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THE CONTENT OF PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY OF THE BUTCHER'S BROOM PLANT (*RUSCUS ACULEATUS* L)

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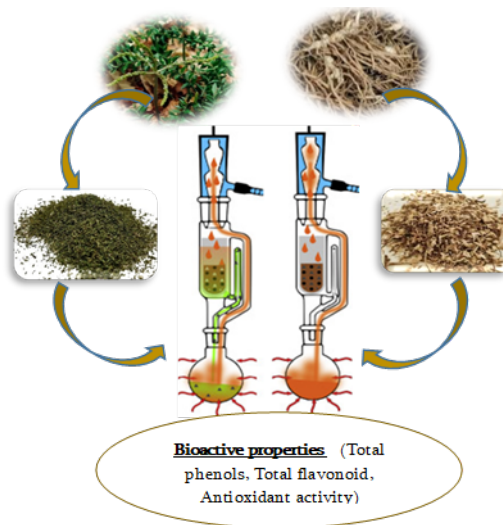
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Total Phenols, Total Flavonoids, Antioxidant Activity, Butcher's Broom Root, Butcher's Broom Herbs.

ABSTRACT

The nature of medicinal products of plant origin is determined by the content of various active components, which makes it possible to use them as therapeutic excipients in traditional medicine.

The objects of the research were the roots and the ground part of butcher's broom wild-growing in Georgia, in the active phase of vegetation: root - in November, the ground part (stem and leaves) in May. The 70% and 40% ethanol alcoholic extracts from root and herbs were prepared, 26.6 - 60.4 mg/g of total phenols were found in all four extracts; the content of flavonoids was 9.2 - 21.2 mg/g and the antioxidant activity was evaluated by the DPPH method at 24.3 - 68.2%.



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Introduction.

The nature of medicines of plant origin is determined by the content of various active components, and the potential of these components in each plant determines the possibility of their use as therapeutic excipients in traditional medicine [1]. According to the World Health Organization, wild-growing or cultivated species are used as therapeutic remedies in both traditional/folk medicine and complementary medicine [2].

Butcher's broom (*Ruscus aculeatus L.*) is an evergreen bushy plant of the Asparagus family up to 20-60 cm high.

The studies have established that the roots of butcher's broom contain steroidal saponins - ruscosides: aglycone - an isomer of ruscogenin or its neoruscogenin, which stipulates its pharmacological effect. Other components are also isolated from the plant, including sterols, triterpenes, flavonoids, coumarins, sparteine, tyramine, glycolic acid, carbohydrates (rhamnose, glucose, arabinose); proteins, sucrose, trace elements (magnesium, aluminum, potassium, iron etc.); vitamins PP and C, plant fiber. The highest concentration of ruscogenins is found in runners [3, 4, 5, 17].

According to literary, the content of ruscogenin in the ground parts of butcher's broom is different: according to Turkish researchers, its content ranges from 0.03 to 1.48% [6,7]. In stems and leaves of *R. aculeatus*, p-coumaric acid and quercetin have been identified [8,9].

The ground parts of butcher's broom are traditionally used as a diuretic [10], the alcoholic extract of butcher's broom roots has anti-inflammatory properties [11], reduces capillary permeability, has a vasoconstrictor effect on peripheral blood vessels, relieves pain in the muscles of the leg, heaviness and cramping spasms, as well as itchiness and swelling. [9, 12]. It is characterized by a cytostatic effect [13].

It is used in the case of blood circulation disorders, gout, hemorrhoids, arthritis, diabetes (prevention of ophthalmological complications), as well as decreased activity of kidneys and gall bladder [14].

Studies have established a correlation between the content of total phenolic compounds and antioxidant activity of plant extracts [9, 15, 16].

Antiseptic, anti-inflammatory, antimicrobial and skin cell regenerating properties of butcher's broom extract are widely used in cosmetology.

The herbal remedy has a positive effect on eye contour care, in the fight against cellulite and stretch marks. When used, blood circulation and metabolic processes are activated, harmful substances are released and cell regeneration is stimulated. It is recommended to put certain combinations together with other plants to enhance the effect, for example with extracts of ivy, chamomile and other plants [20].

Substances and methods

We collected samples of butcher's broom growing wildly in the Imereti region of western Georgia. The ground parts of the plant were collected in the month of May 2022, the underground part (root with runners) in November. The ground and underground parts of the plant were dried at 20 °C before use and stored in paper boxes.

Extraction from finely crushed plant raw materials was made with a 70% ethanol (C₂H₅OH) in a ratio of 1:7, and a 40%-ethanol (C₂H₅OH) in a ratio of 1:7. The extractant was evaporated using a vacuum evaporator. Dry extracts were obtained:

1. From herbs *R. Aculeatus* (RAH) 70%
2. From runner *R. aculeatus* (RAR) 70%
3. From herbs *R. Aculeatus* (RAH) 40%
4. From runner *R. aculeatus* (RAR) 40%

The obtained extracts were dissolved in water. To the extracts of the herbs (RAH) (40, 70%), , we added CH₂Cl₂ (dichloromethane) to remove chlorophyll, and the aqueous fraction was separated. Then, we determined the content of phenolic compounds, total flavonoids and antioxidant activity in all four aqueous extracts.

Determination of the amount of total phenols using the Folin-Ciocalteu reagent - the amount of total phenols was determined by the spectrophotometric method of Folin-Ciocalteu. 1 milliliter taken from the volume of aqueous extracts, we placed in a 25-ml volumetric flask, then we added 5 ml H₂O, 1 ml of Folin-Ciocalteu reagent, retained for 8 minutes at room temperature, then we added 10 ml of 7% Na₂CO₃, retained for 2 hours in the dark, at room temperature. It was determined at a wavelength of 750 nm [18, 19].

We recalculated the data obtained as a result of determination on the calibration curve of gallic acid.

The content of total phenols is calculated by the formula:

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

where: X - total content of phenols, mg/kg;

D - optical density;

K – a conversion factor in gallic acid;

F – dilution factor;

V – total volume of extract, ml;

m - mass of raw materials taken for extraction, g.

Quantification of total flavonoids with $AlCl_3$ reagent, using a spectral method: 1 ml taken from the total volume of the extract was placed in a 10-ml flask, then we added 5 ml H_2O , 0.3 ml of 5% $NaNO_2$, retained for 5 minutes, then we added 0.3 ml of 10% $AlCl_3$, retained for 6 min, then we added 2 mL of 1N $NaOH$ and determined at a wavelength of 510 nm. As a control, we took 1 ml of the corresponding extractant. We recalculated the data obtained as a result of the determination on the calibration curve of the rutin.

The content of total flavonoids is calculated by the formula:

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

where: X - total content of flavonoids, mg/kg;

D - optical density;

K – a conversion factor in rutin;

F – dilution factor;

V – total volume of extract, ml;

m - mass of raw materials taken for extraction, g [23].

The DPPH method for determining antioxidant activity represents a stable free radical with maximum absorption at 515 - 517 nm, whose methanolic extract's purplish violet color changes to light yellow as a result of recovery.

Spectrophotometric determination of the optical density of the sample was performed at a wavelength of 515 nm. The DPPH solution is a control solution, and a 96%-ethyl alcohol is a background.

Inhibition of free radical (DPPH) activity is calculated by the following formula: $In \% = A_C - A_S / A_C \cdot 100$, where A_C - absorption of DPPH alcoholic solution, and A_S - absorption of analyzed extract. [18, 19].

Table 1. Total phenols, flavonoids and antioxidant activity of butcher's broom herbs and root extracts.

Objects under study	Total phenols, mg/g	Total flavonoid mg/g	Antioxidant activity, %
(RAH) 70%	39,2	13,4	34,6
(RAR) 70%	60,4	21,2	68,2
(RAH) 40%	26,6	9,2	24,3
(RAR) 40%	43,7	13,9	53,2

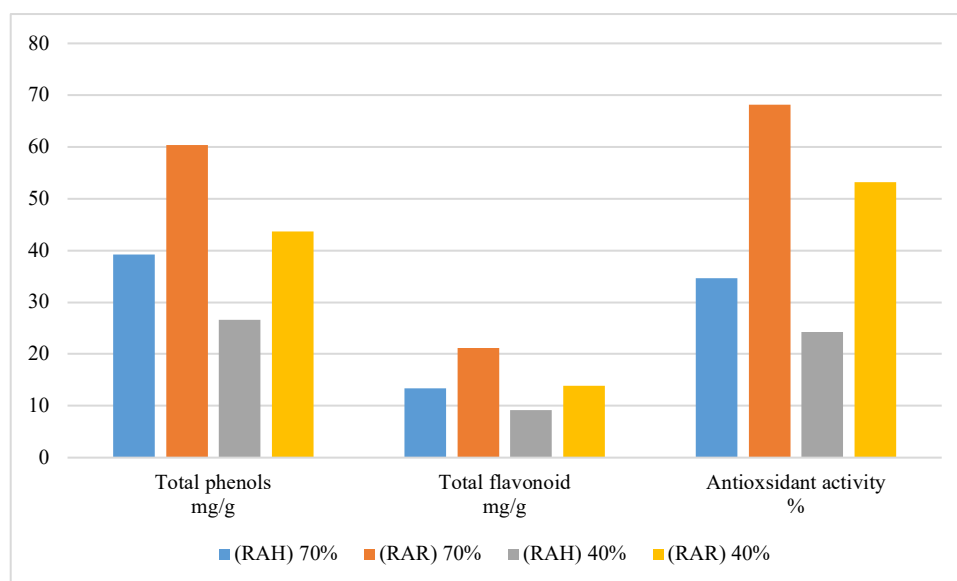


Fig.1. Total phenols, flavonoids and antioxidant activity of butcher's broom herbs and root extracts.

Conclusion.

The polyphenolic composition and antioxidant activity of butcher's broom herbs and root growing in the territory of western Georgia, were investigated. It was proved that:

1. All four extracts of the butcher's broom plant (herbs, root) contain significant amounts of both common phenols and flavonoids, which leads to a high level of antioxidant activity.
2. In a 70%-alcoholic extract of the herbs and root of the butcher's broom plant, the content of total phenols and flavonoids is twice as high as in a 40%-alcoholic extract.
3. Due to the synergistic quality of antioxidants, it is possible to mix the extracts together to obtain an extract with higher antioxidant activity and use it as a cheap, natural alternative source for the pharmaceutical and cosmetic industries.

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