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Research Article

Optimization of Ultrasound-Assisted Extraction of Tannin from *Cynomorium songaricum*

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Abstract: To optimize ultrasound-assisted extraction technology of tannin from the succulent stem of *Cynomorium songaricum*. The content of tannin in *C. songaricum* samples of different source was comparatively studied. Extraction rate of tannin as indexes, extraction process was optimized by single factor experiment and $L_9(3^4)$ orthogonal experiment. The best extraction technology for ultrasonic extraction were 75% as the extraction solvent ethanol concentration, 600 w as the ultrasonic power, 1:65 as the solid-liquid ratio, 105 min as the extraction time and extraction 2 times. According to the results of content determination, *C. songaricum* samples from Guazhou has the highest tannin content than samples from Xinjiang and Inner Mongolia and in the samples of Guazhou which were harvested in April and May has more content than three-nine *C. songaricum* (harvested in the third nine-day period after the winter solstice). This study could provide scientific basis for rational development and quality control of *C. songaricum*.

Keywords: Content determination, *cynomorium songaricum*, process optimization, tannin, ultrasonic extraction

INTRODUCTION

Cynomorium songaricum, referred to as Elixir of Life, is dried fleshy stems from *Cynomorium songaricum Rupr. Cynomorium songaricum Rupr* is succulent perennial herbs as well as achlorophyllous holoparasite belonging to Cynomoriaceae in conjunction with *Cynomorium Songaria* genera. *C. songaricum* always parasitize root of plants which is Zygophyllaceae together with Natraria L (Yoo *et al*., 2014; Zhao *et al*., 2010; Yu *et al*., 2010). Reports show that *C. Songaria* tannin is one of effective constituents among its compositions, steroids, triterpenes and so on. Meanwhile, in addition to effect of hemostatic convergence, it plays important role in anti-oxidation, reducing hematic fat, falling blood pressure as well as anti-tumor. Currently, there are few study about *C. songaria* tannin and no report on ultrasound-assisted extraction technology of tannin from the succulent stem of *C. songaricum.* As a consequence, our study uses ultrasound-mediated method (Capote and de Castro, 2007) to extract *C. Songaria* tannin and optimize conditions by single-factor and orthogonal test (Wang *et al*., 2014). At the same time, we measure tannin content in *C. songaricum* sample from different sources. The recommended study established a precise and effective extraction method of tannin's extraction, ulteriorly, provided references for extraction of *C. songaria* tannin as well as formulating Quality standards. Moreover, it can be supplement to scientific foundations for exploitation and utilization of *C. songaricum.*

MATERIALS AND METHODS

Apparatus and reagents: Numeric control ultrasonic cleaning machine for this study was purchase in Kunshan ultrasonic instrument Co., LTD (KQ-600DE). All the reagents were weighed by electronic balance (AB104-N, Mettle-Toledo). Eco pure water meter was provided by Chengdu corning experimental water factory (Exceed-Da-20). All the reagents in the experiments were analytical grade. The solutions were prepared with distilled water.

Sample: *Cynomorium songaricum* was provided by Guazhou Yide biological technology co., LTD. Sample was dried at 50°C to constant weight, was subsequently crushed through 100 mesh sieve. Afterwards, the crushed material is sealed to preserve.

Preparation for reagents: 0.6 g indicarminum (Tianjin Guangfu fine chemical industry, China) was precisely

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weighed and dissolved by sulfuric acid (Baiyin Liangyou chemical reagent, China) with 50% volume fraction. Then the solution was diluted to 100 mL with distilled water. 25 g gelatin (Shanghai gelatin factory, China) was soaked in saturated sodium chloride (Tianjin Baishi Chemicals, China) and dissolved completely by heating. Acidic sodium chloride was prepared by adding 25 mL concentrated sulfuric acid into 975 mL saturated sodium chloride. 0.79 g potassium permanganate (Tianjin Baishi Chemicals, China) was accurately weighed and dissolved, whereafter, diluted to 500 mL in volumetric flask.

Titration with potassium permanganate: 10 mL extract of *C. songaricum* together with 5 mL indigo carmine indicator was diluted to 100 mL with distilled water. The solution was titrated with 0.01 mol/L potassium permanganate standard solution until its color changed from green to drab yellow. The letter "a" was used to be volume of potassium permanganate consumed.

10 mL extract of *C. songaricum* and 10 mL of Gelatin solution and acidic sodium chloride, respectively. In addition, 1.0 g kaolin (Zhongqin in the Shanghai chemical reagent co., LTD) was added into the solution. Afterwards, the mixture was shaken for many minutes and filtered. The residue was washed for two or three times with distilled water. 5 mL indigo carmine indicator was added in the mix containing filtrate as well as washings. Then solution was diluted to 100 mL with distilled water. The solution was titrated with 0.01 mol/L potassium permanganate standard solution until its color changed from green to drab yellow. The letter "b" was used to be dosage of potassium permanganate consumed.

Calculation of tannin content:

$$\text{Tannin content (\%)} = \frac{(a - b) \times N \times 0.042}{W \times D} \times 100\%$$

In this formula: "a" represents the total volume of potassium permanganate standard solution consumed by sample. "b" is dosage of potassium permanganate standard solution used by non-tannin in the sample. "N" is on behalf of the concentration of potassium permanganate standard solution. The number "0.042" is the weight of tannin per gram (g). "W" stands for the weight of sample (g). "N" is on behalf of the dilution factor.

RESULTS AND DISCUSSION

Single factor test: Extraction solvents, extraction frequency, ultrasound power, ratio of raw material to water and ultrasonic duration were selected to be testing factors on extraction efficiency. Our study indicated that the optimum condition of single factor was: 70% ethanol as extraction solvent, extracting for two times, at 800 W ultrasonic power for 120 min and ratio of raw material to water being 1:70.

Orthogonal test: Comprehensive evaluation of technical parameters (extraction solvents, extraction frequency, ultrasound power, ratio of raw material to water and ultrasonic duration) of ultrasonic extraction process of tannin from *Cynomorium songaricum* were investigated by orthogonal experiment. Extraction efficiency of tannin was used to be assessment index. There were nine experiments in accord with four columns (A, B, C, D) as well as nine rows (1 to 9). As revealed in Table l to 3 the effects of various factors presented an overall order of B (ultrasonic power) >A (ethanol concentration) >D (ultrasonic duration) >C (ratio of raw material to water). Analysis of variance manifested that the four factors have great significance on extraction efficiency. The optimal conditions match was $A_3B_3C_1D_1$ (ethanol concentration = 80%, ultrasonic power = 600 W, ratio of raw material to water = 1:65, ultrasonic duration = 105 min).

Table 1: Orthogonal factors and levels

Level factor	A (ethanol concentration)	B (ultrasonic power)	C (ratio of raw material to water)	D (ultrasonic duration)
1	60%	480 w	1:65	105 min
2	70%	540 w	1:70	120 min
3	80%	600 w	1:75	135 min

Table 2: Results of orthogonal test

Number	A	B	C	D	Extraction efficiency of tannin (%) (n = 3)
1	1	1	1	1	2.94
2	1	2	2	2	2.98
3	1	3	3	3	3.13
4	2	1	2	3	2.80
5	2	2	3	1	3.34
6	2	3	1	2	3.40
7	3	1	3	2	2.86
8	3	2	1	3	3.43
9	3	3	2	1	3.56
K1	3.018	2.866	3.258	3.279	
K2	3.182	3.252	3.116	3.081	
K3	3.283	3.366	3.110	3.123	
R	0.265	0.500	0.148	0.198	

Table 3: Analysis of variance

Factor (SV)	Sum of square of deviation (SS)	Degree of freedom (f)	Mean square (S)	F value	P
Ethanol concentration	0.323	2	0.162	70.182	*
Ultrasonic power	1.237	2	0.619	268.495	**
Ratio of raw material to water	0.126	2	0.063	27.410	*
Ultrasonic duration	0.195	2	0.098	42.386	*
Error	0.041	18	0.002		
	$F_{0.01}(2, 2) = 99.00$, $F_{0.05}(2, 2) = 19.00$				

Table 4: Results of tannin content in various samples

Samples number	Source region of samples	Acquisition time	Tannin content (n = 3)
Three and nine	Guaprefecture	2011.01	3.35%
01	Guaprefecture	2009.05	1.88%
02	Guaprefecture	2009.05	3.35%
03	Guaprefecture	2011.05	3.47%
04	Guaprefecture	2011.05	3.55%
05	Guaprefecture	2011.04	3.51%
06	Guaprefecture	2010.04	3. 64%
Sinkiang	Jimsar County	2012.03	3.27%
Neimenggu	Ejin Banner	2012.03	3.18%

Verification test: *Cynomorium songaricum* from different origin were determined in three replications, as shown in Table 4. Result suggested that content of tannin in all samples except number "01" was more than 3.3%. Our study showed that the optimal conditions were reasonable, feasible and stable.

CONCLUSION

Ultrasound-mediated extraction is time-saving, effective and energy-saving. Meanwhile, it can increase extraction efficiency. It has been widely used in determination of plants' active ingredient. Study indicated that ethanol concentration, ultrasonic power, ratio of raw material to water together with ultrasonic duration had markedly effect on extraction efficiency of tannin. Moreover, the optimal condition was as follows: Ethanol concentration was 80%. Ultrasonic power is equal to 600 W. Ratio of raw material to water was 1:65. Ultrasonic duration was the same with 105 min. *Cynomorium songaricum* tannin content was measured by titration with potassium permanganate. The titration method was easy to operate and with great repeatability. Verification test showed that tannin concentration in *Cynomorium songaricum* differed because of their origin as well as growing period: *C. songaricum* samples from Guazhou has highest tannin content than others; tannin content in the samples of Guazhou which were harvested in April and May was highest. This study provided a precise and effective extraction method for tannin's exploitation and utilization (Capote and de Castro, 2007; Yoo *et al*., 2014; Wang *et al*., 2014; Yu *et al*., 2010; Zhao *et al*., 2010).

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