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

N. Tabanca,[1] F. Demirci,[1] T. Ozek,[1]
G. Tumen,[2] and K. H. C. Baser[1]

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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%),* β*-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%), *β*-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.

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

RRI	Compound	%	RRI	Compound	%
1000	Decane	0.01	1773	δ-Cadinene	0.4
1032	α-Pinene	1.8	1776	γ-Cadinene	0.1
1035	α-Thujene	0.2	1784	(*E*)-β-Bisabolene	0.02
1076	Camphene	0.1	1802	Cuminaldehyde	0.1
1118	β-Pinene	0.8	1827	(*E*,*E*)-2,4-Decadienol	0.1
1132	Sabinene	0.9	1830	β-Damascone	0.1
1136	Isoamyl acetate	0.1	1838	(*E*)-β-Damascenone	0.1
1159	δ-3-Carene	0.3	1844	(*E*)-Anethole	0.1
1174	Myrcene	1.2	1849	*cis*-Calemene	0.1
1188	α-Terpinene	0.3	1864	*p*-Cymen-8-ol	0.02
1203	Limonene	6.4	1868	(*E*)-Geranyl acetone	0.2
1213	1,8-Cineole	2.3	1900	Epicubebol	0.03
1218	β-Phellandrene	0.4	1933	Tetradecanal	0.1
1246	(*Z*)-b-Ocimene	0.1	1941	α-Calacorene-I	0.02
1255	γ-Terpinene	1.9	1953	Palustrol	0.04
1266	(*E*)-β-Ocimene	0.2	1957	Cubebol	0.03
1280	*p*-Cymene	4.1	1958	(*E*)-β-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	(*E*)-Nerolidol	0.2
1406	α-Fenchone	0.01	2065	10-*epi*-Elemol	0.1
1430	α-Thujone	0.02	2069	Cermacrene D-4-ol	0.3
1451	β-Thujone	0.02	2098	Globulol	0.7
1452	α,*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	α-Copaene	0.4	2179	3,4-Dimethyl-5-penthylidene-2(5H)-furanone	0.2
1506	Decanal	0.03	2192	Nonanoic acid	0.4
1528	α-Bourbonene	0.1	2198	Thymol	0.7
1535	β-Bourbonene	2.1	2209	T-muurolol	0.1
1544	α-Gurjunene	0.02	2239	Carvacrol	2.9
1547	(*E*)-2-Nonenal	0.01	2247	*trans*-α-Bergamotol	0.8
1547	β-Cubebene	0.1	2255	α-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-α-ol	0.1
1600	β-Elemene	0.4		(=Caryophylladienol-II)	
1612	β-Caryophyllene	13.0	2353	Caryophylla-2(12),6-dien-5-α-ol	0.1
1638	Menthol	0.02		(=Caryophyllenol-I)	
1661	*allo*-Aromadendrene	0.3	2384	Farnesylacetone	0.2
1668	(*Z*)-β-Farnesene	0.1	2392	Caryophylla-2(12),6-dien-5-β-ol	0.3
1671	(*E*)-β-Farnesene	0.01		(=Caryophyllenol-II)	
1674	γ-Gurjunene	0.1	2500	Pentacosane	0.6
1687	α-Humulene	0.8	2524	Phytol	0.3
1700	Heptadecane	0.2		Monoterpene hydrocarbons	12.41
1706	α-Terpineol	0.3		Oxygenated monoterpenes	14.8
1708	Ledene	0.2		Sesquiterpene hydrocarbons	49.3
1709	α-Terpinyl acetate	0.1		Oxygenated sesquiterpenes	12.1
1726	Germacrene D	10.8		Other	3.3
1755	Bicyclogermacrene	19.9			
				Total	**92.0**

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

Microorganism	Essential oil	Standard*
Escherichia coli (ATCC 25922)	250	62.5
Staphylococcus aureus (ATCC 6538)	125	7.81
Pseudomonas aeruginosa (ATCC 27853)	125	250
Enterobacter aerogenes (NRRL 3567)	125	125
Proteus vulgaris (NRRL 123)	125	31.25
Salmonella typhimurium (NRRL 4420)	125	62.5
Candida albicans**	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 β-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 × 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 10^8 CFU/ml. Sterile distilled water served as growth control. 100 ml 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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