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GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of *Moringa peregrina* (Forssk.) Fiori leaves

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ABSTRACT

Objective: To perform phytochemical screening, estimate total phenolics, flavonoids and to evaluate antioxidant potential of *Moringa peregrina* (*M. peregrina*) leaves.

Methods: The dried powdered leaves of *M. peregrina* (150 g) were extracted exhaustively by Soxhlet with ethanol and then fractionated into hexane, chloroform, ethy alacetate and methanol. All the prepared extracts were also analyzed by gas chromatography-mass spectrometry to identify and characterize the chemical compounds present in the crude extracts. Folin- Ciocalteu reagent and aluminium chloride colorimetric methods were used to estimate total phenolic and flavonoid content of extracts. Hydrogen peroxide and 1,1 diphenyl -2-picrylhydrazyl were used to determine *in vitro* antioxidant activity.

Results: Phytochemical analysis of ethanol extract showed presence of major classes of phytochemicals. Gas chromatography-mass spectrometry results revealed presence of 19 phytoconstituents in hexane extract, 6 in ethyl acetate and 7 compounds in methanolic extract. Methanol extract was found to contain the highest phenolic content and flavonoids. *In vitro* antioxidant activities of all crude extracts were significant and comparable with the standard ascorbic acid.

Conclusions: Results of this study show that the leaves of *M. peregrina* are the rich source of phenolic compounds that can play an important role in preventing the progression of many diseases.

1. Introduction

The *Moringa*ceae is a monotype family of sole genus *Moringa* with 10 to 12 species in tropical world[1]. *Moringa* trees are an important food commodity as almost all plant parts are edible and consumed as nutritive vegetable in many countries[2]. Many medicinal plants of this family are used in traditional medicine globely for the treatment of various illnesses. *Moringa peregrina* (Forssk.) Fiori (*M. peregrina*) is commonly known as horseradish tree or drum stick tree in English and Habb Elyasar or Yen in Arabic, which grows wildly in Oman. It is grown in many areas of the world like Northeastern tropical Africa, Madagascar and Arabia[3]. It is a small, deciduous tree, 6-10 m tall that grows in the most arid locations such as in drained places in a wadi and on the hillsides[4]. It is reported that *M. peregrina* is more drought tolerant than *Moringa oleifera* (*M. oleifera*), which is widely cultivated in tropical and subtropical areas[5]. Its leaves are large (up to 30 cm long) and pinnately divided into opposite or alternate blue green leaflets[3].

The young leaves of *M. peregrina* are rich in vitamins, minerals, proteins and low in fat and carbohydrates, which can be eaten as a vegetable. The leaf extract of *M. peregrina* is rubbed over skin to treat skin rashes and paralysis in folk medicine[4]. The juice from

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the bark of *M. peregrina* is used as a disinfectant to speed up wound healing[6]. In the Northern Oman, oil extracted from the pods is used in treatment of infantile paralysis or convulsions[3]. In Egypt, it is used locally to treat slimness, constipation, headache, fever, burns, back and muscle pains[7]. Its seed oil contains approximately 70% oleic acid and almost all other fatty acids that are found in olive oil. Its seeds possess economic value and medicinal importance due to its unique composition of micro and macronutrients[8].

A detailed literature survey about the pharmacological actions of *M. peregrina* leaves revealed it to possess antimicrobial[7,9], anticancer[10], antioxidant[11], analgesic and anti-inflammatory activities[9]. Various phytochemicals previously isolated from the aerial parts of *M. peregrina* include quercetin, quercetin-3-O-rutinoside, chryseriol-7-O-rhamnoside, and 6, 8, 3',5'-tetramethoxy apigenin[9], lupeol acetate, β-amyrin, -amyrin, β-sitosterol-3-O-β-D-glucoside, rhamnetin, caffeoylquinic acid, *etc*[10]. The pharmacological actions were attributed to the presence of phenolic compounds such as flavonoids and other phenolics in its leaves extract.

To the best of our knowledge, no antioxidant activities have been done on *M. peregrina* leaves grown in Oman. Therefore, it is worthwhile to screen the alcoholic extract of *M. peregrina* leaves for the presence of phytochemicals, to identify and characterize phytoconstituents in its various crude extracts for chemical profiling by gas chromatography-mass spectrometric (GC-MS) analytic technique and to evaluate its antioxidant potential by using *in vitro* methods to correlate with the phenolic and flavonoid content.

2. Materials and methods

2.1. Chemicals

Quercetin, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and gallic acid were purchased from Sigma-Aldrich, USA. Folin- Ciocalteu reagent was obtained from Merck, Germany. All other chemicals and solvents used in the study were of analytical grade procured locally.

2.2. Collection of plant material

M. peregrina leaves were collected in June 2013 from Al-Batinah Region of Sultanate of Oman and authenticated by a botanist from College of Higher Technology, Muscat. The leaves were washed thoroughly with water to remove dust and dried under the shade at room temperature for 5 d. The dried leaves were ground using kitchen blender to obtain the course powder and kept in an air tight container till further use.

2.3. Preparation of extracts

The dried powdered leaves of *M. peregrina* (150 g) were exhaustively extracted with 70% ethanol (400 mL) by using soxhlet extractor at 90 ℃ for 16 h. The polar extract was evaporated at low pressure to obtain crude ethanol extract which was then fractionated into hexane, chloroform, ethyl acetate and methanol extracts by Kupchan's partitioning method[12]. After that, each extracts were evaporated to dryness in fuming hood to obtain corresponding semi solid extracts.

2.4. Phytochemical screening of leaves extract

The freshly prepared crude ethanolic extract of leaves was subjected to qualitative chemical tests to identify various classes of bioactive chemical constituents present in the leaves using standard procedures[13].

2.5. Determination of total phenols by Folin-Ciocalteu reagent method

Folin-Ciocalteu reagent was used to determine the total phenolic content (TPC) of the various organic crude extracts[14]. Gallic acid was used as a reference standard (20-100 μg/mL) for plotting calibration curve. A volume of 0.5 mL of the plant extract (100 μg/mL) was mixed with 1.5 mL of Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and were neutralized with 3 mL of sodium carbonate solution (7.5%, w/v). The reaction mixture was kept in dark at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured by using double beam UV-Vis spectrophotometer (UV Analyst-CT 8200) at fixed wavelength of 765 nm. The TPCs were determined using linear regression equation obtained from the standard plot of gallic acid. The content of total phenolic compounds was calculated as mean±SD (n=3) and expressed as mg/g gallic acid equivalent (GAE) of dry extract.

2.6. Estimation of total flavonoid content (TFC) by aluminum chloride colorimetric method

TFC in crude extracts was determined by the reported procedure of Madaan *et al.* and quercetin was used as a standard to construct the calibration curve[15]. Briefly 10 mg of quercetin was dissolved in 80% ethanol and then diluted to 20, 40, 60, 80 and 100 μg/mL. The diluted standard solutions of quercetin or plant extracts (0.5 mL) of different concentration were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 mol/L potassium acetate and 2.8 mL of distilled water in a test tube. The test tubes were incubated for 30 min at room temperature to complete the reaction. The absorbance of the reaction mixture was measured at 415 nm with double beam UV-Vis spectrophotometer against blank. A typical blank solution contained all reagents except aluminium chloride which is replaced by the same amount of distilled water. The amount of flavonoid was calculated from linear regression equation obtained from the quercetin calibration

curve. The flavonoid content was calculated as mean±SD (n=3) and expressed as mg/g of quercetin equivalent (QE) of dry extract.

2.7. Assay of free radical scavenging activity by DPPH method

The free radical scavenging activity of different concentrations of crude extracts of *M. peregrina* leaves and of standard ascorbic acid was evaluated by using DPPH radical scavenging method as per reported method[14]. The various crude extracts or standard ascorbic acid solution of 1 mL at different concentrations (10, 25, 50, 100 and 200 μg/mL) were taken in separate test tubes. Two milliliter of 1.0 mmol/L DPPH radical solution, prepared in methanol, was added to each test tube. The solution was rapidly mixed and allowed to stand in dark at 37 °C for 30 min. The blank was prepared in a similar way without extract or ascorbic acid. The decrease in absorbance of each solution was measured at 517 nm using UV-Vis spectrophotomer. The percentage of radical scavenging activity of tested extracts and positive control ascorbic acid was calculated by using the following formula:

$$\text{Free radical scavenging activity } (\%) = \frac{[A_c - A_s]}{A_c} \times 100$$

Where A_c=Absorbance of control at 517 nm and A_s=Absorbance of sample.

The concentration of sample required to scavenge 50% of DPPH free radical (IC_{50}) was determined from the curve of percent inhibitions plotted against the respective concentration.

2.8. Hydrogen peroxide (H_2O_2) scavenging assay

The ability of the various leaves extracts of *M. peregrina* to scavenge H_2O_2 was determined according to the reported method of Nabavi *et al*[16]. The concentration of H_2O_2 solution (40 mmol/L) prepared in 50 mmol/L phosphate buffer (pH 7.4) was determined by measuring absorption at 230 nm. Absorbance of assay mixture, containing 1 mL of different concentrations (5, 10, 25, 100 ug/mL) of crude extracts or standard ascorbic acid solution along with 2 mL of H_2O_2 was determined after 10 min against a blank solution containing phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenged was calculated using following formula:

$$H_2O_2 \text{ scavenge } (\%) = \frac{OD_{control} - OD_{test}}{OD_{control}} \times 100$$

2.9. GC-MS analysis

GC-MS analysis of various crude organic extracts of *M. peregrina* leaves was performed on a PerkinElmer Clarus 600 GC System, fitted with a Rtx-5MS capillary column (30 m×0.25 mm inner diameter, ×0.25 μm film thickness; maximum temperature, 350 °C), coupled to a Perkin Elmer Clarus 600C MS. Ultra-high purity helium (99.99%) was used as carrier gas at a constant flow rate of 1.0 mL/min. The injection, transfer line and ion source temperatures were all 290 °C. The ionizing energy was 70 eV. Electron multiplier voltage was obtained from autotune. The oven temperature was programmed from 60 °C (hold for 2 min) to 280 °C at a rate of 3 °C/min. The crude samples were diluted with appropriate solvent (1/100, v/v) and filtered. The particle-free diluted crude extracts (1 μL) were taken in a syringe and injected into injector with a split ratio 30:1. All data were obtained by collecting the full-scan mass spectra within the scan range 40-550 amu. The percentage composition of the crude extract constituents was expressed as a percentage by peak area.

The identification and characterization of chemical compounds in various crude extracts was based on GC retention time. The mass spectra were computer matched with those of standards available in mass spectrum libraries.

2.10. Data analysis

The results are expressed as mean±SD. Student's t-test and One-way ANOVA were applicable and used to analyze level of statistical significance between groups. P<0.05 were considered statistically significant.

3. Results

3.1. Percentage yield of crude extracts

Approximately, 29.44 g (27.01%) viscous mass was obtained from 150 g powder leaves of *M. peregrina* after 16 h of continuous hot extraction in ethanol. Upon Kupchan's partitioning method of crude alcoholic extract, different amounts of hexane, chloroform, ethyl acetate and methanol obtained are shown in Table 1.

Table 1

Amount and % yield of organic extracts from *M. peregrina* leaves.

Extract	Amount (g)	Yield (w/w)
Hexane	10.10	9.27%
Chloroform	1.40	1.28%
Ethyl acetate	0.48	0.45%
Methanol	18.01	16.52%

3.2. Preliminary phytochemical screening

Preliminary phytochemical analysis showed the presence of major classes of secondary metabolites such as tannins, alkaloids, flavonoids, cardiac glycosides, *etc*. in alcoholic extract (Table 2). Saponins and proteins were found to be absent in the extract.

3.3. TPCs

The TPC of the various leaves extract is expressed in terms of GAE and presented in Table 3. The TPCs were calculated using the following linear regression equation obtained from the standard plot

of gallic acid:

y=0.001x+0.021, r^2=0.991

Where y is absorbance and x is the amount of gallic acid in μg.

Table 2

Results of preliminary phytochemical screening of ethanolic extract.

Phytochemical test	Name of the test	Ethanol extract
Tannins	FeCl3 test, lead acetate test	+
Steroids	Salkowski test	+
Flavonoids	Ammonia test, alkaline reagent test	+
Saponins	Frothing test	-
Proteins and amino acids	Ninhydrin test	-
Alkaloids	Hager's, Meyer's and Wagner's tests	+
Carbohydrates	Molisch's test	+
Glycosides	Nitroprusside test	+
Cardiac glycosides	Keller-Killiani test	+
Terpenoids	Salkowski test (modified)	+

+: Detected; -: Not detected.

Table 3

Total phenolics and flavonoid content of various extracts of *M. peregrina* leaves (n=3).

Crude extract	Hexane	Chloroform	Ethyl acetate	Methanol	P
Total phenolics	Nil	75.53±1.65	81.26±3.90	94.56±3.53	0.001*
Total flavonoids	Nil	6.55±0.55	8.39±1.83	20.81±4.02	0.001*

Total phenolics: mg of GAE/g of dry extract; Total flavonoids: mg of QE/g of dry extract; Values are mean±SD; *: P<0.05.

Methanol extract was found to contain the highest amount of phenolic compounds followed by ethyl acetate and chloroform. However, no phenolic compounds were detected in hexane extract. A significant difference in the phenolic contents of different extracts was observed by ANOVA single factor test.

3.4. TFCs

The TFCs of the various crude extracts are expressed in terms of QE and are presented in Table 3. The TFCs were calculated using the following linear regression equation obtained from the standard plot of quercetin:

y=0.0083x+0.003, r^2=0.996

Where y is absorbance and x is the amount of quercetin in μg.

Among four organic extracts, maximum amount of flavonoid content was found in methanol followed by ethyl acetate and chloroform extracts. A significant difference in the flavonoid contents of different extracts was observed by ANOVA single factor test.

3.5. In vitro antioxidant activity

The antioxidant activity of four crude extracts of *M. peregrina* leaves was investigated by commonly used radical scavenging methods such as DPPH and H_2O_2. The scavenging effect of leaves extracts on the DPPH and peroxide free radicals were expressed as % inhibition and they were compared with standard antioxidant, ascorbic acid. All the four extracts showed a dose dependent scavenging activity of DPPH comparable to standard antioxidant. The IC_{50} value (in μg/mL) of the extracts was found in the order of hexane>ethyl acetate>chloroform>methanol extract (Table 4). It was interesting to note that the scavenging ability of methanol extract at concentration of 25, 50 and 100 μg/mL was greater than ascorbic acid although IC_{50} value of ascorbic acid (13.68 μg/mL) was much less than the methanol extract (17.07 μg/mL).

Tabel 4

Percentage inhibition of DPPH free radical by crude extracts/ ascorbic acid at 517 nm (n=3).

Concentration (ug/mL)	Ascorbic acid	Hexane	Chloroform	Ethyl acetate	Methanol
10	42.18±3.46	28.20±1.39	27.40±0.98	27.90±1.20	33.80±0.35
25	70.06±2.09	62.50±2.09	61.35±0.28	63.23±0.27	70.89±0.60
50	92.89±0.81	82.73±0.26	83.77±0.16	85.93±1.99	96.18±0.14
100	93.83±0.19	86.30±1.52	85.83±0.77	87.54±0.36	98.22±0.06
200	99.87±0.58	91.20±0.30	89.86±0.53	90.80±0.64	98.43±0.03
IC_{50} value	13.68	22.36*	17.44	21.87*	17.07

Values are mean±SD; *: P<0.05 compared with ascorbic acid.

The H_2O_2 scavenging activity of prepared extracts were found in the following order of ethyl acetate>methanol> hexane>chloroform, significantly different from the standard ascorbic acid. The maximum antioxidant activity by H_2O_2 assay was observed at 100 μg/mL. The percentage of inhibition produced by ascorbic acid at concentration of 25 μg/mL was greater than the scavenging activity of four extracts at a concentration of 100 μg/mL.

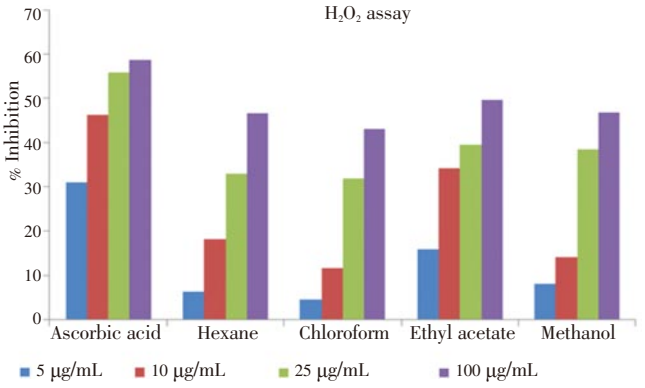

Figure 1. H_2O_2 scavenging activity (%) of leaves extracts and standard ascorbic acid.

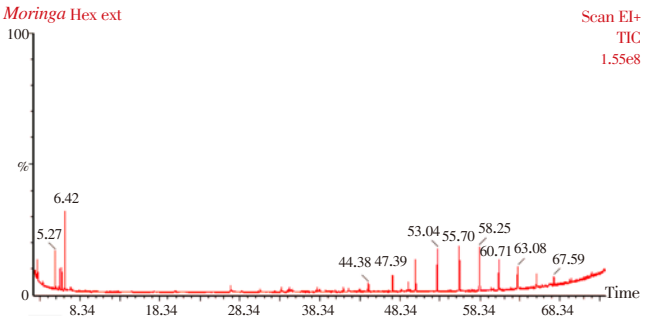

Figure 2. GC-MS chromatogram of hexane extract of *M. peregrina* leaves.

3.6. Chemical composition of extracts by GC-MS analysis

The hexane crude extract showed nineteen peaks in the GC-MS

chromatogram (Figure 2 and Table 5) which were indentified except compound No. 6 according to their retention time on fused silica capillary column. These compounds mainly comprised of hydrocarbons, esters, alcohols and ketones. Ethanone, 1-cyclohexyl- was identified as a major chemical constituent (27.264 81%) followed by pentacosane (17.112 32%), hexacosane (16.577 75%), tetracosane (15.452 81%), heptacosane (13.029 13%), *etc*.

Table 5

Chemical composition of various extracts of *M. peregrina* leaves.

S. No.		Compound name	Retention time (min)	% content
Hexane extract	1	Cyclopentanol, 1-methyl-	3.00	8.081 92
	2	2-Heptanone, 3-methyl-	5.27	7.361 11
	3	Hydroperoxide, 1-ethylbutyl	5.80	4.390 96
	4	Hydroperoxide,1-methylpentyl	6.06	5.214 93
	5	Ethanone, 1-cyclohexyl-	6.42	27.264 81
	6	Unidentified	30.87	1.090 23
	7	Heptadecanoic acid, methyl ester	34.46	1.597 03
	8	Nonadecane	37.92	1.766 76
	9	Eicosane	41.22	1.631 05
	10	Heneicosane	44.35	2.707 88
	11	1-Docosene	47.39	6.068 10
	12	Tricosane	50.28	11.792 62
	13	Tetracosane	53.05	15.452 81
	14	Pentacosane	55.71	17.112 32
	15	Hexacosane	58.24	16.577 75
	16	Heptacosane	60.73	13.029 13
	17	Octacosane	63.11	9.107 29
	18	Nonacosane	65.36	5.936 08
	19	Triacontane	67.59	4.330 88
Ethyl acetate extract	20	Acetic acid, butyl ester	3.26	1.335 53
	21	Oxirane, 2,2-dimethyl-3-propyl-	4.09	1.793 81
	22	Unidentified compounds	4.28	1.951 83
	23	Unidentified compounds	39.24	0.987 23
	24	Hexadecanoic acid, ethyl ester	44.26	2.056 88
	25	9-Octadecenoic acid ethyl ester	49.39	2.985 43
Methanol extract	26	Ethylbenzene	4.04	4.763 87
	27	*p*-Xylene	4.18	10.674 42
	28	*o*-Xylene	4.65	3.817 35
	29	Phenyl acetaldehyde	8.87	0.329 45
	30	C10-hydrocarbon	10.82	1.227 12
	31	1-Hexadecanol	46.92	0.892 59
	32	Phytol	57.85	1.019 57

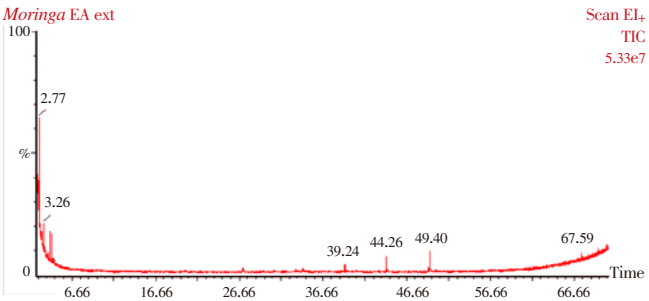

Figure 3. GC-MS chromatogram of ethyl acetate extract of *M. peregrina* leaves.

The ethyl acetate extract showed six peaks in the chromatogram (Figure 3), however, only four compounds could be identified and characterized (Table 5). The major chemical compound identified was 9-octadecenoic acid ethylester (2.985 43%).

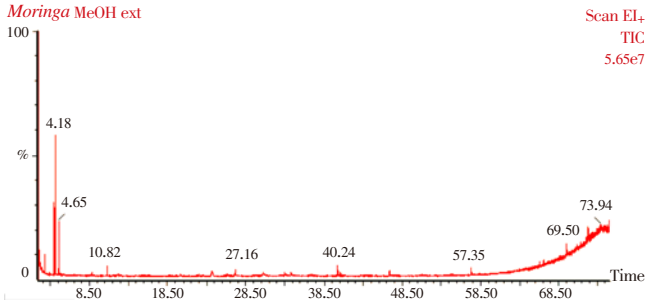

Figure 4. GC-MS chromatogram of methanol extract of *M. peregrina* leaves.

The most polar methanol extract of *M. peregrina* leaves led to the identification of seven chemical compounds by GC-MS analysis (Figure 4 and Table 5). Phytol, c10-hydrocarbon and o-xylene were identified as the main chemical constituents.

4. Discussion

Plants contain numerous phytochemical constituents, many of which are known to be biologically active compounds and are responsible for exhibiting diverse pharmacological activities[17]. Some of these secondary metabolites of plants are important source of natural antioxidants that are preferred over synthetic ones because of safety concerns[18]. The bioactive secondary metabolites have been shown to reduce the risk and progression of diseases such as cancer, cardiovascular, neurodegenerative diseases, *etc*. by scavenging free radicals through various biological mechanisms[19].

The results of preliminary chemical testing confirmed the presence of various classes of bioactive chemical constituents in ethanolic extract of *M. peregrina* leaves including polyphenols (tannins and flavonoids), steroids, alkaloid, carbohydrate glycosides, cardiac glycosides, and terpenoids. Numerous reports available on phenolic compounds have demonstrated their usefulness in exhibiting potential biological activities such as antioxidant, antidiabetic, hepatoprotective, anti-inflammatory, antimicrobial, anticancer *etc*[20,21]. The antioxidant activity of phenolic compounds is mainly due to their reduced properties which allow them to act as metal chelators, absorb and neutralize free radicals[22]. Flavonoids and tannins are considered to be the most promising polyphenolic compounds among plant secondary metabolites[23]. Therefore, based on the phytochemical screening results, the total phenolic and flavonoid contents of different extracts of *M. peregrina* leaves were estimated and also its antioxidant potential were investigated by *in vitro* DPPH and H_2O_2 assay methods.

Methanol, the most polar extract, was found to contain the highest content of total phenol (94.56 mg/g) and flavonoid (20.81 mg/g) as compared to ethyl acetate/chloroform extracts (81.26/75.53 and 8.39/6.55 mg/g, respectively). A significant

difference was observed (P<0.001) in the phenolic and flavonoid content of various crude extracts. Though many studies conducted elsewhere have reported the presence of total phenolics in hexane extract[18], in this study, no phenolic compounds were detected in hexane extract. Dased on the results of total phenol and flavonoid content in the leaves of *M. peregrina*, it can be proposed that biological activity of this species could be due to the presence of flavonoids and other phenolics in it.

The antioxidant activity of the *M. peregrina* leaves was investigated by two *in vitro* models of screening, namely, DPPH and H_2O_2 free radical scavenging method. DPPH and H_2O_2 radical scavenging activity of various crude extracts was compared with standard ascorbic acid. During the DPPH free radical reaction, the degree of discoloration (decrease in absorbance) of the DPPH solution indicates the scavenging potentials of the sample antioxidant. The crude extracts of *M. peregrina* contain plant secondary metabolites such as alkaloids, tannins, saponins, glycosides, *etc*. All these bioactive compounds have the ability to discolor DPPH solution by their hydrogen donating ability[24]. The results of antioxidant activity of the extracts expressed in percentage DPPH activities are presented in this study. All the four extracts exhibited high antioxidant activities comparable to ascorbic acid in concentration dependent manner and maximum activity was observed at 200 μg/mL. Methanol extract exhibited the highest antioxidant activity among all extracts and no statistically significant difference was noted between it and ascorbic acid IC_{50} value. Surprisingly, hexane extract which did not reveal the presence of phenolics also exhibited good free radical scavenging activity, suggesting that hexane extracts contain some non phenolic constituents which are responsible for its antioxidant activity. The antioxidant activity of *M. peregrina* leaves was found to be better than that of *M. oleifera* flowers and stem, the most commonly used species of genus *Moringa* in tropical and subtropical countries for various ailments[14,25]. However, leaves extract of *M. oleifera* showed significant antioxidant activity against DPPH radical and similar to previously reported results for *M. oleifera* leaves, but no correlation between antioxidant activity and TPC was observed for *M. peregrina* leaves[26].

H_2O_2 is involved in the generation of hydroxyl radicals which can initiate cytoxicity. Therefore, any substance that can remove H_2O_2 will protect the living system[27]. The results of free radical scavenging activity by H_2O_2 method also showed a concentration dependent activity. All the extracts scavenged H_2O_2 probably by donating electrons to the hydrogen peroxidase, thereby converting it into water. The antioxidant activity by this method was in the order of ethyl acetate>methanol>hexane>chloroform extract. Thus, it can be concluded that the antioxidant activity of this plant is due to the presence of phytoconstituents, mainly polyphenols.

A total of 29 volatile chemical compounds, belonging to hydrocarbons, esters, alcohols, fatty acids, ketones, *etc*. were identified and characterized in hexane, ethyl acetate and methanol extracts through GC-MS analysis. These compounds have previously been isolated from other medicinal plant species and were believed to play an important role in plant defense system[28].

The results of GC-MS and preliminary photochemical testing indicated that *M. peregrina* leaves contained numerous bioactive phytoconstituents belonging to various classes such as tannins, glycosides, alkaloids, flavonoids, steroids, *etc*. The leaves extract upon quantification by colorimetric methods were found to be rich in phenolic compounds (tannins and flavonoids) and therefore exhibited very good scavenging activity against DPPH and H_2O_2 free radicals. The antioxidant activity by both methods was concentration dependent and comparable to standard antioxidant, ascorbic acid. Based on the results, it can be concluded that *M. peregrina* leaves could be used as a natural source of antioxidants and its regular consumption in diet could provide health benefits to humans by the protection against oxidative stress. Further detailed *in vitro* and *in vivo* correlation studies along with isolation of active constituents are needed to unravel novel treatment strategies for free radical induced diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Dhakar RC, Maurya SD, Pooniya BK, Bairwa N, Gupta M, Sanwarmal. *Moringa*: the herbal gold to combat malnutrition. *Chron Young Sci* 2011; **2**(3): 119-125.

[2] Oluduro AO. Evaluation of antimicrobial properties and nutritional potentials of *Moringa oleifera* Lam. leaf in South-western Nigeria. *Malays J Microbiol* 2012; **8**(2): 59-67.

[3] Miller AG, Morris M, Sruart-Smith S. *Plants of Dhofa: The southern region of Oman-traditional, economic and medicinal uses*. Oman: Office of the Adviser for Conservation of the Environment, Diwan of Royal Court, Sultanate of Oman; 1988, p. 210-211.

[4] Ghazanfar SA, Al-Sabahi AMA. Medicinal plants of Northern and

Central Oman (Arabia). *Econ Bot* 1993; **47**(1): 89-98.

[5] Osman HE, Abohassan AA. Morphological and analytical characterization of *Moringa peregrina* populations in Western Saudi Arabia. *Inter J Theor Appl Sci* 2012; **4**(2): 174-184.

[6] Marwah RG, Fatope MO, Al Mahrooqi R, Varma GB, Al Abadi H, Al-Burtamani SKS. Antioxidant capacity of some edible and wound healing plants of Oman. *Food Chem* 2007; **101**(2): 465-470.

[7] Abdel-Rahman Tahany MA, Hegazy AK, Sayed AM, Kabiel HF, El-Alfy T, El-Komy SM. Study on combined antimicrobial activity of some biologically active constituents from wild *Moringa peregrina* Forssk. *J Yeast Fungal Res* 2010; **1**(1): 15-24.

[8] Hanif MA, Al-Maskri AY, Al-Mahruqi ZM, Al-Sabahi JN, Al-Azkawi A, Al-Maskari MY. Analytical evaluation of three wild growing Omani medicinal plants. *Nat Prod Commun* 2011; **6**(10): 1451-1454.

[9] El-Batran SA, Abdel-Salam OM, Abdelshfeek KA, Nazif NM, Ismail SI, Hammouda FM. Phytochemical and pharmacological investigation on *Moringa peregrina* (Forssk) Fiori. *Nat Prod Sci* 2005; **11**(4): 199-206.

[10] El-Alfy TS, Ezzat SM, Hegazy AK, Amer AM, Kamel GM. Isolation of biologically active constituents from *Moringa peregrina* (Forssk.) Fiori. (family: *Moringa*ceae) growing in Egypt. *Pharmacogn Mag* 2011; **7**(26): 109-115.

[11] Dehshahri S, Wink M, Afsharypuor S, Asghari G, Mohagheghzadeh A. Antioxidant activity of methanolic leaf extract of *Moringa peregrina* (Forssk.) Fiori. *Res Pharm Sci* 2012; **7**(2): 111-118.

[12] Muhit MA, Tareq SM, Apu AS, Basak D, Islam MS. Isolation and identification of compounds from the leaf extract of *Dillenia indica* Linn. *Bangladesh Pharm J* 2010; **13**(1): 49-53.

[13] Jeyaseelan EC, Jashothan PT. *In vitro* control of *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (ATCC 25922) by *Ricinus communis* L. *Asian Pac J Trop Biomed* 2012; **2**(9): 717-721.

[14] Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content, *in-vitro* antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera*. *Asian Pac J Trop Biomed* 2013; **3**(8): 623-627.

[15] Madaan R, Bansal G, Kumar S, Sharma A. Estimation of total phenols and flavonoids in extracts of *Actaea spicata* roots and antioxidant activity studies. *Indian J Pharm Sci* 2011; **73**(6): 666-669.

[16] Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A, Bekhradnia AR. Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica* Mey. *Pharmacologyonline* 2008; **2**: 560-567.

[17] Gu R, Wang Y, Long B, Kennelly E, Wu S, Liu B, *et al.* Prospecting for bioactive constituents from traditional medicinal plants through ethnobotanical approaches. *Biol Pharm Bull* 2014; **37**(6): 903-915.

[18] Ifeson BOT, Fashakin JF, Ebosele F, Oyerinde AS. Antioxidant and antimicrobial properties of selected plant leaves. *European J Med Plants* 2013; **3**(3): 465-473.

[19] Ansari M, Khodagholi F. Natural products as promising drug candidates for the treatment of Alzheimer's disease: molecular mechanism aspect. *Curr Neuropharmacol* 2013; **11**(4): 414-429.

[20] Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. *ScientificWorldJournal* 2013; doi: 10.1155/2013/162750.

[21] Sulaiman S, Ibrahim D, Kassim J, Sheh-Hong L. Antimicrobial and antioxidant activities of condensed tannin from *Rhizophora apiculata* barks. *J Chem Pharm Res* 2011; **3**(4): 436-444.

[22] Mishra SL, Sinhamahapatra PK, Nayak A, Das R, Sannigrahi S. *In vitro* antioxidant potential of different parts of *Oxoxylum indicum*: a comparative study. *Indian J Pharm Sci* 2010; **72**(2): 267-269.

[23] Tomczyk M, Pleszczy ska M, Wiater A. Variation in total polyphenolic contents of aerial parts of *Potentilla* species and their anticariogenic activity. *Molecules* 2010; **15**(7): 4639-4651.

[24] Waheed I, Ahmed M, Syed NH, Ashraf R. Investigation of phytochemical and antioxidant properties of methanol extract and fractions of *Ballota limbata* (Lamiaceae). *Indian J Pharm Sci* 2014; **76**(3): 251-256.

[25] Kumbhare MR, Guleha V, Sivakumar T. Estimation of total phenolic content, cytotoxicity and *in-vitro* antioxidant activity of stem bark of *Moringa oleifera. Asian Pac J Trop Dis* 2012; **2**: 144-150.

[26] Shih MC, Chang CM, Kang SM, Tsai ML. Effect of different parts (leaf, stem and stalk) and seasons (summer and winter) on the chemical compositions and antioxidant activity of *Moringa oleifera. Int J Mol Sci* 2011; **12**: 6077-6088.

[27] Van WR, Van WEP, Wiegant FA, Ives J. Free radicals and low-level photon emission in human pathogenesis: state of the art. *Indian J Exp Biol* 2008; **46**(5): 273-309.

[28] Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy*. 42nd ed. India: Nirali Prakashan; 2008.