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Volatile components from aerial parts of *Centaurea spinosociliata* Seenus ssp. *cristata* (Bartl.) Dostál and *Centaurea spinosociliata* Seenus ssp. *spinosociliata* growing wild in Croatia

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Abstract. The paper reports on the volatile components oil from aerial parts of two Asteraceae growing wild in Croatia: *Centaurea spinosociliata* ssp. *cristata* (syn. *Centaurea cristata* Bartl. subsp. *rabenhorstiana* (Sch.Bip.) Nyman) (CSC) and *C. spinosociliata* ssp. *spinosociliata* (CSS). The volatile components were obtained by hydrodistillation from selected plants and were determined by the GC-MS system on two fused-silica capillary columns of different polarity. The oil content was 0.08 % (CSC) and 0.07 % (CSS) on a dry weight basis. Altogether 73 compounds were identified accounting for 90.8 % (CSC) and 92.8 % (CSS) of the total oil, that were characterized mainly by hydrocarbons (37.9 %; 30.4 %), sesquiterpene hydrocarbons (14.1 %; 29.1 %), oxygenated sesquiterpenes (22.2 %; 13.1 %). The major components of the samples were heptacosane (11.2 %; 12.6 %), cyclosativene (2.1 %; 6.4 %), caryophyllene oxide (10.2 %; 4.7 %). Monoterpenes, fatty acids and phenolic compounds were absent or present in low amount in both oils. The chemotaxonomy of the subgenus *Acrolophus* is briefly discussed.

Keywords: Centaurea spinosociliata ssp. *cristata, C. spinosociliata* ssp. *spinosociliata,* Asteraceae, volatile components, heptacosane, cyclosativene, caryophyllene oxide, GC-MS

INTRODUCTION

The genus *Centaurea* L. is a polymorphous genus belonging to the Cardueae Cass. tribe of the Asteraceae family, and comprises 400–700 species of annual, biennial and perennial grassy plants, rarely dwarf shrubs predominantly distributed in Europe and Asia.[1–5] The delimitation of *Centaurea* as a genus is problematical because in its traditional circumscription of Flora Europaea it is obviously a non-monophyletic taxon.[6,7] *Centaurea* species have been mostly studied for sesquiterpene lactones, flavonoids and alkaloids. The antifungal[8] antibacterial[9] and cytotoxic[10,11] activities of its sesquiterpene lactones have been reported. A number of papers have been published on *Centaurea* volatiles.[12–18] In the present study, as a continuation of our researches on this genus,[18–22] we have investigated the volatile components of the aerial parts of *Centaurea spinosociliata* Seenus ssp. *cristata* (Bartl.) Dostál and *Centaurea spinosociliata* Seenus ssp. *spinosociliata* for which, at the best of our at the best of our knowledge, no analyses have been previously reported. The species, endemic for Croatia, belong to the sect. Arenariae (Hayek) Dostàl, which includes in Europe c. 40 herbaceous taxa with not spiny branches and usually spiny appendages, the lower fimbriae are confluent into a hyaline margin or forming auricles.[23]

EXPERIMENTAL

Plant

C. spinosociliata ssp. cristata aerial parts (CSC) were collected at the full flowering in Skradinski Buk, near the National Park of Krka, Croatia in August 2008. *C. spinosociliata ssp. spinosociliata* aerial parts (CSS) were collected at the full flowering near Pag City, Island of Pag, Croatia, in August 2008 and identified by Prof.

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S. Bancheva. Voucher specimens (CR 08/03, CSC, and CR 08/02, CSS) have been deposited in the Herbarium Neapolitanum (NAP), Dipartimento di Biologia Vegetale, Università degli Studi di Napoli "Federico II", Italy.

Isolation of the Volatile Components

The air-dried samples (lots of 25 g) were ground in a Waring blender and then subjected to hydrodistillation according to the standard procedure described in the European Pharmacopoeia[24] for 3 hours using *n*-hexane as a solvent. The oils were dried over anhydrous sodium sulfate and stored under N_2 at +4 °C in the dark until tested and analyzed. The hydrodistillation yielded 0.08 % (*w*/*w*, CSC) and 0.07 % (*w*/*w*, CSS) of yellowish oils.

Gas Chromatography

Analytical gas chromatography was carried out on a Perkin-Elmer Sigma-115 gas chromatograph equipped with a FID and a data handling processor. The separation was achieved using a HP-5MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Column temperature: 40 °C, with 5 min initial hold, and then to 270 °C at 2 °C min^{-1}, 260 °C (20 min); injection mode splitless (1 μL of a 1:1000 *n*-pentane solution). Injector and detector temperatures were 250 °C and 290 °C, respectively. Analysis was also run by using a fused silica HP Innowax polyethylenglycol capillary column (50 m × 0.20 mm, 0.25 μm film thickness). In both cases helium was used as carrier gas (1.0 mL min^{-1}).

Gas Chromatography – Mass Spectrometry

Analysis was performed on an Agilent 6850 Ser. II apparatus, fitted with a fused silica DB-5 capillary column (30 m × 0.25 mm i.d.; 0.33 μm film thickness), coupled to an Agilent Mass Selective Detector MSD 5973; ionization voltage 70 eV; electron multiplier energy 2000 V; scan range 40–550. Gas chromatographic conditions were as reported above; transfer line temperature, 295 °C.

Identification of Components

Most constituents were identified by gas chromatography by comparison of their linear retention indices (LRI) with either those of the literature[25,26] or with those of authentic compounds available in our laboratories. The linear retention indices were determined in relation to a homologous series of *n*-alkanes ($C_8 - C_{28}$) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with either those stored in NIST 02 and Wiley 275 libraries or with mass spectra from the literature[25,27] and a home made library. Components relative concentrations were obtained by peak area normalization. No response factors were calculated. All determinations were performed in triplicate and averaged.

RESULTS AND DISCUSSION

The hydrodistillation of aerial parts of *C. spinosociliata* subsp. *cristata* (CSC) and *C. spinosociliata* subsp. *spinosociliata* (CSS) collected in Croatia gave two pale yellow oils. Overall, 73 compounds were identified (49 in CSC and 60 in CSS), representing 90.8 % and 92.8 % of the total components, respectively. The components are listed in Table 1 according to their retention indices on HP-5MS column and are classified on the basis of their chemical structures in 9 classes. The composition of the two oils is different although their profiles are quite similar. In fact, the composition and the concentrations of hydrocarbons are enough comparable (37.9 % and 30.4 %) with heptacosane present as the most abundant component of both oils (11.2 % and 12.6 %, respectively). Nonacosane is the second most abundant component in CSC but its concentration (10.5 %) is almost five times with respect to CSS (2.0 %) and the same feature can be observed for caryophyllene oxide, the third most abundant compound product in CSC (10.2 %), whose concentration is double with respect to CSS (4.7 %). As regard the different classes of compounds, sesquiterpenes are the most abundant but, although the percentage in the two oils is quite similar (36.3 % in CSC, 42.2 % in CSS), their profile differs significantly. In fact, sesquiterpene hydrocarbons represent the 29.1 % in CSS oil, about the double of CSC oil (14.1 %), with cyclosativene (6.4 %) as the most abundant component. On the other hand the percentage of the oxygen containing sesquiterpenes is nearly double in CSC (22.2 %) with respect to CSS (13.1 %), being, in the last one, spathulenol (4.9 %) and caryophyllene oxide (4.7 %) the main compounds. Carbonylic compounds show a similar concentration (13.9 % and 12.5 %) and it is worthy to point out the presence of 2,4,6-trimethylbenzaldehyde (3.6 % and 5.0 %) that could arise from thermic rearrangements of ferulol derivatives during the hydrodistillation procedure.[28] Finally, it is noteworthy the absence or the low amount of monoterpenes, fatty acids and phenolic compounds in both oils.

Centaurea spinosociliata subsp. *spinosociliata* (CSS) and *C. spinosociliata* subsp. *cristata* (CSC) belong to Sect. Arenariae (subgenus *Acrolophus*) and, up to our knowledge, no previous reports have been published on species of this Section. However several papers concerning the study of the essential oils of aerial parts of species included in closely related Sections such

Table 1. Volatile components from aerial parts of *Centaurea spinosociliata* ssp. *cristata* (CSC) and *Centaurea spinosociliata ss*p. *spinosociliata* (CSS) growing wild in Croatia

Component	LRI [(a)]	LRI [(b)]	CSC	CSS	Identification[(c)]
HYDROCARBONS			37.9	30.4	
1,4,6-Trimethyl-1,2-dihydro-naphthalene	1373	1549	0.5		R_i, MS
Cadalene	1766	2256	0.8		R_i, MS
Guaiazulene	1773	2125		1.9	R_i, MS
Tricosane	2300	2300	2.7	1.4	Ri, MS, Co-GC
Tetracosane	2400	2400	1.0	0.6	Ri, MS, Co-GC
Pentacosane	2500	2500	3.7	2.7	R_i, MS, Co-GC
Hexacosane	2600	2600	1.1	0.8	R_i, MS, Co-GC
Heptacosane	2700	2700	11.2	12.6	R_i, MS, Co-GC
Octacosane	2800	2800	2.5	3.5	R_i, MS, Co-GC
Nonacosane	2900	2900	10.5	2.0	R_i, MS
Triacontane	3000	3000	0.8	0.9	R_i, MS
Hentriacontane	3100	3100	3.1	1.2	R_i, MS
Tritriacontane	3300	3300		2.8	R_i, MS
CARBONYLIC COMPOUNDS			13.9	12.5	
Benzaldehyde	963	1543		0.2	R_i, MS, Co-GC
1,2,4-Trimethylbenzene	971			0.1	R_i, MS
(*E,E*)-2,4-Heptadienal	1015			t	R_i, MS
Phenyl acetaldehyde	1030	1203		0.6	R_i, MS, Co-GC
Decanal	1206	1510	0.2		R_i, MS, Co-GC
2,4,5-Trimethylbenzaldehyde	1308	1895	0.4	0.6	R_i, MS, Co-GC
2,4,6-Trimethylbenzaldehyde	1336	1928	3.6	5.0	R_i, MS, Co-GC
(*E*)-β-Damascenone	1382	1838	1.0	0.5	R_i, MS
(*E*)-Geranylacetone	1453	1867	1.6		R_i, MS
(*E*)-β-Ionone	1484	1958	2.2	3.0	Ri, MS, Co-GC
Tetradecanal	1618	1934	1.0		R_i, MS
Hexahydrofarnesyl acetone	1845	2131	1.9	1.5	R_i, MS
(*E,E*)-Farnesylacetone	1918	2387	2.0	1.0	R_i, MS
TERPENOIDS					
MONOTERPENE HYDROCARBONS				1.2	
α-Pinene	936	1075		t	R_i, MS, Co-GC
δ^3-Carene	1009			0.1	R_i, MS
p-Cymene	1025	1278		t	R_i, MS, Co-GC
Terpinolene	1086	1265		1.1	R_i, MS, Co-GC
SESQUITERPENE HYDROCARBONS			14.1	29.1	
α-Cubebene	1352	1466	2.2	1.6	R_i, MS
Cyclosativene	1363	1492	2.1	6.4	R_i, MS
α-Copaene	1377	1497	0.3	1.0	R_i, MS
β-Cubebene	1380			0.5	R_i, MS
(*E*)-Caryophyllene	1415	1612	2.1	1.0	R_i, MS, Co-GC
Aromadendrene	1437	1628	0.3	1.7	R_i, MS
Widdrene	1444			0.6	R_i, MS
α-Humulene	1455	1689	0.2	0.5	R_i, MS

[(a)]HP-5 MS column.
[(b)]HP Innowax column.
[(c)]Ri: retention index, MS: mass spectrum, Co-GC: co-injection with authentic compound; t: traces (less than 0.05%).

Table 1. (continued) Volatile components from aerial parts of *Centaurea spinosociliata* ssp. *cristata* (CSC) and *Centaurea spinosociliata ss*p. *spinosociliata* (CSS) growing wild in Croatia

Component	LRI[(a)]	LRI[(b)]	CSC	CSS	Identification[(c)]
γ-Selinene	1456	1687		1.0	R_i, MS
allo-Aromadendrene	1461	1661	0.4		R_i, MS
β-Selinene	1475	1715	0.5	1.5	R_i, MS
γ-Muurolene	1478	1704	0.8	0.2	R_i, MS
Epizonarene	1485	1675		2.1	R_i, MS
α-Amorphene	1487	1679	0.8	0.7	R_i, MS
α-Muurolene	1498	1745	1.1	1.8	R_i, MS
α-Chamigrene	1506	1689	1.5	0.6	R_i, MS
β-Guaiene	1516			t	R_i, MS
δ-Cadinene	1526	1773	1.1	2.6	R_i, MS
α-Calacorene	1542	1918	0.7	3.2	R_i, MS
Calarene	1629	1611		2.1	R_i, MS
OXYGENATED MONOTERPENES			0.9	2.8	
cis-Verbenol	1144			t	R_i, MS
cis-Chrysanthenol	1163			0.1	R_i, MS
p-Cymen-8-ol	1185	1856	0.5	1.8	R_i, MS
Safranal	1201	1613	0.3		R_i, MS
Cuminaldehyde	1239	1802	0.1	0.4	R_i, MS
Piperitenone	1343	1748		0.5	R_i, MS
OXYGENATED SESQUITERPENES			22.2	13.1	
cis-α-Copaen-8-ol	1553	2076	2.7		R_i, MS
Spathulenol	1578	2150		4.9	R_i, MS
Caryophyllene oxide	1579	2208	10.2	4.7	R_i, MS, Co-GC
Guaiol	1593	2103	1.6		R_i, MS
Widdrol	1598	2108	3.8		R_i, MS
Cedrenol	1603	2108		2.1	R_i, MS
β-Oplopenone	1608	2100	1.3		Ri, MS
Caryophylla-4(12),8(13)-dien-5β-ol	1640	2316	0.9		R_i, MS
Aromadendrene oxide	1648	2399		1.2	R_i, MS
α-Cadinol	1652	2253	1.7	0.2	R_i, MS
FATTY ACIDS			0.6	1.3	
Hexadecanoic acid	1958	2931	0.2	1.3	Ri, MS, Co-GC
(*Z,Z*)-9,12-Octadecadienoic acid	2104	3160	0.4		Ri, MS, Co-GC
PHENOLIC COMPOUNDS			0.3	0.5	
Thymol	1295	2197	0.1	t	R_i, MS, Co-GC
Carvacrol	1299	2239	0.2	t	R_i, MS, Co-GC
p-Vinyl guiacol	1313	2180		0.5	R_i, MS, Co-GC
OTHERS			0.9	1.9	
2-Pentylfuran	1002	1243		0.5	R_i, MS
Squalene	2829	3048	0.9	1.4	R_i, MS
TOTAL AMOUNT OF COMPOUNDS			90.8	92.8	

[(a)]HP-5 MS column.
[(b)]HP Innowax column.
[(c)]Ri: retention index, MS: mass spectrum, Co-GC: co-injection with authentic compound; t: traces (less than 0.05%).

as Acrolophus, Dissectae and Cylindracea, all belonging to the subgenus *Acrolophus*, are present in literature. The comparison of the data reported herein with those available in literature allows to do some chemotaxonomic considerations. *C. calolepis*, *C. cariensis* subsp. *maculiceps* and subsp. *microlepis*,[29] C. *consanguinea*[30] and C. *dicroa*,[31] all belonging to the Acrolophus section, and *C. thessala* subsp. *drakiensis*[17] (Cylindracea section) have a very similar profile, but quite different with respect to CSC and CSS. In fact all of them have among the main compounds hexadecanoic acid and spathulenol, the former absent in *C. spinosociliata* sps. and the latter present only in CSS. A closer relationship can be observed, on the other hand, with *C. sicana* and *C. giardinae*[32] (Dissectae section), both having good amount of heptacosane, the main compound in both CSC and CSS, and nonacosane also present in *C. spinosociliata* sps. In conclusion, section Arenariae seems to be more similar to sect. Dissectae than to the other related sections. Nevertheless some peculiar characteristics of the essential oil composition of CSC and CSS, such as the presence of trimethylbenzaldehydes, can justify the inclusion of these species in the section Arenariae.

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SAŽETAK

Hlapljive komponente iz nadzemnih dijelova *Centaurea spinosociliata* Seenus ssp. *cristata* (Bartl.) Dostál i *Centaurea spinosociliata* Seenus ssp. *spinosociliata* koje divlje rastu u Hrvatskoj

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U ovom radu opisane su hlapljive uljne komponente dobivene iz nadzemnih dijelova dvije vrste Asteraceae koje divlje rastu u Hrvatskoj: *Centaurea spinosociliata* ssp. *cristata* (syn. *Centaurea cristata* Bartl. subsp. *rabenhorstiana* (Sch.Bip.) Nyman) (CSC) i *C. spinosociliata* ssp. *spinosociliata* (CSS). Hlapljive komponente dobivene su hidrodestilacijom odabranih biljaka, a zatim su određene sustavom GC-MS s dvije kolone od silikatnih kapilara različite polarnosti. Dobiveni udjeli ulja u suhoj tvari su 0,08 % (CSC) i 0,07 % (CSS). U uljima su ukupno identificirane 73 različite tvari koje zajedno sudjeluju s 90,8 % (CSC) i 92,8 % (CSS) u ukupnoj masi ulja, a karakterizirane su uglavnom kao ugljikohidrati (37,9 %; 30,4 %), seskviterpenski ugljikohidrati (14,1 %; 29,1 %), te oksigenizirani seskviterpeni (22,2 %; 13,1 %). Glavne komponente u uzorcima bile su heptakozan (11,2 %; 12,6 %), ciklosativen (2,1 %; 6,4 %), te kariofilen oksid (10,2 %; 4,7 %). Monoterpeni, masne kiseline i fenoli bili su odsutni ili prisutni u vrlo niskim količinama u oba ulja. Ukratko je razmotrena i kemotaksonomija podroda *Acrolophus*.