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Research Article

Characterization of Polyphenols, Polysaccharides by HPLC and Their Antioxidant, Antimicrobial and Antiinflammatory Activities of Defatted Moringa (*Moringa oleifera* L.) Meal Extract

Alaa A Gaafar[1*], Eman A Ibrahim[1], Mohsen S Asker[2], Ahmed F Moustafa[3], Zeinab A Salama[1]

[1]*Plant Biochemistry Department, National Research Centre, P.O. Box 12622, 33-El Behouth St. Dokki-Giza-Egypt.*
[2]*Microbial Biotechnology Department, National Research Centre, P.O. Box 12622, 33-El Behouth St. Dokki-Giza-Egypt.*
[3]*Environmental Impact Assessment Unit & Environmental monitoring laboratory Unit Beni-Suef Governorate, Egypt*

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ABSTRACT
This study describes some active substances of hot and cold water extract from defatted cake *Moringa oleifera* seed. Phenolic (TP), flavonoids (TF), tannins (TT) compounds, and polysaccharide and their antioxidants, antimicrobial and anti-inflammatory activities were examined. The results showed that total phenolics of 15.76, 15.01 mg/g DW, flavonoid 3.11, 3.09 mg/g DW and tannins 4.99, 5.48 mg/g DW for cold and hot water extract respectively. According to the phytochemical assay, defatted cake *Moringa oleifera* showed the highest antioxidant activity, which could be related with their contents of phenolic compounds. The antimicrobial assay was examined. The results show that *Moringa oleifera* polysaccharide extract exhibited broad spectrum activity against the test organisms. Seven phenolic compounds (ellagic, tannic, coumarin, benzoic, catechin, and caffiec) were identified and quantified in both extracts. The polysaccharide were analyzed sugars by HPLC. Maltose, raffinose and lactose were the most abundant sugars. Profiles of 15 different natural mono sugars are identified. The results demonstrated that, defatted cake *Moringa oleifera* seed concentrate is an exceptionally encouraging of bioactive constituents. Therefore, it could be concluded that polyphenols present in the *Moringa oleifera* extract may be responsible for the antioxidant, antimicrobial and anti-inflammatory activities which could be a good source of antioxidants, antimicrobial and anti-inflammatory for food and pharmaceutical industries.

Keywords: Phenolic, polysaccharides, antioxidant, antimicrobial, anti-inflammatory, *Moringa olifera*

INTRODUCTION

Flora has a significant role in maintaining human health and in improving the quality of life for thousands of years. Recently, the focus on plant research is increased throughout the world due to their extensive applications. *Moringa oleifera* is the most widely cultivated species of the genus *Moringa oleifera* which considers the only genus in the family *Moringaceae.* The plant seeds contain hypotensive activity, strong antioxidant activity and chelating property against arsenic toxicity[1-3]. Defatted cake *Moringa oleifera* is widely used as a natural coagulant for water treatment in developing countries[4]. It has an impressive range of medical uses with high nutritional value[5]. The seeds of *Moringa oleifera* are used in traditional system of medicine to treat arthritis, rheumatism, gout, sexually transmitted diseases and boils. Roasted seeds used for their treatment of epilepsy, skin infections and as a diuretic[6]. The chemical constituents seeds are crude protein, crude fat, carbohydrates, methionine, cysteine, benzyl glucosinolate), moringyne, mono palmitic and di-oleic triglycerides[7,8,9]. The mucilage from the pods is called drum stick polysaccharide, consists of galactose, dextrose, xylose and potassium, sodium, magnesium, calcium salts of glucoronic acid. Natural polysaccharides are widely used as excipients in pharmaceutical industry as they are easily available, biocompatible and biodegradable[10]. These polysaccharides showed multiple and complex biological activities such as antitumor, anticancer, antioxidant and anti-inflammatory[11]. Binders enable compressibility of a drug and make it convenient to administer. They can also be used for modifying drug release. There are limited information on the antioxidant compounds and their antioxidant, anti-inflammatory and antimicrobial activities of the water extract of defatted seed and characterization of the polyphenols and polysaccharides from the seeds of *moringa olifera* L.as antioxidant, antimicrobial and anti-inflammatory sources.

MATERIALS AND METHODS

Chemicals

ABTS$^{\cdot+}$ (2, 2'-azinobis (3-ethyl benzothiazoline - 6 - sulfonic acid)), Folin-Ciocalteau reagents, Gallic acid, quercetin, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, Ferrozine: (3- (2 - pyridyl) - 5, 6- bis- (4-phenylsulfonic acid)-1, 2, 4-triazine, BHT: Butyl Hydroxy toluene and,

*Author for Correspondence

potassium ferricyanide, were from (Sigma Chemical Co., St. Louis, MO, USA).

Preparation of defatted seed Moringa olifera extract

Hundred gram of seeds were defatted with hexane, then ten grams of defatted seeds *Moringa olifera* was dispensed in 100ml of hot and cold distilled water overnight at room temperature using shaker. The mixture was filtered through whatman No.1 filter paper and the extraction step was repeated twice. The filtrate was then concentrated to dryness at 40C in a rotary evaporator. The crude extracts were stored in a refrigerator until further analysis

Phytochemicals analysis

Total phenolic content

The total phenolic content (TPC) of were spectrophotometrically determined by Folin Ciocalteu reagent assay using gallic acid for the preparation of calibration curve (20 – 120 mg/l) according to the method of[12]. A suitable aliquot (1 ml) of cold and hot water defatted seeds *Moringa olifera* extracts or standard solution was added to 25 ml volumetric flask, containing 9 ml of distilled water. One milliliter of Folin Ciocalteu`s phenol reagent was added to the mixture and shaken. After 5 min. 10 ml of 7 % Na_2CO_3 solution were added to the mixture. The solution was diluted to 25 ml with distilled water and mixed. After incubation for 90 min. at room temperature, the absorbance was determined at 750 nm with spectrophotometer (Unicum UV 300) against prepared reagent as blank. A total phenolic content in samples was expressed as mg gallic acid equivalents (GAE)/g dry weight. All samples were analyzed in triplicates.

Total flavonoid content

Total flavonoid content (TFC) was spectrophotometrically determined by the aluminum chloride method using quercetin as a standard[13]. One ml of cold and hot water defatted seeds *Moringa olifera* extracts or standard solution (quercetin, 20–120 mg/l) was added to 10 ml volumetric flask, containing 4 ml of distilled water. To the flask 0.3 ml 5 % $NaNO_2$ was added and after 5 min 0.3 ml 10 % $AlCl_3$ was added. At 6th min, 2 ml 1M NaOH were added and the total volume was made up to 10 ml with distilled water. The solutions were mixed well and the absorbance was measured against prepared reagent blank at 510 nm by using spectrophotometer (Unicum UV 300). Total flavonoids in sample wase expressed as mg quercetin equivalents (QE)/ g dry weight. Samples were analyzed in triplicates.

Total tannins content

Total tannins content (TTC) was measured using the Folin-Ciocalteu reagent assay according to[14]. One ml of cold and hot water defatted seeds *Moringa olifera* extracts or standard solution of (tannic 20-120 mg/l) was added to 7.5 ml distilled water (dH_2O) then add 0.5 ml of Folin reagent and 1 ml of 35% sodium carbonate solution. The volume was made up for 10 ml with distilled water and absorbance was measured against prepared reagent blank at 775 nm by using spectrophotometer (Unicom UV e300). Total tannins in sample were expressed as mg tannic acid equivalent (TAE)/g dry weight. All samples were analysed in triplicates.

Determination of total carbohydrates

Defatted seeds of *Moringa olifera* (0.03 g) were placed in a test tube, and then 10 ml, 1N H_2SO_4 were added. The tubes were sealed and placed in water bath at 100˚C for 2h. The solutions were then filtered into a measuring flask (100 ml) and completed to the mark with distilled water. The total hydrolysable carbohydrates were spectrophotometrically determined using phenol-sulphuric acid method[15].

Antioxidant activity

In-vitro DPPH˙ Free radical scavenging assay

The method described by[16] was used to assess the DPPH˙ (2, 2-diphenyl-1-picryl hydrazyl) radical scavenging activity. 0.1 mM of DPPH˙ in methyl alcohol was prepared and 0.5 ml of this solution was added to 1 ml of cold and hot water defatted seeds *Moringa olifera* extracts at different concentrations (25, 50, 75, 100 µg/ml). Methanol was used as blank. The mixture was shaken vigorously and allowed to stand at room temperature. Butyl Hydroxytoluene (BHT, Sigma) was used as positive control; and negative control contained the entire reaction reagent except the extracts. Then the absorbance was measured at 515 nm against blank. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

The capacity to scavenge the DPPH˙ radical was calculated using the following equation:

DPPH˙ scavenging effect (Inhibition %) = $[(A_c – A_s / A_c) \times 100]$

Where:

A_c was the absorbance of the control reaction. A_s the absorbance in the presence of the plant extracts.

In-vitro Metal chelating activity assay

Metal chelating effects on ferrous ions was carried out as described by[17]. One ml of both extracts, or EDTA solution as a positive control at different concentrations (25, 50, 75, 100 µg/ml) were spiked with 0.1 ml of 2 mM $FeCl_2$-$4H_2O$ and 0.2 ml of 5 mM ferrozine solution and 3.7 ml methanol were mixed in a test tube and reacted for 10 min, at room temperature then the absorbance was measured at 562 nm. Mixture without extract was used as the control. A lower absorbance indicates a higher ferrous ion chelating capacity. The percentage of ferrous ion chelating ability was calculated using the following equation: Iron chelating activity (Inhibition %) = $[(A_c – A_s / A_c) \times 100]$

Where:

A_c was the absorbance of the control reaction.

A_s the absorbance in the presence of the plant extracts.

In-vitro Reducing power assay

The reducing power was assayed as described in[18]. Different concentrations (25, 50, 75, 100 µg/ml) of cold and hot water defatted seeds moringa extracts (1.0 ml) were mixed with 2.5 ml of phosphate buffer (50 mM, pH 7.0) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50 ºC for 20 min. After, 2.5 ml of trichloroacetic acid (10 %) were added to the mixture, centrifuged at 3000 rpm for 10 min. Finally, 1.25 ml from the supernatant was mixed with 1.25 ml of distilled water and 0.25 ml $FeCl_3$ solution (0.1%, w/v). The absorbance was measured at 700 nm. The assays were carried out in

triplicate and the results were expressed as mean values ± standard deviations. Increased absorbance values indicate a higher reducing power. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against the extract concentration. BHT was used as standard.

In-vitro ABTS˙⁺ radical scavenging activity

ABTS˙⁺ assay was generated byoxidation of ABTS˙⁺ with potassium persulphate according to[19]. ABTS˙⁺ was dissolved in deionized water to 7. 4 Mm concentration, and potassium persulphate added to a concentration of 2.6 Mm. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12-16 h at room temperature in the dark. The solution was then diluted by mixing 1ml ABTS˙⁺ solution with 60 mL methanol to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using the spectrophotometer. Fresh ABTS˙⁺ solution was prepared for each assay. Moringa hot and cold aqueous extracts (150 µl) at different concentration (25, 50, 75, 100 µg/ml) were allowed to react with 2850 µl of the ABTS˙⁺ solution for 2 h in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer. Results were expressed as in comparison with standard BHT. A bigger antioxidant capacity of the sample exhibited a smaller production of free radicals. Percent activity was calculated using the equation:

% Inhibition = $[(A_0 - A_1) / A0] \times 100$ %

Where:

A_0 is the ABTS˙⁺ absorbance of the control reaction.

A_1 is the ABTS˙⁺absorbance in the presence of the sample.

Identification of phenolic compounds for defatted moringa meal by HPLC

Phenolic compounds of cold water defatted seeds *Moringa olifera* extract were identified using HPLC according to[20]. All chemicals and solvents used were HPLC spectral grade, and obtained from Sigma (St. louis, USA (Merck–Shcuchrdt) Munich, Germany).

The HPLC system is Agilent 1100 series coupled with UV-Vis detector (G1315B) and (G1322A) DEGASSER. Sample injections of 5 µl were made from an Agilent 1100 series auto-sampler; the chromatographic separations were performed on ZORBAX-Eclipse XDB-C18 column (4.6×250 mm, particle size 5 µm). A constant flow rate of 1 ml/min was used with two mobile phases: (A) 0.5% acetic acid in distilled water at pH 2.65; and solvent (B) 0.5% acetic acid in 99.5% acetonitrile. The elution gradient was linear starting with (A) and ending with (B) over 50 min, using an UV detector set at wavelength 280 nm. Phenolic compounds of banana peel extract were identified by comparing their relative retention times with those of the standard mixture chromatogram. The concentration of an individual compound was calculated on the basis of peak area measurements, and then converted to µg phenolic/ g dry weight.

Extraction of polysaccharides

Seeds of *Moringa olifera* were soaked in cold distilled water at room temperature for 24 hours with shaking. The extract was filtered using Whatman No. 1 filter paper under reduced pressure. Polysaccharides was precipitated with ethanol absolute and centrifuged at 5,000 rpm for 20 minutes to pool it[21].

Identification of monosugars by HPLC

Sugar analysis was carried out according to[21]. The polysaccharide class (0.4 mg) was hydrolyzed with 2N HCl (1 ml) in sealed tube at 100 ºC for 1 h. After the hydrolysis, the acid was removed by flash evaporated on water bath at a temperature of 40 ºC. Then, the hydrolyzed monosaccharides were extracted with petroleum ether plus water. The purified hydrolyses monosaccharaides were analyzed by HPLC (Agilate Pack, serics1200), equipped with Aminex carbohydrate Hp-87c column (300mm × 7.8 mm). Deionized water was used as the mobile phase at flow rate 1 ml/ min. Chromatographic peaks were identified by comparing the retention times with the respective retention times of known standard reference material. Retention time and peak area were used to calculation of sugar concentration by the data analysis of Agilat Packard.

In-vitro anti-microbial activity of defatted Moringa olifera meal extract

Different bacteria, including gram positive (*Bacillus subtilis* NRRL B-94, *Staphylococcus aureus* NRRL B-313), gram negative (*Escherichia coli* NRRL B-3703, *Pseudomonas aeruginosa* NRRL B-32), *Aspergillus niger* NRRL 599, *Aspergillis fluves* NRC, *Candida albicans* NRRL477 and *Saccharomyces cerviciea* strains, were used, where in the measurement of growth inhibition was carried out as previously described[22]. Cells from cultures grown on nutrient slopes were inculated using a sterile loop into fresh nutrient broth and incubated overnight at 30 ºC. One mL of each culture were transferred to Petri dishes (120 mm in diameter) to which 50 mL molten nutrient agar (45 ºC) was added. Wells of 6 mm diameter were then mode in the solidified agar using proper sterile tubes. Antimicrobial action at diameter of inhibition zone was measured as the clear area centered on the agar well containing the sample, well with non-inhibition zone were recorded 0.00. Plates were undisturbed for 30 min to allow diffusion of the sample (200, 400 µg/ml) into the agar, then incubation inverted at 30 ºC for 24 h with yeast, 24-48 h for bacteria and 72 h for fungi. Inhibition zones were measured, wherein the evaluation of antibacterial activity was carried out in triplicate with three replicates on each sample.

In-vitro anti-inflammatory activity of defatted Moringa olifera meal extract

Anti-inflammatory of different extracts from cold and hot aqueous extracts were tested using the method of[23]. The different concentration of cold and hot water defatted seeds *Moringa olifera* extracts or standard drug diclofenac sodium (50, 100, 150, 200 µg/ml) was mixed with 0.45ml bovine albumin serum. The sample extracts were incubated at 37 °C for 20 min and then heated to 57 °C for 3 min after cooling the samples was added 2.5ml phosphate buffer pH 6.4. The absorbance was measured using UV visible spectrophotometer at 255nm.

Statistical analysis

Data were statistically analyzed using Costat statistical package[24].

RESULTS AND DISCUSSION

Total phenolic (TP), total flavonoids (TF) and total tannins (TT) of defatted Moringa olifera meal extracts

The results of the phytochemical composition (Table 1) revealed that defatted meal of *Moringa olifera*, as well as other legumes, is a good source of active constituents. In this study the content of total phenols in both extracts, expressed as gallic acid equivalents per gram of dry extract 15.76 ± 0.20 and 15.01 ± 0.24 mg/g DW) in cold and hot water extract and is lower than that from reported by[25] in *Tordylium maximum* 74.59 ± 0.004 mg/g DW. Water extract was used to achieve extraction of active substances with diversity in their polarity. The content of TPC, TFC, TTC is greater than that found by[26] was 10.18 ± 2.89 mg/g DW which is higher compared to flavonoid 2.90 ± 0.0002 (mg/g DW) and tannic acid of 0.89 ± 0.02 mg/g DW. According to[27-31] as well as, phytochemical components are responsible for both pharmacological and toxic activities in plants. Phenolics have been found as strong antioxidants against hindering the influence of free radicals and ROS, which is the basis of several chronic human infections[33]. The presence of phenolic compound from *Moringa olifera* seeds will encourage the utilization of the seeds for many purposes[6]. These metabolites are useful to a plant itself but can be toxic to animals and mankind. The presence of these chemical constituents in this plant is an indication that the plant, if properly screened, could yield drugs of pharmaceutical significance. The concentration of flavonoids in cold and hot water extract of defatted meal of moringa was determined using spectrophotometric method. The concentrations of flavonoids in both extracts were 3.11± 0.03 and 3.09 ± 0.03 mg/g DW. Flavonoids are important components of human and animal diet. Due to the different biological activities of plant secondary metabolites, their regular consumption may have serious consequences for health, both positive and negative effects[34]. *Moringa olifera* seeds may contain tannins, which are important phytochemicals with a wide range of medicinal properties, including anticancer, anti-inflammatory, antioxidant, and antibacterial activities[35]. The highest content was observed with hot water extract. The differences between our results of total phenolics, flavonoids and tannins contents and other investigators may be attributed to plant species, environmental condition and sample preparation. The differences could be related to the part of plant used for making the extract and type of solvent.

Antioxidant activity

Reactive Oxygen Species (ROS play an important role in oxidative stress related to pathogenesis of various important diseases. The production of free radicals is balanced by the antioxidative defense system. The antioxidant properties of extracts of defatted meal of *Moringa olifera* were determined using four different methods were: $DPPH^{\cdot}$, Fe^{2+}-chelating, Reducing power, and $ABTS^{\cdot+}$ assays. The results showed significant differences in the antioxidant activity between the two types of extracts.

$DPPH^{\cdot}$ scavenging activity

Free radical scavenging capacities of the tested extract was measured by $DPPH^{\cdot}$ assay and results are shown in (Table 2). According to the results obtained, the activity in cold water was higher than that of hot water extract of *Moringa olifera* defatted meal was found active with IC_{50} value 54.17±0.31 µg/ml. A lower IC_{50} value indicates higher antioxidant activity. IC_{50} values of the synthetic antioxidant BHT was 11.59 µg/ml. Usually, antioxidant properties of plant extracts have been attributed to the presence of polyphenolic compounds, which have great potential as antioxidant agents. It is known that phenolic and flavonoid contents are directly linked to antioxidant properties[36].

Table 1: Total phenol, flavonoids and Tannins of defatted Moringa meal extra

Extracts	TPC(mg/g DW)	TFC(mg/g DW)	TTC(mg/g DW)
Cold water	15.76^{b} ± 0.20	3.11^{b} ± 0.03	4.99^{a} ± 0.06
Hot water	15.01^{a} ± 0.24	3.09^{a} ± 0.03	5.48^{b} ± 0.15
LSD at 0.05	0.49	0.07	0.26

All values with the same letters are not significantly different at $p \leq 0.05$.

Table 2: IC_{50} of $DPPH^{\cdot}$ radical scavenging of moringa defatted meal extract

Extracts	IC_{50} µg/ml
Cold water	54.17^{b} ± 0.31
Hot water	63.43^{c} ± 0.33
BHT	11.59^{a} ± 0.33
LSD at 0.05	0.65

All values with the same letter are not significantly different at $p>0.05$

Fe^{2+}chelating activity

Iron is known as the most important lipid oxidation due to its high reactivity. Iron is essential for oxygen transport, respiration, and enzyme activity; it is a reactive metal that catalyzes oxidative damage in living tissues and cells[37]. Ferrozine can quantitatively form complexes with Fe^{2+}. Results indicate that *Moringa olifera* cold water extract exhibited a significantly higher Fe^{2+}-chelating activity (82.94±0.37 µg/ml) compared to hot water extract (74.39±0.37 µg/ml) at 100 µg/ml (Table 3).

Reducing power

It was observed that cold water extract of *Moringa olifera* defatted meal had a higher reducing power (EC_{50} = 41.55 ± 0.51). While the hot water extract of *Moringa olifera* showed the lowest potential activity (EC_{50} = 59.11 ± 0.19 (Table 4 and Fig 1). Fig. (1). Reducing power of moringa defatted meal extract. The reducing power activity may be due to the presence of reductions as electron donors and are capable of converting them into a more stable product

Table 3: Iron chelating activity of moringa defatted meal extracts

Extracts	Chelating activity %			
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Cold water	$15.19^b \pm 0.29$	$35.64^b \pm 0.31$	$68.88^b \pm 0.29$	$82.94^b \pm 0.37$
Hot water	$10.72^a \pm 0.52$	$28.37^a \pm 0.37$	$54.62^a \pm 0.60$	$74.39^a \pm 0.37$
EDTA	$69.71^c \pm 0.45$	$76.60^c \pm 0.23$	$82.20^c \pm 0.23$	$91.40^c \pm 0.37$
LSD at 0.05	0.86	0.71	0.75	0.97

All values with the same letters are not significantly different at $p \leq 0.05$.

Table 4: Reducing power of moringa defatted meal extracts

Extracts	Absorption at 700 nm				EC_{50} µg/ml
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	
Cold water	$0.366^b \pm 0.004$	$0.583^b \pm 0.002$	$0.743^b \pm 0.002$	$0.874^b \pm 0.002$	$41.55^b \pm 0.51$
Hot water	$0.263^a \pm 0.00$	$0.446^a \pm 0.004$	$0.616^a \pm 0.003$	$0.764^a \pm 0.002$	$59.11^c \pm 0.19$
BHT standard	$0.614^c \pm 0.003$	$0.803^c \pm 0.002$	$1.015^c \pm 0.002$	$1.207^c \pm 0.002$	$11.01^a \pm 0.32$
LSD at 0.05	0.02	0.07	0.05	0.08	0.83

All values with the same letters are not significantly different at $p \leq 0.05$.

Table 5: $ABTS^{\cdot+}$ scavenging activity of defatted moringa meal extracts

Extracts	Scavenging activity %			
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Cold water	$24.34^b \pm 0.32$	$38.78^b \pm 0.32$	$61.42^b \pm 0.30$	$77.84^b \pm 0.25$
Hot water	$15.35^a \pm 0.30$	$27.84^a \pm 0.30$	$42.60^a \pm 0.36$	$65.12^a \pm 0.18$
Trolox	$64.80^c \pm 0.24$	$75.82^c \pm 0.25$	$81.30^c \pm 0.30$	$93.72^c \pm 0.42$
LSD at 0.05	0.71	0.73	0.84	0.65

All values with the same letter are not significantly different at $p>0.05$

and terminating the free radical reaction. The reducing power ability of BHT was ($EC_{50} = 11.01 \pm 0.32$ µg/ml). The reducing power of *Moringa olifera* seeds extracts is probably due to the action of hydroxyl group of the phenolic compounds which might act as electron donors. This may be attributed to the low viscosity of the solvent have low density and high diffusivity that allows them to easily diffuse into the pores of the plant materials to leach out the bioactive constituents[38]. Based on results of this study, the extract with the highest antioxidant activity had the highest concentration of phenols. Phenolic compounds are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore, the phenolics in plants contribute directly to their antioxidant[39].

$ABTS^{\cdot+}$ scavenging activity of defatted moringa meal

The antioxidant capacities of Moringa defatted meal in cold and hot water extract were determined according to the $ABTS^{\cdot+}$ discolorations method. The results were shown in (Table 5). At the highest concentration (100 µg/ml), the cold water extract of *Moringa olifera* showed the highest antioxidant activities (77.84% ± 0.25) compared to hot water (65.12 % ± 0.18).

Identification of Phenolic and flavonoids compounds of defatted moringa Meal

It is obvious that the total phenolic content measured by the Folin-Ciocalteu procedure does not give a full picture of the quality or quantity of the phenolic constituents in the extracts[36,40]. Various factors affect HPLC analysis of phenolics, including sample purification, mobile phase, column types and detectors[41]. The HPLC analysis of the

Table 6: HPLC identification of phenolic in defatted moringa meal extract

Phenolic compounds mg/100gDW	Moringa cold water extract
Ellagic	41.10
Tannic acid	1.59
Quercetin	6.30
Coumarin	0.06
Benzoic	26.01
Catechin	2.17
Caffiec acid	0.29

Table 7: Total carbohydrate % and fractionation of sugar contents of defatted moringa meal by HPLC

Sugars	%
Glucuronic	0.156
Stachyose	0.119
Galacturonic	0.132
Raffinose	0.289
Sucrose	0.08
Maltose	0.759
Lactose	0.221
Glucose	0.086
Xylose	0.043
Galactose	0.018
L-Raminose	0.07
Fructose	0.088
Sorbitol	0.003
Ribose	0.011
Total Carbohydrate %	15.14

Table 8: Antimicrobial activity of moringa defatted meal extracts

Extracts	Conc. µg/ml	Diameter of inhibition (mm)							
		Bacteria				Fungus		Yeast	
		B. subtilis	*St. aureus*	*E. coli*	*P. aueginosa*	*A. niger*	*A. fluves*	*S. cervisiae*	*C. albicans*
Cold water	200	10.40	15.00	09.70	11.20	09.50	10.00	11.00	08.80
	400	22.40	23.50	19.70	21.60	16.40	15.80	15.70	16.90
Hot water	200	09.30	08.40	09.00	07.90	09.20	08.60	09.00	08.50
	400	24.50	19.50	22.70	21.50	21.70	22.00	19.80	21.60
Poly-saccharide	200	11.20	13.00	10.50	09.80	08.90	09.30	08.50	09.00
	400	24.10	23.80	25.20	24.70	19.70	17.90	16.70	15.80

Table 9: Anti-inflammatory activity of moringa defatted meal extracts

Extracts	50 µg/ml	100 µg/ml	150 µg/ml
Cold water	$11.70^a \pm 0.61$	$22.49^a \pm 2.28$	$37.63^a \pm 1.96$
Hot water	$25.70^b \pm 2.39$	$39.01^b \pm 2.64$	$57.28^b \pm 1.58$
st. sodium	$74.05^c \pm 0.37$	$81.74^c \pm 0.64$	$85.50^c \pm 0.21$
LSD at 0.05	2.8	4.09	2. 92

Statistical analysis is carried out using Assistat Computer program, where unshared letter is significant at $p \leq 0.05$

phenolic compounds in water extracts were employed using the previous condition and were compiled in (Table 6). Data presented in (Table 6) reveals that the cold water had the highest amounts of ellagic, benzoic and qurectein acids (41.10, 26.01, 6.30 mg/100 g DW) respectively. It has been established that the phenolics and flavonoids content of *Moringa olifera* extracts are strongly depend on the type of the solvent on the different concentrations used as well as the solvent polarity[42,43].

Monosugars of defatted moringa meal

The monosugar in polysaccharide fraction are shown in (Table 7). The most abundant monosugar in the defatted meal of *Moringa olifera* is inulin (3.803%), maltose 0.759 %, Raffinose 0.289 %, lactose 0.221 %, glucuronic 0.156%, galacturonic 0.132%, fructose 0.088%, glucose 0.086% sucrose 0.08%, L-raminose 0.07%, xylose 0.043%, galactose 0.018%, ribose 0.011% and sorbitol 0.003%. The high percentage of mono sugars of the defatted meal extract of *Moringa olifera* has some nutritional advantages, these polysaccharides showed multiple and complex biological activities such as antitumor, anticancer, antioxidant and anti-inflammatory effectas as previously reported by[11] due to presence not only phenolics ,other factors such as polysacharides ,some peptides as well as proteins may display the chelation power as antioxidant mechanism of the extracts of defatted *Moringa olifera* meal extract[33,44]

Antimicrobial activity of defatted Moringa olifera meal extracts

The results presented in (Table 8) showed that polysaccharides extract of *Moringa olifera* enhanced the inhibition of bacterial species at 400 ppm against Gram-positive bacteria including *B. ubtilis* (24.10 mm), *S. aureus* (23.80 mm), *E. coli* (25.20 mm) and *P. auegino* (24.70 mm) respectively. Antimicrobial activity and preservative of *Moringa olifera* extract are believed to be associated with phytochemical components of the *Moringa olifera* seeds like phenolics, tannins[45]. The presence of tannins showed some antimicrobial activity against three of the

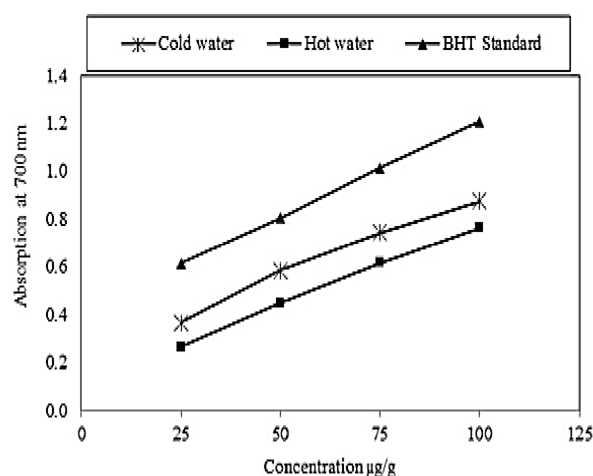

Figure 1: Reducing power of moringa defatted meal extract.

four microorganisms tested: *E. coli, S. aureus*, and *P. aeruginosa.* When we compared the previously published results with those of the current study, it is well known that the concentration of biologically active constituents varies with the plant parts, and the polarity of the solvent used, which directly reflects this activity[46]. Findings of the current study indicated that the microbial inhibition potency of the extracts is related to their TPC. This is in agreement with the work of some co-authors who suggested that the inhibition of microorganisms by phenolic compounds may be due to iron deprivation or hydrogen bonding with vital proteins such as microbial enzymes[47]. Phenolic compounds show generally a good antimicrobial effectiveness against Gram positive bacteria with their effect dependent on their amount: at low concentrations they can induce the denaturation of proteins until an irreversible modification of the cell and death[48]. Antimicrobial activity may also be due to the presence of short peptides that may act directly on microorganisms and result in growth inhibition by disrupting cell membrane synthesis or synthesis of essential enzymes[49]. Therefore,

there is a need to develop alternative antimicrobial drugs for the treatment of infections obtained from various sources such as medicinal plants. The antibacterial activity found in this present study may be attributed to the presence of secondary metabolites of various chemical types present in the plant material either individually. The discovery of a potent remedy from plant origin will be great advancement in microbial infection therapies.

Anti-inflammatory activity of moringa defatted meal extracts

In-vitro anti-inflammatory effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds. Denaturation of proteins is a well-documented cause of inflammation. The ability of *Moringa oleifera* hot water extract in inhibiting heat induced albumin denaturation was studied (Table 9). Maximum inhibition (57 28%.) was observed from defatted *Moringa oleifera* hot water extract followed by cold water extract (37.63) respectively at 150 µg/ml. Diclofenac Sodium, a standard anti-inflammation drug showed the maximum-inhibition (74.05%). Hence, the presence of bioactive compounds in the extract of defatted meal of *Moringa oleifera* may contribute to its, antioxidant and anti-inflammatory activity. The present investigation has shown that strong antioxidant and anti-inflammatory properties were confirmed in the water extract. These activities may be due to strong occurrence of polyphenolic compounds such as flavonoids, tannins, phenolics[46].

CONCLUSION

The results obtained concluded that the defatted meal of *Moringa oleifera* are considered as a rich source of phenolic, flavonoids, tannins, and polysaccharides which contributes to the antioxidant, antimicrobial and anti-inflammatory activities which can be used as potent source of pharmaceutical ingredient**.**

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