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Volatile compounds analysis and antioxidant, antimicrobial and cytotoxic activities of *Mindium laevigatum*

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ABSTRACT

Objective(s): *Mindium laevigatum* is an endemic plant of Iran and Turkey and is widely used as blood purifier, antiasthma and antidyspnea in traditional medicine. Chemical composition of volatile materials of the plant and its antioxidant, antimicrobial and cytotoxic activities were reported in this study.

Materials and Methods: Simultaneous distillation-extraction (SDE) and GC-Mass-FID analysis were used for the plant volatile materials chemical composition identification and quantification. Several antioxidant tests including DPPH radical scavenging, hydrogen peroxide scavenging, reducing power determination, β-carotene-linoleic acid and total phenolic content tests were used for antioxidant activity evaluation. Antimicrobial and anticancer activities were also estimated using microbial strains, cancer cell lines and brine shrimp larva.

Results: GC-Mass-FID analysis of volatile samples showed a total of 74 compounds of which palmitic acid (7.4-33.7%), linoleic acid (6.6-18.6%), heneicosane (1.3-9.6%) and myristic acid (1.4-6.0%) were detected as main volatile components. Moderate to good results were recorded for the plant in *β*-carotene-linoleic acid test. Total phenolic content of the extracts as gallic acid equivalents were estimated in the range of 15.7 to 79.6 µg/mg. Some microbial strains showed moderate sensitivities to plant extracts. Brine shrimp lethality test and cytotoxic cancer cell line assays showed mild cytotoxic activities for the plant.

Conclusion: Moderate to good antioxidant activities in β-carotene-linoleic acid test and presence of considerable amounts of unsaturated hydrocarbons may explain the plant traditional use in asthma and dyspnea. These findings also candidate it as a good choice for investigating its possible modern medical applications.

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Introduction

Flowering plants with an estimated population of 500000 species are sources of diverse secondary metabolites of which many have useful preventive and/or curative potentials against pathologic conditions (1). The application of these plants in traditional medicine is a consequence of these potentials. These abilities also have led to the interest of modern health investigational programs toward them. But, despite this interest, only a small fraction of the plants was scientifically investigated and most of them are still waiting for researcher's consideration.

Iran is a rich country in plant natural flora. These plants were frequently used in Iranian folk medicine as main or supplementary therapeutic agents. Despite this, most of them are not scientifically studied so far and their potential useful medical, food, cosmetic etc. applications remain to be discovered. The genus *Mindium* (family, Campanulaceae) consists of perennial plants growing Wild in Iran, Iraq, Turkey, eastern Mediterranean regions and Caucasus mountains (2, 3). Genus *Mindium* has 7 species in the world and three in Iran (2-5). In traditional medicine, the plants of Campanulaceae family are frequently used to treat various diseases such as tonsillitis, laryngitis, bronchitis and warts (6). They also possess refreshing and stimulant properties and are used as emetic; antiallergic, antiphlogistic, antioxidant, spasmolytic, antiviral and antimicrobial remedies (6). *Mindium laevigatum* (Vent.) Rech.f. & Schiman-Czeika is one of the endemic species of Turkey and Iran (Persian name: ghole shekafteh, former scientific name: *Michauxia laevigata* Vent.). It is a herbal plant growing wild in

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north, west, northwest and central parts of Iran (2, 7). The plant decoction is widely used orally as blood purifier, antiasthma and antidyspnea in the western parts of Iran (8). Asthma and dyspnea are pulmonary disorders with known inflammatory pathophysiologic basis (9). Antioxidant potentials of the plant may play a role in its antiasthma and antidyspnea activities through blocking inflammatory processes in the respiratory tract. Thus, the present study was organized for determination of the chemical composition of the plant volatile materials and estimation of its antioxidant, antimicrobial and cytotoxic potentials in order to explore its possible beneficial medical applications.

Materials and Methods

Solvents, chemicals, microbial strains and brine shrimp eggs

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical, *β*-carotene, linoleic acid, 2,6-di-*tert*-butyl-4-methylphenol (butylated hydroxyl toluene, BHT) and gallic acid were procured from Sigma–Aldrich (Steinheim, Germany). All other chemicals including solvents and culture media were obtained from Merck (Darmstadt, Germany). Microbial strains were provided by Iranian Research Organization for Science and Technology (IROST). Brine Shrimp (*Artemia salina*) eggs were obtained from Advanced Hatchery Technology, INC, Salt Lake City, UTAH 84126, USA. Double distilled water was used in the experiments.

Plant material

Aerial parts of *M. laevigatum* were collected during the plant fruiting seasons (spring and summer 2015) from Shahsavaran valley and Rahagh area of Kashan (Isfahan province, Iran) at altitudes of 1850 m and 2100 m, respectively. The plant materials were botanically identified by Dr. Hossain Batooli. Stems and fruits were separated, dried in the shade, ground (80 mesh), packed in well closed containers and stored in refrigerator. Authenticated specimens of the plant were also deposited in the Herbarium of the Kashan Botanical Garden, Isfahan Center for Research and Education of Agricultural Science and Natural Resources, Isfahan, Iran (Voucher No. KBGH 1091).

Isolation of volatile components

One hundred grams samples of the plant stem and fruit were separately subjected to simultaneous distillation-extraction (SDE) for 1.5 hr using an all-glass Seidel and Lindner type SDE apparatus. *n*-Pentane (50 ml) was used as extraction solvent (10). Every sample was dried over anhydrous sodium sulphate and filtered. After room temperature evaporation of *n*-pentane, obtained volatile materials were stored in amber vials at low temperature (4 ˚C) for future analysis.

Preparation of methanol extracts

Twenty grams samples of the plant stem and fruit were individually transferred to cellulose thimbles and subjected to soxhlet extraction with methanol for 8 hr at the boiling temperature of the solvent. The extracts were concentrated using a rotary evaporator (Buchi Rotavapor R-200, Flawil, Switzerland) at maximum temperature of 45 ˚C and dried overnight in vacuum oven (Memmert, VO400, Germany, set at 45 ˚C). All extractions were repeated three times.

Chromatographic analysis

Samples containing volatile constituents of the plant were analyzed on an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a HP-5MS 5% phenyl methyl siloxane capillary column (30 m×0.25 mm, 0.25 µm film thickness; Restek, Bellefonte, PA). The column outlet was simultaneously connected to an Agilent HP-5973 mass selective detector (MSD) in the electron impact mode (ionization energy: 70 eV) and a flame ionization detector (FID) using a Y type 1:10 post column splitter (Agilent part No: 0101-0595). Oven temperature was kept at 60 ˚C for 3 min, then programmed to 246 ˚C at a rate of 3 ˚C/min. Injector temperature was set at 220 ˚C and both of detectors (MSD and FID) temperatures were set at 240 ˚C. Ultra-high pure helium (flow rate: 1.2 ml/min), hydrogen (flow rate: 40 ml/min) and nitrogen (flow rate: 50 ml/min) were used as carrier, fuel and make up gases, respectively. Compressed air (flow rate: 450 ml/min) was used for combustion. Diluted samples (1/1000 in *n*-pentane, v/v) of 2.0 µl were injected manually in the split mode (split ratio: 1/10). Retention indices (RI) were calculated for all components using a homologous series of *n*-alkanes injected in conditions identical to the samples injections. Identification of samples components were made based on their retention indices (RI) relative to *n*-alkanes, computer matching of their mass spectra with Wiley275.L and Wiley7n.L libraries and comparison of the fragmentation pattern of the mass spectra with the data published in the literature (11). Peak area percent of each compound relative to the area percent of the entire FID spectrum (100%) was used for obtaining its quantitative data. All injections were repeated three times.

Evaluation of antioxidant activity

DPPH radical scavenging assay

Radical-scavenging activities of the plant volatile materials and methanol extracts were determined using a published DPPH radical-scavenging activity assay method (12) with minor modifications. Inhibition percentages (I%) of DPPH radicals were calculated using following equation:

$$I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where A_{blank} is the absorbance value of the control reaction (containing all reagents except test compounds) and A_{sample} is the absorbance value of the test compounds. The sample concentrations providing 50% inhibition (IC_{50}) were calculated by plotting the inhibition percentages against concentrations of the samples. All tests were carried out in triplicate and IC_{50} values were reported as means±SD of triplicates.

Hydrogen peroxide (H_2O_2) scavenging assay

The ability of the plant volatile materials and methanol extracts to scavenge H_2O_2 was determined using the method described by Singh *et al* (13) with minor modifications. The percentages of scavenging of H_2O_2 were calculated by employing the equation:

$$\% \text{ scavenging of } H_2O_2 = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the control solution (H_2O_2 in phosphate buffer without sample and positive control) and A_1 is its absorbencies in the presence of the samples or positive control. Sample concentrations providing 50% inhibition (IC_{50}) were calculated by plotting the scavenging percentages against concentrations of samples. All tests were carried out in triplicate and IC_{50} values were reported as means±SD of triplicates.

Reducing power determination

The ability of the *M. laevigatum* volatile materials and methanol extracts to reduce iron (III) was determined according to the method of Tounsi *et al* (14). The EC_{50} values (µg/ml) were reported as the samples concentrations at which the absorbances were 0.5 for reducing power. They were calculated from the absorbance graph at 700 nm against samples concentrations. Ascorbic acid was used as positive control. Values were presented as means±SD of triplicate analyses.

β -Carotene/linoleic acid bleaching assay

The method described by Miraliakbari an Shahidi (15) was used for the evaluation of *β*-carotene/linoleic acid bleaching ability of the plant samples with slight modifications. Antioxidant activities (inhibition percentages, I%) of the samples and positive control were calculated using the equation:

$$I\% = (A_{\beta\text{-carotene after 2 h}}/A_{\text{initial } \beta\text{-carotene}}) \times 100$$

where $A_{\beta\text{-carotene after 2 hr}}$ is the absorbance values of *β*-carotene remaining after 2 hr in the samples and $A_{\text{initial } \beta\text{-carotene}}$ is the absorbance values of *β*-carotene at the beginning of the experiments. All tests were carried out in triplicate and inhibition percentages were reported as means±SD of triplicates.

Assay of total phenolic compounds

Total phenolic compounds constituents of methanol extracts of *M. laevigatum* were determined using published procedure involving Folin–Ciocalteu phenol reagent and gallic acid standard (16). According to this test, total phenolic compounds content of each extract, as gallic acid equivalent, were determined using its absorbance measured at 760 nm as input to the obtained standard curve and equation. All tests were carried out three times and obtained values as gallic acid equivalents were reported as mean±SD of three determinations.

Cytotoxicity evaluation

Brine shrimp lethality test

Brine shrimp lethality test was preformed according to Meyer *et al* (17) method with minor adaptations. Experiments were conducted along with control and different samples concentrations in a set of three tubes per extracts doses. The lethality percent was determined by comparing the mean number of dead larva in the test and control tubes. Half maximal lethal concentration (LC_{50}) values were obtained from the best fit line of concentration-lethality percentage plots.

Cancer cell lines assay

The effects of plant extracts on cancer cell viability were determined by an adapted Scudiero *et al* 3-(4,5-Dimethylthiozol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method using human colon (HCT116) and prostate (PC-3) cancer cell lines (18). The extent of MTT reduction was measured at 540 nm using a Titertek Multiscan microElisa (Labsystems, Helsinki, Finland) equipment. Cytotoxicity was expressed as the concentration of extract inhibiting cell growth by 50% (IC_{50}). Experiments were conducted in triplicate and cisplatin was used as reference compound.

Antimicrobial activity estimation

Microbial strains

Methanol extracts of *M. laevigatum* were individually tested against a set of 11 microorganisms. Following microbial strains, provided by Iranian Research Organization for Science and Technology (IROST), were used in this research: *Escherichia coli* (ATCC 10536), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29737), *Klebsiella pneumoniae* (ATCC 10031), *Staphylococcus epidermidis* (ATCC 12228), *Shigella dysenteriae* (PTCC 1188), *Proteus vulgaris* (PTCC 1182), *Salmonella paratyphi-A serotype* (ATCC 5702), *Candida albicans* (ATCC 10231), *Aspergillus niger* (ATCC 16404) and *Aspergillus brasiliensis* (PTCC 5011). Bacterial and fungal strains were cultured at 37 °C in nutrient agar (NA) and 30 °C in sabouraud dextrose agar (SDA), respectively.

Disc diffusion assay

Disc diffusion method reported by Murray *et al* (19) were used for initial sensitivity determination of microbial strains towards the plant methanol extracts. Gentamicin (10 µg/disc) and rifampin (5 µg/disc) for

bacteria and nystatin (100 IU) for fungi were used as positive controls. The diameters of inhibition zones were used as a measure of antimicrobial activity and each assay was repeated twice.

Micro-well dilution assay

Microbial strains sensitive to the plant extracts in disc diffusion assay were studied for their minimal inhibitory concentration (MIC) values using published micro-well dilution assay method (20). Gentamicin and rifampin for bacteria and nystatin for yeast were used as standard positive controls in the conditions identical to that of the test materials. The MIC values were defined as the lowest concentration of the plant extracts required for inhibiting the growth of microorganisms. All tests were repeated two times.

Results

Chemical composition of volatile materials

Volatile materials of *M. laevigatum* from Shahsavaran valley and Rahagh area were obtained by SDE. Samples were analyzed by a double detector (FID and Mass) gas chromatograph system and their components identity and quantity were characterized simultaneously (Table 1). Seventy-four components were identified in the plant samples consisting 83.2 to 92.5 percent of them. Palmitic acid (7.4-33.7%), linoleic acid (6.6-18.6%), heneicosane (1.3-9.6%) and myristic acid (1.4-6.0%) were recorded as major volatile constituents. These compounds and most of other (sub-major) constituents of the plant are belong to unsaturated fatty acid and hydrocarbon families of natural products and the plant is essentially poor in classical essential oil components such as monoterpenes, sesquiterpenes and phenylpropanoids (21).

Antioxidant activity

Antioxidant activities of the volatile materials and methanol extracts of *M. laevigatum* have been estimated using a panel of antioxidant tests including DPPH radical scavenging test, hydrogen peroxide scavenging assay, reducing power determination test and *β*-carotene/linoleic acid bleaching test. The results are presented in Table 2.

Cytotoxic activity

Lethal concentrations (LC_{50}) recorded in the brine shrimp lethality bioassay and human colon (HCT116) and prostate (PC-3) cancer cell line assays carried out on the methanol extracts of *M. laevigatum* were >1000 *μ*g/ml and >750 *μ*g/ml, respectively (Table 3).

Antimicrobial activity

Antimicrobial activity of *M. laevigatum* methanol extracts were evaluated against a panel of 11 microorganisms and their potency were assessed both qualitatively and quantitatively by the presence or absence of inhibition zones, zone diameters and MIC values. The results are given in Table 4.

Table 1. Chemical composition of *Mindium laevigatum* volatile materials

			Composition (%)			
			Shahsavaran valley		Rahagh area	
Compound[a]	RI[b]	RI[c]	Stem	Fruit	Stem	Fruit
n-Hexanal	804	801	0.6	-	-	-
(E)-2-Hexenal	847	846	0.6	-	-	-
(*Z*)-3-Hexen-1-ol	849	854	-	1.2	-	-
n-Hexanol	865	863	2.3	3.4	2.3	-
Heptanal	901	901	-	0.4	-	-
1-Octen-3-ol	977	974	0.6	-	-	0.8
Benzene acetaldehyde	1047	1036	0.5	0.4	0.9	0.5
2-Octenal	1066	1061	-	-	0.4	-
1-Octanol	1070	1063	0.6	0.6	-	-
p-Cresol	1077	1071	1.7	1.6	-	-
Linalool	1102	1095	0.4	2.3	-	2.8
3,5-Octadiene-2-one	1104	1093	-	-	1.5	-
Nonanal	1108	1100	0.5	1.8	2.2	1.0
Camphor	1156	1141	-	-	0.5	-
(E)-2-Nonenal	1169	1157	-	-	0.7	-
Pelargonic alcohol	1176	1165	-	0.9	-	0.8
Caprylic acid	1188	1180	-	-	-	3.1
α-Terpineol	1198	1186	-	1.0	3.1	2.1
Decanal	1209	1201	0.5	0.8	0.6	0.7
p-Vinyl phenol	1222	1219	-	-	-	1.0
β-Citronellol	1235	1236	0.5	-	0.6	-

Geraniol	1252	1249	0.8	1.3	-	2.8
Pulegone	1252	1237	-	-	0.7	-
(*E*)-2-Decenal	1266	1260	0.7	0.4	0.9	1.5
4-Methoxy-benzaldehyde	1268	1270	-	-	1.2	-
5-Pentyl-2 (3H)-furanone	1268	1266	-	-	-	0.5
(*E*)-Anethole	1299	1282	-	-	1.9	-
Pelargonic acid	1294	1281	1.6	-	4.2	3.3
Dihydrocarveol acetate	1304	1306	1.2	2.9	-	4.5
Undecanal	1309	1305	-	0.8	-	-
p-Vinyl guaiacol	1318	1309	-	-	-	4.9
(*E, E*)-2,4-Decadienal	1322	1315	0.9	1.6	1.1	-
5-Pentyl-2 (5H)-furanone	1350	1345	0.5	-	1.1	0.7
Eugenol	1364	1359	3.1	1.9	1.8	2.0
(*E*)-2-Undecenal	1365	1357	1.4	-	-	-
Caprinic acid	1387	1380	-	-	1.9	1.4
β-Damascenone	1389	1383	-	-	-	0.9
Methyl eugenol	1407	1401	0.8	-	-	-
Dodecanal	1411	1408	-	0.8	-	0.6
α-Cedrene	1420	1410	-	-	-	1.4
Geranyl acetone	1458	1453	0.9	0.8	0.8	1.1
β-Ionone	1489	1487	0.8	-	-	-
α-Curcumene	1496	1479	-	-	1.4	-
Pentadecane	1500	1500	-	0.6	-	-
Tridecanal	1512	1509	0.4	0.7	-	0.7
Dihydroactinolide	1535	1538	0.4	0.6	-	1.6
Lauric acid	1578	1565	2.2	1.6	2.2	1.5
Caryophyllene oxide	1599	1596	-	-	1.3	-
Hexadecane	1600	1600	0.7	0.9	-	-
Tetradecanal	1614	1611	0.6	1.0	-	0.5
Humulene oxide	1627	1625	-	-	1.0	-
τ-Cadinol	1659	1648	-	-	0.50	-
Heptadecane	1701	1700	1.2	1.1	-	0.8
Myristic acid	1779	1775	2.7	2.4	6.0	1.4
Octadecane	1801	1800	0.7	0.8	-	0.5
Cyclopentadecanolide	1819	1832	-	-	-	0.5
Palmitaldehyde	1825	1822	-	0.8	-	-
Perhydrofarnesyl acetone	1851	1847	2.00	2.8	0.9	3.9
Pentadecanoic acid	1893	1878	-	-	3.0	-
Nonadecane	1902	1900	1.0	3.5	-	2.4
Methyl palmitate	1932	1927	0.8	1.9	0.8	1.9
Palmitic acid	1995	1991	33.7	14.1	30.9	7.4
Ethyl palmitate	1998	1992	5.3	-	-	0.6
Eicosane	2002	2000	-	1.1	-	1.1
Methyl linoleate	2095	2092	0.5	-	-	1.4
Heneicosane	2104	2100	2.2	7.5	1.3	9.6
Linoleic acid	2151	2140	6.6	18.6	10.3	-
Ethyl linoleate	2166	2159	2.8	-	-	0.4
Docosane	2199	2200	0.8	0.6	-	-
9-Tricosene	2272	2271	0.8	0.6	-	1.1
Tricosane	2303	2300	1.5	1.4	0.5	1.4
Tetracosane	2399	2400	1.5	1.3	-	-
(*Z*)-12-Pentacosene	2474	2496	-	0.8	-	1.7
Pentacosane	2503	2500	2.6	2.2	1.4	4.4
Total			92.5	91.8	92.1	83.2

[a] Compounds listed in order of elution from HP-5MS column; [b] Relative retention indices to C_8-C_{32} *n*-alkanes on HP-5MS column; [c] Literature retention indices

Table 2. Antioxidant activity and total phenolic compounds contents of *Mindium laevigatum*

Sample		Antioxidant tests				
Methanol extracts	Plant organ	DPPH (IC_{50}, µg/ml)	β-Carotene/linoleic acid (% Inhibition)	Hydrogen peroxide scavenging (IC_{50}, µg/ml)	Reducing power (IC50, µg/ml)	Total phenolic contents (µg/mg)
Shahsavaran	stem	1366.1±7.1	20.2±0.3	417.2±4.3	633.1±5.2	15.7±1.7
Shahsavaran	fruit	344.3±2.3	39.2±0.6	100.5±2.1	237.6±3.5	79.6±3.6
Rahagh	stem	1052.7±6.1	16.3±0.3	275.4±3.8	679.3±4.8	27.1±2.1
Rahagh	fruit	561.5±4.5	37.4±0.8	218.7±2.4	270.5±3.0	44.6±1.9
Volatile materials						
Shahsavaran	stem	>2000	73.2±0.7	608.2±3.3	683.1±6.2	NT
Shahsavaran	fruit	>2000	64.5±0.4	544.5±4.2	473.5±4.5	NT
Rahagh	stem	>2000	67.8±0.8	739.0±5.4	839.7±6.1	NT
Rahagh	fruit	>2000	55.2±0.5	583.4±3.7	543.2±8.1	NT
Standards						
BHT	-	20.3±0.8	91.3±5.0	NT	NT	NA
Ascorbic acid	-	NA	NT	61.2±1.3	10.2±1.1	NA

NA (Not applicable), NT (Not tested)

Table 3. Cytotoxic activity of *Mindium laevigatum* methanol extracts

Sample		Cytotoxicity test		
Habitat	Plant organ	Brine shrimp bioassay (LC_{50}, µg/ml)	Colon (HCT116) cells (LC_{50}, µg/ml)	Prostate (PC-3) cells (LC_{50}, µg/ml)
Shahsavaran	stem	>1000	>1000	>1000
Shahsavaran	fruit	>1000	753.8±2.5	795.3±3.7
Rahagh	stem	>1000	>1000	>1000
Rahagh	fruit	>1000	833.4±3.3	901.8±2.2
Cisplatin	-	NT	2.3±0.2	3.9±0.4

NT (Not tested)

Table 4. Antimicrobial activity of methanol extracts of *Mindium. laevigatum*

	Plant extract								Antibiotic					
	Rahagh area				Shahsavaran valley									
	Fruit		Stem		Fruit		Stem		Rifampin		Gentamicin		Nystatin	
Microbial strain	DDa	MICb	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC
Gram-positive bacteria														
B. subtilis	13	>500	-	NTc	-	NT	-	NT	13	15.62	21	500	NA	NA
S. epidermidis	24	>500	12	>500	13	>500	22	>500	40	250	35	500	NA	NA
S. aureus	-	NT	-	NT	-	NT	-	NT	10	250	21	500	NA	NA
Gram-negative bacteria														
E. coli	-	NT	-	NT	-	NT	-	NT	11	500	20	500	NA	NA
k. pneumonia	10	>500	-	NT	8	>500	18	>500	7	250	22	250	NA	NA
S. dysenteriae	10	>500	-	NT	11	>500	-	NT	8	250	18	500	NA	NA
P. vulgaris	12	>500	-	NT	10	>500	-	NT	10	125	23	500	NA	NA
S. paratyphi-A serotype	-	NT	-	NT	-	NT	-	NT	-	NT	21	500	NA	NA
Fungi														
C. albicans	-	NT	-	NT	-	NT	-	NT	NA	NA	NA	NA	33	NT
A. niger	-	NT	-	NT	-	NT	-	NT	NA	NA	NA	NA	27	NT
A. brasilienis	-	NT	-	NT	-	NT	-	NT	NA	NA	NA	NA	23	NT

A dash (-) indicate no antimicrobial activity. [a] Inhibition zone in diameter (mm) around the impregnated discs; [b] Minimal Inhibition concentrations (as µg/ml); [c] Samples with no activity in disc diffusion test and nystatin were not entered into the MIC test. NT (Not tested); NA (Not applicable)

Discussion

Chemical composition of volatile materials

Palmitic acid (7.4-33.7%), linoleic acid (6.6-18.6%), heneicosane (1.3-9.6%) and myristic acid (1.4-6.0%) were recorded as major volatile constituents of the plant. These compounds and most of other (sub-major) constituents are belong to unsaturated fatty acid and hydrocarbon families of natural products and the plant is essentially poor in classical essential oil components such as monoterpenes, sesquiterpenes and phenylpropanes (21). These types of secondary metabolites are usually classified as non-classical (non-terpene and non-phenylpropane) essential oils (21). Chromatographic and mass spectral data of many of these compounds were recorded in Adams (11) as the most famous plants essential oils component reference. Our results also confirm the only other work conducted on the plant volatile materials using a combination of experimental GC-Mass and calculative chemometric method (22).

Antioxidant activity

The plant volatile materials showed moderate to good antioxidant activities in *β*-carotene/linoleic acid bleaching assay, but, its activities in other antioxidant tests were negligible. This finding is in accordance with the plant volatile materials chemical compositions which are mainly unsaturated fatty acids and hydrocarbons (see above section). According to these data, antiasthma and antidyspnea effects of the plant decoctions are probably a consequence of the presence of these unsaturated compounds. Inhibition of oxidation processes through hydrogen atom transfer (a well-known capability of unsaturated hydrocarbons) may be suggested as possible mechanism of antioxidant and, subsequently, anti-inflammatory activities of the plant. The plant methanol extracts, on the other hand, only exhibited weak antioxidant activities in DPPH, hydrogen peroxide scavenging and reducing power determination tests. Weak antioxidant activities of the plant extracts may be due to their low phenolic compounds contents reflected in the plant Folin–Ciocalteu's phenol constituents test results reported in Table 2. Inhibition values recorded for *M. laevigatum* stem and fruit methanol extracts in *β*-carotene/linoleic acid bleaching assay were also less than forty percent (Table 2). This is a direct result of vacuum oven drying of the plant methanol extracts which essentially removes almost all volatile materials of the samples. Thus, observed values are solely belong to nonvolatile hydrogen atom donor compounds present in the dried plant extracts. Collection of both volatile and nonvolatile antioxidant compounds in the plant decoction probably intensifies its antiasthma and antidyspnea effects.

Our findings in *β*-carotene/linoleic acid bleaching assay on the plant methanol extracts are similar to another recent report (23). But, our DPPH anti-oxidant activity and phenolic compounds content tests results are completely different from two other studies (5, 23). The first report shows very low activities (IC_{50} = 18.94 to 71.64 mg/ml) in DPPH test despite considerable total phenolic compounds contents (61.0 to 349.3 mg/g). The second one estimates significant antioxidant potentials (IC_{50}= 0.175 to 0.250 mg/ml) but very low total phenolic compounds content (0.158 to 0.182 mg/g). Plant collection conditions and climate differences normally affect plant secondary metabolite compositions and activities. But, unfortunately, we found clues of drawbacks in these reports test procedures which can explain their complicated results and big differences between them and our findings. For example, the first study uses plant extract concentrations up to 10 mg/ml for DPPH test but, surprisingly, reports IC_{50} values of up to 71.64 mg/ml for herbal parts and even up to 287.69 mg/ml for roots of the plant. The second report has an unclear procedure for this test at all. Collectively, we believe that our results are more close to truth than these works.

Cytotoxic activity

High lethal concentrations (LC_{50}, Table 3) in cytotoxic activity evaluations suggest an insignificant cytotoxic activity for the plant. However, fruit extracts from both Rahagh area and Shahsavaran valley showed a slight cytotoxic activity against tested cancer cell lines.

Antimicrobial activity

The fruit methanol extract of the plant from Rahagh area showed moderate antimicrobial activities against five species of the tested microorganisms (Table 4). Maximum inhibition zones and MIC values for microbial strains sensitive to fruit extract were in the range of 10–24 mm and >500 µg/ml, respectively. Other extracts of the plant showed weak antimicrobial activities in both disc diffusion and micro-well dilution tests. Literature only has a single other report on the antimicrobial activity of *M. laevigatum* extracts showing fairly moderate antimicrobial activity for the plant (23).

Conclusion

Literature is poor about the plant *M. laevigatum.* There are few studies about its chemical composition and biological potentials. Moderate to good antioxidant activities of the volatile materials and methanol extracts of the plant in *β*-carotene/linoleic acid bleaching assay and presence of unsaturated hydrocarbons in it may explain its long history of use in traditional medicine. Future works on the plant will hopefully lead to the discovery of useful medical applications for its preparations. Our work may also be considered as a correction for other two unclear and probably wrong published data on the plant.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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