Phytochemical Study of the North Caucasian *Rosa Spinossima L.* Fruit

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Qualitative composition and content of anthocyanins in the *Rosa spinossima L.* fruit were studied. The anthocyanin sum was isolated by talc adsorption with subsequent desorption. The presence of anthocyanins in the resultant fraction was confirmed by thin layer chromatography. Three spots corresponding to anthocyanins were found. Anthocyanins were identified by mass spectrometry. Ion peaks corresponding to glycoside cyanidin and delphinidin were found. Position of sugar in the glycoside structure was determined by UV spectrophotometry with aluminum chloride. The content of anthocyanins was measured by direct UV spectrophotometry by the anthocyanins absorption. Anthocyanin content in raw material in conversion to cyanidin-3-glucoside was 1.46±0.03%.

Key Words: *anthocyanins; Rosa spinossima L.; cyanidin; delphinidin*

Natural flavonoid compounds are highly prevalent in the flowering plants, their chemistry and pharmacology is sufficiently well studied, and they are easily available sources of therapeutic means. These compounds include anthocyanidins and their glycosides anthocyanins, flavilium cation derivatives responsible for the most bright colors of the plants (intensely red, purple, and blue flowers and fruits).

Anthocyanins belong to the polyphenol compounds of plant origin most important for humans. They are used as food dyes and as bioactive compounds, as components of drugs and bioactive additives. Abnormally high antiradical activity of anthocyanins several-fold surpassing this activity of flavonoids of other classes [4] is worthy of note.

The following pharmacological effects of anthocyanins have been proven: vasoprotective (reducing capillary friability and permeability, thus improving endothelial function), antioxidant (LPO inhibition), anti-inflammatory (promoting stabilization of collagen production, inhibiting platelet aggregation, and stimulating prostaglandin production by the endothelium, this, in turn, leading to vasodilatation), and antiedematous (reducing vascular permeability) [2].

Few medicinal plants contain anthocyanins, and hence, studies and detection of prospective sources of anthocyanins is an important problem.

We studied *Rosa spinossima L.* as a prospective source of anthocyanins; this plant is little studied and grows in the Northern Caucasus. It is used in popular medicine for therapy of neurosis, hepatobiliary diseases, nephritis, essential hypertension and atherosclerosis, asthenia, anemia, acute and chronic infections, hyperthyrosis, hemorrhages. The plant is effective in enteric diseases: pectins protect the mucosa from harmful and toxic products forming in putrefactive processes, arrest inflammation, and reduce peristalsis [1].

We studied the qualitative and quantitative composition of anthocyanins in *Rosa spinossima L.* fruit.

MATERIALS AND METHODS

The anthocyanin sum was isolated by talc adsorption [3]. Raw material (50 g) dried in air, fragmented, and sifted through a sieve with 3-mm holes, was poured

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over with 1% hydrochloric acid in 96% ethanol and extracted for 24 h. The extract was removed and a new portion of the extracting agent was poured. Five extracts were thus prepared. After filtration all five extracts were pooled and the resultant extract was condensed under vacuum on a IR-1 rotation evaporator to the minimum volume. In order to extract pure anthocyanin sum, the resultant fraction was subjected to selective adsorption on talc. It was mixed with a sufficient amount of talc to obtain a sort of a liquid paste. This mixture was transferred to Buchner funnel with a paper filter. The funnel was connected to Bunsen flask with an aspirator and the mixture washed in vacuum until washout water was transparent. Washout water was removed from Bunsen flask and anthocyanins were adsorbed with 1% hydrochloric acid solution in 96% ethanol from the precipitate remaining on the filter and washed till the water was colorless. The resultant solution was evaporated in vacuum on an IR-1 rotation evaporator. Anthocyanin-rich complex was thus obtained. The resultant fraction was subjected to qualitative analysis for anthocyanins.

The presence of anthocyanins was verified by thin layer chromatography on Silufol plates in the ethylacetate:glacial acetic acid:formic acid:water system (100:10:10:26). The mass spectrum was detected on an Autoflex II MALDI TOF/TOF mass spectrometer (Bruker Daltonics) – a vacuum device based on the physical laws of charged particles movements in magnetic and electric fields – using laser ionization.

RESULTS

Three red spots were seen on the chromatogram. The upper spot presumably corresponded to the anthocyanin aglycon. These components were identified by mass spectrometry. An important feature differing mass spectrometry from other analytical physicochemical methods is that it directly detects the particles of the substance, while optical, X-ray, and some other methods detects the radiation or energy absorption by molecules or atoms.

A specimen of the resultant anthocyanin sum was applied onto the MTP 384 target plate matt steel T F, dried, and a droplet of the matrix was applied onto it. α-Cyanocinnamic acid served as the matrix. The spectra were recorded using Flex Control software. The data were processed using Flex Analysis software. The resultant spectrum showed intense peaks of ions with m/z=287.316 charge, corresponding to cyanidin aglycon, and m/z=449.214 charge, corresponding to its glycoside form (3-glycoside) (Fig. 1).

In addition to cyanidin-3-glucoside, mass spectrometric analysis detected delphinidin glycoside. The spectrum showed two intense peaks of the ion with

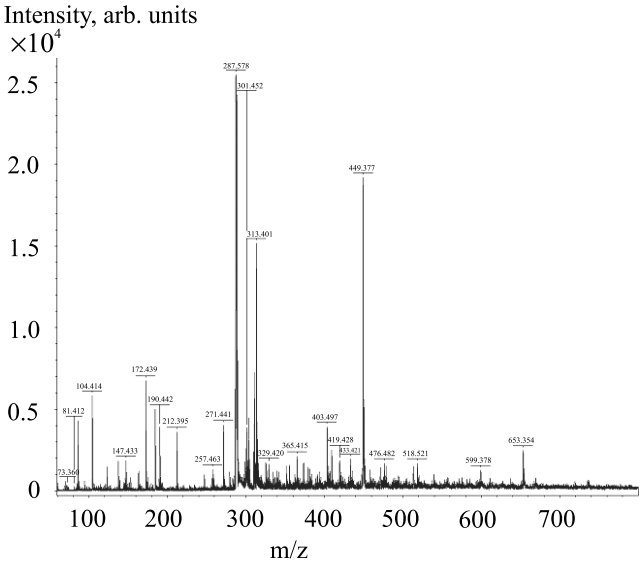

Fig. 1. *Rosa spinossima L.* cyanidin glycoside mass spectrum.

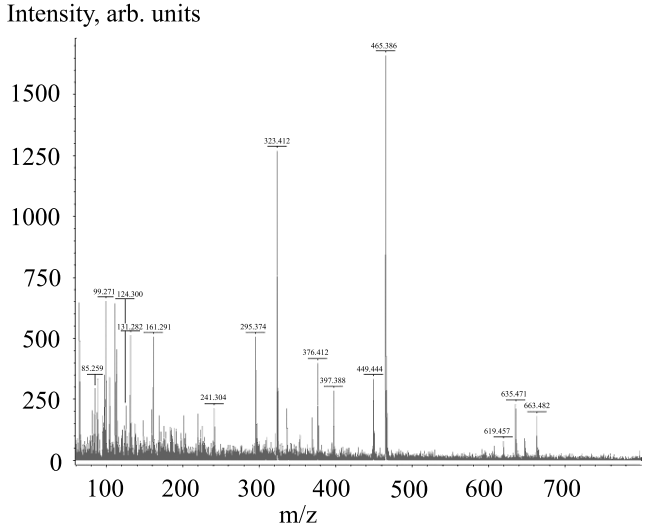

Fig. 2. Mass spectrum of *Rosa spinossima L.* delphinidin glycoside.

charges m/z=323.420, corresponding to delphinidin aglycon sodium form, and m/z=465.390, corresponding to its glycoside form (Fig. 2).

Sugars were located in the anthocyanin glycoside structure by UV spectrophotometry with a shift reagent (5% ethanol solution of aluminum chloride). It is known that complex compounds form during the reaction of flavonoids with aluminum chloride, as a result of which a strong bathochromic shift of absorption bands is seen in the absorption spectra. A 40-50 nm shift was seen for anthocyanins with a free *ortho*-dioxygroup in ring B during the reaction with aluminum chloride.

In order to detect the free *ortho*-dioxygroup in ring B, the UV spectrum of the net anthocyanin sum was first recorded; a single absorption maximum was

recorded at λ=530 nm. Addition of 5% aluminum chloride in 96% ethanol led to a 40 nm shift of the absorption maximum (Fig. 3), indicating the presence of a free *ortho*-dioxygroup in ring B, while the sugar part of cyanidin and delphinidin was located presumably in position 3.

Measurements of anthocyanins in the studied raw material were carried out by direct UV spectrophotometry by the characteristic absorption at λ=490-550 nm. The material (1.0 g), fragmented and sifted through a sieve with 3 mm holes, was put into a flat-bottomed flask (100 ml) and poured over with 20 ml solvent (1% hydrochloric acid in 96% ethanol), connected to a heater, and warmed in water bath for 30 min from the moment of boiling beginning in the bath. The resultant extract was cooled and filtered into a 100-ml volumetric flask. The residue was extracted 4 times more by 20-ml portions, the filtered extract was collected into the same 100-ml flask. After cooling the volume of the extract was brought to 100 ml with the extracting agent and mixed (solution A). Aliquots of 2 ml were collected with a pipette from solution A, transferred into a 25-ml volumetric flask, and the volume was brought to 25 ml with the extracting agent (solution B). Solution B was subjected to photometry on an SF-56 spectrophotometer at λ=450-600 nm; 1% hydrochloric acid solution in 96% ethanol served as the reference solution.

The anthocyanin sum in the raw material in conversion to cyanidin-3-glycoside was calculated by the formula:

$$X=\frac{A\times W_1\times W_2\times M\times 100}{\varepsilon\times 1\times a\times V\times(100-B)\times 100},$$

where X is anthocyanin content in conversion to cyanidin-3-glucoside (in %), A is optical density, W_1 is total volume of extract from raw material (ml), W_2 is volume of the extract after dilution (ml), a is weight of raw material (g), V is aliquot collected for dilution (ml), M is molecular collected for dilution (ml), M is molar weight of cyanidin-3-glycoside, equal to 449.17, 1 is the thickness of the cuvette (cm), ε is molar coefficient of absorption (26,900), and B is wetness of raw material (7.9-8.1%).

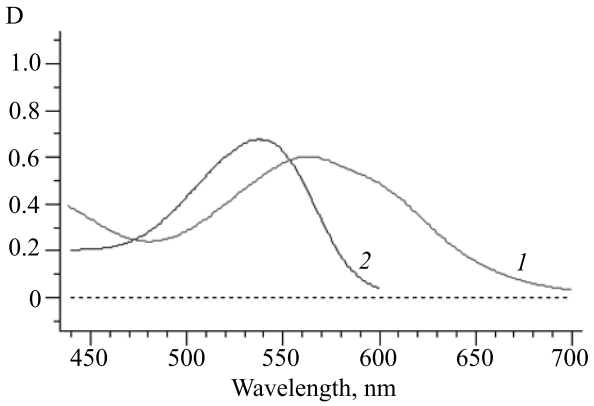

Fig. 3. UV spectrum of absorption of *Rosa spinossima L.* anthocyanin sum (*1*) reacting with aluminum chloride (*2*).

The content of anthocyanin sum in the raw material in conversion to cyanidin-3-glucoside was 1.46±0.03%.

Our data indicated good prospects of using *Rosa spinossima L.* fruit as a source of anthocyanins in medicine.

REFERENCES

1. *Valuable Wild Plants of Russia*, Eds. A. L. Budantsev, E. I. Lesiovskaya [in Russian], St. Petersburg (2001).
2. D. Yu. Korulkin, Zh. A. Abilov, R. A. Muzychkina, and G. A. Tolstikov, *Natural Flavonoids* [in Russian], Novosibirsk (2007).
3. A. V. Ptitsyn, A. P. Kaplun, E. I. Mukhtarov, and V. I. Shvets, *Biotekhnologiya*, No. 2, 22-28 (2007).
4. N. A. Tyukavkina, S. E. Zurabyan, V. L. Beloborodov, *et al.*, *Organic Chemistry. Manual for Higher School*, Ed. N. A. Tyukavkina [in Russian], in 2 books, Book. 2: *Special Course*, Moscow (2008).