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RESEARCH ARTICLE

Analysis of Endophytic Fungal Extracts of Therapeutically important Hydrophytes for different Chemical Compounds

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ABSTRACT:

Endophytic fungi were isolated from leaves of five different therapeutically important hydrophytes. A total of 46 endophytic fungal species were present and their crude extracts were tested for the production of alkaloid, flavonoid, terpenoid, tannin and steroid. All the endophytic fungi produced alkaloid in culture. Flavonoid was produced by 44 endophytic fungi and terpenoid produced by 39 species of endophytic fungi. Twenty five endophytic fungi produced tannin and 24 endophytic fungi produced steroid in culture. Further, out of the 46 endophytic fungi 23 endophytic fungi (Almost 50%) produce all the five chemical groups tested. *Chrysosporium* sp and sterile form III produced only alkaloids in culture.

KEYWORDS: Alkaloid, flavonoid, terpenoid, qualitative test, crude extract, endophytic fungi.

1. INTRODUCTION:

Several years of research indicated that maximum, if not all, plants in the natural environments are symbiotic with either endophytic fungi or mycorrhizal fungi[1]. Most of the endophytic fungi reported from plants are belong to the hyphomycete, ascomycete and coelomycete.[2] Endophytic fungi display complex interactions with their host colonized such as mutualism, latent pathogen, antagonism and occasionally parasitism[3]. Endophytic fungi play an imperative role in the physiological function of the host plants; protect the plants from insects, pests, nematodes and phytopathogens by Vellingiri Manon Mani *etal*., 2015[4]. Now it has been well recognized that endophytic fungi of diverse plant hosts are a prospective and novel source of bioactive secondary metabolites, industrially important enzymes, plant growth promoting substances etc[5],[6],[7].

Endophytic fungi considered as microbial factories for the production of various chemical and bioactive compounds. Moreover, the endophytic fungi have the capability to produce a range of secondary metabolites showing varied bio-activities and resulted in the exploitation of these fungi for biotechnological applications[8]. Recently, the effect of endophytic fungi on green house plant was studied for the growth and its chemical constituents.[9]

MATERIALS AND METHODS:

Inoculation of surface sterilized leaf segments:

The undamaged and healthy plant leaves of *Alternanthera philoxeroides* (Amaranthaceae), *Centella asiatica* (Apiaceae), *Aponogeton natans* (Aponogetonaceaea), *Hygrophila auriculata* (Acanthaceae), *Ipomoea aquatic* (Convolvulaceae) and *Ceratophyllum demersum* (Ceratophyllaceae) were collected and washed thoroughly in running tap water. The leaf segments were surface sterilized in 70% ethanol for 5 sec, immersed in 4% Sodium hypochlorite (NaOCl) for 30 sec, rinsed in autoclaved double distilled water. The 2-5 mm leaf segments were plated on Potato Dextrose Agar (PDA) medium amended with Chloroamphenicol (100 mg/L). The inoculated plates

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were incubated in light chamber for 2- 4 weeks for the growth of endophytic fungi. The light regime was 12 hours light followed by 12 hours darkness.[10]

Qualitative test for chemical compounds:
The crude extracts of all endophytic fungi were qualitatively tested for alkaloid, flavonoid, terpenoid, tannin and steroid[11],[12].

Test for alkaloid:
2mg/ml of the endophytic fungal extracts is treated with 2 drops of Dragendroff's reagent (Bismuth nitrate and tartaric acid – solution A and potassium Iodide – Solution B). Solution A and B mixed together in equal volume) red or orange precipitate indicates the presence of alkaloids.

Test for flavonoid:
10% $NaOCl_3$ in alcohol is treated with 2 mg/ml of the endophytic fungal extracts and heated at 150°C. Blue colour shows the presence of flavonoids.

Test for terpenoid:
2 mg/ml of the endophytic fungal extracts is treated with Puncal reagent (ammonium heptamolybedate, ceric sulphate in concentrated sulphuric acid) and heated at 150°C. Blue colour shows the presence of terpenoid.

Test for tannin:
2mg/ml of the endophytic fungal extracts is treated with saturated solution of ferric chloride; blue colour indicates the presence of tannins.

Test for steroid: Salkowski reaction: -
2ml of crude extract add 2ml chloroform and 2ml of conc H_2 So_4 was added, the appearance of red and greenish yellow fluorescence colour indicates the presence of steroids.

Lieberman's reaction: -
3ml of crude extract add 3ml of acetic anhydride. Then heat the mixture and bring down to room temperature, then add few drops of concentrated H_2 SO_4. Observe for the formation of blue colour indicate the presence of steroid.

RESULTS AND DISCUSSION:

Endophytic fungal research of trees, shrubs and herbs engaged the interest of many Mycologists in temperate and tropical region. However, very scant attention has been given to the study of endophytic fungi of hydrophytes particularly from tropics. A Survey of the leaves of six hydrophytes for the presence of endophytic fungi has revealed that the entire host studied had endophytic fungi. Apart from understanding the diversities of endophytes in a host, another motivation for Mycologists to study these fungi in their ability to produce various and novel chemical and bioactive compounds. These include antibiotics, anti-insect chemicals, antimicrobial, anticancer substances and antinematicial and anti-inflammatory compounds[13-19]

Table 1. Qualitative analysis of crude extracts of endophytic fungi for various chemical groups

Name of the Endophyte	Chemical groups tested				
	Alkal-oid	Flavon oid	Terpen oid	Tannin	Steroid
Hyphomycete					
Acremonium sp	+	+	+	+	+
Alternaria alternata	+	+	+	+	+
Alternaria longipes	+	+	+	-	-
Arthrobotrys oligospora	+	+	+	-	-
Aspergillus flavus	+	+	+	+	+
Aspergillus fumigatus	+	+	+	+	+
Aspergillus japonicas	+	+	+	+	+
Aspergillus niger	+	+	+	+	+
Aspergillus stellatum	+	+	+	+	+
Bactrodesmium sp	+	+	-	-	+
Bipolaris sp	+	+	+	+	-
Botryodiplodia theobromae	+	+	+	+	+
Botrytis sp	+	+	-	-	-
Chrysosporium sp	+	-	-	-	-
Cladosporium sp	+	+	+	-	-
Cladosporium cladosporioides	+	+	+	+	+
Cladosporium macrocarpum	+	+	+	+	+
Cladosporium sphaerospermum	+	+	+	+	+
Curvularia sp	+	+	+	-	-
Curvularia ovoidea	+	+	+	+	+
Curvularia lunata	+	+	+	+	+
Cytospora sp	+	+	+	-	-
Drechslera halodes	+	+	+	-	-
Drechslera hawaiiensis	+	+	+	+	+
Fusarium mairei	+	+	-	-	-
Fusarium oxysporum	+	+	+	-	-
Fusarium solani	+	+	+	-	-
Geotrichum sp	+	+	-	-	-
Gliocladium sp	+	+	+	-	-
Graphium sp	+	+	+	-	-
Ascomycete					
Chaetomium incomptum	+	+	+	+	+
Chaetomium indicum	+	+	+	+	+
Chaetomium globosum	+	+	+	+	+
Emericella nidulaus	+	+	+	+	+
Glomerella sp	+	+	+	+	+
Sporormiella minima	+	+	+	+	+
Aureobasidium sp	+	+	+	-	-
Coelomycete					
Colletotrichum sp	+	+	+	-	-
Pestalotiopsis microspora	+	+	+	-	-
Pestalotiopsis sp	+	+	+	+	+
Phoma sp	+	+	+	+	-
Phomopsis sp	+	+	+	+	+
Phyllosticta sp	+	+	+	-	-
Sterile Mycelia					
Sterile form I	+	+	-	-	-
Sterile form II	+	+	+	+	+
Sterile form III	+	-	-	-	-
Total	**46**	**44**	**39**	**25**	**24**

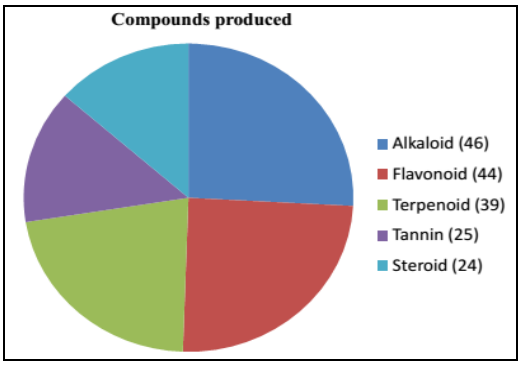

Fig 1: Number of chemical compounds produced by endophytic fungi

Endophytic fungi isolated from various tropical hosts produce several compounds in culture reported the production of alkaloid, flavonoid, terpenoid, tannin and steroid etc. by the endophytic fungi.[17],[18]. Hence, in the present investigation the crude extracts of all 46 endophytic fungi qualitatively analyzed for the production of alkaloid, flavonoid, terpenoid, tannin and steroid. The results are presented in (Table 1) (Fig 1). All the 46 endophyte produced alkaloid in culture. Flavonoid produced by 44 endophytic fungi except *Chrysoporium* sp and Sterile form III. Terpenoid produced by 39 endophytic fungi except sterile formI & II, *Bactrodesomium* sp, *Botrytis* sp, *Chrysosporium* sp, *Fusarium mairei* and *Geotrichum* sp.

Twenty five endophytic fungi produced tannin in culture. Out of 46 endophytic fungi only 24 endophytic fungal species produced steroid in culture (Table 1). Further, out of the 46 endophytic fungi 23 endophytic fungi (Almost 50%) produce all the five chemical groups tested. Some endophytic like *Chrysosporium* sp. and sterile form III produced only alkaloid in culture (Table 1) indicates its specificity in producing particular chemical compound. Out of 4 major groups of fungus all ascomycete isolate produced all the test chemicals (Table 1) followed hyphomycete and coelomycete[20]. Strobel and Daisy (2003) reported that several chemical compounds produced by ascomycete which are antimicrobial in nature[21]. Sanjana Kaul *et al*., 2012 reported that endophytic fungi from medicinal plants produced different chemical compounds like terpenoids, steroids, quinones, phenols, coumarins etc.[22,23]. Hence, endophytic fungi are found to be a rich source of functional metabolites. The discovery of endophytic fungus *Taxomyces andreana* also produce the anti-cancer drug paclitaxel a alkaloid was unexpected that endophyte isolated from a host produce similar compounds as host.

CONCLUSION:

To conclude, the current study showed that a high number of endophytic fungi distributed in hydrophytes and crude extracts exhibited the presence of different chemical groups. Therefore, the hydrophytes of tropical region should be another potential cradle of endophytic fungi and reservoir of chemical compounds. However, the chemical compounds of all endophytic fungi isolate should be studied in depth to isolate pure compounds and to elucidate the metabolite structure.

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