ISBN: 978-93-95847-47-6

Research and Reviews in Plant Science Volume II

EDITORS:
DR. MADHU PRAKASH SRIVASTAVA
DR. MEENAKSHI A. BANGAR
DR. DEVANGI CHACHAD
DR. AVERINENI RAVI KUMAR

BHUMI PUBLISHING, INDIA
FIRST EDITION: FEBRUARY 2024

Research and Reviews in Plant Science Volume II

(ISBN: 978-93-95847-47-6)

Editors

Dr. Madhu Prakash Srivastava

Department of Botany,

Maharishi University of

Information Technology,

Lucknow U.P.

Dr. Meenakshi A. Bangar

Department of Botany,

Netaji Subhashchandra Bose, Arts

Commerce and Science College,

Nanded. Dist. Nanded, M.S.

Dr. Devangi Chachad

Department of Botany,

Jai Hind College (Empowered Autonomous),

Churchgate, Mumbai, M.S.

Dr. Averineni Ravi Kumar

Department of Pharmacognosy,

Nimra College of Pharmacy,

Vijayawada, Andhra Pradesh

Bhumi Publishing

February, 2024

Title: Research and Reviews in Plant Science Volume II

Editors: Dr. Madhu Prakash Srivastava, Dr. Meenakshi A. Bangar,

Dr. Devangi Chachad, Dr. Averineni Ravi Kumar

First Edition: February, 2024

ISBN: 978-93-95847-47-6

Published by:

Bhumi Publishing

BHUMI PUBLISHING

Nigave Khalasa, Tal – Karveer, Dist – Kolhapur, Maharashtra, INDIA 416 207

E-mail: bhumipublishing@gmail.com

PREFACE

In the vast landscape of scientific inquiry, plant science stands as a cornerstone of our understanding of life on Earth. From the intricacies of photosynthesis to the complexities of plant-microbe interactions, the field encompasses a rich tapestry of research that continues to unravel the mysteries of the botanical world.

This inaugural volume of Research and Reviews in Plant Science represents a dedication to the pursuit of knowledge and innovation within the realm of plant biology. Our aim with this publication is to provide a platform for the dissemination of cutting-edge research, insightful reviews, and thought-provoking perspectives that advance our understanding of plants and their significance to the broader ecosystem.

The diverse array of topics covered within this volume reflects the breadth and depth of contemporary plant science. From the molecular mechanisms underlying plant development to the ecological dynamics shaping plant communities, each contribution offers a unique lens through which to explore the wonders of the botanical realm.

As editors, we are deeply grateful to the authors whose scholarly endeavors have enriched this volume with their expertise and dedication. Their commitment to advancing the frontiers of plant science is evident in the quality and rigor of their work, and we commend them for their contributions to the field.

We also extend our appreciation to the reviewers whose thoughtful feedback and constructive criticism have helped to ensure the integrity and excellence of the manuscripts presented herein. Their expertise and insights have been invaluable in shaping the content of this volume and maintaining the highest standards of scholarly inquiry.

Finally, we would like to express our gratitude to the readers of Research and Reviews in Plant Science. It is our sincere hope that this volume will serve as a source of inspiration and knowledge for students, researchers, and enthusiasts alike, fostering a deeper appreciation for the beauty and complexity of the plant kingdom.

As we embark on this journey of exploration and discovery, we invite you to join us in celebrating the marvels of plant science and the boundless opportunities it presents for understanding and stewarding the natural world.

Editors

TABLE OF CONTENT

Sr. No.	Book Chapter and Author(s)	Page No.
1.	**DIVERSE ENDOPHYTIC FUNGI FROM *HIBISCUS ROSA-SINENSIS* LINN. LEAVES: ENZYMATIC INSIGHTS** Nandini Desai and Devangi Chachad	1 – 9
2.	**NATURAL FIBERS IN INDIA: EXPLORING THE FRONTIERS OF ORGANIC COMPOSITES** C Manasa and Sandhya Rani Nagarajaiah	10 – 20
3.	**UNVEILING THE SYNERGISTIC EFFECT OF *COUROUPITA GUIANENSIS*** Rajaruban M D S, Shanthini P and Thirisha Bharathi GK	21 – 29
4.	**PIONEERING SUSTAINABLE AGRICULTURE: THE JOURNEY FROM APOMIXIS TO SYNTHETIC APOMIXIS** Shreya Singh and Sourav Ranjan Nanda	30 – 41
5.	**A PRELIMINARY REPORT ON ETHNOMEDICINAL USES OF SPICES BY THE NATIVES OF BARGARH MUNICIPALITY, ODISHA, INDIA** Alok Ranjan Sahu, Subhashree Sonalika Pradhan, Sandhyarani Sahu and Puspitarani Barik	42 – 50
6.	**SHORT NOTE ON MIYAWAKI FORESTS** Meenakshi A. Bangar	51 – 53
7.	**AN OVERVIEW OF MIYAWAKI PLANTATION** Meenakshi A. Bangar	54 – 56
8.	**A CONCISE REVIEW ON AZOLLA CULTIVATION AND ITS APPLICATIONS** Mohammed Mujahed, Parvati R. Solanke and Baig Zeba Rafat	57 – 64
9.	**PHARMACEUTICAL PROSPECTS OF THE MANGROVE *HERITIERA FOMES*: A REVIEW** Shreyas S. Shelar, Varsha S. Mistry, Sharda K. Patil and Sonal M. Manohar	65 – 76
10.	**HYBRIDIZATION: DETAILED REVIEW** Kiruthika M, Ranjana J, Neelavathi U and Senthil J	77 – 88

11.	**PRELIMINARY FLORAL DIVERSITY ANALYSIS OF KUSHI VILLAGE AND ADJOINING AREAS DIST. – SATARA, MS (INDIA)** Neeta Salunkhe and Swapnaja Deshpande	89 – 95
12.	**ISOLATION OF PLANT FIBRE DEGRADING STRAINS FOR ENHANCED BIOMASS UTILIZATION AND ENVIRONMENTAL SUSTAINABILITY** Syed Nisar Ahmed	96 – 100
13.	**FUNGAL AND PLANT PHENYLALANINE AMMONIA-LYASE (PAL): PLANT-PATHOGEN INTERACTION** Aboli Kshirsagar	101 – 114
14.	**SCREENING FOR PHYTOCHEMICALS, ANTIMICROBIAL AND ANTICOAGULANT ACTIVITY OF AQUEOUS EXTRACT OF *TRIDAX PROCUMBENCE*** Nihar Ranjan Nayak, Anisma Pattnayak and Alok Ranjan Sahu	115 – 126
15.	**NATURAL IMMUNE-BOOSTING HERBS: A GUIDE TO HERBAL REMEDIES** Niyati Shah, Mamta Kumari, Piyushkumar Sadhu, Chitrali Talele and Dillip Kumar Dash	127 – 131

DIVERSE ENDOPHYTIC FUNGI FROM *HIBISCUS ROSA-SINENSIS* LINN. LEAVES: ENZYMATIC INSIGHTS

Nandini Desai and Devangi Chachad

Department of Botany,

Jai Hind College, Mumbai 400020

Corresponding author E-mail: nandini.desai@jaihindcollege.edu.in;

devangi.chachad@jaihindcollege.edu.in

Abstract:

Endophytic fungi are defined as a population of micro-organisms which may present themselves in the plant tissues without causing any apparent infection or disease symptoms to the host. These endophytes are found in almost all angiosperms. *Hibiscus rosa-sinensis* Linn. leaves have been used widely for their therapeutic uses as described in traditional medicine. It has various phyto-components that can confer properties such as anti-diabetic, antioxidant, and even anti-cancer. In the present study, *H. rosa-sinensis* Linn. leaves were used for isolating endophytic fungi. These isolated fungal organisms, after identification, were qualitatively assessed for the production of enzymes such as amylase, protease, lipase and L-asparaginase. These enzymes have pharmaceutical and industrial significance. This study sheds valuable insight into the diversity of these microorganisms from *Hibiscus rosa-sinensis* L. which produces important host plant associated enzymes.

Keywords: Endophytic Fungi, *Hibiscus rosa-sinensis*, enzymes

Introduction:

Within the domain of interactions between plants and microbes, endophytic fungi have surfaced as captivating and underexplored companions, presenting a rich source of possible bioactive substances and enzymes. These fungi which inhabit the medicinal plants are of great interest since the isolation of Taxol- producing endophyte *Taxomyces andreanae* from the Pacific yew tree *Taxus brevifolia* by Strobel and his team in 1993. These myco-endophytes are essential, yet the relatively understudied group of microbial plant endosymbionts. These fungi live asymptomatically, and sometimes systemically, within plant tissues (Carroll.,1988). On the other hand, the endophytes would produce several bioactive constituents for helping the host plants to resist external biotic and abiotic stresses and benefits for the host growth in return. (Zhao *et al.,* 2011)

Endophytic fungi harbouring in medicinal plants are found to produce bioactive compounds that may be the same or structurally like the secondary metabolites produced by their host. These fungi can produce these compounds independent of their host. (Venieraki *et al.,* 2017*)*

Fungi produce various enzymes to aid in colonization of plant tissues, nutrient mobilization inside host plants and for their survival within the host. (Borges *et al.* 2009). These enzymes are also used by humans in various industries like food and beverage, medicine etc. Enzymes like Amylase, Lipase and Proteases are commonly used by the fungi to assimilate primary metabolites. Whereas L-asparaginase is used for inhibiting protein synthesis and hence is used for treatment of acute lymphocytic leukaemia.

Hibiscus rosa-sinensis is one of the most common ornamental members of the *Malvaceae* family. The leaves of the plant have been used for properties ranging from analgesic (Sawarkar *et al.,* 2009), emmenagogue (Al-Snafi, 2018), abortifacient (Jadhav *et al.* 2009), antimicrobial (Uddin *et al.,* 2010).

The plant has been studied extensively as a host for plant pathogen as it of very high horticultural value. It has been screened for different fungi and bacterial hosts which could be responsible for causing diseases. For the present study, the leaves of the above-mentioned plant were screened for myco-endophytes. After isolation of the fungal organisms, and identification of the isolates, further screening of these fungal isolates was carried out. Four different extracellular enzymes were chosen for this study such as amylase, protease, lipase and l-asparaginase which are known for their significance in biotechnological applications.

Materials and Methods:

- **Selection of plant material and isolation of fungal endophytes**

Fresh leaves of Shoe flower were collected from the local gardens in Mumbai. The plant specimen of *Hibiscus rosa sinensis* L. was authenticated from Blatter Herbarium, Mumbai and was found to be matching with *Hibiscus rosa sinensis* L. (K. V. S. 3522).

- **Isolation of endophytic fungi:**

Fresh leaves (collection and processing within 5 - 6 hrs.) were used to isolate the endophytic fungi (EF). The surfaces of the leaves were sterilized using Na-hypochlorite solution (4% free chlorine) and 70% ethanol (Petrini *et al.,* 1993) along with multiple washings with distilled water. They were then dried using sterile tissue papers and cut into small pieces of 5mm x 5mm. These pieces were placed on sterile potato dextrose agar (PDA) plates supplemented with streptomycin (200µg/mL) (Strobel, 2003). These were incubated at 37°C till growth of

mycelium was observed (approximately 10-15 days). Control plates were also incubated after surface sterilization protocol. Leaf imprints (dorsal /ventral) were taken on the Petri Plates amended with streptomycin to rule out any surface contaminants which may appear post-incubation.

Identification of isolated EF was carried out at NFCCI, Agarkar Research Institute, Pune.

- **Qualitative Enzymatic assay: Amylase, Protease, Lipase, L-asparaginase:**

The isolated fungi were qualitatively analysed for production of extracellular enzymes like amylase, protease, lipase and L-asparaginase using agar plate technique.

Amylase:

For amylase producers, the isolates were inoculated on starch agar plates. These organisms were allowed to grow for 5 days. Producers were identified by using Lugol's Iodine as an indicator as described by Patil *et al.* (2015) with slight modifications.

Ingredients	**g/L**
Glucose	1.0
Yeast extract	0.1
Peptone	0.5
Agar	16.0
Distilled water	1 Litre
starch	1%
pH	6.0

Protease:

For Protease assay, the isolates were inoculated 1% skim milk and incubated for 5 days to check for protease producers. Enzymatic production was observed as clear zones where milk casein was hydrolysed due to proteolytic activity as described by Ahmad, M. S., *et al.* (2014) with slight modifications.

Ingredients	**g/L**
Glucose	1.0
Yeast extract	0.1
Peptone	0.5
Agar	16.0
Distilled water	1 Litre
Skim milk powder	1%
pH	6.0

Lipase:

For Lipase assay, the isolates were inoculated in Peptone agar medium containing 1% Tween 20 and allowed to grow for nine days. The results were observed by precipitation of calcium salts in the medium as mentioned by Sunitha *et al.* (2013)

Ingredients	**g/L**
NaCl	5
$CaCl_2.2H_2O$	0.1
Peptone	10
Agar	16
Distilled water	1 Litre
Tween 20	1% (sterilized separately)
pH	6.0

L-asparaginase:

For L-asparaginase assay, the isolates were inoculated on Modified Czapek Dox's medium and allowed to grow for ten days. The positive activity was observed by a change indicator from yellow at acidic pH to blue due to increase in pH as prescribed by Mahajan *et al.* (2013).

Ingredients	**g/L**
Na_2HPO_4	6
KH_2PO_4	2
NaCl	0.5
L- Asparginase	20
glycerol	2
$MgSO_4.\ 7H_2O$	0.2
$CaCl_2.2H_2O$	0.005
Bromothymol Blue (BTB)	0.007%
Agar	2%
Distilled water	1 Litre

Results and Discussion:

Six endophytic fungi were isolated from *Hibiscus* leaves and were identified using the 18S rRNA molecular identification method as shown in Table 1. These endophytic fungi were then screened to produce various enzymes such as amylase, protease, lipase and l-asparaginase

and their results are tabulated in Table 1 and 2. Out of six isolated fungi, only *Chaetomium globosum* was unable to produce any of the four enzymes chosen for this study.

Aspergillus niger and other species of *Aspergillus* have been isolated from *Hibiscus sabdariffa* by Khalil, D., *et al.* (2020). *Penicillium citrinum* has also been isolated by Ahmad *et al.* (2014) which was found to produce a fibrinolytic protease.

Amylase production in most EF has been correlated to its economic importance in food, fermentation, and pharmaceutical industries. (Gupta, 2003) Protease is a widely significant enzyme produced by the EF which is routinely employed in food processing, pharmaceutical, leather, detergent and waste processing industries. (Rao *et al.* 1998) Fungal lipases are utilized in dairy, pharmaceutical, cosmetic and biodiesel industries. (Singh and Mukhopadhyay, 2012) L-asparaginase is one of the most interesting enzymes which is found useful in oncological treatment plans of leukaemia and lymphoma. (Sarquis *et al.,* 2004) These extracellular enzymes produced by the EF can be engineered to have increased yield and easier downstream processing including purification.

Endophytes may produce these extracellular enzymes in response to their relationship with the host plant. These fungal enzymes may be conferring resistance to plants and thereby aiding their growth. It is also possible that the fungi could be producing the enzymes as a part of their life cycle to remain inside the host as a latent pathogen or saprophobe. These enzymes may also help the endophytes to easily metabolize the nutrients or metabolites present in the plant. This complex yet interesting aspect of plant-microbe relationship sheds light on the interaction spectrum ranging from phytopathogen to mutualistic symbiosis.

Table 1: Endophytic fungi isolated from *Hibiscus* leaves and production of enzymes

Code	Identified Name	Amylase	Protease	Lipase	L-Asparginase
HR1	*Aspergillus niger*	P	A	A	P
HR2	*Alternaria alternata*	A	A	A	P
HR3	*Chaetomium globosum*	A	A	A	A
HR4	*Penicillium citrinium*	P	P	A	P
HR5	*Syncephalastrumracemosum*	A	A	A	P
HR6	*Gymnoascusdankaliensis*	P	P	P	P

AMYLASE PRODUCERS

HR1: Aspergillus niger *HR4: Penicillium citrinium* *HR6: Gymnoascus dankaliensis*

PROTEASE PRODUCERS

HR6: Gymnoascus dankaliensis *HR4: Penicillium citrinium*

LIPASE PRODUCER

HR6: Gymnoascus dankaliensis

L-ASPARAGINASE PRODUCERS

HR1:Aspergillus niger *HR2:Alternaria alternata* *HR4:Penicillium citrinium*

HR5:Syncephalastrum racemosum *HR6:Gymnoascus dankaliensis* Control plate

Conclusion:

The vibrant and ornamental exterior of this plant hides a rich and diverse community of endophytes that play essential roles in its health, growth, and ecological interactions. The

research aimed to uncover the hidden contributions of these microorganisms and explore the potential applications of their extracellular enzymes.

Through a combination of isolation techniques, microscopy, and molecular identification, various genera of fungi were identified inhabiting the internal tissues of *Hibiscus rosa-sinensis*. This diversity underscores the complex and symbiotic relationships that exist within this plant. Our investigation into the qualitative presence of extracellular enzymes revealed a wide array of enzymes, including amylases, proteases, lipases and L-asparaginase, being produced by these endophytic microorganisms. These enzymes are essential for various physiological processes within the plant, such as nutrient cycling and defence mechanisms.

The findings of this study have both theoretical and practical significance. On a fundamental level, they contribute to our understanding of the intricate relationships between plants and their associated microorganisms. The presence and enzymatic capabilities of endophytes in Hibiscus rosa-sinensis emphasize the plant's ability to host a diverse microbial community and harness their enzymatic potential. This symbiosis is vital for the overall health and ecological function of the plant.

On a practical level, the qualitative analysis of extracellular enzymes produced by these endophytes opens up new avenues for applications in biotechnology, agriculture, and environmental management. Enzymes play critical roles in processes such as organic matter decomposition and nutrient acquisition, which are crucial for sustainable agriculture and environmental remediation. The enzymatic potential of these endophytes presents an opportunity to develop innovative solutions for improving crop productivity and addressing environmental challenges.

In conclusion, this study highlights the significance of endophytic microorganisms in Hibiscus rosa-sinensis and their capacity to produce extracellular enzymes. The diverse community of endophytes and their enzymatic capabilities contribute to the plant's overall health and open up exciting possibilities for various fields of science and industry. As we continue to explore the intricate relationships between plants and their microbial partners, we uncover new pathways for sustainable agriculture and environmental stewardship, ultimately advancing our knowledge and improving our world.

Acknowledgement:

The authors are grateful to NFCCI, ARI, Pune for molecular identification of mycoendophytes.

References:

1. Ahmad, M. S., Noor, Z. M., & Ariffin, Z. Z. (2014). Isolation and identification fibrinolytic protease endophytic fungi from Hibiscus leaves in Shah Alam. International Journal of Biological, Veterinary, Agricultural and Food Engineering, *8*(10), 1070-3.
2. Al-Snafi, Ali. (2018). Chemical constituents, pharmacological effects and therapeutic importance of *Hibiscus rosa-sinensis*- A review. 8. 101-119.
3. Borges, W. D. S., Borges, K. B., Bonato, P. S., Said, S., & Pupo, M. T. (2009). Endophytic fungi: natural products, enzymes and biotransformation reactions. Current Organic Chemistry, 13(12), 1137-1163.
4. Carroll, G. C. (1988). Fungal endophytes in stems and leaves: From latent pathogen to mutualistic symbiont. Ecology 69:2–9.
5. Gupta, R., Gigras, P., Mohapatra, H., Goswami, V. K., & Chauhan, B. (2003). Microbial α-amylases: a biotechnological perspective. Process biochemistry, *38*(11), 1599-1616.
6. Jadhav, V. M., Thorat, R. M., Kadam, V. J., & Sathe, N. S. (2009). Traditional medicinal uses of *Hibiscus rosa-sinensis*. J Pharm Res, *2*(8), 1220-1222.
7. Jia, M., Chen, L., Xin, H. L., Zheng, C. J., Rahman, K., Han, T., & Qin, L. P. (2016). A friendly relationship between endophytic fungi and medicinal plants: a systematic review. Frontiers in microbiology, 7, 906.
8. Khalil, D., El-Zayat, S. A., & El-Sayed, M. A. (2020). Phytochemical screening and antioxidant potential of endophytic fungi isolated from *Hibiscus sabdariffa*. Journal of Applied Biotechnology Reports, *7*(2), 116-124.
9. Mahajan, R. V., Saran, S., Saxena, R. K., & Srivastava, A. K. (2013). A rapid, efficient and sensitive plate assay for detection and screening of L-asparaginase-producing microorganisms. FEMS microbiology letters, 341(2), 122-126.
10. Patil, M. G., Pagare, J., Patil, S. N., & Sidhu, A. K. (2015). Extracellular enzymatic activities of endophytic fungi isolated from various medicinal plants. Int J Curr Microbiol App Sci, 4(3), 1035-1042.
11. Petrini, O., Sieber, T. N., Toti, L., & Viret, O. (1993). Ecology, metabolite production, and substrate utilization in endophytic fungi. Natural toxins, 1(3), 185-196.
12. Rao, M. B., Tanksale, A. M., Ghatge, M. S., & Deshpande, V. V. (1998). Molecular and biotechnological aspects of microbial proteases. Microbiology and molecular biology reviews, *62*(3), 597-635.
13. Rodriguez, R. J., White Jr, J. F., Arnold, A. E., & Redman, A. R. A. (2009). Fungal endophytes: diversity and functional roles. New phytologist, 182(2), 314-330.

14. Sarquis, M. I. D. M., Oliveira, E. M. M., Santos, A. S., & Costa, G. L. D. (2004). Production of L-asparaginase by filamentous fungi. Memorias do Instituto Oswaldo Cruz, *99*, 489-492.
15. Sawarkar, A., Jangde, C. R., Thakre, P. D., Kadoo, R., & Shelu, S. (2009). Analgesic Activity of *Hibiscus rosa sinensis* Linn in Rat. Veterinary world, *2*(9).
16. Singh, A. K., & Mukhopadhyay, M. (2012). Overview of fungal lipase: a review. Applied biochemistry and biotechnology, *166*, 486-520.
17. Stierle, A., Strobel, G., & Stierle, D. (1993). Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science, *260*(5105), 214-216.
18. Strobel, G. A. (2003). Endophytes as sources of bioactive products. Microbes and infection, 5(6), 535-544.
19. Sunitha, V. H., Nirmala Devi, D., & Srinivas, C. (2013). Extracellular enzymatic activity of endophytic fungal strains isolated from medicinal plants. World Journal of Agricultural Sciences, 9(1), 1-9.
20. Uddin, B., Hossan, T., Paul, S., Ahmed, T., Nahar, T., & Ahmed, S. (2010). Antibacterial activity of the ethanol extracts of *Hibiscus rosa-sinensis* leaves and flowers against clinical isolates of bacteria. Bangladesh J Life Sci, *22*(2), 65-73.
21. Venieraki *et al.*, (2017). Endophytic fungi residing in medicinal plants have the ability to produce the same or similar pharmacologically active secondary metabolites as their hosts, Hellenic Plant Protection Journal 10: 51-66 DOI 10.1515/hppj-2017-0006
22. Zhao *et al.*, (2011). Plant derived bioactive compounds produced by endophytic fungi, Mini-Reviews in Medicinal Chemistry, Vol. 11, No. 2 159 - 168

NATURAL FIBERS IN INDIA: EXPLORING THE FRONTIERS OF ORGANIC COMPOSITES

C Manasa*[1] and Sandhya Rani Nagarajaiah[2]

[1]Department of Chemistry,

[2]Department of Physics,

Vidyavardhaka College of Engineering, Mysore-570 002, Karnataka, India

*Corresponding author E-mail: manasac@vvce.ac.in

Abstract:

Natural fibers offer a sustainable, eco-friendly substitute for synthetics, increasingly employed in organic composites. This study underscores their advantages, like affordability, widespread availability, biodegradability, and renewability. It surveys Indian organic composites crafted from natural fibers, notably jute, coir, sisal, and bamboo, highlighting their apt properties for distinct uses. The applications span automotive, construction, packaging, and furniture sectors. The paper outlines India's current organic composite landscape, spotlighting industry challenges and government/industry incentives for natural fiber utilization. In summary, natural fibers and organic composites stand as India's eco-conscious option, necessitating more research, processing tech, quality control, and standardization for wider adoption.

Keywords: Natural fibre; Biocomposite; Eco-friendly; Biodegradable.

Graphical abstract:

Introduction:

Natural fibers have gained considerable traction as a sustainable and environmentally friendly substitute for synthetic fibers, a trend particularly evident in the burgeoning field of

organic composites [1]. This paper seeks to elucidate the manifold benefits intrinsic to natural fibers, encompassing their cost-effectiveness, widespread availability, biodegradability, and renewable nature. Delving into the realm of Indian organic composites, the study showcases the application of natural fibers such as jute, coir, sisal, and bamboo, illuminating their distinct properties that render them well-suited for specific applications. Notably, these applications span diverse sectors ranging from automotive and construction to packaging and furniture, attesting to the versatility of these materials [2]. By scrutinizing the current landscape of organic composites in India, this paper underscores the challenges faced by the industry while also highlighting the proactive measures undertaken by both the Indian government and industry stakeholders [3]. This analysis underscores the compelling potential of natural fibers and organic composites, positioning them as a formidable and sustainable alternative to synthetic materials. However, the realization of this potential hinges upon continued research endeavors and investment in processing technologies, quality control mechanisms, and standardization protocols to render these composites not only viable but also attractive across a spectrum of applications [4].

Natural fiber composites

Natural Fiber Composites (NFCs) blend plant, animal, or mineral-derived natural fibers with thermoset or thermoplastic resins, resulting in versatile composite materials. NFCs are classified by fiber type, polymer matrix, and manufacturing process. Fiber types range from plant-based options like jute, sisal, hemp, flax, bamboo, or kenaf, to animal-based variants using wool, silk, or leather fibers, and mineral-based choices incorporating asbestos or basalt fibers. Polymer matrices include thermosets like epoxy or phenolic, and thermoplastics such as polypropylene, polyethylene, or polylactic acid. Manufacturing methods encompass compression molding, injection molding, and extrusion. This intricate interplay shapes NFCs, rendering them sustainable and versatile for various industries.

Processing biocomposite materials

The process of making biocomposites involves several essential stages. It starts with carefully choosing the right raw materials, like natural fibers (such as hemp, jute, or flax) and compatible polymer matrices (like PLA or PHA). These materials are picked based on the qualities needed for the final product. After the materials are chosen, the fibers and matrices are prepared. Natural fibers are cleaned and processed to enhance their strength and suitability for the composite. At the same time, polymer matrices are readied using methods like melting or dissolving, ensuring they're ready for the next steps. During the mixing and compounding phase, the prepared fibers and matrices are blended to create a uniform mixture. This blending can be done through techniques like melt compounding or solution compounding, ensuring a well-

integrated combination. After blending, the materials move to shaping. This step involves using manufacturing methods like injection molding, compression molding, or extrusion to give the biocomposites their desired forms. These techniques use controlled temperature and pressure to shape the materials accurately. The final step is post-processing, where the biocomposites are refined. This includes activities like cooling, cutting, trimming, and surface treatments to achieve the desired properties and appearance. These processes ensure that the biocomposites meet the required standards both in terms of function and looks.

Natural fibre characteristics

Natural fibers in composites offer a wide array of favorable physical and chemical properties, suited for diverse applications. Physically, they tend to have lower densities compared to synthetics, providing lightweight advantages for weight-sensitive contexts. Their robust tensile strength often matches or surpasses synthetic counterparts, showcasing inherent durability. Elasticity allows them to recover shape after stretching, and notable moisture absorption aids in moisture management. Low thermal conductivity benefits insulation, while abrasion resistance enhances durability in high-wear scenarios. Moreover, they demonstrate dimensional stability against temperature and humidity variations. Notably, certain fibers like wool and silk possess built-in flame-retardant properties, augmenting safety.

Chemically, natural fibers' hydrophilicity enables moisture absorption, aiding in moisture control. However, extended moisture exposure can lead to degradation. Their interaction with diverse chemicals, including acids and bases, has both potential applications and challenges that require careful handling. While their biodegradability aligns with sustainability goals, it may limit long-term durability. Compatibility with polymer matrices is pivotal for robust bonding, with certain fibers even displaying heightened resistance to UV light—an asset for sun-exposed settings. To conclude, the rich blend of physical and chemical properties exhibited by natural fibers highlights their versatile and promising role in composite materials across industries.

Variables that affect biocomposites

The characteristics of biocomposites are intricately linked to a multitude of variables that encompass the type of natural fiber, polymer matrix, and methods of processing. Among the pivotal factors that significantly influence the properties of biocomposites are: the specific type of natural fiber employed, which imparts unique physical and chemical attributes impacting overall performance – fibers with elevated tensile strength and stiffness, for instance, contribute to the enhanced robustness of the resulting biocomposites; the length of these natural fibers, where longer strands generally bolster the strength and stiffness of the biocomposites, although processing techniques play a role as well; the precise orientation of the fibers within the

composite material, where intentional alignment can amplify strength in preferred directions; the proportion of natural fiber present in the composite, exerting a notable influence on vital traits such as strength, stiffness, and overall weight; the choice of polymer matrix, as the diverse array of polymers exhibit distinct physical and chemical properties that impart specific characteristics to the composite; and lastly, the method by which natural fibers and polymer matrices are combined during processing, with parameters such as temperature, pressure, and curing duration significantly shaping the final attributes of the product. A comprehensive understanding and skillful manipulation of these multifaceted variables enable the precise tailoring of biocomposites to meet the exacting demands of various applications.

Benefits and drawbacks of NFs

Natural fibers (NFs) offer a mix of advantages and drawbacks in composite materials. NFs stand out for being renewable, cost-effective, and lightweight, while certain types like flax and hemp bring robust mechanical properties suitable for demanding applications. Their moisture absorption capability is valuable for moisture management. However, NFs exhibit variability in properties due to sourcing and processing, and some types like cotton and sisal are less durable and moisture-resistant than synthetics, limiting their use. Compatibility issues with certain polymer matrices can affect bonding and mechanical properties, and processing NFs can be more intricate and costly. The availability of specific NFs may be inconsistent across regions. Deciding on NF use in composites requires a careful consideration of their merits and limitations, aligned with application-specific needs for optimal property and performance outcomes.

Biodegradation

Biodegradation is the natural breakdown of organic substances by microorganisms, into simpler, non-toxic forms, playing a vital role in ecosystem balance and environmental protection [5]. This process employs microorganism-produced enzymes to dismantle complex organics into compounds like water, carbon dioxide, and methane. Speed and efficiency hinge on factors like material type, microorganism presence, and environmental conditions [6]. Biodegradation finds utility in waste management, bioremediation, and biotechnology, curbing pollutants' impact. It also crucially contributes to the carbon cycle, recycling carbon and upholding the Earth's ecosystem.

NFC in India

Natural fiber composites (NFCs) amalgamate natural fibers like jute, sisal, coir, bamboo, and hemp with a polymer matrix, e.g., polypropylene or polyethylene, yielding a range of benefits including cost efficiency, reduced weight, and heightened environmental sustainability

(figure 1). In India, NFCs have gained traction, particularly in automotive and construction domains, spurred by the government's push for eco-friendly materials [7]. The automotive sector is a prominent adopter of NFCs, aiming to enhance fuel efficiency by decreasing vehicle weight. This is witnessed in the integration of NFCs in vehicle components like interior trims, door panels, and seat backs [8]. Likewise, India's construction industry explores NFCs as a sustainable alternative to traditional materials like concrete and steel [9], paving the way for applications spanning wall panels, roofing, and flooring. Indian entities like Greenply Industries, Polybond India Pvt Ltd, and Gujarat State Fertilizers & Chemicals Ltd contribute to NFC production [10]. Furthermore, the Indian government fosters NFC usage by establishing research centers such as the Indian Plywood Industries Research & Training Institute and the Indian Institute of Technology, Delhi [11].

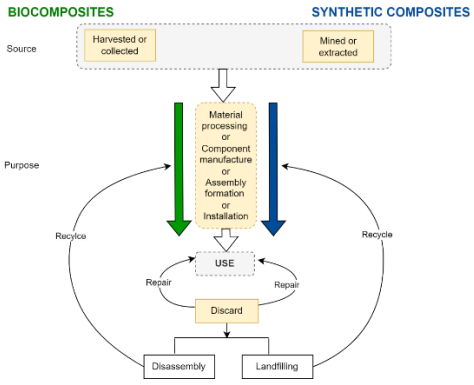

Figure 1: Biocomposites and synthetic composites

Jute-based biocomposites

White Corchorus capsularis (white jute) and Tossa jute are herbaceous annual plants yielding jute fiber. Widely cultivated in countries like Bangladesh, China, India, Indonesia, and Brazil, jute ranks as the second most abundant natural fiber, succeeding cotton. Jute's exceptional growth adaptability, thriving in challenging environments like river flats, depressions, and saline alkali soils, marks it as an environmentally friendly agricultural product requiring no pesticides or fertilizers. Jute fiber, with its solid specific strength, stiffness, and modulus, proves advantageous for enhancing composites. Harvesting techniques and maturity influence fiber attributes. The Birla Jute Industries Ltd. showcases jute composite application in car interiors [12], and the National Institute of Research on Jute and Allied Fibre Technology (NIRJAFT) has innovated distinct jute and NFC products [13]. Jute composites find utility in everyday items, lampshades, luggage, pre-fabricated buildings, and more, aligning with environmental and affordability considerations. Jute-based biocomposites, popularized by India's sustainable drive, exhibit strengths across industries such as automotive and construction. This growth is evident in

Indian entities like the National Jute Board, Jute Corporation of India, and Indian Jute Industries' Research Association.

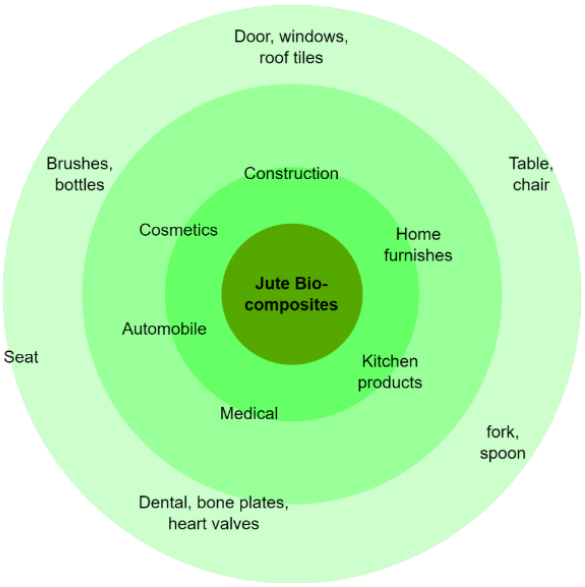

Figure 2: Bio composites of jute

Coir- based biocomposites

Coir, a lignocellulosic natural fiber from coconut husk, primarily sourced in India, resists saltwater and has some waterproof properties. Harvested from mature coconuts, coir fibers are reddish-brown strands of cellulose and lignin. While used in nylon reinforcement, coir's lower cellulose (36–43%) and higher lignin (41–45%) content limit its use in polymer composites. Research aims to find new coir applications, including polymer reinforcement. Strength-wise, cellulose-rich fibers like sisal, jute, and pineapple surpass coir, used in various products like floor furnishings, yarn, and rope. Coir-based biocomposites (figure 3) blend coir fibers with binders and other materials, creating eco-friendly, robust materials for automotive parts and building materials. Natural binders like starch, soy protein, and lignin are cost-effective and biodegradable, while synthetic options lack eco-friendliness. Coir-based biocomposites offer strength, durability, and sustainability as alternatives to conventional composites.

Figure 3: Coir based biocomposites

Sisal- based biocomposites

Dr. Richard Hindorf introduced sisal (*Agave sisalana*) to East Africa in 1893. Sisal, a sturdy drought-resistant plant, yields strong, flexible, water-resistant fiber. Extracted from leaves through mechanical means or retting, sisal fiber comes in lower, medium, and higher grades.
Sisal's robustness makes it a potential filler for thermosets, elastomers, and thermoplastics. Tests included short sisal and glass fiber blends. Sisal is used in carpets, handicrafts, ropes, and twines, finding applications in various industries.

Sisal-based biocomposites (figure 4) mix fibers with binders for improved properties. Sourced from Agave sisalana leaves, sisal offers strength, affordability, and biodegradability. It's used in automotive parts, packaging, and more. Binders can be natural (starch, soy protein, lignin) or synthetic (epoxy, polyester), with these eco-friendly composites offering durability and sustainability, meeting demand for biodegradable materials.

Figure 4: Sisal plant and fibre

Areca- based biocomposites

Arecanut husk fibers are primarily cellulose, along with hemicellulose, lignin, pectin, and protopectin. In Kannada, the well-known nuts of the areca palm are called "Adike" or "Adika." This tall, upright palm grows to varying heights across different environments. Arecanut cultivation covers about 0.78 lakh acres, yielding an average of 5.48 lakh tonnes annually. It thrives in the southern transition, hilly, and coastal zones, growing up to 1000 m above sea level. Husk fiber comprises mainly cellulose, with varying hemicellulose (35–64.8%), lignin (13.1–26.0%), pectin, and protopectin. Its primary constituents are cellulose (43%), crude fiber (33%), and ash (5%), with lignin providing rigidity and color. Researchers have created a water-resistant wastepaper-based paper board, superior to coconut leaf sheath-made boards.

Areca-based biocomposites (figure 5) combine fibers with binders for improved properties. Areca, or betel nut, grows in tropical regions, with its fibers used in rope and twine. These biocomposites are eco-friendly, offering strength, durability, biodegradability, and sustainability. However, further research is needed to optimize their properties and applications.

Figure 5: Areca based bio products

Banana- based biocomposites

Bananas are popular for their high potassium and carb content. "Banana" translates to "finger" in Arabic. Around 20 out of 300 Musaceae species are fit for human consumption. Globally, tropical regions yield roughly 40 million metric tonnes of bananas annually. Al-Qureshi achieved remarkable bonding between fibers and a polymeric matrix, constructing a composite car panel. Soy protein and banana fiber composites were tested for shape, water resistance, and biodegradation, with alkali treatment enhancing mechanical qualities [14]. Durable banana fiber resists decay. Composites blended banana bunch fibers with unsaturated polyester resin. Commercial banana varieties were tested for fiber industry potential. In India, bananas are called "Kalpatharu," reflecting diverse applications. All parts are used, including fruit, leaves, and corm, often as a baby's first solid food. India leads in banana production. NRCB identified banana fiber as a future source, even for plywood and boards.

Banana-based biocomposites (figure 6) combine fibers with binders, enhancing properties. These eco-friendly alternatives repurpose banana byproducts for textiles, paper, and composites, reducing waste. Further research is needed for optimal properties and applications.

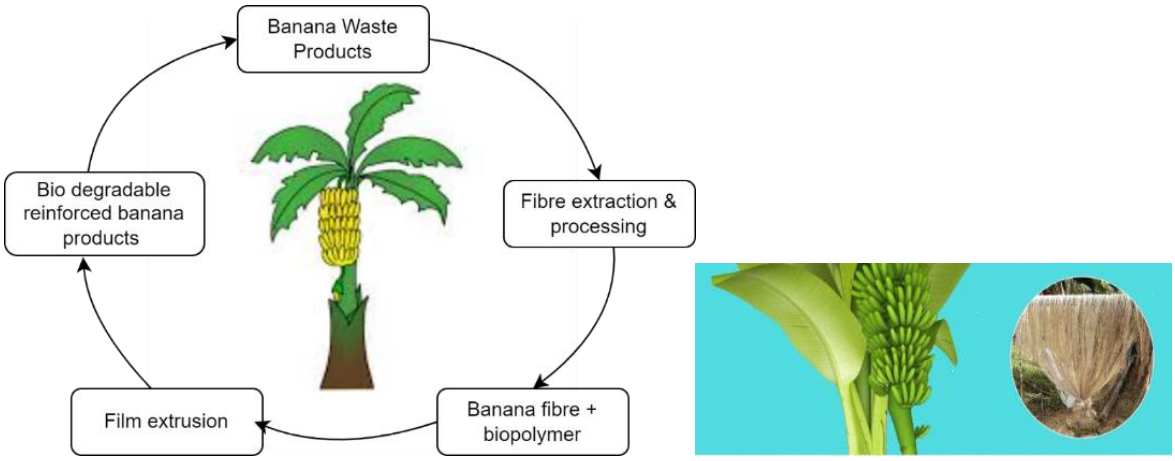

Figure 6: Banana fibre

Applications:

Natural fibers' characteristics are influenced by factors like growing conditions, plant age, species, temperature, humidity, and soil quality. These fibers find use in various industries, such as structural and non-structural composites, geotextiles, packaging, and more. In the automotive sector, natural fiber-reinforced polymers offer advantages like biodegradability, reduced weight, and cost. They're employed for both interior and exterior car components, enhancing fuel efficiency without compromising safety. Train applications include interior paneling, seat panels, soundproofing, luggage racks, and door leaves. Biocomposites are also used in aircraft and naval sectors for interior panels, seat covers, carpeting, insulation, reinforcement, decking, ropes, lines, soundproofing, and more. These natural fiber applications align with sustainability efforts and material innovations, contributing to various industries' advancement.

Conclusion:

In conclusion, using natural fibers in eco-friendly materials is a smart and sustainable idea for India. These fibers are renewable, can break down naturally, and are strong, making them a good option instead of man-made materials. They're useful in many areas like making cars, planes, and buildings. India has a history of using these fibers in things like clothes and crafts. As we focus more on taking care of the environment, using natural fibers in materials becomes more important. India has lots of different fibers like jute, coir, bamboo, and hemp that can be used in materials. Using natural fibers in materials can also help rural areas by creating new jobs and things to sell. But there are challenges, like not having enough places to process the fibers, and making sure the quality is good. Still, the benefits are big – it can help the environment, reduce pollution from making things, and help people in the countryside find work. With support, India can become a leader in making and using these natural fiber materials, and help make the world eco-friendlier. When it comes to transportation, like cars, planes, and ships, a lot of the materials used are not good for the environment. But there are newer materials, like natural fiber composites, that are better. These materials can be made from plants and can be used in different parts of vehicles. They're lighter and can be recycled. Even though it can be a bit harder to make things from these materials, they are better for the Earth. As more people use these materials, it could also help countries like India by creating jobs and using their own resources.

In the future, we could see more and more of these natural fiber materials being used in different parts of India's industries. It's a step towards a greener and more sustainable future.

References:

1. Abdollahiparsa, H., Shahmirzaloo, A., Teuffel, P., & Blok, R. (2023). A review of recent developments in structural applications of natural fiber-Reinforced composites (NFRCs). *Advances in Composite Materials*, 32, 26349833221147540. https://doi.org/10.1177/26349833221147540
2. Markandan, K., & Lai, C. Q. (2023). Fabrication, properties and applications of polymer composites additively manufactured with filler alignment control: A review. *Composites Part B: Engineering*, 256, 110661. https://doi.org/10.1016/j.compositesb.2023.110661
3. Agrawal, R. Y., Majumdar, V. S., Kumar, A., Luthra, S., & Garza-Reyes, J. A. (2023). Opportunities for disruptive digital technologies to ensure circularity in supply Chain: A critical review of drivers, barriers and challenges. *Computers & Industrial Engineering*, 178, 109140. https://doi.org/10.1016/j.cie.2023.109140
4. Aziz, T., Haq, F., Farid, A., Kiran, M., Faisal, S., Ullah, A., & Show, P. L. (2023). Challenges associated with cellulose composite material: Facet engineering and prospective. *Environmental Research*, 223, 115429. https://doi.org/10.1016/j.envres.2023.115429
5. Shelar, A., Nile, S. H., Singh, A. V., Rothenstein, D., Bill, J., Xiao, J., & Patil, R. (2023). Recent advances in nano-enabled seed treatment strategies for sustainable agriculture: Challenges, risk assessment, and future perspectives. *Micro & Nano Letters*, 15(1), 54. https://doi.org/10.1007/s40820-023-01025-5
6. Karimi, H., Mahdavi, S., Asgari Lajayer, B., Moghiseh, E., Rajput, V. D., Minkina, T., & Astatkie, T. (2023). Insights on the bioremediation technologies for pesticide-contaminated soils. *Environmental Geochemistry and Health*, 44(4), 1329-1354. https://doi.org/10.1007/s10653-021-01081-z
7. Kamarudin, S. H., Mohd Basri, M. S., Rayung, M., Abu, F., Ahmad, S. B., Norizan, M. N., & Abdullah, L. C. (2022). A Review on Natural Fiber Reinforced Polymer Composites (NFRPC) for Sustainable Industrial Applications. *Polymers*, 14(17), 3698. https://doi.org/10.3390/polym14173698
8. Akhil, U. V., Radhika, N., Saleh, B., Aravind Krishna, S., Noble, N., & Rajeshkumar, L. (2023). A comprehensive review on plant-based natural fiber reinforced polymer composites: Fabrication, properties, and applications. *Polymer Composites*. https://doi.org/10.1002/pc.27274

9. Karthik, A., Sampath, P. S., Thirumurugan, V., & Prakash, C. (2023). Effect of sample cutting angle on mechanical properties of jute/cotton fabric epoxy composite laminates. *Biomass Conversion and Biorefinery*, 1-11. https://doi.org/10.1007/s13399-023-04166-0
10. Bijalwan, A., Upadhyay, A. P., & Dobriyal, M. J. (2015). Agroforestry for Biodiversity Conservation. *International Journal of Current Research and Biosciences Plant Biology*, 2(6), 214-217.
11. Kain, V., Sinha, D. K., Singh, D., & Asnani, C. K. (2022). Atomic minerals: journey of India to self-sufficiency. *Current Science*, 123(3), 293-309. https://doi.org/10.18520/cs/v123/i3/293-309
12. Kumar, R., Mishra, S. K., & Kumar, K. (2023). A Critical Review on Finite Element Models Towards Physico-Mechanical Properties of Bamboo Fibre/Filler-Reinforced Composite Materials. In *Bamboo and Sustainable Construction* (pp. 241-261). https://doi.org/10.1007/978-981-99-0232-3_9
13. Bavan, D. S., & Kumar, G. M. (2010). Potential use of natural fiber composite materials in India. *Journal of Reinforced Plastics and Composites*, 29(24), 3600-3613. https://doi.org/10.1177/0731684410381151
14. Sathishkumar, T. P., Navaneethakrishnan, P., Shankar, S., Rajasekar, R., & Rajini, N. (2013). Characterization of natural fiber and composites–A review. *Journal of Reinforced Plastics and Composites*, 32(19), 1457-1476. https://doi.org/10.1177/0731684413495322

UNVEILING THE SYNERGISTIC EFFECT OF *COUROUPITA GUIANENSIS*

Rajaruban M D S*, Shanthini P and Thirisha Bharathi GK

Department of Food Technology, Paavai Engineering College

(Affiliated by Anna University, Namakkal, India)

*Corresponding author E-mail: rajaruban195@gmail.com

Introduction:

In the intricate tapestry of plant interactions, the synergistic effect emerges as a pivotal phenomenon, embodying the amplified benefits and growth outcomes resulting from the collaborative interplay among diverse plant species. The synergies of Cannonball tree arise from its diverse array of compounds that work together to potentially enhance remedial effect. As we embark on the exploration of plant synergies, this book chapter seeks to unravel the synergistic effect of *Couroupita guianensis*. This plant exhibits an elevated concentration of bioactive compounds, earning it the recognition as the sacred plant of India. *Couroupita guianensis* ("Nagalinga" in Hindi, "Cannonball tree" in English) is a fast-growing gigantic tree, widely screened for its various pharmacological activities. It is common among tribes and rural people and widely used in traditional medicine, while all plants share similar therapeutic activities, this plant demonstrates specialized therapeutic effects against malarial disease. Hence, it is bestowed with the title of the sacred plant of India. However, its crucial to note that more research is needed to confirm and detailed these synergistic interactions in this plant.

Taxonomy

Kingdom	Plantae
Clade	Tracheophytes
Order	Ericales
Family	Lecythidaceae
Genus	*Couroupita*
Species	*C. guianensis*

Figure 1: *Couroupita guianensis*

Description

Couroupita guianensis, also known as the cannonball tree, is a majestic and towering species that can reach heights of up to 30-35 meters. Recognizable by its distinctively clustered leaves at the branch ends, these leaves typically measure between 8 and 31 centimeters in length, occasionally extending to an impressive 57 centimeters. The tree is renowned for its remarkable spherical fruits resembling cannonballs, which house seeds and add to its unique allure. The flowers are large and flashy, show up in various colors like orange, scarlet or pink with a pleasant fragrance, almost throughout the year. Native to the rainforests of South and Central America, *Couroupita guianensis* is not only a botanical marvel but also holds cultural significance in various regions. Its charismatic presence, coupled with fascinating reproductive features, makes it a captivating subject for exploration and study within the realm of botany and natural history.

Table 1: Chemical constituents of *C. guianensis.*

Part of the plant	Chemical constituents
Fruit	Couroupitine A (tryptanthrin), Couroupitine B (indirubin), malic acid, isocitric acid, stigmasterol, campesterol, hopane, rutin, quercetin, kaempherol, farmaricetin, luteolin and ursolic acid
Flower	Eugenol, linalool, (E, E)-farnesol, nerol, geraniol, (Z, E)-farnesol, vanillin, limonene and geranial, (E, E)- farnesyl acetate, trans ocimene, nootkatone, geraniol, 2-isopropenyl-5-methyl-4-hexenyl acetate), cedr-8- en-13-ol, (E, Z)-farnesyl acetate, methyl (11E)-11-hexadecenoate, isatin, cycloart-24-en3-ol-3'-exomethylene heptadeconate, stigmasterol, p-coumaric acid, o-coumaric acid, caffeic acid, quercetin, octyl 4-(nonanoyloxy) benzoate, myristoleic acid, linoleic acid, (8E, 10E, 12E)-icosa-8, 10, 12-triene)
Leaf	Triterpenic ester β-amirin palmitate, hydroxycinnamic acids, caffeic acid, rosmarinic acid, kaempferol-3- O-neohesperidoside, 20, 40-dihydroxy-60-methoxy-30,50-dimethylchalcone,7-hydroxy-5-methoxy-6, 8- dimethylflavanone, 4-hydroxybenzoic acid
Seed	Indigo, indirubin, stigmasterol, campesterol, linoleic acid, nerol, tryptanthrin
Stem and Bark	Phytosterol, β-amyrin, betulin-3β-caffeate and lupeol-3β-caffeate, couropitone (stigmasta-4,23(E)-dien-3-one 1), β-amyrin, β-amyrone, β-amyrin acetate, stigmasterol, ergosta-4,6,8(14), 22-tetraen-3-one, β-sitosterol and its glycoside

Synergistic effects

Almost all the parts of Cannonball tree, namely Leaves, Flowers, Roots, Fruits, Stem, Seed and Bark exhibits various effect.

Floral:

Silver nanoparticles, when introduced, demonstrate significant antibacterial effects on both Gram positive (*B.* subtilis) and Gram negative (*Escherichia coli*) bacteria. Notably the synergistic combination with the extract from the fruits of *Lageretroemia speciose* and flowers of *Couroupita guianensis* flower petals enhances their antibacterial properties, yielding a novel and cost-effective formulations with increased therapeutic potential. The mixture from fruits of *Lageretroemia speciose* and flowers of *Couroupita guianensis* with effective antibacterial effect where *C.guianensis* with Istatin compound is more effective. Isatin is one of the effective components of the medicinal plant *Couroupita guianensis*, known well for its cytotoxic action contrary to certain lines of tumor cells and it is found to be a potential source of new chemotherapy agents. It is used to treat hypertension, tumors, pain, inflammatory processes, cold, stomachache (norovirus), skin diseases, malaria (rotavirus).

Leaves:

In the synthesis of metal oxide nanoparticles (NPs), plant materials, particularly leaves of *Couroupita guianensis* are selected. This preference is attributed to the abundance of effective phytochemicals in the extracts, including ketones, flavones, aldehydes, amides, phenols, ascorbic acids, and terpenoids. These phytochemicals present in the extract may act as a reducing agent and plays a crucial role in the synthesis process, influencing the characteristics of the resulting ytterbium oxide ($Yb_2O_{3)}$ nanoparticles (NPs)**.**

Depending on the phytochemical composition of the plant extract, the nanoparticle may exhibit antioxidant property, which could be beneficial for certain biological applications. The choice of *Coroupita guianensis* leaves extract adds a botanical dimension to the synthesis process. The antibacterial activity of Yb_2O_3 nanoparticles assisted by ionic liquid (IL) demonstrates greater inhibition against *E. coli* and *S. aureus* compared to NPs and the standard amikacin. This suggests that the presence of the ionic liquid enhances the antibacterial efficacy of Yb_2O_3, making it a potentially effective antimicrobial agent against both *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*).

Fruit:

Examining an excision wound model, the study carried out by (*L. Anna sebha etal.*,2023) assessed the wound healing potential of the ethanolic extract from *C. guianensis* fruit pulp. This extract was then formulated into ointments with varying concentrations (2.5%, 5%, and 10%) using PEG (Polyethylene glycol).

Three Wistar albino rats were chosen based on dosage applied to the rats as test subjects due to their suitability for surgical procedures and the ease of measuring wound healing parameters. Thus, high dose *Couroupita guianensis* fruit pulp ointment accelerates wound healing more rapidly in 15 days than other mid and low dose ointments which takes around 20 days. The CGEE ointment had shown a wound closure of 80.27% with low and mid dose treated groups in 15 days while the complete closure was accomplished in 20 days. The standard betadine ointment had shown 91% closure and takes up to 20 days.

The increasing preference for herbal shampoo is driven by the belief in its safety and the desire for products free from side effects. Herbal shampoos derived from fruit pulp of *Couroupita guianensis* offer added advantage such as reduced hair loss, long-lasting color, lustrous and fortified hair, anti-irritant properties, and moisture retention. Their functions include lubrication, conditioning, promoting hair growth, maintaining hair color, and even providing medicinal benefits. The desired properties encompass easy application, effective debris removal, pleasant fragrance, low irritation, good preservation, and stability.

Table 2: Pharmacological activities of *C. guianensis*

Plant part	**Pharmacological activities**
Flower and bark	Analgesic and anti-inflammatory, Antiulcer
Leaves	Anti-inflammatory, Antidiarrheal, ovicidal, Antinociceptive, Antifeedant and Larvicidal
Fruit pulp	Antibacterial
Oil	Antibacterial
Flowers	Antioxidant, Antimicrobial, Immunomodulatory
Dried flowers	Anticancer and antioxidant
Root	Anti-depressant
Bark and flowers	Antifertility
Fruits	Antimicrobial, Anti-mycobacterial, Antibiofilm, Antifungal
Flower, Bark	Antipyretic

Toxicity

Ingesting this plant can be toxic and handling it may lead to skin irritation or allergies. The fruit is considered edible, yet it's uncommonly consumed by humans due to its disagreeable odor, despite the intense fragrance of its flowers. Instead, it is often given to animals like pigs and domestic fowl. The various parts of the *Couroupita guianensis* tree may have different applications, but it's essential to note that scientific research on its medicinal properties and potential side effects is limited. Here are some general uses of different parts,

- **Flowers:**

The flowers are often admired for their beauty and fragrance. In some traditional practices, extracts from the flowers have been used for their aromatic qualities, but medicinal uses and potential side effects are not well-documented.

- **Leaves:**

Limited information is available on the medicinal uses of the leaves. Traditionally, certain communities may have utilized them for various purposes, but it's crucial to approach such uses with caution without scientific validation.

- **Fruits:**

The cannonball-like fruits of *Couroupita guianensis* are not typically used for medicinal purposes. They are more known for their ornamental value.

- **Bark:**

In some traditional practices, the bark of the tree may have been used for various purposes. However, scientific evidence supporting its medicinal benefits or detailing potential side effects is lacking. Again, it's vital to consult with a healthcare professional or an herbalist before using a part *Couroupita guianensis* for medical application.

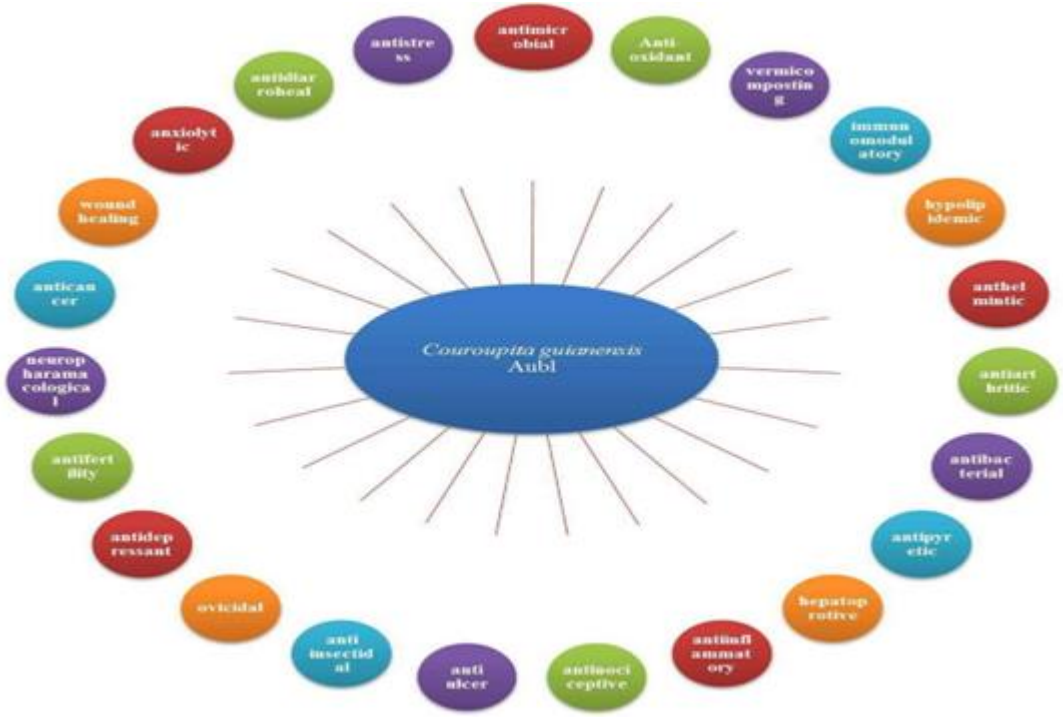

Figure 2: Activities of *C. guianensis*

Silver Nanoparticles vs *C. guianensis*

The current study showcases the synthesis of stable silver nanoparticles (AgNPs) mediated by *Couroupita guianensis* flower bud extract. The extract from buds aged 5 days exhibited greater efficiency in terms of its ratio to the silver solution (1:3000), which is

significantly lower compared to previous findings (Usha Rani and Rajasekharreddy *et al*., 2011), along with a faster rate of reduction. Therefore, the *Couroupita* flower bud extract demonstrates superior efficacy in reducing silver ions and stabilizing the resultant AgNPs compared to earlier reports on green synthesis (Logeswari *et al.*, 2012). This could be attributed to the higher concentration of reducing agents or more effective compounds and stabilizing agents present in the extract during that specific stage of bud development.

The potent antibacterial effectiveness of the synthesized AgNPs against both Gram-positive and Gram-negative bacteria suggests the possibility of formulating synergistic bactericides for biomedical applications by combining the antibacterial properties of *Couroupita* flower bud extract with silver salts. The proteins found in the aqueous extract of *Couroupita* flower buds demonstrated effective reduction of silver ions and stabilization of nanoparticles. These spherical nanocrystals had an average size of 17 nm and exhibited mono dispersity. The combination of silver nanoparticles in nano form with phytochemical coating showed synergistic antimicrobial activity.

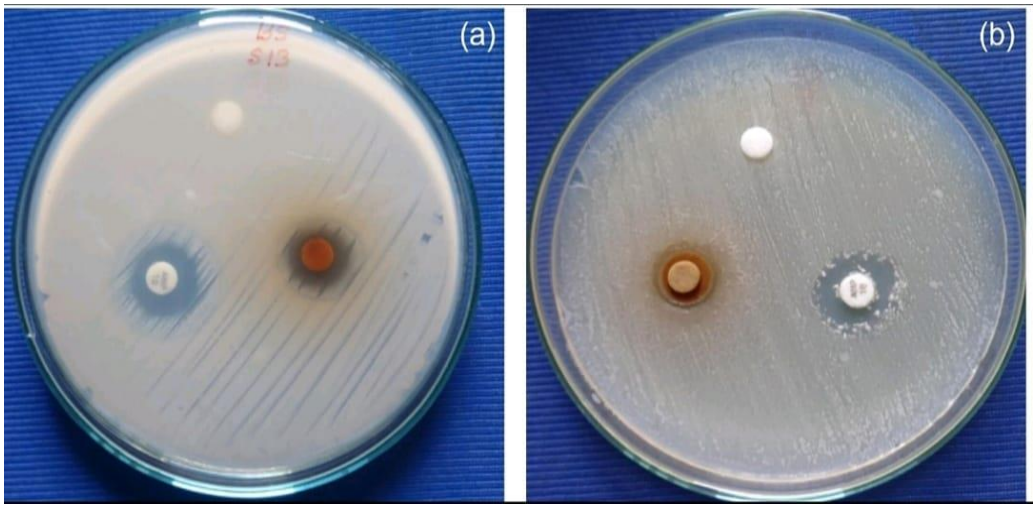

Figure 4: (a-b). Antibacterial activity of AgNPs against Gram Positive (*Bacillus subtilis*) and Gram Negative (*Escherichia Coli*) stains.

Recently, there has been a proliferation of plant-based synthesis methods for producing metal nanoparticles. In a study carried out by (Logambal *et al.*, 2022) the extract from the medicinal plant *Couroupita guianensis* (CG) petals was employed to synthesize biogenic silver nanoparticles in an eco-friendly manner. Various techniques, such as ultraviolet-visible spectroscopy (UV-vis) and Fourier Transform Infrared (FTIR) spectroscopy, were utilized to characterize the nanoparticles**.**

In the present investigation, it was found that the extracts derived from the flower petals of *Couroupita guianensis* are capable of rapidly synthesizing silver nanoparticles in an environmentally friendly, cost-effective, and sustainable manner. The adequate reduction and

stabilization of silver nanoparticles are due to phytochemicals, such as flavonoids, proteins, and phenolic compounds, which are produced in the extract of flower petals from *Couroupita guianensis*. Silver nanoparticles had remarkable antibacterial action against gram-positive bacteria when compared to Gram-negative bacteria (*Escherichia coli*) (*Bacillus subtilis*).

Emerging trends

In recent years, there has been a surge in research exploring the pharmacological potential of *Couroupita guianensis*, commonly known as the cannonball tree. Studies have investigated its antimicrobial properties, revealing its effectiveness against a range of bacteria and fungi.

Additionally, researchers have found that extracts from *Couroupita guianensis* possess antioxidant properties, making them potentially valuable in combating oxidative stress-related diseases. Research into *Couroupita guianensis* has highlighted its potential in combating oxidative stress-related diseases due to its antioxidant properties. Oxidative stress occurs when there is an imbalance between free radicals and antioxidants in the body, leading to cellular damage and the development of various diseases such as cancer, cardiovascular diseases, neurodegenerative disorders, and inflammatory conditions.

Furthermore, there is growing interest in *Couroupita guianensis* in the field of nanotechnology. Researchers have successfully utilized extracts from the tree in the synthesis of silver nanoparticles, which have shown potential for various biomedical applications due to their antimicrobial properties.

In a study carried out by (Swetha *et al.,* 2020) the anti-malarial activity of five distinct extracts from *Couroupita guianensis*, flowers were evaluated using an in vitro β hematin formation assay, alongside High-Performance Thin-Layer Chromatography (HPTLC) fingerprinting analysis. Among the different extracts of *Couroupita guianensis* (CG) flowers, the ethyl acetate extract exhibited significant anti-malarial activity in the β-hematin formation assay. Subsequent isolation and characterization of the active compound revealed it to be Stigmasterol.

In addition to its pharmacological significance, *Couroupita guianensis* holds cultural and ecological importance. It is revered in traditional medicine and is used in various cultural practices. However, due to factors such as habitat loss and overexploitation, conservation efforts are underway to preserve this valuable plant species. Overall, recent trends in *Couroupita guianensis* research encompass its pharmacological potential, applications in nanotechnology, and efforts towards its conservation and ecological preservation.

Conclusion:

In conclusion, the synergistic effects observed in *Couroupita guianensis* underscore the intricate interplay of its bioactive compounds, showcasing its potential for diverse therapeutic

applications. From enhancing the antibacterial properties of silver nanoparticles to accelerating wound healing and even contributing to the formulation of herbal shampoos, the collaborative synergy among the plant's components amplifies its remedial effects. While this chapter has shed light on the synergistic interactions of *Couroupita guianensis*, further research is imperative to fully elucidate and harness the potential synergies within this sacred plant of India. As we delve deeper into understanding these synergistic mechanisms, we pave the way for harnessing the full therapeutic potential of *Couroupita guianensis* in both traditional and modern medicinal contexts.

References:

1. Abdelghany, T.M., *et al.* (2018). Recent Advances in Green Synthesis of Silver Nanoparticles and Their Applications: About Future Directions. A Review. *Bionanoscience, 8*, 5–16.
2. Baskar, K., S., *et al.* (2015). Toxic effects of Couroupita guianensis against *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). *Neotropical entomology*, *44*, 84-91.
3. Benelli, G., & Govindarajan, M. (2017). Green-Synthesized Mosquito Oviposition Attractants and Ovicides: Towards a Nanoparticle-Based "Lure and Kill" Approach? *J. Clust. Sci., 28*, 287-308.
4. Costa, D.C.M., *et al.* (2017). In vitro anti-MRSA activity of *Couroupita guianensis* extract and its component Tryptanthrin. *Natural Product Research, 31*(17), 2077-2080.
5. Desal T, Golatakar SG, *et al.* (2003). Larvicidal property of *Couroupita guianensis* Abul. *Indian Drugs, 40*, 484-6.
6. Elangovan, K., *et al.* (2015). Phyto Mediated Biogenic Synthesis of Silver Nanoparticles Using Leaf Extract of Andrographis Echioides and Its Bio-Efficacy on Anticancer and Antibacterial Activities. *J. Photochem. Photobiol. B, 151*, 118–124.
7. Gothai, S., *et al.* (2018). In vitro-scientific evaluation on anti-*Candida albicans* activity, antioxidant properties, and phytochemical constituents with the identification of antifungal active fraction from traditional medicinal plant *Couroupita guianensis* Aubl. Flower. *Journal of Complementary Medicine Research*, *8*(2), 85-85.
8. Huy, T.Q., & Huyen, P.T.M., *et al.* (2019). Recent Advances of Silver Nanoparticles in Cancer Diagnosis and Treatment. *Anticancer Agents Med. Chem., 20*, 1276–1287.
9. Jayapal Subramaniam, *et al.* (2016). Multipurpose effectiveness of *Couroupita guianensis*-synthesized gold nanoparticles: high antiplasmodial potential, field efficacy against malaria vectors and synergy with Aplocheilus lineatus predators. (pp. 7543-7558).
10. Kavitha R, *et al.* (2011). In vitro antimicrobial activity and phytochemical analysis of Indian medicinal plant *Couroupita guianensis* Aubl. *J Chem Pharm Res, 3*(6), 115-21.

11. Netala, V.R., *et al.* (2015). First Report of Biomimetic Synthesis of Silver Nanoparticles Using Aqueous Callus Extract of *Centella Asiatica* and Their Antimicrobial Activity. *Appl. Nanosci., 5*, 801–807.
12. Pradhan D, Panda PK, Tripathi G., *et al.* (2009). Evaluation of immunomodulatory activity of methanolic extract of *Couroupita guianensis* Aublet. Flowers in rats. *Nat Prod Radiance, 8*(1), 37-42.
13. Premanathan M, *et al.* (2012). Antioxidant & anticancer activities of isatin (1H-indole-2,3-dione), isolated from the flowers of *Couroupita guianensis* Aubl. *Indian J Med Res, 136*(5), 822-6.
14. Rajesh Kumar, T., *et al.* (2016). Evaluation of silver nanoparticles synthetic potential of *Couroupita guianensis* Aubl., flower buds extract and their synergistic antibacterial activity. *3 Biotech, 6*, 1-9.
15. Rane JB, Vahanwala SJ, *et al.* (2001). Chemical examination of the flowers of *Couroupita guianensis* Aubl. *Indian J Pharm Sci, 63*(1), 72-3.
16. Saratale, Ganesh Dattatraya, *et al.* (2017). Anti-diabetic potential of silver nanoparticles synthesized with Argyreia nervosa leaf extract high synergistic antibacterial activity with standard antibiotics against foodborne bacteria. *Journal of Cluster Science, 28*, 1709-1727.
17. Sathishkumar, G., *et al.* (2018). Green synthesis of magnetic Fe3O4 nanoparticles using *Couroupita guianensis* Aubl. fruit extract for their antibacterial and cytotoxicity activities. *Artificial cells, nanomedicine, and biotechnology, 46*(3), 589-598.
18. Sheba, L.A., & Anuradha, V. (2019). An updated review on *Couroupita guianensis* Aubl: a sacred plant of India with myriad medicinal properties. *Journal of Herbmed Pharmacology, 9*(1), 1-11.
19. Subramaniam, J., *et al.* (2016). Multipurpose effectiveness of Couroupita guianensis-synthesized gold nanoparticles: high antiplasmodial potential, field efficacy against malaria vectors and synergy with Aplocheilus lineatus predators. *Environmental Science and Pollution Research, 23*, 7543-7558.

PIONEERING SUSTAINABLE AGRICULTURE: THE JOURNEY FROM APOMIXIS TO SYNTHETIC APOMIXIS

Shreya Singh and Sourav Ranjan Nanda*

Birsa Agriculture University, Ranchi, Jharkhand

*Corresponding author E-mail: srnanda5143@gmail.com

Abstract:

Apomixis, a form of asexual reproduction in plants, offers unique advantages such as genetic stability, trait perpetuation, and accelerated variety development. However, natural apomixis is limited to few crop species, prompting the exploration of synthetic apomixis through innovative molecular strategies. This chapter explores the evolutionary significance, genetic mechanisms, and potential applications of apomixis in plant breeding and crop improvement. It also discusses advancements in creating synthetic apomixis, including the MiMe strategy and genome editing techniques, highlighting their potential to revolutionize clonal seed production, accelerate breeding programs, and enhance crop resilience. By elucidating the genetic and evolutionary implications of apomixis and its synthetic counterparts, this chapter provides insights into harnessing apomixis for sustainable agriculture.

Understanding apomixis: An overview

In flowering plants, apomixis is a form of asexual reproduction in which the embryos develop without the requirement of fertilization. This asexual process usually takes place in the maternal tissues of the ovule, resulting in the formation of the embryo.

Why apomixis?

Sexual mechanisms are vital in plant breeding, facilitating genetic recombination. However, manipulating these mechanisms for breeding purposes presents various challenges. Continuous selection and breeding for specific traits can lead to diminishing heterozygosity, reducing genetic diversity within populations and rendering them more susceptible to environmental stresses. Additionally, the segregation of gene combinations during sexual reproduction can result in the loss of favorable gene combinations, disrupting desirable traits in subsequent generations. This process, along with intensive breeding practices, can lead to genetic erosion, gradually depleting valuable alleles and genetic resources, further limiting the adaptability and resilience of plant populations. This limitation underscores the importance of exploring alternative reproductive strategies such as apomixis, which bypasses the recombination process and produces clonal offspring with identical genetic makeup to the parent plant.

Apomixis plays a pivotal role in plant breeding as an alternative reproductive strategy with unique advantages. By enabling asexual reproduction via seeds, apomixis allows for the perpetuation of specific genotypes without the genetic reshuffling typical of sexual reproduction. This trait is invaluable for maintaining desirable genetic combinations associated with traits like high yield and disease resistance, ensuring their stability and uniformity across generations. Moreover, the genetic stability inherent in apomixis streamlines the preservation of consistent traits in plant populations, crucial for crop production where predictability is key. Additionally, apomixis accelerates variety development by bypassing the lengthy processes of sexual hybridization and trait stabilization, facilitating the rapid deployment of improved cultivars. Furthermore, apomixis contributes to the conservation of genetic resources by preserving specific genotypes and rare alleles, safeguarding genetic diversity. In essence, apomixis offers a promising avenue for enhancing breeding programs and developing genetically stable plant varieties, making it a valuable tool in modern plant breeding efforts.

Gaining insights into natural apomixis is crucial for its potential applications in crop improvement. Most major crops lack natural apomixis, making it essential to study its origin and maintenance in natural plant systems. Understanding the genetic mechanisms underlying apomixis can inform the development of breeding strategies to introduce this reproductive mode into crop plants, enhancing their genetic stability and uniformity. Furthermore, by studying the factors governing natural apomixis, breeders can identify optimal genetic backgrounds and environmental conditions for developing apomictic cultivars with improved traits like yield and disease resistance. Moreover, natural apomixis contributes to preserving genetic diversity by maintaining specific genotypes and rare alleles, aiding conservation efforts. Additionally, integrating apomixis into crop plants can promote sustainable agriculture by reducing the need for chemical inputs and resources associated with traditional breeding methods, thereby fostering long-term soil health and biodiversity. Overall, understanding natural apomixis holds promise for enhancing crop improvement efforts and fostering the development of more resilient and productive agricultural systems.

Types of apomixis

A. Diplospory- Diplospory, a type of apomixis, encompasses the creation of two unreduced megaspore mother cells (MCs) via either restitutional meiosis or mitotic division. This deviation from meiosis bypasses the typical reduction division, facilitating the development of unreduced female gametophytes and subsequent seed formation without the need for fertilization. This unique reproductive strategy enables plants to reproduce asexually, circumventing the conventional sexual reproductive process.

B. Apospory- Apospory involves the development of an embryo sac from a somatic, unreduced cell of the nucellus, circumventing the traditional sexual reproductive pathway. By sidestepping fertilization, apospory allows for the generation of embryos and subsequent seed production without the need for pollination. This mechanism represents another avenue of apomixis, providing plants with a means of asexual reproduction independent of sexual processes.

C. Adventitious embryony- Adventitious embryony represents an alternative developmental route to apomixis, commonly found in diverse taxa without interfering with sexual reproduction. This mechanism, frequently observed in polyploids and hybrids, can result in the production of multiple seedlings within a single seed, termed polyembryony. By enabling plants to reproduce clonally and produce seeds containing numerous embryos, adventitious embryony amplifies the capacity for asexual reproduction within specific plant populations.

Biology of natural apomixis

How does an apomictic individual arise?

The emergence of an apomictic individual can manifest through two primary scenarios: seed dispersal and spontaneous shifts within a sexually reproducing population. In the former, seeds from an apomictic source population are dispersed, giving rise to seedlings that establish a new apomictic population. Alternatively, a spontaneous shift to apomixis may occur within an otherwise sexually reproducing individual, resulting in the development of an apomictic plant amidst a sexual population. Hybridization, particularly between different cytotypes, has long been recognized as a significant trigger for apomixis. This process can lead to the formation of unreduced female gametophytes, a critical step toward apomixis. Genetic studies further support the notion that many natural apomictic taxa have hybrid origins, with complex genetic interactions governing the transition to asexual reproduction. Spontaneous emergence of apomixis, particularly in diploid species and hybrid populations, underscores the flexibility of plant reproductive systems.

How does an apomictic population establish in a natural plant population?

During the establishment phase, the uncoupled expression of apomixis developmental steps becomes functional in the establishment of a polyploid apomictic population. This phase requires the coordinated activation of apomeiosis (reduction division bypass) and parthenogenesis (development of an unfertilized egg cell) to produce clonal seeds. The establishment of an apomictic population often involves shifts in ploidy levels, with polyploid individuals stabilizing the expression of apomixis elements and facilitating the production of clonal seeds. Triploid intermediaries may contribute to the formation of polyploid individuals, similar to mechanisms observed in sexual systems. The presence of partial apomixis and

uncoupled parthenogenesis can lead to diverse outcomes during the establishment of apomictic populations, contributing to genetic and phenotypic diversity. Additionally, polyploidy, common in plant evolution, indirectly promotes apomixis by creating reproductive barriers against diploid populations, disrupting genetic self-incompatibility systems, and enhancing adaptive potentials. Population dynamics, including interactions between cytotypes and mixed ploidy levels, influence the establishment and persistence of apomictic populations, highlighting the importance of understanding genetic diversity and ecological interactions within these populations for studying long-term evolutionary trajectories.

In what ways do apomicts expand their range and undergo speciation?

During the diversification phase, a crucial stage in the evolutionary trajectory of apomixis, apomictic populations diversify, expand their ranges, and potentially give rise to new species. This phase is characterized by geographical parthenogenesis, where apomictic populations colonize new ecological niches, aided by their ability to produce clonal seeds. Polyploidy, often associated with apomictic plants, enhances their adaptive potential by increasing genetic diversity and facilitating rapid adaptation to diverse environmental conditions. As apomictic populations expand their ranges, they may undergo genetic and morphological changes, accumulating genetic diversity, developing unique traits, and acquiring barriers to gene flow, ultimately leading to speciation. Additionally, the independent evolution of apomictic populations from their parental sexual species contributes to the formation of distinct lineages and potentially new species. Successful diversification and speciation depend on various ecological factors, highlighting the importance of understanding the ecological dynamics of apomictic populations for predicting their long-term evolutionary outcomes.

Mechanism of apomixis

The phenomenon of apomixis relies on three pivotal developmental components:

1. Apomeiosis: This phase initiates the formation of cells capable of generating embryos without undergoing meiotic division. Unlike sexual reproduction, where meiosis shuffles genetic material to produce genetically diverse offspring, apomeiosis ensures the preservation of the maternal genotype. Through this mechanism, specific cells acquire the ability to develop into embryos without the genetic recombination characteristic of sexual reproduction.

2. Parthenogenesis: Parthenogenesis facilitates the development of embryos from unfertilized egg cells within the ovule. In contrast to sexual reproduction, where fertilization by male gametes is essential for embryo formation, parthenogenesis enables the autonomous development of embryos. This process allows apomictic plants to reproduce asexually, producing seeds that contain embryos genetically identical to the maternal plant. By bypassing

fertilization, parthenogenesis ensures the perpetuation of specific genotypes and eliminates the need for pollination.

3. Endosperm Development: Endosperm, a nutritive tissue surrounding the embryo within the seed, plays a crucial role in seed formation and development. In apomictic plants, endosperm development can occur autonomously or pseudogamously. Autonomous endosperm formation involves the independent development of endosperm without fertilization, providing nutrients and support to the developing embryo. Alternatively, pseudogamous endosperm development mimics the process of sexual reproduction, where fusion of male and female gametes results in endosperm formation. This flexibility in endosperm development allows apomictic plants to successfully produce seeds in the absence of sexual reproduction.

These key developmental components collectively constitute the mechanism of apomixis, enabling plants to reproduce asexually and produce seeds containing genetically identical offspring.

Genetic and evolutionary implications of apomixis

Genetic implications- Clonal propagation, a hallmark of apomixis, enables plants to replicate offspring that mirror the genetic makeup of the parent plant. Unlike sexual reproduction, which involves genetic recombination, apomixis ensures the faithful preservation of specific genotypes. This characteristic proves advantageous in agriculture and horticulture, facilitating the perpetuation of desirable traits in crops and ornamental plants.

Apomixis plays a pivotal role in fixing heterosis, also known as hybrid vigor, which refers to the enhanced fitness and performance of hybrid offspring compared to their parents. By perpetuating successful hybrid combinations without the need for sexual recombination, apomixis serves to stabilize and perpetuate heterosis. This phenomenon holds particular significance in agriculture, where hybrid crops often exhibit superior traits such as increased yield and disease resistance.

Furthermore, the application of apomixis in crop seed production holds significant promise for revolutionizing agricultural practices. By generating seeds without fertilization, apomictic crops offer the potential to maintain specific genotypes consistently across generations. This innovation could lead to enhanced crop uniformity, reduced seed production costs, and a more dependable yield.

Evolutionary implications- Apomixis fosters reproductive isolation within asexual populations by enabling the development of seeds without the need for pollination. This autonomous seed formation results in genetic isolation from sexually reproducing populations, leading to the establishment of distinct gene pools and independent evolutionary trajectories over time.

Furthermore, apomixis contributes significantly to biodiversity and speciation by facilitating the evolution of apomictic lineages. As these lineages evolve independently, they accumulate genetic variations, fostering ecological speciation as they adapt to diverse niches or habitats. Consequently, apomixis serves as a mechanism for generating diversity within plant species, contributing to the overall richness of biodiversity.

Moreover, apomixis provides stability in challenging environments by ensuring the persistence of successful genotypes even under adverse conditions. The ability to reproduce asexually allows apomictic lineages to thrive when pollinators are scarce, environmental conditions are harsh, or disturbances occur. This resilience enhances the long-term survival prospects of apomictic populations, contributing to ecosystem stability and biodiversity conservation.

Limitations of natural apomixis and need for synthetic apomixis

Aspect	Natural Apomixis	Synthetic Apomixis
Genetic Diversity	Offsprings are genetically identical to mother plant.	Aims to combine reliable trait transmission with genetic diversity. Introduces genetic variation while retaining apomictic reproduction.
Complexity of Mechanisms	The underlying genetic and molecular mechanisms are intricate and not fully understood.	Researchers engineer synthetic apomixis by introducing specific genes to mimic apomictic processes, simplifying the system and allowing targeted manipulation.
Crop Species Availability	Only a few crop species exhibit natural apomixis, such as grasses like Poaceae and some citrus varieties.	Synthetic apomixis has the potential to extend this reproductive mode to a broader range of crops, enhancing breeding efficiency.
Seed Production Challenges	Seed production can be erratic due to environmental conditions and genetic instability.	Engineered synthetic apomixis ensures consistent seed production, benefiting agriculture and crop improvement.
Heterosis Utilization	Does not exploit heterosis (hybrid vigor) as offspring are genetically identical.	Allows the stable transmission of superior traits across generations by combining apomixis with heterosis, enhancing crop performance.

In conclusion, the comparison between natural and synthetic apomixis underscores the inherent limitations of natural apomixis. These limitations emphasize the need for synthetic apomixis as a viable alternative, offering solutions to overcome these challenges.

Synthetic apomixis: An introduction

The phenomenon of apomixis has long fascinated researchers and breeders, as it offers the potential for replicating desirable traits in a predictable and efficient manner. One approach to harnessing the power of apomixis is through synthetic apomixis, a technique that aims to induce apomixis in plants that do not naturally exhibit this trait. Synthetic apomixis involves manipulating the reproductive process in plants to mimic the natural occurrence of apomixis.

Advancements in creating synthetic apomixis

Creating synthetic apomixis involves manipulating a plant's reproductive mechanisms at the molecular level to induce a form of asexual reproduction resembling natural apomixis. This innovative technique aims to produce clonal seeds carrying desired traits by circumventing the processes of meiosis and fertilization. Here are the key stages involved in engineering synthetic apomixis:

1. **Apomeiosis induction**: A crucial aspect of synthetic apomixis is initiating apomeiosis, where unreduced (diploid) gametes are generated directly from somatic cells without undergoing meiosis. This bypassing of meiosis enables plants to produce gametes containing the same genetic information as the parent plant.

2. **Autonomous embryo formation:** In synthetic apomixis, embryos develop autonomously without the need for fertilization. This process triggers the formation of embryos from cells within the ovule, resulting in the production of clonal seeds that are genetically identical to the maternal plant.

3. **Autonomous endosperm development:** Endosperm, a vital tissue supporting embryo development in seeds, is developed autonomously in synthetic apomixis, independent of fertilization. This ensures that the clonal seeds have all the essential nutrients required for germination and growth.

Mitosis Instead of Meiosis (MiMe) for creating synthetic apomicts

The goal of the MiMe (Mitosis instead of Meiosis) strategy is to develop a plant system that bypasses the process of meiosis during gamete formation, resulting in the creation of clonal seeds containing the same genetic material as the parent plant. By substituting meiosis with mitosis in gamete development, the MiMe strategy seeks to achieve synthetic apomixis. This method simplifies the production of clonal seeds, eliminates genetic recombination, improves

reproductive efficiency, streamlines breeding efforts, and holds promise for enhancing crop traits by efficiently propagating and distributing desirable genetic characteristics in crop plants.

The key steps in MiMe:

1. Inhibition of Double-Strand Breaks (DSBs)- Preventing double-strand breaks (DSBs) is a critical step in synthetic apomixis to avoid the mixing of chromosomes during the creation of clonal gametes. During normal meiotic recombination in sexual reproduction, cells undergo two rounds of division after DNA replication, with DSBs initiating genetic mixing between matching chromosomes, leading to diversity in offspring. However, in synthetic apomixis, scientists aim to produce clonal gametes identical to the parent plant by halting DSB formation, ensuring chromosomes remain unchanged and the parent plant's genetic makeup is preserved in the clonal gametes. This process is regulated by specific genes, such as Sporulation-defective11 (SPO11) in eukaryotes, which catalyzes DSB formation, and targeting these genes effectively prevents chromosome mixing, promoting the creation of genetically identical offspring in synthetic apomixis, crucial for maintaining desired traits and genetic integrity.

2. Promotion of premature separation of sister chromatids- Unlike normal meiosis, where sister chromatids are held together until meiosis II, inducing their separation at meiosis I leads to the production of unreduced gametes with the full complement of chromosomes. This process mimics mitosis, ensuring that the clonal gametes retain the same genetic information as the parent plant. By targeting specific genes and protein complexes involved in regulating chromatid separation, researchers can orchestrate this premature separation, facilitating the generation of clonal offspring with desired traits in synthetic apomixis. This strategic manipulation of chromatid separation is essential for maintaining genetic uniformity and preserving the parent plant's genetic integrity in clonal progeny, thus advancing the application of synthetic apomixis.

3. Skipping the second division in meiosis- Skipping the second division in meiosis is a pivotal concept in synthetic apomixis, particularly within the "Mitosis instead of Meiosis" (MiMe) strategy, aimed at producing undiminished (diploid) gametes. Unlike normal meiosis, where two successive divisions result in haploid gametes, skipping the second division prevents the separation of sister chromatids, yielding gametes with the complete set of chromosomes. This process mirrors mitosis, where sister chromatids remain together, leading to genetically identical daughter cells. By generating undiminished gametes, researchers ensure the retention of desired traits from the parent plant, facilitating the production of clonal offspring. This manipulation of meiotic division is regulated by specific genes and protein complexes, offering a promising avenue for synthetic apomixis and clonal seed production in plant breeding and crop improvement.

Other approaches besided the MiMe strategy

In addition to the MiMe strategy, there are alternative approaches and mechanisms that can be employed to achieve apomicts. These include: -

1. Genome sditing: Genome editing technologies can be employed to target and modify specific genes involved in the apomixis pathway, such as those regulating embryo development, endosperm formation, or reproductive cell differentiation. By editing key genes associated with apomixis, one can potentially induce the asexual reproduction process in sexual plants, leading to the production of clonal seeds.

2. Haploid induction: Haploid induction techniques can be used to generate haploid plants as part of the synthetic apomixis process. By inducing haploids and manipulating their reproductive pathways, we can explore ways to bypass meiosis and fertilization, ultimately leading to the development of clonal seeds with fixed genetic traits.

3. Apomixis: Natural apomixis, a process of asexual seed production, can serve as a model for developing synthetic apomixis. By understanding the genetic and molecular mechanisms underlying apomixis in plants that naturally exhibit this trait, we can potentially engineer similar reproductive pathways in sexual crop species to achieve clonal seed production.

4. Endoreduplication: Inducing endoreduplication in plant tissues can alter the ploidy level and potentially contribute to the development of polyploid plants with unique traits. Manipulating endoreduplication processes in reproductive organs opens up opportunities to explore novel ways to generate clonal seeds through altered reproductive pathways.

5. Mutagenesis: Mutagenesis techniques can be used to introduce genetic variations in plant genomes, which may lead to the discovery of novel genes or pathways associated with asexual reproduction. Screening mutagenized populations can help identify mutants with apomictic traits or altered reproductive processes that mimic synthetic apomixis.

6. Hybridization: Hybridization can be utilized to introduce genetic diversity and novel traits into plant populations. Crossing different plant varieties and selecting for individuals with apomictic characteristics, gives potential to create hybrid plants capable of producing clonal seeds through synthetic apomixis mechanisms.

The next phase in attaining clonal progeny involves either removing the male genome from the zygote post-gamete fusion, a process known as genome elimination, or bypassing fertilization altogether through parthenogenesis. Both of these alternatives suffice to initiate embryo development without the involvement of male gametes.

Parthenogenesis- Parthenogenesis, a form of asexual reproduction, enables embryo development from an unfertilized egg cell, bypassing the need for male gamete involvement. In

this process, the egg cell undergoes division and embryogenesis, giving rise to a new individual devoid of genetic contribution from a male parent. Natural occurrences of parthenogenesis in certain plant species are triggered by specific environmental conditions or genetic factors. It manifests in various forms, including apomixis, where seeds develop without fertilization, and parthenocarpy, leading to seedless fruit formation. Artificial induction of parthenogenesis in plant biotechnology allows for the production of clonal offspring, aiding in the propagation of desirable traits and the rapid multiplication of elite varieties. Additionally, parthenogenesis finds utility in breeding programs to fix genetic traits and ensure uniformity in plant populations.

Genome elimination- Genome elimination, a process utilized in plant biotechnology, selectively removes genetic material from one parent in offspring, altering the genome composition of resulting plants. This technique aims to generate offspring with a singular parental genome, either haploid or diploid, by targeting specific genes or proteins involved in chromosome segregation, kinetochore formation, or DNA replication during cell division. By modulating the expression or function of centromere-specific histone 3 (CENH3), crucial for kinetochore formation and chromosome segregation, one can induce genome elimination during cell division, leading to haploid or diploid offspring with a single parental genome.

The integration of parthenogenesis and genome elimination techniques signifies a significant stride forward in synthetic apomixis, offering a viable route to generate clonal offspring with consistent genetic traits.

Potential value of synthetic apomixis in agriculture

1. **Clonal seed production**: Synthetic apomixis enables the generation of clonal seeds identical to the parent plant, ensuring the consistent inheritance of desirable traits and maintaining crop quality across successive generations.

2. **Accelerated breeding programs:** By circumventing the complexities of meiosis and fertilization, synthetic apomixis expedites breeding programs, allowing researchers to swiftly develop new plant varieties with fixed genetic traits, thus saving time and resources compared to conventional breeding methods.

3. **Preservation of hybrid vigor:** Synthetic apomixis helps sustain hybrid vigor over multiple generations by producing clonal seeds with uniform genetic makeup, ensuring the perpetuation of superior traits derived from hybridization.

4. **Genetic stability:** Clonal seed production via synthetic apomixis promotes genetic stability and uniformity in crops, minimizing the risk of genetic drift and preserving valuable traits, which leads to more consistent and reliable crop yields.

5. **Crop improvement:** Synthetic apomixis facilitates crop improvement by enabling the fixation of complex traits such as disease resistance, stress tolerance, and yield potential, thereby enhancing overall crop performance and productivity.

6. **Reduced reliance on seed production:** Synthetic apomixis reduces the dependency on purchasing seeds each season, offering cost savings and enhanced seed availability, particularly in regions with limited access to quality seeds, thus promoting seed security for farmers.

7. **Environmental sustainability:** By promoting genetic uniformity and stability, synthetic apomixis contributes to sustainable agricultural practices, leading to more efficient resource utilization, reduced input requirements, and improved environmental sustainability.

8. **Expansion of crop diversity:** Synthetic apomixis facilitates the preservation and propagation of rare or endangered plant species and enables the creation of novel plant varieties with unique traits that may not be attainable through traditional breeding methods, thereby enhancing crop diversity and genetic resources.

Despite advancements, challenges such as low frequency of clonal offspring and reduced fertility in haploid inducers persist, underscoring the imperative for further optimization of the technology. Collaborative research endeavors and ongoing studies are crucial to address these hurdles comprehensively and unlock the full potential of synthetic apomixis in enhancing crop improvement and agricultural sustainability. Future research aims to enhance efficiency, develop high-throughput selection techniques, facilitate field application, and leverage genetic engineering advances to bolster the efficacy of synthetic apomixis. By refining technology and scaling up production, synthetic apomixis holds promise to revolutionize plant breeding and contribute significantly to global food security. Cooperative efforts involving multidisciplinary teams are indispensable for expediting technology development and widespread adoption. To propel synthetic apomixis forward, it is recommended to prioritize research into understanding underlying mechanisms, optimize technology integration, conduct rigorous validation studies, facilitate seamless technology transfer, invest in capacity building, and address pertinent regulatory considerations. By adhering to these recommendations, we can accelerate the adoption of synthetic apomixis, fostering the emergence of improved crop varieties, heightened agricultural productivity, and sustainable food production systems.

References:

1. Barcaccia, G., Palumbo, F., Sgorbati, S., Albertini, E., & Pupilli, F. (2020). A reappraisal of the evolutionary and developmental pathway of apomixis and its genetic control in angiosperms. *Genes*, *11*(8), 859.

2. Bicknell, R. A., & Koltunow, A. M. (2004). Understanding apomixis: recent advances and remaining conundrums. *The Plant Cell*, *16*,228-245.
3. Brukhin, V., & Baskar, R. (2019). A brief note on genes that trigger components of apomixis. *Journal of biosciences*, *44*, 1-6.
4. Fiaz, S., Wang, X., Younas, A., Alharthi, B., Riaz, A., & Ali, H. (2021). Apomixis and strategies to induce apomixis to preserve hybrid vigor for multiple generations. *GM crops & food*, *12*(1), 57-70.
5. Hojsgaard, D., & Hörandl, E. (2019). The rise of apomixis in natural plant populations. *Frontiers in Plant Science*, *10*, 358.
6. León-Martínez, G., & Vielle-Calzada, J. P. (2019). Apomixis in flowering plants: Developmental and evolutionary considerations. *Current topics in developmental biology*, *131*, 565-604.
7. Mahlandt, A., Singh, D. K., & Mercier, R. (2023). Engineering apomixis in crops. *Theoretical and Applied Genetics*, *136*(6), 131.
8. Schmidt, A. (2020). Controlling apomixis: shared features and distinct characteristics of gene regulation. *Genes*, *11*(3), 329.
9. Underwood, C. J., & Mercier, R. (2022). Engineering apomixis: clonal seeds approaching the fields. *Annual review of plant biology*, *73*, 201-225.
10. Wang, K. (2020). Fixation of hybrid vigor in rice: synthetic apomixis generated by genome editing. *Abiotech*, *1*(1), 15-20.
11. Xiong, J., Hu, F., Ren, J., Huang, Y., Liu, C., & Wang, K. (2023). Synthetic apomixis: the beginning of a new era. *Current Opinion in Biotechnology*, *79*, 102877.

A PRELIMINARY REPORT ON ETHNOMEDICINAL USES OF SPICES BY THE NATIVES OF BARGARH MUNICIPALITY, ODISHA, INDIA

Alok Ranjan Sahu*, Subhashree Sonalika Pradhan,
Sandhyarani Sahu and Puspitarani Barik
Department of Botany, Vikash Degree College, Bargarh, Odisha-768040
*Corresponding author E-mail: alok.btgene@gmail.com

Abstract:

An ethno-medicinal survey was conducted during January 2023 to December 2023 to investigate the traditional knowledge of natives of Bargarh municipality in the state of Odisha, India. Data were collected by interviewing native and elderly people engaged in health practice. The investigation reveals that 16 different important plant species belonging to nine families are being commonly used. Maximum numbers of five species are reported each from family Apiaceae followed by three species by members of Zingiberaceae, two species from the family Lauraceae and one species each from the other six families. During the investigation it was observed that people of Bargarh municipality still continue to depend on plant resources to meet their day-to-day needs and use plant-based formulations from generation to generation for treatment of health-related problems. There is no documentation of such knowledge and it is expected that with the death of elderly people the knowledge may be lost. Hence, the present paper aims to documentations of medicinal uses of spices by the natives of the study area.

Keywords: Spices, Ethnomedicine, Natives, Bargarh Municipality.

Introduction:

Spices always play an important role in the kitchen as well as in certain medicinal activity like diuretic, carminative aperients, expectorant, and many more. Spices have been used medicinally since early. Spices are being used as a revile for health in many diseases, for example, fenugreek, coriander, turmeric, cinnamon, cumin, clove, and others. Traditionally spices, as part of the diets, have holistic effects on human health. In Indian cuisine, all spices are used from ancient times in daily food as well as used in traditional manner (Sachan *et al.*, 2018). India is a great producer of plenty of spices, from 80 types of spices grown in the world whereas about 50 types are grown in India. Spices not only improve the taste of food but also a good source of vitamins B and C, iron, calcium, and other antioxidants. Spices drawn from various parts of plants like bud, bark, root, flower, and fruits. Spices are being used by many medical industries like cosmetic, pharmaceutical, and aromatic as perfumery (Rathore and Shekhawat, 2008). The different Indian kitchen flavors are explicitly against multidrug-safe clinical seclusion

of enterococci having various hereditary apparatus of harmful variables. Seven kinds of the run of the mill Indian flavors and herbs to be specific cumin, fenugreek, cinnamom, cardamom, cloves, and turmeric. The rough ethanolic concentrate of cinnamon, cloves, turmeric, cardamom, and cumin indicated critical antibacterial movement against all the clinical disconnects of enterococci (Vasanthi and Parameswari, 2010). Indian spices have been reported to exhibit a wide range of physiological and pharmacological properties that produce beneficial health promoting/protective effects for various chronic diseases. Indian spices as a biotherapy have become important in the developed and developing world with specific spices such as cinnamon and curcumin involved in the control of the immune system and the antimicrobial therapy. Cinnamon has been shown to regulate insulin levels (Martins, 2018).

The cooking world would be inert without flavors. Flavors, similar to their organic verdant partner's herbs, confer assorted flavor, shading, and taste to different nourishments around the globe. They likewise offer a large group of incredible phytonutrients that can upgrade human wellbeing and prosperity. While culinary flavors are having been utilized from many years for their various wellbeing benefits, broad research over the most recent two decades Flavors are the chief wellspring of spore forming microscopic organisms in huge volumes of sustenance, for example, soups, meals, stews, and sauces created by cooking foundations; under great conditions, they develop and duplicate to infective and harmful levels (Banerjee and Sarkar, 2006). Flavors are an essential piece of both veggie lover and non-vegan Indian cooking. They are normal nourishment added substances that confer flavor and fragrance. A typical Indian kitchen with onion, garlic, ginger, turmeric, tejpattar, coriander, pepper, Ajwain, Jeera, tea, tulsi and neem leaves, and so on is really a little home-grown medication store. Flavors can be the buds (cloves), bark (cinnamon), roots (ginger), berries (peppercorns), fragrant seeds (cumin), and even the disgrace of a bloom (saffron). A portion of the dynamic cancer prevention agent parts in flavors incorporates carnosic corrosive, carnosol, rosmarinic corrosive, thymol, carvacrol, 6-gingerol, 6-shogaol, zingerone, curcumin, capsaicin, vanillin, eugenol, caffeic corrosive, and ferulic corrosive (Gupta *et al.,* 2013). Flavors, for example, mint, garlic, ajowan, fennel, and coriander, are the typical elements of such stomach-related energizer arrangements both business and as home cures. Flavors have been for the most part accepted to increase salivary stream and gastric juice discharge, in this way helping absorption. The stomach-related stimulatory activity of flavors is likewise most likely through the incitement of exercises of compounds that take an interest in acid reflux. In excess of 400 flavors have been utilized on the planet, generally in hot atmosphere nations. Phenolic mixes of flavors, which contain a high level of eugenol, carvacrol as well as thymol, are essentially answerable for bactericidal/bacteriostatic properties (Tajkarimi *et al.,* 2010).

Spices are being used in Indian Ayurveda and folk medicine to treat many diseases like gynecological problems, gastric problems, hepatic disorders, infectious diseases, and blood disorders (Siviero *et al.,* 2015). The Indian arrangement of comprehensive medication known as Ayurveda utilizes fundamentally plant-based medications or definitions to treat different diseases including malignant growth. In Ayurvedic drug, curcumin is a well-recorded treatment for different respiratory conditions (e.g., asthma, sensitivity, etc.) just as for liver issue, anorexia, ailment, diabetic injuries, runny nose, hack, and sinusitis (Gupta *et al.,* 2013). In the conventional Indian arrangement of medication Ayurveda and Siddha, different flavors and herbs are depicted to have restorative properties, for example, being antithrombotic, antiatherosclerotic, hypolipidemic, hypoglycemic, calming, and antiarthritic. Clove is also used since a long time in Ayurveda as it maintains the heat system in the human body; hence, it is used according to region as well as season (Bhowmik *et al.,* 2012). Fenugreek has been alluded to as a therapeutic herb in Indian Ayurvedic. Coriander is thus a successive fix in the planning of Ayurvedic drugs and is a conventional home treatment for an assortment of sicknesses (Bhat and Kempraj, 2009). In the Indian Ayurvedic arrangement of the natural drug, turmeric is known to fortify and warm the entire body (Tilak *et al.,* 2004). Keeping the importance in mind, the present study deals with the documentation of ethnobotanical uses of spices by the native of Bargarh municipality, Odisha, India.

Materials and Methods:

Study site

Bargarh district lies between 19° 23' North latitude and 82° 55' East longitudes and having 5,837 Sq Km of geographical area, most of the area is covered with dense forest. Bargarh district is a tribal dominated district having a population of 12,20,946 (Census of India, 2011). The population density of this district is 230 inhabitants per square K.M. There are 12 Blocks, one municipality *i.e.* Bargarh and three N.A.C. in the Bargarh district. Bargarh municipality was selected as the study site of the present work. This municipality was declared on 28.08.1951. At present the area of this municipality is 16.72 sq. K.M. having 19 numbers of words and a population of about 80440 as per the 2011 census. This study area is bounded north by the villages Dang, Khaliapali and Padhanpali; south by the village Ruhunia; east by Village Tora and west by the villages Haldipali, Sountpur and Dumberpali.

Collection of data and analysis

Before starting fieldwork, a literature survey of ethnomedicinal work in Odisha was carried out (Sahu *et al.,* 2010; Sahu *et al.,* 2013). The study area was frequently visited and close interaction was made with the native peoples of Bargarh municipality. The plant species were collected and identified by using local flora books (Haines, 1925; Saxena and Brahman, 1996).

The local names were cross checked by using earlier published literature of different districts of Odisha (Sahu *et al.*,2010; Sahu *et al.*, 2013; Sahu *et al.*, 2016; Sahu *et al.*, 2020a, 2020b, 2020c; 2020d; Sahu *et al.*, 2021a, 2021b, 2021c; Sahu and Sahu, 2017a, 2017b, 2019, 2020, 2022; Rana *et al.*, 2020; Behera *et al.*, 2021, Sahu and Sahu, 2023; Rout and Sahu, 2023; Behera and Sahu, 2023; Behera *et al.*, 2023). The authority plant names were confirmed at http://worldfloraonline.com. In the following enumeration, the species are arranged alphabetically, botanical name and family name in parenthesis, followed by local name in inverted comma, parts used and the mode of utilization. The histogram for number of species vs family was drawn by using MS-EXCEL 2016.

Enumeration

A total of 16 plants from 15 genera and nine families were reported here (Figure 1). The family Apiaceae contributed five species (*Coriandrum sativum* L., *Cuminum cyminum* L., *Foeniculum vulgare* Mill., *Pimpinella anisum* L.**,** and *Trachyspermum ammi* L.) and dominated over other families, followed by three species (*Curcuma longa* L., *Elettaria cardamomum* (L.) Maton, and *Amomum subulatom* Roxb.) in Zingiberaceae, two species (*Cinnamomum verum* J.S. Presl, and *Cinnamomum tamala* (Buch. -Ham.) Th. G.G. Nees) in Lauraceae, while rest six families (Brassicaceae, Fabaceae, Myristicaceae, Myrtaceae, Piperaceae, and Solanaceae) contributed one species each. The enumerations of above said plants were described as follows:

1. *Amomum subulatom* Roxb., (Zingiberaceae), 'Bad Eliaichi', Seeds (Pods)
   Uses: Black cardamom are the natural detox which help flush out toxin, free radical and dangerous fluids in the body; improve blood circulation throughout the body and improves skin complexion; provides nourishment to the scalp and hair follicles.
2. *Brassica campestris* L., (Brassicaceae), 'Surso', Seeds,
   Uses: Seeds are excellent for digestion, improves cardio vascular health, helps to control diabetes, prevent from asthma.
3. *Capsicum annuum* L., (Solanacea) 'Mircha', Pods,
   Uses: It prevents cancer and diabetes; contains anti-oxidant, anti-inflammatory and pain-relieving properties; fights against fungal infection, colds and flu.
4. *Cinnamomum tamala* (Buch.-Ham.) Th. G.G. Nees, (Lauraceae) **'**Tejpatar', Leaves;
   Uses: Bay leaf poweder can be mixed with hair oils for hair growth, help to prevent kidney stones, help improve cholesterol levels.
5. *Cinnamomum verum* J.S. Presl, **(**Lauraceae) 'Dalchini', Bark,
   Uses: It improves blood sugar regulation and insulin sensitivity, and relieves flatulence abdominal pain
6. *Coriandrum sativum* L., **(**Apiaceae) 'Dhania', Leaves and fruits,

Uses: Leaves prevent anaemia, improve skin health when consumed regularly, good for digestion, reduce blood sugar levels, has anti-inflammatory properties.

7. *Cuminum cyminum* L., (Apiaceae) 'Jeera', Seeds,
   Uses: It improves digestion, improves gastrointestinal tract activity, helps to maintain fluctuating blood pressure level in the body, help to maintain and restore memory, good for hormones and skin healing.
8. *Curcuma longa* L., (Zingiberaceae) 'Haldi', Rhizome,
   Uses: It improves skin health, natural anti-inflammatory, antioxidant.
9. *Elettaria cardamomum* (L.) Maton, (Zingiberaceae) 'Chhoti elachi', Seed pods,
   Uses: Cardamom contain fibre which maintain the health of digestive tract, maintain healthy mouth and throat.
10. *Foeniculum vulgare* Mill., (Apiaceae) 'Pan muhuri', Seeds,
    Uses: Fennel seeds are known to trigger the secretion of digestive juices and enzymes which aid with digestion, Its powder works as an excellent laxative, helps treat respiratory ailments like bronchitis, cough and nasal congestion, promote health vision.
11. *Myristica fragrans* Houtt., (Myristicaceae), 'Jaiphala', Waxy seed covering,
    Uses: It boots our immune system against foreign particles; gives relieve toothache and other form of dental pains; soothes inflammation and eliminates pain such as aching joint, muscle pain, bruises and sore.
12. *Pimpinella anisum* L.**,** (Apiaceae) 'Anais', Seeds,
    Uses: It helps protect the skin from damage caused by free radicals and environmental factor; rich in antioxidant such as flavonoids and polyphenols which reduce chronic diseases like cancer, heart disease and diabetes; helps to relieve menstrual cramps and other menstrual problem; used to treat various fungal infection; also help to boost the immune systems.
13. *Piper nigrum* L., (Piperaceae), 'Golmarich', Fruits,
    Uses: It contains potent antioxidant; help neutralise harmful free radicals in the body; prevents skin damage and wrinkles; inhibit the growth of bacteria, virus and fungi; help regulate blood sugar metabolism in body.
14. *Syzygium aromaticum* (L) Merr. & Perry, **(**Myrtaceae) 'Laung', Buds,
    Uses: It helps in digestion and cures respiratory illnesses, promotes weight loss, helps to relieve toothache

15. *Trachyspermum ammi* L., (Apiaceae) 'Juwani', Seeds,

    Uses: Its seed used to control high blood pressure; seed can treat various digestive condition like acidity, indigestion and flatulence; helps to treat asthma and bronchitis; drinking carom seed water regularly enhance metabolism and burn fat.

16. *Trigonella foenum-graecum* L., (Fabaceae), 'Methi', Seeds,

    Uses: Seed are used to promotes health skin and hair; used to reducing cholesterol level and blood pressure condition; helps in the management of diabetes.

Results and Discussion:

A total of 16 plants from 15 genera and nine families were reported in this present finding (Figure 1). The family Apiaceae contributed five species and dominated over other families, followed by three species in Zingiberaceae, two species in Lauraceae, while rest six families *i.e.*, Brassicaceae, Fabaceae, Myristicaceae, Myrtaceae, Piperaceae, and Solanaceae contributed one species each. The ethnomedicinal study of earlier reports supported the medicinal uses of spices by the native of Bargarh municipality. Sahu *et al.* (2010) reported the ethnomedicinal uses of 209 plant species including five spices by the native of Bargarh district, Odisha, India. Sahu *et al.* (2013) reported a total of 117 medicinal plants including four spices by the native of Sohela block of Bargarh district, Odisha, India. Sahu and Sahu (2017a) reported the use of 57 plants including five spices for dental and oral healthcare by the peoples of Bargarh district. Sahu *et al.* (2020a) reported about the use of 49 species of plants including two plants for oral care by the tribal people in Kalahandi district of Odisha. Sahu and Ekka (2021a) reported a total of 39 plant species including three spices as leafy vegetables by the native of Bargarh district.

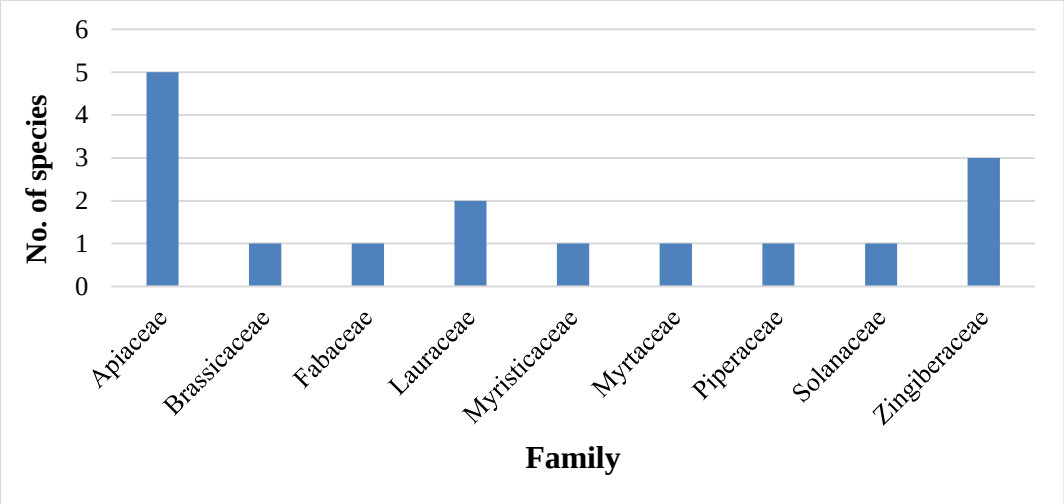

Figure 1: Family-wise distribution of medicinal plant species.

Acknowledgement

The authors of this article are especially thankful to the natives of Bargarh municipality for their cooperation and for voluntarily sharing a wealth of their accumulated knowledge

regarding ethnomedicinal practices. The authors are also grateful to the staff members, Vice-Principal, Principal, and Executive Director of Vikash Degree College, Bargarh for providing necessary facilities, moral support, and encouragement.

References:

1. Banerjee M, and Sarkar PK (2003): Microbiological quality of some retail spices in India. Food Res Int., 36(5): 469–474.
2. Behera A, and Sahu AR (2023): A Preliminary report on traditional use of selected plants of the Fabaceae family at Lathor Village, H.S. Road, Balangir District, Western Odisha. In Frontiers in Life Science Volume X; Parimala B, Mishra P, Yadav KK, and Sahu AR (Ed.). Bhumi Publishing, Nigave Khalasa, Kolhapur 416207, Maharashtra, INDIA. Chapter 10, Pp. 62- 68.
3. Behera C, Swain PK and Sahu AR (2021d): A preliminary Report on Ethno-medicinal Study in Chandli Reserve Forest, Balangir District, Odisha, India. Research Journal of Recent Sciences, 10(3):12-17.
4. Bhat SK, and Kempraj V (2009): Biocidal potential of clove oils against Aedes albopictus–a comparative study. Afr J Biotechnol., 8: 24.
5. Bhowmik D, Kumar KS, Yadav A, Srivastava S, Paswan S, and Dutta AS (2012): Recent trends in Indian traditional herbs Syzygium aromaticum and its health benefits. Journal of Pharmacognosy and Phytochemistry, 1(1):13–22.
6. Gupta SC, Sung B, Kim JH, Prasad S, Li S, and Aggarwal BB (2013): Multitargeting by turmeric, the golden spice: from kitchen to clinic. Mol Nutr Food Res., 57(9): 1510–1528.
7. Martins IJ (2018): Indian spices and biotherapeutics in health and chronic disease. Health, 10(04):374.
8. Rana UK, Sahu M, Mishra S and Sahu AR (2020e): Ethnomedicinal uses of selected plants by the Gond Tribal group of Amlipali Village of Padampur NAC of Bargarh district in western Odisha, India. World Journal of Pharmacy and Pharmaceutical Sciences, 9 (11): 2523-2532 (DOI: 10.20959/wjpps202011-17761).
9. Rathore MS, and Shekhawat NS (2008): Incredible spices of India: from traditions to cuisine. American-Eurasian Journal of Botany, 1(3): 85–89.
10. Rout P, and Sahu AR (2023): Medicinal uses of selected plants by the native of Bhawanipatna of Kalahandi district, South-Western Odisha, India. In Frontiers in Life Science Volume X; Parimala B, Mishra P, Yadav KK, and Sahu AR (Ed.). Bhumi Publishing, Nigave Khalasa, Kolhapur 416207, Maharashtra, INDIA. Chapter 7, Pp. 39-46.

11. Sachan AK, Kumar S, Kumari K, and Singh D (2018): Medicinal uses of spices used in our traditional culture: worldwide. J Med Plants Studies, 6(3): 116–22.
12. Sahu AR and Sahu M (2017b): A Preliminary Report on the Indigenous Knowledge on Animal Health Care Practices in Bargarh District of Odisha, India. Innovative Farming, 2(3): 154-161
13. Sahu AR and Sahu M (2019): A preliminary report on home garden for nutritional and primary health security of rural people of Bargarh District in Western Odisha, India. World Journal of Pharmacy and Pharmaceutical Sciences, 8(7):1383-1394. (DOI: 10.20959/wjpps20197-14174).
14. Sahu AR and Sahu M (2020): A preliminary report on the ethnobotanical plants used for dental care by the tribal of Bargarh District, Western Odisha. World Journal of Pharmacy and Pharmaceutical Sciences, 9 (2):1020-1028. (DOI: 10.20959/wjpps20202-15463).
15. Sahu AR and Sahu M (2022): Green leafy vegetables used by the Tribal Peoples of Jharigaon Block of Nabarangpur District, Odisha, India. In Ecology Research, Jachak *et al.* Bhumi Publishing, Nigave Khalasa, Kolhapur 416207, Maharashtra, INDIA. Volume V, Chapter 7:52-59.
16. Sahu AR, and Ekka NJ (2021a): A preliminary report on the use of leafy vegetables by the native of Bargarh district, Western Odisha, India. International Journal of Applied Research, 7(5): 218-223 (DOI: https://doi.org/10.22271/allresearch.2021.v7.i5d.8567)
17. Sahu AR, and Sahu M (2023): A preliminary Report on Ethnomedicinal Study of Plants Used to Treat Asthma by the Gond Tribes of Nabarangpur District, Odisha, India. In Frontiers in Life Science Volume X; Parimala B, Mishra P, Yadav KK, and Sahu AR (Ed.). Bhumi Publishing, Nigave Khalasa, Kolhapur 416207, Maharashtra, India. Chapter 1, Pp. 1- 10.
18. Sahu AR, Behera N and Mishra SP (2010): Use of Ethnomedicinal Plants by Natives of Bargarh District of Orissa, India. Ethnobotanical Leaflets, 14: 889-910.
19. Sahu AR, Mishra S, Sahu M and Nayak NR (2020a): A preliminary report on ethnomedicinal uses of different plants for oral care in Kalahandi district, Odisha. International Journal of Applied Research, 6 (6): 265-268 (DOI: 10.22271/allresearch.2020.v6.i6e.6793).
20. Sahu AR, Mishra S, Sahu M, Pradhan SN (2020d): Utilization of some selected summer seasonal plants in the rural areas of Bargarh District, Western Odisha. International Journal of Botany Studies, 5(5): 203-206
21. Sahu AR, Nayak AK and Panigrahi SK (2013): Survey of some important ethno-medicinal plants of Sohela Block, Western Odisha, India. Life Sciences Leaflets, 11(11): 1-9.

22. Sahu AR, Panigrahi J and Mishra SP (2016): A preliminary report on the Ethnoveterinary Medicinal Plants of Bargarh District, Western Odisha, India. In: Conservation, Cultivation, Diseases and Therapeutic Importance of Medicinal and Aromatic Plants, Chourasia HK and Roy AK (eds.). Today & Tomorrows Printers and Publishers, New Delhi, Chapter-19, pp.315-325.
23. Sahu AR, Sahu M and Mishra S (2020c): A preliminary Report on Traditional Use of Selected Plants of Fabaceae family at Bargarh District, Western Odisha. International Journal of Herbal Medicine, 8 (9): 109-113.
24. Sahu AR, Sahu M, and Raal A (2021c): An Ethnobotanical Study on Native Plants of Bargarh of Western Odisha, India in relieving Urogenital ailments. Ethnobotany Research & Applications, 21:29 (http://dx.doi.org/10.32859/era.21.29.1-11).
25. Sahu AR, Sahu M, Mishra S, Ekka NJ (2021b): A preliminary Report on Ethnomedicinal uses of Selected Plants by *Sahara* Tribal Groups of Kangaon Village of Bargarh District in Western Odisha. Journal of Medicinal Plants Studies, 9(3): 238-242 (DOI: https://doi.org/10.22271/plants.2021.v9.i3c.1300)
26. Sahu AR, Sahu M, Nayak NR and Sahoo TR (2020b): Medicinal Plants of Saraswati +3 Science College, Bhawanipatna Campus and it's adjacent areas, Kalahandi district, South-Western Odisha, India. World Journal of Pharmacy and Pharmaceutical Sciences, 9 (1): 1738-1752 (DOI: 10.20959/wjpps20201-15408)
27. Sahu M and Sahu AR (2017a): A Preliminary Report on the Traditional Practice for Dental and Oral Health Care in Bargarh District of Western Odisha, India. Journal of Medicinal Plants Studies, 5(5):120-125.
28. Siviero A, Gallo E, Maggini V, Gori L, Mugelli A, Firenzuoli F, and Vannacci A (2015): Curcumin, a golden spice with a low bioavailability. Journal of Herbal Medicine, 5(2): 57–70.
29. Tajkarimi MM, Ibrahim SA and Cliver DO (2010): Antimicrobial herb and spice compounds in food. Food control., 21(9): 1199–1218.
30. Tilak JC, Banerjee M, Mohan H, and Devasagayam TPA (2004): Antioxidant availability of turmeric in relation to its medicinal and culinary uses. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 18(10): 798–804.

SHORT NOTE ON MIYAWAKI FORESTS

Meenakshi A. Bangar
Department of Botany,
Netaji Subhashchandra Bose College, Nanded, M.S.

Miyawaki forests or Miyawaki technique, refer to a unique approach to afforestation and ecological restoration developed by Japanese botanist Dr. Akira Miyawaki. The methodology was developed in the 1970s, with the basic objective to densify green cover within a small parcel of land. The method involves creating dense, multi-layered forests that grow rapidly and mimic the natural biodiversity of native forests. This method involves planting two to four different types of indigenous trees within every square meter. The plants used in the Miyawaki method are mostly self-sustaining and don't require regular maintenance like manuring and watering. In this method, the trees become self-sustaining and they grow to their full length within three years.

K**ey principles of the Miyawaki method**

- **Biodiversity**: Planting numerous native species in close proximity to encourage natural competition, symbiotic relationships, and the development of a self-sustaining ecosystem.
- **Dense planting**: High-density planting of trees ensures that the canopy closes quickly, minimizing sunlight reaching the ground. It reduces the weed growth, thus enhancing the growth of tree saplings.
- **Soil preparation**: The soil is carefully prepared by adding organic matter, nutrients, and microorganisms to create a fertile and conducive environment for tree growth.
- **Mulching:** Mulch is applied to the forest floor to retain moisture, suppress weed growth, and enhance soil fertility.
- **Regular maintenance**: Proper care and maintenance, such as watering, weeding, and monitoring, are crucial during the initial years to ensure the successful establishment of the forest.

Benefits of Miyawaki method

- **Rapid forest development**: Miyawaki forests have a remarkable growth rate, with trees reaching maturity in a much shorter time compared to traditional plantations.
- This is due to the dense planting and carefully prepared soil, which promote rapid canopy closure and efficient resource utilization.

  A study revealed that Miyawaki forests grow 10x faster, are 30 x denser and contain 100x more biodiversity.

- **High biodiversity**: The Miyawaki Method emphasizes the planting of a wide variety of native species in close proximity. These forests become thriving habitats for birds, insects, and other wildlife, contributing to overall ecosystem health and resilience.
- **Enhanced carbon sequestration**: The dense vegetation and rapid growth of these forests enable efficient carbon absorption, helping to mitigate climate change and reduce greenhouse gas emissions.
- **Improved soil quality**: The careful soil preparation in the Miyawaki Method results in the enrichment of soil fertility and structure.
- **Noise and air pollution reduction**: Miyawaki forests planted in urban areas can help mitigate noise pollution by acting as sound barriers and absorbing sound waves. Additionally, they contribute to improving air quality by absorbing pollutants and particulate matter, thereby reducing air pollution levels.
- **Sustainable water management**: The dense vegetation of Miyawaki forests act as natural sponges, absorbing rainfall and reducing runoff, thus contributing to better water retention and preventing water pollution.
- **Regulates surface temperature:** Miyawaki forests can help regulate surface temperatures, particularly in urban areas where the urban heat island effect is a concern. The urban heat island effect refers to the phenomenon where urban areas experience higher temperatures compared to surrounding rural areas. This is due to human activities, the presence of buildings and pavement, and the lack of vegetation.

Scope and importance of adopting Miyawaki method in today's era

Taking several hundred years to complete the process of forest restoration is too long for us, because we live in a world where industry and urbanization are developing very rapidly, for instance, 110 cars are manufactured per minute whereas 36 football ground area of forest is being lost so improvement of an alternative reforestation technique that reduces the time could be a useful tool (Miyawaki., 1999).

One reliable forest restoration method is the **''native forests by native trees''** based on the vegetation ecological theories proposed by Prof. Akira Miyawaki and applied first in Japan. According to this method, restoring native green environments, multilayer forests and well-developed ecosystems can be quickly established because of the simultaneous use of intermediate and late successional species in plantations through careful ecological engineering and human intervention in an organic and sustainable manner.

The Miyawaki technique has been followed in Japan, South American countries, far East and Malaysia in the environmentally degraded lands as well as urban landscapes. In shorter span

of time, more urban forests were developed. The urban forests have multi facet advantages such as reduction in temperature, air quality improvement, CO_2 sequestration, improvement of wellbeing indicators and also hike in real estate prices. That's why many urban real estate builders are announcing green projects in metros so as to attract more clients.

References:

1. Hattori, D., & Miyawaki, A. (2013). Restoration of degraded urban lands with citizen participation: the roles of private and public sectors in greening Tokyo. *Ecological Restoration, 31*(3), 292-300.
2. Miyawaki, A. (1992). Restoration of evergreen broad-leaved forests in Japan. *Restoration Ecology, 1*(1), 1-7.
3. Miyawaki, A. (1993). Search for the restoration of natural forests in Japan. *Bulletin of the Forestry and Forest Products Research Institute, 357*, 3-15.
4. Miyawaki, A. (2004). Restoration of indigenous forests with minimum inputs of external materials and energy. *Journal of Forest Research, 9*(4), 307-315.
5. Miyawaki, A., & Fujiwara, K. (2007). Restoration of urban green spaces destroyed by construction works. *Urban Biodiversity and Design*, 191-204.
6. Miyawaki, A., & Okuda, S. (1978). On the species diversity of the natural vegetation of Japan from the viewpoint of community ecology. *Japanese Journal of Ecology, 28*(4), 241-253.
7. Soni, P., & Purohit, A. (2019). Miyawaki forests: A case study of Ahmedabad. *Journal of Urban Management, 8*(1), 39-45.

AN OVERVIEW OF MIYAWAKI PLANTATION

Meenakshi A. Bangar

Department of Botany,

Netaji Subhashchandra Bose College, Nanded, M.S.

Prime Minister Narendra Modi during his latest, 'Mann ki baat' episode, 18/06/2023 spoke about Miyawaki plantation, the Japanese method of creating dense urban forests in a small area. The PM also cited the example of a Kerala-based teacher, Raafi Ramnath, who used the Miyawaki technique to transform a barren land into a mini forest called Vidyavanam by planting 115 varieties of trees. Meanwhile, to fight climate change, curb pollution levels, and increase the green cover of the financial capital, the Brihanmumbai Municipal Corporation (BMC) has been creating Miyawaki forests in several open land parcels of Mumbai.

Miyawaki plantation method

Named after Japanese botanist Akira Miyawaki, this method involves planting two to four different types of indigenous trees within every square meter. In this method, the trees become self-sustaining and they grow to their full length within three years. The methodology was developed in the 1970s, with the basic objective to densify green cover within a small parcel of land. The plants used in the Miyawaki method are mostly self-sustaining and don't require regular maintenance like manuring and watering.

How is Miyawaki useful?

The dense green cover of indigenous trees plays a key role in absorbing the dust particles of the area where the garden has been set up. The plants also help in regulating surface temperature. Some of the common indigenous plants that are used for these forests include Anjan, Amala, Bel, Arjun and Gunj.

With several infrastructure projects like real estate metro rail construction in progress in Mumbai over the past few years, it was recorded that the surface temperature in certain pockets of Mumbai has increased. Therefore, to fight this challenge, such forests are being created.

What is the Miyawaki method?

Japanese botanist Akira Miyawaki endowed the Miyawaki technique to create dense forests with native plants. This unique method is used worldwide for urban afforestation by growing a forest in someone's backyard.

Requirements for afforestation through the Miyawaki Method

- The Miyawaki afforestation method requires quite a small space, at least 20 square feet.

- One must seed plants very close to save space and dense plant growth. This will also allow young trees to protect each other and block sunlight from hitting the forest's ground, preventing parasitic plant growth.
- This process must attain plant growth 10 times faster, and vegetation 30 times denser than usual.
- One must maintain such a forest for at least 3 years, according to the **Miyawaki method**.

Process of afforestation through Miyawaki Method

An Indian pioneer and expert of afforestation, Shubhendu Sharma, explained everything one needs to learn about the Miyawaki method. He also unveils every step of afforestation under this method, which are as follows:

Step 1: Examine the soil texture and measure biomass

- Examining soil texture is essential as it helps determine fertility, water retention, percolation, etc. All these elements determine the growth and longevity of the forest.
- Then one must measure the existing biomass of the soil. An afforest can add the following biomass to prepare the earth:

1. **Organic fertilizers-** The ground requires fertilizer to provide nutrients for plant growth. Some organic fertilizers are cowpat, goat muck and vermicompost.
2. **Perforating materials-** These materials are helpful for plants to penetrate their roots deeper into the ground. Rice husk, wheat husk, or groundnut shells can be an excellent resource to increase perforation.
3. **Water retainers-** A ground must have significant water retention power to develop a forest. An afforest can add coconut coir and peat moss to strengthen the soil's water retention power.
4. **Mulch-** It is usually layered over the ground to protect it from the scorching sun. It is vital, especially for saplings, as their growth may be affected in dried soil. Afforests can use decaying leaves, dried bark, or even composts.

Step 2: Select native species for plantation

- Afforests must select the native plant species and identify their genus (deciduous or evergreen), height and influence on nature.
- Foresters must allocate those plants in layers, depending on all the above factors.
- 40 to 50 per cent of the total number of trees must comprise the most commonly found species in one's neighborhood. Foresters must choose at least 5 different genera that would be the significant species in that forest.
- Some moderately found native species will compose 25 to 40 per cent as supporting plants. Finally, some other minor species will constitute the rest of the forest.

- Afforests need to collect saplings of these species, which must be in a minimum height of 60 to 80 cm.

Step 3: Prepare the ground and equip the forest area

- Before starting the planting process, afforests must inspect the ground to determine the possibilities and practicality of this project.
- The soil of this area must be clean from any debris and weed.
- It also must catch sunlight for at least 8-9 hours a day to start afforestation under the Miyawaki method**.**
- Foresters must install irrigation facilities, create 100 sq metre mounds and demark those before sowing.

Step 4: Start the plantation process

- One should dig small holes in the soil and remove the root bag of the seedlings to plant them.
- He must put these saplings in those holes and lightly level the soil around their stem.
- It is crucial to choose proper supporting sticks for these plants, according to their height.

Step 5: Take care of the forest for the next 3 years

- Monitoring the forest involves watering it daily, cleaning the weeds and plastics, and maintaining proper drainage.
- Foresters must maintain the mulch level and keep changing it for a minimum of 1 year. They also must have an eye on the plant's growth to ensure the mulch does not immerse it.
- They must not trim the forest, use chemical pesticides and fertilizer, and clean the shed leaves.

To sum it up, the Miyawaki method helps create self-sustaining vegetation within 2 to 3 years, whereas a traditional procedure takes nearly 100 years. Nevertheless, some environmentalists question this technique, despite its benefits for nature. According to them, such woods do not have medicinal properties as natural woods. Also, they speak against the forceful speeded photosynthesis of plants.

References:

1. Miyawaki, A., & Fujiwara, K. (2007). Restoration of urban green spaces destroyed by construction works. Urban Biodiversity and Design, 191-204.
2. Singh, A., & Sharma, S. (2020). Miyawaki Forest: A Green Antidote for Urban Areas. International Journal of Management, Technology, and Social Sciences, 5(1), 121-127.
3. Soni, P., & Purohit, A. (2019). Miyawaki forests: A case study of Ahmedabad. Journal of Urban Management, 8(1), 39-45.

A CONCISE REVIEW ON AZOLLA CULTIVATION AND ITS APPLICATIONS

Mohammed Mujahed*[1], Parvati R. Solanke[1] and Baig Zeba Rafat[2]

[1]Department of Biotechnology,

[2]Department of Botany,

D.S.M's College of ACS, Parbhani-431401, Maharashtra, India

*Corresponding author E-mail: mdmujahed483@gmail.com

Abstract:

Azolla is a free-floating aquatic fern also known as duck weed. Azolla is a good source of protein and contains almost all essential amino acids and minerals for instance iron, calcium, magnesium, phosphorus, potassium, manganese etc. apart from considerable quantities of β-carotene (vitamin A precursor) and vitamin B12. It is known for its short doubling time, nitrogen fixing and bioremediation potential. It has several applications such as green manure, N biofertilizer, cattle, chicken, and fish feed supplements because of its high protein content. Azolla is one of nature's protein-rich wonder plants. It can grow on wastewater improving its quality. Azolla biomass can be utilized for energy production. Azolla biomass has been used for biodiesel, biogas, and biohydrogen production.

Keywords: Azolla, green manure, feed supplement, biodiesel.

Introduction:

Azolla is a small floating fern and also referred as duck weed. It is a genus of seven species of aquatic ferns belongs to the family Salviniaceae. The name Azolla is derived from the Greek words azo (to dry) and allyo (to kill), implying that the plant will die if allowed to dry. Azolla contains about seven or eight living species and forty extinct species (Small *et al.*, 2011).

Azolla the small, free-swimming, fast-growing fern can be found all over the world. It is a dichotomously branching, free-swimming aquatic fern (Ahmed *et al.*, 2021). It is typically found all across India in places with standing water, such as ponds, streams, canals, and other areas where water is present for longer periods of time under sunlight or shade of tree (Masoodi and Khan, 2012; Katole *et al.*, 2017). Indian species are triangular in shape, measuring 1.5 to 3.0 cm long and 1 to 2 cm wide. Fronds have small roots that are frequently associated with microphylla (Ahmed *et al.*, 2021), as well as a short branched stem called the rhizome that is covered in small, alternately overlapping leaves. The sporophyte is dorsiventrally organised, with dorsal and abdominal lobes on each leaf (Pratte *et al.*, 2021). Roots of Azolla plants remain

suspended in water. Azolla is one of the fastest growing plants on the globe, and it can double its surface area every 5 to 10 days, making it an extremely valuable resource.

Azolla is rich source of proteins, amino acids, vitamins (A, B12, beta-carotene), growth promoter intermediates, and minerals (calcium, phosphorus, potassium, iron, copper, and magnesium) (Herath *et al.,* 2023). On a dry weight basis Azolla contains around 23-27 % crude protein and 10 % carbohydrates (Pullin and Almazan 1983; Cherryl *et al.,* 2014; Kathirvelan *et al.,* 2015).

Azolla grows with blue green algae *Anabaena azollae*. Symbiotic relationship exists between Azolla and algae (Reynaud and Franche, 1987; Aber *et al.,* 2016). Some strains of Azolla can fix as much as 2- 3 kg of nitrogen/ha/day. Azolla provides carbon and favorable environment for growth of blue green algae which fixes and assimilates the atmospheric nitrogen and decomposes it by enzymatic activity and further convert into soluble ammonia (Van Hove, 1989). *Anabaena azollae*, a cyanobacteria, which is harboured in the leaf lobe of Azolla, have the ability to fix atmospheric nitrogen while making it accessible to crop plants. Therefore, the Azolla-Anabaena relationship is important in agronomy. The presence of a symbiotic cyanobacterium, *A. azollae,* which occupies the dorsal lobe of the leaves, contributes to the system's nitrogen-fixing capabilities. As a result of this characteristic, it has been widely used as a biofertilizer for rice plants (Herath *et al.,* 2023). The use of Azolla also increases organic matter and potassium contents of the soil (Bhuvaneshwari and Singh, 2015).

Due to easy cultivation and high biomass yield, Azolla can be an ideal feed substitute for cattle, buffalo, sheep, goat, poultry and fish (Becerra *et al.*, 1995; Indira *et al.*, 2009; Leterme *et al.*, 2010).

Classification:

Kingdom : Plantae

Division : Pteridophyta

Class : Pteridopsida

Order : Salvinales

Family : Salvinaceae

Genus : Azolla

Requirements for azolla growth:

The mandatory requirements for the growth of Azolla are as follows

- **Water**: 10-15 cm fresh current water is necessary in multiplication pond. Maintenance of adequate water level at least 4 inches in the pond is necessary.

- **Temperature**: Day/night temperatures ranging between 20ºC and 32ºC have found to be most suitable. For luxurious growth of Azolla the optimum temperature is 25-30˚C.
- **Light**: Azolla prefers to grow well under partial shade.
- **Relative humidity**: 85 to 90 % relative humidity is optimum for its growth.
- **Soil pH**: It grows well in slightly acidic soil having 5.2 to 5.8 pH.
- **Nutrition**: Being an N fixing fern it does not require nitrogenous fertilizer for its growth. It requires Phosphorous @20 kg/ha for good bio-mass production.

Cultivation of azolla:

The procedure for cultivation of Azolla is as below

1. For cultivation of Azolla small ponds of suitable size should be made in low land field.
2. Sufficient water should be added about 10-15 cm standing water should be there in the ponds.
3. The culture of green Azolla 50-200 g/sqm along with single super phosphate (20 kg/ha) as a phosphorus source should be mixed and release into the pond containing water level of 15 cm.
4. Within 14-21 days the rapid multiplication of Azolla plants forms a green color mat just like carpet in the ponds.
5. This green mat of Azolla then can be harvested and released in the rice field or can be used after through washing and drying as an animal feed.
6. This Azolla can also be used as a bio-fertilizer by converting it into compost.
7. During summer season Azolla can be harvested at regular interval of 21 days.
8. However, during winter season growth rate of Azolla plant slow down due to low temperature and moisture stress. Therefore, Azolla should be harvested after 30 days of interval during this season.

Advantages of azolla cultivation:

There are several advantages of Azolla cultivation, some of which are as follows

1. It can grow under controlled conditions as well as readily in the wild.
2. It can easily be produced in both the seasons Kharif and Rabi in large quantity required as green manure.
3. It can fix atmospheric CO_2 and nitrogen to synthesize carbohydrates and ammonia respectively. It adds available nitrogen for crop uptake and organic carbon content to the soil after decomposition.
4. The oxygen released by oxygenic photosynthesis aids in the respiration of other soil microbes and the crop root system.

5. Azolla solubulises Zn, Fe and Mn and make them available to the rice.
6. Azolla suppresses tender weeds such as Chara and Nitella in a paddy field.
7. It releases plant growth regulators and vitamins which enhance the growth of the rice plant.
8. It can be a substitute for chemical nitrogenous fertilizers to a certain extent.
9. It increases the utilisation efficiency of chemical fertilizers.
10. It reduces evaporation rate from the irrigated rice field.
11. It has low input cost
12. It is a food source for waterfowl, fish, shrimp, insects, worms, snails, crustaceans etc. and provides habitat to them.

Limitations of azolla cultivation:

1. Water is pre-requisite for it's multiplication hence it is not suitable for upland crop.
2. Huge quantity of inoculums is required which is difficult for transplanting action during rainy days.
3. Temperature more than 35°C and Extreme low temperature is also not suitable.
4. We can not use Azolla as dry inoculum.
5. Ignorance of people about benefit of Azolla and also the market for azolla is not so popular.

Applications of azolla:

Azolla as a green fertilizer

Because of its high potential for biological N fixation, Azolla biofertilizer may be a promising approach to improving N use efficiency (NUE) in paddy rice fields. Azolla as a cover crop can benefit water bamboo, arrowhead, taro, wheat and rice (Kimani *et al.,* 2021). Incubating Azolla in moist soil as a green manure resulted in rapid mineralization, with 60-80% of the nitrogen released after two weeks (Ahmad *et al.*, 2021; Chandrababu and Parvathy, 2022).

Azolla as a livestock nutritional supplement

Azolla is consumed as a dietary supplement by cattles, rabbits, chickens, ducks and fish (Verma *et al.,* 2021). It was reported that feeding Azolla to broilers produced growth and body weight values comparable to corn-soybean meal (Ouedraogo *et al.,* 2021). Azolla is harvested in huge quantities and fed to cattle as feed (Tarrif *et al.,* 2021). Digested Azolla manure left over from biogas production was suitable as fish pond fertiliser and in a study of lactating cows (Abdelatty *et al.,* 2021), it was found that Azolla could be used as a feed ingredient, with milk yield and fat percentage remaining at the same levels as when fed conventional feed (Chandrababu and Parvathy, 2022).

Azolla for bioremediation

A. pinnata and Lemma minor are used to remove heavy metals such as iron and copper from contaminated water. The water pollutants even in low concentrations can be harmful. It is processed through ponds and can be reused for a variety of purposes (Annisa *et al.*, 2021). This is utilized for agricultural purposes. Tolerance and acceptance have proven to be advantageous. Azolla biomass also acts as a pollutant biofilter. Azolla has a remarkable ability to directly concentrate metals from impurities such as Cu, Cd, Cr, Ni, Pb and nutrient effluents (Prabhakaran *et al.*, 2022).

Azolla as a mosquito repellent/ control

By laying a thick mat of Azolla on the water's surface, we can prevent mosquito hatching and adult emergence (Hossain *et al.*, 2021).

Azolla as a space diet ingredient

Azolla has been proposed as part of the space diet while on Mars in collaboration with the Space Agriculture Task Force and it has been discovered that Azolla meets human nutritional needs on Mars (Katayama *et al.*, 2008). They used combination of Azolla, spirulina and carbohydrate.

Azolla as a source of bioenergy

The production of biofuels for bioenergy, which would not only reduce the need for fossil fuels but would also deliver high-energy fuels at a lower cost compared to other forms of biofuels, could be the most signi8cant possible use for the collected Azolla biomass (Roy *et al.*, 2016).

Azolla in biogas production

The anaerobic fermentation of Azolla (or a combination of Azolla and rice straw) produces methane gas, which can be used as fuel and the remaining effluent, which contains all of the nutrients originally incorporated into plant tissues with the exception of a small percentage of lost nitrogen, can be used as fertilizer (Thiruvenkatachari *et al.*, 2021).

Conclusion:

Azolla's biomass can be utilized as animal feed as it is rich in nutrients, compost for organic farming and kitchen gardening, or bioethanol production. Azolla species are the most attractive, sustainable, and universal feedstock for a wide range of renewable biofuels due to their high productivity, ability to grow on wastewater, and unique chemical makeup. Azolla is considered to be the most promising because of the ease of cultivation and good nutritive value. Thus, Azolla appears to be a potential source of nutrients and has a considerably high feeding value to the animals.

References:

1. Abdelatty, A.M., Mandouh, M.I., Mousa, M.R., Mansour, H.A., Ford, H., Shaheed, I.B. and Bionaz, M. (2021). Sun-dried Azolla leaf meal at 10% dietary inclusion improved growth, meat quality and increased skeletal muscle Ribosomal protein S6 kinase β1 abundance in growing rabbit. Animal. 15(10): 100348.
2. Aber JA, Aber SW and Penner II RL (2016). Rapid Environmental Changes in the Nature Conservancy Wetland at Cheyenne Bottoms, Kansas: A review 2002- 2015. Transactions of the Kansas Academy of Science, 119(1): 33-48.
3. Ahmad, N. and Tariq, H. (2021). Azolla as Waste Decomposer and Bio-fertilizer: A review. Journal of Applied Research in Plant Sciences (JOARPS). 2(1): 108-116.
4. Ahmed, I.M., Saad, S.M., Abd, A.E.Y., Suliman, M.A. and Mustafa, T.M. (2021). A new record for the invasive aquatic fern Azolla filiculoides Lam. in the White Nile, Sudan. DysonaLife Science. 2(2): 18-24.
5. Annisa, K., Sutarno, S. and Santosa, S. (2021). *Azolla microphylla* and *Pseudomonas aeruginosa* for bioremediation of bioethanol wastewater. Biodiversitas Journal of Biological Diversity. 22(4).
6. Becerra M, Preston TR and Ogle B (1995). Effect of replacing whole boiled soya beans with Azolla in the diets of growing ducks. Livestock Research for Rural Development, 7(3).
7. Bhuvaneshwari K and Singh PK (2015). Response of nitrogen-fixing water fern Azolla biofertilization to rice crop. 3 Biotech, 5(4): 523-529.
8. Chandrababu, K.A. and Parvathy, U. (2022). Water Fern- Azolla: A Review. Agricultural Reviews. DOI: 10.18805/ag.R-2529
9. Cheng, W. (2021). Poultry litter biochar application in combination with chemical fertilizer and Azolla green manure improves rice grain yield and nitrogen use efficiency in paddy soil. Biochar. 1-12.
10. Cherryl DM, Prasad RMV, Jagadeeswara Rao S, Jayalaxmi P and Srinivas Kumar D (2014). A study on the nutritive value of Azolla pinnata. Livestock Research International, 2(1): 13-15
11. Herath, Bimal Manuranga & Yapa, Neelamanie & Karunarathna, Samantha. (2023). Azolla as the multifunctional fern in organic agriculture: Prospects and challenges: A Review Article. 19. 63-82.

12. Hossain, M.A., Shimu, S.A., Sarker, M.S.A., Ahsan, M.E. and Banu, M.R. (2021). Biomass growth and composition of Azolla (*Azolla pinnata* R. BR.) supplemented with inorganic phosphorus in outdoor culture. SAARC Journal of Agriculture. 19(1): 177-184.
13. Indira D, Sarjan Rao K, Suresh J, Venugopal Naidu K and Ravi A (2009). Azolla (*Azolla pinnata*) as feed supplement in buffalo calves on growth performance. Indian Journal of Animal Nutrition, 26: 345-348.
14. Katayama, N., Yamashita, M., Kishida, Y., Liu, C.C., Watanabe, I., Wada, H. and Force, S.A.T. (2008). Azolla as a component of the space diet during habitation on Mars. Acta Astronautica. 63(7-10): 1093-1099
15. Kathirvelan C, Banupriya S and Purushothaman MR (2015). Azolla-an alternate and sustainable feed for livestock. International Journal of Science, Environment and Technology, 4(4): 1153-1157.
16. Katole S.B., Lende S.R. and S.S. Patil (2017). A Review on Potential Livestock Feed: Azolla. Livestock Research International, Vol. 05(01), Pages 01-09.
17. Kimani, S.M., Bimantara, P.O., Kautsar, V., Tawaraya, K. and Chandrababu, K.A. and Parvathy, U. (2022). Water Fern- Azolla: A Review. Agricultural Reviews. DOI: 10.18805/ag.R-2529.
18. Leterme P, Londoño AM, Muñoz JE, Súarez J, Bedoya CA, Souffrant WB and Buldgen A (2009). Nutritional value of aquatic ferns (*Azolla filiculoides* Lam. and *Salvinia molesta* Mitchell) in pigs. Animal Feed Science and Technology, 149(1-2): 135-148.
19. Masoodi A and Khan FA (2012). A new record to the invasive Alien Flora of India: Azolla cristata. National Academy Science Letters, 35: 493-495
20. Ouedraogo, S.W. N., Komoe, K., Lompo, D.J.P., Da, S.S., Isimemen, O. and Masse, D. (2021). Effect of invasive aquatic plants (*Azolla a*., *Myriophyllum a*. and *Cyperus a*.) biochar amendment on maize growth: An assessment. Journal of Agricultural Studies. 9(3): 336-351.
21. Prabakaran, S., Mohanraj, T., Arumugam, A. and Sudalai, S. (2022). A state-of-the-art review on the environmental benefits and prospects of Azolla in biofuel, bioremediation and biofertilizer applications. Industrial Crops and Products. 183: 114942.
22. Pratte, B.S. and Thiel, T. (2021). Comparative genomic insights into culturable symbiotic cyanobacteria from the water fern Azolla. Microbial Genomics. 7(6).
23. Pullin RSV and Almazan G (1983). Azolla as fish food. ICLARM Newsletter, 1: 6-7
24. Reynaud PA and Franche (1987). Azolla pinnata var. Africana. De la biologie moleculaire aux applications agronomiques. ORSTOM edition, 1-15.

25. Roy, D. Kumar, V. and M. Kumar, "Effect of feeding *Azolla pinnata* on growth performance, feed intake, nutrient digestibility and blood bio chemical's of Hariana heifers fed on roughage-based diet," Indian Journal of Dairy Science, vol. 69, pp. 190–196, 2016.
26. Small, E. and Darbyshire, S.J. (2011). 35. Mosquito Ferns (Azolla species)-tiny 'superplants'. Biodiversity. 12(2): 119-128.
27. Tarif, S.T. (2021). Use of azolla as a substitute poultry feed and its effect on growth and production: A review. Plant Archives. 21(1): 947-950.
28. Thiruvenkatachari, S., Saravanan, C.G., Geo, V.E., Vikneswaran, M., Udayakumar, R. and Aloui, F. (2021). Experimental investigations on the production and testing of Azolla methyl esters from *Azolla microphylla* in a compression ignition engine. Fuel. 287: 119448.
29. Van Hove C (1989). Azolla and its multiple uses with emphasis on Africa. FAO, Rome. pp. 53.
30. Verma, D. and Dey, K.P.S.D. (2021). Effect of supplementation of Azolla (*Azolla pinnata*) on productive performance in cattle and economics of farmers: A field study. The Pharma Innovation Journal. 2021: 10(4): 336-339.
31. Verma, K. A. Prakriti, S. Babu *et al.*, (2022). Implications and future prospects of Azolla as a low-cost organic input in agriculture. Agriculture, vol. 1, no. 6, pp. 1–7.

PHARMACEUTICAL PROSPECTS OF THE MANGROVE *HERITIERA FOMES*: A REVIEW

Shreyas S. Shelar[1], Varsha S. Mistry[2], Sharda K. Patil[2] and Sonal M. Manohar*[3]

[1]Department of Microbiology, Bhavan's College, Andheri (West), Mumbai - 400058, India

[2]Department of Microbiology, K. K. Wagh Arts, Commerce, Science & Computer Science College, Panchavati, Nashik - 422003, India

[3]Department of Biological Sciences, Sunandan Divatia School of Science, SVKM's NMIMS (Deemed-to-be) University, Vile Parle (West), Mumbai - 400056, India

*Corresponding author E-mail: sonal.manohar@nmims.edu

Abstract:

Mangroves form unique plant communities along the coastal and estuarine areas as they have special adaptations to survive and thrive in hostile, salty environments. They are known to produce an array of secondary metabolites which have attracted the attention of scientists worldwide for exploring their bioactivities. *Heritiera fomes* Banks (family Malvaceae) is a mangrove that has a dense, local distribution in the parts of Indian sub-continents and South East Asia. This mangrove has numerous local applications. Its parts are used as timber in furniture and boat-building industries and by the traditional healers to treat diabetes, gastro-intestinal disorders, skin diseases and so on. Like other mangroves, *H. fomes* is well known for producing a variety of phytochemicals. Extracts and compounds isolated from it have exhibited impressive biological activities. An attempt has been made to compile the up-to-date information on this mangrove plant from scientific databases. This review could serve as an authentic basis for formulating the proposed application of this easily found mangrove in standard as well as complementary medicine.

Keywords: mangroves, pharmacognosy, ethno-medicine, bioactivities, phytomedicine

Introduction:

Natural products have been playing a significant role in traditional ethno-medicine. Phytochemicals are unique compounds, comprising mainly of secondary metabolites that are derived from plants. They have served as a key source of lead molecules in various drug discovery programmes (Murugan *et al.*, 2021).

Mangroves are plants blessed with special ability to tolerate harsh, saline habitats. They not only survive in such hostile environment but also give rise to enormously productive communities. They have numerous applications and their therapeutic importance has been well documented (Bandaranayake, 2002; Manohar, 2021; Nabeelah Bibi *et al.*, 2019).

Heritiera fomes Banks is an important member of mangrove community. It is commonly found mangrove along the coastal lines of South East Asian countries like Myanmar, Malaysia, Thailand and forests of Sundarbans and Bhitarkanika in Bangladesh and India (Giesen *et al.,* 2006). In fact, the name Sundarban, literally meaning "beautiful forest" is believed to be derived from Sundari or Sundri (*Heritiera fomes*), one of the most abundant tree species found in this forest (Naskar, 2004). Forming a vast ecosystem, it supports a variety of fauna; acts as a defensive barrier against natural calamities such as floods and tsunamis; and also prevents the soil erosion.

This mangrove has been widely used by the locals in the ethno-medicine to treat a number of health-ailments. Therefore, the present review would compile latest, up-to-date information about studies done to delineate the pharmaceutical potential of *H. fomes*.

Taxonomy and classification

This mangrove species is taxonomically classified as follows:

Kingdom: Plantae
Sub-kingdom: Viridiplantae
Infra-kingdom: Streptophyta
Phylum: Tracheophyta
Sub-phylum: Spermatophytina
Class: Magnoliopsida
Super-order: Rosanae
Order: Malvales
Family: Malvaceae
Genus: *Heritiera*
Species: *Heritiera fomes*

There lies ambiguity when it comes to classifying *H. fomes* into a family. Many researchers continue to include it in Sterculiaceae family as *H. fomes* Buch. Ham. However, as per the World Mangrove Database, its classification is now revised as *H. fomes* Banks (Malvaceae family). Other mangrove members from the genus *Heritiera* include *H. littoralis* Aiton, *H. macroptera* Kosterm, *H. kanikensis* Majumdar & Banerjee, *H. globosa* Kosterm, and *H. peralata* Kosterm. (Dahdouh-Guebas 2024, World Mangrove database website link)

It is identified by the local names such as *Sundari / Sundri* in the Sundarban forests by Bangla speaking native people, as *Bada Sundari* in Bhitarkanika forest of Odisha state and as *Kanazo* in Myanmar (former Burma) (Anonymous, 2023; Giesen *et al.,* 2006).

Botany:

H. fomes is a Medium-sized evergreen tree that grows up to 25 m, and has numerous erect, pointed, peg-like pneumatophores (around 50 cm long). The trunk is straight, has a rough surface, and is brown in colour. The leaves are of alternate, simple type, dark greenish in colour and have a 1 cm long petiole. Leaf blade is elliptic-lanceolate in shape, is pointed, thick and hard, about 10-20 cm by 5-10 cm. Upper surface appears pale green in colour and is smooth, lower surface is whitish grey and feels rough, having very short, scurfy hair and a net-like venation. Flowers are present in clusters. They are pendulous and are much branched. Flowers are unisexual in *H. fomes*. They are small, cream-orange coloured to pale brownish, having a 6 mm, flower stalk. The number of sepals present is generally 4-5, appear cup-shaped, having a tube beneath, with 4-5 lobes. Calyx lobes are ovate, pointed, hairy, having leathery surfaces on both internal and external sides. Petals are not present. The male flower in *H. fomes* shows the presence of 5 stamens which are fused together. The female flower generally has 4 carpels which are loosely attached and are 2-3 mm long. The style is terminal, long, white but turns brown in colour post maturity. The fruit is sized 2 to 4 cm and appears as a cluster of woody, and winged ripe carpels, which on peeling, it looks knob like having a ventral ridge underneath, and a transverse cum circular ridge. Germination is of hypogeal type. Flowering and fruiting period slightly varies from region to region. In Sundarbans, flowering takes place from May to October whereas in Bhitarkanika, it blooms from March to August. Fruiting is generally observed from May to August. Seeds are solitary and do not exhibit vivipary, but are buoyant, helping in their dispersal (Anonymous, 2023; Giesen *et al.*, 2006; Tomlinson, 1986)

Habitat and distribution

H. fomes is a species that is often seen growing along the landward edges of mangrove forests away from the direct seawater and along brackish tidal streams. It prefers lower salinity. Regions with heavy annual rainfall of 1600 mm to 5334 mm and a warm equable climate of 7.22 °C to 37.78 °C are considered to be conducive for the growth of this species. It prefers well drained soil and grows prolifically in areas where fresh water supply is abundant.

Local and ethno-medicinal uses

A perusal of literature indicates that *H. fomes* has numerous medicinal applications. A decoction is prepared from seeds and leaves to treat various gastro-intestinal disorders such as indigestion, constipation, diarrhoea, dysentery, bloating, colic acidity and lack of appetite (Mollik *et al.*, 2010). Piles are treated using wood powder (Parikh & Datye, 2003). A paste prepared from the bark and stem is applied for curing skin diseases such as eczema, scabies, boils, scars, dermatitis, rashes etc. (Nawaz *et al.*, 2009). Hot decoction of bark is used against diabetes and goitre (Ali *et al.*, 2011; Thatoi & Patra, 2013). Traditional health practitioners use it

to cure fever and pain. Twigs are chewed to clean the teeth (Rahmatullah *et al.,* 2010). Local community has been using the wood of *H. fomes* is for building boats, making poles, and for other construction purpose also as fuel (Giesen *et al.,* 2006). Seed is consumed only in emergency in case of food scarcity. Fish poison is prepared by tribals using bark and roots. Pickle is also made from fruits as a supplementary food (Das, 2020; Mahmud *et al.,* 2014).

Phytochemistry

Both primary and secondary metabolites have been identified from different parts and extracts of *H. fomes* (Table 1). Majority studies have focussed on qualitative analysis to detect different phytochemical classes, and carbohydrates, proteins, fats, reducing sugars, vitamins, minerals, alkaloids, glycosides, anthraquinones, saponins, phenols, flavonoids, steroids, tannins and terpenoids, gums and resins have been reported by various researchers (Feda, 2022; Hossain *et al.,* 2013; Patra & Thatoi, 2015; Ripa *et al.,* 2022; Tabassum, 2020).

Table 1: Quantitative phytochemical contents in *H. fomes* leaf & stem (Patra & Thatoi, 2015)

	Leaf	**Stem**
Primary metabolites		
Carbohydrates	6.45	10.38
Proteins	8.31	9.13
Secondary metabolites		
Phenol	2.47	2.25
Flavonoid	1.88	1.48
Tannin	2.4	2.13
Alkaloid	1.88	1.26
Saponin	15.2	10.8
Vitamins		
Thaimine	0.184	0.203
Riboflavin	0.028	0.027
Minerals		
Nitrogen content	1.33	1.46
Phosphate content	0.07	0.08
Potassium content	1.00	0.99
Values in % dry weight		

Composition of chlorophyll a, chlorophyll b, carotenoids, polyphenols, tannins and proteins has been identified from the leaves (Basak *et al.,* 1996). Bark has been found to be rich

in tannins (Naskar & Guhabakshi, 1987). Proanthocyanidins, a class of polyphenols are present in higher amounts in stem and bark (Dang *et al.*, 2007; Patra & Thatoi, 2015). Flavonol such as epicatechin and phytosterols such as β-sitosterol, stigmasterol, and stigmast-4-en-3-one have been also reported from *H. fomes* extract (Wangensteen *et al.*, 2009). Three unique compounds viz. 2, 5-Anhydrogluconic acid, aminopyrine, and palmitic acid were identified from the root exudates of *H. fomes* using paper chromatography followed by GC-MS (Kumar *et al.*, 2009). Quercitrin-like flavonoid derived glycosides with anti-diabetic activities were identified in the hot water extract of *H. fomes* (Ansari *et al.*, 2022).

Proximate composition and nutrient analysis of *H. fomes* fruits was studied by Hosen *et al.*, (2020). Researchers reported that the fruits are a rich source of proteins, lipids, and vitamin C along with some important micro-nutrients (Table 2).

Table 2: Analysis of nutrients from *H. fomes* fruits (Hosen *et al.*, 2020)

Nutrient / Element / Mineral	**Concentration**
Carbohydrates	8.7 %
Proteins	18.6 %
Lipids	3.1 %
Vitamin C	87.7 mg AA/ 100 g powder
Ca	52.3 mg/kg powder
Cu	10.4 mg/kg powder
Fe	83.8 mg/kg powder
Mg	332.2 mg/kg powder
Mn	8.4 mg/kg powder
P	953.5 mg/kg powder
Zn	10.3 mg/kg powder
K	15.3 g/kg powder
Na	1.3 g/kg powder

Pharmacological activities

Antibacterial

Pneumatophore-extracts prepared in ethanol showed encouraging activity against the tested bacterial strains with zones of inhibitions being greater than 10 mm against majority. *Enterobacter aerogenes* was found to be the most susceptible with a zone of inhibition recorded at 21 mm. (Mondal *et al.*, 2008). The ethanol bark extract was found to be antibacterial in nature when tested against *S. aureus, K. rhizophilia, B. subtilis* and *P. aeruginosa* (Wangensteen *et al.*,

2009) *H. fomes* methanol: ethanol 1:1 fruit extract exhibited relatively weaker anti-bacterial activity against *E. coli*, *Klebsiella* sp., *Shigella boydii*, *S. sonnei*, and *S. aureus* (Hosen *et al.*, 2021). Silver and zinc oxide nano-particles synthesized from the aqueous extracts of *H. fomes* reported zone of inhibition in the range of 9 to 16 mm against the tested pathogenic bacteria (Thatoi *et al.*, 2016). Ethyl acetate extracts of different parts showed highest antibacterial activity against *Shigella dysenteriae* and *Shigella boydii* (Tabassum, 2020). Moderate antibacterial activity of extracts prepared in different solvents has been also reported by Patra & Thatoi (2015) and in acetone by Kalyani *et al.*, (2020).

Antifungal

Weak antifungal activity of methanol and aqueous leaf extracts of *H. fomes* was observed against *Candida kruzii* and *Fusarium* sp. (Patra & Thatoi, 2015).

Anti-oxidant

Wangensteen *et al.*, (2009) reported a promising DPPH radical scavenging and 15-lipoxygenase inhibitory activity and attributed it to the procyanidins present in the ethanol bark extract of *H. fomes*. Hosen *et al.*, (2020) reported high antioxidant activity of *H. fomes* fruit extract with 86.6% DPPH scavenging at 50 µg powder /ml and a reducing power OD of 1.87. Total antioxidant capacities were observed at 102.1 mg GAE/ g powder and 75.9 mg AAE/g powder. Moderate *in vitro* activity of extracts prepared in different solvents has been reported by Patra & Thatoi (2015) with antioxidant capacity recorded between 41.31 to 175.89% dry weight basis. Leaf methanol exhibited the most potent antioxidant activity. Uddin *et al.*, (2004) reported moderate antioxidant potential with free radical scavenging activity RC_{50} values of 2.5 X 10^{-2} and 8.1 X 10^{-3} mg/ml for leaf ethanol and bark ethanol extracts respectively. Synthesized silver and zinc oxide nanoparticles exhibited moderate free radical scavenging activities (Thatoi *et al.*, 2016). Leaf and bark extracts prepared in acetone showed impressive antioxidant activity with IC_{50} values of 26.30 µg/mL and 22 µg/mL respectively (Kalyani *et al.*, 2020). DPPH radical scavenging assay showed encouraging IC_{50} values of 82.24 and 124.98 µg/ml for ethanol bark and leaf extracts respectively. A strong correlation was recorded between total phenolic and flavonoid content and antioxidant capacities (Islam *et al.*, 2019). Pneumatophore ethanol extract was found to have high antioxidant activity with scavenging capacity SC_{50} values of 49 µg/ml and 87 µg/ml for DPPH radical scavenging assay and hydrogen peroxide radical scavenging assay respectively (Sultana *et al.*, 2022).

Analgesic

Ethanol extract prepared using leaves and roots of *H. fomes* was found to have *in vivo* analgesic (pain relieving) activity at par with the standard drug indomethacin. Percentage (%)

inhibition of induced pain in mice was recorded at 84.67 and 83.54 for leaves and roots extracts respectively (Alam, 2022).

Anti-inflammatory

Silver and zinc oxide nano-particles prepared from the aqueous extracts were found to have promising anti-inflammatory activity of approximately 69% and 79% respectively (Thatoi *et al.,* 2016). In another *in vitro* study, bark and leaf ethanol extracts of *H. fomes* indicated their anti-inflammatory potential by inhibiting the haemolysis of erythrocytes. This was further vindicated by an *in vivo* study which showed a 44% decrease in mice paw oedema after they were administered with the plant extract (Islam *et al.,* 2019).

Anti-nociceptive

It a process involving blocking the detection of pain stimulus. Methanol bark extract and ethanol leaf extract were reported to have *in vivo* anti-nociceptive activity (Ali *et al.,* 2011; Hossain *et al.,* 2013)

Anti-diarrhoel

Fruit extract prepared in 1:1 methanol : ethanol at a dosage of 250 mg/Kg of body weight was found to be anti-diarrhoel in nature as it drastically inhibited diarrhoea in tested mice (induced with castor oil administration) (Hosen *et al.,* 2021).

Anti-diabetic

Ansari *et al.,* (2022) reported significant improvement in glucose tolerance and in plasma insulin releasing response. Hot aqueous extract of *H. fomes* was tested *in vitro* and *in vivo*. Significant reduction in glucose absorption was observed in rats which were orally administered with the extract. Sarkar *et al.,* (2019) reported promising *in vitro* anti-diabetic activity of leaf extract based on the inhibitory capacity of enzyme α-amylase, transport of glucose molecules across yeast cells and increased glucose adsorption capacity. *In vivo* anti-hyperglycaemic activity of methanol bark extract has been reported. A significant reduction in the glucose concentrations in serum was recorded after 60 and 90 min of dose administration at 250 and 500 mg/kg body weight (Ali *et al.,* 2011). Synthesized silver and zinc oxide nanoparticles exhibited moderate α-amylase inhibitory potential (Thatoi *et al.,* 2016). Ethanol extract of leaves, bark, and roots exhibited highly promising hypoglycaemic activity when administered in alloxan-induced diabetic Swiss albino mice at 200 and 400 mg/kg body weight (Feda, 2022). Ethanol extract of pneumatophores was also found to have significant *in vivo* anti-hyperglycaemic activity when tested in streptozotocin-induced diabetic rats (Sultana *et al.,* 2022).

Anti-arthritic

It is measured with respect to percentage inhibition of Bovine serum protein denaturation by BSA method. More the % inhibition, more the activity. Leaf ethanol, root chloroform, and

bark ethanol extracts of *H. fomes* showed moderate yet promising (being crude extracts) anti-arthritic efficacy with 49.49%, 49.06%, and 47.83% respectively as compared to 96.91% by the standard diclofenac sodium (Eusufzai *et al.,* 2020). In another *in vitro* study, 63.28% protein inhibition by chloroform root extract was reported by Ripa *et al.,* (2022).

Anti-obesity

Mirza *et al.,* (2017) orally administered leaf methanol extract to high fat cafeteria diet (HFCD) fed obese wistar rats and observed significant lowering of physic-chemical parameters such as gained body and organ weight, cholesterol, triglycerides and glucose levels. Elevated enzymatic liver markers due to induced obesity also resumed their normal levels post treatment with *H. fomes* extract. Overall results of this *in vivo* study clearly highlighted the anti-obesity potential of *H. fomes*.

CNS depressant

Central Nervous System depressant activity of *H. fomes* in mice models was studied (Taslim, 2022). The ethanol extract of *H. fomes* given at a dose of 250 mg/kg and 500 mg/kg significantly reduced the locomotor activity of mice when analyzed using open field and hole-cross tests as compared to the control distilled water and the commercial drug diapzepam. Statistically significant increase in immobility time force was recorded after the administration of plant extract.

Thrombolytic

Leaf, root, bark extracts prepared using ethanol, petroleum ether, chloroform, and ethyl acetate showed noteworthy thrombolytic activity with clot lysis percentage ranging of 14.55 to 22.98%. Leaf petroleum ether extract was observed with highest activity of 22.98% which was not very less than 24.05% exhibited by the standard clopidogrel (Eusufzai *et al.,* 2020). Ripa *et al.,* (2022) also reported moderate thrombolytic activity of different *H. fomes* extracts/fractions with highest % clot lysis of around 33.12 shown by ethanol leaf extract as compared to 67.77% exhibited by the standard streptokinase.

Cytotoxic

Bark extract showed no cytotoxic effect when analysed through the brine shrimp lethality assay (Wangensteen *et al.,* 2009). However, Hosen *et al.,* (2021) reported a promising cytotoxicity of the fruit extract with IC_{50} value of 74.1 µg/ml. Leaf chloroform and bark methanol extracts had shown LC_{50} values of 234.77 and 47.09 mg/ml respectively against *Artemia salina* naupli (Rahmatullah *et al.,* 2010). Weak cytotoxicity of extracts prepared from different parts was also reported by Tabassum (2020). Chloroform was found to demonstrate relatively higher mortality rate than other extracts.

Anticancer

Anticancer activity of leaf and stem methanol extract was reported by Patra & Thatoi (2013). A 40% inhibition of B16 mouse melanoma with IC_{50} values of 75 and 100 µg/ml was observed in the *in vitro* experiment whereas experiments done in the Swiss albino mice showed an inhibition of 41% against Ehrlich Ascites Carcinoma (EAC).

Anthelmintic and insecticidal

Petroleum ether, chloroform, and ethyl acetate soluble fractions of ethanol extract prepared from leaf, bark, and root of *H. fomes* were tested against Helminth *Pheretima posthuma* and notorious pest beetle *Tribolium castaneum*, respectively. All the tested extracts showed a promising anthelmintic activity at concentrations of 25–75 mg/mL and resulted in the paralysis and death of earthworms in comparison with standard chemical albendazole. The ethanol bark extract was found to be the most potent. In case of insecticidal test, leaf ethanol extract exhibited the most lethal mortality rate (73%) (Ripa *et al.*, 2022)

Conclusion:

The present review clearly highlights the pharmaceutical potential of mangrove *H. fomes* as indicated by the diverse range of bioactivities showcased by this plant. More emphasis has to be given on the isolation of novel bioactive compounds from this plant which would have far potent activities than the crude and un-purified extracts.

References

1. Alam F. (2022) Evaluation of analgesic activity of different parts of *Heritiera fomes* on Swiss albino mice. B. Pharm. Thesis, School of Pharmacy, Brac University, Bangladesh.
2. Ali M., Nahar K., Sintaha M., Khaleque H. N., Jahan F. I., Biswas K. R. *et al.,* (2011) An evaluation of anti-hyperglycemic and antinociceptive effects of methanol extract of *Heritiera fomes* Buch- Ham. (Sterculiaceae) barks in Swiss albino mice. Advances in Natural and Applied Sciences, 5(2): 116-121.
3. Anonymous (2023) Mangroves of Odisha. A pictorial guide. Mangrove Forest Division (Wildlife), Rajnagar Forest & Environment Department, Government of Odisha, pp. 1-60.
4. Ansari P., Flatt P. R., Harriott P. & Abdel-Wahab Y. H. A (2022) Insulin secretory and antidiabetic actions of *Heritiera fomes* bark together with isolation of active phytomolecules. PLoS ONE 17(3): e0264632.
5. Bandaranayake W. M. (2002) Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetl. Ecol. Manag. 10: 421-452.
6. Basak U. C., Das A. B. & Das P. (1996) Chlorophylls, carotenoids, proteins and secondary metabolites in leaves of 14 species of mangrove. Bulletin of Marine Science, 58(3): 654-659.

7. Dahdouh-Guebas F. (Ed.) (2024) World Mangroves database. *Heritiera fomes* Banks. Accessed through: World Register of Marine Species at: https://www.marinespecies.org/aphia.php?p=taxdetails&id=235117 on 2024-02-10
8. Dang H. C. T. (2007) Antioxidants in *Heritiera fomes*, a medicinal plant from the Mangrove Forest of Bangladesh. M.S. thesis, School of Pharmacy, University of Oslo, Oslo, Norway.
9. Das S. (2020) A review on the pharmacological properties of *Heritiera fomes*. B. Pharm. Thesis, School of Pharmacy, Brac University, Bangladesh.
10. Eusufzai M. (2020) *In vitro* thrombolytic and anti-arthritic activities of *Heritiera fomes*. B. Pharm. Thesis, School of Pharmacy, Brac University, Bangladesh.
11. Giesen W., Wulffraat S., Zieren M. & Scholten L. (2006) Mangrove Guidebook for Southeast Asia. FAO Regional Office for Asia and the Pacific, Bangkok, Thailand: Wetlands International, Bangkok, Thailand.
12. Hosen M. Z., Biswas A., Islam M. R. & Hossain S. J. (2021) Anti-bacterial, anti-diarrheal, and cytotoxic activities of edible fruits in in the Sundarbans mangrove forest of Bangladesh. Prev. Nutr. Food Sci. 26(2): 192-199.
13. Hosen M. Z., Biswas A., Islam M. R., Bhuiyan M. N. I. & Hossain S. J. (2020) Comparison of physicochemical and antioxidant properties of edible fruits in the Sundarbans' Mangrove forest, Bangladesh. Bangladesh J. Bot. 49(3): 671-678.
14. Hossain, M., Panthi, S., Asadujjaman, M., Khan, S., Sadhu, S. & Ferdous, F. (2013) Phytochemical and pharmacological assessment of the ethanol leaves extract of *Heritiera fomes* Buch. Ham. (Family-Sterculiaceae). Journal of Pharmacognosy and Phytochemistry, 2(3): 95-101.
15. Islam Md. E., Islam K. M. D., Billah Md. M., Biswas R., Sohrab Md. H. & Rahman S. M. M. (2019) Antioxidant and anti-infammatory activity of *Heritiera fomes* (Buch.-Ham), a mangrove plant of the Sundarban. Oriental Pharmacy and Experimental Medicine. 20: 189-197.
16. Kalyani C., Tulasi C. D. S. L. N., Swathi M. S., Geetha A., Narasu M. L. & Saida L. (2020) Screening of antimicrobial and antioxidant activity of acetone extracts of *Heritiera fomes* whole plant against pathogens. Int. J. Pharm. Investigation, 10(4): 564-568.
17. Kumar T., Ray S., Brahmachary R. L., & Ghose M. (2009) Preliminary GC–MS analysis of compounds present in the root exudates of three mangrove species. Acta Chromatographica, 21(1): 117-125.

18. Mahmud, I., Islam, M. K., Saha, S., Barman, A. K., Rahman, M. M., Anisuzzman, M., *et al.*, (2014) Pharmacological and ethnomedicinal overview of *Heritiera fomes*: Future Prospects. International Scholarly Research Notices, 2014(July): 1-12, Article ID 938543.
19. Manohar S. M. (2021) A review of the botany, phytochemistry and pharmacology of mangrove *Lumnitzera racemosa* Willd. Pharmacog. Rev. 15(30): 107-116.
20. Mirza M. S., Ali S. A. & Sanghvi I. (2017) Anti-obesity potential of *Heritiera fomes* leaves methanolic extract in a preclinical model. IOSR Journal of Pharmacy and Biological Sciences, 12(4):1-5.
21. Mollik M. A. H., Hossan M. S. H., Paul A. K., Taufiq-Ur-Rahman M., Jahan R. & Rahmatullah M. (2010) A comparative analysis of medicinal plants used by folk medicinal healers in three districts of Bangladesh and inquiry as to mode of selection of medicinal plants. Ethnobotany Research and Applications, 8:195-218.
22. Mondal S., Paul S. K., Uddin S. J., Nahar L., Auzi A. A. & Sarker S. D. (2008) A comparative study on the *in vitro* antibacterial activity of the pneumatophores of *Heritiera fomes* and *Xylocarpus moluccensis*. Ars Pharmaceutica, 49(1): 77-82.
23. Murugan P., Salim A., Chenthamara D., Robert B. & Subramaniam S. (2021) Therapeutic and pharmacological efficacy of selective Indian medicinal plants – A review. Phytomedicine Plus, 1(2): 100029.
24. Nabeelah Bibi S., Mahomoodally M. F., Zengin G., Jeewon R., Nazurally N., Rengasamy Kannan R. R., *et al.*, (2019) Ethnopharmacology, phytochemistry, and global distribution of Mangroves—A comprehensive review. Marine Drugs, 17(4): 231.
25. Naskar K. & Guhabakshi D. N. (1987) Mangrove swamps of Sunderbans, an ecological perspective. Naya Prakash, Calcutta, India.
26. Naskar K. R. (2004) Manual of Indian Mangroves. Daya Publishing House, Delhi, India.
27. Nawaz A. H. Md. Mahabub, Hossain M., Karim M., Khan M., Jahan R. & Rahmatullah M. (2009) An ethnobotanical survey of Jessore district in Khulna division, Bangladesh. American-Eurasian Journal of Sustainable Agriculture, 3(2): 238-243.
28. Parikh J. & Datye H. (2003) Sustainable management of Wetlands: biodiversity and beyond. SAGE Publications, New Delhi, India.
29. Patra J. K. & Thatoi H. (2013) Anticancer activity and chromatography characterization of methanol extract of *Heritiera fomes* Buch. Ham., a mangrove plant from Bhitarkanika, India. Oriental Pharmacy and Experimental Medicine, 13(2): 133-142.
30. Patra J. K. & Thatoi H. (2015) *In vitro* bioactive potential of an ethnomedicinal mangrove plant, (*Heritiera fomes* Buch. Ham.) from Odisha coast, India. Indian Journal of Geo-Marine Sciences, 44(5): 704-713.

31. Rahmatullah M., Sadeak M. I., Bachar S. C., Hossain M. T., al-Mamun A., Montaha, Jahan N., *et al.*, (2010) Brine shrimp toxicity study of different Bangladeshi medicinal plants. Adv. Nat. Appl. Sci. 4(2): 163-173.
32. Ripa F. A., Hossain M. J., Nesa L., Zahan S., Mitra S., Rashid M. A., Roy A., Alghamdi S., Almehmadi M. & Abdulaziz O. (2022) Phytochemical and pharmacological profiling of *Heritiera fomes* Buch. Ham. deciphered thrombolytic, antiarthritic, anthelmintic, and insecticidal potentialities via *In vitro* approach. Evidence-Based Complementary and Alternative Medicine, 2022: 1-9, Article ID 2594127.
33. Sarkar B. K., Barman S. K., Akhter S., Akter R., Das J., Sarkar A. P., Akter R. & Kundu S. K. (2019) Evaluation of *in vitro* anti diabetic activity of two mangrove plant extracts: *Heritiera fomes* and *Sonneratia apetala*. Journal of Pharmacognosy and Phytochemistry, 8(6): 2376-2380.
34. Sultana M. S., Golder M., Biswas B., Karmakar U. K., Bokshi B., Alam M. J., Shahriar M. & Sadhu S. K. (2022) Antioxidative and antidiabetic potentials of the pneumatophores of *Heritiera fomes* Buch. Ham. Dhaka Univ. J. Pharm. Sci. 20(3): 283-291.
35. Tabassum R. (2020) Phytochemical screening, antimicrobial and cytotoxicity studies of *Heritiera fomes*. B. Pharm. Thesis, School of Pharmacy, Brac University, Bangladesh.
36. Taslim U. (2022) CNS depressant activity of various parts of *Heritiera fomes*. B. Pharm. Thesis, School of Pharmacy, Brac University, Bangladesh.
37. Thatoi P., George Kerry R., Gouda S., Das G., Pramanik K., Thatoi H., & Patra J. K. (2016) Photo-mediated green synthesis of silver and zinc oxide nanoparticles using aqueous extracts of two mangrove plant species, *Heritiera fomes* and *Sonneratia apetala* and investigation of their biomedical applications. Journal of Photochemistry and Photobiology B: Biology, 163: 311-318.
38. Tomlinson P. B. (1986) The Botany of Mangroves. Cambridge University Press, UK.
39. Uddin S. J., Shilpi J. A., Delazar A., Nahar L. & Sarker S. D. (2004) Free radical scavenging activity of some Bangladeshi plant extracts. Oriental Pharmacy and Experimental Medicine, 4(3): 187-195.
40. Wangensteen H., Dang H. C. T., Uddin S. J., Alamgir M. & Malterud K. E. (2009) Antioxidant and antimicrobial effects of the mangrove tree *Heritiera fomes*. Natural Product Communications, 4(3): 371-376.

HYBRIDIZATION: DETAILED REVIEW

Kiruthika M, Ranjana J, Neelavathi U and Senthil J*

Department of Food Technology,

Paavai Engineering College (Autonomous), Namakkal, Tamil Nadu, India

*Corresponding author E-mail: senthilj25@gmail.com

Abstract:

Hybridization has played a major role in the evolution of different lineages, facilitating speciation, adaptability to novel habitats, and phenotypic variation. The study of gene flow between divergent taxa has become easier to do thanks to advances in genomic technologies. This has led to the discovery of both short-term impacts, like hybrid Vigor, and long-term ones, like local adaptation and the emergence of new hybrid species. Plant hybridization is the process of employing one plant to be the male and another as the female. The outcome is F1 hybrid seeds, which are used for transgressive and combination breeding. The study of how hybridization affects biological variety has centuries-long historical roots. Modern genomic and genetic technologies have completely changed how we can record and examine plant hybridization. Based on genetic variations between parents, two types of plant hybridization are identified: distant hybridization and inter-varietal hybridization. Using a dataset spanning several locations, the study explores large-scale evolutionary patterns of hybridization in vascular plants, exposing hybridization variance and widespread occurrence throughout families and species. Taxon species richness influences hybridization propensity, indicating intrinsic group traits are important, and a substantial phylogenetic signal suggests non-uniform distribution among plant orders. Comparative testing on the variables influencing hybridization propensity are made easier by an understanding of hybridization behaviour, which aids in the prediction of characteristics and evolutionary trajectories.

Keywords: Different lineages, Phenotype, Genotype, Transgressive plant, Phylogenetic signals.

Introduction:

In the fields of genetics and molecular biology, hybridization is the blending or combination of various genetic material or traits of a wild two different plants. When organisms from distinct populations, varieties, or species interbreed, the outcome is offspring that have characteristics from both parent sources. Many biological domains, including plants, animals, and microbes, exhibit hybridization. In genetics, hybridization relate to various processes like,

1. **Plant hybridization:** The deliberate crossing or breeding of two distinct plant species or kinds is known as hybridization in the field of plant breeding. The purpose of this

deliberate genetic mixing is to produce hybrid plants that have desired characteristics, such higher yield, disease resistance, or environmental adaptability.

2. **Animal hybridization:** Just like plants, animals may also cross-pollinate, producing hybrid offspring that frequently combine traits from both parent species. Animals that are hybrids can arise spontaneously or by deliberate breeding with the aim of producing animals with special qualities or augmenting particular attributes.
3. **Molecular biology and DNA hybridization:** The process of joining complementary DNA strands from various origins is known as DNA hybridization. This procedure is commonly employed in genetic material detection, analysis, and manipulation techniques such as Southern blotting, DNA microarrays, and polymerase chain reaction (PCR).
4. **Genetic hybridization in microorganisms:** Bacteria, fungus, and other microorganisms are capable of genetic hybridization, which results in the exchange of genetic material and the development of hybrid strains with unique traits.

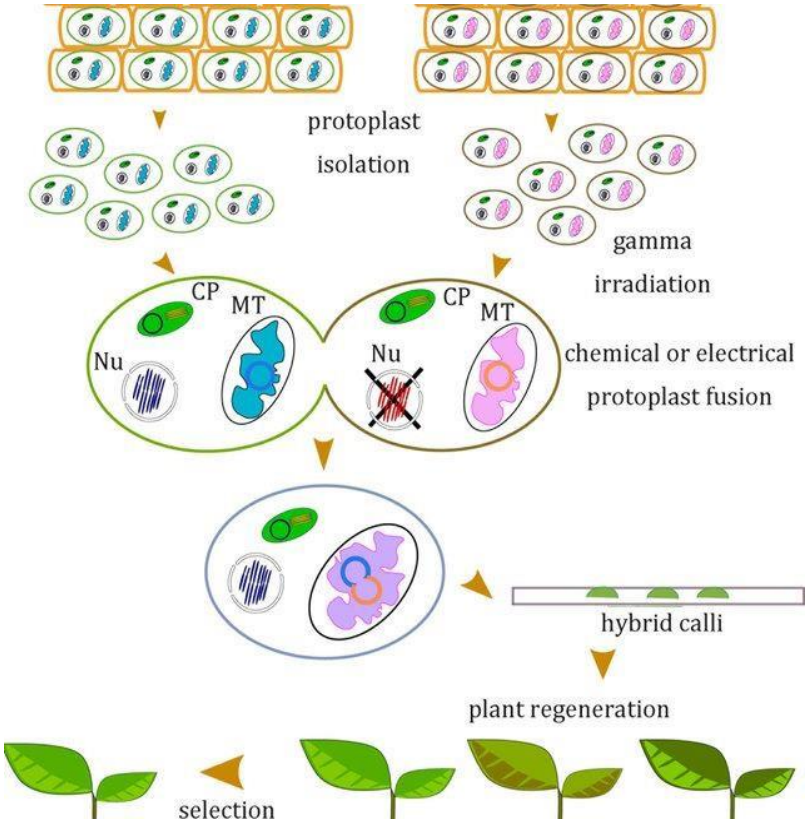

Figure 1: General mechanism of hybridization

In evolutionary processes, hybridization is crucial because it increases genetic variety within populations. It may result in the emergence of beneficial features that help organisms adapt to shifting environmental conditions. Hybridization is also a strategy utilized by scientists and breeders in agriculture, medicine, and other research domains to accomplish certain objectives.

General steps for the Hybridization in Plants:

1. Selection and Preparation of Parents
2. Emasculation
3. Bagging
4. Pollination
5. Selection.

Evolutionary consequences of hybridization

Evolutionary consequences of hybridization refer to the impact that The long-term evolutionary paths of some organisms are impacted by the interbreeding of various species or populations. Genetic diversity, environmental adaptability, and the possible formation of new species are all impacted by hybridization. The following are some significant effects of plant hybridization on evolution are,

1. **Enhanced genetic diversity**: New gene combinations from several parent plants are introduced through hybridization, which increases genetic diversity within the population. Increased diversity can operate as a supply of raw material for natural selection, which may lead to populations that are more adaptive.
2. **Adaptation to new habitats:** Plants with characteristics that enable them to flourish in unfamiliar or shifting habitats might arise as a result of hybridization. Hybrids may show greater fitness and adaptability by integrating features from multiple parent species or variations, increasing their chances of surviving in a variety of ecological niches.
3. **Hybrid strength (Heterosis):** The occurrence of hybrid plants displaying superior features over those of their parent species is referred to as heterosis. Higher yields, disease resistance, and faster growth rates are a few examples of this. In agriculture, hybrid vigor is frequently used to create crops with desired qualities.
4. **Introgression of novel alleles**: Genetic material from one parent species may be introduced into another through hybridization. Through this mechanism, new alleles may be introduced into a population, possibly leading to new adaptations and influencing the species' overall genetic composition.
5. **Transgressive segregation**: The manifestation of features outside the range seen in the parental species might result from hybridization. Transgressive segregation is a phenomena that can produce plants with unusual or severe phenotypes, adding to the population's total diversity.
6. **New hybrid species formation**: Recurrent hybridization events followed by stabilizing processes may occasionally result in the emergence of new hybrid species. These hybrid

species may contribute to biodiversity by having special combinations of traits and qualities that set them apart from their parent species.

7. **Reproductive isolation and speciation:** The evolution of reproductive isolation between populations can also be attributed to hybridization. Over the course of evolutionary time scales, hybrids that have lower fertility or are reproductively isolated from their parent species may help give rise to new species.
8. **Ecological and evolutionary innovations:** Plants with novel ecological strategies or evolutionary innovations can arise as a result of hybridization. Hybrids may acquire new characteristics that enable them to fill ecological niches, changing the dynamics of plant communities.

Classes of hybrids

Hybrids can be classified into various categories based on the nature of the parent organisms and the resulting genetic combination. They are

1) Intraspecific hybrids:
    - Hybrids formed between individuals of the same species.
    - Crossing two different varieties of tomatoes.
2) Interspecific hybrids:
    - Hybrids formed between individuals of different but closely related species.
    - Mules, which result from crossing a horse and a donkey.
3) Intergeneric hybrids:
    - Hybrids formed between individuals of different genera.
    - Tangelos, which are a cross between tangerines (*Citrus reticulata*) and grapefruits (*Citrus paradisi*).
4) F1 hybrids:
    - The first generation of offspring resulting from the cross between two genetically distinct parents.
    - F1 hybrid tomatoes produced by crossing two different tomato varieties.
5) Backcross hybrids:
    - Hybrids formed by crossing an individual with its parent or an organism genetically similar to its parent.
    - Crossing an F1 hybrid with one of its parent plants.
6) Synthetic hybrids:
    - Hybrids formed by crossing individuals from different populations to create a genetically diverse population.

- Creating a population with desirable traits by crossing individuals from various sources

7) Cytogenetic hybrids:
    - Hybrids with different chromosome numbers due to chromosome doubling or reduction.
    - Triploid watermelons, which have three sets of chromosomes.
8) Somatic hybrids:
    - Hybrids formed by combining the somatic cells of two organisms.
    - Production of hybrid plants by fusing plant cells from different varieties.
9) Allopolyploid hybrids:
    - Hybrids formed by the combination of different species with subsequent chromosome doubling.
    - Triticale, a hybrid between wheat (Triticum) and rye (Secale) with doubled chromosomes.
10) Heterosis or hybrid vigor:
    - Hybrids that exhibit greater fitness, growth, or other desirable traits compared to their parents.
    - Hybrid corn varieties that often show increased yield compared to inbred lines.

Types of hybridization

There are two types of hybridization:

1. Somatic hybridization - A relatively new method that is carried out *in vitro* condition.
2. Sexual hybridization - faces the obstacle of sexual compatibility.

1) Somatic Hybridization:

Through the genetic engineering process of somatic hybridization, plants of multiple species or types can be created by fusing protoplasts of the cells without cell walls combine together. Somatic hybridization allows the direct cellular fusion of the genetic material of two parent plants, in contrast to traditional hybridization techniques that entail sexual reproduction and seed development. A general description of the somatic hybridization procedure is provided below:

- **Separation of protoplasts:** Plant tissues are broken down enzymatically to release the protoplasts. Typically, stems, leaves, or other plant parts are used to obtain protoplasts.
- **Fusion of protoplasts:** Different plant species or variations' protoplasts are fused together via chemical or physical means. Protoplast formation can be induced by electric fields, polyethylene glycol (PEG), or other fusogenic substances.

- **Plant regeneration in hybrids:** The genetic material from both parent plants is now present in the fused protoplasts, which are grown in a way that encourages cell proliferation and the growth of new plants.
- **Picking and filtering:** To find and separate the cells that have successfully merged and regenerated into hybrid plants, selection techniques are applied. This could entail using markers, selective media, or other methods.
- **Establishment and adaptation:** After the chosen hybrid cells are encouraged to grow roots, the resultant plantlets are adjusted to their new surroundings.
- **Hybridity verification:** To validate the hybrid nature of the regenerated plants, their genetic makeup is checked. Molecular methods, including DNA markers, can be employed to guarantee that the genetic material of both parent plants is included.

Advantages of somatic hybridization

1. Overcoming barriers to cross ability.
2. Quick evolutionary advancement.
3. Preserving particular qualities.
4. Development of new varieties.

Limitations of somatic hybridization

While somatic hybridization is a useful and innovative method in plant biotechnology for enhancing crops, there are certain drawbacks as well, which are listed below:

1. It is possible that somatic hybridization does not always result in plants with fruitful seeds.
2. Because of chromosomal loss, organelle segregation, somaclonal variances, etc., the plants produced using this procedure may vary. Sometimes it is impossible to produce viable somatic hybrids between distinct species or genera.
3. The effectiveness of hybrid selection techniques is not without restrictions.
4. This technique does not guarantee the expression of any certain character.
5. It is not advantageous when two diploids undergo somatic hybridization because an amphidiploid is created.

2) Sexual hybridization

Plants can breed naturally or under control through a process called sexual hybridization, which is the interbreeding of two different plant kinds, species, or even genera to create offspring that have a combination of genetic features from both parent plants. Sexual hybridization is dependent on the fusing of male and female gametes, usually through the processes of pollination and fertilization, in contrast to asexual reproduction, which involves the generation of offspring without the involvement of gametes. Some important facts of plant sexual hybridization are:

- **Pollination and fertilization:** In sexual hybridization, pollen, which contains male gametes or sperm cells, is transferred from one plant's male reproductive organs (anthers) to another plant's female reproductive organs (stigma). Fertilization is the next step, in which the male and female gametes (egg cells) combine to form a zygote.
- **Genetic variation:** Within populations, sexual hybridization increases genetic diversity. Offspring that receive genetic material from two parent plants have a distinct set of features. For plant species to be adaptive and evolve over time, there must be genetic variety.
- **Natural hybridization**: In natural environments, pollen is transferred between plants by the wind, insects, birds, or other pollinators, leading to sexual hybridization. Plant populations with a variety of genetic makeup are developed as a result of this process.
- **Controlled breeding:** To create new cultivars or varieties with particular desirable qualities, plant breeders frequently use sexual hybridization as a controlled breeding technique. Breeders can affect the genetic composition of the progeny by choosing parent plants with complementing traits.
- **Hybrid vigor:** Some hybrid plants show characteristics that are amplified in comparison to their parent plants, such as increased growth, Vigor, and productivity.

Advantages of sexual hybridization

1. A greater diversity of genetic types: flexibility in changing environments
2. Heterosis - Hybrid Vigor
3. Creation of New Types
4. Preserving Appealing Qualities
5. Processes of Natural Evolution
6. Development of New Species
7. Supporting Programs for Plant Breeding

Limitations of sexual hybridization

1. The compatibility of genes
2. Dual Suitability
3. Reproductive Seclusion
4. Environmental Restrictions
5. Moral and Sociological Aspects

Patterns of plants

In plants, hybridization can lead to a variety of patterns and results depending on the genetic composition of the parent plants. Several trends that occur during plant hybridization include:

- **Recessiveness and dominance**: When at least one parent plant provides the dominant allele, dominant features are displayed. Only when the recessive alleles are contributed by both parent plants can recessive features manifest.
- **Segregation**: According to Mendel's law of segregation, each gamete carries a single allele and that two alleles for a trait segregate, or separate, from one another during the creation of gametes. This raises the prospect of various allele combinations in the progeny.
- **Independent assortment:** Mendel's law of independent assortment is applicable when genes corresponding to distinct phenotypes are found on distinct chromosomes. A characteristics inheritance does not affect the inheritance of another trait; therefore the children may acquire different combinations of traits.
- **Incomplete dominance:** In certain instances, the hybrid exhibits an intermediate phenotype since neither allele is fully dominant.
- **Codominance:** When both alleles are expressed in the heterozygous state, a phenotype that reflects traits from both parents are produced.

Outbreeding depression and genetic rescue in hybridization

Concepts like genetic rescue and outbreeding depression pertain to the results of hybridization, especially when individuals from diverse populations or species are involved.

1) Depressive outbreeding

When individuals from two distinct populations or closely related species cross, and the hybrid offspring show less fitness or adaptability than the parent populations, this phenomenon is known as outbreeding depression. This occurrence may occur because of the introduction of novel and harmful genetic combinations, the disruption of co-adapted gene complexes, or incompatible genetic interactions. In hybrids, reduced fitness can take the form of decreased survival, lower success rates during reproduction, or other characteristics that impair the population's ability to function as a whole.

2) Genetic rescue

Genetic rescue is the term used to describe the process wherein higher fitness and population recovery are achieved through the introduction of genetic material from one population into another, frequently through hybridization. When a population's adaptive capability is increased by the introduction of additional genetic variety, it can overcome environmental obstacles or the depressive effects of inbreeding. This phenomenon is known as genetic rescue. Improved fitness, increased adaptation, increased survival, and successful reproduction within the population can all result from increased genetic variety.

Factors affecting the hybridization process

Plant breeding methods used to create new plant varieties with desired features can also be included in hybridization procedures, in addition to nucleic acids.

1) Genetic diversity

Many factors contribute to a hybrid's reduced fitness, including negative interactions between genes derived from the genomes of the two parental species (hybrid incompatibilities), ecological selection against hybrids, and variations in the number of harmful variants carried by the hybridizing species (hybridization load).

2) Plant species and genotype

Plant transformation is genotype dependent, meaning that a restricted number of plant species or cultivars can be genetically transformed. A strategy that works well for one variety of plants may not always work well for another.

For instance, B73 cannot be treated as maize inbred B104.

3) Ploidy level

The quantity of chromosomal sets within a cell is referred to as its ploidy level. Incompatible mating between parent plants that have different ploidy levels (e.g., tetraploid, diploid) can result in unsuccessful embryo development.

4) Timing of flowering

Proper pollination and hybridization depend on parent plants' flowers blooming at the same time. The likelihood of effective fertilization and pollen transport is increased when flowering timings coincide.

5) Pollination technique

The success of hybridization is influenced by the type of pollination, be it natural or artificial (hand-pollination, for example). In order to guarantee certain crossings and avoid unintentional pollen contamination, controlled pollination procedures are used.

6) Environmental factors

The vitality of pollen and the susceptibility of stigma to it are influenced by temperature, humidity, and light, which in turn affects the success of hybridization. A favorable environment is conducive to effective fertilization.

7) Pollinator behavior

Pollinator behavior affects the flow of pollen from one variety of plant to another. By promoting pollen transmission, an understanding of pollinator preferences and behavior helps maximize hybridization outcomes.

8) Genetic barriers

Plant species or genotypes may find it difficult to successfully hybridize if there are genetic barriers, such as prezygotic (incompatible gametes) or postzygotic (hybrid inviability).

9) Parental line selection

To create hybrids with the required qualities, careful consideration of parental lines with complementing traits like yield and disease resistance is necessary.

10) Breeding methods

Breeders cross two genetically different parent plants through hybridization to produce offspring that combine attractive features from both parents. By using this technique, new genetic diversity is introduced and complementing features can be combined.

Conclusion:

In the development processes of various plant, evolutionary biologists recognize hybridization as a widespread phenomenon across diverse taxa. From early taxonomic studies to the genomic era, botanists have explored the evolutionary implications of hybridization. Access to extensive genomic data reveals complex patterns of ancestry within genomes, shedding light on the varying degrees of gene flow between populations. Integrating modern genetic techniques with classical experimental methods allows researchers to understand the mechanisms and consequences of hybridization, including heterosis and transgressive segregation. By combining new and traditional approaches, scientists can infer the history of gene flow and selection, uncovering insights into the evolutionary dynamics of plant lineages. Moreover, recent discoveries in small RNAs and epigenetics offer new avenues for exploration, enriching our understanding of hybridization's role in plant evolution.

References:

1. Dobzhansky T, Ehrman L, Pavlovsky O, Spassky B (1964): The super species *Drosophila paulistorum*, Proc Natl Acad Sci, 51: 3–9.
2. Dobzhansky T, Koller PC (1938): An experimental study of sexual isolation in *Drosophila*, Biol Zentralblatt, 58: 589–607.
3. Dong S, Adams KL (2011): Differential contributions to the transcriptome of duplicated genes in response to abiotic stresses in natural and synthetic polyploids, New Phytol, 190: 1045–1057.
4. Mayr E (1942): Systematics and the origin of species from the viewpoint of a zoologist. Columbia University Press, New York.
5. Mayr E (1986): Joseph Gottlieb Kölreuter’s contributions to biology, Osiris, 2: 135–176.
6. McNeilly T, Antonovics J (1968): Evolution in closely adjacent plant populations. IV. Barriers to gene flow, Heredity, 23: 205–218.

7. Müntzing A (1930): Outlines to a genetic monograph for the genus *Galeopsis* with special reference to the nature and inheritance of partial sterility, Hereditas, 13: 185–341.
8. Ng DWK, Lu J, Chen ZJ (2012): Big roles for small RNAs in polyploidy, hybrid vigor, and hybrid incompatibility, Curr Opin Plant Biol, 15: 154–161.
9. Nielsen R, Wakeley J (2001): Distinguishing migration from isolation: a Markov chain Monte Carlo approach, Genetics, 158: 885–896.
10. Nilsson-Ehle H (1911): Kreuzungsuntersuchungen an hafer und weizen, Lunds Univ Areskripft, 7: 1–84.
11. Rieseberg LH, Sinervo B, Linder CR, Ungerer MC, Arias DM (1996): Role of gene interactions in hybrid speciation: evidence from ancient and experimental hybrids, Science, 272: 741–745.
12. Rieseberg LH, Widmer A, Arntz AM, Burke JM (2003): The genetic architecture necessary for transgressive segregation is common in both natural and domesticated populations, Philos Trans R Soc Lond B Biol Sci, 358: 1141–1147.
13. Rosenthal DM, Rieseberg LH, Donovan LA (2005): Re-creating ancient hybrid species' complex phenotypes from early-generation synthetic hybrids: three examples using wild sunflowers, Am Nat, 166: 26–41.
14. Rosenzweig BK, Pease JB, Besansky NJ, Hahn MW (2016): Powerful methods for detecting introgressed regions from population genomic data, Mol Ecol, 25: 2387–2397.
15. Ru D, Mao K, Zhang L, Wang X, Lu Z, Sun Y (2016): Genomic evidence for polyphyletic origins and interlineage gene flow within complex taxa: a case study of *Picea brachytyla* in the Qinghai-Tibet Plateau, Mol Ecol, 25: 2373–2386.
16. Saether SA, Saetre GP, Borge T, Wiley C, Svedin N, Andersson G, Veen T, Haavie J, Servedio MR, Bures S (2007): Sex chromosome-linked species recognition and evolution of reproductive isolation in flycatchers, Science, 318: 95–97.
17. Sankararaman S, Sridhar S, Kimmel G, Halperin E (2008): Estimating local ancestry in admixed populations, Am J Hum Genet, 82: 290–303.
18. Scascitelli M, Whitney KD, Randell RA, King M, Buerkle CA, Rieseberg LH (2010): Genome scan of hybridizing sunflowers from Texas (*Helianthus annuus* and *H. debilis*) reveals asymmetric patterns of introgression and small islands of genomic differentiation, Mol Ecol, 19: 521–541.
19. Schumer M, Cui R, Boussau B, Walter R, Rosenthal G, Andolfatto P (2013): An evaluation of the hybrid speciation hypothesis for *Xiphophorus clemenciae* based on whole genome sequences, Evolution, 67: 1155–1168.

20. Schumer M, Cui R, Rosenthal GG, Andolfatto P (2015): Reproductive isolation of hybrid populations driven by genetic incompatibilities, PLoS Genet, 11: e1005041.
21. Schumer M, Rosenthal GG, Andolfatto P (2014): How common is homoploid hybrid speciation?, Evolution, 68: 1553–1560.
22. Servedio MR (2009): The role of linkage disequilibrium in the evolution of premating isolation, Heredity, 102: 51–56.
23. Shivaprasad PV, Dunn RM, Santos BA, Bassett A, Baulcombe DC (2012): Extraordinary transgressive phenotypes of hybrid tomato are influenced by epigenetics and small silencing RNAs, EMBO J, 31: 257–266.
24. Shull GH (1908): The composition of a field of maize, J Hered Os, 4: 296–301.
25. Shull GH (1911): The genotypes of maize, Am Nat, 45: 234–252.
26. Smadja CM, Loire E, Caminade P, Thoma M, Latour Y, Roux C, Thoss M, Penn DJ, Ganem G, Boursot P (2015): Seeking signatures of reinforcement at the genetic level: a hitchhiking mapping and candidate gene approach in the house mouse, Mol Ecol, 24: 4222–4237.
27. Smith JE (1804): Flora Britannica, Vol. 3. J Taylor, London.
28. Snow AA, Pilson D, Rieseberg LH, Paulsen MJ, Pleskac N, Reagon MR, Wolf DE, Selbo SM (2003): A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers, Ecol Appl, 13: 279–286.
29. Solís-Lemus C, Ané C (2016): Inferring phylogenetic networks with maximum pseudo likelihood under incomplete lineage sorting, PLoS Genet, 12: 1509.06075v1.
30. Wen D, Yu Y, Hahn MW, Nakhleh L (2016): Reticulate evolutionary history and extensive introgression in mosquito species revealed by phylogenetic network analysis, Mol Ecol, 25: 2361–2372.
31. Whalen M (1978): Reproductive character displacement and floral diversity in *Solanum* section Androceras, Syst Bot, 3: 77–86.
32. Whitney KD, Broman KW, Kane NC, Hovick SM, Randell RA, Rieseberg LH (2015): Quantitative trait locus mapping identifies candidate alleles involved in adaptive introgression and range expansion in a wild sunflower, Mol Ecol, 24: 2194–2211.
33. Whitney KD, Randell RA, Rieseberg LH (2010): Adaptive introgression of abiotic tolerance traits in the sunflower *Helianthus annuus*, New Phytol, 187: 230–239.

PRELIMINARY FLORAL DIVERSITY ANALYSIS OF KUSHI VILLAGE AND ADJOINING AREAS DIST. – SATARA, MS (INDIA)

Neeta Salunkhe[1] and Swapnaja Deshpande*[2]

[1]Department of Botany, Lal Bahadur Shastri College, Satara, M.S.

[2]Department of Botany, D. P. Bhosale College, Koregaon, M.S.

*Corresponding author E-mail: deshpandeswapnaja85@gmail.com

Abstract:

The floral diversity helps in the magnitude of biodiversity of an area. In the present investigation floristic components of Kushi village from Satara district are enumerated. The preliminary assessment of study area results into identification floral components from 56 families comprising of 112 taxa. Among 57 families Fabaceae (8 spp.), Acanthaceae (7 spp.), Malvaceae (6 spp.), Apocynaceae & Convolvulaceae (5 spp. each) and Asteraceae (4 spp.) are dominant in the study region. Among the studied taxa (45) are herbs, (21) shrubs, (18) climbers and (28) trees. The area harbors some endemic species *Viz. Delphinium malabaricum*, *Ceropegia bulbosa*, *Ceropegia hirsuta* etc.

Keywords: Kushi, Satara, floristic, biodiversity, Endemic

Introduction:

India is one of the mega-biodiverse countries of the world. It has diverse biogeography, and consists of about 17,6768 plant species, which is almost 12.5% of total species in the world. Western Ghats comprises more than 7,400 plant species of which 1,270 are endemic (Nayar et al.2014). Floristic diversity refers to the variety and variability of plants in given region. Satara district lies in Northern Western Ghats of Maharashtra (Sahyadri Ranges) which is rich in biodiversity. The main system of hills in the Satara district are the 'Sahyadri ranges and 'Mahadeo hills. The forts on hilltops of the Sahyadri (Northern Western Ghats) have seen a turbulent historical past and are famous for their architectural style and cultural heritage. Besides this, these hilltops are home to an incredible plant diversity. Satara district is located in the western part of Maharashtra. It lies between the north latitudes of 17.5 and 18.11 and east longitude of 73.33 and 74.54. It has spread over an area of 10,480 sq. km (3.4% total area of Maharashtra). The climate ranges from rainiest Mahabaleshwar to drier regions of Man Tehsil. of Satara is cool and healthy with average annual rainfall of 1033 mm. The study region Kushi is situated 13 kms. away from Satara. The total geographical area of village is 613.82 hectares. It is

located between three hills around which are the storehouse of endemic and unique floral diversity.

Material and Methods:

Extensive seasonal field visits were done for the collection of plant specimens. The collected material was identified with Flora of Mahabaleshwar and adjoining, (Deshpande et. al.1993), Flora of Maharashtra State Dicotyledons and Monocotyledons (Singh & Karthikeyan 2001). The identified plants were enlisted in- table with necessary information viz. family, common name, habit and IUCN status or endemism. The botanical names are as per database on POWO and TROPICOS.

Results:

The Kushi village shows rich floristic diversity within a small geographical area of 613.82 hectares. The area harbors a variety of plants like legumes, wild edibles, ornamentals, parasites along with some rare, endangered and threatened species. The area comprises of 112 species of flowering plants belonging to 57 families 100 genera. The dominant families Fabaceae (8 spp.), Acanthaceae (7 spp.), Malvaceae (6 spp.), Apocynaceae & Convolvulaceae (5 spp. each) and Asteraceae (4 spp.). Among the studied taxa (45) are herbs, (21) shrubs, (18) climbers and (28) trees. The region supports 36 IUCN Red Listed plant species like Critically Endangered (1 spp.) Endangered (2 spp.) Vulnerable (2 spp.) and Least Concern (31 spp.) which are under threat and conservation measures needs to be taken to maintain the diversity of these taxa. The area harbors some endemic species *viz. Delphinium malabaricum*, *Ceropegia bulbosa*, *Ceropegia hirsuta* etc.

Discussion:

Among 112 enlisted taxa 36 are categorized under IUCN Red List of Threatened Species. The area supports variety of wild edible, wild ornamental and endemic species which needs immediate attention from conservation point of view. Wild edible species can be domesticated and utilised to provide the economic stability to the local people. Lack of awareness about the unique diversity, threats and proper utilization of these bioresources are the major threats to the local flora.

Table 1: List of flowering plants from the study region

Sr. No.	Botanical Name	Family	Common Name	Habit	Status
1.	*Asystasia dalzelliana* Santapau.	Acanthaceae	Neelkantha	H	
2.	*Barleria cristata* Roxb.	Acanthaceae	Pandhri Koranti	S	
3.	*Barleria prionitis* L.	Acanthaceae	Pivali Koranti	S	
4.	*Crossandra infundibuliformis* (L)Nees.	Acanthaceae	Aboli	H	LC
5.	*Eranthemum roseum* R. Br	Acanthaceae	Dasmuli	H	LC
6.	*Justicia adhatoda* L.	Acanthaceae	Adulsa	S	LC
7.	*Neuracanthus sphaerostachyus* (Nees) Dalz.	Acanthaceae	Golgonda	H	-
8.	*Achyranthes aspera* L.	Amaranthaceae	Aghada	H	
9.	*Cleosia argentea* L.	Amaranthaceae	Kardeai	H	
10.	*Annona squamosa* L.	Annonaceae	Sitaphal	T	LC
11.	*Portulaca olearacea* L.	Annonaceae	Ghol	H	
12.	*Catharanthus roseus (*L.) G. Don	Apocynaceae	Sadaphuli	H	
13.	*Ceropegia bulbosa* Roxb.	Apocynaceae	Ankalodya	C	
14.	*Ceropegia hirsuta* Wight&Arn	Apocynaceae	Haman	C	
15.	*Thevetia peruviana* K. Schum	Apocynaceae	Bitti	T	
16.	*Wattakaka volubilis* (L.f.) Stapf	Apocynaceae	Hirandodi	C	
17.	*Tylophora dalzellii* Hook. F	Asclepidaceae	Pitmari	C	
18.	*Agave americana* L.	Asparagaceae	Ghayapat	H	LC
19.	*Chlorophytum borivilianum* Sant. f	Asparagaceae	Safed Musali	E	CR
20.	*Aloe vera* (L.) Burm.f.	Asphodelaceae	Korpad	H	
21.	*Galinsoga parviflora* Cav.	Asteraceae	Potato Weed	H	
22.	*Senecio bombayensis* N. P. Balakar.	Asteraceae	Sonaki	H	
23.	*Tridax procumbens* L.	Asteraceae	Ekdandi	H	
24.	*Xanthium strumarium* L.	Asteraceae	Landaga	H	
25.	*Impatiens balsamina* L.	Balsaminaceae	Terda	H	
26.	*Dolichandrone falcata* (wall ex Dc.) Seem.	Bignoniaceae	Medhshingi	T	

27.	*Tecoma stans* (L.) Juss ex kunth.	Bignoniaceae	Phutani	T	LC
28.	*Bombax ceiba* L.	Bombaceae	Kate-Saveri	T	LC
29.	*Brassica campestris* L.	Brassicaceae	Mohari	H	
30.	*Canna indica* L.	Cannaceae	Kardal	H	
31.	*Caesalpinia decapetala* (Roth)Alston.	Cesalpiniaceae	Chilar	S	LC
32.	*Cleastrus paniculatus* Willd	Cleastraceae	Kanguni	C	
33.	*Gloriosa superba* L.	Colchicaceae	Flame lily	C	LC
34.	*Iphigenia indica* (L.) A. Gray. ex Kunth	Colchicaceae	Jambhale bhuichkra	H	LC
35.	*Iphigenia stellata* Blatt.	Colchicaceae	Gulabi bhuichkra	H	EN
36.	*Anogeissus latifolia* Wall. Ex Bedd.	Combretaceae	Dhawada	T	
37.	*Terminalia crenulata (*Heyne) Roth.	Combretaceae	Ian	T	
38.	*Cyanotis fasciculata (*B. Heyne ex Roth) Schult and Schult.f.	Commelinaceae	Nilwanti	H	LC
39.	*Hymenocallis littoralis* (Jacq.) Salisb	Commelinaceae	Spider lily	H	
40.	*Ipomea alba* L.	Convolvulaceae	Moonflower	C	
41.	*Ipomea carnea* Jaacq.	Convolvulaceae	Besharam	S	
42.	*Ipomea hederifolia* L.	Convolvulaceae	Lal Pungali	C	
43.	*Ipomea purpurea (*L) Roth.	Convolvulaceae	Morning Glory	C	
44.	*Ipomea quamoclit* L.	Convolvulaceae	Ganeshvel	C	
45.	*Momordica dioica* Roxb. ex Willd	Cucurbitaceae	Kartoli	C	
46.	*Cyprus rotundus* L.	Cyperaceae	Lavhala	H	
47.	*Dioscorea bulbifera* L.	Dioscoreaceae	Dukarkand	C	
48.	*Eriocaulon tuberiferum*A. R. Kulk. & Desai	Eriocaulaceae	Pangenda	H	VU
49.	*Euphorbia geniculata* Ortega.	Euphorbiaceae	Dudhani	H	
50.	*Euphorbia hirta* L.	Euphorbiaceae	Dudhani	H	
51.	*Abrus precatorius* L.	Fabaceae	Gunj	Climber	
52.	*Alysicarpus tetragonolobus* Edgew.	Fabaceae	Lal shevra	H	

53.	*Bauhinia racemosa* Lam.	Fabaceae	Apata	T	
54.	*Clitoria ternatea* L.	Fabaceae	Gokarn	C	
55.	*Crotalaria pallida* Aiton	Fabaceae	Jumgli tag	S	
56.	*Desmodium gangeticum* (L.) DC	Fabaceae	Shalparni	U	
57.	*Indigofera cassioides* DC.	Fabaceae	Unhali	S	
58.	*Millettia pinnata* (L.) Panigrahi	Fabaceae	Karanj	T	LC
59.	*Tamarindus indica* L.	Fabaceae	Chinch	T	LC
60.	*Canscora diffusa (*Vahl.) R . Br	Gentianaceae	Kilwar	H	
61.	*Exacum pedenculatum* L.	Gentianaceae	Stalked Persian violet	H	
62.	*Curculigo orchioides* Gaertn.	Hypoxidaceae	Kali musali	H	
63.	*Anisomeles indica* (L.) Kuntze	Lamiaceae	Gopali	H	
64.	*Lavandula bipinnata* (Roth)Kuntze	Lamiaceae	Ghodeghui	Herb	LC
65.	*Ocimum sanctum* L.	Lamiaceae	Tulsi	Herb	
66.	*Ledebouria revoluta* (L. f.) Jessop	Liliaceae	Khajkanda	Herb	
67.	*Lawsonia inermis* Linn	Lythraceae	Mhendi	Tree	LC
68.	*Woodfordia fruticosa* (L) Kurz.	Lythraceae	Dhyati	Shrub	LC
69.	*Abutilon indicum (*L.) Sweet	Malvaceae	Mudra	Shrub	
70.	*Alcea rosea* L.	Malvaceae	Chitrasevati	Shrub	
71.	*Hibiscus rosa-sinensis* L.	Malvaceae	Jaswand	Shrub	
72.	*Sida acuta* Burm.f	Malvaceae	Jangalimethi	Herb	
73.	*Sterculia urens* Roxb.	Malvaceae	Bhutya	Tree	
74.	*Urena lobata* L.	Malvaceae	Vanbhendi	Shrub	LC
75.	*Azadirachta indica* Juss.	Meliaceae	Kadulimb	Tree	LC
76.	*Cocculus hirsutus* (L) Diels.	Menispermaceae	Vasanvel	Climber	
77.	*Tinospora cordifolia (*Wild) Miers ex Hook.	Menispermaceae	Gulvel	Climber	
78.	*Acacia catechu* (L.f) Willd	Mimosaceae	Khair	Tree	LC
79.	*Acacia leucophloea* Willd.	Mimosaceae	Hivar	Tree	LC
80.	*Acacia nilotica* (L.) Willd ex Delile.	Mimosaceae	Babhool	Tree	LC

81.	*Mimosa pudica* L.	Mimosaceae	Lajalu	Herb	LC
82.	*Ficus benghalensis* L.	Moraceae	Vad	Tree	
83.	*Ficus racemosa* L.	Moraceae	Umbar	Tree	LC
84.	*Ficus religiosa* L.	Moraceae	Pimpal	Tree	LC
85.	*Moringa oleifera* Lam.	Moringaceae	Drumstick	Tree	LC
86.	*Pisidium guajava* L.	Myrtaceae	Peru	Tree	
87.	*Syzygium cumini* (L.) Skeels	Myrtaceae	Jambhul	Tree	LC
88.	*Bougainvillea spectabilis* Willd	Nyctaginaceae	Kagdiphul	Shrub	
89.	*Mirabilis jalapa* L.	Nyctaginaceae	Gulmus	Herb	
90.	*Jasminum malabaricum* Wight	Oleaceae	Ranmogra	Shrub	
91.	*Jasminum sambac* Ait.	Oleaceae	Mogara	Shrub	
92.	*Nyctanthes arbor-tristis* L.	Oleaceae	Parijatak	Tree	LC
93.	*Sopubia delphiniifolia* (L.) G.Don	Orbachaceae	Dudhali	Herb	
94.	*Oxalis corniculata* L.	Oxalidaceae	Ambushi	Herb	
95.	*Argemone mexicana* L.	Papaveraceae	Pivaladhotra	Herb	
96.	*Cryptolepis buchananii* R.Br.	Periplocaceae	Kavali	Shrub	
97.	*Plumbago zylanica* L.	Plumbaginaceae	Chitrak	Shrub	
98.	*Cymbopogon citratus* (DC.) Stapf	Poaceae	Gavtichaha	Herb	
99.	*Clematis gouriana* Roxb.	Ranunculaceae	Morvel	Climber	
100.	*Delphinium malabaricum* (Huth) Munz	Ranunculaceae	Nilambari	Herb	
101.	*Ixora brachiata* Roxb.	Rubiaceae	Lokhandi	Tree	
102.	*Murraya koenigii* (L.) Spreng	Rutaceae	kadipatta	Tree	LC
103.	*Santalum album* L.	Santalaceae	Chandan	Tree	VU
104.	*Cardiospermum halicacabum* L.	Sapindaceae	Kanphuti	Climber	LC
105.	*Striga densiflora* (Benth.) Benth.	Scrophulariaceae	Aagya	Herb	
106.	*Gnidia glauca* (Fres.) Gilg	Thymelaeaceae	Datpadi	Shrub	
107.	*Grewia hirsuta* Vahl.	Tiliaceae	Kirmid	Shrub	LC
108.	*Rotheca serrata* (L.) Steane & Mabb	Verbenaceae	Bharangi	Shrub	
109.	*Tectona grandis* L.	Verbenaceae	Sagvan	Tree	EN
110.	*Vitex negundo* L.	Verbenaceae	Nirgudi	Tree	LC
111.	*Curcuma aromatica* Salisb.	Zingiberaceae	Jangali haldi	Herb	

112.	*Zingiber neesanum* (J. Graham) Ramamoorthy	Zingiberaceae	Ran ale	Herb	

CR= Critically Endangered; EN = Endangered; LC = Least Concern; VU = Vulnerable;

H= Herb; E = Ephimeral; S= Shrub; T= Tree; C= Climber; U= Undershrub

Acknowledgements:

The authors are thankful to Principal L. B. S. College, Satara and Principal, D. P. Bhosale College, Koregaon.

References:

1. Deshpande S. (1993). Flora of Mahabaleshwar and Adjoinings, Maharashtra. Botanical Survey of India. pp. 776.
2. POWO: https://powo.science.kew.org/
3. Singh N. P., Lakshminarasimhan P, Karthikeyan S and Prasanna P. V. (2001). Flora of Maharashtra State, Dicotyledones. Vol. II. BSI, Calcutta. pp. 916.
4. TROPICOS: https://www.tropicos.org/
5. Nayar, T.S., M. Sibi & A.R. Beegam. (2014). Flowering Plants of the Western Ghats, India, Vol.1 and Vol.2. Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Kerala, India.

ISOLATION OF PLANT FIBRE DEGRADING STRAINS FOR ENHANCED BIOMASS UTILIZATION AND ENVIRONMENTAL SUSTAINABILITY

Syed Nisar Ahmed

Department of Microbiology,

Sir Sayyed College of Arts, Commerce and Science, Aurangabad (MS), India

Corresponding author E-mail: nisarmicro46@gmail.com

Abstract:

A plant fibre is a biomolecule and natural polymer which contain hundreds of carbon, hydrogen and oxygen atoms. It is the main substance of plant cell wall exist as a linear homopolymers of glucose with β- 1,4 glycosidic linkage. A plant fibre (cellulose) in the plants readily available in atmosphere as a important plant biomass. A major obstacle for enzymatic hydrolysis is its crystalline and insoluble nature. Cellulose can be converted into glucose with the assistance of cellulolytic system. The bioconversion of cellulosic material mainly depends on the nature of cellulose source of cellulolytic enzyme, optimal condition for catalytic activity and production of enzymes. Endoglucanase is responsible for random cleavage of β- 1,4 glycosidic bonds along a cellulose chain. Exoglucanase is necessary for cleavage of nonreducing end of cellulose chain and splitting of the elementary fibrils from the crystalline cellulose. Agricultural waste contained unwanted parts of crops and weeds which serves as a nutrient for many cellulose degrading bacteria. The current study sought at the possible utilization of A plant fibre degrading bacteria from agricultural waste to explore its maximum cellulolytic potential at optimum conditions such as pH, temperature. Isolate W-3 showed maximum hydrolytic capacity of 6.7 mm.

Keywords: A plant fibre, cellulolytic potential, endoglucanase, exoglucanase, cellulose degrading bacteria.

Introduction:

A plant fibre is a biomolecule and natural polymer which contain hundreds of carbons, hydrogen and oxygen atoms Cellulose is a linear homopolysaccharide of 3000 or more repeating glucose residues with β- 1,4 glycosidic linkage. A plant fibre is abundantly available in the environment in the form of plants as it is a major biomass of plants. It comprises 33 percent of all the vegetable biomass. Plants produce 4 X 10^9 tons of cellulose annually (6). Its crystalline nature and insoluble nature represent a big challenge for enzymatic hydrolysis. With the help of cellulolytic system cellulose can be converted to glucose. Different micro-organism play an

important role in conversion of lignocellulose waste into valuable products like biofuels produced by fermentation (7). Successful bioconversion of cellulosic material mainly depends on the nature of cellulose source of cellulolytic enzyme, optimal condition for catalytic activity and production of enzymes (8). Cellulase enzyme system comprises three classes of soluble extracellular enzymes ie β- 1,4 endoglucanase, β- 1,4 exoglucanase and β – glucosidase. Endoglucanase is responsible for random cleavage of β- 1,4 glycosidic bonds along a cellulose chain. Exoglucanase is necessary for cleavage of non-reducing end of cellulose chain and splitting of the elementary fibrils from the crystalline cellulose while β- 1,4glucosidase hydrolyses cellobiose and water soluble cellodextrin to glucose. (9,10). Farmland contained unwanted parts of crops and weeds which serves as a nutrient for cellulose degrading bacteria. The current study focused to examine the possible utilization of CDB from farmland for maximum cellulolytic potential at optimum conditions such as pH, temperature.

Materials and Methods:

Sample collection

1-gram soil sample was directly collected from agricultural waste in Aurangabad region in sterilized screw cap tubes randomly. Sample was sieved and brought to laboratory for the isolation of cellulose degrading bacteria (K0). Isolation of cellulolytic bacteria: 1 gm of collected soil sample was mixed in 100 ml of sterile distil water. Serial dilutions up to 10^{-9} was prepared. A pour plate technique was used to isolate the cellulose degrading bacteria (CDB). 1 ml of diluted sample was mixed with cellulose agar media composed of KH_2PO_4-0.5 gm, $MgSO_4$0.25 gm, cellulose-2.0 gm, gelatin-2.0 gm, agar- 15 gm and distil water- 1 L at pH 6.8 to 7.2 and 30 ^{0}C of temperature. After 48 hours of incubation selected colonies were streaked on Congo red agar media for the confirmation of cellulose degrading activity. Composition of Congo red agar is of KH_2PO_4-0.5 gm, $MgSO_4$-0.25 gm, cellulose-2.0 gm, gelatin2.0 gm, agar- 15 gm, congo red -0.2 gm, distil water – 1L, pH- 6.8 to 7.2. Congo red used in the media act as an indicator for cellulose degradation as it provides rapid and sensitive test for cellulolytic bacteria. Colonies showing discoloration of congo red were taken as positive cellulose degrading bacteria (14). Hydrolysis capacity of positive isolates were determined by measuring diameter of clearing zone of colony (15).

Enzyme production

Positive isolates of cellulose degrading bacteria were cultured at 37^{0}C at 150 rpm in an enzyme production media composed of of KH_2PO_4-0.5 gm, $MgSO_4$-0.25 gm, gelatin-2.0 gm, distil water- 1L and whatman filter paper No 1 (1 X 6 cm strip, 50 mg per 20 ml) at pH 6.8 to 7.2. After three days of incubation the broth culture was centrifuged at 5000 rpm for 15 minutes

and supernatant was collected and stored as a crude enzyme preparation. Enzyme assay: Cellulose activity was assayed by using 3,5- dinitrosalisic acid (DNS) reagent and released reducing sugar from filter paper was estimated. The cellulolytic activity was determined by incubating 0.5 ml of supernatant with 1.0 ml of 0.05 M sodium citrate buffer pH 4.8 containing whatman filter paper No 1 strip(1.0 X 6 cm ie 50 mg). After one hour incubation at 50^0C temperature the reaction was terminated by adding 3 ml of 3,5- dinitrosalisic acid (DNS) and 1 ml of reaction mixture. The amount of reducing sugar was estimated spectrophotometrically (16). One unit of enzymatic activity was defined as the amount of enzyme that releases 1µ mol reducing sugar per ml per minute.

Results and Discussions:

Isolation and screening of cellulose degrading bacteria: degradation of cellulosic material is a complex process and require participation of microbial cellulolytic enzymes. Habitats of the substrates are the best source for the isolation of cellulose degrading bacteria. Bacterial colonies cultured on cellulose agar media were selected to determine their cellulolytic potential on congo red agar media. Discoloration of colonies on media was observed in five colonies which are considered cellulose degrading bacteria and named, W-1,W-2, W-3,W-4,W-5. Out of these five isolates W-3 showed maximum hydrolysis capacity (HC) ie 6.7. The range of HC value is similar to range reported by Lu *et al.* (18).

Table 1: Maximum clearing zone and Hydrolytic capacity (HC) of cellulose degrading bacteria (W) on cellulose congo red agar media

Isolated Bacteria	Maximum Clearing Zone (mm)	HC Value (Hydrolytic capacity)
W1	32	5.8
W2	28	4.2
W 3	39	6.7
W 4	24	3.9
W 5	15	2.4

Cellulolytic potential: All the five isolates ie. W-1 to W-5 were selected for enzyme production and their respective cellulolytic activity was estimated. The enzyme assay showed maximum cellulolytic activity for W-3 with 0.43 IU/ml for endoglucanase and minimum for W-2 with 0.15 IU/ml, similarly FPcase activity was observed maximum for W-5 with 0.21 IU/ml and minimum of 0.04 for W-1. The current study suggest that agricultural waste contain diverse type

of microorganisms able to degrade plant fibre (cellulose) by producing cellulose degrading enzyme.

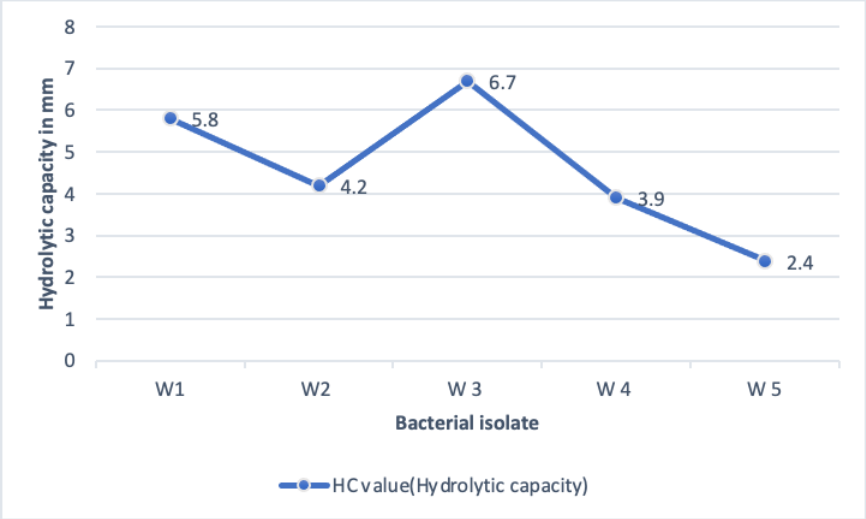

Figure 1: Maximum hydrolytic capacity in mm of isolated micro organisms on cellulose Congo red media

Acknowladgement:

Author is very much thankful to Dr. Shaikh Kabeer Ahmed, principal, Sir Sayyed College, Aurangabad for providing all the necessary facilities for this work.

References:

1. Apiwatanapiwat W., (2009). Bioethanol production from enzymatically saccharified sunflower talks using steam explosion as pre treatment, *Proceedings of World Academy of Science, Engineering and Technology*, vol. 37, p. 140–143.
2. Murali L. (2010). Production of cellulase from a thermophilic bacillus species isolated from cow dung American-Eurasian J Agric Environ Sci; 8(6):685-691.
3. Chakrabortya S, Khopadea *et al.* (2009). Isolation and characterization of novel α-amylase from marine *Streptomyces* sp. D1.J. Molecular Catalysis B: Enzymatic; 58: 17–23.
4. Satheesh Kumar G., *et al.* (2009). Cellulolytic enzymes from submerged fermentation of different substrates by newly isolated *Bacillus Spp.* FME., *Journal of Korean Society of Applied Biological Chemistry*, vol. 52, pp. 17–21.
5. Hatami S., H. A. Alikhsni, *et al.* (2008). Investigation of aerobic cellulolytic bacteria in some of north forest and farming soils, *The American-Eurasian Journal of Agricultural & Environmental Sciences*, vol. 5, pp. 713–716.
6. Gupta P., K. Samant, A. Sahu (2012). Isolation of cellulose degrading bacteria and determination of their cellulolytic potential International journal of Microbiology.

7. Wang, S. J. Yang, Z. C. Wang, and Y. F. Nie, (2006). Isolation and characterization of mesophilic cellulosedegrading bacteria from flower stalks-vegetable waste co-composting system, *Journal of General and Applied Microbiology*, vol. 51, no. 6, pp. 353–360.
8. Apun K, Jong BC and Salleh M A (2000). Screening and isolation of a cellulolytic and amylolytic *Bacillus* from sago pith waste. J Gen Appl Microbilo; 46: 263-267.
9. Gao, J., H. Weng, D. Zhu, M. Yuan, (2008). Production and characterization of cellulolytic enzymes from the thermoacidophilic fungal Aspergillus terreus M11 under solid state cultivation of corn stover. Bioresour. Technol., 99: 7623-7629.
10. Woodward J. and A. Wiseman, (1983). Fungal and other β-dglucosidases:their properties and applications, Enzyme and Microbial Technology, vol. 4, no. 2, pp. 73–79.
11. Ryu D. D. Y. and M. Mandels, (1980). Cellulases: biosynthesis and applications, Enzyme and Microbial Technology, vol. 2, no. 2,. 91–102.
12. Hanif, A., A. Yasmin and M.I. Rajoka. (2004). Induction, production, repression and de-repression of exoglucanase synthesis in *Aspergillus niger*. *Bioresour. Technol*., 94: 311-319.
13. Jamil, A., S. Naim, S. Ahmed and M. Ashraf (2005). Production of Industrially important enzymes using molecular approaches; cellulases and xylanases. In: *Genetic resources and Biotechnology* II, Volume Two, (Eds.): D. Thangadurai, T. Pullaiah, Pedro and A. Balatti. Regency publications, New Delhi.
14. Kaufmann, A., J. Fegan, P. Doleac, (1976). Identification and characterization of a cellulolytic isolate. J. gen. Microbiol., 94: 405-408.
15. W. J. Lu, H. T. Wang, Y. F. Nie *et al.*, (2004). Effect of inoculating flower stalks and vegetable waste with ligno-cellulolytic microorganisms on the composting process, *Journal of Environmental Science and Health, Part B*, vol. 39, no. 5-6, pp. 871–887.
16. Hendricks C. W., J. D. Doyle, and B. Hugley, (1995) A new solid medium for enumerating cellulose-utilizing bacteria in soil, *Applied and Environmental Microbiology*, vol. 61, no. 5, pp.2016–2019, 1995.
17. Miller G. L., (1959). Use of dinitrosalicylic acid reagent for determinationof reducing sugar, *nalytical Chemistry*, vol. 31, no. 3, pp. 426–428, 1959.
18. Lu W. J., H. T. wang, Y. F. Nie *et al.* (1998). Effect of inoculating flower stalk and vegetable waste with lingo cellulolytic micro organism on composting process, Journal of Environmental science and health, part B, vol.39, no 5-6, pp1399-1404.

FUNGAL AND PLANT PHENYLALANINE AMMONIA-LYASE (PAL): PLANT-PATHOGEN INTERACTION

Aboli Kshirsagar
Department of Botany,
Arts & Science College, Pulgaon
(Affiliated to RTM Nagpur University, Nagpur)
Corresponding author E-mail: kshirsagaraboli@gmail.com

Abstract:

One of the essential amino acids, L-phenylalanine, cannot be synthesised by mammals in sufficient quantities to meet the needs of protein synthesis. The shikimic acid pathway is capable of producing phenylalanine in both fungi and plants. Plants either use L-phenylalanine directly for protein synthesis or metabolise it via the phenylpropanoid pathway. It is derived from the shikimic acid pathway. Numerous phenylpropanoid secondary products are biosynthesized as a result of this phenylpropanoid metabolism. Phenylalanine ammonia-lyase is involved in the first step of this metabolic process (PAL). It was shown that some fungi can degrade phenylalanine via a pathway involving an initial deamination to cinnamic acid, as occurs in plants, thanks to the discovery of the PAL enzyme in fungi and the detection of 14CO2 production from 14C-ring-labeled phenylalanine and cinnamic acid. In this review, we give PAL's historical context as well as a current update on the discovery of PAL genes in fungi.

Keywords: Fungi, Phenylalanine ammonia-lyase (PAL), Plant-Pathogen Interaction

Introduction:

L-phenylalanine is nonoxidatively deaminated by phenylalanine ammonia-lyase (PAL; E.C. 4.3.1.5) to produce trans-cinnamic acid and a free ammonium ion (Fig. 1) [1]. The first step in plants' channelling of carbon from primary metabolism into phenylpropanoid secondary metabolism is the conversion of the amino acid phenylalanine to trans-cinnamic acid. Because of its function in plant development and response to a wide range of environmental stimuli, PAL has been the subject of extensive research. The vast variety and abundance of phenylpropanoid products found in plant materials [2] serve as evidence of the significance of this enzyme in plant metabolism. No direct evidence exists to support the significance of this enzyme in fungi other than its catabolic role [3].

Since its discovery [1], reports of the presence of PAL in various plants [4, 5] have been made, including some algae, such as *Dunaliella marina* [6], fungi [7–10], and a few prokaryotic organisms, such as *Streptomyces* [11, 12]. Numerous species of plants, including monocots,

dicots, gymnosperms, ferns, and lycopods, have been found to exhibit PAL activity [13]. Only a small number of basidiomycetes, deuteromycetes, and one ascomycete, Nectriacinnabarina, have shown evidence of PAL activity in fungi [7, 14]. Animal PAL has not been mentioned in any reports.

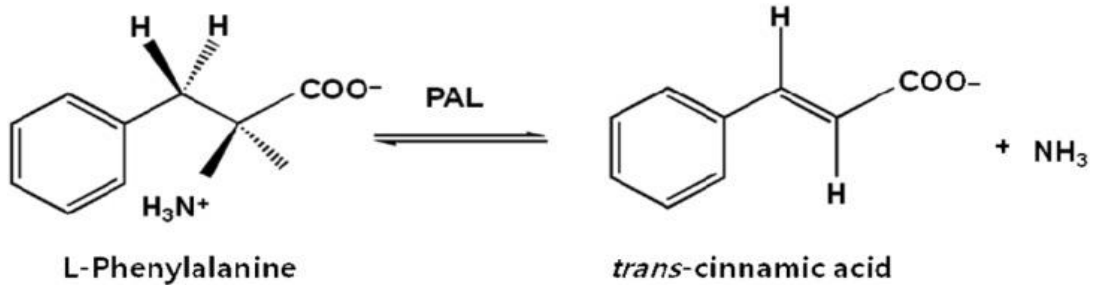

Figure 1: Deamination of L-phenylalanine by L-phenylalanine ammonia-lyase (PAL)

In this review, we give PAL's historical context as well as a current update on the discovery of PAL genes in fungi.

Structural properties of PAL proteins

A variety of plant species, some fungi, and a few bacteria have all contributed to the isolation and characterization of PAL. Diverse source tissues are used for PAL isolation. They include prokaryotic cells [12], seedlings [15], shoots [1], leaf sheaths [16], cell culture [17–19], fruit [20], and cell culture [17–19]. Most well-documented PAL sources for enzyme isolation and its characteristics can be found in several reviews [4, 23, 24]. Purification challenges are frequently experienced, in part due to the low abundance of PAL in cells and changes in size and properties that take place during purification. A seemingly homogeneous protein preparation can frequently be produced under non-denaturing conditions, but under denaturing conditions, additional polypeptide bands are typically found in analytical polyacrylamide gel electrophoresis gels. This can create confusion in the estimation of PAL subunit sizes.

Most reported PALs have a native molecular mass between 300 and 340 kDa. The bacterium *Streptomyces* [11], the mass of 152 kDa in *Ocimumbasilicum* [25], the mass of 226 kDa in *Alternaria* [21], the mass of 250 kDa in *Helianthus annuus* [5], the mass of 266 kDa in *Fragaria ananassa* [20], the mass of 320 kDa in *Ustilago maydis* [26], and the mass of PAL normally consists of four identical subunits that form a homo-tetrameric protein. It has been reported that *H. annuus* (2 58 kDa and 2 68 kDa) and Rhizoctonia solani (2 70 kDa and 2 90 kDa) both produce hetero-tetrameric PAL as a complex of two hetero-dimers. According to Neumann and Schwemmle [27],*Oenothera* seedlings have two PAL isoenzymes with four identical subunits each of 75.5 kDa and 79.2 kDa. *Rhodosporidiumtoruloides* PAL has been reported to be a dimer composed of two identical subunits with a mass of 80 kDa [28]. Iso-electric points (pIs) for PAL typically fall between 2.5 [27] and 6.3 [26] in the acid range. There

have been reports of isoforms with various pIs from a few sources, including three isoforms in the *Leptosphaeria maculans* fungus [29], numerous isoforms between pI 5.1 and 6.1 in *alfalfa* [17], and two isoforms between pI 4.8 and 5.4 in beans [30]. It is interesting to note that two isoforms with various pIs were produced by the expression of a single cDNA of poplar PAL in a *baculovirus* expression system [31].

The majority of PALs are thought to be hydrophobic proteins. This characteristic has caused the purification of PAL from cotton [32] and Rhodotorulaglutinis to be accomplished using hydrophobic affinity column chromatography[33]. *Alfalfa* PAL has been reported to be highly hydrophobic [17]. Consistent with this, the hydropathy profile of the protein sequence deduced from the cDNA sequence also predicted that alfalfa PAL would be hydrophobic [34]. For the maize and potato enzymes, the association of carbohydrate with PAL has been documented [35, 36]. Potential glycosylation sites have been found in the PAL gene sequences of beans [37] and parsley [38], but the role of glycosylation in PAL function has not been thoroughly investigated. The fact that *Escherichia coli* cells transformed with PAL genes from the *parsley* [40, 41] and yeast *Rhodosporidium* [39] produced active PAL suggests that glycosylation is not likely to affect PAL catalysis. Glycosylation may play a role in both the localization of the enzyme within cells and its stability [35, 36]. The three-dimensional structure of the red yeast *R. toruloides* PAL has been described using X-ray crystallography [42]. This homotetrameric protein contains 716 residues per subunit with a molecular mass of 76.88 kDa. A seahorse-like shape of each subunit interlocks with two other subunits, thereby maximizing adjacent subunit interactions and resulting in tetramer formation.

PAL active site and enzyme mechanism

One of the few amino acid-transforming enzymes lacking pyridoxal 5-phosphate is PAL. Dehydroalanine is an unusual prosthetic group that is present in PAL [9]. The activation of the phenylalanine amino group to form a better leaving group than NH3+ is thought to be the function of this post-translationally modified amino acid in catalysis [43]. The enzyme is completely rendered inactive when an electrophilic centre at the active site of PAL is altered by substances like borohydride, cyanide, bifulfite, or nitromethane. The presence of dehydroalanine in the active site is demonstrated by the identity of [3H]-alanine and [14C]-aspartic acid released after acid hydrolysis of PAL enzyme inactivated with radiolabeled reagents, NaB3H4 and 14CN- [9, 44].

Further proof that dehydroalanine is present in the active site is provided by studies on the ability of substrates and substrate analogues of PAL to thwart inactivation by these reagents [9, 44]. Dehydroalanin's function in PAL catalysis is also the subject of a different model [45].

Dehydroalanine is formed, but the exact mechanism of this formation is unknown. A serine residue is thought to be the precursor of dehydroalanine in the cases of other proteins that contain this amino acid, such as subtilin [46], thyroglobulin [47], and pyruvoyl enzymes [48].

The serine residue found in the PAL amino acid sequences is completely conserved across all species [49] and is thought to be connected to the enzyme's active site. Serine has recently been found to be the precursor of the dehydroalanine residue in parsley [45] and poplar PAL [31]. Though a direct role for serine as the precursor of dehydroalanine has not yet been proven, it is likely that a similar process would account for the formation of the active site dehydroalanine from serine in fungal PAL. In cells where PAL was not normally produced, expression of PAL in *E. coli* led to the production of active PAL enzymes [40, 47]. When compared to endogenous PAL from other sources, the expressed PAL proteins displayed similar enzyme characteristics. This suggests that the formation of dehydroalanine may be an autocatalytic process, although it cannot be ruled out that a widespread modifying enzyme is involved in the dehydroalanine formation.

Functional properties of PAL

Trans-p-coumaric acid can be produced by PAL enzymes from a variety of sources, including monocots and specific fungi. These enzymes have activity towards L-tyrosine. Tyrosine Ammonia Lyase (TAL) activity has been labelled as the cause of this [4, 13, 50]. The majority of PAL preparations have very little TAL activity. In PAL preparations, the ratio of PAL to TAL ranges from 1.35 to 5 in *Sporobolomyces pararoseus* fungus [51], from 4 to 20 in wheat [13], and from 0.6 to 1.3 in beans [52]. Multiple plant species' PAL: TAL ratios varied even more, according to reports [53]. No TAL enzyme has ever been purified without PAL activity. It has been established that the same polypeptide contains the PAL and TAL activities in maize PAL expressed in *E. coli* [54]. It has been reported that PAL preparations from various sources only have one Michaelis constant (Km), but the kinetic characteristics of other preparations imply that the enzyme is uncooperative with regard to substrate binding [55]. Numerous sources have reported two different Km values for PAL [23]. The kinetic analysis of each isoform revealed typical Michaelis-Menten saturation kinetics, and individual isoforms were highly purified [17, 56]. According to reports, the Km values for L-phenylalanine range from 0.011mM [57] to 1.7mM [1]. Although it has been reported that metal ions like Mg2+ and Ba2+ can slightly stimulate PAL activity, the majority of PALs do not require them [5]. Numerous substances, such as carbonyl, sulfhydryl, and others, can cause PAL activity to be inhibited and thiol reagents, phenolic acids, and heavy metal ions [24]. Most PALs tested are sensitive to synthetic PAL inhibitors such as (*S*)-2-aminooxy-3-phenylpropanoic acid (AOPP), (*R*)-(1-amino-2-phenylethyl)phosponic (APEP) acid, and 2-aminoindan-2-phosphonic acid

(AIP). Thus, these inhibitors have often been used to block the biosynthesis of phenylpropanoid compounds in plant cells and tissues [23, 58].

The pH optimum for PAL is generally in the range from 8.2~9.0 [21, 23]. The temperature optimum for PAL has been reported to be 35℃ in tobacco [57], 55℃ in sunflower [5], and 44~46℃ in *Rhizoctonia* [22]. Plant PAL enzymes are generally sensitive to repeated freezing and thawing and lose activity as the temperature approaches 60℃. In contrast, fungal PAL is more thermally stable too [22]. *Rhodotorula* PAL is apparently stable for at least 6 months when it is kept at -60℃ [59].

Structural properties of PAL-encoding genes

Following the isolation of PAL cDNA from bean [37], parsley [38] and sweet potato [60], PAL genes have been isolated from many sources. In most plants, PAL is encoded by a small gene family of 3~5 genes. Exceptions to this are the potato PAL gene family, which is made up of 40~50 genes [61], and the loblolly pine PAL, which has been reported to be encoded by a single gene [62]. Currently, either partial or full fungal genome data is available from more 50 species. When we searched through the DNA databases of the Broad Institute (Cambridge, MA, USA) and National Center for Biotechnology Information (Bethesda, MD, USA) for the PAL motif [GS]-[STG]-[LIVM]-[STG]-[SAC]-S-G-[DH]-L-x-[PN]-L-[SA]-x(2,3)-[SAGVTL], 45 potent PAL sequences were found in 28 fungal species . In red yeasts such as *Rhodosporium spp*., PAL is generally encoded by a single gene [63]. In filamentous fungi, PAL is encoded by a single, two, three, and four genes . The *Aspergillus nidulan* genome contains four PAL genes. The presence of introns has been reported in both plant and fungal PAL genes. Plant PAL genes generally contain only one intron, while yeast PAL genes have five [63] or six introns [64]. Two introns have been found in the *Arabidopsis* PAL gene [65], while no introns occur in jack pine and loblolly pine and *U. maydis* PAL genes [66-68]. The 45 PAL gene sequences from fungi were analysed, and the results revealed that different species have different numbers of introns. *A. flavus* and *A. oryzae* are two examples of species that have different numbers of introns in their PAL genes. Ascomycota have intron counts ranging from zero to six, while basidiomycota have intron counts ranging from zero to thirteen. The PAL genes of the rust fungus *Puccinia* have the most introns. Except for *Botrytis cinerea*, which has 1,131 amino acids, the inferred PAL protein length varies from 595 to 750 amino acids. There is not much difference between species in the location of the PAL motif on the PAL protein sequence. Most frequently, the fungal PAL motif is found between 123 and 244 amino acids,, except for *Neurospora* and *Neosartorya* sequences, which position near the C-terminus and N-terminus, respectively. The active site serine residue that is bolded in the PAL motif in is very well-conserved both in ascomycota and basidiomycota.

Table 1: Fungal species having PAL motif sequences properties

Fungal species	Gene name	PAL motif	Position of motif	Length of protein	No. of introns
Ascomycota					
Aspergillus clavatus NRRL 1	ACLA_080920	GSISA**S**GDLIPLSYIAA	123-139	664	1
A. flavus NRRL 3357	AFL2G_05505	GSISA**S**GDLIPLSYIAG	189-205	721	2
A. flavus NRRL 3357	AFL2G_00533	GSISA**S**GDLTPLAYIAG	202-218	714	1
A. flavus NRRL 3357	AFL2G_06214	GSISA**S**GDLSPIAYISG	155-171	671	3
A. fumigatus Af293	Afu2g09110	GSISA**S**GDLMPLSYIAG	181-197	728	2
A. nidulans FGSC A4	ANID_03897	GSISA**S**GDLTPLAYIAG	182-198	687	1
A. nidulans FGSC A4	ANID_06075	GSISA**S**GDLMPLSYIAG	187-203	702	2
A. niger ATCC 1015	e_gw1_15.39	GSISA**S**GDLTPLAYIAG	194-210	719	1
A. niger ATCC 1015	e_gw1_3.237	GSISA**S**GDLSPLSYIGG	181-197	720	2
A. niger ATCC 1015	fge1_pm_C_13000016	GTISA**S**GDLMPLAYVVG	184-200	704	2
A. oryzae RIB 40	AO090005000532	GSISA**S**GDLTPLAYIAG	202-218	714	1
A. oryzae RIB 40	AO090011000788	GSISA**S**GDLIPLSYIAG	189-205	721	2
A. oryzae RIB 40	AO090026000586	GTISA**S**GDLMPLAYVTG	183-199	696	2
A. oryzae RIB 40	AO090701000601	GSISA**S**GDLSP**I**AYISG	228-244	744	4
A. terreus NIH 2624	ATEG_09127	GSISA**S**GDLTPLAYIAG	177-193	695	0
A. terreus NIH 2624	ATEG_10006.1	GSVSA**S**GDLMPLSYIAG	181-197	698	2
Botrytis cinerea B05.10	BC1G_05296.1	GSISA**S**GDLSPLSYIGG	240-256	1131	6
Chaetomium globosum CBS 148.51	CHGG_02399.1	GSISA**S**GDLSTLSYIAG	190-206	707	3
Fusarium. graminearum NRRL 31084	FGSG_09311	GTISA**S**GDLMPMSYIAG	180-196	721	0
F. oxysporium f. sp. *lycopersici* 4287	FOXG_05927	GTISA**S**GDLMPLSYIAG	180-196	750	0
F. verticillioides 7600	FVEG_03798	GTISA**S**GDLMPLSYIAG	180-196	724	0
F. verticillioides 7600	FVEG_10552	GSISA**S**GDLIPLSYIAG	196-212	706	1
Gaeumannomyces graminis R3-111a-1	GGTG_00837.1	GSISA**S**GDLSPLSYIAG	204-220	743	2
Magnaporthe oryzae 70-15 (MG8)	MGG_10036.7	GSISA**S**GDLSALAWIAA	207-223	627	0
M. poae ATCC 64411	MAPG_07598.1	GSISA**S**GDLSPLSYIAG	198-214	730	2
Neurospora crassa OR74A (NC10)	NCU09391.5	GSISA**S**GDLSTLSYIAG	610-623	763	1
Neosartorya fischeri NRRL 181	NFIA_084640	GSISA**S**GDLMPLSYIAG	48-64	595	0
Stagonospora nodorum SN15	SNOG_08528.1	GSISA**S**GDLSPLSYICG	190-206	700	4
S. nodorum SN15	SNOG_09914.1	GSISA**S**GDLSALAWIGA	179-195	610	0
S. nodorum SN15	SNOG_16362.1	GSISA**S**GDLSPLSYVGG	191-207	772	2
Uncinocarpus reesii 1704	UREG_04219.1	GTISA**S**GDLMPLAYIVG	185-201	710	2
Verticillium albo-atrum VaMs.102	VDBG_08166.1	GTISA**S**GDLMPLSYIAG	181-197	676	1
V. dahliae VdLs.17	VDAG_100581.1	GTISA**S**GDLMPLSYIAG	181-197	696	0
V. dahliae VdLs.17	VDAG_05831.1	GSISA**S**GDLSALAWICA	218-234	645	0
Basidiomycota					
Coprinus cinerea okayama7#130	CC1G_06838.3	TSISA**S**GDLSPLSYIAG	212-228	734	9
C. cinerea okayama7#130	CC1G_14161.3	GSISA**S**GDLSPLSYIAG	252-268	770	6
Laccaria bicolor S238N-H82	LACBIDRAFT_291120	GTISA**S**GDLAPLSYIAG	163-179	688	5
L. bicolor S238N-H82	LACBIDRAFT_184628	GSISA**S**GDLSPLSYIAG	201-217	731	11
Puccinia triticina 1-1 BBBD Race 1	PTTG_02413.1	GSISA**S**GDLMPLSYVAA	175-191	653	13
P. graminis tritici CRL 75-36-700-3	PGTG_12283.2	GSISA**S**GDLMPLSYVAA	190-206	691	13
Rhodosporidium toruloides	AAA33883	GTISA**S**GDLSPLSYIAA	207-213	693	6
R. toruloides CBS 14	P11544	GTISA**S**GDLSPLSYIAA	184-200	716	6
Rhodotorula graminis WP1	CAD23828	GSISA**S**GDLSPLSYIAG	213-229	713	1
R. mucilaginosa NRRLY-15597	CAA31486	GTISA**S**GDLSPLSYIAA	213-229	720	5
Ustilago maydis 521	UM00078	SSISA**S**GDLSPLSYVAG	201-217	724	0

(Table 1 PAL, phenylalanine ammonia-lyase report courtesy by NRRL, Northern Regional Research Laboratory; FGSC, Fungal Genetics Stock Center; ATCC, American Type Culture Collection; RIB, Research Institute of Brewing; NIH, National Institutes of Health; CBS, Centraal bureauvoor Schimmel cultures)

The 45 PAL sequences underwent phylogenetic analysis, which revealed that PAL could be subdivided into three main groups: ascomycota I, ascomycota II, and basdiomycota. In

comparison to PAL of ascomycota II, PAL of ascomycota I is more closely related to PAL of basidiomycota. The variation in the PAL protein sequence among fungi was identified by the cladogram. Comparing the inferred protein sequences of PAL from various fungal species revealed that the protein sequences of the different species were 33–77 percent similar. Ascomycota PALs had the highest identity at 65%, while basidiomycota PALs had the highest identity at 97%. The percentage of identity between ascomycota and basidiomycota that has so far been discovered is 41%. A large number of fungi's genomes have shown that many of their species have the PAL gene (s) and the structural properties of the PAL gene vary within a species and among species.

PAL in fungi

While the metabolism of phenylalanine in vascular plants and animals has been well documented, much less is known about the fungal degradation of phenylalanine, according to the genome information on many fungi. Microorganisms use a few of the same phenylalanine metabolism pathways as plants and animals do. As in the case of animals, some microorganisms convert phenylalanine to homogentisic acid by first forming phenylpyruvic acid and p-hydroxyphenylpyruvic acid by transamination and hydroxylation [69]. While the discovery of a PAL enzyme in fungi and the observation of 14CO2 production from 14C-ring-labeled phenylalanine, cinnamic acid, and benzoic acid [71] have shown that some fungi can degrade phenylalanine by a pathway involving an initial deamination to cinnamic acid, as happens in plants, the genome information on many fungi has revealed that. Several basidiomycete fungi, including *Rhodotorula* [72], *Ustilagohordei* [71], *Schizophyllum commune* [71], and *Sporobolomyces roseus* [8], have been found to have a metabolic pathway for the metabolism of phenylalanine via cinnamic, benzoic, p-hydroxybenzoic, and protocatechuic acids. Phenylpyruvic acid, phenylacetic acid, and o-hydroxyphenylacetic acid are additional pathways through which *S. commune* can metabolise phenylalanine [71]. It's interesting to note that *Lentinus lepideus*, a different basidiomycete, has been reported to produce phenylpropanoid compounds like p-coumaric acid, caffeic acid, and isoferulic acid, phloretic acid, and *p*-methoxycinnamic acid) via cinnamic acid derived from phenylalanine [73]. It's interesting to note that *Lentinus lepideus*, a different basidiomycete, has been reported to produce phenylpropanoid substances (such as p-coumaric acid, caffeic acid, isoferulic acid, Numerous of these substances build up in the medium of this fungus as methyl esters, but it is unknown what these substances mean physiologically. Deuteromycete fungi like *Alternaria, R. solani*, and *Penicillium brevicompactum* have all been found to convert phenylalanine to benzoic acid derivatives via cinnamic acid. Gliotoxin, an antibiotic and antiviral cyclic peptide produced by the fungus *Gliocladium*, is modified by the addition of sulphur across the peptide ring and derived in part from phenylalanine [77].

Commercial and medical potential of PAL

The PAL enzyme's selectivity for phenylalanine has been linked to its therapeutic potential for treating neoplasms [78]. In vitro, PAL significantly reduced the growth of cancerous cells [79], and it also cured some mice that had received an injection of lymphoblastic leukaemia [80]. However, the potential of PAL to treat the inherited metabolic disorder phenylketonuria is what attracts clinicians' attention in particular. Patients were advised to follow a regular diet as part of a treatment plan that involved oral administration of PAL [81]. A mouse model has been used for preclinical testing of various species of PEGylated recombinant phenylalanine ammonia lyase for the treatment of phenylketonuria [82]. In 2011, BioMarin Pharmaceutical has announced PEG-PAL (PEGylated recombinant phenylalanine ammonia lyase) is currently in Phase II clinical development for the treatment of PKU.

The commercial demand for L-phenylalanine has resulted in its being produced in large quantities due to the rising consumption of the aspartic acid-phenylalanine dipeptide artificial sweetener, aspartame [83]. PAL can be used in a large-scale bio-conversion to produce L-phenylalanine from trans-cinnamic acid and ammonium salts acid because the reaction is reversible [84]. R. glutinis can produce PAL in large quantities for commercial use (Sigma-Aldrich, St. Louis, MO, USA).

Conciusions:

The structure, expression, and function of PAL in plants have accumulated a wealth of knowledge, but the biological function of PAL in fungi has not been established, and there is very little known about PAL in fungi in general. Fungal PAL has most frequently been proposed to have a catabolic function, in which the enzyme is used to obtain carbon and nitrogen from outside sources of amino acids. However, fungi can also use phenylalanine aminotransferase or amino acid oxidase to convert L-phenylalanine to carbon and nitrogen. What specific benefit does PAL provide that has allowed it to persist in this population of organisms? Cinnamic acid synthesis appears to play a significant role in the life cycle of fungi. It is now possible to compare and predict the effective pathways for phenylalanine degradation among various fungal species thanks to the full sequencing of the fungal genome(s) in diverse fungal species, including human and plant pathogens, saprophytes, and mushrooms. If PAL is necessary for fungal physiology, as well as any connections, if any, exist between PAL activity and pathogenesis, development, and secondary metabolic processes, should be revealed by molecular genetic studies like gene replacement. We still need to do more research to build the knowledge and resources that will help us explain why PAL exists in some fungi.

Acknowledgements:

Author would like to thank Professor A.D. Choudhary Ex-Head PGTD Botany RTMNU Nagpur for critical reading of the manuscript and helpful discussions on the genetic analysis

of PAL and *Fungal species*. Author apologizes to colleagues whose work was not included. This work was supported and fund grant by BRNS from BARC Mumbai as a research project.

References:

1. Koukol J, Conn EE. The metabolism of aromatic compounds in higher plants. IV. Purification and properties of the phenylalanine deaminase of *Hordeum vulgare*. *J Biol Chem.* 1961;236:2692–2698.
2. Jones DH. Phenylalanine ammonia-lyase: regulation of its induction, and its role in plant development. *Phytochemistry.* 1984;23:1349–1359.
3. Marusich WC, Jensen RA, Zamir LO. Induction of L-phenylalanine ammonia-lyase during utilization of phenylalanine as a carbon or nitrogen source in *Rhodotorulaglutinis*. *J Bacteriol.* 1981;146:1013–1019.
4. Camm EL, Towers GHN. Review article: phenylalanine ammonia lyase. Phytochemistry. 1973; 12:961-973.
5. Jorrín J, López-Valbuena R, Tena M. Purification and properties of phenylalanine ammonia-lyase from sunflower (*Helianthus annuus* L.) hypocotyls. *BiochimBiophys Acta.* 1988;964:73–82.
6. Czichi U, Kindl H. Formation of *p*-coumaric acid and *o*-coumaric acid from L-phenylalanine by microsomal membrane fractions from potato: evidence of membrane-bound enzyme complexes. *Planta.* 1975;125:115–125.
7. Bandoni RJ, Moore K, Subba Rao PV, Towers GH. Phenylalanine and tyrosine ammonia-lyase activity in some Basidiomycetes. *Phytochemistry.* 1968;7:205–207.
8. Moore K, Subba Rao PV, Towers GH. Degradation of phenylalanine and tyrosine by *Sporobolomyces roseus*. *Biochem J.* 1968;106:507–514.
9. Hodgins DS. Yeast phenylalanine ammonia-lyase: purification, properties, and the identification of catalytically essential dehydroalanine. *J Biol Chem.* 1971;246:2977–2985.
10. Sikora LA, Marzluf GA. Regulation of L-phenylalanine ammonia-lyase by L-phenylalanine and nitrogen in *Neurospora crassa*. *J Bacteriol.* 1982;150:1287–1291.
10. Emes AV, Vining LC. Partial purification and properties of L-phenylalanine ammonia-lyase from *Streptomyces verticillatus*. *Can J Biochem.* 1970;48:613–622.
11. Xiang L, Moore BS. Inactivation, complementation, and heterologous expression of encP, a novel bacterial phenylalanine ammonia-lyase gene. *J Biol Chem.* 2002;277:32505–32509. 13. Young MR, Towers GH, Neish AC. Taxonomic distribution of ammonia-lyases for L-phenylalanine and L-tyrosine in relation to lignification. *Can J Bot.* 1966;44:341–349.
12. Vance CP, Bandoni RJ, Towers GHN. Further observations on phenylalanine ammonia-lyase in fungi. *Phytochemistry.* 1975;14:1513–1514.

13. Nari J, Mouttet Ch, Pinna MH, Ricard J. Some physico-chemical properties of L-phenylalanine ammonia-lyase of wheat seedlings. *FEBS Lett.* 1972;23:220–224.
14. Havir EA, Hanson KR. L-phenylalanine ammonia-lyase (maize and potato); evidence that the enzyme is composed of four subunits. *Biochemistry.* 1973;12:1583–1591.
15. Jorrin J, Dixon RA. Stress reponses in alfalfa (*Medicago sativa* L.). II. Purification, characterization, and induction of phenylalanine ammonia-lyase isoforms from elicitor-treated cell suspension cultures. *Plant Physiol.* 1990;92:447–455.
16. Bernards MA, Ellis BE. Phenylalanine ammonia-lyase from tomato cell cultures inoculated with *Verticillium albo-atrum*. *Plant Physiol.* 1991;97:1494–1500.
17. Campbell MM, Ellis BE. Fungal elicitor-mediated responses in pine cell cultures. III Purification and characterization of phenylalanine ammonia-lyase. *Plant Physiol.* 1992;98:62–70.
18. Given NK, Venis MA, Grierson D. Purification and properties of phenylalanine ammonia-lyase from strawberry fruit and its synthesis during ripening. *J Plant Physiol.* 1988;133:31–37.
19. Pridham JB, Woodhead S. Multimolecular forms of phenylalanine-ammonia lyase in *Alternaria*. *Biochem Soc Trans.* 1974;2:1070–1072.
20. Kalghatgi KK, Subba Rao PV. Microbial L-phenylalanine ammonia-lyase: purification, subunit structure and kinetic properties of the enzyme from *Rhizoctonia solani*. *Biochem J.* 1975;149:65–72.
21. Hanson KR, Havir EA. Phenylalanine ammonia-lyase. In: Stumpf PK, Conn EE, editors. *Biochemistry of plants: a comprehensive treatise.* Vol. 7. New York: Academic Press; 1981. pp. 577–625.
22. Schomburg D, Salzmann M. *Enzyme handbook 1. Class 4: lyases, phenylalanine ammonia-lyase.* Berlin, Heidelberg: Springer-Verlag; 1990.
23. Hao Z, Charles DJ, Yu L, Simon JE. Purification and characterization of a phenylalanine ammonia-lyase from *Ocimumbasilicum*. *Phytochemistry.* 1996;43:735–739.
24. Kim SH, Kronstad JW, Ellis BE. Purification and characterization of phenylalanine ammonia-lyase from *Ustilago maydis*. *Phytochemistry.* 1996;43:351–357.
25. Neumann G, Schwemmle B. Flavonoids from *Oenothera* seedlings: identification and extranuclear control of their biosynthesis. *J Plant Physiol.* 1993;142:135–143.
26. Adachi O, Matsushita K, Shinagawa E, Ameyama M. Crystallization and properties of L-phenylalanine ammonia-lyase from *Rhodosporidiumtoruloides*. *Agric Biol Chem.* 1990;54:2839–2843.
27. Dahiya JS. Isolation and chalacterization of phenylalanine ammonia-lyase enzyme from the fungus *Leptosphaeria maculans*. *Indian J Exp Biol.* 1993;31:874–877.

28. Bolwell GP, Rodgers MW. L-Phenylalanine ammonia-lyase from French bean (*Phaseolus vulgaris* L.): characterization and differential expression of antigenic multiple Mr forms. *Biochem J.* 1991;279 (Pt 1):231–236.
29. McKegney GR, Butland SL, Theilmann D, Ellis BE. Expression of poplar phenylalanine ammonia-lyase in insect cell cultures. *Phytochemistry.* 1996;41:1259–1263.
30. Dubery IA, Smit F. Phenylalanine ammonia-lyase from cotton (*Gossypium hirsutum*) hypocotyls: properties of the enzyme induced by a *Verticillium dahliae* phytotoxin. *Biochim Biophys Acta.* 1994;1207:24–30.
31. D'Cunha GB, Satyanarayan V, Nair PM. Purification of phenylalanine ammonia lyase from *Rhodotorulaglutinis*. *Phytochemistry.* 1996;42:17–20.
32. Gowri G, Paiva NL, Dixon RA. Stress responses in alfalfa (*Medicago sativa* L.) 12. Sequence analysis of phenylalanine ammonia-lyase (PAL) cDNA clones and appearance of PAL transcripts in elicitor-treated cell cultures and developing plants. *Plant Mol Biol.* 1991;17:415–429.
33. Havir EA. L-phenylalanine ammonia-lyase: binding of polysaccharide by the enzyme from maize. *Plant Sci Lett.* 1979;16:297–304.
34. Shaw NM, Bolwell GP, Smith C. Wound-induced phenylalanine ammonia-lyase in potato (*Solanum tuberosum*) tuber discs: significance of glycosylation and immunolocalization of enzyme subunits. *Biochem J.* 1990;267:163–170.
35. Cramer CL, Edwards K, Dron M, Liang X, Dildine SL, Bolwell GP, Dixon RA, Lamb CJ, Schuch W. Phenylalanine ammonia-lyase gene organization and structure. *Plant Mol Biol.* 1989;12:367–383.
36. Lois R, Dietrich A, Hahlbrock K, Schulz W. A phenylalanine ammonia-lyase gene from parsley: structure, regulation, and identification of elicitor and light responsive cis-acting elements. *EMBO J.* 1989;8:1641–1648.
37. Orum H, Rasmussen OF. Expression in *E. coli* of the gene encoding phenylalanine ammonia-lyase from *Rhodosporidiumtoruloides*. *Appl MicrobiolBiotechnol.* 1992;36:745–747. 40. Schulz W, Eiben HG, Hahlbrock K. Expression in *Escherichia coli* of catalytically active phenylalanine ammonia-lyase from parsley. *FEBS Lett.* 1989;258:335–338.
38. Appert C, Logemann E, Hahlbrock K, Schmid J, Amrhein N. Structural and catalytic properties of the four phenylalanine ammonia-lyase isoenzymes from parsley (*Petroselinum crispum* Nym.) *Eur J Biochem.* 1994;225:491–499.
39. Calabrese JC, Jordan DB, Boodhoo A, Sariaslani S, Vannelli T. Crystal structure of phenylalanine ammonia lyase: multiple helix dipoles implicated in catalysis. *Biochemistry.* 2004; 43:11403–11416.

40. Hanson KR, Havir EA. The enzymic elimination of ammonia. In: Boyer PD, editor. *The enzymes.* New York: Academic Press; 1972. pp. 75–166.
41. Hanson KR, Havir EA. L-Phenylalanine ammonia-lyase. IV. Evidence that the prosthetic group contains a dehydroalanyl residue and mechanism of action. *Arch Biochem Biophys.* 1970;141:1–17.
42. Schuster B, Rétey J. The mechanism of action of phenylalanine ammonia-lyase: the role of prosthetic dehydroalanine. *Proc Natl Acad Sci U S A.* 1995;92:8433–8437.
43. Banerjee S, Hansen JN. Structure and expression of a gene encoding the precursor of subtilin, a small protein antibiotic. *J Biol Chem.* 1988;263:9508–9514.
44. Ohmiya Y, Hayashi H, Kondo T, Kondo Y. Location of dehydroalanine residues in the amino acid sequence of bovine thyroglobulin: identification of "donor" tyrosine sites for hormonogenesis in thyroglobulin. *J Biol Chem.* 1990;265:9066–9071.
45. Recsei PA, Snell EE. Pyruvoyl enzymes. *Annu Rev Biochem.* 1984;53:357–387.
46. Taylor RG, Lambert MA, Sexsmith E, Sadler SJ, Ray PN, Mahuran DJ, McInnes RR. Cloning and expression of rat histidase: homology of two bacterial histidases and four phenylalanine ammonia-lyases. *J Biol Chem.* 1990;265:18192–18199.
47. Neish AC. Biosynthetic pathways of aromatic compounds. *Annu Rev Plant Physiol.* 1960;11:55–80.
48. Parkhurst JR, Hodgins DS. Phenylalanine and tyrosine ammonia-lyase activity in *Sporobolomyce spararoseus*. *Phytochemistry.* 1971;10:2997–3000.
49. Scott DA, Hammond PM, Brearley GM, Price CP. Identification by high-performance liquid chromatography of tyrosine ammonia-lyase activity in purified fractions of *Pheseolus vulgaris* phenylalanine ammonia-lyase. *J Chromatogr.* 1992;573:309–312.
50. Jangaard NO. The characterization of phenylalanine ammonia-lyase from several plant species. *Phytochemistry.* 1974;13:1765–1768.
51. RÖsler J, Krekel F, Amrhein N, Schmid J. Maize phenylalanine ammonia-lyase has tyrosine ammonia-lyase activity. *Plant Physiol.* 1997;113:175–179.
52. Nari J, Mouttet C, Fouchier F, Ricard J. Subunit interactions in enzyme catalysis. *Eur J Biochem.* 1974;41:499–515.
53. Bolwell GP, Bell JN, Cramer CL, Schuch W, Lamb CJ, Dixon RA. L-Phenylalanine ammonia-lyase from *Phaseolus vulgaris*: characterization and differential induction of multiple forms from elicitor-treated cell suspension cultures. *Eur J Biochem.* 1985;149:411–419.
54. Nagai N, Kojima Y, Shimosaka M, Okazaki M. Effect of kinetin on L-phenylalanine ammonia-lyase activity in tobacco cell culture. *Agric Biol Chem.* 1988;52:2617–2619.

55. Zoñ J, Amrhein N. Inhibitor of phenylalanine ammonia-lyase: 2-aminoindan-2-phosphonic acid and related compounds. *Liebigs Ann Chem.* 1992;1992:625–628.
56. Fritz RR, Hodgins DS, Abell CW. Phenylalanine ammonia-lyase: induction and purification from yeast and clearance in mammals. *J Biol Chem.* 1976;251:4646–4650.
57. Tanaka Y, Matsuoka M, Yamanoto N, Ohashi Y, Kano-Murakami Y, Ozeki Y. Structure and characterization of a cDNA clone for phenylalanine ammonia-lyase from cut-injured roots of sweet potato. *Plant Physiol.* 1989;90:1403–1407.
58. Joos HJ, Halhbrock K. Phenylalanine ammonia-lyase in potato (*Solanum tuberosum* L.): genomic complexity, structural comparison of two selected genes, and modes of expression. *Eur J Biochem.* 1992;204:621–629,
59. Whetten RW, Sederoff RR. Phenylalanine ammonia-lyase from loblolly pine: purification of the enzyme and isolation of complementary DNA clones. *Plant Physiol.* 1992;98:380–386.
60. Anson JG, Gilbert HJ, Oram JD, Minton NP. Complete nucleotide sequence of the *Rhodosporidiumtoruloides* gene coding for phenylalanine ammonia-lyase. *Gene.* 1987;58:189–199.
61. Vaslet CA, Strausberg RL, Sykes A, Levy A, Filpula D. cDNA and genomic cloning of yeast phenylalanine ammonia-lyase reveal genomic intron deletions. *Nucleic Acids Res.* 1988; 16 :11382.
62. Wanner LA, Li G, Ware D, Somssich IE, Davis KR. The phenylalanine ammonia-lyase gene family in *Arabidopsis thaliana*. *Plant Mol Biol.* 1995;27:327–338.
63. Campbell MM. *Elicited phenylpropanoid metabolism in pine cell cultures [dissertation]* Guelph : University of Guelph; 1991.
64. Sederoff R, Campbell M, O'Malley D, Whetten R. Genetic regulation of lignin biosynthesis and the potential modification of wood by genetic engineering in loblolly pine. *Recent Adv Phytochem.* 1994;28:313–355.
65. Kim SH, Virmani D, Wake K, MacDonald K, Kronstad JW, Ellis BE. Cloning and disruption of a phenylalanine ammonia-lyase gene from *Ustilago maydis*. *Curr Genet.* 2001;40:40–48.
66. Wat CK, Towers GH. Metabolism of the aromatic amino acids by fungi. *Recent Adv Phytochem.* 1977;12:371–432.
67. Power DM, Towers GH, Neish AC. Biosynthesis of phenolic acids by certain wood-destroying Basidiomycetes. *Can J Biochem.* 1965;43:1397–1407.
68. Moore K, Subba Rao PV, Towers GH. Degradation of phenylalanine and tyrosine by Basidiomycetes. *Life Sci.* 1967;6:2629–2633.

69. Uchiyama K, Kawaguchi K, Tochikura T, Ogata K. Metabolism of aromatic amino acids in microorganisms. Part III. Metabolism of cinnamic acid in *Rhodotorula*. *Agric Biol Chem.* 1969;33:755–763.
70. Towers GH. Metabolism of cinnamic acid and its derivatives in Basidiomycetes. In: Harborne JB, Swain T, editors. *Perspectives in phytochemistry*. New York: Academic Press; 1969. pp. 179–191.
71. Nambudiri AM, Subba Rao PV, Bhat JV. Metabolism of aromatic compounds by an *Alternaria species*. *Phytochemistry.* 1970;9:687–693.
72. Kalghatgi KK, Nambudiri AM, Bhat JV, Subba Rao PV. Degradation of L-phenylalanine by *Rhizoctonia solani*. *Indian J BiochemBiophys.* 1974;11:116–118.
73. Campbell IM, Gallo MA, Jones CA, La Sitis PR, Rosato LM. Role of cinnamate in benzoate production in *Penicillium brevicompactum*. *Phytochemistry.* 1987;26:1413–1415.
74. Griffin DH. *Fungal physiology.* Toronto: Wiley-Less; 1994.
75. Stith WJ, Hodgins DS, Abell CW. Effects of phenylalanine ammonia-lyase and phenylalanine deprivation on murine leukemic lymphoblasts *in vitro*. *Cancer Res.* 1973;33: 966–971.
76. Abell CW, Stith WJ, Hodgins DS. The effects of phenylalanine ammonia-lyase on leukemic lymphocytes *in vitro*. *Cancer Res.* 1972;32:285–290.
77. Abell CW, Hodgins DS, Stith WJ. An *in vitro* evaluation of the chemotherapeutic potency of phenylalanine ammonia-lyase. *Cancer Res.* 1973;33:2529–2532.
78. Hoskins JA, Jack G, Wade HE, Peiris RJ, Wright EC, Starr DJ, Stern J. Enzymatic control of phenylalanine intake on phenylketonuria. *Lancet.* 1980;1:392–394.
79. Sarkissian CN, Gámez A, Wang L, Charbonneau M, Fitzpatrick P, Lemontt JF, Zhao B, Vellard M, Bell SM, Henschell C, *et al.* Preclinical evaluation of multiple species of PEGylated recombinant phenylalanine ammonia lyase for the treatment of phenylketonuria. *Proc Natl Acad Sci U S A.* 2008;105:20894–20899.
80. Klausner A. Building for success in phenylalanine. *Bio/Technology.* 1985;3:301–307.
81. Hamilton BK, Hsiao HY, Swann WE, Anderson DM, Delent JJ. Manufacture of L-amino acids with bioreactors. *Trends Biotechnol.* 1985;3:64–68.

SCREENING FOR PHYTOCHEMICALS, ANTIMICROBIAL AND ANTICOAGULANT ACTIVITY OF AQUEOUS EXTRACT OF *TRIDAX PROCUMBENCE*

Nihar Ranjan Nayak[1,3], Anisma Pattnayak[1] and Alok Ranjan Sahu*[2]

[1]Department of Botany, Guru Ghasidas Vishwavidyala, Bilaspur, Chhattisgarh

[2]Department of Botany, Vikash Degree College, Bargarh, Odisha

[3]Department of Botany, Maa Manikeshwari University,

Manikya Vihar, Bhawanipatna, Odisha

*Corresponding author E-mail: alok.btgene@gmail.com

Abstract:

Tridax procumbens a widely available medicinal plant, has been traditionally used in various folk medicines for its purported antimicrobial properties. Present study aimed to screening of phytochemicals, activity of the aqueous extract of *T. procumbens* against isolated microbes and anticoagulant activity. The extract shows the presence of alkaloids, tannins, terpenoids and flavonoids. The extract demonstrated significant zones of inhibition against isolated Gram-positive bacteria, like *Staphylococcus aureus*, Gram-negative bacteria, like *Escherichia coli and Pseudomonas*, and pathogenic fungi like *A. niger*, *A. flavous*, and *Fusarium*. Further, the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values were determined for the most susceptible bacterial and fungal strains. The MIC and MFC values indicated the concentration of the extract required to completely inhibit microbial growth and induce fungal death, respectively. Moreover, the extract highly inhibited the growth of *Pseudomonas* and *A. flavous*. These findings highlight the promising antimicrobial potential of *T. procumbens* extract, supporting its traditional use as a medicinal plant in managing infectious diseases caused by bacteria and fungi. The natural origin of the extract and its demonstrated efficacy against clinically relevant pathogens underscore its potential as a source for developing novel antimicrobial agents or as a complementary treatment option. However, further studies are warranted to elucidate the active compounds responsible for the observed antimicrobial activity and to evaluate the safety and efficacy of *T. procumbens* extract for clinical applications.

Keywords: *Tridax procumbens*, antimicrobial activity, *Acinetobacter*, Antibiotic

Introduction:

Plant diseases caused by various pathogens, including bacteria, fungi, and viruses, contribute to significant yield losses in agriculture. Conventional methods of disease control

often involve the use of synthetic chemicals, such as antibiotics and fungicides. However, the excessive and indiscriminate use of these chemical agents has led to several adverse consequences, such as environmental pollution, the development of resistance to pathogens, and negative impacts on human health (Micoli *et al*., 2021). Therefore, there is an urgent need to explore alternative approaches that are effective, safe, and environmentally friendly.

One of the major concerns associated with the excessive use of synthetic chemicals is environmental pollution (Naidu *et al.*, 2021). Additionally, the persistence of synthetic chemicals in the environment can have unintended consequences for non-target organisms, including beneficial insects, birds, and soil microorganisms, disrupting the delicate balance of ecosystems. Moreover, the overreliance on synthetic chemicals has led to the emergence and spread of antimicrobial resistance (AMR) in plant pathogens. The rise of antibiotic-resistant plant pathogens further complicates disease control strategies, leaving farmers with limited options for effective management (Micoli *et al.*, 2021). Natural and eco-friendly solutions, such as plant extracts with antimicrobial properties, have gained significant attention in recent years. Plant extracts contain a diverse range of bioactive compounds, including phenolics, flavonoids, alkaloids, and terpenoids, which possess inherent antimicrobial activity against pathogens (Eljounaidi and Lichman 2020).

These plant-derived compounds offer several advantages over synthetic chemicals. Firstly, they are biodegradable and pose minimal risks to the environment, reducing pollution and long-term ecological impacts. Secondly, the complex nature of plant extracts makes it challenging for pathogens to develop resistance, as they contain multiple bioactive compounds that act synergistically. This reduces the likelihood of resistance emergence and ensures long-term effectiveness in disease management (Ayilara *et al.*, 2023). Furthermore, plant extracts have the potential to be integrated into organic farming systems, aligning with sustainable agricultural practices and meeting consumer demands for chemical-free produce.

Tridax procumbens a promising medicinal plant commonly known as coat buttons or Tridax daisy, is a perennial herbaceous plant belonging to the Asteraceae family. It is native to tropical regions and widely distributed across the globe. Traditionally, *T. procumbens* has been used in folk medicine to treat various ailments due to its diverse pharmacological activities. It possesses antimicrobial, anti-inflammatory, antioxidant, wound-healing, and immunomodulatory properties. These characteristics make *T. procumbens* an intriguing candidate for exploring its potential as a natural antimicrobial agent against plant pathogens. Sahu *et al.* (2010) reported the leaf of *T. procumbens* was used as antiseptic cream for healing cut, while roots were used as dysentery by the natives of Bargarh district, Odisha. Sahu *et al.* (2013) reported the leaf of *T. procumbens* was used as antiseptic cream for healing cut by the natives of Sohela block of

Bargarh district, Odisha. The leaf of the plant was used as antiseptic cream for healing cut by the natives of Bhawanipatna, Kalahandi District, Odisha (Sahu *et al.*, 2020).

Several studies have investigated the antimicrobial activity of *T. procumbens* extract against a range of microorganisms, including both human pathogens and plant pathogens. The extract has exhibited significant inhibitory effects against various bacteria, fungi, and viruses. The bioactive compounds responsible for its antimicrobial properties include flavonoids, alkaloids, terpenoids, phenols, and saponins. These compounds have been reported to disrupt the cellular structure and metabolic processes of the pathogens, leading to their growth inhibition and eventual death (Ased *et al., 2020*). *T. procumbens* extract contains a diverse array of bioactive compounds, which are primarily responsible for its antimicrobial properties. These compounds include flavonoids, alkaloids, terpenoids, phenols, and saponins. Flavonoids, such as quercetin and luteolin, are known for their potent antimicrobial activity against a wide range of pathogens (Varsharani *et al.*, 2022). The findings of this study have significant implications for plant disease management and sustainable agriculture. If *T. procumbens* plant extract demonstrates potent antimicrobial activity against pathogenic bacteria, it could serve as an eco-friendly and cost-effective alternative to synthetic chemicals. By harnessing the natural antimicrobial properties of *T. procumbens*, farmers and agricultural practitioners can potentially reduce their reliance on conventional pesticides, thereby minimizing the adverse environmental impacts associated with their use (Varsharani *et al*., 2022).

In conclusion, this research study explores the antimicrobial activity of *T. procumbens* plant extract against isolated plant pathogens. The potential of this medicinal plant as a natural alternative for plant disease management is investigated, highlighting the importance of sustainable agriculture and reducing reliance on synthetic chemicals. The findings of this study have implications for both agricultural practices and the development of eco-friendly solutions for plant disease control. By tapping into the vast potential of plant-derived antimicrobial agents, we can move towards a more sustainable and resilient agricultural system.

Materials and Methods:

Plant materials collection site

The plant was taken from the garden of the Botany department of Guru Ghasidas Vishwavidyala, Bilaspur district of Chhattisgarh. First the plant was uprooted and then the leaves were cleaned carefully with tap water. The leaves were shade dried for 7-8 days.

Extraction of plant material

The extraction process was carried out by the method describe by Sowmya *et al.* (2015) with some modification. *Tridax procumbens* was collected and the leaves and stem parts were separated. The separated leaves were well washed with tap water 3 to 4 times and placed in the

shade dry for 7-8 days. The dried leaves were crushed with the help of a mortar and pestle to a fine powder. 10g of *T. procumbens* leaves and stem powder was combined with 100 ml of distilled water in a 250 ml conical flask, individually dissolved, and heated for 5 minutes. To obtain the extract, the mixture was filtered through 8 layers of muslin cloth (Kaviya and Jeevitha, 2023). The filtered extract was poured into a medium-sized Petri plate and was placed in a water bath at 80℃ up to powder formation. Then the powder was scratched from the surface of a plate with the help of a sterile spatula to obtain a powder.

Phytochemical analysis of *T. procumbens*

Phytochemical analysis of T. procumbens for determinations of different secondary metabolites present in plant extract was done as mentioned below:

Alkaloid's test (Wagner's reagent): 5ml of the extract was added to 1-2 drops of Wagner's reagent (Along the sides of the test tube). And a test tube where extract without reagent for control. Formation of reddish-brown precipitate indicated the presence of alkaloids.

Tannin test: 1ml of extract added to 0.1% of FeCl3 (Ferric chloride) solutions. And a test tube where extract without reagent for control. The presence of tannins was finalised by formation of white precipitate.

Terpenoid test: 1ml of extract was added in 2ml of chloroform along with 5ml of H2SO4. And a test tube where extract without reagent for control. Formation of yellow colour indicated the presence of terpenoids.

Flavonoid test: 1ml of extract was added to 1ml of 10% lead acetate. The colour change and formation of yellow precepted indicate the presence of the flavonoid.

Selection of bacterial and fungal strains

Staphylococcus aureus, Aspergillus niger, E. coli, Fusarium, Pseudomonas, and *Aspergillus flavus* pathogens are obtained from Botany department, GGV, Bilaspur, Chhattisgarh. Further these pure sample were preserved in Nam slant for future use.

Antimicrobial Assay

The Agar Well Diffusion Assay, with changes published by Irshad *et al.* (2012), was used. To make the Agar Well Diffusion media, molten Mueller-Hinton agar was poured into petri dishes and allowed to harden. Following that, 100µl of inoculums (about 1.5 X 108 cells/ml) were spread into Mueller-Hinton agar. This was allowed to settled before making 5 mm holes in the agar with a sterile cork borer. For each pathogen, a 100 mg/ml stock of crude plant extract was generated by dissolving 100 mg of dried plant extract in 1 ml of sterilized distilled water. A concentration of 10 mg per hole was achieved by pipetting 100 µL of the stock extract into the holes. In another plate we have taken streptomycin disk. This was a positive control, and 100 µL of distil water was pipetted into one of the holes as the negative control. This whole

setup was done in three different plates. Then the place in the incubator for 24-48hr for bacteria at 37℃ ± 2, and for 3-4 days for fungus.

Anti-coagulant activity test:

This method was described by (Gursamy *et al.,* 2020) with some modifications. The sample of blood was collected from a healthy volunteer in a blood collection tube. Then 2 drop of blood was taken in 2 glass slides and labelled as blank and T1. Blank is for Normal blood was kept as a control. 2 drops of aqueous leaf extract were added to T1 as shown in Figure 5. The clotting was checked by a needle. Using a stopwatch, the clotting activity of each tube was monitored every 30 seconds and the clotting time was noted.

Results:

Preparation of plant extract

Tridax procumbens has already been recognized as a plant used to cure a variety of illnesses (Taddei *et al*., 2000). Additionally, *T. procumbens* has yielded several bioactive chemicals that have been isolated and characterized. This plant contains the phytochemicals alkaloid, tannin, coumarin, and saponin, among others (Prasad *et al*., 2008). And these bioactive or secondary metabolites compounds were responsible for the antimicrobial property of the plant extract, so this plant is chosen as the sample.

Secondary metabolites of *T. procumbens*

Table 1: Test shows result for presence of Secondary metabolites of *T. Procumbens*

Sl. No	Phytochemicals	Presence
1	Alkaloids	+
2	Tannins	+
3	Terpenoids	+
4	Flavonoids	+

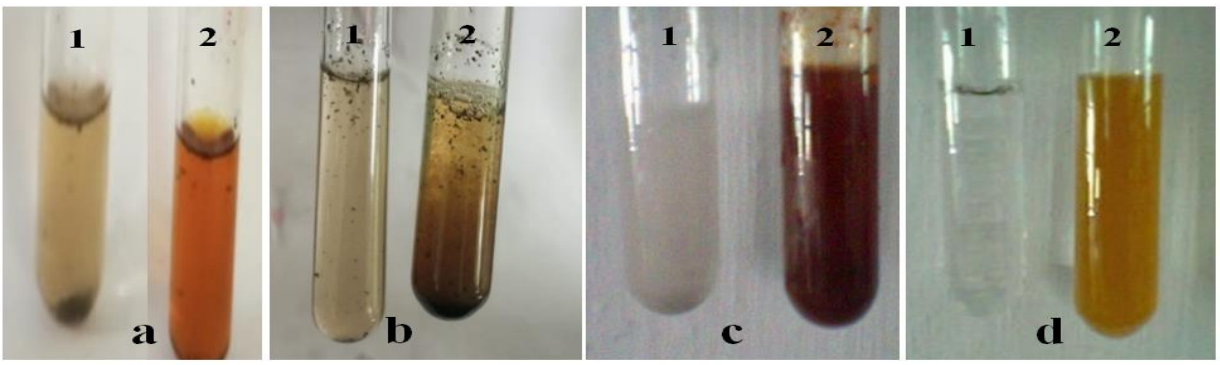

Figure 1: Shows the positive test for alkaloids
(a), Tannins test (b) Terpenoid test (c) and (d) Flavonoid test

In the present study, leaf aqueous extract from *T. procumbens* was screened for various Secondary metabolites. The selected leaves shown the presence of Secondary metabolites such as, alkaloids, tannins, terpenoids, flavonoids as shown in Figure 1. Earlier, *T. procumbens* has been declared as a medicinal plant to treat various diseases, and the secondary metabolites such as tannins and alkaloids presence was detected it has antimicrobial properties result represent in Table 1.

Antimicrobial activities

From our research we have found out that the plant extract contains different secondary metabolites such as alkaloids, tannins and terpenoids, these are the metabolites which are responsible for antimicrobial activity. The result shows that the aqueous extract had inhibitory activity against *Staphylococcus aureus, Pseudomonas*, and *Escherichia coli*, at a concentration of 200 mg/ml of plant extract. The antimicrobial activity of plant material extract was studied. Streptomycin disc was used as the reference standard for the inhibition zone 20mm when evaluating the antibacterial activity of the plant extracts (Table 2). Among bacterial pathogens, the Plant extract shown a high zone of inhibition against *Pseudomonas* by the dimeter of 2.3 followed by *Staphylococcus aureus* by dimeter of 2.2 In *E. coli* strain there is low zone of inhibition 2.0 was shown by *Tridax* extract. In case of fungus, among all fungus the plant extract shown a high zone of inhibition against *A. flavus* of diameter 2.3 followed by *Fusarium* and lowest in *A. niger* 1.2 Zone of inhibition in Muller Hinton media as shown in Figure 3 and 4

The current study is important since bacteria are developing resistance to the medications that are currently being used. And these natural drugs which are purely unrecognized by the bacteria were now used for the drug making companies. So, pharmaceutical companies making plant-based antibiotic medications. As expected, the zone produced by antibiotics was larger and clearly visible. In comparison to the zone of extract, all the antibiotics zone was high from the plant extract zone.

Table 2: Comparative study of effect of plant extract and antibiotic on different microbes on the basis of zone of inhibition formation

S. No	**Organism**	**Activity**	**Zone of Inhibition in mm**	
			Plant extract	**Antibiotics** (*Streptomycin* disc)
1	*E. coli*	+	2 ± 0.1	3.1 ± 0.1
2	*Pseudomonas*	+	2.3 ± 0.1	2.6 ± 0.2
3	*S. aureus*	+	2.2 ± 0.2	2.8 ± 0.1
4	*A. niger*	+	1.2 ± 0.1	1 ± 0.1
5	*A. flavous*	+	2.3 ± 0.1	2 ± 0.2
6	*Fusarium*	+	1.3 ± 0.1	1 ± 0.2

'+' Represent positive activity; Value represent mean ± Standard Deviation

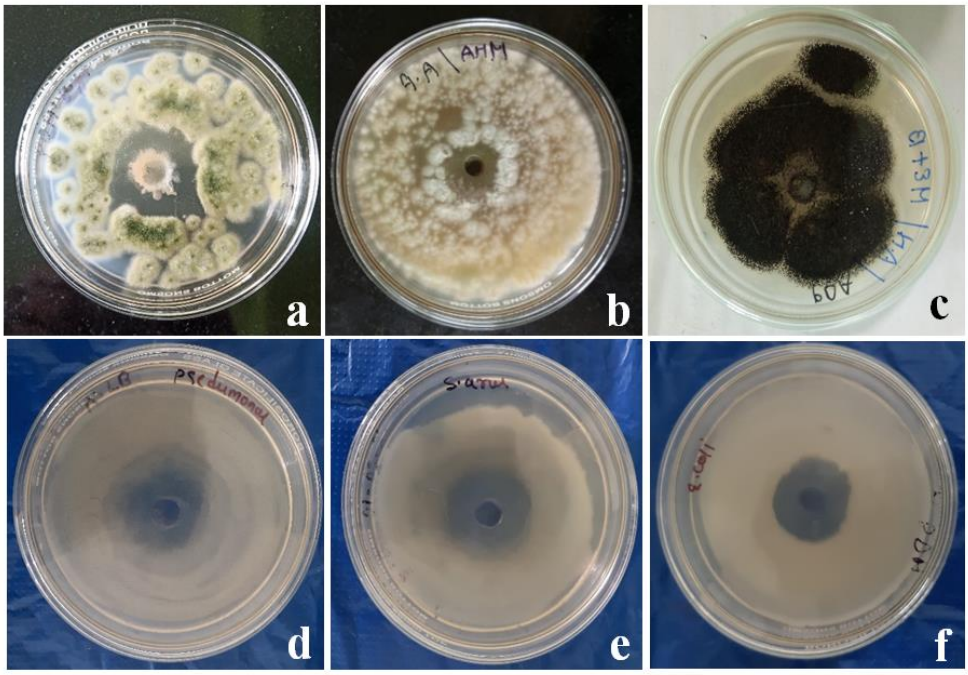

Figure 3: Antimicrobial effect of *T. procumbens* plant extract on different microbes Effect on *A. flavus* (a), Effect on *Fusarium* (b), Effect on *A. niger* (c), Effect on *Pseudomonas* (d), Effect on *S. aureus* (e), Effect on *E. coli* (f).

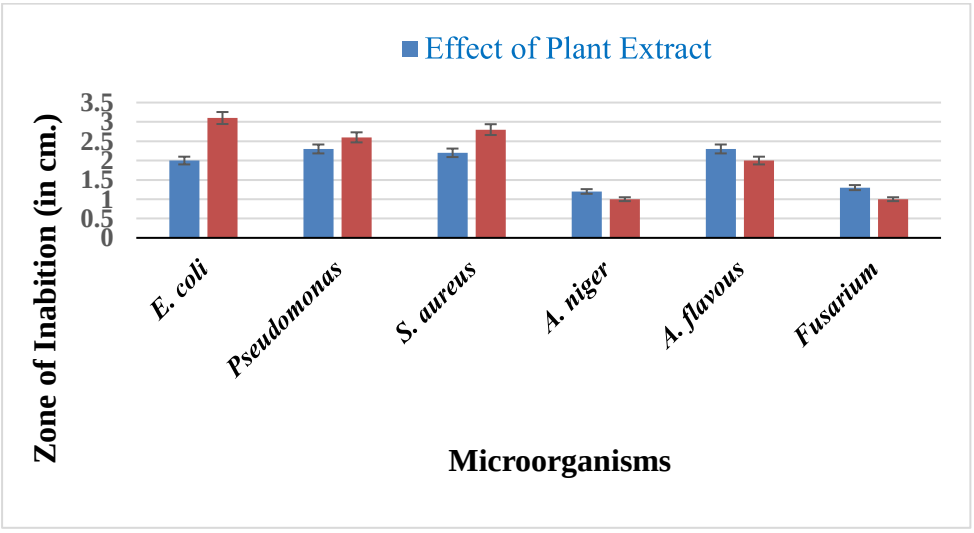

Figure 4: Antimicrobial effect of *Tridax procumbens* in comparison to antibiotic effect on different microbes

Anticoagulant activity

The sample of blood was collected from a healthy volunteer in a blood collection tube. Then 2 drop of blood was taken in 2 glass slides and labelled as blank and T1. Blank is for Normal blood was kept as a control. 2 drops of aqueous leaf extract were added to T1. The result was impressive as the blank or control slide clotting time was 06min and 52 sec whereas the T1

slide clotting time was 05 min and 37 sec as view in Figure 5. When compared to a control, the *Tridax procumbens* extract show a reduced clotting time difference of 01min and 05sec.

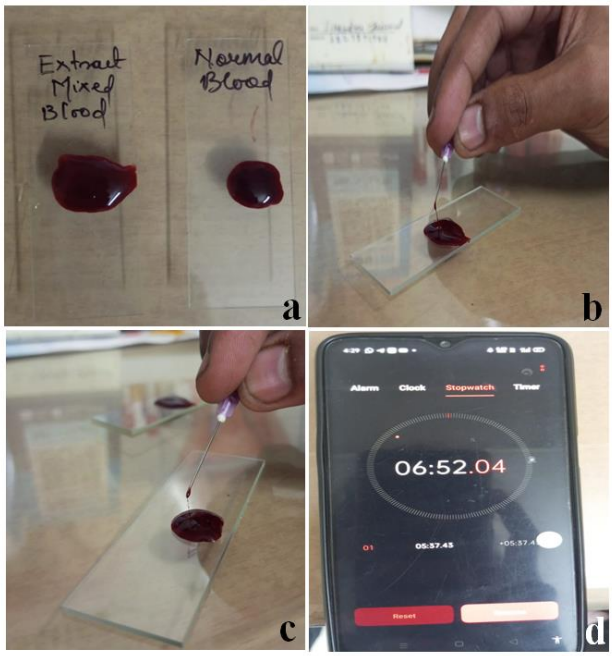

Figure 5: Blood sample taken in slides for test (a), Blood was mixed with extract (b), Blank slide where blood sample without extract was there (c), the blood clotting time difference between control and test (d)

Discussion:

Almost every one of the ancient civilizations has used plants since ancient times, including wild and cultivated species. It has long been known that Ayurveda has been practiced in the Indian subcontinent. Traditional healers and practitioners of the Unani and Ayurvedic medical systems have employed *Tridax procumbens* as a phytomedicine. *Tridax* is linked to antibacterial action, which accounts for its traditional use (Mundada and Shivhare, 2010).

In plant extract the presence of secondary metabolites such as alkaloids, flavonoids and several aromatic compounds are the phytochemicals that reduce the pathogenic activity (Lutterodt *et al*., 1999). The development of multidrug resistance in microbes has encouraged the search for new, safe, and efficient bioactive agents of herbal origin. Resistance in microbes to many antimicrobials has led to morbidity and mortality from failed treatments and increased healthcare costs. According to reports by Taddei *et al.* (2000), *T. procumbens* medicinal plants have been used to cure a variety of illnesses. In our research, the aqueous extract of the plant has strong antimicrobial properties against the bacteria in the agar well diffusion method after an incubation period of 24-48 hr at 37℃. It inhibits growth by making zone against *E. coli, Pseudomonas, S. aureus, A. niger, A. flavous,* and *Fusarium* respectively. According to result

highest zone of inhibition was shown against *E. coli* and *S. aureus* as 2.0cm and 2.2cm. In fungus, the highest zone of inhibition shown in, the *A. flavus* zone of 2.3cm followed by *A. Niger*, Fusarium. It is possibly caused by the active secondary components which prevent bacterial growth, colonization (Al-Bayati and Al-Mola, 2008), inhibiting enzyme synthesis and breaking bacterial membranes (Ogunwenmo *et al*., 2007). The result of the MIC test at different concentrations shown that the increase in concentration increased the zone of inhibition. It means the concentration of extract was directly propositional; to activity against the pathogens. In the anti-blood coagulation tests the meantime without adding the extract was 6.52 ± 0.31 min, while the mean coagulating time after treatment with the extract was 5.37 ± 0.70 min at a dose of 50 μg/ml and 5.11 ± 0.69 min at a dose of 10 μg/ml, achieving 12% of the decrease in normal coagulating time result. Thus, the extract has anticoagulant activity as it decreased the bleeding time. This might mean that one or more steps in the cascade of clotting were skipped. *T. procumbens* extract may activate prothrombin by a mechanism different from factor Xa, indicating that both intrinsic and extrinsic pathways are skipped. This is because clotting factors occur in trace amounts, unlike prothrombin and fibrinogen, which occur in large amounts. Secondly Blood coagulation rate depends on balance of procoagulants and anticoagulants; extract may inhibit physiological anticoagulants like heparin and antithrombin III. This study shows that, at various doses, *T. procumbens* significantly affects TCA (Tricyclic anti depression), TP (Total Protein), and platelet count. The extract of *T. procumbens* indicated the presence of cardiac glycosides, terpenoids, terpenoids, saponins, tannins, and terpenoids. *T. procumbens* leaf extracts may have obtained antibacterial action due to certain of these plant secondary metabolites were present in *T. procumbens* components that were under study in large amounts. The findings concur with those of (Mir *et al.* 2013) and Ikewuchi (2009) on *T. procumbens* leaf. The observation that *E. coli, Pseudomonas, S. aureus, A. niger, A. flavous* and *Fusarium* are susceptible to *T. procumbens* water extracts suggests that the plant has the potential to be a source of medicines that can be used to treat the test pathogens. *T. procumbens* extracts have been shown to have antibacterial action, which could be the explanation for some of the earlier statements that the plant is traditionally used to treat fever, typhoid fever, cough, asthma, epileptic seizures, dysentery (Tejaswini *et al*., 2011) and antiseptic cream for healing cut (Sahu *et al.,* 2020). The results of this research have provided us with the scientific justification for using these plants in traditional medical treatments and as an anticoagulant agent.

Conclusion:

This research journal has extensively explored and validated the antimicrobial property of *Tridax procumbens*, a traditional medicinal plant that has long been used in various folk

medicine practices. The findings presented in this study have provided significant evidence supporting the effectiveness of *T. procumbens* extracts against a wide spectrum of microorganisms, including bacteria, fungi, and even some drug-resistant strains. Through the employment of various experimental methods, the researchers demonstrated the potency of *T. procumbens* in inhibiting microbial growth and demonstrated promising potential for the development of alternative antimicrobial agents. The diverse bioactive compounds present in *T. procumbens* extracts, such as flavonoids, alkaloids terpenoids and alkaloids, have been identified as the key contributors to their antimicrobial activity. *T. procumbens* leaf aqueous extract has antimicrobial properties because it has inhibited the growth of *E. coli, Pseudomonas, S. aureus, A. Niger, A. flavous,* and *Fusarium*. The extract has the well effective anticoagulant activity that decrease the blood time and blood clotting time. Therefore, an effective alternative that both effectively decreased the bleeding and increases blood clotting. The results from this investigation hold great importance in the field of medicine and pharmacology, particularly in the ongoing battle against antimicrobial resistance. With the escalating global health crisis posed by resistant pathogens, *T. procumbens* presents a natural and sustainable solution for combating infectious diseases and reducing the dependency on conventional antimicrobial agents. It is evident from the comprehensive research presented here that *T. procumbens* exhibits significant potential as a valuable source of novel antimicrobial agents. However, further studies are necessary to explore the exact mechanisms of action and to isolate and identify individual bioactive compounds responsible for the observed antimicrobial activity. In conclusion, the antimicrobial property of *T. procumbens* holds great promise and warrants further investigation and development as a potential therapeutic option to combat infectious diseases and address the pressing global health challenges posed by antimicrobial resistance. This research opens up new avenues for pharmaceutical development and underscores the significance of traditional medicinal plants in modern medicine.

References:

1. Abubakar A, Ogbadoy E, Okugon JI, Ghodi TI, and Ibikunle GI (2012): The identification of putative antitrypanosomal compounds in *Tridax procumbens* extracts, Thermofiser, 2(1): 185-194.
2. Al-Bayati FA, and Al-Mola HF (2008): Antibacterial and antifungal activities of different parts of Tribulus terrestris L. growing in Iraq. J Zhejiang Univ Sci B., 9(2): 154-159.
3. Asead S, Natarajan B, Abdullah A, Alyousef, Abdulaziz A, Mohamme A (2000): In-vitro antibacterial, antioxidant potentials and cytotoxic activity of the leaves of *Tridax procumbens,* Saudi Journal of Biological Sciences, 27(2): 757-761.

4. Ayilara S, Adeleke S, Akinola A, Fayose A, Adeyemi T, Gbadegesin A, Omole K, Johnson M, Uthman O, and Babalola O (2023): Biopesticides as a promising alternative to synthetic pesticides: A case for microbial pesticides, phytopesticides, and nanobiopesticides. Frontiers in Microbiology, 14:1040901. doi: 10.3389/fmicb.2023.1040901.
5. Eljounaidi K, and Lichman BR (2020): Nature's Chemists: The Discovery and Engineering of Phytochemical Biosynthesis. Front. Chem., 8: 596479. doi: 10.3389/fchem.2020.596479
6. Gursamy R, Ramya S, Balasubramaniyan G, Saravanaraj A, Sudhamohan S and Subramani K (2020): Testing of Anti-coagulance activity in *Tridax procumbens*, IJRAR 7 (2): 346-356.
7. Ikewuchi JC (2012): An Aqueous Extract of the Leaves of *Tridax procumbens* Linn (Asteraceae) Protected Against Carbon Tetrachloride Induced Liver Injury in Wistar Rats, The Pacific Journal of Science and Technology, 13(1): 519-527.
8. Irshad M, Zafaryab M, Singh M, and Rizvi MM (2012): Comparative Analysis of the Antioxidant Activity of Cassia fistula Extracts. Int J Med Chem., 157125. doi: 10.1155/2012/157125.
9. John LK, Loewenstein G, and Prelec D (2012): Measuring the Prevalence of Questionable Research Practices with Incentives for Truth Telling. Psychological Science, 23(5): 524–532. https://doi.org/10.1177/0956797611430953.
10. Kaviya K, and Jeevitha M (2023): Preparation of *Tridax procumbens* Leaves and Stem Extract and Analysis of Its Antimicrobial Activity, Eur. Chem. Bull. 2023, 12 (S2): 434 – 441.
11. Lutterodt G, Ismail A, Basheer R, and Baharudin H (1999): Antimicrobial effects of Psidium guajava extracts as one mechanism of its antidiarrhoeal action. Malaysian J Med Sci, 6 (2): 17-20.
12. Micoli .F, Bagnoli F, and Rappuoli R *et al.* (2021) The role of vaccines in combatting antimicrobial resistance. Nat. Rev. Microbiol., 19: 287–302.
13. Mir A, Sawhney S, and Jassal M (2013): Qualitative and quantitative analysis of phytochemicals of Taraxacum officinale. Wudpecker Jou dpecker Journal of Pharmacy and Pharmocology, 2(1): 1-5.
14. Mounyr B, Moulay I, and Saad I (2016): Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 6(2): 71-79. doi: 10.1016/j.jpha.2015.11.005.
15. Mundada S, and Shivhare R (2010): Pharmacology of *Tridax procumbens* a Weed, Review. International Journal of Pharm. Tech. Research, 2(2): 1391-1394.

16. Ogunwenmo KO, Idowu OA, Innocent C, Esan EB, and Oyelana OA (2007): Cultivars of *Codiaeum variegatum* (L.) Blume (Euphorbiaceae) show variability in phytochemical and cytological characteristics. Journal of. Biotechnology, 20: 2400-2405.
17. procu
18. Sahu AR, Behera N and Mishra SP (2010): Use of Ethnomedicinal Plants by Natives of Bargarh District of Orissa, India. Ethnobotanical Leaflets, 14: 889-910.
19. Sahu AR, Nayak AK and Panigrahi SK (2013): Survey of some important ethno-medicinal plants of Sohela Block, Western Odisha, India. Life Sciences Leaflets, 11(11): 1-9.
20. Sahu AR, Sahu M, Nayak NR and Sahoo TR (2020): Medicinal Plants of Saraswati +3 Science College, Bhawanipatna Campus and it's adjacent areas, Kalahandi district, South-Western Odisha, India. World Journal of Pharmacy and Pharmaceutical Sciences, 9 (1): 1738-1752 (DOI: 10.20959/wjpps20201-15408)
21. Sowmya BJ, Gajdhane SB, Kasabe PJ, Bhagwat PK, and Dandge P (2015): Phytochemical screening and in vitro antimicrobial activity of *Tridax procumbens* L. Research Journal of Life sciences, Bioinformatics, Pharmaceutical, and Chemical Sciences, 1(1): 42-53.
22. Taddei A, and Rosas-Romero AJ (2000): Bioactivity studies of extracts from *Tridax procumbens*. Phytomedicine.7(3): 235-238. doi: 10.1016/S0944-7113(00)80009-4.
23. Tejaswini K, Vishwanath P, Rudramani D, Shylaja S, and Jyothsna K (2011): Phytochemical screening and antimicrobial activities of plant extract of *Tridax procumbens*. The Bioscan, 6(2): 321-323.
24. Varsharani I, Pravin C, and Sushma K (2022): Phytochemistry and pharmacological aspects of *Tridax procumbens* (L.): A systematic and comprehensive review. Phytomedicine Plus, 2(1): 100199. https://doi.org/10.1016/j.phyplu.2021.100199
25. Zambare AV, Chakraborthy GS and Banerjee SK (2010): Pharmacognostic Studies of Potential Herb – *Tridax Procumbens* Linn, International Journal of Pharmaceuical Sciences and Research, 1Research, 1(9): 58-62.

NATURAL IMMUNE-BOOSTING HERBS: A GUIDE TO HERBAL REMEDIES

Niyati Shah*, Mamta Kumari, Piyushkumar Sadhu,
Chitrali Talele and Dillip Kumar Dash
Department of Pharmacy,
Sumandeep Vidyapeeth Deemed to be University, Piparia, Vadodara-391760, Gujarat.
*Corresponding author E-mail: niyatishah25594@gmail.com

Abstract:

In an era marked by increased health consciousness and a growing interest in natural remedies, "Natural Immune-Boosting Herbs: A Guide to Herbal Remedies" emerges as a comprehensive resource for those seeking to enhance their immune system through the power of herbs. This chapter offers a detailed exploration of the world of herbal remedies, shedding light on their vital role in fortifying the body's defense mechanisms. It not only provides insight into the historical significance of herbal medicine but also shows the relevance of these age-old practices in the modern world. The chapter features an array of herbs known for their immune-boosting properties. It provides in-depth information about each herb's active compounds and mechanisms of action, empowering with the knowledge to make informed choices in their pursuit of better health. "Natural Immune-Boosting Herbs" emphasizes the importance of a holistic approach to health. It underscores that herbal remedies are most effective when integrated into a well-rounded lifestyle that includes a balanced diet, regular physical activity, and adequate rest."Natural Immune-Boosting Herbs" is a guiding light for those who believe in the potential of herbs to contribute to a stronger, more resilient immune system, paving the way to a healthier and more vibrant life.

Keywords: Natural Healing, Bioactive Compounds, Immune-Boosting, Holistic Wellness

Introduction:

In an era where safeguarding our health has become a top priority, the role of herbal remedies in strengthening our immune systems has gained significance. The quest for natural, effective ways to enhance our body's defense mechanisms has led to a resurgence of interest in traditional healing practices and plant-based solutions. Herbal remedies have been a cornerstone of holistic healthcare for centuries, with diverse cultures harnessing the therapeutic power of plants to combat illness and maintain vitality. While the modern medical landscape continues to advance, herbal remedies remain an integral part of the complementary and alternative health toolkit. These remedies offer a natural and time-tested approach to supporting the immune system, often with fewer side effects than pharmaceutical alternatives."Essential Herbal

Remedies to Boost Immunity" sheds light on the vital role that nature's pharmacopeia plays in fortifying our immune defenses. It explores a curated selection of herbs, each chosen for its potential to enhance immunity, combat infections, and promote overall health. Immune health is not solely about what we ingest it's also about how we live. It emphasizes the value of a well-rounded approach, combining herbal remedies with a nutritious diet, regular exercise, adequate sleep, and stress management for optimal results.

A few significant herbs which are much of the time utilized to improve immunity.

***Ocimum sanctum* (Tulsi)**

In the Indian language, *Ocimum sanctum* is called ‘tulsi’ or in the English language, it is called ‘holy basil’. Several portions of *Ocimum sanctum* have been utilized in medicine to treat conditions like chronic fever, skin problems, and respiratory issues, Insect bite and joint ache. Prior studies additionally claimed *Ocimum sanctum* to possess anti fertility properties, antimicrobial, non-mutagenic, glucose, liver, and heart preservation, antitoxin, and analgesic, stimulatory as well as attributes. Ocimun sanctum leaves are a good remedy against common colds. The essential components of oils obtained by steam distillation from *Ocimum sanctum* leaflets are carvacrol, methyl eugenol, eugenol, and caryophyllene. However, Eugenol is the oil's most effective constituent and the reason occimum sanctum is used medicinally.

***Zingiber officinale* (Ginger)**

It is commonly known as Ginger belongs to the genus Zingiber from Zingiberaceae. According to reports, it possesses biological properties such as antibacterial, anti-inflammatory, anticancer, and antioxidant properties. It may be used to prevent and treat a wide range of illnesses, including induced nausea, headaches, diabetes mellitus, obesity, respiratory conditions, and emesis. Patients with chronic immune-mediated inflammatory illnesses are more susceptible to infection because chronic inflammation reduces immunological response, which raises the risk of disease. Its potent anti-inflammatory and antibacterial qualities aid in lowering inflammation, which in turn strengthens the immunological response. Because of these anti-inflammatory qualities, it can be utilized to strengthen immunity against Covid-19.

***Allium sativum* (Garlic)**

It is commonly referred to as garlic because the most prevalent ingredient contains metabolites that are antioxidants. It contains sulfur-containing amino acids and other substances that, according to numerous scholars, not only boost immune system activity but also the body's antiviral defenses against a variety of viruses, including influenza B and simplex. Numerous things, including as bodily harm, psychological stress, poor diet, chemical pollution, and extreme strain, are constantly upsetting the immune system. Nutrients like garlic, which can support immune system function and maintain health against infectious diseases, can make up for it.

***Glycyrrhiza glabra* L. (Licorice)**

In China, licorice is a common herb that is traditionally used for medicinal purposes. According to research, the therapeutic properties of licorice are attributed to its nearly twenty triterpenoids and three hundred flavonoids. These constituents demonstrate attributes such as antiviral, antimicrobial, anticancer, and anti-inflammatory capabilities.

The liquorice plant's roots and rhizomes are the sections that are most frequently utilized in medicine. Due to its exceptional properties, liquorice attracted a lot of attention in this context. With the increasing development of viral and microbial infections in many nations, it is imperative to produce some antiviral or antibacterial drugs.

Glycyrrhizic acids (GL), 18beta-Glycyrrhetinic acid (GA), 7,4'-Dihydroxy flavanone (LTG), chalconoid (LCA), retrochalcone, and glabridin are the active ingredients in licorice flavonoids and triterpenoids that combat viral infections.

***Camellia sinensis* (Green Tea)**

Green tea has been used for medicinal and pharmacological purposes for over 5000 years. Green tea's primary chemical components are polyhydroxybenzoids, caffeine, and protein components. Flavonoids are believed to have antioxidant qualities that have a number of positive effects, including reducing inflammation and serving as a disinfectant.

The photochemical epigallocatechin-3-gallate (EGCG), which is a latent component of green tea, is also evaluated by the latest inquiry. EGCG has a wide range of activities, including accelerating immune response durability, reducing nonspecific antibody synthesis, lightening oxidative and inflammatory stress, and accelerating the regularity of the circulatory system.

***Cassia angustifolia* (Sennamakkahi)**

Sennamakkhi has many benefits, which make it widely utilised. It is a member of the Leguminosae family. The traditional medicinal usage of *Cassia angustifolia* is widespread in China, India, Pakistan, Africa, and additionally in the allopathic medical system of the West. It is mostly used to treat depression; gastrointestinal disorders, skin conditions, and respiratory illnesses. The dried leaves of *Cassia angustifolia* are powdered into a powder and used to treat respiratory ailments.

Citrus and Limon (Lemon)

From the Rutaceae family; Limon (L.) and *Citrus Burma* F. is a bush with evergreen leaves and tasty golden fruits. The essential juices and oils taken from this pod's unprocessed raw material. Such Fruits have incredibly high nutritional content; however it is noteworthy because of its beneficial biological processes. According to Davis, vitamin C builds up. Citrus fruits, which supports the body's inflammatory reaction by turning on the mechanism that delivers vitamin C to cells during stressful moments. Vitamin C is necessary for the best possible growth

for maintenance as well as having immune-modulating properties. A powerful antioxidant, vitamin C can strengthen your body's defense mechanisms. Studies have indicated that an elevated intake of vitamin C can increase the quantity of antioxidant enzymes in bodily fluids by more than 30%. This strengthens your body's defenses against oxidative stress and shields you from both acute and long-term illnesses. There is evidence to show that fish immune systems are significantly impacted by oil produced from citrus lemons. Citrus fruits have antioxidant properties, while citrus peel oils have potent antibacterial properties.

Conclusion:

The world of essential herbal remedies for boosting immunity is a valuable and age-old resource that offers a holistic approach to fortifying our body's natural defense mechanisms. It provides insights into a variety of herbs, each with its unique immune-enhancing properties and rich historical backgrounds. Herbal remedies like Echinacea, Elderberry, Garlic, Astragalus, and many others have been explored, shedding light on their potential to support and strengthen the immune system. These natural allies from the plant kingdom, with their bioactive compounds and therapeutic benefits, offer an alternative or complementary approach to conventional medicine. However, it's essential to remember that immune health is not achieved through herbs alone. A holistic approach to well-being, which combines these remedies with a balanced diet, regular physical activity, adequate sleep, and stress management, is crucial. Our immune system thrives when the entire body is in balance, and these practices play a significant role in achieving that balance. By incorporating these essential herbal remedies into our daily lives and recognizing their role as complementary components in our overall health regimen, we have the potential to achieve a stronger and more resilient immune system. To harness the benefits of nature to enhance their immunity and overall well-being. It encourages individuals to explore the age-old remedies that have stood the test of time and to become more self-sufficient in managing their health. In a world where our well-being is of utmost importance, the wisdom of essential herbal remedies acts as guiding light, guiding us toward a healthier, more vibrant life. It is our hope that this guide has provided valuable insights and inspired you to explore the healing power of nature as you embark on your journey to optimal health and wellness.

References:

1. Abdullah, T.H., Kandil, O., Elkadi, A., & Carter, J. (1988). Garlic revisited: therapeutic for the major diseases of our times. *Journal of the National Medical Association, 80*(4), 439.
2. Abuajah, C.I., Ogbonna, A.C., & Osuji, C.M. (2015). Functional components and medicinal properties of food: a review. *Food Science and Technology*, *52*, 2522-2529.
3. Abu-Reidah, I.M., Ali-Shtayeh, M.S., Jamous, R.M., Arráez-Román, D., & Segura-Carretero, A. (2015). HPLC–DAD–ESI-MS/MS screening of bioactive components from Rhus coriaria L.(Sumac) fruits. *Food Chemistry, 166,* 179-191.

4. Akinyemi, A.J., Thome, G.R., Morsch, V.M., Stefanello, N., Goularte, J.F., Belló-Klein, A., Oboh, G., & Schetinger, M.R. (2015). Effect of dietary supplementation of ginger and turmeric rhizomes on angiotensin-1 converting enzyme (ACE) and arginase activities in L-NAME induced hypertensive rats. *Journal of Functional Foods, 17*, 792-801.
5. Azhir, D., Zakeri, A., & Kargare-Rezapour, A. (2012). Effect of ginger powder rhizome on humoral immunity of broiler chickens. *Journal of Experimental Biology, 2*(6), 2090-2092.
6. Baba, E., Acar, Ü., Öntaş, C., Kesbiç, O.S., & Yılmaz, S. (2016). Evaluation of Citrus limon peels essential oil on growth performance, immune response of Mozambique tilapia Oreochromis mossambicus challenged with Edwardsiella tarda. *Aquaculture, 465*, 13-18.
7. Bagwe, A., Bangi, S., Nete, S., Maiti, S., & Ragade, V. (2019). Evaluation of Cassia angustifolia Vahl as an immunomodulatory agent. *World Journal of Pharmacy and Pharmaceutical Sciences*, 107-113.
8. Calder, P.C., & Kew, S. (2002). The immune system: a target for functional foods. *British Journal of Nutrition, 88*(S2), S165-S176.
9. Schett, G., Sticherling, M., & Neurath, M.F. (2020). COVID-19: risk for cytokine targeting in chronic inflammatory diseases. *Nature Reviews Immunology, 20*(5), 271-272.
10. Sharangi, A.B. (2009). Medicinal and therapeutic potentialities of tea (Camellia sinensis L.)–A review. *Food Research International, 42*(5-6), 529-535.
11. Schett, G., Sticherling, M., & Neurath, M.F. (2020). COVID-19: risk for cytokine targeting in chronic inflammatory disease. *Nature Reviews Immunology, 20*(5), 271-272.
12. Salman, H., Bergman, M., Bessler, H., Punsky, I., & Djaldetti, M. (1999). Effect of a garlic derivative (alliin) on peripheral blood cell immune responses. *International Journal of Immunopharmacology, 21*(9), 589-597.
13. Sharma, R., Sharma, A., Kumari, A., Kulurkar, P.M., Raj, R., Gulati, A., & Padwad, Y.S. (2017). Consumption of green tea epigallocatechin-3-gallate enhances systemic immune response, antioxidative capacity and HPA axis functions in aged male Swiss albino mice. *Biogerontology, 18*, 367-382.
14. Zou, Z., Xi, W., Hu, Y., Nie, C., & Zhou, Z. (2016). Antioxidant activity of Citrus fruits. *Food Chemistry, 196*, 885-896.
15. Wang, L., Yang, R., Yuan, B., Liu, Y., & Liu, C. (2015). The antiviral and antimicrobial activities of liquorice, a widely-used Chinese herb. *Acta Pharmaceutica Sinica B, 5*(4), 310-315.

Research and Reviews in Plant Science Volume II (ISBN: 978-93-95847-47-6)

About Editors

Dr. Madhu Prakash Srivastava is working as an Associate Professor in the Department of Botany, Maharishi University of Information Technology, Lucknow UP, India. He has successfully completed his Ph.D (Botany) from the Department of Botany at Lucknow University, Lucknow. He is actively engaged in teaching and research in the arena of Botany and Plant Pathology for the last 10 years. His area of specialization is Mycology, Plant Pathology and Biological Formulation development. He has shown excellent performance during research work and teaching. He has presented more than 40 papers in National /International seminars and conferences. He has 38 research papers published in National /International journals with impact factor and also co-authored book chapters to his credit. He is a member of various societies, and several National and International organizations and is on the editorial board of various journals. Dr. Srivastava widely travelled both nationally and state level is an academically acclaimed expert in her field. Based on these finding certain scientific recognition as Best Teacher Award (2019), Young Scientist Award (2019), Young Environmentalist Award (2019), Vishisht Nagrik Sammaan (2019), Young Scientist Award (2018), Ruchi Ram Sahni Award (2012), Best Poster award (2017, 2018) have also been granted.

Dr. Meenakshi Arjunrao Bangar is working as Associate Professor and Head, at Department of Botany, Netaji Subhashchandra Bose, Arts Commerce and Science College, Nanded. Dist. Nanded. (Maharashtra). She has more than 16 years of teaching experience at undergraduate level. She was awarded with Ph. D. in 2003 by Dr. Balasaheb Ambedkar Marathwada University Aurangabad. Her research topic for esearch was “Morphological Studies In Some Verbenaceae”. One Research Student is working under her guidance. She has published more than 16 research papers in research journals. Dr. Bangar has presented two papers in international Conferences (Online). She has published 5 Articles in Books. She was awarded with two Best Teacher and Role Model Teacher in 2019 and 2020 respectively.

Dr. Devangi Chachad, born in August 1979, serves as an Associate Professor at Jai Hind College (Empowered Autonomous) in Churchgate, Mumbai. With over 23 years of teaching experience, she earned her Bachelor's (1999) and Master's (2001) degrees in Botany from Mithibai College and obtained her Ph.D. from K.V. Pendharkar College in 2005. Dr. Chachad boasts a portfolio of more than 20 research papers published in journals indexed in Scopus, Web of Science, UGC CARE, and other peer-reviewed publications. Additionally, she has contributed chapters to various books. Her noteworthy achievements include the 2015 Joseph Lister Award in recognition of her work in ethnobotany. Acknowledged as a reputable Ph.D. mentor, she continues to guide students for their research work. Furthermore, she was awarded the fellowship from the Indian Botanical Society. Her research pursuits primarily focus on Ethnobotany, Phytochemistry, Pharmacognosy, and Pharmacology.

Dr. Averineni Ravi Kumar is working as Professor, Department of Pharmacognosy in Nimra College of Pharmacy, Vijayawada, Andhra Pradesh. He has Completed Doctorate in Biotechnology from Bharathidasan University, Tiruchchirapalli, Tamil Nadu in 2009 on thesis Entitled" Ethnopharmacological Studies of Selected Plants of Caesalpiniaceae". He has Obtained M.Pharmacy Specialization in Pharmacognosy Secured Highest Marks in Final Semester in Completion and B.Pharmacy Degrees from A U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh. He has About 31 Years of Teaching Experience. He has worked as Lecturer to Dean Positions in Pharmacy Colleges he has worked. He has Participated in Webinars as Organizer, Guest Speaker, Attended Workshops QIP in Faculty Development Programmes and Presented Papers in Various Sessions. He is Life Member of IPA OPF APTI ABAP. He has Published about more than 100 Research Papers in National and International Journals. He is Author of Books of Industrial Pharmacy II, Biopharmaceutic and Pharmacokinetics, Pharmaceutical Jurisprudence and Pharmacognosy I & II & Cosmetic Science He has about 2 Patents Works on Herbs.

Bhumi Publishing

ISBN 978-93-95847-47-6

9 789395 847476