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Editorial

The PG and UG Department of Botany, Bethune College, Kolkata successfully organized a two-day National Level seminar in collaboration with the Government of West Bengal on 13th and 14th November, 2025. The seminar entitled 'Unravelling the mysteries of Plant Science' aimed at scientific interaction among researchers across various disciplines of plant science and dissemination of scientific knowledge to students. We are pleased to present this special issue of the Heritage journal featuring 15 full length research and review articles from among the several oral and poster presentations made at the seminar. The studies published in this edition stand as a testimonial to the academic pursuits undertaken at different colleges, universities and research institutes.

The articles featured herein include approaches for agricultural advancement such as application of plant growth promoting rhizobacteria (PGPR) through nanoencapsulation, seed biopriming with PGPR, management of seed borne mycoflora and salinity resistance. Articles focusing on medicinal botany includes plant derived anti-microbial peptides, ethno-pharmacological significance of mangrove associated species, neutraceuticals based on plant enzymes mentioned in Ayurveda and usage of *Saraca asoca* in modern healthcare. Ecological and taxonomic studies include effect of climate on wood anatomy, morphometric analysis of plant trichome diversity and species survey at Sunderbans. Interdisciplinary studies focus on neuro-transmitter receptors of humans and plants and astro-botanical documentation.

The editorial team remains committed to maintaining high standards of publication ethics and peer review. The publication of this volume has been possible due to the encouragement of Dr. Rajyasri Roy, Principal, Bethune college; members of the Heritage subcommittee and faculty members of our department. We sincerely acknowledge the efforts put in by the authors, reviewers, publisher and editorial team in maintaining the quality of the issue. We extend our gratitude to our readers and supporters for continuous association with the journal, and hope that they will be immensely benefitted by the articles compiled in this special issue. We further look forward to their valuable feedback and future submission in this journal.

With best regards,

December 2025

Kolkata

Dr. Sritama Mukherjee

Dr. Smita Ray

[Guest Editors for the Special Volume of Heritage]

Contents

1. Plant-Derived Antimicrobial Peptides as Novel Therapeutic Agents against Biofilm-Associated Infections and Antimicrobial Resistance
– *Agnish Roy and Subhendu Bandyopadhyay* 9 – 18

2. Impact of Salinity Stress on *Vigna radiata* (L.) Wilczek (Leguminosae) : Studying different parameters
– *Biswajit Mukherjee and Kunal Sen* 19 – 31

3. A review on Ethnobotanical and Pharmacological properties of a Widespread Mangrove associate species: *Acanthus ilicifolius* Linn. (Acanthaceae)
– *Antara Saha and Seemanti Ghosh* 32 – 42

4. Nano-Encapsulation of PGPR: Advancing Molecular Insight and Enhancing Biofertilizer and Biopesticide Efficacy
– *Satabdi Mallick and Amit Ghosh* 43 – 54

5. Seed-Borne Mycoflora and Eco-Friendly Management Using Medicinal Plant Extracts: A Comprehensive Review
– *Puspita Karmokar and Sritama Mukherjee* 55 – 64

6. Stress Tolerance Mechanisms in *Sorghum bicolor* (L.) Moench (Poaceae) under Abiotic Stress Conditions: A Comprehensive Review
– *Saheli Ghosh and Sritama Mukherjee* 65 – 72

7. Investigating Similarities between Human and Plant through the Light of Chemical Characterizations of Neurotransmission Receptors
– *Subhadip Chakraborty and Antara Sengupta Sen* 73 – 80

8. Ancient Catalysts in Modern Plates: Integrating Ayurvedic Plant Enzymes in Food Biotechnology
– *Tanisa Ghosh, Joydeep Paul and Subhendu Bandyopadhyay* 81 – 91

9. The Mythological significance of *Saraca asoca* (Roxb.) Willd.: An Insight into its traditional folklore usage to modern Healthcare
– *Selima Khatun, Dipan Adhikari, Sourajit Roy and Kashfa Ummiya* 92 – 99

10. Seed Biopriming with Plant Growth Promoting Rhizobacteria
– *Umesh Laha and Amit Ghosh* 100 – 111

11. Decoding Wood Anatomical Trade-offs Underpinning Climate Adaptation in Phylogenetically Distant Mangroves of the Indian Sundarbans
– *Tanmay Santra, Subrata Mondal and Saikat Naskar* 112 – 124

12. Plant species diversity and their different utilities in Sunderban area of West Bengal
– *Sayan Das, Anmwesha Mondal and Subhradip De* 125 – 136

13. Nature's Resilience: A Multi-Locational Study of Pollution-Driven Alterations in the Pharmacological Potential of *Amaranthus viridis*
– *Jyoti Kumari Das, Nitika P. Mishra, Swati Thapa and Saswati Laha* 137 – 152

14. Navagraha Vatika And Nakshatra Vatika : Introducing Astro-ethnobotany Through Plant–planet Correlations, Sacred Garden Conservation, And Traditional Ecological Knowledge
– *Sakshi Mishra and Saswati Laha* 153 – 164

15. Morphometric Attributes of Plant Trichome Diversity of Bethune College And Immediate Surroundings
– *Tanisha Malik, Torsha Bhowmik and Debjani Sinha Ray* 165 – 192

Plant-derived antimicrobial peptides as novel therapeutic agents against biofilm-associated infections and antimicrobial resistance

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Abstract:

In the 21st century, one of the most prominent health issues is Antimicrobial resistance (AMR), with estimates going as high as 10 million deaths per year by 2050. AMR occurs when pathogenic microorganisms become resistant to therapeutic agents, which enables them to survive and successfully multiply within the host by various resistance mechanisms. One of these is the formation of biofilms. In biofilms, microorganisms are enveloped within a self-produced extracellular polymeric substance matrix, which consists mainly of polysaccharides, supplemented by proteins, extracellular DNA, and other polymers. The three-dimensional matrix allows microbial adhesion to biotic and abiotic surfaces, protects the pathogens against most environmental stresses, including desiccation, and drastically reduces their susceptibility to antibiotics and antifungal agents. In addition to this, biofilms enhance horizontal gene transfer, enabling the rapid diffusion of resistance genes. The issues of especially difficult treatment and connection with increased patient morbidity and mortality, prolonged hospitalisation, frequent failures in treatment, and a heavy economic burden in the form of chronic infections and those associated with devices are all being attributed to biofilm-associated infections. These kinds of consequences, which are increasing day by day calls for the development of alternative, more effective therapeutic strategies.

Antimicrobial peptides (AMPs), possessing a molecular weight of 2 to 10 kDa, have been of noticeable attention in recent days due to the fact that they are potential therapeutic candidates owing to their broad-spectrum activity, quick mechanism of action, and reduced chance of resistance development compared to others. This review focuses on antimicrobial peptides derived from five distinct plant sources, highlighting their antibio film properties, proposed mechanisms of action, and potential role as novel therapeutics in combating AMR and biofilm-associated infections.

Keywords:

Antimicrobial Resistance (AMR), plant-derived antimicrobial peptides, novel antibiofilm agents, antibiofilm activity

Introduction

Antimicrobial Resistance (AMR) has become a global health concern in the 21st century. An increasing number of pathogens have and continue to develop resistance against one or multiple antimicrobial agents. Treatments that were once effective in addressing infectious diseases are becoming ineffective with the growing number of these multidrug-resistant pathogens, leading to prolonged hospitalisations, increased medical expenses and higher rates of mortality (Jiang et al., 2024). If left unchecked, estimations suggest that the spread of AMR will cause more than 10 million deaths annually and economic losses of 100 trillion dollars by 2050 (Despotovic et al., 2023). It is, of course, human behaviour that plays a substantial role in this specific phenomenon. The abuse, misuse, or overuse of antibiotic consumption, along with human tourist flow, are considered to be at the roots of the rise of bacterial species displaying multidrug resistance (Baran et al., 2023). Thus comes an increasing demand for novel therapeutic strategies to address the AMR-associated infections (Kok et al., 2022).

Bacteria are often present in biofilms, which makes infections difficult to treat as resistance to antimicrobials and biocidals increases (Pai et al., 2023). Encased in an extracellular polymeric (EPS) substance matrix, which can be derived from the host or self-produced, these complex communities of microorganisms, known as biofilms, can attach to both biotic (living) and/or abiotic (non-living) surfaces. According to the National Institutes of Health and the United States Centers for Disease Control and Prevention, 80% of microbial infections, of which 60% are nosocomial infections, are caused by biofilms (Price and Skaar, 2025). Microbial biofilms often infect medically implanted devices, including orthopaedic implants, pacemakers, catheters, contact lenses, vascular grafts, and many other devices. Microorganisms present in biofilms are often seen to have altered genetic and phenotypic expressions, resistance to antibiotics, and even producing virulence associated factors. As depicted in Fig. 1, the formation of biofilm involves the free-swimming planktonic bacteria transforming into sessile forms. External factors like the temperature, pH, nature of the inhabiting surfaces, quorum sensing, and other signalling molecules govern this complete process of biofilm formation (Rather et al., 2021). Biofilms often show resistance to the defence mechanisms of the host and traditional antibiotics, becoming a reservoir for continued infection (Chang et al., 2022).

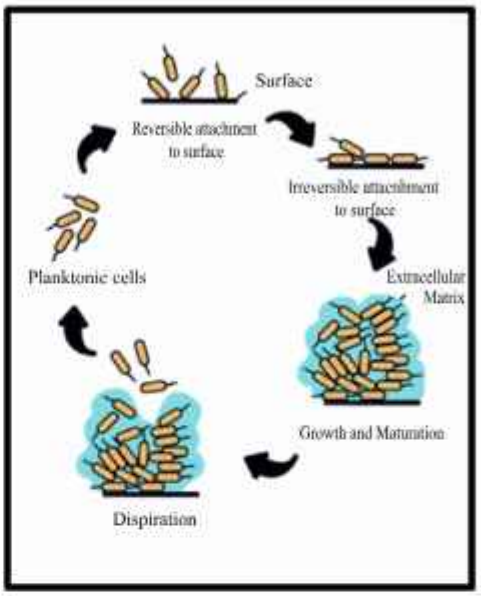


Fig. 1. The biofilm life cycle: the conceptual model of biofilm formation.

Having a molecular weight of 2-10 kDa (Singh et al., 2022) and ranging from 12 to 50 amino acids in length, the AMPs consist of two or more positively charged residues, such as histidine, arginine or lysine, and hydrophobic residues (Hemshekhar et al., 2016). AMPs attack multiple targets, which are of low affinity, reducing the progression of antimicrobial resistance (Mahlapuu et al., 2020). AMPs are also open to peptide engineering and mutagenesis, which have already resulted in many compounds being synthesised, possessing strengthened bioactivity and decreased cytotoxicity (Bin Hafeez et al., 2021). In this review, we examine five different antimicrobial peptides derived from plants (Table 1) that exhibit antibiofilmic properties.

Plant Peptides

The defense peptides produced by plants are mainly of two categories: antimicrobial peptides (AMPs) and plant elicitor peptides (PEPs), which function as the chemical arsenal of plants against the biotic stresses encountered by plants. PEPs, significant endogenous cell signalling molecules, trigger the defense signalling by interacting with membrane receptors and various components of

defense response, like hormone production, and enhanced expression of defensive genes is induced subsequently. Mostly, the AMPs in plants are cationic biomolecules that are bactericidal on interaction with the negatively charged cell membrane. There are successful isolations of AMPs from the seeds, roots, leaves, stems, and flowers of plants that comprise an immense number of AMPs. Due to the wide array of biological activities shown by plant AMPs, they become important candidates for the treatment of different infections in humans (Shirsat et al., 2025). Among the different species, AMPs show variations in structural forms but have similar attributes such as shorter size, hydrophobic and hydrophilic domains, and positive charges (Ciumac et al., 2019).

The ability of plant AMPs to tolerate hypervariable regions while employing a conserved framework enables them to recognize different targets by the alteration of the sequence of non-cysteine residues (Bakare et al., 2022). Disulfide bonds are also present in most plant AMPs, playing a key role in their increased stability towards chemical, thermal and enzymatic degradation, hence protecting the tertiary and quaternary structures (Maan et al., 2020). As shown in Fig. 2, the anti-biofilm mechanisms of AMPs are: i) Membrane-associated activity, involving pore formation and/or disruption of the bacterial cell membrane, ii) Penetration into the bacterial cytoplasm and inhibition of cell wall, enzyme, or protein synthesis, iii) Degradation or disruption of the extracellular matrix, iv) Prevention of biofilm formation and induction of existing biofilm dispersion during the early biofilm formation process (Vladkova et al., 2024).

Gurmarin

The gurmarin polypeptide is obtained from the Gymnema sylvestre (Gs) plant, which has been used traditionally in Ayurvedic medicine for the treatment of diabetes mellitus. The gurmarin polypeptide is a 35-amino acid polypeptide belonging to the family of knottin polypeptides. Crystal violet staining was used to select compounds that affected *Staphylococcus aureus* biofilm growth but did not affect the bacterial growth. A preparative isoelectric focusing (IEF) technique was employed to purify the active principle, as it was proteinaceous. In the IEF technique, charge differences under the electric field are used to separate the polypeptides.

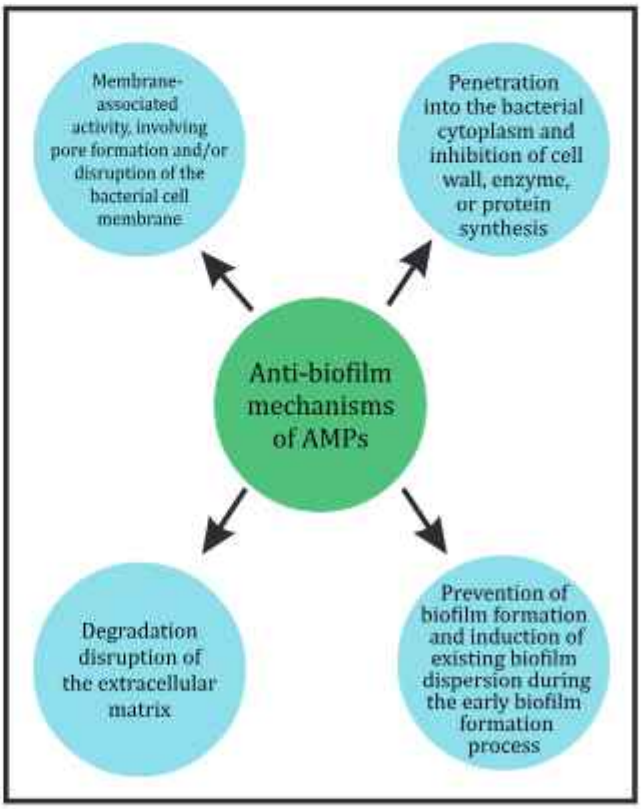


Fig. 2. The Anti-biofilm mechanisms of Antimicrobial Peptides (AMPs).

Gurmarin consists of antiparallel ß-hairpin residues, multiple prominent ß turns and a cystine knot characteristic of toxic and inhibitory polypeptides. A pair of disulfides out of the three disulfide linkages in Gurmarin creates a protease-resistant and heat-stable structure called the inhibitor cystine knot by forming a loop through which the passing of the third disulfide bond occurs. The 6 cystines present in Gurmarin are responsible for the formation of the 3 disulfide bonds present at 3-18, 10-23, and 17-33, along with two antiparallel beta strands (Chang et al., 2022).

The team investigated the purified form of gurmarin's role in the growth rate of *Staphylococcus aureus* bacteria and the inhibitory component on the biofilm. The results indicated that there was no impact on the bacterial viability due to gurmarin, and there were no instances of red blood cell hemolysis. The biofilms were found to be adhering to the walls as well as the bottom of the control test tubes, which were maintained in gentle conditions of shaking. The absence of biofilm on the glass surface was noted in the absence of *S. aureus* bacteria when they were cultured in the presence of gurmarin. The inhibition of *Staphylococcus aureus* biofilm growth was also tested on plastic surfaces. The presence of a thick biofilm with an extracellular polysaccharide fibril in the control was absent in the Gurmarin-treated biofilm, which confirmed the result of immunoblot analysis of PNAG (Chang et al., 2022).

Nodule-specific cysteine-rich peptides

The legume-rhizobia interaction leads to root nodule formation and fixation of nitrogen due to terminal differentiation of bacteroids. Depending on the plant effectors, the endosymbiont differentiation is terminable or reversible. In the case of the inverted repeat lacking clade legumes, the N-nodule specific cysteine-rich proteins regulate the termination of the bacteroid differentiation. About 700 NCR coding genes were identified in *Medicago truncatula.* (Zhang et al., 2023). The NCR peptides bear a resemblance to defensins, which are small cysteine-rich peptides. NCR peptides have a great diversity of amino acid sequences with four to six conserved cysteine residues (Alhhazmi et al., 2024). In the infected cells of the nodule, bacterial cells are encapsulated inside a plant cell membrane due to the development of subcellular compartments referred to as symbiosomes. Bacteria proliferate and undergo morphological and metabolic changes to adapt from the free-living style of life to their advanced endosymbiotic form called bacteroids (Horváth et al., 2023).

The researchers used three antimicrobial prediction models, ClassAMP, AntiBP and AMPA in the prediction of probable peptide sequence from 22 NCR sequences from *Medicago truncatula,* which were selected from GenBank (Alhhazmi et al., 2024). Three promising NCR peptides, namely NCR094, NCR888, and NCR992, were chemically produced. All three NCR peptides chosen for study contained four cysteines in their sequences. The killing percentage for Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) was found to be 6.57% (±1.42) and 20.30% (±2.51) for 25 µM of NCR094 and NCR992, respectively. Gram-negative *Klebsiella pneumoniae* was eradicated by NCR094 at a lower concentration of 25 µM compared to NCR992, which eradicated *Klebsiella pneumoniae* at 50 µM. The wild type and other variants of NCR888

were not considered for downstream assays, as neither MRSA nor *Klebsiella pneumoniae* was killed or inhibited by NCR888 at the tested concentrations.

A biofilm-forming assay was employed to determine the impact of NCR peptides on *Klebsiella pneumoniae* biofilm formation. Increased inhibition of biofilm growth was found to exist for all cysteine replacement mutants of NCR peptides compared to those of the wild-type counterparts. Assessment of human red blood cell toxicity for NCR094 and NCR992 and its analogs was also conducted. The cutoff to denote whether a peptide is toxic or not was set at twenty percentage and more. The hemolysis % activity of NCR094.3 was reported to be 0.69%, ±0.69; however, NCR992.1 showed a toxicity of 63.67% against human erythrocytes. The toxicity of the peptides against the leukaemia (K562) cell line was also performed, where no toxicity was reported for NCR992.1 (11.02%; SD ± 0.32, independent t-test, P-value =0.007) and NCR094.3 had no toxicity as well (Alhhazmi et al., 2024).

CSP37

The Capsicum storage peptides (CSP) are derived from *Capsicum baccatum*. *Staphylococcus epidermidis* biofilm formation was reduced by 80% by an aqueous extract of *C. baccatum* seeds, hence showing the ability of red pepper in the making of antibiofilm and antiadhesive compounds. The genus Capsicum comprises more than 200 varieties of peppers, exhibiting a great diversity in their fruits, as well as in terms of flavour, shape, and size. Indigenous to Central and South America, the peppers growing in the tropical and humid regions are of great economic value. Alongside being consumed as food or spice, they are also used in Native American, Indian, and traditional Chinese medicine for the treatment of wounds, snake or dog bites, and skin rashes.

The group of researchers developed surface CSP coatings and evaluated their effects. Noticeably, CSP did not affect bacterial growth at a comparable concentration. Moreover, bacterial growth curves further confirmed the lack of any antibiotic action of CSP. Despite the inhibition of biofilm formation, the planktonic bacteria remained viable, and the resulting colonies displayed normal phenotypes. Secondly, the anti-adhesive property of CSP was investigated. The spin-coating method was employed to coat CSP on Permanox™ slides that had been subjected to Staphylococcus epidermidis cultures for a period of 24 hours. The adhesion of bacteria, aggregation and accumulation were prevented by the CSP-coated surfaces (Borowski et al., 2020). The uncoated control, which was exposed to a concentration of 2.0 mg/mL of soluble CSP in the culture medium, did not exhibit any bacteria adhering to the surface. The surfaces coated with a concentration of 10.0 mg/mL of CSP demonstrated a complete absence of bacteria as well as the extracellular matrix. The control surfaces (vehicle-coated surfaces and uncoated surfaces without the CSP solution) failed to inhibit the process of bacterial adherence, aggregations, and accumulations that would lead to the formation of a potential biofilm. The CSP MS/MS data previously reported by the researchers and sequence alignment were used to determine the sequence of the detected peptide with higher intensity in the active fraction of CSP, which is RSCQQQIQQAQQLSSCQQYLKQRVQSEEGEDQISQRE (European Patent n EP19305205). This peptide predicted was named "Capsicum storage peptide 37 (CSP37)", which was produced and experimentally tested too. CSP did not show cytotoxicity on lymphocytes after a duration of 24 hours, where a reduction of 7.7% in cell number was observed. Moreover, CSP was found to be non-

toxic to lymphocytes using the Lactate Dehydrogenase assay and the high content analysis (HCA) assay demonstrated that there was no cytotoxicity of CSP37 against 7 different human cell lines.

Most interestingly, both CSP and CSP37 inhibit the formation of the Staphylococcus epidermidis biofilm without effects like antibiotics. Therefore, the antibiofilm ability of CSP and CSP37 relies on the use of an action mechanism that is different from cell killing and that of antibiotics. For this reason, nonantibiotic-based strategies were proposed and are in use nowadays, representing an alternative to bacterial resistance and tolerances, and among which is the use of targeting extracellular bacterial virulence factors. This technique is quite effective because it does not easily disturb the process of gene expressions within the bacteria and can even employ antibiofilm agents to inhibit the bacterial attachment. For instance, the prototype red pepper coat or CSP spin-coated slides inhibit the attachment or biofilm formation by the bacteria. This is likely a concentration-dependent phenomenon. CSPs primarily consist of polar amino acids, which are responsible for the hydrophilic characteristics. The analysis of the angle contact results reported enhanced hydrophilicity on the coated surface when compared with the controls. Since the bacterial attachment depends mainly on the hydrophobic and electrostatic physicochemical characteristics along with the surfaces that are involved, these interactions are reduced by the hydrophilicity of CSP coating (Borowski et al., 2020).

PN5

PN5 is an immunopeptide that is an antimicrobial peptide from the pine needle (PN) of *Pinus densiflora.* Antimicrobial properties were found to be possessed by four different peptides, out of which the peptide PN5 (FKFLARTGKFL) was analyzed based on the Edman degradation method. Antimicrobial action exhibited by peptide PN5 on Gram-positive, Gram-negative, as well as multidrug-resistant (MDR) *E. coli* strains, remained untested by the study authors. Peptide PN5 also suppressed the biofilm formations of the resistant strains *E. coli* and *S. aureus* to carbapenem.

The retention time of PN5 was found to be 22.22 minutes in the reverse-phase high-performance liquid chromatographic procedure carried out on a C18 column. The estimated MW of PN5 confirmed to be 1,326.6 Da as found by matrix-assisted laser desorption ionization-time of flight mass spectrometry analysis of the peptide chain. Thus, the data obtained from PN5 showed a random coil structure with a negative minimum of 190-200 nm when simulated under aqueous conditions represented by 10mM sodium phosphate buffer solution (Kang et al., 2022).

These unique peptide molecules bind to the bacterial cell wall components, while the outer membrane of Gram-negative bacteria consists of LPS, an endotoxin, triggering the innate immunity response because of the endotoxic activity of LPS. These aggregated or accumulated endotoxins are suppressed or neutralised by the binding properties of the AMPs, thereby inhibiting the endotoxic action of LPS. To determine whether the anti-inflammatory potential of the peptide PN5 is mediated by binding interactions with LPS, experiments were conducted to investigate whether the peptide binds to LPS, adopting an alpha-helical conformation, thereby clearly confirming the anti-inflammatory property of the compound itself. Expression of the pro-inflammatory cytokines TNF-α and IL-6, the RAW 264.7 cells, induced by the *E. coli* LPS, were modulated by the compound that modulates the expression of the said pro-inflammatory cytokines, which are involved in the innate immunity response because the Toll-like receptor-4 belongs to the family 'Pattern recognition receptor' (Kang et al., 2022).

Chia-derived peptides

The chia peptides were prepared by hydrolyzing the protein-rich fraction of chia seeds with a method devised and performed by a research team, involving a pepsin-pancreatin two-step process and ultrafiltration with membranes of a 1, 3, and 5 kDa cut-off. The researchers prepared the chia peptide fractions of 1 kDa and below, 1-3 kDa, and 3-5 kDa, with F < 1 kDa having a higher antimicrobial activity towards the gram-positive bacteria. Based on the results of this research, a total of 1067 novel peptides were purified with the aid of a nano-liquid chromatography system, and a system of computer-aided software for identification of amino acid sequences which tested for antimicrobial, antibiofilm, and antioxidants properties respectively with a view of enhancing peptide functions.

Deep-AmPEP30 is the method proposed for the prediction of small AMPs given a small dataset and uses the deep CNN approach as the learning strategy and RAAC as the features. In the proposed method, the approach uses the combination of the scores given by the evolutionary information, score of substitution, hydrophobicity, and contact potential energy of the five reduced amino acid sets. In contrast, the approach to prediction in the proposed Random forest technique takes into account the distribution of patterns in terms of the structure attributes given by the charge, hydrophobicity, polarisability, and the polarisability with normalized Van der Walls volume. In the training data, the limit of the sequence given is 5-30 amino acids. However, within this approach, an enormous number of sub-optimal peptide sequences were eliminated in an effort to achieve high accuracy and specificity for the identification of small-length antimicrobial peptides with not more than 30 amino acids. The AxPEP peptide classifier was applied on the list of 1067 peptide sequences, which were previously selected in a chia peptide fraction. The classification for the peptide sequence was made between antimicrobial peptides and non-antimicrobial peptides using the deep learning approach as well as the random forest approach. The antimicrobial-positive sequence was labeled with 1, the negative sequence with 0, but the invalid sequence was labeled

Table 1 : A comparative overview of the five antimicrobial peptides

Features	Gurmarin	Nodule-specific cysteine rich peptides	Capsicum storage peptide (CSP37)	PN5	Chia-derived peptides
Source	*Gymnema sylvestre*	*Medicago truncatula*	*Capsicum baccatum* (Red pepper)	*Pinus densiflora*	*Salvia hispanica* L.
Peptide Type	cyclic peptide of Knottin family	Nodule-specific cysteine -rich (NCR) peptide variant	Capsicum Storage Peptide (CSP), derived from a 2SS protein	Alpha-helical AMP	Optimized short peptide sequence (derived)
Target organism	*Staphylococcus aureus*	*Klebsiella pneumoniae*	Methicillin resistant *Staphylococcus epidermidis*	Multidrug-resistant (MDR) *E. coli* and *Staphylococcus aureus*	Predicted to be broad-spectrum

with 1. Therefore, the peptide sequence with the highest level of antimicrobial properties was identified based on the sequence with the highest prediction score closest to 1. Based on this approach, the peptide sequence with the score > 0.7 was categorized either as an antimicrobial peptide sequence or as a non-antimicrobial peptide sequence. As per the AxPEP report, the top-ranked predictions contained the sequences YACLKVK, KLGSALLA, and KLKKNL. This order was made following the highest to lowest value of the product of probabilities. These sequences were found to have the highest level of antimicrobial activity in the peptide F<1, where the product probabilities are more than or equal to 0.71. AMPs are water-soluble molecules, and the secondary structure of the active proteins and peptides is a major part in water solubility, which shouldn't be a tightly packed structure because it is not suitable for increasing the functional potential. This lack of characteristic rendered the exclusion of the KLGSALLA peptide, along with other peptides showing high antimicrobial potential.

The Scrambled computational tool was used to create 999 sequence variants for YACLKVK and 59 variants for KLKKNL following which the BIOFIN peptide prediction module was used to test the antibiofilm activity of YACLKVK, KLKKNL and the permutations. The selected peptides with a high score, CVLKKYA, KKLLKN, and KLLKKN were optimized using BIOFIN until the prediction score was greater than or equal to 0.9 by substituting the amino acids. Thus, the optimisation procedure ensured the selection of peptides, KLLKKYL and KKLLKI with prediction scores of over 0.9 for antibiofilm properties and the presence of antioxidant fragments was also tested by BIOPEP-UWM. The importance of obtaining such multifunctional peptides from chia relies on the finding of alternative molecules to food preservatives, which have been associated with cytotoxic and genotoxic effects (León Madrazo & Segura Campos, 2022).

Conclusion

Plant-derived peptides are a fast-growing and extremely promising class of bioactive molecules with much potential to combat two of the biggest challenges-AMR and biofilm-associated infections-of the century. The collected evidence in this review gives an account of the astonishing structural variability, polychronicity, and biological specificity of plant peptides-essentially AMPs-that permit them to act via different mechanisms than conventional antibiotics do, a critical factor in the present clinical scenario, where resistance against traditional antimicrobials has brought down treatment options.

Plant AMPs represent an intrinsic chemical defense against biotic stress. AMPs often perform direct antimicrobial or antibiofilm activity without killing planktonic cells. Their cationic nature, amphipathic structures, and ability to interact with negatively charged microbial membranes or extracellular matrix components make them effective modulators of microbial virulence rather than mere bactericidal agents. This antivirulence strategy reduces selective pressure for resistance development, a key advantage over traditional antibiotics.

The results from the individual case studies in this review cumulatively support this paradigm. Gurmarin is a representative of a knottin peptide extracted from the plant *Gymnema sylvestre* and a prototype of a non-bactericidal antibiofilm agent targeting the process of biofilm assembly mediated by *S. aureus* through disrupting the production of Poly N-Acetyl Glucosamine (PNAG), a crucial component of the extracellular matrix of biofilms. The increased stability and focus on a

non-cytotoxic molecule targeting biofilms specifically illustrate the potency in biofilm targeting through the use of conformationally constrained cystine knot-containing peptides. Nodule-specific cysteine-rich (NCR) peptides from legume rhizobia collaborate in exemplifying the importance of cysteine substitutions in the molecule to increase antibiofilm activity without significantly raising toxicity in host cells. Capsicum peptides, specifically CSP37, represent yet another area where plant peptides can be applied not only for their solubility and use as antimicrobial peptides but can now be applied as surface-active antimicrobial biofilm materials. Based on the alteration in the hydrophilic properties of the surface and the subsequent decrease in the bacterial colonization, coatings made out of CSPs are effective in the inhibition of Staphylococcus epidermidis biofilm without affecting the bacterial viability or causing cytotoxicity to human cells.

The peptide PN5 derived from pine needles appears to be an exciting example of a multitasking AMP possessing the combined effects of being an antibacterial, antibiofilm, and an immune modulator. The capability of PN5 to cause toroidal-pore mediated permeabilisation in the bacterial cell membranes, along with the ability to bind and inactivate the LPS makes it a dual-acting therapeutic molecule having the potency to treat both infection and inflammation caused by the bacterial infection.Chia-derived peptides bring a new dimension by showcasing how plant peptides derived from food sources can be systematically harnessed, optimised, and repurposed using modern computer-based models.

Together, this review makes it clear that plant peptides are more than just additional alternatives for antibiotics but are in fact a completely novel way of microbial control. Their usability in preventing biofilm formation, influencing the host immune system,focusing on virulence factors, and performing these functions without stressing microbes too heavily towards developing resistance are factors that make them extremely preferable as novel agents and agents for industry applications. The future of research will be centred on establishing better structure-function correlations, optimising peptide stability, and translating laboratory results obtained from in vitro experiments into more practical forms for clinical and industrial applications. As biotechnology, peptide engineering, and computational biology continue to advance, plant-derived peptides will form the backbone of a paradigm shift in efforts aimed at combating antimicrobial resistance, biofilm infections, and offering alternative, non-antibiotic approaches within the healthcare and food sectors.

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Impact of salinity stress on *Vigna radiata* (L.) Wilczek (Leguminosae) : studying different parameters

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Abstract:

Salinity stress is a major constraint on legume productivity, affecting growth and metabolism through osmotic imbalance and ion toxicity. In this study, *Vigna radiata* (L.) Wilczek (Leguminosae) seedlings were subjected to graded NaCl concentrations (10–40 mM) for seven days, and their biochemical and physiological responses were evaluated. Germination percentage and radical length declined progressively with increasing salinity, while nucleic acid metabolism showed differential regulation, with DNA peaking at moderate stress and RNA increasing under severe stress. Protein and carbohydrate contents decreased, whereas amino acids accumulated sharply under high salinity, reflecting osmolyte-based adaptation. Sterol content increased under mild stress but declined at higher concentrations, while enzyme activities such as catalase and phosphatases exhibited biphasic regulation. IAA levels decreased consistently, indicating hormonal disruption. These findings highlight that moderate salinity triggers adaptive mechanisms including osmolyte accumulation, sterol stabilization, and antioxidant activation, whereas severe stress leads to metabolic imbalance and growth inhibition. The identification of biochemical markers such as amino acid buildup, sterol variation, catalase activity, and auxin reduction provides practical tools for screening salinity tolerance, offering valuable implications for crop improvement in legumes under saline conditions.

Keywords:

Vigna radiata; salinity stress; NaCl; nucleic acids; protein; carbohydrate; amino acids; sterols and IAA

Introduction

Plants in both natural and agricultural ecosystems are frequently exposed to abiotic stresses, among which salinity stress is one of the most detrimental factors affecting growth, metabolism, and reproduction (Zhang et al., 2025). Stress is defined as an external factor exerting disadvantageous influences on plant physiology. High concentrations of salts, particularly Na^+, K^+, and Ca^{2+}, lead to salinity stress, while stress specifically due to Na^+ is termed sodicity (Greenway & Munns, 1980). Elevated Na^+ levels displace Ca^{2+} from plasma membranes, impairing permeability and causing leakage of K^+, thereby disrupting cellular homeostasis (Watanabe, 2004).

Plants differ widely in their tolerance to salinity. Greenway and Munns (1980) classified species into groups: Group IA halophytes such as *Suaeda martinea* and *Atriplex nummularia* show stimulation at Cl^- levels below 400 mM; Group IB halophytes including Spartina townsendii and Beta vulgaris tolerate salt but exhibit reduced growth; Group II species such as Festuca rubra and tomato (*Lycopersicon esculentum*) are intermediate, while soybean (*Glycine max*) and common bean (*Phaseolus vulgaris*) are sensitive; Group III species, including citrus, avocado, and stone fruits, are highly sensitive and severely inhibited even at low salt concentrations.

Salinity stress alters enzymatic activity across species. Phosphatase activity increases in Parmillate seeds (Jain et al., 2004) and Medicago sativa under osmotic stress (Ehsanpour & Amini, 2003). In *Arabidopsis thaliana*, ornithine-α-aminoacid activity rises under salinity (Petrovic et al., 1999). In maize, shoot-to-root dry mass ratio and leaf Na^+ content showed poor correlation with

tolerance, but Na^+ and organic solute accumulation in roots emerged as reliable markers (Watanabe, 2004). Salt stress also reduces RNA content in maize roots (Atanassova et al., 1997) and affects sterol biosynthesis in black yeast (*Hortaea werneckii*) via HMGR regulation (Petrovic et al., 1999).

Growth and hormonal regulation are also impacted. In *Vigna unguiculata*, NaCl treatments significantly reduced root and shoot elongation, while peroxidase activity inversely correlated with hypocotyl growth (Pujari & Chanda, 2002). Similarly, IAA oxidase activity increased under stress. Cotton varieties (Dandara & Giza 45) showed reduced growth, altered mineral content, and increased phosphorylase activity under salinity (Rathert, 1983). In broccoli, NaCl levels up to 60 mM maintained normal development, but beyond this threshold, nitrate reductase decreased and acid phosphatase increased, indicating stress-induced nutritional disorders (Lopez-Besenguer et al., 2007).

Photosynthetic and molecular adjustments are evident in C4 plants, where salinity stress enhances PEP carboxylase kinase activity independent of Ca^{2+}, leading to altered phosphorylation and regulation of photosynthesis (Maurino et al., 2001). In *Micrasterias denticulata*, NaCl and KCl stress induced vacuolization, mitochondrial deformation, and autophagy-mediated organelle degradation (Affenzeller et al., 2009). Overexpression of MsK4 kinase in *Medicago sativa* improved tolerance by modifying carbohydrate metabolism and starch accumulation (Erban et al., 2007).

Recent studies further highlight molecular and biochemical adaptations under salinity stress. In turmeric (*Curcuma longa*), curcuminoid depletion was coupled with elevated phenolic antioxidants under NaCl stress (Mohankumar et al., 2026). Radish (*Raphanus sativus*) exhibited genotype-specific differences in germination and growth under salinity (Khadija et al., 2026). Comprehensive reviews emphasize ion homeostasis, osmolyte accumulation, and antioxidant regulation as central mechanisms of tolerance (Zhang et al., 2025; Springer Nature, 2026; MDPI, 2025).

Vigna radiata (mung bean) is an important legume crop widely cultivated in Asia and valued for its high protein content, short life cycle, and ability to enrich soil fertility through nitrogen fixation. It serves as a staple source of dietary protein in many developing countries and is also used in sprout production due to its rapid germination rate. Despite its economic and nutritional importance, *Vigna radiata* is highly sensitive to salinity stress, which adversely affects seed germination, root and shoot growth, photosynthetic efficiency, and yield. Recent work has shown that exogenous application of glycine betaine mitigates salt-induced oxidative stress in mung bean by enhancing antioxidant activity and maintaining growth under NaCl stress (Alamer, 2026). Laboratory studies further confirm that salinity reduces germination and alters biochemical parameters in seedlings (IJASRM, 2023). Broader reviews of *Vigna* sp. highlight adaptive responses such as osmolyte accumulation and antioxidant regulation as key tolerance mechanisms (Sekar Suriya & Selvaraj, 2026).

Experimental salinity treatments at 10–40 mM NaCl provide a controlled gradient from mild to severe stress. At 10 mM, physiological changes are subtle; 20 mM induces moderate reductions in germination and chlorophyll; 30 mM causes pronounced ionic toxicity and oxidative damage;

and 40 mM often exceeds tolerance capacity, leading to severe growth inhibition. These ranges are widely used because they reflect realistic soil salinity levels in semi-arid and irrigated regions, while enabling clear differentiation between adaptive and injury responses (Patel et al., 2024; Sekar Suriya & Selvaraj, 2026).

In summary, salinity stress disrupts plant growth and metabolism through ionic imbalance, osmotic stress, and altered enzymatic activity. Classical findings remain foundational, but recent advances provide molecular insights into adaptive strategies, highlighting the importance of integrating physiological, biochemical, and genetic markers for screening salt tolerance in crops. The objective of the present work is to evaluate the impact of graded salinity stress (10 mM, 20 mM, 30 mM, and 40 mM NaCl) on the growth, physiology, and biochemical responses of *Vigna radiata,* with a focus on identifying key markers of tolerance and sensitivity that can contribute to future crop improvement strategies.

Materials and Methods

Preparation of Salt Concentrations and Stress Application

Salinity stress was imposed using NaCl solutions at 10, 20, 30, and 40 mM. Stock solutions were prepared by dissolving analytical-grade NaCl (58.44 g/mol) in distilled water: 0.584 g, 1.168 g, 1.752 g, and 2.336 g/litre, respectively. Seeds were procured from a local pulse vendor to serve as the experimental material for germination studies.Individual experimental setups were prepared by placing seeds on non-absorbent cotton in five Petri dishes, moistening them with distilled water, and incubating them at approximately 25 °C to facilitate germination and plantlet development. Five-days-old *Vigna radiata* (L.) Wilczek (Leguminosae), plantlets grown in distilled water were transferred to nutrient medium supplemented with these concentrations and maintained under stress for seven days, while untreated seedlings served as controls.

Alkaline Phosphatase

Seedlings of *V. radiata* exposed to 10, 20, 30, and 40 mM NaCl stress were homogenized in ice-cold Tris–HCl buffer (50 mM, pH 8.5; prepared by dissolving Tris base in distilled water and adjusting pH with HCl) at a ratio of 0.125 g tissue per ml buffer; the homogenate was centrifuged at 12,000 × g for 15 min at 4°C, and the resulting supernatant was used as crude enzyme extract for alkaline phosphatase assay with p-nitrophenyl phosphate (PNPP) substrate. The reaction produced a pale-yellow colour measured at 450 nm, and activity was expressed as PNPP consumed per minute (Bioassay of Alkaline Phosphatase, Enzolyte™, 2007).

Acid Phosphatase

Extracts (0.062 g/ml) were assayed in Tris–HCl buffer (pH 5) with PNPP substrate. The reaction yielded a pale-yellow colour at 450 nm, and enzyme activity was calculated using PNPP conversion units (Bioassay of Alkaline Phosphatase, Enzolyte™, 2007).

Sterol

Sterol content was determined by the ferric chloride method (Zlatkis, 1953; Henly, 1957).

Reaction mixtures produced a reddish-brown coloration, measured at 560 nm, and sterol concentration was calculated from OD conversion.

Protein

Protein was estimated using the Lowry method (Lowry et al., 1951). Reaction with Lowry reagent and Folin–Ciocalteu phenol produced a blue colour at 660 nm, quantified against a BSA standard curve.

Carbohydrate

Carbohydrate content was measured using the anthrone method (Hedge &Hofreiter, 1962). Reaction with anthrone reagent in H_2SO_4 produced a green colour at 630 nm, quantified using a dextrose standard curve.

Amino Acids

Amino acid content was determined by the ninhydrin method (Moore & Stein, 1948). Reaction with ninhydrin produced a purple colour at 570 nm, quantified against a standard curve prepared with α-valine.

Phosphorus

Phosphorus was estimated by the colorimetric method of Subba Row (1925). Reaction with ammonium molybdate, H_2SO_4, and ANSA (1-Amino-2-naphthol-4-sulfonicAcid) reagent produced a blue-green colour at 570 nm, quantified using a phosphoric acid standard curve.

DNA

DNA was quantified using the diphenylamine method (Burton, 1956). DNA was extracted by homogenizing 0.125 g seedling tissue in 1 ml ice-cold Tris–HCl buffer (50 mM, pH 7.0), followed by centrifugation at 12,000 × g for 15 min at 4°C to obtain the supernatant used for quantification.Extracts treated with TCA (Trichloro acetate) and PCA (Perchloric acid) were reacted with DPA (Diphenyl amine) solution, boiled, and cooled, producing a bluish-green colour measured at 600 nm. DNA concentration was calculated using the conversion factor of 1 OD = 100 µg DNA.

RNA

RNA was estimated using the orcinol method (Sadashivam et al., 1975).RNA was extracted by homogenizing 0.125 g seedling tissue in 1 ml Tris–HCl buffer (50 mM, pH 7.0), and the supernatant obtained after centrifugation was used for estimation. The prepared extracts were reacted with orcinol acid reagent and alcoholic orcinol, boiled, and cooled, producing a blue colour measured at 660 nm. RNA concentration was calculated using the conversion factor of 1 OD = 50 µg RNA.

Indole-3-Acetic Acid (IAA)

IAA content was determined using Salkowski reagent (Knegt & Bruinsma, 1973). IAA was extracted by homogenizing 0.125 g seedling tissue in 1 ml Tris–HCl buffer (50 mM, pH 7.0), neutralizing the extract, and using the supernatant for assay. Neutralized extracts were incubated with reagent, producing a pink colour measured at 540 nm. Quantification was performed using a standard curve prepared with known IAA.

Catalase Activity

Catalase activity was assayed by titration (Luck, 1974). Catalase enzyme extract was prepared by homogenizing 0.125 g seedling tissue in 1 ml ice-cold Tris–HCl buffer (50 mM, pH 7.0), centrifuging at 10,000 × g for 15 min at 4°C, and collecting the supernatant for activity measurement. Extracts (1ml) were incubated for 15minutes with 1ml of H_2O_2 (5%), and reactions were stopped with 2ml of H_2SO_4 (10%) before titration against 0.2N$KMnO_4$ with 2 to 4 drops of Phenolphthalein (colour indicator). The disappearance of pink coloration was used to assess activity, expressed relative to control and blank sets.

Statistical analyses

Trend analysis is performed on studied parameters against salinity grades (10-40mM) using LOESS smoothing using PAST 5.4 software. Principal Component analysis (PCA) is also performed reduce the dataset of the studied parameters against the salinity stress using XLSTAT 2014.5.03 software.

Results

Germination and Growth

Salinity stress significantly reduced germination and root elongation in *Vigna radiata*. Control seedlings showed 67.74% germination with a mean radical length of 2.51 cm. Germination declined progressively to 50% at 10 mM, 36% at 20–30 mM, and 31% at 40 mM NaCl. Radical length remained relatively stable under mild stress (2.57–2.62 cm) but decreased under severe stress (2.24 cm at 40 mM). The graphical represented is illustrated in Fig. 1a and 1b.

Nucleic Acids

DNA content was highest at 20 mM (90.37 µg/g FW) and 30 mM (74.81 µg/g FW), compared to 37.77 µg/g FW in control. RNA content fluctuated, showing a sharp decline at 10 mM (24 µg/g FW), but increased markedly at 30 mM (64 µg/g FW) and peaked at 40 mM (104 µg/g FW). These results suggest differential regulation of nucleic acid metabolism under graded salinity stress (Fig. 1c and 1d).

Protein, Carbohydrate, and Amino Acids

Protein content decreased sharply under stress (Fig. 1e), from 29.23 µg/g FW in control to 8.45 µg/g FW at 20 mM, before partially recovering at 40 mM (52.78 µg/g FW). Carbohydrate levels declined consistently (Fig. 1f), from 11.3 µg/g FW in control to 5.45 µg/g FW at 30 mM, with slight recovery at 40 mM (10.98 µg/g FW). Amino acid content showed a biphasic response (Fig. 1g): reduced at 10 mM (14.75 µg/g FW), moderate increase at 20 mM (20.35 µg/g FW), and a sharp rise at 40 mM (119.44 µg/g FW), indicating stress-induced accumulation of free amino acids.

Phosphorus and Sterol Content

Phosphorus levels varied (Fig. 1h), with slight increases at 10 mM (1.27 µg/g FW) and 30 mM (5.36 µg/g FW), but declined again at 40 mM (2.96 µg/g FW). Sterol content increased substantially (Fig. 1i) at 10 mM (528 µg/g FW) and peaked at 20 mM (784 µg/g FW), before declining at 30 mM (754 µg/g FW) and dropping sharply at 40 mM (84 µg/g FW).

Enzyme Activities

Acid phosphatase activity (Fig. 1j) was highest at 10 mM (2.4 mM PNPP/min/g extract) but declined at 30 mM (0.8 mM PNPP/min/g extract). Alkaline phosphatase activity decreased steadily (Fig. 1k) from 4 mM PNPP/min/g extract in control to 1.6 mM at 30 mM, with partial recovery at 40 mM (2.4 mM). Catalase activity increased under moderate stress (5.01 mg H_2O_2/min/g extract at 20 mM; 6.07 mg at 30 mM) but declined sharply at 40 mM (2.74 mg), indicating enzyme inactivation under severe oxidative stress (Fig. 1).

Indole-3-Acetic Acid (IAA)

IAA content declined progressively with increasing salinity (Fig. 2). Control seedlings contained 45.77 µg/ml/g extract, which decreased to 37.72 µg/ml/g extract at 10 mM, 34.2 µg/ml/g extract at 20 mM, and reached the lowest value of 23.99 µg/ml/g extract at 30 mM. A slight recovery was observed at 40 mM (41.14 µg/ml/g extract).

Statistical analyses

Trend analysis

The scatter plots of the studied parameters are prepared by locally weighted regression curve (LOESS) with smoothing factor 0.3 (Fig. 1, 2). The LOESS score of germination percentage, total amount of net IAA, alkaline phosphatase and catalase activity is higher at control sets and gradually decreases in 10mM salinity. Moreover, in phosphorus, total sterol and DNA content is lower in control sets but gradually increases up to 20 mM concentration and gradually deceases at 40 mM salinity concentration. The concentration of RNA, protein, amino acid increases with high salinity.

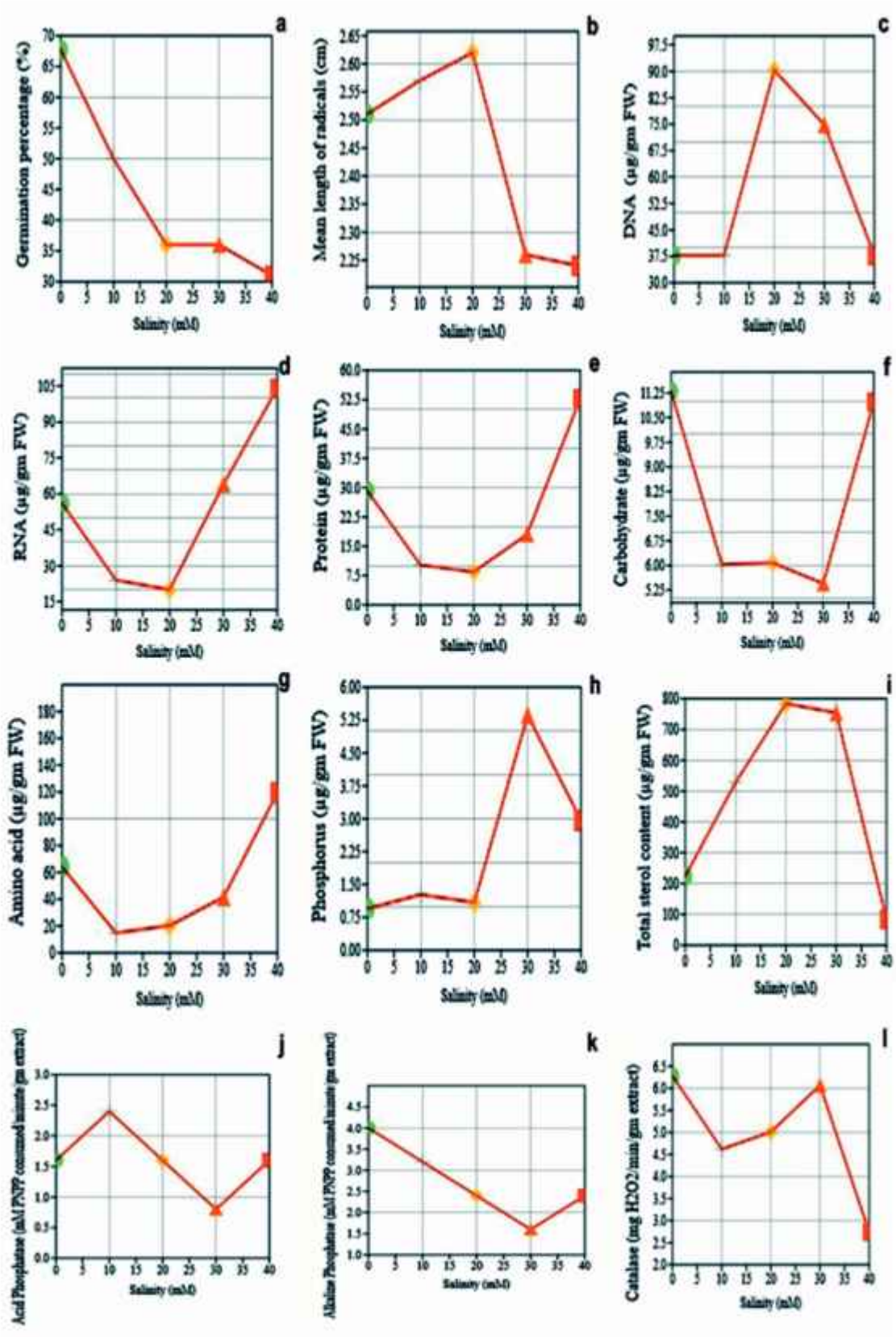


Fig. 1 : Scatter plots showing trends of studied parameters (y-axis) in percentage against different grades of salinity stress in mM (x-axis) using LOESS curves (smoothing factor 0.3)

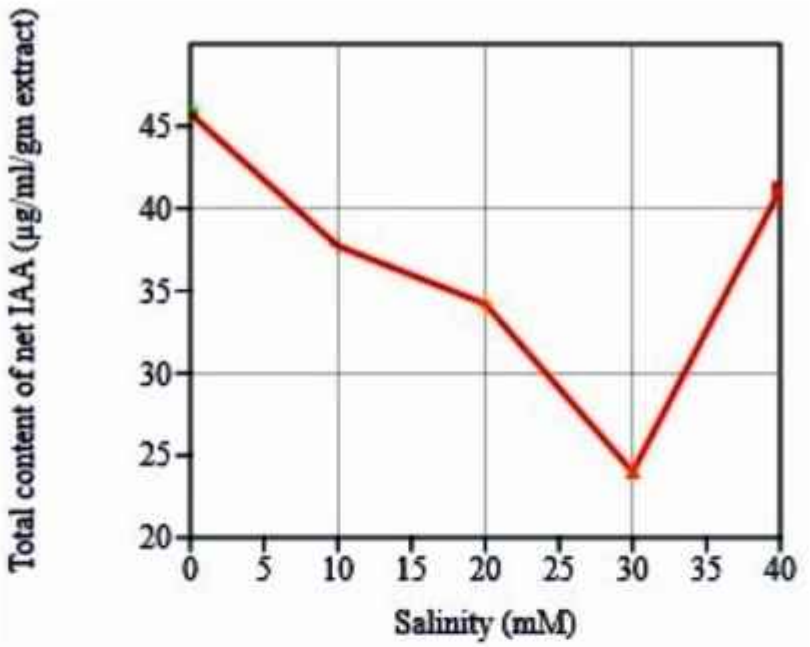


Fig. 2 : Scatter plots showing trends of Total amount of net IAA (y-axis) in percentage against different grades of salinity stress in mM (x-axis) using LOESS curves (smoothing factor 0.3)

PCA biplot

Principal Component Analysis (PCA) of biochemical and physiological parameters in *Vigna radiata* under NaCl salinity stress (10, 20, 30, and 40 mM) revealed that the first two principal components (F1 and F2) explained 83.38% of the total variance, with eigenvalues of 5.9474 and 4.8930, respectively. F1 accounted for 45.75% of variability, while F2 contributed 37.64%. Factor loadings indicated that protein (-0.9104), carbohydrate (-0.9356), amino acids (-0.8783), and RNA (-0.7432) were strongly negatively correlated with F1, whereas DNA (0.8009) and total sterol (0.9955) showed strong positive associations. F2 was positively correlated with phosphorus (0.9097) but negatively associated with alkaline phosphatase (-0.8722), acid phosphatase (-0.7539), germination percentage (-0.7718), and radicle length (-0.8785). Catalase exhibited a strong positive loading on F3 (0.8171), suggesting an independent contribution of antioxidant activity (Fig. 3; Table 1, 2).

Discussion

Salinity stress is known to impair germination and seedling growth in legumes, primarily through osmotic imbalance and ion toxicity. In the present study, mean germination percentage declined from 67.74% in control to 31% at 40 mM NaCl, with radical length also reduced. Similar reductions in germination and root elongation under salinity stress have been reported in *Vigna radiata* by Jothimani and Arulbalachandran (2021), who observed significant inhibition of shoot and root length at NaCl concentrations above 25 mM. Shekhar (2023) also noted decreased shoot height and chlorophyll content in stressed mung bean plants, consistent with our findings of reduced vigor at higher salt levels.

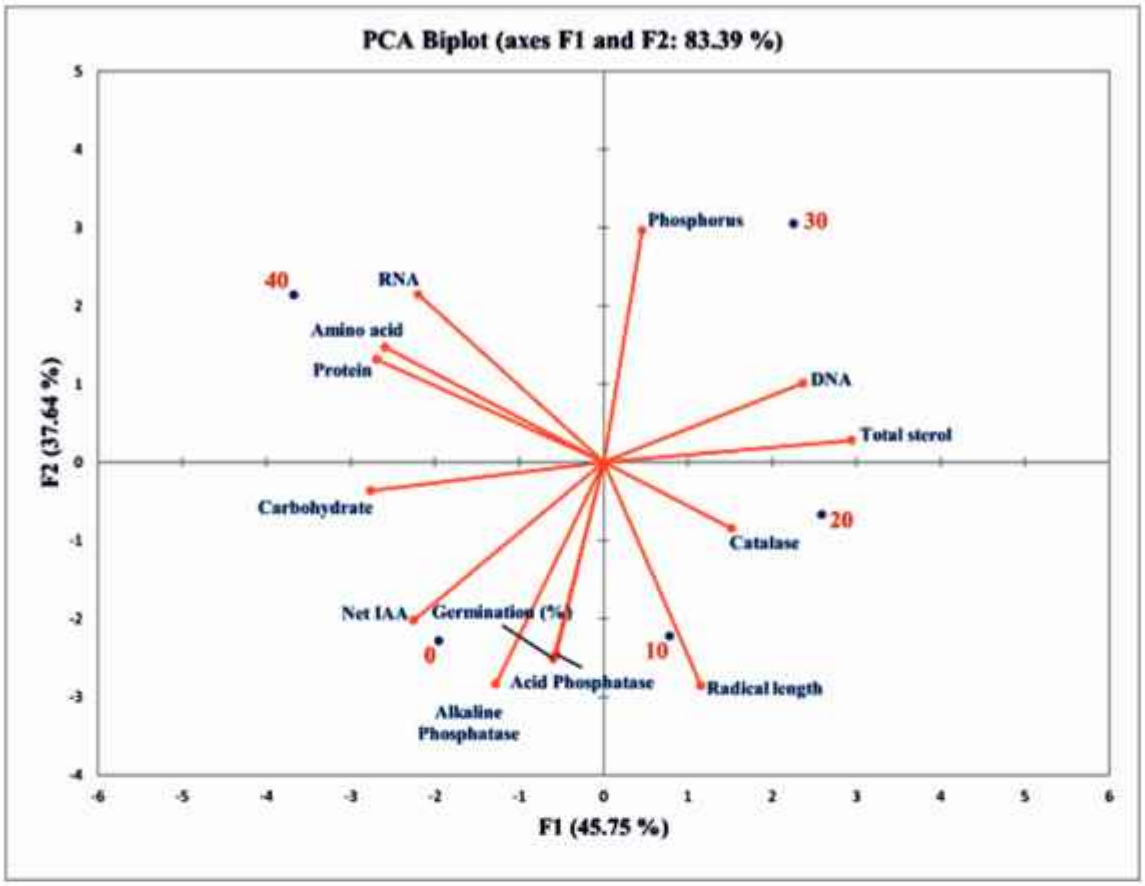


Fig. 3 : PCA biplot showing coordinates for all studied parametersas viriables (DNA, RNA, protein, carbohydrate, amino acid, phosphorus, acid phosphatase, alkaline phosphatase, catalase, total sterol, net IAA, germination (%), mean radical length and the graded salinity as observations (control as 0, 10mM, 10mM, 30mM, and 40mM) along the F1 and F2 axes.

Nucleic acid metabolism showed complex responses. DNA content peaked at 20 mM (90.37 µg/g FW), while RNA content increased sharply at 40 mM (104 µg/g FW). These results suggest stress-induced transcriptional activation under severe salinity. Comparable increases in RNA under stress were reported in maize roots by Atanassova et al., (1997), while Sekar Suriya and Selvaraj (2026) highlighted transcriptional reprogramming as a key tolerance mechanism in *Vigna* species.

Protein content declined under moderate stress but recovered at 40 mM, whereas carbohydrate levels decreased steadily. This pattern reflects metabolic disruption and osmolyte utilization.

Table 1 : Summary results of eigenvalue of Principal Component Anslysis PCA)

	F1	F2	F3	F4
Eigenvalue	5.9474	4.8930	1.5275	0.6322
Variability (%)	45.7491	37.6384	11.7496	4.8629
Cumulative (%)	45.7491	83.3874	95.1371	100.0000

Table 2 : Factor loading recovered from PCA analysis

	F1	F2	F3	F4
DNA	0.8009	0.3111	-0.0200	-0.5113
RNA	-0.7432	0.6587	0.1158	0.0193
Protein	-0.9104	0.4028	-0.0091	-0.0942
Carbohydrate	-0.9356	-0.1131	0.2155	-0.2558
Amino acid	-0.8783	0.4503	0.0108	-0.1603
Phosphorus	0.1551	0.9097	0.2450	0.2973
Acid Phosphatase	-0.1908	-0.7539	-0.5617	0.2825
Alkaline Phosphatase	-0.4330	-0.8722	0.2262	0.0249
Catalase	0.5143	-0.2605	0.8171	-0.0050
Total sterol	0.9955	0.0845	-0.0175	-0.0405
Net IAA	-0.7625	-0.6208	-0.0468	-0.1761
Germination (%)	-0.2004	-0.7718	0.5866	0.1418
Radical length	0.3915	-0.8785	-0.1618	-0.2208

Shekhar (2023) similarly reported reduced carbohydrate accumulation in stressed mung bean plants. Amino acid content, however, increased dramatically at 40 mM (119.44 µg/g FW), indicating osmolyte accumulation as a protective response. Such stress-induced amino acid accumulation has been emphasized as an adaptive mechanism in legumes (Sekar Suriya & Selvaraj, 2026).

Sterol content increased under mild and moderate stress (528–784 µg/g FW) but declined sharply at 40 mM (84 µg/g FW). This suggests sterol involvement in membrane stabilization under moderate stress, but degradation under severe conditions. Comparable sterol alterations under salinity were reported in Hortaeawerneckii (Petrovic et al., 1999).

Enzyme activities showed differential regulation. Acid phosphatase activity peaked at 10 mM, while alkaline phosphatase declined steadily. Catalase activity increased under moderate stress (6.07 mg H_2O_2/min/g extract at 30 mM) but decreased at 40 mM, reflecting antioxidant activation followed by enzyme inactivation under severe oxidative stress. Similar biphasic catalase responses have been documented in mung bean under salinity (Jothimani & Arulbalachandran, 2021).

IAA content declined progressively with increasing salinity, reaching its lowest at 30 mM (23.99 µg/ml/g extract). This reduction in auxin levels under stress is consistent with hormonal disruption reported in mung bean and other legumes (Knegt & Bruinsma, 1973; Sekar Suriya & Selvaraj, 2026).

The PCA analysis also demonstrates that salinity tolerance in *Vigna radiata* is linked to enhanced sterol accumulation and antioxidant activity, while higher salinity levels compromise seedling establishment through suppression of biosynthetic pathways and disruption of phosphorus metabolism. These findings provide insight into the biochemical basis of salt stress responses and highlight potential targets for improving stress tolerance in legumes.

Overall, our findings confirm that *Vigna radiata* exhibits a complex, concentration-dependent response to salinity stress. Mild and moderate stress levels trigger adaptive mechanisms such as sterol accumulation, catalase activation, and amino acid buildup, while severe stress leads to metabolic disruption, reduced germination, and hormonal imbalance. These results align with earlier studies (Jothimani & Arulbalachandran, 2021; Shekhar, 2023; Sekar Suriya & Selvaraj, 2026) and provide new insights into the graded biochemical responses of mung bean, highlighting potential markers for screening salinity tolerance in legumes.

Conclusion

This study demonstrates that *Vigna radiata* responds to graded NaCl stress in a concentration-dependent manner, with germination and radical growth progressively reduced, nucleic acid metabolism showing differential regulation, protein and carbohydrate contents declining, and amino acids accumulating sharply under severe stress. Sterol content increased under mild stress but dropped at higher concentrations, while enzyme activities such as catalase and phosphatases exhibited biphasic regulation, and IAA levels declined consistently, indicating hormonal disruption. Together, these findings highlight that moderate salinity triggers adaptive mechanisms including osmolyte accumulation, sterol stabilization, and antioxidant activation, whereas severe stress leads to metabolic imbalance and growth inhibition. The identification of biochemical markers—such as amino acid accumulation, sterol variation, catalase activity, and auxin reduction—provides practical tools for screening salinity tolerance, with significant implications for breeding and biotechnological strategies aimed at enhancing legume resilience under saline conditions.

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Disclaimer

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Author(s) contribution

Biswajit Mukherjee: Collection and preparation of samples for stress application, data curation, formal analysis, writing manuscript; Kunal Sen: Conceptualization, supervision.

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A review on ethnobotanical and pharmacological properties of a widespread mangrove associate species: *Acanthus ilicifolius* Linn. (Acanthaceae)

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Abstract:

A significant proportion of the Indian mangrove cover is concentrated in West Bengal within the Sundarbans, the world's largest mangrove forest. Substantial mangrove forests are also found along the eastern and western coastlines of the country, supporting a rich diversity of mangrove flora. Within these heterogeneous mangrove ecosystems, *Acanthus ilicifolius* Linn., also known as sea holly or holly mangrove, is a widespread species found across tropical and subtropical coastal regions. This literature survey highlights the global, national (India) and regional (West Bengal) geographic range or distribution of *Acanthus ilicifolius* Linn., as well as the morphological and anatomical features that enable its survival under saline, waterlogged, and anoxic conditions. This study also evaluates and compiles the ethnobotanical, phytochemical and pharmacological relevance of this species.

Keywords:

Acanthus ilicifolius Linn., mangrove associate species, distribution, adaptation, ethnobotany, phytochemistry, pharmacology

Mangroves at a glance

Arroyo (1977) defined mangroves as "A small group of true mangrove plants and associated species belonging to systematically unrelated families, possessing similar physiological characteristics and structural adaptations with common preference to the intertidal habitat". Mangroves are referred to as the meeting point of the land, freshwater and oceans. They are regarded as one of the most complex and endangered ecosystems on earth and are indispensable bio resources (Verma et al., 2020). Generally, mangroves are of two types. True mangroves or exclusive species are restricted to tropical intertidal or mangrove habitats and have specialised adaptations like pneumatophores and salt glands to survive in salty, waterlogged soils, while mangrove associates or non- exclusive species are not strictly mangroves, but they grow in or near mangrove habitats and can tolerate salinity to some extent. Their adaptations are less specialised than true mangroves (Polidoro et al., 2010).

Statistically, 71% of India's mangroves are found in the eastern coasts of India, the largest parts being the Indian Sundarbans and the Andaman and Nicobar Islands. According to the India State of Forest Report (ISFR) 2023, Indian mangrove cover stands at 4,991.68 sq. km making upto 0.15% of its total geographical area. West Bengal accounts for 42.45% of total Indian mangrove coverage, followed by Gujarat (23.66%) and Andaman & Nicobar Islands (12.39%) (Sedyaaw et al., 2024). For the present study, *Acanthus ilicifolius* Linn. has been chosen as it is one of the most widely adapted species across different salinity zones in mangrove forests of West Bengal. This study aims to revise the distribution, structural adaptations and phytotherapeutic significance of *Acanthus ilicifolius* Linn. on the basis of an extensive literature survey and data reported by authors.

Genus *Acanthus*

The genus *Acanthus* Linn. is classified under the Acanthaceae family under the sub-family Acanthoideae and comprises about 30 known species of flowering plants (Table 1), native to the tropics and subtropics (Ragavan et al., 2015). The name of the genus *Acanthus* is derived from Greek ákantha, 'thorn', referring to the spiny or toothed leaves of the plant (Herawati et al., 2020). It is a unique taxon as it includes both terrestrial and mangrove species (Mandal and Naskar, 2008). According to Polidoro, B. A. et al. (2010), four species of *Acanthus* are recognized as true mangroves, namely *Acanthus ebracteatus* Vahl., *Acanthus ilicifolius* Linn., *Acanthus volubilis* Wall., and *Acanthus xiamenensis* R.T. Zhang, out of 30 species in the genus. However, some taxonomists treat *Acanthus xiamenensis* R.T. Zhang as a synonym or variety of *Acanthus ilicifolius* Linn., rather than a distinct species. Wang and Wang (2007) have regarded them as the same species. *Acanthus xiamenensis* R.T.Zhang is endemic to China, and *A. ebracteatus* Vahl, *A. ilicifolius* Linn. and *A. volubilis* Wall. are prevalent in the Indo-West Pacific countries (Ragavan et al., 2015). These species exhibit strong adaptation to high tidal regimes and are resilient pioneers in mangrove coasts. Other species like *Acanthus leucostachyus* Wall. ex Nees and *Acanthus mollis* Linn. grow in damp and arid terrestrial forests respectively (Ma et al., 2023).

Six species found in India as discussed by Karthikeyan et al. (2009) are: *Acanthus ilicifolius* Linn., *Acanthus ebracteatus* Vahl., *Acanthus volubilis* Wall., *Acanthus carduaceus* Griff., *Acanthus mollis* Linn. (cultivated), *Acanthus leucostachyus* Wall. ex Nees. Ragavan et al. (2015) reported three species of *Acanthus* from mangrove habitats of India: *Acanthus ebracteatus* Vahl, *Acanthus ilicifolius* Linn., and *Acanthus volubilis* Wall. Subsequently, Debnath et al. (2013) described a new mangrove associate species, *Acanthus albus* Debnath, B.K. Singh & P. Giri, from the Indian Sundarbans. This species shows strong morphological similarity to *Acanthus ilicifolius,* differing mainly in flower colour, and therefore requires further detailed investigation to confirm its taxonomic status. The comparative morphology to distinguish among these species is discussed in Table 2.

Acanthus ilicifolius Linn.

Acanthus ilicifolius Linn. is a gregarious plant indigenous to wetlands and is extensively found where the river meets the ocean and occupies that place as a mangrove associate species. It is very commonly spotted by the banks of estuaries and lagoons, areas adjacent to seawater, in mangrove and mangrove associated habitats (Herawati et al., 2020). The distribution of *Acanthus ilicifolius* Linn. in India and West Bengal has been summarised in Table 3 and Table 4, respectively.

Morpho-anatomical features and adaptations

Root

Morphology: *Acanthus ilicifolius* Linn. has adventitious aerial roots or stilt roots that develop from the reclining stem, particularly at the nodal regions (Lissy and Simon, 2001).

Anatomy: The outer root layers, i.e., exodermis and hypodermis, are thickened with suberin and lignin. The root cortex is characterised by well-developed aerenchyma. The central cylinder or stele contains the vascular tissues (Pi et al., 2009).

Inference: The stilt roots help the plant to anchor itself in its coastal habitat's muddy and saline soils. The large, well-developed aerenchyma in roots facilitates efficient oxygen diffusion from shoots to roots. The suberin and lignin prevent oxygen from leaking out prematurely into the soil, ensuring its supply to the root apex (Pi et al., 2009).

Stem

Morphology: The stem is thick, cylindrical, glabrous, green to purplish in colour. The plant has opposite phyllotaxy, with each node bearing two leaves, and each petiole base carrying a pair of spines, resulting in four spines per node (Surya et al., 2018).

Anatomy: Stem epidermis is single-layered with a thin layer of cuticle. Within the epidermal layer, sclerenchymatous hypodermis is observed. The outer cortex contains collenchyma, and the inner cortex contains parenchyma cells and large mucilage cells. Vascular bundles are open, conjoint and collateral (Hilal and Hilal, 2019). Parenchymatous cortex and pith contain tannin cells and sclereids (Surya et al., 2018).

Inference: The presence of secondary metabolites such as tannins and oil-filled cells in the cortical region of the stem protects the tissues from dehydration and desiccation, as tannin and oil deposition lower insulation. The sclerenchymatous hypodermis strengthens the stem and is also a protective mechanism against desiccation to mitigate salinity stress in its habitat (Hilal and Hilal, 2019).

Leaf

Morphology: Green, leathery, simple, petiolate, opposite, lanceolate to broadly lanceolate; margin partly entire, partly spiny and dentate; leaf base attenuate, leaf tip acute and narrowly pointed with spiny edge; presence of spines throughout (Ragavan et al., 2015).

Anatomy: Transverse section of the leaf reveals a dorsiventral leaf where the median vein has several irregularly shaped bundles. The epidermis is multi-layered, made up of 2-3 layers of parenchymatous cells and coated with a thick waxy cuticle (Hilal and Hilal, 2019). The epidermis bears salt glands on both adaxial and abaxial sides, and each salt gland structurally has a stalk cell and a head containing more cells. Stomata are sunken and found mostly on the abaxial side (Surya et al., 2018). Within the epidermal layer, two-layered, closely packed palisade cells are present. Three vascular bundles are present, where two smaller ones are on the side, and the large bundle is located at the centre. The vascular bundles are covered by a multi-layered sclerenchymatous bundle sheath. The two small bundles contain a single group of xylem, in turn surrounded by phloem. The large central vascular bundle consists of 4 groups of xylem, and between the two groups of xylem, parenchymatous cells are seen. Spongy parenchyma tissue with little intercellular space is observed below the palisade tissue (Hilal and Hilal, 2019).

Inference: Multi-layered epidermis in the leaf is a xerophytic adaptation to prevent excessive water loss due to transpiration and maintains structural rigidity in leaves to resist shrinking. The presence of thick waxy cuticle and sunken stomata is an additional feature to combat the physiological drought conditions in the mangrove habitat. The upper epidermis contains salt-accumulating glands at intervals (Hilal and Hilal, 2019). These multicellular glands are

responsible for accumulating and expelling the excess salt and help in mineral regulation (Borkar et al., 2009). Reduced intercellular spaces between spongy parenchyma tissues allow the cells to be more packed and retain water to dilute the accumulated internal salt concentration. It also lowers the rate of diffusion, preventing excess water loss. (Hilal and Hilal, 2019).

Fruit

Morphology: Fruit is an ovoid, shiny green capsule with a tough leathery (coriaceous) exterior that dehisces along the dorsi-ventral line. Seed ovate in shape, 3-4 in number (Hilal and Hilal, 2019). Seeds are whitish in appearance and supported on a short hooked retinacula or jaculator (J.S.R. Aluri et al., 2017).

Anatomy: Transverse section of fruit reveals that the outer cortex consists of sclereids followed by chlorophyllated polygonal-shaped parenchyma cells. Inner cortex consists of sclerieds with a groove—tracheids present in the inner cortex (Hilal and Hilal, 2019).

Inference: The capsule splits violently in the dorsiventral plane when ripe and dry. The seed stalk is modified into a hook-shaped jaculator that forcibly ejects the seeds during fruit dehiscence to aid seed dispersal up to a distance of two metres. This adaptation in muddy habitats contributes to the formation of monotypic stands and extension of population (J.S.R. Aluri et al., 2017).

Ethnobotanical and ethnomedicinal uses

Acanthus ilicifolius Linn. is enormously medicinal among all the species of *Acanthus* genus (Zohora et al., 2023). In traditional medicine throughout the world, particularly Chinese and Indian medicine, *Acanthus ilicifolius* Linn. possesses innumerable therapeutic and medicinal properties for addressing a wide range of health issues (Ibrahim et al., 2024).

In Indian and Chinese traditional medicine, *Acanthus ilicifolius* Linn. is employed in the treatment of psoriasis, asthma, rheumatism, paralysis, leukaemia, stomach ailments, diabetes, hepatitis, leprosy, dyspepsia, ringworm, and various skin infections. Moreover, it can also treat oedema and inflammation of the biliary system. The Indo-Chinese and Siamese applied the plant roots in the treatment of nervous disorders, damaged nerves, vaginal infection, paralysis, fluid retention and skin diseases (Ibrahim et al., 2024).

In Ayurveda, *Acanthus ilicifolius* Linn. is also referred to as 'Krishnasaireyaka' or 'Karimkurunji' and is one of the 9 plants associated to the drug 'Sahachara,' which is employed in Ayurvedic medicine to treat rheumatism. The plant decoction mixed with sugar candy and cumin is used to treat dyspepsia with acid eructation. It is also a diuretic and a cure for dropsy and bilious swellings. (Singh and Aeri, 2012).

In Thai folk medicine, it is renowned for anti-inflammatory treatment for rheumatoid arthritis and as an antipyretic, anti-abscess, anti-ulcer and laxative agent. The leaves are used to treat rheumatism, snakebite, paralysis, and asthma (Wu et al., 2023). Extracts from the bark of the plant are used to treat dermatitis and allergies. Aerial parts along with ginger is effective for relieving constipation and soothing sore legs (Ibrahim et al., 2024). In Thai traditional medicine, the plant is known for anti-inflammatory and purgative effects, and its leaves dispensed with *Piper nigrum* are used in tonic pills promoting longevity (Wöstmann & Liebezeit, 2008).

	Species name	Native range of the species	Found in India	Regional distribution in India	References
1	*A. albus* Debnath, B.K.Singh & P.Giri	India	Yes	West Bengal (Sundarbans), Andaman Islands	BSI 2026; POWO 2026; Debnath et al. 2013
2	*A. arboreus* Forssk.	SW. Arabian Peninsula	No	—	POWO 2026; Forsskål 1775
3	*A. austromontanus* Vollesen	SW. Tanzania	No	—	POWO 2026; Vollesen 2007
4	*A. carduaceus* Griff.	East Himalayas	Yes	Assam, West Bengal	BSI 2026; POWO 2026; Griffith 1854
5	*A. caroli-alexandri* Hausskn.	SE. Albania to N. Greece	No	—	POWO 2026; Haussknecht 1886
6	*A. caudatus* Lindau	Angola	No	—	POWO 2026; Lindau 1894
7	*A. dioscoridis* Linn.	Iran, Iraq, Lebanon-Syria, Transcaucasia, Turkey	No	—	POWO 2026; Linnaeus 1756
8	*A. ebracteatus* Vahl.	SW. India, Bangladesh to N. Australia; Carolline Islands	Yes	Andaman & Nicobar Islands, Odisha	BSI 2026; POWO 2026; Vahl 1791
9	*A. eminens* C.B.Clarke	Ethiopia to South Sudan and Central Kenya	No	—	POWO 2026; C.B. Clarke 1899
10	*A. flexicaulis* Bremek.	Sumatra, Malaysia	No	—	POWO 2026; Bremekamp 1955
11	*A. gaed* Lindau	N. Somalia	No	—	POWO 2026; Lindau 1894
12	*A. greuterianus* Snogerup, B.Snogerup & Strid	SE. Albania to N. Greece	No	—	Snogerup, Snogerup & Strid, 2006
13	*A. guineensis* Heine & P.Taylor	West Tropical Africa to Congo	No	—	POWO 2026; Heine & Taylor 1962
14	*A. hirsutus* Boiss.	East Mediterranean	No	—	POWO 2026; Boissier 1844
15	*A. hungaricus* (Borbás) Baen.	SE. Europe	No	—	POWO 2026; Exsiccata 1896
16	*A. ilicifolius* Linn.	Tropical and Subtropical Asia to SW. Pacific	Yes	Gujarat, Maharashtra, Goa, Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, Odisha, West Bengal	BSI 2026; POWO 2026; Linnaeus 1753
17	*A. kulalensis* Vollesen	N. Kenya	No	—	POWO 2026; Vollesen 2007
18	*A. latisepalus* C.B.Clarke	Congo to Gabon	No	—	POWO 2026; C.B. Clarke 1899
19	*A. leucostachyus* Wall. ex Nees	Bhutan to China and Indo-China	Yes	Assam, Meghalaya, E. Himalayas	BSI 2026; POWO 2026; Wallich 1830
20	*A. longibracteatus* Kurz	Myanmar	No	—	POWO 2026; Kurz 1870
21	*A. mayaccanus* Büttner	W. Central Tropical Africa	No	—	POWO 2026; Büttner 1890
22	*A. mollis* Linn.	NW. Africa, Sardinia to E. Mediterranean	Yes	Cultivated in Western Ghats	BSI 2026; POWO 2026; Linnaeus 1753
23	*A. montanus* (Nees) T. Anderson	Sierra Leone, Benin to S. Chad and Zambia	No	—	POWO 2026; T. Anderson 1863
24	*A. polystachyus* Delile	Ethiopia to NW. Tanzania	No	—	POWO 2026; Delile 1826
25	*A. sennii* Chiov.	Ethiopia	No	—	POWO 2026; Chiovenda 1940
26	*A. seretii* De Wild.	South Sudan to NE. DR Congo	No	—	POWO 2026; De Wilderman 1910
27	*A. spinosus* Linn.	SE. Europe to S. Turkey	No	—	POWO 2026; Linnaeus 1753
28	*A. tetraploideus* H.Feng & Y.L.Huang	Hainan	No	—	POWO 2026; Feng & Huang 2024
29	*A. ueleensis* De Wild.	South Sudan to Malawi	No	—	POWO 2026; De Wilderman 1910
30	*A. volubilis* Wall.	Bangladesh to W. Malaysia	Yes	Andaman Islands, Odisha, West Bengal (Sunderbans)	BSI 2026; POWO 2026; Wallich 1831

In Malaysia, seeds of *A. ilicifolius* Linn. and *A. ebracteatus* Vahl. are consumed for curing urolithiasis (Bora et al., 2017). Malay communities in Malaysia use the plant seeds to mediate cough and to deworm infants and young children. The crushed fruit can be used to purify blood and as a dressing for burns. Roots are used to treat shingles. Either the fruit or root extract is applied to affected areas in case of snakebites or arrow wounds (Ibrahim et al., 2024).

Phytochemistry and pharmacology

Through several phytochemical studies, *Acanthus ilicifolius* Linn. has been worked upon owing to the presence of various classes of secondary metabolites, namely alkaloids, benzoxazinoids, lignans, terpenoids, flavonoids, triterpenoids and steroids (Pradhan et al., 2022).

Zohora et al. (2023) has reported that several phytoconstituents have been isolated from the plant parts like alkaloid acanthicifoline from the whole plant extract; 2-benzoxazolinone, pentacyclic triterpenoids, sterols, methyl apigenin 7-o-β-D-glucuronate-flavone glycosides, bisoxazolinone from leaves; lignan, cyclolignan glycosides, benzoxazinoid glucosides, aliphatic alcohol glycoside-ilicifolioside C and two Z-4-coumaric acid glycosides from aerial parts; 1,4-benzoxazinone from pods and saturated fatty acids from stem of the plant.

Table 2: Comparative morphological traits of the *Acanthus* species in India (Ragavan et al., 2014; Ragavan et al., 2015; Ragavan et al., 2016) and eFlora of India (2024).

Species	Habit	Leaf	Bracts & Bracteoles	Corolla
A. ilicifolius Linn.	Erect shrub, often with stilt roots.	Rigid, wavy margins with sharp spines at lobes.	Bracts ovate, bracteoles persistent.	Blue or purplish-blue.
A. ebracteatus Vahl.	Similar to *A . ilicifolius* Linn. but smaller in size.	Similar to *A. ilicifolius* Linn. but with fewer spines.	Bracteoles absent or caducous.	White.
A. volubilis Wall.	Climber.	Oblong/lanceolate, entire margins with no spines.	Bracts deciduous and bracteoles absent; no spines.	White.
A. carduaceus Griff.	Low-growing herb.	Densely spiny, thistle like.	Very spiny, prominent.	White or pale.
A. mollis Linn.	Large perennial herb.	Large, broad, cordate base, usually spineless.	Large, purplish.	White with purple veins.
A. leucostachyu Wall. ex Nees	Prostrate or erect herb.	Elliptic, hairy, usually sub - entire to serrate.	Dense white hairy spikes.	White.
A. albus Debnath, B.K.Singh & P.Giri	Erect shrub.	Similar to *A. ilicifolius* Linn.	White floral parts.	White.

Table 3: Distribution of Acanthus ilicifolius Linn. across India.

State / UT	Locations	River banks	References
West Bengal	Indian Sundarbans	Banks of Matla, Raimangal, and Hooghly rivers.	Kumar et al. (2012)
Odisha	Bhitarkanika National Park and Wildlife Sanctuary	Muddy banks of the Brahmani and Baitarani rivers.	Panda et al. (2017)
Andhra Pradesh	Coringa Mangrove Forest	Gautami-Godavari and Krishna River deltas.	J. S. R. Aluri et al. (2017)
Tamil Nadu	Pichavaram and Muthupet Mangrove Wetland	Vellar-Coleroon estuarine Paminiyar, Koraiyar, and Kilaithankiyar rivers.	Kathiresan (2000); Selvam (2003)
Kerala	Ayiramthengu Mangrove Forest, Kollam, Thiruvananthapuram, Kottayam, Ernakulam, Kochi	Banks of Karamana and Neyyar, Meenachil and Periyar rivers; backwaters.	Surya et al. (2018); Neethu G. Pillai and Harilal C.C. (2015).
Karnataka	Karwar Coast, Kundapura, Mangalore	Estuaries of Kali, Netravati and Panchagangavali rivers.	Khajure and Rathod (2010); Singh et al. (2013)
Goa	Chorao Island & Dr Salim Ali Bird Sanctuary	Banks of the Mandovi and Zuari rivers; Cumbarjua Canal.	Jagtap (1985)
Maharashtra	Dahanu Taluka, Ratnagiri, Thane Creek, Vikhroli	Banks of Suri, Dahanu and Savitri rivers; Mithi River Basin.	Raut et al. (2025); Kulkarni and Bhosale (2021)
Gujarat	Navsari, Gulf of Khambhat, Marine National Park, Jamnagar	Intertidal zones of the Gulf of Khambhat.	Devi and Pathak (2016); Singh, J.K. (2020)
Andaman & Nicobar	South Andaman, Baratang, Middle Andaman, Mayabunder, Havelock	Muddy shores and inland tidal creeks.	Ragavan et al. (2016); Debnath (2004); Dam Roy et al. (2009)

Chemical constituents isolated from the parts of *Acanthus ilicifolius* Linn. were documented in a recent study by Ibrahim et al. (2024). Alkaloids (acanthicifoline, benzoxazin-3-one, benzoxazinoid, glucosides, 2-benzoxazolinone, acanthoside and trigonelline), flavonoids (quercetin, catechin, vitexin, epigalin, apigenin), aliphatic glycosides (ilicifolioside B & ilicifolioside C) were isolated from leaves, roots and aerial parts; terpenoids (lupeol, oleanolic acid, ursolic acid, α-amyrin and β- amyrin), coumaric acid (acancifoliuside), steroids (stigmasterol, stigma-7-en-3β-ol, β-sitosterol, campesterol, cholesterol), fatty acids (2,3dihydroxyhexadecanoic acid propyl ester & 1-monooleoyl-glycerol) and phenylethanol glycosides (acteoside, isoacteoside, martynoside) were isolated from leaves of the plant. Two cyclolignan glycosides, (+)-lyoniresinol

Table 4: Distribution of *Acanthus ilicifolius* Linn. in West Bengal.

District	Locations	River Banks	References
South 24 Parganas	The Sundarbans (Sajnekhali, Sudhanyakhali, Dobanki), Sagar Island, Bakkhali, Henry's Island, Jharkhali, Canning	Banks of Matla, Raimangal, and Datta rivers, Hooghly and Saptamukhi rivers.	Kumar et al. (2012)
East Midnapore	Digha, Shankarpur, Mandarmani, Kanthi	Subarnarekha river and coastal canals.	Das et al. (2015)
North 24 Parganas	Dhamakhali, Minakhan, Sandeshkhali	Banks of the Bidyadhari and Ichamati rivers.	Kumar et al. (2012)

3a-O-β-D-galactopyranosyl-(1 → 6)-β-D-glucopyranoside and (+)-lyoniresinol 2a-O-β-D-galactopyranosyl-3a-O-B-D-glucopyranoside have been reported from aerial parts of *A. ilicifolius* Linn. (Ibrahim et al., 2024).

Continued studies have revealed that *A. ilicifolius* Linn. has significant pharmacological and bioactivities like cytotoxic activity, anti-inflammatory activity, anti-osteoporotic, anti-leishmanial, chemopreventive activity, hepatoprotective activity, antioxidant, antimicrobial, antiulcer and osteoblastic activity due to the presence of these clinically important classes of secondary metabolites. (Pradhan et al., 2022; Ibrahim et al., 2024). *Acanthus ilicifolius* Linn. has potential tumour-reducing and anti-cancer effects, as the compounds from the plant have shown cytotoxic effects and induced apoptosis in various cancer cell lines. These results provide a promising direction for the development of novel pharmaceuticals and natural remedies. The preliminary studies seem promising, but it is necessary to acknowledge that more comprehensive research is required to unlock the full potential of *A. ilicifolius* Linn. (Ibrahim et al., 2024).

Conclusion

Despite being extensively prevalent in the tropical and subtropical regions of India, *Acanthus ilicifolius* Linn. remains surprisingly underutilised in pharmacological and clinical settings. The presence of prominent spines may have served as a morphological deterrent, leading to the underutilisation of a resilient biological resource. Numerous phytochemical studies have reported that *Acanthus ilicifolius* Linn. harbours a wide range of beneficial bioactive compounds and various classes of secondary metabolites. Ethnobotanical records from India, China, Malaysia and Thailand have highlighted the plant's importance in the traditional medicinal framework and attested to its efficacy and therapeutic effects. Yet a critical gap remains between theoretical data and application. Therefore, rigorous and targeted research on this halophytic species is imperative to fully harness its biomedical benefits for the betterment of public health.

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Nano-encapsulation of PGPR: advancing molecular insight and enhancing biofertilizer and biopesticide efficacy

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Abstract:

Chemical pesticides and fertilizers are widely used in modern agriculture to increase crop output, but their indiscriminate and long-term use has become a significant obstacle to sustainable farming. Overuse of these agrochemicals causes environmental contamination, pesticide resistance, loss of beneficial microbes, nutritional imbalance, and soil degradation. In addition to biotic elements like microbial diversity, plant-microbe interactions, and pest dynamics, physicochemical characteristics like soil pH, temperature, moisture, and nutrient availability have a significant impact on soil health, which is a crucial predictor of crop development and production. While biotic factors control soil fertility, disease prevention, and general ecosystem stability, abiotic factors such as severe temperatures, pH changes, and water stress impact nutrient uptake and metabolic processes in plants. Beneficial bacteria known as plant growth-promoting rhizobacteria (PGPR) are essential to sustainable agriculture because they promote plant growth in a number of ways. In contrast to infections, PGPR provides biocontrol, increases nutrition availability, increases abiotic stress tolerance and creates phytohormones. Nano-encapsulation offers a powerful approach in agriculture by protecting bioactive compounds and beneficial microbes from environmental stress while allowing their targeted and controlled release. By reducing input losses and minimizing chemical misuse, nano-encapsulation encourages more precise, sustainable, and environmentally friendly farming practices. By increasing antioxidant activity, nanomaterials promote plant resilience to abiotic stresses. Additionally, microbial nanoparticles aid in the management of plant diseases that could otherwise have a detrimental effect on plant health. This paper looks at the current state of PGPR research, its implementation, and possible future directions to improve its use in promoting un-contaminating agriculture.

Keywords:

Biofertilizer, Encapsulation, PGPR, Nanoparticles, sustainable agriculture, Microorganisms, Technique, Biopesticide.

Introduction

The excess use of chemical fertilisers and pesticides not only decreases the soil fertility but also increases the costs of production and pollutes the environment. This is true especially when plants cannot take up these chemical inputs completely. Continuous reliance on synthetic agrochemicals is likely to disrupt the microbial flora and degrade the overall sustainability of agroecosystems; thus, the need to shift towards eco-friendly alternatives has become a necessity. To protect the biodiversity, the health of the soil and the well-being of the farmers, it is becoming increasingly important to add biofertilizers either as substitutes or as additions. The root soil zone or "rhizosphere" as it was named by Hiltner in 1904, is a microbe-rich environment where microbes can interact with the root either neutral, harmful or extremely beneficial. Plants subjected to stress from living or nonliving factors can use Plant Growth-Promoting Rhizobacteria (PGPR) as an environmentally friendly, self-propagating biological means to restore the balance and promote growth. Their antagonistic as well as synergistic encounters are the main factors that have led to the marked enhancement of the plant growth. These have been widely used across the world to enhance

soil conditions and increase agricultural production. PGPR not only enhance plant growth, but also serve as a means of keeping the soil ecosystem healthy and sustainable for continuous crop production. Besides supplying growth-promoting chemicals, the mechanisms of PGPR in promoting growth are mainly indirect ways, such as rhizo-remediation and stress alleviation . The processes through which PGPR colonise the rhizosphere deeply involve several processes using which they compete with other soil microflora. These include nitrogen fixation, phosphate solubilisation, production of siderophores, indole-3-acetic acid (IAA), ACC deaminase, and hydrogen cyanide'. In addition, many other plant growth-promoting traits have been ascribed to PGPR such as phytoremediation, heavy metal detoxification, salt tolerance, and conferred biological control to plants by combating phytopathogens and insects. Unfortunately, these efficacious benefits do not come without the associated drawbacks of using typical PGPR bioinoculants under field conditions, such as, low survival rate (only little percentage of inoculants), slow growth rate, poor shelf life, and sensitivity to some environmental factors including desiccation and temperature. One of the most important points of integration of microbiology and nanotechnology in the development of desired solutions that overcome these drawbacks of formulation. Nano biofertilizers are known to have slow release characteristics which help in attaining the maximum efficiency of the nutrients added and at the same time limiting the losses. Therefore, the symbiotic union of PGPR with nanoparticles has a great potential of resulting in very effective outputs leading to significantly high levels of bacterial colonisation, enhanced nutrient transfer and positive influences on plant physiological functions.

Molecular Insights into PGPR function

Signal molecules and receptors

Plant growth-promoting rhizobacteria (PGPR) are drawn to the rhizosphere by root exudates . A range of organic substances, such as sugars, amino acids, and secondary metabolites, are present in these exudates and act as chemical cues to helpful microorganisms. For example, root exudates of potato significantly regulate the transcriptome response of *Bacillus mycoides*, suggesting that these exudates are essential in creating advantageous plant-microbe interactions. It also highlights the significance of root exudates in attracting and choosing particular microbial populations by discussing how they affect the expression of bacterial genes involved in microbe-plant interactions. Plants and PGPR communicate through specific signal molecules like flavonoids and strigolactones. For instance, flavonoids also participate in the biosynthesis processes that control interactions between microbes and plants. In addition to drawing advantageous bacteria, these chemicals also alter their behaviour to promote symbiosis and colonisation. Another class of signal molecules called strigolactones contribute to the development of symbiotic connections with mycorrhizal fungi and PGPR. PGPR have particular receptors that identify and react to these signals coming from the plants. These receptors facilitate colonisation by allowing the bacteria to recognise and approach the root exudates.

Colonization and biofilm formation

A crucial stage in the colonisation process is the initial attachment of PGPR to root surfaces. Bacterial surface features like pili and flagella, which enable close contact with the epidermis, frequently mediate this attachment. A key component of PGPR colonisation is biofilm formation, which gives the bacteria a safe haven and increases their capacity to endure in the rhizosphere. In addition to facilitating nutrition exchange, biofilms shield bacteria against antimicrobial substances and environmental stressors. Due to their ability of long-term colonisation and interaction with the host plant, they significantly lead to biofilm formation in the context of plant-microbe interactions. The existence of other microbial communities, soil conditions, and the makeup of root exudates are some of the variables that affect how well PGPR colonisation works, how soil characteristics like pH and moisture stress affect root-microbe interactions and, consequently, colonisation efficiency. Furthermore, harmful bacteria in the rhizosphere may compete with PGPR for nutrients, affecting the success of their colonisation.

Modulation of root architecture

PGPR can have a major impact on root architecture and development, encouraging branching and root growth. The synthesis of plant hormones and other growth-promoting chemicals frequently mediates this regulation. For instance, during interactions with *Azospirillum brasilense*, rice roots exhibit differentially expressed genes, many of which are implicated in hormone signalling pathways that control root development. Through the synthesis and regulation of plant hormones including auxins, cytokinins, and gibberellins, PGPR affects root architecture . Particularly important for root branching and elongation are auxins. According to the study, PGPR can change the hormonal balance in the plant to encourage root growth by highlighting the role of hormone signalling pathways in the interaction between rice roots and *Azospirillum brasilense*. In order to improve plant stress tolerance, PGPR also interacts with plant stress hormones like ethylene and abscisic acid (ABA).

Important PGPR characteristics such the *nif* gene cluster-mediated biological nitrogen fixation improves nitrogen availability in the rhizosphere, resulting in better nitrogen uptake, more biomass buildup, and higher crop yields. Similarly, phosphorus bioavailability is increased by phosphate solubilization, which is fuelled by the secretion of organic acids and phosphatase enzymes encoded by genes like *gcd* and *pho*. This encourages the growth of roots and the effective use of nutrients. Additionally, via generating phytohormones including cytokinins, gibberellins, and indole-3-acetic acid (IAA), PGPR modifies the hormonal balance of plants. By encouraging the development of lateral roots and root hair, IAA biosynthesis pathways alter the molecular structure of the root system and enhance water and stress-relieving enzymes.

A type of plant immunity known as induced systemic resistance (ISR) is brought on by helpful microbes, such as PGPR in the roots, which prepares the entire plant for more robust and quicker defenses against a variety of diseases, including those that are necrotrophic or caused by insects, which involves signaling molecules including ethylene and jasmonic acid. Induced Systemic Resistance (ISR) is initiated when beneficial rhizosphere microbes release Microbe-Associated Molecular Patterns (MAMPs) that are recognized by root receptors, triggering early signaling events like calcium influx and MAPK cascades. Regulated crucially by the transcription factor

MYB72 at the root level, this local recognition generates a salicylic acid (SA)-independent systemic signal that travels to aerial tissues to activate jasmonic acid (JA) and ethylene (ET)-dependent pathways. Relying on the NPR1 regulator, this JA/ET signaling primes the plant for "latent innate immunity," avoiding the metabolic cost of continuous defense activation. Upon subsequent pathogen attack, these primed cells rapidly mount a robust defense by strongly upregulating JA/ET-responsive genes (e.g., PDF1.2, ERF1), reinforcing physical cell wall barriers via lignin and peroxidase synthesis, and deploying chemical defense enzymes like chitinases and β-1,3-glucanases.

Simultaneously, in adverse conditions, plant ethylene levels are lowered by PGPR-produced ACC deaminase, which is expressed by the *acdS* gene. This lessens growth inhibition caused by salinity, drought, or heavy metal toxicity. These molecular-level reactions result in lower disease incidence, higher stress tolerance, and more consistent crop production under less-than-ideal climate conditions. Importantly, the effectiveness of these molecular pathways depends on PGPR's successful colonisation and persistence in the rhizosphere (Fig 1).

Nano-encapsulation techniques and materials

Concept of Nano-Encapsulation:

Bioactive substances (polyphenols, micronutrients, enzymes, antioxidants, and nutraceuticals) were protected by the encapsulation process, which was also used in the final application to shield them from the environment and allow for regulated release at specific locations. Particles with a diameter ranging from 3-800 μm are called microcapsules. Both nanocapsules and nanospheres are examples of nanoparticles, which are colloidal-sized particles having dimensions between 10 and 1,000 nm. Whereas nanospheres are matrix systems where the bioactive chemical is evenly distributed, nano capsules are vesicular systems where the bioactive compound is contained within a cavity encircled by a special polymer membrane. Bioactive

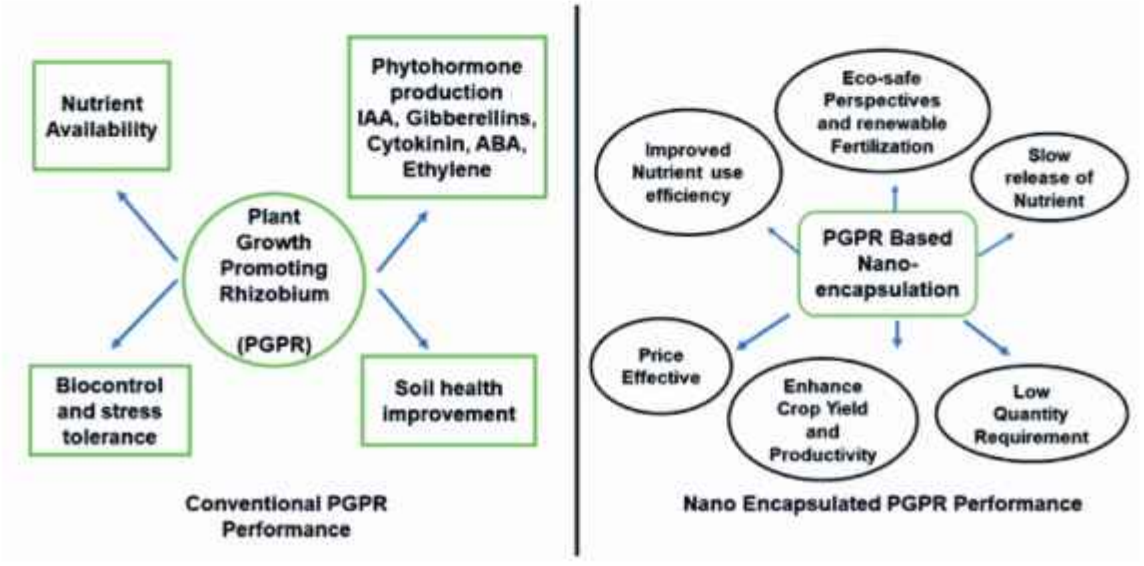


Fig 1: Comparative diagram of conventional and nano-encapsulated PGPR performance

packaging at the nanoscale range is referred to as nanoencapsulation, which is a technology to encapsulate chemicals in small. The particle size has a direct impact on how well any bioactive substance is delivered to different parts of the body. Therefore, compared to microencapsulation, nanoencapsulation has the potential to improve controlled release, increase bioavailability, and enable precise targeting of the bioactive chemicals.

Materials used for Encapsulation

Due to a greater surface area per unit volume and consequently higher biological activity, reducing the particle size may enhance the nutraceuticals' bioavailability, delivery characteristics, and solubility. Hydrophilic substances dissolve in water but remain insoluble in organic solvents and lipids. Ascorbic acid, polyphenols, and other hydrophilic nutraceuticals are among those that are nano encapsulated. Lipophilic substances are soluble in organic solvents and lipids but insoluble in water. The proper combination of diffusion and erosion mechanisms determines the release kinetics of hydrophilic substances, which exhibit faster release rates. The most popular nanocarrier food systems for encapsulation are lipid or naturally biodegradable polymer-based capsules. Three lipid-based nanocarrier systems are used in the food, cosmetic, and pharmaceutical industries: nanoliposomes, archaeosomes, and nanocochleates. For the creation of nano delivery systems, natural polymers such albumin, gelatin, alginate, collagen, chitosan, and α-lactalbumin were utilised (Fig 2).

Enhanced efficacy as biofertilizers and biopesticides

Nano-encapsulated PGPR as biofertilizers

While nanoparticles assist in the slow and steady release of nutrients in accordance with crop need in a synchronized way, PGPR in nano-biofertilizer (a bioorganic component) aids in nitrogen fixation, phosphate solubilization, and soil fertility restoration. By fixing nitrogen, cellulolytic

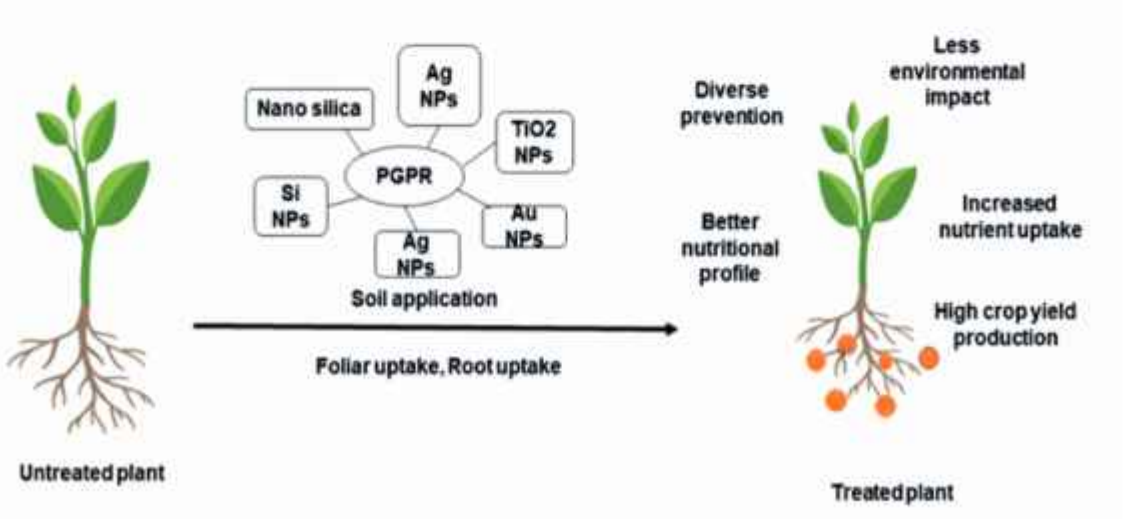


Fig 2: Workflow of showing encapsulation, soil application and plant response

activity, or phosphate, beneficial bacteria found in biofertilizers enhance the chemical and biological properties of soil. They live in the rhizosphere when they are applied to seeds, plant surfaces, roots, or soil. Through their biological activity, they improve nutrient bioavailability, encourage plant growth and boost the microbiota in the soil. As a result, they are preparations that quickly increase soil fertility. *Rhizobium* has symbiotic relationships with legume roots, including rhizobacteria that live in the rhizosphere's soil or on the root's surface. Blue-Green Algae (BGA), *Rhizobium,* and *Azolla* are examples of broad-spectrum biofertilizers; crop-specific bioinoculants include *Azospirillum, Azotobacter,* and phosphorus. *Anabaena,* vesicular-arbuscular mycorrhiza (VAM), and solubilising bacteria (PSB) as cyanobacteria that fix nitrogen. These bacteria are referred to as plant growth-promoting rhizobacteria (PGPR) and biofertilizers. The enzymatic activity of PGPR for crop production carries out competition mechanisms and antagonistic activity, such as the suppression of phytohormones and phytoparasites; it also aids plants in withstanding stress caused by pollution and heavy metal contamination. Biofertilizers are economical, environmentally beneficial, and, if needed, may be generated in large quantities on the farm. Up to 50% of the nitrogen is fixed, and the crop production is raised by 10–40%. Applying biofertilizer to the field consistently for three to four years can preserve fecundity, as a result of the effectiveness of parental inoculums, which could successfully sustain plant growth and multiplication and enhance the pH, texture, and other characteristics of the soil . Renewable sources of plant nutrients that supplement artificial fertilisers. Compared to chemical fertilisers, biofertilizers are less harmful to the environment, may be made from natural sources, assist to develop healthy soil, and, to some extent, cleanse plants of precipitated chemical fertilisers. As a contemporary agricultural tool, biofertilizer is essential for integrated nutrient management, reducing the use of dangerous chemicals, being economical, and providing plants with renewable energy in sustainable agriculture. The process of fixing nitrogen is dynamic and energy-intensive. One method of transforming elemental nitrogen into a form that plants can use is through biological nitrogen fixation. In addition to fixing nitrogen, *Azotobacter* uses plant hormones to create vitamins including riboflavin and thiamine, indole acetic acid (IAA), cytokinins (CK), and gibberellins (GA).

Nano-Encapsulated PGPR as Biopesticides

Biopesticides are garnering international interest as a novel tool for eliminating or managing pest species like weeds, plant diseases, and insects since they are less hazardous to people and the environment than traditional pesticides. The majority of biopesticides are beneficial for increased selectivity and non-target biological safety. Biopesticides are a class of insecticides made from naturally occurring materials that use nontoxic processes to manage pests in an environmentally beneficial manner. Biopesticides originating from microorganisms (*Bacillus thuringiensis*, Trichoderma, and the nucleopolyhedrosis virus), plants (*Azadirachta* and *Chrysanthemum*), and animals (nematodes) comprise their byproducts (semiochemicals) and products (microbial products and phytochemicals), as well as living organisms (natural enemies). Pest-controlled microorganisms (microbial pesticides), naturally occurring pest-controlled compounds

(biochemical pesticides), and plant-controlled pesticides with additional genetic material (PIPs) are the three primary categories of biopesticides . Globally, the usage of biopesticides has grown by almost 10% annually. Similar to chemical pesticides, biopesticides are natural or biologically derived substances that manage pests in an environmentally benign manner. All pest control chemicals, especially microbiological agents, are beneficial, but they must be properly prepared and applied.

Microorganisms including bacteria, fungus, viruses, and protozoa make up microbial biopesticides. Oomycetes, which are typically employed to biologically manage pestiferous insects, weeds, and plant diseases. For a variety of crops, 74% of the market is assured – by bacterial biopesticides, 10% by fungal biopesticides, 10% by viral biopesticides, 8% by predator biopesticides, and 3% by others. Microbial pesticides can reduce a variety of target pests by producing harmful metabolites or in other ways. Microbial insecticides are often harmless and non-pathogenic to all living things, and they are not strictly limited to the pests they are intended to control. The naturally occurring or synthetically generated substances employed in pest management are equivalent to biochemical pesticides. Biochemical pesticides, which are nontoxic in action and either eliminate or target insect pests, have an impact on the growth and development of insect pests.

Safety, challenges and future perspectives

Environmental challenges

The survival, growth, and effectiveness of microbial inoculation are significantly influenced by the pH of the soil. Microbial development can also be strongly influenced by soil properties as water content, texture, cation exchange capability, and nutrient uptake. Because most phosphorus (P) binds with aluminium (Al), iron (Fe), and calcium (Ca), acidic and alkaline soils lack P, which makes it difficult for microorganisms to exist in nutrient-deficient soils. Additionally, eutrophication caused by abuse and misuse of synthetic P fertilisers is one of the P issues. The abundance of salt in saline sodic soils makes it extremely difficult for microorganisms to exist in such hostile environments. Environmental variability, such as soil type, pH, moisture content, temperature changes, and native microbial competition, has a significant impact on the effectiveness of nano-encapsulated plant growth-promoting rhizobacteria (PGPR). Although nano-encapsulation provides defense against abiotic stress, little is known about how well it works in challenging field situations.

Formulation of shelf life

To prevent inadvertent ecological disruptions and to maintain soil health, long-term interactions between nanocarriers, soil bacteria, and plant roots also need to be carefully studied. According to projections, salt stress will affect roughly 50% of agricultural land by 2050. Microbial development and soil enzyme activity are negatively impacted by these heavy metals. Consistent PGPR performance is severely hampered by seasonal variations in temperature and changes in soil moisture brought on by erratic rainfall patterns. By modifying the osmotic activity of the soil and the mix of plant-root exudates, flooding can significantly change the dynamics of microbial

inoculums. This increases competition among bacteria for nutrients. It is anticipated that as the climate changes, these environmental circumstances will get worse. Numerous native microorganisms can be found in soil, and it is commonly known that microbial inoculants eventually lose their vitality. Because PGPR has a short shelf life, delivering PGPR inoculants is extremely difficult. Each plant may react differently to microbial inoculants and create distinct root exudates that are enticing to particular microorganisms. One of the most important challenges is keeping PGPR viable during formulation, storage, and application. For microbial respiration and progressive release, nano-encapsulation devices must strike a compromise between mechanical stability and permeability. Shelf life and biological efficacy are greatly impacted by variables such carrier composition, encapsulation technique, and storage circumstances. In order to achieve extended storage stability without sacrificing microbial activity upon release, future formulations should concentrate on optimizing carrier porosity, moisture retention, and protective matrices. To get the intended results, inoculated seeds or plants can need certain methods. Furthermore, there is a lack of knowledge on how microorganisms and plants interact and impact agricultural productivity. To limit the effects of climate change, food demand, and environmental security, agricultural settings must overcome these obstacles and optimise PGPR inoculant methods .

Regulatory and adaption barriers

High output yield, improved crop production, and soil fertility in an environmentally responsible way are all necessary in today's world. Because there are few regulations addressing nano-enabled biological inputs, nano-encapsulated PGPR formulations risk regulatory uncertainty despite their potential advantages. Approval procedures are complicated by issues with environmental safety, nanomaterial persistence, and effects on non-target creatures. Widespread adoption is further hampered by high manufacturing costs, low field-level validation, and a lack of farmer awareness. Commercialization and farmer acceptability will be facilitated by clear legal frameworks, standardized testing procedures, and stakeholder participation. Therefore, the research must concentrate on the novel idea of rhizo-engineering, which is based on the advantageous partitioning of exotic biomolecules that produce a special environment for the interaction between microorganisms and plants. Future rhizosphere biology research will rely on the advancement of molecular and biotechnological tools in order to improve our comprehension of rhizosphere biology and achieve an integrated control of soil microbial populations. New bioinoculant possibilities should be looked into for other high-value crops like fruits, vegetables, and flowers. Applying a multi-strain bacterial consortium rather than a single inoculation may be a useful strategy for lessening the detrimental effects of stress on plant development. An efficient method for boosting plant development at low temperatures could be the injection of ice-nucleating rhizobacteria. Research on phosphate solubilisation and nitrogen fixation by rhizobacteria that promote plant growth is progressing, but little is known about potassium solubilisation, the third key necessary macronutrient for plant growth. Additionally, the creation of the biosafety data needed for the registration of rhizobacterial agents that promote plant development will be necessary for the sale of bioinoculant products in the future and the release of these transgenics into the environment as environmentally friendly alternatives to agrochemicals. Natural nanoparticles, both primary particles and agglomerates/aggregates, are most abundant in soil. With the emergence of nanotechnology, novel nanodevices (such as biosensors and enzyme encapsulation) and

nanomaterials (such as nanotubes, nanowires, fullerene derivatives, and quantum dots) will likely find use in the fields of agriculture and life sciences . These materials are exceptional and essential in many areas of sustainable agriculture development because of their special size-dependent features. In plant pathology, nanoparticles target particular agricultural issues in plant-pathogen interactions and offer novel approaches to crop protection.

Future research directions

The creation of biodegradable, bio-based nanocarriers suited to rhizosphere-specific conditions should be the main focus of future research. Multi-scale validation, including greenhouse and long-term field studies in various agroecosystems, should be prioritized. Combining cutting-edge methods like systems biology, omics technology, and data-driven modelling can enhance comprehension of PGPR–carrier–plant interactions and facilitate the logical development of next-generation formulations.The current situation presents a fantastic chance to create environmentally acceptable substances that can be readily substituted for chemical pesticides in the context of nano bio-fertilizers. Insecticides, fungicides, or nematicides can be nano encapsulated or microencapsulated to create a formulation that effectively controls pests without leaving residue in the soil. Herbicide compounds like pendimethalin and metolachlor that are encapsulated with polymers like poly styrene sulphonate (PSS) and poly allylamine hydrochloride (PAH) can be readily regulated because they only rupture in wet conditions. The long-term release of active chemicals in these encapsulated herbicides guarantees efficient weed management. Genetic modifications can be used to develop superior or innovative PGPR strains by enhancing the aforementioned features.

Conclusion

Increased antagonism, improved nutrient uptake, and overall improved plant growth result from the symbiotic connections they have with millet plants. As a result, millet plants treated with PGPR consistently yield more, show increased resistance to environmental stresses, and enhance soil fertility. This sustainable strategy supports international initiatives to advance food security and sustainable agriculture in addition to helping farmers by increasing crop output.

By facilitating carrier–microbe matching and performance prediction, developments in systems biology, omics-based microbial profiling, and data-driven optimization techniques are anticipated to further improve nano-encapsulation design. In the end, interdisciplinary cooperation between microbiologists, nanotechnologists, agronomists, and legislators will be necessary for the effective incorporation of nano-encapsulated PGPR into sustainable agriculture. Nano-enabled PGPR formulations have great potential as environmentally benign substitutes for traditional agrochemicals, supporting resilient crop production and long-term soil sustainability when combined with economical manufacture and transparent regulatory frameworks.

Long-term field trials are a critical and effective technique to evaluate their combined performance under realistic agricultural settings. Plant-microbe interactions may be impacted by the nanoparticles in the nano-biofertilizer's detrimental effects on the soil microbiome. Future study should focus on explaining interaction processes across various soils, particularly low-organic

matter tropical and sub-tropical environments. These insights will make it possible to develop customized application strategies that strengthen resilient food production systems and increase sustainability.

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Seed-borne mycoflora and eco-friendly management using medicinal plant extracts: a comprehensive review

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Abstract:

Seed-borne mycoflora constitute one of the most serious biological constraints affecting seed quality, germination, seedling vigor and crop productivity worldwide. Seeds serve as efficient vehicles for the survival and dissemination of fungal pathogens such as *Aspergillus, Fusarium, Penicillium, Alternaria, Rhizoctonia, Curvularia* and many others across agroecosystems. These fungi not only reduce seed viability but also cause seedling diseases and mycotoxin contamination, posing significant threats to food security and human health. Although chemical fungicides are widely used for seed treatment, their continuous application has raised concerns regarding environmental pollution, pathogen resistance and residual toxicity. Consequently, eco-friendly alternatives have gained attention in recent years. Medicinal plant extracts rich in phenolics, flavonoids, alkaloids and organosulfur compounds have demonstrated strong antifungal activity against a wide range of seed-borne fungi. In addition, biocontrol agents such as *Trichoderma* species suppress pathogens through competition, mycoparasitism and antibiosis. This review critically synthesizes existing literature on seed-borne mycoflora across major crop groups and evaluates the potential of medicinal plant extracts and biological agents as sustainable alternatives for seed health management.

Keywords:

Seed-borne mycoflora, medicinal plant extracts, *Trichoderma*; seed health, sustainable agriculture

Introduction

Seed quality is a fundamental determinant of crop establishment and agricultural productivity, as nearly ninety percent of global food crops are propagated through seeds. Seed-borne diseases are responsible for significant yield losses by reducing germination capacity, seedling vigor and plant stand in the field. Among seed-associated pathogens, fungi constitute the largest and most destructive group. Seed-borne fungi may survive during storage and later infect seedlings under favorable environmental conditions, acting as primary sources of inoculum. Several studies across cereals, pulses, oilseeds and vegetables have reported the dominance of fungal genera such as *Aspergillus*, *Fusarium* and *Penicillium*, particularly in tropical and subtropical regions where high temperature and humidity favor fungal proliferation (Ghosh et al., 2018; Khan et al., 2020; Neme et al., 2023). In addition to causing seed deterioration, many of these fungi are known producers of mycotoxins, posing serious risks to human and animal health (Agarwal et al., 2015; Khan et al., 2020).

Chemical fungicides are commonly used to manage seed-borne pathogens; however, their indiscriminate use has resulted in environmental contamination, development of resistant fungal strains and negative effects on beneficial microorganisms. These limitations have prompted the search for safer and sustainable alternatives. Medicinal plant extracts and biological control agents have emerged as promising eco-friendly options due to their biodegradability, availability and broad-spectrum antifungal activity (Griffin & Masih, 2021; Sharma et al., 2019; Xin et al., 2023).

Microorganisms are employed as biological control agents for seed treatments to shield seeds and seedlings from seed-borne infections. Over the past 20 years, there has been a surge in the

scientific development of biological control agents due to the growing interest in organic crop production technology and search for alternatives to chemical pesticides and. agents of biological control within the previous two decades. More knowledge of the control mechanisms employed by these agents, particularly in the soil, has led to advancements throughout this time (Ghosh et al., 2018). *Streptomyces, Pseudomonas, Bacillus,* and *Enterobacter* were prevalent among the bacterial species. Among the fungi, *Phomopsis, Ectomycorrhiza, Gliocladium, Trichoderma* and *Cladosporium* were employed (Anonymous, 2000).

Humanity has always been driven by scientific research and inventions, which are largely responsible for the standard of living today. A nation's natural resources are of primary importance for its economic development. This review critically synthesizes the body of literature on seed-borne mycoflora across major crop groups and assesses the potential of biological agents and medicinal plant extracts as sustainable alternatives for seed health management.

Sample Collection and study methods

Seed samples of various cereal crops were collected to study the prevalence and diversity of seed-borne microflora. Samples were obtained from different sources including farmers' fields, local agricultural markets and seed storage facilities to ensure representation of natural field and post-harvest storage conditions. The selection of multiple sources was intended to capture variability arising from differences in cultivation practices, harvesting time, storage duration and environmental exposure. Sampling was carried out during the post-harvest period, when the incidence of seed-borne fungal contamination is generally high. Mature seeds were randomly collected from different seed lots to minimize sampling bias. Both apparently healthy and visibly infected seeds were included to assess latent as well as externally evident fungal infections. Each sample consisted of an adequate quantity of seeds to allow repeated observations and statistical reliability (Ghosh et al., 2018; Khan et al., 2020; Neme et al., 2023).

The collected seed samples were placed in clean, sterile polythene bags or paper envelopes and properly labelled with relevant details such as crop name, place of collection, date of sampling and storage condition. Care was taken during handling and transportation to prevent secondary contamination. Samples were transported to the laboratory within a short period after collection. Prior to analysis, seeds were air-dried under shade when necessary to remove excess surface moisture. The samples were then stored under ambient laboratory conditions in a dry place until further processing (Mohana et al., 2011; Patekar & Kadam, 2018; Vikhe et al., 2021). These standardized procedures of sample collection and handling ensure that the seed-borne mycoflora observed during analysis accurately reflected the natural infection status of the seeds.

Agar plate method (PDA)

In agar plate method, 25ml of sterilized PDA medium was poured in glass petri dishes. After cooling, seed samples were transferred to the plates. Twenty seeds per petri plate were kept at equidistantly in a circle and incubated at room temperature (25 ± 2ºC) under 12 hours alternating cycles of light and darkness for seven days and has to be observed every day for the growth of fungi

(ISTA, 2005; Ghosh et al., 2018). The seed mycoflora percentage and percentage frequency of various fungal species should be calculated. The incidences of fungi on seed performed under these methods are calculated by this formula:

Plates were incubated at room temperature for seven days. The seeds should also be examined under microscope for preliminary determination of seed mycoflora. The seed-born fungi found on each and every seed were isolated and identified, brought in to pure culture and maintained on PDA plates (Gawande, 2024).

Moist blotter plate method (BPT)

In moist blotter plate; the seeds were placed on water soaked here layered white blotter papers of 8.0 cm diameter and placed in pre sterilized petridishes. 1gm sterilized seeds were placed at equal distance in duplicates and unsterilized seeds kept as control in moist blotter paper placed in pre sterilized petridish. It was then placed under microscope for the preliminary determination of seed mycoflora. The seed born fungi found on each and every seed were isolated and identified, brought in to pure culture and maintained on PDA plates. In both Agar plate method and Moist blotter method the frequency of seed-borne fungi was examined during incubation (Gawande 2024) . The result was expressed in percentage:

$$\% \text{ incidence} = \frac{\text{Number of infected seeds}}{\text{Number of plated seeds}} \times 100$$

Identification of Seed Mycoflora

Across the reviewed studies, identification of seed-borne fungi following colony development was primarily achieved through detailed assessment of cultural (colony) characteristics, which provide the first level of taxonomic resolution in seed mycology. When seeds were incubated on nutrient media such as Potato Dextrose Agar or blotter substrates, distinct fungal colonies emerged, each exhibiting characteristic growth patterns governed by species-specific physiological and morphological traits. These colony-level variations enabled preliminary differentiation among fungal genera prior to microscopic confirmation.

Colony colour, observed on both the surface and reverse side of the culture medium, was one of the most consistently used diagnostic features. Storage-associated fungi such as Aspergillus and Penicillium typically produced dark-coloured or greenish colonies with intense sporulation, whereas field-associated fungi like *Fusarium* and *Alternaria* initially developed white colonies that later acquired pink, violet, grey or brown pigmentation. Reverse pigmentation, resulting from diffusible metabolites, further assisted in narrowing fungal identity and distinguishing closely related taxa (Patekar & Kadam, 2018).

Texture and surface appearance of colonies served as additional diagnostic criteria. Powdery or granular colonies indicated abundant conidial production, commonly associated with *Aspergillus* and *Penicillium*, while cottony or fluffy colonies suggested dominance of vegetative mycelium, characteristic of *Fusarium* and *Rhizopus*. Velvety textures and concentric zonation patterns were frequently linked with *Alternaria* species. Such textural differences reflect variations in sporulation intensity and hyphal architecture, which are genetically determined and taxonomically informative (Patekar & Kadam, 2018; Gawande et al., 2024).

Growth rate and colony expansion pattern were also critical parameters in colony-based identification. Rapidly spreading colonies that covered the culture plate within a short incubation period were indicative of fast-growing fungi such as *Rhizopus* and certain *Aspergillus* species, whereas moderate growth rates were typical of *Alternaria* and *Curvularia*. Observation of colony margins, whether smooth, lobed or filamentous, further contributed to genus-level discrimination, as margin morphology reflects differences in hyphal branching and substrate colonization strategies (Neel et al., 2021).

In addition, special colony features such as concentric ring formation, radial grooves, pigment diffusion and exudate production were documented in several studies and used as supplementary identification markers. These features, when assessed collectively rather than in isolation, allowed reliable grouping of fungal isolates and facilitated comparison with standard mycological descriptions (Patekar & Kadam, 2018).

Importantly, the reviewed literature emphasizes that colony morphology alone was not used for definitive identification, but rather as a preliminary and essential screening step. Final confirmation of fungal identity was consistently achieved through microscopic examination of reproductive structures. Nevertheless, colony-based identification played a critical role in organizing isolates, narrowing taxonomic possibilities and ensuring accurate and efficient microscopic analysis.

Most prevalent seed borne fungi affecting economically important plants

Across the analysed studies covering diverse crops such as rice, maize (*Zea mays*), brinjal, spinach, mustard and other oilseed and vegetable crops, a striking similarity emerges in the composition and dominance of seed-borne microflora. Irrespective of crop species, geographical location, or experimental methodology (PDA, blotter plate, agar plate methods), the fungal communities associated with seeds show a highly conserved pattern (Ghosh et al., 2018; Khan et al., 2020; Neme et al., 2023). These studies collectively demonstrate that seed-borne fungi remain one of the most critical biological constraints affecting seed health, germination, seedling vigour and ultimately crop productivity. The recurring isolation of similar fungal genera across multiple hosts highlights the ecological adaptability of these fungi and underscores the universal vulnerability of seeds to fungal colonisation during both pre- and post-harvest stages.

Among the recorded mycoflora, species belonging to the genus *Aspergillus* consistently appear as the *most* dominant and prevalent fungi across all examined crops. *Aspergillus niger*, *A. flavus* and *A. fumigatus* were repeatedly isolated from cereal grains, leafy vegetables, oilseeds and vegetable crops, indicating their strong association with stored seeds and their ability to thrive under low moisture and suboptimal storage conditions. Their dominance is particularly alarming due to their capacity to cause seed deterioration, reduce germination percentage and produce toxic secondary metabolites such as aflatoxins (Agarwal et al., 2015; Khan et al., 2020; Gawande et al., 2024). The prevalence of *Aspergillus* spp. across both sterilized and unsterilized seed samples further indicates their resilience and persistent nature, making them the most problematic storage-associated mycoflora affecting agricultural systems (Ghosh et al., 2018; Vikhe et al., 2021).

Closely following *Aspergillus*, species of *Fusarium* emerged as another major and widely distributed group of seed-borne fungi. *Fusarium oxysporum*, *F. moniliforme* and related species were commonly associated with cereals and vegetables, where they contribute significantly to seed rot, seedling blight, damping-off and wilting diseases. Unlike purely storage fungi, *Fusarium* spp. bridge the gap between seed-borne and soil-borne pathogens, enabling them to initiate infection at the seed stage and continue disease development throughout plant growth (Mohana et al., 2011; Khan et al., 2020; Neme et al., 2023). Their repeated detection across multiple studies highlights their epidemiological importance and their role in long-term field infections originating from infected seeds (Singh et al., 2018; Ghosh et al., 2018).

The genus *Alternaria*, particularly *Alternaria alternata* and *A. brassicicola*, was frequently reported as a prevalent pathogen in oilseed and vegetable crops. These fungi are strongly associated with seed discoloration, poor germination and foliar diseases such as leaf spots and blights following seed transmission (Khan et al., 2018; Neme et al., 2023). Their ability to persist on seed surfaces and transmit efficiently from seed to seedling makes them significant contributors to early crop establishment failure. Similarly, *Curvularia* spp. were consistently reported in cereals and vegetables, especially under warm and humid conditions, where they cause internal seed infection, seed blackening and reduced seedling vigour (Ghosh et al., 2018; Vikhe et al., 2021). Though sometimes considered secondary pathogens, their repeated occurrence across studies confirms their ecological importance.

Storage-related fungi such as *Penicillium* spp. and *Rhizopus stolonifer* were also commonly isolated, particularly from improperly stored seeds. These fungi act as opportunistic colonizers, rapidly invading mechanically damaged or aged seeds and accelerating deterioration during storage. While they may not always initiate field disease, their impact on seed longevity, market quality and germination potential is substantial (Patekar & Kadam, 2018; Gawande et al., 2024). Additionally, crop-specific pathogens such as *Bipolaris, Helminthosporium, Cladosporium* and *Macrophomina* were reported in cereals and oilseeds, indicating that alongside dominant genera, host-specific fungi contribute to disease complexity and regional variation in seed health (Singh et al., 2018; Khan et al., 2020).

Taken together, the collective analysis of all reviewed papers clearly indicates that the most prevalent and agriculturally significant microflora affecting plants are seed-borne fungal pathogens, with *Aspergillus* spp. ranking as the most dominant, followed by *Fusarium, Alternaria, Curvularia* and *Penicillium* (Ghosh et al., 2018; Khan et al., 2020; Neme et al., 2023). Their consistent occurrence across multiple crops and environments suggests that seed contamination is governed more by ecological adaptability and storage practices than by host specificity alone. This widespread prevalence not only explains recurring problems of poor germination and seedling mortality but also justifies the growing emphasis on eco-friendly seed management strategies, particularly the use of plant extracts as sustainable alternatives to chemical fungicides (Griffin & Masih, 2021; Sharma et al., 2019; Xin et al., 2023).

Eco-Friendly Management Using Medicinal Plant Extracts

Extraction and application methods

Extracts were typically prepared via solvent extraction (e.g., ethanol or water) or cold

pressing. For instance, neem seed oil was diluted (1-5%) and applied as a seed treatment. Studies show efficacy at concentrations of 5-10% for garlic bulb extract against *Fusarium* in tomato seeds (El-Gazzar et al., 2017). Application involves soaking seeds for 30-60 minutes or spraying during storage, often combined with bioagents like *Trichoderma* for synergistic effects.

Efficacy Evidence from Studies: (Table 1)

- Neem (*Azadirachta indica*): A trial on sunflower seeds infected with *Alternaria* showed 70-85% reduction in fungal incidence and improved germination by 25% (Khan et al., 2018). In maize, neem extracts controlled *Aspergillus,* with mycotoxin levels dropping by 60% (Agarwal et al., 2015).

- Garlic (*Allium sativum*): Applied to wheat seeds, it reduced *Fusarium* head blight by 50%, outperforming some chemical controls (El-Gazzar et al., 2017). In legumes, garlic extract inhibited *Rhizoctonia solani*, enhancing seedling vigor (Sharma et al., 2019).

- Turmeric (*Curcuma longa*): Effective against *Penicillium* in stored grains, with 80% inhibition at 2% concentration (Singh et al., 2021). A meta-analysis of 15 studies (2020-2023) indicates average efficacy of 65-75% across crops, comparable to low-toxicity fungicides like captan.

- Other Plants: Extracts from eucalyptus (*Eucalyptus globulus*) and ginger (*Zingiber officinale*) show promise against seed-borne fungi in rice and pulses, with synergistic blends (e.g., neem-garlic) achieving 90% control (Patel et al., 2022).

Management using biocontrol agents:

With the growth of green agriculture methods nowadays people are urgently looking for safe, efficient, and eco-friendly plant disease control methods. Biological control primarily uses helpful organisms and their products to combat plant diseases and to efficiently limit the use of chemical fertilizers and pesticides (Harman et al., 2021). *Trichoderm*, a fungus of Deuteromycotina phylum primarily inhabits soil, air, plant surfaces fungus that is commonly employed to treat plant pests. It is capable of efficiently managing a wide range of plant diseases including soil-borne and diseases associated with leaves and spikes (Samuels et al., 2006; Vicente et al., 2020; Abbas et al., 2022). *Trichoderma* can boost plant resistance, increase nutrient usage efficiency, prevent disease, encourage plant development, and clean up agrochemical pollution (Al-Surhanee, 2022; Tyśkiewicz et al., 2022; Xin et al., 2023).

Researches conducted all over the world reported the use of two species of *Trichoderma, T. viride* and *T. harzianum* to control plant diseases caused by around 29 species of plant pathogenic fungus belonging to 18 genera, including *Botrytis, Fusarium,* and *Rhizoctonia.* Numerous plant pathogenic fungi, including *Rhizoctonia solani, Pythium ultimum, Fusarium oxysporum, Sclerotinia sclerotiorum, Botrytis cinerea, Pseudocercospora* spp., and *Colletotrichum* spp., are controlled by *Trichoderma* (Zhang Y. et al., 2022; Xin et al., 2023). *Trichoderma* has a competitive role in the soil environment, can parasitize about 18 genera of *Pythium, Phytophthora, Rhizoctonia,* and *Peronospora*, have antibiosis effect and can also induce systemic resistance. Different type of commercial treatments for *Trichoderma* spp. is expanding along with the advancement of

Table 1 : Medicinal plant-based control of seed-borne pathogens

Plant extract	Studied seed	Analyzed pathogen	Disease suppression efficiency	Seed germination	References
Azadirachta indica A. Juss *Aegle marmelos* Corr. *Calotropis procera* L. *Catharanthus roseus* L. *Datura metel* L. *Moringa oleifera* Lam. *Murraya koenigii* L. *Polyalthia longifolia* Sonn.	*Spinacia oleracea* L.	*Aspergillus flavus*., *Aspergillus niger*, *Aspergillus fumigatus*, *Alternaria alternata*, *Fusarium oxysporum*, *Rhizopus stolonifera*, *Penicillium digitatum*, *Cladosporium* spp. *Curvularia* spp.	Yes spp.	Positive	Patekar & Kadam, (2018).
Abrus precatorius L. *Aegle marmelos* (L.) Corr. *Balanites aegyptiaca* Delile. *Datura metel* L. *Dioscorea bulbifera* L. *Helicteres isora* L. *Sapindus laurifolius* Vahl. *Semecarpus anacardium*	*Solanum melongena* L.	*Alternaria tenuis*, *Curvularia lunata*, *Fusarium moniliforme*	Yes	Positive	Survase and Khandare (2025).
Azadirect indica L. *Allium sativum* L. *Piper betle* L.	*Arachis hypogea* L. *Brassica*	*Aspergillus niger* *Aspergillus flavus* *Aspergillus*	Yes	Positive	Gawande et al. (2024).
Vitex negundo L. *Parthenium hysterophorous L.* *Azadirachta indica* Juss. *Datura metel* f.	*Zea mays* L.	*Aspergillus niger* *Trichoderma viride* *Aspergillus flavus* *Fusarium oxysporum* *Mucor* spp. *Helminthosporium* *Rhizopus* spp.	Yes	Positive	Neel et al., (2021).
Acacia nilotica *Argemone mexicana* *Caesalpinia coriaria* *Emblica officinalis* *Euphorbia tirucalli* *Lawsonia inermis* L. *Leucas aspera* *Mimosops elengi* *Phyllanthus amarus* *Tinospora cordifolia*	*Oryza sativa* L.	*Alternaria alternata*, *Aspergillus flavus*, *Curvularia lunata*, *Drechslera oryzae*, *D. halodes*, *Fusarium moniliforme*, *Pyricularia oryzae* *Trichoconis*	Yes	Positive	Mohana et al. (2011).

Cinnamomum zeylanicum Blume *Ocimum basilicum* L.	*Capsicum annum* L.	*Aspergillus sp., Curvularia lunata* (Wakker) Boedijn, *Rhizopus stolonifer* (Ehrenb.) Vuill, *Fusarium* sp.	Yes	Positive	Dourado et al., (2020).
Azadirachta indica A. Juss. *Momordica charantia* L	*Moringa oleifera* Lam.	*Aspergillus* spp. *Penicillium* spp.	Yes	Positive	Lima et al., (2020).
Crassiphycus birdiae (E.Plastino & E.C.Oliveira) Gurgel	*Sesamum indicum* L.	*Aspergillus* sp., *Aspergillus* sp. *Penicillium* sp	Yes	Positive	Silva et al., (2021).
Cuminum cyminum Wall., *Zingiber officinale* Roscoe *Citrullus colocynthis* (L.) Schrad	*Hibiscus esculentus* L.	*Macrophomina phaseolina* (Tassi) Goid.	Yes	Positive	Abdulhassan, et al., (2020).
Aloe vera (L.) Burm.f. *Morinda citrifolia* L.	*Daucus carota* L.	*Alternaria alternata* (Fr.) Keissl *Alternaria radicina* Meier, Drechsler &	Yes	Positive	Górski et al., (2020).
Cinnamomum verum J. Presl *Coriandrum sativum* L. *Syzygium aromaticum* (L.)	*Lactuca sativa* L.	*Cercospora longissima* Cooke & Ellis	Yes	Positive	Carmello et al., (2018).
Allium sativum L.	*Cucurbita moschata* Duch.	*Alternaria* sp., *Epicoccum* sp., *Fusarium* sp., *Nigrospora* sp. *Phoma* sp. *Rhizopus* sp.,	Yes	Positive	Sousa et al., (2018).
Agapanthus caulescens Spreng. *Allium sativum* L., *Carica papaya* L. *Syzygium cordatum*	*Phaseolus vulgaris* L. *Vigna unguiculata* (L.) Walp.	*Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara *Colletotrichum*	Yes	Positive	Masangwa et al., (2017).
Ateleia glazioviana Baill	*Lycopersicon esculentum* Mill	*Penicillium* sp.	Yes	Negative	Mauri et al., (2019).
Ocimum gratissimum L.	*Lycopersicon esculentum* Mill	*Penicillium* sp.	Yes	No effect	Mauri et al., (2019).

Azadirachta indica A. Juss	*Raphanus sativus* L.,	*Alternaria brassicae* (Berk.) Sacc.	Yes	Positive	Arefin et. al., (2019)
Allium sativum L., *Allamanda cathartica* L. *Tagetes* spp. *Polygonum hydropiper* L.	*Corchorus capsularis* L. *Corchorus olitorius* L.	*Colletotrichum corchori Ikata* & I. Tanaka, *Macrophomina phaseolina* (Tassi) Goid	Yes	Positive	Ahad et al., (2018).

biological control techniques. They are: (1) Wettable powders are created by combining humectant, powdered carriers, and conidia powder. (2) Conidia and carrier are combined and stirred to create granules. (3) A concoction of chemical fungicides and spore powder, proportionately combined on an appropriate carrier. (4) Conidia are suspended in a lotion made of vegetable oil, mineral oil, emulsifier, etc. to create suspenso-emulsion (Xin et al., 2023).

Conclusion

Seed-borne mycoflora remain a major challenge to sustainable agriculture. Medicinal plant extracts and biological control agents offer effective, economical and environmentally safe alternatives to chemical fungicides. Their integration into seed treatment programs can improve seed quality, reduce disease incidence and promote sustainable crop production.

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Stress tolerance mechanisms in *Sorghum bicolor* (L.) Moench (Poaceae) under abiotic stress conditions: a comprehensive review

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Abstract:

Abiotic stresses such as drought and salinity represent major constraints to crop productivity worldwide, particularly in arid and semi-arid regions. *Sorghum bicolor* is a stress-tolerant cereal crop that exhibits notable resilience to these adverse environmental conditions, making it an important model for studying plant stress adaptation mechanisms. This review summarizes findings from studies conducted over the past fifteen years on the responses of Sorghum to drought and salinity stress. It discusses physiological, biochemical, and molecular responses, including growth regulation, metabolic adjustments, and stress-responsive gene expression. Due to its inherent tolerance, *Sorghum bicolor* serves as a valuable system for understanding plant adaptation to unfavorable environments and for developing stress-resilient crop varieties. Such knowledge is essential for breeding programs and biotechnological approaches aimed at improving drought and salinity tolerance in sorghum.

Keywords:

Sorghum, abiotic stress, drought, salinity, stress-responsive genes

Introduction

Agricultural productivity is severely affected by abiotic stresses resulting from changing environmental conditions. Among these, drought and salinity are major limiting factors for crop production worldwide, particularly in regions characterized by low rainfall and saline soils. These stresses disrupt plant water relations, nutrient uptake, photosynthesis, and cellular metabolism, ultimately leading to oxidative damage and reduced yields (Zhu, 2002; Mittler, 2002; Borrell et al., 2019; Tari et al., 2020). Its resilience makes sorghum a key crop for food security in arid and semi-arid regions of the world (Fracasso et al., 2021). Recent advances in genomics and molecular biology have improved understanding of stress tolerance mechanisms in this crop (Varoquaux et al., 2019; Reddy et al., 2021).

Sorghum bicolor L. Moench is an important cereal crop cultivated globally for food, animal feed, and industrial applications. It is recognized for its ability to tolerate drought and high temperatures, allowing cultivation in environments that are unsuitable for many other crops. However, prolonged exposure to severe drought or salinity can still negatively affect sorghum growth and productivity.

Understanding the mechanisms underlying stress tolerance in *Sorghum bicolor* is therefore essential for sustaining crop production under changing climatic conditions. Sorghum employs multiple adaptive strategies, including modifications in root architecture, improved water-use efficiency, and enhanced antioxidant defense systems. Over the past decade, advances in genomic, transcriptomic, and proteomic approaches have significantly improved our understanding of these stress-responsive mechanisms.

Drought stress severely affects sorghum growth by limiting water availability and impairing photosynthesis. However, drought-tolerant sorghum genotypes exhibit adaptive mechanisms such as the accumulation of osmoprotectants, including proline and soluble sugars, which contribute to

cellular protection. These genotypes also possess efficient antioxidant defense systems that reduce abiotic stress-induced damage and help maintain chlorophyll content under water-limited conditions. (Zhu, 2002; Mittler, 2002; Borrell et al., 2019; Tari et al., 2020).

Salinity stress, caused by excessive salt accumulation in soil, disrupts ionic balance and induces cellular toxicity in plants. In *Sorghum bicolor*, salinity tolerance is associated with selective ion uptake, sodium sequestration, and enhanced antioxidant defenses. These mechanisms enable sorghum plants to mitigate salt-induced oxidative damage and maintain growth under saline conditions. Although drought and salinity impose significant stress, sorghum exhibits strong adaptive capacity to withstand these challenges (Fracasso et al., 2021).

At the molecular level, stress tolerance in sorghum is regulated by a wide range of genes and proteins involved in signal transduction, hormonal regulation, and detoxification processes. Calcium-mediated signaling and abscisic acid play key roles in stress perception and response. Transcription factor families such as DREB, NAC, MYB, and WRKY regulate stress-responsive gene expression. Under severe or combined stress conditions, sorghum activates complex molecular networks to enhance survival (Li et al., 2024; Liu et al., 2024; Wang et al., 2026).

This review summarizes research conducted over the past fifteen years on the responses of *Sorghum bicolor* to drought and salinity stress. It focuses on experimental approaches, physiological and biochemical responses, and underlying molecular mechanisms to provide a comprehensive understanding of stress tolerance mechanisms in *Sorghum bicolor*.

Experimental methods used for abiotic stress research of Sorghum

Most studies employed controlled drought or salinity treatments to evaluate stress responses in *Sorghum bicolor*. Drought stress in controlled experiments is commonly simulated using polyethylene glycol (PEG-6000), typically applied at concentrations ranging from 10–20% (w/v) to create osmotic stress conditions that mimic water deficit in plants (Fracasso et al., 2021; Tari et al., 2020). Salinity stress is commonly induced using 100–200 mM sodium chloride (NaCl) in controlled experiments to simulate saline soil conditions and evaluate plant tolerance responses (Tari et al., 2020; Kumar et al., 2020). These treatments were combined with physiological, biochemical, and molecular analyses to assess stress-induced changes (Fracasso et al., 2016; Johnson et al., 2014).

Several studies have emphasized that understanding abiotic stress responses in *Sorghum bicolor* requires investigation at both whole-plant and molecular levels, integrating physiological observations with transcriptomic and proteomic analyses (Varoquaux et al., 2019; Reddy et al., 2021). Methods such as polyethylene glycol (PEG) treatment for drought stress and sodium chloride (NaCl) treatment for salinity stress allow researchers to control stress conditions in a simple and reproducible way. However, these approaches represent artificial conditions and may not fully reflect what plants experience in natural environments.

Because of this, several researchers have used soil-based drought experiments that more closely resemble field conditions (Fracasso et al., 2016; Mace et al., 2013). By examining plant growth traits, physiological responses, gene expression patterns, and protein changes together, researchers are able to understand how *Sorghum bicolor* responds to stress at different levels. This combined approach helps identify stress-related pathways and genes involved in tolerance

mechanisms, linking morphological traits, physiological parameters, transcriptomic data, and proteomic observations.

Gene expression analyses were conducted using techniques such as RNA sequencing, microarray analysis, and PCR-based methods. Proteomic approaches, including two-dimensional gel electrophoresis and mass spectrometry, were used to identify stress-responsive proteins. Integration of transcriptomic and proteomic data provided insights into molecular mechanisms underlying stress tolerance (Johnson et al., 2014; Kosová et al., 2011; Fracasso et al., 2016; Reddy et al., 2021; Abdel-Ghany et al., 2020).

Some of the experimental approaches used to study abiotic stress in *Sorghum bicolor* are summarized in Table 1, which outlines the commonly used methods and findings.

Physiological and biochemical responses of Sorghum under abiotic stresses

Drought stress reduces plant water content, chlorophyll stability, and photosynthetic efficiency. Drought-tolerant sorghum genotypes counteract these effects through the accumulation of osmoprotectants such as proline and soluble sugars, which contribute to osmotic adjustment and cellular protection (Fracasso et al., 2016; Reddy et al., 2004). These genotypes also exhibit increased activity of antioxidant enzymes, including superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase. Enhanced antioxidant capacity helps mitigate oxidative damage caused by drought-induced reactive oxygen species. They show a better ability to maintain internal stability under stress conditions. These genotypes are able to retain higher water content and lose chlorophyll more slowly compared to stress-sensitive genotypes. As a result, tolerant plants are able to maintain photosynthesis, which is essential for survival under prolonged stress.

Salinity stress disrupts ionic homeostasis and induces oxidative stress. Salt-tolerant sorghum genotypes maintain favorable potassium-to-sodium ratios through selective ion uptake and intracellular sodium compartmentalization. Increased antioxidant enzyme activity further contributes to improved tolerance under saline conditions (Munns & Tester, 2008; Fracasso et al., 2016). Under stress conditions, tolerant sorghum genotypes accumulate higher levels of osmoprotectants such as proline and soluble sugars, which help protect cells from dehydration.

Together, these physiological and biochemical responses allow tolerant sorghum genotypes to sustain growth and productivity during extended periods of drought and salinity stress. The ability to adjust physiological and biochemical processes therefore plays a key role in stress tolerance in *Sorghum bicolor*.

Molecular mechanisms and Stress-Responsive Genes

Studies on *Sorghum bicolor* over the past decade have shown that its tolerance to drought and salinity is controlled by multiple interacting molecular processes. These include stress sensing, regulation of gene expression, and changes in metabolic activity. When *Sorghum bicolor* experiences stress, signaling components such as calcium ions, protein kinase pathways, and the hormone abscisic acid play important roles in sensing stress and transmitting signals within the plant. These signals activate stress-responsive genes that help the plant adapt to unfavorable conditions (Zhu, 2016., 2011; Johnson et al., 2014)

Table 1. Compiled Methods and Key Findings on Abiotic Stress Tolerance in *Sorghum bicolor*

SL. No.	Methods Adopted	Key Molecular / Physiological Findings	Study (Author, Year – short title)
1	RNA-seq under graded drought (control, mild, severe); DEG analysis; GO/KEGG enrichment	883 (mild) & 1582 (severe) DEGs; upregulation of CIPK6, CIPK16, ORR4; enrichment of MAPK signaling, hormone signaling, flavonoid biosynthesis	Fracasso et al., 2016 – Drought transcriptome
2	PEG-6000 osmotic stress at seedling stage; physiological assays; RNA-seq	Increased proline, soluble sugars; 164 DEGs; ABA signaling genes (PYR/PYL–PP2C–SnRK2–ABF) differentially regulated	Zhang et al., 2019 – PEG-induced drought
3	PEG stress; RNA-seq comparison of contrasting genotypes	Upregulation of osmoprotectant genes (α-amylase, glycosyl hydrolase, GAD, aldose reductase); activation of DREB2A, LEA, dehydrin genes	Liu et al., 2018 – Genotype comparison
4	Progressive drought (25–100% FC); qRT-PCR; physiological assays	Induction of SORBI genes; increased CAT, POD, SOD; reduced chlorophyll and RWC	Makita et al., 2015 – Meta-gene validation
5	RNA-seq on tolerant vs sensitive cultivars	Higher expression of SOD1/2, VTC1, MDAR1, MSRB2, CIPK1, SAUL1 in tolerant genotype	Abdel-Ghany et al., 2020 – SC56 vs Tx7000
6	Growth-chamber stress; microarray (~28,500 probes)	~20% transcriptome altered; induction of HSPs, MYB78, ATAF1, polyamine genes	Johnson et al., 2014 – Heat + drought
7	PEG drought; 2-DE + MALDI-TOF-MS; enzyme assays	49 drought-responsive proteins; increased SOD, POD, PPO; metabolic pathway activation	Ngara et al., 2012 – Root proteomics
8	Field-based drought + RNA-seq transcriptomics	Tissue-specific expression differences (roots vs leaves); activation of ABA, ROS, and stress signaling pathways	Varoquaux et al., 2019 – Field transcriptomics
9	Genome-wide expression profiling + physiological integration	Identification of drought-responsive regulatory genes; integration of transcriptomics with physiological traits	Reddy et al., 2021 – Drought-responsive genes
10	Morphological and physiological index for 88 grain sorghum germplasm accessions	Variability in salt tolerance among the sorghum germplasm	Wang et al., 2025- Screening and identification of grain sorghum germplasm ubder salt stress
11	Multi-parent crossing approach	Post-rainy season sorghum genotypes with enhanced yield potential and improved tolerance to drought, shoot fly, and charcoal rot	Patroti et al., 2024- Development of high yielding and stress resilient post-rainy season sorghum cultivars
12	DEGs were identified *via* RNA-seq differential expression analysis	Flavonoid biosynthesis pathway is an important regulatory pathway in the sorghum response to drought stress	Yue et al., 2025- Metabolomic and transcriptomic analyses of drought resistance mechanism

Table 2. Major *Stress*-Associated Genes and Pathways Identified in *Sorghum bicolor*

SL. No.	Functional Category	Representative Genes	Stress Context	Key References (Recent)
1	Signal transduction	CIPK6, CIPK16, ORR4	Drought sensing & signaling	Reddy et al., 2021; Abdel-Ghany et al., 2020
2	Hormone signaling	PYR/PYL, PP2C, SnRK2, ABF	ABA-mediated drought response	Sharma et al., 2019; Tari et al., 2020
3	Transcription factors	DREB2A, MYB78, ATAF1, LEA, dehydrins	Stress-responsive regulation	Fracasso et al., 2021; Reddy et al., 2021
4	Transcription factor	WRKY	Salt tolerance	Wang, et al. 2026
5	Transcription factor	*SbMYBHv33*	Negative regulation of salt tolerance in sorghum by regulating ABA signaling pathway	Zheng et al. 2023
6	ROS detoxification	SOD1/2, CAT, POD, VTC1, MDAR1	Oxidative stress control	Kiranmai et al., 2020; Tari et al., 2020
7	Osmotic adjustment	α-amylase, GAD, aldose reductase	Compatible solute accumulation	Fracasso et al., 2021; Kumar et al., 2020
8	Protein protection	HSPs, MSRB2	Heat & drought tolerance	Kiranmai et al., 2020; Mace et al., 2020
9	Validated candidate genes	SORBI_3002G225100, SORBI_3003G368300	Multi-study drought markers	Reddy et al., 2021; Abdel-Ghany et al., 2020
10	QTLs	qSH1, qRL10-2, qSFW4, qTFW1, and qTFW4	Salt tolerance	Wang, et al. 2020
11	Genes	MAPK cascade genes	Drought tolerance	Zhou et al., 2022
12	Genes	GPAT5	Regulation of root epidermis architecture under salt stress	Zhang et al., 2022
13	Gene	*SbAUX*	Response to salt and drought stresses	Ding et al., 2026
14	Genes	*SbSOD1, SbAPX2, SbCAT3, SbHKT1; 4, SbDREB2A, SbDHN3,* and *SbPRP1*	Response to salt and drought stresses	Alzahrani et al., 2025

Exposure to drought and salinity stress results in differential expression of hundreds to thousands of genes in *Sorghum bicolor* (Table.2). Genes involved in calcium signaling, mitogen-activated protein kinase pathways, and abscisic acid-mediated responses are commonly upregulated. Transcription factor families such as DREB, NAC, MYB, and WRKY play central roles in regulating stress-responsive gene networks. These transcription factors control the expression of genes involved in water regulation, protection against cellular damage, and stress adaptation. Their coordinated activity ensures that appropriate stress-response genes are activated during drought and salinity conditions proteomic analyses have identified increased abundance of proteins involved in carbohydrate metabolism, amino acid biosynthesis, energy production, and antioxidant defense. Protective proteins such as late embryogenesis abundant proteins and dehydrins contribute to cellular stability under stress conditions. (Li et al., 2024; Liu et al., 2024; Wang et al., 2026).

Stress conditions also lead to changes in protein production related to carbohydrate metabolism, amino acid synthesis, and energy generation, indicating adjustments in plant metabolism to support survival (Kosová et al., 2011; Johnson et al., 2014; Wang et al., 2007). In addition, protective proteins such as late embryogenesis abundant proteins and dehydrins accumulate during stress and help maintain membrane stability and protein structure during dehydration. These molecular responses together show how *Sorghum bicolor* coordinates gene expression, protein production, and metabolism to improve tolerance to drought and salinity stress.

Recent advances in genome editing technologies, particularly CRISPR–Cas systems, offer promising opportunities for improving abiotic stress tolerance in Sorghum bicolor. Genome editing enables precise modification of stress-responsive genes involved in osmotic adjustment, antioxidant defense, and signaling pathways. Integrating CRISPR-based approaches with conventional breeding and genomic selection could accelerate the development of sorghum cultivars with enhanced tolerance to drought and salinity (Reddy et al., 2021; Mace et al., 2020).

Combined stress responses

When exposed to combined stresses, such as drought with heat or drought with salinity, sorghum exhibits distinct response patterns compared to individual stress conditions. These include activation of specific transcription factors like heat shock proteins, and polyamines, indicating the presence of integrated regulatory networks that enable adaptation to multiple concurrent stresses. When sorghum faces both drought and salinity simultaneously, the stress becomes more complex. Crosstalk between stress signaling pathways occurs, often involving shared stress-responsive elements such as DREB and NAC transcription factors. Stress induces the expression of late embryogenesis abundant (LEA) proteins, dehydrins, and other protective molecules. Understanding this molecular crosstalk is key to engineer multi-stress tolerant sorghum cultivars (Zandalinas et al., 2018).

Traditional breeding programs have focused on selecting genotypes with improved drought tolerance, using traits like root architecture, stay-green phenotype, and osmolyte accumulation. With the advent of genomics and molecular markers, marker-assisted selection (MAS) and QTL mapping are increasingly used. Additionally, transgenic approaches involving overexpression of genes like P5CS (for proline synthesis) or SOD/CAT (for ROS detoxification) have shown promise (Rosenow et al., 2002; Mace et al., 2020; Borrell et al., 2019; Kumar et al., 2021).

Conclusion

The review demonstrates that *Sorghum bicolor* exhibits tolerance to different abiotic stresses through coordinated physiological, biochemical, and molecular mechanisms. These include osmotic adjustment, ionic homeostasis, antioxidant defense, and regulation of stress-responsive gene expression. Understanding these integrated responses provides valuable insights for breeding and biotechnological strategies aimed at developing Sorghum varieties capable of maintaining productivity under drought- and salinity-prone conditions.

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Investigating similarities between human and plant through the light of chemical characterizations of neurotransmission receptors

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Abstract:

In animals, ionotropic glutamate receptors regulate excitatory neuronal transmission. In plants, glutamate-like receptors, which are homologs of vertebrate glutamate receptors found in synapses, play important roles in wound signaling, stomatal regulation, and pollen tube growth. It has been reported that AtGLR clade I and ATGLR clade II of a plant *Arabidopsis thaliana* are more similar to human iGluR. A close relationship exists in the amino acid transport mechanisms among both the organisms. Hence, the neurotransmission mechanism exists in humans, as vertebrates, that shares similarities exclusively with *Arabidopsis thaliana* may also present across various other species within the plant kingdom. With this notion, in this present study primary protein sequences of five different species of plant organisms and 18 primary protein sequences from ionotropic Glutamte are taken as dataset. A thorough investigation is being conducted to determine whether the neurotransmission mechanism exists in humans, as vertebrates, shares similarities exclusively with *Arabidopsis thaliana* or is also present across various other species within the plant kingdom. The proximity between the protein sequences is calculated based on the percentage distribution of each chemical group within them, and phylogenetic trees are then constructed to illustrate their evolutionary relationships.

Key words:

Neuronal transmission, plants, glutamate like receptors, amino acid, chemical group.

Introduction

Vertebrates have very well-structured nervous systems. It was hypothesized that plant has an alternative mode of sensitive nervous system also. There is close relationship of the neurotransmission mechanism of animal with that of the plant and suspect a close relationship in amino acid transport mechanism among both the organisms. Neurotransmitters are endogenous metabolites that play a crucial role within an organism, at the chemical synapses. Sir John Scott Burdon-Sanderson in his experiment (in 1873) first experienced the existence of a current from the proximal to the distal end of the leaf which was termed as "normal leaf-current". After few years, the great biologist Charles Darwin talked about the nervous impulse in insectivorous plants. In early 1900 s the term "plant nerve" is first introduced by the great physicist Acharya Jagdish Chandra Bose. He also reported that the nervous impulse transmitted in plant is responsible for various physiological functions viz. growth, photosynthesis, respiration, motor activity, environmental responses etc. Neurotransmission refers to a process by which signaling molecules are released by the axon terminal of a neuron, and then react with the receptors on the dendrites of another neuron by binding with them. In vertebrate two types of receptors are there which act as neurotransmitters for the central nervous system (CNS), viz. Ionotropic glutamate Receptors (iGluRs) and gamma-aminobutyric acid (GABA) receptors. The balance between inhibitory neuronal transmission via GABA and excitatory neuronal transmission via glutamate keep the cell membrane stable and help to perform proper neurologic functions. Ionotropic glutamate Receptors are a kind of receptors which have the ability of synapses to modify their synaptic strength in response to activity. There are four classes of ionotropic glutamate receptors in animals, viz. NMDA (N-methyl-D-aspartate) receptors, AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole

propionic acid) receptors, Delta receptors and kainate receptors. AMPARs are cation-permeable ionotropic glutamate receptors. They are located at excitatory glutamatergic synapses and expressed throughout the brain. The delta receptors play important roles in cerebellar function. The KAINATE receptors are localized to the presynaptic and postsynaptic sides of excitatory synapses. They have huge similarities with AMPA receptor proteins. NMDA receptors play various important roles, like development of the central nervous system (CNS), generating rhythms for breathing and locomotion. Malfunctioning of NMDA receptors lead to neurological disorders. Non-NMDA receptors are composed ofAMPA, DELTA and KAINATE. Plants also have glutamate receptor-like (GLR) channels, which are plant homologs of mammalian ionotropic glutamate receptors. Thus, a large family of GLR proteins exist across all kingdoms of life. GABA is formed from glutamate and it is the chief inhibitory neurotransmitter in the vertebrate central nervous system. Two classes of GABA receptors are there, GABA A and GABA B. GABA A is considered in fast synaptic inhibition, whereas, GABA B receptors are considered slow synaptic inhibitors. GABA is synthesised in a pathway known as the GABA shunt, which operates not only in the animals, but in plants too. A plant 'GABA receptor' inhibits anion passage through the aluminium-activated malate transporter family of proteins (ALMTs). GABA regulation of aluminium activated malate transporter (ALMT) activity could function as a signal that modulates plant growth, development, and stress response. The discovery of plant GLRs was an unexpected revelation .

Scientists have tried in different ways to understand the existence of neurotransmission in plants and compare them with nervous system existing in human. Mousavi et.al have investigated leaf-to-leaf wound signalling mechanism in bio-molecular level and found similarities of plant genes with those of responsible for synaptic activity in animals to perform organ-to-organ wound signaling. Authors have tried to establish evolutionary relationships between the iGLuR gene family of mammals and GLRs of plants like Arabidopsis thaliana. According to Jack C. Schultz plant's behaviour is largely biochemical. Researchers find a close relationship of the neuro-transmission mechanism of animal with the plant protein sequences and suspects close relationship in amino acid transport mechanism among both the organisms. It is known that each of 20 standard canonical amino acids contain specific chemical properties. Although the basic structure is same for all but only differ due to the physical and chemical properties of their side chains . Thus, 20 amino acids can be classified into 8 groups based on 8 distinct chemical properties of their side chains. According to the biologists along with the abilities of determining protein structure and various functions, the chemical properties of amino acids also have great contributions in molecular evolution and even in disease occurrences . For example, aliphatic group of amino acids decrease insulin secretion and thus increase the risk of Type 2 Diabetes Mellitus. Reports said the proteins involved in the activities of olfactory receptors are influenced by aromatic, sulfur-containing, and hydroxyl groups of amino acids. Some reported research works witness importance of the chemical properties in characterization of protein families .

Now it is important to carry out the experiment based on the distribution of amino acids in the receptor protein sequences associated to animals and plants based on the chemical properties of the amino acids. As already mentioned, in our previous research works we have conducted research works using a small number of human iGluR gene families and *Arabidopsis thaliana* GLRs from the plant world. Areas of convergence are found. This raises the question of whether the neurotransmission process found in humans, as vertebrates, is unique to *Arabidopsis thaliana* or is

also shared by a number of other plant species. To investigate further, the protein sequences of GABA A receptor of human (from vertebrate) and five more glutamate receptor protein sequences of plants from five different species are considered as datasets.

Material and methods

Dataset specification

The datasets taken are specified in the Table 1. Instead of only proposing new methodologies and applying on a dataset to assess their efficiency, the prime objective of the experiment here is to characterize the primary protein sequences in human and plants and investigate whether they have any evolutionary relationships among them or not. Instead of studying only the amino acid sequences, it is also worthy to see the chemical nature of the amino acids as the structure, stability and functions of a protein are maintained by different biochemical information contained in the

Table 1: Detailed data set of all the genes used for this study

	Accession No.	**Glutamate like receptor Gene Name**	**Accession No.**
Human		**Plant**	
AMPA Sub family		PmuGLR1.4	XP_016649716
GRIA1	P42261	GhGLR2.2	XP_040958698
GRIA2	P42262	CcGLR 3.3	PHU26821.1
GRIA3	P42263	CaGLR 3.4	XP_047249840.1
GRIA4	P48058	CbGLR 3.3	PHT56419.1
DELTA Sub family			
GRID1	Q9ULK0		
GRID2	O43424		
KAINATE Sub family			
GRIK1	P39086		
GRIK2	Q13002		
GRIK3	Q13003		
GRIK4	Q16099		
GRIK5	Q16478		
NMDA Sub family			
GRIN1	Q05586		
GRIN2A	Q12879		
GRIN2B	Q13224		
GRIN2C	Q14957		
GRIN2D	O15399		
GRIN3A	Q8TCU5		
GRIN3B	O60391		

Table 2: Proximity between the primary protein sequence of human and plant based on the percentage distribution of 8 chemical groups

23	GRIA1	GRIA2	GRIA3	GRIA4	GRID1	GRID2	GRIK1	GRIK2	GRIK3	GRIK4	GRIK5	GRIN1	GRIN2A	GRIN2B	GRIN2C	GRIN2D	GRIN3A	GRIN3B
GRIA1	0.00	4.34	3.10	5.24	7.91	6.28	3.66	4.96	5.28	7.03	11.41	7.58	13.70	12.59	16.90	23.72	10.46	26.76
GRIA2		0.00	6.90	4.96	9.56	7.32	3.90	4.92	7.22	8.88	10.97	8.77	14.80	12.52	16.52	23.94	10.61	26.98
GRIA3			0.00	6.89	9.88	7.10	5.26	5.46	6.82	9.07	12.65	7.29	12.72	11.85	18.84	25.36	9.40	27.36
GRIA4				0.00	9.87	8.61	4.45	4.49	4.45	7.29	6.76	7.72	15.23	12.95	12.89	20.05	10.18	22.51
GRID1					0.00	6.45	6.71	6.93	9.19	6.34	8.36	6.76	10.71	10.66	15.03	23.95	6.43	25.73
GRID2						0.00	4.84	5.58	8.96	7.55	12.43	7.06	10.18	9.71	17.06	26.04	8.30	29.08
GRIK1							0.00	1.44	4.88	7.14	9.57	5.15	11.80	9.52	15.54	23.78	6.97	26.82
GRIK2								0.00	4.24	7.08	8.53	4.95	11.26	8.98	15.54	23.48	6.53	26.32
GRIK3									0.00	6.44	7.89	7.19	13.24	10.96	15.78	21.60	9.67	23.44
GRIK4										0.00	6.76	7.74	14.03	12.26	13.13	21.23	8.69	24.27
GRIK5											0.00	9.07	15.79	14.33	9.27	17.05	7.66	18.51
GRIN1												0.00	11.36	8.92	15.72	23.50	7.08	24.80
GRIN2A													0.00	3.49	23.06	31.00	10.16	32.34
GRIN2B														0.00	21.60	29.54	8.92	30.88
GRIN2C															0.00	11.29	12.90	13.86
GRIN2D																0.00	20.83	9.27
GRIN3A																	0.00	23.36
GRIN3B																		0.00

amino acid pattern embedded in the primary protein sequence . Total 18 sequences are taken from four different sub-families (AMPA, DELTA, KAINATE, and NMDA) of iGluR protein family of human. All the sequences are collected from UniPort database (https://www.uniprot.org/). Primary protein sequences of five different species are taken from plants. Those are *Gossypium hirsutum* L. (upland cotton), *Prunus mume* (Siebold) Siebold & Zucc. (Japanese apricot), *Capsicum annuum* L. (bell pepper), *Capsicum baccatum* L. (chili pepper in South America), and *Capsicum chinense* Jacq. (habanero-type pepper). All the sequences are collected from NCBI GenBank (https://www.uniprot.org/).

Percentage-wise calculation of amino acid compositions in all the protein sequences

Amino acids are considered as the building blocks of a protein and serve multiple purposes beyond it. These are the precursors of many bio-molecules also. Many amino acids are reported to give rise to specialized products in the body. Evidences are there that some of the canonical amino acids play significant roles in modulating neurotransmission in animals.

Identifying the conserved regions present in the set of all protein sequences

To identify the conserved regions of primary protein sequences based on occurrence of individual amino acid in the primary protein sequences, conventional multiple sequence alignments (MSA) method Clustal Omega is used (Sievers et al, 2013). Multiple sequence alignments (MSA) method play a key role in comparative study of structure and function of biological sequences. It provides a biological insight in evaluating relationships among various protein families. In Clustal Omega MSA based on character count, gaps of different lengths are inserted within the sequences to allow homologous positions to be aligned with each other . Thus, the identical amino acids among the sequences are identified with the corresponding locations. To identify the conserved regions present in the protein sequences based on the eight chemical groups (as shown in Fig. 1) the FASTA format of the aligned sequences are collected using Clustal Omega (https://www.ebi.ac.uk/jdispatcher/msa/clustalo?stype=dna) and conserved regions are investigated based on eight chemical groups (GAVLI, FYW, CM, ST, KRH, DE,NQ, and P) using colour alignment properties. The programme used in this website investigate each residue and compares it to the other residues in the same column. The identical and similar residues among the sequences are identified using coloured background according to their chemical groups.

To construct the phylogenetic trees the phylip formats of the distance matrices are obtained using the online software http://phylogeny.lirmm.fr/phylo_cgi/ data_converter.cgi and then the tree formats are obtained using another online software named http://www.trex.uqam.ca/.

Results

Percentage-wise calculation of amino acid compositions in all the protein sequences taken as dataset. In this subsection the percent distribution of each of 20 amino acids present in the primary protein sequences of these above-mentioned genes are calculated (Fig. 2). Identifying the conserved regions present in the data set.

According to Fig. 2, in all the human ionotropic glutamate receptor, primary protein sequences contain highest amount of Glycine (G) and Leucine (L). Other most occurred amino acids are Valine (V), Alanine (A), Isolucine (I) and Serine (S). The figure (Fig. 2) shows that in five plant glutamate receptor protein sequences large amount of Leucine(L), Valine (V) and Serine (S)

Table 3. Proximity between each primary protein sequence of human iGluR sequences with plant dataset.

	PmuGLR1.4	GhGLR2.2	CcGLR 3.3	CaGLR 3.4	CbGLR 3.3
GRIA1	12.27	14.21	13.93	8.93	12.93
GRIA2	9.47	14.73	12.23	7.23	11.23
GRIA3	12.85	13.77	15.87	10.87	14.87
GRIA4	8.42	14.04	11.94	7.44	10.94
GRID1	10.42	18.60	10.56	8.30	9.56
GRID2	12.65	14.95	12.09	10.05	11.09
GRIK1	11.25	13.81	12.03	7.67	11.03
GRIK2	10.99	14.13	12.31	8.17	11.31
GRIK3	11.17	13.85	14.23	10.55	13.23
GRIK4	11.58	16.44	10.96	8.74	9.96
GRIK5	8.82	17.38	12.34	9.34	11.34
GRIN1	9.23	17.89	14.91	11.91	13.91
GRIN2A	15.93	18.93	17.39	14.99	16.39
GRIN2B	14.11	16.43	16.63	13.63	15.63
GRIN2C	12.49	19.41	14.49	13.49	15.49
GRIN2D	19.98	24.16	22.16	21.16	23.16
GRIN3A	10.23	15.49	11.77	8.77	10.77
GRIN3B	21.33	26.17	24.17	23.17	25.17

amino acids are present. Glycine (G) and Isolucine (I) are also exist with huge amount. The amino acid compositions of all NMDA receptor protein sequences of Human and glutamate like receptors receptor protein sequences of those five plants also have some commonalities such that they all have huge amount of Leucine (Leu), Valine (Val), and Serine (Ser). The proximity among all possible pair of sequences are calculated using Equation 2 (Sengupta et al, 2022) and two separate distance matrices for the two different datasets associated to ionotropic glutamate receptors and NMDA receptors. Pictorial representation of those similarity matrices are stated in the form of phylogenetic tree. The Neighbour Joining method is used to do so (Fig. 3, Fig. 4, Table 2, Table 3). Based on the occurrence of 20 amino acids in the 18 primary protein sequences of ionotropic receptors and 5 primary protein sequences of glutamate like receptors, the conserved regions are identified using multiple sequence alignment method of Clustal omega.

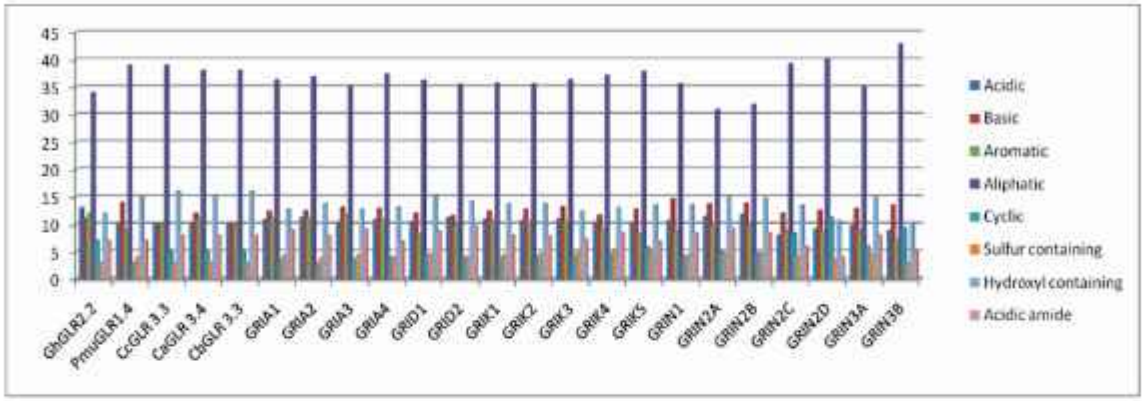


Fig. 1. Distribution of amino acid in primary protein sequences of Human and plants.

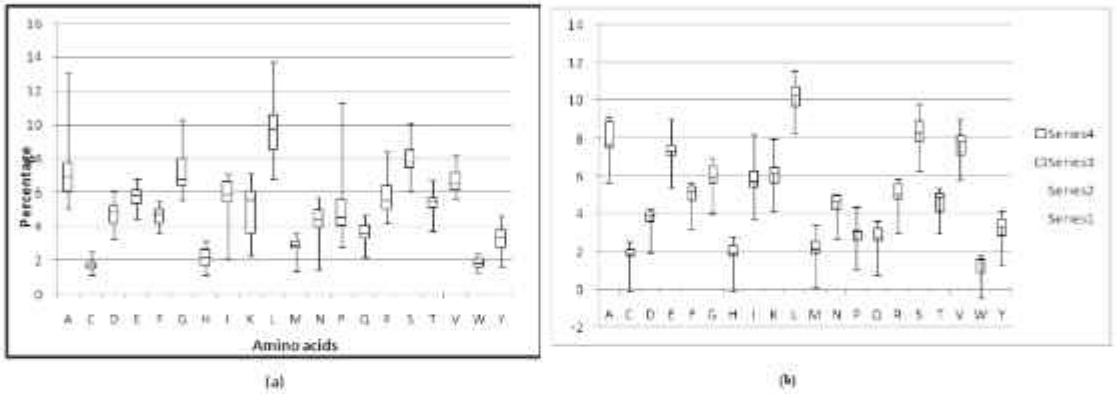


Fig. 2. Box-and-whisker plot showing propensity of 20 amino acid (AA) compositions present in all the protein sequences of human and plants (a) Amino acid composition present in all iGLuR primary protein sequences (b) Amino acid composition present in plant primary protein sequences.[Plant protein names: PmuGLR1.4, GhGLR2.2, CcGLR 3.3, CaGLR 3.4, CbGLR 3.3 ; Human Protein names: GRIA1, GRIA2, GRIA3, GRIA4, GRID1, GRID2, GRIK1, GRIK2, GRIK3, GRIK4, GRIK5, GRIN1, GRIN2A, GRIN2B, GRIN2C, GRIN2D, GRIN3A, GRIN3B]

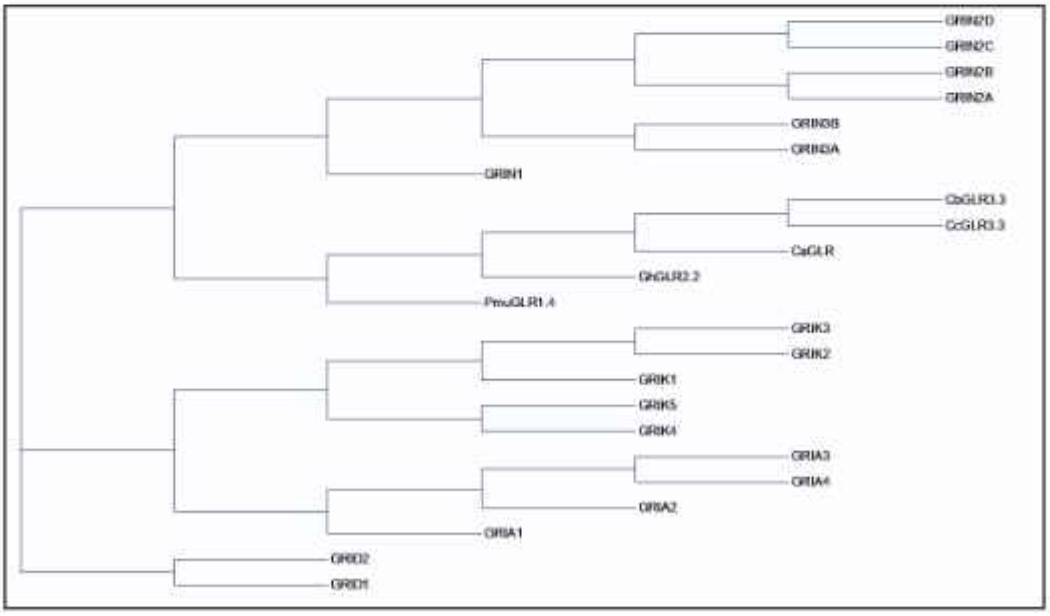


Fig. 3. Phylogenetic trees obtained for all the primary protein sequences using Clustal omega software.

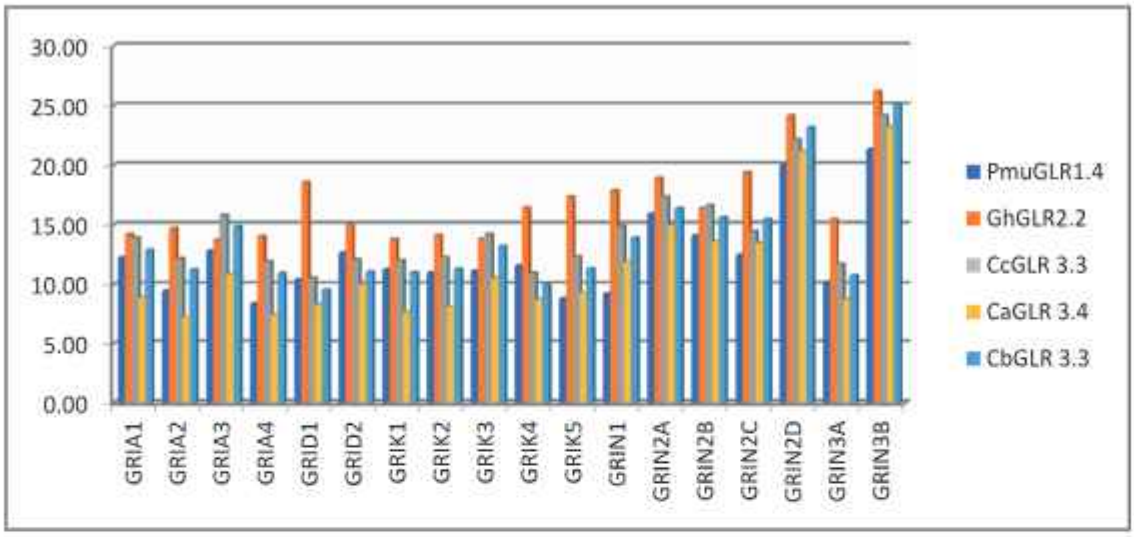


Fig. 4. Proximity between each primary protein sequence of human iGluR sequences with plant dataset.

Discussion

In human ionotropic glutamate receptors make control over excitatory neuronal transmission. Plants have glutamate like receptors which are plant homologs of glutamate receptors in vertebrate synapses and involved in wound signalling, stomatal regulation, and pollen tube growth. Moreover, the human ionotropic glutamate receptor genes belong to four different sub families and have different functional roles in excitatory neurotransmission. Previously we reported that the conserved regions among the protein sequences of *Arabidopsis thaliana* and four different human subfamilies separately. Therefore, the in-silico analysis performed in this study with other five different plant receptors not only claim some common functionalities between human, a vertebrate and plant in neurotransmission, but also initiates a new horizon to the biologists for better understanding of both human and plant physiology. This is a primary finding hence the plan is to investigate the same to other plant groups including cryptogams and phanerogams.

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Ancient catalysts in modern plates: integrating ayurvedic plant enzymes in food biotechnology

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Abstract:

In the embrace of Ayurvedic wisdom, each plant whispers a tale of healing enzymes, waiting to be unveiled by the tools of modern science, to nourish and sustain humanity. Integrating Ayurvedic plant enzymes into food biotechnology offers a promising avenue for developing sustainable and health-promoting food systems. The widespread use of synthetic food additives has heightened consumer awareness of the potential risks. This has caused the food industry to seek natural alternatives that will not only improve the shelf life and quality of the product but also enhance its overall nutritional value. Medicinal and aromatic plants, especially those rooted in Ayurveda, have gained prominence due to their rich nutritional profiles, antioxidant capacity, and antimicrobial properties.

Ayurveda originated in India over 5,000 years ago and continues to thrive to this day as a holistic approach to health. This science of life, which is named Ayurveda, is based on principles of balance between mind, body, and spirit. Ayurveda enhances the concept of biological individuality by offering personalized treatments tailored to each person's unique constitution. Central to this system is the doctrine of the three doshas - Vata, Pitta, and Kapha, which represent the fundamental energies that govern bodily functions. Illness, according to Ayurvedic theory, results from imbalances among these doshas.

The application of ayurvedic plants ranges from medicinal to modern use as concentrated extracts and bioactive compounds. These plants serve as natural food additives, preservatives, and functional ingredients, playing a key role in organic and functional food production. Among these bioactive constituents, plant-derived enzymes from Ayurvedic herbs present a promising frontier in food biotechnology.

Keywords:

Ayurvedic plant enzymes, antioxidants, synthetic additives, doshas.

Introduction

Ayurvedic plants encompass diverse applications from traditional therapeutics to modern nutraceuticals, functioning as natural preservatives, flavour enhancers, and bioactive food ingredients. Rich in phytochemicals and plant-derived enzymes, these botanicals hold transformative potential in food biotechnology, improving functional food quality, safety, and therapeutic efficacy.

The growing use of synthetic food additives has caused consumer concerns about potential risks, causing the food industry to lean towards natural alternatives. These natural enzymes prove to enhance the shelf life and quality of the product, along with the nutritional value. Ayurveda has found its place in every sphere, from medicinal to modern food, in the form of concentrated extracts and bioactive compounds. Ayurvedic enzyme possess the potential to revolutionize food biotechnology .

This study aims to explore the role of Ayurvedic plant-derived enzymes in advancing modern food technology. Emphasis is placed on understanding their antioxidant, antimicrobial, and anti-

inflammatory properties, as well as their contribution to the development of functional and sustainable food systems that bridge ancient wisdom with modern scientific precision.

Rationale and scope of integrating Ayurvedic plant enzymes in food biotechnology

Ayurveda, also well known as the science of life, originated in India 5000 years ago. The sages believed Ayurveda to be passed down from the gods. Ayurveda comprises the knowledge of herbs, foods, aroma, gems, colours, yogas, mantras, and surgery. These methods were used for healing, prevention, and longevity. There are some basic doctrine principles in Ayurveda, Panchbhutas (five elements): Prithvi, jala, agni, vayu, and Akasha. These elements influence human health. Tridosha, which consists of Vata, pitta, and Kapha, the balance of these doshas regulates health, Sapta dhatu (seven tissues), Agni (digestive fire), Malas (waste products), Prakriti (constitution), and Vikriti (imbalance). Dinacharya (Daily routines). Ahara (diet) and asudha (herbs and medicine), Lastly, srotas (channels), these are the pathways via which substances flow through the body. Fig1. describes the contributions of Ayurveda in various aspects. The central image throws light on the traditional practice of Ayurveda with its emphasis on herbal formulations. The surrounding elements are the four cornerstones of contemporary relevance: medicinal plants and traditional pharmacopeia, holistic understanding of human physiology, laboratory-based drug development and validation, and prospects for integration with modern pharmaceutical and molecular research.

Ayurveda is based on the balance of the three doshas, Vata, Pitta, and Kapha. These three doshas are managed by reducing Váta is reduced through heat and moistness. Pitta is reduced through leisure and cold. Kapha is reduced through heat, dryness, and lightness.

In Ayurveda these doshas are heavily related to food and digestion, In parallel to western medicine Vata produces gas in colon (carbohydrates become fermented and proteins become



Fig 1. Conceptual overview of Ayurveda's integration in different fields

putrefied), Meanwhile pitta becomes excess in duodenum when food gathers for a long time in duodenum and small intestine, from the western medicine point of view pitta can be considered as digestive enzymes secreted by pancreas, liver, etc. that are active in the duodenum and small intestine. Kapha becomes excessive in the stomach right after a meal, kapha moistens the food and passes it into the small intestine for the action of pitta. Mucoid secretion and saliva are the parallels of kapha in Western medicine terminology.

Policy and Regulations Supporting Ayurvedic Integration in Food Biotechnology

India's policy and institutional framework actively support the integration of Ayurvedic principles into food biotechnology. This is achieved primarily through government initiatives emphasizing sustainable medicinal plant use, quality standards, and biotech innovation.

Key policies

The Department of Biotechnology launched the BIOE3 policy (Biotechnology for Economy, Environment, and Employment), which integrates Ayurveda into modern biomanufacturing, promoting research on plant-derived bioactives and enzymes for food and nutraceutical applications.

National Ayurveda research roadmap (2026 – 2040)

Vision

To transform Ayurveda into a globally recognized, evidence-based, innovation-driven health science that contributes to global traditional medicine leadership, national health outcomes. This aligns with the Government of India's broader AYUSH policy goals of scientific validation, quality, integration, and international recognition. This aims to integrate modern biomedical research methods with classical Ayurvedic paradigms, strengthen infrastructure, human capital, along with institutional capacity for interdisciplinary research, as AYUSH systems are integrated in 6,155 Community Health Centres (CHCs), 26,636 Primary Health Centres (PHCs), and 759 District Hospitals (DH) in the country. This also supports digitalization of Ayurvedic knowledge, integration with data science, and translational research for personalized healthcare.

Central coordination and governance

The Ministry of Ayush, government of India, will continue to serve as the central coordinating authority, providing strategic directions for policy making, funding allocation, international collaboration, and recognition with nationwide implementation of Ayurveda research initiatives in alignment with national health priorities.

The Central Council for Research in Ayurvedic Sciences (CCRAS) functions as the apex research organization responsible for planning, coordinating, and advancing scientific research in Ayurveda through a structured, evidence-based approach.

Regulations

FSSAI Ayurveda Ahara Regulations (2022 regulations) standardize Ayurvedic food preparations, enabling licensing for biotech- processed products like enzyme-enhanced formulations, which ensure safety and personalized nutrition.

The Food Safety and Standards (Ayurveda Aahara) Regulations, 2022, establish a dedicated regulatory framework for foods formulated on Ayurvedic principles, bringing them under the purview of the Food Safety and Standards Authority of India (FSSAI). These regulations aim to ensure safety, authenticity, standardization, and consumer protection, while clearly distinguishing Ayurveda Aahara from Ayurvedic drugs and nutraceuticals. The regulatory scope can be broadly categorized into formulation and ingredients, use of additives and nutrients, and provisions related to processing aids and enzymes.

Products recognized as Ayurvedic Ahara must be formulated strictly in accordance with recipes, ingredients, and dietary principles as described in authoritative Ayurvedic texts, which are enumerated in Schedule A of the regulations. The texts include Charaka Samhita, Sushruta Samhita, Ashtanga Hridaya, and other recognized Ayurvedic literature.

Table 1 : The table represents the enzymes obtained from their plant sources.

Enzyme	Source	Used In	Product	References
Actidin	Kiwi fruit, pineapple, mango, banana, papaya	Food, dairy products, meat tenderization	Fresh cheese	Kaur et al., 2022, Katsaros et al., (2010)
Papain	Pawpaw latex	Meat	Tenderized meat	Kang & Warner, 1974
Ficin	Fig latex	Food	Protein hydrolysates, controlled tenderization, traditional cheese	Aider, 2021 Raskovic et al., 2016
Bromelain	Pineapple latex	Brewing	Overtenderization of meat	Madhusankha & Thilakarathna, 2021
Lipases	Lupin, avocado, pine nut, walnut, lentils, coconut, chickpea, oats, mungbean, castor bean, eggplant	Cheese, dairy, Bakery, beverage, lipid modifications	Monoglycerides and fatty acids	(Chandra et al., 2020), (Ali et al., 2023)
Lipoxygenase	Soybeans	Food	Fatty acid hydroperoxides	(Tang et al., 2024),
Pectinase	Commonly all fruits	Fruit juice and wine clarification	Fruit juice	(Sandri et al., 2011)
α-Amylase	Malted barley	Brewing	Glucose and maltose	*The Chemistry Of Enzymes* 2024
Invertase	Phloem of higher plants	Food	Glucose and fructose	Kulshrestha et al., 2013Tauzin & Giardina, 2014

National AYUSH Mission Guidelines (2021- 2026)

The operation guidelines for National AYUSH Mission (NAM) were approved for continuation with a funding of Rs. 4607.30 crores, outlining objectives like equitable AYUSH services aimed at holistic wellness via health and wellness centres and infrastructure upgrades. It covers components such as AYUSH services (hospitals, dispensaries, co-location at PHCs/CHCs/DHs), educational institutions, public health programs (e.g., osteoarthritis management, NPCDCS integration), and flexible funding for yoga/telemedicine.

Central sector scheme for conservation, development, and sustainable management of medicinal plants.

The scheme was introduced to focus on quality research and development along with capacity building through training. The scheme aims to support a program for quality assurance and standardization through the development of Good Agriculture and Collection Practices (GACPs). The development of monographs that define standards for quality, safety, and efficacy, along with formulations of region-specific agro-techniques. Additionally, it envisages the establishment of a credible institutional mechanism for certifying drugs, seeds, and planting material. The scheme seeks to enhance the livelihoods of local communities, with special attention to populations in the forest fringe.

Strategies for the medicinal plants scheme

- Establishment of Medicinal Plant Conservation Areas through systematic surveys and geo-referencing of populations of medicinal plant species.
- In-situ and ex-situ conservation strategies strengthen conservation efforts, including resource augmentation and artificial regeneration of local plant populations.
- Promote research and development to bridge technology gaps in quality evaluation, documentation, identification of substitutes, and bioactivity-guided phytochemical studies, with special emphasis on Rare, Endangered, and Threatened (RET) species.
- Improve production and post-harvest management through the adoption of quality standards, Good Agricultural Practices (GAP), Good Field Collection Practices (GFCP), Good Storage Practices (GSP), along with value addition and marketing infrastructure.
- Monitor international developments affecting conservation, trade, quality assurance, and availability of medicinal plant resources.

Ayurvedic enzymes in food biotechnology

The growing advancement in food biotech has led to extensive integration of plant-derived enzymes in food products; these enzymes are known to enhance shelf life, nutritional value, and therapeutic effect of food. Enzyme science evolution has been a cornerstone for the development of the food biotechnology sector. This development started in 1878 when a German physiologist named Wilhelm Kühne first coined the word enzyme. Since then, a series of discoveries have taken place in enzyme evolution to this date. In the food industry, enzymes act as biological catalysts for chemical reactions. These enzymes used in food industries are categorized in six major classes:

Table 2 : Ayurvedic plants and their enzymes. The table below depicts the various ayurvedic plants and their enzymes, enzymatic action, therapeutic uses giving a brief view into Ayurvedic plant's healing potential.

Sl no.	Sanskrit Name	Part Used	Enzymes Present	Actions	Therapeutic Uses	References
1	Rasonam (garlic)	Bulb and oil	Allinase, peroxidase, catalase, superoxide dismutase, and polyphenol oxidase	Alterative, anthelmintic, antiparasitic, carminative, disinfectant, expectorant, rejuvenative, stimulant	Garlic is used in Ayurveda as a cardioprotective, antimicrobial, and detoxifying agent, effective in conditions such as atherosclerosis, hypertension, respiratory infections, digestive disorders, parasitic infestations, and inflammatory diseases	(Chhabria & Desai, 2018; Salehi et al., 2019)
2	Ardrakam (Ginger)	Rhizomes	Zingibain (ginger protease), amylase, lipase	Analgesic, antiemetic, aromatic, aphrodisiac, carminative, diaphoretic, digestive, expectorant, nervine, sialagogue, stimulant	Indigestion, flatulence, colic, vomiting, spasms, fever, cough, cold, asthma, stomach and bowel pain, sore throat, , nausea, retching, diabetes (types 1 & 2), appetite stimulant	(Ali et al., 2024; Tirtha, n.d.; Verma et al., 2025)
3	Arjuna	bark	SOD, peroxidase, esterase	Cardiac stimulant, rejuvenative, astringent, hemostatic, alterative	A potent cardiotonic herb beneficial in the prevention and management of heart disease and angina; it supports healing of cardiac tissue following surgery, promotes healthy bile flow, and helps reduce edema.	(Kumar et al., 2023; Swain et al., 2023)
4	Tulsi(Basil)	herbs	peroxidase, catalase, superoxide dismutase, polyphenol oxidase, and esterase, which contribute to its antioxidant, anti - inflammatory, antimicrobial, detoxifying, and tissue-protective effects recognized in Ayurveda and supported by modern studies.	Antibacterial, antiseptic, analgesic, antispasmodic, diaphoretic, febrifuge, nervine, vermifuge	A powerful respiratory and immune tonic, useful in cough, cold, asthma, allergic bronchitis, fever, tuberculosis support, and sinus congestion	(Baliga et al., 2013; Cohen, 2014)
5	Haridrā, Gauri (Turmeric)	Rhizome, tubers	Curcumin synthase, PAL, peroxidase, ascorbate peroxidase	anthelmintic, antibiotic, aromatic, carminative, stimulant, tonic, vulnerary	Amenorrhea, anemia, arthritis, blood purification, improved circulation, culinary spice, relief from cough, diabetes management, anthelmintic (worms), jaundice, eye disorders, fevers, wound healing, natural antibiotic properties, inflammatory bowel syndrome (IBS), Crohn's disease, hepatitis, asthma, psoriasis, and acne.	(Tian et al., 2025; Tirtha, n.d.)
6	Āmalakī (Indian Gooseberry)	Fruit	Superoxide dismutase (SOD), ascorbate oxidase, polyphenol oxidase, and peroxidase	Aphrodisiac, astringent, hemostatic, laxative, nutritive tonic, refrigerant, rejuvenative (for Pitta), stomachic	Urinary conditions, anemia, biliousness, constipation, diabetes, gastritis, gout, premature graying/balding, hepatitis, hemorrhoids, liver and spleen weakness, regulates blood sugar, increases RBC's	(Tirmanwar et al., n.d.; Tirtha, n.d.)
7	Isapghula (Ispaghula)	Seeds	Mucilage-producing enzymes (polysaccharides), β-glucanase	Demulcent, emollient, laxative, diuretic	Constipation, diarrhea, intestinal issues, dysentery, bladder and kidney issues, cystitis, gastritis, ulcers, fevers, gonorrhea, coughs and colds	(Sakthivel et al., 2024; Tirtha, n.d.)

transferase, lyase, isomerase, hydrolase, ligase , transferase. . Here, Table 1 represents the enzymes obtained from their plant sources.

Ayurvedic plants as a source of enzymes

Ayurvedic plants can synthesize a varied range of enzymes like proteases, amylases, oxidoreductases, etc. These enzymes directly contribute to their therapeutic actions that are traditionally attributed to the plants. For example, plants like *Carica papaya* produce papain, a proteolytic enzyme known for its role in protein digestion and anti-inflammatory activity. Similarly, *Zingiber officinale* (ginger) contains protease and amylase enzymes that support digestive health and improve nutrient absorption. In *Withania somnifera* (ashwagandha), enzymatic activity is associated with antioxidant defense and metabolic regulation .

Ayurvedic plants and their enzymes

Table 2 below depicts the various ayurvedic plants and their enzymes, enzymatic action, and therapeutic uses giving a brief view into Ayurvedic plant's healing potential. Here, Table 2 gives an account of ayurvedic plant enzymes.

Application potential of Ayurvedic herbs in food industry

Ayurvedic herbs serves as the largely untapped resource for modern food technology, this is due to their rich enzymatic system. Their traditional usage for digestion, preservation, detoxification, fermentation with the enzymes that are required in excess in today's food industry like proteases, amylases, peroxidases, etc. The growing consumer awareness has caused a demand in clean label ingredients and plant-based processing instead of synthetic enzymes. Given below are few Ayurvedic plants that have extensive use in today's modern food technology.

Chemicals end up in food intentionally as well as unintentionally for technological purpose or for various pollution factors, The chemicals in food have posed as a worldwide health concern in today's view when pesticides are indispensable in agricultural practices and Pollution of soil and water are posing potential detrimental effects on humans. Elevated levels of ROS can cause biochemical damages which can lead to increased oxidative stress. Some pesticides are well known for directly generating ROS this when accumulated in vegetables which are ingested by humans can cause depletion of body's antioxidant defences. These can lead to mutations, can impair electron transport chain. Exposure to certain toxins can set out cascade of events within cells which may cause cancer. This is where ayurvedic herbs come in play to create functional meals and nutraceuticals.

Methodology of Enzyme Extraction from Ayurvedic plant source

Enzyme design has emerged as a powerful tool to improve enzyme yield and performance by using an approach that combines experimental as well as computational data, which aims at tailoring enzyme activity, stability, and selectivity. Integration of rational design with directed evolution, along with protein engineering, gives researchers the power to develop enzymes with

enhanced substrate binding and specificity. This enables more efficient and biocatalytic substrate binding.

Enzyme designing is a multistep approach that requires the selection of suitable protein variants by the identification of critical and non- critical residues. This is then followed by the mapping of energetically and structurally permissible mutations, as it allows prioritization of favourable sequence variations and also validates the outcome with the help of an experimental approach. The recent advancement in machine learning and deep learning approaches has caused significant improvement in the accuracy of individual design components, yet a multifaceted framework remains necessary for achieving meaningful enhancements in enzyme activity, specificity, and stability. Hence, effective enzyme designing remains heavily dependent on intensive experimental validation along with computational algorithms to decipher relationships between sequence, structure, and functions. Regardless of the importance, widespread adoption of enzyme design remains constrained by conceptual, financial, and technical challenges.

Methodologies for the extraction, characterization, and purification of enzymes that are derived from Ayurvedic plants such as *Ocimum sanctum* (Tulsi) and *Terminalia arjuna* (Arjuna), etc. Commonly reported protocols involve mechanical disruption of plant tissues followed by enzymatic hydrolysis using optimized pectinase–cellulase blends under controlled conditions (typically pH 5.0 and temperatures around 50 °C for 2–4 h) to improve yield and activity. The downstream processes include centrifugation, filtration, and chromatographic purification methods such as DEAE-Sepharose ion-exchange chromatography. After this, the enzymatic activity is assessed by using standard assays (e.g., the DNS method for amylase activity), while molecular characterization through SDS-PAGE and mass spectrometry ensures enzyme identity, purity, and stability. These methodologies used for processes required for synthesizing Ayurvedic plant-derived enzymes align perfectly with food grade safety and regulatory requirements.

Scientific limitations of Ayurvedic enzyme research

There are some critical limitations faced by Ayurvedic plant- derived enzymes for food and health applications which restrict their advancements in clinical and industrial adoption worldwide. One of the major constraints being the lack of evidence-based research this is mainly due to the fact that most studies remain confined to just in vitro assays and preclinical observations. This limits the enzyme's ability to substantiate functional claims in real-world dietary or therapeutic contexts. Inadequate standardization of raw materials, extraction protocols, and formulations further compromises reproducibility, as enzymatic activity can vary substantially due to differences in plant chemotypes, harvesting conditions, and processing methods. Complex formulations pose as another huge challenge, as the presence of various components in herbal formulations can cause complexities in further pharmacokinetic studies and in effective drug delivery strategy development. The gap between research and industry ecosystem causes fragmentation and limited development in real-life advancements of Ayurvedic plant-derived enzymes in food biotechnology.

Over reliance on empirical testing coupled with weak experimental design, small sample size and inconsistent statistical validations undermines the evidentiary strength of data. The limited integration of modern analytical, diagnostics and bioinformatics tools further restrict the understanding of mechanistic insight, while overgeneralized claims often exceed the scope of the presented data. Addressing these gaps is essential for advancing credibility, regulatory acceptance,

and translational impact. Addressing these challenges requires interdisciplinary collaborations among ayurvedic scholars, practitioners, scientists in pharmaceuticals, and biotech developers.

There are also some major threats that need to be addressed, i.e. Primarily the regulatory hurdles as the present global regulatory frameworks provide limited and inadequate provisions for traditional medicine systems which result in complex, prolonged pathways. Along with this, there are also biopiracy concerns; the risk of misappropriation of traditional medicinal knowledge without equitable benefit distribution poses a strong concern. There are concerns regarding market distrust as false perceptions regarding the safety, efficiency, or scientific validity of Ayurvedic practices can face heavy rejections from healthcare professionals as well as consumers. Cultural and philosophical misalignment may cause over-simplification of the holistic principles of Ayurveda to focus immensely on isolated bioactive compounds .

Conclusion and future prospects

In the ancient medical system of ayurveda over 70,000 plant species have been incorporated. Till this date several plants mentioned in ayurvedic scripture remain undiscovered some like *Clerodendrum serratum, Hyptis suaveolens* (L.) Poit., *Mucuna pruriens* var. utilis (Kapikachhu), *Cardiospermum halicacabum* (Balloon Vine), *Boerhavia diffusa* (Punarnava), *Desmodium gangeticum* (Shalaparni), *Evolvulus alsinoides* (Shankhapushpi), *Aerva lanata* (Gorakha ganja) etc. Despite the scientific investigations, the gap in the knowledge of their phytochemical properties and enzyme modulating activities remain limited, which underscores the need to more systemic research for full exploration of their therapeutic potential. Systemic research should be integrated to characterize and validate the plant derived enzymes. .

Future studies should include traditional Ayurvedic knowledge with approaches in genomics, proteomics, and metabolomics to clarify the depth of biosynthesis, activity and stability in food processing conditions. Emphasis should be given to enzyme–phytochemical interactions, which may offer multifunctional benefits such as improved digestibility, enhanced bioavailability, and extended shelf life.

The limitations in Ayurvedic plant–derived enzymes need to be addressed through systemic standardization of plant raw materials using chemo profiling, controlled cultivation, and validated extraction protocols to ensure consistency and reproducibility. Regulatory challenges require early alignment with food safety frameworks through robust toxicological, stability, and efficacy assessments. Scalability can be improved by integrating green extraction technologies and pilot-scale validation. Conduction of in vitro and in vivo studies is necessary to determine pharmaco-kinetics, pharmaco-dynamics and toxicity of enzymes. Integration of modern analytical and bioinformatics tools for developing a strong foundation of mechanistic understanding is heavily required Finally, interdisciplinary collaboration combining Ayurvedic scholarship and industrial expertise is essential to translate traditional enzymatic knowledge into scientifically validated, ethical, and sustainable solutions for modern food systems (Jalalpure, 2025).

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The Mythological significance of *Saraca asoca* (Roxb.) Willd.:An Insight into its traditional folklore usage to modern Healthcare

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Abstract:

Given our traditional, religiously oriented society and intense interest in God and spirituality, "Mythology" has a stronger place in our heritage nation of India than it does in other nations. According to an examination of mythological literature, the "man-plant relationship" is a long-standing cultural concept that is directly or indirectly present in many fundamental facets of human existence. The Ashoka tree, *Saraca asoca* (Roxb.) Willd., was significant in the epic "Ramayana" because Devi Sita spent her traumatic days beneath it following her kidnapping by Lankan King Ravana. Buddhists believe that the hallowed Ashoka tree is where the Buddha Bhagavan Goutam was born. This blossoming tree is therefore native to our nation, which is home to many different spiritual traditions. According to these mythological viewpoints, *S. asoca* is frequently employed by ethnic cultures for female reproductive health care and has ethnomedical value in treating gynecological issues. According to ethnobotanical investigations, ethnic women use a decoction and extraction of stem bark, root, flower, and unopened floral buds to lessen bleeding, excessive menstrual pain (dysmenorrhea), vaginal inflammation following childbirth, vaginal itching with white discharge, and other sexual problems. Every component present in leaf, bark and flowers of *S. asoca* naturally contains several special bioactive phytocomponents and secondary metabolites that have been widely accepted by biomedical scientists and doctors to use the crude decoction for several ailments. Its anti-inflammatory, anti-menorrhea, anti-diabetic, and analgesic properties are attributed to a variety of phytochemical compounds found in various plant parts, including bark, dried bark, flowers, seeds, and pods. The most sought-after components reported till date are catechin, epicatechin, procyanidin, glycosides, lynoside, gallic acid, kaempferol, linolenic acid, linoleic acid, palmitic acid, oleic acid, stearic acid, and others. In addition to its qualities to treat gynecological syndromes, this plant species is used to treat ulcers, dyspepsia, piles, and other gastrointestinal disorders. Flavonoids including quercetin, β-sitosterol, and kaempferol-3-O-α-L-rhamnoside, have found to possess potent cardioprotective actions. In addition, β-sitosterol alone had augmented anti-cancer activity by inducing apoptosis in colon cancer cells whereas glycosides could bring forth CNS depressant activities. The primary focus of this exploratory study is *S. asoca's* significance in female reproductive problems in addition to its potent pharmacological activities for overall wellbeing of human subjects, with particular attention to its intricate phytochemistry and pharmacological properties which could serve as supplemental medications in future.

Keywords:

Saraca asoca (Roxb.) Wild., Mythology, Ethnomedicines, phytochemicals, phytopharmacology and Gynecological disorders.

Introduction

The *Saraca asoca* (Roxb.) Willd., commonly known as the Ashoka tree is a native Indian tree that has been an integral part of the Indian culture across centuries. The tree has held a significant position as a subject of mythological and cultural significance along with its role as an ethnomedical powerhouse. The tree has occupied positions in the mythological stories of Hinduism, Jainism, and Buddhism with various events being centered on this tree. Moreover, in recent day scientific developments, and extensive research of this plant, we have found promising results in its various plant components, that can be effective in playing a significant role in solving female health and hygiene crises, gastroenterological problems and a lot more. This paper emphasizes upon *Saraca asoca* (Roxb.) Willd. as a valuable ethnobotanical resource that can be an effective tool in solving female health crises.

Botanic description: *Saraca asoca* (Roxb.) Willd. is an evergreen tree with 7–10-meter height in average. Leaves are parapinnate 15-20 cm long and the leaflets 6-12, oblong and rigidly sub-coriaceous. Leaves are narrowly lanceolate; cork like at the base and with a shot pestistipules are intra-petiolar and completely united. The bark is dark brown or grey or almost black with warty surface. Stem bark are rough and uneven due to the presence of rounded or projecting lenticles. Bark channeled, smooth with circular lenticles and traversely ridged, sometimes cracked. Fracture splinting exposing striated surface, a thin whitish and continuous layer is seen beneath the cork leaver. Flowers are fragrant. Flowers are Polygamous apetalous, yellowish orange turning to scarlet, in short laterally placed corymbose, axillary panicles, bract small, deciduous, calyx petaloid. Seeds are 4-8, ellipsoid-oblong and compressed (Ayurvedic Pharmacopoeia of India, 2001; Ali, 2008; Rastogi, 2003 and Jain, 1968)

There is a confusion between *Saraca asoca* and *Saraca indica* for their geographical distribution and origin. Eminent Swedish Taxonomist Carolus Linnaeus first described the species *S. indica* L., originated from Java. This name was inappropriately applied to the actual Indian species *S. asoca* (Roxb.) W.J.de Wilde. These two species can be simply identified and distinguished by following characters: (a) During anthesis stage, the bracteoles of *S. indica* do not clasp the pedicels, whereas in *S. asoca* the bracteoles clasp the pedicels, (b) the two species have different geographical distribution throughout the World. *S. asoca* is widely distributed in Sri Lanka, India and Myanmar (Bandyopadhyay et al., 2024)

Classification (Biswas, 1972)

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Fabales
Family : Fabaceae
Subfamily : Detarioideae
Genus : *Saraca*
Species : *asoca*

Ecology and Cultivation (https://talkingmyths.com/the-story-of-the-ashoka-tree)

1. Soil and climate: The plant requires slightly acidic to neutral soils for good growth with medium to deep well drained fertile soils. It grows well in tropical to sub-tropical situations under irrigation.
2. Nursery raising and planting: The crop can be propagated by seeds and stem grafting. The seedlings are planted in the well manured field during the rainy season.
3. Thinning and weeding: Weeding and thinning of the plants may be done as and when required usually after 15-30 days for better growth.
4. Manures, fertilizers and pesticides: The medicinal plants have to be grown without chemical fertilizers and use of pesticides. Organic manures like, Farm Yard Manure (FYM), Vermi-

Compost, Green Manure etc. may be used as per requirement of the species. To prevent diseases, bio-pesticides could be prepared (either single or mixture) from Neem (kernel, seeds & leaves), Chitrakmool, Dhatura, Cow's urine etc.

5. Irrigation: Normally grown as rainfed crop but for better yield irrigation may be done as per requirement (weekly/fortnightly).
6. Harvesting/ post harvesting operation: Bark is removed from about ten years or older tree and then it has to be sun dried.

Ethnobotanical Value

Hindu Iconography (https://commons.wikimedia.org/wiki/Category:Saraca_indica)

The *Saraca asoca* (Roxb.) Willd. tree first appears in the Bhavishya Purana with an interesting story surrounding its origin and its powerful role in Ramayana. There lived a cannibal among the Bhil Tribe called Sashoka who roamed among the forests of Central India, killing travelers and consuming them. Once he encountered a calm Rishi in the forest, calmly focused in his meditation. He was moved by the Rishi's peaceful demeanor, and was struck by intense remorse and desire for penance. The sage blessed him to be reborn as the "Ashoka" tree and would provide Sita with comfort and shelter during her hostage in Lanka. Therefore we get the origin story of the "Ashok tree" which means "remover of sorrows" in Sanskrit.

We find the Ashoka tree again in the Sundara Kanda of Ramayana, where Sita is kept hostage in the Ashoka Vatica by Ravana. Sita passes her days of painful separation, grief and mourning, in the shelters of the Ashoka tree, thereby emphasizing its role in providing comfort and shelter to suffering women. Moreover, we find Lord Hanuman bringing Goddess Sita, the message of Ram's arrival by throwing a ring from the branches of the same Ashoka tree. Thus, it amplifies the role of *Saraca asoca* (Roxb.) Willd or the Ashoka tree in being a beacon of hope for women of our society.

Buddhist Iconography

The *Saraca asoca* (Roxb.) Willd. tree occupies a central theme in the Buddhist scriptures as well. The story revolves around the birth of Gautama Buddha being associated with the comforting presence of the Ashoka Tree. We find Queen Maya being on her journey when the labour pain struck her and thereby, she held on to the branch of the *Saraca asoca* (Roxb.) Willd. tree seeking support from her intense pain amplifying the tree's role as a pillar of support to the women of our society.

Ethnomedicinal Use Ayurveda (Verma et al., 2010)

In Ayurveda, doshas are defined as "Tridosha". These are primarily "Vata", "Pitta", and "Kapha". These are the fundamental forces of biological energy in nature, and are comprised of the five elements of nature. They govern all forms of physical, psychological, and emotional functioning of the body. By means of diet, they ensure that the various biological processes are regulated thereby helping us maintain the healthy life.

The three types of Doshas are explained here:

Vata (Ether and air): Vata governs the force that regulates our communication and our movement. These elements are critical in maintenance of healthy breathing, pulsations, muscular movement and nerve impulses. These are explained as cool, light elements.

Pitta (Fire and water): Pitta governs the force that regulates our metabolism and our digestion. These elements are critical in maintenance of proper functioning of our digestive system, body temperature, assimilation and absorption. These are explained as hot, heavy elements.

Kapha (Earth and water): Kapha governs the force that regulates our physical structure and body stability. It is believed to have been critical in maintenance of the muscles, tendons and proper functioning of our skeletal system. These are explained as smooth and stable elements.

By means of Rasa Panchaka, the various body elements of our body governed by the different Doshas, *S. asoca* produces medicinal effects.

In Ayurveda School of Medicine, the pharmacological benefits of any substance are classified under the five aspects of the Rasa Panchaka system.

Rasa Panchaka is a fivefold system that is designed to understand the effects a medicinal element has upon the interaction with human senses.

The different aspects of Rasa Panchaka are (Table.1):

a) Rasa (Taste): This is the immediate taste that has effects on the tongue. The 6 main Rasas in Ayurveda are, Sweet (Madhura), Sour (Amla), Salty (Lavana), Pungent (Katu), Bitter (Tikta), and Astringent (Kashaya). The Rasa of *Saraca asoca* (Roxb.) Willd. are described as Tikta (Bitter), Kashaya (Astringent), and Madhura (Sweet).

b) Guna (Quality): This is the physical attribute of a medicinal substance. Ayurveda comprises 20 opposite pairs of Gunas. The Gunas of *Saraca asoca* (Roxb.) Willd are described as Guru (Heavy).

c) Virya (Potency): This is the energy of the medicinal substance that has considerable effects on the human body. Ayurveda classifies this as of 2 main types, Ushna (Hot) and Shita (Cold). *Saraca asoca* (Roxb.) Willd. is described as being of Ushna (Hot) type.

Table:1. Different aspects of Rasa Panchaka.

Aspect	**Classification**	**Therapeutic Implication**
Rasa (Taste)	*Tikta* (Bitter), *Kashaya* (Astringent), *Madhura* (Sweet)	Clears toxins and tones tissues.
Guna (Quality)	*Guru* (Heavy)	Sustaining and nourishing to the system.
Virya (Potency)	*Shita* (Cold)	Reduces *Pitta* (heat) and inflammation.
Vipaka (Post-Digestive)	*Katu* (Pungent)	Stimulates metabolism after digestion.
Prabhava (Special Action)	*Sonisthapana*	Stabilizes blood flow and prevents hemorrhaging.

d) Vipaka (Post Digestive Effect): This is the post digestive taste of a medicinal taste, the affects of which are felt upon ingestion of a medicinal substance. The Vipaka is usually of 3 types, Madhura (Sweet), Amla (Sour), and Katu (Pungent). *Saraca asoca* (Roxb.) Willd. is described as being Katu (Pungent) in terms of its Vipaka.

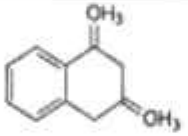
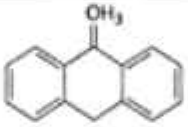
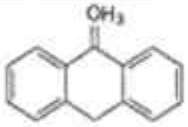
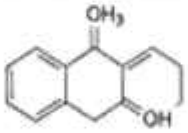
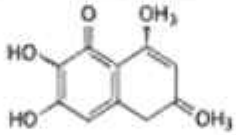
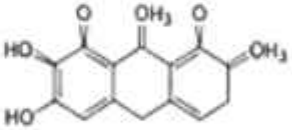
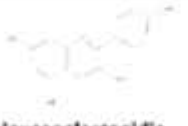
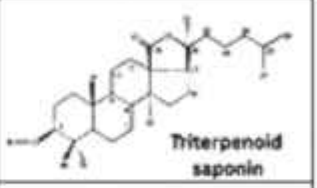
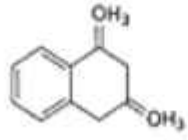
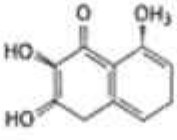
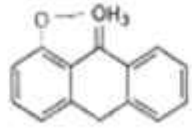
e) Karma/Prabhava (The essential action): The qualities of *Saraca asoca* (Roxb.) Willd. in terms of its Karma/Prabhava is described as being effective in Sonisthapana (Blood stabilizing), Kaphapittashamaka (Balance of Kapha and Pitta elements in our body), Grahi (absorbent) and Varnya (Complexion enhancing).

Thus, Ayurveda describes *Saraca asoca* (Roxb.) Willd, as being qualified in the fivefold Rasa Panchaka system, thereby approving it as a medicinally useful plant with benefits upon multiple health problems.

Phytochemical Constituents (Table.2)

The therapeutic benefits as have been established in classical Ayurveda have been supported by modern day scientific research, giving us important insights into the plants medicinal value.

Table:2. Phytochemical constituents of S*araca asoca*

Phytohmeical Class		Key Constituents	Chemical Structure
Tannins & Polyphenols	 Epicatechin	 Catechin	 Catechin
Phytosterols	 B-Sitoterol	 Stigmasterol	 Stigmasterol
Saponins and glycosides	 leucopelargonidin 3-o-glucoside	 Quercetin-3-O-glucoside	 Triterpenoid saponin
Flavorids	 Querctein	 Kaempforel	 Pelargonidin
Minerals	Organic Calcium Organic Magnesium	Ca^2 Mg^2	Ca^2 Often complexed with organic ligands

1. Tannins & Polyphenols (The Regulator for Hemostasis)

Constituents: Condensed tannins, Epicatechin, and Catechin.

Found in: Stem Bark

Effect on the Body: These act as astringents. When they come into contact with mucous membranes or wounded tissue, they precipitate proteins, creating a protective "tannin-protein" layer.

Clinical Result: This layer constricts local blood vessels and capillaries, effectively stopping internal bleeding (Menorrhagia). This is the scientific validation of the Sonitsthaapana (blood stabilizing) karma.

2. Phytosterols (The Regulator for Endocrine System)

Constituents: Beta sitosterol, Stigmasterol, and Ketosterols.

Found in: Stem Bark, Seed, Flowers and Leaves.

Effect on the Body: These compounds are "phytoestrogens." These are so called because of their likeliness to the human estrogen structure and they function by attaching to the estrogen receptors in the uterus, and playing an active role in supplementing low estrogen levels or blocking excess estrogen.

Clinical Result: They stimulate the endometrium and ovarian tissues, regulating the menstrual cycle and reducing the pain associated with Dysmenorrhea.

3. Saponins & Glycosides (The Tonic for Uterine System)

Constituents: Phenolic and Flavonoid Glycosides.

Found in: Leaves

Effect on the Body: They stimulate the smooth muscles of the uterus.

Clinical Result: The increased muscle tone of the uterine muscles makes it easier for more efficient contraction, helping in "cleaning" the uterine cavity and reducing Leucorrhea (abnormal discharge).

4. Flavonoids (The Anti-Inflammatory & Antioxidant)

Constituents: Quercetin, Kaempferol, and Pelargonidin.

Found in: Flowers.

Effect on the Body: They inhibit enzymes like COX-2, which are responsible for inflammation and pain. They also neutralize free radicals that cause cellular damage.

Clinical Result: This helps us understand Varnya (complexion enhancing) property of *Saraca asoca* (Roxb.) Willd. as it helps to deal with skin oxidative stress and also reduces pelvic inflammation.

5. Minerals (The Structural Catalysts)

Constituents: Organic Calcium and Magnesium.

Found in: Stem Bark

Effect on the Body: The Calcium ions play an essential role in blood clotting and muscle contraction.

Clinical Result: The high calcium content in Ashoka Bark acts as a natural coagulating agent. When combined with tannins, it ensures minimal blood and helps maintain a healthy tension in the uterine muscles.

Medicinal Value

In classical ayurveda, *Saraca asoca* (Roxb.) Willd. is popular in treating a myriad of diseases. In this paper we try to capture the vast efficacies that *Saraca asoca* (Roxb.) Willd. has upon different health problems.

Gynecological Issues: *Saraca asoca* (Roxb.) Willd. is an acclaimed solution to gynecological problems like dysmenorrhea (painful periods or Kashtartava), menorrhagia (heavy bleeding or Raktapradar), metrorrhagia, leucorrhea (Pradar) and uterine disorders like fibroid. It regulates Vata and Pitta Doshas to promote healthy function of the ovary, strengthen the uterine muscles and reduce menstrual pain due to heavy discharge.

Digestive Issues : The bark of *Saraca asoca* (Roxb.) Willd. plays a significant role in preventing problems like bloating, flatulence, diarrhea and a lot more owing to its astringent and dyspeptic qualities. In traditional Ayurveda, *Saraca asoca* (Roxb.) Willd. is known for being used in a variety of more diseases and disorders like, piles, dysuria and as an analgesic for Vata related pain.

Anti-keratinizing : Andangam Purath Shahid et al., studied the anti-proliferative and anti-keratinizing effect of *Saraca asoca* (Roxb.) Willd. on rat uterus having artificially induced keratinizing metaplasia by estradiol. It was observed that methanolic extract of *Saraca asoca* (Roxb.) Willd. was helpful in reducing the thickness of the epithelial layers, which got thickened due to estradiol. It also reduced the serum estrogen level, which showed its significance as an anti-keratinizing agent (https://commons.wikimedia.org/wiki/Category:Saraca_indica (https://commons. wikimedia.org/wiki/Category:Saraca_indica)

Anti-pyretic : The study conducted on Wistar rat models having artificially induced pyrexia by Brewer's yeast to check the anti-pyretic activity of *Saraca asoca* (Roxb.) Willd. demonstrated that its seeds are a good antipyretic agent at a particular dose (Verma et al., 2010).

Cardioprotective : Viswanatha Swamy et al., studied the cardioprotective activity of *Saraca asoca* (Roxb.) Willd. in rat models having induced cardiotoxicity by cyclophosphamide. It was found from the study that alcoholic extract of the bark reversed the changes which were induced by cyclophosphamide in the rats (Saravanan et al., 2011).

Anti-ulcer : Maruthappan V. et al., conducted a study on albino rat models to check the anti-ulcer activity of *Saraca asoca* (Roxb.). Willd. Pyloric ligation and aspirin-induced gastric ulcer were the two models used of albino rats. It was observed that the aqueous suspension of flowers is associated with anti-ulcer activity. Aqueous suspension reduced the ulcer index, which suggests its use as an anti-ulcer agent (Hazarika et al., 2021).

Anti-helminthic : As per the reported study, methanol, chloroform, and aqueous extract of *Saraca asoca* (Roxb.) Willd. is associated with the anti-helminthic activity. This activity was checked against adult earthworms *Pheretima posthuma*. All these extracts of *Saraca asoca* (Roxb.) Willd. exhibited much better anti- helminthic activity than the standard anti-helminthic drugs used in study (Srinivasa et al., 2018).

Analgesic : Angad Verma et al., conducted a study on albino rat models. Pain was induced in the models by using two methods which were the tail immersion method and formalin-induced pain

method. The extracts which were checked for analgesic activity were petroleum ether, chloroform, methanol, and water extracts. The dosage of extracts used was 200 and 400 mg/kg. It was found that methanol extract was a more potent analgesic agent (Panchawat et al., 2011).

Conclusion

Therefore, we can claim that *Saraca asoca* (Roxb.) Willd. or Ashoka tree is a plant that has been of considerable value in Indian ethnic traditions. The tree has shelter, comfort, and been a symbol of strength to indigenous women for centuries, as is clearly indicated by its eminent presence in the scriptures of Hinduism and Buddhism. Moreover, the tree occupies a central role in Ayurvedic treatment and has been used in providing cure to variety of diseases in humans. Thus, this amplifies its position as a medicinally valuable tree that can treat diseases ranging from female health and hygiene problems to digestive problems and much more. The tree that has been of significance towards women is still effectively and relevantly continuing to do so in our society. Contemporary phytochemical studies have given us more knowledge about its ethnomedicinal values and thereby show highly promising prospects in future of medicine and health care, as a sustainable organic solution to our critical diseases that torment our citizens. With judicious exploitation, the traditional knowledge and modern scientific studies can contribute to the use of *Saraca asoca* (Roxb.) Willd. as a highly medicinally beneficial alternative to synthetic drugs.

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Seed biopriming with Plant Growth Promoting Rhizobacteria

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Abstract:

Plant growth, promoting rhizobacteria (PGPR), based seed biopriming is fundamentally a green and sustainable mode of agriculture production with the least negative impacts on nature. It is also helpful in seed vigor, crop establishment, and overall plant productivity increase. Biopriming is a process where seed is manually moistened and a beneficial microbe is inoculated along with it. The method permits microbial colonization to occur at a very early stage but the seeds are not yet germinating. The main biocontrol agents for seed biopriming are the bacteria of the genera *Pseudomonas, Bacillus, Azospirillum,* and *Rhizobium.* These bacteria can perform a multitude of methods, both direct and indirect, to help plant growth. One of the methods is by providing more nutrients to plants. Thus, they fix nitrogen, dissolve phosphates and produce siderophores. Apart from that, they synthesize plant hormones such as indole-3- acetic acid, gibberellins, and cytokinins which assist seed germination and root growth. This technique of seed biopriming with PGPR benefits in treatment seed germination, seedling growth and also the resistance to diseases. PGPR adhere to plant roots, enhance nutrient uptake, synthesize growth hormones and provide protection against pathogens. All this helps the plants grow well, and provides good crop yields in sustainable agriculture. Moreover, biopriming with PGPR will make the crop tolerant of drought, salinity, and high and low temperature stressors. This is enabled by activation of the antioxidant defense system and stress, inducible genes. Biopriming with PGPR is less expensive, decomposes naturally, and is a more environmentally friendly product. In addition, biopriming also facilitates the establishment of root microbe interactions in early stages of dormant plant seed growth. However, by finding possible functions of specific rhizobial strains that promote seed dormancy alleviation, this review paper contributes to an overall understanding of microbial biostimulants for sustainable agricultural production.

Keywords :

Seed Biopriming, PGPR (Plant Growth-Promoting Rhizobacteria), Sustainable agriculture, Microbial colonization, Nutrient uptake, Stress tolerance, Biocontrol agents, Eco-friendly crop production

Introduction

In today's food agricultural system of modern agriculture confronts many challenges, due to the increasing demand of food production and climate change, and environmental pollution. The unsustainable agriculture with the increased reliance of chemical fertilizers, pesticides and herbicides provided by modern agricultural practices to boost crop yields, causes negative environmental both soil degradation, water pollution and biodiversity losses (Gabilondo et al., 2023). Moreover, crops are always under various biotic stresses (such as pests, diseases and weeds) and abiotic stresses including drought, high salinity and extreme temperature that further compromise agricultural productivity. Consequently, there is a strong demand for environmentally friendly farming practices that diminish the use of chemicals and make crops resistant to (abiotic) stresses (Mickan et al., 2021). One such encouraging remedy can be used to overcome this problem is the application of seed priming, which is a pre-sowing technique where in seeds are imbibed and

subsequently re-dried to enhance germination, seedling vigour and stress tolerance. Primed seeds have quicker and more uniform germination which enables better seedling growth especially under adverse environmental conditions (Zamljen et al., 2023). Nevertheless, although traditional seed priming techniques are mostly based on physical or osmotic treatments; the utilization of biological agents like PGPR provides a more sustainable and efficient alternative for priming called biopriming. Biopriming as a seed germinating technique can be used as the treatment for dormant seeds with beneficial rhizobacteria prior to sowing. These bacteria, which are indigenous to plant rhizosphere, have been reported to promote plant growth through wide range of mechanisms that include increasing nutrient uptake, synthesizing different types of hormones and protecting against soil-borne pathogens (Gómez et al., 2024). It is worth noting that the addition of chemical fertilizers and pesticides for crop production results in increased costs and environmental hazards and, therefore, PGPRs can be offered as an ecological-friendly and economic alternative to mitigate such adverse effects because they contribute to promoting plant health. The goals are to assess how PGPR aids in the development of seeds and seedlings, investigate the advantages of biopriming in sustainable agriculture, and draw attention to the difficulties and potential avenues for further study (Juárez-Maldonado et al., 2024). This study aims to provide a thorough understanding of biopriming's potential to increase crop productivity while reducing environmental impact by compiling and evaluating the most recent studies.

Seed priming and its type

Seed priming refers to the process of partially hydrating seeds in controlled conditions so to activate the metabolic processes of germination, but thereafter dehydrating the seeds again before the radicle (embryonic root) is allowed to emerge. The technique enables seeds to germinate faster, more uniformly, and with higher vigor when eventually planted in field. Activation of enzymes, DNA repair, and protein synthesis are among the physiological and biochemical activities that are initiated during priming as seed is 'made up' for rapid growth when conditions are favorable (Ghasemi et al., 2023). Therefore, primed seeds generally exhibit better emergence, seedling establishment, and are less sensitive to various stresses such as drought, salinity, and temperature extremes. There also exist different types of seed priming that depend on the materials and methods used (Waqar et al., 2023). Hydropriming is the easiest method of seed priming, where seeds are soaked in water for a fixed period and then dried before planting. Osmo priming on the other hand is the process of soaking the seeds in osmotic solutions such as polyethylene glycol (PEG) or salts, which regulate water uptake and prevent over, imbibition. Halopriming is an Osmo priming method that uses inorganic salt solutions, for example, sodium chloride or potassium nitrate to increase germination and stress tolerance (Wen et al., 2024). Biopriming involves seed hydration process, during which beneficial microorganisms, like bacteria or fungi, are added. These microorganisms not only provide the seedlings with protection against pathogens but also help to their growth. Hormonal priming implements the use of plant growth regulators, for instance, gibberellic acid or

salicylic acid, to intensify germination and seedling vigor. Nutri priming is a practice of seed soaking in nutrient solutions for the purpose of facilitating early nutrient availability. In general, seed priming is a low, cost and environmentally friendly technique that has a significant place for contemporary sustainable and agricultural crop production (Fahde et al., 2023).

Plant growth promoting Rhizobia (PGPR)

PGPR constitute a varied set of beneficial soil bacteria that live on the roots' surface or the root, soil interface of plants and promote plant growth and development through multiple direct and indirect mechanisms. In the frame of sustainable agriculture and environment conservation, PGPR have attracted considerable interest as green and safe substitutes instead of chemical fertilizers and pesticides since they help to produce more crops without destroying the soil (Gómez et al., 2024).

These microbes form intimate relationships with the roots of plants and the manner by which they deliver their beneficial impact is through enhancing nutrition, secretion of plant hormone, like substances, and shielding plants against biological and non, biological stresses. PGPR are generally classified regarding their method of interaction and the place of activity into extracellular (non-symbiotic or rhizospheric) and intracellular (symbiotic or endophytic) categories, both of which contribute considerably to plant growth promotion (Guzmán et al., 2024).

Extracellular PGPR are bacteria which reside either in the rhizosphere or on the root surface. Their main plant growth promoting action involves plant growth secretion of metabolites, which comprise; phytohormones, organic acids, siderophores, as well as antimicrobial compounds. They also assist the plants gaining more nutrients through solubilizing phosphorus, potassium and zinc by synthesizing organic acids hence transforming the insoluble ones into the plant-available ones. The bacteria also perform non-symbiotic fixation of the nitrogen in the atmosphere to provide more nitrogen to the plants particularly in a place where there is a scarcity of nitrogen. Intracellular PGPR, on the other hand, are those bacteria with which the plants are very closely associated, and often infect the root tissue to the extent of establishing a non-parasitic association. The best-known example of an intracellular PGPR is the development of root nodules which possess a highly efficient and regulated mechanism of atmospheric nitrogen fixation, i.e. the Rhizobium species (Zeng et al., 2023).

Moreover, the development of antibiotic, hydrogen cyanide as well as lytic enzyme by the PRGP inhibits any ill effects of pathogenic organisms in seeds and soil thus establishing a shield to the newly sprout seedlings. Some of the PGPR also secrete the enzyme, ACC deaminase, which reduces plant ethylene, a stress hormone, which under adverse environmental conditions like salinity, drought or stress by heavy metals may inhibit seed germination and premature growth of seedlings. PGPR can regulate ethylene levels to induce the seedlings to come out of the dormancy caused by stress and to attain successful germination. In addition to germination, the role of the PGPR is important in the nutrient uptake during the plant life cycle, the improvement of the root structure, and the augmentation of the root surface area. Plants can explore more volume of soil and

make better use of water and nutrients through the use of improved root systems which are identified by more lateral roots and root hairs (Gong et al., 2023).

Solubilization of phosphorus and movement of micronutrients by PGPR make sure the supply of any essential element needed in metabolic processes, photosynthesis and biomass building constant. The production of siderophore by PGPR binds the iron in the soil and it is therefore accessible to the plants and on the other hand restricts its accessibility to pathogenic microorganisms, thus indirectly enhancing plant health. Moreover, the synergistic interaction of mycorrhizal fungi and the use of the PGPR will result in increased nutrient absorption and especially phosphorus, indicating the integrative effect of the PGPR in the microbial communities of the soils. (Añibarro et al., 2025) By these processes, not only nutrient use efficiency is enhanced by the use of PGPR, but also there is minimized reliance on the use of synthetic fertilizers, which has led to sustainable crop production systems.

PGPR are an important part of the plant-microbe interactions and they include extracellular and intracellular bacteria that form an associative and symbiotic relationship with the plants. The fact that they increase seed germination, root and shoot development, and nutrient uptake by various biochemical and physiological processes makes them very important in contemporary farming practices. PGPR are important to the future of sustainable agriculture because it can be used as an alternative sure-footed solution to boost agricultural production and maintain environmental quality through the promotion of natural, sustainable growth of plants. Plant growth promoting rhizobia (PGPR) are symbiotic soil bacteria which improve crop productivity in a variety of direct and indirect ways. In addition to biological fixation of nitrogen, rhizobia solubilize phosphorus, make siderophores, which enhance getting of iron, and make phytohormones, including indole-3-acetic acid, cytokinins, and gibberellins, that induce root architecture (Flores et al., 2025).

Although PGPR is a best for supportive technology but not a replacement of good soil management as because plant growth, PGPR show host specificity by only colonizing the rhizosphere of certain plant species that they are compatible with through root exudates (Basu et al., 2021). This host, specific interactions make it possible for PGPR to improve the growth of plants through different ways like nutrient mobilization, phytohormone production, and pathogen suppression (Basu et al., 2021). Therefore, PGPR work best when they are used on their compatible host plants resulting in increased growth and productivity. PGPR tend to be less effective in high, fertility soils where there is a high content of nitrogen and phosphate, for example. Under such scenarios, the plants are less dependent on microbes for nutrient acquisition, which in turn makes PGPR activity less effective (Goswami & Deka, 2020). Consequently, the growth, promoting effect of PGPR is significantly higher in soils that are deficient in nutrients than in those that are nutrient, rich. PGPR cause a carbon cost for the host plant since these bacteria use root exudates as an energy source (Goswami & Deka, 2020). The plant is to a certain extent releasing its photosynthetically fixed carbon to these microbial populations in the rhizosphere. This carbon expenditure by the plant

can be seen as a limitation of resources of the plant; however, it is in many cases compensated by the advantages like higher nutrient uptake and stress tolerance. The overall outcome is thus dependent on the surrounding conditions as well as the functioning of the PGPR plant interaction (Sarker et al., 2021).

Methodology for the seed biopriming technique

In the first step as shown in Figure 1, one should select a healthy leguminous plant that will provide a vast source of Rhizobial bacteria. Leguminous plants are different from other plants as they have the ability to enter into a symbiotic relationship with certain species of *Rhizobium* through root nodules (or specialized plant structures that are developed on roots). Root nodules develop on the roots is after the process of signal exchange between leguminous plants and soil, *borne Rhizobium* species, which result in the establishment of nitrogen, fixing symbiosis. Rhizobial bacteria in root nodules fix atmospheric nitrogen into forms that can be used biologically, which leads to a healthy growth of legumes and an increase in soil fertility. So, at this stage, care should be given in finding and taking root nodules together with roots from a leguminous plant so as not to damage the other plant tissues (Flores-Félix et al., 2025).

In Step 2 as shown in Figure 1, the harvested root nodules were first cleaned thoroughly by washing with water to get rid of soil particles and other debris stuck on the surface. An initial cleaning of this nature is very important in the isolation process to get rid of loosely attached microorganisms which, if not removed, may cause contamination. Next, the nodules are separated from the roots and submitted to surface sterilization treatments to be sure that only the bacteria inside the nodules are isolated. The nodules are first immersed in 70% ethanol for about 10 seconds which serves to break down the contaminants on the surface and the microbes that are only lightly attached. This is then followed by a 5-minute exposure to 0.5% sodium hypochlorite, which is a strong disinfectant that can kill even resistant surface microbes. Afterward, the nodules are washed thoroughly several times with sterile water to be sure that no sterilizing agents were left which might have harmful effects on the bacterial growth (Añibarro-Ortega et al., 2024).

In Step 3 as shown in Fig. 1, the attention is primarily placed on verification that the surface disinfection process was successful. The last sterile water used for rinsing is plated onto different culture media, and checked for microbial growth. If there is no growth, it means the surface sterilization was very thorough, so bacterial colonies later on will only be those from the inside of the nodules. After sterility is certain, the nodules are broken up in sterile water without contaminations, which results in the endosymbiotic bacteria becoming free in the solution.

This is a very important step because it makes it possible to get Rhizobial cells out and still keep them alive for culturing. In Step 3 as shown in Fig. 1, the attention is primarily placed on verification that the surface disinfection process was successful. The last sterile water used for rinsing is plated onto different culture media, and checked for microbial growth. If there is no growth, it means the surface sterilization was very thorough, so bacterial colonies later on will only

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The crushed nodule suspension is then spread on Yeast Mannitol Agar (YMA) medium with Congo Red dye, where the pH is adjusted between 6.8 and 7.0. Such selective and differential media are commonly used for isolating *Rhizobium* species. Rhizobial colonies usually absorb very little or no Congo Red, thus they look pale or white, whereas non, rhizobial contaminants generally take up the dye and appear red. The inoculated plates are kept at 30°C for 2, 3 days and microbial growth is checked every 24 hours. This incubation time allows the development of separate bacterial colonies that are ready for further analysis (Panda et al., 2024).

The target colonies in Step 4 as shown in Fig. 1 are divided into Rhizobial isolates and non, Rhizobial isolates based on the appearance of the colony, the absorption of Congo Red, and the growth features. Typically, Rhizobial colonies are circular, transparent, mucilaginous, and slow, growing, while non Rhizobial colonies show characteristics such as rapid growth and strong color. This phase of differentiation is critical for the selection of beneficial Rhizobial strains only for further testing.

Step 5 as shown in Fig. 1 includes selection of colonies, strain purification and identification. Repeatedly sub culturing of individual Rhizobial colonies yield contamination-free pure cultures. Purification guarantees the genetic and functional uniformity of the bacteria strain. Recognizing microorganisms requires sensitive methods that can involve biological techniques and 16S rRNA gene sequencing. The Rhizobial PGPR strains are verified and shown to be suitable for plant growth–promoting applications through identity and authenticity.

During step 6 as shown in Fig. 1, a special purified strain of rhizobium displaying analytic properties is chosen Nitrogen fixation efficiency; production of phytohormones like indole-3-acetic acid (IAA); phosphate solubilization; production of an iron chelator called siderophore; stress tolerance. All these characteristics work together to help the bacterium to act as effective PGPR.

Step 7 as shown in Fig. 1 involves biostimulation, which requires the application of PGPR Rhizobial strain on seeds or soil. The activity of microbes enhances plant physiological processes. This is called biostimulation. Rhizobial PGPR strains enhance nutrient availability and root development stimulates the diversity of microorganisms in the rhizosphere and improves plant growth. Early-life development stages of seeds and seedlings have enhanced metabolic activity due to their interaction. Finally, PGPR effects on the germination of dormant seeds Step 8 as shown in Fig. 1. The use of Rhizobial PGPR boosts seed germination, reduces dormancy, enhances root-shoot growth and tolerance to environmental stress conditions. Thus, Rhizobial PGPR have agricultural importance and can be used as a substitute for chemical fertilizers and growth regulators.

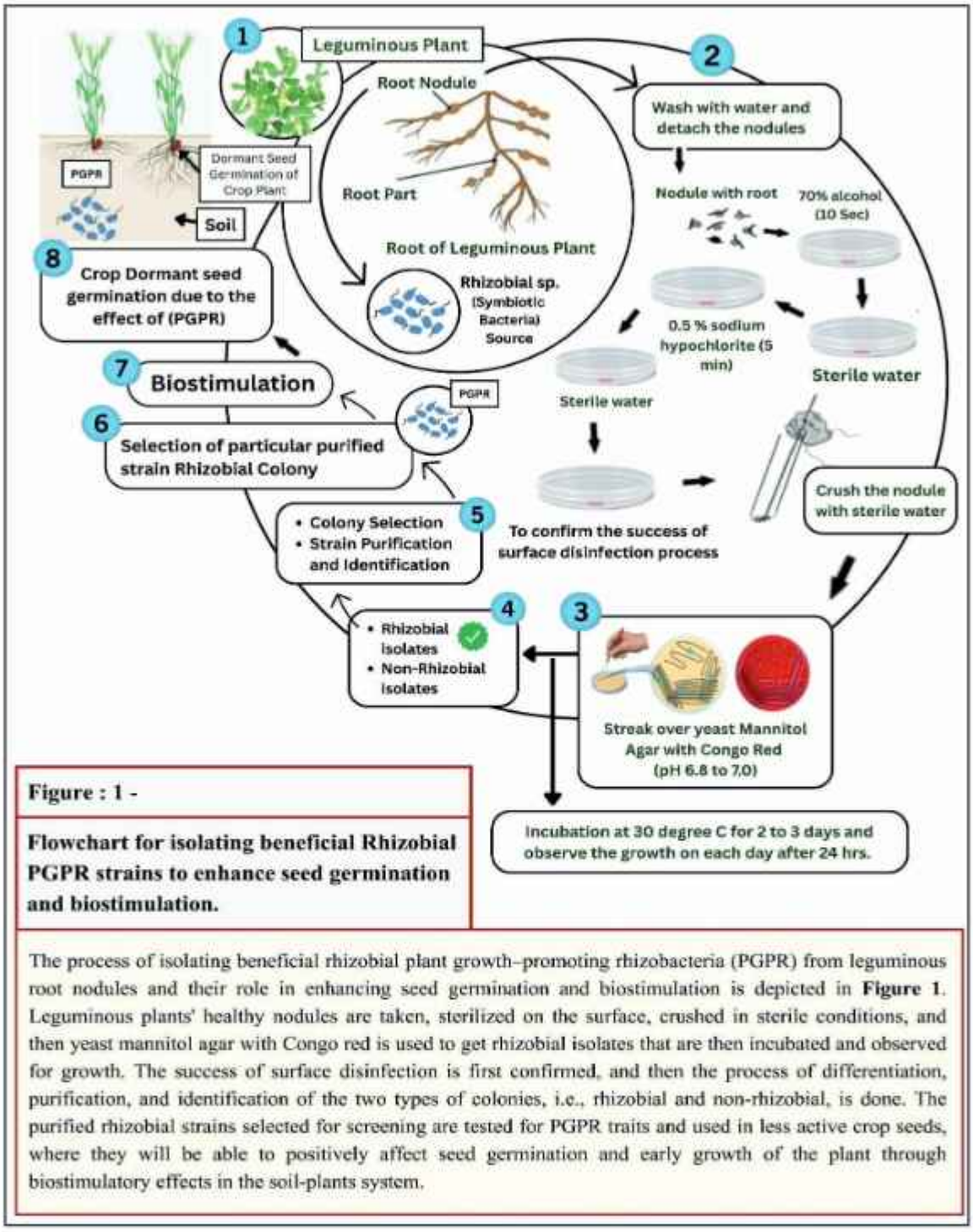


Figure : 1 -

Flowchart for isolating beneficial Rhizobial PGPR strains to enhance seed germination and biostimulation.

The process of isolating beneficial rhizobial plant growth–promoting rhizobacteria (PGPR) from leguminous root nodules and their role in enhancing seed germination and biostimulation is depicted in **Figure 1**. Leguminous plants' healthy nodules are taken, sterilized on the surface, crushed in sterile conditions, and then yeast mannitol agar with Congo red is used to get rhizobial isolates that are then incubated and observed for growth. The success of surface disinfection is first confirmed, and then the process of differentiation, purification, and identification of the two types of colonies, i.e., rhizobial and non-rhizobial, is done. The purified rhizobial strains selected for screening are tested for PGPR traits and used in less active crop seeds, where they will be able to positively affect seed germination and early growth of the plant through biostimulatory effects in the soil-plants system.

Fig. 1. Flowchart for isolating beneficial Rhizobial PGPR strains to enhance seed germination and biostimulation.

Mechanism of action of seed biopriming with PGPR

The process of seed biopriming with beneficial PGPR is an environmentally friendly and effective approach for agricultural purpose that is employed by soaking the seeds in water along

with PGPR. In this technique, the seeds are exposed to moisture and select strains of PGPR are used to treat these seeds so that the microbes can colonize the surface of seeds without causing the emergence of the radicle (Guzmán et al., 2024). The PGPR which have been applied interact very closely with the seed and emerging roots and induce various physiological, biochemical, and molecular responses that promote plant growth and help to combat biotic and abiotic stresses. The seed biopriming with PGPR has the mechanism of action involving which enhance germination, nutrient acquisition, hormonal regulation and disease suppression (Lu et al., 2023).

PGPR biopriming allows for higher nutrient accessibility and absorption which is of great importance during the initial plant life cycle. A number of PGPR strains have been reported to possess the capability of turning insoluble phosphorus into soluble forms by secreting organic acids that include gluconic and citric acids. Likewise, some strains are able to bacteria which are nitrogenase activity, the process through which atmospheric nitrogen is converted into biologically usable forms; fix the nitrogen from the atmosphere (Lu et al., 2023). The same or other PGPR strains are known to mobilize the micronutrients as zinc, iron, and potassium. When the seeds are bio primed, these bacteria that are associated with nutrient mobilization come into the existing rhizosphere very fast thereby enabling the plants to absorb nutrients easily as well as support their initial growth and development especially in tired soils (Guzmán et al., 2024). The use of bio primed seeds for plant growing will result in the application of bacterial production of phytohormones such as indole-3-acetic acid (IAA), gibberellins, cytokinins, and abscisic acid coming from the lab. The application of IAA results in root lengthening and making of new lateral roots which will lead to an increase of the root surface area for water and nutrients absorption. Gibberellins get to work and seeds requirement and stem elongation whereas cytokinins promote cell division and tissue maintenance (Jiménez et al., 2020). The interactions among these hormones lead to the biopriming with PGPR bacteria being the one steering the plant developmental processes and hence the plant vigor (Gómez et al., 2024).

In spite of the fact that PGPR are a source of eco, friendly benefits for boosting crop yield and soil fertility, their consistent effectiveness is constrained due to a few limitations (Muñoz et al., 2023). PGPR are usually host, specific and hence, they deliver maximum performance only with those plant species with which they are compatible (Muñoz et al., 2023). Therefore, their application on a wider variety of crops is limited. In environments with rich soils, especially those with high levels of nitrogen and phosphate, their effectiveness is reduced. This is because the soil's natural fertility decreases the reliance on microbial activity(Muñoz et al., 2023). Another factor that limits the use of PGPR is the carbon cost to the host plant since plants give 5 - 20% of their photosynthates to microbes, which may eventually lead to lower growth.

PGPR are not compatible with all crops universally and might not be able to establish on some plant species. The environment is a major factor in how well they work. Things like soil pH, moisture levels, temperature, and conditions like drought or waterlogging can negatively affect how well they can settle and function (Muñoz et al., 2023). On the one hand, chemicals in

agriculture such as pesticides and herbicides are detrimental to microbes; on the other hand, inoculum quality is directly responsible for performance (Anand et al., 2022). In addition, PGPR need a slow establishment period and yield responses under field conditions are generally variable. Therefore, these limitations highlight the need to combine PGPR with good soil management and specific farming practices to achieve consistent results (Muñoz et al., 2023).

Future Aspects

Seed biopriming along with PGPR is receiving the attention of sustainable agricultural technology and its future is closely tied to the global demand for environment-friendly ways of producing crops (Juárez-Maldonado et al., 2024). PGPR-based seed biopriming is expected to be a significant factor in the reduction of chemical inputs and, at the same time, in crop productivity's maintenance or even enhancement in the light of the growing global concerns about soil degradation, climate change, and the excessive application of fertilizers and pesticides. It is projected that the primary focus of future research will be the refinement of the biopriming techniques tailored to diverse crops, soil varieties, and agro-climatic conditions to achieve maximum effectiveness on the field level (Sati et al., 2023).

The breakthroughs in microbial biotechnology and molecular biology are going to be instrumental in the efficient selection and development of PGPR strains that can be applied for biopriming. The application of genomic and metagenomic techniques will facilitate the identification of specific functional genes that confer the microbes with the capabilities of tolerating stresses, solubilizing nutrients, and resisting diseases (Gómez et al., 2024). The idea of developing multi-strain or consortium-based PGPR formulations is another aspect that will dominate the future since the synergy of the interactions among the beneficial microbes may result in a wider and more uniform promotion of plant growth despite the changing environmental conditions.

Seed biopriming with PGPR carries considerable potential in the future to enhance the resistance of crops to various abiotic stresses such as drought, salinity, heat, and heavy metals. As the adverse effects of climate change increasingly put stress on crops, PGPR strains that are resistant to stress can be included in the seed biopriming methods to improve the plants' adaptability and survival (Gómez et al., 2024). It is believed that the coming studies will delve into the specifics of PGPR for different stress types and the gene and antioxidant system controlling plant stress responsiveness that is helping in the case of climate-smart agriculture (Guzmán et al., 2024).

Discussion and Conclusion

Seed biopriming using PGPR is one of the best and most environmentally friendly methods that have been developed recently for crop establishment, growth, and yield enhancement. This method results in rapid, efficient, and extensive colonization of the root system by PGPR due to seed microbial inoculation taking place simultaneously with controlled seed hydration (Gómez et

al., 2024). The interaction of seeds with beneficial microbes right at the start significantly helps in mollifying the disparities in germination, increasing seedling vigor, and overall early plant development, which are the major factors deciding the crop yield at the end of the season. Advantage of the PGPR, based seed biopriming in several aspects of plant growth and development makes it indispensable in the current agricultural scenario (Sati et al., 2023). Through the different mechanisms like solubilization of nutrients, biological nitrogen fixation, production of phytohormones, and induced systemic resistance, PGPR helps to get more nutrients by the plant, to be better able to cope with stresses, and effectively suppressing pathogens in the soil (Boutasknit et al., 2024). These living processes are not only helping the plant to grow well but also lowering the use of chemical fertilizers and pesticides which are the main contributors to environmental pollution and soil degradation (Gómez et al., 2024).

Although the PGPR is a low-cost system and is effective in phosphate deficient soil and is a sustainable farming but it does not suit all plants since each plant species releases different root exudates and has different rhizosphere conditions (Vurukonda et al., 2016). Certain PGPR strains are so specific that they only colonize and function with their particular host plant, thus limiting their application worldwide. If PGPR are applied to incompatible crops, they might not establish themselves or offer any growth benefits to the plants. In other words, a thorough selection of PGPR strains should be done for the positive performance of crops (Koskey et al., 2021). Effectiveness of PGPR depends to a large extent on the quality of the inoculum used. Good root colonization depends on high viability, correct strain identity, and sufficient population density (Naamala & Smith, 2020). A bad quality inoculum is a major reason for weak establishment and inconsistent plant growth responses (Koskey et al., 2021). The application of agricultural chemicals - which includes pesticides and fertilisers, has a detrimental effect on populations and activity levels of PGPR (Anand et al., 2022). There are numerous soil environmental conditions considered to be planting growth conditions for PGPR, including but not limited to, soil pH, moisture and temperature (Sarker et al., 2021). Due to certain differences in the quality of soil, crop types, and weather conditions, with various differences in the environmental and biological factors the viability and functioning of PGPR might get affected and as a result of this differences PGPR will not all provide the same benefits to agricultural production (Sarker et al., 2021). Therefore, PGPR will not develop or establish successfully on plant roots under adverse environmental conditions (e.g., drought or flood) (Sarker et al., 2021). Successful introduction of PGPR will be determined by managing both climatic conditions in addition to the application method and the establishment method (Sarker et al., 2021).

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Decoding wood anatomical trade-offs underpinning climate adaptation in phylogenetically distant mangroves of the Indian Sundarbans

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Abstract:

Mangroves persist in intertidal saline environments through specialized structural adaptations of all parts of their body.The adaptive traits of mangroves collectively support carbon acquisition, water transport, salt management and mechanical stability to survive in saline, waterlogged substrate. Wood of mangroves particularly important as it integrates long-term functional responses and thus provides a valuable record of environmental adjustment. This study examines wood anatomical trade-off mechanisms in three dominant mangrove species—*Avicennia marina, Rhizophora mucronata,* and *Sonneratia apetala*—from two sites of the Indian Sundarbans, theregion experiencing increasing salinity and climatic variability. Vessel traits, fiber characteristics, tissue allocation, and derived functional indices were quantified through anatomical studies. Significant interspecific differences were detected in vessel size, vessel density, vessel grouping, and fiber traits, indicating contrasting strategies for hydraulic conduit and mechanical strength. *Rhizophora mucronata* exhibited larger vessels and greater fiber investment, consistent with enhanced mechanical support. *Avicennia marina* showed narrower vessels, moderate vessel density, and higher rigidity, reflecting a safety-oriented hydraulic strategy. In contrast, *Sonneratia apetala* displayed higher vessel density, greater vessel area, and lower fiber investment, indicative of a growth-oriented strategy. Multivariate analyses revealed distinct species separation driven by coordinated anatomical variation, demonstrating that wood anatomy functions as an integrated system for mitigating environmental stress. These findings highlight species-specific trade-offs for navigating diverse coastal conditions—knowledge essential for formulating conservation strategies for mangrove ecosystems under shifting climate scenarios.

Key words :

Climate change; Indian Sundarbans; Mangroves; Trade-off mechanisms; Wood anatomy

Introduction

Woody intertidal saline forests, known as mangroves, cover only ~0.1% of the Earth's land surface, yet they play a disproportionate role in coastal protection, climate regulation, and carbon sequestration. Despite their limited spatial extent, mangroves store exceptionally large carbon stocks, often exceeding those of tropical rainforests, and act as effective natural buffers against sea-level rise and storm surges (Donato et al.., 2011; Alongi, 2015).In addition to their role in climate mitigation, mangroves support high biological diversity and provide critical ecosystem services to coastal communities (Lovelock et al.., 2015; Ahmed et al.., 2023;Mondal & Naskar, 2024a).

Mangroves persist in intertidal saline environments through specialized adaptations to high salinity, periodic inundation, and anoxic soils (Mandal & Bar, 2012).These include aerial root systems for gas exchange, modified reproductive strategies, and highly efficient salt-regulation mechanisms. Salt balance is maintained through selective exclusion at the root level, internal compartmentalization, increasing leave succulence and active secretion via leaf salt glands (Tomlinson, 1994; Shi et al.., 2005; Naskar et al.., 2021; Mondal & Naskar, 2025).

The dominant mangrove genera *Avicennia, Rhizophora,* and *Sonneratia* exhibit contrasting ecological strategies. Avicennia combines salt exclusion with active secretion, whereas *Rhizophora* and *Sonneratia* primarily restrict salt entry at the root–vascular interface (Tomlinson, 2016). These genera also differ in reproductive modes and root architecture, with pneumatophores in *Avicennia* and *Sonneratia* facilitating gas exchange, and stilt roots in *Rhizophora* providing mechanical stability in unstable substrates (Tomlinson, 1994). These ecological and morphological contrasts likely shape stem wood anatomy, specifically traits that govern water transport and mechanical support.

Wood anatomical traits are central to plant hydraulic function and structural integrity. Xylem architecture represents an integrated balance between hydraulic efficiency and hydraulic safety, governed by coordinated variation in vessel diameter, vessel density, vessel connectivity, and fiber investment (Tyree & Zimmermann, 2002; Carlquist, 2001; Choat et al.., 2012). Large vessels enhance transport efficiency but increase vulnerability to cavitation, whereas smaller and more numerous vessels reduce risk of embolism at the expense of conductivity (Sperry et al.., 2008). In mangroves, these trade-offs are particularly pronounced because salinity induces physiological drought even in water-saturated soils.

Previous studies have documented safety-oriented xylem traits in mangroves, including narrow vessels, high vessel density, vessel grouping, and elevated wood density (Schmitz et al.., 2006; Robert et al.., 2009). However, most investigations have focused on single traits or individual species, limiting insight into how multiple anatomical features interact to form integrated functional strategies. Increasingly, wood anatomical trade-offs are recognized as key determinants of species performance, niche differentiation, and distribution across environmental gradients (Zanne et al.., 2010; Reich, 2014). Comparative analyses integrating vessel traits, fiber characteristics, and tissue allocation within a unified trade-off framework remain scarce, particularly for South Asian mangroves

The Sundarbans, the world's one of the largest contiguous mangrove forest, provides an exceptional setting to examine these relationships under changing climatic conditions (Naskar 2004; Mondal et al., 2025). This deltaic ecosystem experiences strong spatial and temporal gradients in salinity, tidal inundation, sedimentation, and freshwater input, which has intensified due to sea-level rise, reduced upstream discharge, and increasing climatic extremes (Gopal & Chauhan, 2006; Hazra et al.., 2010). Salinity is a primary driver of mangrove distribution and performance in the region, influencing growth, mortality and species composition (Mitra et al.., 2011; Lovelock et al.., 2017). While physiological responses to salinity have been documented, wood anatomical responses—particularly those integrating long-term environmental stress—remain poorly understood.

Within the Indian Sundarbans, A*vicennia marina, Rhizophora mucronata,* and *Sonneratia apetala* occupy distinct positions along salinity and tidal gradients and represent contrasting life-

history strategies (Mandal and Bar, 2019; Mondal et al.., 2025). *Avicennia marina* is highly salt tolerant and broadly distributed (Mandal & Naskar, 2025), *R. mucronata* exhibits strong mechanical investment suited to dynamic tidal environments (Ankure et al., 2023), and S. apetalais associated with relatively freshwater-influenced zones and rapid growth(Mondal & Naskar, 2024b). Together, these species provide a robust framework for examining how coordinated variation in vessel and fiber traits underpins trade-offs between hydraulic efficiency, hydraulic safety, and mechanical stability.

In this context, the present study aims to decode wood anatomical trade-off mechanisms in these three dominant mangrove species from the Indian Sundarbans. By quantifying vessel characteristics, fiber morphology, tissue allocation, and derived functional indices, this study seeks to identify interspecific differences in hydraulic and mechanical strategies and to evaluate how these strategies reflect adaptive responses to salinity-driven and climate-mediated stress. The findings contribute to a trait-based understanding of mangrove resilience and provide insights relevant to predicting species responses under continuing environmental change.

Materials and Methods

Study species and sampling design

Three true mangrove species—*Avicennia marin*a (Forssk.) Vierh., Rhizophora mucronate Poir., and Sonneratia apetala Banks.—were selected for wood anatomical analysis because they are dominant components of the Indian Sundarbans. Species identification was confirmed using standard field manuals (Mondal & Bar, 2019; Tomlinson, 2016). Wood samples were collected from two sites of Indian Sundarbans:Balikhal and Jharkhali of South 24 Parganas, West Bengal, where at least five mature individuals of each species occurred within a 50 m radius within the intertidal mudflat. The sites were selected to minimize variation due to salinity fluctuations and tidal inundation.

Wood samples were collected from healthy, well-branched individuals with a diameter at breast height (DBH, 1.3 m) greater than 30 cm. From each tree, a 20 cm long branch segment with a minimum diameter of 10 cm was collected, labeled in the field, and transported to the laboratory. In the laboratory, a 0.5 cm-thick stem disc was cut from each branch, and small wood blocks (1 cm × 1 cm) were prepared from the disc. These blocks were soaked overnight in distilled water and then boiled for 30 min in a glycerine–water solution (1:10), following Jansen et al. (1998) for the softening the tissue.

Sectioning and staining

Radial longitudinal section (RLS), tangential longitudinal section (TLS), and transverse sections (TS) were prepared from the wood blocks using a hand microtome (Model: United Scientific MCT001). Sections with a thickness of 3–5 µm were selected and washed with double-distilled water. The sections were immersed in 4% sodium hypochlorite for 10 min followed by

thorough washing. Double staining was performed following Tolivia and Tolivia (1987). Stained sections were photographed using an Olympus CH20i light microscope to obtain qualitative and quantitative anatomical data.

Anatomical measurements and trait quantification

Wood anatomical characterization was done following standard protocols (IAWA Committee, 2006; Carlquist, 1988; Tyre & Zimmermann, 2002). From TS, vessel area (%), fiber area (%), vessel diameter, and fiber diameter were measured. Ray cell arrangement was assessed from RLS, while fiber wall thickness and fiber length were measured from TLS. The wood traits were calculated by the following formulae :

$$1.\ \text{Vessel density (VD)} = \frac{\text{(Number of vessel)}}{\text{mm}^2}$$

$$2.\ \text{Vessel grouping index (VGI)} = \frac{\text{(Mean number of Vessel)}}{\text{(Vessel group)}}$$

$$3.\ \text{Coefficient of rigidity (CR)} = \frac{\text{(Fibre wall thickness)}}{\text{(Fibre width)}}$$

$$4.\ \text{Flexibelity ratio (FR)} = \frac{\text{(Fibre lumen thickness)}}{\text{(Fibre width)}}$$

Statistical analyses

All statistical analyses were conducted using R statistical environment. One-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test, was used to assess interspecific differences at a significance level of $p < 0.05$. Principal component analysis (PCA) was performed to examine multivariate patterns among the studied species.

Results

Wood anatomical characterization

The wood anatomy of Avicennia marina, Rhizophora mucronata, and Sonneratia apetala exhibits distinct structural differentiation across transverse, radial longitudinal, and tangential longitudinal sections, reflecting contrasting ecological strategies in saline intertidal environments.

In transverse sections, vessel architecture varies significantly among species (Table 1). Vessel length is highest in *R. mucronata* (512.03 ± 82.01 µm), followed by *S. apetala* (374.52 ± 34.56 µm), and lowest in *A. marina* (232.81 ± 42.98 µm). In contrast, vessel diameter is greatest in *R. mucronata* (72.5 ± 10.01 µm) and *S. apetala* (71.2 ± 5.59 µm), while *A. marina* exhibits narrower vessels (60.95 ± 7.66 µm). Vasicentric axial parenchyma forming a continuous sheath around vessels is present in *A. marina* (Fig. 1 A) and *R. mucronata* (Fig. 1 B) but absent in *S. apetala* (Fig. 1

C). Structural reinforcement also differs, with *R. mucronata* showing densely packed thick-walled fibers, and *A. marina* characterized by successive phloem and sclereid rings (Fig. 1 A).

Table 1. Quantitative anatomical characters of three true mangrove species from the Indian Sundarbans. VDS, vessel density; VDM, vessel diameter; VGI, vessel grouping index; FWT, fiber wall thickness; FL, fiber length; FW, fiber width; CR, coefficient of rigidity; FR, flexibility ratio; VA, vessel area; FA, fiber area. Data are presented as mean ± SD.

Anatomical Traits	**Species**		
	Avicennia marina	***Rhizophora mucronata***	***Sonneratia apetala***
VDS	38 ± 6.5	21 ± 4	69 ± 13
VDM (µm)	60.95 ± 7.66	72.5 ± 10.01	71.2 ± 5.59
VGI	1-3	1-2	2-3
FL (µm)	376.84 ± 96.73	779.78 ± 319.09	371.3 ± 132.66
FW (µm)	30.26 ± 4.55	66.91 ± 17.39	21.2 ± 2.02
FWT (µm)	2.63 ± 0.42	9.79 ± 1.24	6.8 ± 0.96
CR	0.16 ± 0.21	0.17 ± 0.05	0.3 ± 0.03
FR	0.81 ± 0.02	0.72 ± 0.02	0.32 ± 0.02
VA (%)	4.72 ± 0.2	6.38 ± 0.08	15.32 ± 2.56
FA (%)	28.2 ± 6.7	39.7 ± 9.23	22.08 ± 4.6

Radial longitudinal sections reveal further species-specific variation. Ray cells are thickest (8-16 µm) in *R. mucronata* (Fig. 1 E), thinnest (2-6 µm) in *S. apetala* (Fig. 1 F), and intermediate in *A. marina* (Fig. 1 D). Vessel elements are longest in *S. apetala*, shortest in *R. mucronata*, and intermediate in *A. marina.*

Tangential longitudinal sections demonstrate notable variation in ray structure and fibre characteristics among the investigated mangrove species. Both homocellular and heterocellular rays are observed, with heterocellular rays occurring more prominently in certain taxa. *Sonneratia apetala* is characterized by homocellular rays, whereas *Avicennia marina* and *Rhizophora mucronata* possess heterocellular rays, which may represent adaptive modifications to ecological stress conditions through variation in ray dimensions and frequency. Rays are entirely uniseriate in *S. apetala* (Fig. 1I), range from uni- to multiseriate (2–4-seriate) in *A. marina* (Fig. 1G), and are consistently multiseriate (6–16-seriate) in *R. mucronata* (Fig. 1H). Fiber dimensions also vary markedly among species, with *R. mucronat*a exhibiting more than twofold greater fiber length (779.78 ± 319.09 µm) and width (66.91 ± 17.39 µm) compared with the other species. *Avicennia*

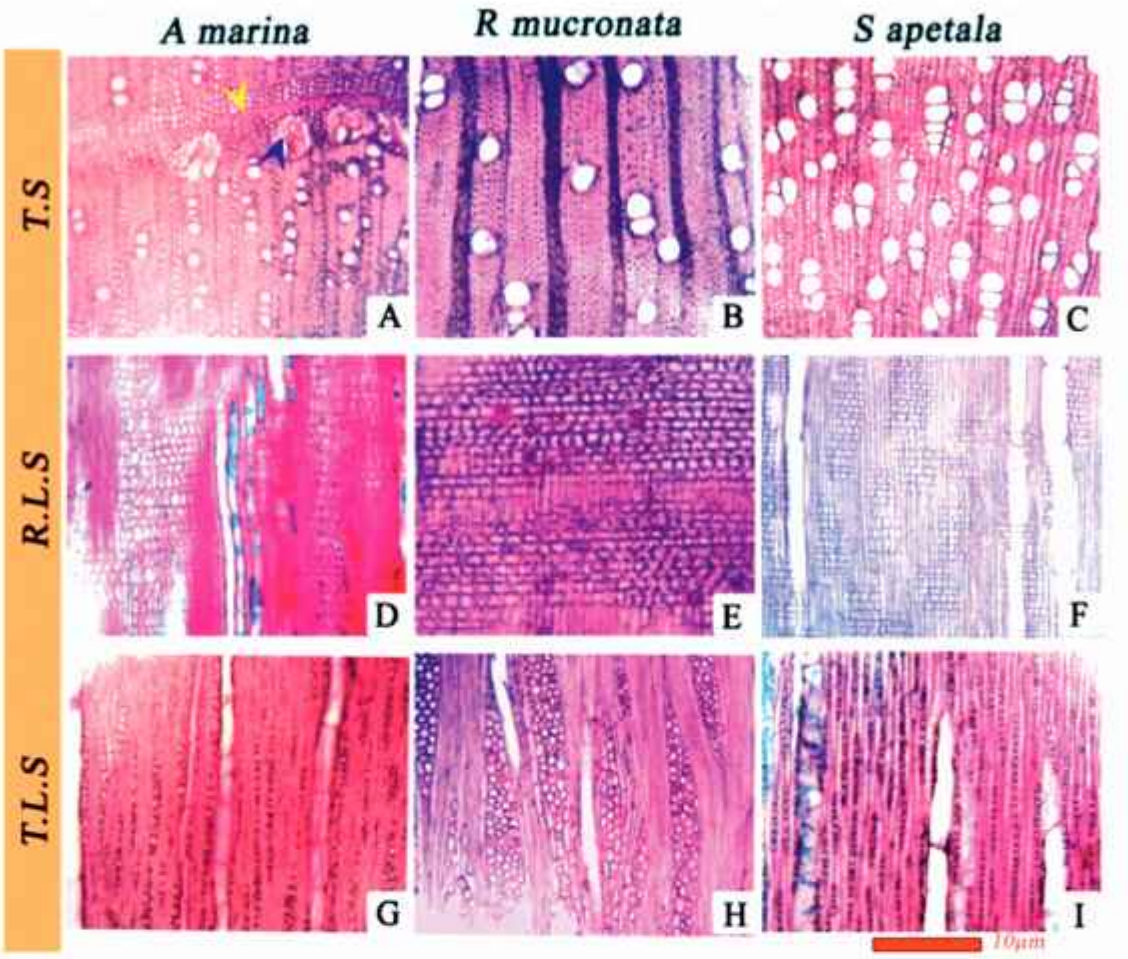


Fig. 1. Wood anatomical features of three true mangrove species from the Indian Sundarban observed in transverse (TS), radial longitudinal (RLS), and tangential longitudinal (TLS) sections. In TS, *Avicennia marina* (A) shows patchy successive phloem (blue arrow), facilitating continuous photosynthate transport, with sclereid ring (yellow arrow) provide mechanical support. The larger vessel diameters in *R. mucronata* (B) than *Sonneratia apetala* (C), enhancing water conduction efficiency. In RLS, *R. mucronata* (E) displays thicker ray cells supporting radial transport and storage compared to *A. marina* (D) and *S. apetala* (F). In TLS, *S. apetala* (I) shows uniseriate rays, *A. marina* (G) exhibits rays ranging from uniseriate to multiseriate, and *R. mucronata* (H) consistently shows multiseriate rays, indicating species-specific adaptations for storage and lateral transport.

marina shows broader fibers (30.26 ± 4.55 µm) than *S. apetala* (21.2 ± 2.02 µm), whereas fiber wall thickness is lowest in *A. marina* (2.63 ± 0.42 µm) and higher in *S. apetala* (6.8 ± 0.96 µm), indicating an inverse pattern between fiber width and wall thickness.

Interspecific variation in measured anatomical traits

Species show a significant effect (ANOVA, $p < 0.05$) on wood anatomical traits among *Avicennia marina, Rhizophora mucronata,* and *Sonneratia apetala* (Table 2 & 3). Vessel density differs strongly among species ($F = 70.23$, $p = 2 \times 10^{-11}$), as does vessel diameter ($F = 5.69$, $p =$

0.00868). Vessel grouping index (VGI) also shows a significant species effect ($F = 30.85$, $p = 1.06 \times 10^{-}$).

Table 2. One-way ANOVA result considering "Species" (*Avicennia marina, Rhizophora mucronata* & *Sonneratia apetala*) as independent variable and different anatomical traits like "Vessel density, Vessel diameter, VGI, Fiber width, Fibre Wall Thickness, Coefficient Rigidity, Fiber Length" as response variables. Statistical significance is indicated as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, and ns = not significant.

Interaction	Df	Sum Sq	Mean Sq	F value	p
Species × Vessel density	2	12118	6059	70.23	2×10^{-11} ***
Residuals	27	2329	86		
Species × Vessel diameter	2	801.5	400.7	5.688	0.00868 **
Residuals	27	1902.3	70.5		
Species × VGI	2	4.786	2.3932	30.85	1.06×10^{-07} ***
Residuals	27	2.095	0.0776		
Species × Fiber width	2	11700	5850	48.22	1.23×10^{-09} ***
Residuals	27	3275	121		
Species × Fibre Wall Thickness	2	259.07	129.54	131.6	1.2×10^{-14} ***
Residuals	27	26.58	0.98		
Species × Fiber Length	2	1097493	548746	11.51	0.000243 ***
Residuals	27	1287758	47695		
Species × Coefficient Rigidity	2	0.0851	0.04257	2.241	0.126 *ns*
Residuals	27	0.5128	0.01899		
Species × Flexibility ratio	2	1.3961	0.6980	2132	$<2 \times 10^{-16}$ ***
Residuals	27	0.0088	0.0003		

Table 3: One-way ANOVA result considering "Species" (*Avicennia marina, Rhizophora mucronata* & *Sonneratia apetala*) as independent variable and different anatomical traits like "Vessel density, Vessel diameter, VGI, Fiber width, Fibre Wall Thickness, Coefficient Rigidity, Fiber Length" as response variables. Statistical significance is indicated as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, and ns = not significant. Tukey's HSD test was used for post hoc pair wise comparisons. While values with distinct superscript letters differ significantly, values with the same letters do not.

Anatomical traits	Species			p
	Avicennia marina	*Rhizophora mucronata*	*Sonneratia apetala*	
Vessel density	$37.9^{b} \pm 6.79$	$20.4^{c} \pm 4.22$	$69^{a} \pm 13.96$	2×10^{-11} ***
Vessel diameter	$60.95^{b} \pm 7.66$	$72.5^{a} \pm 10.01$	$71.2^{a} \pm 5.59$	0.00868 **
VGI	$1\text{-}3^{a}$	$1\text{-}2^{b}$	$2\text{-}3^{a}$	1.06×10^{-07} ***
Fiber width	$30.26^{b} \pm 4.55$	$66.91^{a} \pm 17.39$	$21.2^{b} \pm 2.02$	1.23×10^{-09} ***
Fibre Wall Thickness	$2.63^{c} \pm 0.42$	$9.79^{a} \pm 1.24$	$6.8^{b} \pm 0.96$	1.2×10^{-14} ***
Fiber Length	$376.84^{b} \pm 96.73$	$779.78^{a} \pm 319.09$	$371.3^{b} \pm 132.66$	0.000243 ***
Coefficient Rigidity	$0.16^{a} \pm 0.21$	$0.17^{a} \pm 0.05$	$0.3^{a} \pm 0.03$	0.126 *ns*
Flexibility Ratio	$0.81^{a} \pm 0.02$	$0.72^{b} \pm 0.02$	$0.32^{c} \pm 0.02$	$<2 \times 10^{-16}$ ***

Fiber traits vary markedly among species. Fiber width ($F = 48.22$, $p = 1.23 \times 10^{-}$), fiber wall thickness ($F = 131.6$, $p = 1.2 \times 10^{-1}$), and fiber length ($F = 11.51$, $p = 0.000243$) all differ significantly (Fig. 2 A). In contrast, the coefficient of rigidity does not differ significantly among species ($F = 2.24$, $p = 0.126$). These results show clear species-specific differences in both hydraulic and mechanical wood traits among three dominant true mangrove species.

The proportion of fiber and vessel area differs clearly among species. *Rhizophora mucronata* shows the highest fiber area (≈40%), followed by *Avicennia marina* (≈29%), while *Sonneratia apetala* shows the lowest fiber area (≈22%). In contrast, vessel area is lowest in *A. marina* (≈5%), intermediate in *R. mucronata* (≈6–7%), and highest in *S. apetala* (≈15–16%). These patterns indicate that *R. mucronata* and *A. marina* allocate more wood area to fibers, whereas *S. apetala* allocates more area to vessels.

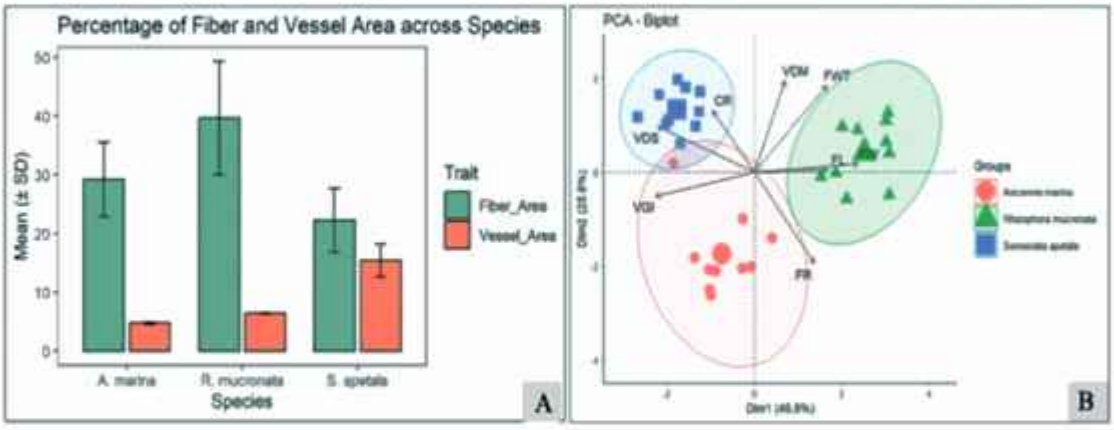


Fig. 2. (A) Proportion of fiber and vessel area in three true mangrove species from the Indian Sundarbans. (B) Principal component analysis (PCA) based on quantitative wood traits.

Variation in derived anatomical and functional indices

Significant interspecific differences in the derived indices ($p < 0.05$) suggest variation in hydraulic architecture and adaptive strategies among the mangrove species. *Sonneratia apetala* shows a greater vessel density (69 ± 13 vessels mm^{-2}) compared with *Avicennia marina* (38 ± 6.5 vessels mm^{-2}), while *Rhizophora mucronata* exhibits the lowest values (21 ± 4 vessels mm^{-2}). Vessel grouping index (VGI) indicates stronger vessel clustering in *S. apetala* (2–3) relative to *A. marina* (1–2) and *R. mucronata* (1–2).

Wood rigidity, expressed as the coefficient of rigidity (CR), is more pronounced in *R. mucronata* (0.17 ± 0.05) than in *A. marina* (0.16 ± 0.21) and *S. apetala* (0.13 ± 0.03). In contrast, *Sonneratia apetala* exhibited the lowest fibre flexibility ratio (FR = 0.32 ± 0.02), indicating comparatively rigid fibres, whereas higher flexibility values were recorded in *Avicennia marina* (0.81 ± 0.02) and *Rhizophora mucronata* (0.72 ± 0.02), suggesting greater fibre flexibility and mechanical adaptability. These patterns reflect species-specific differences in hydraulic safety and mechanical performance among the three mangrove taxa.

Multivariate trait patterns

Principal Component Analysis (PCA) explains 71.0% of the total variation, with PC1 accounting for 49.2% and PC2 for 21.8%. The three species—*Avicennia marina, Rhizophora mucronata,* and *Sonneratia apetala*—form distinct clusters (Fig. 2 B) in ordination space. The variation of *Rhizophora mucronata* associates mainly with fiber width, fiber length, and vessel grouping index, indicating greater investment in fiber traits and vessel grouping. *Avicennia marina* shows strong associations with vessel density and coefficient of rigidity, reflecting higher hydraulic safety and mechanical strength. *Sonneratia apetala* separates primarily along PC2 and associates with larger vessel diameter and thicker fiber walls. Overall, the PCA demonstrates clear anatomical differentiation among the three mangrove species based on coordinated variation in vessel and fiber traits.

Discussion

Mangrove wood anatomy provides a reliable archive of long-term environmental change and reveals strong links between xylem structure, growth strategies, and climatic stressors. Variation in key anatomical traits—particularly vessel characteristics, fiber traits, and wood density—reflects shifts in salinity, temperature, and moisture availability over the lifespan of mangrove trees (Menezes et al.., 2003; Verheyden et al.., 2005; Santini et al.., 2012). These coordinated structural adjustments represent fundamental trade-offs among hydraulic efficiency, mechanical stability, and growth in saline coastal environments.

Distinct wood anatomical strategies are evident among *Avicennia marina, Rhizophora mucronata,* and *Sonneratia apetala*. *Rhizophora mucronata* exhibits the longest vessel elements and largest vessel diameters (Fig. 1 B), indicating high hydraulic conductivity supported by substantial mechanical reinforcement. This species also shows densely packed, thick-walled fibers and thick multiseriate rays (Fig. 1 H), reflecting strong investment in stem rigidity and storage capacity, which is advantageous in wave-exposed intertidal habitats. In contrast, *Sonneratia apetala* relies on high vessel density rather than vessel size, resulting in greater vessel area allocation and hydraulic redundancy. This configuration supports rapid water transport and aligns with its fast-growing, acquisitive strategy.

Avicennia marina displays a more conservative hydraulic architecture, characterized by shorter and narrower vessels and moderate vessel density, traits associated with enhanced hydraulic safety under high xylem tension. The presence of vasicentric axial parenchyma, sclereid rings, and successive phloem tissue indicates a flexible stem design that facilitates radial transport and embolism repair rather than mechanical rigidity. This combination likely underpins the ecological success of *A. marina* across a wide range of salinity conditions.

Salinity emerges as a dominant driver of xylem structure in mangroves. High salinity generally promotes reduced vessel diameter and, in some cases, lower vessel number, which decreases hydraulic conductivity but reduces vulnerability to cavitation. Narrow vessels are less

prone to embolism under negative pressure, enhancing hydraulic safety. Along salinity gradients higher vessel density in seaward *A. marina* stands compared with landward sites suggests either phenotypic plasticity or adaptive differentiation in response to saline stress (Dahdouh-Guebas et al., 2004). Seasonal and within-tree variation in vessel density in *R. mucronata* further highlights the plasticity of mangrove hydraulic architecture under changing hydrological conditions (Verheyden et al., 2005).

Wood density integrates variation in vessel and fiber traits and reflects contrasting ecological strategies among species. Vessel density serves as a proxy for wood density, which is strongly influenced by climate-driven variability. Radiocarbon dating and X-ray densitometry have shown that climatic fluctuations can significantly alter mangrove wood density, as demonstrated by the decline in *A. marina* wood density associated with increased temperature and reduced rainfall in Western Australia (Santini et al., 2013a). Similar climatic trends in the Indian Sundarbans suggest that comparable anatomical shifts may be occurring, favoring hydraulic safety and survival under increasing environmental stress.

Across species, *Sonneratia* occupies the acquisitive end of the functional spectrum, with low wood density supporting rapid radial growth and canopy expansion. In contrast, *Rhizophora* and *Avicennia* represent more conservative strategies, characterized by higher wood density and greater mechanical strength (Quadros et al., 2021). These anatomical strategies correspond with observed differences in growth rates and stress tolerance.

Hydraulic safety is further shaped by intervessel pit traits and vessel grouping. Species-specific pit characteristics—such as reduced pitfield fraction and vestures in *Avicennia*, scalariform pits in *Rhizophora*, and vestured pits in *Sonneratia*—limit air seeding and help maintain water transport under fluctuating salinity and tidal regimes (Lopez-Portillo et al.., 2005; Schmitz et al.., 2007, 2008a). Vessel grouping enhances hydraulic redundancy by enabling water to bypass embolized conduits, although excessive connectivity may increase embolism spread. In *A. marina,* moderate vessel grouping combined with reduced pitfield fraction appears to balance hydraulic efficiency and safety.

Fiber traits further differentiate mechanical strategies. *Rhizophora mucronata* shows the greatest fiber length, width, wall thickness, and fiber area proportion, consistent with high rigidity in mechanically dynamic environments. In contrast, *Sonneratia* apetala exhibits lower fiber investment, resulting in lighter, more flexible wood that supports rapid growth but offers reduced mechanical resistance. *Avicennia marina* occupies an intermediate position, balancing flexibility and reinforcement through coordinated fiber traits and specialized tissues. Wood rigidity, indicated by the coefficient of rigidity, is highest in *Rhizophora mucronata*, while *Avicennia marina* and *R. mucronata* display greater stem flexibility compared with *Sonneratia apetala*. These patterns highlight species-specific differences in mechanical properties and suggest contrasting strategies to balance hydraulic safety and structural support in intertidal environments.

Multivariate analysis confirms that wood anatomy functions as an integrated system rather than as isolated traits. Principal Component Analysis clearly separates the three species based on coordinated variation in vessel and fiber characteristics. *Rhizophora mucronata* clusters with fiber traits and vessel grouping, reflecting mechanical reinforcement; *Avicennia marina* associates with vessel density and rigidity, indicating hydraulic safety and resilience; and *Sonneratia apetala* aligns with vessel diameter and fiber wall thickness, consistent with a growth-oriented strategy. Together, these patterns highlight how wood anatomical trade-offs shape functional diversity and resilience of mangrove species in the Indian Sundarbans.

Conclusion

This study shows that wood anatomy in mangrove species reflects coordinated adaptive strategies that balance hydraulic efficiency, mechanical stability, and growth under saline intertidal conditions. Distinct anatomical trait combinations in *Avicennia marina, Rhizophora mucronata,* and *Sonneratia apetala* demonstrate that wood structure functions as an integrated system rather than as isolated traits. *Rhizophora mucronata* exhibits a mechanically reinforced strategy, *Sonneratia apetala* prioritizes hydraulic efficiency and rapid growth, and *Avicennia marina* combines hydraulic safety with structural flexibility across broad salinity gradients. Salinity and climate-driven variability strongly influence vessel traits and wood density, highlighting the sensitivity of mangrove xylem architecture to environmental change. These findings emphasize the importance of wood anatomical trade-offs in shaping mangrove resilience and functional diversity and provide insights into how mangrove forests of the Indian Sundarbans may respond to ongoing climatic and hydrological changes.

Declaration of Competing Interest

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

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Credit Author Statement

TS: Conceptualization, Data curation, Funding acquisition, Writing – original draft SM: Investigation, Funding acquisition, Writing – original draft. SN: Supervision, Software, Visualization, Writing – review & editing

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Plant species diversity and their different utilities in Sunderban area of West Bengal

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Abstract:

Mangroves may be defined as the 'coastal tropical forest formation encircled or separated by the tidal rivers and or the sea water flooded frequently by the tidal water. Mangrove species are uniquely adapted to tropical and subtropical coasts found in estuaries and along river banks and lagoons. Tropical mangrove ecosystems are highly productive and provide extensive ecosystem services. The term 'mangrove' describes both the ecosystem and the plant families. The Sundarban is the world's largest contiguous mangrove patch covering an estimated area of 10,000 Km² spanning in the Ganga-Brahmaputra-Meghna delta. Sundarban is spread over two countries, of which 60% is in Bangladesh and 40% is in India. Sundarban is recognized internationally as 'World Heritage Site' by UNESCO. Sundarban is the home of about 350 species of vascular plants, 250 species of fishes, 300 species of birds, numerous specious of phytoplankton, fungi, bacteria, zooplankton, benthic invertebrates, molluscs, reptiles, amphibians, and mammals. Sundarbans, the queen of the natural forests attracted people from the medieval period. Although it possesses only 1% of total area of biodiversity, consisting of wide variety of plant species that provide important habitats for a variety of animals including mammals, birds, reptiles, fish and molluscs. The Sundarban has been extensively exploited for agriculture, timber, fish and prawn culture etc. Therefore, it also contributes to the livelihoods locally (and globally) by providing forest resources such as timber, firewood and thatching materials as well as non-timber products. Mangroves are also recognized as an important greenbelt and carbon sink that protects coastal areas from natural disasters such as tsunamis, cyclones and erosion resulting from sea level rise etc. This is a remarkable ecosystem – highly productive forest built by a small group of trees and shrubs that have adapted to survive in the harsh interface between the land and sea they are host to a rich and complex array of associated species, and in turn provided considerable services to human communities, both locally and globally. New research from around the world has shown that mangroves have higher levels of primary productivity than most other tropical or temperate forests. Their standing biomass can also be very high, even in low-stature forests, as a result of high levels of below-ground biomass. This, combined with considerable storage of organic carbon in mangrove soils, means that mangroves, despite their small global extent, may have important role to play in global budgets and in process of mitigating climate change. Preliminary estimates indicate that the total above-ground biomass for the world's mangrove forests maybe over 3700 Tg of carbon, and further sequestration of organic matter directly into mangrove sediments is likely to be in the range of 14–17 Tg of carbon per year. Among the most remarkable observations are those that highlight the intense utilization of mangroves by people. Increasingly, there is an awareness among both coastal peoples and leaders of the indirect benefits provided by mangroves in protecting coastlines from erosion and form the impacts of storm surges. There is good evidence that mangroves even reduced the impact of the 2004 Indian Ocean tsunami in a number of locations. More and more, the plantation of and restoration of mangroves is being undertaken specifically to protect coastal population.

Keywords :

Mangroves, Sundarbans, Biosphere reserve, benefits.

Introduction

Mangroves-the threatened coastal intertidal halophytic flora play very dominant and important roles in the estuarine mouths, sea-land interphase areas or deltaic ecosystems of both the

tropical and sub-tropical zones, especially in the highly populated South-East Asian countries. Inspite of their importance in conserving the floral and faunal biodiversity of deltaic ecosystems and protection of the coastline from natural calamities, these mangrove ecosystems are mostly ignored. Indiscriminate exploitation and degradation of the ecosystems have taken place, by clearing these forests and converting them into agricultural fields, brackishwater farms, salt pans and harbours. Recently these ecosystems attracted the interest of scientist, and developmental workers, who felt the urgency for conserving them.

The Sundarban Biosphere Reserve (SBR) in the sea-land interphase regions of the Ganga-Brahmaputra deltaic complex is located between 21°32′ to 22° 40′ N and 89° 00′ E within the districts of North and South 24-Paraganas of West Bengal covering an area of 9630 square km. south to the Dampier-Hodge's line. (Map 1 & Map 2) It is the largest and most remarkable sheltered part of the wet coastal ecosystem in India. This deltaic complex is developed by the enormous silt and sediments deposition carried down by the river Ganga and Brahmaputra from the great Himalayas.

The Sundarban SBR is the world's largest and most biodiverse mangrove swamp which comprises about 6% of the total area of all mangroves on earth (Khan 2002, Iftekhar and Islam 2004). It is an essential and high-quality wildlife conservation area of regional and international importance (Seidensticker 2004). In Bengali the word 'Sundarbans' means 'beautiful forests', but the name could have derived from the common tree 'sundri' (Heritiera fomes), which means 'beautiful lady' (Hussain and Acharya 1994, Sarkar 2010).The whole Indian Sundarban area, south of the Dampier-Hodges line (that demarcates the inward limit of tidal influence), including 5,366 km^2 of reclaimed lands, has also been selected as the Sundarban Biosphere Reserve.

Materials and Methods

Botanical surveys were carried out from 2024 to 2025 following the standard methodology of Jain and Rao (1977). Near about in this survey the authors selected and visited remote areas. Interaction was done mainly on Bengali language a total of fifty men and women were interviewed. Each interview was made with semi-structured or open ended questionnaire. Each plant was documented in detail by their local names in Bengali language. Scientific names, parts used and main uses were recorded. Each studied plant was collected in their reproductive season and dried and preserved as herbarium specimen following the conventional herbarium techniques (Jain and Rao, 1977) and kept it in the Herbarium of Department of Botany, Taki Govt. College for future references. Moreover, by conducting regular/ periodical visits to the Sundarbans area, the plant material was collected and carefully handled for identification by authenticated source. Most of the plant materials were preserved by making herbaria and all the voucher specimens were carefully numbered and deposited. The value of each plant was enumeration the following pattern a. Botanical name, b. Family, c. Vernacular name, d. Plant parts used with their utilities. The identification of plants was done using Bengal Plants (Vol-1&2).

In the present review, information about, mangroves and their economic and ecological uses was gathered from scientific databases including www.google.com, Springer etc. and related books either online or offline. All the plants have been systematically arranged, using standard format like family, scientific name, utilities etc.

Results and Discussion

The results have been presented with the help of a table depicting their scientific names with family followed by vernacular names (Vernacular name). Plant parts used with their utilities (Table-1).

The word 'mangrove' (a combination of the Portuguese 'mangue' and English 'grove) can refer to an ecological group of halophytic plant communities belonging to 12 genera in 8 families, or a complex of plant communities fringing sheltered tropical shores, or more specifically by some authors, the vegetation formation below the high tide mark (Seidensticker and Hai 1978).

The total mangrove area of the world has been estimated to be around 166,700 km^2 (Choudhury et al. 2001) and the entire Sundarbans is about 10,000 km^2, of which roughly 60% lies in the southwest of Bangladesh (between 21°30′-22°30′ N latitudes and 89°00′-89°55′ E longitudes) and the other 40% in the southeast of the Indian state of West Bengal (between 21°32′-22°40′ N latitudes and 88°05′-89°00′ E longitudes). (Map 1 & Map 2)

The mangrove tree species are generally of two types: 1) basic mangroves growing along the areas inundated by high and medium tides, able to tolerate high salinity in water, mainly the members of the families Rhizophoraceae and Myrsinaceae, and 2) associated or back mangroves, growing on comparatively higher and firmer ground, and less tolerant to high tides and salt, mainly the members of the families Sterculiaceae, Verbenaceae, Lythraceae, Meliaceae and Palmae. Therefore, a rich and diverse associated mangrove community is seen in the Sundarbans with no other mangrove forest in the world offering such a variety of associated mangrove species (Choudhury et al. 2001).

Sundarban is obviously of great economic importance to West Bengal as a prime source of a wide range of valuable natural products such as timber, fish, honey, and nipa (*Nypa fruticans*) leaves for thatching (Blower 1985). It also has an important buffer function protecting the densely settled villages and agricultural areas to the north from the full force of cyclone and tidal waves by mitigating their destructive force (Helalsiddiqui 1998). The tourism policy of West Bengal proposed that, because of its unique and diverse attractions of international renown, the Sundarbans should be developed as the springboard for the tourism industry for the country as a whole.

The following are the most important ecological and economic services of the mangroves: The coastal vegetation, especially the mangrove, serves in two ways: supplying the energy requirement to the local population and by acting as a huge sink of unlimited capacity for absorbing pollutants from air and water which makes the surrounding environment free from pollution. The pollutant

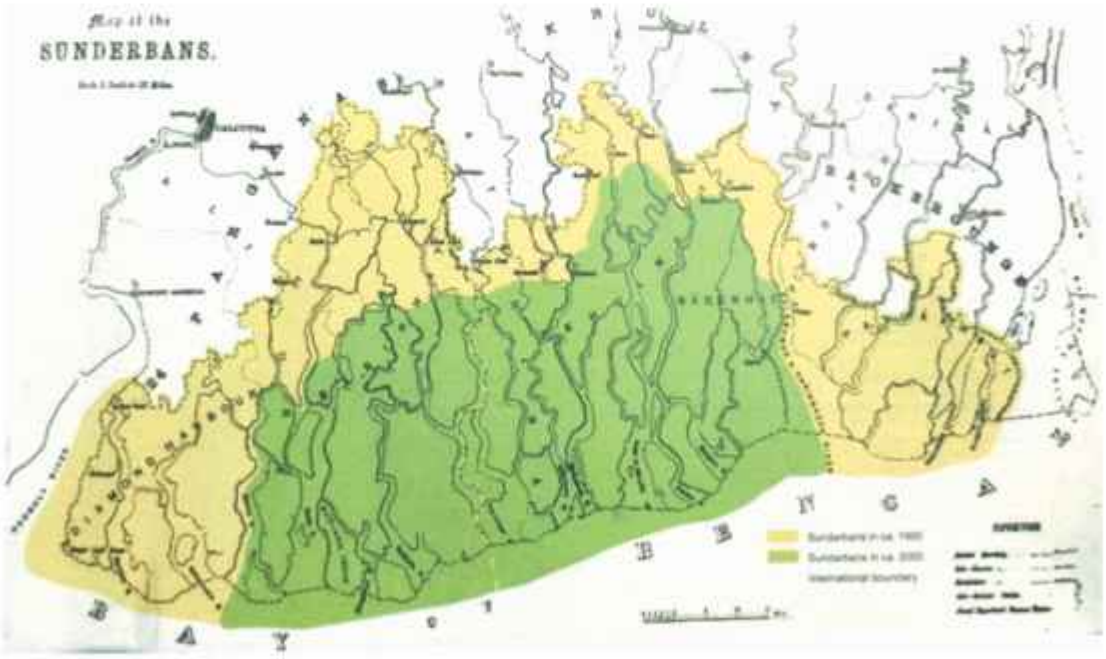
Table:1 Some common mangrove species of Indian Sundarban and their economic services:

Sl.No.	Name of the plant species	Family	Vernacular name (Bengali name)	Plant parts used with their utilities
1.	*Acanthus ilicifolius* L.	Acanthaceae	Hargoza / Harkuch kanta	1. Application of fermented leaves on swelling gland and rheumatic swelling gives good result. 2. Root decoction applied on polio affected limbs. It is used in curing leucorrhoea. 3. The crushed fruits act as good blood purifier and used in dressing for boils and snakebites.
2.	*Aegiceros corniculatum* (L.) Blanco	Myrsinaceae	Khalsi	1. Fruit is poisonous to fish due to presence of saponine and tannin and hence used in aquacultural farms to kill crabs and unwanted predatory fish. 2. Bark is a good source of tannin. 3. The flowers of this tree are good sources of high quality honey.
3.	*Avicennia alba* Blume	Acanthaceae	Baen	1. Used for firewood. 2. Ash obtained after burning the dried bark is used as antacid. 3. Paste of green (raw) fruits is applied to cure boils and skin diseases. 4. Efficient bio-accumulator of heavy metals like Cu and Pb.
4.	*Avicennia officinalis* L.	Acanthaceae	Baen	1. Used for firewood. 2. Ash obtained after burning the dried bark is used as antacid. 3. Paste of green (raw) fruits is applied to cure boils and skin diseases. 4. Efficient bio-accumulator of heavy metals like Cu and Pb.
5.	*Sonneratia apetala* Buch-Ham.	Sonneratiaceae	Keora	1. Wood used in house building and packing cases. 2. Yields excellent fuel. 3. Fruit contains 28g/100gm of Vitamin C and hence helps to fight cough and cold. 4. Efficient bio-accumulator of heavy metals (Zn, Cu, Pb and Mn).
6.	*Bruguiera gymnorrhiza* (L.) Lam. ex Savigny.	Rhizophoraceae	Kakra	1. Bark decoction with coconut oil is applied to cure dermatological problems. 2. Unique source of tannin (36%). 3. Wood used for house post. 4. Yields excellent fuel
7.	*Ceriops decandra* (Griff.) Ding Hou.	Rhizophoraceae	Goran	1. Source of tannin (29%). 2. Provides fuel charcoal. 3. Used for boat building. 4. Obstetric and haemorrhage cases are treated with an infusion of *Ceriops* bark.
8.	Excoecaria agallocha L.	Euphorbiaceae	Gewa / Gewagach	1. Latex applied on leprotic wounds. 2. Leaf decoction is claimed as medicine in epilepsy. 3. Heart wood and pneumatophores give scent, but not commercially used. 4. Efficient bioaccumulator of heavy metals from the ambient media.
9.	*Heritiera fomes* Buch.-Ham.	Sterculiaceae	Sundari	1. Wood fetches a high price in the international market, as it is of demand for making furniture. 2. Indicator of salinity profile of an area. 3. Source of tannin (11%). 4. Rich in astaxanthin.
10.	*Nypa fruticans* Wurmb.	Arecaceae	Golpata	1. Ash prepared after burning tender leaves is used in stomach ulcers. 2. Dried leaves are often used for making roofs in thatched houses. 3. Source of alcohol. 4. Rich in astaxanthin.

load in the Sundarban estuary, estimated over 20,000 kg/day, is alarming for the existence of these mangroves. Therefore, it is felt very much urgent to tackle this acute situation and maintain the balance in this complex but interesting ecosystem. The metropolitan and industrial discharge containing nickel, chromium, chloride and excess nutrients like nitrate and phosphate along with high alkalinity acts adversely on phytoplanktons and ultimately affects the higher plants and animala. Mangrove also acts as the guard or buffer agent and prevents the coast lands from soil and water erosion, which helps in controlling the balance of ecoclimatic factors. Besides all these, mangroves provide fish protein to the urban areas.

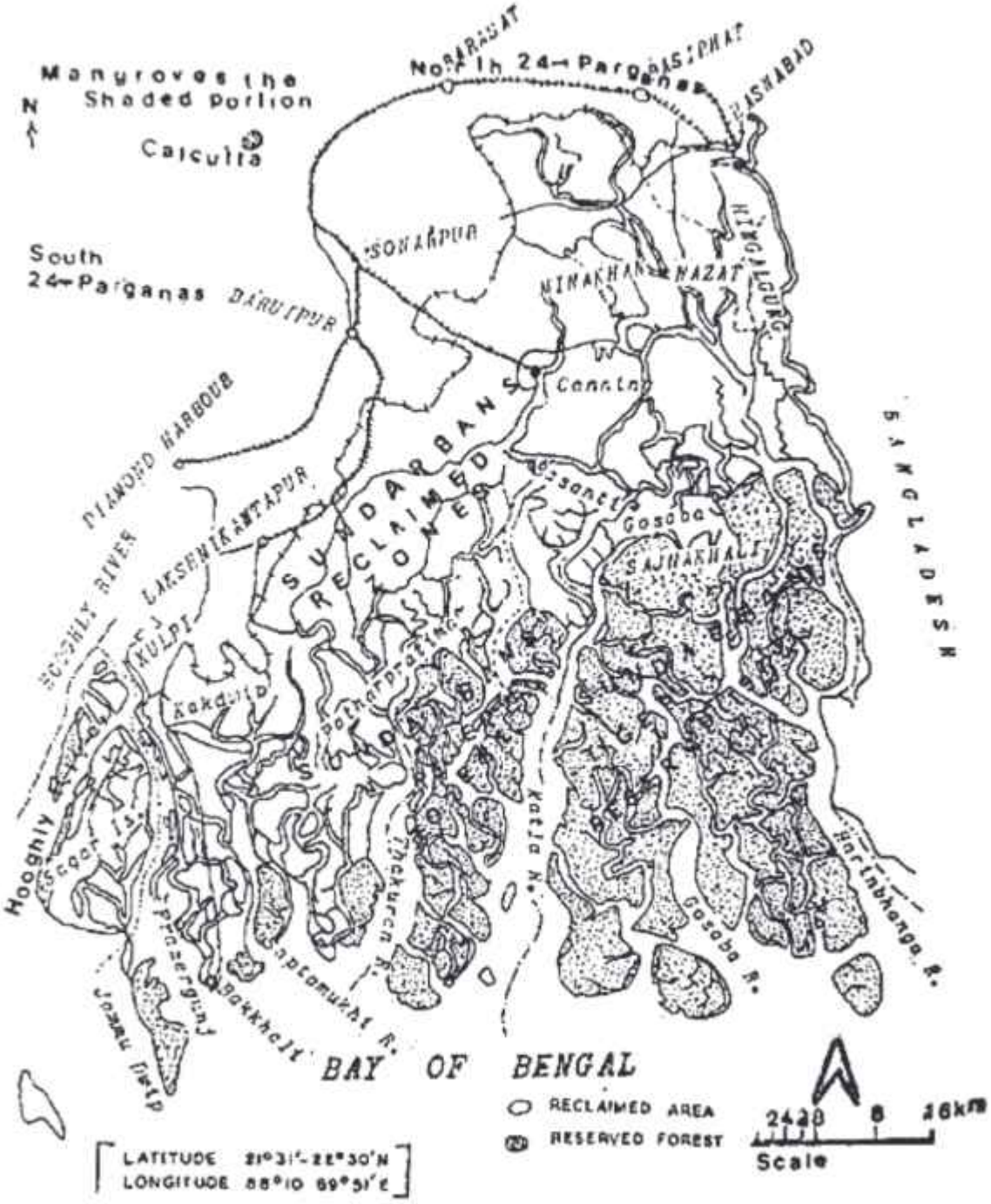
Mangroves serve as breeding, feeding and nursery grounds for many fishes in the offshore and inshore waters. They also provide feeding and breeding grounds for birds, reptiles and mammals. Sundarban also acts as a source of honey and provide employment to more than 2.000 households engaged in extracting 111 tonnes of honey annually. The mangroves also have bioprospecting potential such as black tea beverage, mosquito repellents, microbial fertilizers and medicines for various diseases. These ecosystems play a major role in the global cycle of carbon, nitrogen as well as sulphur and act as reservoirs in the tertiary assimilation of wastes (Kathiresan and Qasim, 2005).

The Indian Ocean Tsunami of December 2004 and massive cyclones along east coast have brought to focus the role of coastal ecosystems in stabilizing coastlines. This is a critical function in tropical countries like India which has long coastline which is periodically battered by extreme weather events. Evidences from the Indian Ocean tsunami indicated that mangroves along with casuarina plantations reduced the impact of waves and protected shorelines against damage along Tamil Nadu coastline (Kathiresan and Rajendran, 2005; Danielsen et al., 2005). Badola and



Map 1 : Extent of the Sundarbans in ca. 1900 and ca. 2000 showing 50% decline of the forest cover (original map from Pran 1905)

Hussain (2005) carried economic assessment of storm protection function of Bhitarkanika mangrove ecosystem and estimated cyclone damage avoided in three selected villages, taking cyclone of 1999 as reference. Economic assessments indicated highest loss in village that was not sheltered by mangroves but by embankments, with the least per capita damage in village with mangroves as barrier. Das and Vincent (2009) validated the storm protection function of mangroves



Map 2: Indian Sundarbans (W.B.) - showing the reclaimed areas and present day's mangrove forest zones.

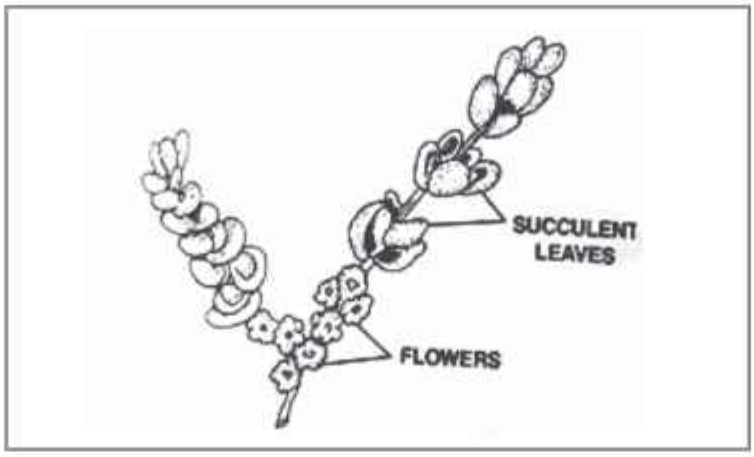


Fig 1; Shoot of *Salsola baryosma* showing succulent leaves.

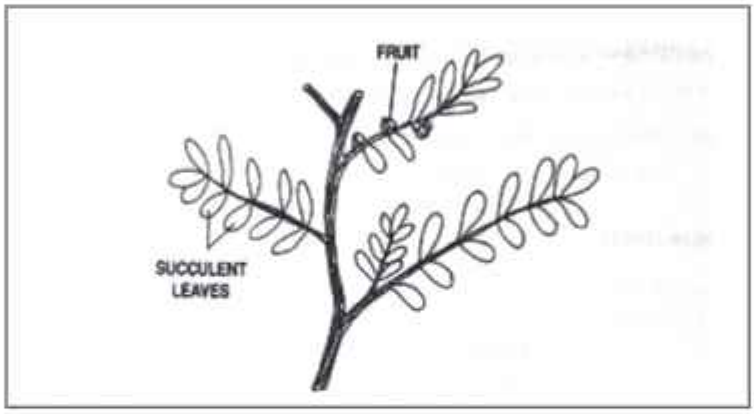


Fig 2. A shoot of *Sueda fruticosa* showing leaves and fruits.

of Odisha on Indian east coast and established that villages with wider mangroves between them and the coast experienced significantly fewer deaths than ones with narrower or no mangroves. These benefits are not expected with concrete coastal protection structures (Danielsen et al., 2005).

The complex structure of mangroves enables them to constrain water movements and to trap sediments, while their high productivity also enables them to extract nutrients from surrounding waters. This has enabled mangroves to perform a valuable service by removing excess nutrients and other pollutants, notably from sewage and aquaculture effluents. This function has been actively evaluated in a number of studies. Of course, such functions may require large areas of mangroves. For the treatment of aquaculture effluents.

Deforestation is a major contributor to greenhouse gas emissions: contributing an estimated 20 per cent of annual greenhouse gas emissions through the 1990s (UNFCCC, 2009). Preventing further forest loss will reduce projected carbon dioxide (CO2) increases, and arguments are growing for a policy known as Reducing Emissions from Deforestation and Forest Degradation (REDD) (Martin, 2008), where political, legal or economic means might be employed as encouragement for countries to maintain existing forest areas.

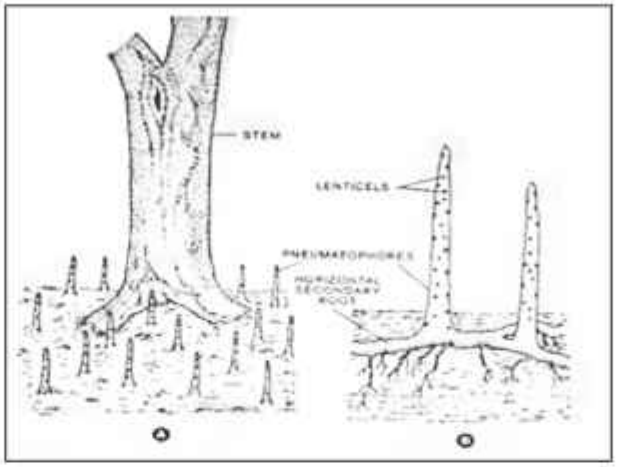


Fig 3. A. Basal portion of mangrove tree- Sonneratia showing a large number of pneumatophores coming out of mud. B. Pneumatophores attached to horizontal roots.

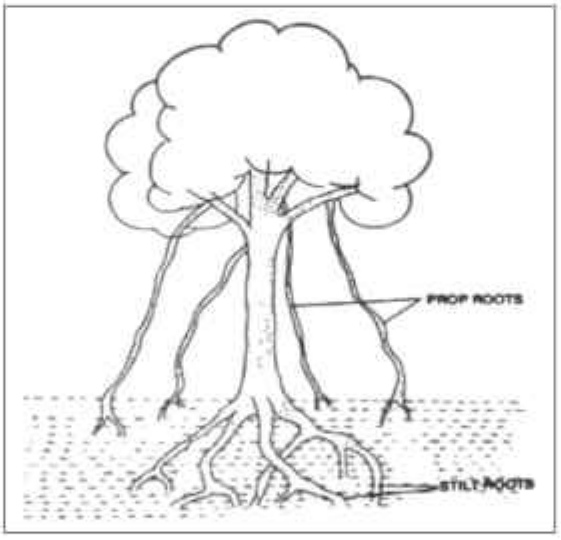


Fig 4. Diagrammatic representation of prop roots and stilt roots developing from the trunk of mangrove plant.

The role of mangroves in global carbon cycles has been somewhat overlooked in this regard, perhaps because of their relatively small total area and often lower physical stature than many adjacent tropical moist forests. In fact, they may be of considerable importance. Their biomass is comparable to higher-canopy terrestrial forests due to their larger proportion of below-ground biomass. They may also play a greater role in sequestration of new CO_2 from the atmosphere than other forests due both to higher rates of productivity and higher rates of long-term carbon deposition in soils and potentially offshore.

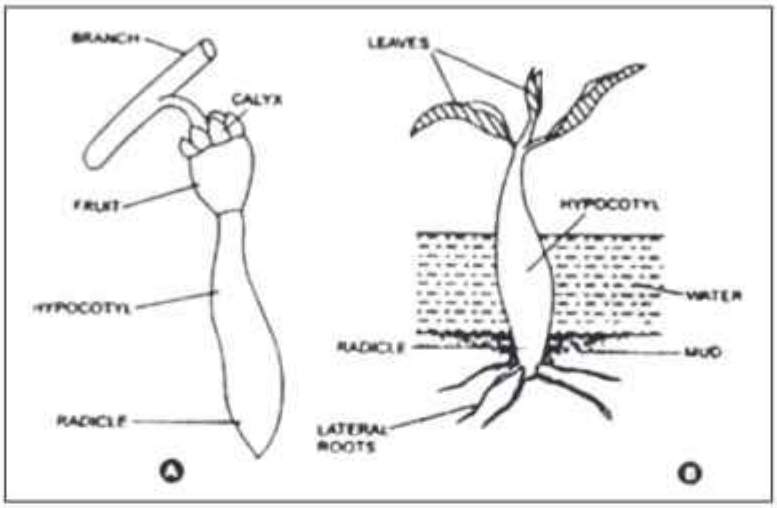


Fig.5. Vivipary in Rhizophora A. A branch with fruit showing seed germination, B. Seedling in the mud.

Mangroves play an important role in wave attenuation, while their complex root systems help in binding and consolidating sediments. In this way they reduce rates of erosion and provide a critical buffer during more extreme storm events. Of course, mangroves are not immune from erosion; but their presence can nevertheless make the difference between a stable or progressive coastline versus an eroding coastline.

Although they can form tall trees, reaching heights of 30m or more in ideal conditions, in many areas mangroves are lower stature, and the most widespread timber products from mangrove forests are poles rather than sawn-wood planking. The dense timber of many species is sought after for construction, particularly because of its resistance to termite attack. It is also widely used for boat building and for much traditional fishing gear such as traps because of its resistance to rotting in saline waters, And, of course, in many areas there is little choice involved. In arid regions and in densely populated countries mangroves often represent the only available timber because adjacent land was either never forested or has been entirely converted to agriculture. Another less sustainable use of mangrove wood is in the production of wood chip and pulp, variously used for paper and chipboard production and, for conversion to rayon for the textile industry.

Throughout this review we use the term fuelwood to cover firewood and mangrove-derived charcoal. Both are widely used in most mangrove countries but are of particular importance in those countries where there are few alternative energy sources and where few other forests remain. Due to the dense nature of mangrove wood, the derived charcoal is high quality and forms the basis for large commercial-scale production. The bark of several trees of *Rhizophora* and *Bruguiera* is rich in tannins and historically has played a critical role in the preparation of leather. Nypa palms are widely used as a form of thatch for more traditional housing throughout its range, with the leaves often woven together for this purpose.

A few societies make use of mangrove fruits for food, notably *Sonneratia*, but also *Avicennia, Bruguiera* and *Kandelia*, although in most cases some degree of cooking or soaking is required to

remove tannins or otherwise make the fruit palatable (Bandaranayake, 1998; Tan, 2001). In Sri Lanka, a pulp from *Sonneratia* has been used to make fruit drinks and ice cream (Jayatissa et al, 2006). Leaves from a number of species are also used to make herbal teas. Probably the most widespread use is of Nypa, whose flower stalks are cut and produce a rich supply of sweet sap (0.5-1 litre per day for up to three months). This liquid can be consumed directly, but is often fermented into an alcoholic drink or given a longer fermentation to produce vinegar. Sugar can also be extracted from the same Nypa sap. The jelly-like endosperm from within Nypa fruits is also quite widely eaten and are even gathered at a commercial scale. Nectar from a number of man-grove species produces excellent honey and is widely utilized by bee-keepers even commercially.

Almost every traditional society in tropical coastal areas has used mangrove leaves, fruits, bark or other products for traditional medicine. The full array of uses is vast, including treatment of bowel disorders, stomach ulcers, hepatitis, tumours, external sores and haemorrahages, asthma and as a contraceptive. Tannin-rich species from the Rhizophoraceae are particularly singled out for the treatment of bowel disorders, with the astringent properties likely to have consistent and measureable effects. Typically, such traditional uses are abandoned when modern medicine becomes more widely available; but recent studies have begun to identify a large array of complex metabolites in mangroves and mangrove-associated plants. It seems likely that future research will find valuable new compounds from mangroves or that they may provide a novel source for existing compounds for medicinal use or potentially even for cosmetic or agricultural applications.

The high-tannin and saline leaves of mangroves do not make for high nutritional value; nevertheless, mangroves are used in many arid countries, notably throughout the Middle East, as a dietary supplement for camels and goats. Some societies have strict regulations on the use of mangroves for browsing livestock in order to ensure that sufficient supplies can be utilized during times of particular need.

Mangroves are among the most important intertidal habitats for marine and coastal fisheries. Mangrove-related species have been estimated to support 30 per cent of fish catch and almost 100 per cent of shrimp catch in South-East Asian countries. Mangroves have often been shunned by tourists. They are hard to access; mosquitoes can be a nuisance; and anaerobic conditions can lead to foul odours. However, this is not always the case, and mangrove areas are being opened up to tourism in many countries. Boat tours are perhaps the most popular means of access; but boardwalks have also been built through many mangrove forests, offering mud-free, tide-independent access to many areas. Recreational fishing and bird-watching are among more specialist uses.

Mangrove ecosystem is a very specialised environment occurring in between the land and the sea in the tropical and sub-tropical regions. It is a transitional ecotone which supports a unique variety of plant community collectively known as the mangrove. The mangrove plants are specially adapted to thrive under varying salinity, recurrent tidal inundation and periodic desiccation. The

mangrove forests with their typical root-system play an important role in protecting and extending the coastline, by trapping sediments and binding soils. The mangrove ecosystem is also known to support fishery, by developing a detritus-based food-web and help regulating regional climate. It is the natural abode of many a group of animals and a large number of species are known to feed, breed and take shelter in this ecosystem.

Conclusion

Mangrove ecosystem is a highly valued ecosystem in terms of economy, environment and ecology. Moreover, mangrove ecosystem is a unique, fragile, highly productive ecosystem in the sea land inter phase, Mangroves are constal forests feard in sheltered ensure and along river banks and fagoons in the trupics and subtropics. The term 'mangrove' describes both the ecosystem and the plant famílles that have developed specialized adaptations to live in this tidal environment (Chowdhury, 2014).Sundarban constitutes majority of all timber and fuel wood output of W.B. The Sundarban serves as 'bio-shields' against the cyclones (tropical storms) and high tidal surges. It also provides protection against coastal erosion and damage. It stabilizes the coastal land by accumulating sediments. Moreover, the Sundarban could be a large carbon sink to diminish global warming and offset the greenhouse gas emissions. The belt of Sundarban mangroves is capable of absorbing 70-90 percent of the energy of a normal wave and 30-40 percent of the total force of a tsunami or cyclone-generated shock-waves before they hit the inhabited areas. More than 3.5 million people depend directly or indirectly for their livelihood on Sundarban's resources. Destruction of the forest will not only affect the ecologybut will also have far reaching impact on the national economy, and cause immense damage to the many unexplored and unutilized marine resources of the Bay of Bengal (2011).

Acknowledgement

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Nature's resilience: a multi-locational study of pollution-driven alterations in the pharmacological potential of *Amaranthus viridis*

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Abstract:

Amaranthus viridis demonstrates remarkable resilience to environmental pollution. This study investigated pollution-driven alterations in its pharmacological potential across three sites in Kolkata, West Bengal, representing distinct pollution gradients: Dhakuria (AQI: 50–65); (less polluted), Dum Dum (AQI: 95–110); (moderately polluted) and Howrah (AQI: 126–150); (highly polluted). Morphological analysis revealed a progressive decline in internode length (3.2 to 0.8 cm) and leaf area (12.4 to 4.2 cm^2) with increasing pollution. Total chlorophyll content declined (55.7% reduction), while ascorbic acid increased significantly (27.93 to 53.11 mg/g) and relative water content rose (91.1 to 96.77%), indicating antioxidant upregulation and osmotic adjustment. The Air Pollution Tolerance Index (APTI) increased from 36.58 to 51.04, confirming high pollution tolerance. Root biomass declined sharply (5.714 to 0.854 g; 85.1% reduction). Phytochemical screening confirmed alkaloids, flavonoids, tannins and glycosides at all sites; tannins peaked at moderate pollution (OD_{725} 0.386), suggesting a hormetic response. Antibiotic susceptibility assays demonstrated the strongest antibacterial activity in less polluted samples, with a complete loss of activity in highly polluted specimens. These findings establish a critical medicinal window and position *A. viridis* as a bioindicator, phytoremediator and pollution-sensitive medicinal resource that requires source-specific quality monitoring.

Keywords:

Amaranthus viridis, environmental pollution, Air Pollution Tolerance Index, phytochemical composition, bioindicator, phytoremediation, antioxidant activity

Introduction

Environmental pollution is among the most pressing challenges of the twenty-first century, threatening the balance between human civilisation and natural ecosystems. Freshwater, fertile land and clean air form the foundation of life on Earth, yet these resources face unprecedented degradation from anthropogenic activity (WHO, 2021). The release of hazardous compounds — industrial waste, vehicular emissions and agricultural runoff — produces a cascade of adverse effects on soil health, water quality and air composition (Boman et al., 2003).

Plants are among the first organisms affected by pollution. Exposure induces physiological stress and altered pigment metabolism, including disruption of carotenoid biosynthesis (Panda & Choudhury, 2005). Such changes directly influence the capacity of plants to synthesise the bioactive compounds essential for therapeutic applications, making the study of pollution responses crucial for both environmental conservation and the development of sustainable health solutions.

Amaranthus viridis (green amaranth), a member of the Amaranthaceae, is a widely distributed annual herb of tropical and subtropical regions, including India. It has been used in ethnobotanical practice for centuries for wound healing, fever management, pain relief and as an anti-

inflammatory agent (Kirtikar & Basu, 1987). Amaranth leaves are exceptionally nutrient-dense, containing far higher levels of ascorbic acid, calcium, provitamin A and iron than lettuce (Sarker & Oba, 2020), and the plant is documented in traditional Indian, Nepalese and Philippine medicine as an antipyretic and a topical remedy for skin disorders (Quisumbing, 1951).

Recent phytochemical investigations attribute the antioxidant, antimicrobial and anti-inflammatory properties of *A. viridis* to flavonoids, phenols and other secondary metabolites (Sarker et al., 2020). More than twenty-five phenolic and flavonoid compounds have been identified, including gallic acid, quercetin, rutin and isoquercetin, alongside triterpenoids, carotenoids, vitamins and minerals (Kaur et al., 2006). This flavonoid-rich profile underpins the therapeutic potential of the species in stress-affected environments.

Pollution markedly modifies plant physiology. Spectral studies show that air pollution reduces leaf water and carotenoid content while paradoxically increasing chlorophyll and anthocyanin levels as an adaptive response, with polluted plants boosting secondary-metabolite and antioxidant production for defence (Mate et al., 2016). Heavy-metal contamination, however, threatens food safety: *A. viridis* growing near highways in Lagos accumulated lead and cadmium above WHO limits despite compliant soil levels (Atayese et al., 2010), while related species absorb cadmium efficiently under controlled conditions (Zhang et al., 2010), underscoring a dual accumulator–remediator role. The Air Pollution Tolerance Index (APTI), computed from ascorbic acid, total chlorophyll, leaf pH and relative water content, quantifies tolerance to air pollution (Singh & Rao, 1983); *A. viridis* has recorded among the highest APTI values of tested species near Coimbatore, positioning it as a bioindicator and phytoremediation candidate (Karthiyayini et al., 2005).

Despite this, the impact of environmental pollution on the pharmacological potential of A. viridis remains largely unexplored. Because air pollution directly affects photosynthetic efficiency, stomatal function and overall physiology, it was hypothesised that populations growing under different pollution gradients would exhibit differential phytochemical profiles and medicinal potential (Mate et al., 2016), since pollutant accumulation may either compromise or enhance bioactive compounds depending on adaptive mechanisms (Atayese et al., 2008). The present study therefore assessed structural and physiological changes in *A. viridis* across pollution gradients through morphological, anatomical and phytochemical analyses; evaluated its antioxidant and antimicrobial potential; compared variation across low, moderate and high pollution; and examined its potential as a bioindicator and phytoremediator for environmental monitoring.

Materials and Methods

Study Sites and Plant Collection

Fresh young and mature plants of *A. viridis* (approximately five kg from each site) were collected from three locations in Kolkata, West Bengal, representing distinct pollution gradients: a

less polluted residential area with minimal industrial activity (Dhakuria), a moderately polluted mixed residential–commercial zone (Dum Dum) and a highly polluted industrial area adjacent to factories (Howrah) (Fig. 1). The Air Quality Index (AQI) of each site was assessed using the Purelogic Labs AQI Detector application (Google Play Store), which provides real-time pollution monitoring based on GPS coordinates.

Morphological and anatomical analysis

Morphological traits — plant height (cm), stem diameter (mm), total leaf number, leaf area (cm^2) and internodal length (cm) — were systematically recorded for each specimen. For the anatomical study, thin transverse sections (approximately 20–40 µm) of stem and leaf tissue were prepared, mounted on glass slides and examined under a light microscope at 40× and 100× magnification. Cell size and shape, cell-wall thickness, intercellular air spaces, and the organisation of vascular tissues including the arrangement of xylem and phloem elements were recorded.

Phytochemical screening

Preliminary qualitative screening of leaf extracts was performed to detect alkaloids, flavonoids, tannins, saponins and glycosides following established protocols (Harborne, 1998). Alkaloids were identified by heating the dried extract in dilute hydrochloric acid followed by the addition of Mayer's reagent, turbidity or a yellowish precipitate indicating a positive result. Flavonoids were detected by the Shinoda test, in which magnesium shavings and concentrated hydrochloric acid yielded a pink to red colouration. Tannins were confirmed using 10% ferric chloride solution, which produced a blue to dark-green colouration. Saponins were assessed by the froth test, persistent foam on vigorous shaking with distilled water indicating their presence. Glycosides were identified after acid hydrolysis and treatment with Fehling's reagent, a brick-red precipitate on boiling confirming the result.

Chlorophyll content estimation

Fresh leaf tissue (1 g) was crushed in 80% acetone (20 mL), centrifuged at 5000 rpm for 5 min, and the supernatant collected; extraction was repeated until the residue was colourless, and the final volume was adjusted to 100 mL. Absorbance was measured at 645 and 665 nm using a UV–Vis spectrophotometer, and chlorophyll content was calculated following Arnon (1949):

$$\text{Chlorophyll a (mg/g)} = [12.7(A_{665}) - 2.69(A_{645})] \times (V/1000W)$$

$$\text{Chlorophyll b (mg/g)} = [22.9(A_{645}) - 4.68(A_{665})] \times (V/1000W)$$

$$\text{Total chlorophyll (mg/g)} = [20.2(A_{645}) + 8.02(A_{665})] \times (V/1000W)$$

where A = absorbance, V = final volume (mL) and W = fresh weight of tissue (g).

Ascorbic acid content estimation

A dye solution was prepared from sodium bicarbonate (42 mg) and 2,6-dichlorophenolindophenol (DCPIP; 52 mg) in 200 mL of distilled water, and a working standard was obtained by diluting an ascorbic acid stock (100 mg per 100 mL of 4% oxalic acid) to 100 µg/mL. The working standard (5 mL) was titrated against the dye and the volume recorded (V_1). Plant tissue (5 g) was extracted in 4% oxalic acid, made up to 100 mL and centrifuged at 6000 rpm for 30 min; the supernatant (5 mL) was titrated against the dye (V_2). Ascorbic acid content was then calculated as:

$$\text{Ascorbic acid (mg/g)} = [0.5 \times V_2 / (V_1 \times 5)] \times [100 / (\text{sample weight} \times 100)]$$

Leaf pH and relative water content

For pH determination, fresh leaves (3 g) were crushed in 30 mL of distilled water, filtered through linen cloth, and the pH of the extract measured with a calibrated pH meter. For relative water content (RWC), fresh leaf samples (1 g) were weighed to obtain fresh weight (FW), immersed in distilled water overnight to record turgid weight (TW), and then oven-dried at 80 °C to constant weight to determine dry weight (DW). RWC was calculated as:

$$\text{RWC (\%)} = [(FW - DW)/(TW - DW)] \times 100$$

Air Quality and Air Pollution Tolerance Index

Daily AQI readings for each site were recorded throughout the study period using the same mobile application, which aggregates real-time data from government and validated community sensors; this longitudinal approach averaged short-term fluctuations to provide a robust estimate of ambient air quality. The Air Pollution Tolerance Index (APTI) was computed from four leaf parameters using the equation:

$$APTI = [A(T + P) + R]/10$$

where A is ascorbic acid content (mg g^{-1}), T total chlorophyll content (mg g^{-1}), P the pH of the leaf extract and R the relative water content (%).

Root biomass analysis

Mature root systems were carefully extracted, rinsed with distilled water and air-dried, then oven-dried at 80 °C to a constant weight, after which the dry weight (g) was recorded.

Total phenol and tannin estimation

For both assays, leaves (1 g) were ground in 80% methanol (10 mL) and the homogenate centrifuged at 6000 rpm for 20 min. For total phenol, the extract (2 mL) was diluted to 3 mL with distilled water, Folin–Ciocalteu reagent (0.75 mL) was added, and after 5 min 10% sodium

carbonate (0.75 mL) was added; after 1 h of dark incubation, the optical density was measured at 725 nm. For total tannin, the extract (0.1 mL) in 7.5 mL of distilled water received Folin reagent (0.5 mL) and 35% sodium carbonate (1 mL), with the volume adjusted to 10 mL; the optical density was read at 725 nm after 30 min. Both parameters were quantified against a gallic acid standard curve (20–100 µg/mL) (Harborne, 1998).

Antibiotic susceptibility testing

Fresh plant tissue (1 g) was homogenised in 10 mL of double-distilled water and centrifuged at 6000 rpm for 20 min, and the supernatant was stored at 4 °C until use. Mueller–Hinton Broth (MHB) and Mueller–Hinton Agar (MHA; MHB supplemented with 2% w/v agar) were prepared and autoclaved at approximately 121 °C for 15 min. Bacterial strains were grown overnight in MHB at 37 °C with shaking, and lawns were prepared by spreading 0.1 mL of suspension onto MHA plates. Sterile filter-paper discs saturated with plant extract were applied to the seeded plates, with standard antibiotic discs (azithromycin and Clavam, 0.5–1.0 µg of active ingredient) serving as positive controls. Plates were incubated at 37 °C for 24 h, and the diameter of the zone of inhibition (mm) was measured for each disc.

Results

Plant collection and air quality assessment

The Air Quality Index confirmed distinct pollution gradients across the three study sites (Fig. 1). *A. viridis* was recorded at all sites irrespective of pollution level and appeared more abundant at the moderately and highly polluted locations than at the less polluted site (Table-1, Fig. 2 and 3).

Morphological and anatomical changes

A. viridis showed clear sensitivity to air pollution. Plants from the less polluted Dhakuria site exhibited normal growth, with longer internodes (3.2 ± 0.15 cm), larger green leaves (12.4 ± 0.8 cm^2) and flexible stems. Moderately polluted Dum Dum plants were intermediate, with reduced internode length (2.1 ± 0.12 cm) and leaf area (8.9 ± 0.6 cm^2). Plants from the highly polluted

Table 1. Air Quality Index (AQI) values for the study locations in Kolkata, representing the distinct pollution gradients used in the investigation.

Location	Pollution Level	AQI Range	Classification
Dhakuria	Less Polluted	50 – 65	Satisfactory
Dum Dum	Moderately Polluted	95 – 110	Unhealthy for Sensitive Groups
Howrah	Highly Polluted	126 – 150	Unhealthy



Fig. 1. Collection sites of *Amaranthus viridis* (1A.a; b; c, 1B.a; b; c; & 1C.a; b; c) across the three study locations in Kolkata — Dhakuria (AQI: 50–65) (less polluted), Dum Dum (AQI: 95–110); (moderately polluted) and Howrah (AQI: 126–150) (highly polluted). The Air Quality Index (AQI) was assessed using the Purelogic Labs AQI Detector app (Google Play Store), which provides real-time pollution monitoring based on GPS coordinates.



Fig. 2. A- B. AQI detector app (Google Play Store) and description of the app on Google Play Store.



Fig. 3. A- C Air Quality Index (AQI) of the less polluted area (Dhakuria, 3A); (Dum Dum, 3B); (Howrah 3C) recorded using the AQI Detector App.

Howrah site displayed severe stunting, with markedly shorter internodes (0.8 ± 0.09 cm), smaller discoloured leaves (4.2 ± 0.4 cm²) and a woody stem texture (Fig. 4)

Transverse sections paralleled this gradient. Less polluted specimens showed distinct vascular bundles with regular cell sizes, well-defined xylem–phloem differentiation and prominent intercellular spaces. Moderately polluted specimens displayed partially compressed vascular tissue with denser, more intensely stained xylem and reduced air spaces. Highly polluted plants showed the most pronounced modifications — compact cortical cells, thickened and darkly stained vascular bands, diminished air spaces and an overall lignified structure — the classic hallmarks of chronic pollution stress (Fig. 5).

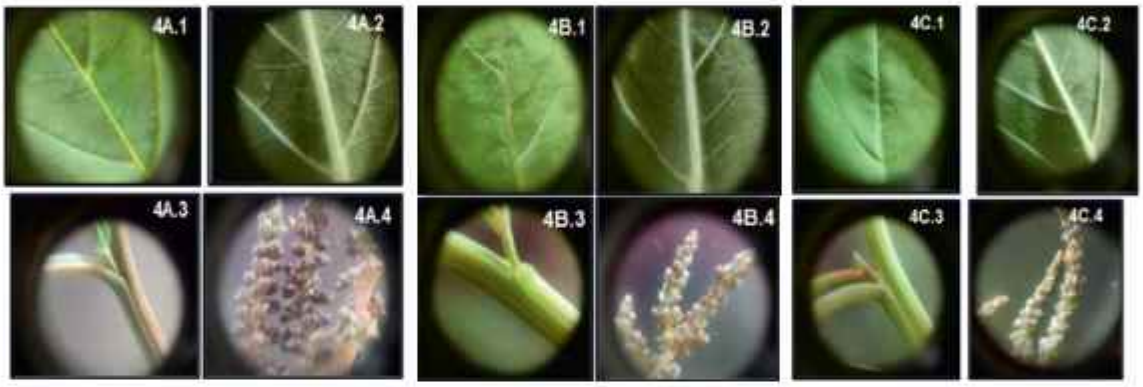


Fig. 4. A-C. Morphological features of *Amaranthus viridis* from the less polluted, moderately polluted and highly polluted area: (1) ventral leaf surface, (2) dorsal leaf surface, (3) internode and stem, and (4) inflorescence.

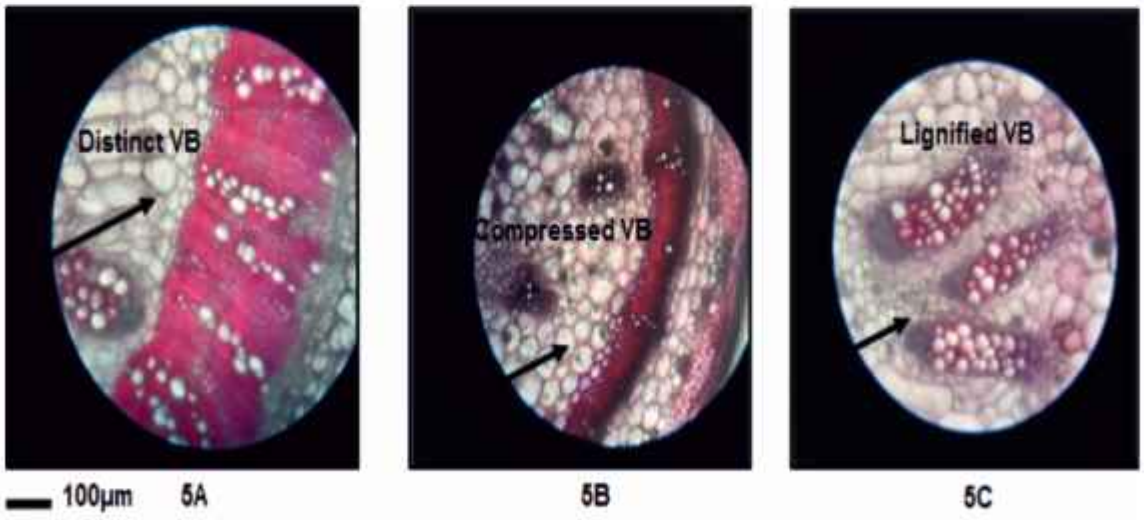


Fig. 5. Transverse section of *Amaranthus viridis* stem from less polluted site, 5A (Dhakuria) showing distinct vascular bundles with regular cell arrangement, clear xylem-phloem differentiation, and prominent intercellular spaces. 5B, moderately polluted site (Dum Dum) exhibiting compressed vascular bundles with denser xylem, reduced intercellular spaces, and early cell wall thickening.5C, highly polluted site (Howrah) displaying compact lignified anatomy featuring markedly thickened vascular tissues, diminished air spaces, and stress-induced structural reinforcement.

Phytochemical qualitative profile

Qualitative screening confirmed a diverse array of bioactive phytochemicals in *A. viridis* leaf extracts. Alkaloids, flavonoids, tannins and glycosides were present at all three sites, whereas saponins were absent throughout (Table 2). Flavonoids contribute anti-inflammatory and antioxidant activity, alkaloids analgesic and antibacterial activity, tannins astringent and wound-healing effects, and glycosides various physiological functions — a profile that positions *A. viridis* as a promising source of natural therapeutic agents.

Table 2. Qualitative phytochemical screening of *Amaranthus viridis* leaf extracts across the pollution gradient. (+) = present; (−) = absent.

Phytochemical	Less Polluted	Moderately Polluted	Highly Polluted
Alkaloids	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Saponins	-	-	-
Glycosides	+	+	+

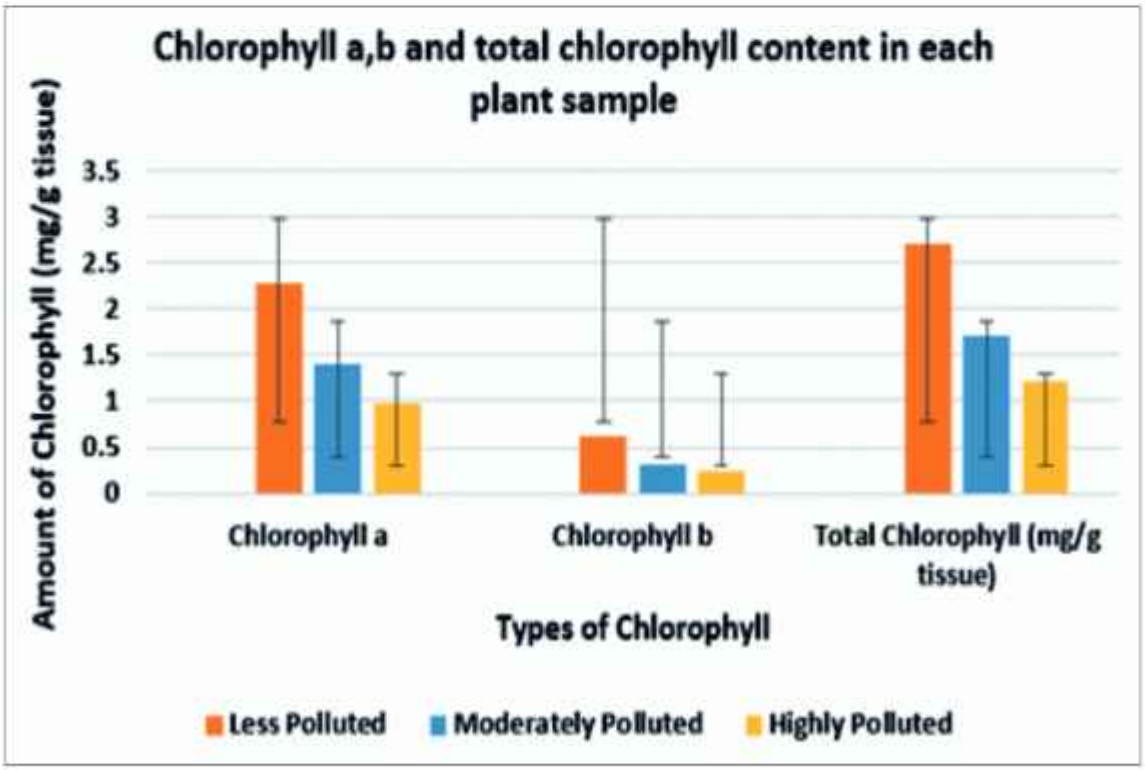


Fig. 6. Chlorophyll a, chlorophyll b and total chlorophyll content (mg/g fresh tissue) in *A. viridis* leaves across the pollution gradient.

Chlorophyll Content

Total chlorophyll content declined significantly with increasing pollution intensity (Figure 6). This reduction is attributable to enhanced chlorophyllase activity, metal-induced inhibition of chlorophyll biosynthesis and oxidative damage to pigment–protein complexes; airborne pollutants accelerate photo-oxidation, destabilise light-harvesting complexes and impair photosystem II efficiency.

Ascorbic Acid Content

Ascorbic acid showed a compensatory stress response, increasing progressively from 27.925 ± 0.124 mg/g at Dhakuria to 42.636 ± 0.216 mg/g at Dum Dum and 53.108 ± 0.081 mg/g at Howrah (Fig. 7), reflecting enhanced antioxidant synthesis may be as an adaptive defence.

Leaf Extract pH

The leaf-extract pH showed a pollution-dependent alkalinisation pattern across the study sites (Dhakuria < Dum Dum < Howrah). This gradual increase indicates stress-induced metabolic shifts may be that enhance biochemical defence activation while maintaining physiological homeostasis under adverse conditions.

Relative Water Content

Contrary to typical stress responses, the relative water content increased with pollution severity, rising from 91.1% at the less polluted site to 96.77% at the highly polluted site (Fig. 7).

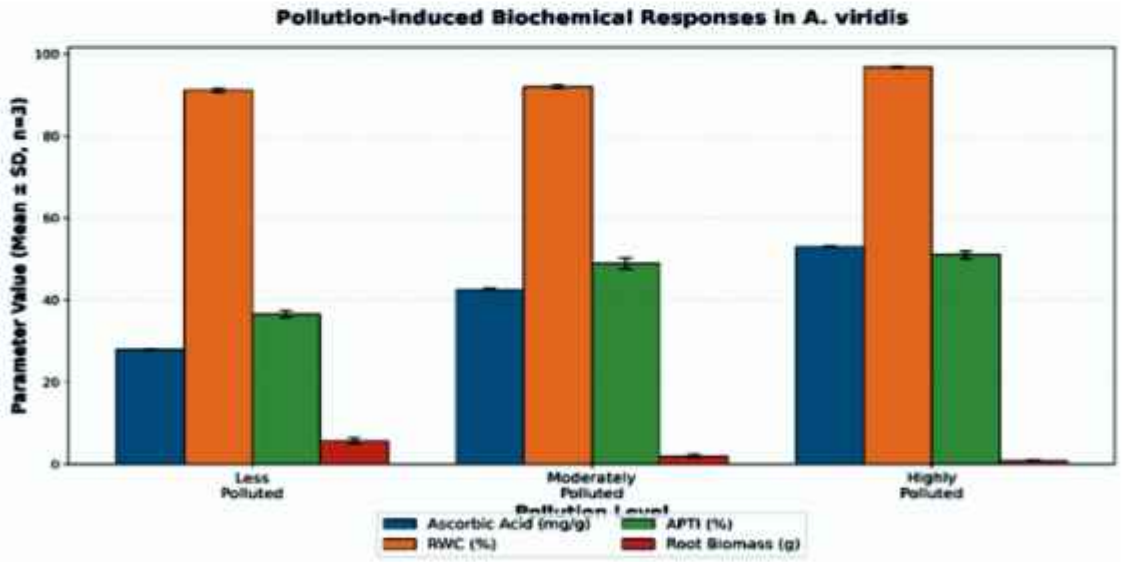


Fig.7. Pollution-induced biochemical and physiological responses in *A. viridis* — ascorbic acid content, relative water content, Air Pollution Tolerance Index and root biomass (mean ± SD, n = 3).

This indicates an adaptive hydration strategy in which stressed plants maintain elevated tissue water content to dilute toxicants and sustain turgor pressure.

Air Pollution Tolerance Index

APTI values increased progressively from 36.584 ± 0.874 (less polluted) to 48.904 ± 1.319 (moderately polluted) and 51.044 ± 0.952 (highly polluted) (Figure 7). Values consistently exceeding 36 classify *A. viridis* as highly pollution tolerant and suitable for urban phytoremediation applications.

Root biomass

Root dry biomass declined sharply with pollution intensity, decreasing from 5.714 ± 0.729 g (less polluted) to 1.944 ± 0.495 g (moderately polluted) and 0.854 ± 0.089 g (highly polluted) — an 85.1% reduction (Fig. 7) — indicating resource reallocation that prioritises aboveground stress defence over belowground growth.

Quantitative phytochemical analysis

Total phenol content remained relatively stable across the pollution gradient (Fig. 8), demonstrating constitutive antioxidant protection independent of stress intensity. In contrast, total

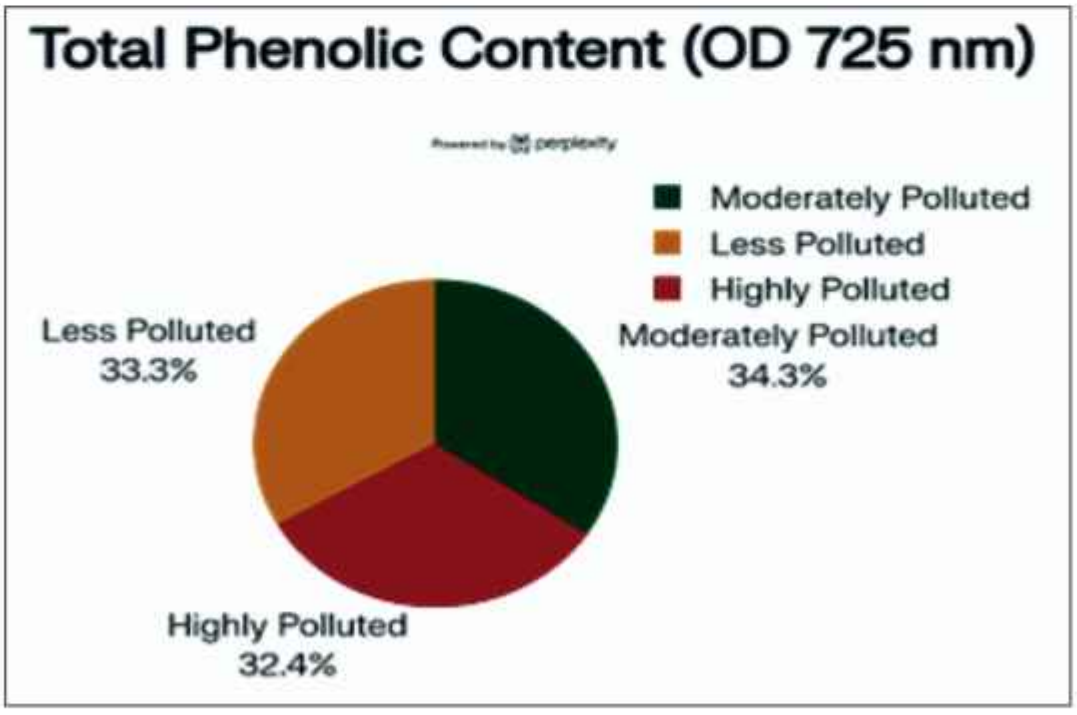


Fig.8. Distribution of total phenolic content (optical density at 725 nm) in *A. viridis* across the three pollution levels.

tannin content exhibited a hormetic response, with peak synthesis at moderate pollution (OD_{725} 0.386) (Fig. 9), suggesting an optimal stress-triggered production threshold before metabolic overload at severe pollution levels.

Antibiotic susceptibility

Both bacterial strains, *Priestia aryabhattai* and *Rossellomorea marisflavi,* were first tested against the reference antibiotics azithromycin and Clavam, respectively, and produced clear zones of inhibition, thereby validating the assay conditions. Azithromycin yielded inhibition zones of approximately 1.0–1.5 cm against *P. aryabhattai* across the concentrations tested, while Clavam produced zones of 1.0–1.4 cm against *R. marisflavi.*

The antibacterial potential of *A. viridis* water and ethanolic extracts showed strong site-dependent differences. Against *P. aryabhattai,* extracts from the less polluted site produced distinct inhibition zones (up to approximately 1.0 cm for the ethanolic extract), extracts from the moderately polluted site showed only weak or inconsistent inhibition (approximately 0.2–0.3 cm), and extracts from the highly polluted site failed to produce any inhibition in either solvent, indicating a complete loss of activity. The pattern was more pronounced against *R. marisflavi*, for which only the ethanolic extract from the moderately polluted site produced a very small zone (approximately 0.2 cm), while all other site–solvent combinations were inactive. Overall, *A. viridis*

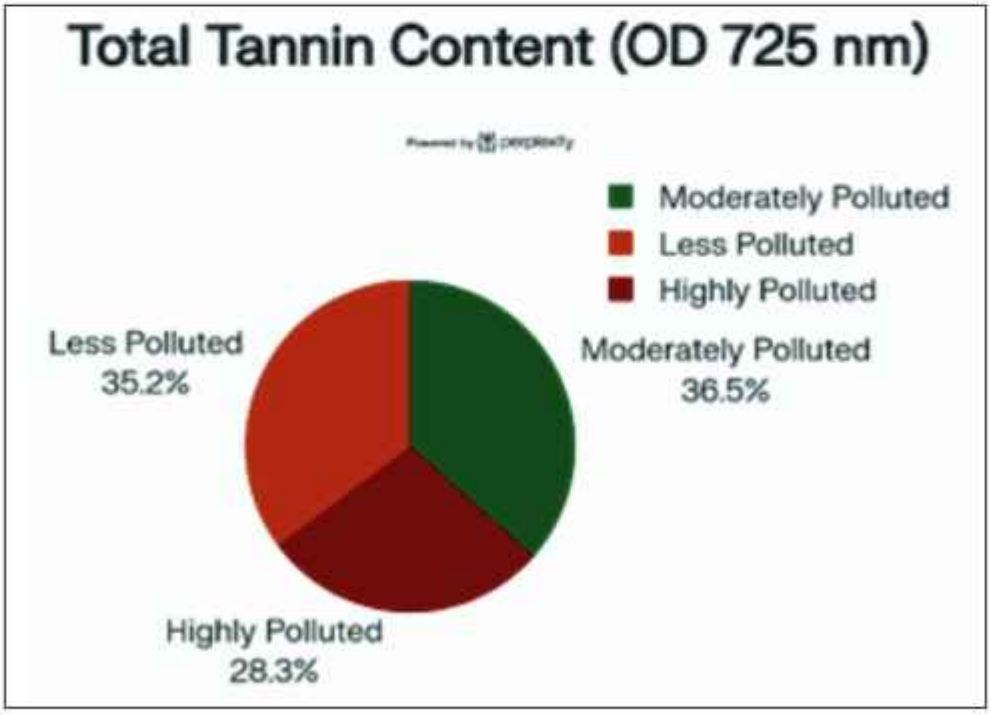


Fig. 9. Distribution of total tannin content (optical density at 725 nm) in *A. viridis* across the three pollution levels.

from less polluted environments retained appreciable antibacterial activity, material from moderately polluted sites showed only sporadic activity, and samples from highly polluted locations were completely inactive under the tested conditions (Fig. 10).

Discussion

Morphological and Anatomical Adaptations

The progressive reduction in internode length (3.2 to 0.8 cm) and leaf area (12.4 to 4.2 cm^2) with increasing pollution reflects photosynthetic inhibition and growth suppression, closely associated with photosystem II impairment under heavy-metal stress (Panda et al., 2005). The woody stem texture of highly polluted plants represents mechanical reinforcement that

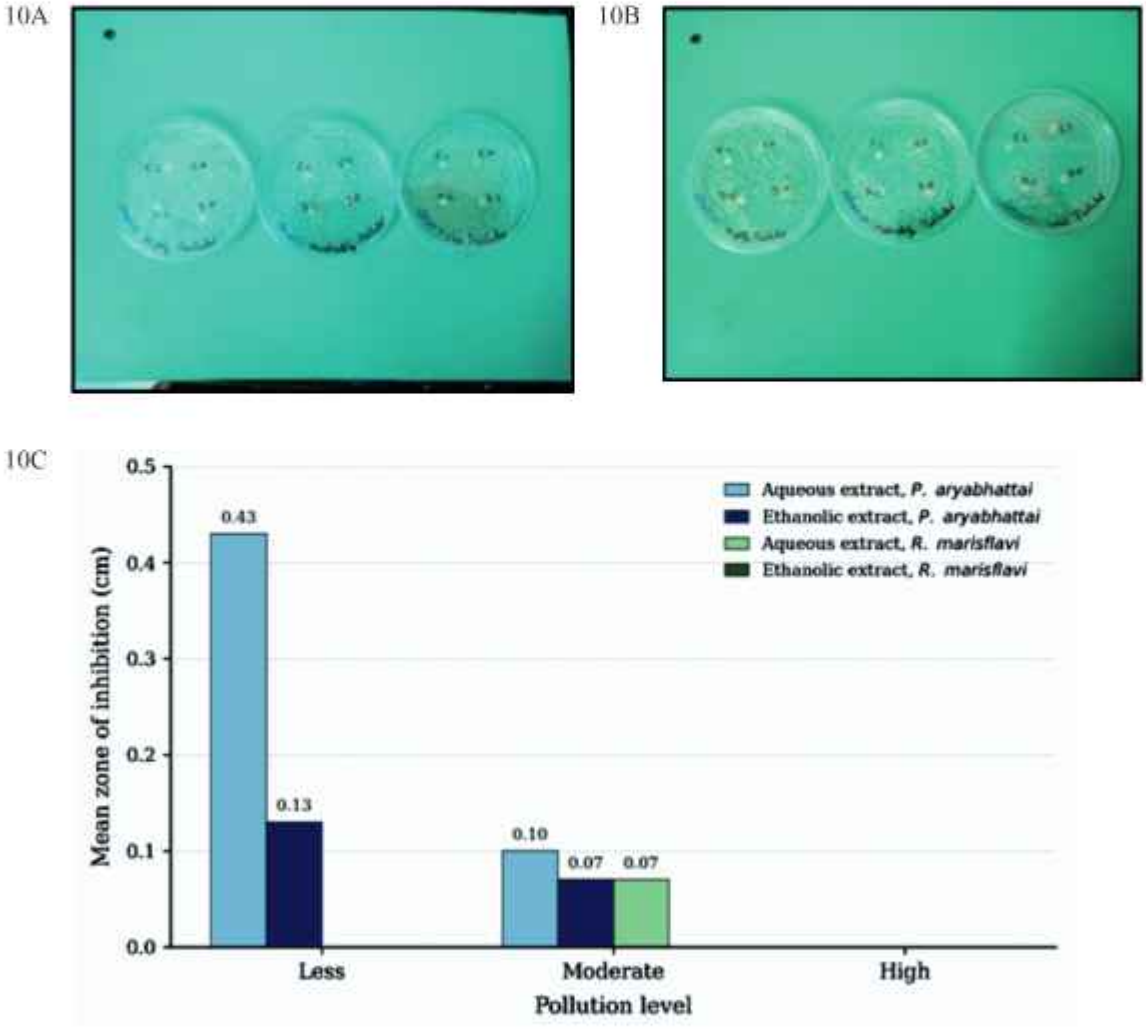


Fig. 10. Antibacterial activity of the bacterial extracts against the test organism. (A, B) Representative antibacterial assay plates showing zones of inhibition. (C) Mean zone of inhibition (cm) of the aqueous and ethanolic extracts of *Priestia aryabhattai* and *Rossellomorea marisflavi* across less-, moderately- and highly-polluted sites.

compensates for reduced biomass allocation and increased structural stress. Anatomical changes — from distinct vascular bundles with prominent intercellular spaces to compressed xylem, thickened cell walls and diminished air spaces — minimise pollutant penetration and water loss while compromising gas exchange (Ashraf & Harris, 2013).

Chlorophyll dynamics and photosynthetic stress

The 55.7% decline in total chlorophyll indicates severe photosynthetic impairment. Particulate matter (PM_{10} and $PM_{2.5}$) deposited on leaf surfaces physically obstructs stomata and reduces gas exchange, while pollutant-induced oxidative stress triggers chlorophyll photo-oxidation through the overproduction of reactive oxygen species (ROS) (Rao et al., 2025). Although moderate stress can paradoxically increase chlorophyll as a compensatory response, this was not observed here, most likely because pollution levels at Howrah exceeded the critical tolerance threshold of the species.

Ascorbic acid and antioxidant upregulation

The counterintuitive increase in ascorbic acid with pollution intensity — a 90% rise from the less to the highly polluted site — represents a critical adaptive mechanism. Ascorbic acid is a key non-enzymatic antioxidant of the ascorbate–glutathione cycle, directly detoxifying hydroxyl radicals, superoxide anions and hydrogen peroxide generated under pollution stress (Noctor & Foyer, 1998). Plants exposed to air pollution enhance the expression of ascorbate-biosynthesis genes and antioxidant enzymes such as superoxide dismutase, catalase and ascorbate peroxidase (Mishra et al., 2023). Consistent with the stress-induced secondary-metabolism paradigm, plants prioritise the production of defensive compounds under adverse conditions, enhancing short-term survival at the cost of growth and reproduction (Levitt, 1980). The elevated relative water content of highly polluted plants (96.77% versus 91.1%) reflects osmotic adjustment through the accumulation of compatible solutes that maintain turgor and support antioxidant enzyme activity (Levitt, 1980).

APTI as a bioindicator and pollutant accumulator

APTI increased with pollution (36.58–51.04), placing *A. viridis* firmly in the high-tolerance category and indicating low pollution sensitivity together with a high pollutant-accumulation capacity. Comparable studies in tropical urban environments confirm that *A. viridis* records among the highest APTI values of herbaceous species (Karthiyayini et al., 2005). By integrating ascorbic acid, chlorophyll, pH and RWC, the APTI provides a comprehensive bioindication tool, qualifying *A. viridis* for air-quality monitoring networks and for urban greening in industrial areas. The inverse relationship between APTI and antimicrobial efficacy, however, suggests that the species functions

simultaneously as a pollutant accumulator and a bioindicator, requiring differentiated management depending on the intended application.

Root biomass decline and resource allocation

The 85.1% reduction in root biomass reflects a fundamentally altered resource-allocation strategy under chronic stress, in which plants direct finite photosynthetic resources towards aboveground tissues, antioxidant defence and protective secondary metabolites rather than towards energetically expensive root development (Ramakrishna & Ravishankar, 2011). While this trade-off enhances short-term tolerance, it compromises long-term nutrient and water uptake and reduces reproductive potential. Although Amaranthus is recognised for superior root stress tolerance, extreme pollution evidently exceeds even its physiological limits, as the dramatic biomass reduction at Howrah demonstrates.

Phytochemical hormesis

Tannins peaked at moderate pollution (OD_{725} 0.386), exemplifying hormesis: moderate stress upregulates the phenylpropanoid pathway through transcription factors that enhance the synthesis of tannins and other polyphenolic defence compounds, whereas extreme pollution exceeds the adaptive capacity of the plant and triggers metabolic overload (Rao et al., 2025). Phenols, by contrast, remained stable, providing constitutive protection. This "optimal stress threshold" implies a quantifiable medicinal window — a specific pollution range within which pharmacological activity is optimised.

Antimicrobial properties and phytochemical quality

The strongest inhibition zones occurred in less polluted samples and declined to zero in highly polluted specimens. The antimicrobial compounds of *A. viridis* — phenolic acids, tannins and flavonoids — are produced through the same biosynthetic pathways as ROS-scavenging molecules; under extreme pollution, competition for precursors and energy favours immediate survival functions over antimicrobial-compound synthesis, thereby reducing phytochemical efficacy (Ramakrishna & Ravishankar, 2011). Thus, while *A. viridis* serves as a valuable bioindicator at high APTI values, its utility as a medicinal agent is inversely correlated with pollution intensity.

Implications for medicinal use and phytoremediation

These findings call for an urgent reconsideration of the traditional use of *A. viridis* in the context of rapid urban industrialisation. Consumers in industrial zones may ingest plants with compromised antioxidant, antimicrobial and nutritional profiles, and meta-analyses additionally document elevated heavy-metal accumulation — particularly of cadmium, lead and mercury — in

Amaranthus grown in contaminated soils (Tőzsér et al., 2023). This "double burden" of reduced therapeutic benefit and increased toxicological risk underscores the need for quality-control certification specifying acceptable pollution-source areas, consumer-awareness campaigns, regulatory limits on harvesting from high-AQI areas, and pollution-adjusted cultivation protocols. Conversely, the high APTI and bioaccumulation capacity of the species support its use in Phyto stabilisation and phytoextraction at contaminated industrial sites. Because medicinal efficacy and remediation capacity diverge, strategic compartmentalisation is essential: *A. viridis* should not be used as a food or medicinal crop where it is deployed for phytoremediation, and distinct cultivation zones should be maintained for therapeutic and remediation purposes.

Limitations and future eirections

Certain limitations of the present study should be acknowledged. Air quality at the three sites was characterised using application-based AQI values rather than direct instrumental monitoring of individual pollutants, which may constrain the precision of site classification. Sampling was restricted to three populations and a single growing season, so seasonal and inter-annual variation in pollutant load and plant response could not be captured. The investigation did not include direct quantification of heavy-metal accumulation in plant tissues or formal inferential statistical testing of the measured parameters. Future studies incorporating instrumental air monitoring, larger sample sizes, multi-season sampling, tissue heavy-metal analysis and statistical validation would strengthen and extend these findings.

Conclusion

This multi-locational investigation demonstrates that *Amaranthus viridis* exhibits exceptional resilience to environmental pollution, making it a valuable species for urban greening, environmental monitoring and phytoremediation. Its high Air Pollution Tolerance Index across the study gradient (36.58–51.04) confirms its status as a superior bioindicator. However, the study also reveals a critical trade-off: while *A. viridis* maintains physical integrity and upregulates certain antioxidant compounds under moderate pollution stress, extreme pollution substantially compromises its medicinal and nutritional potential, as shown by a 55.7% reduction in chlorophyll content, an 85.1% reduction in root biomass, peak antioxidant synthesis at moderate rather than extreme pollution, and a marked decline in antimicrobial efficacy. These results indicate an optimal pharmacological range at moderate pollution levels, beyond which therapeutic efficacy diminishes. For medicinal use, sourcing from less polluted areas remains preferable, whereas for environmental remediation the exceptional tolerance of the species makes it a valuable management tool in polluted zones. By bridging ethnobotanical knowledge with contemporary environmental science, this study advances the understanding of medicinal-plant resilience and provides evidence-based guidance for the sustainable cultivation and use of *A. viridis* in polluted urban environments.

Acknowledgement

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Navagraha Vatika and Nakshatra Vatika : introducing Astro-ethnobotany through plant–planet correlations, sacred garden conservation, and traditional ecological knowledge

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Abstract:

Plants occupy a central place in Vedic cosmology, where every Graha and Nakshatra was assigned a specific tree or herb of healing value. The present paper proposes the term Astro-ethnobotany for the structured study of these plant–planet relations, examined together with ethnographic and pharmacological evidence. Drawing on classical Sanskrit sources (Rigveda, Atharvaveda, Brihat Samhita, Charaka Samhita, Ashtanga Hridayam and Vrikshayurveda), field work with 28 Santhali Ojhas of Birbhum district (West Bengal) carried out during 2023–2025, and photographic documentation of the Navagraha Vatika and Nakshatra Vatika maintained by the Department of Forests and Wildlife, Chandigarh, the study assembles four datasets. These are: a botanical and pharmacological profile of all nine Navagraha plants with their directional placement; a complete 27-Nakshatra plant correlation table with bioactive compounds and classical references; identification of six dual-role species occupying positions in both garden systems; and a conservation argument grounded in the IUCN, CBD and UNESCO frameworks. Tribal Ojhas reported the highest Fidelity Levels (85–100 percent) for the dual-role species, which strengthens the view that these plants form the pharmacological core of the Vedic materia medica. Moraceae alone accounts for 18.5 percent of the Nakshatra trees, suggesting an empirically built conservation logic in favour of keystone fig species. The *Calotropis procera*–Surya assignment is examined as a case in which Vedic solar symbolism, xerophytic ecology, vitamin D photobiology and recent PI3K/AKT/p53 anticancer findings converge. Sacred plant gardens, the paper argues, are ex-situ conservation systems that anticipate modern botanic-garden practice by several millennia and should be recognised under Article 8(j) of the Convention on Biological Diversity.

Keywords:

Astro-ethnobotany; Navagraha Vatika; Nakshatra Vatika; Vedic plants; dual-role species; sacred garden conservation; Santhali Ojha; Traditional Ecological Knowledge.

Introduction

Plants have served as a foundation of Indian civilisation since the earliest phases of cultural history. Vedic literature treats them as living agents within an interconnected web of cosmic, ecological and physiological relations (Jain and Kapoor, 2007). Beyond their utilitarian roles in food, fibre, fuel and medicine (Usman et al., 2014), plants occupy a central position in Vedic cosmology, where their form, habitat and chemistry are read in relation to planetary and stellar influences.

The Vedic celestial system is built around nine Grahas (planetary bodies), twelve Rashis (zodiac signs) and twenty-seven Nakshatras (lunar mansions). Each unit is linked to specific deities, directions, colours and plant species (Soni et al., 2023). Birth charts (Janampatrika) have for centuries guided the selection of therapeutic plants according to an individual's planetary

constitution, a practice grounded in long-term empirical observation rather than in superstition. As Frawley (2000) observed, every planet, during its orbit, gathers and releases forces and so transmits a specific wavelength of energy that helps to maintain order within the solar system.

The Navagraha Vatika is a formal garden of nine sacred plants arranged geometrically by planetary direction. The Nakshatra Vatika is a living collection of twenty-seven trees, one for each lunar mansion. Earlier work treats these gardens as repositories of pharmacologically active species within the Indian materia medica (Soni et al., 2023; Sharma, 1981). The Vrikshayurveda of Surapala (Sircar and Sarkar, 1996), the Brihat Samhita of Varahamihira (Bhat, 1981) and the Charaka Samhita (Sharma, 1981) supply the canonical textual basis for these plant–planet correlations.

There is a meeting point between Vedic cosmology and modern ecology. Trees absorb solar and atmospheric energy, translocate it through phloem and root, and return transformed energy to the atmosphere through transpiration and respiration. The cycle closely resembles the Vedic idea of prana flow (Frawley, 2000). Photosynthesis, the carbon cycle and transpiration represent the plant's measurable contribution to cosmic energy exchange, in keeping with the Vedic concept of a continuous energy continuum (Usman et al., 2014). This convergence permits us to read Vedic botanical knowledge as encoded ecological intelligence rather than as mythology alone.

Field documentation among the Santhali tribal community of Birbhum supplies a living, empirical grounding for these textual associations. The Santhali Ojhas, with 25 of their 31 documented healing plants showing confirmed ethnomedicinal properties (Pradhan and Mondal, 2023), preserve one of the most intact traditions of Graha-linked plant healing in eastern India. Their use of plant selection aligned with planetary categories for the treatment of childhood illness, particularly the conditions classed under Balagraha, points to an operational continuity of the system across millennia.

Recent global research in ethnobotany and natural-product chemistry has turned increasingly towards bio-cultural heritage, plant–microbe–host interactions, AI-assisted phytochemical screening and climate-resilient sacred landscapes (Heinrich et al., 2022). A structured re-examination of Vedic sacred gardens is timely. The present paper integrates field ethnography, photographic garden documentation from the Navagraha and Nakshatra Vatikas at Chandigarh, and a wide review of classical and modern literature to give an integrated account of both garden systems. Six dual-role species are identified, and the conservation value of these living collections is examined through the IUCN and CBD frameworks.

Methods

Field Area and Ethnographic Survey

Primary fieldwork was carried out in five Santhali-inhabited villages of Birbhum district, West Bengal (23°32′ to 24°35′ N latitude; 87°05′ to 88°01′ E longitude), between July 2023 and

January 2025. Birbhum is ecologically distinctive for its semi-arid scrubland, Sal forest patches and rich ethnomedicinal heritage (Pradhan and Mondal, 2023). Supplementary photographic documentation of the Navagraha Vatika and Nakshatra Vatika was carried out at the botanical garden maintained by the Department of Forests and Wildlife, Chandigarh Administration (30°44′ N, 76°47′ E), one of the most scientifically curated sacred plant garden complexes in India.

Data Collection

Data were collected from 28 Ojhas and senior community members (45–82 years; 18 male, 10 female) through semi-structured interviews and participatory observation. Free and Prior Informed Consent was obtained in accordance with the Nagoya Protocol (CBD, 2010). Specimens were collected in flowering or fruiting condition, pressed, dried and identified using standard regional and national floras. The ethnobotanical indices Relative Frequency of Citation (RFC) and Fidelity Level (FL percent) were computed for plants used in Graha-based healing rituals. Photographic documentation of the official signage at the Chandigarh garden gave direct institutional confirmation of plant–Graha and plant–Nakshatra assignments.

Textual and Literature Analysis

Classical sources consulted include the Rigveda (Griffith, 2001), Atharvaveda (Bloomfield, 1997), Vrikshayurveda of Surapala (Sircar and Sarkar, 1996), Charaka Samhita (Sharma, 1981), Brihat Samhita of Varahamihira (Bhat, 1981) and Ashtanga Hridayam of Vagbhata (Nadkarni, 2002). A systematic search of PubMed, Scopus and Google Scholar was performed for the period 2000 to 2025 with the search terms Navagraha plants, Vedic ethnobotany, Nakshatra Vatika, astrological herbalism and Calotropis procera pharmacology. Species nomenclature follows APG IV (2016).

Ethics Statement

The work was conducted in line with the ethical guidelines of the Society of Ethnobiology. All field photographs were taken with explicit informed consent. No endangered species were collected. The tribal knowledge holders are acknowledged as co-contributors and their intellectual heritage is protected under the Protection of Plant Varieties and Farmers' Rights Act, 2001.

Results

Navagraha Vatika : Photographic Documentation and Botanical Profile

The Navagraha Vatika at Chandigarh is a geometric arrangement of the nine sacred plants of the Hindu cosmological system (Fig. 1). Surya (Sun) holds the centre; Brihaspati (Jupiter) the north; Budha (Mercury) the north-east; Shukra (Venus) the east; Chandra (Moon) the south-east; Mangala (Mars) the south; Rahu the south-west; Shani (Saturn) the west; and Ketu the north-west.

The garden's signage agrees with the prescriptions of the Brihat Samhita (Ch. 54) and Vrikshayurveda (Bhat, 1981; Sircar and Sarkar, 1996), and the same layout is reported by Soni et al. (2023) for the Gargi College Vatika at New Delhi.

Table 1 lists all nine Navagraha plants with their direction, family, key bioactive compounds and pharmacological activity. A regional variant was recorded: the Chandigarh garden assigns

Table 1. Navagraha plants: botanical, directional and pharmacological profile.

Graha	Common Name	Botanical Name	Family	Direction / Colour	Key Bioactive Compounds	Pharmacological Activity
Surya (Sun)	Aak / Madar	*Calotropis procera*	Apocynaceae	Centre / Light pink	Calotropin, calotropagenin, giganteol, giganteones A and B, flavonoids, tannins	Anticancer (PI3K/AKT/p53), anti-inflammatory, wound healing, antimicrobial, antidiabetic
Chandra (Moon)	Palash / Dhaak	*Butea monosperma*	Fabaceae	S-East / White	Butein, butin, isobutin, palasonin, monospermoside	Antidiabetic, anti-fertility, hepatoprotective, anthelmintic, anti-inflammatory
Mangala (Mars)	Khair / Cutch tree	*Acacia catechu*	Fabaceae	South / Deep red	Catechin, epicatechin, catechu-tannic acid, quercetin	Antimicrobial, anti-obesity, antioxidant, astringent, anti-tussive
Budha (Mercury)	Apamarga / Prickly chaff	*Achyranthes aspera*	Amaranthaceae	N-East / Green	Saponins, ecdysterone, oleanolic acid, betaine, alkaloids	Diuretic, anti-inflammatory, antimicrobial, expectorant, antipyretic
Brihaspati (Jupiter)	Peepal / Sacred fig	*Ficus religiosa*	Moraceae	North / Yellow	Phytosterols, lanosterol, tannins, β-sitosterol, n-octacosanol	Anti-asthmatic, antidiabetic, neuroprotective, dosha balancing
Shukra (Venus)	Gular / Cluster fig	*Ficus racemosa*	Moraceae	East / White	Lupeol, β-sitosterol, glucanase, leucocyanidin, flavonoids	Antibacterial, antifungal, wound healing, urinary tonic, antidiabetic
Shani (Saturn)	Shami / Ghaf	*Prosopis cineraria*	Fabaceae	West / Black	Prosogerin A–E, spicigerine, tannins, mucilage, amino acids	Digestive, detoxifying, anti-neurotic, wound healing
Rahu (North Node)	Doob / Bermuda grass	*Cynodon dactylon*	Poaceae	S-West / Brown	Triterpenoids, cynodin, flavonoids, chlorophyll derivatives	Wou nd healing, diuretic, anti-inflammatory, haemostatic, anti-convulsant
Ketu (South Node)	Dabh / Cogon grass	*Imperata cylindrica*	Poaceae	N-West / Brown / Grey	Flavone glycosides, campesterol, sitosterol, luteolin, cylindrin	Diuretic, cooling, antipyretic, Vata–Pitta balancer, haemostatic

Sources: Bhat (1981); Bloomfield (1997); Sharma (1981); Sircar and Sarkar (1996); Soni et al. (2023); Habeeb et al. (2024); Calotropis Procera Review (2025). The Chandigarh garden designates Imperata cylindrica for Ketu; the Atharvaveda and Vrikshayurveda place Desmostachya bipinnata (Kusha) in the same slot, with a closely matched pharmacological profile.



Fig. 1. Navagraha Vatika, Chandigarh. Composite plate showing the directional layout of the nine sacred plants and representative species photographed in situ — *Ficus religiosa* (Brihaspati / Jupiter, North), *Achyranthes aspera* (Budha / Mercury, NE), *Ficus racemosa* (Shukra / Venus, East) and *Acacia catechu* (Mangala / Mars, South). Source: field photographic documentation by the authors (2024); directional diagram adapted from official signage of the Department of Forests.



Fig. 2. Nakshatra Vatika, Chandigarh. Composite plate of representative tree species arranged in numerical order of the Nakshatras, from *Strychnos nux-vomica* (Ashwini) to *Madhuca longifolia* (Revati). Asterisks mark dual-role species also present in the Navagraha Vatika. Source: field photographic documentation by the authors (2024).

Imperata cylindrica (Dabh) to Ketu, while the Atharvaveda and Vrikshayurveda place Desmostachya bipinnata (Kusha) in that slot. Both grasses share a closely matched pharmacological profile (Bloomfield, 1997; Sircar and Sarkar, 1996; Soni et al., 2023).

Nakshatra Vatika and the 27-Tree Correlation

The Nakshatra Vatika at Chandigarh houses all twenty-seven trees of the lunar mansion system. Each species carries numbered signage giving its Nakshatra, vernacular name, botanical name and family. Each Nakshatra spans roughly 13°20′ of the ecliptic. Representative species are presented in Fig. 2; the full correlation, cross-checked with the Brihat Samhita (Ch. 54, Nakshatracharyadi), Charaka Samhita and current pharmacological literature, is given in Table 2.

Dual-role species are marked with an asterisk. Five of the twenty-seven Nakshatra species belong to Moraceae (18.5 percent): *Ficus religiosa, Ficus racemosa, Ficus rumphii, Ficus benghalensis* and *Artocarpus heterophyllus*. Their over-representation is consistent with the ecological role of figs as keystone mutualists for frugivores and fig wasps, and with the Vedic association of latex-bearing trees with prana flow.

Dual-Role Species: The Pharmacological Core

Cross-referencing Tables 1 and 2 reveals six species in both garden systems (Table 3, Fig. 3). These dual-role plants represent the pharmacological core of the Vedic botanical canon. Field documentation in Birbhum confirmed that all six are in active use by Santhali Ojhas and recorded Fidelity Levels of 85 to 100 percent, the highest among all documented species (Pradhan and Mondal, 2023).

Plants Associated with the Seven Chakras

The chakra system, recorded in the Ashtanga Hridayam (Nadkarni, 2002), corresponds to measurable bioelectric phenomena (Motoyama, 1981). The nervous system operates through a balance of excitatory (glutamatergic) and inhibitory (GABAergic) signalling, a polarity that mirrors the chakra doctrine of excitation–inhibition balance. Table 4 gives plant associations for each chakra together with key bioactive compounds and modern pharmacological evidence (Soni et al., 2023; Habeeb et al., 2024).

Balagrahas and a Proto-Microbiological Reading

The Balagraha, recorded in Ashtanga Hridayam (Uttarasthana 3) and Charaka Samhita (Sharirasthana 8), are childhood illnesses attributed to invisible pathogenic entities. Vagbhata's tripartite scheme of Himsa (disease-causing), Rati (pathogen completing its life cycle in the host) and Bali (commensal organisms) reads naturally as an early proto-microbiological framework, separating pathogens, endoparasites and commensals (Frawley, 2000). Ayurvedic prescriptions of

Table 2. Complete 27-Nakshatra plant correlation.

No.	Nakshatra	Common Name	Botanical Name	Family	Ruling Planet	Pharmacological Significance
1	Ashwini	Kuchla	*Strychnos nux-vomica*	Loganiaceae	Ketu	Nerve tonic (controlled dose), analgesic, anti-rheumatic
2	Bharani	Amla	*Emblica officinalis*	Phyllanthaceae	Venus	Immunomodulatory, anti-aging, antidiabetic, Rasayana tonic
3	Krittika *	Gular	*Ficus racemosa*	Moraceae	Sun	Antibacterial, antifungal, wound healing, urinary tonic
4	Rohini	Jamun	*Syzygium cumini*	Myrtaceae	Moon	Antidiabetic, astringent, anti-diarrhoeal, antioxidant
5	Mrigashirsha *	Khair	*Acacia catechu*	Fabaceae	Mars	Antimicrobial, anti-obesity, antioxidant, anti-tussive
6	Ardra	Agar	*Aquilaria agallocha*	Thymelaeaceae	Rahu	Sedative, anti-anxiety, anti-asthmatic, sacred incense
7	Punarvasu	Bamboo / Baans	*Dendrocalamus strictus*	Poaceae	Jupiter	Haemostatic, anti-inflammatory, wound healing, antioxidant
8	Pushya *	Peepal	*Ficus religiosa*	Moraceae	Saturn	Anti-asthmatic, antidiabetic, neuroprotective, dosha-balancing
9	Ashlesha	Nagkesar	*Mesua ferrea*	Clusiaceae	Mercury	Antibacterial, anti-inflammatory, wound healing, expectorant
10	Magha	Banyan / Vata	*Ficus benghalensis*	Moraceae	Ketu	Antidiabetic, anti-diarrhoeal, antioxidant, immune-modulating
11	Purva Phalguni *	Dhaak	*Butea monosperma*	Fabaceae	Venus	Antidiabetic, hepato-protective, anthelmintic, anti-inflammatory
12	Uttara Phalguni	Pilkhan	*Ficus rumphii*	Moraceae	Sun	Antioxidant, anti-inflammatory, hepato-protective, antipyretic
13	Hasta	Juhi / Jasmine	*Jasminum auriculatum*	Oleaceae	Moon	Anxiolytic, anti-depressant, sedative, skin-healing
14	Chitra	Bael	*Aegle marmelos*	Rutaceae	Mars	Anti-diarrhoeal, anti diabetic, cardio protective, antioxidant
15	Swati	Arjuna	*Terminalia arjuna*	Combretaceae	Rahu	Cardioprotective, anti-anginal, hypolipidaemic (validated for CHF)
16	Vishakha	Wood Apple	*Limonia acidissima*	Rutaceae	Jupiter	Antibacterial, antifungal, anti-diarrhoeal, liver tonic
17	Anuradha	Maulsari	*Mimusops elengi*	Sapotaceae	Saturn	Dental health, antipyretic, anti-inflammatory, astringent
18	Jyestha	Semal	*Bombax ceiba*	Bombacaceae	Mercury	Aphrodisiac, tonic, anti-diarrhoeal, wound healing, galactagogue
19	Moola	Saphed Dammar	*Vateria indica*	Dipterocarpaceae	Ketu	Antioxidant, anti-inflammatory, fever reduction, Ayurvedic resin
20	Purva Ashadha	Sita Ashoka	*Saraca asoca*	Fabaceae	Venus	Uterine tonic, anti-menorrhagic, anti-inflammatory, antioxidant
21	Uttara Ashadha	Kathal	*Artocarpus heterophyllus*	Moraceae	Sun	Antidiabetic, immunomodulatory, antioxidant, anti-platelet
22	Shravana *	Aak	*Calotropis procera*	Apocynaceae	Moon	Anticancer (PI3K/AKT), anti-inflammatory, wound healing
23	Dhanishtha *	Shami	*Prosopis cineraria*	Fabaceae	Mars	Digestive, detoxifying, wound healing, gastro-intestinal tonic
24	Shatabhisha	Kadam	*Neolamarckia cadamba*	Rubiaceae	Rahu	Antibacterial, antipyretic, anti-malarial, hepatoprotective
25	Purva Bhadrapada	Neem	*Azadirachta indica*	Meliaceae	Jupiter	Broad-spectrum anti-microbial, immunomo dulatory, anti -parasitic
26	Uttara Bhadrapada	Aam / Mango	*Mangifera indica*	Anacardiaceae	Saturn	Antidiabetic, antioxidant, anti-inflamma tory, immunostimulant
27	Revati	Mahua	*Madhuca longifolia*	Sapotaceae	Mercury	Liver tonic, skin health, joint pain relief, Vata–Kapha balancer

* = Dual-role species present in both the Navagraha and Nakshatra Vatikas. Sources: Field documentation by the authors (Chandigarh, 2024); Bhat (1981); Bloomfield (1997); Sharma (1981); Sircar and Sarkar (1996); Soni et al. (2023).

Table 3. Dual-role plants shared between the Navagraha and Nakshatra Vatika systems.

Botanical Name	Common Name	Navagraha Role	Nakshatra Role	Significance of Dual Assignment
Calotropis procera	Aak	Surya (Sun)	Shravana (22)	Sun–Vishnu axis; xerophytic solar biology with Vishnu's preserving function. Anticancer (PI3K/AKT) and antimicrobial activity now confirmed (Joshi et al., 2025; Habeeb et al., 2024).
Ficus religiosa	Peepal	Brihaspati (Jupiter)	Pushya (8)	Jupiterian wisdom (Navagraha) with Saturnine nourishment (Pushya). Pharmacological breadth across neuroprotection, anti-asthma and antidiabetes.
Butea monosperma	Palash / Dhaak	Chandra (Moon)	Purva Phalguni (11)	Lunar fertility and fluidity (Navagraha) with Venusian creativity (Nakshatra); anti-fertility and hepatoprotective profile aligns with lunar physiology.
Acacia catechu	Khair	Mangala (Mars)	Mrigashirsha (5)	Consistent Mars attribution across both systems; blood-purifying astringent catechin chemistry mirrors the Martian archetype of heat and blood.
Ficus racemosa	Gular	Shukra (Venus)	Krittika (3)	Venus–urinary health alignment (Navagraha) with Agni-fire purification (Krittika deity); pharmacology bridges both symbolic frames.
Prosopis cineraria	Shami	Shani (Saturn)	Dhanishtha (23)	Saturn in both systems; arid ecology, slow growth and alkaloid chemistry directed at chronic, deep-acting conditions.

Sources: Bhat (1981); Sharma (1981); Sircar and Sarkar (1996); Soni et al. (2023); Habeeb et al. (2024); Joshi et al. (2025); Pradhan and Mondal (2023).

Table 4. Plants associated with the seven chakras and their bioactive mechanisms.

Chakra	Associated Plants	Key Bioactive Compounds	Pharmacological Evidence
Root (Muladhara)	Dandelion root, Hibiscus, Red clover, Cedarwood	Taraxacin, hibiscin, cedrol	Adaptogenic, cortisol-reducing, grounding effect on the HPA axis
Sacral (Svadhishthana)	Marigold, Calendula, Cinnamon, Damiana	Isorhamnetin, cinnamaldehyde, damianin	Anti-inflammatory, oestrogen regulation, mild aphrodisiac
Solar Plexus (Manipura)	Sunflower, Ginger, Turmeric, Fennel, Chamomile	Gingerols, curcumin, anethole, apigenin	Curcumin inhibits NF-κB; ginger modulates serotonin; digestive stimulants
Heart (Anahata)	Rose, Hawthorn berry, Jasmine, Lavender	Linalool, vitexin, rosmarinic acid	Anxiolytic (linalool), cardioprotective (hawthorn OPCs)
Throat (Vishuddha)	Peppermint, Eucalyptus, Bluebells, Clover	Menthol, cineole, formononetin	Bronchodilatory, antimicrobial, expectorant
Third Eye (Ajna)	Lavender, Blue lotus, Rosemary, Mugwort	Linalool, nuciferine, rosmarinic acid, thujone	Anxiolytic, cognitive enhancer, mild sedative
Crown (Sahasrara)	Lotus, White lilies, Lavender, Frankincense	Nuciferine, nelumboside, boswellic acids	Neuroprotective, anti-neuroinflammatory, antioxidant

Sources: Motoyama (1981); Soni et al. (2023).

Shuchi (asepsis) and Shuddhi (antisepsis) anticipated nineteenth-century germ theory by several millennia. Modern clinical equivalents are summarised in Table 5.

Calotropis procera, Surya and the Sun–Vitamin D Convergence

Calotropis procera (Apocynaceae; Aak/Madar) holds the central Surya position in the Navagraha Vatika and is also the Shravana Nakshatra tree associated with Vishnu's preserving function. Its biological alignment with the Sun is multi-layered. It is xerophytic and thrives under intense solar radiation; it exudes milky latex on injury, in a manner often compared with solar radiation; and it features prominently in Surya offerings and healing rituals (Rigveda 1.50; 3.62.10). The Atharvaveda contains hymns invoking *Calotropis* for wound healing and reduction of fever (Bloomfield, 1997).

All parts of *C. procera* carry cardenolide glycosides (calotropin, calactin, uscharin), triterpenoids (α- and β-amyrin), flavonoids, tannins and alkaloids. The aqueous leaf extract has documented antimicrobial, antidiabetic, anti-hyperbilirubinaemic and wound-healing activity (Habeeb et al., 2024). A 2025 review in the South African Journal of Botany confirms its phytochemical breadth and ethnomedicinal range (*Calotropis procera* Review, 2025). Calotropin, calotropagenin and giganteones A and B trigger apoptosis and cell-cycle arrest through ROS production and modulation of the PI3K/AKT/p53 pathway (Joshi et al., 2025). A plant venerated since Vedic times now stands at the frontier of natural-product oncology.

Table 5. Balagraha entities and their modern clinical correlates.

Balagraha	Modern Clinical Equivalent	Pathobiological Parallel
Skandagraha	Polio, facial palsy, hemiplegia	Motor neuron / peripheral nerve infection
Skandapasmara	Convulsions, epilepsy	Neuro-electric dysregulation
Meshagraha	Meningitis	CNS bacterial or viral invasion
Shwagraha	Rabies (hydrophobia)	Neurotropic zoonotic viral infection
Pitrigraha	Diarrhoea with dehydration	Enteric pathogen / electrolyte imbalance
Shakuni	Impetigo, stomatitis	Staphylococcal / Streptococcal skin infection
Putana	Diarrhoea with hypokalaemia	Secretory enterotoxin-producing bacteria
Shitaputana	Diarrhoea with hypocalcaemia / hypomagnesaemia	Malabsorption and mineral deficiency
Andhaputana	Diarrhoea with vitamin A deficiency	Nutritional immunodeficiency
Revati	Diarrhoea with anaemia	Iron deficiency with enteric blood loss
Mukhamandika	Childhood cirrhosis, nephrotic syndrome	Metabolic liver / renal disorders
Shushkarevati	Marasmus, abdominal tuberculosis	Protein-energy malnutrition with mycobacterial infection

Sources: Sharma (1981); Nadkarni (2002); Frawley (2000).

From a photobiological standpoint, the Surya–Calotropis link recalls the most basic health mechanism attributable to solar radiation: the cutaneous synthesis of vitamin D_3. UVB radiation converts 7-dehydrocholesterol to pre-vitamin D_3, which is then isomerised to vitamin D_3 and metabolised in the liver and kidney to its active form, calcitriol. Calcitriol regulates over two hundred genes governing calcium and phosphorus metabolism, immune modulation and neuromuscular co-ordination. The Rigvedic reverence for Surya as a source of vitality and disease prevention thus encodes, in metaphysical language, the practical observation that solar exposure is fundamental to human health (Frawley, 2000).

Synthesis : Astro-Ethnobotany as a Working Sub-Discipline

Earlier studies treated Navagraha plants in isolation (Soni et al., 2023) or focused on a single garden system. The framework proposed here links astrological symbolism, pharmacological evidence and field documentation in a single matrix. Photographic evidence from a government-maintained garden gives institutional weight to plant–planet associations that until now relied largely on oral tradition and classical text. The accumulating pharmacological evidence in Tables 1 to 3 indicates that the plant–planet associations of Vedic literature were not arbitrary but reflected long-term empirical observation encoded in cosmological metaphor.

The variation in Ketu's plant assignment between the Chandigarh garden (Imperata cylindrica) and the Atharvaveda / Vrikshayurveda (*Desmostachya bipinnata*) is instructive. Both species are sacred Poaceae grasses with documented diuretic, cooling and antipyretic activity. Vedic plant associations therefore appear to operate through pharmacological archetypes —



Fig. 3. Dual-role species serving both Navagraha and Nakshatra functions. Composite plate of the six dual-role species: *Calotropis procera, Ficus religiosa, Butea monosperma, Acacia catechu, Ficus racemosa* and *Prosopis cineraria.* Source: authors' field photographs (2024); nomenclature verified against APG IV (2016).

cooling diuretic grasses, in this case — rather than through rigid species fixation, which permits regional botanical availability while keeping functional consistency intact. This is the ethnobotanical principle of functional substitution (Jain and Kapoor, 2007).

Navagraha and Nakshatra Vatikas function as culturally protected ex-situ conservation systems for pharmacologically valuable trees and predate formal botanic gardens by several millennia. *Aquilaria agallocha* (Ardra) is listed in CITES Appendix II for overharvesting of agarwood; *Mesua ferrea* (Ashlesha) is under increasing anthropogenic pressure; *Vateria indica* (Moola) is IUCN Vulnerable; and Butea monosperma (Purva Phalguni and Chandra) faces habitat loss across its range. The religious proscription against harming a Nakshatra Vriksha — the birth tree of community members born under that lunar mansion — has acted as a community-based conservation mechanism rooted in personal identity rather than in external regulation. Recognition of these gardens under Article 8(j) of the Convention on Biological Diversity and the UNESCO Intangible Cultural Heritage provisions would institutionalise their protection.

International natural-product chemistry groups are actively searching for novel molecules from medicinal plants. The development of sacred plant gardens such as the Navagraha and Nakshatra Vatikas is therefore a useful first step in identifying the traditional plant resources most likely to be of value against human ailments. A Nakshatra Garden, in our view, should be established on the campus of every educational institution where Botany is taught. Such living collections would serve as teaching resources, ex-situ conservation units and reference points for student-led research in Indian traditional plant knowledge.

Conclusion

This paper proposes Astro-ethnobotany as a working sub-discipline and offers the first photographically validated account of the complete Navagraha Vatika (nine species) and Nakshatra Vatika (twenty-seven species) maintained by the Department of Forests and Wildlife, Chandigarh. Read together with classical Sanskrit texts and current pharmacological literature, the gardens function simultaneously as pharmacological archives, conservation systems, repositories of Traditional Ecological Knowledge and monuments of India's astronomical heritage.

Six dual-role species — *Calotropis procera, Ficus religiosa, Butea monosperma, Acacia catechu, Ficus racemosa* and *Prosopis cineraria* — emerge as the pharmacological core of the Vedic botanical canon; their dual assignments reflect a medicinal breadth that crossed individual classification systems. The over-representation of Moraceae in sacred gardens (18.5 percent of Nakshatra species) records a quietly empirical conservation heuristic. The *Calotropis procera*–Surya convergence, which links Vedic solar symbolism, xerophytic ecology, vitamin D_3 photobiology and recent PI3K/AKT/p53 anticancer findings, illustrates how Vedic botanical knowledge functioned as distributed empirical wisdom carried across multiple mnemonic frameworks.

The Santhali Ojhas of Birbhum supply living evidence that the system remains in working use, with RFC values of 0.89 and FL values of 100 percent for key Navagraha plants. Sacred plant

garden traditions therefore deserve formal recognition as living heritage conservation systems under Article 8(j) of the CBD and the UNESCO Intangible Cultural Heritage provisions. A Nakshatra Garden should be established on every campus that teaches Botany. Future research priorities include HPLC-MS and NMR-based phytochemical profiling of the twenty-seven Nakshatra plants; clinical trials of Graha-formulated herbal combinations; GIS-based national mapping of Vatikas; genomic conservation studies for IUCN-listed Nakshatra species; and comparative archaeo-astronomical plant classification across Mesopotamian, Greco-Roman and Mayan traditions.

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Morphometric attributes of plant trichome diversity of Bethune College and immediate surroundings

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Abstract:

Trichomes represent important epidermal structures that exhibit wide morphological diversity and functional specialization across angiosperms. The present investigation analyzed trichome diversity in fifty plant species collected from the Bethune College campus and its surrounding areas to evaluate their morphometric variation and taxonomic relevance. Leaf and petiole samples were processed using epidermal peeling and surface scraping techniques. Prepared slides were examined under a compound light microscope. Trichome characteristics including type, cellular organization, length, branching pattern, and surface distribution pattern were recorded through photomicrography and micrometric measurements. The study revealed considerable interspecific and interfamily variation in trichome morphology. Non-glandular trichomes were predominant across most of the taxa, while glandular trichomes were mainly restricted to selected members of Lamiaceae and Solanaceae. Morphometric analysis showed significant differences in trichome length among species and plant organs. The abaxial leaf surfaces generally exhibit longer trichomes than adaxial surfaces. The results show strong family-level stability of trichome traits along with sufficient species-level variation which highlights the importance in their diagnostic value. Overall, this study confirms that, trichome morphology and quantitative attributes provide reliable micromorphological characters that can support plant identification and taxonomic classification.

Keywords :

Trichome morphology; Plant taxonomy; Micromorphology; Epidermal structures; Angiosperms; Light microscopy; Leaf surface; Species identification.

Introduction

Trichomes are epidermal outgrowths that display considerable structural diversity across plant taxa. They may occur as unicellular or multicellular structures and are broadly classified into glandular and non-glandular types. These structures perform multiple functions, including mechanical protection, reduction of transpiration, secretion of secondary metabolites, and defense against herbivores. Due to their structural stability and taxon-specific distribution patterns, trichomes have been widely used as diagnostic characters in plant taxonomy and systematics (Werker, 2000; Santos-Silva et al., 2013).

Previous studies have demonstrated that trichome micromorphology often exhibits consistent patterns at the genus and family levels, making them valuable tools for resolving systematic relationships (Navarro & El Oualidi, 1999; Zhao et al., 2021). Investigations on genera such as *Mimosa sp.* and *Dyckia sp.* revealed strong correlations between trichome traits and phylogenetic groupings (Dorneles et al., 2014). Indian studies have further emphasized the taxonomic significance of trichomes in families including Malvaceae, Fabaceae, and Convolvulaceae (Leelavathi & Ramayya, 1983; Gopi & Krishnakumar, 2020).

Although some studies have focused on specific plant families, comprehensive multi-family surveys within urban ecosystems remain limited. The present study is therefore designed to document trichome diversity in angiosperm species growing within the campus of Bethune College and its immediate surroundings. The objective of the study was to analyse morphometric variation and assess the taxonomic relevance of trichome characteristics in a heterogeneous plant community.

Materials and Methods

Study area and sample collection

The study was conducted within the Bethune College campus, Kolkata, West Bengal, India (approximately 22.5882°N, 88.3679°E). The region experiences a subtropical climate with moderate to high rainfall and seasonal temperature variation. The campus includes garden areas, roadside vegetation, and semi-natural patches that support diverse angiosperm flora.

A total of fifty plant species representing herbaceous and woody forms were selected to ensure broad taxonomic representation. Healthy and fully expanded leaves were collected during early morning hours to minimize dehydration. Species identification was carried out using standard floristic literature and confirmed through departmental herbarium when necessary.

Slide preparation and microscopy

Collected leaf samples were washed with distilled water to remove surface debris. Small segments of lamina (1–2 cm^2) were excised by avoiding major veins. Epidermal peels were obtained using fine forceps or sterile blades. When direct peeling was not possible then surface scraping techniques were employed to isolate trichomes from leaf and petiole regions.

Prepared samples were mounted in distilled water on clean glass slides and covered with coverslips. Observations were performed using a compound light microscope under 10x and 40x objective. Care was taken to avoid air bubble formation and distortion of epidermal structures.

Observation, measurement and documentation

Trichomes were categorized based on morphological features such as cellular organization, branching pattern, and glandular nature. Measurements of trichome length and glandular head diameter were obtained using a calibrated ocular micrometer. For each species, measurements were recorded from at least three randomly selected trichomes. Photomicrographs were captured and organized systematically for comparative analysis.

Results

Trichome distribution and morphometric variation

The present study investigated trichome morphology and morphometric variation in fifty angiosperm species collected from the Bethune College campus and its immediate surroundings (22.5882°N, 88.3679°E).

Distribution of trichome types

The angiosperm species investigated considerable diversity in trichome morphology. Non-glandular trichomes were dominant across most families, particularly in Malvaceae and Asteraceae, where multicellular stellate, acicular, dendritic, and bifurcate trichomes were frequently observed. Glandular trichomes, including capitate and peltate types, were comparatively less abundant and were mainly restricted to members of Lamiaceae and Solanaceae (Table 1).

Family-level consistency was evident in several taxa. Species of Malvaceae commonly displayed stellate and branched trichomes, while Lamiaceae were characterized by the presence of glandular peltate structures. These patterns indicate taxonomic stability of trichome traits within higher plant groups.

Morphometric variation of trichomes

Morphometric analysis revealed significant variation in trichome length among species as well as plant organs. In most taxa, trichomes present on the abaxial leaf surface were longer than those on the adaxial surface. Trichomes of petiole generally exhibited intermediate to higher length values.

Species such as *Blumea lacera* (Burm.f.) DC. and *Terminalia catappa* L. recorded maximum trichome lengths exceeding 900 µm, whereas shorter trichomes were observed in *Thespesia populnea* (L.) Sol. ex Corrêa and *Solanum lycopersicum* L., with measurements ranging between 50 and 70 µm. These variations reflect species-specific developmental patterns and positional differences in trichome distribution (Table 2).

Taxonomic and ecological significance

Distinct trichome morphologies were associated with specific taxa. Stellate trichomes were prominent in *Hibiscus rosa-sinensis* and *Grewia asiatica,* dendritic trichomes were characteristic of *Withania somnifera* (L.) Dunal, T-shaped trichomes were observed in *Vernonia cinerea,* and hooked trichomes were recorded in *Commelina diffusa* Burm.f.. Glandular trichomes in species such as *Ocimum gratissimum* L. and *Justicia adhatoda* L. were associated with secretory functions and secondary metabolite production.

The observed combination of morphological stability at family levels and quantitative variation at species levels highlights the usefulness of trichomes as reliable micromorphological tools for taxonomic studies.

Conclusion

The present investigation documented significant morphometric and structural diversity of trichomes in fifty angiosperm species from the Bethune College campus and surrounding areas. Application of epidermal peeling, surface scraping, and light microscopic analysis enabled systematic characterization of both glandular and non-glandular trichomes across different plant organs.

Distinct family-level patterns were observed, with Malvaceae predominantly exhibiting stellate trichomes and glandular peltate structure in Lamiaceae. Specialized trichomes such as dendritic, hooked, and T-shaped forms further demonstrated taxon-specific consistency, supporting the evolutionary stability of trichome traits.

Morphometric measurements revealed substantial variation in trichome length among species and plant parts, indicating adaptive and developmental influences. The presence of multiple trichome types within certain species suggests functional specialization and ecological significance.

Overall, the findings confirm that trichome morphology combined with quantitative analysis provides an effective tool for plant identification and taxonomic classification. Future integration of micromorphological data with molecular approaches may further enhance systematic resolution and understanding of evolutionary relationships.

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Appendices

Trichome Observed

PLATE 1- *Commelina diffusa Burm.f.*



Fig. 1. Multicellular trichome of *Commelina diffusa*



Fig. 2. Hooked trichome of *Commelina diffusa*



Fig. 3. Hooked trichome of *Commelina diffusa*



Fig. 4. Multicellular trichome of *Commelina diffusa*

PLATE 2- *Cannabis sativa L.*



Fig. 1. Unicellular acicular trichome of *Cannabis sativa*



Fig. 2. Unicellular acicular trichome of *Cannabis sativa*



Fig. 3. Unicellular acicular trichome of *Cannabis sativa*

PLATE 3- *Trema orientalis (L.) Blume*



Fig. 1. Unicellular acicular trichome of *Trema orientalis*



Fig. 2. Unicellular acicular trichome of *Trema orientalis*



Fig. 3. Unicellular acicular trichome of *Trema orientalis*

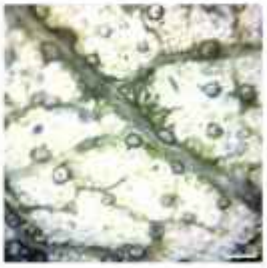


Fig. 4. Capitate glandular trichome of *Trema orientalis*

PLATE 4- *Artocarpus lacucha Buch. -Ham.*



Fig. 1. Unicellular acicular trichome of *Artocarpus lacucha*



Fig. 2. Unicellular acicular trichome of *Artocarpus lacucha*



Fig. 3. Unicellular acicular trichome of *Artocarpus lacucha*

PLATE 5- *Ficus hispida L. f.*



Fig. 1. Unicellular acicular trichome of *Ficus hispida*



Fig. 2. Unicellular acicular trichome of *Ficus hispida*



Fig. 3. Unicellular acicular trichome of *Ficus hispida*

PLATE 6- *Cucurbita maxima Duchesne*



Fig. 1. Multicellular acicular trichome of *Cucurbita maxima*



Fig. 2. Multicellular acicular trichome of *Cucurbita maxima*



Fig. 3. Multicellular acicular trichome of *Cucurbita maxima*

PLATE 7- *Lagenaria siceraria (Molina) Standl.*



Fig. 1. Multicellular acicular trichome of *Lagenariasiceraria*



Fig. 2. Multicellular pilate trichome of *Lagenaria siceraria*



Fig. 3. Multicellular pilate trichome of *Lagenaria siceraria*



Fig. 4. Multicellular acicular trichome of *Lagenaria siceraria*



Fig. 5. Multicellular pilate trichome of *Lagenaria siceraria*

PLATE 8- *Trichosanthes dioica Roxb. [Fig. 1, 2, 3= Multicellular, Acicular Trichome]*



Fig. 1. Multicellular acicular trichome of *Trichosanthes dioica*



Fig. 2. Multicellular acicular trichome of *Trichosanthes dioica*



Fig. 3. Multicellular acicular trichome of *Trichosanthes dioica*

PLATE 9- *Turnera ulmifolia L.*



Fig. 1. Unicellular filiform trichome of *Turnera ulmifolia*



Fig. 2. Unicellular filiform trichome of *Turnera ulmifolia*



Fig. 3. Unicellular filiform trichome of *Turnera ulmifolia*

PLATE 10- *Acalypha hispida Burm. f.*



Fig. 1. Multicellular non-glandular trichome of *Acalypha hispida*



Fig. 2. Multicellular non-glandular trichome of *Acalypha hispida*



Fig. 3. Multicellular non-glandular trichome of *Acalypha hispida*



Fig. 4. Capitate glandular trichome of *Acalypha hispida*

PLATE 11- *Tragia involucrata L.*



Fig. 1. Stinging trichome of *Tragia involucrata*



Fig. 2. Glandular trichome of *Tragia involucrata*



Fig. 3. Unicellular acicular trichome of *Tragia involucrata*



Fig. 4. Unicellular acicular trichome of *Tragia involucrata*



Fig. 5. Stinging trichome of *Tragia involucrata*

PLATE 12- *Terminalia catappa L.*



Fig. 1. Unicellular filiform trichome of *Terminalia catappa*



Fig. 2. Unicellular filiform trichome of *Terminalia catappa*



Fig. 3. Unicellular filiform trichome of *Terminalia catappa*

PLATE 13- *Combretum indicum (L.) DeFilipps*



Fig. 1. Unicellular acicular trichome of *Combretum indicum*



Fig. 2. Capitate glandular trichome of *Combretum indicum*



Fig. 3. Unicellular acicular trichome of *Combretum indicum*



Fig. 4. Unicellular acicular trichome of *Combretum indicum*

PLATE 14- *Hibiscus rosa-sinensis L*



Fig. 1. Unicellular acicular trichome of *Hibiscus rosa sinensis*



Fig. 2. Multicellular stellate trichome of *Hibiscus rosa sinensis*



Fig. 3. Unicellular acicular trichome of *Hibiscus rosa sinensis*



Fig. 4. Bifurcate trichome of *Hibiscus rosa sinensis*



Fig. 5. Multicellular stellate trichome of *Hibiscus rosa sinensis*

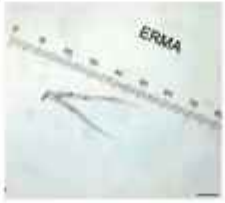


Fig. 6. Bifurcate trichome of *Hibiscus rosa sinensis*



Fig. 7. Unicellular acicular trichome of *Hibiscus rosa sinensis*

PLATE 15- *Hibiscus mutabilis L.*



Fig. 1. Capitate glandular trichome of *Hibiscus mutabilis*



Fig. 2. Stellate trichome of *Hibiscus mutabilis*



Fig. 3. Stellate trichome of *Hibiscus mutabilis*



Fig. 4. Trifurcate trichome of *Hibiscus mutabilis*



Fig. 5. Stellate trichome of *Hibiscus mutabilis*

PLATE 16- *Hibiscus vitifolius L.*



Fig. 1. Multicellular stellate trichome of *Hibiscus vitifolius.*



Fig. 2. Multicellular pilate trichome of *Hibiscus vitifolius.*



Fig. 3. Multicellular stellate trichome of *Hibiscus vitifolius.*

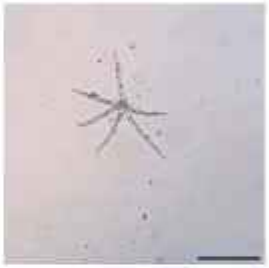


Fig. 4. Multicellular stellate trichome of *Hibiscus vitifolius.*

PLATE 17- *Alcea rosea L.*



Fig. 1. Capitate glandular trichome of *Alcea rosea*



Fig. 2. Capitate glandular trichome of *Alcea rosea*



Fig. 3. Unicellular acicular trichome of *Alcea rosea*



Fig. 4. Unicellular acicular trichome of *Alcea rosea*



Fig. 5. Stellate trichome of *Alcea rosea*

PLATE 18- *Thespesia populnea (L.) Sol. ex Corrêa*

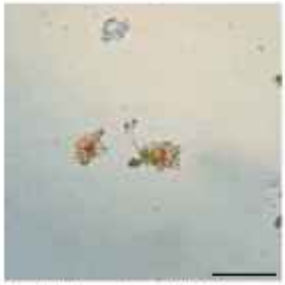


Fig. 1. Star-shaped trichome of *Thespesia populnea*



Fig. 2. Star-shaped trichome of *Thespesia populnea*



Fig. 3. Star-shaped trichome of *Thespesia populnea*

PLATE 19- *Grewia asiatica L.*



Fig. 1. Multicellular pilate trichome of *Grewia asiatica*



Fig. 2. Stellate trichome of *Grewia asiatica*



Fig. 3. Stellate trichome of *Grewia asiatica*



Fig. 4. Stellate trichome of *Grewia asiatica*



Fig. 5. Stellate trichome of *Grewia asiatica*



Fig. 6. Stellate trichome of *Grewia asiatica*

PLATE 20- *Pterospermum acerifolium Willd.*



Fig. 1. Multicellular stellate trichome of *Pterospermum acerifolium*



Fig. 2. Multicellular stellate trichome of *Pterospermum acerifolium*



Fig. 3. Multicellular stellate trichome of *Pterospermum acerifolium*



Fig. 4. Multicellular stellate trichome of *Pterospermum acerifolium*

PLATE 21- *Brassica nigra (L.) W.D.J. Koch*



Fig. 1. Unicellular acicular trichome of *Brassica nigra*

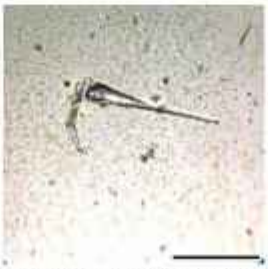


Fig. 2. Unicellular acicular trichome of *Brassica nigra*

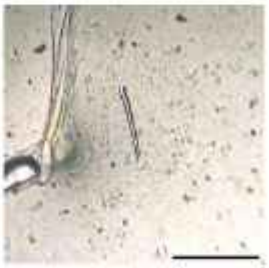


Fig. 3. Unicellular acicular trichome of *Brassica nigra*

PLATE 22- *Heliotropium indicum L.*



Fig. 1. Unicellular acicular trichome of *Heliotropium indicum*


Fig. 2. Capitate glandular trichome of *Heliotropium indicum*


Fig. 3. Unicellular acicular trichome of *Heliotropium indicum*



Fig. 4. Unicellular acicular trichome of *Heliotropium indicum*

PLATE 23- *Ipomoea carnea Jacq.*


Fig. 1. Unicellular acicular trichome of *Ipomoea carnea*


Fig. 2. Peltate glandular trichome of *Ipomoea carnea*


Fig. 3. Peltate glandular trichome of *Ipomoea carnea*


Fig. 4. Unicellular acicular trichome of *Ipomoea carnea*

PLATE 24- *Ipomoea corymbosa (L.) Roth*



Fig. 1. Unicellular acicular trichome of *Ipomoea corymbosa*


Fig. 2. Unicellular acicular trichome of *Ipomoea corymbosa*



Fig. 3. Unicellular acicular trichome of *Ipomoea corymbosa*

PLATE 25- *Solanum nigrum L.*



Fig. 1. Multicellular acicular trichome of *Solanum nigrum*



Fig. 2. Multicellular acicular trichome of *Solanum nigrum*


Fig. 3. Multicellular acicular trichome of *Solanum nigrum*

PLATE 26- *Solanum melongena L.*



Fig. 1. Multicellular stellate trichome of *Solanum melongena*



Fig. 2. Multicellular stellate trichome of *Solanum melongena*

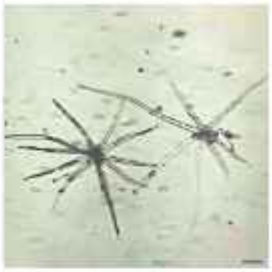


Fig. 3. Multicellular stellate trichome of *Solanum melongena*

PLATE 27- *Solanum lycopersicum L.*



Fig. 1. Multicellular acicular trichome of *Solanum lycopersicum*



Fig. 2. Multicellular acicular trichome of *Solanum lycopersicum*



Fig. 3. Multicellular acicular trichome of *Solanum lycopersicum*

PLATE 28- *Nicotiana plumbaginifolia Viv.*



Fig. 1. Multicellular pilate glandular trichome of *Nicotianaplumbaginifolia*



Fig. 2. Multicellular pilate glandular trichome of *Nicotiana plumbaginifolia*



Fig. 3. Multicellular pilate glandular trichome of *Nicotiana plumbaginifolia*

PLATE 29- *Withania somnifera (L.) Dunal*



Fig. 1. Multicellular dendritic trichome of *Withania somnifera*



Fig. 2. Multicellular dendritic trichome of *Withania somnifera*



Fig. 3. Multicellular dendritic trichome of *Withania somnifera*

PLATE 30- *Nyctanthes arbor-tristis L.*



Fig. 1. Unicellular acicular trichome of *Nyctanthes arbortristis.*



Fig. 2. Unicellular acicular trichome of *Nyctanthes arbortristis.*



Fig. 3. Unicellular acicular trichome of *Nyctanthes arbortristis.*



Fig. 4. Unicellular acicular trichome of *Nyctanthes arbortristis.*



Fig. 5. Unicellular acicular trichome of *Nyctanthes arbortristis.*



Fig. 6. Capitate glandular trichome of *Nyctanthes arbortristis.*

PLATE 31- *Tabernaemontana divaricata (L.) R.Br. ex Roem. & Schult.*



Fig. 1. Multicellular peltate glandular trichome of *Tabernaemontana divaricata*



Fig. 2. Multicellular peltate glandular trichome of *Tabernaemontana divaricata*

PLATE 32- *Scoparia dulcis L.*



Fig. 1. Multicellular peltate glandular trichome of *Scoparia dulcis*



Fig. 2. Unicellular acicular trichome of *Scoparia dulcis*



Fig. 3. Multicellular peltate glandular trichome of *Scoparia dulcis*



Fig. 4. Multicellular peltate glandular trichome of *Scoparia dulcis*

PLATE 33- *Lindenbergia indica (L.) Vatke*



Fig. 1. Multicellular glandular pilate trichome of *Lindenbergia indica*



Fig. 2. Multicellular glandular pilate trichome of *Lindenbergia indica*



Fig. 3. Multicellular glandular pilate trichome of *Lindenbergia indica*



Fig. 4. Multicellular glandular pilate trichome of *Lindenbergia indica.*

PLATE 34- *Justicia adhatoda L.*



Fig. 1. Multicellular acicular non-glandular trichome of *Justicia adhatoda*



Fig. 2. Multicellular acicular non-glandular trichome of *Justicia adhatoda*



Fig. 3. Multicellular peltate glandular trichome of *Justicia adhatoda*



Fig. 4. Multicellular acicular non-glandular trichome of *Justicia adhatoda*



Fig. 5. Multicellular peltate glandular trichome of *Justicia adhatoda*

PLATE 35- *Hygrophila auriculata (Schumach.) Heine*



Fig. 1. Unicellular acicular trichome of *Hygrophila auriculata*



Fig. 2. Unicellular acicular trichome of *Hygrophila auriculata*



Fig. 3. Unicellular acicular trichome of *Hygrophila auriculata*

PLATE 36- *Lantana camara L.*



Fig. 1. Unicellular acicular non-glandular trichome of *Lantana camara*



Fig. 2. Multicellular pilate glandular trichome of *Lantana camara*



Fig. 3. Unicellular acicular non-glandular trichome of *Lantana camara*



Fig. 4. Unicellular acicular non-glandular trichome of *Lantana camara*



Fig. 5. Basal portion of non-glandular trichome of *Lantana camara*

PLATE 37- *Lippia geminata Kunth*



Fig. 1. Multicellular non-glandular trichome of *Lippia geminata*



Fig. 2. Unicellular acicular trichome of *Lippia geminata*



Fig. 3. Unicellular acicular trichome of *Lippia geminata*



Fig. 4. Unicellular acicular trichome of *Lippia geminata*

PLATE 38- *Ocimum sanctum L.*



Fig. 1. Multicellular acicular non-glandular trichome of *Ocimum sanctum*



Fig. 2. Multicellular acicular non-glandular trichome of *Ocimum sanctum*



Fig. 3. Multicellular acicular non-glandular trichome of *Ocimum sanctum*



Fig. 4. Multicellular acicular non-glandular trichome of *Ocimum sanctum*



Fig. 5. Multicellular acicular non-glandular trichome of *Ocimum sanctum*

PLATE 39- *Ocimum gratissimum L.*



Fig. 1. Multicellular acicular non-glandular trichome of *Ocimum gratissimum*



Fig. 2. Multicellular acicular non-glandular trichome of *Ocimum gratissimum*



Fig. 3. Multicellular acicular non-glandular trichome of *Ocimum gratissimum*



Fig. 4. Multicellular acicular non-glandular trichome of *Ocimum gratissimum*

PLATE 40- *Ocimum tenuiflorum L.*



Fig. 1. Multicellular acicular non-glandular trichome of *Ocimum tenuiflorum*



Fig. 2. Multicellular acicular non-glandular trichome of *Ocimum tenuiflorum*



Fig. 3. Multicellular acicular non-glandular trichome of *Ocimum tenuiflorum*



Fig. 4. Multicellular peltate glandular trichome of *Ocimum tenuiflorum*



Fig. 5. Multicellular peltate glandular trichome of *Ocimum tenuiflorum*



Fig. 6. Multicellular acicular non-glandular trichome of *Ocimum tenuiflorum*

PLATE 41- *Ocimum kilimandscharicum Gürke*



Fig. 1. Multicellular peltate glandular trichome of *Ocimum kilimandscharicum*



Fig. 2. Unicellular acicular non-glandular trichome of *Ocimum kilimandscharicum*



Fig. 3. Unicellular acicular non-glandular trichome of *Ocimum kilimandscharicum*

PLATE 42- *Clerodendrum infortunatum L.*



Fig. 1. Multicellular acicular non-glandular trichome of *Clerodendrum infortunatum*



Fig. 2. Multicellular acicular non-glandular trichome of *Clerodendrum infortunatum*



Fig. 3. Multicellular acicular non-glandular trichome of *Clerodendrum infortunatum*



Fig. 4. Peltate glandular trichome of *Clerodendrum infortunatum*

PLATE 43- *Clerodendrum chinense (Osbeck) Mabb.*



Fig. 1. Multicellular acicular non-glandular trichome of *Clerodendrum chinense*



Fig. 2. Multicellular acicular non-glandular trichome of *Clerodendrum chinense*



Fig. 3. Peltate glandular trichome of *Clerodendrum chinense*



Fig. 4. Multicellular acicular non-glandular trichome of *Clerodendrum chinense*

PLATE 44- *Vernonia cinerea (L.) Less.*



Fig. 1. T-shaped non-glandular trichome of *Vernonia cinerea*



Fig. 2. Peltate glandular trichome of *Vernonia cinerea*



Fig. 3. Peltate glandular trichome of *Vernonia cinerea*



Fig. 4. T-shaped non-glandular trichome of *Vernonia cinerea*



Fig. 5. T-shaped non-glandular trichome of *Vernonia cinerea*

PLATE 45- *Wedelia chinensis (Osbeck) Merr.*



Fig. 1. Multicellular acicular non-glandular trichome of *Wedelia chinensis*



Fig. 2. Multicellular acicular non-glandular trichome of *Wedelia chinensis*



Fig. 3. Multicellular acicular non-glandular trichome of *Wedelia chinensis*



Fig. 4. Multicellular acicular non-glandular trichome of *Wedelia chinensis*



Fig. 5. Multicellular acicular non-glandular trichome of *Wedelia chinensis*

PLATE 46- *Blumea lacera (Burm.f.) DC.*



Fig. 1. Multicellular acicular non-glandular trichome of *Blumea lacera*



Fig. 2. Multicellular acicular non-glandular trichome of *Blumea lacera*



Fig. 3. Multicellular acicular non-glandular trichome of *Blumea lacera*

PLATE 47- *Tridax procumbens L.*



Fig. 1. Multicellular acicular non-glandular trichome of *Tridax procumbens*



Fig. 2. Capitate glandular trichome of *Tridax procumbens* with bicellular stalk.



Fig. 3. Capitate glandular trichome of *Tridax procumbens* with bicellular stalk.



Fig. 4. Multicellular acicular non-glandular trichome of *Tridax procumbens*



Fig. 5. Multicellular acicular non-glandular trichome of *Tridax procumbens*

PLATE 48- *Chromolaena odorata (L.) R.M. King & H. Rob.*

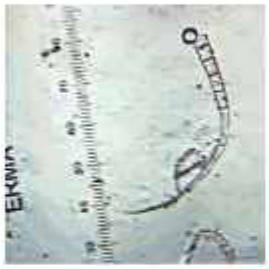


Fig. 1. Multicellular acicular non-glandular trichome of *Chromolaena odorata*



Fig. 2. Multicellular acicular non-glandular trichome of *Chromolaena odorata*



Fig. 3. Multicellular acicular non-glandular trichome of *Chromolaena odorata*



Fig. 4. Multicellular acicular non-glandular trichome of *Chromolaena odorata*

PLATE 49- *Eclipta prostrata (L.) L.*



Fig. 1. Acicular non-glandular trichome of *Eclipta prostrata*



Fig. 2. Acicular non-glandular trichome of *Eclipta prostrata*



Fig. 3. Acicular non-glandular trichome of *Eclipta prostrata*

Table 1. Types of trichomes studied according to APG IV classification

ORDER NAME	FAMILY NAME	PLANT NAME	TYPE OF TRICHOME
Commelinales	Commelinaceae	*Commelina diffusa* Burm.f.	Non-glandular, multicellular (3-4 celled), acicular or hooked shaped apex, uniseriate, thick wall with double layer. [PLATE 1]
Rosales	Cannabaceae	*Cannabis sativa* L.	Non-glandular, unicellular, acicular, uniseriate, slightly ornamented, thick wall with double layer. [PLATE 2]
		Trema orientalis (L.) Blume	Non-glandular, unicellular, pointed head, uniseriate, swollen or round base, Glandular, unicellular or multicellular, peltate [PLATE 3]
	Moraceae	*Artocarpus lacucha* Buch. -Ham.	Non-glandular, multicellular (2-3 celled), uniseriate, acicular, heavily ornamented, thick walled [PLATE 4]
		Ficus hispida L. f.	Non-glandular, unicellular, uniseriate, acicular, spiky appearance, heavily ornamented, swollen or round base. [PLATE 5]
Cucurbitales	Cucurbitaceae	*Cucurbita maxima* Duchesne	Non-glandular, multicellular (3-4 celled), uniseriate, acicular, pointed apex, swollen at the 2nd cell. [PLATE 6]
		Lagenaria siceraria (Molina) Standl.	Non-glandular, multicellular (3-4 celled), uniseriate, pointed apex, acicular glandular, multicellular (3-6 celled), pilate. [PLATE 7]
		Trichosanthes dioica Roxb.	Non-glandular, multicellular (5-6 celled), uniseriate, slightly ornamented, pointed apex and swollen base. [PLATE 8]
Malpighiales	Passifloraceae	*Turnera ulmifolia* L.	Non-glandular, unicellular, uniseriate, filament like appearance, spiky apex thick wall with moderate ornamentation [PLATE 9]
	Euphorbiaceae	*Acalypha hispida* Burm. f.	Non-glandular, multicellular (2-3 celled), thick-walled, double-layered, bulbous at the base Glandular, unicellular, capitate [PLATE 10]
		Tragia involucrata L.	Non-glandular, unicellular, syringe like pointed apex on leaf, thick wall with moderately ornamentation, glandular, unicellular, unicellular, capitate. [PLATE 11]
Myrtales	Combretaceae	*Terminalia catappa* L.	Non-glandular, unicellular, uniseriate, threadlike appearance [PLATE 12]
		Combretum indicum (L.) DeFilipps	Non-glandular, unicellular, uniseriate, wall with moderately ornamentation Glandular, multicellular, peltate [PLATE 13]
Malvales	Malvaceae	*Hibiscus rosa sinensis* L.	Non-glandular, multicellular, bifurcate (2 armed), trifurcate (3 armed), stellate (multiple arms joined at central base). [PLATE 14]
		Hibiscus mutabilis L.	Non-glandular, multicellular, bifurcate (2 armed), trifurcate (3 armed), stellate (multiple arms joined at central base). Glandular, multicellular (3-4 celled), pilate [PLATE 15]

		Hibiscus vitifolius L.	Non-glandular, unicellular, stellate (multiple arms joined at the central base) Glandular, multicellular (3-4 celled), capitate [PLATE 16]
		Thespesia populnea (L.) Sol. ex Corrêa	Non-glandular, multicellular, predominantly stellate (Star-shaped), arms are unicellular tapered. [PLATE 18]
		Alcea rosea L.	Non-glandular, multicellular, bifurcate (2 armed), trifurcate (3 armed), stellate (multiple arms joined at central base). Glandular, unicellular, capitate [PLATE 17]
		Grewia asiatica L.	Non-glandular, multicellular, stellate (multiple arms joined at central base). [PLATE 19]
		Pterospermum acerifolium Willd	Non-glandular, multicellular, stellate (multiple arms joined at central base). [PLATE 20]
Brassicales	Brassicaceae	*Brassica nigra* (L.) W.D.J. Koch	Non-glandular, unicellular, uniseriate, acicular, pointed apex and rounded base. [PLATE 21]
Boraginales	Boraginaceae	*Heliotropium indicum* L.	Non-glandular, unicellular, uniseriate, acicular, pointed apex, moderately ornamented, Glandular, unicellular, capitate [PLATE 22]
Solanales	Convolvulaceae	*Ipomoea corymbosa* (L.) Roth	Non-glandular, uniseriate, unicellular, acicular, thick wall with two layers. [PLATE 24]
		Ipomoea carnea Jacq.	Non-glandular, uniseriate, unicellular, acicular, bulbous appearance at the base. [PLATE 23]
	Solanaceae	*Solanum melongena* L.	Non-glandular, multicellular, stellate (multiple arms joined at central base). [PLATE 26]
		Solanum nigrum L.	Non-glandular, multicellular (2-6 celled), uniseriate, pointed apex, broader base. [PLATE 25]
		Solanum lycopersicum L.	Non-glandular, unicellular or multicellular (2-4 celled), uniseriate, acicular, broader base [PLATE 27]
		Withania somnifera (L.) Dunal	Non-glandular, multicellular (3-6 celled), dendrite (branching like tree), thick wall with two layers, moderately ornamented [PLATE 29]
		Nicotiana plumbaginifolia Viv	Glandular, uniseriate, multicellular (3-6 celled), pilate, wall two layered. [PLATE 28]
Lamiales	Oleaceae	*Nyctanthes arbor tristis* L.	Non-glandular, unicellular, uniseriate, acicular, bulbous appearance at the base Glandular unicellular, capitate [PLATE 30]
		Tabernaemontana divaricata (L.) R.Br. ex Roem. & Schult.	Glandular, multicellular, peltate [PLATE 31]
	Scrophulariaceae	*Scoparia dulcis* L.	Non-glandular, unicellular, uniseriate, acicular, spiky appearance Glandular, multicellular, peltate [PLATE 32]
		Lindenbergia indica (L.) Vatke	Glandular, multicellular (4-6 celled), pilate, thick wall with two layers, acicular, uniseriate [PLATE 33]
	Acanthaceae	*Justicia adhatoda* L.	Non-glandular, multicellular (3-4 celled), uniseriate, acicular, pointed apex, thick wall with two layers, highly ornamented.

			Glandular, multicellular, capitate [PLATE 34]
		Hygrophila auriculata (Schumach.) Heine	Non-glandular, unicellular or multicellular, uniseriate, acicular, spiky appearance [PLATE 35]
	Verbenaceae	*Lantana camara* L.	Non-glandular, uniseriate, unicellular, acicular, pointed apex, broader base, thick wall with two layers, glandular, multicellular (3-4 celled), pilate. [PLATE 36]
		Lippia geminata Kunth	Non-glandular, unicellular, uniseriate, acicular thick wall with moderate ornamentation [PLATE 37]
	Lamiaceae	*Ocimum sanctum* L.	Non-glandular, multicellular (6-8 celled), uniseriate, acicular, filament-like appearance, double layered wall [PLATE 38]
		Ocimum gratissimum L.	Non-glandular, multicellular (6-8 celled), uniseriate, acicular, pointed apex, broader base, thick wall with two layers. Glandular, multicellular, peltate [PLATE 39]
		Ocimum tenuiflorum L.	Non-glandular, multicellular (6-8 celled), uniseriate, acicular, pointed apex, broader base, thick wall with two layers. Glandular, multicellular, peltate [PLATE 40]
		Ocimum kilimandscharicum Gürke	Non-glandular, unicellular, uniseriate, acicular, spiky appearance Glandular, multicellular, peltate [PLATE 41]
		Clerodendrum chinense (Osbeck) Mabb.	Non-glandular, multicellular (6-8 celled), uniseriate, acicular, thick wall with two layers, slightly ornamented Glandular, multicellular, peltate [PLATE 43]
		Clerodendrum infortunatum L.	Non-glandular, multicellular (6-8 celled), uniseriate, acicular, thick wall with two layers, slightly ornamented Glandular, multicellular, peltate [PLATE 42]
Asterales	Asteraceae	*Wedelia chinensis* (Osbeck) Merr.	Non-glandular, multicellular (2-3 celled), uniseriate, pointed head, bulbous base, thick wall, highly ornamented. [PLATE 45]
		Vernonia cinerea (L.) Less.	Non-glandular, multicellular (6-8 celled), t-shaped Glandular, multicellular, peltate [PLATE 44]
		Eclipta prostrata (L.) L.	Non-glandular, multicellular (2-3 celled), uniseriate, pointed head, bulbous base, thick wall, highly ornamented. [PLATE 49]
		Blumea lacera (Burm.f.) DC	Non-glandular, multicellular (2-8 celled), uniseriate, acicular, pointed apex, broader base. [PLATE 46]
		Chromolaena odorata (L.) R.M. King & H. Rob.	Non-glandular, multicellular (6-10 celled), uniseriate, acicular, pointed apex, broader base, spiky appearance. [PLATE 48]
		Tridax procumbens L.	Non-glandular, multicellular (3-4 celled), uniseriate, acicular, bulbous base, pointed apex. Glandular, bicellular stalk, capitate. [PLATE 47]

Table 2. Measurement of trichome studied according to APG IV classification

Plant Name	Type of Trichome Studied	Trichome Length of Upper Surface of Leaf (µm)	Average (µm)	Trichome Length of Lower Surface of Leaf (µm)	Average (µm)	Trichome Length of Petiole (µm)	Average (µm)
Commelina diffusa Burm.f.	Non-glandular	142.8 156 136	144.9333	187 174.5 179	180.1667	204 205.6 190.5	200.0333
Cannabis sativa L.	Non-glandular	119 125 136	126.6667	140.5 136 155	143.8333	125.8 130 140.9	132.2333
Trema orientalis (L.) Blume	Non-glandular	760.65 812 800.6	791.0833	898.95 860.5 915.6	891.6833	345.75 450.8 412.6	403.05
	Glandular	30.6 46 35.4	37.33333	-	-	-	-
Artocarpus lacucha Buch. - Ham.	Non-glandular	184.6 190.2 170	181.6	196 204.6 164	188.2	496.8 500.2 484.05	493.6833
Ficus hispida L. f.	Non-glandular	345.75 375 384.6	368.45	484.05 460 475.6	473.2167	553.2 520 525.6	532.9333
Cucurbita maxima Duchesne	Non-glandular	497.88 484.05 470	483.9767	691.57 760.65 719.16	723.7933	857.46 880.4 836	857.9533
Lagenaria siceraria (Molina) Standl.	Non-glandular	204 221 210.8	211.9333	250.64 248.62 260.7	253.32	376.6 350 340.6	355.7333
	Glandular	204 210 215	209.6667	296.5 280 285	287.1667	345.75 330 325.8	333.85
Trichosanthes dioica Roxb.	Non-glandular	70 84.6 54	69.53333	57.8 65 60.5	61.1	102 115 120	112.3333

	Glandular	-	-	-	-	-	-
Turnera ulmifolia L.	Non-glandular	204.5 196 210	203.5	238 250 296	261.3333	414.9 420 345.75	393.55
Acalypha hi spidaBurm. f.	Non-glandular	330 345 310	328.3333	414.5 404.6 396	405.0333	441.6 455.43 483.78	460.27
Tragia irvducrata L.	Non-glandular	122.4 135.4 155	137.6	150 136.4 180	155.4667	179.79 207.45 235.11	207.45
Terminalia catappa L.	Non-glandular	829.8 850 896.4	858.7333	814.56 856.14 896.4	855.7	829.29 857.94 886.59	857.94
Combretum indicum (L.) DeFilipps	Non-glandular	210 215 245	223.3333	245 250.6 278.5	258.0333	276.6 304.26 331.92	304.26
	Glandular	50 56 37.4	47.8	50 54 42.5	48.83333	-	-
Hibiscus rosasinensis L.	Non-glandular	204 264 289	252.3333	287 296.45 312	298.4833	304.26 331.92 356.4	330.86
Hibiscus mutabilis L.	Non-glandular	345.6 386.4 390	374	355 365.4 414.5	378.3	362.58 390.24 417.9	390.24
	Glandular	-	-	-	-	414.5 425 396.8	412.1
Hibiscus vitifolius L.	Non-glandular	207.45 220 236.4	221.2833	245 265.8 278.5	263.1	276.6 304.26 331.92	304.26
	Glandular	-	-	-	-	296.8 304.5 265.4	288.9
Alcea rosea L.	Non-glandular	187	199.4833	210	216.3333	263.97	291.63

		204		226.5		291.63	
		207.45		212.5		319.29	
	Glandular	34		34		-	-
		37.6	38.86667	37.5	32.16667		
		45		25			
Thespesia populnea (L.) Sol. ex Corrêa	Non-glandular	50		45		55.32	
		56	50.33333	56.5	55.5	69.15	69.15
		45		65		82.98	
	Glandular	-	-	-	-	-	-
Grewia asiatica L.	Non-glandular	276.6		296.8		376.41	
		284	272.3667	312.5	313.2667	404.07	404.07
		256.5		330.5		431.73	
Pterospermum acerifolium Willd.	Non-glandular	296.4		325		469.38	
		310	296.95	345.6	350.4	497.04	497.04
		284.45		380.6		524.7	
Brassica nigra (L.) W.D.J. Koch	Non-glandular	104		145.6		165.96	
		120	110.6667	156	146.1333	193.62	193.62
		108		136.8		221.28	
Heliotropium indicum L.	Non-glandular	345		362.58		404.07	
		350	353.4833	376.41	376.41	417.9	417.9
		365.45		390.24		431.73	
	Glandular	414		362.58		-	-
		425.6	402.6667	376.41	376.41		
		368.4		390.24			
Ipomoea carnea Jacq.	Non-glandular	829.4		857.94		900.54	
		916	865.3	871.77	871.77	912.64	889.8267
		850.5		885.6		856.3	
Ipomoea corymbosa (L.) Roth	Non-glandular	750		752.97		812.65	
		796	768.8333	766.8	766.8	825.9	813.0667
		760.5		*780.63*		*800.65*	
Solanum nigrum L.	*Non-glandular*	*155*		*207.45*		*215.6*	
		160.5	*163.3333*	221.28	*221.28*	236.5	*242.9667*
		174.5		235.11		276.8	
Solanum melongena L.	Non-glandular	96.4	100.9667	110.64	124.47	159.6	174.1333
		104.5		124.47		186.3	

		102		138.3		176.5	
Solanum lycopersicum L.	Non-glandular	70	73.66667	138.3	115.1467	145.8	146
		85.6		96.5		138.2	
		65.4		110.64		154	
Nicotiana plumbaginifolia Viv.	Non-glandular	187	191.2667	207.45	225.89	156.45	215.91
		190		221.28		245.68	
		196.8		248.94		245.6	
Withania somnifera (L.) Dunal	Non-glandular	153	134.6667	134	143.6667	165.89	141.31
		130.2		152		135.64	
		120.8		145		122.4	
Nyctanthes arbortristis L.	Non-glandular	345.6	367.2	362.58	381.02	356.4	382.6
		360		376.41		386.9	
		396		404.07		404.5	
	Glandular	204	220.6667	-	-	-	-
		213					
		245					
Tabernaemontana divaricata (L.) R.Br. ex Roem. & Schult.	glandular	96.5	99.9	-	-	-	-
		100.8					
		102.4					
Scoparia dulcis L.	Non-glandular	85	86.7	135	118.65	146.5	139.3333
		96.5		114.5		116.9	
		78.6		106.45		154.6	
	Glandular	57.6	58.13333	-	-	-	-
		61					
		55.8					
Lindenbergia indica (L.) Vatke	Non-glandular	-	-	-	-	-	-
	Glandular	157	167.6333	207.45	208.51	265.4	243.6
		180.5		221.28		224.8	
		165.4		196.8		240.6	
Justicia adhatoda L.	Non-glandular	136	138.8	138.3	179.79	196.8	210.6333
		145.2		207.45		210.5	
		135.2		193.62		224.6	
	Glandular	25	22.8	-	-	-	-
		17.4					
		26					

Hygrophila auriculata (Schumach.) Heine	Non-glandular	240 256.4 246	247.4667	263.97 277.8 291.63	277.8	312.5 298.5 278	296.3333
Lantana camara L.	Non-glandular	304.26 345 325.6	324.9533	362.58 376.41 390.24	376.41	386.5 345.6 368.5	366.8667
	Glandular	-	-	-	-	335 342.5 376.41	351.3033
Lippia geminata Kunth	Non-glandular	207.45 210 196	204.4833	207.45 221.28 235.11	221.28	245.6 257.8 246.5	249.9667
Ocimum sanctum L.	Non-glandular	276.6 285 256.8	272.8	263.97 291.63 319.29	291.63	256.8 298.6 276.4	277.2667
Ocimum gratissimum L.	Non-glandular	138.3 145 156	146.4333	221.28 235.11 179.79	212.06	234.86 265.4 235.4	245.22
	Glandular	65.4 70 57.4	64.26667	57.6 70.5 65.4	64.5	-	-
Ocimum tenuiflorum L.	Non-glandular	276 256.38 240	257.46	291.63 277.8 319.29	296.24	319.29 304.56 298.7	307.5167
	Glandular	57.4 65 87.5	69.96667	57.8 45.8 60	54.53333	-	-
Ocimum kilimandscharicum Gürke	Non-glandular	196 204.5 186	195.5	207.45 221.28 248.94	225.89	238.6 245.6 264.5	249.5667
	Glandular	77 87 65	76.33333	85 65 90	80	-	-
Clerodendrum infortunatum L.	Non-glandular	414.9 396 385.6	398.8333	404.07 431.73 511.86	449.22	458.6 436 426.9	440.5
	Glandular	207.45	204.4833	-	-	-	-

		210					
		196					
Clerodendrum chinense (Osbeck) Mabb.	Non-glandular	85	122.6667	110.64	124.47	134	125
		138		124.47		126.8	
		145		138.3		114.2	
Vernonia cinerea (L.) Less.	Non-glandular	210	191.3333	165.96	179.79	207.45	221.28
		198.2		179.79		221.28	
		165.8		193.62		235.11	
	Glandular	57	47.5	57.4	47.1	-	-
		48		38.9			
		37.5		45			
Wedelia chinensis (Osbeck) Merr.	Non-glandular	622.35	615.4167	609.75	628.19	-	-
		575		623.58			
		648.9		651.24			
Blumea lacera (Burm.f.) DC.	Non-glandular	829.8	815.4333	857.94	871.8267	913.43	941.09
		814		871.77		941.09	
		802.5		885.77		968.75	
Tridax procumbens L.	Non-glandular	533.2	532.6	511.86	553.71	609.75	642.02
		554.6		553.35		651.24	
		510		595.92		665.07	
	Glandular	51	53.15	56	44.53333	-	-
		65		42			
		43.45		35.6			
Chromolaena odorata (L.) R.M. King & H. Rob.	Non-glandular	622.35	627.85	650.8	665.2333	712	704.3333
		650.4		678.9		705	
		610.8		666		696	
Eclipta prostrata (L.) L.	Non-glandular	622.35	625.95	650.5	659.7	721	709.8333
		610		678		710.5	
		645.5		650.6		698	