

Study on Optimization of Ultrasonic Extraction of Flavonoids from *Bidens Bipinnata* by Orthogonal Experiment

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Abstract

In this paper, ultrasonic assisted extraction of Flavonoids from *Bidens pilosa* was studied. On the basis of four single factor experiments of solvent ratio, ultrasonic extraction time, material-liquid ratio and particle size, orthogonal experiment was designed to optimize the ultrasonic-assisted extraction conditions of Flavonoids from *Bidens pilosa*. The optimum extraction conditions of Flavonoids from *Bidens pilosa* were as follows: ultrasonic extraction time 25 min, particle size 100 mesh, liquid-material ratio 1:2. Under these conditions, the extraction rate of Flavonoids from *Bidens pilosa* reached 0.41%.

Keywords

Orthogonal experiment; Ultrasonic extraction; *Bidens pilosa*.

1. Introduction

The main active components of *Bidens pilosa* include flavonoids, polyene alkynes, coumarins, etc. [1-3]. At present, the extraction technology of active components from natural products has been developed from traditional solvent extraction to modern auxiliary technology, such as supercritical carbon dioxide extraction, microwave-assisted extraction and ultrasonic-assisted extraction. Ultrasonic-assisted extraction has been widely used in the extraction of flavonoids, alkaloids and other bioactive substances from natural products due to its low heat consumption, high efficiency and no destruction of active components [4-7]. In order to study the content of flavonoids in *Bidens bipinnata*, ultrasonic assisted extraction technology was used to obtain the optimal combination of extraction conditions through orthogonal test, so as to provide experimental basis for the further development and utilization of *Bidens pilosa*.

2. Experimental Part

2.1. Materials, Reagents and Instruments

The main instruments used in this experiment are shown in Table 1, and the main reagents are shown in Table 2.

Table 1. Main experimental instruments

Instruments	Equipment Model	Manufacturer
Ultraviolet visible spectrophotometer	TU-1901	Beijing perseer General Instrument Co., Ltd
High-speed universal crusher	300gyf-114	Joyoung Co., Ltd
Electronic balance	Yp602n	Henan guangfan Instrument Equipment Co., Ltd
Ultrasonic device	Kq-500	Kunshan Ultrasonic Instrument Co., Ltd

Table 2. Main reagents

Reagent	Specifications	Manufacturer
Anhydrous ethanol	Analytical purity	Sinopharm Reagent Chemical Reagent Co., Ltd
anhydrous ether	Analytical purity	Sinopharm Reagent Chemical Reagent Co., Ltd
Bidens Bipinnata	Medicinal	Purchased in Zhoukou tonghetang pharmacy

2.2. Preparation of Standard Solution

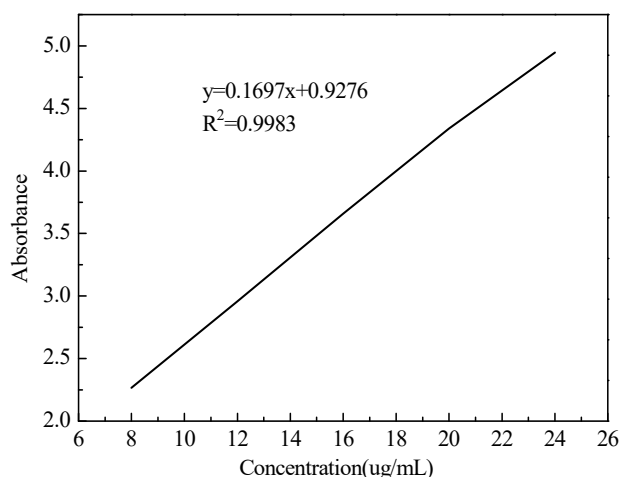
The Rutin Standard Sample (10 mg) was weighed by electronic balance, then added some anhydrous ethanol and dissolved in ultrasonic equipment. Five volumetric flasks (10 mL) were filled with 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL, 0.6 mL standard sample solutions respectively. Then add 1 mL of NaNO_2 with volume fraction of 5%, shaken and placed for 6 min. Then add 1 mL of $\text{Al}(\text{NO}_3)_3$ with 10% volume fraction and shake it for 6 min, then add 1 mL NaOH solution with 4% volume fraction, fix the volume in a 25mL volumetric flask and shake it for standby. Select five 10 mL volumetric flasks, put 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL and 0.6 mL standard sample solution respectively, then add 1 mL of NaNO_2 with volume fraction of 5%, shake well and place for 6 min; Then add 1 mL of $\text{Al}(\text{NO}_3)_3$ with a volume fraction of 10%, shake well and place for 6 min; Then add 1 mL of NaOH solution with volume fraction of 4%, and finally fix the volume with absolute ethanol to 10 mL. Finally, the volume of the volumetric flask was fixed to 10 mL by anhydrous ethanol.

2.3. Determination of Maximum Absorption Wavelength

Using distilled water as reference, the concentration of 0.4 mg/mL rutin standard sample solution was scanned in the wavelength range of 200-700 nm by UV photometer, the maximum absorption wavelength was 358 nm.

2.4. Drawing of Standard Curve

Using distilled water as contrast solution, the absorbance values of Rutin Standard Solution with concentration of 8 $\mu\text{g/mL}$, 12 $\mu\text{g/mL}$, 16 $\mu\text{g/mL}$, 20 $\mu\text{g/mL}$ and 24 $\mu\text{g/mL}$ were determined at 358 nm. Using the absorbance as the vertical coordinate and the concentration of Rutin as the horizontal coordinate to make the standard curve, as shown in Figure 1, the equation of the standard curve is $y = 0.1697x + 0.9276$, $r^2 = 0.9983$. The linear relationship of Rutin Standard sample in the concentration range of 8-24 $\mu\text{g/mL}$ was obtained from the standard equation.

**Figure 1.** Rutin standard curve

2.5. Determination of Absorbance of the Extract of Bidens Pilosa

The raw materials purchased from Zhoukou Tonghe Tang Drugstore were put into the electric blast drying oven and dried at 40 °C for 24 h. Put the raw materials into a high-speed grinder for grinding, screening, particle size of 40 mesh, 60 mesh, 80 mesh, 100 mesh respectively. Take 1g 40 mesh, 80 mesh and 100 mesh powder and put them into corked extraction bottle. The filtrate was filtered again through a microporous filter. The filtrate was kept in a 20 mL volumetric flask, and the absorbance value (A_{test}) of the sample was measured by UV-vis spectrophotometer, so as to calculate the yield of flavonoids in Bidens Pilosa. Its calculation formula is as follows:

$$Yield = \frac{m_F}{m_B} \times 100\% = \frac{C_{test} * 20mL}{m_B} \times 100\% = \frac{(A_{test} - 0.9276) * 20mL}{169.7 * 10^3 mg/mL} \times 100\% \quad (1)$$

m_B is the mass of Bidens bipinnata

m_F is the mass of flavonoids extracted from Bidens bipinnata

C_{test} is the concentration of extracted flavonoids,

A_{test} is the absorbance of extracted flavonoids.

2.6. Design of Single Factor Experiment

When the volume ratios of ethanol and ether were 0:1, 1:1 and 1:0, respectively. When the particle size of raw materials is 40 mesh, 80 mesh and 100 mesh respectively. The ultrasonic time was 10 min, 25 min and 40 min respectively. When the solid-liquid ratio was 1:10 (g:mL), 1:30 (g:mL), 1:50 (g:mL), the effects of four single factors on the yield were studied.

2.7. Design of Orthogonal Test

L_9 (3^4) orthogonal table was used to determine the yield of Bidens pilosa flavonoids according to the orthogonal table conditions, and the orthogonal experimental analysis was carried out to find out the best process conditions.

3. Results and Analysis

3.1. Effect of Solvent Ratio on the Yield of Flavonoids from Bidens Pilosa

The effects of different volume ratios of ethanol and ether on the yield of flavonoids are shown in Figure 2.

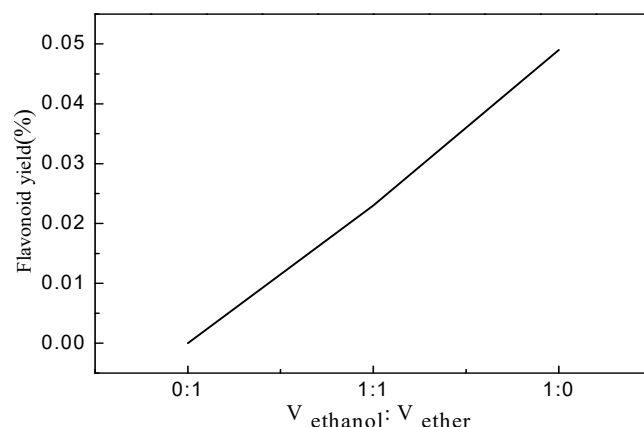


Figure 2. Effect of solvent ratio on yield of flavonoids

It can be seen from Figure 2 that the yield of flavonoids gradually increases at the stage when the volume ratio of ethanol to ether ranges from 0:1 to 1:0. The yield is the highest at 1:0. This may be because the solubility of raw materials in ethanol is greater than that of ether, the extraction is more sufficient, and the boiling point of ether is lower than that of ethanol, which is more volatile, resulting in low yield of chemical components.

3.2. Effect of Raw Material Particle Size on the Yield of Flavonoids from *Bidens Pilosa*

The effects of different *Bidens pilosa* particle sizes on the yield of flavonoids are shown in Figure 3.

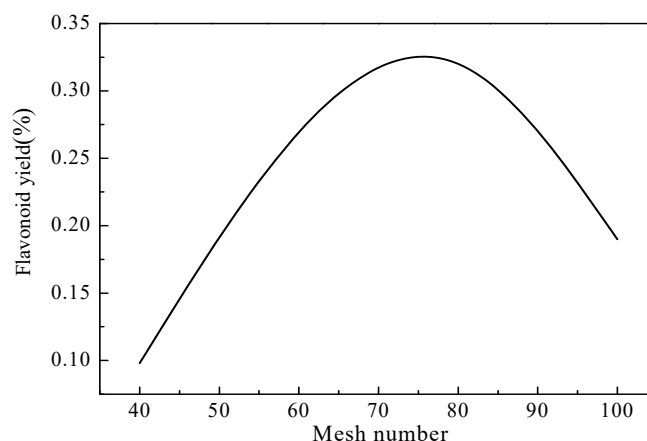


Figure 3. Effect of raw material particle size on chemical yield of Flavonoids from *Bidens pilosa*

It can be seen from Figure 3 that the yield of chemical components gradually increases at the stage of raw material particle size from 40 mesh to 80 mesh, and decreases at the stage of raw material particle size from 80 mesh to 100 mesh. The maximum yield was obtained when the particle size of raw material was 80 mesh. This may be that when the particle size of the raw material is 40 mesh to 80 mesh, the specific surface area of the raw material increases and the contact between the raw material and the solvent is more sufficient with the increase of the particle size of the raw material. When the raw material particles are too small, the mass transfer resistance will increase, resulting in the decrease of yield.

3.3. Effect of Ultrasonic Time on the Yield of Flavonoids

The effects of different ultrasonic time on the yield of *Bidens pilosa* flavonoids are shown in Figure 4.

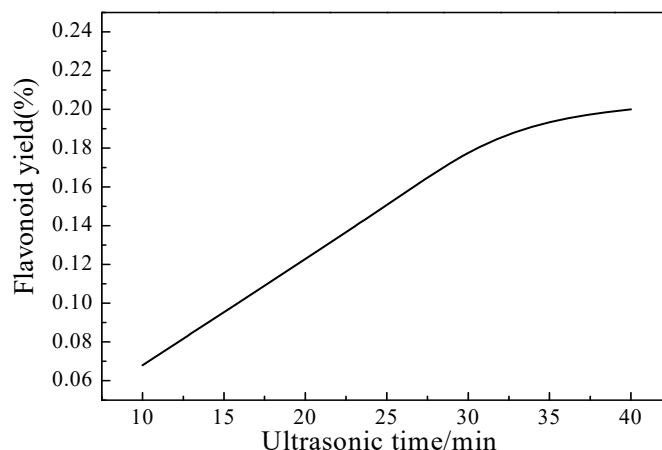


Figure 4. Effect of ultrasonic extraction time on the yield of flavonoids

It can be seen from Figure 4 that the ultrasonic extraction time is 10 min to 40 min, and the yield of chemical components increases gradually. The increase rate of yield slowed down from 30 min to 40 min. The reason may be that with the extension of ultrasonic time, the concentration of flavonoids in cells decreased, resulting in the slow increase of yield.

3.4. Effect of Material-liquid Ratio on Yield of Flavonoids

The effect of material-liquid ratio on the yield of flavonoids was shown in Figure 5.

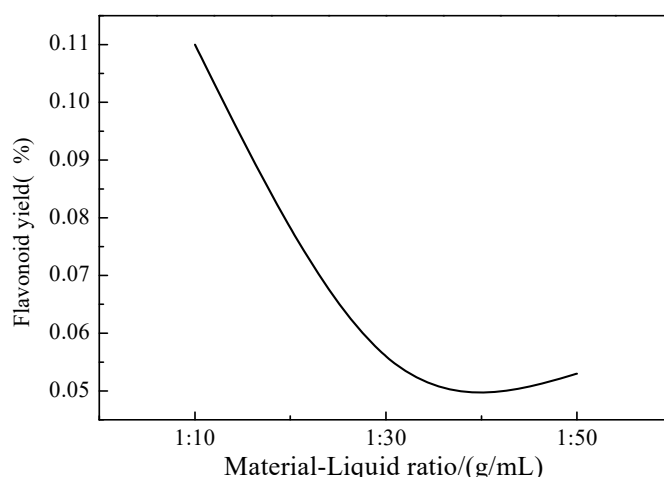


Figure 5. Effect of material-liquid ratio on chemical yield of Flavonoids from *Bidens pilosa*

It can be seen from Figure 5 that the yield of flavonoids decreased gradually from the material liquid ratio of 1:10 (g:mL) to the material liquid ratio of 1:40 (g:mL). The yield of flavonoids increased gradually when the material liquid ratio was between 1:40 (g:mL) and 1:50 (g:mL). The yield of flavonoids was the highest at 1:10(g:mL). The extraction rate of flavonoids decreases with the increase of material liquid ratio, which may be because the dissolution rate of flavonoids slows down with the increase of material liquid ratio, resulting in the decrease of chemical yield.

3.5. Orthogonal Experimental Design

Table 3. Results of orthogonal test

Experiment No.	Raw material particle size (Mesh)	Ultrasonic time (min)	Material-Liquid ratio (g:mL)	Flavonoid yield (%)
1	60	20	1:10	0.05
2	60	30	1:20	0.15
3	60	40	1:30	0.22
4	80	20	1:20	0.36
5	80	30	1:30	0.19
6	80	40	1:10	0.18
7	100	20	1:30	0.39
8	100	30	1:10	0.40
9	100	40	1:20	0.41
K1	0.140	0.267	0.210	
K2	0.243	0.247	0.307	
K3	0.400	0.270	0.267	
R	0.260	0.023	0.097	

It can be seen from table 3 that among the three factors, the particle size of raw materials has the greatest impact on the yield of chemical components, the material liquid ratio is the second, and the ultrasonic extraction time is the smallest. The effects of three factors on the yield of Flavonoids from *Bidens pilosa* were as follows: raw material particle size > solid-liquid ratio > ultrasonic extraction time. The optimum technological condition was A3B3C2, and the chemical yield of *Bidens pilosa* flavonoids was 0.41%.

4. Conclusion

On the basis of single factor experiment, ultrasonic assisted extraction was designed by orthogonal experiment to optimize the extraction process of Flavonoids from *Bidens bipinnata*. Among the three factors of material-liquid ratio, ultrasonic time and raw material particle size, the particle size of raw material has the greatest influence on the yield of chemical components, followed by material-liquid ratio, and ultrasonic time has the least influence on the yield of chemical components. The experimental results showed that the yield of *Bidens pilosa* flavonoids was 0.42%.

Acknowledgments

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