Original Article

Evaluation of chelating ability of aqueous extract of *Tetracarpidium conophorum* (African walnut) in vitro

Olabinri BM¹*, Eniyansoro OO¹, Okoronkwo CO¹, Olabinri PF², Olaleye MT³

¹Department of Biochemistry, Faculty of Basic Medical Sciences, Ladoke Akintola, University of Technology, Ogbomoso, Nigeria. ²Break New Frontier Research Communication, Aha Aroje, Ogbomoso, Nigeria. ³Department of Biochemistry, The Federal University of Technology, Akure, Nigeria.

Summary: The chelating ability of aqueous extract of *Tetracarpidium conophorum* was assessed in vitro. The plant extract showed a dose dependent decrease in chelating ability in vitro. The values of chelating ability for graded doses (2%, 4%, 6%, 8% and 10%, w/v) were 97.38, 90.56, 89.00, 87.46 and 82.80%, respectively. The dose (2%, w/v) had the highest chelating ability. The antioxidant activity of the extract ranged from –3.8% to 71.9%. At 8% concentration, a strong positive significant correlation was observed between chelating ability and total phenolics concentration (r = 0.89; P = 0.01). At 2% concentration, the chelating ability of the extract showed a high positive significant correlation with antioxidant activity (r = 0.68; P = 0.001). In conclusion, the aqueous extract of *Tetracarpidium conophorum* could be used in the treatment of iron-overload disorders due to its high chelating ability in vitro at low doses.

Industrial relevance: *Tetracarpidium conophorum* extract may be explored in the industrial production of iron chelators due to its high chelating ability in vitro at low doses, which will be of clinical relevance in the treatment of iron-overload disorders such as thalassemia, a group of genetically inherited blood disorders characterized by defective globin chain of haemoglobin and iron overload. Iron chelators from the plant extract will decrease iron availability in the blood circulation of thalassemic patients.

Keywords: Iron-overload disease; medicinal plant; chelating activity; natural product and clinical medicine.

Introduction

*Tetracarpidium conophorum* is an economic plant widely cultivated for the production of nuts and is used as delicacies (Adebona, 1998). *T. conophorum* is used as a male-fertility agent (Ajaiyeoba and Fadare, 2006). *Tetracarpidium conophorum* is known in the littoral and the western Cameroon as kaso or ngak and serves as an edible nut eaten between meals (Tchiegang et al, 2007). Two isolectins, Agglutin I and Agglutin II were characterized from the seed extract of the plant (Animashaun et al, 1994). Also, the presence of oxalates, phytates, tannins as well as proteins fibres, oil and carbohydrate in *Tetracarpidium conophorum* has been reported (Enujiugha and Ayodele-Oni, 2003).


Phenolics are characterized by at least one aromatic ring (C6) bearing one or more hydroxyl groups (Michalak et al, 2006). Chelation of metal ions and quenching of singlet oxygen are the major characteristics of antioxidant activity.
activity (Hall and Cuppett, 1997). Studies have shown that specialized phenol storing cells occur in several plant species (Martyn et al, 1976). A major disorder associated with iron overload is thalassemia. Thalassemia usually result in under production of normal globin proteins, often through mutations in regulatory genes (Maggio et al, 2002).

This study was carried out to evaluate the chelating ability of aqueous extract of *Tetracarpidium conophorum* (African walnut) against iron (ii) ion (Fe²⁺) generated in vitro and to investigate the relationships (positive, negative or no correlation) between chelating ability, antioxidant activity and total phenolic concentration.

**Materials and Methods**

**Sources of chemical reagents:** Phenanthroline, Tris HCl, Folin-Ciocalteau and iron(ii) sulphate heptahydrate were products of BDH,UK. 2,2-diphenyl-l-picryl hydrazyl (DPPH) used was a product of Sigma.

**Preparation of plant extract:** African walnut (*Tetracarpidium conophorum*) was purchased at Odo-Oba area Ogbomoso South Local Government Area, Oyo State, Nigeria. The plant was authenticated in the Biology Department of Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The nut was cracked with hammer and then grind in a mortar using a clean pestle. Aqueous extracts of the plant sample of different concentrations were prepared. For 2% aqueous extract (w/v), 0.5g of walnut powder was dissolved in 25ml of distilled water; For 4% aqueous extract (w/v), 1g of walnut was dissolved in 25ml of distilled water; For 6% aqueous extract (w/v), 1.5g of walnut was dissolved in 25ml of distilled water. For 8% concentration, 2g of walnut was dissolved in 25ml of distilled water and 10% concentration, 2.5g of walnut was dissolved in 25ml of distilled water. The different concentrations were shaken for 30min and then filtered. The filtrate of each concentration was stored inside a refrigerator at -20°C and then used for analysis of parameters of interest.

**Preparation of curcumin solution (stock):** 0.2g of curcumin was dissolved in 20ml of deionized water. The solution was incubated in water bath at 38°C for 5mins because curcumin was not completely soluble in water. Different concentrations (grade doses) (500 – 3000ug/ml) of curcumin were prepared from the stock curcumin (10mg/ml).

**Biochemical assays:**

- **Estimation of chelating ability:** The chelating ability of the plant extract was determined according to the modified method of Minotti and Aust (1987). In this assay, the plant extract binds with Fe²⁺ ion generated in vitro using 500uM iron (ii) sulphate as ion donor. 0.2ml of sample of different concentration (2 – 10%, w/v) of the plant extract was mixed with 0.336ml of Tris HCl(0.1M, pH7.4), followed by the addition of 0.436ml (saline, 0.9% NaCl w/v). The mixture was left to stand at room temperature of 5min. 0.26ml of 0.25% aqueous 1,10-phenanthroline was added. The absorbance of the solution was read on uv/visible spectrophotometer at 510nm against control which consists of Tris HCl, saline and phenanthroline without the plant extract. Curcumin was used as a standard chelating agent.

  \[
  \text{Chelating ability (\%) = } \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
  \]

- **Antioxidant activity determination:** The antioxidant activity of aqueous extract walnut was determined according to the method of Blois (1958).This is based on the ability of the extract to inhibit stable diphenyl picryl hydrazyl radical (DPPH). DPPH accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH can be oxidized only with difficulty and then irreversibly. It possesses an odd electron and hence shows a strong absorption band at 517nm.0.5ml of methanolic solution of DPPH (1mM) was added to 0.05ml of graded doses (2 - 10%) of plant extract ,followed by addition of 2ml methanol. The mixture was left for 30min in the dark. The absorbance of the solution was read at 517nm on a uv/visible spectrophotometer against blank(distilled water). Curcumin was used as standard antioxidant.

  \[
  \text{Antioxidant activity (AA,\%) was expressed in terms of inhibition of DPPH free radical in percent and was calculated by the formula}
  \]

  \[
  \text{AA(\%) = } \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
  \]

- **Determination of total phenolic content:** The total phenolic content of the extract was determined according to the method described elsewhere (Hung et al; 2001). In this assay, the phenolic group present in plant extract interacts with Folin – Ciocalteau in alkaline medium using Na₂CO₃ solution giving a blue colour, which has maximum absorption at 685nm and correlates with total phenolic content. 0.1ml of aqueous extract of the plant of different concentrations (2 – 10%) was rapidly mixed with 0.1ml of Folin Ciocalteau reagent ,followed by the addition of 0.3ml sodium carbonate(7.5%,w/v) solution. The mixture was incubated in the dark for 30mins.The absorbance of the blue colour was read at 685nm after 30mins on a spectrophotometer. The total phenolic content was extrapolated from a standard curve using tannic acid (graded doses, 500 – 3000ug/ml) as a standard phenol.
**Statistical analysis:** Student’s t-test was used for statistical analysis. P values less than or equal to 0.1, 0.05, 0.01 or 0.001 were considered significant.

**Results**

The result of this work shows that the chelating ability of the aqueous extract of *Tetracarpidium conophorum* shows a dose-dependent binding with ferrous ion generated in vitro.

**Table 1:** Changes in the levels of chelating ability, antioxidant activity and total phenolic concentration of aqueous of *Tetracarpidium conophorum*.

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Chelating ability (%)</th>
<th>Antioxidant activity (%)</th>
<th>Total phenolic concentration (ug/ml)</th>
<th>Total phenolic concentration (mg/10ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>97.38±1.49</td>
<td>62.80±13.73</td>
<td>1720.00±941.20</td>
<td>172.00±94.12</td>
</tr>
<tr>
<td>4</td>
<td>90.56±2.18</td>
<td>34.40±8.06</td>
<td>1140.00±392.80</td>
<td>114.00±39.28</td>
</tr>
<tr>
<td>6</td>
<td>89.00±5.09</td>
<td>38.00±8.20</td>
<td>2115.00±983.50</td>
<td>211.50±98.35</td>
</tr>
<tr>
<td>8</td>
<td>87.46±7.39</td>
<td>71.94±5.30</td>
<td>1900.00±398.40</td>
<td>190.00±39.84</td>
</tr>
<tr>
<td>10</td>
<td>77.40±11.62</td>
<td>18.46±7.23</td>
<td>1580.00±311.50</td>
<td>158.00±31.15</td>
</tr>
</tbody>
</table>

Values are mean ± S.D of 5 analyses per concentration.

The order of increasing chelating ability of the plant extract is 10% < 8% < 6% < 4% < 2%. The chelating ability values were 97.38±1.49, 90.56±2.18, 89.00±5.09, 87.46±7.39, 77.40±11.62 %, respectively for doses examined (2%, 4%, 6%, 8%, and 10%). The aqueous extract of the plant has the highest chelating ability at 2%, with 97.3% activity when compared with a standard phenolic compound (curcumin) which has the highest chelating ability at 1500ug/ml with 83.26% (Table 1 and Table 2).

**Table 2:** Changes in the levels of chelating ability, antioxidant activity and total phenolic concentration of curcumin.

<table>
<thead>
<tr>
<th>Concentration (ug/ml)</th>
<th>Chelating ability (%)</th>
<th>Antioxidant activity (%)</th>
<th>Total phenolic concentration (ug/ml)</th>
<th>Total phenolic concentration (mg/10ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>77.84±5.68</td>
<td>19.54±4.26</td>
<td>575.00±22.61</td>
<td>57.50±7.26</td>
</tr>
<tr>
<td>1000</td>
<td>62.17±5.74</td>
<td>48.54±4.98</td>
<td>390.00±25.47</td>
<td>39.00±2.55</td>
</tr>
<tr>
<td>1500</td>
<td>83.26±4.88</td>
<td>49.78±4.94</td>
<td>530.00±33.51</td>
<td>53.00±3.35</td>
</tr>
<tr>
<td>2000</td>
<td>24.97±4.88</td>
<td>39.56±3.21</td>
<td>635.00±34.85</td>
<td>63.50±3.49</td>
</tr>
<tr>
<td>2500</td>
<td>29.92±4.92</td>
<td>79.94±1.12</td>
<td>830.00±31.04</td>
<td>83.00±3.10</td>
</tr>
<tr>
<td>3000</td>
<td>48.24±5.53</td>
<td>66.70±3.20</td>
<td>995.00±21.68</td>
<td>99.50±2.17</td>
</tr>
</tbody>
</table>

Values are mean ± S.D of 5 analyses per concentration.

The antioxidant activity of the plant aqueous extract was dose independent. 8% concentration of the extract has the highest antioxidant activity with 71.94% which was slightly lower than the antioxidant activity of curcumin with 79.9% at 2000 ug/ml. The increasing order of antioxidant activity is 6% < 10% < 4% < 2% < 8% concentration of the extract (Table 1 and Table 2). The graphical plot of antioxidant activity of the aqueous extract of the plant against graded doses (different concentrations) yielded three IC₅₀ values (3%, 7% and 8.9%) IC denotes inhibitory concentration. IC₅₀ is the concentration of the aqueous extract of the plant that scavenged 50% of DPPH free radical. The mean value for the three IC₅₀ values was calculated to be 6.4% and that of curcumin is 2225ug/ml.

The chelating and the total phenolic concentration of the aqueous extract of *Tetracarpidium conophorum* were found to be higher than that of curcumin. The values of chelating ability and total phenolic concentration were 97.36% and 2115ug/ml respectively for *Tetracarpidium conophorum* and 83.26% and 995ug/ml for curcumin.

**Table 3:** Pearson correlation between chelating ability and total phenolic concentration of aqueous extract of *Tetracarpidium conophorum*.

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Correlation coefficient (r)</th>
<th>T value</th>
<th>P(0.1)</th>
<th>P(0.05)</th>
<th>P(0.01)</th>
<th>P(0.001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.48</td>
<td>0.87</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>4</td>
<td>0.44</td>
<td>1.38</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>6</td>
<td>0.25</td>
<td>2.82</td>
<td>SG</td>
<td>SG</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>
A strong positive significant correlation was observed between the chelating ability and the total phenolic concentration of the plant extract at 8% concentration \( r = 0.89; P = 0.01 \) as shown in Table 3.

<table>
<thead>
<tr>
<th>Concentration (%</th>
<th>Correlation</th>
<th>T value</th>
<th>P(0.1)</th>
<th>P(0.05)</th>
<th>P(0.01)</th>
<th>P(0.001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0.89</td>
<td>7.03</td>
<td>SG</td>
<td>SG</td>
<td>SG</td>
<td>NS</td>
</tr>
<tr>
<td>10</td>
<td>-0.87</td>
<td>4.20</td>
<td>SG</td>
<td>SG</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 4: Pearson correlation between antioxidant activity and total phenolic concentration of aqueous extract of *Tetracarpidium conophorum*.

The correlation between antioxidant activity and the total phenolic concentration showed negative correlations at all concentrations of the aqueous extract of the plant [For 2% concentration, \( r = -0.25, P = 0.05 \), not significant and weak; for 6% concentration, \( r = -0.61, P = 0.05 \), high significant correlation]. Also, a strong non-significant positive correlation was observed between the antioxidant activity and total phenolic concentration of the aqueous extract of the plant \( r = -0.93; P = 0.05 \) (Table 4).

Table 5: Pearson correlation between chelating ability and antioxidant activity of aqueous extract of *Tetracarpidium conophorum*.

<table>
<thead>
<tr>
<th>Concentration (%</th>
<th>Correlation</th>
<th>T value</th>
<th>P(0.1)</th>
<th>P(0.05)</th>
<th>P(0.01)</th>
<th>P(0.001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.68</td>
<td>6.19</td>
<td>SG</td>
<td>SG</td>
<td>SG</td>
<td>SG</td>
</tr>
<tr>
<td>4</td>
<td>-0.55</td>
<td>1.62</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>6</td>
<td>-0.45</td>
<td>2.06</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>8</td>
<td>-0.41</td>
<td>4.08</td>
<td>SG</td>
<td>SG</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>10</td>
<td>0.44</td>
<td>8.08</td>
<td>SG</td>
<td>SG</td>
<td>SG</td>
<td>NS</td>
</tr>
</tbody>
</table>

A high positive significant correlation was also observed between the chelating ability and antioxidant activity of aqueous extract of the plant at 2% \( r = 0.68; P = 0.001 \) (Table 5).

**Discussion**

In this work, there is overwhelming evidence that the aqueous extract of *Tetracarpidium conophorum* (African walnut) shows high chelating ability in vitro. The maximum in vitro chelating ability of the plant extract is 97.4% at 2% concentration while the minimum in vitro chelating ability is 77.4% at 10% concentration. *T. conophorum* has more than 70% chelating ability on ferrous ion (500uM). Chelation property may afford protection against oxidative damage and iron-overload (Lai et al, 2001). Chelating ability of the plant extract investigate in the present work is dose-dependent. Chelating ability of plant extract provides a strategy to avoid free-radical generation and iron-overload by chelation of metal ion (Robak et al, 1985). Antioxidant action of phenolic compounds is due to their high tendency to chelate metals (Michalak et al, 2006). Phenolics possess hydroxyl group, able to particularly iron and copper (Jung et al, 2003).

This work also reveals that the aqueous extract of *Tetracarpidium conophorum* possesses high antioxidant activity in vitro. The maximum antioxidant activity is 71.9% at 8% concentration in vitro. Historically, natural products have been the most successful source of new drugs (Newman et al, 2003). Antioxidant activity of many plants is useful in unravelling its bioactivity (Babu et al, 2001). Sources of natural antioxidants are primarily plant phenolics which may occur in virtually all parts of plants (Pratt and Hudson, 1990). The reaction of DPPH is monitored by decrease of the absorbance of DPPH radical at 510nm but upon reduction by antioxidants, the absorption disappears (Brand-Williams et al, 1995). The position and degree of hydroxylation of phenolic compounds especially in the B-ring play a major role in antioxidant activity (Fukumoto and Mazza, 2000). The stable free radical DPPH method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extract (Koleva et al, 2002).

The mean IC50 value for three IC values for graded doses (2-10%, w/v) of aqueous extract of *Tetracarpidium conophorum* observed in this work is 6.4%. IC50 is the concentration of plant extract sufficient to obtain 50% of a maximum scavenging activity (Choi et al, 2002).
In conclusion, the aqueous extract of *Tetracarpidium conophorum* demonstrates dose-dependent decrease in chelating ability in vitro and that the positive correlations between chelating ability, antioxidant activity and total phenolics concentration depend on the dose of the plant extract used.

**References**


