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REGULAR ARTICLE

ANTIOXIDANT ACTIVITY OF ENDOPHYTIC FUNGUS *PHYLLOSTICTA* SP. ISOLATED FROM *GUAZUMA TOMENTOSA*

K. Srinivasan*, L.K. Jagadish, R. Shenbhagaraman and J. Muthumary

Center for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai – 6000 25, India

SUMMARY

Antioxidant property of *Phyllosticta* sp. 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 Phyllosticta sp. showed potent antioxidant activity against both ABTS and DPPH radicals with the EC_{50} value of 580.02 ± 0.57µg/ml and 2030.25±0.81 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 *Phyllosticta* sp. may have potential source of natural antioxidant.

Key words: Antioxidant, ABTS, DPPH, phenol, *Phyllosticta* sp.

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*Corresponding Author, Email: vksrini267696@gmail.com, Tel: +91-9566225086; Fax: +91 044 22352494

1. Introduction

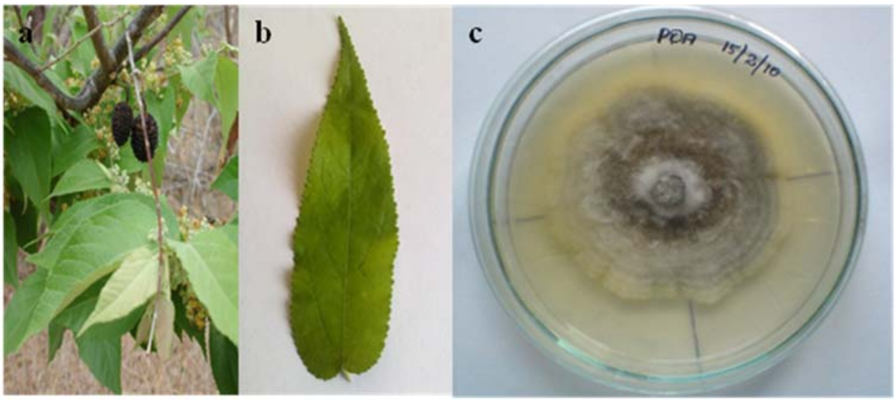
Reactive oxidant species (ROSs) plays an important role in degenerative condition such as aging cancer, neuron degenerative disorders, atherosclerosis and inflammations [1]. These free radicals occur in the body during an imbalance between ROSs (Reactive Oxygen Species) and antioxidants. Hence, the dietary 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 flavanoid 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 *Phyllosticta* sp. an endophytic fungi isolated from the medicinal plant, the isolated fungus was cultivated under submerged culture condition was evaluated for their antioxidant activity.

2. Materials and Methods

Endophytic fungi

Phyllosticta sp. an endophytic fungi isolated from the leaves of the medicinal plant *Guazuma tomentosa* H.B and K (Sterculiaceae) collected from Chennai (Plate 1). The isolated fungus was identified [7, 8, 9and 10]. The pure culture was maintained in potato dextrose agar. The identified culture was deposited to the culture collection centre, CAS in Botany, University of Madras.

Plate 1. *Phyllosticta* sp. Isolated from the host plant *Guazuma tomentosa*; a)Host plant *Guazuma tomentosa*; b) Healthy leaf of *Guazuma tomentosa*; c) Culture morphology of *Phyllosticta* sp grown on potato dextrose agar (PDA).



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 50 ml 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:

ABTS 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.

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]. EC50 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:

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% aqeous 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.

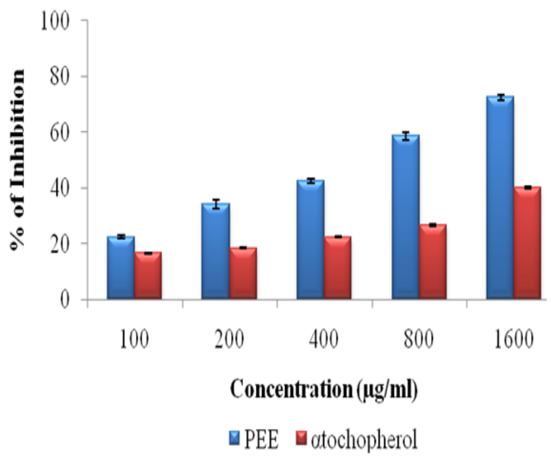
3. Results and Discussion

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 $ABTS^{\cdot +}$ at 734 nm. The ethanolic extract of *Phyllosticta* sp. showed a maximum decolourization of 72.38% at a maximum concentration of 1600 µg/ml with the Ec_{50} value 580.02±0.57 µg/ml (Table 1). The extend reduction of decolourization is directly proportional to the increased concentration of the extract illustrated in Figure 1.

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].

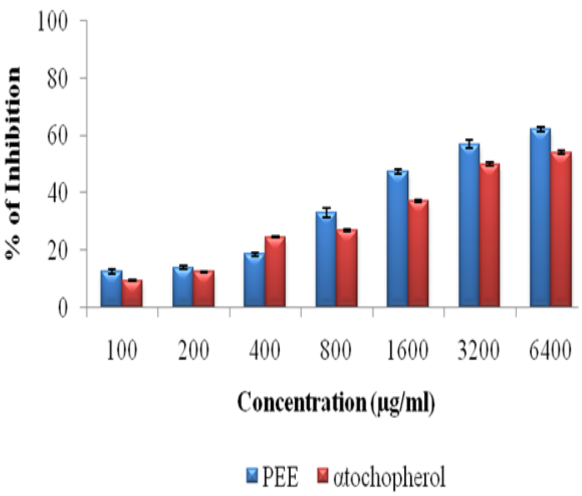
Figure 1.Free radical scavenging effect of *Phyllosticta* sp ethanolic extract (PEE) against ABTS.



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 decolourization 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 *Phyllosticta sp*.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 *Phyllosticta* sp ethanolic extract (PEE) against DPPH radicals.



Determination of antioxidant compounds

Phenolic and Flavanoid compound seems to have an important role in stabilizing lipid oxidation, associated with antioxidant activity [19] shown in the table 1. Total phenol found to be of 18.33±0.68mg GAE/g dry weight and flavanoid content was 6.44±1.24 µg/mg of quercetine equivalent. The results revealed that ethanolic extract of *Phyllosticta* sp. contains significant amount of phenols and flavanoids. *Liu et al.,* [20] have reported total phenolic content in the range of 54.51 mg/g and flavanoid content of 86.76 mg/g in intracellular extract of *Xylaria sp*. The antioxidant content range was more when compared with the current study may be due to the different in extraction process.

Table 1. EC_{50} values and bioactive compounds obtained from the *Phyllosticta* sp culture filtrate extracted with ethanol

Sample	EC_{50} (µg/ml)		Phenol (mg/g)	Flavonoid (mg/g)
	ABTS	DPPH		
Phyllosticta sp.	580.02±0.57	2030.25± 0.81	18.33	6.44

Each value is expressed as mean ± S.E. (n=3).

The data presented in the study demonstrated that endophytic fungus have phenolic and flavanoid 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.

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