www.shd.org.rs

Journal of the Serbian Chemical Society

JSCS@tmf.bg.ac.yu • www.shd.org.rs/JSCS

J. Serb. Chem. Soc. 74 (1) 35–44 (2009)
JSCS–3806

UDC **Ramonda serbica* Panč.:
:556.132.6:665.52/.54
Original scientific paper

Volatiles from vegetative organs of the palaeoendemic resurrection plants *Ramonda serbica* Panč. and *Ramonda nathaliae* Panč. *et* Petrov.

NIKO S. RADULOVIĆ[1]*, POLINA D. BLAGOJEVIĆ[1], RADOSAV M. PALIĆ[1], BOJAN K. ZLATKOVIĆ[2] and BRANKA M. STEVANOVIĆ[3]

[1]*Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš,* [2]*Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš and* [3]*Department of Plant Ecology and Phytogeography, Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade, 11000 Belgrade, Takovska 43, Serbia*

(Received 16 July 2008)

Abstract: GC and GC/MS analyses of the essential oils hydrodistilled separately from fresh leaves and roots of *Ramonda serbica* and *Ramonda nathaliae*, together with diethyl ether extracts of their roots, enabled the identification of 82 constituents accounting for between 88.9 and 94.5 % of the oils and extracted compounds. Although phenylacetaldehyde was one of the major contributors (20.5–57.1 %) of all the oils, it was only a minor contributor to the extracts. The latter were characterized by a large amount of squalene (*R. serbica* – – 36.0 %; *R. nathaliae* – 59.4%) and steroids (*R. serbica* – 27.4 %; *R. nathaliae* – – 14.1 %). Squalene was also the most abundant compound in *R. nathaliae* root oil (29.0 %), but was not detected in the corresponding *R. serbica* oil. While the root oils and extracts of both species contained comparable amounts of volatile fatty acids, there were significant differences in their contents in the oils hydrodistilled from the leaves of *R. serbica* and *R. nathaliae* (18.7 % and 0.6 %, respectively). The presented results provide the first insight into the unique sets of volatiles produced by these distinctive, closely related, relict taxa, which disclose their specific adaptive advantages.

Keywords: Ramonda serbica; *Ramonda nathaliae*; essential oil; ether extract; squalene; phenylacetaldehyde.

INTRODUCTION

The genus *Ramonda* (Gesneriaceae) includes three relict palaeoendemic species of the Tertiary Period, surviving as the rare resurrection angiosperms of the Northern hemisphere in refugia habitats on the Balkan (*Ramonda nathaliae* Panč.

* Corresponding author. E-mail: vangelis0703@yahoo.com
doi: 10.2298/JSC0901035R

et Petrov. and *Ramonda serbica* Panč.) and Iberian (*Ramonda myconi* (L.) Rchb.) Peninsulas. Their current distribution is restricted to the northern rocky slopes of gorges and canyons, mainly on foothills, sometimes reaching the alpine belts.[1,2] They all prefer limestone rocks, while *R. nathaliae* also settles on ophiolitic bedrock.[1,3] The ranges of the Balkan *Ramonda* species, discovered at the beginning of the 19th century by the Serbian botanists Pančić and Petrović, overlap in SE Serbia, constituting a sympatric zone with mixed or spatially very close populations. The distribution of *R. serbica* thus extends from NW Bulgaria, SE and SW Serbia, NE and SE Montenegro to Albania, W FYR Macedonia and NW Greece, while *R. nathaliae* is spread in N and C FYR Macedonia and N Greece, with a disjunct part of the range in SE Serbia.[1,2,4,5] It is noteworthy that the natural hybridization, proposed to occur between *R. serbica* and *R. nathaliae*, has been confirmed by genome size analysis in the sympatric populations from their localities in SE Serbia.[6]

Ramonda species have been the subject of several biological, genetic, and biochemical studies concerning the defence mechanism and the physiological changes that occur during dehydration and rehydration, their genome size and variation of the ploidy level, polymorphism, *etc*.[6–13] The volatile constituents of the vegetative organs of the *Ramonda* species, however, have not been studied. The aim of this study was to determine the chemical composition of the essential oils of the leaves and roots and of the root diethyl ether extracts of *R. serbica* and *R. nathaliae*, and, if possible, to clarify the differentiation in the taxonomic relationship between the two taxa, from their volatile profiles.

EXPERIMENTAL

Plant material

Leaves and roots of the two Balkan *Ramonda* species were collected at the beginning of May, 2007. Sample sites included two distant populations, one located on the slopes of Suva planina (*R. nathaliae*, voucher No. 16208) and the other in Sićevačka klisura (*R. serbica*, voucher No. 20639) in SE Serbia. Voucher specimens were deposited in the Herbarium of the Institute of Botany and Botanical Garden (BEOU) "Jevremovac", Department of Plant Ecology and Phytogeography, Faculty of Biology, University of Belgrade.[14]

Isolation of the essential oils and preparation of the extracts

The minimal amount (500 g) of plant material (fresh leaves or roots of *R. serbica* and *R. nathaliae*) was subjected to hydrodistillation for 2.5 h using a Clevenger-type apparatus. The oils obtained were separated, dried over anhydrous sodium sulphate and immediately analyzed. The yields (%, w/w) were the following: *R. serbica* root oil, 0.002; *R. serbica* leaf oil, 0.002; *R. nathaliae* root oil, 0.004 and *R. nathaliae* leaf oil, 0.003.

Roots of *R. serbica* and *R. nathaliae* (50 g) were cut into small pieces and extracted in sealed vessels with 250 mL of diethyl ether in an ultrasonic bath (Bandelin electronic, GmbH & Co. KG, Germany) for 5 h at room temperature. The extracts were gravity filtered through small columns packed with 1 g of Celite® (Merck, Germany), to remove all the insoluble material, and then concentrated to 10 mL at room temperature using a stream of nitrogen be-

fore GC and GC/MS analyses. The yields of dry extracts (%, w/w), obtained by complete evaporation of the solvents *in vacuo*, were 0.037 (*R. serbica*) and 0.052 (*R. nathaliae*).

Gas chromatography and gas chromatography/mass spectrometry

The GC/MS analyses (three repetitions of each sample) were performed using a Hewlett--Packard 6890N gas chromatograph equipped with a fused silica capillary column HP-5MS (5 % phenylmethylsiloxane, 30 m×0.25 mm, film thickness 0.25 μm, Agilent Technologies, USA) and coupled with a 5975B mass selective detector from the same company. The injector and interface were operated at 250 and 300 °C, respectively. The oven temperature was raised from 70 to 290 °C at a heating rate of 5 °C/min and then held isothermally for 10 min. Helium at 1.0 mL/min was used as the carrier gas. 1 μL of the oil solution in diethyl ether (1:100) or the extract, prepared as earlier mentioned, was injected in a pulsed split mode (the flow was 1.5 mL/min for the first 0.5 min and then set to 1.0 mL/min throughout the remainder of the analysis; split ratio 40:1). The MS conditions were: ionization voltage 70 eV, acquisition mass range 35–500, scan time 0.32 s. The oil and extract constituents were identified by comparing their linear retention indices (relative to C_7–C_{33} alkanes[15] on the HP-5MS column) with literature values[16] and their mass spectra with those of authentic standards, as well as those from Wiley 6, NIST02, MassFinder 2.3, and a homemade MS library with the spectra corresponding to pure substances and components of known oils. Wherever possible, the identity of the constituents was verified by co-injection with an authentic sample. GC (FID) analysis was performed under the same experimental conditions using the same column as described for the GC/MS measurements. The percentage composition of the oil was computed from the GC peak areas without any corrections.

RESULTS AND DISCUSSION

GC and GC/MS analyses of *R. serbica* and *R. nathaliae* essential oils and extracts enabled the overall identification of 82 components, listed in Table I. The common feature of the oils was their high content of phenylacetaldehyde (from 20.5 to 57.1 %), a constituent found only as a minor contributor to the root extracts from the two plant species. The diethyl ether extracts, on the other hand, were characterized by large amounts of squalene (36.0 % in *R. serbica* and 59.4 % in *R. nathaliae*) and steroids (27.4 % in *R. serbica* and 14.1 % in *R. nathaliae*). In addition, constituents belonging to the following classes: terpenoids, alkanes, fatty acids, carotenoid derived compounds and "green leaf" volatiles, were present in almost all the oils and extracts (Table I). It might be assumed that certain volatiles listed in Table I could be considered as artefacts of the isolation procedure and not as direct products of plant metabolism. For example, 3-(methylthio)propanal and 2-acetylthiazole (identified only in the volatile oils) could be products of Maillard-type reactions including the thermal fragmentation of amino acids and sugars, alone or in conjunction, during hydrodistillation.[17] The "green leaf" volatiles, on the other hand, are most probably produced by enzymatic degradation of unsaturated fatty acids, such as in desiccation, *i.e.*, as a stress-induced response of the plants, produced during collection and preparation of the plant samples.[18] Some scent compounds identified in the analyzed essential oils and extracts of *R. serbica* and *R. nathaliae* could be related to their pollination

biology, since it has been shown that a blend containing phenylacetaldehyde, 2-phenylethanol, and benzaldehyde (compounds present in the analyzed oils and extracts, Table I) is attractive to halictid bees.[19]

TABLE I. Percentage composition of *R. serbica* and *R. nathaliae* leaf and root essential oils and root diethyl ether extracts

RI[a]	Compound	Class	Method[b]	*R. serbica*			*R. nathaliae*		
				Root oil	Leaf oil	Root extract	Root oil	Leaf oil	Root extract
753	2-Methylpropanoic acid	F	a, b, c						0.1
763	Butanoic acid	F	a, b, c			0.2			
765	3-Methyl-2-buten-1-ol	T	a, b		0.4				
827	3-Methylbutanoic acid (syn.[c] Isovaleric acid)	T	a, b, c			2.9			
828	Furfural	GL	a, b, c	3.0	0.4			tr[d]	
832	2-Methylbutanoic acid	T	a, b, c			0.7	0.3		0.3
833	3-Methylpentanol	GL	a, b			tr			
844	(*E*)-3-Hexenol	GL	a, b		0.2				
908	3-(Methylthio)propanal	O	a, b	1.0	0.4				
965	Benzaldehyde	O	a, b, c	3.3	0.2	0.2	1.0	1.8	tr
970	Hexanoic acid	F	a, b, c			tr			
978	1-Octen-3-ol	GL	a, b		5.0	tr		8.1	
995	2-Pentylfuran	GL	a, b			tr			
1014	(*E*,*E*)-2,4-Heptadienal	GL	a, b					tr	
1021	2-Acetylthiazole	O	a, b		0.2				
1037	Benzyl alcohol	O	a, b, c			0.2	0.7	0.5	
1047	Phenylacetaldehyde	O	a, b, c	57.1	41.6	tr	20.5	52.2	0.2
1056	5-Methyldecane	A	a, b	tr	tr	tr			
1061	4-Methyldecane	A	a, b	2.3	tr	tr			
1070	Acetophenone	O	a, b, c	1.7	0.4	tr			
1100	Undecane	A	a, b, c	3.6	tr	tr			
1102	Linalool	T	a, b, c		1.3		0.6	0.8	
1108	(*Z*)-6-Methyl-3,5--heptadien-2-one	CR	a, b	3.3				1.2	
1118	2-Phenylethanol	O	a, b, c			0.8	0.6	tr	tr
1126	Isophorone	CR	a, b					tr	
1163	Benzoic acid	O	a, b, c			tr			
1167	*o*-Hydroxyacetophenone	O	a, b, c		tr				
1170	3,5-Dimethylphenol	O	a, b	4.0	2.1		tr		
1172	Borneol	T	a, b, c				0.9	1.1	
1196	α-Terpineol	T	a, b, c		tr			tr	
1252	Phenylacetic acid	O	a, b			1.8			tr
1257	Geraniol	T	a, b, c				0.8		
1280	3-Methyldodecane	A	a, b	tr	2.3	0.2			
1294	Thymol	T	a, b, c		0.6		1.8		
1300	Tridecane	A	a, b, c	tr	tr				

TABLE I. Continued

RI[a]	Compound	Class	Method[b]	*R. serbica*			*R. nathaliae*		
				Root oil	Leaf oil	Root extract	Root oil	Leaf oil	Root extract
1318	*p*-Vinylguaiacol	O	a, b	0.7	0.2		tr	tr	
1320	(*E*,*E*)-2,4-Decadienal	GL	a, b				0.9	0.8	
1405	Vanillin	O	a, b, c			tr			
1500	Pentadecane	A	a, b, c	3.6	1.9	0.2	0.4		
1525	1,2-Diphenylethane	O	a, b				0.4	5.7	
1585	4,6,8-Megastigmatrien--3-one[e]	CR	a, b		1.1				
1631	4,6,8-Megastigmatrien-3-one[e]	CR	a, b		0.8				
1661	(*E*)-4-Oxo-*β*-ionone	CR	a, b			tr			
1667	Syringaldehyde (syn. 3,5-Dimethoxy--4-hydroxybenzaldehyde)	O	a, b			tr			
1694	1-Heptadecene	AE	a, b					0.6	
1700	Heptadecane	A	a, b, c					0.3	
1755	5-Methylheptadecane	A	a, b	tr	1.1	tr	tr		
1762	Tetradecanoic acid	F	a, b, c		0.6	tr			
1796	Dehydrovomifoliol (syn. 7-Hydroxy-3--oxo-*α*-ionone)	CR	a, b			0.4			
1800	Octadecane	A	a, b, c				tr		
1823	Syringic acid (syn. 3,5-Dimethoxy--4-hydroxybenzoic acid)	O	a, b						1.0
1841	Neophytadiene Isomer I	T	a, b			tr			
1847	Hexahydrofarnesyl acetone	CR	a, b		0.6		0.7	1.7	
1944	(*E*)-9-Hexadecenoic acid (syn. (*E*)-palmitoleic acid)	F	a, b			1.0			
1952	(*Z*)-9-Hexadecenoic acid (syn. (*Z*)-Palmitoleic acid)	F	a, b			0.7			
1963	Hexadecanoic acid (syn. Palmitic acid)	F	a, b, c	5.3	18.1	5.8	6.2	0.6	3.3
1994	1-Eicosene	AE	a, b		0.8				
2000	Eicosane	A	a, b, c		tr		tr	tr	
2100	Heneicosane	A	a, b, c		0.4		tr	0.5	
2136	(*Z*,*Z*)-9,12-Octadecadienoic acid (syn. Linoleic acid)	F	a, b			6.0			7.2
2141	(*E*)-9-Octadecenoic acid (syn. Elaidic acid)	F	a, b, c			3.1			
2143	(*E*,*Z*)-9,12-Octadecadienoic acid	F	a, b						4.8

TABLE I. Continued

RI[a]	Compound	Class	Method[b]	*R. serbica*			*R. nathaliae*		
				Root oil	Leaf oil	Root extract	Root oil	Leaf oil	Root extract
2146	(*Z*)-9-Octadecenoic acid (syn. Oleic acid)	F	a, b, c			3.2			
2163	Octadecanoic acid (syn. Stearic acid)	F	a, b, c			0.5			
2194	1-Docosene	AE	a, b		0.8				
2200	Docosane	A	a, b, c	tr				tr	
2300	Tricosane	A	a, b, c				1.9	1.2	
2400	Tetracosane	A	a, b, c				0.4	tr	
2469	4-Methyltetracosane	A	a, b		0.6				
2500	Pentacosane	A	a, b, c			0.2	13.0	7.7	0.3
2600	Hexacosane	A	a, b, c				1.1	0.5	0.2
2662	4-Methylhexacosane	A	a, b				1.5	0.5	
2686	3-Methylhexacosane	A	a, b	tr	4.6				
2694	1-Heptacosene	AE	a, b				0.3		
2700	Heptacosane	A	a, b, c			0.6	9.0	4.2	0.3
2834	Squalene (all *E*)	T	a, b, c			36.0	29.0		59.4
2900	Nonacosane	A	a, b, c			1.2	1.1	0.5	0.2
3000	Triacontane	A	a, b, c		1.9				
3100	Hentriacontane	A	a, b			1.2			
3187	Stigmast-5-en-3β-ol (syn. β-Sitosterol)	S	a, b			22.7			10.8
3200	Dotriacontane	A	a, b, c		2.1				
3237	Stigmast-4-en-3-one	S	a, b			4.7			3.3
	Total			88.9	90.7	94.5	93.1	90.5	91.4
	Terpenoids (T)				2.3	39.6	33.4	1.9	59.7
	Alkanes (A)			9.5	14.9	3.6	28.4	15.4	1.0
	n-Alkanes			7.2	8.6	3.6	26.9	14.9	1.0
	Branched alkanes			2.3	6.3	tr	1.5	0.5	
	1-Alkenes (AE)				1.6		0.3	0.9	
	Fatty acids (F)			5.3	18.7	20.3	6.2	0.6	15.3
	"Green leaf" volatiles (GL)			3.0	5.6	0.2	0.9	8.9	0.1
	Carotenoid derived compounds (CR)			3.3	2.5	0.4	0.7	2.9	
	Steroids (S)					27.4			14.1
	Others (O)			67.8	45.1	3.0	23.2	60.2	1.2

[a]Compounds listed in order of elution from a HP-5MS column (*RI* - experimentally determined retention indices on the mentioned column by co-injection of a homologous series of *n*-alkanes C_7–C_{33}); [b]a - constituent identified by mass spectra comparison, b - constituent identified by retention index matching; c - constituent identity confirmed by co-injection of an authentic sample; [c]synonym; [d]trace (<0.05 %); [e]correct stereoisomer not determined

To some extent, the limited production of volatile secondary metabolites by these *Ramonda* taxa is not surprising, knowing that they are palaeoendemic, evo-

lutionarily very old species. Moreover, the similarity in the chemical composition of their analyzed oils and extracts (Table I) was also expected, bearing in mind the close relationship of the species.[7] However, there are certain striking differences, for example, the fatty acids, one of the dominant compound classes in *R. serbica* leaf oil, were found only as a negligible percentage in the corresponding *R. nathaliae* oil. Furthermore, the root oil of *R. nathaliae* was characterized by a significant amount of terpenoid compounds, primarily squalene, while no terpenoids were identified in the *R. serbica* root oil. Even though squalene was by far the most dominant constituent of both extracts, the relative percentage of this compound was markedly higher in that obtained from *R. nathaliae*. A recent study concerning the genome size variation and polyploidy of *Ramonda* species showed that *R. serbica* is a hexaploid ($2n = 6x = 144$) and *R. nathaliae* a diploid species ($2n = 2x = 48$).[7] This difference in the ploidy level could be correlated to the more pronounced production and/or accumulation of squalene in *R. nathaliae*. For instance, the existence of a correlation between the production of proazulene compounds and ploidy level has been confirmed for some *Achillea* species.[20] In addition, squalene has long been known to exhibit antioxidant properties[21] and, therefore, could enhance the antioxidative system, which was essential for the survival of these poikilohydric or resurrection plants during their repeated dehydration/rehydration cycles. Squalene, which is related to cholesterol biosynthesis may be important in membrane conservation in anabiosis. Sterols are found predominantly in cell membranes and are thought to contribute to the correct functioning of membranes by controlling the fluidity characteristics of the membranes.[22,23] Squalene synthase represents a putative branch point in the isoprenoid biosynthetic pathway capable of diverting the carbon flow specifically to the biosynthesis of sterols and, hence, is considered a potential regulatory point for sterol metabolism.[24] Squalene epoxidase converts squalene into oxidosqualene, the precursor of all known plant sterols.[25] The results of a recent study on regulation of squalene synthase in tobacco suggests that sterol biosynthesis is localised to the apical meristems and that the apical meristems may be a source of sterols for other plant tissues.[24] Thus, it is important to stress that only the root volatiles (either the essential oils or extracts) consisted of a large quantity of squalene and steroids. It seems that since the apical meristems are mostly related to the root system, the present findings corroborate the correlation between the sterol biosynthesis and the meristem. Still, further research is required in this direction for the complete understanding of this special feature of resurrection plants to survive anabiosis for months with 2–5 % of relative water content in their leaves.

Another interesting feature of all of the oils and of the *R. serbica* extract was the presence of branched alkanes as minor contributors. Bearing in mind that these are rather rare in the plant kingdom and that related compounds were found

in the essential oils obtained from some other evolutionarily primitive and old higher plant taxa, such as *Equisetum*,[26,27] it could be assumed that the presence of branched alkanes could be a characteristic of old plant taxa.

Moreover, a comparison of the chemical composition of the oils obtained from the root and leaves of both *R. serbica* and *R. nathaliae* shows that there are certain dissimilarities between the production and/or accumulation of plant organ volatiles (Table I). This suggests that different species-specific biosynthetic pathways leading to the volatile constituents are operational, or at least favoured, in plant organs of each of these species.

As was already mentioned, the volatiles extracted from the taxa belonging to the genus *Ramonda* were not previously analyzed in general. In addition, there is only one reference concerning the essential oil of a species (*Sinningia aggregata*) from the same family (Gesneriaceae).[28] However, the volatile profiles of *S. aggregata* (main contributors of the oil were methyl linoleate (28.4 %), 1-octadecanol (16.9 %), (*Z*)-nerolidyl acetate (8.8 %), spathulenol (7.8 %) and (*E*)-nerolidol (6.7 %)) and of the currently analyzed *Ramonda* species differed significantly. For example, sesquiterpenoids were not among the identified volatiles in either *R. serbica* or *R. nathaliae*, while these comprised *ca*. one third of the *S. aggregata* oil. This distinction of the *Ramonda* oils is even more obvious by further comparison of *Ramonda* volatiles to those of plant taxa belonging to the recognized "aromatic" families (*e.g*., Asteraceae, Apiaceae, Lamiaceae). While the "aromatic" species, rich in essential oils, with yields ranging from 0.1–10.0 % (*cf.* to the value ≈ 0.001 % for the presently investigated *Ramonda*), predominantly produce volatile mono- and sesquiterpenoids and/or phenylpropanoids, these compound classes were represented as only minor contributors, or were not identified at all in the oils and extracts of *Ramonda*.[29–31]

CONCLUSIONS

The identification of 82 compounds as constituents of the essential oils hydrodistilled separately from the fresh leaves and roots of *Ramonda serbica* and *Ramonda nathaliae*, together with the diethyl ether extracts of their roots, studied for the first time, revealed phenylacetaldehyde as one of the major contributors (20.5–57.1 %) of all the oils, but only as a minor contributor of the extracts. The latter were characterized by large amounts of squalene (*R. serbica,* 36.0 %; *R. nathaliae,* 59.4 %) and steroids (*R. serbica*, 27.4 %; *R. nathaliae*, 14.1 %). Squalene was also the most abundant compound in *R. nathaliae* root oil (29.0 %), but was not detected in the corresponding *R. serbica* oil. The difference in the ploidy level of the two *Ramonda* species could be correlated to the more pronounced production and/or accumulation of squalene in *R. nathaliae*. Resurrection is a rare phenomenon in the plant kingdom and some volatiles, such as squalene, which is related to cholesterol biosynthesis, may be important in membrane con-

servation in anabiosis. It can be concluded that these essential oil poor *Ramonda* species have not developed an elaborate metabolic pathway for the production of specific volatiles but the identified constituents are made up of compounds originating from non-specific omnipresent pathways (triterpenoid steroid biosynthesis branch, oxylipin metabolism, oxidative degradation of carotenoids and fatty acids' pathways).

Acknowledgements. This work was funded by the Ministry of Science and Technological Development of the Republic of Serbia (Projects 143015 and 142054 B).

ИЗВОД

ИСПАРЉИВИ КОНСТИТУЕНТИ ВЕГЕТАТИВНИХ ОРГАНА ПАЛЕОЕНДЕМИЧНИХ „ВАСКРСАВАЈУЋИХ“ БИЉНИХ ВРСТА *Ramonda serbica* PANČ. И *Ramonda nathaliae* PANČ. *et* PETROV.

НИКО С. РАДУЛОВИЋ[1], ПОЛИНА Д. БЛАГОЈЕВИЋ[1], РАДОСАВ М. ПАЛИЋ[1], БОЈАН К. ЗЛАТКОВИЋ[2] и БРАНКА М. СТЕВАНОВИЋ[3]

[1]Одсек за хемију, Природно–математички факултет, Универзитет у Нишу, Вишеградска 33, 18000 Ниш, [2]Одсек за биологију и екологију, Природно–математички факултет, Универзитет у Нишу, Вишеградска 33, 18000 Ниш и [3]Катедра за екологију и географију биљака, Институт за ботанику и ботаничка башта "Јевремовац", Биолошки факултет, Универзитет у Београду, Таковска 43, 11000 Београд

Етарска уља добијена хидродестилацијом листова и корена балканских ендемореликтних биљака *Ramonda serbica* и *Ramonda nathaliae*, као и етарски екстракти корена ових врста, анализирани су комбинацијом GC и GC/MS. Идентификоване су укупно 82 компоненте које су чиниле од 88,9 до 94,5 % уља, тј. екстраката. У свим анализираним уљима главна или једна од главних компоненти био је фенилетанал (20,5–57,1 %), у екстрактима присутан тек у занемарљивом проценту. Насупрот томе, екстракти обе врсте били су окарактерисани високим садржајем сквалена (36,0% у *R. serbica* и 59,4 % у *R. nathaliae*) и стероида (27,4 % у *R. serbica* и 14,1 % у *R. nathaliae*). Сквален је био и најзаступљенија компонента у етарском уљу корена *R. nathaliae* (29,0 %), али није детектован у одговарајућем уљу врсте *R. serbica*. Поред тога, садржај масних киселина, упоредив у уљима и екстрактима корена обе анализиране врсте, знатно се разликовао у уљима добијеним из листова врста *R. serbica* и *R. nathaliae* (18,7 и 0,6 %). Презентовани резултати анализе испарљивих конституената *R. serbica* и *R. nathaliae* говоре по први пут о фитохемијској различитости ове две сродне, ендемичне и реликтне врсте, указујући истовремено на њихове диференцијалне адаптивне одлике.

(Примљено 16. јула 2008)

REFERENCES

1. N. Kosanin, *Spomenik Srp. Kralj. Akad*. **20** (1939) 1 (in Serbian)
2. K. F. Meyer, *Math. Naturwiss. Reiche* **19** (1970) 401
3. V. Stevanović, B. Stevanović, *Glas. Prir. Muz. B* **40** (1985) 75 (in Serbian)
4. V. Stevanović, M. Niketić, B. Stevanović, *Glas. Prir. Muz. B* **41** (1986) 89 (in Serbian)
5. V. Stevanović, M. Niketić, B. Stevanović, *Glas. Ins. Bot. Univerziteta u Beogradu* **20** (1986) 45 (in Serbian)
6. S. Siljak-Yakovlev, V. Stevanović, M. Tomasević, S. C. Brown, B. Stevanović, *Environ. Exp. Bot.* **62** (2008) 101

7. C. Sgherri, B. Stevanović, F. Navari-Izzo, *Physiol. Plant*. **122** (2004) 478
8. S. Canigueral, B. M. Muller, M. J. Salvia, J. Iglesias, G. Franz, *Planta Med*. **60** (2008) 73
9. S. Canigueral, M. J. Salvia, R. Vila, J. Iglesias, A. Virgili, T. Parella, *J. Nat. Prod.* **59** (1996) 419
10. M. Quartacci, O. Glisić, B. Stevanović, F. Navari-Izzo, *J. Exp. Bot.* **53** (2002) 2159
11. H. L. Citerne, M. Moller, Q. C. B. Cronk, *Ann. Bot.* **86** (2000) 167
12. F. X. Pico, M. Molller, N. J. Ouborg, Q. C. B. Cronk, *Plant Biol*. **4** (2002) 62
13. B. J. M. Zonneveld, I. J. Leitch, M. D. Bennett, *Ann. Bot.* **96** (2005) 229
14. P. K. Holmgren, N. H. Holmgren, L. C. Barnett, **120** (1990) 1, continuously updated at http://sciweb.nybg.org/science2/IndexHerbariorum.asp
15. H. Van Den Dool, P. D. Kratz, *J. Chromatogr.* **11** (1963) 463
16. R. P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th Ed., Allured Publishing Corporation, Carol Stream, IL, 2007
17. Y.-C. Oh, T. G. Hartman, C.-T. Ho, *J. Agric. Food Chem.* **40** (1992) 1878
18. B. A. Roy, R. A. Raguso, *Oecologia* **109** (1997) 414
19. A. Hanataka, T. Kajiwara, K. Matsui, *Z. Naturforsch. C* **50** (1995) 467
20. M. Maffei, F. Chailva, A. Codignola, *J. Essent. Oil Res.* **2** (1989) 57
21. M. A. Dessi, M. Deiana, B. W. Day, A. Rosa, S. Banni, F. P. Corongiu, *Eur. J. Lipid Sci. Technol*. **104** (2002) 506
22. M. Hartmann, *Trends Plant Sci*. **3** (1998) 170
23. M. Hartmann, P. Benveniste, *Methods Enzymol.* **148** (1987) 632
24. T. Devarenne, A. Ghosh, J. Chappell, *Plant Physiol.* **129** (2002) 1095
25. J. Rasbery, H. Shan, R. LeClair, M. Norman, S. Matsuda, B. Bartel, *J. Biol. Chem*. **282** (2007) 17002
26. V. Milovanović, N. Radulović, V. Mitić, R. Palić, G. Stojanović, *J. Essent. Oil Res.* **20** (2008) 310
27. N. Radulović, G. Stojanović, V. Milovanović, D. Djoković, V. Randjelović, *J. Essent. Oil Res*. **20** (2008) 437
28. M. Stefanello, A. Cervi, A. Wisniewski Jr., *Braz. J. Pharm*. **15** (2005) 331
29. L. Rodriguez-Hahn, J. Cardens, *Curr. Topics Phytochem.* **2** (1999) 91
30. C. Kapetanos, A. Karioti, S. Bojović, P. Marin, M. Veljić, H. Skaltsa, *Chem. Biodiversity* **5** (2008) 101
31. N. Radulović, B. Zlatković, R. Palić, G. Stojanović, *Nat. Prod. Commun*. **2** (2007) 453.