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IN VITRO ANTIOXIDANT POTENTIAL OF METHANOLIC LEAF EXTRACTS OF *PENTATROPIS MICROPHYLLA*

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Abstract

Aim: To evaluate the antioxidant activity of methanolic leaf extract of *Pentatropis microphylla.*

Methods: *P. microphylla* was analyzed by various *in vitro* assays like total antioxidant, free radical scavenging, reducing power and metal ion chelating activities. Ascorbic acid and BHT were used as standards.

Results: The total antioxidant activity was higher in the methanolic leaf extract (1632. 6 ± 143.6). The DPPH$^{\bullet}$ free radical scavenging activity was well established (IC_{50} at 129µg/ml). Reducing power activity was higher (0.441 ± 0.13) in the higher concentration of the extract, 700µg/ml. Similarly, the metal ion chelating activity was also higher (51.74 ± 1.63) in the higher concentration of the extract, 300 µg/ml with the IC_{50} value, 245 µg/ml.

Conclusion: From the above results it can be concluded that the plant species, *Pentatropis microphylla* can be used as an efficient antioxidant source.

Key words: Antioxidant properties, *Pentatropis microphylla,* methanol leaf extract.

Introduction

Human body has multiple mechanisms especially enzymatic and nonenzymatic antioxidant systems to protect the cellular molecules against reactive oxygen species (ROS) induced damage [1]. So that, several synthetic antioxidants like butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT), etc. are available, but are quite unsafe and their toxicity is a problem of concern. Therefore, in recent years, considerable attention has been directed towards the identification of natural antioxidants (plant derived) that may be used for human consumption.

Plants naturally synthesize several carbon compounds, basically for physiologic functions or for using as chemical weapons against disease organisms, insects and predators [2]. The plant species, *Pentatropis microphylla* (Syn: *P. capensis*) belongs to the family, Ascleapiadaceae is mainly found in undisturbed deciduous forest margins in West Bengal, Gujarat and Peninsular India. The plant has the properties of antifungal, antiseptic and keratolytic. It is used in the treatment of skin problems also. The plant contains octacosanol, alpha-amyrin, friedelin and beta-sitosterol and an appreciable amount of salicyclic acid has also been isolated from this plant. It also reported to have a cardiac glycoside [3].

However, information pertaining to the antioxidant properties of *P. microphylla* is meager. To address this lacuna, the possible antioxidant activity of this plant was investigated in detail by employing different *in vitro* models.

Materials and Methods

Plant materials and extraction

For the present study purpose, the shade dried leaf part of the study species was made into fine powder of 40 mesh size using the pulverizer. 100 g of the powder was filled in the filter paper and successively extracted using 500 mL methanol using the soxhlet extractor for 8 – 10 hours [4]. Then the extract was filtered through Whatman No.1 filter paper to remove all undissolved matter, including cellular materials and other constituents that are insoluble in the extraction solvent.

Chemicals

All the chemicals used in the work were purchased from HI-MEDIA Pvt. Ltd, Mumbai. The chemicals used were of analytical grade.

Determination of antioxidant activity

The antioxidant activity was evaluated on basis of the following assays made:

Total antioxidant activity

The total antioxidant activity of the sample was analyzed by phosphomolybdenum method [5]. Two mg/mL of sample was taken and mixed with 1 mL of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) followed by incubation at 95°C for 90 min. Absorbance of the sample was measured at 635 nm

both for sample and the standard, ascorbic acid. The total antioxidant activity was expressed as ascorbic acid equivalents (mg/g of sample).

Free radical scavenging activity (DPPH$^{\bullet}$ assay)

The scavenging activity for DPPH free radicals was measured according to the procedure described by Blios *et al* [6]. Methanol solution of the sample extract at various concentrations (50, 100, 150, 200 and 250 µg/mL) was added separately to each 5 mL of 0.1 mM methanolic solution of DPPH and allowed to stand for 20 min at 27ºC. After incubation, the absorbance of each solution was determined at 517 nm using spectrophotometer. BHT (butylated hydroxy toluene) was used as standard. The corresponding blank reading was also taken and DPPH radical scavenging activity was calculated by using the following formula:

DPPH$^{\bullet}$ radical scavenging activity (%) = Control OD – Sample OD X100/ Control OD

IC_{50} value is the concentration of the sample required to scavenge the 50% DPPH free radical. It has been determined by using the software SPSS v.16.

Reducing power activity

Total reducing power was determined as described by Yildirim *et al.*[7]. Various concentrations of methanol extract (300µg-700µg/ml) were separately mixed with 1ml of 0.2M sodium phosphate buffer (pH 6.6) and 1ml of 1% potassium ferric cyanide, followed by incubation at 50ºC for 20 min. 1ml of 10% TCA was added to the mixture, which was then centrifuged at 3000rpm for 1min. Finally, 2ml of the supernatant solution was mixed with equal volume of distilled water. Absorbance was measured at 700nm after the addition of 0.5ml of 1% $FeCl_3$. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as standard.

Metal chelating activity

The chelating of ferrous ions by various extracts of *P. microphylla* was estimated by the method described by Dinis *et al* [8]. Various concentrations of the extracts *viz.*, 50, 100, 150, 200, 250 and 300 µg/mL of *P. microphylla* were added with 1 mL of 2mM $FeCl_2$ seperately. The reaction was initiated by the addition of 5mM ferrozine (1mL). Absorbance was measured at 562nm after 10min. BHT was used as standard.

Chelating activity (%) = Control OD – Sample OD X100/ Control OD

Results and Discussion

Total antioxidant assay

The total antioxidant activity for the methanol leaf extracts of *P. microphylla* was determined to be 1632.6 mg/g of ascorbic acid equivalents for the methanolic leaf extracts of *P. microphylla* which is comparable to that of the standard, ascorbic acid equivalents which is (Table 1). The secondary metabolite, flavonoids may be present adequately in this species which may be responsible for higher total antioxidant activity of this species [9,10]. Antioxidants may act as free radical scavengers, reducing agents, chelating agents for the transition of metals, quenchers of singlet oxygen molecules and or activators of antioxidative defense enzyme system to suppress the radical damages in biological systems [11,12]. Sangeetha *et al* [13] observed similar type of results in an Asteraceae member, *Sphaeranthus indicus*.

Table-1: $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radical scavenging activities of methanolic leaf extract of *Pentatropis microphylla.*

$ABTS^{\bullet+}$ radical scavenging activity			
Sample	Total antioxidant Activity (µmol TE/g extract)		
Leaf	1632.6± 143.6		
$DPPH^{\bullet}$ radical scavenging activity			
Sample concentration (µg/mL)	Percentage activity	IC_{50} (µg/ml)	IC_{50} Value of BHT
50	21.13± 0.41	129	34.74 ± 00.26
100	37.15± 0.82		
150	58.39± 0.43		
200	63.31± 1.61		
250	73.21± 0.64		

Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).

DPPH radical scavenging activity

To evaluate the antioxidant activity of the methanolic leaf extract, the radical scavenging capacity based on $DPPH^{\bullet}$ assay was determined and the results are presented in Table 1 for the study species, *P. microphylla*. The percentage of scavenging effect on the $DPPH^{\bullet}$ radical was increased with the increase in the concentrations of the extract from 50 - 250 µg/mL. The percentage of inhibition of the $DPPH^{\bullet}$ radical varied from 21.13% in 50 µg/mL of the extract to 73.21% in 250 µg/mL of extract. The IC_{50} values of the methanolic leaf extract of this species and standard drug, BHT were determined to be 129 and 34.74µg/mL respectively. $DPPH^{\bullet}$ determines the ability of pure substances or crude extracts for trapping the unpaired electron species by donating hydrogen atoms or electrons, and producing in consequence the radical disappearance and the formation of less reactive species derived from the antioxidant [14] . In

the present study, the extracts had significant scavenging effect on the DPPH• radical. It has been observed that the extracts prepared in high polar solvents exhibited strong activities which indicates that varieties of polyphenols, flavanone, and flavanoids trapped may play important role in these activities. The present findings are in agreement with the report of Tepe *et al* [15] and Battu *et al* [16] for *Salvia tomentosa* and *Euphorbia heyneana* respectively.

Reducing power activity

Reducing power characteristic of any compound serves as a significant indicator of its potential as antioxidant and is a supporting feature for its antioxidant activity [17]. Methanolic leaf extracts of the study species, *Pentatropis microphylla* showed the highest reducing power in the higher concentration of 700µg/ml and the values were comparable to that of the standared ascorbic acid (Table 2). Polyphenolic contents of all the sample extracts appear to function as good electron and hydrogen atom donors and therefore should be able to terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable products. Similar trend of observation on polyphenolic constituents' dose dependent reducing power activity has been reported for several other plant extracts [18,19,20].

Table-2 : Reducing power activity of methanolic leaf extract of *Pentatropis microphylla.*

Sample concentration (µg/mL)	Leaf extract (Absorbance at 700 nm)	Sample concentration (µg/mL)	Ascorbic acid (Absorbance at 700 nm)
300	0.091 ± 0.01^{a}	20	0.417 ± 0.03^{a}
400	0.142 ± 0.05^{a}	40	0.648 ± 0.02^{b}
500	0.272 ± 0.20^{b}	60	0.856 ± 0.01^{c}
600	0.348 ± 0.25^{c}	80	1.098 ± 0.05^{d}
700	0.441 ± 0.13^{d}	100	1.393 ± 0.02^{e}

Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).

Metal chelating activity

As observed in DPPH• and reducing power assays, the percentage of metal chelating activity was determined to be sample concentration dependent and it was increasing with the increase in the concentration of extract from 50 to 300µg/mL for the study species, *Pentatropis microphylla* (Table 3). The percentage of inhibition of the metal chelation was varying from 30.12 % (in 50µg/mL of extract) to 51.74% (in 300µg/mL extract). The IC_{50} values of the methanolic leaf extract of this species and the standard drug, BHT were determined to be 245 and 52.31µg/mL respectively.

The higher metal ion scavenging activity found in methanolic leaf extracts than that of the standard is explained that the chelating agents in plant sources may serve as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ions. Accordingly it is suggested that the low to moderate ferrous ions chelating effects of these fractions would be somewhat beneficial to protect the cell against oxidative damage [21]. Similar trend of metal ion scavenging activity was observed in the species, *Acalypha fruticosa* [22], *Naregamia alata* [23] and *Leucas ciliate* [24].

Table 3 – Metal chelating activity of methanolic leaf extract of *Pentatropis microphylla.*

Sample concentration (µg/ml)	Percentage activity	IC_{50} (µg/ml)	IC_{50} Value of BHT
50	30.13± 0.41 [c]	245	50.31± 00.15
100	33.17± 0.82 [c]		
150	42.39± 0.43 [b]		
200	45.10± 1.61 [b]		
250	49.21± 0.64 [a]		
300	51.74± 1.63 [a]		

Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).

Conclusion

From the present study it is found that the alcoholic extracts of the study species, *Pentatropis microphylla* showed concentration dependent free radical scavenging activity effectively. However, pharmacognostical studies are suggested to confirm the antioxidant ability before going for commercialization.

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