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Various chemical groups produced by endophytic fungi isolated from the *Calotropis procera* - A pharmaceutically important xerophytic plant

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ABSTRACT

In the present study leaf tissue of host plant *Calotropis procera* was screened for the presence of endophytic fungi. The crude extract of the isolated endophytic fungi was tested for various chemical groups like alkaloids, flavonoids, diterpenoids and phenols. Our study showed the presence of 25 endophytic fungi isolated from the leaf tissue which constituted15 Hyphomycetes, 3 Ascomycetes, 3 Coelomycetes and 4 sterile forms. The qualitative analysis in all the 25 isolates showed the presence of alkaloid, phenol in 23 fungi, flavonoid in 19 and diterpenoids in 18 fungi. Fifteen endophytic fungi produced all chemical groups tested in the crude extract. *Bipolaris* sp a hyphomycete produced only one compound in culture. Among the four groups endophytic fungi Coelomycetes group able to produce all chemical groups tested.

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INTRODUCTION

In order to minimize the side effects caused by the modern synthetic drugs, the need to develop phytocompounds as drugs against wide range of diseases is a key objective among many scientists today (Mohanasundaram *et al.,* 2017). *Calotropis procera* is an important medicinal wild plant that belongs to Asclepiadaceae family. This plant is used in the traditional Indian medicinal system for the treatment of leprosy, ulcers, tumors, piles, diseases related to spleen, liver and abdomen (Upadhyay, 2014; Mohsin *et al.,* 1989; Larhsini*et al.,* 1997). The aqueous and various organic extracts of this host showed analgesic, antipyretic, anti-inflammatory, antimalarial and antimicrobial activity (Dewan *et al.,* 2000; Mascolo *et al.,* 1988) and many of these properties are linked to the endophytic fungi associated with it Isolation and bioactivity study on the endophytic fungi from *Calotropis procera* was carried out. However not much research has been done on the various chemical groups present in the endophytic fungi isolated from this host. Hence, our investigation throws light in this arena with 25 endophytic fungi isolated from leaf tissue

of this host which are also qualitatively tested for various important chemical groups like alkaloid, flavonoids, diterpenoids and phenols. Because endophytic fungi are omnipresent; they exist in the internal tissue leaf, stem and root of living plants. An endophytic fungus has the vast capacity to produce various chemical compounds as secondary metabolites similar to the host. They develop endosymbiotic relationship with host plants of all lineages. The endophytic fungi occur generally and they have been reported in plants from diverse ecological niches. Moreover, the endophytic fungi have the capability to produce a range of secondary metabolites showing varied bioactivities. These discoveries have led to the exploitation of these fungi for biotechnological applications (Suryanarayanan, 2017).

MATERIALS AND METHODS

Isolation of Endophytic Fungi

The leaves taken from the host plant *Calotropis procera* were washed thoroughly in running water. The leaf segments (0.5 cm^2) cut from the midrib region and were surface sterilized using 70% ethanol for 1 min and sodium hypochlorite 4% for 1 min., About 6 to 8 segments of the surface sterilized leaf segments were inoculated in Petri dish containing Potato Dextrose Agar (PDA) supplemented with Chloramphenicol (100 µg/mL) (to inhibit the growth of bacteria). The inoculated petri dishes were sealed with parafilm and incubated at $27^0C \pm 1^0C$ for 21 days. The endophytic fungi grew out from leaf segments were isolated and sub cultured, periodically on PDA medium (Bills and Polishook, 1992).

Qualitative Analysis of Crude Extract

Different qualitative tests were conducted to detect various chemical groups like alkaloid, flavonoid, diterpenoids and phenols present in the crude extract of endophytic fungi (Prashant Tiwari *et al.,* 2011).

Test for alkaloids: About 2 mg/ml of the endophytic fungal extracts were dissolved individually in dilute hydrochloric acid and filtered.

Dragendroff's Test- The filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Wagner's Test- Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Test for flavonoids: Each endophytic fungal extract was individually tested for flavonoids.

Alkaline Reagent Test: Individual endophytic fungal extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour and subsequent decolourization after adding dilute acid indicates the presence of Flavonoids.

Lead Acetate Test: Endophytic fungal extracts were treated with few drops of lead acetate solution. Presence of flavonoids is indicated by yellow colour precipitation.

Test for diterpenoids: Copper Acetate Test: Each endophytic fungal extract was dissolved in water and on treatment with few drops of copper acetate solution if emerald green is formed, indicates the presence of diterpenes.

Test for phenol: Endophytic fungal extracts were treated with few drops of ferric chloride solution; formation of bluish black color indicates the presence of phenol.

RESULTS AND DISCUSSION

Endophytic fungi are found in very unique and hostile environment. Based on the chemical compounds produced by them, these groups of fungi are recognized as sources of new and novel metabolites useful in medicine, biotechnology and agriculture industries (Bills and Polishook, 1992). A variety of metabolites containing structural compounds like terpenoids, steroids, quinines, phenols, alkaloids, coumarins, and many more are produced by the endophytic fungi produce (Sanjana Kaul *et al.,* 2012). Studies done by Owen and Hundley (2004) has shown that endophytic fungi are the chemical producers inside plants. Many tropical plants harbor endophytic fungi and these are known to produce several chemical compounds in culture (Schulz, 2002; Tan and Zou, 2001). In the current investigation, the crude extract of all 25 endophytic fungi isolated from the leaf tissue of *Calotropis procera* were qualitatively analyzed for the production of alkaloid, flavonoid, diterpenoids and phenols. The results are presented in Table (1). Among the 25 endophytic fungi, alkaloid was produced by all, phenol produced by 23, flavonoid produced by 19 and diterpenoids produced by 18 endophytic fungi. Among the individual species of endophytic fungi 14 species produced all the chemical groups tested (Table 1). *Bipolaris* sp alone produced only one chemical group whereas all other endophytic fungi produced 2 chemical groups in culture (Table 1). Alkaloid is one of the commonest chemical compounds produced by endophytic fungi. All the 25 endophytic fungi produced alkaloid in culture. Zhang *et al.,* (2012) reported that there are many alkaloids produced by endophytic fungi in plants with extraordinary

Table 1: Chemical compounds produced by endophytic fungi isolated from *Calotropis procera* leaf.

Endophytic Fungi	Chemical Groups			
	Alkaloids	**Flavonoids**	**Diterpenoids**	**Phenol**
Acremonium sp	+	+	+	+
Aspergillus niger	+	+	+	+
Aspergillus flavus	+	+	+	+
Botryotrichumsp	+	+	-	-
Aspergillus fumigates	+	+	+	+
Aspergillus nidulans	+	+	+	+
Trichoderma sp	+	+	+	+
Bipolaris sp	+	-	-	-
Alternaria alternate	+	+	+	+
Curvularia lunata	+	+	+	+
Nigrospora oryzae	+	+	+	+
Drechslera haloides	+	+	-	+
Cladosporium cladosporioides	+	+	+	+
Aspergillus clavateus	+	-	+	-
Penicillium sp	+	+	+	+
Ascomycetes				
Thielaviasp	+	+	-	+
Sporormiella minima	+	+	-	+
Chaetomium incomptum	+	+	+	+
Coelomycetes				
*Phoma*sp	+	+	+	+
*Phomopsis*sp	+	+	+	+
*Phyllosticta*sp I	+	+	+	+
Sterile mycelia				
MS II	+	-	+	+
MS III	+	-	+	+
MS V	+	-	-	+
MS VI	+	-	-	+
Total No Compounds Produced	**25**	**19**	**18**	**23**

properties and make them as ideal insecticides, antibiotics, cytotoxins and anticancer. The endophytes are also reported to produce several alkaloids such as quinoline and isoquinoline, amines and amides, indole derivatives, pyridines, and quinazolines. Except the two endophytes Hyphomycetes *Botryotrichum sp* and *Aspergillus clavateus* all other endophytes of the 25 fungal isolates, produced phenol. Production of phenol by endophytes gives an edgeto the host because phenols play a very important role in host defense mechanism and help the plants to tackle any pathogenic attack. *Luehea divaricata* harbors the endophytic fungi *Diaporthe helianthi* and it is known to produce a phenolics compound tyrosol and it was studied for its antagonistic effects on various pathogenic bacteria (Vania Specian*et al.,* 2012). Flavonoids are the most general group of polyphenolic compounds and are found universally in plants. In the present study, all endophytic fungi were screened for the production flavonoid and the findings are presented in Table (1). The results showed that only 19 endophytic fungi produced the flavonoids. Among the various fungi only the *Bipolaris* sp and none of the sterile mycelium produced flavonoids in culture (Table 1). Bioassay-guided fractionation of bioactive flavonoids showed the presence of tricin, 7-*O*-(β-d-glucopyranosyl) tricin, isoorientin and 7-*O*-[α-l-rhamnopyranosyl (1−6)−β-d-glucopyranosyl tricin in the extracts of endophytic fungi of *Poaampla* (Yong Ju *et al.,* 1998). Diterpenes are chemical compounds possessing two terpene units. In the present study, all Coelomycetes, only one ascomycete namely *Chaetomium incomptum* and a maximum number of hyphomycetes produced diterpenes (Table 1). Additional, plant secondary metabolites detected in endophytic fungi include naphthodianthrones hypericin, from *Hypericum perforatum* (Kusari*et al*. 2008).

CONCLUSION

Considering the potentials of endophytic fungi in producing plant compounds the pharmaceutical companies could gain by exploiting endophytic fungi rather than the plants. Using modern fermentation technologies and providing optimum culture conditions such as nutrient media, culture

conditions like pH, temperature, aeration, extraction procedures and use of various solvents could lead to a cost-effective, ecofriendly, sustainable, and reproducible yield of secondary metabolites. However, this area of research still needs to be explored and there is a long way to go.

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