Global Advanced Research Journal of Agricultural Science (ISSN: 2315-5094) Vol. 4(4) pp. 188-199, April, 2015.
Available online http://garj.org/garjas/index.htm

Full Length Research Paper

Biochemical and functional properties of *Moringa oleifera* leaves and their potential as a functional food

Sobhy A. El Sohaimy[1*], Gamal M. Hamad[1], Sameh E. Mohamed[1], Mohamed H. Amar[2] and Rashad R. Al-Hindi[3]

[1]Food Technology Department, Arid Lands Cultivation Research Institute, City for Scientific Research and Technological Applications, Alexandria, Egypt
[2]Egyptian Deserts Gene Bank, Desert Research Center, Cairo, Egypt
[3]Biology Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

Accepted 12 March, 2015

BACKGROUND: Moringa is a tree of a not well-understood plant since it has not been fully studied all over the world. Therefore, the aim of the present study is to evaluate the chemical as well as functional properties of the Egyptian Moringa oleifera leaves. Such leaves can be used as a functional food ingredient in the food and pharmaceutical applications. RESULTS: The proximate analysis showed that moringa leaves are rich in: fiber, protein, carbohydrate and energy contents (11.23±0.16, 9.38±0.23, 56.33±0.27 g.100g-1 and 332.68±0.06 KCal, respectively). Moringa is a good source for essential amino acids especially Lysine (69.13±0.13mg.100g-1), essential minerals such as Na (289.34±0.35), K (33.63±0.24), Mg (25.64±0.25) Ca (486.23±0.11), P (105.23±0.32) and Fe (9.45±0.16) mg.100g -1 respectively and vitamins (A=13.48±0.51, B1=0.05±0.28, B2= 0.8±0.25, B3= 220±0.42, C= 245.13±0.46 and E= 16.80±0.24 mg.100g respectively). It is appeared using HPLC that methanol 70% is the most suitable solvent for extraction of phenolic compounds from moringa leaves (. Scavenging activity results confirmed that Moringa leaves extract might be a potent source of natural antioxidants with a high human health benefits. Antimicrobial activity results indicate that Moringa leaves extracts may be used as an antimicrobial agent with reasonable safety margins to inhibit bacterial growth in pharmaceutical and food applications. CONCLUSION: Moringa is considered as a nutrient-rich plant especially in its leaves. Such leaves might be used to combat malnutrition, especially among infants and nursing mothers.

Keywords: *Moringa oleifera*, biochemical analyses, phenolic content, antioxidant, antimicrobial Pathogens.

INTRODUCTION

Moringa oleifera is a perennial tree, still considered as among underutilized plant and falls under Moringa ceae family. The plant is also known as Drumstick, Sahjan or Sohanjana in India. All plant parts are having remarkable range of some functional and nutraceutical properties (Singh *et al,* 2012) make this plant diverse biomaterials for food and allied uses. The leaves, flowers and fruits of this plant are used in the preparation of several delicacies in Indian subcontinent. Associated with high nutritional value of its edible portions pave a way in making this plant more popular as an important food source in order to combat protein energy malnutrition problem prevailed in most of the under developed and developing countries of the world. Presence of various types of antioxidant compounds make this plant leaves a valuable source of natural antioxidants (Anwar *et al,* 2007) and a good source of nutraceuticals and functional components as well (Makkar and Becker, 1996). There are considerable variations among the

Corresponding Author's Email: elsohaimys@gmail.com;
Tel: +2034593420; Fax: +2034593423

nutritional values of Moringa, which depend on factors like genetic background, environment and cultivation methods (Brisibe *et al*., 2009). Therefore, it necessitates determination of the nutritive value of Egyptian Moringa ecotype, which could assists in the formulation of diets according to nutrients requirements. The nutritional composition of Moringa of the African ecotype has little previously been evaluated; and a relatively recent studied. (Moyo *et al*, 2011) reported the profiling of chemical composition, fatty acids, amino acids and vitamins. Amino acids, fatty acids, minerals and vitamins are essential in animal feed. Such nutrients are used for osmotic adjustment; activate enzymes, hormones and other organic molecules that enhance growth, function and maintenance of life process (Anjorin *et al*, 2010). Nutritional composition of the plant plays a significant role in nutritional, medicinal and therapeutic values (AlKharusi *et al*, 2009). Interestingly, it was reported that nutritional content in the leaves of Moringa varies with location (Anjorin *et al*, 2010). Considering these aspects, the objective of this investigation was to evaluate the chemical as well as functional properties of the Egyptian *Moringa oleifera* leaves. Such leaves can be used as a functional food ingredient in the food and pharmaceutical applications.

MATERIALS AND METHODS

Plant materials and bacterial strains

The leaves of *Moringa oleifera* were collected from Desert Research Institute of Ismailia branch, Egypt. The leaves of *Moringa oleifera* were shade-dried for three days and subsequently ground to powder using blender and used to determine its chemical composition. The bacterial strains: *Streptococcus pyogenes* ATCC12344, *Streptococcus agalactiae* ATCC12296, *Staphylococcus epidermis* ATCC35984, *Salmonella senftenberg* ATCC8400*, Klebsiella pneumonia* ATCC 12296, and the yeast strain *Candidaalbicans* ATCC2091 were purchased from the American type culture collection (ATCC, USA). While, *Bacillus subtilis DB100host* was obtained as a gift from the Faculty of Agriculture, Alexandria University, and *Escherichia coli* O-143was purchased from Statens Serum Institute, Denmark. All bacterial and yeast strains were maintained in Brain Heart Infusion Medium (BHI, Atlas 1993) at 37°C.

Proximate analysis

Moisture content of the sample was determined using the method described by (AOAC1995). One gram of sample in pre-weighed crucible was placed in an oven (105°C) for 24 h, cooled, and reweighed. The percentage moisture was calculated as follows:

$Moisture\ (\%) = w_2\text{-}w_3 / w_2\text{-}w_1 \times 100$

Where, w_1 is the weight of the crucible, w_2 is the weight of the crucible after drying at 105°C and sample, and $w_3$is the weight of the crucible and the sample after cooling in airtight desiccators.

Ash content was determined as described in(Fahey, 2005).The samples (1.0g) were weighed and subjected to dry ashing in a well cleaned proclaim crucible at 550°C in a muffle furnace for 2 h. The ash percentage was calculated as follows:

$Ash\ (\%) = w_2\text{-}w_3 / w_2\text{-}w_1 \times 100$

Where, w_1 is the weight of the crucible, w_2 is the weight of the crucible after drying at 550°C and sample, and $w_3$is the weight of the crucible and the sample after cooling in airtight desiccators.

Crude protein was determined using the micro-Kjeldahl method (Fahey, 2005). The crud protein was calculated by multiplying Nitrogen by the conversion factor of 6.25 [P% = TN x 6.25].

Fat content of the moringa leaves was determined using the method of (Folch and Stanly, 1957).Homogenized tissue (10g) was progressively added to small amounts of a chloroform/methanol 2:1 (v/v) mixture (up to 200 ml), with vigorous shaking, and then the extraction was carried on for a further 2 h, using an electromagnetic stirrer. The mixture was filtered and the filter was rewashed with fresh solvent and pressed. Fifty millilitres of 0.88% potassium chloride were added and the mixture was shaken. The aqueous layer (upper) was removed by aspiration and the washing procedure was repeated. The extract was then dried by adding anhydrous sodium sulphate, which was filtered again before the solvent was removed using a rotary evaporator. The extract was then placed in a desiccator overnight and weighed.

Crude Fiber was determined using the method of (AOAC, 2005).1g of sample (W2) was transferred directly to filter bag and sealed with a heat sealer. Sample and blank bags were immersed in enough amount petroleum ether for 10 minutes to extract fat content from samples. All bags were air dried and transferred to a ANKOM 2000 FIBER ANALYZER using H2SO4 and NaOH and the crude fiber was calculated according the following equation:

% Crude Fiber = 100 x (W3 - (W1 x C1))/W2

Where:

W1 =Bag tare weight

W2=Sample Weight

W3=Weight of Organic Matter (Loss of weight on ignition of bag and fiber)

C1=Ash corrected blank bag factor (running average of loss of weight on Ignition of blank bag/original blank bag)

Carbohydrate content was determined by difference, that is, addition of all the percentages of moisture, fat, crude protein, ash, and crude fiber were subtracted from 100%. This gave the amount of nitrogen-free extract otherwise known as carbohydrate.

Carbohydrate (%)= 100 – [moisture (%)+ Fat (%) + Ash (%) + Crude Fiber (%) + Crude Protein (%)].

Energy value

The sample energy value was estimated (in KCal/g) by multiplying the percentages of crude protein, crude lipid, and carbohydrate with the recommended factors (2.44, 8.37, and 3.57, respectively) as proposed by (Martin and Coolidge, 1978).

Minerals determination

AOAC (2005) methods were used to determine the mineral compositions of the samples. One gram of sample was digested with nitric acid: perchloric acid: sulfuric acid mixture in the ratio 9:2:1, respectively, and filtered. The filtrate was made up to mark in a 5 ml volumetric flask. The filtered solution was loaded to an atomic absorption (Model 703; Perkin Elmer, Norwalk, CT). The standard curve for each mineral, that is, calcium, magnesium, iron, aluminum, lead, copper, manganese and zinc, was prepared from known standards and the mineral value of samples estimated against that of the standard curve. Values of sodium and potassium were determined using a flame photometer (FP 920, PG Instruments) using NaCl and KCl as the standard (AOAC, 2005), while phosphorus was determined using the Vanodo-molybdate method.

Vitamins determination

Vitamins A and E were determined by (AOAC, 2005a,b). Vitamin B1 and B2 were determined by acid hydrolysis method of (Finglas and Faulks, 1984), B3 content of samples were determined by the (Ackurt *et al*. 1999).

Determination of amino acid content

Moringa leaves sample hydrolysates were prepared following the method of (Spackman *et al*. 1958). Each of the defatted samples was weighed (200 mg) into a glass ampoule, 5 ml of 6N HCl/L was added to the ampoule, and the contents were hydrolyzed in an oven preset at 105°C for 22 h. Oxygen was expelled in the ampoule by passing nitrogen gas into it. Amino acid analysis was done by (SYKAM S 433 Amino Acid Analyzer). The analysis was carried out with a gas flow rate of 0.5ml/min at 60°C, and the reproducibility was 3%. The amino acid composition was calculated from the areas of standards obtained from the integrator and expressed as percentages of the total protein.

Preparation of the extract for phenolic compounds and flavonoids

Moringa oleifera extract was prepared according to (Sreelatha and Padma, 2009) with some modifications. The leaves were chopped to small pieces and dried in shade. The dried leaves were powdered and passed through sieve no. 20 and extracted (100 g) successively with 1000 ml of water, 70% ethanol and 70%methanol and stirred for 1h. The extracts were centrifuged at 4000 rpm for 15 min. the supernatant was collected and dried under vacuum in vacuum oven at 50°C to dryness. All analysis was carried out in triplicates.

Determination of total phenolics and flavonoids

The amounts of phenolic compounds in the extracts of the leaves were estimated by using Folin–Ciocalteau reagent according to the method of (El Sohaimy and Masry, 2014). In a series of test tubes, 0.4 ml of the extract in methanol was taken, mixed with 2 ml of Folin-Ciocalteau reagent and 1.6 ml of sodium carbonate. After shaking, it was kept for 2 h and the absorbance was measured at 750 nm using a Shimadzu-UV-160 spectrophotometer. Using gallic acid monohydrate, a standard curve was prepared. The linearity obtained was in the range of 1-10 µg/ml, using the standard curve, the total phenolic compounds content was calculated and expressed as gallic acid equivalent in $g.kg^{-1}$ of extracts.

Flavonoids were extracted and estimated by the method of (AOAC, 2000); an aliquot of the extract was pipetted out and evaporated to dryness. Four ml of vanillin reagent was added and heated for 15 min in a boiling water bath. The standard was also treated in the same manner. The optical density was read at 340 nm. The values are expressed as g flavonoids/kg leaf.

HPLC of phenolic compounds

HPLC (Agilent 1000) analysis of phenolic compounds (antioxidants) was performed on a reverse phase Zorbax Eclipse XDB-C18 column (4.6 x 150 mm, 5 µm), using a gradient program with two solvents system (A: 0.5 % acetic acid in acetonitrile: water (1:1) B: 2% acetic acid in water) at a constant solvent flow rate of 1.2 ml/min (Öztürk *et al*, 2007). Injection volume was 20 µl. The signals were detected at 280 nm by UV-VIS detection .All solutions were prepared with deionized water. (Kolayli *et al*, 2010)

DPPH (2, 2-Diphenyl-1-Picrylhydrazyl)-Scavenging Activity

The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH (Rakesh and Singh, 2010). One ml solution of the extract in methanol was added to 0.5 ml of 0.15 mM DPPH solution in methanol. The contents were mixed vigorously and allowed to stand at 20 °C for 30 min. The absorbance was read at 517 nm. IC_{50} value [the concentration required to scavenge 50(%) DPPH free radicals] was calculated. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%)= (A_0 -A_1/A_0) x100;

Where,A_0 was the absorbance of the control reaction and A_1 the absorbance in the presence of the sample.

Antimicrobial activity

The antimicrobial activity was performed by agar well diffusion method (Perez *et al*., 1995) for solvent extract. One hundred µl of the inoculum (1 x 10^8cfu/ml) was mixed with Hi-media and poured into the Petri plate. A well was prepared in the plates with the help of a cork-borer (0.85cm). One hundred µl of the test compound was introduced into the well. The plates were incubated overnight at 37°C. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain controls were maintained as pure solvents were used instead of the extract. The result was obtained by measuring the zone diameter (mm). The experiment was done three times and the mean values are presented.

Statistical analysis

All analyses were run in triplicates. Data were analyzed by an analysis of variance one-way (ANOVA) ($P<0.05$).

RESULTS

Proximate analysis

The leaves of *Moringa oleifera*were analyzed for proximate analysis and the results in (Table 1) showed that, the *Moringa oleifera* leaves were contained moisture (10.74g.100g^{-1}±0.05), ash (4.56g.100^{-1}±0.13), fiber (11.23g.100^{-1}±0.16), total protein (9.38g.100g^{-1}±0.23), total lipid (7.76g.100g^{-1}±0.21) and total carbohydrate (56.33g.100g^{-1}±0.27) and the total energy of 100 gram of leaves was (332.68±0.06)

Mineral content

Mineral content of *Moringa oleifera* leaves was determined and registered in (Table 2). The results in (Table 2) indicate that *Moringa oleifera* leaves are a promising source of essential minerals. The concentration of sodium was (289.34±0.35 mg.100g^{-1}), potassium (33.63±0.24mg.100g^{-1}), magnesium (25.64±0.25mg.100g^{-1}), phosphorus (105.23±0.32mg.100g^{-1}), iron (9.45±0.16mg.100g^{-1}), zinc (1.63±0.021mg.100g^{-1}), copper (0.88±0.52mg.100g^{-1}), calcium (486.23±0.11mg.100g^{-1}) and manganese (5.21±0.12mg.100g^{-1}).

Vitamin content

The vitamin content of *Moringa oleifera* leaves showed in (Table 3). The concentration of detected vitamins were; 13.48±0.51 mg.100g^{-1}of β-Carotene, 16.80±0.24 mg.100g^{-1} of Vitamin E, 245.13±0.46 mg.100g^{-1} Vitamin C, 0.05±0.28 mg.100g^{-1} of Vitamin B1, 0.8±0.25 mg.100g^{-1} of Vitamin B2 and 220±0.42 mg.100g^{-1} of Vitamin B3.

Amino acids composition

Table 4 showed the amino acid composition of *Moringa oleifera* leaves. It is showed 17 amino acids in different concentrations as follows: Lysine (69.13±0.13mg.100g^{-1}), Histidine (29.56±0.21 mg.100g^{-1}), Valine (62.34±0.19mg.100g^{-1}), Leucine (94.36±0.31mg.100g^{-1}), Isoleucine (46.98±0.15mg.100g^{-1}), Threonine (48.35±0.26mg.100g^{-1}), Alanine (4.93±0.12mg.100g^{-1}), Aspartic acid (13.76±0.15mg.100g^{-1}), Serine (3.13±0.15mg.100g^{-1}), Proline (1.86±0.13mg.100g^{-1}), Glutamic acid (18.03±0.09mg.100g^{-1}), Glycine (2.31±0.21mg.100g^{-1}), Arginine (7.65±0.10mg.100g^{-1}), Cysteine (2.15±0.11mg.100g^{-1}), Tyrosine (2.03±0.13mg.100g^{-1}), Methionine (0.43±0.14mg.100g^{-1}), and Phenyl Alanine (3.42±0.10mg.100g^{-1}).

Total phenolic content and flavonoids

Moringa oleifera leaves extracts showed a high level of phenolic content and flavonoids (Table 5). Methanol extract showed the highest level of phenolic content (48.35±0.05 mg GAE.g^{-1} sample) and flavonoids (0.26±0.07 mg.g^{-1}) ($P>0.05$). In contrary, the ethanol and water extracts contained low levels of phenolic content and flavonoids (28.56±0.03mg GAE.g^{-1} and16.33±0.12mg.g^{-1}) and (24.67±0.03mg GAE.g^{-1} and 0.14±0.09 mg.g^{-1}), respectively. However, there are no significant differences between ethanol and water extracts in the content of phenolics and flavonoids.

HPLC of phenolic compounds

Phenolic compounds in the *Moringa oleifera* extracts have been carried out by high performance liquid chromatography (HPLC). Ten phenolic compounds were analyzed as standards and determined the phenolic compounds in three *Moringa oleifera* extracts (gallic acid, itaconic acid, protocathechuic acid, catechin, esculetin, catechol, tannic acid, ferulic acid, pyrogallol, and cinnamic acid). HPLC chromatograms of phenolic compounds include six phenolic acids and four flavonoids are given in (Figure. 1). The concentrations of the phenolic compounds expressed in(mg.100g^{-1}).Methanol extract contained gallic acid (7.745±0.31), itaconic acid (48.53±0.27); esculetin (230.37±0.28), catechol (30.185±0.21), pyrogallol (440.94±0.24) and cinnamic acid (0.0295±0.23) respectively(Figure. 2).While, ethanol extract contained gallic acid (1.695±0.25), itaconic acid (6.195±0.12), catechin (34.42±0.19), and catechol (7.185±0.25) respectively (Figure. 3); and Water extract contained

Table 1. Proximate analysis of *Moringa oleifera*

Nutrient	Conc. ($g.100g^{-1}$)
Moisture	10.74±0.05
Ash	4.56±0.13
Fiber	11.23±0.16
Protein	9.38±0.23
Lipid	7.76±0.21
Carbohydrate	56.33±0.27
Energy (K Cal)	332.68±0.06

Table 2. Mineral Content of moringa oleifera leaves

Mineral	Conc. ($mg.100g^{-1}$)
Na	289.34±0.35
K	33.63±0.24
Mg	25.64±0.25
P	105.23±0.32
Fe	9.45±0.16
Zn	1.63±0.021
Cu	0.88±0.52
Ca	486.23±0.11
Mn	5.21±0.12

Table 3. Vitamin content

Vitamin	Conc. ($mg.100g^{-1}$)
Vitamin A (β- Carotene)	13.48±0.51
Vitamin E	16.80±0.24
Vitamin C	245.13±0.46
Vitamin B1 (Thiamin)	0.05±0.28
Vitamin B2 (Riboflavin)	0.8±0.25
Vitamin B3- (Nicotinic Acid)	220±0.42

Table 4. Amino Acid composition of crud protein

Amino Acid	Conc. (mg.100g^{-1})
Lysine	69.13±0.13
Histidine	29.56±0.21
Valine	62.34±0.19
Leucine	94.36±0.31
Isoleucine	46.98±0.15
Threonine	48.35±0.26
Alanine	4.93±0.12
Aspartic acid	13.76±0.15
Serine	3.13±0.15
Proline	1.86±0.13
Glutamic acid	18.03±0.09
Glycine	2.31±0.21
Arginine	7.65±0.10
Cysteine	2.15±0.11
Tyrosine	2.03±0.13
Methionine	0.43±0.14
Phenylalanine	3.42

Table 5. Phenolic content and flavonoids

Extract	Total phenolic content (mg GAE.g^{-1})	Total flavonoids (mg.g^{-1})
70% Methanol extract	48.35±0.05	35.64±0.07
70% Ethanol extract	28.56±0.03	16.33±0.12
Water extract	24.67±0.03	14.32±0.09

The values in the table are means of the triplicates ±SD, P>0.05

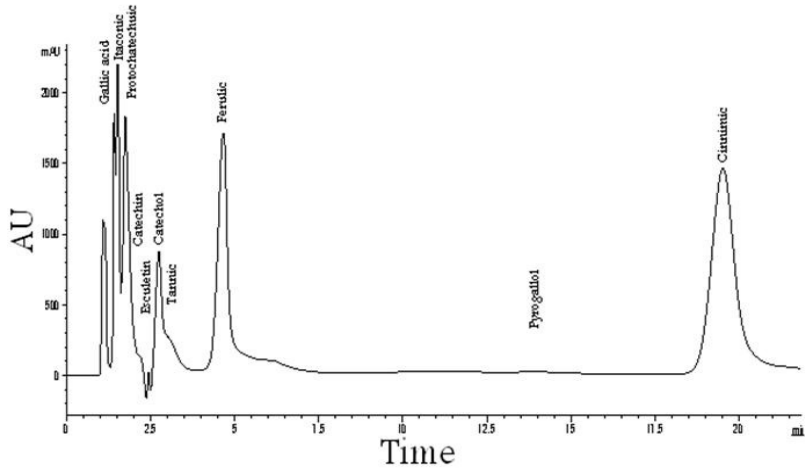

Figure 1. HPLC chromatogram for separating 10 standard phenolic compounds. All peaks were identified by comparison of retention time and UV spectra with commercial standards as follows. Zorbax Eclipse XDB-C18 column (4.6 x 150 mm, 5 µm), gradient eluent acetic acid / acetonitrile/ water/, flow rate1.2 mL/min. Peak identification: (1) gallic acid (2) Itaconic acid, (3) protocathechuic acid, (4) catechin, (5)Esculetin, (6) Catechol, (7) Tannic acid, (8) ferulic acid, (9) Pyrogallol., (10) cinnmaic acid"

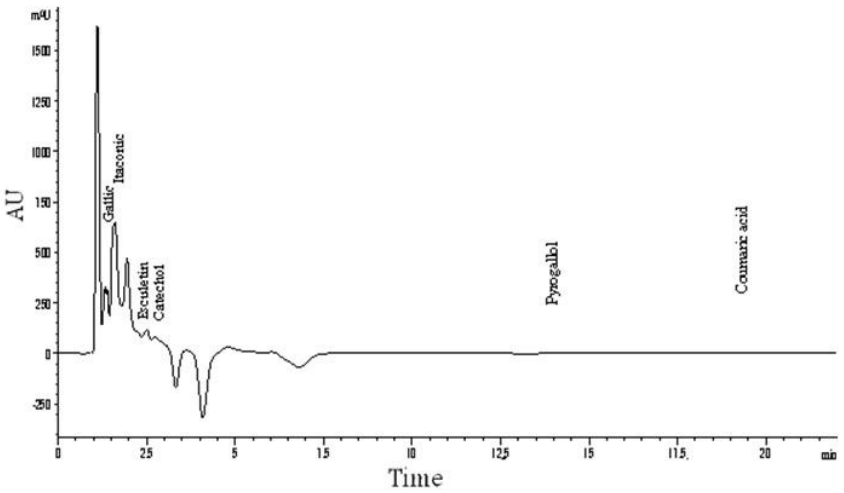

Figure 2. HPLC chromatogram with UV-VIS detection of the methanol extracts: (1) Gallic acid (2) Itaconic acid (3) Esculetin (4)Catechol (5) Pyrogallol (6) Cinnimic acid

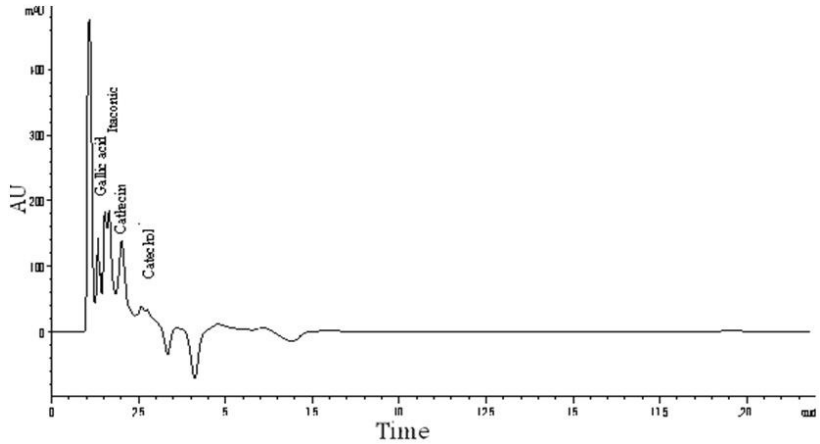

Figure 3. HPLC chromatogram with UV-VIS detection of the ethanol extracts: (1) Gallic acid (2) Itaconic acid (3) Cathecin (4) Catechol

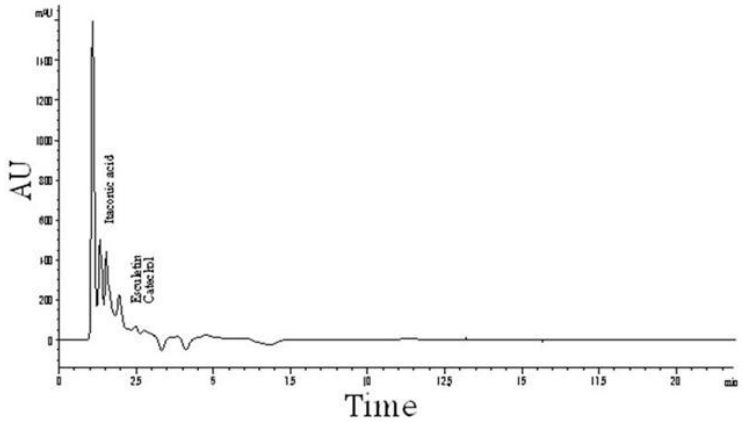

Figure 4. HPLC chromatogram with UV-VIS detection of the water extracts: (1) Itaconic acid (2) Esculetin (3) Catechol

Table 6. 50% inhibition (IC_{50}) of moringa leaf extracts

Extracts	($\mu g.ml^{-1}$)
70% Methanol	33.11±0.08
70% Ethanol	44.10±0.05
100% Water	46.77±0.13
Ascorbic acid	23.44±0.05

The values in the table are means of the triplicates ±SD, P>0.05

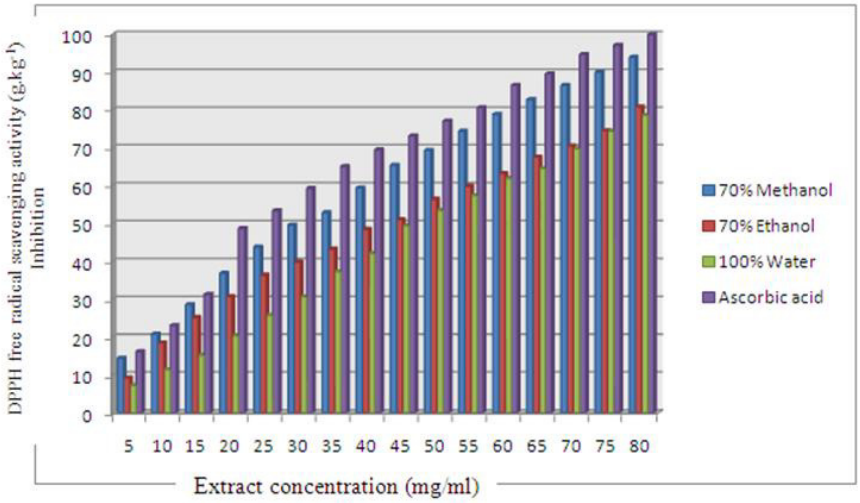

Fig 5 DPPH free radical scavenging activity of *Moringa oleifera* leaves

itaconic acid (25.66±0.23), esculetin (81.145±0.28), and catechol (8.725±0.23) respectively (Figure. 4)

DPPH Free Radical Scavenging Activity

DPPH scavenging activity was carried out for establishing the antioxidant activity of 70% methanol extract, 70 % ethanol extract and water extract of *Moringa oleifera* leaves. The methanol extract showed the highest antioxidant activity which very close of ascorbic acid (lowest IC_{50}at the extract concentration of 33.11±0.08$\mu g.ml^{-1}$)(Table 6, Figure. 5). While; ethanol extract showed a moderate antioxidant activity (IC_{50} at the concentration of 44.10±0.27 $\mu g.ml^{-1}$)(Table 6, Figure. 5).On the other hand, water extract showed the lowest antioxidant activity (highest IC_{50}at the concentration of 46.77±0.13 $\mu g.ml^{-1}$).There is no significant difference in IC_{50} between the ethanol and water extract. While the ascorbic acid as antioxidant standard showed the lowest antioxidant activity (IC_{50} = 23.44±0.05 $\mu g.ml^{-1}$) even over methanol extract. From the results in (Table 6, Figure. 5), there is a positive correlation between the extract concentration, the antioxidant activity and the concentration of phenolic content in the three extracts.

Antimicrobial activity

Figure (6) showed the antimicrobial activity of *Moringa oleifera* leaf extracts against nine pathogenic species of bacteria and fungi. The 70% methanol, 70% ethanol and water extracts were exhibited antimicrobial activities against *Streptococcus pyogenes* ATCC12344, *Streptococcus agalactiae* ATCC12296, *Staphylococcus epidermis* ATCC35984, *Staphylococcus aureus 0006, Salmonella senftenberg* ATCC8400*, Escherichia coli* O-143,*Bacillus subtilis DB100 host*, and *Candidaalbicans* ATCC2091 but no inhibition found against *Klebsiella pneumonia* ATCC 12296. The minimum inhibitory concentration (MIC) of *Moringa oleifera* leaves extracts were carried out and the obtained results confirmed that, the MIC of 70% methanol was (40 $mg.ml^{-1}$) and for 70% ethanol and water was (50 $mg.ml^{-1}$) (Table 7).From the obtained results in our experiment, *Moringa oleifera* leaves extracts possessed a broad range of antimicrobial activity against the most examined bacterial strains and fungi (Figure. 6).70% methanol extract showed the high toxicity

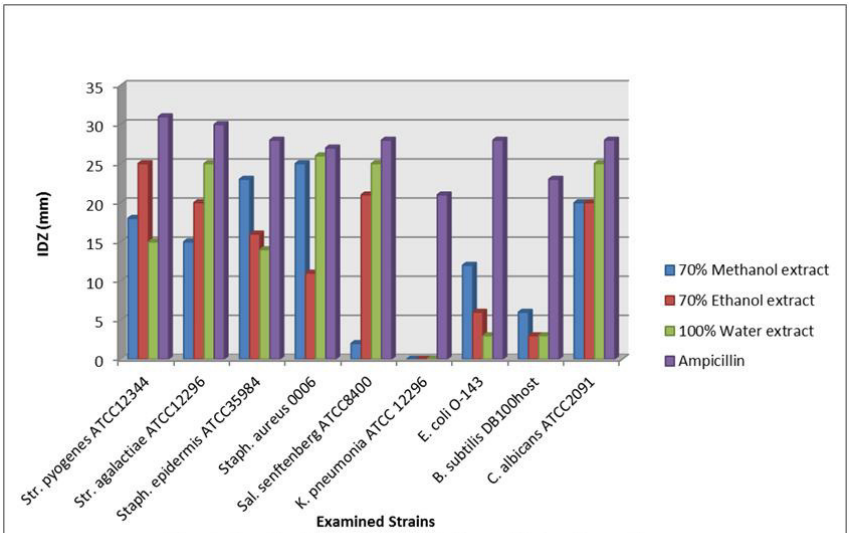

Fig 6 Antimicrobial activity of Moringa leaves

Table7. MIC of Moringa oleifera leaf extract

($mg.ml^{-1}$)	Extract		
	70% Methanol	**70% Ethanol**	**100% Water**
10	-	-	-
20	-	-	-
30	-	-	-
40	++	-	-
50	+++	++	++
60	+++	++	++
70	+++	+++	+++
80	+++	+++	+++
90	+++	+++	+++
100	++++	+++	+++

(+) Inhibition zone detected; (-) No inhibition zone detected

against *Staphylococcus aureus 0006*(25±0.06mm), *Staphylococcus epidermis* ATCC35984(23±0.04mm), *Streptococcus pyogenes*ATCC12344 (18±0.04 mm) and *Candida albicans*ATCC2091 (20±0.14mm), while the same extract showed a moderate toxicity against *Escherichia coli* O-143(12±0.15mm). The lowest antibacterial activity was registered against *Bacillus subtilis* DB100host (6±0.21mm) and *Salmonella senftenberg* ATCC8400 (2±0.05 mm). On the other hand 70% ethanol extract showed the highest antimicrobial activity against *Streptococcus pyogenes* ATCC12344 (25±0.12mm), *Streptococcus agalactiae* ATCC12296 (20±0.08mm), *Salmonella senftenberg* ATCC8400 (21±0.07mm), *Staphylococcus epidermis* ATCC35984 (16±0.22mm) and *Candida albicans* ATCC2091 (20±0.22mm); while the same extract showed a moderate toxicity against *Staphylococcus aureus 0006* (11±0.10mm), *Escherichia coli* O-143 (6±0.08mm) and *Bacillus subtilis* DB100host (3±0.13mm). While water extract registered the highest antimicrobial activity against *Staphylococcus aureus 0006* (26±0.18mm), *Streptococcus agalactiae* ATCC12296 (25±0.19 mm), *Salmonella senftenberg* ATCC8400 (25±0.14mm) and *Candida albicans* ATCC2091 (25±0.16mm); while the same extract reported a moderate toxicity against *Streptococcus pyogenes* ATCC12344 (15±0.09mm). The lowest toxicity was reported against *Escherichia coli* O-143(3±0.11mm) and *Bacillus subtilis* DB100host (3±0.31mm). On the other hand, there was no any toxicity registered against *Klebsiella pneumonia* ATCC12296 for the three kind of *Moringa oleifera* extracts.

DISCUSSION

The results of proximate analyses revealed that the *Moringa oleifera* leaves are an excellent source of nutrition and natural energy for human around the world who lack in many nutritional supplements such as protein (9.38±0.23g.100g^{-1}), carbohydrate (56.33±0.27g.100g^{-1}), lipids (7.76±0.21 g.100g^{-1}) and fibers (11.23±0.16 g.100g^{-1}) (Table 1). 100g of *Moringa oleifera* leaves can provide about 17.5 g of daily requirement. Moisture (10.74±0.05 g.100g^{-1}) in food determines the rate of food absorption and the keeping quality of food. The reported value indicated that *Moringa oleifera* leaf protein might not be stored at room temperature for a long period. Ash (4.56±0.13) in food determines largely the extent of mineral matters likely to be found in food substance, the reported value of ash (4.56±0.13g.100g^{-1}) indicated that moringa leaves are a good source of minerals. *Moringa oleifera* is a good source of fiber (11.23±0.16 g.100g^{-1}) that might be taken as a part of diet to clean the digestive tract by removing potential carcinogens from the body and hence prevents the absorption of excess cholesterol. The fat and carbohydrate content is very valuable as a main source of energy for human body. The same results mentioned by (Sodamade *et al*. 2013), who revealed that *Moringa oleifera* leaves are nutritionally adequate and given the promising source of dietary minerals in most developing countries. It is however important to stress that leaf protein concentrates is not food on their own but it contains nutritional potential that could find application in food ingredient, infant formula, food supplement and food formulation. The Moringa leaves mineral concentrations might be candidate to be one of the important sources of essential elements for human body. Moringa leaves contained a high level of sodium (289.34±0.35 mg.100g^{-1}) (Table 2); while sodium is an important source of electrolytes within the body; potassium (33.63±0.24 mg.100g^{-1}) works with sodium to maintain the water balance in the body and lowering the blood pressure. Magnesium level in moringa leaves was (25.64±0.25 mg.100g^{-1}) that is extremely vital to health by stimulating gastric motility and intestinal function; a high content of phosphorus (105.23±0.32 mg.100g^{-1}) is an important to serve as the main regulator of energy metabolism in cells. Iron (9.45±0.16 mg.100g^{-1}) is very important element as a nucleus of hemoglobin that forms red blood cells in the body. Zinc (1.63±0.021mg.100g^{-1}) can support the immune system and useful for normal growth and development during pregnancy. Copper (0.88±0.52 mg.100g^{-1}) plays a role in the synthesis and maintenance of myelin and as a cofactor for processes that neutralize the dangerous free radicals. Moringa leaves are a very good source of calcium (486.23±0.11mg.100g^{-1}) that very useful for bones and teeth development. Manganese (5.21±0.12mg.100g^{-1}) is very useful for activation of some enzymes that prevent tissue damage and used for digestion and utilization of foods. These obtained results agreed with that registered by (Sodamade *et al,* 2013 and Oluwole *et al,* 2013) whose reported that the moringa leaves are a very promising source for essential elements. *Moringa oleifera* leaves contained a reasonable concentrations of both water-soluble vitamins such as B {Vitamin B1 (Thiamin) 0.05±0.28, Vitamin B2 (Riboflavin) 0.8±0.25, Vitamin B3-(Nicotinic Acid) 220±0.42} and C (245.13±0.46) and fat-soluble vitamins like A (13.48±0.51mg.100g^{-1}) and E(16.80±0.24) mg.100g^{-1} respectively (Table 3). These vitamins could play an important role in improving human health. Vitamin A is a natural antioxidant to inhibit free radicals and very important for improving the immune system. Vitamin E is useful for enhancing the immune system function and skin repair. Vitamin C is very important for cardiovascular health and reducing free radicals in the cells. Vitamin B1 contributes in many cellular functions including carbohydrates metabolism. Vitamin B2 is an important in energy metabolism and folate synthesis. Vitamin B3 plays a role in DNA synthesis and the transfer of methyl groups in the cell metabolism. From the present results, it is clear that *Moringa oleifera* leaves are a powerful vitamin factory in reasonable concentrations for human requirements. The results obtained from the amino acid composition of moringa leaves crude protein confirmed that *Moringa oleifera* leaves contain a high level of Leucine (94.36±0.31mg.100g^{-1}), Lysine(69.13±0.13 mg.100g^{-1}), Valine (62.34±0.19 mg.100g^{-1}), Threonine (48.35±0.26mg.100g^{-1}), and Isoleucine (46.98±0.15mg.100g^{-1}) (Table 4). It is confirmed that moringa leaves are a good source for essential amino acids (Lysine, Methionine, Phenylalanine, Histidine, Leucine, Isoleucine and Valine). The Moringa essential amino acids presence and digestibility scores are more than adequate when measured against the standards (WHO) and (FAO) for small children, the most at-risk population group when it comes to proteins in food. Furthermore, the obtained results showed that moringa leaves contained a high level of lysine, which usually accrued, in a low level in plant materials except legumes and cereals. These results agreed with the results reported by Oluwole *et al* (2013), who have investigated the nutrient composition and phytochemicals of *Moringa oleifera* and revealed that, Moringa could be incorporated into human diet, particularly during infancy, to prevent or reduce protein-energy malnutrition. Phenolic compounds and flavonoids are very important constituents that have antioxidant activity by scavenging free radicals and occurred in several kinds of plants; the total phenolic content determination in moringa leaves revealed that the type of extraction solvent is a limiting factor in the extraction of phenolics and flavonoids. 70% methanol extracted the maximum phenolic content occurred in the moringa leaves (48.35±0.05 GAE.g^{-1} sample) compared to 70% ethanol (28.56±0.03 GAE.g^{-1} sample) and water (24.67±0.03 GAE.g^{-1} sample) ($P>0.05$) (Table 5). These

results disagreed with (Luqman *et al.,* 2012) who reported that, total phenolic content increasing in the concentration-dependent manner, but there is a significant rise in the phenolic content with ethanol extract. The same trend was observed with the extraction of total flavonoids when 70% methanol extracted the maximum level of flavonoids (35.64±0.07 $mg.g^{-1}$), while 70% ethanol extracted (16.33±0.12 $mg.g^{-1}$) and water extracted the minimum level of flavonoids (14.32±0.09 $mg.g^{-1}$) ($P>0.05$) (Table 5).The extraction of antioxidant substances of different chemical structure was achieved using solvents in different polarities. From the obtained results, the highest level of phenolic compounds was obtained by 70 % methanol (6 compounds) and showed the highest concentration of pyrogallol (440±0.24 $mg.100g^{-1}$) and esculetin (230.37±0.28 $mg.100g^{-1}$) (Figure.2). While 70% ethanol extracted four compounds with the maximum concentration of (34.42±0.19 $mg.100g^{-1}$) catechin (Figure. 3) and water extracted only three phenolic compounds with high concentration of (81.145±0.28 $mg.100g^{-1}$) esculetin (Figure. 4). Protocathechuic acid, tannic acid and ferulic acid were not detected in all three extracts. It is appeared that methanol 70% is the most suitable solvent for extraction of phenolic compounds from *Moringa oleifera* leaves (Figure. 2). Numerous investigations of qualitative composition of plant extracts revealed the presence of high concentrations of phenolic compounds obtained using polar solvents (Čanadanović-Brunet *et al*, 2008 and Stanković, 2011).Methanol and water were the stronger extraction media that are able to dissolve most of the phenolic compounds from samples. Methanol is able to extract semi-polar phenolics while water is more favored to polar phenolic acid. The *Moringa oleifera* might be regarded as a promising candidate as a natural plant rich in phenolic compounds. The DPPH free radical scavenging activity current results is similar to that reported by (Gülcin *et al*. 2003), (Sreelatha and Padma (2009), (Huda-Faujan *et al.,* 2009) and (Noriham *et al*. 2004). The all three types of moringa leaves extract showed a considerable antioxidant activity that increased with increasing the concentration of moringa extract (10-80$\mu g.ml^{-1}$) (Figure. 5). 70% methanol extract showed the highest antioxidant activity (lowest IC_{50}=33.11±0.08$\mu g.ml^{-1}$) and the closest one to ascorbic acid (IC_{50}=23.44±0.05) as standard ($P>0.05$, Table 6). In contrast, there is no significant differences were remarked between the antioxidant activity of 70% ethanol and water extracts (IC_{50}= 44.10±0.05 and 46.77±0.13 $\mu g.ml^{-1}$respectively) (Figure. 5). Antioxidant activity of moringa leaves extracts, which comes from phenolic compounds and flavonoids might be involved in human body protection against free radicals causing the damage to the body over time. These results confirmed that *Moringa oleifera* leaves extract might be a potent source of natural antioxidants with a high human health benefits. Moringa leaves extracts recorded a broad spectrum of antimicrobial activity against the most of tested pathogenic strains with MIC between (40 and 50 $mg.ml^{-1}$)(Figure. 6). *Staphylococcus epidermis*, *Streptococcus pyogenes, streptococcus agalactiae and Candida albicans are* the most common pathogens were affected by three types of moringa extracts (70% methanol, 70 % ethanol and water) (Figure. 6). On the other hand, 70% methanol and water extracts have a common effect against *Staphylococcus aureus* while, 70% ethanol and water extracts have a common toxicity against *Salmonella senftenberg* (Figure.6). There was no relation between the concentration of total phenolic content and antimicrobial activity was found. Water extract, which contained the lowest concentration of phenolic compounds showed the highest antimicrobial activity. It might be referred to the type and chemical structure of phenolic compounds in the extract and the ability of these compounds to bind to the bacterial cell and prevent the cell division. The known antibacterial mechanism associated to each class of chemical to which the isolated compounds belong, may explain the antibacterial potency of the crude plant extract (El Sohaimy, 2014). This indicates that *Moringa oleifera* leaves extracts may be used as a natural antimicrobial agent with reasonable safety margins to inhibit bacterial growth in pharmaceutical and food applications. *Moringa oleifera* could be used in curing many diseases like typhoid fever, diarrhea, high blood sugar, hypertension, and gastro-intestinal disorder. It is advised that this plant can be utilized in cooking and making other edible formulations (Olako, 2014).

CONCLUSIONS

Moringa is considered as a nutrient-rich plant and the obtained results in this study indicated that the leaves have immense nutritional value such as phytochemicals, vitamins, minerals, proteins, vitamins and amino acids. So, the leaves might be used to combat malnutrition, especially among infants and nursing mothers. Many of the benefits of *Moringa oleifera* leaves are attributed to rich nutrients like protein elements and rich antioxidants, which come from vitamins, and polyphenols that makes the Moringa leaves an important part of healthy and balanced diet. Our results proved that the *Moringa oleifera* leaves contain good antimicrobial activity agents as presented by the composition of the secondary metabolites of the leaf extract. These results confirmed that *Moringa oleifera* leaves extract might be a potent source of natural antioxidants with a high health benefits. This indicates that *Moringa oleifera* leaves extracts may be used as a natural antioxidant and antimicrobial agent with reasonable safety margins in pharmaceutical and food applications.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Food Technology, Arid Lands Cultivation Research Institute, City for Scientific Research and Technological Application, Alexandria, Egypt for providing the chemicals and facilities of this research work. The authors are thankful for the junior staff especially, Mr. Mohamed Gamal, Mr. Taha Mehany and Mrs. May Mamdouh for their help in this study.

REFERENCES

Ackurt F, Özdemir M, Biringen G, Löker M (1999). Effects of geographical origin and variety on vitamin and mineral composition of hazelnut (*Corylusavellana* L.) varieties cultivated in Turkey. Food Chemistry.65: 309-313.

Al-Kharusi LM, Elmardi MO, Ali A, Al-Said FAJ, Abdelbasit KM, AlRawahi S (2009). Effect of mineral and organic fertilizers on the chemical characteristics and quality of date fruits. Int. J. Agric Biol. **11:** 290-296.

Anjorin TS, Ikokoh P, Okolo S (2010). Mineral composition of *Moringa oleifera* leaves, pods and seeds from two regions in Abuja, Nigeria. Int. J. Agric Biol.12**:** 431-434.

Anwar F, Latif S, Ashraf M, Gilani AH (2007). *Moringa oleifera*: A food plant with multiple medicinal uses, Phytotherapy Res. 21: 17-25.

AOAC (1995).Official Methods of Analysis, Association of Official analytical Chemists. Washington DC, USA.

AOAC (2000). Official Methods of Analysis, Association of Official Analytical Chemists. Washington, DC, USA.

AOAC (2005).Official Methods of Analysis, 17th Ed., Association official Analytical Chemists. Washington DC, USA.

AOAC (2005a). Official method of analysis, 992.06 Vitamin A (Retinol) in milk-based infant formula, *In*: Phifer E (Ed) Official methods of analysis of AOAC International. Chapter50, pp 2.

AOAC (2005b). Official method of analysis, 992.03 Vitamin E activity (All-rac-α-Tocopherol) in milk-based infant formulam. *In*: Phifer E (Ed) Official methods of analysis of AOAC International. Chapter50, pp 4.

Atlas RM (1993). Handbook of microbiological media. p. 147-153, CRC Press, Boca Raton, FL.

Brisibe EA, Umoren UE, Brisibe F, Magalhaes PM, Ferreira JFS, Luthria D, Wu X, Prior RL (2009). Nutritional characterization and antioxidant capacity of different tissues of *Artemisia annua* L., Food Chem.115**:** 1240-1246.

Čanadanović-Brunet J, Ćetković G, Đilas S, Tumbas V, Bogdanović G, Mandić A, Markov S, Cvetković D, Čanadanović V (2008). Radical scavenging, antibacterial, and antiproliferative activities of *Melissa officinalis L.* extracts. J. Med. Food.11**:** 133-143.

El Sohaimy SA (2014).Chemical Composition, Antioxidant and Antimicrobial Potential of Artichoke, The Open Nutraceuticals Journal.7: 15-20.

El Sohaimy SA, Masry SHD (2014). Phenolic content, antioxidant and antimicrobial activities of Egyptian and Chinese Propolis, American-Eurasian J. Agric. And Environ. Sci.14: 1116-1124.

Fahey, J (2005). *Moringa oleifera*: A review of the Medical Evidence for its Nutritional, Therapeutic, and Prophylactic Properties Part 1, Trees for Life Journal.1: 5-10.

Finglas PM, Faulks RM (1984).The HPLC Analysis of Thiamin and Riboflavin in Potatoes, Food Chemistry. 15**:** 37-44.

Folch JM, Stanley GHS (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry, 226: 497-509.

Gülcin I, Oktay M, Kıreçci E, Küfrevio I (2003). Screening of antioxidant and antimicrobial activities of anise (*Pimpellaanisum* L.) seed extracts.Food Chem. 83: 371-382.

Huda-Faujan N, Noriham A, Norrakiah AS,Babji AS (2010). Antioxidant activity of plants methanolic extracts containing phenolic compounds.African Journal of Biotechnology, 8: 484-489.

Kolayli S, Şahin H, Ulusoy E, Tarhan Ö (2010). Phenolic Composition and Antioxidant Capacities of *Helichrysumplicatum Hacettepe*, J. Biol. And Chem. 38: 269-276.

LuqmanS, Srivastava S, Kumar R, Maurya AK, Chanda D (2012). Experimental Assessment of *Moringa oleifera* Leaf and Fruit for Its Antistress, Antioxidant, and Scavenging Potential Using In Vitro and In Vivo Assays. Evidence-Based Complementary and Alternative Medicine**,** 1-12.

Makkar HPS, Becker K (1996). Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. Animal Feed Science Tech.63**:**211-228.

Martin EA, Coolidge AA (1978). Nutrition in action. 4thed. Holt, R and Wilson Co., New York, NY.

Moyo B, Masika PJ, Hugo A, Muchenje V (2011). Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. African Journal of Biotechnology10**:** 12925-12933.

Noriham A, Babji AS, Aminah A (2004). Determination of antioxidative activities of selected Malaysian plant extracts. ASEAN. Food. J. 13**:** 193-199.

Olako EN (2014). Phytochemical Analysis and Antimicrobial Screening of *Moringa oleifera* Leaves Extract. The International Journal of Engineering and Science (IJES)3**:** 32-35.

Oluwole SI, Oluwole AA, Oluwaseun A (2013). Comparative study on nutrient composition, phytochemical, and functional characteristics of raw, germinated, and fermented *Moringa oleifera* seed flour. Food Science & Nutrition, 1**:** 452-463.

Öztürk N, Tuncel M, Tuncel NB (2007). Determination of phenolic acids by a modified HPLC: Its application to various plant materials.J. Liq. Chromatogr. Relat. Technol.30: 587-596.

Perez JM, Lebas F, Gidenne T, Maertens L, Xiccato G (1995). European reference method for *in vivo* determination of diet digestibility in rabbits. World Rabbit Sci.3: 41-43.

Rakesh S, Singh VJ (2010).*In vivo* antioxidant activity of *Moringa oleifera* leaf and pod extracts against carbon tetra chloride induced liver damage in albino mice. J. Chem. Pharm. Res.2**:**275-283.

Singh Y, Jale R, Prasad KK, Sharma RK, Prasad K (2012). *Moringa oleifera*: A Miracle Tree, Proceedings, International Seminar on Renewable Energy for Institutions and Communities in Urban and Rural Settings, Manav Institute, Jevra, India, pp. 73-81.

Sodamade A, Bolaji OS, Adeboye OO (2013). Proximate Analysis, Mineral Contents and Functional Properties of *Moringa oleifera* Leaf Protein Concentrate. IOSR Journal of Applied Chemistry (IOSR-JAC)**,** 4: 47-51.

Spackman DH, Stein EH, Moore S (1958). Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem.**30**:1190-1191.

Sreelatha S, Padma PR (2009). Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. Plant Foods Hum Nutr.64**:** 303-311.

Stanković MS (2011). Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubiumperegrinum* L. extracts. Kragujevac J. Sci.33: 63-72.