

Antioxidant activity of different extracts from the aerial part of *Moringa peregrina* (Forssk.) Fiori, from Jordan

Maher Mahmoud Al-Dabbas*

Department of Nutrition and Food Technology, Faculty of Agriculture, The University of Jordan, Amman, Jordan

Abstract: The antioxidant activities of methanol (M), ethyl acetate (E) and hexane (H) extracts from leaves (L) and seeds (S) of *Moringa peregrina* were evaluated using different model systems *in vitro*. Free radical scavenging activities were assessed by measuring the scavenging activities of leaves and seeds different polar extracts separately using ABTS, Hydroxyl (OH) and DPPH radicals. Effect of extracts on ferrous ions chelating ability and total antioxidant capacity were also investigated for each extract. In addition, total phenolics, flavonoids and flavonols content of *Moringa* leaves and seeds extracts were determined. The leaves methanol (LM) extract showed significantly the highest DPPH radical scavenging activity (IC_{50} value of $5.3 \pm 0.2 \mu\text{g/ml}$), followed by leaves ethylacetate extract (LE) and seeds methanolic extract (SM) with IC_{50} values of 7.1 ± 0.2 and $7.2 \pm 0.4 \mu\text{g/ml}$, respectively. LE extract showed the highest ABTS radical scavenging activity with IC_{50} value of $49.1 \pm 2.7 \mu\text{g/ml}$, followed by LM extract with IC_{50} value of $61.2 \pm 1.2 \mu\text{g/ml}$, whereas the highest hydroxyl radical (OH \cdot) inhibition activity was found for LM and SM extracts with IC_{50} values of 76.9 ± 0.8 and $77.5 \pm 1.2 \mu\text{g/ml}$, respectively. The total antioxidant activity was the highest in LM, LE and SM extracts (294.3 , 244.5 and $231.6 \mu\text{g}$ ascorbic acid equivalent for 1mg extract, respectively). LM, LE and SM extracts at concentration of $100 \mu\text{g/ml}$ showed the highest chelating activity against ferrous ions (98.4 , 91.1 and 90.7% , respectively). All *Moringa* leaves and seeds extracts showed pronounced antioxidant activities in a dose dependent manner and the effects depend strongly on the solvent used for extraction. The results showed that extracts of both leaves and seeds of *Moringa* exhibit antioxidant potential suggesting that *M. peregrina* is a promising plant.

Keywords: *Moringa peregrina*, antioxidant activity, DPPH, ABTS, phenolics.

INTRODUCTION

Herbs, spices and plants have been shown to possess a wide range of pharmacological and therapeutic properties and recently most researches are conducted to isolate and identify the compounds occurring in such plants for their medicinal and biological activities. Many studies have investigated the potential of plant secondary metabolites as antioxidants like phenols, flavonoids, vitamin C and E and tannins...etc against reactive oxygen species (ROS) which are responsible for various diseases including cancer, diabetes mellitus, atherosclerosis, heart diseases, neurodegenerative diseases and many other diseases that induced by free radicals (Broadhurst *et al.*, 2000; Halliwell, 1994; Addis and Warner, 1991).

Synthetic antioxidants like BHT, TBHQ and BHA are usually used in oil or in fat rich food to protect against free radicals damage by scavenging reactive oxygen radicals or terminating radical chain reactions by donating hydrogen from their phenolic hydroxyl group, but due to health concerns resulted from the use of synthetic antioxidants much interest is given to the use of natural antioxidants from plants and herbs (Lu and Foo, 2000; Rice-Evans *et al.*, 1997). It is generally accepted by customers that any medicine derived from plant sources are safer and healthier than their synthetic counterparts (Vongatu *et al.*, 2005; Oluyemi *et al.*, 2007).

Phenolic compounds derived from plants and herbs are one of the major groups of plant secondary metabolites that act as primary antioxidants and free radical scavengers, and flavonoids, in particular, are the most diverse phenolic compounds and probably the most important natural phenolics that possess a broad spectrum of biological and chemical activities (Hayase and Kato, 1984; Dziezak, 1986; Agrawal, 1989; Osawa, 1994).

Moringa is a single genus in the family moringaceae with 14 known species grown in tropical and sub-tropical climates as herbs or massive trees from tropical Africa to the east Indies (Rashid *et al.*, 2008; FAO, 1988). The chemical composition, physicochemical characteristics, protein functional properties, fatty acid composition and medicinal uses of *Moringa peregrina* seeds and seeds oil have been reported by many workers (Somali *et al.*, 1984; Al-Kahtani and Abou-Arab, 1993; Al-dabbas *et al.*, 2010).

In Jordan, *Moringa peregrina* (Forssk) Fiori, occurs naturally in lower Jordan valley, Dead Sea area, Wadi Araba and Wadi Feynan and locally called Al-Ban or Al-Yassar (Al-dabbas *et al.*, 2010). It is a rapidly growing tree of 5-15 meter high and bear long seed pods. The mature seeds have sweet to unpleasant bitter taste. Seeds are of economic, nutritional and medicinal importance; the extracted seed oil is used by Bedouins in cooking, while seeds used as laxative in medicine and in feeding livestock (Hegazy *et al.*, 2008, Al-dabbas *et al.*, 2010).

*Corresponding author: e-mail: m.aldabbas@ju.edu.jo

The antioxidant activity of methanolic extract of *M. peregrina* leaves has been studied and the isolation of rutin was reported (Dehshahri *et al.*, 2012). In addition, antioxidant activities of *Moringa oleifera* leaves and seeds (Lalas and Tsaknis, 2002; Siddiq *et al.*, 2005; Siddhuraju and Becker, 2003) have been reported. The isolation and identification of some flavonoids from *M. oleifera* leaves, such as kaempferol, rhamnetin, isoquercitrin, and kaempferitrin were established (Iqbal and Bhangar, 2006). The aim of this study is to determine the antioxidant properties of different polar extracts from the Jordanian *Moringa peregrina* seeds and leaves with respect to radical scavenging activities, chelating power, total antioxidant activity, phenolics and flavonoids contents.

MATERIALS AND METHODS

Plant materials

The fully mature seeds of *M. peregrina* (500 g) and leaves (500g) were collected from Wadi Fenan at the end of growing season (August 2013). A voucher specimen (JOH3089) was deposited in the herbarium of the National Center for Agricultural Research and Extension (NCARE), Baqa'a, Jordan.

Solvents extraction

The ground sun-dried seeds and/or leaves (300g) were extracted separately with hexane, ethyl acetate and methanol using Soxhlet apparatus at 60°C until the refluxed solvent became colorless. The extracts were separately evaporated to dryness at 40°C using Heidolph rotary evaporator (model 4001, Schwabach, Germany). The yield of hexane seed extract was 16.1% (w/w), whereas for ethyl acetate seed extract was 6.8% and for methanol seed extract was 3.6%. The yields for leaf extracts were 6.6% (w/w) in the hexane extract, 5.3% in the ethyl acetate extract and 4.2% in the methanol extract. All extracts were stored in separate screw cap brown bottles at 4°C before analysis.

Determination of total phenolic compound contents

The total phenolic compounds contents present in seeds or leaves extracts were determined according to the method described by Duh and Yen (1997). Catechole was used as the standard for the calibration curve. Aliquot (100µl) from each extract solution (10 g/l) was transferred into 20 ml volumetric flask and diluted with 10 ml distilled water. Then 1ml of Folin-Ciocalteu and 2 ml of sodium carbonate solution (10%, w/v) were added to each flask and mixed thoroughly by vortex. The volume is completed with distilled water and the absorbance was measured at 760 nm against the reagent blank using a UV-Visible spectrophotometer (Labomed, Model UVD-2900, CA, USA). The total phenolic compound contents (mg/g) were expressed as catechole equivalent and determined from the following regression equation based on the calibration curve

$$Y = 0.044 X, \quad R^2 = 0.96$$

Where Y is the absorbance and X the catechole concentration in mg/l. Triplicate determinations were made for each extract.

Determination of total flavonoids and flavonols

The total flavonoids and flavonols content of leaves and seeds extracts of *M. peregrina* were determined according to the method described by Miliauskas (2004). An aliquot of 1 ml of each plant extract (10 g/l) was mixed with 1 ml of 2 % aluminum trichloride in ethanol. After vortexing the reaction mixture was diluted with 25 ml ethanol and placed in dark place for 40 min at 20°C and the absorbance was measured at 415 nm using a UV-Visible spectrophotometer (Labomed, Model UVD-2900, CA, USA). A mixture of 1 ml from each plant extract and 1 drop of acetic acid diluted with 25 ml ethanol were served as blank and the absorbance was measured separately for each extract as described above. The amount of total flavonoids (mg/g) was calculated as rutin equivalents (RE) from the following regression equation based on the calibration curve.

$$Y=0.001X, R^2 = 0.998$$

Where Y is the absorbance and X the rutin concentration in mg/l. Triplicate determinations were made for each extract.

Total flavonols content was determined as follows: an aliquot of 1 ml of each plant extract (10 g/l) was taken in a test tube and 2 ml of 2% aluminum trichloride solution in ethanol and 6 ml of 5% sodium acetate solution were added to each tube. After vortexing the reaction mixture, the tubes were allowed to stand for 2.5 hr at 20°C and the absorbance was recorded at 440 nm using a UV-Visible spectrophotometer (Labomed, Model UVD-2900, CA, USA). A mixture of 1 ml from each plant extract and 2 ml of ethanol and 6 ml of sodium acetate solution were served as blank and the absorbance was measured separately for each extract as described above. The amount of total flavonols (mg/g) was calculated as rutin equivalents (RE) from the following regression equation based on the calibration curve.

$$Y=0.002X, R^2 = 0.98$$

Where Y is the absorbance and X the rutin concentration in mg/l. Triplicate determinations were made for each extract.

DPPH free radical-scavenging assay

DPPH[•] (1,1-diphenyl-2-picrylhydrazyl) stable radical was used to determine the free radical scavenging activity of leaves and seeds extracts of *M. peregrina* (Hatano *et al.*, 1988). Sample extracts at various concentrations from 0-50 µl (1000 µg/ml) of methanol, ethyl acetate and hexane extracts was added to 1 ml of methanolic solution

of DPPH ($6 \times 10^{-5} \text{M}$). Q After vortexing the reaction mixture, the decrease in absorbance of each extract and/or control (BHT) were measured at 517 nm after 30 minutes. Triplicate determinations were made for each extract. The scavenging activity of the extracts was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Control radical scavenging - Sample absorbance}}{\text{Control absorbance}} \times 100$$

ABTS radical-scavenging activity assay

The ABTS^{•+} [2, 2'-azinobis (3-ethylbenzothiazoline-6 sulphonic acid)] radical-scavenging activity of leaves and seeds extracts of *M. peregrine* was assayed by the method of Ozgen *et al.* (2006). ABTS was dissolved in 20mM acetate buffer (pH 4.5) and ABTS^{•+} radical was produced by reacting ABTS solution with potassium persulfate. Prior to assay The ABTS^{•+} radical solution was diluted with the 20mM sodium acetate buffer (pH 4.5) to give an absorbance of 0.700 ± 0.01 at 734nm in a 1cm cuvette. The extracts of leaves and seeds of *M. peregrine* were diluted to a concentration that can produce between 20% and 80% inhibition of the blank absorbance. After addition of 2.0ml of ABTS^{•+} solution to various concentrations (0-200 μ l) of each extract (1000 μ g/ml) from leaves or seeds, the reaction mixture was vortexed, allowed to stand at room temperature for 20min and the absorbance at 734 nm was recorded. The radical-scavenging activity of extracts was estimated based on percentage of the ABTS^{•+} color reduction by calculating the IC₅₀ (concentration in μ g/ml that cause 50% inhibition of ABTS^{•+} radicals) using a non-linear regression analysis. Triplicate determinations were made for each extract.

Hydroxyl radical scavenging activity assay

The scavenging activity of leaves and seeds extracts of *M. peregrina* on the Hydroxyl radicals (OH[•]) was measured using a modified deoxyribose assay (Menaga *et al.*, 2013; Halliwell *et al.*, 1992). The reaction was generated by Fenton's reaction (from Fe²⁺ - ascorbate - EDTA -H₂O₂ system). The reaction mixture containing 10 μ l of FeCl₃ (10 mM), 100 μ l EDTA (1mM), 100 μ l H₂O₂ (10mM), and 360 μ l of 2-deoxy- D-ribose (10mM), 0-200 μ l (1000 μ g/ml) from each extract separately, 330 μ l of phosphate buffer (50mM, pH 7.4). The reaction was started by adding 100 μ l (100 μ M) ascorbic acid. After incubation at 37°C for 1h, aliquot of 1 ml of incubated mixture was mixed with 1ml of 10% TCA and 1 ml of 0.5% TBA (in 0.025M NaOH containing 0.025% butylated hydroxyl anisole) and the development of pink chromogen was recorded spectrophotometrically at 532 nm against a blank (the same solution but without reagent). The hydroxyl radical (OH[•]) scavenging activity of the leaves and seeds extract was estimated based on percentage inhibition of deoxyribose degradation from the following formula:

$$\text{OH inhibition (\%)} = \frac{\text{Control absorbance (Sample absorbance - Blank absorbance)}}{\text{Control absorbance}} \times 100$$

Triplicate determinations were made for each extract.

Determination of chelating power activity

Chelating power of leaves and seeds extracts of *M. peregrine* was determined according to the method described by Kumar *et al.* (2008). Briefly: aliquot of 100 μ g/ml from each extract was mixed with 0.1ml of 2mM FeCl₂ and 0.2ml of 5mM ferrozine solutions. The reaction mixture was then incubated at 25°C for 10 min. The absorbance of the resulting solution was recorded at 562 nm for each extract. The FeCl₂ and ferrozine mixture was used as control solution. The percentage inhibition (%) of the ferrous ion in the mixture was calculated by comparing the results of leaves and seeds extracts of *M. peregrine* with those of the control using the following formula:

$$\text{Chelating activity (\%)} = \frac{[1 - (\text{absorbance extract} - \text{Extract blank absorbance})]}{\text{Control absorbance}} \times 100$$

Triplicate determinations were made for each extract.

Determination of total antioxidant activity

The total antioxidant activities of leaves and seeds extracts of *M. peregrine* was determined according to the method described by Umamaheswari and Chatterjee, 2008 as follows: an aliquot of 0.1 ml of from each extract was vortexed with 1ml of a reaction solution prepared from 0.6 M H₂SO₄, 28Mm sodium phosphate, 4mM ammonium molybdate. The glass tubes were capped and incubated for 1.5 hr in a water bath at 95°C, and the tubes were cooled to room temperature. The absorbance of the reactant mixtures was recorded at 695 nm using a UV-Visible spectrophotometer (Labomed, Model UVD-2900, CA, USA) against blank. Ascorbic acid was used as standard. The antioxidant activity was expressed as mg equivalents to ascorbic acid. Triplicate determinations were made for each extract.

STATISTICAL ANALYSIS

Statistical calculations were performed using statistical analysis system, SAS program, 2000 (SAS Institute Inc., Cary, NC, USA). Significant differences among means of treatments were determined using LSD test. Differences at $P < 0.05$ were considered significant. Regression equations and correlation coefficients (R) were determined by MS Excel software. All treatments were conducted in triplicate.

RESULTS

Total phenolic, flavonoids and flavonols contents of extracts

The total phenolics, flavonoids and flavonols of different polar extracts obtained from seeds and/or leaves of *M. peregrine* are shown in table 1. The content of phenolic compounds varied between 322 and 46.6 mg/g. It was the highest in the seeds methanolic (SM) extract, followed by the leaves methanolic (LM) extract, leaves ethyl acetate (LE) extract, seeds ethylacetate (SE) extract, seeds hexane

Table 1: Average phenolic, flavonoids and flavonols contents (mg/g) and radicals scavenging activities of *Moringa peregrina* leaves and seeds extracts.

Extracts	Total contents IC ₅₀ (µg/ml) ^a					
	Phenolics (mg/g) (i.e., Catechole equivalent)	Flavenoids (mg/g) (i.e., Rutin equivalent)	Flavonols(mg/g) (i.e., Rutin equivalent)	ABTS	OH	DPPH
Leaves						
Methanol	214.0 ±3.6 ^{b*}	152.2 ±2.3 ^d	56.7 ±1.8 ^c	61.2±1.2 ^c	76.90 ±0.8 ^c	5.3±0.2 ^d
Ethylacetate	149.4 ±2.3 ^c	194.3 ±2.8 ^b	29.3 ±1.2 ^d	49.1±2.7 ^d	102.1 ±1.3 ^b	7.1 ±0.2 ^c
Hexane	46.6 ±1.9 ^f	181.5 ±3.3 ^c	6.0 ±0.7 ^f	ND	94.2 ±1.1 ^c	34.2 ±0.8 ^a
Seeds						
Methanol	322.2 ±4.5 ^a	156.5 ±2.7 ^d	73.3 ±1.9 ^a	72.6 ±1.8 ^b	77.5 ±1.2 ^c	7.2 ±0.4 ^{b,c}
Ethylacetate	68.6 ±2.3 ^d	192.1 ±5.3 ^b	66.1 ±2.1 ^b	92.3 ±1.6 ^a	84.7 ±2.0 ^d	8.2 ±0.6 ^b
Hexane	59.8 ±2.6 ^c	214.2±12.2 ^a T	13.3 ±0.8 ^c	ND	111.8 ±2.8 ^a	ND

* Means within columns followed by different letters are significantly different ($P<0.05$) according to LSD.T = Turbid.

^a Concentrations at which the tested radicals were inhibited by 50%.

Table 2: Average total antioxidant activity and chelating power of various extracts from leaves and seeds of *Moringa peregrina* at concentration of 100µg/ml.

Extracts	Chelating power (Inhibition %)	Total antioxidant activity (µg ascorbic acid equivalent/ mg extract)
Leaves		
Methanol	98.4 ± 1.9 ^a	294.3 ± 13.2 ^a
Ethylacetate	91.1 ± 1.6 ^b	244.5 ± 10.2 ^b
Hexane	44.2 ± 0.7 ^d	13.0 ± 3.3 ^c
Seeds		
Methanol	90.7 ± 1.3 ^b	231.6 ± 14.3 ^b
Ethylacetate	86.3 ± 1.5 ^c	200.3 ± 11.6 ^c
Hexane	24.5 ± 0.8 ^e	94.7 ± 9.4 ^d

Means within columns followed by different letters are significantly different ($P<0.05$) according to LSD.

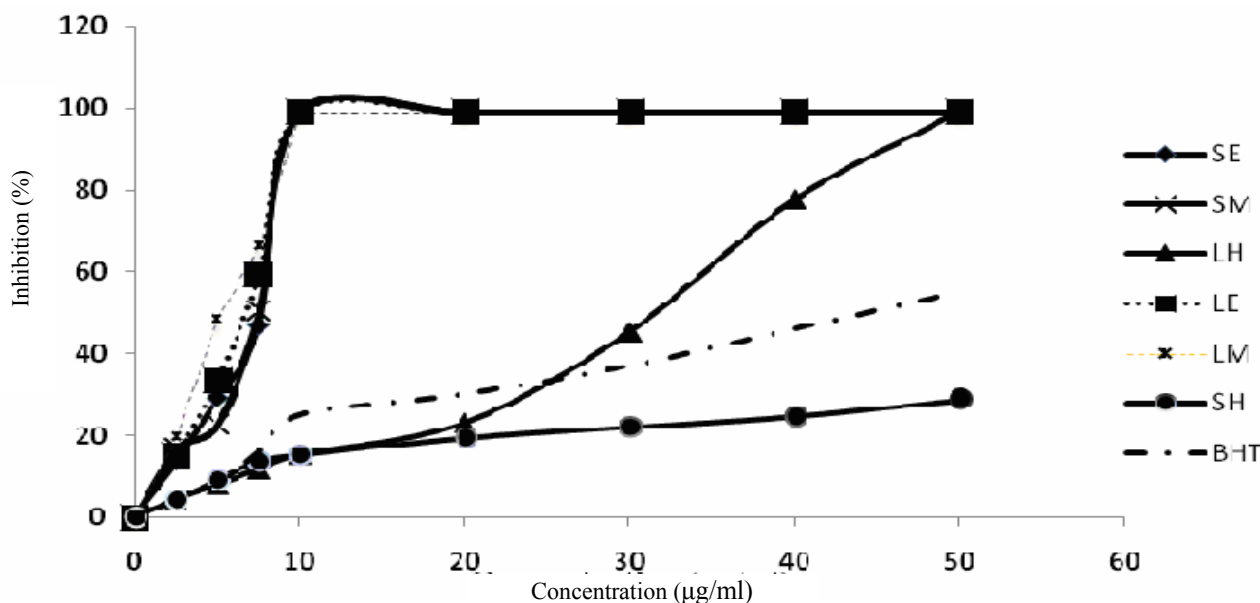


Fig. 1: DPPH free radical scavenging activities (% inhibition) for different extracts from *Moringa peregrina* leaves and seeds (SE (seeds ethylacetate extract), SM (seeds methanol extract), LH (leaves hexane extract), LE (leaves ethylacetate extract), LM (leaves methanol extract) and SH (seeds hexane extracts)).

(SH) extract and leaves hexane (LH) extract in decreasing order. The flavonoids content of SH extract was significantly the highest (214.2 ± 12.2 mg/g) followed by LE extract (194.3 ± 2.8 mg/g) and SE extract (192.1 ± 5.3 mg/g). The flavonols content varied from 73.3 ± 1.9 mg/g in the SM extract to 6.0 ± 0.7 mg/g in the LH extract.

Radical -scavenging activities on DPPH[•], OH[•] and ABTS^{•+}

The DPPH free radical-scavenging activity of various extracts from *M. peregrina* and the standard antioxidant BHT is shown in fig. 1. Results showed that all Moringa extracts (except SH extract) exhibited stronger DPPH radical-scavenging activity than the standard BHT in a dose dependent manner. The results showed that the LM extract exhibited significantly ($P < 0.05$) the highest DPPH scavenging activity in a dose dependent manner (IC_{50} value 5.3 ± 0.2 μ g/ml). At concentration of 7.5 μ g/ml, the inhibition of DPPH radical by LM extract was 66.3%, while for LE, SM, SE, LH and SH extracts the inhibition were 59.1, 50.7, 50.7, 11.7 and 13.7%, respectively. The inhibition of DPPH radical by BHT standard at the same concentration was 16%. The IC_{50} values of various extracts from *M. peregrina* on DPPH radical scavenging activity were in the following decreasing order: LM > LE = SM > SE > LH > BHT > SH (table 1).

The ability of *M. peregrina* extracts to scavenge hydroxyl radical was evaluated using different concentrations from each extract. The IC_{50} values of different *M. peregrina* extracts are shown in table 1. The LM and SM extracts shown significantly (IC_{50} values 76.9 and 77.5 μ g/ml, respectively) the highest activity in quenching the hydroxyl radicals (diminish chromogen formation) and in a dose dependent manner.

In ABTS radical scavenging assay, the IC_{50} values for different extracts from *M. peregrina* seeds and leaves are shown in table 1. The ABTS radical assay was mainly evaluated in this study to check the existence of phenolic compounds with high molecular weight. Hagerman *et al.* (1998) reported that the higher molecular weight of phenolics is the stronger in quenching ABTS radicals. All the studied leaves and seeds extracts showed good ABTS radical scavenging activities and the 50% inhibition of the radical was achieved at concentrations ranged from 49.1 - 92.3 μ g/ml. The inhibitory potential of extracts against the ABTS radical follows the following decreasing orders: LE > LM > SM > SE. However, the activity of LH and SH were not determined due to turbidity.

Chelating power and total antioxidant activities

Table 2 shows the chelating power and the total antioxidant activities of different extracts from Moringa at concentration of 100 μ g/ml. The LM extract has significantly the highest chelating activity on ferrous ion (98.4%) followed by LE, SM and SE extracts (91.1, 90.7

and 86.3%, respectively). In addition, LM extract has significantly the highest total antioxidant activity (294.3 μ g ascorbic acid equivalent/ mg extract) followed by LE, SM and SE extracts (244.5, 231.6 and 200.3 μ g ascorbic acid equivalent/ mg extract, respectively).

DISCUSSION

The antioxidant properties of different extracts from Moringa leaves (L) and seeds (S) have been evaluated using different models. It is evident from the results that the phenolic, flavonoids and flavonols contents were found in considerable amount in all extracts either from leaves or from seeds. Many studies have been suggested that the antioxidant properties of plants and herbs are directly related to their contents of phenolic compounds, flavonoids and flavonols which act by donating hydrogen from the phenolic hydroxyl groups (Lu and Foo, 2000; Miliuskas *et al.*, 2004; Yildirm *et al.*, 2003, Al-Dabbas *et al.*, 2006). Therefore, the relationships between values obtained using different models to evaluate antioxidant activity and the content of phenolic, flavonoids and flavonols compounds were evaluated. With further data analysis a positive correlation ($R = 0.66$) between the total phenolic content of extracts and their DPPH radical scavenging activities was found at concentration of 7.5 μ g/ml, whereas the correlation with the total flavonols was determined to be $R = 0.73$ at the same concentration. These results were in agreement with the results of other researchers whom found a linear relationship between phenolic contents and antioxidant activity of studied plant extracts (Kaur and Kapoor, 2002; Velioglu *et al.*, 1998; Al-Dabbas *et al.*, 2010).

Dehshahri *et al.* (2012) found that the phenolic compound contents (as Gallic acid equivalent) of *M. peregrina* leaves methanolic extract was 88.0 mg/g, in our study the result of phenolic content of LM extract was (214.0 mg/g). The difference in the results between the two studies may due to the difference in the standard used to establish the calibration curve to determine phenolic contents and to the method of extraction.

The hydroxyl radical (OH[•]) is known to be the most reactive free radical that can cause oxidative damage to DNA, proteins and lipids (Kumar *et al.*, 2008). The decolorization effect of extracts in this study reflects the potent capacity of extracts to act as antioxidant by donating hydrogen atoms that inactivate this radical. All the leaves and seeds extracts showed good hydroxyl radical scavenging activities and the 50% inhibition of the radical was achieved at concentrations ranged from 76.9 - 111.8 μ g/ml. The inhibitory potential of extracts against the hydroxyl radical scavenging activity follows the following decreasing orders: LM=SM>SE>LH>LE> SH. With further data analysis a positive correlation ($R = 0.68$) between the total phenolic content of extracts and their

IC₅₀ values of OH radical inhibition (%) was found, whereas strong correlation (R = 0.94) was found between the total flavonoids contents and IC₅₀ values for OH radical inhibition. It is obvious that different polar extracts from *M. peregrina* seeds and leaves shown to possess a potential free radical scavenging activities against different radicals, suggesting the potential of these extracts in contribution in prevention of free radical mediated diseases.

Transition metals like Fe⁺² ions have been proposed as catalyst for the formation of radicals that induced damage to living cells. Chelating agents existed in plant extracts have the ability to reduce radical formation and subsequent lipid per oxidation due to their redox potential that stabilize the oxidized form of the metal ions (Elmastas *et al.*, 2006). The chelating activity of the *M. peregrina* extracts were determined by quantitative measurement of ferrozine complex formation with Fe⁺² ions. The formation of Fe⁺² ferrozine complexes were hindered in the presence of extracts, indicating that Moringa extracts chelate the iron and prevent the completion of the reaction. The 50% chelating activity of the leaf aqueous extract of the same genus *Moringa oleifera* was reported to be 290µg/ml (Akomolafe *et al.*, 2012), thus *M. peregrina* extracts possess higher chelating activity against ferrous ion than *M. oleifera*.

The total antioxidant activity determination of Moringa extracts using Phosphomolybdenum method is based on the formation of green phosphate/Mo (V) complex resulted from the reduction of Mo (VI) to Mo (V) in acidic medium by the extract. This method is quantitative and the total antioxidant activity is expressed as microgram ascorbic acid equivalent (Prieto *et al.*, 1999). With further data analysis it was found that there was a positive correlation between the total phenolic contents of extracts and their total antioxidant activities and chelating power activities (R=0.55 and 0.52, respectively), whereas the correlation with the total flavonols were determined to be R=0.79 and 0.76, respectively. The reason of low antioxidant activities in the leaves and seeds hexane extracts (LH and SH) could be due to the presence of pro-oxidants in leaves and high amount of fat in seeds which dominate the antioxidant compounds present in the extracts.

CONCLUSION

This study showed that different polar extracts from the leaves and seeds of *M. peregrine* exhibited different antioxidant activities according to model system used and these activities are mainly related to their phenolic compounds and flavonoids contents. *M. peregrina* is considered a potential source of natural antioxidants and can inhibit unwanted oxidation processes. Further study is needed to isolate and characterize the antioxidant compounds from *M. peregrina*.

REFERENCES

- Addis P and Warner G (1991). The potential herbal aspects of lipid oxidation products in food. *In: Aruoma OI, Halliwell B* editors. Free radicals and food additives, 1st ed., Tylor and Francis Co., London, pp.77-119.
- Agarwal PK (1989). Carbon-13 NMR of Flavonoids. 1st ed., Elsevier, Amsterdam, p.165.
- Akomolafes, Obboh G, Akindahunsi A, Akinyemi A and Adeyanju O (2012). Inhibitory Effect of Aqueous Extract of *Moringa oleifera* and *Newbuoldia laevis* Leaves on Ferrous Sulphate and Sodium Nitroprusside Induced Oxidative Stress in Rat's Testes *in vitro*. *Open J. Med. Chem.*, **2**: 119-128.
- Al-Dabbas M, Kitahara K, Suganuma T, Hashimoto F and Tadera K (2006). Antioxidant and α -amylase inhibitory compounds from aerial parts of *Varthemia iphionoides*. *Biosci. Biotechnol. Biochem.* **70**: 2178- 2184.
- Al-Dabbas M, Ahmad R, Ajo R, Abulaila K, Akash M and Al-Ismail K (2010). Chemical composition and oil components in seeds of *Moringa peregrina* (Forssk) Fiori. *Crop Res.*, **40**: 161-167.
- Al-Kahtani H and Abou-Arab AA (1993). Comparison of physical, chemical and functional properties of *Moringa peregrine* (Al-Yassar or Al-Ban) and soybean protein. *Cereal Chem.*, **70**: 619-626.
- Broadhurst CL, Polansky MM and Anderson RA (2000). Insulin-like activity of culinary and medicinal plant aqueous extracts *in vitro*. *J. Agric. Food Chem.* **48**: 849-852.
- Dehshahri S, Wink M, Afsharypuor S, Asghari G and Mohagheghzadeh A (2012). Antioxidant activity of methanolic leaf extract of *Moringa peregrina* (Forssk.) Fiori. *Res. Pharm. Sci.*, **7**(2): 111-118.
- Duh P and Yen G (1997). Antioxidative activity of three water extracts. *Food Chem.* **60**: 639-645.
- Dziezak JD (1986). Antioxidants. *J. Food Tech.* **40**: 94-102.
- Elmastaş M, Gülçin D, Beydemir Ş, Küfrevioğlu ÖD and Aboul-Enein HY (2006). A study on the *in vitro* antioxidant activity of juniper (*Juniperus communis* L.) seeds extracts. *Anal. Lett.*, **39**: 47-65.
- FAO (1988). *FAO Yearbook. Traditional Food Plants*. Food and Agriculture Organization of the United Nations, Rome. Pp.369-373.
- Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW and Riechel TL (1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants. *J. Agric. Food Chem.*, **46**(5):1887- 1892.
- Halliwell B (1994). Free radicals, antioxidants and human disease: Curiosity, cause or consequence. *Lancet*, **344**: 721-724.
- Halliwell B, Gutteridge J and Cross C (1992). Free radicals, antioxidants and human disease: Where are we now? *J. Lab. Clin. Med.*, **119**: 598-620.

- Hatano T, Kagawa H, Yasuhara T and Okuda T (1988). Two new flavonoids and other constituents in licorice root; their relative astringency and radical scavenging effects. *Chem. Pharm. Bull.*, **36**: 2090-2097.
- Hayase H and Kato H (1984). Antioxidative components of sweet potatoes. *J. Nutr. Sci. Vitaminol.*, **30**: 37-46.
- Hegazy AK, Hammouda O, Lovett-Doust J and Gomaa NH (2008). Population dynamics of *Moringa peregrina* along altitudinal gradient in the north-western sector of Red sea. *J. Arid Environ.*, **72**: 1537-1551.
- Iqbal S and Bhanger M (2006). Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. *J. Food Comp. Anal.*, **19**: 544-551.
- Kaur C and Kapoor HC (2002). Anti-oxidant activity and total phenolic content of some Asian vegetables. *Int. J. Food Sci. Technol.* **37**(2): 153-161.
- Kumar K, Ganesan K and Rao P (2008). Antioxidant potential of solvent extracts of *Kappaphycus alvarezii* (Doty) Doty An edible seaweed. *Food Chem.*, **107**: 289-295.
- Lalas S and Tsaknis J (2002). Extraction and identification of natural antioxidant from the seeds of the *Moringa oleifera* tree variety of Malawi. *J. Am. Oil Chem. Soc.*, **79**:677-683.
- Lu Y and Foo Y (2000). Antioxidant radical scavenging activities polyphenols from apple pomace. *Food Chem.*, **68**: 81-85.
- Menaga D, Rajkumar S and Ayyasami P (2013). Free radical scavenging activity of methanolic extract of *Pleurotus florida* Mushroom. *Int. J. Pharm and Pharm. Sci.*, **5**: 601-606.
- Miliauskas G, Venskutonis PR and Van-beek TA (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem.*, **85**: 231-237.
- Oluyemi K, Okwuonu U, Baxter D and Oyesola T (2007). Toxic effects of methanolic extract of *Aspilia africana* leaf on the estrous cycle and uterine tissues of Wistar rats. *Int. J. Morphol.*, **25**(3): 609- 614.
- Osawa T (1994). Postharvest biochemistry. In: Uritani I, Garcia VV, Mendoza EM editors. Novel neutral antioxidant for utilization in food and biological systems. Japan, Japan Scientific Societies Press, pp.241-251.
- Ozgen M, Resse R, Tulio A, Scheerens J and Miller R (2006). Modified 2,2'-azino-bis-3-ethylbezthiazoline-6 sulphonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) methods. *J. Agric. Food Chem.* **54**: 1151-1157.
- Prieto P, Pineda M and Aguilar M (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of Vitamin E. *Anal Biochem.* **269**: 337- 341.
- Rashid U, Anwar F, Moser B and Knothe G (2008). *Moringa oleifera*: A possible source of biodiesel. *Bioresour. Technol.*, **99**: 8175-8179.
- Rice-Evans CA, Sampson J, Bramley PM and Holloway DE (1997). Why do we expect carotenoids to be antioxidants *in vivo*? *Free Radic. Res.*, **26**: 381-398.
- Vongtau HO, Abbah J, Chindo BA, Mosugu O, Salawu AO, Kwanashie HO and Gamaniel KS (2005). Central Inhibitory Effects of the Methanol Extract of *Neorautanenia mitis* Root in Rats and Mice. *J. Pharm. Biol.* **43**: 113 – 120.
- SAS Institute (2000). SAS User's Guide in Statistics, (8th edition). Cary, NC., U.S.A., SAS Institutes, Inc.
- Siddhuraju P and Becker K (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J. Agr. Food Chem.*, **51**: 2144-2155.
- Siddiq A, Anwar F, Manzoor M and Fatima A (2005). Antioxidant activity of different solvent extracts of *Moringa oleifera* leaves under accelerated storage of sunflower oil. *Asian J. Plant Sci.*, **4**: 630-635.
- Somali MA, Bajneid MA and Al-Fhaimani SS (1984). Chemical composition and characteristics of *Moringa peregrina* seeds and seed oil. *J. Am. Oil Chem. Soc.*, **61**: 85-86.
- Umamaheswari M and Chatterjee T (2008). *In vitro* antioxidant activities of the fractions of *Coccinnia grandis* L. leaf extract. *Afr. J. Trad. Compl. Altern. Med.*, **5**(1): 61-73.
- Velioglu YS, Mazza G, Gao L and Oomah BD (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agri. Food Chem.*, **46**: 4113-4117.
- Yildirim A, Mavi A and Aydan KA (2003). Antioxidant and antimicrobial activities of *Polygonum cognatum* Meissn extracts. *J. Sci. Food. Agric.*, **83**: 64-69.