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Effects of Different Deproteinization Methods on the Antioxidant Activity of Polysaccharides from *Flos Sophorae Immaturus* Obtained by Ultrasonic Microwave Synergistic Extraction

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Abstract: *Flos Sophorae Immaturus* (FSI) serves as one of the Chinese medicinal herbs of homologous provenance, whose polysaccharides constitute part of the active compounds that exert their pharmacological properties. Single-factor and response surface methodology were employed to investigate optimal extraction conditions for the ultrasonic-microwave synergistic extraction (UMSE) of polysaccharides from FSI (PFSI), which were deproteinized by Sevage, papain, and trichloroacetic acid methods, and the antioxidant potential of PFSI by contrasting deproteinization methods based on free-radical scavenging capacity. The optimum conditions for UMSE extraction of PFSI were 500 W microwave power, 265.887 W ultrasonic power, 20.078 min extraction time, and 94.995:1 liquid-to-material ratio. Meanwhile, the sequence of the single factors on the yield of polysaccharides indicated that microwave power > extraction time > D liquid to material ratio > B ultrasonic power, and that the obtained average value of polysaccharide yield was 37.05%, which was analogous to the predicted value of 37.17%, indicating that the optimization method was reasonable. In vitro, the antioxidant assay demonstrated that PFSI, with or without deproteinization, had a definite capability to scavenge oxidative free radicals. This research provides a theoretical basis for the industrial production of PFSI as a natural antioxidant, and a scientific basis for its industrial development.

Keywords: FSI; polysaccharide; RSM; deproteinized; free-radical scavenging capacity; antioxidant

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1. Introduction

The international academic community has spoken of the 21st century as the 'century of polysaccharides'. Scientifically experimental studies have proved that many plant polysaccharides have biological activities, including immunomodulation, anti-tumor, hypoglycemia, hypolipidaemia, anti-radiation, anti-bacterial, anti-viral, liver protection, etc. [1–3]. This is why plant polysaccharides have long been used widely in the fields of medicine, catering, and everyday life. They are compounds composed of many identical or different monosaccharides with α or β glycosidic bonds and are universally found in plants, including starch, cellulose, polysaccharides, and pectin [4]. The traditional extraction methods are based on solvent extraction and acid-base extraction, but the yield of traditional extraction methods remains low and they can destroy the structure of the active ingredients, which is why the search for a green, low-cost, and high-yield extraction method is an important way to industrialize polysaccharides [5–7]. The prevalent extraction methods for plant polysaccharides are primarily divided into traditional and emerging extraction methods. The ultrasonic-microwave synergistic extraction (UMSE) method [8–10] has been developed as a new technology relying on ultrasonic-assisted and microwave-assisted methods. These have the characteristics of short extraction time, speed, and high efficiency.

Flos Sophorae Immaturus (FSI) generally refers to the dried buds and flowers of *Sophora japonica* L. It is ovate or elliptical in shape, with yellowish-white unopened petals in the upper part of the calyx and several longitudinal stripes on the underside of the calyx. In Chinese medicine, the flowers of *Sophora japonica* frequently feature in the same way as the buds, with FSI being the buds harvested in summer when the flowers are still open, and *Sophora japonica* L. being the buds harvested when the flowers are blooming. First, it is applied to blood in the stool, hemorrhoids, bloody dysentery, collapse, vomiting, epistaxis, liver fever, dizziness, and headache [11,12]. Past studies have suggested that the main components of FSI include flavonoids [13], saponins [14], fatty acids [15], polysaccharides [16], volatile components [17], and other ingredients whose active ingredients have been proven to have definite pharmacological functions [18]. Polysaccharides contribute to the pharmacological effects of FSI, such as hypoglycemia and hypotension, antioxidant and anti-aging, antibacterial and antimicrobial, anti-tumor, and other pharmacological agents. At present, the extraction methods of FSI polysaccharides (PFSI) focus on the hot leaching method, hot water reflux extraction method, ultrasonic extraction method, and complex enzyme extraction method [16]. However, due to the shortcomings of PFSI extraction by traditional extraction methods, such as hot water leaching and ultrasonic extraction, the efficiency of PFSI extraction has been inefficient, with long extraction times and the destruction of active ingredients.

The widely recognized free radical theory of ageing (FRTA) proposes that the accumulation of oxidative damage triggered by reactive oxygen species (ROS) occurs as a principal consequence of aging [19–21]. According to this theory, oxygen-derived free radicals indirectly cause age-related impairments through oxidative damage to biomolecules, with mitochondria being the prime subject of free radical assaults. Recently, during the current campaign to identify new natural antioxidants, various polysaccharides obtained from plants, algae, and microorganisms have been documented to exhibit potent antioxidant properties and promising applications as antioxidants of choice [22–26]. Therefore, protection of that system provided through dietary antioxidant supplementation probably features an appreciable amount in the attainment of healthy aging [27]. It has been shown that the separation and purification of polysaccharides can increase their bioactivity to some extent [28–31]. Hence, in this study, different deproteinization methods of PFSI were isolated and purified separately to screen the process for the best deproteinization method using antioxidant activity (free radical scavenging capacity) as an indicator. The purpose is to establish a theoretical basis for the industrial production of PFSI in such a way as to qualify as an antioxidant in the future.

2. Materials and Methods

2.1. Materials

FSI harvested in August 2021 was authenticated by Zhang Runrong, senior engineer of traditional Chinese medicine, College of Pharmacy and Food Science, Zhuhai College of Science and Technology, Zhuhai, Guangdong Province, China (Production batch: 20210820; Shandong Bozhou R&B Food Sales Co.; Shandong, China); papain (800 U/mg) and Vitamin C (V_C) (Shanghai Yuanye Biotechnology Co., Ltd.; Shanghai, China); phenol, sulphuric acid, anhydrous ethanol, trichloromethane, trichloroacetic acid (TCA), and n-butanol (AR, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China).

2.2. The Process Investigation of the UMSE Method to Obtain PFSI

The rootless and leafless FSIs were separated and degreased overnight by soaking in an 80% ethanol solution. Crushed FSI material was sifted through 40, 60, 80, 100, and 120 mesh sieves, dried at 60 °C and dispensed for consumption. We weighed 0.5 g of pretreated FSI powder in a 50 mL centrifuge tube, added distilled water (temperature: 95 ± 5 °C) as the extraction solution, mixed it with deionized water at a certain ratio, and extracted PFSI by the UMSE method (setting parameter: temperature 100 °C). We centrifuged it at 8000 r/min for 5 min, kept the supernatant, cooled it at room temperature and centrifuged for 10 min

(8000 rpm/min), then transferred the supernatant. Samples were diluted and assayed for the concentration of total saccharides recovered. In this experiment, the PFSI content was determined by the phenol–sulphuric acid approach. It was calculated to retrieve the total saccharide extraction percentage as presented in Equation (1), where Y is the yield of polysaccharide (%), V is the total volume of sample solution (mL), C is the concentration of polysaccharide (mg/mL), D is the dilution factor, and m is the mass of the weighed sample (g).

$$Y\ (\%) = \frac{V \times C \times D}{m \times 10^3} \times 100 \quad (1)$$

The concentrates were deproteinized by three different methods: Sevage reagent 5 times, 80 U/mL papain, and 2.5% TCA. The obtained deproteinized polysaccharide solution was kept in a 3000 Da dialysis bag and permeated under running water for 48 h. The dialyzed polysaccharide resolution gained by centrifugation at 6000 rpm/min for 10 min, with supernatant added to 4 times the volume of 95% ethanol and stored overnight. After discarding the supernatant, the solution containing the precipitate was centrifuged at 6000 rpm for 5 min, then condensed and desiccated under reduced pressure. Finally, the precipitate was purified using a cotton DEAE-52 cellulose column. PFSI-2, PFSI-3, and PFSI-4 was granted after purification, respectively. PFSI-1 was purified in exactly the same way, except for the subsequent isolation without deproteinization. Extraction, isolation, and purification procedures for PFSI-1, PFSI-2, PFSI-3, and PFSI-4 are illustrated in Figure 1.

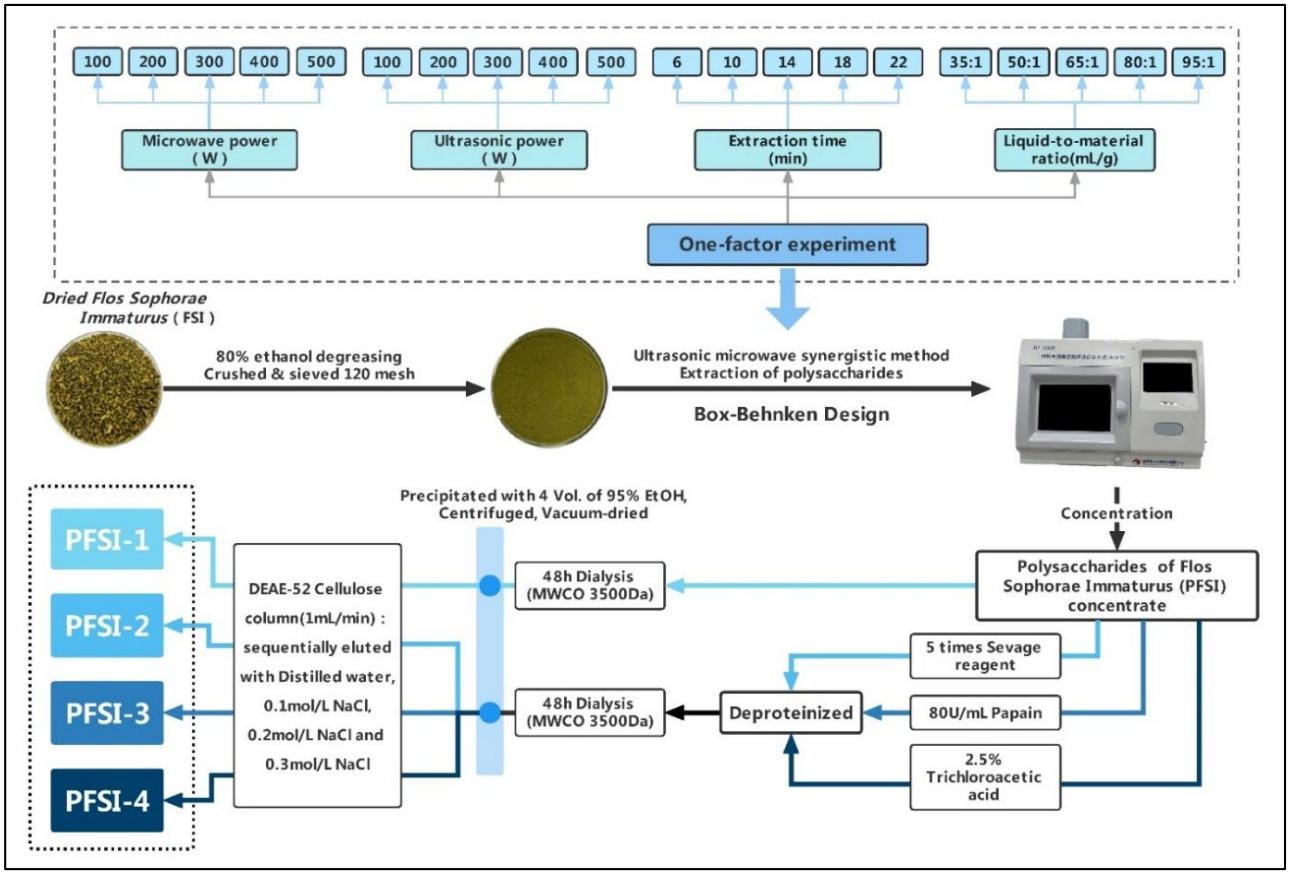

Figure 1. The procedure for extracting and purifying PFSI-1, PFSI-2, PFSI-3, and PFSI-4.

2.3. One-Factor Experiment for Extraction

2.3.1. Effect of Microwave Power on Extraction

We weighed 60 mesh FSI powder 0.50 g in 5 portions; distilled water was used as a solvent; the ratio of material to liquid was 50:1; ultrasonic power was 200 W; with an extraction time of 10 min, followed by extraction at a microwave power of 100 W, 200 W,

300 W, 400 W, 500 W, and 600 W, respectively. Each experiment was repeated three times and the total sugar content was determined by the phenol–sulphuric acid method, which was repeated three times as an average to calculate the percentage of polysaccharide extraction.

2.3.2. Effect of Ultrasonic Power on Extraction

The process was the same as in Section 2.3.1 above, except that the extraction was carried out at ultrasonic power of 100 W, 200 W, 300 W, 400 W, and 500 W.

2.3.3. Effect of Particle Diameter on Extraction

The process was the same as in Section 2.3.1 above, excluding the weighing of screened at 40, 60, 80, 100, and 120 mesh FSI.

2.3.4. Effect of Liquid-to-Material Ratio on Extraction

The process was the same as in Section 2.3.1 above, but with liquid-to-material ratios of 35:1, 50:1, 65:1, 80:1, and 95:1.

2.3.5. Effect of Time on Extraction

The process was the same as in Section 2.3.1 above, but with supplementary extraction times of 6, 10, 14, 18, and 22 min.

2.4. Design of RSM

Based on the findings of the single-factor experiments, the microwave power, ultrasonic power, liquid-to-material ratio, powder particle size, and extraction time were determined as process parameters. The ultimate four-factor, three-level (microwave power, ultrasonic power, extraction time, and liquid-to-material ratio) experimental design of the single-factor experiments was selected in accordance with the limitations of the powder pulverization process to evaluate the polysaccharide extraction yields as the indicators, utilizing Box-Behnken's central combinatorial experimental design principle. The factors affecting the polysaccharide yield were evaluated in terms of their main effects, mutual effects, and quadratic effects.

A multivariate regression equation was fitted to the empirical data with complete quadratic polynomial equations to assess the functional relationships attained between the polysaccharide yields and the individual experimental factors, as shown in Equation (2), where Y is the predicted response value for polysaccharide extraction rate; A_0 is a constant; A_i is a linear coefficient; A_{ii} is a quadratic coefficient; A_{ij} is an interaction regression coefficient; and X and X_i are independent variables.

$$Y = A_0 + \sum_{i=1}^{3} A_{ii} X_i X_i + \sum_{i=1}^{3} \sum_{j=i+1}^{3} A_{ii} X_i X \quad (2)$$

2.5. In Vitro Antioxidant Effect of PFSI

2.5.1. Measurement of DPPH· Radical Scavenging Rate

Sample reaction group: The sample solutions of PFSI-1, PFSI-2, PFSI-3 and PFSI-4 with concentration gradients of 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 8.0 mg/mL were prepared in a 5 mL centrifuge tube and mixed thoroughly with 0.2 mmol DPPH in anhydrous ethanol. The sample solutions were mixed thoroughly in the same volume of anhydrous ethanol solution with 0.2 mmol DPPH in a 5 mL centrifuge tube, and then centrifuged at 6000 rpm/min for 5 min and operated three times in parallel. The supernatant was taken at 517 nm and the absorbance value was measured as A_s. Sample control group: an identical volume of sample solution as the DPPH solution, otherwise operated as in the sample reaction group, with an absorbance of A_{sc}.

Blank reaction group: Evaporated water volume is the equivalent of DPPH solution, other operations as in the sample reaction group, absorbance is A_b. Blank control group: distilled water volume in the same volume as anhydrous ethanol; in other operations as in the reaction group; the absorbance value was A_{bc}.

The same product reaction group of V_C standard solution and the sample control group were operating as a control experiment. Clearance was determined according to Equation (3).

$$Free\ radical\ scavenging\ rate\ (\%) = 1\ \frac{A_s - A_{sc}}{A_b - A_{bc}} \times 100 \quad (3)$$

2.5.2. Measurement of ·OH Radical Scavenging Rate

Sample reaction group: PFSI-1, PFSI-2, PFSI-3 and PFSI-4 were prepared in a 5 mL centrifuge tube with a concentration gradient of 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 8.0 mg/mL, and then 9.0 mmol/L $FeSO_4$ solution, 9.0 mmol/L salicylic acid-ethanol solution and 0.50 mL of 9.0 mmol/L H_2O_2 solution were added to start the reaction. The absorbance was measured at 510 nm as A_s. Sample control group: 0.50 mL of each sample solution was added with 0.50 mL of $FeSO_4$, 0.50 mL of salicylic acid-ethanol solution, and 0.50 mL of H_2O_2 solution, and other operations were performed as in the sample reaction group, and the absorbance value was A_{sc}.

Blank reaction group: 0.50 mL distilled water plus 0.50 mL $FeSO_4$, 0.50 mL salicylic acid-ethanol solution, and 0.50 mL H_2O_2 solution; other operations as for the sample reaction group, absorbance value A_b. Blank control group: 0.50 mL distilled water plus 1.00 mL distilled water and 0.50 mL anhydrous ethanol, otherwise operated as in the sample reaction group, absorbance value A_{bc}. The control group was operated as in Section 2.5.1 above, and the formula was calculated as in Equation (3).

2.5.3. Measurement of ABTS· Radical Scavenging Rate

Sample reaction group: 7.0 mmol/mL of ABTS solution and 2.45 mmol/mL of potassium persulphate solution were mixed and placed in a 100 mL brown flask at room temperature and protected from light overnight to form the ABTS stock solution. Dilute 10.0 mL of ABTS stock solution with phosphate buffer (10 mmol/L, pH = 7.4) until it reaches an absorbance of 0.7 ± 0.02 at $OD_{734\ nm}$. A concentration gradient of 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 8.0 mg/mL of PFSI-1, PFSI-2, PFSI-3 and PFSI-4 was prepared and 0.5 mL of sample solution and 1.50 mL of ABTS working solution was added to each of the 5 mL centrifuge tubes. ABTS working solution and the reaction was carried out at room temperature for 10 min and the absorbance value was measured as A_s. Sample control group: 1.50 mL of potassium persulphate solution was added to each of the different concentrations of the sample solutions, and the other operations were performed as in the sample reaction group, with the absorbance value as A_{sc}.

Blank reaction group: 0.50 mL distilled water plus 1.50 mL ABTS working solution, other operations as for the sample reaction group, absorbance value as A_b. Blank control group: 0.50 mL distilled water plus 1.50 mL potassium persulphate solution, otherwise operated as for the sample reaction group, with an absorbance value of A_{bc}. The control group was operated as in Section 2.5.1 and the formula was calculated as in Equation (3).

2.5.4. Measurement of Ferric-Reducing Antioxidant Power

Sample reaction group: 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 8.0 mg/mL of PFSI-1, PFSI-2, PFSI-3 and PFSI-4 were placed into a 15 mL centrifuge tube, and 1.00 mL of phosphate buffer solution (0.2 mmol/L, pH = 6.6) and 1.00 mL of 1% potassium ferricyanide solution ($K_3Fe(CN)_6$ were sequentially charged. After mixing, the supernatant was centrifuged at 6000 rpm for 10 min. The supernatant was spiked with 3.5 mL of distilled water and 2.5 mL of 0.1% $FeCl_3$ solution, protected from light for 30 min, and the absorbance value of A_S was measured at 700 nm.

Positive control group: 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0 mg/mL of ascorbic acid standard solution, other operations as in the sample reaction group, absorbance value was A_C. Blank reaction group: 0.5 mL of distilled water was taken, other operations were as in the sample group, and the absorbance value was A_b. The ferric-

reducing antioxidant power (FRAP) was estimated by comparing the absorbance values of the sample reaction group ($A_S - A_b$) with those of the positive control group ($A_C - A_b$).

2.6. Statistical Analysis

Data are shown as mean ± SEM, and statistical analysis was performed using Origin 2021, Graphpad Prism 8, Design-Expert 12, and EXCEL 2019. To compare the differences among multiple groups, a one-way analysis of variance (ANOVA) was performed using this software.

3. Results

3.1. Analysis of One-Factor Experiment

3.1.1. Analysis of Microwave Power on Extraction

The influence of microwave power on the extraction ratio of PFSI is shown in Figure 2A. With the microwave power between 100 and 300 W, the concentration of PFSI extraction showed an upwards trend with the increase in microwave power; when the microwave power exceeded 300 W, the withdrawal ratio of PFSI showed a downwards trend with the elevated microwave power. Hence, microwave power of 300 W was considered the optimum extraction parameter.

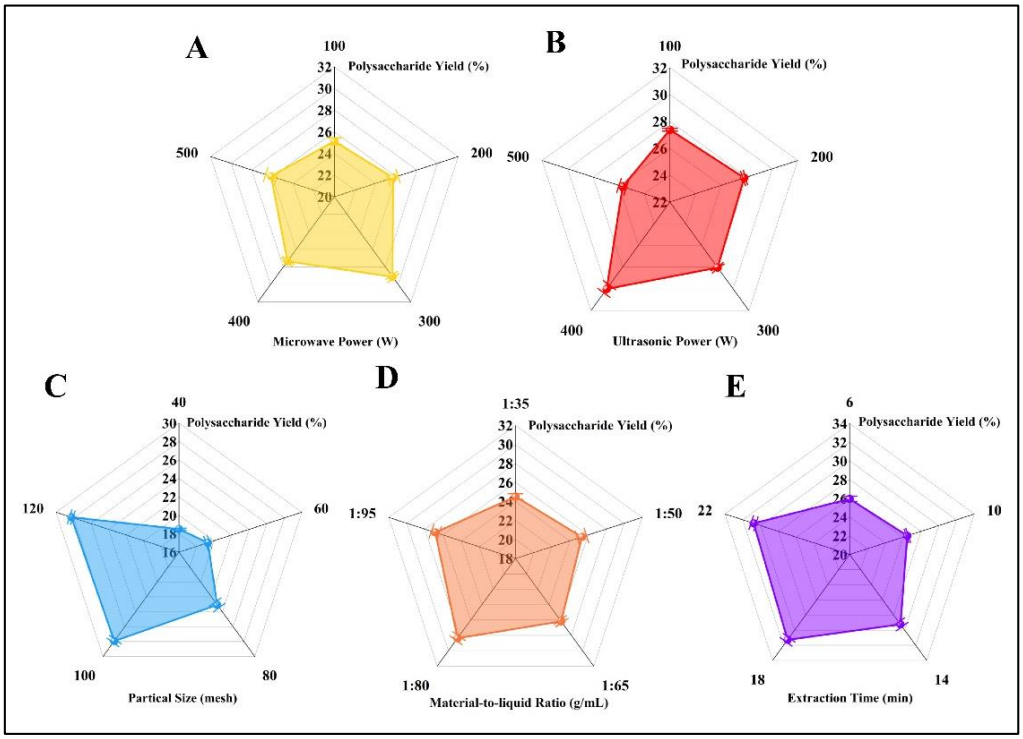

Figure 2. Effect of microwave power (**A**), ultrasonic power (**B**), particle diameter (**C**), liquid-to-material ratio (**D**), and extraction time (**E**) on the polysaccharide yield.

3.1.2. Analysis of Ultrasound Power on Extraction

Figure 2B shows the effect of ultrasound power on the extraction of PFSI. After reaching 400 W, the yield of polysaccharides reached their highest level, then decreased when the ultrasonic power was increased again. Consequently, the best extraction parameter was the ultrasonic power of 400 W.

3.1.3. Analysis of Particle Diameter on Extraction

Figure 2C demonstrates the impact of particle diameter sizes on the extraction rate of PFSI. The yield of polysaccharides rose with the increase in powder particle size, and the contact area of the solute surface area and solution expanded with the increase in particle

diameter, which was more conducive to the dissolution of polysaccharides. This process investigation considered the extraction optimization to be maximized, therefore a sieved 120 mesh FSI powder was selected and was not included in the validation of the four-factor and three-level experiments in the later RSM.

3.1.4. Analysis of Liquid-to-Material Ratio on Extraction

The remarkable effect of the liquid-to-material ratio on the yield of polysaccharides is illustrated in Figure 2D. The yield of polysaccharides increased with the increase in the liquid-to-material ratio from 35:1 to 95:1. Thereafter, the polysaccharide yield did not increase despite the liquid-to-material ratio being continually enhanced. The principal reason was that the leaching of polysaccharides was more favorable because the contact area between the solute and the solution was enlarged with that ratio, but at more than 1:80, the polysaccharides were already solubilized and fully leached out. For this reason, 1:80 was chosen as the optimum extraction parameter for polysaccharide extraction.

3.1.5. Analysis of Time on Extraction

Figure 2E reveals the effect of time of exploitation on the extraction of PFSI-1 at 300 W microwave power, 400 W ultrasonic power, and 95:1 liquid-to-material ratio, which demonstrated that the polysaccharide yield rose with increasing extraction time from 6 to 18 min. After 18 min of extraction, the amount of polysaccharide obtained did not obviously increase. Considering the extraction cost, the extraction time of 18 min was considered the best extraction parameter for polysaccharide extraction.

3.2. RSM Results and ANOVA

On the basis of the results of the single-factor assay, a four-factor, three-level experiment was selected, as shown in Table 1. The Box-Behnken central combination principle was implemented to devise the RSM employing polysaccharide yield as the response value (Table 2), and ANOVA and quadratic polynomial regression equations were fitted (Table 3). The data were calculated using Design expert 12 software to obtain the Y (polysaccharide yield) regression equation ($Y = -103.31 + 0.0233\ A + 0.2738\ B + 4.08\ C + 1.112\ D + 0.000015\ AB - 0.0022\ AC + 0.0013\ AD - 0.0015\ BC - 0.0012\ BD + 0.0013\ CD - 0.00009\ A^2 - 0.00026\ B^2 - 0.067\ C^2 - 0.0078\ D^2$).

Table 1. Design of factors levels for RSM. (A is the microwave power/(W), B is the ultrasonic power/(W), C is the extraction time/(min), and D is the liquid-to-material ratio/(g/mL)).

Level	A	B	C	D
−1	200	300	14	65:1
0	300	400	18	80:1
1	400	500	22	95:1

As can be seen from Table 3, the F value of 12.97, $p < 0.01$, reached a highly conspicuous level and the difference in the misfit term was $p = 0.1964 > 0.05$, which was not prominent, suggesting that the model fit was good and the experimental error was small. This indicated that the model could be applied to the determination of PFSI yields. In addition, it is possible to establish from Table 3 that A, C, D, AD, BD, A^2, B^2, and D^2 are highly conspicuous ($p < 0.01$), C^2 is significantly different ($p < 0.05$), and the remaining terms are not remarkable. Depending on the magnitude of the F-value, one may conclude that the order of the effectiveness of the experimental factors on the polysaccharide yield was A (microwave power) > C (extraction time) > D (liquid-to-material ratio) > B (ultrasonic power).

Table 2. RSM design and results. (A is the microwave power/(W), B is the ultrasonic power/(W), C is the extraction time/(min), and D is the liquid-to-material ratio/(g/mL). In addition, −1 is level 1; 0 is level 2; 1 is level 3.).

No.	A	B	C	D	Y/(%)
1	200	300	18	80:1	28.26795
2	400	300	18	80:1	32.46507
3	200	500	18	80:1	27.97342
4	400	500	18	80:1	32.78722
5	300	400	14	65:1	29.04766
6	300	400	22	65:1	30.80509
7	300	400	14	95:1	31.21825
8	300	400	22	95:1	33.28401
9	200	400	18	65:1	30.32647
10	400	400	18	65:1	31.02944
11	200	400	18	95:1	29.13062
12	400	400	18	95:1	37.66694
13	300	300	14	80:1	27.30151
14	300	500	14	80:1	29.60256
15	300	300	22	80:1	33.58798
16	300	500	22	80:1	33.42231
17	200	400	14	80:1	28.01944
18	400	400	14	80:1	33.07562
19	200	400	22	80:1	33.07255
20	400	400	22	80:1	34.60045
21	300	300	18	65:1	27.97825
22	300	500	18	65:1	30.95466
23	300	300	18	95:1	32.05985
24	300	500	18	95:1	28.08134
25	300	400	18	80:1	33.93774
26	300	400	18	80:1	34.68328
27	300	400	18	80:1	35.24474
28	300	400	18	80:1	33.95615
29	300	400	18	80:1	33.45912

Table 3. The results of the ANOVA for the regression equation fitted the response content. (Note: R^2 = 0.9692, Adj R^2 = 0.9384, Pred R^2 = 0.8474.).

Source	Sum of Squares	df	Mean Square	F-Value	*p*-Value	Significance
Model	186.32	14	13.31	12.97	<0.0001	**
A	51.40	1	51.40	50.10	<0.0001	**
B	0.1123	1	0.1123	0.1095	0.7456	–
C	35.05	1	35.05	34.16	<0.0001	**
D	10.64	1	10.64	10.37	0.0062	**
AB	0.0951	1	0.0951	0.0927	0.7653	–
AC	3.11	1	3.11	3.03	0.1035	–
AD	15.34	1	15.34	14.95	0.0017	**
BC	1.52	1	1.52	1.48	0.2434	–
BD	12.09	1	12.09	11.79	0.0040	**
CD	0.0238	1	0.0238	0.0232	0.8812	–
A^2	5.25	1	5.25	5.12	0.0401	*
B^2	45.25	1	45.25	44.11	<0.0001	**
C^2	7.45	1	7.45	7.26	0.0174	*
D^2	19.95	1	19.95	19.44	0.0006	**
Residual	14.36	14	1.03			
Lack of Fit	12.37	10	1.24	2.49	0.1964	–
Pure Error	1.99	4	0.4966			
Cor Total	200.68	28				
	R^2 = 0.9284		R_{adj}^2 = 0.8569	C.V.% = 3.20		

Note: ** is highly significant, $p < 0.01$; * is significant difference, $p < 0.05$; – represents not significant.

The corresponding response surface and contours were plotted in the regression equation model (Figures 3–8) to visualize the relationship between the response surface and the interaction of the various factors so that the optimum process parameters and the interaction between the parameters could be identified. From the response surface and contour plots, it can be observed that the interaction between microwave power and extraction time has a noticeable contribution to the PFSI yield.

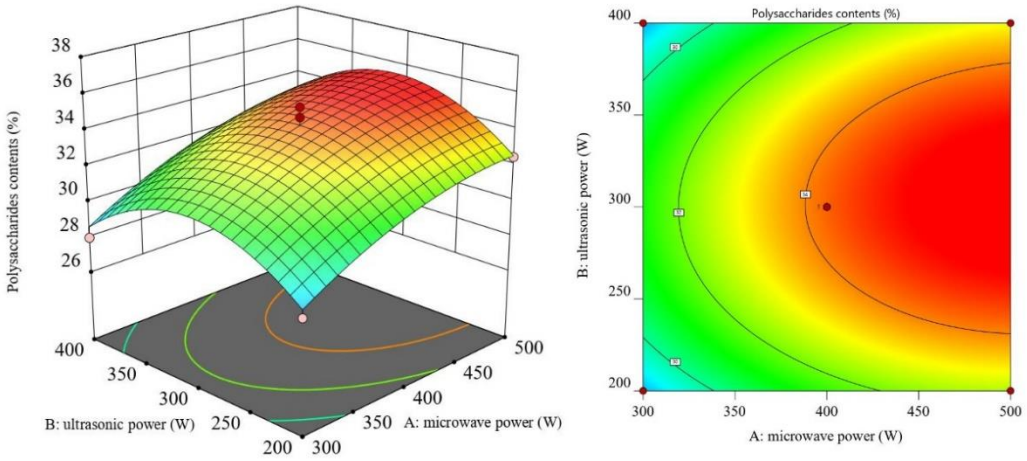

Figure 3. Effect of the interaction of ultrasonic and microwave power on polysaccharide yield. (A is the microwave power/(W), B is the ultrasonic power/(W)).

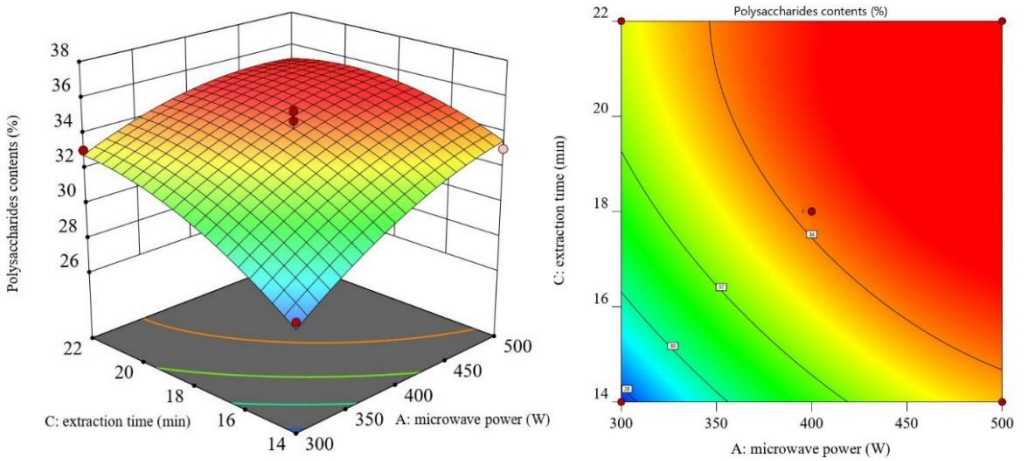

Figure 4. Effect of ultrasound power and extraction time interaction on polysaccharide yield. (A is the microwave power/(W), C is the extraction time/(min)).

3.3. Optimal Process Conditions and Validation Experiments

The optimum conditions for PFSI extraction were obtained by response surface methodology: microwave power 500 W, ultrasonic power 265.887 W, extraction time 20.078 min, liquid-to-material ratio 94.995:1, and the predicted polysaccharide yield was 37.062%. The optimum extraction process was set at 500 W microwave power, 270 W ultrasonic power, 20 min extraction time, and 95:1 liquid-to-material ratio. The average value was 37.05%, which was similar to the predicted value of 37.17%, indicating that the optimized output was reasonable. The optimized conditions for PFSI extraction gained by RSM were retrieved and PFSI-1, PFSI-2, PFSI-3, and PFSI-4 were obtained successively.

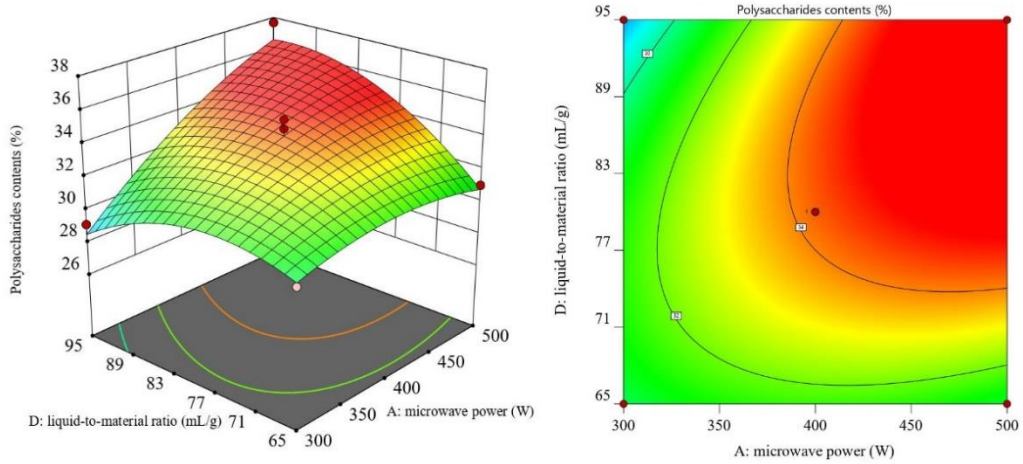

Figure 5. Effect of the interaction of microwave power and liquid-to-material ratio on polysaccharide yield. (A is the microwave power/(W), D is the liquid-to-material ratio/(g/mL)).

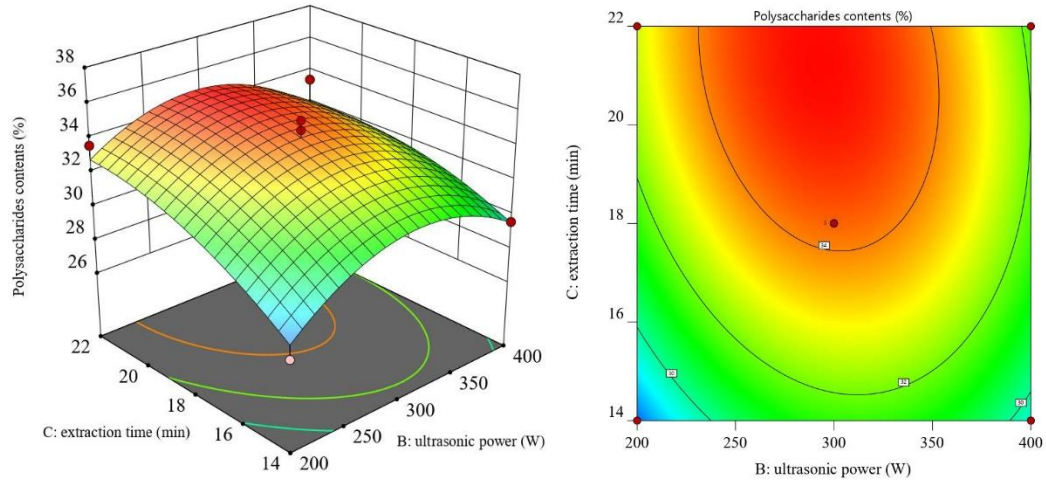

Figure 6. Effect of the interaction of ultrasonic power and time on polysaccharide yield. (B is the ultrasonic power/(W), C is the extraction time/(min)).

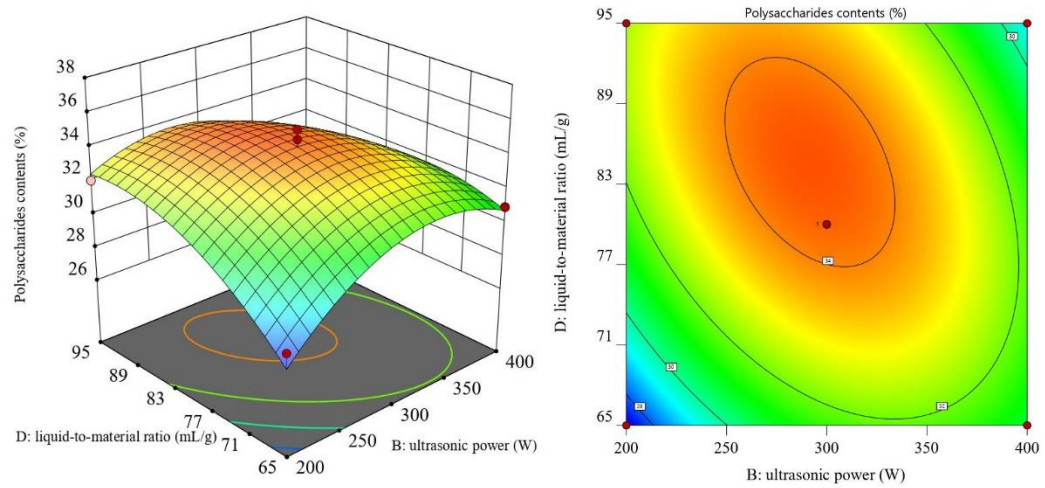

Figure 7. Effect of the interaction of ultrasonic power and liquid-to-material ratio on polysaccharide yield. (B is the ultrasonic power/(W), D is the liquid-to-material ratio/(g/mL)).

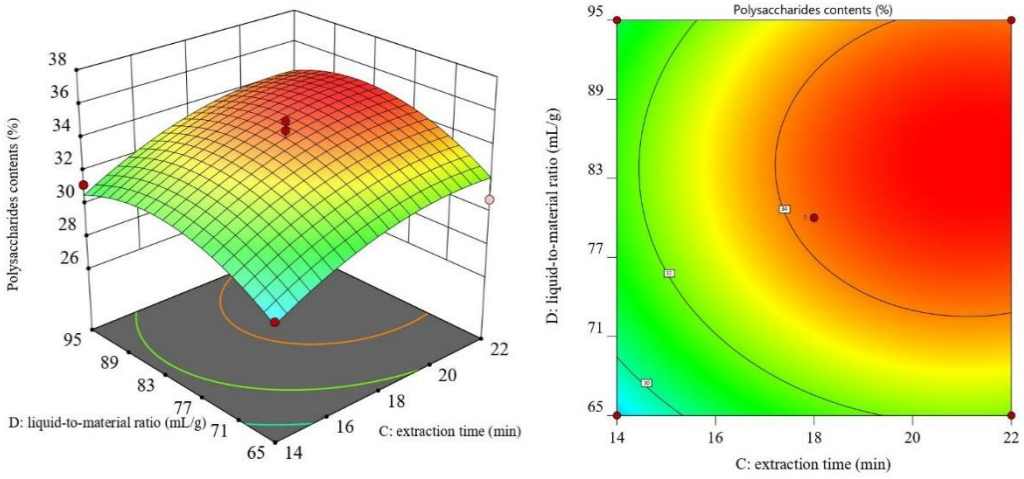

Figure 8. Effect of the interaction of time and liquid-to-material ratio on polysaccharide yield. (C is the extraction time/(min), D is the liquid-to-material ratio/(g/mL)).

3.4. Analysis of PFSI In Vitro Antioxidant

3.4.1. Analysis of DPPH· Radical Scavenging Rate

As illustrated in Table 4 and Figure 9, PFSI-1, PFSI-2, PFSI-3, and PFSI-4 showed significant differences ($p < 0.05$) in DPPH scavenging ability compared with V_C in the concentration range of 0~8 mg/mL, and all of them increased with the rise in concentration and had certain DPPH· radical scavenging ability. The increasing trend of DPPH· radical scavenging rate was stable at a concentration of 4.0 mg/mL for PFSI-1, at a concentration of 0.5 mg/mL for PFSI-3, and at a concentration of 1.0 mg/mL for PFSI-4. With PFSI-2 at a concentration of 8.0 mg/mL, the DPPH· radical scavenging rate compared to V_C achieved a similar DPPH radical scavenging rate. The maximum DPPH radical scavenging rate of PFSI-2 > PFSI-1 > PFSI-4 > PFSI-3 can be judged from the magnitude of the maximum DPPH radical scavenging rate, implying that both have certain DPPH radical scavenging ability.

Table 4. DPPH free radical scavenging by PFSI-1, PFSI-2, PFSI-3, and PFSI-4. (Note: same lowercase letters indicate no significant difference between groups, different lowercase letters indicate a significant difference between groups, $p < 0.05$).

Mass Concentration (mg/mL)	DPPH· Free Radical Scavenging Rate				
	V_C	PFSI-1	PFSI-2	PFSI-3	PFSI-4
0.01	36.90% ± 0.49% ^{d}	17.16% ± 2.66% ^{f}	4.56% ± 0.22% ^{h}	3.95% ± 0.25% ^{i}	0.15% ± 0.15% ^{i}
0.05	95.25% ± 0.18% ^{c}	24.53% ± 1.65% ^{e}	20.75% ± 2.84% ^{g}	20.00% ± 2.41% ^{h}	8.75% ± 1.09% ^{h}
0.1	95.34% ± 0.37% ^{bc}	39.85% ± 1.51% ^{d}	33.25% ± 0.90% ^{f}	32.24% ± 0.65% ^{g}	11.89% ± 1.28% ^{g}
0.25	95.61% ± 0.18% ^{b}	73.30% ± 0.64% ^{c}	72.89% ± 1.22% ^{e}	71.99% ± 1.18% ^{f}	48.76% ± 2.15% ^{f}
0.5	95.13% ± 0.43% ^{c}	85.39% ± 0.75% ^{b}	82.23% ± 0.75% ^{d}	81.90% ± 0.75% ^{e}	70.90% ± 0.33% ^{e}
0.75	95.55% ± 0.30% ^{bc}	85.53% ± 0.61% ^{b}	83.52% ± 0.32% ^{cd}	83.23% ± 0.47% ^{de}	83.52% ± 0.26% ^{d}
1	95.25% ± 0.09% ^{bc}	86.28% ± 0.42% ^{b}	84.78% ± 0.07% ^{cd}	84.34% ± 0.18% ^{cd}	90.96% ± 0.58% ^{c}
1.5	95.64% ± 0.11% ^{b}	87.06% ± 0.54% ^{b}	88.40% ± 3.38% ^{b}	85.06% ± 0.90% ^{cd}	90.99% ± 0.18% ^{c}
2	95.32% ± 0.16% ^{bc}	88.19% ± 0.64% ^{b}	86.79% ± 0.57% ^{bc}	86.50% ± 0.61% ^{bc}	93.80% ± 0.26% ^{b}
3	95.27% ± 0.39% ^{bc}	87.89% ± 2.88% ^{b}	88.73% ± 1.11% ^{b}	88.37% ± 1.08% ^{ab}	95.19% ± 0.22% ^{ab}
4	99.82% ± 0.09% ^{a}	95.55% ± 0.82% ^{a}	89.41% ± 0.50% ^{b}	89.08% ± 0.61% ^{a}	95.30% ± 0.11% ^{ab}
6	100.02% ± 0.21% ^{a}	95.97% ± 1.06% ^{a}	89.95% ± 0.61% ^{b}	89.12% ± 0.65% ^{a}	95.59% ± 0.29% ^{a}
8	99.91% ± 0.09% ^{a}	98.00% ± 2.05% ^{a}	99.64% ± 0.83% ^{a}	90.52% ± 0.75% ^{a}	96.06% ± 0.44% ^{a}

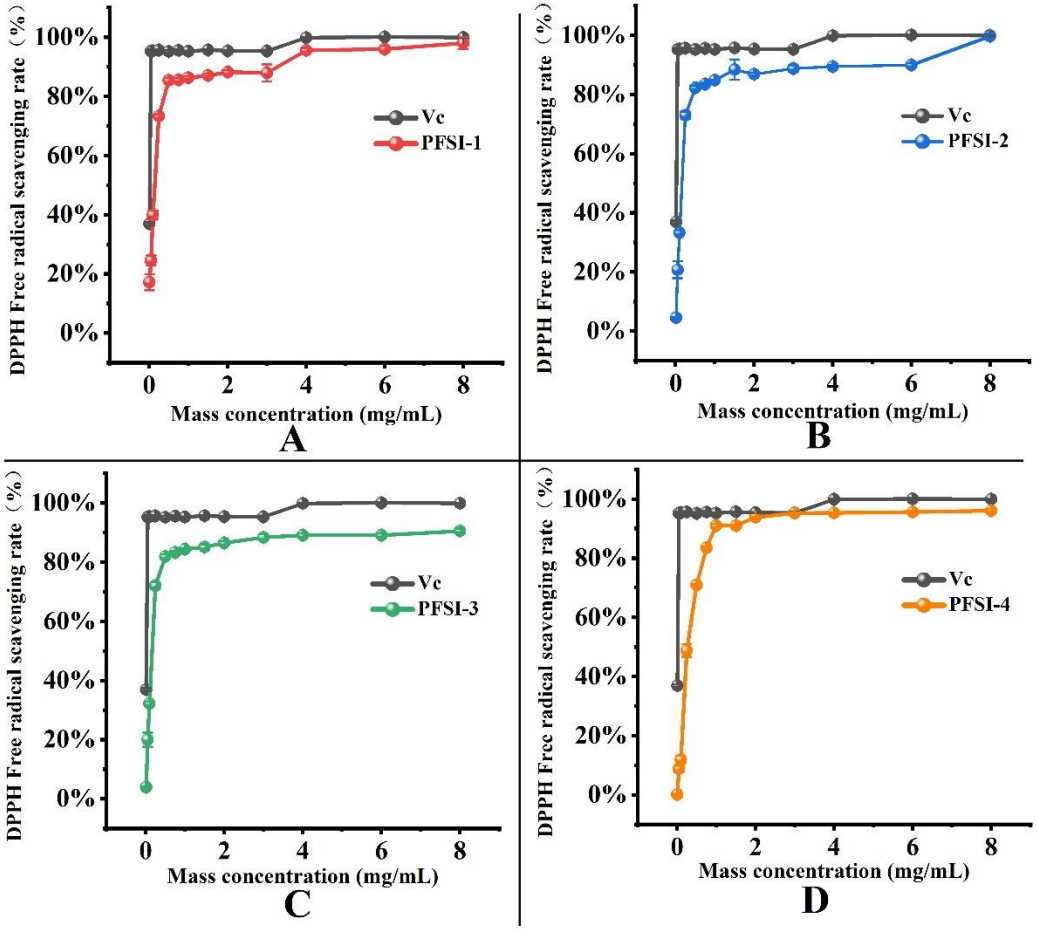

Figure 9. Comparison of the scavenging rate of DPPH· radicals by V_C and PFSI. ((**A**–**D**) represent samples of PFSI-1, PFSI-2, PFSI-3 and PFSI-4, respectively).

3.4.2. Analysis of ·OH Radical Scavenging Rate

As shown in Table 5 and Figure 10, the scavenging rate of ·OH radicals of the four species escalated with an increased polysaccharide concentration range (0.01~8 mg/mL). At a concentration of 4 mg/mL, the ·OH radical scavenging rates of PFSI-2 and PFSI-4 had reached a plateau with no prominent increase. PFSI-1 and PFSI-3 reached 8 mg/mL with similar scavenging rates. The maximum DPPH scavenging rate of PFSI-3 > PFSI-1 > PFSI-2 > PFSI-4 suggested that both had some ·OH radical scavenging ability, whereas the ·OH radical scavenging ability of PFSI-3 was stronger.

3.4.3. Analysis of ABTS· Radical Scavenging Rate

As depicted in Table 6 and Figure 11, the overall trend in the scavenging rates of PFSI-1, PFSI-2, PFSI-3, and PFSI-4 compared to V_C was found to reach similar ABTS· radical scavenging rates. At a concentration of 0.05 mg/mL, PFSI-2, PFSI-,3 and PFSI-4 ABTS· radical scavenging rates reached a plateau. The ABTS· radical scavenging rate of PFSI-1 plateaued at a concentration of 0.1 mg/mL. At concentrations of 1.0~8.0 mg/mL, the ABTS· radical scavenging capacity of PFSI-4 was slightly higher than that of V_C. When concentrations of 0.01~8 mg/mL were compared by the magnitude of the maximum ABTS· scavenging rate, there was almost no difference between V_C and PFSI-1, PFSI-2, PFSI-3, or PFSI-4. It was demonstrated that all had some ABTS· radical scavenging ability.

Table 5. ·OH free radical scavenging by PFSI-1, PFSI-2, PFSI-3, and PFSI-4. (Note: same lowercase letters indicate no significant difference between groups, different lowercase letters indicate a significant difference between groups, $p < 0.05$).

Mass Concentration (mg/mL)	·OH Free Radical Scavenging Rate				
	V_C	PFSI-1	PFSI-2	PFSI-3	PFSI-4
0.01	0.96% ± 0.30% ^{f}	13.85% ± 1.84% ^{h}	1.74% ± 0.09% ^{d}	3.34% ± 0.32% ^{j}	1.06% ± 0.09% ^{g}
0.05	7.32% ± 1.01% ^{e}	14.26% ± 3.19% ^{h}	3.36% ± 0.31% ^{d}	4.00% ± 0.78% ^{ij}	2.06% ± 0.49% ^{g}
0.1	18.56% ± 1.27% ^{d}	17.49% ± 2.92% ^{gh}	3.79% ± 1.92% ^{d}	4.83% ± 1.55% ^{ij}	4.75% ± 1.46% ^{f}
0.25	44.50% ± 0.59% ^{c}	18.66% ± 1.86% ^{g}	4.18% ± 1.44% ^{d}	5.26% ± 0.89% ^{i}	4.63% ± 0.83% ^{f}
0.5	89.33% ± 0.71% ^{b}	19.48% ± 1.14% ^{g}	8.37% ± 0.03% ^{c}	6.93% ± 0.72% ^{h}	6.75% ± 1.06% ^{ef}
0.75	99.86% ± 0.18% ^{a}	20.95% ± 1.04% ^{fg}	8.61% ± 0.37% ^{c}	15.33% ± 1.06% ^{g}	8.47% ± 0.37% ^{de}
1	99.66% ± 0.22% ^{a}	23.19% ± 1.33% ^{f}	9.25% ± 2.57% ^{c}	17.20% ± 0.81% ^{f}	10.61% ± 0.80% ^{d}
1.5	99.92% ± 0.08% ^{a}	29.39% ± 0.97% ^{e}	11.85% ± 1.71% ^{c}	27.44% ± 0.23% ^{e}	10.61% ± 1.57% ^{d}
2	99.80% ± 0.12% ^{a}	34.92% ± 1.18% ^{d}	12.19% ± 1.34% ^{c}	29.80% ± 0.17% ^{d}	15.76% ± 0.37% ^{c}
3	99.92% ± 0.08% ^{a}	39.74% ± 1.62% ^{c}	18.45% ± 1.07% ^{b}	33.07% ± 1.24% ^{c}	19.08% ± 1.54% ^{b}
4	99.80% ± 0.09% ^{a}	41.60% ± 1.39% ^{c}	33.26% ± 1.01% ^{a}	48.03% ± 0.75% ^{b}	21.00% ± 1.52% ^{b}
6	100.00% ± 0.22% ^{a}	46.49% ± 3.96% ^{b}	33.57% ± 1.13% ^{a}	56.43% ± 0.60% ^{a}	24.37% ± 2.60% ^{a}
8	99.84% ± 0.35% ^{a}	53.24% ± 1.35% ^{a}	33.60% ± 1.80% ^{a}	56.57% ± 1.09% ^{a}	24.66% ± 0.26% ^{a}

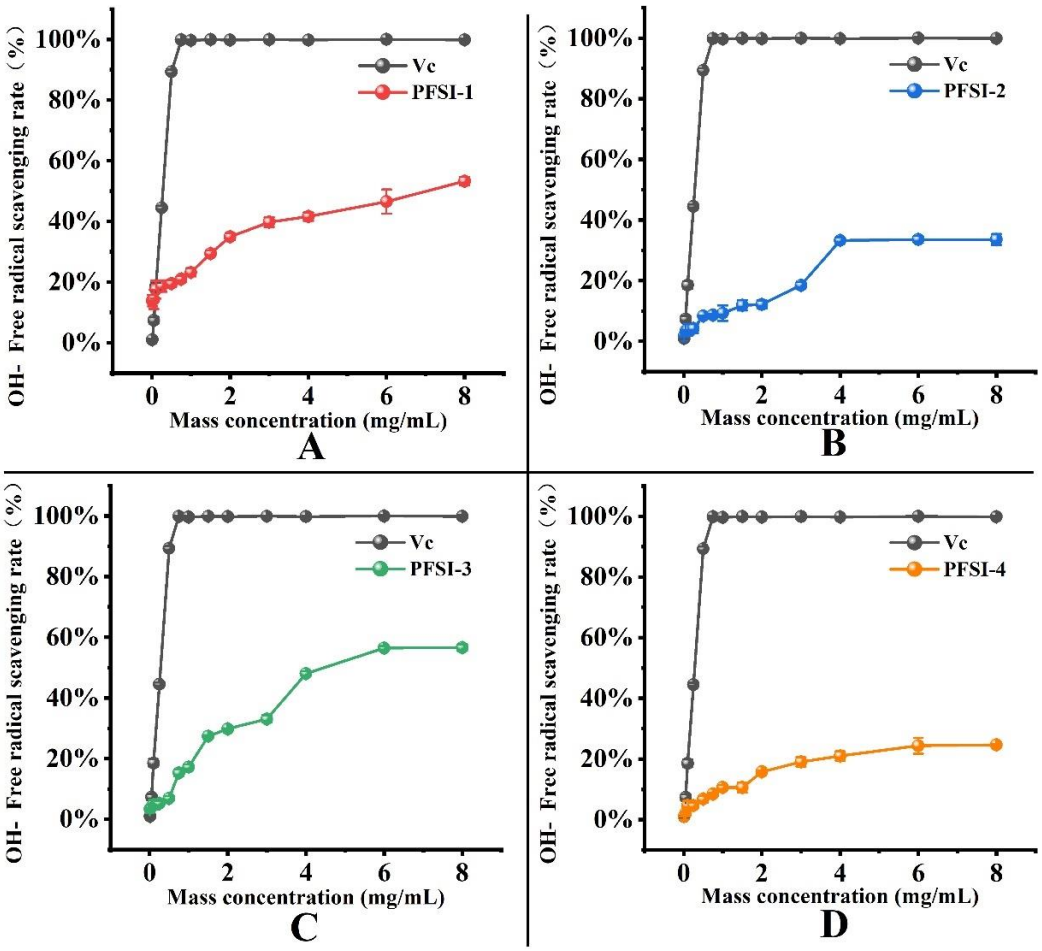

Figure 10. Comparison of the scavenging rate of ·OH radicals by V_C and PFSI·. ((**A**–**D**) represent samples of PFSI-1, PFSI-2, PFSI-3 and PFSI-4, respectively).

Table 6. ABTS· free radical scavenging by PFSI-1, PFSI-2, PFSI-3, and PFSI-4. (Note: same lowercase letters indicate no significant difference between groups, different lowercase letters indicate a significant difference between groups, $p < 0.05$).

Mass Concentration (mg/mL)	ABTS· Free Radical Scavenging Rate				
	V_C	PFSI-1	PFSI-2	PFSI-3	PFSI-4
0.01	46.36% ± 1.71% ^{b}	31.11% ± 1.43% ^{d}	26.81% ± 0.47% ^{d}	32.11% ± 0.08% ^{b}	26.05% ± 0.32% ^{e}
0.05	99.51% ± 0.49% ^{a}	84.99% ± 1.43% ^{c}	99.45% ± 0.16% ^{bc}	99.92% ± 0.16% ^{a}	100.08% ± 0.08% ^{c}
0.1	99.76% ± 0.49% ^{a}	99.54% ± 0.68% ^{b}	100.62% ± 0.16% ^{a}	99.76% ± 0.24% ^{a}	100.16% ± 0.40% ^{c}
0.25	99.45% ± 0.43% ^{a}	99.49% ± 0.86% ^{b}	100.08% ± 0.78% ^{ab}	99.92% ± 0.40% ^{a}	100.40% ± 0.40% ^{c}
0.5	99.33% ± 0.31% ^{a}	100.00% ± 0.23% ^{ab}	100.23% ± 0.23% ^{ab}	100.16% ± 0.16% ^{a}	100.56% ± 0.32% ^{c}
0.75	99.33% ± 0.55% ^{a}	100.68% ± 1.60% ^{a}	100.16% ± 0.16% ^{ab}	100.08% ± 0.32% ^{a}	101.45% ± 0.72% ^{b}
1	99.39% ± 0.37% ^{a}	99.94% ± 0.40% ^{ab}	98.75% ± 1.09% ^{c}	99.36% ± 2.94% ^{a}	101.29% ± 0.80% ^{b}
1.5	99.82% ± 0.31% ^{a}	99.71% ± 0.17% ^{b}	99.92% ± 0.16% ^{ab}	100.08% ± 0.08% ^{a}	101.37% ± 0.16% ^{b}
2	99.57% ± 0.06% ^{a}	99.54% ± 0.91% ^{b}	99.77% ± 0.23% ^{b}	99.84% ± 0.08% ^{a}	102.17% ± 0.24% ^{a}
3	99.08% ± 0.55% ^{a}	100.06% ± 0.51% ^{ab}	99.92% ± 0.16% ^{ab}	100.00% ± 0.01% ^{a}	102.65% ± 0.48% ^{a}
4	99.20% ± 0.43% ^{a}	100.17% ± 0.40% ^{ab}	100.23% ± 0.47% ^{ab}	100.00% ± 0.48% ^{a}	102.57% ± 0.40% ^{a}
6	99.63% ± 0.37% ^{a}	99.60% ± 0.29% ^{b}	100.70% ± 0.47% ^{a}	100.08% ± 0.32% ^{a}	102.89% ± 0.24% ^{a}
8	99.63% ± 0.37% ^{a}	99.24% ± 1.52% ^{b}	100.00% ± 0.23% ^{ab}	99.84% ± 0.08% ^{a}	99.36% ± 0.08% ^{d}

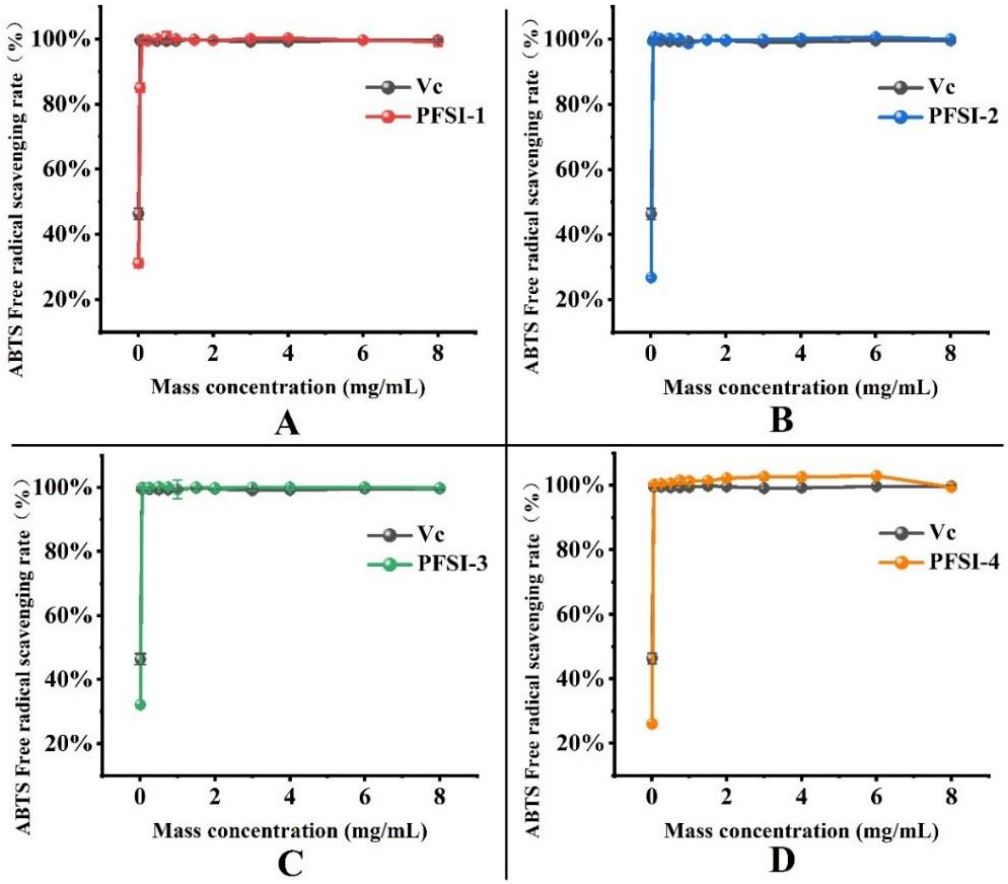

Figure 11. Comparison of the scavenging rate of ABTS· radicals by V_C and PFSI. ((**A**–**D**) represent samples of PFSI-1, PFSI-2, PFSI-3 and PFSI-4, respectively).

3.4.4. Analysis of Ferric-Reducing Antioxidant Power

As shown in Table 7 and Figure 12, the FRAP of PFSI-1, PFSI-2, PFSI-3, and PFSI-4 showed a dose-dependent relationship within the concentration of 0~8 mg/mL. When the concentration reached 6~8 mg/mL, PFSI-2 and PFSI-3 both had little difference in the maximum FRAP/When the concentration reached 8 mg/mL, PFSI-1 reached the maximum

FRAP, which was similar to the V_C comparison. In summary, the order of FRAP was PFSI-1 > PFSI-2 > PFSI-3 > PFSI-4.

Table 7. Total reduction capacity of PFSI-1, PFSI-2, PFSI-3, and PFSI-4. (Note: same lowercase letters indicate no significant difference between groups, different lowercase letters indicate a significant difference between groups, $p < 0.05$).

Mass Concentration (mg/mL)	Absorbance (OD_{700nm})				
	V_C	PFSI-1	PFSI-2	PFSI-3	PFSI-4
0.01	0.049 ± 0.001^{k}	0.014 ± 0.001^{l}	0.060 ± 0.001^{l}	0.062 ± 0.002^{l}	0.058 ± 0.001^{l}
0.05	0.196 ± 0.002^{j}	0.027 ± 0.002^{l}	0.068 ± 0.001^{l}	0.072 ± 0.001^{l}	0.071 ± 0.002^{k}
0.1	0.382 ± 0.005^{i}	0.041 ± 0.001^{k}	0.083 ± 0.001^{k}	0.088 ± 0.001^{k}	0.081 ± 0.001^{k}
0.25	0.862 ± 0.021^{h}	0.075 ± 0.001^{j}	0.121 ± 0.004^{j}	0.136 ± 0.001^{j}	0.118 ± 0.001^{j}
0.5	1.654 ± 0.045^{e}	0.135 ± 0.002^{i}	0.166 ± 0.001^{i}	0.202 ± 0.002^{i}	0.175 ± 0.001^{i}
0.75	1.713 ± 0.030^{f}	0.185 ± 0.001^{h}	0.218 ± 0.005^{h}	0.277 ± 0.004^{h}	0.222 ± 0.002^{h}
1	1.731 ± 0.020^{ef}	0.243 ± 0.005^{g}	0.263 ± 0.001^{g}	0.328 ± 0.004^{g}	0.272 ± 0.004^{g}
1.5	1.762 ± 0.030^{cd}	0.329 ± 0.008^{f}	0.460 ± 0.003^{f}	0.398 ± 0.001^{f}	0.298 ± 0.004^{f}
2	1.772 ± 0.022^{bc}	0.419 ± 0.024^{e}	0.558 ± 0.010^{e}	0.487 ± 0.009^{e}	0.379 ± 0.003^{e}
3	1.803 ± 0.043^{ab}	0.605 ± 0.006^{d}	0.710 ± 0.014^{d}	0.597 ± 0.001^{d}	0.480 ± 0.007^{d}
4	1.811 ± 0.015^{a}	0.751 ± 0.012^{c}	1.084 ± 0.015^{c}	0.731 ± 0.030^{c}	0.569 ± 0.013^{c}
6	1.792 ± 0.023^{ab}	1.215 ± 0.026^{b}	1.274 ± 0.013^{b}	1.249 ± 0.020^{b}	0.734 ± 0.017^{b}
8	1.748 ± 0.019^{a}	1.566 ± 0.025^{a}	1.311 ± 0.012^{a}	1.270 ± 0.008^{a}	0.836 ± 0.024^{a}

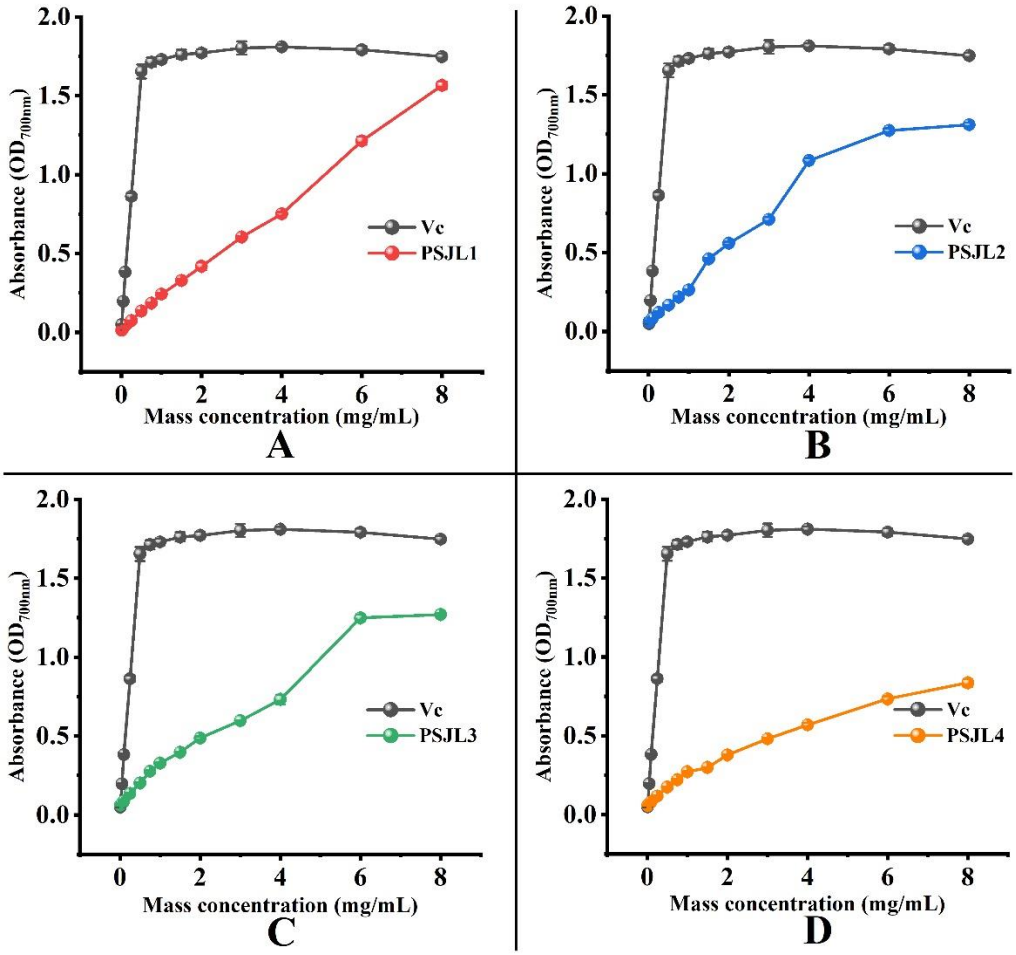

Figure 12. Comparison of FRAP by V_C and PFSI. ((**A**–**D**) represent samples of PFSI-1, PFSI-2, PFSI-3 and PFSI-4, respectively).

4. Discussion

The polysaccharides from plant polysaccharides mostly belong to heterogeneous polysaccharides, and in order to obtain purer polysaccharides, there is a requirement to go through a series of separation and purification experiments such as deproteinization and decolorization. It was found that there is a certain gap between the activity of undeproteinated polysaccharides and the activity of deproteinated polysaccharides, and the deproteination process at a certain intensity has an enhancing function on the activation of polysaccharides [32–34]. The literature [35] reports that both undeproteinated and deproteinated polysaccharides have antioxidant potential, and both show a quantitative effect with increasing polysaccharide concentration. Some authors [36] have also found the antioxidant activity of undeproteinated polysaccharide to be higher than that of deproteinated polysaccharide, while others concluded that the antioxidant activity of deproteinated polysaccharide was higher than that of undeproteinated polysaccharide, pooling the results of the studies to show the inconsistency between the purity of the polysaccharide and the antioxidant action. The implications of different undeproteinization methods on antioxidation are discussed through the utilization of PFSI-1, PFSI-2, PFSI-3, and PFSI-4 obtained by different techniques.

Polysaccharides exist in two main forms, either as pure saccharide chains or as glycopeptides or glycoproteins formed by combining saccharides with proteins. PFSI which is a heteropolysaccharide, whose crude polysaccharide plays an active part in various diseases, may be the sugar component of the crude polysaccharide, the protein component of it, or the combination of saccharides with proteins, and therefore needs to be categorized and explored [37–39]. In this study, the in vitro antioxidant activity of PFSI obtained by three different deproteinization methods (Sevage method, papain method, and TCA method) was investigated using UMSE-extracted PFSI followed by graded alc ·OHolic purification. The in vitro antioxidant analysis of the results yielded that the different deproteinization methods severely reduced, to varying degrees, the in vitro antioxidant activity.

In the conventional Sevage method, the rate of protein removal is relatively inexpensive, and there is considerable polysaccharide loss due to repeated repetitions, and much of the organic solvent is wasted. Since most of the proteins removed by Sevage are free proteins, the glycoconjugate proteins are not destroyed. Despite maintaining favorable activity, it has reduced in vitro antioxidant activity compared to PFSI-1. Papaya proteolysis enzymatically dismantled the glycoconjugate proteins [40], thus partially reducing the bioactivity of Sophora polysaccharides and reducing the in vitro antioxidant activity compared to PFSI-1. TCA caused considerable destruction of the polysaccharide structure when subjected to severe loading conditions, leading to some differences in the in vitro antioxidant activity of the polysaccharide compared to PFSI-1, probably due to a modification of the chemical structure to a certain extent. In conclusion, the in vitro antioxidant activity of *Sophora japonica* polysaccharides without deproteinization was the best in terms of scavenging against oxidative effects [41]. In conclusion, the antioxidant activity of PFSI can be attributed to the combined effect of protein and polysaccharides. For better maintenance of the biological activity of the polysaccharides, it is preferable to develop PFSI antioxidant products without deproteinization, whereas if the product to be developed has to be low in protein, the Sevage method of deproteinization would be the preferable alternative.

The polysaccharide yields of PFSI extracted by UMSE under optimal conditions were observed in comparison with those obtained by Ying Zhou et al. [42] adopting the ultrasonic extraction method, Naxin Li et al. [43] adopting the ultrasonic extraction method, and Changshu Wu et al. [6]. We found that the extraction of PFSI by ultrasonic microcoordinated extraction improved polysaccharide yields in this study. The extent to which polysaccharide yields were influenced by A (microwave power) > C (extraction time) > D (liquid-to material–ratio) > B (ultrasonic power) was also noted. The optimum process parameters for PFSI validation were acquired microwave power 500 W, ultrasonic power 270 W, extraction time 20 min, liquid-to-material ratio 95:1, and the PFSI extraction rate

of the validation experiment reached 37.05% ± 0.12. The proposed technology can be applied to the extraction of PFSI by the ultrasonic microwave extraction method, thereby illustrating the operability of the optimized PFSI extraction process. In this respect, the extraction of PFSI by ultrasonic microwave extraction can provide a promising opportunity to upgrade the economic efficiency of FSI.

5. Conclusions

Notably, the extraction of PFSI by UMSE was first performed in this work, and the extraction process was optimized in terms of the response surface. The optimum extraction procedures were as follows: microwave power 500 W, ultrasonic power 265.887 W, extraction time 20.078 min, and liquid-to-material ratio 94.995:1. The PFSI samples were deproteinated by Sevage, papain, and TCA methods, and then were purified by dialysis and DEAE-52 cellulose column, respectively, and noted to have certain antioxidant effects with or without deproteination, along with the different effects of various deproteination techniques on the in vitro antioxidant potential of PFSI. A comprehensive comparison revealed that PFSI without deproteinization had the maximum in vitro free radical scavenging ability. It was found that the higher the concentration of polysaccharide, the greater the antioxidant capacity, and the stronger the free radical scavenging ability. Additional work should be carried out to provide theoretical and practical guidance for the further exploitation and beneficial application of PFSI antioxidant products.

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Sample Availability: Samples of the compounds are not available from the authors.

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