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Isolation, Molecular Characterization, and Antioxidant Profiling of Endophytic Fungi Associated with *Leucas longifolia* Benth

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ABSTRACT

***Leucas longifolia* Benth. is a medicinally and nutritionally important plant whose endophytic fungal community has not been previously investigated. This study aimed to explore the diversity, distribution, and antioxidant potential of fungal endophytes associated with its leaves, stems, and roots. Fungal endophytes were identified at the molecular level through PCR amplification and sequencing of the ITS region, followed by NCBI BLAST analysis. Eight fungal species were recovered, including *Macrophomina phaseolina*, *Fusarium oxysporum*, *Arcopillus aureus*, *Diaporthe melonis*, and *Colletotrichum gloeosporioides*. *M. phaseolina* species showed the greatest distribution, colonizing the leaves, stems, and roots tissue, while *F. oxysporum* was predominantly isolated from roots and stems. The preliminary phytochemical analysis confirmed the production of secondary metabolites by the endophytic fungi. Antioxidant activity of the isolates was evaluated using the DPPH free radical scavenging assay. Spore suspensions of *A. aureus* and *D. melonis* exhibited the strongest antioxidant potential with scavenging activities of 38.42% and 41.73%, respectively. This first report on the endophytic mycobiota of *L. longifolia* reveals significant variation in tissue colonization among fungal species and identifies *A.* aureus and *D. melonis* as promising candidates for the development of natural antioxidant compounds with potential applications in pharmaceutical and nutraceutical industries.**

Keywords: *Leucas longifolia* Benth, Fungal endophytes, Qualitative phytochemical analysis, ITS sequencing, Anti-oxidant activity, DPPH assay

INTRODUCTION

In recent years, plant-associated endophytes have emerged as promising candidates for the sustainable production of pharmacologically active metabolites. Endophytes are microorganisms, primarily bacteria and fungi, which colonize internal plant tissues without causing apparent disease symptoms (Schulz and Boyle, 2006; Gouda *et al*., 2016). Endophytic fungi, in particular, have gained considerable attention due to their ability to synthesize a wide array of secondary metabolites, including alkaloids, terpenoids, flavonoids, phenols, steroids, and peptides. These compounds exhibit diverse biological activities such as antioxidant, antimicrobial, anticancer, anti-inflammatory, and insecticidal properties (Deshmukh *et al*., 2019; Asomadu *et al*., 2024; Alhadrami *et al.*, 2021). Moreover, endophytes offer several biotechnological advantages over host plants, including faster growth rates, easier genetic manipulation, and simpler large-scale cultivation (Omomowo and Babalola, 2019; Singh and Kumar, 2023).

Reactive oxygen species (ROS), including superoxide radicals, hydroxyl radicals, and hydrogen peroxide, are continuously generated during normal cellular metabolism. Excessive ROS production leads to oxidative stress, which is implicated in the pathogenesis of numerous diseases, including cancer, cardiovascular disorders, neurodegenerative conditions (e.g., Alzheimer's and Parkinson's), inflammation, and aging-related pathologies (Cheung *et al*., 2022; Gogna *et al*., 2024; Liang *et al*., 2025). Several studies have demonstrated that endophytic fungi produce potent antioxidants, with some isolates exhibiting high free radical scavenging activity in DPPH and H_2O_2 assays (Kumar and Prasher, 2024; Jia *et al*., 2025).

Leucas longifolia Benth. (Family: Lamiaceae) is a highly nutritious annual herb native to Western India and Sri Lanka, predominantly found in seasonally dry tropical habitats. Traditionally, various parts of the plant (leaves, stems, and roots) are used in folk medicine for wound healing, fever reduction, inflammation, pain relief, diabetes management, and central nervous system disorders. Other members of

the genus *Leucas* are known for their antioxidant, antimicrobial, and anticancer properties. Despite its ethnomedicinal significance and rich secondary metabolite profile (Pratima *et al.*, 2024), *L. longifolia* remains critically endangered (possibly extinct) in parts of its native range in Sri Lanka and is largely unexplored in modern pharmacological and microbiological research.

To date, no systematic study has investigated the endophytic fungal community associated with *L. longifolia*. Therefore, the present study was undertaken for isolation and molecular characterization of fungal endophytes from the leaves, stems, and roots of *L. longifolia* using ITS region sequencing, and to evaluate their antioxidant potential through the DPPH free radical scavenging assay. This research is the first attempt to explore the endophytic mycobiota and bioactive potential of this understudied medicinal plant.

MATERIALS AND METHODS

2.1 Chemicals and Culture Media

All chemicals and reagents used in this study were of analytical grade. Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) were procured from HiMedia Laboratories (Mumbai, India). Streptomycin, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ascorbic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Preparation of Culture Medium

Potato Dextrose Agar (PDA) was used for the isolation and maintenance of endophytic fungi. The medium was prepared by boiling 200 g of peeled potatoes in 1000 mL distilled water for 30 min. The extract was filtered, and the volume was adjusted to 1000 mL. To this, 20 g dextrose and 20 g agar were added. The pH was adjusted to 5.6 ± 0.2. The medium was sterilized by autoclaving at 121 °C and 15 psi pressure for 15 min. For bacterial suppression, streptomycin (250 mg L^{-1}) was added to the sterilized molten PDA before pouring into Petri plates.

2.3 Plant Material Collection and Authentication

Healthy, disease-free plants of *Leucas longifolia* Benth. were collected from Ramling hills (18.8334°N, 74.3404°E), Shirur tehsil, Pune district, Maharashtra, India, during the monsoon season (2025). Leaves, stems, and roots were carefully separated and immediately transported to the laboratory in sterile polythene bags. The plant specimen was authenticated by the Botanical Survey of India, Western Regional Centre, Pune, and a voucher specimen was deposited with the reference number RRILL1 **(Figure 1)**.

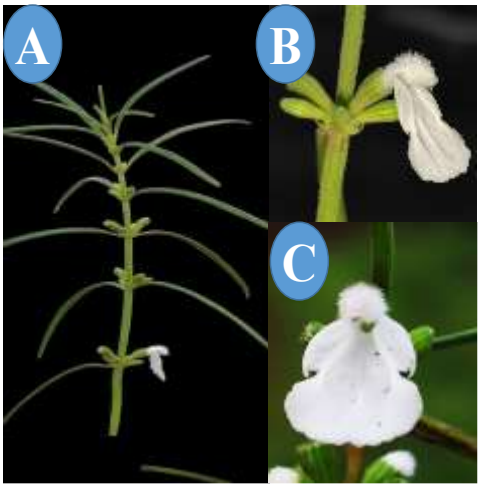


Figure 1: A: Plant body; B: Inflorescence; C: Flower of *Leucas longifolia* Benth.

2.4 Surface Sterilization and Isolation of Endophytic Fungi

Endophytic fungi were isolated following the protocol described by Abdalla *et al.* (2020) with minor modifications. Plant tissues (leaves, stems, and roots) were thoroughly washed under running tap water to remove surface debris. The tissues were cut into small segments (0.5–1.0 cm) and subjected to surface sterilization under aseptic conditions as follows: 70% ethanol for 30–60 seconds, 1–2% sodium hypochlorite solution for 2–4 minutes (depending on tissue type),70% ethanol for 30 seconds, followed by three rinses in sterile distilled water.

The sterilized segments were blot-dried on sterile filter paper and placed onto PDA plates supplemented with streptomycin (250 mg L^{-1}). Plates were incubated at 28 ± 2 °C in the dark for 7–14 days. Emerging fungal hyphae were transferred to fresh PDA plates to obtain pure cultures. All isolations were performed in triplicate.

2.5 Colonization Frequency (Cf%)

The colonization frequency of each endophyte was calculated using the formula:

Colonization Frequency (%) = (Number of segments colonized by fungus / Total number of segments examined) × 100

2.6 Molecular Identification of Fungal Isolates

Pure fungal isolates were grown on PDA for 7 days at 28 °C. Genomic DNA was extracted using a fungal genomic DNA isolation kit. The Internal Transcribed Spacer (ITS) region of the ribosomal DNA was amplified by PCR using universal primers

ITS1 and ITS4. The PCR products were purified and sequenced. The obtained nucleotide sequences were compared with available sequences in the NCBI GenBank database using the BLASTn tool. Phylogenetic analysis was performed using MEGA 12.0 software with the Neighbor-Joining method and 1000 bootstrap replications. Sequences were submitted to GenBank for accession numbers.

2.7 Evaluation of Antioxidant Activity (Dpph Assay)

The antioxidant potential of the fungal isolates was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay according to Osman et al. (2025) with slight modifications. Fungal isolates were grown in PDB for 10–14 days. The culture filtrate was extracted with methanol. Different concentrations of methanolic extracts (10–100 µg mL^{-1}) were mixed with 975 µL of 0.1 mM methanolic DPPH solution. The reaction mixture was incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Ascorbic acid (50 µg mL^{-1}) was used as the positive standard. The percentage of DPPH radical scavenging activity was calculated using the formula:

DPPH Scavenging Activity (%) = $[(A_0 - A_1) / A_0] \times 100$

where,

A_0 = Absorbance of the control (DPPH + solvent),

A_1 = Absorbance of the sample (DPPH + solvent + extract).

2.8 Qualitative Phytochemical Analysis of Spore Suspension Culture of Fungal Endophytes

Qualitative phytochemical screening was conducted on spore suspension culture of endophytic fungi using standard protocols to identify various bioactive compounds. The presence of alkaloids was detected by Wagner's test, in which extract fractions were treated with 3–5 drops of Wagner's reagent (prepared by dissolving 1.27 g iodine and 2 g potassium iodide in 100 mL water), resulting in a reddish-brown precipitate (Singh and Kumar, 2013). Tannins were identified using Braymer's test by mixing 0.5 mL of plant extract with 1 mL of water and 2 drops of 5% ferric chloride solution, which produced a blue or greenish-black coloration (Balasubramanian *et al*., 2024). Polyphenols were detected through the lead acetate test, where 1 mL of fungal extract was treated with a few drops of 1% lead acetate solution, forming a white precipitate. Saponins were verified using the foam test by vigorously shaking 2 mL of extract in a test tube and observing the formation of a stable foamy layer. Lignans were detected with Maule's test by adding a few drops of potassium permanganate solution and concentrated HCl to approximately 1 mL of crude extract, which produced a red coloration. Finally, flavonoids were confirmed using the alkaline reagent test, where 1 mL of extract was mixed with a few drops of dilute sodium hydroxide to produce an intense yellow colour that disappeared upon addition of dilute acid (Parvatham *et al.*, 2026).

2.9 Statistical Analysis

The experiment was conducted in at least four replicates. Data represented as mean of observation ± standard error (S.E). Data from the activity were analysed by using SPSS software.

RESULTS

3.1 Isolation and Molecular Identification of Endophytic Fungi

A total of eight distinct endophytic fungal isolates were successfully recovered from the surface-sterilized leaves, stems, and roots of *Leucas longifolia* Benth. (**Figure 2)**.

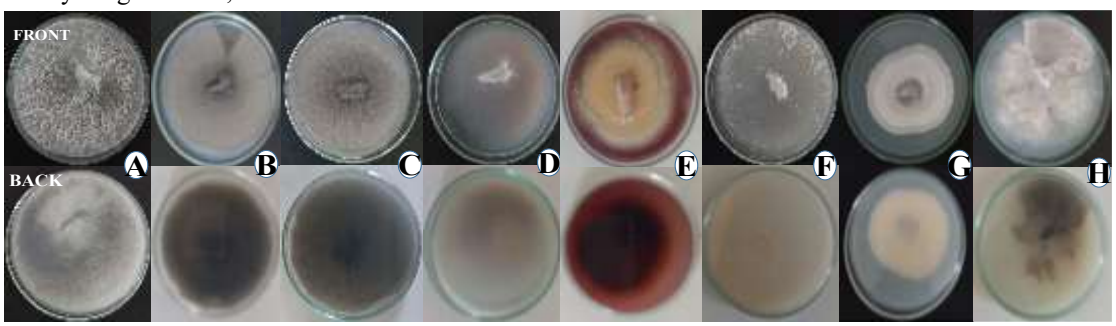


Figure 2: A: *Fusarium oxysporium* N-61-2; B: *Macrophomina phaseolina* RL 136; C: *Macrophomina pseudophaseolina* CMM2709; D: *Fusarium oxysporium*TPYD-6; E: *Arcopilus aureus* CBS: 386.83; F: *Diaporthe melonis* HSS1; G: *Colletotrichum gloeospordoides* MEBP 0008; H: *Macrophomina phaseolina* MP15

Molecular identification was carried out by amplifying the Internal Transcribed Spacer (ITS) region of the ribosomal DNA using universal primers ITS1 and ITS4. The PCR amplicons were visualized on agarose gel electrophoresis under UV transillumination, confirming successful amplification. Subsequent sequencing and BLASTn analysis against the NCBI GenBank database revealed 100% sequence similarity with reference strains for all isolates. The identified fungal species included *Macrophomina phaseolina* (three isolates), *Fusarium oxysporum* (two isolates), *Arcopillus aureus, Diaporthe melonis*, and *Colletotrichum gloeosporioides*. A Neighbour-Joining phylogenetic tree constructed using MEGA 12.0 software clustered all isolates with their respective reference sequences, supported by high bootstrap values, thereby confirming their taxonomic identity (**Figure 3**). The GenBank accession numbers, closest matching sequences, percentage similarity, and strain details are presented in Table 1. This molecular approach provided robust and reliable species-level identification, which is essential for accurate documentation of endophytic diversity**.**

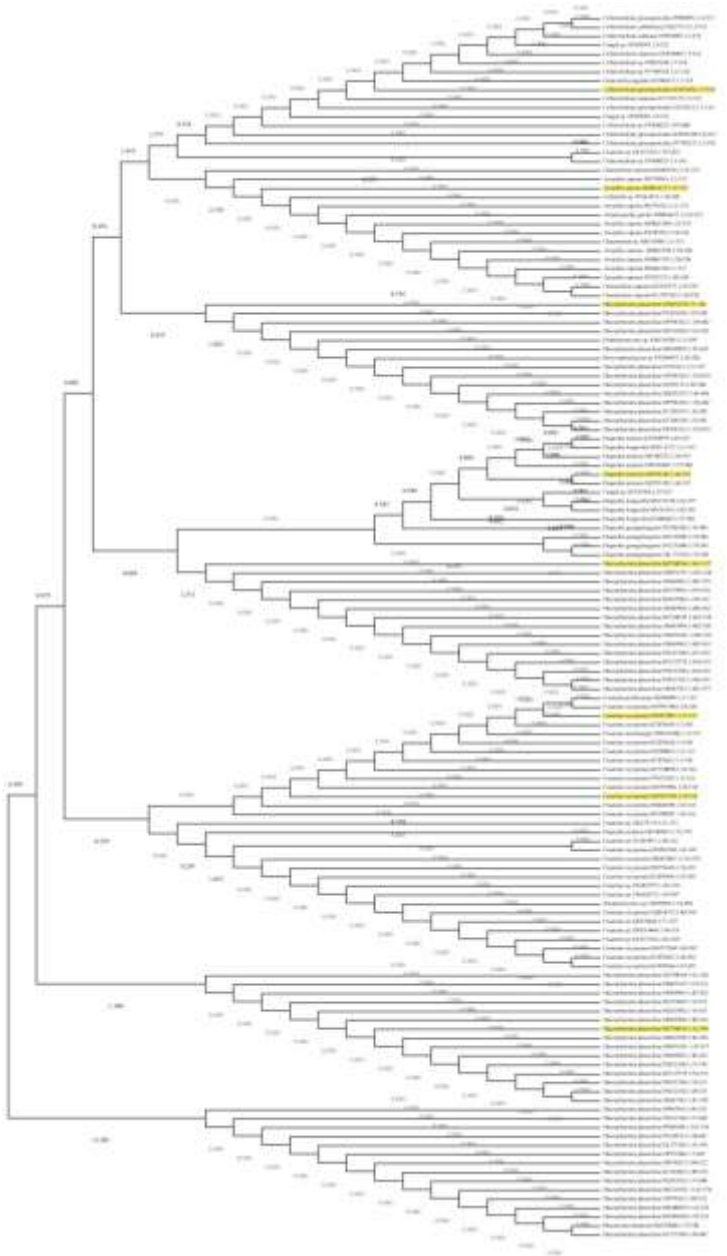


Figure 3: List of ITS rDNA based identified fungal endophytes with accession numbers and similarity percentage (%).

Table 1: List of ITS rDNA based identified fungal endophytes with accession numbers and similarity percentage (%).

Strain id	Strain Name	Similarity (%)	Accession No.
LLR_1	*M. phaseolina*	100	MZ768564.1
LLR_2	*F. oxysporium*	100	MN959987.1
LLS_1	*F. oxysporium*	100	MN545304.1
LLS_2	*M. phaseolina*	100	MZ768545.1
LLS_3	*C. gloeosporioides*	100	KJ676456.1
LLL_1	*A. aureus*	100	MH861615.1
LLL_2	*D. melonis*	100	MZ995183.1
LLL_3	*M. phaseolina*	100	OM674338.1

3.2 Colonization Frequency and Tissue Specificity

Considerable variation was observed in both colonization frequency and tissue distribution of the endophytic fungi within *L. longifolia* (Figure 3 A and B). The genus *Macrophomina* was the most widespread, successfully colonizing all three plant parts like leaves, stems, and roots, indicating its versatile endophytic lifestyle in this host. *F. oxysporum* was predominantly recovered from stems and roots, suggesting a preference for vascular tissues. Various diversity indices, evenness, and species richness of endophytic fungi isolated from *L. longifolia* Benth help to understand ecological diversity of fungal endophytes (Table 2).

Table 2: Diversity indices, evenness, and species richness of endophytic fungi isolated from *L. longifolia* Benth.

Sr. No.	Parameters	Results
1.	Species Richness (S)	08
2.	Abundance	34
3.	Shannon- Weiner diversity (H')	1.9602
4.	Simpson diversity (D)	0.8806
5.	Species Evenness (E)	0.9426
6.	Dominance Index (λ)	0.1449

Overall, leaves and stems showed markedly higher colonization frequencies compared to roots. *F. oxysporum* exhibited the highest colonization frequency at 58.33%, making it the dominant endophyte in the present study. This was followed by *M. phaseolina* and *A. aureus*, each recording a colonization frequency of 16.66%. The remaining isolates displayed relatively lower colonization frequencies (**Figure 5 and 6**).

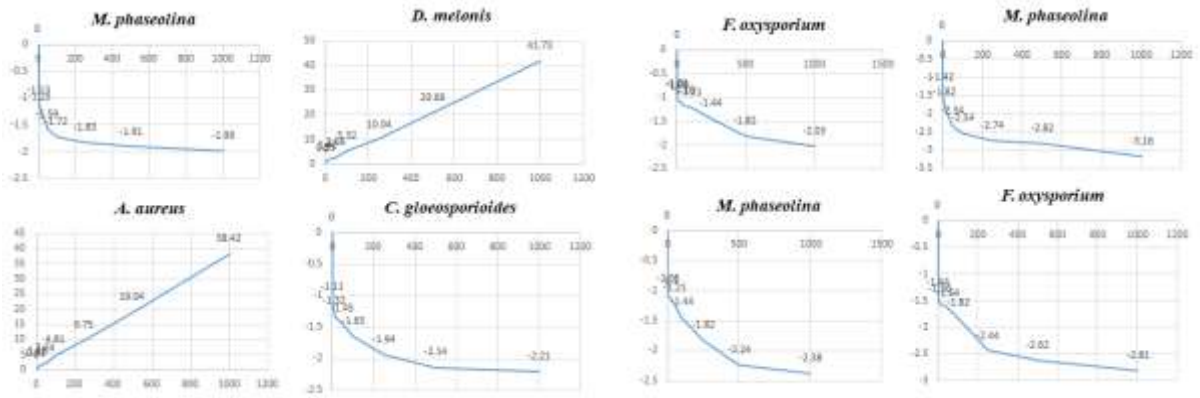


Figure 4: DPPH Scavenging Activity (%)

Tissue-specific isolation frequency analysis (**Figure 3)** further highlighted differential colonization patterns. *F. oxysporum* demonstrated the highest isolation frequency (19.44%), primarily from stem segments. *D. melonis* ranked second with 16.66% isolation frequency, predominantly recovered from leaf tissues. In contrast, the lowest isolation frequency (5.55%) was recorded for *A. aureus*. *C. gloeosporioides* and *M. phaseolina* were mainly isolated from roots with an isolation frequency of 13.88% each, while *M. phaseolina* from leaves showed 11.11% frequency. *F. oxysporum* isolated from roots accounted for 8.33%. These findings clearly demonstrate tissue-specific preferences among the endophytic fungal community associated with *L. longifolia*.

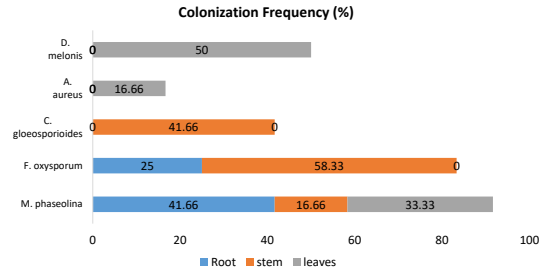


Figure 5: Frequency of colonization (%) of each fungal endophyte isolated from roots, stem, and leaves of *L. longifolia* Benth.

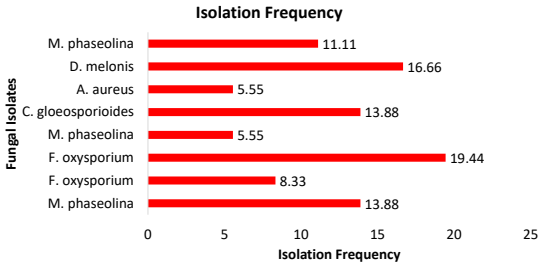


Figure 6: Isolation frequency of each fungal endophyte isolated from roots, stem, and leaves of *L. longifolia* Benth.

3.3 Antioxidant Activity of Endophytic Fungal Extracts

The aqueous extracts obtained from the eight endophytic fungal isolates exhibited prominent and concentration-dependent DPPH free radical scavenging activity (**Figure 4**). Antioxidant potential varied significantly not only among different fungal species but also according to the plant tissue from which the isolates were originally recovered, suggesting possible tissue-specific biosynthesis of bioactive secondary metabolites.

Among all tested isolates, *Diaporthe melonis* displayed the highest DPPH radical scavenging activity with 41.73%, followed closely by *Arcopillus aureus* at 38.42%. Both isolates demonstrated substantially stronger antioxidant capacity compared to the other fungi. A clear dose-dependent response was observed across all concentrations tested (typically 10–100 µg mL^{-1}), with higher extract concentrations resulting in greater decolorization of the stable DPPH radical at 517 nm. This decolorization reflects the efficient hydrogen-donating ability of the fungal secondary metabolites, most likely phenolic compounds, flavonoids, or other reducing agents.

The observed antioxidant activities position the endophytic fungi from *L. longifolia*, particularly *D. melonis* and *A. aureus*, as promising candidates for the development of natural antioxidants. These results are comparable to previous reports on endophytic fungi isolated from other medicinal plants, further supporting the biotechnological significance of exploring endophytes from understudied species.

Qualitative screening of secondary metabolites was carried out in the spore suspension culture filtrates of eight endophytic fungal isolates using standard biochemical tests. The results are summarized in Table 1. Polyphenols were the predominant secondary metabolites, detected in seven out of eight fungal isolates tested. The leaf-derived *M. phaseolina* isolate showed a negative result for polyphenols. Alkaloids were present in four isolates, specifically root and stem mediated *F. oxysporum* and root-derived *M. phaseolina*, and the leaf-derived *A. aureus* isolate. Tannins were detected in four isolates, including both *F. oxysporum* isolates, *A. aureus*, and *D. melonis* (**Table 3)**.

Table 3 Qualitative phytochemical analysis of spore suspension culture of fungal endophytes

Sr.No:	Secondary metabolite	Test name	Results							
			Macrophomina phaseolina	*Fusarium oxysporum*	*Macrophomina phaseolina*	*Fusarium oxysporum*	*Colletotrichum gloeosporioides*	*Macrophomina phaseolina*	*Arcopillus aureus*	*Diaporthe melonis*
1.	Alkaloids	Wagner's test	+	+	-	+	-	-	+	-
2.	Tannins	Braymer's test	-	+	-	+	-	-	+	+
3.	Polyphenols	lead acetate test	+	+	+	+	+	-	+	+
4.	Saponins	foam test	-	+	+	+	-	+	-	-
5.	Lignans	Maule's test	-	-	-	-	-	-	-	-
6.	Flavonoids	alkaline reagent test	-	-	-	-	+	-	-	-

Saponins were recorded in four isolates with an inconsistent pattern across the tested fungi. Flavonoids were detected exclusively in *C. gloeosporioides* and were absent in all other isolates. Lignans were not detected in any of the fungal isolates examined in the present study.

Marked variation in secondary metabolite profiles was observed among different isolates of the same species. Particularly, the three isolates of *M. phaseolina* and the two isolates of *F. oxysporum* showed differences in the presence or absence of alkaloids, tannins, and saponins under identical culture conditions. This indicates considerable intra-specific variability in secondary metabolite production among these endophytic fungi.

DISCUSSION

A total of eight fungal endophytes were isolated from the leaves, stems, and roots of *Leucas longifolia*. Earlier studies on related *Leucas* species, for example *L. aspera* and *L. martinicensis*, have also been shown to harbor diverse endophytic fungal communities with biomedical potential, highlighting the genus as a promising reservoir for bioactive mycoendophytes.

Several of the recovered taxa have been reported as endophytes from various other plant hosts, supporting their classification as host generalists (Kalinga *et al*., 2025; Sun *et al*., 2024; Wary *et al*., 2022; Sevillano-Caño *et al*., 2025; Ayob and Simarani, 2016). Molecular identification via ITS sequencing (Table 1) assigned the isolates as *M. phaseolina* (MZ768564.1), *F. oxysporum* (MN959987.1), *F. oxysporum* (MN545304.1), *M. phaseolina* (MZ768545.1), *C. gloeosporioides* (KJ676456.1), *A. aureus* (MH861615.1), and *D. melonis* (MZ995183.1), *M. phaseolina* (OM674338.1) (**Figure 2**). *M. phaseolina* emerged as the most abundant isolate across all plant tissues.

M. phaseolina was previously documented as a root endophyte of *Brugmansia aurea*, where classical and epigenetic approaches revealed production of bioactive secondary metabolites including phomeolic acid, ergosterol peroxide, and various volatile compounds. Its secretome contains antioxidant enzymes (e.g., thioredoxins, glutathione S-transferase, catalases, and superoxide dismutases) that help mitigate reactive oxygen species (ROS) during plant-fungus interactions. The present study provides the first report of *M. phaseolina* as an endophyte isolated from the leaves and stems of *L. longifolia*. *F. oxysporum* strains were recovered from both roots and stems, aligning with its common endophytic lifestyle in various hosts; non-pathogenic *F. oxysporum* isolates frequently colonize plants asymptomatically and contribute to genetic diversity within the species complex.

Aspergillus aureus has been isolated from leaves of *Vitis vinifera*, where it supports resveratrol production (Dwibedi and Saxena, 2018), and from roots of *Panax notoginseng* (Sun *et al*., 2024). Endophytic *Arcopilus aureus* (syn. *Aspergillus aureus*) demonstrated notable antioxidant activity with low IC_{50} values in DPPH assays. *Diaporthe melonis*, earlier reported from *Annona squamosa,* produces dihydroanthracenone metabolites with anti-inflammatory, neuroprotective, and antimicrobial properties (Ola *et al*., 2014). Broader reviews confirm that *Diaporthe* species are prolific producers of bioactive compounds exhibiting antioxidant, cytotoxic, antifungal, and anti-inflammatory activities. *Colletotrichum gloeosporioides* was previously isolated from *Thevetia peruviana*, with its metabolites applied in the green synthesis of TiO_2 nanoparticles for dye-sensitized solar cells (Singh *et al*., 2025).

The isolation and study of endophytes remain challenging due to their cryptic nature and the complexity of host-microbe interactions. Comprehensive reviews emphasize endophytic fungi as prolific sources of novel secondary metabolites with pharmacological importance, including antioxidants that protect against oxidative stress-related diseases. In the present phylogenetic analysis, the neighbour-joining method based on ITS sequences illustrated close relationships among isolates, consistent with findings in other hosts such as *Alhagi sparsifolia* (Tuerdibieke *et al.*, 2024). Due to morphological similarities and vast fungal diversity, molecular techniques using the ITS region as a primary DNA barcode remain essential for accurate identification (Rehman *et al*., 2026).

Aqueous extracts of the eight isolates were screened for antioxidant activity at varying concentrations using standard assays. *D. melonis* and *A. aureus* exhibited the highest antioxidant potential. These results corroborate broader literature documenting strong antioxidant capacities in endophytic

Aspergillus, *Diaporthe*, *Fusarium*, and *Colletotrichum* species, often linked to phenolic compounds, flavonoids, and polyketides. In previous studies, endophytic fungi *Aspergillus* Sp. 3. (78.17%), *Aspergillus* Sp. 2 (76.8%), *Chaetomium* Sp. (75.42%) and *Penicillium* Sp. 6 (45.1%) isolated from *A. alopecuroides*, *A. setifera*, *A. halimus* L., *H. strobitaceum*, *L. camara*, *M. forsskaollii*, *R. raphanistrum*, *S. vermiculata*, *Z. coccineum* showed the highest antioxidant activity (Osman *et al.,* 2025).

This study offers the first detailed insight into the mycoendophyte diversity of *Leucas longifolia*. It establishes novel host associations, particularly *M. phaseolina* as a leaf endophyte and *M. phaseolina* as a stem endophyte within the genus *Leucas*. These findings provide a foundational baseline for future bioprospecting efforts targeting the Lamiaceae family and underscore the industrial and pharmaceutical potential of these fungi especially *D. melonis* and *A. aureus* as sustainable sources of antioxidant metabolites. Further chemical characterization and in vivo validation of their bioactive compounds are warranted to explore applications in medicine, agriculture, and nanotechnology.

The branches of the phylogenetic tree showed the closeness of the fungal isolates at the molecular level. In the current study, the neighbour-joining method was used to describe closely related species. A similar study was performed by Tuerdibieke *et al.* (2024) on *A. sparsifolia*; the fungal endophytes isolated from this host showed a close relationship in the phylogenetic tree based on ITS sequence using the similar neighbour-joining approach. Fungal identification can be quite challenging due to their vast diversity, limited primary data, and similarities in the physical appearances among different species. Hence, besides morphological identification, molecular phylogenetic techniques were used. These techniques are crucial for the accurate classification of endophytic fungi. The DNA barcoding technique, which utilizes specific DNA sequences for species classification, often relies on the Internal Transcribed Spacer (ITS) region as a widely accepted and most reliable molecular marker for identifying a broad array of kingdom Fungi (Rehman, *et al.,* 2026).

In this study, the aqueous extracts of the 8 different endophytic fungi isolated from *L. longifolia* leaves, stems and roots were screened for their antioxidant efficacy at different concentrations. Out of these, two fungal endophytes significantly showed the highest antioxidant activity. The current study of isolation and molecular identification of fungal endophytes offers an insight into the unexplored diversity of mycoendophytes isolated from the root, stem and leaves of *L. longifolia*. The molecular characterization showed that the fungal strains belong to different genera. This study is the first to report *M. phaseolina* as a leaf-derived endophyte and *M. phaseolina* as a stem-mediated endophyte. These insights will be useful as research of preliminary evaluation and background for the isolation of endophytic fungi from the genus *Leucas* and for further prospecting of their mycoendophytes for diverse roles in different sectors. Both *D. melonis* and *A. aureus* were identified as strong antioxidant fungal endophytes.

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