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POLYPHENOL COMPOSITION OF THE FRUIT AND STEM OF ELDERFLOWER WILD-GROWING IN GEORGIA

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total phenols; anthocyanins; antioxidant activity, elderflower (Sambucus nigra); Caucasian bilberry (Vaccinium artostaphyion).

ABSTRACT
Extending the range of medicinal products produced from plant raw materials and great interest in herbal medicine are totally linked to the availability of plant raw materials, minor toxicity and side effects. The use of medicinal plant raw materials as a source of valuable biologically active substances in the pharmaceutical and food industries is relevant.

The aim of the study was to quantify and evaluate antioxidant activity of biologically active compounds (total phenols, anthocyanins) in the fruit and stem of elderflower (Sambucus nigra) and fruits of Caucasian bilberry (Vaccinium artostaphyion) wild-growing in Georgia.

As raw material under study, we used wild-growing elderflower fruit with the fruit-bearing stem and bilberry fruits collected in August 2020 in the mountainous region of Georgia (Racha-Lechkhumi) in the phase of full ripeness.

It has been confirmed that the elderflower’s fruit stems, as well as the fruit of elderflower anise and bilberry are rich in: phenolic compounds (elderflower’s fruit -13.8 mg/g, stem - 9.2 mg/g; bilberry fruit - 24.9 mg/g); anthocyanins (elderflower’s fruit - 1.4 mg / g, stem - 3.68 mg/g; bilberry fruit - 18.03 mg/g); they are characterized by high antioxidant activity (elderflower’s fruit - 48.2%, stem - 40.5%; bilberry fruit - 60.3%). Based on the results obtained, the fruits of bilberry and elderflower can be considered to be a cheap alternative source of natural antioxidants for the pharmaceutical, cosmetic and food industries.

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Introduction. In addition to their nutritional value, many fruits and berries are known to have medicinal properties and can be used as primary food supplements or to produce medications that is due to the fact that they contain compounds such as bioactive phenolic compounds (flavonoids), organic acids, vitamins and microelements and so on. They have the ability to reduce the amount of free radicals (active oxygen) generated as a result of various adverse external or internal factors in the human body, and protect the body from pathological processes such as atherosclerosis, stroke, diabetes, vascular pathologies, viral infections of various origins and viruses.

The elderflower Sambucus L., a shrub, small tree or a herbaceous perennial plant in the family Adoxaceae, spread in the temperate and subtropical zones, is known for its 40 species. In Georgia, Sambucus nigra is mostly found species. The use of elderflower in folk medicine dates back several centuries. Its fruits and flowers were used as an antipyretic, anti-inflammatory, expectorant, astringent
agent, and the tincture of its flowers and stems was used for the treatment of hemorrhoids, myositis, joint pain, burns, and wounds. Almost all the vegetative organs of elderflower contain biologically active substances; however today, the elderflower’s fruit is what is widely used for producing both foodstuffs and medications [2, 5].

Recently, a lot of work was done to determine the qualitative and quantitative content of biologically active substances. Elderflower fruits contain flavonols, anthocyanins, oxi-arsenic acid, ascorbic acid, terpenes, minerals, and phytosterols, which contribute to its high antioxidant activity [1, 4, 5, 6].

Scientists have published the results of a study which confirmed that the active compounds of elderflower have a direct effect on the virus causing influenza. In addition, biological active substances of elderflower help to increase the effectiveness of a body’s immune response to infection. These properties of elderflower are due to its active ingredients, including anthocyanin-cyanidin-3-glucoside [3].

Bilberry (Vaccinium) is an evergreen shrub that includes 100 species, including 4 species are common in Georgia: mountain bilberry (Vaccinium myrtillus), blueberry (Vaccinium uliginosum), red bilberry (Vaccinium vitis-idaea) and Caucasian whortleberry (Vaccinium vitis-idaea). The area of distribution of mountain bilberries is the subalpine belt. Bilberry fruit is rich in a number of complexes of phenolic compounds, flavonoids, tannins, anthocyanins, vitamins, especially B group vitamins, micro and macro elements: [7, 10].

Bilberry fruits and leaves are used to treat colitis, gastrointestinal problems and diabetes, and for improvement of sight. The regular use of bilberries allows improving visual acuity both in dark and under bright light, day and night.

It is also used in cooking, in many denominations of food products such as jams, various confectionery products.

The aim of the study was to determine phenolic compounds, anthocyanins and antioxidant activity in bilberry fruit (Vaccinium myrtillus), elderflower fruit (Sambucus nigra) and fruit stem wild-growing in Georgia.

Materials and Methods. Raw materials were collected in the mountainous region of Georgia (Racha-Lechkhumi), fruit - in the phase of full ripeness (second half of August).

Sample preparation for the study (determination of total phenols). Samples of mountain blueberry’s fresh fruit, elderflower’s fruit-bearing stem and fresh fruit were treated with a 80%-ethyl alcohol. The extract (sample) and gallic acid (standard) add 100 mQ with 300 mQ 0.02 mol/l of Folin-Ciocalteu reagent, allowed to settle for 8 minutes at a room temperature, then 10 ml of a 6.75%-sodium carbonate solution was added, allowing it to settle for 1 hour in dark, at a room temperature. The optical density was determined at 755 nm. We calculated the total amount of polyphenols in milligrams relative to gallic acid wet weight [8].

Determination of total monomeric anthocyanins, spectrophotometrically, by differential pH method. 5 g of mountain bilberry and elderflower’s fresh stem, fruit-bearing stem were added with an acidified 70%-ethyl alcohol in a ratio of 1:20. Extraction was carried out in water bath for 30 minutes. After decantation, it was filtered and the optical density was determined at 510 nm [9].

To determine antioxidant activity, the alcohol extracts obtained from the objects under study (fresh fruits of mountain bilberry and elderflower, the elderflower’s fruit-bearing stems) were added with 2.85 ml of newly-prepared 0.1 mol/l of diphenyl-1-picrylhydrazine solution, the mixture was settled in dark at a room temperature for 30 minutes, then the optical density was determined at the wave of 515 nm. The reference solution was a 96%-ethyl alcohol. Calculations were performed in accordance with the following formula [10].

$$\text{% of inhibition} = \frac{A - Ax}{A} \times 100\%$$

A – the optical density of the reference solution;
Ax - the optical density of the solution under study.

The optical density was determined using a spectrophotometer (EMC-31PC-UV).

Analysis results.
The content of total phenols was calculated in gallic acid equivalents (mg/g wet weight) using the gallic acid calibration curve.
Table 1. Total phenols, anthocyanins, and antioxidant activity of elderflower fruit, fruit-bearing stem and mountain bilberry fruit

<table>
<thead>
<tr>
<th>Objects under study</th>
<th>Total phenols, mg/g</th>
<th>Anthocyanins, mg/g</th>
<th>Antioxidant activity, %</th>
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</thead>
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<tr>
<td>Elderflower fruit</td>
<td>13,8</td>
<td>1,4</td>
<td>48.4</td>
</tr>
<tr>
<td>Elderflower’s fruit-bearing stem</td>
<td>9,2</td>
<td>3,68</td>
<td>40.5</td>
</tr>
<tr>
<td>Mountain bilberry fruit</td>
<td>24,9</td>
<td>18.03</td>
<td>60.3</td>
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_Fig. 1. The contents of total phenols and anthocyanins in mountain bilberry fruit, elderflower’s fruit and the fruit-bearing stem_

_Fig.2. Antioxidant activity of mountain bilberry fruit, elderflower’s fruit and the fruit-bearing stem_

_Conclusions._ The polyphenolic compositions of mountain bilberry fruit, elderflower’s fruit and the fruit-bearing stem wild-growing in Georgia were studied, and it was confirmed that:

1. The high level of antioxidant activity in mountain bilberry fruit is due to the significant amount of total phenols and anthocyanins.
2. The fruit-bearing stems of elderflower, as well as fruit are characterized by high antioxidant activity, it is possible to collect elderflower fruits together with the fruit-bearing stems.
Due to synergistic property of antioxidants, as well as the coincidence of habitats of the objects under study and the ripening phases of fruit, it is possible to mix them together to obtain the extract with higher antioxidant activity as a cheap, natural alternative source for pharmaceutical, cosmetic and food industries.

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