Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 26, No. (4) : 2024 : 497-506
© EM International
ISSN-0972-3005

DOI No.: http://doi.org/10.53550/AJMBES.2024.v26i04.009

ENDOPHYTIC FUNGI INHABITANTS OF *HULTHOLIA MIMOSOIDES*- ISOLATION, IDENTIFICATION AND ANTIMICROBIAL ACTIVITY

NINGARAJU S. AND MANJULA ISHWARA KALYANI*

Department of Studies and Research in Microbiology, Mangalore University, P.G Centre, Chikka Aluvara, Kodagu 571 232, Karnataka, India

(Received 3 August, 2024; Accepted 8 October, 2024)

Key words: *Hultholia mimosoides, Internal Transcribed Spacer, Antimicrobial, Penicillium glabrum, Cladosporium herbarum.*

Abstract–Fungi that dwell inside the host plant's tissue without damaging it are known as endophytic fungi. Endophytic fungal strains established in medicinal plants can create bioactive chemicals, particularly secondary metabolites. For several biological fields, they have been recognized as possible sources of new natural products. Research into endophytic fungi is still in its early phases, even though all plants seem to have fungi with some bioactive content and activity. In this study, endophytic fungi from *Hultholia mimosoides* were isolated, identified and their anti-microbial activity was assessed. Twelve morphologically distinct endophytic fungi isolated, were identified based on their ITS region of 18s RNA and the same was reported to GenBank. All the isolates subjected to ethyl acetate solvent extraction, in order to determine the anti-microbial activity. The endophytic fungi were identified through both macroscopic and microscopic examinations. The morphological characteristics and the microscopic observations aided in determining the endophytic fungi. These endophytes were further confirmed and classified as *Fusarium chlamydosporum (OQ683919), Fusarium oxysporum (OQ683924), Diaporthe longicolla (OQ685299), Cladosporium herbarum (OQ685410), Aspergillus tubingensis (OQ685748), Fusarium solani (OQ685892), Penicillium madriti (OQ603494), Colletotrichum lindemuthianum (OQ678261), Fusarium oxysporum (OQ680483), Fusarium incarnatum (OQ680522), Fusarium equiseti (OQ680709),* and *Penicillium glabrum (OQ683918).* In order to assess the antibacterial activity of endophytic fungi against six tested bacterial pathogenic organisms, their crude metabolites were extracted using ethyl acetate solvent. The crude extracts of all endophytic fungi demonstrated antibacterial activity. Among them *Penicillium glabrum (OQ683918)* and *Cladosporium herbarum (OQ685410)* exhibited the highest zones of inhibition against the four bacterial pathogens.

INTRODUCTION

Edible plants are highly valued not only for their vital components, synthesizing vitamins and minerals but also for their intrinsic medicinal properties for human consumption. Plant species of various diversity contribute significantly as primary producers in the biotic system. The different components of the plants serve as food, fodder, and also considered one of the intermediate kingdoms balancing the existence of microbes and animal kingdom. Further considering as one of rich natural resources on earth, the plant biologically synthesis and accumulate macromolecules and micronutrients that include species specific carbohydrates, proteins, lipids, that leads to synthesize of qualitative components of alkaloids, terpenoids, flavonoids and steroids. The chemical and structural diversity of the various bioactive metabolites varies significantly between plant genera and species.

Hultholia mimosoides is a tiny spiky tropical tree or climbing shrub belonging to the family of *Fabaceae*. This plant previously known as *Caesalpinia mimosoides,* was replaced taxonomically as *Hultholia mimosoides.* The plant is widely distributed in many parts of Asia. In our study, the region of Chikka Aluvara of Kodagu district in Karnataka, the people in this region are customized of consuming the plant's leaves and fresh young twigs of the plant as an appetizer side dish and locally it's called by name as *Arijinke Kudi*. It is also learned from the local

inhabitants in the area, that the plant is consumed for curing disorders of fainting and dizziness, as well as an anti-flatulent. Additionally, on scientific investigation it has been revealed that *H. mimosoides* leaf extract contains anti-inflammatory, anti-cancer, antioxidant, and antibacterial effects. Numerous phenolic chemicals, including flavonoids and gallic acid, which are well-known antioxidants, have been reported in *H. mimosoides* (Chanwitheesuk *et al*., 2005, 2007; Yodsaoue *et al*., 2010; Rattanata *et al*., 2016).

Also intrinsically and symbiotically plant associated beneficial microorganisms are crucial towards contributing to reservoir of the vital elements in plant. These valuable metabolic constituents have positive impact upon human consumption. It is well understood, that plant-microbial interactions are expressed through different parts of the plant and classified as phyllosphere microbes if microbes are associated with leaves, rhizosphere microorganisms if associated with plant rhizosphere zone, or rhizoplane microorganisms on root surface likewise the endophytes are microorganisms that are found associated symbiotically as intrinsic inhabitants. Endophytes are generally beneficial microorganisms growing asymptomatically within the tissue of plants (Joseph and Priya, 2011). The fungi endophytes are the most frequently dwelling microorganisms compared to bacteria and actinomycetes (Li *et al*., 2012). An endophytic fungus can sustain through mutual biochemical interactions along plant cellular organization. This biochemical interaction along with molecular exchange between endophytic fungi and plant cells could possibly achieved by spreading and penetration of mycelial hyphae in to plant internal tissues and cells. This ineffective mycelial and hypha are the morphological markers for the existence of potential endophytes within any plant spices (Kaul *et al*., 2012).

Thus, endophytic fungi present in a particular plant sp., will definitely contribute specifically and synergistically for biochemical synthesis of unique metabolites in host plants. Various external physical and environmental factors such as aeriation, humidity, temperature, soil qualitative properties and nutrients availability at a particular region selected also contribute the differential diversity of endophytic fungi in plants and differential composition of unique metabolites.

Endophytes are known to produce certain antibiotics, anticancer agents, antioxidants, immunosuppressants, and involved in biological control mechanisms. Notably, endophytic fungi belonging to genera *Colletotrichum, Fusarium, Alternaria,* and *Aspergillus* demonstrate significant antioxidant activity and antimicrobial activity (Idris *et al*., 2013; Kumar and Kaushik, 2013; Nighat Fatima *et al*., 2016; Dhayanithy *et al*., 2019). A compound named "Terrien," displaying antimicrobial and antitumor properties, has been identified in the endophytic fungus *Aspergillus terreus* isolated from *Achyranthes aspera* (Goutam *et al*., 2017). Henceforth there remains a huge demand for the discovery of new novel bioactive metabolites from endophytic sources. For instance, a new derivative of a-tetralone, (3S)-3,6,7-trihydroxy-a-tetralone, along with cercosporamide, b-sitosterol, and trichodermin, has been reported from the endophytic fungus *Phoma* sp. residing within *Arisaema erubescens* (Wang *et al*., 2012).

Hence, in the present study, we aimed an investigation on the isolation of fungal endophytes occurring in *H.mimosoides,* an edible medicinal plant species in the region of Chikka Aluvara Kodagu. The tender meristem part of the plant has been considered as an edible part and having medicinal properties was subjected for endophyte fungal isolation and analysis of their antimicrobial activity.

MATERIALS AND METHODS

Plant materials

The *Hultholia mimosoides* plant was collected from the Mavinahalla forest of the Western Ghats region. The healthy stem and leaves were selected to avoid interferences by plant pathogens and the samples were transferred using sterile polythene bags and processed as early as possible within 24h to the laboratory for endophytic fungi isolation. The *H. mimosoides* plant was identified and authenticated by Dr. Niranjan Raj S, Department of Botany/ Microbiology, KSOU, Mukthagangothri, Mysuru and a voucher specimen *Hultholia mimosoides* KSOUB22S01 was deposited.

Isolation of endophytic fungi from *Hultholia mimosoides*

The protocol followed for the isolation of endophytic fungi from the plant leaves and stem parts in brief involved the removal of dust and debris, the plant material was chopped into small pieces in an aseptic environment using a sterilized

blade. The plant components were surface sterilized with 70% ethanol for 1 min before being submerged for 1 min in a sodium hypochlorite (NaOCl) solution. The samples were washed in sterile distilled water for a minute before being surface-dried on filter paper. Surface sterilized leaf and stem fragments were placed on a PDA plate along with an antibiotic supplement (Chloramphenicol - 1 mg/ml) and cultured for 5 to 7 days at 28 ^{o}C. The pure cultures in PDA slants were stored at 4° C with correct labeling and periodically subcultured (Petrini, 1991).

Calculation of colonizing frequency

The colonizing frequency (CF) of each endophytic fungus was calculated by counting the segments colonized by fungi to that of the number of segments observed (Suryanarayanan *et al*., 2003).

Identification of endophytic fungi

Morphological Characterization of endophytic fungi

The endophyte growth on the PDA media was observed. Each fungal colonies were sub-cultured into a fresh PDA medium and observed for their growth characterization such as their mycelial growth, coloration and texture. Further mycelium colonies were mounted on a clean glass slide for lacto phenol cotton blue staining for their microscopic analysis.

Molecular identification of fungal isolates using ITS-Primer

A volume of 1000 µl of CTAB Extraction Buffer was added to 0.5 g of fungal sample. The mixture was then homogenised and heated in a 60 °C bath for 30 minutes. After the incubation period, subjected the homogenate to vertical centrifugation for 5 min at 14000 x g. Added an equivalent amount of chloroform/isoamyl alcohol in a 24:1 ratio. Achieved phase separation by vertexing the sample for 5 seconds and then centrifuging it at 14,000 x g for 5 minutes. Decanted the liquid top phase into a fresh tube. DNA precipitation was achieved by adding 0.7 volumes of cold isopropanol and incubating at -20 °C overnight. Transfered 750 microlitres each time onto the DNA column. Spin at 12000 rpm for 1 minute. Dispensed 750 µl of wash buffer and spin at 12000 revolutions per minute for 1 minute. Proceeded to repeat the wash buffer cycle. Centrifuged the DNA column at 12000 rpm for 2 minutes. Dispensed 20 µl of elution buffer, allow to stand at room temperature for 3 minutes, and then spin at 12000 rpm for 1 minute. Dispensed 1µl of RNase solution A and then incubated at 37 °C for 30 minutes. After that the PCR conditions was set up as per the kit manufactures protocol. The species-specific primers ITS-1 (5′-TCCGTAGGTGAA CATGCGG-3′), ITS-4 (5′-TCCTCCGCTTATTGA TATGC-3′) were used for PCR to amplify DNA. To evaluate the amplification efficiency and produce multiplex PCR assays for DNA barcode primers, each PCR reaction consists of 1µl of DNA template (25 ng), 2µl of 10X reaction buffer, 0.5µl of $MgCl_2$ (50pM), 1 µl of dNTPs mix (10mM), 1µl of forward primer (10pM), 1 µl of reverse primer (10pM), 0.5 µl of Taq polymerase (5 U/pi), and a final volume of 25 µl. The final volume adjusted to 25 µl. The primers are standard primers used for amplifying the 18srRNA gene. Electrophoresis of the PCR product was performed using 0.8% agarose (Himedia Laboratories,) over TBE buffer at a voltage of 100 V (BioRad Laboratories). The amplified product were submitted to sequencing for subsequent study. Analysis of the findings was conducted using the sequencing analysis software Mega11.

Endophytic fungal and their antimicrobial activity

The antibacterial activity of the endophyte fungal ethyl acetate extracts were tested using the well-diffusion technique. A 90 mm petri plate with nutrient agar medium was inoculated with 0.1 ml of bacterial pathogen. A 9 mm-diameter wells were bored and 10 mg/ml of crude endophytic fungal extract were loaded. Chloramphenicol (1mg/ml) was employed as the control (0.05 ml) the plates were incubated at 37 ^{0}C for 24h.

RESULTS

Isolation of endophytic fungi

Endophytic fungi were isolated from stem and leaf parts of the *H. mimosoides* plant using PDA media. A total of 48 segments were placed to the media from both the stem and leaves of the *H. mimosoides* plant. Upon seven days of growth on PDA media endophytic fungal culture were observed, the colonization rates of the endophytic fungal isolates were 37.5% from the leaf segments and approximately 70.8% from the stem segments. Based on morphological distinctions, twelve endophytic fungal cultures were selected and further subcultured for identification. Each leaf fungus isolate was assigned a unique code, such as HLF-1, HLF-9, HLF-10, HLF-12, HLF-25, and HLF-27. For

stem isolates, codes such as HSF-2, HSF-3, HSF-4, HSF-17(a), HSF-17(b), and HSF-27 were used.

Morphological and Microscopic Identification

The endophytic fungi isolated from the medicinal plant *H. mimosoides* were initially observed for colony morphology, growth pattern and coloration. When observed under 40X with lactophenol cotton blue staining exhibited spore structures and unique shape with distinctive conidiophores, based on these properties isolates were identified belonging to *Fusarium* sp, *Penicillium* sp, *Aspirigillus* sp, *Colletotrichum* sp, and *Cladosporium* sp. Few fungal lack of spore structure thus grouped under sterile mycilia as shown in Figures 1 and 2.

Molecular identification of endophytic fungi

Following electrophoresis, the isolated genomic DNA was clearly visible as a well-defined band on the agarose gel. The integrity of the DNA was verified by the existence of undamaged, high-molecular-weight bands with limited interspersion, suggesting little to no deterioration. Spectrophotometric analysis was used to evaluate the concentration and purity. The A260/A280 ratios fall within the range of 1.8–2.0, suggesting a high level of purity appropriate for subsequent uses. The effective amplification of the PCR products was shown by the presence of well-defined bands on the gel, which correspond to the anticipated sizes of the amplicons. No primer-dimer formations were seen, and the negative control lanes exhibited no amplification, conclusively indicating the lack of contamination. The polymerase chain reaction (PCR) amplicons were separated on a 0.8% agarose gel. Clear, defined bands were detected, with the dimensions of the PCR products aligning with the anticipated target fragments. To verify the accurate amplification of the target regions, a DNA ladder was employed as a size marker. The lack of non-specific bands provided additional confirmation of the specificity of the PCR assay.

After the gel purification the DNA product was subjected to Sanger sequencing PCR. Homologous analysis of the 12 fungal sequences in the current investigation revealed >90% similarity to those in the GenBank database with BLAST hit. A 98.75% sequence similarity between HLF 1 and *Fusarium chlamydosporum* was observed. The resemblance between HLF 9 and *Fusarium oxysporum* is 97.89% and HLF 10 and *Diaporthe longicolla* is 97.72%. HLF 12 and *Cladosporium herbarum* has shown 97.31%, HLF 25 and *Aspergillus tubingensis* has shown 97% and HLF 27 and *Fusarium solani* have 96.80% commonality. Distinct HSFs have been found to have resemblance with distinct endophytic fungi. Where the comparable characteristics of HSF 2 with *Penicillium madriti* was 99.24 %. HSF 3 with *Colletotrichum lindemuthianum* was 98.48%, HSF 4 with *Fusarium oxysporum* was 99.10%. HSF 17(a) and HSF 17(b) have shown 98.77% and 97.81% resemblance with *Fusarium incarnatum* and *Fusarium equiseti* respectively. HSF 27 has shown 97.44% commonality with *Penicillium glabrum* Table 1. To know the fungal history phylogenetic tree was generated depicted in Figures 3 and 4.

Antimicrobial activity

Ethyl acetate is a moderately polar solvent, which helps in selectively extracting a range of secondary

Table 1. Molecular Identification of endophytic fungi with GenBank accession number

Sl No.	Fungal isolates	Microscopic identification	Molecular identification with GenBank Accession number
1	HLF-1	*Fusarium* sp	*Fusarium chlamydosporum* OQ683919
2	HLF-9	Sterile mycilia	*Fusarium oxysporum* OQ683924
3	HLF-10	Sterile mycilia	*Diaporthe longicolla* OQ685299
4	HLF-12	*Cladosporium* sp	*Cladosporium herbarum* OQ685410
5	HLF-25	*Asperigillus* sp	*Aspergillus tubingensis* OQ685748
6	HLF-27	Sterile mycilia	*Fusarium solani* OQ685892
7	HSF-2	*Penicillium* sp	*Penicillium madriti* OQ603494
8	HSF-3	*Colletotricum* sp	*Colletotricum lindemuthianum* OQ678261
9	HSF-4	*Fusarium* sp	*Fusarium oxysporum* OQ680483
10	HSF-17(a)	Sterile mycilia	*Fusarium incarnatum* OQ680522
11	HSF-17(b)	Sterile mycilia	*Fusarium equiseti* OQ680709
12	HSF-27	*Penicillium* sp	*Penicillium glabrum* OQ683918

*HLF= Hultholia Leaf Fungi and HSF= Hultholia Stem Fungi.

metabolites, including terpenoids, alkaloids, and flavonoids. Ethyl acetate was used for extraction of fungal crude extract. To determine the antibacterial activity of fungal ethyl acetate extracts, a well diffusion method was followed to check the efficiency of all the endophytic fungi against antibiotic resistant strains of bacteria, *Bacillus cereus* ATCC 10876, *Proteus vulgaris* ATCC 13315, *Klebsiella pneumonia* ATCC9621, *Salmonella typhimurium* ATCC23564, and *Escherichia coli* ATCC 8739. Isolated endophytic fungi from *H. mimosoides*, *Fusarium chlamydosporum* OQ683919, *Aspergillus tubingensis* OQ685748, showed activity against *Staphylococus aureus* ATCC23565, and *Fusarium oxysporum* OQ683924 showed activity against *Salmonella typhimurium* ATCC23564, also *Fusarium incarnatum* OQ680522 showed activity against *Klebsiella pneumonia* ATCC9621. *Diaporthe longicolla* OQ685299, *Fusarium solani* OQ685892, *Fusarium equiseti* OQ680709 have showed activity for three pathogens. *Penicillium madriti* OQ603494 showed activity for *Klebsiella pneumonia* ATCC9621 and *Staphylococus aureus* ATCC23565. *Cladosporium herbarum* OQ685410, *Colletotricum lindemuthianum* OQ678261, *Fusarium oxysporum* OQ680483, *Penicillium glabrum* OQ683918 each fungal extracts showed activity for four different pathogens in comparison with the positive control Chloramphenicol. Among all these endophytes, prominent fungi *Cladosporium herbarum* OQ685410 showed significant antibacterial activity against *Protues vulgariou* with a zone of inhibition of 13 mm followed by activity against *Klebsiella neumonia* ATCC9621 with a 11 mm zone of inhibition and 6 mm and a 2 mm zone of inhibition was estimated against *Escherichia coli* ATCC 8739, and *Salmonella typhimurium* ATCC 23564 respectively. *Penicillium glabrum* OQ683918 showed activity against *Klebsiella pneumonia* ATCC9621 with the zone of inhibition 8 mm, followed by a 16 mm of zone of inhibition against *Salmonella typhimurium* ATCC23564, 10 mm zone of inhibition against *Escherichia coli* ATCC8739 and 2 mm zone of inhibition *Staphylococus aureus* ATCC23565 Table 2.

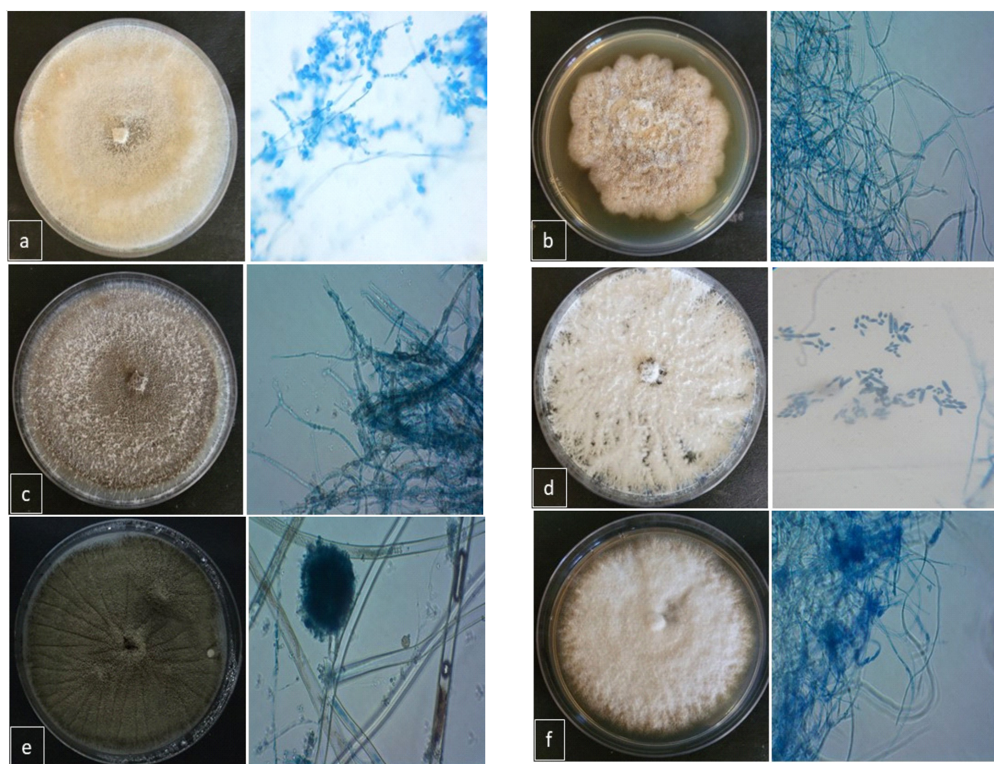

Fig. 1. Morphology and microscopic view of the endophytic fungi isolated from *Hultholia mimosoides* - a) *Fusarium chlamydosporum* OQ683919; Conidiophores are simple, branched irregular, slightly curved. b) *Fusarium oxysporum* OQ683924; Sterile mycilia. c) *DiaportheLongicollaI*OQ685299; Sterile mycilia. d) *Cladosporium herbarum* OQ685410; Conidia ovoid to cylindrical and irregular, lemon-shaped branched chains. e) *Aspergillus tubingensis* OQ685748; Conidiophores upright, single, conidia single celled, globose. f) *Fusarium solani* OQ685892; Sterile mycilia.

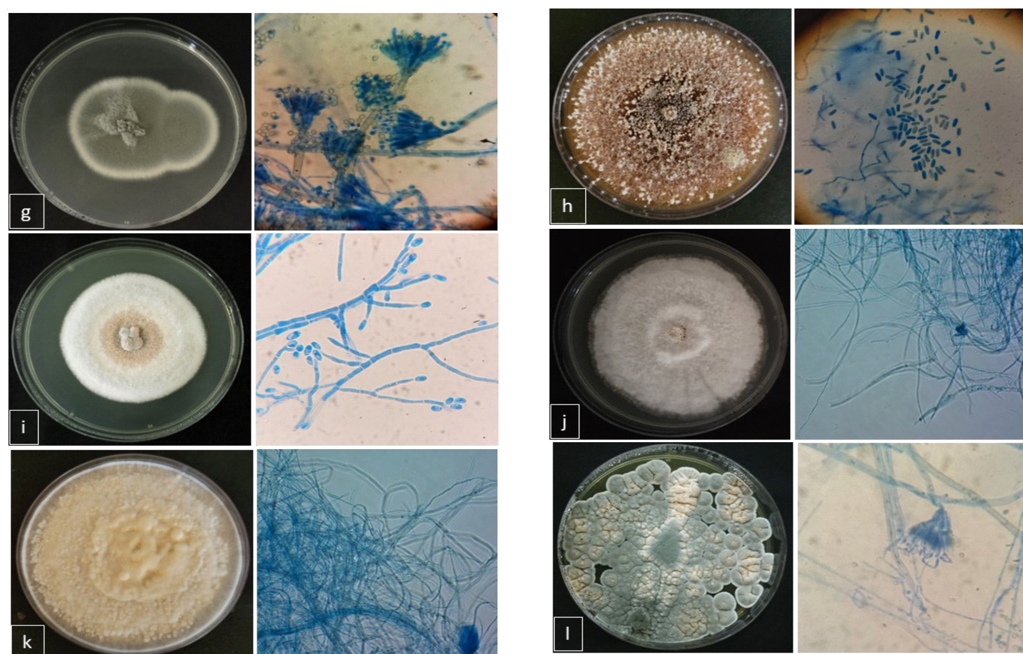

Fig. 2. Morphology on PDA media and microscopic view of the endophytic fungi isolated from *Hultholia mimosoides* - g) *Penicillium madriti* OQ603494; Conidiophores arising from the mycilium, branched near apex, brush like cluster. h) *Colletotrichum lindemuthianum* OQ678261; Conidia hyaline, single celled, ovoid.i) *Fusarium oxysporum* OQ680483; Conidia hyaline, variable, slightly curved, 1- celled ovoid. j) *Fusarium incarnatum* OQ680522; Sterile mycilia. k) *Fusarium equiseti* OQ680709; Sterile mycilia. l) *Penicillium glabrum* OQ683918; Conidiophores arising from the mycilium, branched near apex, brush like cluster.

DISCUSSION

Medicinal plants harbour a vast repertoire of essential components that could contribute to human health benefits. Edible medicinal plants are a significant resource for many of the important therapeutic molecules. These molecules of therapeutic importance contribute immensely to the physiological well-being and health benefits when consumed through diet or in the form of their

Table 2. Antimicrobial activity from ethyl acetate extract from endophytic fungi

Sl No	*H. mimosoides* Endophytic Fungi	Zone of inhibition (mm)					
		K. pneumonia ATCC9621	*S. typhemurum* ATCC23564	*E. coli* ATCC8739	*P. vulgaris* ATCC13315	*B. cereus* ATCC10876	*S. aureus* ATCC23565
1	*Fusarium chlamydosporum* OQ683919	-	-	-	-	-	8
2	*Fusarium oxysporum* OQ683924	-	8	-	-	-	-
3	*Diaporthe longicolla* OQ685299	-	4	11	-	-	4
4	*Cladosporium herbarum* OQ685410	**11**	**2**	**6**	**13**	-	-
5	*Aspergillus tubingensis* OQ685748	-	-	-	-	-	4
6	*Fusarium solani* OQ685892	7	-	-	4	-	4
7	*Penicillium madriti* OQ603494	4	-	-	-	-	8
8	*Colletotricum lindemuthianum* OQ678261	4	-	-	6	4	8
9	*Fusarium oxysporum* OQ680483	6	2	10	-	-	2
10	*Fusarium incarnatum* OQ680522	7	-	-	-	-	-
11	*Fusarium equiseti* OQ680709	11	7	6	-	-	-
12	*Penicillium glabrum* OQ683918	**8**	**16**	**10**	-	-	**2**

* Positive control Chloramphenicol (1mg/ml)-20mm (zone of inhibition).

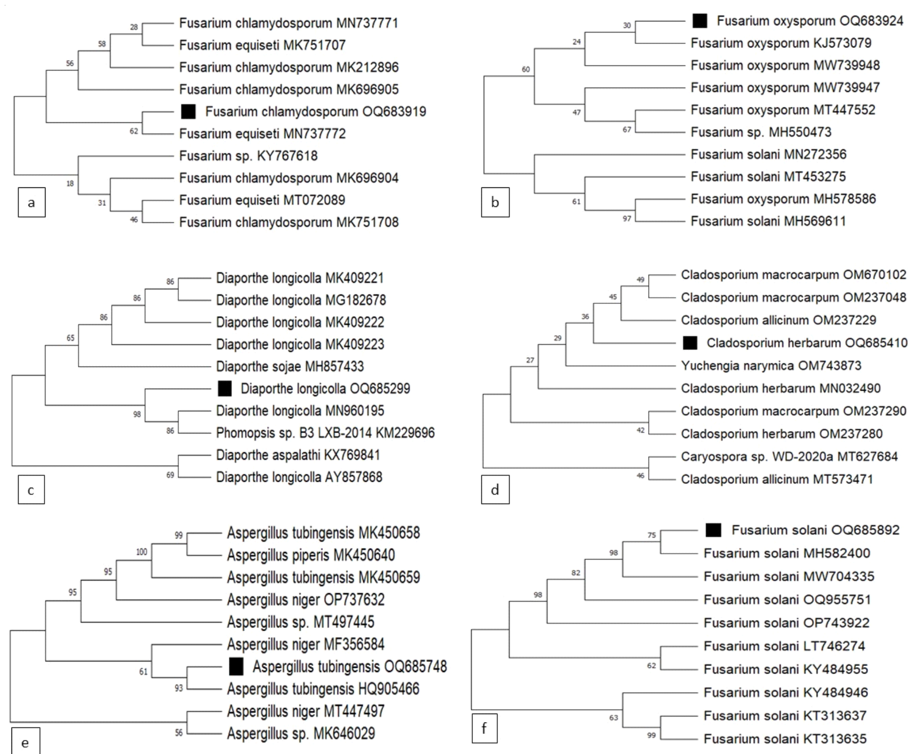

Fig. 3. Phylogenetic tree of endophytic fungus isolated from *H. mimosoides* using the ITS sequencing. The tree was built using the maximum likelihood approach in MEGA 11 with the default parameters, and bootstrap values were computed after 1,000 replicates. a) *Fusarium chlamydosporum* OQ683919; b) *Fusarium oxysporum* OQ683924; c) *Diaporthe longicolla* OQ685299; d) *Cladosporium herbarum* OQ685410; e) *Aspergillus tubingensis* OQ685748; f) *Fusarium solani* OQ685892;

extract. As the diversity of endophytic fungi varies among different plant species, the biosynthetic pathways and their metabolite composition could also be affected seasonal, regional, and other variations in environmental factors. Thus in consideration of the above facts, the present investigation aimed on the selection of a medicinal plant *H. mimosoides* extensively grown in the region of Chikka Aluvara, Kodagu that is frequently consumed by the inhabitants in the region for its health benefits. The plant consumed as food, alternatively possesses various positive health benefits for controlling skin rashes, cold, and fever.

Occurrence of endophytes in a particular plant species are required for mutualistic sustenance and existence of both counterparts. It is well known that all plant species belonging to different genera inhabit within them a vast diversity of endophytes. In this study, fungal endophytes from *H. mimosoides* were identified and screened for antimicrobial activity. Endophytic fungi were isolated and examined in different media to assess their growth and morphology. PDA medium was used to isolate the fungal endophytes from the stems and leaves of the plant. Identification of fungal isolates was initially carried out through microscopic observation and molecular identification. During microscopic observation, the characteristics of the fungal isolates, such as the shape of conidia and mycelia, were noted. Further confirmation of fungal species was conducted through ITS sequencing. Six fungal isolates were obtained from the leaves of *H.mimosoides*, including *Fusarium chlamydosporum* (OQ683919), which exhibited simple conidiophores with irregular and slightly curved spores. Other isolates included *Fusarium oxysporum* (OQ683924), *Fusarium solani* (OQ685892), and *Diaporthe longicolla* (OQ685299), which displayed sterile mycelia that were colourless or transparent in culture without fruiting structures. *Cladosporium herbarum* (OQ685410) showed ovoid to cylindrical conidia and lemon-shaped branched chains, while *Aspergillus tubingensis* (OQ685748) exhibited single-celled conidia with a globose structure and

unbranched conidiophore. From the stems of *H. mimosoides*, six fungal endophytes were obtained. Among them, *Fusarium incarnatum* (OQ680522) and *Fusarium equiseti* (OQ680709) were identified as sterile fungi. *Penicillium madriti* (OQ603494) displayed conidiophores arising from the mycelium, branching near the apex in a brush-like cluster. *Colletotrichum lindemuthianum* (OQ678261) showed single-celled, ovoid-shaped, and hyaline conidia. *Fusarium oxysporum* (OQ680483) had variable, hyaline, slightly curved, and single-celled ovoid spores. *Penicillium glabrum* (OQ683918) exhibited conidiophores arising from the mycelium, branching in a brush-like cluster. In the present study, twelve fungal endophytes were identified based on microscopic and ITS sequencing belonging to the genera of *Fusarium* sp, *Aspergillus* sp, *Penicillium* sp, *Colletotrichum* sp, *Diaporthe* sp, and *Cladosporium* sp.

In another study, eight morphologically distinct endophytic fungal cultures from *Elaeocarpus sphaericus* and *Myristica fragrans*, were identified by microscopic spore structure characteristics. The remaining four fungal cultures were sterile mycelia. The microscopic analysis revealed sterile mycelium, and BLAST revealed the highest number of hits to *Nigrospora* species, *Colletotrichum* species, and *Fusarium* species (Deepthi *et al*., 2018).

Based on various research these endophytic fungi were successfully used in the pharmaceutical industry, biological pests control and plant diseases. The endophytic fungi have immense medicinal

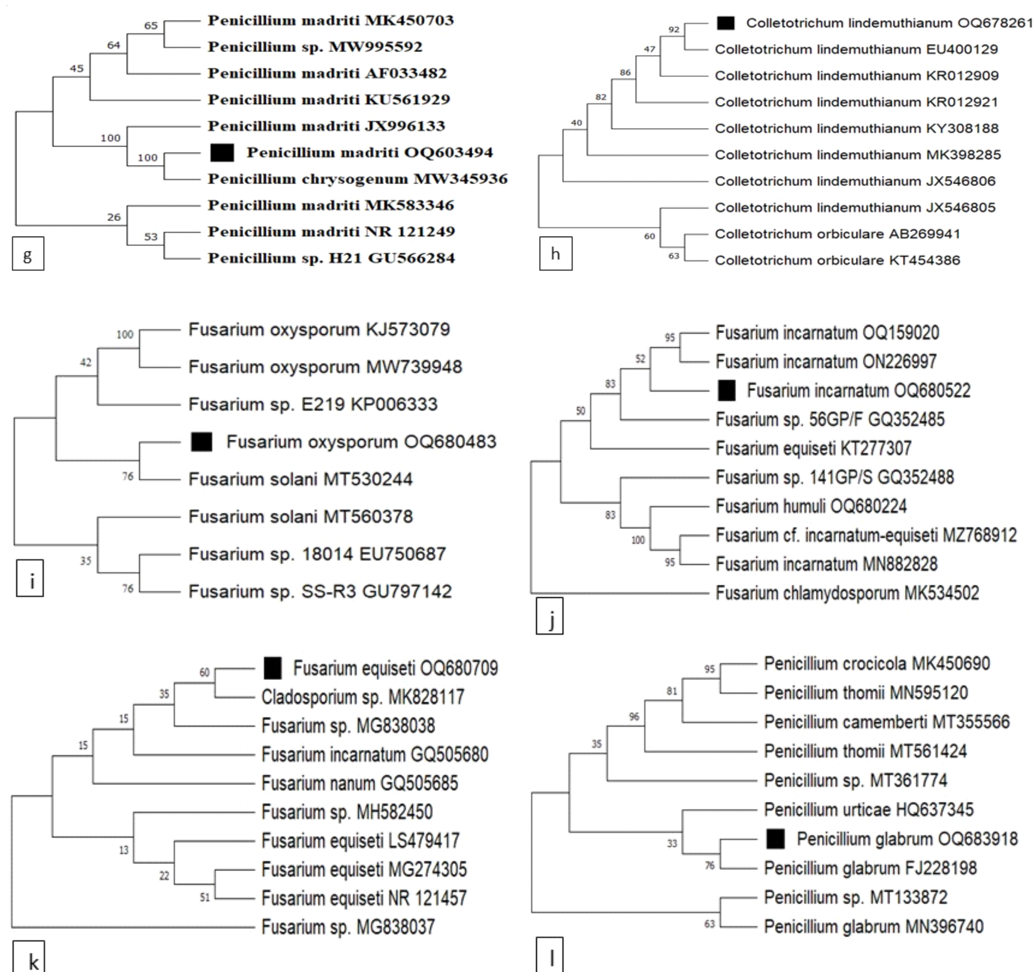

Fig. 4. Phylogenetic tree of endophytic fungus isolated from *H. mimosoides* using the ITS sequencing. The tree was built using the maximum likelihood approach in MEGA 11 with the default parameters, and bootstrap values were computed after 1,000 replicates. g) *Penicillium madriti* OQ603494; h) *Colletotrichum lindemuthianum* OQ678261; i) *Fusarium oxysporum* OQ680483; j) *Fusarium incarnatum* OQ680522; k) *Fusarium equiseti* OQ680709; l) *Penicillium glabrum* OQ683918;

value with numerous active biological compounds as a novel drug that helps towards the betterment of human health issues (Parekh and Chanda, 2007). Currently, there is a significant demand for new antimicrobial agents due to the increasing resistance of pathogens to existing drugs. While new synthetic drugs are being developed, they may have negative environmental impacts. To investigate on the active metabolites, various solvents were used to extract secondary metabolites, and endophytic fungal cultures are typically extracted using ethyl acetate. This solvent is favoured because of its medium polarity, which allows it to dissolve both polar and non-polar active compounds (Bhardwaj *et al.,* 2015; Rahmawati *et al.,* 2018). Also in a study, isolated endophytic fungi from the medicinal plant *Crescentiacujete* L. molecularly characterized and identified four isolates as *Nigrospora sphaerica*, *Fusarium oxysporum*, *Gibberellam oniliformis*, and *Beauveria bassiana* against bacterial human pathogens, the fungal extracts exhibited a potent growth inhibitory impact. The medicinal plant *Aegle marmelos*' an endophytic fungus, which was later identified as *Aspergillus flavus*, was isolated. Against typical human bacterial pathogens *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella abony*, *Bacillus subtilis*, and *Staphylococcus aureus*, this culture exhibited good antibacterial activity (Patil *et al*., 2015). In this work, the antibacterial activity of the endophytic ethyl acetate extract was evaluated against human pathogenic bacteria. The results indicated that all endophytic fungal crude extracts exhibited significant antibacterial activity against at least one pathogenic bacterium. Notably, extracts from *Colletotrichum lindemuthianum* OQ678261, shown activity against *Klebsiella pneumonia* ATCC9621, *Proteus vulgaris* ATCC13315, *Bacillus cereus* ATCC10876, *Staphylococus aureus* ATCC23565. *Cladosporium herbarum* OQ685410, showed the activity against *Klebsiella pneumonia* ATCC9621, *Salmonella typhemurum* ATCC23564, *Escherichia coli* ATCC8739 and *Proteus vulgaris* ATCC13315. *Penicillium glabrum* OQ683918 also demonstrated activity against four different pathogens such as, *Klebsiella pneumonia* ATCC9621, *Salmonella typhimurium* ATCC23564, *Escherichia coli* ATCC8739 and *Staphylococus aureus* ATCC23565. Among these three fungal crude extract, the extracts from *Cladosporium herbarum* OQ685410 showed the hightest zone of inhibition against *P.vulgaris* ATCC13315 (13 mm) and *Penicillium glabrum* OQ683918 showed the highest zones of inhibition against *S.typhimurium* 16 mm and 10 mm against *E.coli* ATCC8739, as detailed in Table 2. These findings suggest that *Cladosporium herbarum* OQ685410 and *Penicillium glabrum* OQ683918 are potential endophytic fungi with promising antimicrobial properties.

CONCLUSION

In conclusion, the current study on identification of endophytic fungi from the edible *H. mimosoides* plant could be explored further for pharmaceutical potential because they produce antimicrobial compounds, and the medicinal properties of this plant may be attributed to the endophytic microorganisms' ability to produce biologically active secondary metabolites.

ACKNOWLEDGEMENTS

The authors would like to express gratitude to the Department of Microbiology, Chikka Aluvara, Mangalore University, and the Molecular Research Laboratory (SERB, Govt. of India 2017-2022, CISEE-VGST Gvot. of Karnataka 2019-2023) for helping to conduct this research work and would like to thank to Dr. Krishnamurthi Chamaram, Assistant Professor, Department of Botany, KSOU, Mukthagangothri, Mysuru for helping to submit the plant herbarium.

Authors' contributions

Ningaraju S performed all of the tests, including gathering the plant sample, and produced the study paper. Prof. Manjula I.K supervised and guided for writing the publication.

Conflict of interests: The authors declare that there is no conflict of interest.

REFERENCES

Bhardwaj, A., Sharma, D., Jadon, N. and Agrawal, P.K. 2015, Antimicrobial and phytochemical screening of endophytic fungi isolated from spikes of *Pinus roxburghii. Arch Clin Microbiol.* 6(3): 1-9.

Chanwitheesuk, A., Teerawutgulrag, A., Kilburn, J.D. and Rakariyatham, N. 2007, Antimicrobial gallic acid from *Caesalpinia mimosoides* Lamk. *Food Chemistry*. 100(3): 1044-1048, doi:10.1016/j.foodchem.2005.11.008.

Chanwitheesuk, A., A. Teerawutgulrag, and N. Rakariyatham, 2005, Screening of antioxidant activity and antioxidant compounds of some edible plants

of Thailand. *Food Chemistry*. 92(3): 491-497.

Deepthi, V., Sumathi, S., Faisal, M. and Elyas, K.K. 2018. Isolation And Identification Of Endophytic Fungi with Antimicrobial Activities from the Leaves of *Elaeocarpus sphaericus* (Gaertn.) K. Schum. and Myristica Fragrans Houtt.

Dhayanithy, G., Subban, K. and Chelliah, J. 2019. Diversity and biological activities of endophytic fungi associated with Catharanthus roseus. *BMC Microbiology.* 19(1): 22, doi:10.1186/s12866-019-1386-x.

Goutam, J., Sharma, G., Tiwari, V. K., Mishra, A., Kharwar, R.N., Ramaraj, V. and Koch, B. 2017. Isolation and characterization of “Terrein” an antimicrobial and antitumor compound from endophytic fungus *Aspergillus terreus* (JAS-2) associated from Achyranthus aspera Varanasi, India. *Frontiers in Microbiology*. 8: 1334.

Idris, A., Al-tahir, I. and Idris, E. 2013. Antibacterial activity of endophytic fungi extracts from the medicinal plant Kigelia africana. *Egyptian Academic Journal of Biological Sciences, G. Microbiology*. 5(1): 1-9.

Joseph, B. and Priya, R.M. 2011. Bioactive Compounds from Endophytes and their Potential. *Am. J. Biochem. Mol. Biol*. 1(3): 291-309.

Kaul, S., Gupta, S., Ahmed, M. and Dhar, M.K. 2012. Endophytic fungi from medicinal plants: a treasure hunt for bioactive metabolites. *Phytochemistry Reviews*. 11 (4): 487-505, doi:10.1007/s11101-012-9260-6.

Kumar, S. and Kaushik, N. 2013. Endophytic fungi isolated from oil-seed crop Jatropha curcas produces oil and exhibit antifungal activity. *PloS One*. 8(2): e56202.

Li, H.Y., D.Q. Wei, M. Shen, andZhou, Z.P. 2012. Endophytes and their role in phytoremediation. *Fungal Diversity*. 54, doi:10.1007/s13225-012-0165-x.

Nighat Fatima, N. F., Usman Mukhtar, U. M., Ihsan-ul-Haq, I.H., Qazi, M.A., Muniba Jadoon, M.J. and Safia Ahmed, S.A. 2016. Biological evaluation of endophytic fungus Chaetomium sp. NF15 of Justicia adhatoda L.: a potential candidate for drug discovery.

Parekh, J. and S. Chanda, 2007. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turkish Journal of Biology*. 31(1): 53-58.

Patil, M.P., Patil, R.H. R.H. and Maheshwari, V.L. 2015. Biological Activities and Identification of Bioactive Metabolite from Endophytic Aspergillus flavus L7 Isolated from Aegle marmelos. *Current Microbiology.* 71(1): 39-48, doi:10.1007/s00284-015-0805-y.

Petrini, O. 1991. Fungal Endophytes of Tree Leaves. In: J.H. Andrews and S.S. Hirano, eds., *Microbial Ecology of Leaves*. New York, NY, Springer New York, Brock/Springer Series in Contemporary Bioscience, p. 179-197, doi:10.1007/978-1-4612-3168-4_9.

Rahmawati, N. I., Sunarva, S. and Rumidatul, S. 2018, Exploration of potential bioactive compounds of endophytic microbial culture isolated from gall rust sengon (*Falcataria moluccana*). *J Pharm Res Sci*. 10 (11): 156-169.

Rattanata, N., Klaynongsruang, S., Daduang, S., Tavichakorntrakool, R., Limpaiboon, T., Lekphrom, R., Boonsiri, P. and Daduang, J. 2016. Inhibitory Effects of Gallic Acid Isolated from Caesalpinia mimosoides Lamk on Cholangiocarcinoma Cell Lines and Foodborne Pathogenic Bacteria. *Asian Pacific Journal of Cancer Prevention.* 17(3): 1341-1345, doi:10.7314/APJCP.2016.17.3.1341.

Suryanarayanan, T.S., Venkatesan, G. and Murali, T. 2003. Endophytic fungal communities in leaves of tropical forest trees: diversity and distribution patterns. *Current Science.* 489-493.

Wang, L.W., Xu, B.G., Wang, J.Y., Su, Z.Z., Lin, F.C., Zhang, C.L. and Kubicek, C.P. 2012, Bioactive metabolites from Phoma species, an endophytic fungus from the Chinese medicinal plant Arisaema erubescens. *Applied Microbiology and Biotechnology.* 93(3):. 1231-1239, doi:10.1007/s00253-011-3472-3.

Yodsaoue, O., Karalai, C., Ponglimanont, C.,Tewtrakul, S. and Chantrapromma, S. 2010, Potential anti-inflammatory diterpenoids from the roots of Caesalpinia mimosoides Lamk. *Phytochemistry*. 71(14-15): 1756-1764, doi:10.1016/j.phytochem.2010.06.016.