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ROUND-LEAVED WINTEGREEN (PYROLA ROTUNDIFOLIA) AS A VALUABLE MEDICINAL PLANT RAW MATERIAL

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The phytochemical and mineral composition of BAS of round-leaved wintergreen (Pyrola rotundifolia) growing in the Lori Region of Armenia has been studied. It is established that the plant extracts are rich in a wide range of pharmacologically active and antioxidant (AO) substances, in particular, arbutin, tannins, naphthoquinones, triterpenoids, organic acids, micro- and macroelements, etc. The plant extracts were found to contain 10 amino acids and 20 vital micro-and macroelements.

On the basis of the research carried out, extracts and broths of *round-leaved wintergreen* can be recommended as a source of amino acids, as well as a preventive and corrective agent when insufficiency or imbalance of macro- and microelements in tissue- and cellular structures in various pathological processes of the body. Extracts of wintergreen can be used as an environmentally friendly source of arbutin in urinary tract infections (UTI) and antioxidants against early aging.

Fig. 1, tables 3, references 18.

Phytotherapy is one of the most ancient sciences. Its history began more than six thousand years ago [1]. Already at the earliest stages of mankind development, herbs were not only a source of human nutrition, but also a remedy for the treatment of diseases. The mildness of the action of most plant preparations and the absence of toxic manifestations with their use (which is related to their naturalness, proximity to the human body) allow us to assume their significant importance in the prevention of various diseases. The undoubted merit of some species of medicinal plant raw materials is also the variety of biologically active substances that are capable of providing the polyvalence of pharmacological effects.

In modern life, it is impossible to avoid the development of various diseases including urological, the share of which is 10-12% in the total structure of morbidity of the world population [2]. Uncomplicated urinary tract infections are one of the most frequent diseases in women of reproductive age. According to statistics, annually 150 million cases of acute cystitis are registered in the world [3]. This high incidence testifies to the urgency and necessity of searching for safe, effective, side-effects free drugs to which phytotherapeutic means primarily belong.

In this regard, one of the promising plants is round-leaved wintergreen. The traditional areas of this plant spread are forests in the temperate zones of the Northern Hemisphere (from the Arctic to Mexico and the Himalayas). The plant grows well in the Russian Federation (Altai Territory) and Transcaucasian republics; specifically, as a wild plant it grows in the forest landscapes of the Lori Region. It is a perennial herb of the Pyroleae family with a long branched creeping rhizome, from the nodes of which additional roots and aboveground sprouts spread. Medicinal raw materials are the leaves of the wintergreen, its flowers, stem that contain a large amount of BAS (iridoids, tannin, phenol, naphthoquinone, triterpenoids, ericoline, chymaphylin), organic acids, micro- and macroelements, resins, essential oils, glycosides, etc.

It is known that the composition and properties of plant raw materials of the same biological species can vary significantly depending on the place of their growth, time of collection, methods of processing and other factors [4-7]. In this regard, prior to the use of this raw material for medicinal purposes, or as a source of AO, it is necessary to study the properties of extracts of plant raw materials growing in a given geographical area.

Hence, study of the qualitative and quantitative composition of biologically active substances (BAS) of round-leaved wintergreen growing in the Lori Region of Armenia is of great interest.

Purpose and objectives of the study. The purpose of the present study is investigation of the phytochemical composition of BAS of round-leaved wintergreen, which provides a wide range of pharmacological effects of the plant.

Experimental Part

Materials and methods

Collection and preparation of raw material. Raw material (leaves, stems and flowers of wintergreen) was collected in June-August 2017 in the forest landscapes in the vicinity of Vanadzor city, far from highways and settlements. The raw material was dried to an air-dry state in a drying chamber at 313*K*, packed in paper bags and stored at room temperature. To obtain the extract, the dried raw material was ground in a ceramic mortar to a powdery state (particle size ≤ 1 *mm*) and passed through a sieve with holes 1 *mm* in diameter.

Determination of moisture, ash content, extra active substances in the analyzed samples was carried out according to the standard procedures [8]. The results of studies are presented in Table 1.

The amino acid, macro-, microelement composition of the plant was studied, the amount of BAS of the phenolic origin (flavonoids, tannins arbutin, vitamin P, vitamin C) displaying the highest physiological and therapeutic activities was determined.

For quantitative determination of arbutin, 5 *g* (exact weight) of the crushed leaves of wintergreen was placed in a 100 *ml* flask, 50 *ml* of water was poured and the whole was boiled for 5 *min*. The extract was filtered into a 100 ml volumetric flask [9]. 25 ml of water was poured into the flask with raw material and boiled for 20 *min*, after which the extract was filtered into the same flask; the raw material was transferred to a filter and washed twice with 10 *ml* hot water, connecting the rinsing water to the filtrate. Then 3 *ml* of a solution of basic lead acetate was added to the extract, mixed and after cooling, was adjusted with water to a mark. The flask was placed in a boiling water bath until the precipitate was completely coagulated. The hot liquid was filtered into a dry flask. To remove the excess of the basic lead acetate, 0.8 *g* of sodium sulfate was added. The solution was filtered (solution A). 0.08 *g* of sodium sulfacyl was dissolved in 10 *ml* of water, 10 *ml* of 0.1*N* hydrochloric acid was added and the volume was adjusted to 100 *ml* (solution B). 2 *ml* of solution B, 2 *ml* of a 0.02% water solution of sodium nitrite were introduced into a test tube, left for 3 *min.* Then 0.5 *ml* of solution A, 0.04 *ml* of a 10% water solution of sodium hydroxide were added, the volume was adjusted to 6 *ml* with water and kept in a warm water bath for 1 *min.* After 20 *min*, the optical density was measured on a UV1800PC spectrophotometer at 490 *nm* wavelength.

The quantitative content of arbutin is calculated by the formula: X% = D x 0.938 x 6 x100/E x a x b, where D is the optical density of the test solution (D = 1.0501); 0.938 - conversion factor for anhydrous arbutin; 6 – total volume of the test solution, *ml*; 100 – volume of a volumetric flask, *ml*; E – specific absorption index of arbutin at a wavelength of 490 *nm*, equal to 221.5; a – weight of raw material (a = 0.5 *g*); b – extraction volume taken for analysis (b = 0.5 *g*).

The results are inserted in the formula:

$$X\%=1.0501\times0.938\times6\times100/22.5\times0.5\times0.5=10.67\%$$

For the quantitative analysis of flavonoids, anthocyanins, carotenoids, rutin, sugars, spectrophotometry methods were used [10-12]. The amount of vitamin C, carboxylic acids and tannins was determined by a titrimetric method [8]. The results are shown in Table 1.

Table 1

The quantitative content of biologically active substances in the extract of round-leaved wintergreen

Moisture,%	7.2
Extractive substances, %	28.59
Arbutin, *mg*%	10.67
Flavonoids, %	1.23
Anthocyanins, %	0.085
Rutin, *mg*%	22.59
Vitamin C, *mg*%	235.72
Carboxylic acids, %	2.35
Tannins, in terms of tannin, %	19.55
Water-soluble polysaccharides	11.25
Carotenoids, in terms of β-carotene, *mg* %	24.128

Determination of the elemental composition was carried out by atomic emission spectrometry with inductively coupled plasma, using the IRISIntrepid spectrometer (ThermoElectron, USA). The sample weights were preliminary held in a muffle furnace at 450-500^{o}C for 4 *h*. After cooling, the ash residue was treated twice with 5 *ml* of 6N HCL with slow evaporation in a water bath. The residue was dissolved by heating in 0.1N HCl and filtered off [13]. The content of chemical elements in the leaves of round-leaved wintergreen is shown in Table 2.

Table 2

The content of chemical elements in the samples of round-leaved wintergreen growing in the Lori Region (in *mg/kg* of absolutely dry raw material)

Macro-microelements	Certain indicators *mg* / *kg*	Macro-microelements	Certain indicators *mg* / *kg*
Fe	124.26	Mn	1.594
Cu	4.75	Co	<0.0047
Zn	8.05	Se	1.389
Ca	8857.4	Cd	0.052
Mg	8324.74	V	0.795
K	13158.3	Cr	0.596
Na	603.12	Ni	0.0413
Al	162.38	Pb	<0.0046
P	398.25	As	0.723
S	352.38	Si	211.02

Qualitative and quantitative analysis of free amino acids (Table 3 and Figure) was carried out using the amino acid analyzer Nexera X2 (Shimadzu, Japan).

Table 3

The content of free amino acids in hydrolysate of round-leaved wintergreen

Amino acids	L-Asp	L-Glu	L-Ser	L-His	Gly	L-Thr	L-Arg	L-Ala	L-Leu	L-Lys
Content of amino acids in terms of dry raw material *mg*/*g*	4.47	4.768	2.384	1.788	7.505	2.09	2.98	2.95	2.98	2.98

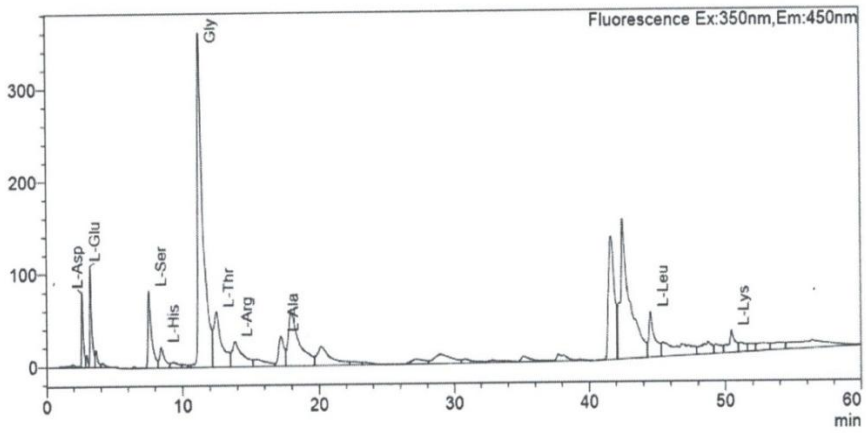

Fig. Chromatogram of free amino acids in the aboveground part of wintergreen.

The device sensitivity is 0.1 *μmol*. The calculation was carried out by comparing the peak areas of the samples under study with the peak areas of a standard amino acid mixture (Sigma, USA). Preparation of the test samples for amino acid analysis was performed as follows: a sample (5 *g*) of the herb dried at 60°C was placed in a 50 *ml* round-bottomed flask made of heat-resistant glass, 20 *ml* of *6N* HCl was added, the flask was closed with a stopper and fixed with a steel clamp. Hydrolysis of the dry sample was carried out in a vacuum drying chamber at 110°C for 22 *h*. After hydrolysis, the contents of the ampoule were cooled, filtered, evaporated and recrystallized from a solution of C_2H_5OH/H_2O = 1/1. After repeated filtration and drying, the amino acid mixture was dissolved in a citrate buffer pH 2.2 [14]. The content of free amino acids in the aboveground part of wintergreen is given in Table 3 and Figure.

To determine the antioxidant activity of wintergreen, to 1 *g* (exact weight) of the raw material 50 *ml* of 30% ethyl alcohol is poured and extracted with a reflux condenser for 30 *min*. Then, the contents of the flask are filtered through a paper filter, cooled and the volume of extraction is adjusted to 50 *ml* with 30% alcohol. 8 *ml* of freshly boiled and cooled distilled water, 1 *ml* of a 20% solution of sulfuric acid, 1 *ml* of 0.05 *N* solution of potassium permanganate are introduced into a 50 *ml* beaker. The whole is mixed and titrated with a 30% alcohol infusion of wintergreen

from a microburette (volume 1 *ml* with a fission rate of 0.01 *ml*) until the pink color disappears. For the control experiment, about 0.0500 *g* (exact weight) of quercetin (FS 42-1290-79) is dissolved in 40 *ml* of ethanol, transferred to a 100 *ml* volumetric flask, made up to the mark with alcohol and stirred. 8 *ml* of freshly boiled and cooled distilled water, 1 *ml* of a 20% solution of sulfuric acid, 1 *ml* of a 0.05 *N* solution of potassium permanganate are mixed into a 50 *ml* titration beaker. The whole is mixed and titrated from a microburette (volume 1 *ml* with a division value of 0.01 *ml*) with a quercetin solution until the disappearance of the pink color. 1 *ml* of a 0.05 *N* solution of potassium permanganate corresponds to 0.25 *mg* of quercetin.

The calculation of the indicator of the antioxidant activity (AOA, which corresponds to concentration of BAS of a reducing nature in terms of quercetin (in *mg/g*), is carried out according to the formula: $B=C_k x V_k x V_o/V_x x m$, where B is the concentation of BAS of the reducing nature of the object under study, used for titration of 1 *ml* of 0.05 *N* potassium permanganate solution, *mg/g*; C_k – the concentration of quercetin in the solution used for the titration of 1 *ml* of 0.05 *N* potassium permanganate solution, *mg/g* (0.5 *mg*/*ml*); V_k – the volume of quercetin solution, spent on titration of 1 *ml* of 0.05 *N* solution of potassium permanganate, *ml* (1.4 *ml*); V_o – the volume of the investigated solution, *ml* (50 *ml*); V_x – the volume of the solution of the object under study, spent on the titration of 1 *ml* of 0.05 *N* solution of potassium permanganate, *ml* (0.4 *ml*); m is the mass of the sample of the object under study, *g* (1*g*).

$$B = 0.5 \times 1.4 \times 50/0.4 \times 1 = 87.5 \ mg/g$$

Thus, the total amount of BAS of a reducing nature in terms of quercetin in 1 *ml* or 1 *g* of the drug was determined.

According to the results obtained, during the titration in the case of quercetin, the consumption was more (1.4 *ml*) than with the extract of wintergreen (0.4 *ml*), which indicates that the wintergreen extract can be treated as an effective preventive means against antioxidant aging of the body [15].

Results and discussion

The research results of the elemental composition of the leaves of round-leaved wintergreen show the availability of 20 elements. Round-leaved wintergreen contains vital macroelements (Na, K, Ca, Mg), essential (Fe, Cu, Zn, Mn, Cr, Se, Co) and conditionally essential microelements (As, Ni, Cd) that ensure proper operation of the main systems of the body (muscle – participate in the process of muscle contraction, digestive and cardio-vascular).

According to [16], the content of cadmium in plants collected in environmentally friendly growing areas is 0.05-0.3, arsenic 1.0-5/0 *mg/kg*. The excess content of mercury is considered to start from 1.0 *mg/kg*. On the whole, the level of concentrations of the investigated elements in the researched extracts of

round-leaved wintergreen is within the range of background values, which makes it possible to classify this plant as ecologically pure [17,18].

As a result of the performed study, 10 free amino acids were found in the hydrolysate (Table 2). The presence of such an amount of amino acids provides a wide range of pharmacological effect of this phytopreparation.

From Table 2 it follows that the content of glycin, which has a positive effect on the CNS, prevails in round-leaved wintergreen. The plant is also rich in glutamic and aspartic acids. Among the numerous functions of these acids, the most significant are the regenerating and immunomodulating with a simultaneous beneficial effect on the hormonal status of the body. As follows from Table 3, the leaves of wintergreen contain a lot of tannins, vitamin C, rutin that have a bactericidal, anti-inflammatory, antioxidant effect. From a medical point of view, arbutin is valuable. Arbutin has an antiseptic property; it is used in chronic kidney diseases and purulent inflammation of the bladder and urinary tract, in inflammation of the prostate gland, chronic pyelonephritis, cystitis, urolithiasis.

High antioxidant activity of wintergreen extracts can be explained by the fact that the investigated extracts contain multifunctional BAS with the presence of easily oxidizable functional groups (for example, -SH, $(CH_3)_2CH$-), which bind free radicals formed in living organisms relatively faster.

Thus, preliminary studies of round-leaved wintergreen (Pyrola rotundifolia) growing in forest landscapes of the Lori Region have shown that it contains various classes of biologically active substances ensuring a wide range of pharmacological effect of the plant: immunomodulating, anti-inflammatory, wound healing, antioxidant, etc.

ԿԼՈՐԱՎՈՒՆ ՏԱՆՁԱՏԵՐԵՎԸ ՈՐՊԵՍ ՀԵՌԱՆԿԱՐԱՅԻՆ ԴԵՂԱԲՈՒՍԱՅԻՆ ՀՈՒՄՔ

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Հետազոտվել է Լոռու մարզում հավաքված կլորավուն տանձատերևից ստացված էքստրակտների (ԿԱՆ, ազատ ամինաթթուներ, հանքային տարրեր և այլն) ֆիտոքիմիական բաղադրությունն ու հանքային կազմը: Էքստրակտների թթվային հիդրոլիզատում հայտնաբերվել են սպիտակուցային ծագման 10 ազատ ամինաթթուներ, իսկ ատոմա-էմիսիոնային սպեկտրոմետրիայի եղանակով պարզվել է, որ բույսի վերգետնյա օրգաններում առկա են 20 հանքային տարրեր, որոնցից ծանր մետաղների (Co, Cd, Cr, V, Ni, Pb, As և այլն) պարունակությունը գտնվում են էկոլոգիապես մաքուր բուսահումքերի համար նախատեսված պահանջների կամ ղրանց ֆոնային արժեքների տիրույթում:

Հետազոտվել է նաև կլորավուն տանձատերևից ստացված թուրմերի և էքստրակտների հակաօքսիդանտային ակտիվությունը, համեմատելով այն հայտնի հակաօքսիդանտային նմուշի՝ կվերցետինի նույնանուն տվյալների հետ: Պարզվել է, որ Լոռու մարզի Վանաձորի տարատածաչրջանի սաղարթախիտ անտառների լանդշաֆտներից հավաքված կլորավուն տանձատերևի հակաօքսիդանտային ակտիվությունը մոտ 3 անգամ գերազանցում է հայտնի հակաօքսիդանտ կվերցետինի հատկությանը:

Ստացված արդյունքների հիման վրա հետազոտված կլորավուն տանձատերև բուսահումքը կարելի է առաջարկել, որպես օրգանիզմների հակաօքսիդանտային ծերացման դեմ պայքարի արդյունավետ կանխարգելման էկոլոգիապես մաքուր միջոց: Բուսահումքից պատրաստված թուրմերն ու էքստրակտները կարելի է կիրառել նաև մարդու օրգանիզմի տարբեր ախտաբանական պրոցեսների՝ հյուսվածքային և բջջային կառուցվածքներում առաջացող մակրո- և միկրոտարրերի անբավարարության կանխարգելման նպատակով:

Իսկ արքուտինի մեծ քանակության առկայությունը հնարավորություն է ընձեռում նաև այդ բուսահումքերի հալենային պատրաստուկներն օգտագործել նաև միզասեռական օրգանների բորբոքումների կանխարգելման նպատակով:

ГРУШАНКА КРУГЛОЛИСТНАЯ В КАЧЕСТВЕ ПЕРСПЕКТИВНОГО ЛЕКАРСТВЕННОГО СЫРЬЯ

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Изучены фитохимический и минеральный составы экстрактов (БАВ, свободные аминокислоты, минеральные элементы и др.) грушанки круглолистной, собранной в Лорийском марзе Республики Армения.

В кислотном гидролизате экстрактов обнаружены 10 свободных белковых аминокислот. С помощью атомно-эмиссионной спектрофотометрии установлено, что в органах надземной части растения присутствуют 20 минеральных элементов, в том числе тяжелые металлы (Co, Cd, Cr, V, Ni, Pb, As и др.), содержание которых соответствует требованиям, предъявляемым к экологически чистым растениям, или находится в пределах фоновых значений.

Изучена также антиоксидантная активность настоев и экстрактов грушанки круглолистной по сравнению с аналогичными значениями известного антиоксиданта – кверцетина. Установлено, что антиоксидантная активность образцов ГРУшанки круглолистной, отобранных из ландшафтов густолиственных лесов в окрестностях г. Ванадзор Лорийского марза, почти в три раза превышает таковую кверцетина.

На основании полученных результатов исследований биомассу грушанки круглолистной можно рекомендовать в качестве эффективного антиоксидантного средства, предупреждающего преждевременное старение. Настои и экстракты растения также можно применять для профилактики и коррекции недостатка или дисбаланса макро- и микроэлементов в тканевых и клеточных структурах при различных патологических процессах в организме человека.

В тоже время присутствие в грушанке круглолистной большого количества арбутина позволяет использовать галеновые препараты растения в качестве противовоспалительного средства при заболеваниях мочеполовых органов.

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