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COMPOSITION OF THE ESSENTIAL OILS OF TWO *Sideritis* SPECIES FROM TURKEY AND ANTIMICROBIAL ACTIVITY

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The genus *Sideritis* (Lamiaceae) is represented in Turkey by 46 species and altogether 55 taxa, 42 taxa being endemic [1–3]. Some species of *Sideritis* are used as medicinal and aromatic plants. *S. perfoliata* L. is known as "adacayi, dagcayi, and Kandil cayi" in different regions of Turkey and are widely used as diuretic and in the treatment of coughs and gastrointestinal disorders [4]. *S. trojana* Bornm. is an endemic species for Turkey and is known as "kazdagi cayi" [4, 5].

The essential oil compositions of some *Sideritis* species have been subjected to previous studies. Previous phytochemical investigations of *S. perfolita* and *S. trojana* have revealed the presence of diterpenes [6, 7], fatty acids [8], and essential oils [9, 10].

The composition of the essential oil of *S. perfoliata* was investigated and limonene was identified as the major constituent [9]. In our previous work, 8α-13-hydroxy-14-en-epilabdane (26–29%), limonene (19–24%), viridiflorol (14%), sabinene (11%), and β-caryophyllene (10%) were reported as the main constituents in the oil of *S. perfoliata* [10]. The essential oil composition of *S. trojana* has also been investigated. β-Pinene (12–17%) and α-pinene (8–14%) were identified as the major components [10]. In another work, the fatty acid composition was investigated in seed oils of *S. perfoliata*. Linoleic, oleic, 6-octadecynoic, palmitic, and linolenic acids were identified as the main fatty acid components of the seed oil [8].

To the best of our knowledge, this is the first report on the antimicrobial activity of *S. perfoliata* and *S. trojana* essential oils.

In this present work, the hydrodistilled essential oils from aerial parts of *Sideritis perfoliata* and *S. trojana* were analyzed by gas chromatography and gas chromatography-mass spectrometry. The compounds characterized are given in Table 1 with their relative percentage amounts. In the oil of *S. perfoliata* (0.36%), 60 compounds were identified, representing 98.6% of the oil, with monoterpene hydrocarbons (76.2%) dominating, together with oxygenated monoterpenes (1.3%), sesquiterpene hydrocarbons (6.2%), oxygenated sesquiterpenes (1.9%), diterpenes (12.8%), and other compounds (0.2%). The main constituents were found to be limonene (37.7%) and sabinene (18.8%). *S. trojana* oil (0.04%) was rich in β-pinene (18.4%) and α-pinene (13.2%). Sixty-two compounds were characterized, representing 87.9% of the oil. The monoterpene hydrocarbon (46.7%) content of this oil was rather low compared to *S. perfoliata*.

The antibacterial and anticandidal activities of the oils are presented in Table 2. The results of the antimicrobial assays indicated that *E. coli*, methicillin-resistant *S. aureus* (MRSA), *E. aerogenes*, *B. cereus*, and *C. albicans* were inhibited by the oil of *Sideritis trojana* moderately with MIC values of 125 to 250 μg/mL, which were lower than that of the standard antimicrobial agent. The oil showed strong inhibitory effect against *S. epidermidis* with a MIC value of 62.5 μg/mL. *S. perfoliata* oil, on the other hand, was less active (125 to 500 μg/mL) against the test microorganisms except for *C. albicans*. The occurrence of a higher content of oxygenated derivatives of mono and sesquiterpenes (20%) in the oil of *S. trojana* may be responsible for the better antimicrobial activity.

Plant Material and Isolation of the Essential Oils. Dried aerial parts of the plant materials were hydrodistilled for 3 h using a Clevenger apparatus to obtain essential oils in dry weight yield.

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TABLE 1. The Composition of the Essential Oils of *Sideritis* Species, %

Compound	RRI	*S. perfoliata*	*S. trojana*	Compound	RRI	*S. perfoliata*	*S. trojana*
α-Pinene	1032	3.9	13.2	α-Terpineol	1706	0.1	0.6
α-Thujene	1035	1.2	0.8	α-Zingiberene	1726	Tr.	-
Hexanal	1093	Tr.	-	Germacrene D	1726	1.1	5.3
β-Pinene	1118	3.3	18.4	β-Bisabolene	1741	-	0.9
Sabinene	1132	18.5	1.1	Bicyclogermacrene	1755	1.1	0.7
δ-2-Carene	1146	-	1.5	*cis*-Piperitol	1758	Tr.	-
δ-3-Carene	1159	-	1.5	(*E*,*E*)-α-Farnesene	1758	0.2	-
Myrcene	1174	2.2	-	Naphthalene	1763	-	0.4
α-Terpinene	1188	0.1	1.0	δ-Cadinene	1773	-	0.2
Limonene	1203	**37.7**	3.7	γ-Cadinene	1776	-	0.2
β-Phellandrene	1218	8.7	1.1	*ar*-Curcumene	1786	0.3	-
(*Z*)-3-Hexenal	1225	Tr.	-	*p*-Methyl acetophenone	1797	0.1	-
(*Z*)-β-Ocimene	1246	Tr.	0.5	Myrtenol	1804	-	0.8
γ-Terpinene	1255	0.2	0.2	*trans*-*p*-Mentha-1(7),8-dien-2-ol	1811	Tr.	-
(*E*)-β-Ocimene	1266	0.1	0.1	(*E*, *E*)-2,4-Decadienal	1827	Tr.	
p-Cymene	1280	0.2	3.4	(*E*)-β-Damascenone	1838	Tr.	0.3
Terpinolene	1290	0.1	0.2	*trans*-Carveol	1845	0.1	-
Hexanol	1360	-	0.2	*p*-Cymen-8-ol	1864	0.1	-
3-Octanol	1393	-	0.1	(*E*)-Geranyl acetone	1868	Tr.	-
Nonanal	1400	-	0.7	*cis*- Carveol	1882	Tr.	-
1-Octen-3-ol	1452	-	0.4	*cis*-*p*-Mentha-1(7),8-diene-2-ol	1896	Tr.	-
α-Cubebene	1466	-	0.1	*epi*-Cubebol	1900	0.1	-
trans-Sabinene hydrate	1474	0.2	-	(*E*)-β-Ionone	1958	Tr.	0.1
Bicycloelemene	1495	0.1	0.1	Isocaryophyllene oxide	2001	Tr.	-
α-Copaene	1497	Tr.	-	Caryophyllene oxide	2008	0.4	2.8
α-Campholene aldehyde	1499	-	0.4	Salvial-4(14)-en-1-one	2037	-	0.4
Decanal	1506	0.1	0.2	Pentadecanal	2041	-	0.2
α-Bourbonene	1528	Tr.	-	*p*-Mentha-1,4-dien-7-ol	2073	Tr.	-
β-Bourbonene	1535	0.6	0.4	Hexahydrofarnesyl acetone	2131	0.1	1.2
Benzaldehyde	1541	-	0.1	Spathulenol	2144	0.6	-
Linalool	1553	0.1	0.6	Valeranone	2145	-	4.8
cis-Sabinene hydrate	1556	0.1	-	β-Bisabolol	2170	0.1	-
Octanol	1562	-	0.6	α-Bisabolol	2232	-	3.2
Pinocarvone	1586	-	1.3	Carvacrol	2239	-	0.7
β-Ylangene	1589	0.1	0.1	9-Geranyl-*p*-cymene	2312	-	1.1
trans-β-Bergamotene	1594	0.1	-	Eudesma-4(15),7-dien-1β-ol	2369	-	0.1
Bornyl acetate	1597	-	0.1	Manoyl oxide	2376	12.6	-
β-Elemene	1600	0.1	0.3	Caryophylla-1(12),6-dien-5β-ol	2392	0.7	0.2
β-Caryophyllene	1612	2.2	4.9	(=Caryophyllenol II)			
β-Cyclocitral	1638	-	0.1	Kaur-16-ene	2438	0.2	-
trans-*p*-Mentha-2,8-dien-1-ol	1639	0.1	-	Pentacosane	2500	-	0.3
Myrtenal	1648	-	1.2	Phytol	2622	-	0.1
Alloaromadendrene	1661	0.1	-	Benzyl benzoate	2655	-	0.1
Pulegone	1662	-	0.3	Heptacosane	2700	-	0.4
(*Z*)-β-Farnesene	1668	-	0.5	Nonacosane	2900	-	1.4
trans-Pinocarveol	1670	-	1.3	Monoterpene hydrocarbons		76.2	46.7
cis-*p*-Mentha-2,8-dien-1-ol	1678	0.1	-	Oxygenated monoterpenes		1.3	8.1
trans-Verbenol	1683	-	0.7	Sesquiterpene hydrocarbons		6.2	13.7
Cryptone	1690	0.1	-	Oxygenated sesquiterpenes		1.9	11.5
p-Mentha-1,8-dien-4-ol	1700	0.2	-	Diterpenes		12.8	1.1
(=Limonen-4-ol)				Others		0.2	6.8
γ-Curcumene	1704	0.2	-	Total		98.6	87.9

RRI: Relative retention indices calculated against *n*-alkanes; % calculated from FID data.

Tr.: trace (<0.1%).

S. perfoliata: C6 Hstay: Antakya-Yayladag yolu, Senkoy-Kislak arasi, 36°00′N, 36°07′E; *S.trojana*: B1 Balikesir: Edremit, Kazdagi, Nanekiri tepesi cevresi, 39°42′N, 26°53′E.

TABLE 2. The Antimicrobial Test Results of *Sideritis* Essential Oils (MIC/μg/mL)

	E. coli	MRSA	*E. aerogenes*	*S. typhimurium*	*B. cereus*	*S. epidermidis*	*C. albicans*
Sideritis trojana	125	250	250	250	125	62.5	125
Sideritis perfoliata	500	250	500	500	125	250	62.5
STD	3.9	62.5	3.9	7.8	7.8	3.9	31.25

Antimicrobial Assay. The microorganisms were refreshed in Mueller Hinton Broth (Merck) at 35–37°C, and inoculated on Mueller Hinton Agar (Mast Diagnostics, Merseyside, U.K.) media for preparation of inoculums. *Escherichia coli* (NRRL B-3008), methicillin-resistant *Staphylococcus aureus* (MRSA, Clinical isolate, Osmangazi University, Faculty of Medicine, Department of Microbiology), *Enterobacter aerogenes* (NRRL 3567), *Salmonella typhimurium* (NRRL B-4420), *Bacillus cereus* (NRRL B-3711), *Staphylococcus epidermidis* (ATCC 12228) and *Candida albicans* (Clinical Isolate, Osmangazi University, Faculty of Medicine, Eskisehir, Turkey) were used as the test microorganisms.

Microdilution Broth Method. Micro-dilution broth susceptibility assay [11, 12] was used for the antimicrobial evaluation of the essential oils. Stock solutions of oils were prepared in DMSO (Carlo-Erba). Dilution series were prepared from 2 mg/mL to 0.001 mg/mL in sterile distilled water in micro-test tubes from where they were transferred to 96-well micro-titer plates. Overnight grown bacterial suspensions in double strength Mueller-Hinton broth (Merck) was standardized to approximately 108 CFU/mL using McFarland No. 0.5 (106 CFU/mL for *C. albicans*); 100 μL of each bacterial suspension was then added to each well. The last row containing only the serial dilutions of antimicrobial agent without microorganism was used as negative control. Sterile distilled water and medium served as a positive growth control. After incubation at 37°C for 24 h the first well without turbidity was determined as the minimal inhibition concentration (MIC). Chloramphenicol (Sigma) and Ketoconazole (Sigma) were used as standard antimicrobial agents.

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