Optimization of Extraction Process of Fagopyri Dibotryis Rhizoma by Orthogonal Experiment

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Abstract [**Objectives**] To optimize the water extraction process of Fagopyri Dibotryis Rhizoma. [**Methods**] The entropy weight method was used to determine the weight of epicatechin extraction rate and dry extract rate and calculate the comprehensive score. The water extraction process of Fagopyri Dibotryis Rhizoma was optimized by orthogonal design with the comprehensive score as the indicator and the amount of water, extraction time and extraction times as the factors. [**Results**] The optimum extraction process of Fagopyri Dibotryis Rhizoma was as follows: adding 10 times of water, extracting 3 times, and extracting for 60 min each time. [**Conclusions**] The optimized extraction process is stable and feasible, and can be used for the extraction of Fagopyri Dibotryis Rhizoma.

Key words Fagopyri Dibotryis Rhizoma, Orthogonal experiment, Epicatechin, Dry extract

1 Introduction

Fagopyri Dibotryis Rhizoma is a commonly used traditional Chinese medicine, derived from the dried rhizome of *Fagopyrum dibotrys* (D. Don) Hara[1], and has the effects of clearing away heat and toxic materials, expelling pus and removing blood stasis. Modern studies[2-5] have shown that Fagopyri Dibotryis Rhizoma mainly contains flavonoids and a small amount of triterpenoids and steroids, which have antioxidant, antibacterial, anti-inflammatory, analgesic and anti-tumor activities. Flavonoids epicatechin is one of the main active components of Fagopyri Dibotryis Rhizoma, and also one of its quality control indicators[1,6-7]. At present, the research on the extraction process of Fagopyri Dibotryis Rhizoma mainly focuses on the single indicator of epicatechin or total flavonoids, and the extraction process is optimized by organic solvents such as methanol and ethanol[8-10]. However, due to the complexity of the active component of Fagopyri Dibotryis Rhizoma[3-5], and the use of water as the extraction solvent in the production process of preparations, it is difficult to effectively evaluate the advantages and disadvantages of the actual production process of preparations by optimizing the extraction process with single chemical component or effective part as the indicator of organic solvent. And it is difficult to ensure the safety and the effectiveness of the clinical use of the preparation. In view of this, in this study, epicatechin and dry extract rate were used as evaluation indicators, and the water extraction process of Fagopyri Dibotryis Rhizoma was optimized by orthogonal experimental design based on the comprehensive score obtained by entropy weight method, so as to provide a certain experimental basis for the better use of Fagopyri Dibotryis Rhizoma.

2 Materials

2.1 Instruments TDP-800 powerful crusher (Tianjin Bohai Xinmao Pharmaceutical Equipment Co., Ltd.); 1260 high performance liquid chromatography-ultraviolet variable wavelength detector (Agilent Technologies Co., Ltd.); XSR204 electronic balance (Mettler - Toledo Instruments Co., Ltd.).

2.2 Raw materials and reagents Reference substance of epicatechin (content calculated at 99.7%, batch No.: 110878-201703, China National Institute for Food and Drug Control); glacial acetic acid (Tianjin Kemiou Chemical Reagent Co., Ltd., HPLC≥99.8%); acetonitrile and methanol are chromatographically pure, ultrapure water (self-made), and other reagents are analytically pure; Fagopyri Dibotryis Rhizoma decoction pieces (batch number 2207012, Chongqing Taiji Chinese Medicinal Materials Planting Development Co., Ltd.) identified by quality inspection department of Taiji Group Chongqing Fuling Pharmaceutical Factory Co., Ltd. as dried rhizome of *Fagopyrum dibotrys* (D. Don) Hara.

3 Methods and results

3.1 Optimization of extraction process design by orthogonal experiment Taking coarse powder of Fagopyri Dibotryis Rhizoma medicinal materials, we crushed the coarse powder into coarse particles using a TDP-800 strong crushing machine set, averagely dividing the coarse particles into 9 pieces, taking the same amount of coarse particles from each piece. The extraction rate and dry extract rate of epicatechin were determined by $L_9(3^4)$ orthogonal table with water addition, extraction times and extraction time as extraction factors (Table 1). The extraction rate of epicatechin and the comprehensive score of dry extract rate were used as evaluation indicators to select the optimal extraction process.

Table 1 Factor level

Level	Factors		
	Water addition (A) // folds	Extraction time (B) // min	Extraction times (C) // times
1	8	30	1
2	10	60	2
3	12	90	3

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3.2 Determination of dry extract rate Precisely pipetted 50 mL of each sample extracted by the orthogonal experiment, placed them in an evaporating dish that has been dried to constant weight, concentrated and evaporated in a water bath, dried in an oven at 105 ℃ for 3 h, took them out, cooled down in a desiccator for 0.5 h, quickly took them out and weighed, and calculated the dry extract rate.

Dry extract rate = Dry extract weight (g) × Total extract weight (g)/[Sample weight (g) × Crude drug weight (g)] × 100%.

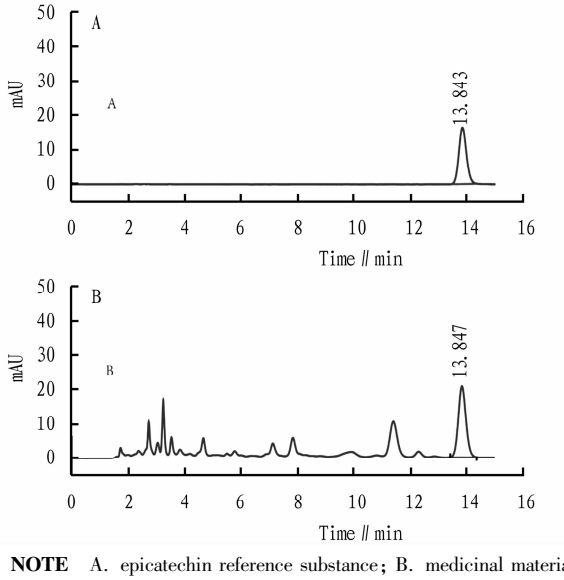
3.3 Determination of epicatechin[3-4]

3.3.1 Chromatographic conditions. Chromatographic column: ODS-H 5 μm × 4.6 m × 150 mm; mobile phase: acetonitrile-0.004% phosphoric acid solution (10 ∶ 90); wavelength: 280 nm, column temperature: 30 ℃, flow rate: 1.0 mL/min.

3.3.2 Preparation of reference substance solution. Precisely weighed a proper amount of epicatechin reference substance, put it into a measuring flask, and added dilute ethanol to prepare a solution at concentration of 25 μg/mL.

3.3.3 Preparation of test solution. (i) Preparation of the medicinal material test solution: took 2 g of Fagopyri Dibotryis Rhizoma coarse powder, weighed it accurately, put it into a conical flask with a stopper, added 50 mL of ultrapure water accurately, sealed the stopper, weighed it accurately, place it for 1 h, heated it and refluxed for 1 h, cooled down, weighed it again, used ultrapure water to make up for the weight loss, shook up and then obtained the extraction solution. (ii) Preparation of the test sample solution of the extraction solution: took the extraction solution to obtain the test solution.

3.3.4 Methodological investigation. (i) System suitability test. Separately pipetted 20 μL of epicatechin reference solution, medicinal material test solution and extract test solution to inject according to the chromatographic conditions in Section **3.3.1**, the symmetry of epicatechin main peak was 1.0, and the number of theoretical plates was calculated as 11 000 according to epicatechin peak. The resolution between the main peak and the impurity peak was greater than 1.5 (Fig. 1).



NOTE A. epicatechin reference substance; B. medicinal material.

Fig. 1 HPLC chromatogram of Fagopyri Dibotryis Rhizoma

(ii) Linear relationship. Precisely pipetted 0.5, 1, 2, 3, and 5 mL stock solutions of epicatechin reference substance with a concentration of 0.102 4 mg/mL into a 10 mL volumetric flask, prepared reference substance solutions with concentrations of 5.12, 10.24, 20.48, 30.72, and 51.20 μg/mL with the mobile phase, and shook up. Injected 20 μL of the above reference solution separately, determined according to the chromatographic conditions in Section **3.3.1**, and plotted a standard curve with the injection volume of epicatechin (μg) as the abscissa *X* and the corresponding peak area as the ordinate *Y*. The regression equation was $y = 1.218\ 9x + 3.102\ 7$, $R^2 = 0.999\ 9$ (Fig. 2), indicating that there was a good linear relationship between epicatechin injection volume and peak area integral value in the range of 102.4 – 1 024.0 ng.

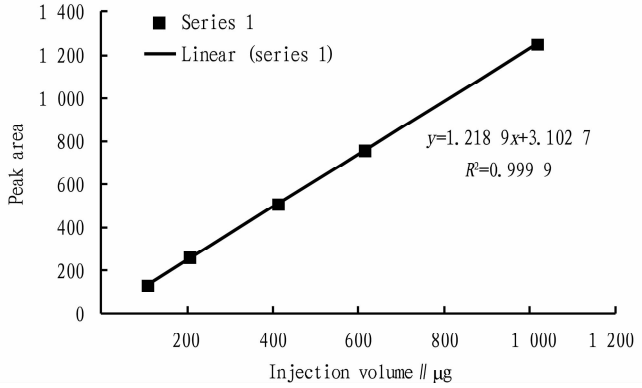


Fig. 2 Linear relationship

(iii) Precision test. Pipetted he epicatechin reference solution (concentration: 30.72 μg/mL), and injected the sample for 6 times according to the chromatographic conditions in Section **3.3.1**. The *RSD* of the chromatographic peak area of epicatechin was calculated to be 0.63%, indicating that the precision was good.

(iv) Stability test. Precisely pipetted the test solution of the same batch, injected the sample at 0, 2, 6, 12 and 24 h, respectively according to the chromatographic conditions in Section **3.3.1**, and determined the peak area. The *RSD* of epicatechin peak area was 0.86%, indicating that the test solution was stable within 24 h.

(v) Repeatability test. Took 6 portions of coarse powder of the same Fagopyri Dibotryis Rhizoma of medicinal materials, prepared the medicinal material test solution according to the method in Section **3.3.3**, and determined according to the chromatographic conditions in Section **3.3.1**. The average content of epicatechin was 0.061%, *RSD* was 0.81% ($n = 6$), indicating that the method was stable and reliable.

(vi) Sample recovery test. Took 6 portions of coarse powder of the same Fagopyri Dibotryis Rhizoma (known content 0.061%), 1 g for each portion, weighed accurately, and placed in a conical flask with a stopper. Separately added 5 mL of epicatechin reference solution (C: 0.102 4 mg/mL), accurately added 45 mL of ultrapure water, prepared the test solution according to the preparation method in Section **3.3.3**, determined and calculated the recovery rate and *RSD* value. As shown in Table 2, the

average recovery was 98.9% and *RSD* was 0.94%.

3.4 Determination results The dry extract rate and epicatechin were determined according to the above measurement methods. The results are shown in Table 3.

Table 2 Results of sample recovery test

Sample No.	Sample weight//g	Original epicatechin weight//mg	Added epicatechin//mg	Measured weight//mg	Recovery rate//%	Average recovery rate//%
1	1.023 4	0.624 3	0.512 0	1.126 4	98.1	98.9
2	0.996 5	0.607 9	0.512 0	1.120 3	100.1	
3	1.054 3	0.643 1	0.512 0	1.149 4	98.9	
4	0.998 4	0.609 0	0.512 0	1.120 4	99.9	
5	1.056 8	0.644 6	0.512 0	1.145 7	97.9	
6	1.024 4	0.624 9	0.512 0	1.128 8	98.4	

Table 3 Results of orthogonal experiment

Experiment No.	Factor			Dry extract rate//%	Epicatechin extraction rate//%	Comprehensive score points
	A	B	C			
1	1	1	1	6.83	0.032	48.74
2	1	2	2	10.26	0.040	69.29
3	1	3	3	14.58	0.046	93.19
4	2	1	2	14.25	0.050	93.53
5	2	2	3	14.68	0.059	100.00
6	2	3	1	10.84	0.053	78.43
7	3	1	3	13.62	0.052	91.45
8	3	2	1	7.02	0.041	54.04
9	3	3	2	13.68	0.050	90.77
K_1	70.41	77.91	60.40			
K_2	90.65	74.44	84.53			
K_3	78.75	87.46	94.88			
R	20.25	13.02	34.48			

With reference to the method in the literature, using SPSS AU online platform, we used the entropy weight method to determine the weight of each indicator and calculated the comprehensive score (M). The weights of epicatechin extraction rate and dry extract rate were 0.286 9 and 0.713 1, respectively. The comprehensive score (M) was calculated by the formula M = (Epicatechin extraction rate/Max epicatechin extraction rate) × 0.286 9 + (Dry extract rate/Max dry extract rate) × 0.713 1.

The size of the range *R* value can intuitively reflect the impact of the change of each factor level on the comprehensive score. The greater the range value, the greater the impact, and *vice versa*. The results of variance analysis are shown in Table 4. The results showed that the order of the three factors affecting the extraction rate of epicatechin and the amount of dry extract rate was C > A > B, and the optimal extraction process was $A_2B_2C_3$, that is, adding 10 times of water, extracting 3 times, 60 min each time.

3.5 Verification experiment Took the coarse powder of Fagopyri Dibotryis Rhizoma and extracted 3 times for 60 min each time according to the optimal extraction process, to conduct the verification experiment. Results indicated that the average extraction rate of epicatechin was 0.058%, and the average dry extract rate was 14.63%, which was close to the higher data in the orthogonal test results, and the *RSD* value was less than 5.0%, indicating that the process was stable and reliable (Table 5).

Table 4 Results of variance analysis

Variance source	Sum of squares of deviations	Freedom	Mean square	*F* value	*P* value
A	621.204	2	310.602	1329.19	<0.01
B	272.845	2	136.422	583.81	<0.01
C	1 877.859	2	938.930	4 018.06	<0.01
Error	0.467	2	0.234		

Table 5 Verification results of extraction process %

No.	Epicatechin extraction rate	Dry extract rate
1	0.057	14.59
2	0.058	14.68
3	0.058	14.62
Mean	0.058	14.63
RSD	1.0	0.31

4 Conclusions and discussion

Through the comprehensive evaluation and verification experiment of epicatechin and dry extract rate determination results, it was concluded that the optimal water extraction process of Fagopyri Dibotryis Rhizoma was to add 10 times of water, extract 3 times, and extract 60 min each time. The optimized extraction process was stable and feasible.

4.1 Selection of scoring indicators The epicatechin belongs to a flavanol flavonoid component, and modern researches[6-7] show that the epicatechin has a wide range of pharmacological activities such as antioxidation, hypoglycemic, insulin resistance inhibition, cardiovascular protection, neuroprotection, anti-inflammatory, bacteriostasis, immunity improvement and so on, and it is a main active component of Fagopyri Dibotryis Rhizoma. In addition, dry extract rate is often used to evaluate the quality and extraction effect of traditional Chinese medicines. Therefore, in this study, we optimized the water extraction process of Fagopyri Dibotryis Rhizoma by epicatechin and dry extract rate scores.

4.2 Selection of scoring methods At present, there are three main types of weight calculation methods: subjective weighting method, objective weighting method, and subjective and objective weighting method[11]. Among them, the entropy weight method is one of the commonly used objective weighting methods. This method uses information entropy to calculate the entropy weight according to the variation degree of the index, and further obtains the

weight of the indicator. It has the characteristics of strong practicability and adaptability, and is objective and scientific[12]. In recent years, it has been widely used in the weight calculation of the comprehensive evaluation of multi-indicator components of traditional Chinese medicines[13-16]. In this study, the extraction rate of epicatechin and the comprehensive score obtained by the standard weight of dry extract rate were determined by entropy weight method, and the amount of extraction solvent, extraction time and extraction times were investigated by orthogonal experimental design. The extraction process of Fagopyri Dibotryis Rhizoma was optimized. The optimized process is stable and reliable, and is expected to provide a reference for the further development and utilization of Fagopyri Dibotryis Rhizoma.

References

[1] National Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China[M]. Beijing: China Pharmaceutical Science and Technology Press, 2020: 228. (in Chinese).

[2] WANG LY, HUANG J, CHEN QF, *et al.* Research progress of *Fagopyrum dibotrys*[J]. Journal of Chinese Medicinal Materials, 2019, 42(9): 2206-2208. (in Chinese).

[3] YANG XW, ZHANG Y, LI LY. Advances in studies on medicinal plant of *Fagopyrum dibotrys*[J]. Modern Chinese Medicine, 2019, 21(6): 837-846. (in Chinese).

[4] YAN J, YUAN JJ, LIU LN, *et al.* Progress of research on pharmacological effects and clinical application of *Fagopyrum dibotrys*[J]. Shandong Journal of Traditional Chinese Medicine, 2017, 36(7): 621-624. (in Chinese).

[5] SHENG HG, ZHU LQ, LIN GT. Progress of research on chemical composition and pharmacological effects of *Fagopyrum dibotrys*[J]. Northwest Pharmaceutical Journal, 2011, 26(2): 156-158. (in Chinese).

[6] ZHANG XL, ZHOU YB, LIU CW. (-)-Epicatechin pharmacological activity and its mechanism progress research[J]. Tea in Fujian, 2018, 40(9): 3-6. (in Chinese).

[7] TONG GZ, FU XP, YANG Y, *et al.* Advances in research on the distribution and pharmacological activities of epicatechin[J]. Journal of Yunnan Agricultural University (Natural Science), 2018, 33(2): 343-349. (in Chinese).

[8] CAO TW, ZHAO L, LUO H, *et al.* Study on the extraction and purification technology for active ingredients of JinQiaoMai by taking epicatechin as the marker[J]. Western Journal of Traditional Chinese Medicine, 2019, 32(3): 36-40. (in Chinese).

[9] RUAN HS, CAO L, CHEN ZB, *et al.* Optimization of extraction technology for *Fagopyrum dibotrys* by central composite design and response surface methodology[J]. Herald of Medicine, 2013, 32(2): 226-229. (in Chinese).

[10] WANG WZ, TIAN Y, WU X, *et al.* Optimisation of the extraction process of total flavonoids from *Fagopyrum dibotrys* by quadratic regression orthogonal design method[J]. Journal of Chinese Medicinal Materials, 2012, 35(11): 1861-1863. (in Chinese).

[11] LIU QY, WU XN. Review on the weighting methods of indexes in the multi-factor evaluation[J]. Knowledge Management Forum, 2017, 2(6): 500-510. (in Chinese).

[12] WANG R, YANG LY, WU ZF, *et al.* Effect of drying method on quality attribute of benchmark samples of Huagai Powder Powder based on entropy weight method[J]. Chinese Traditional and Herbal Drugs, 2023, 54(3): 768-778. (in Chinese).

[13] FU Q, XIAO XF, ZHOU YT, *et al.* Optimization of extraction process of iridoids from Gardenia based on entropy weight method and multiple indexes[J]. Chinese Journal of New Drugs, 2022, 31(13): 1265-1272. (in Chinese).

[14] ZHENG WW, HUI SY, LIN JD, *et al.* Optimization of extraction process of Jingshi Mixture based on multi-index comprehensive evaluation of UPLC-MS/MS and G1-entropy weight method[J]. Chinese Journal of Hospital Pharmacy, 2022, 42(24): 2581-2588. (in Chinese).

[15] LIU XL, ZHANG R, WANG X, *et al.* Optimization of extraction process for Qushi Qingfei Recipe by combination of central composite design-response surface methodology with entropy weight method and its anti-inflammatory activity[J]. Chinese Journal of Hospital Pharmacy, 2022, 42(24): 2600-2606. (in Chinese).

[16] LI X, LIU W, SONG XL, *et al.* Optimizing the extraction process of Shaoyao Gancao Decoction based on entropy weight method and Box-Behnken response surface method[J]. Asia-Pacific Traditional Medicine, 2022, 18(12): 53-58. (in Chinese).

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[4] JIN LH. The determination of mineral elements in honey by ICP-MS and its application to discriminate Northeast-China black honey and linden honey[D]. Tai'an: Shandong Agricultural University, 2016. (in Chinese).

[5] LI CS, ZHONG N. Yangshuo: Chasing flowers and honey to strengthen the bee industry[N]. Guangxi Daily, 2018-10-11. (in Chinese).

[6] QIN HR, BI ZH, ZHOU DW, *et al.* Investigation and research on honey bees utilising the nectar plant *Bauhinia championii* in Guangxi[J]. Apiculture of China, 2016(67): 40-42. (in Chinese).

[7] LIN L. *In vitro* immunotoxicity evaluation of 5-HMF and its dimer OMBF using RBL-2H3 cells and immunogenicity of three traditional Chinese medicine injections using PLNA[D]. Beijing: Peking Union Medical College, 2018. (in Chinese).

[8] ZHANG XX, TANG YT, TANG L, *et al.* Research progress of processing and quality analysis of *Polygonatum sibiricum*[J]. China Modern Doctor, 2018, 56(22): 165-168. (in Chinese).

[9] National Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China (a)[M]. Beijing: China Pharmaceutical Science and Technology Press, 2020: 374-376. (in Chinese).

[10] FALLICO B, ARENA E, ZAPPALA M. Degradation of 5-Hydroxymethylfurfural in honey[J]. Journal of Food Science, 2008, 73(9): 625-631.

[11] DONG YY, CHI ZZ, CHI YS. Comparative study on the quality of sugar source honey, flower source honey and commercial honey[J]. Food Research and Development, 2021, 42(1): 48-54. (in Chinese).

[12] WANG TH, ZHANG SB, ZHANG HM, *et al.* Determination of volatile components in buckwheat honey and correlation analysis with honey maturity[J]. Food Science, 2020, 41(22): 222-230. (in Chinese).

[13] European Community. Council Directive 2001/110/EC of December 2001 relating to honey[S]. Official Journal of the European Communities, 2002, L10/47-L10/52.

[14] ZHANG LY, CHEN RY, ZHANG M, *et al.* Identification of syrup adulterated honey based on solid phase extraction-thin layer chromatography image analysis[J]. Journal of Chinese Institute of Food Science and Technology, 2021, 21(3): 263-273. (in Chinese).

[15] LU HX, WANG YH, YU YS, *et al.* Research and analysis of Yunnan honey HMF[J]. Journal of Bee, 2016, 36(8): 4-7. (in Chinese).