



Enhancing moringa nutritive value for impoverished populations and healthy wellbeing through proper nutrient enrichment

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Abstract

Taking into account the many cases of malnutrition related deaths in especially sub-Saharan Africa, the current research effort was conceived with the objective of appraising the nutritive potential of *Moringa oleifera* L. plant through the evaluation of proper agronomic practice that will enhance its nutritive potential. Measured quantity of dried roots, stem, and leaves portions of 5 months old *Moringa oleifera* L. seedlings, grown under various applied phosphorus (P) and potassium (K) rates were analysed for essential and non-essential amino acids content, total antioxidants, secondary metabolites (phenolics and flavonoids), and mineral nutrient elements. The high-performance liquid chromatography (HPLC) analysis revealed the presence of 9 essential and 7 non-essential amino acids, with a range of 4.24 to 10.04 gkg⁻¹ recorded for leucine, threonine, histidine, glutamic acid, aspartic acid, and glycine. The 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and the ferric reducing antioxidant power (FRAP) assay revealed strong antioxidants presence (up to 68% DPPH scavenging activity and a high level FRAP potential of 2500 mg ascorbic acid equivalent (AAE) / 100g dry weight (DW)). Folin-Ciocalteu's and aluminium chloride calorimetric assay revealed high levels of secondary metabolites (ranging from 1000 to 1350 mg gallic acid equivalent (GAE)/100g DW total phenolics and up to 2400 mg quercetin equivalent (QE)/1mg DW total flavonoids), and the nitric acid wet digestion procedure revealed a lot of mineral nutrient elements (with significant values of up to 19.13 g kg⁻¹ maximum content acquired from calcium (Ca) and up to 9.90 g kg⁻¹ recorded for iron (Fe)). These nutritive components are noted to be highest in the leaves portion, followed by the roots, and then the stem parts. Based on its recognised rich base nutritive content, *Moringa oleifera* L. is a tree plant of nutraceuticals importance for impoverished communities.

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Keywords

Moringa

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Mineral elements

Introduction

Recognition of crop plants nutritional composition is fundamental as they constitute the building blocks of human nutrition globally. Plants are recognized to be endowed with abundance of nutritional metabolites that improve human health and longevity. The well-known classes of nutrients ranging from carbohydrates, proteins, fats, vitamins, and minerals are reported to be present in significant quantity in the moringa plant. The demand for health-

giving foods has been increasing in recent years, for that purpose the food industry is continuously looking for new sources of nutritive and health-giving constituents, making moringa a prime area of research. *Moringa oleifera* L. has served as an important staple food for especially the people of Africa and Asia for decades, and is widely reported to be enriched with health benefitting nutrients essential to human wellbeing (Nikkon *et al.*, 2003; Anwar *et al.*, 2006). In many impoverished localities, such as is commonly found in sub-Saharan Africa, where

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often many deaths are reported due to malnutrition, moringa leaf and fruit supplement could effectively be utilized to provide a protein rich diet. The proteins are of utmost significance for the continuous survival of humans – they virtually catalyse almost all the cellular chemical reactions: amino acids are their building blocks. The ability of protein pack foods to meet the amino acid requirements serves as a basis for measuring their nutritional value, which is dependent on the concentration and balance of the amino acids and on their digestibility and availability for metabolic processes (Humayum *et al.*, 2007). Animal foods have been recognised to contain more concentrations of essential amino acids than plant based diets.

Antioxidants naturally produced by the human body, such as glutathione, alpha-lipoic acid, and ubiquinone, and those obtained from food or supplements, such as resveratrol, carotenoids (carotenes and xanthophylls), astaxanthin, vitamin C and vitamin E combine to make humans notably enjoy the longest natural lifespans within the animal kingdom by providing their cellular entities with adequate defence against attack by reactive oxygen species (ROS) and repairing damaged molecules (Passwater, 1998). The secondary metabolites produced by plants, such as alkaloids, terpenoids, and phenolics are known to have powerful physiological effects in humans and thus popularly utilized for medicinal purposes. Secondary metabolism is a unique activity in the growth and development of plant species. The numerous beneficial effects of crop plants secondary metabolites, such as polyphenols and flavonoids have been linked to their antioxidant activities. The phenolic compounds, as well as sulphites, functions by quenching free radicals, thus, antioxidants which contain a phenol ring structure and one or more hydroxyl groups, act by scavenging reactive oxygen species (ROS) to form a more stable phenoxyl radical that assist to lower down radical-driven reactions (Gostner *et al.*, 2015). The production of fruits and seeds in crop plants is known to be influenced by some key mineral nutrients, notably phosphorus, potassium and calcium, that are known to be vital to seed formation, fruit setting, and fruit quality. The mineral nutrients serve as catalyst to normal plant growth and development and in the resultant setting of the fruits and seeds.

With all the aforementioned benefits, the literatures of research work conducted to further advance the understanding of this important plant are scanty. This study was therefore undertaken with the objectives of exploring and evaluating the possible potential of various plant growing parts of

moringa (leaves, stem, and roots) for use as natural plant sources of amino acids, secondary metabolites, antioxidants, and mineral nutrients and subsequent recommendation for utilization as nutraceuticals in impoverished communities.

Materials and Methods

Experimental location

The experiment was undertaken at the Faculty of Agriculture Experimental farm #2, Universiti Putra Malaysia (2°56'14.92"N, 101°42'40.87"E) nursery shade house. The seedlings were grown inside polybags (23 cm × 36 cm size) containing beach ridges interspersed with swales (BRIS) soils. The soil contains >95% sand, obtained from eastern Malaysia state of Terengganu (Rhu Sepuluh, 5°35'32.92"N, 102°49'24.40"E, altitude of 13 meters above sea level) with a low nutrient, low organic matter content, low water retention capacity and a high hydraulic conductivity. The irrigation of the seedlings was manually done using the surrounding tap water that was found, after analysis, to be in conformity with Jones (2005) recommendation on water considered appropriate for utilisation in high quality irrigation. The seeds were germinated first inside the BRIS soil spread in a rectangular (30 cm × 45 cm) open plastic container and afterwards transplanted to polybags (36 cm depth and 23 cm diameter) 3 weeks after germination. The trial was conducted for a period of 5 months.

Experimental design

The experiment with 125 plant materials was done during the 2011 growing season. The experimental plant materials were arranged in a completely randomized design (CRD) with 10 treatment units, incorporating 5 levels of P, and 5 levels of K, with the P supplied in the form of triple super phosphate (TSP) at the rate of 0, 50, 100, 150, and 200 kg P ha⁻¹ and the K supplied in the form of muriate of potash (MOP) at the rate of 0, 50, 100, 150, and 200 kg K ha⁻¹. Uniform rate of 50 kg ha⁻¹ N was applied through the use of urea (46% N) for all the 125 experimental units. Application of micronutrients was achieved through the use of a standard Hoagland solution preparation (solution of 10 liters distilled water containing 0.2, 0.0014, 0.0384, 0.0035, 4.930, 4.930, 0.0268, and 0.0002 grams of iron, copper, manganese, zinc, magnesium, sulphur, boron, and molybdenum respectively) uniformly sprayed on the growing plants leaves. At the end of the experimental period, the harvested leaves, stems, and roots parts were oven dried at 65°C, weighed, and grounded for

further laboratory analysis to determine the amino acids, secondary metabolites, antioxidants, and mineral nutrients content.

Amino acids analysis

The amino acids determination was performed following the procedure outlined by Zarkadas *et al.* (2007), where digestion of 5 g of treatment samples was effected through the mixing of each of the respective sample with 6N HCl (5 mL) and heating to a temperature constant of 110°C for 24 h inside an oven. The resulting hydrosylates were then mixed with 50 µmol/mL of L- α -amino-n-butyric acid serving as internal standard (400 µL; AABA). This was followed by making up of the mixture to 100 mL level using distilled deionized water, after which filtering of the resultant mixture was done with Whatman No. 1 filter paper. The amino acid derivation was then conducted using 10 µL of sample aliquot and 20 µL AccQ reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) before being injected into the HPLC column. Analysis was performed using Waters- HPLC System (Milford, MA, U.S.A.) consisting of Waters 1525 binary HPLC pump, 717 plus auto sampler, 2475 multi λ fluorescence detector; wavelength excitation 250 nm, emission 395 nm and a AccQ Tag™ amino acid analysis column (3.9 mm \times 150 mm; packing material: silica based bonded with C18), which were all based according to the AccQ Tag™ method. The column was maintained at 37°C and the eluent (flowed at a rate of 1 mL/min.). Chromatographic peaks were plotted and evaluated using Breeze™ Workstation version 3.20, with two different eluents used (AccQ Tag™ concentrate and Acetonitrile/Water, 60/40%). Amino acids standard mixture was prepared using commercial mixture of standard H (Pierce Chemical, U.S.) which was used as a reference to calibrate the HPLC-system.

Extraction of plant material for secondary metabolites and antioxidants content determination

Dried parts (leaves, root and stem) of *M. oleifera* were put in 50 mL conical flask covered with parafilm (Pechiney plastic packaging Menasha, Wisconsin U.S.A) and wrapped with aluminium foil (Diamond Reynolds, Richmond, U.S.A.) and extracted with deionized water in a temperature- controlled water bath shaker (WNB 7- 45, Memmert, Germany) at a constant speed, solid-solvent ratio, extraction time and temperature. Crude extracts were then filtered through Whatmann No. 1 filter paper (Whatmann International Ltd, England). Filtrates were collected in amber bottles and used directly for the estimation

of phenolic antioxidant and evaluation of antioxidant activities using various biochemical assays (Thoo *et al.*, 2010).

Determination of total phenolic content

Total phenolic content (TPC) was determined using Folin-Ciocalteu's (FC) method as reported by Thoo *et al.* (2010) with slight modifications. Briefly, 500 µL of dilute crude extract was mixed with 500 µL of 10-fold diluted Folin-Ciocalteu reagent. After 3 minutes, 400 µL of sodium carbonate anhydrous was added and vortexed. After 2 hours of incubation in dark at room temperature, absorbance was determined at 765 nm against a blank (prepared by replacing plant extract with deionized water) using a UV/VIS spectrophotometer (Lambda 25, ParkinElmer, Singapore). Measurements were calibrated to a standard curve of prepared gallic acid solution (10 – 100 µg/mL) with equation $y = 0.01x - 0.009$ ($R^2 = 0.999$) and TPC was then expressed as milligram of gallic acid equivalent (GAE) per 100g of dry weight (DW).

Determination of total flavonoid content

Total flavonoid content (TFC) was determined using aluminium chloride calorimetric assay reported by Kaur and Mondal (2014) with slight modifications. Measured portion of 125 µL of crude extract was mixed with 625 µL deionized water and 37.5 µL of 5% sodium nitrite. The mixture was allowed to stand for 6 minutes and 75 µL of 10% aluminium chloride-6-hydrate was added thereafter. After 5 minutes, 250 µL sodium hydroxide solution was added. 137.5 µL deionized water was added and mixed. Absorbance was measured immediately at 510 nm against a blank (prepared by replacing plant extract with deionized water). Measurements were calibrated to a standard curve of prepared quercetin solution (0 – 800 mg/mL) with equation $y = 0.0000x + 0.003$ ($R^2 = 0.981$) and TFC was then expressed as milligram quercetin equivalent (QE) per 1g dry weight (DW).

2,2'- diphenyl-1- picrylhydrazyl radical scavenging activity

Antioxidant capacity through 2,2'- diphenyl-1-picrylhydrazyl (DPPH) scavenging activity was determined according to the protocol of Musa *et al.* (2010) as reported by Tan *et al.* (2012) with slight modifications. Methanolic DPPH stock solution was prepared by dissolving 4 mg DPPH powder into 100 mL absolute methanol. Working solution was obtained by mixing 50 mL stock solution with 20 mL methanol to obtained absorbance of 1.00 ± 0.02 unit at 517 nm wavelength. 100 µL plant extract was mixed

with 900 μ L methanolic DPPH solution and allowed to stand in dark at room temperature for 30 minutes. After 30 minutes incubation, absorbance was taken at 517 nm using UV/VIS spectrophotometer (Lambda 25, ParkinElmer, Singapore) against blank (methanol). The 2,2-Diphenyl-1-picrylhydrazyl, a stable organic free radical with an absorption band of 517 nm, loses its absorption by accepting an electron or a free radical species, resulting in a visually noticeable discoloration from purple to yellow. Percentage DPPH scavenging activity was determined using the relation:

$$\text{DPPH scavenging activity (\%)} = [(A_0 - A_{30})/A_0] \times 100$$

Where A_0 = Absorbance at time 0; A_{30} = Absorbance after 30 minutes.

Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) assay was carried out using the protocols of Benzie and Strain (1996) as reported by Thaipong *et al.* (2006). The stock solution included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 mL $C_2H_4O_2$), pH 3.6, 10 mM 2,4,6-Tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ solution. Fresh FRAP working solution was prepared by mixing acetate buffer, TPTZ solution and $FeCl_3 \cdot 6H_2O$ solution in the ratio of 10:1:1, respectively. The FRAP solution was warmed at 37°C for 30 minutes in water bath (GFL 1004 Burgwedel, Germany) before using. The 100 μ L plant extract was allowed to react with 1000 μ L FRAP solution in dark for 30 minutes. Absorbance of the colour product (ferrous tripyridyltriazine complex) was measured at 593 nm wavelength using UV/VIS spectrophotometer (Lambda 25, ParkinElmer, Singapore) against blank (prepared by replacing plant extract with deionized water). Measurements were calibrated to a linear standard curve of prepared ascorbic acid solution (5 – 35 mg/mL) with equation $y = 0.045x + 0.395$ ($R^2 = 0.996$) and results expressed as milligram ascorbic acid equivalent (AAE) per 100 g DW. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

Wet digestion

Wet digestion involving decomposition of a measured sample was conducted through the addition of liquid reagents (mainly acids for increased solubility of the digest) for enhanced solubility. The procedure (AOAC, 1990) comprises decomposition of organic materials by means of a boiling oxidizing

acid or its mixture that finally produces salts or acids of the inorganic constituents of the sample, in addition to other by-products of carbon dioxide, water, and other volatile gaseous compounds. The leaf samples were first oven dried and ground to ease the dilution process; they were afterwards measured into 0.25 g portions and placed in a fume chamber (block digester). Then measured 5 mL of concentrated H_2SO_4 were pipetted to each sample and heated to a temperature of 150°C, later increased to 310°C, until the white fumes produced were visibly dispersed. Drops of H_2O_2 were added until the solution change from coloured to colourless. The resulting product was allowed to cool, filtered, then make up, with a deionized water, to 100 mL using a volumetric flask. The determination of N, P, and K was performed using auto-analyser (AA) (Quikchem® FIA+ 8000 series USA) and Ca, Mg, Mn, Cu, Fe, and Zn were then effected through the use of atomic absorption spectrophotometer (AAS) (PerkinElmer PinAAcle 900T, USA).

Statistical analysis

The results were analysed using SAS and expressed as mean \pm standard deviation. A difference was considered statistically significant if $p \leq 0.05$. Pearson correlation coefficient was utilized for further analysis to determine the relationship between the flavonoids and phenolics content with the different antioxidant assays, with students t-test used to test level of significance (defined as $p < 0.05$).

Results

Amino acids

Amino acids play a central role of serving as the building blocks of proteins and ensuring the achievement of a balanced metabolism. Their level of content is an indicator of the nutritional value of food and fodder proteins. Based on P applications, the HPLC analysis conducted with the moringa leaves samples revealed the concentration of 9 essential (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine) and 7 non-essential (alanine, aspartic acid, glutamic acid, glycine, proline, serine, and tyrosine) amino acids, with histidine, leucine, and threonine, recording the highest proportion levels of 8.32, 6.76, and 4.94 g kg^{-1} , respectively out of the 9 essential amino acids identified, while glutamic acid, aspartic acid, and glycine recorded the highest proportion levels of 10.04, 4.99, and 4.24 g kg^{-1} out of the 7 non-essential amino acids discovered (Table 1). Generally, there were notably a higher percentage amino acids

Table 1. Amino acids content as influenced by P levels (*Moringa oleifera* L. leaves).

	Leaves Samples				
	P1	P2	P3	P4	P5
Essential amino acids (g kg⁻¹)					
Arginine	1.55	5.22	5.44	2.03	5.56
Histidine	6.74	5.73	7.45	8.32	8.31
Isoleucine	1.72	1.83	1.76	1.89	1.91
Leucine	4.91	4.79	4.75	5.16	4.82
Lysine	0	0.07	0.07	0.03	0.02
Methionine	0.37	0.52	0.68	0.52	0.83
Phenylalanine	2.34	2.21	0.37	0.88	0.89
Threonine	4.84	4.29	4.43	4.94	4.79
Valine	2.25	2.29	0	2.44	2.46
Non-essential amino acids (g kg⁻¹)					
Alanine	1.94	2.78	2.92	2.2	3.04
Aspartic acid	3.31	2.24	2.58	4.02	2.71
Glutamic acid	4.38	8.8	9.92	4.85	10.04
Glycine	3.53	3.22	3.56	3.63	3.47
Proline	1.82	0.89	1	0.88	1.08
Serine	2.15	1.92	1.84	2.33	1.97
Tyrosine	1.57	0.7	0.87	1.62	0.84
Total	43.36	47.48	47.62	45.74	52.74

concentration with higher levels of P applications and a lower amino acids concentration with a higher K application. A range of 105.48 to 121.63% increase in the amino acids concentration was noted between the various increased P levels and the control. The results portrayed no notable influence of increased K levels with respect to improvement of amino acids concentrations.

Secondary metabolites

The phenol content of a plant is dependent on both intrinsic (genetic and extracting solvent) and extrinsic (environment and handling) factors. Plant phenol content affects the level of its antioxidant activity, with reports specifying high level antioxidant activity of plants usually linked to the phenols (Mooza *et al.*, 2014). Considered as a plant secondary metabolite, the phenols are very essential in chelating redox-active metal ions and preventing hydroperoxide conversions into reactive oxyradicals (Arash *et al.*, 2015). The TPC of the 3 plant extracts (leaves, stem and roots), expressed as gallic acid equivalents, portrayed all the plant portions of possessing a high phenolic content (Figure 2), ranging from 1000 – 1350 mg GAE/100 g DW, with leaves recording the highest TPC (1350 mg GAE/100 g DW), followed by the roots (1100 mg GAE/100 g DW), and stem (1000 mg GAE/100 g DW). The results depicted a lower range of diversity in the TPC of the 3 plant extracts examined.

Flavonoids have long been accorded recognition of enhancing plants protective effect against invasion by microbes. For centuries, human diseases have been controlled by flavonoid-rich plant extracts (Mooza *et al.*, 2014). The TFC, expressed in mg quercetin per 1mg dry weight, as per result from our calorimetric assay portrayed the leaves with a high TFC value (2400 mg QE/1 mg DW). The TFC

contained from the various extracts (leaves, roots, and stem) varied considerably, ranging from 500 to 2400 mg QE/1 mg DW, with the roots and stem extracts recording lower values of 600 and 500 mg QE/1 mg DW, respectively. Similar observations (Amoo *et al.*, 2012) on flavonoids and phenolic content fluctuations in twenty four flowering plants have been made and reported.

Antioxidants content

The wide scale utilization of various plant parts for pharmaceutical purposes have over the years aroused the interest of researchers into discovering the bioactivities of plants from various geographic locations. It can evidently be understood from the obtained results that *Moringa oleifera* L. have outstanding antioxidant activities. The antioxidants are believed to be an essential nutraceuticals on account of their several medicinal uses and are currently popularly utilized by the food industries as inhibitors (Scherer and Godoy, 2009). The result conforms to an earlier report (Farooq *et al.*, 2007) that recommended its consideration as a plant species with great prospect of utilization for natural medicine, based on their findings about its possessing antihypertensive, cholesterol lowering, hepatoprotective, and diuretic activities. The report pointed out the presence of nitriles and glycosides that possess blood pressure lowering effect and the existence of quercetin, a recognised flavonoid with hepatoprotective activity.

Past trials have established the antioxidative properties of moringa leaves, our research is an endeavour to ascertain whether such anti-oxidative property is existent in the stem and roots portion of the moringa tree and to further evaluate and make comparison of available content within each portion through the utilization of two standard analytical procedures: DPPH radical scavenging activity and FRAP assay. The noted variations from the results in the free radical scavenging ability of the aqueous moringa extracts of roots, stem, and leaves on the basis of percent inhibition is illustrated in Figure 1. It is evident from this result, that the aqueous extract of all the 3 parts studied portrayed strong antioxidant potential, with the leaves and roots possessing the highest antioxidant potential (68 and 67% DPPH scavenging activity respectively), and the stem depicting a lower DPPH scavenging activity percentage of 45%. The FRAP antioxidant activity on the moringa aqueous plant parts portrayed the leaves with maximum FRAP potential (2500 mg AAE/100 g DW), with the roots and stem parts displaying lower FRAP activity (800 and 700 mg AAE/100 g

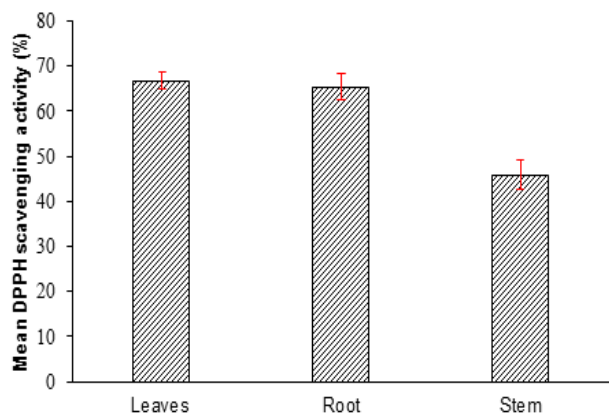


Figure 1. The DPPH radical scavenging activity of *Moringa oleifera* L. roots, stem, and leaves portions

DW, respectively). Ferric tripyridyltriazine (Fe (III) – TPTZ) was reduced to ferrous tripyridyltriazine (Fe (II) – TPTZ) via a reductant (antioxidant). The FRAP serves as an indicator of the reducing power of the aqueous extract, which serves as the reductant by bringing about the reduction of Fe^{+3} to Fe^{2+} form, resulting in an increase of the absorbance power to 700 nm. The intensity of colour change of the aqueous extract is an indicator of the degree of antioxidant activity (Jones, 2005). The FRAP assay is an easy and economical procedure that provides the total available antioxidant content of the plants or their parts. Generally, the higher the FRAP value obtained the bigger is the antioxidant activity. Based on this assay, greater antioxidant activity was displayed by the leaves compared to roots and stem, signifying that the moringa leaves extract possesses substantial redox properties that makes them adsorb and neutralize free radicals.

Mineral nutrient elements content

A plant requires all the essential elements in the right amount and correct ratio for its proper development and productivity. Some of the elements function in promoting vegetative growth and root development, while others, serve in the reproduction process and fruit production. Over time, researchers have been manipulating plant growth aspects and development for improvement of yield and quality. Results from this trial depict P manipulation to significantly affect the leaves mineral nutrient content with respect to iron (Fe), manganese (Mn), and zinc (Zn), with highest recorded values of 5.18, 0.90, and 1.21 g kg^{-1} for Fe, Mn, and Zn respectively, equalling 57.9 and 136.8% variation on the leaves mineral nutrient content as compared to the control treatment with respect to Fe and Mn respectively. Manipulating K levels gave a significantly higher leaves mineral nutrient content values in terms of calcium (Ca) and Fe values (Table 3).

Table 2. Correlation analysis between phenolic antioxidant and antioxidant activities observed in different parts of *Moringa oleifera*.

R ²	TFC	DPPH	FRAP
TPC	0.765	0.597	0.933**
TFC		0.594	0.900**
DPPH			0.554

Correlation between flavonoids and phenolics with antioxidant activities

Correlation coefficients of TPC and TFC with the DPPH and FRAP assay depicted both the TPC and TFC significantly correlating with antioxidant activities (Table 2). The flavonoid and phenolic contents had revealed excellent relationship with DPPH, and FRAP scavenging activities. A close relationship between the flavonoids and phenolics content of red clover (*Trifolium pratense* L.) extract and radical scavenging activity have been reported earlier (Arash *et al.*, 2015). Similar results (Sahreen *et al.*, 2010) stressed the presence of a strong correlation between the phytochemical contents and ABTS, DPPH, and superoxide radical scavenging.

Discussion

Amino acids

All living creatures require taking in nutrients for their survival. The nutrients serve as energy source for body metabolic processes that promote growth, maintenance, and repair. Enhancing the nutritive value of plant materials for human well-being can prevent the progression of many diseases. “We are what we eat”. *Moringa* tree is already serving as an important nutritive vegetable in many impoverished communities of Africa and Asia. Nutritional value of protein-rich foods is dependent upon their ability to meet the amino acids requirement (Humayum *et al.*, 2007). Humans can produce 10 of the 20 amino acids, signifying that the remaining 10 must be supplied in the diet, and, unlike fat and starch, the human body does not store excess amino acids for later use - the amino acids must therefore be supplied in the food every day. The fact that moringa leaves was found to contain a high concentration of these essential (histidine, leucine, threonine) and non-essential (glutamic acid, aspartic acid, glycine) plus a range of various other amino acids strongly indicates the nutritive value of this important tree crop. Histidine is known to be an important constituent of hemoglobin (oxygen carrying pigment of red blood cells), while leucine is known to serve as a nutrient for regulating protein metabolism and also plays a functional role in regulating target of rapamycin

Table 3. Effect of P and K levels on mineral nutrients content of *Moringa oleifera* L.

Treatment kg ha ⁻¹	g kg ⁻¹								
	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
P0	69.67 ^a	25.86 ^a	58.28 ^a	13.41 ^a	4.80 ^a	0.28 ^a	3.28 ^b	0.38 ^{ab}	1.18 ^a
P50	50.33 ^a	22.16 ^a	70.93 ^a	12.60 ^a	6.82 ^a	0.20 ^a	3.97 ^{ab}	0.68 ^{ab}	0.50 ^b
P100	76.53 ^a	32.00 ^a	70.17 ^a	12.29 ^a	4.44 ^a	0.23 ^a	4.58 ^{ab}	0.64 ^{ab}	1.27 ^a
P150	67.40 ^a	23.38 ^a	69.21 ^a	11.13 ^a	6.14 ^a	0.03 ^a	4.75 ^{ab}	0.90 ^a	1.06 ^{ab}
P200	48.93 ^a	23.14 ^a	73.35 ^a	12.00 ^a	4.39 ^a	0.14 ^a	5.18 ^a	0.18 ^b	1.21 ^a
K0	46.67 ^a	28.40 ^a	56.65 ^a	3.80 ^b	4.10 ^a	0.29 ^a	6.90 ^{ab}	0.26 ^a	1.11 ^a
K50	49.53 ^a	31.40 ^a	44.09 ^a	19.13 ^a	4.54 ^a	0.13 ^a	4.58 ^b	0.50 ^a	1.49 ^a
K100	55.60 ^a	33.00 ^a	42.14 ^a	11.65 ^{ab}	4.95 ^a	0.23 ^a	6.99 ^{ab}	0.38 ^a	0.94 ^a
K150	41.80 ^a	22.91 ^a	67.60 ^a	12.95 ^{ab}	3.92 ^a	0.14 ^a	9.65 ^a	0.24 ^a	0.96 ^a
K200	32.47 ^a	20.93 ^a	63.02 ^a	8.68 ^{ab}	4.94 ^a	0.15 ^a	9.90 ^a	0.52 ^a	0.76 ^a

Note: Means sharing similar letter in a column for different levels of the same nutrient are statistically non-significant according to Duncan's Multiple Range Test (DMRT) at $\alpha=0.05$; values are the means of five replicates \pm standard error.

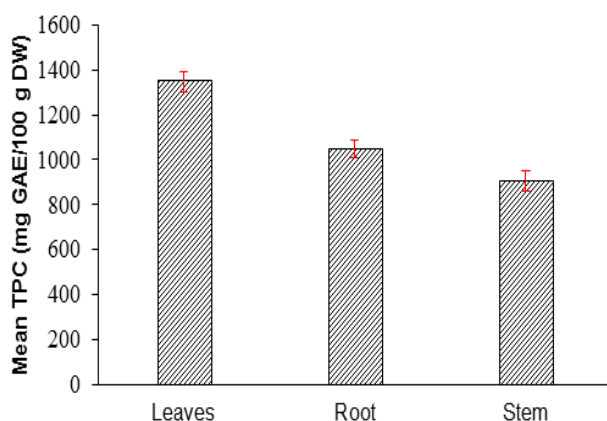


Figure 2. Total phenolic content of *Moringa oleifera* L. roots, stem, and leaves portions.

(TOR) activity (Stipanuk, 2007).

Aspartic acid, another non-essential amino acid, plays a significant part in the citric acid cycle (Krebs cycle), whereby a number of other amino acids and biochemicals are generated. It is also involved in the movement of the coenzyme nicotinamide adenine dinucleotide (NADH) molecules from the main body of the cell to its mitochondria, where it is used to generate adenosine triphosphate (ATP), the power that energize all cellular activity. Glutamic acid is regarded as a neurotransmitter (transmitting nerve impulses) and also serves as a key mediator of numerous metabolic pathways that lead to the generation of glutathione antioxidant (Meister and Tate, 1976). The basis for the influence of increased P levels on amino acids improvement and the corresponding non-influence of K for same could easily be linked to the former's role as being a component of certain enzymes and proteins.

Secondary metabolites

A number of plants secondary metabolites are noticed to be valuable basis of natural antioxidants and the presence of such bioactive phytoconstituents comprising of alkaloids, steroids, polyphenols

(flavonoids and tannins), carbohydrate glycosides, and terpenoids was established in *Moringa peregrina* leaves. Mooza *et al.* (2014) revealed the existence, inside the crude extracts of *Moringa peregrina* leaves, of such secondary metabolites, as tannins, alkaloids, glycosides, and saponins. They further observed that the most promising of the polyphenolic compounds among the plants secondary metabolites are flavonoids and tannins that function as metal chelators, absorbing and neutralizing free radicals. A high phenolic value was obtained from all the extracts under study (ranging from 1000 – 1350 mg GAE/100 g DW), while the flavonoids content varied considerably (ranging from 500 to 2400 mg QE/1 mg DW) as per obtained results (Figure 2).

Phenolic compounds are secondary metabolites in plants which play an important role in human health and nutrition, and are associated with higher antioxidant activities (Jaykumar *et al.*, 2013). Flavonoids are a major class of oxygen containing heterocyclic natural products that are widespread in green plants. Generally, they are found as plant pigments in a broad range of fruits and vegetables. The rich-flavonoid plants could manifest themselves as good sources of antioxidants that would assist in the enhancement of the overall antioxidant capacity of an organism and protection against lipid peroxidation (Arash *et al.*, 2015).

Antioxidants

The term "antioxidant" implies that a compound can quench radicals or diminish the oxidation of other molecules. Free radicals normally occur as by-products of metabolic processes or as a result of radiation. These free radicals capture electrons by attacking other compounds in order to attain stability. The compounds attacked then become new free radicals attacking other compounds with a resultant chain reaction. The net effect of all these is damage to living cells and development of diseases

and other body disorders such as cardiovascular disease, cancer, and aging. Antioxidant compounds preservation until their internalization inside the cells is of utmost importance, but in particular many phytochemicals are only partially bioavailable, so they might not reach relevant concentrations within a cell. Additionally, within the cell, the compound must not be converted into an inactive metabolite (DelRio *et al.*, 2013). Plants are generally regarded as the natural potential sources of antioxidants, and within the last few years lots of studies have been undertaken to determine the antioxidant properties of a number of plants (Chorage *et al.*, 2013), and their capacity in controlling lots of human diseases promoted by free radicals (Hou *et al.*, 2003).

The DPPH assay from result of our study portrayed the roots and leaves with highest antioxidant activity (67 and 68%, respectively) and the FRAP assay depicted the leaves with a high antioxidant potential of 2500 mg AAE/100 g DW. Generally, our findings showed that the antioxidant activity was in the order, leaves extract > roots extract > stem extract (Figure 3 and 4). Measuring the wide array of antioxidants possessed by plants and the capacity of each individual compound is of utmost importance. The antioxidants scavenging properties are commonly linked with their ability to form stable radicals after the DPPH radical accepts hydrogen ion from the plant aqueous extract with a resultant bleaching of the DPPH solution (Chorage *et al.*, 2013) from violet coloration to yellow. The greater the bleaching action (reduction in absorbance) on the DPPH solution, the greater will be the antioxidant potential. The use of the DPPH assay offers a fast and easy approach of assessing antioxidants by spectrophotometry (Eugenio *et al.*, 2012), thus it is a suitable process of evaluating several substances at a time. The method has the advantage of incorporating many samples in a short time span and is vulnerable enough to distinguish active ingredients at low concentrations (Arash *et al.*, 2015).

Mineral nutrient elements

Several elements are involved in plant growth and development. Those absorbed from soil are the mineral nutrients (categorized as macronutrients, secondary nutrients, and micronutrients), while those obtained from air and water are termed non-mineral nutrients (carbon, hydrogen, and oxygen). These elements constitute the raw materials required for normal plant growth and development, and subsequent production of fruits and seeds. The mineral nutrient content of the moringa leaves were generally found not to be affected by the various P and

K levels, with significant variation ($P < 0.05$) obtained only with respect to Fe, Mn, Zn, and Ca, while the remaining mineral nutrient elements were found to be non-significantly ($P > 0.05$) affected (Table 3). The result conforms to what have been reported earlier (Janick and Paull, 2008) that the moringa leaves are a good source of vitamins A, B, and C, protein, and minerals such as Ca and Fe.

Plants are known to employ various uptake kinetics to selectively accumulate mineral nutrients within their cells (Marschner, 1995). Varying these nutrient levels during the growth processes of the moringa tree apparently doesn't influence the mineral nutrient composition of the leaves. The plant being a dynamic living system is prompted in its growth process by a number of factors existent in its growing environment. The various plant nutrient elements are known to undertake distinct functions in the plant growth cycle. They are considered relatively essential where they partake to be part of a plant chemical composition or serve in regulating its numerous physiological processes. When these mineral elements availability falls below the quantity required for optimum plant growth, the effect became manifested in the plant growth, development, and yield.

Conclusion

Moringa plant parts could be exploited to make the greatest nutraceuticals contribution amongst impoverished communities globally. Our findings underlined the importance of various moringa plant parts (roots, stem, and leaves) with respect to essential nutritive materials. There are strong indications that with further studies, molecular extracts of this important plant can be utilized for biochemical and pharmacological benefits. The trial demonstrates the existence, within the *Moringa oleifera* L. specie, of substantial level of amino acids, as well as establishes the presence of antioxidants in various plant parts (roots, stem, and leaves). Equally noted is the considerable variation of antioxidant activity, with the aerial leaves portion depicting high level activity, followed by the underground root portion, with the stem portraying the lowest level of activity. These antioxidant activities are directly linked to the existence in the aqueous extract portions of secondary metabolites, mainly phenolics and flavonoids. Having established the presence of these various nutritive constituents (amino acids, antioxidants, secondary metabolites, and mineral nutrients, especially Fe and Ca), and taking into account how this important tree has served for decades as an important staple food

to the impoverished populations of Africa and Asia, *Moringa oleifera* L. can be strongly recommended for utilization as a realistic source of essential and non-essential amino acids, natural antioxidants, calcium and iron, with resultant gains in general wellbeing. Future trials will further suggest ways of improving these nutritive constituents (for example, improving the antioxidant status of the stem parts that were noted to be low), as well as establishing the specific biochemical constituents of this important tree for possible utilization in the formation of new medications.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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