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COMPARATIVE PHYTOCHEMICAL ANALYSIS OF *MORINGA OLEIFERA* AND *MORINGA PEREGRINA*

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Abstract

Moringa peregrina and *Moringa oleifera* are used in folk medicine and grows widely in the tropical and subtropical regions of Africa and India. The objectives of this investigation were to determine the phytochemical composition and nutritive contents of *Moringa peregrina* and *Moringa oleifera* extracts from the leaves and fruits. The powdered plant samples were examined separately for their phytochemical constituents like total phenol content, micro and macro elements using appropriate methods. They were also subjected to proximate analysis such as ash content, dry matter and crude protein. All the elements content were determined by using ICP AES. According to the nutritional values obtained, the phytochemical and proximate analysis results showed the high percentage in *M. oleifera.* There was no significant difference in dry matter content between two species. The ash content, total phenol content, protein, macro elements (Ca, K, Mg, Na, P) and micro elements (Cu, Co, Fe, Mn, Zn) were higher in *M. oleifera* when compared to *M. peregrina*. The extracts from both plants showed the presence of active phytochemicals may provide substantial basis for the use of these plants in ethaomedicine. Their nutritional properties in different parts may provide incentive for proper evaluation of these plants as dietary food as well as medicinal agents essential for human health. Further studies are required to ascertain this conclusion, which are underway in our lab.

Keywords: Phytochemicals, *Moringa peregrina, Moringa oleifera,* proximate analysis

Introduction

Throughout the world, plants have been a rich source of nutrients and antioxidants as they contain lot of bioactive molecules and compounds. Out of the bioactive molecules, most of them are as produced as chemical defense against stresses or infections. Native plants usage in traditional as well as modern medicine is gaining a lot of attention now a days, and the recent studies showed that a number of plant products and herb extracts exert potent antioxidant actions (1). *Moringa* species are one of the most useful trees in the tropics and subtropics of Asia and Africa, with multiple uses. *Moringa peregrina* is native to the region extending from the DeadSea to Southern Arabia and northern Somalia (2). In the past, *M. peregrina* was both an indigenous and a cultivated tree in Sudan (3). *M. peregrina* leave is an excellent source of protein, minerals and essential amino acids (4). These species are the most widely cultivated of the Moringaceae family and was utilized by the ancient Egyptians, southern Arabia and northern Somalia. *M. Oleifera* and *M. peregrina* are the best known and utilized species (5). Traditionally, almost all parts of *Moringa* flower, fruits and roots are edible, and have long been consumed as vegetable and used to treat many diseases such as abdominal tumors, hysteria, scurvy, paralytic attacks, helminthic bladder, prostate troubles, sores and skin infections (6).

Drumstick (*Moringa oleifera*, Family: Moringaceae) is a native Indian medicinal plant that grows widely in the tropics and subtropics of Asia and Africa. *M. oleifera* was used as a nutritional supplement and remained popular among the lower socio-economic class (7). It has been reported as the significant sources of vitamins (A, B, C, E, riboflavin, nicotinic acid, folic acid, pyridoxine, ascorbic acid, and beta carotene), iron, calcium and alpha tocopherol (8). *M. oleifera* has been investigated for its fast growth, high nutritional attributes, and utilization as a livestock fodder crop. It can be grown as a crop on marginal lands with high temperatures and low water availability, where cultivation of other agricultural crops is difficult (9). The leaves are highly nutritious, which contain more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than oranges and more potassium than bananas and more protein than milk and eggs (10). The leaves of *M. oleifera* extract also exhibited strong anticancer potential (10). The hydro-ethanolic extract of *M. oleifera* leaves prophylactically and therapeutically protects against acetaminopheninduced hepatotoxicity in experimental rats through their antioxidant nature (11).

Many native fruits and Vegetables are the cheapest and most available sources of important nutrients, supplying the body with minerals salts, vitamins and certain hormone precursors, protein, energy and essential amino acids (12). Hence, proximate and nutrient analysis of wild edible plants plays a crucial role in assessing their nutritional significance (13). Until now a full depiction of the extract the leaves and fruit of *M. peregrine* has not been reported. Moreover, the use of different methods of phytochemical and proximate analysis has not been studied. Hence in this study we aimed to compare the phytochemical and proximate composition of *M. peregrina* with cultivated species of *M. oleifera* plant parts.

Materials and Methods

Plant Material

The fully matured fruits and leaves of *Moringa peregrina* and *Moringa oleifera* samples were collected from Al-Foah Experimental Station of College of Food and Agriculture, UAEU. The plants were identified and authenticated at UAEU. The fresh plant leaves were washed thoroughly and carefully with distilled water and air dried for 7 days.

Preparation of extracts

The parts of plants were dried in the shade and powdered so that all the material could passed through a mesh not larger than 0.5 mm. The powdered materials of each plant species (1000 g) were soaked in 3L of methanol (Sigma-Aldrich, USA) for 1 day, and the steps were repeated twice, followed by Soxhlet extraction by using methanol for 72 hrs. At the end of extraction, it was passed through Whatman filter paper No.1 (Whatman Ltd., England). The extract was concentrated to dryness under vacuum on rotary evaporator at 40°C then stored at 4°C for further use (1).

Total Phenol

Quantification of total phenolic content in ethanolic plant extract was carried out according to the method of Slinkard & Singleton (14) with slight modification (14a). The ethanolic plant extract powder will be dissolved in 25% ethanol (v/v) to obtain concentration of 0.5% (w/v). The solution (0.5

mL) was added to 100 µL of Folin–Ciocalteu reagent (two-fold diluted with de-ionized water) and mixed thoroughly. After 3 min, 1.5 mL of 2% sodium carbonate solution was added. The reaction mixture was mixed thoroughly and placed in the dark for 40 min and the absorbance was read at 760 nm. The total phenolic content was calculated from the standard curve of tannic acid (0–0.1 mg/mL) and expressed as mg tannic acid per gram of dry ethanolic plant extract after blank substraction. Blank was prepared in the same manner, except that distilled water was used instead of Folin–Ciocalteu reagent.

Dry matter

This was determined by the standard methods of the Association of Official Analytical Chemists (15). 1g of sample was weighed into a clean dried porcelain evaporating dish. This was placed on an oven and the temperature maintained at 105°C for 6 hrs. The evaporating dish was cooled in a desiccator to room temperature then reweighed and recorded. Weight of moisture was calculated by subtracting the weight of dried sample from the fresh as follows:

%dry matter = fresh weight-dried weight/weight of fresh sample x 100/1

Ash content

The percentage composition of ash was estimated by the furnace method. 20g of the sample was weighed into a preheated and weighed porcelain crucible. The crucible was inserted into a furnace and regulated to a temperature of 630°C and heated for 3hrs. The set up was then allowed to cool to room temperature and weighed again. Percentage composition of ash was then obtained as follows:

%Ash = (weight of crucible + ash –weight of crucible)/weight of sample x100/1

Protein

Total nitrogen contents of samples was determined using an Elemental Analyzer (Vario MICRO Cube, Elementar Analysensysteme GmbH, Donaustraße, Germany), by mean of ignition of 50 mg samples. Sulfanilamide, the manufacturer recommended calibration standard, was run to compute the daily factor. The amount of Crude protein was estimated by multiplying the sample.

percent nitrogen content by a factor 6.25

%Protein = % Nitrogen × 6.25

Microelements and Macroelements

Samples were prepared accurately by weighing 0.5 grams of sample into the microwave digestion vessels and 10 ml of concentrated nitric acid (HNO3) and 2 ml hydrochloric acid (HCL) were added (Method 3015A, US Environmental Protection Agency, 2008). The vessels were capped and place in the microwave digestion system. The analysis was conducted using ICP-OES - Agilent Technologies, 710.

Results and Discussion

As shown in Figure 1 the amount of total phenol is higher in *M. oleifera* fruit extract (MOFE) and is 1.474 when compared to *M. Oleifera* leaf extract (MOLE), *M. peregrina* fruit extract (MPFE) and *M. peregrina* leaf extract (MPLE). The dry matter seems to be approximately equal. The proximate analysis showed the dry content of MOLE to be 98.275 and 97.46 in MOLF and more when compared to MPLE content 98.4 and 97.76 in MPFE. The results of physico-chemical analysis of plant ash are given in Figure 1. The Ash content is higher in leaf rather than fruit in both samples. Percentage of loss on drying was highest in MOLE followed by MPLE, MOFE and MPFE. All the samples were found alkaline in pH. Ash content is generally taken to be a measure of the mineral content of the original food (16). Natural food products should have a general ash content of about 5% while processed food can have ash content ranging over 10%. This study shows that *Moringa* species have acceptable levels of ash content as natural food products. According to A.O.A.C (15), mineral contents are usually needed in small proportions. This is because high ash content signifies low food quality while low ash content signifies high food quality.

The protein content in *M. oleifera* was higher than *M. peregrina*. The crude protein of MOLE was 13.69 and MOFE 16.88 respectively with MPLE content of 8.22 and MPFE of 12.07. The high level protein content *M. oleifera* is a further confirmation of its use as vegetable. Protein which would serve as enzymatic catalyst, mediate cell responses, control growth and cell differentiation (17). For most of the studies the protein content are considered as the main determinants of food type and less is known about elemental composition of various wild edible species (18). The recommended dietary allowance (RDA) for protein is 56g for individual weighing 70kg

and 46g for adult weighing 50kg, children may consume 2kg/day (19).

The micronutrient and macronutrients analysis of the *Moringa* species showed variation among different elements. As shown in table 1, the content of microelements in both species and nutritional significance of elements were high when compared with the standard recommended dietary allowance. According to the nutritional value obtained the presence of macro elements (Ca, K, Mg, Na, P) and micro elements (Cu, Co, Fe, Mn, Zn) in MPFE, MPLE shows higher amount when compared to MPFE and MPLE. Especially in *M. oleifera* reveals the Ca, Co is higher in leaves and K, Cu is higher in fruit. Copper is a very powerful pro-oxidant and catalyzes the oxidation of unsaturated fats and oils as well as ascorbic acid. The copper content of MOLE is 4.441 and 5.695 in MOFE which is higher than 2.040 and 3.288 respectively for MPLE and MPFE. The zinc content of MOLE was 26.65 and 19.20 mg/kg in MOFE. The Recommended Dietary Allowance (RDA) for zinc is 13mg/kg.

Increased contents of flavonoids may help in providing oxidative stress protection by contributing along with other antioxidant vitamins, and enzyme to the total antioxidative defense system of the human body. Many studies have attributed that antioxidant properties are due to the presence of flavonoids (20). Moisture content determination is one of the most fundamental and important analytical procedure. According to Yisa et al. (21) high moisture content increases perishability as the fruits are more susceptible to microbial infections. This result indicated low shelf life of the fresh plant hence extended storage would lead to spoilage due to susceptibility to microbial attack. This supports the practice of storage in dry form by users. Moisture content is among the most vital and mostly used measurement in the processing, preservation and storage of food (16).

Sodium and potassium are important intracellular and extracellular cations respectively. Sodium is involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction (22). Zinc is required for the proper functioning of the reproductive system was found to be present in the high amount in *M. oleifera* when compare to *M. peregrine* (23). Low sodium diet has been reported to be beneficial in the prevention of high blood pressure and high potassium has been reported to have a protective effect against excessive sodium intake (24). Calcium is good for growth and maintenance of bones, teeth and muscles (25).

Copper is a very powerful pro-oxidant and catalyzes the oxidation of unsaturated fats and oils as well as ascorbic acid. The content of copper was very high in *Moringa* species. Copper is very vital in diet because it is involved in the proper usage of iron (Fe) and especially for the synthesis of cytochrome oxidase, which contains both iron (Fe) and copper (Cu).

Excess copper can lead to jaundice (Wilson's disease) (26). Zinc is essential in the activation of certain enzymes. These include dehydrogenase, alkaline phosphatase and carboxypeptidase. Zinc containing organic compounds is employed as astringent and anti-fungal agents. It aids wound healing and metabolism of nucleic acid and insulin. Zinc in excess causes anemia and if deficient in the body can lead to dermatitis.

The iron content of MOLE was 483.58 and 211.51 mg/kg in MPLE. According to Bolt et al. (27), the recommended daily requirement of iron for man is 6 – 40mg/kg. Iron is very important in the formation of haemoglobin in red blood cells and deficiency of iron leads to anaemia.

Energy and nutrient values of medicinal plant samples are mainly used to translate medicinal samples intakes as intakes of food components. Thus, the use of *Moringa* species leaves and fruits in our diet could help boasting in blood level especially in anemic conditions (28).

Phytochemical properties are the high levels of phenolic compounds found in plants, as antioxidants which are considered to be beneficial to human health. Our findings showed that leaves of *Moringa* species are very rich in nutrients and chemical substances which offer great potential for food and pharmaceutical companies. This study proved the edible wild plants as rich sources of dietary supplements and they contain essential nutrients like protein, mineral, moisture and ash needed by the human body (and animals) for proper growth and maintenance. Because it is frequently prescribed by non-professionals and taken concomitantly with conventional medicine, it poses a potential risk for herb drug interactions.The result highlighted significance of wild edible plant as a cheap source of nutrient for the rural poor.

Nutritional values of wild plant foods are of considerable significance, as they help to pinpoint traditional food resources of tribals. Due to lack of awareness and negative approach towards the wild food plants, it is important to create community awareness to accept wild food plants as useful as the cultivated ones. It may be concluded that promotion

of consumption and processing of these plant species, to various products, may help to improve the diet and alleviate nutrient deficiencies. Its conservation is therefore very important for further studies on its medicinal and other benefits. Lastly, more information on the phytochemical analysis of the plants with medicinal importance to determine their relevance as raw materials for drug manufacture should be made. In addition, further studies are needed to determine, isolate and characterize the structure of the bioactive compounds from the leaves and fruit of *Moringa* species which are underway in our lab.

Author Contributions: SAA & FYS conducted the experiment as part of their Senior Project under the supervision of AJC at College of Food and Agriculture, UAEU. AIA assisted in lab works. KK assisted in preparation of manuscript. MASY & SSK assisted in overall planning of experiment and manuscript review.

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Figure 1. Pytochemical and Proximate composition of *Moringa* species. MOFE- *M. oleifera* fruit extract, MOLE - *M. Oleifera* leaf extract, MPFE - *M. peregrina* fruit extract MPLE - *M. peregrina* leaf extract.

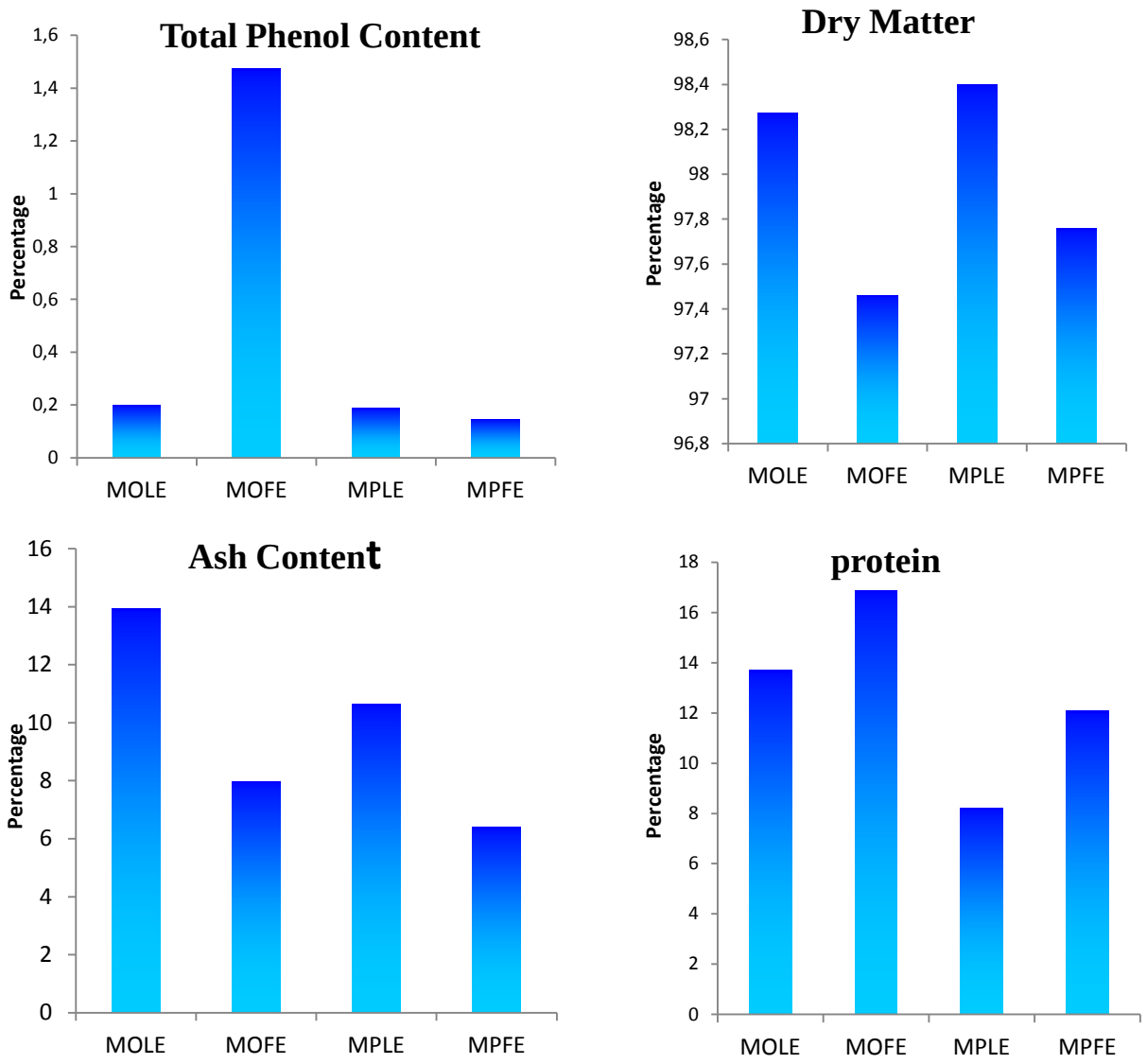

Table 1. Mineral composition of Moringa species. MOFE- *M. oleifera* fruit extract, MOLE - *M. Oleifera* leaf extract, MPFE - *M. peregrina* fruit extract MPLE - *M. peregrina* leaf extract.

Sample ID	Macroelements (%) mg/Kg (ppm)						Microelements (%) mg/Kg (ppm)				
	Ca	K	Mg	Na	S	P	Cu	Co	Fe	Mn	Zn
MOLE	3.341	0.955	0.762	0.273	0.452	0.208	4.441	0.684	483.58	53.79	26.65
MOFE	0.283	2.047	0.263	0.052	0.723	0.390	5.695	<0.003	77.13	12.44	19.20
MPLE	1.640	0.809	1.193	0.463	1.119	0.231	2.040	<0.003	211.51	25.00	11.74
MPFE	0.296	1.520	0.303	0.067	0.694	0.254	3.288	<0.003	58.02	8.68	18.25