Chemical composition of ultrasonic-assisted *n*-hexane extracts of *Sideritis scardica* Grieseb. and *Sideritis raeseri* Boiss. & Heldr. (Lamiaceae) from Macedonia and Albania

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Abstract

Chemical composition of *n*-hexane extracts obtained from dried over-ground parts of two species of *Sideritis*, *S. scardica* Grieseb. and *S. raeseri* Boiss. & Heldr. (Lamiaceae) was analyzed using GC/FID/MS. The collection of plants was made on different locations in the western part of Macedonia and the southern part of Albania, comprising twelve different samples of plant material. The ultrasonic-assisted extraction process was used for preparation of the *n*-hexane extracts yielded 0.73-3.33 % and 9.11-10.44 % of extracts for *S. scardica* and *S. raeseri*, respectively. Over one hundred constituents of the extracts were identified, belonging to several classes of components: diterpenes, hydrocarbons, dominantly present in each of the extracts, followed by fatty acids, aliphatic and aromatic alcohols, sterols, triterpene alcohols, and monoterpenes and sesquiterpenes, which were found in much smaller amounts or only in traces. The most abundant constituents of the extracts of both species of *Sideritis* were two diterpene components, both with *M*=286, which were not fully identified. Large percentages of nonacosane (1.71-12.22% and 7.46-19.68% for *S. scardica* and *S. raeseri*, respectively) and hentriacontane (4.48-20.79% and 8.09-30.31 % for *S. scardica* and *S. raeseri*, respectively) were also found in the extracts of both species.

Key words: *Sideritis scardica*, *Sideritis raeseri*, *n*-hexane extracts, ultrasonic-assisted extraction, composition, GC-MS analysis.

Introduction

Sideritis scardica Griseb. and *Sideritis raeseri* Boiss. & Heldr. (Lamiaceae) are wild, hardy flowering perennials that have adapted to survive with little water and little soil, also known as Mountain tea, Ironwort, Shepard's tea or Pirin tea. Both plants are very popular in Macedonia, Bulgaria, and Greece as well as throughout the Eastern Mediterranean as refreshment but also to cure common cold. The herbs are often used to prepare teas widely believed to alleviate sinus congestion, aches, pains and viruses including flu and common cold. A very aromatic variant of the tea includes combining the *Sideritis* plant with spices common to the Mediterranean cuisine. Scientists recently suggested that the traditional Greek promotion of mountain tea as a panacea for most illnesses may be remarkably close to the truth.

In past decades species of genus *Sideritis*, especially species from Spain, Turkey, Bulgaria, Albania, Greece, and Serbia, have been extensively investigated on chemical composition of flavonoids, phenylpropanoids, cinnamic acid derivatives and phenylethanoids (Palomino et al., 1996; Aboutabl et al., 2002; Ozkan et al., 2005; Erkan et al., 2011; Alipieva et al., 2010; Koleva et al., 2003; Gabrieli et al., 2005; Armata et al., 2008; Pljevljakusic et al., 2011) as well as on essential oils composition, chemistry of diterpeneoids, iridoids and other terpenoids or related components (Gomez-Serranillos et al., 1997; Gomez-Serranillos et al., 2004; Rodriguez-Garcia et al., 2004; Topku et al., 2001; Topku et al., 2002; Alipieva et al., 2009). Recently, data on flavonoids, phenylethanoids and phenylpropanoid acids and their esters have been reported for native Macedonian *Sideritis* (*S. scardica* and *S. raeseri*) (Janeska et al., 2007; Petreska et al., 2011a, 2011b), as well as data on essential oil composition of the same species (Kostadinova et al., 2007).

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In most of the studies conducted on *Sideritis*, scientists suggested that phenolic and/or polyphenolic components are responsible mostly for the antioxidant activity of alcohol extracts, while terpene components from the essential oils and even more different diterpeneoids are found to be responsible for the anti-inflammatory, analgesic, antiulcer, antibacterial, antifungal, cytotoxic and antitumor activity (Gomez-Serranillos et al., 2004). Therefore, preparation of an *n*-hexane extracts of dried herbs of *Sideritis* is the most preferable type of extraction if terpenoid components represent research interest (Alcaraz et al., 1989; Gomez-Serranillos et al., 1998; Gomez-Serranillos et al., 1997; Aboutabl et al., 2002; Menghini et al., 2005; Tsaknis and Lalas, 2005; Kostadinova E. et al., 2008). Usually, the process of extraction is carried out via methods of percolation and maceration, characterized by small efficiency and long drawing (Lysyansky and Grebenyuk, 1987). Nowadays, the use of different electro-physical methods (ultrasound, electro-flotation, etc) allows increasing the extraction rate and yield of biologically active compounds (Sulman et al., 1997; Semagina et al., 2000).

Taking into account the consideration mentioned above, the aim of this work was preparation of *n*-hexane extract of *Sideritis scardica* and *S. raeseri* by ultrasonic-assistance extraction and determination of the chemical composition of the extracts using GC/FID/MS method.

2. Experimental

2.1. Plant material

Samples of *Sideritis* were collected in a flowering stage in the western, central and southern areas in R. Macedonia and southeastern parts of Albania. *Sideritis scardica* was collected from 7 different regions in Macedonia: Shar Planina (Ljuboten) (V.No. S1/08), Suva Gora (near Gostivar) (V.No. S2/08), Prilep (cultivated plant, V. No. S3/08), Bistra (Lazaropole) (V.No. S4/08), Kozuf (Dve ushi) (V.No. S5/08), Nidze (Kajmakchalan) (V. No. 14/08) and Ilina mountain (S 16/08), while the samples of *Sideritis raeseri* were collected from Galicica mountain (V. No. 15/08) and Baba mountain (V.No. S6/08) in Macedonia and from Gramoz mountain (V. No. S7/08), Tepele-na (V. No. S8/08) and Logora mountain (V. No. S18.08) from south Albania. The collections were made in 2008 and 2009. A voucher specimen of each species was deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, University SS Cyril and Methodius, Skopje, Republic of Macedonia. Plant material was left to dry at room temperature and, after drying it was packed in paper bags and left in dark, cool and dry place until analysis.

2.2. Extraction

Plant extracts were obtained using ultrasonic-assisted extraction process at room temperature. *n*-Hexane was

used for the extraction in 2 portions in ratio to plant material 1:20 (1 g plant material was extracted twice with 10 ml solvent). The duration of the extraction was 2 x 30 minutes. The extracts obtained after filtration were concentrated to dryness below 35°C. The residue were measured as yield of the extraction and then dissolved in *n*-hexane to obtained solution with concentration of 1 g plant material in 1 ml solution. For GC/FID/MS analysis additional 1 µg/ml dilutions were prepared.

2.3. Gas chromatography

n-Hexane extracts were analyzed on Agilent 7890A Gas Chromatography system equipped with FID detector and HP-5ms 5% phenyl 95% dimethylpolysiloxane bonded phase capillary column (30 m x 0.25 mm, film thickness 0.25 µm). Operating conditions were as follows: oven temperature at 60°C for 5 min, then increased to 80°C at rate of 1°C/min and held 2 min and at the end increased to 280°C at rate of 5°C/min and held 5 min; helium as carrier gas at a flow rate of 1ml/min; temperature of the injector 260°C and that of the FID detector 270°C; the GC split ratio 1:1. 1 µl of each sample of the essential oil, dissolved in xylene (1: 1000 v/v) was injected per GC run.

The percentage composition of *n*-hexane extracts were computed by the normalization method from the GC/FID peak areas (relative amount of each compound to the total amount present), calculated by means of three injections from each extract.

The extracts were analyzed on Agilent 7890A Gas Chromatography system interfaced to an Agilent 5975C mass spectrometer. The gas chromatographic conditions were the same as reported for GC analysis and the same column was used. The mass spectrometry conditions were: ionization voltage 70 eV, ion source temperature 230°C and mass range from 50 - 500 Da.

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

3. Results and discussion

The ultrasonic-assisted extraction yielded different amount of dry extracts (dry residues after evaporation) ranged from 0.73-3.33 % for the samples of *S. scardica* and from 9.11-10.44 % for the samples of *S. raeseri* (Table 1.). The yields of *n*-hexane extracts of *S. raeseri* were almost the same for different samples of the plant regardless of the collection site. In the case of *S. scardica* much more variations in the extraction yields appeared. The higher yield of

Table 1. The yields of *n*-hexane extracts of *Sideritis* obtained by ultrasonic-assisted extraction

<i>S. scardica</i>	<i>n</i> -hexane extr. (%)	<i>S. raeseri</i>	<i>n</i> -heksane extr. (%)
Shar Planina (Ljuboten)	0.73	Baba mountain.	9.11
Suva Gora (Gostivar)	0.89	Galicica mountain	9.87
Prilep (cultivated)	1.78	Gramoz mountain (Albania)	10.44
Bistra (Lazaropole)	3.33	Tepelena (Albania)	9.53
Kozuf (Dve ushi)	0.82	Logora mountain (Albania)	9.34
Nidze (Kajmakalan)	1.23		
Ilina mountain	1.52		

(n = 3)

n-hexane extract for *S. raeseri* against *S. scardica*, probably due to the larger amounts of components extractable in hexane in *S. raeseri*. Bulgarian scientists reported 0.50-0.58% yields of the *n*-hexane extracts for both species, *S. scardica* and *S. raeseri*, which is significantly lower from our findings (Kostadinova et al., 2008). The reason of lower yield probably lays in the differences in geographical origin of the samples, but it is worth mentioning that those scientists have used 24 h maceration as extraction procedure while in our case it was ultrasonic-assisted extraction. According to literature data, ultrasonic-assisted extraction has been pointed out as more effective extraction procedure (Sulman et al., 1997, Samagina et al., 2000).

Gas-chromatography analysis showed presence of over 100 individual components identified by GC/MS. The results of chemical composition of *n*-hexane extract of *S. scardica* are presented in Table 2 and for *S. raeseri* in Table 3. The chemical composition of the *n*-hexane extracts of both species showed qualitative and quantitative similarities. Mono and sesquiterpenes were presented in very small amounts, almost in traces, but apart from that, 25 components were identified. In *S. scardica* the sesquiterpene: *trans*-caryophyllene, δ -cadinene, caryophyllene oxide and α -cadinol+ τ -muurolol denoted the main part of this fraction. In *S. raeseri* additionally α - and β -pinenes, myrtenol, *trans*-cadin-1,4-diene, viridiflorol, valeranone and α -bisabolol were found. These data differ a lot from the previously reported data of chemical composition of essential oils of *S. scardica* (Galicnik) and *S. raeseri* (Galicica) from Macedonia (Kostadinova et al., 2007). For both species, α -cadinol was defined as the dominant component and elemol acetate and germacron additionally for the *S. raeseri*. Interesting results for the chemical composition of essential oils of cultivated *S. raeseri* subsp. *raeseri* were recently reported for the plant cultivated in Serbia. The main mono and sesquiterpene components of those oils were bicyclogermacrene and spatulenol (Plevljak-isic et al., 2011). In both cases essential oils were obtained by hydro-distillation and besides mono and sesquiterpenes contained fatty acids and their esters, hydrocarbons and several diterpenes. All these components were also found in *n*-hexane extracts of our specimens of *Sideritis*.

The fractions of fatty acids and fatty acid-ester compounds were found in larger amount than fractions of mono and sesquiterpenes. Within these fractions, the main components were methyl, isopropyl hexadecanoate and hexadecanoic acid in *S. scardica* as well as octadecanoic acid methyl ester and hexadecanoic acid in *S. raeseri*. Large fraction of the *n*-hexane extracts belonged to hydrocarbons comprising aliphatic components with long carbon-chains (C5-C33). The hydrocarbons represented 13.33-47.24 % of *n*-hexane extract of *S. scardica* (Fig. 1) and 26.24-68.12 % of *n*-hexane extracts of *S. raeseri* (Fig. 2). The most abundant components were heptacosane, nonacosane, hentriacontane and tritriacontane. Among them, hentriacontane was the predominant hydrocarbon in the extracts of both species, with 4.48-20.79 % in *S. scardica* and 8.09-30.31 % in *S. raeseri*. Very small part of extracts contained oxidized products of hydrocarbons such as different alcohols, rarely some esters. Kostadinova et al. (2008) in *n*-hexane extracts of *S. scardica* and *S. scardica* $\times$ *S. syriaca* hybride also found nonacosane and hentriacontane as predominant hydrocarbons. Seasonal variation in the content of some hydrocarbons, mainly hentriacontane and nonacosane, was found for both species (Table 2 and 3).

Besides hydrocarbons, second predominant fraction, of the *n*-hexane extracts of the investigated species of *Sideritis*, were diterpeneoids. This fraction represented 23.00-60.80 % of the *S. scardica* (Fig. 1) and 22.13-45.44 % of the *S. raeseri* *n*-hexane extracts (Fig. 2). (+)-Beyeren, pimara-8(14),15-diene, kaur-15-en, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,1,4a,7-tetramethyl-2-phenanthrenol and manyol oxide were identified and in addition eight more components were tentatively identified as diterpeneoids. We were unable to identify the two dominant components in this fraction (in some cases the most dominant components in the whole extract). These components, both with M=286, are probably pimara derivatives. Besides them, there were three diterpene components with M=306, M=346 and M=286, also not identified. Few of them were probably components with kaurane skeleton, as kauranes were often identified until now in different species of *Sideritis* as the predominant

Table 2. Chemical composition of hexane extracts of *Sideritis scardica* from different location from Macedonia

Components	KIE	Shar 2008	Planina 2009	Suva 2008	Gora 2009	Prilepsko** 2008	2009	Bistra 2008	2009	Kozuf 2008	Nidze 2008	Ilina Mtn. 2008
MONO and SESQUITERPENS												
α -Pinene	951.1	tr	tr	-	-	-	-	0.01	-	-	tr	-
Camphene	960.7	-	-	-	-	-	-	0.01	-	-	-	-
β -Pinene	980.8	tr	-	-	-	-	-	0.01	-	-	-	-
β -Myrcene	992.6	-	-	-	-	-	-	0.01	-	-	-	-
D-Limonene + β -Phellandren	1024.2	tr	tr	tr	tr	-	-	0.02	tr	tr	tr	tr
<i>trans</i> -Pinocarveole	1145.1	tr	-	tr	tr	tr	tr	-	-	-	-	-
<i>trans</i> -Sabinol	1152.9	tr	-	tr	tr	tr	-	-	-	-	tr	
Myrtenol	1212.9	0.05	0.02	tr	tr	tr	tr	0.01	tr	tr	tr	tr
(-)-Verbenone	1231.5	tr	-	tr	tr	0.06	tr	0.01	tr	-	tr	-
α -Cubebene	1403.6	0.02		-	tr	tr	-	-	-	-	tr	-
α -Copaene	1437.4	-	0.01	tr	tr	tr	-	-	0.05	tr	0.07	0.05
Muurolo-4(14),15-diene (<i>cis</i>)	1454.2	0.03	-	-	-	-	-	-	-	-	-	-
<i>trans</i> -Caryophyllene	1488.7	0.09	0.10	0.14	0.26	0.05	0.08	0.01	0.07	0.30	0.31	0.08
Muurolo-3,5-diene (<i>trans</i>)	1524.7	-	-	tr	tr	tr	-	-	-	-	-	-
α -Hummulene	1528.9	0.01	-	-	-	-	-	-	tr	0.07	0.05	-
Germacren D	1560.7	0.03	0.01	-	-	-	-	0.01	tr	0.04	0.05	0.08
Bicyclogermacrene	1578.1	0.03	0.01	-	-	-	-	-	-	-	-	-
δ -Cadinene	1606.4	0.05	0.02	-	-	-	-	tr	0.08	-	0.15	0.03
Cadina-1,4-diene (<i>trans</i>)	1617.3	0.04	0.02	-	-	0.06	-	tr	-	-	0.04	0.05
(-)-Spatulenol	1669.8	0.05	0.02			-	-	tr	-	0.04	0.04	-
Caryophyllene oxide	1677.0	0.12	0.10	0.13	0.12	-	0.11	-	0.04	0.05	0.12	0.07
Viridiflorol	1686.1	0.03	0.01	-	-	-	-	-	0.05	-	-	-
α -Cadinol + τ -Muurolol	1739.1 1739.5	0.07	0.05	-	-	-	-	-	0.30	0.04	0.03	0.05
Valeranone	1775.4	-	0.03	-	-	-	-	tr	tr	-	-	0.03
Total mono- and sesquiterpens:		0.71	0.43	0.35	0.47	0.30	0.22	0.21	0.67	0.57	0.95	0.49
DITERPENS												
(+)-Beyerene	2056.7	0.07	0.05	-	-	0.06	-	0.01	tr	-	-	-
Pimara-8(14),15-diene	2072.4	0.07	0.05	-	-	-	-	tr	-	-	-	-
Kaur-15-en	2126.0	0.10	0.08	-		0.16	-	0.05	0.07	0.05	-	-
Manoyl oxide	2146.2	0.04	0.02	-		-	-	-	tr	-	-	-
3 α -Hydroxymanool (M=288)*	2327.3	0.22	0.20	0.24	0.09	0.23	0.21	0.08	0.29	0.08	0.06	0.06
7-ethenyl- 1,2,3,4,4a,4b,5,6,7,9,10,10a- dodecahydro-1,1,4a,7- tetramethyl-2-phenanthrenol (M=306)	2335.8	0.26	0.24	0.23	0.24	0.23	0.13	0.08	0.18	0.13	0.10	0.15

Components	KIE	Shar Planina		Suva Gora		Prilepsko**		Bistra		Kozuf	Nidze	Ilina Mtn.
		2008	2009	2008	2009	2008	2009	2008	2009	2008	2008	2008
Diterpene (M=306)	2374.2	0.22	0.19	0.12	0.13	0.19	-	0.07	0.09	0.07	-	0.10
Diterpene (M=306)	2388.5	-	-	-	-	0.24	-	0.06	0.19	0.15	0.04	0.12
Diterpene (M=306)	2458.8	0.64	0.62	0.28	0.22	0.76	0.28	0.25	0.44	0.46	0.23	0.14
Diterpene (M=286)*	2487.7	10.27	10.49	6.70	7.78	13.14	6.11	8.72	12.65	8.52	4.15	4.68
Diterpene (M=286)*		36.02	37.12	24.08	28.25	42.43	19.92	15.86	31.15	28.66	20.00	17.04
Diterpene (M=346) *	-	-	1.32	-	0.92	-	0.89	0.67	0.30	0.92	0.55	0.59
Diterpen (M=286) *	-	-	-	0.74	1.86	3.59	1.50	1.13	2.23	2.02	1.47	0.11
Total diterpens:		48.91	50.38	32.31	39.49	61.03	29.04	38.04	47.61	41.06	27.50	23.15
ESTERS												
Benzyl benzoate	1924.3	0.05	0.02	-	-	-	0.05	tr	0.04	-	-	-
Isopropyl tetradecanoate	1978.5	0.05	0.03	0.08	-	0.04	0.11	-	0.08	0.06	0.25	0.32
Di-isobutyl phthalate*	2029.2	0.05	0.01	-	-	-	0.09	tr	0.07	-	-	-
Methyl hexadecanoate	2103.3	0.05	0.03	-	-	-	0.11	0.01	0.06	0.04	-	-
Hexadecanoic acid ethylester	2135.6	-	-	-		0.06	-	0.03	0.07	-	-	-
Isopropyl hexadecanoate	2243.4	-	0.03	-	-	-	-	-	0.04	-	-	-
Methyl octadecanoate*		-	0.10	0.10	0.14	-	0.12	0.04	0.05	-	-	-
9,12-Octadienoic acid ethylester*		-	-	-	-	-	-	-	0.10	0.15	0.10	0.15
Total esters:		0.20	0.22	0.18	0.14	0.10	0.48	0.14	0.52	0.25	0.35	0.47
HYDROCARBONS												
Heptadecane	1790.5	0.05	0.01	-	0.10	-	-	0.22	0.03	0.05	-	-
Octadecane	1896.4	tr	0.01	-	-	-	-	-	0.02	-	-	-
Nonadecane	2002.0	0.03	0.01	-	-	-	-	0.03	0.04	0.04	0.05	-
Eicosane	2108.2	0.06	0.04	-	-	-	0.13	-	0.03	-	0.03	0.03
Heneicosane	2213.4	0.07	0.05	-	-	-	0.09	tr	0.06	0.04	0.06	0.06
Docosane	2318.5	0.16	0.14	-	-	0.05	0.18	-	0.08	-	0.15	0.10
Tricosane	2423.2	0.27	0.25	0.57	0.39	0.38	0.32	0.11	0.35	0.26	0.59	0.31
Tetracosane	2528.7	0.07	0.05	0.14	0.10	0.16	0.36	0.04	0.41	0.10	0.18	0.09
Pentacosane	2631.0	0.36	0.34	0.82	0.64	0.80	0.82	0.20	0.90	0.79	1.47	0.84
Hexacosane	2691.5	-	0.07	-	-	-	0.09	-	0.12	-	0.04	0.07
Heptacosane	2784.4	1.66	1.62	3.17	2.73	1.68	3.12	0.47	2.19	2.17	4.10	3.86
Octacosen*	2810.3	-	0.27	-	0.44	0.56	0.68	0.08	0.48	0.18	0.55	0.18
Octacosane	2822.2	0.30	0.28	0.46	0.49	0.50	0.72	0.11	0.72	0.52	0.61	0.52
Nonacosane	3024.6	7.42	7.41	12.22	10.41	3.48	11.01	1.71	7.20	9.59	0.13	8.59
Triacontane	3025.1	0.26	0.24	0.83	0.68	0.16	0.79	0.18	0.75	0.87	1.08	1.05
Hentriacontane	3132.3	12.35	12.30	20.79	16.72	4.48	17.92	13.36	12.0	18.63	9.96	18.13
Dotriacontane*		0.50	0.47	0.93	0.79	0.07	0.60	0.14	0.46	1.19	1.04	1.19
Tritriacontane *		2.00	3.14	7.38	5.97	1.01	5.30	1.43	3.66	5.78	7.85	6.78
Total hydrocarbons:		25.57	26.61	47.24	39.34	13.33	42.13	18.09	26.50	40.21	48.27	26.55

Components	KIE	Shar Planina 2008 2009	Suva Gora 2008 2009	Prilepsko** 2008 2009	Bistra 2008 2009	Kozuf 2008 2009	Nidze 2008 2009	Ilina Mtn. 2008
FATTY ACIDS								
Dodecanoic acid	1643.2	0.03 0.01	- -	- -	- -	- -	- -	-
Tetradecanoic acid	1860.6	0.04 0.02	- -	- -	- 0.02	0.05	0.10	0.08
Hexadecanoic acid	2070.2	0.75 0.74	1.60 1.91	0.89 3.09	0.63 1.66	1.38	2.10	0.91
9,12-ocatadecadienoic acid	2258.0	- -	0.42 1.03	0.10 1.84	0.20 0.32	1.52	1.49	0.93
9,12,15-octadecatrenoic acid*		0.60 0.42	0.73 1.23	0.20 3.17	0.41 1.24	-	0.98	0.88
Octadecanoic acid *		0.16 0.14	0.55 0.45	0.04 -	- 0.27	0.41	0.75	0.63
Total fatty acids:		1.58 1.33	3.30 4.62	1.23 8.10	1.24 3.51	3.36	5.42	3.43
ALCOHOLS								
2,4-di-tert-butylphenol	1590.3	- -	- -	0.04 -	0.04 0.12	0.08	0.03	0.03
Tetradecanol	1765.2	- 0.01	- -	- 0.13	- tr	-	-	-
Phytol (isomer)*	1939.1	- -	- -	0.09 0.26	0.05 0.25	0.05	0.03	0.03
2-Hexadecen-1-ol	1981.0	- 0.05	- -	0.03 0.12	- 0.09	0.01	0.04	-
Oleyl alcohol	2091.1	2.88 2.86	1.75 1.98	3.88 1.28	1.31 2.12	1.90	0.75	0.45
Octadecanol	2198.8	0.23 0.21	0.08 -	0.15 -	0.04 0.69	0.12	0.12	0.07
Total alcohols:		3.11 3.13	2.06 1.98	3.99 1.79	1.44 3.29	2.17	0.97	0.58
STEROLS								
(-)-Cholesterol*		- -	- -	0.09 0.29	- 0.29	0.26	0.28	-
22,23-dihydrobrassicasterol*		0.80 0.78	- -	0.27 0.90	0.26 0.47	0.47	0.65	0.25
Stigmasterol*		0.90 0.88	0.34 0.45	0.47 1.37	0.39 0.69	0.85	0.94	0.61
γ -Sitosterol*		- 1.97	1.49 1.91	1.55 3.86	0.82 2.75	1.84	3.30	2.05
Total sterols:		1.70 3.63	1.83 2.36	2.38 6.42	1.47 4.20	1.58	5.17	2.95
TRITERPENS								
Squalene*		0.17 0.15	0.48 0.54	0.40 0.66	0.07 1.01	0.52	0.66	0.37
β -Amyrine*		0.43 0.41	0.29 0.46	0.73 0.36	0.28 0.39	0.63	2.10	0.99
α -Amyrine*		0.40 0.38	0.78 -	0.40 0.41	0.19 0.28	0.35	0.25	0.30
Triterpene alcohol* [†]		0.18 0.16	- -	tr -	0.01 -	0.19	0.37	0.37
Total triterpens:		1.18 1.10	1.55 1.00	1.54 1.53	0.55 1.68	1.69	3.38	2.03
OTHER COMPOUNDS								
Hexahydrofarnesil acetone	1946.2	0.09 0.07	- -	0.08 0.09	0.01 -	0.07	0.14	0.10
TOTAL		83.06 86.90	88.67 89.40	83.98 89.90	60.14 87.98	90.96	91.25	59.95

* - identification according NIST or Wiley (without AMDIS), [†] - tentative identification (AMDIS); (-) - not found; (/) - not available; KIE – estimated retention indices, cultivated plant

Table 3. Chemical composition of hexane extracts of *Sideritis raeseri* from different location from Macedonia and Albania

Components	KIE	Baba Mtn. Macedonia 2008 2009	Galicica Macedonia 2008	Gramoz Mtn Albania 2008 2009	Tepelena Albania 2008	Logora Mtn. Albania 2008		
MONO and SESQUITERPENS								
α -Pinene	951.1	0.10	tr	0.05	0.26	0.05	0.18	tr
Camphene	960.7	-	-	-	-	-	-	-
β -Pinene	980.8	1.20	0,08	0.11	0.58	0.90	0.36	tr
β -Myrcene	992.6	-	-	-	-	-	-	-
D-Limonene + β -Phellandren	1024.2	tr	tr	tr	tr	tr	tr	tr
<i>trans</i> -Pinocarveole	1144.8	0.10	0,04	tr	tr	tr	-	tr
<i>cis</i> -Verbenol	1152.6	tr	tr	tr	tr	tr	-	tr
Myrtenol	1212.9	0.10		tr	0.02	0.04	-	tr
(-)-Verbenone	1230.5	0.08	0,02	tr	tr	tr	tr	tr
α -Cubebene	1403.6	0.05	0,02	0.05	0.10	0.12	-	-
α -Copaene	1437.4	0.20	0,24	0.12	tr	0.10	0.05	0.04
Muurola-4(14),15-diene (<i>cis</i>)	1454.2	-	tr	0.08	tr	tr	tr	-
<i>trans</i> -Caryophyllene	1488.7	0.20	0,34	0.11	0.79	0.56	0.52	0.31
Muurola-3,5-diene (<i>trans</i>)	1524.7	0.01	-	-	-	-	-	-
α -Hummulene	1528.9	0.10	0,12	0.06	0.04	0.06	tr	-
Germacren D	1560.7	0.02	0,06	0.06	0.06	0.02	0.02	0.04
Bicyclogermacrene	1578.1	-	-	0.08	-	-	0.04	-
α -Muurolene	1580.3	0.01	0,01	-	-	tr	-	-
β -Bisabolene	1588.8	0.04	0,06	0.04	0.10	tr	-	-
δ -Cadinene	1606.4	0.22	0,35	0.16	0.15	0.15	0.11	0.10
Cadina-1,4-diene (<i>trans</i>)	1617.3	0.05	0,05	0.04	-	-	-	0.29
α -Calacorene	1630.2	0.02	0,02	0.03	-	tr	-	-
(-)-Spatulenol	1670.2	tr	0,02	0.06	0.07	0.05	0.03	tr
Caryophyllene oxide	1677.0	0.15	0,15	0.12	0.18	0.12	0.08	0.11
Viridiflorol	1686.1	0.09	0,24	0.08	0.06	0.10	-	tr
β -Gurjunenepoxide	1690.0	tr	0,01	-	tr	tr	-	tr
α -Cadinol + τ -Muurolol	1739.1 1739.5	0.50	1,27	0.24	0.24	0.58	0.04	tr
Valeranone	1776.5	0.05	0,05	0.11	-	-	0.06	tr
α -Bisabolole	1780.6	0.06	0,05	0.03	-	-	0.02	tr
Total mono and sesquiterpens:		3.36	3.32	1.68	2.72	3.94	1.56	1.02
DITERPENS								
(+)-Beyerene	2057.7	0.08	0,03	-	0.05	0.03	0.05	0.04
Pimara-8(14),15-diene	2072.4	-	-	-	-	-	-	-
Kaur-15-en	2125.2	0.10	tr	0.03	0.09	0.07	0.12	0.09
Manoyl oxide	2145.9	0.05	0.03	-	0.02	0.01	0.01	-
3a-Hydroxy manool (M=288)	2327.5	0.20	0,21	0.10	0.33	0.37	0.47	0.23

Components	KIE	Baba Mtn. Macedonia 2008 2009	Galicica Macedonia 2008	Gramoz Mtn Albania 2008 2009	Tepelena Albania 2008	Logora Mtn. Albania 2008		
7-ethenyl-1-,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,1,4a,7-tetramethyl-2-phenanthrenol (M=306)	2335.8	0.24	0,22	0.07	0.21	0.24	0.37	0.33
Diterpene (M=306)	2374.2	0.22	0,15	0.11	0.14	0.20	-	0.27
Diterpene (M=306)	2387.9	-	-	-	-	-	-	-
Diterpene (M=306)	2459.0	-	0,20	0.18	0.33	0.20	0.68	0.50
Diterpene (M=286)	2487.7	10.36	8.99	5.40	21.07	15.87	18.22	13.16
Diterpene (M=286)		17.53	11.49	3.41	18.36	18.02	25.64	23.96
Diterpene (M=346)		1.20	0,20	0.08	0.65	0.45	0.25	0.21
Diterpen (M=286)		1.35	0,82	0.24	1.45	1.31	-	2.13
Total diterpens:		31.31	22.35	9.62	42.73	36.65	45.81	14.92
ESTERS								
Benzyl benzoate	1924.3	tr	tr	0.02	-	0.05	0.02	0.03
Isopropyl tetradecanoate	1978.5	0.05	tr	0.03	tr	0.35	0.03	0.03
Di-isobutyl phthalate*	2029.2	0.06	0,04	0.41	0.05	0.06	0.06	0.11
Methyl hexadecanoate	2103.3	-	0,08	0.10	0.02	0.08	0.21	0.19
Hexadecanoic acid ethylester	2135.6	0.04	0,10	0.06	-	0.12	0.20	-
Isopropyl hexadecanoate	2243.4	-	-	-	-	-	-	0.54
Methyl octadecanoate	1924.3	1.35	2,54	0.07	0.62	2.86	0.06	-
9,12-Octadienoic acid ethylester	1978.5	0.18	0,14	0.15	-	0.12	0.12	0.11
Dodecenol acetat *			0,15	0.03	1.10	0.79	0.27	0.28
Myristyl tetradecanoate *		0.12	0,17	0.18	0.04	0.26	0.09	0.23
Total esters:		1.81	3.24	1.05	0.84	4.69	1.06	1.52
HYDROCARBONS								
Heptadecane	1790.5	0.02	0,03	0.04	tr	0.04	-	tr
Octadecane	1896.4	0.02	0,01	0.02	tr	0.03	-	tr
Nonadecane	2002.0	-	-	-	-	-	-	-
Eicosane	2108.2	0.09	0,08	0.06	0.06	0.06	0.09	0.04
Heneicosane	2213.4	-	0,04	0.06	-	0.04	0.07	0.04
Docosane	2318.5	0.26	0,21	0.10	tr	0.02	0.15	0.15
Tricosane	2423.2	0.41	0,21	0.17	0.40	0.34	0.43	0.36
Tetracosane	2528.7	0.21	0,10	0.11	0.16	0.15	0.35	0.11
Pentacosane	2631.0	0.75	0,47	0.45	0.79	0.54	0.97	0.81
Hexacosane	2691.5	0.08	3.52	0.05	0.13	0.11	0.17	0.15
Heptacosane	2784.4	3.97	0.74	4.29	3.81	2.67	2.98	3.06
Octacosen*	2810.3	1.15		0.63	1.16	0.78	1.66	0.97
Octacosane	2822.2	0.53	0,66	0.74	1.08	0.83	0.92	0.53
Nonacosane	3024.6	11.35	15.55	19.68	8.87	8.20	7.79	7.46
Triacontane	3025.1	0.56	0,93	1.24	0.48	0.87	0.65	0.66
Hentriacontane	3132.3	14.50	25.40	30.31	8.09	13.25	10.39	9.14
Dotriacontane*		0.72	0.97	1.35	-	0.78	0.34	0.51

Components	KIE	Baba Mtn. Macedonia 2008 2009	Galicica Macedonia 2008	Gramoz Mtn Albania 2008 2009	Tepelena Albania 2008	Logora Mtn. Albania 2008		
Tritriacontane *		4.05	8.02	8.94	4.27	4.05	2.68	2.23
Total hydrocarbons:		38.67	59.94	68.12	29.33	32.76	29.64	26.24
FATTY ACIDS								
Dodecanoic acid	1643.2	tr	tr	0.06	-	-	-	0.03
Tetradecanoic acid	1860.6	0.06	0,02	0.10	0.04	0.05	-	0.16
Hexadecanoic acid	2070.2	0.92	1,87	2.23	0.03	2.72	0.55	3.68
9,12-ocatadecadienoic acid	2258.0	0.40	0,54	1.80	0.55	0.68	0.06	0.83
9,12,15-octadecatrienoic acid*	-	-	-	-	-	-	-	0.30
Octadecanoic acid*	-	-	-	0.73	-	-	-	0.30
Total fatty acids:		1.39	2.44	4.92	0.62	3.45	0.61	5.30
ALCOHOLS								
2-Nonen-1-ol	1072.3	tr	0,02	tr	-	-	-	-
2,4-di-tert-butylphenol	1590.3	0.06	tr	0.12	-	0.07	0.09	0.11
Tetradecanol	1765.2	0.12	0,17	0.24	0.05	0.17	0.07	0.25
Phytol (isomer) [†]	1939.1	0.05	0,20	0.07	0.16	0.23	0.08	0.04
2-Hexadecen-1-ol	1981.0	0.04	0,07	0.03	-	-	-	-
Oleyl alcohol *	2091.1	1.82	1,04	0.28	1.51	1.23	2.07	2.80
Octadecanol	2198.8	1.12	0,06	-	0.07	0.06	0.10	
Total alcohols		3.22	1.50	0.75	1.79	1.76	2.41	3.20
STEROLS								
(-)-Cholesterol*		0.16	0,24	-	0.13	0.23	0.28	0.36
22,23-dihydrobrassicasterol *		0.52	0,43	0.26	0.36	0.42	0.28	0.20
Stigmasterol*		0.75	0,77	0.61	0.79	0.55	0.46	0.49
γ-Sitosterol*		2.27	2,36	1.88	2.07	1.91	1.69	1.35
Total sterols		3.70	3.80	2.75	3.35	3.11	2.71	2.40
TRITERPENS								
Squalene *		0.36	0,48	0.52	0.45	0.52	0.31	1.41
β-Amyrine*		0.59	0,36	0.82	0.56	0.31	0.56	0.44
α-Amyrine*		0.39	0,28	0.23	0.57	0.41	0.53	0.42
Triterpene alcohol [†] *		0.33	0,22	0.27	0.24	0.13	0.17	0.11
Total triterpens		1.67	2.01	1.84	1.82	1.37	1.57	2.38
OTHER COMPOUNDS								
Hexahydrofarnesil acetone	1946.6	-	0,08	0.17	0.12	0.09	0.10	0.08
TOTAL		85.15	98.68	90.90	83.29	80.94	85.47	83.06

* - identification according NIST or Wiley (without AMDIS), [†] - tentative identification (AMDIS); (-) - not found; KIE – estimated retention indices

Fig. 1. The content of different types of components in the *n*-hexane extracts of *Sideritis scardica*

Fig. 2. The content of different types of components in the *n*-hexane extracts of *Sideritis raeseri*

type of diterpenes (Gomez-Serranillos et al., 1997, 1998; Topku et al., 2001, 2002). Seasonal variation in the content of some diterpenes were registered and the most variable was the most dominant diterpenes, mainly in the *n*-hexane extracts of *S. scardica* (Table 2 and 3).

In *n*-hexane extracts of *S. scardica* and *S. scardica* x *S. syriaca* hybride, Kostadinova et al. (2008) found predom-

inant presence of diterpene fractions in both species, in amounts from 13.8-54.1 %. Ten diterpene components were registered and only three were fully identified (manoyl oxide, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,1,4a,7-tetramethyl-2-phenanthrenol and pimara-8,15-diene-3-ol. The other diterpene components had following molar mass: 288, 286, 290, 304 and 346. The most abun-

dant was M=346 diterpene. Comparing these data with our results, some similarities could be found. We also registered diterpene M=346, but this component was present in a very small amount in comparison to other components and especially to components with M=286. As there were no other literature data considering diterpene components in *S. scardica* and *S. raeseri*, we made some comparisons with other *Sideritis* species. Thus, Topcu et al. (2001) have defined ten diterpenes in *Sideritis argyrea*: candol, 7-epicandicanol, *ent*-7 α -acetoxy-18-hydroxykaur-16-ene, foliol, linearol, sidol, 7-epocandicanol 18-monoacetate, siderol and 11,12-sideridiol, all of them with kaurane skeleton and only one labdane diterpene named *ent*-6 α ,8 α -dihydroxyabda-13(16)14-diene. Later, the same research group published that, besides above mentioned components, *Sideritis sipilea* and *S. dichotoma* contain also different kaurane isomers, but also some beyerane components (Topcu et al., 2002). In Iberian's *Sideritis* species, Gomez-Serranillos et al. (2004) determined the content of ten diterpenes: serradiol, linearol, conchtriol, foliol, isofolol, andalusol, lagascatriol, tobarrol, sidol and siderol, seven of them kauranes.

In general, diterpenes represented very interesting group of components because of their chemistry and their distribution in plants, the possibilities for their use as markers in order to find or approve some taxonomy solutions, but much more because of their possible biological and pharmacological activity and further use in healing illnesses or other use. For these reasons, diterpenes of *Sideritis scardica* and *S. raeseri* should be further more investigated, for full identification and determination and assessment of possible biological activity.

Conclusion

The ultrasonic-assisted extraction process with *n*-hexane on dried over-ground parts of two species of *Sideritis* collected on different locations in western part of Macedonia and south-eastern part of Albania, yielded 0.73-3.33 % and 9.11 - 10.44 % of extracts for *S. scardica* Grieseb. and *S. raeseri* Boiss. & Heldr., respectively. Over one hundred constituents of the extracts were identified using gas-chromatography (FID) and gas-chromatography/mass spectrometry methods. The components belonged to several classes of components such as diterpenes and hydrocarbons, dominantly presented in each of the extracts, followed by fatty acids, aliphatic and aromatic alcohols, sterols, triterpene alcohols and different monoterpenes and sesquiterpenes, found in much smaller amounts or traces. The most abundant constituents of the extracts of both species of *Sideritis* were two diterpene components, both with M=286, which were not fully identified. Large percentages of nonacosane (1.71-12.22% and 7.46-19.68% for *S. scardica* and *S. raeseri*, respectively) and hentriacontane (4.48-20.79% and 8.09-30.31 % for *S. scardica* and *S. raeseri*, respectively) were also found in the extracts of both species. Season-

al variation in the content of some diterpenes and hydrocarbons was found for the both species of *Sideritis*.

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Резиме

Хемиски состав на *n*-хексански екстракти добиени со ултразвучна екстракција од *Sideritis scardica* Griseb. и *Sideritis raeseri* Boiss. & Heldr. (Lamiaceae) од Македонија и Албанија

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Клучни зборови: *Sideritis scardica*, *Sideritis raeseri*, *n*- хексански екстракти, ултразвучна екстракција, состав, GC-MS анализа.

Хемиски состав на *n*-хексански екстракти добиени од суви надземни делови од два вида *Sideritis*, *S. scardica* Griseb. и *S. raeseri* Boiss. & Heldr. (Lamiaceae) е испитуван со помош на гасна хроматографија (GC/FID) и гасна хроматографија/масена спектрометрија (GC/MS). Собирањето на растенијата е извршено на различни локалитети во западниот дел на Македонија и во јужниот дел на Албанија, вкупно дванаесет различни примероци на растителен материјал. Ултразвучната екстракција е користена за подготовка на *n*-хексански екстракти, а добиениот принос се движи од 0,73-3,33% и 9,11-10,44% на екстракти на *S. scardica*, односно *S. raeseri*. Во екстрактите се идентификувани повеќе од сто компоненти, кои што припаѓаат на неколку класи соединенија како што се дитерпени и јаглеводороди, главно присутни во секој екстракт, проследени со масни киселини, алифатични и ароматични алкохоли, стероли, тритерпенски алкохоли и разни монотерпени и сесквитерпени, што се најдени во многу помали количества или само во траги. Најзастапени компоненти на екстрактите од двата видови *Sideritis* се две дитерпенски компоненти, двете со *M* = 286, кои што не се целосно идентификувани. Голем процент од наонакозан (1,71-12,22% и 7,46-19,68% за *S. scardica*, односно *S. raeseri*) и хентриаконтан (4,48 - 20,79% и 8,09-30,31% за *S. scardica*, односно *S. raeseri*) се најдени во екстрактите од двата вида.