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Research Article

Optimization Extracting Technology of *Cynomorium songaricum* Rupr. Saponins by Ultrasonic and Determination of Saponins Content in Samples with Different Source

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Abstract: Extraction process was optimized by single factor and orthogonal experiment (L_9 (3^4)). Moreover, the content determination was studied in methodology. The optimum ultrasonic extraction conditions were: ethanol concentration of 75%, ultrasonic power of 420 w, the solid-liquid ratio of 1:15, extraction duration of 45 min, extraction temperature of 90°C and extraction for 2 times. Saponins content in Guazhou samples was significantly higher than those in Xinjiang and Inner Mongolia. Meanwhile, Guazhou samples harvested in April and May were higher than that in Guazhou *Cynomorium songaricum* numbered "the three-nine". In addition, saponin content in various samples grown in separate years is different. This ultrasonic extraction process can significantly improve the saponins extraction efficiency. Determination by this method is fast, easy-to-operate. The result of this method is reliable. The conclusion of study could provide scientific basis for rational development and quality control of *Cynomorium songaricum Rupr*.

Keywords: Content determination, *Cynomorium songaricum Rupr*, process optimization, saponins, ultrasonic extraction

INTRODUCTION

In recent years, *Cynomorium songaricum Rupr.* (*cs*) has been playing core role in health-care and has become a new material for developing and producing health foods and medicines (Zhao *et al*., 2010). Up to now, there have been a few study of *Cynomorium songaricum Rupr.* saponins (*cs* saponins); in addition, literature concerning extraction as well as determination content in *cs* saponins has not been reported (Zhao *et al*., 2010; Wang *et al*., 2014; Yoo *et al*., 2014; Capote and de Castro, 2007). Therefore, in this study, ultrasonic extraction was applied for extraction of cs saponins and the optimal conditions of extraction process were investigated by orthogonal experiment. The *cs* saponins content in different samples from various origins also were determined in this study to provide basis for establishment extraction methods, content determination together with quality standard of *cs* saponins (Nickrent *et al*., 2005).

MATERIALS AND METHODS

Reagents: Ursolic acid, Vanillic aldehyde, glacial acetic acid, perchloric acid and other chemicals were analytical grade reagents and the purity of Ursolic acid was 98%.

Sample: *Cs* provided by GuaZhou YiDe biotechnology. Ltd. Samples dried to constant weight at 50°C, crushing and through 100 mesh sieve.

Apparatus: A KQ-600DE ultrasonic equipment from Kun Shan Ultrasonic Instrumants co., Ltd, a AB104-N electronic scales from Mettle-Toledo, a CARIN Austrilia RTY, Ltd and a Exceed-Da-20 Aike Barnstead from Chengdu Kangning Experimental water Factory.

The preparation of the standard solution: Ursolic acid Standard solution (0.10192 mg/mL) was prepared by dissolving 2.6 mg which dried to constant weight in methanol and made to volume in a 25 mL volumetric flask.

The preparation of the test sample solution: *Cs* powder was sophisticated weighted 1.0000 g, reflux extracted with ethyl alcohol and obtained *cs* extracting solution, then ethanol was recycled by reduced pressure and distilled with 40 mL hot water and extracted with 40 mL pertroleum ether 2 times. The above solution

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extracted with 40 mL water saturation n-butyl alcohol, then combined the n-butyl alcohol liquid and evaporated to dryness. The residue dissolved in methanol and transferred to 50 mL volumetric flask with methanol constant volume and shook well (Jin *et al*., 2012). Further precision took 1 mL to10 mL volumetric flask with methanol constant volume and shook well again as the test sample solution. Drawn the standard curve with absorbance value as the ordinate and ursolic acid concentration as the abscissa. Obtained regression curve equation: Y = 52.5318 X -0.0872 (r = 0.9990) at the determination wavelength at 548 nm, showed that ursolic acid solution was on 8.5~23.5 μg/mL (Yoo *et al*., 2014; Capote and de Castro, 2007).

RESULTS AND DISCUSSION

In this study, the single factor experimental was employed to guide the preliminary range of the variables including the ethanol concentration, ultrasonic power, the solid-liquid ratio and extraction time. In detail, the ethanol concentration was set respectively for 50, 60, 70, 80 and 90%, the ultrasonic power was set respectively for 240, 360, 480 and 600 w, the solid-liquid ratio was set respectively for 1:6, 1:10, 1:20, 1:30, 1:40, the temperature was set respectively for 50, 60, 70, 80, 90 and 95°C, extraction time was set respectively for 30, 60, 90 and 120 min and extraction times was set respectively for 1, 2, 3, 4 times, then examined the influence of their to cs saponins extraction rate.

Orthogonal experiment and the results: According to the values obtained in the single factor experiment, orthogonal experiment was applied to optimum the extraction conditions of saponins from *cs*. Choose the ethanol concentration (A), ultrasonic power (B), the solid-liquid ratio (C) and extraction time (D) as examine factors and designed 4 factors 3 levels orthogonal experiment. Factors and levels were shown in Table 1. The results of orthogonal experiment were shown in Table 2. The results of variance analysis were shown in Table 3.

Table 1: Factors and levels

	Factors			
Levels	A (The ethanol concentration)	B (Ultrasonic power)	C (The solid-liquid ratio)	D (Extraction time)
1	65%	300w	1:15	45 min
2	70%	360w	1:20	60 min
3	75%	420w	1:25	75 min

Table 2: Results of orthogonal experiment

Number	A	B	C	D	Extract ratio (%) (n = 3)
1	1	1	1	1	6.18
2	1	2	2	2	6.09
3	1	3	3	3	6.03
4	2	1	2	3	5.87
5	2	2	3	1	5.78
6	2	3	1	2	6.24
7	3	1	3	2	5.81
8	3	2	1	3	6.28
9	3	3	2	1	6.29
k_1	6.099	5.954	6.236	6.082	
k_2	5.966	6.052	6.084	6.049	
k_3	6.130	6.188	5.874	6.063	
R	0.164	0.234	0.362	0. 033	

Table 3: The results of variance analysis

Soruce of variation (SV)	Sum of squares (SS)	Free (f)	Squares (S)	F value	Significant P
The ethanol concentration	0.137	2	0.069	11.370	0.001
Ultrasonic power	0.247	2	0.124	20.456	2.321×10^{-5}
The solid-liquid ratio	0.592	2	0.296	49.001	5.215×10^{-8}
Extraction time	0.005	2	0.003	0.416	0.666
Error	0.109	18	0.006		

Table 4: Different *cs* saponins samples content from different sources

Sample number	Sample origin	Acquisition time	Saponins content (n = 3)
The three-nine	Guazhou	2011.01	5.70%
001	Guazhou	2009.05	3.69%
002	Guazhou	2009.05	4.93%
003	Guazhou	2011.05	6.25%
004	Guazhou	2011.05	6.69%
005	Guazhou	2011.04	6.48%
006	Guazhou	2010.04	6.40%
Xinjiang	Jimusaer	2012.03	4.47%
Inner Monglia	Ejinaqi	2012.03	5.15%

Orthogonal experiment data (Table 3 and 4) were shown various factors affecting *cs* saponins extraction rate sequence was: the solid-liquid ratio > ultrasonic power > ethanol concentration > extraction time. Got the best conditions was A3B3C1D1, namely the ethanol concentration of 75%, ultrasonic power 420 w and the solid-liquid ratio 1:15 and extraction time of 45 min.

Verified experiment: According to the best extraction conditions what were determined by orthogonal experiment, the ethanol concentration of 75%, ultrasonic power of 420 w, the solid-liquid ratio of 1:15, extraction time of 45 min and extraction temperature of 90°C, extracting 2 times, cs saponins have been measured the average extraction rate was 6.40% (n = 5, RSD = 1.13%), The result shown that a higher content than orthogonal experimental results and the extraction technology can effectively improve the saponins extraction rate and good repeatability.

Determination of saponins content in the samples (Yoo *et al*., 2014): *Cs* powder was sophisticated weighted 1.0000 g in the 100 mL round bottom flask, obtained *cs* saponins extract solution which used extraction conditions by orthogonal experiment, then ethanol was recycled by reduced pressure and residue was dissolved with 40 mL distilled hot water and extracted 3 times with 40 mL pertroleum ether. Different *cs* saponins samples content from different sources shown as Table 4.

The experimental results showed that Guazhou samples saponins content were significantly higher than Xinjiang and Inner Mongolia and Guazhou samples which harvested in April and May were higher than Guazhou three-nine *Cynomorium songaricum*. In addition, the different years samples saponin content is different (Luan and Li, 2010).

CONCLUSION

Ultrasonic extraction have time saving, high efficiency, energy saving etc., sometimes even than modern extraction method, such as supercritical fluid extraction, microwave extraction, has been widely used extraction of effective ingredients in plant and can effectively increase the extraction rate. This study results show that the ultrasound power, the solid-liquid ratio, the alcohol concentration has a significant effect on *cs* saponins extraction rate, screened extraction process can obviously increase *cs* saponins extraction rate.

Ursolic acid as standard, this study adopts the vanillin-perchlorate chromogenic method measured *cs* saponins contents, precision, repeatability, stability and recovery experiment results show that this method is stable and reliable, repeatability well, can be used to *cs* saponins content determination (Zhao *et al*., 2010; Wang *et al*., 2014).

Different sources *cs* saponins results show that the content of saponins with Guazhou *cs* saponins content was significantly higher in Inner Mongolia, Xinjiang samples. *Guazhou cs saponins is number one*, *cs* saponins content and medicinal materials quality have a certain degree of relation, but need combination *cs* saponins active research to clarify. Guazhou samples which harvested in April and May were higher than Guazhou three-nine *Cynomorium songaricum*., suggested that *cs* Saponins content has a tendency to increased gradually at the early stage of the unearthed, unearthed period and exuberant plant growth period. In addition, the different years samples saponin content is different. This may be related to that the growing environment of soil nutrients, water and climate factors (Zhao *et al*., 2010; Wang *et al*., 2014; Yoo *et al*., 2014; Capote and de Castro, 2007). The research results will provide the basis for the establishment of *cs* saponins extraction, content determination and quality standard.

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