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ANTIOXIDANT ACTIVITY OF EXTRACT FROM THREE SPECIES OF MOSSES: *PLAGIOMNIUM CUSPIDATUM, MNIUM SPINOSUM, ANOMODON VITICULOSUS*

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Phytotherapy is one of the most important areas of traditional medicine worldwide and the use of herbal remedies for various treatments is gaining great popularity. There is a growing trend toward the correlation of plant phytochemical constituents with their pharmacological activity. The mosses of *Mnium spinosum, Anomodon viticulosus, Plagiomnium cuspidatum* were collected from Armenia. Antioxidant activities were found for mosses 20,12 µg/ml, 21,12 µg/ml, and 44,06 µg/ml. High catalase activity was found in the aqueous extract of moss *Plagiomnium cuspidatum* - 83.91 µkat/g, further moss extract *Mnium spinosum* 71.3 µkat/g and lowest in moss extract *Anomodon viticulosus* – 25.0 µkat/g. The data obtained indicate that the catalase activity of moss extracts is significantly higher than that of medicinal plants.

Bryophytes – antioxidant activity – DPPH – reactive oxygen species (ROS) – catalase – ascorbic acid

Բուսաբուժությունը ամբողջ աշխարհում ավանդական բժշկության կարևորագույն ուղղություններից է: Բրիոֆիտների վերջին դեղաբանական հետազոտությունները ցույց են տվել, որ այս բույսերում առկա ակտիվ միացությունները բավականին եզակի են և ունեն պոտենցիալ քիմիական կիրառություն և հակաօքսիդանտ ազդեցություն: Ուսունասիրվել են Յայաստանում տարածված *Mnium spinosum, Anomodon viticulosus, Plagiomnium cuspidatum* մամուռների լուծամզվածքների հակաօքսիդանտային ակտիվությունը, որը կազմում է համապատասխանաբար 20,12 µg/ml, 21,12 µg/ml, 44,06 µg/ml: Բարձր կատալազային ակտիվություն հայտնաբերվել է մամուռի ջրային լուծամզվածքում *Plagiomnium cuspidatum* – 83,91 մկաթ/գ, *Mnium spinosum* 71,3 մկաթ/գ և *Anomodon viticulosus* մամուռի լուծամզվածքում՝ 25,0 մկաթ/գ: Ստացված տվյալները ցույց են տալիս, որ մամուռների լուծամզվածքում կատալազային ակտիվությունը զգալիորեն ավելի բարձր է, քան դեղաբույսերինը: Ստացված արդյունքների հիման վրա կարելի է եզրակացնել, որ հետազոտված մամուռների լուծամզվածքները կարող են օգտագործվել որպես բնական հակաօքսիդանտի հեշտ հասանելի աղբյուր:

Բրիոֆիտներ – հակաօքսիդանտային ակտիվություն – ԴՖՊՅ – թթվածնի ակտիվ ձևեր (ԹԱՁ) – կատալազ, ասկորբինաթթու

Фитотерапия является одним из важнейших направлений народной медицины во всем мире, и использование растительных средств для различных лечебных целей приобретает все большую популярность. Наблюдается растущая тенденция к корреляции фитохимических составляющих растений с их фармакологической активностью. Нами исследовались экстракты мхов *Mnium spinosum, Anomodon viticulosus, Plagiomnium cuspidatum* собранных в Армении. Антиоксидантная активность обнаружена у мхов 20,12

мкг/мл, исследовались экстракты мхов *Mnium spinosum, Anomodon viticulosus, Plagiomnium cuspidatum* собраных в Армении. Антиоксидантная активность обнаружена у мхов 20,12 мкг/мл, 21,12 мкг/мл и 44,06 мкг/мл соответственно. Высокая каталазная активность обнаружена у водного экстракта мха *Plagiomnium cuspidatum* – 83,91 мккат/г, далее у экстракта мха *Mnium spinosum* 71,3 мккат/г, а самая низкая у экстракта мха *Anomodon viticulosus* – 25,0 мккат/г. Полученные данные свидетельствуют о том, что каталазная активность экстрактов мхов значительно выше, чем у лекарственных растений.

Фитотерапия является одним из важнейших направлений народной медицины во всем мире, а использование растительных средств для различных лечебных целей приобретает все большую популярность. Нарастает тенденция к корреляции фитохимических составляющих растений с их фармакологической активностью. Недавние исследования по казали, что природные антиоксиданты, полученные из лекарственных растений, защищают от токсического и вредного воздействия свободных радикалов и обладают широким спектром фармакологических эффектов, включая противомикробное, антимутагенное, противоаллергическое, антиоксидантное действие по удалению свободных радикалов и антиканцерогенное действие. В последнее время большое внимание уделяется природным антиоксидантам, полученным из лекарственных или пищевых растений, как многообещающим средствам для снижения риска неврологических заболеваний, вызванных окислительным стрессом. Недавние фармакологические исследования мохообразных доказали, что активные вещества, присутствующие в этих растениях, совершенно уникальны и имеют потенциальное химическое применение и антиоксидантную способность. Из собраны мхи. Антиоксидантную активность мхов *Mnium spinosum, Anomodon viticulosus, Plagiomnium cuspidatum* собранных в Армении проверяли по активности по удалению свободных радикалов (анализ DPPH). Антиоксидантная активность обнаружена у мхов 20,12 мкг/мл, 21,12 мкг/мл, 44,06 мкг/мл. Высокая каталазная активность обнаружена у водного экстракта мха *Plagiomnium cuspidatum* – 83,91 мккат/г, в экстракте мха *Mnium spinosum* 71,3 мккат/г, а самая низкая у экстракта мха *Anomodon viticulosus* – 25,0 мккат/г. Полученные данные свидетельствуют о том, что каталазная активность экстрактов мхов значительно выше, чем у лекарственных растений. На основании полученных результатов предполагается, что экстракт определенных здесь видов мхов может быть использован в качестве легкодоступного источника природного антиоксиданта для лечения.

Мохообразные – антиоксидантная активность – ДФПГ – активные формы кислорода (АФК) – каталаза – аскорбиновая кислота

In connection with the evolution of photosynthesis in cyanobacteria, a significant amount of oxygen appeared in the Earth's atmosphere. Since then, the formation and transformation of active oxygen species (ROS) have become a subtle component of aerobic life. The presence of two unpaired electrons in an oxygen molecule significantly limits its reactivity. In living organisms, in the course of evolution, specialized enzymatic systems for the reduction of molecular oxygen were formed by transferring one, two, or three electrons to it – oxidase. Reactions involving oxidases are one of the main sources of ROS formation. ROS are also formed as by-products of various metabolic pathways localized in many cellular compartments, primarily in chloroplasts, mitochondria, and peroxisomes [3]. Simultaneously with the systems that generate ROS, in the course of biochemical evolution, systems that utilize them, called antioxidants, also appeared. The balance of ROS generation and utilization is tightly regulated by a large network of genes [7].

ROS are considered key regulators (components of signaling systems) and toxic by-products of metabolism [3, 12]. The balance between oxidants and antioxidants (redox balance) is essential in maintaining a healthy cellular microenvironment. The generation of oxidative stress is caused by an alteration in the balance between ROS production and the efficiency of the cell antioxidant defense system. Cells and tissues are continuously being exposed to free radicals derived from the metabolism or external factors, such as pollution, microbes, allergens, radiation, cigarette smoke, and pesticides [6].

An imbalance between the formation and neutralization of ROS causes oxidative damage to biomacromolecules and cell structures. Plant responses to stressors are accompanied by both increased ROS production and activation of antioxidant systems. It is assumed that the interaction of ROS and antioxidants (AO) is an important component of signaling that regulates gene expression and ensures the adaptive flexibility of the organism [3]. AO in living organisms is represented by an extensive group of chemically heterogeneous substances that delay or inhibit the oxidation of substrates, being present in the system at significantly lower concentrations compared to these substrates.

Antioxidants can be classified into three lines of defense according to their mechanism of action. The first line includes antioxidants that prevent the formation of new free radicals, which include enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX); proteins that bind metals such as ferritin and ceruloplasmin; and minerals such as Se, Cu, and Zn. The second group of antioxidants is responsible for capturing free radicals, and thus they prevent oxidative chain reactions. This group is formed by glutathione, albumin, vitamins C and E, carotenoids, and flavonoids. The third line of defense includes antioxidant enzymes that repair the damage caused by free radicals to biomolecules, such as lipases, proteases, DNA repair enzymes, transferases, and methionine-sulfoxide reductases. Exogenous antioxidants constitute a very large and diverse group of molecules in terms of chemical structure and biological properties. This group can be divided into three subgroups: polyphenols, vitamins and derivatives, and antioxidant minerals. There is another group that comprises synthetic antioxidants widely used in the food industry. Exogenous chemicals and endogenous metabolic processes can produce high levels of ROS which are directly linked to hypertension, and cardiovascular diseases. In addition, emphysema, cirrhosis, inflammation, genotoxicity, and cancer have been correlated with ROS effects [7]. Therefore, the development of effective antioxidants of natural origin is of great interest.

Due to increasing concerns about the sustainability of human living, the control of the damaging effects of microorganisms is becoming very important. A wide range of microorganisms exist in a biological balance with the human body and its living environments, but an uncontrolled and rapid growth of microbes can lead to some dangerous problems. Antimicrobial agents are used as antibiotic drugs to control infections in the human body, but they can cause many side effects, especially increasing reactive oxygen species (ROS) in the human body. ROS are very dangerous to human health and well-being and play a role in producing cancer; further, they can increase potential health risks. The herbal materials used as medicinal plants include several types of plants. Many of these herbal materials show medicinal activities such as antioxidant, anticancer, anti-inflammatory, antimicrobial, and antiviral activities [4]. Furthermore, these herbs can play the main role in drug synthesis and development. The identification of plant-based antioxidants is an important aspect that has gained immense importance to protect cells/tissues from the damage caused by free radicals. Phenolic compounds present in plants act as powerful antioxidants which can protect the cells from free radicals by acting as hydrogen donors and radical scavengers. Antioxidants act as free radical scavengers and are thus helping to mitigate the effect of oxidative stress in a variety of diseases such as cardiovascular diseases, Parkinson's disease, Alzheimer's disease, cancerogenesis, neuro-degenerative, nephrotoxicity, diabetes, and aging. Several studies have reported that phenolic compounds, such as flavonoids and phenolic acids present in plants are responsible for their antioxidant nature [4]. Plant phenolics are commonly found in both edible and non-edible plants, and have been reported to have multiple biological effects, including antioxidant activity. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers as the trend of the future are moving toward functional food with specific health effects. Bryophytes belong to phylum bryophyte and include three classes *Hepaticopsida* (liverworts), *Bryopsida* (mosses), and *Anthocerotea* (hornworts). They are small, terrestrial photosynthetic, spore-bearing plants that require a humid environment and can be found all over the world. There are about 20,000species of bryophytes worldwide, which is about five percent of the total of 400,000 plant species on the earth [9]. Bryophytes are considered as a rich reservoir of new, natural products or secondary compounds, many of which have shown interesting biological activity among which are antimicrobial, antifungal, cytotoxic, antitumor, vasopressin antagonist, and cardiotonic. Some of the latest results also predicted the beneficial influence of bryophytes in AIDS therapy (some dibenzyl of liverworts) [7].

The aim of this work was to identify antioxidant properties, study catalase activity, and the presence of vitamin C in moss extracts gathered from Armenia for use in medicine and pharmacology.

Materials and methods. <u>*Plant Materials*</u>.

The bryophytes viz *Mniaceae*, *Mnium spinosum (*Voit) Schwaegr, *Plagiomnium cuspidatum* (Hedw), *Anomontaceae, Anomodon viticulosus* (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, 13456 respectively).

<u>*DPPH radical scavenging activity*</u>

The antioxidant activity was determined by using DPPH radical scavenging activity (free radical method) [1]. Experimental samples contained moss extracts with a concentration of 100 µg/ml, 500 µg/ml, 100 µg/ml, 50 µg/ml, and 10 µg/ml, respectively, and 125 µl of DPPH. The control samples contained 750 µl of ethanol and 125 ml DPPH. Catechin was used as a positive control. Samples were incubated at room temperature, and the absorption spectrum of solutions was measured by GENESYS 10S UV-VIS (Thermo Scientific, Germany). The percentage of inhibition activity was calculated using the following equation: % Inhibition = (Ac - As/Ac) = 100 where Ac is the absorbance of the control and As is the absorbance of the extract/standard. The free radical scavenging activity of samples was expressed as IC_{50} value, which represented the effective concentration of extract/standard required to scavenge 50% of DPPH radicals.

<u>*Determination of catalase activity*</u>

The determination of catalase activity by this method is based on the extraction of the enzyme from biological material with water, after which an enzymatic reaction is carried out for a certain time when an aqueous extract of catalase is added to a hydrogen peroxide solution. At the end of the enzymatic reaction in the reaction medium, the amount of hydrogen peroxide that has not decomposed under the action of the enzyme is determined by titration in an acidic medium with a solution of potassium permanganate.

Simultaneously with the analyzed sample, the amount of hydrogen peroxide remaining undecomposed after the reaction with hydrogen peroxide of the inactivated enzyme is determined (control variant). In this reaction, partial decomposition of hydrogen peroxide occurs in a non-enzymatic way. The difference between the titration of the control and the analyzed sample is used to determine the amount of hydrogen peroxide that has been decomposed into water and oxygen under the action of catalase, and the result obtained is used to calculate the catalytic activity of the enzyme [13].

Determination of ascorbic acid in moss extracts by the iodate method
When determining vitamin C by this method, the reduction reaction of potassium iodate with ascorbic acid to free iodine is carried out, which is stained with the addition of a starch solution and quantified [13].
Statistical analysis. Data processing was done using Excel 2013 Microsoft program. Statistical analysis was done using one-way analysis of variance (ANOVA). The validity of differences between different series (n=5) was evaluated by Student P-test: the value $P<0.05$ was considered as significant.

Results and Discussion. Plant compounds have been an impressive resource for medicinal compounds and will undoubtedly remain so [5]. Learning more about this resource is essential if we are to avail ourselves of its potential, and screening for bioactivity is an important tool in this learning. As part of this effort, we have screened three species of bryophytes, the mosses *M.spinosum, Anomodon viticulosus,* and *Plagiomnium cuspidatum,* for antioxidant properties. The antiradical activity of moss extracts was evaluated by the free radical method. The data obtained indicate that the ethanol extracts of 3 representatives of mosses that are gathered from Armenia, which we studied, have antiradical activity (tab.1). Lowest half-saturation value (IC_{50}) found in moss extracts *Mnium spinosum* and *Plagiomnium cuspidatum* 20,12 µg/ml and 21,12 µg/ml respectively.

According to literature data, moss species extracts of *C. schmidii* had no antibacterial activity, *L.aduncum* demonstrated very weak antioxidant activity 1329.02 µg/ml, which means < 50% binding of free radicals [10], extract of moss *R.murale* – 67,1% DPPH free radical inhibition [9]. The data obtained by us show that moss extract *M.spinosum* binds 93.44 % free radicals and *P.cuspidatum* - 97.44% respectively.

Table 1. The antiradical activity of the extracts of the mosses (% Inhibition of DPPH, n=3, $p<0.05$)

extract concentration (µg/ml)	% inhibition of DPPH		
	M.spinosum	*A. viticulosus*	*P.cuspidatum*
1000	93,44± 1,4	90,44± 1,4	97,44± 1,4
500	76,52± 2,3	77,25± 2,3	80,30± 1,3
100	66,07± 0,2	63,04± 0,2	56,30± 0,2
50	52,80± 1,2	51,7± 1,2	51,0± 1,2
10	35,13± 0,4	34,5± 0,4	45,0± 0,4
IC_{50} (µg/ml)	20,12	44,06	21,12

Under conditions of oxidative stress, the antioxidant enzymatic system can lose productivity. The reason - is the inactivation of enzymes by free radicals. In this case, the value of low molecular weight non-enzymatic antioxidants increases. Low molecular weight antioxidants interact with oxygen and organic radicals, inhibiting free radical processes in the cell. They take on the impact of oxygen derivatives (O_2*, O_2^-, $^{\cdot}OH$, H_2O_2) oxidases and stop the reaction going on in the cell. But, non-enzymatic low molecular weight antioxidants are not very productive compared to enzymatic ones [11].

In the next series of our experiments, we studied the activity of the antioxidant enzyme catalase in moss extracts. Catalase catalyzes the decomposition of hydrogen peroxide into water and molecular oxygen. The biological role of catalase is to degrade hydrogen peroxide formed in cells as a result of the action of a number of flavoprotein oxidases (xanthine oxidase, glucosidase, etc.) and to provide effective protection of cellular structures from destruction under the action of hydrogen peroxide. Catalase is widely distributed in animal tissues, including humans, plants, and microorganisms. Catalase is present in cells where cellular respiration occurs. Catalase is localized in peroxisomes and glycosomes. In mitochondria was found a special isospecies, the activity of which was also found in plant chloroplasts. Catalase provides oxygen supply to those tissue areas where for one reason or another, oxygen penetration is limited. In mitochondria, a special isospecies was found, the activity of which was also found in plant chloroplasts. Catalase provides oxygen supply to those tissue areas where, for one reason or another, oxygen penetration is limited. Catalase is most active in young plant organs and tissues. Enzymes of the antioxidant system during ontogenesis are involved in the regulation of metabolism and are important for plants, because thanks to them, plants adapt to environmental changes [11].

We studied the catalase activity of aqueous extracts of mosses *Mnium spinosum, Anomodon viticulosus, Plagiomnium cuspidatum.* High catalase activity found in water extract of moss *Plagiomnium cuspidatum* – 83,91 μkat/g, further moss extract *Mnium spinosum* 71,3 μkat/g and lowest in moss extract *Anomodon viticulosus* – 25,0 μkat/g (tab. 2). We also studied the catalase activity extracts of medicinal plants *Matricaria chamomilla and Calendula officinalis.* The data obtained indicate that the catalase activity of moss extracts is significantly higher than that of medicinal plants.

Table 2. Catalase activity (μkat activity/g (n=3, $p<0.05$)

Moss	activity
Plagiomnium cuspidatum	83,91± 2,4
Mnium spinosum	71,3±1,5
Anomodon viticulosus	25,0±1,0
Matricaria chamomilla	1,90±0,10
Calendula officinalis	1,50±0,03

Bryophytes can produce reactive oxygen free radicals to induce oxidative stress in order to form complex enzymatic and non-enzymatic defense systems that will resist cell damage caused by drought stress and thus ensure their survival. The first type removes or reduces free radicals, including enzymes and antioxidants, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and Vitamin C, carotenoids (Cars), and Glutathione These enzymes are the most critical in the enzymatic defense system. In the non-enzymatic defense system, Cars are the most important quenchers of O_2^-, which can prevent peroxidation of unsaturated fatty acid, protecting the membrane system. Antioxidant enzyme activities and Cars content of the three moss species were positively correlated with stress intensity under non-drought stress. In the early stage of stress (i.e., low-stress level), SOD, POD, and CAT can scavenge active oxygen-free radicals by increasing their enzymatic activity to prevent self-inflicted injury. As stress levels increased (i.e., mosses suffering from severe drought damage for 48 h), the balance between ROS generation and the antioxidant system was disrupted, leading to damaged membrane structure and inhibition of enzyme activity [10].

Exogenous antioxidants constitute a very large and diverse group of molecules in terms of a chemical structure and biological properties. In the family of vitamins and their derivatives, vitamins C, E, K, and carotenoids can be distinguished. Vitamins, along with polyphenols and antioxidant minerals, are one of the subgroups of exogenous antioxidants. Vitamin C or ascorbic acid is known by its electron-donating ability, thanks to which it prevents the accumulation of oxidizing agents and free radicals. It is especially efficient in eliminating superoxide anion radicals, hydrogen peroxide, hydroxyl, singlet oxygen, and RNS (reactive nitrogen species) [6]. Plant organisms using solar energy and inorganic compounds form vitamins. Vitamins produced in the plant tissues of the plant are important. It is known that in the absence of minerals, plant roots naturally do not develop regularly, and germination of seeds also becomes impossible. As in the body of animals, in plants vitamins- perform a catalytic function. Ascorbic acid is synthesized in the leaves of plants. The concentration of ascorbic acid in plants depends on climatic conditions as well as plant nutrition from the content of materials. Ascorbic acid is found in the aqueous phase, chloroplasts, mitochondria and other structures, as well as in the intermembrane space. As a reducing agent, ascorbic acid can interact directly with peroxide, hydroxyl, and tocopherol radicals [11].

The data obtained show (Fig.1) that the extracts of the *Plagiomnium cuspidatum* species contain 2.110 mg /%, *Mnium spinosum* – 1.176 mg /%, *Anomodon viticulosus* – 1.100 mg /% ascorbic acid. We also studied extracts of Matricaria chamomilla, Calendulae offcinalis. The data obtained indicate that the amount of ascorbic acid in the P/C moss extract and Matricaria chamomilla is almost the same. Thus, moss extract can serve as an alternative source of vitamin C.

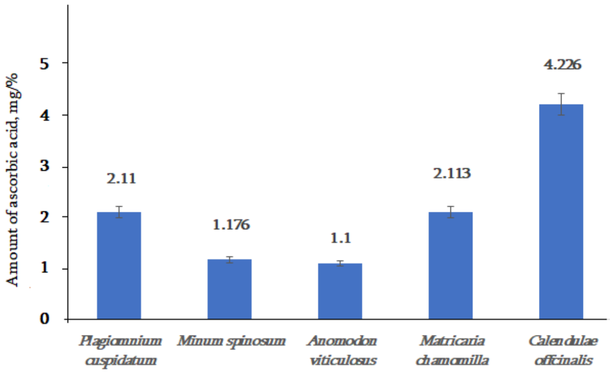

Fig.1. The amount of ascorbic acid in different plants (mg/%, n= 3, p< p <0,05)

Conclusion. Phytochemicals derived from plants are a major source of ntioxidants. These phytochemicals are redox-active molecules and are dynamic to maintain redox balance in the body. Undoubtedly, plant-derived natural antioxidants are supposed to have more progressive effects on the body than synthetic ones. This is because plant constituents are a part of the physiological functions of living flora and are thus well suited to the human body. In recent years, the rising importance of biologically active components of plant origin has gained increased significance as highly promising prophylactic and restorative measures to combat diseases caused by oxidative stress. Higher plants, in particular, angiosperms, are used and explored as antioxidant sources. Cryptogams, especially bryophytes, hold rich reservoirs of unique phytochemicals imparting them a strong defense mechanism to survive under highly diverse habitats despite having a non-lignified structure. There is huge potential to utilize this untapped resource in modern healthcare as eco-friendly antibiotics and antioxidants [2].

The large diversity of bryophytes also acts as a “remarkable reservoir” of natural products or secondary compounds such as terpenoids, flavonoids, alkaloids, glycosides, saponins, anthraquinones, sterols, and other aromatic compounds. Many of them show interesting biological activity and become potential sources of different medicines. They also possess anticancer and antimicrobial activity due to their unique chemical constituents [9].

On the basis of the results, it is suggested that the extract of three moss species determined here could be of use as an easily accessible source of natural antioxidants for the treatment.

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