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Chemical Composition and Antioxidant Activities of Leaf and Flower Essential Oils of *Origanum onites* L. (Lamiaceae) Growing in Mount Ida-Turkey

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Abstract: The chemical composition of leaf and flower essential oils of *Origanum onites* L. were analyzed using Thermo Scientific TSQ GC-MS/MS. Also, antioxidant activities of the leaf and flower essential oils were investigated by using DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity and *β*-carotene linoleic acid assays. BHA (Butylated hydroxyanisole) and BHT (Butylated hydroxytoluene) were used as standards. The essential oil yields of *O. onites* were 1.75% for leaves and 4.25% for flowers. A total of twenty-three compounds representing 99.9% of leaf oil and twenty-four compounds constituted 99.6% of the flower oil were determined. Oxygenated monoterpenes were detected at a high percentage (69.2%) in leaf essential oil, and carvacrol (64.9%) was determined as the main compound. Also, flower essential oil was dominated by sesquiterpene hydrocarbons (73.5%), and α-cubebene (36.4%) was determined as a primary compound. For leaf oil, a high antioxidant capacity was determined, primarily due to carvacrol and *p*-cymene.

Keywords: *Origanum onites*, essential oil, carvacrol, α-cubebene, antioxidant activity.

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INTRODUCTION

Plants have been one of the necessary, indispensable resources of life since ancient times. People have used plants not only for nutrition but also for the treatment of various diseases (1). Nowadays, medicinal and aromatic plants are used in food, cosmetics, paint, textile, medicine, and agriculture (2).

Lamiaceae (Labiatae) family, which is rich in medicinal plants, is usually one or perennial herbaceous plants containing essential oil. Turkey is an important center in terms of gene Lamiaceae plants (3, 4). The genus *Origanum* L. is one of the most widely used genera of the Lamiaceae family. The genus *Origanum* has 21 species (24 taxa) and 13 hybrids in Turkey (5-7). The pharmacological and biological activities of *Origanum* species mostly due to the antioxidant, antimicrobial, anticancer, analgesic, antiradical, antibacterial, cytotoxic, antifungal, and insecticidal activities of their essential oils (8-14).

O. onites L. (Turkish oregano), leaves, flowers, and essential oils of this plant are used in herbal tea, medicine, food, cosmetics, and perfumery industries. It is also named as 'kırkbas kekik', 'bilya kekik', 'tokalı kekik', 'koca lealı kekik', 'arı kekiği' in vernacular (15). The infusion prepared from the above-ground parts of this species is used in the treatment of diseases and symptoms such as gastrointestinal diseases, diabetes, dyspepsia, carminative, bronchitis, respiratory tract diseases, cold & flu, hypertension, and tachycardia by the local people (1, 15, 16). *O. onites* is abundantly

present in the natural habitat of the Mediterranean coastline (17). The essential oil compounds of *O. onites* have been researched earlier from diverse places in the world. The essential oil was comprised of carvacrol as a major compound, followed by thymol, linalool, α-pinene, *p*-cymene, sabinene hydrate, γ-terpinene, α-terpinene (17-23). The essential oil of *O. onites* has antioxidant (23-26), antimicrobial, antifungal (27), insecticidal (28), larvicidal (29), antidiabetic (30), and cholinesterase inhibitory (31) activities.

The literature abounds with reports regarding the detection of chemical components of the essential oil of *O. onites* aerial parts, and no studies differentiating the essential oils of leaf and flower have been reported to date. Although the essential oils are usually procured from the aerial parts of the herb, this report gives us to see the variation among the components of both parts of the herb. Hence, this report was aimed to identify chemical components and antioxidant activities of leaf and flower essential oils of *O. onites*.

EXPERIMENTAL SECTION

Plant material

The aerial parts of *O. onites* (300 g) were collected from Balıkesir, (Edremit, Altınoluk, Kaz Dağı, Bent Picnic Area) 39°34'51.4"N, 26° 45'26.4"E, 100 m, in July 2016. The investigated species was identified by Prof. Dr. Selami Selvi at Balıkesir University. The voucher specimens were deposited at the Herbarium of the Altınoluk Vocational School, Balıkesir University, Balıkesir, Turkey (Herbarium number SV 1567).

Essential oil

Fresh leaves and flowers (40 g each) were dried in the shade, chopped into small pieces, and subjected to hydrodistillation with a Clevenger-type apparatus for 4 h. The yields of essential oils are 1.75% and 4.25% from leaf and flower, respectively. They were stored in amber vials at 4 °C for further analyses.

GC-MS experiments

GC-MS was conducted on Thermo Scientific TSQ GC-MS/MS. The column used was Rtx-5Sil MS, 30 m, 0.25 mm ID, 0.25 µm (32). A detailed procedure was given in the supplementary material.

Antioxidant activity

The antioxidant activities were measured based on DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity (33-36) and *β*-carotene linoleic acid (34-36) assays. The activity tests were carried on at 10, 25, 50, 100 µg/mL concentrations. BHA and BHT were used as standards. IC_{50} values of all samples were calculated. A detailed procedure was given in the supplementary material.

Statistical analysis

Antioxidant activity results were evaluated using a One-way ANOVA test (GraphPad, Software version is 8.4.2). $P < 0.05$ was accepted as the minimum level of significance.

RESULTS AND DISCUSSION

Essential oil

Higher essential oil yield was obtained from the flower (4.25%) compared to the leaf (1.75%) essential oil. Altogether, twenty-three compounds representing 99.9% of leaf essential oil and twenty-four compounds constituted 99.6% of the flower essential oil were determined. The components of essential oils were classified into 4 based on their chemical structures: monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes. The essential oil components of leaves and flowers of *O. onites* are summarized in Table 1.

Table 1: Essential oil compositions of leaf and flower of *O. onites.*

No	Compounds	RT[a]	KI[b]	Leaf (%)	Flower (%)
1	α-thujene	5.25	930	1.5	0.5
2	α-pinene	5.37	939	0.5	0.2
3	camphene	6.08	954	0.3	t[c]
4	sabinene	14.48	975	t[c]	t[c]
5	*β*-pinene	6.70	979	0.1	-
6	α-phellandrene	7.85	1003	1.3	0.7
7	α-terpinene	8.30	1017	2.4	0.9
8	***p*-cymene**	8.53	1025	**11.2**	3.9
9	*β*-phellandrene	8.70	1030	0.6	0.3
10	(E)-*β*-ocimene	9.28	1050	9.1	2.7
11	γ-terpinene	9.39	1060	0.4	0.3
12	sabinene hydrate-cis	17.25	1070	0.4	0.1
13	sabinene hydrate-trans	11.41	1098	0.2	0.1
14	*β*-cis-terpineol	13.20	1144	1.2	0.1
15	camphor	18.22	1146	0.8	0.1
16	carvacrol, ethyl ether	20.10	1298	2.1	-
17	**carvacrol**	20.14	1299	**64.5**	16.0
18	**δ-elemene**	21.77	1338	-	**34.6**
19	**α-cubebene**	22.33	1351	-	**36.4**
20	α-copaene	23.49	1377	0.4	-

21	*β*-bourbonene	24.05	1388	-	0.1
22	aromadendrene	26.27	1441	1.1	1.2
23	Z-*β*-farnesene	26.33	1443	0.1	0.1
24	α-humulene	26.82	1455	0.2	-
25	E-*β*-farnesene	26.92	1457	-	0.1
26	allo-aromadendrene	27.07	1460	1.3	1.0
27	spathulenol	31.96	1578	0.2	0.1
28	α-cadinol	34.93	1654	-	0.1
Monoterpene hydrocarbons				27.4	9.5
Oxygenated monoterpenes				69.2	16.4
Sesquiterpene hydrocarbons				3.1	73.5
Oxygenated sesquiterpenes				0.2	0.2
Total (%)				99.9	99.6

[a] RT: Retention time
[b] KI: Kovats indices
[c] t: trace (<0.1%)

The main components of leaf essential oil were carvacrol (64.5%), *p*-cymene (11.2%), and (E)-*β*-ocimene (9.1%), while α-cubebene (36.4%), δ-elemene (34.6%), and carvacrol (16.0%) were determined as the main compounds in the flower essential oil (Figure 1). The leaf essential oil was qualified by the high content of monoterpenes (96.6%), including hydrocarbons (27.4%) and their oxygenated derivatives (69.2%), while sesquiterpenes (3.3%) were detected in very low amounts.

Sesquiterpene hydrocarbons (73.5%) composed the primary class of compound determined in the flower essential oil with α-cubebene (36.4%) and δ-elemene (34.6%). Oxygenated monoterpenes and monoterpene hydrocarbons were also detected at an average percentage in flower essential oil (16.4% and 9.5%, respectively).

The results indicate that the essential oil content of leaf and flower were dissimilar. For example, carvacrol was found mainly in leaf essential oil at 64.5% against 16.0% in the flower essential oil. In contrast, α-cubebene (36.4%) and δ-elemene (34.6%), significant compounds of flower essential oil were not detected in leaf essential oil. Also, *β*-pinene, carvacrol ethyl ether, α-copaene, and α-humulene were detected only in the leaf essential oil. Also, *p*-cymene and (E)-*β*-ocimene were found to be significant compounds in leaf essential oil at 11.2 and 9.1% against 3.9 and 2.7% in the flower essential oil, respectively. It is well known that different parts of the same plant may include different phytochemicals (37, 38). This variation can be elucidated by the presence of different secretory structures in different plant parts. Dissimilar phytochemicals are available in each of the parts of the plant may explanation for the variation in the pharmacological and biological properties.

Carvacrol, the most abundant compound of the leaf essential oil, was reported in the essential oils of aerial parts of *O. onites* from Turkey (18, 24, 29, 39, 40) and Greece (22, 23, 41, 42). However, α-cubebene and δ-elemene were not reported previously as significant components of *O. onites*. α-Cubebene was detected in low quantities of aerial parts of *O. onites* from Greece (42). Also, Figuérédo et al. reported that *O. onites* was the linalool types (42). Ceylan et al. (2003) reported the essential oil compounds of *O. onites* from eighteen different localities of Turkey, while generally, carvacrol was found to be a significant compound, and only one locality had linalool-rich (43). Lukas et al. (2010) reported that chemotypes of *O. onites* from ten different locations of Turkey and Greece. In Greece location of *O. onites* was found to be "cymyl"-chemotypes. In Turkey location of *O. onites* was characterized by linalool and "cymyl"-chemotypes (44). These differences in the chemical composition of essential oil may be due to the environmental, climate conditions, drying methods, harvest period, extraction methods, extraction time, and temperature. These variables affect the vegetative cycle of the herb and subscribe to the chemical variations of its essential oil.

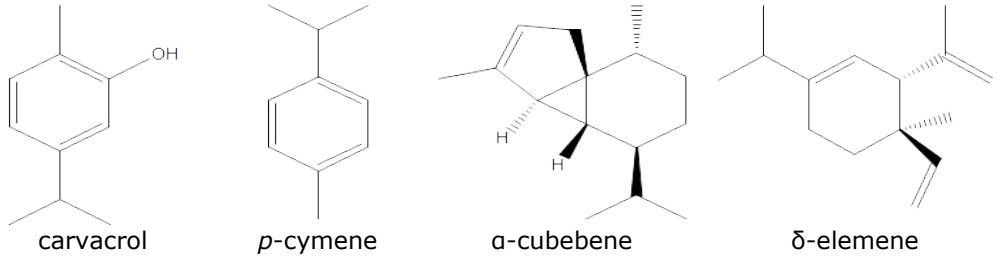

Figure 1: Chemical structures of the main compounds of leaf and flower essential oils.

Antioxidant activity

DPPH free radical scavenging activity and *β*-carotene linoleic acid assays were used to determined antioxidant activities of leaf and flower essential oils of *O. onites*. The 50% inhibition concentrations (IC_{50}) results are given in Table 2. Leaf and flower essential oils have an excellent antioxidant capacity for both tested assays. IC_{50} values for the DPPH of leaf and flower essential oils were found to be 19.05 ± 3.96 µg/mL and 29.95 ± 5.86 µg/mL, respectively. Besides, BHA and BHT IC_{50} values were found to be 11.73 ± 2.27 µg/mL and 17.22 ± 1.55 µg/mL. IC_{50} values of leaf and flower essential oil were found to be 22.39 ± 3.88 µg/mL and 30.29 ± 0.84 µg/mL in the *β*-carotene linoleic acid assay, respectively. None of the leaf and flower essential oils showed higher antioxidant activity than BHA or BHT. Low IC_{50} values reflect a high antioxidant activity. These results revealed that leaf essential oil showed better antioxidant capacity when compared to flower essential oil. Carvacrol and *p*-cymene were reported to play a important role in antioxidant capacity (45, 46).

Table 2: Antioxidant capacity of leaf and flower essential oils (IC_{50}).

	***β*-carotene**	**DPPH**
Leaf	22.39 ± 3.88	19.05 ± 3.96
Flower	30.29 ± 0.84	29.95 ± 5.86
BHA	14.21 ± 1.16	11.73 ± 2.27
BHT	17.73 ± 2.43	17.22 ± 1.55

IC_{50} values are mean ± SD (n = 3).

CONCLUSION

The chemical contents of leaf and flower essential oils of *O. onites* were investigated. Also, the antioxidant activity of the essential oils was determined. In this study, it was found that the leaf essential oil was found as carvacrol type, and flower essential oil was the α-cubebene type. It can be said that the quantitative and qualitative differences of essential oils depend on different secretory structures in different plant parts. To the best of our knowledge, this is the first report on the chemical components and antioxidant activities of leaf and flower essential oils of *O. onites*. The leaf and flower essential oils of *O. onites* have the good antioxidant capacity. Thus, the essential oils from both parts of *O. onites* may be regarded as possible natural antioxidant agents for cosmetic, food and pharmaceutical industries.

Supplementary data

Inhibition (%) of lipid peroxidation and DPPH free radical scavenging activity, Gas chromatography-Mass spectrometry conditions and antioxidant activities procedures were given in supplementary material.

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SUPPLEMENTARY DATA

Chemical Composition and Antioxidant Activities of Leaf and Flower Essential Oils of *Origanum onites* L. (Lamiaceae) Growing in Mount Ida-Turkey

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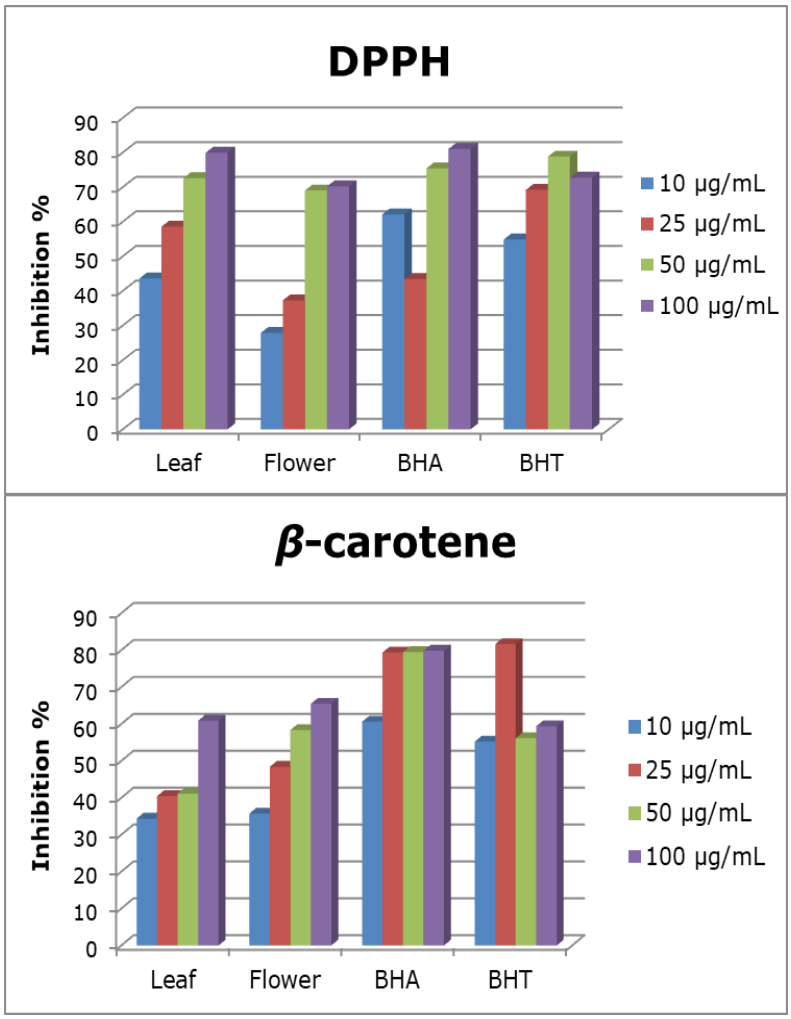

Figure S1. Inhibition (%) of lipid peroxidation and DPPH free radical scavenging activity of essential oils.

Gas chromatography-Mass spectrometry (GC-MS) conditions

Helium was used as carrier gas at a constant flow rate of 1 mL/min (20 psi). 1 µL of the sample was injected (100 µL of essential oil dissolved in 1900 µL of dichloromethane). The GC temperature program was set as follows; 150 °C hold for 5 min, ramp to 250 °C at 3 °C/min, and hold for 10 min. The temperature of the MS transfer line was set at 230 °C. A mass range from 50 to 650 *m/z* was scanned. The column used was an Rtx-5Sil MS, 30 m, 0.25 mmID, 0.25 µm. Thermo Scientific TSQ GC-MS/MS was used in this study. A homologous series of n-alkanes was used as a reference in the calculation of Kovats Indices (KIs). Identification of the compounds was based on the comparison of their relative retention indices and mass spectra with those obtained from authentic samples and the NIST and Wiley spectra as well as the literature data (1).

Antioxidant activity

DPPH free radical scavenging method

The free radical scavenging activity of the extracts was determined spectrophotometrically by the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay (2-5). In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 0.1 mM solution of DPPH in methanol was prepared and 160 µL of this solution was added to 40 µL of sample solutions in methanol at different concentrations (10, 25, 50, and 100 µg/mL). These tubes were left in the dark for 30 min. The measurements were made at 517 nm. BHA and BHT were used as standard compounds.

***β*-carotene bleaching method**

The antioxidant activity was evaluated using *β*-carotene-linoleic acid model system (3-5) *β*-carotene (0.5 mg) in 1 mL of chloroform was added to 25 µL of linoleic acid, and 200 mg of Tween 40 emulsifier mixture. After evaporation of chloroform under vacuum, 100 mL of distilled water saturated with oxygen, was through vigorous shaking. A mixture of 4000 µL was transferred into different test tubes containing different concentrations of the sample (10, 25, 50, and 100 µg/mL). As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. The emulsion system was incubated for 2 h at 50 °C. A blank, devoid of *β*-carotene, was prepared for background subtraction. BHA and BHT were used as standard compounds.

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