



## Phytochemical and Nutritional Evaluation of Southeastern Nigerian Grown *Moringa oleifera* Leaf Extract

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### Authors' contributions

This work was carried out in collaboration between both authors. Author ICE designed the study, wrote the protocol, wrote the first draft of the manuscript and managed the literature searches while author ORE managed the analyses of the study, performed the spectroscopy and managed the experimental process. Both authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/JOCAMR/2016/30018

#### Editor(s):

(1) Sabyasachi Chatterjee, Burdwan University, India.

#### Reviewers:

(1) Paul Eme, University of Nigeria, Nigeria.

(2) Kanokporn Boonsirichai, Thailand Institute of Nuclear Technology, Thailand.

(3) Sunisa Sirimongkolvorakul, Mahanakorn University, Thailand.

Complete Peer review History: <http://www.sciencedomain.org/review-history/17203>

Original Research Article

Received 12<sup>th</sup> October 2016  
Accepted 5<sup>th</sup> December 2016  
Published 10<sup>th</sup> December 2016

### ABSTRACT

Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illnesses. The plant kingdom represents a rich store house of organic compounds, many of which have been used for medicinal purposes. In recent times, focus on plant research has increased all over the world and a large body of evidence has shown immense potentials of medicinal plants in various traditional systems. The aqueous leaf extract of *M. oleifera*, obtained during the dry season (October, 2015) from the Southeastern Nigeria, was subjected to qualitative and quantitative screening for chemical constituents using standard procedures. The result showed the presence of flavonoids, alkaloids, saponins, terpenoids, carotenoids, phenols and tannins. The proximate analysis showed the presence of carbohydrates (57.71±0.01%), protein (16.09±0.02%), fats (2.84±0.02%), crude fiber (9.11±0.01%),

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moisture ( $4.08 \pm 0.04\%$ ) and ash ( $10.06 \pm 0.04\%$ ). The mineral constituents showed nitrogen ( $6.03 \pm 0.02$  g/100 g), calcium ( $3.09 \pm 0.01$  g/100 g), magnesium ( $0.04 \pm 0.03$  g/100 g), potassium ( $2.6 \pm 0.02$  g/100 g), phosphorus ( $0.48 \pm 0.04$  g/100 g), zinc ( $0.09 \pm 0.00$  g/100 g), copper ( $0.06 \pm 0.03$  g/100 g), iron ( $0.82 \pm 0.21$  g/100 g) and sulphur ( $0.92 \pm 0.06$  g/100 g). The chemical compounds found in the leaf extracts of *M. oleifera* grown in Southeastern Nigeria have good pharmacological properties. In Nigeria, the plant is widely used for the treatment of bacterial infection, fungal infection, diabetes, anti-inflammation, sexually-transmitted diseases, malnutrition and diarrhoea. This finding validates the claims of *M. oleifera* as possessing nutritional and ethno-medicinal properties.

**Keywords:** *Phytochemical; proximate; mineral contents; Southeastern Nigerian grown Moringa oleifera.*

## 1. INTRODUCTION

For a long time, plants have been an important source of natural products for human health. The practice of complementary and alternative medicine is now on the increase in developing countries. This is in response to World Health Organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine [1,2]. Immense benefits have been derived by man from using medicinal herbs in disease management because they are relatively safer, more affordable and sometimes offer better therapeutic value than synthetic drugs. The increasing discovery of more medicinal plants has demanded for increased scientific scrutiny of their bioactivity so as to provide data that will help physicians and patients make wise decision before using them. *M. oleifera* is a well documented world renowned plant herb for its extraordinary nutritional and medicinal properties. It belongs to the family of *Moringaceae*, a fast growing drought-resistant tree, native of Sub-Himalayan tracts of Northern India but now distributed worldwide in the tropics and sub tropics [3]. *M. oleifera* is referred to as a "miracle tree" or a "wonder tree" of significant socio-economic importance because of its several nutritional [4,5], pharmacological [6,7] and industrial [8] applications. The leaves of this plant contain a profile of important trace elements, and are a good source of proteins, vitamins, beta-carotene, amino acids and various phenolics [9]. It is a common vegetable consumed in Nigeria. The tree's leaf and seed pod are widely consumed as food. The bark, leaf and root have ethno-medicinal properties [10]. It is a natural antihelmintic, antibiotic, detoxifier, outstanding immune builder and is used in many

countries to treat malnutrition and malaria [7,11]. The powdered seed of *M. oleifera* is used as coagulant for water purification and therefore helps in reducing the incidence of water borne diseases [12,13,14,15]. Apart from ethno-medicinal and nutritional uses, there are several reports on biological activities of *M. oleifera* in literature which include hypotensive activities [16], hypocholesterolemic effects [17,18], anti-inflammatory [19] and anti-helmintic, analgesic, management of heart diseases, dyspepsia and ulcers [16].

*M. oleifera* is rich in phytochemicals which are chemical products of various parts of the plants. Chemical constituents of the aqueous extract of the fresh leaves is found to be rich in tannins, saponins, flavonoids, cardiac glycosides, alkaloids, terpenoids, anthraquinone, anthocyanin, carotenoids, steroid and phenol [20,21,22]. The proximate and mineral contents have also revealed the presence of moisture, lipid, fiber, ashes, crude protein, carbohydrates, iron, calcium, magnesium, potassium, phosphorus, zinc, copper and sulphur [5,22]. These compounds have various pharmacological and nutritional benefits. However, a number of environmental factors may affect the growth of the plants which in turn affect the quality of herbal ingredients present in a particular species even when it is obtained from the same country. These factors include climate, altitude, rainfall and other conditions that may result to major variations in the bioactive compounds. Although, much have been done on the plant particularly in India; yet not much information is available for the Southeastern Nigerian grown *M. oleifera*. This research work is centered on investigating, analyzing and validating the ethno-medicinal and nutritional claims of Southeastern Nigerian grown *M. oleifera* plant.

## **2. MATERIALS AND METHODS**

### **2.1 Collection and Identification of Plant Materials**

Fresh leaf samples of *M. oleifera* were harvested from the Botanical Garden of the Biology Department, Abia State Polytechnic, Aba, Nigeria. A plant taxonomist in the Department identified and authenticated the leaves as *M. oleifera* with voucher number BGBD 101.

### **2.2 Extraction of Plant Materials**

The methods of Oyagade et al. [23] were employed in the preparation of the plant extracts. The leaves of *M. oleifera* were plucked, rinsed with tap water and air-dried at room temperature for 3-4 days. The dried leaves were pulverized using a milling machine to obtain fine powder. 50 g of finely grounded leaf of the plant materials was suspended in 250 mL of hot distilled water for 48 hours, to achieve pure dissolution and extraction of the sample. The samples were filtered using Whatman's No 1 filter paper and the filtrate obtained was concentrated in water bath at 40°C for about 12-14 hours.

### **2.3 Phytochemical Screening of the Leaves of *M. oleifera***

The aqueous extracts of *M. oleifera* were subjected to qualitative and quantitative screening for chemical constituents using standard procedures [24,25,26]. The methods of James [27] and AOAC [28] were employed in evaluating the mineral and proximate compositions of the whole leaf extract.

### **2.4 Qualitative Methods of Analysis [24,25,26]**

#### **2.4.1 Test for flavonoids**

About 0.5 g of each portion was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of conc. HCl. A pink, orange, or red to purple coloration indicates the presence of flavonoids.

#### **2.4.2 Test for saponins**

Crude extract (10.0 mL) was mixed with 5 ml of distilled water in a test tube and shaken

vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

#### **2.4.3 Test for tannins**

About 0.5 g of the dried powdered sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration. This confirmed the presence of tannins.

#### **2.4.4 Test for steroids**

Crude extract was mixed with 2 mL of chloroform and concentrated H<sub>2</sub>SO<sub>4</sub> was added sidewise. A red color produced in the lower chloroform layer indicated the presence of steroids.

#### **2.4.5 Test for terpenoids**

Crude extract was dissolved in 2 mL of chloroform and evaporated to dryness. To this, 2 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was added and heated for about 2 minutes. A grayish color indicated the presence of terpenoids.

#### **2.4.6 Test for alkaloids**

1 mL of the extract was mixed with 2.0 mL of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

#### **2.4.7 Test for phenols**

The extract (1.0 mL) was added into 1.0 mL of 10% ferric chloride. The formation of a greenish-brown or black precipitate or color confirmed the presence of phenols.

### **2.5 Quantitative Methods of Analysis [24,25,26]**

#### **2.5.1 Determination of alkaloids**

The gravimetric method was used in determining the alkaloids. About 5 g of the sample was dispersed in 50 mL of 10% acetic acid solution in ethanol. The filtrate was evaporated to quarter of its original volume. Concentrated NH<sub>4</sub>OH was added drop-wise to precipitate the alkaloids. The precipitate was filtered off and washed with 1% NH<sub>4</sub>OH solution and weighed. The precipitate was dried at 60°C for 30 minutes and reweighed.

By weight difference, the weight of alkaloid was determined.

### **2.5.2 Determination of tannin**

0.5 g of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 mL of 0.1 M FeCl<sub>3</sub> in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

### **2.5.3 Determination of saponins**

A pre-weighed round bottom flask was fitted unto the soxhlet apparatus (bearing the sample containing thimble) and methanol poured into the flask. The methanol should be enough to cause the reflux. The saponins was exhaustively extracted for 3 hours by heating the flask on a hot plate after which the difference between the final and the initials weights of the flask represents the weight of saponins extracted.

### **2.5.4 Determination of steroids**

Five grams (5.0 g) of the powdered leaf sample was hydrolyzed by boiling in 50mL hydrochloric acid for 20 minutes and filtered using Whatman's filter paper (No 42). Equal volume of ethyl acetate was added. The content was properly mixed and allowed to separate into 2 layers. The ethyl acetate (extract) was recovered, while the aqueous layer was discarded. The extract was heated at 100°C for 5 minutes in a steam bath and later heated with concentrated amylalcohol to extract the steroids. The turbid mixture was filtered and dried. The dry extract was cooled and used to determine the steroids.

### **2.5.5 Determination of flavonoids**

Five grams of the sample was mixed with 1mL of diluted HCl in a ratio of 1:10w/v. The mixture was boiled for 30 minutes. The boiled extract was allowed to cool and filtered. 20 mL of the filtrate was treated with ethyl acetate to precipitate the flavonoids. The precipitate was measured and determined by weight difference.

### **2.5.6 Determination of phenols (Spectrophotometric method)**

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic

component for 15 min. 5mL of the extract was pipetted into a 50 mL flask, and then 10mL of distilled water was added. 2 mL of ammonium hydroxide solution and 5 mL of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30 min for color development. It was measured at 505 nm.

## **2.6 Determination of Proximate Composition [27,28]**

### **2.6.1 Determination of moisture content**

Five grams (5.0 g) of the sample was weighed into weighed moisture can. The can and content were dried in the oven at 150°C for 3 hours. The difference in weight after drying was used for the calculation of moisture content.

### **2.6.2 Determination of ash content**

Five grams of the sample was weighed in a previously weighed porcelain crucible and allowed to burn for 2- 3 hours in a muffle furnace at 550°C. The difference in weight was used for calculation.

### **2.6.3 Determination of crude fiber**

Five grams of the sample was defatted and boiled in 200 mL of 1.25% H<sub>2</sub>SO<sub>4</sub> solution under reflux for 30 minutes. This was later washed with hot water using a two-fold muslin cloth to trap the sample particles. The sample was carefully transferred to a flask and 20 mL of 1.25% NaOH solution was added before boiling for 30 minutes. The sample was later dried and reweighed and the difference in weight was used for calculation.

### **2.6.4 Determination of protein**

The micro Kjeldahl method was used for Nitrogen estimation. Sample was digested with concentrated sulfuric acid in the presence of copper sulphate. The ammonia was distilled by the addition of excess sodium hydroxide. Released ammonia was collected in boric acid and titrated with standard hydrochloric acid using methylene blue as an indicator. Total protein was calculated by multiplying nitrogen percentage by 6.25.

### **2.6.5 Determination of fat content**

The apparatus used for estimation of fat is Soxhlet extractor. To determine the percentage of fat the dried sample of plant was extracted

with petroleum ether. It was then distilled off completely and dried. The oil weight and percentage of oil was calculated.

### 2.6.6 Determination of carbohydrates

The carbohydrate content was calculated by difference as the nitrogen free extraction (NFE). The NFE was calculated using the formula:

$$\%NFE = 100 - (\%MC + \%ASH + \%CF + \%EE + \%CP),$$

where MC= moisture content; CF= crude fiber; EE= ether extract and CP= crude protein.

### 2.6.7 Determination of minerals

The sample for the determination of the elements was subjected to acid digestion using concentrated hydrochloric acid and subsequently, the different elements were determined using appropriate methods as described by James [27].

## 2.7 Statistical Analysis

The data obtained from this study were analyzed using descriptive statistics and presented as mean  $\pm$  standard error of mean of three (3) determinants (mean  $\pm$  SEM) using the statistical software package (SPSS) for window version. The proximate analysis was expressed in percentage (%) while the phytochemical and mineral contents were expressed in g/100 g.

## 3. RESULTS

The qualitative and quantitative screening of the phytochemical constituents of the leaf of *M. oleifera* is shown in Table 1. The result showed that tannins (4.36 $\pm$ 0.04 g/100 g) and terpenoids (4.30 $\pm$ 0.05 g/100 g) were highly present relative to the other components; flavonoid (3.46 $\pm$ 0.03 g/100 g), alkaloids (3.30  $\pm$  0.00 g/100 g) and steroids (3.22 $\pm$ 0.00 g/100 g) were moderately present while carotenoids (1.18  $\pm$  0.05 g/100 g), saponins (0.95  $\pm$  0.03 g/100 g) and phenols (0.26 $\pm$ 0.04 g/100 g) were slightly present.

The quantitative proximate composition of leaf extract of *M. oleifera* is shown in Table 2. The results from this study showed *M. oleifera* had 57.71 $\pm$  0.01% carbohydrate content, protein (16.09 $\pm$ 0.02%), fat (2.84 $\pm$  0.02%), crude fiber (9.11 $\pm$  0.01%), moisture (4.08 $\pm$ 0.04%) and ash (10.06 $\pm$ 0.04%).

**Table 1. Qualitative and quantitative screening of the phytochemical constituents of *M. oleifera* leaf extract**

Phytochemical group	Qualitative composition	Quantitative composition (g/100 g)
Flavonoids	++	3.46 $\pm$ 0.03
Alkaloids	++	3.30 $\pm$ 0.00
Saponins	+	0.95 $\pm$ 0.03
Steroids	++	3.22 $\pm$ 0.00
Tannins	+++	4.36 $\pm$ 0.04
Carotenoids	+	1.18 $\pm$ 0.05
Terpenoids	+++	4.30 $\pm$ 0.05
Phenols	+	0.26 $\pm$ 0.04

Key: += slightly present; ++ = moderately present; +++ = highly present (relative to the other components)

**Table 2. Quantitative screening of proximate composition of *M. oleifera* leaf extract**

Proximate composition	Quantitative composition (%)
Carbohydrates	57.71 $\pm$ 0.01
Protein	16.09 $\pm$ 0.02
Fat	2.84 $\pm$ 0.02
Crude fiber	9.11 $\pm$ 0.01
Moisture	4.08 $\pm$ 0.04
Ash	10.06 $\pm$ 0.04

Table 3 shows the quantitative mineral composition of leaf extract of *M. oleifera*. Nitrogen was 6.03  $\pm$  0.02 g/100 g, calcium was 3.09  $\pm$  0.01 g/100 g and potassium was 2.68  $\pm$  0.02 g/100 g. Others include sulphur (0.92  $\pm$  0.06 g/100 g), iron (0.82  $\pm$  0.21 g/100 g), phosphorus (0.48  $\pm$  0.04 g/100 g), magnesium (0.04 $\pm$  0.03 g/100 g), zinc (0.09 $\pm$ 0.00 g/100 g) and copper (0.06 $\pm$ 0.03 g/100 g).

**Table 3. Quantitative mineral composition of leaf extract of *M. oleifera***

Mineral composition	Quantitative composition (g/100 g)
Nitrogen	6.03 $\pm$ 0.02
Calcium	3.09 $\pm$ 0.01
Magnesium	0.40 $\pm$ 0.03
Potassium	2.68 $\pm$ 0.02
Phosphorus	0.48 $\pm$ 0.04
Zinc	0.09 $\pm$ 0.00
Iron	0.82 $\pm$ 0.21
Copper	0.06 $\pm$ 0.03
Sulphur	0.92 $\pm$ 0.06

## 4. DISCUSSION

The practice of complementary and alternative medicine is now on the increase in developing countries. *M. oleifera* is a widely used plant

because of its nutritional and medicinal potentials. All parts of this plant are edible and have been consumed by humans. It is considered a complete food because of its high nutritional value with impressive range of medicinal uses and this necessitated this scientific study. The qualitative and quantitative screenings of the phytochemical constituents of the leaf extracts of *M. oleifera* in this study, showed tannin and terpenoid were present in high concentration relative to the other components; flavonoid, alkaloid and steroid were moderately present while saponnin, carotenoid and phenol were slightly present. This finding is comparable with the results obtained by Kwaghe and Ambali [20], Azubuogu [22], and Nweze and Nwafor [29]. So far researchers have reported that different extraction solvents have different extraction capabilities and spectrum of solubility for phyto-constituents [22,29,30]. The findings of Azubuogu [22], using ethanol, n-Hexane, ethyl acetate and water, observed water to be a better solvent in the extraction of the bioactive agents. Similarly, Nweze and Nwafor [29] obtained higher values of phytochemicals with water compared to ethanol. Rockwood et al. [31] also using three different solvents (de-ionized water, inorganic ethanol and organic ethyl acetate) as extraction agents for seeds and leaves of *M. oleifera*, observed that de-ionized water was the only solvent capable of extracting plant constituents which conferred bacterial inhibition.

The quantitative proximate composition also showed the leaf to be rich in carbohydrate ( $57.71 \pm 0.01\%$ ) and protein ( $16.09 \pm 0.02\%$ ). Other nutrients present in moderate concentrations include fats, crude fiber, moisture and ash. This result is in agreement with the values reported by Nweze and Nwafor [29] on *M. oleifera* leaf extract obtained from Southeastern Nigeria. However a study carried out in India by Verma and Nigam [32] gave higher protein (34.93%), fiber (22.90%) and fat (16.07%) contents but lower in carbohydrates (7.4%) when compared with this result. The variations between our result and the reports obtained by Verma and Nigam [32] from India and Valdez-Solana [5] from the Mexican cultivars may depend on the seasonal variations, the plants' stages of development and the techniques employed before analyses. Even when the cultivars are from the same country, differences may still exist. For example, the proximate composition obtained from Northern Nigeria by Oghe and Affiku [33] on fiber, fat and ash were higher compare to the results obtained from this study. The rich proximate composition

of *M. oleifera* leaf extract justifies its direct use in development of balanced diets for human and animal nutrition. Thus, *M. oleifera* leaves could be used to prevent malnutrition.

The present work also revealed the leaf extract of *M. oleifera* to be rich in some macro-elements like nitrogen ( $6.03 \pm 0.02$  g/100 g), calcium ( $3.09 \pm 0.01$  g/100 g) and potassium ( $2.68 \pm 0.02$  g/100 g). Other macro-elements present in smaller concentrations include magnesium, phosphorus and sulphur. The micro-elements tested and found present include iron, zinc and copper. The values obtained from the Mexican cultivars [5] were lower compared to our reports on the minerals composition of Southeastern Nigerian cultivars. Variations in bioactive compounds are affected by environmental factors which in turn affect the quality of herbal ingredients present in a particular species [34]. These minerals and phytochemicals may contribute to the therapeutic properties of *M. oleifera*.

## 5. CONCLUSION

To validate the ethno-medicinal and nutritional potentials of *M. oleifera*, we investigated the phytochemical, proximate and mineral compositions of the plant. The results showed *M. oleifera* to possess potentials that could serve as excellent source of useful drugs. The pharmaceutical companies should make use of the information on the excellent medicinal value of this plant, to produce cheaper and effective alternative to the synthetic drugs. The plant is also rich in proximate and mineral contents and thus can be used to solve malnutrition problems.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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