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Contributed Paper

Optimization of Ultrasonic-assisted Extraction Using Taguchi Design and *In vivo* Activities of Polysaccharides from *Flos populi*

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ABSTRACT

There is an increasing demand for developing simple and efficient extraction of active polysaccharides from herbs, and ultrasound-assisted extraction (UAE) has attracted considerable interest. In this study, UAE of polysaccharides from *Flos populi* (PFP) was optimized using Taguchi L_{27} orthogonal design. The statistical analysis showed that the most important factors contributing to the extraction yield of PFP were liquid to solid ratio, extraction temperature, ultrasonic power and time, respectively, and the optimum conditions were at 25 mL/g, 50 °C, 400 W and 30 min which result the maximum yield of PFP (59.8 mg/g). At the above conditions, the extraction yield of 61.9 ± 2.5 mg/g was close to the predicted value. Moreover, the effect of extraction cycle (1-5) on the PFP yield was tested, and extraction cycle of 2 was determined. The UAE and conventional solvent extraction methods (CSE) were compared for extracting PFP. The UAE technique was very efficient in the extraction of PFP compared with CSE, for the similar yield of PFP was obtained at 60 and 150 min, respectively. Additionally, the antioxidant activities of PFP were accessed *in-vivo*. The results revealed that PFP had markedly enhanced the levels of catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), while decreased the level of malonaldehyde (MDA) in serums and livers of mice compared to CCl_4 treated group. The study revealed that PFP possess potent antioxidant activity and could be useful for the development of new antioxidant agents.

Keywords: *Flos populi*, polysaccharides, ultrasonic-assisted extraction, taguchi experimental design, antioxidant activity

1. INTRODUCTION

Polysaccharides have been attracting increasing focus because of their various activities used in medicine, anti-cancer, antioxidant, immunostimulatory, antitumor, and health-care food [1, 2]. Many herbs contain largely untapped source of powerful new pharmaceutical products. Particularly, they represent an unlimited source of polysaccharides with antioxidant and immunostimulating properties. In present, lots of active polysaccharides have been extracted from various herbs and some of them are now used in clinics, e.g. *Astragalus mongholicus* polysaccharides (ASP) from *Astragalus mongholicus* [3], *Ganoderma lucidum* (Reishi) polysaccharides from *Ganoderma Lucidum* [4] and antioxidant activities of polysaccharides from *Prunella vulgaris* L. [5]. These polysaccharides enhanced and stimulated the immune system, and are thus considered to be the biological response modifiers.

Flos populi is a traditional Chinese medicinal which grows in the whole nation of China, is often used for curing diseases such as diarrhea, bacterial infection, inflammation, pain, hyperlipidemia and oxidative stress [6, 7]. This herb is rich in flavonoids, phenolics and polysaccharides. In our previous research, we have optimized the extraction and enrichment process of flavonoids from *Flos populi* [8, 9]. However, no detailed investigation has been conducted on optimization of polysaccharides extraction from *Flos populi* so far. For exploiting the resource of *Flos populi*, a simple and efficient method should be used to extract polysaccharides from *Flos populi.*

The extraction of active polysaccharides from plants is usually carried out in a variety of ways, such as ultrasound assisted extraction (UAE), microwave assisted extraction (MAE), supercritical fluid extraction, soxhlet extraction and reflux extraction [10, 11]. The extraction efficiency is usually low using conventional methods, such as soxhlet and reflux extraction. So it needs to develop some efficient techniques to improve the extraction efficiency. UAE is widely used for polysaccharides extraction form natural plants due to its high extraction efficiency [12]. Owing to acoustic cavitation, extraction solvent is easy to penetrate cell walls and promote the release of soluble ingredients from the plant body [13]. Therefore, extraction efficiency of the compounds from natural plant is accelerated by ultrasound. In the foretime, UAE was chiefly exploited on the laboratory scale rather than used in factory due to his powerful noise and special the equipment; however, it has already been found in industrial applications with the development of equipment [14, 15]. The new technology has been attracting increasing attention in the department of extraction and separation in recent years.

It is important to improve the performance of the systems and increase the extraction yield of the processes without increasing the cost. To improve quality, reduce cost and provide robust design solutions, Taguchi method has been widely used in many applications of engineering. Taguchi design is a special design of an orthogonal matrix that improves efficiency and quality, and gives a optimal design solutions. This method provides an established approach for accessing interaction effects when ranking and screening various experimental parameters. Furthermore, Taguchi design is suitable to solve various problems involving discrete, continuous and qualitative design variables [16, 17].

In view of little information on the UAE for polysaccharides from *Flos populi*, we report here in detail the optimization

of extraction conditions for polysaccharides from *Flos populi*. Several factors that could potentially affect the extraction yield were tested and optimized using Taguchi design. Additionally, the antioxidant activities of PFP were assessed *in vivo*, including the levels of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) and malonaldehyde (MDA) in serums and livers of mice.

2. MATERIALS AND METHODS

2.1 Materials and Apparatus

Flos populi (male inflorescence of *Populus tomentosa Carr.*) was purchased from a medicinal herbs store (Anguo, Hebei Province, China) and authenticated by Associate Professor Junkai Wu (Heilongjiang University of Traditional Chinese Medicine, Harbin, China). The voucher specimen (Accession no. 1009015ch) has been deposited at the Herbarium in the College of Veterinary Medicine, Northeast Agricultural University. *Flos populi* was dried at 60 °C in a vacuum oven for 4 h and then ground up using a mechanical grinder. Then the powder were refluxed with 95% ethanol at 70 °C in a water bath for 2 h to inactivate the endogenous enzymes and remove some soluble materials, including free sugars, amino acids and phenols. Finally, the extraction solvent was evaporated to obtain dry powder.

The standards (D-Glucose, Vitamin C) were obtained from the Chinese Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purity of standards exceeded 98% (w/w). Experiments were performed with deionized water provided by Milli-Q academic water purification system (Millipore, Bedford, MA, USA). Commercial kits obtained from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China) were employed to test the levels of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) and malonaldehyde (MDA). All other analytical grade reagents were obtained from Hangzhou Company (Hangzhou, PR China).

Ultrasonic irradiation was performed using a BILON-S650CT high-power ultrasonic generator (30 kHz, 0-650 W; Shanghai Bilang Instrument Making Limited Company, China) equipped with a flat tip probe transducer. The frequency of the ultrasound waves was kept at 30 kHz in the UAE. A brown glass tubes (4 cm diameter × 7 cm height) was used as the extraction cell which treated with a thermostatic water bath to control the temperature. A 1.2 cm probe was located 1 cm from the top liquid level of extraction solvent. The ultrasonic pulse sequence was 1 s on and 3 s off to deal with the bursting of the bubbles. UV-1800 spectrophotometer (Shimadzu Corporation, Japan) was employed to test the content of PFP.

2.2 Ultrasound-assisted Extraction (UAE)

Each dried sample (2.00 g) was extracted by deionized water in the extraction cell. The effects of four parameters, including ultrasonic power (100 - 600 W), time (10 - 60 min), temperature (40 - 90 °C), and solvent to solid ratio (5 - 30 mL/g), on extraction yield of PFP were evaluated. When complete, the extraction solution was concentrated at 60 °C under vacuum. The proteins in the extraction solution were removed by Sevag method [18]. And then the extraction solution was precipitated by the addition of dehydrated alcohol to a final concentration of 80% (v/v) at 4 °C for 8 h. After centrifugation at 3000 rpm for 10 min, the precipitate was freeze-dried at -40 °C under vacuum to get the crude polysaccharides. Anthrone-sulfuric method was used to determine the content of

polysaccharides and D-glucose was adopted as a standard [19]. The extraction yield (%) was calculated as follows:

$$Yield(\%) = \frac{weight\ of\ polysaccharides\ in\ the\ crude\ polysaccharides}{weight\ of\ the\ related\ Flos\ Populi\ powder} \quad (1)$$

2.3 Conventional Soxhlet Extraction (CSE)

CSE was also employed for PFP preparation as a control according to the reported method [20]. Each dried pretreated sample (2.00 g) was extracted by deionized water in a designed solvent to solid ratio, temperature and time. The treatment process of the extraction solution was required as the method above.

2.4 Taguchi Design

Four variables used in this study were, ultrasonic power (300 - 500 W, A), ultrasonic time (20 - 40 min, B), extraction temperature (50-70 °C, C) and solvent to solid ratio (15 - 25 mL/g, D), with three levels for each variable, while the dependent variable was the yield of PFP. The factors and their levels are shown in Table 1. A L_{27} orthogonal matrix generated by Minitab 16 software was adopted which needs 27 experiments to optimize the extraction process [21]. The extraction results and the signal-to-noise (S/N) ratio performed under orthogonal design conditions are shown in Table 2.

The signal-to-noise (S/N) ratio is used as statistical measure in Taguchi method [22]. The S/N ratio can be divided into three categories: the higher-the-better, the nominal-the-better and the lower-the-better. In our case the experimental target was the yield of PFP, so the higher-the-better option was adopted:

$$S/N = -10\log\frac{1}{n}\left(\Sigma\frac{1}{y^2}\right) \quad (2)$$

Where n is the measurement, S/N is signal-to-noise ratio and y is the yield of PFP.

Table 1. Factors and levels for the Taguchi experimental design (A-D are the respective codes for each factor).

Level	[A] ultrasonic power (W)	[B] extraction time (min)	[C] extraction temperature (°C)	[D] liquid-solid ratio (mL/g)
1	300	20	50	15
2	400	30	60	20
3	500	40	70	25

Table 2. The results of Taguchi test L27 (3^4).

Test	A	B	C	D	Extraction yield (mg/g)	Means (mg/g)	S/N ratio
1	300	20	50	15	41.08	41.27	32.31
2	300	20	50	15	40.71	*	*
3	300	20	50	15	42.09	*	*
4	300	30	60	20	44.54	45.10	33.08
5	300	30	60	20	45.47	*	*
6	300	30	60	20	45.31	*	*
7	300	40	70	25	52.30	51.80	34.29

Table 2. Continued.

Test	A	B	C	D	Extraction yield (mg/g)	Means (mg/g)	S/N ratio
8	300	40	70	25	51.62	*	*
9	300	40	70	25	51.48	*	*
10	400	20	60	25	50.20	50.97	34.14
11	400	20	60	25	50.78	*	*
12	400	20	60	25	51.90	*	*
13	400	30	70	15	47.42	48.00	33.62
14	400	30	70	15	47.57	*	*
15	400	30	70	15	49.03	*	*
16	400	40	50	20	53.58	53.70	34.59
17	400	40	50	20	55.13	*	*
18	400	40	50	20	52.41	*	*
19	500	20	70	20	48.86	48.03	33.63
20	500	20	70	20	46.89	*	*
21	500	20	70	20	48.34	*	*
22	500	30	50	25	52.88	54.27	34.69
23	500	30	50	25	55.08	*	*
24	500	30	50	25	54.83	*	*
25	500	40	60	15	34.97	33.80	30.58
26	500	40	60	15	33.12	*	*
27	500	40	60	15	34.33	*	*

2.5 Optimization of Cycle

Extraction cycle is directly correlated with the extraction yield, work efficiency and energy cost [23]. Under the optimized process, 2.00 g of dried sample was extracted by deionized water in the extraction cell. Then the residual sample was separated from extraction solution by centrifugalization, which was employed to extract another baths of the dried sample. Five batches of samples were treated according to the optimized conditions. Therefore, the effects of extraction cycles (1-5) on extraction yield of PFP were tested.

2.6 *In vivo* Antioxidant Properties of PFP

2.6.1 Animals

Thirty BALB/c mice (20 ± 2 g weight, 8 weeks old) of both sexes (bisexual each half) were procured from the Laboratory Animal Center of Heilongjiang University of Traditional Chinese Medicine (Harbin, China). They were housed at ambient temperature of 25 ± 2 °C in polypropylene cages on 12 hr light-dark cycle at a humidity level of 50 - 60%. All mice were given water and feed *ad libitum* thought the experiment. Mice were adapted to housing conditions for a week before experiments. The trial was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), and was permitted by the Institutional Animal Ethical Committee of Northeast Agricultural University (No. SRM - 08).

2.6.2 *In vivo* antioxidant properties

The mice were randomly divided into five groups (six mice per group): normal

control group (NCG), and three PFP treatment groups. Mice in NCG were fed with 1% CMC (10 mL/kg bw/day, p.o.). by gavage. Mice in PFP extract treatment groups were respectively fed with PFP extract in three different doses (300, 600 and 1200 mg/kg body weight/day, p.o.) by gavage. The different doses were administered once daily for 30 consecutive days. Twenty four hours after the last dose of CCl_4, mice were sacrificed humanely by cervical dislocation. Blood samples were centrifuged at 4000 rpm for 10 min and the serums were collected. The livers were dissected out from mice, washed carefully and homogenized. In order to remove cellular debris, the homogenate was centrifuged again, and the supernatant was collected for analysis.

Glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) and malonaldehyde (MDA) were measured in serums and liver homogenates according to the instructions of kits purchased from Nanjing Jiancheng Bioengineering Institute. Briefly, GSH-Px activity was measured on the basis of the reaction of GSH and 5, 5 -dithio-bis-(2-nitrobenzoic acid) [24]. SOD activity was measured based on the inhibition of hydroxylamine oxidation by the superoxide radicals generated in the xanthine-xanthine oxidase system. The data obtained were expressed as units per milliliter (U/ml) in serum or units per milligram of protein (U/mg protein) in liver [25]. The activity of CAT was tested on the basis of the rate of H_2O_2 reduction [26]. The MDA levels were determined in serum samples according to the method described [27]. The principle of the assay is based on the reaction of MDA with thiobarbituric acid (TBA) and forming a pink colored compound with a maximum absorbance at 532 nm. The data obtained were expressed as nmole per milliliter (nmoL/mL) in serum or nmole per milligram of protein (nmoL/mg protein) in liver. The protein content in the liver supernatants was determined by the Lowry method using bovine serum albumin as the standard [28].

2.7 Statistical Analysis

All the experiments values were expressed as means ± SD of three parallel measurements and the data of Taguchi were analyzed using Minitab 16 software. Fischer's test was employed to determine the type of mode equation followed by Tukey test. Statistical analysis of the experimental data in the antioxidant test was performed using ANOVA (one-way analysis of variance) followed by the Tukey test.

3. RESULTS AND DISCUSSION

3.1 Effects of Operation Parameters of UAE and CSE on the Yield

3.1.1 Effects of ultrasonic power on the yield of polysaccharides

To determine the central point of ultrasonic power, different ultrasonic power (100, 200, 300, 400, 500 and 600 W) were prepared when other experimental parameters were set as follows: extraction temperature 60 °C, solvent to solid ratio 15 mL/g, and extraction time 30 min.

It can be seen from the Figure 1A that the extraction yield of PFP increased significantly with increasing extraction power from 100 W to 400 W, and then decreased dramatically when the extraction power was over 400 W. An explanation for this was that the larger the amplitude of ultrasound wave traveling through a mass medium, the more violently the bubbles collapse [29]. However, after the ultrasonic intensity reached a certain value, the increase in the density of bubbles undergoing

cavitation near the radiating surface that would hamper the transmission of the ultrasonic energy into the liquid [30]. This may explain why the extraction yield of PFP raised in advance then fell with the increase of ultrasonic power. The remarkable differences of yield ($p < 0.05$) were found among these ultrasonic powers with the value of 400 W versus others. Thus, 400 W was used for the following experiments.

3.1.2 Effects of extraction time on the yield of polysaccharides

Extraction time is associated with the final concentration of polysaccharides, the efficiency of extraction and the energy cost. To determine the central point of ultrasonic time, different extraction time (10, 20, 30, 40, 50 and 60 min) were prepared when other experimental parameters were set as follows: ultrasonic power 400 W, extraction temperature 60 °C and solvent to solid ratio 15 mL/g. The control experiments of CSE were also performed in different time (30, 60, 90, 120, 150 and 180 min) at the 90 °C.

Using the two extraction methods, the highest extractive rate of PFP was given when the extraction time was 60 or 150 min, respectively (Figure 1B). The extraction yield of PFP given by CSE method increased significantly with increasing extraction time from 30 min to 180 min, and then decreased dramatically when the extraction time was over 180 min, for the structures of PFP were destroyed when the extraction time was over 150 min. Although the extraction yield of PFP was insignificantly improved compared with CSE, UAE was found to save the extraction time. This behaviour has been explained by the cavitational effects. For one thing, cavitational effects caused the intensification of mass transfer and thus closed interaction between the solvent and the plant tissues. For another, the collapse of cavitation bubbles near tissue surfaces produces micro-jets, causing tissue disruption and a good penetration of the solvent into the tissue matrix [31]. However, there is an insignificant increase ($p > 0.05$) in the PFP yield with the extraction time from 30 min to 60 min. Other similar research was reported by Zhang et al. that the optimal ultrasound-assisted extraction of polysaccharides from *Ginkgo biloba* leaves was 40 min in the single-factor experiment [22]. Take work efficiency into account, 30 min was adopted as central point of the parameter.

3.1.3 The effect of extraction temperature on the polysaccharides yield

Figure 1C showed the effect of extraction temperature on the yield of PFP. By UAE, the extraction yield of PFP displayed a positive linear increase ($p < 0.05$) when the temperature increased from 40 to 60 °C, and then it declined gradually ($p > 0.05$) after the temperature was higher than 60 °C. An explanation for this was that temperature affected many physical properties such as acoustic cavitation, diffusion, vapor pressure and surface tension. Increase of temperature enhanced diffusion through the cell walls and acoustic cavitation. However, excessive temperature caused the increase of vapour pressure and the decrease within micro bubbles, leading to the damping of the ultrasonic wave [22]. Thus, the extraction yield of PFP firstly increased and then decreased with the increase of extraction temperature. According to the results, 60 °C was adopted as the central point of the extraction temperature.

The disadvantages of the CSE method can be seen by comparison with the results from UAE. CSE get the same extraction

yield compared with UAE, however, the energy consumption of the former increased significantly. On the other hand, long extraction time excessively at high temperature may induce the degradation of polysaccharides and therefore lead to the change of activities [32].

3.1.4 The effect of solvent to solid ratio on the polysaccharides yield

The effects of solvent to solid ratio on the release of polysaccharides by the two methods were shown in Figure 1D. With the UAE method, the extraction yield of PFP increased significantly ($p < 0.05$) when the solvent to solid ratio ranged from 5 mL/g to 20 mL/g, and after that it was gradually trending down ($p > 0.05$). And here is the reason: the concentration difference between the solvent and the interior plant cells was enlarged with the increase in solvent volume, and then the polysaccharides are easier to diffuse quickly [33]. However, the too much extraction solvent can restrain the cavitation effect and generation of aerosol [34]. To avoid the wasting consumption of solvents and bulky handling in the subsequent processes, 15-25 mL/g was used for Taguchi experimental design.

Figure 1D showed that extraction yield of PFP increased significantly ($p < 0.05$) when the solvent to solid ratio is ranged from 5 to 15 mL/g. However, the extraction yield of PFP increased insignificantly ($p > 0.05$) if the solvent to solid ratio increased from 15 to 30 mL/g.

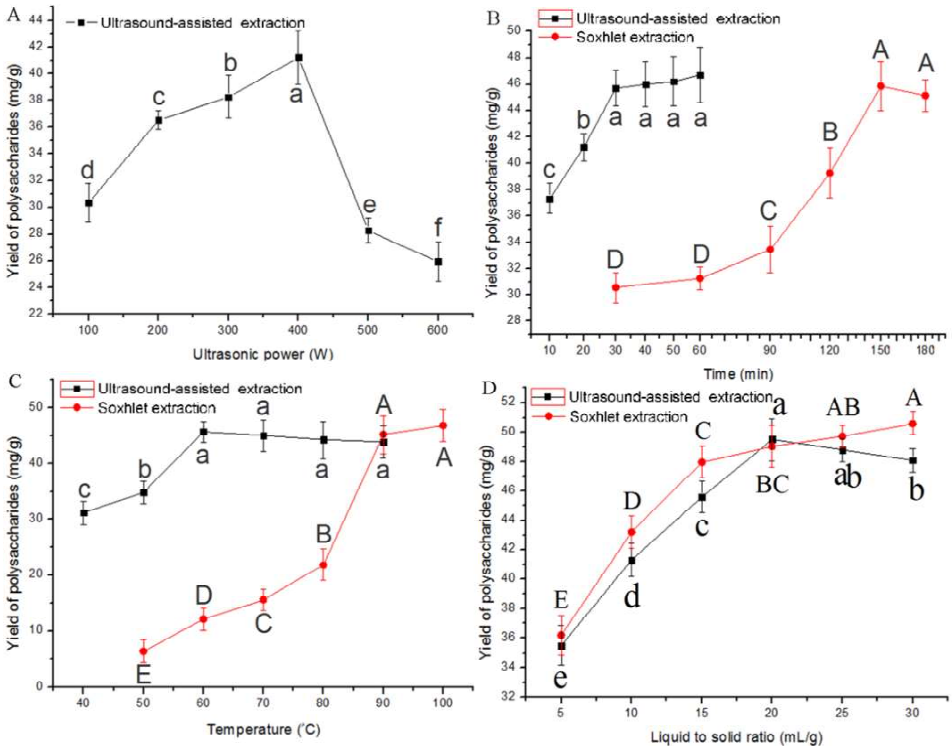

Figure 1. Effects of different extraction parameters on the yield of PFP (ultrasonic power, W; extraction time, min; extraction temperature, C; solvent to solid ratio, mL/g). Values were expressed as mean ± SD (n = 3), and evaluated by one-way AVONA followed by the Tukey test. Different letter and same letter were considered to be statistically significant ($p < 0.05$) and statistically insignificant ($p > 0.05$), respectively.

3.2 Model Building and Statistical Significance Test

Based on the preliminary experiments above, the UAE technique was shown to be very efficient in the extraction of PFP compared with CSE. So, the operational parameters of UAE were further optimized using Taguchi design method. Table 2 showed the experimental design, corresponding response data and signal-to-noise (S/N). The S/N responses were used to evaluate the effects of each factor on S/N and were shown in Table 3. The relative difference showed that liquid to solid ratio (D) has the most important influence on the yield of PFP, followed by temperature (C), ultrasonic power (A) and time (B) in decreasing order. The optimal extraction condition for PFP was $A_2B_2C_1D_3$ (ultrasonic power = 400 W, time = 30 min, temperature = 50 °C and liquid to solid ratio = 25 mL/g). The effects of the different levels of the four parameters on the yield of PFP can also be visualized in Figure 2.

Table 3. S/N response table.

Levels	A	B	C	D
1	33.23	33.36	33.86	32.17
2	34.12	33.80	32.60	33.77
3	32.96	33.15	33.84	34.37
Delta	1.16	0.65	1.26	2.20
Effect	3	4	2	1

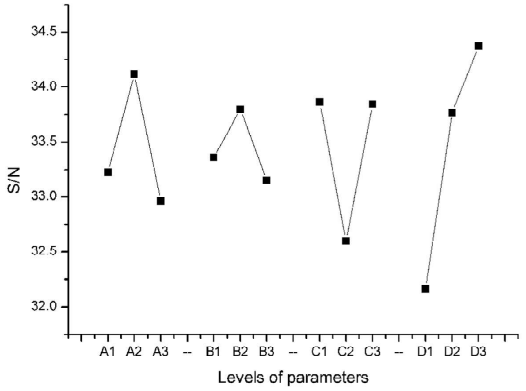

Figure 2. Effect of different levels of L_{27} orthogonal array (Taguchi experimental design) for each parameter on the yield of PFP.

The optimal values of the selected variables were obtained using Minitab 16 software. The predicted value of S/N is 35.8 and the predicted yield is 59.8 mg/g. To validate the predicted result, the validation experiments were repeated three independent replicates under the optimal conditions. The yield was 61.9 ± 2.5 mg/g, which agree with the predicted value.

Statistical analysis of variance (ANOVA) was use to evaluate the effects of the process parameters on extraction yield of PFP. As seen in Table 4, all the parameters are statistically significant for the extraction of PFP (p <0.01). Furthermore, it also showed that the factor which made the greatest contribution to the extraction yield of PFP was liquid to solid ratio (57.49%), followed by temperature (22.07%), ultrasonic power (15.42%) and finally time (3.67%).

3.3 Effect of Extraction Cycle on Extraction Yield of PFP by UAE

Generally, it is difficult to take PFP out of the sample completely by one extraction cycle. As it can be seen in Figure 3, there is a significant increase (p < 0.05) in the PFP

yield (from 61.9 mg/g to 75.8 mg/g) with the extraction cycle from 1 to 2, and then the yield remained constant, indicating that the polysaccharides in *Flos Populi* power was exhausted. Therefore, in order to make better use of the solvent, extraction number 2 was adopted.

Table 4. ANOVA for extraction yield.

Source	df	Seq SS	Adj MS	*F*	*P*	*P* (%)
A	2	162.99	81.50	103.11	< 0.01**	15.42
B	2	38.81	19.40	24.55	< 0.01**	3.67
C	2	233.28	116.64	147.57	< 0.01**	22.07
D	2	607.54	303.77	384.34	< 0.01**	57.49
Error	18	14.23	0.79			
Total of source	26	1056.84				

Different were considered to be statistically significant if $p < 0.05$.

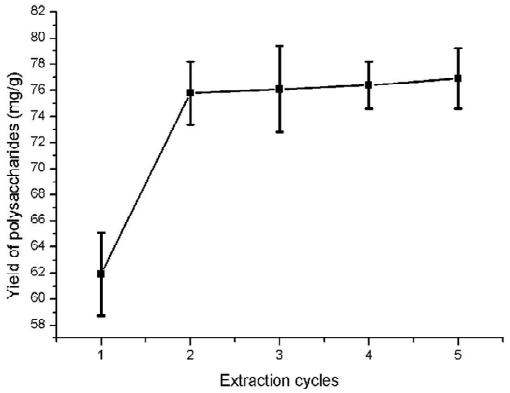

Figure 3. Effect of extraction cycles on the yield of PFP.

3.4 Antioxidant Activity of PFP *In vivo*

In vitro antioxidant assays often used for the evaluation of antioxidant capability of natural polysaccharides, however, it has been noticed that the *in vitro* results often could not agree with *in vivo* findings due to bioavailability. Generally, some adverse factors, such as short half-life in plasma, low absorption in organism, and its extensive metabolism in liver reduce the capability of dietary polysaccharides *in vivo*. To evaluate the prospect of PFP in future production and practice, measurement *in vivo* antioxidant activity potential of PFP is incredibly necessary.

SOD, GSH-Px and CAT are referred as primary antioxidant enzymes which act mutually to exert a major part in preventing cells against reactive oxygen species (ROS) [2]. Generally, SOD can transform superoxide radicals into hydrogen peroxide by catalytic reaction. Hydrogen peroxide is harmful to the body as it has active oxygen, while GSH-Px and CAT detoxifies hydrogen peroxide into water and oxygen. To assess the *in vivo* antioxidant potential of PFP, the activities of antioxidant enzymes (SOD, GSH-Px and CAT) were examined due to its involvement in detoxification [2]. *In vivo* antioxidant potential of PFP was studied in mice under elevated oxidative stress. Moreover, the increasing doses of 300, 600 and 1200 mg/kg body weight, p.o. were used to assess the dose-depended response of PFP. Effects of PFP on the activities of SOD, CAT and GSH-Px in serums and livers mice are shown in Tables 5. A significant decrease in the activities of enzyme was observed in CCl_4 treated mouse when compared with control ($p < 0.01$). Administration of PFP to CCl_4 treated mouse with 600 and 1200 mg/kg significantly increased the activities of these

antioxidant enzymes (SOD, GSH-Px and CAT) both in serums and liver ($p < 0.05$). Especially the levels of SOD in serums and livers, and GSH-Px in serums returned to normal after administration of PFP to CCl_4 treated mice. SOD, CAT and GSH-Px enzymes activities also enhanced (statistically not significant) in 300 mg/kg treated groups ($p > 0.05$). Yu et al. found a similar result that mice given *Angelica sinensis* polysaccharides (150-300 mg/kg) obtained a significant increase of the main enzymatic antioxidant defense (SOD, GSH-Px and CAT) compared with CCl_4-treated mice [35].

In addition, MDA is a key by-product of lipid peroxidation leading to oxidative stress. So the amount of MDA was calculated to determine lipid peroxidation level [36]. In this study, effects of PFP on the levels of MDA in serums and livers of mice are also observed in Table 5. A significant increase in the amount of MDA was observed in CCl_4 treated mouse when compared with control ($p < 0.01$). Although MDA level decreased dose-dependently, the differences were not statistically significant for 300 mg/kg and 600 mg/kg PFP compared with the MDA level in CCl_4 treated group ($p > 0.05$). Significant decrease ($p < 0.01$) was seen in the doses of 1200 mg/kg for PFP group. The *in-vivo* results indicated that PFP had the capacity to inhibit the peroxidation of lipids in a dose-dependent manner. A similar report was found that polysaccharides from *Hyriopsis cumingii* inhibited significantly the formation of malondialdehyde in mice livers and serums and raised the activities of antioxidant enzymes and total antioxidant capacity in a dose-dependent manner (200-800 mg/kg) [37].

Table 5. Effects of PFP on the activities of SOD, CAT, GSH-Px and CAT in serums and livers mice.

Treatment	serous				Liver			
	SOD (U/mL)	GSH-Px (U/mL)	MDA (nmol/mL)	CAT (U/mL)	SOD (U/mL)	GSH-Px (U/mL)	MDA (nmol/mL)	CAT (U/mL)
Control (1% CMC, 0.5 mL/kg)	87.08±5.08	90.53±7.30	3.50±0.32	84.77±6.90	216.44±18.82	130.71±8.77	5.28±0.64	223.16±10.71
CCl_4 treated (0.5 mL/kg)	56.01±6.52$^{\#\#}$	56.58±4.97$^{\#\#}$	9.03±0.65$^{\#\#}$	51.73±3.14$^{\#\#}$	159.67±8.85$^{\#\#}$	77.97±8.95$^{\#\#}$	13.88±1.60$^{\#\#}$	150.61±11.79$^{\#\#}$
Extract (300 mL/kg)+CCl_4 treated	60.53±3.16$^{\#\#}$	67.12±3.32$^{\#\#}$	7.82±0.89$^{\#\#}$	61.15±5.07$^{\#\#}$	173.30±7.61$^{\#}$	87.18±10.25$^{\#\#}$	11.42±1.14$^{\#\#}$	159.52±6.52$^{\#\#}$
Extract (600 mL/kg)+CCl_4 treated	73.35±7.15*	78.87±4.06**	7.39±0.85$^{\#\#}$	65.81±2.94$^{**\#}$	177.07±5.41*	91.50±6.43$^{\#\#}$	10.42±1.18$^{*\#\#}$	161.41±9.11$^{\#\#}$
Extract (1200 mL/kg)+CCl_4 treated	79.02±2.64**	82.19±3.36**	6.45±0.73$^{\#}$	69.84±4.85$^{**\#}$	184.76±8.88*	103.48±9.01**	8.16±0.94**	175.27±6.76$^{*\#\#}$

Values were expressed as mean ± SD (n = 6), and evaluated by one-way ANONA. Different were considered to be statistically significant if $p < 0.05$.

$^{*}p < 0.05$ compared to CCl_4 treated group; $^{**}p < 0.05$ compared to CCl_4 treated group;

$^{\#}p < 0.05$ compared to Control group; $^{\#\#}p < 0.01$ compared to Control group.

4. CONCLUSION

Taguchi design was employed to determine the optimal parameters for UAE of PFP in the present study. A maximum yield of 75.8 mg/g was obtained with the following conditions: ultrasonic power of 400 W, extraction time of 30 min, extraction temperature of 50 °C, liquid to solid ratio of 25 mL/g, and extraction cycles of 2. Compared with CSE, UAE was better to extract PFP. The method showed less extraction time and lower temperature, although the yield of polysaccharides was similar to the yield of polysaccharides by CSE. Additionally, the antioxidant activities of PFP *in vivo* including SOD, GSH-Px, CAT and MDA in serums and livers of mice were evaluated. Based on the results, it is revealed that the PFP possess potent antioxidant activity and could be useful for the development of antioxidant agents.

AUTHOR CONTRIBUTIONS

Ying Zhang carried out most of the studies. Jiaxuan Li, Zheng Jia, Jianqing Chen participated in the animal experiments. Chunbo Gao revised the language of manuscript. Zunlai Sheng designed the study and wrote the manuscript. All authors have read and approved the final version.

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