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Evaluation of Proximate, Minerals, Vitamins and Phytochemical Composition of *Moringa oleifera* Lam. Cultivated in Ado Ekiti, Nigeria

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Abstract: *Moringa oleifera* is a plant that is in high demand for its nutritional and medicinal values. This study was aimed at assessing the nutritional and phytochemical constituents of the leaf of *Moringa oleifera* cultivated in Ado Ekiti, Nigeria. Samples of leaf of *M. oleifera* were obtained from six different moringa cultivating locations in Ado Ekiti. The proximate analysis of dried leaf of *M. oleifera* showed that it is highly rich in protein (28.00±0.33%) and metabolizable energy (2625.25±79.30 Kcal/Kg), with appreciable levels of fat (3.88%), ash (9.88%), crude fibre (12.57%) and carbohydrate (37.87%). The leaf was found to be rich in minerals and vitamins. It contained high concentrations of Zn^{2+}, Mg^{2+}, Ca^{2+}, K^{+} and PO_4^{3-}, respectively, 64.17±2.04, 643.33±6.06, 82.50±6.89, 430.00±8.37 and 50.43±2.05mg/100g. Low, but appreciable levels of Fe^{2+}, ascorbic acid, thiamin, riboflavin and niacin were equally obtained. Phytochemical analysis revealed high concentrations of alkaloids, flavonoids and saponins; 446.67±12.11, 846.67±11.07 and 844.17±27.82mg/100g respectively. The concentrations of phytates and tannin were high, but with low oxalate content. No cyanogenic glycoside was detected. A satisfactory oxygen radical absorption capacity level of 37.23% and very low anti-protease activity (0.13±0.06mg/100g) were obtained. The rich array of results obtained in this study displays that *M. oleifera* is a multipurpose plant that could contribute immensely towards meeting both human and livestock nutritional requirement.

Key words: *Moringa oleifera* leaf · Nutritional and Phytochemical Constituents

INTRODUCTION

Moringa oleifera Lam. is native to Africa, Arabia, South Asia, South America, the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan and it is the most widely cultivated out of the fourteen species in the Moringaceae family [1, 2]. *M. oleifera* has been naturalized in many region of the world and as such is now referred to by a number of names such as horseradish tree, drumstick tree, ben oil tree, miracle tree and "Mothers best friend" [2]. According to Roloff *et al.* [3] and Vinoth *et al.* [2] *Moringa oleifera* is a small, fast-growing evergreen or deciduous tree that usually grows up to 10 to 12m in its height, open crown of drooping fragile branches, feathery foliage of trip innate leaves and thick corky, whitish bark. When grown in well-drained soils, *Moringa* grows rapidly reaching high heights, however it can tolerate sandy soils, clay soils and water limited conditions [2]. *Moringa* tree is cultivated for foods and medicinal purposes [4-6]. According to Oluduro [7], all parts of the *Moringa* tree are edible and have long been consumed by humans. For example, the leaves of *M. oleifera* Lam. are eaten in African countries, such as Ghana, Ethiopia, Nigeria, East Africa and Malawi.

Based on some of the antioxidant and phytochemical properties possessed by the leaves of *Moringa,* it is capable of being used as a natural anticancer, antihypertensive, diuretic, antispasmodic, antiulcer, antihelmintic, antibiotic, detoxifing and immune building agent and also possess some cholesterol lowering activities [1,7-10]. Nutritionally, the leaves have been found to be a valuable source of both macro and micronutrients, rich source of β-carotene, calcium and

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potassium. It also contain high content of highly digestible protein, carotenoids, minerals and vitamins especially vitamin C, hence it can be used as an ideal nutritional supplement which have been used to combat malnutrition, especially among infants and nursing mothers. The leaves and pods have also been indicated to be helpful in increasing breast milk in nursing mothers during breast feeding [7,12]. In animal husbandry, feeding broilers with diet supplemented with moringa leaf has been found to improve the performances of the birds significantly [5]. The *Moringa oleifera* plant has also be used in the treatment of psychosis, eye diseases, asthma, back pain, rheumatism and fever and as an aphrodisiac [13].

According to Fugile [14] and Oluduro [7], other uses to which *M. oleifera* plant can be applied include alley cropping for biomass production, the leaves and treated seedcake can be used as animal forage, the leaves can be used for biogas production, the seed cake can be used as fertilizer. Juice expressed from leaves can be used as foliar nutrient etc. This study was designed to evaluate the proximate, minerals and physicochemical properties of *Moringa oleifera* leaves obtained from the authors vicinity.

MATERIALS AND METHODS

Sample Collection: *Moringa* leaves used in this study were obtained from six different *Moringa* cultivating locations in Ado Ekiti, including ABUAD Farm, the teaching and research farm of Afe Babalola University Ado Ekiti, Nigeria.

Preparation of Sample: The harvested *M. oleifera* leaves were allowed to air dry for a while after which the leaves were ground into fine powder form using an electric blender. The fine powder was then used in the analysis.

Analysis of Sample: Proximate analysis was carried out on the powdered *Moringa oleifera* leaf to determine the presence of moisture, protein, ether extract (fat), ash, crude fibre, carbohydrate (by difference) and metabolizable energy using standard analytical methods as described by the Official Methods of Association of Official Analytical Chemists [15]. Minerals and vitamin such as iron, zinc, magnesium, calcium, potassium, phosphate, ascorbic acid, thiamine, niacin and riboflavin content of dried *Moringa oleifera* leaf was determined using the method described by the Official Methods of Association of Official Analytical Chemists [15].

Phytochemical, antioxidant and anti-protease components of *Moringa oleifera* leaf was determined using the method described by Harbone [16] and Mayuri [17].

Proximate Analysis

Ash Content: A dry ashing method was used to determine the ash content. First, the crucible was measured; then, the weight of the sample + crucible was taken before the sample was incinerated in a Muffle furnace at 400°C. The remaining inorganic material was cooled in a desiccator, weighed and the ash content was determined.

$$\% \text{ Ash} = \frac{\text{weigh of sample reamaining}}{\text{weigh of original sample}} \times \frac{100}{1}$$

Determination of Moisture Content: Moisture content was measured using oven dry method [15]. The oven was used to dry the samples till constant weight then removed and measured. The percentage of moisture content was calculated as:

$$\% \text{ moisture} = (1 \frac{\text{moisture}}{\text{weigh of sample}} \times 100)$$

Determination of Crude Fiber: This is the non-digestive component of food sample. This test involves two stages, acid and alkaline solution using method described by AOAC [15] and the crude fibre is calculated thus:

$$\% \text{ crude fibre} = \frac{\text{weigh of sample} - (\text{weight of sample+weight of crucible})}{\text{weight of sample}} \times 100$$

Determination of Protein: The crude protein was determined using the Kjeldahl Nitrogen method [15]

$$\% \text{ Nitrogen} = \frac{\text{v2} - \text{v1} \times \text{ca} \times 0.014 \times 100}{\text{ws} \times 10(\text{ml of sample})} \times 100$$

Mineral Composition: The mineral contents were determined on aliquots of the solution of the dried leaf sample by UV/Visible and atomic absorption spectrophotometers [15]. A NOVA 400 atomic absorption spectrometer (Analytik Jena AG, Jena, Germany) with an air/acetylene flame and respective hollow-cathode lamps was used for absorbance measurements. Wavelengths, slits and lamp current used for the determination of six elements were 213.9 nm, 0.5 nm, 4.0 mA (zinc); 422.7 nm, 1.2 nm, 4.0 mA (calcium); 248.3 m, 0.2 nm, 6.0 mA (iron) and 766.5 nm, 0.8 nm, 4.0 mA (potassium), respectively. The results for mineral contents were expressed as mg/100 g DW.

Quantitative Phytochemical Analysis: Tests for the presence of the following plant secondary metabolites including alkaloids, flavonoids, steroids, saponins, phenols, tannins, glycosides and cardiac glycosides and phytates were carried out on the powdered samples following the method of Herboone [16] and Mayuri [17].

Test for Flavonoids: One gramme of the sample was weighed into 10ml of 80% Methanol; left to stand for 2 hours, filtered into a weighed Petri dish and left to dry in the oven at 40°C until it attained a constant weighed. The weight of the Petri dish was recorded.

Determination of Total Flavonoids: A volume of 0.5ml of 2% $AlCl_3$ Methanol solution was added to 0.05ml sample solution. After 1hr at room temperature, the absorbance was measured at $A_{420.}$ Calculate Flavonoids content was obtained with the aid of a calibration graph.

Tannins: One gramme of the sample was extracted with 25ml of the solvent mixture of 80:20 Acetone: 10% Glacial Acetic Acid for 5hours. The supernatant was filtered and the Absorbance of the filtrate as well as the reagent blank measured at 500nm Absorbance. A standard graph was produced with 10, 20, 30, 40, 50 mg/100g of Tannic Acid. The concentration of Tannin was read off taking into consideration dilution factors.

Alkaloids: One gramme of the sample (W) was added to 20ml of 10% Acetic Acid in Ethanol, shaken, allowed to stand for 4 hours and filtered. The filtrate was allowed to evaporate to about a quarter of its original volume and one drop of concentrated Ammonia added. The precipitate formed was filter through a weighed (W1) filter paper. The filter paper dried in the oven at 60°C, weighed when it has attained a constant weight (W2).

$$\%\text{Alkaloids} = \frac{W2 - W1}{W} \times 100$$

Phenols/Phenolics and Antioxidants – (Extraction): Extract 2g with 20ml of 80:20 Acetone: 0.5% formic Acid for 2min and filter.

For Phenols/Phenolics/Phenolic Acids: 2ml of the extract is mixed with 0.5ml of Folin-Ciocalteau Reagent and 1.5ml Sodium Carbonate (20%). Mix for 15 sec and allow to stand at 40°C for 30min to develop colour. Measure $A_{765.}$ Express as GAE/g (Gallic Acid Equivalent).

Saponins: One gramme of sample was added to 5ml of 20% Ethanol in a conical flask and placed in a water bath at 55°C for 4hours. This was followed by filtering and washing the residue with 20% Ethanol twice and reducing the extract to about 5ml in the oven. The extract was further treated successively Petroleum Ether, butanol and 5% Sodium Chloride.

Steroids: Five gramme of the sample was added to 100ml of water and drops of 0.1M Ammonium Hydroxide was added to take the pH to 9.1. then 2ml Pet Ether, 3ml Acetic Anhydride and conc. H_2S0_4 were added and the absorbance measured at 420nm.

Cardiac Glycosides/Cardenolides: One gramme of the sample was extracted with 40ml water and place in the oven 100°C for 15min. add 1ml extract plus 5ml water to 2ml Glacial Acetic Acid plus one drop of $FeCl_{3.}$ Add 1ml Conc. H_2S0_4. Measure the absorbance of the resulting solution at 410nm.

Phytates: Extract One gramme of the sample was added to 10ml 3% TCA. The Phytate was precipitated as Ferric Phytate with 0.1% Ammonium Ferric Sulphate. The Ferric Phytate was converted to Ferric Hydroxide and Sodium Phytate by adding 10ml 0.5M Sodium Hydroxide. The precipitate was boiled and dissolved with the addition of dilute acid. The absorbance of the solution was taken at 519nm.

Protease Inhibitor Determination: A 2% solution of egg albumin and 0.1% solution of Bromelain were prepared, both in pH 7 Phosphate buffer. Then 5ml of the egg albumin substrate and 1ml of the Bromelain enzyme were incubated at 55°C for 10 min. A 5ml of 10% TCA was added to stop the reaction. The precipitate was filtered off with Whatman No 1 Filter paper and the absorbance of the filtrate was measured at 280nm As.

The entire procedure was repeated but incubating with the enzyme and substrate mixture 1ml of the extract of the material for Protease Inhibitor determination. Also the absorbance of the filtrate was measured at 280nm (Ai).

$$\%\text{ Protease Inhibition is: } \frac{As - Ai \times 100}{As}$$

Determination of Antioxidants (Oxygen Radical Scavenging Activity): The oxygen radical scavenging activity was carried out by method described by Cuatrecasas and Anfisen [18] and Anosike *et al.* [19] with some modifications. The method is based on measurement of loss of colour of DPPH solution by the

change of absorbance at 517nm caused by the reaction of DPPH with the test sample. To 0.2ml extract add 2.8ml of freshly prepared 20mg/dm^3 DPPH in Methanol, incubated for 20min at room temperature. Inhibition in % RSC (Radical Scavenging Capacity) as follows:

RSC % = (1 - A_{sample}/A_{blank}) x 100%

$A_{blank}$is Abs for Control while $A_{sample}$is Abs for test sample.

RESULTS

Samples of leaf of *M. oleifera* were obtained from six different moringa cultivating locations in Ado Ekiti, were analysed for their nutritional and phytochemical constituents. The proximate analysis of dried leaf of *M. oleifera* showed that it is highly rich in protein (28.00±0.33%) and metabolizable energy (2625.25±79.30 Kcal/Kg), with appreciable levels of moisture (7.88%), fat (3.88%), ash (9.88%), crude fibre (12.57%) and carbohydrate (37.87%) (Table 1).

The leaf was found to be rich in minerals and vitamins. It contained high concentrations of Zn^{2+}, Mg^{2+}, Ca^{2+}, K^+ and PO_4^{3-}, respectively, 64.17±2.04, 643.33±6.06, 82.50±6.89, 430.00±8.37 and 50.43±2.05mg/100g. Low, but appreciable levels of Fe^{2+}, ascorbic acid, thiamine, riboflavin and niacin were equally obtained (Table 2).

Phytochemical analysis revealed high concentrations of alkaloids, flavonoids and saponins; 446.67±12.11, 846.67±11.07 and 844.17±27.82mg/100g respectively. The concentrations of phytates and tannin were high, but with low oxalate content. No cyanogenic glycoside was detected. A satisfactory oxygen radical absorption capacity level of 37.23% and very low anti-protease activity (0.13±0.06mg/100g) were obtained (Table 3).

Table 1: Proximate analysis of *Moringa oleifera* leaves

Parameter	N	Minimum	Maximum	Mean ± SD
Moisture %	6	7.50	8.20	7.88±0.29
Protein %	6	27.60	28.50	28.00±0.33
Ether Extract (Fat) %	6	3.70	4.00	3.88±0.12
Ash %	6	9.50	10.20	9.82±0.32
Crude Fibre %	6	12.20	12.90	12.57±0.28
Carbohydrate (By difference) %	6	36.60	38.90	37.87±1.09
Metabolizable Energy (Kcal/Kg)		2540.50	2699.00	2625.25±79.30

Table 2: Minerals and vitamins composition of *Moringa oleifera* leaf

Parameter	N	Minimum	Maximum	Mean ± SD
Fe^{++} (mg/100g)	6	0.50	0.70	0.58±0.076
Zn^{++}(mg/100g)	6	60.00	65.00	64.17±2.04
Mg^{++}(mg/100g)	6	635.00	650.00	643.33±6.06
Ca^{++}(mg/100g)	6	70.00	90.00	82.50±6.89
K^+(mg/100g)	6	420.00	440.00	430.00±8.37
PO_4^-(mg/100g)	6	48.40	52.40	50.43±2.05
Ascobic Acid (mg/100g)	6	0.60	0.80	0.72±0.10
Thiamin (mg/100g)	6	0.20	0.30	0.22±0.04
Niacin (mg/100g)	6	1.40	1.60	1.48±0.08
Riboflavin (mg/100g)	6	1.40	1.60	1.48±0.08

Table 3: Phytochemical, antioxidant and anti-protease components of *Moringa* oleifera leaf

Parameter	N	Minimum	Maximum	Mean ± SD
Alkaloids (mg/100g)	6	430.00	460.00	446.67±12.11
Cyanogenic Glycosides (mg/100g)	6	ND	ND	ND
Phytates (mg/100g)	6	730.00	865.00	794.17±65.22
Tannins (mg/100g)	6	480.00	525.00	501.67±19.15
Saponins (mg/100g)	6	815.00	880.00	844.17±27.82
Oxalates (mg/100g)	6	23.00	24.30	23.65±0.58
Total Flavonoids (mg/100g)	6	835	865	846.67±16.07
Anti Protease Activity (mg/100g)	6	0.1	0.2	0.13±0.06
Oxygen Radical Absorption Capacity (% Inhibition)	6	36.9	37.8	37.23±0.49

ND – Not detected

DISCUSSION

In the last few years, *M. oleifera* has been in the limelight of research and many researchers have reported that it is a potential source of outstanding and highly digestible protein, Ca, Fe, Vitamin C and carotenoids suitable for exploitation in many of the developing countries globally, where undernourishment is a major concern; basically for its nutritional, prophylactic and therapeutic potentials which has been reported in various experimental studies in animals and humans, although limited in number [1, 20, 21]. The nutritional and medicinal potentials of *M. oleifera* was assessed in this study through the determination of the proximate analysis, minerals and vitamin composition as well as the relative distribution of phytochemicals, antioxidant and anti-protease components.

The moisture content in leaves of *M. oleifera* in this study is within the expected range [22]. High moisture contents in leaves make them highly perishable and susceptible to microbial spoilage during storage [23]. The relatively low moisture content in *M. oleifera* would prevent the growth of microorganisms and prolong storage life. The protein value obtained from our sample suggests that *M. oleifera* are proteins of good quality and as such are suitable for animal feeds and human diet and can effectively contribute to the daily protein needed [24]. Dietary proteins are important for natural synthesis and maintenance of body tissues, enzymes and hormones as well as other substances required for healthy functioning [25]. Plant food that provides more than 12 % of its caloric value from protein is considered a good source of protein [23].

The ash value obtained in this study suggests that the *M. oleifera* are a good source of inorganic minerals [26]. High ash content in food is a measure of high deposit of mineral contents [27]. Crude fibre has little nutritional value, all the same, adequate intake of dietary fibre can lower the serum cholesterol level and aids absorption of trace elements in the gut as well as reduced the risk of coronary heart disease, diabetes, constipation, hypertension, colon and breast cancer [23, 25, 28]. The fibre content of *M. oleifera* in this study is adequate in relation to diet and it is in agreement with previous studies [26]. The ether extract (Fat) content reported in this study is moderate when compared to those from other plants. Dietary fats function in the increase of palatability of food by absorbing and retaining flavours [29]. A diet providing 1 – 2 % of its caloric of energy as fat is said to be sufficient to human beings as excess fat consumption is implicated in certain cardiovascular disorders such as atherosclerosis, cancer and aging [30].

The carbohydrate content of *M. oleifera* is high compared to that reported by some authors and suggests that it could be a good supplement as well as a source of energy and structural materials [31]. Generally, carbohydrates add to the bulk of the diets, they play a pivotal role as they provide energy to cells such as brain, muscles and blood. They contribute to fat metabolism and spare proteins as energy source and act as mild laxative for human beings [32]. The high calorie content of *M. oleifera* can contribute significantly to the daily caloric requirement of the body [26]. The contribution of energy from plants is important, since sufficient supply of energy in the diet is required for protein to be fully utilized [33].

Samples of *M. oleifera* analysed in this study reveals that it possess abundant mineral constituents such as Fe, Mg, Ca, K and PO_4 [34]. The zinc content of *M. oleifera* is an indication that it can play an important role in the management of diabetes, which results from deficiency in insulin secretion, insulin action or both. Zinc is important for the synthesis of DNA, RNA, insulin and function of several enzymes, zinc is also required for cell reproduction and growth especially the sperm cells [22]. To prevent anaemia and other related diseases in infants, pregnant and nursing women as well as elderly people, diet rich in Fe should be taken regularly. Added to this, Fe also plays a pivotal role in immune function, cognitive development, temperature regulation and energy metabolism. Fe is essential for the synthesis of haemoglobin and myoglobin, its deficiency results could in anaemia [28].

The K, Mg and Ca level compares favourably to most values reported for medicinal plants in literatures. Mg is required in over 300 enzymes that use adenosine triphosphate. It contributes to DNA and RNA synthesis during cell proliferation. It is also important for nerve and heart function as well as release of insulin and ultimate insulin action on cells. It decreases blood pressure by dilating arteries and preventing abnormal heart rhythm. Deficiencies in animals lead to irritability, convulsion and even death. In like manner, Ca along with P is required for formation and maintenance of bones and teeth. It is also required in blood clothing and muscle contraction [28]. Potassium is very important in regulation of water and electrolyte balance and acid-base balance in the body, as well as responsible for nerve action and functioning of the muscles. Deficiency of potassium leads to muscle paralysis [33].

The results obtained for the selected vitamins, namely ascorbic acid, thiamine, niacin and riboflavin are in agreement with that previously reported for *M. oleifera* [1]. Vitamins are essential in the body as their deficiencies affect metabolism in the body. Ascorbic acid is an excellent antioxidant and free radical scavenger, capable of protecting the cells from oxidative damage by oxidants. It required for connective tissue metabolism especially the scar tissues, bone and teeth. Added to this, it prevents scurvy and enhances iron absorption from the intestine [35].

Niacin and riboflavin, though in trace amount are important for the body metabolism. Niacin prevents pellagra. Riboflavin is unique among the water-soluble vitamins; riboflavin deficiency has been implicated as a risk factor for cancer, although this has not been satisfactorily established in humans [36,37]. Thiamin is an essential nutrient for humans, its deficiency causes beriberi, which disturbs the central nervous and circulatory systems [38].

Examination of the phytochemicals of *M. oleifera*, suggests that it is a rich source of phytochemical. Phytochemicals possess biological functions which include anti-inflammatory, antioxidative, antiviral and anti-carcinogenic properties (flavonoids). Some act as pain relievers and tranquilizers (alkaloids) while some confer protection against platelet aggregation and oxidative damage as a result of free radicals [1,36,39]. *M. oleifera* also possesses anti-protease activity as well as free radical destroying or neutralising power as depicted by its ORAC value in this present study.

In conclusion, rich array of results obtained in this study displays that *M. oleifera* is a multipurpose plant that could contribute immensely towards meeting human nutritional requirement. Its high mineral, vitamins and protein content could be explored in the development of nutraceuticals. Added to this, because of its active bioconstituents if taken as a supplement, it could confer adequate protection in managing, preventing or delay oxidative stress/damage that has been implicated in the pathogenesis of various diseases or illness.

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