyeriana* leaf extract to inhibit bacterial growth is likely due to compounds with antibacterial properties but with a narrow or specific spectrum. The effectiveness of the extract is probably due to the phenolic compounds contained in the extract. Several studies proved phenol has potent antibacterial action against various organisms, including bacteria, yeasts, and molds[19, 27, 28]. Phenolic compounds efficiently reduced the growth of *Bacillus subtilis, Escherichia coli*, and *Pseudomonas aeruginosa*[29].The mechanism of antimicrobial activity of phenolic compounds is usually caused by hydrogen bonding from the phenolic compound, resulting in intracellular function changes that lead to changes in cell permeability. The increased lipophilic character of the phenolic compound will increase antimicrobial activity by supporting the interaction of phenolic compounds with cell membranes, causing permanent damage to the cytoplasmic membrane resulting in lysis[30].

Several *Strobilanthes* species, notably *S. formosanus* and *S. kunthiana*, have been shown to have antibacterial activity[31,32]. Additionally, *S. urticifolia* methanolic extract was also found to have antibacterial activities against *E. coli, Micrococcus luteus, S. aureus,* and *S. typhi*[33], while dichloromethane extract of *S. crispus* inhibited *B. subtilis* and *S. aureus*[34]. Recently, *S. ciliatus* was found to have antibacterial activity against *S. aureus, B. subtilis, E.coli, P. aeruginosa, Klebsiella, Cornybacterium, A. niger, C. albicans, Trichophyton rubrum, Microsporum gupseum,* and *Monascus purpureus*[2].

4. CONCLUSION

Phytochemical screening of *S. auriculata* var. *dyeriana* leaf extract showed the presence of phenols. In addition, the total phenol content is high by 82.9±0.86µg/100µg,

but no anthocyanin was found. The leaves also showed excellent antioxidant activity (IC_{50} value of 96.17 ppm) and strongly inhibited the growth of *S. typhimurium*. This study reveals that *S. auriculata* var. *dyeriana* leaves are potent antibacterial and antioxidant agents.

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According to the contribution made in this paper, the authors state that IPAHW and ASL are the main contributors, while PSA, INL, and VS are the co-contributors.

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Effect of Foliar Micronutrient Application on Phytoconstituents and Mineral Composition of Carrot Grown in Trans-Himalayan Region

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ABSTRACT

The cropping season in trans-Himalayan region of Ladakh is limited to six months. Soil in the region is sandy, coarse textured, pH 7.79±0.2 and deficient in micronutrients. Carrot is one of the major root vegetable crops growing in this region. Therefore, a study investigating the effects of mineral supply on the nutritional quality (phytoconstituents and micronutrients) of carrot roots was undertaken. Maximum values of carotene and total flavonoids were recorded under the foliar application of $ZnSO_4$ @ 0.5 % (T_3), which was at par with application of $ZnSO_4$ @ 1.0 % (T_4). However, the maximum value of total phenol concentration (6.59±0.34 mg GAE/g DW) was recorded under foliar application of $ZnSO_4$ @ 1.0 % (T_4), which was at par with $ZnSO_4$ @ 0.5 % (T_3). Zinc and boron application influenced the mineral content of carrots. During plant growth, adding small amounts of zinc and boron to the feeding solutions affected the Cu, Mn and Zn concentration, in roots. Applying different amounts of minerals nutrients has the potential to improve the nutritional value and morpho-physical quality of carrots. This present study will help to understand the utilisation of optimal quantity of micronutrients to improve carrot cultivation in cold desert of Ladakh. Taking into consideration of variables like soil micronutrient deficiencies, this research opens the door to the biofortification of necessary minerals in crops.

Keywords: Antioxidant; Carrot; Carotenoid; Cold desert region; Foliar application; Phenolics compound

NOMENCLATURE

mMTE : Millimole Trolox Equivalent
GAE : Gallic Acid Equivalent
RE : Rutin Trihydrate Equivalent
rpm : Revolution Per Minute
FW : Fresh Weight
DW : Dry Weight

1. INTRODUCTION

Carrot, botanically named as *Daucus carota* L.*,* is a popular root vegetable in the *Apiaceae* family. A carrot is a crop that grows in cool temperatures and can be planted in temperate climates during spring or in subtropical climates during autumn or winter. Carotene, a precursor to vitamin A, is abundant in orange-coloured carrots, which also have significant amounts of thiamine and riboflavin. Carrots are consumed by many people due to their rich nutritional content and many health benefits. The carrots are considered as a rich source of fibre, vitamins, carbohydrates, and various mineral salts[1]. In Leh district, vegetable production occupies 5.5 % of the total 10,319 hectares of agricultural land, with carrots accounting for only 2.0 % of the total vegetable production (Hill Council of Ladakh, 2018).

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Micronutrients are important in agricultural crop production. These are imporstant for plant growth, yield, and quality[2]. The application of micronutrient concentrations at optimum levels alleviates plant biotic and abiotic stress. Micro/macronutrients serve as cofactors of various enzymes and help in the biosynthesis of many biomolecules. Biofortification has been used to increase the nutritional value of crops. Improvement in agronomic qualities can be observed upon the administration of these components in edaphic and/or foliar doses, and it also tends to increase the number of important elements in different edible sections of plants[3].

Zinc is essential for the growth and development of plants, chlorophyll and carbohydrate biosynthesis and serves as a cofactor for enzyme systems. It also helps plant tissues withstand cold temperatures. Zinc deficiency in crops can be found in every part of the world and almost all respond positively to the application of Zn[4]. In this regard, some findings suggest that applying edaphic and foliar zinc fertilisers together can boost crop yield and enhance the concentration of zinc in agricultural commodities[5].

Boron is the seventh major element needed to support plant growth and production and is required

for cell division, cell wall formation, cell elongation, protein metabolism, sugar translocation and improved phytohormone transport[6].

Ladakh is a trans-Himalayan region and geographical condition allows cropping season of only six months during the summer. The agro-climatic region during summer favours the cultivation of European carrots in this region. Soil of Ladakh is sandy, coarse textured and high pH 7.79±0.2 and micronutrient deficiency has been found in the soil[7]. According to previous reports, the soil of Ladakh is deficient in zinc and boron[8]. Enhancing the bioavailability of mineral elements in consumable crops can help overcome mineral deficiencies. Mineral fertilisers and/or increases in mineral element solubilisation and mobilisation in the soil, boost mineral element concentrations in edible tissues. Bio-fortification is the agricultural practice of incorporating the required micronutrients into a plant, seed, or grain. Balanced nutrient content has different roles during various stages of development, growth, and reproduction. Hence, keeping these factors in mind, the present study was investigated to analyse the influence of foliar application of zinc and boron of different concentrations and at different time intervals of the growing stage of carrot to improve the productivity and mineral composition of the carrot. On the other hand, biochemical constituents such as ascorbic acid, carotenes, total flavonoids, total phenols, and total antioxidant activity in the Early Nantes variety of carrots were observed.

2. MATERIAL AND METHODS

2.1 Experimental Details

Carrot var. Early Nantes seeds were procured from the local market and field trials were conducted in randomised block design at the Agriculture Research Unit (11526±32.30 ft. AMSL), DIHAR, Ladakh during the summer season in the years 2020-21and 2021-22. Recommended doses of FYM were applied in the carrot experimental field. Foliar application of zinc sulfate and borax in different treatment combinations *viz., viz.,* (T_0-Control, T_1-Borax @ 0.1 %, T_2-Borax @ 0.2 %, T_3-$ZnSO_4$ @ 0.5 %, T_4-$ZnSO_4$ @ 1.0 %, T_5-Borax @ 0.1 % + $ZnSO_4$ @ 0.5 %, T_6-Borax @ 0.2 % + $ZnSO_4$ @ 1.0 %, T_7-Borax @ 0.1 % + $ZnSO_4$ @ 1.0 %, T_8-Borax @ 0.2 % + $ZnSO_4$ @ 0.5 %) were applied at 45 and 90 days of sowing. All the standard agronomical practices were followed during the growing period of carrots.

Figure 1. Foliar application of zinc and boron on carrot.

2.2 Ascorbic Acid

The method described by Rangana[9] was followed for the determination of ascorbic acid content in carrots. Five grams of root pulp were pulverised with a 3 % metaphosphoric acid buffer, filtered, and diluted to 100 ml. The aliquot of 5 ml was titrated with 2, 6-dichlorophenol indophenol dye till colour changed to light pink. It was expressed as mg/100 g of root pulp.

2.3 Carotene

To analyse the carotene content, the crude pigment was extracted in organic solvent as described by Rangana[10]. A 5 g sample was gently crushed with 10 ml of petroleum ether and diluted with 3 % acetone in water containing 5 % sodium sulfate (Na_2SO_4). A 10 ml aliquot was placed in a separating funnel. Aliquot mixed with distilled water, and left at room temperature for 5 minutes. This extraction was repeated 3 to 4 times to enhance carotenoid recovery. After draining the aqueous layer, the supernatant was collected in a test tube and using UV-Visible Spectra Max i3x Spectrophotometer the absorbance was measured at 452 nm with petroleum ether as the blank. A β-carotene standard curve was used to estimate total carotenoids, reported as µg/100 g.

2.4 Determination of Total Flavonoids Content (TFC) and Total Phenolic Content (TPC)

One-gram pulverized dry sample was homogenized in 20 ml methanol, followed by centrifugation for 10 min at 10,000 rpm. The extraction was done thrice at room temperature in dark conditions. The supernatant was collected using filter paper (WhatmanNo.1 for further quantification.

The extract was used to assess TFC following aluminium chloride method[11], using Rutin Trihydrate as standard. Extracts (300 µl) at various concentrations of Rutin trihydrate were diluted with 1200 µl of distilled water. Then, 90 µl of sodium nitrite reagent (0.724 M) was added and incubated for 5 minutes at room temperature. 90 µl of aluminium chloride (0.749 m) was added and mixture was incubated for 6 minutes. Next, 600 µl of sodium hydroxide (1.0 M) and 720 µl of deionised water were added, to make up total volume to 3000 µl. At 510 nm, absorbance was measured and results were expressed in mg rutin trihydrate equivalent (RE) per g dry weight.

TPC from extracted samples was evaluated by using FC reagent method with minor changes[11]. A mixture of 9 ml of deionized water and 1 ml of extracts at various concentrations was prepared, then 1 ml of FC reagent was added and incubated for 5 minutes at room temperature. Further 2 ml of 20 % sodium carbonate solution and set to incubation at room temperature for 60 minutes in the dark. Absorbance at 750 nm was measured. A standard curve of gallic acid was prepared with different concentrations. Results were expressed in mg (gallic acid equivalent) per g dry weight.

2.5 Total Antioxidant Activity

The total antioxidant activity was determined using Apak[12] method. Trolox was used to create a standard curve and results of total antioxidant activity were expressed as mMTE/L. Carrot Juice was extracted from a 20 g root sample and filtered using filter paper (Whatman No. 1). In a test tube, 1 ml each of copper chloride (1.705 g/L), neocuproine (1.562 g/L ethanol), and ammonium acetate buffer (pH 7, 19.27 g/250 ml) were combined with 0.1 ml of the extracted juice. 6 ml of volume was adjusted with distilled water. 30 minutes incubation at room temperature was done and absorbance at 450 nm was measured.

2.6 Determination of Micronutrient

The mineral content of the carrot samples was determined using the AOAC method[13] with some modifications. 200 mg of samples were digested (microwave digestion, Analytik Jena) with 8 ml of acid mixture (2.0ml HCl and 6.0ml HNO_3). A clear digest was diluted with distilled water up to 50 ml. This prepared sample solution was used to determine sodium, magnesium, manganese, iron, copper, and zinc using AAS (Analytik Jena). Mineral concentrations were calculated using standard calibration curves and expressed as mg/100g.

2.7 Statistical Analysis

All data were tested by Analysis of Variance (ANOVA) and significance of the mean difference test was analysed by using SPSS, Inc. version 22.0 ($p<0.05$). The data were expressed as mean ± standard deviation.

3. RESULT AND DISCUSSION

3.1 Ascorbic Acid

The foliar application of zinc and boron with various concentrations had no significant impact on the ascorbic acid content of carrot roots (Table 1). Ascorbic acid in carrots is less than other vegetables like peas, brassicas, and spinach. Hence are not considered to be a significant source of ascorbic acid[14]. The ascorbic acid in carrots was observed from 7.17 to 9.01 mg/100 g FW among the treatments. But statistically, significant change was not observed in the ascorbic acid content of carrot after foliar application of zinc and boron.

3.2 Carotenes

The concentrations of carotenoids found in the carrot var. Early Nantes grown under the various zinc and boron treatments were presented in Table 1. A significant influence on carotene content was observed by the application of zinc and boron. The maximum value of carotenes (4298.78 µg/100 g FW) was found in the foliar application of $ZnSO_4$ @ 0.5 % (T_3), which was statistically at par with the application of $ZnSO_4$ @ 1.0 % (4294.41 µg/100 g FW). A minimum value of carotenes (3533.04 µg/100 g FW) was also found in the foliar application of Borax @ 0.2 %. The overall phytochemical content in carrots may be affected by genetic and abiotic stresses due to the high-altitude condition. The high or low carotene concentration for given treatments depends on several factors, including morphological and physiological traits of the cultivar, as well as growth factors. In the present study, it was found that boron affected carotenes concentration. More specifically, a larger dose of boron might have decreased the level of carotenoids in plants. Whereas, adequate doses of zinc applied in carrots, might increase carotenes in plants. Zinc treatment increases carotenoid content, stomatal conductance, antioxidant enzyme activities and chlorophyll content, while decreasing electrolyte leakage and water loss in dry conditions[15]. The application of zinc enhances carotenoid contents that have an important role to overcome photo oxidative damage.

Table 1. Combined and individual effects of foliar application of boron and zinc treatments on phenolics compounds and antioxident content in carrot

Treatments	Ascorbic acid (mg/100gFW)	Carotenes (µg/100g FW)	TFC (mgRE/g DW)	TPC (mgGE/g DW)	Antioxidant (mMTE/L FW)
T_0	7.17±0.43^{a}	4105.64±51.64^{d}	1.60±0.31^{bc}	5.61±0.86^{abc}	59.06±1.92^{a}
T_1	7.42±0.43^{a}	3696.98±12.80^{b}	1.28±0.09^{abc}	5.33±0.10^{abc}	64.37±2.14^{ab}
T_2	7.63±0.69^{a}	3533.04±42.68^{a}	0.90±0.09^{a}	4.14±0.81^{a}	63.80±0.74^{ab}
T_3	9.01±0.6^{9}a	4298.78±91.94^{e}	1.75±0.22^{c}	6.27±0.39^{c}	64.17±2.09^{ab}
T_4	8.51±1.08^{a}	4294.41±24.81^{e}	1.73±0.09^{c}	6.59±0.34^{c}	66.62±1.88^{b}
T_5	8.97±0.69^{a}	3764.59±61.53^{b}	1.41±0.13^{abc}	6.00±0.37^{bc}	67.81±1.92^{b}
T_6	8.51±0.47^{a}	3928.86±47.55^{c}	0.90±0.36^{a}	4.50±1.00^{ab}	67.44±1.63^{b}
T_7	8.97±0.69^{a}	4208.23±48.30^{de}	1.05±0.11^{ab}	5.32±0.09^{abc}	66.42±2.87^{b}
T_8	8.97±0.69^{a}	3815.10±25.45^{bc}	0.87±0.24^{a}	4.42±0.52^{ab}	65.03±1.32^{b}

According to Tukey's test, different letters within each column indicate significant differences (P = 0.05). All data are presented as mean ± standard deviation n=3

3.3 Total Flavonoids Content (TFC)

In contrast to ascorbic acid, the flavonoid content was significantly different among the treatments. Individually zinc application produced significantly higher values of TFC in foliar application of $ZnSO_4$ @ 0.5 % compared to boron. The highest TFC (1.75±0.22mg RE/100g DW) was recorded in the foliar application of $ZnSO_4$ @ 0.5 % (T_3), which was statistically at par with $ZnSO_4$ @ 1.0 %. While the lower TFC values 0.87, 0.90, and 0.90 mg RE/100 g DW content were found in foliar application of Borax @ 0.2 % + $ZnSO_4$ @ 0.5 % (T_8), Borax @ 0.2 % + $ZnSO_4$ @ 1.0 % (T_6) and Borax @ 0.2 % (T_2), respectively. Boron concentration seems to affect flavonoid levels. Sarafi[16], reported that boron toxicity considerably boosted flavonoid content in cultivar Odysseo while dramatically decreased it in cultivars Arlequin, Century, Imperial, and Salomon, showing a distinct genotypic response and harvesting time-dependent variation. This could be explained by the increased photosynthesis and sugar accumulation followed by zinc sprays, which might promote the synthesis of phenolic compounds, particularly flavonoids[17].

3.4 Total Phenolic Content (TPC)

Total phenol content in carrot roots increased significantly ($p > 0.05$) by foliar application of different combinations of boron and zinc. It was also observed that total phenol content in carrot roots increased significantly by foliar application of boron and zinc without combination Table 1. Foliar application of zinc showed an increase in Total Phenolic Contents with respect to control. Data shows that total phenolic contents were significantly affected by foliar application of zinc treatments. The highest value of total phenol concentration (6.59±0.34 mg GAE/100 g DW) was recorded under foliar application of $ZnSO_4$ @ 1.0 % (T_3), which was on at par with $ZnSO_4$ @ 0.5 %. There was a reduction in total phenol concentration with an increase in boron application rate compared with the control. Whereas, minimum TPC was observed in foliar application of Borax @ 0.2 % (T_2). This proposes that boron application leads to the reduction of phenols. It indicates that a specific level of boron causes the maximum reduction in the concentration of phenols[18]. However, our result was found similar to Song[19] that noted the accumulation of total phenols upon foliar application of zinc in berry.

3.5 Total Antioxidant Activity

In plants, vitamin C functions as an antioxidant and protects plants from oxidative stress/abiotic stress[20]. Foliar application of zinc and boron significantly affected carrot antioxidant activity compared with the control. The maximum antioxidant (67.81±1.92 mMTE/L DW) was observed in foliar application of Borax @ 0.1 % + $ZnSO_4$ @ 0.5 % (T_5), which is statistically at par with $ZnSO_4$ @ 1.0 % (T_4), Borax @ 0.2 % + $ZnSO_4$ @ 1.0 % (T_6), Borax @ 0.1 % + $ZnSO_4$ @ 1.0 % (T_7),and Borax @ 0.2 %+ $ZnSO_4$ @ 0.5 % (T_8). While the lowest value (59.06±1.92 mMTE/L DW) of antioxidants was found in control (T_0). In Tiwari[21] similar results of antioxidant activity was observed in carrot root. In this study, it was found that the antioxidant activity increases with the foliar spray of zinc and boron. An increase in antioxidant capacity was also reported by Majdoub[22] with the foliar application of zinc.

3.6 Micronutrients

Significant differences with respect to micronutrient content using the foliar application of different concentrations were observed (Table 2). The manganese concentration ranges between 1.30 to 1.84 mg/100 g DW, with the lowest value found with the $ZnSO_4$ @ 0.5 % (T_3), while the Borax @ 0.1 % +$ZnSO_4$ @ 0.5 % (T_5) foliar dose produced the highest value (1.84 mg/100 g DW).

The application of Borax @ 0.2 %+ $ZnSO_4$ @ 0.5 % produced highest zinc concentrations 11.17 mg/100 g DW,

Table 2. Combined and individual effect of foliar application of boron and zinc on accumulation of Mn, Zn, Cu, Na and Fe in carrot

Treatments	Mn (mg/100g)	Zn (mg/100g)	Cu (mg/100g)	Na (mg/100g)	Fe (mg/100g)
T_0	1.36±0.1^{9a}b	5.49±0.66^{a}	0.62±0.06^{b}	310.73±16.88^{a}	8.66±0.47^{a}
T_1	1.39±0.14^{ab}	5.38±0.22^{a}	0.43±0.08^{ab}	341.36±4.69^{a}	9.79±0.45^{a}
T_2	1.69±0.10^{ab}	5.54±0.33^{a}	0.51±0.12^{ab}	373.68±22.34^{a}	10.30±1.01^{a}
T_3	1.30±0.08^{a}	10.16±0.44^{c}	0.46±0.06^{ab}	374.86±58.49^{a}	9.54±0.95^{a}
T_4	1.39±0.04^{ab}	7.14±0.31^{b}	0.41±0.07^{a}	322.94±10.28^{a}	9.82±0.77^{a}
T_5	1.84±0.39^{b}	10.24±0.16^{c}	0.50±0.05^{ab}	406.43±24.31^{a}	10.24±0.51^{a}
T_6	1.74±0.16^{ab}	8.16±0.37^{b}	0.41±0.05^{a}	331.47±5.10^{a}	9.57±1.05^{a}
T_7	1.38±0.18^{ab}	7.97±0.57^{b}	0.44±0.05^{ab}	337.29±5.88^{a}	10.20±0.36^{a}
T_8	1.42±0.13^{ab}	11.17±0.32^{c}	0.49±0.08^{ab}	334.10±12.65^{a}	10.15±0.53^{a}

According to Tukey's test, different letters within each column indicate significant differences (P = 0.05). All data are presented as mean ± standard deviation n=3

which was statistically at par with Borax @ 0.1 % + $ZnSO_4$ (10.24 mg/100 g DW) and $ZnSO_4$ @ 0.5 % (10.16 mg/100 g DW) whereas, foliar dose of Borax @ 0.1 % reported minimum value 5.38 mg/100 g DW. In the present study, zinc was found the best absorbed micronutrient. Gupta[23] reported that due to chelation of Zn^{2+}; Zn is known to be better transported by phloem compared to xylem. Many studies have revealed a negative association of Zn with Cu, Fe, and Mn.

The copper concentration was highest (0.62 mg/100 g DW) in control compared to all the treatments. Minimum copper concentration (0.41 mg/100 g DW) was observed with foliar application of $ZnSO_4$ @ 1.0 % and Borax 0.2 % + $ZnSO_4$ @ 1.0 %. This relationship occurs because of the competition between cations for absorption sites[24]. In this study, it was observed that zinc and boron had a negative correlation with copper but with other cationic micronutrients (Fe and Mn) applications of zinc and boron were not correlated either positive or negative. The absorption of Cu and Mn in carrots was affected by boron individually and by a combination of boron and zinc. The sodium (Na) and iron (Fe) values were presented in table 2, and showed no significant effect of boron and zinc application on carrot roots and the values ranged between 310.73 to 406.43 mg/100 g DW and 8.66 to 10.24 mg/100 g DW, respectively.

Esringü[25] found comparable results in strawberry, where the Fe content in the roots increased and decreased when applied with lesser and higher concentration of boron, respectively. These findings indicate an affinity of boron and iron that might be a synergistic interaction between the two nutrients. Rajaie[26] also observed a positive correlation between the concentrations of iron with an increase in boron concentration in *Citrus aurantifolia.* This study of foliar application of zinc and boron was not influenced by the absorption of Fe content in carrot roots. Our result was similar[27] that micronutrient content in apple leaves and fruit increases after foliar applications of micronutrient. It was reported that an increase in zinc levels in the leaves and fruit of pomegranate spraying with $ZnSO_4$[28]. Minerals like Zn, Cu and B in roots were found higher correlated with the increased concentration of these minerals in leaves that is linked with the foliar spray boron and zinc. These findings suggested the movement of micronutrients from phloem to other parts of the plant[27] that established the source-sink relationship of minerals movement. The higher concentration of Mn in roots is usually considered to be an imperfectly mobile element[29] however foliar application of zinc and boron helps in the movement of Mn from roots to other parts of plants. This enhanced micronutrient concentration in roots after foliar zinc and boron application individually and in combination is highly desired because it can overcome the widespread micronutrient deficiencies in the food chain[30].

4. CONCLUSION

It was found that the supplementation of $ZnSO_4$ in the nutrient application regimes in carrots had positive effect on TFC, TPC and carotene content whereas the opposite effect was observed with the application of boron. There was no significant effect on ascorbic acid content. Uptake of Fe and Na was not influenced by $ZnSO_4$ and boron. The uptake of Zn and Mn was increased by the addition of $ZnSO_4$ and boron in the feeding solution. Therefore, mineral nutrient applications are helpful to manipulate the nutritional value of carrot crops. This will minimize fertiliser use efficiency and reduce deleterious impacts on the environment. Foliar application of micronutrients overcomes the deficiency of micronutrients in crops. This type of agricultural practice helps in the development of biofortification research and improves soil fertility.

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Poly-Herbal Extract: A Promising Approach for Mitigating Gastric Ulcers in Rat Models

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ABSTRACT

A prevalent condition that affects humans is stomach ulcers. The constraints and rapid expansion of civilization, particularly a stressful lifestyle, are contributing to an increase in the occurrence of ulcers. New synthetic pharmaceuticals and herbal drugs must fulfil global quality, safety, and efficacy criteria as Western medicine progresses. The stomach mucosa breaks down into gastric ulcers, which have a diameter greater than 5 mm and extend into the muscularis mucosa. Changes in the stomach's defence mechanisms may alter the gastric mucosa, resulting in erosion and ultimately ulceration. This study aims to explore the potential of tamarind seed and aloe vera extracts in mitigating gastric ulcers, presenting a natural alternative to NSAID-induced ulcer treatment. The herbal extract's effectiveness in reducing oxidative stress and gastric mucosal damage was assessed in comparison to omeprazole. The anti-ulcer activity was assessed using approximated biochemical parameters, and in a dose-dependent manner, the outcomes were statistically noteworthy in contrast to the rats in the ulcer group.

Keywords: Gastric ulcer; Aloevera; Tamarind seeds; Indomethacin; Omeprazole

NOMENCLATURE

CAT : Catalase
CCSEA : Committee for control and supervision of experiments on animals
GI : Gastrointestinal
GPx : Glutathione peroxidase
IAEC : Institutional animal ethics committee
MDA : Malondialdehyde
MPx : Myeloperoxidase
NSAIDs : Nonsteroidal anti-inflammatory drugs
PG : Prostaglandin
PPIs : Proton pump inhibitors
SOD : Superoxide dismutase

1. INTRODUCTION

Peptic ulcers, characterized by disruptions in the mucosal integrity of the stomach, duodenum, or esophagus, present a significant health challenge[1]. While conventional treatments such as Proton Pump Inhibitors (PPIs) like omeprazole are effective in reducing gastric acid secretion, they come with their own set of drawbacks, including long-term dependence, potential drug interactions, and side effects like nutrient malabsorption and an increased risk of gastrointestinal infections[2]. Moreover, patients on chronic Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) therapy, such as aspirin and indomethacin, face a heightened risk of developing ulcers due to the suppression of mucosal prostaglandin synthesis, leading to weakened mucosal defenses[3].

The use of NSAIDs remains essential for managing pain and inflammation, but their associated Gastrointestinal (GI) complications significantly limit their therapeutic scope[4]. The primary concern with NSAIDs is their ability to cause erosive and ulcerative lesions in the gastric lining, particularly in chronic users. This has led to the search for safer, natural alternatives that can address both the root cause of ulcer formation and the side effects caused by NSAIDs.

In this context, the exploration of traditional medicinal plants like *Tamarindus indica* (tamarind) and *Aloe barbadensis* (aloe vera) offers a promising avenue[2]. Both tamarind seeds and aloe vera have long been recognised for their anti-inflammatory, antioxidant, and wound-healing properties. Tamarind seeds, commonly used in traditional medicine across Asia and Africa, have been reported to alleviate gastric discomfort and provide protective effects against mucosal damage[5,6]. Aloe vera, known for its soothing and tissue-repairing properties, has demonstrated the potential to reduce inflammation and promote ulcer healing[7].

This study aims to assess the anti-ulcerogenic potential of tamarind seeds and aloe vera in mitigating NSAID-induced gastric ulcers. By focusing on these natural

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alternatives, we seek to address the gaps in conventional therapies, particularly the limitations and side effects associated with synthetic drugs like omeprazole.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

The local farmers of Mallapurain Chitradurga, and Hullekere in Tumkur, India, provided the Tamarind Seeds (*Tamarindus indica Linn.*).Aloe Vera powder purchased from Patanjali Ayurveda store, Bagalagunte, Bangalore-560073. We obtained the medications indomethacin and omeprazole from Bharani Medicals, located at Bhuvenshwari Nagar, Bagalagunte, Bangalore-560057.Normal saline, Methanol, Petroleum ether, and Hydrogen peroxide 100V 30 % Solution w/v, were procured from KFC, Sigma-Aldrich, Bengaluru.

2.2 Animals

The experiment was approved by the Acharya & BM Reddy College of Pharmacy's institutional animal ethics committee (Approved IAEC no: 997/PO/Re/S/06/ CPCSEA) and carried out in compliance with CCSEA, New Delhi, India norms. Wistar albino rats weighing 150-200g were chosen for in vivo studies. Normal environmental conditions were maintained for the animals, including a 12-hour light/dark cycle, a controlled humidity level, and normal feed pellets and water available for consumption.

2.3 Authentication of Seeds

The indigenous farmers of Mallapura, Chitradurga, and Hullekere, Tumkur, India, provided the dried seeds of *Tamandus indica Linn*, which were verified for authenticity by Dr. Kanupriya, Senior Scientist at the Indian Institute of Horticultural Research, Bengaluru-560089, Division of Fruit Crops.

2.4 Extraction of Seeds for Crude Drug

To detach their outer brown covering, the dried *Tamarindus indica* seeds were roasted to 140 °C in a hot air oven for 45 minutes, cooled, and then cracked. The only seed coat to be gathered and pulverized into a fine powder was the brown-red one. Petroleum ether was then used to defeat them. Following a 48-hour methanol extraction and defatting process using petroleum ether, the powdered seed coat was filtered with filter paper (Whatman No. 4). A second extraction of the residues using 100 milliliters of methanol was performed. The solvent in the combined extract was taken out with less force (34–36 kPa) by employing a rotating vacuum heater set at 40 °C. Subsequently, the contents underwent air drying[2].

2.5 Indomethacin-Induced Ulcer Model

The animals were fasted for 18-24 hours before the experiment, with access to water only, to ensure empty stomachs and enhance ulcer induction sensitivity. Following the fasting period, indomethacin, an ulcerogenic agent, was administered orally via gavage at a dose of 20 mg/kg, suspended in saline. The animals were then kept in their cages for 4-6 hours, during which gastric ulcers developed as a result of indomethacin-induced inhibition of prostaglandin synthesis, leading to gastric mucosal damage.

After ulcer induction, the combination of *Tamarindus indica Linn* and *Aloe Vera* was administered orally at two different dose levels: a low dose of 200 mg/kg and a high dose of 400 mg/kg. The standard drug, omeprazole, was administered at a dose of 20 mg/kg. These treatments were given daily for 15 consecutive days to evaluate their therapeutic effects on the indomethacin-induced ulcers.

The animals were grouped according to Table 1, which categorized them based on different treatment regimens. This allowed for a clear assessment of the efficacy of the treatments across different groups.

At the end of the treatment period, the animals were sacrificed humanely using an overdose of CO_2 asphyxiation. A midline incision was made, and the stomachs were carefully dissected out and opened along the greater curvature to expose the gastric mucosa for ulcer assessment and further analysis[7,12].

Table 1. Grouping of experimental animals (n=6)

Group	Treatment(Dose and route of administration)
Normal control	Normal Saline (2 ml/kg, p.o.)
Positivecontrol	Indomethacin[3] (20 mg/kg, p.o.)
Standard treatment	Omeprazole[2] (20 mg/kg, p.o.)
Treatment low dose	Methanolic extract of Tamarind Seeds + Aloe Vera (200 mg/ kg, p.o.)
Treatment high dose	Methanolic extract of Tamarind Seeds + Aloe Vera (400 mg/ kg, p.o.)

2.6 Parameters

2.6.1 Estimation of Gastric Juice

The measuring cylinder was used to determine the total volume of the stomach content[15].

2.6.2 Total Acidity Estimation

A 1-milliliter sample of gastric juice, diluted with an equal amount of distilled water, was introduced into a conical flask with a 50-milliliter capacity. Add two drops of phenolphthalein indicator, and titrate the liquid using 0.01N NaOH until a consistent pink hue appears. Note the quantity of 0.01N NaOH utilized and indicate the overall acidity in milliequivalents per liter (mEq/L)[16,17].

The formula for estimation of Total acidity:

$$\text{Acidity} = \text{NaOH volume} \times N \times 100$$

2.6.3 Free Acidity Estimation

Topfer's reagent replaced phenolphthalein as the indicator in the titration of a sample of stomach juice mixed with 0.01N NaOH. There was a noticeable canary

yellow coloring, and the quantity of 0.01N NaOH used was recorded. The free acidity was computed with the same procedure that was used to determine the total acidity in an ulcer caused by ethanol[18].

2.6.4 Catalase (CAT) Estimation

To prepare a 10 % w/v homogenate, stomach tissue underwent homogenization using a homogenizer in ice-cold Tri's buffer. Following homogenization, the resultant mixture was centrifuged for 15 minutes at 4 °C at 10,000 rpm. Next, 1.9 ml of 50 mM phosphate buffer and 0.1 ml of the supernatant were mixed in a cuvette. One milliliter of recently made 30-millimeter hydrogen peroxide was added to this mixture. Utilizing anultraviolet spectrophotometer, the alteration in absorbency was measured over three minutes at 240 nm, with 30-second intervals. The blank value was determined using 0.1 ml of supernatant-free distilled water. The catalyst concentration required to maintain a 50 % change in absorbance for the control sample within one minute is defined as one unit of enzyme activity. Units of enzyme activity per milligram of protein are used to express catalase activity[19].

The formula for estimation of CAT

Catalase = (Absorbance×Volume of reaction mixture)/(43.6 ×Volume of sample)×1/(mg protein)

2.6.5 Superoxide Dismutase (SOD)Estimation

To prepare a 10 % weight/volume homogenate, stomach tissue underwent homogenization using a homogenizer in a Tris buffer that was frozen. After that, the homogenate was centrifuged for 30 minutes at 4 °C at 5000 rpm. After adding 0.1 ml of stomach supernate, 0.1 ml of phenazine methosulphate, 0.3 ml of nitroblue tetrazolium, 0.2 ml of 0.052 M sodium pyrophosphate buffer (pH 8.3), and 0.2 ml of NADH, the reaction was stopped with 1 milliliter of glacial acetic acid and heating the mixture for 90 minutes at 300 degrees Celsius. Following stirring, 4 ml of n-butanol was added before centrifuging for 10 minutes at 4000 rpm. Using the blank value, the organic layer's absorbance at 550 nm was calculated.Determined with 0.1 ml of tissue supernatant-free distilled water. Enzyme activity was quantified based on the quantity of enzyme required to reduce the chromogen absorbance in the control sample by 50 % during testing[20].

The formula for estimation of SOD:

SOD = (Control absorbance-Sample absorbance)/(Control absorbance/2)×1/(mg protein)

2.6.6 Estimation of Malondialdehyde (MDA)

To prepare a 10 % w/v homogenate, stomach tissue underwent homogenisation with a homogenizer in an ice-cold Tris buffer. The combination was composed of 0.1 ml of homogenate, 1.5 ml of 0.8 % thiobarbituric acid solution, 0.2 ml of 0.1% sodium dodecyl sulfate, and 1.5 ml of 20 % acetic acid solution. After adding distilled water to make a total volume of 5 mL, the mixture was heated to 95 °C for 60 minutes, utilizing a condenser made of glass,following cooling under running tap water, 5 ml of a 15:1 n-butanol and pyridine combination were added, followed by vigorous shaking. After a 10-minute after 4000 rpm of centrifugation, the organic layer was separated, then the intensity of absorption at 532 nm was determined compared to a reference blank. Using the standard curve, the tissue MDA level was determined and interpreted as nmol/g wet tissue[21].

The formula for estimation of MDA:

x = y-0.0162⁄0.0654

Where, y = absorbance of the sample

2.6.7 Estimation of Glutathione peroxidase (GPx)

To create a 10 % w/v homogenate, stomach tissues were uniformized using a polytron homogenizer in a cold phosphate buffer. The resulting homogenate was centrifuged for 15 minutes at 4 °C and 5,000 rpm, after which the supernatant was collected. For GPx estimation. For the preparation of a 0.4 M phosphate buffer solution (pH 7), test tubes were loaded with 0.2 M EDTA, 0.1 M sodium azide, 0.1 M GSH, 0.1 M H2O2 solution, and 0.2 M tissue supernatant, followed by incubation for ten minutes at 37 °C. The tubes were then heated to ambient temperature, and before centrifugation at 2000 rpm for 10 minutes, 0.5ml of 10 % TCA was added. After centrifugation, 0.1 milliliters of 0.04 % DTNB solution was introduced to the supernatant, and the solution's absorbance at 420 nm was calculated in comparison to the blank. A blank reaction mixture was created by omitting the tissue supernatant. Theμ moles of GSH oxidized/min/mg protein was the expression for the GPx activity[22].

The formula for estimation of GPx:

GPx = ((change in absorbance/min×GSH std×volume of total mixture))/((absorbance of sample×307.32×volume of sample source)×protein(mg/ml))

2.6.8 Estimation of Myeloperoxidase (MPx)

Using a homogenizer with a volume of 1/10 of the stomach weight stomach tissue was homogenized in ice-cold phosphate buffer containing 0.5 % hexadecyl trimethyl ammoniumbromide. The homogenate was centrifuged for 30 minutes at 4ºC at 15000 rpm. After adding 40 µl of the supernatant to 960 µl of phosphate buffer that contained hydrogen peroxide (0.0005%) and o-dianisidine dihydrochloride (0.167 mg/ml), the concoction was intensely agitated. For three minutes, every 60 seconds, the absorbance at 460 nm was measured. An activity's single unit of measurement was the quantity of MPx needed to cause a change in absorbance measured at 460 nm for three minutes. The units/ml for MPx activity statistics are displayed[23].

The formula for estimation of MPx:

$MPX = (\Delta A \times Vt \times 4)/(E \times \Delta t \times Vs)$

Where, Vt = Total volume (ml)
Vs = Sample volume (ml)
Δ A = Change in absorption
E = Extinction coefficient
Δ t = Measuring time

2.6.9 Evaluation of gastric mucosal injury (Ulcer index)

Ulcer indexing was completed using the following modified Adami et al. (1997) grading system: Lesions are represented by the numbers 0 = no lesions, 1 = hemorrhagic suffusions, 2 = small ulcers up to 3 mm in size ranging from 1 to 5, 3 = 5 numerous small ulcers exceeding 5 or 1 ulcer exceeding 3 mm, 4 = many numerous ulcers exceeding 3 mm, and 5 = perforated ulcers. The results were analyzed after the mean score for each group was calculated[24].

2.6.10 Statistical Analysis

The mean ± SEM was employed to showthe values (n = 6). ANOVA was used forthe data analysis, and a statisticallysignificance level of P≤0.05 for the Dunnett test indicates a notable variation between the treatment and disease groups.

3. RESULTS

3.1 Effects of Tamarind and Aloe Vera Extracts on Gastric Volume and Acidity Levels - Free and Total

When indomethacin was given to ulcerated rats, there was a statistically significant rise in gastric volume compared to normal controls as shown in Table 2. However, in rats treated with 200 mg/kg and 400 mg/kg b.w. of polyherbal extracts of methanolic extract of tamarindseeds combined with aloe vera, the observed increases in gastric volume were significantly decreased to 1.06±0.04 and 0.85±0.05, respectively.

Regarding acidity levels, the estimated levels of free and total acidity in the group treated with indomethacin (disease control) were 87±1.155 mEq/L and 14±1.155 mEq/L, respectively. Pre-treatment with 200 mg/kg b.w. and 400 mg/kg b.w. of the polyherbal extracts significantly reduced free acidity to 56±2.309 and 45.67±1.202, respectively, and total acidity to 8±0.5774 and 6.333±0.8819, respectively (p≤0.001). These reductions were comparable to the standard treatment with omeprazole.

3.2 Impact of Tamarind and Aloe Vera Extracts on Tissue Antioxidants - MDA, SOD, CAT, GPx, and MPx Levels

The combined results of the effects of polyherbal extracts of tamarind seed methanolic extract and aloe vera on tissue antioxidants in an indomethacin-induced model are as follows:

The ulcerated group (disease control) exhibited significantly higher levels of MDA (P≤0.001), while rats given indomethacin showed a substantial Decrease in CAT and SOD activities (P≤0.001) as shown in Table 3. However, the polyherbal extracts of tamarind seed and aloe vera, administered in a dose-dependent manner, significantly improved the parameters of ratstomach mucosa lipid peroxidation, SOD, and CAT activity with Indomethacin ulcers. These effects were comparable to those of the standard drug, Omeprazole, and the normal control group. Additionally, in the ulcerated group, the GPx level was considerably lower, while there was a significant increase in MPx activity in rats given indomethacin. Impressively, the polyherbal extracts of tamarind seed methanolic extract and aloe vera significantly improved the GPx and MPx activity of the gastric mucosa in rats with Indomethacin ulcers. These effects also compared favorably with the standard drug (omeprazole) used in the study and the normal control.

Table 2. Gastric volume and acidity levels-free and total

Parameters	Normal control	Disease control (Indomethacin20mg/kg)	Treatment low dose (Tamarind Extract + Aloe vera Extract-200mg/kg)	Treatment high dose (Tamarind Extract + Aloe vera Extract400mg/kg)	Standard treatment (Omeprazole 20mg/kg)
Gastric volume	0.45±0.05	1.75±0.05	1.06±0.04***	0.85±0.05***	0.65±0.05***
Free acidity	32.67±1.453	87±1.155	56±2.309***	45.67±1.202***	50.67±1.453***
Total acidity	6±0.5774	14±1.155	8±0.5774***	6.333±0.8819***	10.33±0.8819***

The values for n=6was expressed as Mean ± SEM. Statistical analysis included ANOVA, and significance, denoted by *, was determined through the Dunnett test when comparing treatment groups with the disease group.**

Table 3.Tissue antioxidant parameters

Parameters	Normal control	Disease control (Indomethacin 20 mg/kg)	Treatment low dose (Tamarind Extract + Aloe vera Extract 200 mg/kg)	Treatment high dose (Tamarind Extract + Aloe vera Extract 400 mg/kg)	Standard treatment (Omeprazole 20 mg/kg)
MDA	2.446±0.381	12.2±0.6612	7.997±0.5012***	5.953±0.5506 ***	4.037±0.1846***
SOD	42.35±1.272	25.75±0.3252	32.93±0.6944***	36.69±0.6458***	41.59±1.366***
CAT	24.75±0.7491	13.18±0.6412	18.67±0.3782***	20.81±0.332***	23.22±0.5969***
GPx	57.62±1.054	26.27±0.944	33.5±1.181***	41.81±0.7102***	55.02±0.5173***
MPx	16.01±0.8285	36.88±0.9135	22.31±0.6536***	19.63±0.5259***	17.35±0.3018***

The values for n=6was expressed as Mean ± SEM. Statistical analysis included ANOVA, and significance, denoted by ***P<0.001, was determined through the Dunnett test when comparing treatment groups with the disease group.

3.3 Ulcer Index and % Ulcer Inhibition

The effects of the extract on the percent inhibition against ulcer and ulcer index in experimental animals are shown in Table 4 and Fig. 1, respectively.

The ulcer index, which measures the degree of ulceration increased significantly in rats treated orally with indomethacin at a dose of 20 mg/kg b.w. The animals treated with aloe vera and tamarind seed methanolic extract polyherbal extracts showeda notable increase in the degree of inhibition against ulcer formation. Taking 200 mg/kg b.w., the extract compared favorably with the usual medication (omeprazole) used and provided superior protection against ulcers.

Table 4. Ulcer index and % ulcer inhibition

Parameters	Normal control	Disease control (Indomethacin 20 mg/kg)	Treatment low dose (Tamarind Extract + Aloe vera Extract 200 mg/kg)	Treatment high dose (Tamarind Extract + Aloe vera Extract 400 mg/kg)	Standard treatment (Omeprazole 20 mg/kg)
Ulcer index	0	1.887±0.1145	0.6367±0.0120***	0.5367±0.01453***	0.51±0.02082***
% Ulcer inhibition	0	107%	50%	62%	66%

The values for n=6was expressed as Mean ± SEM. Statistical analysis included ANOVA, and significance, denoted by *, was determined through the Dunnett test when comparing treatmentgroups with the disease group.**

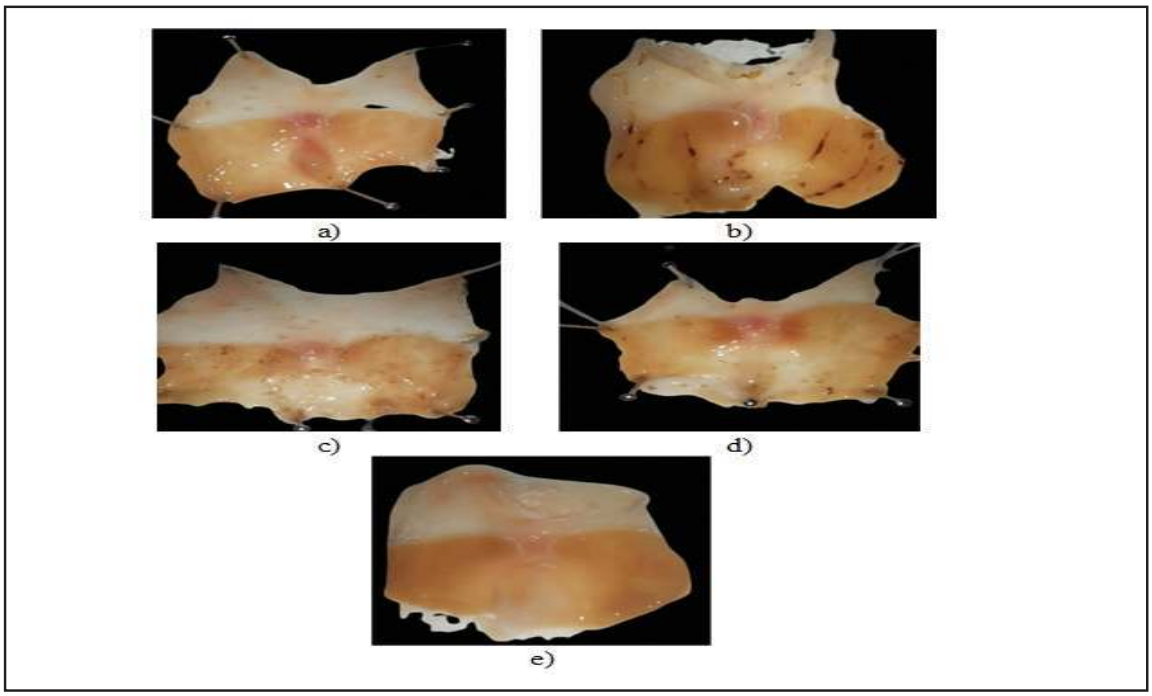

Figure 1. Open stomach of Indomethacin-induced gastric ulcer rat model, a) Normal control, b) Disease control (Indomethacin 20 mg/kg), c) Treatment low dose (Tamarind Extract + Aloe vera Extract 200 mg/kg), d) Treatment high dose (Tamarind Extract + Aloe vera Extract 400 mg/kg), e) Standard treatment (Omeprazole 20 mg/kg).

4. DISCUSSION

An ulcer is described as active inflammation that results in a local defect or excavation by disrupting the mucosal integrity of the stomach and/or duodenum. Ulcers in the stomach or duodenum are common and often chronic. Stomach and duodenal ulcers are frequent and frequently chronic. Gastric ulcers are the result of an imbalance between the destructive forceand the gastro-duodenal mucosal defence mechanism[2].

It is widely acknowledged that an imbalance between aggressive forces and endogenous defence mechanisms that maintain mucosal integrity leads to stomach ulcers. Prostaglandin (PG)-induced excess stomach acid production is accompanied by a reduction in aggressive components, mainly pepsin and acid, as well as an increase in mucosal resistance[25].

Because of their analgesic, antipyretic, and anti-inflammatory properties, NSAIDs are frequently utilised. The bulk of side effects from NSAID medication are associated with the digestive system. The primary limitation on the use of NSAIDs as anti-inflammatory drugs is ulcerative lesions, which include bleeding, perforation, and stomach ulcers[4].

Consequently, the current investigation examined the anti-ulcer effect of polyherbal extract on rat models of stomach ulcers caused by indomethacin[26].

The study's findings indicate the potential of polyherbal extracts, specifically a combination of tamarind extract and aloe vera extract, in reducing gastric volume and acidity in rat models of stomach ulcers caused by indomethacin. This suggests a promising anti-ulcer effect of these extracts, which may be related to their capacity as antioxidants. The study also emphasizes how crucial oxidative stress is to the pathophysiology of indomethacin-induced gastric lesions and the potential role of Myeloperoxidase (MPx) in this process.

The findings regarding the effects of Tamarind extract and Aloe vera extract on the activities of Superoxide Dismutase (SOD) and Catalase (CAT) in the stomach mucosa of rats with indomethacin-induced ulcers are particularly noteworthy. These extracts were found to significantly enhance the activities of these antioxidant enzymes, which could contribute to their anti-ulcer effects. Additionally, the study suggests that the combination of tartar extract and aloe vera extract may have a greater impact on ulceration compared to the individual extracts alone.

Overall, the findings of this study underscore the significance of oxidative stress in the pathogenesis of stomach ulcers and offer insightful information on the possible processes underpinning the anti-ulcer benefits of polyherbal extracts. To completely comprehend the medicinal potential of these extracts and their modes of action in the management of stomach ulcers, more investigation is required.

5. CONCLUSION

The study demonstrated that a combination of tamarind and aloe vera extracts significantly reduced the likelihood of stomach ulcers induced by indomethacin. The treatment also led to a notable reduction in stomach capacity, particularly at 200 mg/kg and 400 mg/kg body weight dosages. The combination of tamarind and aloe vera resulted in a significant decrease in Malondialdehyde (MDA) levels and a significant increase in Glutathione Peroxidase (GPx) and Metalloproteinase (MPx) activities.

Moreover, the combination significantly reduced the ulcer index, indicating its potential as a preventive measure against stomach ulcers. The anti-inflammatory, antioxidant, anti-infective, laxative, anthelmintic, anti-microbial, anti-bacterial, anti-diabetic, and wound-healing properties of tamarind and aloe vera further support their efficacy in treating stomach ulcers.

The methanolic extract of tamarind and aloe vera at a dose of 400 mg/kg showed noteworthy anti-ulcer efficacy in contrast to the pharmaceutical Omeprazole, suggesting its potential as a natural alternative for ulcer treatment.

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Ability of Machine Learning and Deep Learning Models for Multiclass Classification of Kidney Stone and Lung Cancer from Computed Tomography Images: A Comparative Study

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ABSTRACT

Feature extraction is crucial in biomedical image classification because it determines the accuracy of image representations and significantly impacts the effectiveness of classification models. Deep neural network classification architectures have gained significant interest due to their ability to automatically extract important features from input images, resulting in significant progress in diverse image classification tasks in recent years. However, with the rise of deep learning techniques, traditional machine learning approaches have been largely overshadowed. This study aims to close this gap by undertaking a rigorous comparative analysis of three important machine learning models, namely Gaussian Naïve Bayes, Support Vector Machine, and Random Forest Classifier, and three advanced deep learning models, namely VGG16, InceptionV3, and Xception. The comparison is based on their ability to do multiclass classification, using two datasets kidney stone and lung cancer. Each dataset consists of four different target classes. Both machine learning and deep learning frameworks are trained separately on the datasets, with deep learning models utilizing transfer learning techniques. The performance of each model across the varied output classes is assessed using evaluation measures such as precision, recall, and F1 scores. The results of the simulation analysis reveal that both machine learning and deep learning models perform equally well, as indicated by similar F1 scores across all output classes for both datasets. This study represents a major step towards simplifying classification efforts by promoting the use of machine learning models instead of deep learning models for classifying kidney stone and lung cancer datasets. This approach helps reduce the workload and computing requirements for training.

Keywords: Deep learning; Machine learning; Biomedical image classification; Computed tomography; Biomedical image processing; Feature extraction

1. INTRODUCTION

Accurate classification of biomedical images, particularly Computed Tomography (CT) scans, is vital for early detection and precise diagnosis of medical conditions such as kidney stones and lung cancer. Feature extraction, a fundamental technique in biomedical image classification[1,2], involves identifying and highlighting crucial image elements necessary for distinguishing between different disease classes. The effectiveness of any classification model heavily relies on the proficiency with which these features are extracted, directly impacting the accuracy and reliability of the classification process. Deep Neural Networks (DNNs)[3,4] have emerged as formidable contenders in the realm of biological image classification due to their capability to autonomously learn intricate patterns from input images. These models have demonstrated outstanding performance across various image classification tasks, often surpassing traditional Machine Learning (ML)[5] models. However, traditional ML techniques remain pertinent in biomedical image classification, offering results that are interpretable and computationally efficient, albeit facing challenges with complex datasets.

Deep learning models, particularly DNNs, excel at detecting complex patterns and correlations within extensive and high-dimensional datasets. Their ability to learn directly from raw input data enables them to perform intricate tasks without necessitating domain-specific knowledge. Despite their computational demands, DL models exhibit exceptional generalisation ability, adaptability to changing conditions, and capacity to learn from new data. In contrast, ML techniques, while less computationally intensive, may require additional support when handling large and intricate datasets.

Feature extraction is pivotal in effectively representing image information, thereby playing a crucial role in image classification. Researchers have proposed various comparative approaches and techniques for feature extraction, aiming to enhance classification accuracy by identifying the most suitable method. Moreover, studies have explored the efficacy of deep learning methods in the diagnosis

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of kidney stones[6–9] and lung cancer[10–13], demonstrating significant advancements in automated kidney stone and lung cancer classification. Additionally, researchers have investigated methods to improve image quality and enhance diagnosis using ML and DL techniques.Within the field of medical research, experts are always working to improve diagnostic models specifically designed for the automated classification of kidney stones[14,15] and lung cancer[16,17]. The consistent and determined effort highlights the pressing necessity to improve medical diagnostics[18,19] specifically in crucial fields such as oncology. Kanavati[20] performed a crucial effort in this field, specifically studying the complex distinctions between the main histological kinds of lung cancer. By carefully developing and training a deep learning model utilizing H&E-stained Whole Slide Images of tiny trans bronchial lung biopsy specimens, scientists achieved a significant improvement in classification accuracy, paving the way for more accurate and efficient diagnosis. Wang and Dong[21] introduced an innovative method for identifying lung cancer by utilising the capabilities of CT imaging. Their application of transfer learning, along with a complex neural network structure, not only demonstrates technological progress but also emphasizes the integration of varied datasets and approaches in the pursuit of diagnostic excellence.

On the other hand, Marentakis and Karaiskos[22] undertook an extensive investigation, examining various methods for classifying tumors using CT images. Their comprehensive analysis not only enhances our comprehension of the subtle interplay between various approaches but also underscores the intrinsic difficulty of medical imaging jobs. Moreover, the ground breaking research conducted by Adriana and Dinh-Hoan [23] in the automation of kidney stone classification represents a major achievement in the discipline, introducing a novel age of precision and effectiveness through the utilisation of supervised learning methodologies. However, in the middle of all this innovation, there remains a significant gap - there is no comparative study that explains the ability of ML and DL models to extract features for classifying kidney stones and lung cancer. This unexplored domain invites investigation, offering the possibility for profound understanding and potentially significant progress in the field of medical diagnostics.

This study aims to compare ML and DL models in classifying CT images for kidney stone and lung cancer detection. The evaluated ML models include Support Vector Machines (SVM[24]), Random Forest Classifier (RFC[25]), and Gaussian Naïve Bayes (GNB[26]), while DL models comprise VGG16[27], InceptionV3[28], and Xception[29]. By leveraging transfer learning, DL models utilize pre-trained weights to enhance feature extraction. Performance evaluation is conducted using precision, recall, and F1 score metrics across multiple output classes. Our objective is to provide insights into choosing suitable ML or DL models for multi-class classification using CT scans, focusing on kidney stone and lung cancer detection. Additionally, we aim to elucidate the feature extraction capabilities of these models in the context of biological image classification, identifying their comparative strengths and limitations. The findings of this study have the potential to improve patient outcomes and healthcare effectiveness by developing more accurate and reliable classification models for early disease detection using CT scans and potentially expanding to other medical conditions.

The subsequent sections of this paper are organised as follows: Section 2 outlines the proposed methodology and provides an overview of the simulation study. Section 3 delves into the empirical findings and evaluates performance, while Section 4 discusses the outcomes of the experiment. Finally, Section 5 concludes by suggesting directions for future research.

2. MATERIAL AND METHODS

Fig. 1 represents the flow diagram of the proposed framework for kidney and lung cancer classification. It has five phases. The first phase is the data collection. The second phase is data pre-processing. The third phase corresponds to the model training where three ML and three DL algorithms are implemented. The result evaluation is done in the fourth phase. Model comparison over the selected parameters is done in the fifth phase.

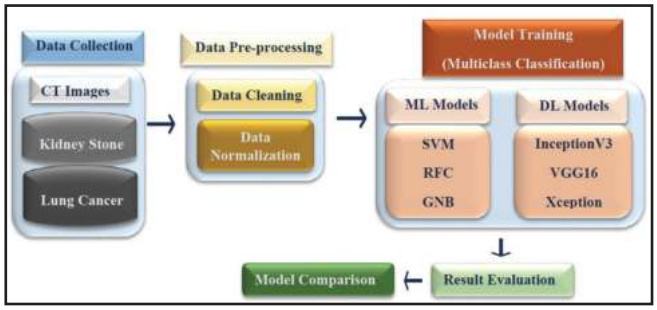

Figure 1. The flow diagram of the proposed classification framework.

2.1 Data Collection

This study utilised two distinct datasets to perform multiclass classification tasks. One dataset focused on categorizing kidney stones, while the other centered on classifying lung cancer. The kidney dataset comprised 12,446 CT images, categorised into four groups: cyst (3,709 images), normal (5,077 images), stone (1,377 images), and malignant (2,283 images). Conversely, the lung cancer dataset contained 1,000 CT images, distributed among four categories: adeno carcinoma (338 images), large cell carcinoma (260 images), squamous carcinoma (187 images), and normal cell (215 images). Both datasets were collected from Kaggle, a publicly available data repository. Fig. 2 visually represents the class distribution of the lung cancer (left) and kidney stone (right) datasets. Within the lung cancer dataset, adeno carcinoma emerges as the most prevalent subtype, constituting nearly 40% of all lung cancer cases. Large cell carcinoma, characterised by atypical features compared to other types, and squamous cell carcinoma, often associated with a history of smoking and primarily affecting central

lung regions, are also depicted. Figure 3 shows sample CT scans from the lung cancer dataset, providing visual insights into various lung cancer pathologies. Moving to the kidney dataset, four distinct classes were identified: kidney tumor, cyst, stone and normal, each reflecting specific clinical conditions impacting kidney health. Kidney tumors denote abnormal growths within kidney tissue, which can be either benign or malignant. Conversely, kidney cysts represent fluid-filled sacs, typically harmless and asymptomatic. Kidney stones, composed of calcium oxalate, and other compounds, can form in the kidneys, leading to various health issues. Fig. 4 exhibits sample CT scans from the kidney stone dataset, illustrating different disease manifestations related to kidney health.

2.2 Data Pre-Processing

Data cleaning is the process of detecting and rectifying discrepancies in a dataset to ensure its integrity and dependability for analysis. The images which are not labelled are discarded. Data normalisation aims to scale the values of features in a dataset to a comparable range, hence enhancing the performance of machine learning algorithms. The dimensionality of each image varies, in this stage, all the images are resized into one dimension for both the datasets. The input pixels are between range (0-255) and all the image pixels are normalised to the range (0-1).

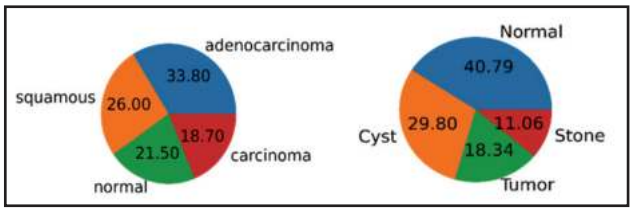

Figure 2. Class distribution for lung cancer dataset(left) and kidney stone dataset(right).

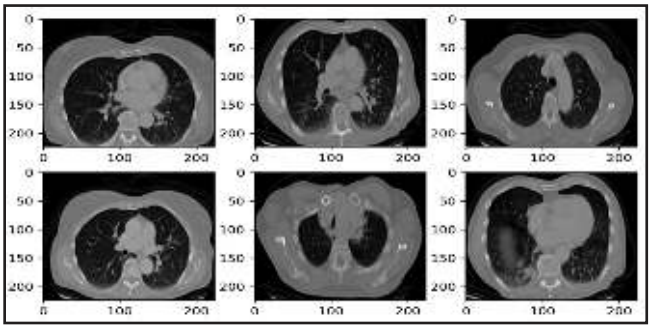

Figure 3. The sample CT images for lung cancer dataset.

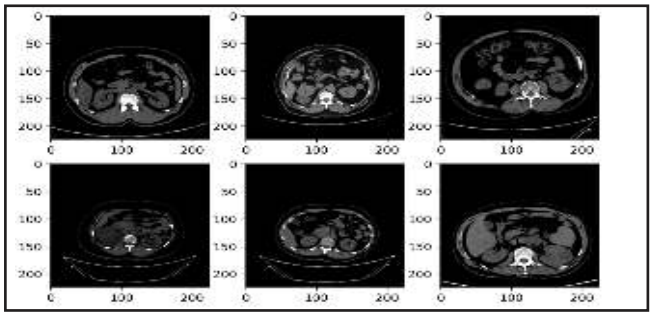

Figure 4. The sample CT imges for kidney stone dataset.

2.3 The Model Training

The model training phase of this study involves the investigation and use of different ML and DL algorithms. More precisely, we are considering three supervised ML algorithms: SVM, RFC, and GNB. The selection of these algorithms is based on their effectiveness in classification tasks, and they are then utilised on the dataset to evaluate their performance. In addition, deep learning models, particularly CNNs, are used because of their exceptional capacity to acquire intricate patterns from input images. This study employs three widely-used CNN architectures, specifically InceptionV3, VGG16, and Xception. The CNN models utilised in this work have been pre-trained on extensive ImageNet dataset. And then further implemented on the dataset used in this research using transfer learning method. CNNs are composed of several layers that perform convolution and pooling operations. These operations allow the network to extract important features from input images at various levels of abstraction. The convolutional layers utilize filters to analyze the input images, identifying patterns such as edges, textures, and forms. Afterwards, the pooling layers gather the input from the convolutional layers, decreasing the spatial dimensions of the features while maintaining their fundamental qualities. CNNs utilize a hierarchical feature extraction technique, enabling them to effectively capture complex patterns and representations contained in the input data. This makes them very suitable for image classification. The model training phase consists of implementing and evaluating a variety of ML and DL algorithms. Each algorithm is selected based on its specific strengths and capacities to handle classification task being performed. The work seeks to determine the best efficient algorithm for appropriately classifying the dataset using a comprehensive approach. Following are the pseudo codes for each algorithm implemented in this work.

Pseudo Code for InceptionV3

```
1. base_model ← InceptionV3(include_top=False, weights=
   'imagenet', input_shape= IMG_SIZE)
2. x ← base_model.output
3. x ← GlobalAveragePooling2D()(x)
4. x ← Dense(256, activation='relu')(x)
5. predictions ← Dense(4, activation='softmax')(x)
6. model ← Model(inputs=base_model.input, outputs=predictions)
7. for layer in base_model.layers:
       layer.trainable =False
```

Pseudo Code for VGG16

```
1. base_model ← VGG16(include_top=False, weights= 'imagenet',
   input_shape=IMG_SIZE)
2. x ← base_model.output
3. x ←GlobalAveragePooling2D()(x)
4. x ← Dense(256, activation='relu')(x)
5. predictions ←Dense(4, activation='softmax')(x)
6. model ← Model(inputs=base_model.input, outputs=predictions)
7. for layer in base_model.layers:
       layer.trainable = False
```

Pseudo Code for Xception

```
1. base_model ← Xception(include_top=False, weights= 'imagenet',
   input_shape= IMG_SIZE)
2. x ← base_model.output
3. x ← GlobalAveragePooling2D()(x)
4. x ← Dense(256, activation='relu')(x)
5. predictions ←Dense(4, activation='softmax')(x)
6. model←Model(inputs=base_model.input, outputs=predictions)
7. for layer in base_model.layers:
       layer.trainable = False
```

Pseudo Code for SVM

```
1. model ← SVC()
2. model.fit(train_x,train_y)
3. predictions =← model.predict(val_x)
```

Pseudo Code for GNB

```
1. model ← GaussianNB()
2. model.fit(train_x,train_y)
3. predictions ← model.predict(val_x)
```

Pseudo Code for RFC

```
1. model ←RandomForestClassifier()
2. model.fit(train_x,train_y)
3. predictions ← model.predict(val_x)
```

The remaining phases of the proposed framework corresponds to the results evaluation and model comparison and are discussed in Section 3.

3. RESULTS

The experimental results from the simulation study are analysed in this section. It has two subsections. The section 3.1 contains the result analysis for kidney stone classification and section 3.2 contains the result analysis for lung cancer classification. All the implemented models are evaluated using three metrics: precision, recall and F1 score. A model's precision measures how well it predicts the True Positives (TP) out of all the occurrences predicted to be positives for a given class. It is determined as the proportion of TP to all other true and False Positives (FP). Recall measures a model's ability to pick out TP from all the other positive examples in that class. It is calculated as the proportion of TP to all true positives and False Negatives (FN). The harmonic mean of accuracy and recall, or the F1 score, is a measurement of the precision against recall trade-off. A higher F1 score denotes greater performance in terms of both accuracy and recall. It gives a single value that combines both precision and recall.

3.1 Result Analysis Of Kidney Stone Classification

Table 1-3 displays the Precision, Recall, and F1 scores for various models in a classification problem involving kidney stones. The table consists of the models GNB, RFC, SVM, VGG16, InceptionV3, and Xception, along with their respective scores for the classes cyst, normal, stone, and tumor. Table 1 displays the precision scores of the RFC, SVM, VGG16, InceptionV3, and Xception models, all of which have achieved a perfect score of 1.00 for all classes. This indicates that these models have accurately predicted all positive cases without any false positives. The GNB model has an average precision score of 0.86, while the RFC, SVM, VGG16, InceptionV3, and Xception models have an average precision score of 1.00.

Table 1. Precision score for kidney stone classification

Models	Cyst	Normal	Stone	Tumor	Average F1 score
GNB	0.97	0.83	0.95	0.69	0.86
RFC	1.00	1.00	1.00	1.00	1.00
SVM	1.00	1.00	1.00	1.00	1.00
VGG16	1.00	0.99	1.00	1.00	1.00
InceptionV3	1.00	0.99	1.00	1.00	1.00
Xception	1.00	1.00	1.00	0.99	1.00

Table 2 demonstrates that the RFC, SVM, VGG16, InceptionV3, and Xception models exhibit recall scores of 1.00 for the majority of classes, signifying their ability to accurately identify all positive examples without any false negatives. Nevertheless, the VGG16 and InceptionV3 models have a recall score of 0.99 for the tumor class, suggesting that they failed to detect certain positive cases for that particular class.

Table 2. Recall score for kidney stone classificatin

Models	Cyst	Normal	Stone	Tumor	Average F1 score
GNB	0.76	0.93	0.65	0.87	0.84
RFC	1.00	1.00	1.00	1.00	1.00
SVM	1.00	1.00	1.00	1.00	1.00
VGG16	1.00	1.00	1.00	0.99	0.99
InceptionV3	1.00	1.00	1.00	0.98	0.99
Xception	1.00	0.99	1.00	0.99	1.00

Table 3 shows the F1 scores for the classes cyst, normal, stone, and tumor. In the GNB model, the F1 scores for these classes are 0.85, 0.88, 0.77, and 0.77, respectively. The RFC, SVM, VGG16, InceptionV3, and Xception models have perfect F1 scores of 1.00 for the majority of classes, indicating their effective ability to maintain a balance between recall and precision for certain classes. Nevertheless, the VGG16 and InceptionV3 models exhibit F1 scores of 0.99 for the tumor class, suggesting a somewhat diminished performance for that class when considering the balance between precision and recall. The GNB model has an average F1 score of 0.84, while the RFC, SVM, VGG16, InceptionV3, and Xception models have an average F1 score of 1.00.

Table 3. F1 score for kidney stone classification

Models	Cyst	Normal	Stone	Tumor	Average F1 score
GNB	0.85	0.88	0.77	0.77	0.84
RFC	1.00	1.00	1.00	1.00	1.00
SVM	1.00	1.00	1.00	1.00	1.00
VGG16	1.00	1.00	0.99	1.00	1.00
IncetionV3	1.00	0.99	1.00	0.99	0.99
Xception	1.00	1.00	1.00	0.99	1.00

3.2 Result Analysis Of Lung Cancer Classification

Table 4-6 displays the Precision, Recall, and F1 scores for various models used in the classification of lung cancer. The tables in this section consist of models GNB, RFC, SVM, VGG16, InceptionV3, and Xception, along with their respective scores for the classes adenocarcinoma, carcinoma, normal, and squamous, similar to the previous section.

The precision scores for the classes adeno carcinoma, carcinoma, normal, and squamous are displayed in Table 4. The GNB model yields precision scores of 0.58, 0.34, 0.79, and 0.50 for the adeno carcinoma, carcinoma, normal, and squamous classes, respectively. The RFC, SVM, VGG16, InceptionV3, and Xception models exhibit differing precision scores across various classes. The precision scores for different models vary between 0.56 and 0.89 on average. Higher precision scores indicate greater accuracy in predicting relevant outcomes for the respective groups.

According to Table 5, the GNB model demonstrates recall scores of 0.20, 0.76, 0.93, and 0.40 for the classification of adeno carcinoma, carcinoma, normal, and squamous, respectively. The RFC model achieved recall scores of 0.78, 0.61, 0.98, and 0.81 for the classes of adenocarcinoma, carcinoma, normal, and squamous, respectively. For several models, the average recall scores vary between 0.51 and 0.88.

According to Table 6, the GNB model achieves F1 scores of 0.29, 0.47, 0.85, and 0.45 for the adeno carcinoma, carcinoma, normal, and squamous classes, respectively. The RFC model achieves F1 scores of 0.78, 0.72, 0.95, and 0.72 for the classification of adeno carcinoma, carcinoma, normal, and squamous, respectively. The F1 scores for several models in this situation vary between 0.48 and 0.88 on average. Higher F1 scores signify a superior balance between precision and recall, resulting in enhanced overall model performance for the relevant classes in terms of both FP and FN.

4. DISCUSSION

Tables 3 and 6 present the F1 scores attained by different models in classifying kidney stones and lung cancer, respectively. The F1 score, a composite measure of precision and recall, is used to evaluate the accuracy of a model. When it comes to both classification challenges, models like RFC, SVM, VGG16, InceptionV3, and Xception continuously demonstrate high performance, routinely achieving F1 scores above 0.76. In contrast, the GNB model has comparatively lower F1 scores in both situations, which suggests a decrease in accuracy. VGG16 and Xception demonstrate the highest F1 scores in lung cancer classification, indicating their greater accuracy in discriminating between various forms of lung cancer. However, choosing the best model requires careful evaluation of other criteria such as computational complexity, interpretability, and practical application. This necessitates comprehensive study and testing for each unique use case.

Table 4. Precision score for lung cancer classification

Models	Adeno carcinoma	Carcinoma	Normal	Squamous	Average F1 score
GNB	0.58	0.34	0.79	0.50	0.56
RFC	0.78	0.89	0.93	0.64	0.80
SVM	0.72	1.00	0.93	0.62	0.80
VGG16	0.88	1.00	0.91	0.77	0.89
InceptionV3	0.96	0.64	0.70	0.77	0.77
Xception	0.96	0.76	0.91	0.84	0.88

Table 5. Recall score for lung cancer classification

Models	Adeno carcinoma	Carcinoma	Normal	Squamous	Average F1 score
GNB	0.20	0.76	0.93	0.40	0.51
RFC	0.78	0.61	0.98	0.81	0.79
SVM	0.84	0.46	0.95	0.74	0.77
VGG16	0.96	0.88	0.84	0.87	0.88
InceptionV3	0.96	0.44	0.82	0.74	0.77
Xception	1.00	0.81	0.82	0.91	0.88

Table 6. F1 score for lung cancer classification

Models	Adeno carcinoma	Carcinoma	Normal	Squamous	Average F1 score
GNB	0.29	0.47	0.85	0.45	0.48
RFC	0.78	0.72	0.95	0.72	0.79
SVM	0.78	0.63	0.94	0.67	0.76
VGG16	0.92	0.93	0.88	0.82	0.88
InceptionV3	0.96	0.52	0.76	0.76	0.76
Xception	0.98	0.79	0.86	0.87	0.88

The results of the simulation study highlight the complex and diverse parameters that affect the effectiveness of ML and DL models in tasks involving image classification. The kidney stone dataset, which consists of 12,446 CT images, shows enhanced performance in both ML and DL models. However, the smaller lung cancer dataset, which includes 1000 CT scans, produces relatively inferior results. Factors like as the relevance of the data, the presence of noise, the size of the dataset, and the quality of the features have significant impacts on the performance of the model. Images that are of excellent quality and clearly labeled, and that accurately depict real-life situations, have a tendency to improve the accuracy of models. On the other hand, images that are of poor quality, containing a lot of noise, arti facts, or inconsistencies, might negatively impact the accuracy of classification. The presence of noise in image data might introduce irrelevant information or mask important patterns, which can negatively impact the effectiveness of the model. Likewise, when there is a large amount of variation in the image data, it becomes difficult for the model to apply what it has learned to new and unseen images, resulting in reduced accuracy. The size of the image dataset used for training is crucial, as larger and more diverse datasets result in more accurate and representative features. However, this comes at the cost of requiring extensive resources for data collecting and management. Moreover, the accuracy of the model is greatly influenced by the quality of the features used for training, specifically the image representations. Relevant features that enhance the ability to distinguish and accurately classify, contribute to the model's effectiveness. Conversely, poorly chosen or irrelevant features decrease the model's effectiveness. This research highlights the important relationship between data quality, noise, data variance, dataset size, and feature quality in determining the effectiveness of ML and DL models in image classification tasks. Although larger datasets that are of good quality often result in more accurate models, it is important to consider and address aspects such as noise and feature quality in order to enhance the performance of the model for various classification tasks.

5. CONCLUSIONS

This work has produced numerous noteworthy contributions that have been clarified by the collected results: Firstly, it thoroughly evaluates the effectiveness of ML and DL models in classifying images of two different medical illnesses, specifically kidney stones and lung cancer. This analysis offers vital insights into how these models compare in terms of their usefulness in healthcare applications. Additionally, the analysis presents the F1 scores of several models used in the classification tasks. It demonstrates that models like RFC, SVM, VGG16, InceptionV3, and Xception consistently exhibit enhanced performance in both kidney stone and lung cancer classification. Furthermore, the study finds key characteristics that have a substantial impact on the performance of image classification models. These elements include the quality of the data, the level of noise present in the data, the variation in the data, the size of the dataset, and the effectiveness of feature selection. It is important to note that noise in image data negatively affects the accuracy of models, while large variation makes it difficult to apply the models to new, unseen images. Moreover, the size of the image dataset utilised for model training is a significant factor, since larger datasets enable more accurate feature extraction and improved accuracy. However, this comes with the drawback of requiring extensive resources for data collecting and management. Furthermore, the selection of high-quality features for model training is crucial. Relevant features improve model accuracy, while irrelevant or poorly chosen features reduce the model's ability to distinguish and overall effectiveness.

The study highlights the crucial role of image quality in datasets, emphasizing the importance of clear, accurately labelled, and representative images in improving the accuracy of model performance. It also emphasizes the negative impact of low-quality images on misclassifications and reduced accuracy of both ML and DL models. Furthermore, the results highlight the need of taking these characteristics into account when choosing the most suitable model for particular use cases, while balancing computational complexity, interpretability, and real-world applicability requirements. It emphasizes the significance of conducting further analysis and testing to ascertain the most suitable model for certain situations. Future research should prioritize overcoming the problems outlined in order to improve the accuracy and dependability of image classification models for classifying kidney stones and lung cancer. Furthermore, there is a requirement for the advancement of more efficient feature extraction techniques that can accurately capture significant image attributes to enhance classification results.

Future work will be focused on improving the accuracy and reliability of image classification models for kidney stones and lung cancer. Efforts will be directed towards enhancing image quality, mitigating noise in data, collecting larger and diverse datasets, and developing efficient feature extraction techniques

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In Vitro Antibiotic Potential and Antioxidant Activity of Ethanol and Acetone Excerpts of *Sesbania grandiflora* (L.) Pres. (Agastya) Bark

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ABSTRACT

Sesbania grandiflora (L.) Pres. is a commonly used ethnomedicinal plant belonging to the family Fabaceae. In this study, the antibiotic potential of bark extracts from this plant was evaluated against one fungus *(Candida albicans)*, four gram-positive bacteria (*Lactobacillus acidophilus, Bacillus subtilis, Enterococcus faecalis, & Streptococcus mutans*), and one gram-negative bacteria (*Pseudomonas aeruginosa*). The bark extracts exhibited the highest zone of inhibition against *Candida albicans* (230.81 mm^2) in ethanol solvent, and the lowest against *Enterococcus faecalis* (30.70 mm^2). In acetone solvent, the highest inhibition was observed against *Bacillus subtilis* (136.79 mm^2), and the lowest against Pseudomonas aeruginosa (13.96 mm^2). Antibiotic potential against *Lactobacillus acidophilus, Bacillus subtilis*, & *Streptococcus mutans* is not reported yet. The values of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) displayed variability, reaching the highest point against *Bacillus subtilis* (acetone extracts) and the lowest against *Lactobacillus acidophilus* (ethanol extracts). Moreover, the antioxidative potential of the ethanol and acetone bark extracts was evaluated through DPPH and CUPRAC antioxidant assays. The ethanol extracts demonstrated maximal inhibition against *Candida albicans* (41.08 %) and minimal inhibition against *Enterococcus faecalis* (11.15 %), whereas the acetone extracts exhibited maximal inhibition against *Enterococcus faecalis* (50.02 %) and minimal inhibition against *Lactobacillus acidophilus* (4.58 %). Additionally, the most significant scavenging activity against DPPH was observed at a concentration of 30 µg/ml (19.50 % for ethanol extracts and 15.60 % for acetone extracts), while the least activity was observed at 5 µg/ml (1.96 %) for both solvents. Similarly, in the CUPRAC assay, the highest scavenging activity was noted at a concentration of 100 µg/ml, with the lowest at 2 µg/ml for both ethanol and acetone extracts. The primary objective of this investigation is to explore the phototherapeutic antibiotics and antioxidants inherent in the extracts derived from the bark of Sesbania grandiflora, which offer significant benefits with minimal or negligible adverse effects.

Keywords: *Sesbania grandiflora* "(L.)"; Antibacterial; MIC; MBC; Antioxidant

1. INTRODUCTION

In contemporary healthcare practices of the 21st century, the significance of medicinal plants is on the rise as an increasing number of individuals seek safer remedies and healthcare approaches. The global demand for various herbal products, including herbal medicines, health products, pharmaceuticals, nutraceuticals, supplements, and cosmetics, is witnessing a notable surge. This trend is fuelled by the growing recognition of these products as predominantly non-toxic, with fewer side effects, better compatibility with physiological flora, and affordability. Throughout history, the world population has relied significantly on medicinal plants, with approximately 80 %[1-3] of the population utilizing them since ancient times. Among these, *Sesbania grandiflora* (L.) Pres. stands out as a well-known ethnomedicinal plant of the Fabaceae family,[4] renowned for its pharmacological activities [5-12] and nitrogen-fixing ability[13,14]. It is found scattered across tropical and subtropical regions, including Thailand, India, the Philippines, Sri Lanka, Indonesia, and Malaysia[15]. In India, it is predominantly found in northeast states, Bihar, West Bengal, Jharkhand, Karnataka, and Assam.16 Known by various names such as August, Hummingbird tree, Agastya, Turi, and Agathi, this plant holds rich medicinal properties documented in Ayurvedic, Unani, and Chinese literature. The leaves, flowers and bark of this plant are visualised in photograph, which is shown in Fig. 1.

The rampant misuse and overuse of antibiotics have led to the emergence of antimicrobial resistance, rendering many existing medications ineffective. Consequently, the discovery of new antibiotics has become an urgent objective. Plant-based products are pivotal in this pursuit, considering the potential negative consequences associated with prolonged usage of allopathic medications. The quest for plants exhibiting potent antimicrobial properties has become a central focus of research endeavours aimed at addressing the risks linked with infectious diseases, while simultaneously reducing or eliminating side effects.

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Free radicals such as singlet oxygen species, hydroxyl ions, superoxide ions, and hydrogen peroxide are the result of the unpairing of electrons from any molecules and atoms. These free radicals are the main cause of the imbalance of our immune system which leads to many deadly diseases such as inflammation, ageing, diabetes, heart problems, and many more. This toxic and highly reactive free radical are overcome by antioxidant-rich food and medicine. The word antioxidant comes from the Greek word 'anti' and the English word 'oxidant' which means the molecules/substances which are oxidizing in nature.[17] These antioxidants either balance the unpaired electrons of free radicals or scavenge them. Antioxidants are of two types synthetic (retrieved from chemical processes) and natural (retrieved from nature). While both types of antioxidants are dependable, plant-based antioxidants are preferable due to their minimal occurrence of side effects.

Antioxidants gained prominence in the 1990s when scientists recognized their ability to mitigate the damage inflicted by free radicals, which play a pivotal role in the initial stages of atherosclerosis—a condition characterized by the narrowing of arteries. This pathological process has been linked to various chronic diseases, such as cancer, vision impairment, and others. Studies have demonstrated that individuals with a low consumption of antioxidant-rich fruits and vegetables are at a higher risk of developing these chronic conditions compared to those who include such foods abundantly in their diets. As a result, research into the protective effects of antioxidants continues to be a global priority.[18]

According to estimates by the World Health Organization (WHO), approximately 140 million people worldwide suffer from alcohol dependence, with the disease accounting for 3.8% of global mortality and 4.6 % of Disability-Adjusted Life Years (DALYs). Alcohol-related Liver Disease (ALD) is a consequence of liver damage resulting from the accumulation of acetaldehyde and oxidative stress induced by alcohol consumption.[19]

2. MATERIAL AND METHODS

2.1 Collection and Identification

The bark of *Sesbania grandiflora* was sourced from Vidya Nagar, Harmu, Ranchi, Jharkhand. The plant was identified and authenticated by the National Institute of Science Communication and Policy Research, New Delhi, in 2022, with the authentication number NIScPR/RHMD/Consult/2022/4258-59-2.

2.2 Extract Preparation

The fresh bark was collected, washed, and air-dried for approximately 1-1.5 months. Once dried, the bark was ground into powder. Subsequently, 20 grams of the bark powder was mixed with 200 ml of 80 % methanol solution prepared in distilled water. The mixture was then placed in a shaker incubator at 34 °C and 100 rpm for 3-4 days. After incubation, the solvent-powder mixture was filtered using What man filter paper 1 and then placed in an incubator at 38°C until the methanol was completely evaporated. The resulting dry bark extract was then collected, scratched, and stored in centrifuge tubes inside a refrigerator (stock). This extraction method, commonly referred to as the cold extraction method, was utilized for both the antibiotic and antioxidant assays of *Sesbania grandiflora* bark.[21]

2.3 Anti-microbial Assay

2.3.1 *Preparation of Extract Solution*

The antimicrobial efficacy of *Sesbania grandiflora* (L.) Pres. bark was evaluated according to the methodology delineated by Bauer, A. W. et al. Initially, 8 mg of *Sesbania grandiflora* bark extract was mixed with 1000 µl of both ethanol and acetone solutions. This mixture was then subjected to agitation using a thermo-mixer set at 30 °C and 1000 rpm for a duration of 30 minutes. Following agitation, the resultant mixture underwent centrifugation at 28 °C and 1800 rpm for 20 minutes,

Figure 1. a-plant, b-leaves, c-flowers and d- bark.

after which the supernatant was meticulously collected for subsequent analysis.

2.3.2 *Microbial Strains and Inoculation*

The antimicrobial activity of *Sesbania grandiflora* bark extract was evaluated against one fungus, *Candida albicans* (MCC 1152), and four gram-positive bacteria: *Lactobacillus acidophilus* (MCC LA), *Bacillus subtilis* (MCC 2511), *Enterococcus faecalis* (MCC 3040), and *Streptococcus mutans* (MCC SM), as well as one gram-negative bacterium, *Pseudomonas aeruginosa* (MCC 3973). These microorganisms were chosen due to their Biosafety level 1 classification, ensuring safety during experimentation.

2.3.3 *Antibiotic Assay Procedure*

In this assay, 50 µl of different microbial inoculums were spread onto Petri plates containing nutrient agar (for bacteria) and PDA media (for fungus) using a glass rod spreader. Following this, 6 mm paper discs made from Whatman filter paper 1 were positioned onto the media. Various volumes (5, 10, and 15 µl) of *Sesbania grandiflora* bark extracts, for a concentration of 8 mg/ml, were applied onto sterilized Petri plates alongside positive controls (90 % ethanol for ethanol extracts and 90 % acetone for acetone extracts) and negative controls (tetracycline for bacteria and thioquest for fungus). The loaded Petri plates were then incubated for 18 hours at 36°C to allow the diffusion of the plant extracts into the media, and inhibition of microbial growth was observed using the Disc diffusion method[22]. Subsequently, the diameters of inhibition zones and the percentage of inhibition were measured. The concentrations of the drugs present in 5, 10, and 15µl were 40, 80, and 120µg, respectively.

2.3.4 *Calculations*

Triplicates of each Petri plate was meticulously prepared following the standard protocol. The zone of inhibition was quantified in square millimetres (mm^2) employing the following formula:

$$\text{Zone of inhibition } (mm^2) = \text{Area of inhibited zone } (\pi r_1^2) - \text{Area of disc } (\pi r_2^2)$$

Standard Deviation (SD), Standard Error (SE), and p-values were calculated using one-way ANOVA. The percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \frac{Zone\ of\ sample}{Zone\ of\ standard} * 100$$

(Where Control Diameter represents the diameter of growth in the negative control)

2.4 MIC and MBC

A separate experiment was conducted to ascertain the Minimum Inhibitory Concentration (MIC) and Maximum Bacterial Concentration (MBC) values of ethanol and acetone bark extracts of *Sesbania grandiflora* (L.) Pres. For this method, the following materials were necessary: 100 µl of nutrient broth, 20 µl of bacterial inoculum, and 0.5 % of 5 µl of TTC (2,3,5-triphenyl tetrazolium chloride) freshly prepared in distilled water.[22]

2.4.1 *Procedure*

1. Different volumes, ranging from 2 4, 6, 8, 10, 12, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 & 40 µl of the concentration 5 mg/ml of the bark extracts were placed into 96-well microtiter plates which is equivalent to 10, 20, 30, 40, 50, 60, 80, 90, 100, 110,120,130,140, 150, 160,170,180,190 & 200µg.
2. Each well was then supplemented with 100 µl of nutrient broth and 20 µl of bacterial inoculum.
3. Subsequently, 5 µl of 0.5 % TTC solution was added to each well.
4. The microtiter plates were then incubated for eighteen hours at 36°C inside the incubator.
5. After incubation, the plates were examined for the presence or absence of pink colouration. The appearance of pink colour indicated microbial growth, while the absence of colour indicated microbial inhibition.

2.4.2 *Interpretation*

The MIC value was noted as the lowest concentration of the bark extract at which no pink colour was observed, indicating complete inhibition of microbial growth.

The culture wells where no colour was observed were subculture onto nutrient agar media and incubated until no microbial growth was observed. The concentration of the bark extract corresponding to this well represented the MBC value, indicating the concentration at which microbial growth was completely inhibited.

2.5 Anti-oxidant Assay

2.5.1 *DPPH Antioxidant Assay*[23,24]

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) antioxidant assay is widely recognized as a reliable method for evaluating the antioxidant activity of sample extracts. In executing this assay, 5 to 30 µg of *Sesbania grandiflora* bark stock was meticulously mixed with 1000 µl of ethanol and acetone solvents, utilizing the same extraction procedures as those applied in the antimicrobial assay.

A fresh stock solution of DPPH (0.5 mM) was meticulously prepared in a 90 % methanol solvent and utilized in the experiment. To test the sample extracts, 100 µl of this fresh DPPH stock solution was combined with 10 µl of extracts of varied concentrations, prepared in the two distinct solvents. Following an incubation period of 30-35 minutes in darkness to prevent light interference, absorbance was gauged at 517 nm. It's crucial to note that DPPH exhibits high sensitivity to light; thus, all experimental procedures were conducted under dark conditions. DPPH served as the control, while ascorbic acid was employed as the standard.

DPPH antioxidant assay: DPPH (0.5mM) + Test sample= absorbance (517nm)

Mena, SD, SE, and IC^{50} values were computed, and

the p-value was determined through one-way ANOVA. The percentage inhibition in this assay was calculated using the following formula:

$$\text{Inhibition \%} = \frac{\text{Control Absorbance} - \text{Plant Extract Absorbance}}{\text{Control Absorbance}} X\ 100$$

2.5.2 CUPRAC Antioxidant Assay[25]

The CUPRAC (cupric reducing antioxidant capacity) antioxidant assay was performed to assess the antioxidant activity of *Sesbania grandiflora* bark extracts. Extracts ranging from 2 to 100 µg were combined with 1000 µl of two polar solvents (ethanol and acetone), following the same preparation steps outlined in the antimicrobial activity assay.

For this assay, three reagents were required: ammonium acetate (1 mM), neo-cuproine (7.5 mM), and copper II chloride dihydrate (10 mM). Reagents 1 and 3 were dissolved in distilled water, while reagent 2 was dissolved in ethanol. 150 µL of plant extracts of varying concentrations were mixed with 150 µL each of 7.5 mM neo-cuproine, 1 mM ammonium acetate, and copper (II) chloride dihydrate in a centrifuge tube, and then incubated for 30 minutes at room temperature. After incubation, the absorbance of the reaction mixture was measured at 450 nm using a spectrophotometer.

A calibration curve was constructed using standard concentrations of ascorbic acid ranging from 25 µg/ml to 100 µg/ml, plotted against the absorbance recorded at a wavelength of 450 nm. The total CUPRAC reducing capacity was determined using the standard calibration curve based on linear regression analysis. The concentration of the analyte in the extracts was calculated from the equation derived from the calibration curve and expressed in terms of ascorbic acid equivalents. The detailed protocol of this method (slide modification) is provided below:

CUPRAC antioxidant assay: Test sample + Reagent 1+ Reagent 2 + Reagent 3 = Absorbance (450nm)

3. RESULTS

3.1 Antimicrobial Activity

All selected bacteria and fungi exhibited positive responses against both ethanol and acetone bark extracts (refer to Fig. 2 & 3). In ethanol solvent, the bark extracts demonstrated the maximum zone of inhibition against MCC 1152, with a diameter of 230.81 mm^2, and the minimum against MCC 3040, with a diameter of 30.70 mm^2 (see Table 1). Conversely, in acetone solvent, the maximum inhibition was observed against MCC 2511, with a diameter of 136.79 mm^2, while the minimum was against MCC 3973, with a diameter of 13.96 mm^2 (see Table 2).

Ethanol solvent extracts exhibited higher antibiotic potential against MCC 1152, MCC LA, MCC SM, and MCC 3973 compared to acetone extracts (see Table 3). Additionally, the maximum percentage inhibition was reported against *Candida albicans*, with a value of 41.08 %, and the minimum against *Enterococcus faecalis*,with a value of 11.15 %, in ethanol extracts (see Table 4). In contrast, acetone extracts demonstrated the maximum inhibition against *Enterococcus faecalis*, with a value of 50.02 %, and the minimum against *Lactobacillus acidophilus*, with a value of 4.58 % (see Table 5).

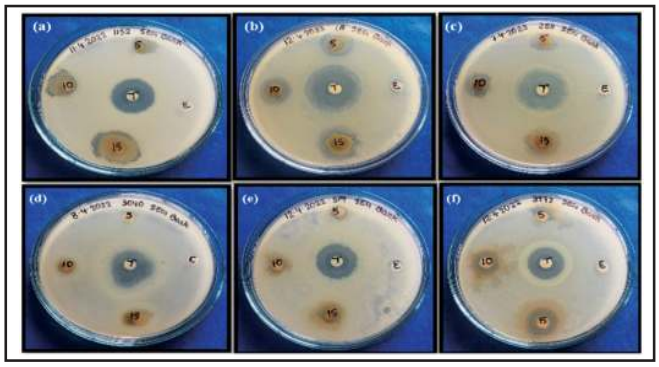


Figure 2. Antibiotic capacity of ethanol bark excerpts against a- MCC 1152 (p-value: $6.28*10^{-5}$), b- MCC LA (p-value: 0.410), c- MCC 2511(p-value: 0.259), d- MCC 3040(p-value: 0.0014), e- MCC SM (p-value: 0.0011) and f- MCC 3973 (p-value: $2.38*10^{-5}$).

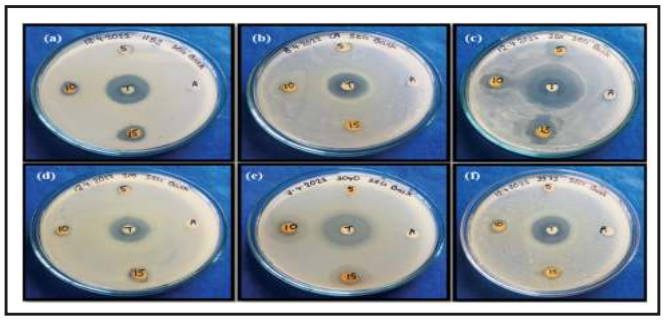
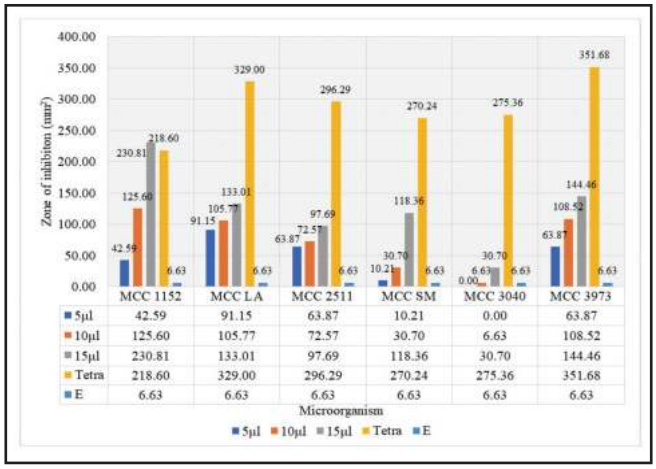
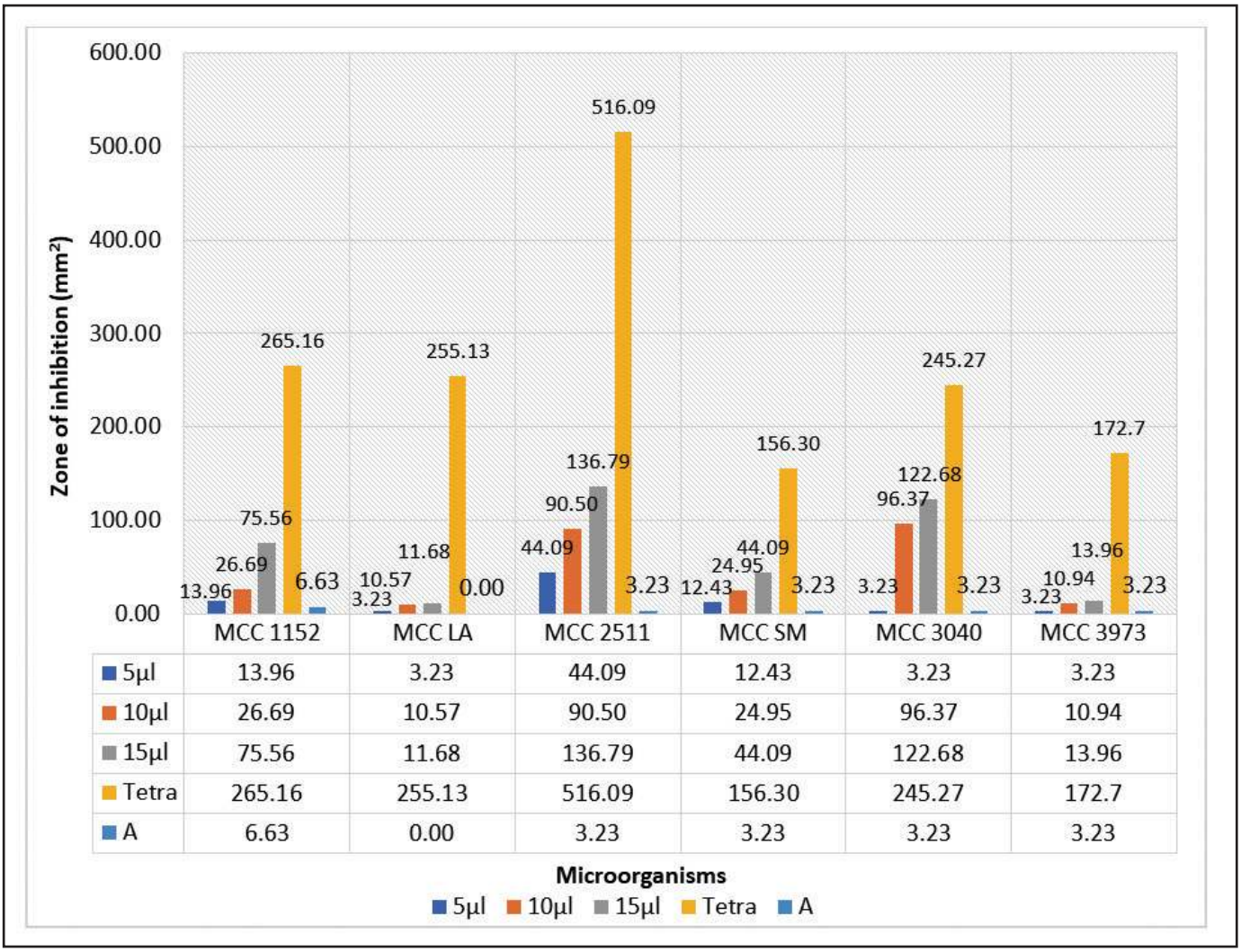


Figure 3. Antibiotic capacity of acetone bark excerpts against a- MCC 1152 (p-value: $2.7*10^{-5}$), b- MCC LA (p-value: 0.055), c- MCC 2511(p-value: $7.62*10^{-5}$), d- MCC 3040 (p-value: 0.0008), e- MCC SM (p-value: $1.62*10^{-5}$) and f- MCC 3973 (p-value: 0.096).



	MCC 1152	MCC LA	MCC 2511	MCC SM	MCC 3040	MCC 3973
5µl	42.59	91.15	63.87	10.21	0.00	63.87
10µl	125.60	105.77	72.57	30.70	6.63	108.52
15µl	230.81	133.01	97.69	118.36	30.70	144.46
Tetra	218.60	329.00	296.29	270.24	275.36	351.68
E	6.63	6.63	6.63	6.63	6.63	6.63

Table 1. Zone of inhibition of ethanol solvent bark excerpts



	MCC 1152	MCC LA	MCC 2511	MCC SM	MCC 3040	MCC 3973
5µl	13.96	3.23	44.09	12.43	3.23	3.23
10µl	26.69	10.57	90.50	24.95	96.37	10.94
15µl	75.56	11.68	136.79	44.09	122.68	13.96
Tetra	265.16	255.13	516.09	156.30	245.27	172.7
A	6.63	0.00	3.23	3.23	3.23	3.23

Table 2. Zone of inhibiton of acetone solvent bark exerpts

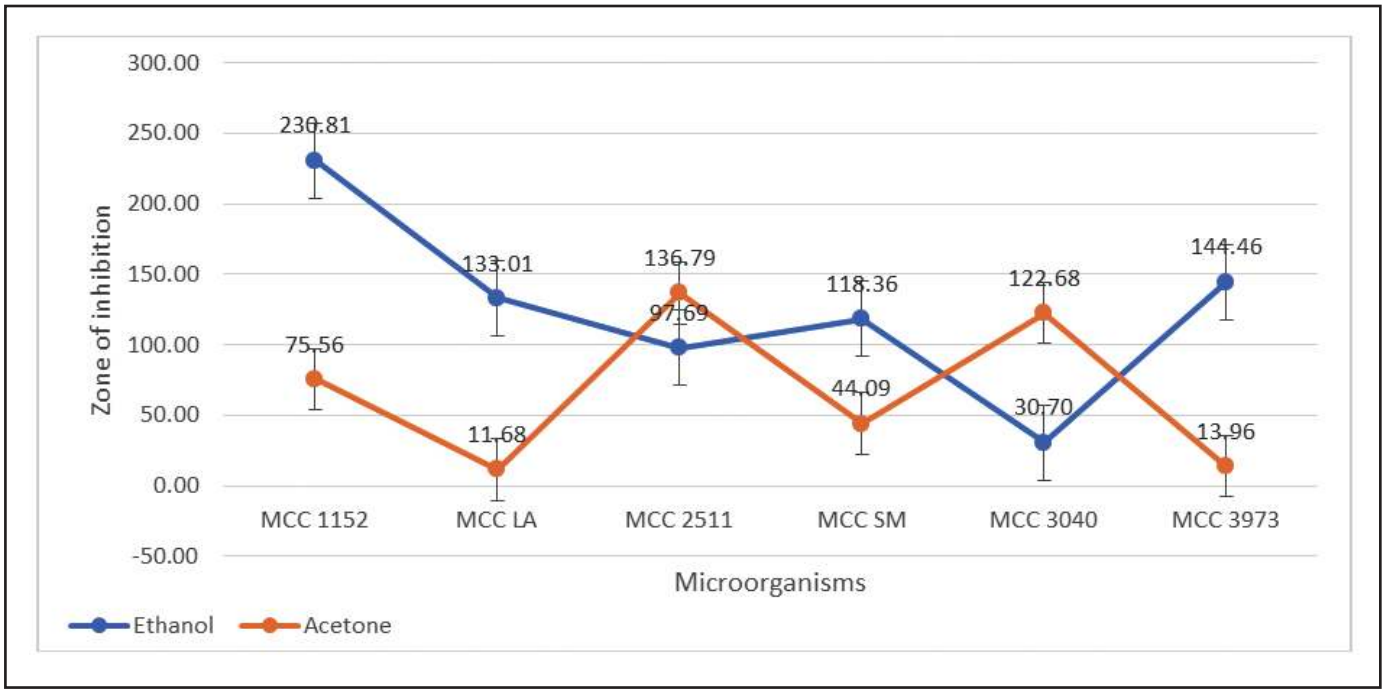


Table 3. Comparison of the zone of inhibition of ethanol and acetone solvent bark excerpts

Table 4. % inhibition of ethanol-solvent bark excerpts

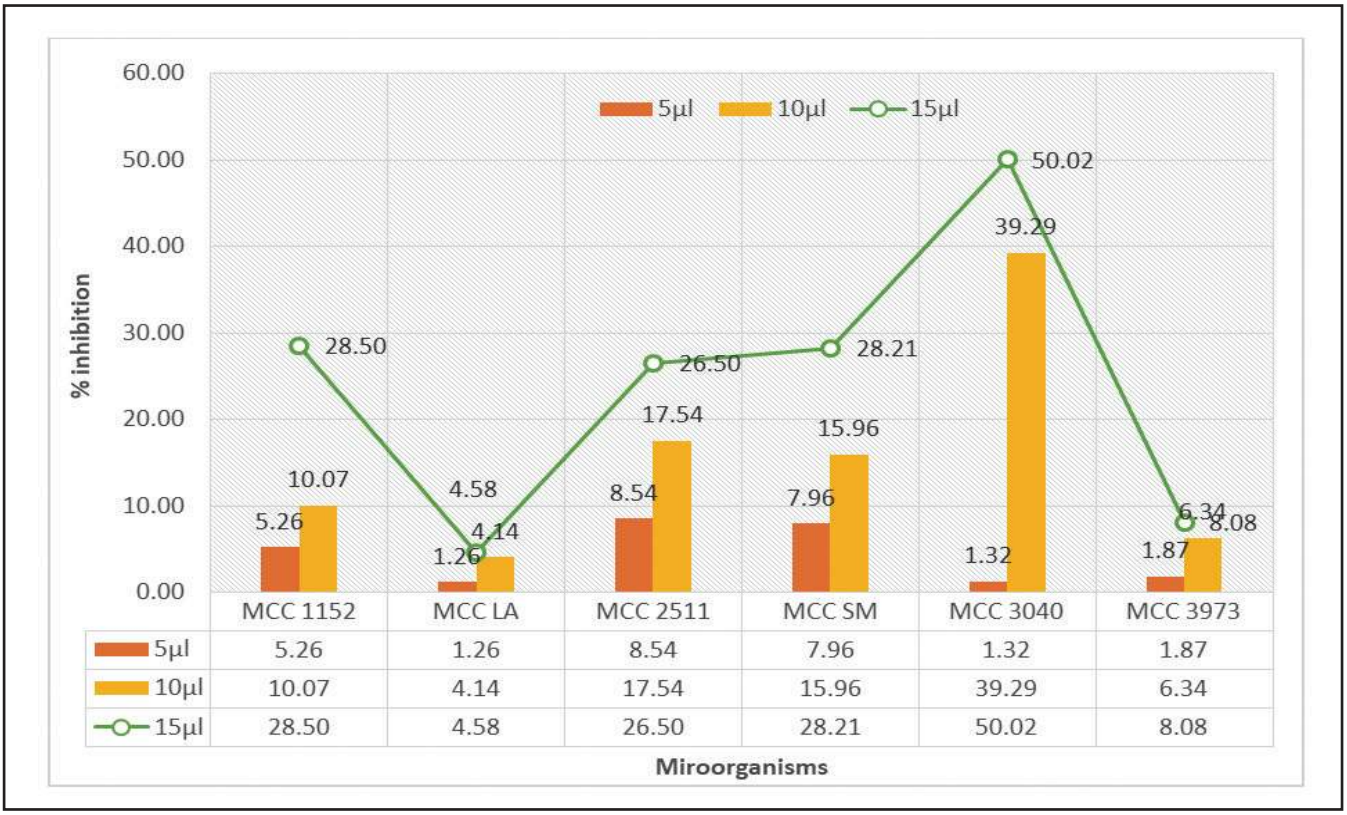


Table 5. % inhibition of acetone-solvent bark excerpts

3.2 MIC and MBC

The MIC and MBC values were determined for *Sesbania grandiflora* bark extracts at concentrations ranging from 10 to 200 µg.

In ethanol extracts, the maximum MIC was observed against MCC SM, while the minimum was against MCC LA. Similarly, in acetone extracts, the maximum MIC was observed against MCC 2511, while the minimum was against MCC SM (refer to Table 6). Additionally, the maximum MBC value for ethanol extracts was reported against MCC SM, while the minimum was against MCC LA. In acetone extracts, the maximum MBC value was observed against MCC 2511, while the minimum was against MCC SM.

Tetracycline was used as the standard antibiotic for comparison.

Table 6. MIC and MBC value of bark excerpts against diferent microoganisms.

S. NO.	Microorganisms	Concentration: 5mg/ml			
		Volume (µl)			
		MIC		MBC	
		Ethanol	Acetone	Ethanol	Acetone
1	MCC 1152	11	18	21	24
2	MCC LA	10	20	18	28
3	MCC 2511	16	24	22	30
4	MCC SM	20	14	28	22
5	MCC 3040	18	18	24	26
6	MCC 3973	14	22	26	28

3.3 DPPH free Radical Scavenging Activity

The DPPH free radical scavenging activity assay was conducted to assess the antioxidant potential of *Sesbania grandiflora* ethanol and acetone bark extracts, a method commonly employed to evaluate the ability of plant extracts to counteract free radicals.

In the ethanol extracts, the highest scavenging activity was observed at a concentration of 30 µg/ml, demonstrating a percentage inhibition of 19.50 %, while the lowest activity was recorded at a concentration of 5 µg/ml, with a percentage inhibition of 1.96 %. Similarly, in the acetone extracts, the maximum scavenging activity was noted at a concentration of 30 µg/ml, exhibiting a percentage inhibition of 15.60 %, while the minimum activity occurred at a concentration of 5 µg/ml, with a percentage inhibition of 1.96 % (see Table 7).

The IC_{50} value, representing the concentration of the extract required to scavenge 50% of the free radicals, was higher for acetone extracts (93.60 µg/ml) compared to ethanol extracts (60.35 µg/ml) (refer to Table 7).

3.4 CUPRAC Antioxidant Assay

The CUPRAC antioxidant assay was utilized to assess the free radical scavenging activity of Sesbania grandiflora ethanol and acetone bark extracts, employing a standard calibration curve (refer to Table 8).

In both ethanol and acetone extracts, the highest free radical scavenging activity was observed at a concentration of 100 µg/ml. Specifically, ethanol extracts displayed a percentage scavenging activity of 8 %, while acetone extracts exhibited a percentage scavenging activity of 2.28 %. Conversely, the lowest scavenging activity was noted at a concentration of 2 µg/ml for both ethanol and acetone extracts. Ethanol extracts demonstrated a scavenging activity of 0.31 %, while acetone extracts exhibited a scavenging activity of 0.25 % (refer to Table 9).

Table 7. DPPH free radical scavenging activity

Free radical (DPPH antioxidant assay) scavenging activity						
Concentrations (µg/ml)	Ethanol excerpts		Acetone excerpts		Ascorbic acid	
	Mean SD (±)	% Inhibition	Mean SD (±)	% Inhibition	Mean SD (±)	% Inhibition
5	1.85±0.028	1.96	1.85±0.028	1.96	0.82±0.002	38.88
10	1.82±0.025	3.68	1.83±0.022	3.33	0.72±0.002	46.07
15	1.75±0.02	7.32	1.80±0.00	5.01	0.63±0.00	52.96
20	1.63±0.014	13.86	1.73±0.012	8.51	0.53±0.004	60.57
25	1.57±0.017	16.83	1.67±0.017	11.55	0.46±0.003	65.24
30	1.52±0.02	19.50	1.60±0.00	15.60	0.38±0.02	71.54
p-value	$8.7*10^{-6}$		$1*10^{-5}$		$2.2*10^{-12}$	
IC_{50} value	60.35		93.60		11.59	

Table 8. Standard curve (Asorbic acid) for CUPRAC free radical scacenging activity

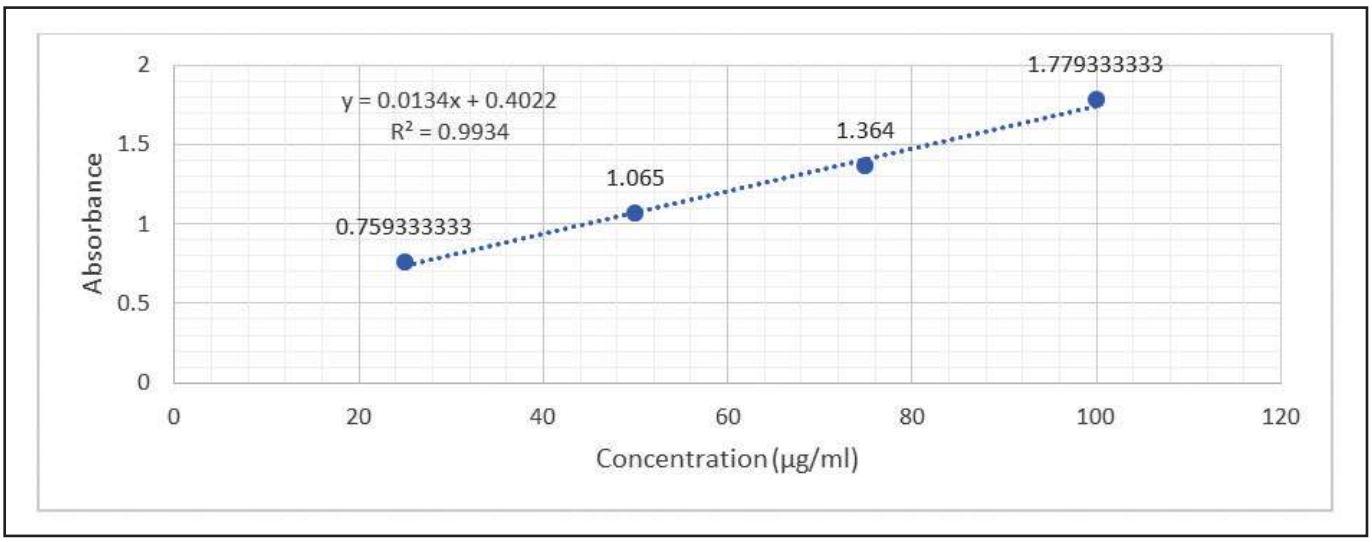


Table 9. CPPRAC free radical scavenging activity

CPPRAC free radical scavenging activity (%)													
Ascorbic acid equivalence antioxidant capacity (mg AAE/g)													
Concentration (µg/ml)													
2		5		10		20		40		80		100	
E	A	E	A	E	A	E	A	E	A	E	A	E	A
0.31 ± 0.02	0.25 ± 0.03	0.84 ± 0.02	0.29 ± 0.04	2.26 ± 0.04	0.60 ± 0.02	3.47 ± 0.03	1.07 ± 0.02	4.49 ± 0.04	1.35 ± 0.02	6.72 ± 0.02	1.74 ± 0.03	8.00	2.28 ± 0.07

***E= Ethanol and A= Acetone**

4. DISCUSSION AND CONCLUSIONS

Sesbania grandiflora is abundant in both non-enzymatic and enzymatic antioxidants, effectively scavenging free radicals[26]. The Minimum Bactericidal Concentration (MBC) value of its bark extracts is lower than the Minimum Inhibitory Concentration (MIC) value for gram-negative bacteria such as *S. sonnei & S. typhi,* and for gram-positive bacteria including *B. cereus, S. epidermidis, E. faecalis, & S. aureus,* where the MIC value closely matches the MBC value.[27] Ethyl acetate extracts of this bark demonstrate efficacy against *Staphylococcus aureus* (methicillin-resistant), *enterococci* (vancomycin-resistant), *Pseudomonas aeruginosa* & *Escherichia coli*[28]. Moreover, bark extracts of the Agastya plant exhibit promising antibiotic activity against *Candida albicans, Staphylococcus aureus, Escherichia coli* & *Pseudomonas aeruginosa,* with Amphotericin-B for fungus and Streptomycin for bacteria serving as standards.[29] The present study also highlights effectiveness against *Pseudomonas aeruginosa & Candida albicans,* while antibiotic potential against *Lactobacillus acidophilus, Bacillus subtilis, & Streptococcus mutans* remains unexplored.

In addition to its antimicrobial properties, *Sesbania grandiflora* bark extracts demonstrate significant antioxidant activity. Antioxidants have garnered scientific interest due to their numerous health benefits, including anti-aging, anti-inflammatory, and anticancer effects. Our study contributes to the existing body of evidence supporting the role of plant-based antioxidants in promoting health and reducing the risk of various diseases.[30] Research suggests that a higher intake of dietary antioxidants is associated with reduced mortality from all causes and cardiovascular diseases among adults with diabetes.[31] Furthermore, individuals who consume antioxidants have a 21 % lower mortality risk from respiratory diseases compared to non-users.[32] This finding, derived from a study involving 62,063 participants from the Singapore Chinese Health Study, underscores the importance of a diet rich in antioxidant nutrients. Public health recommendations advocate increased consumption of plant-based foods abundant in antioxidants.[33]

Plant-based antioxidants offer several advantages, including reliability, cost-effectiveness, renewability, and easy availability. The consistent effectiveness of *Sesbania grandiflora* bark, supported by numerous pieces of literature, underscores its reliability. The bark of this plant is rich in glycosides, alkaloids, flavonoids, tannins, and saponins. The total phenolic content in methanolic and aqueous extracts varies between 54.83, 58.06, 33.87, 54.19 µg/ml respectively, expressed in gallic acid equivalents (GAE).

The high phenol content in the bark extract explains its potent free radical scavenging activity.[34]

Results of DPPH scavenging activity indicate that the IC^{50} value of the ethanol extract (60.35 µg/mL) is significantly lower than that of the acetone extract (93.60 µg/mL). This difference may be attributed to the strong hydrogen donating ability of polyphenols present in the ethanol extract, leading to the reduction of DPPH, compared to the weaker abilities observed in the acetone extracts. The present results reflect the high polyphenol content present in the bark of this plant. While the antioxidant potential of this plant bark remains underexplored, our study demonstrates that bark extracts in both solvents efficiently inhibit free radicals. Overall, our findings suggest that *Sesbania grandiflora* bark extracts possess promising antimicrobial and antioxidant properties, warranting further investigation in animal models infected with drug-resistant bacteria. Further research is necessary to explore the therapeutic potential of this plant in combating infectious diseases and oxidative stress-related conditions.

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Comparative Assessment of Acute Toxicity of *Sanjivani Vati* Prepared With Two Different Species of Aconite Through Fish Embryo Toxicity Test

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ABSTRACT

The presented study deals with evaluating the Acute toxicity of *Sanjivani Vati* **(**SV), an ayurvedic medicament prepared with two different species of Aconite *viz.* naturally sourced sample and marketed sample employing Fish Embryo Toxicity (FET) using OECD guideline 236.All the raw botanicals were authenticated before the preparation of the drug. Two groups of *Sanjivani Vati* namely G-1 and G-2 were prepared from *Aconitum napellus* L., and *Aconitum balfourii* Holmes ex Stapf. respectively. Their aqueous extraction was done using a soxhlet apparatus**.** They were used as test drugs further. The study was conducted at CSIR-IHBT, Himachal Pradesh. No indications of toxicity were detected in the initial limit test, OECD 236 for both the groups of SV. The median lethal concentration of G-1 (naturally sourced sample) i.e. 535.53 µg/mL was found less than the median lethal concentration of G-2 (marketed sample) i.e. 652.81 µg/mL. The findings suggest that both the tested species of Aconite could be utilized for drug preparation following thorough processing as per the classical guidelines. Nonetheless, caution is advised regarding dosage administration. Preferably, the drug sourced from natural origins should be utilized owing to variations in potency.

Keywords: Aconite; Acute toxicity; FET; OECD 236; *Sanjivani Vati***;** Zebrafish embryo

NOMENCLATURE

SV	: Sanjivani vati
FET	: Fish embryo toxicity
OECD	: Organization for economic cooperation and development
API	: Ayurvedic pharmacopoeia of india
LC_{50}	: Median lethal concentration
EC_{50}	: Median effective concentration

1. INTRODUCTION

The evaluation of the toxicity profile of traditional medicines, despite their long-standing history of safe usage, is imperative to uphold public health standards and ensure continued trust in these therapeutic practices. While traditional medicines, such as Ayurveda, have been relied upon for generations and are often perceived as safe due to their natural origins, modern scientific scrutiny offers a deeper understanding of potential risks and benefits. Comprehensive toxicity evaluation provides valuable insights into the safety margins and potential adverse effects of traditional medicines, enabling informed decision-making by healthcare professionals and consumers alike. Thus, the evaluation of toxicity for Ayurvedic medicaments is necessary for globalization to ensure compliance with international trade regulations, protect consumer health, harmonize safety standards, facilitate market access, address health concerns, and promote cultural sensitivity and acceptance of traditional medicine systems.

Sanjivani Vati (SV) is an extensively used multi component Ayurvedic medicine, outlined in the Ayurvedic Formulary of India[1]. It is widely recommended by Ayurvedic practitioners for addressing a range of conditions such as fever, indigestion, dysentery, Gastroenteritis, etc. It comprises ten ingredients of herbal origin including a couple of Schedule E-1 drugs viz. *Vatsanabha*(*Aconitum ferox* Wall. Ex Ser.) and *Bhallataka*(*Semecarpus anacardium* Linn.). Furthermore, Cow's urine holds significant importance as one of the primary constituents, utilized for *Bhavana* (impregnation)[2]. Despite its established History of Safe Use (HoSU), SV lacks a comprehensive toxicity study to ascertain its safety more precisely. Additionally, concerns arise regarding one of its ingredients i.e. *Vatsanabha*, historically recognized as one of the most poisonous plants. It is being sold as a mixture of various species in Indian markets. Previous research has highlighted significant qualitative and quantitative variations in the total alkaloid content among different species of *Vatsanabha*[3]. However, the potential impact of utilizing different species on the safety profile of various formulations remains unexplored.

The adoption of the European policy REACH i.e. Registration, Evaluation, Authorisation, and Restriction

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of Chemicals[4] underscores a clear protocol to actively encourage the development of alternative methodologies as per the 3Rs principle, which emphasizes "Replacement, Reduction, and Refinement[5]." This directive prioritizes the utilization of data generated through validated alternative methods whenever feasible. Consequently, there is a growing impetus to replace conventional animal-based approaches for assessing acute toxicity and teratogenicity with innovative approaches such as adverse outcome pathway-based biomarker experiments[6] and in vitro testing[7]. Additionally, there is a push for the adoption of more 3Rs-compatible in vivo models[8,9]. Notably, alternative in vitro studies and in vivo assays, including those utilizing fish embryos, offer the potential for high-throughput testing[10], aligning with the overarching goal of promoting ethical and efficient methods for chemical safety assessment. The Zebrafish embryo acute toxicity test is one such method utilised for evaluating acute toxicity, employing embryonic and larval models. This approach is deemed appropriate due to the resemblances observed between the human and zebrafish genomes, as well as similarities in the physiology of their, nervous, digestive, and vascular systems. Therefore, the current experiment deal with the evaluation of Acute toxicity of SV prepared with two different species of *Vatsanabha* employing Fish Embryo toxicity (FET) in the Zebrafish model using OECD guideline 236.

2. MATERIAL AND METHODS

2.1 Procurement of Raw materials

All requisite raw botanicals including a sample of *Vatsanabha* were purchased from a local vendor, Jaipur, in dried form. Another sample of *Vatsanabha* was collected from Chicham Khas, Himachal Pradesh (Elevation - 4300m, Lat. 32.339601^{0}, Long. 77.997743^{0}). Fresh *Gomutra* (Cow's urine) was collected from nearby cowsheds in the early morning.

2.2 Authentication of Raw material

The procured samples of individual crude drugs were authenticated by the Raw Materials Herbarium and Museum, Delhi, under National Institute of Science Communication and Policy Research (CSIR). The authentication numbers for its constituents issued by the institute are as follows: *Vidanga* (4081-82-2), *Nagara* (4081-82-8), *Krishna* (4081-82-9), *Pathya* (4081-82-6), *Amala*(4081-82-5), *Bibhitaka*(4081-82-4),*Vacha* (4081-82-3), *Guduchi* (4081-82-7), *Bhallataka* (4081-82-1). Apart, the fresh sample of *Vatsanabha* was identified and authenticated as *Aconitum napellus* L. (BSI/BGIR/1/TECH./2021/027/80) by Botanical Survey of India, Botanical Garden of Indian Republic, Noida. The marketed sample of *Vatsanabha* was identified and authenticated as *Aconitum balfourii* Holmes ex Stapf (RRDR/AIIA/129) at Taxonomy and Herbarium laboratory, Regional Raw Drug Repository (RRDR), All India Institute of Ayurveda, New Delhi. The voucher specimens and herbarium have been preserved in the respective Institutes. Furthermore, the confirmation of species *Vatsanabha* was also done by DNA fingerprinting(BLAST RID: *Aconitum napellus* L.-1RWPUC26013 and *Aconitum balfourii* Holmes ex Stapf – 130MZKGG013).

2.3 Preparation of *SanjivaniVati*

Two groups of *Sanjivani Vati* namely G-1 and G-2 were prepared in the Pharmaceutical Laboratory, *Rasashastra* and *Bhaishajya Kalpana* Department, All India Institute of Ayurveda as per the official reference[11]. G-1 and G-2 can be stated as *Sanjivani Vati* prepared from *Aconitum napellus* L. and *Aconitum balfourii* Holmes ex Stapf respectively. The formulation composition is depicted in Table 1.All the relevant physicochemical parameters for both of the test drugs were found within permissible limit as per the Ayurvedic Pharmacopoeia of India (API Part I, Volume III).The analytical study was also conducted at All India Institute of Ayurveda, New Delhi.

Table 1. Table 1: Formulation Composition of *Sanjivani Vati*

S. No.	Ingredient	Latin name	Part used	Ratio
1.	Vidanga	*Embeliaribes* Burn.	Dried Fruit	1
2.	Nagara	*Zingiber officinale* Rosc.	Dried Rhizome	1
3.	Krishna	*Piper longum*Linn.	Dried Fruit	1
4.	Pathya	*Terminalia chebula* Retz.	Dried Pericarp	1
5.	Amala	Embelicaofficinalis Gaertn.	Dried Pericarp	1
6.	Bibhitaka	*Termenalia bellirica* Roxb.	Dried Pericarp	1
7.	Vacha	*Acoruscalamus* Linn.	Dried Rhizome	1
8.	Guduchi	*Tinospora cordifolia* Miers ex Hook. & Thoms.	Dried Stem	1
9.	Shuddha Bhallataka	Processed *Semecarpusanacardium* Linn.	Dried Fruit	1
10.	Shuddha Visha	Processed *Aconitum napellus* L. (G-1) /*Aconitum balfourii* Holmes ex Stapf (G-2)	Dried Root tuber	1
11.	Gomutra	Cow urine		Q.S. for Bhavana

2.4 Preparation of Test Samples

The extraction for both G-1 and G-2 was carried out in the soxhlet apparatus using water as a solvent. The resultant filtrates were evaporated to dryness in an oven at 40 ^{0}C. The extracts thus obtained were used as test samples for the Zebrafish Embryo Acute Toxicity Test.

2.5 Test Organism and Ethical Approval

The test subjects employed for the study were the embryo of *Danio rerio* i.e. short fin Zebrafish, wild-type. The test was performed at the Zebrafish Research Facility, Pharmacology and Toxicology Laboratory, Institute of Himalayan Bio-resources and Technology under the Council of Scientific and Industrial Research (CSIR-IHBT). Approval for the study was granted by the Institutional Animal Ethics Committee on August 18, 2023, under CPCSEA(1381/GO/ReBiBt/S/10/CPCSEA).

The experimental procedure involved dispersing the test sample in conditioned water(Temperature: 25-27 °C, Conductivity: 400-600µS, pH: 7.0-7.5, Oxygen saturation: 95-98 %)maintained by the fish system i.e. Zebtec, Tecniplast, Italy to attain various concentrations as mentioned below. The sample after dispersion in the water was sonicated for 5 minutes, and the solution of the final test concentration was changed every 8 h during incubation to avoid precipitation. Initially, the limit test was conducted at 100 mg/L following OECD TG 236 guidelines. In this test, newly fertilized eggs (20 for each observation) were exposed to 100 mg/L of the test drugs for 96 hours at 26 ± 1 °C in a BOD incubator, maintaining conventional water conditions as prescribed in OECD 236; S. No. 12. An additional group of 20 embryos were exposed to solvent, that served as a solvent control. Four observations were made on each tested embryo at interval of 24-hour to assess toxicity, including(A) coagulation of fertilised embryos; (B) Lack of somite formation; (C) Lack of detachment of tail-bud from the yolk sac; and (D) absence of a heartbeat.

Subsequently, the trial was conducted at various concentrations of the test sample (200-1000 µg/mL) to determine LC_{50}. Before commencing the test, the test chambers were conditioned with the experimental solutions for 24 hours. Then, 20 embryos were incubated at each concentration in conventional inert chambers as per OECD 236; S. No. 1 for 96 hours. Throughout incubation, consistent changes were made using a semi-static renewal procedure, ensuring embryos remained covered with a small amount of prior test solutions to prevent dehydration. Efforts were made to reduce stress while handling and observing them. Embryos were examined under a microscope, and images were captured every 24 hours until 96 hours for signs of toxicity. Notably, the developmental phase never surpassed the threshold for unprotected phases of development outlined by existing animal welfare laws by the European Union[12,13].Mortality of embryos/larvae was recorded at all tested concentrations throughout the 96-hour incubation period. This procedure was employed for both the test drugs.

3. RESULTS

No indications of toxicity were found in embryos in the limit test for both tested samples. On incubating at a concentration of 100 mg/L G-1 and G-2, all the embryos exhibited normal growth without any mortality. The developmental stages during a limit test for G-1 and G-2 have been shown in Fig. 1 and 2 respectively. Also, solvent control group exhibited complete survival of the embryos without any adverse effect.

In brief, by the 24-hour mark (Fig. 1-A & Fig. 2-A), the basic organization of the embryo is evident, with identifiable anatomical features like somites, otoliths, notochord, eye anlage, and heart anlage. Also, the tail curling of the embryo can be seen. By 48 hpf(Fig. 1-B & Fig. 2-B), the development of important sensory organs namely the eye and ear have been observed. The caudal fin was also formed. Also, different partitions of the brain and spine over the notochord are distinguishable. By 72 hpf(Fig. 1-C & Fig 2-C), the whole anatomy got predominantly developed. Also, the embryos were poised for hatching. The fins have undergone further development. By 96 hpf(Fig. 1-D & Fig 2-D), the volume of yolk sac resorption has significantly decreased along with the development of the swim bladder. Also, the intestinal tract is completely formed. All the observations at each stage were found to correspond with the normal development of zebrafish embryos[14].

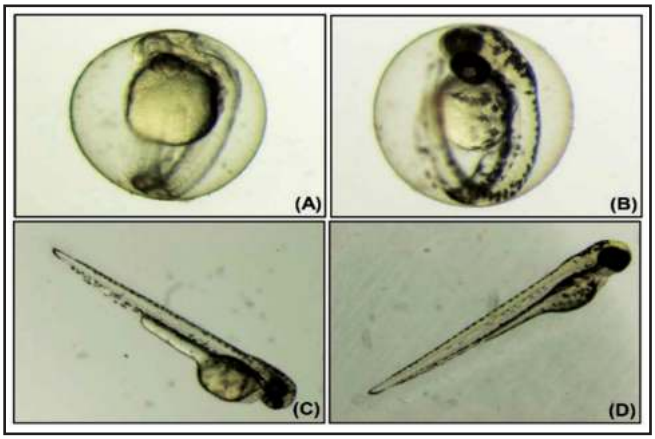

Figure 1. Development of embryos in limit test (100 mg/L of G1) @ (A) 24 hours, (B) 48 hours, (C) 72 hours, and (D) 96 hours.

Figure 2. Development of embryos in limit test (100 mg/L of G2) @ (A) 24 hours, (B) 485 hours, (C) 72 Hours, and (D) 96 hours.

However, mortality and abnormalities were observed at higher tested concentrations of test samples, based on which the LC_{50} was calculated. The toxicity observed at various tested concentrations of G-1 and G-2 during different observational endpoints have been depicted in Fig. 3 (A to E) and Fig. 4 (A to E) respectively. A representative image from each tested concentration is shown in the figure at a time point where maximum toxicity was observed. At a concentration of 200 µg/mL, blood congestion within the pericardial region was observed, resulting in the absence of heartbeat at 96h. At 400 µg/mL, reduced or lack of somite formation was seen at 48 h. Also, impaired development was evident at 48h with a concentration of 600 µg/mL. Further, on increasing the concentration to 800 µg/mL, defects were noticed in the early stages of development with a short tail or no tail noted at 24 h. Likewise, a lack of tail detachment was found with 1000 µg/mL at 24 h. Both the experimental groups exhibited comparable morphological alterations at different concentrations.

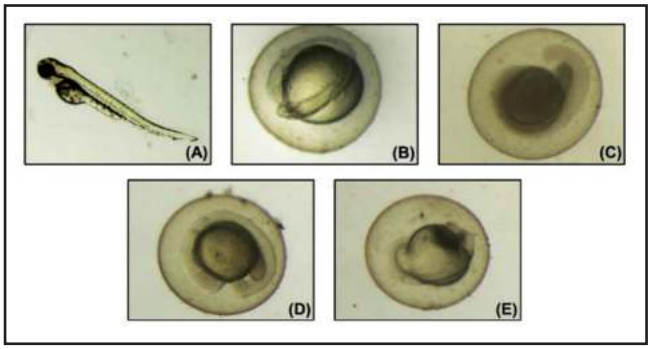

Figure 3. Toxicity observed at various tested concentrations of G1 during different observational endpoints. A representative image from each tested concentration is shown in the figure at a time-point where maximum toxicity was observed (A: 200 µg/mL at 96 h; B: 400 µg/mL at 48 h; C: 600 µg/mL at 48 h; D: 800 µg/mL at 24 h and; E: 1000 µg/mL at 24 h).

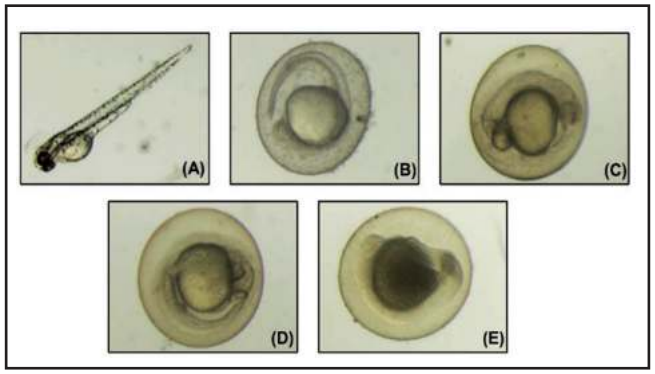

Figure 4. Toxicity observed at various tested concentrations of G2 during different observational endpoints. A representative image from each tested concentration is shown in the figure at a time-point where maximum toxicity was observed (A: 200 µg/mL at 96 h; B: 400 µg/mL at 48 h; C: 600 µg/mL at 48 h; D: 800 µg/mL at 24 h and; E: 1000 µg/mL at 24 h).

3.1 Data Analysis

The data for mortality was utilized to compute LC_{50} employing the Probit analysis[15] with minor modifications. The data for mortality at each tested concentration was transformed into corresponding probit values using a probit table[16]. To determine the probit, the percentage of larvae dead within the 96-hour incubation period for both 0 % and 100 % were adjusted as follows: 0 % dead as 100 (0.25/n) and 100 % dead as 100 (n-0.25/n). Here, 'n' represents the total number of larvae[17].The probit values were then plotted on the Y-axis graphically against log concentrations on the X-axis. The concentration corresponding to 50 % mortality on the logarithmic scale was noted. The antilogarithm of this value yielded the LC_{50} measurement. This analysis has been represented in Fig. 5 and Fig. 6 for tested samples. The calculated LC_{50} value of G1 was found to be 535.53 µg/mL i.e. antilog 2.72878 of corresponding Probit 5. For G-2, the LC_{50} value was found to be 652.81 µg/mL i.e. antilog 2.81479 of corresponding Probit 5.

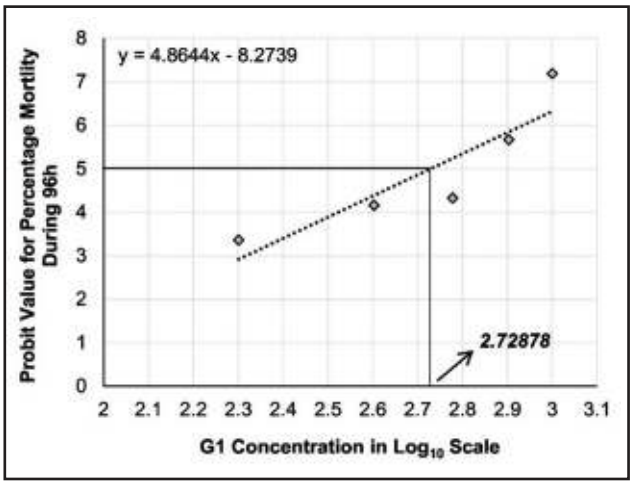

Figure 5: Probit analysis for G-1.

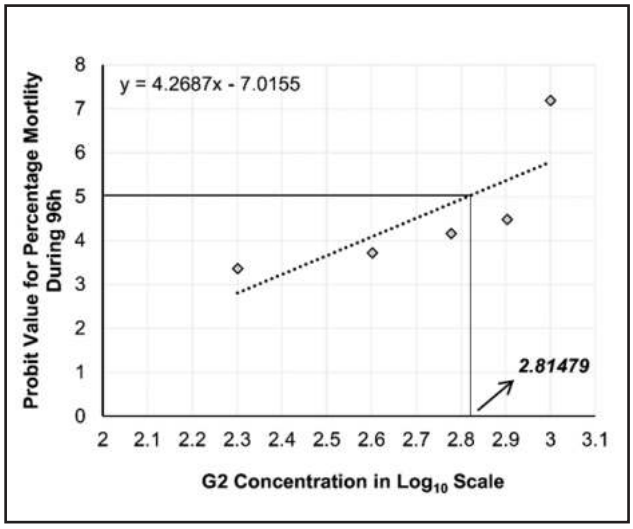

Figure 6. Probit analysis for G-2.

4. DISCUSSION

The Fish Embryo Acute Toxicity (FET) test employing Zebrafish embryos, as outlined in OECD test guideline (TG) 236, serves as an alternative to conventional acute fish toxicity testing like the OECD Acute Fish Toxicity Test (TG 203). To ensure comparable sensitivity to the acute fish test, the original FET test was streamlined to focus solely on four morphological core endpoints as mentioned above. These endpoints were chosen due to their direct or indirect correlation with mortality, making them practical for screening by proficient technical personnel and involving relatively simple morphological alterations[14].This FET test has gained wide recognition in scientific circles and even proved to have a greater sensitivity and closer correlation to humans compared to other models[18,19].Thus, this model has been employed in the current study to assess the acute toxicity of a frequently used medicament i.e. *Sanjivani Vati* prepared with two distinct species of Aconite.

On reviewing the outcomes, it can be inferred that both the test drugs were entirely safe in the initial limit test, suggesting their suitability for drug formulation. However, these findings necessitate validation through alternative and diverse models. Furthermore, the signs of toxicity were noted with increasing concentrations. At the highest concentration of 1000 µg/mL, the toxicity was evident in the early stages of development while defects in later stages of development were manifested in lower concentrations of the drug. Thus, both the test drugs exhibited concentration-dependent mortality. All these alterations were similar at different time points in both groups, indicating a similar nature among different species within the same genus. Looking over the toxicity signs closely, the cardiotoxic potential of the drug could be understood. The possible reason behind it may be its crucial ingredient i.e. *Vatsanabha* which consists of Aconitine. Aconitine is classified as a diester-diterpene alkaloid, well-known for its arrhythmogenic effects. A study on zebrafish embryos reported that aconitine-induced cardiac dysfunction and apoptosis were related to Ca^{2+} signaling pathway[20].When discussing additional indicators of toxicity, the failure of tail detachment is observed during the initial stage of development and can either serve as an indicator of general delayed development The reasons behind the failure of the tail to detach are still unclear, and it is essential to focus not only on its incidence and severity but also on the possibility of its recovery[14].These indications of toxicity could also be linked to Piperlongumine, an alkaloid present in another component of the test drug i.e. long pepper which has been observed to stimulate a rise in the number of inter segmental vessels and impede early-stage development in *Daniorerio* embryos. Additionally, it has been noted to impact heart formation and heart rate[21]. Apart, the absence or lack of somite formation indicates severe lethality. Somites play a crucial role in the formation of various structures such as the vertebrae, ribs, skeletal muscles, and skin, emphasizing their significance in the subsequent development of the embryo[22].

In addition, the LC_{50} value for both groups was determined as it provides a quantitative measure of the toxicity of a substance, indicating the concentration at which it is lethal to 50 % of the test population within a specified exposure period[23]. Moreover, the LC_{50} values frequently serve as the cornerstone for numerous advanced assays in higher tiers, including behavioral assessments and more targeted and mechanistic evaluations of toxicity using adult animals[14].On comparing the values, it was observed that the median lethal concentration of G-1 was found less than the median lethal concentration of G-2. It indicates the variation in potency between a naturally sourced drug sample and one procured from the market. Also, aconitine was identified and quantified in both the Aconitum species through High Performance Thin Layer Chromatography (Mobile Phase: Toluene: Ethyl acetate: Diethyl amine-70:20:10 v/v/v) as: *Aconitum napellus* L.-440.32 ug/100 mg and *Aconitum balfourii* Holmes ex Stapf.-16.90ug/100mg.Moreover, these results could be further substantiated through the calculation of median effective concentration i.e. EC_{50}.

5. CONCLUSION

No indications of toxicity were detected in the initial limit test, OECD 236 for *Sanjivani Vati* prepared from *Aconitum napellus* L., and *Aconitum balfourii* Holmes ex Stapf. This suggests that both the tested species of Aconite could be utilized for drug preparation following thorough processing as per the classical guidelines. Nonetheless, caution is advised regarding their dosage administration. Moreover, additional studies may also be required to elucidate the findings.

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He contributed to the conception and design of the work and in the supervision of the study.

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The Role of Mindfulness on The Relationship Between Personality Factors and Cognitive Failures: A Mediation Study

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ABSTRACT

Cognitive failures such as minor lapses in perception, memory and action are common in daily life. Several factors including personality and mindfulness are instrumental in bringing about individual differences in cognitive failures. Personality factors might be indicative of these slips. The present research aims at exploring the association between personality factors and cognitive failures, and the mediating role of mindfulness. The sample consisted of 419 participants between the age range of 18 to 74years (M= 29.06 years, SD = 12.55) and were assessed on personality factors, cognitive failures and mindfulness. Findings from hierarchical regression analysis suggest that the personality factors and mindfulness account for 29.2 % of variance in cognitive failures. On the other hand, mediation analysis of mindfulness on the relationship between personality factors and cognitive failures suggest that mindfulness is a partial mediator for the relationship between extraversion and cognitive failures; and a complete mediator for the relationship between neuroticism and cognitive failures. This study has attempted to further the understanding of the interactions between personality traits and mindfulness, and their consequent impact on cognitive failures in the adult Indian population.

Keywords: Cognitive failures; Mindfulness; Psychoticism; Extraversion; Neuroticism; Mind wandering

1. INTRODUCTION

Cognitive failure, a prevalent phenomenon in our routine tasks[1], is defined as the general lapses in perception, memory and action[2]. The frequency of cognitive failures differs from person to person, with some people being more vulnerable to these slips than others[3], which may, to some extent be explained by genes[4,5], the structure of the brain[6,7] , lifestyle[8], poor sleep quality and low mood[9,10].

Certain types of personality also make an individual susceptible to cognitive failures. It was [11]found that the personality profile of a person prone to cognitive failures would have higher levels of self-consciousness and anxiety, as its major components with self-consciousness partially mediating the positive relationship between Cognitive Failure Questionnaire (CFQ) scores and anxiety. It was pointed out[12] that cognitive failures played an essential role in individual safety behaviour, especially when conscientiousness was low. It was also observed that cognitive failures were associated with high level of self-directedness trait[13].

However, various factors have been identified to aid in reducing the frequency of cognitive failures among individuals. Stress management interventions[14], mindfulness-based interventions[15], workplace flexitime[16] etc, have been effective in reducing cognitive failures. Higher degrees of neuroticism and cognitive failures scores were significantly associated with lower self-reported mindfulness scores, indicating that mindfulness may be a useful tactic to mitigate the negative impact of neuroticism on cognitive failures.[17,18].

Links have also been established between personality traits and mindfulness. For instance, a study was conducted[19] that aimed at extending the understanding of the relationship between mindfulness and personality trait (conscientiousness) on a sample of 458 individuals using the Sustained Attention to Response Task (SART) framework for mindfulness[20]. The self-regulation was found to be positively associated to openness, negatively associated with neuroticism and positively associated with conscientiousness, and "conscientious confusion" cluster, showed a mixed relationship between conscientiousness and mindful self-regulation. This suggests that people will be able to participate with daily life more fully the more dispositional mindfulness they possess.

The self-regulation cluster implies that mindful tendencies help in adaptive regulation of behavior and thoughts, which allow for improved participation in pursuits and activities that are more in line with one's own ideals, which in turn promote greater psychological well-being, consequently explaining the inconsistency

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with negative emotionality. Mindfulness was negatively related to neuroticism and positively to conscientiousness[21].

In essence, personality traits of the individuals can be used to infer about their characteristic thoughts, feelings and behaviors. Possessing certain personality traits can make a person vulnerable to cognitive failures[22] which are general lapses or failures in perception, memory and action[2]. These errors, though very common in everyday life, can have dire consequences like dangers to patient safety[23] and automobile accidents[22]. Though dispositional mindfulness has emerged as an important factor that can account for the individual differences in the occurrence of cognitive failures[24,25], the paucity of research conducted on how mindfulness affects the relationship between personality traits and cognitive failures, especially for Indian population, warrants the present research. In order to address the gap in the current literature, the following hypotheses were formulated:

- **H1** There will be significant relationships among mindfulness, personality factors and cognitive failures.
- **H2** Mindfulness will mediate the relationship between personality factors and cognitive failures.
- **H2a** Mindfulness will mediate the relationship between extraversion and cognitive failures.
- **H2b** Mindfulness will mediate the relationship between neuroticism and cognitive failures.
- **H2c** Mindfulness will mediate the relationship between psychoticism and cognitive failures.

2. MATERIALS AND METHODS

2.1 Participants

The sample was drawn using the snowball sampling method, from the adult Indian population. The online survey link was circulated amongst the participants. The sample constituted 419 participants (M= 29.06 years, S.D.= 12.55), of which 167 were males (M= 33.22 years, S.D.= 10.06) and 252 were females (M= 26.32 years, S.D.= 14.66). Nearly 57 % of the participants had an educational qualification of post-graduation and beyond. About 49 % of the participants were students, followed by 30 % employees, 9 % self-employed, 7 % research scholars, 4 % unemployed and 2 % retired. The majority of the participants reside in urban areas (about 69 %), followed by 20 % in semi urban and 11% in rural areas. A vast majority of the participants were unmarried (69 %), followed by married (31 %) and separated individuals (0.24 %).

2.2 Procedure

The data was collected using snowball sampling technique. The responses of the participants were collected through an online mode consisting of three questionnaires chosen for the present study. The general background and purpose of the study was conveyed to the participants, along with a general set of instructions. Terms of informed consent were specified appropriately. The basic demographic details of the participant were also collected: age, gender, educational qualification, designation, area of dwelling and marital status. A general set of instructions was also provided to aid the participants in recording their responses wherein participants were asked to read the items carefully and select one of the various given options pertaining to them, by checking the box of the preferred response. The survey could be completed in within duration of 20-30 minutes. The contact details of the researcher were also provided in case of requirement of a clarification. 426 participants filled the questionnaire forms, and after eliminating responses with missing data, 419 of these were found to be eligible.

2.3 Measures

2.3.1 Personality

Eysenck's Personality Questionnaire-Revised (Short Form) (EPQR-S)[26] was used to assess personality traits. It consists of 48 dichotomous items which are responded to as Yes/No, divided into 4 subscales: Neuroticism, Extraversion, Psychoticism and Lie scale. Each subscale has 12 items. The Lie Scale is used to determine the reliability of the responses given by the participants. Cronbach's alpha for psychoticism, extraversion and neuroticism scales were respectively 0.398, 0.746 and 0.808. The responses "Yes" and "No", scores of 1 and 0 are assigned respectively. There is a total of 18 negatively worded items, for which reverse scoring is used: 2, 6, 8, 12, 18, 20, 24, 26, 27, 28, 29, 33, 35, 37, 40, 41, 43 and 47. For these items, scores of 0 and 1 are assigned respectively for the responses of "No" and "Yes". The score range for each dimension is 0-12. The highest score in any of the three subscales of Neuroticism, Extraversion and Psychoticism indicate that the person is dominant in that personality trait.

2.3.1 Cognitive Failures

The Cognitive Failures Questionnaire (CFQ) has 25 items and yields a single score[2]. The Cronbach alpha was determined to be 0.904. A 5-point Likert scale, ranging from 0-Never to 4-Very often, is used to record responses. The range of scores is 0 to 100. Higher scores indicate more cognitive failures.

2.3.2 Mindfulness

The Five Facet Mindfulness Questionnaire (FFMQ) is a 39-item scale used to assess mindfulness[27]. The facets were also found to be moderately inter-correlated[28], and alpha coefficients ranged from 0.73 (non-reactivity) to 0.91 (describing), which sufficiently indicated internal consistency. Cronbach's alpha was determined to be 0.822. Participants are asked to answer on a five-point Likert scale, where 1 represents never or extremely rarely true and 5 represents very often or always true. Nineteen negatively phrased, reverse-scored items total. The scale goes from 39 to 195, where higher numbers correspond to more mindfulness.

3. RESULTS

The purpose of the current study was to investigate how mindfulness may mediate the association between personality traits and cognitive failures.

As seen in Table 1, the mean value of psychoticism is 3.49 (SD= 1.735), extraversion is 7.45 (SD= 2.860), neuroticism is 5.84 (SD= 3.279), mindfulness is 126.09 (SD= 15.838) and cognitive failures is 36.71 (SD= 14.867).

Table 1. Average (Mean) and standard deviation(SD) for the variables (N=419)

Variables	Average (Mean)	SD
Psychoticism	3.49	1.735
Extraversion	7.45	2.860
Neuroticism	5.84	3.279
Mindfulness	126.09	15.838
Cognitive failures	36.71	14.867

Table 2 depicts the Pearson product moment correlations between the variables of study. Neuroticism exhibits the strongest positive association with cognitive failure, meaning that people high on neuroticism tend to experience higher frequency of cognitive failures. This is in line with the previous literature[29-33]. Mindfulness has the strongest negative association with neuroticism, indicating that people with higher levels of mindfulness tend to have lower levels of neuroticism. This is in congruence with previous studies[34-38]. Cognitive failures and mindfulness also exhibit the strongest negative association, implying that people experiencing higher frequency of cognitive failures tend to have lower levels of mindfulness[24-25,39-41]. Psychoticism and cognitive failures were found to be significantly positively correlated, but a weak correlation ($r=0.144$, $p<0.001$). Result thus implied that people reporting higher levels of cognitive failures are likely to report higher levels of psychoticism as well, and vice versa. Significant correlation was also found between cognitive failures and psychoticism[30]. However, contrary to the present finding[42], no significant correlation between psychoticism and cognitive failures was found. The variations in the sample characteristics, notably the nationality, cultural differences, sample size and age, could be the cause of these discrepancies in the results.

Hierarchical linear regression was performed for evaluating how much the personality traits could explain the variation produced in cognitive failures. It was also explored as to how much more could mindfulness contribute to this variation. The Table 3 shows the summary of the output of the hierarchical linear regression. For the first block analysis, all the three predictor variables of psychoticism, extraversion and neuroticism were utilized. The results of the analysis revealed a statistically significant model labelled as Model 1 ($p<0.001$). The R^2 value of 0.208 associated with this regression model revealed that the three predictor variables of psychoticism, extraversion and neuroticism explained 20.8 % of the variance in cognitive failures with $F(3, 415)= 36.387$, $p<0.001$, while the rest 79.2 % of the variation may not be explained by these predictor variables. The findings showed that psychoticism positively predicted cognitive failures ($\beta= 0.164$, $p=0.001$), extraversion negatively predicted cognitive failures ($\beta= -0.098$, $p<0.05$) and neuroticism positively predicted cognitive failures ($\beta= 0.398$, $p<0.001$). A slightly different outcome was observed from the second block analysis.

For the second block analysis, mindfulness was also added as a predictor. The results of this analysis also revealed a statistically significant model. The R^2 value of 0.292 associated with this regression model, labelled as Model 2, revealed that the personality traits acting as predictors: psychoticism, extraversion and neuroticism, along with mindfulness significantly explained 29.2 % of the variance in cognitive failures, with $F (1, 414) = 49.111$, $p<0.001$. The findings revealed that the psychoticism positively predicted cognitive failures ($\beta= 0.143$, $p= 0.001$) and neuroticism also positively predicted cognitive failures ($\beta= 0.250$, $p<0.001$) along with mindfulness ($\beta= -0.355$, $p<0.001$), which negatively predicted cognitive failures. However, extraversion did not predict cognitive failures ($\beta= -0.046$, $p= 0.291$). The $\Delta R2$ value of 0.084 explained 8.4 % variance of Model 1 and Model 2 with $\Delta F (3, 414)= -12.724$, $p<0.001$. The regression weights for personality traits differed subsequently from Model 1 to Model 2: for psychoticism, it reduced from 0.164 to 0.143 ($p<0.05$), for extraversion, reduced from 0.235 to 0.226 ($p= 0.001$) and for neuroticism, reduced from 0.398 to 0.250 ($p<0.001$).

Hence, the results of hierarchical linear regression demonstrated that though personality traits could explain the variation produced in cognitive failures to a certain extent, including mindfulness as a predictor in this model could better account for the variation produced in cognitive failures, thereby accounting for hypothesis 1.

Table 2. Pearson product moment correlations

Variables	Psychoticism	Extraversion	Neuroticism	Mindfulness	Cognitive failure
Psychoticism	1				
Extraversion	0.035	1			
Neuroticism	-0.041	-0.254**	1		
Mindfulness	-0.039	0.266**	-0.477**	1	
Cognitive failures	0.144**	-0.193**	0.416**	-0.473**	1

Note: **p<0.001.

Table 3. Summary of hierarchical linear regression for the effect of mindfulness and personality traits on cognitive failures

Model	Variables	B	95% C.I.	SE B	B	R^2	ΔR^2
1	Constant	25.073**	[19.632, 30.513]	2.768		0.208	0.208**
	Extraversion	-0.511*	[-0.973, -0.050]	0.235	-0.098	0.037	0.037**
	Neuroticism	1.804**	[1.401, 2.207]	0.205	0.398	0.173	0.173**
	Psychoticism	1.407**	[0.671, 2.144]	0.375	0.164	0.021	0.021*
2	Constant	62.273**	[54.364, 80.182]	6.567		0.292	0.084**
	Psychoticism	1.227**	[.528, 1.926]	0.356	0.143	0.021	0.021*
	Extraversion	-0.239 (p= 0.291)	[-0.682, 0.205]	0.226	-0.046	0.037	0.037**
	Neuroticism	1.135**	[0.710, 1.560]	0.216	0.250	0.173	0.173**
	Mindfulness	-0.315**	[-0.403, -0.227]	0.045	-0.335	0.223	0.223**

Note: **p<0.001.

To have a deeper comprehension of how mindfulness affects the relationship between extraversion and neuroticism separately in relation to cognitive failures, Model 4 of PROCESS Macro by Andrew Hayes was used in association with SPSS, which aids in analysing simple mediation models with a single predictor variable, mediating variable and outcome variable. Two models were examined for the mediation analysis, the first one with extraversion and the second one with neuroticism as predictors of cognitive failures, with mindfulness as the mediator. Since statistically significant associations between psychoticism and cognitive failures were not found, mediation analysis was not conducted on that factor (Refer to Tables 5-9 for relevant values and Fig. 1 and 2 for mediation models).

According to the extraversion mediation model (Fig. 1), extraversion does not directly cause cognitive failure, but it does have an indirect effect through mindfulness, suggesting that mindfulness serves as a full mediator between extraversion and cognitive failures. Table 4 shows the direct and indirect impacts of extraversion on cognitive failures.

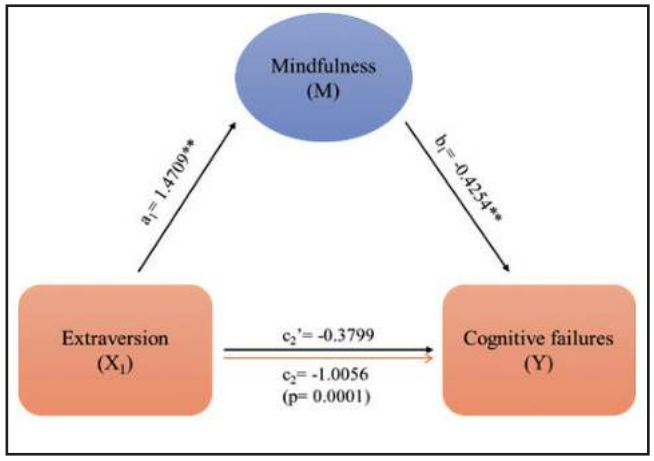

Figure 1. Mediation model for extraversion.

Note: **p<0.001. a_1= Direct effect of Extraversion on Mindfulness. b_1= Direct effect of Mindfulness on Cognitive Failures. C_2'= Direct effect of Extraversion on Cognitive Failures. C_2= Total effect of extraversion on Cognitive Failures.

To examine the direct effect of extraversion on mindfulness, extraversion was considered as the predictor variable and mindfulness as the outcome variable (Refer to Table 4). It was observed that extraversion was a predictor of mindfulness (b= 1.4709, s.e.= 0.2614, p< 0.001), therefore, extraversion was considered to have a direct effect on mindfulness within the path model. Mindfulness was also observed to be a significant predictor of cognitive failures (b= -0.4254, s.e.= 0.0419, p<0.001), by virtue of it having a direct effect on cognitive failures. Extraversion was not found to be a significant predictor of cognitive failure (b= -0.3799, s.e.= 0.2322, p= 0.1026), implying that extraversion did not have a direct effect on cognitive failures.

To calculate the indirect impact of extraversion on cognitive failures through mindfulness, extraversion is considered the predictor variable, cognitive failures the outcome, and mindfulness the mediator. Extraversion was found to have an unstandardised indirect effect on cognitive failures that was statistically significant [Effect= -0.6257, 95 % C.I. (-0.8811, -0.3984)]. Using bootstrap standard errors and confidence levels, the standardised indirect effect of extraversion on cognitive failures was examined for a sample size of 5000. Additionally, it was discovered that extraversion's fully standardised indirect effect on cognitive failures was statistically significant [Effect= -0.1204, 95 % C.I. (-0.1692, -0.0769)]. Therefore, extraversion uses mindfulness to indirectly affect cognitive failures.

Additionally, extraversion's direct impact on cognitive failure is coupled with its indirect impact through mindfulness, which was found to be -1.0056, to get the overall impact of extraversion on cognitive failures through mindfulness. Given that extraversion has no direct impact on cognitive failure but rather indirectly through mindfulness, it may be said that mindfulness acts as a complete mediator in the relationship between extraversion and cognitive failures.

Hence, pertaining to hypothesis 2a, it was found that mindfulness fully mediates the relationship between

Table 4. Mediation analysis for extraversion

Path	Direct effect of X_1 on M- (a_1)	Direct effect of M on Y- (b_1)	Direct effect of X_1 on Y (c'_1)	Total effect of X_1 on Y (c_1)
Extraversion(X_1)-> Mindfulness(M)-> Cognitive failures(Y)	1.4709**	-0.4254**	-0.3799 (p= 0.1026)	-1.0056 (p= 0.0001)

Note: **p<0.001.

extraversion and cognitive failures. This implies that the mechanism by which extraversion predicts cognitive failures can be completely explained by mindfulness. In other words, the effect of extraversion is completely transmitted to cognitive failures through mindfulness. People who are extraverted tend to be gregarious, expansive, lively, fun-oriented, interested in other people, and have a tendency to feel good about themselves.[43,44]. Two major characteristics of being mindful is being able to engage non-judgementally with both external and internal environments and being able to share and articulate/describe one's experiences with others, devoid of inhibitions. Hence, this can explain why extraversion may predict higher levels of mindfulness. Though significant but weak negative correlation was observed between extraversion and cognitive failure, extraversion could not be directly established as being the causal factor of cognitive failures, as shown by the path analysis in mediation.

The mediation model for neuroticism (Fig. 2) shows that neuroticism has both direct and indirect effect on cognitive failure (through mindfulness), which implies shows that the association between neuroticism and cognitive failures is partially mediated by mindfulness.. The direct and indirect effects of neuroticism on cognitive failures are depicted in Table 5. (Refer to tables 6, 7 and 8 for the direct effects, completely standardized indirect effects and total effects of extraversion and neuroticism).

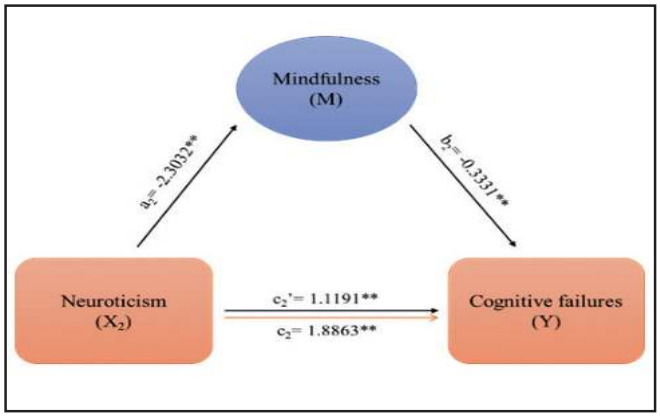

Figure 2. Mediation model for neuroticism.

Note: **p<0.001. a_2= Direct effect of Neuroticism on Mindfulness. b_2= Direct effect of Mindfulness on Cognitive Failures. c_2'= Direct effect of Neuroticism on Cognitive Failures. c_2= Total effect of Neuroticism on Cognitive Failures.

Table 5. Mediation analysis table for neuroticism

Path	Direct effect of X_2 on M- (a_2)	Direct effect of M on Y- (b_2)	Direct effect of X_2 on Y (c_2')	Total effect of X_2 on Y (c_2)
Neuroticism->Mindfulness->Cognitive failures	-2.3032**	-0.3331**	1.1191**	1.8863**

Note: **p<0.001.

Table 6. Direct effect of predictor on the outcome variable

Predictor variable	Effect	S.E.	t	p	LLCI	ULCI
Extra -version	-0.3799	0.2322	-1.6358	0.1026	-0.8363	0.0766
Neuro-ticism	1.1191	0.2160	5.1802	0.0000	0.6944	1.5473

Table 7. Completely standardized indirect effect of predictor on outcome variable, through mediator

Predictor variable	Effect	BootS.E.	BootLLCI	BootULCI
Extraversion	-0.1204	0.0233	-0.1692	-0.0769
Neuroticism	0.1692	0.0262	0.1198	0.2231

Table 8. Total effect of predictor on the outcome variable

Predictor variable	Effect	S.E.	t	p	LLCI	ULCI
Extra -version	-1.0056	0.2497	-4.0265	0.0001	-1.4965	-0.5147
Neuro-ticism	1.8863	0.2019	9.3424	0.0000	1.4894	2.2832

Neuroticism was found to have a direct effect on mindfulness within the path model (b= -2.2032, s.e.= 0.2079, p< 0.001). It was also observed that mindfulness (b= -0.3331, s.e.= 0.0447, p<0.001) was a significant predictor of cognitive failures. Hence, it was discovered that cognitive failures were directly impacted by mindfulness. Neuroticism is the predictor variable, cognitive failures

are the result, and mindfulness is the mediator variable in order to compute the indirect relationship between neuroticism and cognitive failures through mindfulness. The unstandardised indirect effect of neuroticism on cognitive failures was found to be statistically significant [Effect= 0.7672, 95 % C.I. (0.5362, 1.0229)]. The standardized indirect effect of neuroticism on cognitive failures was tested using bootstrap standard errors and confidence levels, for a sample size of 5000. The completely standardized indirect effect of neuroticism on cognitive failures was also found to be statistically significant [Effect= 0.1692, 95 % C.I. (0.1198, 0.2231)]. Hence, neuroticism has an indirect effect on cognitive failures via mindfulness.

Hence, pertaining to hypothesis 2b, it was found that mindfulness partially mediates the relationship between neuroticism and cognitive failures. Neuroticism emerges as one of the most commonly explored personality trait with cognitive failures. Nervousness, emotional instability, moodiness, tension, irritability, propensity to worry, anxiety, sadness, and anger are traits of people with high degrees of neuroticism[42,43].

4. DISCUSSION

This study aimed to investigate how mindfulness influences personality traits and cognitive failures. The relationship between extraversion and cognitive failures was fully mediated by mindfulness, while the relationship between neuroticism and cognitive failures was partially mediated by mindfulness.

Extraverted people are characterized as sociabile, expansive, lively, fun and interested in other people[43] along with a propensity to experience positive affect[44]. Two major characteristics of being mindful is being able to engage non-judgementally with both external and internal environments and being able to share and articulate/describe one's experiences with others, devoid of inhibitions. Hence, this can explain why extraversion may predict higher levels of mindfulness. Though a significant weak negative correlation was observed between extraversion and cognitive failure, extraversion could not be directly established as being the causal factor of cognitive failures, as shown by the path analysis in mediation.

However, it has been reported that people having higher levels of assertiveness, excitement-seeking and cheerfulness, meaning, those high on extraversion tend to be more inclined towards engaging in social situations that are complex, which in turn keep them involved with cognitively rich activities[45], that consequently support their cognitive functioning and guard them against cognitive failures[46]. Also, extraverted individuals may be biased in self-reporting cognitive failures, owing to their greater self-efficacy and positive evaluations they have about their lives[47].

Moreover, people who are disposed to be mindful are capable of "paying and maintaining attention to present-moment experiences with an open and non-judgemental attitude"[48], thereby enabling extraverts to accurately report the cognitive failures they experience in daily life. The result could also be explained by two-factor model theory of mindfulness[49]and the cognitive model of mindfulness[50] which explain that high mindfulness individuals are able to focus more and make the correct decisions as they are more open and have receptive attitude toward the feelings and experiences of the present.[51,52]. This explains the full mediation model for extraversion that has emerged through this study.

The present study also established that mindfulness acted as a partial mediator for the relationship between neuroticism and cognitive failures. Neuroticism emerges as one of the most commonly explored personality trait with cognitive failures. Individuals having high levels of neuroticism may be more likely to ruminate (repetitively think about the same issue), which may consequently distract them from the on-going behaviour and action[53]. It was also reported that with low level of mindfulness, neuroticism predicts increase in cognitive failures, whereas for average and high mindfulness, it predicts decrease in cognitive failure[18]. Similar impact of mindfulness (dispositional) was reported for executive functioning[54].

Furthermore, high neuroticism has been linked with poor sleep[55], leading to daytime sleepiness that may ultimately be instrumental in impairing mental processes, resulting in cognitive failures. In addition to this, people having higher levels of neuroticism may tend to be more critical of themselves and their cognitive functioning[56,57]. Moreover, it[58] also showed higher neuroticism to be linked with more real-time cognitive failures. This sufficiently explains why neuroticism is capable of predicting cognitive failures.

Mindfulness, however, endows a person with clarity of mind, which is helpful in enhancing self-regulation and mental health[59]. Hence, it can be safely concluded that mindfulness is capable of acting as a protective factor against the consequences of negative emotional reactivity that is characteristic of neuroticism[60]. These concepts can also be explained by the two-factor model of mindfulness[48] and the cognitive model of mindfulness[50]. This clarifies how neuroticism indirectly contributes to cognitive deficits through mindfulness.

5. CONCLUSIONS

Mindfulness based interventions can be implemented to help elevate trait/dispositional mindfulness, ultimately mediating the interaction between personality traits and cognitive failures. Organizations and academicians could hence incorporate mindfulness in their general working culture and curriculum respectively as an effective way to reduce the incidences of cognitive failures, consequently leading to potential improvements in performance in various aspects along with reductions in stress and accidents.

The present study will help pioneer the understanding of the relationships among personality traits, mindfulness and cognitive failures in Indian settings. People in a variety of settings have been interested in minimizing accidents

and harms, and consequently improving performance in all domains of life. Since cognitive failure is one of the reasons that can lead to potentially fatal accidents, it has become crucial to understand its nuances in connection with individual differences, fundamentally, with personality. Through our study, it was found that mindfulness could fully mediate the relationship between extraversion and cognitive failures, while it could only partially mediate the relationship between neuroticism and cognitive failures.

This study has attempted to further the understanding of the interactions between personality traits and mindfulness, and their consequent impact on cognitive failures in the adult Indian population. The potentially negative consequences of cognitive failures may be reduced by improving mindfulness, which is a trainable and improvable construct, as being mindful has a multitude of benefits in a variety of situations, from reducing workplace accidents to enhancement in general wellbeing[61]. Reduced susceptibility to minor lapses of attention can benefit people from all walks of life.

The relationship between the personality trait of psychoticism and mindfulness needs to be explored further. Future research on similar lines may be conducted with a larger sample size. Moreover, racially diverse samples may be used to explore if the findings of the present study can be replicated. Replicating this study on more specific samples may aid in understanding the nuances of such relationships on those samples. Further, the outcomes of mindfulness training should be explored as a way of reducing cognitive failures, which can be of potential benefit to the community at large.

Despite uncovering important insights, this research study has its own limitations. First, survey research design of the study may be unable to rule out extraneous variables. Second, the relatively small sample size may not be capable of representing the adult population in India. Third, the sample was racially homogeneous. Fourth, since all the measures used were self-report questionnaires, there is a high chance of subjective bias emerging in the responses of the participants. Fifth, despite the usage of well validated measures, survey fatigue may have influenced the responses of the participants since the survey form was quite lengthy.

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Harnessing Plants for Ciprofloxacin Pollution: A Green Approach

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ABSTRACT

Antibiotic pollution is a major environmental risk that is contributing to the emergence of antibiotic resistance and threatening public health. This review addresses the sources of antibiotic contamination, focusing on ciprofloxacin, a commonly used human & veterinary antibiotic. Some of the main sources are pharmaceutical manufacturing, agricultural runoff, hospital discharges, and improper medication disposal, which lead to a significant cause of increase in environmental antibiotic levels. Ciprofloxacin has been found in various environmental matrices, such as soil, water, and sediments, with concentrations varying at both Indian and global levels. The review discusses phytoremediation as an effective, green approach for eliminating antibiotics from the environment. Various plant species have shown the capacity to absorb and degrade ciprofloxacin, decreasing the amount of the antibiotic in the surrounding environment. The goal of this review is to evaluate phytoremediation as a potential mitigation method for ciprofloxacin pollution and to comprehend the extent of this pollution.

Keywords: Antibiotic; Antibiotic resistance; Ciprofloxacin; Phytoremediation

1. INTRODUCTION

Soil is one of the major components of the environment essential for supporting life. The continuous release of harmful chemicals, fertilizers, industrial and pharmaceutical waste has led to the depletion of the soil ecosystem. Lately, the usage of antibiotics for treating infectious and harmful diseases in humans, animals, and livestock has increased globally. According to Van Boeckel[1], *et al.* (2015) by the year 2030, the consumption of antibiotics will increase by 67 % in some of the major countries including India. Depending on the class of antibiotics, around 40-90 % of the administered antibiotics reach the environment. In today's healthcare, antibiotics are frequently used agents for treatment. In just over a century, antibiotics have profoundly changed modern medicine and extended human longevity by 23 years[1]. Antibiotics are compounds intended to target bacteria with the purpose of preventing and treating bacterial illnesses. They hinder bacterial cell division or alter essential cellular functions within the cell, which ultimately leads to the bacterial cell's destruction. According to their in vitro effects on bacteria, antimicrobial drugs are divided into two broad categories: bacteriostatic and bactericidal. Bactericidal antibiotics such as Aminoglycosides, Beta-lactams, Fluoroquinolones, and glycopeptides, etc. "kill" the bacteria whereas bacteriostatic antibiotics such as Tetracyclines, Sulfonamides, Macrolides, and Oxazolidinones, etc. "prevents the growth" of bacteria[2].

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Antibiotic pollution in the environment has become a significant issue as a result of its potential threat to humans, plants, animals, and microorganisms. It is one of the most harmful pollutants to the environment. The constant release of even a small amount of antibiotics into the soil and water could result in the emergence of resistance in the various living organisms[3]. Antibiotics present in the environment at low concentrations also accumulate in human bodies through long-term exposure. The majority of antibiotics are excreted in their unmetabolised form because they are not completely metabolised by the body. They then reach soil and water through manure, sewage wastewater, and biosolids. Antibiotic contamination causes the depletion of microbial communities which includes various bacteria responsible for essential ecological functions[4]. It also results in changes in the water and soil properties such as pH, nutrients, and moisture. Increasing antibiotic pollution leads to various toxic impacts on humans, plants, and animals. The main causes of antibiotic resistance are typically attributed to drug overuse and misuse. Antibiotic pollution can have detrimental effects on human health and contribute to the emerging global health risk of Antimicrobial Resistance (AMR). Antibiotic resistance develops in bacteria, viruses, fungi, and parasites, making antibiotics less effective or useless in treating diseases. Fig. 1, represents that the extensive usage of antibiotics has led to a selection pressure that has aided in the evolution of isolates with resistance[5]. Over time, many infectious organisms have

developed resistance to the medications meant to kill them, decreasing the effectiveness of the agents. Antimicrobial drugs that were once utilized to treat pathogens are no longer effective against an increasing number of them[6].

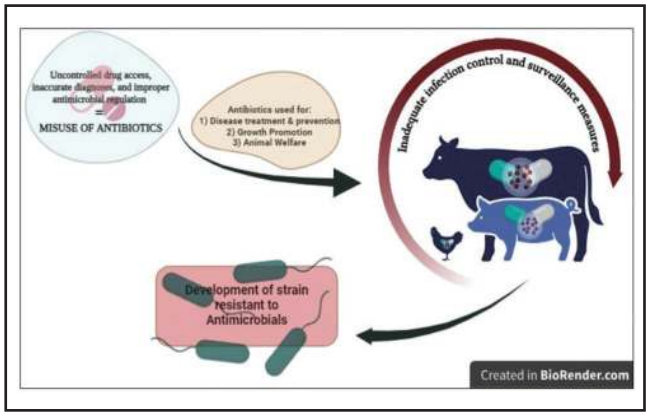

Figure 1. Development of antibiotic resistance strain[5,6].

Keeping in mind the negative impacts of antibiotic contamination, their removal from the environment has become very essential. The most befitting treatments compared to physicochemical and conventional methods are bioremediation and phytoremediation. These are considered economical and sustainable approaches for effectively reducing environmental contaminants by utilizing microbes and plants respectively[7]. Due to the expensive cost of physicochemical methods, alternative biological technologies are becoming more and more popular, especially those that rely on the remediation potential of plants and microorganisms[7].These remediation techniques don't require any expensive equipment, skills, labour, and management therefore, the best approach to eliminate antibiotics from the environment is using antibiotic-remediating plants. This method is eco-friendly, can be applied easily on large contaminated fields, and improves soil properties[8].

This review focuses on the significant issue of antibiotic pollution, with particular attention to the commonly used fluoroquinolone antibiotic i.e. Ciprofloxacin. To prevent the spread of antibiotic resistance and manage environmental antibiotic pollutants, the present review attempts to emphasize the role of phytoremediation strategy. The bioremediation and phytoremediation techniques, highlighting the potential of phytoremediation to mitigate ciprofloxacin pollution were also analysed.

2. ANTIBIOTIC POLLUTION SOURCES IN THE ENVIRONMENT

Since the antibiotics were discovered, there has been an exponential increase in demand for and production of them globally. As a result, of increased production, they are also continuously released into the environment as metabolites and degradation products. Agricultural runoff, anthropogenic effluents, livestock farms, slaughterhouses, landfill runoff, and Wastewater Treatment Plants (WWTPs) are all potential sources of antibiotic pollution, as shown in Fig. 2. The illustration also depicts the closed-loop entry of antibiotics into various ecosystems and their eventual return to the source[9]. The main source of antibiotic pollution in the environment is considered to be WWTPs. Antibiotic residue-containing wastewater is produced by pharmaceutical industries, residential areas, livestock farms, and hospitals which ultimately ends up in WWTPs. However, over-the-counter use and improper disposal of expired antibiotics from residential areas, hospitals, and industries also contribute to WWTP systems[9]. The application of manure containing antibiotic residues in agricultural fields contaminates the soil, which in turn contaminates surface water and groundwater. Antibiotic residues are also present in the sludge produced by WWTPs, which is either dumped in landfills or used as a soil conditioning agent in fields. There are various sources of antibiotic pollution, some of the major sources are discussed below.

2.1 Residential Wastewater

Residential wastewater, also known as domestic or household wastewater, is widely acknowledged as a major contributor to antibiotic contamination[10]. The disposal of unused antibiotics and the excretion of unmetabolised antibiotics by humans are two ways that antibiotics enter the wastewater system. No proper wastewater treatment can lead to the introduction of antibiotics into natural water bodies and further to the soil and food chain. Such kind of environmental contamination can cause a significant risk to public health, as it may reduce the effectiveness of antibiotics in treating infections by promoting the spread of antibiotic-resistant bacteria.

2.2 Hospital Wastewater

Conventional wastewater treatment methods are generally incapable of adequately treating the wastewater discharged by hospitals, which increases the risk of release of antibiotics into the ecosystem. Due to the widespread use of antimicrobial drugs in healthcare institutions, hospital wastewater is a significant source of antibiotic pollution. Together with other medications, disinfectants, and pathogens, this wastewater has significant concentrations of antibiotics[11]. These substances are flushed into the sewage system through cleaning procedures, laboratory operations, and patient care. Hospital wastewater has a higher percentage of antibiotics than normal wastewater[10].

2.3 Slaughterhouse Wastewater Discharge

Antibiotics are commonly utilised in livestock farming to enhance growth and prevent disease, which causes an accumulation of antibiotics in animal tissues. Wastewater produced during animal slaughter may contain residues of antibiotics that are subsequently introduced into the environment. Antibiotics can remain in treated water because traditional wastewater treatment plants are usually not built to efficiently eliminate pharmaceutical compounds[10]. As a result, these contaminants may find their way into water bodies, endangering aquatic life by disturbing ecosystems and encouraging the growth of bacteria resistant to antibiotics.

2.4 Agricultural Effluent

The key cause of antibiotic pollution in agriculture is the extensive usage of antibiotics in livestock farming. Antibiotics are frequently given to livestock animals, such as cattle, pigs, and poultry, to cure infections, promote growth, and prevent diseases[12]. Although animals can metabolize most antibiotics, a significant amount is excreted in the undigested form in their urine and feces. The use of antibiotic-treated animal manure as fertilizer on fields increases the risk of antibiotics entering into the soil or water bodies through irrigation or rainfall.

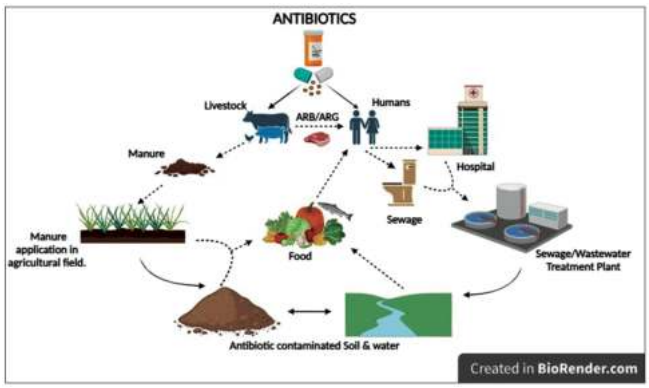

Figure 2. Reoresentation of the various source of antibiotic contamination in environment[10,11].

3. CIPROFLOXACIN & ITS CONCENTRATION IN WATER: GLOBAL & INDIAN SCENARIO

Lately, the concerns regarding the ecological effects of antibiotics have grown immensely as they inhibit major functions like nutrient regeneration and the carbon and nitrogen cycles in the environment[13]. Fluoroquinolone (FQ) antibiotics are currently among the most extensively utilised antibiotics in medical practice. It has been estimated that the antibiotic market is valued at around 2.2 billion USD, which is 16.8 % of the total pharmaceutical sales in India[14]. FQs account for up to 25 % of all antibiotic usage in India, where the greatest proportion of antibiotic intake is 3.75 specified daily dose/1000 population daily[15]. For human administration, the three main FQs—ciprofloxacin, norfloxacin, and ofloxacin—are authorised. The most widely utilised & effective fluoroquinolone is ciprofloxacin. Ciprofloxacin had the largest global value at 1.3 billion dollars, followed by ofloxacin at 900 million dollars[16].

Based on their pharmacokinetic profile and spectrum of activity, fluoroquinolones are categorised into four generations[17]. Table 1 provides an overview of different generations of Fluoroquinolone[17].Ciprofloxacin is a second-generation fluoroquinolone. Its molecular formula is [$C_{17}H_{18}FN_3O_3$] and has a 331.3 g/mol molecular weight[18]. It is a widely used human & veterinary drug to treat various bacterial infections and is also used as a growth promoter for livestock. Ciprofloxacin shows activity against many gram-positive and negative bacteria. It acts by blocking bacterial DNA synthesis, resulting in bacterial cell death. It functions via two mechanisms; either by inhibiting the activity of major target DNA gyrase or secondary target DNA Topoisomerase IV[19]. It easily enters into the environment through sewage, treated water from wastewater treatment plants, manure applied to agricultural fields, livestock farming, and leachate from landfills. It has been observed that around 50-60 % of CIP gets excreted in unmetabolised form through human urine and 15-25 % through faeces[13]. Antibiotics like ciprofloxacin are known to be very stable and persistent within the environment.

Table 1. Overview of the different fluoroqunolone antibiotic generations

Generation	Examples	Spectrum of activity	Clinical use
Ist	Nalidixic Acid Cinoxacin	Effective against Gram-negative bacteria	Restricted usage because of resistance development & narrow range. Commonly used for urinary tract infections.
IInd	(a) Norfloxacin Ciprofloxacin (b) Ofloxacin Lomefloxacin	Active againstall Gram-negative bacteriaas well as some Gram-positive bacteria	Effective enough to combat a broader spectrum of illnesses, such as skin, gastrointestinal, and respiratory infections.
IIIrd	Levofloxacin Sparfloxacin Gatifloxacin	Increased efficacy against Gram-positive bacteria & improved coverage against Gram-negative bacteria	Used to treat sinusitis, bronchitis, pneumonia, and other respiratory diseases.
IVth	Moxifloxacin Trovafloxacin Gemifloxacin	Effective against certain anaerobic bacteria as well as a broad spectrum of Gram-positive and Gram-negative bacteria.	Effective for severe respiratory & intra-abdominal infections. Moxifloxacin showed increased activity against anaerobes.

Antibiotic contamination of the environment has been reported in several Asian countries, including China, India, and Japan, in recent years. Fig.3 summarises the ciprofloxacin concentrations found in various sources across several nations[20]. Antibiotic concentrations in sewage sludge are greater than those in sediments. Due to their potent adsorption onto sewage sludge.

It has been reported that the concentration of ciprofloxacin in Indian wastewater treatment plants (WTP) is about 40 times higher in comparison to countries such as North America, Australia, Europe, and Asia[21]. The concentration of CIP in the outlet at Okhla WTP, Delhi was 2.5-15 times higher as compared to the outlets in Australia, Italy, U.S.A., Canada, and China wastewater treatment plants[21]. In a cross-sectional study performed in a hospital, seven antibiotics were detected on the outskirts of Ujjain, an Indian city, out of which Ciprofloxacin was one of the antibiotics used most frequently in the hospital. Also, the residue levels of CIP in wastewater were highest[22]. Table 2 summarizes the various reported ciprofloxacin levels in water and wastewater.

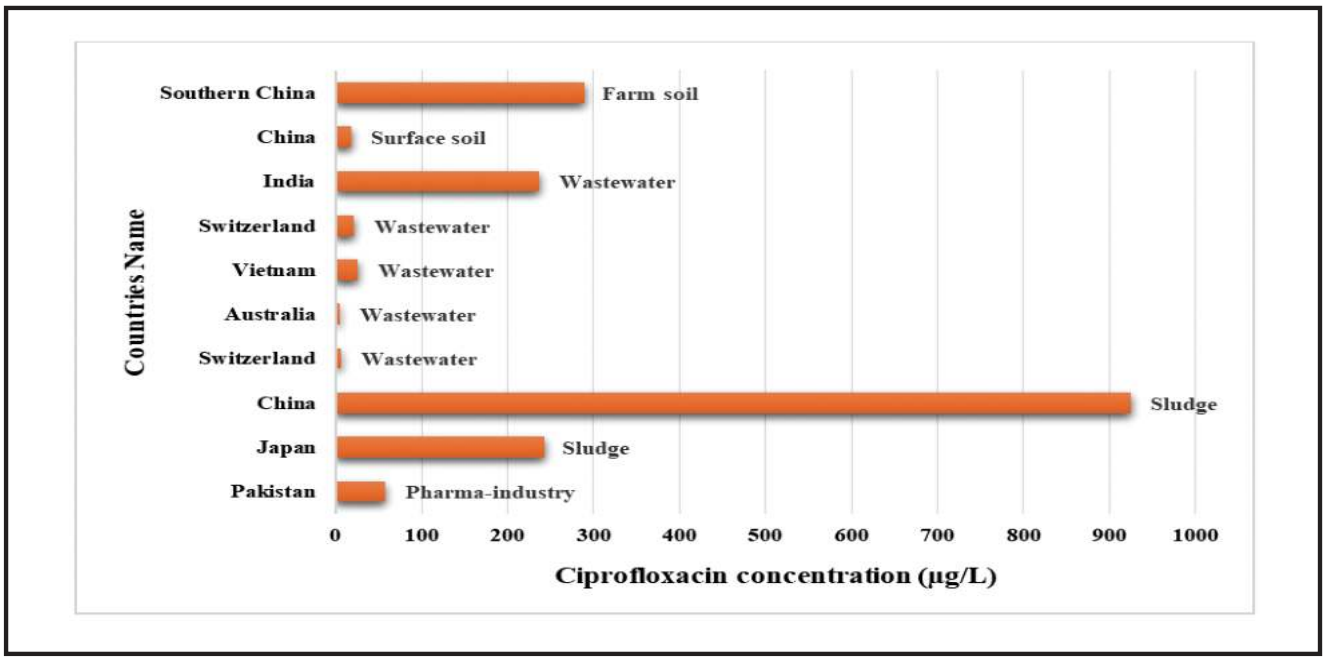

Figure 3. Illustration of the ciprofloxacin concentration detected in different countries from various sources.

Table 2. Reported concentration of ciprofloxacin in river, lakes, wastewater/sewage treatment plants, surface water, and wells of India

Site of sample collection	Year of study	Detected concentration of CIP (ng/L)	Reference
Chennai City & Suburbs; Tamil Nadu: Sewage treatment plants, Groundwater	2022	20.48	23
Nagpur; Maharashtra: Sewage treatment plants, Nag & Pili River	2014	5,080-28,230	24
Hyderabad; Patancheru Enviro. Tech Ltd. Plant:	2009	25,00,000-65,00,000	25
Lakes		>5000-10,000	
Surface & groundwater		14,000	
Wells		12,000-11,00,000	
Isakavagu-Nakkavagu River		1,40,00,000	
Effluent treatment plant		2,80,00,000- 3,10,00,000	
Patancheru Wastewater Treatment Plant	2007		26
Ujjain; Madhya Pradesh: Hospital Effluent-R.D. Gardi Medical College	2010	2,36,600	22
Delhi;	2013		27
Yamuna River (winter):		1440-1190	
Okhla Sewage Treatment Plant:			
Influent		20,100	
Effluent		8000	

4. BIOREMEDIATION

Worldwide attention is focused on the rise of antibiotic-contaminated soil. As it easily enters into the natural ecosystem and has become a matter of concern for animals, plants, microbes, and humans. Therefore, an effective remediation method for antibiotic-polluted soil is the need of an hour. Various conventional physicochemical methods are not practiced due to their damaging effect on soil, high expenditure, and labour cost. Technologies like chemical oxidation, electro-kinetic remediation, extracted washing, and nanomaterial remediation cause vast changes in the pH and moisture content of soil[28]. It has been observed that in comparison to physicochemical technologies, biological technologies such as bioremediation and phytoremediation are more reliable, practiced, promising, low cost, and environment friendly. Bioremediation utilizes naturally occurring microbes, fungi, and plants to break down or detoxify the harmful environmental pollutants into less toxic forms, that pose a threatto human health and the environment[29].

4.1 Types of Bioremediation Techniques

Based on the application site, bioremediation techniques can be classified as either

4.1.1 In-situ Bioremediation

In-situ bioremediation is the process of detoxifying, degrading, or eliminating the toxins by improving the metabolic properties of the microbial community present in the contaminated site[30]. The most efficient bioremediation technique is in situ because it needs less mechanical work to remove toxins that are spreading and stop pollutants from traveling or being carried away to other treatment facilities[31].

4.1.2 Ex-situ Bioremediation

Ex-situ bioremediation, as an alternative, treats pollutants in the samples that have been excavated. Due to the significant work involved in removing contaminated soil from the site and moving it offsite, this categorisation is not widely used. To perform Ex-situ remediation, the proper soil oxygen, moisture, and nutrient conditions must be introduced offsite[29,31].

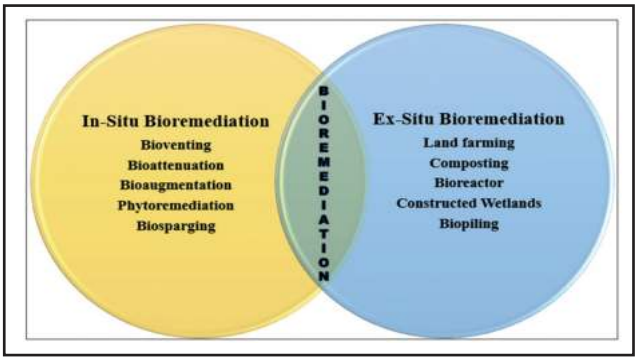

Figure 4. Different types of techniques used for In-situ & Ex-situ bioremediation methods[31,32].

Fig. 4, illustrates the various types of techniques under each category of bioremediation. Each one of these methods has specific applications, advantages, and disadvantages. Out of all the bioremediation techniques, phytoremediation is a widely utilised approach due to its economic and environmental benefits. In the following discussion, we have explored its type, pros and cons.

5. PHYTOREMEDIATION: A GREEN SOLUTION FOR ANTIBIOTIC POLLUTION

Phytoremediation is an eco-friendly technique that includes the usage of associated microbes and plants. It is one of the promising technologies that utilize the plant's capability to remediate harmful contaminants like antibiotics from the soil. It's a much cheaper and more sustainable method. It is a substitute technique that can be utilised instead of mechanical conventional clean-up methods, which frequently have large capital and energy requirements. This technique uses plants to degrade, remove, extract, or sequester the antibiotics[33]. Various plants, such as (*Ocimum basilicum*, *Brassica juncea*, *Lactuca sativa*, and *Zea mays*), are commonly used to remove various contaminants from the environment[34]. The pros and cons of this method, are listed in Table 3.

Table 3. Pros and cons of phytoremediation[35,36]

PROS	CONS
It is an eco-friendly process and not expensive.	Only restricted to shallow soil and water.
It can preserve the soil and water ecosystem	It requires a long period for the complete elimination of pollutants from the environment.
Suitable for contamination levels that are medium to low.	Cannot be applied in developed areas where there is less availability of land.
It is an in-situ remediation process	Not applicable in environment that have high pollutant concentrations.
Helps in improving the soil quality and promotes productivity	Relies on soil characteristics and environmental factors.

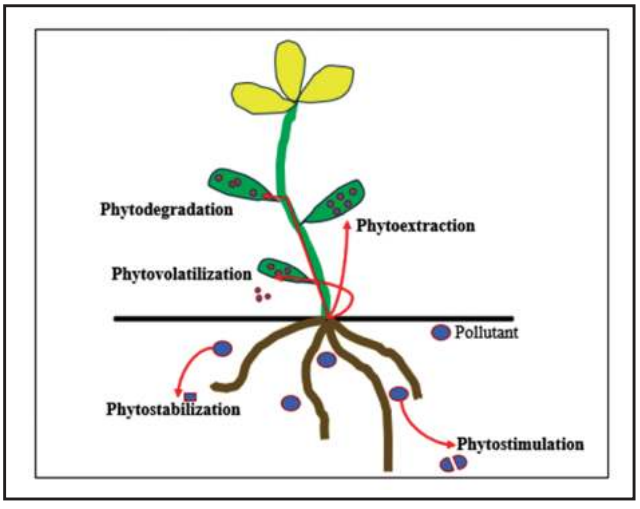

Figure 5. Illustrates the different types of phytoremediation techniques[29].

There are five categories of phytoremediation techniques; phytostabilization, phytovolatilization, phytodegradation, phytostimulation, and phytoextraction as shown in Fig. 5.

5.1 Phytoextraction

It is the method by which plants store toxins in their roots, aboveground shoots, or leaves. This method gathers minimal concentrations of pollutants from a large area.

5.2 Phytodegradation

It explains how organic contaminants are absorbed from sediments, soil, or water and subsequently change into a form that is less mobile, less harmful, or more stable.

5.3 Phytostabilisation

Plants use this approach to lower the movement and migration of pollutants in the soil.

5.4 Phytovolatilisation

Pollutants are absorbed by plants and released as volatile chemicals into the atmosphere via their leaves.

5.5 Phytostimulation

Natural compounds released by plant roots encourage the growth of microbes, which in turn break down pollutants in the soil.

6. PHYTOREMEDIATION OF CIPROFLOXACIN USING VARIOUS REMEDIATOR PLANTS

Phytoremediation of antibiotics can be achieved by using different species of antibiotic- remediating plants like Daucus carota L. (carrot), Brassica juncea (L.) Czern (mustard),Lactuca sativa L. (lettuce), Vigna radiata L. (Mung bean), Cicer arietinum L. (Chickpea), and Cymbopogon citratus (lemon grass) etc. Phytoremediation helps in restoring the natural habitat, removing the contaminants without transporting soil to another place[35]. Various investigations and studies have been performed to analyze the remediation potential of different plant species. The use of phytoremediation has demonstrated the enormous potential for ciprofloxacin removal from the environment. Table 4 summarizes reports where phytoremediation technology is used for treating ciprofloxacin contaminated environment.

The results of these investigations suggest that ciprofloxacin concentrations can be efficiently reduced by plant-based remediation techniques utilizing microbial interactions in the rhizosphere, root uptake, and accumulation in plant tissues. Comparative studies of several plant species reveal the variation in their effectiveness based on variables such as ciprofloxacin concentration, surrounding circumstances, and the plant's natural detoxifying capacity.

7. CONCLUSION & FUTURE PROSPECTS

The production and widespread usage of fluoroquinolones in human and animal medicine has become a matter of great concern. Out of all antibiotics, ciprofloxacin is now becoming the number one antibiotic contaminant, as it is commonly found in the environment. Ciprofloxacin pollution causes toxic impacts on plants, and the emergence of antibiotic resistance among microbes, humans, and animals. According to several studies, a high level of ciprofloxacin is detected in wastewater/sewage treatment plants and rivers of India. There are several ways through which ciprofloxacin enters the environment; the main pathway through which it does so is when the antibiotic is not totally absorbed by the gut of humans or animals and hence gets eliminated into the surrounding environment. Due to the slow rate of fluoroquinolone biodegradation, residues and metabolites are easily detectable in soil and water sources. Antibiotics are susceptible to biological degradation processes by microorganisms and plants present in soil and water. It is recommended that an

Table 4. Summary of research studies using various phyto-remediator plants for the removal of cipfofloxacin

Selected Plant	CIP concentration	Results	References
*Eichhornia crassipe*s-Grown in Hydroponic conditions	10 ,100, and 1000 µg/L	84.38%, 67.95%, and 62.36 % of total CIP accumulated in roots within 7 days of theexperiment. After 14 days in response to CIP stress Superoxide dismutase & catalase activities showed an increase. After 21 days decrease in chlorophyll content was seen. Leaves turned white and shriveled.	37
Cicer arietinum-Grown in Hydroponic conditions	15-25 mg/l	After 7 days, a remediation rate of 60% was observed for CIP. A decrease inthe length ofthe root and shoot of the plant due to toxicity.	38
Acrostichum aureum L. & *Rhizophora apiculate Blume*- Pot Experiment	10 mg/kg	After 14 days, a remediation rate of 65% was observed for CIP. After 21 days, no effect on growth rate; CIP found mainly in root tissue samples	39
Brassica juncea L. Czern- Grown in Hydroponic conditions	5-10 mg/l	After completion of 12 days remediation rate of 50%-63.2% was observed for CIP. Whereas at higher concentrations of 20 to 60 mg/l; decrease in percentage remediation, root & shoot length.	40

environment-friendly approach for making an antibiotic free environment like phytoremediation is used. The plant's root and shoot act as a hyperaccumulator and eliminate the contaminants from the natural environment. A prospect for enhancing the effectiveness of phytoremediation involves the synergistic use of microbes and plants. Integrating the complementary abilities of both biological systems, microbial communities, and plant-based remediation can improve the degradation and uptake of antibiotics. Antibiotics can be broken down by microorganisms into less toxic substances that plants can absorb and further detoxify. This combination strategy may lead to the more thorough and effective removal of antibiotics from contaminated areas. It can be stated that the plant-microbe synergy is the upcoming future in the field of biological remediation methods. Although it has theoretically well-known aspects, research studies for the remediation of antibiotics are needed for a better understanding and implementation of this synergistic technology.

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She has contributed to the study conception & design, analysis, thorough checking of the manuscript, reviewed and approved the final version of the manuscript.

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A Systemic Review of Beta-Caryophyllene

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ABSTRACT

A naturally occurring sesquiterpene known as Beta-Caryophyllene (BCP) is present in many essential oils, spices, and plants including black pepper, cloves, and cannabis. A thorough assessment of the pharmacological traits, biological functions and therapeutic potential of BCP is given in this article. Due to its singular capacity to interact with the CB2 receptor of the endocannabinoid system without having any psychoactive effects, BCP has drawn a lot of attention. Consequently, it has shown a variety of intriguing medicinal applications.

Keywords: Beta-caryophyllene; Phytoconstituents; Biological activities

NOMENCLATURE

BCP : Beta-caryophyllene
CB2 : Cannabinoid receptor type 2
THC : Tetrahydrocannabinol

1. INTRODUCTION

BCP, also known as beta-caryophyllene or -caryophyllene, is a fascinating and adaptable sesquiterpene molecule that is widely present in many different plants, especially in the essential oils of numerous herbs and spices. Its distinctive chemical structure, which also adds to its extraordinary features and possible health advantages, makes it unique[1]. This detailed introduction will develop deep into the world of beta-caryophyllene and examine its history, molecular makeup, and wide range of uses. Beta-caryophyllene is a naturally occurring hydrocarbon that is widely distributed in the natural environment. It is a botanical treasure since it is notably present in plants like hops, cannabis, black pepper, cloves, and peppermint. Its importance in the Flavors and fragrance industries has grown as a result of the distinctive spicy, woody, and peppery scent it emits[2]. Notably, it is essential in determining the distinctive aroma of black pepper, which affects both the flavour and the appeal of the aroma. The distinctive structure of beta-caryophyllene, which consists of a bicyclic ring system with a smaller cyclobutene ring bonded with a bigger cyclohexane ring, distinguishes it from other compounds which is shown in (fig. 1). The secret to its wide range of biological activities and interactions with the human body is in its unique configuration[3].

The extraordinary capacity of beta-caryophyllene to preferentially interact with the endocannabinoid system, particularly the CB2 receptors, is what sets it apart from other compounds. Due to its possible medicinal uses, this particular characteristic has generated a great deal of interest in the substance[4].

Numerous promising health benefits of beta-caryophyllene have been discovered via extensive investigation. It has antioxidant, analgesic (pain-relieving), and anti-inflammatory effects, making it a hot topic of research in the area of alternative medicine. Additionally, its interaction with CB2 receptors suggests that it has a role in controlling immunological responses and may be useful in treating diseases like arthritis, autoimmune disorders, and neurodegenerative problems that are associated with inflammation[5].What sets beta-caryophyllene apart from other chemicals is its unique structure, a bicyclic ring system with a larger cyclohexane ring connected to a smaller cyclobutene ring[6].

In summary, beta-caryophyllene is a remarkable sesquiterpene with an excellent chemical structure and a wide range of prospective applications in the pharmaceutical, flavouring, and fragrance sectors. Scientific research is constantly being sparked by its special relationship with the endocannabinoid system and its favourable therapeutic properties, highlighting its promise as an important natural substance for improving human health and general well-being[7]. Beta-caryophyllene may become an essential resource for holistic health and the larger scientific community as this field of study develops.

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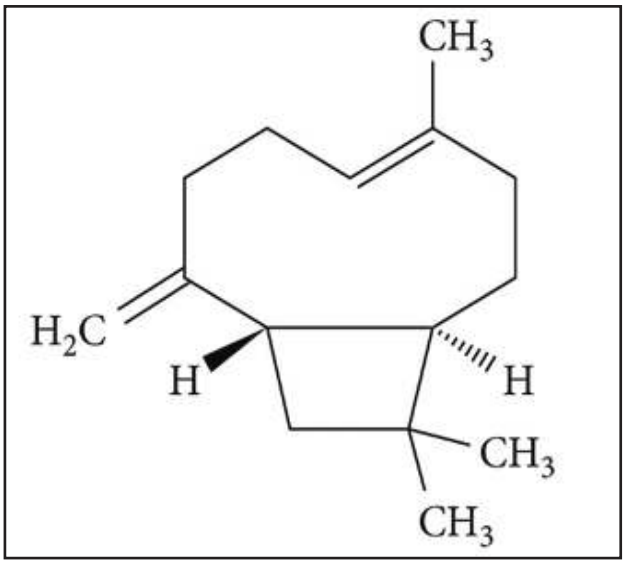

Figure 1. Structure of beta-caryophyllene chemical name :4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene.

2. PLANT DESCRIPTION

2.1 Chemical Structure

- A bicyclic sesquiterpene, beta-caryophyllene is also referred to as -caryophyllene or BCP. The hydrocarbon has a unique tricyclic structure and the chemical formula C15H24.

2.2 Aroma and Flavour

- A spicy, woodsy, peppery scent with undertones of clove and black pepper characterizes beta-caryophyllene. It has a flavour that is similar to these spicy undertones[8].

2.3 Natural Sources

- Numerous plant species essential oils include beta-caryophyllene. A few noteworthy sources include:
- Cannabis sativa, also known as marijuana or hemp, contains this important terpene.
- Black pepper (Piper nigrum): The high beta-caryophyllene content in black pepper makes it well-known.
- Cloves (Syzygium aromaticum): The beta-caryophyllene content of clove essential oil is substantial.
- Copaiba (Copaifera spp.): Copaiba trees produce an oleoresin that is high in beta-caryophyllene.
- This terpene is also present in the essential oil of oregano (Origanum vulgare)[9,10].

2.4 Medicinal and Therapeutic Uses

A lot of interest has been paid to beta-caryophyllene because of its possible therapeutic uses.

2.5 Some of Its Reported Properties Include

- Anti-inflammatory: Studies have shown that beta-caryophyllene has anti-inflammatory characteristics, which may be useful for illnesses that include inflammation.
- Analgesic (pain-relieving): It might aid in easing discomfort and suffering.
- As an antioxidant, it can aid in preventing oxidative stress and cell damage.
- Potentially reducing anxiety and depression: Some research points to a possible interaction between beta-caryophyllene and the endocannabinoid system, which is involved in mood regulation[11,12].

2.6 Interaction with the Endocannabinoid System

- Because it selectively activates the CB2 (cannabinoid receptor type 2) receptor, one of the receptors in the endocannabinoid system, beta-caryophyllene stands out among terpenes. Its possible therapeutic benefits are influenced by this interaction[13].

2.7 Common Uses

- Because of its distinctive perfume and potential therapeutic effects, beta-caryophyllene is frequently used in aromatherapy, where it is added to essential oil mixes.
- It can also be found in a variety of cosmetic and personal care items, especially those that are touted as having calming or anti-inflammatory effects[14].

3. PHYTOCONSTITUENTS

The primary components of the phytoconstituents that make up beta-caryophyllene are carbon, hydrogen, and occasionally oxygen atoms organised in a certain molecular structure. Beta-caryophyllene has a molecular weight of around 204.35 grams per mole and the chemical formula C15H24. The cyclobutane ring, a bicyclic ring structure, is fused to a bigger cyclohexane ring in its structural formula[15].

Some key points about the phytoconstituents of beta-caryophyllene include

- **Terpenes:** These are fragrant molecules that are present in a variety of plants, and beta-caryophyllene is one form of terpene. Many plants and essential oils have distinctive aromas and fragrances that are attributed to terpenes.
- **Hydrocarbons:** Beta-caryophyllene is a hydrocarbon since it only has carbon and hydrogen atoms. Its stability and function as a volatile aromatic molecule are both facilitated by the chemical structure's simplicity[16].
- Alpha-caryophyllene and beta-caryophyllene are the two isomers of beta-caryophyllene. The most prevalent and biologically active type is beta.
- **Cannabimimetic Activity:** The interaction of beta-caryophyllene with the endocannabinoid system is one of its special characteristics. It functions as a selective agonist of the CB2 cannabinoid receptor, which is mostly present in peripheral tissues and immune cells. The modulation of the endocannabinoid system by beta-caryophyllene may have therapeutic uses, according to this relationship[17].

- **Pharmaceutical Qualities:** Numerous research have shown that beta-caryophyllene possesses anti-inflammatory, analgesic (pain-relieving), and antioxidant activities. Its potential application in treating ailments like pain, inflammation, and anxiety is also being researched.
- **Food &Flavouring:** Spices and herbs used to flavour food and beverages frequently contain beta-caryophyllene. Foods like black pepper and cloves benefit from the spiciness and pepperiness in their flavour characteristics[18].

4. BIOLOGICAL ACTIVITIES

4.1 Cannabinoid Receptor Interaction

Although beta-caryophyllene is a naturally occurring terpene present in many plants, including cannabis, it is not a cannabinoid in and of itself. Beta-caryophyllene interacts with the endocannabinoid system in a special way despite not being a cannabinoid. The human body's intricate endocannabinoid system regulates a number of physiological functions, including the perception of pain, mood, appetite, and inflammation[19].

The CB2 receptor, one of the endocannabinoid system's components, is where beta-caryophyllene interacts with the body the most. CB1 and CB2 receptors are the two primary types found in the endocannabinoid system. While CB2 receptors are more prevalent in immune cells and peripheral organs, particularly in regions linked to inflammation, CB1 receptors are largely present in the brain and central nervous system.

Because it can only bind to CB2 receptors, beta-caryophyllene stands out among terpenes as a CB2 receptor agonist. It can cause the body to react in a number of ways when it binds to these receptors, including the control of immunological response and the lowering of inflammation. Beta-caryophyllene has potential therapeutic benefits due to its interaction with CB2 receptors, especially in the treatment of diseases marked by inflammation and immune system dysregulation[20].

Beta-caryophyllene can activate CB2 receptors, but it does not have the same psychoactive effects as cannabinoids like THC, which primarily act on CB1 receptors in the brain. This is a crucial distinction to make. It is an intriguing substance for further research in the fields of natural medicine and cannabis-based therapies because it instead offers possible therapeutic benefits without mind-altering effects[21].

4.2 Anti-Inflammatory Effects

A natural substance called beta-caryophyllene is present in many plants, including cannabis, black pepper, and cloves. It is well known for having possible anti-inflammatory properties. The body's endocannabinoid system, specifically

The CB2 receptors, which are mostly present in immune cells and tissues linked to inflammation, are influenced by beta-caryophyllene[22].

Beta-caryophyllene can affect the immune system and lessen inflammation when it binds to CB2 receptors. Through this interaction, inflammation-related symptoms including pain and swelling could be lessened.

While studies on the anti-inflammatory effects of beta-caryophyllene have shown promise, further research is necessary to completely comprehend these effects and their potential therapeutic uses. Additionally, the source and concentration of the chemical can affect beta-caryophyllene's efficacy[23].

4.3 Analgesic (Pain Relief)

A naturally occurring substance called beta-caryophyllene is present in many different plants, including cannabis and several spices like black pepper and cloves. It is well known for having possible pain-relieving properties, principally as a result of its interactions with the endocannabinoid system of the body.

The control of pain and inflammation in the body is largely dependent on the endocannabinoid system. Because it selectively activates the CB2 receptors in the endocannabinoid system, beta-caryophyllene stands out from other terpenes (aromatic chemicals present in plants). The majority of peripheral tissues, particularly those connected to the immune system, contain CB2 receptors.

Beta-caryophyllene may lessen pain and inflammation when it binds to CB2 receptors. Effects on inflammation: Inflammation, which can be a source of discomfort in illnesses like arthritis or muscular injuries, may be reduced by beta-caryophyllene[24].

Analgesic (pain-relieving) properties: Beta-caryophyllene may help reduce pain signals delivered to the brain and provide relief from a variety of pain via regulating CB2 receptors.

Effects on neuroprotection: Some studies indicate that beta-caryophyllene may have protective effects on the nervous system, which may be advantageous for diseases that cause nerve pain or injury[25].

4.4 Neuroprotective Properties

Natural sesquiterpenes like beta-caryophyllene, which can be found in plants like cannabis and black pepper, are thought to have neuroprotective qualities. Through a number of ways, this substance has shown the potential to shield brain and nervous system nerve cells. First, it reduces chronic inflammation that can damage neurons by interacting with the CB2 cannabinoid receptor, which is largely located in immune cells. Beta-caryophyllene also functions as an antioxidant, scavenging damaging free radicals that result in oxidative stress and cellular damage, two factors that might contribute to neurodegenerative disorders. Although continuing studies are highlighting its potential, particularly in diseases like Alzheimer's, Parkinson's, and multiple sclerosis, more research is required to fully comprehend its mechanisms and possible therapeutic uses. Whether beta-caryophyllene is consumed through diet or supplements, its effectiveness may differ depending on the source and route of

delivery. Prior to contemplating its usage for potential neuroprotection, as with any natural substance or supplement, it is imperative to speak with a healthcare practitioner[26,27].

4.5 Antioxidant Activity

A natural substance called beta-caryophyllene is present in many plants, including cloves, black pepper, and cannabis. It has drawn attention because of its potent antioxidant qualities, which are essential for shielding the body from oxidative stress and free radical damage. By scavenging dangerous free radicals from the body, beta-caryophyllene functions as a powerful antioxidant that lowers the risk of a number of chronic diseases and aging-related ailments. Its capacity to activate the CB2 cannabinoid receptor, a component of the endocannabinoid system, which controls a number of physiological processes, including immune response and inflammation, is credited with its antioxidant action. Beta-caryophyllene is a promising natural substance with potential health advantages since it modulates this receptor, which can help fight oxidative stress and inflammation. Beta-caryophyllene's antioxidant abilities are still being studied, and there is considerable interest in its possible therapeutic uses in situations like cancer prevention, cardiovascular disease, and neurological diseases. To completely comprehend its mechanics and realize its therapeutic potential, more research is necessary[28,29,30].

4.6 Anti-Anxiety and Anti-Depressant Effects

A natural substance called beta-caryophyllene is present in many plants, including cloves, black pepper, and cannabis. Its possible anti-anxiety and anti-depressant benefits have attracted a lot of interest. The CB2 receptors, which are mostly present in the immune system and peripheral tissues, are thought to be the target of beta-caryophyllene's interactions with the endocannabinoid system. Beta-caryophyllene may have anti-inflammatory and neuroprotective effects through binding to these receptors, which can indirectly support its anti-anxiety and anti-depressant qualities. The release of neurotransmitters like serotonin and dopamine, both of which are essential for controlling mood and emotions, may also be modulated. Potentially, this modulation will lessen anxiety and depressive symptoms. Beta-caryophyllene's natural origin and encouraging preliminary results make it an appealing possibility for future study into complementary therapies for anxiety and depression, even if further studies are required to fully understand its mechanisms and therapeutic potential. The effects of beta-caryophyllene, however, may differ from person to person, therefore people should seek the advice of medical specialists before using it as a treatment for mental health issues[31,32].

4.7 Gastro protective Effects

The natural substance beta-caryophyllene, which is present in many plants including cloves, black pepper, and cannabis, is renowned for its possible gastroprotective properties. Due to its capacity to shield the gastrointestinal tract from harm and inflammation, this sesquiterpene has drawn interest. According to research, beta-caryophyllene preferentially targets the CB2 receptors, which are prevalent in the gut, in its interactions with the endocannabinoid system.

Beta-caryophyllene is a potential therapeutic agent for a variety of gastrointestinal conditions such gastritis, ulcers, and inflammatory bowel disease because activating these receptors can reduce oxidative stress and inflammation in the digestive tract. Additionally, by preventing the release of pro-inflammatory chemicals and encouraging tissue repair, beta-caryophyllene's anti-inflammatory and antioxidant capabilities may contribute to its gastroprotective benefits. Although more investigation is required to fully comprehend the scope of beta-caryophyllene's gastroprotective advantages and its ideal therapeutic uses, it shows promise as a natural substance that might support digestive health and possibly supplement current treatments for gastrointestinal conditions[33,34].

4.8 Antimicrobial Activity

Natural sesquiterpene beta-caryophyllene is mostly found in cannabis and spices like black pepper, cloves, and rosemary. According to research, beta-caryophyllene is antimicrobial, which means it has the power to prevent the development and spread of germs including bacteria, fungi, and even some viruses. Its distinctive molecular structure, which enables it to engage with certain cellular targets in these diseases, is credited with its antibacterial efficacy. In order to kill or inhibit these microorganisms, beta-caryophyllene works by interfering with the fungal cell wall and compromising the integrity of the cell membrane in bacteria. Additionally, it might have potential uses in the creation of new antimicrobial drugs, offering an alternate strategy for dealing with infections that are resistant to treatment and supporting continuing efforts to address the global problem of antimicrobial resistance. To completely comprehend the scope of beta-caryophyllene's antibacterial activities and its potential therapeutic benefits, more study is necessary[35].

5. CONCLUSION

A prospective participant in the field of natural medicines is beta-caryophyllene, a naturally occurring chemical that may be found in a variety of plants but is notably prevalent in cannabis and spices. The scientific community is becoming more and more interested in it because of its special qualities, which include selective binding to CB2 receptors and a wide variety of possible health advantages. Beta-caryophyllene may potentially play a significant role in the development of novel and organic treatments for a range of health ailments as research continues to uncover its secrets.

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A Solitary *Plexiform Neurofibroma* Mimicking *Parotid Tumor*: A Rare Case Report

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ABSTRACT

Our case report describes an uncommon occurrence of a solitary plexiform neurofibroma in the salivary gland, which is generally associated with neurofibromatosis-1 (NF-1) but, in this case, arose independently. A 6-year-old girl presented with a painless, progressively enlarging mass in the right parotid region over a span of five years. Histopathological analysis revealed a variety of cellular elements, such as Schwann cells, perineurial cells, axons, and fibroblasts, with S100 immunohistochemistry confirming the diagnosis. This case highlights the importance of identifying solitary plexiform neurofibromas in the salivary gland, even without NF-1, and stresses the need for prompt diagnosis and treatment for optimal management.

Keywords: Head and neck-cancer; Plexiform neurofibroma; Parotid malignancy; Neurofibromatosis type 1

NOMENCLATURE

PN : Plexiform neurofibroma
IHC : Immunohistochemistry

1. INTRODUCTION

Neural-origin tumors in salivary glands are extremely rare, making up only 0.401 % of all salivary gland tumors. Plexiform Neurofibromas (PN), benign tumors from Schwann cells, are rarely found in salivary glands. They are more commonly observed in regions such as the ocular area, neck, back, and inguinal zones. These tumors usually present as single or multiple lesions and are often linked to neurofibromatosis-1 (NF-1).Due to their diffuse and infiltrative growth pattern that affects multiple nerve bundles and adjacent tissues, the complete surgical removal of plexiform neurofibromas is notably challenging [2].

Although PN is a rare manifestation of NF-1 and is pathognomonic of this condition, its isolated occurrence is even less common[3]. Here, we describe a case of a PN presenting as a parotid swelling in a 6-year-old female, who showed no other features of NF-1.

2. CASE REPORT

A 6-year-old female presented to the ENT OPD with swelling in the right infraauricular region, slowing growing over the course of 5 years. Onexamination, a right-sided infraauricular swelling measuring 7x 8x 5 cm was observed.

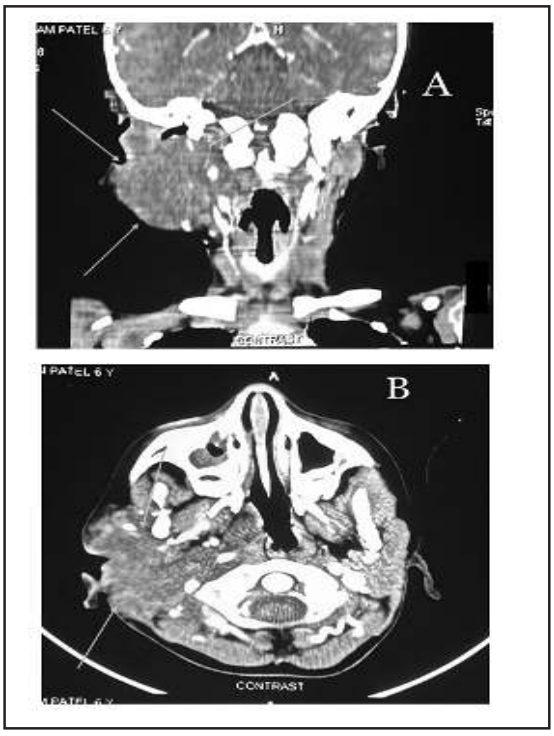

Figure 1. Contrast enhanced computed tomography scan coronal (A) and axial (B) cut - ill-defined heterogeneously enhancing mass arising in the Rparotid gland without any bony involvement or intracranial extension, anteriorly extending in the mastercator space; posteriorly till the right stemocleidomastoid; lateral to right external audiotory canal; medially reaching till the right parapharyngeal space.(as depicted via the arrows in yellow).

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The swelling was non-tender, Rubbery in consistency, and the surrounding skin appeared non adherent with no scars or sinuses. There was no family history of neurofibromatosis or any other genetic condition. The CECT scan demonstrated heterogeneously enhancing mass lesion, measuring 7 x 8 x 6.1 cm, with distinct margins originating within the right parotid gland, involving both the superficial and deep lobes without any intracranial extension or bony involvement (Fig. 1).

Fine-needle aspiration was inconclusive. After informed consent was obtained, the patient underwent a right total parotidectomy. Intraoperatively, a well-defined, pinkish-purple mass was found closely adhering to the lower trunk of the facial nerve. The mass was carefully dissected and removed with all necessary precautions.(Fig. 2).

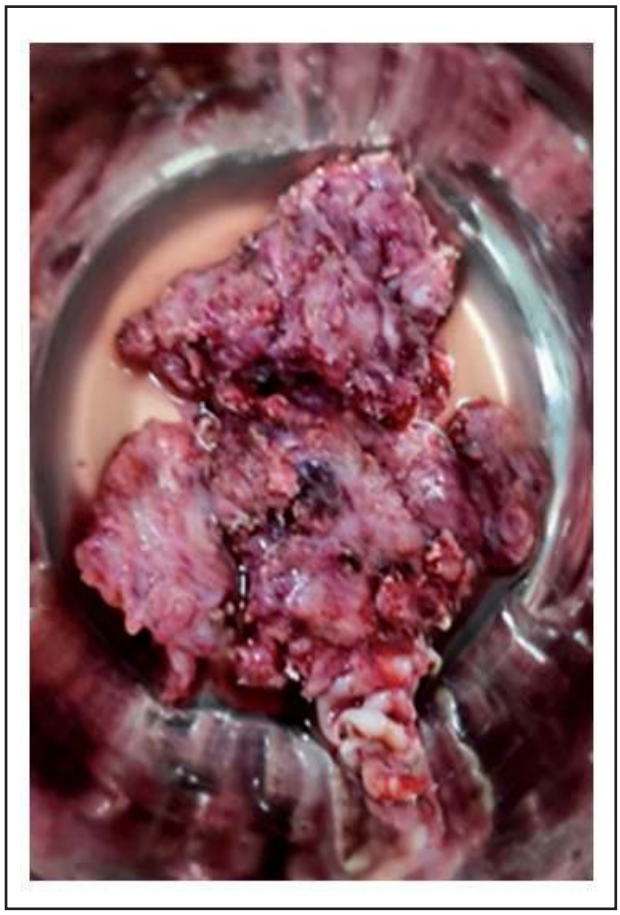

Figure 2. Intraoperative specimen following total parotidectomy.

Gross examination revealed a reddish brown-colored, firm mass measuring 7 × 8 × 6 cm with irregular, tumor nodules within which convoluted nerve bundles are present. Histopathological analysis at low magnification revealed multiple thickened nerve bundles interspersed with normal serous salivary gland tissue. Higher resolution and magnification revealed convoluted nodules showing peripheral spindle shaped cells with wavy comma shaped nuclei and eosinophilic cytoplasm and show a central myxoid stroma with collagen fibrils and palisading nerve fibres (Fig. 3).

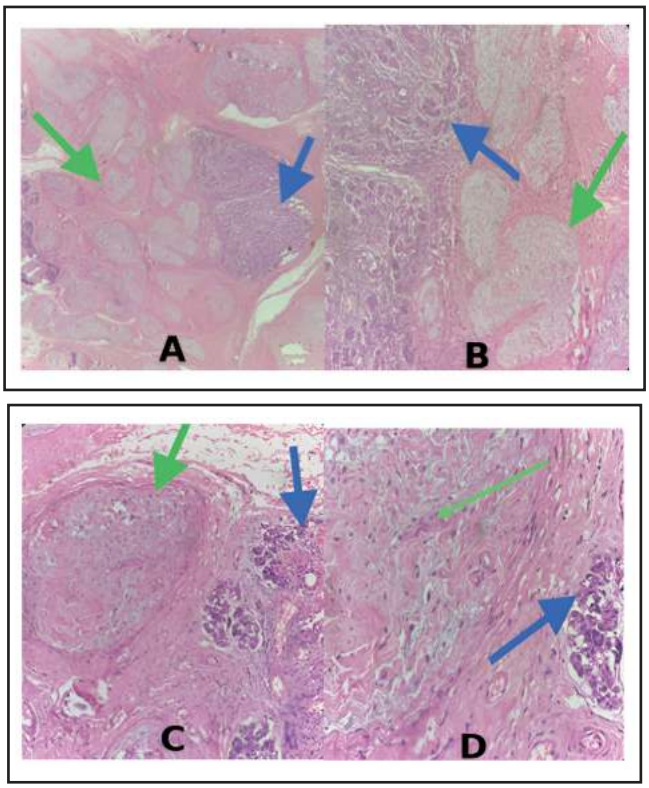

Figure 3. Haematoxylin and eosin-stainedslides, scanner view (A) (4x magnification) show multiple nodules (green arrow) of varying sizes and are seen enclosing lobules of salivary gland (blue arrow) tissue, a lymph node and within peripheral adipose tissue. (B) At 10x magnification showing better delineation of nerve bundles arranged in plexiform pattern (green arrow) surrounded by normal salivary glands(blue arrow). (C) at 20x magnification and (D) at 40x magnification these nodules show peripheral spindle shaped cells with wavy comma shaped nuclei eosinophilic cytoplasm and show central myxoid stroma with collagen fibrils and palisading nerve fibres (green arrow). salivary gland (blue arrow)shows lymphoplasmacytic infiltrates.

S100 immunostaining revealed both nuclear and cytoplasmic positivity, confirming the tumor's neural origin. Consequently, the diagnosis of PN was confirmed through histopathological examination, including IHC(immunohistochemistry).

Following surgery, patient had a smooth recovery and was discharged in good condition, with only mild marginal mandibular nerve palsy. The patient was monitored for six months post-surgery, with no signs of recurrence.

3. DISCUSSION

Neurofibromas are benign nerve tumors that can appear as solitary or multiple growths, occurring sporadically or with neurofibromatosis types I or II. The WHO classifies them as grade I tumors, either dermal, affecting a single nerve, or plexiform, involving multiple nerve bundles[3].

PN account for 15 % of benign mesenchymal tumors and 11 % of nonepithelial salivary gland tumors. They typically grow slowly, infiltrate surrounding tissues,

and are generally painless[5]. Clinical symptoms vary based on the tumor's location and can include pain and neurological deficits. Plexiform neurofibromas in the parotid gland are extremely rare, with benign tumors like pleomorphic adenomas being more frequently encountered in this region[5].

As a significant feature of neurofibromatosis 1 syndrome, plexiform neurofibromas require careful monitoring for specific clinical signs indicative of the condition. Histopathologically, plexiform neurofibromas are characterised by a diffuse tubular enlargement of nerve fascicles, hypocellularity, and a matrix containing Schwann cells and other components. Imaging techniques such as computed tomography can assist in diagnosing these tumors, which appear as non-encapsulated, tortuous nodular growths along nerve branches[6-7].

Surgical excision is the primary treatment for plexiform neurofibromas. Recurrence rates are approximately 20 % after complete resection and up to 44 % following subtotal removal[8]. It is crucial to monitor for signs of malignant transformation, such as rapid growth or changes in consistency, as there is a 2–5 % risk of malignancy in plexiform neurofibromas. Histopathological examination, including IHC for S100, is vital for confirming the diagnosis. Understanding the clinical and pathological characteristics of plexiform neurofibromas is essential for effective management and ensuring optimal patient outcomes[9,10,11].

4. CONCLUSION

In conclusion, the presence of plexiform neurofibromas in atypical locations such as the salivary glands underscores the importance of thorough evaluation and consideration of less common aetiologies in cases of isolated glandular swellings. The association of these tumours with neurofibromatosis type 1 (NF-1) emphasizes the need for comprehensive genetic investigations in affected individuals. Early detection and vigilant monitoring are crucial in patients with NF-1-associated PN, as they face an elevated mortality risk. Long-term follow-up is essential to track disease progression, detect recurrences, and identify potential malignant transformations. Maintaining a proactive surveillance approach enables healthcare providers to deliver personalized care, optimize outcomes, and ensure timely interventions for individuals with plexiform neurofibromas.

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She coordinated closely with the other authors to ensure the manuscript's integrity and quality.

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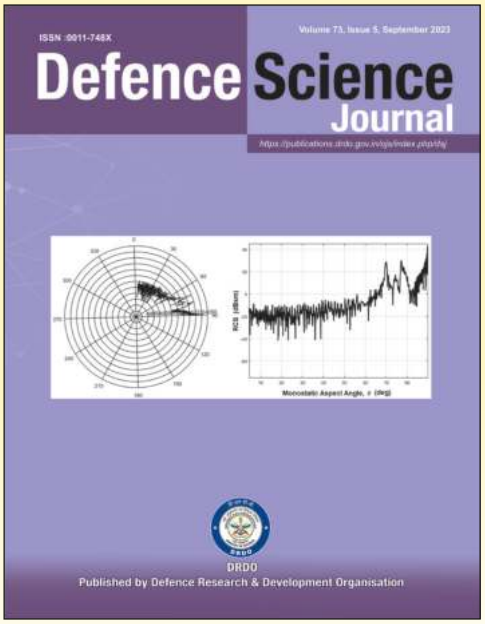

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2. Wang, Z.J.; Birch, J.M. & Dickinson, M.H. Unsteady forces and flows in low Reynolds number hovering flight: Two-dimensional computations vs robotic wing experiment. *J. Experimental Biol.,* 2004, **207**(3), 449-60. doi: 10.1242/jeb.00739 [Accessed on 17 November 2007]. The electronic sources should include the URL and date of access.

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Chapter from a Book
4. Bodony, D.J. &Lele, S.K. Applications and results for large-eddy simulations for acoustics: Far-field jet acoustics. *In* LES for eddy acoustics, edited by C. Wagner, T. Huttl& P. Sagaut. Cambridge University Press, Cambridge, England, UK, 2005. pp. 289-310. doi: 10.1017/CBO9780511546143.008
5. Lokesha, B.N. Advanced avionics and electronic warfare system for fighter aircraft. *In* DRDO Technology Spectrum. Defence Research and Development Organisation, Ministry of Defence, India, 2008. pp. 10-26.

Conference Paper
6. Ekstein, J.; Freitag, E.; Hirsch, C. &Sattelmayer, T. Experimental study on the role of entropy diffusion waves in low-frequency oscillations for a diffusion burner. *In* Proceedings of the ASME Turbo Expo 2004: Power for Land, Sea, and Air, ASME, Fairfield, NJ, 2004. doi:10.1115/GT2004-54163

Report
7. Savage, S.J. Defence applications of nanocomposite materials. FOI-SwedeshDefence Research Agency, User Report No. FOI-R-1524-SE. December 2004.

Patent
8. Man, T.Y.; Leung, C.Y.; Leung, K.N.; Mok, P.K.T. & Chan, M. Single-transister-conrol low-dropout regulator. US patent 7285952, 23 October 2007.

Standard
9. International Organisation for Standardisation. Document Management–Electronic document file format for long-term preservation–Part1: Use of PDF 1.4 (PDF/A-I). ISO 19005-1:2005, Geneva, Switzerland. ISO, 2005.

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10. De Roek, W. Hybrid methodologies for the computational aeroacoustic analysis of confined, subsonic flows. Katholieke University, Leuven, Belgium, 2007. (PhD Thesis).

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