The Study of Composition and Content of Anthocyanins and Flavonoids of Fruits of the *Sambucus Nigra* L. (*Sambucaceae* Botsch ex Bork. Family)

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It was investigated the quantitative content and qualitative composition of anthocyanins and flavonoids of mature fruits of *Sambucus nigra* L. growing in Azerbaijan. It was established that the content of anthocyanins varies from 3.12 to 4.02% depending on the site of occurrence, and flavonoids vary from 108 to 186 mg%. It was found the presence of 3 derivatives of cyanidin - cyanidin-3,5-di-glucoside, cyanidin-3-glucoside, cyanidin-3-sambubioside of anthocyanins. In the flavonoid composition, 4 flavonoids were identified, of which 3 were isolated in an individual form and found quercetin derivatives - quercetin-3-glucoside, quercetin-3-rutinoside.

Keywords: *Sambucus nigra L., anthocyanins, flavonoids, chromatographic and spectral analysis*

INTRODUCTION

Plants rich in polyphenolic compounds, in particular anthocyanins and flavonoids, are widely used in the medical industry as a raw material for the preparation of a medicinal product. Flavinoids and anthocyanins have the ability to show the strength and elasticity of blood vessels, especially capillaries, to provide preventive and curative effects in atherosclerosis, hypertension, radiation damage, capillary toxicosis (Hertog et al., 1995; Wu et al., 1997) Along with P-vitamin activity, they possess antiradical (Makarova et al., 2005; Cody et al., 1998), antioxidant (Novruzov, 1998; Zhao et al., 2003; Bagchi et al., 2004; Gulcin et al., 2005; Kano et al., 2005), antimutagenic (Junior et al., 2005) anticarcinogenic (Cody et al., 1998), insulin-active (Jayaprakasam et al., 2005) and interferon activity (Asadullaev et al., 1987). Preparations and biologically active additives containing flavonoids and anthocyanins are used in the treatment of various cardiovascular and other diseases.

It is known that various elderberry organs are used in folk medicine to treat a number of diseases (Kiosyev et al., 2000; Schwaiger et al., 2011). The most widely used mature fruit in food and medicinal purposes (Schwaiger et al., 2011; Youdim et al., 2000). Anthocyanins of elderberry fruits are very effective in vascular diseases (Harborne, 1958; Novruzov, 2010) and diseases associated with stress (Youdim et al., 2000). The extract of elderberry fruits has an inhibitory effect on cyclooxygenase-2, which is very important for cancer chemotherapy, especially colorectal adenocarcin (Jing et al., 2008).

Flora of Azerbaijan is rich with wild medicinal plants (1547 species). Most of them contain a sufficient number of biologically active substances (BAS). Among them, a special place is occupied by elderberry species, which are widely distributed in Azerbaijan and have a sufficient quantity of raw materials (fruits).

The purpose of this work is to study the content and quality of anthocyanins and flavonoids in mature black elderberry fruits.

MATERIALS AND METHODS

The material of the study was the ripe black elderberry fruits harvested in the Uzen village of the Guba region in 2016. Freshly picked ripe fruit (1.0 kg) was passed through a juicer. The residue was treated 3 times with ethyl alcohol containing 1% concentrated hydrochloric acid in a ratio of 1: 2, at a temperature of 45-50°C. The extraction time was 2 hours. The combined juice and extract were filtered and evaporated in vacuo at 30°C to an aqueous residue. The aqueous residue was successively treated with ether and ethyl acetate. The content of flavonoids was determined by the method of Petrechenko (Petrecenko et al., 2002) the anthocyanins by the method E.N.Novruzov (Novruzov, 2005). The qualitative composition was determined by paper chromatography. Individual anthocyanins were prepared by column chromatography. Paper FN-15 (Germany) was used for paper chromatography. Adsorbent for the separation of anthocyanins was a cellulose powder activated with hydrochloric acid, given in the work of E.N.Novruzov (Novruzov et al., 1988). The flavonoids were separated on a polyamide powder Wolem. The following solvent systems were used to separate the anthocyanins and flavonoids by paper chromato-

graphy: I butanol-acetic acid-water 4: 2: 1, II acetic acid-conc. HCl-water 15:3:82, III formic acid-conc. HCl-water 5:2:3, IV acetic acid-conc. HCI-water 30:3:10, V water: conc. HCl 97:3. The qualitative composition of anthocyanins and flavonoids was determined by two-dimensional on paper chromatography in systems I and II, and aglycons in systems III and IV. Individual compounds were purified by rechromatography on paper in systems I and II.

Individual anthocyanins and flavonoids were subjected to chromatographic and spectral analysis (Harborne, 1958; Jurd, 1962; Novruzov, 2005). Spectral analysis, complete and partial acid hydrolysis, alkaline cleavage was carried out according to Harbour (Harborne, 1958). The qualitative composition and the amount of sugars in the molecule were determined according to Chandler and Harper (Bryant, 1950). The sugar portion of the hydrolyzate was neutralized with an ion exchange resin and examined by paper chromatography in system I. Sugar spots on chromatograms were detected by Patridge's reagent (Hayes et al., 1962).

Individual anthocyanins and flavonoids were determined by the color of the spots in the visible and UV light treated with ionizing and complexing reagents, based on the results of complete and partial hydrolysis. UV spectra were recorded using a Specol 1500 spectrophotometer.

RESULTS AND DISCUSSION

Chromatographic analysis of the sum of anthocyanins by the method of two-dimensional paper chromatography showed availability of 4 substances of anthocyanin nature. The color of the spots on the chromatogram in visible light is the same as fuchsin, UV light the spots 1 and 3 were dull-fuchsin and 2 is gray-fuchsin. This indicates that all three spots refer to the derivatives of cyanidin. According to the value of Rf spots on the chromatogram, one of them is a monoglycoside, and two are diglucoside.

From the sum of anthocyanins obtained by column chromatography method filled with cellulose powder (Novruzov et al., 1988), elution of system II received 5 fractions. Individually pure anthocyanins were obtained by rechromatography on paper in system III I, III and V contain one substance, II and IV with 2 substances. The second fraction contains the substance corresponding to the spot 1 and 2, and the fourth. The anthocyanins on the chromatogram were recovered with methanol containing 0.1% hydrochloric acid. Thus, 3 chromatographically pure anthocyanins were obtained and designated as A, B, C.

Substance A - dark red powder, Rf is 0.37 (system I), 0.25 (system II), 0.06 (system III), the color of the spots in visible light is fuchsin, in UV light is dim-fuchsin; λ_{max} nm in methanol 280, 525, in ethanol 535, +aluminum chloride 542, E_{440}/E_{max}=22.

Substance B - red powder, Rf is 0.25, 0.40, 0.18 (respectively in systems I, II, III), the color of spots in visible light is fuchsin, in UV light is gray-fuchsin; λ_{max} nm in methanol 278, 524, in ethanol 531, +aluminum chloride 540, E_{440}/E_{max}=13.

Substance C - violet-red powder, Rf - 0.36, 0.61, 0.15 (system I, II, III), the color of the spots in visible light is fuchsin, in UV light is dim-fuchsin; λ_{max} nm in methanol 283, 526, in ethanol 534, +aluminum chloride 542, E_{440}/E_{max}=25.

With complete acid hydrolysis of the sugar part of substance A and B, 1 substance was found, chromatographic analysis of which showed its identity with D-glucose. A substance accounts for 65% of the aglucone, and 42% for the substance. This indicates that the A-substance contains one glucose molecule, based on the results of chromatographic and spectral acid analyzes and comparing them with authentic and published data (Novruzov, 2005) substance A is identified as cyanidin-3-glucoside.

Judging by the content of the aglycon on the hydrolyzate, substance B has 2 glucose molecules. The number and positions of the sugar residue are recognized during staged and enzymatic hydrolysis. In stepwise acid hydrolysis, 3-di-glucoside initially forms monoglycoside, then aglycone, 3,5-diglucoside initially forms 2 monoglucoside, and at the end of hydrolysis aglucone. In the stepwise hydrolysis substance B after 60 minutes, 2 monoglycosides were detected, the second one which fluoresces with yellow color. According to the literature, 5-monoglucosides have yellow fluorescence (Harborne, 1958). This substance is identified as cyanidin-3,5-diglucoside. After 80 minutes, two spots were found in the hydrolyzate, one corresponding to cyanidin-3-glucoside and the second to cyanidin. After 100 minutes, only the aglycone was detected. Based on the results of chromatographic, spectral and acid hydrolysis, substance B was identified as cyanidin-3,5-diglucoside.

Substance C. With complete acid hydrolysis in the sugar portion of the hydrolyzate, L-xylose and D-glucose were detected. The ratio of aglycons to sugar is 1:2. This indicates that substance B is a cyanidin bioside. With partial hydrolysis, monoglycoside and glucose are first formed, and aglycone and xylose are then used. Based on the results of chromatographic, spectral and acid analyzes, substance C was identified as cyanidin-3-sambuobioside (ksilosil glucoside).

Analysis of the sum of anthocyanins showed that the main mass is cyanidin-3,5-di-glucoside (59%), cyanidin-3-glucoside content 12.0%, cyanidin-3-sambubioside 29%.

The study of the quantitative content of anthocyanins in mature black elderberry fruits showed that it depends on the condition of growing plants. The plant collected on open conditions anthocyanin content is 4.02%, and growing in the forest is 3.12%.

As a result of two-dimensional chromatography on paper in systems I and II of the ethel fraction was observed one, an ethyl acetate fraction 4 substances of a flavonoid nature.

Preparative chromatography on silicagel from ethel extraction isolated one individual substance. From the ethyl acetate fraction, 3 substances were isolated by means of column chromatography on a polyamide powder Wolem, which were designated A, B, C, D. After crystallization in methanol and chromatography on paper in systems I and II, a single spot was obtained, indicating their individuality. On the basis of the Briant test (Hayes et al., 1962), the substance from the ethel fraction and substance A are assigned to aglycons, and substances B, C to glycosides.

Substance A - yellow needle crystals, readily dissolved in ethanol, methanol, acetone, weakly in ether, insoluble in hexane and chloroform, Rf - 0.33; 0.39 (in systems I and VI, respectively). In the UV spectrum, λ_{max} nm 258, 300, 370 in ethanol, +CH_3COONa 273, 375; +CH_3COONa+H_3BO_4 - 260, 385; +$AlCl_3$ 270, 420. Shifts that occur when ionizing and complexing reagents are added indicate the presence of free hydroxyl groups at the positions C3, C5, C7, C3', C4'. Under alkaline degradation, substance A forms floroglucin and pyrocatechic acid, which proves the identity with 3,5,7,3',4'-pentahydroxyflufonom (quercetin).

Substance B - yellow powder, UV spectrum λ_{max} nm in methanol: 255, 265, 362; +CH_3COONa 258, 270, 379; + CH_3COONa+H_3BO_4 - 256, 266, 373; + $AlCl_3$ 258, 261, 395. Changes in the addition of ionizing and complexing reagents indicate the presence of free hydroxyl groups at positions 5, 7, 3', 4'. The substance in acid hydrolysis forms an aglycone identical to quercetin, and sugar is D-glucose. The obtained data allow identifying substance B as 5,7,3',4'-tetrahydroxyflavone-3-O-β-D-glucopyranoside (isoquercitrin).

Substance C - pale yellow crystals, readily soluble in ethanol, methanol, weakly in acetone, insoluble in ether. In the UV spectrum, λ_{ma}x nm in methanol: 258, 300, 356: +CH_3COONa 271, 328, 385: +CH_3COONa + H_3BO_4 - 263, 378: +$AlCl_3$ 275, 290, 350. Spectral data indicate the presence of free OH groups at positions 5, 7, 3', 4'. With xylitol hydrolysis, the yield of the aglycon is 46.8%. Spectral and chromatographic data indicate the identity of this aglycon with quercetin. In the sugar portion of the hydrolyzate, sugar was found identical to D-glucose and L-rhamnose. Chromatography and spectral data and comparison with authentic samples indicate the identity of substance C with routine (quercetin-3-rutinoside).

The content of flavonoids in mature fruits, depending on the growth of plants, varies from 108-186 mg% (recalculation to wet weight) and 399.6-688.2 mg% on a dry basis.

CONCLUSION

The content and qualitative composition of anthocyanins and flavonoids in mature fruits of Sambucus nigra L., grown in Azerbaijan, was studied chromatographically by spectrophotometric method. It was established that the content of anthocyanins varies from 3.12 to 4.02%, and flavonoids from 108 to 186 mg%, depending on the site of occurrence. The presence of 3 derivatives of cyanidin - cyanidin-3,5-di-glucoside, cyanidin-3-glucoside, cyanidin-3-sambubioside was found in the anthocyanins. The composition of flavonoids content quercetin and 2 derivatives of quercetin - quercetin-3-glucoside, quercetin-3-rutinoside.

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Sambucus Nigra L. (*Sambucaceae* Botsch ex Bork. Ailəsi) Növü Meyvələrinin Antosian və Flavonoidlərinin Keyfiyyət Tərkibinin və Miqdarlarının Öyrənilməsi

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Xromato-spektrofotometrik metodla Azərbaycanda bitən *Sambucus nigra* L. növünün yetişmiş meyvələrinin antosian və flavonoidləri miqdarı və keyfiyyət tərkibi tədqiq edilmişdir. Müəyyən edilmişdir ki, yetişmiş meyvələrlə antosianın miqdarı bitdiyi şəraitdən asılı olaraq 3,12-4,02%, flavonoidlər isə 108-188 mq% arasında dəyişilir. Alınmış antosian cəmində 3 sianidin törəməsi – sianidin-3-glikozid, sianidin-3,5-diglükozid və sianidin-3-sambubiozid aşkar edilmişdir. Flavonoid cəmində kversetin və 2 kversein törəməsi – kversetin-3-glükozid, kversetin-3-rutinozid müəyyən edilmişdir.

Açar sözlər: *Sambucus nigra* L., *antosianlar, flavonoidlər, xromatoqrafik və spectral analiz*

Изучение Состава и Содержания Антоцианов и Флавоноидов Плодов *Sambucus Nigra* L. (Сем. *Sambucaceae* Botsch ex Bork.)

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Хромато-спектрофотометрическим методом исследовано содержание и качественный состав антоцианов и флавоноидов в зрелых плодах *Sambucus nigra* L., произрастающей в Азербайджане. Установлено, что содержание антоцианов в зависимости от местопроизрастания изменяется от 3,12 о 4,02%, а флавоноидов от 108 до 186 мг%. В составе антоцианов установлено наличие 3-х производных цианидина - цианидин-3,5-диглюкозид, цианидин-3-глюкозида, цианидин-3-самбубиозида. В составе флавоноидов установлено наличие 2-х производных кверцетина – кверцетин-3-глюкозид и кверцетин-3-рутинозид.

Ключевые слова: *Sambucus nigra* L., *антоцианы, флавоноиды, хроматографический и спетральный анализ*