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RESEARCH ARTICLE

Chemical composition and antioxidant activity of essential oils of six Lamiaceae plants growing in Southern Turkey

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Abstract

Plants of the Lamiaceae family are rich in polyphenolic compounds, and a large number are well known for their antioxidant properties. In the present study, the essential oils of six species were evaluated for their chemical composition and antioxidant activity. The species used in the present study were namely *Origanum dubium, Origanum vulgare* subsp*. hirtum, Thymbra spicata, Thymus sipyleus, Satureja cilicica* and *Ziziphora clinopodioides,* respectively. The essential oil contents for *Origanum dubium* was 4.6%, for *Origanum vulgare* subsp*. hirtum 3.4%, Thymbra spicata* 1.28%, *Thymus sipyleus* 1.78%, *Satureja cilicica* 1.64% and for *Ziziphora clinopodioides* was 0.35%. The essential oils compositions were determined by GC-MS. Major identified components were carvacrol for *O. dubium, O. vulgare* subsp*. hirtum,* and *T. spicata; α*-pinene for *T. sipyleus;* and borneol for *Z. clinopodioides.* Total phenolic contents of essential oils have a very high equivalent amount compared to gallic acid, however, low radical scavenging properties.

Keywords: Antioxidant, *Lamiaceae*, Essential oils, Chemical composition

Introduction

The Lamiaceae family is spread all over the world, mostly in North-Western Asia and the Mediterranean region, and comprises mostly herbs and shrubs consisting of annual or perennial aromatic plants. Members of the Lamiaceae can grow in almost all habitat types and all altitudes (Watson & Dallwitz, 1978). This family with about 220 genera and 3500, is represented by 45 genera, 565 species and 735 taxa in our country (Başer, 1994; Davis, 1988; Morgariset al., 1982; Watson and Dallwitz, 1978). Lamiaceae is the family with the highest number of endemic species in Turkey and the endemism rate is 45% (Güner at al, 2000).

At the intersection of different climate zones, Turkey is a very rich country in terms of plant species and diversity. Herbs and spices of the *Lamiaceae* constitute an important proportion of the export items to the world markets generating an important trade income. Plants of this family are also used for various purposes, mostly as tea or spices (Baytop, 1999). In addition, Turkey is an important gene center for *Lamiaceae* members, which have been used in traditional medicine for very long times (Kocabaş & Karaman, 2001; Özkan, 2007). Biological and pharmacological activities of species belonging to this family have been known for years. The bioactivity of these plants are also associated to their essential oil components (Bozinet al., 2006). Members of this family have also been the focus of research in terms of plants with antioxidant activity in recent years. *Rosmarinus officinalis* and *Salvia spp*. are currently known and used in the world (Göger, 2006). Their main use is in tea form. *Lamiaceae* plants are among the most known and studied plants for essential oils all over the world (Başer, 1994; Göger, 2006).

Antioxidant supplements or antioxidant-containing foods may be used to help the human body to reduce singlet oxidative damage (Halliwell & Gutteride, 2007). There are two basic categories of antioxidants namely synthetic and natural ones. However, the use of these synthetic antioxidants has been restricted in some countries, mainly because they are suspected to be carcinogenic (Bartosz, 1997). Many researchers have focused on natural antioxidants, and in the plant kingdom numerous crude extracts and pure natural compounds have previously been reported to have antioxidant properties. Therefore, the development and utilization of more effective antioxidants of natural origin are desired.

The aim of this study was to determine chemical composition and antioxidant potential of the essential oils of *Origanum dubium, Origanum vulgare* subsp. *hirtum, Thymbra spicata, Thymus sipyleus, Satureja cilicica,* and *Ziziphora clinopodioides* growing in southern Turkey.

Materials and Methods

Plant Material

The aerial parts of *Origanum dubium, Origanum vulgare* subsp *hirtum, Satureja cilicica, Thymbra spicata, Thymus sipyleus* and *Ziziphora clinopodioides* were collected at bloom stage in July, 2012 from Ermenek (Southern part of Turkey). The plant specimens were identified at the Department of Agronomy of Agricultural Faculty of Çukurova University. Vouchers of the specimens are deposited.

Essential Oil Isolation

Air- dried plant material (25 g) was hydrodistilled for 3 h using a Clevenger type apparatus. The resulting oils were kept in sealed vials at 4 °C until analysis.

GC–MS Analysis

Essential oils were analyzed by GC-MS. A Perkin Elmer apparatus equipped with CPSil5CB (25 m × 0.25 mm *i.d.*, 0.4 µm film thickness) fused-silica capillary column. The flow rate of helium as carrier gas was 1 mL/min. The injector temperature was 250ºC, set for splitless injection. The column temperature was 60ºC/5ºC/min/260ºC for 20 min. Mass spectra were taken at 70 ev. Mass range was between *m/z* 30–425. A library search was carried out using the Wiley GC-MS Library and the Flavor Library of Essential Oil Constituents. The mass spectra were also compared with those of reference compounds and confirmed with the aid of retention indices from published sources. Relative percentage amounts of the separated compounds were calculated from total ion chromatograms by the computerized integrator.

DPPH Radical Scavenging Assay

The hydrogen atom or electron donation ability of the corresponding extract and some pure compounds were measured from the bleaching of a pure –colored methanolic solution of DPPH. This spectrophotometric assay uses the stable radical 2,2'–diphenylpicrylhydrazyl ($DPPH^{\bullet}$) as a reagent (Hwang et al., 2001). In brief, 0.1ml of extract or essential oil solution was mixed with 3.9 ml of $DPPH^{\bullet}$ solution.The mixture was incubated for 30 min at 25 ^{o}C. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. Inhibition of free radical DPPH (%I) was calculated as;

$I\% = A_{blank} – A_{sample} / A_{blank} \times 100$; where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and Asample is the absorbance of the test compound. The extract concentration providing 50% inhibition (IC_{50}) was calculated from graph of inhibition percentage against extract concentration. Tests were carried out in triplicate.

Assay for Total Phenolics

Total phenolic content was estimated by the Folin-Ciocalteu method (Singleton and Rossi, 1965). 100 microliters of 1:10 diluted sample were added to 1 ml of 1:10 diluted Folin-Ciocalteu reagent. After 4 min, 800 µL of sodium carbonate (75 g/L) were added. After 2 h of incubation at room temperature, the absorbances at 765 nm were measured. Gallic acid (0-500 mg/L) was used for calibration of a standard curve. The results were expressed as gallic acid equivalents (GAE) / g dry weight of plant material. Triplicate measurements were taken and mean calculated.

Results and Discussion

The essential oil content of *O. dubium* hydrodistilled by Clevenger apparatus was determined as 4.6 %. Eighteen compounds, representing 96.96 % of the total oil, were characterized with carvacrol (63.1%) and gamma-terpinene (11.1%) being the main compounds whereas other significant compounds were pcymene (9.3%), alpha-phellandrene (2.7%) and myrcene (2.6%) as listed in Table 1. It was reported that the chemical composition of *Origanum* essential oils were very variable (Scheffer *et al.* 1986; Barata*et al.* 1998; Baser *et al.* 2003). Our finding is in agreement with Baser *et* al., 2011, who reported that *Origanum dubium* oil was rich in carvacrol (78.27%).

The essential oil content of *O. vulgare* subsp. *hirtum* was determined as 3.4%. GC-MS analysis of O. *vulgare* subsp. *hirtum* resulted in the identification of seventeen compounds, representing 96.82% of the oil. The results showed that the essential oil was rich in carvacrol (60.8%), linalool (9.2%), *p*-cymene (7.5%), ⍰-terpinene (6.1%), myrcene (3.4 %) and isoeugenol (2.8%) as main components. According to Russo et al. (1998), there are two main chemotypes of oregano (either the thymol or the carvacrol chemotype), but there are also two intermediary chemotypes (thymol/carvacrol and carvacrol/thymol). The environmental conditions are able to influence which biosynthetic pathway (thymol or carvacrol) may be dominant. The results reported in the present work suggest that the oregano that we analyzed belonged to a carvacrol/thymol chemotype.

The essential oil content of *T. spicata* was determined as 1.28%. sixteen components were identified representing 97.44% of total oil. The major constituents were carvacrol (27.4%), α-terpinene (27.0%), p-cymene (14.5%), thymol (13.1%) and alpha- terpinene (4.1%). Our results verify that, in previous studies the major essential oil components of *T. spicata* were carvacrol, γ-terpinene and *p*-cymene (Kizil, 2013; Barakat et al., 2013; Ravid and Putievsky, 1985; Baydar et al., 2004; Unlu et al., 2009; Markovic et al., 2011; Kizil et al., 2015). Barakat *et al.* (2013) found that the main components of *T. spicata* oil of Lebanese origin were carvacrol (16.1–62.9%), α-thujene (1.7–4.8%), myrcene (1.1–5.1%), γ-terpinene (11.4–24.1%) and *p*-cymene (8.1–46.8%), respectively.

Seventeen compounds, representing 88.87 % of the total oil, were identified in *T. sipyleus* essential oil, where α-pinene (20.9%), (*Z, E*)-farnesol (16.1%) and 1,8-cineole (9.5%) were the main compounds (Table 1). The other important compounds were determined as limonene (7.6%), linalool (6.6%), α-terpineol (5.2) and isoborneol (isomer 1) (4.6%). The compositional data shows that α-pinene was the main compound of essential oil of *T. sipyleus.*

The major constituents of the *Z. clinopodioides* essential oil were borneol (24.4%), followed by α-pinene, (17.7%), camphene (8.9%), camphor (8.6%) and cis-verbenol (4.7%), isoborneol (21.2%). Meral *et, al*. reported that main component of the essential oils of *Z. clinopodioides* is borneol. Pulegone is the major component of several *Ziziphora* species (Sezik et al., 1991; Meral et al., 2002; Başer 2002; Shahla, 2012).

Table 1. GC/MS analysis results (relative %) of essential oils

RT	Compound	1*	2*	3*	4*	5*
3.28	Tetradecane	-	-	-	-	0.45
7.02	α-phellandrene	2.74	2.09	3.10	-	-
7.24	α-Pinene	1.38	1.10	1.10	20.88	17.65
7.76	Camphene	0.25	0.16	0.20	4.29	8.86
8.84	β-Pinene	0.24	-	0.24	1.99	0.97
8.84	sabinene	-	0.20	-	1.08	0.48
9.53	Myrcene	2.59	3.40	2.39	1.86	-
10.00	α -phellandrene	0.36	0.26	0.40	0.71	-
10.52	α-terpinene	2.36	1.30	4.06	-	-
10.87	*p*-Cymene	9.25	7.53	14.47	0.41	1.71
11.03	Limonene	0.61	0.49	0.70	7.59	2.98
11.13	1,8-cineole (Eucalyptol)	0.25	-	-	9.49	3.32
12.42	α - terpinene	-	-	26.95	-	-
12.39	γ-Terpinene	11.08	6.08	-	-	0.43
12.70	Linalylbutyrate	1.00	-	-	-	-
12.70	δ-3-carene	-	0.91	0.30	-	-
14.27	Linalool	0.23	9.20	0.32	6.61	0.43
15.30	α-Methylbenzylalcohol	-	-	-	0.28	-
16.08	Farnesol	-	-	-	16.10	-
16.21	ocimene	-	-	-	4.26	0.48
16.58	*trans*- Sabinene	-	-	-	-	2.04
17.09	Isoborneol	0.302	0.182	0.22	4.60	24.42
17.66	terpinene	0.425	0.235	-	0.27	-
18.29	α-Terpineol	0.189	-	-	5.24	-
18.35	Camphor	-	-	-	-	8.57
20.73	Isoeugenol	-	2.834	-	-	-
21.79	Verbenol	-	-	-	-	3.33
22.64	*cis*-Verbenol	-	-	-	-	4.67
22.98	Thymol	-	-	13.14	-	-
23.46	Carvacrol	63.09	60.57	27.42	-	-
28.06	β-Caryophyllene	0.631	0.283	2.39	3.21	-
	Total (%)	18	17	16	17	16
	Number of components	96.96	96.82	97.44	88.87	80.34

*1*Origanum dubium, 2* Origanum vulgare subsp. hirtum, 3* Thymbra spicata, 4* Thymus sipyleus, 5* Ziziphora clinopodioides*

Antioxidant Activity

DPPH radical-scavenging activity: The DPPH radical is a stable free radical, commonly used as a substrate, to evaluate in vitro antioxidant activity of extracts of fruits, vegetables and medicinal plants. Antioxidants can scavenge the radical by hydrogen donation, which causes a decrease of DPPH absorbance at 517 nm. Table 2 shows the DPPH radical-scavenging activity of the different the essential oils. The concentration of sample at which the inhibition percentage reaches 50% is defined as the IC_{50} value. Thus, IC_{50} value is negatively related to the antioxidant activity, the lower the IC_{50} value, higher is the antioxidant activity of tested sample. The radical scavenging effect with the DPPH method was found to be too low for each essential oil. Their 50%

inhibition concentration is very high. Compared with essential oil extracts, the highest radical cleaning feature showed essential oil extract of *Origanum vulgare subsp. hirtum. Origanum dubium and Thymbra spicata* are slightly less than *Origanum vulgare subsp. hirtum. Ziziphora clinopodioides* and *Thymus sipyleus* have the lowest radical scavenging effect.

Table 3. Radical scavenging activity of *Lamiaceae essential* oils in terms of IC_{50} values (g/mL)*1000

Essential Oil	**Radical Scavenging Activity**
Thymus sipyleus	> maximum concentration
Origanum dubium	1340
Origanum vulgare subsp. *hirtum*	8886
Thymbra spicata	1319
Ziziphora clinopodioides	> maximum concentration
Satureja cilicica	196800

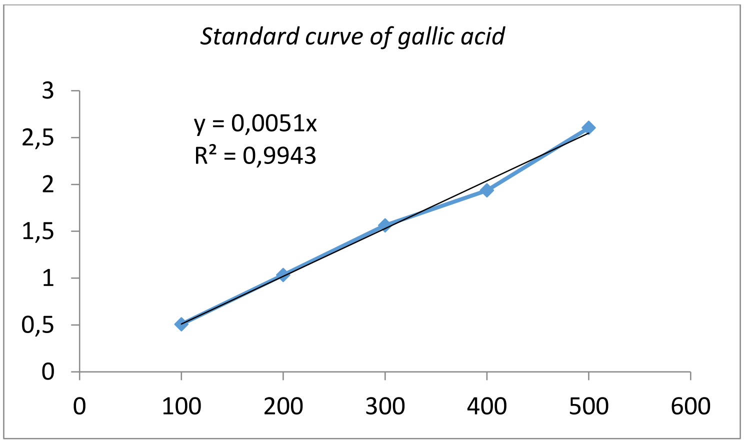
*Values are mean of six different samples of each Lamiaceae species, analyzed individually in triplicate.

In the present study, the Folin-Ciocalteu reagent was used to obtain a crude estimate of the amount of phenolic compounds present in the extracts. As results, essential oil extracts showed very high total phenolic content. Equivalent amounts of gallic acid are given on Table 3. The order of the extracts is given on the table. *Origanum dubium*, *O. vulgare* subsp. *hirtum*, and *Thymbra spicata* have shown high total phenolic effect because carvacrol and other oxygenated compounds are abundant in these species.

Table 4. Phenolic contents of Lamiaceae essential oils

Essential Oil	**mg-GA/mg-extract**
Thymus sipyleus	75,91
Origanum dubium	344,24
Origanum vulgare subsp. *hirtum*	307,92
Thymbra spicata	302,18
Ziziphora clinopodioides	41,18
Satureja cilicica	232,84

Figure 1. Antioxidant activity



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