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Full Length Research Paper

Phytochemical, Proximate Composition and Minerals contents of *Moringa Oleifera*

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The aim of the present study was to evaluate the proximate composition, phytochemicals, and minerals contents of Moringa oleifera seeds , family Moringaceae. The proximate analysis reveal the dry matter 94.60%, ash content 2.13%, crude protein 58.32% , oil 11.3% , crude fiber 4.75% and total carbohydrate content is 23.5% . The phytochemicals analysis of Moringa seeds in this study are phytic acid (351.12 mg/100g), tannin (0.13 mg/100g) and total polyphenols (629.70 mg/100g) . The minerals content are Ca (42.13 mg/100g), phosphorus (1311.20 mg/100g) and iron (55.98 mg/100g).

Keywords: *Moringa seeds, Proximate Composition, Photochemical analysis, Minerals content*.

INTRODUCTION

Moringa oleifera is one of the species of family Moringaceae, native to, Africa, Arabia, South Asia, South America, Himalaya region, India, Pakistan, the pacific and Caribbean Islands. *Moringa oleifera* has been naturalized in many tropic and subtropics regions worldwide, the plant is referred to number of names such as horseradish tree, drumstick tree, ben oil tree, miracle tree, and “Mothers best friend” (Julia coppin, 2008). *Moringa oleifera* is commonly known as “Drumstick”. It is a small or medium sized tree, about 10m height, found in the sub-Himalayan tract (Trupti Rastogi *et al*., 2009). *Moringa oleifera* is a small, fast-growing evergreen or deciduous tree that usually grows up to 10 to 12m in its height, open crown of drooping fragile branches, feathery foliage of trip innate leaves and thick corky, whitish bark (Roloff A, 2009).The Moringa oleifera plant provides a rich and rare combination of zeatin, quercetin, kaempferom and many other phytochemicals (Pal K *et al*, 1995). *Moringa oleifera* leaves as rich in protein source, which can be used by doctors, nutritionists and community health cautious persons to solve worldwide malnutrition or under nutrition problems Thurber and Fahey, (2009).Some articles and research studies have reported that the dry leaves of *M. oleifera* contain 7 times more vitamin C than orange, 10 times vitamin A than carrot, 17 times calcium than milk, 15 times potassium than bananas, 25 times iron than spinach and 9 times proteins than yogurt (Fuglie, 1999). Numerous studies now point to the elevation of a variety of detoxication and antioxidant enzymes and biomarkers as a result of treatment with moringa or with phytochemicals isolated from *moringa* have shown, antiulcer, effect on immune response, spasmolytic activities, hypercholesterolemia effects, antibacterial activity. *Moringa oleifera* was claimed to boost immune systems (Olugbemi *et al.,* 2010). Apart from the medicinal uses, *Moringa oleifera* was reported to be a good source of vitamins and amino acids (Olugbemi *et al*;2010). *M. oleifera* is a miracle tree with a great indigenous source of highly digestible proteins, Ca, Fe and Vitamin C. It contains all the essential nutritional elements that are essential for livestock and human beings (Fahey, 2005). *Moringa* seeds contain between 30 –

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42% oil and press cake obtained as a by-product of the oil extraction process contains a very high level of protein, some of these protein (approximately 1%) are active cationic polyelectrolyte having molecular weights between 7 – 17 K Dalton . this protein can therefore be used as a non – toxic natural polypeptide.

MATERIALS AND METHODS

Materials

The *Moringa* seeds was cultivated at my home, Sudan- Khartoum state (Abo-Adm), then dried in a hot air oven at 75℃ for 2-3 hours and ground to fine particles using house blender and mortar to passed through 0.4mm and stored in polyethylene bags at 4℃ for further use. All chemicals used in this study were of reagent grade.

Proximate analysis:

Moisture content:

Moisture was determined by the AOAC method (2000). Two grams of well-mixed samples were weighed accurately using sensitive balance in a clean dry and pre-weighed crucible and then placed in an oven at 105^0C. The crucible was transferred to a desiccator and allowed to cool and then weighed. Additional placements in the oven were carried out until a constant weight was obtained. Moisture was calculated using the following formula:

$$\textbf{MC (\%)} = \frac{(W2 - W1) - (W1 - W3)}{(W2 - W1)} \times 100$$

Where:
MC = Moisture content

W_1 = Weight of empty crucible

W_2 = Weight of crucible with sample

W_3 = Weight of crucible with dry sample

Ash content determination:

Ash content of the sample was determined according to the method of AOAC(2000). Two grams of sample were placed in a clean dry pre- weighed crucible, and then the crucible with it is content ignited in a muffle furnace at 550℃ for 3 hours or more until light grey ash was obtained. The crucible removed from the furnace to a desicator to cool and then weighed. Ash content was calculated using the following equation:

$$\textbf{AC} = \frac{W2 - W1}{W3} \times 100$$

Where:

AC = Ash content

W_1 = Weight of empty crucible

W_2 = Weight of crucible with ash

W_3 = Weight of sample

Crude protein determination:

The crude protein was determined by using the micro-kjeldahl method according to AAOC(2000) as follows:

Digestion:

About 0.2 gram of the sample was weighed and placed in small digestion flask (250ml). Two catalysts tablets (anhydrous sodium sulphate + copper sulphate) were added to the sample, 3.5 ml of approximately 98%H_2SO_4wasadded. The content of the flask was then heated on electrical heater for two hour till the colour changed to blue – green. The tubes were then removed from digester and allowed to cool.

Distillation:

The digested sample was transferred to the distillation unit and 15 ml of 40% NaOH were added. The ammonia was received in 100ml conical flaskcontaining 10 ml of boric acid plus 3-4 dropps of methyle red indicator. The distillation was continued until the volume reached 50ml.

Titration:

The content of the flask were titrated against 0.02N HCl. The titration reading was recorded. The crude protein was calculated using the following equation:

$$CP\% = \frac{(T - B) \times N \times 14 \times 100 \times 6.25}{WS \times 1000}$$

Where:

CP = Crude protein
T = Titration reading
B = Blank titration reading
N = Normality of HCl
WS = Weight of sample
1000 = To convert to mg
6.25 = Protein factor

Fat content determination:

Fat content was determined according to the method of AOAC (2000) using Soxhlet apparatus. An empty clean and dry extraction round bottomed flask was weighed. About two gram of sample was weighed and placed in a clean extraction thimble and covered with cotton wool. The thimble was placed in extractor. Extraction was carried out for 8hours with petroleum either. The heat was regulated to obtain at least 15 siphoning per hour. The residual ether was dried by evaporation. The flask was placed in an oven at 105℃ till it dried completely and then cooled in a desicator and weighed. The fat content was calculated using the following equation:

$$FC = \frac{W2-W1}{W3} \times 100$$

Where:

FC= Fat content
W1= Weight of extraction flask
W2= Weight of extraction flask with fat
W3= Weight of sample

Crude fiber determination: Crude fiber was determined according to AOAC (2000). Two grams of defatted sample were treated successively with boiling solution of $H_2SO_4$of 0.26 N and KOH of 0.23 N. The residue was then separated by filtration, washed and transferred into a crucible then placed into an oven adjusted to 105℃

for 18-24 hours . The crucible with the sample was weighed and ashed in a muffle furnace at 500℃ and weighed . The crude fiber was calculated using the following equation:

$$\mathbf{FC} = \frac{\mathbf{W2} - \mathbf{W1}}{\mathbf{W3}} \times 100$$

Where:

CF= Crude fiber

W1= Weight of crucible with sample before ashing

W2= Weight of crucible with sample after ashing

W3= Weight of sample

Determination of Carbohydrates:

Carbohydrates were determined by difference. The sum of moisture, fat, protein and ash contents was subtracted from 100 to obtain the total carbohydrates by difference (Pearson, 1976).

Carbohydrates = 100 - (Ash % + moisture % + CP % + oil % + fiber %).

Phytochemical analysis:

Determination of tannin content:

Quantitative estimation of tannins was carried out using the modified vanillin – HCl methanol, according to Price *et al*, (1978). The vanillin HCl reagent was prepared by mixing equal volumes of 8% concentrated HCl in methanol and 1% vanillin in methanol, the two solvents of the reagent were mixed just prior to use, it was discarded if a trace of colour appeared.Catechin was used as reference standard.

Determination of phytic acid:

The physic acid content was determined according to the method of Wheeler and Ferrel (1971).Tow grams of finely ground samples were weighed in 50 ml tube. The sample was extracted with 50 ml of 3% trichloroacetic acid (TCA) for 3 hr with shaking. The suspension was centrifuged for 5 min . at 2500 rpm.Ten milliliters aliquot of the supernatant were transferred to 50 ml tube, 4 ml $FeCl_3$ (Solution containing 2 mg Fe^{+3} iron/ml 3% TCA were added to the aliquot by blowing rapidly from the pipette. The tubeand contents was heated in a boiling water bath for 45 min. One or two drops of 3% Na_2SO_4 in 3% TCA were added to develop a precipitate. Then tube was cooled and centrifuged for 10-15 min at 2500 rpm. The clear supernatant was decanted and the precipitate was washed twice by dispersing well in20- 25 ml 3% TCA, and heated for 10-15 min in boiling water bath, then cooled and centrifuged. The precipitate was washed one or two times with distilled water, and was dispersed in a few ml of distilled water. Three milliliters of 1.5N NaOH were then added and the volume completed to 30 ml with distilled water. The tube was heated in a boiling water bath for 30 min, and hot filtered using Whatman No. 1 filter paper. The precipitate was washed with hot 60-70 ml of distilled water and the washings were decanted. The precipitate was dissolved from the filter paper with 40 ml hot 3.2N HNO_3 into 100 ml volumetric flask and the paper was washed again with a hot distilled water in the same flask and completed to the volume with distilled water. A volume of the above suspension was transferred into 10ml volumetric flask. Two milliliters of 1.5NKSCN (potassium thiocyanate) were added and completed the volume with distilled water, then immediately (within one minute) the absorbance was read using spectrophotometer (JENWAY 6305 UV) at 480 nm.

A standard curve of different Fe $(NO_3)_3$ concentrations was plotted to calculate the ferric iron concentration. The phytate phosphorus was calculated from the ferric iron concentration assuming 4:6 iron: phosphorus molar ratio.

Calculation:

$$\text{Phytate (mg/ 100g)} = \frac{6}{4} \times \frac{A \times C \times 20 \times 10 \times 50 \times 100}{1000 \times 2}$$

A= Optical density

C= concentration corresponding to the optical density

Total Polyphenols determination:

Total Polyphenols were determined by use of Purssion bluespectrophotometric method (Price and Bulter, 1977). Sixty mg of sample were shaken manually for sixty second with 3 ml of absolute methanol in a test tube. The mixture was filtered, then the tube quickly rinsed with 3 ml of methanol and the contents poured at once into the funnel. The filtrate was mixed with 50 ml of distilled waterand analyzed within one hour. Three ml of 0.1M $FeCl_3$ in 0.1N HCl were added to 1 ml of filtrate, followed immediately by timed addition of 3ml of0.008M $K_3Fe(CN)_6$. The absorbance was read on spectrophotometer (Jenway 6306 uv/vis spectrophotometer) at 720nm after 10 min. Tannic acid was used

to make the standard curve following the same steps in the procedure above. The polyphenol content was calculated as follows:

Total polyphenol% (Tannic equivalent) $=\frac{C\times 56\times 100}{60}$

C= Concentration corresponding to optical density

56= Volume of extract.

60= Weight of sample

Minerals determination :

Minerals of samples were extracted according to the dry-ashing method
as described by Pearson (1981). Two grams of each sample were placed in
Porcelain dish and burnt inmuffle furnace at 550°Cand placed in the a sand bath for 10 minutes after addition of 5 N HCl . Then the solution was carefully filtered in a 100ml volumetric flask and finally distilled water was added up to mark. From this extract, the elements calcium and iron were determined using Perkin Elmer Atomic Absorption Spectroscopy.

Phosphorus determination:

The phosphorus for both total and HCl-extractable were carried out according to the method of Champman and Pratt (1982). Five ml of the ash extract were pipetted in to a 50 ml volumetric flask. Ten ml of the ammonium molybdate ammonium vanadate reagent (22.5g) of (NH_4) MO_7 O_{24} $4H_2O$ in 400ml distilled water + 1.25 gram ammonium vanadate in 300 ml boiling water + 250 ml concentrated HNO_3 then diluted to liter) were added.The content in the flask were mixed and diluted to the volume. The density of the color was read after 30 minutes at 470 nm suing spectrophotometer (JENWAY 6305 UV/ Vis). Phosphorus was determined from the standard curve.

Calculation:

P (mg/ 100 g) $=\frac{mg/L\times volume\times 100}{1000\times Wt}$

Where:

Mg/ L = ppm (curve reading)

Volume used = volume of extract

1000 = conversion form mg/ L to mg /m

Wt. of sample = 2 grams

Statistical Analysis

All data were subjected to statistical analysis, each determination was carried out and analyzed in triplicate then averaged. Data was assessed by the analysis of Variance (ANOVA) Gomez and Gomez (1984).

RESULTS AND DISCUTION

Proximate composition of defatted moringa seeds :

The proximate compositions of moringa seeds are presented in Table 1. The dry matter of moringa seeds (MS) was 94.60% which was higher than that reported by Abdulkarim *et al*., (2005) who reported 92.20%. The ash content of MS was 2.13% which was lower than 6.5% ash content of whole moringa seeds reported by Abdulkarim *et al*., (2005) .The protein content of (MS) was 58.32% which was higher than the value 38.30% reported by Abdulkarim *et al*., (2005). The oil content of (MS) was 11.3%. The crude fiber content of (MS) was 4.75% which was slightly higher than that reported by Abdulkarim *et al*., (2005) who reported 4.5% .The carbohydrate content of (MS) was 23.5% which was greatly higher than the value 16.50% of carbohydrate moringa seeds , reported by Abdulkarim *et al*., (2005.

Table (1): Proximate composition of defatted moringa seeds :

Parameter (%)	Dry matter	Ash content	Crude protein	Oil content	Crude fibre	Carbohydrate
Moringa seeds	94.60 (±0.05)	2.13 (±0.02)	58.32 (±0.32)	11.3 (±0.17)	4.75 (±0.25)	23.5 (±0.29)

Values are means(±SD) of three replicates.

Phytochemical analysis (Phytic acid , Tannins and Total Polyphenols):

The Phytochemicals of moringa seeds are shown in Table 2. The phytic acid content of moringa seed (MS) was found to be 351.12 mg/100g which was lower than the value 1018mg/100g phytic acid reported by Anhwange *et al*., (2004. The tannin content of (MS) was 0.13% which was significantly lower than that reported by Anwange *et al*., (2004) who reported 2.13%. The total polyphenol of (MS) was 629.7mg/100g.

Table (2): Phytochemicals (Phytic acid , Tannin and Total Polyphenols)

Of defatted Moringa seeds

Sample	Phytic acid	Tannin (%)	Total polyphenols
moringa seeds	351.12	0.13	629.7

	(±3.14)	(±0.002)	(±2.25)

Values are means (±SD) of three replicates.

Total minerals contents of defatted moringa seeds.

The total minerals contents moringa seed are shown in Table 3. Calcium content of moringa seeds was 42.13 mg/100g, while phosphorus was found to be 1311.20 mg/100g and iron content was 55.98 mg/100g.

Table (3): Total (mg /100g) minerals contents of defatted moringa seeds :

Samples	Calcium00	Phosphorus	Iron
Defatted moringa seeds	42.13 (±0.10)	1311.20 (±0.01)	55.98 (±0.13)

Values are means (±SD) of three replicates.

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