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Research Article

Antioxidant Activity of Endophytic Fungi isolated from *Lannea coromendalica*

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ABSTRACT

The aim of the present study was to evaluate the antioxidant potential of the ethyl acetate extract of the endophytic fungi isolated from *Lannea coromandelica* (Houtt.) Merr (Anacardiaceae).The dominant fungi isolated from *Lannea coromandelica* were *Aspergillus niger, Aspergillus flavus and Alternaria alternata,* they were subjected to antioxidant activity by diphenylpicrylhydrazyl (DPPH) method. All the extracts showed significant antioxidant potential and the antioxidant nature of the extracts were dependent on the concentration.. There was a positive correlation between the phenolic content and the antioxidant capacity of the endophyte extracts. Further work is needed to isolate the exact compound which is responsible for antioxidant activity and further characterization will be done in the future.

Keywords: Antioxidant activity; ethyl acetate extract; *Lannea coromendalica;* phenolic compounds

INTRODUCTION

Reactive oxygen species (ROS) comprising superoxide (O_2•-), hydrogen peroxide (H_2O_2), hydroxyl radical (•OH), and singlet oxygen ($(1)O_2$) are generated as by-products of normal aerobic respiration. Superoxide, a reactive oxygen by-product generated by leakage of electrons from the electron transport chain (ETC) to oxygen, is a precursor to many other forms of ROS.ROS are vital for cells as molecular and key regulators but they are destructive for cells in high extent of their production. These reactive oxidants when accumulated in large quantities lead to oxidative stress which in turn leads to various diseases like Alzheimer's, Parkinson's, rheumatoid arthritis, cardiovascular diseases, etc. (Nunomora et al., 2006). They are produced in organelles of chloroplasts, mitochondria and peroxisomes in different metabolic pathways and they are exterminated by antioxidant defence mechanisms (Apel and Hirt, 2004).

Nature has provided living systems with numerous antioxidant molecules. An antioxidant is a stable molecule which donates an electron to a rampaging free radical and terminates the chain reaction before vital molecules are damaged. Free radical scavenging property of antioxidants delays or inhibits cellular damage (Halliwell, 1995). Antioxidants may protect the body against ROS toxicity either by averting the formation of ROS by bringing disruption in ROS attack, by converting them to less reactive molecules or by scavenging the reactive metabolites (Hegde and Joshi, 2009). Synthetic antioxidant like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propylgallate (PG) and tertiary butyl-hydroquinone (TBHQ) are known to ameliorate oxidative damages but they have been restricted due to their carcinogenic and harmful effect on the lungs and liver (Gokhan Zengin et al., 2011). Therefore, researchers have focused on natural antioxidants especially plant phytochemicals. and the microorganisms that produce them. Recent research studies have shown endophytic fungi derived secondary metabolites have a high antioxidant activity against foreign particles (Kaul et al., 2013).

Endophytic fungi, by definition, are the fungi which spend the whole or part of their lifecycle colonizing inter-and/or intracellularly inside healthy tissues of the host plants, typically without causing apparent symptoms of disease (Azevedo, 2007). They have the ability to produce a range of secondary metabolites, providing researchers with numerous leads for compounds of pharmaceutical significance and possible development as new drugs (Strobel, 2003). Endophytes provide a broad variety of bioactive secondary metabolites belong to diverse structural groups including alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, xanthones, and others (Tan and Zou 2001). Hence, endophytic fungi provides an enormous opportunity to explore the relatively untapped source of information regarding biological activity.

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Medicinal plants are known to harbour endophytic fungi that are believed to be associated with the production of pharmaceutical products (Zhang et al., 2006).Therefore, it is important to explore the endophytic mycoflora in the medicinal plants. Hence *Lannea coromandelica*, an important medicinal plant was investigated for their endophytic community and their biological activity. *Lannea coromandelica* which is commonly known as "The Indian Ash Tree" is a deciduous tree which grows up to 14 metres high. It belongs to the family Anacardiaceae. The bark of the tree and leaves are used as traditional medicine to cure sprains, bruises, skin eruptions, heart diseases, dysentery, mouth sores., toothache and diabetes (Farhana Islam *et al*., 2012) In the present study, in vitro antioxidant potential of ethyl acetate crude extracts of endophytic fungi isolated from *Lannea coromandelica* were investigated.

MATERIALS AND METHODS

Isolation and identification of the fungus

The healthy plant were washed in running tap water The leaf segments were cut into 2 mm^2 segments and were surface sterilized by sequentially plunging into 70% ethanol for 5 seconds, followed by 4% sodium hypochlorite for 90 seconds and then rinsed with sterile water for 10 seconds. The excess moisture was blotted in a sterile filter paper.In each petri dish, 4 --- 5 segments were placed on Potato Dextrose Agar (PDA) supplemented with antibiotics streptomycin (100 µg/mL concentration), (Sigma, St. Louis, MO, USA) to suppress the growth of bacteria After inoculation the petri dishes were sealed with parafilm and incubated at 27°C ± 10°C for 7 days.Another segment of the same origin without surface sterilization was cultured as a negative control to check the presence of contaminated microbes on the segment surface. The plates were examined for fungal growth, the fungus grown out from explant were sub cultured in PDA plates. Later the purified endophytic fungi were transferred to PDA slants separately and were kept at 4°C after being cultured at 28°C for 7 days.

The endophytic fungi grew out from the leaf and bark were identified on the basis of cultural characteristics like texture, color, surface, elevation reverse side and margin, and the morphology of fruiting bodies and spores, using standard monographs **(**Rajagopal, 2004**)**

Fermentation, extraction, and antioxidant activity test

The purified fungi (a small park) were transferred into 500 ml Erlenmeyer flasks containing 250 ml of potato dextrose broth (PDB) and incubated at 28ºC for 15 days on shaker at 150rpm. The broth cultures were filtered to separate the mycelia from broth. All cell free culture filtrates were extracted three times with equal volume of ethyl acetate (EtOAc) in a separating funnel. The EtOAc phase was collected and the extract was concentrated by evaporation of solvent in vacuum, the resulting residue was subjected to antioxidant activity test.

1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity

DPPH radical is a relatively stable free radical and has been widely used to evaluate the antioxidant activities of various biological samples. This method is based on the reduction of the stable 2, 2- diphenyl- 1-picryl-hydrazyl radical (DPPH) in the presence of a radical scavenger or hydrogen donors due to the formation of non radical form of DPPH-H. The DPPH radical contains an odd electron, which is accountable for the absorbance at 517 nm and also for a visible deep purple color. DPPH is decolorized when it accepts an electron donated by an antioxidant compound, which can be quantitatively measured from the changes in absorbance (Jao and Ko, 2002). Various concentrations of the ethyl acetate extracts of endophytic fungi (50-250 µg/ml, 2.5 ml) were mixed with methanolic solution containing DPPH radicals (0.1 mM, 0.5 ml). The mixture was shaken vigorously and left to stand in dark for 30 min. The reduction in the DPPH radical concentration was determined by measuring the absorbance at 517 nm Methanol was taken as blank, and DPPH solution without the extracts was taken as control (Dandamudi and Rao, 2011) and ascorbic acid was used as standard. The percentage of DPPH scavenged was calculated using the equation:

$$\% \text{ Scavenged} = [(Ac-As)/Ac] \times 100,$$

where Ac is the absorbance of control, and As is the absorbance of solution containing sample extracts.

RESULT AND DISCUSSION

In present study, three dominant fungi were isolated from *lannea coromendalica* which were identified as *Aspergillus niger, Aspergillus flavus* and *Alternaria alternata*. Ethyl acetate crude fungal extracts of these endophytic fungi were investigated for antioxidant potential by using DPPH radical scavenging activity method. Ethyl acetate extraction is most efficient method of isolating fungal secondary metabolites. Ethyl acetate as an extraction solvent selectively extracts low molecular weight phenolic compounds and high molecular weight polyphenols (Scholz and Rimpler 1989) All the three extracts showed antioxidant activity up to varying extent, ranging from 47% to 70%. (Table-1) *Aspergillus flavus* showed a high antioxidant capacity value of 69.8%.Not much difference was seen between *Aspergillus flavus* and *A. niger* strain which were having 69.8% and 68.8% of reducing potential respectively. *Alternaria alternata* showed slightly lesser antioxidant activity with 52.1%. Ascorbic acid was taken as standard showing 98.7% antioxidant activity. Percentage of DPPH radical scavenging activity of endophytic fungi and ascorbic acid is shown in Figure 1. Even though the DPPH scavenging capacity of the extracts was found to be

Table 1: Antioxidant activity of ethyl acetate extract of endophtytic fungi

Fungal extract/ Conc. (µg\ml)	50	100	150	200	250
Aspergillus flavus	54.6	59.5	62	69.3	69.8
Aspergillus Niger	54	59.5	65.5	68.6	68.8
Alternaria alternata	47.2	47.7	48.7	50.5	52.1
Ascorbic acid	80.3	90.2	92.8	95.9	98.7

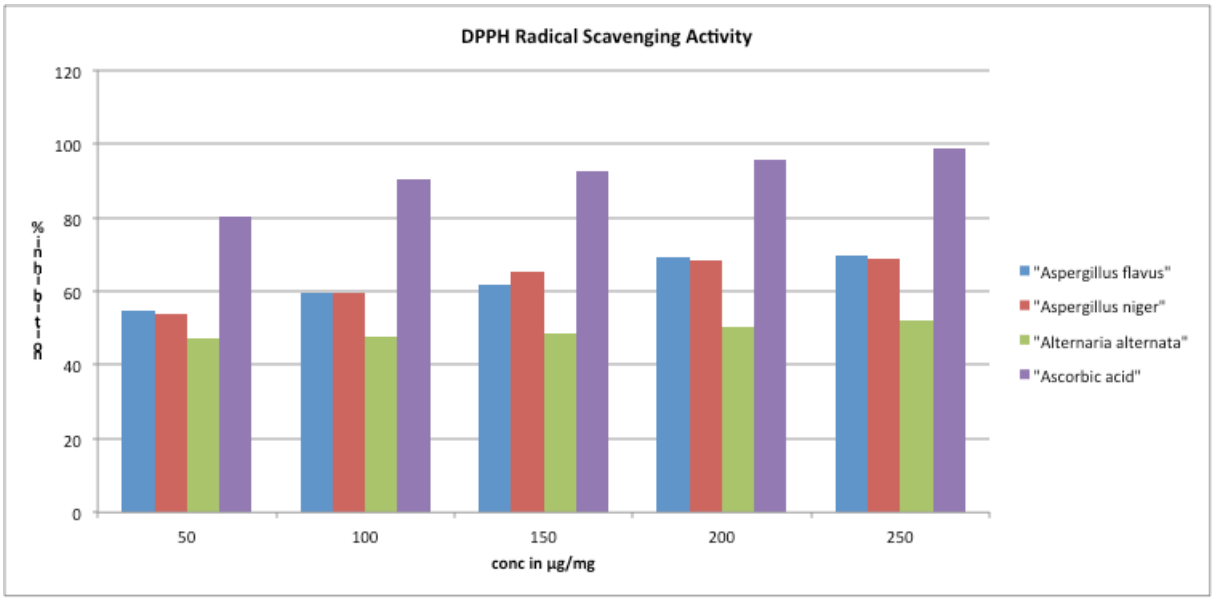

Figure 1: Effects of fungal extracts on scavenging DPPH radicals

lower than that of the activity of commercial antioxidant, ascorbic acid at same concentrations, it still showed significant inhibition at 250µg/ml concentration. This study shows that the extract have proton donating ability and could serve as free radical scavenging, acting as primary antioxidant. This study coincides with the earlier work done by (Rukachaisirikul, 2010).

A list of endophytic fungi isolated from a number of medicinal plants have been claimed to possess antioxidant potential. Similar to our study ethyl acetate extract of *Aspergillus terreus* isolated from *Ocimum sanctum* exhibited 34.83% antioxidant activity with (14.96 ± 0.07) mg/g GAE phenolic content (Sharma and, Vijaya Kumar, 2013) In another study by.Sadananda et al., 2011; have shown endophytes *Aspergillus niger* and *Penicillium* sp. of *Tabebuia* sp. to possess biological properties related to antioxidant mechanisms. Fernandes et al*.,* 2009; have found the endophytic fungus *A. alternata* to possess antioxidant activity. Ravindran et al., 2012; reported *A. flavus* a dominant endophyte from mangrove species as having antioxidant potential. Khennouf *et al.,* 2010; have reported that endophytic fungi *Phoma*, *Cladosporium,* and *Chaetomium* have antioxidant activity.

Similarly Sadrati et al., 2013; have reported antioxidant activity of *Chaetomium sp*. isolated from wheat as 38% by using the β-carotene/ linoleic acid system oxidation. Yet in another study *Chaetomium sp*. isolated from *Nerium oleander* also possessed the highest antioxidant capacity and phenolic content (13.95 ± 0.11) mg/GA ([Huang etal., 2007). Likewise *Cephalosporium* sp., an endophytic fungus isolated from the root of *Trachelospermum jasminoides* (Apocynaceae) produced a phenolic compound (graphislactone A) with strong free radical scavenging and antioxidant activity (Song et al., 2005). Harper et al., 2003; reported two antioxidants, pestacin and isopestacin produced *by Pestalotiopsis microspora*, an endophytic fungus residing in *Terminalia morobensis* in Papua New Guinea. Another antioxidant compound phenyl propanoid amide has been isolated from endophytic fungus *Penicillium brasilianum* that reside in *Melia azedarach* (Fill et al., 2010).

Studies have shown that extracts having high phenolic content show good antioxidant activity. The free radical scavenging ability of phenols is attributed to the occurrence of hydroxyl groups. (Sultana et al., 2007) The mechanisms of antioxidant activity of polyphenols is characterized by direct scavenging or quenching of oxygen free radicals and inhibition of oxidative enzymes that generate reactive oxygen species (Terao, 2009).

In our study ethyl acetate solvent is used to extract the secondary metabolites of endophytic fungi and since ethyl acetate solvent selectively extracts low molecular weight phenolic compounds and polyphenols, therefore, antioxidant activity of the endophytes may

be due to the presence of phenolic compounds in the extracts. The results of this study represent that endophytic fungi may serve as a potential source of natural antioxidants.

CONCLUSION

The present study reports the antioxidant properties of endophytic extracts, isolated from *Lannea coromenadilica.* Further studies are needed to isolate and characterize the exact compound which is responsible for antioxidant activity and elucidate their antioxidant mechanisms. This scientific information would serve as an important platform to facilitate a better understanding towards the development of natural antioxidant agent as safe and effective natural medicine against various diseases.

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