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Antimicrobial Activity and Essential Oil Composition of Five *Sideritis* taxa of *Empedoclia* and *Hesiodia* Sect. from Greece

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Abstract: Dried aerial parts of five taxa of Greek *Sideritis* were subjected to hydrodistillation and the oils obtained were analyzed by using GC and GC-MS. A total of 82 compounds were identified and the analysis showed important differences between the samples not only quantitatively but also qualitatively. The microbial growth inhibitory properties of the essential oils were determined using the broth microdilution method against eight laboratory strains of bacteria - Gram positive: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Enterococcus faecalis*, *Bacillus subtilis* and Gram negative: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and two strains of the yeast *Candida albicans*. The tested essential oils exhibited considerable activity against certain strains of the microorganisms tested, with *S. lanata* oil presenting MIC values to *S. aureus* and *M. luteus* comparable to those of the reference antibiotics.

Keywords: Antimicrobial activity; chemical composition; essential oils; *Sideritis* spp.

1. Introduction

The genus *Sideritis* L. (Labiatae) comprises of 150 species distributed in North temperate regions, occurring mainly in the Mediterranean area [1-3]. The genus name derives from the Greek word ‘sideros’ (iron) in reference to these vulnerary plants that heal the wounds caused by iron weapons [4]. This genus is represented in the Greek Flora by 15 taxa (species and subspecies), 6 of which are endemic in Greece [5,6]. These taxa are further divided into two sections; *Empedoclia* (Rafin.) Bentham (10 taxa) and *Hesiodia* Bentham (5 taxa) [2,3].

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In González-Burgos *et al*. [7] review work on *Sideritis* species is reported that these plants have been traditionally used as teas for feeding, flavoring agents and in folk medicine as anti-inflammatory, antiulcerative, antimicrobial, vulnerary, antioxidant, antispasmodic, anticonvulsant, analgesic and carminative agents. *Sideritis* species are generally known in Greece under the name 'tsai tou vounou' (mountain tea) and comprises one of the most frequently traded herbs [6]. The dried flowering aerial parts are used in Greek folk medicine to prepare an odoriferous infusion or/and decoction to treat dyspepsia, anaemia, influenza, common cold, cough, sore throat, having calmative, diuretic, antipyretic and stimulant properties [8-11].

The aims of this work were to determine the chemical composition of the essential oils from five taxa of *Sideritis* growing in Greece: *S. clandestina* (Bory & Chaub.) Hayek subsp. *peloponnesiaca* (Boiss. and Heldr.) Baden, *S. clandestina* (Bory & Chaub.) Hayek subsp. *clandestina* and *S. euboea* Heldr., perennial herbs that belong to the section *Empedoclia*, and *S. romana* L. subsp. *purpurea* (Talbot ex Bentham) Heywood and *S. lanata* L. annual herbs of *Hesiodia* section, and to evaluate their antimicrobial activity *in vitro*.

2. Materials and Methods

2.1. Plant Material

Aerial parts of full flowered plants of *S. clandestina* subsp. *peloponnesiaca*, *S. clandestina* subsp. *clandestina*, *S. euboea*, *S. romana* subsp. *purpurea* and *S. lanata* were collected during the years 2006-2008 from different wild growing populations in Greece (Table 1). Voucher specimens were authenticated by Dr Bazos and have been deposited in the Herbarium of the University of Athens (ATHU). Air-dried aerial parts were cut in small pieces and subjected to hydrodistillation for 3 h, using a modified Clevenger-type apparatus. The oils, yellowish of pleasant scent, were obtained using *n*-pentane as a collecting solvent and subsequently they were dried over anhydrous sodium sulphate and stored under N_2 atmosphere in amber vials at 4 °C until they were analyzed.

Table 1. Collection data and oil yields of *Sideritis* taxa investigated

Species	Voucher no.	Collection Period	Region	Oil yield (% v/dry weight)
S. clandestina subsp. *peloponnesiaca* (SCPS)	[OT-90]	July 2007	Mt. Saitas, Achaia, Peloponnisos	0.60
S. clandestina subsp. *peloponnesiaca* (SCPC)	[OT-86]	July 2006	Mt. Chelmos, Achaia, Peloponnisos	0.46
S. clandestina subsp. *clandestina* (SCC)	[OT-87]	July 2006	Mt. Taygetos, Laconia, Peloponnisos	0.26
S. euboea (SE)	[OT-93]	July 2007	Mt. Dirfis, Euboea	0.10
S. romana subsp. *purpurea* (SRP)	[IB 4476]	May 2008	near the village of Arachamites, Arcadia, Peloponnisos	0.55
S. lanata (SL)	[Z 2693]	April 2007	Kalymnos island	0.94

2.2 GC and GC/MS analysis

GC analyses were carried out using an SRI (Brooks, Hatfield, PA, USA) model 8610C GC-flame ionization detector (FID) system, equipped with a DB-5 capillary column (30 m×0.32 mm; film thickness, 0.25 μm) and connected to a FID detector. The injector and detector temperatures were 280 °C. The carrier gas was He, at flow rate of 1.2 mL/min. The thermal program was 60-280 °C at a rate of 3 °C/min; split ratio 1:10. The essential oils obtained by hydrodistillation were dissolved in *n*-

hexane (100 μL/mL). The injected volume was 1 μL. The percentage composition of the essential oil is relative, computed from peak areas without correction factors.

GC-mass spectrometry (GC-MS) analyses were carried out using a Hewlett Packard (Hewlett Packard GmbH, Waldbronn, Germany) model 5973-6890 GC-MS system operating in electron ionization mode at 70 eV, equipped with a split-splitless injector (200 °C). The transfer line temperature was 250 °C. Helium was used as a carrier gas (1 mL/min). The capillary column used was an HP 5MS (30 m×0.25 mm; film thickness, 0.25 μm; Agilent, Palo Alto, CA, USA). The temperature program was the same as that used for the GC analyses; split ratio 1:10. Total scan time 83.33 min. Acquisition mass range 40-400 amu. Identification of the constituents was based on comparison of the retention times with those of authentic compounds, comparing their linear retention indices relative to the series of *n*-hydrocarbons and on computer matching against commercial (Wiley) (available through Hewlett Packard) and the literature [12-15]. Authentic references chemicals were purchased from Sigma-Aldrich Co (Sigma-Aldrich, Buchs SG, Switzerland).

2.3 Optical rotations of the oils

Optical rotations of the oils were recorded with a Perkin-Elmer Polarimeter 341 (UV-Vis).

2.4 Antimicrobial activity

The antimicrobial activity was evaluated using eight different laboratory control strains of bacteria - Gram positive: *Staphylococcus aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *Micrococcus luteus* (ATCC 9341), *Enterococcus faecalis* (ATCC 29212), *Bacillus subtilis* (ATCC 6633) and Gram negative: *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (NCIMB 9111), *Pseudomonas aeruginosa* (ATCC 27853), and two strains of yeast: *Candida albicans* (ATCC 10259 and ATCC 24433). Microorganisms were provided by the Institute of Immunology and Virology, Torlak, Belgrade. Active cultures for experiments were prepared by transferring a loopful of cells from the stock into tubes that contained 10 mL of Mueller-Hinton broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for fungi. After incubation for 24 h at 37 °C and 25 °C respectively, the cultures were diluted with fresh Mueller-Hinton and Sabouraud dextrose broth in order to achieve optical densities corresponding to $1.5x10^6$ colony forming units (cfu/mL) for bacteria and $1.6x10^7$ cfu/mL for fungi which was used as inoculum.

A broth microdilution method was used to determine the minimum inhibitory concentration (MIC) according to Clinical and Laboratory Standards Institute (CLSI) [16]. The inoculum of the bacterial strain was prepared from overnight broth culture and suspension was adjusted to 0.5 McFarland standard turbidity. The tested oils were dissolved in 1% dimethylsulphoxide (DMSO) and then diluted to the highest concentration. Two-fold serial concentrations of the essential oil were prepared in a 96-well microtiter plate.

In the tests, triphenyl tetrazolium chloride (TTC) (Aldrich Chemical Company Inc. USA) was also added to the culture medium as a growth indicator. The final concentration of TTC after inoculation was 0.05%. The microbial growth was determined by absorbance at 620 nm using the universal microplate reader (ThermoLabsystems, Multiskan EX, Software for Multiscan ver.2.6.) after incubation at 37 °C for 24 h for bacteria, and at 26 °C for 48 h for fungi. The MIC is defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. All determinations were performed in triplicate and two positive growth controls were included. The MICs of the reference antibiotics were determined in parallel experiments.

3. Results and Discussion

3.1 Chemical composition of the essential oils

Quantitative and qualitative analytical results of the studied essential oils of *Sideritis* species by GC and GC-MS are shown in Table 2. Two samples, SCPS and SCPC, which are endemic to South Greece, *S. clandestina* subsp. *peloponnesiaca* (syn. *S. clandestina* subsp. *cyllenea*) showed significant

differences. Monoterpenes were the dominant group (46.6%) in sample SCPS oil, with *α*-pinene being the principal compound (35.4%) followed by *β*-pinene (8.2%). Sesquiterpenes were presented in a high percentage (35.6%), with *δ*-cadinene (9.3%) and bicyclogermacrene (8.9%) being the main constituents, while diterpenes were detected in traces. On the contrary, sample SCPC oil was characterized by the presence of diterpenes (25.0%), with the labdane derivative isoabienol being the major constituent (18.3%). Monoterpenes and sesquiterpenes were presented almost in equal amount (18.9% and 18.5%, respectively) in SCPC oil, with *α*-pinene (14.3%) and *ar*-curcumene (3.8%) being the main components respectively. In a previous study on the essential oil of this subspecies the total percentage of *α*- and *β*-pinene was also high (22.73%) and *ca*. 30% of the oil content was not identified [17].

S. clandestina subsp. *clandestina* essential oil (sample SCC) was also characterized by a high content of monoterpenes (44.4%), with *α*-pinene (12.4%) being the major monoterpene. Sesquiterpenes were present in 39.3% with derivatives of caryophyllene being the most abundant metabolites (34.5%), while diterpenes were present in small amount (1.1%). Comparing the essential oils of two *S. clandestina* subspecies, it seems that sample SCC is closer to sample SCPS (subsp. *peloponnesiaca* collected from Mt Saitas). Koedam [18] reported for *S. clandestina* subsp. *clandestina* oil (collected in a different location in Taygetos Mt) that sesquiterpenes overlapped monoterpenes (54.3% and 43.5% respectively), with *β*-copaene (13.49%), *δ*-cadinene (11.49%) and *β*-caryophyllene (9.07%) being the main constituents, followed by the monoterpenes, *α*-pinene (12.16%) and *β*-pinene (11.92%). In a previous study on the essential oil composition of this subspecies collected at Parnon Mt, the oil was rich in monoterpenes, with *α*- and *β*-pinene being the major components (42.84% in total), while *ca*. 30% of the oil content was not identified [19]. In the analysis of *S. clandestina s.l.* oil by Ntalli *et al.* [20] monoterpenes and sesquiterpenes were present in almost equal amount (~35%) and *ca*. 25% of the oil content was not identified. Our experimental data are in agreement with Aligiannis *et al*. [19], with the exception of germacrene D and *α*-bisabolol, which were found in 6.1% and 7.1% respectively, were not present in SCC sample oil.

The oil of the Greek endemic *S. euboea* has not been studied previously. Even though *S. euboea* (sample SE) belongs to *Empedoclia* sect., monoterpenes were present in a significant low percentage (4.8%) in the oil comparing to those of previous taxa, with *α*-pinene being the only monoterpene in a measurable amount. *S. euboea* oil was dominated by the presence of sesquiterpenes (55.4%), with the oxygenated sesquiterpene valeranone (12.1%) being the main component, followed by *β*-caryophyllene (9.0%) and *γ*-muurolene (9.0%). Diterpenes were present in a considerable amount (21.7%), with isoabienol (13.1%) and abienol (8.6%) being the principal ones.

Kirimer *et al.* [21] classified taxa of *Empedoclia* sect. according to their main class of metabolites in the essential oils. Most of them are characterized by the presence of relatively high amounts of monoterpenes and specifically of *α*- and/or *β*-pinene. Two studied subspecies of *S. clandestina*, samples SCPS and SCC, could be characterized as monoterpene hydrocarbon-rich oils, whereas sample SCPC is a diterpene-rich oil and sample SE is a sesquiterpene- rich oil.

The oil of *S. romana* subsp. *purpurea*, an endemic of the W. part of Balkan peninsula, has not been previously investigated. This oil (sample SRP) was predominated by sesquiterpenes (83.6%) with bicyclogermacrene (48.9%) being the major constituent of the total oil. Monoterpenes were present in 16.2% with *β*-pinene (7.9%) being the main metabolite, while diterpenes were not detected. It is worth noting that *S. romana* subsp. *romana* of Turkish origin was characterized as an oxygenated monoterpene-rich oil with thymol (24.9%) being the most abundant component of the oil [22].

S. lanata essential oil (sample SL) of Greek origin has not been studied earlier. The oil was characterized by the abundance of sesquiterpenes (46.3%), the poor content in monoterpenes (3.5%) and the presence of diterpenes (10.9%), with spathulenol (12.7%), *β*-phellandrene (3.5%) and ent-2*α*-hydroxy-8(14),5-pimaradiene (9.7%) being the major metabolites, respectively. Turkish *S. lanata* essential oil had also spathulenol (9.45%) as the main metabolite of the sesquiterpene group [22].

The essential oils of these two annual *Sideritis* species of sect. *Hesiodia* were quite distinct. All the studied oils of *Sideritis* taxa contained diterpenes except the oil of *S. romana* subsp. *purpurea*. Many diterpenoids have been isolated from *Sideritis* extracts, whereas only few diterpenes have been identified from the essential oils. Diterpenes from *Sideritis* taxa are known to possess antimicrobial and anti-inflammatory activity [23-27]. The composition of *Sideritis* species essential oils seems to be

influenced by the geographical location, season, climatological variations, plant variety, experimental conditions and a possible existence of chemotypes or ecotypes [17, 28-30].

Table 2. Chemical composition (%) of *Sideritis* species essential oils

RI[a]	Compound[b]	SCPS*	SCPC*	SCC*	SE*	SRP*	SL*
924	*α*-Thujene	-	-	2.5	-	t	-
932	*α*-Pinene	35.4	14.3	12.4	4.8	2.3	-
952	Benzaldehyde	-	-	-	-	-	6.5
969	Sabinene	t	t	1.8	-	t	-
974	*β*-Pinene	8.2	3.4	4.5	t	7.9	-
975	1-Octen-3-ol	-	-	-	-	-	2.1
988	*β*-Myrcene	t	t	1.9	t	t	-
1001	*δ*-2-Carene	-	-	4.4	t	-	-
1002	*α*-Phellandrene	t	-	1.5	t	t	t
1014	α-Terpinene	-	-	1.7	t	t	-
1020	*p*-Cymene	t	t	3.3	t	t	-
1024	Limonene	3.0	1.2	4.2	t	t	t
1025	*β*-Phellandrene	t	t	6.2	t	3.3	3.5
1032	(*Z*)-*β*-Ocimene	-	-	-	t	-	-
1044	(*E*)-*β*-Ocimene	-	-	-	t	t	-
1054	*γ*-Terpinene	-	t	t	t	t	-
1086	Terpinolene	t	t	t	t	t	t
1100	Nonanal	-	-	-	t	t	3.5
1111	6-Camphenol	-	-	-	t	-	-
1122	*α*-Campholenal	t	t	-	t	-	-
1155	Isoborneol	t	-	-	-	-	-
1340	Verbanol acetate	t	-	-	-	2.7	t
1345	*α*-Cubebene	t	t	-	t	t	-
1374	*α*-Copaene	5.0	0.6	-	t	t	1.9
1383	(*E*)-*β*-Damascenone	t	t	-	-	-	-
1387	*β*-Bourbonene	t	t	-	t	t	t
1387	*α*-Isocomene	-	-	0.8	-	-	-
1390	7-*epi*-Sesquithujene	-	t	-	-	-	-
1389	*β*-Elemene	t	-	-	t	t	t
1410	*α*-Cedrene	-	-	-	t	-	-
1417	*β*-Caryophyllene	4.5	2.5	14.3	9.0	12.7	t
1429	*cis*-Thujopsene	-	t	-	t	t	-
1430	*β*-Copaene	t	-	-	t	t	t
1439	Aromadendrene	t	-	-	t	t	t
1452	*α*-Humulene	t	t	t	t	t	-
1454	(*E*)-*β*-Farnesene	-	t	-	t	4.4	t
1458	*allo*-Aromadendrene	t	-	-	-	t	t
1460	Dehydroaromadendrane	t	-	-	-	1.8	-
1464	*α*-Acoradiene	-	0.9	-	t	-	-
1464	9-*epi*-(*E*)-Caryophyllene	t	-	12.5	-	-	-
1478	*γ*-Muurolene	5.3	1.4	2.0	9.0	11.9	t
1479	*ar*-Curcumene	-	3.8	-	3.1	t	-
1481	*γ*-Curcumene	-	0.6	-	-	t	-
1493	*α*-Zingiberene	-	1.5	-	2.8	-	-
1500	Bicyclogermacrene	8.9	-	t	2.2	48.9	4.4
1501	*α*-Muurolene	t	-	-	-	-	t
1505	*β*-Bisabolene	t	t	t	t	t	-
1513	*γ*-Cadinene	t	-	-	-	-	t
1414	*β*-Curcumene	-	t	-	t	t	-

1521	*trans*-Calamenene	-	1.5	-	-	-	t
1522	*δ*-Cadinene	9.3	0.9	-	2.4	t	2.1
1544	*α*-Calacorene	t	t	-	t	-	t
1577	Spathulenol	2.6	2.5	-	4.1	3.9	12.7
1582	Caryophyllene oxide	t	t	5.8	6.6	-	3.2
1590	*β*-Copaene-4-α-ol	t	-	-	t	t	t
1592	Viridiflorol	t	t	-	-	t	1.0
1630	Muurola-4,10(14)-dien-1-*β*-ol	-	-	-	-	t	4.7
1636	*β*-Acorenol	-	-	-	-	-	1.7
1644	*α*-Muurolol	t	-	-	-	t	1.9
1668	14-Hydroxy-9-*epi*-(*E*)-caryophyllene	t	-	1.9	t	-	-
1674	Valeranone	-	-	-	12.1	-	-
1685	*α*-Bisabolol	-	-	-	4.1	-	-
1759	Benzyl benzoate	-	-	-	t	-	t
1778	(*Z*)-Lanceol	-	t	2.0	-	-	7.0
1849	Hexahydrofarnesyl acetone	t	t	t	t	t	5.7
1854	(*Z*)-Lanceol acetate	-	1.4	t	-	-	-
1890	(*E*)-Lanceol acetate		0.9	t	-	-	-
1905	Isopimara-9(1)-15-diene	t	1.7	t	t	-	-
1948	Pimaradiene	t	t	-	t	-	-
1962	M270 [1]	-	1.3	-	-	-	-
1964	M272 [1]	8.3	-	-	t	-	2.7
1967	M270 [2]	6.4	15.9	9.8	6.5	-	7.6
1982	M272 [2]	-	-	1.2	8.6	-	-
1997	Kaur-15-ene	t	-	-	-	-	-
1998	(*Z*,*E*)-Geranyl linalool	-	5.4	1.6	t	-	-
2020	Manoyl oxide	t	1.7	1.1	t	-	-
2042	Kaurene	t	-	-	-	-	-
2113	Isoabienol	-	18.3	-	13.1	-	-
2149	Abienol	-	3.3	-	8.6	-	-
2180	12*β*-Hydroxy-9,11-dehydrokaurene	-	-	-	-	-	1.2
2190	ent-2*α*-Hydroxy-8(14),5-pimaradiene	-	-	-	-	-	9.7
2196	9-Octadecen-1-ol**	-	1.5	-	-	-	-
2300	Tricosane	-	-	-	-	t	1.5
-	Pentacosane	-	-	-	-	t	1.4
-	Heptacosane	-	1.0	-	-	-	7.3
-	Nonacosane	-		-	-	-	2.5
	Total identified %	82.2	70.3	86.4	81.9	99.8	85.5
	$[\alpha]_D^{20}$	-8.98° ($CHCl_3$ *ca*. 1.18)	-9.74° ($CHCl_3$ *ca*. 1.25)	+3.91° ($CHCl_3$ *ca*. 1.28)	-1.09° ($CHCl_3$ *ca*. 1.28)	+4.62° ($CHCl_3$ *ca*.0.65)	-0.27° ($CHCl_3$ *ca*. 0.3)
	Grouped components						
	Monoterpenes	46.6	18.9	44.4	4.8	16.2	3.5
	Sesquiterpenes	35.6	18.5	39.3	55.4	83.6	46.3
	Diterpenes	t	25.0	1.1	21.7	-	10.9

[a]RI: Retention indices on HP-5 MS column relative to C_9-C_{23} *n*-alkanes.
[b]Constituents listed in order of elution from a HP-5 MS column.
t, trace (<0.1%).
* For species abbreviations, see Table 1.
** Correct isomer not identified.

MS data: M270 [1] [*m/z* (rel. int., %)]: 270(8), 227(13), 159(16), 145(45), 132(72), 119(100), 105(33), 91(25), 69(33); M270 [2] [*m/z* (rel. int., %)]: 270(5), 227(17), 159(12), 145(58), 132(78), 119(100), 105(32), 91(17), 69(25); M272[1] [*m/z* (rel. int., %)]: 272(9), 227(9), 159(18), 145(36), 132(64), 119(100), 105(45), 91(36), 69(42); M272 [2] [*m/z* (rel. int., %)]: 272(13), 227(4), 159(15), 145(22), 132(60), 119(100), 105(31), 93(60), 69(58).

3.2 Antimicrobial activity of the essential oils

In the antimicrobial screening (Table 3), the oils of samples SCPS, SCC, SE, SRP and SL were tested as they were in a sufficient amount, enough for all the assays. Generally, Gram positive bacteria were more sensitive than Gram negative.

Table 3. Antimicrobial activity of *Sideritis* taxa essential oils
* for species abbreviations, see Table 1.

Microorganism	Minimal inhibitory concentration MIC (μg/ml)							
	SCPS*	SCC*	SE*	SRP*	SL*	Amikacin	Ampicillin	Nystatin
S. aureus ATCC 25923	6.91	7.56	6.51	6.16	2.22	2.00	1.00	n.t.
S. epidermidis ATCC 12228	>110.50	15.12	6.51	24.62	8.88	n.t.	0.20	n.t.
M. luteus ATCC 9341	27.62	30.25	3.25	6.16	2.22	n.t.	2.80	n.t.
E. faecalis ATCC 29212	55.25	121.00	3.25	>98.50	>142	2.40	n.t.	n.t.
E. coli ATCC 25922	>110.50	>121.00	>104.16	>98.50	>142	8.60	4.40	n.t.
K. pneumoniae NCIMB 9111	>110.50	>121.00	>104.16	>98.50	>142	6.40	n.t.	n.t.
P. aeruginosa ATCC 27853	>110.50	>121.00	>104.16	>98.50	>142	2.80	n.t.	n.t.
C. albicans ATCC 10259	13.81	15.12	26.05	24.62	17.75	n.t.	n.t.	3.80
C. albicans ATCC 24433	>110.50	>121.00	>104.16	>98.50	>142	n.t.	n.t.	6.20

n.t.: not tested

Tested essential oils exhibited significant antimicrobial activity against Gram positive bacteria and one strain of *Candida albicans*. The most sensitive bacterial strain to all the tested essential oils was *S. aureus* with MIC values ranging from 2.22 to 6.91 μg/mL, whereas *E. coli*, *K. pneumoniae* and *P. aeruginosa* strains were found to be resistant to the tested oils. Sample SL oil exhibited the best antimicrobial activity against *S. aureus*, *S. epidermidis* and *M. luteus* (MIC values: 2.22, 8.88 and 2.22 μg/mL respectively). In particular, the MIC values of SL oil against *S. aureus* and *M. luteus* were comparable to the reference antibiotics. Sample SE oil also presented a high activity against *S. aureus*, *S. epidermidis*, *M. luteus* and *E. faecalis* (MIC values: 6.51, 6.51, 3.25, 3.25 μg/mL, respectively). Especially, this oil showed similar activity to the ampicillin and amikacin against *M. luteus* and *E. faecalis*, respectively. Interestingly, one strain of *Candida albicans* (ATCC 10259) was more sensitive compared with the other, which was found to be resistant to all tested oils. In particular the oil of SCPS and SCC samples exhibited good antifungal activity against the most sensitive Candida strain (MIC values: 18.81 and 15.12 μg/mL, respectively). Gergis *et al.* [31] reported that essential oils obtained from Greek *S. sipylea, S. euboea, S. clandestina* subsp. *cyllenea* and *S. clandestina* subsp. *clandestina* were less effective against Gram-negative bacteria than Gram-positive ones which is in accordance with our results.

More studies are required to elucidate the structure of the unidentified diterpenes in the oils and evaluate their contribution in the antimicrobial activity.

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