 DOI: 10.19601/j.cnki.issn1943 - 9903.2025.08.006

Optimization of Ultrasonic-Assisted Extraction Process for Anthocyanins from *Lycium ruthenicum* Murray

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Abstract [**Objectives**] To investigate the optimal extraction conditions for anthocyanins from defatted *Lycium ruthenicum* Murray using ultrasonic-assisted solvent extraction. [**Methods**] Anthocyanins were extracted from wild *L. ruthenicum* in Qinghai Province using ultrasonic-assisted ethanol extraction. Through single-factor and orthogonal experiments, the optimal extraction conditions were determined as follows: temperature 50 ℃, solid-liquid ratio 1∶15 (g/mL), ethanol concentration 60% (*v/v*), and ultrasonic extraction time 25 min. Under these conditions, the anthocyanin content of *L. ruthenicum* was quantified by UV-Vis spectrophotometry at 280 nm. [**Results**] The extraction yield of anthocyanins from wild Qinghai *L. ruthenicum* was 17.0 mg/g, which is superior to the yield of 10.0 mg/g obtained by water solvent extraction, representing a 0.7% increase in extraction rate. The anthocyanin content in *L. ruthenicum* from different regions was determined, revealing that samples from the Chaidamu area in Qinghai had the highest content (17.3 mg/g), while samples from the Gansu area had the lowest (12.0 mg/g). [**Conclusions**] Ultrasonic-assisted ethanol extraction technology offers advantages including rapid operation, low energy consumption, high extraction yield, simple detection, and safety.

Key words *Lycium ruthenicum* Murray, Anthocyanins, Ultrasonic-assisted solvent extraction, Extraction yield

0 Introduction

Medicinal plants have long been indispensable resources in healthcare. According to the Food and Agriculture Organization of the United Nations (FAO), approximately 25% of the active ingredients in medicines globally are derived directly from plants, with the annual market value of botanical pharmaceutical preparations reaching as high as 43 billion USD. Diverse traditional medicinal systems worldwide predominantly utilize plant extracts[1]. Within the food additive industry, synthetic pigments are commonly employed. However, compared to natural pigments, synthetic alternatives often possess varying degrees of toxicity. Long-term consumption of these synthetic pigments may pose health risks. Consequently, extracting healthy natural pigments from plants, purifying them, and studying their stability has become a significant research trend.

Since ancient times, China has maintained a tradition of utilizing herbal medicines to treat diseases. This rich history produced many renowned pharmacopoeias, such as Li Shizhen's *Compendium of Materia Medica*, which systematized herbal knowledge and offered therapeutic solutions for their era. However, the relatively slower onset of action of traditional Chinese medicine (TCM) and the larger quantities of material often required per dose, combined with the advent and growing popularity of Western medicine, led to a decline in TCM's mainstream market share in certain areas. Western medicine gained widespread acceptance largely due to its faster symptomatic relief and more convenient dosing. Over time, as the duration of Western medicine use increased, people observed that while it often acts quickly, it can also carry significant side effects. Long-term use of certain types may even increase the risk of other health complications, causing concern for patients. In contrast, although TCM typically exerts its effects more gradually, it is generally perceived to have a more favorable safety profile when used appropriately according to TCM principles. Advances in scientific research have deepened the understanding of TCM. The slower action is now recognized not merely as a deficiency, but as potentially linked to its characteristic holistic approach involving multiple active components working synergistically on multiple targets within the body. Consequently, modern research focuses not only on identifying and extracting bioactive compounds but also on elucidating the complex mechanisms of TCM formulas, improving standardization and delivery methods, and rigorously evaluating their efficacy and safety to integrate the strengths of both traditional wisdom and modern science.

L. ruthenicum has rich nutritional value. In recent years, with the deepening of research on small berries, many scientists began to study the functional substances in *L. ruthenicum*, which are rich in minerals, polysaccharides, anthocyanins, flavonoids and other substances, and have anti-lipid, anti-oxidation and other effects. With the improvement of people's living standards, arteriosclerosis and hypertension have become a high incidence. Many researchers at home and abroad have studied the functional components in the fruits and leaves of *L. ruthenicum*, and found that *L. ruthenicum* has great value[2]. Because of its special natural and climatic conditions, Qinghai Province has abundant animal and plant resources, especially the wild wolfberry in the Qaidam Basin of Haixi Prefecture. The wolfberry grains are large, the calcium content is about 8 times that of the general wolfberry, the phosphorus content is about 3 times that of the general wolfberry, and the iron content is about 5 to 9 times that of the general wolfberry. Its amino acids, sugar content and vitamin types are superior to those of "Ningxia wolfberry". It has rich resources, high

Received: May 6, 2025 Accepted: July 1, 2025

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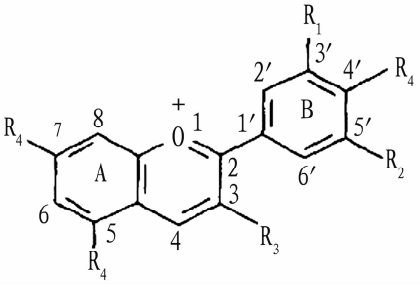
edible and medicinal value, and is widely liked and favored by people[3].

1 Anthocyanins in *L. ruthenicum*

Wild *L. ruthenicum* is rich in nutrients including vitamins, proteins, and trace elements. These nutrients participate in various enzymatic activities involved in physiological functions, immunity, and protein synthesis. The purple peel of wild *L. ruthenicum* is particularly abundant in anthocyanins, which effectively scavenge free radicals in the body. Its anti-aging properties are highly valued. The health and medicinal value of wild *L. ruthenicum* significantly surpasses that of common red goji berries (*Lycium barbarum*), earning it the nickname "soft gold". Long-term consumption of wild *L. ruthenicum* infused in water offers substantial benefits for vision protection and is also effective in preventing and managing diabetes. Furthermore, its diverse functions, such as combating fatigue, protecting the liver, preventing cancer, reducing blood viscosity and thrombosis, effectively lowering blood pressure, and aiding in the protection of the cardiovascular and cerebrovascular systems and overall health, have garnered widespread attention[4].

1.1 Structure of anthocyanins The basic structural parent nucleus of anthocyanins is 2-phenylbenzopyrylium, as shown in Fig. 1



NOTE R1 and R2 are H, OH or OCH3, R3 is glycosyl or H, R4 is glycosyl or OH.

Fig. 1 Basic structure of anthocyanins

For most anthocyanins, the hydroxyl groups are primarily substituted at the 3-, 5-, and 7-carbon positions of the core structure, as illustrated in Fig. 1. The remarkable diversity of anthocyanins arises from variations in the substituents attached to the carbon atoms on the B ring. These substituents can be hydroxyl groups, methoxy groups, or others, leading to distinct chemical profiles. To date, over 200 distinct anthocyanins, categorized into 22 groups, have been identified in nature. Table 1 highlights the six major anthocyanin derivatives most significant in foods: Pelargonidin, Cyanidin, Delphinidin, Petunidin, Peonidin, and Malvidin derivatives[5].

1.2 Properties of anthocyanins Anthocyanins (also known as anthocyanidin glycosides) are water-soluble flavonoid compounds. While the aglycone forms (anthocyanidins) exhibit limited solubility in water, both anthocyanins and anthocyanidins are soluble in polar solvents like ethanol. Conversely, they are generally insoluble in non-polar organic solvents such as chloroform. Anthocyanins can be adsorbed by activated carbon. Their characteristic absorption spectra show distinct peaks: one in the visible region (465 – 560 nm) and another in the ultraviolet region (270 – 280 nm). Due to their polarity, plant anthocyanins are commonly extracted using acidic ethanol or similar polar solvent systems[6].

Table 1 Structural characteristics of six common anthocyanin derivatives

Name	Substituents						
	3	5	6	7	3′	4′	5′
Pelargonidin	OH	OH	H	OH	H	OH	H
Cyanidin	OH	OH	H	OH	OH	OH	H
Pelunidin	OH	OH	H	OH	OMe	OH	OH
Delphinidin	OH	OH	H	OH	OH	OH	OH
Malvidin	OH	OH	H	OH	OMe	OH	OMe
Peonidin	OH	OH	H	OH	OMe	OH	H

When the pH is different, the anthocyanin solution will show different colors with different pH, the anthocyanin solution will show red when the pH is less than 7, the anthocyanin solution will show purple when the pH is between 7 and 8, and the anthocyanin solution will show blue when the pH is higher than 11. Anthocyanins are abundant in angiosperms. According to the statistics, there are 27 families and 73 genera of angiosperms, and anthocyanins have been detected in different levels in these plants. For example, the tissues of cherry, grape, blueberry, red berry, mulberry, morning glory and hawthorn all contain different contents of anthocyanins. Red pigment from grape skin is the first anthocyanin extracted by researchers.

The 20th century is widely recognized as the era of vitamins and antibiotics; now, the discovery and utilization of anthocyanins are ushering in a new era focused on these compounds. Anthocyanins are water-soluble flavonoid pigments widely distributed in plants. They confer significant benefits to human health, exhibiting multiple functional properties such as potent antioxidant activity, free radical scavenging, anti-aging effects, inhibition of inflammation and allergy, and enhancement of blood vessel elasticity. *L. ruthenicum* is notably rich in anthocyanins. Fundamentally, anthocyanins act as powerful antioxidants. Since free radicals are harmful substances, anthocyanins protect the human body from their damaging effects. Research indicates that the free radical scavenging capacity of anthocyanins is 50 times higher than that of vitamin E and 20 times higher than vitamin C. Anthocyanins are readily bioavailable and can be completely absorbed by the human body. Their presence is detectable in human blood within 20 min of ingestion and can be maintained for up to 27 h. Furthermore, anthocyanins possess the distinctive capability to cross the blood-brain barrier, enabling them to provide direct protective effects on the brain's central nervous system-a property not shared by many other antioxidants[8].

1.3 Feasibility of resource utilization of *L. ruthenicum* *L. ruthenicum* (black goji berry) is a highly resilient plant, tolerant of both salt and drought, enabling it to thrive in harsh environments

like the Helan Mountains of Ningxia, eastern Qinghai, and northern Xinjiang. Data indicates its total cultivation area has reached thousands of hectares, particularly in regions such as the eastern foothills of the Tianshan Mountains, Ruoqiang, and the shores of Ebinur Lake. These areas share key characteristics conducive to *L. ruthenicum* growth: significant diurnal temperature variations, prolonged daily sunlight exposure, and high altitude. These conditions promote the accumulation of nutrients and flavonoids, especially anthocyanins. In fact, *L. ruthenicum* is recognized as the wild shrub with the highest known anthocyanin content. When soaked in water, the berries release these pigments, resulting in a distinctive natural sky-blue color. Consumption of this infusion over several days is reportedly associated with a ruddy complexion, highlighting its potential as a cost-effective source of raw materials. Consequently, the rich nutritional profile of *L. ruthenicum* is becoming a pathway to prosperity for local communities, fostering regional economic development.

2 Anthocyanin extraction methods

2.1 Organic solvent extraction method This method represents a widely employed approach for extracting natural pigments in China. Organic solvent extraction is extensively utilized for the extraction and separation of anthocyanins from fruits such as grape seeds, mulberries, blueberries, and others. Within this method, selecting an effective solvent is paramount. The chosen organic solvent must effectively extract the active ingredients present in plant fruits while minimizing the dissolution of other impurities. To enhance the extraction yield of anthocyanins, a small quantity of organic acid or inorganic acid can be incorporated into the organic solvent to adjust the pH of the extraction solution. Commonly used inorganic acids include hydrochloric acid, sulfuric acid, and carbonic acid. Commonly used organic acids include formic acid and acetic acid.

2.2 Water solvent extraction method In the process of extracting anthocyanin with organic solvents, toxic residues typically persist in most cases, and environmental pollution is readily caused during production. Consequently, developing extraction methods devoid of organic solvents has become a research priority. Against this backdrop, the aqueous extraction method emerged. The specific procedure for this method involves: first, thoroughly soaking the plant material in hot water under normal pressure or pressurized conditions for a period; subsequently, adsorbing and extracting using macroporous resin, or alternatively, performing direct hot water extraction under oxygen-free conditions. This approach features straightforward operation and low equipment requirements, though its primary drawback is the poor purity of the extracted product.

2.3 Supercritical fluid extraction Supercritical fluid extraction (SFE) is a method that extracts effective components by altering the solubility of supercritical fluids through variations in temperature and pressure. When materials are processed this way, the extraction yield of active ingredients is higher, though the equipment costs for this method are also increased. Sun Chuanjing *et al.* employed this technique to extract anthocyanins from black currant seeds and grape seeds, optimizing the experimental process. These studies demonstrated that the carbon dioxide and modifiers used in this method can be recycled without causing environmental pollution.

2.4 Microbial fermentation method Microbial fermentation is an extraction method that effectively integrates chemical production and life science. This approach primarily utilizes the catalytic action of microorganisms or enzymes produced during microbial growth to degrade and separate cell walls containing anthocyanins in raw materials. This process enables anthocyanins within the cells to fully dissolve into the extract, thereby accelerating extraction efficiency. Currently, researchers have successfully extracted anthocyanins by degrading the cell walls of *Malva sylvestris* through microbial action combined with cellulase. This method offers the advantages of stable operational performance and no environmental pollution.

2.5 Subcritical water extraction Compared with other methods, subcritical water extraction is a new extraction method. Its main principle is to increase the pressure of the experiment properly, heat the temperature of water to more than 100 ℃, and the critical temperature is below 374 ℃, so as to promote the polarity of water to change with the change of temperature, so as to achieve the purpose of extracting anthocyanins from plants.

2.6 Ultrasonic-solvent extraction Ultrasound is widely employed across diverse fields due to its high-frequency characteristics and also finds extensive application in substance extraction. This technique offers numerous advantages, such as simple operation, high efficiency and speed, clean production processes, absence of pollution, and broad application prospects, which have been affirmed by many scholars. Li Wenpeng *et al.* [7] extracted anthocyanins from blackcurrant using ultrasonic-assisted solvent extraction. Concurrently, they employed single-factor analysis to investigate the influence of parameters including extraction solvent concentration, solvent type, ultrasonic extraction frequency, solid-to-liquid ratio, and ultrasonic extraction duration on anthocyanin yield in blackcurrant. The antioxidant capacity of the anthocyanins was assessed via the TBARS method, leading to the conclusion that anthocyanins exhibit significant antioxidant capacity.

2.7 Cavitation of ultrasonic wave In a fluid, when the local pressure falls below the separation pressure of dissolved gases, gas dissolved in the fluid is released, forming bubbles within or on the fluid surface. These bubbles are termed cavities. The process encompassing the formation, development, and collapse of these cavities (bubbles) is called cavitation. During ultrasonic propagation in a liquid medium, the wave induces oscillatory motion of medium molecules about their equilibrium positions. During the compression phase of the sound wave, intermolecular distances decrease; during the rarefaction phase (*i. e.*, negative pressure phase), intermolecular distances increase. For an ultrasonic wave of intensity I, the resulting sound pressure exerted on the medium is expressed by Equation (1):

$$P_a = P_A \sin\omega t \tag{1}$$

where P_A denotes sound pressure amplitude, and ω denotes angular frequency of sound waves.

$$I = \frac{p_A^2}{2\rho c} \tag{2}$$

where ρ refers to the density of the medium, and c denotes the speed of sound.

In the rarefied phase of the sound wave, $(P_h - P_a)$ can represent the force on the medium in the rarefied phase, and P_h refers to the static pressure of the liquid. Therefore, from Equations (1) and (2), we can conclude that if the sound pressure amplitude increases with the increase of sound intensity, that is to say, the pressure on the medium will increase accordingly. If the pressure increases to a certain extent, it can make the distance between molecules exceed the limit distance, which can destroy the original complete structure of the liquid, thus forming a cavity[9].

3 Experiment

3.1 Raw materials and pre-treatment *L. ruthenicum* collected from Chaidamu (Golmud, Qinghai Province), Xinjiang, and Gansu was used as the experimental material in the single-factor and orthogonal experiments. Clean *L. ruthenicum* was placed in a mortar and ground into powder. The powder was sieved using a 60-mesh sieve, and 6 g of the sieved *L. ruthenicum* powder was weighed. This powder was mixed with petroleum ether at a ratio of 1∶10 (v/v) and then fully soaked for 1 h. Finally, the mixture was heated and refluxed in a water bath at 50 ℃ for 2 h, filtered under reduced pressure, and the filter residue was dried in a cool place to obtain powder for extracting anthocyanin.

3.2 Reagents Anhydrous ethanol was purchased from Yantai Shuangshuang Chemical Co., Ltd., petroleum ether was purchased from Tianjin Yongsheng Fine Chemical Co., Ltd., and acetone was purchased from Baiyin Chemical Reagent Factory. The reagents were all analytically pure.

3.3 Instrument and equipment The instruments and equipment used include the T6 New Century UV-Visible Spectrophotometer (Beijing Purkinje General Instrument Co., Ltd), SHB-III Circulating Water Multi-purpose Vacuum Pump (Shanghai Bilon Instrument Co., Ltd.), KQ-400KDE High-Power CNC Ultrasonic Cleaner (Kunshan Ultrasonic Instrument Co., Ltd.), TDL-40B Low-Speed Benchtop Centrifuge (Shanghai Anting Scientific Instrument Factory), DF-101Z Thermostatic Heating Magnetic Stirrer with Heat Collection (Zhengzhou Greatwall Scientific Industrial and Trade Co., Ltd.), W·S·206B Rotary Evaporator (Shanghai Yukang Science and Education Equipment Co., Ltd.), W·S·206B Constant Temperature Water/Oil Bath (Shanghai Yukang Science and Education Equipment Co., Ltd.), 101A-2E Electric Blast Drying Oven (Shanghai Experimental Instrument Co., Ltd.), AL204 Electronic Balance (METTLER TOLEDO), and HH-6 Digital Display Constant Temperature Water Bath (Changzhou Guohua Electric Appliance Co., Ltd).

3.4 Determination method of anthocyanins There are many methods to determine the content of anthocyanins. In this experiment, the content of anthocyanins was determined by UV-Vis spectrophotometry to evaluate the efficiency of ultrasonic extraction. Anthocyanins exhibit a maximum absorption peak at 280 nm and ethanol was used as the solvent for determination[10-13].

3.5 Anthocyanin quantitative method

3.5.1 Extraction method of anthocyanins. (i) Water solvent extraction method. Weighed out 1 g of defatted *L. ruthenicum* powder. Added distilled water at a solid-to-liquid ratio of 1∶15 (g/mL) and allowed it to soak thoroughly for a specified period. Centrifuged the mixture at 4 000 rpm for 15 min and collected the supernatant. Repeated the extraction process 2-3 times on the residue under the same experimental conditions. Combined all the supernatants obtained from the extractions. Finally, diluted the combined extract to volume with distilled water. The anthocyanin extraction yield obtained using the aqueous solvent extraction method was 10.0 mg/g, equivalent to 1.0%.

(ii) Ultrasound-assisted extraction method. Defatted *L. ruthenicum* powder (1 g) was weighed and mixed with an ethanol solution of specified concentration at a designated solid-to-liquid ratio for thorough soaking. The soaked mixture was transferred to a flat-bottomed flask equipped with a reflux condenser and placed in an ultrasonic bath. Ultrasonic extraction was performed at constant power for a predetermined duration. Following extraction, the mixture was centrifuged at 4 000 rpm for 15 min, resulting in phase separation, after which the supernatant was collected. The residue was re-extracted 2-3 times under identical conditions. All supernatants were combined and the resulting extract was diluted to volume with absolute ethanol. The effects of single factors, including the *L. ruthenicum*-to-ethanol ratio, ethanol volume fraction, extraction temperature, and extraction time, were investigated on extraction yield. Suitable conditions were selected based on these single-factor experiments, and an orthogonal array design was implemented using these parameters to determine the optimal process conditions for anthocyanin extraction from *L. ruthenicum*[14].

3.5.2 Plotting of standard working curve of anthocyanins. (i) Preparation of standard solution. Accurately weighed 0.010 0 g of anthocyanin reference substance, dissolved it with absolute ethyl alcohol, and accurately diluted it to 100.00 mL to prepare a standard stock solution with a concentration of 0.10 mg/mL. Diluted with absolute ethanol to prepare a standard solution with a concentration of 10.0 μg/mL.

(ii) Plotting of working curve. Aliquots of the above anthocyanin standard working solution (2.00, 4.00, 6.00, 8.00, and 10.00 mL) were transferred into separate 10.0 mL volumetric tubes. Absolute ethanol was added to each tube, diluted to the 10.0 mL mark, stoppered, and thoroughly mixed to prepare standard series with concentrations of 2, 4, 6, 8, and 10 μg/mL, respectively. As anthocyanins exhibit a maximum absorption peak at 280 nm wavelength, absorbance measurements were performed at 280 nm using absolute ethanol as the blank to zero the instrument and subtract background values. The relationship between standard solution concentration and absorbance was determined, and a calibration curve of anthocyanin concentration (μg/mL) versus absorbance was plotted (Fig. 2).

The concentration of anthocyanidin in the solution was linear with the absorbance in the range of 2-10 μg/mL. The regression equation of the mass of anthocyanidin (μg) and absorbance curve calculated by the least square method was $Y = 0.021\ 5x - 0.020\ 2$, and the correlation coefficient was 0.888 1.

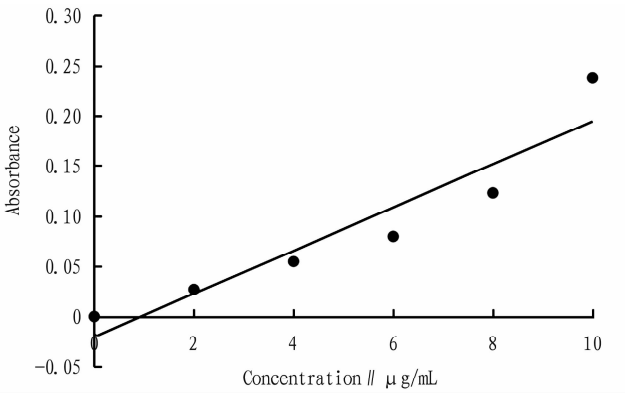


Fig. 2 Working curve of anthocyanin concentration and absorbance

3.5.3 Calculation of anthocyanin extraction yield. (i) Sample determination. Weighed *L. ruthenicum* extract dry powder and dissolved in absolute ethanol to a constant volume of 100.00 mL, centrifuged at a speed of 4 000 r/min for 10 min, and took the supernatant was taken. Diluted it 10 times with absolute ethyl alcohol, then took 2.00 mL of it and fixed the volume to 10.00 mL with absolute ethyl alcohol, shook it up, determined the absorbance value, calculated the concentration of anthocyanin according to the working curve, and calculated the content of anthocyanin in the dry powder of *L. ruthenicum* extract.

(ii) Calculation formula:

$$\text{Anthocyanin}\ (\%) = \frac{m \times V_2 \times d}{10^3 \times G \times V_1} \times 100\% \qquad (3)$$

where m represents the content of anthocyanin corresponding to the working curve, expressed in μg; V_1 denotes sampling volume of the sample solution to be tested, expressed in mL; V_2 denotes volume of the sample at constant volume, expressed in mL; G means the mass of sample measured, expressed in g, and d represents the dilution factor of sample solution.

4 Results and analysis

4.1 Single factor experiment

4.1.1 Effects of ethanol volume fraction on extraction yield of anthocyanins. Weighed 1 g of defatted *L. ruthenicum* powder and add 20%, 40%, 60% and 80% ethanol solution. According to the ultrasound-assisted extraction method in Section **3.5.1** (ii), ultrasonically extracted in a water bath at 50 ℃ for 25 min, and repeated the operation steps twice.

The experimental results are shown in Fig. 3. When the volume fraction of ethanol is 20%, the extraction yield of anthocyanin is 10.7 mg/g; when it is 40%, the yield is 11.4 mg/g; when it is 60%, the yield is 12.2 mg/g; and when it is 80%, the yield is 11.8 mg/g. Since ethanol has lower polarity and water has higher polarity, the polarity of the ethanol-water solution gradually decreases as the ethanol content increases. As shown in Fig. 3, the volume fraction of ethanol significantly impacts the extraction efficiency. Specifically, as the ethanol volume fraction increases, the anthocyanin extraction yield from wild *L. ruthenicum* also increases. However, when the volume fraction reaches 60%, the yield begins to decline. Therefore, 60% ethanol is optimal as the extractant.

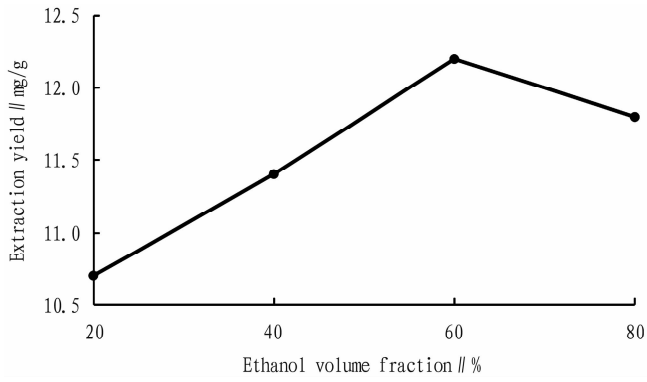


Fig. 3 Effects of ethanol volume fraction on extraction yield of anthocyanins

4.1.2 Effects of extraction temperature on extraction yield of anthocyanins. Weighed 1 g of degreased *L. ruthenicum* powder, and added 60% ethanol solution (volume fraction) according to the solid-liquid ratio of 1∶15 (g/mL). According to the above steps, ultrasonic treatment was carried out for 25 min in water baths at 30, 40, 50 and 60 ℃, respectively, and repeated twice.

The experimental results are shown in Fig. 4. When the extraction temperature is 30 ℃, the anthocyanin extraction yield is 10.2 mg/g; when the temperature is 40 ℃, the yield is 11.1 mg/g; when the temperature is 50 ℃, the yield is 12.3 mg/g; and when the temperature is 60 ℃, the yield is 11.3 mg/g. Generally, as temperature increases, molecular motion accelerates, leading to faster permeation, diffusion, and dissolution rates. The structure of cell membranes may be affected by temperature, and high temperatures may cause damage, allowing anthocyanins to enter the solvent through the compromised membranes. As shown in Fig. 4, temperature significantly impacts the extraction efficiency. The anthocyanin extraction yield from *Lycium ruthenicum* varies with temperature: as temperature rises, the yield increases accordingly. However, when the extraction temperature exceeds 50 ℃, the yield begins to decline. This decrease may occur because higher temperatures intensify oxidation, partially degrading anthocyanin structures and consequently reducing the extraction yield. Therefore, 50 ℃ was determined to be the optimal extraction temperature for the experiment.

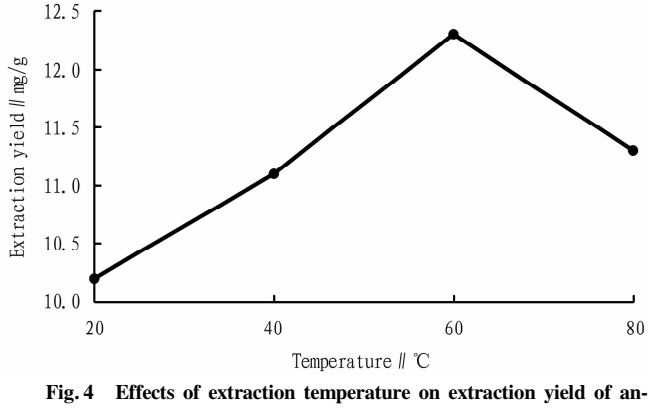


Fig. 4 Effects of extraction temperature on extraction yield of anthocyanins

4.1.3 Effects of solid-to-liquid ratio on extraction yield of anthocyanins. Weighed 1 g of degreased *L. ruthenicum* powder, added 60% ethanol solution at the ratio of 1 ∶ 10, 1 ∶ 15, 1 ∶ 20 and 1 ∶ 25, respectively, and carried out ultrasonic treatment for 25 min in 50 ℃ water bath according to the above steps, and repeated twice.

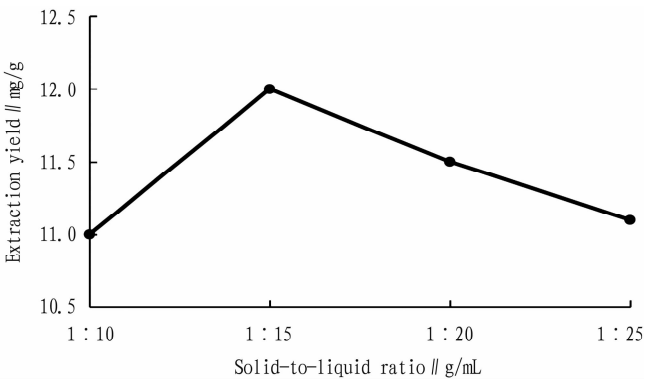


Fig. 5 Effects of solid-to-liquid ratio on extraction yield of anthocyanins

The experimental results are shown in Fig. 5. When the solid-to-liquid ratio of *L. ruthenicum* and ethanol is 1 ∶ 10, the extraction yield of anthocyanin is 11.0 mg/g; when the solid-to-liquid ratio of *L. ruthenicum* and ethanol was 1 ∶ 15, the extraction yield of anthocyanin was 12. 0 mg/g; when the solid-to-liquid ratio of *L. ruthenicum* to ethanol was 1 ∶ 20, the extraction yield of anthocyanin was 11.5 mg/g; when the solid-to-liquid ratio of *L. ruthenicum* to ethanol was 1 ∶ 25, the extraction yield of anthocyanin was 11.1 mg/g. It can be seen from Fig. 3 that the solid-to-liquid ratio of *L. ruthenicum* and ethanol has a certain influence on the extraction effect of anthocyanin. At the beginning, with the increase of the solid-to-liquid ratio, the extraction yield of *L. ruthenicum* anthocyanin also increased, but when the solid-to-liquid ratio of *L. ruthenicum* and ethanol reached 1 ∶ 15 (g/mL), The extraction yield of anthocyanin began to decrease. Considering that too large solid-to-liquid ratio will cause the waste of ethanol and increase the recovery cost of the experiment, the solid-to-liquid ratio of *L. ruthenicum* and ethanol should be selected as 1 ∶ 15 (g/mL).

4.1.4 Effects of extraction time on extraction yield of anthocyanins. Weighed 1 g of degreased *L. ruthenicum* powder, and added 60% ethanol solution at a solid-to-liquid ratio of 1 ∶ 15 (g/mL). According to the above steps, ultrasonic treatment was repeated twice in 50 ℃ water bath for 10, 15, 20 and 25 min, respectively.

As shown in Fig. 6, when the extraction time was 10 min, the extraction yield of anthocyanin was 10.6 mg/g; when the extraction time was 15 min, the extraction yield of anthocyanin was 12.0 mg/g; when the extraction time was 20 min, the extraction yield of anthocyanin was 11.7 mg/g; when the extraction time was 25 min, the extraction yield of anthocyanin was 10.9 mg/g. In general, the complete degree of anthocyanin dissolution is proportional to the ultrasonic treatment time, and the extraction yield will increase accordingly. It can be seen from Fig. 6 that the extraction yield of *L. ruthenicum* anthocyanin increased significantly with the increase of ultrasonic extraction time at the beginning. However, the extraction yield of anthocyanins decreased slightly when the ultrasonic extraction time exceeded 15 min. Therefore, the ultrasonic extraction time should be selected as 15 min[15-18].

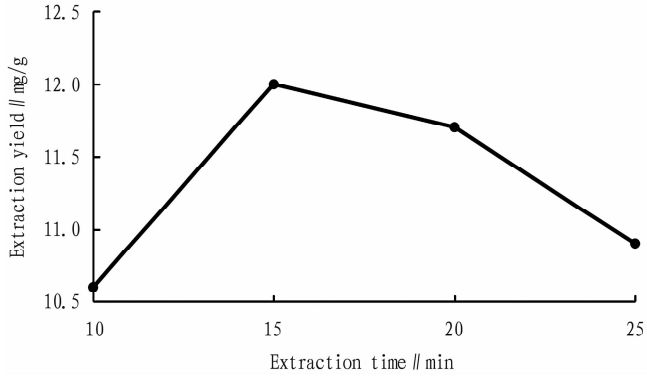


Fig. 6 Effects of extraction time on extraction yield of anthocyanins

4.2 Orthogonal experiment Through the single factor experiment, the volume fraction of ethanol, the solid-to-liquid ratio of *L. ruthenicum* and ethanol, the temperature of ultrasonic extraction, the time of ultrasonic extraction and other factors were selected for the orthogonal experiment (see Table 2 for the specific factors and levels). The optimum process conditions for extracting anthocyanin from *L. ruthenicum* were determined, and the experimental results are shown in Table 3.

Table 2 Factor level of orthogonal experiment

Level	Factor			
	A Temperature ℃	B Ethanol concentration // mg/g	C Time min	D Solid-to-liquid ratio
1	30	60	20	1 ∶ 20
2	40	40	10	1 ∶ 15
3	60	20	15	1 ∶ 25
4	50	80	25	1 ∶ 10

Table 3 Orthogonal experimental design and results

No.	A	B	C	D	Extraction yield // mg/g
1	1	1	1	1	10.2
2	1	2	2	2	10.2
3	1	3	3	3	15.1
4	1	2	4	4	11.4
5	1	1	2	3	15.8
6	1	2	1	4	13.4
7	1	3	4	1	11.0
8	1	2	3	2	12.4
9	3	1	3	4	10.2
10	3	2	4	3	14.3
11	3	3	1	2	10.0
12	3	2	2	1	14.2
13	4	1	4	2	17.0
14	4	2	3	1	12.8
15	4	3	2	4	10.5
16	4	2	1	3	14.5

The optimal experimental combination was determined to be $A_4B_1C_4D_2$, that is: under an extraction temperature of 50 ℃, a solid-to-liquid ratio of *L. ruthenicum* to ethanol at 1 ∶ 15 (g/mL), using a 60% ethanol solution by volume, with ultrasonic extraction for 25 min. Following these conditions, anthocyanin extraction experiments were conducted on *L. ruthenicum* from Xinjiang, Gansu, Golmud, and Qaidam regions. The calculated extraction yields for anthocyanins in different regions were as follows: Xinjiang: 15.4 mg/g, Gansu: 12.0 mg/g, Golmud: 15.7 mg/g, Qaidam: 17.3 mg/g[19-21]. This indicates that *L. ruthenicum* from the Qaidam region contains the highest anthocyanin content, while that from the Gansu region contains the lowest.

5 Conclusions

The optimum extraction conditions for anthocyanins from defatted *L. ruthenicum* using ultrasonic-assisted solvent extraction were investigated. Ultrasound-assisted ethanol extraction offers advantages including rapid processing, low energy consumption, high extraction yield, and simple/safe operation. Results demonstrated that the optimal conditions were: ethanol concentration 60%, extraction temperature 50 ℃, solid-to-liquid ratio 1 ∶ 15 (g/mL), and extraction time 25 min. Ethanol was selected as the extractant due to its relative safety and food-grade suitability. Ultrasonic waves generate solid-liquid vibrational effects, while cavitation disrupts cellular walls. This enhances solute diffusion from cells to solvent, facilitating anthocyanin extraction from *L. ruthenicum* with a yield of 17 mg/g (equivalent to 1.7%). Comparative experiments showed this outperformed traditional methods (10 mg/g or 1.0%), representing a 0.7% absolute yield increase. As anthocyanins are heat- and light-sensitive, experiments were conducted at low temperature under light-protected conditions. To enhance extraction efficiency based on raw material properties, *L. ruthenicum* was soaked in solvent prior to ultrasound-assisted extraction.

As the crushing degree of wild *L. ruthenicum* particles increases (*i.e.*, particle size decreases), the anthocyanins in wild *L. ruthenicum* become easier to extract. Therefore, in this experiment, wild *L. ruthenicum* passed through a 60-mesh sieve was used as the experimental material. The degreasing pretreatment with petroleum ether is necessary, as it can simplify subsequent refinement processes of the extract. Analysis of the experimental results shows that the *L. ruthenicum* from the Qaidam Basin region of Qinghai had the highest content at 17.3 mg/g, indicating superior quality; samples from Golmud and Xinjiang followed with 15.7 and 15.4 mg/g, respectively; while those from Gansu had the lowest content at 12.0 mg/g.

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