BIOLOGICA NYSSANA

8 (1) • September 2017: 99-103

DOI: 10.5281/zenodo.964345

Original Article

Received: 23 Mart 2017
Revised: 25 May 2017
Accepted: 25 July 2017

First insight into the chemical composition of essential oils and head space volatiles obtained from fresh leaves and flowers of *Peucedanum longifolium* Waldst. & Kit.

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***Abstract*:**

Stojanović, G., Jovanović, O., Zlatković, B., Jovanović, S., Zrnzević, I., Ristić, N.: *First insight into the chemical composition of essential oils and head space volatiles obtained from fresh leaves and flowers of Peucedanum longifolium Waldst. & Kit.. Biologica Nyssana, 8 (1), September 2017: 99-103.*

For the first time, chemical composition of *P. longifolium* essential oils (EO) and head space (HS) volatiles obtained from the fresh leaves, harvested in different phases of the plant development, and inflorescences was compared. The major contributor of leaves essential oil in vegetative phase (LD) was β-elemene (44.1%). On the contrary, β-elemene (22.5%) was the second most abundant component, while (*E*)-β-ocimene (26.7%) was the first and *cis*-lanalool oxide (furanoid, 21.9%) was the third most represented compound in the leaves essential oils collected in blossoming phase (LF). (*E*)-β-Ocimene was the major compound in both HS leaf samples but in very varying amounts (LD 81.8% and LF 27.4%). Beside (*E*)-β-ocimene, *p*-cymene and limonene were represented more than 10% (13.6% and 15.2% respectively). The essential oil and HS volatiles of the inflorescences was characterized by a high content of β-phellandrene (16.4% and 20.4% respectively), α-phellandrene (22.5% and 13.9% respectively) and myrcene (23.1% and 22.1% respectively). The presented data showed that there was a significant difference in the composition of volatile components from different plant organs and also from the same organ in different stages of development.

Key words: *Peucedanum longifolium*, essential oil composition, head space volatiles

***Apstrakt*:**

Stojanović, G., Jovanović, O., Zlatković, B., Jovanović, S., Zrnzević, I., Ristić, N.: *Prvi uvid u hemijski sastav etarskog ulja i "head space" (HS) isparljivih komponenti svežih listova u različitim fazama razvoja biljke i svežih cvetova Peucedanum longifolium Waldst. & Kit.. Biologica Nyssana, 8 (1), Septembar 2017: 99-103.*

Po prvi put je određen i upoređen hemijski sastav etarskog ulja (EO) i "head space" (HS) isparljivih komponenti *P. longifolium* dobijenih iz svežih listova u različitim fazama razvoja biljke i svežih cvetova. Najzastupljenija komponenta etarskog ulja listova biljke u vegetativnoh fazi (LD) je bio β-elemen (44.1%). U etarskom ulju listova biljke u fazi cvetanja (LF) redosled zastupljenosti je bio: (*E*)-β-ocimen (26.7%), β-elemene (22.5%) i *cis*-lanalol-oksid (furanoid, 21.9%). (*E*)-β-Ocimen je bio najzastupljeniji medju "head space" (HS) isparljivim komponentama oba HS uzorka listova (LD 81.8% i LF 27.4%). Pored (*E*)-β-ocimena, *p*-cimene i limonene su bili zastupljeni preko 10% (13.6% i 15.2% respektivno). Etarsko ulje i HS isparljive komponente cvetova je karakterisao visok sadržaj β-felandrena (16.4% i 20.4%, respektivno), α-felandrena (22.5% i 13.9%, respektivno) i mircena (23.1% and 22.1%, respektivno). Prezentovani rezultati pokazuju da postoji razlika u sastavu isparljivih komponenti listova i cvetova *P. longifolium* kao i listova biljke u fazi razvića i cvetanja.

Ključne reči: *Peucedanum longifolium*, sastav etarskog ulja, sastav "head space" isparljive komponente

Introduction

About 120 *Peucedanum* L. species are widespread in Europe, Asia and Africa (S p a l i k et al., 2004). Of this number, 29 species and subspecies grow in the territory of Europe, of which 14 in Serbia (N i k o l i ć , 1973). Some of the members, e. g. *P. longifolium* Waldst. & Kit., *P. cervaria* (L.) Lapeyr., *P. officinale* L., *P. oreoselinum* (L.) Moench and *P. arenarium* Waldst. & Kit. are indicated in cardiovascular, respiratory, genitourinary and nervous system disorders, as used in traditional medicine of some Balkan countries (P e t k o v & M a n o l o v , 1978; S a r i ć , 1989; М и т р е в , 1995). *Peucedanum longifolium* (sect. *Peucedanum*) is glabrous perennial plant with solid (60-200 cm high), branched steams and small, yellow flowers arranged in several compound inflorescences. The species inhabits dry grasslands and rocky, usually calcareous slopes of medium altitudes in the C, S & E parts of Balkan Peninsula, extending to the mountains of C Romania (S p a l i k et al., 2004). However, this species is mainly reported for limestone habitats (N i k o l i ć , 1973), while we have surprisingly found it growing over silicate bedrock.

Previous phytochemical studies found coumarins and flavonoids in the roots, steams, flowers and fruits of *P. longifolium* (K u z m a n o v et al., 1980). Evaluation of antioxidant and antimicrobial activity of essential oils (T e p e et al., 2011; I l i ć et al., 2015) and extracts (M a t e j i ć et al., 2013) was also reported.

To the best of the authors' knowledge, there are only few papers (T e p e et al., 2011; I l i ć et al., 2015; K a p e t a n o s et al., 2008) referring to the chemical composition of essential oil of air-dried aerial parts of *P. longifolium* in flowering phase and one report related to the volatiles of fresh aerial parts from initial stage of the plant development (J o v a n o v i ć et al., 2015). Recently, chemical composition of the essential oil and head space volatiles obtained from the fresh root of *P. longifolium* was published (S t o j a n o v i ć et al., 2017). The present study reports, for the first time, chemical composition of the essential oil (EO) and head space (HS) volatiles obtained from the fresh leaves and flowers of the *P. longifolium* growing in unusual natural environments i.e. on silicious substrate.

Material and methods

Plant material

Plant material was collected from thermophilous grasslands at siliceous, rocky grounds of Mt. Stara Planina. All specimens were collected from the same population, habitat and locality (Topli Do, E Serbia, 43°17'59" N and 22°36'42" E). 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 11780 (vegetative stage; 05.06.2015.) and 11781 (flowering stage; 05.07.2015.).

Isolation and analysis of volatiles

Isolation and analysis of volatiles was done as previously described (J o v a n o v i ć et al., 2015). The light yellow oil, which has pleasant odor, was obtained in a yield of 0.09%, 0.07%, and 0.38% from fresh leaves in the developing and flowering phase and from the fresh flowers (respectively).

Results and discussion

Oil yield of both leaf samples (0.09% LD and 0.07% LF) was many times lower than the oil yield obtained from the flower (0.38%).

A total of 42 compounds, whose representation in at least one sample was greater than or equal 0.1, were identified in six examined samples: essential oils (EO) and head space (HS) volatiles of leaves in vegetative and flowering phase (LD and LF, respectively) as well as inflorescence samples (F) (**Tab. 1**).

Monoterpenes were predominant in all samples except in sample EO LD which was dominated by sesquiterpene β-elemene (44.1%). Twenty compounds were identified in the HS LD,

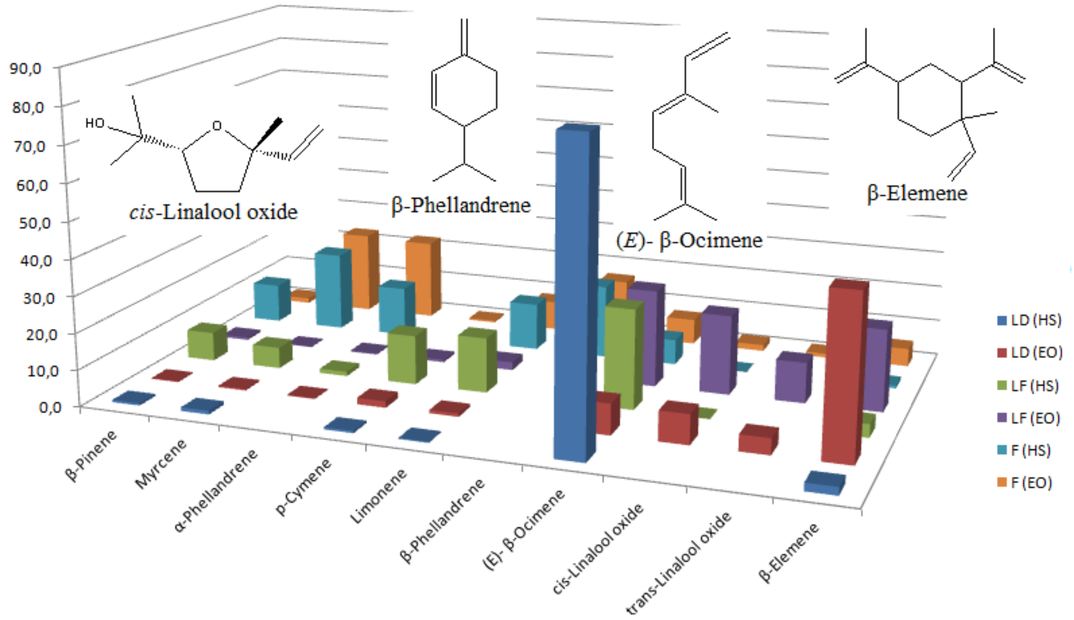

Fig. 1. Variation of the percentage content of the *P. longifolium* essential oil (EO) and head space volatile (HS) components (LD-leaf, developing phase; LF-leaf, flowering phase; F-flower)

accounting 98.9% of the HS volaties, of which almost four fifth was (*E*)-β-ocimene (81.8%). Its presence was reduced to 27.4% in the HS LF sample (**Fig. 1**). Opposite to that, percentage representation of α-pinene (1.3% LD, 4.6% LF), sabinene (0.5% LD, 6.0% LF), β-elemene (2.2% LD, 3.7% LF), β-pinene (0.6% LD, 8.0% LF), myrcene (1.0% LD, 5.9% L), *p*-cymene (0.6% LD, 13.6% L) and limonene (0.3% LD, 15.2% L) was increased in LF sample.

Regarding LD oil, it was characterized by β-elemene (44.1%) whose participation was approximately twice decreases in the LF sample (22.5%). In contrast to β-elemene presence of the following components was greatly increased in the LF sample: (*E*)-β-ocimene (8.5% LD, 26.7% LF), *cis*-linalool oxide (8.2% LD, 21.9% LF), and *trans*-linalool oxide (4.5% LD, 11.2% LF).

Comparing mutual composition of HS and EO, it is evident that the *cis*-linalool oxide and *trans*-linalool oxide were not detected, even as a trace, in samples of HS. Further, presence of β-elemene was many times more in EO samples than in corresponding HS samples. It can be assumed that the oxides are formed from the acyclic monoterpenes during hydrodistillation (Schofield et al., 2002) while lower temperature of preparation of HS samples in relation to the essential oils obtaining (80 °C vs. 100 °C) could be the reason for a lesser presence of β-elemene in HS samples.

Composition of HS F and EO F was different from the corresponding leaves samples, particularly regarding to the β-phellandrene. This *p*-menthane monoterpene was represented with 20.4% and 16.4% in HS F and EO F samples, respectively, while it was not detected in leaf samples at all.

α-Phellandrene was identified in the both flower and leaf samples, but in different quantities (1.2% HS LF, 0.3 % EO LF, 13.9% HS F and 22.5% E OF). Myrcene was the most abundant compound in the HS F and EO F. Comparing mutual composition HS F and EO F it could be noticed a difference in the composition of α-pinene (3.8% and 1.8%, respectively) and β-pinene (10.9% and 1.4%, respectively) as well as β-elemene (0.8% and 5.0%, respectively).

Previous studies have been done with the entire dry above-ground part on the flowering stage (Kapetanos et al., 2008; Tepe et al., 2011; Ilić et al., 2015) or fresh above-ground part in the developing stage (Jovanović et al., 2015). 8-Cedren-13-ol (33.7%), myrcene (15.9%), α-pinene (36.3%) and β-elemene (24.9%) (Kapetanos et al., 2008; Tepe et al., 2011; Ilić et al., 2015; Jovanović et al., 2015), respectively, published as the most represented components of essential oils. Oil yield of both leaf samples was approximate each other (0.09% LD and 0.07% LF) many times lower than the oil yield obtained from the flower (0.38%). Chemical composition of the EO and HS volatiles obtained from the fresh root of *P. longifolium* growing on the same substrate as here examined sample (Stojanović et al., 2017) was totally different relative to composition of leaves and flowers. Namely, α-pinene was the most abundant compound in root EO (60.3%) and root HS volatiles (76.3%) while β-elemene (EO 0.1%; HS bellow

Table 1. Chemical composition (%) of *P. longifolium* essential oils (EO) and head space volatile (HS) constituents

	RI	AI	Compound	Content (%)					
				LD		LF		F	
				HS	EO	HS	EO	HS	EO
1.	802	801	Hexanal	0.4	-	0.5	-	-	-
2.	840	831	(*E*)-2-Hexenal	0.4	-	-	-	-	-
3.	854	850	(*Z*)-3-Hexenol	-	tr	2.3	6.7	-	-
4.	867	863	Hexanol	-	-	0.9	0.2	tr	-
5.	929	924	α-Thujene	tr	0.1	3.7	0.1	2.6	1.3
6.	936	932	α-Pinene	1.3	0.2	4.6	tr	3.8	1.8
7.	951	946	Camphene	0.1	-	0.3	-	0.9	0.3
8.	975	969	Sabinene	0.5	0.3	6.0	0.4	-	4.5
9.	979	974	β-Pinene	0.6	0.3	8.0	0.7	10.9	1.4
10.	992	988	Myrcene	1.0	0.4	5.9	0.1	**22.1**	**23.1**
11.	993	991	*trans*-Dehydroxy-linalool oxide	-	0.1	-	-	-	-
12.	1007	1002	α-Phellandrene	-	0.2	1.2	0.3	13.9	22.5
13.	1008	1006	*cis*-Dehydroxy-linalool oxide	-	0.1	-	-	-	-
14.	1020	1014	α-Terpinene	-	-	tr	-	0.2	tr
15.	1027	1020	*p*-Cymene	0.6	1.8	13.6	0.7	-	0.5
16.	1031	1024	Limonene	0.3	0.9	15.2	2.2	13.2	8.2
17.	1039	1025	β-Phellandrene	-	-	-	-	20.4	16.4
18.	1041	1032	(*Z*)-β-Ocimene	5.5	0.8	3.2	1.2	1.3	0.8
19.	1050	1044	(*E*)-β-Ocimene	**81.8**	8.5	**27.4**	**26.7**	7.0	7.1
20.	1061	1054	γ-Terpinene	0.2	0.5	2.0	2.7	1.4	1.6
21.	1075	1067	*cis*-Linalool oxide (furanoid)	-	8.2	tr	21.9	tr	1.7
22.	1090	1084	*trans*-Linalool oxide(furanoid)	-	4.5	-	11.2	-	1.1
23.	1091	1086	Terpinolene	-	-	tr	-	0.4	-
24.	1101	1095	Linalool	-	0.2	-	-	tr	0.1
25.	1180	1174	Terpinen-4-ol	-	0.1	-	-	tr	0.1
26.	1389	1389	iso-Longifolene	-	2.4	-	-	-	-
27.	1397	1389	β-Elemene	2.2	**44.1**	3.7	22.5	0.8	5.0
28.	1426	1417	(*E*)-Caryophyllene	0.2	1.5	tr	-	0.3	0.1
29.	1461	1452	α-Humulene	0.2	7.9	tr	-	tr	-
30.	1481	1476	α-Chamigrene	-	0.4	-	-	-	-
31.	1489	1478	γ-Muurolene	0.3	-	-	-	-	-
32.	1488	1484	Germacrene D	-	3.6	tr	0.4	0.3	0.6
33.	1494	1489	β-Selinene	0.3	1.1	tr	-	0.3	0.7
34.	1502	1498	α-Selinene	0.3	0.7	0.3	-	tr	-
35.	1503	1500	Bicyclogermacrene	-	0.7	-	-	-	0.1
36.	1514	1508	Germacrene A	2.7	5.3	0.6	0.8	-	0.2
37.	1529	1522	δ-Cadinene	-	0.1	-	-	tr	-
38.	1585	1577	Spathulenol	-	0.3	-	-	-	-
39.	1591	1582	Caryophyllene oxide	-	0.2	-	-	-	-
40.	1618	1608	Humulene epoxide II	-	0.6	-	-	-	-
41.	1648	1640	epi-α-Murrolol (≡ τ-Muurolol)	-	0.1	-	-	-	-
42.	1663	1658	Selin-11-en-4-α-ol	-	1.1	-	-	-	-
			Monoterpenoids	91.9	27.2	91.1	68.2	98.1	92.5
			Sesquiterpenoids	6.2	70.1	4.6	23.7	1.7	6.1
			Other	0.8	**0**	3.7	6.9	0	0
			Total (%)	**98.9**	**97.3**	**99.4**	**98.8**	**99.8**	**99.2**

HS – head space volatiles; EO – essential oil; LD–leaf, vegetative phase; LF – leaf, flowering phase; F – inflorescences; RI – experimental linear retention indices relative to C8-C40 alkanes on the HP-5MS; AI- Adam's retention indices; tr– trace (< 0.05%) and not detected (-); The most represented components are in bold.

0.1%), (*E*)-β-ocimene (EO and HS 1.2%) and myrcene (EO 2.3%; HS 2.1%) were manifold less represented comparing to their representation in EO and HS of here examined leaves and flowers.

Conclusion

The results of this and previous studies show that *P. longifolium*, like most other species, has a different composition of the volatile components in various organs in the same vegetative phase and in the same organ in different stages of development. In accordance with that, depending on application, should be chosen in which stage of development and which plant organ should be collected. In the case of *P. longifolium,* if the intention is to use *P. longifolium* in perfumery, it is best to use its flower because of their pleasing aroma originating from high content of phellandrenes and ocimenes. If there is intention for utilization of *P. longifolium* in the pharmaceutical industry, it is the best to use leaf, especially in the development phase, because of the high content of β-elemene for which benefits for cancer treatment have been previously reported (Z h u et al., 2011).

Acknowledgements. The research was supported by the Serbian Ministry of Education, Science and Technology Development (Grant No. 172047).

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