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

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**Abstract**

**Objectives(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 7.96 mg/g. 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.

**Keywords:** Antimicrobial activity, Antioxidant activity, Cytoxic activity, Mindium laevigatum, Volatile compounds

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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, spasmyloytic, 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, V0400, Germany, set at 45 °C). All extractions were repeated three times.

**Chromatographic analysis**

Samples containing volatile components 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 20 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:

\[
I% = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}}\right) \times 100
\]
where $A_{\text{blank}}$ is the absorbance value of the control reaction (containing all reagents except test compounds) and $A_{\text{sample}}$ 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:

$$\% \text{ scavenging of } H_2O_2 = \left( \frac{A_0 - A_1}{A_0} \right) \times 100$$

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 a positive control. Values were presented as means±SD of triplicate analyses.

$\beta$-Carotene/linoleic acid bleaching assay

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

$$1\% = \left( \frac{A_{\beta\text{-carotene after 2 h}}}{A_{\text{initial } \beta\text{-carotene}}} \right) \times 100$$

where $A_{\beta\text{-carotene after 2 h}}$ is the absorbance values of $\beta$-carotene remaining after 2 h in the samples and $A_{\text{initial } \beta\text{-carotene}}$ is the absorbance values of $\beta$-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 means±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-Dimethylthiazol-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 dispiat 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 (LC50) 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 μg/ml and >750 μg/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.
| Compound                        | Relative Retention Indices |
|--------------------------------|-----------------------------|
| **Geraniol**                   |                             |
| **Pulegone**                   |                             |
| (E)-2-Decenal                  |                             |
| 4-Methoxy-benzaldehyde         |                             |
| 5-Pentyl-2 (3H)-furanone        |                             |
| (E)-Anethole                    |                             |
| Pelargonic acid                 |                             |
| Dihydrocarveolacetate           |                             |
| Undecanal                       |                             |
| p-Vinyl guaiacol                |                             |
| (E, E)-2,4-Decadienal           |                             |
| 5-Pentyl-2 (5H)-furanone        |                             |
| Eugenol                         |                             |
| (E)-2-Undecenal                 |                             |
| Caprinic acid                   |                             |
| β-Damascenone                   |                             |
| Methyl eugenol                  |                             |
| Dodecanal                       |                             |
| a-Cedrene                       |                             |
| Geranylacetone                  |                             |
| β-Ionone                        |                             |
| α-Curcumene                     |                             |
| Pentadecane                     |                             |
| Tridecanal                      |                             |
| Dihydroactinolide               |                             |
| Lauric acid                     |                             |
| Caryophyllene oxide             |                             |
| Hexadecane                      |                             |
| Tetradecanal                    |                             |
| Humulene oxide                  |                             |
| γ-Cadinol                       |                             |
| Heptadecane                     |                             |
| Myristic acid                   |                             |
| Octadecane                      |                             |
| Cyclopentadecanamide            |                             |
| Palmitaldehyde                  |                             |
| Perhydrofarnesyl acetone        |                             |
| Pentadecanoic acid              |                             |
| Nonadecane                      |                             |
| Methyl palmitate                |                             |
| Palmitic acid                   |                             |
| Rhyl palmitate                  |                             |
| Eicosane                        |                             |
| Methyl linoleate                |                             |
| Heneicosane                     |                             |
| Linoleic acid                   |                             |
| Rhyl linoleate                  |                             |
| Docosane                        |                             |
| 9-Tricosene                     |                             |
| Tricosane                       |                             |
| Tetracosane                     |                             |
| (2)-12-Pentacosene              |                             |
| Pentacosane                     |                             |
| **Total**                       |                             |

* Compounds listed in order of elution from HP-SMS column; | Relative retention indices to C<sub>10</sub>-C<sub>14</sub> n-alkanes on HP-SMS column; <sup>c</sup> Literature retention indices.
Table 2. Antioxidant activity and total phenolic compounds contents of Mindium laevigatum

| Sample | Antioxidant tests |
|--------|------------------|
|        |                  |
| Methanol extracts | Plant organ | DPPH (IC<sub>50</sub>, μg/ml) | β-Carotene/linoleic acid (% Inhibition) | Hydrogen peroxide scavenging (IC<sub>50</sub>, μg/ml) | Reducing power (IC<sub>50</sub>, μg/ml) | Total phenolic contents (μg/mg) |
| Shahsavaran stem | 136.1±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 | 7.9±3.6 |
| Rahagh stem | 512.7±5.1 | 61.2±0.3 | 275.4±3.8 | 679.3±4.8 | 27.1±1.1 |
| Rahagh fruit | 561.5±4.5 | 37.4±0.8 | 218.7±2.4 | 270.5±3.0 | 4.6±1.9 |

Volatiles materials

| Sample | Content |
|--------|---------|
| Shahsavaran stem | >2000 | 73.2±0.7 | 608.2±3.3 | 681.1±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 | 585.4±3.7 | 543.2±8.1 | NT |

Table 3. Cytotoxic activity of Mindium laevigatum methanol extracts

| Sample | Cytotoxicity test |
|--------|------------------|
|        |                  |
| Methanol extracts | Plant organ | Brine shrimp bioassay (LC<sub>50</sub>, μg/ml) | Colon (HCT116) cells (LC<sub>50</sub>, μg/ml) | Prostate (PC3) cells (LC<sub>50</sub>, μg/ml) |
| Shahsavaran stem | >1000 | >1000 | >1000 |
| Shahsavaran fruit | >1000 | 753.8±2.5 | 793.5±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 |

Table 4. Antimicrobial activity of methanol extracts of Mindium laevigatum

| Sample | Microbial strain |
|--------|------------------|
|        | Plant extract | Plant organ | Shahsavaran valley | Antibiotic |
|        | Fruit area | Stem | Fruit | Stem | Rifampin | Gentamicin | Nystatin |
|        | DDx | MIC<sub>b</sub> | DD | MIC | DD | MIC | DD | MIC | DD | MIC | DD | MIC |
| Gram-positive bacteria |
| B. subtilis | 13 | >500 | NT<sup>c</sup> | NT | NT | NT | 13 | 15.6±2 | 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 | NT | 11 | 500 | 20 | 500 | NA | NA |
| k. pneumonia | 10 | >500 | NT | NT | 8 | >500 | 18 | >500 | 7 | 250 | 22 | 250 | NA | NA |
| S. dysenteriae | 10 | >500 | NT | NT | 11 | >500 | 11 | >500 | 8 | 250 | 18 | 500 | NA | NA |
| P. vulgaris | 12 | >500 | NT | NT | 10 | >500 | 19 | >500 | 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 | NT | NT | 27 | NA | NA | NA | NA | NA |
| A. niger | - | NT | NT | NT | NT | NT | NT | 27 | NA | NA | NA | NA | NA |
| A. brassicaiis | - | NT | NT | NT | NT | NT | NT | 27 | NA | NA | NA | NA | NA |

A dash (·) indicate no antimicrobial activity. *Inhibition zone in diameter (mm) around the impregnated discs; †Minimal Inhibition concentrations (as μg/ml); ‡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 (14.4-6.0%) 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 antioxidant 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 cytotoxicity evaluations suggest an insignificant cytotoxic activity for the plant. However, fruit extracts from both Rahagh area and Shahasavar 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.

Acknowledgment

The results described in this paper were part of student thesis. Financial support (Grant number: 211037-12) made by the Research Chancellor of the University of Kashan, Kashan, Islamic Republic of Iran is gratefully acknowledged.

Conflict of interest

The authors declare that there are no conflicts of interest.

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