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Chaerophyllum aureum L. Volatiles: Composition, Antioxidant and Antimicrobial Activity

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Abstract: The present study reports the chemical composition on the essential oil of fresh flowering aerial parts and headspace (HS) volatiles obtained from fresh stem and flower of *Chaerophyllum aureum* L. For hydrodistilled oil, 45 components were identified representing 99.1 % of the total, while 23 components, representing 99.9 % of total HS stem volatiles and 25 components, representing 99.9 % of total HS flower volatiles were found using GC and GC/MS method. The main constituents of *C. aureum* hydrodistilled oil, stem and flower HS volatiles were: sabinene (40.8 %, 53.5 %, 58.5 %) and terpinolene (19.1 %, 23.8 %, 11.2 %) respectively. The results of antibacterial assay showed that the essential oil was not active at concentration of 3 and 5 mg per disk. Also, the examined oil was almost inactive in applied antioxidant assays.

Keywords: *Chaerophyllum aureum* L.; chemical composition; essential oil; headspace volatiles; GC/MS.

1. Plant Source

The plant material (flowering stage) was collected on Vlasina plateau (Serbia), at an altitude of 1250 m, in June 2013. The plant material was identified by Bojan Zlatković and the voucher specimen was deposited in the Herbarium Moesiacum Niš (HMN), Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš under the acquisition number 7233.

2. Previous Studies

The genus *Chaerophyllum* L. (Apiaceae) is taxonomically complex genus and comprises about 40 species which occur commonly throughout Europe, Asia and North America. *Chaerophyllum aureum* L. is a perennial herb growing in the mountainous sub-alpine regions of Europe [1-2]. The plants of this genus contain essential oil in the secretory canals in all vegetative and reproductive organs. Previous phytochemical investigation of *Chaerophyllum* species have revealed the presence of secondary plant metabolites like lignans [3], phenyl propanoids and polyacetylenes [4], phenolic acids [5], and flavonoid glycosides [6-8]. Previous reports on the essential oils of *Chaerophyllum* species from different regions showed variety of terpenoids and aliphatic volatile compounds. The

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composition of the essential oil from the leaves and fruits of *Chaerophyllum aromaticum* L. showed that the leaf oil was dominated by γ-terpinene, while the fruit oil had γ-terpinene and β-phellandrene as main compounds [9]. Distillation of the fresh flowering tops of *Chaerophyllum prescottii* DC showed major components consisting of (*E*)-β-ocimene, (*Z*)-β-ocimene, γ- terpinene, myrcene and terpinolene, representing 89.0 % [10]. The oil of *Chaerophyllum macropodum* was found to contain α-pinene (23.0 %) and β-pinene (17.3 %) as the major constituents [11]. The main constituents of the essential oils of the leaves and flowers of *C. macropodum* were *trans*-β-farnesene, *trans*-β-ocimene, β-pinene, limonene, spathulenol and myrcene, constituting 49.6–73.1% of the oils [12]. In the microdistilled oil of the fruits of *C. macropodum* Boiss. the main component was *p*-cymene (39.3%) [13]. In the hydrodistilled essential oils from flower, leaf and stem of *C. macropodum* Boiss. myristicin and *trans*-β-ocimene were major components [14]. The essential oil of *C. crinitum* was characterized by a higher amount of (*E*)-β-ocimene (50.5 %) [11]. The main components of the oil of *C. byzantinum* Boiss. were sabinene (30.0 %) and *p*-cymene-8-ol (16 %) [15]. The essential oils of *C. macrospermum* were dominated by (*E*)-β-ocimene [16-17]. Previous investigation of the oils of the aerial parts and fruits of *C. coloratum* L., an endemic species of the Balkan Peninsula, revealed that the major compound was (*E*)-β-farnesene (68.5-79.2 %) [18]. The leaf essential oil of *C. villosum* was dominated by γ-terpinene (74.9 %); whereas carvacrol methyl ether (31.1 %) and thymol methyl ether (18.6 %) were noticed as the major constituents in rhizome essential oil of *C. villosum* [19-20]. Comparison of the essential oils from different plant parts of *Chaerophyllum hirsutum* shows that the β-pinene was the main constituent of the fruit oil (25 %). In the stem and leaf oils, sabinene was found in 58.2% and 25.9%, respectively. The main constituents of the root oil were monoterpene hydrocarbons (94%) [21]. The major components of the water-distilled essential oil from crushed fruits of *Chaerophyllum aksekiense* were heptacosane (10.1%) and humulene epoxide II (7.8%) [22]. Of the oil isolated from the epigeal part of *C. bulbosum* growing in Azerbaijan, linalool (18.0 %) was the main component [23], while (*E*)-β-farnesene (22.3 %), (*Z*)-β-ocimene (18.8 %), and myristicin (17.1 %) were the major components in the oil of *C. bulbosum* from Iran [24]. The analysis of the volatile fraction of *Chaerophyllum bulbosum* L. growing wild in Greece was dominated by apiol (37 %) [25]. In the essential oils of the aerial parts and fruits of *Chaerophyllum aureum* L., collected from two mountains in Serbia, the sabinene (18.5-31.6 %), *p*-cymene (7.9-25.4 %) and limonene (1.9-10.9 %) were characterized as the main constituents [26]. The biological activity of the essential oils of some *Chaerophyllum* species, such as antimicrobial and antioxidant activity have been investigated [12, 14-15, 19, 24, 26-29].

3. Present Study

The fresh aerial parts of the plant were chopped and hydrodistillated (224 g) for 2.5 h using a Clevenger type apparatus. The oil was extracted with diethyl ether and the yield was 0.14 % (w/w). For HS experiments, 300 mg of milled fresh plant material was put into 20 mL HS vial than soaked with 2 mL of distilled water. The sample was heated at 80°C for 20 minutes with the next mixing program: shaking for 5 seconds, pause for 2 seconds. 500 μL of vapor generated from the aerial parts was drawn out from the vial using a gas-tight syringe (90°C) and injected directly in the chromatographic column via a transfer line (75°C).The GC-FID and GC/MS analyses were performed as previously described [30].

Chemical compositions of hydrodistillated essential oil as well as flower and stem headspace volatiles of *C. aureum*, obtained by GC and GC/MS, are presented in Table 1. For hydrodistilled oil, 45 components were identified and representing 99.1 % of the total oil, while 23 identified components, representing 99.9 % of total HS volatiles of stem and 25 identified components, representing 99.9 % of total HS volatiles of flower. In all samples, the most dominant components were monoterpene hydrocarbons: sabinene (40.8 %, 53.5 %, 58.5 %) and terpinolene (19.1 %, 23.8 %, 11.2 %), respectively in hydrodistilled oil, stem and flower HS volatiles.

Table 1. Chemical composition of the *C. aureum* volatiles achieved by GC and GC/MS.

RI_{ref}	RI_{exp}	Compound	Relative amount %			Class
			Sample A	Sample B	Sample C	
924	928	α-Thujene	0.2	0.1	0.2	M
932	935	α-Pinene	2.6	4.0	2.2	M
946	950	Camphene	t	t	t	M
969	978	Sabinene	40.8	53.5	58.5	M
974	980	β-Pinene	2.1	0.4	4.4	M
988	993	Myrcene	3.5	3.8	4.7	M
998	1004	n-Octanal	t	-	-	O
1002	1007	α-Phellandrene	0.4	0.4	0.4	M
1014	1019	α-Terpinene	0.6	0.1	0.3	M
1020	1027	*p*-Cymene	0.3	0.3	0.5	M
1024	1031	Limonene	5.7	5.3	t	M
1025	1032	β-Phellandrene	4.0	2.1	7.1	M
1032	1039	(*Z*)-β-Ocimene	2.8	1.1	1.4	M
1036	1045	Benzene acetaldehyde	0.1	-	t	O
1044	1050	(*E*)-β-Ocimene	4.8	3.1	5.8	M
1054	1061	γ-Terpinene	3.0	1.1	2.6	M
1065	1069	*cis*-Sabinene hydrate	0.6	t	0.1	MO
1086	1092	Terpinolene	19.1	23.8	11.2	M
1089	1092	*p*-Cymenene	1.0	t	-	M
1098	1099	*trans*-Sabinene hydrate	0.4	-	0.1	MO
1108	1114	1,3,8-p-Menthatriene	t	t	t	M
1118	1123	*cis*-*p*-Menth-2-en-1-ol	0.1	-	-	MO
1174	1180	Terpinen-4-ol	1.9	t	0.2	MO
1179	1186	*p*-Cymen-8-ol	0.1	-	t	MO
1186	1192	α-Terpineol	0.1	-	-	MO
1195	1198	*cis*-Piperitol	t	-	-	MO
1207	1209	*trans*-Piperitol	t	-	-	MO
1215	1221	*trans*-Carveol	t	-	-	MO
1335	1342	δ-Elemene	t	-	-	S
1374	1382	α-Copaene	t	-	-	S
1389	1397	β-Elemene	t	-	-	S
1417	1427	(*E*)-Caryophyllene	0.3	0.1	t	S
1434	1438	γ-Elemene	t	-	-	S
1452	1462	α-Humulene	0.4	t	t	S
1484	1489	Germacrene D	2.5	0.6	0.2	S
1493	1499	α -Zingiberene	0.1	-	-	S
1500	1504	Bicyclogermacrene	0.6	0.1	t	S
1505	1510	(*E*,*E*)-α-Farnesene	t	-	-	S
1505	1513	β-Bisabolene	0.1	-	-	S
1517	1526	Myristicin	0.2	-	-	O
1522	1529	δ-Cadinene	0.1	-	-	S
1559	1566	Germacrene B	0.1	-	-	S
1640	1650	*epi*-α-Muurolol	0.1	-	-	SO
1652	1662	α-Cadinol	0.1	-	-	SO
1677	1687	Apiole	0.3	-	-	O
		Total	**99.1**	**99.9**	**99.9**	
		Monoterpenoids	94.1	99.1	99.7	
		Hydrocarbons(M)	90.9	99.1	99.3	
		Oxygenated(MO)	3.2		0.4	
		Sesquiterpenoids	4.5	0.8	0.2	
		Hydrocarbons (S)	4.2	0.8	0.2	
		Oxygenated (SO)	0.2			
		Others (O)	0.6			

Compounds are listed in order of elution from a HP-5 MS column; RI_{ref}: literature retention indices; RI_{exp}: experimental Retention indices relative to C_8-C_{32} *n*-alaknes; t: traces (<0.1%); (-): not detected**.** Sample A - aerial parts (oil), Samples B - stem HS, C - flower HS.

During identifying the components, there was an interesting case where terpinolene and *p*-cymenene occurred as a single peak. Their relative ratio was successfully determined applying selected ion monitoring (SIM) mode measuring the current intensity of the ions at *m/z* 136 for terpinolene and 132 for *p*-cymenene.

Content of monoterpenoids, which are more volatile compounds, is little higher in headspace volatiles then in essential oil, while some oxygenated monoterpenoids and sesquiterpenoids, which have relatively high retention times, were only found in traces or not found at all. The oil contained 0.6 % of oxygenated sesquiterpenes that were not even detected in samples B and C. As can be noticed, the chemical compositions of headspace volatiles in sample B and sample C were very similar regarding their qualitative and quantitative pattern. They differ by content of *p*-cymen-8-ol and *trans*-sabinene hydrate which were detected only in sample C. Further, *cis*-sabinene hydrate and terpinen-4-ol, represented 0.1 % and 0.2 % of sample C, respectively, were detected in trace amount in sample B.

Comparing the results of the sample A and reference [26], sabinene was the most abundant constituent in both studies, while terpinolene Lakušić et al. found only in trace amount. The observed differences in essential oil composition could be explained by different habitat conditions (temperature, moisture, soil, vegetation type and other environmental variables), as well as the fact that in our survey fresh plant material was hydrodistillated immediately after collection, while Lakušić et al. distilled dried specimens.

Determination of ferric reducing power. This assay was performed according to the method of [31]. The ferric reducing power of the oil was found to be weak, because 1 mg of oil was equivalent to 0.044 μg ascorbic acid (FRP was 0.044 μg AAE/mg oil).

DPPH "scavenging" radical capacity of samples was determined using DPPH radical method [31]. Regarding antioxidant activity examined oil was found almost inactive in scavenging $DPPH^{\bullet}$ radical, namely scavenging capacity was 1.13 % on the $DPPH^{\bullet}$ (0.017025 μg TE/mg oil).

ABTS radical "scavenging" activity was measured using a modification of the method of [32]. The antioxidant capacity, estimated in terms of the $ABTS^{\bullet+}$ radical scavenging activity, was 1.385 μg TE/mg oil.

The *in vitro* antibacterial activity of the essential oil was determined against a panel of laboratory control strains belonging to the American Type Culture Collections, Maryland - USA. Antibacterial activities of mentioned samples were estimated according to disk diffusion assay [33]. Antibacterial activity was evaluated against two Gram-positive and three Gram-negative bacteria. The Gram-positive bacteria used were: *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538. The Gram-negative bacteria utilized in the assay were: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* ATCC 6017. The results of antibacterial assay showed that the oil was not active at concentration of 3 and 5 mg per disk. All positive control showed significant antibacterial activity, while hexane showed no activity at all. The above results differ from the results of Lakušić and collaborators [19]. They found that the samples of the oil were active in relation to the *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Escherichia coli* and *Klebsiella pneumoniae* and not active to *Pseudomonas aeruginosa*. But the paper did not include information of the disks diameters used and the volume of oil solution on them. For these reasons, comparison of the results is questionable.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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