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## COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *Origanum* × *dolichosiphon* P. H. DAVIS\*

N. Tabanca,<sup>1</sup> F. Demirci,<sup>1</sup> T. Ozek,<sup>1</sup>  
G. Tumen,<sup>2</sup> and K. H. C. Baser<sup>1</sup>

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The essential oil obtained by hydrodistillation from aerial parts of *Origanum* × *dolichosiphon* P. H. Davis (Lamiaceae), a hybrid of *O. amanum* Post x *O. laevigatum* Boiss., was analyzed by GC/MS. Ninety-five compounds were characterized representing 92% of the oil. The major compounds were bicyclogermacrene (19.9%),  $\beta$ -caryophyllene (13.0%), and germacrene D (10.8%). The antimicrobial activity of the oil was also determined.

**Key words:** *Origanum* × *dolichosiphon* P. H. Davis, Lamiaceae, essential oil, bicyclogermacrene, antimicrobial activity.

The genus *Origanum* (Lamiaceae) is represented by 22 species, 7 hybrids, and altogether 32 taxa in Turkey [1, 2].

Several *Origanum* species are known as «Kekik» and widely used as herbal tea and in folk medicine in various regions of Turkey [3]. The species *O. x dolichosiphon* P. H. Davis is a hybrid of *O. amanum* Post and *O. laevigatum* Boiss. [1]. *O. amanum* is an endemic species which grows in Adana and Hatay provinces located in the Southern part of Turkey. *O. laevigatum* is distributed in Southern (Adana, Hatay), South-Eastern Anatolia (Maras, Gaziantep), and Cyprus [1]. These species are also cultivated in gardens in the United States [4]. *O. x dolichosiphon* is distributed in Adana: Bahce: Duldul mountain, however the study material was collected from Hatay province [1].

In previous studies, we have reported the compositions of essential oils of three *Origanum* hybrids. *O. x adanense* Baser et Duman is an endemic hybrid of *O. bargyli* Mouterde and *O. laevigatum* Boiss. The main components of this hybrid were carvacrol (17.3%) and bicyclogermacrene (9.3%) [5]. *O. x intercedens* Rech. fil. is a hybrid of *O. vulgare* L. subsp. *hirtum* (Link) Ietwaart and *O. onites* L. This oil was reported as rich in carvacrol (46%) [6]. *O. x majoricum* Cambess. is a hybrid of *O. majorana* L. and *O. vulgare* L. subsp. *virens* (Hoffm. et Link) Ietswaart. This hybrid is cultivated in gardens and used as condiment. The oil was characterized with *cis*-sabinene hydrate (24-37%) and terpinen-4-ol (6-13%) [7] as the main components.

Here, we report on the essential oil composition of *O. x dolichosiphon* and antimicrobial activity of the oil for the first time.

The results of the GC/MS analyses of the essential oil are given in Table 1. Ninety-five compounds were found to represent 92.0% of the oil. The oil yield of *O. x dolichosiphon* (0.04%) was very poor compared to other *Origanum* species. The major compounds were found as bicyclogermacrene (19.9%),  $\beta$ -caryophyllene (13%), and germacrene D (10.8%). Overall consideration of the essential oil showed high amounts of sesquiterpene hydrocarbons (49%) followed by oxygenated monoterpenes (15%) as seen in Table 1 (RRI: Relative retention indices).

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1) Medicinal and Aromatic Plant and Drug Research Centre (TBAM), Anadolu University, 26470, Eskisehir, Turkey;  
2) Faculty of Science and Letters, Department of Biology, Balikesir University, 10100 Balikesir, Turkey. Published in *Khimiya Prirodnykh Soedinenii*, No. 3, pp. 204-206, May-June, 2001. Original article submitted June 19, 2001.

TABLE 1. The Composition of the Essential Oils of *Origanum x dolichosiphon*

RRI	Compound	%	RRI	Compound	%
1000	Decane	0.01	1773	$\delta$ -Cadinene	0.4
1032	$\alpha$ -Pinene	1.8	1776	$\gamma$ -Cadinene	0.1
1035	$\alpha$ -Thujene	0.2	1784	( <i>E</i> )- $\beta$ -Bisabolene	0.02
1076	Camphene	0.1	1802	Cuminaldehyde	0.1
1118	$\beta$ -Pinene	0.8	1827	( <i>E,E</i> )-2,4-Decadienol	0.1
1132	Sabinene	0.9	1830	$\beta$ -Damascone	0.1
1136	Isoamyl acetate	0.1	1838	( <i>E</i> )- $\beta$ -Damasconone	0.1
1159	$\delta$ -3-Carene	0.3	1844	( <i>E</i> )-Anethole	0.1
1174	Myrcene	1.2	1849	<i>cis</i> -Calemene	0.1
1188	$\alpha$ -Terpinene	0.3	1864	<i>p</i> -Cymen-8-ol	0.02
1203	Limonene	6.4	1868	( <i>E</i> )-Geranyl acetone	0.2
1213	1,8-Cineole	2.3	1900	Epicubebol	0.03
1218	$\beta$ -Phellandrene	0.4	1933	Tetradecanal	0.1
1246	( <i>Z</i> )- <i>b</i> -Ocimene	0.1	1941	$\alpha$ -Calacorene-I	0.02
1255	$\gamma$ -Terpinene	1.9	1953	Palustrol	0.04
1266	( <i>E</i> )- $\beta$ -Ocimene	0.2	1957	Cubebol	0.03
1280	<i>p</i> -Cymene	4.1	1958	( <i>E</i> )- $\beta$ -Ionone	0.1
1290	Terpinolene	0.1	2001	Isocaryophyllene oxide	0.2
1345	3-Octanyl acetate	0.2	2008	Caryophyllene oxide	3.5
1386	1-Octenyl acetate	0.01	2025	Perilla alcohol	0.1
1393	3-Octanol	0.01	2045	Norbourbonene	0.2
1400	Tetradecane	0.1	2050	( <i>E</i> )-Nerolidol	0.2
1406	$\alpha$ -Fenchone	0.01	2065	10- <i>epi</i> -Elemol	0.1
1430	$\alpha$ -Thujone	0.02	2069	Cermacrene D-4-ol	0.3
1451	$\beta$ -Thujone	0.02	2098	Globulol	0.7
1452	$\alpha,p$ -Dimethylstyrene	0.03	2104	Viridiflorol	0.3
1475	Menthone	0.1	2131	Hexahydrofarnesyl acetone	0.3
1495	Bicycloelemene	0.6	2144	Spathulenol	4.9
1497	$\alpha$ -Copaene	0.4	2179	3,4-Dimethyl-5-penthylidene-2(5H)-furanone	0.2
1506	Decanal	0.03	2192	Nonanoic acid	0.4
1528	$\alpha$ -Bourbonene	0.1	2198	Thymol	0.7
1535	$\beta$ -Bourbonene	2.1	2209	T-muurolol	0.1
1544	$\alpha$ -Gurjunene	0.02	2239	Carvacrol	2.9
1547	( <i>E</i> )-2-Nonenal	0.01	2247	<i>trans</i> - $\alpha$ -Bergamotol	0.8
1547	$\beta$ -Cubebene	0.1	2255	$\alpha$ -Cadinol	0.3
1553	Linalool	0.5	2300	Decanoic acid	0.3
1565	Linalyl acetate	0.8	2300	Tricosane	0.1
1597	Bornyl acetate	0.03	2324	Caryophylla-2(12),6(13)-dien-5- $\alpha$ -ol (=Caryophylladienol-II)	0.1
1600	$\beta$ -Elemene	0.4			
1612	$\beta$ -Caryophyllene	13.0	2353	Caryophylla-2(12),6-dien-5- $\alpha$ -ol (=Caryophyllenol-I)	0.1
1638	Menthol	0.02			
1661	<i>allo</i> -Aromadendrene	0.3	2384	Farnesylacetone	0.2
1668	( <i>Z</i> )- $\beta$ -Farnesene	0.1	2392	Caryophylla-2(12),6-dien-5- $\beta$ -ol (=Caryophyllenol-II)	0.3
1671	( <i>E</i> )- $\beta$ -Farnesene	0.01			
1674	$\gamma$ -Gurjunene	0.1	2500	Pentacosane	0.6
1687	$\alpha$ -Humulene	0.8	2524	Phytol	0.3
1700	Heptadecane	0.2		Monoterpene hydrocarbons	12.41
1706	$\alpha$ -Terpineol	0.3		Oxygenated monoterpenes	14.8
1708	Ledene	0.2		Sesquiterpene hydrocarbons	49.3
1709	$\alpha$ -Terpinyl acetate	0.1		Oxygenated sesquiterpenes	12.1
1726	Germacrene D	10.8		Other	3.3
1755	Bicyclogermacrene	19.9			
			<b>Total</b>		<b>92.0</b>

TABLE 2. Antimicrobial Activity (MIC) of *Origanum × dolichosiphon* Essential Oil

Microorganism	Essential oil	Standard*
<i>Escherichia coli</i> (ATCC 25922)	250	62.5
<i>Staphylococcus aureus</i> (ATCC 6538)	125	7.81
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	125	250
<i>Enterobacter aerogenes</i> (NRRL 3567)	125	125
<i>Proteus vulgaris</i> (NRRL 123)	125	31.25
<i>Salmonella typhimurium</i> (NRRL 4420)	125	62.5
<i>Candida albicans</i> **	250	125**

\*Chloramphenicol. \*\*Ketoconazole.

The essential oil of *O. laevigatum* was previously reported by Tucker [8] and later by us [9]. In both cases, bicyclogermacrene (24.6% and 37.9%), germacrene D (20.5% and 21.7%), and  $\beta$ -caryophyllene (16.8% and 4.5%) were found as the main components. The occurrence of bicyclogermacrene in the oil of the hybrid is enough evidence to prove that *O. laevigatum* is one of the parents, since this species contains bicyclogermacrene as the main component in the oil. This work necessitates investigation of the essential oil composition of the other parent *O. amanum*. Previous microbiological investigations of oregano species resulted in strong inhibition of various pathogens [10-13]. The antimicrobial evaluation of *O. x dolichosiphon* essential oil against the common pathogenic bacteria and yeast resulted in moderate activities as seen in Table 2. When compared to standard drugs the oil showed comparable inhibition against *Enterobacter aerogenes* (MIC 125 mg/ml). *Salmonella typhimurium* and *Pseudomonas aeruginosa* were inhibited in strength close to the standard.

## EXPERIMENTAL

**Plant Material.** The plant was collected from Hatay: Amanos Mountain, 700 m, July 1995 in Turkey. Voucher specimens are kept at the Herbarium of the Faculty of Pharmacy of Anadolu University in Eskisehir, Turkey (ESSE: 11997)

**Distillation.** Aerial parts of the air dried plant material were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to yield the essential oil (0.04%).

**Analysis of Essential Oils.** The oils were analyzed by GC/MS using a Hewlett Packard GCD system. Innowax FSC column (60m  $\times$  0.25 mm, with 0.25 mm film thickness) was used with helium as a carrier gas (1 ml/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, then kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min.

Alkanes were used as reference points in the calculation of relative retention indices (RRI). The split ratio was adjusted at 50:1. The injector temperature was at 250°C. MS were taken at 70 eV. Mass range was from 35 to 425 *m/z*. Library search was carried out using the Wiley GC/MS Library and the TBAM Library of Essential Oil Constituents. Relative percentage amounts were calculated from Total Ion Chromatogram (TIC) by the computer.

**Antimicrobial Assay.** Microdilution broth susceptibility assay was used for the antimicrobial evaluation of the essential oil [14]. Stock solutions of the essential oil and compounds were prepared in DMSO. Serial dilutions were prepared in sterile distilled water in a 96-well microtiter plate from 2000 mg/ml up to 1.94 mg/ml for the essential oils and 1000 mg/ml up to 0.97 mg/ml for the pure compounds (standard drugs). Freshly grown bacterial suspensions in double strength Mueller-Hinton broth and yeast suspension of *Candida albicans* in yeast medium were standardized to 10<sup>8</sup> CFU/ml. Sterile distilled water served as growth control. 100  $\mu$ l of each microbial suspension was then added to each well. The last row containing only serial dilutions of the antimicrobial agents (chloramphenicol and ketoconazole for *C. albicans*) without microorganism was used as negative control. After incubation at 37°C for 24 h the first well without turbidity was determined as the minimal inhibition concentration (MIC). Human pathogens *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Proteus vulgaris*, and *Salmonella typhimurium* were obtained from the culture collection of the Microbiology Department in Anadolu University, and *Candida albicans* was obtained from the culture collection of Osmangazi University, Faculty of Medicine, Microbiology Department (Table 2).

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