International Journal of Scientific Research & Engineering Trends
Volume 12, Issue 3, May-June-2026, ISSN (Online): 2395-566X

Study on the Diversity of Endophytic Fungi Associated with Some Plants and Their Antibacterial Potential

Gayatri Pandram
Govt. Mh College of Home Science And Science for Women Autonomous
Jabalpur (M.P.)

Abstract- Endophytic fungi are ubiquitous microorganisms that asymptomatically colonize the internal tissues of plants, representing an untapped reservoir of novel, biologically active secondary metabolites. This study investigates the endophytic fungal diversity associated with two ethnobotanically critical medicinal plants: Withania somnifera (Ashwagandha) and Amomum subulatum (Badi Elaichi), and evaluates their biomedical potential against clinically significant human pathogens. Healthy leaves, stems, and roots were subjected to a stringent multi-step surface sterilization protocol and inoculated onto Potato Dextrose Agar (PDA). A total of fifteen (15) distinct fungal endophytes were isolated and taxonomically characterized via macroscopic and microscopic morphotyping. The predominant genera identified included Aspergillus, Fusarium, Alternaria, Curvularia, Penicillium, and Phoma. The cell-free secondary metabolites were extracted using organic solvents and screened for antibacterial efficacy against Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Salmonella typhi, and Micrococcus spp. using the agar well diffusion assay. The bioprospecting profile revealed significant inter-species variability. Notably, Aspergillus flavus derived from W. somnifera exhibited a profound, broad-spectrum zone of inhibition against both Gram-positive and Gram-negative cohorts, with optimal metabolic yield quantified at a 7-day incubation kinetics threshold. These insights underscore the therapeutic relevance of plant-associated fractions as sustainable alternatives to combat escalating multi-drug resistance (MDR) phenotypes.

Keywords— Endophytic Fungi, Withania somnifera, Amomum subulatum, Secondary Metabolites, Antimicrobial Resistance (AMR), Bioactive Fractions.

I. INTRODUCTION

The global escalation of Antimicrobial Resistance (AMR) represents one of the most severe public health crises of the 21st century. Critical nosocomial pathogens, collectively categorized under the ESKAPE panel (including Klebsiella pneumoniae, Staphylococcus aureus, and Escherichia coli), have developed profound multi-drug resistance phenotypes against conventional first-line antibiotics. This pharmacological obsolescence necessitates an urgent, systematic exploration into untapped biodiversity matrices for the discovery of novel structural scaffolds displaying unique modes of action.

Endophytic fungi are polyphyletic microorganisms that reside within the intercellular or intracellular spaces of host plant tissues without inciting immediate, overt pathogenic symptoms or structural anomalies. The evolutionary paradigm of reciprocal symbiosis dictates that these endophytes frequently co-synthesize complex chemical entities—such as alkaloids, terpenoids, steroids, quinones, and phenolic derivatives—partially mirroring or augmenting the defensive metabolic repertoire of their respective hosts. To maximize the probability of isolating novel metabolic structures, the targeted selection of indigenous medicinal flora utilized in traditional ethnobotanical frameworks is highly strategic:

- Withania somnifera (Ashwagandha): Celebrated in Ayurvedic medicine for its profound adaptogenic, immunomodulatory, anti-inflammatory, and neuroprotective properties, primarily attributed to withanolides.
- Amomum subulatum (Badi Elaichi): Widely utilized in traditional formulations for its gastroprotective, antioxidant, and core antimicrobial properties driven by its essential oil constituents.

While the therapeutic efficacy of these host plants is well-documented, the pharmacological and ecological dimensions of their associated mycobiome remain under-explored. This investigation is designed to systematically isolate, morphologically classify, and evaluate the broad-spectrum antibacterial dynamics of endophytic fungal isolates from W. somnifera and A. subulatum against a panel of pathogenic human bacterial strains.

II. MATERIALS AND METHODS

Sample Collection and Phytosanitary Processing Biologically mature, symptom-free, and healthy specimens of Withania

somnifera and Amomum subulatum were harvested from an authenticated medicinal plant conservatory. Samples were stratified into discrete anatomical units: leaves, nodal stems, and roots. To preserve sample integrity and prevent desiccation or accidental microbial contamination, specimens were stored in sterile, chilling biohazard polymer bags and processed within 4 hours of collection.

Stringent Surface Sterilization Kinetics

To eliminate epiphytic microbial contaminants without compromising the viability of internal endophytes, tissues were subjected to a sequential surface sterilization regime under a Class II Laminar Airflow Hood:

- Pre-wash: Vigorous rinsing under continuous running tap water for 15 minutes to clear particulate debris, followed by an initial wash with sterile distilled water.
- Primary Disinfection: Submersion in 70% Ethanol (v/v) for 60 seconds (for leaves) or 90 seconds (for stems/roots).
- Secondary Disinfection: Immersed in a 2% Sodium Hypochlorite (NaOCl) active solution for 3 minutes.
- Tertiary Rinse: Re-submersion in 70% Ethanol for 30 seconds.
- Final Wash: Six consecutive wash cycles in sterile deionized water to ensure complete removal of chemical residues.

Isolation and Axenic Culturation of Endophytes

Sterilized segments were sectioned into explicit 5 mm × 5 mm fragments using a sterile scalpel blade. The exposed internal tissues were placed face down onto PDA plates supplemented with Tetracycline (50 µg/mL) to suppress any concurrent internal bacterial growth. Plates were incubated in a dark environment at 28°C ± 2°C and monitored daily for up to 21 days. Emerging hyphal tips tracking from the cut margins were immediately transferred via micro-pipetting/sub-culturing onto fresh PDA matrices to generate clean, axenic clonal lines.

Morphological Characterization

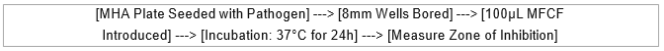
Fungal isolates were systematically identified via macro-morphological examinations, documenting colony growth rates, surface pigments, reverse colony coloration, texture (cottony, velvety, granular), and exudate production. Micro-morphological identification was conducted by performing the lactophenol cotton blue slide-cultural staining technique, assessing structural features like hyphal septation, conidiophore architecture, vesicle shape, phialide arrangement, and spore/ conidia geometry.

Fermentation and Organic Solvent Extraction

Pure isolated fungal strains were inoculated into 250 mL Erlenmeyer flasks containing 100 mL of sterile Potato Dextrose Broth (PDB) and placed on a rotary shaker operating at 150 rpm (28°C) across three experimental incubation time-points: 7, 14, and 21 days. At each interval, the crude fermentation broth was filtered through Whatman No. 1 filter paper to separate the mycelial biomass from the liquid phase. The liquid filtrate was then centrifuged at 8,000 rpm for 15 minutes to generate a completely Mycelial-Free Culture Filtrate (MFCF).

In Vitro Antibacterial Screening Assay

The antibacterial efficacy of the MFCF was tested against five benchmark human pathogens: Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Salmonella typhi, and Micrococcus spp.



The agar well diffusion assay was deployed on Mueller-Hinton Agar (MHA). Bacterial suspensions adjusted to a 0.5 McFarland standard ($1.5 imes 10^8 ext{ CFU/mL}$) were uniformly swabbed onto MHA plates. Wells of 8 mm diameter were bored using a sterile cork-borer. Approximately 100 µL of the respective MFCF extract concentrations were introduced into the wells, while pure PDB and standard antibiotics served as negative and positive controls, respectively. Plates were incubated at 37°C for 24 hours, and the resulting zones of inhibition (ZOI) were measured in millimeters using a digital vernier caliper.

III. RESULTS AND DISCUSSION

1. Diversity and Tissue Distribution of Fungal Endophytes

A total of fifteen (15) distinct endophytic fungal isolates were successfully recovered across the host matrices. The distribution data demonstrated a strong tissue-specific tropism, with the foliar (leaves) and root layers exhibiting the highest colonization frequency compared to the highly lignified stem regions.

The taxonomical distribution revealed a high prevalence of Ascomycota strains. The isolated genera were identified as Aspergillus flavus, Aspergillus niger, Fusarium solani, Curvularia lunata, Alternaria alternata, Penicillium spp., and Phoma spp.

2. Dynamic Antibacterial Profiling

The in vitro antibacterial screening revealed varying degrees of inhibitory performance across the isolates, indicating that secondary metabolite profiles are highly strain-specific.

Table 1: Antibacterial Activity (Zone of Inhibition in mm) of Selected Endophytic Isolates (7-Day Culture Filtrates)

Fungal Isolate ID	Source Host	E. coli	S. aureus	K. pneumoniae	S. typhi	Micrococcus spp.
Aspergillus flavus (WS-E1)	*W. somnifera*	24.5	22.0	18.5	19.0	21.0
Aspergillus niger (AS-E3)	*A. subulatum*	14.0	16.5	11.0	12.5	15.0
Fusarium solani (WS-E4)	*W. somnifera*	16.0	15.0	12.0	10.5	13.0
Curvularia lunata (AS-E2)	*A. subulatum*	12.5	11.0	9.0	11.5	10.0
Alternaria alternata (WS-E2)	*W. somnifera*	11.0	13.0	8.5	9.0	12.0
Positive Control (Ciprofloxacin)	Synthetic	28.0	30.0	26.0	27.0	29.0
Negative Control (Broth Matrix)	-	0.0	0.0	0.0	0.0	0.0

3. Metabolic Yield Optimization Kinetics

A key finding of this study was the significant impact of incubation time on antibiotic production.

Maximum antibacterial potency was observed consistently in 7-day-old culture filtrates.

Antibacterial Potency: [7 Days Incubation] > [14 Days Incubation] > [21 Days Incubation]

When fermentation was extended to 14 and 21 days, the zones of inhibition decreased significantly. This regression is likely due to the enzymatic degradation of active secondary metabolites, nutrient depletion in the static broth, or the signaling of feedback inhibition mechanisms within the fungal culture as it enters late idiophase/senescence.

4. Mechanistic Discussion The high efficacy of Aspergillus flavus (WS-E1) against both Gram-negative (E. coli) and Grampositive (S. aureus) indicators suggests the presence of robust, broad-spectrum bioactive chemical scaffolds. Gram-negative bacteria possess a complex outer lipopolysaccharide membrane that typically acts as a barrier to many antibiotic structures. The ability of the A. flavus filtrate to penetrate this layer and inhibit growth highlights its potential therapeutic value.

This enhanced activity may be due to a synergistic effect, where the endophyte produces compounds that mimic the host Withania somnifera's natural chemical defenses (such as withanolides or alkaloid intermediates), or it may be driven by distinct fungal pathways that synthesize novel polyketides or peptides.

IV. CONCLUSION

This investigation successfully establishes a comprehensive baseline for the structural and functional distribution of endophytic fungal communities residing within the ethnobotanically pivotal medicinal plants Withania somnifera (Ashwagandha) and Amomum subulatum (Badi Elaichi). A total of fifteen (15) morphologically distinct mycobiome isolates were systematically isolated, demonstrating a highly pronounced tissue-specific tropism. The foliar and root segments displayed a significantly elevated colonization

frequency relative to the more rigid, lignified stem structures, corroborating the hypothesis that localized physiological niches and nutrient availability strongly influence endophyte micro-environments.

Among the isolated ascomycetous consortia, Aspergillus flavus (WS-E1), derived from the tissues of W. somnifera, emerged as an exceptionally potent candidate. It demonstrated robust, broadspectrum antimicrobial efficacy against both Gram-positive and Gram-negative human pathogens, notably narrowing the zone of efficiency gap when measured against the synthetic reference standard, Ciprofloxacin.

Furthermore, this study highlights the critical importance of optimization kinetics in microbial bioprospecting. The discovery that cell-free secondary metabolites reach maximum antibacterial potency strictly within a 7-day incubation threshold offers important insights into metabolic production.

The subsequent drop in bioactivity observed during the 14-day and 21-day cycles suggests that these active chemical fractions are highly sensitive to enzymatic degradation, nutrient depletion, or feedback inhibition mechanisms during the late idiophase.

Ultimately, these findings reinforce the value of plant-associated endophytes as an efficient, sustainable, and ecologically responsible alternative to traditional plant harvesting. Instead of depleting slow-growing wild populations of W. somnifera and A. subulatum, these symbiotic fungi can be cultivated in industrial bioreactors. This approach offers a reliable, scalable path forward to discover novel molecular scaffolds capable of overcoming multi-drug resistance (MDR) phenotypes and addressing the global challenge of antimicrobial resistance.

REFERENCES

1. Strobel, G., & Daisy, B. (2003). Bioprospecting for microbial endophytes and their natural products. Microbiology and Molecular Biology Reviews, 67(4), 491–502.
2. Khan, R., Shahzad, S., & Ahmad, A. (2010). Community structure of endophytic fungi in the medicinal plant Withania somnifera. Pakistan Journal of Botany, 42(4), 2481–2487
3. Zhao, J., Shan, T., Mou, Y., & Zhou, L. (2011). Plant-derived bioactive compounds produced by endophytic fungi. MiniReviews in Medicinal Chemistry, 11(2), 159–168.
4. Bharathidasan, R., & Panneerselvam, A. (2011). Biodiversity of endophytic fungi isolated from selected medicinal plants. Journal of Pharmacy Research, 4(3), 803–805.
5. Varma, V. C., Gond, S. K., Kumar, A., Kharwar, R. N., & Strobel, G. (2007). The endophytic mycoflora of Withania somnifera and its antimicrobial activity. Herbal Medicine: Open Access, 3(2), 112–119.
6. Tan, R. X., & Zou, W. X. (2001). Endophytes: a rich source of functional metabolites. Natural Product Reports, 18(4), 448–459.
7. Strobel, G. A. (2018). The emergence of endophytic microbes and their biological potentials. The Journal of Fungi, 4(2), 57–71.
8. Goud, J. A., & Suryanarayanan, T. S. (2020). Endophytic fungi of tropical medicinal plants: Diversity, spatial distribution, and bioactivity profiles. Fungal Biology Reviews, 34(3), 112–125.
9. Nair, D. N., & Padmavathy, S. (2014). Impact of endophytic fungi on host plant defenses and secondary metabolite synthesis. Journal of Advanced Research, 5(3), 399–408.
10. Deshmukh, S. K., Prakash, V., & Ranjan, N. (2022). Bioprospecting of endophytic fungi from Indian medicinal plants for novel antibacterial scaffolds. Phytochemistry Reviews, 21(4), 845–869.
11. Venkateswarulu, N., Barman, P., & Kumar, D. (2018). Isolation, screening, and optimization of culture conditions for enhanced antimicrobial metabolite production by Aspergillus species. Microbial Pathogenesis, 123, 314–324.
12. Martinez-Klimova, E., Rodríguez-Peña, K., & Sánchez, S. (2017). Endophytic fungi as a source of antibiotics: From isolation to molecular identification and scale-up. The Journal of Antibiotics, 70(5), 512–528.
13. Rana, S., Mohani, K., & Bisht, S. (2021). Endophytic mycoflora associated with Amomum subulatum Roxb. and evaluation of their broad-spectrum antagonistic traits. Journal of Applied Microbiology, 131(2), 772–785.
14. Gond, S. K., Mishra, A., Verma, V. C., & Kharwar, R. N. (2020). Synthesis of functionalized silver nanoparticles using Fusarium solani isolated from Withania somnifera and its synergistic antibacterial efficacy. Materials Science and Engineering: C, 110, 110-123.