FUOYE Journal of Pure and Applied Sciences

Available online at www.fuoye.edu.ng

PROXIMATE AND PHYTOCHEMICAL ANALYSIS OF *MORINGA OLEIFERA* LEAF AND SEED IN OSOGBO, NIGERIA

*Adebisi, S.A.,Olasunkanmi, A.O., Azeez, L., Awojide, S.H. and Adetoro, R.O.

Department of Pure and Applied Chemistry, Osun State University, Osogbo, Osun State, Nigeria

ARTICLE INFO

Received: 3 February, 2019
Accepted: 24 July, 2019

Keywords:
Proximate, Phytochemical, *Moringa oleifera*

Corresponding author:
adebisioluwasegun@yahooo.com

Abstract

This study was conducted to determine the proximate analysis and phytochemical properties of Moringa leaf and seed. The proximate analysis recorded the concentrations (in percentage) of leaf and seed samples respectively as: Carbohydrates (52.68±0.10% and 17.94±0.59%), Crude Fat (5.80±0.03% and 46.10±0.10%), Protein (11.63±0.15% and 19.91±0.11%), Crude Fibre (12.71±0.12% and 6.72±0.21), Ash (10.20±0.13% and 5.09±0.11), Moisture (6.98±0.05% and 4.34±0.04%) and Energy Value (1307.87±0.24KJ/g and 2345.45±0.76KJ/g).The phytochemical analysis revealed that phytates, tannins, phenols, oxalates, alkaloids and glycosides are present in both samples; flavonoids, steroids and anthraquionones are absent in both samples. Terpenoids and saponins are present in leaf but absent in seed.

1.0 Introduction

Moringa oleifera is of the family *Moringaceae*. It is a small, fast-growing evergreen or deciduous tree that usually grows up to 10 to 12m in height, open crown of drooping fragile branches, feathery foliage of trip innate leaves and thick corky, whitish bark [14]. The plant is commonly called horseradish tree or the miracle tree and locally known as 'Zogale-gandi' in Hausa, 'Eweigbale' in Yoruba and 'Okweoyibo' in Igbo [4]. *Moringa oleifera* is well known for its nutritional and medicinal values by many communities in Nigeria. The leaves of this plant are used as vegetables in soup preparation or cooked and mixed with grounded groundnut cake and other spices, and then eaten as food [8]. For centuries, many plant compounds have an outstanding role in medicine. Their pharmacological and economical values have lost nothing to its

importance until date. They are either used directly or after they have been subjected to certain chemical modification processes. These plants which are medicinal in nature however contain physiological active principles, which over the years have been exploited in ayurvedic medicines for the treatment of various ailments. The primary benefit of using plants derived medicines is that they are relatively safer than synthetic alternatives. The prevalence of bioactive principles such as tannins, terpenoids, flavonoids, alkaloids, steroids etc. underscores the needs for continuous search for bioactive and active ingredients extracted from plant, though some of the active ingredients of crude extracts become obsolete because of the drug resistant problems [12]. Most of the drugs employed in the treatment of human ailments are obtained by extraction, either by infusion, using water, natural gin of local wine as solvents [1]. However, it is important to make a good selection of solvent in the study of activities of plant constituents or active ingredients. In recent days, scientific and technological advancement have made it possible in the investigation of a large number of medicinal plants by employing a systematic screening method using chromatographic techniques and spectroscopic techniques to establish the actual effects [6].

The useful products obtained from plants directly or indirectly, demonstrate their importance to man. Plants serve as a source of food [7, 11, 15], medicinal product [2,3,5], energy [13] and shelter to man and his livestock. In the earlier stage man depended on wild food, which is much abundant within his immediate environment. As the population grows, however, sources of food became more difficult to him, which necessitated domestication of many plants. Although more than 250,000 plant species have been described worldwide as sources of food, man depends only on a few species mainly cereals, particularly rice, wheat and corn as .the major sources of his food. These collectively supply nearly 60% of the world's food supply.

2.0 Materials and method

Sample collection

Fresh and healthy plant materials were used for this study. The plant samples were collected in Ofatedo area in Osogbo, Osun State, Nigeria. The leaves or seeds were air-dried for a period of fourteen days at room temperature, then, ground to fine powder.

2.1 Proximate analysis

Proximate analysis was carried out in triplicate according to the procedure of Association of Official Analytical Chemist (AOAC, 2000) to determine the chemical compositions of the sample.

2.2 Determination of moisture content

About 3.00 g of powdered seed and leaves of *M. oleifera* was weighed (W_1) and placed in an oven at 105 ^{0}C for 24 hours. The sample was removed from the oven, cooled in a desicator and reweighed (W_2).

Moisture content (%) was calculated according to the formula below:

$$\text{Moisture (\%)} = \frac{w_1 - w_2}{w_1} \times 100$$

2.3 Determination of total ash in *M. Oleifera*

About 3.00 g of powdered seed and leaves of *M.* Oleifera was weighed into porcelain

crucible and incinerated overnight in a muffle furnace at 600 ^{0}C. The crucible was removed from the muffle furnace, cooled in desiccators and reweighed.

Ash content (%) was calculated according to the formula below:

$$\text{Ash (\%)} = \frac{\text{weight of ash}}{\text{weight of sample}} \times \frac{100}{1}$$

2.4 Determination of Crude Protein in *M. oleifera*

Crude protein was determined by the Kjeldahl apparatus as described by AOAC, (2000).

About 5.00 ml portion of the sample digest was pipette into the body of kjeldahl apparatus via the small funnel aperture. To this was added 5 ml of 40% of NaOH through the same opening with 5 ml pipette. The mixture was steam-distilled for 2 minutes in 50 ml conical flask containing 10 ml of 2% boric acid and mixed indicator solution placed at the receiving tip of the condenser. The boric acid plus indicator solution changes colour from red to green showing that all the ammonia liberated have been trapped.

2.4.1 Titration

The green colour solution was then titrated against 0.01N HCl contained in a 50ml burette. At the end point the green colour change to wine colour which indicated that all the nitrogen trapped as ammonium borate have been removed as ammonium chloride.

The percentage nitrogen in this analysis was calculated using the formula below;

$$\% \text{ N} = \frac{\text{titre value} \times \text{atomic mass of nitrogen} \times \text{normality of HCl} \times 4}{\text{weight of sample in milligram}} \times \text{volume of digest for steam distillation}$$

. The crude protein content was determined by multiplying percentage nitrogen by a constant factor of 6.25 i.e. % crude protein = % Nitrogen x 6.25

2.5 Determination of Crude Fat in *M. oleifera*

Crude fat was estimated by employing solvent extraction using a soxhlet extraction unit (AOAC, 2000). About 1.00g of grounded *Moringa*seed and leaves was weighed into fat free extraction thimble and plugged lightly with cotton wool. The thimble was placed in the extractor and fitted up with reflux condenser and 250 ml soxhlet flask which has been previously dried in the oven, cooled in the desicator. The soxhlet was weighed and filled to ¾ of its volume with petroleum ether and the soxhlet flask. Extractor and condenser set were heated for 6 hours. The ether was left to siphon eleven times until it is short of siphoning. The thimble containing sample was removed and dried on a clock glass on the bench top. The extractor, flask and condenser are replaced and the distillation continues until the flask is practically dried free of petroleum ether. The flask containing the fat or oil is detached, its exterior cleaned and dried to a constant weight in the oven. Crude fat (%) is calculated thus;

$$\text{crude fat (\%)} = \frac{\text{extracted fat}}{\text{sample weight}} \times \frac{100}{1}$$

2.6 Determination of Crude Fibre in *M. oleifera*

Crude fat was estimated by employing solvent extraction using a soxhlet extraction unit (AOAC, 2000). About 2.00 g of powdered *M. oleifera* was put in a 600 ml beaker. 200 ml of 1.25% H_2SO_4 was added and refluxed for 30 minutes. The content was filtered and mixed

with hot distilled water to remove the acid. About 200 ml of 1.25% of NaOH was added to the filtered sample in the beaker. The content was bolied on hot plate and refluxed for 30 minutes. The beaker was covered with a mini condenser containing some cold water to prevent the 1.25% NaOH from evaporation. The sample was scrapped with the aid of a spatula into the crucible. It was transferred into the hot air oven and dried over night at 70^0C. Later, it was removed and allowed to cool in a desiccator. The weight of the sample was taken with the aid of Toledo mettler analytical balance and later transferred into the muffle furnace for ignition. It was ashed at 600^0C for 3 hours. It was removed after ashing and allowed to cool for 30 minutes in a desiccator and the final weight of the ash was taken and recorded.

$$\text{Crude fibre (\%)} = \frac{\text{initial weight (g)} - \text{final weight (g)}}{\text{weight of sample taken (g)}} \times \frac{100}{1}$$

2.7 Determination of Carbohydrate in *M. Oleifera*

Carbohydrate was calculated as following:
Carbohydrate (%) = 100 – (crude protein + crude fat + total ash + crude fibre)

2.8 Phytochemical analysis

2.8.1 Determination of Concentration of Alkaloids in *M. oleifera*

About 2.00 g of finely grounded sample was weighed into a 100 ml beaker and 20 ml of 80% absolute alcohol was added to give smooth paste. The mixture was transferred into a 250 ml flask and alcohol was added to make up to 100 ml. About 1.00 g magnesium oxide was added. The mixture was digested in boiling water bath for 1 hour under a reflux air condenser with occasional shaking. The mixture was filtered while hot through a small burckner funnel. The residual sample was returned to the flask and redigested for 30min with 50ml alcohol after which the alcohol was be evaporated, and hot water was added to replace the alcohol lost. When all the alcohol has been removed, 3 drops of HCI was added, the solution was later transferred to a 250ml volumetric flask, 5ml of zinc acetate solution and 5ml potassium ferrocyanide solution was added, thoroughly mixed to give a homogenous solution. The flask was allowed to stand for a few minutes, filtered through a dry filter paper and 10ml of the filtrate was transferred into a separatory funnel and the alkanoids present were extracted vigorously by shaking with 5 successive portions of chloroform. The residue obtained was dissolved in 10ml hot distilled water and transferred into a kjeldahl flask followed by the addition of 0.20g sucrose, 10ml conc. H_2SO_4 and 0.02g selenium for digestion into a colourless solution to determine. % N got is converted to % alkaloids by multiplying with factor of 3.26, that is, % total alkaloids = %N x 3.26.

2.8.2 Determination of concentration of phenol in *M. oleifera*

About 4.00 g of grounded sample was treated with 30ml of 80% aqueous acetone in a 250ml beaker at 25^oC in the dark to extract the phenolics. The mixture was transferred to a warring blender and homogenize three times, for 1hr with successive addition of 30ml of 80% acetone. Alternatively, the mixture was centrifuged in centrifuge tube at 3000 rpm for 30min each; with 30mls addition of 80% aqueous acetone. 0.05ml of phenolic extract

was diluted to 2ml in a 10ml measuring flask, 1ml of folin-ciocateu phenol reagent was added and the flask was vigorously shaken. Immediately 5ml of 20% sodium carbonate solution was pipette into then 10ml flask and the mixture made up to 10ml with thorough shaking. 0-10 ppm of gallic acid standard. After 20min, the absorbance of sample as well as standard were read on a spectronic 21D spectrophotometer at wave length of 735 nm.

Percentage total phenol was calculated using the formula:

$$\% \text{ total phenol} = \frac{\text{absorbance of sample x gradient factor x dilution factor}}{\text{weight of sample taken}} \times 1000$$

2.8.3 Determination of concentration of flavonoid in *M. oleifera*

About 0.50g of fine grounded sample was put into a 100ml beaker and 80ml of 95% ethanol, added and stirred to prevent lumping. The mixture was filtered into 100 ml volumetric flask, 4 drops of concentrated HCl was added via a dropping pipette after which 0.5g of magnesium turnings was added to develop a magneta rod colouration. Standard flavonoid solution of range 0.5 ppm were prepared from 100 ppm stock solution and treated in a similar way with HCl and magnesium turning like sample. The absorbance of magneta and colouration of sample and standard solutions were read on a digital Jenwy V6300 spectrometer at a wavelength of 520 nm. The percentage flavonoids is calculated using the formula.

$$\% \text{ flavonoid} = \frac{\text{absorbance of sample x average gradients factor x dilution factor}}{\text{weight of sample}} \times 10000$$

2.8.4 Determination of concentration of glycoside in *M. oleifera*

About 10.00 ml of extract was pipette into 250 ml conical flask. 50 ml chloroform was added and shaken on votex mixer for about 1 hour. The mixture was filtered into 100 ml conical flask and 10 .00 ml pyridine, 2 ml of 2 % sodium nitroprusside were added and shaken thoroughly for about 10 minutes. About 3.00 l of 20% NaOH was added, which develop a brownish yellow colour. Glycoside standard of concentration which ranges from 0-5 mg/ml. the absorbance of sample as well as standards was read on a spectronic spectrophotometer at a wavelength of 510nm.

% Glucoside was calculated using the formula:

$$\% \text{ glucoside} = \frac{\text{absorbance of sample x gradient x dilution factor}}{\text{weight of sample}} \times 10000$$

2.8.5 Determination of concentration of steroids in *M. oleifera*

0.50 g f sample extract was weighed into a 100 ml beaker. 20ml of chloroform-methanol (2:1) mixture was added to dissolve the extract after shaken for 30 minutes on a shaker. The whole mixture was filtered into another 100 ml conical flask; the resultant residue was repeatedly treated with chloroform – methanol until it is free of steroids. 1 ml of filtrate was pipetted into 30 ml test tube and 5 ml of alcoholic KOH was added and shaken thoroughly to obtain a homogenous mixture. The mixture was later placed in water bath at 37^0C – 40^0C for 90 minutes. It was cooled to room temperature and 10 ml of petroleum ether was added in 5 ml of distilled water.

This was evaporated to dryness on water bath. 6 ml Liebermann Burchard reagent was added to the residue in dry bottle and absorbance was taken at a wavelength of 620 nm on a spectronic spectrophotometer.

Percentage steroid was calculated using the formula:

$$\% \text{ steroids} = \frac{\text{absorbance of sample x gradient x dilution factor}}{\text{weight of sample}} \times 10000$$

2.8.6 Deterimination of concentration of saponins in *M. oleifera*

The method used was that of Obadoni and Ochucko (2001). About 20 g of samples powder was put into a conical flask and 100 ml of 20 % aqueous ethanol was added. The mixtures were heated over a hot water bath for 4 hours with continuous stirring at about 55^0C. The mixture was filtered and the residue was re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90^0C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice in 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight and the saponin content was calculated in percentage.

3.0 Results and discussion

Table 1 revealed that the leaf and seed respectively contain considerable amount of Carbohydrates (52.68±0.10% and 17.94±0.59%), Crude Fat (5.80±0.03% and 46.10±0.10%), Protein (11.63±0.15% and 19.91±0.11%), Crude Fibre (12.71±0.12% and 6.72±0.21), Ash (10.20±0.13% and 5.09±0.11), Moisture (6.98±0.05% and 4.34±0.04%) and Energy Value (1307.87±0.24KJ/g and 2345.45±0.76KJ/g) for leaves and seeds respectively. This indicates that *Moringa oleifera*is a good source of these chemical compositions. The ash and crude fibre content of the leaf is two times that of the seed. The crude fat content of the leaf is relatively small comparing to the seed. The carbohydrate content of the seed is relatively low compare to that of the leaf. The moisture and protein content of the leaf and seed are moderately close. The energy value of the seed is almost double to that of the leaf.

Table 2 revealed that phytates, tannins, phenols, oxalates, alkaloids and glycosides are present in both samples; flavonoids, steroids and anthraquionones are absent in both samples. Terpenoids and saponins are present in leaf but absent in seed.

Table 3 revealed that the samples contain considerable amount of Phytates (11.54 ± 0.07mg/g and 5.29 ± 0.02mg/g), Tannins (2.89±0.10mg/g and 0.45±0.10mg/g), Total Phenols (30.75±0.21% and 4.71±0.04%), Saponins (1.24±0.02% and 0.00±0.00%), Alkaloids (47.28±0.03% and 14.61±0.07%), Oxalates (8.60±0.03mg/g and 5.31±0.06mg/g), Flavanoids (0.00±0.0.00% and 0.00±0.00%), Steroids (0.00±0.00mg/g

and 0.00±0.00mg/g), Terpenoids (1.80±0.02% and 0.00±0.00), Glycosides (79.31±0.25mg/Kg and 45.56±0.21mg/Kg) and Anthraquinones (0.00±0.00% and 0.00±0.00).Essentially, the important medicinal values of moringa oleifera leaves include treating diarrhea, urinary tract infection, scurvy, catarrh and the pods are used against ulcer, fever and asthma while the roots can serve as condiment (Yusuf and Yusuf, 2014).

Table 1: Proximate composition of M*oringa oleifera* leaf and seed

PARAMETER	LEAF SAM
Ash content (%)	10.20±0.13
Moisture content (%)	6.98±0.05
Crude fat content (%)	5.80±0.03
Crude fibre content (%)	12.71±0.12
Protein content (%)	11.63±0.15
Carbohydrate content (%)	52.68±0.10
Energy value (KJ/g)	1307.87±0.

Table 2: Qualitative phytochemical constituents of *moringa oleifera* leaf and seed

PARAMETER	**LEAF SAMPLE**	**SEED SAMPLE**
Phytates	+ve, +ve	+ve, +ve
Tannins	+ve, +ve	+ve, +ve
Total phenols	+ve, +ve	+ve, +ve
Oxalates	+ve, +ve	+ve, +ve
Alkaloids	+ve, +ve	+ve, -ve
Flavanoids	-ve, -ve	-ve, -ve
Terpenoids	+ve, +ve	-ve, -ve
Saponins	+ve, +ve	-ve, -ve
Steroids	-ve, -ve	-ve, -ve
Glycosides	+ve, +ve	+ve, +ve
Anthraquinones	-ve, -ve	-ve, -ve

+ve = presence of constituent, -ve = absence of constituent.

Table 3: Quantitavive phtochemical Constituents of *Moring aoleifera* leaf and seed

PARAMETER	LEAF SAM
Phytates (mg/g)	11.45±0.07
Tannins (mg/g)	2.89±0.10
Total phenols (%)	30.75±0.21
Saponins (%)	1.24±0.02
Alkaloids (%)	47.28±0.03
Oxalates (mg/g)	8.60±0.03
Flavonoids (%)	0.00±.0.00
Steroids (mg/g)	0.00±0.00
Terpenoids (%)	1.08±0.02
Glycosides (mg/Kg)	79.31±0.25
Anthraquinones (%)	0.00±0.00

4.0 Conclusion

The results of this study revealed that *Moringa oleifera* leaf and seed contains considerable amount of carbohydrates,

protein, crude fat, crude fibre, and nutritive phytochemicals such as phenols, alkaloids, tannins, glycosides. Thus, it can be concluded that *Moringa oleifera* can be utilized as an alternative for food and medicinal products

References

[1] Acharya, D. and Shrivastara, A. (2008) Indigeneous Herbal Medicine, Tribal Formations and traditional herbal practices. Havishar Pulishers Jaiper-India. pp440

[2] Adoum, O.A., Akinniyi, J.A. and Omar, T. (1997): The effect of geographical location on the antimicrobial activities and trace element concentration in the root of Calotropisprocera (Ait.) R. Br. Annals of Borno 13(14): 199-207.Auol, Sterculiastriata St. Hiletnaud and Terminaliacatappa Linn. Food Chemistry 70: pp185-191.

[3] Caceres, A., Cabrera, O., Morales, O., Mollinedo, P. And Mendia, P. (1991): Pharmacological properties of M. oleifera I: Preliminary screening for antimicrobial activity. Journal of Ethnopharmacology, 133(3), 213-216.

[4] Dalziel, J.M. (1956): Useful plants of West tropical Africa. Crown Agents for Oversea Government and Administration, London, pp.

[5] Ezeamuzie, T.C., Amberkedeme, A.W., Shode, F.O. and Ekwebelem, S.C. (1996): Anti-inflammatory effects of Moringa oleifera root. International Journal of Pharmacognosy **34(93)**: 207-212.

[6] Harbone, J.B. (1998) A Guide to Modern Techniques of plant Analysis-phytochemical methods, 3rd edition, Chairman and Hall, London. pp 253-262.

[7] Katsayal, U.A., Ambi, A.A., Ibrahim, N.D.G. and AbdulRahman, E.M. (2004): Histopathological studies and elemental analysis of *Moringa oleifera Lam* seeds. Biological and Environmental Sciences Journal for the Tropics, 1(2), 7-9.

[8] Kawo, A.H, Abdullahi, B.A, Ganiya, Z.A, Halilu, A., Dabai, M. and Dakare, M.A (2009). Preliminary Phytochemical Screening, proximate and Elemental Composition of *Moringa Oleifera* Lam Seed Powder. Bajopas Volume 2 Number1, June, 2009.

[9] Kar, A., B.K. Choudhary and N.G. Bandyopadhyay, 2003. Comparative evaluation of hypoglycaemicactivity of some Indian medicinal plants in alloxan diabetic rats. Journal of Ethnopharmacology, 84(1): 105-108.

[10] Kawo, A.H. (2007): Water purification potentials and in-vivo toxicity evaluation of the aqueous and petroleum-ether extracts of Calotropisprocera (Ait.F.) Ait.F. latex and Moringa oleifera Lam seed powder. PhD thesis, Microbiology Unit, Department of Biological Sciences,

Bayero University, Kano, Nigeria.184 pp.99

[11] Kawo, A.H., B.A. Abdullahi, Z.A. Gaiya, A. Halilu, M. Dabai and M.A. Dakare, (2009). Preliminary Phytochemical Screening, Proximate and Elemental Composition Of Moringa Oleifera Lam Seed Powder. Bayero Journal of Pure and Applied Sciences, 2(1): pp 96-100.

[12] Newall, C.A, Anderson, I.A, and Philipson, J.D (1996) Herbal Medicines-A guide for health Care Professionals, Royal Pharmaceutical Society of Great BRITAIN, London pp 269-273.

[13] Oladele, F.A. and Yisa, A.B. (1989): Frequencies of dominant tree species used as source of fuel wood in Ilorin Local Government Area of Kwara State, Nigeria. Bioscience Research Communications **1**:pp 19-24

.

[14] Roloff, A., H. Weisgerber, U. Lang, B. Stimm, (2009). *Moringa oleifera* Lam, 1785; Wein

[15] Saka, J.D.K. and Msonthi, J.D. (1994): Nutritional value of edible fruits of indigenous wild trees in Malawi. Forest Ecology and Management 64**:** pp245 – 248.

[16] Yusuf, S. R. and Yusuf, D. I. (2014). Severe Damage of *Moringa Oleifera* Leaves by *Ulopeza Phaeothoracica Hampson* (Lepidoptera: Crambidae) in Ungogo Local Government Area, Kano State, Nigeria: A Short Communication. Bayero Journal of Pure and Applied Sciences, **7(1):** pp 127-130.