Chemical Composition of the Essential Oil, Total Phenolic Content and Antioxidant Activity in *Origanum Majorana* L. (Lamiaceae) Cultivated in Iran

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ABSTRACT

The chemical composition of essential oil isolated by hydrodistillation from the aerial part of *Origanum majorana* was determined using Gas Chromatography (GC) and Gas Chromatography/Mass Spectrometry (GC/MS). The main components of the oil in *Origanum majorana*, were trans Sabinene hydrate (39.2% ± 3.7), Terpinene-4-ol (16.8% ± 2.3), cis-Sabinene hydrate (7.5% ± 0.4), δ-Terpinene (6.6% ± 0.9), Sabinene (5.2% ± 0.1), α-Terpineol (4.6 ± 0.5), α-Phellandrene (3.6 ± 0.4), p-Cymene (2.6 ± 0.8) and β-Caryophyllene (2.5 ± 0.3). The total phenolic contents and the antioxidant activity of plant extract were determined, respectively, by Folin-Ciocalteau and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays. Plant extract of *Origanum majorana* had more phenolic content and radical scavenging effect. A significant correlation was found between phenolic contents and antioxidant activity ($R^2=0.98$). The results showed that *Origanum majorana* possesses significant sources of natural antioxidant properties for culinary and possible medicinal use.

Key words: *Origanum majorana*, Essential oil, Trans Sabinene hydrate, phenolic compounds, Antioxidant.

Introduction

Medicinal plants are among the main sources of human drugs, and play a key role in world health care systems. Natural components of these plants are used as food additives, pigments, dyes, insecticides, cosmetics, perfumes and fine chemicals. These compounds belong to a group collectively known as secondary methabolites [21].

The labiatae family include about 250 genera and 6700 species and plants spread in the warm and temperate regions all over the world [19]. They are mainly grasses and shrubs, very fragrant and rich in medicinal properties and widely used in spices, perfumes, and medicinal products [22]. *Origanum* is an important genera in lamiaceae family as herbal tea, flavoring agents (condiment and spice) and medicinal plants [29].

*Origanum vulgare* L. is the endemic *Origanum* species in Iran (Common Persian name: Marzanjoosh.

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or Marzangoosh) [24]. In this research, we cultured *Origanum majorana* as a new importation medicinal plant in Iran. Herbal parts of *Origanum* species are aromatic and are used as condiment or herbal tea. Dried *Origanum* species are also used for the production of essential oil, and an aromatic water or hydrosol. Oregano water rich in carvacrol is taken orally for gastrointestinal disorders to reduce blood cholesterol and glucose levels and also for cancer [4].

Previous study on essential oil composition in *Origanum majorana* showed that, terpinene-4-ol (38.4%), cis-sabinene hydrate (15.5%), p-cymene (7.0%) and δ-terpinene (6.9%), were the main components in this plant [32].

*Origanum majorana* has two chemotypes, Sweet marjoram and white oregano. White oregano, is the component in the oil of *O. majorana*. Carvacrol (78.27%) was found to be the major components in this plant [4].

In this research, we cultured *Origanum majorana* and investigated essential oil composition of this plant. This study focuses on influence of environments of Iran on essential oil composition and bioactive substances of *Origanum majorana* as possible sources for valuable components and determines the total phenolic content and antioxidant activity in this medicinal plant as possible new sources for phenolic content and natural antioxidant. This research also demonstrates a possible relationship between phenolic content and antioxidant activity.

**Materials and methods**

**Chemical Reagents:**

The chemical reagents DPPH (2, 2-diphenyl-1-picrylhydrazyl), gallic acid monohydrate [3, 4, 5-trihydroxybenzoic acid] and sodium carbonate were purchased from Sigmaaldrich chemicals (St. Louis, USA). Folin Ciocalteau reagent, methanol and acetone were purchased from Merck (Darmstadt, Germany). Trolox and Quercetin hydrate were purchased from Acros organics (New Jersey, USA).

**Plant Material:**

Seed of *Origanum majorana* was obtained from Institute of Medicinal Plants, Isfahan, Iran and was grown in green house conditions. Seed of medicinal plant was grown in sterile soil. One seedling of each plant were transplanted into pots (20×20 cm) and arranged in randomized complete block design in four replicates. Each replicate was contained 8 pots. Green house plants were irrigated to pot capacity and maintained at day/night temperatures of 26-30 and 18-22 ºC respectively. Green house plants were harvested in preflowering stage. Each plant was vouchedered, weighed, bulked and placed in paper bag and dried in room temperature for 15 days befor analysis.

**Essential oil Extraction and Analysis:**

Essential oil was obtained from dried aerial parts of *Origanum majorana* by steam distillation using Clevenger type apparatus for 3 hours. The extracted essential oil was dried with anhydrous sodium sulfate and stored in sealed amber flasks at 4ºC until analysis.

Gas chromatography analysis was performed on an Agilent technologist model (6990 USA) series II gas chromatograph was equipped with flame ionization detector and capillary column HP (30 m 0.25 mm, 0.25 µm film thickness). The chromatographic conditions were as follows: The oven temperature increased from 60 ºC to 240 ºC at a rate of 3 ºC/min. The injector and detector temperatures were 240 ºC and 250 ºC, respectively.
Helium used as the carrier gas was adjusted to a linear velocity of 32 cm/s. The samples were injected using split sampling technique by a ratio of 1:50. Quantitative data was obtained from electronic integration of peak areas without the use of correction factors. Essential oil was also analysed by Hewlett-Packard GC-MS (model 6890 series II) operating at 70eV ionization energy, equipped with a HP-5 capillary column phenyl methyl siloxane (30 m × 0.25 mm, 0.25 µm film thickness) with Helium as the carrier gas and a split ratio of 1:20. The retention indices for all the components were determined according to the Van Den Dool method using n-alkanes as standard. The compounds were identified by comparison of retention indices (RRI-AP-5) with those reported in the literature and by comparision of their mass spectra with the Wiley and Mass finder 3 libraries or with the published mass spectra.

Preparation of Extract:

For preparation of extracts, grounded air dried plant material (7.5 g) was weighed in a glass and then defatted with petroleum benzine for 3 hours. Each Sample was twice extracted with 200 ml of 90% aqueous methanol (methanol: water, 90:10), each time for 24 hours at room temperature. Each extraction was filtrated through whatman filter paper (Whatman Ltd., England) and evaporated to dryness using a rotary evaporator to a volume of about 1 ml. These concentrated extracts were freeze-dried in Eyela lyophilizer (model FD 100) and weighed to determine the yield. These samples were stored at 4°C for further experiments.

Total Phenolic Content:

The total phenolic content was determined with the Folin-Ciocalteau reagent as described previously [28]. Briefly, 200 μl of plant extract dissolved in methanol (1 mg/ml) were mixed with 2.5 ml of Folin-Ciocalteau reagent (diluted 10 times in distilled water) in glass tubes in triplicate. The samples were incubated at room temperature for 5 min and vortex mixed at least 2 times. Then, 2 ml of Na2CO3 (7.5%) was added and the glass tubes were incubated in the dark for 90 min with continuous shaking. The absorbance of samples were measured at 765 nm using a Spectrophotometer (Perkin-Elmer UV/Vis double beam lambda 1, USA) against a blank of distilled water. Different concentrations of gallic acid in methanol were tested to obtain a standard curve. Total phenolic content was expressed as milligrammes of gallic acid equivalent per gram of dry weight (mg GAE/g dw).

The antioxidant activity was determined by DPPH free radical scavenging assay as described previously with some modifications [7]. Briefly, 4 different concentrations of the plant extract dissolved in methanol were incubated with a methanolic solution of DPPH 100 mM in a total volume of 4 ml. After 30 min of incubation at room temperature, the absorbance was recorded at 517 nm. Methanol was used as blank and all measurements were carried out in triplicate. Trolox, a water-soluble equivalent of vitamin E, and quercetin were used as reference compounds. All solutions were made daily. The percent inhibition of DPPH free radical was calculated by the formula:

\[
\text{Percentage inhibition} = \left( \frac{\text{A blank} - \text{A sample}}{\text{A blank}} \right) \times 100
\]

Where, A blank is the absorbance of the control reaction (DPPH alone), and A sample is the absorbance of DPPH solution in the presence of the test compound.

IC50 values denote the concentrations of the sample, required to scavenge 50% of DPPH free radicals.

Results and Discussion

Fresh and Dry Weight, essential Oil Yield and Composition:

Fresh and dry weights in Origanum majorana are presented in Table 1. The amounts of fresh and dry weight are 21.05 and 4.65 (g/plant) respectively. Chemical composition of the essential oil of O. majorana are given in (Table 2). The essential oil isolated by hydrodistillation of the aerial part of O. majorana was found to be a pale yellow oil, obtained in yield of 1.39 (v/w) based on dry weight and essential oil efficiency was 64.74 (mg/plant) respectively (Table 1).

Twenty-five components were identified in the essential oil of O. majorana, that represented 99.05% of the oils. The major components were, trans-sabinene hydrate (39.2%), terpinene-4-ol (16.8%), cis-sabinene hydrate (7.5%), δ-terpinene (6.6%), sabinene (5.2%), α-terpineol (4.6%) and α-Phellandrene (3.5%), p-cymene (2.6 ± 0.8) and β-caryophyllene (2.5 ± 0.3) (Table 2).

The main components of essential oil of O. vulgare was carvacrol [14,33]. In our study in O. majorana there is no representation of carvacrol but this plant was rich as trans-sabinene hydrate, terpinene-4-ol, cis-sabinene hydrate and δ-terpinene.

Total Phenolic Content:

In this study, we used Thymus vulgaris, which is an important medicinal plant in the Labiatae.
family, as a reference plant for comparisons of the total phenolic content and antioxidant activity. Phenolic compounds is a class of antioxidant agents which act as free radical scavengers and are responsible for the major part of the antioxidant activity of medicinal plants [27]. Total phenolic content was measured by the folin-Ciocalteau reagent and expressed as gallic acid equivalent (Standard curve equation: \( y = 0.0287x + 0.2384 \), \( R^2 = 0.9949 \) (Data not shown). \( O. majorana \) and \( T. vulgaris \) with total phenolic contents of 44.07 and 19.55 mg GAE/g DW, respectively, had the highest and the lowest levels of total phenolic content among the tested plants in this study (Table 3).

The results show that the total phenolic content in \( O. majorana \) is about more than two times that in \( T. vulgaris \) in this study. Similarly, Zheng and Wang [34] have previously reported that the oregano herb (\( O. vulgare \)) had antioxidant activity and total phenolic content 3-20 times higher than the other studied herbs assessed by the ORAC method.

### Free Radical Scavenging:

Damage induced by free radicals such as super oxide, hydroxyl and peroxyl radicals, and single oxygen could cause or aggravate many diseases such as cancer and coronary heart disease [8, 17]. Much plant extracts containing bioactive compounds including phenolics and flavonoids exhibit efficient antioxidant properties, and could potentially be protective against free radical damages [15, 18]. The antioxidant activity can be exerted by different mechanisms such as prevention of chain initiation, decomposition of proxides, and prevention of continued hydrogen abstraction, free radical scavenging, reducing capacity and binding of transition metal ions [6, 20].

In the present study, we used DPPH stable free radical scavenging capacity as an index of antioxidant activity. The DPPH method is an easy, rapid and sensitive way to study the antioxidant activity of plant extracts and specific compounds [15].

In this assay, when DPPH encounters proton radical scavengers, its purple colour fades rapidly. The ability to scavenge DPPH radicals by antioxidant is determined by measuring the reduction of DPPH absorbance at 517 nm [23]. Some variations of plant extracts were used for antioxidant activity in this study.

The radical scavenging activity of \( O. majorana \) and \( T. vulgaris \) extracts increased along with increasing in concentrations of plant extracts. The highest radical scavenging activity was shown by \( Origanum majorana \). \( IC_{50} \) value which is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50% was determined (Table 3). Antioxidant activity in \( Origanum majorana \) and \( Thymus vulgaris \) were (\( IC_{50} = 6.88 \mu g ml^{-1} - 8.07 \mu g ml^{-1} \)) respectively.

A significant linear relationship was observed between DPPH scavenging \( IC_{50} \) values and the total phenolic content in the studied plants (\( R^2 = 0.98 \)) (Tables 4). In fact, \( Origanum majorana \) showed the highest phenolic content as well as the highest antioxidant activity. On the other hand, \( Thymus vulgaris \) showed the low phenolic content and was the weakest in antioxidant activity (Tables 2 and 3). These results suggest that the DPPH radical scavenging ability could be mainly due to the presence of the phenolic compounds in the plant extracts.

### Conclusion:

This study shows that the \( Origanum majorana \), is the main source for trans sabinene hydrate, Terpinene-4-ol and other valuable components such as \( \delta \)-terpinene, \( p \)-cymene, sabinene, \( \alpha \)-terpineol, \( \alpha \)-phellandrene, and \( \beta \)-caryophyllene. Also this plant possesses strong activities and could potentially be used as natural source of phenolics and antioxidants. These results showed that environmental condition of Iran is suitable for sowing oregano.

### Acknowledgments

This research was supported by Estahban branch, Islamic Azad University, Estahban Iran, and Medicinal and Natural Products Chemistry Research Center of Shiraz Medical Sciences. Shiraz, Iran. We would like to thank Professor Ramin Miri for his help and the use of laboratory facilities.

### Table 1: Fresh and dry weight, essential oil yield and efficiency in \( Origanum majorana \).

<table>
<thead>
<tr>
<th>Fresh weight * (g/plant)</th>
<th>Dry weight * (g/plant)</th>
<th>Essential oil yield † (%)</th>
<th>Essential oil efficiency ‡ (mg/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.05 ± 0.27</td>
<td>4.65 ± 0.13</td>
<td>1.39± 0.06</td>
<td>64.74± 0.003</td>
</tr>
</tbody>
</table>

Each value in the table was obtained by calculating the average of four experiments ± standard deviation.

* Fresh and Dry weight. Data expressed as grams.

† Essential oil yield. Data expressed as mg per 100 g dry weight (DW).

‡ Essential oil efficiency. Data expressed as mg per g dry weight (DW).
Table 2: Essential oil yield and composition in *Origanum majorana*.

<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
<th>RI</th>
<th>% in Oil Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-Thujene</td>
<td>928</td>
<td>0.3 ± 0.01</td>
</tr>
<tr>
<td>2</td>
<td>α-Pinene</td>
<td>934</td>
<td>0.5 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>Sabinene</td>
<td>973</td>
<td>5.2 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>β-Pinene</td>
<td>978</td>
<td>0.3 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>Myrcene</td>
<td>990</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>6</td>
<td>α-Phellandrene</td>
<td>1002</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td>7</td>
<td>α-Terpinepin</td>
<td>1015</td>
<td>0.3 ± 0.01</td>
</tr>
<tr>
<td>8</td>
<td>P-Cymene</td>
<td>1024</td>
<td>2.6 ± 0.08</td>
</tr>
<tr>
<td>9</td>
<td>β-Phellandrene</td>
<td>1033</td>
<td>0.05 ± 0.04</td>
</tr>
<tr>
<td>10</td>
<td>Sabinene hydrate</td>
<td>1061</td>
<td>7.5 ± 0.4</td>
</tr>
<tr>
<td>11</td>
<td>Terpinolene</td>
<td>1087</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>12</td>
<td>Trans sabinene hydrate</td>
<td>1100</td>
<td>39.2 ± 3.8</td>
</tr>
<tr>
<td>13</td>
<td>P-cymene</td>
<td>1126</td>
<td>1.2 ± 0.02</td>
</tr>
<tr>
<td>14</td>
<td>Trans-p-cymene-2-en-1-ol</td>
<td>1143</td>
<td>0.5 ± 0.03</td>
</tr>
<tr>
<td>15</td>
<td>Terpinolene-4-ol</td>
<td>1190</td>
<td>0.1 ± 0.04</td>
</tr>
<tr>
<td>16</td>
<td>α-Terpineol</td>
<td>1224</td>
<td>0.2 ± 0.04</td>
</tr>
<tr>
<td>17</td>
<td>P-menth-8-yl-acetate</td>
<td>1240</td>
<td>0.2 ± 0.01</td>
</tr>
<tr>
<td>18</td>
<td>Trans-sabinene hydrate</td>
<td>1289</td>
<td>0.1 ± 0.02</td>
</tr>
<tr>
<td>19</td>
<td>Geranyl acetate</td>
<td>1336</td>
<td>0.2 ± 0.06</td>
</tr>
<tr>
<td>20</td>
<td>β-Caryophyllene</td>
<td>1381</td>
<td>0.1 ± 0.01</td>
</tr>
<tr>
<td>21</td>
<td>α-Humulene</td>
<td>1417</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>22</td>
<td>Bicyclogermacrene</td>
<td>1496</td>
<td>1.7 ± 0.08</td>
</tr>
<tr>
<td>23</td>
<td>Bicycloelemene</td>
<td>1500</td>
<td>0.2 ± 0.06</td>
</tr>
<tr>
<td>24</td>
<td>Geranyl acetate</td>
<td>1530</td>
<td>0.2 ± 0.04</td>
</tr>
<tr>
<td>25</td>
<td>P-Menth-8-yl-acetate</td>
<td>1544</td>
<td>0.1 ± 0.01</td>
</tr>
</tbody>
</table>

Total 99.05%

Table 3: Total phenolic content and antioxidant activity in *Origanum majorana* and *Thymus vulgaris*.

<table>
<thead>
<tr>
<th>Chemical fertilizer Treatment</th>
<th>Total phenolic content (mg GAE/g DW.)</th>
<th>( IC_{50} ) (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Origanum majorana</em></td>
<td>44.07 ± 0.08 a</td>
<td>6.88 ± 0.06 a</td>
</tr>
<tr>
<td><em>Thymus vulgaris</em></td>
<td>19.55 ± 0.115 b</td>
<td>8.07± 0.17 b</td>
</tr>
</tbody>
</table>

1Data expressed as mg of gallic acid equivalents per g dry weight (DW).

2\( IC_{50} \): Data expressed as μg per millilitre. Lower \( IC_{50} \) values indicated the highest radical scavenging activity.

Means with different letters were significantly different at the level of \( p<0.05 \).

Each value in the table was obtained by calculating the average of four experiments ± standard deviation. Values followed by the same letter under the same row, are not significantly different (\( p>0.05 \)).

Table 4: Pearson correlation between traits in *Origanum majorana*.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Fresh weight</th>
<th>Dry weight</th>
<th>Essential oil Yield</th>
<th>Essential oil efficiency</th>
<th>Total phenolic content</th>
<th>Antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh weight</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Dry weight</td>
<td>0.89 **</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Essential oil Yield</td>
<td>0.82 **</td>
<td>0.65**</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Essential oil efficiency</td>
<td>0.99 **</td>
<td>0.88 **</td>
<td>0.82**</td>
<td>---</td>
<td>0.79*</td>
<td>---</td>
</tr>
<tr>
<td>Total phenolic content</td>
<td>0.79**</td>
<td>0.52 *</td>
<td>0.67 **</td>
<td>0.87 **</td>
<td>0.98**</td>
<td>---</td>
</tr>
<tr>
<td>Antioxidant activity</td>
<td>0.87 **</td>
<td>0.62 *</td>
<td>0.74 **</td>
<td>0.87 **</td>
<td>0.98**</td>
<td>---</td>
</tr>
</tbody>
</table>

*: Significant at 5% probability

**: Significant at 1% probability

References


