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# *In vitro* antioxidant activity of various extracts of aerial parts of *Premna tomentosa*

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**ABSTRACT**

The study was designed to examine the *in vitro* antioxidant activities of various extracts of aerial parts of *Premna tomentosa.* The antioxidant activity was evaluated by DPPH (α,α-diphenyl-β-picrylhydrazyl) radical scavenging activity, Iron chelating activity with reference standard rutin, EDTA and total phenolic content respectively. The methanolic extract of *Premna tomentosa* was found to more effective in the DPPH radical scavenging activity. The $IC_{50}$ of the methanolic extract of *Premna tomentosa* and Rutin were found to be 245µg/ml and 480µg/ml respectively. The Iron chelating activity of the methanolic extract of *Premna tomentosa* was found to most effective than that of petroleum ether & ethyl acetate extract. An $IC_{50}$ value of *Premna tomentosa* and EDTA were found to be 78µg/ml and 65µg/ml respectively. But when compare to the all the two extracts with rutin (standard), the methanolic extract of the *Premna tomentosa* showed the similar result. In addition, the methanolic and ethyl acetate extract of *Premna tomentosa* was found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidants. It is concluded that a aerial parts of methanolic extract of *Premna tomentosa,* which contains large amounts of phenolic compounds, exhibits high antioxidant and free radical scavenging activities. These *in vitro* 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:** *Premna tomentosa*, *In vitro* antioxidant, DPPH assay, Iron chelating activity, Total phenol.

## 1. INTRODUCTION

Vegetables, fruits, for example, tea, coffee, and wine are unequivocally prescribed for wellbeing support because of their high antioxidant activity[1]. These antioxidant- rich nourishments and drinks may assume a real part in the prevention of chronic diseases, for example, tumor, obesity, type 2 diabetes and hypertension[2,3,4,5]. It is obvious that a good diet is favourable to human wellbeing, yet these general suggestions leave us to more noteworthy confusion, as to which foods and drinks to consume. It is, subsequently, still an extraordinary test for researchers to recognize the antioxidant capacity of food components, and as well to develop quick, simple, convenient and economic evaluation methods for screening antioxidants.

These days, the part of free radicals in numerous ailments and illnesses including inflammation, rheumatoid joint inflammation, tumor and cardiovascular diseases has been broadly established[6]. Free radicals which have one or more unpaired electrons are delivered during normal and pathological cell metabolites. Reactive oxygen species (ROS) respond effectively with free radicals to become radicals themselves. ROS are different types of activated oxygen, which incorporate free radicals, for example, superoxide anion radicals ($O_2^-$) and hydroxyl radicals ($OH^-$), as well as non-free radicals species ($H_2O_2$) and the singlet oxygen ($^1O_2$)[7]. Antioxidants give protection to living organisms from harm created by uncontrolled generation of ROS and associated lipid peroxidation, protein damage and DNA stand breaking[8]. A few substances from natural sources have been indicated to contain antioxidants and are under study. Antioxidants compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals, for example, peroxide, hydroperoxide or lipid peroxyl and accordingly inhibit the oxidative mechanisms that prompt

degenerative diseases[9]. Ethnomedical literature contains an extensive number of plants that can be utilized against diseases, in which reactive oxygen species and free radical play significant role. There is a plenty of plants that have been found to have strong antioxidant activity[10]. Recent reports demonstrate that there is a reverse relationship between the dietary ingestion of antioxidant-rich foods and the rate of human diseases[11].

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[12]. Its bark extract is claimed to have a lasting cure for hepatic disorders[13]. Extracts from *P. tomentosa* leaves are known to have diuretic[14], hepatoprotective[15], antioxidant[16], lipid-lowering[17], immunomodulatory activities[18] and protective against acetaminophen induced mitochondrial dysfunction properties[19]. 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 [20,21].

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.

## 2. MATERIAL AND METHODS

### 2.1. Collection and identification of plant materials

The aerial parts of *Premna tomentosa* were collected from Shencottai, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The aerial parts of *Premna tomentosa,* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

### 2.2. 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[22] for 24 hrs. 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 lyophilizer till dry powder was obtained.

### 2.3. Evaluation of antioxidant activity by *in vitro* techniques

#### 2.3.1. DPPH photometric assay

The effect of extract on DPPH radical was assayed using the method of Mensor *et al* (2001)[23]. 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.

#### 2.3.2. Iron chelating activity

The method of Benzie and strain (1996) [24] was adopted for the assay. The principle is based on the formation of *O*-Phenanthroline-$Fe^{2+}$ complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 mL of 0.05% O-Phenanthroline in methanol, 2 mL ferric chloride (200µM) and 2 mL of various concentrations ranging from 125 to 1000µg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

#### 2.3.3. Total phenol

The measurement of total phenol is based on Mallick and Singh (1980) [25]. To 0.25g of sample, added 2.5 mL of ethanol and centrifuged at 2°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.

## 3. RESULTS AND DISCUSSIONS

The various extracts of *Premna tomentosa* were subjected to screening for its phytochemical constituents. The phytochemical screening results

are shown in table 1. The petroleum ether extract of *Premna tomentosa* contains phytosterols and fixed oils & fats. Ethyl acetate extract contains alkaloids, carbohydrates, protein and amino acid compounds, phenolic compounds, and fixed oils & fats. The methanolic extract contains alkaloids, carbohydrates, glycoside, phenolic compounds, saponins, tannins, protein and amino acids, coumarins & flavonoids.

### 3.1. DPPH scavenging activity

The percentage of DPPH radical scavenging activity of petroleum ether extract of *Premna tomentosa* depicted in Table 2. The $IC_{50}$ values of the petroleum ether extract of *Premna tomentosa* and Rutin were found to be 1320µg/ml and 480µg/ml respectively.

The percentage of DPPH radical scavenging activity of ethyl acetate extract of *Premna tomentosa* depicted in Table 3. The $IC_{50}$ values of the ethyl acetate extract of *Premna tomentosa* and Rutin were found to be 950µg/ml and 480µg/ml respectively.

The percentage of DPPH radical scavenging activity of methanolic extract of *Premna tomentosa* depicted in Table 4. The $IC_{50}$ values of the methanolic extract of *Premna tomentosa* and Rutin was found to be 245µg/ml and 480µg/ml respectively.

From the result indicated the $IC_{50}$ values of methanolic extract of *Premna tomentosa* exhibits significant antioxidant activity when compared to that standard Rutin. $IC_{50}$ value of plant extract and Rutin was recorded as 245µg/ml and 480µg/ml respectively. But other two extracts showed lower activity when compared to that of methanolic extract of *Premna tomentosa* and standard Rutin.

### 3.2. Iron chelating activity

Iron binding capacity of the petroleum ether extract of *Premna tomentosa* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in table 5. The $IC_{50}$ value of plant extract and EDTA was recorded as 460µg/ml and 65µg/ml respectively.

Iron binding capacity of the ethyl acetate extract of *Premna tomentosa* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values are presented in table 6. The $IC_{50}$ value of plant extract and EDTA was recorded as 350µg/ml and 65µg/ml respectively.

Iron binding capacity of the methanolic extract of *Premna tomentosa* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values are presented in table 7. The $IC_{50}$ value of plant extract and EDTA was recorded as 78µg/ml and 65µg/ml respectively.

From the result indicated the $IC_{50}$ value of methanolic extract was found more effective than that of EDTA as a standard. Based on the above results indicated, the methanolic extract of *Premna tomentosa* exhibited significant antioxidant activity was comparable to that of petroleum ether & ethyl acetate extracts of *Premna tomentosa*.

### 3.3. Total phenol

The total phenolic content of various extract of aerial parts of *Premna tomentosa* are presented in Table 8.

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

**Table - 1: Phytochemical analysis of various extracts of *Premna tomentosa***

| Test | Petroleum ether | Ethyl acetate | Methanol |
|---|---|---|---|
| Alkaloids | - | + | + |
| Carbohydrates & Glycosides | - | + | + |
| Phytosterols | + | - | + |
| Coumarins | - | - | + |
| Flavonoids | - | - | + |
| Phenolic compounds & Tannins | - | + | + |
| Protein & Amino Acid | - | + | + |
| Saponins | - | - | + |
| Fixed oil & Fats | + | + | - |

+ Positive; - Negative

**Table - 2: Effect of Petroleum ether extract of *Premna tomentosa* on DPPH assay**

| Concentration (µg/ml) | % of activity(±SEM)* | |
|---|---|---|
| | Sample (Pet. ether extract) | Standard (Rutin) |
| 125 | 19.37 ± 0.080 | 18.85 ± 0.076 |
| 250 | 21.65 ± 0.050 | 22.08 ± 0.054 |
| 500 | 26.87 ± 0.045 | 52.21 ± 0.022 |
| 1000 | 35.55 ± 0.061 | 69.83 ± 0.014 |
| | **$IC_{50}$ = 1320 µg/ml** | **$IC_{50}$ = 480 µg/ml** |

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

**Table - 3: Effect of Ethyl acetate extract of *Premna tomentosa* on DPPH assay**

| Concentration (µg/ml) | % of activity(±SEM)* | |
|---|---|---|
| | Sample (Ethyl acetate extract) | Standard (Rutin) |
| 125 | 16.74 ± 0.098 | 18.85 ± 0.076 |
| 250 | 27.73 ± 0.054 | 22.08 ± 0.054 |
| 500 | 41.56 ± 0.024 | 52.21 ± 0.022 |
| 1000 | 51.78 ± 0.034 | 69.83 ± 0.014 |
| | **$IC_{50}$ = 950 µg/ml** | **$IC_{50}$ = 480 µg/ml** |

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

**Table - 4: Effect of Methanolic extract of *Premna tomentosa* on DPPH assay**

| Concentration (µg/ml) | % of activity(±SEM)* | |
|---|---|---|
| | Sample (Methanolic extract) | Standard (Rutin) |
| 125 | 41.78 ± 0.072 | 18.85 ± 0.076 |
| 250 | 50.89 ± 0.036 | 22.08 ± 0.054 |
| 500 | 61.45 ± 0.068 | 52.21 ± 0.022 |
| 1000 | 67.74 ± 0.054 | 69.83 ± 0.014 |
| | **$IC_{50}$ = 245 µg/ml** | **$IC_{50}$ = 480 µg/ml** |

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

**Table - 5: Effect of Pet. ether extract of *Premna tomentosa* on Iron-chelating method**

| Concentration (µg/ml) | % of activity(±SEM)* | |
|---|---|---|
| | Sample (Pet. ether extract) | Standard (EDTA) |
| 125 | 41.52 ± 0.034 | 58.68 ± 0.007 |
| 250 | 44.56 ± 0.054 | 65.87 ± 0.018 |
| 500 | 53.35 ± 0.012 | 83.83 ± 0.012 |
| 1000 | 62.45 ± 0.043 | 97.90 ± 0.019 |
| | **$IC_{50}$ = 460 µg/ml** | **$IC_{50}$ = 65 µg/ml** |

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

**Table - 6: Effect of Ethyl acetate extract of *Premna tomentosa* on Iron-chelating method**

| Concentration (µg/ml) | % of activity(±SEM)* | |
|---|---|---|
| | Sample (Ethyl acetate extract) | Standard (EDTA) |
| 125 | 16.83 ± 0.046 | 58.68 ± 0.007 |
| 250 | 28.34 ± 0.061 | 65.87 ± 0.018 |
| 500 | 71.65 ± 0.078 | 83.83 ± 0.012 |
| 1000 | 83.23 ± 0.028 | 97.90 ± 0.019 |
| | **$IC_{50}$ =350 µg/ml** | **$IC_{50}$ = 65 µg/ml** |

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

**Table - 7: Effect of Methanolic extract of *Premna tomentosa* on Iron-chelating method**

| Concentration (µg/ml) | % of activity(±SEM)* | |
|---|---|---|
| | Sample (Methanolic extract) | Standard (EDTA) |
| 125 | 54.45 ± 0.022 | 58.68 ± 0.007 |
| 250 | 64.13 ± 0.046 | 65.87 ± 0.018 |
| 500 | 71.30 ± 0.048 | 83.83 ± 0.012 |
| 1000 | 80.34 ± 0.018 | 97.90 ± 0.019 |
| | **$IC_{50}$ = 78 µg/ml** | **$IC_{50}$ = 65 µg/ml** |

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

**Table - 8: The total phenolic content of various extracts of aerial parts of *Premna tomentosa***

| Extracts | Total phenol content (mg/g of Catechol) (±SEM)* |
|---|---|
| Petroleum ether extract of *Premna tomentosa* | 0.30 ± 0.028 |
| Ethyl acetate extract of *Premna tomentosa* | 2.15 ± 0.022 |
| Methanolic extract of *Premna tomentosa* | 3.60 ± 0.049 |

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

## 4. CONCLUSION

From the results obtained in the present study, it is concluded that a aerial parts of methanolic extract of *Premna tomentosa,* which contains large amounts of phenolic compounds, exhibits high antioxidant and free radical scavenging activities. These *in vitro* assays indicate that this plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract. Furthermore, the *in vivo* antioxidant activity of this extract needs to be assessed prior to clinical use.

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