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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 (Javanmardi *et al*., 2003). These antioxidants play a role in delaying intercepting, or preventing oxidative reactions catalysed by free radicals (Velioglu *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* L 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 Anacardiaceae and Gentianaceae families, especially in the leaves and the bark of *Mangifera indica* (Yoshimi *et al*., 2001).

Mangifera indica is used medically in some ailment such as asthma, cough, diaorrhea, dysentry and malaria (Madunagu *et al*., 1990). It possesses anti-inflammatory (Aggarwal *et al*., 2006; Briones *et al*., 2002), anti-tumor a (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 antioxidative potentials of polyphenol fractions of *Mangifera indica* and *Carica papaya* leaves have not been investigated to date in Therefore, this study was designed to: assess *in vitro* the hydroxyl radical

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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 (111) 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), $FeSO_4.7H_2O$(BDH) and 1,10-phenanthroline (BDH).

Collection of plant material: The leaves of *Mangifera indica* was 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 extracts of *Mangifera 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 7.2%, 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-Ciocalteau (10%, w/v,) was added to 0.1 mL sample, followed by the addition of 0.4 mL of aqueous Na_2CO_3 (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 (Genesy 10vis, Thermoelectric 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 (Genesy 10vis, Thermoelectronic Incorportion, USA) after 30 min against the blank (distilled water).

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

Where:

$A_{control}$ = Absorbance of methanolic DPPH solution

A_{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 $FeSO_4.7H_2O$ (1 mM) was added 90 μL of aqueous 1, 10- phenanthroline. About 2.4 mL of 0.2 M Na_2HPO_4 (pH 7.8) was added the mixture followed by the addition of 150 μL of H_2O_2 (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 (Genesy 10vis, Thermoelectronic Incorportion, USA) then using distilled water as blank.

$$\text{Hydroxyl radical scavenging } (\%) = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

Where:

$A_{control}$ = Absorbance of the control

A_{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 aaded followed by the addition of 0.2 mL of conc. H_2SO_4 which gave yellow colour indicating the presence of flavonoids.

Qualitative test for tannin: About 2 mL of ferric chloride (1% $FeCl_3$) 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.

Table 1: Changes in the level total phenolic concentration and antioxidant activity of aqueous extract of *Magnifera indica* and *Carica papaya*

	Mangifera indica		*Carica papaya*	
Time (min)	Total phenolic concentration (mg mL^{-1})	Antioxidant activity (%)	Total phenolic concentration (mg mL^{-1})	Antioxidant activity (%)
10	99.80±19.14	80.14±0.03	25.00±2.0	83.42±0.07
20	96.20±7.800	82.44±0.03	25.40±2.7	84.85±0.03
30	29.20±16.39	86.95±0.03	35.00±2.1	86.12±0.02
40	71.60±5.600	84.64±0.08	23.20±1.7	84.64±0.03
50	128.20±22.00	86.95±0.07	29.00±2.5	89.70±0.03

Values are mean +/-SD of 5 analyses per time

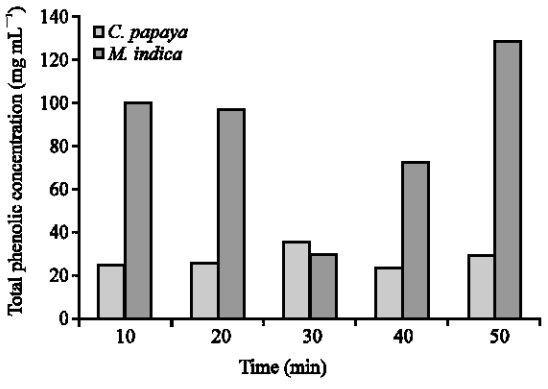

Fig. 1: The bar chart of total phenolic concentration of both *M. indica* and *C. papaya*

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 *Mangifera 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 30 and 50 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 phtocontituents including phenolics (Larson, 1998). The antioxidant activity of the polphenol 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.

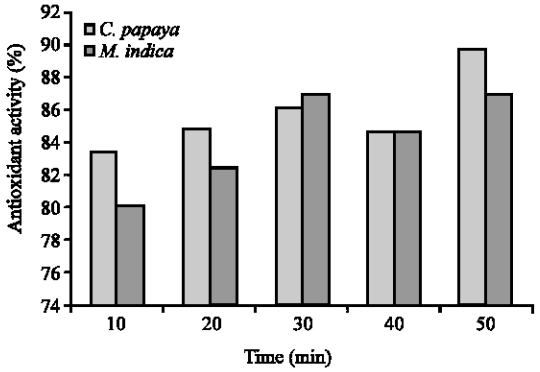

Fig. 2: The bar chart of antioxidant activity of both *M. indica* and *C. papaya*

Table 2: Changes in the level antioxidant and hydroxyl radical scavenging activity of polphenol extract of *Mangifera indica* and *Carica papaya*

	Polyphenol extract of *Mangifera indica* leaves (%)		Polyphenol extract of *Carica papaya* leaves (%)	
Concentration (µg mL^{-1})	Antioxidant activity	OH radical scavenging	Antioxidant activity	H radical scavenging
50	12.16±10.61	-13.25±12.22	21.22±6.750	4.20±7.00
100	13.96±14.92	-12.59±13.81	23.18±8.110	1.81±3.90
150	8.65±13.73	-28.97±6.900	35.59±8.100	-5.43±3.60
200	7.51±13.60	-27.49±3.640	11.67±11.34	-4.61±2.40
250	10.41±12.04	-39.51±5.930	6.74±8.000	-9.55±1.90

Values are mean±SD of 5 analysis per concentration

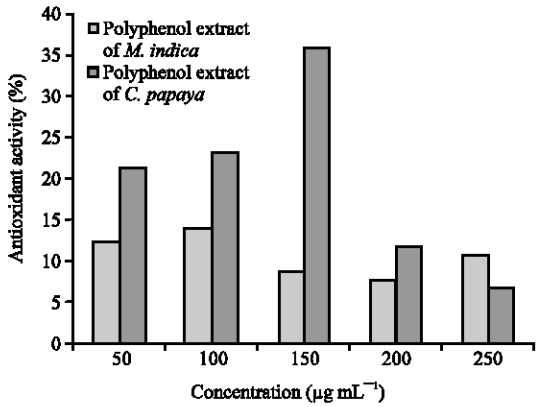

Fig. 3: The bar chart of antioxidat of polyphenol extract of *M. indica* and *C. papaya*

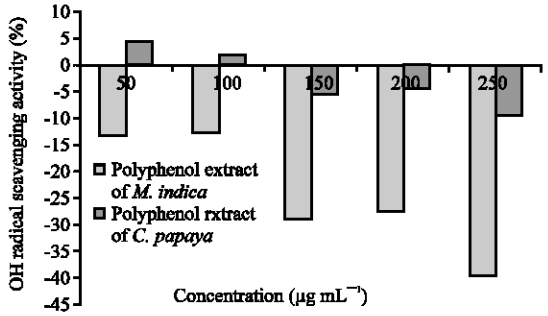

Fig. 4: The bar chart of OH redical scavenging activity of polyphenol extract of both *M. indica* and *C. papaya*

The polyphenol extract of *M. indica* produced hydroxyl radical at all doses as shown in Fig. 4. The polyphenol extract from *Mangifera indica* did not scavenge hydroxyl radical at all doses investigated (50-250 µg mL^{-1}). Medicinal plants are a potential source of antioxidant and Reactive Oxygen Species (ROS) scavenger molecules (Arora *et al*., 2005) (Table 3-5). Due to the presence of conjugated ring structures and hydroxyl groups, most phenolic compounds have potential to function as antioxidants by scavenging hydroxyl radical (Schieber *et al*., 2000).

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

Phytoconstituent screenings	*Mangifera indica* aqueous extracts	*Carica papaya* aqueous extract
Flavonoids using		
KOH	++	+
Dil.ammonia + Conc. H_2SO_4	++	+
Tannin using		
1% $FeCl_3$	++	+

++ 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*

Time (min)	Pearson correlation (r)	t-value	p (0.1)	p (0.05)	p (0.01)	p (0.001)
10	-0.646	-1.466	NS	NS	NS	NS
20	0.066	0.114	NS	NS	NS	NS
30	-0.139	-0.243	NS	NS	NS	NS
40	0.592	1.272	NS	NS	NS	NS
50	-0.256	-0.459	NS	NS	NS	NS

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

Time (min)	Correlation (r)	t-values	p (0.1)	p (0.05)	p (0.01)	p (0.001)
10	0.476	0.937	NS	NS	NS	NS
20	0.469	0.920	NS	NS	NS	NS
30	0.157	0.275	NS	NS	NS	NS
40	0.255	0.457	NS	NS	NS	NS
50	0.434	0.834	NS	NS	NS	NS

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.

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