Journal of Applied Pharmaceutical Science Vol. 3 (05), pp. 110-113, May, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.3521
ISSN 2231-3354

Optimized Ultrasonic-Assisted Extraction of Polysaccharides from Chinese Traditional Medicinal Plant *Paris fargesii* Using Orthogonal Design

Gang Xiang [#], Dan Wang [#], Gui-Hua Zou, Xing Huang, Meng-Liang Tian*, and Feng Gao*
Department of Chinese traditional herbal, Agronomy College, Sichuan Agricultural University, No.211, Huiming Road, Wenjiang Region, Chengdu 611130, China.

ARTICLE INFO

Article history:
Received on: 09/03/2013
Accepted on: 07/05/2013
Available online: 30/05/2013

Key words:
Paris fargesii; total polysaccharides; ultrasonic-assisted extraction; orthogonal design.

ABSTRACT

Paris fargesii is a famous Chinese traditional medicinal plant used as antipyretic, antidotal, antiphlogistic and analgesic. This research aimed at the determination of the total polysaccharides contents in *P. fargesii*, which were collected from Baoxing country of Sichuan Province in order to get the optimized extraction technology. Ultrasonic method was adopted in the extraction of total polysaccharides from *P. fargesii*. Through single factor experiment and orthogonal test, the effects of ultrasonic extraction conditions on the total polysaccharides extraction were measured, including solid-liquid ratio, ultrasonic temperature, ultrasonic power and ultrasonic time. Then colorimetric method was applied for the assaying. The optimized extraction conditions of total polysaccharides from *P. fargesii* were as follows: ultrasonic power: 600w, solid-liquid ratio: 1:20, ultrasonic extraction time: 1.5h and ultrasonic temperature: 90℃. Under the optimum parameters, the total polysaccharides extraction efficiency was 0.0715%.

INTRODUCTION

Polysaccharides, polymers connected by monosaccharide, are of significance molecules with bioactivity other than nucleic acid and proteins. Polysaccharides also are important components for all living creatures, which are widely distributed in animal, plant and microbial cell walls. After a long history of scientific research, researchers have already found many therapeutic activities of polysaccharides, including anti-tumor (Zong *et al.*, 2012), anti-oxidative damage (Jiang *et al.*, 2012), immune (Zhang *et al.*, 2012; Xu *et al.*, 2010), reducing blood sugar and anti-virus, and nearly conduct no side effect on human body (Li *et al.*, 2012). In this research, Chinese traditional medicinal herb, Paris fargesii, as the raw material, is the rhizome of several plants in *liliaceae* Genus *Paris*. It was included in "Sheng Nong's herbal classic" (Wen *et al.*, 2012) firstly and has a long history been used for medicinal purposes. Since the last century, researches on main bioactive ingredients in Genus *Paris* have been conducted around steroids and saponins. Whereas, due to the complex chemical components, strong pharmacological activities and wide clinical application of Genus *Paris*, it is necessary to further strengthen on the extraction, isolation, identification and pharmacological evaluation of compounds from *Paris*. As is well known, polysaccharides are water soluble, which is consistent with ingredients got from decoction of herbal reagents. Therefore, investigation on polysaccharides from Paris is of practical significance.

Many researchers have been keeping on the research of extraction of polysaccharides from various resources, including medicinal plants (Gabriela *et al.*, 2003; Wu *et al.*, 2013) and animal organs (Yu and Chao, 2013). Several extractive methods of polysaccharides were studied, from the traditional aqueous extraction (Zhou *et al.*, 2002; Hou and Chen, 2008) to modern ultrasonic method (Chen *et al.*, 2012; Tian *et al.*, 2012). After a comprehensive consideration, combination of phenol-sulfuric acid method and ultrasonic extraction was conducted to make the results more accurate and reasonable. Single factor experiment and orthogonal design were also adopted to investigate the optimized extraction conditions of polysaccharides from *Paris fargesii*, which

# These two authors are thought to have equal contributions.
*Corresponding author
E-Mail: gaofeng@sicau.edu.cn;
Tel.: +86-28-8629-0870; Fax: +86-28-8629-0780.

would provide theoretical basis for plant chemotaxonomy as well as the preservation, development and utilization of the famous traditional medicinal resources. Herein, we reported the optimized ultrasonic extraction technology of polysaccharides from the *Paris fargesii* firstly, which was established by the methodology of single factor and orthogonal design experiments.

MATERIALS AND METHODS

Plant material

The whole herb of *Paris fargesii* was collected from Baoxing Country of Sichuan, China in Nov. 2012, and indentified by Prof. Meng-Liang Tian of Sichuan Agricultural University. A voucher specimen (No. 2012-11-02) was prepared from the entire plants of *Paris fargesii* and deposited in the herbarium of the Department of Chinese Traditional Herb, Agronomy College, Sichuan Agricultural University.

Preparation of standard glucose solution

Anhydrous glucose (0.0502 g) was dried to constant weight at 105℃, to formulate into 50 ml 100.4μg/ml glucose solution. 1.00, 2.00, 3.00, 4.00, 5.00, 6.00 and 7.00ml of glucose solutions were pipetted into seven 50ml volumetric flasks respectively. They were used as glucose standard solution after been diluted to constant volume.

Determination of testing wavelength

1ml of test solution of different concentrations were added into seven test tubes with stopper respectively, then sequentially added 1ml phenol solution, and 5ml concentrated sulfuric acid into each of the seven test tubes respectively. Shook it up and bathed in boiling water for 15min. After cooled to room temperature, we conducted colorimetric determination under maximum absorption wavelength with reagent blank as reference. The standard curve was drawn by glucose concentration on the horizontal axis and absorbance on the vertical, and obtained the regression equation as $Y = 8.4586X-0.3671$ (R^2=0.9987）.

RESULTS AND DISCUSSION

Impact of the four ultrasonic extraction conditions

Except for the variable in the four single factor tests, other experimental conditions of the tests were chosen as 70 ℃, 800w, 30min and 1:20, three of which were set as constants in the tests respectively. From Figure 1, the changing trend of absorbance under four variables was shown independently, in other words, the effecting line of the four ultrasonic extraction conditions on extraction ratio of polysaccharides could be seen from the four pictures. According to the first picture, we should know that if the solid-liquid ratio was too low, it would lead to overly viscous solution, which was adverse to extraction; If the

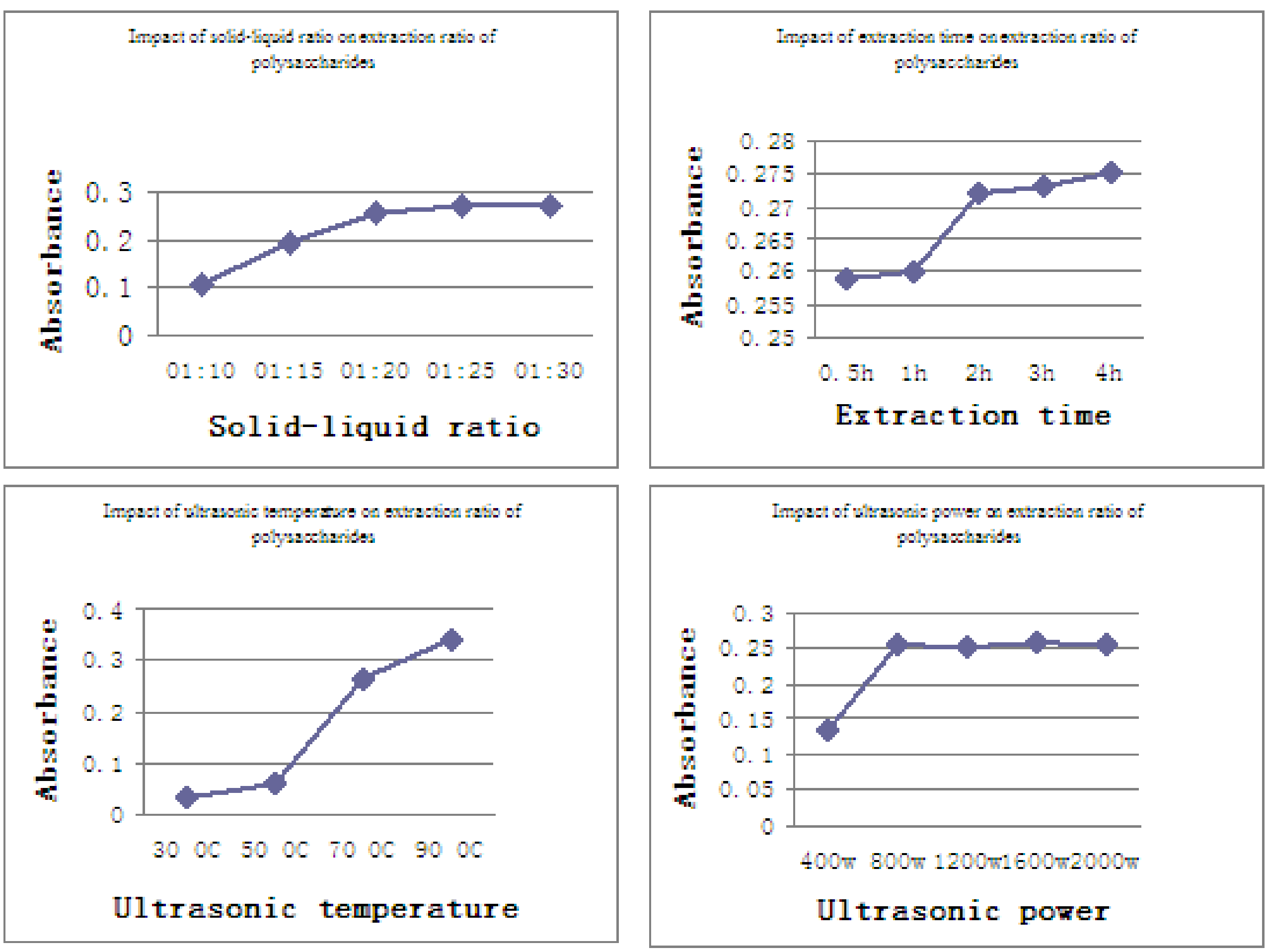

Fig. 1 Impacts on extraction ratio of polysaccharides.

solid-liquid ratio was too high, it would make it uneasy to concentrate, which increased costs. 1:20 was then considered to be a proper solid-liquid ratio. In the second picture, it was known that the amount of leaching solute was generally proportional to the extraction time (Zuzana et al., 2013; Ying et al., 2011), however, after diffusion reached equilibrium, time didn't work. In the third one, we chose 90℃ as the upper limit temperature, for that polysaccharides were active substances, the prolonged high temperature would reduce the activity. In the last picture, the absorbance remained constant under ultrasonic power beyond 800w.

Multi-factor test and results

Referring to the single factor test results, we listed the factors and levels of multi-factor test in Table 1 $L_9(3^4)$ orthogonal combination design was adopted, and set three levels for extraction time, extraction temperature, solid-liquid ratio and ultrasonic power respectively. As a result, the test had nine treatments, and three replications.

Table 1. Factor levels of orthogonal test.

Disposition	Factors			
	A (h) **Extraction time**	**B(℃)** **Ultrasonic temperature**	**C** **Solid-liquid ratio**	**D (w)** **Ultrasonic power**
1	0.5	70	1:15	600
2	1	80	1:20	800
3	1.5	90	1:25	1000

It could be drawn from Table 2 that combination 9 got the highest absorbance of 0.466, which means the highest glucose concentration.

Table 2. Orthogonal test and results.

Dispose	Factors				Absorbance
	A (h) **Extraction time**	**B(℃)** **Ultrasonic temperature**	**C** **Solid-liquid ratio**	**D (w)** **Ultrasonic power**	
1	0.5	70	1:15	600	0.247
2	0.5	80	1:20	800	0.348
3	0.5	90	1:25	1000	0.442
4	1	70	1:20	1000	0.292
5	1	80	1:25	600	0.408
6	1	90	1:15	800	0.419
7	1.5	70	1:25	800	0.350
8	1.5	80	1:15	1000	0.301
9	1.5	90	1:20	600	0.466
Optimal group	A9B9C9D9				

Stabilities

We took the same copy of test solution, and sequentially added 1ml test solution, 1ml phenol solution, and 5ml concentrated sulfuric acid into a test tube with stopper. Shook it up and bathed in boiling water for 15min. After cooled to room temperature, we conducted colorimetric determination under maximum absorption wavelength with reagent blank as reference. Determined the absorbance under 0, 10, 20, 30, 40, 50, 60 min, and calculated the RSD as 0.546%.

Precision test

We took six pieces of the test solution, sequentially added 1ml test solution, 1ml phenol solution, and 5ml concentrated sulfuric acid into six test tubes with stopper respectively. Shook it up and bathed in boiling water for 15min. After cooled to room temperature, we conducted colorimetric determination under maximum absorption wavelength with reagent blank as reference. Determined the absorbance, and calculated the RSD as 2.035%.

CONCULSION

In current investigation, we adopted single factor test to measure the effects of ultrasonic extraction conditions on the extraction of total polysaccharides, including solid-liquid ratio, ultrasonic temperature, ultrasonic power and ultrasonic time. Then optimized the four factors using multi-factor test combined with L^9 (34) orthogonal combination design. In addition, we separately conducted the stability test and precision test, and finally determined that the ultrasonic extraction conditions of combination nine were most conducive to the total extraction ratio of polysaccharides from *Paris fargesii*, which were ultrasonic power 600w, solid-liquid ratio 1:20, ultrasonic extraction time 1.5h and ultrasonic temperature 90℃. According to the formula, the total extraction ratio of 0.0715% was obtained under the optimized conditions. Comparing with other reports, the extraction ratio of polysaccharides on current investigation was relatively lower than that from other materials, which could be attributed to the fact that contents of polysaccharides was lower in *Paris fargesii* than in other materials. In summary, the optimized ultrasonic-assisted extraction of polysaccharides from *Paris fargesii* was investigated for the first time.

ACKNOWLEDGEMENT

We are grateful to the NSFC (No. 81001383) and Science and Technology Support Plan of Science& Technology Department of Sichuan Province (No. 2011BAI13B02-6).

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How to cite this article:

Gang Xiang, Dan Wang, Gui-Hua Zou, Xing Huang, Meng-Liang Tian, and Feng Gao. Evaluating the incidence, risk factors and glycaemic control of new-onset diabetes mellitus in kidney transplant recipients: a single centre study. J App Pharm Sci. 2013; 3 (05): 110-113.