SHORT REPORT

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Influence of Polish Climate Conditions on Content and the Chemical Variation of Volatiles in the Roots of Six *Eleutherococcus* Species and Their Potential Use

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Abstract: The aim of this study was the term of the climate influence on essential oil and aroma components of six *Eleutherococcus* species [*E. senticosus* (Rupr. & Maxim.) Maxim., *E. setchuensis* (Harms) Nakai, *E. sessiliflorus* (Rupr. & Maxim.) S. Y. Hu, *E. gracilistylus* (W. W. Smith) S. Y. Hu, *E. henryi* Oliv., *E. divaricatus* (Siebold & Zucc.) S. Y. Hu] cultivated in Poland. The hydrodistilled volatiles of the samples were ranged from 0.2% to 0.4%. The components of the determined volatiles were analyzed by GC/MS/MS. Thirty of the same compounds were present in all samples. Major components of the samples were (*E,E*)-farnesol (43.6-6.9%), (*E,Z*)-farnesol (7.2-0.7%), (*Z,E*)-farnesol (1.4-0.1%), tetradecanoic acid (9.91-2.08%), and pentadecanoic acid (12.8-3.5%). Highest (*E,E*)-farnesol content (43.6%) was determined in the roots of *E. divaricatus.* This compound may be considered as chemical marker of the species. This is the first time, when the analysis of volatiles in the roots of *Eleutherococcus* spp. cultivated in Poland was performed. This study provides a platform for further investigation for the isolation and pharmacological activity of active principles.

Keywords: *Eleutherococcus*; GC/MS/MS; Essential oil; Farnesol; Phytotherapy; Climate.

1. Plant Source

The *Eleutherococcus* Maxim. [*Acanthopanax* (Decne. et Planch) Witte] genus comprises about 40 species growing in Eastern Asia (Chine, Korea, Japan) and Russia. The most known species of this genus is *E. senticosus*. The main chemical substances are eleutherosides (glycosides of coumarins, lignans, triterpenic acids and sterols). Eleutherosides have been shown to have various levels of activity such as anticancer, adaptogenic, antibacterial, antiinflammatory, antioxidant, antidepressant, immunostymulatory and hypocholesterolemic effect. It is interesting, *E. senticosus* belongs to the adaptogenic plants, similarly to *Panax ginseng* and *Schisandra chinensis* [1-4].

The roots of *E. senticosus* (Rupr. & Maxim.) Maxim., *E. setchuensis* (Harms) Nakai, *E. sessiliflorus* (Rupr. & Maxim.) S. Y. Hu, *E. gracilistylus* (W. W. Smith) S. Y. Hu, *E. henryi* Oliv. and *E. divaricatus* (Siebold & Zucc.) S. Y. Hu were obtained from arboretum in Rogów (Poland). Catalog

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number were marked as EE1, EE2, EE3, EE4, EE5, EE6, respectively. Voucher specimens were deposited at the Department of Pharmaceutical Botany, Medical University of Lublin, Poland (EE1, Ro-E10a; EE2, Ro-10b; EE3, Ro-13c; EE4, Ro-10d; EE5, Ro-10e; EE6, Ro-10f). The roots were collected in October 2010. The ages of all plants were 20 years old.

2. Previous Studies

There are no previous studies on the volatiles composition of the *Eleutherococcus* roots cultivated in Poland. Only limited reports were on the roots of *E. senticosus* and *E. sessiliflorus* growing in Asia [3, 5, 6].

3. Present Study

The extraction of volatiles was carried out according to the Polish Pharmacopoeia VIII [4]. 10 g of each roots' sample were air–dried and powdered (0.5 mm). The volatiles were obtained by distillation with Clevenger–type apparatus. 200 mL of water was added into a flask of 1000 mL, containing appropriate root's sample. 0.3 mL of xylene was placed over the water before running destillation. The duration of distillation process was 3 hrs with flow speed of 4 mL water/min. From the solution of xylene-volatiles the 0.27 mL of xylene was substracted and the quantity of volatile in sample calculated in percentage (v/d.w.). The oil was stored at 4 ^{o}C in a brown vial.

A highly sensitive and accurate multiplex gas chromatography-linear ion trap technique was used to identify components of the volatiles. GC/MS/MS was performed using Varian 4000 GC/MS/MS chromatograph equipped with Flame Ionization Detector (FID). The GC conditions were as follows: VF-5 ms fused silica capillary column (30 m x 0.25 mm, film thickness 0.25 μm), the oven temperature was programmed at a rate of 1 ^{o}C from 50 (held for 5 min.) to 250 ^{o}C, injector and detector kept at 250 ^{o}C, split ratio 1 : 100. Helium was used as the carrier gas with a constant flow rate of 0.5 mL/min. The volume of injection was 1 μL of a pentane-volatiles solution (1:1). Mass spectra were recorded at 70 eV. Mass range was from *m/z* 40 to 1000. The identification of individual compounds resulted on the basis of Kovats linear retention index (LRI) determination relative to (C_6–C_{40}) *n*–alkanes and retention time (RT). Compounds were further identified using their MS data compared to homemade library mass spectra built up from pure substances (Main Lab Library).

4. Results and Discussion

4.1. Essential oil and aroma composition

Volatiles extracted from the roots of *Eleutherococcus* spp. cultivated in Poland were analyzed by GC/MS/MS. Volatiles were pale-yellow to light-blue colour with aromatic-spicy odour. The yield was 0.2% (*E. senticosus*, *E. gracilistylus*), 0.3% (*E. sessiliflorus*, *E. henryi*), 0.4% (*E. divaricatus, E. setchuensis*), (v/d.w.). Such a yield, according to the pharmacopoeial requirements (>0.1%) classifies these species as an essential oil material.

In the literature there is not much information about the amount of EO and aroma in these plants. The EO yield in the present study is different than the amount obtained by Yu et al. [5]. According to Yu et al. [5], the yield of EOs from the roots of *E. senticosus* growing in China was 0.05%, in comparison to *E. senticosus* growing in Poland (0.2%). Such a variation in the EO yield of *E. senticosus* may be due to the varied climatic conditions of the regions. The analyzed species are cultivated at the botanical garden in Rogów, which lies in the Central Polish Lowlands region with geographic data such as 51° 49′N and 19° 53′E. The average, long-term temperature is -20.1°C, what classified the garden to the 6bth sub-climate (according to USDA Frost Hardiness Zones) and to the second zone according to the Kórnik's category. These plants are grown on the acidic, luvic, and sandy soils. It is important the *Eleutherococcus* species cultivated in Poland are frost resistance to -25°C [6].

The volatiles of the six root samples have showed distinct similarities and differences with respect to their composition. In total, 125 compounds were identified, accounting on average for

99.0% - 99.6% of the composition. In *E. setchuensis* 87 compounds were identified, in *E. gracilistylus* 72, in *E. senticosus*, *E. sessiliflorus* and *E. henryi* 69, in *E. divaricatus* 65.

Table 1. Volatile Constituents present in all samples of *Eleutherococcus*.

No	Compound	[a] LRI	Relative amount (%) [b]					
			1	2	3	4	5	6
1	heptanal	919	0.3	0.2	t	t	0.1	0.4
2	cumene	932	1.9	1.1	1.6	1.1	1.7	1.9
3	**α- pinene**	939	0.3	12.9	0.5	**18.8**	1.5	1.1
4	1,2,3 trimethyl benzene,	974	2.2	1.4	1.5	1.2	1.9	2.0
5	*β*- pinene	981	0.2	0.4	0.2	2.9	0.3	t
6	2-pentyl furan,	993	0.8	0.8	0.5	0.8	0.5	0.2
7	octanal	1009	0.8	1.5	0.8	0.9	0.5	1.7
8	nonanal	1107	0.4	0.2	0.2	0.1	t	0.4
9	*α*-campholenal	1129	0.1	4.3	0.2	7.1	1.9	1.0
10	*trans*-pinocarveol	1147	t	3.1	t	6.1	0.6	0.7
11	myrtenal	1215	0.1	2.0	t	4.2	0.4	0.8
12	decenal	1238	0.1	0.0	t	t	0.2	0.1
13	bornyl acetate	1291	t	0.1	t	3.8	1.2	0.4
14	trans-myrtanol acetate	1390	0.3	0.3	0.3	1.7	0.7	0.4
15	geranyl acetone	1459	0.4	0.2	0.3	0.1	0.4	0.4
16	allo-aromadendrene	1469	6.3	1.8	2.7	1.2	2.8	7.8
17	(*E*)-nerolidol	1568	0.5	0.3	0.5	0.1	0.7	0.5
18	spathulenol	1586	2.5	2.4	0.9	0.8	2.1	2.8
19	caryophyllene oxide	1591	0.7	0.5	0.3	0.3	1.3	0.9
20	globulol	1595	0.3	0.3	t	t	0.3	0.3
21	viridiflorol	1603	0.3	0.6	0.1	0.1	0.4	0.4
22	*α*-cadinol	1653	1.7	0.7	0.1	0.1	1.4	0.9
23	*α*-bisabolol	1700	1.5	2.0	7.3	2.1	4.4	2.5
24	(*Z,E*)-farnesol	1713	1.4	0.3	0.7	0.1	0.8	0.4
25	**(*E,E*)-farnesol**	1724	**33.7**	**13.1**	**43.6**	6.9	7.0	**26.3**
26	(*E,Z*)-farnesol	1747	7.2	1.9	2.1	0.7	1.7	3.7
27	*cis,cis*- 7,10-hexadecadienal	1792	0.7	0.6	1.2	0.3	0.8	0.5
28	tetradecanoic acid	1835	2.0	3.4	9.9	2.4	5.3	2.6
29	**pentadecanoic acid**	1865	6.2	6.6	4.5	3.5	**12.3**	12.8
30	ethyl hexadecanoate	1891	1.7	4.0	4.9	1.8	6.5	2.5

1. *E. senticosus*, 2. *E. setchuensis*, 3. *E. divaricatus*, 4. *E. gracilistylus*, 5. *E. henryi*, 6. *E. sessiliflorus*. [a] LRI: linear retention indices (HP-5 column). [b] Average values (peak area relative to total peak area) from three replicate sample analyses. t - trace, for less than 0.05%.

Thirty compounds were present in all samples (Table 1). Major components of the samples were (*E,E*)-farnesol (43.6-6.9%), (*E,Z*)-farnesol (7.2-0.7%), (*Z,E*)-farnesol (1.4-0.1%), tetradecanoic acid (9.9-2.0%), and pentadecanoic acid (12.8-3.5%). (*E,E*)-Farnesol was detected as the major compound in four samples, ranging from 6.9% to 43.6%. Most (*E,E*)-farnesol was determined in the roots of *E. divaricatus* (43.6%), in *E. senticosus* (33.7%), in *E. sessiliflorus* (26.3%), in *E. setchuensis* (13.1%). *α*-

pinene (18.8%) was reported as the main component in *E. gracilistylus*, whereas pentadecanoic acid (12.3%) dominated in *E. henryi*. The details of these results are described in the supporting information (S1).

Our results presented distinct chemical profile from those obtained from *E. senticosus* growing in China. Based on the results of the analysis of Yu et al., *E. senticosus* growing in China contains more caryophyllene oxide (16.4%), (2*E*,4*Z*)-decadienal (7.9%), *α*- pinene (7.1%), *β*- pinene (1.1%) and *p*-cymene (3.5%), [5]. According to the studies of Richter et al., *E. senticosus* contains a lower amount of farnesol (0.5%), tetradecanoic acid (1.2%), pentadecanoic acid (2.0%) and *δ*3-carene (1.0%) than *E. senticosus* cultivated in Poland [3]. These differences may be related to Polish climatic conditions. Different geographical zone might have affected, to some extent, the chemical components of these Asian species.

Its very important that, we did not assay *t*-thujone in *E. senticosus*, *E. setchuensis*, *E. divaricatus*, *E. henryi* and *E. sessiliflorus* at all, which is neurotoxic and neurodegenerative. *t*-Thujone has been detected in *E. senticosus* (0.09%) and *E. sessiliflorus* (2.5% - from the one year roots and 0.2% - from the three years roots) growing in China [3, 7].

It is worth noting that in four species cultivated in Poland, the main compound is (*E*,*E*)-farnesol most of which can be found in *E. divaricatus* (43.6%). Farnesol is an effective antibacterial compound against wide strains of opportunistic human bacteria and fungi. Many *in vitro* studies report a high efficacy of EOs against pathogens, especially bacteria (*E. coli*, *Staph. aureus*, *Staph. typhimurium*, *B. cereus*) and fungi (*A. fumigatus*, *A. nidulans*, *C. albicans* ans *Sacch. cerevisiae*). Mechanism of action farnesol involves induction of generation of reactive oxygen species (ROS) in microorganisms. Beside, according to recent studies farnesol activates apoptosis in bacteria [8, 9]. There are known antileukemic, antihepatoma and antimelanoma properties of farnesol. It plays an important role in the induction of apoptosis in leukemic cells and is a more effective inhibitor of cancer cells than nerolidol, perillyl alcohol and geraniol. The induction of apoptosis is suggested through mitochondrial pathway [10, 11]. According to Voziyan et al. farnesol inhibits the growth of the human leukemic CEM- C1 cell line by decreasing cholinephosphotransferase activity (CTP). The inhibitory concentration ranges between 25-250 μM [12]. An interesting thing to notice is that *E. divaricatus* has more farnesol (43.6%) than commonly used in medicine oil linden inflorescence *Tilia cordata* (0.2-0.3%), *Pittosporum undulatum* (10.9%) or *Anthemis melampodina* (16.5%), [9, 13, 14].

The findings found in this work may present an important factor in the choice of the *Eleutherococcus* volatiles, especially those rich in the farnesol. For this reason, especially *E. divaricatus* may become an alternative source of biologically active farnesol, as well as may be considered as chemical marker of the species.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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