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In vitro Antimicrobial and Antioxidant activities and Chemical Composition of Essential Oils of the Leaf and Flower of *Origanum minutiflorum* O. Schwarz et. P. H. Davis

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Abstract: It was aimed to determine chemical structure and *in vitro* antimicrobial and antioxidant activities of the essential oil at the leaf and flower of *Origanum minutiflorum* O. Schwarz et. P. H. Davis. The essential oils of *Origanum minutiflorum* (Lamiaceae) were analyzed via GC-MS method. Leaves were composed of carvacrol 44.96 % while the oil of the flowers was composed of 34.04 % carvacrol. The essential oils in leaves and flowers showed strong antimicrobial activity against to all bacteria except *Pseudomonas aeruginosa.* Antioxidant activity was analyzed systems of β-carotene/linoleic acid and DPPH. IC_{50} values of the essential oils were found 110µg/ml and 105µg/ml respectively. Inhibition percentage of *Origanum minutiflorum* essential oil was found as 66% and 71% respectively.
Keywords: *Origanum minutiflorum*, Essential oils, Antimicrobial activity, Antioxidant activity

Origanum Minutiflorum O. Schwarz et. P. H. Davis Yaprak ve Çiçeklerinin Uçucu Yağlarının Kimyasal Kompozisyonu ile İnvitro Antimikrobiyel ve Antioksidan Aktivitelerinin Belirlenmesi

Özet: *Origanum minutiflorum* O. Schwarz et. P. H. Davis yaprak ve çiçeklerinin uçucu yağlarının kimyasal kompozisyonu ile invitro antimikrobiyel ve antioksidan aktivitelerinin belirlenmesi amaçlanmıştır. *Origanum minutiflorum* (Lamiaceae) GS-MS metodu ile analiz edilmiştir. Etken maddesi karvakrol yapraklarda 44.96% iken çiçeklerde 34.04% olarak bulunmuştur. Bitkinin yaprak ve çiçeklerinin uçucu yağları *Pseudomonas aeruginosa* hariç tüm bakterilere karşı güçlü antimikrobiyel aktivite göstermiştir.Antioksidan aktivite β-karotene/linoleik asid ve DPPH metotlarıyla belirlenmiştir. IC_{50} değerleri sırasıyla yapraklarda 110µg/ml ve çiçeklerde 105µg/ml olarak belirlenmiştir. İnhibisyon oranları ise 66% ve 71% tespit edilmiştir.
Anahtar Kelimeler: *Origanum minutiflorum*, antioksidan aktivite, antimikrobiyel aktivite

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1. INTRODUCTION

Recently, the essential oils and different kind of plant extracts have been started to search as sources of natural products. Especially the antimicrobial, antioxidant activities of essential oils have used in for many practices, including alternative and natural medicines, pharmaceuticals, raw and processed food preservatives (3,4,24).

Origanum minutiflorum (O.M) is regarding Labiatae family including 3,000 plants grown in warm areas through the world (13, 16-18). *O.M* was an endemic at mountain habitats in Turkey (8).

Origanum species have been used for the cold and stomach ache (6,19,23,28), abdominal pain, rheumatism, and as an antiseptic (28), antigenotoxic (19), antibacterial and antifungal (23). Most chemical component of the essential oil of these species is carvacrol, gamma-terpinene, thymol and p-cymene (6-8,15,20).

Many researchers have been reported antimicrobial and antioxidant activity of the essential oil in *Origanum* species and *O.M* recently (2,7,11,15,20, 27). But there isn't any study about essential oils of flowers and leaves at *O.M*.

The purpose of the study was to seek the antioxidant and antimicrobial activities and compare the chemical formation of essential oils between flowers and leaves at *O.M* which grown in Turkey.

2. MATERIAL AND METHODS

Material of Plant: *O.M* plant has been gathered in Senirkent-Isparta, Turkey and the taxonomic identification was done by Dr. Erol Donmez (Ph.D.) at the Department of Biology, Cumhuriyet University, Faculty of Science, (Sivas-Turkey) during flowering and has been stored at the Herbarium of the Department of Biology, Cumhuriyet University Faculty of Science, Sivas, Turkey (CUFH-Voucher No: ED 11001/ OM).

Isolation of the Essential Oil: The parts of *O.M* were treated for 3 h to water distillation via Clevenger-type apparatus (Flowers; yield 2.3 % v/w and Leaves yields 2.9% v/w). After the process the products were stored at +4°C until analysed.

Analysis of Gas Chromatography/mass Spectrometry (GC/MS): The chemical composition in essential oil of the *O.M* was analysed via gas chromatograph/mass spectrometer (Shimadzu QP5000, Kyoto, Japan) with a 70 eV EI quadrupole detector and a GL Science capillary column TC-5 (30 m × 0.25 mm i.d., 0.25 mm). The components identification was compared by of Kovats as described in. NBS75K-MS Library and with MS Data were used for identification (1).

Antimicrobial Activity

Microbial Strains: Antimicrobial and antifungal activities of the oil were evaluated against 3 Gram-positive bacteria and 5 Gram-negative bacteria and 1 fungi as shown in Table2 by the disk diffusion method in the Department of Contagious Diseases Research, Refik Saydam Hygiene Center, Ankara-Turkey. Bacterial cultures were studied in Mueller Hinton Agar (MHA-Oxoid-CM337) and the yeast was cultured in Sabouraud Dextrose Agar (Oxoid-CM41). The tests were repeated in three times. Average and standard deviation (SD) of the inhibition zone diameters were calculated.

Antimicrobial Assay (Disc Diffusion Assay): It has been done for to evaluate antimicrobial activities of the essential oil (21, 22). Suspensions of the tested microorganisms (0.1 ml 10^8 cells per ml) were spread on the solid media plates. The filter paper disks (6 mm in diameter) were placed on the plate after being treated with 10 µl of oil, incubated at 4°C for 2h and at 37°C for 24 h respectively and at 30°C for 48 h for the yeast. The

diameters of the inhibition zones were characterized as millimetres.

Antioxidant Activity

The principle of assay in the presence of a hydrogen donating antioxidant reduction of radical solutions in alcoholic 2,2- diphenylpicryl-hydrazyl (DPPH). It observed a band at 517 nm for DPPH (10,12). Butylatedhydroxytoluene (BHT) was used as a positive control. Each of tests was done in three times.

The reducing of the yellow colour of β-carotene caused by its reaction with radicals which are composed by linoleic acid oxidation (10,12). The rate of β-carotene decolorizing can be reducing in the presence of BHT as an antioxidant as shown in 490 nm.

3. RESULTS

About 33 compounds were formed (93.19%) of the complete oil from *O.M* flowers. The ratio of the essential oil contents is carvacrol (34.04%), p-cymene (12.42%), 1-borneol (9.17%) and terpinen-4-ol (5.12%) and the total (60.75%). *O.M* leaves; GC/MS analysis resulted in the identification of 30 compounds (97.15%) of the total oil carvacrol (44.96%), endo-borneol (9.42%), terpinen-4-ol (6.68%) and m-cymene (5.74%) the components (66.80%) of the essential oil was the main component as shown Table1.

Table1. Chemical composition of *Origanum minutiflorum* essential oil

Retention time	Compounds	*Origanum minutiflorum* flowers components%	*Origanum minutiflorum* leaves componenets %
12.508	α-thujene	1.10	0.65
12.770	α-pinene	1.97	1.1
13.555	Camphene	1.23	0.56
15.183	β-pinene	0.35	0.24
16.418.	β-myrcene		1.45
17.500	delta-3-carene	0.15	
18.000	α-terpinene	0.62	1.26
18.815	m-cymene		5.74
18.531	1,8 cineole	2.62	6.09
18.910	D,l-limonene	0.42	0.29
18.968	p-cymene	12.42	
20.667	gamma-terpinene		3.39
21.203	cis-sabinene-hydrate		1.80
21.220	trans-sabinene-hydrate	1.28	1.80
21.875	ethyl amyl carbinol	2.32	0.28
23.808	Linalool	2.78	2.54
23.900	Thujylalkol	0.29	0.31
24.09	farnestylalchol	0.18	
24.1	amyl vinilcarbinol	0.68	1.14
25.300	Camphor		0.18
26.115	Cuminol	0.27	
27.712	1-borneol	9.17	
28.500	terpinen-4-ol	5.12	6.68
28.650	caren-4-ol	0.50	
29.785	dihidrocarvone	0.27	
29.910	d-carvone		0.54
32.283	endo-borneol		9.42
33.408	methyl-thymyl ether	0.56	
36.092	Carvone	1.01	
38.625	Carvacrol	34.04	44.96
37.258	Thymol	0.83	1.03
39.215	myrceneacetate	0.25	
39.750	sabinylasetate	0.22	0.69
40.845	α-terpinylasetate	0.45	

41.441	Cuminol		0.13
41.978	dihidrocarveolasetat	0.32	
45.983	trans caryophylene	1.84	3.18
46.327	Junipene		0.30
46.600	d-nerolidol	2.45	0.98
47.992	α-humulene	0.23	
53.543	Spathulenol		0.88
56.125	Farnesol	3.3	0.25
58.366	p-terbuthylcatechol	3.95	0.84
Total		93.19%	97.15%

Table2. Antimicrobial activity of the essential oils of *Origanum minutiflorum* flowers and leaves using agar disc diffusion method and Minimum inhibitory concentrations (MIC).

Microorganisms	*Origanum minutiflorum* leaves[a]		*Origanum minutiflorum* flowers[a]		Gentamycin[d]	Nystatin[e]
	Disc diffusion method[b]	MIC[c]	Disc diffusion method[b]	MIC[c]		
Staphylococcus aureus	90±1.52	26.50	90 ±1.62	12.25	23±0.54	-
Escherichia coli	50±0.88	16.25	34 ±0.23	21.5	16±0.20	-
Pseudomonas aeruginosa	18±1.01	45.50	11±0.75	40.10	20±0.28	-
Salmonella typhi	42±1.16	26.50	53±1.43	34.60	10±0.18	-
Klebsiella pneumoniae	65±1.18	38.60	65±1.05	32.25	20±0.40	-
Proteus vulgaris	64±1.68	65.10	33±1.75	50.50	22±1.45	-
Bacillus subtilis	90±1.35	62.50	72±1.30	90.50	29±0.80	-
Corynebacterium diphteriae	90±1.22	46.25	51±1.57	30.10	23±0.15	-
Candida albicans -	50±1.43	26.40	46±1.54	30.50	-	25±0.16

[a]Results are means of three different measurements.
[b]Agar disc diffusion method, diameter of inhibition zone (mm) including disk diameter of 6 mm;
[c]Minimum inhibitory concentrations (MIC)
[d]Antibacterial; [e]Antifungal.

Table3. Effects of essential oil of *Origanum minutiflorum* and positive control (butylated hydroxytoluene) on the *in vitro* free radical DPPH scavenging and β-carotene-linoleic acid systems[a]

Sample	Inhibition IC_{50} (µg/ml) with DPPH scavenging	% Inhibition (µg/ml) with β-carotene-linoleic acid system
Origanum minutiflorum (Leaves)	110	66
Origanum minutiflorum (Flowers)	105	71
Butylated hydroxytoluene (BHT)	10.5	100
[a]Results are means of three different measurements.		

4. DISCUSSION AND CONCLUSION

It wasn't observed any study about leaf and flower during the literature reviews. But carvacrol (68.23%) was found to be the main component of essential oil a study related with aerial parts of *O.M* which was carried out by Dadalıoğlu et al., (2004), Bayramoğlu (2005) and Vardar-Unlu et al., (2007) reported that essential oil of *O.M* which grown in Turkey contained carvacrol (78.8%), γ-terpinen (3.7%) and p-cymene (3.5%) as major components. This finding was similar to our finding of flowers (34.04%), leaves (44.96%) and total carvacrol (79.0%). Baydar (2005) reported effects of essential oil content and formation in *O.M* different harvest times. According to these reports, the major content of the essential oil was carvacrol (60.3-92.3%).

Antimicrobial activities of 10 µl amount of the

essential oils of *O.M* leaves and flowers oils had a superiority effect. The essential oils in leaves and flowers observed strong antimicrobial activity against to all bacteria except *Pseudomonas aeruginosa* as shown Table2. P-cymene biological precursor of carvacrol, does not act as antibacterial when used single (5,26). But when used with carvacrol, a synergism against to *B. cereus* has been recorded (26). These study results are similar to Dadalıoğlu et al., (2004), Vardar-Unlu et al., (2007) and Başer et al. (1993) as antimicrobial activity.

We could find a small number of studies about to the essential oils compositions of *O.M.* (2,8,15,27). But there aren't any researches about antimicrobial and antioxidant activities of essential oils from the flowers and leaves of *O.M.* This study was evaluated the *in vitro* antimicrobial activity of the essential oil of treatment against to micro-organisms and pathogenic yeast.

Although a few studies are report about the chemical composition, activities of antioxidant, antifungal, antiradical and antibacterial in *Origanum* species (2, 14, 24, 25) only a few studies have been figure out in *O.M* (15,27).

Because of it has strong antibacterial activity in antioxidant activity tests *O.M*., this plant can be used as a natural food protective against microorganisms that produce toxic and unwanted chemicals which can cause poisoning in the food industry. Antibiotic-resistant microorganisms are rapidly spreading and new antibiotics are needed. Plants are an important resource for finding new antimicrobial compounds.

According to our knowledge, this is the first research about the essential oil composition of *O.M* leaves and flowers separately and the antioxidant, antimicrobial activities of this species. These findings supporting that the plant of essential oil can be use in pharmaceutical and food industries.

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