

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Rose Flower Petals: Rich Source of Anthocyanins.

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ABSTRACT

Dried flower petals of "Tornado" floribunda rose cultivar are a highly abandoned source of anthocyanins with more than 4.0 g of anthocyanins per 100 g of the dried plant material. The main components of the "Tornado" flower anthocyanin composition were found to be 3,5-diglucosides of cyanidin (80.9 %) and peonidin (13.9 %). While in flower petals of "Shock Versilia", "New Fashion" and "Black Magic" hybrid tea rose cultivars cyaniding-3,,5-diglucoside was also the main anthocyanin, that of "Corvette" cultivar is a proper source to get the same pelargonidin derivative (68.4 %). An optimized method for anthocyanin extraction and partial purification in a "simulated moving-bed" solid phase extraction mode was proposed to get a concentrate in a mixture of 1 part of 0.1 M HCl water solution and 3 parts of ethanol with the overall anthocyanins concentration of 3.7 g/l as a cyanidin-3-glucoside chloride equivalent.

Keywords: *Rosa*, flowers, anthocyanins, extraction, purification

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INTRODUCTION

Anthocyanins belong to a subgroup of water-soluble flavonoids with health promoting properties [1]. For the great variety of plant fruits, flowers and leaves flavylum forms of the substances provides coloration ranging from orange-red to bluish-red [2]. The high antioxidant power of anthocyanins permits to regard them as prominent natural food colorants [3, 4]. While edible fruits may be utilized directly in a human diet, the other plant parts remain rarely claimed for anthocyanin extraction. However, it should be mentioned that the use of edible flowers of ornamental plants for human nutrition is an old tradition in some parts of the world [5], among the plants one can emphasize roses. Roses are the plant material that can be grown up in any climate and even on the polluted soil because the anthocyanins may be cleaned up from heavy or radioactive metal ions by solid phase extraction on reversed-phase sorbents. These are roses that are known as industrial sources of essential oil [6], hot water infusions of dried rose petals may have high antioxidant power [7] and roses petals were investigated as material for extraction and microencapsulation of red pigment [8].

According to the survey [9] anthocyanin types of roses flowers petals depend upon plant variety, being mostly cyanidin-3,5-diglucoside (Cy3,5dG), pelargonidin-3,5-diglucoside (Pg3,5dG) or peonidin-3,5-diglucoside (Pn3,5dG). The list of possible anthocyanins includes 3-glucosides (Cy3G, Pg3G) of the same anthocyanidins and the list was markedly spread after the investigation of roses petal of forty-four taxa of three sections [10] by 3-sophorosides, 3-rutinosides as well as by 3,5-diglucosides acylated with *p*-coumaric acid for cyanidin and peonidin moieties. Some uncommon blue pigments with structures similar to pyroanthocyanins were also found [11, 12].

According to literature data the overall anthocyanin accumulation in rose flowers petals may reach 600 mg per 100 g of fresh material [13] and as high as 1 % for dried petals [7], proving them to be among the most abandoned plant sources. By the way, black-colored fruit of *Rosa spinosissima* L. contains even more than 0.7 g (per 100 g of fresh fruits) of anthocyanins with cyanidin-3-glucoside (Cy3G) as a dominant type, while concentration of Cy3,5dG is more than ten-fold lower [14].

The aim of the present study was to estimate flower petals of some Rose cultivars from collection of Belgorod National Research University Botanical Garden as a source for anthocyanin production and to work out an optimized methods for extraction and purification.

MATERIALS AND METHODS

Sampling

Rose flowers were picked in the open air flower bed in Belgorod National Research University Botanical Garden and they were brought to the laboratory for investigation within 0.5 hour. The list of selected cultivars with some characteristics is presented in Table 1.

Table 1: Hybrid rose cultivars selected for the investigation

No	Cultivar	Type	Petal color
1	Shock Versilia	Hybrid tea	Pink
2	New Fashion	Hybrid tea	Earthy red with beige reverse
3	Black Magic	Hybrid tea	Dark red
4	Corvette	Hybrid tea	Orange
5	Tornado	Floribunda	Red-orange

Petals were detached, spread in a thin layer in a cartoon box and dried without direct sunlight access in the laboratory for four-five days at the room temperature (25 to 35°C). The dried material was crashed in a coffee grinder and stored in a tightly closed opaque bank in the same laboratory.

For extraction, grinded plant material was mixed with 0.1 M solution of HCl in distilled water and macerated overnight or placed into an orbital shaker at the room temperature (25°C). Then supernatant was separated from solid residue by filtration through paper filter using Büchner funnel and Bunsen flask under vacuum.

Partial purification of anthocyanins was performed by solid phase extraction on the reversed-phase sorbent of DIAPAC C18 cartridge (BIOCHEMMACK, ST, Moscow, RF) with subsequent desorption with mixture of 0.1 M HCl water solution and ethanol. Before sorption cartridges were washed with acetone (5 ml) and conditioned with 0.1 M HCl water solution (15 ml).

Qualitative and quantitative determination of anthocyanins

Overall anthocyanin concentration in solutions was determined by the spectroscopic differential method [15] as a cyanidin-3-glucoside chloride equivalent.

Analytical HPLC was performed with utilisation of Agilent Infinity 1200 equipment with diode array (DAD) and MS (6130 Quadrupole LC/MS) detectors. Chromatographic column: 4.6×250 mm Symmetry[®]C₁₈; the mobile phase: 7 vol. % of acetonitrile in distilled water, acidified with 10 vol. % of formic acid, 1 ml·min⁻¹. The electronic spectra of the anthocyanin peaks were recorded in DAD cell with a range step 0.50 nm. Mass spectra were recorded at positive ESI-mode when column 2.1×150 mm Kromasil 100-3.5C18 was used with mobile phase 10 vol. % of HCOOH and 8 vol. % of CH₃CN in distilled water, 150 ml·min⁻¹. Fragmentor voltage of 100 V was applied to get molecular ions and 150 or 200 V was applied to get fragmented ions of the corresponding anthocyanidins.

Chemicals and equipment

The mobile phases for HPLC were composed of distilled water, acetonitrile (Super Gradient, LAB-SCAN), and reagent grade formic acid (SPECTR-CHEM Ltd, RF).

Reagent grade acetone, ethanol, concentrated water HCl solution and distilled water were used in for extraction and partial purification of anthocyanins.

RESULTS AND DISCUSSION

The choice of rose cultivar

The choice was made by petals color intensity and the number of flowers per one plant to get the most anthocyanin-rich samples as well as by type of color – to find sources for different types of anthocyanins: for the roses with essential fraction of pelargonidin derivatives the petals coloring should have an orange shade. Indeed, the HPLC profiles of the petal anthocyanin complexes revealed somewhat difference between the samples under investigation, Fig.1. The main component in four cases according to electronic spectra as well as to mass-spectra (see Fig.2 and Table 2) was cyanidin-3,5-diglucoside (Cy3,5diG), while for rose petals of “Corvette” cv the pelargonidin-3,5-diglucoside (Pg3,5diG) peak area exceeded that for Cy3,5diG. The anthocyanin complexes were rather constant for all period of each cultivar flowering (from July to September). The data of the investigation are summarized in Table 2. Consequently, four (end even five) Rose cultivars are suitable sources of Cy3, 5diG, while only one cultivar may be explored for Pg3,5diG extraction.

Table 2: Relative composition of flower petal anthocyanin complexes of some rose cultivars

No	Rose variety	Mole fraction* (%) (mean ± SD)			
		Cy3,5dG	Cy3G	Pg3,5dG	Pn3,5dG
1	Shock Versilia	98.5 ± 0.3	0.9 ± 0.2	0.5 ± 0.2	tr.
2	New Fashion	93.4 ± 0.6	1.8 ± 0.4	4.8 ± 0.2	tr.
3	Black Magic	97.2 ± 0.2	1.3 ± 0.2	0.4 ± 0.1	tr.
4	Corvette	28.1 ± 1.2	0.4 ± 0.2	68.4 ± 1.0	tr.
5	Tornado	80.9 ± 0.8	3.3 ± 0.2	0.9 ± 0.3	13.9 ± 0.4
Peak parameters					
m/z		611.3 (287.1)	449.2 (287.1)	595.2 (271.1)	625.3 (301.1)
λ _{max} , nm		514.0	515.5	500.5	514.0

* - calculated as a fraction of peak areas, detected at 515 nm.

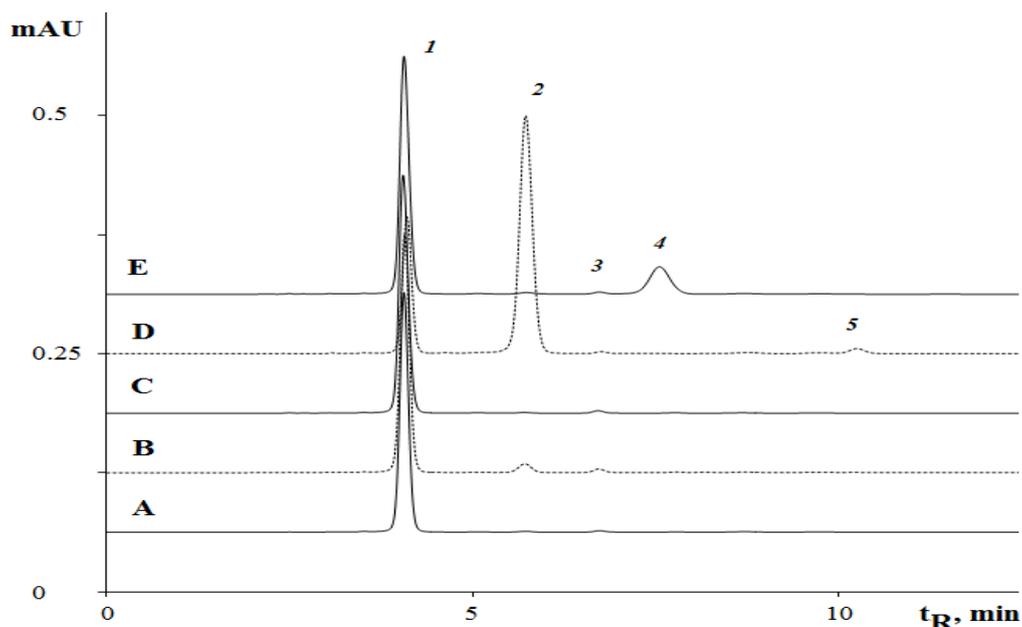


Figure 1: Chromatograms of anthocyanin complexes of rose cultivars flowers: A – “Shock Versilia”, B – “New Fashion”, C – “Black Magic”, D – “Corvette” and E – “Tornado”; 1 – Cy3,5dG, 2 – Pg3,5dG, 3 – Cy3G, 4 – Pn3,5dG, 5 – Pg3G.

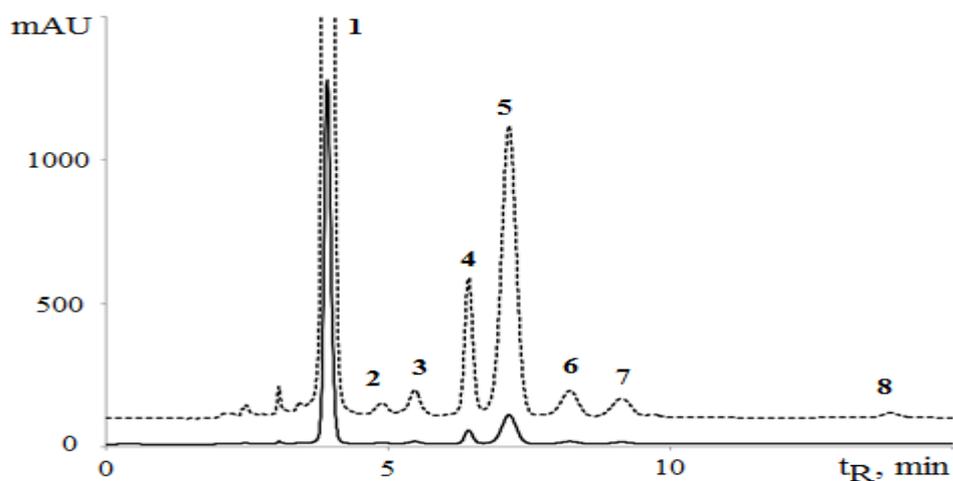


Figure 2: Chromatogram of “Tornado” cultivar rose petal anthocyanins

The “Tornado” cultivar was chosen for the further investigation because of the abundance of flowers covering plants and their dark red color indicating a high anthocyanin accumulation. HPLC profile of the extract revealed an intensive biosynthesis of 3,5-diglucosides of two main anthocyanidins, cyanidin and peonidin (Fig. 2), the latter being a particularity of the cultivar anthocyanin complex. Solutes: cyanidin-3,5-diglucoside (peak 1), pelargonidin-3,5-diglucoside (peak 3), cyanidin-3-glucoside (peak 4), peonidin-3,5-diglucoside (peak 5), unidentified components – (peaks 6 and 7), peonidin-3-glucoside (peak 8). The fraction of Pg3,5dG was less than 1%, and some other components of a trace amount were found, among them, cyanidin-3-sophoroside (peak 2 according to comparison of electronic spectra and retention times of the component and that of cyanidin-3-sophoroside from red raspberry fruit extract [16]) may be present, but cyanidin-3-rutinoside was undoubtedly absent according to comparison of retention times of the component and cyanidin-3-rutinoside from black currant fruit extract [17]).

The choice of anthocyanin extraction mode

A quick extraction of anthocyanins may be performed by homogenization of wet plant material with silica powder under acidic water media in a porcelain mortar. Acidification is necessary to prevent possible

anthocyanin destruction in contact with liberated during homogenization some cell substances. According to our experience the attempt to extract anthocyanins from purple carrot by extraction with 0.1 M HCl water solution after sample grinding was unsuccessful because of anthocyanin disappearance during grinding stage. The method has disadvantage as a consequence of extract contamination with polymeric substances that will interfere with extract filtering. To prevent pectin to be extracted together with anthocyanins alcohol may be added, though alcohol must be evaporated before solid phase extraction of anthocyanins. On the other hand it is possible to cut the sample into small pieces for a subsequent maceration. The procedure also leads to destruction of some part of cell's membranes, but the extraction of polymers may be significantly reduced. The disadvantages of the method may be a necessity of controlled time to reach equilibrium and of additional maceration stages for full extraction.

For anthocyanin extraction from plant material "acidic water – alcohol" mixtures with different ratios were investigated. Acidic water was 0.1 M solution of HCl devoted to keep anthocyanins in the most stable flavilium form (pH < 4.5), while alcohol was added to precipitate pectin. Maceration of dried petals in the solutions was performed at solvent-to-solid ratio 0.75 : 50 (g·ml⁻¹). The mixture was left for maturation overnight. The results for duplicate sample maceration are presented in Table 3.

Table 3: Influence of solvent composition upon efficiency of anthocyanins extraction

No	Vol. % of solvent fraction			Anthocyanins yield (g per 100 g of dried material)*		
	0.1 M HCl in water	Ethanol	Acetone	1 step	2 step	Summ
1	100	0	0	3.53 ± 0.07	0.38 ± 0.03	3.91 ± 0.05
2	80	20	0	3.99 ± 0.12	0.20 ± 0.06	4.19 ± 0.08
3	60	40	0	4.11 ± 0.05	0.14 ± 0.02	4.25 ± 0.04
4	40	60	0	3.91 ± 0.09	0.14 ± 0.02	4.05 ± 0.07
5	20	80	0	4.12 ± 0.07	0.21 ± 0.02	4.33 ± 0.05
6	20	0	80	2.07	0.11	2.18

* - mean values for two parallel measurements

It becomes evident that maceration for the taken solvent-to-solid ratio by one step is not acceptable because of low yield of anthocyanins. But according to the data the yield of anthocyanins for two-step extraction was close to all ratios of water 0.1 M HCl – ethanol mixtures. Moreover, all extracts were easily filtered under vacuum. Thus, there is no sense of ethanol addition – it is a first evident and favorable feature of rose anthocyanin extraction. By the way, acetone should be used carefully for extraction because of possibility of direct reaction with anthocyanins [18] in spite of proposition [19]. The losses of anthocyanin may reach 50 % at least for the subject under investigation.

The choice of "solid to solvent ratio"

For the choice of efficient "solid to solvent ratio", extraction was performed with 0.1 M water HCl solution without ethanol addition. The mixture of dried petals and solvent was left for maceration overnight.

Table 4: Influence of "solid to solvent ratio" upon efficiency of anthocyanins extraction

N	Solid to solvent ratio (g:ml)	Anthocyanins yield,* (g per 100 g of dried material)			
		1 step	2 step	3 step	Summ
1	1:100	3.98 ± 0.12	0.29 ± 0.07	0.06 ± 0.03	4.33 ± 0.15
2	1:50	3.82 ± 0.09	0.36 ± 0.07	0.12 ± 0.04	4.30 ± 0.12
3	1:25	3.48 ± 0.11	0.47 ± 0.09	0.21 ± 0.02	4.16 ± 0.15
4	1:12.5	2.60 ± 0.07	0.90 ± 0.09	0.45 ± 0.08	3.96 ± 0.15

* - mean values for two parallel measurements

According to the data summarized in Table 4 the ratio 1:100 may be explored quite effectively with two extraction steps, while for another ratios examined the third stage of extraction must be utilized to avoid significant anthocyanin losses.

It should be mentioned that no difference between anthocyanin type compositions for consecutive extraction stages products was found. Moreover, a complete anthocyanin extraction may be controlled by entirely white color of the flower petals solid residue.

The choice of maceration time

For the choice of effective maceration time extraction of the powdered dried petals in 0.1 M HCl water solution was investigated under reflux with utilization of orbital shaker at the room temperature (25°C). After the determined time refluxing was stopped and the mixture was filtered under vacuum before measurement of the overall anthocyanin concentration. The results of the investigation for solid-to-liquid ratio 1 : 100 are summarized in Fig 3.

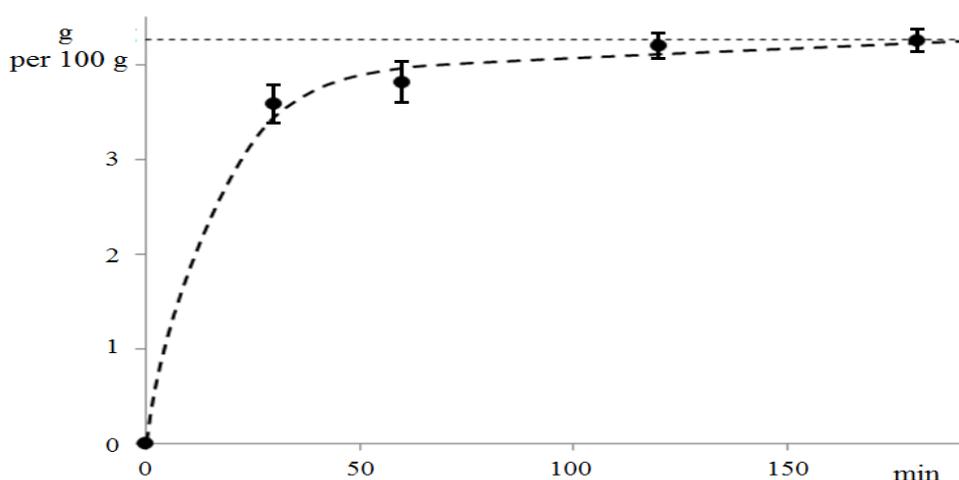


Figure 3: Yield of anthocyanins as a function of maceration time

It is evident that duration of maceration may be restricted to two hours without previously utilized overnight maceration.

Partial purification by solid phase extraction in “simulated moving bed” technique

For solid phase extraction DIAPAC C18 syringe cartridges were applied in a “simulated moving-bed” technique [20]. The cartridges are usually used for solid phase extraction by passing the solution of the anthocyanins through the cartridge until the appearance of the first colored drops of eluent. This technique permits to get representative results after subsequent exhausting re-extraction of the solute from the stationary phase by a proper eluent, though a full sorption ability of the sorbent is not reached. Meanwhile it is possible to connect a new cartridge in a series with the first one just before the appearance of the colored eluate, so the saturation of the first cartridge may continue, especially in the case of utilization of a third cartridge by the same manner. Then before the connection of the fourth cartridge the first one that becomes saturated may be detached for re-extraction of the solute.

We used a mixture of ethanol – 0.1 M HCl water solution (3 : 1 vol.) for the elution of anthocyanins from the cartridges to get a solution with anthocyanin concentration of 3.7 g/l as cyanidin-3-glucoside chloride equivalent.

The cartridge after anthocyanin removal was rewashed by acetone (5 ml), then conditioned by 0.1 M HCl solution in water (10 ml) solution and so prepared for cyclic utilization for anthocyanin clean up.

CONCLUSIONS

Dried petals of flower of Tornado rose cultivar are the abandoned source of anthocyanins that permits a simple extraction and partial purification for production of food colorant.

ACKNOWLEDGMENT

The investigation was performed under financial support of Task № 4.875.2014/K of the Ministry of Education and Science of Russia.

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