



Original Research

Structural identification and *in vitro* antioxidant activities of anthocyanins in black chokeberry (*Aronia melanocarpa* Elliot)

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ABSTRACT

Anthocyanins is a natural edible pigment with many health benefits. The aim of this work was the identification of anthocyanins present in *Aronia melanocarpa* using mass spectrometric features. The anthocyanins of the *A. melanocarpa* were analyzed by UV-Vis, HPLC-DAD and LC-EIS/MS methods. The four important anthocyanins were identified as follows: cyanidin-3-galactoside (68.68%), cyanidin-3-arabinoside (25.62%), cyanidin-3-glucoside (5.28%) and cyanidin-3-xyloside (0.42%). Among the four anthocyanin monomers, three anthocyanins with the highest content of *A. melanocarpa* were selected, and the antioxidant activity was studied with the total anthocyanins. The antioxidant capacity was cyanidin-3-galactoside > total anthocyanin > cyanidin-3-arabinoside > cyanidin-3-glucoside. The activity of the four anthocyanin samples was greater than ascorbic acid. The methodology described in this study will provide an effective tool for anthocyanins identification. Our results suggested that anthocyanins from *A. melanocarpa* exhibited effective antioxidant activity. These findings may be crucial in future research concerning chokeberry based functional food products.

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1. INTRODUCTION

A. melanocarpa is Rosaceae, a perennial deciduous shrub with spherical fruit, purple-black peel and dark ruby red juice. It is rich in cellulose [1], flavonoids [2, 3], phenolic compounds [4, 5], organic acids [6], minerals [7], vitamins [8, 9], sterols [10], etc. According to research reports [1, 2], *A. melanocarpa* is the plant with the highest content of anthocyanins, 80-180 times that of grapes and 1000-2000 times that of bananas.

Anthocyanin of *A. melanocarpa* is a natural edible pigment with nutritional and pharmacological effects [11, 12]. It has great application potential in the development of food and medicinal products. Research shows that anthocyanin has antiaging [13] antioxidant [14, 15], antiproliferative [16], anti-inflammatory [17–19], cardiovascular disease treatment [20, 21], neuroprotective [22], cholesterol reduction [23, 24], anticancer [25], stomach preservation [26], liver protection [27], and anti-ultraviolet radiation b (UVB) irradiation activity [28] and so on.

Rational development and efficient use of anthocyanins related products in *A. melanocarpa* is an urgent problem to be solved. The aim of this paper is to analyze and identify the composition and

molecular structure of anthocyanins from *A. melanocarpa* by using the separation and detection technologies of ultraviolet spectrum (UV), liquid chromatography and liquid chromatography-mass spectrometry combined with retention time and reference documents, so as to improve the identification and detection limit and accuracy of anthocyanins. At the same time, the *in vitro* antioxidant activity of *A. melanocarpa* anthocyanins is also studied, which is of great significance for improving the basic research of *A. melanocarpa* anthocyanins in China and developing and utilizing the natural pigment in food industry.

2. MATERIALS AND METHODS

2.1. Materials and reagents

Black chokeberry (The cultivar was identified as *A. melanocarpa* Elliot by Prof. Guang Xin from Shenyang Agricultural University) was purchased and stored at -20 °C in Haicheng City, Liaoning Province. The crude anthocyanin of *A. melanocarpa* was extracted by ultrasonic wave and purified by HP-20 macroporous adsorption resin to obtain lyophilized powder of *A. melanocarpa* anthocyanin.

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Acetonitrile and formic acid are chromatographic pure. Catechol, ferric chloride, potassium ferricyanide, acetic acid, DPPH, ferrous sulfate, anhydrous ethanol, ascorbic acid, water-soluble vitamin E, pyrogallol, hydrogen peroxide, salicylic acid and purified water.

2.2. Preparation of anthocyanin monomer

The sample (1.000 g) was purified by HP-20 macroporous adsorption resin. A solution was prepared with a concentration of 40 mg/mL with acetonitrile containing 0.01% acetic acid, a liquid phase was prepared with Agilent 1260, respectively collected effluent according to retention time, measured the purity of each group of effluent by peak area normalization method, and prepared a liquid phase system under the same gradient elution conditions as above. The effluent was collected from each group and was vacuum concentrated at 30°C. At last, the samples were frozen dry at -40°C for 50 h to obtain high purity anthocyanin monomer.

2.3. Analysis and identification of anthocyanins

2.3.1. UV-vis analysis

The freeze-dried sample was prepared into a certain concentration with 0.01% HCl-methanol solution, and its UV-visible spectrum was measured by UV765 ultraviolet-visible spectrophotometer (Shanghai Yidian analytical instrument Co.,) by scanning at 200–800 nm. Observe whether it has absorption peak was observed between 300–330 nm and fluorescence under ultraviolet lamp. Dropped 3–5 drops of aluminum chloride methanol solution into the sample solution, immediately scanned the ultraviolet-visible spectrum, and record the deviation of the maximum absorption peak in the visible light region; added a small amount of sodium methoxide solid to the sample solution and observed the shift of the maximum absorption peak.

2.3.2. HPLC-DAD analysis

Using Agilent 1200 liquid chromatograph (Agilent Technology Co.,) and diode array detector, the composition and proportion of anthocyanin monomer in *A. melanocarpa* were detected under the following chromatographic conditions:

Column: Eclipse XDB-C18 (4.6 × 150 mm, 5 μm) column (Agilent Technologies, Santa Clara, CA, USA), mobile phase: (a) 2.5% acetic acid-water (v/v), (b) acetonitrile; Gradient elution conditions: 0–10 min, 5%–8% B; 10–15 min, 8%–12% B; 16–20 min, 12%–5% B, 5 μm; Column temperature: 30°C; Flow rate: 1 mL/min; Detection wavelength: 520 nm; Injection volume: 5 μL.

2.3.3. Ultra-high performance liquid chromatography-mass spectrometry (UPLC-MS analysis and identification)

The freeze-dried sample was dissolved in methanol (containing 0.01% HCl) to prepare a pigment solution with a certain concentration. The structure of the sample was analyzed by using

a UltiMate 3000 liquid chromatography and LTQ Orbitrap mass spectrometer (Thermo Fisher Company, U.S.)

UPLC parameters: chromatographic column: Eclipse XDB-C18 (4.6 × 150 mm, 5 μm) Column (Agilent Technologies, Santa Clara, CA, USA), mobile phase: (a) 2.5% acetic acid-water (v/v), (b) acetonitrile, gradient elution conditions: 0–10 min, 5%–8% B; 10–15 min, 8%–12% B; 16–20 min; 12%–5% B, 5 μm; Column temperature: 30°C; Flow rate: 1 mL/min; Detection wavelength: 520 nm; Injection volume: 1 μL.

MS parameters: MS scanning three times, ESI ion source, positive ion mode, dryer temperature 300°C, ion transfer tube temperature 320°C, spray voltage 3.5 KV, sprayer pressure 0.2 MPa, ion scanning range 100–1000 (m/z).

2.4. Determination of total antioxidant capacity of anthocyanins in *A. melanocarpa*

Ferric reducing antioxidant power (FRAP) assay was evaluated according to the method of Benzie and Strain [29] with slight modifications. The different anthocyanins were weighed, dissolved and diluted properly, with sample concentration gradient of 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL pipettes took 3 mL, added 15 mL of preheated FRAP working solution, mixing evenly, reacting at 37°C for 20 min, added anhydrous ethanol into FRAP working solution as blank control, and took water-soluble vitamin E as positive control. The absorbance was read at 593 nm, and each group of samples was parallel three times. The antioxidant capacity of the samples was expressed by FRAP value: 1 FRAP unit = 1 mmol/LFeSO₄, the antioxidant capacity of the samples was equivalent to the mmol/L number of FeSO₄ [30].

2.5. DPPH scavenging capacity of anthocyanins from *A. melanocarpa*

2.5.1. Experimental method

Xu's method was selected and modified [30]. Twenty-five mg of DPPH powder was weighed and dissolved with 95% ethanol to 250 mL to prepare 2.5 × 10⁻² mg/mL DPPH solution, respectively took 1 mL of different concentrations of *A. melanocarpa* anthocyanin sample solution and 3 mL of 2.5 × 10⁻² mg/mL DPPH solution, added them into the same test tube with plugs, shook well, and reacted for 30 min in the dark at room temperature. The absorbance of the reaction solution at 517 nm was measured. The control group used absolute ethanol instead of anthocyanin sample solution, and Vc was used as the positive control. The concentration gradient of the sample was 2, 4, 6, 8, and 10 μg/mL. The average value was taken for each group of experiments three times. The DPPH scavenging rate was calculated according to the following formula:

$$\text{DPPH radical scavenging rate (I\%)} = [(A_1 - A_2) / A_1] \times 100\%$$

In the formula A₁ is the light absorption value of the control group; A₂ is the absorbance value of the sample set.

2.5.2. Calculation of semi-inhibition amount and evaluation of scavenging ability

According to the DPPH radical scavenging curves of different anthocyanin monomers of *A. melanocarpa*, a linear equation is fitted. After calculation, when the original concentration of DPPH radical is reduced to 50%, the added amount of anthocyanin EC_{50} of *A. melanocarpa* is obtained. The scavenging capacity of free radicals is judged according to the size of EC_{50} . The larger the EC_{50} value, the weaker the scavenging capacity of free radicals is.

2.6. Scavenging activity of anthocyanin from *A. melanocarpa* on peroxyanions

The $O_2^{\cdot -}$ scavenging activity was determined by referring to Li [31] in the literature. One milliliter of anthocyanin solution and 9 ml of phosphate buffer solution with pH of 8.2 of 0.1 M were taken, uniformly mixed, reacted for 15 min in a constant temperature water bath at 25°C, 4.5 mL of that mix solution was taken into a colorimetric tube with plugs, added 0.15 mL of 45 mM pyrogallol solution, uniformly mixed, added 80 μ L 10 M hydrochloric acid solution after 4 min, terminated the reaction, and measured the light absorption value (A_1) of the mixed solution at 325 nm. At the same time, distilled water was used instead of pyrogallol solution to measure the absorbance value (A_2) of the mixed solution, and sample solvent was used instead of anthocyanin solution to measure the absorbance value (A_3) of the mixed solution. Each group of experiments was repeated three times with Vc as the positive control. The concentration gradient of the sample is: 0.1, 0.2, 0.3, 0.4, 0.5 mg/mL. The average $O_2^{\cdot -}$ clearance rate of three times for each group of experiments shall be calculated according to the following formula:

$$\text{Clearance rate (\%)} = [L - (A_1 - A_2) / A_3] \times 100.$$

2.7. Data statistics

The surface area of signal response value and retention time were compared with UPLC-MS mass spectrogram, and the obtained data were analyzed by peak time and molecular ion fragment information. Data from antioxidant research were statistically processed by Origin 8.0 software and Microsoft Excel 2003.

3. RESULTS AND DISCUSSION

3.1. UV-Vis analysis results

As can be seen from **Figure 1**, the ultraviolet-visible spectrum of the anthocyanin solution of *A. melanocarpa* contains two obvious absorption peaks, with peak 1 at 280 nm and peak 2 at 520 nm, which is a typical anthocyanin absorption wavelength. There is no absorption peak at 300–330 nm, indicating that the anthocyanin substance does not contain acyl. There is no fluorescence under the ultraviolet lamp, indicating that there is no substituent at position 5 of the anthocyanin. After dropping aluminum chloride, the maximum absorption wavelength of the sample changed from 520 nm to 552 nm, showing obvious red shift, which indicates that

in the structure of anthocyanins, ring B contains ortho-phenolic hydroxyl [32], which is due to the decrease in the density of electrons on hydroxyl after adding aluminum chloride solution, resulting in the decrease of excitation energy and the shift of absorption peak to long wavelength, while anthocyanins without ortho-phenolic hydroxyl will not show obvious red shift. After sodium methoxide was added, the absorption peak at 520 nm of the sample showed red shift, which indicated that the glycoside substituent in the anthocyanin structure of *A. melanocarpa* was at position 3 of the C ring [33].

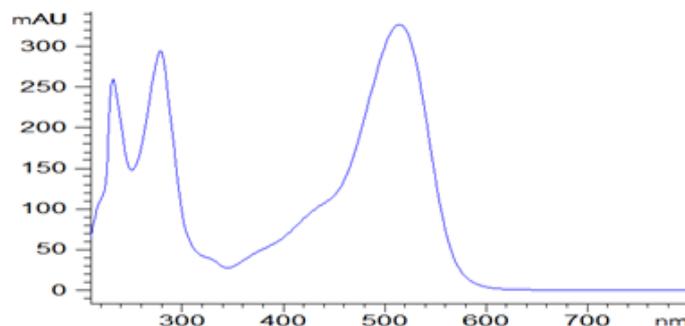


Figure 1 UV spectrogram of *Aronia melanocarpa* anthocyanins.

3.2. Results of HPLC-DAD analysis

The crude anthocyanin extract was purified by HP-20 macroporous adsorption resin, and the impurities such as sugar, organic acid and protein were removed. The HPLC-DAD chromatogram of the purified sample at 520 nm is shown in **Figure 2**.

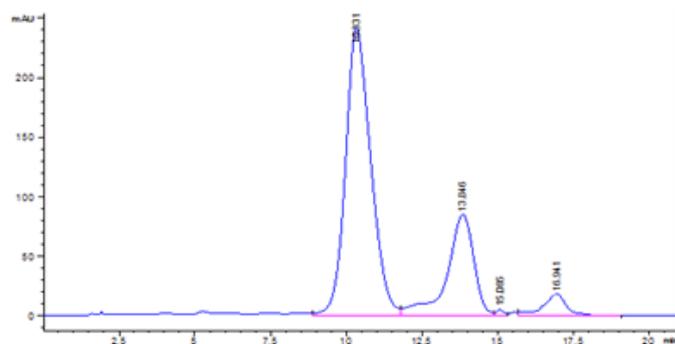


Figure 2 HPLC chromatogram of *Aronia melanocarpa* anthocyanins after purified by HP-20 resin at 520 nm.

According to the HPLC conditions in this experiment, considering the number of peaks and the degree of separation between each peak, the best HPLC chromatogram obtained is shown in **Figure 2**. It can be seen that the anthocyanin of *A. melanocarpa* purified by HP-20 macroporous adsorption resin has four absorption peaks at 520 nm, representing four anthocyanin monomers in fruits, representing the content of each anthocyanin by peak area, and the content of each anthocyanin was attained. The proportions of these anthocyanin monomers were 68.68%, 25.62%, 0.42%, 5.28%.

3.3. Analysis and identification of anthocyanins

In this experiment, the structure of anthocyanin from *A. melanocarpa* was identified by UPLC-MS. The analysis was fast and agile, and expensive standard products were not needed. Its mass spectrometry data can provide the type, molecular weight, number and other information of glycosides [34]. The mass spectrometry information of molecular ions and fragment ions of each peak is shown in the following figure (Figure 3 a, b, c, d).

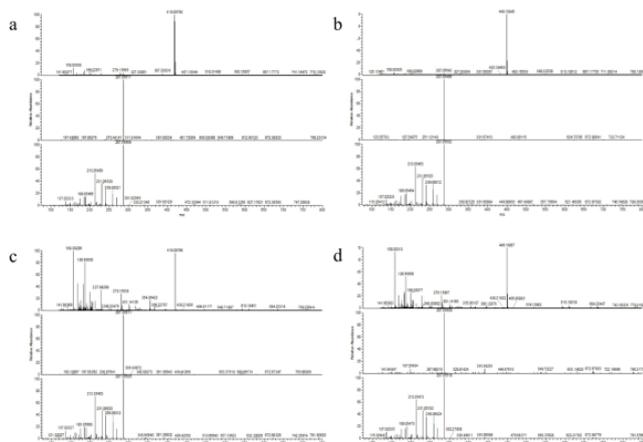


Figure 3 Spectra of MSn to peak 1, 2, 3, 4.

Table 1 Results of UPLC-MS analysis of *Aronia melanocarpa* anthocyanins.

Peak	Retention time (min)	[M+H] ⁺ (m/z)	(M) ²	Anthocyanin identification	
1	10.331	455.8	287	Cyanidin-3-galactoside	[35]
2	13.846	485.4	287	Cyanidin-3-arabinoside	[33]
3	15.065	455.8	287	Cyanidin-3-xyloside	[36]
4	16.694	485.4	287	Cyanidin-3-glucoside	[36]

From Figure 3 a to Figure 3 d and Table 1, it can be seen that the molecular ion m/z obtained from peak 1 and peak 3 is 419, and the fragment ion m/z is 287, which is obtained by anthocyanin molecular ion losing a neutral fragment with a mass of 132. The retention time of peak 1 is 10.331min, and that of peak 3 is 15.065min. Combining with reference [36], it can be inferred that peak 1 is cyanidin-3-galactoside, and peak 3 is cyanidin-3-xyloside. The molecular ion m/z obtained from peak 2 and peak 4 is 449, and the fragment ion m/z is 287, which is obtained by the anthocyanin molecular ion losing a neutral fragment with a mass of 163. the anthocyanin aglycon m/z is 287, which can be inferred as procyanidin [37], i.e. the anthocyanin aglycon with a m/z of 287 is obtained by the cyanidin aglycon molecule losing a hexose aglycon, the retention time of peak 2 is 13.846 min, and the retention time of peak 4 is 16.694 min. According to the retention time and literature [37, 38], it can be inferred that peak 2 is cyanidin-3-

arabinoside and peak 4 is cyanidin-3-glucoside. The structure of each anthocyanin is shown in Figure 4 a, b, c, d.

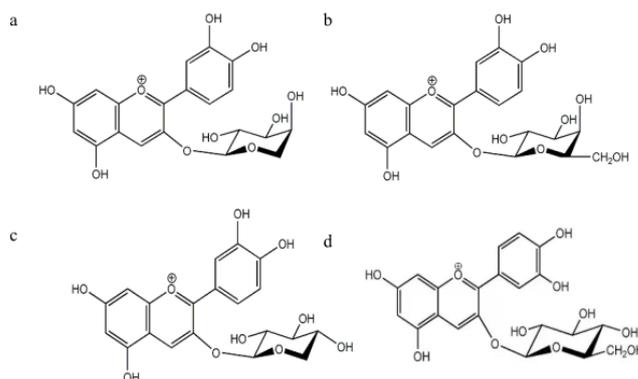


Figure 4 Chemical structure of peak 1 (cyanidin-3-galactoside), peak 2 (cyanidin-3-arabinoside), peak 3 (cyanidin-3-xyloside) and peak 4 (cyanidin-3-glucoside) of *Aronia melanocarpa* anthocyanins.

3.4. Determination of total antioxidant capacity of anthocyanins in *A. melanocarpa*

The principle of FRAP method is based on the redox reaction of the system. Under acidic conditions, trivalent iron ions can react with TPTZ to form a complex. After antioxidant is added, trivalent iron ions are reduced to bivalent iron ions, which show blue color and have maximum absorption at 593 nm, while the content of bivalent iron ions is proportional to the light absorption value of the system. Therefore, FRAP method is widely used to measure the total antioxidant activity of natural products.

3.5. FeSO₄ standard curve

FeSO₄ standard curve was shown in Figure 5. As can be seen from Figure 5, in the range of 0.3-1.5 mmol/L, at 593 nm, the concentration of FeSO₄ has a linear relationship with the light absorption value of the solution. the fitting equation is y (light absorption value) = 0.6861x + 0.0158, and the correlation coefficient R² = 0.9909. Therefore, it is feasible to convert the light absorption value at 593 nm into the equivalent concentration of FeSO₄. The equivalent concentration unit is mmol, and the greater the equivalent concentration, the stronger the antioxidant capacity of the sample.

3.6. Determination of antioxidant capacity of anthocyanin samples

In a certain concentration range, the antioxidant capacity of each anthocyanin can be found by comparing the anthocyanin of *A. melanocarpa* with Trolox. As can be seen from Figure 6, the absorbance value of the solution increased with the increasing concentration of anthocyanin. Generally speaking, cyanidin-3-galactoside has the strongest antioxidant capacity, while total

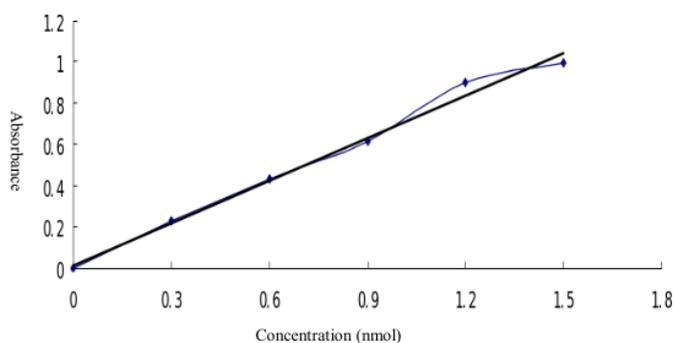


Figure 5 | Standardcurve of FeSO_4 .

anthocyanin and cyanidin-3-arabinside are weaker.

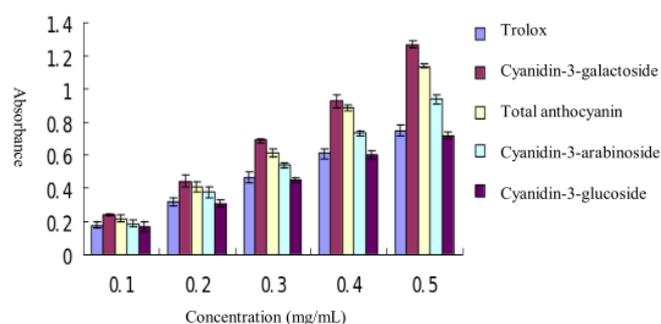


Figure 6 | Theantioxidant ability of different *Aronia melanocarpa* anthocyanins.

However, the antioxidant capacity of these anthocyanins at the same concentration is still greater than that of the control Trolox, and cyanidin-3-glucoside has the lowest antioxidant capacity. It can also be concluded from the figure that when the concentration is lower than 0.1 mg/g, the antioxidant capacity of anthocyanins is similar, and with the increasing concentration, the antioxidant capacity of each anthocyanin gradually differs significantly. The absorbance value of the reaction system is represented by Y, and the mass concentration of anthocyanin is represented by X. Linear fitting standard curve equations by Origin 8.0 software were as follows:

$$\text{Trolox: } y = 1.421x + 0.0396, R^2 = 0.9978$$

$$\text{Cyanidin-3-galactoside: } y = 2.526x - 0.0442, R^2 = 0.9911$$

$$\text{Total anthocyanin: } y = 2.301x - 0.0377, R^2 = 0.9939$$

$$\text{Cyanidin-3-arabinside: } y = 1.852x + 0.0004, R^2 = 0.9987$$

$$\text{Cyanidin-3-glucoside: } y = 1.396x + 0.0304, R^2 = 0.9987$$

The fitting equation of anthocyanin showed that the antioxidant capacity of anthocyanin from *A. melanocarpa* was linearly related to its content. According to the fitting equation, the equivalent concentrations of Trolox and FeSO_4 of each anthocyanin were cyanidin-3-galactoside ($3.46 \text{ mmol} \cdot \text{g}^{-1}$) > total anthocyanin ($3.17 \text{ mmol} \cdot \text{g}^{-1}$) > cyanidin-3-arabinside ($2.64 \text{ mmol} \cdot \text{g}^{-1}$) > trolox ($2.12 \text{ mmol} \cdot \text{g}^{-1}$) > cyanidin-3-glucoside ($2.07 \text{ mmol} \cdot \text{g}^{-1}$).

3.7. DPPH scavenging capacity of anthocyanins from *A. melanocarpa*

The free radical scavenging ability can be expressed by measuring the DPPH radical scavenging strength of the sample. The ethanol solution containing DPPH radical is purple. After anthocyanin is added, the color of the system changes from purple to yellow, because proton atoms contained in anthocyanin molecules can be paired with lone pair contained in DPPH molecules. The DPPH radical scavenging capacity of the sample can be measured by examining the degree of reduction in the absorbance of the system at 517 nm. The stronger the scavenging capacity, the smaller the absorbance of the system, and the stronger the antioxidant capacity of the sample [35].

The antioxidant activities of total anthocyanins and three monomeric anthocyanins were determined in the experiment. Vitamin C was taken as the positive control, and DPPH scavenging effect was shown in **Figure 7**.

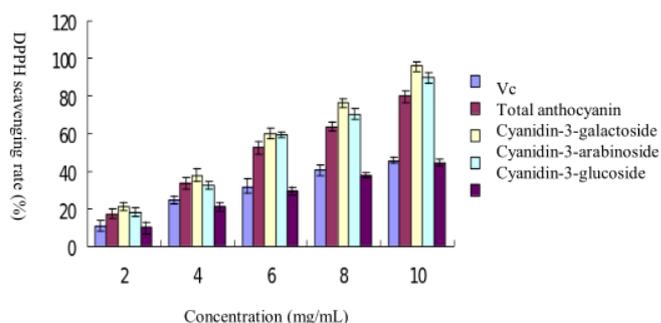


Figure 7 | DPPH radical scavenging rate of different *Aronia melanocarpa* anthocyanins.

As can be seen from **Figure 7**, the anthocyanin samples of different *A. melanocarpa* have certain DPPH scavenging capacity, and the scavenging capacity is proportional to the concentration. In the range of 2-4 $\mu\text{g}/\text{mL}$, the scavenging capacity of total anthocyanin and each anthocyanin monomer is not different. When the sample concentration reaches 10 $\mu\text{g}/\text{mL}$, the DPPH scavenging rate of cyanidin-3-galactoside reaches 95.721%, which is 2.15 times higher than that of cyanidin-3-glucoside, and the DPPH scavenging capacity of cyanidin-3-glucoside is slightly higher than Vc. The experimental results show that: *A. melanocarpa* anthocyanin has strong hydrogen supply capacity and strong DPPH radical scavenging effect. The scavenging efficiency is Y and the concentration of anthocyanin is X. The linear regression equations fitted by SPSS are as follows:

$$\text{Ascorbic acid: } y = 4.2458x + 3.0978, R^2 = 0.9923$$

$$\text{Cyanidin-3-galactoside: } y = 2.526x - 0.0442, R^2 = 0.9911$$

$$\text{Total anthocyanin: } y = 9.0677x - 0.405, R^2 = 0.9872$$

$$\text{Cyanidin-3-arabinside: } y = 7.7271x + 3.1296, R^2 = 0.9945$$

$$\text{Cyanidin-3-glucoside: } y = 4.2649x + 5.3809, R^2 = 0.9704$$

The IC_{50} values of each sample obtained from the fitting equation are respectively: cyanidin-3-galactoside ($5.12 \text{ mg} \cdot \text{L}^{-1}$),

total anthocyanin ($5.56 \text{ mg}\cdot\text{L}^{-1}$), cyanidin-3-arabinoside ($6.06 \text{ mg}\cdot\text{L}^{-1}$), ascorbic acid ($11.06 \text{ mg}\cdot\text{L}^{-1}$), cyanidin-3-glucoside ($10.46 \text{ mg}\cdot\text{L}^{-1}$). The larger the IC_{50} value, the weaker the DPPH radical scavenging ability.

3.8. Experimental results of scavenging effect of anthocyanins from *A. melanocarpa* on superoxide anion

In the whole reaction system, alkaline conditions will cause pyrogallol to generate superoxide anion, which in turn will accelerate the self-oxidation reaction rate of pyrogallol. The intermediate product of the reaction has color. The concentration of the intermediate product has a good linear relationship with the light absorption value of the solution at 325 nm. When anthocyanin antioxidants were added, hydrogen atoms generated by anthocyanin molecules combine with superoxide anion in the system to reduce the concentration of superoxide anion in the system. Anthocyanin inhibited the self-oxidation rate of pyrogallol and prevented the formation of intermediate products. The scavenging efficiency of anthocyanins on superoxide anion was evaluated by examining the absorbance value of the whole system at 325 nm [39].

The scavenging effects of total anthocyanin and three anthocyanin monomers on superoxide anion in the concentration range of $0.1\text{--}0.5 \text{ mg}\cdot\text{mL}^{-1}$ were determined by experiments respectively. Ascorbic acid was taken as the positive control. The results are shown in Figure 8.

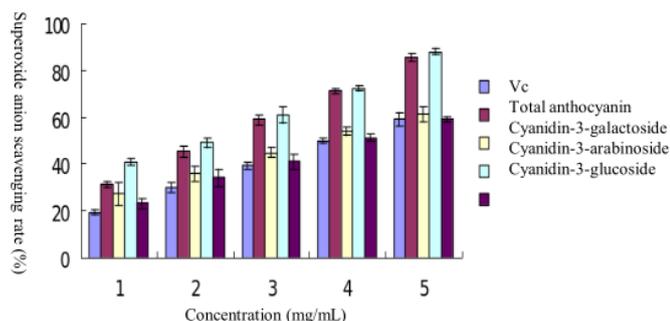


Figure 8 Superoxide anion radical scavenging rate of Vc and different *Aronia melanocarpa* anthocyanins.

Ascorbic acid and anthocyanin samples have certain scavenging ability to superoxide anion, and the scavenging rate to superoxide anion increases with the increase of concentration in the measurement range. Total anthocyanin and cyanidin-3-galactoside have obvious scavenging effect on superoxide anion. When the concentration was $0.5 \text{ mg}\cdot\text{mL}^{-1}$, the scavenging efficiency on superoxide anion reaches 88.5% and 88.2%. On the whole, the ability of cyanidin-3-arabinoside and cyanidin-3-glucoside to remove superoxide anion is similar to that of positive control Vc. There is a good linear relationship between the scavenging rate of superoxide anion and the mass concentration of the sample. Y represents the scavenging rate of superoxide anion and X represents the mass concentration of the sample. The linear

regression equations fitted by SPSS are as follows:

Ascorbic acid: $y = 0.99x + 0.1002$, $R^2 = 0.9995$

Cyanidin-3-galactoside: $y = 0.859x + 0.1899$, $R^2 = 0.9985$

Total anthocyanins: $y = 1.3408x + 0.1832$, $R^2 = 0.9994$

Cyanidin-3-arabinoside: $y = 0.859x + 0.1899$, $R^2 = 0.9985$

Cyanidin-3-glucoside: $y = 0.889x + 0.1521$, $R^2 = 0.9964$

From the above regression equation, the IC_{50} values of Vc and anthocyanin samples can be calculated: ascorbic acid ($0.403 \text{ mg}\cdot\text{mL}^{-1}$), cyanidin-3-galactoside ($0.195 \text{ mg}\cdot\text{mL}^{-1}$), total anthocyanin ($0.236 \text{ mg}\cdot\text{mL}^{-1}$), cyanidin-3-arabinoside ($0.361 \text{ mg}\cdot\text{mL}^{-1}$), and cyanidin-3-glucoside ($0.391 \text{ mg}\cdot\text{mL}^{-1}$). The scavenging capacity of superoxide anion is cyanidin-3-galactoside > total anthocyanin > cyanidin-3-arabinoside > cyanidin-3-glucoside > ascorbic acid.

4. CONCLUSIONS

The structure of anthocyanins in *A. melanocarpa* was identified by UV-Vis spectroscopy, HPLC-DAD and LC-MS coupled with spiking method. HPLC-DAD results detected anthocyanins contained four monomers. The pure product of each peak was obtained by preparing liquid phase. The four anthocyanins were found to be cyanidin-3-galactoside (68.68%), cyanidin-3-arabinoside (25.62%), cyanidin-3-xyloside (0.42%) and cyanidin-3-glucoside (5.28%) by ultra-high performance liquid chromatography-mass spectrometry. The antioxidant effect of anthocyanins from *A. melanocarpa* was evaluated by studying its total antioxidant ability and scavenging ability to DPPH radical and superoxide anion radical. There was a certain correlation between each experiment. Overall, the antioxidant effect of anthocyanins from *A. melanocarpa* was better than Vc. The results of each experiment were summarized as follows: the antioxidant ability of anthocyanins from *A. melanocarpa* was in the order of cyanidin-3-galactoside > total anthocyanin > cyanidin-3-arabinoside > cyanidin-3-glucoside > ascorbic acid. In scavenging superoxide anion and DPPH test, the free radical scavenging capacity is similar to the antioxidant activity results.

5. ABBREVIATIONS

FRAP: Ferric reducing antioxidant power; UV: ultraviolet spectrum; UVB: ultraviolet radiation b; UPLC-MS: Ultra-high performance liquid chromatography-mass spectrometry

CONFLICTS OF INTEREST

All the authors declare that there were no conflicts of interest.

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