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Isolation and identification and phytochemical screening of endophytes from medicinal plants

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Abstract
Medicinal plants are known to harbour endophytic fungi that are believed to be associated with the production of pharmaceutical products. Therefore, it is important to explore endophytic mycoflora in the medicinal plants.In the present study, 15 medicinal plants were selected for the presence of endophytic community. The Medicinal plants, were collected from STET Herbal Garden, STET Women's college, Mannargudi, Thiruvarur (DT), Tamil Nadu. Medicinal plants namely, *Alpinia galangal, Ludwigia perennis, Ocimum basilicum, Caesalpinia pulcherrima, Cissus quadrangulaircs, Bryophyllum pinnatum, Piper betle, Sansevieria laxburghii, Phyllanthus acidus, Cymbopogon citrates, Sauropus audrogynus, Rauvolfia tetraphyhlla, Clitoria ternatea, Tridax prochumbens, Ficus nervosa*. Endophytic fungi were isolated from the leaves of above said medicinal plants by Lactophenol Cotton Blue Staining techniques and identified using standard manuals. Isolated and identified endophytic fungi are such as *Aspergillus oryzae, A. flavus, A. fucus, A. fumicates, A.niger, A.terreus, A. nidulans, A. rugulosus, Alternaria tenuis, Rhizopus species, Penicillum citrinum, P. javanicum, Helminthosporum, H. oryzae, Fusarium, Bipolaris.* Maximum number of endophytic fungi belonging to the class Deutromycetes (9 genera), Ascomycetes (2 genera), Phycomycetes (2 genera) and Hypomycetes (3 genera) were recorded. Totally 8 species of *Aspergillus* was dominant in the medicinal plants followed by 2 species of *Penicillum* were recorded. In Phytochemical screening, all the compounds are present in *Tridax procumbens and Ficus nervosa*, colonization frequency was high in *Piper betle*, (77.27) relative percentage occurrence was high in *Bryophyllum pinnatum* (43.55%), endophytic infection rate are highly present in are *Ficus nervosa* (57.14%) detected in the medicinal plants.

Keywords: medicinal plants, endophytes, phytochemicals, colonization frequency

Introduction
India is commonly called the Botanical garden of the world, owing to the wealth of herbal medicines. India with its great topographic and climatic diversity has a very rich and diverse flora and funna. The uses of plants as medicines have been practiced from ancient time. Tamil Nadu is ethanobotanically very rich, having a wide variety of medicinal plants. With its (Cauvery) diverse topographical condition, the region is well situated for a range of medicinal plant species. Mannargudi is located at 380km North of Chennai, 80km east of Tiruchirappalli, 35km east of Thanjavur (1 hour) and 40km west of Kumbakonam. The region is covered with mainly alluvial or black soil which is conducive for rice cultivation.

Endophytes
Endophytes are microorganisms that are presents in living tissues of various plants (root, fruit, steem, seed, leaf etc). Establishing mutual relationship without apparently any symptom of disease. This endophytes protect their hosts from infectious agents and adverse condition by secreting bioactive secondary metabolites.
The term "endophyte'' originally introduced by De Bary (1886) refers to the any organisms occuring within plant tissues, distinct from the epiphytes that live on plant surfaces. Endophytes have been defined by various scientists as mutualists that colonize aerial parts of living plant tissues and do not cause symptoms of disease. Endophytes are microbes which colonize living, internal, tissues of plants without causing any harm to their host (Bocan & White, 2000). All vascular plants harbor endophytic organisms (Zhang *et al*., 2006) [56]. These endophytes protect their hosts from infectious agents and adverse conditions by secreting bioactive secondary metabolites (Azevedo *et al*., 2000; Carroll, 1978) [4, 12].
Endophyte includes a group of microorganisms that grow intra or intercellularly in the tissues of higher plants in which they live, or fungal endophytes are organisms living in a plant host for at least a part of their life, without cause any apparent disease. Such mutualistic interaction between, endophyte and host plant results in fitness benefits for both partners. The microbes residing in the internal parts of plant tissues called "endophyte'' Constitute a group of plant symbionts and are a component of microbial diversity. Endophytes offers plethora of unknown advantages to the host with immense applications in agricultural and medicine (Clay *et al.*, 2002) [13].
Endophytic fungi protect their host against insects, pests, pathogens and even from herbivores (Mainowski & Belesky 2006) [33]. Medicinal plants are reported to harbor endophytes (Strobel, 2002) [43] which in turn protect their host from infectious agents and survive the plants from adverse environmental conditions (Dandu Anita, *et al.*, 2013). Endophytic fungi are able to produce antimicrobial, anticancer such as Taxol (Walker and Croteau 2001) and antimicrobial activities (Wiyakrutta *et al.*, 2004). Endophytic fungi are a

group of fungi that colonize living, internal tissues of plants without causing any immediate, overt negative effects (Hirsch and Braun, 1992).

Medicinal Plants

Medicinal plants traditionally occupied an important position in rural and tribal lives of India and are considered as one of the most important source of medicine since the down of human cultivation. Medicinal plants constitute the basis of the population of India and are a great sources of income for rural population.

Plants with ethano botanical history are generally expected to be powerful sources of endophytes producing active natural products. As more than 3000 disease are clinically described today less than that needs new therapeutic agent with infectious disease control (Strobel and Daisy, 2003) [44].

Medicinal plants are reported to harbor endophytes (Strobel & Daisy, 2014) [45], which in turn provide protection to their host from infectious agents and also provide adaptability to survive in adverse environmental conditions (Jones *et al.,* 2000) [25]. Medicinal plants are known to harbor endophytic fungi that are believed to be associated with the production of pharmaceuticals products (Zhang *et al.,* 1986). Medicinal plants have been recognized as a respiratory of fungal endophytes with novel metabolites of pharamaceuticals importance (Strobel *et al.,* 2004) [46]. The present work planned to investigate the diversity of endophytic fungi from medicinal plants, S.T.E.T Herbel Garden, S.T.E.T Women's College, Thiruvurur (DT).

Materials and Methods

Sample collection

15 Medicinal plants were collected from STET Herbal Garden, STET Women's college, Mannargudi, Thiruvarur (DT), Tamil Nadu. Healthy and mature leaf samples were segregated and brought to the Microbiology laboratory, PG & Research department of Microbiology with utmost care and kept in room temperature for further experiments. The plants are listed below,

Alpinia galanga, Ludwigia perennis, Ocimum basilium, Caesalpinia pulcherrima, Cissus quadrangulaircs, Bryophylum pinnatum, Piper betle, Sansevieria laxburghi, Cicca acida, Cymbopogon citrates, Sauropus audrogynus, Rauvolfia tetraphyhla, Clitoria ternatea, Tridax prochumbins, Ficus nervosa.

Plants with no visible symptoms of disease were carefully selected after physical examination. The plant materials was brought to the laboratory in sterile bags and processed within hours after sampling.

Isolation of endophytic fungi

The collected plant Samples (leaves) were immediately brought to the laboratory and used within 8 hours for isolation of fungal endophytes. Sterile paper bags and stored at 4°C till further use. The voucher specimens were collected and kept in the herbarium of S.T.E.T. Women's College, Mannargudi. These plants samples were throughly washed in running tap water to remove soil particles and adhered debris, and finally with distilled water. From each samples sub samples were prepared for further isolation of endophytes.

Surface sterilization of leaves (Ellis, 1976)

In order to isolate the endophytic fungi, the collected healthy leaves were throughtly washed in tap water. Then the leaves were cut into small segments (about $1cm^2$) including midrib portion. The leaf samples were surface sterilized 0.1 %mercury chloride for 60 seconds and then rised in sterile distilled water for 10 seconds (three times). Freshly prepared PDA medium supplemented with antibiotic Chloramphenicol (50mg/liter).

Culture of leaf samples on agar plates

Five leaf segments of a centimeter square, size range 3-4 cm×0.5-1 cm pieces with and without midrib.were placed on the PDA media plates equidistantly by the help of sterile forceps and pressed later on followed by incubation for 3 to 7days. Endophytes fungi usually began to produce hypal filaments after 5-6 days of incubation at 30 °c. The hypal tips appeared were carefully transferred to fresh potato dextrose agar plates (Gillman, 1857) [21]. Petridishes were sealed, incubated for 2 weeks at 25°c.The pure endophytic fungi strains medium maintained by repeated subculturing.

Colonization frequency (CF)

$$CF = \frac{\text{No. of species isolated}}{\text{No. of segments screened}} \times 100$$

Relative Percentage Occurance (RPO), of different groups of fungi

$$RPO = \frac{\text{Density of colonization of one group}}{\text{Total Density of colonization}} \times 100$$

Endophytic Infection Rates (EIR)

$$EIR = \frac{\text{Number of infected segments}}{\text{Total number of segments screened}} \times 100$$

Identification using standard manuals

The fungal isolates were identified based on their macroscopic features (i.e. the colour, shape and growth of cultured colonies) as well as microscopic characteristics (i.e. the structure of hyphae, conidia, and conidiophores).The identification of the fungi is done using standard laboratory manuals, The genus Aspergillus (Raper and Fennell, 1965), Manual of Penicilla (Raper and Thome, 1949), The manual of soil fungi (Gillman, 1857) [21] and Dematiaceous, Hyphomycetes and more Dematiaceous Hyphomycetes (Ellis,1976), Morphologies ofcultured fungi (Watanable, 2002), Higher fungi (Kohlmeyer, 1979), Soil fungi (Domsch *et al.,* 1980), Hyphomycetes (Subramaniyan,1971), A manual of soil fungi (Gillman, 1857) [21], Inventory of fungi (Rossman, 1998), Fungi of India (Goswami and Ojha, 2001) [24], Microbiology (Pandey *et al.,* 2004) [36]. Physiology of fungi (Lilly,1951), Genera of imperfect fungi (Barnett, 1998) [7], The *Fusarium* laboratory manual (Leslie, 2006), Isolation of fungi (Suryanarayanan *et al.,* 2011) [48], Microbial endophytes (Bacon, 2000) [6], The genus of *Fusarium* (Booth,

1971), Illustrated genera of fungi (Barnett & Hunter, 1987) [7], Identifying filamentous fungi (St-Germain & Summerbell, 1996), Microbial application (Benson, 1998), communities of endophytic fungi (Gamboa and Bayman, 2001), Microbial ecology (Promputtha *et al.*,2005).

Phytochemical analysis was also the standard methods done by (Safoware, 1993), (Trease, Erans, 1989) [53], (Harborne, 1857) [26].

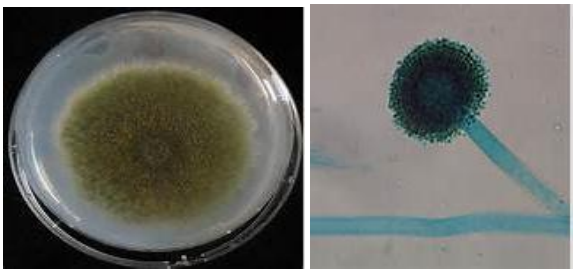
Results

Collection of medicinal plants

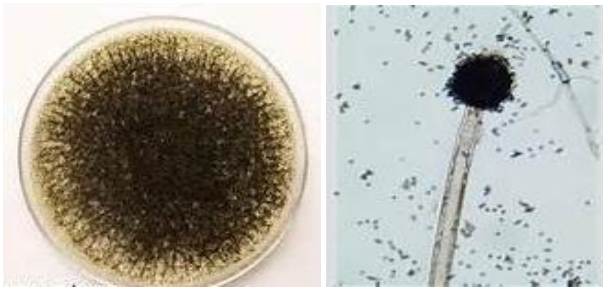
The present study was planned to isolate the endophytic flora from the leaves of the medicinal plants in around STET herbal Garden, Mannargudi, Thiruvarur (DT). A total of 15 medicinal plants were screened for the presence of endophytic fungi.

The fresh leaves of *Alpinia galangal, Ludwigia perennis, Ocimum basilicum, Caesalpinia pulcherrima, Cissus quadrangulaircs, Bryophyllum pinnatum, Piper betle, Sansevieria laxburghi, Phyllanthus acidus, Cymbopogon citrates, Sauropus audrogynus, Rauvolfia tetraphyhlla, Clitoria ternatea, Tridax prochumbens, Ficus nervosa*. Medicinal plants were collected from STET Herbal Garden, STET Women's college, Mannargudi, Thiruvarur (DT). Endophytic fungi were isolated from these plants (Fig1).

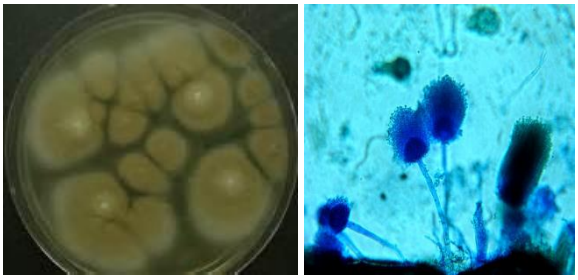
Aspergillus oryzae



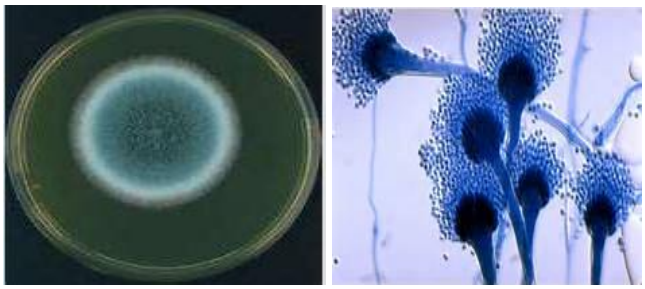
A. flavus



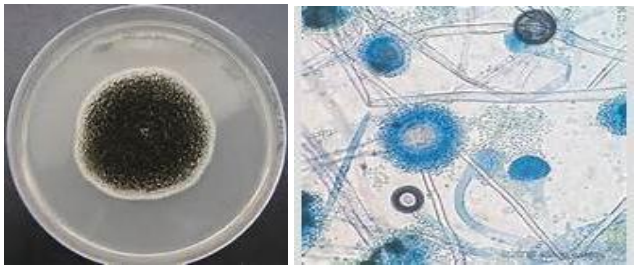
A.terreus



A. fumicates

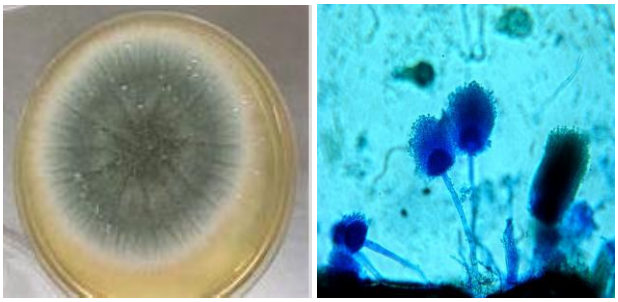


A.niger



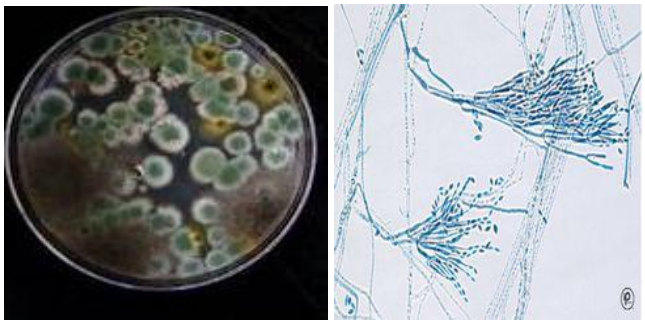
A. nidulans

Alternaria tenuis

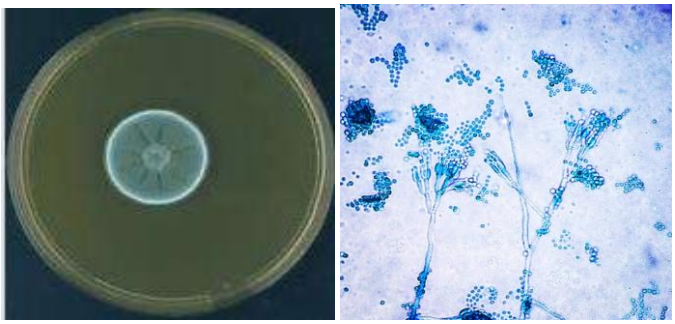


***Rhizopus* sps**

Penicillum citrinum

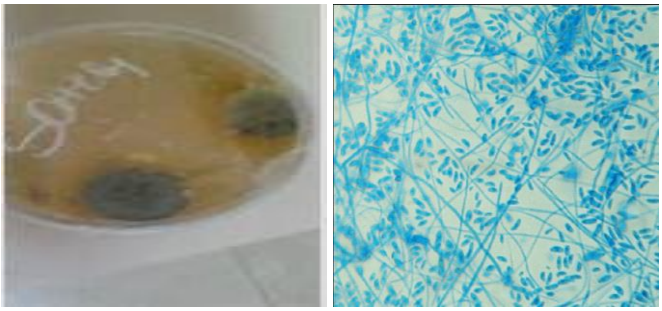


Penicillum citrinum

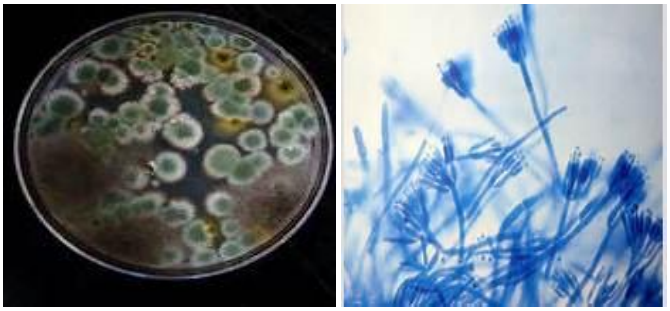


Alternaria tenuis

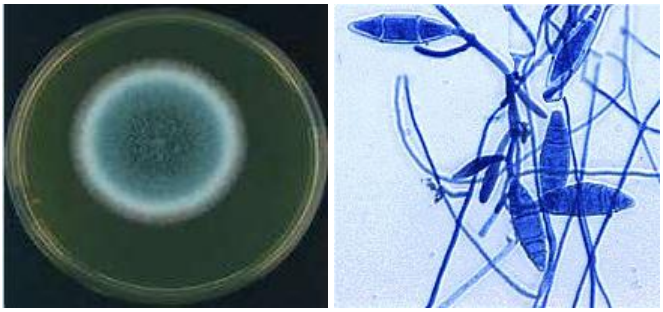
H. oryzae



P. notatum



Bipolaris



Fusarium

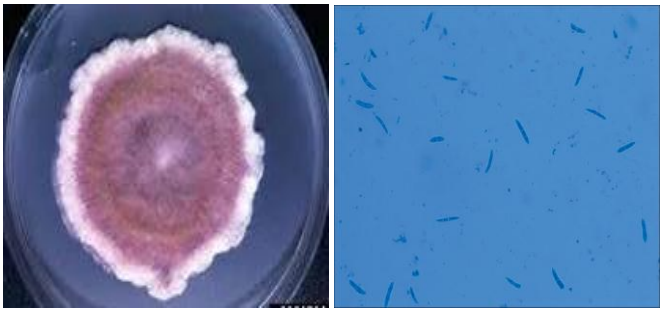


Fig 3: Identification of endophytic mycoflora of medicinal plants

Isolation and identification of endophytic fungi

Totally 15 medicinal plants were used for isolation, endophytic fungi, collected from STET Herbal Garden, STET Women's college, Mannargudi, Thiruvarur (DT). The isolated and identified endophytic fungi such as *Aspergillus oryzae, A. flavus, A. fucus, A. fumicates, A.niger, A. nidulans, A. rugulosus, A.terreus, Alternaria tenuis, Rhizopus* sps, *Penicillum citrinum, P. notatum, Helminthosporum, H. oryzae, Fusarium, Bipolaris.*(Table 1).The medicinal plants were preserved as harberium in terms for future reference.

Table 1: Details of isolated endophytic fungi from medicinal plants

Organisms	Size	Colour	Conidial structure
Aspergillus oryzae	500-2500	Un coloured	Smooth conidia
A. flavus	400-800	Pale brown	Smooth hyphae andrough conidia
A. fucus	200-300	Yellow or green	finely roughened
A. fumicates	200-400	Grayish color	Smooth conidia
A.niger	400-3000	Slightly brown	Very rough conidia
A. nidulans	70-150	Brown in age	Smooth and slightly rough
A. rugulosus	500-2300	Yellow coloured	Rough conidia
A.terreus	100-250	Un coloured	Smooth conidia
Alternaria tenuis	300-400	Light brown colour	Longest conidia
Rhizopus sps	22 - 343	Growing dark green color	Septate conidium
Penicillum citrinum	260-280	Green and pale yellow	Septate hyphae
P. notatum	260-280	Olive green and off white	Smooth hyphae
Helminthosporum	430–580	Brown	Straight conidia
H. oryzae	430–580	brown or olivaceous brown	Straight to flexuousconidia
Fusarium	3.2 - 4.5	White cream gray surface	Micro or macro conidia
Bipolaris	2–14	fast growing grey toblackish brown	Large conidia

Maximum number of endophytic fungi belonging to the class Deutromycetes (4 genera) and Phycomycetes (2 genera) were recorded. Nearly 8 species of *Aspergillus* was dominant and 2 species *Penicillum* are recorded. All other genera were represented in terms of species, they are recorded. Morphology of the isolated organisms are listed in the Table 2

Table 2

S.no	Host plants	Tamil name	Parts collection	Fungi
1	*Tridax procmbens*	Vettukaya thalai		*Aspergillus oryzae*
2	*Phyllanthus acidus*	Aranellai		*Bipolaris A. flavus*
3	*Ficus nervosa*	Neer all		*A. fucus* *A. terreus*
4	*Alpinia galangal*	Setherathi		*A. fucus* *A. terreus*
5	*Cissus quadrangularis*	Pirandai	Leaves	*A. fumicates* *A. nige*
6	*Ludwigia perennis*	Muyal kathilai		*A. nidulans*
7	*Cymbopogon citrates*	Lemon grass		*A. rugulosus* *Alternaria tenuis*
8	*Bryophyllum pinnatum*	Rana kelli		*Rhizopus* sps
9	*Ocimum basilicum*	Thirunetru pachilai		*Penicillum citrinum*
10	*Sanseveria raxburghii*	Marul		*Alternaria tenuis*
11	*Piper betle*	Vettrilai		*P. notatum*
12	*Clitoria ternatea*	Sangu poo		*Fusarium*
13	*Caesalpinia pulcherrima*	Mauir kondri		*Penicillum citrinum*
14	*Rauvolfia tetraphylla*	Aval pori		*H. oryzae*
15	*Sauropus androgynus*	Davasi murangi		*Bipolaris*

List of endophytic fungi from medicinal plants

During the present method study *Aspergillus oryzae, Bipolaris* are present in *Tridax procmbens*, next *A. flavus* present in *Phyllanthus acidus*, *A.fucus* and *A.terreus* present in *Ficus nervosa, A. fumicates* present in *Alpinia galangal*. In *Cissus quadrangularis* was persent *A.niger. A. nidulans* present in *Ludwigia perennis, A.rugulosus* and *Alternaria tenuis* present in *Cymbopogon citrates*, Similarly *Rhizopus* sps was persent in *Bryophyllum pinnatum, Penicillum citrinum* present in *Ocimum basilicum, Alternaria tenuis* was present in the *Sanseveria raxburghii*, *P. javanicum* present in the *Piper betle.*Moerover *Fusarium* was present in the *Clitoria ternatea*, *Penicillum citrinum* present in the *Caesalpinia pulcherrima, H. oryzae* present in *Rauvolfia tetraphylla, Helminthosporum* present in *Sauropus androgynus.*

Phytochemical screening of medicinal plants

In phytochemical screening steriods are commonly present in the plants namely, *Tridax procmbens, Ficus nervosa, Ludwigia perennis, Bryophyllum pinnatum, Ocimum basilicum, Sauropus androgynus.* Trepenoids was present in the *Tridax procmbens, Ficus nervosa, Cissus quadrangularis, Cymbopogon citrates, Bryophyllum pinnatum, Ocimum basilicum, Piper betle, Clitoria ternatea, Caesalpinia pulcherrima, Rauvolfia tetraphylla, Sauropus androgynus.*Glycosides present in the *Tridax procmbens, Ficus nervosa, Cissus quadrangularis, Ludwigia perennis, Ocimum basilicum, Piper betle, Clitoria ternatea, Caesalpinia pulcherrima, Rauvolfia tetraphylla.* Flavonoides are also persent in the *Tridax procmbens, Ficus nervosa, Alpinia galangal, Cissus quadrangularis, Ludwigia perennis,*

*Cymbopogon citrates, Bryophyllum pinnatum, Ocimum basilicum, Clitoria ternatea, Caesalpinia pulcherrima, Rauvolfia tetraphylla, Sauropus androgynus.*Next saponins present in the *Tridax procmbens, Ficus nervosa, Phyllanthus acidus, Cissus quadrangularis, Ludwigia perennis, Cymbopogon citrates, Bryophyllum pinnatum, Ocimum basilicum, Caesalpinia pulcherrima, Rauvolfia tetraphylla*. Over all aspects steriods, Trepenoids, Glycosides, Flavonoides, Saponins are commonly present in *Tridax procmbens, Ficus nervosa.* (Table 3).

Table 3

Plants	**Steroids**	**Trepenoids**	**Glycosides**	**Flavonoids**	**Saponins**
Tridax procmbens	+	+	+	+	+
Phyllanthus acidus	_	_	_	_	+
Ficus nervosa	+	+	+	+	+
Alpinia galangal	_	_	_	+	_
Cissus quadrangularis	_	+	+	+	+
Ludwigia perennis	+	_	+	+	+
Cymbopogon citrates	_	+	_	+	+
Bryophyllum pinnatum	+	+	_	+	+
Ocimum basilicum	_	+	+	+	+
Sanseveria raxburghii	_	_	_	_	_
Piper betle	+	+	+	_	_
Clitoria ternatea	+	+	+	+	_
Caesalpinia pulcherrima	_	+	+	+	+
Rauvolfia tetraphylla	_	+	+	+	+
Sauropus androgynus	+	+	_	+	_

(+) = Positive
(-) = Negative

Colonization frequency

Moreover it was observed that the colonization frequency (CF) was noticed in *Sanseveria* raxburghii (43.42%),*Alpinia galangal*(12.05%), *Ludwigia perennis* (23.54%), *Ocimum basilium*(9.33%), *Caesalpinia pulcherrima*(18.54%),*Cissus quadrangulaircs*(11.11%), *Bryophylum pinnatum*(4.45%),*Piper betle*(77.27%), *Phyllanthus acidus* (10.05%), *Cymbopogon citrates*(43.33%), *Sauropus audrogynus* (10.45), *Rauvolfia tetraphyhlla*(17.13%), *Clitoria ternatea*(20.10%), *Tridax procumbens* (9.33%), *Ficus nervosa*(20.05%).(Table 4)

Table 4

Plants	**Colonization frequency (%)**
Tridax procmbens	9.33%
Phyllanthus acidus	10.05%
Ficus nervosa	20.35%
Alpinia galangal	12.05%
Cissus quadrangularis	11.11%
Ludwigia perennis	23.54%
Cymbopogon citrates	43.33%
Bryophyllum pinnatum	4.45%
Ocimum basilicum	9.33%
Sanseveria raxburghii	43.42%
Piper betle	77.27%
Clitoria ternatea	20.10%
Caesalpinia pulcherrima	18.54%
Rauvolfia tetraphylla	17.13%
Sauropus androgynus	10.45%

Relative percentage occurance (RPO)

Similarly Relative percentage occurance (RPO**)** was also obtained in the selected medicinal plants in our study. Maximum PRO was noticed in *Alpinia galangal* (23.55%), *Ludwigia perennis* (12.32%), *Ocimum basilicum*(34.23%), *Caesalpinia pulcherrima*(13.33%),*Cissus quadrangulaircs*(20.23%), *Bryophyllum pinnatum*(43.55%), *Piper betle*(10.56), *Sansevieria laxburghi*(15.02%), *Phyllanthus acidus*(15.33%), *Cymbopogon citrates* (24.55%), *Sauropus audrogynus*(26.6%), *Rauvolfia tetraphyhlla* (20%), *Clitoria ternatea*(23%), *Tridax prochumbens* (14.54%), *Ficus nervosa*(12.53%).(Table 5)

Table 5

Plants	**Relative percentage occurance (%)**
Tridax procmbens	14.54%
Phyllanthus acidus	15.33%
Ficus nervosa	12.53%
Alpinia galangal	23.55%
Cissus quadrangularis	20.23%
Ludwigia perennis	12.32%
Cymbopogon citrates	24.55%
Bryophyllum pinnatum	43.55%
Ocimum basilicum	34.23%
Sanseveria raxburghii	15.02%
Piper betle	10.56%
Clitoria ternatea	23%
Caesalpinia pulcherrima	13.33%
Rauvolfia tetraphylla	20%
Sauropus androgynus	26.6%

Endophytic infection rate (EIR)

Among the isolated endophytic fungi Endophytic infection rate (EIR) was also noticed *Alpinia galangal* (20%), *Ludwigia perennis*(42.8%), *Ocimum basilium* (20.32%), *Caesalpinia pulcherrima* (22.43%),*Cissus quadrangulaircs* (26.6%), *Bryophyllum pinnatum*(15.45%), *Piper betle*(18.54%), *Sansevieria laxburghi* (13.34%), *Phyllanthus acidus*

(13.33%), *Cymbopogon citrates* (13.33%), *Sauropus audrogynus* (12.54%), *Rauvolfia tetraphyhlla* (32.5%), *Clitoria ternatea* (19.20%), *Tridax prochumbins* (33.3%), *Ficus nervosa* (57.14%). (Table6)

Table 6

Plants	Endophytic Infection Rate (%)
Tridax procmbens	33.3%
Phyllanthus acidus	13.33%
Ficus nervosa	57.14%
Alpinia galangal	20%
Cissus quadrangularis	26.6%
Ludwigiaperennis	42.8%
Cymbopogon citrates	13.33%
Bryophyllum pinnatum	15.45%
Ocimum basilicum	20.32%
Sanseveria raxburghii	13.34%
Piper betle	18.54%
Clitoria ternatea	19.20%
Caesalpinia pulcherrima	22.43%
Rauvolfia tetraphylla	32.5%
Sauropus androgynus	12.54%

Aspergillus species are frequently isolated from *Tridax procmbens, Phyllanthus acidus, Cissus quadrangularis, Ficus nervosa.* Moreover all the Phytochemical screening compounds are present in *Tridax procmbens, Ficus nervosa.* Colonization frequency (CF) was high in *Piper betle* (77.27%), Relative percentage occurance (RPO) was high in *Bryophylum pinnatum* (43.55%), Endophytic infection rate (EIR) was highly present in *Ficus nervosa* (57.14%) are also high in these medicinal plants. Totally 16 endophytic fungi were isolated belonging to the class Deutromycetes and Phycomycetes.

Discussion

Our results were correlated to the findings of Rezwana Khan *et al.,* (2010) communities of endophytic fungi in medicinal plant *Withania somnifera.* Thirty-three fungal strains of 24 different species were isolated. The endophytic fungal communities found at three different sites were different. Almost all the isolates were recovered from older plant samples than younger once. The highest species richness, as well as frequency of colonization was found in stems. With exception of *Aspergillus niger, Aspergillus terreus, and Alternaria alternate*, all the fungi were found to be organ specific. In our study *Aspergillus* was the highest species from *Tridax procmbens, Phyllanthus acidus, Ficus nervosa, Alpinia galangal, Cissus quadrangularis, Ludwigia perennis* plants leaves.

Our results were agreed with Ashok (2016) endophytic mycoflora of some medicinal plants that study totally 35 species belonging to 11 genera of endophytic fungi were isolated from medicinal plants collected from Kumbakonam, Thanjavur (DT). The isolated and identified endophyticfungi such as *Aspergillus candidus, A.flavus, A.fucus, A.fumatigatus, A.ellipticus, A.granulosus, A.luchuensis, A.niger, A.nidulans, A.nutans, A.pulvinus, A. rugulosus, A.sulphureus, A.sydowi, A.stromatoides, A. terreus, A.thomii, A.ustus, A. variecolor, Absidia glauca, Rhizopus sp, Syncephala strum, and Verticillum terrestre.*In our study 8 species of *Aspergillus,* 2 species of *Penicillum* were isolated from *Ocimum basilicum, Piper betle, Caesalpinia pulcherrima, Tridax procmbens, Phyllanthus acidus, Ficus nervosa, Alpinia galangal, Cissus quadrangularis, Ludwigia perennis* plant leaves.

Similar to Banerjee *et al.,* (2009) studied endophytes of three medicinal plants of Lamiaceae *(Ocimum sanctum Linn, O.basilicum L. and Leucas aspera* (willd) link and *Vitex negundo L.* (Verbenaceae), respectively. Krishnamurthy *et al.,* (2008) [27] nine medicinal herbs for fungal communities from Bhandra river project, Malnad region, Sourthern India during wet and dry seasons. In their study, colonization frequency of fungal species varied significantly between two seasons. In our study colonization frequency was high in *Piper betle* (77.27%).

Moreover Manisha shukla *et al., (*2012) fungal flora of some medicinal plants. In present study different species of fungi were isolated from different medicinal plants. *Aspergillus, Curvularia, Penicillum, Alternaria, Fusarium* are some of common and abdundant fungi in all most all medicinal plants. In the present study, endophytic fungi *Aspergillus oryzae, A. flavus, A. fucus, A. fumicates, A.niger, A. nidulans, A. rugulosus, A.terreus, Alternaria tenuis, Rhizopus* sps, *Penicillum citrinum, P. notatum, Helminthosporum, H. oryzae, Fusarium, Bipolaris.*

Our results were similar to the funging of Bijaya Kumar Nayak (2015) isolation and identification of phylloplane and endophytic fungi one ornamental plant, *Mangifera indica.* Among the phylloplane and endophytic fungal population *Aspergillus niger, Tricoderma* sp. Our results were highlighted that isolated endophytic fungi in particluar to *Deuteromycetes* and *Ascomycetes*.

Monnanda Somaiah Nalini *et al.,* (2014) reported endophytic fungal diversity in medicinal plants of Western Ghats, India. From 5200 segments of plants materials a total of 1529 isolated were obtained; these were grouped into 31 taxa. Mycelia the fungal taxa that failed to sporulate, were also reported from this study. This fungal group is prevalent in endophytic studies. The fungal endophytes were analyzed from four plant parts, namely, stem, root, rhizome and infloresence; however, their occurance in root and infloresence was investigated for few plant species only, as the phenology and sampling of plants never correlated with seasons. The leaves were not considered for isolations since some of the plants were climbers and stagglers with delicate hairy surfaces and stringent surface sterilization techniques would render them unsuitable for plating on agar medium. Relative precentage of endophytic isolations from stems segments were greater (80.37%) than isolations from roots (19.22%). In our study, the relative percentage occurance (RPO) are highly present in the plants are *Ocimum basilicum* (34.23%), *Bryophyllum pinnatum* (43.55%).

Conclusion

Many of pharmaceutical compounds produced by medicinal plants are reportedly produced by their endophytic fungi. Hence, it is important to study medicinal plants for their endophytic mycoflora for biodiversity and then to determine their medicinal properties. Medicinal plants have been recognized as the repository of fungal endophytes with novel

metabolites of pharmaceuticals importance. The future work pertaining to isolation endophytic fungi from other plant parts, carried out for broad spectrum medicinal properties. Medicinal plants are chosen to study because they come in abundant source, easily available and some of them are already being utilized in traditional medicinal. By studying the presence of phytochemical in these plants in traditional treatment can be explained scientifically.

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