

**DIVERSITY OF LEAF SURFACE FUNGI ON THE MEDICINAL PLANT,
PLECTRANTHUS AMBOINICUS LOUR. AND ITS ANTIMICROBIAL EFFICACY
AGAINST MTCC PATHOGENS**¹Ranadive Ananth G., *¹Dr. Bijaya Kumar Nayak, ¹Surendra S., ²Gayathri Parivallal and ³Anima Nanda¹Department of Botany, Kanchi Mamunivar Govt. Institute for Postgraduate Studies and Research (Autonomous),
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Phylloplane and endophytic fungi are considered as the best source of novel biocompounds and have a high level of structural diversity on the leaf surfaces. Bioactive complexes produced by these phylloplane and endophytes have publicized gifted prospective towards human health, for which, it is essential to capture and operate this vital microbial resource and make it more helpful for the welfare of manhood. During the present study, isolation and enumeration of Ectophytic (phylloplane) and endophytic fungi were carried out from one medicinal plant, *Plectranthus amboinicus* Lour. with the host relationship based on the methodology i.e., agar plate method. Overall, 16 phylloplane and endophytic fungal species of 8 genera were isolated from the medicinal plant, *Plectranthus amboinicus* Lour. In distribution of leaf surface fungi, phylloplane contributed the maximum (70%) followed by the endophytes (30%) in *Plectranthus amboinicus* plant. In the phylloplane and endophytic fungal population, *Aspergillus awamori*, *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus terreus*, *Penicillium oxalicum*, *Penicillium citrinum*, *Penicillium chrysogenum*, Gray sterile mycelia were the most dominant fungal species in the agar plate method. Fungi isolated from both the upper and sub-surface of leaf were more or less similar to each other. Antimicrobial potential of the leaf surface fungal extracts was very effective against *E. coli*, *Pseudomonas aeruginosa*, *Shewanella putrefaciens* and *Candida albicans* but *Staphylococcus aureus* was found to be resistance towards the fungal extracts. The host relation favored and tissue description sign was recognized between the phylloplane and endophytes based on the fungal community dispersal and structure.

KEYWORDS: Phylloplane and Endophytic fungi, Diversity, Medicinal plant, MTCC pathogens, *Plectranthus amboinicus* Lour.**INTRODUCTION**

The leaf surface (phylloplane and endophyte) fungi are the microbes that inhabit the tissues of plants during their life cycle without producing any disease signs and many are noteworthy in biotechnological processes within. In over-all, vascular plants harbor phylloplane and endophytic organisms.^[1] The fungi who exist in the internal part of plant tissues called endophytes, which constitute a group of plant symbionts and are a component of microbial diversity and those who reside over the leaf surfaces are recognized as phylloplane fungi^[1, 2]. Endophytic fungi that are existing in living tissue of diverse plant parts viz., root, fruit, stem, seed, leaf etc. establishing a mutual relationship without making seemingly any dysfunction in the host. Fungal endophytes are skilled of living in host plant without causing any types of symptoms.^[3,4] Endophytic fungi are

ubiquitous in nature and found in the plants, residing intercellular or intracellular at least for a portion of their life without causing apparent symptoms of infection.^[5,6] Fungi are group of microbes having a great biodiversity and they are the largest group of microbes of tropical ecosystems throughout the world. They are present in most of the plant parts, especially the leaves where the tissue is apparently healthy.^[4,5,6] It is beneficial for us to study the relations between the phylloplane and endophytes with their host plants and to develop an compatible approach for competently producing these unusual and valuable bioactive compounds.^[6-10] The phylloplane and endophytes produce a number of bioactive compounds for helping the host plants to resist external biotic and abiotic stresses and benefiting for the host growth in return.^[3,11] Limited phylloplane and endophytic fungi have developed the aptitude to produce

the same or similar bioactive materials as those created from the host plants. During our present study, isolation, identification and enumeration of phylloplane and endophytic fungi were carried out from one medicinal plant species viz., *Plectranthus amboinicus* Lour. collected from KMGIPSR and TGASC campus, Pondicherry- 605008, India employing agar plate methods. The potential relationships of the ectophytes and endophytes with the host plants were also discussed with their antimicrobial efficacy against MTCC pathogens.

MATERIALS AND METHODS

Collection of sample

Different leaves of medicinal plant, *Plectranthus amboinicus* Lour. were collected in fresh condition from our KMGIPSR and TGASC campus, Puducherry-605008, India. Mature leaves were carefully segregated and brought to the Microbiology Laboratory, Department of Botany with utmost care and kept in room temperature for the isolation of leaf surface fungi by agar plate method.

Description of the plant

Binomial name : *Plectranthus amboinicus* Lour.
 Family : Lamiaceae
 Common name : Indian borage
 Vernacular name : Indian Mint



Plate I: *Plectranthus amboinicus* Lour.

Plectranthus amboinicus (Lour.) Spreng. (Plate I) is a perennial herb belong to the family Lamiaceae which finds naturally throughout the warm regions and tropics of Asia, Australia and Africa. This herb has nutritional and therapeutic properties ascribed to its natural phytochemical compounds which are highly prized in the pharmaceutical industry. As well, it has horticultural possessions due to its aromatic nature and essential oil generating capability. It is widely used in folk medicine to treat conditions like asthma, cold, headache, constipation, fever, skin and cough diseases. The leaves of the plant are regularly eaten raw or employed as flavoring agents, or incorporated as ingredients in the home work of traditional food. It contains the phytochemicals such as monoterpenoids,

sesquiterpenoids, diterpenoids, triterpenoids, phenolics, flavonoids, esters, alcohols and aldehydes. Studies have cited numerous pharmacological properties including antimicrobial, anti-inflammatory, wound healing, larvicidal, anti-epileptic, antioxidant antitumor and analgesic activities.

Isolation of Phylloplane and Endophytic fungi

For the isolation of endophytic fungi, the collected healthy leaves were thoroughly washed in running tap water. Then the leaves were cut into small segments (about 1cm²) including midrib portion. The leaf samples were surface sterilized by 0.1 % mercuric chloride for 60 seconds and then rinsed in sterile distilled water for 10 seconds (three times). For phylloplane mycoflora study, the leaf segments were not surface sterilized since phylloplane fungi grown on the surface of the leaves. Without washing the segments, they were placed on the PDA plates equidistantly.

Culture of leaf samples on agar plates

After sterilization, the excess water was blotted out by sterile filter paper from the leaf segments and kept separately. Then the surface sterilized segments were placed in petridishes containing PDA supplemented with streptomycin. Five (5) leaf such as Young, Mature, Yellow and Litter segments of a centimeter square, both sterile and unsterile were placed separately on the PDA media plates equidistantly by the help of sterile forceps and pressed later on followed by incubation for 3 to 7 days.

Incubation for the growth of fungi

All the plates were incubated at 25±3°C temperature in the incubation chamber. Incubation time was maintained differently since, 7-8 days is meant for the fungal growth of fungi in agar plate method, but in moist chamber method, 1 to 3 weeks are required for the growth of fungi. Every day watch of the petriplates and check the growth of fungi was almost necessary in our present study after 3rd day of incubation.

Identification of fungi

After three days of incubation, the fungal colonies were counted for individual species and the total number was enumerated. Microscopic slides stained with lacto phenol cotton blue were prepared from each colony of the fungus and observed microscopically under the trinocular digital photography microscope to identify up to species level. The colony which was not been identified directly from plates was sub cultured in SDA/PDA media again and identified later on. The laboratory experience and taxonomic literature were employed to identify the fungal CFUs up to species level.^[12-16] The presence and absence based on the occurrence of individual fungus in the phylloplane and endophytic were determined and plotted in the form of tables and figures.

Antibacterial activity of the active agar plugs of dominant fungi

The active agar plugs of 6mm size were taken carefully from the three-day pure culture plates of the leaf surface fungi and were studied for antimicrobial activity against pathogenic bacteria and one fungus using agar plug assay method. The test organisms used were from MTCC cultures i.e., *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shewanella putrefaciens* and *Candida albicans*. The microbes were grown in nutrient broth for 12 h. Lawns of pathogenic bacteria and *Candida albicans* were prepared on nutrient agar plates using sterile cotton swabs. Active agar plugs were placed on nutrient agar plates and each plug was placed inside the wells prepared earlier by cork borer. The plates containing bacteria and active plugs of endophytic fungi with extracts were incubated at 37°C for 24 to 48 hours in the BOD incubator. The plates were examined for the zone of inhibition after 24 hrs, which appeared as clear area around the wells. Inhibition zone diameter was measured in mm by the HI-Media scale.

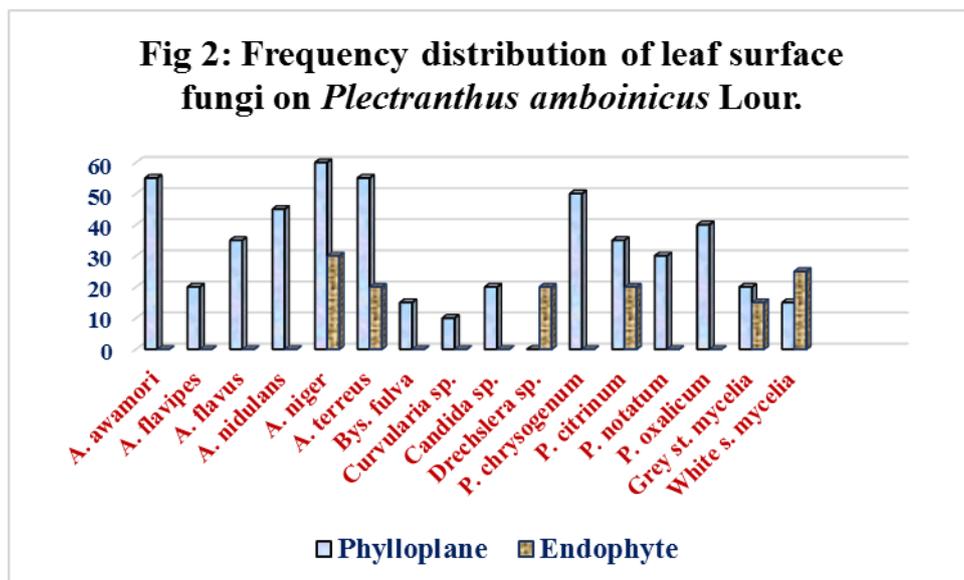
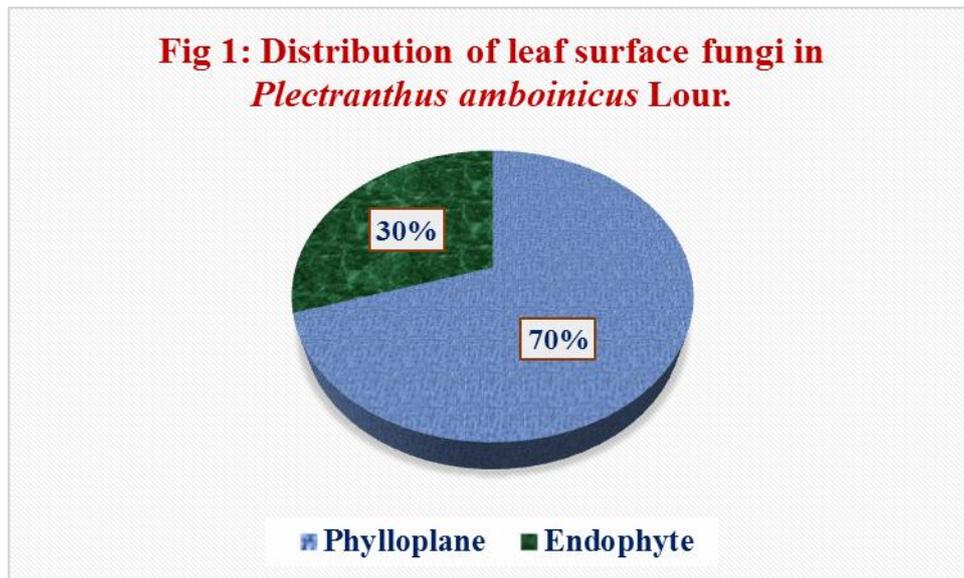
RESULTS AND DISCUSSION

In our present study, overall a total of 16 leaf surface fungal species under 8 genera were isolated and identified from mature leaf samples of the medicinal plant, *Plectranthus amboinicus* (Lour.) by agar plate method. The phylloplane fungi were recorded under 15 species and 7 genera whereas endophytic fungi were of 6 species under 5 genera. Abundance and percentage occurrence of phylloplane and endophytic fungi isolated by agar plate method from the medicinal plant,

Plectranthus amboinicus (Lour.) is given in Table 1. Distribution and frequency of phylloplane and endophytic fungi are given in Fig 1 & 2. In fungal distribution, phylloplane supported the growth of the maximum fungi (70%) in comparison to endophyte (30%) (Fig 1). Leaf surface fungi like, *Aspergillus awamori*, *Aspergillus flavipes*, *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus terreus*, *Curvularia* sp., *Candida* sp., *Drechslera* sp., *Byssoschlamys fulva*, *Penicillium citrinum*, *P. notatum*, *P. oxalicum*, *Penicillium chrysogenum*, Gray sterile mycelia and White sterile mycelia were isolated from the mature leaves of *Plectranthus amboinicus* (Lour.). *Aspergillus awamori*, *A. niger*, *A. flavus*, *A. nidulans*, *A. terreus*, *Penicillium citrinum*, *P. oxalicum* and *P. notatum* were recorded as dominant phylloplane fungi. *Aspergillus terreus*, *Aspergillus niger*, *Drechslera* sp., *Penicillium citrinum*, Gray sterile mycelia and White sterile mycelia were documented as dominant endophytes. Two distinct fungi, *Drechslera* sp. was recognized as special endophytes but not recorded as phylloplane (Table 1). Five fungi of the endophytes were also included with phylloplane fungi but ten of phylloplane were not recorded as endophytes, they remained as phylloplane only (Table 1, Fig 2). Bharathidasan & Panneerselvam.^[10] recorded a total 10 fungal species and they also described that among the endophytic flora, *Phoma* sp. was the most prominent genus. In their work, no endophytes were isolated from 110 leaves samples and overall colonization frequency from surface in their work.

Table 1: Percentage occurrence of leaf surface fungi on *Plectranthus amboinicus* Lour.

Sl. No.	Leaf surface fungi	Phylloplane	Endophyte
1	<i>Aspergillus awamori</i>	55	-
2	<i>A. flavipes</i>	20	-
3	<i>A. flavus</i>	35	-
4	<i>A. nidulans</i>	45	-
5	<i>A. niger</i>	60	30
6	<i>A. terreus</i>	55	20
7	<i>Byssoschlamys fulva</i>	15	-
8	<i>Curvularia</i> sp.	10	-
9	<i>Candida</i> sp.	20	-
10	<i>Drechslera</i> sp.	-	20
11	<i>Penicillium chrysogenum</i>	25	-
12	<i>Penicillium citrinum</i>	35	20
13	<i>P. notatum</i>	30	-
14	<i>P. oxalicum</i>	40	-
15	Grey sterile mycelia	20	15
16	White sterile mycelia	15	25



Ectophytic and endophytic microbes have acknowledged significant attention as they are found to protect their host against pest pathogens and even domestic herbivorous.^[1] Most of the isolated fungi belonged to anamorphic fungi in particular to Deuteromycetes and Ascomycetes.^[3] The isolated fungi of the endophytic and phylloplane origin may lead to the production of special

compound within the host, medicinal plant, *Plectranthus amboinicus*.^[7,9] Fungi have been widely known as a source of bioactive compounds, an excellent example for the anti-cancer drug taxol, which was previously supported to occur only in the plant.^[9] *Plectranthus amboinicus* (Lour.) is a plant having a broad spectrum of medicinal properties.



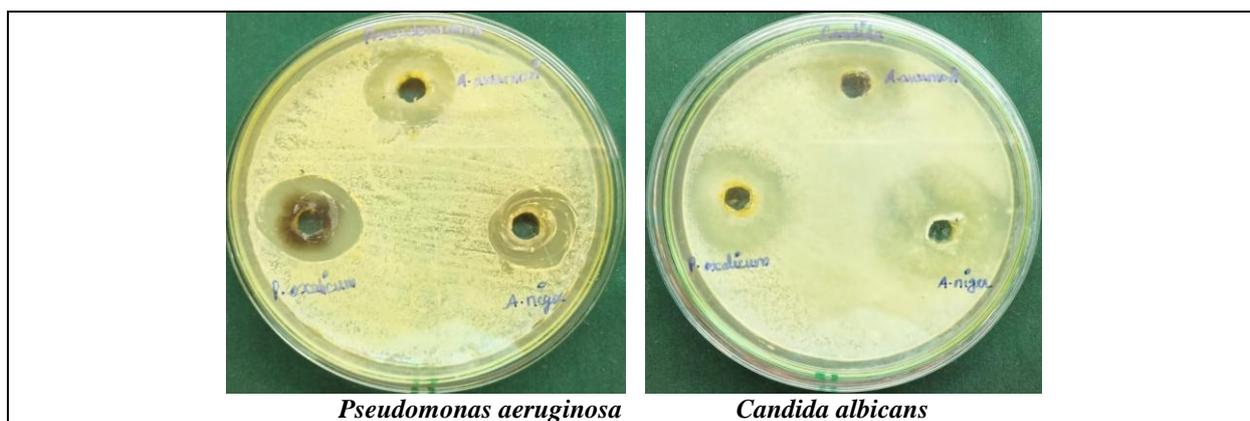
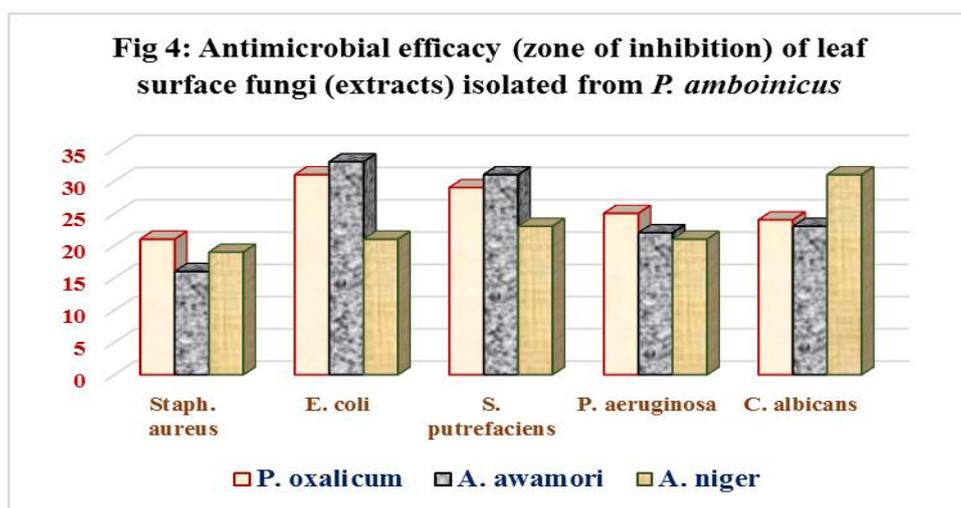


Fig. 3: Antimicrobial potential of leaf surface fungal extracts based on their active agar plugs.



Antimicrobial potential of the leaf surface fungal extracts and their active plugs were found to be good against the MTCC bacteria and *Candida albicans* (Fig 3 and 4). Extracts of *Aspergillus awamori* and *Penicillium oxalicum* showed good efficiency against the pathogens in comparison to *Aspergillus niger*. *Escherichia coli* and *Shewanella putrefaciens* were found to be more susceptible towards the fungal plugs and extracts compared to other pathogenic microbes (Fig 4).

Each part of the plants are used in one or the other ways as medicine. Isolation of only 16 taxa of leaf surface fungi showed that the medicinal property as antimicrobial agents and has some role in order to play in the colonization of fungi in the plants [11]. New studies have been carried out about the endophytic host ecology, taxonomy, biodiversity, reproduction and their power on host. [1,2,11,18] Leaf surface fungi are now considered as an outstanding source of bioactive natural products, because they occupy unique biological niches as they grow in so many unusual environments. [10,11] Studies on endophytic and phylloplane biodiversity of medicinal plant in Puducherry, India was conducted by the Nayak and his teammates. [17] They have reported diversity of fungal species ranging within 30 and they documented the common endophytic fungal isolates and similarity coefficient studies on different medicinal plants by agar

plate method. [1-9] Isolation of different configuration of fungal species from phylloplane and endophytes from medicinal plants may differ but the studies of their involvement in varied applications for the human welfare is really recommendable. [18]

CONCLUSION

Antimicrobial properties of leaf surface fungi with their natural products serve the humankind as the basic remedies of most of the sicknesses. *Plectranthus amboinicus* (Lour.) is one of the medicinal plants which have broad spectrum medicinal properties. In the present study, a total of 16 phylloplane and endophytic fungal species under 8 genera were recorded by agar plate method. The most of the fungi isolated belonged to the class Deuteromycetes and Ascomycetes. Among the phylloplane and endophytic fungi, *Aspergillus awamori*, *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus terreus*, *Drechslera* sp., *Penicillium oxalicum*, *Penicillium citrinum*, *Penicillium chrysogenum*, Gray sterile mycelia were recorded as the most dominant ones. It was also confirmed that the leaf surface fungi of the medicinal plant, *Plectranthus amboinicus* have antimicrobial potential that inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shewanella putrefaciens* and *Candida albicans* in our present study.

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