Optimization of Extraction of *Chaenomeles lagenaria* Polysaccharide and its Antibacterial Activity

Yahua Wu,[a] Xinyue Yin,[a] Jie Li,[a] and Shiquan Qian [b,*]

A water-soluble polysaccharide from *Chaenomeles lagenaria* (CLWSP-1) was purified and structurally identified. Then, response surface methodology (RSM) was performed to optimize the hot water extraction (HWE) of CLWSP-1. In addition, the antibacterial activity of CLWSP-1 was also evaluated. The results indicated that the polysaccharide CLWSP-1 mainly contained galacturonic acid, arabinose, and galactose, and its molecular weight was 1.23×10^2 kDa. The optimal HWE for extraction of CLWSP-1 was a ratio of water to solid of 48.9 mL/g, temperature of 91 °C, and an extraction time of 114 min; an ethanol concentration of 81% and a 1.24% concentration of CLWSP-1 were achieved. Moreover, the obtained CLWSP-1 had strong antibacterial activity when exposed to *Escherichia coli* and *Staphylococcus aureus*, suggesting that CLWSP-1 may potentially contribute to the development of a natural preservative in the food industry.

Keywords: Chaenomeles lagenaria; Polysaccharide; Antibacterial activity

*Contact information: a: College of Food and Biological Engineering, Bengbu University, Bengbu, Anhui 233030, China; b: Jiangsu Key Laboratory for Eco-Agricultural Biotechnology Around Hongze Lake, Jiangsu Collaborative Innovation Center of Regional Modern Agriculture and Environmental Protection, Jiangsu Key Laboratory for Food Safety and Nutrition Function Evaluation, Huaiyin Normal University, Huaian 223300, China; *Corresponding author: qianshq@163.com*

INTRODUCTION

Chaenomeles lagenaria is a large perennial evergreen cork herb from fruit trees (Crous *et al.* 2013). As a medicinal and food homologous plant, *C. lagenaria* is rich in nutrients, such as polysaccharides, organic acids, pectin, protein, nitrogen acids, vitamins, minerals, steroids, flavonoids, triterpenes, and other active substances. It displays biological activities, such as bacteriostasis, antioxidant qualities, immune regulation, and cancer prevention; thus, it has a high medical value and broad market application prospects (Parikh *et al.* 2014; Bhattacharjee and Perumal 2019; Suroowan *et al.* 2019). However, at present, there are few reports on the extraction of polysaccharides from *Chaenomeles lagenaria*.

Many methods have been developed to extract polysaccharides from plants, including hot water extraction (Zhou *et al.* 2020), ultrasound-assisted extraction (Chen *et al.* 2019), and microwave extraction (Hu *et al.* 2019). Previous studies have shown that excessive ultrasound radiation leads to the degradation of soluble polysaccharides (Xu *et al.* 2018; Dou *et al.* 2019). Microwave radiation could also destroy structures of polysaccharides and decrease the activity of polysaccharides, which makes it difficult to achieve large-scale industrial applications (Singh *et al.* 2012; Zhou *et al.* 2013). Hot water extraction could retain polysaccharides in plants to a great extent, and experimental operation is simple and safe, which provides clarity on the effects of relevant factors on the extraction of plant polysaccharides (Liu *et al.* 2016; Yun *et al.* 2019). In the present study,

Chaenomeles lagenaria was used as the research object. A water-soluble polysaccharide named CLWSP-1 was purified from *Chaenomeles lagenaria*, and its structural analysis was conducted. Then, the hot water process of extraction CLWSP-1 was performed under optimal conditions. Moreover, the antibacterial assays were investigated to evaluate the bacteriostatic effects of CLWSP-1.

EXPERIMENTAL

Materials

Chaenomeles lagenaria was harvested from a local farm in Xuancheng, China. *Chaenomeles lagenaria* was deseeded and cut into thin slices with thicknesses of 1 cm to 2 cm. The thin slices were dehydrated at 65 °C for 1.5 h to keep constant and were crushed through 60-mesh sieves. All chemicals used in this study were purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China.

Methods

Microorganism and culture mediums

Escherichia coli (CICC 10899) and *Staphylococcus aureus* (CICC 10001) obtained from the China Center of Industrial Culture Collection, Beijing, China were used as indicator bacteria. The Luria-Bertani (LB) medium (g/L) contained 10 g of NaCl, 10 g of trypsin, 5 g of beef extracts, and 10 g of agar. It was maintained at a pH 7.0 and a temperature of 121 °C, and was sterilized for 20 min.

Extraction of polysaccharides from Chaenomeles lagenaria

A degreasing treatment was performed on 5 g of the *Chaenomeles lagenaria* sample with anhydrous ether evenly blended with 100 mL of deionized water. The mixed solution was treated at 90 °C for 2 h, and the supernatant was obtained at 6000 r/min for 10 min. Twice the volume of ethanol was added to the supernatant, and the temperature of the solution was maintained at 4 °C for 12 h. After centrifugation, crude polysaccharides from *Chaenomeles lagenaria* (CPCL) were collected.

The crude polysaccharides were dissolved in distilled water, and the proteins were removed using the Sevag method (Staub 1965). A 3,500 g/mole M_w cutoff membrane was used to dialyze the polysaccharides at 4 °C for 12 h, and the dried polysaccharides were obtained after vacuum drying.

Purification of polysaccharides

The crude polysaccharides were mixed evenly with deionized water at 8000 r/min for 10 min to obtain the supernatant. The solutions with 0 to 0.3 mol/L of NaCl concentrations were used to elute the supernatant with 2.0 mL/min of flow velocity using a flow column (15 mm × 180 mm). An amount of 0.15 mol/L of NaCl was selected to elute the separate fractions. The separate fractions were purified using gel-filtration (1.8 cm × 100 cm) equipped with a Sephadex G-100 column (Thermo Fisher Scientific, Waltham, MA, USA) at 0.4 mL/min of flow velocity. The major peaks were obtained as the pure polysaccharide and named CLWSP-1. The extraction rate of CLWSP-1 was calculated as follows: Extraction rate (%) = a / b, where a is the mass (g) of the obtained CLWSP-1 and b is the mass (g) of the dehydrated *Chaenomeles lagenaria* sample.

Analysis of CLWSP-1

The analysis of the total carbohydrates and proteins of CLWSP-1 was performed by the previous method of Zhou *et al.* (2020). The carbazole method (Kosakai and Yosizawa 1979) was used to assay the content of uronic acid with the standard of galacturonic acid. Gel permeation chromatography (GPC, model 600 pump; Agilent Technologies, Santa Clara, CA, USA) was adopted to analyze the molecular weight of CLWSP-1. Approximately 20 μL of the sample was assayed on a column of TSK-GEL G3000Wxl (7.5 mm × 300 mm) and detected with a refractive index detector (RID). The monosaccharide composition of CLWSP-1 was analyzed by the previous method of Hu *et al.* (2019). Additionally, 10 mg of CLWSP-1 was hydrolyzed by 3 mL of 2 mol/L of trifluoroacetic acid (TFA) (Thermo Fisher Scientific, Waltham, MA, USA). The composition analysis of CLWSP-1 was performed by the high-performance liquid chromatography (HPLC) method. Approximately 20 μL of sample was eluted according to the previous work (Qian *et al*. 2018) and assayed on an operation system (Agilent 1260; Agilent Technologies, Santa Clara, CA, USA). Fourier transform-infrared (FT-IR) analysis of CLWSP-1 was performed on a Thermo Fisher IS5 (Thermo Fisher Scientific, Waltham, MA, USA), and the carbon chain groups of CLWSP-1 were protracted.

Optimization of the extraction process of CLWSP-1

The effects of the ratio of water to solid (20 to 90 mL/g), temperature (60 to 100 °C), time (60 to 180 min), and ethanol concentration (60 to 100%) on the extraction rate of CLWSP-1 were investigated. All the experiments were repeated in triplicate.

The response surface methodology (RSM) was performed to optimize the hot water process for CLWSP-1 extraction. The ratio of liquid to solid (A, mL/g), temperature (B, °C), time (C, min), and ethanol concentration (D, %) was taken as independent variables with extraction rate (Y, %) as the response value. The Design Expert software (Stat-Ease, Inc., version 8.0.6, Minneapolis, MN, USA) and a Box–Behnken Design (BBD) were further used to optimize the hot water process for the extraction of CLWSP-1 according to the results of the single factor experiments. The ratio of water to solid, temperature, time, and ethanol concentration was selected as four independent variables and nominated as *A*, *B*, *C*, and *D*, respectively, with the response of extraction rate of CLWSP-1 (*Y*). The model showed the relation of variables and the response was constructed using the following equation,

$$Y = \alpha_0 + \alpha_1 A + \alpha_2 B + \alpha_3 C + \alpha_4 D + \alpha_{11} A^2 + \alpha_{22} B^2 + \alpha_{33} C^2 + \alpha_{44} D^2 + \alpha_{12} AB + \alpha_{13} AC + \alpha_{14} AD + \alpha_{23} BC + \alpha_{24} BD + \alpha_{34} CD \quad (1)$$

where Y is the response variable, α_0 is the intercept, α_1, α_2, α_3, and α_4 are the linear coefficients, α_{11}, α_{22}, α_{33}, and α_{44} are the square coefficients, α_{12}, α_{12}, α_{13}, α_{14}, α_{23}, α_{24}, and α_{34} are the interactive coefficients, and A, B, C, and D are the independent variables.

Antibacterial activity assay

The antibacterial activity was assayed by the recently published method (Wang *et al.* 2019). Briefly, 25 to 200 mg/mL of CPCL and CLWSP-1 concentrations were prepared using sterilized distilled water and filtered through a 0.22-μm millipore filter. Indicator strain cultures amounting to 100 μL (10^6CFU/mL) were spread evenly onto LB agar medium, and a 10-mm filter paper disk containing 50 μL CLWSP-1 samples was well placed, with 50 μL of 0.9% sterilized saline as controls. The indicator strains were cultured at 37 °C for 16 h. The indicator strains in each plate were observed, and the diameter of the

inhibition zone was measured by a vernier caliper. The minimum inhibitory concentration (MIC) of CLWSP-1 was assayed by the double dilution method. CLWSP-1 samples in the amount of 50 μL with concentrations of 200, 100, 50, 25, 12.50, 6.25, and 3.125 mg/mL were filtered through a 0.22-μm millipore filter and mixed with 100 μL cultures (10^6 CFU/mL) of indicator strains in a 96-well microplate, with 50 μL of 0.9% sterilized saline as controls. The treated microplate was cultured at 37 °C for 16 h and 600 nm absorbance of culture broth was detected to evaluate the MIC of the bacteriostasis effect of CLWSP-1 on indicator strains.

Data analysis

All data in the present study were analyzed by an analysis of variance (ANOVA) and shown as means ± SD (n = 3) and estimated by SPSS 20.0 software (IBM Corporation, Armonk, NY, USA).

RESULTS AND DISCUSSION

Chemical Analysis of CLWSP-1

The content of the total carbohydrates and galacturonic acid in CLWSP-1 were 98.43% and 50.17%, respectively. However, there was no protein detected in in CLWSP-1. Figure 1 shows that the fraction at 16.33 min indicated the purity of CLWSP-1. The average molecular weight of CLWSP-1 was 1.23×10^2 kDa. As shown in Table 1, the compositions of CLWSP-1 contained five types of monosaccharides, including arabinose, galactose, glucose, mannose, and xylose. Arabinose and galactose were the key constituents of CLWSP-1.

Table 1. Monosaccharide Composition of the Polysaccharide Purified from *Chaenomeles lagenaria*

Monosaccharide	Composition (%)
Glucose	1.03 ± 0.02
Mannose	1.24 ± 0.03
Arabinose	2.9 ± 0.0.07
Xylose	0.28 ± 0.00
Galactose	2.29 ± 0.06

All experiments were repeated in triplicate and data was expressed as mean ± standard deviation

IR Analysis of CLWSP-1

Figure 2 shows the FTIR spectrum of CLWSP-1, in which 3429 cm^{-1} might be the infrared absorption peak of the O-H stretching vibration; 2973 cm^{-1} to 2932 cm^{-1} might be the infrared absorption peak of the C-H stretching vibration; 1735 cm^{-1} to 1629 cm^{-1} might be the infrared absorption peak of the C=O stretching vibration; and 1438 cm^{-1} might be the infrared absorption peak of the C-H bending vibration. The infrared characteristic absorption peak of C-O-C might occur between 1094 cm^{-1} to 1025 cm^{-1}, and 886 cm^{-1} might be the infrared characteristic absorption peak of C-H. The results indicated that CLWSP-1 had the typical carbon chain groups in polysaccharide structures.

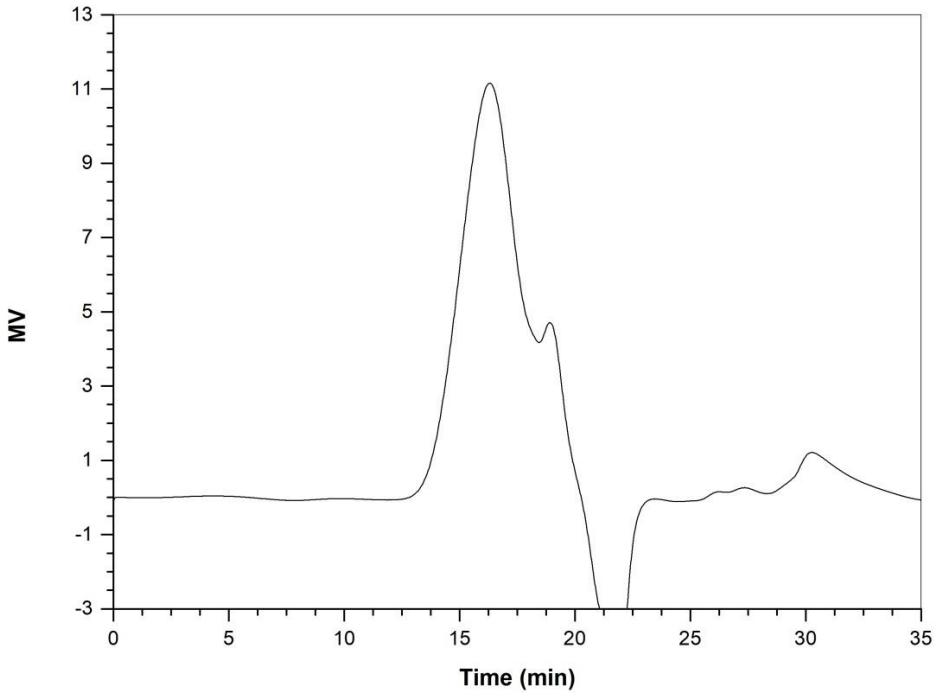


Fig. 1. GPC chromatogram of CLWSP-1

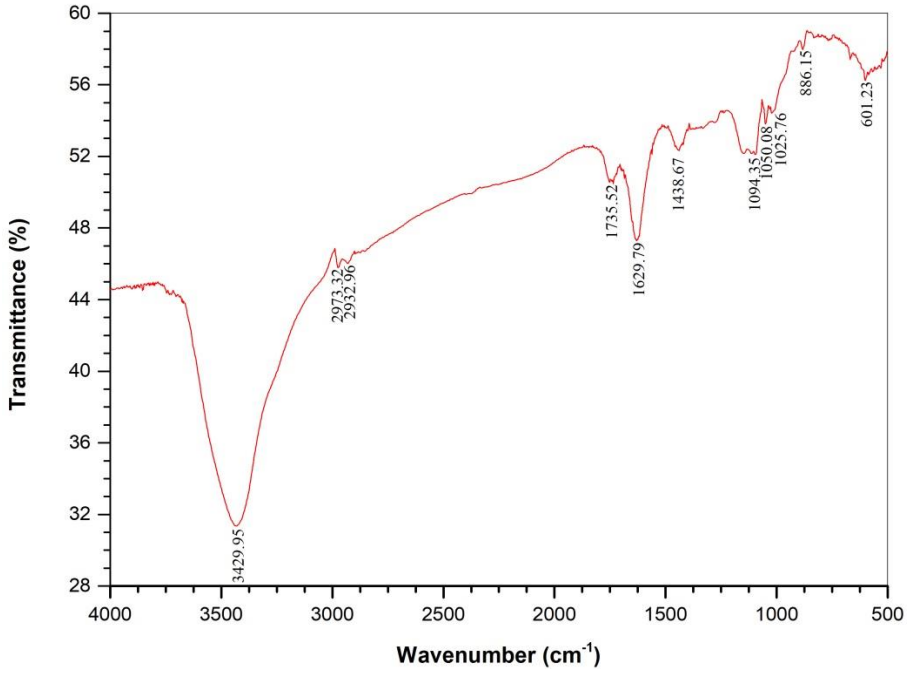


Fig. 2. FT-IR spectra of CLWSP-1

Effects of Hot Water Process on Extraction of CLWSP-1

Ratio of water to solid

As shown in Fig. 3(A), the extraction rate of CLWSP-1 increased significantly with the increase of the ratio of water to solid from 20:1 mL/g to 50:1 mL/g and reached the maximum extraction rate at the ratio of water to solid of 50:1 mL/g. Then, when the ratio of water to solid continued to increase over 50:1 mL/g, the extraction rate of CLWSP-1

decreased significantly. It was concluded that the excessive ratio of water to solid was unfavorable for dissolution of polysaccharides.

Temperature

Figure 3(B) shows that the highest extraction rate of CLWSP-1 occurred when the temperature increased to 80 °C. The extraction rate of CLWSP-1 decreased while the temperature was in the range of 80 to 100 °C. Considering that a high temperature might affect the structure and biological activity of polysaccharides, an excessively high temperature would reduce the extraction rate of polysaccharides. Thus, 80 °C was chosen as the suitable temperature for the extraction of CLWSP-1.

Time

Figure 3(C) shows that when the extraction time ranged from 60 to 120 min, the yield of CLWSP-1 markedly increased and reached the maximum value at 120 min. When the extraction time was over 120 min, the extraction rate of CLWSP-1 began to decrease significantly. According to the results of Fig. 3c, an appropriate extraction time for the extraction of CLWSP-1 should be limited within 110 to 130 min.

Ethanol concentration

Figure 3(D) shows that the extraction rate of CLWSP-1 gradually increased with ethanol concentration in the range of 50% to 80%, and the extraction rate was the highest when the ethanol concentration reached 80%. When ethanol concentration ranged between 80 to 95%, the extraction rate of CLWSP-1 showed a downward trend. Therefore, it was advisable to control the ethanol concentration between 80% to 85%. As a binary solvent, water-ethanol changed the polarity of the solvent, which facilitated the dissolution of the target substance.

Optimization of CLWSP-1 Extraction

The results are shown in Table 2 and the multivariate regression-fitting model was used to obtain the quadratic regression equation of the extraction rate of CLWSP-1 (Y) for four independent variables:

$$Y = -0.19 + 0.02A + 0.02B + 4.63E - 003C + 2.23E - 003D + 3.00E - 005AB - 4.17E - 007AC + 6.25E - 006AD - 2.00E - 005BC - 3.00E - 005BD + 1.75E - 005CD - 1.96E - 004A^2 - 6.71E - 005B^2 - 1.91E - 005C^2 - 1.20E - 005D^2 \quad (2)$$

The data in Table 3 were summarized and analyzed by an ANOVA. The results are shown in Table 3. From Tables 2 and 3, the linear coefficients (A, B, and C), were significant, which indicated that the ratio of liquid to solid, temperature, and time greatly influenced CLWSP-1 extraction. The interaction terms (AB, BC, and CD) and quadratic terms (A2, B2, C2, and D2) were significant ($P < 0.001$). The model for CLWSP-1 extraction was significant ($P < 0.001$). The lack of fit was not significant ($P = 0.1101 > 0.005$), indicating that the fitting model was good. The $R^2 = 0.9976$ and indicated that the model could explain 99.76% variation of the extraction rate of CLWSP-1. The regression equation was significant, and the relationship between each factor and response value could be described accurately. Therefore, the equation could determine the optimum extraction process of extraction of CLWSP-1.

Table 2. Results of Response Surface Analysis

Run	Ratio of Liquid to Solid (A, mL/g)	Temperature (B, °C)	Time (C, min)	Ethanol Concentration (D, %)	Extraction Rate (Y, %)
1	-1 (30)	-1 (80)	0 (120)	0 (90)	1.16 ± 0.03
2	1 (70)	-1 (80)	0 (120)	0 (90)	1.13 ± 0.02
3	-1 (30)	1 (100)	0 (120)	0 (90)	1.13 ± 0.02
4	1 (70)	1 (100)	0 (120)	0 (90)	1.13 ± 0.02
5	0 (50)	0 (90)	-1 (90)	-1 (80)	1.22 ± 0.01
6	0 (50)	0 (90)	1 (150)	-1 (80)	1.19 ± 0.01
7	0 (50)	0 (90)	-1 (90)	1 (100)	1.20 ± 0.03
8	0 (50)	0 (90)	1 (150)	1 (100)	1.20 ± 0.02
9	-1 (30)	0 (90)	0 (120)	-1 (80)	1.15 ± 0.01
10	1 (70)	0 (90)	0 (120)	-1 (80)	1.14 ± 0.02
11	-1 (30)	0 (90)	0 (120)	1 (100)	1.14 ± 0.03
12	1 (70)	0 (90)	0 (120)	1 (100)	1.13 ± 0.02
13	0 (50)	-1 (80)	-1 (90)	0 (90)	1.20 ± 0.02
14	0 (50)	1 (100)	-1 (90)	0 (90)	1.20 ± 0.02
15	0 (50)	-1 (80)	1 (150)	0 (90)	1.20 ± 0.03
16	0 (50)	1 (100)	1 (150)	0 (90)	1.18 ± 0.03
17	-1 (30)	0 (90)	-1 (90)	0 (90)	1.14 ± 0.01
18	1 (70)	0 (90)	-1 (90)	0 (90)	1.13 ± 0.02
19	-1 (30)	0 (90)	1 (150)	0 (90)	1.12 ± 0.01
20	1 (70)	0 (90)	1 (150)	0 (90)	1.11 ± 0.02
21	0 (50)	-1 (80)	0 (120)	-1 (80)	1.21 ± 0.02
22	0 (50)	1 (100)	0 (120)	-1 (80)	1.21 ± 0.03
23	0 (50)	-1 (80)	0 (120)	1 (100)	1.22 ± 0.03
24	0 (50)	1 (100)	0 (120)	1 (100)	1.20 ± 0.01
25	0 (50)	0 (90)	0 (120)	0 (90)	1.22 ± 0.03
26	0 (50)	0 (90)	0 (120)	0 (90)	1.22 ± 0.03
27	0 (50)	0 (90)	0 (120)	0 (90)	1.22 ± 0.02
28	0 (50)	0 (90)	0 (120)	0 (90)	1.22 ± 0.03
29	0 (50)	0 (90)	0 (120)	0 (90)	1.22 ± 0.02

All experiments were repeated in triplicate and data were expressed as mean ± standard deviation.

Table 3. Variance Analysis

Source	Sum of Squares	df	Mean Square	F-value	P-value
Model	0.043	14	3.09E-003	423.59	< 0.0001
A	4.32E-004	1	4.32E-004	59.2	< 0.0001
B	2.80E-004	1	2.80E-004	38.41	< 0.0001
C	4.32E-004	1	4.32E-004	59.2	< 0.0001
D	6.53E-005	1	6.53E-005	8.95	0.0097
AB	1.44E-004	1	1.44E-004	19.73	0.0006
AC	2.50E-007	1	2.50E-007	0.034	0.8558
AD	6.25E-006	1	6.25E-006	0.86	0.3704
BC	1.44E-004	1	1.44E-004	19.73	0.0006
BD	3.60E-005	1	3.60E-005	4.93	0.0434
CD	1.10E-004	1	1.10E-004	15.11	0.0016
A^2	0.04	1	0.04	5471.5	< 0.0001
B^2	2.92E-004	1	2.92E-004	40	< 0.0001
C^2	1.92E-003	1	1.92E-003	263.21	< 0.0001
D^2	9.47E-006	1	9.47E-006	1.3	0.2737
Residual	1.02E-04	14	7.30E-006		
Lack of fit	9.22E-05	10	9.22E-006	3.69	0.1101
Pure error	1.00E-005	4	2.50E-006		
Corrected total	0.043	28			
R^2	0.9976				
R^2adj	0.9953				
C.V.%	0.23				

Df: degrees of freedom; R^2adj: R^2 adjusted; C.V.%: coefficient of variation

Results of Response Surface Analysis

From the regression equation, the response surface and contour maps of ratio of liquid to solid (A), temperature (B), time (C), and ethanol concentration (D) affecting extraction rate of CLWSP-1 (Y) were obtained, as shown in Fig. 4(A) to 4(F). By observing the response surface graph formed by the interaction effects of any two factors, it could be seen that the steeper the trend of the graph curve, the more significant influence on extraction CLWSP-1; the gentler the trend of the curve, the less this factor was able to influence the extraction rate of CLWSP-1. Figure 4(A) shows that the effect of the ratio of liquid to solid and temperature on the extraction rate of CLWSP-1 was parabolic. The extraction rate of CLWSP-1 increased first and then decreased with the increase of ratio of liquid to solid and temperature. Figure 4(B) shows that with the increase of ratio of liquid to solid and extraction time, the extraction rate of CLWSP-1 increased first and then decreased. According to Fig. 4(C), the extraction rate of CLWSP-1 increased first and then tended to be flat with the increase of ratio of liquid to solid and ethanol concentration. From plots of response surface and contours, the extraction rate of CLWSP-1 did not increase after a certain value, as shown in Fig. 4(D), when temperature and ethanol concentration increased.

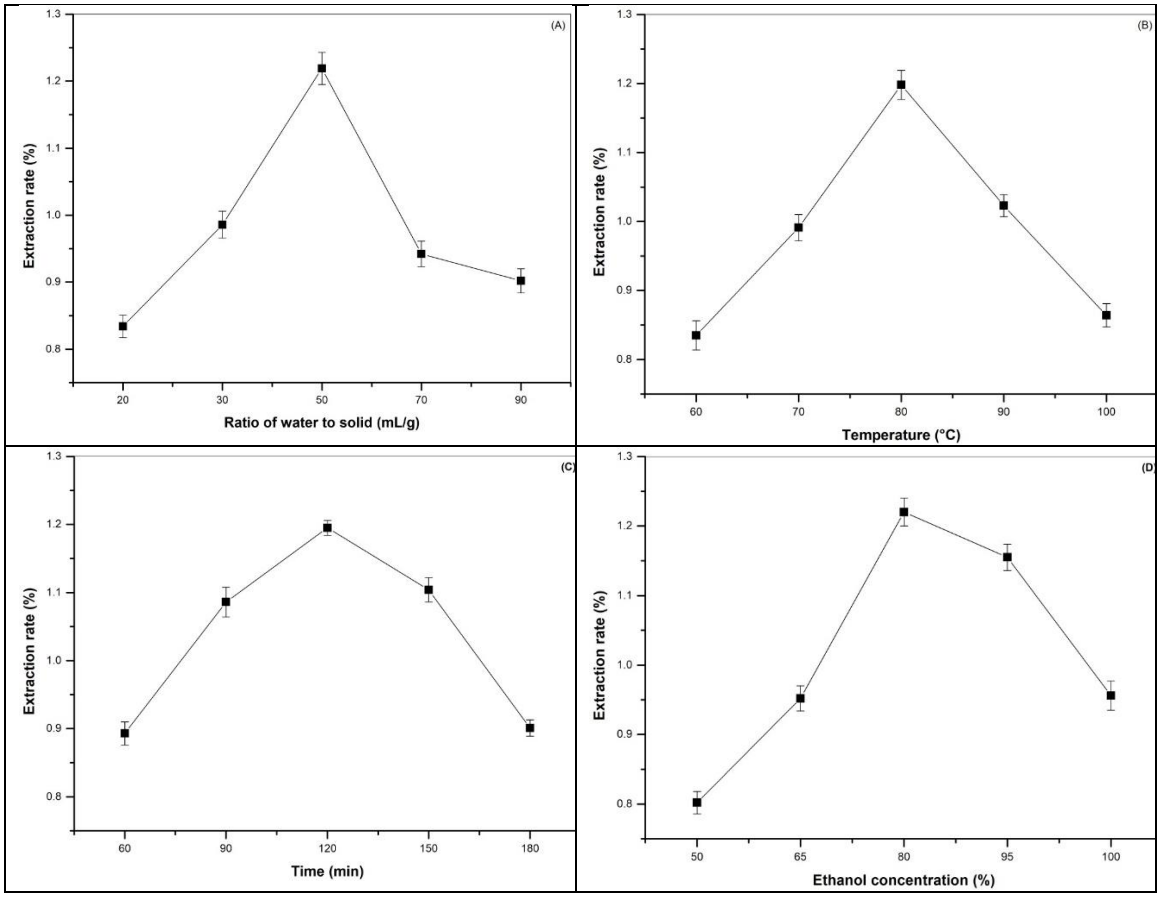
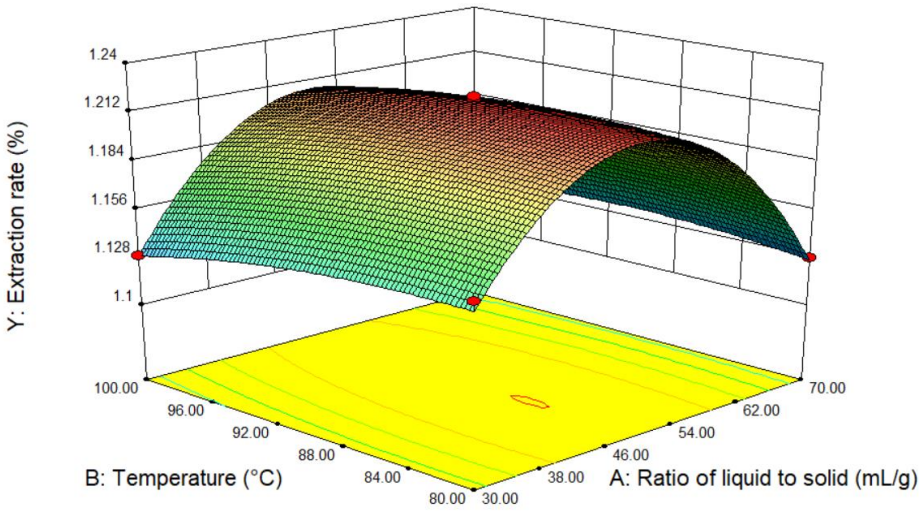
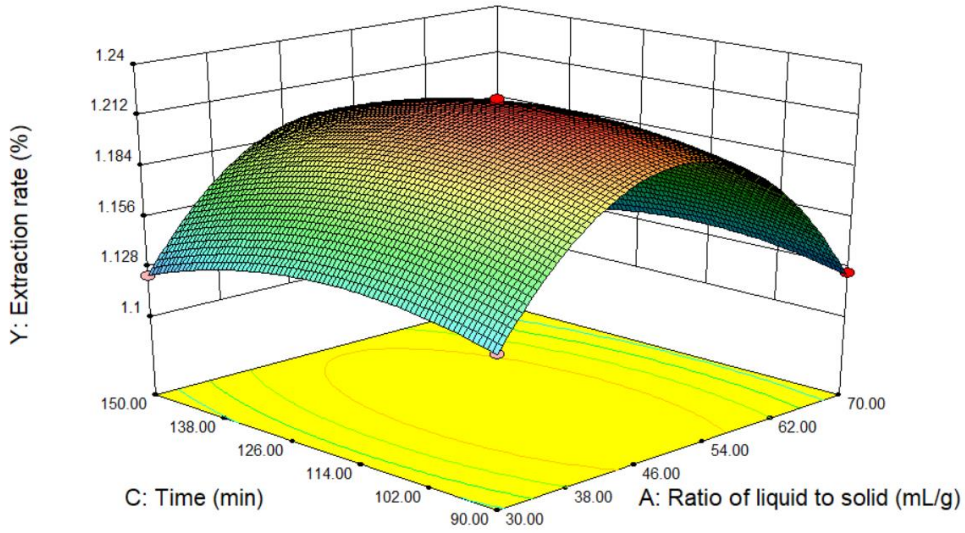


Fig. 3. Effects of hot water process on extraction of CLWSP-1 : (A) ratio of water to solid, (B) temperature, (C) time, and (D) ethanol concentration ; all experiments were replictaed in triplicate and data were expressed as mean ± standard deviation.

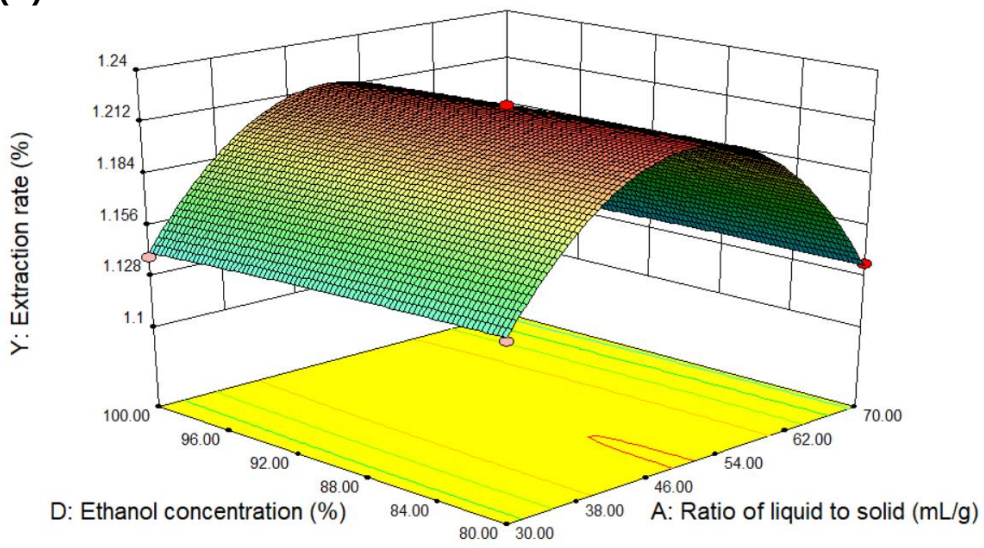
As shown in Fig. 4(E), with the increase of temperature and ethanol concentration, the extraction rate of CLWSP-1 increased gradually and then tended to be flat. As shown in Fig. 4(F), with the increase of extraction time and ethanol concentration, the extraction rate of CLWSP-1 increased and then decreased to a constant value.



(A)


Y: Extraction rate (%)
1.24
1.212
1.184
1.156
1.128
1.1
C: Time (min)
150.00
138.00
126.00
114.00
102.00
90.00
A: Ratio of liquid to solid (mL/g)
30.00
38.00
46.00
54.00
62.00
70.00

(B)


Y: Extraction rate (%)
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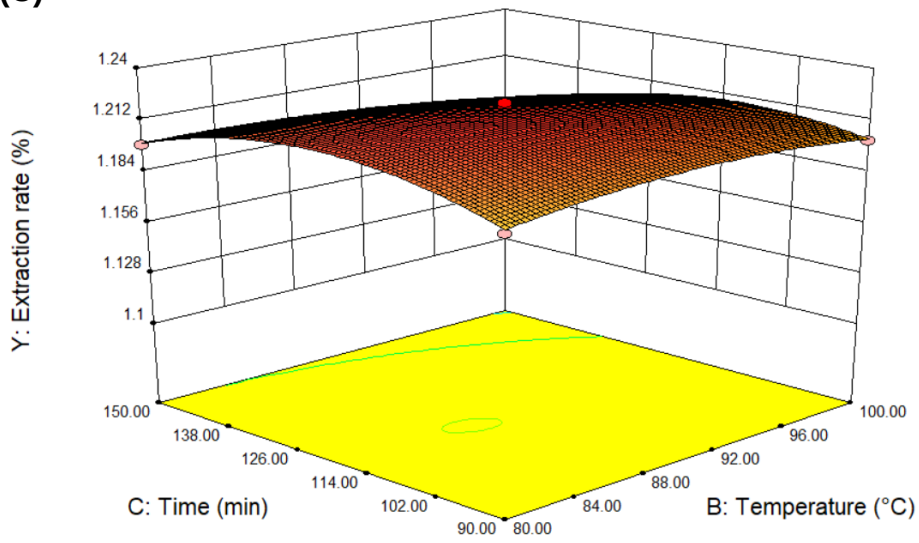
1.212
1.184
1.156
1.128
1.1
D: Ethanol concentration (%)
100.00
96.00
92.00
88.00
84.00
80.00
A: Ratio of liquid to solid (mL/g)
30.00
38.00
46.00
54.00
62.00
70.00

(C)


Y: Extraction rate (%)
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1.1
C: Time (min)
150.00
138.00
126.00
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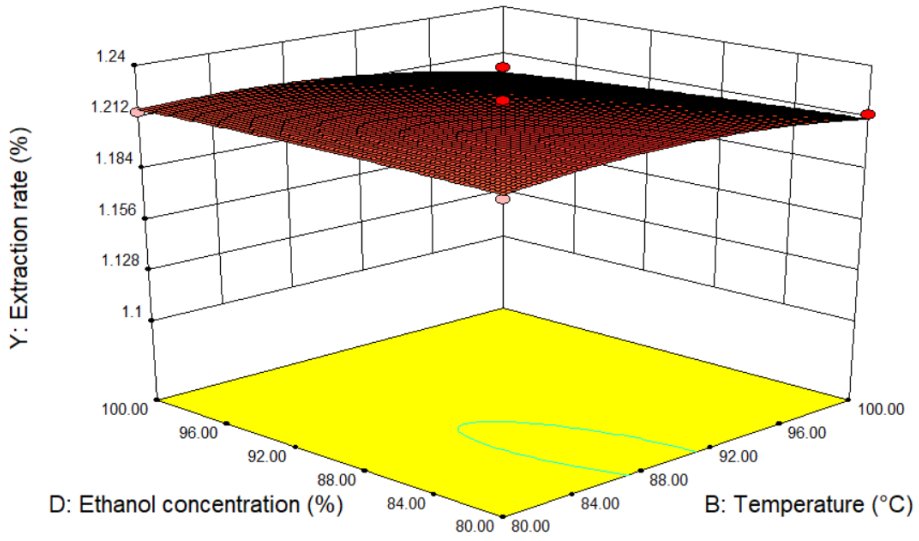
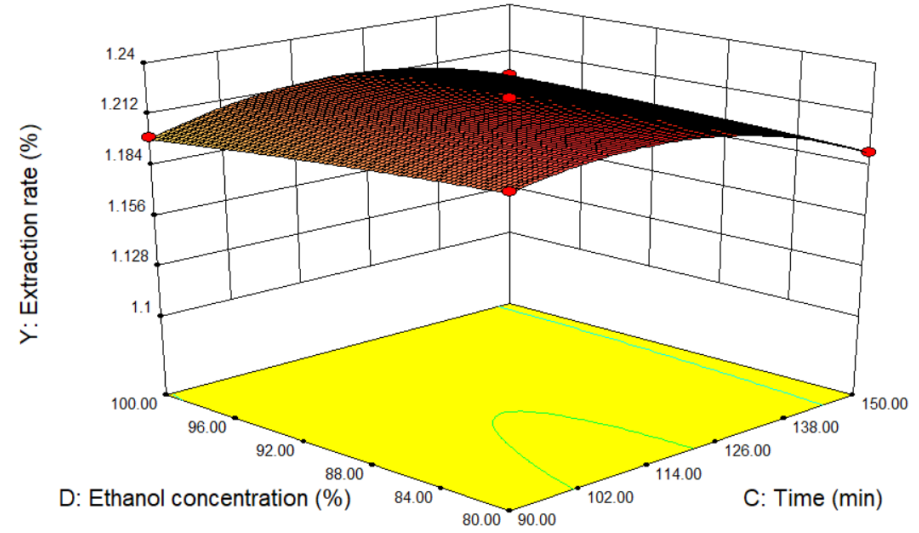
100.00

(D)



(E)



(F)

Fig. 4. Interaction effects of hot water process on extraction of CLWSP-1 : (A) ratio of water to solid and temperature, (B) ratio of water to solid and time, (C) ratio of water to solid and ethanol concentration, (D) temperature and time, (E) temperature and ethanol concentration, and (F) time and ethanol concentration; all experiments were repeated in triplicate, and data were expressed as mean ± standard deviation.

The hot water extraction has been widely used as an efficient process to extract polysaccharides from natural plants. Reasonable control of process conditions, such as temperature and time, can improve the extraction efficiency. The previous papers evidenced that taking reasonable control of the process conditions, such as ratio of water to solid, temperature and time, could improve the extraction efficiency (Yun *et al.* 2019; He *et al.* 2020). In this study, within the limits of the factors affecting the extraction rate of CLWSP-1, the optimum hot water process conditions were predicted to be a ratio of liquid to solid of 48.9 mL/g, temperature of 91.1 ℃, extraction time of 114.3 min, and ethanol concentration of 81.0% with a CLWSP-1 yield of 1.2%. Considering the actual laboratory extraction conditions, the optimum extraction conditions for extraction

CLWSP-1 were adjusted to be a ratio of liquid to solid of 48.9 mL/g, temperature of 91 °C, extraction time of 114 min, and ethanol concentration of 81%. To verify and prove the optimum extraction conditions, the extraction of CLWSP-1 was performed in triplicate and the average extraction rate of CLWSP-1 was calculated as 1.24 ± 0.02% (n = 3), which was highly similar to the predicted value (1.22%). This showed that the optimized hot water process was reliable and reproducible for CLWSP-1 extraction.

Antibacterial Activity Analysis

Table 4 shows that CLWSP-1 had a strong inhibition effect on *Escherichia coli* and *Staphylococcus aureus*. The bacteriostatic activity increased with the increase of CLWSP-1 concentration. When the concentration of CLWSP-1 was 50 mg/mL, the inhibitory zone diameter of *E. coli* and *S. aureus* were 13.00 mm and 16.8 mm, respectively, and that of 14.5 mm and 20.0 mm when at 200 mg/mL of CLWSP-1. According to the results of the bacteriostasis activity test, the concentration of CLWSP-1 was positively correlated with the bacteriostasis effect. The minimum inhibitory concentrations (MIC) of CLWSP-1 to *E. coli* and *S. aureus* are shown in Table 5. When the concentration of CLWSP-1 was 50 mg/mL, no growth of *E. coli* was found. Therefore, the MIC of *E. coli* was 50 mg/mL. When the concentration of CLWSP-1 was diluted to 12.50 mg/mL, *S. aureus* did not grow, so the MIC of *S. aureus* was 12.50 mg/mL. The results showed that CLWSP-1 had stronger bacteriostatic activity of *S. aureus* than that of *E. coli*.

Table 4. Bacteriostasis Effects of CPCL and CLWSP-1 Against *Escherichia coli* and *Staphylococcus aureus*

Concentration (mg/mL)	Inhibition Zone Diameter (mm)			
	Escherichia coli		*Staphylococcus aureus*	
	CPCL	CLWSP-1	CPCL	CLWSP-1
25	-	-	6.48 ± 0.19	15.40 ± 0.43
50	5.42 ± 0.13[a]	13.00 ± 0.33	7.98 ± 0.24	16.75 ± 0.47
100	6.03 ± 0.16	13.85 ± 0.35	9.35 ± 0.29	18.35 ± 0.34
200	8.19 ± 0.22	14.50 ± 0.34	10.23 ± 0.31	20.04 ± 0.49
Control (0.9% sterile saline)	0	0	0	0

All experiments were performed in triplicate and data were expressed as mean ± standard deviation; -, No inhibition zone diameter was detected.

Table 5. Minimal Inhibitory Concentration of CLWSP-1 Against *Escherichia coli* and *Staphylococcus aureus*

Concentration (mg/mL)	*Escherichia coli*	*Staphylococcus aureus*
200	++	+++
100	+	+++
50	+	++
25	-	++
12.50	-	+
6.25	-	-
3.125	-	-

All experiments were performed in triplicate and data were expressed as mean ± standard deviation; Note: -, No inhibition zone diameter was detected; +, inhibition zone diameter was 10 to 14 mm; ++, inhibition zone diameter was 4 to 18 mm; +++, inhibition zone diameter was above 18 mm.

CONCLUSIONS

1. A novel polysaccharide CLWSP-1 was isolated from *Chaenomeles lagenaria*. The key constituents of CLWSP-1, which had a molecular weight of 1.23×10^2 kDa, were galacturonic acid, arabinose, and galactose.
2. The optimal hot water process for the extraction of CLWSP-1 was finally determined to be a ratio of water to solid of 48.9 mL/g, temperature of 91 °C, extraction time of 114 min, and ethanol concentration of 81%. A 1.24% extraction rate of CLWSP-1 was obtained.
3. The polysaccharide CLWSP-1 exhibited satisfactory antibacterial activity to *Escherichia coli* and *Staphylococcus aureus* and the antibacterial activity of *Staphylococcus aureus* was stronger than that of *Escherichia coli*.

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