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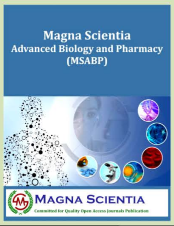

(RESEARCH ARTICLE)

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Study on ultrasonic assisted extraction of polysaccharide from Angelica Dahurica

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Abstract

Taking Bozhou *Angelica dahurica* as the raw material, taking the polysaccharide yield as the evaluation index, the ultrasonic-assisted extraction of *Angelica dahurica* polysaccharide was studied. The effects of ultrasonic power, ultrasonic treatment time, solid-liquid ratio and temperature on the polysaccharide yield were investigated. Based on single factor experiment, the orthogonal experiment was carried out to optimize it. The results showed that the optimum technological conditions were as follows: solid-liquid ratio of 1:40, ultrasonic power of 500 W, ultrasonic time of 45 min, and the highest yield of *Angelica dahurica* polysaccharide was 15.8%. Ultrasonic time has a significant effect on the yield of *Angelica dahurica* polysaccharide. The ultrasonic power and the ratio of material to liquid have a significant effect, and the temperature has a low effect on polysaccharide extraction.

Keywords: *Angelica dahurica*; Polysaccharide; Ultrasound; Process optimization

1. Introduction

Angelica dahurica, is the Chinese name of a herb. It is 1-2.5 m in height, with columnar roots, 2-5 cm in diameter at the base of the stem, 1-5 cm at the base, 1 pinnate division at the base, terminal or lateral compound umbels, and oblong to ovate fruits. The main varieties are Hang *Dahurica*, Qi *Dahurica*, and Taiwan *Dahurica*. Its rhizome can be used for treating diseases, removing dampness, activating blood circulation and relieving pain. It is also cultivated in some northern provinces, mostly for self-production and sale, with a small amount exported to foreign countries. It is often found in the forest understory, forest edge, streamside, thickets, and valleys(Zhao et al., 2022).

Angelica dahurica is a pungent and warm food, mainly used to treat cold and headache, brow and arch bone pain, nasal congestion, toothache, leucorrhoea, sore and swelling pain, etc. Studies at home and abroad have shown that *Angelica dahurica* has anti-inflammatory, antiseptic, whitening, antibacterial, and ulcer healing properties (Ji et al., 2020). The effectiveness and scope of application of *Angelica dahurica* are recorded in the Compendium of Materia Medica, which is the most popular herb of all time (Wang et al., 2020).

Polysaccharides of *Angelica dahurica* are the main constituent of the Chinese herb *Angelica dahurica*, which is known to have antiviral, antioxidant, and immunomodulatory effects. It has been reported that the hydrolysis product of *Angelica dahurica* polysaccharide contains six monosaccharides, namely rhamnose, arabinose, xylose, mannose, glucose, and galactose, with a molar ratio of 1.19:1.19:0.765:1:8.08:3.34. Its peak molecular weight is 88538, and the UV and IR absorption peaks of the polysaccharide are visible, and it contains an amide structure, indicating that it is a class of amino polysaccharide(Kim et al., 2013). The biological activities of *Angelica dahurica* polysaccharides are antitumor activity, antioxidant activity, antiviral activity and immune-modulating ability(Hu et al., 2017; Yang et al., 2020; Zheng et al., 2016)

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The application of ultrasound in plant extraction is usually magnetic substances generated by a transducer, and its effective frequency is usually between 20 and 50 kHz. Extraction of herbs and Cyperus species extracts by ultrasonic extraction is superior to conventional extraction methods (Yin et al., 2007). The ultrasound extraction technique uses the mechanical, cavitation, and thermal properties of ultrasound, which is a physical crushing process(Liu et al., 2020), realizing cell wall fragmentation and efficient and rapid extraction of substances from cells. Using the increased rate of movement of the medium and enhancing the penetration of the medium, the extraction of bioactive substances is achieved (Tian et al., 2019; Li et al., 2023).

Polysaccharides are a common herbal medicine of plant, animal and fungal origin, which are primarily water-soluble polysaccharides(Dong et al., 2014). The polysaccharides are usually extracted by hot water leaching, whose extraction time is long and the glycopeptides in polysaccharides are prone to structural breakage during extraction and separation.Yang (Yang et al., 2014) studied three-factor-three-level Box-Behnken design was employed to optimize the ultrasonic power, extraction time and ratio of water to raw material to obtain a high AGP yield. The analysis of variance and response surface plots indicated that ultrasonic power was the most important factor affecting the extraction yield. Lin (Lin et al., 2021) investigated the ultrasonic extraction process of polysaccharides from the peel of red heart dragon fruit, and this paper was based on ultrasonic technology. It was concluded that the leaching amount was similar to the effect of ultrasonic treatment with different ultrasonic treatment times and different liquid phase ratio and different liquid phase content. Yu (Yu et al., 2022) concluded the effect of ultrasonic leaching time, leaching temperature and sulfuric acid concentration on the copper leaching effect using ultrasonic-assisted acid leaching process with copper-containing black mica. The ultrasonic-assisted acid leaching method extracted copper from copper-bearing black mica better than the conventional acid leaching method.

The research studied in this paper is devoted to ultrasonic-assisted extraction, and fewer studies have been conducted on ultrasonic extraction of *dahurica* polysaccharides, as a physical crushing process using ultrasonic waves to intensify the treatment of plant polysaccharides. Consequently, its implementation of the extraction of traditional Chinese medicine and the extraction of various plant active ingredients, in place of the conventional cutting process, can achieve the purpose of high efficiency, energy saving and environmental protection. In addition, ultrasonic waves cause many side effects, such as thermal effects, emulsification, diffusion, and crushing. This study was conducted to determine the process flow of polysaccharide extraction from *Angelica dahurica* and its optimal process parameters to provide better practical guidance for industrial production.

2. Material and methods

2.1. Materials and instruments

Angelica dahurica Bozhou Heyitang Trade Co., Ltd; Centrifuge (H1850R) Hunan Xiangyi Laboratory Instrument Development Co., Ltd; Suction filter (TS-02PVACUUMPUMP) Henan Aride Instrument and Equipment Co., Ltd; Ultrasonic cleaner (JK-500B) Hefei Jinnick Machinery Manufacturing Co., Ltd; Electric blast drying oven (DHG-70L) Hefei Youke Instrument Equipment Co., Ltd.

2.2. Raw material treatment

The purchased *Angelica dahurica* tablets are made into *Angelica dahurica* powder by a pulverizer and sifted through a 100-mesh sieve. To avoid moisture, they need to be stored in a sealed bag for standby.

2.3. Key points of experiment operation

- Weighing. Accurately weigh 1 g of *Angelica dahurica* powder, put it into a 50 mL centrifuge tube, add distilled water in proportion, stir evenly, and wait for use.
- Ultrasonic water extraction. The centrifuge tube is put into the ultrasonic cleaner, and the ultrasonic power, time, temperature and other parameters are set, and then the ultrasonic water bath method is used for soaking.
- Centrifuge. Place the sample after ultrasonic treatment in a centrifuge and centrifuge it at 6000 r/min for 15 min to obtain the supernatant.
- Alcohol precipitation (Wu et al., 2021). Add 3 times the volume of absolute ethanol into the supernatant after centrifugation, put it into a conical flask, and refrigerate it in a refrigerator at 5 °C for 12 hours.
- Suction filtration. Use a suction filter to separate *Angelica dahurica* polysaccharide solution and impurities.
- Dry. Put the beaker containing *Angelica dahurica* polysaccharide solution into the electric blast drying oven to dry for 3 hours, and the obtained substance is pure *Angelica dahurica* polysaccharide.

- Calculation. Weigh the purified *Angelica dahurica* polysaccharide. The yield of *Angelica dahurica* polysaccharide is calculated according to the following formula. In the formula, m1: mass of *Angelica dahurica* polysaccharide (g), m0: mass of *Angelica dahurica* powder (g).

$$\text{Yield of } \textit{Angelica dahurica} \text{ polysaccharide} = \frac{m1}{m0} \times 100\% \quad \text{........(1)}$$

2.4. Optimization of Extraction Process of *Angelica dahurica* Polysaccharide

2.4.1. Single factor experimental design

Effect of material to liquid ratio on extraction of *Angelica dahurica* polysaccharide

Based on 1 g of *Angelica dahurica* powder, when the ultrasonic power is 240 W, the temperature is 36 °C, and the ultrasonic time is 10 minutes, under this condition, the ratio of material to liquid is set to 1:10, 1:20, 1:30, 1:40, 1:50, and the best ratio of material to liquid is determined according to the above process.

Effect of ultrasonic power on extraction of *Angelica dahurica* polysaccharide

According to the above experiments, when the optimum ratio of material to liquid is 1:30, the temperature is 36 °C, and the ultrasonic time is 10 min, set the ultrasonic power to 240 W, 280 W, 320 W, 360 W, 400 W, and 500 W respectively, and determine the optimum ultrasonic power according to the above process flow.

Effect of temperature on extraction of *Angelica dahurica* polysaccharide

According to the previous single-factor test, the optimum ratio of material to liquid of *Angelica dahurica* polysaccharides is 1:30, the optimum ultrasonic power is 400 W, and then on the basis of the ultrasonic time of 10 min, the temperature is set at 26 °C, 36 °C, 46 °C, 56 °C, 66 °C respectively to determine the optimum temperature.

Effect of ultrasonic time on extraction of *Angelica dahurica* polysaccharide

According to the determination of the optimum addition amount, the effects of different ultrasonic time of 10 min, 20 min, 30 min, 40 min and 50 min on polysaccharide extraction were studied to determine the optimum ultrasonic time.

2.4.2. Orthogonal experiment

After single-factor experiments, the material-liquid ratio, ultrasonic power and ultrasonic time were obtained as the three main factors affecting the ultrasonic effect. According to the single-factor experiment of ultrasound-assisted method, the material-liquid ratio, ultrasonic treatment time and power were investigated by using three-level orthogonal experiments with the yield of polysaccharide as the index. The orthogonal experimental factors and level design are shown in Table 1.

Table 1 Factors and level design of orthogonal test for *Angelica dahurica* polysaccharide

Level	**A**	**B**	**C**
	Material-liquid ratio/(g/mL)	**Ultrasonic power/W**	**Ultrasound time/min**
1	1：20	300	35
2	1：30	400	40
3	1：40	500	50

3. Results and discussion

3.1. Effect of material to liquid ratio on extraction of *Angelica dahurica* polysaccharide

240W ultrasonic wave, 36 °C temperature, 10 min ultrasonic wave, set five groups of different material-liquid ratios 1:10, 1:20, 1:30, 1:40, 1:50 to carry out the experiment. The effect of the material-liquid ratio on the extraction of *Angelica dahurica* polysaccharide is shown in From Figure 1, it can be observed that the yield of polysaccharide was increasing from 1:10 to 1:30, and the yield was 15.2% at the feed-liquid ratio of 1:30, but the leaching rate of *dahurica* polysaccharide from 1:40 to 1:50 decreased with the increase of the solid-liquid ratio. As the higher solid-liquid ratio increases the viscosity of the extraction system, thereby affecting the cavitation effect of ultrasound, which is detrimental to the dissolution of polysaccharides; the lower the solid-liquid ratio, the less the influence of ultrasound on the unit volume, and the lower the leaching rate of *dahurica* polysaccharides . At this time, the best solid-liquid ratio was 1:30. Therefore, the three levels of 1:20, 1:30, and 1:40 were selected for the orthogonal test comprehensively.

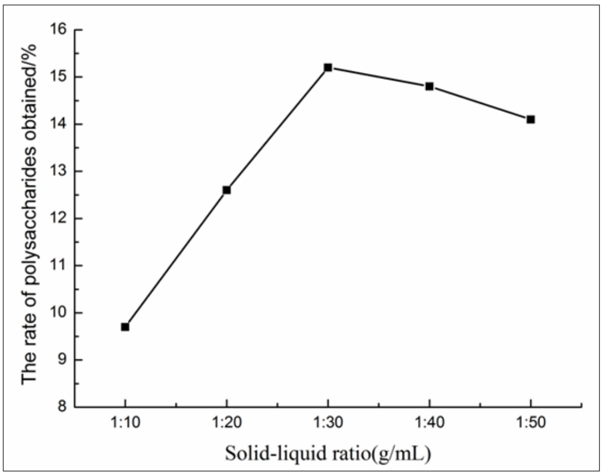

Figure 1 Effect of solid-liquid ratio on the yield of *dahurica* polysaccharide

3.2. Effect of ultrasonic power on extraction of *Angelica dahurica* polysaccharide

The optimum ratio of material to liquid determined from Figure 1 is 1:30. With other factors unchanged, the temperature is 36 °C and the ultrasonic time is 10 minutes. Six groups of different ultrasonic powers of 240 W, 280 W, 320 W, 360 W, 400 W and 500 W were tested. The effect of ultrasonic power on the extraction of *Angelica dahurica* polysaccharides is shown in Figure 2. As shown in Figure 2, the yield of polysaccharide increased and then decreased with the increase of ultrasonic power; the higher the ultrasonic power, the greater the amount of *dahurica* polysaccharide leached, but when the ultrasonic power exceeded 400 W, its leaching amount decreased. The extracted amount almost did not increase. Due to the special frequency of ultrasound and the special radiation surface. The energy increases, and as the sound pressure increases, the amplitude and pressure of the sound pressure in the fluid increases accordingly. The time required for cavitation bubble rupture is reduced. This is beneficial for increasing the extraction efficiency. However, it is possible to increase the force indefinitely. If the acoustic intensity is increased, the cavitation bubble will not have time to collapse in the compressed phase due to the excessive intensity of the acoustic field. Therefore, it is not beneficial for extraction and reduces the amount of extraction (Wen et al., 2019) .Therefore, the optimum ultrasonic power for extraction of polysaccharides was determined to be 400 W, when the extraction rate of polysaccharides was up to 15.6% at 400 W. Too high ultrasonic power would damage the structure of the cells and therefore, the polysaccharide yield decreased. Therefore, in the orthogonal experiment, three aspects were compared from 300 W, 400 W and 500 W.

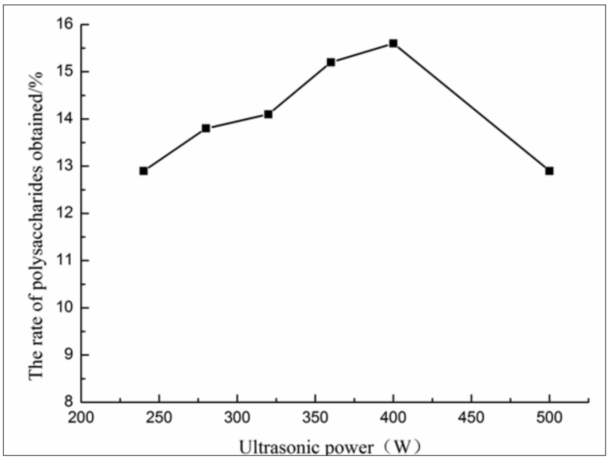

Figure 2 Effect of ultrasonic power on the yield of *dahurica* polysaccharide

3.3. Effect of temperature on extraction of *Angelica dahurica* polysaccharide

The first two groups of experiments showed that the best solid-liquid ratio and ultrasonic power were 1:30 and 400 W respectively, and the ultrasonic time was fixed at 10 minutes. Five groups of different temperatures were set at 26 °C, 36 °C, 46 °C, 56 °C and 66 °C to conduct the experiment. The effect of temperature on the extraction of *Angelica dahurica* polysaccharides is shown in Figure 3. It can be seen from Figure 3 that that the optimum temperature is 36 °C, and the yield of polysaccharide is 13.9%. From Figure 3, it seems that temperature has little effect on the extraction of *Angelica dahurica* polysaccharide, and this factor can be omitted in the orthogonal test.

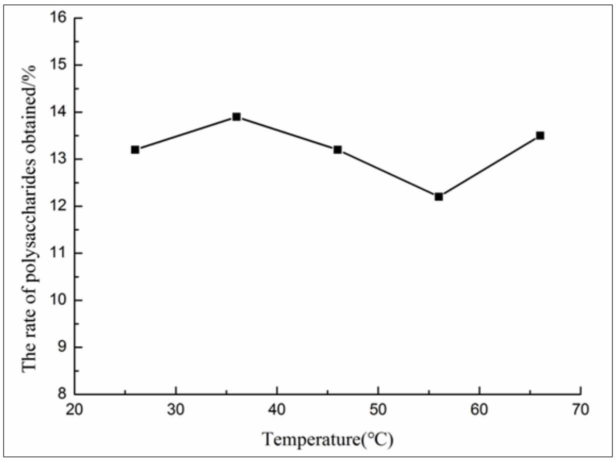

Figure 3 Effect of temperature on the extraction of polysaccharides from *Angelica dahurica*

3.4. Effect of ultrasonic time on extraction of *Angelica dahurica* polysaccharide

In the previous three groups of experiments, the optimum ratio of material to liquid was 1:30, the ultrasonic power was 400 W and the temperature was 36 °C. Five groups of different time were set for 10 min, 20 min, 30 min, 40 min, and 50 min for the experiment. The effect of ultrasonic action time on the extraction of *Angelica dahurica* polysaccharides is shown in Figure 4. As shown in Figures 4, the leaching of polysaccharides first decreased, and then rose and gradually decreased with the continuation of ultrasonication. The highest polysaccharide yield of 14.5% was obtained under the action of ultrasound for 40 min, which gradually decreased with the duration of time. This was because the action of ultrasound would be more persistent, and its cavitation and mechanical vibration effects would lead to the rupture of *dahurica* cells, thus causing further destruction of *dahurica* cells and thus improving the quality of the extraction. However, after some time, if a longer extraction cycle is sustained, the mechanical shearing effect of ultrasound causes the decomposition of macromolecular glycans at long-term temperature, resulting in post-processing losses and affecting the extraction effect. Therefore, the extraction time was selected as 40 min. the best extraction timing of polysaccharides was determined by ultrasonic method as 40 min. therefore, from all factors, three different levels of 20 min, 30 min and 40 min were selected for orthogonal experiments.

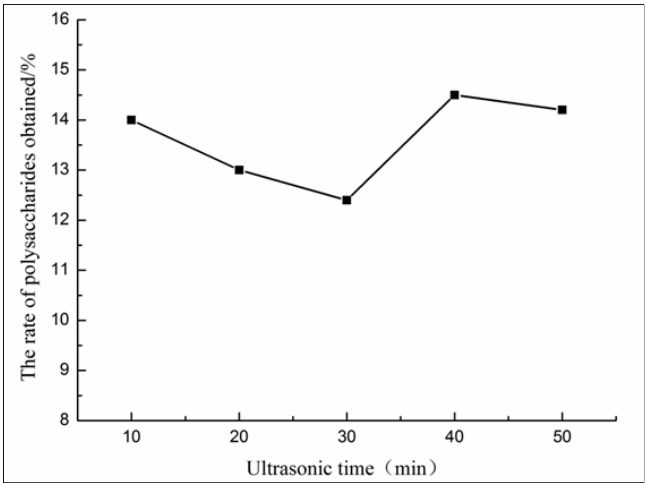

Figure 4 Effect of ultrasonic time on extraction of *Angelica dahurica* polysaccharide

3.5. Analysis of orthogonal test results

Comparing the K values and the extreme difference R values, it was found that the three factors of ultrasonic-assisted extraction of *dahurica* polysaccharide were: ultrasonic time > ultrasonic power > liquid ratio, and ultrasonic time had a significant effect on the yield of polysaccharide, while ultrasonic power and liquid ratio had a lower effect. On the basis of this conclusion, the best factor for *dahurica* polysaccharide extraction was A3B3C3, 1:40 material-liquid ratio, 500 W ultrasonic power and 45 min ultrasonic time in tube 9. According to this combination, the yield of polysaccharide reached 15.8%, which was the highest rate.

Table 2 Results of orthogonal test

Experiment No	A/（g/mL）	B/W	C/min	The rate of polysaccharides obtained/%
1	1：20	300	35	7.5
2	1：20	400	45	11.3
3	1：20	500	40	11.9
4	1：30	300	45	11.4
5	1：30	400	40	13
6	1：30	500	35	12.5
7	1：40	300	40	11.3
8	1：40	400	35	7.6
9	1：40	500	45	15.8
K_1	30.7	30.2	27.6	
K_2	36.9	31.9	36.2	
K_3	34.7	40.2	38.5	
R	6.2	10	10.9	

4. Conclusion

Combining the results of specific single-factor tests, it can be seen that different material-liquid ratios, ultrasonic power, temperature and ultrasonic time have certain effects on the yields. The results of the orthogonal tests were combined to confirm the optimum values. According to the experimental results, it can be seen that the relationship between the material-liquid ratio, ultrasonic power and ultrasonic action is significant. The orthogonal experiment and single-factor experiment were used to optimize the ultrasonic-assisted extraction of *dahurica* polysaccharides. The best polysaccharide extraction effect was 15.8% with the feed-liquid ratio of 1:40, ultrasonic power of 500 W and ultrasonic time of 45 min. In addition, there are few studies on *dahurica* polysaccharides, and subsequent studies can be conducted in the following aspects: preliminary exploration of its antioxidant and antibacterial effects, and further exploration of its structure and mechanism of action. In conclusion, this method has some reference value for the extraction of *dahurica* polysaccharides.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors have no conflict of interest.

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