In Vitro Antioxidant Activity Of The Hexane And Methanolic Extracts Of Cordia Wallichii And Celastrus Paniculata

H Makari, N Haraprasad, P Ravikumar

Abstract
In-vitro antioxidant effects of the hexane and methanolic leaf extracts of Cordia wallichii and Celastrus paniculata were tested. The methanolic extracts of C. wallichii had shown good DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging activity. The methanolic extract C. paniculata also exhibited promising result at higher concentration. BHA was used as standard antioxidant and positive control. The DPPH radical scavenging activity of the extract was increased with the increasing concentration. The methanolic extract of C. wallichii was found to be most effective than hexane extract. The Reducing power of extracts was carried out with ascorbic acid as a standard reducing agent. C. wallichii exhibited higher reducing power than C. paniculata. All the analysis was made with the use of UV-Visible Spectrophotometer (Systronics 117, INDIA). In these two plant leaf extracts there was a remarkable concentration dependent DPPH scavenging and reducing power was exhibited.

INTRODUCTION
Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias (Polterat, 1997) Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties. (Nakayoma and Yamada, 1995).

Plants are potent biochemical factories and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plants based natural constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, seeds, etc (Gordon and David, 2001) i.e. any part of the plant may contain active components. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct (Wink, 1999).
Antioxidant-based drugs/formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer have appeared during the last 3 decades (Devasagayam et al 2004). This has attracted a great deal of research interest in natural antioxidants. Subsequently, a worldwide trend towards the use of natural phytochemicals present in berry crops, tea, herbs, oilseeds, beans, fruits, and vegetables has increased. Several herbs and spices have been reported to exhibit antioxidant activity, including rosemary, sage, thyme, nutmeg, turmeric, white pepper, chili pepper, ginger, and several Chinese medicinal plants extracts (Lee et al 2003). The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, beta-carotene, and tocopherol are known to possess antioxidant potential (Prior, 2003). The systematic record of the relative antioxidant activity in selected Iranian medicinal plant species extracts was recorded by Pourmorad et al in 2006. With this background and abundant source of unique active components harbored in plants, the present study was taken up on two alternative medicinal plants namely Cordia wallichii belongs to the family moraginaceae and Celastrus paniculata belongs to the family Celastraceae.

MATERIALS AND METHODS
PLANT MATERIAL
Plant samples of the selected species viz. Cordia wallichii and Celastrus paniculata were collected from Hassan, Mysore district, Karnataka. Further identified by DR.
Krishnappa, Department of Applied Botany and voucher specimens were deposited at the same department, Kuvempu University, Karnataka, India. Leaves were separated and dried under shade for three days. Dried leaf samples were ground into a uniform powder using a blender and stored in polythene bags at room temperature.

**PREPARATION OF EXTRACTS**

10 g of the dried powdered samples from plants Cordia wallichii and Celastrus paniculata species were taken separately in a paper cone and placed into Soxhlet apparatus. 100 ml of hexane a polar solvent was taken in the round bottom flask attached to the Soxhlet apparatus. A condenser was attached to this set up. Then the whole setup was placed on a heating mantle. The temperature was set in the range of 25-30°C. Hexane gets vaporized and rises up to the condenser where it condenses back into liquid. This liquid falls into the plant sample in the cone and extracts certain compounds and falls back into the round bottom flask. This process was continued till all the compounds that can be extracted from the plant by ether gets extracted and finally only clear liquid of ether starts falling into the round bottom flask. The same procedure was repeated with polar solvent such as methanol. The extracts got from the above process was evaporated over night and stored in screw cap vials.

**ANTIOXIDANT ASSAY**

The antioxidant activity of Plant extracts were determined by different in-vitro methods such as, the DPPH free radical scavenging assay and reducing power methods. The different extracts were dissolved in methanol at the concentration of 2mg/ml. all the assays were carried out in triplicate and average value was considered.

(A) **DPPH RADICAL SCAVENGING ACTIVITY:**

DPPH scavenging activity of the plant extract was carried out according to the method of Koleva et al 2002; Mathiesen et al 1995. 0.2 ml of methanolic solution of plant extract samples at different concentration (20-100µg ml⁻¹) was mixed with 0.8 ml of Tris Hcl buffer (100Mm, pH 7.4). One ml DPPH (500 M in methanol) solution was added to above mixture. The mixture was shaken vigorously and incubated for 30 min in room temperature. Absorbance of the resulting solution was measured at 517 nm UV-Visible Spectrophotometer (Systronics UV-Visible Spectrophotometer 117, INDIA). All the assays were carried out in triplicates with BHA (Butylated Hydroxy Anisole) as a positive control. Blank was prepared without the addition of DPPH and for control 0.2 ml of methanol (without plant extract) was added. Percentage of DPPH scavenging activity determined as follows.

\[
\% \text{ DPPH radical-scavenging} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of test Sample}}{\text{Absorbance of control}}\right) \times 100
\]

Control was the DPPH solution without plant extract.

Purified sample 2mg/ml in Methanol of Cordia wallichii and Celastrus paniculata extracts were taken for antioxidant activity with a standard BHA (Butylated Hydroxy Anisole) antioxidant. Decreased absorbance of the reaction mixture indicates stronger DPPH radical-scavenging activity. In this study, hexane and methanolic leaf extracts of both C. wallichii and C. paniculata were used.

(B) **REDUCING POWER**

This was carried out as described previously (Yildirim et al 2001; Lu and Foo). 1 ml of plant extract solution (final concentration 100-500 mg/l) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide \( [K_3Fe(CN)_6] \) (10 g/l), then mixture was incubated at 50 degree C for 20 minutes. Two and one-half, 2.5 ml of trichloroacetic acid (100 g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml FeCl3 (1 g/l) and absorbance measured at 700 nm in UV-Visible Spectrophotometer (Systronics UV-Visible Spectrophotometer 117, INDIA). As a control, ascorbic acid was used (final concentration 10 mg/ml).

Increased absorbance of the reaction mixture indicates stronger reducing power. In this study, hexane and methanolic leaf extracts of Cordia wallichii and Celastrus paniculata were used.

**RESULT AND DISCUSSION**

**ANTIOXIDANT ASSAY**

1. **DPPH SCAVENGING ACTIVITY**

The percentage of DPPH radical scavenging activity presented in Table 1(a). Like reducing power, the DPPH radical scavenging activity of the extract increases with increasing concentration, only 19.27% DPPH radical scavenging. Nevertheless, it was 72.32% in the presence of 100 mg/l BHA (Butylated Hydroxy Anisole). Although this plant extract shows lower scavenging activity in comparison...
to BHA, it is remarkably higher than those of essential oils from the leaves of L. nobilis and the gum of A. cilicia (Alma et al. 2003).

**Figure 1**
Table 1a: Antioxidant activity of hexane extract of

<table>
<thead>
<tr>
<th>Concentration</th>
<th>OD 517nm Sample</th>
<th>OD 517nm Standard</th>
<th>% of activity Sample</th>
<th>% of activity Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>50μl</td>
<td>1.048</td>
<td>2μl</td>
<td>0.973</td>
<td>5.58</td>
</tr>
<tr>
<td>100μl</td>
<td>1.044</td>
<td>4μl</td>
<td>0.679</td>
<td>8.64</td>
</tr>
<tr>
<td>150μl</td>
<td>0.983</td>
<td>6μl</td>
<td>0.482</td>
<td>11.40</td>
</tr>
<tr>
<td>200μl</td>
<td>0.944</td>
<td>8μl</td>
<td>0.359</td>
<td>14.95</td>
</tr>
<tr>
<td>250μl</td>
<td>0.896</td>
<td>10μl</td>
<td>0.274</td>
<td>19.27</td>
</tr>
</tbody>
</table>

The methanolic extract of Celastrus paniculata was found to be most effective than hexane extract. The DPPH radical scavenging activity of the extract increases with increasing concentration, only 25.85% DPPH radical scavenging. Nevertheless, it was 75.32% in the presence of 100 mg/l BHA.

**Figure 2**
Table 1b: Antioxidant activity of Methanolic extract of

<table>
<thead>
<tr>
<th>Concentration</th>
<th>OD 517nm Sample</th>
<th>OD 517nm Standard</th>
<th>% of activity Sample</th>
<th>% of activity Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>50μl</td>
<td>1.030</td>
<td>2μl</td>
<td>0.973</td>
<td>5.58</td>
</tr>
<tr>
<td>100μl</td>
<td>1.010</td>
<td>4μl</td>
<td>0.679</td>
<td>8.64</td>
</tr>
<tr>
<td>150μl</td>
<td>0.953</td>
<td>6μl</td>
<td>0.482</td>
<td>11.40</td>
</tr>
<tr>
<td>200μl</td>
<td>0.914</td>
<td>8μl</td>
<td>0.359</td>
<td>14.95</td>
</tr>
<tr>
<td>250μl</td>
<td>0.863</td>
<td>10μl</td>
<td>0.274</td>
<td>19.27</td>
</tr>
</tbody>
</table>

The % of DPPH radical scavenging activity of hexane extract of C. wallichii presented in Table 1(c). Like reducing power, the DPPH radical scavenging activity of the extract increases with increasing concentration, only 16.75% DPPH radical scavenging was present for 250 l. This result found to be lower than that of hexane extract of C. paniculata. Nevertheless, it was 75.32% in the presence of 100 mg/l BHA. Although this plant extract shows lower scavenging activity in comparison to BHA. Plant extract exhibited antioxidative potential and increased concentration of plant extract has shown increased antioxidative potential.

**Figure 3**
Table 1c: Antioxidant activity of hexane extract of

<table>
<thead>
<tr>
<th>Concentration</th>
<th>OD 517nm Sample</th>
<th>OD 517nm Standard</th>
<th>% of activity Sample</th>
<th>% of activity Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>50μl</td>
<td>1.078</td>
<td>2μl</td>
<td>0.973</td>
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<td>100μl</td>
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<td>0.679</td>
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<td>150μl</td>
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<td>6μl</td>
<td>0.482</td>
<td>11.40</td>
</tr>
<tr>
<td>200μl</td>
<td>0.803</td>
<td>8μl</td>
<td>0.359</td>
<td>14.95</td>
</tr>
<tr>
<td>250μl</td>
<td>0.694</td>
<td>10μl</td>
<td>0.274</td>
<td>19.27</td>
</tr>
</tbody>
</table>

The methanolic extract of Cordia wallichii was found to be most effective than hexane extract (1 (d)). The DPPH radical scavenging activity of the extract increases with increasing concentration exhibited only 28.28% DPPH radical scavenging activity was noted. Nevertheless, it was 75.40% in the presence of 100 mg/l BHA. These results suggest that methanolic extracts of C. wallichii exhibited little better than other extracts.

**2. REDUCING POWER**

Different extracts of Celastrus paniculata exhibited good reducing power. The reducing power of methanolic extract of Celastrus paniculata along with that of ascorbic acid at concentrations between 100-500 mg/ml. The reducing power of the plant extract was determined by the method of Ozai (1986). High absorbance indicates high reducing power. The reducing power of the plant hexane extract of C. paniculata leaf as the amount of extract increases Table 2(a). However, this reducing power is lower than that of ascorbic acid which was used as control. Therefore, the absorbance of ascorbic acid in a sample was (10 mg/l) 0.96 while at the 500mg/l methanolic extract concentration it was 0.44. Nevertheless the reducing power of methanolic extract of C. paniculata was 0.54 and it was considerably higher than those of hexane extract Table. 2(b).
The reducing power of Cordia wallichii leaf extract has shown good reducing power than Celastrus paniculata. As the amount of extract increase, the reducing power also increases. Table 2(c). However, this reducing power is lower than that of ascorbic acid which was used as control. Therefore, the absorbance of ascorbic acid in a sample was (10 mg/l) 0.96 while at the 500mg/l methanolic extract concentration it was 0.52. Nevertheless the reducing power of extract of C. wallichii was higher (0.61) Table 2(d) than those of C. paniculata (0.53). In both cases of C. paniculata and C. wallichii there is a remarkable concentration dependent reducing power was exhibited. This variation in reducing activity may be due to crude nature of plant extracts and availability of different phytochemicals in these plants.

**CONCLUSIONS**

Against the backdrop of many known medicinal properties of these plants, results from the present work suggest that relatively low values of antioxidant and reducing power may not imply a low medicinal value. Emerging trends in antioxidant research point to the fact that low levels of phenolics (and other phytochemicals) and low value of antioxidant indices in plants do not translate to poor medicinal properties. The present investigation indicates that through Cordia wallichii and Celastrus paniculata has been described as plants of low economic values, these are not worthless. Use of these plants in traditional medicine attests to this. There is prospectus for the commercial utilization especially in the view abundant and widespread nature. The toxic compounds in these plants could be removed through appropriate extraction and processing methods making extracts and products of these plants safe for the utilization of animal and man.

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**References**

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