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ISOLATION AND IDENTIFICATION OF ENDOPHYTIC FUNGI WITH ANTIMICROBIAL ACTIVITIES FROM THE LEAVES OF *ELAEOCARPUS SPHAERICUS* (GAERTN.) K. SCHUM. AND *MYRISTICA FRAGRANS* HOUTT.

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ABSTRACT: Endophyte fungi reside in the tissues beneath the epidermal cell layers and live within a plant for at least part of its life without causing apparent diseases. Eight morphologically distinct endophytic fungi were selected from the medicinal plants *Elaeocarpus sphaericus* coded as ELSPF 1 to 4 and *Myristica fragrans* coded as MYFRF 1 to 4. The highest colonization frequencies (CF %) were found to be 12.69%, and 8.33% for ELSPF 1 and MYFRF 2. Internal transcribed spacer- polymerase chain was performed for molecular identification and ~600 bp amplified products were obtained. The sequences were analysed by BLAST and the phylogenetic tree was constructed with neighbour joining method. The fungi were cultured on Potato Dextrose broth for production metabolites. The metabolites were extracted with ethyl acetate and constituent phytochemicals were analyzed. The crude extracts were tested for antimicrobial activity by agar well diffusion method. The test organisms used for the antimicrobial activity studies were *E. coli*, *Staphylococcus aureus* and *Klebliella pneumonia*. The ethyl acetate extracts of all endophytic fungi showed antimicrobial activity except MYFRF-1 against *E. coli*, *Staphylococcus aureus*, *Klebliella pneumonia*.

INTRODUCTION: An endophyte is an endosymbiont, often a fungus, which lives within a plant for at least part of its life without causing any apparent disease. They form inconspicuous infections within tissues of healthy plants for all or at least a part of their life cycle [1]. Endophytes are ubiquitous and have been found in all the species of plants and their tissues such as stem, leaves, roots and petioles *etc*. Endophytes may benefit host plants by preventing pathogenic organisms from colonizing them.

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The relationship between host-plant and endophytes are usually symbiotic, in which endophytes get nutrients from plants [2]. The symbiotic relationship might sometimes turn into opportunistic pathogen when host plant becomes weakened.

Elaeocarpus sphaericus is commonly known as Rudraksha. It is a large, evergreen broad leaved tree found in tropical and sub-tropical areas and is grown in Assam and Himalayan region of India. Tree is about 50 - 200 feet high and the leaves are ovate with tithed edges [3]. Rudraksha is known as King of herbal medicine and is used in folk medicine for the treatment of stress, anxiety, depression, palpitation, nerve pain, epilepsy, migraine, lack of concentration, asthma, hypertension, arthritis and liver diseases. *Myristica fragrans* is a small evergreen tree of 5 - 13 m tall.

It is commonly called Nutmeg and belongs to the family Myristicaceae. It is found to have various health benefits and significant herb in Ayurvedic medicine. It is an aromatic spreading plant and is a source of many oils, proteins, starch, minerals and resins. It is digestive, appetizer, aromatic and astringent. Ayurveda also attributes various other efficacies to it like anti-inflammatory, anti-diarrheal and analgesic.

Sporulating structures of fungi were considered as diagnostic features for identification. However, apart from cultured characteristics the identity of the certain non-sporulating strains required molecular tools. For the identification of endophytic fungi, molecular phylogeny in combination with morphological and cultural characteristics was used. Internal transcribed spacer (ITS) refers to the spacer DNA situated between the small-subunit and large-subunit of ribosomal RNA (rRNA), is the most widely used fungal identification tool [4, 5]. Microorganisms are important sources of bioactive natural products with enormous potentials of new molecules for drug discovery, industrial use and agricultural applications. The bioactive compounds of medicinal plants are products of the plant itself or of endophytes living inside the plants. Recently endophytes are viewed as an outstanding source of secondary metabolites, bioactive compounds, natural antimicrobial products and aid in nutrients uptake process [6]. Endophytic fungi can produce plant-derived novel bioactive compounds, which could be developed into novel antimicrobial and anticancer agents [7, 8].

MATERIALS AND METHODS:

Collection of Plant Sample: The leaf samples of *Elaeocarpus sphaericus* and *Myristica fragrans* were collected from botanical garden University of Calicut, Kerala, India. The symptomless and apparently healthy leaves were collected in pre-sterilized polythene bags and processed within 24 hrs of collection.

Isolation of Endophytic Fungi: The leaf samples were surface sterilized by the modified method [9]. The samples were washed initially with running tap water for 10 min, then with 1% sodium hypochlorite for 3 - 4 min, followed by 70% ethanol wash for 1 min and then finally rinsed in sterile distilled water three times. The excess moisture was blotted using a sterile filter paper. The rinsed water was streaked on to the PDA medium and sterilized segments were imprinted on PDA medium to ensure the surface sterilization. The surface- sterilized segments were cut in to 1 cm × 1 cm length were placed in petri dishes containing PDA (HiMedia, Mumbai, India) medium supplemented with chloramphenicol (100 mg/L). The petri dishes were monitored every day to check the growth of fungal colonies from the leaf segments. Individual hyphal tips that emerged from the edges of each treated plant bits were transferred separately onto fresh PDA medium. The pure cultures were maintained in cryovials on PDA layered with 15% glycerol (v/v) at -80 °C in an ultra-low temperature freezer (New Brunswick, eppendorf).

Taxonomy of Endophytic Fungi: Morphological and microscopic identification of endophytic fungi: Fungal identification were based on the morphology of the cultures, the mechanisms of spore production, and characteristics of the spores Morphological studies were done by plating the fungi on PDA and incubating it for 7 days. The growth appearances were observed both the top and bottom sides of the culture plates. For tentative identification, microscopic slides of each fungal endophyte were prepared. Slides were prepared by tease mount method using lactophenol cotton blue staining and observed under microscope [10].

Colonization Frequency of Endophytic Fungi: The colonization frequency (CF) percentage of the endophytic fungi were calculated as described below [11].

$$CF\% = \frac{\text{Number of segments colonized by endophytes}}{\text{Total number of segments analyzed}} \times 100$$

Molecular Identification of Fungal Isolates using ITS-PCR: Molecular identification fungal DNA was extracted using the method described [12]. Approximately 0.05 gm of 5 - 8 days old fungal mycelia were scraped from fresh cultures growing on PDA plates homogenized in liquid nitrogen using sterile motor and pestle. The powdered mycelium was ground with 1 ml of extraction buffer (100 Mm Tris, 1.4 M NaCl, 20 mM EDTA, 2% CTAB). The homogenate incubate at 65 ºC for

10 min, and then centrifuged at 12000 rpm for 5 min. The supernatant was transferred in to fresh eppendorf tube added equal volume of chloroform isoamyl alcohol (24:1) and centrifuged at 12,000 rpm for 5 min. The supernatant was transferred to fresh tube and added with two volumes of ice cold isopropanol and incubated at -20 ºC for 10 min. The pellet was washed in 500 µL of 70% ethanol and centrifuged at 10000 rpm for 5 min. The pellet was air dried and resuspended in 30 µL of TE buffer (10 mM Tris Cl pH 8.0, 1 mM EDTA). 1 µl of RNase A (20mg/ml) was added and incubated at 37 ºC for 1 h, the DNA was stored at -20 ºC. The DNA quantity was checked by electrophoresis on 1 % agarose (low EEO grade; HiMedia, India) gel supplemented with ethidium bromide to a final conc. of 0.5 mg mL^{-1} for 2 h at 50 V in tris-acetate EDTA buffer (40 mM Tris; 2 mM EDTA; 20 mM glacial acetic acid, pH 8) [13] and visualized under UV Transilluminator.

The ITS region of the ribosomal DNA was amplified by PCR with the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (TC-CTCCGCTTATTGATATGC) [14]. PCR amplifications were performed in a total reaction volume of 20 µl containing 10 µl of 2X PCR master mix, 1 µl of 0.5 µM of forward and reverse primer and 7 µl of sterile water. PCR amplifications were performed in a Gradient thermal cycler with an initial denaturation at 95 °C for 3 min, followed by 35 amplification cycles at 92 °C for 1 min for denaturation, 50 °C for 1 min for annealing, 72 °C for 2 min for extension 72 °C for 10 minutes for final extension. Following amplification, the PCR products were subjected to electrophoretic analysis by horizontal agarose gel electrophoresis through 1% agarose gel supplemented with ethidium bromide as described earlier and along with 100 bp DNA marker.

The bands were visualized using gel documentation system. The PCR products were purified by using Quick gel extraction and PCR purification combo kit. The purified products were sequenced using an automated DNA sequencer and the sequences were submitted to GenBank on the NCBI website (http://www.ncbi.nlm.nih.gov). Sequences obtained in this study were compared with the GenBank database using the BLAST software (http: //www. ncbi.nlm.nih.gov/BLAST/).

Production of Secondary Metabolites: Mycelia from 7 days old actively growing endophytic cultures were inoculated in 300 ml potato dextrose broth in 1 L Erlenmeyer flasks. The inoculated flasks were incubated at 28 ºC for 21 days with intermediate shaking at 150 rpm. Culture broths were filtered through muslin cloth and filtrates were extracted thrice with an equal volume of ethyl acetate (1: 1, v/v and the mycelial mats were washed with distilled water and dried at 50 ºC and powdered. The powdered mycelium was extracted and both mycelia and culture filtrate extracts were pooled together and dried using rotary vacuum evaporator.

Antibacterial Activity: The agar well diffusion method was employed for preliminary screening of endophytic extracts against selected bacteria. The clinical isolates of the bacterial strains *E. coli*, *Staphylococcus aureus, Klebsiella pneumoniae*, were grown in 10 ml of nutrient broth (NB) medium for 24 h at 37 ºC. The turbidity of bacterial suspension was compared to 0.5 McFarland turbidity standards [0.05 ml of 1.175% Barium chloride ($BaCl_2.2H_2O$) in 9.95 ml of 1% Sulphuric acid (H_2SO_4)]. This level of turbidity is considered equivalent to approximately 1.5×10^8 CFU/ml.

Hundred mirolitre of bacterial suspension was inoculated in 90 mm petri plate containing nutrient agar. Plates were prepared with 20 ml of Nutrient Agar medium and inoculated with standardized inoculum. About 3 mm diameter wells were cut out using cork borer and crude endophytic fungal extracts (10 mg/mL) was added to the well. 20 µl of standard antibiotic chloramphenicol (1µg/ml) was used as control and an equal quantity of ethyl acetate was used as a negative control. The plates were incubated at 37 ± 1 ºC for 24 h. The diameter of inhibition zone measured after 24 h of incubation and the mean values were calculated.

RESULTS AND DISCUSSION: Endophytic fungi, the most unexplored organism with rich source of biodiversity and biological product. The symbiotic associations of the endophytic fungi with plants produce beneficial substances for host. Several studies have been carried out regarding the biodiversity, taxonomy, ecology and the symbiotic relationship with the host [15].

Morphological and Microscopic Identification: Eight morphologically different endophytic fungal cultures were isolated from 26 isolates of *Elaeocarpus sphaericus* and 14 isolates of *Myristica fragrans* **Fig. 1**, **Table 1**. Each fungal isolate was given a unique code ELSPF 1 to 4 (*Elaeocarpus sphaericus* fungus 1 to 4) and MYFRF 1 to 4 (*Myristica fragrans* fungus 1 to 4) for the identification and maintained in the laboratory. The isolation and identification of endophytic fungi from Makassar fruit isolated four genera, including *Trichoderma* sp., *Fusarium* sp., *Penicillium* sp. and *Aspergillu*s sp.

The diversity of endophytic fungi was investigated in the susceptible and resistant clones of cocoa plant isolated *Fusarium* sp., *Colletotrichum* sp., *Aspergillus* sp, *Geotrichum* sp, *Curvularia* sp, 16 and 14 fungal species were isolated from endemic medicinal plants of Tirumala hills are *Fusarium oxysporum*, *Pestalotiopsis* species, *Aspergillus flavipes, Colletotrichum falcatum* and sterile mycelia *etc*. [17] *Aspergillus flavipes*, *Aspergillus niger, Aureobasidium pullulans, Bipolaris nodulosa, Cladosporium epiphyllum, Colletotrichum* sp., were isolated and identified based on the morphology of the spores [18].

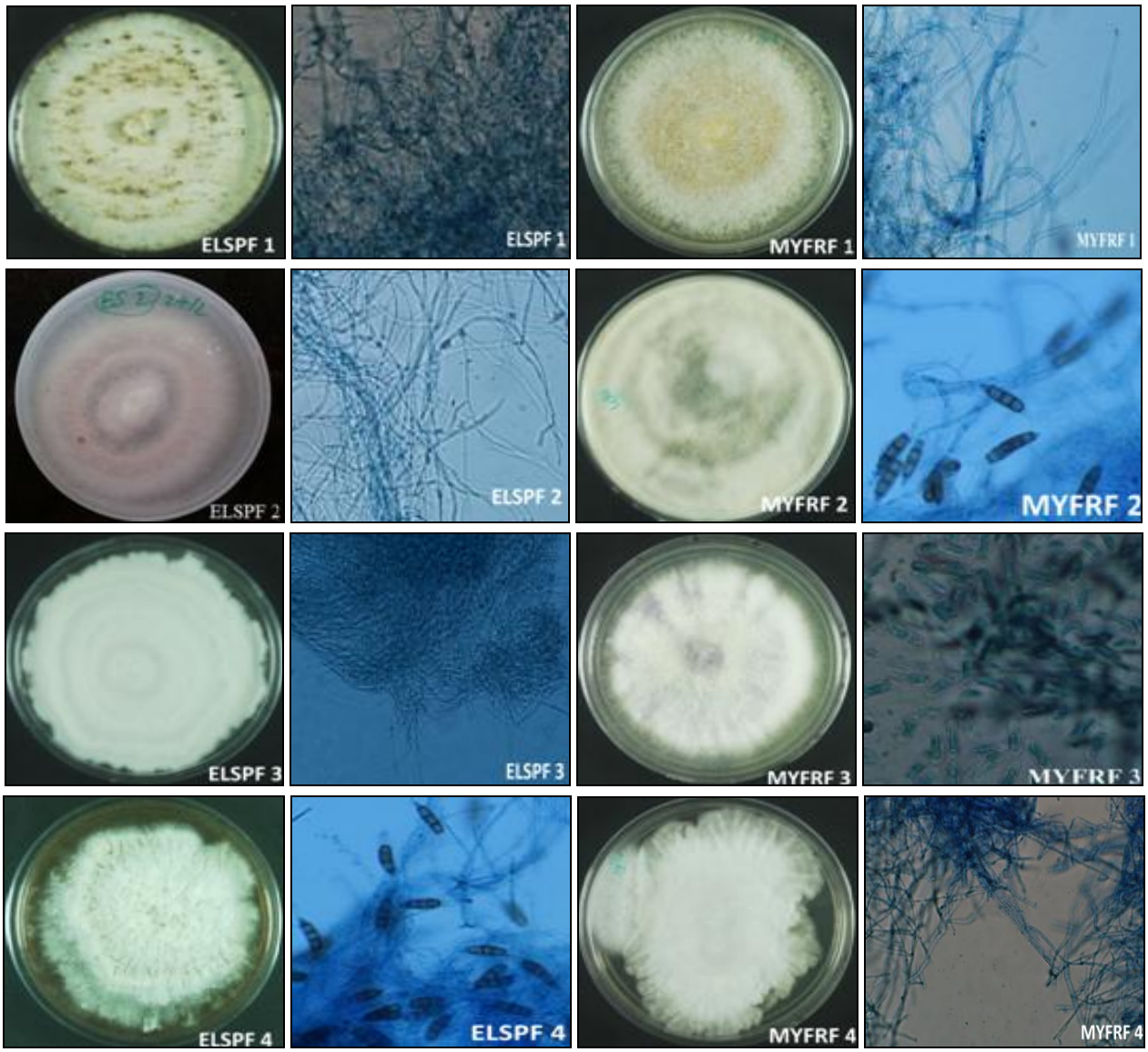

FIG. 1: MORPHOLOGY AND MICROSCOPIC VIEW OF THE ENDOPHYTIC FUNGI ISOLATED FROM *ELAEOCARPUS SPHAERICUS* AND *MYRISTICA FRAGRANS*

Colonization Frequency (%) of Fungal Endophytes: The colonization frequencies of the fungal isolates from *Elaeocarpus sphaericus* (ELSPF 1 to 4) were from 1.58 % to 12.69% and the colonization frequencies of the endophytic fungi isolated from *Myristica fragrans* (MYFRF 1-4) were from 1.38 % to 8.33% **Table 2**.

TABLE 1: MORPHOLOGICAL AND MICROSCOPIC IDENTIFICATION

S. no.	Endophytic fungi	Macroscopic characteristics	Microscopic characteristics
1	ELSPF 1	White colour colonies later turned in to cream colour. Circular form with flat elevation	Sterile mycelia
2	ELSPF 2	Pink colour colonies, crateriform elevation and filamentous form	Sterile mycelia
3	ELSPF 3	White cottony colonies, nmbonate elevation and irregular form	Sterile mycelia
4	ELSPF 4	White colour colonies, flat elevation and filamentous form	Conidia is multi-celled with three darkly pigmented centre cells and clear pointed end cells, two or more clear, whisker-like appendages arising from the end cells
5	MYFRF 1	Yellow colour colonies, flat elevation and circular form	Sterile mycelia
6	MYFRF 2	White cotton colonies. black colour on back side	Conidia is multi-celled with three darkly pigmented centre cells and clear pointed end cells, two or more clear, whisker-like appendages arising from the end cells
7	MYFRF 3	White colour colonies turned in to violet colour. Flat elevation and filamentous form	Macroconidia were ovoid, 1-2 celled and slightly curved.
8	MYFRF 4	White colour colonies with a yellow colour back view. Flat elevation and undulate margin	Sterile mycelia

The overall colonization frequency of endophytes in both leaf and stem was 3.44% in medicinal plants of Tirumala hills and *Calotropis procera* from Thai region was 35.1% [19]. The medicinal properties of the plant have some role in the colonization of endophytic fungi. The low rate of colonization may be due to the secretion of phytochemicals that contain certain antibacterial and antifungal components [20].

TABLE 2: COLONIZATION FREQUENCY (%) OF ENDOPHYTIC FUNGI FROM *ELAEOCARPUS SPHAERICUS* AND *MYRISTICA FRAGRANS*

S. no.	Name of the Endophytic fungus	Colonization frequency (%)
1	ELSPF 1	12.69 %
2	ELSPF 2	9.52 %
3	ELSPF 3	1.58 %
4	ELSPF 4	4.76 %
5	MYFRF 1	2.77 %
6	MYFRF 2	8.33 %
7	MYFRF 3	1.38 %
8	MYFRF 4	4.16 %

Molecular Identification Endophytic Fungi: The development of molecular techniques for the identification of endophytic fungi has been opening a new perspective for taxonomic characterization and relationship between complex groups of organisms. Endophytic fungal isolates were subjected to molecular characterization based on ITS sequencing. Genomic DNA was isolated from the endophytic fungal cultures were amplified by using internal transcribed spacer polymerase chain reaction (ITS PCR). The primers amplified the entire target ITS region. A single band at ~600 bp was observed in all lanes after electrophoresis of the PCR product. Molecular methods were used to authenticate the identification of 8 endophytes isolated from *Elaeocarpus sphaericus* and *Myristica fragrans* **Fig. 2**.

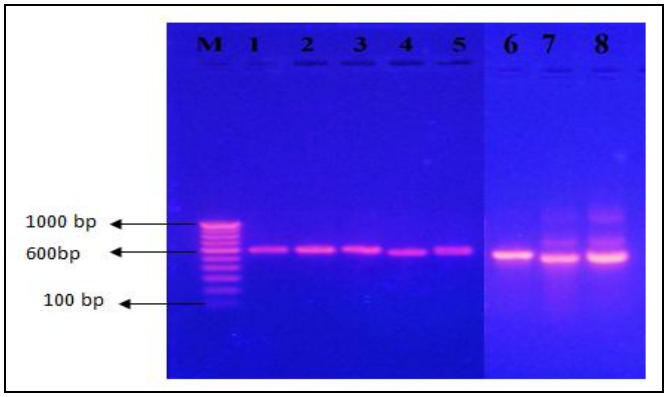

FIG. 2: PCR AMPLIFIED PRODUCTS OF ENDOPHYTIC FUNGI ISOLATES AMPLIFIED WITH PRIMERS ITS-1 AND ITS-4. LANE M: 100 bp MARKER, LANE 1- 8: PCR AMPLIFIED PRODUCT ~600 bp

ITS Sequence and Phylogenetic Analysis of Endophytic Fungi: In the present study Homologous comparison of the eight fungal sequences in which seven of them showed >90% identity to those present in the GenBank database and none were exactly identical to the sequences obtained from the database **Table 3**. ELSPF 3 was excluded for further analysis as the sequenced data contained very short sequence stretch but returned

with BLAST hit for *Sordariomyctes* sp. Out of 7 samples; three isolates were identified by microscopic spores (ELSPF 4, MYFRF 2 and 3) and supported the best blast hit. The rest four were sterile (ELSPF 1 and 2, MYFRF 1 and 4) and the top BLAST hit was considered as identification and were taken for further analysis.

TABLE 3: SEQUENCE ANALYSIS OF ENDOPHYTIC FUNGI

S. no.	Sequence name	Microscopic Identification of spores	Nucleotide Blast Results – Top Hit only				
			Accession ID with organism Name	Total Score	Query coverage	E-Value	Identity
1	ELSPF1	Sterile	KU504314.1 Nigrospora Sp.	929	98%	0.0	99%
2	ELSPF2	Sterile	KU504328.1 *Nigrospora* Sp.	826	96%	0.0	97%
3	ELSPF3*	Sterile	KX908901.1 *Sordariomycetes Sp.*	553	94%	1e-153	99%
4	ELSPF4	*Pestalotiopsis* sp.	KM998724.1 *Pestalotiopsis* Sp.	891	98%	0.0	99%
5	MYFRF1	Sterile	KP748194.1 *Colletotrichum* Sp.	739	96%	0.0	92%
6	MYFRF2	*Pestalotiopsis* sp.	KJ787111.1 *Pestalotiopsis* Sp.	695	76%	0.0	98%
7	MYFRF3	*Fusarium* sp.	HQ846580.1 *Fusarium* Sp.	436	57%	2e-118	97%
8	MYFRF4	Sterile	FR682078.1 *Fusarium* Sp.	571	60%	6e-159	99%

* was excluded in the phylogenetic analysis due to short sequence stretch

The phylogenetic relationships among top most hit against GenBank were investigated. Phylogenetic tree shows the closely related nucleotide sequences of fungal isolates. As indicated in the phylogenetic tree **Fig. 3** ELSPF 1 and 2 showed close similarity to *Nigrospora* species with accession Ids KU504314.1, KU504328.1. The microscopic identification showed sterile mycelium, BLAST showed maximum number and top most hits to *Nigrospora* sp. This could be an identification mark that the ELSPF 1 and ELSPF 2 could be *Nigrospora* sp.

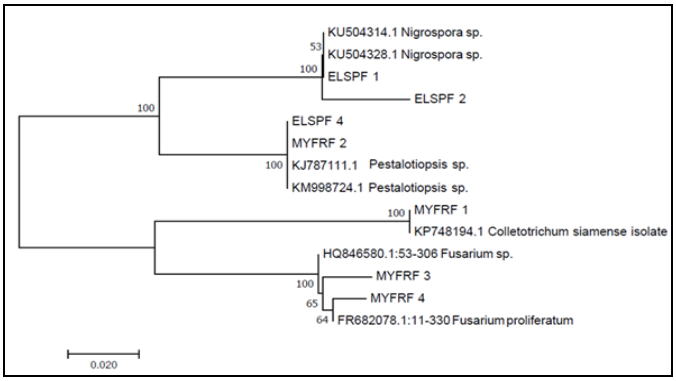

FIG. 3: PHYLOGENETIC TREE OF THE ENDOPHYTIC FUNGI FROM *ELAEOCARPUS SPHAERICUS MYRISTICA FRAGRANS*

The Microscopic inference validated that ELSPF 4 and MYFRF 2 and 3 were backed up by the BLAST hit and the organisms were identified as *Pestalotia* sp., *Fusarium* sp. However, MYFRF 1 and 4 showed sterile mycelium and showed BLAST hit against *Colletotrichum* sp. KP748194.1 and CFR682078.1 *Fusarium* sp. respectively. *Endophytic filamentous* fungi from coffee plants (*Coffea arabica* and *C. robusta*) deposited in the Brazilian Collection of Environmental and Industrial Microorganisms (CBMAI) were characterized taxonomically by using molecular tools Thirty - seven out of 39 CBMAI strains investigated were identified to at least at genus level by ITS and ribosomal DNA D1/D2 sequencing and phylogenetic analyses [21]. Gherbawy and Gashgari, 2014 [25] Fungal endophytes were isolated from the leaves of *Calotropis procera* were collected from Taif region; thirty-three different taxa were recovered.

A total of 161 isolates were obtained and identified into 33 distinct operational taxonomic units and identified based on the sequencing of the ITS region of rRNA, indicated a full correspondence between the molecular and morphological identification of fungal isolates. The most prevalent fungi were *Aspergillus flavus, Chaetomium globosum, Cochliobolus lunatus, Fusarium dimerum, F. oxysporum,* and *Penicillium chrysogenums*. The amplified ITS I -5.8-ITS II region of ribosomal DNA have also been previously used for molecular characterization of endophytic fungi from two traditional medicinal plants, *Ocimum sanctum* and *Sapindus detergens* 22. 57 Endophytic fungal isolates were separated from the root epidermis and remnant tissues of *S. miltiorrhiza*. According to the macro and microscopic characteristics 14 fungal isolates were selected and identified by ITS sequencing as Alternaria, Pleosporeles, Leptosphaeria, Peyronellaea, Phoma, Xylomelasma, Bionectria,

Fusarium, Sarocladium, Aspergillus, Petrinella, and Cadophora. The ITS1-5.8S-ITS4 partial sequences of 14 distinct isolates were submitted to the GenBank and the closest related species were got by BLAST analysis and had homology greater than or equal to 99 % to their closest related species [23].

Species Diversity Indices: The data collected from the leaf segments of two medicinal plants *Elaeocarpus sphaericus* and *Myristica fragrans* were analysed based on the Shannon-weiner diversity index and Simpson's diversity index. The diversity index showed H′ 1.095, D 0.67 with an evenness of 0.79 for endophytic fungi from *Elaeocarpus sphaericus* and index H′ 1.17, D 0.72 with an evenness of 0.84 for endophytes from *Myristica fragrans* **Table 4**. The Shannon-Wiener diversity index (H') was only 1.808 (the H' index is usually between 1.5 and 3.5) [24] and the reason to account for this is the lower number of sampled trees, which would significantly affect the number of isolated species [25].

TABLE 4: DIVERSITY INDICES AND EVENNESS OF *ELAEOCARPUS SPHAERICUS* AND *MYRISTICA FRAGRANS*

S. no.		*Elaeocarpus sphaericus*	*Myristica fragrans*
1	Shannon-weiner diversity index (*H')*	1.095	1.17
2	Simpson's diversity index (D)	0.67	0.72
3	Evenness	0.79	0.84

Antimicrobial Activity of Crude Extracts of Endophytic Fungi: Antibacterial activity of the crude extracts of all endophytic fungi against the test organisms was determined by well diffusion method. All most all crude extracts showed anti microbial activity against the test organisms *E. coli*, *K. pneumonia*, *S. aureus* except MYFRF 1 **Fig. 4**, **Table 5**. With respect to the positive control (chloramphenicol) ethyl acetate extracts from fungal isolates of *Myristica fragrans* showed comparatively less antimicrobial activity because it may have active compounds, but probably in smaller amounts.

Endophytic fungus *Nodulisporium* sp. PT11 isolated from the leaves of *Mitragyna javanica* exhibited the strongest antimicrobial activity against all test microorganisms (*Bacillus subtilis*, *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli*, *Saccharomyces cerevisiae* and *Candida albicans* [26]. *C. pallescens*, the endophytic fungus in *C. procera* showed antibacterial against *B. subtilis*, *Klebsiella pneumoniae, S. epidermidis*, and *E. coli* [27]. Antibacterial activity may be due to the active components present in the fungal extracts and the solubility of the active compounds in the solvent used (ethyl acetate) [28]. The difference in inhibition of the test organism by the crude extracts may be due to the number and concentration of the active compounds in them [29].

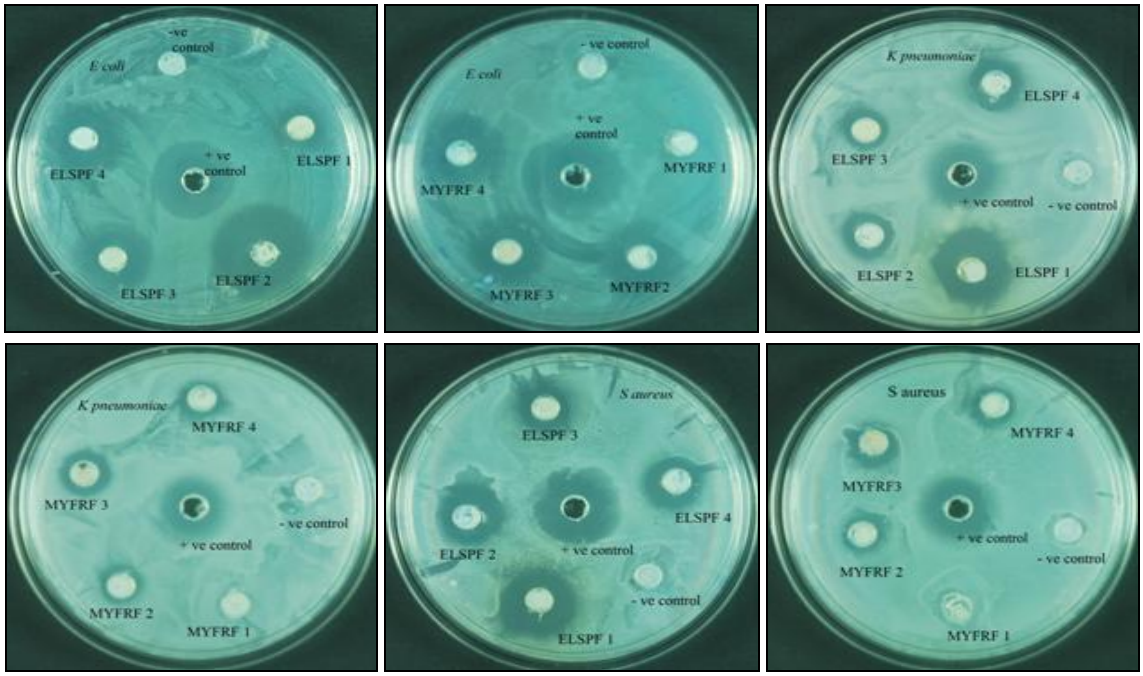

FIG. 4: ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS OF ENDOPHYTIC FUNGI FROM *ELAEOCARPUS SPHAERICUS* AND *MYRISTICA FRAGRANS*

TABLE 5: ANTIBACTERIAL ACTIVITY (INHIBITION ZONE mm) OF CRUDE EXTRACTS OF ENDOPHYTIC FUNGI

		Test organism		
S. no.	**Name of the endophytic fungi**	***E. coli***	***K. pneumoniae***	***S. aureus***
1	ELSPF1	9 mm	19 mm	18 mm
2	ELSPF 2	24 mm	13 mm	16 mm
3	ELSPF 3	17 mm	16 mm	11 mm
4	ELSPF 4	14 mm	14 mm	13 mm
6	MYFRF 1	-	-	-
7	MYFYF 2	13 mm	10 mm	9 mm
8	MYFRF 3	15 mm	12 mm	12 mm
9	MYFRF 4	19 mm	11 mm	10 mm
10	Positive Control	19 mm	17 mm	20 mm

CONCLUSION: *Elaeocarpus sphaericus* and *Myristica fragrans* are known to have good medicinal properties. The data produced in the study provide valuable insight into the diversity of endophytic fungi from *Elaeocarpus sphaericus* and *Myristica fragrans*. Molecular techniques including sequencing and phylogenetic analyses were successful for the identification to at least at genus level of eight endophytic fungi isolates analysed. The antibacterial activities of the isolates were studied against the test organisms, these isolates could be further considered for anticancer assays. These endophytic fungi have potential source as producers of natural antimicrobial compounds and could used as an agent for antibacterial activity. Further investigation will focus on the isolation of bioactive compounds from these endophytic fungi for anticancer assays.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interests.

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