

INVESTIGATION OF AMINO ACID COMPOSITION  
OF SOME MOSSES GATHERED FROM ARMENIA

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Bryophytes are rich in a variety of secondary biological active compounds, thus provide a great potential for biotechnological and biopharmaceutical applications. Bryophytes contain potentially useful natural products, including polysaccharides, terpenoids, lipids, amino acids and phenylpropanoids. Bryophytes isolated compounds and extracts have cytotoxic, antimicrobial activities. The result of our research indicate the presence in the extracts of bryophytes collected in Armenia, the entire spectrum of amino acids, especially essential ones. Also revealed in some species a certain amount of nitrogen – 840 mg/g, which indicates a more intensive synthesis of non-essential amino acids. The results obtained can be used in medicine, pharmacology.

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**Keywords:** bryophytes, amino acid, nitrogen.

**Introduction.** Medical plants play an important role in health care and medicine, as a treatment for both traditional and alternative medicine. World Health Organization data indicate that approximately 80% of the world's population use plants as a treatment for various diseases [1–3].

Bryophytes considered the first land plants, are a group of over 24 000 species worldwide. They have been used since ancient times for the treatment of diseases such as tuberculosis, pneumonia, burns, seizures, snakebites, as an antibacterial agent etc. [4, 5]. Interest in bryophytes increases with the discovery of a large number of biologically active compounds that have a positive effect in the treatment of tumors of various etiologies [1, 6], have antibacterial potential [7, 8]. The study of biologically active compounds (polysaccharides, amino acids), as a well as antioxidant potential, will expand the range of use of these plants as an affordable alternative to existing drugs. Herbal medicines are a viable, safe and very rarely have side effects. They contain organic compounds that have important physiological effects on the human body. These substances include tannins, alkaloids, carbohydrates, amino acids, terpenoids, steroids, flavonoids and phenolic compounds [8, 9].

Bryophytes are subdivided into 3 groups: anthocyanins, liverworts and green mosses. These plants are used in the treatment of various diseases. Of the greatest interest are bryophytes in recent years antibacterial, antifungal, antiviral and

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antitumor properties of these plants have been discovered. The antitumor effect of bryophytes is due to the activation of genetic and biological pathways that lead to apoptosis and necrosis [7].

In plant organisms amino acid derivatives amides and betaines perform important functions. Asparagine and glutamine, glycolbetaine are well studied. Asparagine and glutamine are involved in the formation of a protein molecule, are important metabolites, due to which are synthesis of all nitrogenous bases, alkaloids, some lipids, vitamins, chlorophyll, phytohormones (auxins, cytokinins) of plant organisms. Plants and microorganisms can synthesize all the necessary amino acids from other organic compounds in contrast to humans and animals, the body of which is not able to synthesize some proteinogenic amino acids. These amino acids are called essential (lysine, tryptophan, methionine, threonine, leucine, valine, isoleucine, phenylalanine) and they must be ingested with food. In the organism, with a lack of amino acids, protein synthesis is inhibited, with can be the cause of severe pathologies [10].

In plants the content of free amino acids depends on the organ or tissue, age, environmental conditions, and especially on the course of biochemical processes (synthesis of proteins, nucleic acids and some nitrogenous compounds). The concentration of some amino acids can increase due to metabolic disorders in the body, also under stress. For example, with a lack of water in plant cells, an accumulation of proline occurs, and with an excess of ammonium substances, an accumulation of asparagine, glutamine and arginine [5].

Bryophytes are a rich reservoir of biological active compounds such as terpenoids, flavonoids, alkaloids, glycosides, saponins, anthroquinons, sterols and other aromatic compounds. They also possess anticancer and antimicrobial activity due to their unique chemical constituents. In the present investigation on Phytochemicals and antimicrobial activity of Bryophytes [3]. Amino acids readily interact with reducing sugars to form melanoidins (dark colored compounds). During the enzymatic oxidation of tyrosine and phenylalanine, melanin is formed and interacting with atmospheric oxygen, from darkening on peeled potato roots. Melanins are synthesized from pyrochatechol and dioxyphenylalanine (DOPA) [4].

In this paper we study the amino acid composition of bryophytes common in Armenia for use in medicine and pharmacology.

#### **Materials and Methods.**

**Plant Materials.** The bryophytes viz *Mniaceae*, *Mnium spinosum* (Voit) Schwaegr, *Plagiomnium cuspidatum* (Hedw), *Brachytheciaceae*, *Brachythecium salebrosum* (Web. et Mohr) B.S.G., *Thuidiaceae*, *Thuidium recognitum* (Hedw) Lindb, *Dicranaceae*, *Dicranum scoparium* (Hedw), *Anomontaceae*, *Anomodon viticulosus* (Hedw), *Amblystegiaceae*, *Drepanocladus aduncus* (Hedw) were collected from Armenia (at a height of ~1450 m). The plants were identified by Dr. A. Poghosyan (Department of Botany and Mycology, Yerevan State University, Armenia) and deposited in the Takhtadjyan Herbarium of the Department of Botany and Mycology, YSU (Vouchers no. 13450, 13451, 13452 and 13453, 13454, 13456, 13457, respectively).

Air dried and powdered mosses (0.5 g) were hydrolyzed with 5 mL 6 N HCl (2 h, 105°C). The precipitate was placed in an evaporation dish (washed until neutral pH).

**Determination the Amino Acid Composition.** The amino acid composition of mosses was determined by chromatography on paper. For this, the concentrated aqueous solution was evaporated to dryness and washed of quantitatively with 10 mL 70% ethanol solution. The 0.03 mL obtained extract was applied with a micropipette on chromatographic paper FN-6 (Germany) and chromatographed by the descending method in the solvent system *n*-butanol–acetic acid–water (4 : 1 : 5) for 16 h. The amino acid zones were detected with a 0.2% freshly prepared ethanol solution of ninhydrina, followed by heating the chromatogram in an oven at 60°C for 30 min [9]. The amino acids were used as control see Tab. 1.

The quantitative determination of amino acids in the extracts of the studied mosses was carried out by the spectrophotometric method. It is based on the measurement of the optical density of diketonehydrindeliketonehydramine (DUDA), a copper derivative. The quantitative trace of the amino acid and the optical density of its color are in a linear and relationship within the range of 0.025–0.25  $\mu\text{mol}$ . In a weakly acidic medium as a result of the interaction of ninhydrina with an amino acid, the resulting blue DUDA transforms into a absorption spectrum of which is 530 nm [10].

**Results and Discussion.** We investigated the qualitative and quantitative amino acid composition amino acid of mosses common in Armenia. During the hydrolysis of proteins, all the amino acids presented by use were found. The results are given in Tab. 1.

Table 1

Amino acid composition of some mosses common in Armenia  
(amino acids in 100 g by dry biomass,  $n=5$ ,  $p<0.05$ )

Amino acid	<i>A. viticulosus</i>		<i>P. cuspidatum</i>		<i>D. aduncus</i>		<i>M. spinosum</i>		<i>B. salebrosum</i>		<i>T. recognitum</i>		<i>D. scoparium</i>	
	mg	%	mg	%	mg	%	mg	%	mg	%	mg	%	mg	%
<i>cys</i>	1.60±0.1	2.2	0.42±0.2	0.4	0.60±0.01	0.5	–	–	0.72±0.01	0.8	0.62±0.01	0.7	–	–
<i>lys</i>	3.20±0.01	4.5	4.41±0.01	4.8	5.08±0.02	4.9	2.88±0.04	4.9	4.71±0.01	5.3	4.60±0.03	5.4	3.28±0.01	5.2
<i>his</i>	3.78±0.2	5.4	2.82±0.01	3.1	5.40±0.03	5.2	0.76±0.01	1.3	3.12±0.1	3.5	3.01±0.01	3.5	11.6±0.03	1.8
<i>arg</i>	1.40±0.03	2.0	2.06±0.02	2.2	4.88±0.05	4.7	–	–	2.36±0.2	2.7	3.25±0.02	3.8	–	–
<i>asp</i>	2.04±0.01	2.9	7.42±0.03	8.2	8.48±0.10	8.2	3.18±0.01	5.5	7.72±0.1	8.8	7.61±0.02	9.0	3.58±0.01	5.7
<i>glyc</i>	6.70±0.02	9.5	5.96±0.02	6.5	6.80±0.1	6.6	5.28±0.02	9.1	6.26±0.3	7.1	6.15±0.03	7.3	5.68±0.02	9.0
<i>ser</i>	5.52±0.01	7.8	4.84±0.03	5.3	6.46±0.3	6.2	4.76±0.03	8.2	5.14±0.5	5.2	5.13±0.02	6.4	5.16±0.04	8.2
<i>gln</i>	12.78±0.04	18.2	11.70±0.05	12.3	14.69±0.06	14.2	7.86±0.01	13.6	14.70±0.4	16.8	11.89±0.1	14.1	8.26±0.01	9.6
<i>thr</i>	5.04±0.03	7.2	6.68±0.02	7.3	10.60±0.08	10.3	5.64±0.06	9.7	8.98±0.3	10.2	6.89±0.03	8.1	6.04±0.01	19.5
<i>ala</i>	10.70±0.02	15.2	12.52±0.05	13.8	12.68±0.3	12.3	11.82±0.03	20.5	12.88±0.3	14.7	12.73±0.01	15.1	12.22±0.03	6.9
<i>tyr</i>	4.68±0.1	6.6	5.88±0.02	6.5	6.98±0.01	6.7	3.96±0.01	6.8	6.18±0.01	7.0	6.09±0.02	7.2	4.36±0.01	–
<i>met</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>val</i>	3.80±0.03	5.4	7.02±0.01	7.7	6.78±0.03	6.5	3.04±0.01	5.2	7.32±0.01	8.3	7.23±0.02	8.5	3.44±0.01	5.5
<i>phe</i>	2.02±0.02	2.8	1.80±0.1	7.9	2.46±0.01	2.3	2.60±0.01	4.5	2.10±0.01	2.4	2.01±0.01	2.3	3.0±0.01	4.7
<i>leu</i>	67.4±0.145	9.6	6.82±0.01	7.5	10.86±0.04	10.5	5.84±0.02	1.01	7.12±0.02	8.1	7.03±0.01	8.3	6.34±0.02	10.1

The study showed that the amount of the total amino acid value in the extract of *D. aduncus* moss – 102.75 mg/100 g of dry biomass. The data on the amino acid composition of proteins indicate that in the total amount of amino acids the content of glutamic acid, alanine, threonine prevails over the rest. Of the essential amino acids in *D. aduncus* moss extracts the threonine content is significant. The mosses under study are distinguished by their amino acid composition, quantitatively and nor quantitatively. From the studied bryophytes according to the total number of

amino acids *D. aduncus* – 107.7 mg/100 g, *P. cuspidatum* – 90.35 mg/100 g, then *B. salebrosum* – 62.98 mg/100 g, *T. recognitum* – 84.24 mg/100 g, *A. viticulousus* – 62.98 mg/100 g, in last place moss *M. spinosum* – 52.62 mg/100 g. All studied bryophytes lack the essential amino acids methionine. The species *D. aduncus* revealed the presence of essential amino acids: threonine – 10.8 mg/100 g, leucine – 10.6 mg/100 g, valine – 6.7 mg/100 g, histidine – 5.4 mg/100 g, lysine – 5.08 mg/100 g. The share of essential amino acids in the total number is 6.3%, significant amount is made up of nonessential amino acids, the content of glutamic acid is especially high – 14.6 mg, alanine – 12.6 mg, aspartic acid – 18.4 mg, tyrosine – 6.9 mg which is the lowest value in all studies types. From this point of view, the species *M. spinosum* can be distinguished in the extracts of with the proportion of essential amino acids is 5.1%, a relatively large amount of leucine, threonine and lysine was found *D. scoparium* moss extract contains less histidine, and the amino acid arginine was absent. On the nonessential amino acids in the extract of *A. viticulousus*: glutamic acid – 12.78 mg/100 g, alanine – 10.70 mg/100 g, in extract of mosses *D. scoparium* – glutamic acid – 14.69 mg/100 g, alanine – 12.68 mg/100 g was found. The research results indicate the content of equal amounts of essential amino acids – 5.7% extract of mosses *B. salebrosum* and *T. recognitum*. The extract of these species have a particularly high threonine content of 8.9 mg/100 g and 6.08 mg/100 g. The species *Brachytheciaceae* and *Thuidiaceae* are distinguished by the content of nonessential amino acids, in particular glutamic acid.

Table 2

Amount of nitrogen in mosses (n=5, p<0.05)

Moss	Content in mg/g
<i>Anomodon viticulosus</i>	840 ± 0.1
<i>Plagiomnium cuspidatum</i>	17.92 ± 0.3
<i>Drepanocladus aduncus</i>	4.5 ± 0.01
<i>Mnium spinosum</i>	3.64 ± 0.01
<i>Brachythecium salebrosum</i>	840 ± 0.1
<i>Thuidium recognitum</i>	2.59 ± 0.01
<i>Dicranum scoparium</i>	1.540 ± 0.02

Further, was investigated the nitrogen content in mosses. Nitrogen is the main constituent of proteins, which is the predominant metabolite in the body. The nitrogen cycle in nature is the main condition without which life on our planet would be impossible. Nitrogen is a part of proteins, which are the main constituents of the cytoplasm and nucleus, nucleic acids, chlorophyll, enzymes, many vitamins and other organic nitrogenous compounds that play an important role in metabolic processes in plants. Climatologists note that bryophytes, lichens, cyanobacteria are the best nitrogen consumers on our planet. Mosses and lichens recycle 7% of nitrogen throughout the year, using it to synthesize the necessary compounds. In the course of research it was revealed that mosses, lichens absorb 14.36 billion liters of CO<sub>2</sub>, which is approximately 7% of the total CO<sub>2</sub>. In addition, scientists have found that mosses and lichens are the most productive nitrogen absorbers, they are also the main sources of nitrogen in the biosphere, on average they contribute from 30–80% of biological nitrogen, depending on climatic conditions and environmental conditions. Atmospheric deposition is considered to be the main nutrient source for

forest mosses and they efficiently absorb deposited nutrients [11]. We can say that they are a reservoir that accumulates nutrients and represent a reserve of important nutrient for the ecosystem [12].

Studies have shown that mosses are sensitive to the presence of nutrients, they can effectively trap nutrients and be important sources of N and F [13, 14].

The data indicate that the extract of studied bryophytes contain a certain amount of nitrogen (see Tab. 2). As shown in the table 2 840 mg/g of nitrogen was found in the extracts of *A. viticulosus* and *B. salebrosum* mosses, which is a high indicator in comparison with other species. Thus, it can be stated that in these two representatives of bryophytes the biosynthesis of nonessential amino acids occurs more intensively. It should also be noted that a certain amount of nitrogen was also found in other species, which is very important for metabolic processes occurring in plants (synthesis of new amino acids, formation of biologically active compounds).

The results of our research indicate that the studied mosses have a rich amino acid composition and contain a sufficient amount of nitrogen, which indicates that these plants due to the content of biologically active compounds, are advisable to use in pharmacology, medicine and the food industry (as a packaging for perishable foods).

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#### ՀԱՅԱՍՏԱՆՈՒԽ ՏԱՐԱԾՎԱԾ ՈՐՈՇ ՄԱՄՈՒՆՄԱՆՆԵՐԻ ԱՄԻՆԱԹՅՎԱՅԻՆ ԿԱԶՄԻ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆԸ

Մամուլները հարուստ են բազմազան կենսաբանական ակտիվ միացություններով, ինչը թույլ է տալիս դրանք օգտագործել կենսատեխնոլոգիական և կենսաբժշկական նպատակների համար: Մամուլները պարունակում են պոլիսախարիդներ, տերպենոիդներ, լիպիդներ, ամինաթթուներ, ֆենիլպրոպանոիդներ: Մամուլների լուծամզվածքներն օժտված են ցիտոտոքսիկ, հակամանրէային ակտիվությամբ: Մեր հետազոտությունների արդյունքները վկայում են, որ Հայաստանում տարածված մամուլների լուծամզվածքները պարունակում են ամինաթթուներ, որոնց թվում զգալի է անփոխարինելիների քանակը: Հայտնաբերվել է նաև ազոտի որոշակի պարունակություն հետազոտվող լուծամզվածքներում, որոշ ներկայացուցիչների մոտ այն կազմում է 840 մգ/գ, ինչը վկայում է փոխարինելի ամինաթթուների ինտենսիվ սինթեզի մասին: Ստացված արդյունքները կարող են կիրառվել բժշկության և դեղաբանության մեջ:

Г. Г. СЕМЕРДЖЯН, И. Г. СЕМЕРДЖЯН

#### ИССЛЕДОВАНИЕ АМИНОКИСЛОТНОГО СОСТАВА НЕКОТОРЫХ МОХООБРАЗНЫХ, СОБРАННЫХ В АРМЕНИИ

Мохообразные богаты множеством вторичных биологически активных соединений, что позволяет применять их для биотехнологических и биофармацевтических целей. Мохообразные содержат полисахариды, терпеноиды, липиды, аминокислоты, фенилпропаноиды. Экстракты бриофитов обладают цитотоксической, антимикробной активностью. Результаты наших исследований свидетельствуют о наличии в экстрактах мохообразных, собранных в Армении, всего спектра аминокислот, в особенности незаменимых. Также выявлено наличие азота, у некоторых представителей оно составляет 840 мг/г, что свидетельствует о более интенсивном синтезе заменимых аминокислот. Полученные результаты можно использовать в медицине, фармакологии.