ANTIMICROBIAL ACTIVITY IN CULTURES OF ENDOPHYTIC FUNGI ISOLATED IN SOME MEDICINAL PLANT SPECIES

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Abstract: *Fungal endophytes have been studied from five medicinal plants of Caesalpinia sappan, Alternanthera sessil, Sapindus laurifolius, Basala alba and Acalypha indica All the plants were found with endophytic fungi. The endophytes were isolated using three different mycological media namely potato dextrose agar (PDA), malt extract agar (MEA) and water agar (WA). Maximum endophytes were isolated in PDA and minimum on WA medium. Altogether 12 fungal endophytes belonging to differentn genera were isolated from the three medicinal plants. Out of which, all endophytes were obtained as filamentous forms and some of them colonies. Species Colletotrichum spp, Aspergillus spp, Bispora spp., Geotrichum spp., Trichoderma spp. Cladosporium spp. Aspergillus spp. Gliocladium spp., Trichoderma spp. Fusarium spp. Trichosporoniodes spp Trichoderma spp of antimicrobial activity inhibiting at least one of the test pathogens. Among the potent strains, 19.3% displayed antibacterial showed antimicrobial activity against all the test pathogens. An endophytic fungus identified as Trichoderma spp.5.2 Aspergillus spp.4.8 sp. Fusarium spp.4.8, Trichoderma spp. 3.9 zone of inhibition, displayed significant antimicrobial activity. whereas other fungal isolates revealed less zone of inhibition The crude extract of this endophytic strain was very effective against the bacterial pathogens and moderately active against the fungal pathogens. The study reinforced the assumption that endophytes of 5 medicinal plants could be a promising source of antimicrobial substances.*

Key words: *Antimicrobial activity, endophytic fungi, medicinal plant, Phytochemical*

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INTRODUCTION

The search for new antibiotics to overcome the growing human problems of drugs resistance in microorganisms and appearance of new diseases has been rapidly increasing around the world. Realizing the capability of microorganisms to produce diverse bioactive molecules and the existence of unexplored microbial diversity, research is underway to isolate and screen microbes of diverse habitat and unique environment for discovery of novel metabolites. One such unexplored and less studied microorganism is the endophytic fungi, which are defined as those microbes that colonize healthy tissues of plants, at least for a part of their life cycle, without causing apparent disease symptoms in their host (Petrini, 1991; Wilson, 1995). Different works carried out so far regarding the role of endophytes in host plants indicate that they can stimulate plant growth, increase disease resistance, improve plant's ability to withstand environmental stresses and recycle nutrient (Sturz and Nowak, 2000). Besides these, endophytes are also recognized as rich sources of bioactive metabolites of multifold importance (Tan and Zou, 2001; Strobel and Daisy, 2003).

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. However, recently it has been reported that fungal endophytes residing within these plants could also produce metabolites similar to or with more activity than that of their respective hosts (Strobel, 2002). Therefore, it is believed that search for novel compounds should be directed towards plants that commonly serve indigenous populations for medicinal purposes and plants growing in unique environmental setting or interesting endemic locations as they are expected to harbor novel endophytes that may produce unique metabolites with diversified applications (Strobel and Daisy, 2003). With this knowledge, the present investigation was carried out to study fungal endophytes associated with five medicinal plants used as medicine by the people communities of India and to evaluate these endophytes for antimicrobial activity against some human pathogens.

The nature and biological role of endophytic fungi with their plant host is variable. Endophytic fungi are known to have mutualistic relations to their hosts, often protecting plants against herbivory, insect attack or tissue invading pathogens (Siegel MR *et al*. 1985, Clay K.1986, Yang X *et al*. 1993); and in some instances the endophyte may survive as a latent pathogen, causing or quiescent infections for a long period and symptoms only when physiological or ecological conditions favors virulence (Carroll GC., 1986, Bettucci L *et al*., 1993).

In Malaysia, extract from many types of local plants are used in traditional manner for treatments of various ailments (Ong HC *et al*., 1998,1999). The question is whether they are produce by the plant itself or as a consequence of a mutualistic relationship with beneficial organisms in their tissue. Many reports showed that in a microbe-plant relationship, endophytes contribute substances that possess various types of bioactivity, such as antibacterial and antifungal. Thus in this study, we focus on the isolation of endophytic fungi and screening them for bioactivity (Son Radu *et al.* 2014).

PRODUCTION OF ANTIBACTERIAL COMPOUND FROM MEDICINAL PLANTS

Antibacterial activity of crude extract, alcoholic extract and extracted phenol from various parts of tropical pteridophyta, Hemionitis arifolia were tested by agar diffusion and tube dilution assay. Both the crude and alcoholic extracts of vegetative and reproductive leaves of H. arifolia showed considerable antibacterial activity against Gram negative test strain of *Escherichia coli* (MTCC-739) (Karmakar J *et al*., 2011).

The antidiabetic potential of *Alternanthera sessilis* Red was investigated using the obese type 2 diabetic rats induced by high fat diet and streptozotocin. Three fractions (hexane, ethyl acetate, and water) were obtained from the crude ethanol extract of Alternanthera *sessilis* Red. *Alternanthera sessilis* Red ethyl acetate fraction (ASEAF) was found to possess the most potent anti hyperglycemic effect through oral glucose tolerance test. The ASEAF was subsequently given to the diabetic rats for two weeks. It was found that two-week administration of ASEAF reduces the fasting blood glucose level, triglyceride level, and free fatty acid level of the rats. ASEAF-treated diabetic rats showed higher pancreatic insulin content and pancreatic total superoxide dismutase activity compared to the untreated diabetic rats. Also, the insulin sensitivity indexes suggested that ASEAF ameliorates the insulin resistant state of the diabetic rats. In conclusion, ASEAF could be developed into a potential antidiabetic agent for the management of type 2 diabetes (Kok Keong Tan *et al*., 2013**).**

Eextracts from the leaves of *Acalypha indica* were tested against Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis, Bacillus cereus*, *Streptococcus faecalis*) and Gram-negative (*Klebsiella pneumoniae*, *Escherichia coli*, *Proteus vulgaris, Pseudomonas aeruginosa*) bacteria. All the extracts exhibited antibacterial activity against Gram-positive organisms with minimum inhibitory concentrations (MIC) between 0.156 to 2.5 mg/ml. Among the Gram-negative bacteria, only the *Pseudomonas aeruginosa* was susceptible to the extracts (Govindarajan M *et al*., 2008).

Production of secondary metabolites by endophytic fungi

Some species of endophytic fungi have been identified as sources of anticancer, antidiabetic, insecticidal and immunosuppressive compounds (Strobel, G. *et al.* ,2006). Further, endophytic fungi may also produce metabolites with thermo protective role. For example, plants in some volcanic areas in USA were found colonized by an endophytic fungus *Curvularia* sp.(Redman *et a*l., 2002,).

Antimicrobial activity of endophytic fungi isolated from the leaves, stems, and roots of 54 species of Orchidaceae collected in a Brazilian tropical ecosystem. In total, 382 filamentous fungi and 13 yeast isolates were obtained and cultured to examine the production of crude extracts. Thirty-three percent of the isolates displayed antimicrobial activity against at least one target microorganism. The multivariate statistical analyses conducted indicate that the extracts of endophytic fungi isolated from leaves of terrestrial orchids in semi deciduous forest were more active against *Escherichia coli*, whereas extracts of endophytic fungi from roots of rupicolous orchids collected in rock fields were more active against *Candida krusei* and *Candida albicans*. Among the fungi that were screened in the study, 22 isolates held their antimicrobial activities after replication and were therefore selected for assessment of the minimum inhibitory concentration (MIC), which ranged from 62.5 to 250 microg/mL and 7.8 to 250 microg/mL against bacteria and fungi, respectively. One isolate of *Alternaria* sp. and one isolate of *Fusarium oxysporum* presented the strongest antibacterial activity. Three *Fusarium* isolates, *Epicoccum nigrum*, and *Sclerostagonospora opuntiae* showed the greatest MIC values against the pathogenic yeasts(Vaz AB *et al*. 2009).

Endophytic fungi inhabit vegetable tissues or organs, without causing them any harm. Endophytes can co-evolve with plant hosts and possess species-specific interactions. They can protect the plant from insect attacks and diseases, and are also able to produce substances of biotechnological interest. In folk medicine, the bark, roots and fruits of *Sapindus saponaria* is used to produce substances with anxiolytic, astringent, diuretic and expectorant properties, as well as tonics, blood depuratives and cough medicine. This study evaluated the diversity of endophytic fungi present in the leaves of *S. saponaria* L. and observed the colonization of host plants by endophytes, using light and scanning electron microscopy. The genera of some isolates of *S. saponaria* were identified mainly by sequencing of ITS region of rDNA and, when possible, also by their microscopic features, as follows: *Cochliobolus, Alternaria, Curvularia*, *Phomopsis, Diaporthe and Phoma*. Phylogenetic analysis showed the existence of genetic variability of the genera Phomopsis and Diaporthe and interspecific variation among the *Curvularia, Alternaria* and *Phoma*, belonging to family Pleosporaceae (García A *et al.* ,2012).

Anticestodal activity of one of the endophytic fungi Pestalotiopsis sp. from Neem plant was observed on protoscoleces of hydatid cysts of Echinococcus *granulosus.*(Verma VC., *et al*., 2013).

Endophytic fungi from three commonly found seagrasses in southern Thailand were explored for their ability to produce antimicrobial metabolites. One hundred and sixty endophytic fungi derived from *Cymodoceaserrulata* (Family ymodoceaceae), *Halophilaovalis* and *Thalassiahemprichii* (Family Hydrocharitaceae) were screened for production of antimicrobial compounds by a colorimetric broth microdilution test against ten human pathogenic microorganisms including Staphylococcus *aureus* ATCC 25923, a clinical isolate of methicillin-resistant S*. aureus*, Escherichia *coli* ATCC 25923, Pseudomonas *aeruginosa* ATCC 27853, Candida *albicans* ATCC 90028 and NCPF 3153, Cryptococcus *neoformans* ATCC 90112 and ATCC 90113 and clinical isolates of Microsporum *gypseum* and Penicillium *marneffei*. Sixty-nine percent of the isolates exhibited antimicrobial activity against at least one test strain. Antifungal activity was more pronounced than antibacterial activity (Supaphon P *et al*., 2013).

New metabolites produced by endophytic fungi from Thai medicinal plants, two new triene fatty acid amides, bipolamides A (1) and B (2), were discovered from the endophytic fungus Bipolaris sp. MU34. had moderate antifungal activity against Cladosporium *cladosporioides* FERMS-9, Cladosporium *cucumerinum* NBRC 6370, Saccharomyces *cerevisiae* (Siriwach R,*et al*., 2014).

Endophytes, which reside in plant tissues, have the potential to produce novel metabolites with immense benefits for health industry. Cytotoxic and antimicrobial (Katoch M *et al*.,2014).

Bamboos, regarded as therapeutic agents in ethnomedicine, have been used to inhibit inflammation and enhance natural immunity for a long time in Asia, and there are many bamboo associated fungi with medical and edible value. In the present study, a total of 350 fungal strains were isolated from the uncommon moso bamboo (Phyllostachys edulis) seeds for the first time. The molecular diversity of these endophytic fungi was investigated and bioactive compound producers were screened displayed broad-spectrum activity against clinical bacteria and yeasts by the agar diffusion method. antimicrobial activity of endophytic fungi associated with moso bamboo seeds, and the results show that they could be exploited as a potential source of bioactive compounds and plant defense activators(Shen XY *et al*., 2014).

Antibacterial activity and cytotoxicity of endophytes isolated from Scapania verrucosa Heeg., which belongs to the liverwort class. A total of 49 endophytic fungi were isolated from S. verrucosa and classified into seven genera and one family in our previous study. In this study, the cytotoxic activity of the endophytes was assessed using the brine shrimp lethality bioassay, seven of which showed potent toxicity against the brine shrimp. T-27 exhibited the strongest antibacterial activity against Staphylococcus aureus, with minimal inhibitory concentrations below 0.25 and 4 mg/mL, which can inhibit the growth of two standard strains - ATCC 25923 (methicillin-sensitive S. aureus) and ATCC 43300 (methicillin-resistant S. aureus). Endophytes in S. verrucosa are the sources for the production of natural bioactive products (Wu JG *et al*., 2013).

Endophytes of medicinal plants have the capacity to synthesis same or similar active substances with their hosts. To investigate the diversity and capacity to produce saponins of endophytic fungi of *Panax ginseng*, thirty-eight strains of were isolated. The culture filtrate of Pg30 exhibited its antibacterial activity *Staphylococcus aureus* and Pg42-1 showed strong inhibition against *Klebsiella pneumonia (*Wu H et al., 2013).

Drug resistance in bacteria has become a global concern and the search for new antibacterial agents is urgent and ongoing. Endophytes provide an abundant reservoir of bioactive metabolites for medicinal exploitation, and an increasing number of novel compounds are being isolated from endophytic fungi. Ophiopogon japonicus, containing compounds with antibacterial activity, is a traditional Chinese medicinal plant used for eliminating phlegm, relieving coughs, latent heat in the lungs, and alleviating diabetes mellitus. We investigated the antimicrobial activities of 30 strains of O. japonicus (Liang H., *et al.*,2012).

As part of our ongoing efforts towards finding novel anti-oxidant and anti-microbial agents, and other bioactive chemicals from natural resources, we had recently investigated the secondary metabolites of endophytes isolated from 29 tradjtional Chinese medicinal plants in Hong Kong, and found some endophytic fungal isolates exhibited strong anti-oxidant capacity and anti-microbial activity (Kumar and Hyde, 2004; Kumar *et al.,* 2005; Huang *et al.,* 2007a,b). It is important and necessary to identify and classify these potential bioactive fungi for a better understanding of the characteristics of the relevant endophytic communities.

The main aim of the project is to screen antimicrobial properties in the Endophytic fungi present in the 5 medicinal plants. These endophytes in symbiotic relationship with fungi may produce certain antimicrobials which can be cultured in mass to produce the product. It is more economical and easily cultivable than totally depending on the medicinal plants.

MATERIALS AND METHODS

Sample collection:

The root hairs were collected from the five different plant. The sample was collected from Dhanvantri Vana, a unit of Government Ayurvedic College, Bangalore. The samples were collected from June 2013 to September 2013. The plants with no visible symptoms of disease were carefully selected after physical examination. The samples were kept in sterile containers and processed within few hours after sampling.

Root hair processing

The root hairs of selected medicinal plants were collected and cut into 1 cm long pieces. It was disinfected with 75% alcohol for 1 minute followed by immersion in 1-5% sodium hypo chloride for 3-10 minutes. They were again immersed in 75% alcohol for 30 seconds. The root bits were then rinsed in sterile distilled water and blot dried on sterile blotting paper. The root bits were placed on Potato Dextrose Agar (PDA) supplemented with Streptomycin. The plates were sealed with parafilm and incubated at 28ºC for 4-6 weeks.

Microscopic features:

The morphology of different endophytic fungi was studied by staining with methylene blue of the isolates from Potato Dextrose Agar (PDA) after 4-6 week incubation at 28ºC.

Physiological characteristics:

Method involved for the study:

The selective fungal cultures are stored as stock cultures, which can be used for the study of antibacterial property.

Antibacterial activity:

The antibacterial activity of the endophytic fungi was determined by application of fungal broth by tube dilution technique with three different bacteria. It is also done by the Agar well diffusion method.

Tube dilution technique:

Three types of bacteria were selected to study antibacterial property. A gram positive bacteria, *Staphylococcus aureus* and two gram negative bacteria*, Escherichia coli* and *Klebsiella* spp. were incubated in nutrient broth for 48 hours. Equal ratio's of 48 hrs bacterial culture, sterile nutrient broth and the extract (1:1:1) were taken in sterile tube and incubated at 37ºC for 48 hrs. the tubes were observed for bacterial growth indicating turbidity by UV spectrophotometer. The clear broth indicated that the filtrate contained antibacterial activity.

Agar-Plate technique:

This is an alternative method of using tubes, where antibacterial potency can be studied with solid media, showing areas of inhibition zone. A plate of nutrient agr medium was inoculated with the test organism by spread plate technique. Using a 2 mm cork borer the agar block was removed at the centre. About 5µl of extract was loaded in the centre (well) and the plates were incubated at 37ºC for 36-48 hours and observed for the zone of inhibition around the well.

Organic Analysis of Fungal broth:

The crude extract of broth was studied for the presence of antibacterial compounds like phenols, aldehydes, esters, etc. the tests were performed under laboratory conditions for organic analysis. Organic compounds may be aliphatic of aromatic. They may be saturated or unsaturated. Depending upon the functional group they contain, they show different solubility and give characteristic reactions. Solubility tests were performed with dilute HCl, 1% NaOH and concentrated sulfuric acid.

Organic analysis chart to detect organic compounds

Group 1: Water soluble compounds; (a) acetic acid, (b) acetone.

(a) Acetic acid: Physical state and odour, colourless

Experiment	Observation	Inference
A little of the compound is ignited in a spatula.	Burns with a non-smoky flame	It is an aliphatic compound
To 3 drops of the compound a little sodium bicarbonate solution is added and shaken	Effervescence takes place and carbon dioxide gas is liberated.	The compound is an acid.

(b) Acetone: Physical state and odour; Colourless liquid, alcohol smell

Experiment	Observation	Inference
4 drops of compound + sodium bicarbonate solution	No effervescence	The compound is not an acid.

Group (Aniline) 2: Physical state and color; Brown liquid, fishy smell

Experiment	Observation	Inference
2 drops of the liquid shaken well with 2ml of dil. HCl and allowed to stand.	The compound dissolved	It belongs to III solubility group. It is an amine.
Diazotization: 3 drops of the liquid dissolved in 1 ml of dil. HCL. The solution is cooled in ice. To the ice cold solution 0.2 gm of sodium nitrite is added and solution of beta nephthol is added.	Red dye is produced	It is a primary aromatic amine.

Group 3: (a) Benzoic acid, (B) Salicylic acid, (c) Phenol

(a) Benzoic acid; Physical state: colorless, crystalline solid.

Experiment	Observation	Inference
0.1 g of thee solid is shaken with 2ml of cold dil. HCl	The compound does not dissolve.	The compound does not belong to III solubility group.
Experiment	**Observation**	**Inference**
0.1 ml of the liquid is shaken well with 2ml of 5% NaOH solution.	The compound dissolves to give a clear solution.	The compound belongs to IV solubility group. May be phenol or acid.
0.1 ml of the liquid is shaken	Effervescence takes place	The compound is an acid.

with 2ml of sodium bicarbonate solution	and Carbon dioxide gas is liberated.	
0.2 ml of the liquid is treated with neutral ferric chloride solution.	No violet colour is produced.	It is not salicylic acid, hence it is benzoic acid.

(b) Salicylic acid: Physical state; Colourless crystalline acid.

Experiment	Observation	Inference
0.2 ml of the liquid is shaken with neutral ferric chloride solution.	A violet colour is produced	The compound is a phenolic acid (salicylic acid)

(c) Phenol: Physical state; Colourless crystalline solid with phenolic odour.

Experiment	Observation	Inference
0.2 ml of the liquid + 2 ml of sodium bicarbonate	No effervescence and no Carbon dioxide gas liberated.	It is not an acid
Experiment	**Observation**	**Inference**
Liebermann's reaction: to 2 drops of the liquid taken in a dry test tube a little sodium nitrite is added. The mixture is warmed, shaken and cooled to the mixture 4 drops of conc. Sulfuric acid is added. The mixture is added to plenty of cold water.	A red solution is formed	It is phenol.

Group 4: (a) Benzaldehyde (b) Acetophenone (c) Ethyl benzoate

(a) Benzaldehyde: Physical state: Colourless liquid; smell of bitter almounds.

Experiment	Observation	Inference
3 drops of the compound is shaken with 2 ml of conc. Sulfuric acid	The compound dissolves	The compound belongs to IV solubility group. It may be and aldehyde, ketone, ester or alcohol.
Schiff' reagent test: 2 drops o the liquid is shaken with 1 ml of Schiff's reagent.	An orange yellow crystalline solid is formed.	The compound may be an aldehyde or ketone.
Tollen's reagent test: 2 drops of the liquid, 2ml of Tollen's reagent is added and the mixture is shaken well and gently heated.	A bright silver mirror is formed on the side of the test tube.	The compound is an aldehyde.

(b) Acetophenone: Physical state: colourless liquid with a pleasant smell.

Experiment	Observation	Inference
2 drops of the liquid 1ml of Schiff's reagent is added and the mixture is shaken.	No pink colour is produced	It is not an aldehyde. So it is ketone.
2 drops of the compound 2 ml of Tollen's reagent is added and shaken well and gently heated.	No silver mirror is formed.	It is not an aldehyde. So it is a ketone.

(c)Ethyl benzoate: Physical state: colourless liquid with a fruity smell.

Experiment	Observation	Inference
Saponification: 5 drops of solution, 5 ml of NaOH is added Mixture is boiled for 5 minutes. Then it is added and acidified with dilute sulfuric acid.	A white precipitate is formed.	The compound is an ester.

RESULTS AND DISCUSSION

The endophytic fungi are very unique in their properties, which infect only the plant parts like root. They differ from the rhizosphere soil in their qualitative microflora niche. They can be considered obligatory parasites infecting only the plants. Very few can be facultative in existence surviving in the rhizosphere soil and also as an endoparasite in plants. The isolated fungi were maintained as a stock on PDA slants. The fungi isolated in endophytic are as follow:

Table 1 Medicinal plants selected for endophytic studies.

Sr. No.	Plant name	Medicinal Properties	Endophytic Fungi
1.	*Caesalpinia sappan*	Diarrhoea	*Colletotrichum spp.* *Aspergillus spp.* *Bispora spp.* *Geotrichum spp.*
2.	*Alternanthera sessilis*	Skin rashes, Liver disorders	*Trichoderma spp.* *Cladosporium spp.* *Aspergillus spp.*
3.	*Sapindus lourifolius*	Diarrhoea, White patches on skin	*Gliocladium spp.* *Trichoderma spp.*
4.	*Basala alba*	Mouth Ulcer, body energy	*Fusarium spp.* *Trichosporoniodes spp*
5.	*Acalypha indica*	Skin disease , cough	*Trichoderma spp.*

The zone of inhibition is taken in three different directions and mean has been calculated of that measures. The zone of inhibition is taken in centimeter and they are following:

Table 2 Antibacterial activity of endophytic fungi by Agar diffusion method:

Medicinal plants	Endophytic fungi	*Staphylococcus aureus*	*E. coli*	*Klebsiella*
Caesalpinia sappan	*Colletotrichum spp.*	3.1 cm	0	0
	Aspergillus spp.	4.8cm	4.3 cm	0
	Bispora spp.	3.5 cm	2.2 cm	0
	Geotrichum spp.	4.0 cm	0	1.8 cm
Alternanthera sessil	*Trichoderma spp.*	5.0 cm	4.6 cm	3.9 cm
	Cladosporium spp.	3.4 cm	0	0
	Aspergillus spp.	2.7 cm	3.3 cm	0
Sapindus laurifolius	*Gliocladium spp.*	3.6 cm	3.0 cm	0
	Trichoderma spp.	5.2 cm	3.5 cm	5.0 cm
Basala alba	*Fusarium spp.*	4.8 cm	0	1.9 cm
	Trichosporoniodes spp.	0	1.2 cm	0
Acalypha indica	*Trichoderma spp.*	5.0 cm	3.7 cm	2.8 cm

Antibacterial study by tube method and Agar diffusion method:

The tube method is the study of the exposure of any bacteria to the fungal broth with the test culture, showed results with presence or absence of turbidity. This helped in predicting their antibacterial activity studies done turbiditometric analysis indicated their property of antibacterial.

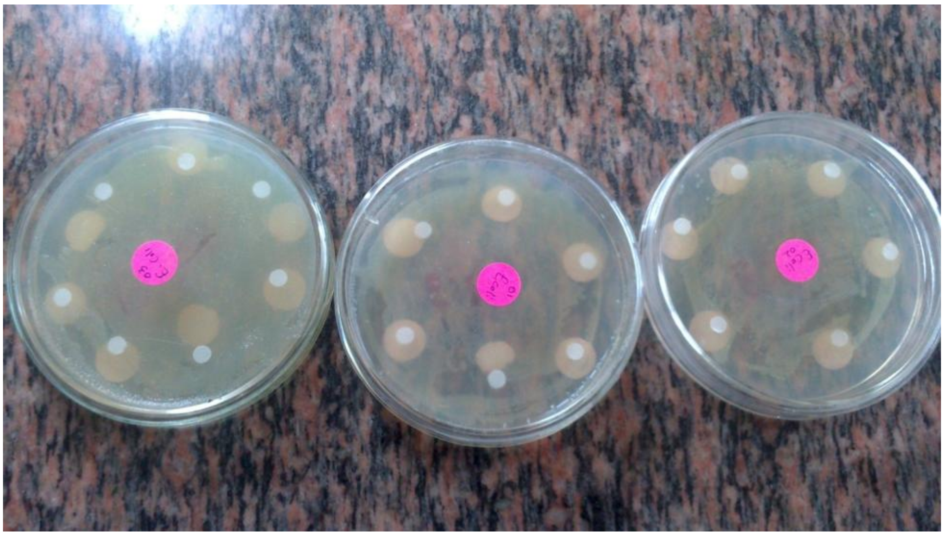
Table 3 Antibacterial activity of endophytic fungi by tube method:

Sr. no.	Medicinal plant	Endophytic fungi	Effect of fungal extract on bacteria (+) indicates Growth (-) indicates No Growth		
			Staphylococcus spp.	*E. coli.*	*Klebsiella spp.*
1	*Caesalpinia sappan*	*Colletotrichum spp.* *Aspergillus spp.* *Bispora spp.* *Geotrichum spp.*	- - - -	+ - - +	+ + + -
2	*Alternanthera sessilis*	*Trichoderma spp.* *Cladosporium spp.* *Aspergillus spp.*	- - -	- + -	- + +
3	*Sapindus laurifolius*	*Gliocladium spp.* *Trichoderma spp.*	- -	- -	+ -

4	*Basala alba*	*Fusarium spp.* *Trichosporoniodes spp*	- +	+ -	- +
5	*Acalypha indica*	*Trichoderma spp.*	-	-	-

The root decoctions of these plants were taken and studied for the similar antibacterial properties and it was found that the following results were obtained.

Medicinal plant	*Staphylococcus aureus*	*E. coli.*	*Klebsiella*
Caesalpinia sappan	1.8 cm	2.0 cm	1.0 cm
Alternanthera sessilis	3.0 cm	2.0 cm	1.8 cm
Sapindus laurifolius	2.4 cm	2.3 cm	1.7 cm
Basala alba	3.8 cm	3.1 cm	1.6 cm
Acalypha indica	3.0 cm	2.8 cm	1.3 cm



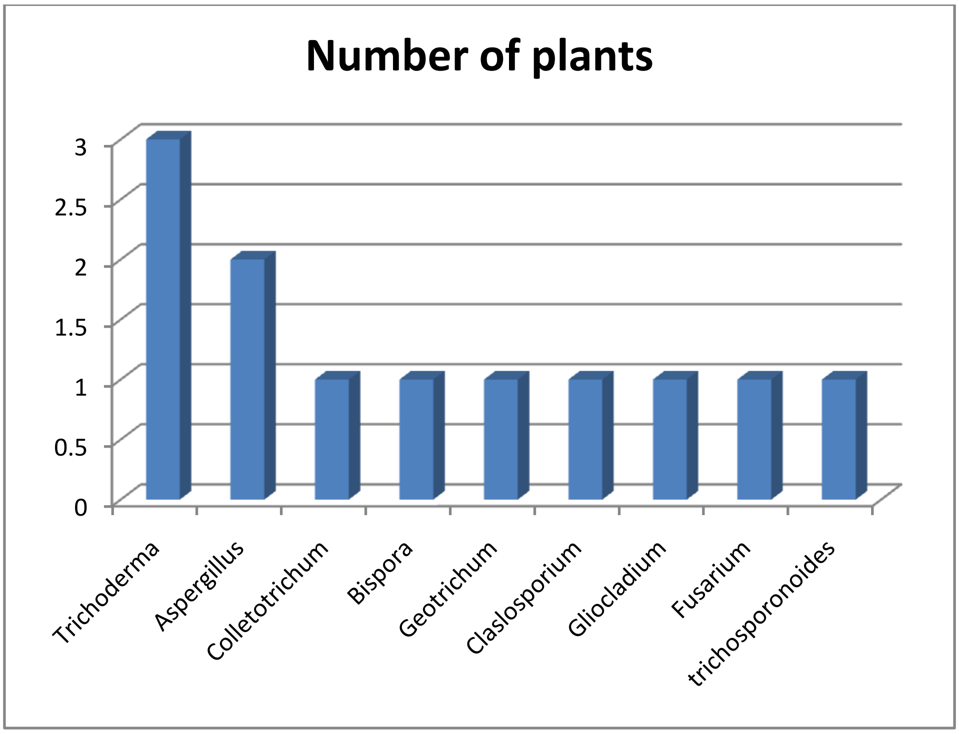
Antibacterial activity of endophytic fungi

By this experiment we could understand that the fungus harboring in the root could produce antibacterial substances and the isolated fungus could be as industrially important ones. They can be characterized further at molecular level and used as a stock culture. These inoculants can be grown in surface or submerged fermentation process to release the metabolite and this metabolite can be purified by various techniques like chromatography and became an industrially important product under the pharmaceuticals.

Endophytic fungal relationship with host plants

Few endophytic fungi are found as multiple plant parasites in relationship and a few fungi were fungi were found more specific to a particular medicinal plant. This property was studied among plants and the following data was collected.

Host nature	Number of plants	Endophytes
Multiple	3	*Trichoderma*
	2	*Aspergillus*
Single	1	*Colletotrichum, Bispora, Geotrichum, Claslosporium, Gliocladium, fusarium, Trichosporonoides*



Organic Analysis

Medicinal plant	Endophytic Fungus	Aliphatic compound	Aromatic compound	Acid	Amine	Aldehyde	Ester	Ketone	Phenol
Caesalpinia sappan	*Colletotrichum spp.*	-	+	-	-	+	+	+	+
	Aspergillus spp.	-	+	-	-	+	+	+	+
	Bispora spp.	-	+	-	-	+	+	+	+
	Geotrichum spp.	-	+	-	-	+	+	+	+
Alternanthera sessil	*Trichoderma spp.*	-	+	-	+	-	+	+	+
	Cladosporium spp.	-	+	-	-	+	+	+	+
	Aspergillus spp.	-	+	-	+	-	+	+	+
Sapindus laurifolius	*Gliocladium spp.*	-	+	-	+	-	+	+	+
	Trichoderma spp.	-	+	-	+	+	+	+	+
Basala alba	*Fusarium spp.*	-	+	-	+	-	+	+	+
	Trichosporoniodes spp	-	+	-	+	-	+	+	+
Acalypha indica	*Trichoderma spp.*	-	+	-	+	+	+	+	+

All the plant species were found colonized with endophytic fungi. The endophytes were isolated using three different mycological media namely potato dextrose agar (PDA), malt extract agar (MEA) and water agar (WA). In many instances these media were commonly used for isolation of endophytes (Mahesh et al., 2005; Tejesvi et al., 2005). Maximum endophytes were obtained in potato dextrose agar medium and minimum in water agar media (Table 2).

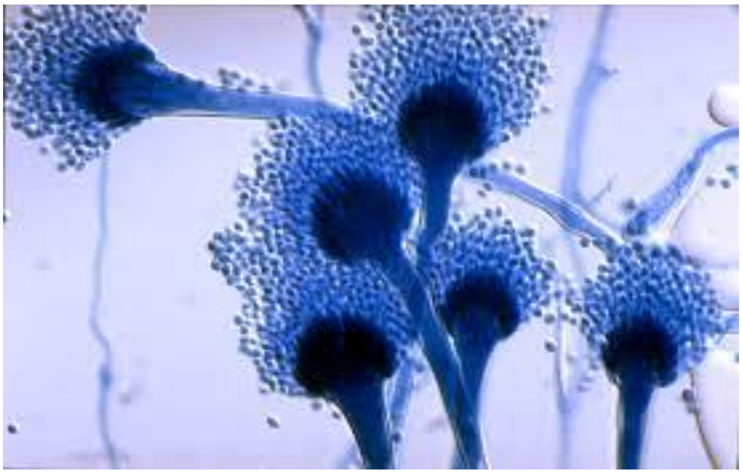
Altogether 12 fungal endophytes belonging to different genera were isolated from the five them (48.33%) as medicinal plants (Table 1). Out of which obtained as filamentous forms and some of colonies. Mycelia of endophytes was found to be variable in the five medicinal plants. The highest numbers and mycelia *Caesalpinia sappan* forms were isolated in lowest numbers. In *Acalypha indica* less same in numbers. Despite the broad occurrence of endophytes in plant organs, many endophytic fungi appear specialized to particular host tissues (Arnold et al., 2000). Furthermore, fungal endophytes are reported to be host specific at the same time several species can also be isolated from different host (Suryanarayanan et al., 2002). Such host specific endophytes have also been observed in our present study in the five medicinal plants.

Fungal endophytes have been recognized as repository of novel secondary metabolites for potential therapeutic use (Tan and Zou, 2001). Further, Daisy and Strobel (2003) necessitated that medicinal and endemic plants should use for endophytic studies as they are expected to harbor rare and interesting endophytes with novel bioactive metabolites. This has lead to the discovery of several bioactive compounds from fungal endophytes and wealth of literature on antimicrobial activity of endophytic fungi isolated from medicinal plants (Raviraja et al., 2006; Li et al., 2006; Tayung and Jha, 2006). In our present study also, we have demonstrated crude metabolites extracts of fungal endophytes isolated from the three medicinal plants showed considerable antimicrobial activity against a panel of

human pathogenic microorganisms. Out of the 12 fungal endophytes isolated as filamentous forms, could display antimicrobial activity inhibiting at least one of the test pathogens (Table 4). Among the potent strains, 19.3% displayed both antibacterial (Gram-positive & Gram-negative) and antifungal activity inhibiting at least one of the test pathogens. However, only 6.4% of the strains showed antimicrobial activity against all the test pathogens. Among the potent strains, crude metabolite of an endophytic fungus identified as Trichoderma sp.

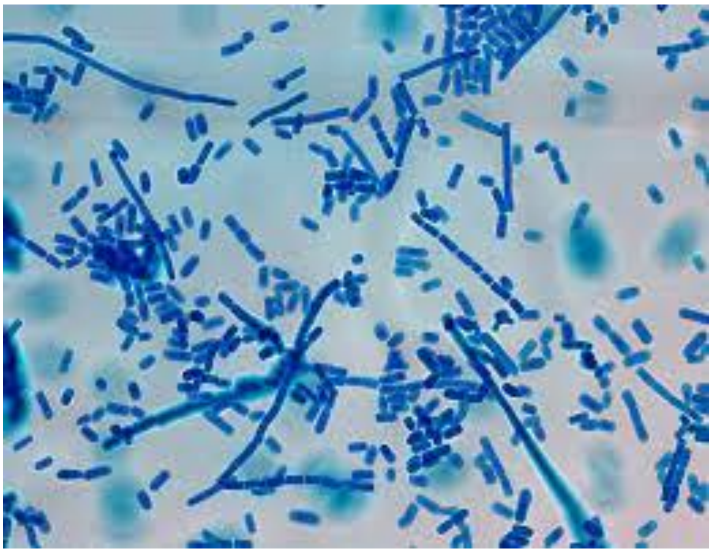
displayed significant antimicrobial activity against entire test pathogens. The extract was significantly effective against both Gram-positive and Gram-negative bacteria (Table 2) and moderately effective against the fungal pathogens. This showed the broad-spectrum nature of the metabolite

The fungus was isolated from *Basala alba and Acalypha indica* which is a plant of medicinal importance. *Colletotrichum spp* as endophyte has been reported from several plant species (Mahesh et al., 2005; Nalini et al., 2005) and its antimicrobial potential have been studied and reported (Wang et al., 2007). However, to our knowledge this is the first report of *Trichoderma spp* as endophyte and its antimicrobial activity. 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 in use in one or more countries (Balick et al., 1996). However, due to over exploitation of these genetic resources and other biotic interferences, many plants used as medicines have become critically endangered or are in verge of extinction. Since, it is believed that these plant species may harbor quite distinct and potential fungal endophytes that might produce novel metabolites with multifold applications, research priority should be directed to study them because disappearance of any of these plant species will also disappears the entire suite of associated endophytes. The study of plant-associated endophytes .could therefore provides the best possible way of acquiring novel metabolites. The present study thus, reinforced the assumption that endophytes of ethno medicinal plants could be a promising source of antimicrobial substances. We are currently working

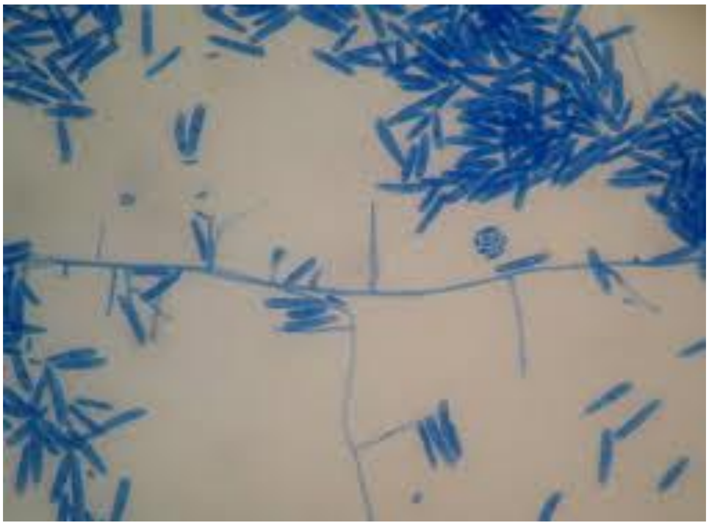


Aspergillus *spp.*

Bispora



Geotrichum



Fusarium

Morphological characteristic:

The morphology of isolated fungi was studied under microscope after staining with methylene blue stain.

The characteristics of fungal isolates are tabulated as below:

Fungal Isolate	Characteristic features
Aspergillus spp.	Branched septet mycelium, conidiophores erect, globosa vesicle with uniseriate sterigmata which produces chain of conidia by basipetal succession.
Bispora spp.	Mycelium dark; conidiophores dark, short simple, sparingly branched; conidia dark, oblong to ellipsoid, 2-celled or less with thick black septa, saprophytic.
Colletotrichum spp.	Acervuli is disc shaped, waxy. Conidiophores are simple, elongated, conidia hyaline, ovoid and parasitic.
Cladosporium spp.	Septate mycelium, conidiophores tall, dark and clustered. Conidia (blastospores) dark, 1-2 celled, ovoid to cylindrical, lemon shaped. Saprophytic or parasitic.
Fusarium spp.	Mycelium extensive with slender conidiophores, whorl of phialids grouped as sporodochium, micro conidia 1-celled ovoid, macor conidia is 2-3 celled, canoe or sickle shaped.
Gliocladium spp.	Conidiophores like penicillate brushes, conidia in mucilaginous droplets. Shows a stage Verticillium. Saprophytic in soil.
Geotrichum spp.	Mycelium white, septate; conidiophores absent; conidia hyaline, single celled, short cylindrical with truncate ends, mostly saprophytic.
Trichoderma spp.	Conidiophores branched, non vermiculate, conidia in terminal clusters. Saprophytic in soil and wood.
Trichosporoniodes spp.	True and pseudo mycelium, conidiophores simple with swollen globose apex bearing conidia (botryo blastspores) on sterigmata, conidia hyaline single celled yeast like.

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