Teucrium polium L. essential oil: phytochemical component and antioxidant properties

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Abstract

The essential oil (EO) obtained by hydrodistillation from Teucrium polium L. was analyzed by gas chromatography and gas chromatography mass spectrometry (GC/MS). Antioxidant activity was evaluated through DPPH assay and Beta-carotene/linoleic acid assay. Twenty one components were identified representing (90.4%) of the EO. Main constituents of the essential oil were found as Spathulenol (15.06%), Beta-Pinene (11.02), Beta-Myrcene (10.05), Germacrene B (10.11%), Germacrene D (8.15%), Bicyclogerma (8.25%) and Linalool (4.02). This essential oil was able to reduce the stable free radical DPPH with an IC$_{50}$ of 9200 μg/ml. In Beta-carotene/linoleic acid assay, these samples were not effectively able to complete inhibit the linoleic acid oxidation, exhibiting only 61% inhibitions at 2 mg/ml. These results demonstrate that the T. polium EO possesses antioxidant activity in vitro. Further investigations are needed to verify whether this antioxidant effect occurs in vivo.

Introduction

Teucrium is a genus of perennial plants which belongs to the family Lamiaeae is represented by more than 340 species widespread all around the world and comprises about 12 species in Iran. They are most common in Mediterranean climates and Middle East. An unusual feature of this genus compared with other member of Lamiaeae is that flower completely lack of the upper lip of corolla. Its flower is small and range from pink to white and its leaves are used in cooking and for medicinal purposes (Rechinger, 1982; Zargari, 1992; Mozaffarian, 1996). T. polium L. is a plant that has been used for over 2000 years in traditional medicine due to its diuretic, diaphoretic, tonic, antipyretic, antispasmodic and cholagogic properties (Galati et al., 2000; Said et al., 2002). In addition, the plant possesses hypoglycemic, insulinotropic, anti-inflammatory activities, hypolipidemic, antinociceptive and antioxidant properties (Couladis et al., 2003; Esmaeili et al., 2004). The therapeutic benefit of medicinal plants is often attributed to their antioxidant properties (Rice, 2004; Dixon et al., 2005). In this regard, a methanolic extract of T. polium protected red blood cells (RBCs) against lipid peroxidation induced by 10 mmol hydrogen peroxide (Souh et al., 2004). In another study, Kadifkova-Panovska et al. (2005) demonstrated that extracts of T. polium prepared using different organic solvents (diethyl ether, ethyl acetate and n-butanol) were effective inhibitors of b-carotene oxidation. Ljubuncic et al. (2005) showed that the aqueous extracts prepared from the foliage of T. polium suppressed iron (Fe2)-induced lipid peroxidation in rat liver homogenates to the same extent as Trolox, the water soluble analog of vitamin E. Additionally, this extract was not cytotoxic because it did not adversely affect cell membrane integrity or suppress mitochondrial respiration of cultured Hep G2 and PC12 cells following 24 h exposure (Ljubuncic et al., 2005). In another study, Azaizeh et al. (2005) reported that adding fertilizer caused a significant concentration-dependent increase in antioxidant activity of the cultivated T. polium compared with the wild-type (Azaizeh et al., 2005). In the present work we have studied the chemical composition and antioxidant properties of the T. polium EO that grow in Kerman province in Iran.

Material and Methods

Plant material

The T. polium L. was collected during flowering stage from West of Iran (Kerman province) and identified by the Herbarium of faculty of Pharmacy, University of Tabriz, Tabriz, Iran. Then shade dried and grinded into powder. The prepared powder was kept in tight containers protected completely from
light.

**EO preparation**

Dry aerial parts (100 g) of *T. polium* L. were subjected to the hydrodistillation of 2.5 h, using a cleavenger-type apparatus, according to the method recommended by the European Pharmacopia to produce oils (Maissoneuve, 1983). The obtained essential oil was dried over anhydrous sodium sulphate and stored at +4°C until tested and analyzed.

**Gas chromatography–mass spectrometry (GC/MS)**

The EO was analyzed by gas chromatography. The chromatograph (Agilent 6890 UK) was equipped with an HP-5MS capillary column (30 × 0.25 mm ID × 0.25 mm film thickness) and the data were taken under the following conditions: initial temperature 50°C, temperature ramp 5°C/min, 240°C/min to 300°C (holding for 3 min), and injector temperature at 290°C. The carrier gas was helium and the split ratio was 0.8 mL⁻¹/min. For confirmation of analysis results, essential oil was also analyzed by GC/MS (Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass-selective detector; Agilent UK) and the same capillary column and analytical conditions as above. The MS was run in electron-ionization mode with ionization energy of 70 eV (Mahmoudi et al., 2012).

**Antioxidant assay**

**DPPH assay**

The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple colored methanol solution of DPPH. This spectrophotometer assay (Pharmacia, Uppsala, Sweden) uses stable radical DPPH (Sigma–Aldrich) as a reagent. Aliquots (50 µl) of various concentrations of the essential oil were added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percent (I %) was calculated in following way:

\[
I\% = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}}\right) \times 100
\]

Where \(A_{\text{blank}}\) is the absorbance of the control reaction (containing all reagents except the test compound), and \(A_{\text{sample}}\) is the absorbance of the test compound. Essential oil concentration providing 50% inhibition (IC₅₀) was calculated from the graph plotted inhibition percentage against essential oil concentration (Cuendet et al., 1997; Burits and Bucar, 2000).

Synthetic antioxidant reagent butylated hydroxytoluene (BHT) was used as the positive control and all tests were carried out in triplicate.

**Beta-Carotene–linoleic acid assay**

In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius et al., 1998). A stock solution of B-carotene/linoleic acid (Sigma–Aldrich) was prepared as follows. First, 0.5 mg of B-carotene was dissolved in 1 ml of chloroform (HPLC grade), then 25 µl of linoleic acid and 200 mg of Tween 40 (Merck) were added. The chloroform was subsequently evaporated using a vacuum evaporator (Buchi, Flawil, Switzerland). Then 100 ml of distilled water saturated with oxygen was added with vigorous shaking. Aliquots (2.5 ml) of this reaction mixture were transferred to test tubes, and a 350 µl portion of the essential oil was added before incubating for 48 h at room temperature. The same procedure was repeated with BHT at the same concentration and a blank containing only 350 µl of ethanol. After the incubation period, the absorbencies of the mixtures were measured at 490 nm. Antioxidant capacities of the samples were compared with those of BHT and the blank.

**Assay for total phenolics**

Total phenolic constituent in *T. polium* EO was performed employing the literature methods (Slinkard and Singleton, 1977; Chandler and Dodds, 1983). Involving Folin-Ciocalteu reagent and gallic acid (both Sigma–Aldrich) as standard. Briefly, an aliquot (0.1) ml of extract solution containing 1 mg of extract was transferred to a volumetric flask, 46 ml distilled water and 1 ml Folin-Ciocalteu reagent was added and the flask was shaken thoroughly. After 3 min, 3 ml of solution 2% Na₂CO₃ was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. The same procedure was repeated for all standard gallic acid solutions (0–1000 mg in 0.1 ml) and a standard curve was obtained according to the equation

\[
\text{Absorbance} = 0.0012 \times \text{Gallic acid (µg)} + 0.0033
\]

**Results**

The EO was extracted by the hydrodistillation of dried parts of *T. polium* were analyzed by GC and GC–MS, the oil yields were calculated on a dry weight basis as 0.50% (w/w). GC and GC–MS analysis enabled the identification of a total of 21
Table 1. Constituents of T. polium EO

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Compound Name</th>
<th>RT (minute)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Verbenene</td>
<td>6.97</td>
<td>1.23</td>
</tr>
<tr>
<td>2</td>
<td>Beta-Pinene</td>
<td>7.59</td>
<td>1.12</td>
</tr>
<tr>
<td>3</td>
<td>Beta-Myrccene</td>
<td>7.93</td>
<td>10.05</td>
</tr>
<tr>
<td>4</td>
<td>Benzene, 1-methyl</td>
<td>8.88</td>
<td>1.66</td>
</tr>
<tr>
<td>5</td>
<td>Cyclohexene, 1-methyl(14-(1-methyllethyl)</td>
<td>9.01</td>
<td>2.00</td>
</tr>
<tr>
<td>6</td>
<td>2,3,3-Triethyl-3-cycloptene acetaledehyde</td>
<td>11.80</td>
<td>1.71</td>
</tr>
<tr>
<td>7</td>
<td>Bicyclgermacrene</td>
<td>12.49</td>
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</tr>
<tr>
<td>8</td>
<td>Benzene</td>
<td>13.87</td>
<td>1.46</td>
</tr>
<tr>
<td>9</td>
<td>Bicyc[3.1.1]hept-3-ene, 4,6,6-trimethyl</td>
<td>14.27</td>
<td>1.31</td>
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<tr>
<td>10</td>
<td>1,6,10-Dodecatriene, 7,11-dimethyl-1-methyle</td>
<td>20.71</td>
<td>1.23</td>
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<tr>
<td>11</td>
<td>Germacrene D</td>
<td>21.42</td>
<td>8.15</td>
</tr>
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<td>Germacrene B</td>
<td>23.28</td>
<td>10.11</td>
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<td>13</td>
<td>sesquiaslbene hydrate</td>
<td>23.46</td>
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<td>o-Menth-8-ene</td>
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<td>1.33</td>
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<td>16</td>
<td>3-Cyclohexene-1-methanol, alpha,4-dimethyl</td>
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<td>1.23</td>
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<td>17</td>
<td>1H-3a,7-Methanoindene</td>
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<td>1.84</td>
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<td>Naphthalene</td>
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<td>1.21</td>
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<td>19</td>
<td>Linalool</td>
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<td>4.02</td>
</tr>
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<td>20</td>
<td>Cycloleinglockene oxide, dehydo</td>
<td>26.22</td>
<td>1.05</td>
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<tr>
<td>21</td>
<td>Benzenemethanol,4(1-methylethyl)</td>
<td>26.98</td>
<td>1.30</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>90.40</td>
</tr>
</tbody>
</table>

RT: Retention Time (minute)

Discussion

The EO of Teucrium species has been studied in Iran and in the world. A scientific study of the oil of T. stocksianum subsp. Stocksianum in April 2003 found thirty-eight compounds with 97.7% of the oil were identified. The oil yield obtain 0.7% on a dry weight basis. The major component of the oil as Camphene (20.6%), alpha pinene (19.7%), Myrcene (10.2%) and Carvacrol (9.90%) (Jaimand et al., 2006). The studies made have reported the volatile constituents of T. flavum leaves from Iran at the full flowering stage in May 2001, eighteenth compounds were identified constituting about 99% with 0.2% yield of the oil that major components were beta-caryophyllene (30.7%), Geranicne (21.3%) and alpha-humulene (8.4%) (Baher and Mirza, 2003).

A study on the oil obtained from T. polium grow in Iran (Eikani et al., 1999) revealed the presence of sesquiterpenes as major components. Germacrene D (13.2%), beta-caryophyllene (18%), Spathulenol (10.4%) and bicyclgermacrene (9.0%) being the major components identified. The main and important ingredient studied in different regions of the world in Italia on T. fruticans include p-Cymene (7.0%) (Cozzani et al., 2005). Chemical composition of the EO of the aerial parts of T. polium L. grow in Jordan was determined by GC/MS. The oil obtained by hydrodistillation was found to contain 39 components, of which 37 were identified. The major components determined were 8-cedren-13-ol(24.8%), beta-caryophyllene (8.7%), Germacrene D (6.8%) and Subinene (5.2%) (Aburja et al., 2006). Thirty-seven components were detected in the EO obtained from the aerial part of Turkish T. polium (Cakir et al., 1998). The major components identified were beta-pinene (18%), beta-caryophyllene (17.8%), alpha-pinene (12%), Caryophyllene oxide (10%), Myrcene (6.8%), Germacrene D (5.3%), Limonene (3.5%) and Spathulenol (3.3%). It is the first time that a T.polium has been found to contain Spathulenol (14.65%) at such high percentage, Spathulenol was reported as an important constituents of T. polium (10.4%)
(Eikani et al., 1999) and in the EO obtained from the aerial parts of Turkish T. polium (3.3%) (Cakir et al., 1998). Based on the results of GC/MS analysis of T. polium EO in our study, beta-Pinene, beta-Mycene, Germaocene B, Germaocene D, bicyclogermacone and Linalool were the major components and also these component has been reported as the major compounds of EO obtained from Teucrium species in other scientific researches (Cakir et al., 1998; Eikani et al., 1999; Baher and Mirza, 2003, Amiri, 2003; Aburjai et al., 2006).

The EOs of Teucrium species were characterized by a higher content of sesquiterpenoids, in accordance with the results reported by previous studies (Cavalerio et al., 2002; Kucuk et al., 2006; Hachicha et al., 2007; Saroglu et al., 2007). So the Predominance of the sesquiterpenoids (5.26%) in this Teucrium species is not surprising. It is possible to say that the difference in the quality or quantity of the composition of volatile oils may be due to genetic, differing chemotype, dring conditions, mode of distillation and or extraction and geographic or climatic factors.

The results of the Antioxidant activity T. polium EO are in agreement with those reported by other groups of investigators using a variety of other assays to assess the antioxidant activity of extracts of T. polium (Couladis et al., 2003, Suboh et al., 2004; Kadikofka-Panovska et al., 2005). In their study, Couladis et al. (2003) screened Greek aromatic plants from the Lamiaceae family for the antioxidant activity. Of the 21 plants tested, they found ethanol extracts prepared from T. polium, as well as 9 other plants, exhibited the same antioxidant activity as alpha-tocopherol in their ability to inhibit bleomycin-Fe(II) complex-induced arachidonic acid superoxidation to MDA. Regrettably, these authors did not include any data about the plant extract concentration(s) in their report. In another study, Suboh et al. (2004) investigated the antioxidant activity effects of various concentrations of methanol extracts of T. polium and the six other medicinal plants as high as 1 mg ml⁻¹, the identical concentration to the highest one used in our study. They reported that the extract significantly reduced 10 mmol hydrogen peroxide-induced lipid peroxidation in human erythrocytes. In contrast, the extract failed to protect erythrocytes against protein oxidation and loss of cell deformability of the oxidatively stressed erythrocytes. Kadikofka-Panovska et al. (2005) also investigated the antioxidant activity of extracts of T. chamaedrys, T. montanum and T. polium prepared using different organic solvents, namely diethyl ether, ethyl acetate and n-butanol. Using such extracts, they assessed their antioxidant abilities in a series of assays that included inhibition of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH assay), inhibition of hydroxyl radical (d-ribose assay) and inhibition of B-carotene oxidation. They found that a 0.4 mg ml⁻¹ extract of T. polium was very effective in inhibiting b-carotene oxidation. Based on our results and these reports, there can be little doubt that extracts of T. polium, irrespective of the mode of preparation, can inhibit oxidative processes leading us to conclude that the extract has substantial antioxidant activity in vitro. We did not attempt to identify the chemical constituents that could account for the antioxidant action of the extract. However, other investigators have reported that the aerial parts of T. polium are rich in flavonoids (Rizk et al., 1986; Harborne et al., 1986). Therefore, it would be reasonable to assume that the antioxidant properties of an aqueous extract of T. polium can be attributed to presence of these bioactive components.

**Conclusion**

Based on the results of this study, we conclude that EO of T. polium possesses remarkable antioxidant activities. Accordingly, we propose that the therapeutic benefit of the essential oil as used in traditional medicine might be due to its antioxidant activity. Further investigations are needed to verify whether this antioxidant effect occurs in vivo.

**References**


Cavalerio, C., Salguerio, L. R., Antunes, T., Sevinate-


