Study on Extraction and Purification of Total Flavonoids from Mappianthus iodoies

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Keywords: Mappianthus iodoies; total flavonoids; orthogonal test; D-101 macroporous resin.

Abstract: Objective To optimize the extraction and purification of total flavonoids from Radix. Methods The rutin was used as the reference substance. The ethanol concentration, extraction multiple, extraction time and extraction times were used as the influencing factors. The orthogonal test was also carried out. At the same time, the total flavonoids of D. chinensis were purified by D-101 and AB-8 macroporous resin respectively. The purification process uses ultraviolet spectrophotometry to determine the total flavonoid content. The results were as follows: 60% ethanol, extraction ratio 12, extraction time 2 h, extraction times 3 times, the extraction scheme was the best, and the total flavonoids were better purified with D-101 macroporous resin. The resin volume (V): the crude drug amount (M)=1:1, the loading flow rate is 2 BV/h, the elution flow rate is 2-3 BV/h, and 70% ethanol is used as the elution solvent. When 5 column volumes are eluted, the purity of total flavonoids is 62.30%. . Conclusion The extraction and purification of this process is effective, the feasibility is strong, and the results are sTable, which can provide theoretical basis for further research.

1 Introduction

Mappianthus iodoies is a woody vine of the Plantplant-hus Hand.-Mazz. plant of the genus Iceacinaceae [1-2]. The roots of the vines or the old vines have the effect of reducing wind, dehumidification and anti-inflammatory, and are often used in the treatment of snake bites and jaundice [1,3]. Studies have confirmed that the heart contains vines, organic acids, tannins, alkaloids, phenolic components, amino acids, flavonoids and other chemical components [4-8] flavonoids have antiviral, anti-viral Inflammation, anti-tumor, regulation of endocrine, liver protection, etc. [9-10], in addition, through the preliminary exploration of the Institute's pharmacology room, Dingxin also has a good anti-platelet aggregation effect, through the literature review combined with pharmacological test preliminary judgment, The flavonoids in this plant may be effective sites. In this experiment, the extraction and purification process of the total flavonoids from the vines was studied.

2. Instruments and reagents

2.1 Materials

Dingxin Teng (Kunming, Yunnan) was appraised by Professor Zhang Qinde of Shandong College of Traditional Chinese Medicine.

2.2 Main test drugs

Rutin reference (100082, Nanjing), methanol (AR), sodium nitrite (AR), aluminum nitrate (AR), sodium hydroxide (AR), ethanol (AR), D-101 macroporous resin, AB-8 Macroporous resin.

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2.3 Instruments

Ultraviolet spectrophotometer (754 PC type, Shanghai Jinghua), vacuum pump (SHZ-DIII type, Gongyi City to China), vacuum drying box (ZKXF-1 type, Shanghai established).

3. Methods and results

3.1 Preparation of reference solution

Weigh accurately the rutin reference substance 5.0 mg, put it in a 25 mL volumetric flask, add 75% ethanol to dissolve and dilute to the mark, that is, obtain a 0.2 mg/mL reference solution.

3.2 Preparation of standard curve

Accurately measure the reference solution 1.0 mL, 2.0 mL, 4.0 mL, 6.0 mL, 8.0 mL, 10.0 mL, respectively, in a 25 mL volumetric flask, add methanol to 10.0 mL each, add 5% sodium nitrite solution 1 mL Mix well, place for 10 min, add 1 mL of 10% aluminum nitrate solution, mix well, place for 10 min, add 1% NaoH 10 mL, add methanol to the mark, shake well, place for 20 min, then get. The absorbance was measured for each sample at a wavelength of 512 nm with a blank solution. Linear regression analysis was carried out with rutin concentration (X) and absorbance (Y). The regression equation was Y=10.284x+0.0002 (R2=0.9998). In addition, the blank sample had no absorption at 512 nm, indicating no interference, and the method was exclusive. Strong. The results showed that the rutin standard solution was in the range of 0.008-0.08 mg/mL, and the linear relationship was good. The rutin standard curve is shown in Figure 1.

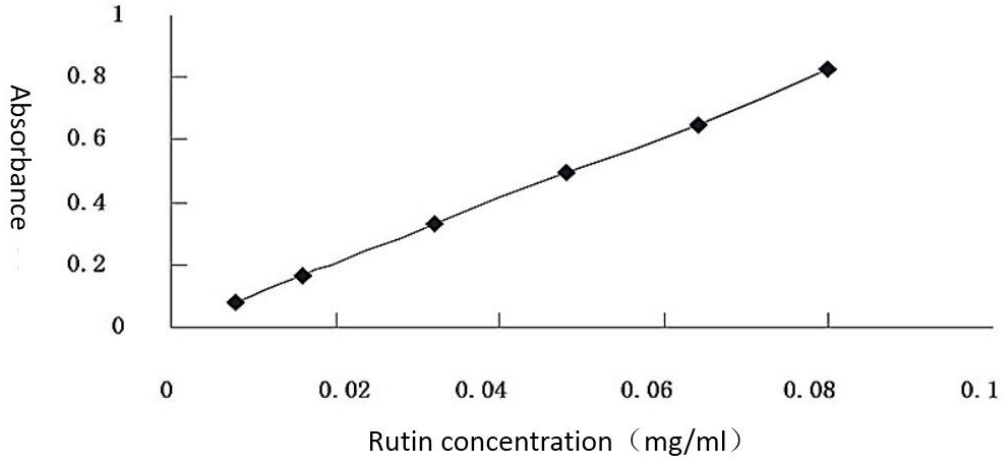

Figure 1 rutin standard curve

3.3 Precision inspection

Weigh accurately 6 parts of rutin reference solution, each 6.0 mL, placed in a 25 mL volumetric flask, add 1 mL of 5% sodium nitrite solution, mix well, then press “2.1.2” The method was operated to measure the absorbance of each solution. The results are shown in Table 1. From the Table (RSD < 5), it can be seen that the precision is good under these conditions.

Table 1 Precision survey data

sample	1	2	3	4	5	6	RSD(%)
absorbance	0.496	0.487	0.494	0.485	0.491	0.499	1.05

3.4 Preparation and determination of the test solution

Weigh accurately 9 parts of Dingxin vine powder, each 10.0 g, reflux extraction according to the level of Table 2, suction filtration, constant volume to 250 mL, respectively accurately draw 2 mL solution, according to the method of “2.1.2”, The absorbance of each sample solution was measured,

and the total flavonoid content in the test solution was calculated according to the regression equation.

3.5 Orthogonal test design

In order to further optimize the process parameters of ethanol extraction, the experiment conducted orthogonal design on ethanol concentration (A), extraction multiple (B), extraction time (C) and extraction times (D). The total flavonoid content in the extracted samples was taken as an indicator. L9 (34) orthogonal Table design [11] is selected, the level of each factor is shown in Table 3, and the orthogonal test design and result analysis are shown in Table 5.

Table 2 Factor Level Table

Levels	Factors			
	A/%	B/times	C/h	D/times
	60	8	1	1
	75	10	2	2
	90	12	3	3

Table 3 Orthogonal test and results

No.	Factors				Extraction rate of flavonoids(%)
	A	B	C	D	
1	1	1	1	1	0.7229
2	1	2	2	2	0.7324
3	1	3	3	3	0.7495
4	2	1	2	3	0.7155
5	2	2	3	1	0.7059
6	2	3	1	2	0.7165
7	3	1	3	2	0.7169
8	3	2	1	3	0.7056
9	3	3	2	1	0.7263
K_1	0.7349	0.7184	0.7150	0.7115	
K_2	0.7126	0.7146	0.7247	0.7219	
K_3	0.7163	0.7308	0.7241	0.7304	
R	0.0223	0.0161	0.0097	0.0190	

It can be obtained from Table 3, through orthogonal design experiments and data analysis, the optimal process conditions are A1B3C2D3. Therefore, it is the best method to extract 3 times with 60% ethanol, 12 times each time.

3.6 Best Process Verification

Accurately weigh 3 parts of dry dried vine powder 5 g, balance the operation 3 times according to the optimal process conditions, measure the absorbance according to the method of “2.3”, and calculate the total flavonoid content according to the regression equation. The results are shown in Table 4.

Table 4 Best Process Verification Results

No.	Extraction rate of flavonoids(%)	Average extraction rate(%)	RSD(%)
1	0.7546		
2	0.7683	0.7566	1.44
3	0.7468		

It can be seen from Table 4 that the total flavonoid content of the extract is higher than other orthogonal test values (Table 5) and the RSD<5, indicating that the total flavonoid content of the extract is sTable and the extraction process is reproducible. , in line with production requirements.

3.7 Determination of sample recovery

Precisely draw 6 parts of the test solution extracted by the optimal process conditions, 2.0 mL per serving, 3 parts of which are added to the reference solution 2 mL, and the remaining 3 parts are added to the reference solution 4 mL respectively; according to the above preparation method of the test sample Preparation, the absorption wavelength was measured at 512 nm, the measurement results are shown in Table 5, from the Table can be found that the recovery rate of the sample is 98.98%.

Table 5 Sample recovery rate

Total Flavonoids in Samples(mg)	Addition amount(mg)	Measurements(mg)	Rate of recovery(%)	Average recovery(%)	RSD (%)
0.4656	0.40	0.8594	98.45	98.98	1.25
0.4656	0.40	0.8569	97.825		
0.4656	0.40	0.8678	100.55		
0.4656	0.80	1.2463	97.5875		
0.4656	0.80	1.2598	99.275		
0.4656	0.80	1.2672	100.2		

3.8 Purification Process Research

Through the literature review, the total flavonoids were generally purified by non-polar macroporous adsorption resin. The commonly used non-polar resins D-101 and AB-8 were investigated.

3.8.1 Comparison of static adsorption of two resins

Two kinds of macroporous adsorption resins were weighed and placed in an Erlenmeyer flask. Accurately weigh 0.2 g of the extract prepared above, dissolve it with 10 mL of water, transfer to a conical flask and sonicate with the resin for 30 min, filter the solution separately, wash with water and transfer to a 250 mL volumetric flask to obtain D-101. Water eluate and AB-8 water eluate; the remaining resin was added to 10 mL of 70% ethanol, sonicated for 30 min, filtered, and the filtrate was transferred to a 250 mL volumetric flask, diluted with water to the mark to obtain D-101 alcohol. Eluate and AB-8 alcohol eluate; respectively, the sample was accurately pipetted 2 mL, transferred to a 25 mL volumetric flask, and determined by content determination method. The results are shown in Table 6.

Table 6 Resin Selection - Static Adsorption Experimental Data

	Total flavone content(mg)	
Resin type	D-101	AB-8
WATER WASH	7.3468	1.3268
Alcohol washing material	9.3564	1.9472
Adsorption rate (%)	57.35	60.20
Desorption rate (%)	94.72	79.71

Comparing the static adsorption capacity and desorption rate of the two resins by Table 6, the adsorption rate of AB-8 macroporous resin is higher than that of D-101 macroporous resin, but the desorption rate is poor. Comprehensive comparison, D-101 macropores The resin is suiTable as a purified filler, so D-101 macroporous resin was selected as a purification filler.

3.8.2 D-101 macroporous resin purification process

3.8.2.1 Investigation of resin leakage curve

Accurately measure 20 mL of D-101 macroporous resin after treatment (measured resin column volume is about 10 mL), add water to balance and wait for use; accurately weigh 3.5 g of centering rattan extract extracted from the pilot test (about equivalent 40 g) of Dingxin vine medicine, 100 mL of water was added to dissolve the solution, and the dissolved sample was applied to a well-balanced D-101 macroporous resin, and eluted at a flow rate of 1 BV/h to collect the eluate. The first bottle was connected to 20 mL, and after each bottle was connected to 10 mL, the total flavonoid content in the eluate was determined. The leakage curve is shown in Fig. 2.

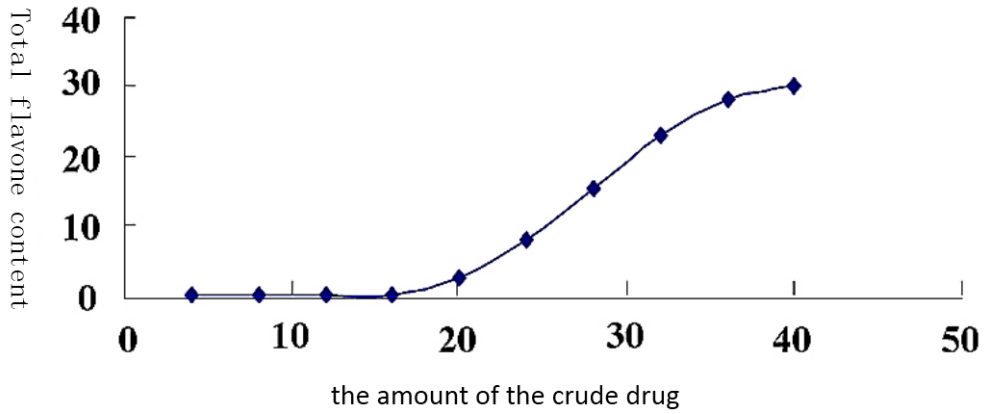

Figure 2 resin leakage curve

It can be seen from Fig. 2 that when the ratio of the volume of the resin to the quality of the medicinal material is 1:1, the flavonoids slightly leak but not obvious, but when the amount of the medicinal materials is 24 g, the total flavonoids begin to leak, and the leakage rate reaches 26.4%. When applied to 40 g of medicinal material, the resin is almost not adsorbed. It can be seen that when the resin and the medicinal material are kept at a V (resin volume): M (raw medicinal amount) = 1:1, it is ensured that the total flavonoids are absorbed without substantially leaking.

3.8.2.2 Investigation of the concentration of the sample

Accurately weighed 1.75 g of each sample of Sagittaria sinensis extract (about 20 g of Sagittarius sinensis), and added water 20 mL, 40 mL, 60 mL, and 80 mL, respectively, to prepare an aqueous solution for processing. Four 20 mL macroporous resin columns were eluted with water and then eluted with 70% ethanol. 70% ethanol eluate was collected and the total flavonoid content was calculated as shown in Table 7. The sample can be sampled from the Table. A solvent with a concentration of 3 times the amount of the drug can achieve a better adsorption effect, so the concentration of the sample is determined to be 1:3.

Table 7 Investigation of the loading concentration

Sample concentration(g/ml)	1	0.5	0.3	0.25
Adsorption capacity of total flavones(mg)	98.65	124.6	140.23	140.35

2.8.2.3 Investigation of the loading flow rate

Accurately weighed 1.75 g of each sample of Sagittarius sinensis extract (about 20 g of Sagittaria sinensis), dissolved in 60 mL of distilled water, and applied to three well-treated 20 mL macroporous resin columns. Above, at a flow rate of 1 BV/h, 2 BV/h, 3 BV/h, eluted first with water, then with 70% ethanol, and 70% ethanol eluate was collected. The results are shown in Table 8. The total

flavonoid content can be obtained. When eluted at 2 BV/h, it can adsorb flavonoids well and save time and improve efficiency, so the loading flow rate is 2 BV/h.

Table 8 Investigation of the loading flow rate

Elution flow rate(BV/h)	1	2	3
Adsorption capacity of total flavones(mg)	140.36	139.78	133.69

3.8.2.4 Investigation of elution solvent

Accurately weighed 1.75 g of each sample of Sagittaria sinensis extract (about 20 g of Sagittarius sinensis), dissolved in 60 mL of distilled water, and loaded on three well-treated 20 mL macroporous resin columns. Above, use 50%, 70%, 90% ethanol at a flow rate of 2 BV/h first, then elute with 50%, 70% and 90% ethanol respectively, and collect the eluate. The results are shown in Table 9 The total flavonoid content in the Table is 70% ethanol and 90% ethanol elution is basically the same, 50% ethanol may have some flavonoids incomplete elution, the content is slightly lower, therefore, 70% ethanol is selected as the elution solvent.

Table 9 Investigation of elution solvent

Elution solvent(%)	50	70	90
Adsorption capacity of total flavones(mg)	101.68	142.05	142.14

3.8.2.5 Investigation of elution multiples

Accurately weigh about 3.75 g of the sample of Sagittarius sinensis extract (about 20 g of Sagittaria sinensis), dissolve it in 60 mL of water, and load it onto the treated 20 mL macroporous adsorption resin column (column volume). Approximately 10 mL), eluted with water, 70% ethanol at a flow rate of 2 BV/h, eluted first with water, eluted until the eluent was colorless, and the eluate was collected. The eluent was about 85 mL, and then used. 70% ethanol was eluted and collected in a column volume. As a result, the total flavonoid content eluted was as shown in Table 10. It can be seen from Table 10 that the elution rate can reach about 92.23% when eluted to 5 column volumes, and the flavonoids are significantly reduced by the sixth column volume, so it is determined that 70% ethanol elutes 5 column volumes. .

Table 10 Investigation of elution multiples

Column volume	1	2	3	4	5	6	7
Total Flavonoids Content(mg)	59.68	34.62	20.95	14.36	9.64	3.65	1.1

3.8.2.6 Determination of D-101 macroporous resin purification process

After the above conditions were explored, the sample loading was determined as: V (resin volume): M (raw dose) = 1:1; the sample concentration was diluted to 3 times the amount of the drug; the sample flow rate was 2 BV/ h; elution flow rate was 2 to 3 BV / h; with 7% ethanol as elution solvent, elution 5 column volumes.

3.9 Purification of total flavonoids from Dingxiang

According to the above optimized extraction process, 100 g of medicinal herbs were extracted and concentrated under reduced pressure to obtain 8.469 g. Purification was carried out according to the procedure under “2.7.2.6” to obtain 1.095 g of dry paste, and the purified centering vine was accurately weighed. The dry paste was 0.1095 g, the absorbance was measured according to the method of “2.3”, and the total flavonoid content in the solution was calculated according to the regression equation. The results are shown in Table 11.

Table 11 Total flavonoids in purified dry paste

sample	Contrast	Purified Dry Ointment
absorbance	0.1639	0.2236
concentration(mg/ml)	0.016	0.02183

As can be seen from Table 11, the concentration of the sample is 0.02183 mg/mL, and the purity (P) of the flavonoids after purification of the dried vines is calculated by the following formula:

$$P\ (\%)=W/Wo\times 100\%$$

Where w is the content of flavonoids in solution (g); Wo is the weight after solution drying (g)

$$P = 0.02183 \times 25 / 2 \times 250 / 0.1095 \times 1000 \times 100\% = 62.30\%$$

It can be obtained from the above formula that the extraction and purification process of total flavonoids of Radix Paeoniae Alba is feasible, and the purity of total flavonoids in the dry paste is 62.30%.

4. Discussion

The experiment systematically studied the method for determining the total flavonoids content of Dingxin, and obtained a reliable and stable method for determination. Firstly, the orthogonal design experiment was carried out to optimize the extraction process, and the optimal extraction process was determined. The extraction process was carried out for 2 h and extracted with 60% ethanol for 3 times. Secondly, D-101 and AB-8 macroporous resin were investigated, and the purification process of D-101 macroporous resin was optimized, and a stable purification process was obtained. The amount of D-101 macroporous resin was resin volume. (V): the amount of crude drug (M) = 1:1, the loading flow rate is 2 BV / h, the elution flow rate is controlled at 2 ~ 3 BV / h, with 70% ethanol as the elution solvent, eluting 5 column volumes The purification effect is better. Through the investigation and verification of the extraction and purification process, the extraction and purification process is feasible, stable and reproducible, and can be used as a process condition for production amplification, and has high clinical application value.

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