Assessment of the antioxidant activity of acetone, ethyl alcohol and aqueous extracts of the aerial roots of *Pothos aurea* (Linden ex Andre) climbed over *Lawsonia inermis* and *Areca catechu*

P. Arulpriya* and P. Lalitha

*Department of Chemistry, Avinashilingam, Institute for Home Science and Higher Education for Women University, Coimbatore*

**ABSTRACT**

Antioxidant activity testing of three different extracts (acetone, ethyl alcohol and aqueous) of the aerial roots of *Pothos aurea* intertwined over on *Lawsonia inermis* (MM) and *Areca catechu* (MP) were carried out by two different methods namely 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay and reducing power test. Higher antioxidant potential of the extracts was observed in both DPPH scavenging and reducing power assay. The appreciable free radical scavenging activity of the extracts might be attributed to the secondary metabolites alkaloids, flavonoids, tannins, phenols and anthraquinones present in the extracts. The plants may be considered as good sources of natural antioxidants for medicinal uses such as against aging and other diseases related to radical mechanisms.

**Key words:** *Pothos aurea*, Antioxidant properties, DPPH assay, reducing power assay.

**INTRODUCTION**

Antioxidants play a major role in protecting biological systems against many diseases. Over the last few years, reasonable supplementations of antioxidants have been widely practiced in different fields of industry and medicine to prevent and delay oxidative stress. Antioxidants are also used in dermocosmetic formulations in order to fight against oxidative stress which causes irreversible damages on skin, like psoriasis, cancer, premature aging [1]. Antioxidants are inhibitors of the process of oxidation, even at relatively small concentrations and thus have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers and helps in converting the radicals to less reactive species [2].

Many oxidative stress related diseases are as a result of accumulation of free radicals in the body. A lot of researches are going on worldwide directed towards finding natural antioxidants of plants origins. Currently, there is a great interest in the study of antioxidant substances mainly due to the findings concerning the effects of free radicals in the organism [3]. Phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites are rich in antioxidant activity [4].

Plants are potent biochemists and have been components of phytomedicine since times immemorial. Many plant species have been utilized as traditional medicines but it is necessary to establish the scientific basis for the
therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs [5].

Natural antioxidants occur in all parts of plants. Plants may contain many different antioxidant components such as phenolic compounds, nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity. There is currently immense interest in natural antioxidants and their role in human health and nutrition [6]. *Pothos aurea* (Money plant) is a popular house plant and wonderful evergreen plant, popularly grown for its beautiful and attractive carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity. There is currently immense interest in natural antioxidants and their role in human health and nutrition [6].

*Pothos aurea* (Money plant) is a popular house plant and wonderful evergreen plant, popularly grown for its beautiful and attractive carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity. There is currently immense interest in natural antioxidants and their role in human health and nutrition [6]. *Pothos aurea* (Money plant) is a popular house plant and wonderful evergreen plant, popularly grown for its beautiful and attractive carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity. There is currently immense interest in natural antioxidants and their role in human health and nutrition [6].

The aim of this study is to evaluate the antioxidative activity of acetone, ethyl alcohol and aqueous extracts of *Pothos aurea* (MM and MP) using DPPH (1, 1 diphenyl-2-picrylhydrazyl) radical quenching and reducing capacity assay.

**EXPERIMENTAL SECTION**

**Collection of plant**
Aerial roots of *Pothos aurea* intertwined over the *Lawsonia inermis* were collected from Coimbatore and that intertwined over *Areca catechu* (betal nut palm) was collected from Palakkad District.

**Preparation of extracts**
Solvent extracts of the aerial roots of MM and MP (acetone, ethyl alcohol and aqueous) were prepared by refluxing for 12 hours.

**Assessment of Antioxidant activity**

**DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay:**
The H-donor activity of the extracts was measured by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (Blois method) [8]. 2 ml of DPPH (0.1Mm in methanolic solution) was added to different concentrations (3µg to 9µg) of the MM and MP extracts (acetone, ethyl alcohol and aqueous) and observed at 517 nm against a blank. The percentage (%) of inhibition was calculated as,

\[
\% \text{ of DPPH Scavenged} = \frac{B_0 - B_1}{B_0} \times 100
\]

Where, \(B_0\) = the absorbance of control, \(B_1\) = the absorbance of sample

**Reducing Power assay**
The relative reducing activity of *Pothos aurea* roots of MM and MP extracts was determined by Oyaizu method [9]. 1 ml (300µg/ml) of MM and MP extracts (acetone, ethanol and aqueous) was prepared in distilled water and mixed with 2.5 ml of phosphate buffer(0.2M dibasic sodium phosphate, 0.2M monobasic sodium phosphate) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Then 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged. From the centrifugate, 1ml of aliquot was taken and mixed with 3 ml of distilled water and 0.5 ml of ferric chloride. By using colorimeter the absorbance was measured at 700 nm. Increase in absorbance was interpreted as increased reducing activity.
RESULTS AND DISCUSSION

DPPH assay
The DPPH radical scavenging assay is an easy and sensitive method for the antioxidant screening of plant extracts. A number of methods are available for the determination of free radical scavenging activity but the assay employing the stable DPPH has received the maximum attention owing to its ease of use and its convenience [10]. The invitro antioxidant study of petroleum ether, chloroform and ethyl acetate extracts of aerial roots of *Pothos aurea* intertwined over *Lawsonia inermis* and *Areca catechu* have been reported to show higher antioxidant potential (table 1) [11].

In the present study the acetone, ethanol and aqueous extracts of the aerial roots of *Pothos aurea* (MM and MP) were studied for its antioxidant activity by DPPH assay. The results are tabulated (table 1) and given in Fig. 1 and 2. Comparing the percentage inhibition (Fig. 2) of antioxidant potential, it is seen that among the three extracts, acetone extract of MM has highest activity (62.12%), while other extracts of MM and MP showed activity in the range 58-60%. The aqueous extract of *Pothos aurea* showed least inhibition against the stable DPPH radical.

![Figure 1: DPPH scavenging activity of ascorbic acid](image1)

**Figure 1:** DPPH scavenging activity of ascorbic acid

![Figure 2: DPPH scavenging activity of Pothos aurea extracts](image2)

**Figure 2:** DPPH scavenging activity of *Pothos aurea* extracts

Fig. 1 illustrates the decrease in the concentration of DPPH radical due to the scavenging ability of standard ascorbic acid, as a reference compound. The DPPH activity was found to vary from ~63-69% with increase in concentration (30µg/ml to 150µg/ml).
The extracts of the aerial roots of *Pothos aurea* MP also showed good antioxidant activity. Ethyl acetate extract MP showed higher activity compared to the other extracts. The aqueous extract of MP showed comparatively better activity (20%) than MM extract (14%). Around 10-15mg of the extracts gave 68% DPPH scavenging activity. From the present results it may be postulated that the extracts of *Pothos aurea* reduces the DPPH radical to corresponding hydrazine when its reacts with hydrogen donors in the antioxidant principles.

### Table 1: Antioxidant activity of *Pothos aurea* extracts

<table>
<thead>
<tr>
<th>Extracts</th>
<th>% of increase DPPH scavenging activity</th>
<th>Absorbance value of Reducing power assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>67.56</td>
<td>64.86</td>
</tr>
<tr>
<td>Chloroform</td>
<td>59.45</td>
<td>58.10</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>56.75</td>
<td>64.86</td>
</tr>
<tr>
<td>Acetone</td>
<td>62.12</td>
<td>60.81</td>
</tr>
<tr>
<td>Ethyl Alcohol</td>
<td>60.81</td>
<td>58.10</td>
</tr>
<tr>
<td>Aqueous</td>
<td>14.86</td>
<td>20.27</td>
</tr>
</tbody>
</table>

**Reducing Power Assay**

The relative reducing activity of the acetone, ethanol and aqueous extracts of the aerial roots of *Pothos aurea* of MM and MP were determined according to the Oyaizu method [6]. Literature reports are evident that the reducing powers of bioactive compounds are associated with antioxidant activity [12,13]. Thus a relation should be located between reducing power and the antioxidant effect. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [14]. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. In this assay, the yellow colour of the test solution changes to various shades of green and blue depending upon the reducing power of the test solution. The presence of reductones (i.e. antioxidantst) in plant extracts causes the reduction of the \( \text{Fe}^{3+} / \text{ferricyanide} \) complex to the ferrous form. Therefore, the \( \text{Fe}^{2+} \) can be monitored by measuring the formation of Prussian blue at 700 nm [15]. From the results it is clear that the extracts consist of reductones that contributes towards its reducing power. In other words, the \( \text{FeCl}_3 / \text{K}_3[\text{Fe(CN)}_6] \) system offers a sensitive method for the “semi-quantitative” determination of dilute concentrations of polyphenolics, which participate in redox reaction [16].

**Figure 3: Reducing power assay capacity of ascorbic acid**

In the presence study the, acetone, ethanol and aqueous extracts of aerial roots of *Pothos aurea* of claimed over *Lawsonia inermis* and *Areca catechu* were carried out at concentration of 3mg/ml. The reductive capabilities of the standard ascorbic acid (300µg/ml) are illustrated (Fig.3).
Figure 4: Reducing power assay capacity of *Pothos aurea* extracts

From the results it is obvious that the reducing power of the standard ascorbic acid increased with concentration (600µg/2ml to 2100µg/7ml). When the concentration of the extract was increased thrice, the reducing power too increased proportionately. Comparing the extracts of aerial roots of money plant climbed over *Lawsonia inermis* and *Areca catechu*, the extract of aerial roots climbed over *Lawsonia inermis* showed better reducing power than extract of the aerial roots climbed over *Areca catechu*. The order of increasing absorbance of the roots extracts of *Pothos aurea*, was found to be aqueous > ethanol > acetone. The antioxidant activity has been reported to be concomitant with the development of reducing power. Phytochemical screening of the extracts of *Pothos aurea* reveals the following:

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>alkaloids, tannins, steroids, anthraquinone</td>
</tr>
<tr>
<td>Ethyl Alcohol</td>
<td>alkaloids, flavonoids, saponins, steroids, anthocyanin, glycosides, phenols, anthraquinone</td>
</tr>
<tr>
<td>Aqueous</td>
<td>alkaloids, flavonoids, saponins, phenols, anthraquinone, anthocyanin</td>
</tr>
</tbody>
</table>

The fine antioxidant activity of the extracts as obvious from the results may be attributed to the phytochemical constituents present in the extracts. Flavonoids are very good antioxidants. The presence of flavonoids in the aqueous and ethanol extracts may be the cause for the extracts having more antioxidant activity as compared to acetone extract.

In conclusion this work describes for the first time *in vitro* antioxidant activity of the extracts of the aerial roots of *Pothos aurea*. Most of the extracts showed excellent free radical scavenging activity. Determination of the natural antioxidant compounds of plant extracts will help to develop new drug candidates for antioxidant therapy. The plants may be considered as good sources of natural antioxidants for medicinal uses such as against aging and other diseases related to radical mechanisms. Literature reports are evident that the reducing power of bioactive compounds is associated with antioxidant activity thus a relation is evidenced between reducing power and the antioxidant effect.

**Acknowledgement**

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