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Plant Physiology

Content of Active Metabolites in some Species of Mosses of Georgia

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Content of active metabolites: ascorbic acid, tocopherol, proline and soluble phenols, as well as total proteins, soluble carbohydrates and nitrates, and peroxidase and total antioxidant activity in three species of mosses: *Anomodon* viticulosus (Hedw.) Hook. et Tayl, *Hypnum cupressiforme* Hedw. and Leucodon sciuroides Schwägr., widely spread in Georgia, has been investigated for the first time. Amount of ascorbic acid and tocopherol was statistically similar in all three species. *Hypnum cupressiforme* was distinguished by the content of soluble phenols, proline and carbohydrates. Experimental results contain interesting information from pharmaceutical point of view and should be taken into account while appreciating the medicinal value of the studied plants. *© 2020 Bull. Georg. Natl. Acad. Sci.*

Active metabolites, antioxidants, mosses

Interest in medical plants is stipulated by the presence of various biological active substances in their organs. Drugs prepared on the base of natural raw material gain more and more popularity, as more effective, with fewer side effects. This fact is supported with real numbers: 22% of new drugs are from natural products, i.e. is semi synthetic; 4% are purely of natural origin and only 13% are fully synthetic [1]. Vascular plants are regarded to be well studied taxon from pharmaceutical and pharmacological points of view. Mosses – evolutionary older plants, paid the scientists' attention later, after the biologically active compounds with multifaceted activity were discovered in their organs [2]. Different class active metabolites revealed in mosses possess antibacterial, antifungal, cytotoxic, antitumor and insecticidal activity [3-5]. Some representatives of mosses have been widely used for treatment of different diseases in the east medicine from ancient [6].

The qualitative and quantitative composition of the biologically active compounds of mosses growing in Georgia is actually undiscovered. Accordingly, the purpose of the surveys was the quantitative investigation of biologically active compounds, among them of antioxidants, in widely spread in our country species of mosses.

Species of mosses *Anomodon viticulosus* (Hedw.) Hook. et Tayl, *Hypnum cupressiforme* Hedw., and *Leucodon sciuroides* Schwägr. were selected as test-objects. Plant material was supplied and identified by the specialists of the department of lower plants of the Institute of Botany of Ilia state University. Voucher material of studied species is stored in the herbarium of the Institute of Botany. Activity of peroxidase, content of low molecular antioxidants: ascorbic acid, tocopherol, proline and total phenols, as well as proteins, soluble carbohydrates and nitrates, and the total antioxidant activity have been studied.

Content of ascorbic acid was determined by the titration method with 0.001 M solution of dichlorphenolindophenol [7].

Content of tocopherol was studied spectrophotometrically at 470 nm (SPEKOL 11, KARL ZEISS, Germany) [8].

Proline was investigated after Bates at al. spectrophotometerically at 520 nm [9].

Soluble phenols were determined using Folin-Ciocalteu reagent. Optical density was measured at 765 nm [10].

The content of proteins was studied after Lowry [11].

Soluble carbohydrates were tested with anthrone reagent at 620 nm with a spectrophotometer (SPECOL 11, KARL ZEISS, Germany) [12].

Activity of peroxidase was determined spectrophotometricaly, using guaiacole. Optical density of guaiacole oxidized products was measured at wavelength of 470 nm over a period of 2 min [7]. Results are given in conditional units per one gram of fresh weight.

Nitrates were discovered with disulphophenolic acid. Optical density was determined at 410 nm [13].

The total antioxidant activity of leaves was determined using the modified DPPH (diphenylpicrylhydrazine) method [14]: to 0.01 ml of the test-extract was added 40 μM solution of DPPH and after 30 min of incubation in dark the optical density was measured at 515 nm. The percent of inhibition was calculated.

Mean values of three biological replicates and their standard deviations are given in the Table.

According to obtained results it is clear that studied species of mosses are quite rich in ascorbic acid (Table). Content of this substance in all three species was statistically similar, as well as of the vitamin E (tocopherol) ($p<0.05$).

Ascorbic acid is a very significant substance taking an active part in many metabolic processes. It is an antioxidant which protects organism against the damage with free radicals. As a medicine it is used in cases of different diseases and disorders [15]. Ascorbic acid is associated with the reduction of vitamin E. The latter protects cell membrane from damage [16].

Table. Content of ascorbic acid, tocopherol, soluble phenols, proline, total proteins, soluble carbohydrates, nitrates, and peroxidase and antioxidant activity in mosses

Species	Ascor. acid, mg/%	Tocoph. mg/g	Soluble phenols, mg/g	Proline, μg/g	Soluble carbohydr. mg/g	Peroxidase, per 1g of fresh matter	Total proteins. mg/g	Nitrates, mg/100g	Antioxidant activity, % of inhibition
Anomodon viticulosus	33.56±1.7	1.6±0.1	5.55±0.3	72.2±3.8	32.1±1.6	1.0±0.05	80.39±4.0	1.1±0.1	7.8±0.4
Hypnum cupressi-forme	43.42±2.2	1.4±0.1	11.3±0.6	211.96±6,4	40.6±2.0	2.39±0.1	90.54±4.5	0.9±0.04	7.2±0.4
Leucodon sciuroides	39.48±2.0	1.0±0.05	6.87±0.3	94.4±4.7	17.32±0.9	2.89±0.1	45.05±2.2	1.3±0.1	7.4±0.4

These data are probably acceptable while studying the medical value of these species.

Hypnum cupressiforme distinguished with the high content of phenols among tested species. This index was almost twice higher compared to other results (Table).

Phenolic substances are popular group of metabolites, widely spread in plant kingdom. Their positive role for health has been substantiated many times [17]. Thus, the high content of phenols in *Hypnum cupressiforme* adds one more positive point to its medical value.

Hypnum cupressiforme prevailed the studied species of mosses by the content of proline and soluble carbohydrates as well.

Proline plays a role of metabolic signal by means of modulation of responses to biotic or abiotic stresses, which regulates the metabolic pull and expression of great number of genes, effecting this way plant's growth and development [18]. In humans amino acid proline is irreplaceable in collagen and cartilage formation. It keeps muscles and joints flexible.

Soluble sugars reveal a dual activity with respect to reactive oxygen species (ROS). They can be involved in ROS-producing metabolic pathways, as well as and can feed NADPH-producing metabolic pathways, such as the oxidative pentose-phosphate (OPP) pathway, which can contribute to ROS scavenging [19]. Thus, received data on proline and carbohydrates content in *Hypnum cupressiforme* are definitely important in case of its medicinal use.

Low activity of the enzyme peroxidase was mentioned in leaves of *Anomodon viticulosus* compared to other tested ones.

Peroxidases (EC 1.11.1) form a large group of enzymes, which play multiple functions in cell metabolism [20]. In particular, they neutralize the hydrogen peroxide formed under the stress conditions, as well as regulate content of phenols and auxins, take part in plant growth and development, etc. [21].

High content of total proteins was mentioned in *Anomodon viticulosus* and *Hypnum cupressiforme* (Table).

Proteins may serve as excellent antioxidant additives in food, because of their ability to inhibit lipid oxidation by means of reactive oxygen species inactivation, scavenging free radicals or chelates formation [22]. Accordingly, quantitative investigation of total proteins in experimental plants was interesting from this point of view. Content of nitrates was statistically identical in all tested species of mosses (Table). Since the content of antioxidant substances in experimental species was not low, the total antioxidant activity of the plant material appeared to be significantly low (Table), which is difficult to explain.

Summarizing the obtained results it may be concluded that *Hypnum cupressiforme* distinguished by the high content of most studied active metabolites among the three tested species of mosses. Experimental results may be taken into account while appreciating the pharmaceutical value of studied plants.

მცენარეთა ფიზიოლოგია

აქტიური მეტაბოლიტების შემცველობა საქართველოს ხავსების ზოგიერთ სახეობაში

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პირველადაა შესწავლილი აქტიური მეტაბოლიტების: ასკორბინის მჟავას, ტოკოფეროლის, პროლინის, ჯამური ფენოლების, საერთო ცილების, ხსნადი ნახშირწყლებისა და ნიტრატების შემცველობა, აგრეთვე ფერმენტ პეროქსიდაზასა და ჯამური ანტიოქსიდანტური აქტივობა საქართველოში გავრცელებული ხავსის სახეობებში: *Anomodon viticulosus* (Hedw.) Hook. et Tayl, *Hypnum cupressiforme* Hedw. და *Leucodon sciuroides* Schwägr. ასკორბინის მჟავასა და ტოკოფეროლის შემცველობა სამივე შესწავლილ სახეობაში სტატისტიკურად მსგავსი აღმოჩნდა. ჯამური ფენოლების, პროლინისა და ხსნადი ნახშირწყლების შემცველობით გამოირჩეოდა *Hypnum cupressiforme*. მიღებული შედეგები ყურადსაღებია და გათვალისწინებულ უნდა იქნეს ხავსების შესწავლილი სახეობების სამედიცინო მიზნით გამოყენების შემთხვევაში.

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