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Nutritional Composition and Antioxidant Capacity of *Moringa Oleifera* Seeds, Stem Bark and Leaves

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Abstract

Moringa oleifera tree has attracted a lot of attention in the recent past with a lot of acclaimed nutritional and health benefits. This work set out to verify some of these claims and the nutritional composition and antioxidant capacity of the seeds, stem bark and leaves of the plant (*Moringa oleifera*) were evaluated. Results of the proximate analysis for the plant parts recorded ash content in the range 4.00 - 12.13 %, protein 7.53 - 38.23 %, moisture 29.7 - 76.48 %, crude fat 8.70 - 25.8 % and crude fibre 21.1- 27.7 %. Vitamin C content of the plant ranged from 7.85 mg/g in the stem bark to 14.42 mg/g in the seeds and 19.34 mg/g in the leaves. Mineral contents were in the range: 4.50 mg / kg - 13.7 mg/kg for copper, 19.7513.7mg/kg-73.25 mg/kg for zinc, 0.00 mg/kg- 0.25 mg/kg for cadmium and 11.75 mg/kg- 23.5 mg/kg for nickel. Lead was not detected in any of the parts. The antioxidant activities of the three parts, determined by DPPH scavenging capacity, showed that at low concentration of extract (5 µg/ml), the seeds have the highest antioxidant activity (64.53 ± 1.96 % inhibition) while the leaves have the highest activity (82.79 ± 0.79% inhibition) at a high extract concentration (125 µg/ml). However, none of the plant parts is as active as the standard antioxidant (BHA) with antioxidant activity of 96.30 – 99.81 %.

Key words: *Moringa oleifera*, nutritional composition, antioxidant activity.

1. Introduction

Over the years, researchers have become convinced that nutrients found in vegetables do more than just prevent deficiency diseases such as beriberi or rickets. In addition to vegetables being a major source of vitamins and micro-nutrients needed for the normal functioning of the human body physiology, they also serve as good source of antioxidants. There is a vast body of literature accumulated over the years suggesting that adequate intake of fruits and leafy vegetables form an important part of a healthy diet and low fruit and vegetable intake constitute a risk factor for chronic diseases such as cancer, coronary heart disease (CHD), stroke and cataract formation (Van Duyn and Pivonka, 2000).

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The generation of free radicals is a vital phenomenon of the normal metabolism of human body. Varieties of free radicals are generated as a byproduct of cellular functions, and normally, all these free radicals are neutralized by the enzymatic and non-enzymatic antioxidants present as an inbuilt antioxidant mechanism in the body and also by the dietary antioxidants supplied in daily diets through fruits and vegetables (Jang *et al*., 2008). Most diseases today are caused by oxidative stress. Accelerated cell oxidation contributes to cardiovascular disease, tumor growth, wrinkled skin, cancer, Alzheimer's disease, and even a decline in energy and endurance (Fresquet *et al*., 2006). The antioxidants play a vital role in delaying, intercepting or preventing oxidative reactions catalyzed by free radicals (Velioglu *et al*., 1998). They also help prevent molecular damage caused by oxidation and the protection offered may help fend off many diseases such as cancer, cardiovascular diseases and muscular degeneration (Islam *et al*., 2002).

Moringa oleifera (Drum stick) is considered as one of the world's most useful trees, as almost every part of the Moringa tree can be used for food, medication and industrial purposes (Khalafalla *et al*., 2010). People use its leaves, flowers and fresh pods as vegetables, while others use it as livestock feed (Anjorin *et al*., 2010). This tree has the potential to improve nutrition, boost food security and foster rural development (Hsu, 2006). *Moringa oleifera* tree is one of the most investigated plants in recent times; the leaves can be eaten raw, cooked or dried and are believed to be rich in biologically active carotenoids, tocopherols and vitamin C which have health-promoting potentials in preventing free-radical damage that can initiate many illnesses (Smolin and Grosvenor, 2013). The whole seeds can also be eaten green, roasted or powdered, or steamed in tea and curries (Fahey, 2005), while the corky stem bark yields a coarse fiber which is utilized in making mat, paper and cordage. It exudes a mucilaginous gum that is used in leather tanning and calico printing (Nautical *et al*., 1987). However, there still exist lack of adequate, reliable information on the nutritional composition and antioxidant capacity of the "miracle plant". This work therefore aims to evaluate the nutritional composition and antioxidant potential of the different parts- leaves, seeds and stem bark of the *Moringa oleifera* plant.

2. Materials and Methods

Collection and preparation of samples

The leaves, stem bark and seeds of *Moringa oleifera* (Drum Stick) were collected from various locations in Ilorin metropolis Kwara State, Nigeria and were identified by a taxonomist in the Department of Plant Biology of the University of Ilorin, Nigeria. The leaves were destalked, rinsed and air-dried with constant turning of the vegetable leaves to avert fungal growth. The seeds were dried through the same process. The stem bark was also air-dried for a period of five (5) weeks before homogenizing using a mortar and pestle. The leaves, stem bark and seeds were later milled using an electric blender and were stored in well labeled air-tight containers and refrigerated prior analysis.

Nutritional composition, Mineral analysis and Antioxidant activity

The proximate analysis – moisture and protein (AOAC, 1984), fat (AOAC, 2000), crude fibre (AOAC, 1990) and ash contents (Indrayan *et al*., 2005 and Gordon *et al*., 1990), were carried out by various standard methods. Vitamin C (Ascorbic acid) content was determined by the method of Ayekyaw (1978).

Mineral analysis was carried out by the triple acid digestion method using nitric acid (HNO_3), sulphuric acid (H_2SO_4), and 60 % perchloric acid ($HClO_4$) (9:2:1 v/v). The digest was analyzed for minerals using Atomic Absorption Spectroscopy (Sahrawat *et al.*, 2002). Antioxidant activities were carried out by the DPPH radical scavenging activity assay (Khalaf *et al*., 2008). All determinations were carried out in triplicates and all data were subjected to the analysis of variance and significant difference was recorded at the 0.05 level. SPSS Version 20.0 (IBM Corp, Armonk, NY) was used for the statistical analysis.

3. Result and Discussion

Table 3.1: Nutritional analysis (g/100g) of different plant parts Moringa *oleifera.*

sample	%moisture	%Ash	%Crude fat	%Crude fibre	%Protein
Seeds	29.70± 5.12[a]	4.00±1.00[a]	25.80±0.04[c]	21.10±2.4[c]	38.23±0.59[c]
Stem bark	76.48±1.05[b]	12.13±4.63[b]	8.70±0.08[a]	27.70±3.03[c]	7.53±0.90[a]
Leaves	73.66±0.57[b]	6.33±4.51[a, b]	16.88±1.26[b]	25.85±5.55[c]	12.97±1.39[b]

Results are means ± s.d. of triplicate determinations. Values in the same column having the same letter superscript are not significantly different at $p < 0.05$. NB: Moisture was done on wet basis while other parameters were determined based on the dry matter.

Table 3.1 shows the proximate composition of the seeds, stem bark and leaves samples. The stem bark was highest in moisture, ash and crude fibre while the seeds were lowest in the same parameters. The highest protein and fat contents were however observed in the seeds while the stem bark had the lowest fat and protein contents. Values recorded for the leaves were somewhere in between the values recorded for seeds and stem bark. The moisture content ranged from 29.70% in the seeds to 76.48% in the stem bark. Moyo *et al*. (2011) reported 9.53% moisture content in dry Moringa leaves. However, the 73.66% reported in this study is very similar to the 76.53% moisture reported for wet moringa leaves by Oduro *et al*. (2008). The three parts contain a considerable amount of ash suggesting a high mineral composition in the various plant parts.

Aja *et al*. (2013) reported the ash content of *Moringa oleifera* seeds and leaves to be (5.00±0.05 and 10.0±0.05%) respectively. Odura *et al*. (2008), also reported the ash content of Moringa leaves to be (7.13±0.03%) while Alikwe and Omotosho (2013) and Karuna *et al*. (2014) reported the ash content of *Moringa oleifera* leaf meal to be 13.67±0.01 and 12.23±0.70 respectively. Also, the crude fat of the stem bark is half that of the leaves while the seeds had the highest value. Aja *et al*. (2013) reported the crude fat content of *Moringa oleifera* leaves to be 20.0±0.5%. Karuna *et al.* (2014) also reported the lipid content of *Moringa oleifera* stem bark and seeds to be 17.47±0.35 % and 16.05±0.25 %; these values correlate with the results obtained in this work. Fats are essential in the diet and increase the palatability of food. Crude fats are principal sources of energy but should not exceed the daily recommended dose of 30 calories as to avoid obesity and other related diseases. The seed contains the highest protein content compared with the leaves and stem bark but it is the lowest in crude fibre while the stem bark was highest in crude fibre but lowest in protein. The leaves were higher than the stem bark in protein content but lower in crude fibre. Karuna *et al*. (2014) reported crude fibre contents in stem bark, leaves and seeds to be: 25.73±0.15, 22.90±0.25 and 16.27±0.25% respectively. Crude fibre in diet is known to enhance digestibility, reduce the blood cholesterol and reduce the risk of cancer. Dietary fibre also plays an important role in decreasing the risk of many disorders such as constipation, diabetes, cardiovascular diseases and other health challenges (Slinkard and Singleton, 1977).

The high protein content of the seeds may suggest that the seeds can be used as protein supplements for other food types. Protein deficiency plays a part in the disease called Kwashiorkor (Jeffery *et al.*, 2003) with symptoms including apathy, diarrhea, inactivity, failure to grow, flaky skin, fatty liver, and edema of the belly and legs. Protein deficiency can also lead to poor growth and body development (Jeffery *et al.*, 2003). Hence, the importance of protein in diets cannot be over-emphasized.

Estimation of Vitamin C (Ascorbic Acid)

Table 3.2: Vitamin C content of *Moringa oleifera*.

sample	Vitamin C (mg/g)
Leaves	19.34^{c} ±0.26
Seeds	14.42^{b} ±2.03
Stem bark	7.85^{b} ±0.54

Data are means ± standard deviation of triplicate determinations

Moringa leaves have the highest vitamin C content while the stem bark has the lowest content of Vitamin C and all the values are significantly different from each other. Vitamin C is an anti-scurvy vitamin. It facilitates the transformation of cholesterol into bile acid in the liver. The presence of vitamin C hastens the healing of wounds and it enhances the absorption of iron thus it has a role in reducing iron deficiency and anemia (Potter and Hotchkiss, 1995). The leaves contain high content of ascorbic acid in the leaves make it a possible food supplement that can be incorporated into infant formulae and weaning foods especially in countries where the rate of infant mortalities are high as a result of malnutrition, kwashiorkor and scurvy.

Vitamin C participates in many reactions by donating electrons as hydrogen atoms. In a reducing reaction, the electron in the hydrogen atom donated by vitamin C combines with other participating molecules, making vitamin C a reducing agent, essential to the activity of many enzymes. By neutralizing free radicals, vitamin C may reduce the risk of heart disease, certain forms of cancer, and cataracts (Kushi *et al.*, 1996 and Johnston, 2001). Vitamin C is needed to form and maintain collagen, a fibrous protein that gives strength to connective tissues in skin, cartilage, bones, teeth, and joints.

There are insufficient data to establish a tolerable upper intake level for vitamin C, but in 1991, COMA recommended a daily intake of 40 mg/day of ascorbic acid for adults, with an increase in pregnancy to 50 mg/day, and during lactation to 70 mg/day (COMA, 1991).

Mineral Analysis

Table: 3.3a: Macro elements of *Moringa oleifera* (g/kg).

Sample	Na	K	Ca	Mg	Fe
Seeds	8.880	9.518	0.914	2.240	0.145
Stem bark	12.950	12.960	23.377	3.377	0.183
Leaves	16.095	17.620	236.253	38.751	0.189

The mineral analysis (Table 3.3a) shows a high concentration of sodium, potassium, calcium, magnesium and iron in moringa leaves. The three parts (seeds, stem bark and leaves) contain considerable amounts of macro nutrients but in varying concentrations. The leaves are superior in mineral contents to the stem bark and seeds, suggesting that the leaves can contribute significantly to the nutrient requirements of human and should be recommended in diets.

The body requires a large quantity of sodium in order to maintain acid-base balance, osmotic balance between cells and interstitial fluid and nerve functions. (Akpabio *et al.*, 2012). The recommended daily allowance of sodium is 115-750 mg/kg body weight /day for infants, 324-975 mg/kg/ body weight/ day for children and 1100-3300 mg/kg body weight/ day for adults (Akpabio *et al.,* 2012). The body requires potassium in a large quantity for the maintenance of acid-base balance, body water balance and nerve function and its absence may result in muscular weakness and paralysis. The recommended daily allowance for an adult in good health is 2,500 mg/kg body weight/day (Akpabio *et al.,* 2012). Calcium is important for blood clotting and muscle contraction. It is also involved in enzyme reaction, hormonal signal transmission and glucose metabolism. Calcium helps to regulate the acid-base status of the blood (Olomu, 1995). The recommended daily allowance of calcium by the National Institute of Health (NIH, 2011) is 200mg/day for infants, 1,300 mg/day for adults and 1500mg/day for pregnant or lactating mothers. Phosphorus is also required for nearly every metabolic process in the body. It is good for kidney function (Uchegbu and Okwu, 2012). Magnesium helps to reduce cholesterol (Uchegbu and Okwu, 2012) and it also plays fundamental roles in most reactions involving phosphate transfer; believed to be essential in the structural stability of nucleic acid and intestinal absorption while its deficiency in man is responsible for severe diarrhoea and migraines (Appel, 1999). The recommended dietary allowance (RDA) for magnesium is 80-240mg/day for children, 360-410mg/day for adolescents and 320-420 mg/day for adults. Iron is part of the haemoglobin molecule involved in oxygen transport to and within the cells (Michael, 1997). Iron is required for blood haemoglobin formation which also involves energy metabolism. It is a trace element needed by the body. The deficiency of iron in the body results to anemia (Adeyeye and Fagbohon, 2005). The WHO recommended dietary allowance for iron in adult and children is 10 mg/day while female adult is 15 mg/day.

Table: 3.3b: Micro elements of *Moringa oleifera* (mg/kg).

Sample	**Cu**	**Zn**	**Cd**	**Pb**	**Ni**
Seeds	5.75	73.25	0.00	0.00	23.50
Stem bark	4.50	19.75	0.00	0.00	11.75
Leaves	13.75	39.00	0.25	0.00	13.25

Table 3.3b indicated that the seeds were highest in zinc and nickel; while the leaves were highest in copper and cadmium. The stem bark gave values between these ranges. Cadmium was not present in the seeds and stem bark while lead was absent in all the parts. This indicated that as far as lead poisoning was concerned, the plant is safe for human consumption.

Copper and iron are required in the formation of red blood cells while iron is required for cellular metabolism (Sarwar *et al*., 2009). WHO recommended dietary allowance for copper is 3mg/day for adult and 2 mg/day for children. Zinc is an essential trace element for protein and nucleic acid synthesis and normal body development, vital during periods of rapid growth such as infancy, adolescence and during recovery from illness (Akinyele, 1989). WHO recommended standard for zinc in adult and children as 15 mg/day and 10 mg/day respectively. Cd is hazardous both by inhalation and ingestion and can cause acute and chronic intoxications. (Nordberg *et al*., 2007). Lead is a poisonous metal that can damage nervous connection especially in young children. It also affects their faculty of reasoning. Increased lead absorption may affect both the central and peripheral nervous systems (Lin-Fu, 1976). In adults, it damages brain, kidney and ultimately cause death while in pregnant women it causes miscarriage (Needlepoint *et al.,* 1990); The Environmental Protection Agency (EPA) has detected and declared that there is no safe level of lead intake (Montague and Peter, 2010). Nickel (a trace element), has only recently been considered essential in human diets (Belay *et al*., 2014). Non-essential components of foods can still have significant impacts on health and can either be beneficial or toxic. These non-essential elements are however frequently consumed and accumulated in living organisms, though are not required. Thus, the body requires less than twenty (20mg/day) of each trace elements (Mader, 2006), and the concentration acceptable level by WHO is 4.0 mg/kg body weight/day. However heavy metals like Cu, Zn, Cd, Pb, Ni, etc. are of great concern because of their potential effects on human health, on agriculture and on the environment.

DPPH radical scavenging activity

Table 3.4: DPPH Assay of Moringa Leaves, Seeds and Stem bark.

Conc (μg/ml**)**	**% Inh(LV)**	**% Inh(SD)**	**% Inh(SB)**	**% Inh(BHA)**
5	58.80±.1.38[a]	64.53±.1.96[b]	61.40±.0.54[a, b]	99.81±.2.74[c]
10	63.73±4.07 [a]	67.43±1.99 [a]	63.35±1.86 [a]	97.24±1.99[b]
25	78.57±0.97 [b]	71.88±1.80 [a]	70.22±1.76 [a]	96.78±2.19 [c]
50	76.24±1.58 [b]	67.90±0.86 [a]	82.69±0.59 [c]	96.30±0.88 [d]
125	82.79±0.79 [c]	71.69±1.50 [a]	78.99±0.43 [b]	98.24±2.06 [d]

SD (Moringa seed), LV (Moringa leaves), SB (Moringa stem bark), BHA (Butylated Hydroxy Anisole) Means ± standard deviations in the same **row having the same letter are not significantly different from each other at p < 0.05**

The antioxidant activity of *Moringa oleifera* plant extracts were determined using methanolic solution of DPPH reagent. The results summarized in table 3.4 indicate that the leaves, seeds and stem bark extracts were found to interact with the DPPH radicals and thereby stabilize their hyperactivity. Among the tested parts, the leaves were found to be more effective (82.78%) as DPPH radical scavenger at the highest concentration (125 μg/ml) while minimum effect was observed in the seeds (71.69%) at the same concentration. The stem bark was between these ranges (78.99%) and all values were lower than the 98.24 % recorded for BHA, a standard free radical stabilizing agent. The result of the inhibition also shows that all the parts have a potential to inhibit oxidative reactions in the body. The table also revealed that the activities of the plant parts is concentration dependent i.e. at low concentrations, the activity is lower than what is observed at high concentrations. There is also a little difference between the activities of the three parts, especially at low concentrations.

The principle of DPPH assay is based on the reduction of DPPH radical in the presence of a hydrogen donating antioxidant. Extracts reduce the colour of DPPH due to the hydrogen donating ability (Blois, 1958). DPPH is one of the compounds that possess a proton free radical with a characteristic absorption, which decreases significantly on exposure to free radical scavengers (Yamaguchi *et al*., 1998). Antioxidants may guard against reactive oxygen species (ROS) toxicities by scavenging reactive metabolites and converting them to less reactive molecules. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Blazovics *et al*., 2003).

4. Conclusion

The results of this study has shown that both leaves and stem bark of *Moringa oleifera* contain appreciable amounts of crude fibre, moisture and ash, while the seeds have relatively low value of the same parameters. The seeds contain high protein and crude fat thus can be incorporated into infant formulae and weaning foods especially in countries where the rate of infant mortalities are high as a result of mal-nutrition, kwashiorkor and scurvy. This could also be utilized as a food / nutritional supplement to improve growth performance and health status.

The results also revealed highest amounts of macro elements (Na, K, Ca, Mg and Fe) mg/kg in the leaves whereas the seeds showed the highest amounts of the micro elements (Cu, Zn,Cd,Pb and Ni) mg/kg. The leaves also showed the highest antioxidant activity which indicate that this plant contains antioxidant substances of high importance and can contribute to human health by reducing many disorders in humans such as atherosclerosis, arthritis, Alzheimer disease, cancer etc. It can also be concluded that *Moringa oleifera* leaves and seeds can contribute significantly to the nutrient requirements and health management of man and should be recommended in human diet.

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