In vitro Comparative Antioxidative Potentials of Mango and Pawpaw Leaf Extracts

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Abstract: The influence of concentration on hydroxyl radical scavenging and antioxidant activities of polyphenol extracts of Mango and Pawpaw leaves were assessed in vitro. The polyphenol extract from Mango leaves failed to scavenge hydroxyl radical at all the concentrations (50-250 μg mL⁻¹) while Pawpaw leaves failed to scavenge hydroxyl radical at three different concentrations out of five concentrations investigated (50-250 μg mL⁻¹). The polyphenol extract from Pawpaw leaves was a poor scavenger of hydroxyl radical in vitro (4.2% maximum scavenging activity). The polyphenol extracts of Mango and Pawpaw leaves exhibited weak antioxidant activities in vitro at all the concentrations investigated. Mango leaves had the highest total phenolic concentration (128 mg mL⁻¹) at the maximum extraction time (50 min). At 50 min extraction time both the aqueous extracts of the two plants demonstrated maximum antioxidants activity (86.95% for aqueous extract of Mangifera indica and 89.70% for Pawpaw aqueous extract). A non-significant moderate positive correlation was observed between total phenolic concentration and antioxidant activity of aqueous extract of Mangifera indica and that of Pawpaw leaves (r = 0.592; p = 0.05 for Mangifera indica; r = 0.469; p = 0.05 at 20 min extraction time).

Key words: Bioactive compound, scavenger, reactive oxygen, active principle, biomarker, phytoconstituents

INTRODUCTION

Many natural products have been reported to contain large amounts of antioxidants other than vitamin C, E and carotenoids (Javannardi et al., 2003). These antioxidants play a role in delaying intercepting, or preventing oxidative reactions catalysed by free radicals (Veligolu et al., 1998). This antioxidant activity may be mainly due to the presence of phenolic components such flavonoids (Pietta, 1998), phenolic acids and diterpenes (Shahidi and Wanasundara, 1992).

The pawpaw plant (Carica papaya) is widespread throughout tropical Africa; it belongs to the group (Caricaceae) (Starley et al., 1999). The bioactive compounds of C. papaya stems, leaves and fruits are papain, chymopapain, leukopapain and the alkaloidal compound, carpaine (Starley et al., 1999). Carica papaya extracts possess antibacterial (Emeruwa, 1982), anti-inflammatory activity (Gupta et al., 2000), antifertility (Udoh and Kehinde, 1999), anti-hypertensive agent (Eno et al., 2000) and anti-cancer (Kuwahara et al., 2004, Galati et al., 2000) properties. In addition, it possesses anti-ulcer (Hewitt et al., 2000), diuretic (Sripanidkulchai et al., 2001) and anti-sickling (Iyamu et al., 2002) effects. Mango (Mangifera indica) belongs to the family Anarcardiaceae. Mangifera indica is a large evergreen tree, which has been introduced wherever the climate is sufficiently warm and damp and is now completely naturalized in many parts of tropics and subtropics (Ross, 1999). The pharmacologically-active compound of Mangifera indica, mangiferin is widely distributed in the Anarcardiaceae and Gentianaceae families, especially in the leaves and the bark of Mangifera indica (Yoshimi et al., 2001).

Mangifera indica is used medically in some ailments such as asthma, cough, diarrhoea, dysentery and malaria (Madunagu et al., 1990). It possesses anti-inflammatory (Aggarwal et al., 2006, Brones et al., 2002), anti-tumor activity (Chen and Kong, 2005, Peng et al., 2004; Yoshimi et al., 2001), anti-diabetic, immunomodulatory (Christman et al., 2000), antiviral (Guha et al., 1996), antibacterial and antifungal (Stoilova et al., 2005) properties.

Known to the researchers, a comparative in vitro study of antioxidant potentials of polyphenol fractions of Mangifera indica and Carica papaya leaves have not been investigated to date. Therefore, this study was designed to: assess in vitro the hydroxyl radical
scavenging activity and antioxidant activity of polyphenol fractions of Mangifera indica and Carica papaya leaves (11) investigate the influence of extraction time on total phenolic concentration and antioxidant activity of aqueous extracts of both leaves of the two plants and (11) assess the relationship between the total phenolic concentration and antioxidant activity of the aqueous extracts of both leaves of the two plants.

**MATERIALS AND METHODS**

**Chemicals:** The chemicals used in this study were 2, 2-diphenyl-2-picrylhydrazyl (DPPH) (Sigma product), tannic acid (BDH), FeSO₄·7H₂O (BDH) and 1,10-phenanthroline (BDH).

**Collection of plant material:** The leaves of Mangifera indica were obtained in front of Physics Department of Ladoke Akintola University of Technology, Ogbomoso, Nigeria while the Carica papaya leaves was obtained from Ogbomoso North Local Government Area, Ogbomoso, Nigeria.

**Preparation of aqueous extracts:** The leaves were washed with distilled water and dried for 12 days and grounded to powder using blender. Aqueous extracts of the plant leaf powder were prepared by adding 50 mL of distilled water to 0.05 g of the powder (0.1%, w/v) and centrifuged (5000 rpm) at different time intervals (10, 20, 30, 40 and 50 min) for each 5 replicates.

**Preparation of the polyphenol fraction:** The polyphenol extract of Mango indica and Carica papaya leaves were prepared according to the method of Chu et al. (2002). About 25 g of the Mangifera indica leaf and 25 g of Carica papaya leaves powder were soaked in 75 and 100 mL of acetone, respectively, for 24 h and filtered. The filtrates were allowed to evaporate. The final residue obtained were the polyphenol contents of the two plants. They were weighed and found to be 2.2 g for Mangifera indica and 1.8 g for Carica papaya. Therefore percent yield was 8.8 and 7.29%, respectively. About 0.2 g of the polyphenol extracts were weighed and mixed with 20 mL of 70% ethanol for each. About 1 mL of these stocks were taken mixed with 9 mL of 70% ethanol to obtain 1000 μg mL⁻¹ stock from which different concentrations (50-250 μg mL⁻¹) were made for the two polyphenol extracts with 5 replicates for each concentration.

**Biochemical assays**

**Total phenolic estimation:** Total phenolic content was determined according to the method of Hung et al. (2002). The total phenolic content was determined using the Folin-Ciocalteu reagent. The phenolic compounds are oxidized to phenolates by the reagent at alkaline pH in a saturated solution of sodium carbonate resulting in a blue molybdenum-tungstate complex. About 0.5 mL of Folin-Ciocalteu (10%, w/v) was added to 0.1 mL sample, followed by the addition of 0.4 mL of aqueous Na₂CO₃ (7.5%, w/v). The mixture was allowed to stand in the dark for 30 min. The absorbance of the blue color solution was read at 765 nm on a UV visible spectrophotometer (Genesys 10vis, Thermo electronic corporation, USA) against blank (distilled water). Total phenolic concentration (mg mL⁻¹) of the sample was extrapolated from a standard curve, constructed using tannic acid as a standard.

**DPPH-based antioxidant activity estimation:** Antioxidant activity of the sample was estimated according to the method of Blois (1958). In the presence of an antioxidant, DPPH radical obtains one or more electron and the absorbance decreases. About 0.3 mL of 0.1 mM 70% methanolic DPPH solution was added to 0.1 mL of the sample in a test tube. The mixture was allowed to stand in the dark at room temperature for 30 min. The absorbance of the yellow colour solution was read at 517 nm on a UV/visible spectrophotometer (Genesys 10vis, Thermo electronic Incorporation, USA) after 30 min against the blank (distilled water).

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

Where:

- \( A_{\text{control}} = \) Absorbance of methanolic DPPH solution
- \( A_{\text{sample}} = \) Absorbance of sample in the presence of other reagents in the antioxidant activity assay

**Hydroxyl radical scavenging estimation:** The hydroxyl radical scavenging activity of the samples was determined according to the method of Yu et al. (2004). About 60 μL of aqueous FeSO₄·7H₂O (1 mM) was added 90 μL of aqueous 1, 10- phenanthenol. About 2.4 mL of 0.2 M Na₂HPO₄ (pH 7.8) was added the mixture followed by the addition of 150 μL of H₂O₂ (0.17M) and 1.5 mL of extract (50-250 μg mL⁻¹). The mixture was incubated for 5 min at room temperature. The absorbance of the mixture was read at 560 nm on a UV/visible spectrophotometer (Genesys 10vis, Thermo electronic Incorporation, USA) then using distilled water as blank.

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

Where:

- \( A_{\text{control}} = \) Absorbance of the control
- \( A_{\text{sample}} = \) Absorbance of sample
Phytochemical screening

Qualitative test for flavonoids: To 2 mL of the aqueous extracts of *Mangifera indica* and *Carica papaya*, 1 mL of conc. KOH was added to both extracts and the colour change from green to yellow. To the both aqueous extracts 0.5 mL of diluted ammonia was added followed by the addition of 0.2 mL of conc. H₂SO₄, which gave yellow colour indicating the presence of flavonoids.

Qualitative test for tannin: About 2 mL of ferric chloride (1% FeCl₃) was added to 2 mL of the both aqueous extracts. The colour changed to blue which shows the presence of tannin.

RESULTS AND DISCUSSION

The total phenolic concentration of *M. indica* was at highest level at 50 min extraction time with 128.20±22.00 mg mL⁻¹ and that of *C. papaya* at 30 min extraction time with 35.00±2.1 mg mL⁻¹. The lowest level *M. indica* and *C. papaya* was at 30 and 10 min with 29.20±16.39 mg mL⁻¹ and 25.00±2.0 mg mL⁻¹, respectively as shown in Table 1.

The total phenolic concentration of both *M. indica* and *C. papaya* was compared and it showed high phenolic concentration at 50 min of extraction time with 28.20±22.00 and 29.00±2.5 mg mL⁻¹ as shown in Fig. 1. *Magnifera indica* plant is a rich source of phenolic compound consistent with the findings of Schieber et al. (2003). Dried aromatic herbs are rich sources of antioxidants in particular from the group of phenolic compounds.

In this research, the leaves extract of *Magnifera indica* and *Carica papaya* revealed the presence of flavonoids, polyphenols and tannins. The antioxidant effect of plant products is mainly due to radical scavenging activity of phenolic compounds such as flavonoids, polyphenols and tannins (Rahman and Moon, 2007).

The antioxidant activity of both *Magnifera indica* and *Carica papaya* showed high antioxidant activity at the same time at 50 min of extraction time with 89.70±0.03 and 86.95±0.07. The antioxidant activity of *C. papaya* was at highest level at 50 min with 89.70±0.03 and that of *M. indica* was at 50 and 30 min with 86.95±0.03 and 86.95±0.07, respectively as shown in Table 1 and Fig. 2.

Phenolic antioxidants are potent free radical terminators. The high potential of phenolics to scavenge free radicals may be due to the many phenolic hydroxyl groups (Sawa et al., 1999). The aqueous extract of plants leaves demonstrated maximum antioxidants activity. Many plants extract exhibit efficient antioxidant properties due to their phytocomponents including polyphenols (Larson, 1998). The antioxidant activity of the phenol extract of *M. indica* has highest level at the dose of 100 μg mL⁻¹ with 13.96±14.92 while that of *C. papaya* was at dose of 150 μg mL⁻¹ as shown in Table 2.

Polyphenol is capable of acting as an antioxidant through many mechanisms available in vitro primarily as potent scavenger of free radical (Leiro et al., 2003). The antioxidant activity of both *M. indica* and *C. papaya* was low at the same dose of 100 (μg mL⁻¹) with 8.65±13.73 and 23.18±8.11 as shown in Fig. 3.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Total phenolic concentration (mg mL⁻¹)</th>
<th>Antioxidant activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>95.00±13.14</td>
<td>80.14±6.03</td>
</tr>
<tr>
<td>20</td>
<td>96.20±7.86</td>
<td>82.44±6.03</td>
</tr>
<tr>
<td>30</td>
<td>29.20±16.39</td>
<td>35.00±2.1</td>
</tr>
<tr>
<td>40</td>
<td>71.60±5.60</td>
<td>84.64±6.08</td>
</tr>
<tr>
<td>50</td>
<td>128.20±22.00</td>
<td>89.70±6.07</td>
</tr>
</tbody>
</table>

Values are mean ±SD of 3 analyses per time

Fig. 1: The bar chart of total phenolic concentration of both *M. indica* and *C. papaya*

Fig. 2: The bar chart of antioxidant activity of both *M. indica* and *C. papaya*
Table 2: Changes in the level antioxidant and hydroxyl radical scavenging activity of polyphenol extract of *Mangifera indica* and *Carica papaya*

<table>
<thead>
<tr>
<th>Concentration (µg mL⁻¹)</th>
<th>Antioxidant activity (%)</th>
<th>OH radical scavenging (%)</th>
<th>Antioxidant activity (%)</th>
<th>OH radical scavenging (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>13.96±14.92</td>
<td>-12.59±3.81</td>
<td>23.18±8.110</td>
<td>1.81±3.90</td>
</tr>
<tr>
<td>150</td>
<td>8.65±13.73</td>
<td>-28.97±6.900</td>
<td>35.59±8.100</td>
<td>-5.43±3.60</td>
</tr>
<tr>
<td>200</td>
<td>7.51±13.60</td>
<td>-27.49±3.640</td>
<td>11.67±11.34</td>
<td>-4.61±2.40</td>
</tr>
<tr>
<td>250</td>
<td>10.41±12.04</td>
<td>-39.51±5.930</td>
<td>6.74±8.000</td>
<td>-9.55±1.90</td>
</tr>
</tbody>
</table>

Values are mean±SD of 5 analysis per concentration.

Table 3: Phytochemical screening result of aqueous extracts of *Mangifera indica* and *Carica papaya*

<table>
<thead>
<tr>
<th>Phytoconstituent screenings</th>
<th><em>Mangifera indica</em> aqueous extracts</th>
<th><em>Carica papaya</em> aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids using</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>KOH</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Dilammonia + Conc. H₂SO₄</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Tannin using</td>
<td>1% FeCl₃</td>
<td>++</td>
</tr>
</tbody>
</table>

**++ indicating that the phytochemical is higher and positive; + is indicating that the phytochemical is low and positive**

Table 4: Pearson correlation between total phenolic concentration and antioxidant activity of aqueous extract of *Mangifera indica*

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Pearson correlation (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>10</td>
<td>0.646</td>
<td>-1.466</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>20</td>
<td>0.066</td>
<td>0.114</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>30</td>
<td>-0.139</td>
<td>-0.243</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>40</td>
<td>0.592</td>
<td>1.272</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>50</td>
<td>-0.256</td>
<td>-0.459</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 5: Pearson correlation between the total phenolic concentration and antioxidant activity of aqueous extract of *Carica papaya*

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Correlation (r)</th>
<th>t-values</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>10</td>
<td>0.476</td>
<td>0.937</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>20</td>
<td>0.469</td>
<td>0.920</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>30</td>
<td>0.157</td>
<td>0.275</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>40</td>
<td>0.255</td>
<td>0.457</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>50</td>
<td>0.434</td>
<td>0.834</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

CONCLUSION

In this study, the polyphenol extract from *Mangifera indica* did not scavenge hydroxyl radical at all concentrations investigated (50-250 µg mL⁻¹), while polyphenol extract from *Carica papaya* leaves also fail to scavenge hydroxyl radical at three different doses out of five doses investigated in *vitro*. The aqueous extracts of *Mangifera indica* had the highest total phenolic concentration (128 mg mL⁻¹) at the maximum extraction time (50 min).

At 50 min extraction time both the aqueous extracts of the two plants demonstrated maximum antioxidants activity (86.95% for aqueous extract of *Mangifera indica* and 89.70% for *Carica papaya* aqueous extract). A non significant moderate positive correlation was observed between total phenolic concentration and antioxidant activity of aqueous extract of *Mangifera indica* (r = 0.592, p = 0.05). There was a moderate non-significant correlation between total phenolic concentration and antioxidant activity of aqueous extract of *Carica papaya* (r = 0.469; p = 0.05) at 20 min extraction time.
REFERENCES


