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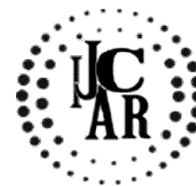

Research Article

ANTIOXIDANT ACTIVITY OF FUNGAL ENDOPHYTE *ASPERGILLUS SYDOWII* ISOLATED FROM *ANANUS COMOSUS* L.

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A B S T R A C T

The study documented a comparative evaluation of antioxidant potential of crude extract of fungal endophyte in different growth media and extraction solvents through free radical scavenging assay. Endophytic fungal isolate of *Aspergillus sydowii* was obtained from the leaf of *Ananus comosus* L. Three different fungal growth media were used. Two different solvents were used for extraction of secondary metabolite from mycelial mat and culture filtrate. Differences in antioxidant activity in respect of growth media and extraction solvents were observed. Ethyl acetate extract of mycelial mat harvested from CYB medium found to have highest antioxidant activity.

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INTRODUCTION

Plants are considered as a complex and dynamic system composed of interacting microbial populations. This type of interactions exists on the surface of plants and in the internal tissues (Andrews and Harris, 2000). Endophytes are microbes that colonize the living internal tissues of plants without causing any immediate overt negative effects (Bacon and White, 2000). Endophytes are regarded as an important source of bioactive natural products. Some of the endophytes are considerd as the chemical synthesizers inside the plants (Owen and Hundley, 2004).

Fungi were considered as the new sources of antioxidants in the form of their secondary metabolites (Rodrigues *et al.*, 2005). A wide range of bioactive secondary metabolites including alkaloids, benzopyranones, flavonoids, phenolic acids, quinones, terpenoids, steroids etc. were characterized from endophytes (Tan and Zou, 2001). This diverse array of secondary metabolites produced by fungi can be exploited for Pharmaceuticals activities including antioxidant activity (Fox and Howlett, 2008). The detection and isolation of bioactive compounds are usually done using single culture condition for assessing bioactive potential of fungi (Ramos and Said, 2011). But microorganisms synthesize various secondary metabolites which depend on their environment and the available nutritional resources (Bode *et al.*, 2002).

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Different organic solvents are used for extracting secondary metabolites from endophytic fungi (Rebbapragada and Kalyanaraman, 2016). *Ananas comosus* leaves extract reported to have significant antidiabetic and antioxidant activity in STZ induced diabetic rats (Subramanian *et al.*, 2014). As Endophytic fungi have the ability to produce similar bioactive secondary metabolites of their respective hosts (Aly *et al.*, 2010; Kusari *et al.*, 2012). Therefore, the screening of antioxidant activity of endophytic fungal isolate isolated from the host plant has the relevance.

The specific goal of this study was to evaluate the antioxidant activity of endophytic fungal metabolites by using different culture medium.

MATERIALS AND METHODS

Isolation of endophytic fungi

Explants were selected from leaf of healthy and disease free *Ananas comosus* L. plant from Tripura. Isolation of fungal endophytes was done according to the standard protocol (Schulz *et al.*, 1998; Strobel and Daisy, 2003) with slight modification. Four to five segments of plant tissues were placed on Potato Dextrose Agar (PDA) plate supplemented with streptomycin (100 μg/ml), and incubated in a BOD incubator for 21 days at 25± 2°C. In order to ensure proper surface sterilization, the sterilization protocol was validated using leaf imprint method (Schulz *et al.*, 1998). Hyphal tips from fungus growing out from the samples were subsequently transferred onto fresh PDA plates to isolate pure colony.

Identification of endophyte

The microscopic identification of the isolates was carried out by lacto phenol staining technique. On the basis of macroscopic and microscopic characteristics, the fungus was identified with the help of standard manuals (Raper and Fennell, 1965). Identification was authenticated by Molecular identification (ITS sequence of rDNA) by Agharkar Research Institute (NFCCI, Pune). The rDNA sequence of fungal strain was submitted to NCBI Gene Bank database and the accession number (KP233747) was obtained.

Cultivation and extraction of fungal metabolites

Three different culture media, i.e. Potato Dextrose Broth (PDB), Malt Extract Broth (MEB) and Czapek dox Yeast Extract Broth (CYB) were used to select the best suitable growth medium. Selected endophytic fungal isolates were further inoculated into 250 ml Erlenmeyer flasks containing 100 ml of each PDB, MEB and CYB medium separately and incubated at 25±2ºC for 15 days under stationary conditions. After completion of incubation, the broth cultures from each type of medium were filtered to separate the mycelia and filtrate. For solvent optimization two different solvents i.e. ethyl acetate and methanol were used. Separated mycelia were dried at 40°C. Dried mycelium was pulverized and extracted with two types of solvents. Extraction was repeated for three times. The separated culture broths were also extracted with ethyl acetate and methanol. Equal volume of the cell free culture filtrate and solvent was taken in a separating funnel and shaken vigorously for 10 min and kept for 5 min till the two clear immiscible layers formed. The upper layer of solvent containing the extracted compounds was separated. The samples were extracted separately with two solvents. Fractions collected after extractions were composited. All the Ethyl acetate and Methanolic extract were concentrated separately under reduced pressure conditions in Rotary evaporator (Rotavap: PBV-7D) to yield the final extract. The bioactive compound extracted from *Aspergillus sydowii* was assessed by measuring the activity of crude extract against DPPH. All the extracts obtained from tested fungal species were stored in dark at 4 °C before being used for the in vitro DPPH radical scavenging assay.

Antioxidant assay: DPPH radical scavenging activity

Radical scavenging activities of fungal extracts were measured by slightly modified method of Miliauskas *et al.*, (2004). Methanolic stock solutions of extracts were prepared and various concentrations of extracts were obtained by serial dilution. 0.5ml of methanolic solution of DPPH (1mM) was added to the extract solutions. The reaction mixture was shaken and allowed to stand at room temperature for 30 minutes. Absorbance was read at 570 nm using methanol as blank reference in spectrophotometer (Eppendorf AG 22331Hamburg). Ascorbic acid was used as control.

DPPH scavenging activity (%) of the standard and extracts were determined by the formula: Percentage of inhibition: [(A Blank – A Sample) / A Blank] ×100, Where, A Blank and A Sample denotes the absorbance of control and test compound respectively. EC_{50} value (mg/ml) is the effective concentration at which DPPH radicals were scavenged by 50% and the value was obtained by interpolation from linear regression analysis.

Statistical analysis

All assays were performed in triplicate in three independent and separate experiments. The data were presented as means ± standard deviations (SD) from three independent analyzes.

RESULTS AND DISCUSSION

DPPH was used to study the radical scavenging effects of extract. As antioxidant donate proton to the DPPH, the absorption of reaction mixture was decreased. The sample was tested against this radical at different concentrations. The scavenging effects of the sample were compared with the standard Ascorbic acid (Fig. 1).

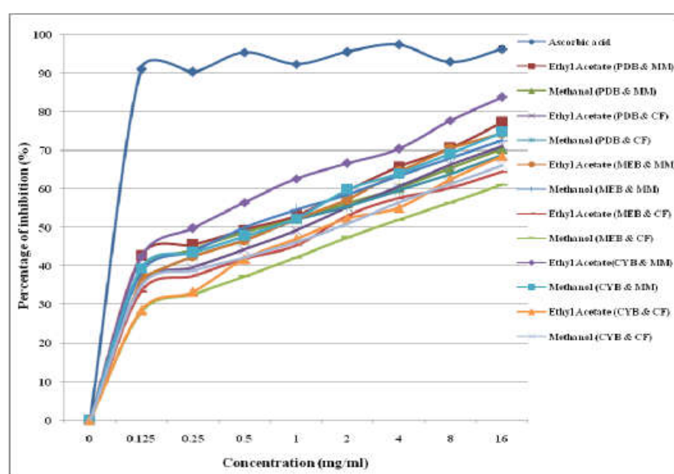

Fig 1 Concentration dependent DPPH radical scavenging activity exhibited by different test samples.

Lowest EC_{50} value was recorded in case of Ethyl acetate extract of mycelial mat harvested from CYB medium which correlated with the findings where Czapek dox's medium was considered most effective for metabolite production responsible for antioxidant activity (Arora and Chandra, 2010). In case of methanolic extract of mycelial mat, lowest EC_{50} was obtained for mycelial mat grown in MEB medium. For Ethyl acetate and Methanolic extracts of culture filtrate obtained from PDB medium exhibited lower EC_{50} value in comparison to other experimental sets. However, Methanolic extract of culture filtrate collected from PDB medium showed lowest EC_{50} value (Table 1).

Table 1 EC_{50} values (mg/ml) of tested endophytic fungi and control.

Fungal isolate	Growth media	Solvent used				
		Ethyl acetate (MM)	Methanol (MM)	Ethyl acetate (CF)	Methanol (CF)	Ascorbic acid (Control)
Aspergillus sydowii	PDB	0.592	0.722	1.19	0.764	
	MEB	0.851	0.506	1.66	3.077	0.075
	CYB	0.259	0.72	1.57	1.877	

The antioxidant activity recorded for methanolic extracts might be due to presence of flavonoids as secondary metabolite. Phytochemical screening of methanolic extracts of *Aspergillus* sp. isolated from *Azadirachta indica* contained flavonoids as well as exhibited high free radical scavenging activity (Preethi *et al.*, 2015) which supported present findings. Much lesser EC_{50} value was recorded for standard ascorbic acid than the other test samples (Table 1). This might be due to that, the standard antioxidant was used in purified form and the crude extracts were the mixture of various compounds where some compound exerted inhibitory effect (Yadav *et al.*, 2012).

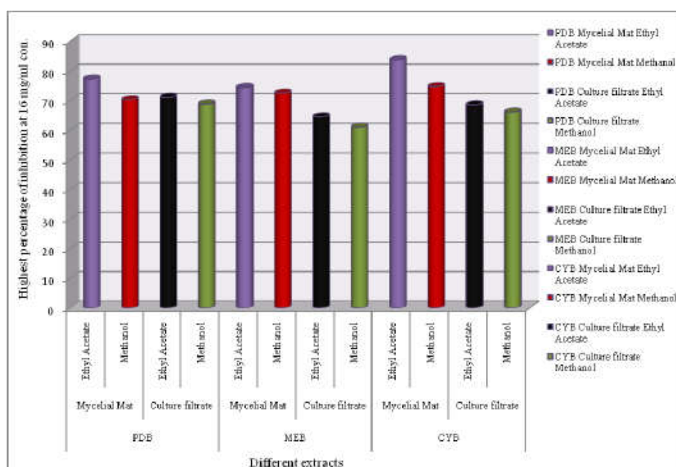

Fig. 2 Maximum inhibition percentage recorded for different test samples.

Maximum percentage of inhibition against DPPH radical was observed at maximum concentration of 16mg/ml of both extracts of mycelial mat and culture filtrate of fungal strain. Maximum percentage of inhibition was recorded in case of ethyl acetate extracts of mycelial mat obtained from CYB medium (83.75±0.311). For methanolic extracts mycelial mat grown in CYB medium also exhibited higher inhibition percentage (74.717±0.123). For extracts produced from culture filtrate, maximum percentage of inhibition (71.1±0.071) was recorded for ethyl acetate extracts obtained from PDB media (Fig. 2).

Earlier study revealed that the, supernatant and mycelial extracts from the static culture of the fungus FS3 (*Aspergillus sydowii* strain W4-2) isolated from *Agelas* sp, exhibited the free radical scavenging activity against DPPH radical. In GC/MS analysis 38 compounds were identified, of them 22 compounds from supernatant static and 23 compounds from mycelia of the fungus which include flavone, isoflavone, coumeric acid, sterol etc. (Abd El-Hady *et al.*, 2014). Media conditions had different effects on the production of fungal secondary metabolites (Miao *et al.*, 2006). The variations of culture conditions optimized the yields of a specific compound i.e. the active metabolite in a medicinal fungus (Xu *et al.*, 2006). Productions of antioxidant compounds like phenols and flavonoids depended upon media components and their conditions (Rebbapragada and Kalyanaraman, 2016). Variations in physiochemical and nutritional parameters had an effect on secondary metabolite production (Thakur *et al.*, 2009). The media dependent differences in EC_{50} value of culture filtrate and mycelial mat recorded in present study correlated with these earlier findings. The differences in EC_{50} value obtained for different sample sets using different solvents supported the fact that the bioactive components may also differ in their solubility depending on the extractive solvents used (Arora and Chandra, 2010). As ethyl acetate solvent selectively extracts low molecular weight phenolic compounds and polyphenols, therefore, antioxidant activity exhibited by ethyl acetate extracts of the tested fungus may be for presence of phenolic compounds in the extracts (Premjanu and Jaynthy, 2014).

CONCLUSION

The present study emphasizes the effect of different growth media and extraction solvent in the production of secondary metabolites having antioxidant properties by *Aspergillus sydowii*. The growth medium plays a role in the secondary metabolite production. The different type of extraction solvents showed difference in inhibition percentage. Therefore proper selection of media and solvent may lead to enhancement of antioxidant metabolite production and their activity. However, isolation and characterization and structural elucidation of metabolites produced by the fungi will pave the way for further progress to exploit the potency of antioxidant compounds.

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Conflict of Interest Statement

The authors declare that they have no competing interests.
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