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Original scientific paper

Essential oil composition of different parts of endemic species *Seseli gracile* Waldst. & Kit. (Apiaceae) from natural and cultivated conditions

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Abstract: The chemical composition of the essential oils of *Seseli gracile* Waldst. & Kit. from natural habitat (Đerdap Gorge, Serbia) and from cultivated plants (Belgrade, Serbia) were characterized. The essential oils of the root, aerial parts, inflorescence and fruit were analyzed by GC/MS and GC/FID. Monoterpene hydrocarbons were the main compounds in the essential oil of aerial parts (45.2–93.0 %), inflorescences (84.1 and 90.0 %) and fruit (85.0 %). Polyacetylenes (38.8 and 87.6 %) were dominant in the essential oil of root. The cluster analysis revealed that there were significant differences in the chemical composition of the *S. gracile* oils at different phenological stages. On the other hand, essential oils from the aerial parts from natural and cultivated plants showed quite uniform qualitative composition. The aerial parts essential oil from natural habitat contained higher content of *para*-cymene (mean values 17.3 *vs*. 6.5 %) and lower amounts of terpinolene (mean values 23.1 *vs*. 49.9 %). Also polyacetylene falcarinol was present only in the aerial parts samples from natural habitat. The essential oil of inflorescences from natural habitat contained higher concentration of terpinolene, quite similar amount of *para*-cymene and lower content of *α*-pinene.

Keywords: *Seseli gracile*; GC/MS; GC/FID; natural habitat; cultivated plants; cluster analysis.

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INTRODUCTION

Seseli gracile Waldst. & Kit. (Apiaceae) is an endemic species from south-west Romania and northeast Serbia. In Serbia, it grows only in Đerdap Gorge on Mali and Veliki Štrbac.[1] Species of the genus *Seseli* are traditionally used for the treatment of digestive disorders, rheumatism and as anti-inflammatory agents.[2,3] The essential oils of these species are located in endogenous secretory canals and have shown antimicrobial, antifungal and antigenotoxic activity.[4–6]

The biosynthesis of the terpenes is highly species specific. Variation in the chemical composition of the essential oils may be observed with regard to the origin and the developing stage of the collected plant material.[4,7–9] The change of the environmental conditions has shown a very strong influence on the essential oil profile.[10] The previous investigation of *S. rigidum* Waldst. & Kit. essential oils, showed the marked effect of climate on the oil's composition, while the impact of the substrate was less pronounced.[11]

The aim of the present work was to characterise and compare essential oils of different plant parts of the same genotype of *S. gracile*, collected from the natural habitat (Mt. Štrbac, Serbia) and from the cultivated plants (Belgrade, Serbia). According to the available literature this is the first report of the essential oil composition of this endemic species.

EXPERIMENTAL

Plant material

Whole plants (aerial parts and root) of *S. gracile* were collected on Mali Štrbac in Djerdap Gorge, Serbia (Table I). Voucher specimens have been deposited at the Herbarium of the Department of Botany, University of Belgrade, Faculty of Pharmacy (3702 HFF). Part of material was transferred to private garden in Belgrade and cultivated there, while another part was used for analysis.

TABLE I. Collection sites of *S. gracile* samples

Locality	Plant part	Date
Mali Štrbac, Djerdap Gorge	Root	September 2013
		July 2015
	Aerial parts	September 2013
		July 2015
	Inflorescences	July 2015
Belgrade, private garden	Aerial parts	August 2014
		August 2015
	Inflorescences	August 2015
	Fruit	September 2014

Isolation of the essential oil

The essential oils were isolated from dried plant material. Isolation of the essential oils was obtained by hydrodistillation in a Clevenger-type apparatus according to the procedure given in the European Pharmacopoeia 7.0.[12] The essential oils yields were calculated on dry-weight basis (mL/g).

GC/MS and GC/FID analysis

The volatile constituents were determined by GC/FID and GC/MS. The GC analysis was performed on Agilent 6890N GC system equipped with 5975 MSD and FID, using HP-5 MS column (30 m×0.25 mm, 0.25 μm film thickness). The injection volume was 2 μL and the injector temperature was 200 °C with a 10:1 split ratio. Helium was the carrier gas and its flow rate was 1.0 mL/min (constant flow mode). The column temperature was linearly programmed in the range 60–280 °C at a rate of 3°/min and held at 280 °C for 5 min. The transfer line was heated at 250 °C. The FID detector temperature was 300 °C. EI mass spectra (70 eV) were acquired in the *m*/*z* range 35–550. The retention indices were experimentally determined using *n*-alkanes (C_8–C_{20} and C_{21}–C_{40}) injected under the same chromatographic conditions. The identification of the compounds was based on the comparison of their retention indices (*RI*), their retention times (t_R) and mass spectra with those obtained from authentic samples and/or the NIST AMDIS (Automated Mass Spectral Deconvolution and Identification System) software, Wiley libraries, Adams data base and literature.[13] Relative percentages of the identified compounds were computed from the GC/FID peak area.

Statistical analysis

The analysis of variance (ANOVA) was used to research the impact of different plant organ on the composition of essential oils. ANOVA was applied to calculate critical value from *F*-test (F) and *p*-statistical significance ($p < 0.05$). The cluster analysis was used to group the samples on the basis of their similarities or differences in the essential oils composition. Initial data set used for analysis included all components of investigated essential oils (78 components×9 samples). Cluster analysis of essential oil samples, that are complex mixtures of high number of components is based on the distances calculations between the samples in a multidimensional space. The overall similarity between the measured units was described by Pearson's distances in the UPGMA (unweighted pair-group method using arithmetic averages) clustering method. The statistical analyses were performed with the package Statistica 5.1.[14]

RESULTS AND DISCUSSION

In the essential oil of different parts of *S. gracile* 78 compounds were identified, that represented 91.3–99.0 % of the total oil (Table II). Monoterpene hydrocarbons were the main compounds in the essential oil of aerial parts (45.2––93.0 %), inflorescences (84.1 and 90.0 %) and fruit (85.0 %). Terpinolene (6.1––57.5 %), γ-terpinene (3.3–24.2 %) and *para*-cymene (1.3–25.2 %) mostly dominated in the essential oil of all aboveground parts. The essential oil of aerial parts also contained β-pinene (4.3–6.1 %) and *para*-cymen-8-ol (0.2–5.5 %). The contents of γ-terpinene (24.2 %), α-pinene (9.6 %), β-pinene (7.6 %) and δ-cadinene (5.9 %) were the highest in the fruit oil.

TABLE II. Chemical composition of the essential oil of different parts of *S. gracile*

Constituent[a]	*KI*[b]	Root		Aerial parts				Inflorescence		Fruit
		St[c]		St		Bg[d]		St	Bg	Bg
		2013	2015	2013	2015	2014	2015	2015	2015	2014
Heptanal	900	1.7	t[e]	t	t	–	–	–	–	–
α-Thujene	926	t	t	t	0.3	0.2	0.3	t	t	0.2
α-Pinene	933	0.5	0.3	1.1	2.1	1.6	2.9	1.1	5.0	9.6

TABLE II. Continued

Constituent[a]	*KI*[b]	Root		Aerial parts				Inflorescence		Fruit
		St[c]		St		Bg[d]		St	Bg	Bg
		2013	2015	2013	2015	2014	2015	2015	2015	2014
Camphene	948	t	0.2	t	0.1	t	0.2	t	0.7	0.9
Sabinene	973	t	t	0.8	1.7	1.0	1.4	0.8	1.0	1.7
β-Pinene	978	0.8	t	4.3	6.1	4.6	5.7	1.9	2.0	7.6
2-Pentylfuran	989	0.9	t	t	–	–	–	–	–	–
Myrcene	990	–	–	0.4	1.2	1.2	1.4	1.1	2.0	2.2
n-Octanal	1002	10.1	0.5	0.4	–	–	–	–	–	–
α-Phelandrene	1006	t	–	t	0.3	0.4	0.3	0.3	0.3	0.2
α-Terpinene	1017	t	–	t	0.2	0.1	t	0.1	t	0.1
para-Cymene	1020	5.0	t	25.2	9.4	1.3	11.7	7.7	6.8	4.1
Limonene	1028	0.9	t	3.4	3.7	3.9	4.7	3.7	5.3	4.5
(*Z*)-*β*-Ocimene	1036	t	t	0.6	2.8	3.7	2.3	2.0	2.6	2.9
(*E*)-*β*-Ocimene	1046	t	–	t	0.6	0.2	t	1.0	t	t
(*E*)-2-Octen-1-al	1055	0.4	–	–	–	–	–	–	–	–
γ-Terpinene	1060	1.0	t	3.3	23.0	17.3	11.6	18.4	13.7	24.2
Terpinolene	1091	1.4	0.2	6.1	40.0	57.5	42.3	51.6	44.5	26.8
Linalool	1100	t	–	0.2	–	t	–	0.2	0.3	0.1
6-Camphenone	1100	t	–	0.2	–	–	–	–	–	–
n-Nonanal	1102	1.1	t	t	–	–	–	–	–	–
1-Octen-3-yl-acetate	1112	t	–	t	0.2	t	0.1	t	t	t
1,3,8-*p*-Menthatriene	1113	t	–	t	0.2	t	0.2	0.3	0.2	t
2-Propenyl-phenol	1136	t	–	t	0.4	0.1	0.2	0.2	0.1	t
trans-Pinocarveol	1138	t	–	0.9	–	–	0.1	–	t	t
cis-para-Mentha-2, 8-dien-1-ol	1145	0.6	t	3.6	0.7	0.2	2.0	1.6	1.8	0.2
(*E*)-2-Nonen-1-al	1157	0.6	t	t	–	–	t	–	–	–
Pinocarvone	1162	t	–	0.5	t	–	t	–	t	–
1,8-Menthadien-4-ol	1177	t	–	2.6	0.1	t	4.0	0.4	3.9	0.6
Terpinen-4-ol	1177	–	–	–	0.2	–	t	0.2	–	–
Dec-1-en-3-ol	1178	0.6	t	t	–	–	–	–	–	–
para-Methyl-aceto-phenone	1185	–	–	1.2	t	–	1.0	t	t	–
para-Cymen-8-ol	1185	0.7	t	5.5	1.2	0.2	3.3	1.1	3.7	0.3
Myrtenal	1196	t	–	1.2	t	–	0.2	–	t	–
β-Cyclocitral	1204	–	–	t	0.1	t	0.2	0.4	0.2	t
(*E*)-2-Decenal	1260	1.1	t	t	–	–	–	–	–	–
Thymol	1291	t	–	0.8	t	–	t	t	t	t
(*E,Z*)-2,4-Decadienal	1292	4.2	0.2	–	–	–	–	–	–	–
Carvacrol	1301	t	–	2.4	0.2	t	0.2	t	t	t
(*E,E*)-2,4-Decadienal	1316	8.8	0.5	t	–	–	–	–	–	–
(*E*)-2-Undecenal	1362	0.8	t	t	–	–	–	–	–	–
α-Copaene	1376	t	t	0.7	0.2	0.2	0.1	0.2	0.3	0.9
β-Elemene	1389	t	–	0.8	0.2	–	–	0.2	–	–
2-*epi*-*β*-Funebrene	1414	t	0.2	–	–	–	–	–	–	t
(*E*)-Caryophyllene	1420	–	–	0.4	0.6	1.1	0.6	0.4	0.7	0.7

TABLE II. Continued

Constituent[a]	*KI*[b]	Root		Aerial parts				Inflorescence		Fruit
		St[c]		St		Bg[d]		St	Bg	Bg
		2013	2015	2013	2015	2014	2015	2015	2015	2014
β-Cedrene	1422	t	0.2	–	–	–	–	–	–	–
β-Barbatene	1443	t	0.4	t	–	–	–	–	–	t
(*E*)-*β*-Farnesene	1456	0.6	1.2	1.8	1.3	–	–	0.6	t	t
β-Acoradiene	1467	t	0.2	t	–	–	–	–	–	–
γ-Muurolene	1477	–	–	t	t	t	t	t	t	0.2
Germacrene D	1482	–	–	–	0.3	0.6	0.2	0.3	0.2	1.5
ar-Curcumene	1482	6.2	1.5	0.8	–	–	–	–	–	–
Bicyclogermacrene	1496	–	–	–	t	t	t	t	t	t
Cuparene	1505	1.1	0.2	t	–	–	–	–	–	–
Germacrene A	1505	–	–	t	0.1	–	–	0.2	–	–
β-Bisabolene	1508	t	0.3	t	–	–	–	–	–	t
β-Curcumene	1510	2.1	3.2	t	–	–	–	–	–	–
β-Sesquiphellandrene	1523	–	0.2	–	–	–	–	–	–	–
δ-Cadinene	1524	t	–	1.6	1.2	1.5	0.5	1.5	1.4	5.9
γ-Cuprenene	1533	t	0.2	–	–	–	–	–	–	t
Elemicin	1543	0.7	t	t	–	t	–	–	–	–
Dodecadienol	1551	0.8	–	–	–	–	–	–	–	–
Germacrene B	1557	t	t	1.0	t	t	t	–	–	t
Spathulenol	1579	t	–	0.4	–	–	t	t	t	–
Caryophyllene oxide	1584	t	–	1.7	0.1	t	0.7	t	0.4	t
Muurola-4,10(14)-di--en-1-β-ol	1629	t	–	0.6	t	–	t	t	0.2	0.3
Amorpha-4,9-dien-8--ol	1718	–	–	0.7	–	–	–	t	–	–
Tetradecanoic (myristic) acid	1762	t	–	0.7	–	–	–	–	–	–
Neophytadiene	1836	–	–	t	t	0.1	–	–	–	t
Hexahydrofarnesyl acetone	1842	t	–	0.9	–	–	–	–	t	–
Hexadecanoic acid	1961	2.4	–	1.8	–	t	–	–	–	–
Falcarinone[f]	2000	t	0.6	–	–	–	–	–	–	–
Falcarinol	2034	38.8	87.0	7.6	t	–	–	–	–	–
9-Octadecen-1-ol (oleyl alcohol)	2059	t	–	0.7	t	0.3	t	–	t	t
2,13-Octadecadien-1--ol	2073	t	–	4.4	0.2	1.2	0.3	0.2	0.6	1.6
Linoleic acid	2125	2.2	–	t	t	–	–	–	–	–
Oleic acid	2130	1.4	0.1	t	–	–	–	–	–	–
Ethyl linoleate	2149	0.7	0.6	t	–	–	–	–	–	–
Monoterpene hydrocarbons		9.6	0.7	45.2	91.7	93.0	85.0	90.0	84.1	85.0
Oxygenated monoterpenes		1.3	0	17.9	2.5	0.4	10.0	3.9	9.9	1.2
Sesquiterpene hydro-carbons		10.0	7.8	7.1	3.9	3.4	1.4	3.4	2.6	9.2
Oxygenated sesquiterpenes		0	0	3.4	0.1	0	0.7	0	0.6	0.3

TABLE II. Continued

Constituent[a]	*KI*[b]	Root		Aerial parts				Inflorescence		Fruit
		St[c]		St		Bg[d]		St	Bg	Bg
		2013	2015	2013	2015	2014	2015	2015	2015	2014
Polyacetylenes		38.8	87.6	7.6	0	0	0	0	0	0
Other		38.5	1.9	10.1	0.8	1.7	1.6	0.4	0.7	1.6
Identified		98.2	98.0	91.3	99.0	98.5	98.7	97.7	97.9	97.3

[a]Content of compound expressed as percentage of the total oil composition; [b]Kovat's retention index determined relative to two series of *n*-alkanes (C_8–C_{20} and C_{21}–C_{40}) on a HP-5 MS column; [c]Natural habitat Mali Strbac, Djerdap Gorge; [d]private garden in Belgrade; [e]trace (<0.1 %); [f]tentative determination

On the other hand, polyacetylenes (38.8 and 87.6 %) were dominant in the essential oil of root. Polyacetylene falcarinol (38.8 and 87.0 %), aldehydes *n*-octanal (10.1 and 0.5 %), (*E*,*E*)-2,4-decadienal (8.8 and 0.5 %) and sesquiterpene *ar*-curcumene (6.2 and 1.5%) were main compounds in the root oil.

The analysis of variance (ANOVA) was used to investigate the differences between the essential oils isolated from various plant parts. In the root essential oil the content of polyacetylenes was significantly ($p < 0.05$) higher, while monoterpene carbohydrates were present in markedly ($p < 0.05$) lower amount. The main differences were in the content of root oil main compounds falcarinol, (*E*,*E*)-2,4-decadienal, (*E*,*Z*)-2,4-decadienal and *ar*-curcumene, that were present in small amounts or even absent from the aboveground parts. On the other hand, the root oil contained smaller amounts of terpinolene (1.4 and 0.2 %) and *γ*-terpinene (1.0 % and traces) that dominated in other oils.

Another focus of this research was to determine the influence of different habitat on the composition of *S. gracile* aerial parts essential oils. Plants from the natural habitat were transferred to Belgrade and the composition of essential oil was compared. Although there was a high variability in the quantitative composition, their qualitative composition was quite uniform. The aerial parts essential oil from natural habitat contained higher content of *para*-cymene (mean values 17.3 *vs*. 6.5 %) and lower amounts of terpinolene (mean values 23.1 *vs*. 49.9 %). Also, polyacetilene falcarinol was present only in the samples from natural habitat (7.6 %). The essential oil of inflorescences from nature contained higher concentration of terpinolene (51.6 *vs.* 44.5 %), quite similar amount of *para*-cymene (7.7 *vs.* 6.8 %) and lower content of *α*-pinene (1.1 *vs.* 5.0 %). Falcarinol was not detected in natural or in the cultivated *S. gracile* inflorescence and fruit essential oil.

The cluster analysis was used to observe similarities and relationships between different samples of *S. gracile* oils (Fig. 1). Two clusters were formed; essential oils from all aboveground plant parts were in the first and the essential oils of root were in the second cluster.

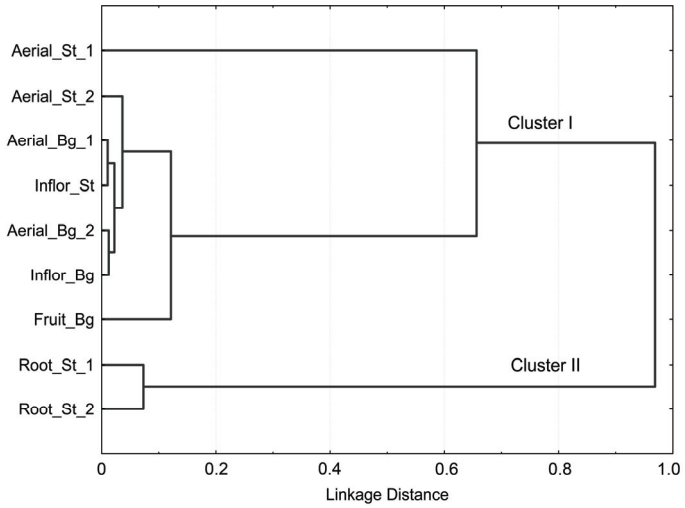

Fig. 1. Cluster analysis (CA) of the chemical composition of *S. gracile* essential oils for 78 constituents presented as UPGMA based on Pearson's distances. Aerial_St_1 – aerial parts Strbac 2013; Aerial_St_2 – aerial parts Strbac 2015; Aerial_Bg_1 – aerial parts Belgrade 2014; Aerial_Bg_2 - aerial parts Belgrade 2015; Inflor_St – inflorescences Strbac 2015; Inflor_Bg – inflorescences Belgrade 2015; Fruit_Bg – fruit Belgrade 2014; Root_St_1 – root Strbac 2013; Root_St_2 – root Strbac 2015.

Similarly to ANOVA, cluster analysis confirmed that the essential oils of root are clearly separated and chemically different from other plant parts oils. In the first cluster the oil of aerial parts from the natural habitat (Štrbac, 2013) was separated from other aboveground parts oils. The chemical differentiation of aerial parts essential oil from natural habitat (Štrbac, 2013) is based on lower amounts of terpinolene (6.1 %) and *γ*-terpinene (3.3 %) as well as the higher content of *para*-cymene (25.2 %), falcarinol (7.6 %) and *para*-cymen-8-ol (5.5 %) in the oil. The reason for such a differentiation was probably a phase of plant development. The sample from natural habitat Štrbac was collected in 2013 in a fruiting phase (September) while other natural or cultivated samples were obtained in a flowering phase (July and August). Previous investigations showed that the content and composition of essential oil can be significantly changed during the plant development and fruit maturation.[7,8,15]

In the cluster analysis the fruit oil was also slightly separated by higher content of *γ*-terpinene (24.2 %), *α*-pinene (9.6 %), *β*-pinene (7.6 %) and *δ*-cadinene (5.9 %).

Within the species of the genus *Seseli*, essential oils of aerial parts and fruits were mostly researched. The main compounds in the essential oils of aerial parts of species: *S. rigidum* Waldst. & Kit., *S. pallasii* Besser (syn. *S. varium* Trevir.), *S. peucedanoides* (M. Bieb.) Koso-Pol., *S. montanum* L., *S. globiferum* Vis., *S.*

tortuosum L. and *S. campestre* Besser were monoterpenes α-pinene, β-pinene and sabinene.[2,4,16–20] Similarly, in the fruit oils of *S. rigidum*, *S. pallasii*, *S. tortuosum*, *S. globiferum*, *S. campestre* and *S. montanum* subsp. *peixotoanum* (Samp.) M. Laínz *α*-pinene, *β*-pinene or sabinene were predominant.[3,4,16,21,22] Other group of essential oils of *Seseli* species was characterised by high sesquiterpene content. The sesquiterpenes (*β*-selinene, germacrene A, spathulenol, bicyclogermacrene and carotol) were dominant constituents in the essential oil of aerial parts or fruits of *S. annuum* L., *S. libanotis* (L.) W. D. J. Koch., *S. gummiferum* Sm., *S. petraeum* M. Bieb. and *S. andronakii* Woronow.[9,23–25]

The main compounds of *S. gracile* essential oils of aboveground parts were monoterpenes terpinolene and *γ*-terpinene. Although it seems like these oils are chemically different from other essential oils of the species of the genus *Seseli*, there is a certain biochemical similarity. Monoterpenes terpinolene and *γ*-terpinene, as well as *α*-pinene, *β*-pinene and sabinene are biosynthesised from the same precursor *α*-terpinyl acetate in a methylerythritol phosphate (MEP) pathway. Terpene synthase catalyses proton loss of *α*-terpinyl cation that leads to terpinolene. From the same precursor by hydride shifts, followed by proton losses terpinene or phellandrene are synthetised. After additional cyclizations of the *α*-terpinyl cation, the pinyl or sabinyl cation can be generated, the precursors of *α*-pinene/*β*-pinene or sabinene.[26,27]

The *S. gracile* root essential oil contained polyacetylene falcarinol, fatty acid derived metabolite and quite common constituent of the roots of the members of the Apiaceae family.[28] Previous researches showed that falcarinol dominated in the *S. rigidum* root essential oil (88.8 %).[4] Falcarinol and falcarindiol were isolated from *S.annuum* root extract[29], while falcarindiol from the aerial parts extract of *S. mairei* H. Wolff.[30]

CONCLUSION

The *S. gracile* essential oils of aerial parts, inflorescences and fruits were characterised by high content of monoterpenes (terpinolene, *γ*-terpinene and *para*-cymene) and on the other hand the oil of underground parts by polyacetylene falcarinol. Meanwhile, there were no prominent differences in the composition of the essential oils between the plants from natural habitat and cultivated ones.

These results show that cultivation of rare or endemic plants and their comparison with natural samples could provide information about influence of cultivation on the chemical composition of plant secondary metabolites and enable us for proper conservation of plants.

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ИЗВОД

САСТАВ ЕТАРСКОГ УЉА РАЗЛИЧИТИХ БИЉНИХ ДЕЛОВА ЕНДЕМИЧНЕ ВРСТЕ *Seseli gracile* WALDST & KIT. (APIACEAE) ИЗ ПРИРОДНИХ И ГАЈЕНИХ УСЛОВА

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Испитивање хемијског састава етарских уља ендемичне врсте *Seseli gracile* Waldst. & Kit је урађено на биљкама из природног станишта (Ђердапска клисура) и из гајених услова (Београд). Етарска уља корена, хербе, цвасти и плода су анализирана помоћу GC/MS и GC/FID методе. Монотерпенски угљоводоници представљају главне компоненте у етарским уљима хербе (45,2–93,0 %), цвасти (84,1 и 90,0 %) и плода (85,0 %). Насупрот њима, полиацетилени (38,8 и 87,6 %) доминирају у етарском уљу корена. Кластер анализа је показала да постоје значајне разлике у хемијском саставу етарских уља у различитим фенофазама. Насупрот томе, у квалитативном саставу етарских уља хербе биљака са природних станишта и из гајених услова нису уочене значајније разлике. Етарско уље хербе са природног станишта је садржало већу количину *para*-цимена (средње вредности 17,3 према 6,5 %), као и мању количину терпинолена (средње вредности 23,1 према 49,9 %). Такође, полиацетилен фалкаринол је био присутан само у узорцима хербе са природног станишта. Етарско уље цвасти са природног станишта је садржало вишу концентрацију терпинолена, сличну *para*-цимена, а нижу α-пинена.

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