Extraction Process of Zhuang Medicine Fumigation Lotion

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Abstract [**Objectives**] To establish the extraction process and quality standard method of Zhuang medicine fumigation lotion. [**Methods**] The orthogonal design method was employed to optimize the water extraction process with the amount of water added, decocting time and extraction times as factors, and syringin content and dry extract yield as indexes. The content of syringin was determined by high performance liquid chromatography. [**Results**] The best water extraction process was: soaking in water for 1 h, decocting twice, added 10 times the amount of water each time, decocting for 1 h. The average content of syringin in 3 batches was 0.98 mg/g, and the average dry extract yield was 26.07%. [**Conclusions**] The project adopts water extraction method to prepare Zhuang medicine fumigation lotion, which has the characteristics of high efficiency and suitable for large-scale production. The quality control method is reliable, rapid and accurate, and can effectively control the quality of the lotion.

Key words Zhuang medicine fumigation lotion, Extraction process, Content determination

1 Introduction

Zhuang medicine fumigation lotion is an ethnic medicine prescription developed by the Dermatology Department of Guangxi International Zhuang Medical Hospital. The prescription is made of 14 kinds of traditional Chinese medicine, such as Cortex Ilicis Rotundae, Herba Violae, Herba Polygoni Perfoliati, Radix et Rhizoma Rhei, Radix Sangusorbae, Herba Portulacae, *etc.* More than 10 years of clinical application results show that the lotion has the effects of clearing heat and detoxification, dispersing knots and detumescence, treating various types of facial dermatitis, subacute eczema, folliculitis, skin furuncle and other skin diseases with erythema, papules, nodules and cysts as clinical manifestations, and it has obvious curative effect. In their long-term medical practice, the Chinese people have created a variety of methods to treat dermatosis, which is a general term for diseases occurring on the skin and its accessory organs[1-2].

Zhuang medicine fumigation lotion is made by decocting and boiling Chinese medicine, and the original medicine liquid is decocted in clinical application, which is extremely inconvenient to use. It is neither conducive to promptly controlling the disease, nor easy to carry and store. In order to overcome the above shortcomings, based on the compatibility of the original prescription, this study explored Zhuang medicine fumigation lotion according to the relevant requirements of medical institutions, which not only retains the effectiveness of the original prescription, but also conforms to the characteristics of the lotion, and is easy to carry and preserve, while improving the stability of the preparation. The pure traditional Chinese medicine preparation with good quality has a broad clinical application prospect and can produce significant social and economic benefits. In this project, the lotion was studied thoroughly and developed into a preparation. In order to further control the quality of this product and ensure its clinical efficacy, the extraction process and quality standard were studied.

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2 Materials

2.1 Instruments Agilent 1260 high performance liquid chromatograph; ME155DU electronic balance (Mettler); Simplicity ultra-pure water system (Millipore China Co., Ltd.); TGL-16G high speed tabletop centrifuge (Shanghai Anting Scientific Instrument Factory); HWS-26 electric-heated thermostatic water bath (Shanghai Qixin Scientific Instrument Co., Ltd.).

2.2 Materials Fourteen kinds of traditional Chinese medicine, such as Cortex Ilicis Rotundae, Herba Violae, Herba Polygoni Perfoliati, Radix et Rhizoma Rhei, Radix Sangusorbae, Herba Portulacae, Herba Taraxaci, Flos Chrysanthemi Indici, *etc.*, were purchased from Guangxi Xianzhu Chinese Medicine Technology Co., Ltd. Syringin reference (batch No.: 111574-201605) and aesculetin reference (batch No.: 110741-202109) were purchased from China Institute for Food and Drug Control. Acetonitrile (batch No.: 170060) was a chromatographically pure produced by Thermo Fisher Scientific; phosphoric acid (batch No.: T200110324) was an analytical pure manufactured by Sinopharm Chemical Reagent Co., Ltd.

3 Methods and results

In this test, the content of syringin, a component of Cortex Ilicis Rotundae, in the extracted medicinal liquid was determined by high performance liquid chromatography (HPLC) at a wavelength

of 265 nm[3-4].

3.1 Preparation of reference solution Appropriate amount of syringin reference was accurately weighed, dissolved in methanol and shaken well to prepare the reference solution with a concentration of 0.957 7 mg/mL.

3.2 Preparation of test solution and its negative reference solution According to the proportion of the prescription, a dose of medicinal materials was weighed, and decocted twice. It was soaked in 10 times the amount of water for 1 h in the first time and was soaked in 8 times the amount of water for 1 h in the second time. The decoction was merged, and the total volume was accurately measured (mL). Appropriate amount of supernatant was filtered through 0.22 μm microfiltration membrane, and the solution obtained was the test solution.

According to the proportion of the prescription, a dose of Cortex Ilicis Rotundae prescription medicinal material was weighed, and the negative control solution was prepared by the same method.

3.3 Chromatographic conditions Chromatographic column: Gemini ®-C_{18}110Å (5 μm, 4.6 mm×250 mm); mobile phase: acetonitrile (A) -0.1% phosphoric acid aqueous solution (B); gradient elution (The elution schedule is shown in Table 1); flow rate: 1 mL/min; column temperature: 30 ℃; injection volume: 10 μL; detection wavelength of syringin: 265 nm; theoretical plate number not be less than 2 000 for the calculation of syringin peak. The syringin component in the prescription was well separated from other components, and other components in the sample did not interfere with the measured components (Fig. 1).

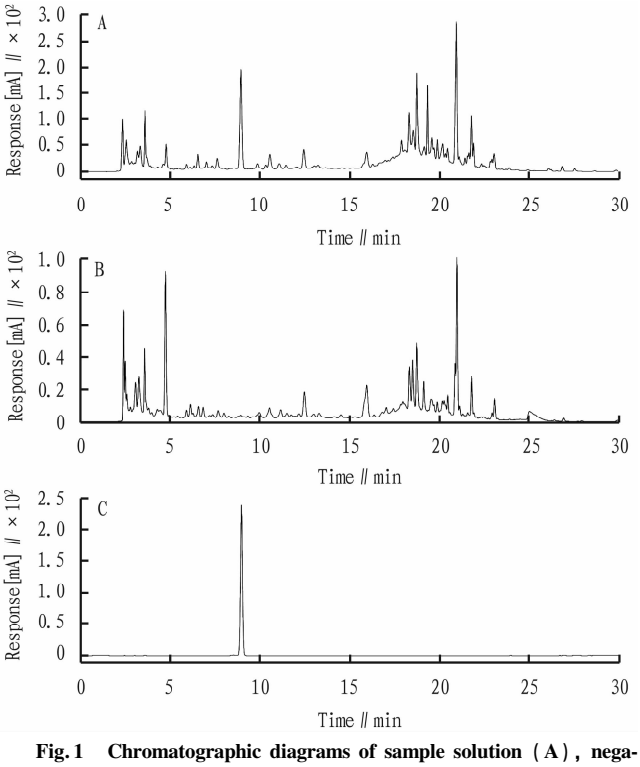

Fig. 1 Chromatographic diagrams of sample solution (A), negative sample of Cortex Ilicis Rotundae (B), and syringin reference (C)

Table 1 Gradient elution schedule

Time // min	Mobile phase	
	Acetonitrile (A) // %	0.1% (*V/V*) Phosphoric acid aqueous solution (B) // %
0	10	90
12	18	82
26	72	28
30	10	90

3.4 Methodology

3.4.1 Linear relationship investigation. Certain amount of above reference solution was precisely absorbed, mixed in a brown volumetric bottle, and then set to a constant volume with methanol. Similarly, 7 different concentrations of Dachengqi decoction mixed reference solution were prepared. The injection volume was 10 μL. The samples were respectively injected and detected by liquid chromatograph. The peak area was measured. The standard curve was plotted with the concentration (mg/mL) as the abscissa and the peak area as the ordinate. The regression equation was calculated as follows: $Y=26\ 306X-42.370$, $R^2=0.999\ 9$, showing a good linear relationship in the concentration range of 0.038 308 – 0.143 655 mg/mL.

3.4.2 Precision test. The same syringin reference solution (0.15 mL of 0.957 7 mg/mL mother liquor is loaded into a 2 mL volumetric bottle and added with methanol to the scale, and the concentration is 0.071 827 5 mg/mL). The samples were injected for consecutive 6 times, and the peak area of syringin in Zhuang medicine fumigation lotion was determined by liquid chromatography described in Section **3.3**. The results showed that the *RSD* of the peak area of syringin in the lotion was 0.10%, and all *RSD* values were less than 3.00%, indicating good precision of the instrument.

3.4.3 Stability test. According to the proportion of the prescription, a copy of Zhuang medicine fumigation lotion was prepared into test solution according to the method described in Section **3.2**, and the sample was injected at 0, 2, 4, 8, 12 and 24 h after the preparation of the test solution. The peak area of syringin in the test solution was determined according to the method described in Section **3.3**. The results showed that the *RSD* of the peak area of syringin was 1.37%, and all *RSD* values were less than 3.0%, indicating that the test solution was stable within 24 h.

3.4.4 Repeatability test. According to the prescription ratio, 6 copies of Zhuang medicine fumigation lotion were weighed and prepared into test solutions according to the method described in Section **3.2**. According to the chromatographic conditions described in Section **3.3**, the peak area of syringin in each test solution of Zhuang medicine fumigation lotion was determined, and the average mass concentration of syringin and the *RSD* of mass concentration in each extract solution were calculated. The average mass concentration of syringin in the samples was 0.925 8 mg/mL, and the *RSD* was 2.15%, indicating good repeatability.

3.4.5 Recovery test. Six copies of Zhuang medicine fumigation lotion (batch No.: 210601) with known content were precisely measured, 5 mL each copy, were placed in 10 mL volumetric bottles. Afterwards, appropriate amount of syringin was added pre-

cisely, and pure water was added to the scale, shaken, and mixed well. Appropriate amount of sample solution was centrifuged at 13 000 r for 10 min, and the supernatant was filtered by 0.22 μm microfiltration membrane. The peak area of syringin was determined according to the chromatographic conditions described in Section **3.3**, and the content of syringin in this product was calculated by external standard one-point method. The calculated recovery rates ranged from 97.10% to 102.20%, and the average recovery rate was 99.62%. The *RSD* was 1.89% ($n=6$), indicating good accuracy of the method (Table 2).

Table 2 Experimental results of recovery rate of Zhuang medicine fumigation lotion ($n=6$)

No.	Sample content μg/mL	Injection content μg/mL	Measured content μg/mL	Recovery rate//%	Average recovery rate//%	*RSD* %
1	34.08	34.09	68.58	101.20	99.62	1.89
2	34.08	34.09	67.18	97.10		
3	34.08	34.09	67.78	98.86		
4	34.08	34.09	68.15	99.94		
5	34.08	34.09	67.63	98.42		
6	34.08	34.09	68.92	102.20		

3.5 Orthogonal test The Chinese medicine was extracted by water boiling method. The extraction efficiency can be affected by the amount of water added, decocting time, soaking time, extraction times and particle size of Chinese medicine. Considering the actual large-scale production, working efficiency and economic cost, the extraction effect was greatly affected by the amount of water added, decocting time and extraction times. Therefore, the amount of water added, decocting time and extraction times were selected for orthogonal test, and the content of syringin and dry extract yield were used as evaluation indexes to optimize the extraction process.

3.5.1 Factor level design. According to the single factor test results and actual production conditions, the factors affecting the extraction process were: the amount of water added, decocting time and soaking time. Combined with the actual production, the amount of water added (A), decocting time (B) and extraction times (C) were selected as the investigation factors in the orthogonal test, and the content of syringin and dry extract yield were taken as evaluation indexes. $L_9(3^4)$ orthogonal design was used to optimize the extraction process of Zhuang medicine fumigation lotion, and the level of factors is shown in Table 3.

Table 3 Factor level of $L_9(3^4)$ orthogonal design for the extraction process of Zhuang medicine fumigation lotion

Level	Factor		
	Amount of water added (A)	Decocting time (B)	Extraction times (C)
1	8	0.5	1
2	10	1	2
3	12	1.5	3

3.5.2 Orthogonal test. According to the factors and levels of orthogonal test designed in Table 3, the medicinal materials were weighed according to the proportion of THE prescription, extracted following the conditions in Table 4, and filtered for later use.

Table 4 Arrangement and results of $L_9(3^4)$ orthogonal design for the extraction process of Zhuang medicine fumigation lotion

Level	A	B	C	D	Syringin mg/g	Dry extract yield//%
1	1	1	1	1	0.752 6	18.94
2	1	2	2	2	0.956 3	24.52
3	1	3	3	3	0.898 2	25.18
4	2	1	2	3	0.646 9	24.95
5	2	2	3	1	0.872 5	25.76
6	2	3	1	2	0.824 8	21.94
7	3	1	3	2	0.859 3	25.72
8	3	2	1	3	0.980 5	21.20
9	3	3	2	1	1.012 4	25.61
Syringin	K_1	0.869 0	0.752 9	0.852 6	0.879 2	
	K_2	0.781 4	0.936 4	0.871 9	0.880 1	
	K_3	0.950 7	0.911 8	0.876 7	0.841 9	
	R	0.169 3	0.183 5	0.024 0	0.038 3	
Dry extract yield	K_1	22.880	23.203	20.693	23.437	
	K_2	24.217	23.827	25.027	24.060	
	K_3	24.177	24.243	25.553	23.777	
	R	1.337	1.040	4.860	0.623	

The ANOVA results of syringin extraction (Table 5) showed that factor B had significant influence on the extraction process ($P<0.05$), and the main influencing factors were B > A > C, that is, the decocting time had the greatest influence, followed by amount of water added and extraction times, and the optimal combination was $A_3B_2C_3$.

Table 5 Analysis of variance table for syringin extraction

Source of variation	Sum of squares of deviations	Degree of freedom	Variance	*F*	*P*
Amount of water added (A)	0.043 0	2	0.021 5	15.063 0	>0.05
Decocting time (B)	0.059 5	2	0.029 8	20.835 4	<0.05
Extraction times (C)	0.001 0	2	0.000 5	0.339 8	>0.05
Error (D)	0.002 9	2	0.001 4		

The ANOVA results for the extraction of dry extract yield (Table 6) showed that the amount of water added (A) and decocting time (B) had no significant influence on the extraction process ($P>0.05$), while the factor of extraction times (C) had significant influence on the extraction process, and the influencing factors successively were: C > A > B, that is, the extraction times had the greatest influence. Combined with the intuitive analysis results, it can be seen that the optimal extraction process combination was: $A_2B_3C_3$.

Table 6 Analysis of variance table for the extraction of dry extract yield

Source of variation	DEVSQ	Degree of freedom	Variance	*F*	*P*
Amount of water added (A)	3.470	2	1.735	5.942	>0.05
Decocting time (B)	1.644	2	0.822	2.815	>0.05
Extraction times (C)	42.675	2	21.337 5	73.074	<0.05
Error (D)	0.58	2	0.29		

Considering the actual large-scale production and economic cost factors, and to adapt to industrial large-scale production,

(To page 41)

mL. Under these conditions, the expected content of quercetin was 34.805 3 mg/g. The mathematical model established in this experiment is reliable and reasonable. It improves the work efficiency, and provides a reference for the further development of rutin in Flos Sophorae Immaturus to transform quercetin.

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(From page 31)

combined with the traditional decocting method, the extraction process of this product was determined as $A_2B_2C_2$, that is, each flavor of medicinal materials was weighed according to the prescription, soaked in water for 1 h, extracted twice, added 10 times the amount of water each time, and decocted for 1 h.

3.5.3 Extraction process verification. The process was verified according to the optimization results of above extraction process. The medicinal materials were weighed according to the prescription, soaked in water for 1 h, extracted twice, added 10 times the amount of water each time, and decocted for 1 h. The extraction solutions were merged. The test was repeated for 3 consecutive times to determine the syringin content and dry extract yield of this product, and the results are shown in Table 7.

Table 7 Verification test results of the extraction process of Zhuang medicine fumigation lotion

No.	Syringin//mg/g	Average value//mg/g	Dry extract yield//%	Average dry extract yield//%
1	1.04	0.98	26.28	26.07
2	0.93		25.79	
3	0.97		26.13	

The experimental results showed that the average content of syringin was 0.98 mg/g and the average dry extract yield was 26.07% according to the optimized process. The better results demonstrated that the process was reasonable and feasible.

4 Conclusions

In this study, 14 kinds of traditional Chinese medicine, such as Cortex Ilicis Rotundae, Herba Violae, Herba Polygoni Perfoliati, Radix et Rhizoma Rhei, Radix Sangusorbae, Herba Portulacae, *etc.*, were used as raw materials to prepare Zhuang medicine fumigation lotion by water extraction process, and a quality standard was preliminarily established to evaluate and control the quality of Zhuang medicine fumigation lotion.

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