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Isolation of Endophytic Fungi from Selected Medicinal Plants

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Abstract

Endophytic fungi are microorganisms that colonize internal plant tissues without causing apparent harm and are recognized as important sources of bioactive compounds. The present study aimed to isolate and identify endophytic fungi from selected medicinal plants, namely Tridax procumbens, Leucas aspera, Mimosa pudica, and Capsicum annuum, under controlled laboratory conditions. Healthy plant samples were surface sterilized using ethanol and sodium hypochlorite to eliminate external contaminants, followed by inoculation on Potato Dextrose Agar (PDA) and incubation at 25–28°C for 5–7 days. Fungal isolates emerging from plant tissues were purified and identified based on macroscopic colony characteristics and microscopic features using lactophenol cotton blue staining. A diverse assemblage of endophytic fungi was recorded, with dominant genera including Alternaria, Chaetomium, Cladosporium, and Colletotrichum. Leaf tissues exhibited the highest colonization frequency and diversity compared to other plant parts. The results confirm that medicinal plants serve as rich reservoirs of endophytic fungi with significant ecological and biotechnological potential. These endophytes may contribute to host plant properties and represent promising sources of bioactive secondary metabolites. Further studies involving molecular characterization and bioactivity evaluation are recommended to fully explore their applications.

Keywords: Alternaria, Chaetomium, Cladosporium, and Colletotrichum, endophytic fungi.

Introduction

Medicinal plants have been an integral part of traditional and modern healthcare systems due to their rich repository of bioactive compounds[1]. In recent years, scientific interest has expanded beyond plant-derived metabolites to include the microorganisms associated with plants, particularly endophytic fungi, which inhabit internal plant tissues without causing any visible harm. These endophytes are now recognized as important contributors to plant health and as potential sources of novel pharmacologically active compounds[2].

Medicinal plants are considered "hotspots" for endophytes with pharmacologically relevant metabolites, and the selection of ethnomedicinal species is recommended for bioprospecting. Endophytic fungi are widely distributed across different plant species and colonize various plant parts such as leaves, stems, roots, and fruits. The association between plants and endophytes is often symbiotic, where the host plant provides nutrients and shelter, while the endophytes contribute to plant growth, stress tolerance, and resistance to pathogens[3]. Importantly, many endophytic fungi have the ability to produce secondary metabolites similar to those found in their host plants, making them valuable in the fields of medicine, agriculture, and biotechnology. So, endophytes can produce compounds identical or similar to host phytochemicals, offering an alternative supply that reduces pressure on overharvested plants[4].

The diversity of endophytic fungi varies depending on several factors, including plant species, tissue type, environmental conditions, and geographical location. Studies have shown that different plant parts harbor distinct endophytic communities, reflecting tissue-specific colonization patterns. For instance, leaves are often more exposed to environmental spores, whereas roots interact closely with soil microorganisms, leading to variations in fungal diversity[5].

Among the numerous fungal genera reported as endophytes, species belonging to Endophytic fungi from medicinal plants are now recognized as micro-factories of structurally diverse bioactive compounds with broad pharmacological potential. Genera such as *Alternaria*, *Chaetomium*, *Cladosporium*, and *Colletotrichum* are frequent in medicinal hosts and repeatedly yield antimicrobial, antioxidant, anticancer, anti-inflammatory, and antidiabetic metabolites. Targeting ethnomedicinal plants like *Tridax procumbens*, *Leucas aspera*, *Mimosa pudica*, and *Capsicum annuum* provides a strong scientific basis for isolating endophytic fungi with promising therapeutic potential while supporting conservation-oriented natural product discovery.

The significance of the present study lies in its contribution to understanding the diversity of endophytic fungi in medicinal plants and their potential applications in drug discovery and biotechnology. By isolating and identifying fungi from different plant parts, this study aims to provide insights into their distribution, diversity, and possible functional roles.

Materials and Methods

In order to separate and identify endophytic fungi from particular medicinal plants, the current investigation was carried out in a controlled laboratory setting. By removing exterior pollutants using the proper surface sterilization processes, the methodology was carefully constructed to guarantee that only interior fungal colonies were studied.

Plant sample collection and selection: Based on their availability and therapeutic significance, four medicinal plants, *Tridax procumbens*, *Leucas aspera*, *Mimosa pudica*, and *Capsicum annuum* were chosen (Figure-1). Healthy, disease-free plants were collected from nearby areas to guarantee the existence of natural endophytic populations. Leaves, stems, roots, and fruits were among the several plant parts that were gathered (Table-1). The samples were put in sterile bags and tagged with the location, date of collection, and name of the plant. Every specimen was sent right away to the lab for additional examination.

Culture media preparation: The fungi were isolated using Potato Dextrose Agar (PDA) medium, which supports the growth of numerous fungal species. In summary, 200 g of peeled potatoes were cooked in distilled water before the extract was filtered. The capacity was reduced to one liter by using distilled water. The mixture was then boiled until the 15–20 g of agar and 20 g of dextrose were completely dissolved. The medium was sterilized by autoclaving it at 120°C for 15 to 20 minutes. After cooling to 57°C, an antibiotic (*streptomycin*) was applied to avoid bacterial infection. The medium was then put aseptically into sterile petri dishes and allowed to solidify.

Surface sterilization of plant samples: In order to eradicate epiphytic microorganisms and guarantee the separation of just endophytic fungi, surface sterilization was performed. To get rid of dirt and debris, plant samples were first cleaned under running tap water. A sterile scalpel was then used to cut the samples into tiny pieces ($<$ 1cm) (Figure-2). The segments were sequentially sterilized by immersing them in 70% ethanol for 30-60 seconds, then immersing them in 1% sodium hypochlorite for two to three minutes, and then rinsing them twice with sterile distilled water to get rid of any remaining sterilants. The sterilized segments were dried on sterile blotting paper under aseptic conditions. Using sterile forceps, the segments were aseptically injected into PDA plates, with three to six segments evenly placed on each plate. To avoid contamination, the plates were incubated at 25°C for 7 days and covered with parafilm. Every day, plates were checked for fungus growth.

Isolation and purification of fungal colonies: Emerging fungal colonies from the plant segments were observed and differentiated based on morphological characteristics. Distinct colonies were subcultured by transferring a small portion onto fresh PDA plates. Subculturing was repeated until pure fungal cultures were obtained.

Identification of fungal isolates: Fungal isolates were identified using standard macroscopic and microscopic techniques. Macroscopic observation included Colony characteristics such as color (white, green, black, or grey), texture (cottony, velvety, or powdery), growth pattern, and margin were recorded. While microscopic observation included a small portion of the fungal culture, which was placed on a clean glass slide, stained with a drop of lactophenol cotton blue, and covered with a coverslip. The preparation was examined under a microscope. Features such as hyphal structure (septate or non-septate), spore shape and arrangement, and the presence of conidiophores or other reproductive structures were carefully observed. Identification was performed by comparing these characteristics with standard fungal identification manuals.

Data recording and documentation: All observations were meticulously documented, encompassing the number of isolates collected from each plant section, the characteristics of the colonies, and their microscopic details. Additionally, photographs were taken to capture both the colony morphology and microscopic structures for precise documentation and analysis.

Results and Discussion

The present study successfully resulted in the isolation and identification of endophytic fungi from different parts (leaves, stems, roots, and fruits) of the selected medicinal plants: *Tridax procumbens*, *Leucas aspera*, *Mimosa pudica*, and *Capsicum annuum* (Figure-1). The results are presented based on growth characteristics, distribution across plant parts, and morphological identification.

Fungal growth: Fungal growth was observed emerging from the inoculated plant segments within 3–5 days of incubation. The growth initiated from the cut edges of the tissues, confirming their internal origin. Leaf segments showed maximum fungal colonization. Root and stem segments showed moderate colonization. Fruit samples (in *Capsicum annuum*) showed comparatively lower colonization. The colonies exhibited diverse colors, textures, and growth patterns, indicating the presence of multiple fungal species (Figure-3).

Morphological characterization and fungal identification: All of the fungal isolates had septate hyphae when examined under a microscope with lactophenol cotton blue staining. Detailed morphological observations enabled preliminary identification of the isolates at the genus level. *Alternaria* species were characterized by multicellular, darkly pigmented conidia possessing both transverse and longitudinal septa. *Chaetomium* exhibited filamentous hyphae along with distinctive reproductive structures, while *Cladosporium* was identified by branched conidiophores bearing chains of conidia. In contrast, *Colletotrichum* species displayed typically curved conidia with characteristic morphology.

In addition to microscopic features, the isolates demonstrated distinct macroscopic characteristics when cultured on Potato Dextrose Agar (PDA) medium. Colonies of *Alternaria* appeared dark brown to black, *Chaetomium* formed white to grey cottony colonies, *Cladosporium* exhibited greenish velvety growth, and *Colletotrichum* produced pinkish to grey colonies often with concentric ring patterns. Morphological characterization, incorporating both macroscopic and microscopic observations, plays a crucial role in the preliminary identification and classification of endophytic fungi (Figure-3). These phenotypic traits serve as reliable indicators for genus-level identification and provide a strong foundation for subsequent molecular confirmation. The use of lactophenol cotton blue staining further enhances visualization of fungal structures, ensuring accurate morphological assessment and supporting reliable taxonomic identification[6].

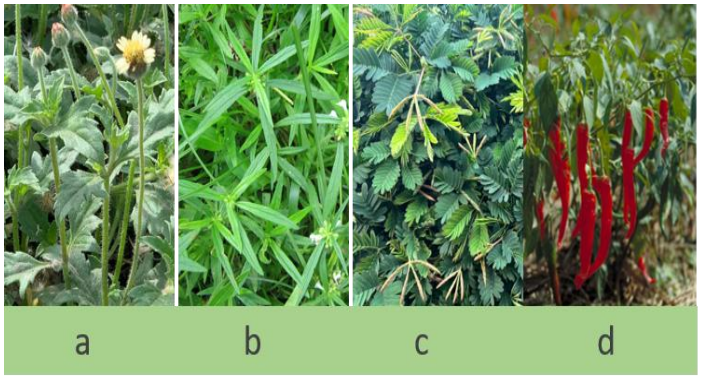


Figure-1: Ethnomedicinal plants studied a. *Tridax procumbens*, b. *Leucas aspera*, c. *Mimosa pudica*, and d. *Capsicum annuum*.

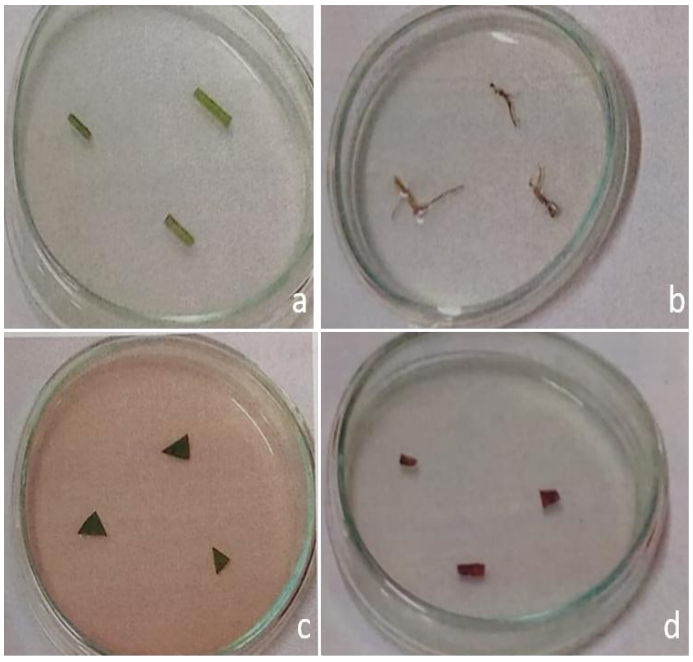


Figure-2: Inoculation of plant samples a. stem, b. root; c. leaf; d. fruit (*Capicum).*

Tissue-specific distribution and colonization patterns: The distribution of endophytic fungi across different plant tissues exhibits distinct patterns that reflect both host factors and environmental influences. The present results demonstrate that leaf segments showed maximum fungal colonization, while root and stem segments showed moderate colonization, and fruit samples showed comparatively lower colonization[7]. This tissue-dependent distribution is consistent with observations from numerous other medicinal plant studies. Research on endophytic communities in sweet potato revealed that the diversity of endophytic fungi followed a root > stem > leaf trend in healthy plants, suggesting tissue-specific factors influence colonization intensity[8].

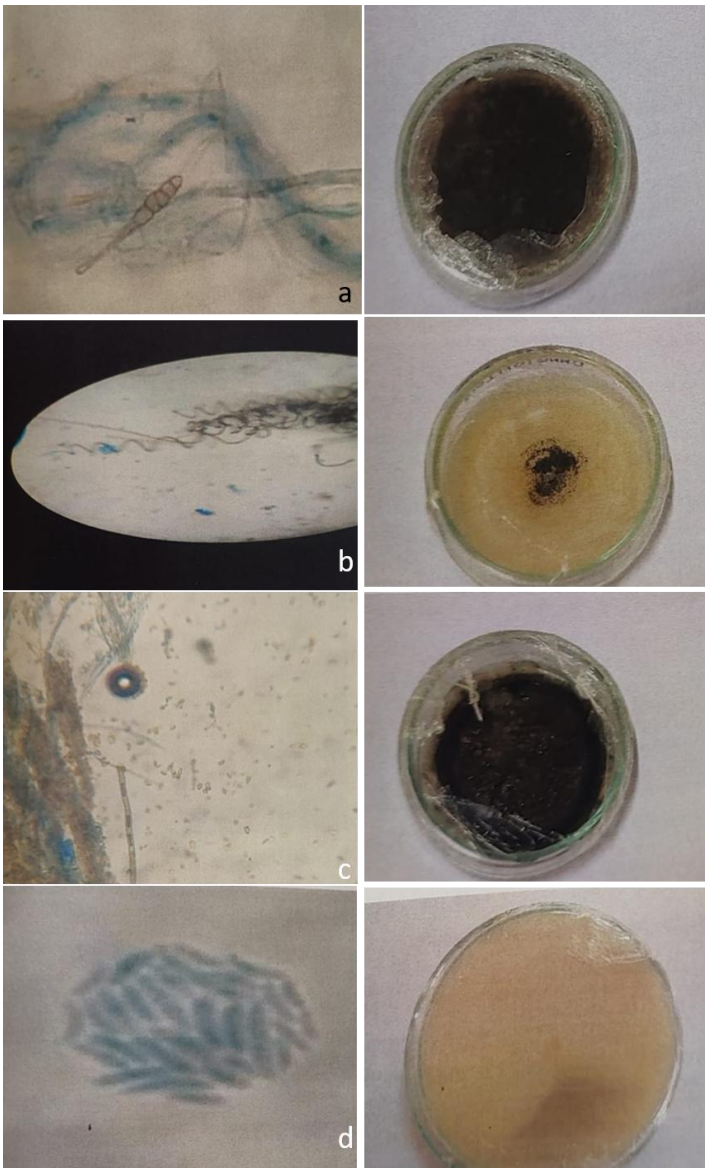


Figure-3: Study of fungal morphology under the light microscope and colony morphology in PDA, a. *Alternaria*, b. *Chaetomium* c. *Cladosporium* d. *Colletotrichum.*

Table-1: Distribution of endophytic fungi in different plant parts.

Plant Name	Plant Part	No. of Segments	No. of Isolates	Identified Fungi
Tridax procumbens	Leaf	10	4	*Alternaria*, *Cladosporium*
	Stem	10	2	*Alternaria*
	Root	10	2	*Chaetomium*
Leucas aspera	Leaf	10	3	*Alternaria*, *Cladosporium*
	Stem	10	2	*Alternaria*
	Root	10	1	*Chaetomium*
Mimosa pudica	Leaf	10	4	*Alternaria*, *Colletotrichum*
	Stem	10	2	*Alternaria*
	Root	10	2	*Chaetomium*
Capsicum annuum	Leaf	10	3	*Alternaria*, *Cladosporium*
	Stem	10	2	*Alternaria*
	Root	10	1	*Chaetomium*
	Fruit	10	2	*Colletotrichum*

Diversity and dominance patterns in endophytic communities: *Alternaria* species emerged as the most prevalent endophytes, found in every plant species and tissue examined. *Cladosporium* and *Colletotrichum* were somewhat widespread, while *Chaetomium* was mainly linked to root tissues. Generally, fungal diversity was highest in leaves, followed by stems, roots, and then fruits. The findings of this study reveal that the selected medicinal plants host a wide variety of endophytic fungi, with different plant parts fostering unique fungal communities. Alternaria species are the predominant endophytes across all plants. The variety of fungi suggests a potential for producing bioactive compounds. These results clearly indicate that medicinal plants are abundant sources of endophytic fungal diversity, holding significant ecological and biotechnological value[9].

The frequency and dominance of fungal isolates revealed important patterns regarding the composition of endophytic communities within the studied medicinal plants. Alternaria species emerged as the most dominant endophytes, present in all plant species and tissues examined[2]. This dominance of Alternaria aligns with findings from multiple studies on endophytic fungi in medicinal plants worldwide. The genus Alternaria demonstrates remarkable adaptability and competitive capacity within plant tissues, contributing to its consistent presence across diverse host plants and environmental conditions[10]. The secondary distribution of *Cladosporium* and *Colletotrichum*, observed as moderately distributed across the plant materials, reflects the typical hierarchical structure of endophytic fungal communities in medicinal plants[11]. While *Chaetomium* was primarily associated with root tissues in the present study, this genus-specific tissue preference suggests ecological niche partitioning within the endophytic community. Studies examining endophytic populations in multiple medicinal plant tissues have similarly documented differential distribution of fungal genera across plant organs, indicating that tissue-specific factors influence colonization by particular fungal taxa[12]. These patterns of fungal distribution provide insights into community assembly processes and host-fungal selectivity within medicinal plants.

The findings of this study also have important implications for biotechnology and drug discovery. Endophytic fungi are increasingly recognized as a promising source of novel natural products with wide-ranging applications. The diversity of fungi observed in this study suggests that these isolates could be explored further for their potential to produce bioactive compounds useful in medicine, agriculture, and industry. From an ecological perspective, the association between medicinal plants and endophytic fungi can be viewed as a mutualistic relationship. The fungi benefit from the nutrients and protection provided by the host plant, while the plant gains advantages such as enhanced resistance to pathogens, improved tolerance to environmental stresses, and possibly increased production of secondary metabolites. However, the study has certain limitations. The identification of fungi was based primarily on morphological and microscopic characteristics, which may not always provide precise species-level identification. Advanced techniques such as molecular characterization (e.g., DNA sequencing) would enhance the accuracy of identification. Additionally, the functional roles of the isolated fungi were not experimentally evaluated, which could be an important area for future research.

Conclusion

This study demonstrated that *Tridax procumbens, Leucas aspera, Mimosa pudica*, and *Capsicum annuum* harbor a diverse community of endophytic fungi, with distribution varying across plant tissues and highest colonization observed in leaves. Dominant genera included *Alternaria*, along with *Chaetomium, Cladosporium,* and *Colletotrichum,* highlighting significant fungal diversity. The applied isolation and identification methods proved effective for recovering and preliminarily

classifying endophytes. These findings reinforce the importance of medicinal plants as reservoirs of endophytic fungi with the potential to produce bioactive secondary metabolites of pharmaceutical and biotechnological relevance. However, further studies involving molecular identification and bioactivity evaluation are necessary to fully explore their functional roles and applications.

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