

RESEARCH ARTICLE

Essential oil composition of two Greek cultivated *Sideritis* spp.

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Abstract

In this present work, essential oil composition of cultivated commercially available *Sideritis raeseri* Boiss. & Heldr. and *Sideritis scardica* Griseb. from three different restricted areas of Kozani (central Greece) were studied. The essential oils were obtained by hydrodistillation in a modified Clevenger-type apparatus, and their analyses were performed by GC-MS. Monoterpene hydrocarbons constituted the main fraction of *S. raeseri* from Polirraxo (Kozani) and of *S. scardica* from Chromio (Kozani), while sesquiterpene hydrocarbons were the main group of *S. raeseri* from Metamorfosis (Kozani) and *S. scardica* from Metamorfosis (Kozani). Despite the different cultivations, some constituents were found even in different percentages in both samples of *S. scardica*: α -pinene, β -pinene, cis-caryophyllene, bicyclogermacrene and germacrene D. It is noteworthy that the two samples of *S. raeseri* have totally different main constituents, which could be related to the different cultivation conditions, as well as to the known tendency of some *Sideritis* species to hybridize, which suggests further research.

Keywords: *Sideritis raeseri*, *Sideritis scardica*, cultivation, composition of volatiles

Introduction

Sideritis L. (Lamiaceae) comprises more than 150 species, indigenous in Central Europe, the Mediterranean, the Balkans, the Iberian Peninsula and the west Asia (González-Burgos et al., 2011). Traditionally, the infusion of herba *Sideritis* (*S. scardica*, *S. clandestina*, *S. raeseri*, *S. syriaca*) has been used against the common cold, mild gastrointestinal disorders and for cough relief (EMA, 2015). Essential oils (EOs) from the genus *Sideritis* L. are widely used in pharmaceutical herbal products, food additives and as important sources in aromatic and cosmetic industries. Numerous studies over the past years have elucidated a plethora of significant biological activities of the *Sideritis* EOs, such as anti-inflammatory, antioxidant, antimicrobial, antifungal and anti-ulcerative (González-Burgos et al., 2011). Intriguingly, the analyses of the essential oils of *Sideritis* spp. have been the subject of several studies and show significant diversity in their chemical compositions, mainly due to hybridism. Therefore, it seemed interesting to investigate the volatile constituents of this genus.

The overall aim of this study was to examine two cultivated *Sideritis* species, originated from different restricted locations of Kozani (Metamorfosis, Polirraxo and Chromio), focusing on determining the effects of organic cultivations on essential oil yield and composition. The chemical analyses of the essential oils of each species were compared with the previously stated in the literature.

Materials and Methods

Plant material

Aerial parts of four *Sideritis* different organic cultivated populations, commercially available, were provided as follows: *S. raeseri*, one sample from Metamorfosis Kozani (SR_M) and another from Polirraxo Kozani (SR_P); *S. scardica*, one sample from Metamorfosis Kozani (SS_M) and a second one from Chromio Kozani (SS_X). The plants were identified by Associate Prof. Th. Constantinidis (Faculty of Biology, National and

Kapodistrian University of Athens). Voucher specimens have been deposited in the Department of Pharmacognosy and Chemistry of Natural Products (Faculty of Pharmacy, NKUA) under the following codes: ATHU Skaltsa & Tomou 002A & 002B for SR_M and SR_P, respectively; ATHU Skaltsa & Tomou 003A & 003B for SS_M and SS_X, respectively.

Extraction and chemical composition of essential oils

25.0 g of air-dried aerial parts of each plant material were cut in small pieces, and the essential oil was obtained by hydrodistillation for 1.5 h in a modified Clevenger apparatus with a water-cooled oil receiver to reduce artefacts produced during distillation by over-heating. The oils, taken in 2 mL of capillary GC grade *n*-pentane and dried over anhydrous sodium sulphate, was subsequently analysed by GC-MS and stored at -20 °C.

The composition of the volatile constituents was established by GC-MS analyses, performed on a Hewlett-Packard 6890/5973 system operating in EI mode (70eV) equipped with a split/splitless injector, using a fused silica HP-5 MS capillary column (30 m x 0.25 mm I.D., film thickness: 0.25 µm). Helium was used as a carrier gas at a flow rate of 1.0 mL/min and the oven temperature was increased from 60°C to 240°C at a rate of 10°C/min. Injection was at 280°C in a split ratio 1:5; the ion source temperature and transfer line temperature were set at 230 and 250°C, respectively. Injection volumes of each sample were 2 µL. Retention indices for all compounds were determined according to the Van den Dool approach (Van den Dool & Kratz, 1986), using *n*-alkanes as standards. The identification of the components was based on comparison of their mass spectra with those of Wiley and NBS Libraries (Massada, 1986) and those described by Adams (2007), as well as by comparison of their retention indices with literature data (Adams, 2007). In many cases, the essential oils were subjected to co-chromatography with authentic compounds (Fluka, Sigma).

Results and Discussion

Sideritis raeseri Boiss. & Heldr.

The yields (v/w) of the essential oils from the aerial parts of cultivated *S. raeseri* from Metamorfofis Kozani (SR_M) and of cultivated *S. raeseri* from Polirraxo Kozani (SR_P) were 0.12% and 0.10 %, respectively. The chemical composition of the essential oil of SR_M showed 41 constituents, where the main components were germacrene D (14.8 %), 9-octadecen-1-ol (12.4%), bicyclogermacrene (11.2 %) and *cis*-caryophyllene (10.0 %) (Table 1). In contrast, the essential oil of SR_P was consisted by 57 compounds with β -pinene (19.1 %) being the major compound followed by germacrene D (15.9 %), α -pinene (12.9 %) and limonene (8.5 %) (Table 1). Furthermore, it is important to note that the two samples demonstrated a totally different chemical load. Sesquiterpene hydrocarbons (46.9 %) were the main group of SR_M, while monoterpene hydrocarbons (45.4 %) were the main fraction of SR_P (Table 3). Comparing our results with previous data (Papageorgiou et al., 1982; Koedam, 1985; Aligiannis et al., 2001 και Kostadinova et al., 2007; Pljevljakušić et al., 2011), we observed some chemical variations between the components of the essential oils. According to Koedam (1985), the key constituents of the essential oil of *S. raeseri* were the monoterpene hydrocarbons α -pinene (16.5%), β -pinene (20.61%) and limonene (6.73%). This is in accordance only with the results of our sample SR_P. In contrast, Kostadinova et al. (2007) reported the sesquiterpene hydrocarbons elemol acetate (15.9%), germacrone (25.0%) and α -cadinol (8.2%) as major components. Despite the fact that sesquiterpene hydrocarbons were the main fraction in our sample (SR_M), its chemical composition differed considerably. Moreover, Pljevljakusic et al. (2011) mentioned that the sesquiterpene fraction of cultivated *S. raeseri* EOs was the principal group in all samples with bicyclogermacrene as main compound. Our analyses revealed that also our samples are abundant in sesquiterpenes; bicyclogermacrene was found in both samples, but in lower

percentages. Furthermore, compared to the study of Aligiannis et al. (2001) *ar-curcumene* was not detected in our samples. Papageorgiou et al. (1982) has mentioned *naphthalene* (22.0%) as major volatile component, while in the present study, *naphthalene* was not detected.

***Sideritis scardica* Griseb.**

The yields (v/w) of the essential oils of the aerial parts of SS_M and SS_X were 0.18 % and 0.12 %, respectively. The chemical composition of the essential oil of SS_M showed 46 constituents, where the main components were α -pinene (8.2%), β -pinene (12.8%), *cis*-caryophyllene (6.6%), bicyclogermacrene (6.6%) and germacrene D (6.6 %) (Table 2). In contrast, the essential oil of the SS_X was consisted of 49 compounds with α -pinene (17.8%), β -pinene (13.1 %), *cis*-caryophyllene (7.6 %), bicyclogermacrene (7.1 %), *m*-camphorene (10.3 %) and germacrene D (2.2 %) being the main compounds (Table 2). It is interesting note that the two different samples of *S. scardica* presented similar chemical compositions with small differences in the quantities of the individual volatile components. Monoterpene hydrocarbons were the main fraction of SS_X (Table 3). This is in accordance with the previous studies (Kokkalou, 1987; Baser et al., 1997; Todorova et al., 2000; Konstadinova et al., 2007; Tredafilova et al., 2013; Todorova & Trendafilova, 2014). According to Todorova et al. (2013), the predominant group of EOs of cultivated *S. scardica* could be mono- or sesquiterpene hydrocarbons which is related to the environmental conditions such as altitude. In sample SS_M, sesquiterpene hydrocarbons were the major fraction of the EO (Table 3). This difference between the major groups of the two samples could be attributed to the different altitude of the two locations. A distinguishing feature between the two samples was the presence of *m*-camphorene in the essential oil of SS_X. It is noteworthy to be mentioned that only in this sample, diterpene hydrocarbons were observed in a considerable amount (11.2 %). Moreover, *m*-camphorene has been previously reported in the essential oil of this species from Bulgaria (Tredafilova et al., 2013). Comparing our results with the literature (Kokkalou, 1987; Baser et al., 1997; Tredafilova et al., 2013), we found a significant uniformity. Todorova et al. (2000) noticed the presence of β -caryophyllene (18.8 %) and nerolidol (12.1 %) in the essential oil of *S. scardica* from Bulgaria. In our study, we found β -caryophyllene in lower percentages, while we didn't identify nerolidol. The research by Kostadinova et al. (2007) supports the presence of α -cadinol and octadecenol as major components of the essential oil of *S. scardica* originated from south Bulgaria and North Macedonia, which were not identified in our samples.

The present study revealed the chemical variations in comparison to the chemical profile of *S. raeseri* and *S. scardica* previously investigated. In the EOs of Greek *Sideritis* species, monoterpenes are the dominant components (Aligiannis et al., 2001; González-Burgos et al., 2011). In the present study, the samples were rich in monoterpenes or sesquiterpenes. Kirimer et al. (2000) indicated a classification of the Turkish *Sideritis* species based on the main components of their essential oils. Regarding to this study, our samples could be categorised as follows: the EOs of SR_P and SS_X are monoterpene-rich oils, while the EO of SR_M and SS_M are sesquiterpene-rich oils. Considering the yields and the chemical variations of our samples, we support that many factors such as hydrodistillation and location could influence the amount of the oil (Pljevljakušić et al., 2011, Todorova et al., 2013; Todorova & Trendafilova, 2014). Taking into consideration the small different altitudes of each location (Polliraxo-Kozani: 567m; Chromio-Kozani: 640m and Metamorfozi-Kozani: 700m), we observed that the monoterpene-rich samples SR_P and SS_X were both located at 567-640 m a.s.l. On the other hand, the sesquiterpene-rich samples SR_M and SS_M were originated from the same location at 700 m a.s.l.. Different distillation methods, as well as various cultivation methods could increase the yield of the essential oil of this high valuable species. Thus, we assume that the aforementioned factors

(altitude, cultivation methods and distillation methods) have played an important role about the chemical variations of our samples.

The scope of this study was to analyse samples of two Greek cultivated species of *Sideritis*, indicating the significant differences in the chemical composition of these essential oils even if the plants are originated from cultivations. In this present study it was underscored also that the EOs of *Sideritis raeseri* showed totally different chemical constituents compared to the already studied Greek *Sideritis raeseri*, therefore more detailed studies should be conducted to examine and clarify its high tendency of hybridism with other species.

Table 1. Chemical composition of the essential oils from samples SR_M and SR_P.

Compound	KI	RI ^a	Composition (%)	
			SR_M	SR_P
α -thujene	924	924	-	1.1
α -pinene	932	930	-	12.9
allylbenzene	934	932	-	0.3
camphene	946	945	-	0.2
β -pinene	974	970	-	19.1
myrcene	988	982	-	1.2
α -phellandrene	1002	998	-	0.4
α -terpinene	1014	1009	-	0.1
<i>p</i> -cymene	1020	1016	-	0.1
limonene	1024	1021	-	8.5
<i>cis</i> -ocimene	1032	1028	-	0.4
γ -terpinene	1054	1048	-	0.2
octanol	1063	1064	-	0.3
terpinolene	1086	1078	-	0.9
linalool	1095	1091	-	0.2
nonanal	1100	1095	-	0.6
α -campholenal	1122	1116	-	0.2
<i>trans</i> -pinocarveol	1135	1127	-	0.2
<i>trans</i> -verbenol	1140	1133	-	0.2
pinocarvone	1160	1150	-	0.4
terpinen-4-ol	1162	1165	-	0.2
α -terpineol	1186	1180	-	0.1
myrtenal	1195	1193	-	0.7
bornyl acetate	1284	1282	-	0.5
δ -elemene	1335	1335	-	0.1
bicycloelemene	1336	1335	0.2	-
eugenol	1356	1354	-	0.1
α -copaene	1374	1372	0.4	1.3
β -bourbonene	1387	1385	0.3	0.5
β -cubebene	1387	1385	-	0.1
β -elemene	1389	1382	0.5	0.5
benzyl isovalerate	1396	1390	-	0.2
<i>cis</i> -caryophyllene	1408	1401	10.0	-

<i>trans</i> - β -caryophyllene	1417	1412	-	3.8
β -copaene	1428	1428	-	0.1
1-butanol, 3-methyl-benzoate	1430	1427	-	0.3
β -humulene	1433	1433	-	0.2
α -humulene	1452	1450	0.3	-
neryl acetone	1453	1451	0.3	-
<i>trans</i> - β -farnesene	1454	1454	6.0	2.4
dodecanal	1465	1463	0.3	-
germacrene D	1484	1484	14.8	15.9
β -ionone	1487	1486	0.6	0.2
bicyclogermacrene	1500	1499	11.2	4.6
β -bisabolene	1505	1500	1.5	0.9
germacrene A	1508	1506	-	0.2
2,4-bis (1,1-dimethylethyl)phenol	1513	1512	1.4	-
phenol, 2,5-bis(1,1-dimethylethyl)	1517	1515	-	0.3
δ -cadinene	1522	1520	1.7	2.3
spathulenol	1577	1575	4.1	1.0
hexyl benzoate	1579	1579	-	0.2
caryophyllene oxide	1582	1580	4.5	0.8
viridiflorol	1592	1591	0.2	0.1
hexadecane	1600	1500	0.4	-
t-cadinol	1632	1629	-	0.2
α -cadinol	1638	1636	-	0.2
isopathulenol	1639	1635	0.2	-
valeranone	1656	1656	-	0.2
6(<i>Z</i>), 9(<i>E</i>)-heptadecadiene	1668	1666	0.3	-
α -bisabolol	1685	1680	1.6	-
pentadecanal	1711	1708	1.7	0.2
benzyl benzoate	1759	1755	2.3	5.6
4-methyl phenyl ester octanoic acid	1777	1769	0.8	-
octadecane	1800	1796	0.3	-
hexadecanal	1822	1819	0.3	-
6,10,14-trimethyl-2-pentadecanone	1847	1842	1.7	0.1
heptadecanal	1920	1915	-	0.2
ent-pimara-8(14),15-diene	1939	1932	0.7	-
(3 <i>Z</i>)-cembrene A	1965	1958	-	2.6
kaur-15-ene	1997	1990	0.8	0.1
<i>n</i> -eicosane	2000	1995	0.4	-
13-epi-manoyl oxide	2009	2001	0.4	-
9-octadecen-1-ol	2077	2074	12.4	1.5
heneicosane	2100	2095	0.6	-
phytol	2111	2108	0.6	-
linoleic acid	2132	2129	0.2	-

iso-dextro-pimaraldehyde	2184	2181	1.4	-
docosane	2200	2197	0.3	-
tricosane	2300	2298	1.1	-
tetracosane	2400	2398	0.3	-
pentacosane	2500	2499	0.9	-
TOTAL			88.0	96.0

^aRetention Index calculated against C₉-C₂₄ *n*-alkanes on the HP 5MS column capillary column; SR_M: *S. raeseri* from Metamorfozis Kozani; SR_P: *S. raeseri* from Polirraxo Kozani

Table 2. Chemical composition of the essential oils from samples SS_M and SS_X.

Compound	KI	RI ^a	Composition (%)	
			SS_M	SS_X
α-thujene	924	924	0.4	0.2
α-pinene	932	930	8.2	17.8
verbenene	961	960	-	0.2
β-pinene	974	970	12.8	13.1
myrcene	988	982	0.6	0.7
α-phellandrene	1002	997	0.5	-
δ-3-carene	1002	1003	0.5	-
<i>p</i> -cymene	1020	1016	0.4	0.1
limonene	1024	1019	3.4	2.7
<i>trans</i> -β-ocimene	1032	1028	-	2.3
<i>cis</i> -β-ocimene	1044	1038	0.4	0.4
γ-terpinene	1054	1048	0.2	1.2
α-terpinolene	1086	1082	0.3	0.3
linalool	1095	1090	0.4	0.3
nonanal	1100	1095	0.8	0.7
α-campholenal	1122	1115	0.2	0.7
<i>trans</i> -pinocarveol	1135	1132	0.5	0.3
<i>trans</i> verbenol	1140	1138	0.3	0.4
pinocarvone	1160	1160	0.5	0.7
myrtenal	1195	1189	0.6	1.0
bornyl acetate	1284	1284	0.2	0.2
δ-elemene	1335	1333	-	0.2
eugenol	1356	1353	-	0.3
α-copaene	1374	1368	2.2	1.1
β-bourbonene	1387	1384	0.6	0.4
β-elemene	1389	1385	0.4	-
<i>cis</i> -β-caryophyllene	1408	1401	7.9	7.6
α-humulene	1452	1448	0.2	0.3
geranyl acetone	1453	1450	-	0.2
<i>trans</i> -β-farnesene	1454	1456	-	1.6
<i>cis</i> -β-farnesene	1440	1440	1.5	-
germacrene D	1484	1480	7.7	2.9
β-ionone	1487	1485	0.3	0.5
bicyclogermacrene	1500	1499	6.6	7.1

β -bisabolene	1505	1501	1.1	0.8
γ -cadinene	1513	1513	0.7	-
δ -cadinene	1522	1524	4.0	1.9
spathulenol	1577	1575	2.1	2.7
caryophyllene oxide	1582	1580	2.4	3.8
viridiflorol	1592	1590	1.4	0.4
hexadecane	1600	1600	-	1.0
<i>t</i> -cadinol	1632	1630	-	0.6
<i>t</i> -muurolol	1644	1644	4.2	
tetradecanol	1671	1668	-	1.9
valeranone	1674	1674	0.9	-
α -bisabolol	1685	1685	1.7	0.5
heptadecane	1700	1700	-	0.4
pentadecanal	1711	1709	0.5	1.2
benzyl benzoate	1759	1756	1.5	2.3
6,10,14-trimethyl-2-pentadecanone	1845	1842	-	0.6
6,10,14-trimethyl-pentan-2-one	1847	1842	0.9	-
ent-pimara-8(14),15-diene	1948	1942	0.4	0.4
m-camphorene	1944	1949	-	10.3
kaur-15-ene	1997	1995	0.8	0.5
13-epi-manoyl oxide	2009	2001	0.2	-
9-octadecen-1-ol	2077	2077	3.0	0.3
heneicosane	2100	2100	0.3	-
tricosane	2300	2299	0.4	-
TOTAL			85.1	95.9

^aRetention Index calculated against C9-C24 n-alkanes on the HP 5MS column capillary column; SS_M: *S. scardica* from Metamorfofis Kozani; SS_X: *S. scardica* from Chromio Kozani

Table 3. Grouped Components (% v/v) of each sample.

Group Components	SR_M	SR_P	SS_M	SS_X
Monoterpene hydrocarbons	-	45.4	27.7	39.0
Oxygenated monoterpenes	-	3.4	3.5	4.6
Sesquiterpene hydrocarbons	46.9	32.9	32.9	23.9
Oxygenated sesquiterpenes	11.5	2.7	13.0	8.7
Diterpenes	1.9	2.7	1.4	11.2
Oxygenated diterpenes	0.6	-	-	-
Hydrocarbons	4.6	-	0.7	2.2
Oxygenated hydrocarbons	22.5	8.9	5.9	6.3

SR_M: *S. raeseri* from Metamorfofis Kozani; SR_P: *S. raeseri* from Polirraxo Kozani;
SS_M: *S. scardica* from Metamorfofis Kozani; SS_X: *S. scardica* from Chromio Kozani

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