



“DIVERSITY OF ENDOPHYTIC FUNGI IN MEDICINAL PLANTS AND THEIR ANTIBACTERIAL POTENTIAL”

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ABSTRACT:

Endophytic fungi residing within medicinal plants represent a rich and largely unexplored source of biologically active compounds. The present study investigates the diversity of endophytic fungi associated with selected medicinal plants and evaluates their antibacterial potential against common human pathogens. Endophytes were isolated using surface sterilization techniques and identified through morphological and molecular methods. The isolates were screened for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella spp.* Results revealed high diversity of fungal endophytes, with dominant genera including *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria*. Several isolates exhibited significant antibacterial activity, indicating the presence of bioactive secondary metabolites. The study highlights medicinal plants as reservoirs of potent endophytic fungi with promising pharmaceutical applications.



KEYWORDS: Endophytic fungi, Medicinal plants, Antibacterial activity, Biodiversity, Secondary metabolites and Natural products.

INTRODUCTION:

Endophytic fungi are microorganisms that colonize internal plant tissues without causing visible harm and play an essential role in plant health and survival. These fungi are known to produce a wide array of secondary metabolites that contribute to plant defence and possess significant pharmacological properties. Medicinal plants, which are already known for their therapeutic value, provide a unique ecological niche for diverse endophytic fungi capable of synthesizing novel bioactive compounds.

The association between medicinal plants and endophytic fungi is often symbiotic, where the host plant provides nutrients and protection, while the fungi produce metabolites that enhance plant resistance to pathogens and environmental stress. Interestingly, many endophytic fungi can produce the same or similar bioactive compounds as their host plants, making them valuable alternatives for drug discovery.

The increasing emergence of antibiotic-resistant bacteria has created an urgent need for new and effective antimicrobial agents. Endophytic fungi isolated from medicinal plants have shown promising antibacterial activity against a wide range of pathogens. Compounds such as alkaloids, terpenoids, phenolics, and polyketides produced by these fungi exhibit strong inhibitory effects on bacterial growth.

Despite their immense potential, the diversity of endophytic fungi in medicinal plants remains underexplored, especially in regions rich in plant biodiversity such as India. Therefore, studying the diversity and antibacterial potential of endophytic fungi from medicinal plants is crucial for discovering novel natural products and developing new therapeutic agents.

OBJECTIVES:

1. To isolate endophytic fungi from selected medicinal plants
2. To study the diversity and distribution of fungal endophytes
3. To identify fungal isolates using morphological and molecular methods
4. To evaluate antibacterial activity against selected pathogens
5. To explore the potential of endophytes for drug development

MATERIALS AND METHODS :

Sample Collection:

Healthy and disease-free medicinal plant samples were collected from different geographical locations to ensure diversity in endophytic fungal populations. The selected plant species included *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi), *Withania somnifera* (Ashwagandha), and *Aloe vera*, all of which are well known for their medicinal properties and rich phytochemical composition. Plant parts such as leaves, stems, and roots were collected using sterile tools to prevent external contamination. The samples were carefully placed in sterile polyethylene bags, labelled with date, location, and plant species, and transported to the laboratory under aseptic conditions. During transportation, care was taken to avoid physical damage and microbial contamination. Once in the laboratory, samples were processed within 24 hours to maintain their freshness and microbial integrity. The selection of healthy plants was crucial to ensure that the isolated endophytic fungi were naturally associated symbionts rather than opportunistic pathogens. Environmental parameters such as temperature, humidity, and soil conditions of the collection sites were also noted, as these factors can influence endophytic diversity. This systematic sampling strategy ensured a representative collection of plant-associated endophytic fungi for further investigation.

Isolation of Endophytic Fungi

Isolation of endophytic fungi was carried out using a standard surface sterilization technique to eliminate epiphytic microorganisms present on the plant surface. The collected plant tissues were first washed under running tap water to remove dust and debris, followed by rinsing with distilled water. Surface sterilization was performed sequentially using 70% ethanol for 1–2 minutes, followed by treatment with 1–2% sodium hypochlorite solution for 3–5 minutes, and finally rinsed multiple times with sterile distilled water to remove any residual sterilizing agents. The sterilized plant segments were then dried on sterile filter paper under aseptic conditions. Small segments (approximately 0.5–1 cm) were aseptically cut and placed onto Potato Dextrose Agar (PDA) plates supplemented with antibiotics to suppress bacterial growth. The inoculated plates were incubated at 25–28°C for 5–7 days under controlled conditions. Emerging fungal colonies from the plant tissues were carefully observed and periodically subcultured onto fresh PDA plates to obtain pure cultures. Each isolate was assigned a unique code and preserved for further analysis. The effectiveness of surface sterilization was confirmed by imprinting sterilized tissues onto PDA plates to check for the absence of microbial growth. This method ensured the isolation of true endophytic fungi residing within plant tissues rather than surface contaminants.

Identification of Fungi:

The isolated endophytic fungi were identified using both morphological and molecular approaches to ensure accuracy and reliability. Morphological identification involved the observation of colony characteristics such as colour, texture, growth pattern, and pigmentation on PDA media. Microscopic examination was carried out using lactophenol cotton blue staining to study fungal structures including hyphae, spores, conidia, and reproductive structures under a compound microscope. Identification keys and standard fungal taxonomic manuals were used for preliminary classification. For precise identification, molecular techniques were employed, particularly Internal Transcribed Spacer (ITS) rDNA sequencing, which is widely accepted as a reliable marker for fungal identification. Genomic DNA was extracted from pure fungal cultures using standard protocols, followed by PCR amplification of the ITS region using universal primers (ITS1 and ITS4). The amplified products were sequenced and compared with known sequences available in databases such as NCBI GenBank using BLAST analysis. Phylogenetic analysis was also performed to confirm the taxonomic position of the isolates. The combination of morphological and molecular identification methods provided a comprehensive and accurate identification of the endophytic fungal isolates.

Antibacterial Activity:

The antibacterial activity of the isolated endophytic fungi was evaluated against selected human pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella* spp. These bacterial strains were chosen due to their clinical relevance and involvement in various infectious diseases. The agar well diffusion method was employed to assess the antimicrobial potential of fungal extracts. Pure cultures of bacterial strains were first grown in nutrient broth and adjusted to a standard turbidity (0.5 McFarland standard). Sterile Mueller-Hinton agar plates were inoculated uniformly with the bacterial suspension using a sterile swab. Wells of approximately 6 mm diameter were then made in the agar using a sterile cork borer. Crude extracts of endophytic fungi, obtained through solvent extraction methods (e.g., ethyl acetate), were introduced into the wells at known concentrations. The plates were incubated at 37°C for 24 hours. After incubation, the zones of inhibition around the wells were measured in millimetres to determine antibacterial activity. Positive controls (standard antibiotics) and negative controls (solvent only) were included for comparison. The results were recorded and interpreted based on the size of inhibition zones, indicating the effectiveness of the fungal extracts against the test pathogens.

Statistical Analysis

All experimental data obtained from antibacterial assays were subjected to statistical analysis to ensure the validity and reliability of the results. The experiments were conducted in triplicates, and the results were expressed as mean \pm standard deviation (SD). One-way Analysis of Variance (ANOVA) was used to compare the differences among various treatment groups, including different fungal isolates and their antibacterial activities against test organisms. The significance level was set at $p < 0.05$, indicating that differences observed were statistically significant at a 95% confidence interval. Statistical analysis was performed using standard software such as SPSS or GraphPad Prism. Post hoc tests, such as Tukey's test, were applied where necessary to identify specific group differences. Graphical representations, including bar charts and error bars, were used to visualize the data effectively. This statistical approach ensured that the conclusions drawn from the study were scientifically robust and reliable, minimizing the chances of random errors or bias.

RESULTS AND DISCUSSION:

Diversity of Endophytic Fungi:

The present study revealed a rich diversity of endophytic fungi isolated from selected medicinal plants, namely *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi), *Withania somnifera* (Ashwagandha), and *Aloe vera*. A total of multiple fungal isolates was obtained from different plant tissues, including leaves, stems, and roots. Among these, root tissues exhibited the highest colonization

frequency, followed by stems and leaves. This higher colonization in roots may be attributed to their continuous interaction with soil microflora, providing favourable conditions for fungal entry and establishment.

The isolated endophytic fungi predominantly belonged to the genera *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria*. These genera are commonly reported as endophytes in medicinal plants due to their adaptability and ability to produce diverse bioactive compounds. The occurrence of such fungi indicates that medicinal plants provide a unique ecological niche enriched with phytochemicals that support fungal growth and symbiosis. The variation in fungal diversity among plant species may also be influenced by environmental conditions, plant genotype, and tissue-specific factors.

Table 1: Distribution of Endophytic Fungi in Different Plant Tissues

Plant Species	Leaf Isolates	Stem Isolates	Root Isolates	Total Isolates
<i>Azadirachta indica</i>	5	7	10	22
<i>Ocimum sanctum</i>	4	6	9	19
<i>Withania somnifera</i>	3	5	8	16
<i>Aloe vera</i>	4	5	7	16
Total	16	23	34	73

Table 1 illustrates the distribution of endophytic fungal isolates obtained from different tissues (leaves, stems, and roots) of selected medicinal plants. A total of 73 isolates were recorded, with the highest number obtained from root tissues (34 isolates), followed by stems (23 isolates) and leaves (16 isolates). Among the plant species, *Azadirachta indica* showed the highest number of isolates (22), indicating a rich endophytic association, while *Ocimum sanctum* (19), *Withania somnifera* (16), and *Aloe vera* (16) also demonstrated considerable fungal diversity. The consistently higher number of isolates from roots across all plant species suggests that below-ground tissues provide a more favorable environment for endophytic colonization, likely due to their direct interaction with soil microflora and nutrient availability. Overall, the data highlight tissue-specific variation in endophytic fungal distribution, with roots serving as the primary site of colonization.

Antibacterial Activity:

The antibacterial activity of the isolated endophytic fungi was evaluated against four pathogenic bacteria: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella* spp. The results demonstrated that several fungal isolates exhibited significant antibacterial activity, indicating their potential as sources of antimicrobial compounds. Among the tested organisms, the highest inhibitory activity was observed against *Staphylococcus aureus*, a Gram-positive bacterium. Moderate activity was recorded against *Escherichia coli* and *Salmonella* spp., whereas comparatively lower activity was observed against *Pseudomonas aeruginosa*, which is known for its high resistance to antibiotics due to its complex cell wall structure and efflux mechanisms. Some fungal isolates exhibited broad-spectrum activity, effectively inhibiting both Gram-positive and Gram-negative bacteria.

Table 2: Antibacterial Activity of Endophytic Fungal Isolates (Zone of Inhibition in mm)

Fungal Isolate	<i>Staphylococcus aureus</i>	<i>E. coli</i>	<i>Salmonella</i> spp.	<i>Pseudomonas aeruginosa</i>
<i>Aspergillus</i> sp.	18 ± 0.5	14 ± 0.6	13 ± 0.4	10 ± 0.3
<i>Penicillium</i> sp.	20 ± 0.7	15 ± 0.5	14 ± 0.5	11 ± 0.4
<i>Fusarium</i> sp.	16 ± 0.4	13 ± 0.3	12 ± 0.6	9 ± 0.2
<i>Alternaria</i> sp.	17 ± 0.6	14 ± 0.4	13 ± 0.5	10 ± 0.3

Table 2 presents the antibacterial activity of different endophytic fungal isolates measured as zones of inhibition (mm) against selected pathogenic bacteria. Among the isolates, *Penicillium* sp. exhibited the highest antibacterial activity, particularly against *Staphylococcus aureus* (20 ± 0.7 mm), indicating its strong efficacy against Gram-positive bacteria. *Aspergillus* sp. and *Alternaria* sp. also showed considerable inhibitory effects, with zones ranging between 17–18 mm against *S. aureus* and moderate activity against *E. coli* and *Salmonella* spp. In contrast, *Fusarium* sp. displayed comparatively lower antibacterial activity across all tested organisms. Overall, all fungal isolates demonstrated reduced effectiveness against *Pseudomonas aeruginosa*, with inhibition zones ranging from 9–11 mm, suggesting its higher resistance. These results indicate that endophytic fungi possess differential antibacterial potential, with stronger activity generally observed against Gram-positive bacteria than Gram-negative bacteria.

DISCUSSION:

The findings of the present study highlight the significant role of endophytic fungi as potential sources of antimicrobial compounds. The antibacterial activity observed can be attributed to the production of secondary metabolites such as alkaloids, terpenoids, phenolics, and polyketides by the fungal isolates. These bioactive compounds are known to interfere with bacterial cell wall synthesis, protein synthesis, and nucleic acid metabolism, ultimately leading to bacterial cell death.

The higher susceptibility of Gram-positive bacteria, particularly *Staphylococcus aureus*, compared to Gram-negative bacteria can be explained by differences in cell wall structure. Gram-positive bacteria possess a relatively simple peptidoglycan layer, allowing easier penetration of antimicrobial compounds. In contrast, Gram-negative bacteria such as *Pseudomonas aeruginosa* have an outer membrane containing lipopolysaccharides, which acts as a barrier to many antimicrobial agents.

The dominance of genera such as *Aspergillus* and *Penicillium* is consistent with earlier reports, as these fungi are well-known producers of antibiotics and other bioactive metabolites. The higher colonization frequency in roots supports the idea that soil-associated microbial interactions play a crucial role in shaping endophytic communities. The results of this study are in agreement with previous research, which suggests that medicinal plant-associated endophytes represent a promising and sustainable source of novel antimicrobial agents. Further studies involving purification, characterization, and structural elucidation of these bioactive compounds are necessary to explore their pharmaceutical potential.

CONCLUSION:

The present study clearly demonstrates that medicinal plants such as *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi), *Withania somnifera* (Ashwagandha), and *Aloe vera* harbor a rich and diverse community of endophytic fungi. These endophytes, isolated from different plant tissues, particularly roots, exhibited significant variation in their distribution and abundance, highlighting the influence of plant type and tissue specificity on microbial colonization. The predominance of fungal genera such as *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* further confirms their ecological adaptability and association with medicinal plants. Importantly, the antibacterial screening revealed that several endophytic fungal isolates possess strong antimicrobial activity against clinically important pathogenic bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., and *Pseudomonas aeruginosa*. The comparatively higher efficacy against Gram-positive bacteria suggests that the bioactive compounds produced by these fungi may have selective mechanisms of action, likely targeting bacterial cell wall synthesis or metabolic pathways. These findings reinforce the concept that endophytic fungi are valuable reservoirs of secondary metabolites with therapeutic potential. This study validates the hypothesis that medicinal plant-associated endophytes can serve as promising sources of natural antimicrobial agents. In the context of rising antibiotic resistance, such alternative sources are of immense scientific and pharmaceutical importance. However, further investigations focusing on the isolation, purification, structural characterization, and mode of action of these bioactive

metabolites are essential. Additionally, in vivo studies and clinical evaluations will be required to establish their safety and efficacy for potential drug development.

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