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Essential oil analysis of two endemic *Eryngium* species from Serbia

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Abstract: The volatile composition of two *Eryngium* species was studied. The essential oils were obtained by hydrodistillation in a modified Clevenger-type apparatus, and their analyses were performed by GC and GC–MS. A total of 58 different compounds were identified. Their main constituents were as follows: *E. serbicum*: germacrene D (19.7 %), *β*-elemene (10.0 %) and spathulenol (6.9 %); *E. palmatum*: sesquicineole (21.3 %), caryophyllene oxide (16.0 %), spathulenol (16.0 %) and sabinene (5.5 %). The main portion in both studied taxa consisted of sesquiterpenes.

Keywords: *E. palmatum* Vis. et Pančić, *E. serbicum* Pančić, *Apiaceae*, volatile constituents, sesquiterpene hydrocarbons.

INTRODUCTION

The genus *Eryngium* L. comprises 220–250 species. It belongs to the tribe Saniculeae, subfamily Saniculoideae of the Apiaceae family. In the flora of Europe, *ca*. 26 species are present,[1] among them five species are found in the flora of Serbia.[2]

In the present study, two endemic taxa were examined: *E. palmatum* Vis. et Pančić and *E. serbicum* Pančić. *E. palmatum* is an endemic perennial herb, growing in dry places and woods, which is distributed in the central part of the Balkan Peninsula (Serbia, Bulgaria, FYROM and Albania).[1,2] *E. serbicum* is an endemic perennial plant growing only in Serbia in dry habitats.[1,2]

Hitherto, the composition of the volatile compounds is known in only three species: *E. billardieri* F. Delaroche,[3] *E. paniculatum* Cav.[4] and *E. foetidum* L.[4–9]

EXPERIMENTAL

Analysis of volatile compounds

The air-dried plant material (100 g) from each population was cut into small pieces and the essential oils were obtained by hydrodistillation in 500 ml H_2O for 2 h, in a modified Clevenger

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apparatus with a water-cooled oil receiver to reduce over-heating artifacts of hydrodistillation.[10] The oils were taken in 2 ml of capillary GC grade *n*-pentane, dried over anhydrous sodium sulfate and stored at –20 °C. The composition of the volatiles was determined utilizing GC and GC–MS analyses. The GC analysis was carried out on a Perkin Elmer 8500 gas chromatograph with a FID, fitted with a Supelcowax-10 fused silica capillary column (30 m × 0.32 mm (i.d.); film thickness: 0.25 μm). The column temperature was programmed from 75 to 260 ºC at a rate of 2.5 ºC min^{-1}. The injector and detector temperatures were set at 230 and 300 ºC, respectively.

GC-MS analyses were performed on a Hewlett–Packard 5973-6890 system operating in EI mode (70 eV) equipped with a split/splitless injector (220 °C), a split ratio 1/10, using two different columns: a fused silica HP-5 MS capillary column (30 m × 0.32 mm (i.d.), film thickness: 0.25 μm) and a HP-Innowax capillary column (30 m × 0.32 mm (i.d.), film thickness: 0.50 μm). The temperature program for the HP-5 MS column was from 60 °C (5 min) to 280 °C at a rate of 4 °C min^{-1} and for the HP-Innowax column from 60 °C to 260 °C at a rate of 3 °C min^{-1}. Helium was used as the carrier gas at a flow rate of 0.8 ml min^{-1}. Injection volume of each sample was 2 μl. Retention indices for all compounds were determined according to the Van den Dool approach,[11] using *n*-alkanes as standards. The identification of the components was based on comparison of their mass spectra with those of the Wiley Library[12] and those described by Adams,[13] as well as by comparison of their retention indices with literature data.[13,14] In many cases, the essential oils were subject to co-chromatography with authentic compounds (Fluka, Sigma).

Optical rotation values were determined at 25 ºC at 589 nm in dichloromethane.

Plant Material

Aerial parts of both taxa were collected from natural populations during the flowering stage as follows: *E. serbicum* Pančić (ery-1) at Kosovska Mitrovica in June 2003 and *E. palmatum* Vis. et Pančić (ery-2) at Ozren in June 2003.

Voucher specimens of the *Eryngium* species were determined by Dr. P. D. Marin and deposited in the Herbarium of Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade, under the code numbers: ES 6039 and EP 60310, respectively.

RESULTS AND DISCUSSION

As shown in Table I, both essential oils were complex mixtures of about fifty constituents in each investigated case, with the contributions of the main compounds never exceeding 20 % of the total. Among them, sesquiterpenes were the main constituents in both studied taxa (Table II).

The main constituents of the investigated *Eryngium* essential oils are the following: in *E. serbicum* germacrene D (19.7 %), *β*-elemene (10.0 %) and spathulenol (6.9 %); in *E. palmatum* sesquicineole (21.3 %), caryophyllene oxide (16.0 %), spathulenol (6.6 %) and sabinene (4.4 %).

Previously investigated *Eryngium* essential oils had different compositions. In the essential oil of *E. paniculatum*, (*E*)-anethole (52.6 %) was found as main component,[4] while in *E. billardieri*, *α*-muurolene was dominant.[3] Previously, several investigations of the essential oils of *E. foetidum* revealed that fatty acids and aldehydes were the main constituents. Thus, Leclercq *et al.*[9] reported (*E*)-2-dodecenal (45.5 %), dodecanoic acid (15.5 %) and 2-dodecenoic acid (8.6 %) as the main compounds. Wong *et al*.[8] found (*E*)-2-dodecenal (59.7 %) in the leaves, while 2,3,6-trimethylbenzaldehyde was dominant in the root (37.6 %). Martins *et al*.[6] found 2,3,6-trimethylbenzaldehyde (37.5 %) and (*E*)-2-dodecenal (23.7 %)

as the main constituents in different plant parts. More recent investigations[5] also revealed 2,4,5-trimethylbenzaldehyde (27.7 %) and (*E*)-2-dodecenal (27.5 %) as the dominant components. In contrast, Pino *et al.*[7] reported that caryophyllene oxide (19.3 %) was one of the main compounds.

TABLE I. Qualitative and quantitative composition (% v/v) of volatile compounds

	RI^a	RI^b	Ery-1	Ery-2
Heptanal	897		–	0.4
α-Thujene	927		1.4	0.3
α-Pinene	935		5.7	4.4
β-Thujene	966		–	0.2
Sabinene	971	1123	1.5	5.5
β-Pinene	975	1112	4.3	–
2-Pentylfuran	987		–	1.2
Myrcene	989	1160	4.3	–
Octanal	999	1289	3.8	4.4
α-Terpinene	1016	1180	–	0.2
p-Cymene	1024	1268	0.5	0.2
Limonene	1027	1201	1.1	0.6
γ-Terpinene	1055	1243	–	0.4
2-Nonanone	1090	1385	0.4	–
Undecane	1100		-	0.3
Nonanal	1101	1390	0.4	0.8
Pinocarvone	1161	1559	0.3	–
Tepinen-4-ol	1174	1591	–	0.1
Dodecane	1200		–	0.6
2-Decenal	1263	1639	1.2	–
Perillaldehyde	1271	1288	–	0.7
(–)-Bornyl acetate	1288	1570	–	1.6
Tridecane	1300		–	0.3
2,4,5-Trimethylbenzaldehyde	1357	1901	2.2	–
α-Copaene	1375	1481	1.1	0.7
β-Bourbonene	1384	1507	1.1	–
β-Elemene	1390	1578	10.0	0.9
β-Caryophyllene	1416	1584	1.2	2.2
Calarene	1431	1559	0.4	–
α-Humulene	1452	1662	0.4	1.1
trans-*β*-Farnesene	1454	1659	0.6	–
Germacrene D	1476	1709	19.7	0.6
Ar-curcumene	1479		–	0.2
β-Selinene	1489	1717	1.7	–
α-Selinene	1497	1722	1.0	–

TABLE I. Continued

	RI^a	RI^b	Ery-1	Ery-2
Bicyclogermacrene	1497	1732	2.4	–
Ledene	1498	1716	–	0.4
Germacrene A	1499		0.8	–
α-Muurolene	1501		–	0.2
β-Bisabolene	1503	1724	0.7	0.2
Sesquicineole	1515		–	21.3
δ-Cadinene	1520	1755	1.2	0.4
Elemol	1550	2082	–	1.5
1,5-Epoxysalvial-4(14)-ene	1564	1924	1.1	1.2
Spathulenol	1578	2128	6.9	6.6
Caryophyllene oxide	1581	1987	2.6	16.0
β-Copaene-4-α-ol	1590		1.2	–
Salvial-4(14)-en-1-one	1593	2013	3.4	0.6
Nor-copaanone	1597	2156	2.2	–
Vulgarol B	1605		1.3	–
β-Oplopenone	1607	1964	0.5	–
Humulene epoxide II	1608		–	4.8
α-Copaen-8-ol	1626		–	2.5
T-muurolol	1642	2236	–	1.5
Ageratochromene	1656		2.2	–
Caryophylla-4(12),8(13)-dien-5- β-ol	1668	2299	0.9	0.3
Eudesma-4(15),7-dien-1-β-ol	1684		3.9	–
α-Bisabolol	1685	2222	–	6.8
TOTAL			95.6	92.2
$[\alpha]_D^{20}$			-2.55 (CH_2Cl_2 *c* 0.10)	-2.12(CH_2Cl_2 *c* 0.07)

[a]Components listed in order of elution from a HP 5MS column.
[b]*RRI*, relative retention indices calculated against C_9–C_{24} *n*-alkanes on the HP 5MS column (1) and HP Innowax (2) capillary columns, respectively.

TABLE II. Grouped components

	Ery-1	Ery-2
Aliphatics		
Alcanes, alkenes	–	1.2
Aldehydes	7.6	5.7
Ketones	0.4	–
Terpenoids		
Monoterpene hydrocarbons	18.8	11.8
Oxygenated monoterpene	0.3	2.4
Sesquiterpene hydrocarbons	42.3	6.9
Oxygenated sesquiterpene	24.0	63.1
Miscellaneous	–	1.2
Compounds with 13 C	2.2	–

According to the present results, the essential oils of *E. serbicum* and *E. palmatum* have several differences between them, as their main components

differ significantly. *E. palmatum* is characterized by an abundance of sesquicineole (21.3 %), which was absent in *E. serbicum*.

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И З В О Д

АНАЛИЗА ЕТАРСКИХ УЉА ДВЕ ЕНДЕМСКЕ *ERYNGIUM* ВРСТЕ ИЗ СРБИЈЕ

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У раду су испитивани испарљиви састојци две *Eryngium* врсте. Етарска уља су изолована хидродестилацијом у модификованом апарату типа Clevenger и анализирана методама GC и GC–MS. Укупно је идентификовано 58 различитих једињења. Главни састојци испитиваних врста су: у *E. serbicum*: гермакрен D (19,7 %), β-елемен (10,0 %) и спатуленол (6,9 %); у *E. palmatum*: сесквицинео (21,3 %), кариофилен-оксид (16,0 %), спатуленол (16,0 %) и сабинен (5,5 %). Велики део обе испитиване јединке састоји се од сесквитерпена.

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