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COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OILS OF TWO ENDEMIC SPECIES FROM TURKEY: *Sideritis cilicica* AND *Sideritis bilgerana*

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Aerial parts of Sideritis cilicica *Boiss. & Bal. and* Sideritis bilgerana *P.H. Davis (Lamiaceae) were hydrodistilled to obtain essential oils that were then analyzed by GC and GC/MS.* β*-Pinene (39%),* α*-pinene (28%), and* β*-phellandrene (20%) were the main components in the oil of* S. cilicica, *while* β*-pinene (48%), and* α*-pinene (32%) were the major constituents in the oil of* S. bilgerana. *The antimicrobial activities of the oils were evaluated by using the microdilution broth method. Both of the oils showed good inhibitory effects on* C. albicans.

Key words: *Sideritis*, essential oil, GC/MS, antimicrobial activity.

The genus *Sideritis* (Lamiaceae) is represented by 46 species and 55 taxa in Turkey, 42 taxa being endemic [1, 2].

These species are generally known as "dag cayi, yayla cayi" and are extensively used as herbal tea and folk medicine in Turkey [3]. In folk medicine, they are used as anti-inflammatory, nervous system stimulant, antispasmodic, carminative, analgesic, antitussive, stomachic, and anticonvulsant [2, 3].

Most of the biological studies on *Sideritis* species are concerned with their antioxidant [4, 5], anti-inflammatory [6–9], and antimicrobial activities [10–13]. Furthermore, the essential oil compositions of some *Sideritis* species have previously been reported by us [14–26].

The two endemic species that are the subject of this study were studied for the first time for their essential oil compositions and antimicrobial activities.

The essential oils of *Sideritis cilicica* and *S. bilgerana* were analyzed both by GC and GC/MS to determine their constituents (Table 1). As a result of GC and GC/MS analyses, 40 and 81 components were identified, representing 98% of the total for both *S. cilicica* and *S. bilgerana* oils, respectively. GC/MS analyses of the oils have revealed the occurrence of β-pinene (39, 48%) and α-pinene (28, 32%) as the main constituents of *S. cilicica* and *S. bilgerana*, respectively. β-Phellandrene (20%) was also characterized as a main component in the oil of *S. cilicica*.

Our group has previously reported on the main constituents of *Sideritis* essential oils belonging to all existing taxa in Turkey [2, 27].

Antimicrobial assay on the essential oils showed moderate to significant inhibitory effect on human pathogenic bacteria from 0.125 to 0.5 mg/mL (Table 2). Significantly enough, *S. cilicica* oil showed promising activity close to that of the standard antimicrobial agent chloramphenicol against MRSA, having an MIC value of 0.125 mg/mL.

Methicillin-resistant *Staphylococcus* strains show resistance to all of the beta-lactams and at the same time macrolides, aminoglycosides, quinolones, rifampin, and chloramphenicol. Glycopeptides are effective antibiotics for MRSA strains but have serious side effects [28]. Therefore, there is a great need for alternative MRSA inhibitory agents.

Furthermore, both oils showed significant inhibitory effects (MIC 0.03 mg/mL) against *Candida albicans*. The MIC values of the oils and the standard antimicrobial agents are given in detail in Table 2. The results of previous investigations on the antimicrobial effects of some *Sideritis* essential oils produced similar results [12].

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TABLE 1. Essential Oil Composition of *Sideritis bilgerana* (A) and *Sideritis cilicica* (B)

RRI	Compound	A, %	B, %	RRI	Compound	A, %	B, %
1014	Tricyclene	0.1	-	1685	α-Humulene	Tr.	-
1032	α-Pinene	31.9	27.9	1687	*trans*-Verbenol	0.4	-
1035	α-Thujene	Tr.	-	1706	α-Terpineol	0.2	0.2
1076	Camphene	0.3	0.2	1709	α-Terpinyl acetate	Tr.	-
1093	Hexanal	-	0.1	1719	Borneol	Tr.	-
1118	β-Pinene	48.4	39.1	1726	Germacrene D	0.1	-
1132	Sabinene	1.7	1.8	1732	Bicyclosesquiphellandrene	Tr.	-
1159	δ-3-Carene	0.1	-	1738	*p*-Mentha-1,5-dien-8-ol	Tr.	-
1174	Myrcene	1.3	0.7	1740	α-Muurolene	Tr.	0.2
1176	α-Phellandrene	-	1.3	1755	Bicyclogermacrene	0.2	1.2
1183	*p*-Mentha-1,7(8)-diene (=pseudolimonene)	Tr.	-	1763	Naphthalene	0.1	-
1203	Limonene	2.4	2.1	1773	δ-Cadinene	0.2	0.5
1218	β-Phellandrene	5.2	20.3	1786	(*E*)-α-Bisabolene	Tr.	-
1232	(*E*)-2-Hexenal	Tr.	-	1799	Cadina-1,4-diene (=cubenene)	0.1	-
1246	(*Z*)-β-Ocimene	-	0.1	1804	Myrtenol	0.2	-
1255	γ-Terpinene	Tr.	-	1808	Isobutyl benzoate	Tr.	-
1257	(*E*)-β-Ocimene	Tr.	-	1838	(*E*)-β-Damascenone	Tr.	-
1280	*p*-Cymene	0.1	0.2	1845	*trans*-Carveol	Tr.	-
1285	Isoamyl isovalerate	Tr.	-	1853	*cis*-Calamenene	0.1	-
1286	2-Methylbutyl-2-methylbutyrate	Tr.	-	1868	(*E*)-Geranyl acetone	Tr.	-
1290	Terpinolene	Tr.	Tr.	1900	*epi*-Cubebol	0.1	0.1
1299	2-Methylbutyl isovalerate	0.1	-	1929	2-Methylbutyl benzoate	Tr.	-
1393	3-Octanol	0.1	Tr.	1936	3-Methylbutyl benzoate	Tr.	-
1400	Nonanal	0.1	-	1957	Cubebol	0.3	0.14
1452	1-Octen-3-ol	0.2	0.2	2008	Caryophyllene oxide	0.1	-
1466	α-Cubebene	Tr.	0.1	2028	2-Pentadecanone	-	0.1
1474	*trans*-Sabinene hydrate	0.1	Tr.	2050	(*E*)-Nerolidol	Tr.	-
1497	α-Copaene	0.1	0.2	2073	Germacrene D 4-ol	Tr.	Tr.
1504	(*E,E*)-2,4-Heptadienal	Tr.	-	2088	1-*epi*-Cubenol	Tr.	0.1
1535	β-Bourbonene	0.1	-	2095	Hexyl benzoate	Tr.	-
1544	α-Gurjunene	0.1	-	2104	Viridiflorol	Tr.	Tr.
1553	Linalool	0.4	0.1	2131	Hexahydrofarnesyl acetone	0.1	-
1556	*trans*-*p*-Menth-2-en-1-ol	Tr.	-	2144	Spathulenol	0.2	0.2
1562	Linalyl acetate	Tr.	-	2152	(*Z*)-3-Hexenyl benzoate	Tr.	-
1586	Pinocarvone	0.1	Tr.	2179	3,4-Dimethyl-5-pentylidene-2(5H)-furanone	Tr.	-
1590	Bornyl acetate	0.5	-	2186	Eugenol	Tr.	-
1594	*trans*-β-Bergamotene	-	0.1	2193	Copaborneol	-	0.4
1611	Terpinen-4-ol	-	0.1	2209	T-Muurolol	1.0	-
1612	β-Caryophyllene	0.4	-	2219	Dimyrcene II-a	-	0.1
1644	Isopropyl propionate	-	Tr.	2247	*trans*-α-Bergamotol	-	Tr.
1648	Myrtenal	0.1	-	2255	α-Cadinol	0.1	-
1651	Bornyl isobutyrate	Tr.	-	2309	9-Geranyl *p*-cymene	-	Tr.
1661	*trans*-Pinocarvyl acetate	Tr.	-	2324	Caryophylla-2(12),6(13)-dien-5α-ol	Tr.	-
1663	*cis*-Verbenol	Tr.	-		(=caryophylladienol II)		
1670	Cryptone	-	Tr.	2347	Kaur-15-ene	Tr.	-
1670	*trans*-Pinocarveol	0.3	-	2392	8α-9-oxy-14en-epilabdane	-	0.1
1672	(*Z*)-β-Farnesene	Tr.	-	2438	Kaur-16-ene	-	0.1
1682	δ-Terpineol	Tr.	-	2931	Hexadecanoic acid	0.3	-

RRI: Relative retention indices on a polar column.

Tr.: < 0.05%.

TABLE 2. Antimicrobial Activity (MIC, mg/mL)

	S. cilicica	*S. bilgerana*	Standard
Escherichia coli (NRRL B-3008)	0.25	0.5	0.015*
MRSA (Clinical isolate)	0.125	0.5	0.062*
Enterobacter aerogenes (NRRL B-3567)	0.25	0.5	0.03*
Salmonella typhimurium (NRRL B-4420)	0.25	0.25	0.015*
Bacillus cereus (NRRL B-3711)	0.125	0.125	0.007*
Staphylococcus epidermidis (ATCC 12228)	0.125	0.125	0.003*
Candida albicans (Clinical isolate)	0.03	0.03	0.03**

*Chloramphenicol; **ketoconazole.
MRSA: Methicillin-resistant *S. aureus*.

EXPERIMENTAL

Plant Material and Isolation of the Essential Oils. *Sideritis cilicica* was collected from Cobanak yaylasi, Kozan, Adana (Turkey) in June 2001. *Sideritis bilgerana* was collected from Ermenek to Mud Road 40th km, Icel (Turkey) in August 2000. Voucher specimens (HD 8585 and HD 8375) are kept at the Herbarium of Gazi University, Faculty of Science, and Department of Biology. The essential oils were obtained by hydrodistillation using a Clevenger-type apparatus for 3 h, from aerial parts of the *Sideritis cilicica and Sideritis bilgerana*. The oil yields were calculated on a dry weight basis as 0.55 and 0.26%, respectively.

Gas Chromatography (GC). GC analysis was carried out using a Hewlett Packard 6890 system. An HP-Innowax FSC column (60 m × 0.25 mm inner diameter, with 0.25 μm film thickness) was used with nitrogen as carrier gas (1 mL/min). The 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. The injector temperature was at 250°C. The percentage compositions were obtained from electronic integration measurements using flame ionization detection (FID, 250°C).

Gas Chromatography-Mass Spectrometry (GC/MS). A Hewlett-Packard GCD system with Innowax FSC column (60 m × 0.25 mm inner diameter, with 0.25 μm film thickness) was used with helium as carrier gas. GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and then kept constant at 220°C for 10 min and programmed to 240°C at a rate of 1°C min. Split flow was adjusted at 50 mL/min. The injector temperature was at 250°C. MS were taken at 70 eV. Mass range was from *m/z* 35 to 425.

The separated compounds were characterized using the in-house Baser Library of Essential oil constituents. Alkanes were used as reference points in the calculation of relative retention indices (RRI).

Microorganisms. The microorganisms were refreshed in Mueller Hinton Broth (Merck) at 35–37°C, and inoculated on Mueller Hinton Agar (Mast Diagnostics, Merseyside, UK) media for preparation of inoculum.

Escherichia coli (NRRL B-3008), MRSA (Methicillin-resistant *Staphylococcus aureus*, 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 pathogen test microorganisms.

Antimicrobial Assay: Microdilution Broth Method. Microdilution broth susceptibility assay [29–32] was used for antibacterial and anticandidal assay. Stock solutions of essential oils were prepared in dimethylsulfoxide (DMSO, Carlo-Erba, France). In sterile distilled water, dilution series were prepared from 4 mg/mL to 0.007 mg/mL in micro-test tubes (Eppendorf) which were transferred to 96-well microtiter plates. Overnight grown bacterial and *C. albicans* suspensions in Mueller-Hinton broth were standardized to (for bacteria and *C. albicans* appr. 10^8 and 10^6 cfu/mL respectively) using McFarland No 0.5 standard solution. Each microorganism suspension was then added into the wells. The last well-column with medium and microorganism served as a positive growth control. After incubation at 37°C for 18–24 h the first well without turbidity was determined as the minimal inhibitory concentration (MIC). Chloramphenicol was used as standard antibacterial agent whereas ketoconazole was used as antifungal.

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