Accumulation of biologically active compounds in Eleuthero (*Eleutherococcus senticosus* /Rupr. et Maxim./ Maxim.) grown in Poland

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**Summary**

Eleuthero (*Eleutherococcus senticosus* /Rupr. et Maxim./ Maxim.) is a shrub native to north-eastern Asia. Underground parts of this plant are classified as a drug with adaptogenic activity. Eleutherosides are regarded as main active compounds of this plant. The studies on cultivation and raw material quality of eleuthero were undertaken in the Department of Vegetable and Medicinal Plants of Warsaw University of Life Sciences – SGGW. The content of eleutherosides B and E and phenolic acids in the raw materials were determined by HPLC. The total content of eleutherosides B and E in the dried rhizomes with roots exceeded considerably the content of these compounds required by the British Pharmacopoeia (80 mg/100 g). In the raw material of two-, three- and four-year-old plants it amounted to 104.7, 167.4 and 292.4 mg/100 g, respectively. The content of eleutherosides in the bark of woody stems was almost twice as high as the content of these compounds in the rhizomes with roots. Both rhizomes with roots and bark of woody stems were characterised by high content of phenolic acids, especially chlorogenic acid (849.8 and 421.0 mg/100 g, respectively).

**Key words:** *Eleutherococcus, eleutherosides, phenolic acids, age of plants*

**INTRODUCTION**

Eleuthero is regarded as one of the most interesting and promising plant classified as an adaptogen. This shrub, 2-3 m high, is native to northeastern Asia. Its underground parts (rhizomes with roots) are usually used as a medicinal raw material. Eleutherosides, considered as active compounds of this plant, belong to
different chemical groups, such as lignans, phenylopropanes, coumarins, saponins and sterols. The most pharmacologically active seems to be eleutheroside B (syringin) and eleutheroside E (syringaresinol-4,4-O-β-D-diglucoside) [1-3].

As yet, raw material for extracts production has been obtained from wild growing eleuthero plants [3, 4]. Taking into consideration the needs of phytopharmaceutical industry, especially concerning standardization of raw materials, in the Department of Vegetable and Medicinal Plants of Warsaw University of Life Sciences – SGGW the studies on cultivation of eleuthero have been undertaken. The investigations were especially focused on accumulation of biologically active compounds in different organs of two-, three-, and four-year-old plants.

MATERIALS AND METHODS

Eleuthero plant material used for the field experiment was taken from local collection of plants originating from natural sites in Siberia. The stem-root cuttings were planted in spring 2002, 2003 and 2004 at 75x75 cm distance. For chemical evaluation two-, three-, and four-year-old plant materials (rhizomes with roots, stem, bark and fruits – see photos) were taken. Fruits were analyzed only from four-year-old plants. Raw materials were collected in late autumn of 2006 and dried at 40°C.

For the determination of eleutherosides and phenolic acids, 1 g of grounded raw material was extracted with 100 ml of ethanol in Büchi B-811 Extraction System. After evaporation of solvent, the residue was dissolved in 10 ml of methanol, filtered through a Supelco IsoDisc PTFE 25 mm x 0.45 μm filter, and subjected to HPLC. The analysis was carried out using the Shimadzu chromatograph with DAD detector. Luna 5 μm C18 (2) 250 x 4.6 mm column was used. Gradient elution of 10% and 55% ACN in water (pH 3.0) was applied. Peaks were identified 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), 254 nm (rutoside), 264 nm (eleutheroside B) and 330 nm (chlorogenic and rosmarinic acids).

The presented results are mean values of ten plants.

RESULTS AND DISCUSSION

Eleuthero plants grown at the experimental field in Warsaw were well adapted to Polish climatic conditions and overwintered without any damages. They were characterized by intensive growth both of under- and above-ground organs (fig. 1 - 8). In the fourth year of plant vegetation flowers and fruits with germinating seeds appeared.
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Values marked with the same letter do not differ at $\alpha=0.01$

Figure 1. Dry mass of underground organs and stem bark (g/plant)

Figure 2. HPLC chromatogram of underground organs extract

Figure 3. HPLC chromatogram of stem bark extract
Figure 4. HPLC chromatogram of fruits extract

Figure 5. Fruits (fot. K. Bączek)

Figure 6. Steams and steam bark (fot. K. Bączek)

Figure 7. 4-year-old plants at the stage of blooming (fot. K. Bączek)

Figure 8. 4-year-old plants at the stage of winter dormancy (fot. K. Bączek)
The presented study is the first trial to evaluate the accumulation of biologically active compounds in cultivated eleuthero during a four-year period of plant development.

The content of two most important eleutherosides (B and E) in underground organs (main raw material of eleuthero) was distinctly higher in three- and four-year-old plants in comparison with two-year-old plants (tab. 1, fig. 2). However, even in the raw material from two-year-old plants the sum of those eleutherosides was higher than the minimum demanded by British Pharmacopoeia [1].

**Table 1.**
The content of eleutherosides in underground organs, stem bark and fruits [mg/100g]

<table>
<thead>
<tr>
<th></th>
<th>2-year-old plants</th>
<th>3-year-old plants</th>
<th>4-year-old plants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>underground organs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>54.39 b</td>
<td>100.51 a</td>
<td>115.07 a</td>
</tr>
<tr>
<td>E</td>
<td>50.29 b</td>
<td>66.84 b</td>
<td>94.11 a</td>
</tr>
<tr>
<td>B+E</td>
<td>104.68 c</td>
<td>167.35 b</td>
<td>209.18 a</td>
</tr>
<tr>
<td><strong>stem bark</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>173.72 c</td>
<td>277.25 b</td>
<td>376.31 a</td>
</tr>
<tr>
<td>E</td>
<td>24.49 b</td>
<td>48.21 a</td>
<td>30.74 ab</td>
</tr>
<tr>
<td>B+E</td>
<td>198.21 c</td>
<td>325.46 b</td>
<td>397.05 a</td>
</tr>
<tr>
<td><strong>fruits</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>–</td>
<td>–</td>
<td>35.61</td>
</tr>
<tr>
<td>E</td>
<td>–</td>
<td>–</td>
<td>29.75</td>
</tr>
<tr>
<td>B+E</td>
<td>–</td>
<td>–</td>
<td>65.36</td>
</tr>
</tbody>
</table>

Values marked with the same letter in rows do not differ at \( \alpha=0.01 \)

Especially rich source of eleutherosides appeared to be stem bark. The content of eleutherosides B and E in this raw material was over twice as high as in the underground organs. Fruits were characterized by considerably lower content of these compounds. The presence of eleutherosides B and E in stem bark of eleuthero has been previously reported by Ryu et al. [5], and in fruits – by Kim et al. [6], but without quantitative data.

In all three investigated organs of eleuthero high content of chlorogenic acid was found (tab. 2). This compound was determined as one of major phenolic compounds of eleuthero stem bark by Nishibe et al. (1990) [7]. In the stem bark and fruits rutoside (quercetin-3-rhamnoglucoside) was also present (tab. 3).

**Table 2.**
The content of phenolic acids in underground organs, stem bark and fruits [mg/100g]

<table>
<thead>
<tr>
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<th>4-year-old plants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>underground organs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>chlorogenic acid</td>
<td>231.45 c</td>
<td>405.86 b</td>
<td>559.37 a</td>
</tr>
<tr>
<td>rosmarinic acid</td>
<td>14.11 b</td>
<td>14.07 b</td>
<td>24.74 a</td>
</tr>
<tr>
<td><strong>stem bark</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>chlorogenic acid</td>
<td>1015.46 c</td>
<td>1028.07 a</td>
<td>849.84 b</td>
</tr>
<tr>
<td>rosmarinic acid</td>
<td>5.53 b</td>
<td>6.62 b</td>
<td>11.40 a</td>
</tr>
<tr>
<td><strong>fruits</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>chlorogenic acid</td>
<td>–</td>
<td>–</td>
<td>409.26</td>
</tr>
<tr>
<td>rosmarinic acid</td>
<td>–</td>
<td>–</td>
<td>84.02</td>
</tr>
</tbody>
</table>

Values marked with the same letter in rows do not differ at \( \alpha=0.01 \)
Table 3.
The content of rutoside in stem bark and fruits [mg/100g]

<table>
<thead>
<tr>
<th></th>
<th>2-year-old plants</th>
<th>3-year-old plants</th>
<th>4-year-old plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>stem bark</td>
<td>125.32 a</td>
<td>94.72 b</td>
<td>66.50 c</td>
</tr>
<tr>
<td>fruits</td>
<td>–</td>
<td>–</td>
<td>143.34</td>
</tr>
</tbody>
</table>

Values marked with the same letter do not differ at \( \alpha = 0.01 \)

CONCLUSIONS

1. During four years of plant vegetation considerable weight increment of both underground and aboveground organs was observed.
2. Analyzed plant organs (rhizomes with roots, stem bark and fruits) differed both quantitatively and qualitatively in respect of analyzed biologically active compounds.
3. Content of eleutherosides B and E in underground organs significantly increased in the period of three years (from the second to fourth year of plant vegetation).
4. Irrespective of the age of plants, stem bark was characterized by threefold higher content of eleutheroside B and chlorogenic acid in comparison with the underground organs.
5. In stem bark and fruits rutoside (flavonoid glycoside) was found.

ACKNOWLEDGEMENT

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REFERENCES

GROMADZENIE SIĘ ZWIĄZKÓW BIOLOGICZNIE AKTYWNYCH W ELEUTEROKOKU KOŁCZYSTYM (*ELEUTHEROCOCUS SENTICOSUS/RUPR. ET MAXIM./MAXIM.*) UPRAWIANYM W POLSCE

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Streszczenie

Eleuterokok kolczysty to krzew występujący głównie na terenie północno-wschodniej Azji. Organy podziemne eleuterokoka wykorzystywane są jako surowiec o działaniu adaptogen-nym. Najważniejszą grupą związków biologicznie czynnych, które występują w tej roślinie, są eleuterozydy. Badania nad uprawą oraz jakością uzyskanych surowców eleuterokoka podjęte zostały w Szkole Głównej Gospodarstwa Wiejskiego w Warszawie. Zawartość eleuterozydów B i E oraz kwasów polifenolowych w surowcach oznaczono za pomocą wysokosprawnej chromatografii cieczowej (HPLC). Suma eleuterozydów B i E w badanych organach podziemnych eleuterokoka przekraczała istotnie zawartość tych związków wymaganą przez Farmakopeę Brytyjską (80 mg/100 g).
W surowcach pozyskanych z dwu-, trzy- i czteroletnich roślin ich zawartość wyniosła odpowiednio 104,7, 167,4 i 292,4 mg/100 g. Zawartość eleuterozydów w korze pędów była prawie dwukrotnie wyższa w porównaniu z organami podziemnymi. Zarówno organy podziemne jak i kora pędów charakteryzowały się wysoką zawartością kwasów fenolowy-
nych, a w szczególności kwasu chlorogenowego (odpowiednio 849,8 i 421,0 mg/100 g).

Słowa kluczowe: Eleutherococcus, eleuterozydy, kwas fenolowy, wiek roślin