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Comparison of the antioxidant activities, phenolic and flavonoids contents of the leaves-crud extracts of *Moringa peregrine* and *Moringa oleifera*

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Abstract

Moringa peregrine and *Moringa oleifera* are used in folk medicine and grows widely in the tropical and subtropical regions of Africa and India. In the present study, the total potential antioxidant activities, reducing powers and total phenolic and flavonoids contents of methanol and water extracts of the leave-crud extracts of the two *moringa* species, *M. peregrine* in comparison to those of *M. oleifera*, were determined. Generally, of the extracts tested, the methanol extracts for the two species showed higher potent antioxidant activities, reducing power and phenolic and flavonoids contents than water extracts. Therefore, the methanol extract of *Moringa peregrine* had the higher antioxidant activity (265.49±4.12mg ascorbic acid equivalent /g extract), higher reducing power (335.89±18.02mg equivalent to ascorbic acid/g extract), total phenolic contents (137.53±8.05mg gallic acid equivalent/g extract) and higher total flavonoids contents (33.40±1.74mg quercetin equivalent/g extract).There was a strong correlation between antioxidant activity and total phenolic contents. Therefore, the increase of the antioxidant activity in *M. pregrena* is mainly by the attribution of the phenolic compounds as major component. Thus, there was a strong correlation between the reducing powers and the total phenolic contents of the extracts. These results combined show that *Moringa peregrine* is better source of antioxidant than *Moringa oleifera*.

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Introduction

Moringa tree (also is known as the "miracle plant) belongs to the flowering plant family *Moringa*ceae that contains 13 species from tropical and subtropical climates that range in size from tiny herbs to massive trees (El-Awady *et al.*, 2015). However, the most widely cultivated species are *Moringa oleifera* and *Moringa peregrine*. *Moringa oleifera* Lam is the best known and most widely distributed species of *Moringa*ceae family, having an impressive range of medicinal uses with high nutritional value throughout the world (Fuglie, 1999; Tahany *et al.*, 2010). Almost every part of this highly esteemed tree have long been consumed by humans and used for various domestic purposes as for alley cropping, animal forage, biogas, domestic cleaning agent, blue dye, fertilizer, foliar nutrient, green manure, gum (from tree trunks), honey and sugar cane juice-clarifier (powdered seeds), ornamental plantings, biopesticide, pulp, rope, tannin for tanning hides, water purification, machine lubrication (oil), manufacture of perfume, and hair care products (Okuda *et al.*, 2001; Siddhuraju and Becker, 2003; Dehshahri *et al.*, 2012; El-Awady *et al.*, 2015). In general, *Moringa peregrina* tree, commonly known as a drumstick tree, that is native to tropical widely naturalized and cultivated in many countries including Malaysia (Okuda *et al.*, 2001). A literature survey indicated that the presence of quercetin flavonoids (Selvakumar and Natarajan, 2008), sterols (Yammuenart *et al.*, 2008), tocopherols (γ and α), β -carotene and other antioxidants (Anwar *et al.*, 2007), have been reported from the plant. The different extracts of the plant were also screened for *in-vitro* anti-inflammatory and antioxidant activities (Anwar *et al.*, 2007). The main product derived from *Moringa peregrina* is seed oil, called 'ben oil'. The use of the oil goes back to antiquity and is already referred to in old Egyptian texts (Abd El-Baky and El-Baroty, 2012). The oil is used for cooking, in cosmetics and in medicine. In southern Sudan and Yemen, *Moringa peregrina* is a bee plant and its leaves are used as fodder and in medicine field. The plant is grown as ornamental in Saudi Arabia and the Middle East (Al-Kahtani, 1994; Afsharypuor *et al.*, 2010; Sadraei *et al.*, 2015).

Most of the previous studies on *Moringa* concentrated on (*M. oleifera*) because of its prevalence in the poor areas in Africa and Asia where most of the people in the rural areas search for edible natural food resources to support their living and fill the stomach in respective of the nutritive value of these resources (Wangcharoen and Gomolmanee, 2011; Ukachi *et al.*, 2015). However, *Moringa peregrine*, the tree that grown as a wild plant in the Arabian desert and known as the Arabian tree of *moringa*, has received less focus and no details studies were traced on its chemical composition and biological activity (Al-Kahtani, 1994). Accordingly, the present study was proposed to investigate the potential antioxidant activities of the leave-crud extracts of the *M. peregrine* in comparison to those of *M. oleifera* using methanol and water as a solvents.

Materials and methods

Collection of Plant Material

Leaves and seeds of *Moringa oleifera* and *Moringa peregrine* were obtained from their original growing places in Egypt by the Desert research center. The species were identified and authenticated to the genus and specie level by specialized botany taxonomist. The collected leaves were air-dried at room temperature for 2 weeks, then ground and stored at -20°C.

Extraction process

The leaves extraction was extracted according to NCCLS (1997), the leaves of the two *moringa* species were dried in shad and finally powdered by electric mill. About 100 g of air-dried powdered leaves was extracted separately by 100% methanol and pure distilled water. For extraction with methanol, the leaves powder (100 g) was soaked in methanol (500 ml) for three days with stirring followed by extraction three times day by day. The filtrate containing extract soluble in methanol was distilled using rotary evaporator under vacuum until dryness affording known weight of crude methanol extract. For extraction with water, the leaves powder (100 g) were soaked in boiling distilled water (500 ml) for six hour with stirring followed by filtration and the plant

material was stored in freezing refrigerator (-20°C) for extraction two another times in the following consecutive days. The aqueous was distilled using rotary evaporator under vacuum till dryness affording known weight of aqueous extract. The alcoholic and aqueous extracts were stored in brown glass bottles and become ready for investigation.

Antioxidant activity

Three different chemical methods were used for evaluation the antioxidant activity of methanol and aqueous extracts; 1,1-diphenyl picrylhydrazyl scavenging activity and phosphomolybdenum method. These assays were performed as described by Abdel-Hameed *et al*., (2012**).**

Scavenging ability towards 1,1-diphenyl picrylhydrazyl (DPPH)· radical

This method depends on the reduction of purple DPPH radicals to a yellow colored diphenyl-picrylhydrazine and the remaining DPPH radicals which show maximum absorption at 517 nm were measured using UV–Vis spectrophotometer. Two ml of different concentrations of each sample were added to 2 ml solution of 0.1 mM DPPH. An equal amount of methanol and DPPH served as control. After 20 min of incubation at 37°C in the dark, the absorbance was recorded at 517 nm. The experiment was performed in triplicates. The DPPH radical scavenging activity was calculated according to the following equation:

% DPPH radical scavenging activity = [1-($A_{sample}/A_{control}$)] x 100

Where A_{sample} and $A_{control}$ are absorbance of the sample and control.

Determination of the total antioxidant capacity by phosphormolybdenum method

The assay is based on the reduction of Mo (VI) to Mo (V) by the antioxidants and subsequent formation of a green phosphate/Mo (V) complex at acid pH.300 µl of each sample solution and ascorbic acid (100 µg/ml) were combined with 3 ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). A typical blank solution containing 3 ml of reagent solution and an appropriate volume of the same solvent was used for the sample. All tubes were capped and incubated in a boiling-water bath at 95°C for 90 min. After the samples were cooled to room temperature, the absorbance of the solution of each sample was measured at 695 nm against the blank using a UV/Vis spectrophotometer. The experiment was performed in triplicates. The antioxidant activity was expressed as the number of equivalents of ascorbic acid.

Estimation of the total phenolic and flavonoid contents

In this study, the total phenolic, flavonoid and flavonol contents of methanol and aqueous extracts were measured according to the methods described by Abdel-Hameed (2009)**.** The total phenolic content of plant extracts was determined using Folin-Ciocalteu's reagent (FCR). 100 µl of each sample solution (100 µg/ml) and also 100 µl of gallic acid (100 µg/ml) were mixed with 500 µl of the FCR and 1.5 ml of 20% sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml using distilled water. The mixture was allowed to stand for 2 h. Then the absorbance at 765 nm was determined against a blank that contained all reagents without the samples or the gallic acid at the same conditions. All determinations were carried out in triplicates. The total phenolic content was expressed as the number of equivalents of gallic acid (GAE).The flavonoids content was determined by aluminium chloride method using quercetin as a reference compound. 100 µl of each sample solution (1 mg/ml) was mixed with 100 µl of 2% aluminum trichloride in ethanol and a drop of acetic acid, and then diluted with ethanol to 5 ml. The absorption at 415 nm was read after 40 min. Blank was prepared from all reagents without the samples. The absorption of the standard rutin solution (100 µg/ml) in methanol was measured under the same conditions. All determinations were carried out in triplicates. The amount of flavonoids in plant extracts in quercetin equivalents (QE) was calculated by the following formula:

$X = (A - m_o)/(A_o - m)$.

Where X is the flavonoid content, mg/mg plant

extract in RE, A is the absorption of plant extract solution, A_o is the absorption of the standard rutin solution, m is the weight of plant extract (mg) and m_o is the weight of rutin in the solution (mg).

The content of flavonols was determined by using quercetin as a reference compound. One ml of each sample solution (1 mg/ml) was mixed with 1 ml aluminiumtrichloride (20 mg/ml) and 3 ml sodium acetate (50 mg/ml). The absorbance at 440 nm was read after 2.5 h. The absorption of the standard quercetin solution (0.5 mg/ml) in methanol was measured under the same conditions. All determinations were carried out in triplicates. The amount of flavonols in plant extracts in quercetin equivalents (QE) was calculated by the same formula used in flavonoids:

$X = (A - m_o)/(A_o - m)$.

Where X is the flavonols content, mg/mg plant extract in QE, A is the absorption of plant extract solution, A_o is the absorption of the standard quercetin solution, m is the weight of plant extract (mg) and m_o is the weight of quercetin in the solution (mg).

Statistical analysis

All determinations were carried out in triplicates and the values are mean ± standard deviation. The statistical analyses were carried out using Microsoft Excel and SPSS 13.0 programs.

Results

The *in-vitro* antioxidant properties of the aqueous and methanol extracts from leaves of wild *Moringa (M. peregrine* and cultivated *Moringa (M. oleifera)* were estimated using three rapid and stable methods (Table 1 and 2).The methanol and aqueous extract of *M. peregrine* leaf samples exerted high free radical scavenging activity toward the artificial free radical *in-vitro* model DPPH with maximal half scavenging activity (SC_{50}) < 50 µg/ml, whereas the methanol and aqueous extract of *M. peregrine* leaf samples exerted moderate and weak radical scavenging activity with SC_{50}> 50 < 150 µg/ml, respectively (Table 1).

Table 1. Yield and 50 % DPPH free radical scavenging concentration (SC_{50}) of methanol (MeOH) and aqueous (H_2O) extracts of *Moringa peregrine* and *Moringa oleifera* leaves.

Extract		Yield (%)	SC_{50} [µg/ml]
Moringa peregrina	MeOH	16.52	29.17±0.12
	H_2O	15.43	49.49±0.53
Moringa oleifera	MeOH	23.33	84.03±1.29
	H_2O	25.17	136.92±1.16
Ascorbic acid		-	7.75±0.10

SC_{50}: Concentration in µg/ml required scavenging the DPPH radical (100 µg/ml) by 50 %. SC_{50} was calculated by probit-graphic interpolation for six concentration levels.

Values of SC_{50} was expressed as mean of triplicate determinations ± standard deviation (n=3).

The methanol extract in each species showed higher DPPH free radical scavenging activity (SC_{50} = 29.17 and 84.03 µg/ml for wild and cultivated *Moringa,* respectively) than the aqueous extract (SC_{50} = 49.49 and 136.92 µg/ml for wild and cultivated *Moringa*, respectively). The determination of total antioxidant capacity using phosphormolybdenum method showed that the methanol extract of *M. peregrine* leaf samples had higher antioxidant capacity (402.22±16.77 mg ascorbic acid equivalent/g extract) followed by the methanol extract of *M. oleifera* (324.44±7.69 mg ascorbic acid equivalent/g extract) (Table 2).On the other hand, the aqueous extracts of *M.peregrina* and *M. oleifera* showed lower activity (255.55±13.87 and 153.33±17.63 mg ascorbic acid equivalent/g extract, respectively). The reducing power activity of the methanol extract of *M. peregrine* leaf samples had higher reducing power activity

(335.89±18.02 mg equivalent to ascorbic acid /g extract) followed by the methanol extract of *M. oleifera* and aqueous extract of *M.peregrina* (324.44±7.69 mg ascorbic acid equivalent/g extract). By contrast, the aqueous extracts of *M. peregrina* and *M. oleifera* showed lower activity (271.12±16.46 and 224.20±5.64 mg ascorbic acid equivalent/g extract, respectively) whereas the aqueous extract of *M. oleifera* showed the lower reducing power activity value (134.30 mg ascorbic acid equivalent/g extract, respectively).Vitamin C was used as a reference antioxidant due to its role as scavenger of free radicals through electron transfer.

Table 2. DPPH free radical scavenging activity, total antioxidant capacity and reducing power activity of methanol (MeOH) and aqueous (H2O) extracts of *Moringa peregrine* and *Moringa oleifera* leaves.

Extract		DPPH free radical scavenging activity [mg ascorbic acid equivalent /g extract][1]	Total antioxidant capacity [mg equivalent to ascorbic acid/g extract][2]	Reducing power activity [mg equivalent to ascorbic acid/g extract][3]
Moringa peregrina	MeOH	265.49±4.12	402.22±16.77	335.89±18.02
	H_2O	156.51±2.88	255.55±13.87	224.20±5.64
Moringa oleifera	MeOH	92.19±2.61	324.44±7.69	271.12±16.46
	H_2O	56.56±0.59	153.33±17.63	134.30±10.10

Values of DPPH free radical scavenging activity, total antioxidant capacity and reducing power activity were expressed as mean of triplicate determinations ± standard deviation (n=3).

1Radical scavenging activity expressed by mg ascorbic acid equivalent /g extract.

2Antioxidant capacity monitored by the phosphomolybdenum method expressed by mg ascorbic acid equivalent /g extract.

3Reducing power activity expressed by mg ascorbic acid equivalent /g extract.

Phytochemical analysis

The extracts from both species showed the presence of phenolic and saponin compounds with different percent as obtained from preliminary phytochemical tests with TLC and PC analysis. Little differences in the kind of compounds appeared between the aqueous and methanol extracts. The total phenolics, flavonoids and flavonols were estimated using the well identified methods (Table 3). It was showed that the methanol and aqueous extracts of *M. peregrine* leaf samples contains higher amount of total phenolic compounds (137.53±8.05 and 92.26±2.30 mg/g GAE, respectively) than the methanol and aqueous extracts of *M. oleifera* leaf samples (63.53±4.40 and 37.6±1.73 mg/g GAE, respectively).

The determination of the total flavonoids that are the major class of phenolics and its major subclass total flavonols of the methanol and aqueous extracts from the dried leaves of *M. peregrine* and *M. oleifera* revealed that the methanol extract for each species had higher flavonoids and flavonols (33.40±1.74 and 19.33±2.10 mg/g QE for flavonoids and 9.59±0.17 & 5.80±0.36 mg/g QE for flavonols, respectively) than the aqueous extract for each species (13.89±0.93 and 8.22±0.44 mg/g QE for flavonoids and 6.91±0.22 & 4.14±0.07 mg/g QE for flavonols, respectively).

Discussion

Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods, cosmetics or medicinal materials to replace synthetic antioxidants, which can include many health risks (Mosquera *et al.*, 2007; El-Awady *et al.*, 2015**).** It was reported that differences in antioxidant activity between the methanol and aqueous extracts, that the kind of extracting solvent played an important role in decreasing or increasing the percentage of compounds responsible for the biological activity (Okuda *et al.*, 2001; Siddhuraju and

Becker, 2003; Wong *et al.*, 2006; Dehshahri *et al.*, 2012). Most of the previous studies on antimicrobial, antifungal, and antioxidant activities were concentrated on (*M. oleifera*) because of its prevalence in the poor areas in Africa and Asia where most of the people in the rural areas search for edible natural food resources (Al-Kahtani, 1994; Afsharypuor *et al.*, 2010; Sadraei *et al.*, 2015). However, *Moringa peregrine*, the tree that grown as a wild plant in the Arabian desert has received less focus and no details studies were traced on its chemical composition and biological activity (Al-Kahtani, 1994). Therefore, this study was conducted to evaluate two *moringa* species using several parameters such as total phenol content, total flavonoid content, total antioxidant capacity, reducing power and hydrogen peroxide scavenging ability.

Table 3. Total amount of phenolic, flavonoid and flavonol compounds of methanol (MeOH) and aqueous (H2O) extracts of *Moringa*peregrinaand *Moringa*oleifera leaves.

Extract		Total phenolics (mg gallic acid equivalent/g extract)[1]	Total flavonoids (mg quercetin equivalent/g extract)[2]	Total flavonols (mg quercetin equivalent/g extract)[3]
Moringa peregrina	MeOH	137.53±8.05	33.40±1.74	9.59±0.17
	H_2O	92.26±2.30	13.89±0.93	6.91±0.22
Moringa oleifera	MeOH	63.53±4.40	19.33±2.10	5.80±0.36
	H_2O	37.6±1.73	8.22±0.44	4.14±0.07

Values of total amount of phenolic, flavonoid and flavonol compounds were expressed as mean of triplicate determinations ± standard deviation (n=3).

[1]Total phenolics expressed by mg gallic acid equivalent/g extract.

[2]Total flavonoids expressed by mg quercetin equivalent/g extract.

[3]Total flavonols expressed by mg quercetin equivalent/g extract.

The methanol extract showed higher antioxidant activity than the aqueous extract that may be attributed that the water not only extracted the compounds responsible for the antioxidant activity but also extracted other compounds that did not contribute in antioxidant activity like; free sugars, organic acids, and salts. The results of this study were found in good agreement with previous studies on the antioxidant activities of some *Moringa sp.* from different localities (Ghebremichael *et al.*, 2005; Gortzi *et al.*, 2007). From the above results the antioxidant activity are attributed to the presence of phenolic compounds as major components. The higher antioxidant activity of the methanol extract is correlated its higher content of phenolic, flavonoid and flavonol contents than the aqueous extract which had lower content. Many studies showed positive correlation between the antioxidant properties and total content of phenolic compounds (Cai *et al.*, 2004; Kumaran and Karunakaran, 2006; Afsharypuor *et al.*, 2010; Sadraei *et al.*, 2015).

Antioxidant activity in *Moringa peregrine* is evaluated by different methods but the most widely used methods are those that generate free radical species which are then neutralized by antioxidant compounds. EGCG had stronger dose-dependent radical scavenging activity against DPPH and superoxide anion radicals in *Moringa peregrine* which agrees with an earlier study (Abbas *et al.*, 2009; Dehshahri *et al.*, 2012). However, leaf extract as well as EGCG revealed dose-dependent anti radical activity. The *Moringa peregrine* phenolic compounds may contribute directly to antioxidative effect. The presence of 3-OH group as well as hydroxyl groups in ring B is related to the superoxide scavenging activity of flavonoids. Epigallocatechin with a hydroxyl group at C-3 and three hydroxyl groups in ring B shows high superoxide scavenging activity (Cos *et al.*, 1998; Abbas *et al.*, 2009; Sadraei *et al.*, 2015). It is also

demonstrated that the principal site of antioxidant reactions in EGCG and epigallocatechin (EGC) was the trihydroxy-phenyl B-ring, regardless of the presence of a 3-galloyl moiety (Valcic *et al.*, 2000; Dehshahri *et al.*, 2012). Therefore, the observed *in vitro* antioxidant activity of the leaf extract of *M. peregrina* may be attributed to its phenolic content and also related to flavonoid rutin isolated from this plant which are in agreement with several reports on flavonoids in *moringa*ceae family including quercetin 3-O- rhamnoglucoside and quercetin 3-O-glucoside in *M. stenopetala* and 3'-methoxylated quercetin, vitexin, quercetin 3-O-glucoside, quercetin 3-O-6"-malonyl-glucoside and lower amounts of kaempferol 3-O-glucoside, and kaempferol 3-O-(6"-malonyl-glucoside) in *M. peregrina* leaves and myricetin in the seeds of *M. peregrina* which showed antioxidant activity (Cos *et al.*, 1998; Valcic *et al.*, 2000; Abbas *et al.*, 2009; Dehshahri *et al.*, 2012; Sadraei *et al.*, 2015).

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