Content available at: https://www.ipinnovative.com/open-access-journals

Journal of Pharmaceutical and Biological Sciences

Journal homepage: https://www.jpbs.in/

Original Research Article

Isolation and identification of endophytic fungi from *Ocimum sanctum*

Renu Namdev[1], **Preeti Dass**[1]*, **Kanchan Khare**[2]

[1]*School of Studies in Botany, Vikram University, Ujjain, Madhya Pradesh, India*
[2]*Dept. of Applied Chemistry, Jabalpur Engineering College, Jabalpur, Madhya Pradesh, India*

ARTICLE INFO

Article history:
Received 10-05-2024
Accepted 07-06-2024
Available online 08-08-2024

Keywords:
Endophytic fungi
Ocimum sanctum
Isolation
Identification
Bioactive compounds

ABSTRACT

Aim: In this study, we aimed to isolate and identify endophytic fungi from the Ocimum sanctum plant, commonly known as Holy Basil and Hindi name- Tulsi.
Objective: 1. To investigate the endophytic mycobiota from Ocimum sanctum. 2. To isolate and identify some endophytic fungi in selected medicinal plant. 3. To study phenotypic characteristics of endophytic fungi in selected medicinal plant.
Materials and Methods: Methods employed for the isolation and identification of endophytic fungal strains encompassed a multi-step approach, involving surface sterilization, tissue maceration, and subsequent fungal isolation. Morphological and microscopic characterization were utilized to identify the isolated fungal strains. As a result, a total of five distinct endophytic fungal strains were successfully isolated and identified.
Result: The results revealed a high diversity of endophytic fungi associated with Ocimum sanctum. Furthermore, several potential bioactive compounds were detected in the isolated fungi, further emphasizing their importance.
Conclusion: This study contributes to the knowledge of endophytic fungi associated with Ocimum sanctum and provides a foundation for further investigation into their biotechnological potential.

1. Introduction

India's abundance of various plant species has led some to believe that it is a major centre of biodiversity. More than 3/4 of the herbal medications and fragrance products used worldwide are produced in India. Therefore, India's vast and diversified plant diversity, particularly the genetic diversity of medicinal and aromatic plants, is one of its primary strengths and the cornerstone for all prospective bio-industrial breakthroughs. One of the world's twelve mega diversity countries, India has a wide variety of biotic resources. In India, one of the countries with the most diverse spectrum of medical and cultural traditions in the world, the medicinal plant industry has a long history and is still held in high esteem today.[1–6]

Endophytic fungi are ubiquitous inhabitants of plants, inhabiting their internal tissues without causing any apparent disease symptoms. These microorganisms form symbiotic relationships with their host plants, providing various benefits such as improved nutrient uptake, enhanced stress tolerance, and protection against pathogens (Alexopoulos, et al. 2007). Furthermore, endophytic fungi have been found to produce a wide range of bioactive secondary metabolites with diverse chemical structures and biological activities (Chandra, 2012). These bioactive compounds have garnered significant attention due to their potential use in medicine, agriculture, and industry, highlighting the importance of exploring the complex

* *Corresponding author*.
E-mail address: dasspreeti@hotmail.com (P. Dass).

https://doi.org/10.18231/j.jpbs.2024.006

relationships between plants and their endophytic fungal associates.

Ocimum sanctum, also known as (Common name) Holy Basil or (Hindi name) Tulsi, is a revered medicinal plant with a rich history in traditional medicine systems across many cultures. This plant possesses numerous pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, and anticancer activities, making it a valuable resource for the development of novel therapeutic agents (Chutulo and Chalannavar, 2018). Despite its prominence in traditional medicine, the associated endophytic fungi of Ocimum sanctum remain largely unexplored, leaving a significant gap in our understanding of their potential for producing bioactive compounds. This study aims to bridge this knowledge gap by isolating and identifying endophytic fungi from Ocimum sanctum and analysing their bioactive compound production capabilities.[7–11]

2. Materials and Methods

2.1. Sample acquisition

To ensure a comprehensive representation of genetic diversity, plant samples were meticulously collected from various locations, with a focus on selecting healthy and disease-free Ocimum sanctum specimens. Leaves were carefully harvested, avoiding any visible signs of damage or disease, to minimize contamination and maximize the integrity of the plant material. The plant material was subsequently subjected to a rigorous washing process under running tap water to eliminate any surface debris and particulate matter, thereby rendering the samples suitable for subsequent sterilization procedures.[12–15]

2.2. Surface sterilization

In order to eradicate epiphytic microorganisms that may have been present on the surface of the plant samples, a multi-faceted approach was employed, utilizing a combination of antiseptics, including ethanol, sodium hypochlorite, and hydrogen peroxide. The sterilization protocols were meticulously optimized to minimize any potential damage to the plant tissue, thereby preserving the integrity and functionality of the samples. This rigorous decontamination process ensured that the plant samples were free from microbial contaminants, thereby providing a reliable foundation for subsequent scientific analysis and experimentation.

2.3. Tissue maceration and fungal isolation

Following thorough surface sterilization, the plant material underwent careful dissection into small sections. These sections were then carefully transferred to sterile petri dishes that were filled with Potato Dextrose Agar (PDA) supplemented with antibiotics to prevent contamination. The plant tissues were delicately macerated using a sterile mortar and pestle, breaking down the cells to release any potential fungal spores or hyphae. The resulting extract was then carefully spread onto the PDA plates to encourage the growth of individual, pure fungal colonies for further analysis.

2.4. Incubation and observation

The prepared plates were moved to an incubator set at a controlled temperature range of 25^0C to 28^0 C. This specific temperature range was selected as it is optimal for the growth of endophytic fungi. Over the course of 5 to 7 days, observations were consistently carried out to closely monitor the development of fungal growth on the plates as well as to note any distinctive colony characteristics such as color, texture, and size. This diligent observation process allowed for the identification and documentation of the different fungal species present in the samples.

3. Sub-culture and Isolate Purification

Following the incubation period and the observation of fungal growth, promising fungal isolates exhibiting distinct morphological characteristics in terms of color, texture, and growth rate were carefully selected for further analysis. These selected isolates were then sub-cultured onto fresh media to ensure their purity and to prevent any potential contamination from other microorganisms (George, et al. 2011). This sub-culturing process involved transferring a portion of the isolated fungal colonies onto new agar plates, providing them with a fresh environment to thrive and grow without interference.

4. Morphological Characterization

Once the selected fungal isolates were successfully sub-cultured onto fresh media, a detailed morphological characterization was conducted to gain a deeper understanding of their physical attributes. The isolated fungal colonies were closely examined for various morphological features including colony color, texture, growth rate, and spore production patterns. To further investigate the structural details of the fungal isolates, slide preparations were meticulously created using lactophenol cotton blue stain. These prepared slides allowed for a detailed microscopic examination of the fungal hyphae and spores present within the isolates. The slides were then carefully observed under both 10X and 40X objective lenses to capture a more detailed view of the fungal structures and identify any unique characteristics. Standard identification manuals were utilized for accurate identification (Barnett and Hunter, 1998).

Figure 1: Surface sterilization

Figure 2: Collection of plant sample

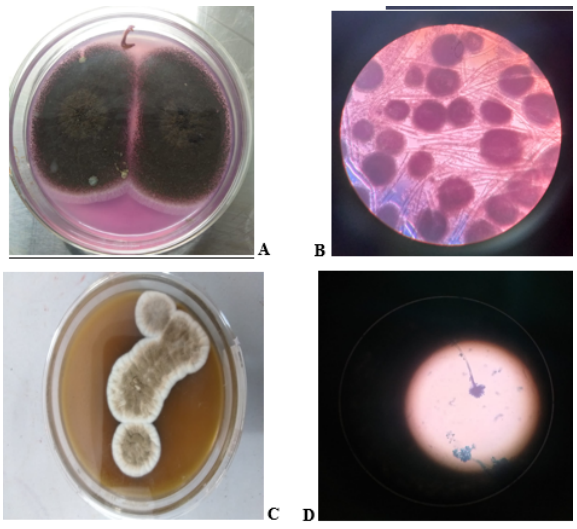

Figure 3: Isolated and identified Endophytic Fungi; **A** (Macroscopic view), **B** (Microscopic view) = Aspergillus niger; **C** (Macroscopic view), **D** (Microscopic view) = Aspergillus versicolor.

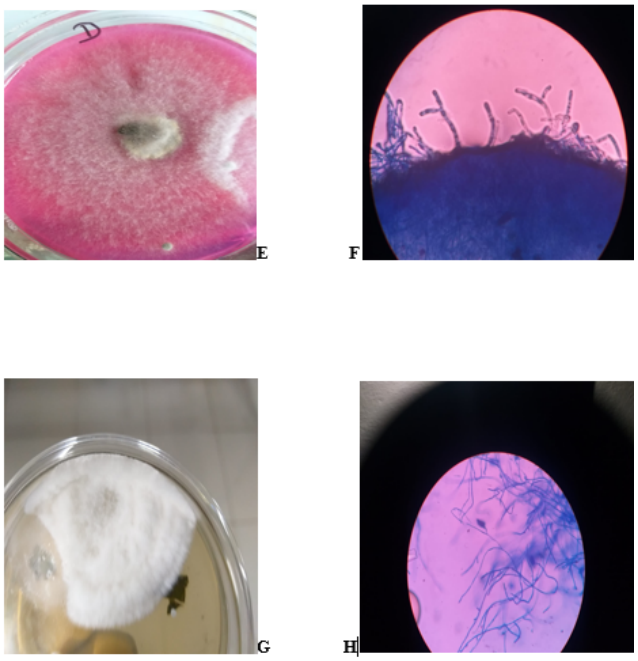

Figure 4: Isolated and identified Endophytic Fungi.; **E** (Macroscopic view), **F** (Microscopic view) = Alternaria spp.; **G** (Macroscopic view), **H** (Microscopic view) = Fusarium solani.

5. Results and Discussion

A comprehensive examination of the internal tissues of *Ocimum* sanctum yielded a total of five endophytic fungal strains, each exhibiting a distinct set of morphological characteristics. The morphological analysis revealed a diverse range of colony colours, textures, and spore morphologies, indicative of the presence of multiple fungal species, including *Aspergillus* spp., *Fusarium* solani, and Alternaria. These findings suggest a high degree of fungal diversity within the plant, potentially harboring novel species or strains that may have evolved in symbiosis with the plant.

Furthermore, the isolated endophytic fungi demonstrated a remarkable capacity for producing bioactive compounds, showcasing a range of biochemical activities. Preliminary screening assays revealed the presence of bioactive compounds possessing antimicrobial, antioxidant, and antitumor properties, thereby underscoring the potential of these endophytic fungi as sources of novel bioactive compounds. The discovery of these compounds highlights the significance of endophytic fungi associated with *Ocimum* sanctum in the pursuit of novel therapeutic agents and bioproducts.

6. Conclusion

The comprehensive study of endophytic fungi inhabiting the medicinal plant Ocimum sanctum has yielded a

plethora of significant findings, shedding light on the vast diversity of these microorganisms. The study's discovery of novel bioactive compounds within these endophytic fungi underscores the importance of investigating these microorganisms for their potential applications in pharmaceuticals and agriculture (Gond, et al. 2007). The detection of bioactive compounds in these fungi has far-reaching implications for the development of novel pharmaceuticals, crop protection agents, and other bioproducts.

This study's findings significantly expand our understanding of the fungal diversity associated with Ocimum sanctum, laying the groundwork for future research into the biotechnological potential of these microorganisms. Furthermore, the elucidation of mechanisms of action and optimization of bioactive compound production are crucial steps towards unlocking their full therapeutic and agronomic potential. This study makes a substantial contribution to the field of endophytic fungi research, highlighting the importance of Ocimum sanctum as a rich source of diverse and potentially valuable bioactive compounds. The discovery of these novel compounds has significant implications for the development of novel pharmaceuticals, crop protection agents, and other bioproducts. Ultimately, this study underscores the need for continued research into the endophytic fungi associated with medicinal plants, as well as the potential benefits that can arise from their exploitation. In conclusion, this study's findings have significant implications for the development of novel pharmaceuticals, crop protection agents, and other bio products. The comprehensive study of endophytic fungi inhabiting Ocimum sanctum has provided valuable insights into the diversity and potential applications of these microorganisms. Future research is warranted to further elucidate the mechanisms of action and optimize the production of bioactive compounds, thereby unlocking their full therapeutic and agronomic potential.

7. Source of Funding

None.

8. Conflict of Interest

None.

References

1. Mims ACJ, Blackwell CW. Introductory mycology. and others, editor. Wiley India (P) Ltd; 2007. p. 1–10.
2. Barnett HL, Barry H. Illustrated Genera of Imperfect Fungi-. and others, editor. Burgess Publishing Company; 1998. p. 94–130.
3. Chandra S. Endophytic fungi: novel sources of anticancer lead molecules. *Appl Microb Biotec*. 2012;95(1):47–59.
4. Chutulo EC, Chalannavar R. Endophytic mycoflora and their bioactive compounds from Azadirachta indica: A comprehensive review. *J Fungi*. 2018;4(2):1–12.
5. Gary S, Bryn D. Bioprospecting for microbial endophytes and their natural products. *Microb Mol Biol Revi*. 2003;67(4):491–502.
6. George T, Kulanthai SS, Vasanthi SN, Kannan KP. Extraction, purification and characterization of chitosan from endophytic fungi isolated from medicinal planta. *World J Sci Technol*. 2011;1(4):43–8.
7. Gond SK, Verma VC, Kumar A, Kumar V, Kharwar RN. Study of endophytic fungal community from different part of Aegle marmelos. Correa (Rutaceae) from Varanasi (India). *World J Microb Biotech*. 2007;23(10):1371–5.
8. Gunasekaran S, Sundaramoorthy S, Anitha U, Sathiavelu M, Sathiavelu A. Endophytic fungi with antioxidant activity- A review. *Res J Pharm Technol*. 2015;8:731–7.
9. Haiyan L, Qing C, Zhang Y, Zhao Z. Screening for endophytic fungi with antitumour and antifungal activities from Chinese medicinal plants. *World J Microb Biotech*. 2005;21:1515–9.
10. Jalgaonwala RE, Vishwas B, Raghunath M, Mahajan T. A review: Natural products from plant associated endophytic fungi. *J Microb Biotech*. 2011;1(2):21–32.
11. Khan R, Shahzad S, Choudhary IM, Khan SH, Ahmad A. Communities of endophytic fungi in medicinal plant Withania somnifera. *Pak J Bot*. 2010;42(2):1281–90.
12. Kharwar NR, Verma CV, Strobel G, David E. The endophytic fungi complex of Catharanthus roseus (L.). *Curr Sci*. 2008;78:228–33.
13. Maheshwari R, Varahalarao V, Ramars A, Suryanarayana MU. Endophytic Fungi as Novel Resources of natural Therapeutics. *Braz Arch Biol Tech*. 2017;60:1–26.
14. Naik SB, Shashikala J, Krishnamurthy YL. Diversity of fungal endophytes in shrubby medicinal plants of malnad region. *J Fung Ecol*. 2008;1(2-3):89–93.
15. Rajagopal K, Suryanarayan TS. Isolation of endophytic fungi from leaves of neem (Azadirachta indica). *Curr Sci*. 2000;78(11):1375–8.

Author biography

Renu Namdev, Research Scholar https://orcid.org/0009-0001-8443-4126

Preeti Dass, Assistant Professor

Cite this article: Namdev R, Dass P, Khare K. Isolation and identification of endophytic fungi from *Ocimum sanctum*. *J Pharm Biol Sci* 2024;12(1):38-41.