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Antiproliferative and antimicrobial activity of anthocyanins after their isolation and freeze-drying

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Abstract

Natural phytochemicals in foods, including anthocyanins, can play an important role in human health. Anthocyanins have been reported to cause many various useful effects, such as reducing cancer cell proliferation, regulating blood pressure, preventing tumour formation, improving eyesight, and preventing diabetes. In this study, we aimed to reveal the qualitative anthocyanin content, antiproliferative and antimicrobial effects of different extracts derived from *Vaccinium myrtillus*, *V. corymbosum*, *Sambucus nigra* and *Aronia melanocarpa*. The anthocyanin content of the plants mentioned in the study was characterized after the freeze-drying process. MTT assay was used to determine the antiproliferative effect of extracts on cancer cells. Antimicrobial effects of extracts were studied on typical and clinical strains of 5 different bacteria. As a result, the anthocyanin content in the extracts obtained was determined to be quite good with the freeze-drying method, and it was also determined that the extracts had various levels of antiproliferative and antibacterial effects.

Keywords : Antimicrobial, berries, cytotoxic, cyanidine, elderberry, lyophilization.

Introduction

Anthocyanins are one of the most common natural pigments into the plant kingdom. They are water-soluble pigments responsible for the colours of plant foods ranging from red to purple and have a positive effect on human health and are very sensitive to changing pH and temperature. Anthocyanins are flavonoid substances belonging to the group of polyphenols. There are about 7,000 different flavonoids found in fruits, vegetables, and beverages (tea, coffee, beer, wine, and juice). Each plant species has a characteristic anthocyanin pigment that exhibits a range of colours from red to blue, hence bearing the colours of fruits and vegetables.

Anthocyanins are found in nature in the form of O-glycosides, their aglycones (non-saccharide part) are called anthocyanidins. The difference between the individual aglycon types depends on a number of linked hydroxylated groups and their methylation level. The most common anthocyanidins are delphinidin, cyanidin, peonidin, petunidin, malvidin, and pelargonidin. Galactose, glucose, arabinose, xylose, and rhamnose are the most common sugars that bind anthocyanidins in their mono-, di-, or trisaccharide forms. The core structure of the anthocyanin is a C6-C3-C6 skeleton containing a benzopyran with a phenolic ring attached to the 2 position of the pyran and is called a flavylium cation.

On the other hand, plants and compounds derived from plants have an important role in the prevention and treatment of various diseases. The beneficial effects of anthocyanins on human

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health have been known since the 16th century. For example, the use of blackberries to treat eye and mouth infections is very old [1]. Also, plant-derived anthocyanins have been extensively studied for their medicinal potential. Anthocyanins have protective effects on cardiovascular diseases as well as anti-inflammatory, anticancer, antimicrobial, anti-obesity and antidiabetic effects [2]. Cancer is an insidious disease with a high mortality rate in the human population. Cancer cells differ from normal cells in terms of morphology and function. Anthocyanins can act on cancer cells due to these differences. The most important feature of cancer cells is their uncontrolled cell division, which leads to continuous division and proliferation. Pure anthocyanins and anthocyanin-rich extracts have been reported to inhibit cell proliferation with the ability to block various stages of the cell cycle in cancer cells [3,4].
The anthocyanin properties mentioned above are the main reason for the increased interest in studies in this field in recent years. Therefore, anthocyanins derived from edible plants can potentially be considered as pharmaceutical ingredients. Among different plant sources, mulberry fruits are particularly rich in different polyphenols, including anthocyanins.
The anthocyanin content of strawberry fruits ranges from 7.5 mg / 100 mg of fresh fruit in currant (*Ribes rubum*) to 460 mg / 100 g of fresh fruit in chokeberry (*Aronia melanocarpa*) [5]. Today, new ways of preserving various biological properties of anthocyanins are sought in the pharmacology and food industries. Freeze drying is considered a suitable method for drying heat sensitive pigments. It is very modern with its freeze drying process, water extraction and product stabilization capability. This process is based on dehydration by sublimation of a frozen product, and during this procedure, the core materials and matrix solutions are homogenized and then lyophilized, resulting in a dry material [6,7].
The purpose of our study is to prepare pure anthocyanins stabilized in powder form as a food supplement and to perform qualitative and quantitative characterization of anthocyanins in selected plant species after lyophilization. In addition, it is to reveal the antiproliferative and antimicrobial effects of anthocyanins derived from different small fruits.

Materials and methods

Fruit Source

High-bush blueberry, *Vaccinium corymbosum* L.,
Bilberry, *Vaccinium myrtillus* L.,
Elderberry, *Sambucus nigra* L.
Black Chokeberry, *Aronia melanocarpa* Wild.
Fresh plant materials were obtained from local farms in Eastern Slovakia. Samples were stored at temperature of -20 ^{o}C until use.

Chemicals

Ethanol, acetone, hydrochloric, oxalic, citric or siccine acids as well as adsorbents (Amberlite XAD-7, Talcum and C18) were purchased from Sigma. All used chemicals were analytical grade.

Preparation of plant extracts

Ethanol extraction: Freeze plant materials were blended in home blender. Samples with weight of 1000 g plant material was mixed with double (weight to volume) volume of 20-96 % (volume to volume) ethanol-water solution acidified by 0-5 % of hydrochloric, oxalic, citric or siccine acids for 0.5-1.5 hour extraction with continuous mixing. Extracts were separated from plant material by filtration through a filter paper with vacuum suction using a Buchner funnel and water-flow pump. Plant material was mixed with fresh ethanol-water

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solution two more times for maximal extraction of anthocyanins. Filtrates were moved to boiling flask and ethanol was removed by rotary evaporator. Purification was carried out by mixing of 30-50 g of solid adsorbents (Amberlite XAD-7, Talcum and C18) which was activated with double volumes of ethanol and than with three volumes of acidified deionized distilled. Filtrate was mixed with 50 g of adsorbent and separated from filtrate by filtration through a filter paper. Adsorbent was flashed by two volumes of acidified water with 1% citric acid (to remove water soluble compounds – colorants, sugars, organic acids etc.) and that with two volumes of ethylacetate (to remove polyphenols). Elute anthocyanin pigment was removed from solid adsorbent by extraction with acidified ethanol-water solution. Ethanol was removed by vacuum evaporation at 38 °C.

Acetone extraction: 1000 g of material was macerated with same amount (weight to volume) of acetone. Filtrate was separated by vacuum suction using filter paper and Buchner funnel. Extraction of anthocyanins was carried by maceration of plant material with 70% (v/v) aqueous acetone (70 % of acetone and 30 % of 1% citric acid water solution) for 30 min. Filtrate was separated by filter paper and Buchner funnel and moved to a separatory funnel and mixed with double volume of chloroform, mixed by turning funnel upside down a few times. Solution was stored overnight at 4 °C. Aqueous phase (upper portion) was separated by boiling flask. Admixtures of acetone and chloroform were removed by rotary evaporation in vacuum at 38 °C.

LC-MS- IT-TOF

Identification and determination quantity of natural anthocyanins in selected plant matrices were performed by liquid chromatography on a reverse phase in conjunction with mass detection. LC-MS-IT-TOF (liquid chromatography on reverse phase coupling atmospheric pressure ionization with Ion-Trap (IT) and Time-of-Flight (TOF) technologies connected with mass detection) was used for qualitative and quantitative analysis extracts anthocyanins of these four plant materials: *Vaccinium corymbosum, Vaccinium myrtillus, Sambucus nigra* and *Aronia melanocarpa*. Technology of hybrid mass spectrometer: ion trap (IT) in conjunction with mass analyzer time-of-flight (TOF) provides high-resolution mass spectra. It also allows to make fragmentation experiments (MSn, n = 1-10), which is the basis of structural analysis of unknown substances. The results of qualitative analysis by LC-MS-IT-TOF include high-resolution mass spectra (resolution of 10 000), which is the molecular weight of each ion (the monoisotopic ion) measured to 4 decimal places of accuracy of weight measurement between 1-10 ppm. In general, mass spectra of anthocyanins obtained by electrospray ionization in positive mode abundant contained the following signals: molecular ion (M)+, the ion of antocyanidin ions representing a gradual breakdown of monosaccharide units - the neutral hexoses straight loss 162.0528 m/z respectively, pentosis with neutral straight loss 132.0423 m/z.

Samples for analysis were prepared by extraction with acetone and ethanol. The acetone extracts were purified before analysis on SPE cartridges (LiChrolut* RP-18 (40-63 um) 1000 mg/6 ml PP tubes, MERCK). The ethanol extracts were purified on sorbent Silica gel 100 C18, FLUKA. Ionization was performed using a conventional ESI source, in the positive ionization mode. The heat block and curved desolvation line (CDL) were maintained at 200 °C. Nitrogen was used as the nebulizing gas and drying gas, set at 1.5 L/min. The ESI source voltage was set at 4.5kV and the detector voltage was set at 1.56 kV.

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Anti-proliferative Activity

The human colorectal cancer cell line DLD1 was obtained from Gülhane Military Medicine Academy and it was routinely cultured in 10 % heat-inactivated fetal bovine serum (FBS), 1 % penicillin-streptomycin supported medium RPMI 1640 medium. Cells were grown at 37 ° C under 5 % CO_2 conditions. DLD1 cells were then harvested and transferred to ELISA plates and no treatment was performed before 24 hours. Anthocyanin samples were prepared with serum free medium and applied to cells at various concentrations (0.125-1 mg/ml) after then they were incubated for two-time intervals (24-48 hours). Cell proliferation experiments were performed with the MTT assay. Viable tumour cells were counted for their ability to reduce yellow dye (MTT) to a blue formazan product [8]. Four hours later, the formazan product of MTT reduction was dissolved in isopropanol and the optical density of the plates was measured at 570 nm using an Elisa microplate reader. The data obtained from the MTT test were subjected to statistical analysis and at least three replicates were performed for each concentration, and the average data were taken into account. The statistical analysis was conducted with using one-way analysis of variance (ANOVA) followed by Dunnett's test for comparison with control cells via SPSS version 23 for Mac OS (SPSS Inc., Chicago, IL, USA) and p less than 0.05 was selected as the level of significance.

Antimicrobial activity

As test cultures, the following bacteria and yeasts from the American Type Culture Collection were used: *Candida albicans* ATCC 885-653; *Staphylococcus aureus* ATCC 25923*; Escherichia coli* ATCC 25922; *Enterococcus faecalis* ATCC 29212; *Streptococcus pyogenes* ATCC 19615. We also used clinical strains of bacteria and yeasts (*S. aureus*, *E. coli*, *S. pyogenes*, *C. albicans*) isolated from the oral cavities of patients suffering from inflammatory periodontium. The sensitivity of microorganisms to anthocyanins was determined by the agar diffusion test [9]. Anthocyanins were dissolved in 1 mL DMSO. The bacterium inocula 100 µL in the physiological solution were adjusted to the equivalent of 0.5 McFarland standard, and evenly spread on the surface of Muller-Hinton agar (incubated at 37±2^{o}C for 24 hours); yeasts – on SDA agar (incubated at 35±2^{o}C for 48 hours). The extracts 20µL were introduced into wells 6 mm in diameter. The diameters of the inhibition zones were measured in millimetres including the diameter of the well. The antimicrobial effect was assessed by presence of growth inhibition zone. Each antimicrobial assay was performed at least three times.

As a positive control were used: gentamicin (10 mg/disk) for Gram-negative bacteria, ampicilin (10 mg/disk) for Gram-positive bacteria, nystatin (100 UI) for *Candida*. As negative control were used DMSO. For the results of experiment, we used statistical software Microsoft Office-Excel (2013) with the calculation of averages, error, and standard deviation.

Results

Freeze-drying is a process where frozen raw materials are placed in a refrigerated vacuum system and, without thawing, are dehydrated. The ice in the product is sublimated into water vapor. During the freeze-dried process, cell structure remains intact. The freeze-dried product also retains the color, shape, flavor and nutritional value of the original raw material better than other drying methods. It consist of four stages: sample pretreatment, deep freezing, primary drying and secondary drying. Ward & Matejtschuk [10] defined freeze drying as a drying process through sublimation. Freeze dried is considered one of the best methods of preserving the organoleptic and nutritional properties of biological products [11]. Freeze dried products

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are characterized by low water activity, low changes in volume, and shape, high rehidratation capacity, increased porosity and presenting a glassy state [12].

Qualitative Anthocyanin Content by LC-MS-IT-TOF Analyses

According to the results of LC-MS-IT-TOF analyses, we can clearly say that the ethanolic extracts contain more anthocyanins than acetone extracts. There were detected 25 anthocyanins detected in ethanol extract and 19 in acetone extract.
In our study, cyanidine-3-glukotide is a common compound in the content of all studied samples. We also detected cyanidine-3-galaktozide, malvidine-3-galactozide, malvidine-3-glukozide, cyanidine-3,5-diglukozide and cyanidine-3-rutinozide in various amounts, respectively. Cyanidin-3,5-di glucoside and cyanidin-3-rutinoside were only found in *S. nigra* samples. In addition, malvidin-3-galactoside and malvidin-3-glucoside were detected only in *Vaccinium* species. The anthocyanine contents of lyophylisates after their ethanolic and acetone extraction and identification by LC-MS-IT-TOF are given in Table 1-4.

Table 1: Anthocyanine contents of Elderberry lyophylisates in regard to ethanolic fruit extract using

Compound Assigned	Rt (min)[a]	Molecular Ions	Fragment Ions	Bilberry *Vaccinium myrtillus*	Highbush Blueberry *Vaccinium corymbosum*	Eldelberry *Sambucus nigra*	Black Chokeberry *Aronia melanocarpa*
cyanidíne-3,5-diglukoside	8,985	611,616	449,112; 287,06	-	-	●	-
cyanidíne-3-sambubiozide-5-glukoside	9,390	743,202	581,158; 449,106; 287,056	-	-	●	-
delfinidíne + hexose	9,545	465,089	303,042	-	●	-	-
delfinidíne-3-galaktoside	9,565	465,102	303,051	●	-	-	-
delfinidín-3-glukozid	10,005	465,102	303,051	●	-	-	-
cyanidín-3-galaktozid	10,300	449,106	287,055	●	●	-	●
delfinidín-3-arabinozid	10,375	435,093	303,050	●	●	-	-
cyanidín-3-glukozid	10,677	449,106	287,055	●	●	●	●
cyanidín-3-sambubiozid	10,750	581,148	287,053	-	-	●	-
petunidín-3-galaktozid	10,820	479,117	317,066	●	-	-	-
petunidín + hexóza	10,800	479,104	317,056	-	●	-	-
cyanidín-3-arabinozid	10,970	419,098	287,055	●	●	-	●
cyanidín-3-rutinozid	11,072	595,164	449,108; 287,050	-	-	●	-
petunidín-3-glukozid	11,105	479,118	317,066	●	-	-	-
pelargonidín-3-glukozid	11,260	433,113	271,060	-	-	●	-
peonidín-3-galaktozid	11,360	463,120	301,070	●	-	-	-
peonidín + hexóza	11,365	463,110	301,060	-	●	-	-
petunidín-3-arabinozid	11,420	449,108	317,067	●	●	-	-
pelargonidín-3-sambubiozid	11,485	565,133	271,046	-	-	●	-
peonidín-3-glukozid	11,690	463,124	301,070	●	-	-	-
malvidín-3-galaktozid	11,704	493,133	331,082	●	●	-	-
cyanidín-3-xylozid	11,815	419,097	287,053	-	-	-	●
peonidín-3-arabinozid	11,965	433,100	301,060	●	●	-	-

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malvidín-3-glukozid	11,955	493,133	331,082	●	●	-	-
malvidín-3-arabinozid	12,265	463,124	331,080	●	●	-	-

Table 2: Anthocyanine contents of Elderberry lyophylisates in regard to acetone fruit extract using

Compound Assigned	**Rt (min)[a]**	**Molecular Ions**	**Fragment Ions**	**Bilberry *Vaccinium myrtillus***	**Highbush Blueberry *Vaccinium corymbosum***	**Eldelberry *Sambucus nigra***	**Black Chokeberry *Aronia melanocarpa***
cyanidín-3,5-diglukozide	9,305	611,160	449,112; 287,060	-	-	●	-
cyanidín-3-sambubiozid-5-glukozid	9,835	743,202	581,158; 449,106; 287,056	-	-	●	-
delfinidín + hexóza	10,015	465,101	303,050	●	●	-	-
cyanidín-3-sambubiozid	11,050	581,148	287,054	-	-	●	-
cyanidín-3-galaktozid	10,671	449,106	287,050	●	●	-	●
delfinidín-3-arabinozid	10,705	435,091	303,050	●	●	-	-
cyanidín-3-glukozid	10,995	449,106	287,050	●	●	●	●
petunidín + hexóza	11,129	479,116	317,065	●	●	-	-
cyanidín-3-arabinozid	11,265	419,098	287,054	●	●	-	●
cyanidín-3-rutinozid	11,315	595,164	449,108; 287,050	-	-	●	-
pelargonidín-3-glukozid	11,559	433,113	271,060	-	-	●	-
peonidín + hexóza	11,660	463,120	301,070	●	●	-	-
petunidín-3-arabinozid	11,685	449,109	317,066	●	●	-	-
pelargonidín-3-sambubiozid	11,725	565,155	271,060	-	-	●	-
malvidín-3-galaktozid	11,973	493,131	331,079	●	●	-	-
cyanidín-3-xylozid	12,035	419,097	287,053	-	-	-	●
peonidín-3-arabinozid	12,215	433,111	301,070	●	●	-	-
malvidín-3-glukozid	12,240	493,131	331,079	●	●	-	-
malvidín-3-arabinozid	12,524	463,122	331,079	●	●	-	-

● – detect anthocyanin.

Table 3**:** Quantitative Anthocynin Content of Ethanol extracts by LC-MS-IT-TOF Analyses [ppm]

Compound Assigned	**Bilberry *Vaccinium myrtillus***	**Highbush Blueberry *Vaccinium corymbosum***	**Eldelberry *Sambucus nigra***	**Black Chokeberry *Aronia melanocarpa***
cyanidine-3-glukotide	153.04±29,64	1.39±0.29	240.51±50.87	52.52±2.60
cyanidine-3-galaktozide	148.91±30,50	35.42±1.03	-	356.83±84.76
malvidine-3-galactozide	43.19±8,87	65.46±11.74	-	-
malvidine-3-glukozide	144.75±31,03	3.64±0.29	-	-
cyanidine-3,5-diglukozide	-	-	46.44±2.78	-
cyanidine-3-rutinozide	-	-	8.96±0.58	-

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Table 4: Quantitative Anthocynin Content of Acetone extracts by LC-MS-IT-TOF Analyses [ppm]

Compound Assigned	**Bilberry *Vaccinium myrtillus***	**Highbush Blueberry *Vaccinium corymbosum***	**Eldelberry *Sambucus nigra***	**Black Chokeberry *Aronia melanocarpa***
cyanidine-3-glukozide	550.60±18.37	1.48±0.09	958.47±139.68	73.97±5.65
cyanidine-3-galaktozide	545.89±37.50	45.57±1.94	-	1103.49±10.99
malvidine-3-galactozide	140.95±5.24	250.49±21.70	-	-
malvidine-3-glukozide	456.42±23.98	13.88±2.54	-	-
cyanidine-3,5-diglukozide	-	-	198.08±3.88	-
cyanidine-3-rutinozide	-	-	21.62±2.65	-

MTT Assay Results

DLD1 human colorectal cancer cell line was used to determine the antiproliferative effects of different anthocyanin samples. MTT analysis shows that the viability of DLD1 cells is significantly reduced by applying anthocyanins at different time intervals. The results show that all anthocyanins have time and dose-dependent inhibitory effect on the viability of cancer cells ($p < 0.05$). Responses of DLD1 cells to increased anthocyanin concentrations were given in Figure 1-2.

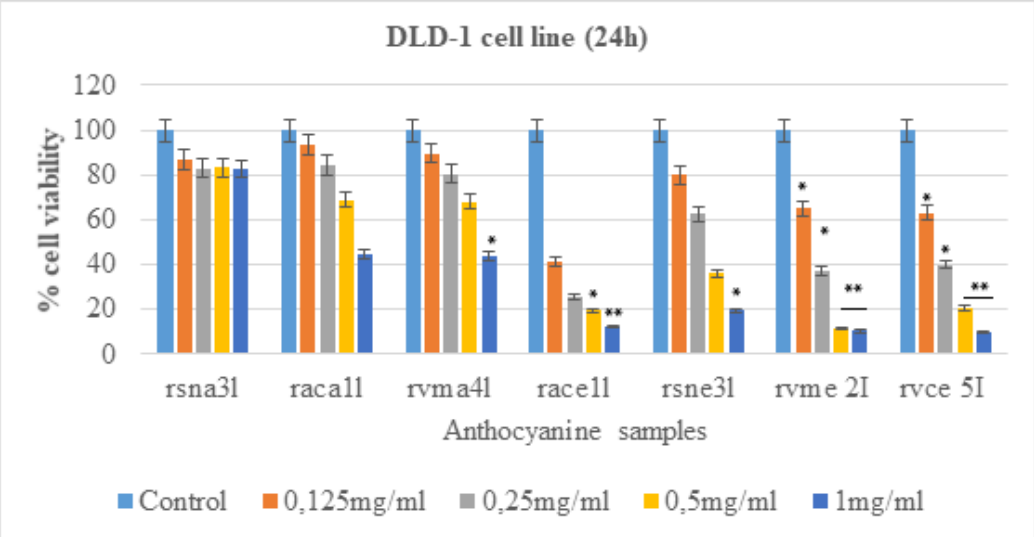

Figure 1: The antiproliferative effects of anthocyanines for 24hour application (* p<0,05; ** p<0,001)

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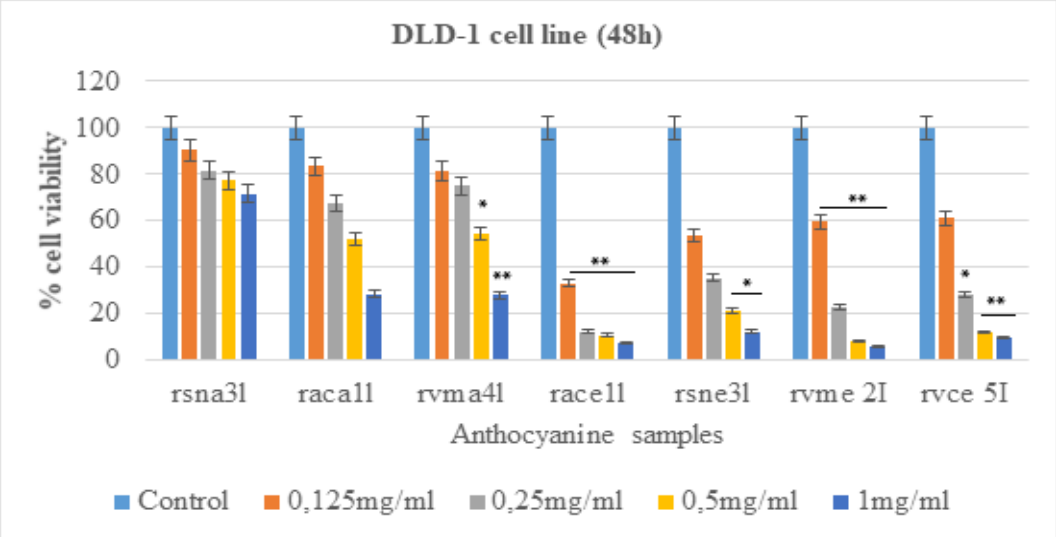

Figure 2: The antiproliferative effects of anthocyanines for 48 hour application (* p<0,05; ** p<0,001)

When we consider MTT results in general, it can be said that RACE 1I is more effective than others. This is followed by RVME 2I and RVCE 5I examples, respectively. As a general contribution, we can clearly say that ethanol extracts have a much better effect than acetone extracts for an antiproliferative effect. The IC_{50} doses of anthocyanins were given Table 5.

Table 5: The IC_{50} doses of anthocyanins on DLD1 Cells

IC_{50}	**RSNA3I**	**RACA1I**	**RVMA4I**	**RACE1I**	**RSNE3I**	**RVME 2I**	**RVCE 5I**
24h	up to 1mg/ml	0.88 mg/ml	0.875 mg/ml	0.108 mg/ml	0.370 mg/ml	0.175 mg/ml	0.196 mg/ml
48h	up to 1mg/ml	0.541 mg/ml	0.576 mg/ml	0.093 mg/ml	0.151 mg/ml	0.159 mg/ml	0.167 mg/ml

Antimicrobial activity of Anthocyanins

The results of the research showed that anthocyanins of *Aronia melanocarpa* and *Vaccinium myrtillus* revealed antimicrobial activity against clinical and reference strains of *S.aureus*. Only *Vaccinium myrtillus (acetone)* was shown to exert antimicrobial activity against reference and clinical strains of *E. faecalis*.

Antimycotic activity of anthocyanins was not revealed. Absence of antimicrobial activity of anthcyanins against *S. pyogenes* and clinical strains of *E. coli* was established.

It was further established that antocyanins of *Sambucus nigra* did not show any antimicrobial activity. Thus, *Vaccinium myrtillus (acetone)* was proved to reveal antimicrobial activity against clinical and reference strains of *S. aureus* and *E. faecalis*.

The antimicrobial activities of extracts against typical and clinical strains were given at Table 6-7 respectively.

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Table 6: Antimicrobial activity of anthocyanins against typical strains, zones inhibition in millimeters including diameter of well, mm (n=3, x ± SD)

№	Samples	*S.aureus ATCC 25923*	*E. coli ATCC 25922*	*E. faecalis ATCC 29212*	*S.pyogenes ATCC 19615*	*C.albicans ATCC 885-653*
1	*Vaccinium myrtillus* (ethanol)	9.33±0.50	-	-	-	-
2	*Vaccinium myrtillus* (acetone)	9.50±0.50	8.66±0.58	17.66±0.58	-	
3	*Aronia melanocarpa* (ethanol)	16.50±0.29	8.33±0.58	-	-	
4	*Aronia melanocarpa* (acetone)	13.33±0.58	-	12.83±0.58	-	-
5	*Sambucus nigra* (ethanol)	-	-	-	-	-
6	*Sambucus nigra* (acetone)	-	-	-	-	-
7	*Vaccinium corymbosum* (ethanol)	-	10.83±0.76	-	-	-

«-» − no inhibition.

Table 7: Antimicrobial activity of anthocyanins against clinical strains, zones inhibition in millimeters including diameter of well, mm (n=3, x ± SD)

№	Samples	*S.aureus*	*E. coli*	*E. faecalis*	*S.pyogenes*	*C.albicans*
1	*Vaccinium myrtillus* (ethanol)	9.11±0.29	-	-	-	-
2	*Vaccinium myrtillus* (aceton)	12.33±0.58	-	11.33±0.58	-	-
3	*Aronia melanocarpa* (ethanol)	13.00±1.00	-	-	-	-
4	*Aronia melanocarpa* (aceton)	10.66±0.58	-	-	-	-
5	*Sambucus nigra* (ethanol)	-	-	-	-	-
6	*Sambucus nigra* (aceton)	-	-	-	-	-
7	*Vaccinium corymbosum* (ethanol)	10.83±0.76	-	-	-	-

«-» − no inhibition.

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Discussion

Anthocyanins are natural plant pigments that have beneficial effects on animals and humans. Revealing the anthocyanin content of herbal preparations is very important both in nutritional and pharmacological terms. According to our results, we can clearly say that *Vaccinium* species possessed the highest amount of anthocyanins. Anthocyanin content may vary between different plant sources, especially berry fruits are very rich in different polyphenols, including anthocyanins. The anthocyanin content of individual fruits may also be affected by environmental factors such as fruit maturity at harvest [13]. Also, the quantity of anthocyanins can be affected by process conditions. As in our study, the freeze-drying method is the best method to protect phenolics and anthocyanins, it has been reported in many studies before [14,15]. Freeze drying is one of the useful methods to preserve colour, flavour, and nutrient compounds due to the lack of water, low pressure and temperature [16].

Anthocyanins are a water-soluble flavonoid class that exhibits a number of pharmacological effects, such as the prevention of various diseases such as cancer. Potential anti-cancer effects are reported to be based on a wide range of biological activities such as antioxidant and anti-mutagenesis effects, inhibition of cell proliferation, induction of cell cycle arrest and apoptosis.

Anthocyanins have been extensively studied for antiangiogenesis based on anticancer properties as well as in vitro and cell culture studies and animal models. Anthocyanins have been studied in detail for their anticancer properties based on in vitro studies and animal models. In the prevention of cancer, antiangiogenesis is a process that prevents the formation of new blood vessels that supply oxygen to tumour cells. Like many other phytochemicals, flavonoids and anthocyanins are potential anticancer and antiangiogenic agents [17]. Anticancer effects of anthocyanins from different herbal sources have been studied in many types of cancer, such as oesophagus, colon, breast, liver, haematological and prostate. Findings from a previous study indicate that F344 rats bound to N-nitrozo methyl benzylamine have a chemopreventive potential in freeze-dried black raspberry and anthocyanin-rich fraction [18]. Colorectal cancer is the second most common cause of cancer death and affects more than one million patients worldwide each year [19]. In this point, anthocyanins have appeared as hopeful compounds that may promote their health benefits in colorectal cancer due to their antioxidant and anti-inflammatory properties [20,21]. In this study, we aimed to reveal the antiproliferative and antimicrobial effects of anthocyanins. When the antiproliferative effect was evaluated, we obtained results consistent with previous reports [22,23]. It has been reported the anthocyanins-rich extracts from Chinese blueberry suppressed the proliferation of colon carcinoma cell lines, DLD1 and COLO-205 cells [24]. In the other study reported that cyanidin and anthocyanins-rich extracts obtained from tart cherry were able to induce a dose-dependent decrease in cell proliferation both HCT-116 and HT-29 cells [25].

Polyphenolic compounds, including anthocyanins, have antimicrobial activity against a wide variety of microorganisms, especially in the growth inhibition of pathogens [26]. Anthocyanins show their antimicrobial activities by stimulating cell damage through various mechanisms and then triggering cell destruction. Antimicrobial activity of plant phenolic compounds against human pathogens has been broadly studied to qualify and develop new useful food contents as well as pharmaceutical products. Although there is limited information on this subject, there are studies done. Burdulis, *et al.* [27] determined the total anthocyanin content in blueberry (*V. myrtillus*) and blueberry (*V. corymbosum*) and identify the antimicrobial properties of their extracts. They reported that, the extracts showed inhibitory effects on the growth of both Gram-positive and Gram-negative strains and while *C. freundii* and *E. faecalis* strains were the most susceptible, *E. coli* showed the greatest resistance among the tested bacteria. In one another study, it was reported that European cranberry (*V. macrocarpon*) extracts inhibited the growth of a wide range of human pathogenic bacteria and *L.*

monocytogenes and *E. faecalis* strains were the most sensitive, *S. enterica* ser. *Typhimurium*, and *S. aureus* were found to be of moderate resistance and *E. coli* rods were the least sensitive [28]. Genskowsky, *et al.* [29] reported maqui berry extracts had an antibacterial activity with the highest sensitivity to *Aeromonas hydrophilia* and *Listeria innocua*. These antimicrobial activities of anthocyanin-containing extracts are possible due to the multiple mechanisms and synergistic effects of various phytochemicals in the extracted content [30, 31]. Therefore, it is necessary to investigate the content well. The results we obtained in our study are compatible with previous studies.

Conclusion

As a conclusion, anthocyanin-rich products can have an important role and a protective effect on human health. However, much more studies are required to determine the true effects of anthocyanins in these health-promoting properties, because in most studies the fruit extract has been used [30]. For this reason, it is very important to know which phytochemicals or phytochemicals are active in the content and to consider the synergistic effects if any.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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