Composition and Antimicrobial Activity of the Essential Oils of Five Taxa of *Sideritis* from Greece

N. Aligiannis, E. Kalpoutzakis, I. B. Chinou, and S. Mitakou*

Division of Pharmacognosy, School of Pharmacy, University of Athens, Panepistimiopolis Zografou, Athens, Greece

E. Gikas and A. Tsarbopoulos

GAIA Research Center, Bioanalytical Department, The Goulandris Natural History Museum, 13 Levidou Street, Kifissia 14562, Athens, Greece

The chemical compositions of the essential oils obtained from the aerial parts of five taxa of *Sideritis* were analyzed using various GC−MS techniques. A total of 99 different compounds was identified, and significant differences (qualitative and quantitative) were observed between the samples. The in vitro antimicrobial activity of the essential oils against six bacteria and three fungi is also reported.

Keywords: *Sideritis essential oils; antimicrobial activity; GC−MS*

INTRODUCTION

The genus *Sideritis* (Lamiaceae) has been difficult to classify because of the strong tendency of a number of species to hybridize (*1*). The genus comprises 8 species and 7 subspecies according to Flora of Greece (*2* and *3*). *Sideritis* species are often used in Mediterranean countries as herbal tea, and the common Greek name of these plants is "mountain tea". Moreover, these plants are used in Mediterranean traditional medicine for their antiinflammatory, antirheumatic, and antimicrobial activities (*4* and *5*). In continuation of our investigation on the composition and antibacterial evaluation of the essential oils of aromatic and edible plants of Greece (*6*), we present here the qualitative and quantitative analysis of the volatile oils of five taxa of *Sideritis* growing in Greece. *Sideritis raeseri* subsp. *attica* Papanic. & Kokkini (Heldr.), a subspecies endemic to central Greece (*3*), has not been investigated previously. On the other hand, a little data on the volatile oils of *S. sipylea* Boiss. (a species of West and Central Anatolia, extending to the East Aegean Islands) and *S. clandestina* subsp. *clandestine* Hayek (subspecies endemic to the South Greece) (*3*) have been reported (*1* and *7*). Finally, the reinvestigation of the oil composition for *S. raeseri* subsp. *raeseri* Boiss & Heldr. and *S. syriaca* subsp. *syriaca* L., which are endemic to the Western Balkan peninsula and the mountains of Crete, respectively (*3*), was necessary because of the confusing and contradictory results of previous studies (*7*−*10*). In addition, the knowledge of the exact composition of the tested samples is of great importance for understanding the antimicrobial activity of the essential oils of these plants.

MATERIALS AND METHODS

Plant Material. Aerial parts (herba in flowering stage) of plants were collected in July 1999. *S. clandestina* subsp. *clandestina* was collected from Mt. Parnon in central-east Peloponnisos; *S. raeseri* subsp. *raeseri* was collected from Mt. Agrafa (west Greece); *S. raeseri* subsp. *attica* came from Mt. Parnis in Central Greece; *S. sipylea* was obtained from Lesvos island; and *S. syriaca* subsp. *syriaca* was from Mt. Idi (Psiloritis) in the island of Crete. Voucher specimens were deposited (KL034, KL034B, KL034C, KL034D, and KL035) at the Herbarium of the Laboratory of Pharmacognosy, Department of Pharmacy, University of Athens.

Isolation of the Essential Oils. The aerial parts of these plants were subjected to steam distillation for 3 h (*11*), and the resulting oils were dried over anhydrous sodium sulfate and stored at 4−6 °C.

Gas Chromatography−Mass Spectrometry. The chemical composition of the essential oils was analyzed using various gas chromatography−mass spectrometric (GC−MS) techniques (electron impact (EI), chemical ionization (CI), and EIMS/MS). Identification of the components was based on the comparison of their mass spectra with those of Wiley275, NBS (*12*) and NST Libraries, as well as with their mass spectra retention indices previously reported (*13*). The mass spectrometer employed for GC−MS analysis was a Hewlett-Packard (HP) 5973 mass selective detector in the EI ionization mode (70 eV), interfaced to an HP 6890 gas chromatograph equipped with a capillary column HP-5 MS (30 m × 0.25 mm; film thickness, 0.25 μm). The temperature program employed was 60 °C (5 min) to 280 °C at a rate of 3 °C/min, with injection temperature of 200 °C; the flow rate of the carrier gas (helium) was 0.8 mL/min. GC−MS analysis was also performed on a Finnigan GCQ Plus ion-trap mass spectrometer with an external ion source in both the EI and CI modes using helium as a carrier gas at a flow rate of 1.0 mL/min, and CH_4 as the CI ionization reagent. GC and EI−MS/MS analysis of certain GC eluting components was carried out on the GCQ ion-trap mass spectrometer operated in the EI ionization mode. The ions characteristic of the target analytes were first selected, isolated, and stored in the ion-trap by applying a notched waveform to the end-cap electrodes. The isolated precursor ions were collisionally activated by a resonant excitation process, and the generated product ions were detected by applying a conventional rf amplitude ramp of the ring electrode.

Antimicrobial Strains and Media. The bacteriostatic activity of the essential oils against two Gram-positive bacteria (*Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 12228)), four Gram-negative bacteria (*Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC

* To whom correspondence should be addressed (telephone/fax 017274594; e-mail mitakou@pharm.uoa.gr).

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Table 1. Main Components (%) of the Five *Sideritis* Taxa

	components	*S. clandestina* subsp. *clandestina*	*S. raeseri* subsp. *raeseri*	*S. raeseri* subsp. *attica*	*S. sipylea*	*S. syriaca* subsp. *syriaca*	K. I.
1	heptanal	–	–	–	–	0.17	899
2	α-thujene	0.13	0.28	2.06	0.21	0.17	929
3	α-pinene	20.11	3.63	24.85	35.21	3.14	937
4	camphene	–	–	–	0.49	–	951
5	benzaldehyde	–	–	–	–	0.12	951
6	sabinene	–	1.01	1.31	2.86	0.32	975
7	*β*-pinene	7.31	9.06	17.99	8.75	1.97	978
8	1-octen-3-ol	0.15	1.01	0.31	–	2.27	980
9	*β*-myrcene	0.37	0.95	2.40	7.63	0.60	989
10	octanol-3	–	–	–	–	0.13	994
11	α-phellandrene	0.29	0.90	4.57	–	1.24	1002
12	*δ*-3-carene	0.30	1.18	5.77	–	0.84	1009
13	α-terpinene	0.38	0.89	5.84	0.23	0.94	1016
14	*o*-cymene	–	0.25	0.18	–	–	1021
15	*p*-cymene	–	2.78	2.46	0.25	1.02	1025
16	*β*-phellandrene	–	6.06	7.60	–	6.84	1029
17	limonene	1.60	[a]	[a]	–	[a]	1029
18	1,8-cineole	–	0.56	0.31	8.43	–	1032
19	*β*-(*Z*)-ocimene	0.11	0.61	2.13	–	0.29	1038
20	benzene acetaldehyde	–	0.30	–	–	0.15	1043
21	*β*-(*E*)-ocimene	–	0.64	0.44	–	–	1048
22	*γ*-terpinene	0.26	0.99	2.92	0.39	0.69	1060
23	1-octanol	0.15	–	–	–	0.23	1069
24	α-terpinolene	0.15	0.95	0.85	–	0.29	1085
25	linalool	0.80	3.18	1.31	–	1.21	1098
26	nonanal	0.17	–	–	–	0.53	1101
27	thujone	–	–	–	0.42	–	1112
28	α-campholenal	–	0.56	–	–	0.11	1123
29	pinocarveol	–	1.54	–	–	–	1137
30	camphor	–	–	–	0.47	–	1140
31	pinocarvone	–	1.34	–	–	0.18	1160
32	borneol	–	–	–	1.53	0.25	1164
33	naphthalene	–	3.83	–	–	–	1179
34	α-terpineol	0.47	0.80	1.07	–	0.51	1189
35	methyl salicylate	0.11	–	–	–	–	1191
36	myrtenal	–	1.28	–	–	0.19	1193
37	estragol	–	–	–	–	0.12	1195
38	myrtenol	–	0.60	–	–	–	1196
39	decanal	–	–	–	–	0.12	1204
40	naphthalene, 1,2,3,4-tetrahydro-	–	0.43	–	–	–	1207
41	pulegone	–	–	–	1.29	–	1233
42	cuminal	–	0.28	–	–	0.53	1236
43	carvone	–	0.22	–	–	0.35	1240
44	nonanoic acid	–	1.25	–	–	–	1280
45	bornyl acetate	–	–	0.23	–	–	1281
46	(*E*)-anethole	–	–	–	–	1.71	1283
47	thymol	–	–	–	0.67	1.00	1293
48	carvacrol	–	0.85	–	4.52	33.68	1301
49	linarodin	–	–	–	–	0.28	1348
50	α-cubebene	0.11	–	–	–	–	1351
51	eugenol	–	0.26	–	–	0.28	1355
52	α-copaene	0.84	3.80	0.23	–	0.60	1373
53	*β*-damascenone	–	0.88	–	–	0.30	1381
54	*β*-burbonene	0.51	–	–	–	–	1384
55	*β*-cubebene	0.19	–	–	–	–	1387
56	*β*-elemene	0.22	–	–	–	0.28	1389
57	naphthalene 1,2,-dihydro-	–	0.68	–	–	–	1389
58	α-curjunene	0.21	–	–	–	–	1406
59	α-cedrene	–	0.36	–	–	–	1407
60	*β*-(*E*)-caryophyllene	3.45	4.17	4.56	3.17	8.47	1416
61	α-*trans*-bergamotene	0.27	–	–	–	–	1432
62	aromadendrene	–	–	–	–	0.61	1436
63	*β*-(*Z*)-farnesene	0.25	–	–	–	–	1440
64	α-humulene	0.56	–	–	–	0.28	1451
65	geranyl acetone	–	0.24	–	–	–	1453
66	*β*-(*E*)-farnesene	0.58	1.67	1.23	0.37	1.51	1456
67	α-acoradiene	0.51	1.20	0.25	–	0.58	1461
68	9-epi-(*E*)-caryophyllene	2.97	–	–	–	–	1466
69	*γ*-curcumene	–	–	1.67	2.20	–	1475
70	α-amorphene	–	2.00	–	–	0.31	1475
71	*γ*-muurolene	–	–	–	–	3.91	1477
72	germacrene D	6.13	–	–	–	–	1480
73	AR-curcumene	0.67	6.14	0.47	0.33	1.13	1481
74	*β*-ionone	–	0.36	–	–	–	1483

Table 1 (Continued)

	components	*S. clandestina* subsp. *clandestina*	*S. raeseri* subsp. *raeseri*	*S. raeseri* subsp. *attica*	*S. sipylea*	*S. syriaca* subsp. *syriaca*	K. I.
75	epi-bicyclosesquiphellandrene	0.29	–	–	–	–	1488
76	bicyclogermacrene	–	3.27	4.02	3.13	5.29	1491
77	α-zingiberene	2.89	–	–	–	–	1493
78	α-muurolene	–	–	–	–	0.18	1497
79	*β*-bisabolene	1.63	0.87	0.22	–	0.57	1506
80	cis-calamenene	2.40	–	–	–	–	1519
81	*δ*-cadinene	–	4.83	0.39	–	1.35	1520
82	cadina-1,4-diene	2.30	0.29	–	–	–	1530
83	α-calacorene	–	0.50	–	–	–	1542
84	nerolidol	0.25	–	–	–	–	1562
85	dodecanoic acid	–	0.20	–	–	0.42	1567
86	(*Z*)-hexenylbenzoate	0.19	–	–	–	–	1568
87	(+)-spathulenol	–	2.19	0.66	1.22	1.54	1573
88	caryophyllene oxide	0.91	2.57	0.58	0.66	2.04	1579
89	viridoflorol	–	–	–	–	0.35	1587
90	isospathulenol	–	–	–	–	0.17	1637
91	α-cadinol	–	–	–	–	0.21	1652
92	valeranone	1.10	–	–	–	–	1670
93	α-bisabolol	7.06	1.25	–	–	0.29	1686
94	benzyl benzoate	1.54	1.48	–	–	0.33	1763
95	2-pentadecanone, 6,10,14-trimethyl	–	0.45	–	–	–	1845
96	2-heptadecanone	0.71	–	–	–	–	1901
97	hexadecanoic acid	–	1.66	–	–	–	1968
98	kaur-15-ene	0.54	–	–	–	–	1988
99	manoyl oxide	–	–	–	–	0.24	2010
	total	72.14	89.53	98.88	84.43	93.43	

[a] Masked by *β*-phellandrene.

13047), *Klebsiella pneumoniae* (ATCC 13883), and *Pseudomonas aeruginosa* (ATCC 227853)), and the antifungal activities of the essential oils against pathogens fungi (*Candida albicans*, *Candida tropicalis*, and *Torulopsis glabrata*) were determined by using the dilution technique (*14*). The culture medium used for bacteria was Müller-Hinton agar, but Sabouraud agar was used for growing the fungi. The incubation conditions used were 24 h at 37° C for the bacteria and 48 h at 28° C for the fungi. These particular strains were standard reference strains (of American Type Culture Collection) that are routinely used for the evaluation of antimicrobial compounds.

Antimicrobial Assay (MICs). The minimum inhibitory concentrations (MICs) were measured as described previously (*6*) for the oils, α-pinene, *β*-pinene, and carvacrol. Initial emulsions of oils were prepared at 10 mg/ml in sterile distilled water with 10% Tween 80. Serial dilutions of the stock solutions in broth medium (100 *μ*l of Müller-Hinton broth or on Sabouraud broth) were prepared in a microtiter plate (96 wells). Then 1 *μ*l of the microbial suspension (in sterile distilled water) was added to each well. For each strain, the growth conditions and the sterility of the medium were checked and the plates were incubated as referred above. MICs were determined as the lowest concentrations preventing visible growth. Standard antibiotics (amphotericine B, 5-flucitocine, intraconazole, netilmicin, amoxicillin, and clavulanic acid) were used in order to control the sensitivity of the tested bacteria, whereas 5-flucytocine, amphotericin B, and intraconazole were used in order to control the tested fungi.

RESULTS AND DISCUSSION

Chemical Composition of the Essential Oils. The yields (v/w) of the essential oils from the air-dried, aerial parts of the five species under study were 0.19% (*S. syriaca* subsp. *syriaca*), 0.12% (*S. raeseri* subsp. *raeseri*), 0,26% (*S. clandestina* subsp. *clandestina*), 0.37% (*S. raeseri* subsp. *attica*), and 0.40% (*S. sipylea*). The chemical composition of the essential oils was analyzed by GC−electron impact (EI)MS, chemical ionization (CI)MS, and EI-tandem MS. CI is a powerful "soft" ionization method yielding only little extent of fragmentation, thus allowing the molecular weight identification of each compound. Nevertheless, there are little applications in the chemical studies of essential oils (*15* and *16*). Tandem mass spectrometry (also referred to as MS/MS) not only provides invaluable data for the structure elucidation of the analyzed compounds, but it also enables the assignment of "seemingly" identical and isobaric compounds contained in separated GC eluting peaks. Qualitative and quantitative analytical results are showed in Table 1.

Monoterpene hydrocarbons were shown to be the main group of constituents in four of the samples. In the oil of *S. sipylea*, *S. raeseri* subsp. *attica*, and *S. clandestina* subsp. *clandestina*, α- and *β*-pinene were the major components, reaching percentages of 43.96%, 42.84%, and 27.42%, respectively. In the sample of *S. raeseri* subsp. *raeseri*, the percentage of the above two pinene components was 12.69%, but the total percentage of monoterpene hydrocarbons was 30.18%. The percentage of monoterpene hydrocarbons from *S. syriaca* was smaller than that observed for other samples (18.35%).

The chemical composition of the oil of *S. sipylea* according to data published by Gergis et al. (*1*) showed 90 identified compounds where a high percentage of α-pinene was indicated. In this study *S. sipylea* was also characterized by the presence of α-pinene (35.21%). 1,8-Cineole (8.43%), *β*-myrcene (7.63%), and carvacrol (4.52%) which were also present in this sample. On the other hand *β*-elemene, germacrene D, *cis*-perillyl alcohol, limonene, and phellandrene, some of the main constituents reported by Gergis et al. (*1*), were not identified in the present study.

The essential oil of *S. raeseri* subsp. *attica* was also characterized by the presence of *β*-phellandrene/limonene (7.60%), α-terpinene (5.84%), *δ*-3-carene (5.77%), α-phellandrene (4.57%), *trans*-*β*-caryophyllene (4.56%), and bicyclogermacrene (4.02%). The identification of *β*-phellandrene and limonene, which have the same

Table 2. Antimicrobial Activity (MIC mg/mL) of the Essential Oils of *Sideritis* Species and Their Main Components

essential oil	*S. aureus*	*S. epidermidis*	*P. aeruginosa*	*E. cloacae*	*K. pneumoniae*	*E. coli*	*C. albicans*	*C. tropicalis*	*T. glabrata*
S. clandestina subsp. *clandestina*	9.75	9.90	6.65	9.12	8.31	3.24	4.95	3.80	2.78
S. raeseri subsp. *raeseri*	–	–	–	–	–	–	–	–	–
S. raeseri subsp. *attica*	9.34	7.46	5.47	9.33	7.35	2.98	3.42	2.98	1.87
S. sipylea	7.5	6.75	4.75	8.75	6.75	2.75	3.5	2.65	1.75
S. syriaca subsp. *syriaca*	0.65	0.60	2.25	1.50	1.25	0.75	1.25	1.20	0.65
α-pinene	7.50	9.50	6	15	8	2	4	4	2
β-pinene	9.50	16.33	9.72	>20	11.38	6.88	9.51	9.22	9.14
carvacrol	<0.1	<0.10	1	0.75	0.50	<0.1	1	1	0.35
intraconazole	–	–	–	–	–	–	1×10^{-3}	0.1×10^{-3}	1×10^{-3}
5-flucytocine	–	–	–	–	–	–	0.1×10^{-3}	1×10^{-3}	10×10^{-3}
amphotericin B	–	–	–	–	–	–	1×10^{-3}	0.5×10^{-3}	0.4×10^{-3}
netilmicin	4×10^{-3}	4×10^{-3}	8.8×10^{-3}	8×10^{-3}	8×10^{-3}	10×10^{-3}	–	–	–
amoxycillin	2×10^{-3}	2×10^{-3}	2.4×10^{-3}	2.8×10^{-3}	2.2×10^{-3}	2×10^{-3}	–	–	–
clavulanic acid	0.5×10^{-3}	0.5×10^{-3}	1×10^{-3}	1.6×10^{-3}	1×10^{-3}	1.2×10^{-3}	–	–	–

retention indices, was achieved with the aid of the GC−tandem-MS technique.

Germacrene D and α-bisabolol were also present in the volatile oil of *S. clandestina* subsp. c*landestina* in high percentages (6.13% and 7.06%, respectively). In a previous study of this oil by Koedam (*7*) the percentage of α- and *β*-pinene was also high (24.04%), but *β*-copaene, *δ*-cadinene, calacorene, and α-cadinol, which were found in high percentages by Koedam were not identified at all in the present study.

The volatile oil of *S. raeseri* subsp. *raeseri* was first investigated in 1982 by Papageorgiou et al. (*17*). They had identified 47 compounds, and naphthalene was the major volatile component (22%). That was the major contradiction with the data reported by Koedam (*7*) where only a minor amount (0.18%) of naphthalene could be identified. In a recent investigation of the volatile oil of the plant by Galati et al. (*8*) 36 compounds were identified, among which camphor and 1,8-cineol were the major components (14.9% and 11.6%, respectively). In the present study the percentage of 1,8-cineol was only 0.56%, and camphor was not identified (similar data were reported by Koedam in ref *7*). In our present study, the presence of AR-curcumene (6.14%), *β*-phellandrene/limonene (6.06%), *δ*-cadinene (4.83%), *β*-caryophyllene (4.17%), and α-copaene (3.80%) characterized the oil of *S. raeseri* subsp. *raeseri*.

The essential oil of *S. syriaca* subsp. *syriaca* was first investigated by Komaitis et al. (*9*) and resulted in the recognition of about 20 terpenes and 10 additional substances (aldehydes, fatty acids, and alcohols). Two samples (from 1987 and 1989) of this plant were investigated in 1995 by Laer et al. (*10*). Plant material harvested in 1987 exhibited qualitative and quantitative differences from the material harvested in 1989. A total of 29 compounds, amounting to 72% of the essential oil, was identified. In our study, with the aid of GC, CIMS and GC−tandem-MS techniques, 59 components were identified, representing 94.6% of the essential oil. Carvacrol showed the highest percentage (33.68%) in the sample, which was also characterized by the presence of *β*-phellandrene/limonene (6.84%), *β*-caryophyllene (8.47%), and bicyclogermacrene (5.29%). It is noteworthy that the identification of *β*-phellandrene and bicyclogermacrene have not been reported in previous studies.

Antimicrobial Activity. The results of the bioassays (Table 2) showed that the oils of *S. clandestina* subsp. *clandestina* and *S. raeseri* subsp. *attica* appeared to have moderate activity against all the tested microorganisms (MIC values of 1.87−9.90 mg/mL), whereas the oil of *S. sipylea* (containing as main compound α-pinene 35.21%) showed stronger activity (MIC values of 1.75−8.75 mg/mL). It is noteworthy that the oil of *S. syriaca* subsp. *syriaca*, which was characterized by the presence of a high percentage of carvacrol (33.68%), exhibited the strongest activities against the tested bacteria (MIC values of 0.75−2.25 mg/mL) and against the pathogenic fungi (MIC values of 0.65−1.25 mg/mL). On the other hand, the sample of *S. raeseri* subsp. *raeseri*, in which the percentage of these two compounds is low (4.48%), appeared totally inactive. In the screening, standards of the pure monoterpenes α-pinene, *β*-pinene, and carvacrol were tested on the same cultures under identical conditions to compare their activity with those of the investigated oils. The results suggest that the activity of the oils can be attributed, to a considerable degree, to the existence of α-pinene and mainly to carvacrol, which appear to possess the strongest activity against all the tested microorganisms.

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