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# Preparation of High-Purity Rutin from *Sophora japonica*

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## ABSTRACT

**High-Purity Rutin (HPR) was extracted from *Sophora japonica* by ultrasonication, decolorized using activated carbon, separated by polyamide chromatography, and recrystallized. An orthogonal design of experiment was used to determine the optimal ultrasonic extraction time, number of ultrasonic extractions, and ultrasonic extraction temperature to maximize the extraction yield of rutin. The optimal conditions were determined as follows: 2000 mL of $CH_3OH$ were used to extract 20 g of the dried *Sophora japonica* flower bud powder for 30 minutes and the extraction was repeated 2 times under ultrasonic conditions (ultrasound frequency: 40 KHZ; water temperature: 40 °C). The decolorization of the $CH_3OH$ extract of *Sophora japonica* using active carbon was studied. The optimal processes are as follows: The filtrate of $CH_3OH$ extract was concentrated to 200 mL by rotary evaporation; 7.2 g of activated carbon powder was added; and the mixture was refluxed in an 80 °C water bath for 0.5 hours (twice). The polyamide chromatography separation conditions for crude rutin were investigated. The influence of particle size and amount of polyaminde used on extraction yield of rutin was studied. It was found that best separation results were obtained when 50 g of polyamide with particle size of 0.170~0.210 mm was used to purify 3 g of crude rutin. The purity of the HPR obtained was 99.8%, the extraction yield of HPR was 18.6%, and the extraction rate of HPR was 70.8%. The structure of the final product was identified by elemental analysis, IR, HPLC and $^1H$ NMR. It was experimentally demonstrated that the proposed process was a safe, mild, low-cost and waste-free procedure.**

**Keywords:** High-Purity of Rutin (HPR), Ultrasound extraction, Activated carbon decolorization, Polyamide chromatography separation.

## INTRODUCTION

*Sophora japonica* is the dried flower bud of *Sophora japonica L.*, which is widely cultivated in China for its favorable medicinal value. It has been reported that *Sophora japonica*, which has many active substances, has various biological activities, including antibacterial capacity, vasodilation, and antioxidation capacity, etc. The dried flower bud of *Sophora japonica L.* has been widely applied in health and food products [1]. There is a large amount rutin (Fig. 1) in the flower buds of *Sophora japonica L*. Rutin, a flavonoid compound, has many important physiological activities in the treatment of inflammation, cerebrovascular diseases, cardiovascular disorders, cancer, etc., attributed to its anti-inflammatory [2], antiviral [3], remarkable antioxidation [4], and antifree radical properties [5].

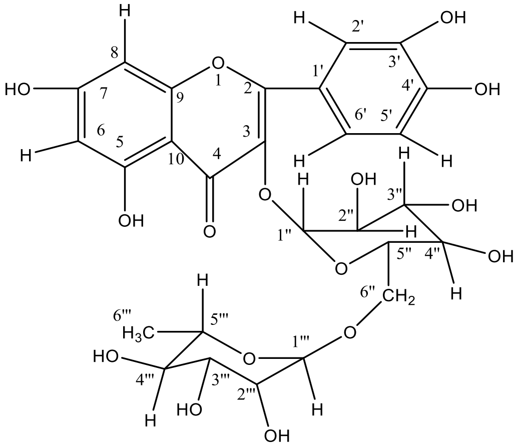

**Fig. 1: Chemical structure of rutin**

The extraction methods of rutin mainly include alkali extraction and acid precipitation [6, 7], hot extraction and cold precipitation [8], and organic solvent extraction [9~12]. However, these methods have some drawbacks, such as thermal degradation of rutin due to prolonged heat at high temperature, potential effects on human health and the environment through the use of large amounts of harmful organic solvents. In order to overcome these problems, the use of environmentally friendly technologies has been highly valued by researchers, including microwave-assisted extraction [13], ultrasound-assisted extraction [14], enzyme-assisted extraction [15], supercritical fluid extraction [16], etc. Rutin purification methods mainly include alkali solubilized acid precipitation method [17], cold water washing method [18], dextran gel method [19], macroporous adsorption resin method [20], polyamide chromatography method [21, 22], macroreticular resin method [23], high-pressure liquid phase preparation method [24], two-step chromatographic process (MCI GEL® CHP20P and Sephadex® LH-20 columns) method [25], etc. Among them, both cold water washing method and alkali solubilized acid precipitation method have the problems of low purification rate and incomplete impurity removal. The latter few methods have the problems of cumbersome operations, low yields, and high costs. These shortcomings limit the wide application of rutin in food, cosmetics, and medicine areas.

*Sophora japonica L.*, which is also widely grown in China, and has become an important source of rutin. The current upper limit purity of rutin available on the market is about 98%. To meet higher needs arising in production and scientific research, as well as to control the quality of rutin products, we have successfully prepared HPR using activated carbon decolorization method polyamide chromatography method, and recrystallization method. The purity of HPR was determined using the HPLC method. The structure of rutin was also characterized by IR, elemental analysis, and $^1$H NMR. Compared with the extraction and purification processes reported in the literature, the method for preparing HPR described in this paper has the advantages of simple process, non-toxic and pollution-free solvents, safety, low cost, and reusable solvents and polyamides, making it suitable for industrial production.

## MATERIALS AND METHODS

### Materials and Instruments

Chromatographic methanol and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical Co., Ltd. (Shanghai, China). Polyamide (particle size: 0.140 mm-0.575 mm) was obtained from Jiangsu Changfeng Chemical Co., Ltd. (Changzhou, China). Powdered activated carbon (particle size: 1.0 μm-150 μm) was obtained from Shanghai Bilang Environmental Protection Technology Co., Ltd. (Shanghai, China). *Sophora japonica* was obtained from Chuzhou, Anhui Province, China.

A Waters e2695 instrument and a 2498UV/Vis detector were obtained from Waters Corporation of Shanghai (Shanghai, China). A Bruker (500 MHz) NMR and Vector 22 type infrared spectrometer were obtained from Brooke Company (Karlsruhe, Germany). A CHN-O-Rapid elemental analyzer was obtained from Heraeus Analytical Instruments (Hanau, Germany). A chromatography column was obtained from Beijing Synthware Glass Instrument Co., Ltd. (Beijing, China).

### Content Determination and Extraction Rate Calculation of Rutin From *Sophora japonica*

Based on previous research methods [26], 55 mg of rutin standard was weighed and placed in a 250 mL volumetric bottle; 60% ethanol was added to the scale; and rutin standard solution was obtained at a concentration of approximately 0.22 mg/mL. A rutin standard solution of 0.22 mg/mL was placed in a 10 mL volumetric flask (0.0, 1.0, 2.0, 3.0, 4.0, and 5.0 mL). Next, 0.5 mL of 5% NaNO2 solution was added and placed for 6 min; 0.5 mL of 10% Al $(NO_3)_3$ solution was added and calmly placed for 6 min. Next, added 4 mL of 4% NaOH solution was added, and the distilled water was diluted to 10 mL, shaken evenly, and then calmly placed for 15 min. Rutin was not added to the blank control group. The absorbance value was measured at 500 nm. The standard curve was plotted using the standard solution absorbance as the ordinate Y and the mass concentration of rutin in the standard solution as the abscissa X. The regressive equation of the curve was Y = 9.5182X-0.0072, R2 = 0.9973. The results showed that its mass concentration and absorbance were quite linear in the range from 22 μg/mL to 110 μg/mL.

Based on a previous research method [27], *Sophora japonica* powder (1 g) and ether (120 mL) were added to a Suo Shi extractor, heated, and refluxed to obtain a colorless extracted liquid. The extracted liquid of ether was discarded after being cooled. Methanol (90 mL) was added to the Suo Shi extractor once again, heated, refluxed to colorless extracted liquid, and transferred to a 100 mL volumetric flask. Methanol was added to the scale, and methanol extract solution was obtained. Five milliliters of methanol extract solutions were placed in a 50 mL volumetric flask, and distilled water was added to the scale. Two milliliters were removed from the previously prepared solution and placed in a 10 mL volumetric flask, and the absorbance was measured according to the standard curve method. The mass fraction (c) of rutin in *Sophora japonica* was calculated as 25.4% based on the concentration-absorbance of the standard sample.

In the subsequent ultrasonic extraction and decolorization process, each extract or decolorization liquid was transferred to a 100 mL volumetric bottle, methanol was added to the scale, and a methanol extract solution was obtained. Five milliliters of methanol extract solutions were placed in a 50 mL volumetric flask, and distilled water was added to the scale and used as the test solution. In the polyamide column layer separation process study, 100 mL of eluate was taken each time. Two milliliters of eluate were transferred to a 50 mL volumetric flask, and 50% ethanol was added to the scale and used as the test solution.

Two milliliters were removed from each test solution and placed in a 10 mL volumetric flask, and the absorbance was measured according to the standard curve method. The mass fraction (c') of rutin in the test solution was calculated according to the standard linear equation of concentration-absorbance of the standard sample, and the extraction rate of rutin was calculated according to the following formula:

$$\text{Extraction rate of rutin (\%)} = (c'/c) \times 100\%$$

## Polyamide Pretreatment and Packing

Polyamide (140 g) mixed with an appropriate amount of anhydrous ethanol was added to a 1000 mL round bottom flask, heated under reflux in a constant temperature water bath (90 °C), filtered and washed with distilled water more than three times after 2 h. The filter cake was transferred to a 1000 mL beaker filled with distilled water (500 mL), stirred well, and then soaked overnight. The next day, the polyamide powders that removed some bubbles in the beaker were poured into the chromatography column filled with absorbent cotton at the bottom of the column and naturally settled. Excess water is released from the chromatography column and then stopped by closing the piston at the bottom of the column until the water surface is slightly higher than the surface of the polyamide powder. The chromatography column was installed and used for the separation and purification of rutin.

## Extraction and Isolation

The dried flower bud powders of *Sophora japonica* (20.0 g) were loaded into a flask (5000 mL) and extracted with $CH_3OH$ (2000 mL × 2) under ultrasonic conditions (ultrasound powder: 40 KHZ; water temperature: 40 °C). Each extraction time was 30 min. The solutions were filtered and merged. The filtrate was concentrated to 200 mL by rotary evaporation; 7.2 g of activated carbon powder was added; and the mixture was refluxed in an 80 °C water bath for 0.5 h (two times), filtered and merged to obtain a brown-yellow liquid. As previously mentioned, the brown–yellow liquid was concentrated and dried under vacuum to obtain a crude extract of rutin (6.2 g). The crude rutin (3.0 g) was separated on a with polyamide chromatographic column using elution solvent with $H_2O$-$C_2H_5OH$ (V/V, 1:1) to afford 30 fractions ($F_1$-$F_{30}$, 100 mL/fraction). Fractions $F_6$–$F_{20}$ were merged, concentrated, dried in vacuum and further recrystallized from 400 mL $H_2O$ to obtain HPR (1.8 g) with a yield of approximately 18.6% from *Sophora japonica*.

A High-performance liquid chromatography (HPLC) system of (Waters) was selected to determine the purity of rutin. The analytical chromatography conditions are listed as follows: rutin (5 mg) was dissolved in methanol in a 100 mL volumetric flask. After filtration with a 0.22 μm filter, purity analysis of rutin was carried out with a $C_{18}$ column (4.6 mm i.d ×250 mm, particle size of 5 μm). The mobile phases were $H_2O$ and $CH_3OH$ (70:30, V/V), the flow rate was 1.0 mL/min, and the sample volume was 10 μL. The temperature of the column was maintained at 30 °C, and the UV detection wavelength was set to 254 nm. Identification of rutin was performed by IR, elemental analysis and $^1$H NMR spectroscopy. IR was performed using a Vevtor 22 infrared spectrometer. The $^1$H NMR experiment was carried out using a Bruker DR×500 NMR spectrometer. Elemental analysis was performed by a CHN-O-Rapid elemental analyzer.

## RESULTS AND DISCUSSION

### Identification of *rutin* Isolated from *Sophora japonica*

The structure of the final product separated was identified by IR, elemental analysis, HPLC and NMR spectroscopy as follows: Purity (HPLC): 99.8% (Fig. 2); appearance: light yellow powder; mp: 195-196 °C. In the infrared spectrum (Fig. 3), the strong and wide absorption peak at 3427.3 cm$^{-1}$ resulted from the -OH stretching vibration.

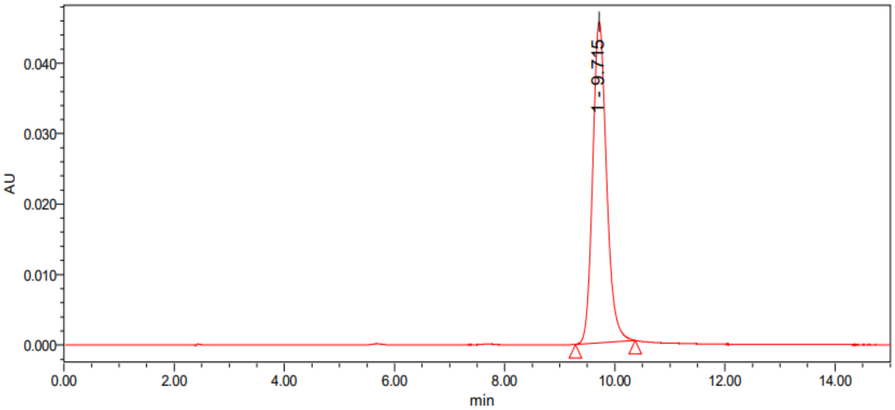

**Fig. 2: HPLC spectrogram of rutin isolated methanol solution**

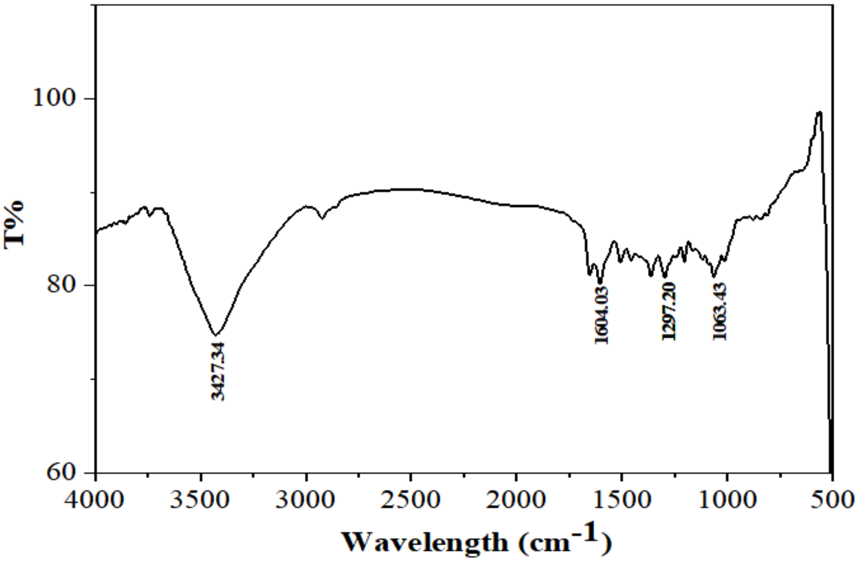

**Fig. 3: Infrared spectrum of rutin**

The weak absorption peak of 2923.7 cm$^{-1}$ resulted from $CH_3$- and -$CH_2$- stretching vibrations. The strong and wide absorption peak of 1652.8 cm$^{-1}$ indicated the presence of a conjugated carbonyl group. The peaks at 1604.6 cm$^{-1}$, 1506.2 cm$^{-1}$, 937.3 cm$^{-1}$, 877.5 cm$^{-1}$ and 811.9 cm$^{-1}$ were caused by aromatic ring skeletons and aromatic ring substitutions. The absorption peak of 1361.5 $cm^{-1}$ showed the presence of methyl group in this compound. The absorption peak of 1456.1 $cm^{-1}$ was caused by C-H bending vibration of methylene. 1297.9 $cm^{-1}$, 1243.9 $cm^{-1}$, 1205.3 $cm^{-1}$, 1164.8 $cm^{-1}$ and 1062.3 $cm^{-1}$ were attributed to various hydroxyl groups in flavonoid glycosides.

The results of element analysis according to $C_{27}H_{30}O_{16}$ were the measured values (calculated values)/%: C, 53.10 (53.07); H, 4.88 (4.91). The structure of the isolated compound and rutin are identical.

The chemical shifts of different types of protons are shown in the $^1H$ NMR spectra (DMSO, 500 MHz) (Fig. 4). Apart from the sharp peak of δ 12.58 (1H, S, 5-OH), the remaining three peaks of 10.83 (1H, S, 7-OH), 9.67 (1H, S, 4′-OH), and 9.18 (1H, S, 3′-OH) hydroxyl protons on the aromatic ring are wide and flat, and all four hydroxyl protons appear in the low field ($\delta > 9.0$). The five protons on the aromatic ring are 7.52 (1H, S, 6′-H), 7.51 (1H, S, 2′-H), 6.83 (1H, S, 5′-H), 6.37 (1H, S, H-8), and 6.17 (1H, S, H-6). These protons appear in the field ($9.0 > \delta > 6.0$). The six –OH proton glycosides are 5.28 (1H, S, 2′′-OH), 5.11 (1H, S, 3′′-OH), 5.08 (1H, S, 4′′-OH), 5.07 (1H, S, 2′′′-OH), 4.53 (1H, S, 3′′′-OH), and 4.35 (1H, S, 4′′′-OH). The three hydroxyl glucose group appear in the lower field because the glucose groups is near the mother nucleus of the flavonoid. The chemical shifts of two hydrogen atoms on the first carbon of glucosylrhamnose are 5.31 (1H, S, H-1′) and 4.39 (1H, S, H-1′′′). The peaks of 0.97, and 0.96 are typical proton-induced chemical shift of rhamnose methyl. The chemical shifts of ten hydrogen atoms on the glucose and pyran ring of rhamnose are located between 3.0 and 4.0, forming overlapping multiple peaks. All data were identical to those of rutin [28].

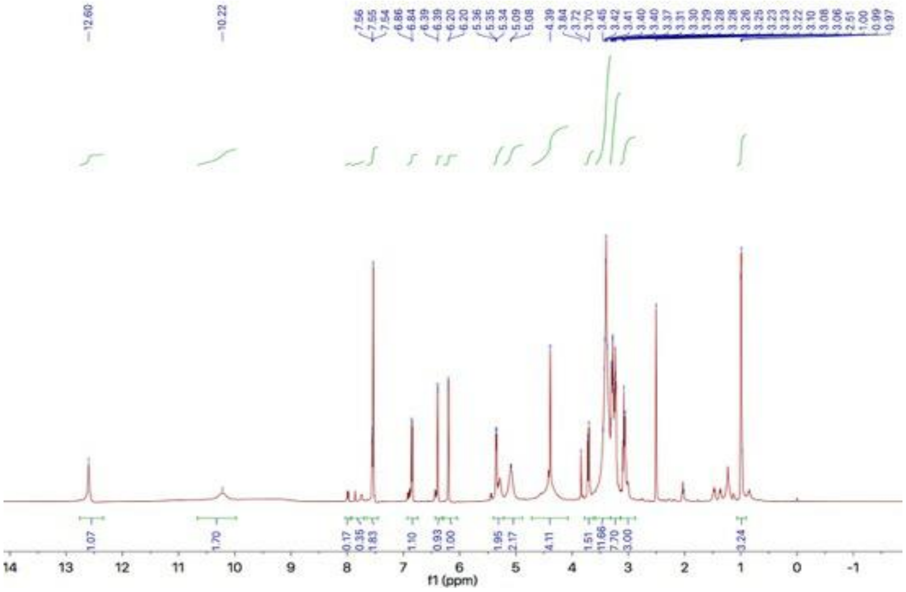

Fig. 4: $^1H$ NMR spectrogram of rutin

## Factors Influencing the Separation and Purification of Rutin

### Factors Influencing Ultrasonic Extraction:

The ultrasonic extraction process has shown many advantages, including a faster extraction rate, lower energy input, lower solvent consumption, and lower temperature in the extraction of natural products [29]. This extraction technology is widely utilized in industry. Ultrasound waves cause cell destruction and ultrasonic jets and reduce the particle size, improving the contact area of the solid-liquid phase and producing more active substances in the extraction solvent. The increasing extraction yield of natural products is devoted to acoustic cavitation phenomena under ultrasound conditions. The expansion cycle could produce numerous microbubbles or cavities in the liquid phase at sufficient ultrasound intensity. These formed bubbles could absorb the energy from ultrasonic waves and increase during the expansion cycles. The pressure and temperature increased due to compression, making the bubbles break up, which produces a shock wave that traversed the solvent, improving the mass transfer effect within the ultrasound extraction system [30].

Rutin is an alcohol-soluble component that is extracted with methanol as the solvent. A three-factor and three-level orthogonal experiment was established with ultrasonic extraction times (A) of 20 min, 30 min and 40 min, ultrasonic extraction times (B) of 1 time, 2 times and 3 times, and ultrasonic extraction temperatures (C) of 20 °C, 40 °C and 60 °C (Table 1). The ultrasonic extraction technology of rutin from *Sophora japonica* was investigated. The extraction rate of rutin was used as a marker. The dried flower bud powders of *Sophora japonica* (1.0 g) were loaded into a flask (50 mL) and extracted with $CH_3OH$ (10 mL) under ultrasonic conditions (ultrasound powder: 40 KHZ) every time. The experimental results are shown in Table 2.

**Table 1: Factors and levels of orthogonal experiments for ultrasonic extraction**

| Levels | Factors | | |
|---|---|---|---|
| | **Time/A(min)** | **Times/B(Times)** | **Temperature/C (℃)** |
| 1 | 20 | 1 | 20 |
| 2 | 30 | 2 | 40 |
| 3 | 40 | 3 | 60 |

**Table 2: Results of ultrasonic extraction orthogonal experiment**

| No | Factors and levels | | | | The extraction rate of rutin (%) |
|---|---|---|---|---|---|
| | **A** | **B** | **C** | **D(Blank)** | |
| 1 | 1 | 1 | 1 | 1 | 64.5 |
| 2 | 1 | 2 | 2 | 2 | 72.4 |
| 3 | 1 | 3 | 3 | 3 | 77.1 |
| 4 | 2 | 1 | 2 | 3 | 84.6 |
| 5 | 2 | 2 | 3 | 1 | 85.1 |
| 6 | 2 | 3 | 1 | 2 | 81.2 |
| 7 | 3 | 1 | 3 | 2 | 79.3 |
| 8 | 3 | 2 | 1 | 3 | 86.2 |
| 9 | 3 | 3 | 2 | 1 | 85.7 |
| I | 214.0 | 228.4 | 231.9 | 235.3 | |
| II | 250.9 | 243.7 | 242.7 | 232.9 | |
| III | 251.2 | 244.0 | 241.5 | 247.9 | |
| R | 37.2 | 15.6 | 10.8 | 15.0 | |

As shown in Table 2, the $A_3B_3C_2$ method (ultrasonic extraction time of 40 minutes, 3 ultrasonic extraction times, and ultrasonic extraction temperature of 40 °C) was the optimal ultrasonic extraction method for rutin from *Sophora japonica*.

With the extended ultrasound time, the extraction rate of rutin gradually increased. When the time exceeded 30 minutes, the effect of time on the extraction rate of rutin was not obvious. As shown in Table 2, the rutin extraction rate was nearly unchanged by ultrasound time at 30 minutes and 40 minutes. The concentration gradient of rutin inside and outside the plant cells tended to 0 in a short time, and the rutin extraction rate exhibited minimal change with increasing extraction time. Under the conditions of constant extraction time and extraction temperature, the ultrasonic extraction time had a significant effect on the extraction rate of rutin. If the number of extractions is too small, rutin leaching will be incomplete. With an increase in extraction times, rutin leaching becomes relatively more complete; however, the production cost is increased. Comprehensive consideration shows that the 2 extraction times yields better results. The increase in ultrasonic extraction temperature can improve the solubility and diffusion coefficient of rutin in the solvent and enhance the extraction rate. However, when the temperature is too high, ultrasonic cavitation may be weakened, which is not conducive to the extraction of rutin [31]. Overall, an ultrasound temperature of 40 °C was selected for subsequent experiments. The method of $A_2B_2C_2$ (ultrasonic extraction time: 30 minutes; number of ultrasonic extractions: 2 times; ultrasonic extraction temperature: 40 °C) was determined as the method of ultrasound extraction. The test was repeated three times according to the optimized process conditions, and the average extraction rate of rutin was 88.6%. The result was better than that of any group in the orthogonal trials. The results showed that the optimal extraction method that was selected was convenient and reliable, had a high extraction rate and was suitable for extracting rutin from *Sophora japonica*.

**Factors Influencing Activated Carbon Decolorization:**

The methanol ultrasonic extract of *Sophora japonica* contains more chlorophyll, which should be decolorized with activated carbon. Systematic research on the method of decolorization of methanol ultrasonic extract of *Sophora japonica* was carried out. The dried flower bud powders of *Sophora japonica* (9.0 g) were loaded into a flask (500 mL) and extracted with $CH_3OH$ (90 mL) under ultrasonic conditions (ultrasound powder: 40 KHZ) according to the optimal methanol ultrasonic extraction process $A_2B_2C_2$ (ultrasonic extraction time: 30 minutes; ultrasonic extraction: 2 times; ultrasonic extraction temperature: 40 °C). The two extracts were filtered, merged, concentrated to 90 mL by rotary evaporation, evenly divided into nine aliquots, and decolorized according to the orthogonal experiment method (Table 3). The extraction rates of rutin, chlorophyll A (at 663 nm) and chlorophyll B (at 645 nm) absorption in decoloring solution were selected as the comprehensive marker, and the $L_9\,(3^4)$ orthogonal table was arranged for the experiment. The experimental results are shown in Table 4.

$$\text{Comprehensive marker (CM)} = 25\%\,(\bar{X}/X) + 25\%\,(\bar{Y}/Y) + 50\%\,(\bar{Z}/Z)$$

X is the absorption of chlorophyll A in 9 parts of the decolorization solution; $\bar{X}$ is the mean of chlorophyll A absorption in 9 decolorization solutions; Y is the absorption of chlorophyll B in 9 parts of the decolorization solution; $\bar{Y}$ is the mean of chlorophyll B absorption in 9 decolorization solutions; Z is the extraction rate of rutin in 9 parts of decolorization solution; and $\bar{Z}$ is the mean extraction rate of rutin in 9 decolorization solutions.

**Table 3: Factors and levels of orthogonal experiments for decolorization process (Refluxing with water bath, 80 ℃)**

| Levels | Factors | | |
|---|---|---|---|
| | Amount of activated carbon/A (%) | Times/B(Times) | Time/C(min) |
| 1 | 12 | 1 | 10 |
| 2 | 24 | 2 | 20 |
| 3 | 36 | 3 | 30 |

Note: The amount of activated carbon refers to the quality fraction of activated carbon in *Sophora japonica*.

**Table 4: Results of decolorization orthogonal experiment**

| No | Factors and levels | | | | Markers | | | CM |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D(Blank) | X | Y | Z | |
| 1 | 1 | 1 | 1 | 1 | 0.194 | 0.121 | 71.1 | 0.70 |
| 2 | 1 | 2 | 2 | 2 | 0.022 | 0.028 | 80.9 | 1.58 |
| 3 | 1 | 3 | 3 | 3 | 0.015 | 0.020 | 69.6 | 1.92 |
| 4 | 2 | 1 | 2 | 3 | 0.174 | 0.119 | 53.2 | 0.57 |
| 5 | 2 | 2 | 3 | 1 | 0.013 | 0.016 | 66.5 | 2.16 |
| 6 | 2 | 3 | 1 | 2 | 0.013 | 0.015 | 61.5 | 2.16 |
| 7 | 3 | 1 | 3 | 2 | 0.015 | 0.021 | 79.5 | 1.97 |
| 8 | 3 | 2 | 1 | 3 | 0.012 | 0.017 | 65.1 | 2.19 |
| 9 | 3 | 3 | 2 | 1 | 0.010 | 0.012 | 34.2 | 2.42 |
| I | 4.20 | 3.24 | 5.05 | 5.28 | | | | |
| II | 4.89 | 5.93 | 4.50 | 5.71 | | | | |
| III | 6.58 | 6.5 | 6.27 | 4.68 | | | | |
| R | 2.38 | 3.26 | 1.77 | 1.03 | | | | |

As show in Table 4, the extraction rate of rutin and the absorption degree of chlorophyll A and chlorophyll B in the decolorization solution: were on the order of B > A > C, namely, decolorization times > activated carbon dosage > decolorization time. The optimal process condition for activated carbon decolorization of *Sophora japonica* methanol extract is $A_3B_3C_3$, that is, 3.6% of activated carbon powder is consumed and 3 reflux decolorization times, with 30 min each time. However, there is minimal difference between $RB_2$ and $RB_3$ according to the orthogonal table analysis. The $B_2$ level was selected based on the reduction in production cost. The best activated carbon decolorization process is $A_3B_2C_3$, namely, 36% of activated carbon powder was consumed and refluxing decolorization was performed twice with 30 min each time. The test was repeated three times according to the activated carbon decolorization process ($A_3B_2C_3$). The extraction rate of rutin, the absorption of chlorophyll A and B in decolorization fluid, and the comprehensive marker (CM) were 82.3%, 0.012, 0.011, and 2.54, respectively. The result was higher than that of any group in the orthogonal test, indicating that the process was stable and feasible.

The activated carbon decolorization effect is good and is widely utilized in the extraction process of various natural ingredients. The amount of activated carbon had a significant effect on the decolorization effect of the *Sophora japonica* methanol extract. When the amount of activated carbon increases, the amount of the adsorbed coloring material is also enhanced. However, excessive activated carbon can cause large amounts of rutin to be adsorbed by activated carbon, resulting in a decreased extraction rate of rutin. When the amount of activated carbon was small, the extraction rate of rutin increased because of the weak adsorption capacity of activated carbon. Simultaneously, the decolorization effect decreases due to the adsorptivity of the activated carbon reducing to coloring material. The test results showed that when 36% activated carbon powder was utilized, the extraction rate of rutin increased. Increasing the activated carbon decolorization time and decolorization time yields an improvement in the adsorption capacity and decolorization ability of the activated carbon coloring material. The test results showed that a good decolorization effect could be achieved by adding 36% activated carbon powder and conducting refluxing decolorization 2 times for 30 min each time. The decolization effect did not significantly change, and the extraction rate of rutin constantly decreased with increasing decolorization time. Overall, the decolization effect for the *Sophora japonica* methanol extract and the extraction rate of rutin improve after reflux decolorization were performed twice for 30 minutes each time.

**Influencing Factors of Polyacrylamide-Packed Chromatographic Column Separation:**
The crude rutin (3.0 g) was obtained according to the previously preferred optimal ultrasonic extraction and activated carbon decolorization process conditions with a polyamide chromatographic column. The particle size of the polyamide was selected based on the extraction rate of rutin and the flow rate of the eluent as markers in the column layer separation process. The results are shown in Table 5.

**Table 5: Influence of particle diameter of polyamide**

| Diameter of polyamide /mm | Extracting rate of rutin/% | Current velocity of elution/mL·min$^{-1}$ |
|---|---|---|
| 0.300-0.575 | 42.6 | 8.1 |
| 0.210-0.300 | 56.8 | 6.2 |
| 0.170-0.210 | 75.2 | 4.2 |
| 0.140-0.170 | 76.8 | 2.2 |

The particle size of polyamide decreases, and the separation effect of rutin is improve. However, small particles could slow the flow rate of eluent and extraction efficiency of rutin and prolong the separation time. With an increase in particle size, the separation degree of rutin from other ingredients decreased, which affected the extraction rate of rutin. Collectively, polyamide with a particle size of 0.170~0.210 mm was used as the separation material. The extraction rate of rutin was near the maximum value, the flow rate of eluent was faster, and the production efficiency was higher.

In the separation process of the polyamide column, if the mass ratio between the crude rutin and the polyamide used is too large, the separation efficiency of rutin will decrease; if this mass ratio is too small, the amount of polyamide used increases, and the production cost of rutin is obviously improved. Collectively, the mass ratio of 1:40-60 is good for the preparation of rutin. The influence of the 1:40-60 mass ratio on the separation of rutin is shown in Table 6.

**Table 6: Influence of the sample amount to the polyamide amount**

| $M_{sample}$ /$M_{polyamide}$ (g/g) | Extracting rate of rutin/% |
|---|---|
| 1:40 | 55.7 |
| 1:45 | 65.3 |
| 1:50 | 76.6 |
| 1:55 | 76.8 |
| 1:60 | 77.5 |

As show in Table 6, when the mass ratio of the crude rutin to polyamide used was 1:50, the extraction rate of rutin did not increase. Therefore, the ratio of 1:50 is selected.

## CONCLUSIONS

In recent years, natural products have been increasingly investigated as anticancer drugs. In this study, HPR (HPLC: 99.8%) was successfully separated from *Sophora japonica* by ultrasonic extraction, activated carbon decolorization, polyamide column chromatography, and recrystallization. The extraction yield of HPR was 18.6%. The chemical structure of the final product separated was identified by IR, element analysis and $^1$H NMR. Compared with the traditional separation process, optimized extraction process of HPR in this paper has the advantages of simple process, environmentally friendly solvent, safe and cheap, and polyamide can be reused. so optimized extraction process is suitable for industrial production. The extraction yield and purity of rutin increased significantly. The research results have provided a strong foundation for the further development and utilization of *Sophora japonica* resources.

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## Conflict of Interest

The authors declare no conflict of interest.

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