Advances in Bioresearch
Adv. Biores., Vol 16 (3) May 2025: 125-130
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Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.16.3.125130

Advances in Bioresearch

ORIGINAL ARTICLE

Exploring Endophytic Fungi for their Antimicrobial Potential from Medicinal Plants: A Study from Northern India

Nidhi[1], Ashwanti Devi[1*], Bharti Arora[2], Vikas Kumar[3] and Meenakshi Baher[4]
[1]Department of Bio-Sciences and Technology, Maharishi Markandeshwer (Deemed to be University), Mullana, Ambala
[2]Department of Microbiology Maharaja Agarsen Medical College, Agroha, Hisar
[3]International Medical School, University of International Business, Almaty, Kazakhstan
[4]OM Global University, Hisar
***Corresponding author's Email:** ashwantidevi@gmail.com

ABSTRACT
The increasing prevalence of drug-resistant bacteria has created a critical need for novel antimicrobial agents. Endophytic fungi, residing in healthy plant tissues, are emerging as potential sources of bioactive compounds. This study aimed to isolate and identify endophytic fungi from medicinal plants in Northern India and evaluate their antimicrobial activity against nosocomial pathogens. The study was conducted over six months in the microbiology laboratory of Maharaja Agrasen Medical College, Agroha, Haryana. Leaves from 11 medicinal plants, including Azadirachta indica, Ocimum tenuiflorum, Carica papaya, and others, were collected. Endophytic fungi were isolated on Sabouraud Dextrose Agar, identified morphologically, and tested for antimicrobial activity using the disk diffusion assay against Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa. Growth optimization and GC-MS analysis were performed to evaluate biomass production and bioactive compound profiles. Twenty-eight fungal isolates were obtained, predominantly Aspergillus (67.86%) and Alternaria (28.57%). Catharanthus roseus exhibited the strongest antibacterial activity, with inhibition zones of 20 mm, 22 mm, and 12 mm against S. aureus, E. coli, and K. pneumoniae respectively. Optimal growth was observed at pH 5.5, with maximum biomass of 6.7 mg achieved by Aspergillus. GC-MS analysis identified bioactive compounds, including tetrahydroauraglausin (20.24%) and L-Threonic Acid (1.36%). Endophytic fungi from medicinal plants demonstrated significant antimicrobial activity and identified bioactive compounds, emphasizing their potential as novel antimicrobial agents. These findings lay the groundwork for further research and pharmaceutical applications targeting drug-resistant pathogens.

Keywords: *Endophytic fungi, Medicinal plants, Antimicrobial activity, Nosocomial pathogens, Bioactive compounds, Aspergillus, Alternaria, Drug resistance, GC-MS analysis, Fungal biomass.*

Received 10.03.2025 Revised 05.05.2025 Accepted 23.05.2025

How to cite this article:
Nidhi, Ashwanti D, Bharti A, Vikas K and Meenakshi B. Exploring Endophytic Fungi for their Antimicrobial Potential from Medicinal Plants: A Study from Northern India. Adv. Biores., Vol 16 (3) May 2025: 125-130.

INTRODUCTION

In recent years, there has been a concerning increase in drug resistance among bacteria, leading to severe health problems worldwide. This has created a critical need for effective antimicrobial agents. Endophytes, which are microorganisms that reside within healthy plant tissues without causing any apparent adverse effects on the host [1], have emerged as promising sources of novel bioactive products. They thrive in millions of unique biological niches and can flourish in various unusual circumstances [2]. Natural bioactive products synthesized by endophytic fungi possess unique structures [3]. Despite this, endophytic fungi remain a poorly investigated group of microorganisms, despite being abundant and reliable sources of bioactive and chemically novel compounds with significant potential for the pharmaceutical industry [4, 5]. A thorough study has revealed that 51% of biologically active substances derived from endophytic fungi were previously unidentified [6-8]. Endophytic fungi possessing antimicrobial activity from natural products can be harnessed for industrial fermentation to produce natural active compounds on a large scale at low cost and without pollution [9]. In this study, several medicinal plants, including *Anisomeles malabarica* (Lamiaceae), *Cardiospermum halicacabum* (Sapindaceae), *Aristolochia indica* (Aristolochiaceae), and *Acacia mangium* (Fabaceae), traditionally used in Ayurvedic and Siddha medicine for treating various conditions such as rheumatism, cancer, wound healing, and pneumonia were selected. It is hypothesized that some of the therapeutic effects of these plants may be attributed to the presence of endophytic fungi. Therefore, this study aimed to identify the endophytic fungal community within these medicinal plants and assess their potential antimicrobial activities against human pathogens. The potentially beneficial endophytic fungi were identified using ITS sequence analysis.

MATERIAL AND METHODS

Study Design and Site

This prospective study was conducted in the Microbiology Laboratory of Maharaja Agrasen Medical College and Hospital, Agroha, Hisar, Haryana. The region's agricultural biodiversity and medicinal plant availability made it an ideal site for isolating endophytic fungi.

Study Duration

The study was carried out over six months, from January to June 2024.

Sample Collection

Plant Samples: Eleven plant species, commonly found in the Hisar region, were selected based on ecological and medicinal significance. A total of 33 leaves (3 per plant species) were collected from healthy, disease-free plants. The selected species included *Azadirachta indica* (Neem), *Ocimum tenuiflorum* (Tulsi), *Carica papaya* (Papaya), *Musa paradisiaca* (Banana), *Catharanthus roseus* (Sadabahar), *Calotropis gigantea* (Aak), *Psidium guajava* (Guava), *Epipremnum aureum* (Money plant), *Ficus religiosa* (Peepal), *Aloe barbadensis miller* (Aloe Vera), and *Tinospora cordifolia* (Giloy).

Nosocomial Pathogen Samples: A total of 100 clinical samples were collected from ICU patients diagnosed with nosocomial infections. Samples included blood, urine, respiratory secretions, and wound swabs.

Isolation of Endophytic Fungi

Surface Sterilization: Collected plant leaves were washed with distilled water, sterilized with 70% ethanol, and cut into sections of ~5 mm. These sections were plated on Sabouraud Dextrose Agar (SDA) medium supplemented with antibiotics and incubated at 25°C for 7 days. Emerging fungal colonies were sub-cultured on fresh SDA plates to obtain pure cultures [10].

Fungal Identification: Isolated fungi were identified based on morphological characteristics, including colony texture, pigmentation, and spore structure, and cataloged using standard identification keys [11].

Antimicrobial Assays

Preparation of Antimicrobial Disks: Sterile paper disks were soaked in fungal filtrates, air-dried, and stored at 4°C.

Disk Diffusion Assay: Fungal filtrate disks were tested against clinical bacterial isolates (*Staphylococcus aureus*, MRSA, *Escherichia coli*, and *Klebsiella pneumoniae*). Nutrient agar plates were inoculated with bacteria and incubated with fungal disks at 37°C for 24 hours. Inhibition zones were measured in millimeters.

Optimization of Fungal Growth

Endophytic fungi with significant antimicrobial activity were grown in SDA broth under varying pH conditions (5.0, 6.0, and 7.0) and incubation periods (3, 5, 7, and 9 days). Growth was assessed by measuring fungal biomass (mg) after filtration and drying.

Molecular Identification

DNA was extracted from *Aspergillus chevalieri* and *Alternaria alternata*, and species-specific primers were used in real-time PCR. Amplification curves were analyzed for cycle threshold (Ct) values to confirm species identity.

GC-MS Analysis

Bioactive compounds from fungal filtrates were extracted using ethyl acetate and analyzed via GC-MS. Compound identification was performed using NIST libraries.

RESULTS

Distribution and Diversity of Fungal Isolates

A total of 28 fungal isolates were obtained from the 11 plant species included in the study. The most dominant genus was *Aspergillus*, accounting for 67.86% of the isolates, followed by *Alternaria*

(28.57%), and *Fusarium* (7.14%). Among the plant sources, *Papaya* (*Carica papaya*) exhibited the highest fungal diversity, yielding four isolates, including *Alternaria* and *Fusarium*. Other plants such as *Neem* (*Azadirachta indica*), *Tulsi* (*Ocimum tenuiflorum*), and *Aloe Vera* (*Aloe barbadensis miller*) predominantly supported *Aspergillus* species. The presence of fungal isolates across multiple genera demonstrates the microbial richness of medicinal plants in the region.

Antimicrobial Activity of Endophytic Isolates

The antimicrobial activity of fungal isolates was assessed against four bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Among the isolates, *Sadabahar* (*Catharanthus roseus*) exhibited the strongest antibacterial activity, with inhibition zones of 20 mm, 22 mm, and 12 mm against *S. aureus*, *E. coli*, and *K. pneumoniae*, respectively. Peepal(*Ficus religiosa*) showed broad-spectrum activity with moderate inhibition zones against all tested bacterial strains, including *P. aeruginosa* (10 mm). Tulsi(*Ocimum tenuiflorum*) and Giloy (*Tinospora cordifolia*) exhibited selective activity, primarily effective against *S. aureus* and *E. coli*. Conversely, isolates from Banana (*Musa paradisiaca*) and Aak (*Calotropis gigantea*) demonstrated no antimicrobial activity. These findings underscore the variability in antimicrobial potential among fungal isolates, with some showing promising broad-spectrum activity (Table 1).

Table 1: Antimicrobial activity zone size in mm of endophytic isolates.

Source Plant	***Staphylococcus aureus***	***Escherichia coli***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***
Tulsi (*Ocimum tenuiflorum*)	15	16	0	0
Sadabahar (*Catharanthus roseus*)	20	22	12	0
Peepal (*Ficus religiosa*)	12	18	15	10
Giloy (*Tinospora cordifolia*)	18	0	0	0
Money plant (*Epipremnum aureum*)	14	0	0	13

Optimization of Growth Conditions

The growth of potent fungal isolates was optimized under varying pH conditions and incubation periods. Both *Aspergillus* and *Alternaria* isolates demonstrated optimal growth at pH 5.5, with steady increases in biomass over nine days (Fig. 1). The second *Aspergillus* isolate exhibited the most robust growth, reaching 6.7 mg biomass by Day 9. *Alternaria* followed closely, with a maximum biomass of 6.3 mg at the same time point. The first *Aspergillus* isolate showed comparatively lower growth, peaking at 3.4 mg. These results highlight the potential of certain isolates for applications requiring enhanced biomass production (Fig. 2).

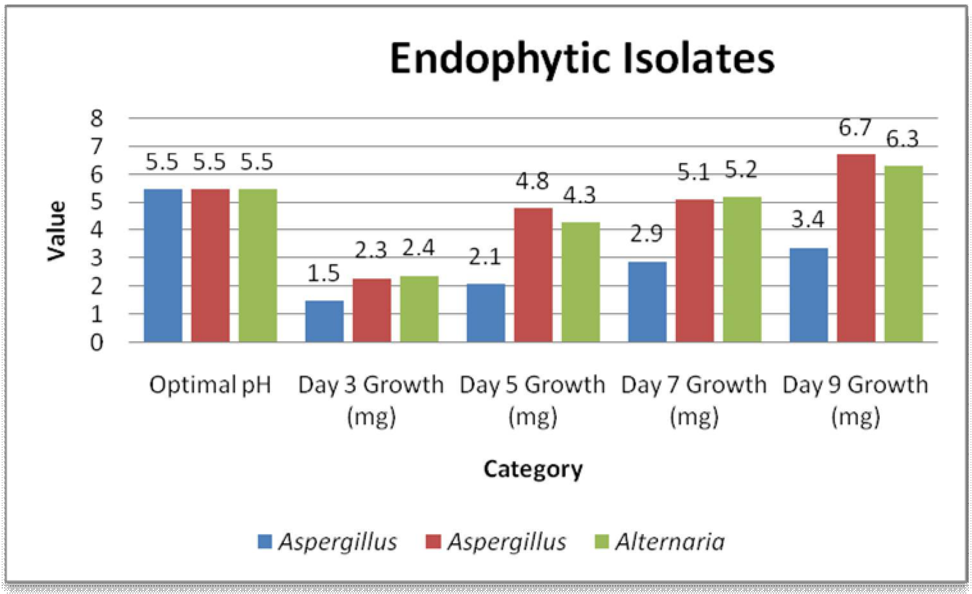

Figure1: Growth conditions of potent endophytic isolates.

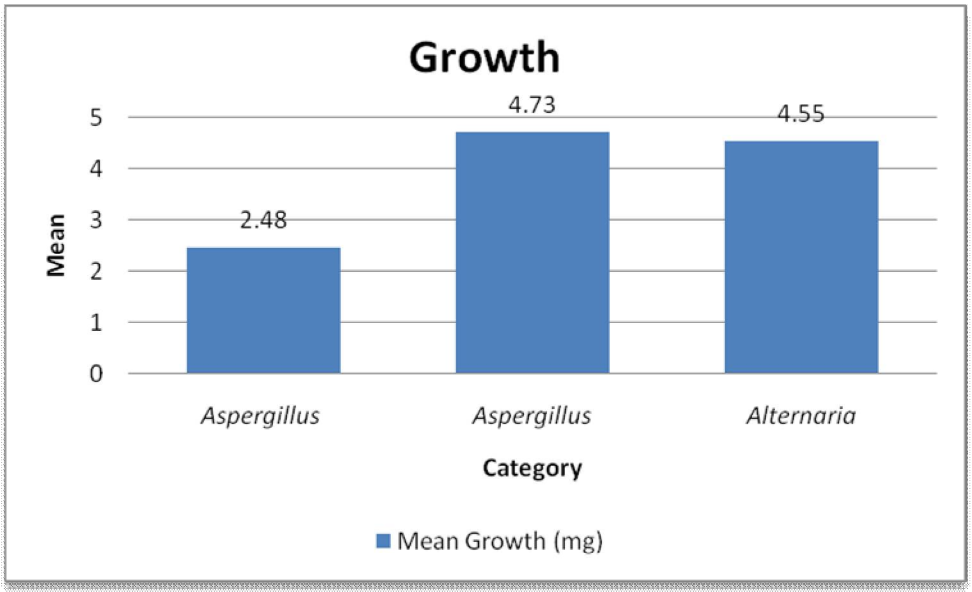

Figure 2: Statistical analysis of growth of Aspergillus and Alternaria.

GC-MS Analysis of Bioactive Compounds

The GC-MS analysis revealed key bioactive compounds in the fungal isolates, emphasizing their antimicrobial potential. In *Aspergillus chevalieri*, tetrahydroauraglausin (20.24% peak area) emerged as the predominant compound, known for its potent activity against Gram-positive bacteria. Other identified compounds, such as flavoglaucin (0.80%), displayed both antimicrobial and antioxidant properties. Similarly, in *Alternaria alternata*, L-Threonic Acid (1.36%) and Glyceric Acid, 3TMS Derivative (1.17%) were the most abundant compounds, with documented antioxidant and antimicrobial activities. The presence of diverse bioactive metabolites underscores the therapeutic potential of these fungal isolates (Figs. 3, 4).

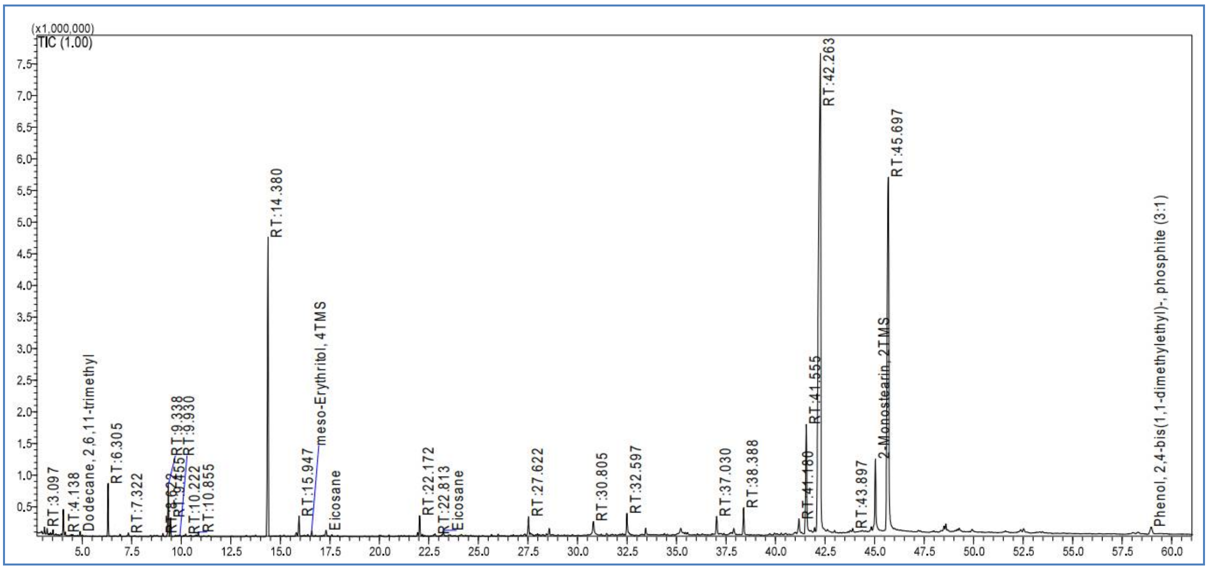

Figure 3: Gas chromatography of *Aspergillus chevaleiri*

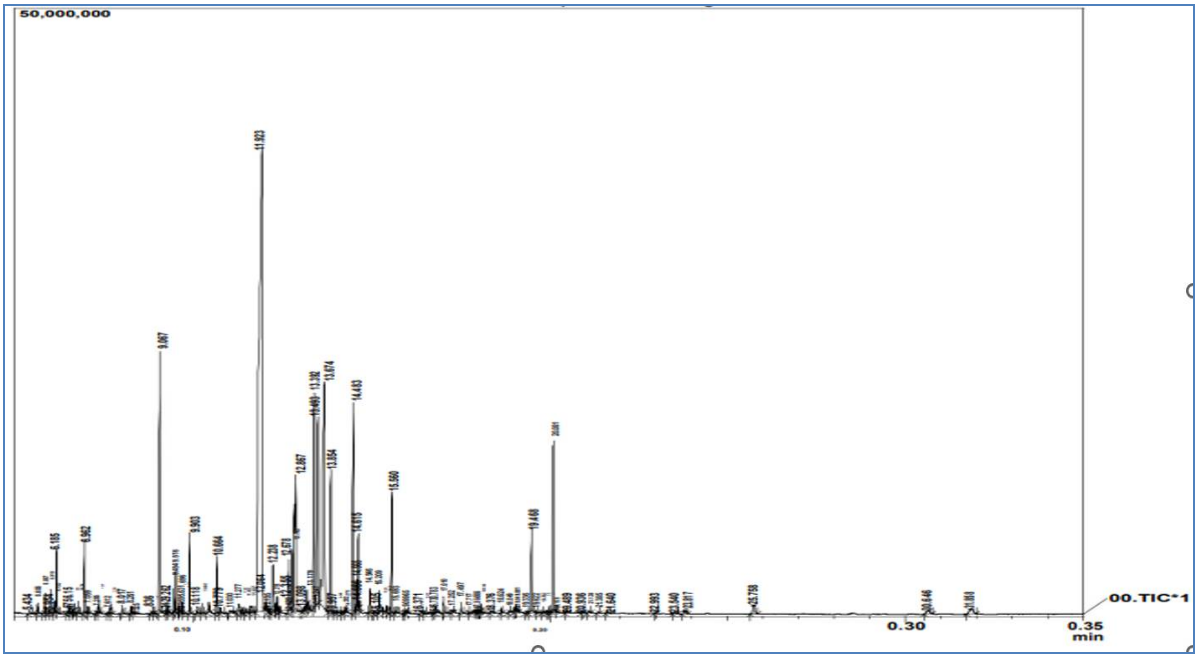

Figure 4: Gas chromatography of *Alternaria alternate*

DISCUSSION

Our study isolated 28 fungal endophytes from 11 medicinal plants, with *Aspergillus* (67.86%) and *Alternaria* (28.57%) being predominant. Notably, *Carica papaya* exhibited the highest fungal diversity, yielding four isolates, including *Alternaria* and *Fusarium*. These findings align with previous research highlighting the rich diversity of endophytic fungi in medicinal plants [12]. The antimicrobial activity of these isolates was assessed against four bacterial strains. The isolate from *Catharanthus roseus* exhibited the strongest antibacterial activity, with inhibition zones of 20 mm, 22 mm, and 12 mm against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*, respectively. This is consistent with studies reporting significant antimicrobial properties of endophytic fungi from medicinal plants [13].

Optimization of growth conditions revealed that both *Aspergillus* and *Alternaria* isolates demonstrated optimal growth at pH 5.5, with steady increases in biomass over nine days. The second *Aspergillus* isolate exhibited the most robust growth, reaching 6.7 mg biomass by Day 9. This suggests that specific pH conditions can enhance the biomass production of endophytic fungi, which is crucial for large-scale cultivation and bioactive compound extraction. GC-MS analysis identified key bioactive compounds in the fungal isolates. In *Aspergillus chevalieri*, tetrahydroauraglausin (20.24% peak area) was predominant, known for its potent activity against Gram-positive bacteria. Similarly, in *Alternaria alternata*, L-Threonic Acid (1.36%) and Glyceric Acid, 3TMS Derivative (1.17%) were abundant, with documented antioxidant and antimicrobial activities. These findings are in line with previous research highlighting the potential of endophytic fungi as sources of bioactive compounds [14]. Statistical analysis of growth data revealed significant variability among the isolates. The second *Aspergillus* isolate and *Alternaria* exhibited higher mean growths of 4.73 mg (SD = 1.83) and 4.55 mg (SD = 1.60), respectively, suggesting their suitability for large-scale cultivation aimed at bioactive compound production. In summary, our findings underscore the rich diversity and antimicrobial potential of endophytic fungi in medicinal plants. The identification of bioactive compounds further highlights their potential for pharmaceutical applications. Future studies should focus on the purification and characterization of these compounds, as well as exploring the mechanisms underlying their antimicrobial activity.

CONCLUSION

This study demonstrated the antimicrobial potential of endophytic fungi isolated from medicinal plants in Northern India, with *Aspergillus* and *Alternaria* being the predominant genera. Fungi from *Sadabahar* (*Catharanthus roseus*) and Peepal (*Ficus religiosa*) showed the highest activity against nosocomial pathogens like *Staphylococcus aureus* and *Escherichia coli*. Optimization of growth conditions enhanced biomass production, and GC-MS analysis identified bioactive compounds such as tetrahydroauraglausin and L-Threonic Acid with significant antimicrobial properties. These findings underscore the potential of endophytic fungi as a source of novel antimicrobial agents, paving the way for future research and pharmaceutical applications against drug-resistant pathogens.

LIMITATION

This study had several limitations. The focus on 11 medicinal plant species from a specific region may have restricted the diversity of isolated fungi. Identification relied largely on morphology, with limited molecular characterization. Antimicrobial activity was tested in vitro against a narrow range of bacterial strains, lacking in vivo validation or testing against fungi and viruses. Potential synergistic effects with antibiotics were not explored, and bioactive compounds identified through GC-MS were not quantified or purified. Additionally, the molecular mechanisms underlying the antimicrobial activity were not investigated. Addressing these limitations could enhance the scope and applicability of future research.

CONFLICT OF INTEREST

The authors state that they have no conflicts of interest to declare.

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