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Free Radical Scavenging Activity of Various Extracts of Aerial Parts of *Macaranga peltata* (Roxb.): An *In Vitro* Evaluation

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Abstract
Macaranga peltata (Roxb.) is belongs to the family *Euphorbiaceae* is commonly known as Chandwar is a medicinal plant associated with diverse biological activities. In the presented study aerial parts of different extracts of the plant was evaluated for its *in-vitro* antioxidant potential by DPPH free radical scavenging activity, superoxide scavenging activity taking ascorbic acid and quercetin as the standard and total phenol content respectively. An IC_{50} value was found that methanolic extract of *Macaranga peltata* is more effective in DPPH radical, superoxide radical scavenging activity than that of ethyl acetate and petroleum ether extract. The methanolic extract of *Macaranga peltata and* ascorbate exhibited free radical scavenging activity possessing IC_{50} values 188µg/ml and 66µg/ml (DPPH Free Radical Scavenging Activity). 210µg/ml and 60µg/ml (Superoxide Scavenging Activity) respectively. Moreover, the results were observed in a concentration dependent manner. In addition, the methanolic and ethyl acetate extract of *Macaranga peltata was* found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidants. All the above invitro studies clearly indicate that the methanolic extract of *Macaranga peltata* has a significant antioxidant activity. These *invitro* assays indicate that this plant extracts is a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords
Macaranga peltata , DPPH radical scavenging activity, Superoxide radical activity, Total Phenol.

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INTRODUCTION
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[1]. 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[2]. 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[3]. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases[4]. 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.

Macaranga peltata (Roxb.) is belongs to the family Euphorbiaceae is commonly known as Chandwar. *Macaranga peltata* Muell. is a small tree commonly found in Indian forests[5].It is found in Bengal, Bihar, Orissa and the Deccan Peninsula, mostly in the hills[6]. Two major centers of distribution are tropical America and Africa. Macaranga genus contains 240 species[7].It is found throughout kerala[8]. These are small dioecious trees; h-12 m, d-30 cm. Bark 10-15 mm thick, surface pale, grayish brown, mottled with white. About 12 species are found in india. It is used for sizing paper and for taking impressions of leaves, coins, medallions etc; it is used also as a substitute for gum Arabic[9]. The gum powder from *Macaranga peltata* has been used in Indian medicine for the treatment of venereal diseases[10]. A decoction of leaves and bark is used as a wash for ulcers. In tribal medicine, gum powder of *Macaranga peltata* bark is used to join fractured bones, the fruit is eaten during the periods of scarcity. The wood of *M. peltata* is pale brown with a mottled appearance and is reported to be suitable for making pencil, matches and paper pulp[11].This plant is used for antibacterial and antifungal activity[12]. However, no data are available in the literature on the antioxidant activity of aerial parts of *Macaranga peltata* (Roxb.). Therefore, we undertook the present investigation to examine the antioxidant activities of various extract of aerial parts of *Macaranga peltata* (Roxb.) through various *in vitro* models.

MATERIAL AND METHODS

Collection and Identification of Plant materials

The aerial plant of *Macaranga peltata* (family Euphorbiaceae) were collected form kulasekaram, Kanyakumari District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The aerial plant of *Macaranga peltata*, were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve[13].

Preparation of Extracts

The above powered materials were successively extracted with Petroleum ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus for 24 hrs[14]. Then the marc was subjected to Ethyl acetate (76-78^{0}C) for 24 hrs and then mark was subjected to Methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilized till dry powder was obtained [15].

Evaluation of Antioxidant activity by in vitro Techniques:

DPPH photometric assay

The effect of extract on DPPH radical was assayed using the method of Mensor et al (2001)[16]. A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavengingactivity(\%) = \frac{A_{518}\ \text{Control} - A_{518}\ \text{Sample}}{A_{518}\ \text{Control}} \times 100$$

Where A_{518} control is the absorbance of DPPH radical+ methanol; A_{518} sample is the absorbance of DPPH radical+ sample extract/ standard.

Superoxide radical scavenging activity

Superoxide radical (O_2^-) was generated from the photo reduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne et al (1975)[17]. The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Quercetin was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Total phenol

The measurement of total phenol is based on Mallick and Singh (1980)[18]. To 0.25g of sample, added 2.5 ml of ethanol and centrifuged at 2^{o}C for 10 mins. The supernatant was preserved. Then, the sample was re-extracted with 2.5 ml of 80% ethanol and centrifuged. The pooled supernatant was evaporated

to dryness. Then, added 3 ml of water to the dried supernatant. To which added 0.5 ml of Folins phenol reagent and 2 ml of sodium carbonate (20%). The reaction mixture was kept in boiling water bath for 1 min. the absorbance was measured at 650 nm in a spectrophotometer. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallicacid equivalent per g dry weight.

RESULTS AND DISCUSSION

DPPH scavenging activity

DPPH is a stable free radical at room temperature often used to evaluate the antioxidant activity of several natural compounds. The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants[19]. The percentage of DPPH radical scavenging activity of petroleum ether extract of *Macaranga peltata* presented in Table 1. The petroleum ether extract of *Macaranga peltata* exhibited a maximum DPPH scavenging activity of 50.38% at 800 µg/ml whereas for ascorbate (standard) was found to be 72.82% at 800 µg/ml. The IC_{50} of the petroleum ether extract of *Macaranga peltata* and ascorbate were found to be 810µg/ml and 66µg/ml respectively.

Table 1: Effect of Petroleum ether extract of *Macaranga peltata* (Roxb.) on DPPH assay

S.No	Concentration (µg/ml)	% of activity(±SEM) *	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	100	12.43±0.036	54.19 ± 0.024
2	200	21.62±0.012	59.24 ± 0.032
3	400	33.78±0.038	65.32 ± 0.054
4	800	50.38±0.076	72.82 ± 0.062
		IC_{50} = 810 µg/ml	**IC_{50} = 66 µg/ml**

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

The percentage of DPPH radical scavenging activity of ethyl acetate extract of *Macaranga peltata* presented in Table 2. The ethyl acetate extract of *Macaranga peltata* exhibited a maximum DPPH scavenging activity of 68.62% at 800 µg/ml whereas for ascorbate (standard) was found to be 72.82% at 800 µg/ml. The IC_{50} of the ethyl acetate extract of *Macaranga peltata* and ascorbate were found to be 432µg/ml and 66µg/ml respectively

Table 2 :Effect of Ethyl acetate extract of *Macaranga peltata* (Roxb.) on DPPH assay

S.No	Concentration (µg/ml)	% of activity(±SEM) *	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	100	32.22 ± 0.023	54.19 ± 0.024
2	200	42.68 ± 0.022	59.24 ± 0.032
3	400	52.34 ± 0.043	65.32 ± 0.054
4	800	68.62 ± 0.055	72.82 ± 0.062
		IC_{50} = 432µg/ml	**IC_{50} = 66 µg/ml**

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

The percentage of DPPH radical scavenging activity of methanolic extract of *Macaranga peltata* presented in Table 3. The methanolic extract of *Macaranga peltata* exhibited a maximum DPPH scavenging activity of 67.41% at 800 µg/ml whereas for ascorbate (standard) was found to be 72.82% at 800 µg/ml. The IC_{50} of the methanolic extract of *Macaranga peltata* and ascorbate were found to be 188µg/ml and 66µg/ml respectively.

Table 3 : Effect of Methanolic extract of *Macaranga peltata*(Roxb.) on DPPH assay

S.No	Concentration (µg/ml)	% of activity(±SEM) *	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	100	42.43±0.032	54.19 ± 0.024
2	200	51.18±0.045	59.24 ± 0.032
3	400	58.72±0.047	65.32 ± 0.054
4	800	67.41±0.052	72.82 ± 0.062
		IC_{50} = 188 µg/ml	**IC_{50} = 66 µg/ml**

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

The methanolic extract of *Macaranga peltata* was found to more effective than petroleum ether and ethyl acetate extract. The DPPH radical scavenging activity of the extract increases with increasing concentration, 67.41% DPPH radical scavenging. Nevertheless, it was 72.82% in the presence of 800µg/ml of ascorbate (standard). The IC_{50} of the methanol extract of *Macaranga peltata* and ascorbate were found to be 188µg/ml and 66µg/ml respectively.

Superoxide anion scavenging activity

Percentage scavenging of superoxide anion examined at different concentrations of petroleum ether extract of *Macaranga peltata* (125, 250, 500, 1000 µg/ml) was depicted in table 4. The percentage scavenging of superoxide radical surged with the enhanced concentration of plant extract. The maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 55.25% and 98.01% respectively. The IC_{50} value of plant extract and Quercetin was recorded as 510µg/ml and 60µg/ml respectively.

Table 4: Effect of Petroleum ether extract of *Macaranga peltata*(Roxb.) on Superoxide anion scavenging activity method

S.No	Concentration (µg/ml)	% of activity(±SEM) *	
		Sample (Petroleum ether extract)	Standard (Quercetin)
1	125	21.24 ±0 .022	73.81 ± 0.006
2	250	38.65 ± 0.013	91.31 ± 0.011
3	500	49.36 ± 0.025	92.99 ± 0.024
4	1000	55.25 ± 0.043	98.01 ± 0.012
		IC_{50} = 510 µg/ml	**IC_{50} = 60 µg/ml**

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

Percentage scavenging of superoxide anion examined at different concentrations of ethyl acetate extract of *Macaranga peltata* (125, 250, 500, 1000 µg/ml) was depicted in table 5. The maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 70.21% and 98.01% respectively. The IC_{50} value of plant extract and Quercetin was recorded as 435µg/ml and 60µg/ml respectively.

Table 5: Effect of Ethyl acetate extract of *Macaranga peltata*(Roxb.) on Superoxide anion scavenging activity method

S.No	Concentration (µg/ml)	% of activity(±SEM) *	
		Sample (Ethyl acetate extract)	Standard (Quercetin)
1	125	26.48 ± 0.021	73.81 ± 0.006
2	250	43.48 ± 0.034	91.31 ± 0.011
3	500	54.76 ± 0.032	92.99 ± 0.024
4	1000	70.21±0.018	98.01 ± 0.012
		IC_{50} = 435 µg/ml	**IC_{50} = 60 µg/ml**

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

Percentage scavenging of superoxide anion examined at different concentrations of methanolic extract of *Macaranga peltata* (125, 250, 500, 1000 µg/ml) was depicted in table 6. The percentage scavenging of superoxide radical surged with the enhanced concentration of plant extract. The maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 69.82% and 98.01% respectively. Superoxide scavenging ability of plant extract might primarily be due to the presence of flavanoids[27]. The IC_{50} value of plant extract and Quercetin was recorded as 210µg/ml and 60µg/ml respectively.

Table 6: Effect of Methanolic extract *Macaranga peltata*(Roxb.) on Superoxide anion scavenging activity method

S.No	Concentration (µg/ml)	% of activity(±SEM) *	
		Sample (Methanolic extract)	Standard (Quercetin)
1	125	37.22 ± 0.012	73.81 ± 0.006
2	250	52.48 ± 0.016	91.31 ± 0.011
3	500	64.42 ± 0.023	92.99 ± 0.024
4	1000	69.82±0.027	98.01 ± 0.012
		IC_{50} = 210 µg/ml	**IC_{50} = 60 µg/ml**

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

The methanolic extract of *Macaranga peltata* was found to more effective than petroleum ether and ethyl acetate extract. The Superoxide anion scavenging activity of the extract increases with increasing concentration, 69.82% Superoxide anion scavenging activity. Nevertheless, it was 98.01% in the presence of 1000µg/ml of quercetin (standard). The IC_{50} of the methanol extract of *Macaranga peltata* and quercetin were found to be 210µg/ml and 60µg/ml respectively.

Total phenol

Phenolic compounds are known as powerful chain breaking antioxidants. The phenolic compounds may contribute directly to antioxidative action. The total amount of phenolic content of various extract of aerial plant of *Macaranga peltata* was present in Table 7.

Table 7: The total Phenolic content of various extracts of aerial plant of *Macaranga peltata* (Roxb.)

S.No	Extracts	Total phenol content (mg/g of Gallic acid) (±SEM) *
1	Petroleum ether extract of *Macaranga peltata*	0.94 ± 0.24
2	Ethyl acetate extract of *Macaranga peltata*	6.27 ± 0.36
3	Methanolic extract of *Macaranga peltata*	18.98 ± 0.14

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

Based on the result the methanolic extract of *Macaranga peltata* was found higher content of phenolic components than that of petroleum ether and ethyl acetate extract of *Macaranga peltata* .

DISCUSSION

Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation[20]. The *in-vitro* antioxidant potential of various extracts was evaluated by DPPH free radical scavenging activity, superoxide anion radical scavenging activity, and estimation of total phenolic content. The studies were carried out taking ascorbic acid and quercetin as the standard antioxidant which is also a natural antioxidant. The results of antioxidant activity by DPPH free radical scavenging activity, superoxide anion radical scavenging activity and Hydrogen peroxide scavenging activity were expressed in terms of % inhibition of generated free radicals respectively with respect to various concentrations. Concentration dependent effects were observed in each case i.e; higher concentrations were found to exhibit higher % inhibition in each protocol of the antioxidant study.

DPPH radical is one of the few stable organic nitrogen free radicals, which has been widely used to determine the free radical scavenging ability of the various samples[21]. The method is based on the reduction of an alcoholic DPPH solution in the presence of a hydrogen donating antioxidant due to

the formation of the non-radical form DPPH–H by the reaction[22]. DPPH radical scavenging activity were examined various extracts and found IC50 value reflects higher scavenging ability. Among the three different plant extracts tested, interestingly, in the DPPH radical scavenging activity of the methanolic extract of *Macaranga peltata* exhibited DPPH radical scavenging potential comparable with that of standard ascorbate. Superoxides could be produced in large amounts by various biological processes. It is known to be very harmful to cellular components as a precursor of the most reactive oxygen species (ROS), contributing to tissue damage and various diseases[23].The methanolic extract of *Macaranga peltata* exhibited higher ability in scavenging superoxide anion radical, when compared to the standard quercetin. methanolic extract of *Macaranga peltata* was found higher content of phenolic components than that of other extracts. It is well known that flavonoids and polyphenols are natural antioxidants but have also been reported to significantly increase SOD, glutathione and catalase activities. Furthermore, it was shown that these compounds act as promoters for SOD, catalase and glutathione and cause the expression of SOD, glutathione and catalase[24].

CONCLUSION

The present study was clearly indicated the methanolic extract of *Macaranga peltata* showed strong antioxidant activity by inhibiting DPPH, Super oxide anion scavenging activity, when compared with standard ascorbate and quercetin. In addition, the methanolic extract of *Macaranga peltata* was found to contain a noticeable amount of total phenols, 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 *Macaranga peltata*.

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