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DETERMINATION OF TOTAL PHENOLS, ANTHOCYANINS AND ANTIOXIDANT ACTIVITY IN THE BUTCHER'S BROOM (*Ruscus aculeatus L*) RIPE FRUIT

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The Butcher's Broom Fruit; Total Phenols; Anthocyanins; Antioxidant Activity.

ABSTRACT

The useful properties of medicinal plants are due to the presence of various biologically active substances in them, such as phenolic compounds, alkaloids, anthocyanins, glycosides, saponins, essential oils, resins, mucus, vitamins and so on, which have a certain effect on physiological processes in the human body. The object of the study was the the butcher's broom fruit growing wildly in Georgia, collected in August-September upon full maturation. Our research confirms that the alcoholic extract of the butcher's broom fruit contains biologically active substances (total phenols - 39 , 4 mg/g; anthocyanins - 39.4 mg/g), which leads to the high antioxidant activity of the said extract - 45% (F=10).

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Introduction.

Plant raw materials are among the sources medicines production.

The useful properties of medicinal plants are due to the presence of various biologically active substances in them, such as phenolic compounds, alkaloids, anthocyanins, glycosides, saponins, essential oils, resins, mucus, vitamins and so on, which have a certain effect on physiological processes in the human body.

Butcher's broom is an evergreen, bushy plant up to 20-60 cm in the Asparagaceae family.

The most attractive part of the plant is the round-shaped, 8-13 mm diameter, red, fleshy, one- or two-seeded fruit.

Anthocyanins are the dominant compounds in the butcher's broom fruit [1].

Anthocyanins are the flavin cation products (2-phenyl benzopyrylium), and important pigments and they cause the color of the plant flowers, fruits, leaves (blue, violet, red, brown, etc.), and these properties are used to obtain natural dyes [2, 3, 4].

The studies have established that the high antioxidant activity of plant extracts is due to the content of anthocyanins [5]. The maximum antioxidant activity of anthocyanins manifests itself at neutral pH. Delphinidin and its anthocyanin delphinidin 3-rutinoside [5], as well as delphinidin 3-glycoside, delphinidin 3-rutinoside and cyanidin 3-glycoside are characterized by higher antioxidant activity among anthocyanins and anthocyanidins found in plants [6].

The biosynthesis of a significant amount of anthocyanins in the butcher's broom fruit makes it possible to use them as components in food, cosmetic and pharmaceutical products.

The above-ground parts of the butcher's broom are traditionally used as a diuretic [7], the alcoholic extract of the butcher's broom roots has anti-inflammatory properties [8], reduces capillary permeability, has a vasoconstrictive effect on peripheral blood vessels, relieves pain in the leg muscle, heaviness and cramping spasms, as well as itching and swelling. [9,10]. It is also characterized by cytostatic action [11].

Substances and methods.

The object of the study was the the butcher's broom fruit growing wildly in Georgia, collected in August-September upon full maturation.

Total phenol content was determined by Folin-Ciocalteu reagent, using the spectrophotometric method. The sample taken for analysis was extracted with 80%-ethyl alcohol. We placed 1 ml taken from the total volume of the extract in a 25-ml volumetric flask, added 5 ml of H_2O, 1 ml of Folin-Ciocalteu, allowed to remain at room temperature for 8 minutes, then we added 10 mL of 7% Na2CO3, filled the flask with H_2O, allowed it to remain in the dark at room temperature for 2 hours. We made a determination at a wavelength of 750 nm. As a control, we took 1 ml of the corresponding extractant. We recalculated the obtained data on the gallic acid calibration curve [13].

The total phenol content was calculated using the formula:

$$X = (D K V F) \cdot 1000 /m,$$

where X – total phenol content, mg/kg;
D – optical density;
K – gallic acid conversion factor;
F – dilution factor;
V – total volume of extract, ml;
m - the mass of raw materials taken for extraction, g.

We determined the total monomeric anthocyanins by the pH-differential method. We took 5 g of the analytical sample and extracted it with 45%-ethyl alcohol. The volume of extract was brought to 100 ml. From the total volume of extract, we took 1-1 ml of extract in two test tubes and added 4-4 ml of buffer solutions. In one test tube, we added 0.025 M potassium chloride, and in 0.4 M sodium acetate we added to the another one, after 20 min we determined the optical density of the analytical solutions at 520 nm and 700 nm wavelengths [14].

The amount of monomeric anthocyanins was calculated using the following formula:

$$X = A \cdot MW \cdot DF \cdot 103 / \varepsilon \cdot L$$

where A is the total absorption index, and it was calculated using the following formula:

$$A=(A520 – A700)\ pH1{,}0 – (A520 – A700)\ pH4.5$$

MW – 449,2 g/mole (mass of cyanidin-3-glucoside);
DF – dilution factor;
E – 2690 molar extinction coefficient;
L – length of cuvette;
X– anthocyanin pigments.

In order to determine the antioxidant activity, we used the colorimetric DPPH method of analysis, added 2.85 ml of newly-prepared 0.1 mole/l 2,2-diphenyl-1-picrylhydrazine solution to the alcoholic extract obtained from the object of study (butcher's broom fruit), left the mixture in the dark at room temperature for 30 minutes, determined the optical density at 515 nm wavelength. The reference solution was 96%-ethyl alcohol. We made the calculation using the formula [12].

$$\% \text{ inhibition} = \frac{A-Ax}{A} \text{x100 \%}$$

A – optical density of the reference solution;
A_x- optical density of the test solution.
These indicators were determined using a spectrophotometer (EMC-31PC-UV).

Results and discussion.
The results of the study are presented in Table 1

Table 1. Total phenols, anthocyanins and antioxidant activity of the butcher's broom ripe fruit extracts.

Study object	Total phenols, mg/g	Anthocyanins, mg/g	Antioxidant activity %, F=10
Butcher's broom ripe fruit	39,4	73,6	45

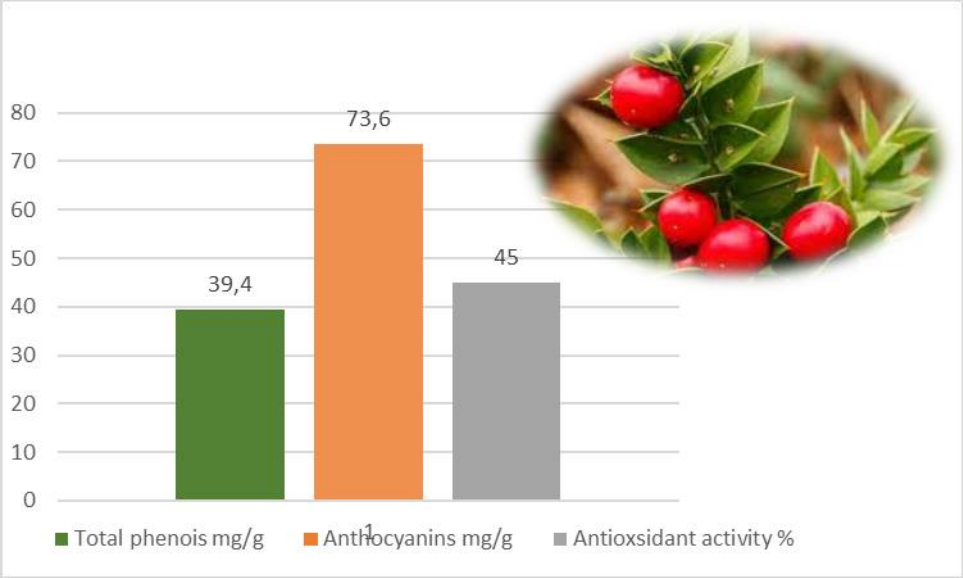

Fig. 1. Total phenols, anthocyanins and antioxidant activity of the butcher's broom ripe fruit extracts.

Conclusions.
Since the middle ages, the aboveground and underground parts of the butcher's broom (R. Aculeatus) were the plant raw materials with medicinal properties, which were widely used to treat various diseases of the venous system. Our research confirms that the alcoholic extract of the butcher's broom fruit contains biologically active substances (total phenols - 39 , 4 mg/g; anthocyanins - 39.4 mg/g), which leads to the high antioxidant activity of the said extract - 45% (F=10) and therefore increases the possibilities of using the said plant in pharmacy and food technology.

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