

Phytochemical Analysis of Anthocyanins Extracted from the Flowers of Two Dahlia Cultivars and their Cytotoxic Properties

Tetiana Gontova¹, Viktoria Mashtaler¹, Svitlana Romanova¹,
Ludmila Maloshtan² and Mariia Shanayda^{3*}

¹Department of Pharmacognosy and Nutriciology, National University of Pharmacy, Ukraine.

²Department of General Pharmacy, National Technical University "Kharkiv Polytechnic Institute", Ukraine.

³Department of Pharmacognosy and Medical Botany, I. Horbachevsky Ternopil National Medical University, Ukraine.

*Corresponding Author E-mail:shanayda@tdmu.edu.ua

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Dahlia Cav. is a genus of ornamental plants that belongs to the Asteraceae family. These plants are visually pleasing and contain biologically active substances such as flavonoids, hydroxycinnamic acids, organic acids, and inulin. Among these substances, anthocyanins are especially noteworthy. These water-soluble vacuolar pigments of a glycoside nature have significant health benefits, including antioxidant, antidiabetic, hypocholesterolemic, anticancer, cardioprotective, and hypotensive properties. The purpose of this study was to evaluate the content of anthocyanins by spectrophotometry and the composition by HPLC in the extracts from flowers of two varieties of dahlias ('La Baron' and 'Colorado Classic') grown in Ukraine, as well as to evaluate the cytotoxic effect of these extracts. According to HPLC analysis, among 18 revealed anthocyanins the flower of 'La Baron' cultivar had the highest concentration of cyanidin-3-O-arabinside (31.85%) and cyanidin-3-O-galactoside (23.01%) while 'Colorado Classic' accumulated more delphinidin-3-O-arabinside (39.80%) and Delphinidin-3-O-galactoside (23.15%). Our study also found that malvidin, peonidin, and petunidin played a minor role in the coloration of the flowers. The total amount of anthocyanins was slightly higher in the flowers of the 'La Baron' cultivar (1.250%) compared to the 'Colorado Classic' one (1.138%). To determine the toxicity of anthocyanins, it was used an *in vitro* model of bone marrow cells (BMC) and found that the cytoprotective and cytotoxic activities of the anthocyanins were dose-dependent. Based on these findings, the study concluded that there is potential for developing new herbal medicinal products using dahlia flowers with a significant content of anthocyanins.

Keywords: Anthocyanins; Cytotoxic Effect; Dahlia; Dry Extract; Flowers; *In Vitro* Study; Spectrophotometry; High-Performance Liquid Chromatography.

Anthocyanins are part of the flavonoid group of phenolic substances and are synthesized from anthocyanidins^{1,2}. They play a crucial role in determining the color of various plant organs, including flowers, fruits, tubers, and leaves.

Anthocyanins are essential for plant survival, as they act as optical filters and protect plant tissues from excessive ultraviolet radiation. Additionally, anthocyanins are effective in inhibiting reactive oxygen species^{3,4}. These compounds are stored in

plant cells in the form of glycosides, and their color (rose, red-purple, dark violet, or blue) depends on various factors such as the pH of the medium, temperature, and the structure of the molecule. The color also depends on the number and position of OH groups, methylation, the nature of the sugar, the amounts of molecules and their location.

There are over 1000 natural anthocyanins obtained from the anthocyanidin aglycones with different glycosylations and acylations⁵. The most common anthocyanidins in nature are cyanidin, pelargonidin, peonidin, delphinidin, petunidin, and malvidin⁶.

Anthocyanins are compounds that have a wide range of pharmacological effects and are used in medicines and as food additives⁷. The anthocyanins that have a hydroxyl group at position 3 of the C ring possess high antioxidant properties, which are further enhanced by the acylation of sugar residues^{8,9}. The ability of anthocyanins to enhance the oxidative function of mitochondria in muscle cells and brown adipose tissue affects the increase in the rate of energy metabolism in the body. Anthocyanins, by suppressing the activity of hydrolytic enzymes, slow down the hydrolysis of food, and as a result, after eating, the level of glucose in the blood rises evenly⁸. Among the delphinidin and cyanidin groups, the representatives exhibit the highest antioxidant activity and can inhibit the inflammatory process⁸⁻¹⁰. Anthocyanins are also known to restore the integrity of genomic DNA, stabilize collagen molecules, and reduce capillary permeability^{8,11,12}. Numerous studies have demonstrated the antidiabetic, hypocholesterolemic, anticancer, cardioprotective, and hypotensive effects of anthocyanins¹³⁻¹⁷. Anthocyanins reduce markers of inflammation; therefore, they are promising in the treatment of obesity and concomitant or independent diseases such as diabetes, cancer, and dysbiosis. Cardiovascular diseases are directly related to oxidative stress, metabolic syndrome, and chronic inflammation in the human body. Consuming natural products containing anthocyanins on a regular basis helps to reduce the lipid profile and blood sugar levels, which is important in the prevention of diseases of the cardiovascular system^{8,11,13,14,17}. Katsube, et al. conducted a study on blueberry extract and found that among the

isolated anthocyanins, delphinidin or malvidin as an aglycone inhibited the growth of HL60 cells by inducing apoptosis, and pure delphinidin and glycoside inhibited the growth of HCT116 cells¹⁶. It was established their ability to suppress the growth of Gram-positive (*Staphylococcus aureus*, *Streptococcus faecalis*, and *Bacillus cereus*) and Gram-negative (*Pseudomonas aeruginosa*, and *Escherichia coli*) bacteria as well as phytopathogenic (*Penicillium spinulosum* and *Rhizopus stolonifer*) and mold (*Aspergillus flavus*, *Aspergillus niger*) fungi¹⁸.

Certain plant species are currently being used as an industrial source of anthocyanins. Among them fruits of grapes (*Vitis vinifera* L.), blackberries (*Morus alba* L.), blueberries (*Vaccinium corymbosum* L.), jambul (*Syzygium cumini* (L.) Skeels), strawberries (*Fragaria x ananassa* Duchesne ex Rosier) and purple corn (*Zea mays* L.) as well as leaves of red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra*), roots vegetable of carrot (*Daucus carota* L.) and radish (*Raphanus sativus* L.) or tubers of purple sweet potato (*Ipomoea batatas* (L.) Lam.)¹⁹. Adding anthocyanins to food products not only imparts different colors, making them visually appealing, but also acts as an antioxidant, protecting the food to which they were added²⁰.

Over the past few years, researchers have taken a keen interest in studying various species of flowers (such as *Clitoria ternatea* L., *Hibiscus sabdariffa* L., *Dahlia*, *Viola* L., and others) for their anthocyanin content²¹⁻²⁵. These anthocyanins can be used in medical treatments, as well as in the food industry. Mishra and his team obtained a purple dye from dahlia flowers that they suggested could be used as an eco-friendly alternative to phenolphthalein²⁶. The anthocyanins in dahlia flowers have also been proposed as an ingredient in yogurt production. In Mexican cuisine, dahlias are used in salads, desserts, and other dishes²⁷.

Scientists are searching for plants with a high concentration of anthocyanins for the development of herbal medicines. Plants of the *Dahlia* genus, which belong to the Asteraceae family, are of particular interest in this field because they can accumulate anthocyanins in their flower petals. This genus is native to Mexico to Colombia (as shown in Fig. 1), but it is now widely distributed

around the world due to its easy cultivation. There are a total of 41 species and 15,000 varieties and cultivars in this genus²⁸.

The Dahlia species is known to contain inulin, which could be beneficial for patients with type 2 diabetes by preventing high blood glucose levels²⁹. Additionally, these plants can accumulate valuable phenolic substances, amino acids, and organic acids³⁰. The various species and cultivars of Dahlia plants allow for the selection of the most promising ones based on their chemical composition and pharmacological effects³⁰⁻³³. The color of dahlia flowers ranges from white-rose to dark purple, which is related to the level of accumulation of anthocyanins. Due to the diversity of species and cultivars, it is crucial to conduct a phytochemical analysis of plant raw materials for the quality assessment and efficacy of herbal medicines³⁴.

This study aimed to conduct a phytochemical analysis of anthocyanins in the flowers of 'La Baron' and 'Colorado Classic' Dahlia cultivars and to determine the *in vitro* cytotoxic activity of their extracts.

MATERIALS AND METHODS

Plant raw material

The study focused on the flowers of the 'La Baron' and 'Colorado Classic' Dahlia cultivars (Fig. 2) that belong to a group of 'Decorative (ornamental) dahlias' (according to the classification of The International Dahlia Register³⁵). From 2016, we have been conducting research on inulin in underground tubers and phenolic substances in above-ground organs of various dahlias^{29,30}. The research objects were selected from the botanical garden at Kharkiv Karazin University in Ukraine, taking into account the tuber weight and flower color. The 'Colorado Classic' flowers (Fig. 2, A) are light pink at the base and bright lilac at the tips, up to 15.0 cm long and 0.6-1.3 cm wide, with an elongated oval shape and smooth, pointed, concave edges. The 'La Baron' cultivar flowers (Fig. 2, B) are dark pink to purple, 7.0-10.0 cm long and 0.6-1.0 cm wide, with a flat or slightly concave shape, elongated lanceolate form, and smooth edges with a blunt top.

The plants' raw materials were collected during the flowering stage in August 2021 at the

collection site of the botanical garden named after V. N. Karazin (Kharkiv, Ukraine). Flowers were air-dried to an air-dry state by the requirements of the State Pharmacopoeia of Ukraine³⁶. The identity of dahlia cultivars has been confirmed by Yuri Gamulya, PhD in Biological Sciences, the curator of the herbarium fund of V. N. Karazin Kharkiv National University.

Spectrophotometric analysis

The total content of anthocyanins was measured using a spectrophotometric method with UV-VIS spectrophotometer Specord 200 (Analytik Jena, Germany)³⁶. Five series of raw materials of each dahlia cultivar were used to prepare the test solution. 5.00 g of raw material was crushed and sifted through a sieve with a hole diameter of 1-2 mm. Then the raw material was placed in a 100 mL conical flask and 95 mL of methanol was added, and stirred evenly for 30 min. The extract was filtered through a paper filter into a 100 mL volumetric flask. The filter was rinsed and the extraction volume was brought to the mark of 100 mL with the same solvent. A 50-fold dilution of the resulting solution was prepared in 0.1% hydrochloric acid in methanol³⁰.

Measurements were carried out at a wavelength of 528±2 nm in terms of cyanidin-3-O-glucoside chloride. A solution of 0.1% hydrochloric acid in methanol was used as a compensation solution.

This formula was used for the calculations of the total content of anthocyanins:

$$(A \times 5000) / (718 \times m),$$

where

A – optical density of the test solution;

m – weight of the sample of raw material;

718 - specific absorption rate of cyanidin-3-O-glucoside chloride.

Chromatographic analysis using high-performance liquid chromatography (HPLC)

The chromatographic analysis of anthocyanins was carried out in the acidified aqueous extracts of the studied raw materials using reverse-phase HPLC. We used a Shimadzu LC-20 Prominence liquid chromatograph (Japan), equipped with a four-channel pump LC-20AD, a column thermostat STO-20A, an automatic sampler SIL-20A, a diode matrix detector SPD20A and

a ChemStation LC20. An Agilent Technologies liquid chromatograph (model 1200) was also used. The chromatograph is equipped with a G1379A vacuum degasser, a G1313A automatic injector, a G13111A four-channel low-pressure gradient pump, a G1316A column thermostat, and a G1316A diode array detector. The results were calculated using ChemStation LC20 software.

To separate anthocyanins, a Luna C18 (2) chromatographic column (250 mm x 4.6 mm, sorbent size 5 μ m); the Phenomenex column was used. The flow rate of the mobile phase during analysis is 1 mL/min. The temperature of the column thermostat is 34° C. The volume of the injected sample is 10 μ L. Detection was performed at a wavelength of 520 nm. A mixture of mobile phases was used for the gradient elution of anthocyanins. Mobile phase A consisted of acetonitrile (for HPLC). Mobile phase B comprised a mixture of glacial acetic acid, acetonitrile, trifluoroacetic acid, and highly purified water (MilliQ) (10:0.2:87.8). The resulting solutions were filtered through a membrane filter with a pore diameter of 0.2 μ m. The elution mode is presented in Table 1.

The acidified aqueous extracts were processed to obtain the anthocyanins from plant raw material. 2.00 g of dried and crushed raw material was sifted through a sieve with a hole diameter of 1-2 mm, placed in a 100 mL round-bottom flask, poured with 50 mL of a 4% phosphoric acid solution, and kept in a boiling water bath for 30 minutes using a reflux condenser. The resulting solutions were cooled to room temperature and filtered through a red ribbon paper filter. Before the chromatographic analysis, the test samples were filtered through a membrane filter with a diameter of 0.45 μ m³⁷.

The absorption spectra were recorded during HPLC analysis. The spectral measurements were carried out in the wavelength range 200-600 nm and the volume of the injected sample was 10 μ L. To determine the total content of anthocyanins, the normalization method was used.

The contents of identified anthocyanins were determined by the area of the peaks. The content of each compound was calculated based on the total amount of anthocyanins³⁶ using the formula:

$$X, \% = \frac{A_i}{\sum A_n} \times 100$$

where: A_i – peak area calculated from chromatograms of the test solution;
 $\sum A_n$ – the sum of the pigment peak areas, calculated from the chromatograms of the test solution.

Pharmacological *in vitro* studies

To obtain a dry extract for pharmacological study, the flowers of both Dahlia cultivars were crushed to a particle size of 2-3 mm. 50% ethanol acidified with hydrochloric acid was used as an extractant. The extraction was carried out three times. The raw material-extractant ratio was 1:9 and the time of each extraction was 1 h. The obtained extracts were filtered, combined, and then evaporated in a vacuum evaporator at a temperature of 50° C. The yield of the dry extract from the flowers of the ‘La Baron’ cultivar was 12.06%, and from ‘Colorado Classic’ it was 11.34%.

The quantitative assessment of the basic *in vitro* cytotoxicity of the test substances was performed using microscopy under SOP /T/001.5^{38,39}. The rat BMC were isolated from the diaphysis of the femoral bones of animals according to the generally accepted method³⁷. The resulting suspension contained single cells in an amount of 2.0-2.1 \times 10⁶ cells/mL. Cells were incubated at 37 \pm 2° C (without CO₂). To obtain the initial solutions of the studied substances, the dry extracts were dissolved in a physiological solution (the concentration of the initial solutions was 2%). Then the initial solutions were titrated in an immunological plate using the rolling method. The following concentrations of the test substances were studied: 1%, 0.5%, 0.25%, 0.125% and 0.0625%.

Equal volumes of rat MBC suspension were added to each well of the immunological plate with the studied extracts. The native rat MBC in suspension with saline was used as a negative control. A quantitative assessment of the basic cytotoxic effect of the samples was carried out after 15, 45 and 90 min of incubation.

The quantitative assessment of included cytotoxicity was conducted by counting the number of viable/non-viable cells in the Goryaev chamber. The results were expressed as the percentage

of non-viable cells comparatively to their total number. The 0.1% trypan blue staining method was used to determine cell viability. Trypan blue is an acidic aniline dye that is unable to penetrate cells through an intact cell membrane; it selectively stains dead cells with damaged cell membranes^{37,38}.

Statistical analysis

The statistical processing of the results of quantitative determinations was carried out by the method of dispersion analysis following the requirements of the State Pharmacopoeia of Ukraine under the Microsoft Excel 7.0 program using the "Statistica" application program package³⁶.

Statistical processing of the obtained pharmacological data was processed using the Statistica 11 program using analysis of the variance of the data at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

The profile and content of anthocyanins in the studied dahlia cultivars

According to the results of spectrophotometric analysis (Table 2), the total amount of anthocyanins was slightly higher in the flowers of the 'La Baron' cultivar (1.250%) compared to the 'Colorado Classic' one (1.138%).

The UV spectra of anthocyanins in raw material of both studied cultivars are presented in Fig. 3,4.

The chromatographic profiles of anthocyanins were analyzed by the HPLC method (Fig. 5,6). The revealed anthocyanins were the derivatives of the delphinidin, cyanidin, petunidin, peonidin, and malvidin groups (Table 3). It was found that the predominant groups of anthocyanins for both studied cultivars were cyanidin and delphinidin derivatives. The content of the petunidin, peonidin, and malvidin groups was significantly lower.

Table 3 shows data on the percentage of each component among all identified. As can be seen from Table 3, a larger total amount of anthocyanins was identified in the flowers of the 'La Baron' cultivar than in the 'Colorado Classic'. Among the representatives of the delphinidin group, four glycosides were identified in the flowers of both cultivars. It should be noted that anthocyanins of the delphinidin group accumulated in a larger amount in the 'Colorado Classic' cultivar (67.7%) compared to 'La Baron' one (25.1%). Delphinidin-3-O-arabinoside and delphinidin-3-O-galactoside accumulated in the largest quantities in both cultivars. Delphinidin-3-O-rutinoside,

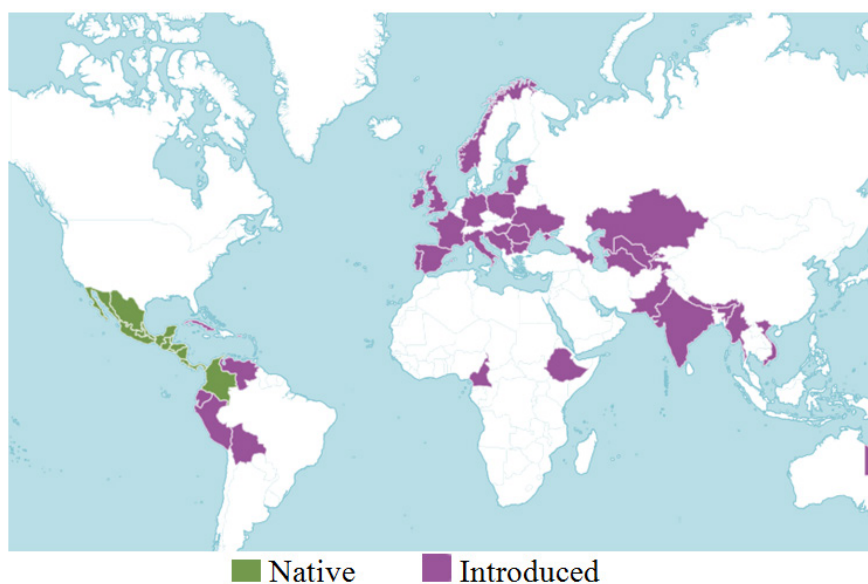


Fig. 1. The geographic distribution of the different species that belong to the *Dahlia* genus all around the world (according to 'Plants of the World online'²⁸)

delphinidin-3-O-xyloside and delphinidin-3-O-glucoside also accumulated in insignificant quantities.

In the flowers of both cultivars, five substances of the cyanidin group were identified. The total content of cyanidins in the flowers of the 'La Baron cultivar was 58.7%, and in the 'Colorado Classic' 25.7%. Among the identified cyanidins, cyanidin-3-O-arabinoside and cyanidin-3-O-galactoside accumulated in large quantities in the flowers of both cultivars compared to the other three cyanidin glycosides. The total content of petunidin glycosides was higher in the flowers of the 'La Baron cultivar (8.3% of the total anthocyanins) compared to the 'Colorado Classic one (2.6%). The total content of peonidin was 4.7% in the 'La Baron' cultivar and 1.5% in the 'Colorado Classic'. Malvidin anthocyanins were represented by a higher content in the flowers of the 'La Baron' cultivar (3.2%) compared to the 'Colorado Classic' (1.5%).

Pharmacological studies

As it is known, the *in vitro* cytotoxicity

assays are used for the initial assessment of the toxic potential of chemical or natural substances. It allows to evaluation the effects of plant extracts on cells' viability, growth, and membrane permeability.

The results of the conducted *in vitro* studies of the effect of the extracts obtained from 'La Baron' and 'Colorado Classic' cultivars of dahlia on the viability of rat BMC are presented in Fig. 7,8. It was revealed that both dry extracts showed a cytoprotective effect on cell viability at concentrations of 0.0625 and 0.125 for all three exposures of 15, 45, and 90 min.

It was concluded that the dry extract from the flowers of the 'La Baron' cultivar had a significant cytoprotective effect at a concentration of 0.0625%. Under the influence of such a minimum concentration, the number of viable cells increased by 8.0–5.0% after exposure for 45 and 90

Table 1. The elution mode in the HPLC analysis of dahlia's anthocyanins

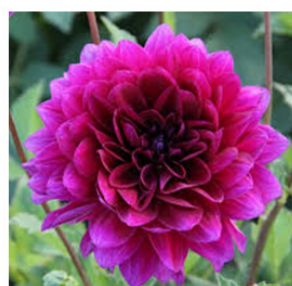
Time (min)	Mobile phase A (%)	Mobile phase Á (%)
0	0	100
5	0	100
20	20	80
25	40	60
30	0	100
35	0	100

Table 2. Quantitative content of anthocyanins in the flowers of 'Colorado Classic' and 'La Baron' cultivars of dahlia (n=5)

Cultivar	Content, %	±ε, %
'Colorado Classic'	1.138 ± 0.002 X _{cp} = 1.138 S ² = 0.0000050 S _{cp} = 0.00100 P = 0.95	0.2442
'La Baron'	1.250 ± 0.002 X _{cp} = 1.250 S ² = 0.0000053 S _{cp} = 0.00102 P = 0.95	0.2289



A



B

Fig. 2. Inflorescences of Dahlia cultivars: 'Colorado Classic' (A) and 'La Baron' (B)

min. With an increase in concentration of 0.125% in test samples using the 'Colorado Classic' extract, the number of stained cells increased to 23% (Fig. 8). The extract from the 'La Baron' flowers showed cytoprotective activity at all exposures at this concentration.

A noticeable increase in cell death by 40% was revealed after the influence of both tested

extracts at a concentration of extract 0.25% and higher (with an exposure of 90 min). A reliable cytotoxic effect of extracts from both cultivars was recorded at a concentration of 0.5%. Moreover, for the 'La Baron' cultivar (with an exposure of 90 min) the death of BMC corresponded to 67%, while for the Colorado Classic variety, it was 50%. At the maximum concentration (1.0%) of extracts,

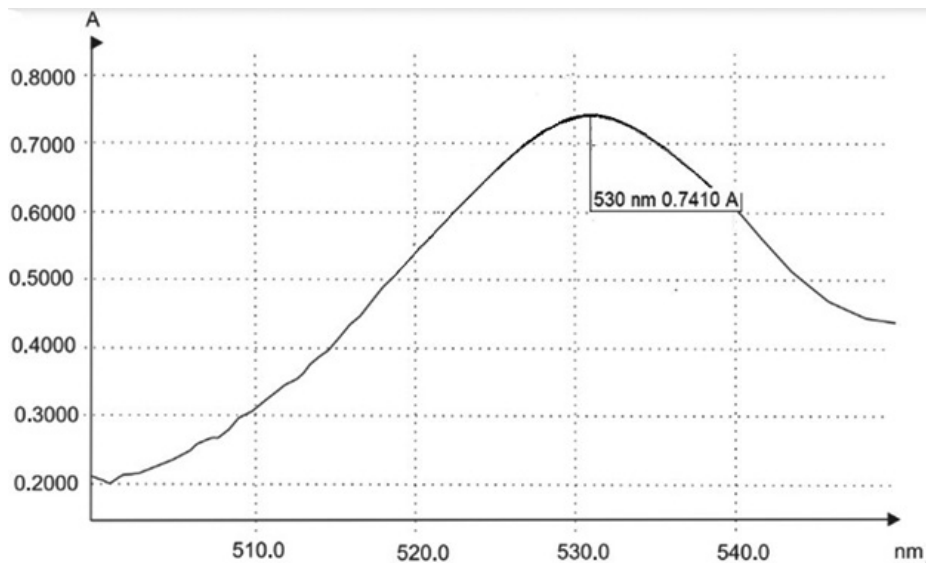


Fig. 3. UV-spectrum of anthocyanins from the flowers of the 'La Baron' cultivar

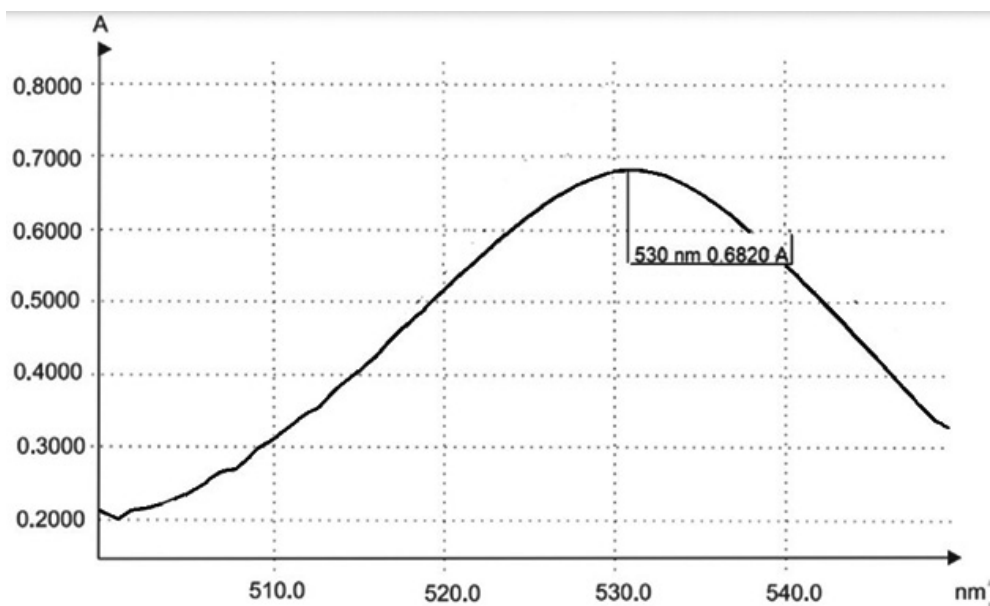


Fig. 4. UV-spectrum of anthocyanins from the flowers of the 'Colorado Classic' cultivar

a cytotoxic effect was observed at all exposures for both cultivars. The percentage of cell death in assays using extracts of ‘Colorado Classic’ and ‘La Baron’ did not differ significantly from each other.

Generally, the conducted studies made it possible to establish the *in vitro* cytoprotective and cytotoxic activities of dry extracts from the flowers of ‘La Baron’ and ‘Colorado Classic’ cultivars on rats’ BMC. The obtained results indicate that the

vital activity of BMC was influenced by both the concentration of the dry extracts studied as well as the time of exposure to the cells. At the lower concentrations of 0.065-0.25%, the ‘La Baron’ and ‘Colorado Classic’ extracts did not have a cytotoxic effect on BMC. On the contrary, they demonstrated cytoprotective activity. Thus, more than 50% of BMC died when it used the concentrations of 0.5-1.0% which revealed the presence of a cytotoxic effect.

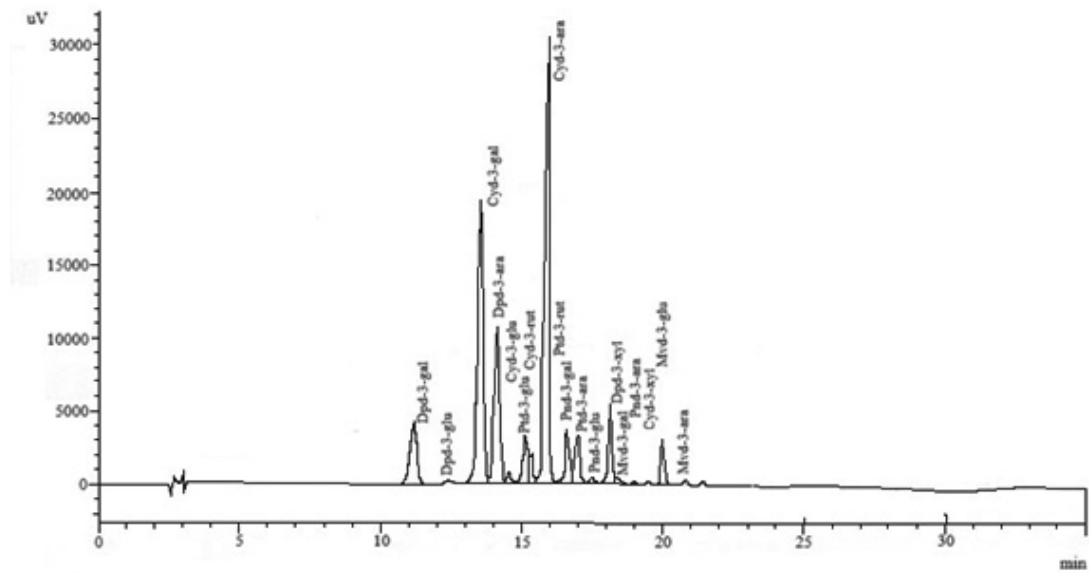


Fig. 5. HPLC chromatogram of anthocyanins extracted from the flowers of the ‘La Baron’ cultivar

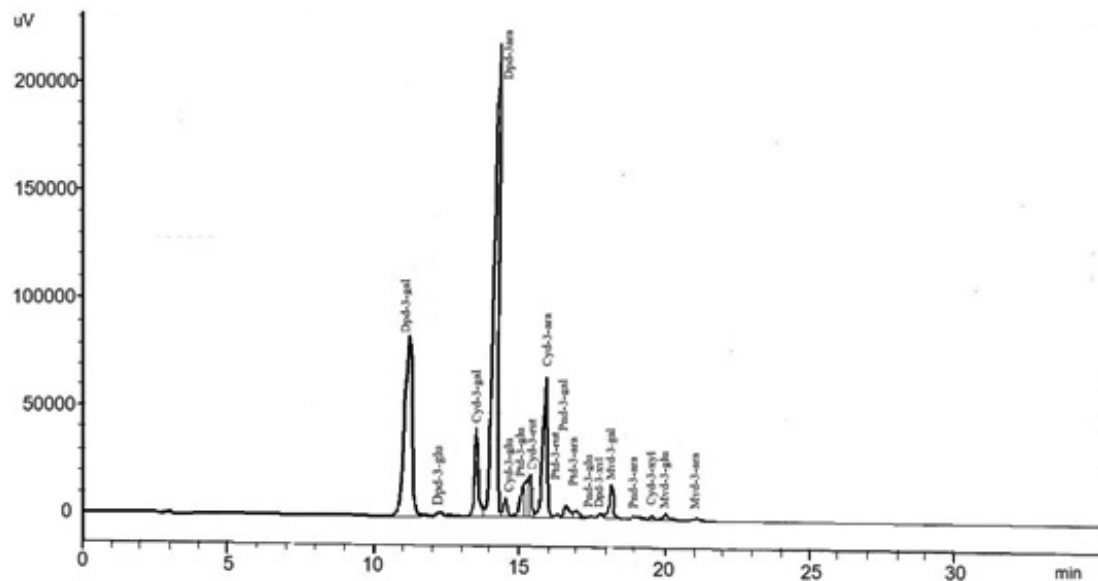
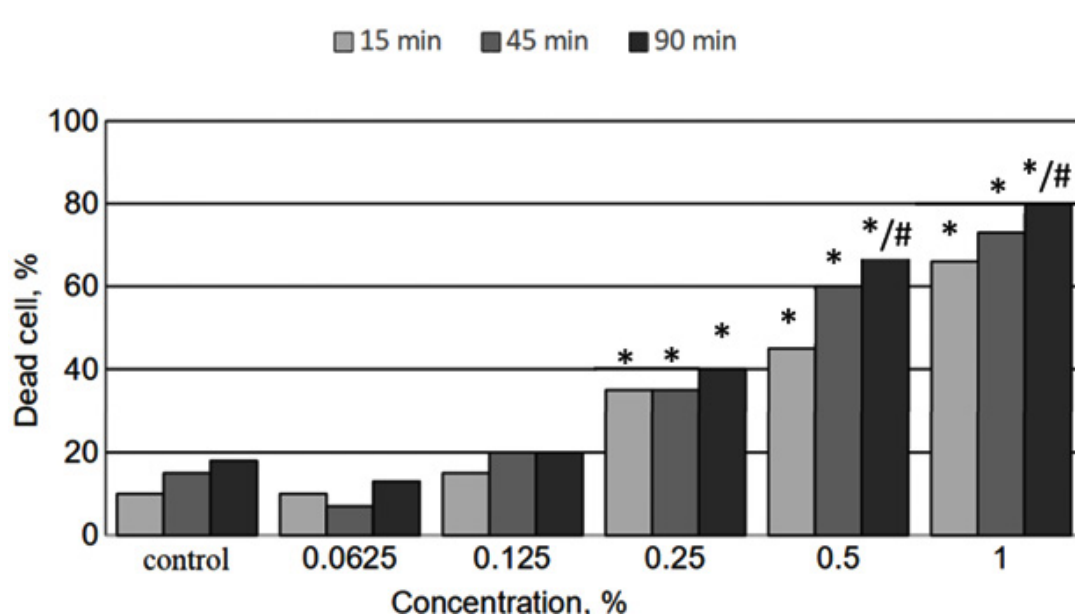


Fig. 6. HPLC-chromatogram of anthocyanins extracted from the flowers of the ‘Colorado Classic’ cultivar

Table 3. Composition of anthocyanins in the flowers of ‘La Baron’ and ‘Colorado Classic’ dahlia cultivars

Compound	Cultivars			
	‘La Baron’		‘Colorado Classic’	
	Retention time, min	Content, %	Retention time, min	Content, %
Delphinidin-3- <i>O</i> -galactoside	11.12	7.23	11.11	23.15
Delphinidin-3- <i>O</i> -glucoside	12.54	0.10	12.36	0.16
Cyanidin-3- <i>O</i> -galactoside	13.46	23.01	13.46	8.42
Delphinidin-3- <i>O</i> -arabinoside	14.05	12.05	14.06	39.80
Cyanidin-3- <i>O</i> -glucoside	14.50	0.73	14.50	1.26
Petunidin-3- <i>O</i> -glucoside	15.07	4.44	15.12	1.70
Cyanidin-3- <i>O</i> -rutinoside	15.32	1.96	15.32	2.80
Cyanidin-3- <i>O</i> -arabinoside	15.83	31.85	15.84	11.41
Petunidin-3- <i>O</i> -rutinoside	16.33	0.21	16.33	0.18
Peonidin-3- <i>O</i> -galactoside	16.58	3.80	16.59	1.28
Petunidin-3- <i>O</i> -arabinoside	16.96	3.50	16.96	0.57
Peonidin-3- <i>O</i> -glucoside	17.48	0.67	17.50	0.11
Delphinidin-3- <i>O</i> -xyloside	18.08	5.29	18.09	0.45
Malvidin-3- <i>O</i> -galactoside	18.30	0.55	18.16	1.86
Peonidin-3- <i>O</i> -arabinoside	19.00	0.14	19.01	0.03
Cyanidin-3- <i>O</i> -xyloside	19.60	0.16	19.60	0.24
Malvidin-3- <i>O</i> -glucoside	19.99	2.36	20.04	0.47
Malvidin-3- <i>O</i> -arabinoside	20.80	0.26	20.82	0.03

**Fig. 7.** Determination of the basic cytotoxicity of a dry extract from dahlia flowers of the ‘La Baron’ cultivar (n=3)

Note: 1. * – differences are significant relative to control, $p < 0.05$; 2. # – significant differences between experimental variants of 15 and 45, 15 and 90 min, successively (depending on incubation time), $p < 0.05$.

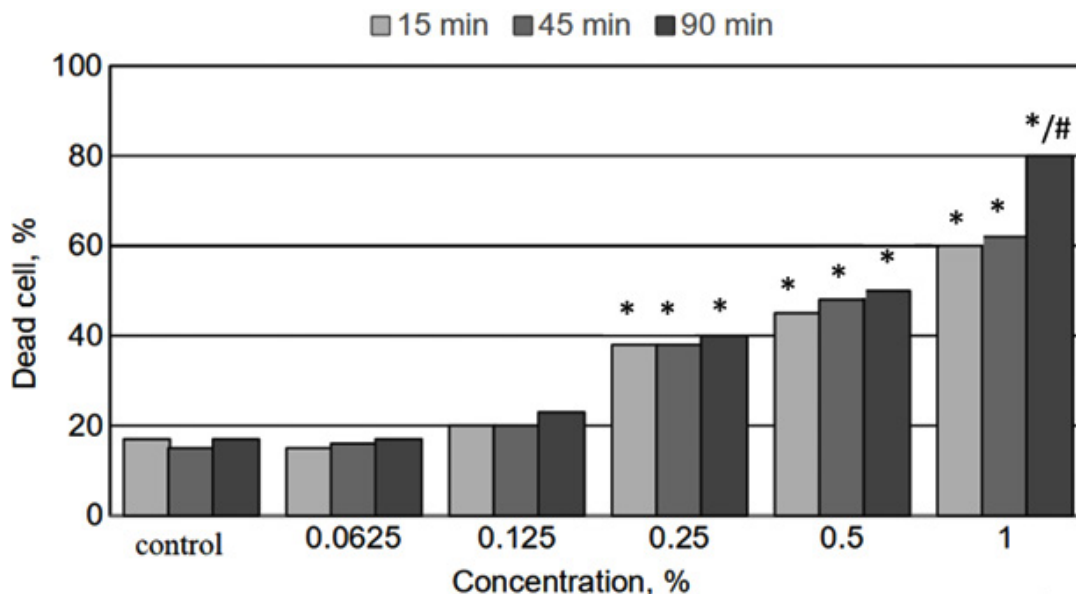


Fig. 8. Determination of the basic cytotoxicity of a dry extract from dahlia flowers of the 'Colorado Classic' cultivar (n=3)

Note: 1. * – differences are significant relative to control, $p < 0,05$; 2. # – significant differences between experimental variants of 15 and 45, 15 and 90 min, successively (depending on incubation time), $p < 0.05$.

DISCUSSION

The most common anthocyanins found in plants are cyanidin, delphinidin, malvidin, petunidin and pelargonidin^{4,20}. In fruits and vegetables, cyanidin accounts for 50% of the distribution, while delphinidin and peonidin account for 12%, and malvidin and petunidin each account for 7%²⁰. Study of black flowers of dahlia cultivars revealed that the dark color of the petals is influenced by a significant number of compounds from the cyanidin group⁴⁰. Specifically, the flowers of the 'Dandy' cultivar contained cyanidin-3-glucoside-5-arabinoside, while the flowers of *Dahlia variabilis* accumulated cyanidin-3-monoside, pelargonidin, and delphinidin⁴².

Pelargonidin glycosides, including diglucoside pelargonin and 3-monoside pelargonin, have been found in *Dahlia coecinea* and *Dahlia coronata*⁴³. Granados-Balbuena and co-authors investigated the anthocyanins profile of *Dahlia pinnata* flowers using UPLC-MS/MS. They identified four major substances, namely delphinidin-3-glucoside, delphinidin-3-rutinoside, pelargonidin-3-sambubioside-5-glucoside, and peonidin-3-sambubioside-5-glucoside²¹.

The color of anthocyanins and anthocyanidins is determined by how they absorb light in the UV and visible parts of the spectrum. When the pH is low, anthocyanins are in the form of flavylium cations, and at neutral pH, uncharged quinones are formed. Changes in pH can destroy anthocyanins and cause them to lose their color. Generally, adding hydroxyl groups to anthocyanins makes them bluer but less stable, while adding methyl groups makes them redder and more stable⁴⁵. Berries that are red, blue, violet, or purple are the richest sources of anthocyanins, containing up to 5 mg/g⁴⁶.

Recent studies have shown that delphinidin and its glycosides represent a promising group of substances for the treatment of lung, skin, liver, prostate, breast, and ovarian cancer⁴⁷, they have antioxidant and anti-inflammatory properties^{5,48-51}. Cyanidin-3-O-galactoside is a monoglycosylated anthocyanin found in fruit of chokeberries, red-skinned apples, blueberry, elderberry, and blackberry and cranberries which are an important part of the human diet. The amount of Cy3Gal varies depending on the species. The accumulation of Cy3Gal and other anthocyanins is significantly

influenced by temperature, light, and air humidity⁵². The presence of this substance is linked to the dark red or dark purple coloration of the fruits⁵³.

Rupasinghe and the co-authors discovered that the concentration of cyanidin-3-O-galactoside in apple juice can reach 39 mg/L. This juice exhibited antioxidant activity in *in vitro* ferric ion reduction antioxidant capacity and oxygen radical absorbance capacity assays. These studies suggested that cyanidin-3-O-galactoside could be used as an effective therapeutic additive⁵⁴. There is evidence that cyanidin-3-O-galactoside improves human cognitive function⁵⁵.

Lim and the co-authors conducted experiments on mice and found that an extract from the fruits of *Aronia melanocarpa*, which was rich in cyanidin-3-O-galactoside, exhibited anti-obesity properties⁵⁶. The supplementation of this extract was linked with a decrease in insulin resistance, serum triglyceride levels, low-density lipoproteins, and total cholesterol. It also resulted in a reduction in the weight of white adipose tissue. Furthermore, studies have demonstrated that anthocyanins from the purple tubers of *Ipomoea batatas* are effective in protecting the kidneys against uric acid-related disorders⁵⁷.

Certain substances act as effective antioxidants, specifically petunidin-3-O-arabinoside and petunidin-3-O-glucoside. These substances are found in high concentrations in blueberries, dark grapes, raspberries, and strawberries⁵⁶. Additionally, peonidin-3-galactoside was found in red raspberry, mango, blackberry, and blueberry, while peonidin-3-O-glucoside was found in red wine, purple onions, and red corn⁵⁸.

Malvidin-3-O-galactoside is a compound found in various blueberry varieties and is known for its antitumor activity. Studies have shown that it can inhibit the proliferative ability of Huh-7 cells¹⁹. Furthermore, it can promote cell apoptosis and inhibit cell proliferation through the regulation of p38/JNK/ERK MAPK and Akt/PTEN signaling pathways⁵⁹. Additionally, malvidin and its glycosides have properties that can help regulate blood sugar, prevent cardiovascular diseases, and improve brain function due to their antioxidant and anti-inflammatory effects.

After analyzing data from scientific sources on the content of anthocyanins in the fruits and seeds of various plants, it was found

that the black soybean varieties contained anthocyanin content ranging from 1.89-26.33 mg/g⁶⁰. The anthocyanin content of flesh berries varies significantly, depending on the species. Thus, the amounts of anthocyanins vary from 75.0 mg/g fresh fruit in *Ribes rubrum* up to 46000 mg/g fresh fruit in *Aronia melanocarpa*⁶¹.

There is scientific data that the properties and reactivity of substances are affected by their spatial structure. Stetsenko and the co-authors utilized quantum-chemical methods with modern computer software to investigate how the spatial arrangement of substances impacts their properties and reactivity⁶². Their research revealed that anthocyanins have a significant ability to counteract free radicals when proton levels are elevated. Anthocyanins are electrophilic compounds, enabling them to readily capture unpaired electrons from free radicals in chemical reactions, which explains their strong antioxidant properties. By scavenging oxygen radicals within cells, anthocyanins as well as many other polyphenols can prevent oxidative stress and protect cell membranes from damage⁶³. This suggests that the membrane-stabilizing and antioxidant effects of anthocyanins can be anticipated and potentially harnessed in certain doses.

Therefore, derivatives of cyanidin, delphinidin, petunidin, peonidin, and malvidin from plant sources could be considered very promising substances for the prevention of chronic non-infectious diseases such as cardiovascular, metabolic, and neurodegenerative disorders.

CONCLUSION

In this study, we used HPLC and spectrophotometric methods to examine the anthocyanin profiles of two dahlia cultivars: 'La Baron' and 'Colorado Classic'. The revealed anthocyanins were classified into delphinidins, cyanidins, petunidins, peonidins, and malvidins groups. A total of 18 components were identified and measured. The main coloring substances in 'Colorado Classic' flowers were the anthocyanins of the delphinidin group, while the 'La Baron' cultivar had a higher concentration of cyanidins. Both cultivars contained small amounts of malvidins, peonidins, and petunidins. Additionally, the study assessed the toxicity of dry extracts

from the flowers of the studied cultivars using the BCM *in vitro* model. The results showed that the cytoprotective and cytotoxic effects of the extracts varied with the dose administered. However, further research is required to fully evaluate the pharmacological properties of the extracts from these two cultivars.

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Conflict of interest

The authors declare no conflict of interest

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