Optimization on Extraction Engineering of the Anti - inflammatory Bioactive Materials from Ainsliaea Fragrans Champ

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Abstract. Ainsliaea fragrans Champ.(A.fragrans) is a traditional Chinese herbal, phenolic compounds was the major anti - inflammatory bioactive constituents .To improve the bioavailability and enhanced the curative effect of A.fragrans, the anti - inflammatory effect of phenolic acids and the "non-active" group of control vectors constitute a new biomedical material, which is of great significance to the treatment of diseases inflammation. Hence, in this thesis, regarding the total phenolic acid transfer rate as the indicator，$L_9(3^4)$ orthogonal design was used to optimize the extraction process of total Phenolic acid from A.fragrans by reflux extraction method on solvent dosage, extraction times and extraction time． The optimal extraction technology was as follows: 15 times of water volume, reflux extraction 3 times, extraction time 60 min. The result of pharmacological activity indicated anti-inflammatory effect: 95% ethanol extraction > water extraction> 30% ethanol extraction > 60% ethanol extraction.

1. Introduction

Biomedical material is used for diagnosis and treatment of organisms, repair or replace the lesion tissues or organs or enhance their functions of materials. They are generally composed of the physiological "activity" material and the "non active" material of the control vector. Therefore, the physiological "activity" material is an important part of the Biomedical materials, and often come from more active constituents of Chinese herbal medicine.

Ainsliaea fragrans Champ. (A.fragrans) is a folk herbal medicine named‘Xingxiang Tuerfeng’in China[1,2]. It is usually used to cure consumptive steaming bone, metrorrhagia and metrostaxis, damp-heat jaundice, scrofular tuberculoderm. A.fragrans contains numerous constituents and phenolic compounds was the major bioactive constituents, such as 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid[3-6]. Anti - inflammatory effect is the characteristic activity of A.fragrans. To improve the bioavailability and enhanced the curative effect of A.fragrans, the anti - inflammatory effect of phenolic acids and the "non - active" group of control vectors constitute a new biomedical material, which is of great

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significance to the treatment of diseases inflammation. Hence, in this thesis, taking anti-inflammatory pharmacological activity (Experiment of ear swelling caused by xylene in mice and Experiment of mice capillary permeability hyperfunction induced by acetic acid) as the principal and combining with the total phenolic acid transfer rate as the index conducted a study on extraction technology of total phenolic acid of A.fragrans.

2. Instruments, Reagents and Animals

Main Instruments. UV-2550 UV-Vis spectrophotometer was purchased from Shimadzu. Mettler AE240 hundred thousandth electronic balance was purchased from Switzerland Mettler. KQ-250DB ultrasonic cleaner was purchased from Kunshan ultrasonic instrument Co.,Ltd.

Main Reagents. 3, 5-dicaffeoylquinic acid (99% purity) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Other reagents are analytically pure.

Animals. Female pathogen-free KM mice (n=50,18-20 g) were provided by the Hunan Silaikejingda Laboratory Animal Co. Ltd. (Changsha, PR China). These animals were housed under controlled conditions (22 ± 2 °C, RH 50± 20%) with a natural light–dark cycle for 3 days before the experiment was carried out. Animal experiments were performed in accordance with the Regulations of the Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China.

3. Methods and Results

3.1 Determination of Total Phenolic Acid

Preparation of Reference Solution. Accurately weighed solid portions of 3, 5-dicaffeoylquinic acid was dissolved in 50% ethanol to prepare separate stock solutions consisting of 9.44 $\mu g \cdot mL^{-1}$.

Preparation of the Testing Solution. Accurately 0.1g extract of total phenolic acid of A.fragrans were weighed and transferred into 500 ml volumetric flask with a proper amount of 50% ethanol, the flask was sealed and processed by ultrasound for 30 min. When the solution was cooled down, 50% methanol was added to the scale mark and shaken well.

Selection of the Detection Wavelength. The testing and reference solution was scanned with wavelength from 200 to 400 nm. The result showed that the maximum wavelength absorbed in both the reference and testing solution was 328 nm, thus the detection wavelength was determined as 328 nm.

Preparation of Standard Curve Accurately 1.0，1.5，2.5，3.0，4.0 and 5.0 ml of spared reference solution were transferred separately to 25 mL volumetric flask, added with 50% ethanol until reaching the scale mark, 50% ethanol as for the blank group, were detected wavelength, with absorbency (A) as the Y-axis and tested concentration (C) as the X-axis. The result showed that the regression equation was obtained: Y=0.1314X － 0.0141，r=0.9992, at the concentration range of 0.0025mg/mL～0.0125 mg/ml exhibited a good linear relationship.

Precision Test. The same tested solution was collected and detected the absorbance, with 6 replications. The result showed that RSD (relative standard deviation) value was 0.09 %(n=6), indicating that the instrument possessed a high accuracy.

Stability Test. The same tested solution was placed for 20, 40, 60, 80, 100 and 120 min at room temperature, then detected the absorbance respectively. The experimental results (RSD=0.11%) indicated that the solution possessed a good stability within 120 min.

Repeatability Test. Six shares same batch of A.fragrans was prepared into tested solution according to the methods described previously, and were measured the absorbance. The RSD value was calculated 0.31% indicating that the method possessed a good repeatability.

Test of Recovery Rate. Six shares of A.fragrans medicinal herbs (0.1 g each) were added accurately with 0.003g reference substance, respectively. The testing solution was prepared according to the methods described previously, and measured the absorbance, then the content of phenolic acids was calculated. Experimental results showed that average recovery rate was 98.3 %; RSD = 0.41%, indicating that the optimized extraction method was well repeatable.

4. Selection of the Extraction Solvent

4.1 Preparation of the Different Solvent Extraction

50.0g A.fragrans was weighed precisely for 4 shares, and reflux extracted respectively by using 10 times of water, 30% ethanol, 60% ethanol and 95% ethanol for 60 min, replicated three times. Then different solvent extraction was vacuum concentrated at 60℃ to dry. According to the method "5.3" measured the content of total phenolic acid and calculated total phenolic acid transfer rate(refer with: Eq. 1),the results are shown in table 1. Referring to the method in "5.1" (Experiment of ear swelling caused by xylene in mice and Experiment of capillary permeability hyperfunction) measured anti-inflammatory pharmacological activity of different solvent extract, the results are shown in table 2, 3.

$$\text{transfer rate} = \frac{total\ phenolic\ acid\ content\ in\ the\ extract}{total\ phenolic\ acid\ content\ in\ extraction\ of\ medicinal\ materials} \times 100\% \quad (1)$$

5. Pharmacological Test Method

5.1 Experiment of Ear Swelling Caused by Xylene in Mice

Mice were administrated by intragastric administration for 6 days, once a day (Intragastrical administration was calculated according to the principle of mice weight 0.2ml /10g). And mice in the model group were fed with the same volume of distilled water. 1 h after mice were fed with drug for the last time, 50μL xylene obtained with sample injector smeared on two sides of the right ear，the left ear was as control. Mice were put to death after 2h. Stainless steel puncher (the diameter was 9 mm) was used to obtain ears at symmetrical parts of ears． They were weighed with one over ten thousand electronic analytical balance． The weight difference of left and right ears was as degree of swelling. The swelling rate and the inhibiting rate were calculated by equation 2,3.

$$\text{swelling rate}(\%) = \frac{weight\ of\ right\ ear - weight\ of\ left\ ear}{weight\ of\ left\ ear} \times 100\% \quad (2)$$

$$\text{inhibiting rate}(\%) = \frac{average\ swelling\ rate\ of control - average\ swelling\ of\ treatment}{average\ swelling\ of\ control} \times 100\% \quad (3)$$

5.2 Experiment of Capillary Permeability Hyperfunction

Mice were randomly divided into model group and treatment group. After the treatment group intragastric administration, the tail was injected with Evans blue by intravenous injection. Later, mice were injected with glacial acetic acid by intraperitoneal injection for hyperfunction model. After 20min, mice were injected with normal saline by intraperitoneal injection. Softly rubbed the abdomen until intraperitoneal fluid mixed completely. Nipped and lifted up the abdominal wall of postabdomen with tweezers，cut a small hole and sucked up peritoneal fluid. Absorbancy of the peritoneal liquid was determined at 590 nm of wavelength with spectrophotometer. It was screened of activity areas in Ainsliaea fragrans Champ with model group.

5.3 Results of the Extraction Solvent

According to the result of various extracts pharmacological activity, compared with model group, results of water extract and 95% ethanol extraction indicated statistical significance, 30% ethanol extraction and 60% ethanol extraction were not significant. It indicated anti-inflammatory effect: 95% ethanol extraction > water extraction> 30% ethanol extraction > 60% ethanol extraction. Therefore, water and 95% ethanol was selected as the extraction solvent of total phenolic acid of A.fragrans.

But according to the result of total phenolic acid transfer rate , the total phenolic acid rate of water extract was 78.1% apparently higher than 95% ethanol extraction. Furthermore, according to the cost, water was selected as the extract solvent of total phenolic acid of A.fragrans.

Table 1 Effects Of Different Extract Solvent On The Extraction Rate

sample	Transfer rate of total phenolic acid(%)
Water extract	78.1
30% extract	98.0
60% extract	97.5
95% extract	31.4

Table 2 Effects Of Different Extracts On Mice Ear Swelling Induced By Xylene (N=7)

Group	Degree of ear swelling (%)	Inhibiting rate (%)
Model group	0.0098±0.0017	
Water extract	0.0054±0.0019**	45.20
30% extract	0.0073±0.0039	26.02
60% extract	0.0080±0.0023	18.75
95% extract	0.0052±0.0031**	47.26

Notice: comparing with model group, *p<0.05,* *p<0.01;

Table 3 Effects Of Different Extracts On Mice Capillary Permeability Hyperfunction Induced By Acetic Acid (N=7)

group	Model group	Water extract	30% extract	60% extract	95% extract
OD	0.38	0.29	0.29	0.38	0.37

5.4 Optimization of Extraction Technology

Based on the extraction solvent study, solvent dosage(A), extraction times (B) and extraction time (C) were taken as the investigation factors， with 3 levels for each factor (Table 4). With the total phenolic acid transfer rate as the index, L_9（3^4） orthogonal design was adopted for the research．The result and analysis of variance showed in Table 5.

Table 4 Factors And Levels

Level	Factor		
	A solvent dosage (times)	B Extraction Times	C Extraction time (h)
1	10	1	0.5
2	12	2	1.0
3	15	3	2.0

Results of orthogonal experiment shown the effects of various factors on the extraction of the total phenolic acid exhibited a descend order of B> A> C. Analysis of variance showed that factor B affected significantly the experimental results. Therefore, the optimal combination of extraction technology of the total phenolic acid of Ainsliaea fragrans conditions was $A_3B_3C_2$.

Table 5 Results Of Orthogonal Experiment

Test No.	A	B	C	D	Total phenolic acid transfer rate (%)
1	1	1	1	1	46.69
2	1	2	2	2	72.62
3	1	3	3	3	77.84
4	2	1	2	3	58.07
5	2	2	3	1	77.69
6	2	3	1	2	83.60
7	3	1	3	2	59.69
8	3	2	1	3	81.68
9	3	3	2	1	89.27
K1	197.15	164.45	211.96	213.65	
K2	219.35	231.99	219.96	215.91	
K3	230.64	250.71	215.22	217.58	
k1	65.72	54.82	70.65	71.22	
k2	73.12	77.33	73.32	71.97	
k3	76.88	83.57	71.74	72.53	
R	11.16	28.75	2.67	1.31	

5.5 Verification Test

Three shares same batch of A.fragrans medicinal materials was collected with 25g for each sample. Extraction was carried out for 3 times under the optimal extraction technology of the total phenolic acid of A.fragrans. With the total phenolic acid transfer rate as the index, the stability of the optimization technology was investigated. According to the Table 6, the optimization extraction technology for total phenolic acid transfer rate was fairly stable, indicating that this technology was stable, good repeatability and reliable.

Table 6 Results Of Confirmation Experiment

Test No.	The total phenolic acid transfer rate (%)
1	86.87
2	89.23
3	87.85

6. Conclusions

The aim of this research was to make full use of Ainsliaea fragrans medicinal materials. With the total phenolic acid of Ainsliaea fragrans medicinal materials transfer rate as the index and combining with anti-inflammatory pharmacological activity investigated the extraction solvent, Solvent dosage, extraction times and extraction time of the total phenolic acid of Ainsliaea fragrans medicinal materials. The optimal extraction technology of Ainsliaea fragrans medicinal materials was as follows: 15 times of water volume, reflux extraction 3 times, extraction time 60 min.

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