

EXPERIMENTAL PAPER

Diversity of *Eleutherococcus* genus in respect of biologically active compounds accumulation

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Summary

Eight species of *Eleutherococcus* genus grown at Rogów Arboretum collection were compared in respect of biologically active compounds accumulation, i.e. eleutheroside B and E, phenolic acids and sterols. For the determination of content of these compounds in underground organs and stem bark high performance liquid chromatography was applied. The highest content of the sum of eleutherosides B and E was observed in underground organs and stem bark of *E. leucorrhizus* (322.0 and 300.8 mg × 100 g⁻¹, respectively) and *E. nodiflorus* (218.9 and 363.5 mg × 100g⁻¹, respectively). In the raw materials from *E. senticosus*, the content of these compounds was significantly lower (177.4 and 159.3 mg × 100 g⁻¹, respectively). *E. divaricatus* and *E. setchuenensis* were characterized by the lowest accumulation of these compounds in underground organs whereas *E. divaricatus*, *E. sessiliflorus* and *E. giraldii* – in stem bark. Four phenolic acids were identified in the investigated species, namely: chlorogenic, rosmarinic, ferulic and caffeic acids. The main phenolic acid in the analyzed species was chlorogenic acid. The content of this compound in underground organs varied from 102.1 (*E. henryi*) to 958.7 mg × 100 g⁻¹ (*E. leucorrhizus*) and in stem bark from 26.7 (*E. giraldii*) to 542.5 mg × 100 g⁻¹ (*E. setchuenensis*). The content of identified sterol compounds (sitosterol 3-O-β-D-glucoside /eleutheroside A / campesterol, stigmasterol) was relatively low, but higher in underground organs in comparison with stem bark.

Key words: *Eleutherococcus* genus, eleutherosides B and E, phenolic acids, sterols, HPLC

INTRODUCTION

In the genus *Eleutherococcus* Maxim., syn. *Acanthopanax* (Decne. & Planch.) Miq. (*Araliaceae*) about 40 species were distinguished. The plants grow freely in northeast Asia, from Himalayas to Vietnam and from Russia to North Philippines [1, 2]. They are thorny shrubs or small trees with flowers settled in 2–6 umbels at the top of erect stems and with divided leaves consisting of 3–5 leaflets [3]. In Poland they are kept almost exclusively at Warsaw University of Life Sciences – SGGW (Rogów Arboretum and scientific collections of Department of Vegetable and Medicinal Plants).

The best known, from among the genus, is eleuthero (*Eleutherococcus senticosus* / Rupr. et Maxim. / Maxim., syn. *Acanthopanax senticosus* / Rupr. et Maxim. / Harms), the misnamed Siberian ginseng. It is harvested from natural sites in Russia and northeast China. Underground organs of this plant (rhizomes with roots) have been used by the Chinese for over 2000 years as a medicinal raw material in general weakness, lassitude, anorexia, lumbar and knee pain, insomnia and dream-disturbed sleep [4]. Currently, it has become a popular medicine also in Eurasia and North America. According to European Medicines Agency, it is a herbal product traditionally used for symptoms of fatigue and weakness [5]. It has been also much prized for its anti-stress action. The extract's actions have been described primarily as immunostimulant, immunomodulatory, stress reducing, stimulating, enhancing work performance, radioprotective, anabolic and adaptogenic. The stimulant and tonic effects of eleuthero preparations are reputedly stronger and longer acting than ginseng (*Panax ginseng*) [4]. Eleutherosides are considered to be the compounds responsible for adaptogenic activity of this plant [6-8]. They cannot be assigned to a particular compound class such as ginsenosides but belong to different chemical groups such as lignans (eleutheroside E, syn. liriiodendrin), phenylpropanoids (eleutheroside B, syn. syringin), sterols (eleutheroside A, syn. sitosterol 3-O- β -D-glucoside) and coumarins. With regard to the activity of isolated constituents from eleuthero underground organs, the most active seem to be eleutheroside B and E [4,7,8].

On its natural sites, eleuthero is endangered by over-harvesting, and this is exacerbated by its slow growth [9]. Other *Eleutherococcus* species are also mentioned as a source of raw materials (roots, root and stem bark) for medicinal purposes [9-12]. Most of them are used due to lack of products from *E. senticosus* [4,9]. The aim of undertaken study was to investigate chemical diversity of 8 *Eleutherococcus* species growing at Arboretum of Warsaw University of Life Sciences – SGGW in Rogów in respect of the contents of eleutherosides B and E, phenolic acids and sterols in underground organs and stem bark.

MATERIAL AND METHODS

Plant material

The objects of the study were eight *Eleutherococcus* species included in the national collection of the *Araliaceae* in Rogów Arboretum. The thorough

identification and documentation of the species was performed by Tumiłowicz and Banaszczak [3]. The data concerning the origin of the accessions and the age of plant shows table 1. Underground organs and stem bark from plants growing at Arboretum in Rogów were collected at the beginning of April 2012. The stems were barked with a sharp knife. Stem bark was collected from 3-4-year-old stems (15 randomly chosen stems for each plant). Rhizomes with roots were harvested from the outside part of underground organs, in a distance of about 1 m from the core of plant. From each plant, 3 samples of fresh raw materials were taken and dried at 40°C.

Table 1.

Origin of investigated *Eleutherococcus* species

Species	Number of accession at Rogów Arboretum	Origin	The year of planting
1. <i>E. senticosus</i> (Rupr. & Maxim.) Maxim.	11857	Russia (Vladyvostock B.G.)	1978
2. <i>E. henryi</i> Oliv.	11856	China (Shanghai B.G.)	1989
3. <i>E. leucorrhizus</i> Oliv.	10770	China (Huanshan Mountains)	1986
4. <i>E. nodiflorus</i> (Dunn) S.Y. Hu	11451	China (Qui Ling Mountains)	1986
5. <i>E. divaricatus</i> (Siebold & Zucc.) S.Y. Hu	8553	Holland (Wageningen Univ. B.G.)	1978
6. <i>E. sessiliflorus</i> (Rupr. & Maxim.) S.Y. Hu	8557	Russia (Vladyvostock B.G.)	1980
7. <i>E. giraldii</i> (Harms) Nakai	12655	China (Shanghai B.G.)	1993
8. <i>E. setchuenensis</i> (Harms) Nakai	11472	China (Shanghai B.G.)	1989

Phytochemical analysis

The studies on chemical composition of the raw materials were undertaken in the laboratories of Department of Vegetable and Medicinal Plants of Warsaw University of Life Sciences – SGGW.

Commercially available standards (ChromaDex®) were separately dissolved in 10 ml volumetric flask with MeOH according to the ChromaDex's Tech Tip 0003: Reference Standard Recovery and Dilution and used as standard stock solutions. Further calibration levels were prepared by diluting these solutions with methanol in 10 ml volumetric flasks (injected volume ranges: 10, 50, 100, 200, 500 and 1000 µl). The working solutions were injected (10 µl) on

a column in six replicates ($n=6$) using SIL-20A to generate a six-point calibration curve for the each standard compound separately, using CLASS VP™ 7.3 chromatography software. The peak table and spectra library (190–450 nm) of individual compounds were created. Standard curve parameters were calculated with statistical service e-stat (<http://www.chem.uw.edu.pl/stat/e-stat/>). Signal-to-noise ratio approach were used to determined LOD (S/N of 3:1) and LOQ (S/N of 10:1).

Raw materials were dried at 40°C. One gram of grounded raw material was extracted with 100 ml of methanol in Büchi Labortechnik AG Extraction System B-811. Soxhlet hot extraction with twenty-five extraction cycles, flushing and drying was used. After solvent evaporation, the residue was dissolved in 10 ml of methanol, filtered with Supelco Iso-Disc™ Syringe Tip Filter Unit, PTFE membrane (diameter 25 mm, pore size 0.45 μm) and subjected to HPLC for determination of eleutherosides B, E, as well as phenolic acids. The analysis was carried out using the Shimadzu chromatograph equipped with auto sampler SIL-20A, photodiode array detector SPD-M10A VP PDA and CLASS VP™ 7.3 chromatography software. Phenomenex Luna C18 (2) 5 μm 250 \times 4.6 mm column was used. Gradient elution of 10% ACN (mobile phase A) and 55% ACN (mobile phase B) (LabScan) in water adjusted to pH 3.0, flow rate 1 ml \times min⁻¹ and temperature 30°C were applied, injection volume: 10 μl . UV-spectra were recorded between 190 and 450 nm. Peak identification was confirmed by comparison of retention time and spectral data with adequate parameters of standards. Quantification was based on the peak area at 206 nm (eleutheroside E), 264 nm (eleutheroside B) and 330 nm (chlorogenic, rosmarinic, caffeic and ferulic acids).

For sterol determination dry raw material (1 g) was extracted with 100 ml of hexane in the Büchi Labortechnik AG Extraction System B-811. Soxhlet hot extraction with twenty-five extraction cycles, flushing and drying was used. After evaporation of solvent, the residue was dissolved in 10 ml of chloroform-methanol (4:1) mixture. The obtained extract was filtered through Supelco Iso-Disc™ Syringe Tip Filter Unit, PTFE membrane, diameter 25 mm, pore size 0.45 μm . The analysis was carried out using Shimadzu chromatograph equipped with auto sampler SIL-20A, photodiode array detector SPD-M10A VP PDA and CLASS VP™ 7.3 chromatography software. Phenomenex Luna C8 (2) 5 μm 250 mm \times 4.6 mm column was used as a solid phase. The following conditions were applied: mobile phase A – methanol (LabScan), mobile phase B – ACN (LabScan), flow rate 1 ml \times min⁻¹, temperature 30°C, injection volume: 20 μl . UV-spectra were recorded between 190 and 450 nm. Peak identification was confirmed by comparison of retention time and spectral data with adequate parameters of standards. Quantification was based on the peak area at 210 nm for all substances (sitosterol 3-O- β -D-glucoside /eleutheroside A/, campesterol and stigmasterol).

Statistical analysis

The results of chemical analysis were subjected to the one-way analysis of variance and Tukey's test at $\alpha=0.05$. The presented results are mean values obtained from 3 replications.

RESULTS AND DISCUSSION

The data on the content of biologically active compounds in the raw materials obtained from *Eleutherococcus* species are scarce [10, 13-19]. It is probably due to fact that the species are wild growing plants occurring in Far East and most of the literature available has been published in Chinese and Korean with only brief English summaries. On the other hand there is much information on the activity of the raw material collected from eleuthero (*E. senticosus*) [4-8, 11-13].

The studies on the content of eleutherosides B and E in stems and roots as well in fruits of *E. koreanum*, *E. senticosus*, *E. senticosus* forma *inermis*, *E. divaricatus* var. *albeofructus* and *E. chiisanensis* were performed earlier by Kang et al. [9]. According to the results, the content of eleutheroside E was higher in the stems than in the roots of investigated species, except from *E. chiisanensis*. Eleutheroside B was identified only in raw material from *E. koreanum* and *E. senticosus* (0.478 and $0.445 \text{ mg} \times \text{g}^{-1}$, respectively). The content of eleutheroside E in roots of both species was similar (0.538 and $0.561 \text{ mg} \times \text{g}^{-1}$, respectively).

The results obtained in presented study indicate that investigated *Eleutherococcus* species differed significantly in respect of the accumulation of biologically active compounds. According to Polish Pharmacopoeia, the content of the sum of eleutheroside B and E (principle active compounds of *Eleutherococcus* species) in dried underground organs of *E. senticosus* should not be lower than $80 \text{ mg} \times 100\text{g}^{-1}$ [6]. Both compounds are considered to be responsible for immunostimulant actions of the extracts from eleuthero. Eleutheroside E shows also protective effect against stress-induced gastric ulcer in rats. In our study (tab. 2), the content of eleutheroside B and E in underground organs and stem bark of *E. leucorrhizus* (322.0 and $300.8 \text{ mg} \times 100\text{g}^{-1}$, respectively) and *E. nodiflorus* (218.9 and $363.5 \text{ mg} \times 100\text{g}^{-1}$, respectively) was very high, as compared with Pharmacopoeia requirements. The content of these compounds was significantly lower in underground organs and stem bark of *E. senticosus* (177.4 and $159.3 \text{ mg} \times 100\text{g}^{-1}$, respectively). In *E. senticosus* stem bark their presence has been previously reported by Nishibe et al. [13], however, without quantitative data. The studies on the accumulation of biologically active compounds in underground organs and in the stem bark from *E. senticosus* were undertaken a few years ago in Department of Vegetable and Medicinal Plants, Warsaw University of Life Sciences – SGGW. According to these results the content of

eleutherosides B and E in underground organs and in stem bark of eleuthero- increased from the second (104.7 and 198.2 mg \times 100 g⁻¹, respectively) to fourth year of plant vegetation (209.2 and 397.1 mg \times 100 g⁻¹, respectively) [14].

Table 2.

Content of eleutherosides B and E in underground organs and stem bark of eight *Eleutherococcus* species (mg \times 100 g⁻¹ DM)

Species	Raw materials	
	Underground organs	Stem bark
<i>E. senticosus</i>	177.4 \pm 7.1 c	159.3 \pm 5.4 c
<i>E. henryi</i>	163.2 \pm 6.7 cd	144.8 \pm 5.2 c
<i>E. leucorrhizus</i>	322.0 \pm 12.8 a	300.8 \pm 11.6 b
<i>E. nodiflorus</i>	218.9 \pm 10.8 b	363.5 \pm 19.8 a
<i>E. divaricatus</i>	79.4 \pm 3.51 e	58.5 \pm 4.2 e
<i>E. sessiliflorus</i>	126.3 \pm 6.8 d	56.4 \pm 5.1 e
<i>E. giraldii</i>	91.1 \pm 4.6 de	69.9 \pm 5.8 de
<i>E. setchuenensis</i>	72.9 \pm 3.2 e	108.6 \pm 6.1 d
mean	155.2	157.7

a–e: values in rows marked with the same letter do not differ significantly at $\alpha = 0.05$
Data are given as mean \pm SD (n=3)

In our study, *E. divaricatus*, *E. sessiliflorus* and *E. giraldii* were characterized by the lowest content of eleutheroside B and E in stem bark. The results obtained by Lee et al. [12] on the chemical composition of stems and roots of *E. senticosus* and *E. sessiliflorus* indicate the lack of eleutheroside B in stems of *E. sessiliflorus* and higher content of eleutheroside E in stems and roots of *E. sessiliflorus* in comparison with *E. senticosus*.

The data on the phenolic acids in raw material obtained from *Eleutherococcus* species concern mainly the identification of selected acids in underground organs of *E. senticosus*. In the study of Kurkin et al. [18], seven phenolic acids, namely chlorogenic, ferulic, caffeic, p-coumaric, syringic and p-hydroxybenzoic acids were identified in roots of *E. senticosus*. In the analyzed raw material, four phenolic compounds were identified, i.e. chlorogenic, rosmarinic, ferulic and caffeic acids. Chlorogenic acid was a dominant compound. However, its content in underground organs and stem bark of investigated species differed significantly. In underground organs, the content of this compound varied from 102.1 (*E. henryi*) to 958.7 mg \times 100 g⁻¹ (*E. leucorrhizus*) and in stem bark from 26.7 (*E. giraldii*) to 542.5 mg \times 100 g⁻¹ (*E. setchuenensis*) (tab. 3). Chlorogenic and rosmarinic acids were also reported to be constituents of underground organs, stem bark, leaves, and fruits of *E. senticosus* [12–14]. The other identified phenolic acids, in both raw materials, were found in much lower quantities (tab. 3). The investigated species differed also significantly in the content of identified

Table 3.

Content of phenolic acids in underground organs and stem bark of eight *Eleutherococcus* species (mg × 100 g⁻¹ DM)

Species	Chlorogenic acid		Rosmarinic acid		Ferulic acid		Caffeic acid	
	Underground organs	Stem bark	Underground organs	Stem bark	Underground organs	Stem bark	Underground organs	Stem bark
<i>E. senticosus</i>	495.2±18.6 c	403.3±12.2 c	76.8±3.8 c	46.0±3.1 a	42.5±3.1 a	68.9±4.4 a	11.5±0.3 c	10.2±0.3 a
<i>E. henryi</i>	102.1±6.2 e	244.2±10.5 d	46.1±5.4 c	19.4±2.1 c	18.3±1.9 b	22.5±2.4 b	9.5±0.6 c	1.1±0.3 b
<i>E. leucorrhizus</i>	958.7±26.6 a	492.3±17.2 b	105.0±5.0 ab	16.4±1.1 c	40.1±2.5 a	8.6±0.8 d	29.8±1.7 a	3.0±0.4 b
<i>E. nodiflorus</i>	599.8±20.4 b	165.5±6.6 e	178.3±7.4 a	23.9±1.6 bc	23.0±1.2 b	20.0±1.4 b	27.2±1.9 a	1.5±0.3 b
<i>E. dhvaricatus</i>	275.0±10.5 d	154.5±6.8 e	75.5±4.9 c	19.2±1.4 c	15.8±0.9 b	29.5±1.9 b	18.6±1.5 b	1.5±0.4 b
<i>E. sessiliflorus</i>	287.3±11.4 d	107.9±6.3 e	42.9±3.5 b	17.8±1.2 c	22.7±1.7 b	13.8±0.8 c	5.9±0.5 d	2.0±0.2 b
<i>E. giraldii</i>	487.1±16.9 c	26.7±1.9 f	52.3±2.8 bc	17.3±0.9 c	18.0±1.0 b	5.6±0.3 d	7.1±0.7 d	6.6±0.6 a
<i>E. setchuensis</i>	287.5±9.9 d	542.5±19.3 a	81.2±4.6 b	34.1±2.7 b	5.8±0.4 c	55.7±3.8 a	22.8±1.0 b	7.1±0.6 a
mean	436.6	267.1	82.3	24.3	23.3	28.1	16.6	4.1

a-f: values in columns marked with the same letter do not differ significantly at $\alpha=0.05$

Data are given as mean ±SD (n=3)

Table 4.

Content of sterols in underground organs and stem bark of eight *Eleutherococcus* species (mg × 100 g⁻¹ DM)

Species	Sitosterol 3-O- β -D-glucoside (eleutheroside a)		Campesterol		Stigmasterol	
	Underground organs	Stem bark	Underground organs	Stem bark	Underground organs	Stem bark
<i>E. senticosus</i>	1.3±0.2 c	1.7±0.3 bc	4.2±0.2 ab	1.4±0.1 c	6.3±0.4 a	0.0 c
<i>E. henryi</i>	3.6±0.4 bc	2.1±0.2 b	5.5±0.3 a	2.7±0.2 b	3.2±0.3 b	0.0 c
<i>E. leucorrhizus</i>	9.6±0.7 ab	7.8±0.5 a	1.9±0.2 b	5.6±0.3 ab	1.6±0.2 c	0.0 c
<i>E. nodiflorus</i>	4.5±0.5 bc	3.1±0.4 ab	2.4±0.3 b	0.8±0.1 cd	1.8±0.2 c	1.2±0.2 b
<i>E. divaricatus</i>	13.3±0.8 a	0.4±0.1 c	1.5±0.2 b	0.4±0.1 d	1.1±0.2 c	2.9±0.4 a
<i>E. sessiliflorus</i>	6.0±0.4 b	1.4±0.2 bc	2.6±0.2 b	7.0±0.3 a	5.5±0.4 a	0.0 c
<i>E. giraldii</i>	2.2±0.4 c	4.0±0.3 ab	1.2±0.2 b	0.3±0.0 d	1.3±0.1 c	0.0 c
<i>E. setchuensis</i>	4.8±0.5 b	2.7±0.1 b	1.4±0.1 b	0.2±0.0 d	1.2±0.1 c	0.0 c
mean	5.7	2.9	2.6	2.3	2.8	-

a-d: values in columns marked with the same letter do not differ significantly at $\alpha=0.05$

Data are given as mean ±SD (n=3)

sterol compounds, i.e. sitosterol 3-O- β -D-glucoside (eleutheroside A), campesterol and stigmasterol (tab. 4) There are no reports on accumulation of sterols in cultivated plants, and almost any on the content of these compounds in wild growing plants [7]. In present study, the sterol compounds were detected both in underground organs and stem bark of analyzed *Eleutherococcus* species, although, its amount was relatively low (tab. 4). The highest content of sitosterol 3-O- β -D-glucoside (eleutheroside A) was found in the underground organs of *E. divaricatus* ($13.3 \text{ mg} \times 100 \text{ g}^{-1}$).

The obtained results indicate that besides underground organs of *E. senticosus*, promising raw materials, with potential adaptogenic activity, seem to be the stem bark of this species, as well as roots and stem bark of *E. leucorrhizus* and *E. nodiflorus*. The stem bark of investigated *Eleutherococcus* species seems to be a new medicinal raw material of special interest. Apart from high accumulation of biologically active compounds, it can be collected every year without much shrub injury [16].

CONCLUSIONS

- Investigated *Eleutherococcus* species grown at Rogów Arboretum differed significantly in the content of biologically active compounds in underground organs and stem bark.
- Underground organs and stem bark of *E. leucorrhizus* and *E. nodiflorus* occurred to be the richest source of eleutherosides B and E.
- In all analyzed *Eleutherococcus* species, the dominating phenolic acid, both in underground organs and stem bark, was chlorogenic acid.
- The highest content of sitosterol 3-O- β -D-glucoside (eleutheroside A) was found in *E. leucorrhizus*.

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ZRÓŻNICOWANIE RODZAJU *ELEUTHEROCOCCUS* POD WZGLĘDEM GROMADZENIA ZWIĄZKÓW BIOLOGICZNIE AKTYWNYCH

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Streszczenie

Osiem gatunków z rodzaju *Eleutherococcus* rosnących w kolekcji Arboretum w Rogowie porównano pod kątem gromadzenia związków biologicznie aktywnych tj. eleuterozydów B i E, kwasów fenolowych i steroli. W celu określenia zawartości tych związków w organach podziemnych i korze pędów zastosowana została wysokosprawna chromatografia cieczowa. Najwyższą zawartością sumy eleuterozydów B i E charakteryzowały się organy podziemne i kora pędów *E. leucorrhizus* (odpowiednio: 322,0 i 300,8 mg × 100 g⁻¹) i *E. nodiflorus* (odpowiednio: 218,9 i 363,5 mg × 100 g⁻¹). W *E. senticosus*, było ich istotnie mniej (odpowiednio: 177,4 i 159,3 mg × 100 g⁻¹). *E. divaricatus* i *E. setchuenensis* charakteryzowały się najniższą zawartością tych związków w organach podziemnych, natomiast *E. divaricatus*, *E. sessiliflorus* and *E. giraldii* – w korze pędów. W badanych gatunkach zidentyfikowano cztery kwasy fenolowe: chlorogenowy, rozmarynowy, ferulowy i kawowy. Kwas chlorogenowy był związkiem dominującym wśród zidentyfikowanych kwasów fenolowych. Jego zawartość w organach podziemnych wahała się od 102,1 (*E. henryi*) do 958,7 mg × 100 g⁻¹ (*E. leucorrhizus*), a w korze pędów od 26,7 (*E. giraldii*) do 542,5 mg × 100 g⁻¹ (*E. setchuenensis*). Zawartość zidentyfikowanych związków sterolowych (3-O-β-D-glukozydu sitosterolu /eleuterozydu A/, kampesterolu, stigmasterolu) była relatywnie niska, przy czym organy podziemne były bogatsze w te związki niż kora pędów.

Słowa kluczowe: *Eleutherococcus*, eleuterozydy B i E, kwasy fenolowe, sterole, HPLC