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ANTIOXIDANT ACTIVITY OF THE ENDOPHYTIC FUNGI ISOLATED FROM MANGROVE ENVIRONMENT OF KARANKADU, RAMANATHAPURAM DISTRICT

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ABSTRACT

Antioxidant property of *Phomopsis amygdali* culture filtrate extracted with ethanol, was evaluated in vitro. ABTS and DPPH radicals were used to evaluate their antioxidant activity. Antioxidant components like total phenol and flavonoid were also determined. The ethanolic extract of *Phomopsis amygdali* showed potent antioxidant activity against both ABTS and DPPH radicals with the EC50 value of 580.02 ± 0.511µg/ml and 2030.25±501 µg/ml respectively. Total amount of phenol and flavonoid quantified were of 18.33 ± 0.68 gallic acid equivalents per gram and 6.44 ± 1.24 µg/mg of quercetin equivalent respectively. In conclusion, the culture filtrate of *Phomopsis amygdali* may have potential source of natural antioxidant.

INTRODUCTION: Reactive oxidant species (ROSs) plays an important role in degenerative condition such as aging cancer, neuron degenerative disorders, atherosclerosis and inflammations [1]. These free radicals occur in the body during an imbalance between ROSs (Reactive Oxygen Species) and antioxidants. Hence, the dietary intake of antioxidant is necessary and important to balance the antioxidant status that would reduce the pathological conditions induced by free radicals.

Plant derived materials have recently become of great interest owing to their multipurpose applications. An enormous variety of plants have been studied for new source of natural antioxidants [2], especially phenolic and flavonoid compounds derived from plants were proved to be potent antioxidant and free radical scavengers [3].

Endophytic fungi are microorganism hidden within healthy host plant were poorly investigated group among other microorganisms, they represent an abundant and dependable source of novel bioactive compounds with huge potential for exploitations in a wide variety of medicinal, agricultural and industrial areas [4]. There are many reports and studies on the biological activities of endophytes like antiviral, anticancer and antimicrobial effects [5 and 6].

Apart from these biological properties, the reports published on antioxidant properties of endophytytic fungi were very few. Hence in the present study *Phomopsis amygdale,* an endophytic fungus isolated from the Mangrove plant, the isolated fungus was cultivated under submerged culture condition was evaluated for their antioxidant activity.

MATERIALS AND METHODS:

Endophytic fungi: The mangrove plants species namely *Avicennia marina, Suaeda monica and Rhizophora mucronata* were collected from mangrove environment of Karankadu, Ramanathapuram District The collected samples were carefully stored in polythene bags and used for further investigation.. The isolated fungus was identified [7, 8, 9 and 10]. The pure culture was maintained in potato dextrose agar.

Cultivation and sampling: The test fungus was grown in 2 litre Erlenmeyer flasks containing 500 ml of PDB medium. The test fungus was inoculated and incubated for 21 days. After incubation the culture filtrate was extracted and filtered through four layers of cheesecloth to remove mycelia. Then the culture filtrate was extracted with three equal volumes of solvent ethanol. The organic phase was collected and the solvent was then removed by evaporation under reduced pressure at 45°C using rotary vacuum evaporator. The dry solid residue was re-dissolved in ethanol and the crude extract was evaluated for their antioxidant property.

Antioxidant Assays:

ABTS Radical Scavenging Activity: The two stock solutions included 7.4 mM ABTS and 2.6 mM potassium persulphate was prepared as described by Arnao, Cano and Asota [11]. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 hr at room temperature in dark. The solution was diluted by mixing with 1 ml ABTS solution prepared using 50ml of methanol, in order to obtain absorbance 1.1 ± 0.02 units at 734 nm. Samples (1.5 ml) were mixed with 2.850 ml of ABTS solution and the mixture was left at room temperature for 2 hr in dark. The absorbance was then measured at 734 nm. The capability to scavenge the ABTS radical was calculated using the following equation:

$$\text{ABTS scavenging effect (\%)} = [(A_0\text{-}A_1 / A0) \times 100]$$

Where, A_0 was the absorbance of the control reaction and A_1 the absorbance in the presence of the sample. The extract concentration providing 50% inhibition (EC50) was calculated was obtained by interpolation from linear regression analysis.

DPPH Radical Scavenging Activity: The free radical scavenging activities of extracts were measured by using 1, 1- diphenyl-2-picryl-hydrazyl (DPPH). Briefly, extract concentration of (0.1–20 mg/ml) in water or ethanol (4 ml) was mixed with 1 ml of methanolic solution containing 1, 1- diphenyl-2-picrylhydrazyl (DPPH, Sigma) radicals of 0.2 mM. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm against a blank [12]. 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. α-tocopherol were used for comparison. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0\text{-}A_1/A_0) \times 100],$$

Where, A_0 was the absorbance of the control reaction and A_1 the absorbance in the presence of the sample. The extract concentration providing 50% inhibition (EC_{50}) was calculated was obtained by interpolation from linear regression analysis.

Determination of Antioxidant Component:

Total Phenol: Total phenolic compounds were determined according to Taga, Miller and Pratt [13] using Folin-Ciocalteu's method. To 5 ml of 0.3% HCl in methanol/deionised water (60:40, v/v), 100 mg of the ethanolic extract was added. From the resulting mixture (100 µl) was added to 2 ml of 2% aqueous sodium carbonate. The mixture was incubated for 2 mins. To that 100 µl of 50% Folin- Ciocalteu's reagent was added and incubated for 30 mins, absorbance was measured at 750 nm against blank. The content of total phenol was calculated on the basis of the calibration curve of gallic acid and the results were expressed as mg of gallic acid equivalents (GAEs) per g of extract.

Flavonoid: Total flavonoid was determined according to Barros *et al.,* [14]. The fungal extract (250µl) was mixed with distilled water (1.25 ml) and $NaNO_2$ solution (5%, 75µl). After 5 mins the $AlCl_3.H_2O$ solution (10%, 150µl) was added. After 6 min, NaOH (1M, 500µl) and distilled water (275µl) were added to the mixture.

The solution was mixed well and the intensity of the pink color was measured at 510 nm against blank. The content of flavonoid was calculated on the basis of the calibration curve of quercetin and the results were expressed as mg of quercetin equivalents per g of extract.

RESULTS AND DISCUSSION

Radical Scavenging Activity against ABTS: ABTS a stable free radical with the characteristic absorption at 734 nm was used to study the radical scavenging effect of extracts. The results demonstrated that the extracts reacted with ABTS at different concentration ranging from 100, 200, 400, 800 and 1600µg/ml respectively and the readings were observed by measuring the reduction of radical cation generated by ABTS. + at 734 nm. The ethanolic extract of *Phomopsis amygdali* showed a maximum decolorization of72.38% at a maximum concentration of 1600 µg/ml with the Ec50 value 580.02±0.57 µg/ml (**Table 1**). The extend reduction of decolorization is directly proportional to the increased concentration of the extract illustrated in **Figure 1**.

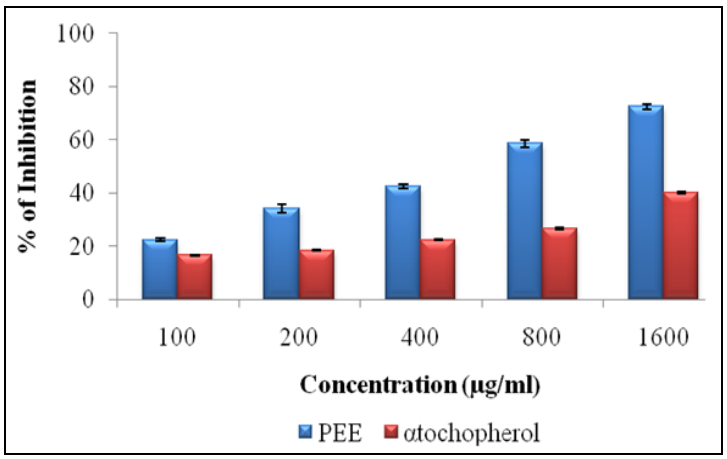

FIGURE 1: FREE RADICAL SCAVENGING EFFECT OF PHOMOSIS SP ETHANOLIC EXTRACT (PEE) AGAINST ABTS

ABTS Assay is an excellent tool for determining the antioxidant activity of phytochemical products [15]. The antioxidant properties of ethanolic extract from edible basidiomycetes assayed against this ABTS radical, reported to have scavenging ability against these radicals [16 and 17].

Radical Scavenging Activity using DPPH: DPPH, a stable free radical with the characteristic absorption at 570 nm, was used to study the radical scavenging effects of extract. As antioxidant donate proton to this radical the absorption decreases. The sample was tested against this radical at different concentrations ranging from (100 to 6400µg) and the readings were observed by decreasing the absorbance taken as a measure indicates the extent of radical scavenging property.

The scavenging effects of the sample were evaluated along with the standard α-Tocopherol. The fungal extracts against DPPH radical showed a maximum decolorization of 2.17% at the maximum concentration of 6400 µg/ml, the EC_{50} value against DPPH radicals found to be 2030.25±0.81µg/ml (**Figure 2**, Table 1). The performance of ethanolic extracts of *Phomopsis amygdali* was higher than the standard α-tocopherol which is an agreement with the previous study made by *Duan et al*., [18].

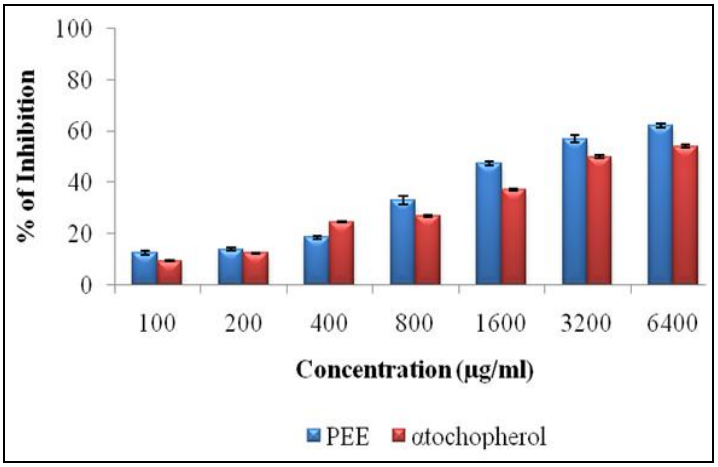

FIGURE 2: FREE RADICAL SCAVENGING EFFECT OF *PHOMOSIS SP*. ETHANOLIC EXTRACT (PEE) AGAINST DPPH RADICALS

TABLE 1: BIOACTIVE COMPOUNDS OBTAINED FROM THE *PHOMOSIS SP*. CULTURE FILTRATE EXTRACTED WITH ETHANOL

Sample	ABTS	DPPH	Phenol (mg/g)	Flavonoid (mg/g)
Phomosis sp.	580.02±0.57	2030.25± 0.81	18.33	6.44

Determination of Antioxidant Compounds: Phenolic and Flavonoid compound seems to have an important role in stabilizing lipid oxidation, associated with antioxidant activity [19] is shown in table 1. Total phenol found to be of 18.33±0.68mg GAE/g dry weight and flavonoid content was 6.44±1.24 µg/mg of quercetin equivalent. The results revealed that ethanolic extract of *Phomopsis amygdali* contains significant amount of

phenols and flavonoids. *Liu et al.,* [20] have reported total phenolic content in the range of 54.51 mg/g and flavonoid content of 86.76 mg/g in intracellular extract of *Phomopsis amygdali*. The antioxidant content range was more when compared with the current study may be due to the different in extraction process demonstrated that endophytic fungus have phenolic and flavonoid content showed excellent activity of against ABTS and DPPH radicals, could be a source of natural antioxidants. In addition, the characteristics of phytochemicals having antioxidants property should be further studied.

REFERENCES:

1. Aruoma, O.I. Free radical, oxidative stress and antioxidants in human health and disease, *Journal of the American Oil Chemists Society* 1998, 75,199–212.
2. Essawi, T., & Srour, M. Screening of some Palestinian medicinal plants for antibacterial activity. *Journal of Ethnopharmacology* 2000, 70, 343–349.
3. Silva, F., Ferreres, J.O., Malva and A.C.P. Dias. Phytochemical and antioxidant characterization of *Hypericum perforatum* alcoholic extracts, *Food Chemistry* 2005, 90 (1–2), 157–167.
4. Tan, R. X., & Zou, W. X. Endophytes: a rich source of functional metabolites. Natural Product Reports 2001, 18, 448–459.
5. Guo, B. Cytonic acids A & B: novel tridepside inhibitors of hCMV protease from the endophytic fungus *Cytonaema* species. *Journal of Natural Product* 2000, 63, 602–604.
6. Strobel, G., Yang, X., Sears, J., Kramer, R.,Sidhu, R. S., & Hess, W. M. Taxol from Pestalotiopsis micrspora, an endophytic fungus of *Taxus wallichiana*. *Microbiology* 1996, 142, 435–440.
7. Guba, E.F. *Monograph of Monochaetia* and *Pestalotia*. Harvard University Press. Cambridge, Massuchettes 1961.
8. Ellis. *Dematiaceous Hyphomycetes*. Common wealth Mycological Institute, Kew, Survey, England 1971.
9. Sutton, B.C. *The Coelomycetes*. *CMI,* Kew, Surrey, England 1980.
10. Onions, A.H.S., D. Allsopp and H.O.W. Eggins. *Smith's Introduction to industrial Mycology*. Edward Arnold Ltd. London 1981. 311-316.
11. Arona, M.B., Cano A Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chemistry* 2001, 73, 239–244.
12. Shimada, K., Fujikawa, K., Yahara, K., Nakamura, T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry* 1992, 40, 945–948.
13. Taga, M.S., Miller, E.E., Pratt, D.E. Chia seeds as a source of natural lipid antioxidants. *Journal of the American Oil Chemists Society* 1984, 61, 928–993.
14. Barros, L., Ferreira, M.J., Queirós, B., Ferreira, C.F.R., Baptista, P. Total phenols, ascorbic acid, β-carotene andlycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chemistry* 2007, 103, 413–419.
15. Leong, L.P., Shui, G. An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry* 2002, 76:69–75.
16. Jagadish. L.K., Krishnan V.V., Shenbhagaraman, R., Kaviyarasan, V. Comparative study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* (J. E. Lange) Imbach before and after boiling. *African Journal of Biotechnology* 2009, 8(4), 654–661.
17. Jagadish, L.K., Shenbhagaraman, R., Krishnan, V.V., Kaviyarasan, V. Studies on the phytochemical, antioxidant and antimicrobial properties of three *Pleurotus* species collected indigenously. *Journal of Molecular Biology & Biotechnology* 2008, 1, 20–29
18. Duan, X. J., Zhang, W. W., Li, X. M., &Wang, B. G. (2006). Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. *Food Chemistry*, 95, 37–43.
19. Yanishlieva-Maslarova, N. V. Inhibiting oxidation. In J. Pokorny, N. Yanishlieva, & M. H. Gordon (Eds.), Antioxidants in food: Practical applications, Cambridge: CRC Press, Woodhead Publishing Limited. 2001, (22–70).
20. Liu, X., Mingsheng Dong., Xiaohong Chen, Mei Jiang., Xin L.v., Guijun, Yan. Antioxidant activity and phenolics of an endophytic Xylaria sp. from *Ginkgo biloba*. *Food Chemistry* 2007, 105, 548-554

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