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The Comparison and Analysis of Three Extraction Methods for Polysaccharides in Purslane

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Abstract In this study, three extraction methods of polysaccharides in Purslane were compared and analyzed, and the best method was ascertained. Purslane polysaccharides were extracted using water, ultrasonic and microwave. By the results of single factor experiments and orthogonal experiments, the optimal extracting conditions were selected and then the best extraction method was determined. In order to calculate the extraction yield of polysaccharides, its content were measured by the phenol–sulfuric acid method using glucose as the standard, and then the efficiency of three extraction methods was evaluated. The results showed that the extraction yield of ultrasonic was 35.42% under the optimal conditions of 60°C, 60 min, 1:20 and 200w. The extraction yield of microwave method was 32.12% under the optimal conditions of 3 min, 1:25 and 500w. The extraction yield of water method was 31.22% under the optimal conditions of 80°C, 2 h, 1:30 and 3 times. It could be concluded that ultrasonic method was the best one in these three methods.

Keywords: *Purslane, polysaccharides, Extraction methods, comparison, analysis*

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1. Introduction

As a kind of ancient wild plant, Purslane is widely distributed in China. Purslane has a variety of biological activities that have a wide range of pharmacological effects, such as antibacterial [1,2], hypolipidemic, anti-aging, anti-inflammatory [3], antioxidative [4], analgesic, and wound healing activities [5,6]. Purslane has been used for thousands of years in traditional Chinese medicine and is a dietetic Chinese medicine which has been officially recognized as a food and a Chinese medicine simultaneously [7]. Known as "vegetable for long life" in Chinese folklore. Purslane is listed in the World Health Organization as one of the most used medicinal plants and it has been given the term 'Global Panacea [8]. For example, it is used in the Arabian Peninsula as an antiseptic, in oral ulcers and in urinary disorders [9]. In addition, this plant has been exhaustively studied in Nigeria and Scotland mainly for its muscle relaxant activity [10,11,12,13]. Therefore, it is necessary to study on Purslane.

In recent decades, polysaccharides isolated from plants and animals have attracted a great deal of attention due to their various biological activities [14], for example strong antioxidant, anticancer, anti-microbial and anti-inflammatory properties. Because of anti-microbial, polysaccharides can be used for the study of natural preservatives. With the improvement of living standard, people pursue healthy life increasingly. However, the chemical preservatives are used in the processing and production of food [15]. It is known that chemical preservatives ingested for a long time may cause adverse effects on health, even lead to cancer [16]. So the natural preservatives will become the focus of social attention. At the same time, the natural preservatives have strong anti-microbial properties, good water solubility and thermal stability. Therefore, the research for development and utilization of natural preservatives has become a focus of food industry. Nowadays, many extraction methods of polysaccharides have been reported. For example water extraction of polysaccharides was done by Gao et al [17]. Ultrasonic extraction of polysaccharides was done by Qu et al [18] and microwave extraction of polysaccharides was done by Qian and Bi [19]. Because Purslane has the characteristics of convenience, fast growth and low cost [20], the polysaccharides extracted from Purslane are feasible.

In spite of extensive studies on the extraction method of polysaccharides, the comparative analysis about extraction method of Purslane polysaccharides has rarely been studied. This study aims to fill in the knowledge gap for this useful plant. It also lays a foundation for further research of polysaccharides extraction.

2. Materials and methods

2.1. Materials

Dried Purslane was purchased from pharmacy in Changchun City, Jilin Province, China. Other reagents were analytical grade and obtained from Jilin Agriculture University in Changchun City, Jilin Province, China.

2.2. Sample Preparation

Dried Purslane plant was defatted in a Soxhlet apparatus with methanol twice, and then filtered the processed sample. The pretreated Purslane was dried at 40°C for 12h and extracted with distilled water in different conditions using single factor experiments. After centrifugation, the supernatant was concentrated and poured into 3 vol. of anhydrous ethanol for 24 h to precipitate the crude polysaccharides. According to the standard curve, the absorbance of glucose was determined, and then the content of the polysaccharide was calculated.

2.3. Determination of Polysaccharides Content

The polysaccharide content was determined according to the phenol–sulfuric acid method [21]. Standard glucose (10 mg) was dissolved with distilled water (100 ml) and then the standard solution was diluted to different concentrations, and finally pure water was added into the diluted solution. 5% phenol (1 mL) was added and mixed up. Then sulfuric acid (5 mL) was added and mixed. The absorbance of the solution was measured at 490 nm with an ultraviolet-visible spectrophotometer, using water as a blank. The standard curve of glucose was: y = 0.0068 + 0.0169 x, R^2= 0.9927 (x represented the concentration of glucose solution, y represented absorbance).

2.4. Water Extraction of Polysaccharides from Purslane

The factors that influenced water soluble polysaccharides extraction included temperature, extraction time, solid-liquid ratio and extraction times. In single factor experiments, the mixture was extracted using various extraction time (1 h, 2 h, 3 h, 4 h, 5 h), temperature (50°C, 60°C, 70°C, 80°C, 90°C), solid-liquid ratios (1:15, 1:20, 1:25, 1:30, 1:35), extraction times (1, 2, 3, 4, 5 times).

On the basis of single factor experiments, four factors and three levels of L9 (3^4) orthogonal test was selected. The factor levels were shown in Table 1.

Table 1. L9 (3^4) Orthogonal table of water extraction

Level	A (temperature)	B (time)	C (solid-liquid ratio)	D (Extraction times)
1	60°C	2h	1:20	2
2	70°C	3h	1:25	3
3	80°C	4h	1:30	4

2.5. Ultrasonic Extraction of Polysaccharides from Purslane

There are many factors that affect the ultrasonic extraction, such as extraction temperature, ultrasonic time, solid-liquid ratio and ultrasonic power. The mixture was extracted using various extraction temperature (40°C, 50°C, 60°C, 70°C, 80°C), time (30 min, 40 min, 50 min, 60 min, 70 min), Solid-liquid ratios (1:15, 1:20, 1:25, 1:30, 1:35), power levels (100 w, 200w, 300w, 400w, 500w). According to the results of single factor experiments, four factors and three levels of L9 (3^4) orthogonal test was chosen and the level of various factors were shown in Table 2.

Table 2. L9 (3^4) Orthogonal table of ultrasonic method

Level	A (temperature)	B (time)	C (solid-liquid ratio)	D (power)
1	50°C	40min	1:20	100w
2	60°C	50min	1:25	200w
3	70°C	60min	1:30	300w

2.6. Microwave Extraction of Polysaccharides from Purslane

Table 3. L9 (3^4) Orthogonal table of microwave method

Level	A (time)	B (solid-liquid ratio)	C (power)
1	2	1:20	300
2	3	1:25	400
3	4	1:30	500

The factors influenced microwave extraction included time, solid-liquid ratio and microwave power. In single factor experiments, the mixture was extracted using various extraction time (1 min, 2 min, 3 min, 4 min, 5 min), solid-liquid ratios (1:15, 1:20, 1:25, 1:30, 1:35), microwave power levels (200w, 300w, 400w, 500w, 600w).

On the basis of single factor experiments, three factors and three levels of L9 (3^4) orthogonal test was chosen and the factor levels were shown in Table 3.

3. Results

3.1. The Results of Water Extraction Method

As can be seen from the results, the extraction yield was increased with the rising of temperature before 70°C. When the temperature reached 70°C, there was no significant change. Extraction yield was proportional to extraction time before 3 h and there was no significant increase after 3 h. When the solid-liquid ratio reached 1:25, the extraction yield was the highest. The extraction times ranges from one to five, but after three times the extraction yield no longer increased. Based on the single factor experiments, the results of orthogonal experiment were shown in Table 4

Table 4. L9 (3^4) Orthogonal experiment results of water extraction method

Number	Temperature (A)	Time (B)	Solid-liquid ratio(C)	Extraction times (D)	Extraction yield (%)
1	A1	B1	C1	D1	19.89
2	A1	B2	C2	D2	21.88
3	A1	B3	C3	D3	22.63
4	A2	B1	C2	D3	24.88
5	A2	B2	C3	D1	20.06
6	A2	B3	C1	D2	25.13
7	A3	B1	C3	D2	31.22
8	A3	B2	C1	D3	19.93
9	A3	B3	C2	D1	24.39
K1	64.40	75.99	64.95	64.34	
K2	70.07	61.87	68.82	78.23	
K3	75.54	72.15	71.15	67.44	
K1i	21.47	25.33	21.65	24.45	
K2i	23.36	20.62	22.94	26.08	
K3i	25.18	24.05	23.72	22.48	
R	1.82	4.71	2.07	3.6	

Note: R refers to the result of extreme analysis.
Extraction yield (%) = (the content of polysaccharide in the sample / the content of polysaccharide in material) × 100.

From Table 4, the optimum conditions were determined by orthogonal test and extreme difference analysis ($\boldsymbol{R} = {}_{\max}K_i - {}_{\min}K_i$). According to the value of R (R_B=4.71>R_D>R_C>R_A), the influence of extraction time on the extraction yield of polysaccharides among the four factors was the biggest. These factors were sequenced by their influence on extraction yield for B > D > C > A. According to the values of K_i (K_{A3}>K_{A2}>K_{A1}, K_{B1}>K_{B3}>K_{B2}, K_{C3}>K_{C2}>K_{C1}, K_{D2}>K_{D3}>K_{D1}), the optimum extraction conditions were $A_3B_1C_3D_2$, temperature 80°C, time 2 h, solid-liquid ratio 1:30 and extraction times 3, the extraction yield reached the optimal level.

In single factor experiments of ultrasonic-assisted method, the extraction yield of polysaccharides was significantly increased with the increase of temperature before 60°C. When the temperature exceeded 60°C, the extraction yield no longer changed. Between 30 min to 50 min, polysaccharides extraction yield was increased with the increase of time, but when more than 50 minutes, the yield was decreased. When the ultrasonic power reached 200 w, the extraction yield reached optimal value. However, when the power exceeded 200 w, the extraction yield was deceased significantly. Solid-liquid ratio 1:25 could obtain best extraction yield. According to the single factor experiments, the results of orthogonal experiment were shown in Table 5.

3.2. The Results of Ultrasonic Method

Table 5. L9 (3^4) Orthogonal experiment results of ultrasonic method

Number	Temperature (A)	Time (B)	Solid-liquid ratio(C)	power (D)	Extraction yield (%)
1	A1	B1	C1	D1	30.24
2	A1	B2	C2	D2	31.57
3	A1	B3	C3	D3	30.83
4	A2	B1	C2	D3	31.21
5	A2	B2	C3	D1	29.98
6	A2	B3	C1	D2	35.42
7	A3	B1	C3	D2	29.93
8	A3	B2	C1	D3	30.14
9	A3	B3	C2	D1	30.22
K_1	92.64	91.38	95.80	90.44	
K_2	96.61	91.69	93	96.92	
K_3	90.29	96.47	90.74	92.18	
K_{1i}	30.88	30.46	31.93	30.15	
K_{2i}	32.20	30.56	31	32.31	
K_{3i}	30.10	32.16	30.25	30.73	
R	2.1	1.7	1.68	2.16	

Note:R refers to the result of extreme analysis.
Extraction yield (%) = (the content of polysaccharide in the sample / the content of polysaccharide in material) × 100.

From Table 5, the results were determined by extreme difference analysis ($\boldsymbol{R} = {}_{\max}K_i - {}_{\min}K_i$). According to the value of R (R_D>R_A>R_B>R_C), the influence of ultrasonic power on the extraction yield of polysaccharide among the four factors was the biggest. These factors were sequenced by their influence on extraction yield for D > A > B > C. According to the value of Ki (K_{A2}>K_{A1}>K_{A3}, K_{B3}>K_{B2}>K_{B1}, K_{C1}>K_{C2}>K_{C3}, K_{D2}>K_{D3}>K_{D1}), the optimum extraction conditions were $A_2B_3C_1D_2$ with temperature 60°C, time 60 min, solid-liquid ratio 1:20 and power 200 w. Under the conditions the extraction yield reached the optimal level.

3.3. The Results of Microwave Method

According to the single factor experiments of microwave-assisted method, the extraction yield was increased before 3 min and decreased after 3 min. Solid-liquid ratio 1:25 was the best ratio in single factor experiments. The power more than 400w had a negative impact on extraction yield. Based on the single factor experiments, the results of orthogonal experiment were shown in Table 6.

Table 6. L9 (3^4) Orthogonal experiment results of microwave method

Number	Time (A)	Solid-liquid ratio (B)	Microwave power(C)	Extraction yield (%)
1	A1	B1	C1	21.45
2	A1	B2	C2	24.18
3	A1	B3	C3	26.73
4	A2	B1	C2	24.31
5	A2	B2	C3	32.12
6	A2	B3	C1	22.77
7	A3	B1	C3	23.08
8	A3	B2	C1	22.11
9	A3	B3	C2	22.72
K1	72.36	68.84	66.33	
K2	79.2	78.41	71.21	
K3	67.91	72.22	81.93	
K1i	24.12	22.95	22.11	
K2i	26.4	26.14	23.74	
K3i	22.64	24.07	27.31	
R	3.76	3.19	5.2	

Note: R refers to the result of extreme analysis.
Extraction yield (%) = (the content of polysaccharide in the sample / the content of polysaccharide in material) × 100.

In Table 6, microwave power had the highest R value (5.2), the effects of the variables on extraction yields followed the order: microwave power > time > solid-liquid ratio. According to the value of Ki, the optimal combinations were $A_2B_2C_3$ with time 3 min, solid liquid ratio 1:25 and power 500w.

Table 7 shows the summary results of these three methods in optimal conditions. Extraction yield of ultrasonic-assisted method was higher than other two methods from Table 7.

Table 7. Comparison the three extraction methods of polysaccharides

Method	Water extraction	Ultrasonic extraction	Microwave extraction
Extraction temperature	80°C	60°C	—
Extraction time	2h	60 min	3 min
Solid-liquid ratio	1:30	1:20	1:25
Extraction power	—	200w	500w
Extraction times	3	1	1
Extraction yield	31.22%	35.42%	32.12%

4. Discussion

In recent years, extraction of polysaccharides from Purslane has been studied. It is reported that there were many methods of extracting polysaccharides, such as water extraction, microwave assisted extraction, ultrasonic assisted extraction and some enzymatic methods [22,23,24]. However, the comparative research on these methods is not reported until now. So polysaccharides from Purslane were extracted using hot water, ultrasonic and microwave in this study, and according to the results of the orthogonal experiment, these three methods were compared in Table 7 and the best method was determined, which will have an important significance to the research and application of polysaccharides.

Water extraction is a traditional method, and its advantage is mild condition and stable easy to control. The molecules of polysaccharides will not be destroyed by water extraction method. But it should be noted that water extraction of polysaccharides was associated with long extraction time and high temperature. Zhang et al. reported that extraction yield was 9.68% by water extraction under the conditions of temperature 95°C, time 60 min, solid-liquid ratio 1:40 [25]. Chen et al. reported that the extraction yield was 12.98% by water extraction under the conditions of temperature 90°C, time 120 min, solid-liquid ratio 1:12 [26]. In our study, by the improvement of the previous method, it was found that according to the results of the orthogonal experiment in Table 4, the optimal conditions of water extraction were 80°C, 2 h, 1:30 and 3 times, and the highest extraction yield was 31.22%. This suggested that our water extraction method had practical significance.

During microwave extraction, it is necessary to optimize the conditions. The results of the microwave orthogonal experiment were shown in Table 6, the optimal process conditions were 3 min, 1:25 and 500w. Liang reported that the extraction yield was 11.57% under the conditions 15 min, 1:35 and 540w [27]. However, in our study, the highest extraction yield was 32.12% after optimization 3 min, 1:25 and 500w. In the microwave extraction method, microwave power has a great impact on material. For example, the high power of microwave extraction can cause selective migration of the target compounds from the material to the surroundings at a more rapid rate. Tao and Xu reported that degradation occurred in the structure of polysaccharides during the microwave heating process [28]. Marshall et al. also studied the possibility of minimizing the disassembly of polysaccharides during microwave extraction [29]. According to the above reports, we could come to a conclusion that the microwave power is an important influence factor of extraction yield.

In the present study, the highest extraction yield was 35.42% under the optimal ultrasonic conditions (60°C, 60 min, 1:20, 200w). Yu reported that the extraction yield was 13.28% under the conditions of ultrasonic (75°C, 55 min, 1:45, 130w) [30]. Zhang studied that the extraction yield was 11.01% during ultrasonic extraction (60°C, 30 min, 1:25, 280w) [31]. This could be attributed to the optimal conditions. With the increase of ultrasonic power, the effect of ultrasonic on the cell wall also increases. In addition, when the power is too strong, the ultrasonic can lead to the liquid flow too fast. This means that the residence time of material is reduced and extraction yield is also decreased by strong power. On the whole, it is also necessary to optimize the conditions during ultrasonic extraction.

As the saying goes, every coin has two sides. Ultrasonic method and microwave method have the advantages of reducing both extraction time and solvent consumption, which compared with water extraction. At the same time, the disadvantages of ultrasonic method and microwave method are strictly conditions. In addition, compared with the ultrasonic method, the extraction time of microwave method is too short, and the extraction temperature of microwave method cannot be controlled. So by comparing the data and condition of three methods, it was easy to come to a conclusion that ultrasonic method was the best.

5. Conclusions

In summary, these three extraction methods have different characteristics. No matter what method, optimization of condition is necessary. When each of these three methods in optimal conditions, ultrasonic method was the best. It lays a foundation for further research of polysaccharides. Further studies on natural preservatives are currently underway.

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