

Extraction process and composition identification of anthocyanins from xinjiang wild *Prunus cerasifera* fruit peel

Jing Shen^{1,2}, Pei Zhang², Xin Zhang², Jun Yao^{1*} and Jun-min Chang^{1*}

¹College of Pharmacy, Xinjiang Medical University, Urumqi, Xinjiang Uygur Autonomous Region, China

²Department of Pharmacy, Hospital of Xinjiang Medical University, Urumqi, Xinjiang Uygur Autonomous Region, China

Abstract: To extract anthocyanins from wild *Prunus cerasifera* fruit peel and to identify the main compositions. The effects of extraction solvent, solid-to-liquid ratio, extraction time and extraction temperature on the extraction rate of anthocyanins were determined using colorimetric method, differential spectrophotometry and L9 (3⁴) orthogonal experiment. The structure of the anthocyanins extracted from wild *Prunus cerasifera* fruit peel was preliminarily identified by liquid chromatography-electrospray ionization-tandem mass spectrometry. And hydroxyl radical scavenging rate and DPPH radical scavenging rate were used to investigate the antioxidant activity of Xinjiang cherry anthocyanin *in vitro*. The highest extraction rate of anthocyanins was acquired under the conditions including 1% HCl-methanol, solid-to-liquid ratio =1:5, extraction temperature 55°C and extraction time 80 minutes. Liquid chromatography-electrospray ionization-tandem mass spectrometry results showed that the main compositions of anthocyanins were cyanidin 3-galactoside, cyanidin 3-glucoside, cyanidin 3-rutinoside and cyanidin 3-xyloside. The results of antioxidant test showed that Xinjiang cherry anthocyanin had certain scavenging effect on 1, 1-diphenyl-2-picrylphenylhydrazine (DPPH·) and hydroxyl radical (·OH), but was weaker than Vc. Findings from this study provide evidence for extraction process and composition identification of anthocyanins from Xinjiang wild *Prunus cerasifera* fruit peel.

Keywords: *Prunus cerasifera*, anthocyanins, extraction, liquid chromatography-electrospray ionization-tandem mass spectrometry, antioxidant activity.

INTRODUCTION

Prunus cerasifera, commonly called wild cherry plum, is a small tree or deciduous shrub that is a valuable wild fruit tree (Li *et al.*, 2013). It strikingly presents in the Xiaoxigou and in particular Daxigou of Huocheng County, Xinjiang Uygur Autonomous Region, China (Li *et al.*, 2010). More than 40 kinds of wild *Prunus cerasifera* have been discovered (Yang *et al.*, 2003). *Prunus cerasifera* fruit has soft, juicy, white flesh with a unique flavor. It also contains rich anthocyanins and a variety of vitamins (Zhang *et al.*, 2004). Wide *Prunus cerasifera* fruit contains six kinds of compounds including alcohols, esters, aldehydes, hydrocarbons, ketones and heterocycles and maintains rich genetic diversity (Liu *et al.*, 2008).

Anthocyanins, mainly found in *Prunus cerasifera* fruit peel, are a kind of flavonoid polyphenols widespread in plants formed by glucosidic bonds

of aglycone and glucose (Sun *et al.*, 2009). They have good physiological functions and excellent coloring effect (Wang *et al.*, 2008). Anthocyanins have many kinds and they are the main pigments affecting fruit and flower color and mainly accumulate in the vacuoles of petal epidermal cells (Yang 2013). Anthocyanins exhibit

multiple pharmaceutical functions. Antioxidant activity is a significant physiological function of anthocyanins (Chen *et al.*, 2021). Anthocyanins have been reported to inhibit endothelial cell injury (Yi *et al.*, 2012). Natural anthocyanins participate in filtering ultraviolet, prevent against pathogens (Fang and Jiao, 2012), exert anti-inflammatory effect (Abdin *et al.*, 2013), prevent against and treat cardiovascular diseases (Fabio *et al.*, 2013), cancer (Herrera-Sotero *et al.*, 2020, Zhao *et al.*, 2019), hypertension (Huang *et al.*, 2020, He *et al.*, 2020) and senile dementia (Afzal *et al.*, 2019). Factors that affect the stability of anthocyanin pigments include pH, temperature, light and nucleophiles (Escobar-Ortiz *et al.*, 2021 and Li *et al.*, 2020). According to a previous report (Wang Y 2016), the level of anthocyanins in the purple *Prunus cerasifera* fruit peel was significantly greater than that in the red fruit peel. Accordingly, in this study, we extracted anthocyanins from the purple *Prunus cerasifera* fruit peel and identified their compositions. The antioxidant activity of anthocyanins from Xinjiang *Prunus cerasifera* was evaluated by hydroxyl radical scavenging and DPPH radical scavenging. The antioxidant activity of plum anthocyanins from Xinjiang cherry was evaluated by hydroxyl radical scavenging and DPPH radical scavenging methods. Based on preliminary experiments, we performed single factor experiment and orthogonal experiment to develop the

*Corresponding author e-mails: 380531866@qq.com; 1617265908@qq.com

technique used to extract anthocyanins from *Prunus cerasifera* fruit peel, providing evidence for in-depth investigation on the pharmacological activity of anthocyanins from *Prunus cerasifera* fruit peel.

MATERIALS AND METHODS

Materials and reagents

Materials

Purple wild *Prunus cerasifera* fruits were harvested from Daxigou of Huocheng County, Ili Kazak Autonomous Prefecture, Xinjiang Uygur Autonomous Region, China, and then quick-frozen for use.

Reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), Ascorbic Acid (Vitamin C), Ferrous sulfate, salicylic acid, Absolute ethanol, methanol, concentrated hydrochloric acid, distilled water, acetic acid, sodium acetate, potassium chloride, citric acid, and sodium dihydrogen phosphate were used. All reagents were of analytical grade.

Instruments and equipment

Electronic balance (FA2004B; Shanghai Precision Scientific Instrument Co., Ltd., China), ultra-violet/visible spectrophotometer (2004; Beijing Persee General Instrument Co., Ltd., China), high-speed centrifuge (TDL-5A; Shanghai Fulgor Analysis Instruments Co., Ltd., Shanghai, China), digital thermostatic water bath instrument (Jintan Medical Instrument Co., Ltd., China), and liquid mass spectrometer (LCQ DECA XP MAX; Thermo Fisher Scientific, USA) were used.

Preparation of anthocyanin extract

Approximately 10 g wild *Prunus cerasifera* fruit peel was soaked in different proportions of solvent for a single factor experiment. Then it was warmed in a thermostatic water bath instrument and filtered. The filtrate was collected and the residues were further soaked with half the amount of the solvent. After warming in the thermostatic water bath instrument for the same duration, the filtrate was collected and mixed with the filtrate collected above. The resulting mixture was centrifuged for later use.

Preparation of anthocyanin sample solution

The anthocyanin extract was diluted with the same amount of solvent to prepare anthocyanin sample solution.

Influential factors

The effects of extraction solvent, solid-to-liquid ratio, extraction time and extraction temperature on the extraction rate of anthocyanins were determined.

Design of orthogonal experiment

According to the results of the single factor experiment,

color scale value and total anthocyanin content were used as indices and L9 (3⁴) orthogonal experiment was performed. Four key factors including extraction solvent, extraction temperature, extraction time and solid-to-liquid ratio were selected. Detailed information is shown in table 1.

Determination of anthocyanin content

Colorimetric method

An aliquot of 2mL anthocyanin sample solution was mixed with 18 mL of buffer solution (pH= 3.0; 0.6 mol/L Na₂HPO₄·12H₂O: 0.3 mol/L citric acid = 100:127, v/v). A mixture of 2 mL solvent and 18 mL buffer solution was used as blank control. The absorbance values at 390-780 nm in the anthocyanin sample solution and the blank control sample were measured using an ultra-violet visible spectrophotometer. Color scale (i.e. specific absorbance) was calculated according to the formula: $E = A \times 10 \times a / W$, in which E is pigment concentration, A is absorbance at peak, W is the weight (g) of sample and a is the dilution multiple of the sample solution.

Differential spectrophotometry

An aliquot of 2mL anthocyanin sample solution was diluted with buffer solution 1 (pH=1.0; 0.2mol/L KCl: 0.2 mol/L HCl =25:67, v/v) and buffer solution 2 (pH=4.5; 0.2mol/L NaAc·3H₂O: 0.2 mol/L HAc=1:1, v/v) to a final solution of 20 mL. A mixture of 2 mL solvent and 18mL corresponding buffer solution was used as blank control. Absorbance at 510nm and 700nm was measured. The content of anthocyanins was calculated based on the level of cyanidin 3-glucoside according to the following formula: $A = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}]$, $ACY(mg/100g) = A \times 449.2 \times 10 \times V \times 100/26900 \times m$, in which V is the total volume (mL) of extract, m is the weight (g) of sample; 26900 is the molar extinction coefficient of cyanidin 3-glucoside and 449.2 is the molar molecular mass of cyanidin-3-glucoside.

Chromatographic condition

Chromatographic column: C18 column (250 mm × 3.0 mm, 5μm); mobile phase: acetonitrile 2% formic acid; flow rate: 0.3 mL/min; column temperature 35°C, sampling volume 20μL.

Mass spectrometry condition: positive ion scan (ESI+); electron energy 30 eV; ion source temperature 300°C; mass-to-charge ratio (m/z) range 100-1,000 m/z; sheath gas flow rate 30 arb; and auxiliary gas flow rate 15 arb.

Determination of Antioxidant Activity of Anthocyanins in purple *Prunus cerasifera* fruit peel

Determination of Hydroxyl Radical Scavenging activity

Accurately add 2 mL of 6 mmol/L ferrous sulfate, 2 mL of anthocyanin solution or Vc solution of different concentrations and 2 mL of 6 mmol/L H₂O₂ solution to the orientation test tube, and shake well. After that, let it

stand for 10 min, then add 2 mL of 6 mmol/L salicylic acid solution, shake well and let stand for 30 min, and measure the absorbance A_{sample} respectively; replace the salicylic acid solution with distilled water, and measure the absorbance A_{control} ; use distilled water instead of anthocyanin solution, measure its absorbance A_{blank} .

$$\cdot\text{OH clearance rate (\%)} = (1 - (A_{\text{sample}} - A_{\text{control}}) / A_{\text{blank}}) \times 100$$

Determination of DPPH free radical scavenging activity

Add 2 mL of anthocyanin solution or Vc solution of different concentrations, 2mL of 0.2mmol/L DPPH·ethanol solution, and 2 mL of distilled water to the test tube and then keep it for 1h in the dark after mixing. Measure the absorbance of A_{sample} and A_{blank} respectively; mix 2mL of 0.2 mmol/L DPPH·ethanol solution and 2mL of distilled water, then stand for 1h in the dark and measure the absorbance of A_{control} .

$$\text{DPPH clearance rate (\%)} = \left(\frac{1 - (A_{\text{sample}} - A_{\text{control}})}{A_{\text{blank}}} \right) \times 100$$

STATISTICAL ANALYSIS

All experiments were repeated 3 times. The measurement data are all expressed, using SPSS16.0 statistical software for result analysis, and one-way analysis of variance for result analysis. $P < 0.05$ is considered statistically significant.

RESULTS

Single factor experiment on extraction of anthocyanins from purple *Prunus cerasifera* fruit peel

Effects of different extraction solvents on the extraction rate of anthocyanins from purple *Prunus cerasifera* fruit peel.

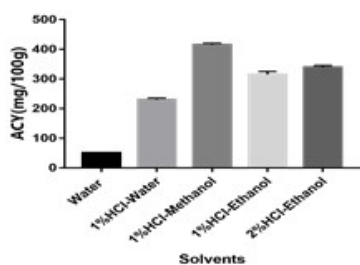


Fig. 1: Effects of extraction solvents on the extraction rate of anthocyanins from purple *Prunus cerasifera* fruit peel

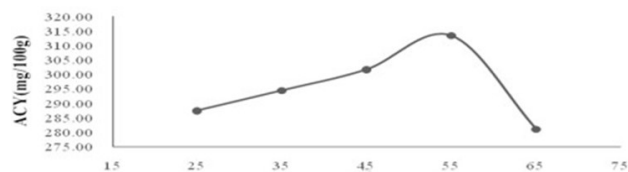


Fig. 2: Effects of extraction temperature on the extraction rate of anthocyanins from purple *Prunus cerasifera* fruit peel

Under the conditions of solid-to-liquid ratio =1:4, extraction temperature 25°C and extraction time 60

minutes, the effects of different extraction solvents on the extraction rate of anthocyanins were investigated (fig. 1). Results revealed that because hydrogen ions penetrated the cell membrane and protected anthocyanins, when H_2O and 1% HCl- H_2O were used low level of anthocyanins was extracted. To ensure high extraction rate, 1% HCl-ethanol, 1% HCl-methanol and 2% HCl-ethanol were selected for orthogonal array design.

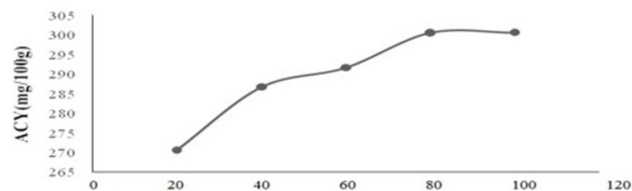


Fig. 3: Effects of extraction time on the extraction rate of anthocyanins from purple *Prunus cerasifera* fruit peel

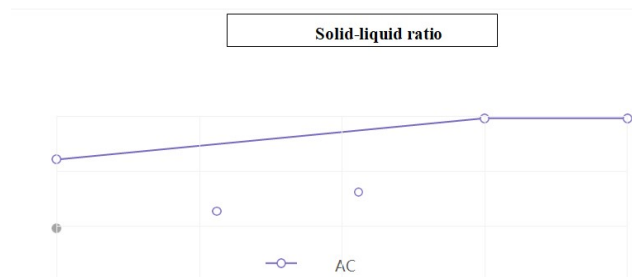


Fig. 4: Effects of solid-to-liquid ratio on the extraction rate of anthocyanins from purple *Prunus cerasifera* fruit peel

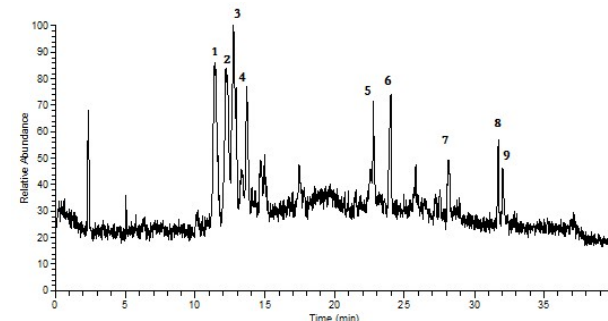


Fig. 5: Total ion chromatogram of purple *Prunus cerasifera* fruit peel

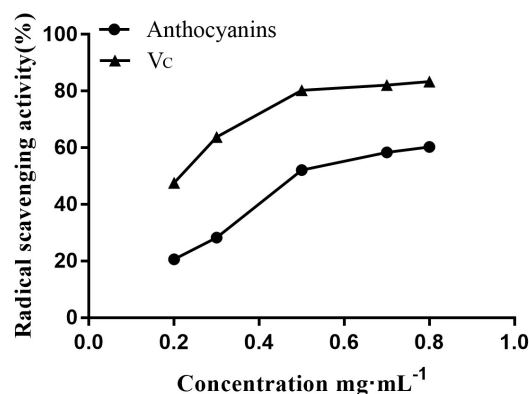


Fig. 6: Anthocyanin hydroxyl radical scavenging activity

Under the conditions of extraction solvent 2% HCl-ethanol, solid-to-liquid ratio 1:4 and extraction time 60 minutes, the effects of extraction temperature on the extraction rate of anthocyanins from purple *Prunus cerasifera* fruit peel were investigated (fig. 2). Results revealed that within a certain temperature range, the extraction rate of anthocyanins increased with increased temperature. But anthocyanins are heat-sensitive and extraction temperature can accelerate the oxidation and degradation of anthocyanins when it exceeded the critical value. Therefore, extraction temperature 35-55°C was selected for the orthogonal array design.

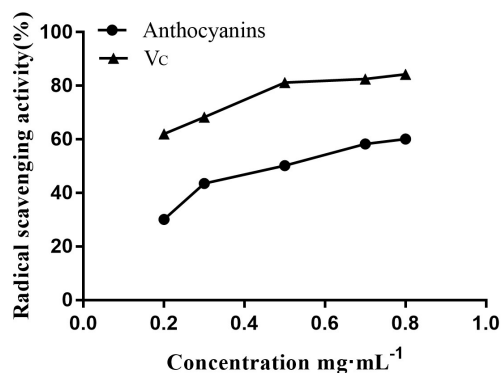


Fig. 7: DPPH radical scavenging activity

Under the conditions of extraction solvent 2% HCl-ethanol, solid-to-liquid ratio 1:4 and extraction temperature 25°C, the effects of extraction time on the extraction rate of anthocyanins from purple *Prunus cerasifera* fruit peel were investigated (fig. 3). Results revealed that longer extraction time yielded more sufficient reaction between raw solution and extract ant. But longer extraction time made more anthocyanins being oxidated and then lost. When designating extraction time, two issues should be considered:

One is whether anthocyanins are fully dissolved, and the other is that too long an extraction time leads to decreased extraction rate of anthocyanins from purple *Prunus cerasifera* fruit peel. So extraction time 60-100 minutes was selected for orthogonal array design.

Under the conditions of extraction solvent 2% HCl-ethanol, extraction temperature 25°C and extraction time 60 minutes, the effects of solid-to liquid ratio on the extraction rate of anthocyanins from purple *Prunus cerasifera* fruit peel were investigated (fig. 4). Results revealed that increased solid-to-liquid ratio can increase molecular diffusion rate and shorten concentration equilibrium time, which increased the content of anthocyanins dissolved in the solvent. This phenomenon tended to be stable when the solid-to-liquid ratio was 1:3. So the solid-to-liquid ratios 1:3, 1:4 and 1:5 were designated in the orthogonal experiments.

Extraction process of anthocyanins from Xinjiang wild *Prunus cerasifera* fruit peel

It can be seen from table 2 that the extraction solvent (A) has the greatest impact on the anthocyanin content, followed by the C time (C) and the extraction temperature (B) and the solid-liquid ratio (D) has a relatively small effect, namely A>C>B>D. Through the analysis of the variance of the orthogonal experiment, it can be seen that different combinations of the four factors have shown significant differences in the extraction effect of anthocyanins, which are basically consistent with the results of the single-factor experiment. Therefore, it can be concluded that the optimal extraction conditions are A₁B₃C₂D₃, 1% HCl methanol as extraction solvent, extraction temperature 55°C, extraction time 80min, solid-liquid ratio 1:5. Under these conditions, the extraction rate of anthocyanins from *Prunus cerasifera* was 480 mg/100g.

Table 3 shows that extraction solvent, solid-to-liquid ratio, extraction time and extraction temperature all greatly affected the extraction rate of anthocyanins from Xinjiang wild *Prunus cerasifera* fruit peel. Regardless of the observation index used color scale of specific absorbance or anthocyanin content, extraction solvent was the most influential factor, followed by solid-to-liquid ratio, extraction temperature and the last extraction time. Analysis of variance of the orthogonal experiment revealed that extraction solvent, solid-to-liquid ratio, extraction temperature, and extraction time affected the extraction of anthocyanins to significantly different levels, which are basically the same as that of single factor experiment. Extraction solvent acquired the highest F value, followed by solid-to-liquid ratio, extraction temperature and the last extraction time. This phenomenon was the same as that of R value. Therefore, the optimal extraction conditions of 1% HCl-methanol, solid-to-liquid ratio 1:5, extraction temperature 25°C, two extractions for 80 minutes each were acquired.

Analysis on anthocyanins from purple *Prunus cerasifera* fruit peel by liquid chromatography-electrospray ionization-tandem mass spectrometry

The compositions of anthocyanins were analyzed using liquid chromatography-electrospray mass spectrometry. According to a previous report (Wang Y, 2016), the possible chemical structures of several active components were identified fig. 5 shows the total ion chromatogram of 20μL purple *Prunus cerasifera* fruit peel sample solution. Positive ion mass spectra of purple *Prunus cerasifera* fruit peel sample solution shows that the m/z of the fragment ions was 287, suggestive of cyanidin anthocyanins. The m/z of the molecular ions was 449 at peaks 1 and 2 in the liquid chromatography-electrospray ionization procedure, and that was 162 [449-287] for glycoside and therefore, cyanidin 3-glucoside and cyanidin 3-galactoside were preliminarily determined.

Table 1: Factors and levels for orthogonal array design

Level	Extraction solvent	Extraction time (min)	Extraction temperature (°C)	Solid-to-liquid ratio
1	1%HCl-methanol	60	35	1:3
2	1%HCl-ethanol	80	45	1:4
3	2% HCl-ethanol	100	55	1:5

Table 2: Orthogonal array design and results

Experiment No.	Extract solvent	Extraction temperature	Extraction time	Solid-to-liquid ratio	Anthocyanin acylation (mg/100 g)
1	1	1	1	1	458.94
2	1	2	2	2	460.63
3	1	3	3	3	462.81
4	2	1	2	3	400.32
5	2	2	3	1	360.03
6	2	3	1	2	379.90
7	3	1	3	2	375.06
8	3	2	1	3	390.47
9	3	3	2	1	414.36
K1	1382.38	1234.32	1229.31	1233.33	
K2	1140.25	211.13	1275.31	1215.59	
K3	1179.89	1257.07	1197.90	1253.60	
R	242.13	45.94	77.41	38.01	

Table 3: Analysis of variance for the total anthocyanin content

Variable	Sum of square	Degree of freedom	Variance	F value
Extraction solvent	33733.49	2.00	16866.74	46.63
Extraction temperature	1055.27	2.00	527.64	1.46
Extraction time	3031.63	2.00	1515.82	4.19
Solid-to-liquid ratio	723.45	2.00	361.72	
total variance	38543.84	8.00		

Note: $P < 0.05$ was considered statistically significant.

Table 4: Mass spectrometric characteristics of purple cherry plum anthocyanins and anthocyanins measured by LC-ESI-MS

Peak	Retention time (min)	M ⁺ ion mass-to-charge ratio	MS ² fragment mass-to-charge ratio	Error ($\times 10^{-6}$)	Formula	Major fragment ion	Compound
1	12.07	449.10784	449.10651	-2.961	C ₂₁ H ₂₁ O ₁₁	287.45657[M-C ₆ H ₁₀ O ₅] ⁺	Cyanidin
2	12.87	595.16575	595.16437	-2.319	C ₂₇ H ₃₁ O ₁₅	287.04739[M-C ₁₂ H ₂₀ O ₉] ⁺	3-galactoside
3	13.33	449.10784	449.10660	-2.761	C ₂₁ H ₂₁ O ₁₁	287.04834[M-C ₆ H ₁₀ O ₅] ⁺	Cyanidin 3-glucoside
4	15.60	595.16575	595.16449	-2.117	C ₂₇ H ₃₁ O ₁₅	287.06873[M-C ₁₂ H ₂₀ O ₉] ⁺	Cyanidin 3-rutinoside
5	15.90	419.09782	419.09687	-2.27	C ₂₀ H ₁₉ O ₁₀	287.04632[M-C ₅ H ₈ O ₄] ⁺	Sagittaria 3-O-xyloside
6	22.81	491.11895	491.11796	-2.02	C ₂₃ H ₂₃ O ₁₂	287.05351[M-C ₈ H ₁₂ O ₆] ⁺	Cornulin (acetyl)-3-o-glucoside
7	23.89	287.05501	287.05411	-3.135	C ₁₅ H ₁₁ O ₆	无碎片离子	Cyanidin
8	28.92	303.04993	303.04916	-2.541	C ₁₅ H ₁₁ O ₇	285.07120[M-H ₂ O] ⁺ , 257.11249[M-H ₂ O-CO] ⁺	delphinin
9	32.1	449.10784	577.01132	-2.961	C ₂₁ H ₂₁ O ₁₁	287.06873	-

The m/z of the fragment ions at peak 3 was 287 and the m/z of the molecular ions was 595 in the liquid chromatography-electrospray ionization procedure, suggestive of cyanidin 3-rutinoside. The m/z of the fragment ions was 287 at peak 4 in the liquid chromatography mass spectrometry procedure and the m/z of fragment ions at peak 4 was 419 in the liquid chromatography-electrospray ionization procedure, suggestive of cyanidin 3-xyloside.

The mass-to-charge ratio m/z of the fragment ions of the peak 5 compound is 287, the mass-to-charge ratio of the first mass spectrum is m/z 419 and the mass-to-charge ratio of the missing glycoside is 132 [419-287], which is the five-carbon sugar missing one fragment obtained from molecular water. Common five-carbon sugars in anthocyanins are arabinose and xylose. Xylose is D-type and arabinose is L-type. Experiments by the research group found that Bluebonnet 3-O-arabinosides are retained with peak 5 at different times, It is speculated that the anthocyanin is Centaurea 3-O-xyloside. The mass-to-charge ratio m/z of the molecular ion of peak 6 is 491, the mass-to-charge ratio of the secondary fragment ion is 287 and 204[491-287] is the missing glycoside. It is speculated that the compound is cornflower (acetyl) 3-O -Glucoside. Peak 9, the mass-to-charge ratio m/z of the primary mass spectrum is 577 and the mass-to-charge ratio m/z of the secondary mass spectrum fragment ion is 287, which is speculated to be a cornflower anthocyanin, but the structure of the sugar has not been identified. See table 4 for details.

Antioxidant Activity

The Results of hydroxyl radical scavenging activity

Since the analyte with scavenging hydroxyl free radicals is added to the reaction system, the generated hydroxyl free radicals are reduced, so that the amount of colored compounds generated is reduced and the absorbance value also changes. Therefore, the change of absorbance can reflect the antioxidant activity of anthocyanins. It can be seen from fig. 6 that the anthocyanins in the purple *Prunus cerasifera* fruit peel have a certain ability to scavenge hydroxyl free radicals, and with the increase of the anthocyanin concentration, the scavenging rate gradually increases, but it is lower than the scavenging rate of the Vc solution of the same concentration.

The Results of DPPH radical scavenging activity

DPPH is an effective free radical scavenger. When there is a free radical scavenger, its absorbance gradually disappears due to its one-electron pairing, so an ultraviolet spectrophotometer can be used for effective measurement. It can be seen from figs. 1-8 that within a certain concentration range, as the mass concentration increases, the scavenging rate of DPPH free radicals also increases and the scavenging rate of Vc is significantly higher than that of the anthocyanin extract in the purple *Prunus cerasifera* fruit peel. The highest clearance rate of

Vc can reach 88.2% and the highest clearance rate of anthocyanins in purple *Prunus cerasifera* fruit peel can reach 63.4%. Therefore, it can be seen that the anthocyanin in purple *Prunus cerasifera* fruit peel has an obvious scavenging effect on DPPH free radicals.

DISCUSSION

This study optimized the extraction conditions of XJP-ACY through a single factor experiment and an orthogonal experiment. The research results show that the best conditions are 1% HCl-methanol as the extraction solvent, solid-liquid ratio 1:5, temperature 55°C and extraction time 80min. The total anthocyanin content of Xinjiang purple *Prunus cerasifera* obtained by the best extraction conditions was 480.81mg/100g, which was higher than that in prunes (Zhou CS, Ma HL *et al.*, 2013), grapes (Liu X, Yang L *et al.*, 2015) and blueberries (Liu HJ, Liu XL *et al.*, 2009).

Use HP2MGL macroporous resin as adsorbent, pH2.5, 80.8% (v/v) ethanol solution as eluent, and eluent flow rate of 2.1ml/min. Purify XJP-ACY extract to obtain refined extract, The content of anthocyanin in the refined extract is 32%, which can be used for later pharmacodynamic experimental research.

The electrostatic field orbitrap high-resolution mass spectrometer has high resolution, fast scanning speed and high sensitivity. It can accurately collect the mass-to-charge ratio of precursor ions and the mass of fragment ions at the same time and realize the detailed information of different chemical components in traditional Chinese medicine by a single needle injection. The information is especially suitable for the qualitative identification of chemical components in the complex system of traditional Chinese medicine lacking reference substances. In this study, liquid chromatography-electrospray ionization-electrostatic field orbitrap high resolution mass spectrometry (LC-ESI-Orbitrap HRMS) technology was used to analyze XJP-ACY components, based on retention time, primary mass spectrometry, secondary mass spectrometry and related literature reports. For structural identification with other information, a total of 8 anthocyanins or anthocyanin components were analyzed.

Studies have shown that anthocyanins have antioxidant activity along with VC, tocopherol and beta-carotene. The purple *Prunus cerasifera* fruit peel Anthocyanin in the skin has the ability to remove hydroxyl free radicals and DPPH free radicals and antioxidant activity is higher, to a certain concentration range, sample concentration of anthocyanins in cherry plum fruit quality had obvious positive correlation with its antioxidant activity, namely with the increase of sample mass concentration, its antioxidant activity is enhanced. Therefore, anthocyanins are a potential antioxidant, so it is of great significance to study the antioxidant activity of anthocyanins in *Prunus*

cerasifera for Individual well-being and later industrial production. This study laid a foundation for the utilization and exploitation of anthocyanins in purple *Prunus cerasifera* fruit peel.

ACKNOWLEDGEMENT

This work was supported by The Xinjiang Key Laboratory of Natural Medicine Active Components and Drug Release Technology(XJDX1713) and the Natural Science Foundation of Xinjiang Autonomous Region (2020D01C227).

REFERENCES

- Abdin M, Hamed YS, Akhtar HMS, Chen D, Chen GJ, Wan P and Zeng XX (2020). Antioxidant and anti-inflammatory activities of target anthocyanins diglucosides isolated from *Syzygium cumini* pulp by high speed counter-current chromatography. *J Food Biochem*, **44**(2): 13209-13222.
- Afzal M, Redha A and AlHasan R (2019). Anthocyanins potentially contribute to defense against Alzheimer's disease. *Molecules*, **24**(23): 4255-4270.
- Chen SS, Zhou HN, Zhang G, Dong Q, Wang ZH, Wang HL and Hu N (2021). Characterization, antioxidant and neuroprotective effects of anthocyanins from *Nitraria tangutorum* Bobr. fruit. *Food Chem*, **353**(1): 129435-44.
- Cui LN, Gao RQ, Sun AQ, Dong ST and Zhang HY(2020). Regularity of Carotenoids and Anthocyanins accumulation in various genotypes of maize kernel. *Zuowu Xuebao*, **36**(5): 818-825.
- Escobar-Ortiz A, Castaño-Tostado E, Rocha-Guzmán NE, Gallegos-Infante JA and Reynoso-Camacho R (2021). Anthocyanins extraction from *Hibiscus sabdariffa* and identification of phenolic compounds associated with their stability. *J Sci Food Agric*, **101**(1): 110-119.
- Fabio G and Luca L (2004). Cyanidins: Metabolism and biological properties. *J. Nutr. Biochem.*, **15**: 2-11.
- Fang H and Jiao Z (2012). Research progress in Anthocyanin biosynthesis and metabolism engineering. *Jiangsu Nongye Kexue*, **40**(7): 5-10.
- He HY, Chen WY, Yang AP, Jiang CY and Zhang R (2020). Effect of blackcurrant polyphenols on lowering blood pressure. *Food and Fermentation Industries*, **46**(3): 97-103.
- Herrera-Sotero MY, Cruz-Hemández CD, Oliart-Ros RM, Chávez-Servia JL, Guzmán-Gerónimo RI, González-Covarrubias V, Cruz-Burgos M and Rodríguez-Dorantes M (2020). Anthocyanins of blue corn and tortilla arrest cell cycle and induce apoptosis on breast and prostate cancer cells. *Nutr. Cancer*, **72**(5): 768-777.
- Huang WY, Hutabarat RP, Chai Z, Zheng TS, Zhang WM and Li DJ (2020). Antioxidant blueberry anthocyanins induce vasodilation via PI3K/Akt signaling pathway in high-glucose-induced human umbilical vein endothelial cells. *Int. J. Mol. Sci.*, **21**(5): 1575-1590.
- Li A, Liu XY and Zhang WG (2020). Effects of fermentation and storage conditions on the stability of anthocyanin in blueberry wine and its antioxidant activity. *China Brewing*, **39**(2): 146-151.
- Li HB, Liu Y, Taxmamat Mahsum, Zhao Y and Iv DK(2013). Characteristics of seedling establishment and distribution of wild endangered *Prunus divaricata* in Xinjiang, China. *Xinjiang Nongye Kexue*, **50**(9): 1612-1619.
- Li J, Xu Z, Zhou L, Zhao JJ and Fu L (2010). Determination of estimation of nutrient compositions of wild *Prunus cerasifera*. *Xinjiang Nongye Kexue*, **47**(11): 2145-2149.
- Liu CQ, Chen XS, Wang JZ, Chen XL, Wang HB, Tian CP and Wu CJ (2008). Studies on genetic diversity of phenotypic traits in wild Myrobalan Plum (*Prunus cerasifera* Ehrh.). **35**(9): 1261-1268.
- Liu HJ, Liu XL and Zhou JZ (2009). Extraction of anthocyanins from blueberry and study on its antioxidant activity. *Jiangsu Nongye Xuebao*, **25**(6): 1347-1350.
- Liu X, Yang L, Zhang FF and Zhang ZW (2015). Changes in fruit texture and anthocyanin content during ripening of wine grape. *Shipin Kexue*, **35**(2): 105-109.
- Sun JX, Zhang Y, Hu XS, Wu JH and Liao XJ (2009). Structural stability and degradation mechanisms of Anthocyanins. *Zhongguo Nongye Kexue*, **42**(3): 996-08.
- Wang F, Deng JH, Tan XH, Zhang LH, Zhong H and Li QM(2008). Research progress on Anthocyanins and Copigmentation. *Shipin Kexue*, **29**(2): 472-476.
- Wang Y (2016). Main anthocyanins compositions of *Prunus cerasifera* Ehrh fruit and their correlation. Shandong: Shandong Agricultural University, 2020.
- Yang HJ, Cui DF, Xu Z and Lin PJ (2003). Analysis on the compositions and resources of seed plants of wild fruit forest in Tianshan mountains of China. *Zhiwu Ziyuan yu Huanjing Xuebao*, **12**(2): 39-45.
- Yang XN (2013). Application of Anthocyanins biosynthesis key enzyme gene in plant gene engineering. *Anhui Nongye Kexue*, **7**: 2866-2869, 2893.
- Yi L, Chen CY, Jin X, Mi MT, Yu B, Chang H, Ling WH and Zhang T(2010). Structural requirements of anthocyanins in relation to inhibition of endothelial injury induced by oxidized low-density lipoprotein and correlation with radical scavenging activity. *FEBS Lett.*, **584**(3): 583-590.
- Zhang SK, Xiao ZC, Zhang GL and Zhang WM(2004). The nutritive value of *Prunus divaricata* and *Vaccinium ashei*. *Zhongguo Yesheng Zhiwu Ziyuan*, **23**(3): 1-3.
- Zhao X, Feng PF, He WQ, Du X, Chen C, Suo LH, Liang M, Zhang N, Na A and Zhang Y (2019). The prevention and inhibition effect of anthocyanins on colorectal cancer. *Curr Pharm Des*, **25**(46): 4919-4927.
- Zhou CS, Ma HL, Yu XJ, Liu WM and Zhou JL (2013). Extracting technology and degradation characteristics of prunes anthocyanins. *Jiangsu Daxue Xuebao*, **34**(5): 536-542.

