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ANTIBACTERIAL ACTIVITY OF FUNGAL ENDOPHYTES OF MEDICINAL PLANTS FROM BANGALORE REGION.

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ABSTRACT

There is an increasing demand and prospect of finding new drugs that may be effective on micro-organisms. Endophytic association with host plants produce a plethora of substances. Endophytic fungi do not cause harm to the host tissue but produce many secondary metabolites known as bioactive compounds which has a potential of being antimicrobial, anticancerous and antibiotic. Therefore endophytic fungi are good targets for new source of antibacterial drugs. In the present study endophytic fungi were isolated from 25 medicinal plants collected from Dhanavantri vana and Siddarabetta in Bangalore, India. 101 endophytic fungi were isolated and studied for antibacterial property. The fungi were cultured in Potato Dextrose Broth with an incubation period of 21 days. The crude extract of fungi was studied for antibacterial property by turbidity method. From a total of 101 types of isolates, (50 isolates) 50% showed antibacterial activity. Analysis of organic compounds resulted in 42% Aromatic compounds, 38% aliphatic compounds, 39%phenols, 16% amines, 23% ketones, 38% acids, 24% Aldehydes with 33 fungal endophytes. Benzoic acid and Salicylic acid was reported in few fungal specimens of *Penicillium*, *Gliocladium* and *Fusarium*, where majority of the fungi did not produce these acids.

KEYWORDS: Endophytic Fungi, Medicinal Plants, Fungal Diversity, Organic Compounds, Anti- microbial activity.

INTRODUCTION

Endophytic fungi reside inside healthy plant tissues without any discernible infectious symptoms. They could be a potential source of novel natural products for medicinal,

agricultural and industrial uses.[3] Because they are relatively unstudied, much attention is now being paid to endophytic biodiversity and the chemistry and bio activity of endophytic secondary metabolites.[7-9]

It has been reported that some the endophytic fungi can promote host plant growth and protect the host from microbes and insects.[5] A variety of relationships exist between these endophytes and their host plants, ranging from mutualistic or symbiotic to antagonistic or slightly pathogenic.[3] There is great potential of finding new drugs from endophytes for treating diseases in human and animals using such novel antibiotics, anti-mycotic, immunosuppressant and anticancer compounds.[1-5] In addition, studies of endophytic fungi and their relationship may shed light on the ecology and evolution of both plants and endophytes; the ecological factors that influence the direction and strength of the endophyte host plant interaction.[13-16] It is necessary to identify and classify these potential bioactive compounds for better understanding of the characteristics of the relevant endophytic communities.

The interaction of endophytes with single or multiple host plants depends on the factors like host specificity, selectivity, recurrence or host – preference. The differences in endophyte assemblages from different hosts might be related to the chemical differences of the hosts[16-19].As a part of ongoing efforts to find bio-active chemicals and antimicrobial agents from natural resources, we investigated the secondary metabolites of endophytes isolated from 25 medicinal plants located in Karnataka state, India and isolated fungal isolates exhibiting strong antimicrobial activity.

The chemical constituents of the endophytic and host plant assemblages were also studied along with the relationship between the compositions of endophytic fungi and bioactive chemicals of their plant hosts.

MATERIALS AND METHODS

Plant material

A total of 25 medicinal plants from different families were collected from Dhanavantari vana and Siddarabetta, located in Karnataka, India. The plants were 3 months to one year old, where the Rhizosphere soil and the root samples were collected and processed within 24 hours. All the species were first identified morphologically and subsequently verified by taxonomists. Digital photos were taken for all the species studied.

Isolation of endophytic fungi

A total of 25 root samples from each plant species were first washed thoroughly with running water. The roots were cut into small pieces (10mm in length) and surface sterilized by processing the root bits in 75% alcohol for 1 minute, followed by 5% Sodium hypochlorite (5 minutes) and again 70% alcohol for 30 seconds. The root bits were washed in sterile distilled water and blot dried aseptically. These root bits were placed on the Potato Dextrose Agar for isolation of endophytic fungi by method of Schulz et al.[16] Antibiotic Streptomycin was added to the culture medium to suppress the growth of bacteria. The pure endophytic fungal strains were identified and preserved in the laboratory.

Identification of endophytic fungal isolates

The morphological identification of endophytic fungal strains was done based on its characteristics. The characteristics of the Hyphae, septate/non-septate mycelium, reproductive structures, their spores or conidial characters. Screening of all fungal characters was made in water mounts and the slides were subsequently mounted on lactophenol cotton blue and sealed with DPX. All experiments and observations were repeated thrice.

Tube dilution technique

Study of antibacterial property of the endophytic fungi was studied against three types of bacteria- Gram positive bacteria, *Staphylococcus aureus* & gram negative *Escherichia coli* and *Klebsiella pneumoniae*. The bacteria were inoculated and incubated in nutrient broth for 48 hours. Equal ratios of bacterial culture, sterile nutrient broth and the fungal extract (1:1:1) were taken in sterile tube and incubated at 37ºC for 48 hrs. The tubes were observed for bacterial growth study indicating turbidity. The clear broth indicated that the filtrate contained antibacterial activity.

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 or aromatic. They may be saturated or unsaturated. Depending upon the functional group the compounds contain, they show different solubility and give characteristic reactions. Solubility tests were performed with dilute HCl, 1% NaOH and specific reagents like neutral Ferric chloride, Tollen's reagent, Schiff's reagent to detect Aldehydes and Ketones.

RESULTS AND DISCUSSION

Plants / Endophytic Fungi	Aliphatic	Aromatic	Acid	Amine	Aldehyde	Ester	Ketone	Phenolic	Salicylic	Benzoic
Premna serratifolia	Sm, Cs	-	-	Cs	Cs	Cs	Cs	Sm		
Lagerstroemia speciosa	Pe, As	-	Pe, As	-	Pe, As	As	-	Pe, As	Pe	-
Passiflora edulis		Pe, Sm						Pe, Sm		
Carrisa carandas	-	-	-	-	-	-	Rt, Ca	Pe	-	-
Sapindus laurifolius	-	-	-	-	-	-	-	-	-	-
Santalum album	Ph, Ch	Ph, Fu, Ha, Co, Ch	-	Ph, Fu, Ha, Ch	Ch, Rt	-	Ph, Fu, Ha, Co, Ch, Rt	-	-	-
Acalypha indica	Gl	Rt, Al, Gl, Co			Rt, Al, Gl, Co		Gl, Co	Gl		
Euphorbia tirucalli	Ni	Ni	-	-	Rt	-	Ni, Rt	Ni	-	-
Aloe vera	Pe, Fu		Pe, Fu					Pe, Fu	Pe	Pe, Fu
Withania somnifera	Ph, Pe, Sm	-	-	-	Sm	-	-	-	-	-
Ruta graveolens	Bi, Ch	Co	Co	-	Co	-	-	Bi, Ch		
Azadirachta indica	Sa, Sm	Pe	sm	sm	Ph	Pe	Pe	-	-	Rt
Alternanthera sessilis	As,Gl, Ba	As, Ba	As, Pe, Ba	As, Ba	-	-	As,Gl, Ba	-	As, Pe	Pe
Caesalpinia sappan	-	Gl, Fu, Bi, Cs, Sm, Myc, As	Gl, Fu, As	Gl, Cs	Fu	-	Rt	Bi, Cs, Sm, Myc, As	Sm, Myc	-
Catharanthus roseus		Al, Ca, Pe, Gl	Al, Ca, Pe, Gl , Sm		Rt		Rt	Al, Ca, Pe, Gl ,		
Ocimum sanctum	Ca, Gl	Ph, Pe, Ha , Sm	Ha, Pe	Gl, Pe	Ca	-	Ca	Ca, Ph, Pe, Ha , Sm	-	-
Hemigraphis colorata	Ha	Ha	-	-	-	St	-	ca		
Cymbopogan citratus	Co, Ch	Cs	Co, Ch,	Cs				Co, Ch, Cs		
Tinospora cordifolia	Ca, Gl, Pe	Ca, Fu, Co, Ch	Ca, Fu, Co, Ch	ca	Ca, Pe, Fu, Co, Ch	-	-	Pe, Ca, Ch	Ch	Ch
Mentha piperita	Sm, Rh	Sm, Co, Rh	Sm, Co, Rh	-	Co	-	-	-	-	-
Tylophora asthmatica	Pe, Sm, Gl, Co	-	Co, Sm	Co, Sm	-	Pe	Pe	Co, Sm	-	sm
Chamaecostus cuspidatus			Bi, Sm							
Basella alba	Ca	-	Ca, Ni	-	-	-	Rt	-	-	-
Chrysanthemum indicum		Fu	Ca, Sm,Fu		Fu					
Bacopa monnieri	Ca, Pe, Ch	sm	Pe	-	Ch	-	Ca	Pe, Rh , Sm, Ca		

Endophytic Fungi	Symbols
Alternaria	Al
Aspergillus	As
Basidiobotrytis	Ba
Bispora	Bi
Balanium	Bn
Cladorrhinium	Ca
Cladosporium	Cs
Chalaropsis	Ch
Cunninghamella	Cu
Colletotrichum	Co
Chaetopsis	Ce
Chaetomium	Cm
Eleutheromyces	El
Excipularia	Ex
Fruiting Body	Fb
Fusarium	Fu
Geotrichum	Ge
Halosporangium	Ha
Humicola	Hu
Nigrospora	Ni
Mortierella	Mo
Phoma	Ph
Pseudobotrytis	Ps
Penicillium	Pe
Rhizopus	Ri
Stachybotrytis	Sa
Sterile mycelium	Sm
Stachylidium	St
Rhizoctinia	Rh
Trichoderma	Tr
Gliocladium	Gl
Root	Rt

Table 1a) The organic analysis of fungal endophytes from roots of 25 medicinal plants. b) The abbrivated list of fungal endophytes

The isolated endophytic fungal isolatesfrom plant roots showed unique properties. Many of the endophytes are observed to be obligatory parasites infecting only particular medicinal plants.Very few can be facultative in nature, their existence in rhizosphere soil as well in plants as an endophyte. The isolated fungi were maintained as a stock on PDA slants. The fungi isolated in endophytic are as follow (Table: 1).

Antibacterial activity of endophytic fungi

The different endophytes were tested for antimicrobial activity against the bacterial pathogens *Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli(Table:2).* Out of 101 fungal isolates 50 isolates exhibited antagonistic activity against at least two of

the three tested pathogens and endophytic fungi of 19 plants were positive against *Staphylococcus aureus*. About one third of isolates, inhibited the growth of all tested pathogens. The crude extracts from *Passiflora edullus, Santalum album, Acalypha indica, Alternanthera sessilis, Sapindus lauifolius and Mentha paperita* exhibited broad spectrum antimicrobial activities and are considered the most promising isolates for further attention. 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.

Table 2: The antimicrobial activities exhibited by the different endophytes and root samples.

Plants	Endophytic Fungi	Staphylococcus	E.coli	Klebsiella
Premna serratifolia	Sterile mycelium	-	+	+
	Cladosporium	-	+	+
Lagerstroemia speciosa	Penicillium	+	-	-
	Aspergillus	+	-	-
	Gliocladium	+	+	+
Ocimum sanctum	Cladorhinium	+	+	+
	Cladorhinium	-	-	-
	Gliocladium	+	-	+
	Halosporangium	-	-	+
Carrisa carandas	Penicillium	+	-	-
	Penicillium	+	-	+
	Cladorrhinium	-	-	+
	Gliocladium	-	-	+
Sapindus laurifolius	Phoma	+	+	+
Santalum album	Phoma	+	+	+
	Fusarium	+	+	+
	Halosporangium	+	+	-
	Colletotrichum	+	-	+
	Chalaropsis	-	-	+
Acalypha indica	Alternaria	+	-	+
	Gliocladium	+	-	+
	Gliocladium	+	-	+
	Colletotrichum	+	-	+
Euphorbia tirucalli	Nigrospora	+	-	-
	Chalaropsis	+	-	+
Basella alba	Sterile mycelium	+	-	-
Chrysanthemum indicum	Cladorrhinium	+	-	-
Alternanthera sessilis	Aspergillus	+	-	-
	Penicillium	+	-	-
	Gliocladium	+	-	-
	Basidiobotrytis	+	-	-
Caesalpinia sappan	Gliocladium	+	-	-
	Fusarium	+	-	-
	Bispora	+	-	-
	Sterile mycelium	+	-	-
	Myce.fb	+	-	-
Catharanthus roseus	Cladorrhinium	+	-	-
	Penicillium	+	-	-
Aloe vera	Gliocladium	-	+	-
	Penicillium	+	-	-
	Fusarium solani	+	+	-
Ruta graveolens	Chalaropsis	-	+	-
Cymbopogan citratus	Colletotrichum	+	+	-
Tinospora cordifolia	Cladorrhinium	-	+	-
	Fusrium(Haem)	+	+	-
	Fusarium	+	+	-
	Penicillium	+	+	-
	Gliocladium	-	+	-
Tylophora asthmatica	Gliocladium	+	-	-
	Sterile mycelium	+	+	-
	Penicillium	+	+	-
Withania somnifera	Phoma	-	+	-
	Penicillium	+	-	-
Azadirachta indica	Penicillium	+	+	-

Host plants - Endophytic fungal relationship

Endophytes associated with medicinal plants are of interest as the producers of important bioactive compounds. 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 could be a promising source of antimicrobial substances.

Fungal endophytes have been recognized as a repository of novel secondary metabolites for potential therapeutic use. Further, the medicinal and endemic plants should be used for endophytic studies as they are expected to harbor rare and interesting endophytes with different bioactive metabolites. This has contributed to the discovery of several chemical compounds from fungal endophytes. In our present study, also, we have demonstrated crude metabolites extracts of fungal endophytes isolated from twenty medicinal plants showed considerable antimicrobial activity against a panel of human pathogenic microorganisms. Out of the 101 isolates, 50 fungal endophyte isolates showed crude antibacterial metabolites (Table :1).

It has been estimated by the World Health Organization (WHO) that approximately 80% of the world's population of 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.[16]

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.[17] 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. The disappearance of any of these plant species will also disappears the entire suite of associated endophytes. Our results emphasize that endophytic association with medicinal plants are an unexplored resource for the discovery of biologically active compounds.

CONCLUSION

From a total of 101 types of fungal isolates, (50 isolates) 50% showed antimicrobial activity. Analysis of organic compounds resulted in 42% Aromatic compounds, 38% aliphatic

compounds, 39% phenols, 16% amines, 23% ketones, 38% acids, 24% Aldehydes with 33 fungal endophytes. Benzoic acid and Salicylic acid was reported in few fungal specimens of *Penicillium, Gliocladium* and *Fusarium*, where majority of the fungi did not produce these acids.

From the results, it is concluded that, *Santalum album, Caesalpinia sappan, Tinospora cordifolia* can be said as a perfect habitat for fungal endophytes which might act as a rich source of bioactive compounds with good antimicrobial activity. The ability of endophytic isolates is to produce Ketones, Aliphatic compounds, Acids, Phenols, Esters and Aldehydes,. The root extracts of *Santalum album, Acalypha indica, Alternanthera sessilis* exhibited broad spectrum of antimicrobial activities and are considered to be the most promising isolates for further attention. The biological activity of the fungal endophytes owing to their secondary metabolites having medicinal properties needs further detailed investigations.

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 could be a promising source of antimicrobial substances.

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