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Phytochemical and antioxidant evaluation of *Moringa oleifera* (Moringaceae) leaf and seed

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Abstract
Moringa oleifera, a plant with a wide range of medicinal, nutritional and economic benefits was examined for phytochemicals and evaluated for antioxidant activities. Phytochemical tests, total phenol and flavonoid contents were determined using standard procedures. Antioxidant activities of the methanol extracts and fractions were evaluated using the 2,2-diphenyl-1-picryl hydrazyl radical scavenging assay. The leaf and seed contained alkaloids, saponins, phenols and flavonoids. The ethyl acetate fraction of the leaf contained significantly ($P < 0.05$) more phenol (78.67 ± 3.40 mgGAE/g Extract) than the crude extract and petroleum ether fraction with total phenolic content of 58.00 ± 1.00 and 26.17 ± 3.88 mg GAE/g Extract respectively while the seed extract had a total phenol content of 17.67 ± 2.02 mg GAE/g Extract. The petroleum ether fraction of the leaf was found to contain significantly ($P < 0.05$) more flavonoid (161.33 ± 19.05 mgQE/g Extract) than the other samples (31.73 ± 2.66, 30.20 ± 10.14 and 20.73 ± 4.16 mgQE/g Extract for the crude leaf extract, leaf petroleum ether fraction and seed extract respectively). The leaf and seed extracts exhibited remarkable and concentration-dependent increase in radical scavenging activities with IC_{50} values ranging from 5.72-42.56 µg/mL. The results therefore support the use of *Moringa oleifera* as a natural plant antioxidant by preventing free radical damage.

Keywords: *Moringa oleifera*; Antioxidant; Phytochemical, Free radical

INTRODUCTION

Oxidative reactions are common in living systems, these reactions which most frequently occur during cellular metabolism and respiration can produce free radicals such as the reactive oxygen and reactive nitrogen species (Cadenas, 1989; Davies, 1995; Victor *et al*., 2006). These radicals often initiate chain reactions that are often toxic to the cells causing several deteriorating effects such as DNA damage, lipid peroxidation, tissue injury and protein degradation which are major contribution to a number of diseases like cancer, arthritis, neurodegenerative disorders, atherosclerosis and aging (D'Angio and Finkelstein, 2000; Rahman *et al.*, 2001; Praveen and Awang, 2007; Chun-Weng *et al*., 2011). The harmful effects of these free radicals causing potential biological damage is termed oxidative stress and occur when the production of these free radicals overwhelm the body's ability to defend against them (Kovacic and Jacintho, 2001; Ridnour *et al*., 2005; Zima, 2001; Valko *et al.*, 2001). The

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body is endowed with a number of antioxidant defense mechanisms such as superoxide dismutase, thioredoxin, catalase, glutathione, uric acid, ascorbic acid that help mop-up free radicals and terminate the chain reactions they initiate (Cadenas, 1997). In recent time, there have been increasing interests in natural antioxidants that may oppose these free radicals with a view of lowering the risk of diseases associated with them. Of such reservoirs of natural antioxidants are products of plant origin.

Moringa oleifera Lam. (Moringaceae) is a tree growing up to 5-12 m with an open umbrella-shaped crown native to India, Africa, Arabia, Southeast Asia, South America, and the Pacific and Caribbean Islands (Iqbal *et al.,* 2006). The species is cultivated in many tropical and sub-tropical regions worldwide, where it is known by various vernacular names as horseradish tree, drumstick tree, bean oil tree, miracle tree, and "Mother's Best Friend" (Shindano and Chitundu, 2008). Various parts of the plant are used culturally for its nutritional and medicinal values. The leaves can be eaten fresh, cooked, or stored as a dried powder for later use as a food flavoring or additive (Abilgos *et al.,* 1999; Lockett *et al.,* 2000; Fahey, 2005; Arabshahi-D *et al.,* 2007, Abdukarima *et al*., 2007) and also as animal feed (Makkar and Becker, 1996; Sarwatt *et al.,* 2002; Soliva *et al*., 2005; Fahey, 2005; Sáncheza *et al.,* 2006; Makkar *et al*., 2007). Studies have indicated that *M*. *oleifera* leaves are a good source of nutrition (Gomez-Conrado *et al.,* 2004; Lako *et al.,* 2007) and exhibit anti-tumor, anti-inflammatory, anti-ulcer, anti-atherosclerotic and anticonvulsant activities (DanMalam *et al.,* 2001; Dahiru *et al.,* 2006; Chumark *et al.,* 2008). The present study will quantify the polyphenolic content (total phenol and flavonoid) and investigate the free radical scavenging activity *viz a viz* the antioxidant activity of the methanol extracts of the leaf and seed of the plant.

EXPERIMENTAL

Plant collection and preparation. Fresh Moringa leaves were collected from Songhai Delta, Amukpe, Delta State in January, 2013. The dried seeds were obtained from a local herbal market in Benin City. The plant materials were identified in the department of Plant Biology and Biotechnology, University of Benin. The leaves were dried at ambient temperature and the samples were reduced to coarse powder by means of mechanical grinder.

Extraction of crude powdered sample. The powdered plant materials (200 g each) were extracted with 1 L of methanol by maceration at room temperature for 5 days. The leaf extract was partitioned using petroleum ether and ethyl acetate to obtain petroleum ether fraction, ethyl acetate fraction and the residual aqueous-methanol fraction. All extracts and fractions were concentrated *in vacuo* and refrigerated at 4^{o}C until use.

Phytochemical screening. Simple chemical tests to detect the presence of alkaloids, tannins, saponins, carbohydrates, reducing sugars, proteins, flavonoids and other phenolic compounds were done in accordance with standard methods (Sofowora, 1982; Evans, 2002).

Determination of antioxidant activity: DPPH radical scavenging assay. The scavenging effect of crude methanol extracts of *Moringa oleifera* leaf and seed and fractions of the leaf extract on DPPH radical was estimated according to the method previously described by Jain *et al.* (2008). A solution of 0.1 mM DPPH in methanol was prepared, and 1.0 mL of this solution was mixed with 3.0 mL of extract in methanol containing 0.001 - 0.2 mg/mL of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm.

Ascorbic acid was used as reference standard. The ability to scavenge DPPH radical was calculated by the following equations:

DPPH radical scavenging activity (%) =
$$[(A_0-A_1)) / (A_0)] \times 100$$
Where A_0 was the absorbance of DPPH radical + methanol; A_1 was the absorbance of DPPH radical + sample extract / standard.

The 50% inhibitory concentration value (IC-50) is indicated as the effective concentration of the sample that is required to scavenge 50% of the initial DPPH free radicals (Jain *et al*., 2008).

Determination of total phenol content. Total phenol contents in the extracts and fractions were determined by the method described by Kim *et al.* (2003). The extract solution (0.5 mL) with a concentration of 1 mg/mL was added to 4.5 mL of deionized distilled water and 0.5 mL of Folin Ciocalteu's reagent (previously diluted with water 1:10, v/v) which was then added to the solution. After mixing the tubes, they were maintained at room temperature for 5 minutes followed by the addition of 5 mL of 7% sodium carbonate and 2 mL of deionized distilled water. After mixing the samples, the samples were incubated for 90 minutes at room temperature. The absorbance was measured by spectrophotometer at 750 nm. The total phenol content was expressed as milligram of gallic acid equivalents (GAE) per gram of extract (mg GAE/g extract). The standard curve was prepared by gallic acid in six different concentrations (12.5, 25, 50, 75, 100 and 150 mg/L).

Determination of total flavonoid content. Total flavonoid contents were estimated according to the method of Ebrahimzadeh *et al.* (2008). Briefly, 0.5 mL of extract sample (1 mg/mL) was mixed with 1.5 mL of methanol and then, 0.1 mL of 10% aluminium chloride was added, followed by 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured by a spectrophotometer at 415 nm. The results were expressed as milligram quercetin equivalents (QE) per gram of extract (mgQE/g extract). The standard curve was prepared by quercetin in six different concentrations (12.5, 25, 50, 75, 100 and 150 mg/L).

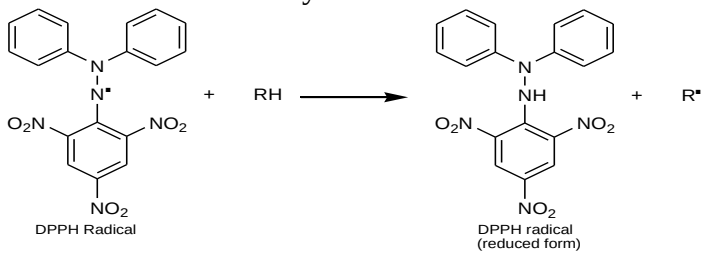
Statistical analysis. The experimental results were expressed as mean ± standard error of mean (SEM) of three replicates. Statistical comparison was established by one-way analysis of variance (ANOVA) and P-value < 0.05 represent significant difference between mean at 95% confidence interval.

RESULTS AND DISCUSSION

Phytochemical analysis. Phytochemical screening have shown the presence of phenols, flavonoids and saponins in the leaves and seeds, alkaloids in the seeds, while tannins and phytosterols where absent in both seeds and leaves (Table 1).

Antioxidant activity. The result of DPPH radical scavenging activity showed that *Moringa oleifera* leaf and seed extracts have appreciable and concentration-dependent increase in scavenging effect with the ethyl acetate fraction of the leaf being the most active. At the highest concentration (200 µg/mL), the percentage inhibition include 71.67 ± 2.79, 66.70 ± 3.93, 69.86 ± 0.86, 90.83 ± 0.34 for methanol extract of the leaf, methanol extract of the seed, petroleum ether fraction and ethyl acetate fraction of the leaf respectively, whereas the reference standard (ascorbic acid) had a percentage inhibition of 98.33 ± 0.74 at 200 µg/mL (table 2). The IC_{50} value denotes the concentration that will scavenge 50% of the initial DPPH radical. Evaluation of the IC_{50} as shown in table 3 demonstrated that the ethyl acetate fraction exhibits the highest antioxidant activity with IC_{50} value of 5.72 µg/mL. This was fairly low but significantly different from that of the reference standard (2.10 µg/mL). The

observed result was similar to the findings of Atawodi *et al*. (2010). The DPPH radical is one of the few stable organic nitrogen radicals, with a deep purple colour. It is the most commonly used measure for the antioxidant activity due to its ease of assay. The assay is based on the measurement of the reducing ability of antioxidants towards DPPH. This ability can be evaluated by electron spin resonance (ESR) spectrometry or by measuring the decrease of its absorbance at about 517 nm (Prior, 2005).



Scheme 1: The reaction of DPPH with antioxidant compound RH

Table 1: Phytochemical composition of *Moringa oleifera* leaf and seed.

Phytochemical	Inference	
	Leaf	Seed
Alkaloids	_	+
Carbohydrates	+	+
Reducing sugars	_	+
Saponins	+	+
Phytosterols	_	_
Tannins	_	_
Phenolic compounds	+	+
Flavonoids	+	+
Proteins	_	+

+ indicates presence - indicates absence of component

Table 2: DPPH-scavenging activity of crude and fractionated extracts at various concentrations

Concn (µg/mL)	Percentage Inhibition				
	Ascorbic acid	Crude Methanol extract (leaf)	Crude Methanol extract (seed)	Petroleum ether fraction (leaf)	Ethyl acetate fraction (leaf)
1	32.77 ± 6.14	21.66 ± 3.28	61.67 ± 7.85	41.39 ± 0.71	49.72 ± 2.83
2	38.47 ±11.98	34.72 ± 0.52	58.50 ± 9.05	42.78 ± 2.19	34.17 ± 2.38
5	91.39 ± 5.28	42.50 ± 1.35	53.50 ± 7.60	43.33 ± 2.57	49.45 ± 1.99
10	98.75 ± 0.59	62.92 ± 4.50	51.90 ± 9.80	49.17 ± 2.07	49.44 ± 1.93
25	97.08 ± 0.34	74.86 ± 1.42	57.30 ± 17.23	56.25 ± 1.56	62.33 ± 2.14
50	99.17 ± 0.34	81.11 ± 2.51	52.40 ± 7.67	53.89 ± 0.52	76.25 ± 2.23
100	97.92 ± 0.90	81.81 ± 1.68	64.70 ± 4.65	67.08 ± 0.34	89.58 ± 0.34
200	98.33 ± 0.74	71.67 ± 2.79	66.70 ± 3.93	69.86 ± 0.86	90.83 ± 0.34

Data represent Mean ± Standard Deviation of triplicate analysis.

Table 3: IC_{50} values of methanol extract of *Moringa oleifera* leaf and seed and the reference standard (ascorbic acid).

Sample	IC_{50}
Ascorbic acid	2.10 µg/mL
Crude methanol extract (leaf)	35.42 µg/mL
Crude methanol extract (seed)	91.13 µg/mL
Petroleum ether fraction (leaf)	42.56 µg/mL
Ethyl acetate fraction (leaf)	5.72 µg/mL

Table 4: Total Phenol and total flavonoid content of *Afzelia bella*

	Crude methanol extract (leaf)	Crude methanol extract (seed)	Petroleum ether fraction (leaf)	Ethyl acetate fraction (leaf)
Total phenol (mg GAE/g extract)	58.00 ± 1.00	17.67 ± 2.02	26.67 ± 3.88	78.67 ± 3.40
Total flavonoid (mg QE/g extract)	31.73 ± 2.66	20.73 ± 4.16	161.33 ± 19.05	30.20 ± 10.14

Data represent Mean ± Standard deviation of triplicate analysis

The scheme below shows the reaction of DPPH radical with an hypothetical antioxidant molecule RH, the reaction is considered to be mainly based on an electron transfer (ET) while hydrogen-atom abstraction is regarded a marginal reaction pathway (Ou, 2005).

Total phenol and flavonoid contents. Table 4 shows the total phenol and flavonoid contents of *Moringa oleifera* leaf and seed. Total phenol content were reported as mg gallic acid equivalent/g of extract by reference to a standard curve ($y = 0.002x + 0.002$; $R^2 = 0.997$). The total flavonoid content were reported as mg quercetin equivalent/g of extract by reference to a standard curve ($y = 0.005x + 0.003$; $R^2 = 0.996$). The result shows that total phenol and flavonoid contents were significantly ($P < 0.05$) higher in the leaf extract (78.67 ± 3.40 mgGAE/g extract) than the seed extract (and17.67 ± 2.02 mgGAE/g extract). Fractionation of the leaf extract shows the ethyl acetate portion of the leaf extract to contain more phenol than the ether soluble portion. On the other hand, evaluation of the total flavonoid content showed that the ether soluble portion was found to contain more flavonoid than the ethyl acetate soluble portion. Solvent extraction has been found to play significant role in the antioxidant activity of extracts depending on their phenolic content (Horax, 2005; Erkan, 2008). The presence of hydroxyl (OH) groups in the phenolic compounds may directly contribute to their antioxidant activity and may be a key determinant of their radical scavenging (Sathishsekar and Subramanian, 2005). However, in the present study, we found no significant correlation between the observed antioxidant activity and the content of phenolic and flavonoid compounds in *Moringa oleifera* leaf and seed ($r^2 = 0.790$, $P = 0.111$ and $r^2 = 0.007$, $P = 0.914$).

Conclusion

The leaves and seeds of *Moringa oleifera* contained high levels of phenols and which may be key determinant of the antioxidant activity. These findings together with previous studies demonstrate that *Moringa oleifera* is an excellent plant candidate to be used to improve the health and nutrition of communities and also appears to be a promising candidate from which specific nutraceuticals and bioactive products could be developed.

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