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FREE RADICAL SCAVENGING ACTIVITY OF VARIOUS EXTRACTS OF AERIAL PARTS OF *PREMNA TOMENTOSA*: AN *IN-VITRO* EVALUATION

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Background: The objective of the present investigation was to evaluate the *in vitro* antioxidant and total flavonoids effect of various extracts from aerial parts of *Premna tomentosa*. **Methods:** The free radical scavenging activity to evaluate by total antioxidant activity**,** FRAP method and Estimation of total flavonoids.**Results:**. Total antioxidant activity of petroleum ether, ethyl acetate, methanolic extract and reference standard ascorbate IC_{50} values was found to be 1020, 620, 320 µg/ml and 410 µg/ml. FRAP method of petroleum ether, ethyl acetate, methanolic extract and reference standard ascorbate IC_{50} values was found to be 1120, 430, 235 µg/ml and 50 µg/ml. The total phenol content of petroleum ether, ethyl acetate and methanolic extract was found to be 0.029, 1.243 and 3.849mg/g respectively. **Conclusion:** Based on the results, we concluded that the methanolic extract of *Premna tomentosa* is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: *Premna tomentosa*, *in vitro* antioxidant, FRAP, total flavonoids, total antioxidant.

INTRODUCTION

Considerable evidence have accumulated to implicate cellular damage arising from reactive oxygen species (ROS), at least in part, in the etiology and pathophysiology of human diseases such as neurodegenerative disorders (e.g. Alzheimer disease, Parkinson disease, multiple sclerosis, Down's syndrome), inflammation, viral infections, autoimmune pathologies, and digestive system disorders such as gastrointestinal inflammation and ulcer[1-3]. Antioxidants are important in the prevention of human diseases. Antioxidant compounds may function as free radical scavengers, complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation [4] .

Antioxidants are often used in oils and fatty foods to retard their autoxidation. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have restricted use in foods as they are suspected to be carcinogenic. Therefore, the importance of search for natural antioxidants has greatly increased in the recent years [5]. Ethnomedical literature contains a large number of plants that can be used against diseases, in which reactive oxygen species and free radical play important role. There is a plethora of plants that have been found to possess strong antioxidant activity[6]. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases [7]. So, many researchers have focused on natural antioxidants and in the plant kingdom numerous crude extracts and pure natural compounds were previously reported to have antioxidant properties.

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The genus Premna (Verbenaceae) comprises a group of more than 200 different trees, distributed in tropical and subtropical areas of the world. *P. tomentosa* (Verbanaceae) is a well-known medicinal plant used extensively for the treatment of various ailments. In Indian system of medicine, all parts of *P. tomentosa* have been employed for the treatment of various disorders [8]. Its bark extract is claimed to have a lasting cure for hepatic disorders [9]. Extracts from *P. tomentosa* leaves are known to have diuretic [10], hepatoprotective [11], antioxidant [12], lipid lowering [13], immunomodulatory activities [14] and protective against acetaminophen induced mitochondrial dysfunction properties [15]. In spite of the various pharmacological uses of *P. tomentosa* extracts, little is known about the chemical constituents. Previous studies on this species have resulted in the isolation of various compounds, including flavonoids, triterpenoids and steroids [16,17].

However, no data are available in the literature on the antioxidant activity of aerial parts of *Premna tomentosa*. Therefore we undertook the present investigation to examine the antioxidant activities of various extracts of aerial parts of *Premna tomentosa* through various in vitro models.

MATERIALS AND METHODS

Collection and identification of Premna tomentosa:

The aerial parts of Premna *tomentosa* was collected from Shencottai, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plants

Unit Siddha, Government of India, Palayamkottai. The aerial parts of *Premna tomentosa* was dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powdered plant materials were stored in an airtight container.

Preparation of various extracts from Premna tomentosa:

The aerial parts of *Premna tomentosa* were dried in shade and powdered. The powdered plant materials were successfully extracted with petroleum ether (40-60°C) by hot continuous percolation method in Soxhlet apparatus [18] for 24 hrs. Then the marc was dried and then subjected to ethyl acetate extraction (76-78°C) for 24 hrs, then marc was dried and then it was subjected to methanol extraction (80ºC) for 24 hrs. The solvent from the extracts was recovered under reduced pressure using rotary evaporator and subjected to freeze drying in a lyophilizer until dry powder was obtained.

Total antioxidant activity (Phosphomolybdic acid method):

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex [19]. An aliquot of 0.4 ml of sample solution was combined in a vial with 4 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95^0C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expresses relative to that of ascorbic acid.

FRAP Assay:

A modified method of Benzie and Strain [20] was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-S-triazine) solution in 40 mM HCl and 20 mM $FeCl_3$. $6H_2O$. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml $Fecl_3$.$6H_2O$. The temperature of the solution was raised to 37^0 C before using. Plant extracts (0.15 ml) were allowed to react with 2.85 ml of FRAP solution for 30 min in the dark condition. Readings of the colored product (Ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $FeSO_4$. Results are expressed in µM (Fe (II) /g dry mass and compared with that of ascorbic acid.

Estimation of total flavonoids: [21]

0.2g of the plant material was ground with ethanol-water in 2 different ratios namely 9:1 and 1:1 respectively. The homogenate was filtered and these 2 ratios were combined. This was evaporated to dryness until most of the ethanol has removed. The resultant aqueous extract was extracted in a separating funnel with hexane or chloroform. The solvent extracted aqueous layer was concentrated 0.5 ml of aliquot of extract was pipette-out in a test tube. 4 ml of the vanillin reagent (1% vanillin in 70% conc. H_2SO_4) was added and kept in a boiling water bath for 15 mins. The absorbance was read at 360 nm. A standard was run by using catechol (110 µg/ml).

RESULTS AND DISCUSSIONS

Total antioxidant activity (Phosphomolybdic acid method):

The percentage of total antioxidant activity of petroleum ether extract of *Premna tomentosa* presented in Table 1. The pet. ether extract of *Premna tomentosa* exhibited a maximum total antioxidant activity of 51.12% at 1000µg/ml whereas for ascorbate (standard) was found to be 65.23% at 1000µg/ml. The IC_{50} values of the petroleum ether extract of *Premna tomentosa* and ascorbate were found to be 1020µg/ml and 410µg/ml respectively.

The percentage of total antioxidant activity of ethyl acetate extract of *Premna tomentosa* presented in Table 2. The ethyl acetate extract of *Premna tomentosa* exhibited a maximum total antioxidant activity of 68.36% at 1000µg/ml whereas for ascorbate (standard) was found to be 65.23% at 1000 µg/ml. The IC_{50} values of the ethyl acetate extract of *Premna tomentosa* and ascorbate were found to be 620µg/ml and 410µg/ml respectively.

The percentage of total antioxidant activity of methanolic extract of *Premna tomentosa* presented in Table 3. The methanolic extract of *Premna tomentosa* exhibited a maximum total antioxidant activity of 74.42% at 1000 µg/ml whereas for ascorbate (standard) was found to be 65.23% at 1000 µg/ml. The IC_{50} of the methanolic extract of *Premna tomentosa* and ascorbate were found to be 320µg/ml and 410µg/ml respectively.

Table-1: Total antioxidant activity of pet. ether extract of *Premna tomentosa* by phosphomolybdic acid method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Pet. ether extract)	Standard (Ascorbate)
1	125	12.66 ± 0.023	26.87 ± 0.076
2	250	28.22 ± 0.045	30.30 ± 0.054
3	500	44.87 ± 0.023	60.64 ± 0.022
4	1000	51.12 ± 0.064	65.23 ± 0.014
		IC_{50} = 1020 µg/ml	**IC_{50} = 410 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table-2: Total antioxidant activity of ethyl acetate extract of *Premna tomentosa* by Phosphomolybdic acid method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	21.52 ± 0.042	26.87 ± 0.076
2	250	32.44 ± 0.056	30.30 ± 0.054
3	500	48.98 ± 0.074	60.64 ± 0.022
4	1000	68.36 ± 0.046	65.23 ± 0.014
		IC_{50} = 620 µg/ml	**IC_{50} = 410 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table-3: Total antioxidant activity of methanolic extract of *Premna tomentosa* by Phosphomolybdic acid method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	35.12 ± 0.038	26.87 ± 0.076
2	250	43.66 ± 0.056	30.30 ± 0.054
3	500	67.78 ± 0.042	60.64 ± 0.022
4	1000	74.42 ± 0.086	65.23 ± 0.014
		IC_{50} = 320 µg/ml	**IC_{50} = 410 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Based on the result clearly indicated the methanolic extract of *Premna tomentosa* was found to more effective than petroleum ether and ethyl acetate extract. But when compare all the extracts with standard the methanolic extract of *Premna tomentosa* was found strong antioxidant activity. The IC_{50} of the methanolic extract of *Premna tomentosa* and Ascorbate were found to be 320µg/ml and 410µg/ml respectively

FRAP assay:

The antioxidant potential of *Premna tomentosa* was ascertained from FRAP assay based on their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). The reducing ability of the petroleum ether extract of *Premna tomentosa* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Table 4. The maximum reducing ability at 1000µg/ml for petroleum ether extract and ascorbate were found to be 43.62% and 98.07% respectively. The IC_{50} values of petroleum ether extract and ascorbate were recorded as 1120µg/ml and 50µg/ml respectively.

The reducing ability of the ethyl acetate extract of *Premna tomentosa* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Table 5. The maximum reducing ability at 1000µg/ml for ethyl acetate extract and ascorbate were found to be 59.80% and 98.07% respectively. The IC_{50} values of ethyl acetate extract and ascorbate were recorded as 430µg/ml and 50µg/ml respectively.

The reducing ability of the methanolic extract of *Premna tomentosa* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Table 6. The maximum reducing ability at 1000µg/ml for methanolic extract and ascorbate were found to be 75.63% and 98.07% respectively. The IC_{50} values of methanolic extract and ascorbate were recorded as 235µg/ml and 50µg/ml respectively.

Table-4: Reducing ability of pet. ether extract of *Premna tomentosa* on FRAP assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Pet. ether extract)	Standard (Ascorbate)
1	125	21.43 ± 0.057	72.04 ± 0.014
2	250	25.47 ± 0.052	82.05 ± 0.034
3	500	34.56 ± 0.034	86.04 ± 0.026
4	1000	43.62 ± 0.047	98.07 ± 0.041
		IC_{50} = 1120 µg/ml	**IC_{50} = 50 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table-5: Reducing ability of ethyl acetate extract of *Premna tomentosa on* FRAP assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	39.45 ± 0.064	72.04 ± 0.014
2	250	44.46 ± 0.023	82.05 ± 0.034
3	500	53.31 ± 0.056	86.04 ± 0.026
4	1000	59.85 ± 0.086	98.07 ± 0.041
		IC_{50} = 430 µg/ml	**IC_{50} = 50 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table-6: Reducing ability of methanolic extract of *Premna tomentosa* on FRAP assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	34.22 ± 0.034	72.04 ± 0.014
2	250	52.87 ± 0.045	82.05 ± 0.034
3	500	63.66 ± 0.076	86.04 ± 0.026
4	1000	75.63 ± 0.056	98.07 ± 0.041
		IC_{50} = 235 µg/ml	**IC_{50} = 50 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table-7: The total flavonoids content of various extracts of aerial parts of *Premna tomentosa*

S.No	Extracts	Total flavonoids content (mg/g)(±SEM)*
1	Petroleum ether extract of *Premna tomentosa*	0.029 ± 0.034
2	Ethyl acetate extract of *Premna tomentosa*	1.243 ± 0.053
3	Methanolic extract of *Premna tomentosa*	3.849 ± 0.042

*All values are expressed as mean ± SEM for three determinations

Based on the above results indicated, the methanolic extract of *Premna tomentosa* was found to most effective than that of petroleum ether & ethyl acetate extract. . But when compare to the all the three extracts with ascorbate (standard), the ethyl acetate extract of the *Premna tomentosa* showed the moderate result.

Total flavonoids:

Flavonoids present in food of plant origin are also potential antioxidants. Most beneficial effects of

flavonoids are attributed to their antioxidant and chelating abilities. The total amount of flavonoids content of various extract of aerial parts of *Premna tomentosa* was presented in Table 7.

CONCLUSION

The present study was clearly indicated the methanolic extract of *Premna tomentosa Mucuna pruriens* showed strong antioxidant activity by inhibiting total antioxidant activity (Phosphomolybdic acid method) and FRAP assay when compared with standard Ascorbate. In addition, the methanolic and ethyl acetate

extract of *Premna tomentosa* was found to contain a noticeable amount of total flavonoids, which play a major role in controlling antioxidants. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Amutha Iswarya Devi, Tharmini, and Rilan were the principle investigators who performed the field trial, preparation of the manuscript, the *in vitro* analysis, and statistical analysis, Kottai Muthu was conceived the idea and prepared the project proposal and helped in the preparation of the manuscript. All authors read and approved the final manuscript.

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