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Variations in the headspace volatile profiles of three different *Achillea coarctata* Poir. (Asteraceae) populations

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***Abstract*:**

Radojković-Kostevski, I., Petrović, G., Stojanović, G., Stamenković, J., Zlatković, B.: ***Variations in the headspace volatile profiles of three different Achillea coarctata Poir. (Asteraceae) populations. Biologica Nyssana, 6 (2), December 2015: 75-80.***

This study presents a detailed compositional analysis of six *Achillea coarctata* Poir. samples obtained by static headspace method and interrelationships based on the volatiles profiles from different plant parts, three different populations and geological substrates, using multivariate statistical analysis. The most dominant components were mutual for aerial vegetative plant parts and inflorescences collected at the same locality. Main compounds differed in percentages for two localities (values in parenthesis refer to aerial plant parts and inflorescences, respectively): 1) 1,8-cineole (40.7%; 39.9%), β-pinene (29.6%; 36.4%) and α-pinene (7.2%; 3.3%); 2) 1,8-cineole (51.8%; 53.3%), β-pinene (18.0%; 28.2%) and α-pinene (5.6%; 4.5%). the most abundant constituents identified in third locality were 1,8-cineole (37.2%; 35.6%), β-pinene (18.6%; 11.7%) and *o*-cymene (11.6%; 11,7%). Samples collected on different geological substrates are qualitative and quantitative various according to agglomerative hierarchical clustering analysis and can be grouped in two clades and two subclades.

Key words: *Achillea coarctata* Poir., headspace, volatile profiles, statistical analysis

***Apstrakt*:**

Radojković-Kostevski, I., Petrović, G., Stojanović, G., Stamenković, J., Zlatković, B.: ***Razlike headspace profila isparljivih komponenti tri različite populacije Achillea coarctata Poir. (Asteraceae). Biologica Nyssana, 6 (2), December 2015: 75-80.***

U ovom radu su predstavljeni rezultati dobijeni ispitivanjem hemijskog sastava lako isparljivih komponenti iz šest uzoraka biljke *Achillea coarctata* Poir. headspace statičkom metodom, i ispitana je zavisnost varijacije hemijskih profila od ispitivanih delova biljke i geološke podloge, korišćenjem multivarijantne statističke analize. Uzorci nadzemnog dela biljke i cveta prikupljani na jednom staništu sadržali su iste glavne komponente. Rezultati su pokazali da je razlika između dva lokaliteta u sadržaju istih glavnih komponenti, čiji su procenti prikazani u zagradama za nadzemni deo biljke i cvet: 1) 1,8-cineol (40.7%; 39.9%), β-pinen (29.6%;

36.4%) i α-pinen (7.2%; 3.3%); 2) 1,8-cineol (51.8%; 53.3%), β-pinen (18.0%; 28.2%) i α-pinen (5.6%; 4.5%). Kod trećeg staništa najdominantnije su bile sledeće komponente: 1,8-cineol (37.2%; 35.6%), β-pinen (18.6%; 11.7%) i *o*-cimen (11.6%; 11.7%), za nadzemni deo biljke odnosno cvet. Prema rezultatima AHC analize, uzorci prikupljeni sa različitih podloga se kvalitativno i kvantitativno razlikuju i grupisani su u dva klastera i dva subklastera.

Key words: *Achillea coarctata* Poir., headspace, profili isparljivih komponenti, statistička analiza

Introduction

The genus *Achillea* L. (Asteraceae) is comprised of about 115 species found in the Northern Hemisphere, mostly in the Euro-Asian continent that are commonly known as yarrows (Benedek et al., 2008; Nemeth & Bernath, 2008; Radulović et al., 2010). The *Achillea* L. species belong to the oldest medicinal plants that are used both for pharmaceutical purposes and in folk medicine. *Achillea* species are diuretic, emmenagogue agents, used for healing wounds, curing stomachache and diarrhea, with antichloristic, antispasmodic, antiseptic and infection preventing properties. They have also been used to reduce sweating and to stop bleeding (Alsohaili & Al-fawwaz, 2014).

The *Achillea* genus has a wide distributional range, and the differences in oil composition may be affected by different environmental factors such as plant genetic type, seasonality, and developmental stage, because it is a chemically polymorphic and perennial plant. Terpenoids (1,8-cineole, camphor, borneol, pinenes, artemisia ketone, santolina alcohol, farnesane, caryophyllene and its oxides, cubebene, germacrenes, eudesmol, α-bisabolol and oxides, farnesene, γ-gurjunene, γ-muurolene and chamazulene) are the principle components of *Achillea* essential oils (Motavalizadehkakhky et al., 2013).

A. coarctata is a perennial herb with yellow ligules growing in dry hillsides and sandy oils, with the range restricted to the Balcan Peninsula, the South Ukraine and the Asia Minor (Gajić, 1975; Simić et al., 1999; Tzakou et al., 2009). As far as we know, there are three reports about chemical composition of *A. coarctata* essential oil. Different components are reported as the main constituents of the oil among these three studies. First known analysis represents caryophyllene oxide, 1,8-cineole and *trans*-linalool oxide as dominant constituents (Simić et al., 1999). Tzakou found 1,8-cineole, camphor and borneol, while Toker reported 1,8-cineole, camphor and viridiflorol to be major components of the essential oil (Tzakou et al., 2009; Toker et al., 2003).

The aims of this study were to perform a detailed compositional analysis of six *A. coarctata* samples obtained by static headspace method and to establish interrelationships based on the volatiles profiles from different plant parts and different populations using multivariate statistical analysis.

Material and methods

Plant material

The plant material (flowering stage) was collected at three different locations in Serbia (Rujan mountain; Preševo (Mitrovac); Pčinja valley (Trgovište)), in June 2014. The plant materials were identified by Bojan Zlatković and the voucher specimens were deposited in the Herbarium Moesiacum Niš (HMN), Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš under the acquisition numbers 9367, 9368 and 9369. Types of geological substrates were identified according to Basic Geological Mapp 1:100.000 (Karajovanović & Hristov, 1976; Babović & Cvetković, 1977).

Sample preparation

300 mg of milled fresh plant material was put into 20 mL HS vial and 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).

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-5MS (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.

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). Some components were identified by co-injection of pure substances.

Multivariate Statistical Analysis

The contents of the components of the *A. coarctata* headspace volatiles obtained in this study were analyzed by agglomerative hierarchical cluster analysis (AHC). The AHC was performed with Euclidean distances as metric and using single linkage method as aggregation criterion using the "Statistica, version 8.1" software.

Results and discussion

Plant materials were collected at three different locations - Rujan mountain (samples RN, RC), Preševo (Mitrovac) (samples PN, PC) and Pčinja valley (Trgovište) (samples TN, TC). Each location has different geological substrate type: 1) serpentine (Preševo), 2) silicate (Rujan mountain), 3) conglomerates and molasse (Pčinja valley). Compositions of aerial plant parts and inflorescences headspace volatiles of *A. coarctata* from three different localities obtained by GC and GC/MS, are presented in **Tab. 1**.

Number of identified compounds in RN, TN, PN, RC, TC and PC samples were 38, 36, 33, 34, 28 and 32, respectively (representing 98.9% (RN); 98.1% (TN); 98.3% (PN); 98.2% (RC); 98.8% (RC) and 98.6% (TC) of total HS volatiles.

The most dominant components were mutual for aerial plant parts and inflorescences collected at the same locality. Main compounds found in RN and RC samples were 1,8-cineole (40.7%; 39.9%), β-pinene (29.6%; 36.4%) and α-pinene (7.2%; 3.3%). The same components were found as the most dominant in samples TN and TC, with following percentage: 1,8-cineole (51.8%; 53.3%), β-pinene (18.0%; 28.2%) and α-pinene (5.6%; 4.5%), for TN and TC respectively. The most abundant constituents identified in PN and PC samples were 1,8-cineole (37.2%; 35.6%), β-pinene (18.6%; 11.7%) and *o*-cymene (11.6%; 11.7%).

As far as we know, there is no any published data on headspace volatiles composition of *Achillea coarctata*. Three papers revealed no different compounds as the most abundant in obtained essential oils compositions: (1) caryophyllene oxide, 1,8-cineole and *trans*-linalool oxide; (2) 1,8-cineole, camphor and borneol and (3) 1,8-cineole, camphor and viridiflorol, respectively (Simić et al., 1999; Tzakou et al., 2009; Toker et al., 2003). Content of monoterpenoids, which are more volatile compounds, is higher in headspace volatiles then in essential oil, while sesquiterpenoids, which have relatively high retention times, were only found in traces or not found at all.

The results of the AHC analysis are depicted in **Fig. 1**. Table 1 lists the identified constituents with their contents of the six *A. coarctata* headspace volatile samples included in the AHC. The dendrogram depicted in **Fig. 1**, obtained as the result of the AHC, indicates the existence of two statistically different classes of samples (C1–C2).

It is obvious that the chemical compositions of the aerial parts and inflorescences of the samples collected at the same locations are almost identical and they are grouped in the same stocks since they have the same main components that differ only in percentages in different parts of plants. Samples PC and PN are separated from the rest of the *A. coarctata* samples and constituted the first clade (C1). The samples TC, TN, RC and RN composed the second clade (C2). There was further subdivision within clade C2, into two more subclades consisted of the samples collected from the same sites. The second clade is more homogeneous than the first one and it is flatter on the dendrogram. Samples TC, TN, RC and RN were characterized by high contents of same compounds (1,8-cineole, β-pinene and α-pinene), reason for their strong association (same clade). Greater dissimilarity level (1.6) observed in this analysis separated samples PC and PN in clade C1 from the rest of the samples considering that the most abundant constituents identified in these samples were 1,8-Cineole, β-Pinene and *o*-Cymene.

Since each population has different geological substrate: serpentine (samples PC and PN, Preševo), conglomerates and molasses (samples TC and TN, Pčinja valley) and biotite gneiss (samples RC and RN, Rujan mountain) it can be concluded that, with respect to the sample origin, different substrates produce various chemotypes. Chemical compositions

Table 1. Chemical composition of the *A. coarctata* volatiles achieved by GC and GC/MS

			Relative amount %						
RI$_{ref}$	**RI$_{exp}$**	**Compound**	**RN**	**TN**	**PN**	**RC**	**TC**	**PC**	**Class**
801	802	Hexanal	0.1	0.3	0.2	tr	0.4	tr	O
846	851	2(*E*)-Hexenal	0.2	1.2	0.4	0.3	0.3	tr	O
850	854	3(*Z*)-Hexenol	0.2	0.7	0.3	-	-	-	O
859	865	2(*Z*)-Hexenol	0.1	0.2	0.8	-	-	-	O
863	867	*n*-Hexanol	0.8	1.1	2.1	0.2	0.2	0.3	O
921	923	Tricyclene	0.2	tr	0.2	0.2	tr	0.2	M
924	928	α-Thujene	0.1	tr	0.3	0.1	0.1	0.3	M
932	935	α-Pinene	7.2	5.6	11.5	3.3	4.5	4.7	M
946	951	Camphene	3.5	1.6	3.2	3.0	1.7	3.1	M
952	962	Benzaldehyde	0.1	0.5	tr	0.2	0.2	0.1	O
969	976	Sabinene	0.7	1.0	2.8	1.5	1.5	3.5	M
974	979	β-Pinene	29.6	18	18.6	36.4	28.2	25.7	M
988	992	Myrcene	0.2	-	-	0.3	-	-	M
988	994	Dehydro-1,8-Cineole	-	0.1	tr	-	0.1	tr	MO
1014	1019	α-Terpinene	0.6	0.9	1.3	0.7	0.7	2.8	M
1022	1027	*o*-Cymene	0.5	0.9	11.6	0.2	0.4	11.7	M
1024	1031	Limonene	0.2	0.2	0.5	0.2	tr	0.5	M
1026	1034	1,8-Cineole	40.7	51.8	37.2	39.9	53.3	35.6	MO
1054	1060	γ-Terpinene	1.0	2.3	0.9	1.1	1.2	1.2	M
1065	1069	*cis*-Sabinene hydrate	0.6	0.6	-	-	-	-	MO
1086	1092	Terpinolene	0.2	0.4	0.2	0.2	0.2	0.2	M
1095	1100	Linalool	-	-	-	0.3	0.4	0.4	MO
1107*	1108	6-Ethenyldihydro-2,2,6-trimethyl-2H-pyran-3(4H)-one	3.7	1.9	0.5	3.1	0.9	1.6	O
1122	1128	α-Campholenal	-	tr	tr	-	-	-	MO
1135	1142	*trans*-Pinocarveol	-	0.1	-	-	-	-	MO
1141	1148	Camphor	3.7	1.5	1.6	1.9	1.1	1.6	MO
1160	1166	Pinocarvone	tr	0.1	tr	tr	tr	tr	MO
1165	1169	Borneol	0.2	0.2	0.3	0.3	0.3	0.4	MO
1170	1176	*cis*-Linalool oxide (pyranoid)	0.1	-	-	-	-	-	MO
1174	1180	Terpinen-4-ol	0.6	1.2	0.4	0.6	0.7	0.6	MO
1186	1193	α-Terpineol	0.8	0.9	0.5	0.9	0.8	0.6	MO
1195	1199	Myrtenal	tr	0.3	-	0.1	0.1	tr	MO
1288	1291	Lavandulyl acetate	-	0.1	0.2	0.1	-	0.4	MO
1374	1381	α-Copaene	0.2	0.9	0.4	0.6	0.2	0.3	S
1417	1427	(*E*)-Caryophyllene	0.4	0.9	1.7	0.4	0.8	1.8	S
1454	1458	(*E*)-β-Farnesene	tr	0.1	-	-	-	-	S
1458	1466	*allo*-Aromadendrene	0.7	-	0.2	0.8	-	0.4	S
1474	1482	10-*epi*-β-Acoradiene	0.2	-	-	0.2	-	-	S
1484	1488	Germacrene D	0.7	2.3	0.3	0.6	0.5	0.2	S
1500	1503	Bicyclogermacrene	0.1	0.1	tr	-	-	-	S
1513	1519	γ-Cadinene	0.1	-	-	0.1	-	-	S
1522	1529	δ-Cadinene	tr	0.1	0.1	0.1	-	0.2	S
1582	1592	Caryophyllene oxide	-	-	-	-	-	0.2	SO
1635	1642	*cis*-Cadin-4-en-7-ol	0.6	-	-	0.3	-	-	SO
		Total	**98.9**	**98.1**	**98.3**	**98.2**	**98.8**	**98.6**	
		Monoterpenoids	90.7	87.8	91.3	91.3	95.3	93.5	
		Hydrocarbons(M)	44.0	30.9	51.1	47.2	38.5	53.9	
		Oxygenated(MO)	46.7	56.9	40.2	44.1	56.8	39.6	
		Sesquiterpenoids	3.0	4.4	2.7	3.1	1.5	3.1	
		Hydrocarbons (S)	2.4	4.4	2.7	2.8	1.5	2.9	
		Oxygenated (SO)	0.6	0	0	0.3	0	0.2	
		Others (O)	5.2	5.9	4.3	3.8	2.0	2.0	

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; (*): identified by NIST Chemistry WebBook Retention indices; tr: traces (<0.1%); (-): not detectid**.** Samples RN, TN, PN- aerial plant parts (collected at Rujan mountain, Pčinje valley (Trgovište) and Preševo (Mitrovac), respectively); samples RC,TC, PC- inflorescences (collected at Rujan mountain, Pčinje valley (Trgovište) and Preševo (Mitrovac), respectively).

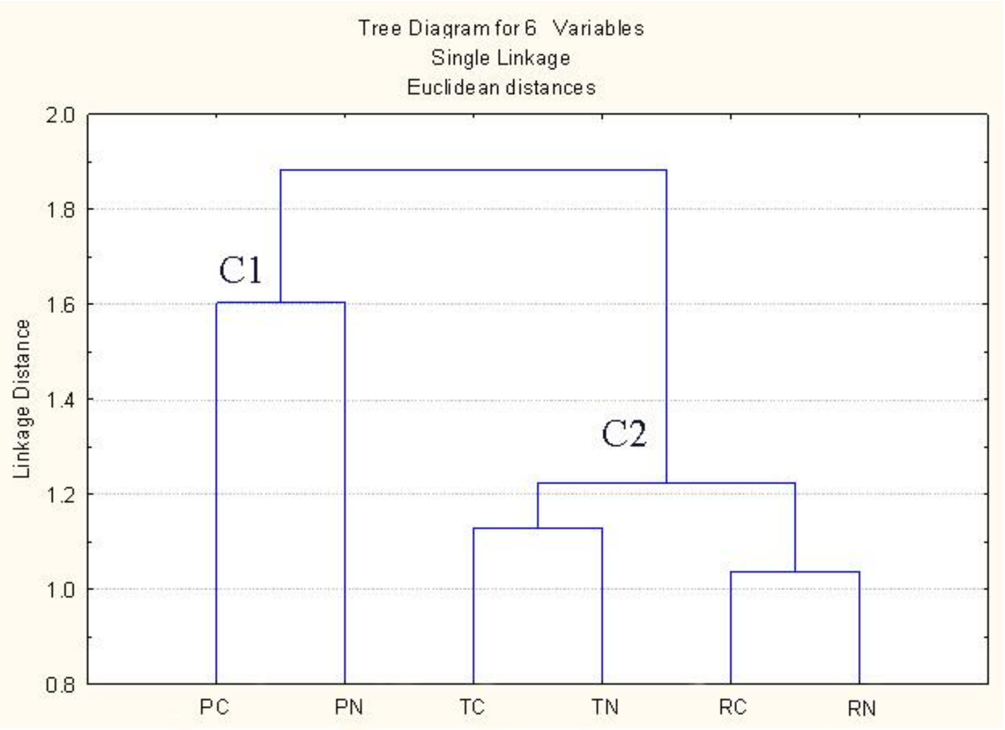

Fig. 1. Dendrogram obtained by agglomerative hierarchical clustering (constituent contents used as cases)

of samples collected on biotite gneiss and conglomerates and molasses do not differ to a great extent while the sample from serpentine is slightly different. It has been already noticed that the deficiency of water and indispensable mineral elements result in numerous structural and functional adaptations of plants species that grow on a serpentine substrate (Stevanović et al., 2003). Therefore, differences in the chemical composition of the investigated samples can probably be considered as another manifestation of the "serpentine syndrome".

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