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ACCUMULATION OF PHENOLICS IN ELEUTHERO (*Eleutherococcus senticosus* (Rupr. et Maxim.) Maxim.) AS AFFECTED BY PLANT DEVELOPMENT

Katarzyna Bączek✉, Jarosław L. Przybył, Olga Kosakowska, Zenon Węglarz

Warsaw University of Life Science – SGGW, Department of Vegetable and Medicinal Plants, Nowoursynowska 166, 02-787 Warsaw, Poland

ABSTRACT

The aim of the study was to determine the effect of plant age and growth phase on the accumulation of phenolics in stem bark, leaves and underground organs of Eleuthero. Their content was assessed using validated HPLC-DAD method. In underground organs and stem bark 7 phenolics were determined, i.e. eleutherosides B and E as well as caffeic, ferulic, chlorogenic, rosmarinic and protocatechuic acids. The content of eleutherosides B was significantly higher in stem bark while eleutherosides E, in underground organs. Accumulation of these compounds was the highest in the 4-year-old plants (87.43 mg·100 g^{-1} DW of eleutherosides B and 85.22 mg·100 g^{-1} DW of eleutherosides E in underground organs; 302.21 mg·100 g^{-1} DW of eleutherosides B and 24.89 mg·100 g^{-1} DW eleutherosides E in stem bark). In these organs, among identified phenolic acids chlorogenic acid was the dominant. In the leaves 4 phenolic acids (caffeic, ferulic, chlorogenic and rosmarinic acids), as well as 2 flavonoids (rutoside and hyperoside), were identified. Flavonoids and caffeic acid occurred in higher amounts at the beginning of leaf senescence, whereas the other phenolic acids – at the full vegetation. Their content was the highest in 2-year-old plants.

Key words: plant age, HPLC analysis, eleutherosides, phenolic acids, flavonoids

INTRODUCTION

Eleuthero (*Eleutherococcus senticosus* (Rupr. et Maxim.) Maxim.; syn. *Acanthopanax senticosus* (Rupr. et Maxim.) Harms) is a woody shrub belonging to *Araliaceae* family, native to Far Eastern Asia [Court 2000, Kim and Sun 2004]. Underground organs of this plant i.e. rhizomes with roots, commonly named roots, have been used in traditional Chinese medicine for over 2000 years. In Western medicine first reports on its usage date back until 1960. Nowadays, its monographies can be found in several Pharmacopoeias, e.g. European, Polish, British, Russian, American, Chinese as well as in WHO and EMA reports. Preparations made of Eleuthero roots are highly valued for their adaptogenic activity [Panossian et al. 1999, Davydov and Krikorian 2000]. According to European Medicines Agency [2014] Eleuthero roots are herbal medicinal product traditionally used to improve general condition. They are considered to increase body resistance to a number of external and internal stressors. Preparations from these raw materials are used as tonics for symptoms of asthenia such as fatigue, weakness, exhaustion or lose of concentration. They stimulate the immune system, improve mental activity and physical capacity. Eleuthero roots reveal radioprotective and anti-ulcer effects and are used during chemotherapy as an agent

✉ katarzyna_baczek@sggw.pl

stimulating body regeneration [Davydov and Krikorian 2000, Rogala et at. 2003, Kimura and Sumiyoshi 2004, Kurkin et al. 2006]. In Japanese or Korean folk medicine also Eleuthero leaves and stem bark are mentioned as tonics marked for reducing liver damage and stimulating alcohol detoxification [Davydov and Krikorian 2000].

The constituents identified in Eleuthero roots have been classified into various chemical groups, among which phenolic compounds are considered to be the most abundant and the most pharmacologically active. The main effects of Eleuthero roots are related to the presence of two substances from this group i.e. eleutheroside B and E. Thus, the standardization of these raw material is based on their content. According to European Pharmacopoeia [2014], it should contain not less than 0.08% for the sum of eleutheroside B and E. Some investigations on pharmacology of these compounds have been carried out. It was confirmed that eleutheroside B (syn. syringin) reveals antioxidant, anti-inflammatory, anti-nociceptive as well as neuroprotective and hepatoprotective effects [Song et al. 2010, Gong et al. 2013]. Syringin was also found to possess anti-allergic effect whereas eletheroside E ((+)-syringaresinol-O-β-D-glucoside) shows stress protective activity [Huang et al. 2011]. Both substances reveal also hypoglycaemic activity [Niu et al. 2008, Ahn et al. 2013]. Among phenolics identified in Eleuthero roots some phenolic acids are also mentioned, namely: caffeic, chlorogenic, rosmarinic and protocatechuic [Davydov and Krikorian 2000, EMA 2014]. They reveal various pharmacological activities, i.e. antibacterial, antiviral, anti-inflammatory, antiulcer, antiatherosclerotic and choleretic. Many authors agree that the most important is the ability to scavenging of free radicals [Gűlçin 2006, Falé et al. 2009, Sato et al. 2011, Muñoz-Muñoz et al. 2013, Kakkar and Bais 2014, Sevgi et al. 2014, Bakota et al. 2015, Sytar 2015, Yilmaz et al. 2015].

So far, the Eleuthero roots are collected almost exclusively from the wild, mainly in China and Russia. As a result, raw material origins from plants of various age, undergoing pressure related to different environmental factors affecting both the development of these plants and accumulation of biologically active compounds. It seems that standardized raw material with expected content of these constituents is possible to be obtained only from field cultivation.

The aim of our study was to determine the accumulation of phenolic compounds in Eleuthero above- and underground organs during second, third and fourth year of plant vegetation, i.e. in the period of the most intensive plant growth.

MATERIAL AND METHODS

Plant material. The investigation was carried out in two four-year cycles (2009–2013 and 2010–2014). The field experiment was established at the organic farm in the East Poland on sandy soil enriched by manure compost and highmoor peat of pH (KCl) 5.6. The plant material (stem-root cuttings) used to start the field experiment originated from maternal plants grown in the collection of medicinal and aromatic plants of Department of Vegetable and Medicinal Plants, Warsaw University of Life Sciences – SGGW. The maternal plants were obtained from the seeds of *E. senticosus* growing in the Polish national collection of the *Araliaceae* in Rogów Arboretum, described by Tumiłowicz and Banaszczak [2006].

Two hundreds of well-rooted cuttings with 3–5 leaves and the height of 15–30cm, were obtained after 3 months of rooting. They were planted out in the field on the area of 100 m^2 in May. Raw materials from 2-, 3- and 4-year-old plants were collected from five randomly chosen plants (one plant = replication). The underground organs and stem bark were harvested in June (at the stage of full vegetation) and in November (at the beginning of winter dormancy), whereas the leaves – in June (at the stage of full vegetation) and in September (at the beginning of leaf senescence) (figs 1 and 2). The raw materials were dried at 40°C and subjected to chemical evaluation. Voucher specimens were deposited in the herbarium of Department of Vegetable and Medicinal Plants SGGW-WULS.

HPLC analysis of phenolic compounds. The analyses were performed using a Shimadzu (Kyoto, Japan) chromatograph equipped with auto sampler

SIL-20A, photodiode array detector SPD-M10A VP PDA and CLASS VP™ 7.3 chromatography software. Separations were achieved using 250 × 4.60 mm C18 (2) 5 µm Luna™ column (Phenomenex®, Torrance, USA). Binary gradient elution of deionized water (Cobrabid Aqua, Warsaw, Poland) adjusted to pH 3 with phosphoric acid and MeCN (Sigma-Aldrich®, St. Louis, USA) was used as follows: 0.01 min – 10% B; 10.00 min – 55% B; 15.00 min – 55% B; 15.50 min – 10% B, total time of analysis 30 min, flow rate 1.0 ml·min^{-1}, temperature 30°C.

Fig. 1. Eleuthero 3-year-old plant at the stage of full vegetation

Fig. 2. Eleuthero 3-year-old plant at the beginning of winter dormancy

Table 1. Validation parameters of the HPLC-DAD analysis (n = 6)

Compound	t_R	Precision intra-day (CV %)	Precision inter-day (CV %)	Regression equation	Linearity (r^2)	Range (µg·ml^{-1})	LOD (µg·ml^{-1})	LOQ (µg·ml^{-1})	Recovery (%)
Eleutheroside B	4.3	1.29	1.78	y = 2 168.7x – 53 492	0.9999	4.97–496.50	0.052	0.172	97.5
Eleutheroside E	7.8	0.88	1.22	y = 6 473.0x + 16 038	0.9999	1.85–184.72	0.016	0.054	99.3
Rosmarinic acid	15.9	2.30	2.54	y = 8 876.1x + 342 158	0.9997	9.86–986.40	0.011	0.037	100.8
Protocatechuic acid	4.8	2.23	2.68	y = 7 102.9x + 43 850	0.9997	3.82–382.00	0.014	0.047	98.4
Chlorogenic acid	5.6	1.93	2.14	y = 2 718.2x – 49 105	0.9998	3.95–394.62	0.021	0.070	99.8
Caffeic acid	9.3	1.66	1.87	y = 3 957.8x + 91 219	0.9998	9.98–998.40	0.027	0.090	100.1
Ferulic acid	14.7	2.11	2.45	y = 5 164.7x – 39 592	0.9998	4.00–399.68	0.032	0.106	101.2
Rutoside	10.8	1.58	1.98	y = 1 696.6x – 67 861	0.9998	3.66–365.60	0.057	0.191	99.7
Hyperoside	11.2	1.84	1.87	y = 2 563.7x – 1 763	0.9998	3.84–384.00	0.040	0.133	98.5

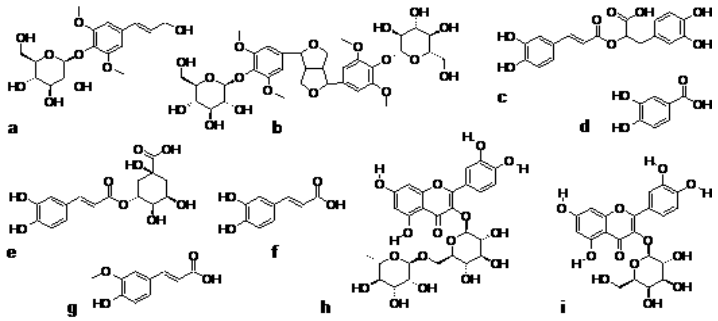

Fig. 3. Chemical structures of the identified phenolics: (a) eleutheroside B; (b) eleutheroside E; (c) rosmarinic acid; (d) protocatechuic acid; (e) chlorogenic acid; (f) caffeic acid; (g) ferulic acid; (h) rutoside; (i) hyperoside

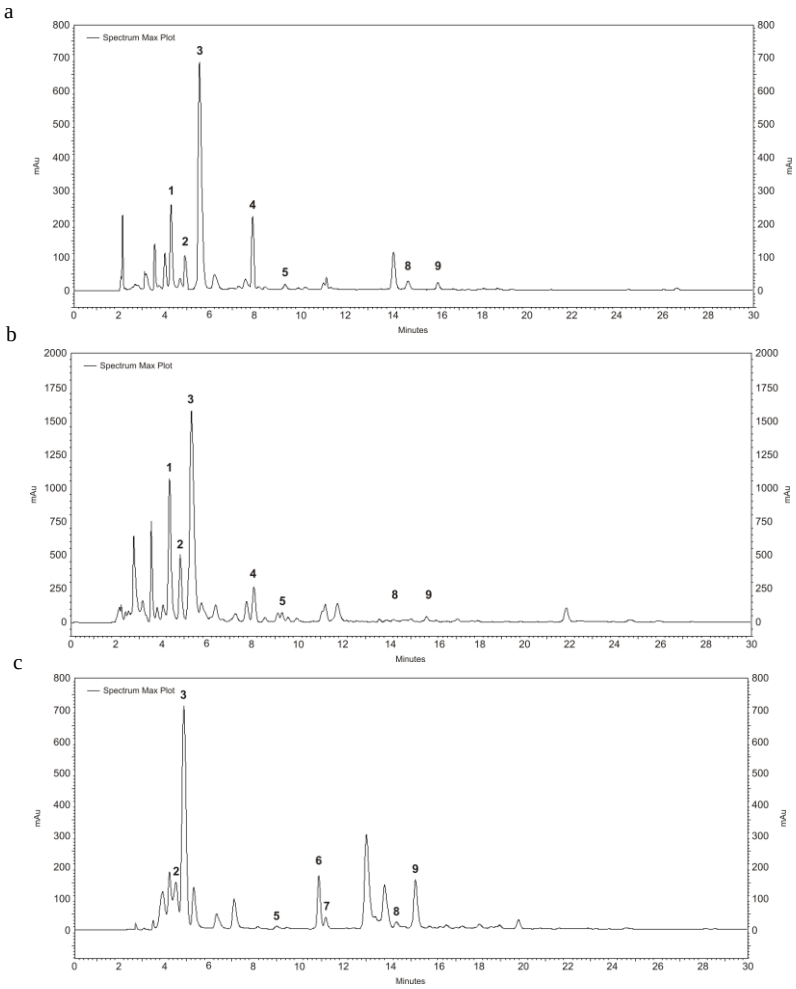

Fig. 4. Chromatograms of Eleuthero underground organs extract (a); stem bark extract (b) and leaves extract (c): 1 – eleutheroside B; 2 – protocatechuic acid; 3 – chlorogenic acid; 4 – eleutheroside E; 5 – caffeic acid; 6 – rutoside; 7 – hyperoside; 8 – ferulic acid; 9 – rosmarinic acid

Validation procedure. Commercially available standards (ChromaDex®, Irvine, USA) were separately dissolved with methanol (Sigma-Aldrich®, St. Louis, USA) in 10 ml volumetric flask according to the ChromaDex's Tech Tip 0003: Reference Standard Recovery and Dilution and used as standard stock solutions (https://www.chromadex.com/media/2126/techtip0003-recoverydilutionprocedures_nl_pw.pdf). Working standard solutions were prepared by dilution 10, 50, 100, 200, 500 or 1000 µl stock solutions of each compound with methanol in 10 ml volumetric flasks. The working solutions were injected (10.0 µl) on a column in six replicates (n = 6) using SIL-20A (Shimadzu, Kyoto, Japan) to generate a six-point calibration curve. Analytical data was recorded at wavelength of 206 nm for eleutheroside E, 254 nm for protocatechuic acid, rutoside and hyperoside, 264 nm for eleutheroside B and 330 nm for chlorogenic acid, caffeic acid, ferulic acid and rosmarinic acid. The peak table and UV-spectra library (190–450 nm) of individual compounds were created. Standard curve parameters were calculated with Microsoft Excel 14 (tab. 1). Signal-to-noise ratio approach were used to determined LOD (S/N of 3 : 1) and LOQ (S/N of 10 : 1).

Sample preparations. Air-dry, finely powdered and homogenized raw materials (1.000 g) were extracted with 100 ml of methanol using Extraction System B-811 (Büchi Labortechnik AG, Flawil, Switzerland). 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 methanol. The obtained extracts were filtered with Supelco (Sigma-Aldrich®, St. Louis, USA) Iso-Disc™ Syringe Tip Filter Unit, PTFE membrane, diameter 25 mm, pore size 0.45 µm. The real samples injection volume was 10 µl. The content of the determined compounds was calculated in mg per 100 g of dry matter.

Statistical analysis. Data were analyzed using Statistica plus software (StatSoft, Tulsa, USA). The mean values were compared by using one way analysis of variance (ANOVA) followed by Tukey's multiple range test at $\alpha = 0.05$ significance level and expressed as mean ± standard deviation (SD). The differences between individual means were deemed to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

Three groups of phenolics i.e.: eleutherosides, phenolic acids and flavonoids in above- and underground Eleuthero organs were determined (figs 3 and 4). The content and composition of these compounds have changed with the age of plants and during the growing season. Among eleutherosides, two substances were determined, i.e. eleutheroside B and E. Both of them were found in underground organs and stem bark, whereas in leaves they were not detected. However, eleutheroside B clearly dominated in stem bark, while eleutheroside E – in underground organs. The content of these compounds increased from full vegetation (June) to the beginning of winter dormancy (November), irrespectively of plant age. With regards to the accumulation of eleutherosides based on the age of plants, it was found that their content, in the conditions of our experiment, increased gradually in following years, reaching the maximum in 4th year (tab. 2). Eleuthreo underground organs are standardized in the content of above mentioned eleutherosides [European Pharmacopoeia 2014]. In the present work, the sum of eleutherosides B and E exceeded pharmacopeial requirements, especially in case of the raw material harvested at the beginning of winter dormancy, from 4-year-old plants (tab. 2).

Numerous authors confirm the occurrence of eleutheroside B and E in Eleuthero underground organs and stem bark [Nishibe et al. 1990, Davydov and Krikorian 2000, Deyama et al. 2001, Bączek 2009, Bączek et al. 2011, Liu et al. 2012, Kim et al. 2013]. Our results show that Eleuthero stem bark is especially rich source of eleutheroside B. Its content was even three times higher in stem bark in comparison with underground organs (tab. 2). The possibility of using stem bark as a raw material was mentioned earlier by Kim et al. [2013].

In the present study, five phenolic acids were identified: caffeic, ferulic, chlorogenic, rosmarinic

Table 2. Accumulation of eleutherosides B and E in Eleuthero organs (mg·100 g^{-1})

Raw material	Compound	Month	Age of plants (years)			Mean
			2nd	3rd	4th	
Underground organs	Eleutheroside B	June	22.17 ±2.44	32.27 ±4.84	71.28 ±9.27	41.91
		November	72.36 ±10.13	75.26 ±9.03	103.57 ±16.57	83.73*
		Mean	47.27b	53.77b	87.43a	
	Eleutheroside E	June	23.42 ±1.87	37.24 ±3.35	65.30 ±9.80	41.99
		November	68.45 ±7.53	69.66 ±9.06	105.14 ±12.62	81.08*
		Mean	45.94b	53.45b	85.22a	
Stem bark	Eleutheroside B	June	85.89 ±7.73	116.58 ±9.93	192.02 ±28.80	131.50
		November	367.07 ±44.05	360.54 ±43.26	412.40 ±57.74	380.00*
		Mean	226.48b	238.56b	302.21a	
	Eleutheroside E	June	3.15 ±0.44	5.22 ±0.47	13.15 ±1.97	7.17
		November	21.24 ±2.76	29.09 ±3.49	36.63 ±4.40	28.99*
		Mean	12.20c	17.16b	24.89 a	

Means marked in rows with different letters differ at P < 0.05; *P < 0.05

Table 3. Accumulation of phenolic acids in Eleuthero underground organs (mg·100 g^{-1})

Compound	Month	Age of plants (years)			Mean
		2nd	3rd	4th	
Caffeic acid	June	5.48 ±0.82	8.82 ±1.15	8.54 ±1.20	7.61
	November	11.18 ±1.23	12.38 ±1.36	14.75 ±1.18	12.77*
	Mean	8.33ab	10.60a	11.65a	
Ferulic acid	June	59.96 ±6.60	36.95 ±5.54	39.14 ±5.87	45.35
	November	63.88 ±8.30	58.11 ±7.55	62.16 ±7.46	61.38*
	Mean	61.92a	47.53b	50.65b	
Chlorogenic acid	June	549.44 ±49.45	409.65 ±45.06	305.68 ±45.85	421.59
	November	811.37 ±97.36	792.99 ±103.09	823.18 ±98.78	809.18*
	Mean	680.41a	601.32b	564.43b	
Rosmarinic acid	June	26.51 ±3.28	35.16 ±4.47	53.12 ±5.84	38.26
	November	65.27 ±5.48	71.25 ±5.57	89.14 ±11.59	75.22*
	Mean	45.89 c	53.21b	71.13a	
Protocatechuic acid	June	26.67 ±3.47	19.46 ±2.53	20.22 ±1.72	22.12
	November	37.48 ±4.12	38.15 ±4.20	29.30 ±3.52	34.98*
	Mean	32.08a	28.81b	24.76c	

Means marked in rows with different letters differ at P < 0.05; *P < 0.05

and protocatechuic acid (tabs 3, 4 and 5). All of them were present in underground organs and stem bark, while in the leaves protocatechuic acid was absent. Chlorogenic acid was the dominant compound in all the examined organs, while rosmarinic and ferulic acids were present in considerable amounts only in the leaves. The content of most of identified phenolic acids in the underground organs and stem bark increased from the period of full vegetation (June) to the beginning of winter dormancy (November) (tabs 3 and 4). The exception was ferulic acid, accumulated in stem bark in significant amounts already during the period of full vegetation (tab. 4).

Accumulation of particular phenolic acids in underground organs and stem bark seems to be related to the age of plants, as well. The content of chlorogenic acid in both organs clearly decreased in subsequent years of plant vegetation. In turn, the content of ferulic acid in stem bark and rosmarinic acid in underground organs gradually increased (tabs 3 and 4).

The obtained results indicate that in Eleuthero leaves (irrespectively of plant age), the accumulation of identified phenolic acids was higher during full vegetation (June) than at the beginning of leaf senescence (September). The content of chlorogenic acid in this organs decreased progressively in following years, what was accompanied by an increase of ferulic acid content (tab. 5). These results correspond with the studies of other authors [Davydov and Krikorian 2000, Deyama et al. 2001, Bączek 2009, Bączek et al. 2010, Liu et al. 2012, Kim et al. 2015].

Table 4. Accumulation of phenolic acids in Eleuthero stem bark (mg·100 g^{-1})

Compound	Month	Age of plants (years)			Mean
		2nd	3rd	4th	
Caffeic acid	June	8.17 ±0.90	7.45 ±0.97	7.19 ±1.01	7.60
	November	45.66 ±7.76	42.73 ±5.55	49.40 ±6.42	45.93*
	Mean	26.92[b]	25.09[b]	28.30[a]	
Ferulic acid	June	54.59 ±8.73	60.25 ±8.44	66.19 ±8.60	60.34*
	November	2.40 ±0.29	2.55 ±0.20	2.89 ±0.46	2.61
	Mean	28.50[c]	31.40[b]	34.54[a]	
Chlorogenic acid	June	451.39 ±67.71	395.58 ±59.34	334.32 ±28.47	393.76
	November	870.49 ±113.16	851.76 ±102.21	742.98 ±89.16	821.74*
	Mean	660.94[a]	623.67[b]	538.65[c]	
Rosmarinic acid	June	3.15 ±0.28	5.22 ±0.44	7.15 ±0.64	5.17
	November	29.09 ±3.78	21.24 ±2.55	20.73 ±2.69	23.69*
	Mean	16.12[a]	13.23[b]	13.94[b]	
Protocatechuic acid	June	29.33 ±4.11	29.28 ±3.81	38.34 ±4.22	32.32
	November	58.29 ±4.66	39.36 ±6.30	39.84 ±5.58	45.83*
	Mean	43.81[a]	34.32[b]	39.09[a]	

Means marked in rows with different letters differ at $P < 0.05$; *$P < 0.05$

Phenolic acids occur in plants commonly in free or bonded forms. In the present work the identified acids represent free compounds (caffeic, ferulic and protocatechuic acids) and depsides (chlorogenic and rosmarinic acids). Protocatechuic acid originates from benzoic acid, while others belong to cinnamic acids derivatives related to caffeic acid [Kohlmünzer 2012]. In general, physiological role of phenolic acids is combined with the response of plants to stress e.g. pathogen attack. They reveal also allelopathic activity [Wu et al. 1998, Inderjit et al. 2002, Zhang et al. 2010]. As components of lignins they incrust cell walls making them stronger and more resistant [Glazener 1982, Lee et al. 1995, Weng and Chapple 2010]. Phenolic acids are precursors of other phenolics such as flavonoids or tannins [Kohlmünzer 2012].

In our study, within flavonoids, two substances were identified, namely: rutoside and hyperoside. Both of them were found only in the leaves, with the clear domination of rutoside (tab. 5). It was shown that their content increased from the full vegetation (June) to the beginning of leaf senescence (September), irrespectively of plant age. The content of both compounds was the highest in 2-year-old plants and decreased gradually to reach the minimum in 4th year (tab. 5). Chen et al. [2002] confirm the presence of these flavonoids, in Eleuthero leaves providing also the occurrence of quercetin and quercitrin. According to Kolesnikov and Gins [2001] this raw material contain six flavonoids, including derivatives of quercetin, isorhamnetin and kaempferol. Flavonoids, as natural antioxidants, play a significant role in plant

Table 5. Accumulation of phenolic compounds in Eleuthero leaves (mg·100 g^{-1})

	Compound	Month	Age of plants (years)			Mean
			2nd	3rd	4th	
Phenolic acids	Caffeic acid	June	3.92 ±0.59	2.70 ±0.40	3.10 ±0.26	3.24
		September	8.40 ±1.09	3.26 ±0.39	4.02 ±0.48	5.23*
		Mean	6.16^{a}	2.98^{c}	3.56^{b}	
	Ferulic acid	June	112.48 ±12.37	113.89 ±14.81	183.68 ±25.72	136.68*
		September	35.72 ±6.07	57.78 ±7.51	112.74 ±14.66	68.75
		Mean	74.10^{c}	85.84^{b}	148.21^{a}	
	Chlorogenic acid	June	812.05 ±113.69	706.61 ±91.86	684.08 ±75.25	734.25*
		September	635.75 ±50.86	216.84 ±34.69	194.04 ±27.17	348.88
		Mean	723.90^{a}	461.73^{b}	439.06^{c}	
	Rosmarinic acid	June	135.33 ±12.18	172.76 ±14.71	163.95 ±14.76	157.35
		September	78.98 ±10.27	88.68 ±10.64	89.00 ±11.57	85.55*
		Mean	107.16^{b}	130.72^{a}	126.48^{ab}	
Flavonoids	Rutoside	June	286.13 ±45.78	245.98 ±34.44	79.62 ± 10.35	203.91
		September	405.07 ±48.61	298.82 ±23.91	154.80 ±24.77	286.23*
		Mean	345.60^{a}	272.40^{b}	117.21^{c}	
	Hyperoside	June	3.34 ±0.53	2.56 ±0.36	2.98 ±0.39	2.96
		September	45.92 ±5.51	31.19 ±2.50	27.70 ±4.43	34.94*
		Mean	24.63^{a}	16.88^{b}	15.34^{c}	

Means marked in rows with different letters differ at $P < 0.05$; *$P < 0.05$

cells. The biosynthesis of this compounds takes place in endoplasmic reticulum, from where they are transported to different cellular compartments directly into the center or proximity of reactive oxygen species generation [Agati et al. 2012]. Considerable amounts of these substances in Eleuthero leaves, especially rutoside, as well as high content of phenolic acids, namely ferulic, chlorogenic, and rosmarinic, make this raw material an interesting from the pharmacological point of view [Marrassini 2011].

Taking into account the physiological role of phenolic compounds in plants, it can be suspected that presence of these substances may be related with plants reaction to stress. All the investigated Eleutero organs collected from younger plants (2-year-old) were characterized with high content of chlorogenic acid. Such phenomena may be explained with the need to protect younger, intensively growing plants against the stressors of different origin. For the same reason it may be assumed that rutoside was accumulated in the leaves in the highest amount by the youngest plants. According to Erlejman et al. [2004] this compound may protect plant cells from damage by interaction with the polar head of phospholipids in cell membrane which leads to the enhancement of its rigidity.

In turn, seasonal changes in phenolics content can be associated with the circulation of these substance in plants during vegetative period. Both short and long distance transport of plants secondary metabolites is a newly developing research area. Secondary metabolites, including phenolics, are often transported from source cells to the neighbouring cells and futher to other tissues, to be stored in preferential organs [Yazaki 2005, 2006, Yazaki et al. 2008]. In the present study, it can be suspected that at the beginning of winter dormancy underground organs have become acceptors of eleutherosides and phenolic acids which could explain a high content of these compounds in the mentioned organs.

Seasonal variation in accumulation of phenolics was observed in other adaptogenic plants as well. In the study on roseroot (*Rhodiola rosea* L.) the content of rosarin and rosavin, the dominant phenolics in underground organs, was the highest in the 1st year of plant vegetation, then decreased gradually until 4th year [Węglarz et al. 2008]. In turn the results obtained by Skiba and Węglarz [1999] show that the total content of flavonoids in the underground organs of maral root (*Rhaponthicum carthamoides* (Willd.) Iljin.) increased from 1st to 3rd year of plant vegetation, while the content of phenolic acids – decreased.

Results obtained in the present study indicate on directional changes of phenolic compounds accumulation in Eleuthero organs. Based on such changes, the pharmacological activity of these organs could be predicted.

CONCLUSIONS

1. The accumulation of biologically active compounds in Eleutheo organs was strictly combined with the age of plants and their growth stage during vegetation period.

2. The highest content of eleutherosides B and E in Eleuthero roots was detected at the beginning of winter dormancy in the 4th year of plant vegetation.

3. Due to extremely high content of eleutheroside B in Eleuthero stem bark, it may be considered as an alternative raw material to the roots.

4. Due to high content of phenolic compounds, especially chlorogenic, rosmarinic and ferulic acids, Eleuthero leaves appear to be an interesting antioxidant raw material.

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