INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

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ISOLATION AND SCREENING OF ENDOPHYTIC FUNGI FROM MEDICINAL PLANTS OF VIRUDHUNAGAR DISTRICT FOR ANTIMICROBIAL ACTIVITY

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ABSTRACT
Fifty one fungal endophytes belonging to twenty one genera were isolated from medicinal plants in Virudhunagar District. The crude metabolite of endophytic fungus, *Cladosporium* sp. displayed a significant antimicrobial activity against all test pathogens. Phytochemical analysis of ethyl acetate solvent extract revealed the presence of saponins, phenolic compounds, anthraquinones, steroids, cardiac glycosides and tannins in *Alternaria alternata and Cladosporium* sp. The extract of *Acyranthus aspera* was effective against all test organisms except *Bacillus cereus, B. subtilis* and *Proteus* sp. Endophyetes can reduce the growth of the harmful bacteria in plants by different mode of action.

KEYWORDS: Endophytic fungi, medicinal plants, phytochemicals, antibacterial activity.

INTRODUCTION

Fungi are group of organism having a great biodiversity. They are the second largest group after insects and key component of tropical ecosystems throughout the world. They are present in most plant parts, especially the leaves, where the tissue is apparently healthy. They may be endophytes, epiphytes or latent pathogens. The term 'endophyte' includes all organisms that grow inside plant tissues without causing disease symptoms (Petrini, 1991 and Chanway, 1996). Endophytic fungi are unexplored group of organisms that has enormous potentials for new pharmaceutical substances. They play an essential role to provide protection to their host against attack by other pathogens and environmental factors. An endophyte is a bacterial (including actinomycete) or fungal microorganism, which spends the whole or part of its life cycle colonizing inter- and/or intra-cellularly inside the healthy tissues of the host plant, typically causing no apparent symptoms of disease (Tan and Zou, 2001). The environmental conditions under which the host is growing also affect the endophyte population and the endophyte profile may be more diversified in tropical areas. Endophytes can influence soil stability (Beglinger *et al.*, 2011) directly by their mycelial networks in the soil as well as indirectly altering roots and physical conditions of the host plants. Medicinal plants and their endophytes are important resources for discovery of natural products. In developing countries, the indigenous communities have been using medicinal plants in different ways for the treatment of various diseases, which in turn has resulted in scientific discoveries, with a wealth of literature on plant extracts and their biological activities. Now a day's herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness, minimal side effects in clinical experience and relatively low cost (Valiathan, 1998). Endophyte-infected plants often grow faster than non-infected plants. They colonise plant tissue and are remain within the tissue, except that fruiting structures may emerge through the surface of the plant tissue. Indeed, leaves may be fully colonised by a variety of fungi within a few weeks of leaf emergence. The colonies remain asymptomatic and some in perennial plant parts may have a very long life. In a microbe-plant relationship, endophytes contribute substances that possess various types of bioactivity, such as antibacterial, antifungal, antibiotic, antitumor, antioxidant, anti-inflammatory, *etc*. The bioactive substances in plants are produced as secondary metabolites. Virudhunagar District is surrounded by the Western Ghats in their western side of Tamil Nadu. Virudhunagar District of Tamil Nadu holds very different vegetation belts from dry lands to thick forests. In the present study we focus on the isolation and identification of endophytic fungi from medicinal plants of Virudhunagar District and screening them for antibacterial and antifungal activities and to identify the phytochemical compounds in the extracts of endophytic fungi.

METHODOLOGY

Study area and collection of samples

Medicinal plant materials were collected from in and around Virudhunagar District, Tamil Nadu, South India. The total area of Virudhunagar District is 3445.73 km^2. Medicinal plant samples were collected during February-March 2010. The mean temperature during the study period was 32±2°C. The plant species were identified (Gamble and Fischer, 1928), authenticated and maintained in our Laboratory. Mature healthy plant leaves were collected by sampling from different parts of the trees. Leaf samples from each plant were randomly cut off with an ethanol-disinfected sickle and placed separately in sterile polythene bags and stored in an icebox, chilled samples (4.5ºC) were used to isolate endophytic fungi within 48 h of collection.

Isolation of endophytic fungi

Leaves were thoroughly washed with mild detergent and running tap water and then air–dried. After which they were surface sterilized by submerging them in 75% ethanol for 2 min. The branch portions were further sterilized sequentially in 5.3% sodium hypochlorite solution for 5 min, and 75% ethanol for 0.5 min. After sterilization, each leaf was divided into three segments and placed on czapek's dox agar (CDA) and water agar (WA) medium supplemented with streptomycin (100mg/L) to suppress bacterial growth. Branch portions were cut to expose their inner tissue and placed on the same medium. All the plates were incubated at 27°C until fungal growth appeared. The plant segments were observed once a day for the growth of endophytic fungi. Hypal tips growing out the plated segments were immediately transferred into potato dextrose agar slant and maintained at 4°C. The fungal isolates were identified based on their morphological characters conidiospore structures using standard identification manuals (Subramanian, 1971; Barnett and Hunter, 1972).

Mass cultivation of endophytic fungi

The fungal endophytes were mass cultivated on potato dextrose broth by placing agar blocks of actively growing pure culture (3mm in diameter) in 250ml Erlenmeyer flasks containing 100ml of the medium. The flasks were incubated at room temperature for 3 weeks with periodical shaking at 150 rpm. After the incubation period, the cultures were taken out and filtered through sterile cheesecloth to remove the mycelia mats. Mycelial mats were dried and the biomass of endophytes was measured.

Extraction of metabolites from endophytic fungi

After mass cultivation of endophytic fungi, the fungal metabolites from different endophytic mycelial mats were extracted by using ethyl acetate. Equal volume of the filtrate and solvent was taken in a separating funnel and shaken vigorously for 10 min. The solution was then allowed to stand, the cell mass got separated and the solvent so obtained, was collected. Ethyl acetate was evaporated and the resultant compound was dried in vacuum evaporator using $MgSO_4$ to yield the crude extract (Raviraja *et al.,* 2006). The crude extracts were then dissolved in Dimethyl sulphoxide (DMSO) for antimicrobial bioassay.

Selection of test organisms

Altogether nine common human pathogens were used to evaluate the antimicrobial activity of endophytic crude extracts. All the test pathogens were obtained from Department of Microbiology, V.V.Vanniaperumal College for Women, Virudhunagar. Gram-positive *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus* and *Streptococcus pyogens,* Gram-negative *Escherichia coli, Klebsiella pneumoniae, Proteus* sp., *Pseudomonas* sp. and *Salmonella typhi* were used for this study. The selected microbial cultures were maintained using sub culturing technique. Nutrient agar slants were used for bacterial culture maintenance. Bacterial cultures after 24 hrs incubation period at 37^{o}C, the tubes were kept under refrigerator condition.

Evaluation of antimicrobial activity of endophytic fungi

Endophytic fungal crude extracts was screened for their antimicrobial activity with nine reference human pathogenic microorganisms. Antimicrobial activity was determined using the paper disc susceptibility test (Wang *et al*., 1999). A sterilized filter paper was dipped into the extracts and then placed on to the lawn of reference microorganisms. The magnitude of antimicrobial activity was assessed by the diameter of inhibition zones relative to those of positive and negative controls. Streptomycin used as positive controls, and 10% DMSO as a negative control. The plates were incubated at 35±1°C for 24 h and the zone of inhibition was measured and compared with the control. Three replicates were maintained in each case.

Phytochemical Analysis

The endophytic fungal crude extracts were subjected to various qualitative chemical tests to determine phytochemical constituents such as saponins, phenolic compounds, anthraquinones, steroids, cardiac glycosides (Bandoni *et al.,* 1976) and Tannins (Thomson, 1987).

RESULTS & DISCUSSION

In the present study, fifty plants belonging to various families were observed in and around Virudhunagar District, Tamil Nadu, South India are presented in Table 1.

TABLE 1. List of Medicinal Plants in and around Virudhunagar District

S.No	Botanical Name	Tamil Name	Family
1	*Abutilon indicum*	Thuththi	Malvaceae
2	*Achyranthes aspera*	Naayuruvi	Amaranthaceae
3	*Acorus calamus*	Vasambu	Araceae
4	*Adhatoda zeylanica*	Adhatodai pavettai	Acanthaceae
5	*Aegle marmelos*	Vilvam	Rutaceae
6	*Aerva wightii*	Sirupeelai	Amaranthaceae
7	*Allium cepa*	Venkayam	Lilliaceae
8	*Aloe vera*	Sortru kartralai	Liliaceae
9	*Andrographis paniculata*	Siriyanangai (or) Nilavembu	Acanthaceae
10	*Arachis hypogaea*	Nilakadalai	Fabaceae
11	*Asparagus racemosus*	Thanneervittan kizhangu	Liliaceae
12	*Azadirachta indica*	Vembu	Meliaceae
13	*Borassus flabellifer*	Panai	Arecaceae
14	*Calotropis gigantea*	Erukku	Asclepiadaceae
15	*Capsicum annuum,*	Milakai	Solanaceae
16	*Cassia auriculata*	Avarai	Caesalpiniaceae
17	*Centella asiatica*	Vallarai	Apiaceae

18	*Citrullus colocynthis*	Kompatikai	Cucurbitaceae
19	*Coccinia grandis*	Kovai	Cucurbitaceae
20	*Cocos nucifera*	Thennai	Arecaceae
21	*Curcuma longa*	Manjal	Zingiberaceae
22	*Enicostemma littorale*	Vellarugu	Gentianaceae
23	*Ferula asafoetida*	Perunkaayam	Apiaceae
24	*Ficus benghalensis*	Aalam	Moraceae
25	*Gloriosa superba*	Kalapaikilangu	Liliaceae
26	*Gymnema sylvestre*	Sirukurinjan	Asclepiadaceae
27	*Hemidesmus indicus*	Nannari	Asclepiadaceae
28	*Ixora arborea*	Marapaavattai	Rubiaceae
29	*Lagenaria siceraria*	Suraikai	Cucurbitaceae
30	*Lawsonia inermis*	Maruthani	Lythraceae
31	*Leucas aspera*	Thumbai	Lamiaceae
32	*Morinda tinctoria,*	Manjanathi	Rubiaceae
33	*Murraya koenigii*	Karuveppilai	Rutaceae
34	*Ocimum basilicum*	Naai thulasi	Lamiaceae
35	*Ocimum sanctum*	Thulasi	Lamiaceae
36	*Pedalium murex*	Aanai mul	Pedaliaceae
37	*Phyllanthus amarus*	Keelanelli	Euphorbiaceae
38	*Phyllanthus emblica*	Nelli	Euphorbiaceae
39	*Phyllanthus reticulatus*	Karinelli	Euphorbiaceae
40	*Ricinus communis*	Aamanaku	Euphorbiaceae
41	*Sesamum indicum*	Ellu	Pedaliaceae
42	*Solanum trilobatum*	Thoothuvalai	Solanaceae
43	*Solanum xanthocarpum*	Kandankathari	Solanaceae
44	*Tephrosia purpurea*	Kozhingi	Fabaceae
45	*Terminalia bellirica*	Thanri	Combretaceae
46	*Thespesia populnea*	Poovarasu	Malvaceae
47	*Tinospora cordifolia*	Seenthil	Menispermaceae
48	*Tribulus terrestris*	Nerunji	Zygophyllaceae
49	*Vigna unguiculata*	Thattanpayaru	Fabaceae
50	*Zingiber officinale*	Ingi	Zingiberaceae

TABLE 2. Useful parts, uses and medicinal properties of the selected medicinal plants

Botanical Name	Useful part of the plant	Medicinal properties and uses
Abutilon indicum	Whole plant	The plant is used as a demulcent, aphrodisiac, laxative, diuretic, pulmonary and sedative (leaves). The bark is astringent and diuretic; The leaves can also be used to treat ulcers, headaches, gonorrhea and bladder infection.
Achyranthes aspera	Whole plant	The plant is bitter, acrid, digestive, diuretic, spermicidal, anti-allergic, cardiovascular, nephroprotective, antiparasitic, hypoglyceamic, analgesic and antipyretic.
Acorus calamus	Rhizomes	The rhizome is acrid, bitter, aromatic, antipyretic, insecticidal, cough, skin diseases, colic, tranquillising, sedative, analgesic and hypertensive.
Adhatoda zeylanica	Leaves	The leaves, flowers, fruits and roots are extensively used for treating cold, cough, whooping-cough and chronic bronchitis and asthma as sedative-expectorant, antispasmodic and as anthelminthic.
Aegle marmelos	Leaves, roots and fruits	The leaves are astringent, laxative, febrifuge and expectorant and useful in diarrhoea, dysentery, constipation.
Aerva wightii	Whole plant	The plant is diuretic, demulcent, decoction is used to remove swellings. *Aerva* plants are used to cure lithiasis, dropsical affections, eye affection, toothache, headache, in disorders of abdomen and inflammation of internal organs.
Allium cepa	Bulb	The bulb is used to regulate blood pressure (hypertension), treat inflammation, diabetes, urinary problems, dysentery, fever, dropsy, colic, renal and biliary calculi, catarrh, chronic bronchitis, scurvy, body heat, spleen enlargement, rheumatic pain and extract is used externally for acne treatment.
Aloe vera	Leaves	The plant is bitter, laxative, wound healing, skin burns. It is used for local application in painful inflammations, chronic ulcers.
Andrographis paniculata	Whole plant	The plant is bitter, acrid, expectorant digestive, and useful in chronic fever, weakness, intestinal worms and release of gas.
Arachis hypogaea	Seeds, Oil	The seeds are sweet, oleaginous, aphrodisiac, galactagogue, constipating and tonic and are useful in agalactia, diarrohea and general debility.
Asparagus racemosus	Root tubers	The roots are bitter, sweet and useful to enhance lactation, general weakness, fatigue and cough.
Azadirachta indica	Leaves	The leaves are bitter, astringent, acrid, depurative, antiseptic, ophthalmic, insecticidal, demulcent and refrigerant.
Borassus flabellifer	Leaves, Roots	The juice of the leaf stalks and young roots is good for gastric catarrh and

	and Fruits	hiccough
Calotropis gigantea	Whole plant	The dried whole plant is a good tonic, expectorant, depurative and anthelmintic. The leaves are useful in the treatment of paralysis, arthralagia, swellings and intermittent fevers.
Capsicum annuum,	Fruits	The fruits are acrid, bitter, thermogenic, digestive, carminative, laxative, expectorant and stimulant. They are useful in malarial and intermittent fevers
Cassia auriculata	Leaves, Seeds	The leaves are depurative and anthelmintic and are recommended for leprosy, skin diseases and ulcers.
Centella asiatica	Whole plant	The plant is diuretic, insomnia, epilepsy, abdominal disorders due to dysentery in children.
Citrullus colocynthis	Fruits and roots	The roots are purgative and are used for treating mammillitis and visceromegaly in children
Coccinia grandis	Leaves, fruits and roots	The leaves are bitter, sweet, astringent and cooling, and are useful in vitiated conditions of kapha and pitta.
Cocos nucifera	Seeds and roots	The water is sweet, cooling, digestive and is useful in dysentery and diarrhoea
Curcuma longa	Rhizome	The rhizomes are bitter, acrid, antiseptic, asthma, cough, skin diseases, general debility and diabetes
Enicostemma littorale	Whole plant	The plant is bitter, digestive, anti-inflammatory, liver tonic and is useful in skin diseases, swellings. The plant is locally applied in snake bite.
Ferula asafoetida	Resinous exudate of root	The oleo resin is bitter, acrid, sedative, expectorant and used in whooping cough and asthma
Ficus benghalensis	Leaves, bark and fruits	The leaves are good for ulcers, leprosy, allergic conditions of skin. The bark is useful in burning sensation and gonorrhoea
Gloriosa superba	Rhizome	The rhizomes are bitter, acrid, intensely poisonous, promotes labour pains and expulsion of placenta.
Gymnema sylvestre	Whole plant	The plant is bitter, acrid and useful in diabetes, hydrocil, asthma, bronchitis, cough, jaundice, renal and vesical calculi.
Hemidesmus indicus	Leaves, Root	The roots are bitter, sweet, appetiser, refrigerant and useful in dysentery, leucorrhoea, fever and general debility.
Ixora arborea	Leaves, roots	The leaves are useful in diarrhoea. The roots are useful in antiseptic, sores and ulcers, gonorrhoea and anorexia.
Lagenaria siceraria	Whole plant	The fruits are bitter, purgative, diuretic and useful in bronchitis, leprosy, fainting and fever.
Lawsonia inermis	Leaves	The leaves are bitter, liver tonic, useful in wounds, boils, greyness of hair and jaundice.
Leucas aspera	Leaves, flowers	The leaves and flowers are bitter, acrid, antibacterial and depurative. They are useful in chronic skin eruptions, cough and catarrh in children.
Morinda tinctoria,	Leaves,	The leaves are digestive, carminative, febrifuge and tonic. They are useful in gastropathy, wounds, inflammation, sarcocele and fever.
Murraya koenigii	Leaves	The leaves are bitter, acrid, astringent, febrifuge useful in skin diseases, inflammations and foul ulcers.
Ocimum basilicum	Whole plant	The plant is bitter, acrid, aromatic, insecticidal, antibacterial and antipyretic. It is useful in cough, asthma, bronchitis and malarial fevers.
Ocimum sanctum	Whole plant	The plant is bitter, acrid, aromatic, digestive diuretic, vermifuge. It is useful in asthma, genitor urinary disorders, ringworm and skin diseases.
Pedalium murex	Whole plant	The plant is sweet, cooling, mucilaginous, diuretic and useful in renal and vesical calculi diseases.
Phyllanthus amarus	Whole plant	The plant is bitter, sweet, cooling, antiseptic and useful in gastropathy, jaundice, dysentery, scabies ulcers and wounds.
Phyllanthus emblica	Fruits, bark	The fruits are sour, anodyne, digestive, and useful in diabetes, greyness of hair, dysentery, colic, vitamin - c, cough, diabetes, cold, laxative, hyper acidity.
Phyllanthus reticulatus	Whole plant	The plant is astringent, diuretic and useful in sores, burns diarrhoea, skin eruptions and obesity.
Ricinus communis	Seeds	Seeds are acrid, thermogenic, digestive, cathartic and aphrodisiac. They are useful in dyspepsia and for preparing a poultice to treat arthralgia.
Sesamum indicum	Seeds	Seeds are sweet, acrid, laxative, hair-restorer and tonic and useful in haemorrhoids, ulcers, burns, obesity and emaciation, leucoderma and alopecia.
Solanum trilobatum	Leaves, fruits	Leaves are used as expectorant and in the treatment of respiratory diseases, asthma, chronic febrile infections, tuberculosis, cardiac and liver diseases. This plant possesses a broad spectrum of antibiotic, antibacterial, antimitotic and anticancer activity.
Solanum xanthocarpum	Whole plant	The plant is bitter, acrid, anti-inflammatory, febrifuge and useful in dental cares, constipation, leprosy, rheumatoid arthritis and bronchitis.
Tephrosia purpurea	Whole plant	The seeds are useful in skin diseases and rat poisoning. The leaves are useful in pectoral diseases syphilis, gonorrhoea and bruises.
Terminalia bellirica	Bark and Fruits	The fruits are astringent, acrid, sweet, anthelmintic, antiemetic and rejuvenating and are useful in cough, insomnia, dropsy, vomitting and ulcer. The mature dry fruit is constipating and is useful in diarrhea and dysentery.
Thespesia populnea	Whole plant	The plant is astringent, acrid, cooling, antidiarrhoeal and antibacterial and is useful in scabies, psoriasis and ringworm. The bark and fruits possess more

		curative properties.
Tinospora cordifolia	Stem	The stem is bitter, astringent, rejuvenating, galacto-purifier and tonic. It is useful in anaemia, asthma, uropathy, burning sensation and chronic fevers.
Tribulus terrestris	Whole plant	The seeds are astringent, strengthening and are useful in epistaxis, haemorrhages and ulcerative stomatitis. The ash of the whole plant is good for external application in rheumatoid arthritis.
Vigna unguiculata	Seeds	The seeds are sweet, appetizer, diuretic and liver tonic and useful in constipation, agalactia, jaundice and general debility.
Zingiber officinale	Rhizome	The dry ginger is acrid, appetizer, laxative, carminative and useful in asthma, expectorant, cholera and nausea. It is also much used in several domestic preparations.

In traditional medicine, the plants are assumed to have some healing power that may be due to unknown bioactive compounds within the plant tissues (Strobel and Daisy, 2003). Medicinal plants are used to cure various diseases like gonorrhoea, rheumatism, cough, asthma and sore throat. Leaf extracts of *Thespesia populnea*, *Centella asiatica* and *Solanum trilobatum* were given in case of diarrhoea, dysentery, leukoderma skin diseases, coughing asthma and other respiratory disorders (Sheela and Kannan, 2003). People in different parts of India exclusively used *Andrographis paniculata* for curing malarial fever and poison bites. However, healers of Bhadravati Taluk of Karnataka used *Andrographis paniculata* for treating skin diseases (Nayak *et al.*, 2004; Vidyarthy and Gupta, 2004). Ten Medicinal plants were selected for the isolation of endophytic fungi on the basis of medicinal importance and availability. All the plant species were found colonized with endophytic fungi. The endophytes were isolated using two different mycological media namely czapek's dox agar and water agar. Maximum endophytes were obtained in CDA medium and minimum in WA media. Altogether 51 fungal endophytes belonging to 21 genera were isolated from 10 different types of medicinal plants in Virudhunagar District (Table 3).

TABLE 3. Isolation of endophytes from medicinal plants on PDA and WA media

S.No	Name of the Plant	Parts Used	Media Used	No. of Colonies Observed	Total No. of Colonies Observed
1	*Achyranthes aspera*	Leaf	CDA	2	
			WA	1	3
2	*Adhatoda zeylanica*	Leaf	CDA	2	
			WA	2	4
3	*Aegle marmelos*	Fruit	CDA	9	
			WA	5	14
4	*Azadirachta indica*	Leaf	CDA	9	
			WA	2	11
5	*Calotropis gigantea*	Stem	CDA	3	
			WA	-	3
6	*Cassia auriculata*	Leaf	CDA	3	
			WA	1	4
7	*Centella asiatica*	Leaf	CDA	2	
			WA	-	2
8	*Leucas aspera*	Leaf	CDA	3	
			WA	2	5
9	*Phyllanthus niruri*	Leaf	CDA	2	
			WA	-	2
10	*Thespesia populnea*	Leaf	CDA	3	3
			WA	-	

CDA – czapek's dox agar Medium, WA – Water Agar Medium

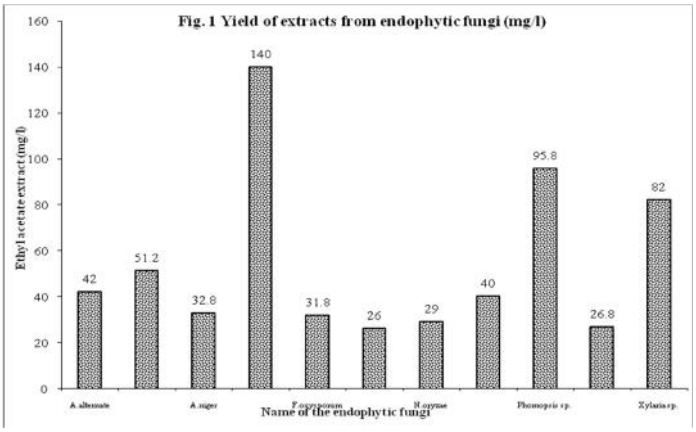
The results of this study showed that endophyte fungi were more prevalent in the leaves. Nearly 13 genera of fungal endophytes were observed from *Aegle marmelos* and 10 different genera were observed in *Azadirachta indica*, *Aspergillus* sp., *Cladosporium* sp. and *Fusarium* sp. are commonly present in most of the medicinal plants. *Cladosporium* sp., *Fusarium oxysporum*, *Nigrospora oryzae*, *Trichoderma* sp. and *Verticillium* sp. are observed in both *Aegle marmelos* and *Azadirachta indica* (Table 4).

TABLE 4. List of endophytic fungi isolated from the selected medicinal plants

S.No	Name of the plants	Name of endophytic fungi
1	*Achyranthes aspera*	*Aspergillus flavus, Cladosporium* sp., *Xylaria* sp.
2	*Adhatoda zeylanica*	*Aspergillus* sp., *Chaetomium spirale, Curvularia clavata, Penicillium* sp.
3	*Aegle marmelos*	*Alternaria alternata, Aspergillus fumigatus, Aspergillus niger, Chaetomium globosum, Cladosporium* sp., *Fusarium oxysporum, Nigrospora oryzae, Penicillium* sp., *Pestalotia macrotricha, Phomopsis* sp., *Rhizoctonia* sp., *Stenella agalis, Trichoderma* sp., *Verticillium* sp.,
4	*Azadirachta indica*	*Acremonium* sp., *Cladosporium* sp., *Curvularia lunata, Fusarium oxysporu, Fusarium solani, Nigrospora oryzae, Pestalotiopsis* sp., *Phoma eupyrena, Phyllostica* sp., *Trichoderma* sp., *Verticillium albo-atrum*
5	*Leucas aspera*	*Alternaria* sp., *Colletotrichum* sp., *Fusarium* sp., *Spicaria* sp., *Stemphylium* sp.

Antibacterial activity of endophytic fungi

Antibacterial activity of various isolated endophytic fungi was observed against selected test organisms. The crude extract of fungi was extracted using ethyl acetate (Fig.1) and the biomass of isolated microorganisms was measured (Fig.2). Depending on the measured values of the complete inhibition diameter of the circle excluding the disc in millimeter, the antibacterial activity can be classified into no activity (0 – 6 mm), not significant (7 – 9 mm), poor activity (10 – 12 mm), low activity (13 - 15 mm), good activity (16 - 18 mm) and above 18 mm is significant. In the present study, crude metabolite extracts of fungal endophytes isolated from the medicinal plants showed considerable antimicrobial activity against human pathogenic microorganisms. Eleven isolates could display antimicrobial activity inhibiting the test pathogens (Table 5 and 6). with inhibition zones that ranged from 6 to 42 mm. The result showed that fungal crude ethyl acetate extract inhibited gram-positive than gram-negative bacteria. Among the potent strains, crude metabolite of an endophytic fungus, *Cladosporium* sp. displayed significant antimicrobial activity against all test pathogens. The extract was significantly effective against both Gram-positive and Gram-negative bacteria. This showed the broad-spectrum nature of the metabolite. Mostly all the samples showed antimicrobial activity against all the test pathogens. Our results correlated with the findings of Corrado and Rodrigues, (2004); Wiyakrutta *et al.,* (2004); Ramasamy *et al.,* (2010). Hiremath *et al.* (1996) stated that the dicot plants especially leaves were found to have wider antibacterial activity, when compared to other plant parts. The fungal cell wall protects the organism against a hostile environment and relies on signal for invasion and infection of a likely plant, animal or human host. Several classes of antifungal proteins are involved in inhibition of the fungal cell wall or disruption of cell wall structure and function and other perturb fungal membrane structure, resulting in cell lysis (Selitrennikoff, 2001).



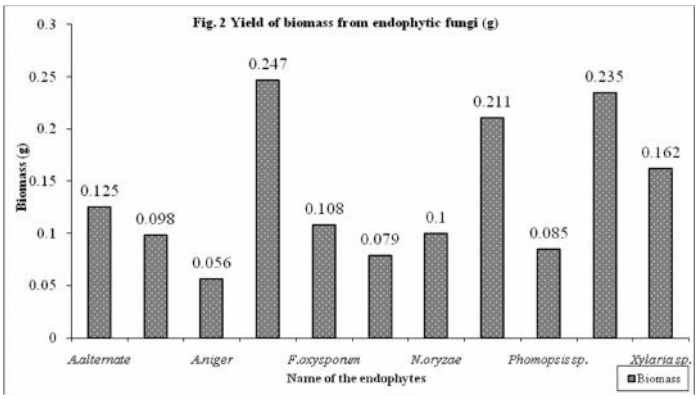


TABLE 5. Zone of inhibition (in mm) of crude extracts of endophytic fungi by disc diffusion method

Bacterial Strains	Diameter of Zone of Inhibition (mm)					
	Aa	Af	An	Cs	Fo	Fs
Bacillus cereus	35	21	19	34	25	10
Bacillus subtilis	24	16	-	40	18	09
Escherichia coli	30	35	34	39	37	25
Klebsiella pneumoniae	28	31	27	36	42	34
Proteus sp.	-	15	23	38	36	30
Pseudomonas sp.	-	-	-	34	08	25
Salmonella typhi	20	19	25	42	31	28
Staphylococcus aureus	40	37	34	39	26	30
Streptococcus pyogens	30	39	30	41	29	38

Aa - *Alternaria alternata*, Af - *Aspergillus flavus*, An - *Aspergillus niger* , Cs - *Cladosporium* sp., Fo - *Fusarium oxysporum* and Fs *Fusarium solani*

TABLE 6. Zone of inhibition (in mm) of crude extracts of endophytic fungi by disc diffusion method

Bacterial Strains	Diameter of Zone of Inhibition (mm)				
	No	Ps	Ps	Ts	Xs
Bacillus cereus	08	02	28	17	30
Bacillus subtilis	11	20	16	09	21
Escherichia coli	28	25	32	26	30
Klebsiella pneumoniae	25	19	-	19	26
Proteus sp.	27	24	-	31	19
Pseudomonas sp.	18	16	29	30	26
Salmonella typhi	23	30	40	15	17
Staphylococcus aureus	39	39	41	40	37
Streptococcus pyogens	35	32	38	38	39

No - *Nigrospora oryzae*, Ps - *Penicillium* sp., Ps - *Phomopsis* sp., Ts - *Trichoderma* sp., Xs - *Xylaria* sp.

Endophytes are believed to carry out a resistance mechanism to overcome pathogenic invasion by producing secondary metabolites (Tan and Zou, 2001). A novel antibiotic, Phomol, from a *Phomopsis* sp. was identified from medicinal plant *Erythrina crista-galli* (Weber *et al.*, 2004). Asperfumin a bioactive metabolites produced by *Aspergillus fumigatus* CY018, an endophytic fungus, has the ability to inhabit *Candida albicans* (Liu *et al*., 2004). Endophytic fungi from teak (*Tectona grandis* L.) and rain tree (*Samanea saman* Merr.) leaves could inhibit the growth of Gram positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* to a greater degree than Gram negative bacteria (*Escherichia coli*) (Chareprasert *et al*., 2006). The antimicrobial compounds can be used not only as drugs by humankind but also as food preservatives in the control of food spoilage and food-borne diseases (Liu *et al.*, 2008).

Phytochemical screening

Phytochemical analysis of ethyl acetate solvent extract revealed the presence of saponins, phenolic compounds, anthraquinones, steroids, cardiac glycosides and tannins.

Extracts from cultures of all (11 different species) endophytic fungi gave a wide variety of biological activities. Presence and absence of phytochemicals in endophytic extracts are given in Table 7.

TABLE 7. Phytochemical screening for the different endophytic fungal extract (ethyl acetate solvent)

S. No.	Name of Fungi	Saponins	Phenolic compounds	Anthra quinones	Steroids	Cardiac glycosides	Tannins
1	*Alternaria alternata*	+	+	+	+	+	+
2	*Aspergillus flavus*	+	+	-	-	-	-
3	*Aspergillus niger*	+	+	+	+	+	-
4	*Cladosporium* sp.	+	+	+	+	+	+
5	*Fusarium oxysporum*	-	+	-	-	+	-
6	*Fusarium solani*	-	+	-	+	+	+
7	*Nigrospora oryzae*	-	+	-	-	-	-
8	*Penicillium* sp.	+	+	-	-	-	+
9	*Phomopsis* sp.	+	+	-	-	-	-
10	*Trichoderma* sp.	+	+	-	-	-	-
11	*Xylaria* sp.	-	+	-	-	-	-

+ = Present; - = Absent

The presence of phytochemicals in endophytes is an indicator that they can be potential source of precursors in the development of synthetic drugs (Jack and Okorosaye-Orubite, 2008). The endophytes has showed the presence of different phytochemicals, saponins (Khanna and Kannabiran, 2008), phenolic compounds (Pelczar *et al.*, 1988; Lai *et al.*, 2010), steroids (Kalyoncu *et al.*, 2009), cardiac glycosides (Ahmed *et al.*, 2005), and tannins (Kaur and Arora, 2009) and they are known to possess strong antimicrobial and antioxidant activity. The antioxidant capacities of the endophytic fungal cultures were correlated with their total phenolic contents, suggested that

phenolics were also the major antioxidant constituents of the endophytes (Wu-Yang *et al.,* 2007). The presence of phytochemicals within endophytes can be potential source for medicinal and industrial use.

CONCLUSION

It has been estimated by the World Health Organization (WHO) that approximately 80% of the world's population from developing countries rely mainly on traditional medicines (mostly derived from plants) for their primary health care and at least 119 chemical compounds, derived from 90 plant species, are important drugs currently is use in one or more countries. The study of plant associated endophytes could therefore provide the best possible way of acquiring novel metabolites. The present study thus, reinforced the assumption that endophytes of ethno medicinal plants of Virudhunagar district could be a promising source of antimicrobial substances. The endophytes of medicinal plants provide a good source for compounds of biological activity and endophytes are an untapped reservoir of potentially novel effective drugs. It can be concluded that the antibacterial activity of endophytic fungi are varied from species to species. Endophytes in host plants can stimulate plant growth, increase disease resistance, improve plant's ability to withstand environmental stresses and recycle nutrient.

ACKNOWLEDGEMENT

This research work was funded by University Grants Commission, South Eastern Regional Office, Hyderabad. I sincerely thank them for their support.

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