Comparison of Antiradical Activities and Compositions of Essential Oils of Two Origanum spp. from Turkey

B. Cosge, M. Kiralan, A. Ipek, A. Bayrak, B. Gurbuz

Abstract

Hydro-distilled essential oils from the aerial parts of Origanum vulgare L. subsp. hirtum (Link) Letsw. (OVH) and Origanum onites L. (OO) were analyzed by GC/MS. Twelve compounds comprising 98.89% of the (OVH) essential oil were characterized, and the main components were thymol (58.35%) and γ-terpinene (23.22%). Twenty compounds representing 94.58% of the (OO) essential oil were identified, among which carvacrol (73.90%) and γ-terpinene (5.96%) were the major ones. Antiradical activities of essential oils investigated were tested using the DPPH radical-scavenging method. DPPH radical scavenging activities of two Origanum species essential oils were very high, and this was obviously related to their chemical compositions which were markedly rich in phenolic components such as thymol and carvacrol.

Key words: Origanum vulgare L. subsp. hirtum, Origanum onites L., carvacrol, DPPH, essential oil, GC/MS, thymol

Introduction

Antioxidants are usually employed in industry as product additives and in food processing and preservation to prevent undesirable changes due to oxidation an important deterioration process for oil and fats. Butylated hydroxytoluene (BHT, E321), butylated hydroxyanisole (BHA, E320), propyl gallate (PG, E310) and tertiary butyl hydroquinone (BHQ) are the most widely used artificial antioxidants in food[16,18]. However, it has been suspected that they have negative effects on health such as decreasing the risk of cancer and heart disease[26,21]. Therefore, antioxidants from natural are preferred rather than from synthetic sources[1] and there is a parallel increase in studies of essential oils of many medicinal and aromatic plants having strong antioxidant activity. Many authors have reported antimicrobial, antifungal, antioxidant and radical-scavenging properties of essential oils from medicinal and aromatic plants. In particular, the Labiatae family includes a large number of plants such as rosemary, sage, thyme or oregano, well known for their antioxidant properties[26,13,28,25,28]. But, antioxidant activity similarly other biological activities and composition of essential oils from herbs change based on the differences in type, cultivation, origin, vegetative stage, growing seasons and collection season of the plants etc.[10,22,31].

Origanum genus which is an annual, perennial and shrubby herb is represented by 22 species and 4 subspecies in Turkey considered as the gene centre.
of this genus. They are grouped into 8 sections and 14 species are endemic to Turkey[31,11]. The plant has been used as a stimulant, analgesic, antitussive, expectorant, sedative, antiparasitic, antihelminthic, and gastrointestinal complaints in Turkish folk medicine[12,4]. Because of the variability in chemical and aroma composition, Origanum plants are widely used as a culinary herb, to flavor food products and alcoholic beverages[35,2,5].

When the prevalence of the main compounds in essential oil composition of Origanum species is taken into consideration, Origanum taxa can be divided in three groups: (a) linalool, terpinen-4-ol and sabinene hydrate group; (b) carvacrol and/or thymol group; (c) sesquiterpenes group[32,17]. O. onites and O. vulgare subsp. hirtum have both high essential oil and high carvacrol in their essential. While carvacrol content in essential oil of O. onites ranged 60 to 82% in the previous study[4], the highest carvacrol content in O. vulgare subsp. hirtum essential oil was recorded as 78.73%[4,14].

Biological activities of Origanum species depended mainly on carvacrol and thymol in their essential oils, and carvacrol is an oxygenated monoterpene with multiple pharmacological actions including antioxidant, antispasmodic, antitumoral, antimicrobial, antifungal, and analgesic activities [13,38,32,27].

The aim of the present work is to evaluate the chemical profile of the essential oils from aerial parts of O. vulgare subsp. hirtum and O. onites and describe their antiradical activities.

Materials and methods

Plant material

This study was carried out at laboratories of Field Crops and Food Engineering Departments, Faculty of Agriculture of Ankara University in 2007. O. vulgare subsp. hirtum (OVH) and O. onites (OO) plants at flowering stage were collected from the experimental field of Field Crops Department and natural area in Mugla-Turkey, respectively. The collected plants were dried in shadow at room temperature.

Isolation of essential oil

The dried aerial parts (approximately 100 g) of (OVH) and (OO) were subjected to hydro-distillation for 4 h in 500 ml water, using a Clevenger-type apparatus. Obtained essential oils were dried with over anhydrous sodium sulfate, and later filtered, and stored at + 4°C in refrigerator until analyzed for essential oil components (GC-MS analysis) and assayed for antioxidant activity (DPPH assay).

Gas chromatographic-mass spectrometric analysis of essential oil

The essential oil was analyzed by GC-MS. The analysis was performed using a Hewlett Packard 6890 N GC, equipped with HP-5 MS capillary column (30 m x 0.25 μm) and HP 5973 mass selective detector. For GC-MS detection an electron ionization system with ionization energy of 70 eV was used. Helium was carrier gas, at a flow rate of 1 ml/min. Injector and MS transfer line temperatures were set at 220 and 290°C, respectively. Column temperature was initially kept at 50°C for 3 min, then gradually increased to 150°C at a 3°C/min rate, held for 10 min and finally raised to 250°C/min. Diluted samples (1/100 in acetone, v/v) of 1.0 μl were injected automatically and in the splitless mode[31]. Individual components were identified by spectrometric analyses using computer library. Also, the library search carried out using Flavor2L, Wiley7n.1 and NIST98. LGC-MS library of essential oil.

Free radical-scavenging activity: DPPH assay

The hydrogen atom or electron donation ability of the (OVH) and (OO) essential oils was measured from bleaching of purple-colored ethanol solution of DPPH (2,2-diphenyl-1-picrylhydrazyl). The concentrations of the tested samples ranged from 0.1 to 0.002 mL. 0.1 mL of various concentrations of the essential oil in methanol was added to a 10⁻⁴ M methanolic solution of DPPH and vortex-mixed. 0.1 mL of sample was added to 3 mL of a 10⁻⁴ M methanolic solution of DPPH and vortex-mixed. After 30 min of incubation, the absorbance was measured at 515 nm, using a UV-Visible spectrophotometer[8] and all tests were carried out in duplicate. Antiradical action toward DPPH radical was estimated from the difference in absorbance with or without sample (control). Inhibition of free radical DPPH in percent (I %) was calculated in following way:

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

were \( A_{\text{blank}} \) is the absorbance of the control reaction and \( A_{\text{sample}} \) is the absorbance of the absorbance of the test compound.

The results obtained from antibacterial bioassays were expressed as means ± standard error of the mean. All data were analyzed by analysis of variance (ANOVA) and mean values were compared with Duncan’s Multiple Range Tests using SPSS vers. 15 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Essential oil content and its compounds

Table 1 shows the content and chemical composition of essential oils from aerial parts of (OVH) and (OO). Essential oil contents of (OVH) and (OO) were obtained 3.2 and 4.2%, respectively.

\[ \text{essential oil content} = \frac{\text{weight of essential oil}}{\text{weight of dried sample}} \times 100 \]
The essential oil content from (OO) was found higher (1%) than one from (OVH). That the ratio of essential oil from dried O. onites L. herb, the most widely traded oregano species in Turkey, collected from Western Anatolia was 2% reported by Dundar et al. (2008). Origanum vulgare L. subsp. hirtum (Link) Ietswaart and O. onites L. essential oil ratio was between 2.9 and 6.5%, and 2% and 4%, respectively in previous studies.[3,49,40]. Also, Esen et al.[14] stated that essential oil contents in wild and cultivated Origanum vulgare subsp. hirtum were obtained 3.0–6.1% and 3.0–5.7%, respectively.

The essential oils of Origanum species basically compose of terpenoids. But, the essential oil compositions may vary significantly among different genotypes. Oregano species are rich in phenolic monoterpenoids such as carvacrol and thymol.

In our study, twelve compounds comprising 98.89% of the (OVH) essential oil were characterized. The main components were thymol (58.35%) and γ-terpinene (23.22%). This essential oil also contained smaller quantities of ρ-cymene (4.23%), α-terpinene (3.00%), benzene (2.07%), β-caryophyllene (2.01%), myrcene (1.55%), carvacrol (1.43%) and β-bisabolene (1.27%). In addition, α-humulene, α-pinene and α-phellandrene were found trace amounts (<0.1%).

Milos et al.[21] stated that the total of 16 compounds were identified representing 97.6% of the oil in oregano (Origanum vulgare L., subsp. hirtum) collected in central Dalmatia. The main components were thymol (40.4%), carvacrol (24.8%) and ρ-cymene (16.8%). The essential oil also contained smaller quantities of γ-terpinene (1.7%), 1-octen-3-ol (2.1%), borneol (1.2%) and terpinen-4-ol (2.1%). In other study carried out by Esen et al.[14], wild and cultivated Origanum vulgare subsp. hirtum oils contained carvacrol (82.9–7.5% and 85.4–5.3%, respectively) and thymol (60.1–0.3% and 68.0–0.3%, respectively) as the main components. Other major components identified in oils from wild and from cultivated samples, respectively, were as follows: ρ-cymene, 31.1–6.4% and 31.6–2.8%; γ-terpinene, 7.8–0.1% and 19.5–3.0%; linalool, 0.4–0.1% and 0.3–0.1%. The basic composition of the essential oils from wild plants was the same as those from cultivated plants. However, the γ-terpinene content was lower in wild collections (trace–7.8%) than those cultivated in Yalova (3.0–19.5%). In the contrary to findings from these two studies, γ-terpinene content (23.22%) in essential oil from (OVH) was found higher in our study.

The essential oil of Origanum vulgare subsp. Hirtum (Link) Ietswaart has been analysed by several authors[3,15,33] and its thymol and carvacrol chemotypes were identified in O. vulgare subsp. hirtump[36]. In addition, it was stated that carvacrol content in essential oil of Origanum vulgare L. subsp. hirtum (Link) Ietswaart increased up to 78.73%[3,2].

Twenty compounds representing 94.58% of the (OO) essential oil were identified, among which carvacrol (73.90%) and γ-terpinene (5.96%) were the major ones. The seven constituents accounted for 1.04 to 3.88% of the total essential oil of the (OO) aerial parts and included: α-terpinene, myrcene, ρ-cymene, isoborneol, terpinen-4-ol, β-caryophyllene and β-bisabolene (listed in order of elution). On the other hand, α-pinene, camphene, β-pinene, terpinolene, carvone, thymol, α-humulene, germacrene-D, δ-cadinene, spathulenol and caryophyllene oxide did not exceed 1%.

It was found that carvacrol (60–82%) was the main component of O. onites essential oil in 13
Table 2: Radical-scavenging activities of *O. vulgare* L. subsp. *hirtum* (Link) Letsw. and *O. onites* essential oils (%)

<table>
<thead>
<tr>
<th>Methanol Concentrations (µL/µL)</th>
<th>Radical-scavenging activity (DPPH) a</th>
<th><em>O. vulgare</em> L. subsp. <em>hirtum</em> (Link) Letsw.</th>
<th><em>O. onites</em> L.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>94.72±0.06 b*</td>
<td>95.69±0.08 b</td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>86.90±0.06 c</td>
<td>91.53±0.08 c</td>
<td></td>
</tr>
<tr>
<td>0.03</td>
<td>80.22±0.12 d</td>
<td>88.31±0.16 c</td>
<td></td>
</tr>
<tr>
<td>0.025</td>
<td>78.02±0.18 e</td>
<td>85.36±0.15 d</td>
<td></td>
</tr>
<tr>
<td>0.016</td>
<td>71.15±0.16 f</td>
<td>78.25±0.00 e</td>
<td></td>
</tr>
<tr>
<td>0.0125</td>
<td>68.19±0.08 g</td>
<td>73.44±0.16 e</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>56.67±0.08 h</td>
<td>71.43±0.08 e</td>
<td></td>
</tr>
<tr>
<td>0.003</td>
<td>28.25±0.06 i</td>
<td>31.46±0.06 e</td>
<td></td>
</tr>
<tr>
<td>0.002</td>
<td>20.82±0.06 j</td>
<td>25.76±0.12 e</td>
<td></td>
</tr>
</tbody>
</table>

* Data represented the means ± standard error (n=4).
* Means with the same letter within columns are not significantly different at P>0.01.

Free radical-scavenging activity: DPPH assay

The potential antioxidant activity of the oils was determined on the basis of the scavenging activity of the stable free radical DPPH (Table 2). DPPH is a stable free radical and accepts hydrogen radical to become a stable diamagnetic molecule, yellow colored diphenylpicrylhydrazine[37]. Relatively stable organic radical DPPH has been widely used in the determination of the antioxidant activity of the different plant extracts[41,7].

The essential oils from (OVH) and (OO) exhibited DPPH radical scavenging activity, and this was obviously related to their chemical compositions. In several reports, thymol and carvacrol, in particular, were found to be main antioxidant constituents of the oils isolated from several *Origanum* species[21,27,29,30]. Our findings in radical scavenging activity are in accordance with these reports, since the percentage of thymol (58.35%) in (OVH) essential oil and carvacrol (73.90%) in (OO) essential oil were remarkably high (Table 1). Besides, the antioxidative effectiveness of about 100 pure components of essential oils has been studied, and the phenols were confirmed to possess the highest antioxidant activity[29]. The antiradical effect of essential oils from aromatic and medicinal plants is dependent on the oil concentration and can be attributed to relative high concentrations of phenol compounds in these ones[34,39,6]. In our study, phenolic constituents in (OVH) and (OO) essential oils, thymol and carvacrol were found 59.78% and 74.19% in total, respectively. Similarly, Sahin et al.[31] reported that DPPH radical scavenging activity of *O. vulgare* ssp. *vulgare* essential oil was...
very low, and this was obviously related to its chemical composition. Because, the percentage of thymol and carvacrol were remarkably low (0.84% and 0.57%, respectively) in O. vulgare ssp. vulgare essential oil.

The essential oil isolated from (OO) showed higher scavenging values than the one isolated from (OVH) at all methanol concentrations (Table 2). In other words, the negative correlation (r = -0.918, P<0.01) was recorded in between the methanol concentration and antiradical activity of essential oils investigated. The oil samples extracted from both species at concentration of 0.1 µL/µL possessed the most effective capacity for free radical scavenging.

**Conclusions**

This study has shown that essential oils from O. vulgare subsp. hirtum and O. onites had differences in their chemical compositions, and the essential oils also showed antiradical activity. The essential oils of these species were markedly rich in phenolic components. The results of the present study suggest that the essential oil of these two *Origanum* species could be in use as potential resource of natural antioxidants for food industry.

**References**


