

Volatile aroma compounds in infusions of stems and rosette leaves of *Sideritis raeseri* Boiss. & Heldr. from R. Macedonia, Albania and Greece

Bujar Qazimi*, Gjoshe Stefkov, Marija Karapandzova, Ivana Cvetkovikj, Svetlana Kulevanova

Institute of Pharmacognosy, Faculty of Pharmacy, University SS Cyril and Methodius, Skopje, Republic of Macedonia

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Abstract

The composition of the volatile aroma components was defined in the infusion prepared by stems of *Sideritis raeseri* (SR-S) from R. Macedonia, Albania and Greece and in the infusion of rosette leaves of *S. raeseri* (SR-R) from R. Macedonia. Analysis was made by gas chromatography (GC/FID/MS) equipped with a headspace (HS) sampler. Twenty components (12 monoterpenes representing 76.70-94.84% and 8 sesquiterpenes representing 5.16-18.55% of the entire volatiles) were identified as aroma components in SR-S samples. The predominant components in all samples were β -pinene, α -pinene and *trans*-caryophyllene. The high abundance of limonene and δ -3-carene were characteristic for the samples from Greece and 1,8-cineole was for the samples from R. Macedonia and Albania. In the infusions of SR-R, ten components were identified, 5 monoterpenes (73.20-83.89%) and 5 sesquiterpenes (16.10-26.80%). Prevailing components in all tested samples of SR-R were β -pinene, α -pinene, 1,8-cineole and *α-epi*-murolool. There was almost no difference in the chemical profiles of the aroma compounds between SR-S and SR-R. Infusion of rosette leaves exhibit very similar aroma compounds profile with the infusion of stems of *S. raeseri*.

Keywords: *Sideritis raeseri*, infusion, volatile aroma components, Headspace, GC/MS.

Introduction

Sideritis raeseri Boiss. & Heldr. is endemic to the Balkan Peninsula and is reported to grow in Greece, R. Macedonia and Albania. Mountain tea is a traditional beverage in the Balkan countries, prepared as a refreshing herbal tea. Aerial parts of this plant are widely utilized in Mediterranean folk medicine in the form of a decoction or infusion. This herb is often used to treat the common cold, to alleviate sinus congestion, pains and virus infections, including influenza (Heywood, 1972; Obon de Castro, 1994; Gabri-

eli et al., 2005; Bojović et al., 2011). Rosette leaves traditionally is not used.

Various extracts and essential oils of *S. raeseri* have shown different types of activities as: antioxidant (Gabrieli et al., 2005; Koleva et al., 2003; Pljevljakusić et al., 2011; Karapandzova et al., 2013; Petreska et al., 2011b), antibacterial (Kostadinova et al., 2008; Menković et al., 2010), spasmolytic (Branković et al., 2011), hypotensive, vasorelaxant and cardiodepressant (Kitić et al., 2012). These activities are due to the presence of flavonoids, phenolic acids, terpenes, iridoids, coumarins, lignans, sterols, phenylpropanoids (Kostadinova et al., 2008; Pljevljakusić et al., 2011; Karapandzova et al., 2013; Janeska et al., 2007; Qazimi et al., 2010; Fraga B.M., 2012; Petreska et al., 2011a;

* bqazimi2003@yahoo.com

Petreska et al., 2011b; Alipieva et al., 2010), and a complex mineral composition (Pljevljakusić et al., 2011; Spaseska et al., 2011; Karapandzova et al., 2013).

However, the specific and particular aroma is maybe the most important reason for the wide use of the tea by the Balkan peoples. Although numerous data have been published about the chemical composition of the essential oils (Kostadinova et al., 2008; Pljevljakusić et al., 2011; Petreska et al., 2011b; Menković et al., 2013; Tzakou, 2002; Galati et al., 1996) and aroma components in the fresh and dried aerial parts of *S. raeseri* (Qazimi et al., 2014), there are no data about the volatile aroma compounds in the infusion of *S. raeseri*. To analyze these compounds a refined method of headspace sampling hyphenated with GC/FID/MS analysis can be utilized (Watson, 2005).

Taking into account all the considerations mentioned above, the aim of this work was the determination of the volatile aroma compounds in the infusion of dried stems and infusion of rosette leaves of mountain tea (*S. raeseri*) using a headspace (HS) method with GC/FID/MS.

Material and methods

Plant material

The aerial parts (stems) (SR-S) of the flowering plants (20–25 cm from the top) of *S. raeseri* were collected in different localities in National Park Galichica (R. Macedonia), Albania and Greece and rosette leaves (SR-R) of *S. raeseri* were collected in different localities in National Park Galichica (R. Macedonia) during the summer of 2012 (Table 1). The plant material was air dried, packed in paper bags and kept in a dark and cold place until analysis. Plant identity was verified and voucher specimens were deposited at the Institute of Pharmacognosy, Faculty of Pharmacy, Skopje, R. Macedonia.

Preparation of the infusion

Infusions were made from 1 g plant material covered with 100 ml boiling water and left for 5 minutes in a closed vessel, stirring and filtered.

GC and GC-MS analyses

5 ml of infusion of the stems or infusion of the rosette leaves was put in sealed vials, warmth for 5 minutes and the gas phase (highly volatile compounds) was investigated on Agilent 7890A Gas Chromatography system equipped with flame ionization detector (FID) and Agilent 5975C Mass Quadrupole detector as well as capillary flow technology which enable simultaneous analysis of the sample on both detectors. HP-5ms (30 m x 0.25 mm, film thickness 0.25 µm) capillary column was used. Operating conditions were as follows: oven temperature 60 °C, 20 °C/min to 280 °C; helium as carrier gas at a flow rate of 1 mL/min; injector temperature 260 °C and FID temperature 270 °C. 1000 µL of gas phase was injected at split ratio 1:1. The mass spectrometry conditions were: ionization voltage 70 eV, ion source temperature 230 °C, transfer line temperature 280 °C and mass range from 50-500 Da. The MS was operated in scan mode.

Head Space method

Incubation temperature was 80 °C, incubation time 5 min, syringe temperature 85 °C and agitator speed 500 rpm.

Identification and quantification of the components

Identification of the components was made by comparing mass spectra of components with those from Nist, Wiley and Adams mass spectra libraries, by AMDIS (Automated Mass Spectral Deconvolution and Identification

Table 1. Specimens of *S. raeseri*

Species	Locality	Voucher specimen	Symbol
<i>S. raeseri</i> -stems	R.M., Galichica, Baba	S1/12	SR-S (1)
	R.M., Galichica, Kazani	S2/12	SR-S (2)
	R.M., Galichica, Korito	S3/12	SR-S (3)
	R.G., Lefkada	S4/12	SR-S (4)
	R.A., Tepelena	S5/12	SR-S (5)
	R.A., Gramoz	S6/12	SR-S (6)
	R.G., Parga	S7/12	SR-S (7)
<i>S. raeseri</i> -rosette leaves	R.M., Galichica, Baba	S1/R-12	SR-R (1)
	R.M., Galichica, Kazani	S2/R-12	SR-R (2)
	R.M., Galichica, Korito	S3/R-12	SR-R (3)

R.M. – R. Macedonia, R.G. – R. Greece, R.A. – R. Albania

System) and by comparing literature and estimated Kovat's (retention) indices that were determined using mixture of homologous series of normal alkanes from C₉ to C₂₅ in hexane, under the same above mentioned conditions.

The percentage ratio of the components was computed by the normalization method of the GC/FID peak areas and average values were taken into further consideration.

Results and discussion

The chemical composition of aroma compounds in the infusion of stems of S. raeseri (SR-S)

Total of 20 individual components were identified in the infusion of stems of *S. raeseri* (SR-S), collected from three different localities in R. Macedonia, two localities in Albania and two localities in Greece, representing 92.25-100% of the total content (Table 2). Data analysis of the chemical composition revealed four different classes of components: monoterpene hydrocarbons (MH)

58.32-94.84%, oxygen containing monoterpenes (OM) up to 29.93%, sesquiterpene hydrocarbons (SH) 3.66-15.55% and oxygen containing sesquiterpenes (OS) up to 8.17% (Fig. 1).

The most abundant components in all SR-S samples were monoterpenes β -pinene (26.83-63.13%) and α -pinene (16.82-28.16%), followed by sesquiterpenes *trans*-caryophyllene (2.36-7.07%), α -copaene (1.08-4.95%) and α -*epi*-murolool (1.14-4.88%). It is important to stress the presence of 1,8-cineole (3.49-9.35%), found only in the samples from R. Macedonia and Albania. The higher abundance of limonene and δ -3-carene (3.67-18.7% and 1.06-10.63%, respectively) were specific for the samples from Greece.

The chemical composition of aroma compounds in the infusion of rosette leaves of S. raeseri (SR-R):

Total of 10 individual components were identified in the infusion of rosette leaves samples of *S. raeseri* (SR-R), collected from three different localities in R. Macedo-

Table 2. Chemical composition of volatile aroma compounds in the infusion of stems of *S. raeseri* (SR-S) (%)

No.	Components	KIL	KIE	SR-S						
				(1)	(2)	(3)	(4)	(5)	(6)	(7)
1	α -Thujene	924	933.7	-	-	-	2.41	-	-	-
2	α -Pinene	932	937.5	16.82	22.66	23.43	18.13	28.16	26.15	26.98
3	Sabinene	969	966.4	-	-	3.30	-	-	-	-
4	β -Pinene	974	973.8	41.50	58.74	48.13	26.83	62.73	60.14	63.13
5	δ -3-Carene	1008	989.7	-	-	1.27	10.63	-	-	1.06
6	Limonene	1024	1005.9	-	-	-	18.70	-	-	3.67
7	1,8- Cineole	1033	1017.2	5.49	9.55	6.26	-	3.49	3.84	-
8	α -Campholenal	1122	1102.0	2.89	-	-	-	-	-	-
9	<i>trans</i> -Pinocarveol	1135	1118.0	7.15	-	-	-	-	1.24	-
10	Pinocarvone	1160	1159.3	5.37	-	-	-	-	1.54	-
11	Myrtenal	1195	1195.2	6.25	-	-	-	-	1.08	-
12	Bornyl acetate	1284	1260.9	2.78	-	1.93	-	-	-	-
13	α -Copaene	1374	1345.9	4.75	4.95	3.24	4.46	-	1.08	2.80
14	<i>trans</i> -Caryophyllene	1417	1387.3	-	-	2.55	7.07	4.20	2.58	2.36
15	Bicyclogermacrene	1500	1501.7	-	-	-	4.02	-	-	-
16	α - Muurolene	1500	1468.0	-	-	1.72	-	-	-	-
17	Caryophyllene oxide	1582	1552.6	-	-	1.61	3.00	-	1.21	-
18	Globulol	1590	-	2.12	-	2.18	-	-	-	-
19	α - <i>epi</i> -Murolool	1640	1613.4	4.88	4.1	4.38	-	-	1.14	-
20	Valeranone	1674	-	-	-	-	-	1.42	-	-
Total (%)				100	100	100	95.25	100	100	100

SR-S (1)-(7) = infusions of stems of *S. raeseri* from Galichica, Baba, R.M., Galichica, Kazani, R.M., Galichica, Korito, R.G., Lefkada, R.A., Tepelena, R.A., Gramoz, R.G., Parga, respectively), (-) - not found; KIL- Kovats (retention) index- literature data (Adams, 2007), KIE – Kovat's (retention) index experimentally determined (AMDIS).

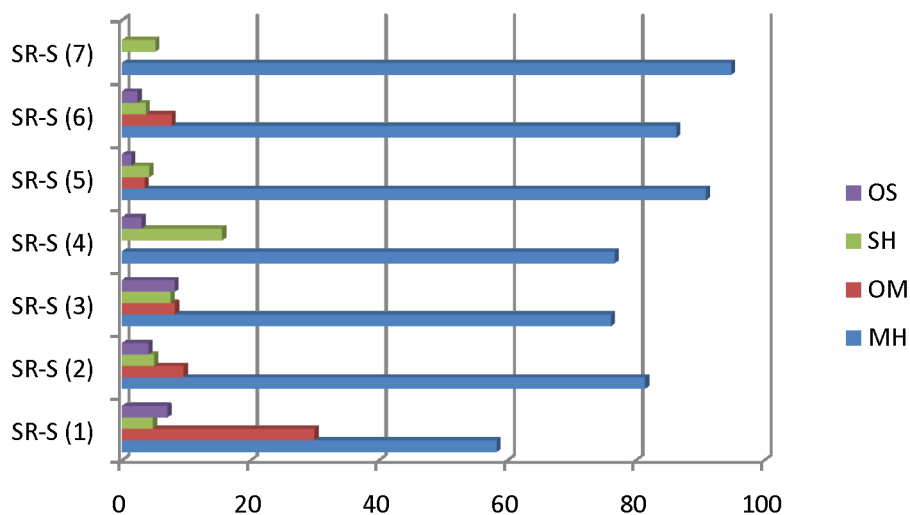


Fig.1. The content of different classes of volatile aroma components in the infusion of stems of *S. raeseri*: MH-monoterpene hydrocarbons, OM-oxygen containing monoterpenes, SH-sesquiterpene hydrocarbons, OS-oxygen containing sesquiterpenes.

Table 3. Chemical composition of volatile aroma compounds in the infusion of rosette leaves of *S. raeseri* (SR-R) (%)

No.	Components	KIL	KIE	SR-R		
				(1)	(2)	(3)
1	α -Pinene	932	937.5	18.11	17.34	19.75
2	Sabinene	969	966.4	5.78	4.48	-
3	β -Pinene	974	973.8	40.85	47.82	53.43
4	1,8- Cineole	1033	-	8.46	10.90	9.91
5	Bornyl acetate	1284	1260.9	-	3.35	-
6	α -Copaene	1374	1345.9	6.26	2.95	-
7	Globulol	1590	-	6.92	-	-
8	α - <i>epi</i> -Murorolol	1640	1613.4	13.62	-	16.91
9	α -Cadinol	1652	1621.8	-	7.91	-
10	Valeranone	1674	-	-	5.24	-
Total (%)				100	99.99	100

SR-R (1)-(3) = infusions of rosette leaves of *S. raeseri* from R.M., Galichica, Baba, R.M., Galichica, Kazani and R.M., Galichica, Korito, respectively; (-) - not found; KIL- Kovats (retention) index- literature data (Adams, 2007), KIE – Kovat's (retention) index experimentally determined (AMDIS).

nia, representing 99.99-100% of the total content (Table 3). Data analysis of the chemical composition revealed four different classes of components: MH (64.74-73.18%), OM (8.46-14.25%), SH (2.95-6.26%) and OS (13.15-20.54%) (Fig. 2).

The prevailing components in all SR-R samples were monoterpenes β -pinene (40.85-53.43%), α -pinene (17.34-19.75%) and 1,8-cineole (8.46-10.90%), followed by sesquiterpene α -*epi*-murorolol (13.62-16.91%).

The number of components found in SR-S was larger than in SR-R. Also, the share of the classes of components in SR-S (MH>OM>SH>OS) differed from SR-R (MH>OS>OM>SH). Infusion of rosette leaves exhibit only smaller differences of aroma compounds profile with the infusion of stems of *S. raeseri*. The Fig. 3 shows the ratio between 5 major aroma volatile components in infusions of stems and rosette leaves, where small variations observed in the content of the dominant components.

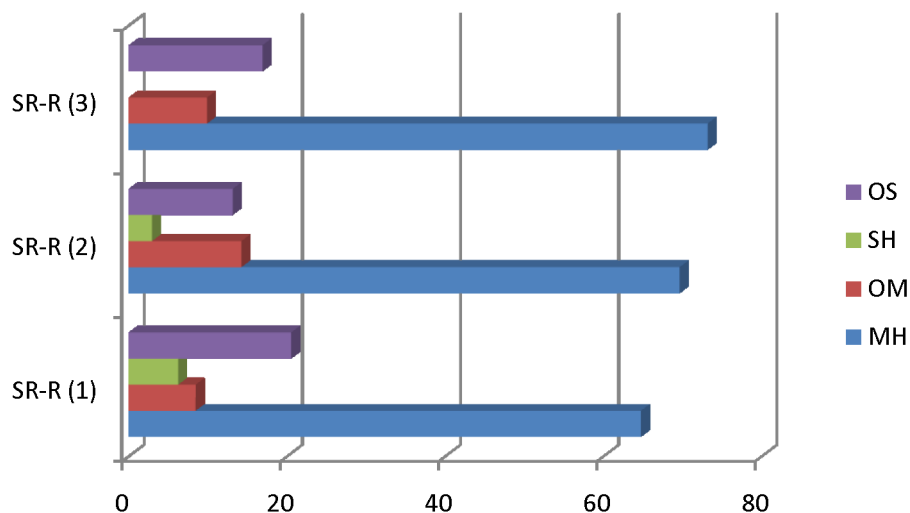


Fig.2. The content of different classes of volatile aroma components in the infusion of rosette leaves of *S. raeseri*: MH-monoterpene hydrocarbons, OM-oxygen containing monoterpenes, SH-sesquiterpene hydrocarbons, OS-oxygen containing sesquiterpenes.

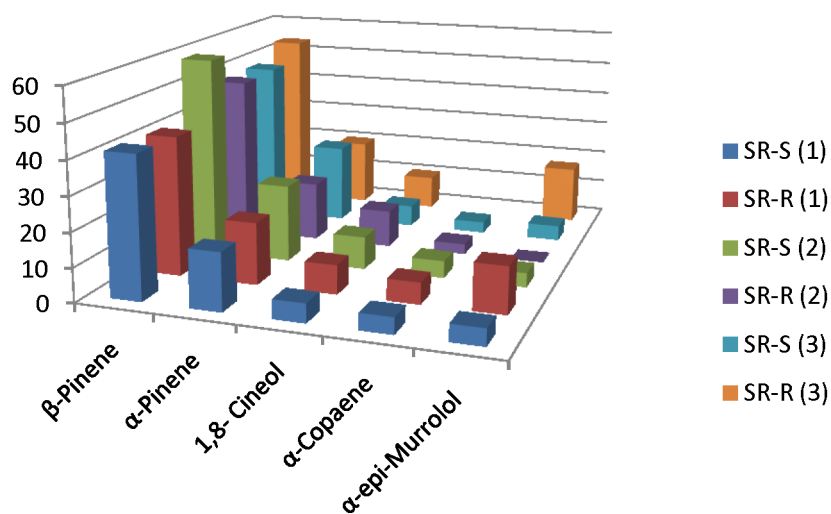


Fig.3. Main volatile aroma compounds in the infusion of stems (SR-S) and rosette leaves (SR-R) of *S. raeseri* ((1)-(3) – samples collected from National park Galichica, R.M, from localities Baba, Kazani and Korito, respectively).

As there are no data for the volatile aroma compounds in the infusion of stems and rosette leaves of *S. raeseri* analyzed by HS-GC/FID/MS, most of our findings were compared with the aroma compounds in the fresh and dried stems of *S. raeseri* and essential oil composition. Similar results were obtained in our previous work where we analyzed the aroma compounds in the dried aerial parts of *S. raeseri* from R. Macedonia, Albania and Greece, and in the fresh aerial parts of *S. raeseri* from R. Macedonia, by HS-

GC/MS-FID. The monoterpene hydrocarbons β -pinene (21.9-48.8% and 37.9-40.7%), α -pinene (17.5-41.0% and 21.5-29.1%), and limonene (1.2-15.7% and 5.1-7.1%), followed by the sesquiterpene hydrocarbons α -copaene (0.9-9.8% and 3.7-8.2%) and *trans*-caryophyllene (1.9-6.5% and 1.4-4.5%, respectively) were the major identified components (Qazimi et al., 2014). These components were identified as major components in the infusions of stems and rosette leaves as well. The infusions were also charac-

terized with high presence of 1,8-cineole.

Pljevljakusic et al. analyzed the chemical composition of the essential oils from cultivated *S. raeseri* aerial parts at four different developmental phases using GC-MS and revealed that the sesquiterpene fraction was the predominant terpenoid group in all samples (58.2-67.8%). The sesquiterpene hydrocarbon bicyclogermacrene was the main constituent of all analyzed oils (35.8-42.5%), followed by spathulenon and β -caryophyllene (5.0-15.0% and 0.1-9.0%, respectively); the major monoterpene hydrocarbon was *cis*- β -ocimene (3.0-4.9%) (Pljevljakusic et al, 2011). Compared to these data, in the HS profile of our samples, bicyclogermacrene exceeded only in the sample SR-S (8) from Lefkada, Greece (4%).

Kostadinova et al. reported the sesquiterpenes germacron and elemol acetate (25.0 and 15.9%, respectively) as dominant components of the essential oil of *S. raeseri* from Galichica Mtn. (Kostadinova et al., 2008). These compounds were not identified in our tested samples.

Conclusion

Specific and particular aroma of *S. raeseri* is one of the reasons for the wide use by the Balkan's people. The chemical composition of the volatile aroma components was defined in the infusion prepared by stems of *S. raeseri* (SR-S) from R. Macedonia, Albania and Greece and in the infusion of rosette leaves of *S. raeseri* (SR-R) from R. Macedonia. Analysis were made by gas chromatography (GC/FID/MS) equipped with a headspace (HS) sampler. In SR-S and SR-R samples were identified 20 and 10 components, respectively. The components belonged to several classes of components such as monoterpene hydrocarbons, oxygen containing monoterpenes, sesquiterpene hydrocarbons and oxygen containing sesquiterpenes. Infusion of the rosette leaves exhibit very similar aroma compounds profile with the infusion of the stems, comprising β -pinene, α -pinene, 1,8-cineole, α -copaene and α -*epi*-murolool as predominate components and accordingly can be consider as a additional plant material source of this endemic aromatic plant. For complete assessment additional phytochemical analysis are require.

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Резиме

Испарливи арома компоненти во инфузи од цветни изданоци и розетни листови на *Sideritis raeseri* Boiss. & Heldr. од Р. Македонија, Албанија и Грција

Бујар Казими, Ѓоше Стефков, Марија Карапанцова, Ивана Цветковиќ,
Светлана Кулеванова

Институт за Фармакологија, Фармацевтски Факултет, Универзитет Св Кирил и Методиј, Скопје, Република Македонија

Клучни зборови: *Sideritis raeseri*, инфуз, арома испарливи компоненти, хедспејс, GC/MS

Составот на испарливи арома компоненти е испитуван во инфузи подготвени од цветни изданоци на *Sideritis raeseri* (SR-S) од Р. Македонија, Албанија и Грција и во инфузи на розетни листови на *S. raeseri* (SR-R) од Р. Македонија. Анализите се направени со помош на гасна хроматографија (GC/FID/MS) опремена со хедспејс (HS) семплер. Дваесет компонентите (12 монотерпени 76,70-94,84% и 8 сесквитерпени 5,16-18,55%) се идентификувани како арома компоненти во SR-S примероци. Доминантни компоненти во сите примероци беа β -pinene, α -pinene и *trans*-кариофилен. Висока застапеност на лимонен и δ -3-карен се карактеристични за примероците од Грција и 1,8-цинеол за примероците од Р. Македонија и Албанија. Во инфузи на SR-R, идентификувани се десет компоненти, 5 монотерпени (73,20-83,89%) и 5 сесквитерпени (16,10-26,80%). Доминантни компоненти во сите испитувани примероци на SR-R се β -pinene, α -pinene, 1,8-цинеол и α -*epi*-муролол. Речиси не постои разлика на хемиските профили на арома компоненти меѓу SR-S и SR-R. Инфузи од розетни листови покажуваат многу слични профили на арома компоненти со инфузи од цветни изданоци на *S. raeseri*.