

INTRODUCTION

An important task of modern medicine is using less toxic, harmless phototherapeutic agents and adaptogenic, antioxidant plants that can increase the human body's natural defenses and resistance to the adverse effects of stress [1- 4]. Medicinal and food plants are the most important sources of biologically active substances (BAS) and natural antioxidants. It is of considerable interest to study the antioxidant properties not only of individual substances isolated in the chemically pure form of plant extracts but also these extracts since they contain hundreds and thousands of components, and their total antioxidant activity and other beneficial properties often exceed those of individual compounds [5 - 11].

For this reason, studying the pharmacochemical and antioxidant properties of the valuable, adaptogenic, and immunomodulatory medicinal plant *Eleutherococcus (E.) senticosus* (Rupr. and Maxim) is of practical interest. The great interest of *E. senticosus* in medicine is due to its BAS, which includes phenylpropanoids, eleutherosids, saponins, terpenoids, phenolic acids, flavonoids, organic acids, and vitamins, which determine its value [12 - 15] —the first studies on *E. senticosus* began in 1960 by A.I. Brekhman at the Institute of Biochemistry in Vladivostok. Initially, *E. senticosus* attracted the interest of researchers as a reliable and cheap substitute for *Panax ginseng*. Further studies showed that the pharmacological properties of *E. senticosus* were no less valuable and even superior to those of *Panax* [16]. Various parts (leaves, stems, fruits, roots) of *E. senticosus* are valuable medicinal raw materials, and their preparations are used to treat cardiovascular diseases, cerebral ischemia, diabetes, depression, Alzheimer's and Parkinson's diseases, etc. [17 - 21]. *E. senticosus* preparations and dietary supplements increase the body's resistance to physical exertion, help adapt to stress, normalize metabolic functions, and improve mental and physical performance [3, 6, 12, 15]. Therefore, the study and inclusion of such medicinal plants with adaptogenic and antioxidant

properties in dietology and phytotherapy are important.

Hydroponic biotechnology promotes the cultivation of medicinal raw materials of various valuable plants with high quality and productivity [22, 27]. Consequently, research on the introduction of *E. senticosus*, the development of its soilless cultivation biotechnology, and the production of high-quality and radio-safe medicinal raw materials are relevant.

MATERIALS AND METHODS

The studies were carried out from 2021 to 2024 under hydroponic (EBB and FLOW) and soil conditions (Experimental Station of the Institute of Hydroponics Problems (IHP) (Armenian Nuclear Power Plant (ANPP) zone with a radius of 30 km) in the Ararat Valley and Dilijan Forest Experimental Station (DFES) (ANPP zone with a radius of 90 km).

The subject of the study was *E. senticosus* (Fig 1). Hydroponic vegetation vessels with an area of 2 m² were used for the experiments. The soil of the IHP experimental station is semi-desert, loamy, and carbonate, with a humus content of between 1.5 and 2.5 %, and is rich in phosphorus and potassium. The DFES is located near the city of Dilijan at an altitude of 1400-1500 m above sea level. The soils of the DFES are brown, rich in humus (9.0 - 9.3 %) and potassium but poor in nitrogen and phosphorus [28]. It is important to note that environmental factors are taken into account when assessing the radioecological safety of medicinal plants in this region. Agrotechnical methods (tilling, fertilizing, loosening, regular watering, and weed control) were used to grow the soil plants, which were the study's control group. In the hydroponics, the plants were fed with Davtyan's nutrient solution (N=200 mg/L, P=65 mg/L, K=350 mg/L, pH 5.8 - 6.5, EC=1.2 - 1.3 mS/cm) twice a day [21]. Volcanic slag (red) with a diameter of 3 to 15 mm was used as a hydroponic substrate.



Figure 1: *E. senticosus* in outdoor hydroponics (a) and soils of IHP (b) and DFES (c)

During the vegetation period, samples of *E. senticosus* plant raw materials (leaf, stem, fruit, root) were collected for pharmaco-chemical studies. Each analysis was done with three replicates.

Determination of eleutherosids, phenolic compounds, and antiradical activity (ARA): Dried samples of plant material were extracted with 70 % ethanol under reflux. In the received extracts, phenolic acids (rosmarinic, gallic, caffeic, chlorogenic), total flavonoids, total eleutherosides, and antiradical activity were determined by the spectrophotometric method [12, 23 - 25] using Agilent Cary 60 UV-Vis spectrophotometer.

Total eleutherosides were expressed as eleutheroside B equivalent (wavelength: $\lambda = 266$ nm), and total flavonoids were expressed as quercetin equivalent ($\lambda = 370$ nm) [23 - 25]. Phenolic acids were determined under 277 - 330 nm wavelengths [21]. 96 % ethanol was used as a reference solution.

The ARA of leaf ethanolic extracts of *E. senticosus* was analyzed using a reduction reaction of 2,2-Diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich). The optical density of the extract solutions was determined at 517 nm. The antiradical activity of the substances in each variant was assessed by the value of the color reaction. The IC_{50} value was determined as the concentration of the extract that neutralizes 50 % of the free radicals in the solution [4, 9].

Radiochemical measurement: The gross β -radioactivity of the samples was determined by radiochemical methods using a UMF-1500 radiometer

with a low background. The obtained results were compared with the Maximum Allowed Concentration (MAC) [26 - 27, 28].

Statistical Analysis: Experimental results were presented as mean \pm standard deviation (SD) of at least three independent replicates ($n = 3$). GraphPad Prism 8 software (t-test, ANOVA) was used for statistical analyses.

RESULTS

Literature sources have shown that most BAS (polyphenols, flavonoids, anthocyanidins, vitamins, etc.) produced by plants possess antioxidant activity [6, 10] and have a protective effect on the human organism at the molecular level [7 - 8, 29]. As many authors have shown [7, 9, 11, 30], the antioxidant activity of medicinal plants was significantly correlated with their content of BAS, mainly phenolic compounds.

Therefore, an important stage of our research is determining the content of phenolic acids, total flavonoids, and eleutherosides in the extracts of various parts of *E. senticosus* and the antioxidant activity of its leaf extracts (Fig 2, 3). Analyses of extracts from leaves, stems, fruits, and roots of *E. senticosus*, cultivated in the Ararat Valley and the DFES, showed that the content of phenolic acids formed an identical descending range: chlorogenic acid > rosmarinic acid > gallic acid > caffeic acid (Fig 2). Regardless of the cultivation methods of hydroponics and soils (Ararat Valley and DFES), the various parts of *E. senticosus* formed the following range according to the content of phenolic acids: leaf > fruit > root > stem.

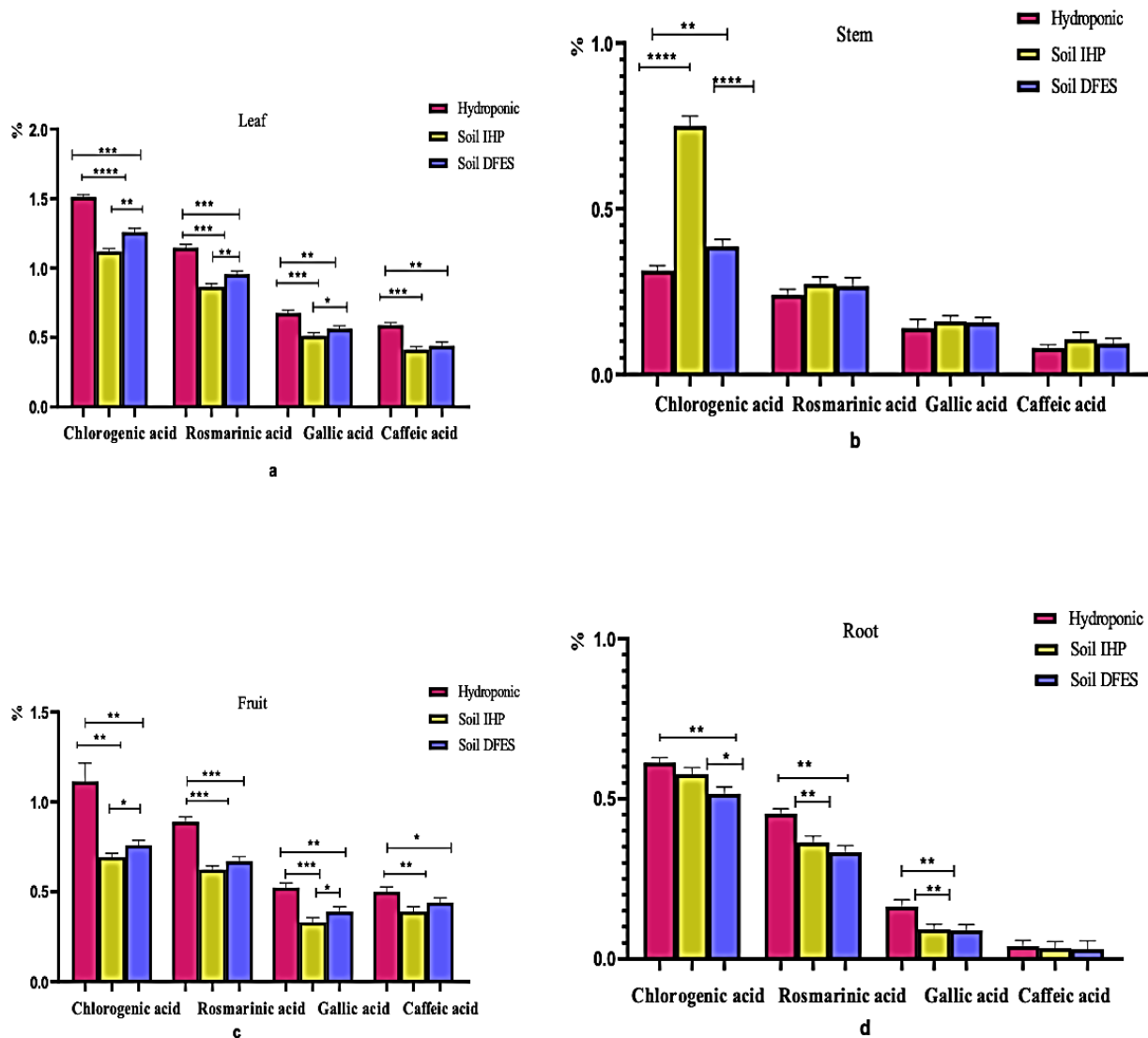


Figure 2: Phenolic acids content in leaves (a), stems (b), fruits (c), and roots (d) of *E. senticosus* under hydroponic and soil conditions (mean ± SD): *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001

The chlorogenic acid, rosmarinic acid, gallic acid, and caffeic acid contents in the leaves of all investigated variants ranged from 1.12 - 1.51 %, 0.87 - 1.15 %, 0.51 - 0.68 %, and 0.41 - 0.59 %, respectively (Fig 2 a). Leaves of hydroponic plants exceeded those of soil plants grown in the Ararat Valley and DFES in the content of chlorogenic, rosmarinic, and gallic acids by 1.3 and 1.2 times and in the content of caffeic acid by 1.4 and 1.3 times, respectively. The chlorogenic acid, rosmarinic acid, gallic acid, and caffeic acid contents in the stems of all variants ranged from 0.31 - 0.75 %, 0.24 - 0.27 %, 0.14 - 0.16 %, and 0.08 - 0.1 %, respectively (Fig 2 b). The chlorogenic acid content in the stems of soil plants grown in IHP and DFES was 2.4 and 1.9 times higher than that in the stems of hydroponic plants. The

range of chlorogenic acid, rosmarinic acid, gallic acid and caffeic acid in the fruits of all studied variants fluctuated within 0.69 - 1.1 %, 0.62 - 0.89 %, 0.33 - 0.52 % and 0.39 - 0.5 %, respectively, and in the roots: 0.52 - 0.61 %, 0.33 - 0.45 %, 0.1 - 0.16 % and 0.03 - 0.04 %, respectively (Fig 2 c, d). The content of phenolic acids in the fruits of hydroponic plants exceeded those of soil plants: in Ararat Valley chlorogenic acid exceeded 1.6 times, rosmarinic acid - 1.4 times, gallic acid - 1.6 times, caffeic acid - 1.3 times, and in DFES these phenolic acids exceeded 1.4, 1.3, 1.3 and 1.1 times, respectively (Fig 2 c). In the roots of hydroponic plants, the levels of rosmarinic and gallic acids exceeded those of the soil variant by 1.2 and 1.4 times in the Ararat Valley and by 1.4 and 1.8 times in the DFES (Fig. 2 d).

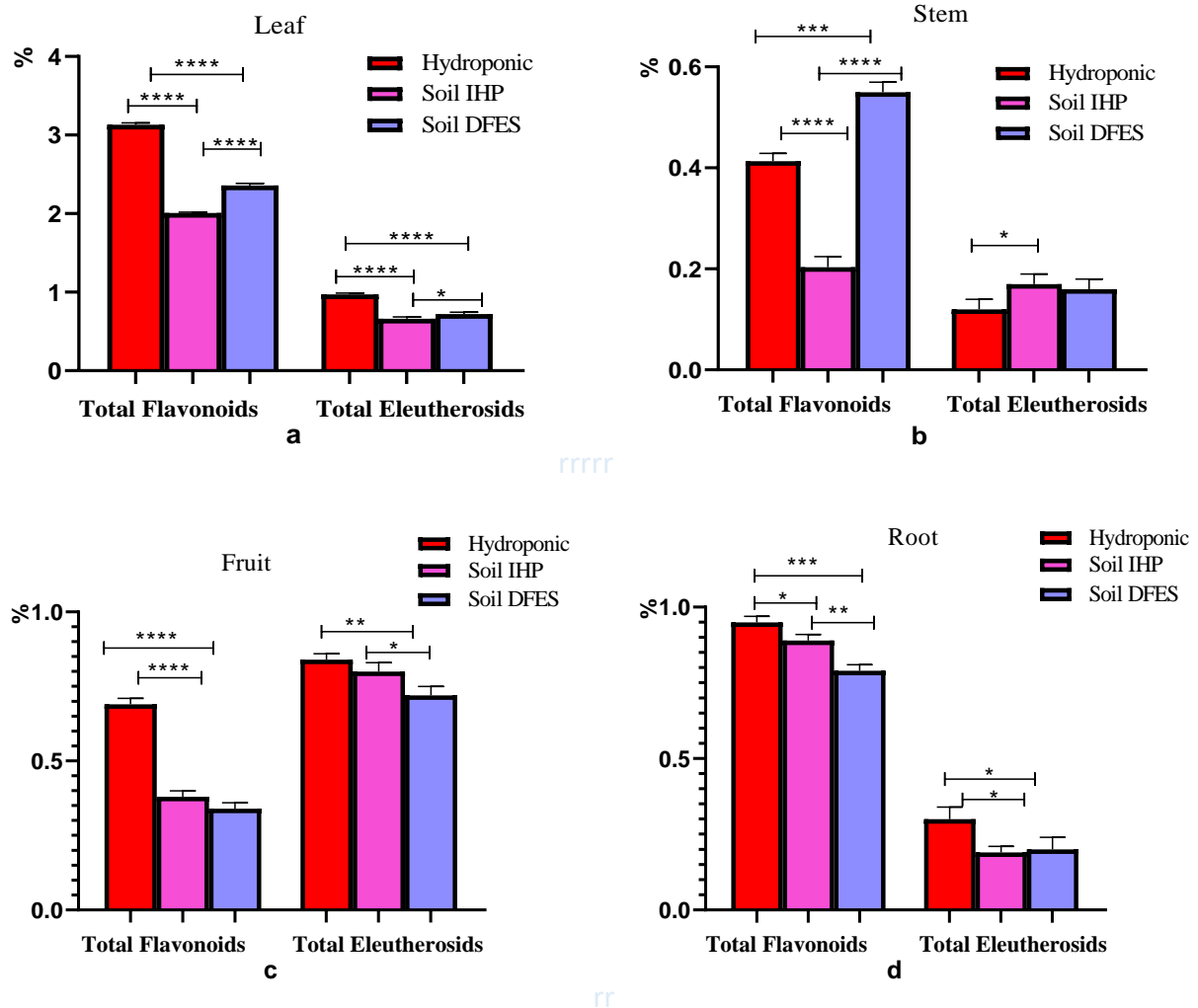


Figure 3: The content of total flavonoids and eleutherosides in leaves (a), stems (b), fruits (c), and roots (d) of *E. senticosus* under hydroponic and soil conditions (mean ± SD): *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001

According to literature data [11] and our studies [21], the dominant component of phenolic acids in *E. senticosus* was chlorogenic acid, and the highest results for phenolic acids, total flavonoids, and eleutherosides were obtained in leaf extracts. Under different growing conditions, the content of total flavonoids and total eleutherosides in medicinal raw materials of *E. senticosus* ranged from 2.01 - 3.13 % and 0.66 - 0.97 % in leaves, from 0.2 - 0.55 % and 0.12 - 0.17 % in stems, from 0.34 - 0.69 % and 0.72 - 0.84 % in fruits and from 0.79 - 0.95 % and 0.12 - 0.3 % in roots, respectively (Fig 3). The data in Figure 3 (a, c, d) show that the content of total flavonoids and eleutherosides in leaves, fruits, and roots of hydroponic plants exceeded that of soil ones in Ararat Valley and DFES by 1.5, 1.3, and 1.5, 1.3 times; 1.8, 2.0 and 2.2, 1.2 times; 1.1, 1.2 and 1.6, 1.5 times, respectively. The exception was the stems of IHP

and DFES soil plants, which exceeded the hydroponic stems in terms of eleutheroside content by 1.4 and 1.3 times, respectively (Fig 3 b). Comparing the results of our experiments with literature data [11, 15], it should be noted that regardless of the cultivation conditions, the content of the total flavonoids in the different parts of *E. senticosus* had the same sequence: leaf > root > fruit > stem. This may probably be due to the biological properties of this species. According to the article of the State Pharmacopoeia (SPH), the content of total eleutherosides in the raw materials of *E. senticosus* equivalent to eleutheroside B should not be less than 0.3 % [23]. The content of total eleutherosides in all variants of leaves, fruits, and hydroponic roots of the analyzed samples of *E. senticosus* grown in Ararat Valley and DFES meets the requirements of the SPH article.

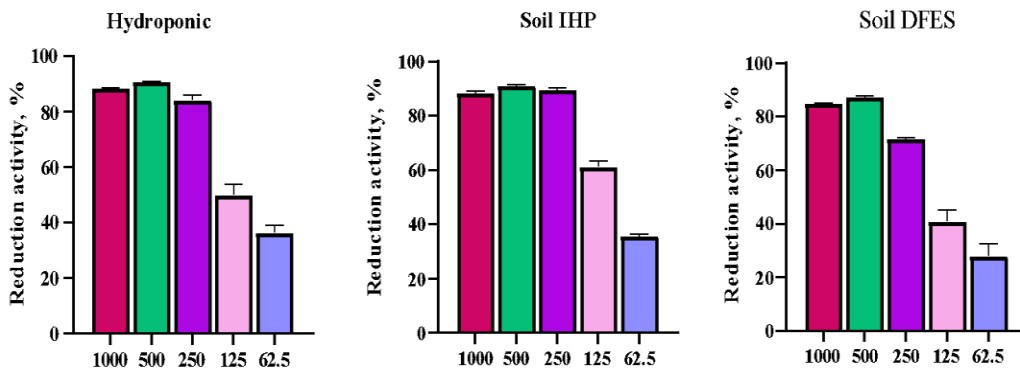


Figure 4: ARA of different concentrations of leaf extracts of *E. senticosus* grown under hydroponic and soil conditions (mean ± SD), %

The ARA of *E. senticosus* leaf extracts were determined by the DPPH reduction rate. It was found that high ARA of leaf extracts (hydroponic and soil plants: IHP, DFES) were obtained starting from a concentration of 250 µg/mL. As shown in Figure 4, the highest ARA of all variants was observed at an extract concentration of 500 µg/mL, which neutralized 87.2 - 90.9 % of free radicals in solutions. The high ARA of *E.*

senticosus leaves is probably due to the rich composition of BAS, mainly phenolic compounds.

Since the experimental station of the IHP is in the zone of technogenic influence of the ANPP (with a radius of 30 km), the analysis of the gross β-radioactivity in the various parts of *E. senticosus* grown there in outdoor hydroponics and soil conditions is of particular interest. It is important to determine the quality of the obtained medicinal raw material.

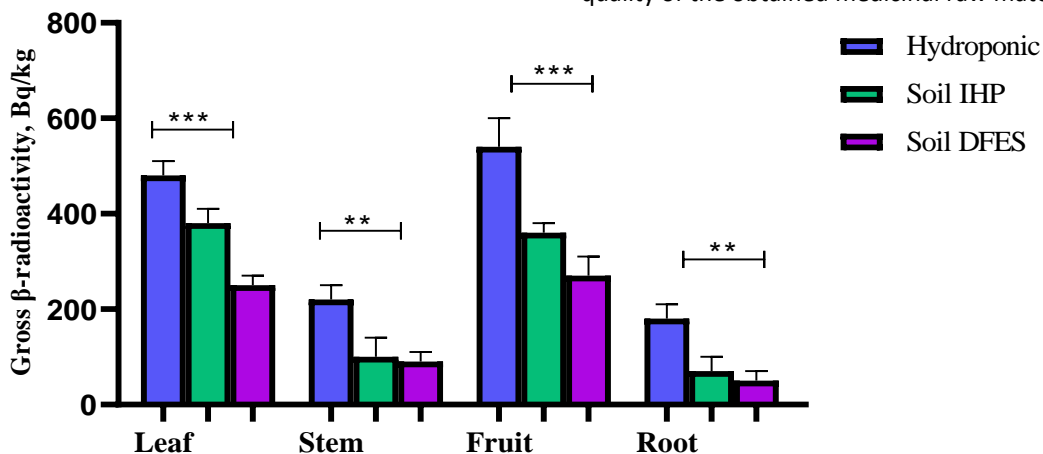


Figure 5: Gross β-radioactivity of various parts of *E. senticosus* in hydroponic and soil conditions (mean ± SD): **p < 0.01, ***p < 0.001

Radiochemical analyses showed that in the continental climatic zone of the Ararat Valley, at the same radioecological tension, the leaves, stems, fruits, and roots of *E. senticosus* plants grown hydroponically exceeded the same parts of plants grown in soil in terms of gross β-radioactivity by 1.3, 2.2, 1.5 and 2.6 times (Fig. 5). However, it is important to note that the indicators of the hydroponic samples did not exceed the MAC [26] and have no potential health implications. Regardless of the radioactive zone (IHP and DFES) and the cultivation method (hydroponic and soil), various parts of *E. senticosus* form the following

decreasing range of gross β-radioactivity: fruit > leaf > stem > root. This shows that the medicinal raw material is radio-safe, as its gross β-radioactivity does not exceed 1.0 Bq/g [26].

CONCLUSION

Pharmacochemical studies of different parts (leaf, stem, fruit, root) of *E. senticosus* revealed the highest content of BAS (total eleutherosides, total flavonoids, phenolic acids) in the leaves. As a result, the leaf extract of *E. senticosus* had high antioxidant activity and the best ARA result was recorded at an extract concentration of 500 µg/mL, where 87.2 - 90.9 % of the

free radicals in the solution were neutralized. The medicinal raw material of *E. senticosus*, containing phenylpropanoids (eleutherosides), phenolic acids, and flavonoids, is a source of antioxidant preparations, irrespective of the area and method of cultivation. The medicinal raw material of *E. senticosus* is radiosafe since the level of its gross β -radioactivity corresponds to the WHO standard of 1.0 Bq/g.

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Abbreviations: ANPP - Armenian Nuclear Power Plant; ARA- Antiradical Activity; BAS - biologically active

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substances; DFES - Dilijan Forest Experimental Station; DPPH - 2,2-Diphenyl-1-picrylhydrazyl; *E. senticosus* - *Eleutherococcus senticosus*; IHP - Institute of Hydroponics Problems; MAC - Maximum Allowed Concentration; RA - Republic of Armenia; SPH - State Pharmacopoeia; SD - standard deviation; WHO - World Health Organization.

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