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EVALUATION OF ANTIOXIDANT ACTIVITY OF TWO ENDOPHYTIC FUNGI ISOLATED FROM *ANANUS COMOSUS* L.

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ABSTRACT

Two endophytic fungi isolated from Ananus comosus L. were evaluated for the in vitro antioxidant potential. Ethyl acetate extracts and methanolic extracts of mycelial mat of two endophytic fungi, Daldinia eschscholtzii and Trichoderma asperellum were examined. Assay of antioxidant activities was done using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) . Among the two lowest EC_{50} value was obtained in case of the ethyl acetate extract of the fungal endophyte Trichoderma asperellum. The lowest EC_{50} value of free radical scavenging activity indicates better ability of the mycelial mat extract to act as DPPH radical scavenger.

KEY WORDS: In vitro, Mycelial Mat, Daldinia Eschscholtzii, Trichoderma Asperellum, DPPH and EC_{50}.

INTRODUCTION

Fungal endophytes are microorganisms colonize living internal tissues of plants without causing any immediate and apparent symptoms or diseases in the host (Owen and Hundley, 2004). Endophytes produce natural products similar to the host plant (Nagda *et al.*, 2017).They can be regarded as important sources of bioactive compounds, presumably due to the symbiotic relationship with their hosts. Metabolic interactions between fungus and plant may facilitate the synthesis of secondary metabolites with novel properties (Schulz *et al.*, 2002). Free radicals occur in the body due to an imbalance between Reactive Oxygen Species and antioxidants. (Bharathidasan and Panneerselvam, 2012) Antioxidants serve as the defensive factor against free radicals in the body (Arora and Chandra, 2010). Endophytic fungi possess antioxidant components like flavonoids, carotenoids, phenolics, tannin and ascorbic acids that were reported to exhibit free radicals scavenging activity (Kumaresan *et al.*, 2015). The natural antioxidants are very effective for preventing the destructive processes due to oxidative stress (Shah, 2015). Use of synthetic antioxidants to prevent free radical damage may involve toxic side effects thus the search for natural antioxidants and free radical scavengers is required. (Radulovic *et al.*, 2007).

Ananas comosus L. (Bromeliaceae) is an herbaceous perennial plant. This plant is also known for its folk medicinal utility in Thailand, China and parts of north-east India. Leaf extract of the plant reported to have good free radical inhibitor or scavenger activity (Kataki, 2010).

MATERIALS AND METHODS

Sampling

Collection of explants was carried out during January 2014 to April 2016 from different districts of Tripura. Plants were identified on the basis of external morphological characters. Leaves and roots from healthy and disease free *Ananas comosus* L. were collected from experimental sites in sterile paper bags and processed within 24 hours of collection.

Isolation of endophytes

Isolation of fungal endophytes was done according to the standard protocol (Schulz *et al*, 1998; Strobel and Daisy, 2003) with slight modification as per requirement of root/leaf tissue. The exposed time length with sterilant could be decided based on tissues strength. Tissues were finally washed in double distilled sterile water to remove chemical and excess moisture and then blotted on sterilized tissue paper. Samples were cut in the dimensions of 0.5 cm ×0.5 cm. 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). The plated segments were monitored on every alternate day. Hyphal tips from fungi growing out from the samples were subsequently transferred onto fresh PDA plates to isolate pure colonies.

Identification of endophytes

The microscopic identification of the isolates was carried out by lacto phenol staining technique. On the basis of macroscopic and microscopic characteristics, the fungi were identified with the help of standard manuals (Ellis 1971; Domsch *et al*. 1980; Watanabe 2002). Identification was authenticated by Molecular identification (ITS sequence of rDNA) by Agharkar Research Institute (NFCCI, Pune, India). The rDNA sequences of two fungi were submitted to NCBI Gene Bank database for accession number.

Cultivation and extraction of fungal metabolites

Selected endophytic fungal isolates were further inoculated into 250 ml Erlenmeyer flasks containing 100 ml Potato Dextrose Broth and incubated at 21ºC for 21 days under stationary conditions. After the completion of incubation, the broth culture was filtered to separate the mycelia and filtrate. Separated mycelia were dried at 40°C. Dried mycelium was pulverized and extracted with ethyl acetate and methanol (1:1, v/v) three times. Fractions were pooled and ethyl acetate and methanolic extract was concentrated separately under reduced pressure conditions in Rotary evaporator (Rotavap: PBV-7D) to yield the final extract. Extracts of all tested fungal species were stored in dark at 4 °C before being used for the *in vitro* DPPH radical scavenging assay.

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

Free radical scavenging (FRS) 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: $[(A_{Blank} – A_{Sample}) / A_{Blank}] \times 100$, 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.

RESULTS AND DISCUSSION

The two endophytes selected for evaluation of antioxidant activities using two different solvent (ethyl acetate and methanol) for extraction were *Daldinia eschscholtzii* which isolated as leaf endophyte and *Trichoderma asperellum* as root endophyte (Fig. 1).

Fig.1: Submerged cultures and separated mycelia mats (a, b- *Daldinia eschscholtzii,* c,d- *Trichoderma asperellum*).

DPPH, a stable free radical was used to study the radical scavenging effects of extract. As antioxidant donate proton to this radical the absorption of reaction mixture decreased. The sample was tested against this radical at different concentrations (Fig. 2). The scavenging effects of the sample were evaluated along with the standard Ascorbic acid.

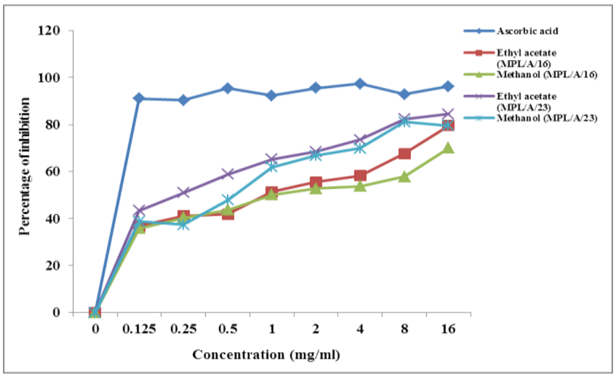

Fig. 2: Free radical scavenging (FRS) activity of ethyl acetate and methanolic extract of mycelial mat of

Daldinia eschscholtzii and Trichoderma asperellum on DPPH at various concentrations.

The fungal extracts against DPPH radical showed a maximum percentage of inhibition at maximum concentration of 16mg/ml. Maximum percentage of inhibition were 84.37 in Ethyl acetate extract of *T. asperellum*, followed by methanolic extract of *T. asperellum* (79.45), but for *D. eschscholtzii* these were 79.56 and 70.01respectively. However, for standard Ascorbic acid it was found to be 96.23 (Fig. 1). Experimental results indicate that for both the endophytic fungal strain ethyl acetate extract of mycelial have more potent scavenging activity.

Table. 1: EC $_{50}$ value (mg/ml) of tested endophytic fungi.

Sl. No.	Name of the fungi with Gene Bank Accession Number	Solvent used	
		Ethyl acetate	Methanol
1.	*Daldinia eschscholtzii*, KU258051	0.983	1
2.	*Trichoderma asperellum*, KU687321	0 .235	0.609

In comparison to *D. eschscholtzii* both methanolic and ethyl acetate extract of *T. asperellum* mycelial mat had lower EC_{50} value. However, the lowest EC_{50} of FRS activity obtained in case of ethyl acetate extract of *T. asperellum* (Table 1). Synthetic antioxidant ascorbic acid, which was used as a standard, had superior EC_{50} (0.072 mg/ml) value in comparison to all the four tested samples.

Antioxidant property of natural products derived from plants is attributed to the secondary metabolites, particularly phenolics and flavonoids. Earlier studies on qualitative evaluation of methanolic extract of endophytic fungi reported flavanoid in the extract (Nagda *et al.,* 2017). (Lopes *et al*., 1999). Various fungal species have been also reported to produce compounds which possess the antioxidant potential as well (Song and Yen, 2002). This secondary metabolite has been reported in different endophytic fungi such as *Aspergillus nidulans* and *A. oryzae* (Qiu *et al.,* 2010) and *A. niger* and *Fusarium oxysporum* (Gobindappa *et al.*, 2011).

Among different extraction solvent ethyl acetate reported to be more effective solvent to elute the components responsible for antioxidant potential rather than Choloroform and Butanol (Arora and Chandra, 2010). The free radical scavenging activity of mycelial mat extracted using ethyl acetate and methanol may be due to presence of different components with different polarities in mycelial mat. The solubility of bioactive components may differ depending on extraction solvent used (Arora and Chandra, 2010) which may attributed to differences in maximum inhibition percentage and EC_{50} of four test samples. Differences in antioxidant activity of ethyl acetate and methanolic extract of two endophytic fungi may be due to extraction of different types of compounds using different solvents. Experimental results may supports the earlier findings where Ethyl acetate crude extract of the endophytic fungal isolate of *Alternaria* isolated from *Tridax procumbens* contain alkaloids, flavonoids, cardiac glycosides, terpenoids and steroids whereas methanolic extract contains flavonoids and cardiac glycosides (Kumar *et al.*, 2015). However, detailed isolation and characterization of compounds may provide understanding of differences in antioxidant activities in test samples.

CONCLUSION

It is concluded from the present investigation that differential free radical scavenging activity exhibited by mycelial mat extracts of *Daldinia eschscholtzii* and *Trichoderma asperellum*. Hence these two endophytic fungi could be a source of natural antioxidants. Ethyl acetate extracts was found to be more potent free radical scavenger. Therefore, different solvents can be used for evaluating pharmacological potential of endophytes through extraction of secondary metabolites. Optimization of endophytic fungal culture condition and extraction of fungal metabolite may be used for identification and isolation of novel and unknown compounds. However, analytical chemistry procedures can be used for isolation and characterization of the bioactive compounds responsible for the antioxidant activity of the endophytic fungal strain.

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REFERENCES

1. Arora, D.S. and Chandra, P. Assay of Antioxidant Potential of Two Aspergillus Isolates by Different Methods under Various Physio-Chemical Conditions. Braz J Microbiol, 2010; 41: 765-777.
2. Bharathidasan, R. and Panneerselvam, A. Antioxidant Activity of the Endophytic Fungi Isolated from Mangrove Environment of Karankadu, Ramanathapuram District. Int J Pharm Sci Res., 2012; 3(8): 2866-2869.
3. Domsch, K.H., Gams, W. and Anderson, T.H. (1980). Compendium of soil fungi, Vol.1., Academic Press (London) Ltd.
4. Ellis, M. B. (1971). Dematiaceous hypomycetes, CAB International.
5. Govindappa, M., Bharath, N., Shruthi, H.B. and Santoyo, G. In vitro antioxidant activity and phytochemical screening of endophytic extracts of

Crotalaria pallida. Free Radic Antioxid, 2011; 1(3): 79-86.
6. Kataki, M.S. Antibacterial activity, in vitro antioxidant activity and Anthelmintic activity of ethanolic extract of Ananus comosus L. tender leaves. Pharmacologyonline, 2010; 2: 308-319.
7. Kumar, A., Jha, P.K., Kumar, R., Kumar, K. and Sedolkar, V. Antibacterial activity, phytochemical and enzyme analysis of crude extract of endophytic fungus, Alternaria Sp. isolated from an ethanobotanical Medicinal Plant Tridax procumbens. Int J Pharm Pharm Res., 2015; 7(6): 1111-1115.
8. Kumaresan, S., Karthi, V., Senthilkumar, V., Balakumar, B.S. and Stephen, A. Biochemical constituents and antioxidant potential of endophytic fungi isolated from the leaves of Azadirachta indica A. Juss (Neem) from Chennai, India. Journal of Academia and Industrial Research, 2015; 3(8): 355-361.
9. Lopes, G.K., Schulman, H.M. and Hermes-Lima, M. Polyphenol tannic acid inhibits hydroxyl radical formation from Fenton reaction by complexing ferrous ions. BBA Gen Subj, 1999; 1472(1-2): 142-52.
10. Miliauskas, G., Venskutonis, P.R. and Van Beek, T.A. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem, 2004; 85: 231-237.
11. Nagda, V., Gajbhiye, A., Kumar, D., Isolation and characterization of endophytic fungi from Calotropis procera for their antioxidant activity. Asian J Pharm Clin Res., 2017; 10(3): 254-258.
12. Owen, N. and Hundley, N. Endophytes-the chemical synthesizers inside plants. Sci Prog, 2004; 87: 79-99.
13. Qiu, M., Xie, R.S., Shi. Y., Zhang, H., Chen, H.M. Isolation and identification of two flavonoid-producing endophytic fungi from Ginkgo biloba L. Ann Microbiol, 2010; 60(1): 143-50.
14. Radulovic, N., Stankov-Jovanovic, V., Stojanovic, G., Smelcerovic, A., Spiteller, M. and Asakawa, Y. Screening of Invitro antimicrobial and antioxidant activity of Nine Hypericum species from the Balkans. Food Chem, 2007; 103: 15-21.
15. Schulz, B., Guske, S., Damman, U. and Boyle, C. Endophyte host interaction II: Defining symbiosis of endophyte host innteraction. Symbiosis, 1998; 25: 212-227.
16. Schulz, B., Boyle, C., Draeger, S., Rommert, A.K. and Krohn, K. Endophytic fungi:a source of novel biologically active secondary metabolites. Mycol Res., 2002; 106: 996–1004.
17. Shah, R.K. Antioxidant activity and estimation of total phenols and flavonoids in extracts of Smilax ovalifolia leaves. Int J Pure App Biosci, 2015; 3(3): 174-177.
18. Song, T.Y. and Yen, G.C. Antioxidant properties of Antrodia camphorata in submerged culture. J Agric Food Chem, 2002; 50(11): 3322-7.
19. Strobel, G.A. and Daisy, B. Bioprospecting for Microbial Endophytes and their Natural Products. Microbiol Mol Biol Rev, 2003; 67(4): 491-502.
20. Watanabe, T. (2002). Pictorial Atlas of soil and seed fungi: Morphololologies of cultured fungi and key to species. 2nd ed. CRC Press, Florida, US.