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Essential oil analysis of different plant parts of *Geocaryum cynapioides* (Guss.) L. Engstrand

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***Abstract*:**

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Phytochemical analysis by GC and GC/MS of the essential oil samples, obtained from fresh aerial parts, shoots and inflorescences of *Geocaryum cynapioides* (Guss.) L. Engstrand, allowed the identification of 55 components in total, comprising 99.5%, 99.7% and 99.0% of the oils compositions, respectively. Regarding the aerial parts essential oil, the major of 52 identified volatile compounds were (*E*)-β-Farnesene (66.6%), 7-epi-*cis*-Sesquisabinene hydrate (17.4%) and (*E,E*)-α-Farnesene (3.7%). The same compounds were dominant among the 48 components detected in the shoots and 42 volatiles in inflorescences oil, but in different proportions. Hydrocarbon sesquiterpenes had the highest contribution in all investigated *Geocaryum cynapioides* essential oils, with a share of about three quarters of all, followed by one quarter of oxygenated sesquiterpenes, while the monoterpenoid and nonterpenoid compounds were detected only in trace amounts.

Key words: *Geocaryum cynapioides*, essential oil, phytochemical analysis, GC/MS

***Apstrakt*:**

Petrović, G., Stamenković, J., Stojanović, G., Zlatković, B., Jovanović, O.: *Analiza etarskih ulja iz različitih biljnih delova vrste Geocaryum cynapioides (Guss.) L. Engstrand. Biologica Nyssana, 9 (1). Septembar, 2018: 31-35.*

Fitohemijskom analizom uzoraka etarskih ulja izolovanih iz svežeg biljnog materijala različitih delova (nadzemnog dela, stabljike i cvasti) biljne vrste *Geocaryum cynapioides* (Guss.) L. Engstrand, pomoću GC i GC/MS metode, identifikovano je ukupno 55 komponenti, što predstavlja 99,5% nadzemnog dela, 99,7% stabljike i 99,0% cvasti. U etarskom ulju izolovanom iz svežeg nadzemnog dela biljke identifikovane su ukupno 52 komponente među kojima su (E)-β-Farnezen (66,6%), 7-epi-cis-Seskvisabinen hidrat (17,4%) i (E,E)-α-Farnezen (3,7%) bile najzastupljenije. Iste te komponente su bile dominantne i u etarskim uljima izolovanim iz stabljike i cvasti ali u različitim procentnim udelima. U svim ispitivanim uzorcima tri četvrtine ukupnog sastava etarskog ulja činili su ugljovodonični seskviterpeni, zatim oksigenovani seskviterpeni koji su činili jednu četvrtinu, dok su monoterpenoidi i neterpenoidne komponente detektovani samo u tragovima.

Ključne reči: *Geocaryum cynapioides*, etarsko ulje, fitohemijska analiza, GC/MS

Introduction

The genus *Geocaryum* L. (Apiaceae) is small genus, comprised of only 8 accepted species (The Plant list, 2012). It belongs to the tribe Scandiceae, subtribe Scandicinae (Spalik & Downie, 2001) with 11 another larger genera like *Chaerophyllum*, *Anthriscus*, *Osmorhiza* etc. *Geocaryum cynapioides* (Guss.) L. Engstrand is endemic species which inhabits pastures and meadows of the high mountains in southern parts of the Balkan Peninsula and central and southern parts of Italy (Ball, 1968). It is a perennial herb, 45 cm tall, with flexuous or curved internodes and undivided uppermost cauline leaves, linear or filiform lamina up to 20 mm, flowering from May to July. According to the Plant list database, *G. cynapioides* has as many as 18 synonymous names classifying it in other genera such as *Bunium*, *Carum* or even *Chaerophyllum*. Downie (Downie et al., 2010) finally hemotaxonomically defined its position within the tribe Scandiceae on the basis of his investigation of nuclear ribosomal DNA.

But, it is very unusual that there are no any previous data about the chemical composition of essential oils, as well as about the extracts of species belonging to this genus, and their biological activities. As far as we know, there is only one report on chemical composition of *G. cynapioides* essential oil (Radulović et al., 2008) which relates to the plant as a whole, including underground tubers, for which it is literally known that they might be completely different in chemical composition.

The aim of this study was to perform a detailed phytochemical analysis of *Geocaryum cynapioides* (Guss.) L. Engstrand essential oils obtained from different plant parts.

Material and methods

Plant material

The plant material (flowering stage) was collected at Vlasina Lake plateau, Serbia, in June 2017 and was identified by one of the authors Bojan Zlatković. 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 13317.

Sample preparation

The essential oils samples were prepared by hydrodistillation of fresh chopped whole aerial plant parts (194 g), shoots (158 g) and inflorescences (54 g), for 2.5 hours using Clevenger type apparatus, according to the method recommended in British Pharmacopoeia (British Pharmacopoeia, 1988). The essential oils were extracted with *n*-hexane, dried over anhydrous sodium sulfate and evaporated. The yields of the oils were 0.06%, 0.05% and 0.08%, based on the weight of fresh plant, respectively. Samples were re-dissolved in *n*-hexane to obtain the desired optimal concentrations and stored at -20 °C in the dark until analyzed.

- Hexane - Analytical reagent grade, Fisher Chemical, UK;
- Na_2SO_4 - Pro analysis grade, Sigma-Aldrich, Germany.

GC and GC/MS analysis

The sample of essential oil (20 mg/ml) was analyzed by a 7890/7000B GC/MS/MS triple quadrupole system in MS1 scan mode (Agilent Technologies, USA) equipped with a Combi PAL sampler. 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^{-1}. The carrier gas was helium with a flow of 1.0 mL min^{-1}. One microliter of the oil solution in hexane was injected (1:100, split ratio 40:1). Post run: back flash for 1.89 minutes, at 280 °C, with helium pressure of 50 psi. MS conditions were as follows: ionization voltage of 70 eV, acquisition mass range 40-440, scan time 0.32 seconds. GC-FID analysis was carried out under the same experimental conditions using the same column as described for the GC/MS. The percentage composition of the sample was computed as an average of the GC peak areas obtained in triplicate, without any corrections.

Identification of volatile compounds

EO constituents were identified by comparison of their linear retention indices relative to C_8-C_{32} alkanes on the HP-5 MS column (Van Den Dool & Kratz, 1963), with literature values and their MS with

Table 1. Chemical compositions of the *G. cynapioides* essential oils obtained by GC/MS

			Relative amount %			
RI_{exp}	**RI_{ref}**	**Compound**	**SO**	**IO**	**AO**	**Class**
998	988	Myrcene	tr	tr	tr	M
1034	1024	Limonene	tr	tr	tr	M
1042	1032	(*Z*)-β-Ocimene	1.5	0.2	1.0	M
1052	1044	(*E*)-β-Ocimene	0.6	0.1	0.4	M
1083	1088*	1-Nonen-3-ol	0.1	-	tr	O
1091	1086	Terpinolene	0.2	-	0.1	M
1101	1095	Linalool	tr	tr	-	MO
1100	1100	Undecane	-	tr	-	O
1106	1100	*n*-Nonanal	tr	0.1	tr	O
1147	1144	2(*Z*)-Nonen-1-al	0.1	-	tr	O
1161	1157	2(*E*)-Nonen-1-al	tr	-	tr	O
1287	1283	Isobornyl acetate	tr	-	tr	MO
1291	1288	Lavandulyl acetate	-	tr	tr	MO
1299	1300	Tridecane	tr	-	tr	O
1278	1374	α-Copaene	tr	tr	tr	S
1383	1379	Geranyl acetate	0.1	-	tr	MO
1388	1387	β-Bourbonene	0.1	-	tr	S
1394	1389	β-Elemene	0.1	-	tr	S
1406	1405	Sesquithujene	0.1	0.1	tr	S
1416	1410	α-Cedrene	tr	tr	tr	S
1423	1417	(*E*)-Caryophyllene	0.8	1.6	1.0	S
1429	1429*	Himachala-2,4-diene	0.2	0.1	0.1	S
1432	1430	β-Copaene	tr	.	tr	S
1437	1432	α-*trans*-Bergamotene	tr	tr	tr	S
1444	1440	(*Z*)-β-Farnesene	tr	tr	tr	S
1461	1454	(*E*)-β-Farnesene	61.0	72.0	66.6	S
1481	1481	γ-Curcumene	-	0.1	0.1	S
1485	1484	Germacrene D	2.7	3.0	2.8	S
1494	1491*	(*Z,E*)-α-Farnesene	1.8	1.2	1.5	S
1500	1500	Bicyclogermacrene	0.6	0.2	0.4	S
1503	1506	(*Z*)-α-Bisabolene	0.1	tr	0.1	S
1508	1505	(*E,E*)-α-Farnesene	4.4	2.6	3.7	S
1513	1514	β-Curcumene	0.5	0.2	0.4	S
1525	1521	β-Sesquiphellandrene	tr	tr	tr	S
1525	1522	δ-Cadinene	0.2	tr	0.1	S
1533	1529	(*E*)-γ-Bisabolene	tr	tr	tr	S
1555	1542	*cis*-Sesquisabinene hydrate	2.1	1.4	1.7	SO
1564	1561	(*E*)-Nerolidol	0.4	0.3	0.3	SO
1579	1574	Germacrene D-4-ol	0.1	-	tr	SO
1583	1577	*trans*-Sesquisabinene hydrate	0.4	0.1	0.1	SO
1593	1586*	7-epi-*cis*-Sesquisabinene hydrate	19.3	13.1	17.4	SO
1611	1611	Tetradecanal	tr	-	tr	O
1634	1632	α-Acorenol	0.6	0.3	0.4	SO
1651	1636	β-Acorenol	0.2	0.1	0.1	SO
1672	1670	epi-β-Bisabolol	0.7	0.4	0.5	SO
1685	1683	epi-α-Bisabolol	0.2	-	0.1	SO
1698	1699	β-Sinensal	tr	tr	tr	SO
1900	1900	Nonadecane	-	0.1	tr	O
2000	2000	Eicosane	-	tr	tr	O
2100	2100	Heneicosane	-	0.1	tr	O
2200	2200	Docosane	-	tr	-	O

RI_{exp}	RI_{ref}	Compound	Relative amount % SO	IO	AO	Class
2300	2300	Tricosane	0.1	0.6	0.2	O
2500	2500	Pentacosane	tr	0.4	0.1	O
2700	2700	Heptacosane	0.1	0.2	0.1	O
2900	2900	Nonacosane	0.3	0.4	0.2	O
		Total	**99.7**	**99.0**	**99.5**	
		Monoterpene hydrocarbons - M	2.3	0.3	1.5	
		Monoterpene oxygenated - MO	0.1	-	-	
		Sesquiterpene hydrocarbons - S	72.6	81.1	76.8	
		Sesquiterpene oxygenated - SO	24.0	15.7	20.6	
		Others - O	0.7	1.9	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*-alkanes; (*): identified by NIST Chemistry WebBook Retention indices; tr: traces (<0.1%); (-): not detected**.** Essential oils: SO - shoots; IO - inflorescences; AO - aerial plant parts.

those of authentic standards, as well as those from Adams (Adams, 2007), 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 EOs by the application of the AMDIS software (Automated Mass Spectral Deconvolution and Identification System, Ver. 2.1, *DTRA*/NIST, 2011).

Results and discussion

Chemical compositions of the aerial plant parts, shoots and inflorescences essential oils of *Geocaryum cynapioides*, obtained by GC and GC/MS analyses, are presented in **Tab. 1**. Number of identified compounds in aerial parts (AO), inflorescences (IO) and shoots (SO) samples were 52, 42 and 48 respectively, representing 99.5%, 99.0% and 99.7% of total volatiles.

The composition of the essential oils of the shoots and the oil obtained from inflorescences was found to be quite comparable. Hydrocarbon sesquiterpenes had the highest contribution in all investigated *G. cynapioides* essential oils, with a share of about three quarters of all, followed by one quarter of oxygenated sesquiterpenes. The shoot essential oil had a little bit lower content of hydrocarbon sesquiterpenes compared to the inflorescences oil, 72.6% and 81.1%, respectively. In both essential oils this class of compounds predominated over the oxygenated sesquiterpenes. A reverse distribution was observed for the oxygenated sesquiterpenes which are prevalent in shoots essential oil over the inflorescences oil (24.0% vs. 15.7%, respectively). Hydrocarbon monoterpenes were present in negligible amounts in both oils, while the oxygenated monterpenes were not detected at all. Obtained results indicate that the composition of the *G. cynapioides* aerial parts essential oil represents the average value of the contents of essential oils derived from inflorescences and shoots essential oils.

Phytochemical analysis by GC and GC/MS of the essential oil samples, obtained from fresh aerial parts, shoots and inflorescences of *G. cynapioides*, allowed the identification of 55 components in total. Regarding the aerial parts essential oil, the major of 52 identified volatile compounds were (*E*)-β-Famesene (66.6%), 7-epi-*cis*-Sesquisabinene hydrate (17.4%) and (*E,E*)-α-Farnesene (3.7%). The same compounds were dominant among the 48 components detected in the shoots and 42 volatiles in inflorescences oil, but in different proportions.

As we already shown, chemical composition of inflorescences could be pretty similar (Kostevski et al., 2016) or essentially various (Petrović et al., 2018; Stamenković et al., 2015) in comparison to the composition of the shoots essential oil. On the other hand, the composition of the volatile components obtained from all the above-ground parts of the plant and root is always completely different (Petrović et al., 2017; Stamenković et al. 2015).

Compared with the previously published results of *G. cynapioides* essential oil (Radulović et al., 2008), certain differences in chemical compositions of aerial parts essential oils can be observed. That can be explained by the fact that sample A consisted of fresh aerial parts (inflorescences, stems and leaves) and underground tubers, while sample B was composed of stems, leaves and umbels full of ripe fruit (fruiting stage). Sample B differs significantly both by the number of identified components and by the relative content of compound classes, especially in relation to monoterpenoids content (hydrocarbon monoterpenes

6.5% and oxygenated monterpenes 4.9%). However, the main difference that can be noticed is that one of the main components identified in our investigation, 7-epi-*cis*-Sesquisabinene hydrate, was not found at all. The probable reason is the similarity of the mass spectra and the proximity of the retention indices of this compound and *trans*-Sesquisabinene hydrate, which was found in a significant amount, which led to a misidentification.

According to the results of this study, the essential oil of *G. cynapioides* consisted mainly of sesquiterpene compounds, implying that there are great differences in composition of investigated essential oil compared to other reports about the Scandicinae species` essential oils. Considering the fact that *Geocaryum* genus is not investigated at all, more studies are needed regarding essential oil constituents of its species. Obtained data could be helpful in future research to clarify the chemotaxonomic relationships between the genera within the tribe Scandiceae and subtribe Scandicinae.

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