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HEADSPACE VOLATILES OF *CHAEROPHYLLUM AUREUM* L. †

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Jelena Stamenković, Ivana Radojković, Goran Petrović*, Gordana Stojanović, Aleksandra Đorđević

Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Serbia

Abstract. *Differences in the headspace volatile profiles (HS) of fresh and air-dried fruits, stems and aerial parts of* Chaerophyllum aureum *L. (Apiaceae) were studied here for the first time using HS-GC-MS (head space – gas chromatography – mass spectrometry). This was done in order to probe to which level HS volatiles of different plant organs were susceptible to air drying. The most dominant headspace volatiles of all samples were monoterpene hydrocarbons. Sabinene was the major volatile of the fresh aerial parts, air-dried fruits, fresh and air-dried stems, representing 47.8%, 31.4%, 67.7% and 73.0% of the total volatiles, respectively. The most abundant headspace volatiles of the fresh fruits were terpinolene (45.3%), γ-terpinene (13.1%) and β-pinene (10.2%). The air-dried aerial parts were characterized by a high amount of limonene (69.0%). The results of HS-GC-MS were subjected to multivariate statistical analysis in order to get a better insight into the similarities/dissimilarities existing between the investigated samples. According to the results of multivariate analysis, the drying process significantly influenced HS volatiles.*

Keywords: *Chaerophyllum aureum, headspace, volatiles, statistical analysis*

1. INTRODUCTION

The genus *Chaerophyllum* L. (Apiaceae) is a 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 to sub-alpine regions of Europe (Cannon, 1968; Duman, 2000). The plants of this genus contain essential oil in the secretory canals in all vegetative and reproductive organs. Previous phytochemical investigations of *Chaerophyllum* species have revealed the presence of secondary plant metabolites such as lignans (Mikaya et al., 1981), phenyl

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* Contacts of the corresponding author: Goran Petrović, Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia, E-mail: peca@pmf.ni.ac.rs

propanoids and polyacetylenes (Rollinger et al., 2003), phenolic acids (Dall'Acqua et al., 2004), and flavonoid glycosides (Gonnet, 1985, 1986). Previous reports on the essential oils of *Chaerophyllum* species from different regions showed a variety of volatile compounds (Başer et al., 2000, 2006; Chizzola, 2009; Ebrahimabadi et al., 2010; Joshi and Mathela, 2013; Joshi, 2013; Kokkalou and Stefanou, 1989; Kubeczka et al., 1989; Kürkçüoğlu et al., 2006; Letchamo et al., 2005; Mamedova and Akhmedova, 1991; Masoudi et al., 2011; Nematollahi et al., 2005; Sefidkon, and Abdoli, 2005; Vajs et al., 1995). 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 (Lakušić et al., 2009).

The present study was carried out in order to determine the chemical composition of the headspace (HS) volatiles of *Chaerophyllum aureum* L obtained from fresh and air dried fruits, stems and aerial parts which have not been previously reported. The aim of this study was to investigate the difference in the composition of volatile components from different plant organs and the changes in the headspace volatile profile provoked by drying of plant material. The obtained results were subjected to PCA (Principal Components Analysis) and AHC (Agglomerative Hierarchical Cluster Analysis) in order to get a better insight into the similarities between the examined samples.

2. MATERIALS AND METHODS

2.1. Plant material

The plant was harvested at the fruiting stage in July 2013, in the region of Vlasina Lake in the southeast of Serbia (Europe). 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.

After harvesting, the plant material was put in clean black polyethylene bags; the bags were tied and transported from the collection site to the laboratory for further handling. The fresh plant material was analyzed immediately after harvest while the rest of the plant material was air-dried at ambient temperature in the shade. The plant was kept in a single layer on trays and dried on laboratory bench at room temperature for 30 days. After 30 days dry plant material was analyzed.

2.2. Sample preparation

Three hundred mg of milled plant material was put into 20 mL HS vial and then soaked with 2 mL of distilled water. The sample was heated at 80 °C for 20 minutes with the following mixing program: shaking for 5 seconds, pause for 2 seconds. 500 μL of vapor generated from the samples 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).

2.3. GC and GC/MS analysis

The samples were analyzed by a 7890/7000B GC/MS/MS triple quadrupole system in MS1 scan mode (Agilent Technologies, USA) equipped with a Combi PAL sampler and Headspace for G6501B/G6509B. The fused silica capillary column HP-5 MS (5% phenylmethylsiloxane, 30 m x 0.25 mm, film thickness 0.25 μm) was used. The injector and interface operated at 250 and 300 °C, respectively. Temperature program: from 50 to 290 °C at a heating rate of 4 °C/min. The carrier gas was helium with a flow of 1.0 mL/min. 500 μL of HS vapor was injected via a transfer line (75 °C). Post run: back flash for 1.89 min, at 280 °C, with helium pressure of 50 psi. MS conditions were as follows: ionization voltage of 70 eV, acquisition mass range 50-650, scan time 0.32 s. GC analysis was carried out under the same experimental conditions using the same column as described for the GC/MS. The percentage composition of the samples was computed from the GC peak areas without any corrections.

2.4. Identification of volatile compounds

HS volatiles were identified by comparison of their linear retention indices (relative to C_8-C_{32} *n*-alkanes on the HP-5MS column) with literature values and their MS with those of authentic standards, as well as those from Wiley 6, NIST11, Agilent Mass Hunter Workstation B.06.00 software and a homemade MS library with the spectra corresponding to pure substances and components of known essential oils by the application of the AMDIS software (Automated Mass Spectral Deconvolution and Identification System, Ver. 2.1, *DTRA/*NIST, 2011).

2.5. Multivariate Statistical Analysis

Data analyses were performed using agglomerative hierarchical cluster analysis (AHC) and principal component analysis (PCA), multivariate statistical techniques widely applied in essential oil studies with constituent contents used as cases. The AHC is performed with Euclidean distances as metric and using single linkage method as aggregation criterion. The PCA technique identifies the directions in which the most information is retained in the hyperspace of the variables. All analyses were performed using the "Statistica, version 8.1" software.

3. Results and Discussion

Composition of HS volatiles: In all samples, the most dominant components were monoterpene hydrocarbons while the sesquiterpene hydrocarbons were found only in the samples of fresh and air dried stem. Sabinene was the major component of the fresh aerial parts, air dried fruits, fresh and air dried stems, representing 47.8%, 31.4%, 67.7% and 73.0% of total volatiles, respectively. The most abundant constituents of fresh fruit volatiles were terpinolene (45.3%), γ-terpinene (13.1%) and β-pinene (10.2%). The air dried aerial parts was characterized by a higher amount of limonene (69.0%). A TIC (total ion chromatogram) chromatograms of the analyzed samples are given in Fig. 1.

Multivariate Statistical Analysis: The results of the AHC and PCA analysis are depicted in Figs. 2 and 3, respectively. Table 1 lists the constituents with their contents of the *Chaerophyllum aureum* headspace (H1-H6) samples included in the AHC and PCA.

Table 1 Chemical composition (%) of *Chaerophyllum aureum* HS volatiles.

			Relative amount %						
RI_{ref}	RI_{exp}	Compound	H1	H2	H3	H4	H5	H6	Class
924	929	α-Thujene	0.2	0.3	0.2	0.4	0.6	-	M
932	936	α-Pinene	6.7	6.9	6.1	3.4	4.4	2.4	M
946	951	Camphene	0.1	-	tr	0.1	-	-	M
969	976	Sabinene	2.1	67.7	47.8	31.4	73.0	0.5	M
974	980	β-Pinene	10.2	1.0	3.7	7.6	1.0	5.9	M
988	993	Myrcene	6.4	4.4	3.3	5.9	2.3	1.5	M
1002	1008	α-Phellandrene	0.5	0.2	0.1	0.3	0.1	-	M
1014	1020	α-Terpinene	0.1	0.2	0.1	0.1	0.4	-	M
1020	1028	*p*-Cymene	3.7	1.0	1.1	8.0	0.7	6.4	M
1024	1032	Limonene	7.4	-	25.3	-	-	69.0	M
1025	1033	β-Phellandrene	tr	8.6	-	31.1	4.3	-	M
1032	1040	(*Z*)-β-Ocimene	0.1	1.4	tr	0.9	1.7	-	M
1044	1050	(*E*)-β-Ocimene	4.0	0.8	1.8	4.2	0.8	0.6	M
1054	1062	γ-Terpinene	13.1	0.8	2.2	4.7	0.9	1.3	M
1065	1069	*cis*-Sabinene hydrate	-	-	0.1	0.4	-	-	MO
1086	1091	Terpinolene	45.2	5.1	8.2	-	8.1	6.3	M
1089	1091	*p*-Cymenene	tr	-	tr	0.8	-	tr	M
1100	1098	*trans*-Sabinene hydrate	-	-	-	0.1	-	-	MO
1108	1115	1,3,8-*p*-Menthatriene	0.1	tr	tr	-	-	-	M
1174	1181	Terpinen-4-ol	-	-	tr	0.1	tr	-	MO
1179	1187	*p*-Cymen-8-ol	-	-	-	-	-	1.2	MO
1417	1427	(*E*)-Caryophyllene	-	0.2	-	-	0.2	-	S
1454	1458	α-Humulene	-	0.1	-	-	-	-	S
1484	1490	Germacrene D	-	0.5	tr	-	0.5	-	S
		Total	99.9	99.2	100	99.5	99.0	95.1	
		Monoterpenoids	99.9	98.4	100	99.5	98.3	95.1	
		Hydrocarbons(M)	99.9	98.4	99.9	98.9	98.3	93.9	
		Oxygenated(MO)	-	-	0.1	0.6	-	1.2	
		Sesquiterpenoids	-	0.8	-	-	0.7	-	
		Hydrocarbons (S)	-	0.8	-	-	0.7	-	

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*-alkanes; tr: traces (<0.1%); (-): not detected**.**
Samples: H1 – fresh fruits, H2 – fresh stems, H3 – fresh aerial parts, H4 – air dried fruits, H5 – air dried stems, H6 – air dried aerial parts.

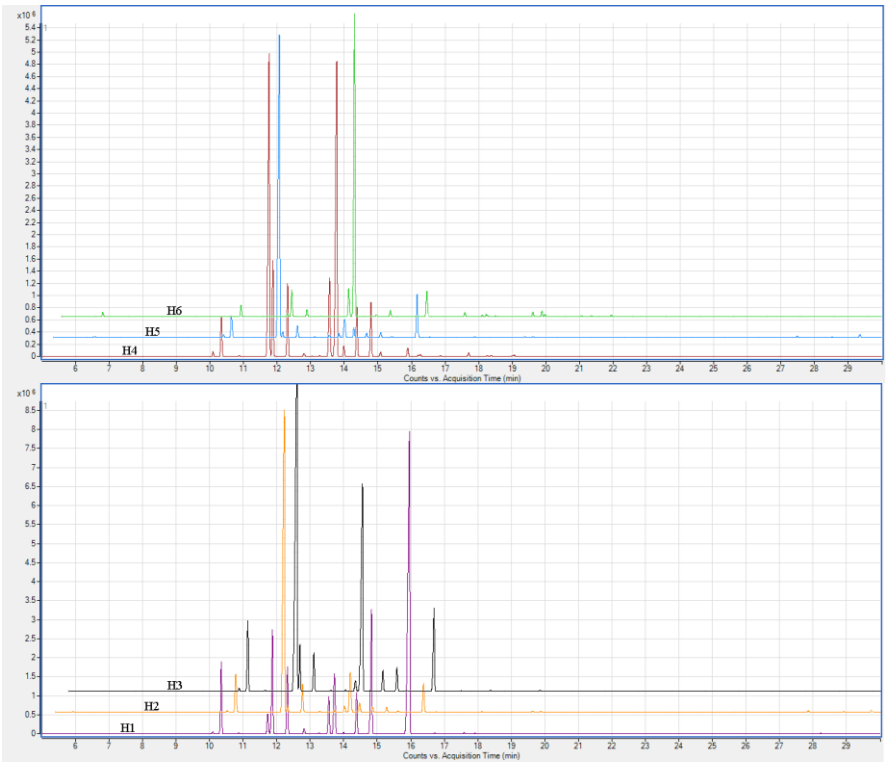

Fig. 1 Total ion current (TIC) of examined samples: H1 – fresh fruits, H2 – fresh stems, H3 – fresh aerial parts, H4 – air dried fruits, H5 – air dried stems, H6 – air dried aerial parts.

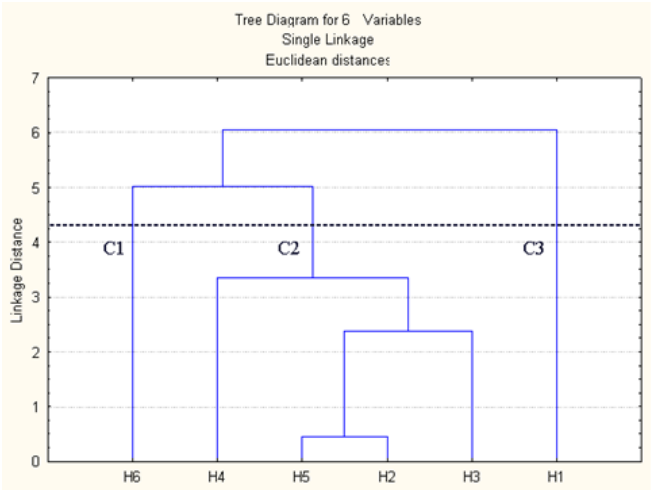

Fig. 2 Dendrogram obtained by agglomerative hierarchical clustering and representing the chemical composition dissimilarity relationships of *Chaerophyllum aureum* samples.

The dendrogram depicted in Fig. 2, obtained as the result of AHC, indicates the existence of three statistically different classes of samples (C1–C3). HS sample H6 (air dried aerial parts) is separated the most from the rest of the *C.aureum* samples and constituted the first clade (C1). The second clade, C2, grouped the samples H4, H5, H2 and H3, while sample H1 is separated as the third clade (C3). The branches of the most numerous group C2 are separated due to significant relative content of sabinene in samples. There was further subdivision within clade C2, which grouped samples H2 and H5 into one subclade. These two samples were the only samples that contained sesquiterpene hydrocarbons, the reason for their strong association (same subclade). AHC further showed that clade C1 was closer related to clade C3.

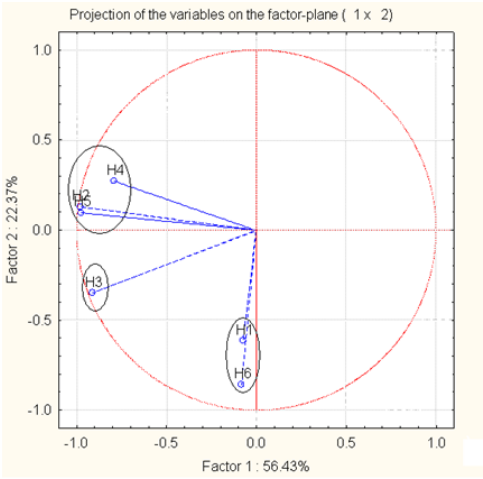

Fig. 3 Plot obtained by principal component analysis of *Chaerophyllum aureum* samples. Axes refer to the ordination scores obtained from the samples. Axes Factor 1 and Factor 2 (the first and second principal component, resp.) account for 56.43% and 22.37% of the total variance.

PCA biplot is given in Fig. 3 and the horizontal axis accounted for 56.43% of the variation, while the vertical axis explained a further 22.37%. The results of both statistical analyses are mostly in agreement, and nearly the same clustering was obtained for PCA as for AHC (Figs. 2 and 3). Samples H6 (clade C1 in the AHC) and H1 (clade C3 in AHC) were clearly distinguished from the other samples, since they are the only samples in which sabinene is not the main component. Samples H2, H4 and H5 (clade C2 in AHC) were grouped together also according to PCA. These samples were dominated by sabinene and they are placed in statistically different class with regard to other samples due to significant presence of β-phellandrene, while limonene was not detected at all. A similar chemical composition (in terms of principal component) was found in sample H3, with sabinene (47.8%) as main constituent, while the main difference lies in fact that this sample also contained considerable amounts of limonene (25.3%). Further, sabinene, accompanied by terpinolene, α-pinene and β-phellandrene make up over 88% of the samples H2 and H5, reason why these samples were closer related to each other than to the rest of the HS samples.

3. CONCLUSIONS

It has been previously shown that the chemical compositions of essential oils isolated from different parts of the same species may differ to a significant level (Jovanović et al., 2015; Stamenković et al., 2015). This is in general agreement with the herein obtained results regarding HSes of different parts of the same species. Nonetheless, the main difference in the head space profiles of different parts of *C. aureum* is in the relative amount, not identity of the main volatiles components. The results of multivariate analysis showed that drying may cause significant changes in the HS profile.

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ISPARLJIVI SASTOJCI BILJNE VRSTE *CHAEROPHYLLUM AUREUM* L.

U ovom radu su, po prvi put, ispitane razlike u headspace *profilima (HS) svežih i na vazduhu sušenih plodova, stabljika i nadzemnih delova biljne vrste* Chaerophyllum aureum *L (Apiaceae) pomoću metode HS-GC-MS (headspace – gasna hromatografija – masena spektrometrija). Na osnovu ovih rezultata moglo bi se utvrditi do koliko intenzivnih promena u HS profilima različitih delova biljke može doći usled sušenja biljnog materijala. U svim uzorcima su dominantni* headspace *isparljivi sastojci bili monoterpeni. Utvrđeno je da je sabinen bio glavni isparljivi sastojak svežeg nadzemnog dela (47,8%), suvog ploda (31,4%), svežeg (67,7%) i suvog stabla (73,0%). Kao glavni isparljivi sastojci svežeg ploda nađeni su terpinolen (45,3%), γ-terpinen (13,1%) i β-pinen (10,2%), dok je suvi nadzemni deo bio okarakterisan značajno većom zastupljenošću limonena (69,0%). Rezultati HS-GC-MS analize su podvrgnuti multivarijantnoj statističkoj analizi kako bi se dobio bolji uvid u sličnosti/razlike među razmatranim uzorcima. Na osnovu rezultata multivarijantne analize sledi da sušenje u velikoj meri utiče na HS profil.*

Ključne reči: *Chaerophyllum aureum, headspace, isparljivi sastojci, statistička analiza*