International Journal of Health Sciences and Research
Volume 14; Issue: 6; June 2024
Website: www.ijhsr.org
Original Research Article *ISSN: 2249-9571*

Quality Assessment of a Locally Processed Nigerian *Moringa Oleifera* Leaf Powder Intended for Use in a Clinical Trial

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DOI: https://doi.org/10.52403/ijhsr.20240628

ABSTRACT

Background: Preclinical evidence indicates that *Moringa oleifera* leaf has antihypertensive effects, making the herbal material an attractive candidate for hypertension clinical trials. The quality of herbal medicines is an important consideration for human usage.
Objective: To assess the quality of a Nigerian *Moringa oleifera* leaf powder by analysing the proximate, heavy metals, microbial and antioxidant contents.
Materials and Methods: *Moringa oleifera* leaves were harvested from cultivated sources, and the harvested leaves were dried under shade. The dried material was milled into powder and was stored in airtight polythene bags. Analyses of the proximate, heavy metals and microbial contents were done using standard methods. Results were compared to WHO guidelines. Phenolic and flavonoid content determination was performed using colorimetric techniques. The antioxidant assay of the herbal material was done using 1, 1-diphenyl-2-picrylhydrazyl.
Results: The proximate composition of the herbal material revealed 2.8% moisture content and 33.3% crude fibre. Heavy metals detected including lead, cobalt, copper, and cadmium were within the permissible limits of WHO standards. Microbial load, including total aerobic bacteria, fungi and indicator organisms, met the WHO standards. The phenolic content was 564.29 (SD=79.17) gallic acid equivalent, while the flavonoid content was 65.54 (SD=2.46) rutin equivalent per g of the test extract. The concentration of the test extract that scavenged 50% DPPH was 15.46 mg/ml.
Conclusion: The locally processed *Moringa oleifera* leaf powder is of quality fit for oral consumption in humans. The appreciable amounts of phenolics and flavonoids in *Moringa oleifera* leaf further highlight its antihypertensive potentiality.

Keywords: *Moringa oleifera*, quality, antioxidant assay, hypertension, Nigeria

INTRODUCTION

Several surveys indicate that *Moringa oleifera* leaf is commonly used for the traditional treatment of hypertension in many countries including Nigeria. [1-4]. In addition, there is increasing evidence from animal studies supporting the antihypertensive effects of *Moringa oleifera* leaf, making the herbal material an important candidate for hypertension clinical trials. [2,5,6] This is as the World Health Organisation promotes the development and integration of traditional herbal medicines into the national health systems in member countries to help improve access to medicines, especially in Low- and medium-income countries. [7] And, locally in

Nigeria, the development and integration of traditional herbal medicines into the national healthcare system for priority diseases like hypertension is part of the core mandates of the recently created Traditional, Complementary and Alternative Department in the Federal Ministry of Health. [8] The actual progress of integrating traditional herbal medicines, like *Moringa oleifera* leaf, into the mainstream clinical practice depends on research data on product quality, and clinical safety and efficacy. [7,9].

Moringa oleifera (Moringaceae, Lam), also known as *Moringa ptergossperma* (Gaerth), is a fast-growing, deciduous, and drought-resistant tree of the monogenous family Moringaceae. [10, 11] *Moringa oleifera* is native to the Indian and Pakistan regions and is naturalised in other tropical and subtropical regions, including Nigeria. [1,11,12] Common names for *Moringa oleifera* include Moringa, Ben oil tree, Drumstick tree (from the long and slender seed pods), Horseradish tree (from the taste of its roots), and miracle tree (due to its versatility and economic importance. [2,5] In Nigeria, *Moringa oleifera* is commonly known as "*Eweile*" "*Okochi egba*" and "*Zogale"* among the Yoruba, Igbo, and Hausa communities respectively.[1] *Moringa oleifera* leaf is rich in constituents comprising vitamins, elements, and phenolic compounds including flavonoids that are claimed to contribute to antihypertensive benefits through antioxidant mechanisms. [6,11] There is substantial evidence of the antihypertensive efficacy, and safety of *Moringa oleifera* leaf in animal studies to support the use of the herbal material in clinical trials. [2,5,13] The present study on the quality assessment of *Moringa oleifera* leaf powder is a preliminary step to the intended use of the herbal product in a clinical trial that is aimed at determining the antihypertensive effectiveness of the herbal product.

The quality of medicines connotes their performance on purity, strength and stability based on their intended route of administration in humans. [9,14] Quality assessment of herbal medicines is a regulatory requirement for all medicines intended for clinical trials and actual clinical practice to safeguard the well-being of the users. [9,14,15] *Moringa oleifera* leaf powder, processed from a local source, is intended to be used in a clinical study. Therefore, its quality in terms of fitness for oral administration to humans and the strength of its putative antihypertensive constituents needs to be assessed. Quality parameters analysed in this study are the proximate, microbial, heavy metals, and antioxidant contents of a locally processed Nigerian *Moringa oleifera* leaf powder.

MATERIALS & METHODS

1.2 Sample Preparation

Moringa oleifera leaves were harvested in December 2017 from cultivated sources at Lohmak village in Langtang-North Local Government Area of Plateau State, Nigeria. The harvested leaves were stripped of stems, and cleaned under running water from a borehole to remove any attached foreign matter and dirt. Drying was accomplished by spreading the cleansed leaves on mats in a house for 4 days. A motor-drive mill ground the dried leaves into a fine *Moringa oleifera* leaf powder. The *Moringa oleifera* leaf powder was stored in air-tight polythene bags, away from sunlight and humid areas, for further use. The *Moringa oleifera* leaf was identified at the Herbarium Unit of the Federal School of Forestry Jos, Plateau State, and a voucher for reference was prepared (Voucher number: FHJ235).

1.2 Proximate Analysis

Proximate analysis was determined at the Kofar Laboratory Services Jos, Nigeria, using the Association of Official Analytical Chemists (AOAC) methods. [16] Ash and moisture contents were determined using the weight difference method. The crucible was weighed alone, followed by weighing it plus the sample material. The sample material was then incinerated in a Muffle furnace at 400^0C for 24 hours. The remaining inorganic material was cooled in a desiccator and weighed. The ash content was determined using Equation 1:

$$\% \, Ash = \frac{Weight\ of\ sample\ remaining}{Weight\ of\ original\ sample} X \frac{100}{1} \quad (1)$$

For moisture content determination, the oven was used to heat the sample material till constant weight was obtained. The difference in weight before and after oven heating represented the moisture content. The percentage moisture content was calculated using Equation 2:

$$\% \, Moisture\ content = \frac{Moisture}{Weight\ of\ original\ sample} X \frac{100}{1} \quad (2)$$

For the determination of the crude fibre content, 1 g of the sample material was weighed and 50 mL of 0.05 M phosphate buffer pH 6 was added. The sample was then placed in the bath at 95 ^{0}C for 30 minutes, and alpha-amylase was added. After that, the pH was adjusted to 4.5, and the enzyme amyl-glucosidase (6 U/mg) was added. After finishing the digestion, ethanol was added to precipitate the fibre before filtering. The crude fibre was determined using equation 3, where R = weight of the sample residue, P = protein from the residue, A = ash from the residue, and W = sample weights:

$$\text{Crude fiber } (\%) = \frac{\frac{R1-R2}{2} - (P+A)}{\frac{W1-W2}{2} X\ 100} \quad (3)$$

Protein content was determined using the Kjeldhal Nitrogen method. For this method, 2 g of the sample was weighed into a tube, and 7 g catalytic mixture and 15 mL of 96% sulfuric acid were added. The tube was placed in a heating block, reaching 450 ^{0}C for 1 hour. After cooling, distillation was done by adding 32% NaOH until the neutralisation of the mixture. The total protein was then calculated using Equation 4 below. In the formula, V represents the total volume used to neutralise the mixture, and W is the weight of the sample:

$$Protein\ (\%) = \frac{V(HCL)\ X\ 0.1\ X\ 1.4\ X\ 5.7}{W} \quad (4)$$

A Soxhlet extractor was used to determine the total fat content of 1 g of the dried sample material. The sample material was kept in a cellulose cartridge, and 50 ml ether was added. The ether was heated to 80 ^{0}C to extract the fat from the sample. The extracted fat dropped into a previously weighed aluminium cup. The fat content was calculated by using the difference in the weight of the aluminium cup. Lastly, the carbohydrate content was determined by the difference method as shown in Equation 5:

$$\text{Carbohydrate}(\%) = 100 - \%\ (protein + fat + fiber + ash + moisture) \quad (5)$$

1.3 Heavy Metals Analysis

The heavy element contamination of *Moringa oleifera* leaf powder was determined at the Kofar Laboratory Services Jos, Nigeria, using AOAC standard methods. [16] The results were reported in parts per million (ppm).

1.4 Microbial Assessment

The microbial assessment of the *Moringa oleifera* leaf sample was done at the Department of Pharmaceutical Microbiology Laboratory, University of Jos, Nigeria. One gram of the powdered material was weighed and suspended in 10 ml of sterile water/peptone. From 1 ml of the suspended preparations lower dilutions (1; 10, 1:100) were made and the aliquots were plated on Nutrient agar (NA) and Sabouraud dextrose agar (SDA) for total bacterial and fungal growth enumeration respectively. For indicator bacteria, MacConkey agar (MCA), Mannitol salt agar (MSA), Salmonella/Shigella agar (SSA) and Cetrimide agar (CA) were used to assess for the presence of *Escherichia* coli, *Staphylococcus aureus*, *Salmonella spp.* and

Pseudomonas aeruginosa respectively. They were then incubated at 37^0 C, and the microbial count was read at 24 hours and 48 hours for sterile containers. All tests were done in duplicates, and the results were expressed as mean colony forming unit (CFU) per gram of culture medium.

1.5 Phenolic and Flavonoid Assessments

The phenolic and flavonoid contents were determined at the Pharmaceutical Chemistry Laboratory of the Faculty of Pharmaceutical Sciences, University of Jos, Nigeria. *Moringa oleifera* leaf powder was extracted with 70% ethanol for 72 hours. The filtrate was filtered twice using Whatman filter paper number 1. The extract was concentrated in-vacuo over a rotary evaporator to afford dark green hydroethanolic extract. The extract was kept for further use.

The total phenolic content of the extract was evaluated by a colorimetric method utilising the Folin-Ciocalteu reagent according to the method described by Adedapo, Jimoh, Afolayan and Masika. [17] In brief, an aliquot of the extracts was mixed with 5 ml Folin-Ciocalteu reagent. The tubes were vortexed for 15 seconds and allowed to stand for 30 minutes at 40^0C for colour development. Samples containing polyphenols are reduced by the Folin-Ciocalteu reagent thereby producing blue coloured complex. The phenolic concentration of the 70% ethanolic extract was evaluated from a Gallic acid calibration curve. 500µL aliquots of 10, 20, 30, 40, 50, and 60 µg/mL methanolic Gallic acid solutions were mixed with 2.5 mL Folin–Ciocalteu reagent (diluted ten-fold) and 2.5 mL (75 g/L) sodium carbonate. The tubes were vortexed for 10 seconds and allowed to stand for 2 hours at 25º C. After incubation at 25 °C, absorbance was measured at 765 nm against reagent blank using the Shimadzu UV-Vis Spectrophotometer 1650 (Japan). Total phenolic content was expressed as mg Gallic acid equivalent/g using equation 6 based on the calibration curve:

$$y = 0.0069x + 0.0673, \quad R^2 = 0.9947 \quad (6)$$

Where x was the absorbance
And y was the Gallic acid equivalent (mg/g)

All determinations were performed in triplicate. Total phenolic content was expressed as milligrams of Gallic acid equivalent (GAE) per gram of extract.

The total flavonoid content of the 70% ethanolic extract was measured using an aluminium chloride colorimetric assay reported by Zhishen, Mengcheng and Jianming. [18] An aliquot (1 mL) of extract (40 mg) or rutin standard solution with the following concentrations (10, 20, 40, 60, 80 & 100 µg/mL) was added to a 10 mL volumetric flask containing 4 mL of distilled water. To the flask, 300 µL of 5% sodium nitrate ($NaNO_2$) and 300 µL of 10 % aluminium dichloride ($AlCl_3$) were added. After 6 minutes, 2 mL of 1 molar sodium hydroxide (NaOH) was added and the total volume was brought to 10 mL by the addition of 2.4 mL water. The solution was vortexed to mix the mixture thoroughly and the absorbance was measured at 510 nm against the reagent blank using the UV-Vis Spectrophotometer 1650 Shimadzu, Japan. The total flavonoid content of the 70% ethanolic extract was expressed as mg Rutin equivalents mg (RE)/g of 70% ethanolic extract. All treatments were carried out in triplicate. The results were calculated using the standard calibration curve of rutin in methanol (R^2 = 0.9957) and expressed as rutin equivalents per gram of the test extract (RE mg/g).

1.6 Antioxidant Assay

The antioxidant activity (free radical scavenging activity) of the extract on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was determined according to the method described in Brand-Williams, Cuvelier and Berset. [19] The following concentrations of 70% ethanolic extract of plant material were prepared 500, 250, 125,

62.50, 31.25, 15.62, 7.8125, 3.91, 1.95 and 0.98µg/mL. All the solutions were prepared with methanol. Two millilitres (2mL) of each prepared concentration were mixed with 4mL of 50µM DPPH solution in methanol. The experiment was done in triplicate. The mixture was vortexed for 10s to homogenise the mixture and test tubes were incubated for 30 minutes at room temperature in the dark, after 30 minutes of incubation the absorbance was measured at 515 nm using UV-vis spectrophotometer (Shimadzu, 1620 Japan). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Ascorbic acid was used as standard with the following concentrations 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.7812, 0.391, and 0.195µM. A blank solution was prepared by mixing 2mL of methanol with 4mL of 50µM DPPH solution in methanol.

The difference in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as percentage scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation 7:

$$\%\ inhibition = \frac{Abs(control) - Abs\ (sample)}{Abs\ (control) X\ 100} \quad (7)$$

Finally, the 50% inhibition (IC_{50}) value, defined as the concentration of the sample leading to a 50% reduction of the initial DPPH concentration, was calculated from the separate linear regression of plots of the mean percentage of the antioxidant activity against the concentration of the test extract (µg/mL).

RESULTS

Proximate Composition of *Moringa oleifera* Leaf Powder

The result presented in **Table 1** is the proximate composition of the locally processed Nigerian *Moringa oleifera* leaf powder. The dry *Moringa oleifera* leaf powder had a moisture content of 2.8% and a crude fibre composition of 33.6%.

Table 1. Proximate Composition of *Moringa oleifera* Leaf Powder

Parameter	Composition (%)
Moisture	2.80
Fat	4.60
Ash	12.94
Protein	22.70
Carbohydrate	23.90
Crude fiber	33.60

Heavy Metals Composition of *Moringa oleifera* Leaf Powder

The heavy metals composition of the locally processed *Moringa oleifera* leaf powder is presented in **Table 2**. Heavy metals analysis detected lead, cobalt, copper, zinc and cadmium in the locally processed *Moringa oleifera* leaf powder. However, all the detected heavy metals were within the WHO permissible limits for herbal medicines for internal use.

Table 2. Elemental Composition of *Moringa Oleifera* Leaf Powder

Element	Quantity (ppm)	Permissible limit (ppm) [14]	Comment
Lead	0.427	10	Within limit
Cobalt	0.370	0.48	Within limit
Cupper	0.310	20	Within limit
Zinc	0.270	50	Within limit
Cadmium	0.019	0.3	Within limit

Microbial Load of *Moringa oleifera* Leaf Powder

The results of the microbial assessment of *Moringa oleifera* leaf powder are shown in **Table 3**. Aerobic bacteria growth was recorded in the plant material and increased in a duration-dependent fashion. There was no fungal growth and no growth for other indicator organisms within the experimental period. The microbial results were within the WHO permissible limits. [14]

Table 3. Microbial Load of *Moringa oleifera* Leaf Powder

Parameter	Attribute	Count (cfu/g)	Reference limit (cfu/g) [14]	Comment
Total aerobic microbial count	Bacteria			Within limit
	24-hour	5.60×10^2	10^5	
	48-hour	1.91×10^3	10^5	
	Fungi			Within limit
	24-hour	Nil	10^3	
	48-hour	Nil	10^3	
Indicator organism	*E. coli*	Nil	10	Within limit
	S. aureus	Nil	Absence	Within limit
	Ps. Aeruginosa	Nil	Absence	Within limit
	Salmonella spp.	Nil	Absence	Within limit

cfu = colony forming unit

Phenolic and Flavonoid Contents of *Moringa oleifera* Leaf

The mean phenolic content of *Moringa oleifera* leaf ethanolic extract was 564.29 (SD = 79.17) milligrams of gallic acid equivalent per gram of the test extract, and the mean flavonoid content was 65.54 (SD =2.46) milligrams of rutin equivalent per gram of the test extract.

Antioxidant Activity of *Moringa oleifera* Leaf

The minimum concentration of *Moringa oleifera* leaf ethanolic extract that scavenged 50% of DPPH radical (IC_{50}) was 15.46 mg/ml (SD=1.18), which was significantly (P<0.05) higher when compared to the IC_{50}value of ascorbic acid as the reference antioxidant (1.61, SD=0.03).

DISCUSSION

With *Moringa oleifera* leaf products showing promising antihypertensive benefits in animal studies, the employment of the herbal product in hypertensive clinical trials is justified to determine clinical safety and efficacy data that will inform the possible development and integration of the herbal product. The clinical use of *Moringa oleifera* leaf powder demands a quality assessment of its fitness for human consumption and the concentration of its claimed antihypertensive chemical constituents. [9,14] This present study assessed the proximate composition, heavy metals and microbial loads. Also determined were the phenolic, flavonoid and antioxidant assay of the locally processed Nigerian *Moringa oleifera* leaf powder.

The moisture content of the locally processed *Moringa oleifera* leaf powder was within the permissible limit of 0.5% set by the National Agency for Food, Drug Administration and Control (NAFDAC) for solid-form herbal medicines intended for oral use. [20] This indicates good stability of the herbal product as a low moisture content slows down microbial growth and cellular enzymatic hydrolysis of medicines. [21] This finding agrees with reports in which dried *Moringa oleifera* leaf powder from the Northern region of Nigeria had moisture content below 5%. [22, 23] Effective drying of *Moringa oleifera* leaf is known to reduce its moisture content which is important for retarding microbial degradation of herbal remedies. [21] Herbal medicines can be contaminated with microbes during cultivation, processing and handling. Microbial contamination of herbal products shortens the shelf-life of the product and can cause serious infections in human consumption. [21]. The results for microbial contents of the locally processed *Moringa oleifera* leaf powder show that the herbal product meets the World Health Organisation's specifications regarding total aerobic bacterial and fungi counts, as well as the absence of indicative pathogenic organisms. [21]. This result indicates that when administered orally, the locally processed Moringa oleifera leaf powder will not significantly introduce pathogenic microbes to humans. This finding is consistent with a study by Adebayo and

Abdul-Salaam that reported good microbial quality of a dried Nigerian *Moringa oleifera* leaf powder, which was attributed to the antimicrobial activity of the herbal material. [23] However, the finding of this study is in contrast to a report by Monera-Penduka and colleagues in which *Moringa oleifera* leaf powders marketed in Zimbabwe had bacterial, fungi and indicative pathogenic bacteria above permissible limits. [24] Differences in cultivation, processing and handling practices might account for the contrast in microbial loads between the studies. Good agricultural practices, good harvesting practices and good manufacturing practices are required in the production of herbal medicines to minimise microbial contamination. [9, 14]

Herbal medicines can be contaminated with heavy metals by polluted soil, water and air during cultivation and processing stages. [14]. Heavy metals have harmful effects on humans, including hypertension pathophysiology.[25] Therefore, the WHO set specifications for heavy metal limits for herbal medicines intended for use in humans. [14] The results for heavy metal contents of the locally processed *Moringa oleifera* leaf powder show it met the permissible limits specified by the WHO. [14] This result indicates that the methods used in processing the *Moringa oleifera* leaf powder successfully limited the heavy metals contamination of the herbal product. Therefore, the consumption of the herbal product will not contribute significant toxic heavy metals to humans. The finding of this study contrasts with that reported by Monera-Penduka and colleagues, in which the concentration of arsenic, cadmium, and nickel in *Moringa oleifera* leaf powder marketed for human use in Zimbabwe was above the permissible limits set by the WHO. [14, 24]

Phenolics including flavonoids are the active constituents partly responsible for the blood pressure-lowering effects of *Moringa oleifera* leaf via antioxidant mechanisms.[6, 26, 27] Therefore, an assay of phenolic and flavonoid contents and antioxidant activity measures the active principle in the herbal product. [9] The results of the present study indicate good antihypertensive potentials of the locally processed *Moringa oleifera* leaf because of the high phenolic content and antioxidant activity. This study's finding is consistent with the report of high flavonoid content in the dry leaf of African, Indian and Indonesian *Moringa oleifera* ecotypes cultivated in Indonesia. [28] The antihypertensive effect of phenolic compounds depends partly on their concentrations. [29,30]

CONCLUSION

The locally processed Nigerian *Moringa oleifera* leaf powder met the permissible limits for microbial and heavy metals contamination, therefore, it is of quality fit for oral consumption in humans. The appreciable amounts of phenolics and flavonoids in *Moringa oleifera* leaf further highlight its antihypertensive potentiality.

Declaration by Authors

Ethical Approval: Not applicable

Acknowledgement: The authors the following: Prof. Kolawale Jacob of the Kofar Services Jos Nigeria for the proximate and heavy metals analysis; Prof. Alemika Taiwo and Dr. Ojerinde Stephen of the Department of Pharmaceutical Chemistry, University of Jos, Nigeria for the phenolic, flavonoid and antioxidant contents analyses; and Prof. Olurunfemi Patrick and Mr. Lamsing Nanpon of the Department of Pharmaceutical Microbiology, University of Jos, for the microbial assessment of the crude drug.

Source of Funding: None

Conflict of Interest: The authors declare no conflict of interest.

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How to cite this article: Shalkur David, Maxwell Lonji Dapar, Nanloh Samuel Jimam. Quality assessment of a locally processed Nigerian Moringa Oleifera leaf powder intended for use in a clinical trial. *Int J Health Sci Res.* 2024; 14(6):190-198. DOI: *10.52403/ijhsr.20240628*

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