

Full Length Research Paper

## Levels of anti-oxidants in different parts of moringa (*Moringa oleifera*) seedling

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**Moringa (*Moringa oleifera*) tree is known as a medicinal plant, with every part of the plant edible and rich in anti-oxidants. However, there is little information on these anti-oxidants distribution in the plant; these compounds are produced at different concentrations in different parts of the plant. This research, investigated the levels of anti-oxidants in different parts of the plant. Seedlings were grown in replication, consisting of four plots, and replicated into three. Pooled plant parts were collected for analysis. There were significant differences in sugar concentration and anti-oxidant distribution in different parts of moringa seedling. The sucrose concentration was the dominant carbohydrate produced in different parts of the seedling, except glucose in plant roots. Raffinose was detected only in leaf, stem and root of the seedling. Whereas the highest anti-oxidant concentration was also recorded in: Total anti-oxidant (TAO) ( $1.8 \text{ mg g}^{-1}$ ), leaf-ascorbic acid (AsA) ( $2.0 \text{ mg g}^{-1}$ ), and total phenols (TP) ( $64.1 \mu\text{g g}^{-1}$ ); stem-TAO ( $1.2 \text{ mg g}^{-1}$ ); root-carotenoids ( $29.7 \text{ mg g}^{-1}$ ), TP ( $57.3 \mu\text{g g}^{-1}$ ); seed-  $\alpha$ -tocopherol ( $28.57 \mu\text{g g}^{-1}$ ). Although the seedlings had substantial amount of total crude protein, seed ( $110.4 \text{ mg g}^{-1}$ ) and leaf ( $76.1 \text{ mg g}^{-1}$ ) had the highest concentrations. Different parts of moringa seedlings had different levels of sugars and anti-oxidants; this contributes to their nutritional qualities and eventually qualifies it as a suitable underutilized crop with every part being edible. The specific aims of this study were to generate analytical data on anti-oxidants concentrations in different parts of the moringa plants and identification of different parts of the plant as potential ingredients in functional food products.**

**Key words:** Anti-oxidants, carbohydrates, moringa, nutrients, protein.

### INTRODUCTION

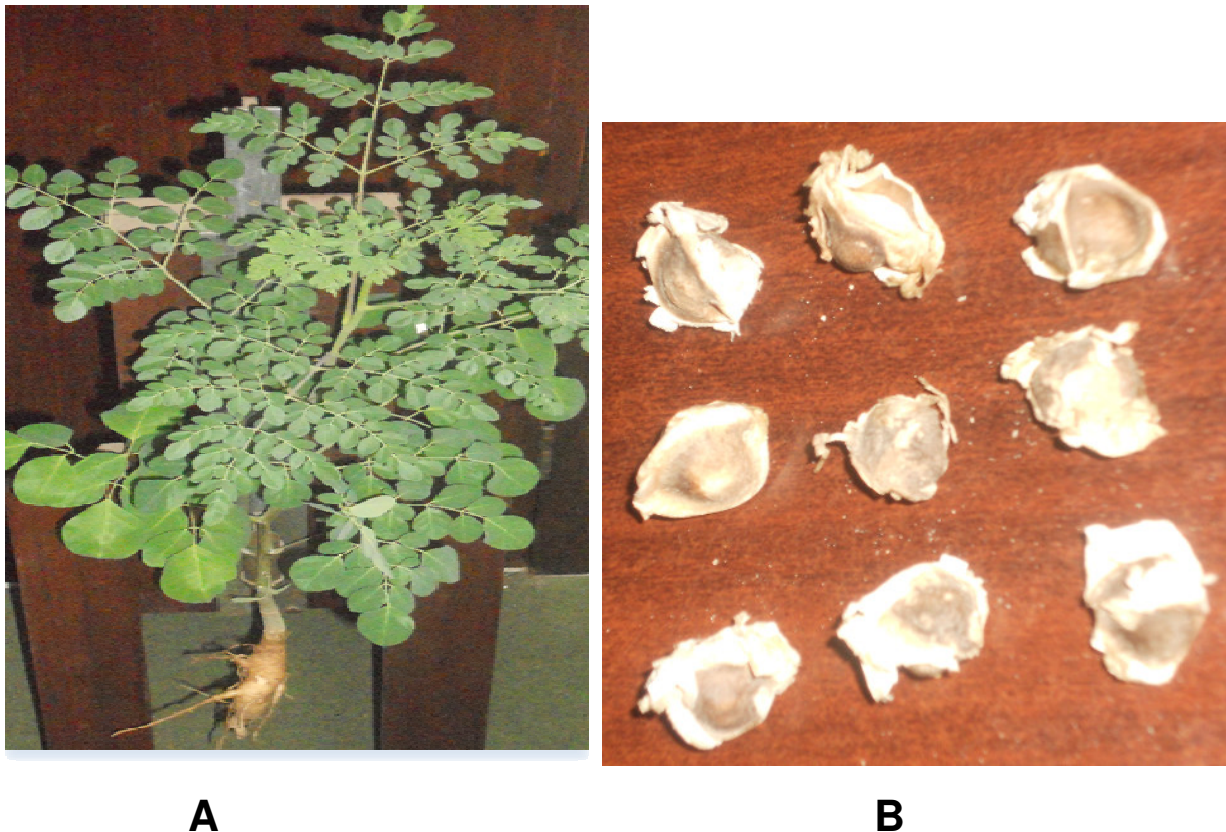
Moringa (*Moringa oleifera*) is a perennial softwood tree, which is known for its traditional medicinal and industrial uses. It is an important crop in India, Ethiopia, Philippines and Sudan, and is extensively being grown in West, East and South Africa, Tropical Asia, Latin America, the Caribbean, Florida and the Pacific Islands. Recently, this tree has become an outstanding indigenous source of highly digestible protein, calcium (Ca), Iron (Fe), Vitamin C, and carotenoids. These nutritional characteristics of the plant may be, potentially beneficial to the developing regions of the world where undernourishment is a major concern. All parts of the moringa tree are edible and have long been consumed by humans, and their anti-oxidant

concentrations warrant the plant's image as a 'healthy' food source (Fahey, 2005).

Moringa is one of underutilized tree species (Fahey, 2005). The large numbers of underutilized species represent an enormous food resource which can narrow the gap to the increasing demand for food and nutrition, energy, medicines and industrial needs. Those species require a thematic approach for their scientific research for development and evaluation, resulted in supplementing nutrients and anti-oxidants to the diet and are sometimes convenience food for low-income urban people in developing countries.

From a physiological viewpoint the purpose of anti-oxidants is to scavenge reactive oxygen species (ROS), most commonly hydrogen peroxide, the superoxide radical and the hydroxide radical (Vranova et al., 2002), produced within a cell due to oxidative stress, before

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**Figure 1.** The moringa (*Moringa oleifera*) seedling, whereby leaf, stem and root tissues were collected (plate A) and seeds with seed coat (plate B).

these oxidize vital cell components. Reactive oxygen species (ROS) are a chemically-reactive molecule that contains oxygen including oxygen ions and peroxides. Due to the presence of unpaired valence shell electrons the reactive oxygen species are highly reactive in the plant tissue leading to undesirable changes. ROS form as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis (Devasagayam et al., 2004). However, during times of environmental stress (e.g., UV or heat exposure), ROS levels can increase dramatically. This may result in significant damage to cell structures. This cumulates into a situation known as oxidative stress. ROS are also generated by exogenous sources such as radiation. In general, harmful effects of reactive oxygen species on the cell are most often damage the DNA, oxidations of polyunsaturated fatty acids in lipids (lipid peroxidation), oxidations of amino acids in proteins and oxidatively inactivate specific enzymes by oxidation of co-factors. Therefore, the occurrence of damaging concentrations of ROS needs to be counteracted to avoid permanent tissue damage (Seifried et al., 2007). Such an accumulation of ROS in the cell can be counteracted by a system of low molecular mass anti-oxidants (e.g., ascorbic acid, Vitamin E, phenols etc) as well as by

certain enzymatic anti-oxidants (Blokhina et al., 2003). Similarly, the plant plays a significant role as an anti-oxidant after they are consumed by human beings.

The plant has, therefore, drawn a special attention and lifted up human health issues due to these anti-oxidants, is consumed as staple food, other than medicinal values. The plant, knowledge of the anti-oxidant content of different parts, therefore, is important from consumers' point of view.

Although there are reports that moringa contains anti-oxidants and that levels vary with different plant parts, little is known about the anti-oxidant systems present in various plant parts and how they compare to each other. The aim of this study was, therefore, to report how different concentrations of moringa anti-oxidant compounds are distributed in the different plant parts.

#### MATERIALS AND METHODS

Moringa seeds were germinated on paper-towel; once root and leaf were produced (two weeks after) the seedlings were transplanted to a seedling bed and grown under wet-walled plastic tunnel for four months (Figure 1A and B). After plant tissues were collected destructively, freeze-dried and kept in cold storage at  $-21^{\circ}\text{C}$  until further analysis.

## Determination of bioactive compounds

### Total anti-oxidant capacity (TAOC) assay

Total anti-oxidant capacity (TAOC) was determined according to Benzie and Strain (1996) with slight modifications. These authors developed the FRAP assay which is based on the reduction of the ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) complex by a reductant, therefore determining the combined anti-oxidant capacity of anti-oxidant molecules present in the tissue under investigation. Aliquots of 0.1 g freeze-dried plant material were extracted with 1 N perchloric acid, vortexed and centrifuged at 12,400 g for 10 min at 4°C. A fresh FRAP reagent solution (300 mM sodium acetate buffer pH 3.6, 10 mM Fe(II)-TPTZ prepared in 40 mM HCl, 20 mM FeCl<sub>3</sub> × 6H<sub>2</sub>O (10:1:1)) was prepared prior to measurement. Subsequently an aliquot of the samples (30 µl) was mixed with 900 µl FRAP reagent solution and the absorbance was measured at 593 nm after 10 min. The total anti-oxidant capacity was expressed as mg FeSO<sub>4</sub> × 7H<sub>2</sub>O × g DW<sup>-1</sup> equivalent.

### Determination of total phenols

Phenols were determined according to Hertog et al. (1992), with slight modifications. Briefly, freeze-dried tissue (1.0 g) was mixed with 10 ml 99.8% (v/v) methanol and vortexed for 30 s. The mixture was then manually shaken using IKA® (ks 130, Staufen, Germany) and left overnight at room temperature to extract the free phenols (Hertog et al., 1992). Subsequently the mixture was centrifuged, the supernatant filtered through Whatman® no. 1 filter paper and the pellet repeatedly rinsed with 10 ml solvent until color was no longer released. Membrane-bound phenols were released from the remaining plant residue by acid hydrolysis. A 10 ml portion of acidified (2 M hydrochloric acid) 60% (v/v) aqueous methanol was added to each sample, which was then incubated at 90°C for 90 min. Samples were allowed to cool before the supernatant was filtered and analyzed filtered free phenol extract. Free and membrane-bound phenols concentrations were determined spectrophotometrically at 750 nm by adding Folin-Ciocalteu reagent to the extract and expressing the results in 'gallic acid equivalents' (GAE).

### Determination of ascorbic acid (AsA)

Ascorbic acid concentrations were determined according to Böhm et al. (2006) by comparing the absorbance of plant tissue extracts at 520 nm with values obtained using an L-ascorbic acid standard curve. The results were expressed in mg AsA g<sup>-1</sup> DW.

### Determination of total carotenoids

Total carotenoids were determined according to Dere et al. (1998) by computing the absorbance values of the plant tissue extracts at wavelengths of 470, 646.8, and 663.2 nm.

### Determination of vitamin E content

Dried plant material (0.5 g) was immersed in 20 ml of ethanol for 30 min in a water bath at 85°C. The solution was allowed to cool and then filtered into a separating funnel. Heptane (10 ml) was added, and the solution was shaken for 5 min. Then, 20 ml of 1.25% sodium sulfate was added and the solution was shaken again for 2 min, and allowed to separate into layers. Total tocopherols were determined by a reaction with cupric ions and complexation with 2, 20-biquinoline (cuproine) according to Contreras-Guzmán and

Strong (1982). A volume of 0.5 ml of α-tocopherols in ethanol was processed in the same way as a sample and used as a standard.

### Determination of sugar concentration

Sugar concentrations were determined according to Liu et al. (1999). Briefly, freeze-dried material (0.05 to 0.10 g) was mixed with 10 ml 80% (v/v) ethanol and homogenized for 1 min. Thereafter, the mixture was incubated in an 80°C water bath for 60 min to extract the soluble sugars. Subsequently the mixture was kept at 4°C overnight. After centrifugation at 12000 g for 15 min at 4°C, the supernatant was filtered through glass wool and taken to dryness in a vacuum concentrator. Dried samples were re-suspended in 2 ml ultra-pure water, filtered through a 0.45 µm nylon filter and analyzed using an isocratic HPLC system equipped with a refractive index detector on a Phenomenex® column (Rezex RCM-Monosaccharide). The concentration of individual sugars was determined by comparison with authentic sugar standards.

### Total soluble protein extraction

Total soluble proteins were extracted according to Kanellis and Kalaitzis (1992) with slight modifications. Frozen leaf tissue powder (1 g DW) was extracted in 5 ml 50 mM Tris-HCl buffer (pH 7.4 containing 0.2 M NaCl, 20 mM MgSO<sub>4</sub>, 1 mM EDTA, 5 mM β-mercaptoethanol, 0.5 mM PMSF, 10 mM leupeptin, and 10 % (v/v) glycerol). The mixture was allowed to stand on ice for 15 min and then centrifuged at 20000 ×g for 20 min. The supernatant was used for Bradford assay and SDS-PAGE after being passed through Miracloth®.

### Total protein assay

The Bradford microassay was used to determine the protein content of the samples (Bradford, 1976). Bradford dye reagent was prepared by diluting the dye concentrate with distilled water 1:4. The dye (1 ml) was added to test tubes containing 20 µl sample extract, mixed and incubated at room temperature for 5 min. Samples were then read spectrophotometrically at 595 nm and the protein concentration determined by comparing results with a standard curve constructed using bovine serum albumin (BSA).

### Statistical analysis

Analyses of variance were performed using GenStat version 12<sup>th</sup> edition (VSN International, Hemel, Hempstead, UK). Standard deviation values were calculated and differences among treatments were separated by the least significant difference at P ≤ 0.05 level.

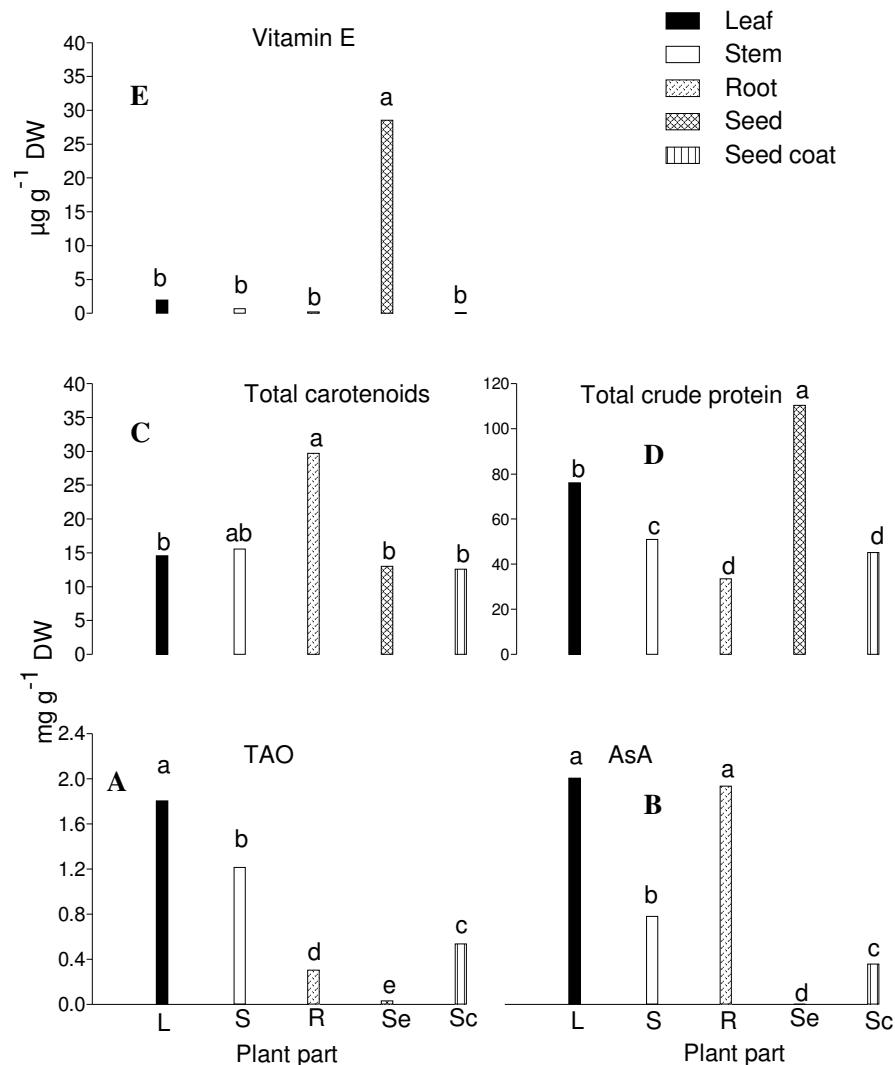
## RESULTS

### Total antioxidant capacity

Total anti-oxidant capacity (TAOC) differed significantly (P ≤ 0.05) between plant parts (Figure 2A). The leaf part of the moringa plant had the highest concentration (1.8 mg g<sup>-1</sup>) of TAOC, followed by stem (1.2 mg g<sup>-1</sup>) and seed coat (0.5 mg g<sup>-1</sup>), respectively.

### Ascorbic acid (AsA)

The ascorbic acid (AsA) concentration also differed



**Figure 2.** Evaluation of total anti-oxidant capacity (TAO,  $\text{LSD}_{0.05} = 0.2$ ) as well as different types of individual anti-oxidant compounds (Ascorbic acid (AsA),  $\text{LSD}_{0.05} = 0.3$ ; total carotenoids,  $\text{LSD}_{0.05} = 16.2$ ; vitamin E,  $\text{LSD}_{0.05} = 8.2$ ; total crude protein,  $\text{LSD}_{0.05} = 11.7$ ). Values followed by a different lower-case letter are significantly different at  $P = 0.05$ . ( $n = 4$ ).

significantly between plant parts (Figure 2B). Similar to the TAOC, AsA was the highest concentration in the leaf ( $2.0 \text{ mg g}^{-1}$ ) followed by the root ( $1.9 \text{ mg g}^{-1}$ ).

### Total carotenoids

The carotenoids concentration was the highest in the root ( $29.7 \text{ mg g}^{-1}$ ) as compared to others (Figure 2C), whereas leaf, stem, seed and seed coat had relatively equal concentration.

### Vitamin E

The vitamin E concentration was the highest in the seed ( $28.57 \text{ mg g}^{-1}$ ) as compared to others (Figure 2E), whereas

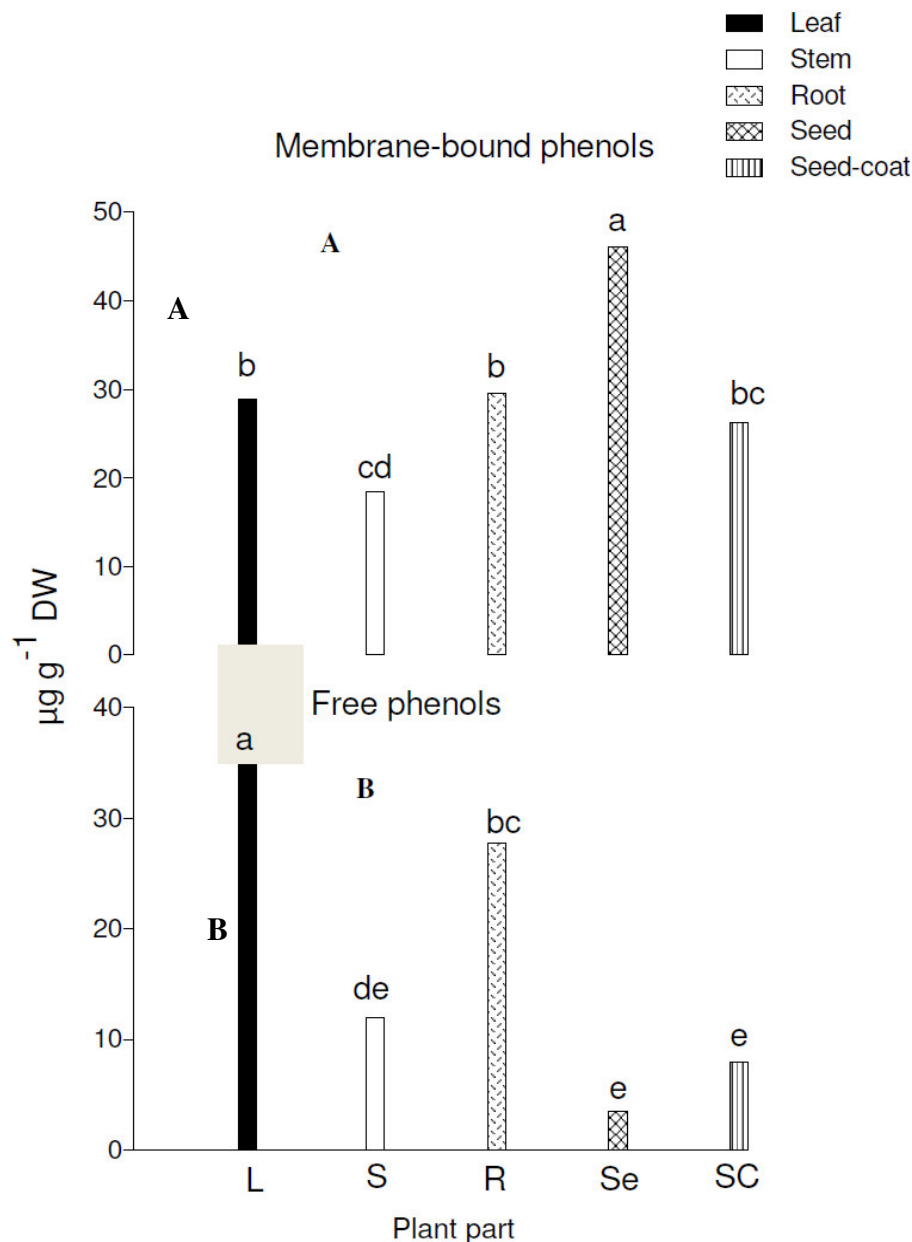
leaf and stem, had relatively equal concentration of vitamin E.

### Total phenols (TP)

The TP concentration was significantly the highest in leaf tissue, followed by root and seed (Figure 3). Different plant parts displayed different forms of phenols between free phenols and compared to membrane-bound ones. The seed ( $46 \text{ mg g}^{-1}$ ) had significantly higher membrane-bound phenols than free ones ( $3.5 \text{ mg g}^{-1}$ ).

### Total crude protein

The Bradford reagent assay resulted in high concentration



**Figure 3.** Free and membrane-bound forms of total phenols produced in leaf, stem, root, seed and seed coat parts of moringa (*Moringa oleifera*) seedling. Values followed by a different lower-case letter are significantly different at  $P = 0.05$ .  $\text{LSD}_{0.05} = 10.08$ . ( $n = 4$ ).

of crude protein in all tissues (Figure 2D). Seed ( $110.4 \text{ mg g}^{-1}$ ) had high concentration of total crude protein, followed by leaf ( $76.1 \text{ mg g}^{-1}$ ). The SDS-PAGE electrophoresis result showed the leaf proteins pattern, loaded in a smaller volume and had expressed protein of molecular weight ( $\approx \text{kDa } 37$ ) (Figure 4).

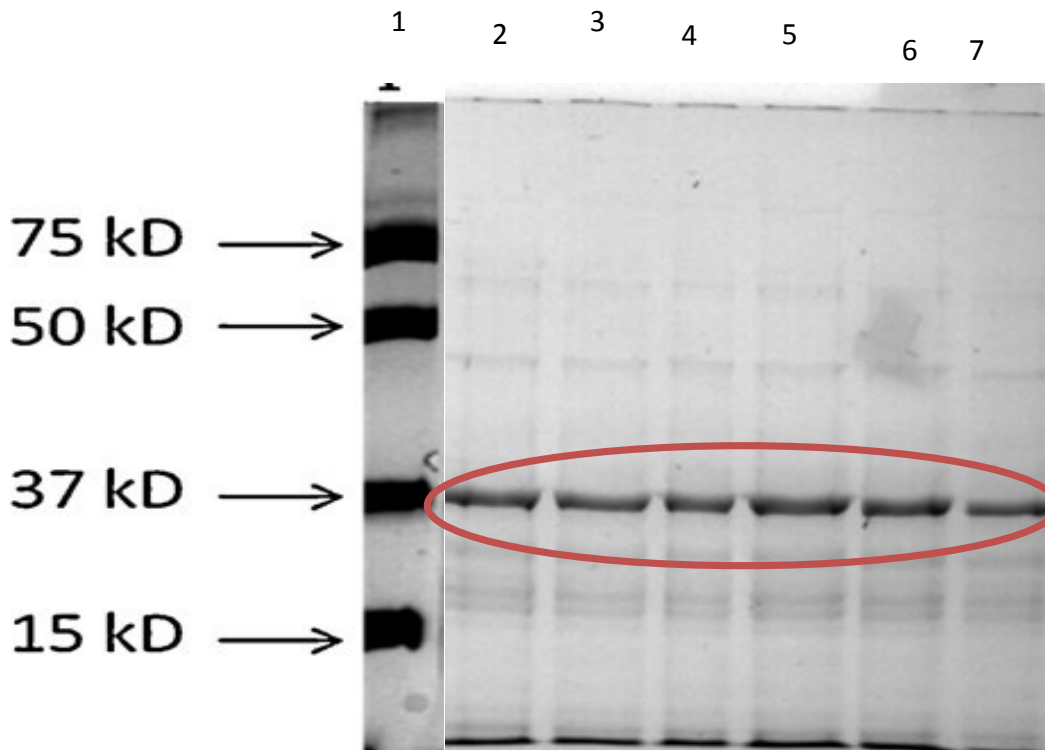
#### Non-structural soluble carbohydrates

There were significant differences on carbohydrate distribution on different parts of moringa seedlings (Table

1 and Figure 5). Root ( $258.90 \text{ mg g}^{-1}$ ) and stem ( $245.72 \text{ mg g}^{-1}$ ) recorded the highest sugar concentration. Sucrose was found as the dominant sugar ( $245.16 \text{ mg}$ ), followed by glucose ( $180.19 \text{ mg g}^{-1}$ ). Raffinose was also detected only in series of stem, root and leaf, respectively.

#### ESEM nutrient content

The ESEM result also showed different nutrients at



**Figure 4.** SDS-PAGE was performed on 12% homogeneous gels using the Bio-Rad system (Bio-Rad Laboratories, CA, USA). Lane 1, molecular marker; Lanes 2 to 7, leaf proteins loaded from ranges of 4, 5, 6, 7, 8, 9  $\mu\text{g}$  of concentrations, respectively.

**Table 1.** Distribution of carbohydrates at various concentrations in leaf, stem, root, seed and seed coat parts of moringa (*Moringa oleifera*) seedling.

Plant parts	Fructose	Glucose	Raffinose	Sucrose	Total
	$\text{mg g}^{-1} \text{ DW}$				
Leaf	12.99 <sup>fg</sup>	14.51 <sup>fg</sup>	8.91 <sup>fg</sup>	42.14 <sup>d</sup>	78.55
Stem	19.85 <sup>ef</sup>	74.81 <sup>abc</sup>	66.32 <sup>c</sup>	84.74 <sup>ab</sup>	245.72
Root	70.69 <sup>bc</sup>	90.04 <sup>a</sup>	20.95 <sup>ef</sup>	77.22 <sup>abc</sup>	258.90
Seed	nd	nd	nd	33.74 <sup>de</sup>	33.74
Seed coat	0.71 <sup>g</sup>	0.83 <sup>g</sup>	nd	7.32 <sup>fg</sup>	8.86
Total	104.24	180.19	96.18	245.16	

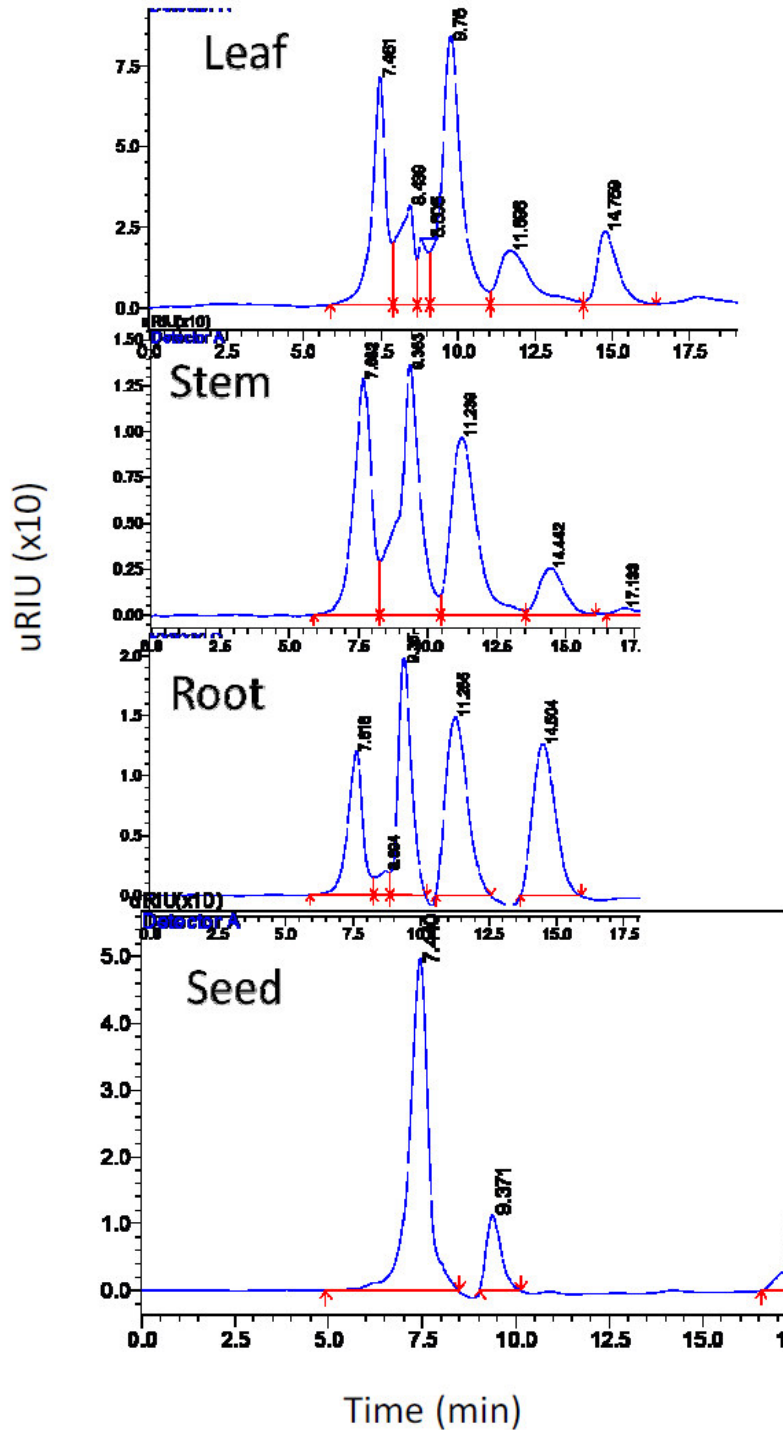
Values followed by a different lower-case letter are significantly different at  $P = 0.05$ .  $\text{LSD}_{0.05} = 15.7$ . ( $n = 4$ ); nd – not detected.

various concentrations on different parts of moringa plant (Table 2). Potassium predominantly accumulated in root, stem, seed coat; high accumulation of phosphorous was found in leaf and seed; sulfur was accumulated in leaf, seed, root; and magnesium also distributed in similar concentrations over different organs of the seedling.

## DISCUSSION

Moringa plant is unique compared with other plants in various aspects. It has been used to combat malnutrition,

especially among infants and nursing mothers in developing countries (Ray-Yu et al., 2006; Anwar et al., 2007). Every part of the plant is edible. The leaves can be eaten as fresh, like others perishable fresh commodities such as lettuce and other vegetables. Different anti-oxidant compounds are produced in substantial amount and well distributed within a plant (Perry et al., 1999). Similarly, confirming the present findings literature clearly shows that high nutrients, antioxidants and glucosinolates, and low oxalate contents are common features of the moringa species (Ray-Yu et al., 2006). *Moringa peregrina* was also found to be reach



**Figure 5.** HPLC chromatographic presentation of non-structural soluble carbohydrates of moringa seedling for different plant organs: Leaf, stem, root and seed (Sugars are in series of: Raffinose-Sucrose-Glucose-Fructose).

in anti-oxidant while *M. oleifera* contains the highest concentrations of highly desirable food nutrient values among other species of the plant (Ray-Yu et al., 2006). The agro-climatic conditions in most sub-Saharan

countries are favorable for the growth of moringa plants while most population in the sub-Saharan countries is currently being affected with protein malnutrition. Moringa with high concentrations of anti-oxidants and other

**Table 2.** ESEM nutrient analysis of moringa (*Moringa oleifera*) seedling on different tissues.

Plant nutrients	Leaf	Stem	Root	Seed	Seed coat
	mg g <sup>-1</sup> w/w				
Magnesium (Mg)	5.65	7.11	4.62	5.95	10.10
Phosphorous (P)	20.63	nd	6.54	20.47	7.30
Sulfur (S)	47.40	13.91	36.40	46.43	16.73
Potassium (K)	21.62	49.32	54.38	22.38	40.00
Calcium (Ca)	4.67	19.82	14.12	4.04	19.00

nd-Not detected.

essential nutritive values would partly play a key role in improving the food security in the region.

The moringa leaf was reported as the main source of nutrition as it contains numerous nutritional anti-oxidant compounds in substantial amount, such as ascorbic acid, carotenoids,  $\alpha$ -tocopherol, phenols (Perry et al., 1999). The result of the present study also confirms this finding. The leaf had high concentration of anti-oxidants, proteins and nutrients (Sreelatha and Padma, 2009). TAOC differed significantly ( $P \leq 0.05$ ) between plant parts. In present study, leaf recorded the highest concentration of AsA, TP as well as TAOC (Figures 2 and 3). There were positive correlations among three components: AsA, TP and TAOC. Due to high concentration of these beneficial compounds, they are regarded as the best, nutritionally rich plant that can be consumed for a long time over a period of growth and development where food crops are scarce. Leaves can be further processed into powder final products and used as one of the ingredient in improving traditional foods or to develop new innovative food products by considering socio-cultural factors in the regions. Leaves can also be eaten fresh, cooked, or stored as freeze-dried powder for many months without refrigeration, and reportedly without significant loss of nutritional value. The consumption of moringa leaf powder is also crucial in situations where starvation is imminent (Mahmood et al., 2010). Moringa plants can be grown during the rainy season and can be dried using appropriate low-cost drying technology and then can be preserved under ambient conditions once packaged in a proper packaging material. The present data then clearly showed that different parts of the plant can be used in the process of food security in Sub-Saharan countries. In view of this, there is a huge potential for dried products from moringa plant parts to be used as one of the ingredient in different functional food product development. In addition, moringa leaves satisfies the definition of functional foods as it is known to have curing effect on some human diseases (Lipipun et al., 2003; Ashok et al., 2003). Similarly, Brett and Pharm (2005) indicated that the addition of moringa into the daily diet shows considerable promise as an adjunct to improving health in a variety of important ways.

It has also been reported that moringa leaves contain more concentration of vitamin A than the concentration of

vitamin A in carrots, more calcium level than the level of calcium in milk, more iron than the amount that has been found in spinach, more vitamin C than concentration of vitamin C in oranges, and more potassium than the concentration in bananas, and that the protein quality of leaves rivals that of milk and eggs (Fuglie, 1999). Eating moringa food products is good for those suffering from malnutrition due to the high protein and fibre content (Lockett et al., 2000; Fahey, 2005). The high concentrations of the micro-nutrients found in the moringa leaves including calcium and protein (Fahey, 2005), provides a huge potential for this product to be used to embark upon reducing malnutrition in sub-Saharan countries where this plants can easily grow.

The seedling stems and stem-barks can also be consumed as fresh as well as dried-powder for the searching of the nutritional compounds that are produced in the leaf. The seedling stem was found to contain higher concentration of TAOC which was found to be second to leaf (Figure 2A) with respect to the concentration of TAOC. In most cases, they are harvested in the form of stem barks from moringa trees. The barks are reported to have different nutritional values as well as medicinal values. As a medicinal plant, the bark are used for treating different diseases, these properties are linked as they have strong anti-oxidant capacity, contain various types of anti-oxidant compounds, this eventually contributes to the plant anti-oxidant pool.

Roots had highest concentrations of total carotenoids, and also recorded high concentrations of AsA and TP, following the leaf (Figure 2). The roots also have numerous medicinal purpose, they are known to have antibacterial activities. The roots of moringa contain high levels of fiber, protein, vitamins and minerals. The harvested roots from the trees serve a wide variety of purposes (Fahey, 2005).

Seed produced significantly highest concentration of  $\alpha$ -tocopherol followed by TP (Figures 2 and 3). The phenols concentration of different forms was not significant, except that the seed membrane-bound phenols concentration was predominantly produced over the free ones. This result supports the speculation that proves the vitamin E are dominantly produced in the seed as oil source and has further function of cell protection. The



seeds are harvested for different purposes, mainly for commercial and industrial purposes. They are used for extraction of edible oil, known for its stability from oxidation; this could be aligned to high anti-oxidant concentration in the seed (Fahey, 2005). In this case, toco-pherol as well as TP might have a strong impact in stabilizing ROS, avoids excessive accumulation of damaging oxidants and extends the storability of moringa oil as the result.

Moringa seed oil (yield 30 to 40% by weight), also known as Ben oil, is a sweet non-sticking, non-drying oil that resists rancidity. It has been used in salads, for fine machine lubrication, and in the manufacture of perfume and hair care products (Tsaknis et al., 1999). In the West, one of the best known uses is the use of powdered seeds to flocculate contaminants and purify drinking water (Berger et al., 1984), but the seeds are also eaten green, roasted, powdered and steeped for tea or used in curries (Gassenschmidt et al., 1995). Recently it has drawn a significant interest; the moringa seeds can also be used as a potential material in production of biodiesel.

## Conclusion

The outcome of this experiment revealed that different parts of moringa contain different compounds: such as anti-oxidants, beneficial nutrients, carbohydrates, and their composition in bio-active elements thus, making the tree a potential plant in minimizing malnutrition as well as source of household income in developing countries. Overall, it could be concluded that moringa tree contains different potent anti-oxidant compounds and their precursors. Every part of the tree has different anti-oxidant potential, and becomes nutritious if they are eaten as a staple food. Leaves are one of the main organs that can be used for human consumption over long seasons. And the significant accumulation of vitamin E in the seed also implies seed characteristics as oil crop. Therefore, the moringa tree as a multi-purpose plant warrants a special attention for further investigation.

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