Optimization of extraction of polysaccharides from *Suaeda salsa* (L.) Pall. by ultrasonic: characterization, purification and antioxidant assessment

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Abstract. Under optimal extraction conditions, characterization and antioxidant activity of polysaccharides from the *Suaeda salsa* (L.) Pall. were investigated. This was the first report that described the composition of the polysaccharide from this plant. Obviously, the yield of 2.19% for extraction of polysaccharides was obtained as ethanol volume fraction of 65%, extraction time of 72 min, ultrasound assisted power of 438 W, and temperature of 85°C, respectively. The structure study was carried out with FT-IR and SEM, and the chemical contents of carbohydrates, proteins, uronic acids and total flavonoids were measured in this study. The crude polysaccharides were purified into two components including SGP-1-1 and SGP-2-1. Moreover, the chemical composition analysis according to HPLC showed that it was viscous polysaccharide, which mainly contained glucose, mannose, xylose, galacturonic acid, glucuronic acid, fucose, rhamnose, arabinose, galactose, respectively. Besides, it was proved that the optimum polysaccharides possessed higher significant potentials ($P < 0.05$) in scavenging ability against DPPH, ABTS⁺ and hydroxyl radical.

1 Introduction

Suaeda salsa (L.) Pall. is a unique salt-tolerant herb of the chenopodiaceae, traditionally, it is a halophyte that growing mainly on sandy beaches and deserted in saline or alkaline soil. The plants are mainly distributed on the coast and north of the Oceania, China's Yellow River Delta beach area. Traditionally, it has been used as a medicine to reduce fever or consumption to increase salinity [1]. Due to the growth in saline soil, the trace elements and oils contents of seeds in *Suaeda salsa* were much higher than that of soybean. In addition, the stems and leaves of it were also rich in amino acids, vitamins and minerals [2]. Typically, the aqueous extraction of *Suaeda salsa* consisted essentially of salts and other components such as flavonoids and polyphenols, which signified that the extraction had certain antioxidant, bacteriostatic [3, 4] and anti-inflammatory functions [5, 6]. Besides, the coastal was the main areas for the growth of this plant. There was sufficient sunshine with large temperature difference between day and night, which was very conducive to the accumulation and transportation of anthocyanins and other secondary metabolites. So far, no studies have been reported to extract polysaccharides from *Suaeda salsa*.

Polysaccharides were a variety of poly-hydroxy macromolecular polymers that were widely found in cells of animals, plants and microorganisms. Generally, it was one of the basic substances that sustained life metabolism, and had physiological functions such as structural support, energy storage [7], and so on.

However, a large number of researches have consistently shown that polysaccharides had widely biological activities, including anti-tumor, anti-virus [8], anti-coagulation, anti-oxidation [9], hypoglycemic and immunomodulatory properties. The study found that plant polysaccharides had obvious enhanced immune function, in which mainly increased it through the following aspects [10-12]: (1) by promoting the body to produce antibodies to enhance humoral immune function; (2) by promoting the proliferation of T and B lymphocytes to enhance the immune function of cells; (3) by increasing the phagocytic activity of macrophages to increase the number of macrophages; (4) by promoting increased transcriptional activity of cytokine mRNA to enhance biological activity of cytokines; (5) by altering the content and relative ratio of cellular signaling molecules such as cAMP, cGMP, NO, and Ca²⁺ in immune cells to regulate immune function. For example, asparagus polysaccharide can activate macrophage to release active medium NO and promote the expression of TNF- α and IL-6 genes, which may be one of the ways for asparagus to enhance the body immunity. Therefore, researches on polysaccharides have been attracting attention in recent years, especially in extracting polysaccharides from salt-tolerant plants of Polygonaceae plants. For example, most of the polysaccharides extracted from *Herba Pyrolae*, *Cordyceps* and *Lactobacillus*, which consisted primarily of arabinose, glucose and galactose.

More recently, an acidic polysaccharide from the plant had been studied for anti-tumor activity. On this

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basis, we carefully studied the extraction, purification, characterization and oxidation resistance in vitro. The main purpose of this study was to obtain maximum yield of polysaccharides from *Suaeda salsa*. The crude polysaccharides were purified by DEAE-52 cellulose and Sephadex G-100. Its physical characterization and chemical component analysis were performed by FT-IR, SEM and HPLC, and analysis of antioxidant properties were also carried out.

2 Materials and methods

2.1 Plant materials

Plants were harvested in July 2018 from Dongying (Long.118°5' E; Lat.38°15' N) of Shandong province in China. The plant was identified as *Suaeda salsa* (L.) Pall. by researcher X. Zhang (Department of Life Sciences, Shandong Normal University) which the sample was No.SD-s-2153327. The following reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA) including DEAE-52 cellulose, 1-phenyl-3-methyl-5-pyrazolone (PMP), Sephadex G-100, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and diammonium salt (ABTS). The TFA, methanol, hexane, ethanol and chloroform were all analytical purity.

2.2 Extraction process of polysaccharide

The whole plants were washed several times with water and frozen rapidly at -37°C with liquid nitrogen to be dried. They were crushed by superfine grinding at 200 orders to be used. As shown in Fig. 1, *Suaeda salsa* plants were treated several times with hexane and ethanol for 24 h at room temperature of 27°C in order to remove oily ingredients and pigments after they were washed and crashed. The rest of residue was used to extract crude polysaccharide by hot water with the ultrasound-assisted (Elmasonic S 40H, Sigen, Germany) extraction (UAE). The enrichment of extracting solution was centrifuged (at 4°C, 10 000 r/min for 20 min) [12] after precipitation of 95% (V/V) ethanol [13]. The precipitation was considered to be crude polysaccharide called SGP. The crude SGP solution was added with 25% (V/V) savage-chloroform shaking for 10 min to remove the protein of each group [14, 15]. Besides, it was dialyzed by membranes (3 kDa) to remove salt ion and depigmented by resin (elution solvent: PBS (0.1 mol/L, pH 5.2), 10 mL/min). Finally, the solution was freeze dried to provide the crude SGP, *Suaeda salsa* polysaccharides.

Fig.1 Steps for polysaccharide extraction of *Suaeda salsa* (L.) Pall. and purification

The yield percentage of polysaccharide was calculated as Eq. (1)

$$\text{Polysaccharide yield } (Y_{\text{SGP}}, \%) = \left[\frac{\text{Weight of extracted after(g)}}{\text{Weight of the whole sample(g)}} \right] \times 100 \quad (1)$$

2.3 Experimental design of response surface methodology

The influence of 4 factors process including ethanol volume fraction (X_a : 50%, 65%, and 80%), extraction time (X_b : 50min, 60min, and 70 min), ultrasound assisted power (X_c : 200W, 320W, and 440W) and extraction temperature (X_d : 65°C, 75°C, and 85°C). The yield of carbohydrates under this extraction factors from *Suaeda salsa* was recorded via the Box-Behnken experimental design (BBD) with 29 order experiments in order to obtain the best combination.

2.4 Purification of crude SGP

Crude SGP solution was centrifuged at 5 000 rpm for 10 min in order to remove insoluble portions after it was dissolved in distilled water with ultrasound assistance at 200 W and 40°C. The SGP supernatant was then placed at room temperature (28°C) for ion exchange chromatography on a DEAE-52 cellulose column (2.6×60 cm) [16]. The samples were separately eluted with distilled water, 0.2 and 0.4 M sodium chloride (NaCl) solution [17]. Data was recorded at 490 nm using sulfuric acid-phenol colorimetry when two completely separated fractions of SGP-1 and SGP-2 were collected, respectively. Then, the above components were concentrated and lyophilized. They were again purified by distillation on a Sephadex G-100 column (1.6×100 cm) [18]. As a result, they were finally separated and obtained: SGP-1 to SGP-1-1, SGP-2 to SGP-2-1, respectively.

2.5 Characterization of SGP

2.5.1 Determination of monosaccharide composition and molecular weights

The monosaccharide composition of the SGP was analyzed by the method of PMP complexation according to published reports [19, 20]. Sample (5 mg/mL, 1 mL) and TFA (4 M, 4 mL) was hydrolyzed at 120°C for 6 h under the confined environment, then it was added to methanol to remove excess TFA by rotary evaporation. The hydrolyzed monosaccharide with PMP (0.5 M, 1-phenyl-3-methyl-5-pyrazolone) expresses significant UV absorption at 290 nm. The monosaccharide composition of SGP were analyzed by HPLC (Waters Breeze2, MA, USA) equipped with a UV detector (Waters 2489). The testing conditions were as follows: Venusil XBP C₁₈ column (Waters Hewlett-Packard, MA, USA; 150 mm×4.6 mm, 5 μm), column temperature at 27°C, mobile phase via phosphate buffer (0.2 mol/L, pH 6.9) - acetonitrile (82:18, V/V), flow rate at 1.0 mL/min, wavelength at 290 nm.

Multi-Angle Laser Light Scattering (MALLS) was used to determine the molecular weight and distribution of products, which was widely used in chemical material analysis and determination of biological macromolecules. The laser wavelength was 490 nm with quartz glass sample cell of MALLS. The data was recorded every 15° during the scan interval of 0°-180° in dynamic light scattering experiment. The molecular weight of SGP and its particle size distribution can be tested according to this study.

2.5.2 Infrared spectral (IR) and Scanning Electronic Microscopy (SEM) analysis of SGP

Physical structure of SGP was performed by the Fourier transform infrared spectrophotometer (Thermo Electron Corp., Waltham, MA, USA). The mixture (with 10 mg sample and 100 mg KBr) was determined from 4000 ~ 400 cm⁻¹ (mid infrared region) to analyze the characterization of SGP [21, 22].

The scanning electronic microscopy of SGP has been reported by the scanning projection electron microscope (Thermo Prisma E., Waltham, MA, USA) [23, 24]. The SGP sample (2 mg) was detected on a tray to analyze the outside shape.

2.6. Antioxidant activities

The antioxidant activity of polysaccharide extracted from *Suaeda salsa* was estimated according to 4 tests (including DPPH radical scavenging ability, ABTS⁺ radical scavenging ability, hydroxyl scavenging ability). Generally, the ascorbic acid was chosen as the standard sample.

2.6.1 DPPH free radical scavenging activity

The method of DPPH radical scavenging of polysaccharides from *Suaeda salsa* were experienced

via the reported essay [25]. 5 mL of DPPH solution (0.1 mM in ethanol) was mixed with different concentrates polysaccharide samples. Then, the mixture was reacted for 45 min in the dark at about 27°C and detected at around 517 nm via UV-vis (UV-2501PC). The experiment was conducted with ascorbic acid as the control. The DPPH radical scavenging rate of the sample was calculated according to Eq. (2):

$$\text{DPPH free radical scavenging rate (\%)} = (1 - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}}) \times 100 \quad (2)$$

2.6.2 ABTS⁺ radical scavenging activity

The ABTS⁺ radical scavenging ability test was mentioned by the previous report [26]. Different concentrations of polysaccharide solution (1 mL with pH 7.1) was added into the blend, which included phosphate buffer (2.5 mL at 0.2 M with pH 6.6) and 1% potassium ferricyanide solution (2.5 mL at 1% (m/v)). In order to fully react, all of these were protected at 50°C under water bath for 40 min in dark room. Then, the ABTS⁺ radical solution was added into trichloroacetic acid solution (2.5 mL at 0.5M), and the steady absorbance was obtained at 700 nm. The ABTS⁺ radical scavenging ability was tested at around 700 nm with the blend of 2.5 mL distilled water and ferric chloride solution (0.5 mL at 0.05 mM) for 20 min after the initial mixing. The scavenging ability was indicated as Eq. (3):

$$\text{Inhibition (\%)} = (1 - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}}) \times 100 \quad (3)$$

2.6.3 Hydroxyl radical scavenging activity

In this study, OH[·] scavenging ability was estimated according to the method [27]. The reaction blend contained FeSO₄·7H₂O solution (2 mL at 6 mM), salicylic acid-ethanol solution (2 mL at 7.5 mM with pH 6.7), H₂O₂ (2 mL at 8 mM) and different concentrates of polysaccharide solution (2 mL). Then, the blend was reacted for 30 min at about 37°C and measured at around 510 nm via UV-vis, ascorbic acid was used as the control. The scavenging ratio of the sample was expressed according to Eq. (4):

$$\text{Scavenging percent} = [1 - (A - A_0) / A_1] \times 100 \quad (4)$$

A = Absorbance of extract sample; A₀ = Absorbance of control (to replace sample with water); A₁ = Absorbance of blank (to replace sample and H₂O₂ solution with water)

2.7 Statistical analysis

The BBD design and data analysis of RSM was calculated by Software Design-Expert (V12). The significance of diversities was assessed by one-way ANOVA analysis (with the significance of 2 level). All tests were repeated for three groups, with three parallel tests of each group (n=3×3). Data of triplicate parallel

experiences were recorded as means $\pm$ standard deviations by Microsoft Office (15.0).

3 Results and discussion

3.1 Extraction yield of SGP

3.1.1 Effect of ethanol volume fraction on extraction yield of SGP

In this test, the ethanol volume fraction was set as 35%, 50%, 65%, 80% and 95%. In the certain ethanol volume fraction range from 35% to 95%, the total extraction yield of SGP was correlated significant ($P < 0.05$) with the condition in Fig.2 (A). The total extraction yield of SGP reached a maximum 1.76 ± 0.09 % when the ethanol volume fraction was 65 %. This situation might be due to the mixing of ethanol and water which promoted the supersaturating of the system [28].

3.1.2 Effect of extraction time on extraction yield of SGP

Longer extraction time would express a positive effect on extraction yield of SGP. In this test, different extraction time was under 30 min, 40 min, 50 min, 60 min and 70 min with the 65% ethanol on 75°C under the ultrasound power of 320 W. It can be seen from Fig. 2 (B) that the extraction yield of SGP increased significant ($P < 0.05$) with the extraction time from 30 min to 70 min. The extraction rate of the SGP did not change with the extension time after 60 min, which may because that it was basically dissolved [27] in the solvent at about 1.96 ± 0.06 %.

3.1.3 Effect of ultrasound assisted power on extraction yield of SGP

Ultrasound assisted power was an important factor to affect the extraction yield of SGP obviously. In the experiments, the ultrasound power was set with 100W, 200W, 320 W, and 400 W as the SGP was reflected at 75°C for 60 min with ethanol volume fraction of 65%. It can be seen from Fig. 2 (C) that the extraction yield of SGP increased significant ($P < 0.05$) with the increasing ultrasound power, which the maximum value was consistently remained at 1.87 ± 0.06 %. It might be dissolved more completely under the action of ultrasonic power (320 W). Conversely, excessive power would cause the glycosidic bond broken, which might increase the destruction of SGP.

3.1.4 Effect of extraction temperature on extraction yield of SGP

To ensure the influence of extraction temperature on yield of SGP, the extraction temperature of 50°C, 65°C, 75°C and 85°C was investigated in this study, as shown in Fig.2 (D). The extraction time and ethanol volume fraction were reflected for 60 min with 65% at the 320W of ultrasound power. From 50°C to 85°C, the total extraction yield of SDF was correlated significant ($P < 0.05$) with the extraction temperature. The total extraction yield of SGP reached a maximum of 1.89 % when the extraction temperature reached 75°C. This situation might be because the high temperature caused its decomposition.

3.2 Optimization of extraction parameters of SGP

3.2.1 Statistical analysis and model fitting

As shown in the Table 1, the data of fitted quadratic multiple regression equation is analyzed by Design-Expert (V8.0.6) as follows:

$$Y(\%) = 2.07 + 0.057X_a + 0.081X_b + 0.12X_c + 0.12X_d + 5.000E-003X_aX_b - 0.020X_aX_c + 0.030X_aX_d + 0.045X_bX_c + 0.027X_bX_d + 0.040X_cX_d - 0.21X_a^2 - 0.065X_b^2 - 0.071X_c^2 - 0.067X_d^2$$

The detailed results about the significance analysis of the binomial regression model coefficients in this experiment were calculated in Table 2. The low P -value ($P < 0.0001$) indicated that the model was significant on yield of polysaccharide at 95% confidence. The *Lack of fit* ($P = 0.6317$) indicated that the model was fitted well and the error of the experimental value was relatively small. The high model determination coefficient ($R^2 = 0.9499$) indicated the regression equation can be used to analyze and predict the results. In addition, the model coefficient of variation (CV) was 5.36%, we can concluded that the test can be used for statistical analysis. The factors of X_a , X_b , X_c , X_d and X_a^2 had extremely significant ($P < 0.0001$) effect on the yield of polysaccharides, while quadratic terms X_b^2 , X_c^2 and X_d^2 reached significant ($P < 0.01$) levels. The significant between X_aX_c , X_bX_c and X_cX_d were considerable. The importance degree of each factor was listed as follows: ultrasound assisted power > extraction temperature > extraction time > ethanol volume fraction.

Table 1 Design and results of response surface experiments for the yield response

Experiments	Factors				Response Yield (%)
	X_a : Ethanol volume fraction (%)	X_b : Time (min)	X_c : Ultrasound power (W)	X_d : Temperature (°C)	
1	80(1)	60(0)	440(1)	75(0)	1.99
2	65(0)	50(-1)	320(0)	65(-1)	1.76
3	80(1)	60(0)	200(-1)	75(0)	1.76

4	65(0)	50(-1)	200(-1)	75(0)	1.78
5	65(0)	50(-1)	440(1)	75(0)	1.99
6	50(-1)	60(0)	320(0)	65(-1)	1.69
7	65(0)	60(0)	440(1)	85(1)	2.15
8	65(0)	60(0)	440(1)	65(-1)	1.81
9	65(0)	60(0)	320(0)	75(0)	2.16
10	50(-1)	60(0)	200(-1)	75(0)	1.57
11	80(1)	60(0)	320(0)	85(1)	1.73
12	50(-1)	60(0)	320(0)	85(1)	1.83
13	65(0)	60(0)	320(0)	75(0)	2.02
14	65(0)	60(0)	320(0)	75(0)	2.03
15	50(-1)	60(0)	440(1)	75(0)	1.88
16	50(-1)	70(1)	320(0)	75(0)	1.81
17	80(1)	50(-1)	320(0)	75(0)	1.71
18	65(0)	70(1)	320(0)	85(1)	2.19
19	65(0)	60(0)	200(-1)	85(1)	1.91
20	65(0)	70(1)	320(0)	65(-1)	1.87
21	80(1)	70(1)	320(0)	75(0)	1.91
22	50(-1)	50(-1)	320(0)	75(0)	1.63
23	80(1)	60(0)	320(0)	85(1)	1.99
24	65(0)	70(1)	200(-1)	75(0)	1.82
25	65(0)	60(0)	200(-1)	65(-1)	1.73
26	65(0)	60(0)	320(0)	75(0)	2.04
27	65(0)	50(-1)	320(0)	85(1)	1.97
28	65(0)	60(0)	320(0)	75(0)	2.09
29	65(0)	70(1)	440(1)	75(0)	2.21

Table 2 The analysis of variance (ANOVA) of the regression model for the yield response

Variable	Yield(%)			
	SS	DF	F-value	P-value
Model	0.79	14	18.97	<0.0001 ^b
X _a	0.039	1	13.00	0.0029 ^a
X _b	0.078	1	26.45	<0.0001 ^b
X _c	0.18	1	59.92	<0.0001 ^b
X _d	0.18	1	59.10	<0.0001 ^b
X _a X _b	0.156	1	0.034	0.1569
X _a X _c	0.056	1	0.54	0.0047
X _a X _d	0.034	1	1.21	0.0891
X _b X _c	0.035	1	2.73	0.0006
X _b X _d	0.215	1	1.02	0.3296
X _c X _d	0.176	1	2.16	0.0039
X _a ²	0.28	1	95.19	<0.0001 ^b
X _b ²	0.027	1	9.20	0.0090 ^a
X _c ²	0.033	1	11.06	0.0050 ^a
X _d ²	0.029	1	9.92	0.0071 ^a
Residual	0.042	14		
Lack of Fit	0.028	10		0.6317
Pure Error	0.013	4	0.63	
Cor Total	0.83	28		
C.V.(%)	5.36			

^a Significant at 0.001 level.^b Significant at 0.01 level.

3.2.2 Analysis of response surface

The best region to examine the interaction was reflected by the three-dimensional response surface with Design-expert (V12) software between every two factors. The optimal extraction conditions were obtained by determining the slope of the three-dimensional map and the degree of circularity of the contour map in Fig. 3. It can be judged that the bending of the six groups of three-

dimensional images was relatively large according to the design principle of the response surface test. So, it reflected the extraction effect of the polysaccharide was also significantly different. Therefore, the optimal isolation parameters were as follows: ethanol volume fraction was 65%, extraction time was 72 min, ultrasound assisted power was 438 W, and temperature was 85°C.

Fig.3 Response surface plots of the interaction of various factors on the yield of SGP. (a)The factors of time (min) and ethanol volume fraction (%). (b) The factors of ethanol volume fraction (%) and ultrasound power (W). (c)The factors of ethanol volume fraction (%) and temperature (°C). (d) The factors of time (min) and ultrasound power (W). (e)The factors of temperature (°C) and time (min). (f) The factors of temperature (°C) and ultrasound power (W).

3.3 Purification and characterization of SGP

3.3.1 Purification of crude SGP

As shown in Fig. 4, crude SGP was purified by DEAE-52 cellulose column to obtain SGP-1(0.2 M NaCl) and

SGP-2 (0.4 M NaCl). The two groups were purified with 0.2 M NaCl solution by size exclusion chromatography of Sephadex G-100 to obtain SGP-1-1 and SGP-2-1, respectively.

Fig.4 Elution curve of crude SGP on DEAE-52 cellulose chromatography column (A) and elution curves of SGP-1 (B) and SGP-2 (C) on Sephadex G-100 chromatography column, respectively.

3.3.2 Composition and chemical property of SGP

Table 3 Composition of crude SGP and pure SGP

Items	Crude SGP	SGP-1-1	SGP-2-1
Protein (%)	1.12±0.06	-	-
Carbohydrate (%)	52.31±2.02 ^a	79.01±2.71 ^b	77.15±2.21 ^c
Total flavonoid (mg GAE/100 mg)	2.16±0.08	-	-
Uronic acid (%)	7.15±0.15 ^a	12.39±1.68 ^b	11.25±1.24 ^c

- was not detected and the different lowercases indicated significant difference ($P_b < 0.05$) in the same line.

The contents of carbohydrates, proteins, uronic acids and total flavonoids in crude SGP and purified SGP samples were summarized in Table 3. The results indicated that the higher content of these samples was

carbohydrate, which were 52.31±0.02 %, 79.01±2.71%, and 77.15±2.21%, respectively. The crude SGP contained lower uronic acid content (7.15±0.15%) while it was absent of protein (1.12±0.06%). The phenomenon

of this difference in composition indicated that the savage produced good deproteinization effect. In addition, the lack of flavonoids detection in the pure

SGP determined the independence of the antioxidants of the polysaccharides.

Fig.6 FPLC of crude SGP (A), SGP-1-1 (B) and SGP-2-1(C) (glucose : Glc, mannose: Man, xylose: Xyl, galacturonic acid: Galu, glucuronic acid: Glcu, fucose: Fuc, rhamnose: Rha, arabinose: Ara and galactose: Gal).

3.3.3 Monosaccharide composition and molecular weight of SGP

The monosaccharide composition analysis of SGP was performed by area normalization of HPLC. Totally, the molecular weight of crude SGP, SGP-1-1 and SGP-2-1 were 6.6×10^5 g/mol, 3.27×10^5 g/mol and 2.89×10^5 g/mol, respectively. The main particle size distribution was shown in Fig. 5. The chromatogram of 9 monosaccharide standards showed that peaks were collected within 65 min named as glucose, mannose, xylose, galacturonic acid, glucuronic acid, fucose, rhamnose, arabinose, galactose, as shown in Fig. 6 (A),

respectively. Then, peaks were determined by matching their retention times to that of standards. SGP-1-1 included 9 monosaccharides, at the molar ratio of 0.27: 0.85: 0.21: 2.13: 0.63: 0.92: 0.23: 1.15: 0.43 in Fig. 6(B), respectively. SGP-2-1 contained 6 monosaccharides, namely glucose, mannose, galacturonic acid, glucuronic acid, fucose, arabinose, at the molar ratio of 0.83: 0.42: 0.56: 1.87: 0.61: 0.63 in Fig. 6(C). This result was some different from previous reports on blackberry polysaccharides [29]. The reasons may be closely related to the growth environment of the plants as well as the extraction and purification methods.

Fig.5 Molecular weight and particle size distribution of crude SGP.

Fig.7 FT-IR spectra of SGP

3.3.4 FT-IR spectrum and SEM of SGP

The IR analysis spectrum of the polysaccharide was shown in Fig. 7. The broad peak at around 3400 cm^{-1} (SGP-1-1: 3365 cm^{-1} , SGP-2-1: 3438 cm^{-1}) were belonged to O-H of associative hydroxyl group, and the peak around 2939 cm^{-1} (SGP-1-1: 2900 cm^{-1} , SGP-2-1: 2988 cm^{-1}) were attributed to C-H stretching vibration and bending vibration. The absorption peak at 1424 cm^{-1} (SGP-1-1: 1420 cm^{-1} , SGP-2-1: 1412 cm^{-1}) belonged to the C-O-H carboxy symmetric stretching vibration, which was ascribed to the uronic acid groups of the polysaccharide [30]. The absorption band on the polysaccharide at 1615 cm^{-1} (SGP-1-1: 1612 cm^{-1} , SGP-2-1: 1623 cm^{-1}) were due to the $\text{C}=\text{O}$ group [31]. The peak at 1022 cm^{-1} (SGP-1-1: 1012 cm^{-1} , SGP-2-1: 1020 cm^{-1}) were attributed to the C-O-H deformation vibration. The adsorption band at 1099 cm^{-1} (SGP-1-1: 1071 cm^{-1} , SGP-2-1: 1054 cm^{-1}) belonged to the C-O-C stretching vibration of the pyranose pentacyclic ring [32]. It indicated the existence of the glycosidic bond contained in pyranose ring. Additionally, the absorption peak at

about 890 cm^{-1} (SGP-1-1: 894 cm^{-1} , SGP-2-1: 897 cm^{-1}) suggested that β -configuration was contained in this sugar structure [33]. Therefore, all these results showed the presence of the characteristic groups of the polysaccharide.

It can be seen from Fig. 8 that the surface of the SGP had a clear pore structure when the magnification was 20.0 K , while it was spherical with a viscosity wire under the magnification of 40.0 K and 50.0 K . The reason for these phenomena may due to that SGP was broken into small molecule polysaccharides under ultrasound [23], and the apparent structural properties led to its higher solubility [34]. In addition, the deproteinization operation also played an important factor on the rough pore structure of the polysaccharide surface [35]. Besides, the alone component polysaccharide appearance after purification including SGP-1-1 and SGP-2-1 can be clearly observed at 50.0 K . The SGP-1-1 and SGP-2-1 lacked protein filament when we compared with the shape of crude polysaccharide. The reason for this phenomenon may be that the polysaccharide was more purify after cellulose chromatography.

Fig. 8 SEM of SGP

3.4 Antioxidant activities in vitro of SGP

3.4.1 DPPH free radical scavenging activity of SGP

The principle of DPPH free radical scavenging activity depended on the disappeared absorption peak at 517 nm , when a free radical scavenger paired with the DPPH radical single electron, and therefore can be performed to analyze its oxidation by spectrophotometers rapidly [24]. As shown in Fig. 9 (A), it can be seen that the crude SGP exhibited a significant ($P < 0.05$) dose-dependent effect on better free radical scavenging ability in the concentration range from 0.2 to 4.0 mg/mL . The DPPH free radical scavenging activities was $55.21 \pm 2.5\%$, it was 0.61 times that of the standard of VC at the concentration of 4.0 mg/mL , where the IC_{50} of crude SGP was 0.16 mg/mL . The scavenging ability of SGP-1-1 and SGP-2-1 expressed lower than that of crude SGP at the same polysaccharide concentration. All of the three groups showed a dose-dependent effect. These phenomena involved in the experiment might due to SGP which can be seen as an electron provider to scavenge DPPH. The color of the system was changed when the electrons provided by the polyhydroxy structure of the polysaccharide connected with the DPPH

radical electrons. This method precisely simulated the state of competition with the body for DPPH free radicals.

3.4.2 ABTS⁺ radical scavenging activity of SGP

The absorbance of ABTS^+ can be determined at 734 nm to calculate the total antioxidant capacity of the samples. Green ABTS^+ provided by ABTS under the action of oxidant when the production of ABTS^+ were inhibited in the presence of antioxidants [36]. The lighter the color of the reaction system, the higher the antioxidant capacity confirmed. As shown in Fig. 9 (B), the ABTS^+ radical scavenging activity were positively correlated ($P < 0.05$) with the sample concentration range. The crude SGP radical scavenging activity of ABTS^+ was higher than SGP-1-1 and SGP-2-1 at every concentration, which was $93.45 \pm 1.5\%$ at the concentration of 4.0 mg/mL , respectively, and the IC_{50} of VC was 2.75 times higher than that of crude SGP. SGP-2-1 showed the worst oxidation resistance when we compared these three groups except for the control. It probably because it lacked fucose which possessed stronger [37] effect than SGP-1-1. The ABTS^+ free radical scavenging activity of SGP might depend on their structural integrity and

molecular size, which was no different from previous reports [38, 39]

3.4.3 Hydroxyl radical scavenging activity of SGP

Gene transcriptional expression process was blocked when the hydroxyl group rapidly reacted with intracellular DNA across the biofilm system. This phenomenon caused by hydroxyl groups which would result in apoptosis and tissue damage. Therefore, removing the hydroxyl radicals was the main protection for the living system. As shown in Fig. 9 (C), the hydroxyl radical scavenging activity of SGP was

concentration dependent. The VC scavenging activity of hydroxyl was $93.24 \pm 2.1\%$ at the concentration of 4.0 mg/mL, as the IC_{50} was 0.32 mg/mL. The trend changes of the three groups were distinguished at 1.0 mg/mL. For example, crude SGP had opposite trend when the concentration was greater than 1.0 mg/mL, compared with SGP-1-1 and SGP-2-1. At the same time, SGP-1-1 and SGP-2-1 also expressed dose-dependent effects. The factor for this phenomenon may be explained by that SGP provided a amount of active hydroxyl groups from polysaccharides under low temperature treatment, which was consistent with previous reports [40].

Fig.9 Antioxidant activities on DPPH free radical scavenging activity, ABTS+ radical scavenging activity and hydroxyl radical scavenging activity of VC, crude SGP, SGP-1-1 and SGP-2-1. Values were present as means $\pm$ SD.

4 Conclusion

In this study, the SGP was purified after extraction with hot water assisted by ultrasound from *Suaeda salsa*. BBD was adopted to increase the extraction yield of SGP, and the optimal conditions were obtained as follows: ethanol volume fraction was 65%, extraction time was 72 min, ultrasound assisted power was 438 W, and temperature was 85°C, respectively. It was coincided with the model predictions under these conditions, which the extraction yield of SGP was $2.19 \pm 0.13\%$. The crude SGP was purified with DEAE-52 cellulose and Sephadex G-100 into SGP-1-1 and SGP-2-1. Crude SGP provided higher significant potentials ($P < 0.05$) in scavenging ability against DPPH, ABTS⁺ and hydroxyl radical under the optimized conditions. Meanwhile, the monosaccharide composition analysis including the molar ratio of crude SGP, SGP-1-1 and SGP-2-1 were measured, respectively. Besides, both SEM and FT-IR clearly showed that the characteristic structure of SGP was viscous polysaccharide with uronic acid groups, which means that the polysaccharide could gives viscous solution. Thus, it can be used as a natural antioxidant source with good development prospects, and further study should concentrate on the use of polysaccharide in nutrition food in the future.

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Compliance with ethics guidelines

The authors declare that they have no conflict of interest.

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References

1. Benwahhoud M, Jouad H, Eddouks M, Lyoussi B Journal of Ethnopharmacology. **76**, 35-38 (2001)
2. Bartolozzi F, Bertazza G, Bassi D, Cristoferi G Journal of Chromatography A. **758**, 99-107 (1997)
3. Leone S, Molinaro A, Dubery I, Lanzetta R, Parrilli M Carbohydr Res. **342**, 1514-1518 (2007)
4. Costa L S, Fidelis G P, Cordeiro S L, Oliveira R M, Sabry D A, Camara R B G, Nobre L T D B, Costa M S S P, Almeida-Lima J, Farias E H C, Leite E L, Rocha H A O Biomedicine & Pharmacotherapy. **64**, 21-28 (2010)
5. Okoli C O, Akah P A Pharmacol Biochem Behav. **79**, 473-481 (2004)
6. Amir M, Shikha K European Journal of Medicinal Chemistry. **39**, 535-545 (2004)
7. Ralet M C, Andre-Leroux G, Quemener B, Thibault J F Phytochemistry. **66**, 2800-2814 (2005)

8. Shakhmatov E G, Toukach P V, Michailowa C, Makarova E N *Carbohydr Polym.* **113**, 515-524 (2014)
9. Thambiraj S R, Phillips M, Koyyalamudi S R, Reddy N *Industrial Crops and Products.* **74**, 950-956 (2015)
10. Zhang J, Wen C, Chen M, Gu J, Zhou J, Duan Y, Zhang H, Ma H *Int J Biol Macromol.* **134**, 172-179 (2019)
11. Xiang B, Yu X, Li B, Xiong Y, Long M, He Q *J Food Biochem.* **43**, e12899 (2019)
12. Li S, Wang A, Liu L, Tian G, Xu F *Food Sci Biotechnol.* **28**, 759-767 (2019)
13. Tang C, Ding R, Sun J, Liu J, Kan J, Jin C *Food Funct.* **10**, 2290-2312 (2019)
14. Scroccarello A, Della Pelle F, Neri L, Pittia P, Compagnone D *Food Res Int.* **119**, 359-368 (2019)
15. Della Pelle F, Scroccarello A, Scarano S, Compagnone D *Anal Chim Acta.* **1051**, 129-137 (2019)
16. Wu D T, Liu W, Han Q H, Du G, Li H Y, Yuan Q, Fu Y, Zhao L, Zhang Q, Li S Q, Qin W *Int J Biol Macromol.* **136**, 891-900 (2019)
17. Abou El Azm N, Fleita D, Rifaat D, Mpingirika E Z, Amleh A, El-Sayed M M H *Molecules.* **24**, (2019)
18. Chen C, Wang P P, Huang Q, You L J, Liu R H, Zhao M M, Fu X, Luo Z G *Food Funct.* **10**, 3684-3695 (2019)
19. Pu X, Ma X, Liu L, Ren J, Li H, Li X, Yu S, Zhang W, Fan W *Carbohydr Polym.* **137**, 154-164 (2016)
20. Wang Z-B, Chen B-B, Luo L, Yan J-K *Journal of the Taiwan Institute of Chemical Engineers.* **67**, 54-60 (2016)
21. Hammi K M, Hammami M, Rihouey C, Le Cerf D, Ksouri R, Majdoub H *Food Chemistry.* **212**, 476-484 (2016)
22. Yuan Q, Xie Y, Wang W, Yan Y, Ye H, Jabbar S, Zeng X *Carbohydr Polym.* **128**, 52-62 (2015)
23. Wefers D, Gmeiner B M, Tyl C E, Bunzel M *Phytochemistry.* **116**, 320-328 (2015)
24. Chen R-Z, Tan L, Jin C-G, Lu J, Tian L, Chang Q-Q, Wang K *Industrial Crops and Products.* **77**, 434-443 (2015)
25. Wang Y, Wang F, Ma X, Sun S, Leng F, Zhang W, Wang X *Industrial Crops and Products.* **77**, 467-475 (2015)
26. Ben Salem Y, Amri S, Hammi K M, Abdelhamid A, Le Cerf D, Bouraoui A, Majdoub H *International Journal of Biological Macromolecules.* **97**, 8-15 (2017)
27. Ballesteros L F, Teixeira J A, Mussatto S I *Carbohydrate Polymers.* **157**, 258-266 (2017)
28. Mzoughi Z, Abdelhamid A, Rihouey C, Le Cerf D, Bouraoui A, Majdoub H *Carbohydr Polym.* **185**, 127-137 (2018)
29. Yin J Y, Nie S P, Zhou C, Wan Y, Xie M Y *J Sci Food Agric.* **90**, 210-217 (2010)
30. Jiang Y Y, Wang L, Zhang L, Wang T, Yu L, Ding C B, Yang R W, Wang X L, Zhou Y H *Int J Biol Macromol.* **70**, 92-99 (2014)
31. Zhao Y M, Song J H, Wang J, Yang J M, Wang Z B, Liu Y H *J Sci Food Agric.* **96**, 4484-4491 (2016)
32. Ono Y, Furihata K, Isobe N, Saito T, Isogai A *Int J Biol Macromol.* **107**, 2598-2603 (2018)
33. Zhao T, Mao G, Feng W, Mao R, Gu X, Li T, Li Q, Bao Y, Yang L, Wu X *Carbohydr Polym.* **105**, 26-33 (2014)
34. Sun Y, Cui S W, Tang J, Gu X *Carbohydrate Polymers.* **80**, 544-550 (2010)
35. You Q, Yin X, Zhang S, Jiang Z *Carbohydr Polym.* **99**, 1-10 (2014)
36. Zhang L, Ye X, Ding T, Sun X, Xu Y, Liu D *Ultrason Sonochem.* **20**, 222-231 (2013)
37. Zhu X, Li W, Li Y, Xu W, Yuan Y, Zheng V, Zhang H, O'Donnell J M, Xu Y, Yin X *Neuropharmacology.* **153**, 20-31 (2019)
38. Zhang S, Yi X, Su X, Jian Z, Cui T, Guo S, Gao T, Li C, Li S, Xiao Q *J Cell Mol Med.* **74**, (2019)
39. Russo D, Faraone I, Labanca F, Sinisgalli C, Bartolo M, Andrade P B, Valentao P, Milella L *Phytochem Anal.* **84**, (2019)
40. Foyer C H, Pellny T K, Locato V, Hull J, De Gara L *Methods Mol Biol.* **1990**, 165-181 (2019)