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Essential Oil Composition of *Centaurea finazzeri* and *C. rupestris* from North Macedonia †

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Abstract: The essential oil composition of fresh flowering heads (capitula) and fresh aerial parts of *Centaurea finazzeri* Adamović and *Centaurea rupestris* L. (Asteraceae, Centaurea sect. *Acrocentron*) from Štip, North Macedonia were analyzed. The essential oils were obtained by simultaneous distillation and extraction using Likens–Nickerson type apparatus and analyzed by GC-FID/GC-MS. In total, 112 compounds were identified representing 97.0-99.2% of the total oil composition. All samples were dominated by aliphatic hydrocarbons (46.3-85.7%). The dominant compounds differed between species. The most abundant compounds of *C. rupestris* essential oils were hexanal (10.7%, 6.3%) for aerial parts and flowering heads, respectively, (2*E*)-hexanal (10.6%) and *α*-pinene (6.0%) for aerial parts, and hexadecanoic acid (7.2%) and 2-methyl hexyl ester butanoic acid (4.5%) for flowering heads. The main volatile constituents of *C. finazzeri* oils were acetophenone (13.5%), (2*E*)-hexanal (12.1%), and hexadecanoic acid (6.9%) for aerial parts, and hexadecanoic acid (21,8%), heptacosane (10.3%), and nonacosane (9.1%) for flowering heads. Taxonomic implications are discussed.

Keywords: Asteraceae; GC-FID/GC-MS; volatile compounds; taxonomy

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1. Introduction

Genus *Centaurea* L. (Cardueae, Centaureinae) is a large member of the Asteraceae family with approximately 250 species mainly distributed in Eurasia, especially in the Irano-Turanian and Mediterranean regions [1,2]. *Centaurea* is a genus known for complex taxonomy [3] due to broad morphological diversity [4] and frequent hybridization [5]. *Centaurea rupestris* L. belongs to the subgenus *Lopholoma* (Cass.) Dobrocz., section *Acrocentron* (Cass.) DC. [6]. It is a perennial plant that inhabits dry grasslands and rocky places in Austria, Italy, and the Western and Central parts of the Balkan peninsula [6,7]. *Centaurea finazzeri* Adamović is a Balkan endemic species [8], earlier treated as a subspecies of *C. rupestris*, but it has acquired species rank according to the plant databases [9,10]. There are several studies concerned with the phytochemistry and biological activity of *C. rupestris*. Extracts of this plant and isolated quercetagetin flavonoid showed antiphytoviral activity against the tomato bushy stunt virus [11]. Although not *C. finazzeri* and *C. rupestris*, several *Centaurea* taxa such as *C. cyanus* L., *C. benedicta* (L.) L., *C. calcitrapa* L., and *C. scabiosa* L. are used in traditional medicine as diuretic, emmenagogue, cholagogue, astringent and antiseptic agents, and in the treatment of fever and tumors [12,13]. Usage of *Centaurea* spp. is supported by the fact that species of this genus synthentize wide range of specialized metabolites [14–17]. Studies on *Centaurea* essential oil are numerous, but the essential oil of *C. rupestris* is scarcely investigated. To the best of our knowledge,

there is no information on *C. finazzeri* essential oil composition as well as *C. rupestris* essential oil from North Macedonia. The aim of this study is to investigate the compostion of essential oil of frozen aerial parts and capitula of *C. finazzeri* and *C. rupestris,* compare results between different plant parts and species, and to compare with other conducted studies.

2. Materials and Methods

Plant Material

Samples of *C. rupestris* and *C. finazzeri* were collected in July 2011 and 2012 from Štip (North Macedonia). Voucher specimens (accessions No. 38444 and 38492) were deposited at the Herbarium of the University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac" (BEOU) [18].

Isolation of Essential Oils

The essential oils were obtained from freshly frozen capitula of *C. rupestris* (RU_C) and *C. finazzeri* (FE_C) and freshly frozen aerial parts of *C. rupestris* (RU_A) and *C. finazzeri* (FE_A) of five individuals each (50 g per samples), using a Likens-Nickerson type apparatus for 2 h [19]. The volatiles were collected in CH_2Cl_2 and stored in amber glass vials at 4 °C until GC-FID and GC-MS analyses.

GC-FID and GC-MS Analyses

The gas chromatography coupled with flame onization detector (GC-FID) and gas chromatography coupled to mass spectrometry (GC-MS) analyses were conducted according to the procedure described in [20].

3. Results and discussion

In the essential oil of investigated species overall, 126 compounds were detected, of which 112 have been identified, representing on average 98.14% of the total oil composition. The compounds and their percentage are shown in Table 1. All essential oils are characterized by the high presence of other compounds (53.5–85.7%). Sesquiterpenes were represented in considerable amounts (9.4–23.8%) with an evident dominance of oxygenated sesquiterpenes (6.8–19.7%) over sesquiterpene hydrocarbons (2.6–6.1%). Sesquiterpenes were in higher amounts in all essential oils, except the essential oil of *C. rupestris* aerial parts (RU_A), where monoterpenes were in higher percentage (24.3%). The considerable presence of monoterpenes was also noticed in all essential oils (3.2–24.3%), with a dominance of monoterpene hydrocarbons in aerial parts of *C. rupestris* (RU_A) and *C. finazzeri (*FE_A) – (18.3% and 12.4%, respectively), and more represented oxygenated monoterpenes in the essential oil of *C. rupestris* flowering heads (RU_C; 5.6%). Diterpenoid compound phytol was also detected in RU_A and FE_A (0.7% and 1.1%, respectively).

There was a difference in dominant components in all investigated essential oils, with non-terpenoid constituents being principal in all samples. As an important observation, the hexadecanoic acid was the dominant compound with the highest percentage in essential oils of flowering heads of both studied species, RU_C and FE_C (7.2% and 21.8%, respectively). Hexadecanoic acid was detected in high abundance in all oils (4.4–21.8%), with the lowest percentage in RU_A and the highest in the essential oil of *C. finazzeri* flowering heads (FE_C). Aliphatic aldehyde hexanal was detected in the highest amount in RU_A (10.7%) and less in RU_C (6.3%). Another aldehyde, (2*E*)-hexenal, was also represented in high abundance in RU_A (10.6%) and FE_C (12.1%) and in lower in RU_C (4.2%). In addition, monoterpene α-pinene was detected in a high percentage in RU_A (6.0%). Besides hexadecanoic acid and hexanal, in the RU_C, aliphatic compound 2-methyl hexyl ester butanoic acid was noted in high abundance (4.5%). An aromatic ketone, acetophenone, was the principal constituent of FE_A (13.5%), and it was detected

only in the aforementioned oil. Terpenoid compounds, monoterpene hydrocarbon *β*-pinene and oxygenated sesquiterpene caryophyllene oxide, were present in high amounts and equal percentages (4.4%). Oil of FE_C was characterized by the dominance of aliphatic compounds with hexadecanoic acid being principal (21.8%) and long-chain alkanes heptacosane and nonacosane being in lesser abundance (10.3% and 9.1%, respectively).

In literature, the most abundant components in essential oils of taxa from *Centaurea* sect. *Acrocentron* were *β*-caryophyllene, caryophyllene oxide, and germacrene D, while sesquiterpenes were the most represented class of compounds [14–19]. Essential oil of *C. rupestris* was the subject of earlier studies conducted in Italy and Croatia [21,22]. The most abundant class of volatile compounds obtained from *C. rupestris* growing wild in Italy were sesquiterpenes (74.5%) which differs from the current study. Germacrene D (42.3%), (*E*)-*β*-farnesene (8.3%), and β-caryophyllene (8.0%) that were principal in *C. rupestris* from Italy were considerably less represented in RU_A and RU_C. In comparison, some dominant components from RU_A and RU_C were present in less than 1% of the oil of *C. rupestris* growing wild in Italy. The prevalent compounds in essential oils of *C. rupestris* from Croatia were germacrene D (24.3%), heptacosane (14.4%), phytol (6.7%), β-caryophyllene (5.0%), and pentacosane (4.5%) in the first sample. In comparison, hexadecanoic acid (18.7 %), heptacosane (13.8 %), *α*-linolenic acid (11.8 %), nonacosane (7.8 %), and germacrene D (5.4 %) were dominant compounds in the second sample. Besides, the most represented classes of compounds differed between two investigated localities, with terpenes dominant in the first and the non-terpenes in the second sample [21]. There is a slight resemblance with the second essential oil sample from Croatia, where non-terpenoid compounds were more represented as well as in the *C. rupestris* oil investigated in the current study. In some cases, essential oils of taxa from *Centaurea*. sect. *Acrocentron* can be used as chemotaxonomic markers [24]. Comparing our results with a reported composition of essential oil in the literature, one can notice exceptions in cases such as *C. rupestris*, with a significantly different composition of essential oil observed in geographically distant populations [21,22]. *C. finazzeri* (syn. *C. rupestris* subsp. *finazzeri* (Adamović) Hayek) also shows a different chemical profile of *C. rupestris*. As a result of our investigation, significant differences were noticed in the content of dominant compounds of essential oils in these two taxa and other investigated essential oils of *C. rupestris*. The data on individually volatile organic compounds (VOCs) may not be useful in phylogeny reconstruction, but these data can provide additional support for clades reconstructed with other types of characters. Other factors besides phylogeny, e.g., pollinator interactions, may influence VOCs composition [27]. To understand fully the evolution of VOCs (complex phenotypes) it is necessary to investigate the genetic background of these compounds [27]. Previous research on capitula essential oils of *Centaurea* species from the *C. atropurpurea* complex, also members of sect. *Acrocentron*, showed that differences in VOCs indicate taxonomic distance between species, but at the same time affiliation to the complex [20]. Essential oils produced in the flowering period are mostly under genetic control due to communication with specialized pollinators, so that it may be significant in the taxonomic interpretation at species level [27]. *C. finazzeri* and *C. rupestris* have not yet been thoroughly investigated from a taxonomic perspective, so we suggest further research of more species and samples to gain a better understanding of chemical diversity within this group.

Table 1. Essential oil composition of *C. finazzeri* and *C. rupestris*. 1

No.	RI[1]	Compound [%][2]	RU_A[3]	RU_C	FE_A	FE_C
1	836	Hexanal	**10.7**	**6.3**	2.5	2.1
2	853	Isovaleric acid	0.4	1.6	-	-
3	855	Furfural	0.1	0.9	0.6	-
4	861	2-methyl-Butanoic acid	0.8	1.7	0.3	-
5	866	(2*E*)-Hexenal	**10.6**	4.2	**12.1**	1.5
6	875	*n*-Hexanol	2.4	0.8	0.7	-
7	888	1-(1-methyl-2-cyclopenten-1-yl)-Ethanone	-	0.2	0.5	-
8	901	Heptanal	1.1	0.4	0.3	-
9	932	*α*-Pinene	**6.0**	1.1	3.5	0.7
10	937	4-butoxy-Butanoic acid	0.2	-	-	-
11	959	Benzaldehyde	0.6	0.3	-	-
12	972	Sabinene	2.2	0.4	1.0	0.2
13	976	*β*-Pinene	2.4	1.0	4.4	0.4
14	986	6-methyl-5-Hepten-2-one	0.2	0.9	-	-
15	991	Myrcene	-	-	0.7	0.1
16	991	2-Pentyl furan	2.4	1.9	-	-
17	998	(2*E*,4*E*)-Heptadienal	1.4	1.5	0.6	-
18	1003	*cis*-2-(2-Pentenyl) furan	0.5	-	-	-
19	1011	(2*E*,4*Z*)-Heptadienal	3.9	0.4	1.1	-
20	1018	2,4 Octadiene	0.9	0.3	-	-
21	1025	*p*-Cymene	0.8	0.3	0.2	-
22	1029	Limonene	4.2	0.8	2.0	0.3
23	1032	Eucalyptol	0.2	-	-	-
24	1045	Benzene acetaldehyde	3.5	2.2	1.9	-
25	1061	*γ*-Terpinene	1.1	0.2	-	-
26	1069	Acetophenone	-	-	**13.5**	-
27	1094	Terpinolene	0.5	0.6	-	-
28	1105	Linalool	0.6	-	0.3	-
29	1109	*n*-Nonanal	1.3	0.4	0.2	-
30	1111	*cis*-Thujone	0.7	0.2	-	-
31	1113	3,5-dimethyl-Cyclohexanol	0.6	-	0.3	-
32	1120	3-Cyclohexene-1-carboxaldehyde, 1-methyl-	1.0	-	-	-
33	1124	dehydro-Sabina ketone	-	-	0.4	-
34	1130	*α*-Campholenal	-	-	0.2	-
35	1141	*trans*-Pinocarveol	-	-	1.9	-
36	1144	1,3,8-*p*-Menthatriene	-	-	0.4	-
37	1147	*trans*-Verbenol	-	-	2.0	-
38	1147	Camphor	0.6	0.3	-	-
39	1165	Pinocarvone	-	-	1.7	-
40	1168	*p*-Mentha-1,5-dien-8-ol	-	-	0.2	-
41	1175	Octanoic acid	-	0.4	-	-
42	1179	Terpin-4-ol	0.8	0.4	0.3	-
43	1186	*p*-Cymen-8-ol	0.4	2.6	0.5	1.6
44	1198	Myrtenal	-	-	2.2	-
45	1201	Safranal	0.4	-	-	-
46	1207	*n*-Decanal	0.4	0.3	-	-
47	1211	Verbenone	-	-	0.8	-
48	1220	*trans*-Carveol	-	-	0.2	-
49	1222	*β*-Cytrocitral	0.7	0.2	0.3	-
50	1237	methyl ether Thymol	-	0.4	-	-
51	1245	Carvone	-	-	0.2	-
52	1293	1-Tridecene	0.6	1.1	-	-
53	1296	Dihydroedulan II	0.7	2.2	-	-
54	1316	Thymol	0.5	0.5	-	-

No.	RI[1]	Compound [%][2]	RU_A[3]	RU_C	FE_A	FE_C
55	1318	(2*E*, 4*E*)-Decadienal	1.4	1.1	-	-
56	1343	Butyl isovalerate	-	1.5	-	-
57	1370	Decanoic acid	-	0.3	-	-
58	1379	*α*-Copaene	-	0.3	-	-
59	1387	(*E*)-*β*-Damascone	0.4	-	-	-
60	1391	*β*-Cubebene	0.6	-	-	-
61	1422	(*E*)-Caryophyllene	1.2	1.0	2.0	0.7
62	1432	*β*-Copaene	-	-	0.3	-
63	1447	Pentanoic acid pentyl ester	-	1.0	-	-
64	1451	Pentanoic acid, 2-propenyl ester	-	0.8	-	-
65	1456	*α*-Humulene	1.1	0.4	0.6	-
66	1460	(*E*)-*β*-Farnesene	0.3	0.4	-	-
67	1481	5-Hydroxy-2-decanoic acid delta-lactone; mas-	-	0.8	-	-
68	1484	Germacrene D	2.8	1.5	2.8	1.3
69	1489	(*E*)-*β*-Ionene	1.4	0.3	0.6	-
70	1493	*n*-Dodecanol	-	0.4	-	0.4
71	1511	*β*-Bisabolene	-	-	0.3	-
72	1526	*δ*-Cadinene	-	0.3	0.2	0.5
73	1532	2(4H)-Benzofuranone,	-	-	-	0.4
74	1550	2-methyl hexyl ester Butanoic acid	-	**4.5**	-	0.8
75	1553	Butanoic acid, 3-methyl-, propyl ester	-	1.7	-	0.2
76	1556	Aristolene epoxide	-	0.7	0.5	-
77	1564	Dodecanoic acid	-	-	-	2.7
78	1566	(*E*)-Nerolidol	0.3	1.3	-	-
79	1580	Spathulenol	2.1	3.2	3.4	1.8
80	1585	Caryophyllene oxide	2.9	3.1	4.4	1.5
81	1596	Salvial-4(14)-en-1-one	0.3	0.8	-	-
82	1612	Humulene epoxide II	1.0	1.1	0.8	0.3
83	1614	*β*-Atlantol	0.8	1.5	0.9	0.8
84	1640	*allo*-Aromadendrene epoxide	0.3	0.8	0.3	-
85	1645	*epi*-*α*-Murrolol (=τ-muurolol)	-	-	0.3	-
86	1650	Ledene alcohol	-	-	0.4	0.6
87	1653	*α*-Eudesmol	2.3	2.2	0.4	0.2
88	1657	*α*-Cadinole	-	-	1.4	0.9
89	1683	Germacra-4(15),5,10(14)-trien-1-*α*-ol	-	-	0.6	-
90	1689	Eudesma-4(15),7-dien-1-*β*-ol (IMPURE)	0.6	2.1	-	-
91	1689	2-*α*-hydroxy-Amorpha-4,7(11)-diene	-	-	1.1	1.0
92	1752	2,2-Dimethylpropionic acid, tridecyl ester	-	2.1	-	-
93	1752	2,2-Dimethylpropionic acid, hexadecyl ester	-	-	-	2.5
94	1756	Butanoic acid, 3-methyl-, hexyl ester	-	0.8	-	1.4
95	1763	Tetradecanoic acid	0.7	1.3	0.5	1.6
96	1849	2-Pentadecanone, 6,10,14-trimethyl-	0.5	-	-	0.3
97	1929	7-Hexadecenoic acid, methyl ester, (*Z*)-	-	0.4	-	-
98	1932	Methyl hexadecanoate	0.4	-	0.3	0.3
99	1945	Cyclohexadecanoolide	-	2.1	-	1.0
100	1969	Hexadecanoic acid	4.4	7.2	**6.9**	**21.8**
101	1996	Ethyl hexadecanoate	-	0.2	-	0.4
102	2082	Methyl linoleate	0.9	1.1	0.5	1.7
103	2088	(*Z*, *Z*, *Z*)-9,12,15-Octadecatrienoic acid, methyl ester	0.9	0.8	0.8	1.1
104	2098	Phytol	0.7	-	1.1	-
105	2121	Linoleic acid	-	-	0.7	6.8
106	2168	(*Z*, *Z*, *Z*)-9,12,15-Octadecatrienoic acid, ethyl ester,	0.6	0.4	0.4	0.5
107	2200	Docosane	-	0.2	-	0.3
108	2300	Tricosane	-	3.2	-	2.2
109	2400	Tetracosane	-	0.6	-	0.6
110	2500	Pentacosane	-	-	-	2.5

No.	RI[1]	Compound [%][2]	RU_A[3]	RU_C	FE_A	FE_C
111	2700	Heptacosane	-	-	-	**10.3**
112	2800	Nonacosane	-	-	-	9.1
		Total monoterpenes	**24.3**	**10.2**	**23.6**	**3.2**
		Monoterpene hydrocarbons	*18.3*	*4.7*	*12.4*	*1.6*
		Oxygenated monoterpenes	*6.0*	*5.6*	*11.2*	*1.6*
		Total sesquiterpenes	**19.8**	**23.6**	**23.8**	**9.4**
		Sesquiterpene hydrocarbons	*5.4*	*3.9*	*6.1*	*2.6*
		Oxygenated sesquiterpenes	*14.5*	*19.7*	*17.7*	*6.8*
		Diterpenes	**0.7**	**0.0**	**1.1**	**0.0**
		Other[4]	**53.5**	**62.4**	**46.3**	**85.7**
		Unknown	**0.8**	**1.4**	**2.2**	**0.4**
		Number of compounds	69	82	68	50
		TOTAL	**99.2**	**97.6**	**97.0**	**98.8**

[1] The retention indices (RI) were experimentally determined using the standard method involving retention times (tR) of n-alkanes, which were injected under the same chromatographic conditions. [2] Contents are given as percentages of the total essential oil composition; tr: trace (0.05 < tr < 0.10%); –: not detected; compounds with contents < 0.05% are not listed; dominant components are in boldface. [3] For detailed information cf. Material and methods. [4] Other: aliphatic hydrocarbons, aliphatic aldehydes and alcohols, aliphatic acids, their esters and aldehydes, aromatic esters with acids, alkyl-aromatic alcohols, and aryl esters of aromatic acids.

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