

Ultrasonic-assisted Extraction, Purification, Antioxidant and Antibacterial Activity of Polysaccharide from *Cornus officinalis* Leaves

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Abstract The ultrasonic-assisted extraction process of polysaccharide from *Cornus officinalis* leaves (COLP) was optimized. After isolation and purification, the antioxidant and antibacterial activities of polysaccharide were studied *in vitro*. The extraction rate was highest under conditions of ultrasonic power 340 W, solvent to solid ratio 26:1(v/w), extraction temperature 71 °C and extraction time 2.3 h, reaching 7.04%, which was consistent with the model predicted value. The crude polysaccharide was isolated and purified by DEAE-52 cellulose chromatography column, and four components COLP-1, COLP-2, COLP-3 and COLP-4 were obtained. Then, Sephadex G-100 chromatography column was used to purify COLP-1 and COLP-3, and two components COLP-1-1 and COLP-3-2 were obtained. COLP-1-1 had strong scavenging ability against DPPH free radicals, against hydroxyl radical, and superoxide anion radical, with the highest clearance rate reaching 81.48%, 79.48% and 72.87% respectively. COLP-3-2 also had strong scavenging ability against DPPH free radicals, against hydroxyl radical, and superoxide anion radical, with the highest clearance rate reaching 88.57%, 76.64% and 86.08% respectively. The results indicated that COLP-1-1 and COLP-3-2 had higher antioxidant activity, and the antioxidant activity of COLP-3-2 was stronger than COLP-1-1. The results of antibacterial activity showed that COLP-1-1 had the highest antibacterial activity on *Bacillus subtilis* and weaker antibacterial activity on *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*; COLP-3-2 had a certain antibacterial activity on *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Bacillus subtilis*.

Keywords: *cornus officinalis* leaves, polysaccharide, ultrasonic-assisted extraction, antioxidant activity, antibacterial activity

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1. Introduction

The traditional Chinese medicine cornel dogwood is dried and ripe fruit of *Cornus officinalis* Sieb.et Zucc., which is first published in "Sheng Nong's herbal classic". It has the function of tonifying liver and kidney, astringent and solidifying, and is effective in treating vertigo, tinnitus, waist and knee pain, impotence and spermatorrhea, urination frequency, heart shaking powder, great sweat collapse, internal heat quench thirst and so on [1]. The fruit of *Cornus officinalis* is one of the traditional Chinese medicines with homologous medicine and food, which is a commonly used medicine in traditional Chinese medicine prescriptions [2]. Modern researches have found that the components of *Cornus officinalis* are complex and diverse, which not only contain abundant active ingredients such as iridoids, saponins, polysaccharides, tannins, organic acids, flavonoids, esters, but also contain a variety of essential trace elements for human body [3,4,5,6]. Modern

pharmacological studies have also found that it is not only effective in protecting liver, anti-inflammatory, antioxidant, anti-fatigue, anti-tumor, protecting myocardium, hypoglycemic, enhancing immunity, regulating heart rate, but also effective in the treatment of senile dementia and infertility [7,8,9].

Polysaccharide is an important active ingredient in *Cornus officinalis*, which has physiological functions such as anti-aging, hypoglycemic, antioxidant, anticancer, anti-virus, and enhancing immunity, which can be widely used in medicine and health food [8,10]. Wang found that a polysaccharide from *Fructus Corni* showed significant hypoglycemic and hypolipidemic effects [11]. Sun found that polysaccharide extracted from *Cornus officinalis* could improve secondary hippocampal damage of epileptic rats due to its antioxidation and antiapoptosis effects [12]. Wang found that polysaccharide of *Fructus corni* could regulate ovarian function-related hormone levels in aging mice, indicating that it had significant anti-aging effects [13].

Fruits are the traditional medicinal part of *Cornus officinalis*, while the cores and leaves are abundant, which are

basically discarded. In recent years, studies had shown that the cornel kernels were rich in tannins, unsaturated fatty acids, ursolic acid, gallic acid, amino acids, vitamins and so on, which were also effective in anti-inflammatory, anti-tumor, myocardial protection, anti-aging, anti-fatigue, etc. [8]. At present, there have been no reports on polysaccharide from *Cornus officinalis* leaves. In this study, the ultrasonic-assisted extraction process of polysaccharide from *Cornus officinalis* leaves was optimized by response surface methodology, and its antibacterial and antioxidant activities were studied in order to provide theory gist for the comprehensive utilization of *Cornus officinalis* leaves.

2. Materials and Methods

2.1. Materials and Reagents

Cornus officinalis leaves were gathered from mountainous area of western Henan Province, which were sieved using a 40 mesh screen after drying and crushing.

Chemical reagents such as trichloromethane, butyl alcohol, phenol, pyrogallol, ethanol, ascorbic acid (V_C), 1,1-diphenyl-2-picrylhydrazyl (DPPH), H_2O_2 were analytical grade, and they were purchased from Tianjin Deen Chemical Reagent Co., Ltd.

2.2. Extraction of Polysaccharide

Quantitatively weighed the ground *Cornus officinalis* leaves, and put them in a Soxhlet extractor degreasing with petroleum ether, and then dried out. Added an appropriate amount of distilled water as the extraction medium and mixed well. Ultrasonic crushed, and then heated in a water bath at a certain time. Centrifuged at 8000 r/min for 10 min and collected the supernatant. Three times the volume of anhydrous ethanol was added to the supernatant, and then it was allowed to stand overnight. The precipitation was centrifuged at 8000 r/min for 15 min. Sevage method was used to remove the protein [14], H_2O_2 method to remove the pigment [15], and then vacuum freeze-dried to obtain crude polysaccharide.

2.3. Determination of Polysaccharide Extraction Rate

The concentration of polysaccharide was determined by phenol-sulfuric acid colorimetry [16]. The polysaccharide concentration was calculated using a linear equation: $Y = 11.93X - 0.0462$, $R^2 = 0.9972$ (Using glucose as the standard curve to get the relationship between sugar concentration and absorbance value).

$$\text{Polysaccharide extraction rate (\%)} = \frac{\left[\frac{\text{Polysaccharide concentration}}{\times \text{Polysaccharide solution volume}} \right]}{\text{Dry weight of sample}} \times 100\%.$$

2.4. Single Factor Test

The effects of ultrasonic power (150, 250, 350, 450, 550, 650 W), solvent to solid ratio (15:1, 25:1, 35:1, 45:1,

55:1 (v/w)), extraction temperature (40, 50, 60, 70, 80, 90°C) and extraction time (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 h) on the extraction effect of polysaccharide from *Cornus officinalis* leaves were investigated.

2.5. Response Surface Test Design

On the basis of single factor test results, the four-factor and three-level central composite design (CCD) was performed: ultrasonic power (X_1), solvent to solid ratio (X_2), extraction temperature (X_3) and extraction time (X_4) (Table 1).

Table 1. Factors and levels of central composite design

Factors		Levels				
		-2	-1	0	1	2
Ultrasonic power (W)	X_1	250	300	350	400	450
Solvent to solid ratio (w/v)	X_2	15:1	20:1	25:1	30:1	35:1
Extraction temperature (°C)	X_3	60	65	70	75	80
Extraction time (h)	X_4	1.0	1.5	2	2.5	3.0

2.6. Separation and Purification of Polysaccharide

The crude polysaccharide from *Cornus officinalis* leaves was prepared as a 5 mg/mL solution, and DEAE-52 chromatography column (2.6 cm×30 cm) was used for chromatography. The sample quantity was 10 mL, and flow rate was 1.25 mL/min. Distilled water, 0.10, 0.20, 0.30, 0.40 mol/L NaCl solution was used for gradient elution respectively. One tube was collected every 5 mL by the automatic fraction collector. Phenol-sulfuric acid method was used to measure the absorbance at 490 nm, and the elution curve was plotted. The peak eluate samples were combined, dialyzed against deionized water for 2 d, and freeze-dried in vacuo.

Lyophilized groups collected from DEAE-52 column chromatography were distributed to make 5 mg/mL solutions, and 2 mL was loaded. Sephadex G-100 column was used for chromatography, and flow rate was 0.5 mL/min with distilled water as eluent. The eluate was collected by automatic fraction collector, and the absorbance was measured to plot elution curve. The peak eluate samples were combined, dialyzed, and vacuum freeze-dried to obtain refined polysaccharide.

2.7. Determination of DPPH Radical Scavenging Ability

According to the method of Yuan [17]: 1.0 mL polysaccharide solution was taken, adding 4 mL 0.004% DPPH solution, and mixed evenly. Then, it was standing in the dark for 30 min. The absorbance was measured at 517 nm. Absolute ethanol was used as blank control and V_C of corresponding concentration was used as the positive control. Each sample was measured 3 times in parallel and the average value was taken. The clearance formula could be expressed as:

$$\text{DPPH radical scavenging rate (\%)} = \left(1 - \frac{A_1}{A_2} \right) \times 100\%$$

In the formula, A_1 was the sample solution or positive control absorbance, A_2 was the blank control absorbance.

2.8. Determination of Hydroxyl Radical Scavenging Ability

According to the method of Yuan [18]: 0.4 mL pH 7.4 PBS, 0.25 mL redistilled water, 0.5 mL FeSO_4 (7.5 mmol/L) and 0.15 mL phenanthroline solution (5 mmol/L) were added into the test tube, and mixed evenly. Then, 1.0 mL polysaccharide solution and 0.1 mL 1% hydrogen peroxide solution was added at last. The absorbance was measured at 536 nm after a water bath at 37°C for 60 min. V_C of corresponding concentration was used as the positive control, the damaged tube with no sample, the control tube with no sample and hydrogen peroxide, and the blank tube with no hydrogen peroxide. Each sample was measured 3 times in parallel and the average value was taken. The clearance rate formula could be expressed as:

$$\begin{aligned} & \text{Hydroxyl radical scavenging rate (\%)} \\ &= \frac{A_{\text{sample}} - A_{\text{damaged}}}{A_{\text{control}} - A_{\text{blank}}} \times 100\%. \end{aligned}$$

In the formula, A_{sample} was the sample tube absorbance, A_{damaged} was the damaged tube absorbance, A_{control} was the control tube absorbance, A_{blank} was the blank tube absorbance.

2.9. Determination of Superoxide Anion Radical Scavenging Ability

According to the method of Yuan [18]: 0.1 mL polysaccharide solution with different mass concentrations was taken, and 2.8 mL Tris-HCl buffer solution (0.05 mol/L), 0.1 mL pyrogallol (3 mmol/L) were added. The control group was distilled water, while the blank group was 2.8 mL Tris-HCl buffer solution, 0.1 mL distilled water, and 0.1 mL HCl (0.01 mol/L). The positive control was V_C . After reaction for 30 s, the absorbance was determined at 320 nm every 30 s, and the reaction was performed for 5 min.

$$\begin{aligned} & \text{Superoxide anion radical scavenging rate (\%)} \\ &= \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100\% \end{aligned}$$

In the formula, A_{control} was the control group absorbance; A_{sample} was the sample solution absorbance.

2.10. Bacteriostatic Experiment

The test bacteria were inoculated on the bacterial medium, incubated in a constant temperature incubator at 37°C for 24 h. The activated bacteria were picked up by inoculating loop and placed in sterilized distilled water, and then mixed to make a bacterial suspension.

Filter paper method [19] was used for the bacteriostatic test: the sterilized round filter paper (Φ 6 mm) was dried and immersed in the prepared polysaccharide solution for 12 h. Then, the prepared various bacterial suspensions (200 μL) were placed on the solid medium surface and spread evenly. The soaked filter paper was pasted on the medium surface with sterilized forceps, and incubated in a constant temperature incubator at 37°C for 18–24 h. The

inhibition zone was observed and its diameter was measured. The blank control was treated with sterile water.

3. Results and Discussions

3.1. Single Factor Test Results

With the increase of ultrasonic power, the extraction rate of polysaccharide first increased and then decreased (Figure 1a). When the ultrasonic power was 250 W, the extraction rate of polysaccharide reached the highest 4.96%; then decreased gradually. Due to the mechanical and cavitation effect of ultrasonic waves, the cell wall and membrane structure of *Cornus officinalis* leaves would be destroyed, so that polysaccharide could be dissolved more easily. Moreover, the more fully cell wall was destroyed with the increase of ultrasonic power, the more fully the polysaccharide would be dissolved. However, high ultrasonic power would not only change the structure of polysaccharide, but also break the bonds between polysaccharide molecules, and cause degradation. Moreover, sound intensity would also increase with the increase of power, leading to the generation of numerous vacuoles, and the energy transmission would be reduced by reflecting acoustic waves, which was not conducive to the extraction process, resulting in a decline of polysaccharide extraction rate [20]. Therefore, the ultrasonic power was chosen to be 250 W.

The extraction rate of polysaccharide increased gradually with the decrease of solvent to solid ratio (Figure 1b). When solvent to solid ratio was 25:1(v/w), the extraction rate was 5.04%, and then gradually stabilized. This was probably because the total amount of dissolved polysaccharide in *Cornus officinalis* leaves would increase with the increase of extract solution amount. In addition, the concentration of protein in the whole extraction system would decrease with the increase of extract solution amount, which would reduce the adsorption effect of protein deposition on polysaccharide and the loss of polysaccharide [21]. However, an overtop solvent to solid ratio would increase raw materials and reagents amount, cause waste and increase costs, as well as increase the difficulty of post-treatment process. Therefore, solvent to solid ratio was chosen to be 25:1(v/w), which could ensure a high extraction rate and reduce the extraction solution amount.

The extraction rate of polysaccharide increased rapidly and then reduced gradually with the rise of extraction temperature (Figure 1c). The extraction rate of polysaccharide reached the highest 5.52% when the temperature was 70°C. This was probably because the solubility of polysaccharide would increase with the extraction temperature rise, and the dissolution amount of polysaccharide would increase, leading to the rise of extraction rate. However, the color of extracted polysaccharide was darker when the extraction temperature was too high, which might be because the high temperature would lead to the destruction of polysaccharide structure, and the degradation of polysaccharide [19]. Moreover, high temperature would accelerate the release rate of impurities in leaves, leading to a reduction in polysaccharide yield. Therefore, 70°C was a suitable extraction temperature.

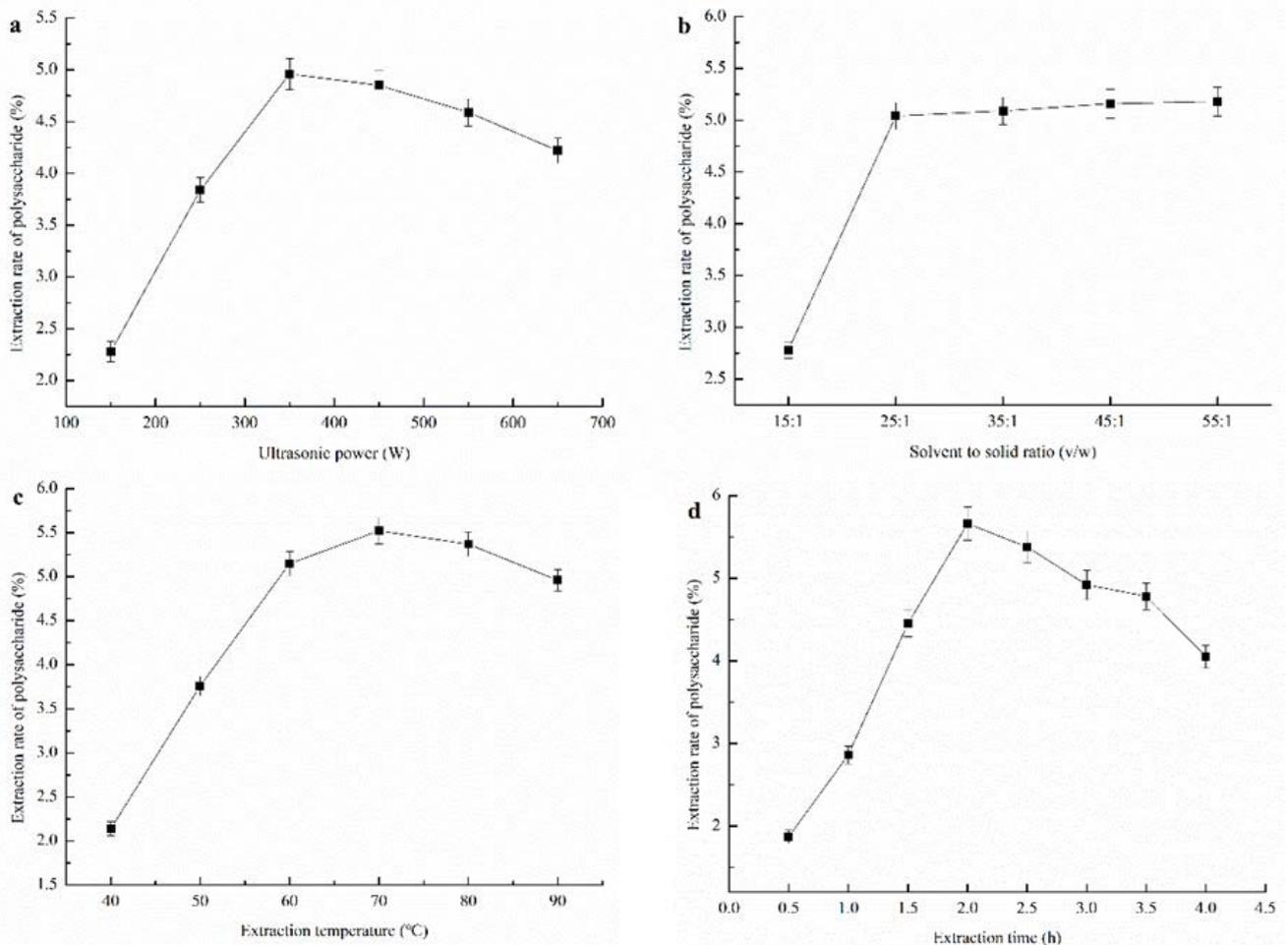


Figure 1. Effects of different factors on the extraction rate of polysaccharide. a ultrasonic power, b solvent to solid ratio, c extraction temperature, d extraction time

Within 0.5~2.5 h, the extraction rate of polysaccharide gradually rose (Figure 1d). The extraction rate reached the maximum value of 5.66% when the extraction time was 2.0 h. However, the extraction rate gradually decreased after 2.0 h. The longer the extraction time was, the more beneficial it was to polysaccharide dissolution, which resulting in the rise of the extraction rate within a certain period. However, a large amount of viscous substances in the leaves would be dissolved if the extraction time is too long, and the impurities and viscosity of the solution would increase, which would affect the dissolution of polysaccharide. In addition, and prolonged heating could break polysaccharide molecular chains into small molecules and degrade the polysaccharide, increasing the loss of polysaccharide during alcohol precipitation process, leading to a decline in polysaccharide extraction rate. Therefore, 2.0 h was a suitable extraction time.

3.2. Results and Analysis of CCD

3.2.1. Results of CCD

Design-expert 8.0 was used to design the four-factor and three-level central combination test. The response value was extraction rate of polysaccharide. The design scheme and results of the 27 experimental combinations were shown in Table 2.

3.2.2. Model Establishment and Significance Test

The Design-expert software was used to analyze the experimental results, and the fitted equation was as follows:

$$Y = 7.00 - 0.23X_1 + 0.11X_2 - 0.011X_3 + 0.28X_4 + 0.055X_1X_2 - 0.17X_1X_3 + 0.054X_1X_4 + 0.23X_2X_3 + 0.000X_2X_4 - 0.020X_3X_4 - 0.57X_1^2 - 0.27X_2^2 - 0.26X_3^2 - 0.25X_4^2.$$

At the same time, the variance analysis results of the regression equation by analysis software were shown in Table 3.

As could be seen from Table 3, the model was highly significant ($p < 0.01$). At the same time, the p values of X_1 , X_2 , X_4 , X_1X_3 , X_2X_3 , X_1^2 , X_2^2 , X_3^2 , X_4^2 in the model were all less than 0.05, indicating that they were all significant; in other words, they had significant effects on the extraction rate of polysaccharide. The lack of fit was 0.1924, and it's not significant ($p > 0.05$), which indicated that the model was properly selected.

In addition, correlation coefficient R^2 of the model was 0.9491, which could explain 94.91% of experimental results, indicating that the model had a good correlation. C.V. of the model was 3.98%, which indicated that the experimental operation was reliable and could accurately

reflect the experimental results. In conclusion, the model had good fitting degree and small experimental error, which could be used to analyze and predict the extraction rate of polysaccharide.

Among the four factors, the influence of solvent to solid ratio was significant, and ultrasonic power and extraction time was extremely significant. The order of four factors affecting the extraction rate was as follows: $X_4 > X_1 > X_2 > X_3$.

Table 2. Scheme and results of central composite design

Runs	Factors and levels				Extraction rate of polysaccharide (%)
	X_1 Ultrasonic power	X_2 Solvent to solid ratio	X_3 Extraction temperature	X_4 Extraction time	
1	2	0	0	0	4.58±0.22
2	0	0	-2	0	6.19±0.31
3	0	-2	0	0	5.88±0.28
4	0	0	0	-2	5.72±0.29
5	1	1	-1	-1	5.18±0.24
6	0	0	0	2	6.69±0.34
7	0	0	0	0	7.11±0.35
8	-1	1	-1	1	5.83±0.28
9	0	0	2	0	6.16±0.32
10	1	-1	1	-1	4.40±0.21
11	-1	-1	-1	-1	5.70±0.27
12	0	0	0	0	6.88±0.35
13	1	-1	1	1	5.12±0.25
14	-1	-1	-1	1	6.04±0.31
15	1	1	-1	1	5.71±0.28
16	0	0	0	0	7.02±0.36
17	1	1	1	1	5.81±0.28
18	-1	1	1	-1	6.08±0.31
19	1	1	1	-1	5.16±0.24
20	-1	1	-1	-1	5.07±0.25
21	1	-1	-1	-1	5.09±0.26
22	1	-1	-1	1	5.97±0.29
23	-1	1	1	1	6.49±0.32
24	-1	-1	1	-1	5.46±0.26
25	0	2	0	0	6.38±0.31
26	-2	0	0	0	5.25±0.25
27	-1	-1	1	1	5.87±0.28

Table 3. Variance analysis (ANOVA) of test results

Source	Sum of squares	df	Mean square	F- value	p- value, Prob>F	
Model	11.97	14	0.86	15.99	< 0.0001	significant
X_1	1.23	1	1.23	23.05	0.0004	**
X_2	0.30	1	0.30	5.59	0.0357	*
X_3	2.817E-003	1	2.817E-003	0.053	0.8224	
X_4	1.84	1	1.84	34.33	< 0.0001	**
X_1X_2	0.048	1	0.048	0.90	0.3603	
X_1X_3	0.46	1	0.46	8.64	0.0124	*
X_1X_4	0.046	1	0.046	0.86	0.3710	
X_2X_3	0.86	1	0.86	15.99	0.0018	**
X_2X_4	1.776E-015	1	1.776E-015	3.320E-014	1.0000	
X_3X_4	6.400E-003	1	6.400E-003	0.12	0.7354	
X_1^2	6.96	1	6.96	130.11	< 0.0001	**
X_2^2	1.53	1	1.53	28.53	0.0002	**
X_3^2	1.40	1	1.40	26.18	0.0003	**
X_4^2	1.32	1	1.32	24.67	0.0003	**
Residual	0.64	12	0.054			
Lack of fit	0.62	10	0.062	4.58	0.1924	not significant
Pure error	0.027	2	0.013			

Note: * significant, $p < 0.05$; ** very significant, $p < 0.01$.

3.2.3. Response Surface Analysis

In order to analyze the interaction between various factors and determine the best experimental point, response surface graph was drawn by Design-expert software to evaluate the interaction of each factor on the extraction effect. The response surface graph could visually see the interaction between various factors and the optimal parameters [22]. Figure 2 was the response surface graph of the interaction between various factors. The response surface slopes of 2b and 2d were both relatively steep, indicating that the interaction between X_1 and X_3 , X_2 and X_3 had a significant effect on the extraction

rate. However, the response surface slopes of 2a, 2c, 2e, and 2f were relatively gentle, indicating that the interaction among other factors had no significant influence on the extraction rate.

3.2.4. Confirmatory Experiment

Through further analysis of software, the corresponding values of each factor (X_1 , X_2 , X_3 , X_4) at the maximum response value (Y) were: $X_1=341.01$ W, $X_2=26.23:1$ (v/w), $X_3=70.64^\circ\text{C}$, $X_4=2.27$ h. Under these conditions, the theoretical extraction rate of polysaccharide was 7.1104%.

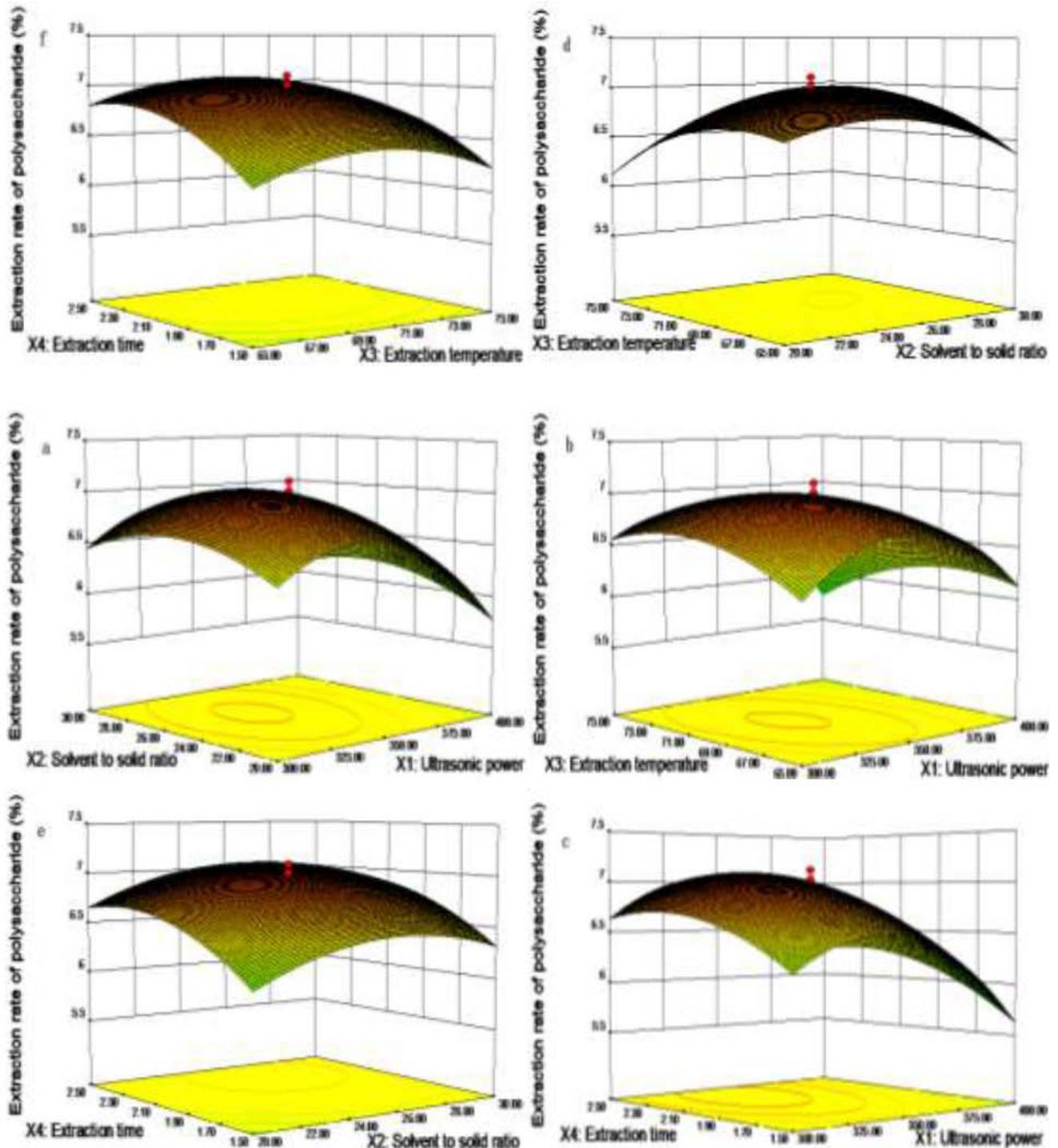


Figure 2. Influence of various factors on extraction rate of polysaccharide

The reliability of the results was verified according to the optimal value of each factor determined by response surface. According to the feasibility of actual operation, the optimal process conditions were modified to ultrasonic power 340 W, solvent to solid ratio 26:1 (v/w), extraction temperature 71°C, and extraction time 2.3 h. Under the modified optimal experimental conditions, multiple repeated experiments were performed, and the average extraction rate of polysaccharide was 7.04%, which was very close to theoretical value, and the relative error was only 0.99%, indicating that the regression model was high accuracy.

3.3. Separation and Purification of Polysaccharide

The separation and purification result of crude polysaccharide by DEAE-52 was shown in Figure 3a. Due to the existence of some charged groups such as sulfuric acid group and uronic acid in polysaccharide components, the components with single charge were preliminarily separated according to ionic strength. COLP-1, COLP-2, COLP-3 and COLP-4 were eluted by 0, 0.10, 0.20, and 0.30 mol/L NaCl solution respectively. Due to the low content of COLP-2 and COLP-4, the components COLP-1 and COLP-3 were dialyzed, vacuum freeze-dried and further purified.

COLP-1 and COLP-3 were further purified by Sephadex G-100 column. Two components, COLP-1-1 and COLP-1-2, were obtained from COLP-1, but the content of COLP-1-2 was very low (Figure 3b). Similarly, two components, COLP-3-1 and COLP-3-2, were eluted from COLP-3, but the content of COLP-3-1 was very low (Figure 3c). COLP-1-1 and COLP-3-2 were dialyzed and vacuum freeze-dried to obtain refined polysaccharide.

3.4. Antioxidant activity of COLP-1-1 and COLP-3-2

DPPH radical is a nitrogen-centered free radical with stable chemical properties, which is relatively difficult to remove. If it can be removed by the subject, it indicates that the subject has strong scavenging ability. The scavenging ability of COLP-1-1 and COLP-3-2 to DPPH radical increased with the rise of sample concentration (Figure 4a). When the sample concentration was 1.2 mg/mL, the clearance rate of COLP-1-1 and COLP-3-2 to DPPH radical reached 81.48% and 88.57%, respectively. COLP-1-1 and COLP-3-2 had lower scavenging ability to DPPH radical than V_C, while COLP-3-2 had stronger scavenging ability than COLP-1-1. The results showed that COLP-1-1 and COLP-3-2 had strong ability to scavenge DPPH radical.

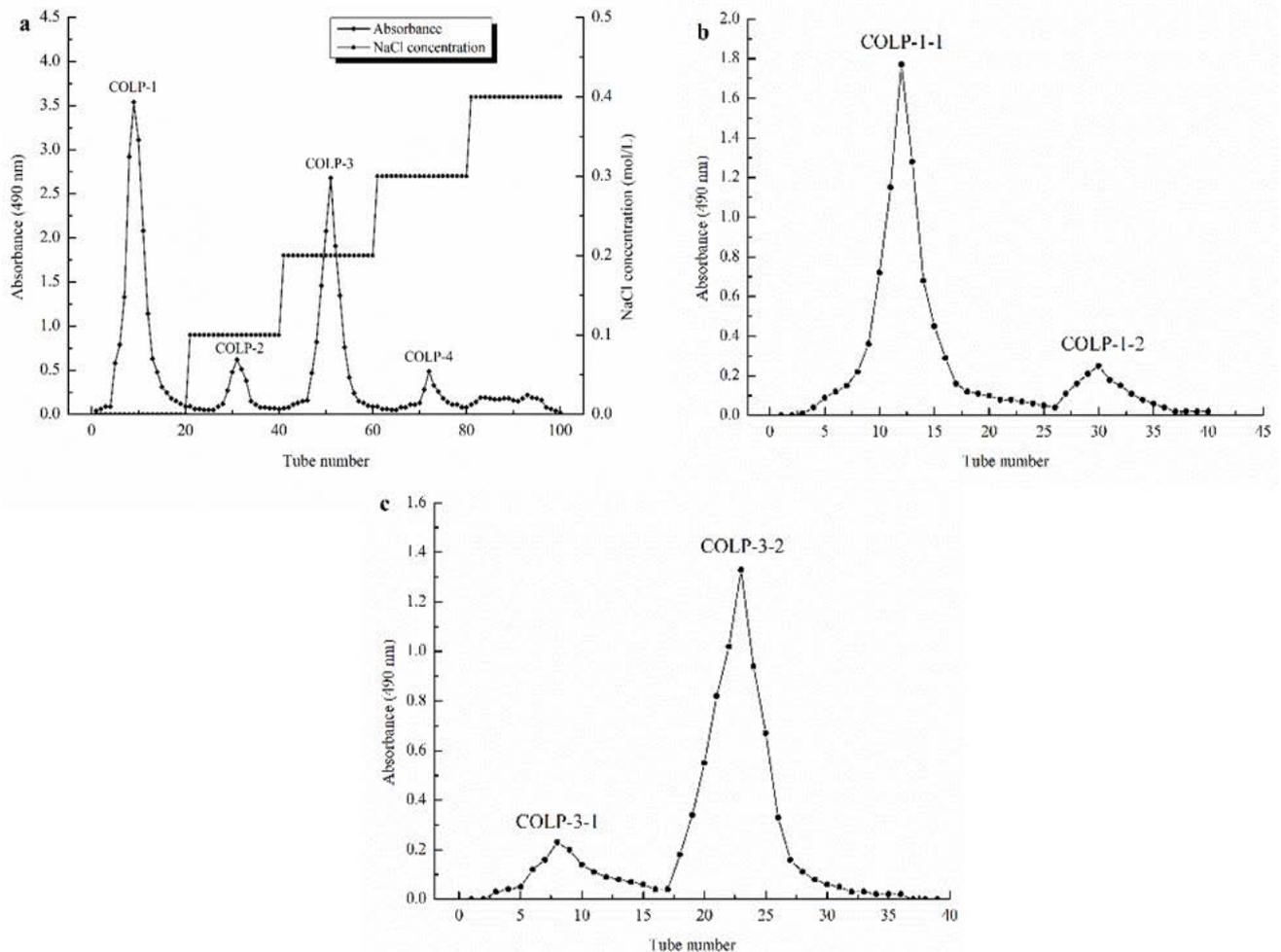


Figure 3. Elution curve of polysaccharide by chromatographic column. a elution curve of DEAE-52 cellulose column; b, c elution curve of Sephadex G-100

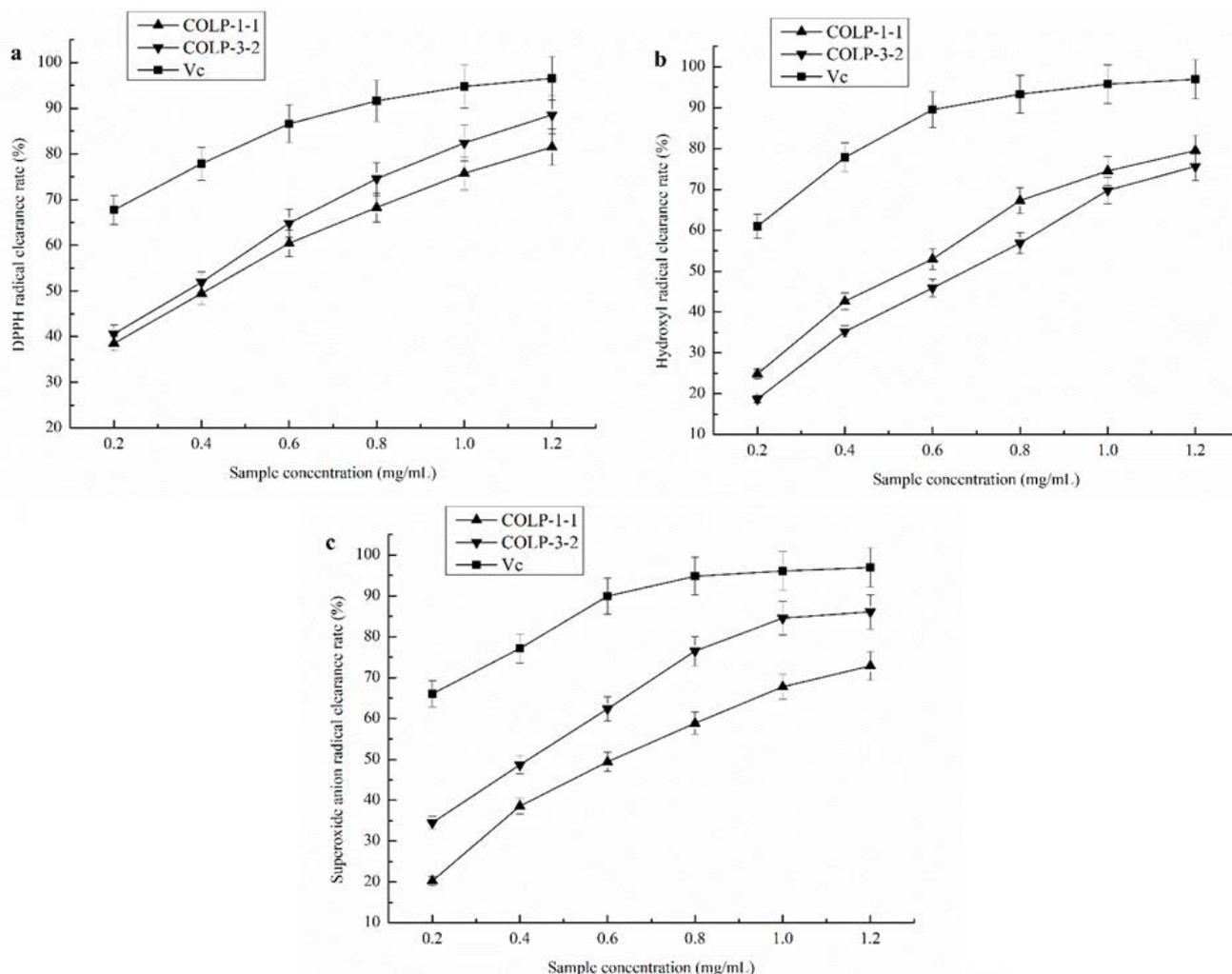


Figure 4. Scavenging ability of polysaccharide. a scavenging ability to DPPH radical, b scavenging ability to hydroxyl radical, c scavenging ability to superoxide anion radical

Hydroxyl radical can cause serious damage to macromolecules such as proteins and nucleic acids, so the scavenging effect of test substances on hydroxyl radical is also an important indicator to measure its antioxidant capacity [23]. The scavenging ability of COLP-1-1 and COLP-3-2 to hydroxyl radical increased with the rise of sample concentration (Figure 4b). When sample concentration was 1.2 mg/mL, the clearance rate of COLP-1-1 and COLP-3-2 to hydroxyl radical reached 79.48% and 76.64%, respectively. COLP-1-1 and COLP-3-2 had lower scavenging capacity to hydroxyl radical than V_C, while COLP-1-1 had stronger scavenging ability than COLP-3-2. The results showed that COLP-1-1 and COLP-3-2 had strong scavenging ability to hydroxyl radical.

The scavenging ability of COLP-1-1 and COLP-3-2 to superoxide anion radical increased with the rise of sample concentration, and showed a good dose-effect relationship (Figure 4c). When sample concentration was 1.2 mg/mL, the clearance rate of COLP-1-1 and COLP-3-2 to superoxide anion radical reached 72.87% and 86.08%, respectively. Both COLP-1-1 and COLP-3-2 had lower scavenging capacity to superoxide anion radical than V_C, while COLP-3-2 had stronger scavenging ability than COLP-1-1. The results showed that COLP-1-1 and COLP-3-2 had strong scavenging ability to superoxide anion radical.

3.5. Antibacterial Activity of COLP-1-1 and COLP-3-2

COLP-1-1 had the strongest antibacterial activity on *B. subtilis*, and the diameter of inhibition zone could reach 20.52 mm; besides, it also had a certain antibacterial activity on *E. coli*, *S. typhimurium* and *S. aureus*, but the inhibition zone was small and the antibacterial activity was weak; it had no inhibitory effect on *P. aeruginosa* and *P. mulfocida* (Table 4). COLP-3-2 had weak antibacterial activity on *E. coli*, *P. aeruginosa*, *S. typhimurium* and *B. subtilis*, but no inhibitory effect on *S. aureus* and *P. mulfocida* (Table 4).

Table 4. Antibacterial activity of COLP-1-1 and COLP-3-2

Strains	Inhibitory zone diameter (mm)	
	COLP-1-1	COLP-3-2
<i>Escherichia coli</i>	9.47±0.18	13.33±0.26
<i>Pseudomonas aeruginosa</i>	—	12.16±0.25
<i>Salmonella typhimurium</i>	11.74±0.24	14.48±0.27
<i>Staphylococcus aureus</i>	16.76±0.33	—
<i>Pastevula mulfocida</i>	—	—
<i>Bacillus subtilis</i>	20.52±0.48	14.36±0.26

Note: "—" indicated no antibacterial activity.

In conclusion, COLP-1-1 had stronger inhibitory effect on gram-positive bacteria and weak inhibitory effect on gram-negative bacteria. While, COLP-3-2 had stronger inhibitory effect on gram-negative bacteria than gram-positive bacteria.

4. Conclusions

In this study, the ultrasonic-assisted extraction process of COLP was optimized by response surface method. Then, antioxidant and antibacterial activities of polysaccharide were studied after isolation and purification. The correlation coefficient of the optimal regression mathematical model for the extraction of polysaccharide was 0.9491 through the central combination design, which indicated that the model had a good correlation and could accurately predict the extraction rate. Among the four factors, ultrasonic power and extraction time had extremely significant effects on the extraction rate, and solvent to solid ratio had significant effect, and the order of four factors affecting the extraction rate was extraction time > ultrasonic power > solvent to solid ratio > extraction temperature. After optimization, the optimal solution for polysaccharide extraction was as follows: ultrasonic power 340W, solvent to solid ratio 26:1(v/w), extraction temperature 71°C and extraction time 2.3h. Under these conditions, the extraction rate was 7.04%, which was basically consistent with the theoretical value.

DEAE-52 cellulose chromatography column was used to isolate and purify crude polysaccharide, and four components, COLP-1, COLP-2, COLP-3 and COLP-4, were obtained. Then, COLP-1 and COLP-3 were further purified by Sephadex G-100 chromatography column, and two components COLP-1-1 and COLP-3-2 were obtained.

Antioxidant activity experiment results showed that COLP-1-1 and COLP-3-2 had strong antioxidant activity, and COLP-3-2 was higher than COLP-1-1. COLP-1-1 had strong scavenging ability against DPPH free radicals, against hydroxyl radical, and superoxide anion radical, with the highest clearance rate reaching 81.48%, 79.48% and 72.87% respectively. COLP-3-2 also had strong scavenging ability against DPPH free radicals, against hydroxyl radical, and superoxide anion radical, with the highest clearance rate reaching 88.57%, 76.64% and 86.08% respectively. The biological activity of polysaccharide is closely related to its primary and advanced structure. The antioxidant activity of polysaccharide is not only related to composition of monosaccharides, type of glycosidic bonds, type, number and position of substituents, and length of branch chains, but also influenced by molecular weight and advanced structure [24]. Therefore, it is necessary to analyze the structure of polysaccharide from *Cornus officinalis* leaves in the follow-up study.

Results of bacteriostatic experiment showed that COLP-1-1 had the strongest antibacterial activity on *B. subtilis* and weaker antibacterial activity on *E. coli*, *S. typhimurium* and *S. aureus*; COLP-3-2 had weak antibacterial activity on *E. coli*, *P. aeruginosa*, *S. typhimurium* and *B. subtilis*.

This study provides theory gist for the comprehensive utilization of *Cornus officinalis* leaves resources, and a

certain theoretical basis for screening natural antibacterial and antioxidant substances.

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Conflicts of Interest

The authors declare no conflict of interest.

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