The phytochemical study of *Eleutherococcus senticosus* (Rupr. & Maxim) leaves in hydroponics and soil culture

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**ABSTRACT**

**Background:** In the medical field *Eleutherococcus senticosus* (Rupr. & Maxim) (*E. senticosus*) or Siberian ginseng is known as a natural adaptogen and immunomodulator. All parts of *E. senticosus*: roots, stems, leaves, and berries, have medicinal properties. In medicine, *E. senticosus* is used to treat depression, Alzheimer’s and Parkinson’s diseases, cardiovascular diseases, cerebral ischemia, and diabetes. The adaptogenic properties of the plant are related to its rich composition of biologically active compounds (phenylpropanoids, eleutherosides, flavonoids, phenolic acids, anthocyanins, vitamins, etc.). *E. senticosus* activates the body’s protection mechanisms, directly affecting tissue metabolism. It increases mental and physical performance, immunity, and protects from stress, making its use important in sports medicine and the military. The use of *E. senticosus* in food and dietary supplements has become popular in recent years, whereas some studies suggest that its potential benefits are the reduction of stress and enhancement of immune system function.

**Objective:** To study the content of the main biologically active compounds of medicinal raw material (leaves) of *E. senticosus*, cultivated in outdoor hydroponics and soil conditions during different stages of growth and development.

**Methods:** The spectrophotometric method was used to determine the content of total phenols, flavonoids, phenolic acids and eleutherosides in a 70 % water-alcohol extract from *E. senticosus* dry leaves at different phases of vegetation
period. The spectra were recorded using an Agilent Cary 60 UV-Vis spectrophotometer. Moreover, the content of extractive substances was determined, and the content of vitamin C, and β-carotene in fresh leaves (State Pharmacopoeia 2015).

**Results:** The results of our study showed that levels of phenolic compounds in the leaves of *E. senticosus* obtained in hydroponics and soil culture are the highest during the flowering phase (August-September). It was observed that during the flowering period, the content of total phenols, phenolic acids, and eleutherosides was 1.3 times higher, and flavonoids - 1.2 times higher compared to the vegetative phase. It is worth noting that the content of vitamin “C”, β-carotene and extractives was also higher during the flowering period. Hydroponic plants had higher content of vitamin “C”, β-carotene, and extractives, respectively by 1.4, 1.2 and 1.2 times compared to soil plants. This could be due to several factors such as the optimal content of nutrients (N=200 mg/L, P=65 mg/L, K=350 mg/L), and the high content of oxygen in the hydroponic system.

**Conclusion:** According to the results of a phytochemical study of *E. senticosus* leaves grown in outdoor hydroponics and soil culture in the Ararat Valley, the highest content of biologically active compounds (eleutherosides, phenols, phenolic acids, vitamins) is recorded in hydroponic plants in the flowering period. In general, the research has practical importance, since the *E. senticosus* plants grown in hydroponic conditions can be a rich source for the production of natural food and dietary supplements.

**Keywords:** biologically active compounds, adaptogen, eleutherosides, flavonoids, phenolic acids, medicinal raw material
INTRODUCTION
The main challenges of the 21st century are the prevention of various human diseases and the optimization of health. One of the ways to solve the problems of modern healthcare is the use of functional foods and harmless natural preparations obtained from medicinal plants. The recent development of pharmacological therapy is characterized by the significant use of phytotherapeutics and medicinal plants, which can influence human physiological processes and increase natural defense and resistance to adverse and stressful conditions [1-6]. There are many natural adaptogens of plant and animal origin that activate the body’s defense mechanisms, directly affecting tissue metabolism. Moreover, those adaptogens increase mental and physical performance, and immunity, preventing disorders caused by stress and other extreme factors [7-9]. Thus, one such valuable adaptogenic, immunomodulatory herb is *E. senticosus* Rupr. and Maxim., belonging to the *Araliaceae* family and widely growing in Northeastern Asia, Japan, China, and Korea [7, 10]. All aerial parts of the plant are valuable medicinal raw materials, and the mainly used ones are 5 – 6 years old plant roots. The roots are collected in October when the content of biologically active substances is at the highest level [11, 12]. The major active compounds of *E. senticosus* include phenylpropanoids, eleutherosides (A, B, C, D, E), acanthoside, chiisanoside, senticoside, triterpenic saponins, flavones, flavonoids, phenolic acids, anthocyanins, sesamine, polysaccharides, vitamins, etc. [13-19]. The extracts and medicines of *E. senticosus* have adaptogen properties, promote human physical and mental activity, and have antidiabetic, restorative, antioxidant, anticancer, immunoregulatory, anti-inflammatory, immunomodulating, antimicrobial and antiviral properties [2, 8, 20 - 26]. The use of *E. senticosus*-containing medicines in medical practice is aimed at the prevention of respiratory diseases and improving visual memory and eye vision. In addition, they are used to alleviate fatigue and severe infectious diseases, to relieve the effects of radiation therapy and for neuroses. The stimulative and adaptogen properties of *E. senticosus* are widely used in sports medicine, as it increases physical and mental efficacy, and immunity, and prevents emotional stress, anxiety, and fatigue. Various studies demonstrate that the plant's phyto-adaptogenic properties have a valuable use in the army to increase the resistance of military personnel in stressful conditions, improve physical and mental performance, and strengthen immunity [3 - 9, 11, 17, 20, 27 - 30]. Therefore, the development of biotechnology for the introduction, cultivation, and determination of the content of the main biologically active compounds (BAC) of medicinal raw materials of *E. senticosus* as an adaptogen for use in public health is relevant and promising.

MATERIALS AND METHODS
The research was conducted in 2021–2023 at the hydroponic experimental station of the Institute of Hydroponics Problems in Ararat Valley. The study involved both soil and hydroponic vegetation vessels, each with an area of 1m² and three replicates. The Ararat Valley is situated at an altitude of approximately 850 – 900 m above sea level. It is important to note that the region is exposed to very dry climate, with an average annual temperature of 11.0 – 11.8 °C, relative humidity of 40 %, and an average annual precipitation of 200 – 300 mm [31]. The surrounding soils of the hydroponic institute are semi-desert, loamy, and carbonate-based, with a humus content of 1.5 – 2.5 %. These soils are rich in phosphorus and potassium. In soil culture, standard agricultural practices were followed, including soil tillage, fertilization, loosening, regular watering, and weed removal. In the hydroponic system, the plants were provided with the nutrient solution recommended by Davtyan (Table 1) [32].
Table 1. The nutrient solution recommended by Davtyan consisted of various essential nutrients necessary for plant growth and development, with their respective concentrations in g/m³ of water.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>The beginning of the vegetation period</th>
<th>Vegetative growth period</th>
<th>Fertility period</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-80-200</td>
<td>80</td>
<td>175</td>
<td>200</td>
</tr>
<tr>
<td>KNO₃</td>
<td>580</td>
<td>580</td>
<td>580</td>
</tr>
<tr>
<td>NH₄NO₃</td>
<td>-</td>
<td>170</td>
<td>170</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>-</td>
<td>175</td>
<td>175</td>
</tr>
<tr>
<td>CO(NH₂)₂</td>
<td>-</td>
<td>-</td>
<td>56</td>
</tr>
<tr>
<td>P-45-65</td>
<td>45</td>
<td>65</td>
<td>65</td>
</tr>
<tr>
<td>H₃PO₄</td>
<td>170</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>K- 310-350</td>
<td>310</td>
<td>310</td>
<td>350</td>
</tr>
<tr>
<td>K₂SO₄</td>
<td>170</td>
<td>170</td>
<td>170</td>
</tr>
<tr>
<td>S-100-150</td>
<td>100</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Ca -150</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>CaSO₄·2H₂O</td>
<td>640</td>
<td>640</td>
<td>640</td>
</tr>
<tr>
<td>Mg- 30-50</td>
<td>30</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>300</td>
<td>400</td>
<td>500</td>
</tr>
</tbody>
</table>

**Microelements**

<table>
<thead>
<tr>
<th>FeCl₃·6H₂O or Fe₂(SO₄)₃·9H₂O</th>
<th>5-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₃BO₃</td>
<td>2-3</td>
</tr>
<tr>
<td>KMnO₄ or MnSO₄·4H₂O</td>
<td>1-2</td>
</tr>
<tr>
<td>ZnCl₂ or ZnSO₄·7H₂O</td>
<td>0.4-0.8</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>0.2</td>
</tr>
<tr>
<td>Na₂MoO₄</td>
<td>0.2</td>
</tr>
<tr>
<td>CoCl₂·6H₂O or Co(NO₃)₂·6H₂O</td>
<td>0.1</td>
</tr>
<tr>
<td>KI</td>
<td>0.2-1.0</td>
</tr>
</tbody>
</table>

Red volcanic slag with a diameter of 3 to 15 mm, was used as the growing media. Prior to use, the filler material was disinfected with a 0.05 % solution of KMnO₄. The object of the study was three-year-old plants of *E. senticosus* (Fig. 1).
Phytochemical study of leaves was implemented in different periods of vegetation: vegetative growth (June) and flowering (August) phases.

**Determination of eleutherosides, total phenols, flavonoids, and phenolic acids:** A spectrophotometric method for determination of the total content of eleutherosides on the bases of eleutheroside B (syringin) [33, 34], phenolic acids (rosmarinic, gallic, caffeic, chlorogenic), total phenols on the bases of gallic acid, total flavonoids on the bases of quercetin [35 - 37] and extractives was used [33, 40].

One g (accurately weighted) of finely ground dry herbs sieved through a 2.0 mm diameter mesh was placed in a 250 mL conical flask and for 60 minutes was infused with 100 mL of 70 % alcohol on bain-marie. The conical flask was connected to the reflux refrigerator. Then the extract was cooled and filtered through filter paper in a 100 mL volumetric flask. The content in the volumetric flask was brought to the mark by adding 70 % alcohol solution (solution A). Solution A (2 mL) was transferred into a volumetric 25 mL flask and 96 % ethanol was added till the mark.

The spectra were recorded using an Agilent Cary 60 UV-Vis spectrophotometer with 10 mm layer thickness cuvette at appropriate wavelengths: λ = 266 nm for eleutherosides; λ = 370 nm for total flavonoids; λ = 277 nm for total phenols and caffeic acid; λ = 327 nm for gallic acid and rosmarinic acids; λ = 330 nm for chlorogenic acid. As a reference solution 96% alcohol was used.

**Determination of Vitamin C:** A sample of the fresh leaves (5 g) was crushed in a mortar with 20 mL of (1 %) HCL solution until a uniform mass was achieved. Then, the mass was transferred into a volumetric flask with 80 mL of oxalic acid (1 %) solution. Two parallel 10 mL samples were taken with a pipette and titrated the micro burette with 0.001N 2.6 dichlorophenolindophenol dye solution [33, 38].

**Determination of β-carotene:** The fresh leaf material (0.5 g) was carefully crushed with pastel in the mortar with NaHCO₃ (for the extraction of beta carotene from plant material, it is necessary to neutralize organic acids) and Na₂SO₄ (to dehydrate). When the mixture was well-grounded, hexane was added to the mortar. Then the content of the mortar was filtered with suction, the mortar was rinsed with hexane and the material left on the filter was washed with small portions of hexane until the color of the flowing hexane disappeared. The resulting extract was transferred to a 25 mL volumetric flask and hexane was added till the mark. The resulting extract was observed with a UV spectrophotometer under λ = 445 nm wave [33, 39].

**Statistical Analysis:** The obtained data were subjected to statistical analysis using Microsoft Excel and Graph Pad Prizm 8.

**RESULTS AND DISCUSSION**

The growth and development of *E. senticosus* under different cultivation conditions (outdoor hydroponics and soil) and the accumulation of the main biologically active substances in the medicinal raw material (leaves) during different vegetation periods were studied.
Figure 2 presents the growth dynamics of *E. senticosus* during vegetation (April - September). The height of hydroponic plants was 1.3-1.6 times higher than soil plants. The correlation coefficients between the growth indices of *E. senticosus* plants in different growing conditions demonstrate that during vegetation there are strong positive comparative connections (correlation coefficient $r = 0.98\pm 0.08$, $t_{\text{actual}} = 12.2$) between growth rates (height) of *E. senticosus* plants in hydroponics and soil conditions [41]. Since $t_{\text{actual}} = 12.2 > t_{\text{theoretical}0.5} = 2.78$, the correlation between the growth indices is considered significant.

![Figure 2](image1.png)

**Fig. 2.** Growth dynamics of *E. senticosus* during vegetation (April - September).

The research results presented in Figure 3 show that during the flowering phase, the vitamin "C" content in hydroponic plants was 1.3-1.4 times higher than in soil plants. Moreover, this difference is more noticeable in June. In the vegetative growth period, hydroponic plants contained 1.2 times more β-carotene than soil plants. However, in the flowering phase, β-carotene content was 1.1 times lower in hydroponic plants in comparison with soil plants. During vegetation, the content of extractive substances in hydroponic plants did not change, but in soil plants, it decreased by 1.1 times. Furthermore, in the flowering phase, hydroponic plants accumulated 1.2 times more extractives than soil ones.

![Figure 3](image2.png)

**Fig. 3.** Comparison of the content of vitamin "C" (a), β-carotene (b), and extractive substances (c) in *E. senticosus* leaves in hydroponics and soil culture.

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![Figure 3](image2.png)

**Fig. 4.** Accumulation of phenolic acids in *E. senticosus* leaves in hydroponics and soil culture.
The data of Figure 4 shows that at the beginning of vegetation, the content of phenolic acids (rosmarinic, gallic, caffeic, chlorogenic) in the leaves of soil plants exceeded that of hydroponic ones, while in the flowering phase, the opposite happened: the content of phenolic acids in soil plants was inferior to those in hydroponic plants. Regardless of the growth conditions, the comparative character of the change of phenolic acids content in the plant material is the same. The content of phenolic acids in the leaves under hydroponic and soil conditions during the vegetation formed the same decreasing range: rosmarinic > chlorogenic > gallic > caffeic acids. This partially coincides with the literature data [16]. The quantitative index of phenolic acids content in plant material is different under hydroponic and soil conditions. As a result of the study, it was found that in soil plants the content of rosmarinic, gallic, and chlorogenic acids exceeded the similar indicators of hydroponic plants 1.1 – 1.2 times during the vegetative growth period and was inferior 1.1 – 1.4 times in the flowering phase. Hydroponic and soil plants did not differ significantly in caffeic acid content at the beginning of vegetation. Meanwhile, in the flowering phase, the content of caffeic acid in hydroponic plants is 1.4 times higher than in soil plants.

According to the data shown in Figure 5, during the vegetation, the content of phenolic compounds and eleutherosids in the leaves of *E. senticosus* formed the same decreasing range in hydroponic and soil conditions: flavonoids > eleutherosides > total phenols. During the vegetative growth, the soil plants exceeded hydroponic ones in flavonoid content by 1.3 times. At the same time, during flowering, the content of flavonoids, total phenols and eleutherosides was 1.3 - 1.4 times lower in soil plants compared with hydroponic plants.

According to the Pharmacopeia requirements [8, 9, 12, 16, 18, 19, 33, 34] the medicinal raw material of *E. senticosus* is the root. Our literature data [20, 42] has shown that the leaves of *E. senticosus* are also a good source of BAC. Thus, the leaves of *E. senticosus* can be used as a medicinal raw material and as a food supplement.

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CONCLUSION: The growth conditions have influenced the content of the BAC in *E. senticosus* plants. According to the results of a phytochemical study of leaves of *E. senticosus* grown in outdoor hydroponics and soil culture in the Ararat Valley, the high content of BAC (eleutherosides, flavonoids, phenolic acids, vitamin “C”, β-carotene, extractives) was recorded in the flowering period. However, the highest content of biologically active substances was observed in hydroponic plants. In general, the research has practical importance, since the *E. senticosus* plants grown in hydroponic conditions can be a rich source for producing natural food, dietary supplements, and pharmaceutical preparations. The hydroponic method of cultivation is recommended to produce medicinal raw material of *E. senticosus*.

**Abbreviations:** BAC – Biological Active Compounds; *E. senticosus* – *Eleutherococcus senticosus*

**Authors Contribution:** All authors contributed to this study.

**Competing interests:** The authors declare no conflict of interest.

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