Chemical Diversity and Antimicrobial Activity of Salvia multicaulis Vahl Essential Oils

Layal Fahed, Didier Stien, Naïm Ouaini, Véronique Eparvier, Marc El Beyrouthy

Chemical Diversity and Antimicrobial Activity of *Salvia multicaulis* Vahl Essential Oils

Layal Fahed[a,b]), Didier Stien*[a,c]), Naïm Ouaini[b]), Véronique Eparvier[a]), Marc El Beyrouthy[b])

[a]) CNRS, Institut de Chimie des Substances Naturelles, UPR 2301, 1 Avenue de la Terrasse, 91198 Gif-sur-Yvette, France (phone: +33-1-69 82 36 10; fax: +33 1 69 82 37 84; E-mail: didier.stien@cnrs.fr)

[b]) Department of Agricultural Sciences, Holy Spirit University of Kaslik, Kaslik, B.P. 446, Jounieh, Lebanon

[c]) Sorbonne Universités, UPMC Univ Paris 06, CNRS, Laboratoire de Biodiversité et Biotechnologies Microbiennes (LBBM), Observatoire Océanologique, 66650 Banyuls/Mer, France

Abstract

The chemical composition and antimicrobial activity of the essential oils (EOs) of aerial parts of *Salvia multicaulis* Vahl, collected during the same week from two different Lebanese regions, were investigated. The EOs were obtained by hydrodistillation using a Clevenger-type apparatus and characterized by GC and GC-MS analyses. The minimum inhibitory concentrations (MICs) of these essentials oils were determined against one Gram-negative and two Gram-positive bacteria, one yeast, and five dermatophytes using the broth microdilution technique. One essential oil was notably active against *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA) and all of the *Trichophyton* species tested. Nerolidol was found to be the major compound in the active oil; nerolidol was also absent from the inactive oil. This study demonstrated that nerolidol is antimicrobial and therefore significantly contributes to the antimicrobial potential of the oil. The chemical diversity of worldwide *S. multicaulis* EOs was analysed, revealing that the EOs of the present study belong to two different chemotypes found in the literature. The nerolidol chemotype appears to be restricted to Lebanon and can be used as an antimicrobial against external bacterial and fungal infections.

Key words: *Salvia multicaulis*, essential oils, chemical diversity, antimicrobial activity, nerolidol.

Introduction. –The incidence and severity of microbial infections has consistently increased in recent years [1]. This increase originates, in part, from the extensive use of antifungal and antibacterial agents to treat infections, regardless of severity. Topical infections could be treated with plant essential oils, and oils are widely used for this purpose in traditional and alternative medicines [2]. Essential oils can also be used in combination with classical drugs to increase activity, reduce toxicity and limit the risk of resistance development [3].

Salvia multicaulis Vahl (Lamiaceae) is endemic to the Middle East region and is known in Lebanon as "kasirat al souq" and "Chafiya", which means the healing plant. This name is in reference to the folk use of the plant against coughs and as a febrifuge and emollient [4]. The plant is also used as an herbal remedy in Turkey to treat many ailments, such as colds [5], the flu and tonsillitis [6]. Two samples of *S. multicaulis* EOs from two different Lebanese regions were investigated in a preliminary antimicrobial screening of 26 Lebanese EOs (unpublished data). One EO showed promising antibacterial and antifungal potential. We therefore sought to study the chemical diversity of *S. multicaulis* EOs based on our work and descriptions from the literature in an attempt to identify the origin of the antimicrobial potential of the active chemotype.

Results and Discussion. – *Chemical Analyses of Essential Oils*. *Salvia multicaulis* from Zahle yielded 0.67% essential oil, while *Salvia multicaulis* from Bechwet yielded 1.29% essential oil. Forty-one components, accounting for 83.7% of the Zahle EO composition, were identified. These 41 components accounted for 86.9% of the

Bechwet EO. All 41 components were identified and quantified by GC and GC-MS analyses (Table 1).

Please insert table 1 here

To understand the chemical diversity of *S. multicaulis* species, a hierarchical clustering analysis (Figure 1) was conducted using all of the previously reported essential oils from aerial plant parts and the essential oils investigated in this study. Compounds accounting for more than 4 % of at least one EO are listed in Table 2. A composition table consisting of compounds that account for more than 1% of an essential oil was used for statistical analysis. Three chemotypes were detected; the EOs studied here belong to two different chemotypes.

Cluster 1 (chemotype 1) is made up of Iranian EOs only. This chemotype is characterized by high relative proportions of β-caryophyllene, borneol and bornyl acetate, with average proportions of 10.3%, 11.8% and 14.7%, respectively. Chemotype 2 contains the Zahle EO from our study and two previously reported Lebanese EOs. EOs in this cluster have the lowest relative proportions of camphene (average of 1%) and camphor (average of 1.2%) compared to chemotypes 1 (camphene and camphor averages of 6.1% and 10.9%, respectively) and 3 (camphene and camphor averages of 7.8% and 11.7%, respectively). Another characteristic of chemotype 2 is the presence of nerolidol; this compound is absent from the other chemotypes. Nerolidol was the major compound in the Zahle EO. Chemotype 3 includes EOs from several Middle Eastern

countries, including Lebanon. The Bechwet EO belongs to this chemotype. The specificity of chemotype 3 originates from the remarkably high proportion of eucalyptol in the oils (20.2% in average). The relative proportion of eucalyptol in the other chemotypes is significantly lower (averages of 5.8% in chemotype 1 and 4.5% in chemotype 2).

Antimicrobial activity. The minimum inhibitory concentrations (MICs) of both *S. multicaulis* EOs are reported in *Table 3*. EOs with MICs of 128 µg/ml and below are considered to be interesting for medicinal purposes [21] [22]. Both strains of *S. aureus* were sensitive to the Zahle EO (MIC of 128 µg/ml) and resistant to the Bechwet EO (MIC > 512 µg/ml). Both EOs were inactive against *P. aeruginosa* (MIC > 512 µg/ml) and *C. albicans* (MIC of 512 µg/mL for the Zahle EO and MIC > 512 µg/ml for the Bechwet EO). The Zahle EO exhibited significant activity against all of the dermatophytes tested (MICs from 8 to 64 µg/ml), while the Bechwet EO was essentially inactive against the dermatophytes (MICs from 64 to 256 µg/ml).

Because nerolidol, the main component of the Zahle EO (12%), was totally absent from the Bechwet EO, the antimicrobial potential of nerolidol alone was evaluated. Nerolidol was found to be active on both *S. aureus* strains and on *T. rubrum* (MICs of 128 and 64 µg/ml, respectively), indicating that nerolidol contributes at least partially to the biological activity of the Zahle EO. The antimicrobial activity of nerolidol has been described previously in the literature [23] [24].

Please insert table 3 here

Conclusions. – To date, three chemotypes of *S. multicaulis* EOs have been described in the literature. One of these chemotypes appears to be restricted to Lebanon. This chemotype was found to have antimicrobial activity due to the presence of nerolidol. EOs of this chemotype could therefore be used in topical applications against microbial infections. However, additional studies are needed to ensure the suitability and safety of these EOs.

Acknowledgments

This work benefitted from an "Investissement d'Avenir" grant managed by the Agence Nationale de la Recherche (CEBA, ref. ANR-10-LABX-25-01) and from the support of CNRS Lebanon.

Experimental Part

Plant Material. Aerial parts were collected in April 2013 in the Bekaa Valley, Lebanon in Zahle (33°50'50.96" N 35°52'28.13" E) and Bechwet (34°07'52.49" N 36°08'16.41" E) at altitudes of 1173 and 1152 m, respectively. Plant identification was based on *La nouvelle flore du Liban et de la Syrie* by Paul Mouterde [25]. The air drying of plant material was performed in a shady place for two weeks at room temperature. A voucher specimen of each plant was deposited in the Herbarium of the Faculty of Agricultural

and Food Sciences of USEK, Lebanon under the registry numbers MNV161a for the Zahle plant and MNV161b for the Bechwet plant.

Essential Oil Extraction. As described in *European Pharmacopoeia* [26], essential oils were extracted by hydrodistillation for three hours using a *Clevenger*-type apparatus.

GC Analyses. Analytical gas chromatography was performed on a Thermo Electron Corporation gas chromatograph fitted with a flame ionization detector (FID), a DB-5 MS capillary column (30 m × 0.25 mm, film thickness 0.10 µm) or a fused silica HP Innowax polyethylene glycol capillary column (50 m × 0.20 mm, film thickness 0.20 µm). Helium was used as the carrier gas (0.7 ml/min). The column temperature was initially set to 35 °C, gradually increased to 85 °C at a rate of 5 °C/min, held for 20 min at 85 °C, raised to 300 °C at 10 °C/min and finally held for 5 min at 300 °C. Diluted 1 µl samples (1/100, v/v) were manually injected at 250 °C in splitless mode. Flame ionization detection (FID) was performed at 310 °C.

GC–MS Analyses. GC/MS analyses were performed using an Agilent 6890 gas chromatograph coupled with a 5975 Mass Detector. The 7683B autosampler injected 1 µL of each oil sample. A fused silica capillary column DB-5 MS (30 m × 0.25 mm internal diameter, film thickness 0.1 µm) or a fused silica HP Innowax polyethylene glycol capillary column (50 m × 0.20 mm, film thickness 0.20 µm) was used. Helium was used as the carrier gas (0.7 ml/min). The oven temperature profile was identical to that described above (*cf.* GC Analyses). Mass spectra were recorded at 70 eV with an

ion source temperature of 310 °C and a transfer line temperature of 320 °C. Acquisitions were recorded in full scan mode (50 – 400 amu).

Qualitative and Quantitative Analyses. Most constituents were identified by gas chromatography by comparing their retention indices (RI) with those from the literature [27] [28] or with those of authentic compounds obtained from Sigma-Aldrich (Lebanon and France). Retention indices were determined relative to a homologous series of *n*-alkanes (C8 to C24) analysed under the same operating conditions. Further identification was obtained by comparing mass spectra on both columns with those provided in the NIST and Wiley 275 libraries, with our homemade library constructed with pure compounds and EOs of known composition or with mass spectra from the literature [27] [29]. The relative concentration of each component was calculated based on the GC peak area without any correction factor and is reported in Table 1.

Statistical analysis. An agglomerative hierarchical-clustering graph was constructed with XLSTAT 2013.4.08 based on Euclidean distance with Ward's aggregation method [30].

Antimicrobial activity. The Gram (–) bacterial strain *Pseudomonas aeruginosa* CIP 82118, the Gram (+) bacterial strains *Staphylococcus aureus* ATCC 29213 and *Staphylococcus aureus* ATCC 33591 (Methicillin Resistant *Staphylococcus aureus*), the yeast *Candida albicans* ATCC 10231 and clinical isolates of dermatophytic fungi, including *Trichophyton rubrum* SNB-TR1, *Tricophyton mentagrophytes* SNB-TM1, *Tricophyton soudanense* SNB-TS1, *Tricophyton violaceum* SNB-TV1 and *Tricophyton*

tonsurans SNB-TT1 [31], were the microorganisms tested for EO sensitivity in this study.

EO antimicrobial activity was measured using a broth microdilution method according to guidelines from the Clinical and Laboratory Standards Institute (CLSI) [32-36]. Essential oils and nerolidol were diluted in DMSO and were tested at concentrations ranging from 512 to 16 μg/ml. Microplates were incubated at 37 °C for 24 h for bacteria, 48 h for yeasts and 5 days for dermatophytes. The minimum inhibitory concentration (MIC) refers to the lowest concentration that prevented visible microbial growth (Table 3). Oxacillin, gentamicin and vancomycin (16 – 0.03 μg/ml) were used as reference antibiotics. Itraconazole (16 – 0.03 μg/ml) and fluconazole (64 – 0.125 μg/ml) were used as positive controls for the antifungal assays. Antimicrobial standards were purchased from Molekula – Dorset, UK. Nerolidol was purchased from Sigma-Aldrich, France.

REFERENCES

[1] G. M. Rossolini, F. Arena, P. Pecile, S. Pollini, *Curr. Opin. Pharmacol.* **2014,** *18,* 56.

[2] B. Ali, N. A. Al-Wabel, S. Shams, A. Ahamad, S. A. Khan, F. Anwar, *Asian Pac. J. Trop. Biomed.* **2015,** *5,* 601.

[3] W. T. Langeveld, E. J. A. Veldhuizen, S. A. Burt, *Crit. Rev. Microbiol.* **2014**, *40*, 76.

[4] M. El Beyrouthy, Ph.D. Thesis, Université Droit et Santé – Lille 2, France, 2008.

[5] U. Çakilcioğlu, M. T. Sengün, I. Türkoğlu, *J. Med. Plants Res.* **2010**, *4*, 567.

[6] F. Tetik, S. Civelek, U. Cakilcioglu, *J. Ethnopharmacol.* **2013**, *146*, 331.

[7] K. Javidnia, R. Miri, M. Soltani, M. Gholami, A. R. Khosravi, *Chem. Nat. Compd.* **2008**, *44*, 654.

[8] H. Amiri, *J. Med. Plants* **2012** , *41*, 111.

[9] L. Ahmadia, M. Mirza, *J. Essent. Oil Res.* **1999**, *11*, 289.

[10] E. Mancini, N. Apostolides-Arnold, L. De Martino, V. De Feo, C. Formisano, D. Rigano, F. Senatore, *Molecules* **2009**, *14*, 4725.

[11] F. Senatore, N. Apostolides-Arnold, F. Piozzic, *J. Chromatogr. A* **2004**, *1052*, 237.

[12] A. Azizi, A. M. Azizi, G. Azizi, *J. Plant Sci. Res.* **2009**, *15*, 39.

[13] M. Altuna, M. Ünalb, T. Kocagözc, A. C. Görend, *J. Essent. Oil Bear. Pl.* **2007**, *10*, 251.

[14] K. Morteza-Semnani, K. Moshirt, M. Akbarzadeh, *J. Essent. Oil Bear. Pl.* **2005**, *8*, 6.

[15] E. Bagci, A. Kocak, *Int. J. Sci. Technology* **2008**, *3*, 13.

[16] M. Mohammadhosseini, A. Pazoki, H. Akhlaghi, *Chem. Nat. Compd.* **2008**, *44*, 127.

[17] B. Tepea, E. Donmeza, M. Unlub, F. Candanc, D. Dafererad, G. Vardar-Unlub, M. Polissioud, A. Sokmena, *Food Chem.* **2004**, *84*, 519.

[18] M. Yousefzadia, A. Sonbolib, F. Karimic, S. N. Ebrahimid, B. Asgharid, A. Zeinalia, *Z. Naturforsch.* **2007,** *62 c*, 514.

[19] M. Paknejadi, F. Foroohi, M. Yousefzadi, *J. Paramed. Sci*. **2012**, *3*, 12.

[20] A. Rustaiyan, S. Masoudi, A. Monfared, H. Komeilizadeh, *Flavour Fragr. J.* **1999**, *14*, 276.

[21] J. L. Rios, M. C. Recio, *J. Ethnopharmacol*. **2005**, *100*, 80.

[22] E. Houel, A. M. S. Rodrigues, A. Jahn-Oyac, J.-M. Bessiere, V. Eparvier, E. Deharo, D. Stien, *J. Appl. Microbiol.* **2013,** *116*, 288.

[23] H. Skaltsa, D. Lazari, A. Mavromati, E. Tiligada, T. Constantinidis, *Planta Med*.**2000**, *66*, 672.

[24] M. J. Park , K. S. Gwak , I. Yang , K. W. Kim , E. B. Jeung , J. W. Chang , I. G. Choi, *Fitoterapia* **2009**, *80*, 290.

[25] P. Mouterde, 'Nouvelle flore du Liban et de la Syrie', Tome III, Dar El Mashreq, Liban, 1970.

[26] 'European Pharmacopoeia', 3rd edition, Council of Europe, Strasbourg, 1997.

[27] W. Jennings, T. Shibamoto, 'Qualitative analysis of flavour and fragrance volatiles by glass capillary gas chromatography', Academic Press, New York, 1980.

[28] N. W. Davies, *J. Chromatogr. A* **1990**, *503*, 1.

[29] R. P. Adams, 'Identification of essential oil components by gas chromatography/Mass spectroscopy' Allured Publishing Corporation, Carol Stream, IL, 2007.

[30] M. Khoury, D. Stien, N. Ouaini, V. Eparvier, N.A. Apostolides, M. El Beyrouthy, *Chem. Biodivers.* **2014**, *11*, 1990.

[31] C. Nirma, V. Eparvier, D. Stien, *J. Nat. Prod*. **2015**, *78*, 159.

[32] Clinical and Laboratory Standards Institute. ‘Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, Approved standard’, 8th Ed. Document M7-A8, CLSI, Wayne, PA, USA, 2009.

[33] Clinical and Laboratory Standards Institute. ‘Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; Approved Standard’, 2nd Ed. Document M38-A2, CLSI, Wayne, PA, USA, 2008.

[34] Clinical and Laboratory Standards Institute. ‘Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved Standard’, 3rd Ed. Document M27-A3, CLSI, Wayne, PA, USA, 2008.

[35] M. Khoury, M. El-Beyrouthy, N. Ouaini, M. Iriti, V. Eparvier, D. Stien., *Chem. Biodivers.* **2014**, *11*, 825.

[36] A. M. S. Rodrigues, P. N. E. T. Theodoro, C. Basset, M. R. R. Silva, J. Beauchêne, L. S. Espindola, D. Stien, *J. Nat. Prod.* **2010**, *73*, 1706.

Table 1. *Chemical Composition of* Salvia multicaulis *Essential Oils*

			Compounds (%)	
RI[a]	**RI[b]**	**Compounds**	**Zahle EO**	**Bechwet EO**
938	1076	α-Pinene	4.9	16.3
953	1076	Camphene	1.0	6.1
980	1118	β-Pinene	1.1	3.5
993	1174	Myrcene	0.5	1.3
1025	1280	*p*-Cymene	0.6	0.6
1034	1213	Eucalyptol	6.5	16.2
1145	1532	Camphor	2.4	6.7
1165	1587	Pinocarvone	0.3	0.3
1167	1719	Borneol	0.9	0.8
1176	1611	Terpinen-4-ol	1.1	0.7
1193	1648	Myrtenal	0.9	0.8
1196	1804	Myrtenol	3.5	6.8
1285	1597	Bornyl acetate	0.4	0.9
1297	1638	Sabinyl acetate	0.9	4.0
1377	1497	α-Copaene	1.3	1.2
1385	1533	β-Bourbonene	0.7	0.2
1415	1612	β-Caryophyllene	2.8	1.6
1437	1628	Aromadendrene	1.4	0.9
1452	1668	α-Humulene	1.8	-
1455	1689	β-Farnesene	1.1	0.3
1463	1661	Alloaromadendrene	0.7	0.6
1478	1704	γ-Muurolene	1.8	0.5
1483	1784	α-Curcumene	-	2.5
1485	1711	β-Selinene	0.6	-
1495	1740	Valencene	1.6	0.5
1500	1740	α-Muurolene	1.2	0.4
1508	1741	β-Bisabolene	1.0	0.4
1515	1776	γ-Cadinene	2.5	1.3
1525	1772	δ-Cadinene	4.6	1.7
1526	1773	α-Cadinene	0.7	0.1
1541	1918	α-Calacorene	0.8	0.2
1566	2050	*trans*-Nerolidol	12.3	-
1577	2008	Spathulenol	4.0	1.5
1580	2152	Caryophyllene oxide	1.1	0.2
1585	2098	Globulol	0.8	0.5
1591	2104	Viridiflorol	1.4	0.1
1629	2025	*epi*-Globulol	1.2	0.5
1640	2188	*T*-Cadinol	3.4	1.3
1645	2145	Torreyol	0.7	0.4
1649	2188	α-Eudesmol	1.1	1.0
1650	2256	α-Cadinol	2.0	1.0
1668	2020	β-Bisabolol	-	2.1
1677	2256	Cadalene	-	0.3
1684	2152	Valeranone	6.1	-
1688	2229	α-Bisabolol	-	0.6
		Total identified	83.7	86.9

[a])[b]) RI[a] and RI[b] are the retention indices determined relative to a series of n-alkanes (C8-C24) on the apolar DB-5 MS and the polar HP Innowax capillary columns, respectively ; -, not detected.

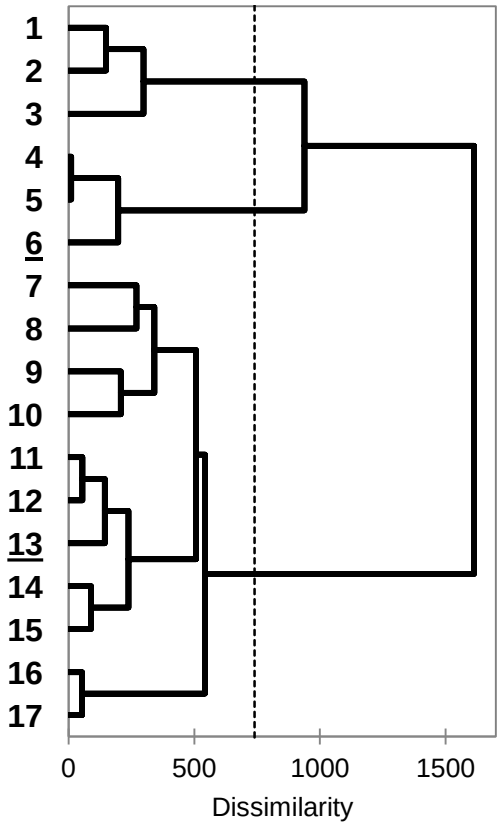

Fig. 1. *Degree of dissimilarity of the chemical compositions of seventeen* S. multicaulis *EOs (Table 2, Entries 1–17). The dendrogram was obtained by agglomerative hierarchical clustering based on Euclidian distances with Ward's aggregation method. For the composition, country of origin and literature source of each EO, cf. Table 2*

Table 2. *Literature Survey of the Chemical Diversity and Chemotypes of* S. multicaulis *Essential Oils*

Entry	Country of collect[a]	Eucalyptol	*α*-Pinene	*β*-Caryophyllene	Borneol	Camphene	Camphor	Bornyl acetate	*β*-Pinene	*trans*-Nerolidol	Valeranone	*α*–Eudesmol	Myrtenol	*trans*-Sabinyl acetate	*α*-Copaene	Aromadendrene	*δ*-Cadinene	*α*-Terpineol	*α*-Ylangene	Limonene	Spathulenol	*p*-Cymene	Caryophyllene oxide	Sabinene	*cis*-Linalool oxide	References
1	I	6.9	8.3	8.5	12	7.8	14	7.3	3.5	-	-	-	0.2	-	-	0.4	0.9	0.5	0.1	-	-	4.4	6.2	-	-	[7]
2	I	2.1	4.8	5.9	17	5.6	14	19	0.6	-	0.4	-	4.8	-	1.2	0.9	0.6	0.5	-	-	0.8	-	0.9	-	-	[8]
3	I	8.4	16	17	6.5	5.1	5	18	2.4	-	-	-	-	-	0.3	-	0.4	-	-	8.3	0.4	0.4	2.1	0.2	-	[9]
4	L	3.1	5.5	4.4	1.2	0.9	0.8	0.5	0.9	1.4	-	-	4.6	4.6	6.6	3.9	3	-	0.3	0.3	0.7	2.3	0.8	0.4	-	[10]
5	L	3.8	6.6	4	1.9	1.1	0.5	0.4	0.7	1.2	-	-	5.7	5.3	8	4.6	2	-	0.8	0.8	-	0.1	-	0.2	-	[11]
6	L	6.5	4.9	2.8	0.9	1	2.4	0.4	1.1	12.3	6.1	1.1	3.5	0.9	1.3	1.4	4.6	-	-	-	4	0.6	1.1	-	-	Our
7	I	25	8.5	-	-	1.8	18	0.7	4.4	-	-	-	-	-	-	-	-	7.1	-	-	-	-	-	8.6	8	[12]
8	T	26	1.8	1	-	14	13	6.6	4.2	-	-	-	-	-	-	-	-	-	-	8.1	-	1.1	0.7	-	-	[13]
9	I	11	7.5	-	8.6	4.7	11	3.6	3	-	-	-	-	-	-	-	4.8	3.7	4.5	-	-	-	-	-	-	[14]
10	T	17	9.3	4.3	1.5	3.5	13	-	-	-	8.5	5.7	-	-	-	0.2	0.2	-	-	-	0.6	-	-	0.4	-	[15]
11	I	25	18	5.8	1.9	8.5	12	7.9	4.6	-	-	-	-	-	0.2	-	-	-	0.5	-	-	-	0.2	0.3	-	[16]
12	T	20	22	4.2	7.3	7.8	11	3.3	4.7	0.2	0.9	0.5	2.2	-	0.2	0.5	0.2	0.1	-	-	0.4	-	0.2	-	-	[17]
13	L	16	16	1.6	0.8	6.1	6.7	0.9	3.6	-	-	1	6.9	4	1.2	0.9	1.8	-	-	-	1.5	0.6	0.2	-	-	Our
14	I	21	17	8.9	8.3	7.5																				[18]
15	I	17	12	8.9	2.7	7.5	-	-	6.6	-	-	-	0.6	-	0.7	-	-	-	-	-	2.3	3.7	1.8	3	-	[19]
16	I	20	26	1.9	4.7	12	19	1.2	3.5	-	-	-	-	-	0.2	-	0.5	2.2	-		-	-	-	-	-	[20]
17	I	25	21	-	5	13	24	5.8	3	-	-	-	-	-	-	-	-	1.3	-	-	-	-	-	-	-	[16]

[a]) I: Iran; L: Lebanon; T: Turkey

Table 3. *Antimicrobial Activity (MIC in µg/ml) of* S. multicaulis *Essential Oils and Nerolidol*

	Zahle EO	Bechwet EO	Nerolidol	Positive control
S. aureus (ATCC 29213)	128	>512	128	1[a]
M. R. *S. aureus* (ATCC 33591)	128	>512	128	1[b]
C. albicans (ATCC 10231)	512	>512	-	1[c]
P. aeruginosa (CIP 82118)	>512	>512	-	0.25[d]
T. rubrum (SNB-TR1)	64	256	64	2[c]
T. mentagrophytes (SNB-TM1)	64	256	-	1[e]
T. soudanense (SNB-TS1)	32	64	-	2[c]
T. violaceum (SNB-TV1)	8	256	-	2[c]
T. tonsurans (SNB-TT1)	64	128	-	4[c]

[a]) Oxacillin; [b]) Vancomycin; [c]) Fluconazole; [d]) Gentamicin; [e]) Itraconazole.