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Chemistry

COMPARATIVE STUDY OF THE CONTENT OF ANTHOCYANINS IN THE FRUITS OF BLUEBERRIES, BLACKBERRIES AND BLACKCURRANTS. DEVELOPMENT OF A TECHNOLOGICAL METHOD FOR OBTAINING PARTIALLY PURIFIED NEW-GALENIC EXTRACTS

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The work is devoted to a comparative study of the content of anthocyanins in the fruits of blueberries, blackberries and blackcurrants to develop a technological method for producing partially purified extracts. The latter, due to the significant content of anthocyanins, compounds with antioxidant, antiaggregate, angioprotective, anti-inflammatory activities stipulate their use in the complex therapy of ophthalmic diseases (myopia, age-related macular degeneration, diabetic retinopathy).

The objects of the study were fresh natural fruits of common blueberries (*Vaccinium myrtillus* L.), blackberries (*Rubus caesius* L.) and blackcurrants (*Ribes nigrum* L.) collected in the foothill landscapes of the city of Aparan of the Republic of Armenia, harvested in 2022.

The purpose of this study was to determine the comparative indicators of the quality and content of anthocyanins in fresh fruit extracts by direct spectrometry and obtain environmentally friendly extracts with the lowest content of macro- (Fe, Cu, Zn, Ca, Mg, etc.) and microelements (Mn, Co, Cd, V, Se, Cr, As, Pb, etc.) by ion-exchange sorption and desorption.

It has been shown that the highest content of the total amount of anthocyanins in terms of cyanidin-3-O-glycoside is determined in the juice of fresh blueberries in an acidic medium at an analytical wavelength of 546 *nm* – 4.21 ± 0.18%.

At the same time, the completeness of extraction of anthocyanins (≥ 95%) from blueberries is achieved at a ratio of raw material to extractant (95% EtOH, 1% HCl) 1 : 50 and when heated in a boiling water bath for 40 *min*.

It has been shown that the content of these macro- and microelements in the extracts after ion-exchange sorption and desorption with Ku-2×8 (H^+) cation exchanger does not exceed the permissible limits.

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***Keywords*:** fruits of blueberries, blackberries, currants, anthocyanins, extract, lozenges, sorbent, macro-and microelements.

Introduction. One of the most important sources for the isolation of biologically active compounds that are widely used, especially in medicine, are plants.

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The distinguishing feature of the majority of preparations of plant origin is the complex and complementary nature of the action of compounds they contain [1–3].

The interest in natural compounds not only does not weaken, but even increases. These compounds include anthocyanins that are contained in relatively large quantities in the fruits of blueberries common, blackberries and currants. These plants have long been used in folk medicine as an astringent. Scientific medicine exhibits special interest in the fruits of these plants as sources of a complex of biologically active substances (BAS), which have an antioxidant effect, improve the rheological properties of blood, strengthen the walls of blood vessels, and accelerate the recovery of decolorized rhodopsin. However, despite the valuable biological properties of fruits of blackberries, blueberries and currants, modern requirements for products intended for both treatment and prophylaxis, that is, dietary supplements, are determined by the qualitative and quantitative content of BAS. Such a requirement can be met by developing an improved technology that meets the conditions for preserving BAS in their native form, as well as reliable methods for standardizing fruits and final products (in particular, anthocyanins) [3].

Subcritical CO_2-extraction and supercritical fluid carbon dioxide extraction (SCF–CO_2) are modern and promising methods for extracting BAS from plant materials [4–11]. Subcritical CO_2-extraction is slow, cold, clean; intact, but less deep extraction matrix; limited output targeting and fractionation options.

Both of them negatively affect the safety of extracted substances – some of the thermolabile compounds decompose, thereby violating the integrity of the extraction matrix, simultaneously contaminating the final product. For example, during supercritical extraction, sugars often become caramelized, which gives the extract an almost irremovable and rather unpleasant smell and taste of burnt sugar. And increased pressure creates a specific environment, in which the substances included in the extract (both native and thermal decomposition products) enter into chemical reactions with each other, the course of which and the effect of their results on the final product is extremely difficult to predict.

Taking into account the high cost of these methods, extraction methods using selective extractants and CO_2 gas cushion to prevent oxidation processes are still relevant [12].

The objects of this study were fresh natural fruits of common blueberries (*Vaccinium myrtillus* L.), blackberries (*Rubus caesius* L.) and blackcurrants (*Ribes nigrum* L.), collected in the foothill landscapes of the city of Aparan of the Republic of Armenia, harvested in 2022.

The objectives of this study were to determine the comparative quality indicators and the total content of anthocyanins by direct spectrometry in an acidic medium at an analytical wavelength of 546 *nm* and to develop a technological method for the partial purification of the resulting extracts of fresh fruits from macro- and microelements.

Materials and Methods.

Preliminary Preparation, Fruit Squeezing and Juice Purification. As an object of study, ripe fruits of blueberries, blackberries and blackcurrants, harvested in the foothills of the city of Aparan in 2022, were used. After sanitizing the production site (class A or B) and washing fresh blueberries, blackberries and

currants (accurately weighed 1 *kg*), they were squeezed on a "Braun 700" juicer, grinding a sample of fruits to a particle size passing through a sieve with holes 1 *mm* in diameter.

Preparation of 70% Ethyl Alcohol Containing 1% Hydrochloric Acid. 6.5 *mL* of hydrochloric acid (State Pharmacopoeia of the Russian Federation, XII edition) is added to 126 *mL* of 95% ethyl alcohol and diluted with water to a volume of 200.0 *mL*.

Quantitative Determination of the Total Content of Anthocyanins in the Studied Fruits. About 1 *g* of crushed raw blueberries, blackberries and blackcurrants (accurately weighed) is placed in a 100 *mL* conical flask with a section, 50 *mL* of 60% ethyl alcohol containing 1% hydrochloric acid is added. The flask is closed with a stopper and weighed on an electronic balance with an accuracy of ± 0.001 *g*. The flask is attached to a reflux condenser and heated in a boiling water bath for 90 *min*. Then the flask is cooled to room temperature for 30 *min*, closed with the same stopper, weighed again and the missing extractant is replenished with 70% ethanol, containing 1% hydrochloric acid. The extract is filtered through a paper filter (brand "Red tape"). 1 *mL* of the obtained extract is placed in a 25 *mL* measuring flask and brought to the mark with 1% hydrochloric acid in 95% ethanol. The optical density is measured in a cuvette with a layer thickness of 1 *cm* at a wavelength of 546 *nm*. 95% Ethyl alcohol is used as a reference solution. The total amount of anthocyanins in common fruits of blueberries, blackberries and currants in percent (X) in terms of absolutely dry raw material and cyanidin-3-O-glycoside is calculated by the formula:

$$X = \frac{A \times 25 \times 50 \times 100}{m \times 1 \times 100 \times (100 - W)},$$

where A is the optical density of the test solution; m is the mass of raw material, *g*; W is the weight loss on drying in percent; 100 is the specific absorption rate of cyanidin-3-O-glucoside.

The results of processing the experiments performed show that the error of a single determination of the amount of anthocyanins in the studied fruits of blueberries, blackberries and blackcurrants with a 95% confidence level is 4.21%, 3.56% and 3.64%, respectively (Tab. 1).

Separation of Anthocyanins from a Mixture of Amino Acids, Metal Cations and Water-Soluble Compounds. For this purpose, 500 *mL* of the extract obtained with 1% hydrochloric acid in 95% ethanol was distilled off to 1/4 of the volume of the original sample (125 *mL*) and the solution was acidified to pH 2–3 by adding 50 *mL* of 2 *N* HCl. Then the resulting mixture was passed through a column with a cation exchanger in the form of Ku-2×8(H^+). After passing the entire solution (an acidic aqueous solution with a probable content of unsorbed anthocyanins was collected), the column was washed with water until neutral pH. Then anthocyanins were desorbed from the cationite surface with 1% hydrochloric acid solution in 95% ethanol. After extracting unsorbed anthocyanins from the acidic aqueous fraction with 1% hydrochloric acid solution in 95% ethanol, organic fractions were added (300 *mL*) and distilled to 1/4 of the volume of the original sample (75 *mL*).

Methods for Quantitative Determination of the Amount of Anthocyanins in the Juice of Fresh Natural Fruits of Blueberries, Blackberries and Blackcurrants. Quantitative determination was carried out using the method of direct spectrometry in an acidic medium at an analytical wavelength of 546 *nm*.

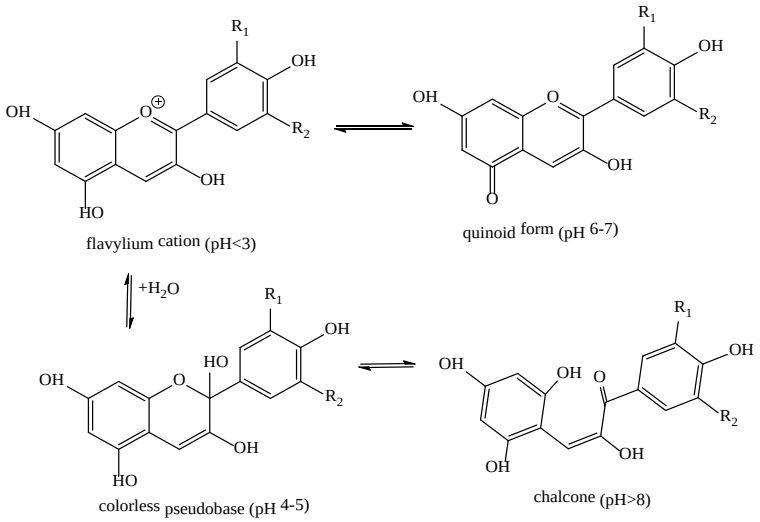
Determination of the Elemental Composition of the Studied Fruits of Blueberries, Blackberries and Blackcurrants. The elemental composition was determined by optical emission spectrometry with inductively coupled plasma using Agilent 5800 VDV ICP-OES ("Agilent Technologies") with axial and radial analysis. The sample injection system consisted of a SeaSpray nebulizer with a single pass cyclone spray chamber and injection torch "DV-d" with a diameter of 1.8 *mm*.

For the study high purity deionized water from the Milli-Q system (resistance > 18.2 *MΩ·cm*, "Millipore", Bedford, USA) was used. Before preparing the solutions, all laboratory materials were decontaminated by soaking in 10% HNO_3, then washed with high purity water. Subsequently, all materials were dried in a drying oven.

All solvents and reagents were of the highest commercially available purity. To dissolve a sample, pure 69% (v/v) HNO_3 and 30% (v/v) H_2O_2 ("Merck", Darmstadt, Germany) were used. High purity calibration standard solution ("Agilent Technologies") contained 500 *mg*/*L* Ca, K, Mg, Na, 200 *mg*/*L* Al, Ba, 100 *mg*/*L* Fe, 60 *mg*/*L* Sb, 50 *mg*/*L* Co, V, 40 *mg*/*L* Ni, 25 *mg*/*L* Cu, 20 *mg*/*L* Zn, 15 *mg*/*L* Mn, 10 *mg*/*L* Ag, As, Cr, Tl, 5 *mg*/*L* Be, Cd, Se, 3 *mg*/*L* Pb, Sr. Pb in 5% nitric acid was also used. 5% nitric acid was prepared to dilute the standard solution. The purity of argon exceeded 99.99%.

Sample Preparation. About 200 *mg* of samples were added to Teflon vessels with 8 *mL* HNO_3 69% (v/v). The mixtures were left at room temperature for about 15 *min*. The microwave heating program was applied as follows: (1) holding 20 *min* at a temperature up to 180°C; (2) 20 *min* at 180°C (Tab. 2). The vessels were then removed from the microwave rotor and cooled to room temperature. After decomposition, deionized water and 2 *mL* of 30% (v/v) H_2O_2 were added to bring the final volume to 50 *mL*. All samples were filtered off with "Blue tape" filters.

Results and Discussion. It is known that anthocyanins can exist in 4 tautomeric forms depending on the medium acidity. In an acidic environment (pH < 3) they are present in solution in the form of red benzopyrylium salts. With an increase in pH (< 4–5), the addition of a hydroxyl group in the second position occurs with the formation of a colorless pseudo-base, and at pH greater than 8.0, the chromene ring opens with the formation of the corresponding chalcone (see Scheme).



Scheme. Tautometric transformations of anthocyanins depending on the pH value.

Consequently, to prevent structural changes in native anthocyanins, all further studies of the effect of factors on the completeness of extraction of anthocyanins from fresh fruits of blueberries, blackberries and blackcurrants were performed in an acidic environment at pH 2–3. The results are given in Tab. 1.

Table 1

Influence of various factors on the completeness of extraction of anthocyanins from fresh fruits of common blueberries (No 1–6), blackberries (No 7–9) and blackcurrants (No 10–12)

No	Concentration of ethyl alcohol	Ratio of raw material : extractant	Time of extraction, *min*	The content of anthocyanins in terms of cyanidin-3-O-glucoside in fresh fruits	Note
	extractant				
1	95% (1% HCl)	1:50	120	2.42± 0.23%	infusion at room temperature
2	95% (0.1% HCl)	1:50	40	2.53± 0.21%	heating in a boiling water bath
3	95% (1% HCl)	1:50	40	4.21± 0.18%	heating in a boiling water bath
4	70% (1% HCl)	1:50	40	3.64± 0.20%	heating in a boiling water bath
5	40% (1% HCl)	1:50	40	3.39± 0.16%	heating in a boiling water bath
6	water (1% HCl)	1:50	40	3.00 ± 0.16%	heating in a boiling water bath
7	95% (1% HCl)	1:50	120	2.14± 0.23%	infusion at room temperature
8	95% (0.1% HCl)	1:50	40	2.33± 0.20%	heating in a boiling water bath
9	95% (1% HCl)	1:50	40	3.56± 0.16%	heating in a boiling water bath
10	95% (1% HCl)	1:50	120	2.39± 0.22%	infusion at room temperature
11	95% (0.1% HCl)	1:50	40	2.43± 0.23%	heating in a boiling water bath
12	95% (1% HCl)	1:50	40	3.67± 0.21%	heating in a boiling water bath

From the results of Tab. 1 it is seen that fresh fruits of common blueberries collected in the foothill landscapes of the city of Aparan contain the highest content of the total anthocyanins (4.21± 0.18%) in terms of cyanidin-3-O-glycoside. They are extracted in a boiling water bath at the ratio of raw material to extractant 1 : 50. The extraction time is no more than 40 *min*.

The worst extraction is observed when fresh unacidified water is used as an extractant (≤ 3.00).

Fig. 1 reflects the transformation of a red-colored cation of anthocyanin (pH < 3) into blue phenolates (pH 6–7), and when the acidity of the medium is stable (Fig. 2, pH < 3), no characteristic tautomeric transformation of the native structure of anthocyanins is observed.

Fig. 1. TLC image of isolated anthocyanin fractions.

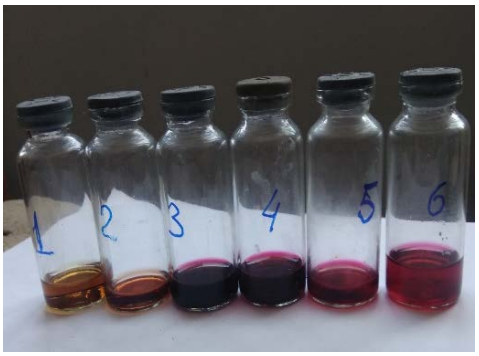


Fig. 2. Isolated fractions of anthocyanins.

To determine the degree of ecological purity of anthocyanin extracts, from acidic fractions (pH < 3) 5 *g* of dry extracts of blueberries, blackberries and blackcurrants were obtained, and according to Sample preparation the content of macro- (Fe, Cu, Zn, Ca, Mg, etc.) and microelements (Mn, Co, Cd, V, Se, Cr, As, Pb, etc.) was determined. The results are shown in Tab. 2.

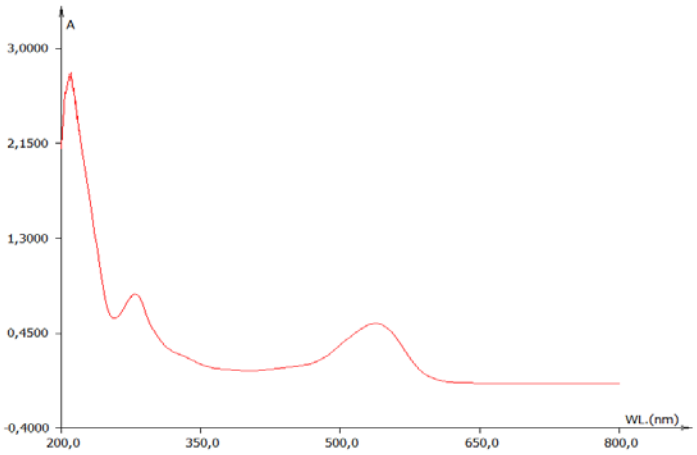


Fig. 3. Electron absorption spectrum of the total anthocyanins isolated at pH < 3 from fresh juice of common blueberries fruits.

The results of studying the elemental composition of dry extracts of fruits of blueberries, blackberries and blackcurrants prove the presence of more than 20 elements, including macro- (Fe, Cu, Zn, Ca, Mg, etc.) and microelements (Mn, Co, Cd, V, Se, Cr, As, Pb, etc.). It is shown that the content of these macro- and microelements in extracts after sorption and desorption does not exceed the permissible norms [13] and the studied extracts can be recommended for the manufacture of partially purified new galenic preparations, in particular, “Blueberry lozenges”.

Table 2

The content of chemical elements (mg/kg) in dry extracts of blueberries, blackberries and blackcurrants

Element	Dry extract of blueberries	Dry extract of blackberries	Dry extract of currants	Wavelength, *nm*
Ag	0.00	0.00	0.00	328.068
Al	49.14	43.74	52.19	396.15
As	0.05	0.09	0.10	234.984
Ba	2.83	2.98	3.06	455.403
Be	0.00	0.00	0.03	313.042
Ca	1517.45	1624.42	1689.71	422.673
Cd	0.00	0.00	0.00	214.439
Co	0.00	0.00	0.05	238.892
Cr	1.15	1.66	2.84	267.716
Cu	6.05	6.20	7.09	324.754
Fe	40.20	41.58	52.93	238.204
K	820.50	890.94	953.34	766.491
Mg	646.30	661.81	677.32	280.270
Mn	2.75	2.66	3.05	257.610
Mo	0.00	0.00	0.00	202.032
Na	1762.37	1917.45	2037.12	589.592
Ni	0.55	0.61	0.70	216.555
Pb	0.00	0.00	0.00	283.305
Sb	3.05	3.12	3.35	206.834
Se	0.00	0.00	0.00	196.026
Sr	9.08	9.14	10.62	421.552
Tl	0.00	0.00	0.00	190.794
V	0.00	0.01	0.00	309.310
Zn	3.39	3.53	4.56	213.857

Conclusion.

1. The comparative indicators of the quality and content of anthocyanins in the fruits of blueberries, blackberries and blackcurrants collected in the foothill landscapes of the city of Aparan, RA, were studied. The highest content of the total anthocyanins in terms of cyanidin-3-O-glycoside was determined in the juice of fresh fruits of blueberries – 4.21 ± 0.18%. It has been established that the completeness of anthocyanins extraction (≥95%) from fruits of blueberries is achieved at the ratio of raw material to extractant (95% EtOH, 1% HCl) 1 : 50 and when heated in a boiling water bath for 40 *min*.

2. The authenticity of comparative indicators of quality and content of anthocyanins in extracts of fresh fruits is confirmed by the method of direct spectrometry in an acidic medium at an analytical wavelength of 546 *nm*.

3. It has been shown that the content of macro- (Fe, Cu, Zn, Ca, Mg, etc.) and microelements (Mn, Co, Cd, V, Se, Cr, As, Pb) in extracts after ion-exchange sorption and desorption with the sorbent in the form of Ku-2×8 (H^+) is within acceptable limits.

4. Based on the studies carried out, the acidic extract of fruits of blueberries collected from the foothill landscapes of the Aparan City, RA, can be recommended for the manufacture of partially purified new galenic preparations, in particular, “Blueberry lozenges”.

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ԱՆՏՈՑԻԱՆՆԵՐԻ ՊԱՐՈՒՆԱԿՈՒԹՅԱՆ ՀԱՄԵՄԱՏԱԿԱՆ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆԸ ՀԱՊԱԼԱՍԻ, ՄՈՇԻ, ՍԵՎ ՀԱՂԱՐՋԻ ՊՏՈՒՂՆԵՐՈՒՄ ԵՎ ՄԱՍՆԱԿԻ ՄԱՔՐՎԱԾ ՆՈՐԳԱԼԵՆԱՅԻՆ ԼՈՒԾԱՀԱՆՈՒԿՆԵՐԻ ՍՏԱՑՄԱՆ ՏԵԽՆՈԼՈԳԻԱԿԱՆ ՄԵԹՈԴԻ ՄՇԱԿՈՒՄ

Աշխատանքը նվիրված է հապալասի, մոշի և սև հաղարջի պտուղներում անտոցիանների պարունակության համեմատական ուսումնասիրությանը՝ մասնակի մաքրված լուծահանուկների ստացման տեխնոլոգիայի մշակման նպատակով: Վերջիններս, շնորհիվ հակաօքսիդանտային, հակաագրեգատային, անոթապաշտպան, հակաբորբոքային ակտիվությամբ օժտված անտոցիանների բարձր պարունակության կիրառվում են ակնաբուժական հիվանդությունների համալիր թերապիայի մեջ (կարճատեսություն, տարիքային մակուլյար դեգեներացիա, դիաբետիկ ռետինոպաթիա):

Հետազոտության առարկա են հանդիսացել Հայաստանի Հանրապետության Ապարան քաղաքի նախալեռնային լանդշաֆտներից հավաքված հապալասի (*Vaccinium myrtillus* L.), մոշի (*Rubus caesius* L.) և սև հաղարջի (*Ribes nigrum* L.) թարմ պտուղները (2022 թ. բերքահավաք):

Հետազոտության նպատակն էր անտոցիանների պարունակության համեմատական ցուցանիշների որոշումը թարմ պտուղներից ստացված լուծահանուկներում՝ ուղղակի սպեկտրոմետրիայի մեթոդով, ինչպես նաև էկոլոգիապես մաքուր լուծահանուկների ստացումը՝ մակրո-(Fe, Cu, Zn, Ca, Mg և այլն) և միկրոտարրերի-(Mn, Co, Cd, V, Se, Cr, As, Pb և այլն) նվազագույն պարունակությամբ, իոնափոխանակային սորբցիայի և դեսորբցիայի մեթոդներով:

Ցույց է տրվել, որ անտոցիանների առավելագույն քանակ՝ ցիանիդին-O-գլիկոզիդի վրա հաշվված (4.21 ± 0.18%), հայտնաբերվել է հապալասի պտուղներից ստացված լուծահանուկում, թթվային միջավայրում, 546 *նմ* երկարության տակ: Միևնույն ժամանակ հապալասի պտուղներից անտոցիանների առավելագույն լուծահանում (≥ 95%) տեղի է ունենում հումք : լուծահանիչ՝ 1:50 հարաբերության դեպքում և եռացող ջրային բաղնիքում 40 *րոպե* տաքացման պայմաններում:

Ցույց է տրվել, որ վերը նշված մակրո- և միկրոտարրերի պարունակությունը Ky-2×8 (H^+) կատիոնափոխանակային խեժով իոնափոխանակային սորբցիայից և դեսորբցիայից հետո չի գերազանցել թույլատրելի սահմանները:

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СРАВНИТЕЛЬНЫЙ АНАЛИЗ СОДЕРЖАНИЯ АНТОЦИАНОВ В ПЛОДАХ ЧЕРНИКИ, ЕЖЕВИКИ И ЧЕРНОЙ СМОРОДИНЫ. РАЗРАБОТКА ТЕХНОЛОГИЧЕСКОГО СПОСОБА ПОЛУЧЕНИЯ ЧАСТИЧНО ОЧИЩЕННЫХ НОВОГАЛЕНОВЫХ ЭКСТРАКТОВ

Работа посвящена сравнительному исследованию содержания антоцианов в плодах черники, ежевики и черной смородины для разработки технологического

способа получения частично очищенных экстрактов, которые благодаря значительному содержанию антоцианов (соединений, обладающих антиоксидантной, антиагрегатной, ангиопротекторной, противовоспалительной активностью) обусловливают их применение при комплексной терапии офтальмологических заболеваний (миопия, возрастная макулярная дегенерация, диабетическая ретинопатия).

Целями настоящего исследования являлись определение методом прямой спектрометрии сравнительных показателей качества и содержания антоцианов в экстрактах свежих плодов черники обыкновенной (*Vaccinium myrtillus* L.), черной ежевики (*Rubus caesius* L.) и смородины (*Ribes nigrum* L.), собранных на предгорных ландшафтах г. Апаран (Республика Армения), урожая 2022 г., а также получение экологически чистых экстрактов с наименьшим содержанием макро- (Fe, Cu, Zn, Ca, Mg и т.д.) и микро- (Mn, Co, Cd, V, Se, Cr, As, Pb и т.д.) элементов способом ионообменной сорбции и десорбции. Показано, что наибольшее содержание суммы антоцианов в пересчете на цианидин-3-О-гликозид обнаружено в соке свежих плодов черники в кислой среде при аналитической длине волны 546 *нм* – 4.21± 0.18%. При этом, полнота извлечения антоцианов (≥ 95%) из плодов черники достигается при соотношении сырье : экстрагент (95% EtOH, 1% HCl)=1:50 и при нагреве на кипящей водяной бане в течение 40 *мин*. Показано, что содержание указанных макро- и микроэлементов в экстрактах после ионообменной сорбции и десорбции катионитом Ку-2×8(H^+) не превышает допустимые нормы.