



Studies on Chemical Constituents and Nutrients Bioavailability in *Moringa oleifera* Leaf and Seed

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OAO and KAT designed the study, Author OAO performed the chemical analysis and wrote the first draft of the manuscript. Authors SOG, BSO and MMI worked on the technical quality of the manuscript and its revision. Author KAT supervised the project. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to evaluate and compare the nutritional composition, antinutritional factors and antioxidant potentials of the bioactive compounds of *Moringa oleifera* (*M. oleifera*) seeds and leaves.

Place and Duration: The study was carried out in the department of Food Science and Technology, Obafemi Awolowo University, Ife, Nigeria, between March-September, 2014

Methodology: *M. oleifera* leaf powder and seed flour were obtained by drying fresh leaves and seeds in a hot air dryer and milled. The seeds and leaves were characterized and analysed using standard methods.

Results: The results showed that the crude protein, total ash, and crude fat contents of *M. oleifera* leaf were 20.29±0.41, 7.27±0.25 and 1.79±0.17% while those of the seed were 33.82±0.21, 5.17±0.41 and 38.69 ±0.38%, respectively. Calcium content of the seed

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(46.93±0.18 mg/100 g) was relatively low when compared with that of the leaf (521.96±0.32 mg/100 g). Zinc and iron concentration (14.72±0.12 and 8.12±0.11 mg/100g) in the seed were higher than in the leaf (12.28±0.02 and 7.86±0.62 mg/100g for Zn and Fe respectively). In the leaf and seed, alkaloids (0.428±0.13 and 0.597±0.04%), saponin (2.860±0.11 and 0.295±0.02%), and tanin (mg/g) were within the consumable limit. Phytate content of the leaf (0.4267±0.02 mg/g) and seed (0.4905 ±0.06 mg/g) were low enough to permit bioavailability of phosphate and not impair calcium and zinc bioavailability but may lower iron absorption in the body. Proportion of phosphate as phytate (11.95 and 13.73 mg/100g for leaf and seed respectively) was low indicating high bioavailability of phosphate. 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activities of the leaf and seed were 83.61±0.31 and 69.84±1.08% respectively implying the seed and leaf possess noteworthy antioxidants activity.

Conclusion: The study implies that *M. oleifera* leaf and seed from South Western, Nigeria are suitable for consumption with great health benefits.

Keywords: *Moringa oleifera*; nutrition; antinutrition; bioavailability; antioxidants.

1. INTRODUCTION

Moringa oleifera belongs to the family of shrubs and tree, it is considered to have its origin in Agra and Oudh, in the northwest region of India, South of the Himalayan Mountains [1]. In English, *M. oleifera* is commonly known as Horseradish tree, Drumstick tree, Never Die tree, West Indian Ben tree, and Radish tree [2] but locally called *Zogeli* among the Hausa speaking people of Nigeria [3]. It is widely grown and cultivated in the northern part of Nigeria and many countries in tropical Africa. This tree (Fig. 1) can be found growing naturally at elevation of up to 1,000 m above sea level. It is a fast growing tree and has been found to grow to 6-7 m in one year. It tolerates a wide range of soil conditions, but prefers a neutral to slightly acidic (pH 6.3 to 7), well drained sandy or loamy soil (abdul, 2007). Minimum annual rainfall requirements are estimated at 250 mm with maximum at over 3000 mm [4,5].

Every part of *M. oleifera* like fruit, pods, flower, leaves, roots and seeds are considered useful and edible [6]. The tender leaves taste like watercress and along with the flower are eaten cooked or raw like spinach. Its leaves are also commonly dried and crushed into powder and used in soups and sauces. The tender pods have a taste very similar to asparagus and are eaten as a nutritious vegetable, either cooked or pickled. The seeds taste like peanuts after frying and also consumed raw or cooked. The seed oil is reported to be similar to olive oil and is rich in palmitic, stearic, behmic oleic acid. The parts have been reported to be rich in protein, vitamins, minerals, essential amino acids, antioxidants and Phytochemicals [7]. *M. oleifera* has enormous medicinal potential which

has long been recognized in the Ayurvedic system [8]. Nearly every parts of this plant including the seed oil have been used for various ailment in the indigeneous medicine. The plant is reported to be anticancer, anti-inflammatory, hepatoprotective, antiulcer and diuretic [4].

Several study has been carried out on nutritional value of *M. oleifera* by researchers from different part of the world and establish the fact that considerable variation exist among its nutritional value. The variation is dependent on factors which range from location to agro-ecologies of different region [9]. Anjorin et al. [3] reported significant variation in macro and micro elements in various organs (leaf, pod and seed) of *M. oleifera* from different regions of Abuja, Nigeria. However, limited report exist on other nutritional components and the antinutritional factors of *M. oleifera* leaf and seed obtained from other parts of the country, western zone inclusive. This study investigated and compared the nutritional, antinutritional components and antioxidant capacity of *M. oleifera* seed and leaf from South West Nigeria. This investigation is important to establish the nutritional value of *M. oleifera* seed and leaf and to increase consumption in the South West Zone of Nigeria. Also, the study determines the bioavailability of the nutrients due to the level of antinutritional factors in both the seed and leaf as it may be used as supplements in human nutrition.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Leaf and Seed Samples

Samples of leaves and seeds were collected from Obafemi Awolowo University Teaching and



Fig. 1. *M. oleifera* Tree (a – immature seed pod; b – flower; c – matured seed pod; d – leaves)

Research Farm, Ile-Ife, Nigeria between February and March 2014. The identification was done at the herbarium of the department of botany, Obafemi Awolowo University. The leaves were harvested green, washed and dried at 45°C to a constant weight immediately after harvest. The matured seeds were dehusked manually and further dried at 45°C for 4 h. Drying of the leaf and seed was done using a locally fabricated hot air dryer. The leaves and seeds were dry milled to flour separately using Marlex Excella dry mill (Marlex appliances PVT, Daman). Each was packaged in air tight polyethylene nylon and stored at room temperature (27±2°C) for further analysis.

2.2 Determination of Nutritional Content

Crude protein, Crude fat, total ash, crude fiber and moisture content of dried *M. oleifera* leaf flour and seed flour were determined using standard methods of analysis [10]. Carbohydrate content was determined by difference of the sum of all the proximate composition from 100. Mineral composition of the leaf and seed was also determined by the standard methods of analysis [10].

2.3 Determination of Antinutritional Factors

Antinutritional factors determined include tannin, phytate, saponin, cyanide and alkaloids. Tannin was determined by the modified vanillin-HCL method [11], using catechin as tannin standard. Phytate was determined according to the method of Harland and Oberleas [12]. Saponin was determined using spectrophotometric method described by Brunner [13]. Alkaloids content was determined by the method of Obomanu et al. [14].

2.4 Determination of Phytate to Mineral Molar Ratio

The mole of phytate and minerals was determined by dividing the weight of phytate and mineral with its atomic weight (phytate: 660 g/mol; Fe: 56 g/mol; Zn: 65 g/mol; Ca: 40 g/mol). The molar ratio between phytate and mineral was obtained after dividing the mole of phytate with the mole of minerals [15]. Phytate phosphorous (P) was calculated by assuming phytate contains 28% phosphorus, and accordingly non phytate phosphorous = total phosphorous – phytate P [16].

2.5 Preparation of Extract of *M. oleifera* Seed and Leaf

M. oleifera seed and leaf extract were prepared according to the method of Zhou et al. [17]. One gram of the sample was dissolved in 10 ml deionized water. The suspension was stirred for 30 min after which the mixture was centrifuged using BOSCH centrifuge (LD-3000, England) at 3000 xg for 30 min. The supernatant was assayed for antioxidant activities.

2.6 Determination of Bioactive Compounds

Total phenolic contents of *M. oleifera* seed and leaf extract were determined using Folin-ciocalteu reagent method as described by Singleton et al. [18] Galic acid standard solution was used to prepare calibration curve and the absorbance read at 725 nm. Concentration of flavonoid was estimated spectrophotometrically and Catechin was used to prepare the standard curve and absorbance taken at 500 nm. The concentration in mgCAT/g extract was obtained using the equation 1. Total carotenoid was determined spectrophotometrically as described by Fish et al. [19]. Ascorbic acid content was determined using indophenols titration method [10].

mgCAT/g extract =

$$\frac{\mu\text{gCAT} \times 1 \text{ mg} \times \text{mL of Solvent used in dissolving the sample} \times \text{dilution factor}}{\text{mL} \times 1000 \mu\text{g} \times \text{mass of the sample used}}$$

(1)

2.7 Determination of Antioxidants Capacity of *M. oleifera* Seed and Leaf

Free radical scavenging activities of *M. oleifera* seed and leaf extracts were determined using stable radical DPPH (2, 2-diphenyl-2-picrylhydracyl hydrate) as described by Pownall et al. [20]. To different concentration of the standard (0.0 – 0.1 mg/ml, vitamin C) and 1 ml of the extract, was added 1 ml (0.3 mM) methanolic extract of DPPH. The resultant mixture was shaken thoroughly and allowed to stand at room temperature in the dark for 30 min. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517 nm. Methanol was used as blank and the percentage inhibition was calculated according to equation 2.

Metal chelating activity of the *M. oleifera* leaf and seed extract was also determined according to

the method of Singh and Rajini [21]. An aliquot of the extract (1 mM) was each mixed with 1 ml of 2 mMFeCl. 4H₂O and incubated for 5 min. The reaction was then initiated by the addition of ferrozine (1 ml). The mixture was shaken vigorously, allowed to stand at room temperature for a period of 10 min and the absorbance measured at 562 nm. The higher the absorbance at 562 nm, the weaker the ferrous ion chelating strength of the sample extract. The percentage inhibition of ferrozine-ferrous ion complex was calculated following the formula in equation 2.

$$\text{Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (2)$$

Where A is the absorbance.

Ferric reducing antioxidant power assay was carried out according to the method of Benzie and Strain [22]. Acetate buffer (300 mM) of pH 3.6, 10 mM of TPTZ (2,4,6-tris 2-pyridyl -1,3,5-triazine) and 20 mM of ferric chloride solution were mixed in ratio 10:1:1 respectively to obtain FRAP working reagent. Sample extract (50 µl) was added to 1ml of the FRAP reagent. After incubation in the dark for 30 min, the absorbance was read at 593 nm. The absorbance of the solution of different concentrations of ascorbic acid (used as standard) was plotted against concentration to obtain standard curve. The ferric reducing antioxidant power was expressed as mg Ascorbic acid equivalent per gram of extract (mgAAE/g extract).

2.8 Statistical Analysis

The statistical significance of the means of triplicate experimental data was analysed, where necessary, by the analysis of variance (ANOVA) statistical technique. This analysis was carried out using SPSS statistical package (version 16.0).

3. RESULTS AND DISCUSSION

3.1 Proximate Composition of *M. oleifera* Seed and Leaf

Table 1 shows the results of the proximate analysis of *M. oleifera* leaf and seed flour. Crude protein contents were 33.82 and 20.29% for *M. oleifera* seed and leaf respectively. Protein contents obtained for both *M. oleifera* seeds and leaves were high with the seeds having more than 50% protein greater than the leaves. Both the seeds and leaves can be used to improve the

protein content of cereal based foods that are of low protein content such as maize based products. Value obtained for *M. oleifera* seeds was within the range obtained by other researchers [23,24] for *M. oleifera* seed from Burkina Faso and Pakistan, respectively. These researchers reported crude protein value of 35.37% for *M. oleifera* seed from Burkina Faso and 29.6 to 31.3% for *M. oleifera* seed from Pakistan. Crude protein value obtained for *M. oleifera* leaves in this study was lower (20.29%) than those obtained by Makkar and Becker [25] and Oduro et al. [26], who reported 25.1% and 27.51% for the leaves obtained from Nicaragua and Ghana respectively. Ogbe and Affiku, [27] also reported a value of 17.01% for dry *M. oleifera* leaves, obtained from northern part of Nigeria, which was lower than the value obtained in this study.

Table 1. The proximate composition (%) of dried *M. oleifera* seed and leaf

	Seed	Leaf
Protein	33.82±0.21 ^b	20.28±0.41 ^a
Ash	5.17±0.41 ^a	7.27±0.25 ^b
Fat	38.69±0.38 ^b	1.79±0.17 ^a
Crude Fibre	5.46±0.49 ^a	8.45±0.04 ^b
Moisture	6.17±0.56 ^b	3.78±0.31 ^a
Carbohydrate	10.68±0.15 ^a	50.25±11.91 ^b

Means of the same row followed by different letters are significantly different ($P=0.05$)

The total ash content of *M. oleifera* leaf (7.27%) was significantly ($P=0.05$) higher than that of the seed (5.17%). Abiodun, et al. [28] obtained 4.70% of ash for *M. oleifera* seed which was closer to the value reported in this study for the seed. Ogbe and Affiku, [27] reported 7.93% and Oduro et al. [26] reported 7.13% for *M. oleifera* leaves. The total ash in food determines largely the extent of mineral matters likely to be found in the food substance. The value of ash obtained in this study indicated that *M. oleifera* leaf and seed may be good sources of minerals.

The crude fat content was higher in *M. oleifera* seed (38.69%) than in the leaf (1.79%) indicating that *M. oleifera* seed is a good source of oil. The oil of *M. oleifera* seed contains more unsaturated fatty acids than saturated fatty acids of which oleic acid account for about 75% of the total fatty acids [23,29]. This implies that *M. oleifera* seed flour has little tendency to deteriorate and become rancid or sticky during storage [29], suggesting that rancidity occurrence in products

with *M. oleifera* seed during storage will be minimal.

Crude fibre was higher in *M. oleifera* leaf (8.45%) than the seed (5.46%) and the values were significantly different ($P=0.05$) from each other. These values indicate, that the materials may be good sources of dietary fibre. Carbohydrate content of the materials was obtained by difference of the sum of all the proximate composition from 100. The least value was obtained in *M. oleifera* seed (10.68%). The value was close to 10.59% reported by Abiodun et al. [28], but higher than 9.12% reported by Compaore et al. [23]. There were variations in proximate composition as compared with the values reported by other researchers [23,25,28, 29,30] for *M. oleifera* seeds and leaves obtained from different part of the world. These variations may be due to difference in geographical location, climatic conditions, and soil composition.

3.2 Mineral Composition of *M. oleifera* Seed and Leaf

The result of mineral composition of *M. oleifera* leaf and seed is presented in Figs. 2 and 3. Among the macro elements, *M. oleifera* leaves contained more concentration (mg/100g) of K (1293.75), Mg (297.51), Ca (521.96) and Na (43.61) than the seed flour. Phosphorus (P) and sulphur (S) content of the seed flour (521.00 and 27.85) were higher than 417.10 (P) and 26.12 (S) of the leaf flour though the sulphur content was not significantly higher ($P=0.05$) than each other. This implies that the leaves are richer in essential macro elements than the seed. The concentrations of trace elements such as zinc, iron and copper were higher in the seeds flour (14.72, 8.12 and 3.35 mg/100 g) than in the leaves (12.28, 7.86 and 0.92 mg/100 g).

This implies that the trace elements in Nigeria soil were more readily available for the seed than available for the leaf i.e. *M. oleifera* seeds were able to absorb more of these trace elements from Nigeria soil. Reverse trend was reported by Ilayas et al. [31] for the trace elements of both *M. oleifera* seed and leaf powder obtained from Pakistan. Likewise, the values reported in this study were lower than 500 (0.5%), 3650 (3.65%), 164 (0.164%), 630 (0.63%) and 86.8 mg/100 g reported for Mg, Ca, Na, S and Mn respectively by Moyo et al. [32] of leaves obtained from South Africa.

The concentration of the essential minerals obtained in this study for both seed and leaf compared favourably with those found in some leafy vegetables (*Amaranthus hybridus*), fruits and leguminous seeds [33,34]. Generally, minerals are structural components of body tissues and play important roles in health and disease states of humans. The results obtained here showed that *M. oleifera* leaves and seeds

may be explored as good sources of dietary minerals that may in turn be helpful in improving human health.

3.3 Antinutrient Contents of *M. oleifera* Seed and Leaf

Antinutrient components of *M. oleifera* leaf and seed are presented in Table 2. The alkaloids

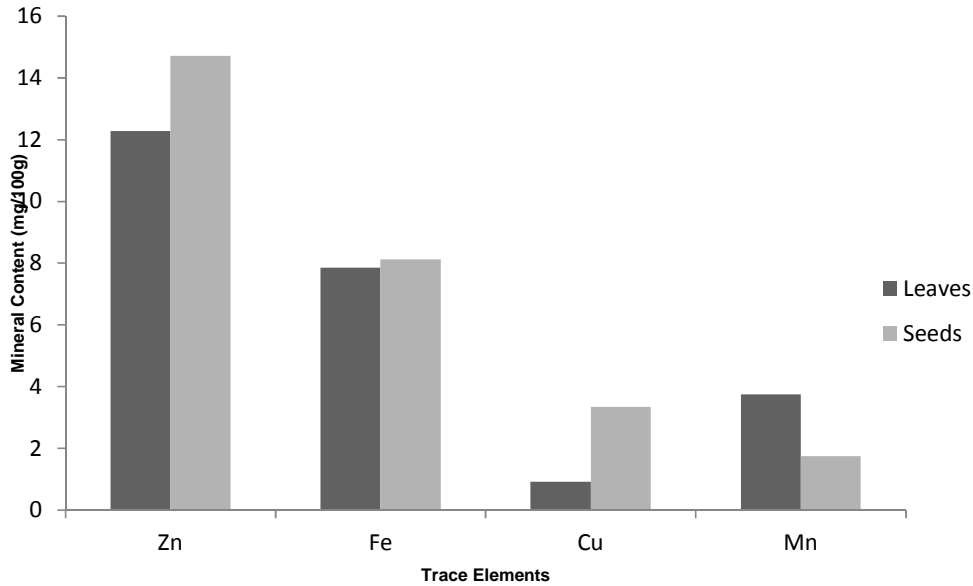


Fig. 2. Trace elements of *Moringa oleifera* seeds and leaves

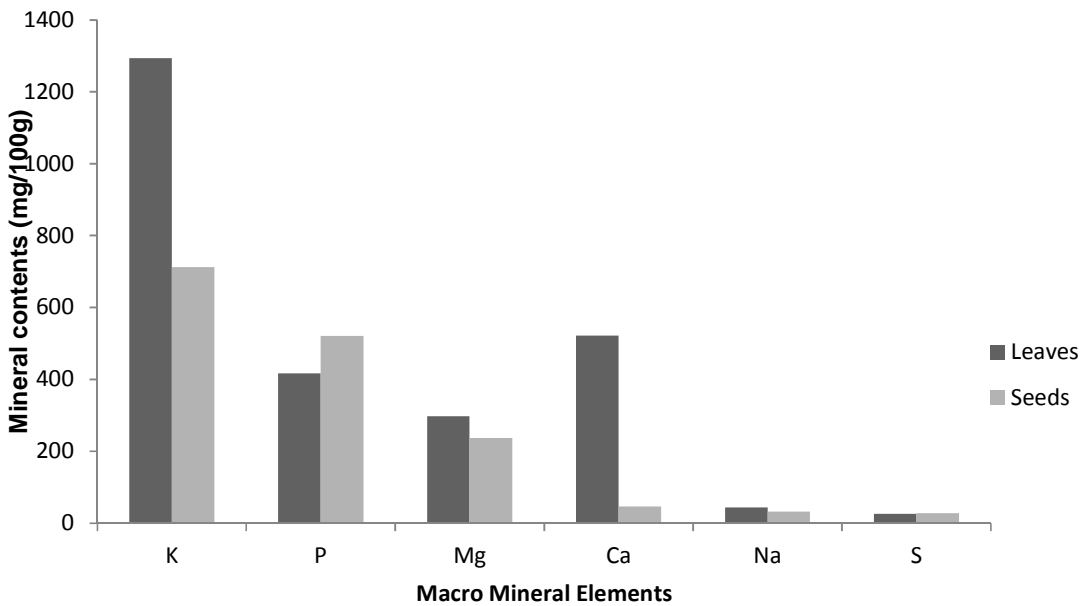


Fig. 3. Macro mineral elements of *M. oleifera* seeds and leaves

contents of *M. oleifera* leaf and seed were 0.428 and 0.597%, respectively. The value obtained for alkaloid in this study is comparable to the 0.58% reported by Ijeh et al. [35] for African bread-fruit that is well consumed in the community. This level of alkaloids may be desirable as alkaloids are known to impart a bitter taste to most foods [35,36]. Low dose of alkaloids has been reported to mediate important pharmacological activities, such as analgesic, reducing blood pressure, killing tumor cells, stimulating circulation and respiration [37].

Table 2. Antinutritional component of dried *M. oleifera* leaf and seed

Antinutrients	Leaf	Seed
Alkaloid (%)	0.428±0.13 ^a	0.597±0.04 ^a
Saponin (%)	2.860±0.11 ^b	0.295±0.02 ^a
Phytate (mg/g)	0.427±0.02 ^a	0.491±0.06 ^a
Tannin (mg/g)	3.742±0.52 ^b	0.324±0.04 ^a

Means of the same row followed by different letters are significantly different ($P=0.05$)

Saponin present in *M. oleifera* leaf and seed were 2.86 and 0.295%, respectively. Makkar and Becker [25] reported 8.1 and 1.06% saponin in *M. oleifera* leaf and seed, respectively, while Foidl et al. [29] reported 5.0 % and 1.1 % for the leaf and seed, respectively. Saponin, because of its structural complexity, has a number of physical, chemical and biological properties, which include sweetness and bitterness foaming and emulsifying properties, pharmacological and medicinal properties [38]. Saponin is reported to possess hypocholesterolemic, immunostimulating and anticarcinogenic properties, therefore is used to control plasma cholesterol, prevent peptic ulcer and osteoporosis and to reduce the risk of heart disease [39].

Phytate content was 0.43 and 0.50 mg/g for *M. oleifera* leaves and seeds respectively which were lower than 3.10 and 2.60% reported by Foidl et al. [29] for *M. oleifera* leaves and seeds respectively. Phytate impact negatively on the bioavailability of divalent, and trivalent mineral ions such as Zn^{2+} , $Fe^{2+/3+}$, Ca^{2+} , Mg^{2+} , Mn^{2+} , and Cu^{2+} . Whether or not, high levels of consumption of phytate containing foods will result in mineral deficiency depends on its effect on the bioavailability of such minerals which include Ca, Fe, and Zn [40]. Phytates have health benefits; they lower the blood glucose response of diabetes patients by reducing the rate of starch digestion and slowing gastric emptying [41].

Tannin content was 3.742 and 0.324 mg/g for *M. oleifera* leaf and seed respectively. The value obtained for tannin in leaf was lower than 1.4% reported by [29]. The total acceptable tannin daily intake for a man is 560 mg [41]. The result obtained here showed that the level of tannin in both *M. oleifera* leaf and seed can be more beneficial than harmful even when up to 100 g of the leaf or seed is consumed.

The balance between beneficial and hazardous effects of plant antinutrients rely on their concentration, chemical structure, time of exposure and interaction with other dietary components. The values of antinutrient contents observed for *M. oleifera* used in this study indicated that both the leaf and the seed may possess some biologically active compounds which could serve as potential source of vegetable drugs for pharmacological and biochemical action in human body.

3.4 Computed Mineral Ratio and Phytate Mineral Molar Ratio

The Computed values for mineral ratio (wt/wt) and phytate to mineral molar ratio (mg/mol) is presented in Table 3. Ratios (wt/wt) of Na/K, Ca/P, and Ca/Mg for *M. oleifera* leaves were 0.0337, 1.25 and 1.75 while those of the seed were 0.0461, 0.0901 and 0.198 respectively. Molar ratios (mg/mol) of Phytate/Ca, Phytate/Fe, Phytate/Zn, and [Phytate][Ca/[Zn] for *M. oleifera* leaf are 0.00498, 0.460, 0.340, and 0.00445 while those of the seed are 0.0625, 0.51, 0.329, and 0.000385 respectively.

The values obtained for the mineral ratios were higher than the values reported by [42] for Fluted Pumpkin Seed flour. Sodium to potassium ratio for both *M. oleifera* seed and leaf were less than the critical value reported by Verla et al. [40]. Sodium and potassium are responsible for maintaining osmotic balance of the body fluid, control glucose absorption and enhance normal retention of protein during growth. It is recommended that potassium should be more than sodium ($Na/K < 1$) in a diet to prevent high blood pressure. The result obtained here therefore indicate that *M. oleifera* leaf and seed may be useful in the management and prevention of high blood pressure. Calcium to phosphorus ratio for *M. oleifera* leaf was more than one while that of *M. oleifera* seed was less than 0.5. It has been reported that high phosphorus intake can lead to loss of calcium in urine therefore any food with Ca/P greater than

Table 3. Computed ratio for mineral and phytate interaction for *M. oleifera* seed and leaf

Mineral/phytate	Calculated values		Critical values
	Leaf	Seed	
Na/K	0.0337	0.0461	< 1
Ca/P	1.25	0.0901	> 1
Ca/Mg	1.754	0.198	=1
[Phytate]/[Ca]	0.00498	0.0625	< 0.24
[Phytate]/[Fe]	0.460	0.510	< 1
[Phytate]/[Zn]	0.340	0.329	< 1.5
[Phytate][Ca]/[Zn]	0.00445	0.000385	< 0.05
Phytate P (mg/100g)	11.95	13.734	-
Non phytate P	405.15	507.27	-

P- Phosphorus

1 is considered good, Ca/P<0.5 is poor while Ca/P >2 will help increase calcium absorption in the small intestine [40]. Phosphorus and calcium function together to contribute to blood formation processes and other supportive structures of the body. Ca/P obtained in this study (Table 4) implies that Ca absorption may be high in the consumers of *M. oleifera* leaf but very poor in the consumers of *M. oleifera* seed. Calcium to magnesium ratio was more than one in *M. oleifera* leaf but less than one in the seed. Calcium and magnesium depends on each other to be assimilated into the body. If calcium is high, then magnesium intake also needs to be high [40]. The ideal ratio for most people's needs is equal amounts of calcium and magnesium [40,43]. Calcium to magnesium ratio implies that consumers of *M. oleifera* leaf may experience magnesium depletion related disorder while those who consume the seed may experience calcium depletion disorder.

Phytate to mineral molar ratio was determined to predict the effect of phytate on bioavailability of some mineral elements such as Ca, Fe, Zn and P (Table 4). Bioavailability is the ability of the body to digest and absorb the mineral in the food consumed. Phytate can form stable complexes with mineral ions rendering them unavailable for intestinal uptake [44]. The phytate/Ca molar ratio for both *M. oleifera* leaf and seed was less than 0.24 predicted critical value [16]. This indicates phytate level of *M. oleifera* leaf and seed may not exert adverse effect on Ca bioavailability when consumed. Report has it that phytate is the main inhibitor of iron absorption in plant-based diets and phytate/Fe molar ratio should be less than one in cereal-based diets for good iron bioavailability and preferably 0.4 to obtain significant increase in absorption [16]. According to Siegenberg et al. [45], ratio as low as 0.2 was reported to exert some negative effect [46] and

phytate/Fe molar ratios greater than 0.15 is regarded as indicative of poor Fe bioavailability. In this study phytate iron ratio (0.46 and 0.51 for leaf and seed respectively) was less than 1 but more than recommended 0.15 [45] indicating that iron bioavailability may be poor in consumers of *M. oleifera* leaves and seeds. Phytate Zn ratio was below the 1.5 critical value implying that phytate concentration was low enough not to impair the Zinc bioavailability. Effect of calcium on zinc absorption in the presence of high phytate intakes has led to the determination of [Phy][Ca]/[Zn] molar ratio as a better index of zinc bioavailability than [Phy]/[Zn] molar ratio alone [47]. [Phy][Ca]/[Zn] molar ratio of *M. oleifera* leaf and seed obtained in this study was less than recommended 0.05 critical value for Zn bioavailability.

Phytate P (mg/100 g) are 11.95 and 13.73 for the leaf and seed respectively while non phytate P are 405.15 and 507.27 for the leaves and seeds respectively. Generally diets are regarded as being adequate in bioavailable phosphate, however the high proportion of phosphate as phytate has consequences for bioavailability of minerals and trace elements. Phytate P for *M. oleifera* seed and leaf is low implying that phosphorus in the leaf and seed may be readily digested and absorbed in the body of consumers of *M. oleifera* leaf and seed.

3.5 Bioactive Components of the Extract of *M. oleifera* Leaves and Seeds flour

M. oleifera leaf and seed extract were analysed for bioactive compounds such as total phenolic, flavonoid, carotenoid and ascorbic acid as shown in Table 4. Total phenolic content of the leaf extract was 48.31±0.98 and 39.01±1.21 mg/gGAE for the seed. The flavonoid, ascorbic acid and total carotenoid content of the leaf were

51.91±1.09 mg/gCE, 3.20±0.85 mg/g, 208.80 µg/g respectively and 43.81±0.38 mg/gCE, 0.50±0.05 mg/g and 47.20±0.69 µg/g for the seeds. The results showed that *M. oleifera* leaf possess the bioactives compounds examined in higher concentration than the seed. The same trend was also reported by Ilyas et al. [31] for *M. oleifera* leaf and seed obtained from Pakistan. Total phenolic and flavonoid obtained in this study for the leaf were higher while the ascorbic content was lower than the values reported for *M. oleifera* tender and mature leaves [30]. The values were also lower than the values reported for *M. oleifera* leaf and seed from Pakistan [31]. Variation in this result when compared with values obtained from other parts of the world may be due to geographical locations and the climatic condition. Ilyas et al. [31] reported that variation in results of bioactive compounds may be due to the method of extraction of polyphenol compound and degree of polarity of the solvents. Some other authors also reported that antioxidants composition varies widely with factors such as part of plant analysed, stage of maturity, variety, postharvest handling, processing and storage [30].

Table 4. Bioactive compounds of *M. oleifera* leaf and seed

Parameter	<i>M. oleifera</i> leaf	<i>M. oleifera</i> seed
Total phenolics (mgGAE/g)	48.31±0.98 ^b	39.01±1.21 ^a
Total flavonoids (mgCE/g)	51.91±1.09 ^b	43.81±0.38 ^a
Total carotenoids (µg/g)	208.80±1.92 ^b	47.20±0.69 ^a
Ascorbic acid (mg/g)	3.20±0.85 ^b	0.50±0.05 ^a

GAE, Gallic acid equivalent; CE, cateching equivalent.
Values are mean ± SD of triplicate determinations
Means of the same row followed by different letters are significantly different ($p < 0.05$)

3.6 Antioxidant Potentials of the Extract of *M. oleifera* Leaves and Seeds Flour

Antioxidant mechanisms such as DPPH, ferric reducing ability and metal chelating activities were determined to ascertain the antioxidant potentials of the extract of the leaves and seeds of *M. oleifera*. The result, as shown in Table 5 reveal that the leaf of *M. oleifera* possesses higher antioxidants activity potentials than the

seed for all the parameters examined. DPPH was significantly ($p=0.05$) higher in *M. oleifera* leaf (83.61±0.31%) than seed (69.84±1.08%) implying that *M. oleifera* leaf has a significantly higher free radical scavenging potentials than the seed. The result followed the same trend and compared favourably with the values reported by Ilyas et al. [31] for *M. oleifera* leaf and seed. Free radical scavenging effects of an agent is usually measured by their ability to scavenge 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radicals. Higher free radical scavenging effect obtained for *M. oleifera* leaf may be due to the concentration of bioactives compound.

Table 5. Antioxidant potential of *M. oleifera* leaf and seed

Parameters	Leaf	Seed
DPPH scavenging activities, %	83.61±0.31 ^b	69.84±1.08 ^a
Metal chelating activities, %	61.81±1.09 ^a	58.29±0.58 ^a
Ferric Reducing ability, mgAAE/g	152.01±1.21 ^b	134.19±0.73 ^a

AAE, ascorbic acid equivalent; Values are mean ± SD of triplicate determinations

Means of the same row followed by different letters are significantly different ($P=0.05$)

Another mechanism of an antioxidant is the chelation of transition metals. The metal chelating potential of *M. oleifera* leaf (61.81±1.09%) and seed (58.29±0.58) as shown in Table 5 were not significantly different ($P=0.05$) from each other. The values obtained for metal chelating potentials in this study compared favourably to 61.5% reported for benzo-hydroxamic acid (synthetic metal chelator) [48]. The result obtained in this study implied that both the seed and leaf of *M. oleifera* can be used as metal chelator and in turn has therapeutic potential in the treatment of disease. The transition metal ion, Fe^{2+} possesses the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions such as reactive oxygen species (ROS). ROS are generated as by-products of metabolic reactions and as intermediates of metal catalysed oxidation reaction [49]. Therefore, chelating of metal ions is a good tool in scavenging ROS generation that is associated with redox active metal catalysis and to reduce the amount of available transition metal.

Another assay examined to determine the antioxidant potentials of *M. oleifera* leaf and seed extract is Ferric Reducing ability. *M. oleifera* leaf (152 mgAAE/g) had significantly higher ferric reducing ability than seed (134 mgAAE/g). High value of ferric reducing ability obtained may be due to high concentration of bioactive compounds that act as antioxidants in *M. oleifera* leaf and in seed. Ferric reducing assay measure the antioxidant potential when iron (Fe^{3+}) is reduced by electron-donating antioxidants present within the sample being analysed.

Carotenoids, as an antioxidant, have been reported to actively quench singlet oxygen (O_2) and prevent lipid peroxidation [50]. Vitamin C also has been referred to as the most important antioxidant in extracellular fluids that protect biomembrane against lipid peroxidation damage. Ascorbic acid can easily give up electrons to provide stability to ROS [50]. Flavonoids, acting as antioxidants, are important tools for protecting the body against reactive oxygen species (ROS) and as a result play important role in liver and cardiovascular diseases [51]. The studies on the antioxidant potential showed that *M. oleifera* leaf and seed possessed noteworthy ability to stabilize highly reactive free radicals, deactivate transition metals as well as reducing ferric ions.

4. CONCLUSIONS

The study has shown that *M. oleifera* leaf and seed from South West, Nigeria are of good nutritional importance such that can be used to combat malnutrition and improve human health. The antinutritional factors are low enough not to impair bioavailability of the nutrient. The leaf possess more bioactive compound than the seed while both the seed and the leaf posses these compounds in quantity that may be use to promote health. Therefore the seed and the leaf should be consumed and considered for use as food supplement for improved human nutrition and health.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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