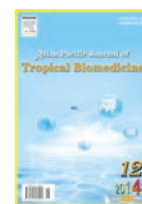




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Document heading

doi: 10.12980/APJTB.4.201414B295

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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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ARTICLE INFO

Article history:

Received 9 Jul 2014

Received in revised form 8 Aug 2014

Accepted 13 Aug 2014

Available online 31 Oct

Keywords:

Antioxidant activity

Flavonoid content

GC- MS analysis

Moringa peregrina

Total phenolics

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, ethyl acetate 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 *Moringaceae* 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 globally 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 wild 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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Foundation Project: Supported by the Department of Pharmacy, Oman Medical College, Muscat, Oman (Grant No. OMC-PHAR/425-05/13).

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 coarse 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 °C 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 μ g/mL) for plotting calibration curve. A volume of 0.5 mL of the plant extract (100 μ g/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 \pm 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 μ g/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 \pm 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 μ g/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 spectrophotometer. 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 μ g/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:

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

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 \times 0.25 mm inner diameter, \times 0.25 μ m 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 μ L) 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 \pm 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	FeCl ₃ 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 \pm 1.65	81.26 \pm 3.90	94.56 \pm 3.53	0.001*
Total flavonoids	Nil	6.55 \pm 0.55	8.39 \pm 1.83	20.81 \pm 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 \pm 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 $\mu\text{g/mL}$) 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 $\mu\text{g/mL}$ was greater than ascorbic acid although IC_{50} value of ascorbic acid (13.68 $\mu\text{g/mL}$) was much less than the methanol extract (17.07 $\mu\text{g/mL}$).

Table 4

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

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

Values are mean \pm 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 $\mu\text{g/mL}$. The percentage of inhibition produced by ascorbic acid at concentration of 25 $\mu\text{g/mL}$ was greater than the scavenging activity of four extracts at a concentration of 100 $\mu\text{g/mL}$.

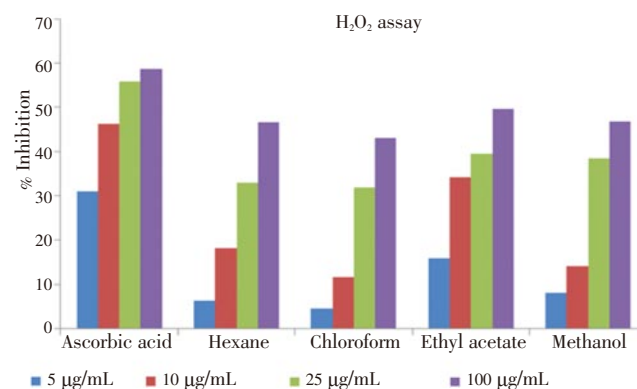


Figure 1. H_2O_2 scavenging activity (%) of leaves extracts and standard ascorbic acid.

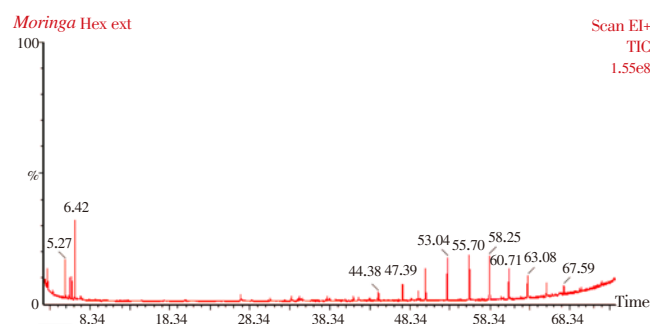


Figure 2. GC-MS chromatogram of hexane extract of *M. peregrina* leaves.

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 identified 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 (27.26481%) followed by pentacosane (17.11232%), hexacosane (16.57775%), tetracosane (15.45281%), heptacosane (13.02913%), *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	8.08192
	2 2-Heptanone, 3-methyl-	5.27	7.36111
	3 Hydroperoxide, 1-ethylbutyl	5.80	4.39096
	4 Hydroperoxide, 1-methylpentyl	6.06	5.21493
	5 Ethanone, 1-cyclohexyl-	6.42	27.26481
	6 Unidentified	30.87	1.09023
	7 Heptadecanoic acid, methyl ester	34.46	1.59703
	8 Nonadecane	37.92	1.76676
	9 Eicosane	41.22	1.63105
	10 Heneicosane	44.35	2.70788
	11 1-Docosene	47.39	6.06810
	12 Tricosane	50.28	11.79262
	13 Tetracosane	53.05	15.45281
	14 Pentacosane	55.71	17.11232
	15 Hexacosane	58.24	16.57775
	16 Heptacosane	60.73	13.02913
	17 Octacosane	63.11	9.10729
	18 Nonacosane	65.36	5.93608
	19 Triacontane	67.59	4.33088
Ethyl acetate extract	20 Acetic acid, butyl ester	3.26	1.33553
	21 Oxirane, 2,2-dimethyl-3-propyl-	4.09	1.79381
	22 Unidentified compounds	4.28	1.95183
	23 Unidentified compounds	39.24	0.98723
	24 Hexadecanoic acid, ethyl ester	44.26	2.05688
Methanol extract	25 9-Octadecenoic acid ethyl ester	49.39	2.98543
	26 Ethylbenzene	4.04	4.76387
	27 <i>p</i> -Xylene	4.18	10.67442
	28 <i>o</i> -Xylene	4.65	3.81735
	29 Phenyl acetaldehyde	8.87	0.32945
	30 C10-hydrocarbon	10.82	1.22712
	31 1-Hexadecanol	46.92	0.89259
	32 Phytol	57.85	1.01957

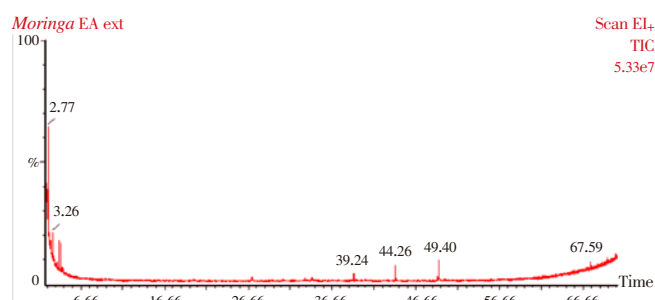


Figure 3. GC-MS chromatogram of ethyl acetate extract of *M. peregrina* leaves.

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 (2.98543%).

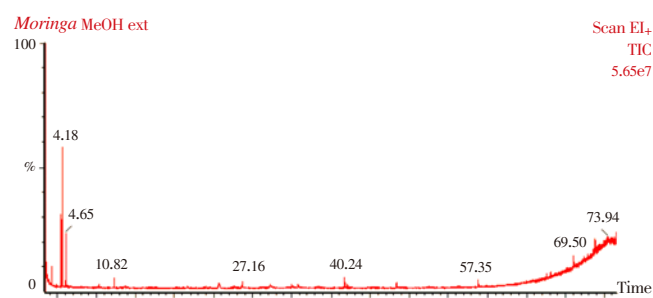


Figure 4. GC-MS chromatogram of methanol extract of *M. peregrina* leaves.

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 H₂O₂ 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. Based 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 $\mu\text{g/mL}$. 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 cytotoxicity. 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.

Acknowledgements

The authors would like to thank Mr. Jamal N. Al Sabahi of Sultan Qaboos University for GC-MS analysis. The authors are also grateful to the dean, associate dean and pharmacy head of Oman Medical College for providing infrastructure and necessary research facilities. This work was supported by the Department of Pharmacy, Oman Medical College, Muscat, Oman (Grant No. OMC-PHAR/425-05/13).

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