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Extraction of *Sanguisorba officinalis* L. polysaccharide by ultrasound-assisted extraction: structural characterization, antioxidant, hemostatic and immunological activity

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ABSTRACT

Sanguisorba officinalis L. (*S. officinalis*) has been widely distributed in various regions of China and holds significant potential for various applications. However, limited studies have focused on *S. officinalis* polysaccharides (SOPs) and their biological activities. Utilizing response surface methodology (RSM), the process of ultrasonic-assisted extraction of SOPs was optimized. The optimal conditions were 270.2 W, 42.4 mL/g, and 47.7 min. Through separation and purification, a neutral polysaccharide named SOUP-1 was obtained. SOUP-1 (17.1 kDa) mainly includes glucose and a few galactose, mannose, and arabinose. The main chain of SOUP-1 is primarily → 4)-α-D-Glcp-(1 → 4,6)-α-D-Glcp-(1 → substituted at C6 with branch chain → 6)-α-D-Glcp-(1 → . Furthermore, SOUP-1 showed antioxidant activity, including scavenging activities of hydroxyl radicals and DPPH. It also enhances macrophage phagocytic activity and modulates the expression of inflammatory cytokines such as TNF-α and IL-6. Additionally, this study first discovered the hemostatic activity of SOUP-1. This study provides theoretical support for the potential applications and further research of SOPs. It provides a basis for the development of SOPs as novel natural-source immunomodulators in the food and pharmaceutical industries.

1. Introduction

Sanguisorba officinalis L. (*S. officinalis*) is the dried root of the plant *Sanguisorba officinalis* L. of the Rosaceae family. This natural resource is abundant and widely distributed in various regions of China [1] and can be used to make S. *officinalis* wine and S. *officinalis* congee. To date, various compounds have been identified in *S. officinalis*, including saponin, tannin, and flavonoids [2]. And exhibit a wide range of biological activities, such as hemostatic [3], immunological activity [4], antibacterial [5], and antitumor activities [6].

Polysaccharide is a natural compound and the essential molecules for various life processes [7]. Due to the diverse biological activities of natural products polysaccharides have attracted increasing attention in recent years [8]. Polysaccharides from *Fritillaria ussuriensis* Maxim. exhibit antioxidant and immunomodulatory activities [9], and the ginger polysaccharides possess antioxidant and immunological activity [10], which could significantly increase the production of immune substances. And polysaccharide from *Pueraria lobata* (Willd.) Ohwi could improve immune function in immunosuppressed mice [11]. *Corn silk* polysaccharide exhibit hemostatic activity, which affected the coagulation indicators activated partial thromboplastin time (APTT), thrombin time (TT) and prothrombin time (PT) [12]. Current research on *S. officinalis* has primarily focused on small-molecule compounds, with relatively few studies exploring *S. officinalis* polysaccharides (SOPs) and their biological activities. Previous studies have shown that SOPs possess significant biological activities including promote burn wound

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healing activities [13] and RSP-3 from *S. officinalis* could effectively ameliorate renal function and had significant protective effect, which could alleviate acute kidney injury [14]. However, studies on other potential activities of SOPs need further research into their bioactive properties.

The most common method for extracting natural polysaccharides is extract with hot water. While simple to operate, it often requires high temperatures and long extraction times, which may result in low extraction yields [9]. Ultrasonic-assisted extraction (UAE) utilizes cavitation bubbles generated by ultrasonic energy to enhance the interaction between the solvent and the sample, thereby improving extraction efficiency. In recent years, UAE has attracted increasing attention due to its advantages of being environmentally friendly and efficient. Hu et al. used UAE to extract polysaccharides from ginkgo leaves, achieving a yield 2.34 times higher than that extract with hot water [15]. Similarly, Wang et al. applied UAE to extract pectic polysaccharides from *Choerospondias axillaris* peel and found that the antioxidant activity of the ultrasound-extracted polysaccharides was significantly higher than that of those extracted with hot water [16]. Guo et al. compared to the traditional hot water extraction method, ultrasound-assisted extract polysaccharide from maca exerted remarkable immune activity [17]. These findings suggest that ultrasonic-assisted extraction is an ideal method for studying natural polysaccharides.

Hemostasis is a physiological phenomenon that accelerates blood clotting, prevents blood leakage from injured tissue sites, and maintains a balance of continuous blood flow in the circulatory system. As early as the 1960s, the clotting cascade theory was proposed, which is currently the most widely recognized coagulation theory [18,19]. The entire coagulation process is carried out through a highly controlled mechanism, and excessive anticoagulation or coagulation can lead to life-threatening diseases. *S. officinalis* has long been used for hemostasis, and studies have shown that saponins are the main component with hemostatic effects [20].

The current development of *S. officinalis* mainly focuses on small molecular components. In order to further realize the development and utilization of *S. officinalis* components and improve the economic value, this study optimized the extraction conditions of *S. officinalis* polysaccharides using ultrasound-assisted methods, and obtained a novel type of neutral polysaccharide SOUP-1, and determined its structure. This study found that SOUP-1 has good antioxidant capacity and significant immunomodulatory ability. In addition, this study has firstly confirmed the hemostatic activity of SOPs. This study provides a basis for the development of economic products from *S. officinalis* and the novel natural source immunomodulators in the fields of food and medicine.

2. Materials and methods

2.1. Extraction process and parameter optimization of SOUP-1

The extraction yield of polysaccharides was utilized as a performance indicator, and response surface methodology (RSM) in conjunction with Box-Behnken design (BBD) were implemented, based on single-factor experiments, to optimize three critical variables: ultrasonic power, extraction time, and liquid-to-material ratio. The technical route for total polysaccharides and SOUP-1 from *S. officinalis* is shown in Fig. S1, with comprehensive details provided in the Supplementary materials.

2.2. Characterization of SOUP-1 polysaccharide

2.2.1. Homogeneity and molecular weight analysis of SOUP-1

Following the method described in our previous report [9], SOUP-1 (0.1 mg/mL) was determined. A standard linear calibration curve was obtained with Dextran T-series standards. The details provided in the Supplementary materials.

2.2.2. Monosaccharide composition analysis of SOUP-1

According to Li et al. method with little modifications [21], SOUP-1 was detected by HPLC-DAD with PMP precolumn derivatization. In brief, the derivatization of SOUP-1 (10 mg) was reacted with PMP (0.5 M) and NaOH (0.3 M) for 1 h (70 °C) after hydrolyzed with trifluoroacetic acid hydrolyzed with trifluoroacetic acid (3 M, 110 °C, 6 h). And after neutralized with HCl (0.3 M), SOUP-1 was analyzed with HPLC-DAD (245 nm, 30 °C) (Cosmosil 5C18-PAQ, 4.6 × 250 mm, 5 μm) with phosphate buffer solution (0.08 M, pH = 6.85). The flow rate is 0.8 mL/min. The gradient elution program of the mobile phase is shown in Table S4.

2.2.3. Spectroscopy analysis of SOUP-1

The ultraviolet spectrum (UV) of SOUP-1 was obtained over a wavelength range of 200–400 nm with a UV-2550 spectrophotometer (Shimadzu, Japan). And FTIR-8400S spectrometer (Shimadzu, Japan) was used to obtained the infrared spectroscopy (IR) of SOUP-1.

2.2.4. Methylation analysis of SOUP-1

The analysis of SOUP-1 was conducted following a slightly modified protocol from Ciucanu et al. [22], the methylation analysis of SOUP-1 was obtained. In brief, 20 mg of SOUP-1 was dissolved in DMSO (2 mL), adding NaOH-DMSO and iodomethane (2 mL) and stirring for 1 h under the nitrogen and dark (three times). After hydrolyzed with 2 mL of TFA at 110 °C for 6 h, 20 mg $NaBH_4$ was added to continuous reaction for 60 min at room temperature. Then, the acetylation reaction was continued with acetic anhydride (2 mL) and pyridine (2 mL) at 110 °C for 2 h. The sample was analyzed by GC–MS using a Shimadzu GCMS-QP 2010 (DB-1701 capillary column, 30 m × 0.25 mm). According to the peak time and mass fragmentation patterns acquired from the CCRC Spectral Database, the partially O-methylated alditol acetates (PMAAs) were identified.

2.2.5. NMR spectrum of SOUP-1

SOUP-1 (50 mg) was analyzed to record the NMR spectrum (Bruker AVANCE III). The ^{1}H NMR, ^{13}C NMR, COSY, HSQC, and HMBC spectra information were used for the structural analysis of SOUP-1.

2.2.6. Atomic force microscopy analysis of SOUP-1 and Congo red test

The AFM (NT-MDT Ntegra, Russia) was used to analyze the molecular morphology of SOUP-1. The triple helix structure of SOUP-1 was detected with the report method with slightly modification [23]. Briefly, after the SOUP-1 (2 mg/mL) sample mixed with Congo red solution, and NaOH solutions of varying concentrations (0, 0.1–0.5 mol/L) were added to the solution. After reacted at room temperature, it was measured with a UV-2550 spectrophotometer (Shimadzu, Japan) (300–700 nm).

2.3. Antioxidant activity of SOUP-1

The antioxidant activity of SOUP-1, including DPPH and hydroxyl radical scavenging activities, were analyzed following a previously described method [9].

2.4. Hemostatic activity of SOUP-1

Activated partial thromboplastin time (APTT) and prothrombin time (PT) tests were used to assess the intrinsic and extrinsic pathways of coagulation. The rate of fibrinogen conversion to fibrin was assessed by the thrombin time (TT) test. All experiments were conducted according to the manufacturer's specifications. Animal experiment has been approved by the Animal Ethics Committee of Harbin Medical University. The blood from wistar rats was collected into tubes containing 3.2 % sodium citrate solution (9:1 v/v) and centrifuged at 3000 rpm for 10 min at room temperature. For PT and TT experiments, 50 μL of the supernatant plasma was incubated with 10 μL of SOUP-1 solution (20, 50, 100

μg/mL) or 10 μL of saline (blank control group) at 37 °C for 3 min. PT or TT reagents (100 μL for PT, 50 μL for TT) were then added. For the APTT test, plasma (50 μL), SOUP-1 (10 μL), and APTT reagent (50 μL) were incubated for 5 min, before adding 50 μL calcium chloride solution (0.25 M), with heparin sodium as the control group [12].

2.5. Immunomodulatory activity of SOUP-1

2.5.1. Cell culture

RAW264.7 cells were cultured in 1 % penicillin–streptomycin, 10 % FBS, and 89 % DMEM at 37 °C in 5 % CO_2. The groups included control group, SOUP-1 at different concentrations groups (62.5–500 μg/mL), and LPS group (1 μg/mL).

2.5.2. Cell viability assay

The MTT method and the neutral red staining method were utilized to measure the effect of SOUP-1 on RAW264.7 cell viability and phagocytic activity, respectively [24]. Detailed information is available in the Supplementary data.

2.5.3. ELISA assay

According to Li et al. [25] method and the manufacturer's specifications, The supernatants from RAW264.7 cells were collected, and the expression levels of TNF-α and IL-6 were determined using the ELISA method for analyzing the immunomodulatory activity of SOUP-1.

2.6. Data analysis

All the experimental results were expressed as mean ± SD, visualized using GraphPad Prism 8, and analyzed by ANOVA ($p < 0.05$).

3. Results and discussion

3.1. Single factor experiments

Single-factor experiments were performed to investigate the factors of power, time, and liquid-to-material ratio on the extraction yield of *S. officinalis* polysaccharides (SOPs). The extraction yield of SOPs exhibited a gradual increase with increasing power, reaching a maximum at 270 W, after which it began to decline, as shown in Fig. S2a. This trend may be due to the fact that, within an optimal range, higher ultrasonic power generates more cavitation bubbles, thereby enhancing the polysaccharide extraction rate. However, when this range is exceeded, excessively high power may lead to hydrolysis of polysaccharides and a decreased extraction rate [26]. Similarly, the excessive time and large liquid-to-material ratio beyond this optimal range lead to decreased extraction efficiency, as shown in Fig. S2b and c [26]. Therefore, it is particularly important to determine the optimal range of extraction conditions. Table S1 shows the BBD for RSM experiment.

3.2. Response surface optimization

These above conditions exceed certain critical values, the extraction rate decreases. Therefore, RSM experiments were conducted based on the above results to determine the optimal extraction conditions for SOPs.

As shown in Table S2, the BBD experiments were divided into 17 groups, enabling the elucidation of relationships between response variables and experimental parameters, which can be described by the following fitting equation:

$$\text{Yields (\%)} = 15.23 + 0.04625\,A + 0.395B - 0.12625C + 0.0825\,AB - 0.05\,AC + 0.1175\,BCE - 1.9175\,A^2 - 0.995\,B^2 - 0.9925\,C^2.$$

A: Power; B: Liquid-to-Material Ratio; C:Time.

According to the analysis of variance (ANOVA) results, the regression model has a p-value < 0.0001. The R-squared value of 0.9730 and the adjusted R-squared value of 0.9382 demonstrate that the model fits the experimental data well. Fig. 1 was generated based on the regression equation.

The response surface plots demonstrated the response values of the variables and the interactions between them. The results indicated that the interactions between the above factors significantly influenced the extraction yield of SOPs. The optimal theoretical parameters for SOPs are 270.2 W, 47.7 min, and 42.5 mL/g. Under these parameters, the theoretical maximum extraction rate of SOPs is 14.61 %. In practical experiments, we extracted SOPs powder using 270 W of ultrasonic power, 42 mL/g, and 48 min, which resulted in an actual experimental yield of 14.82 ± 0.24 %.

3.3. Isolation and purification

Although the optimized extraction process maximized the extraction rate of SOPs, the resulting samples still contained impurities such as proteins. To improve polysaccharide purity and obtain homogeneous samples, it is necessary to separate and purify the extracts further. The technical route for extracting and purifying SOUP-1 from SOPs is illustrated in Fig. S1. After the sample was defatted with 95 % ethanol, extracted with ultrasonic-assisted method, and proteins removed with sevage method, SOPs was obtained. Then, the extract was initially separated by ion-exchange resin to obtain SOP-B. Subsequently, SOP-B was further purified with DEAE-52 chromatography column, with NaCl solution used for elution, to obtain homogeneous polysaccharide SOUP-1.

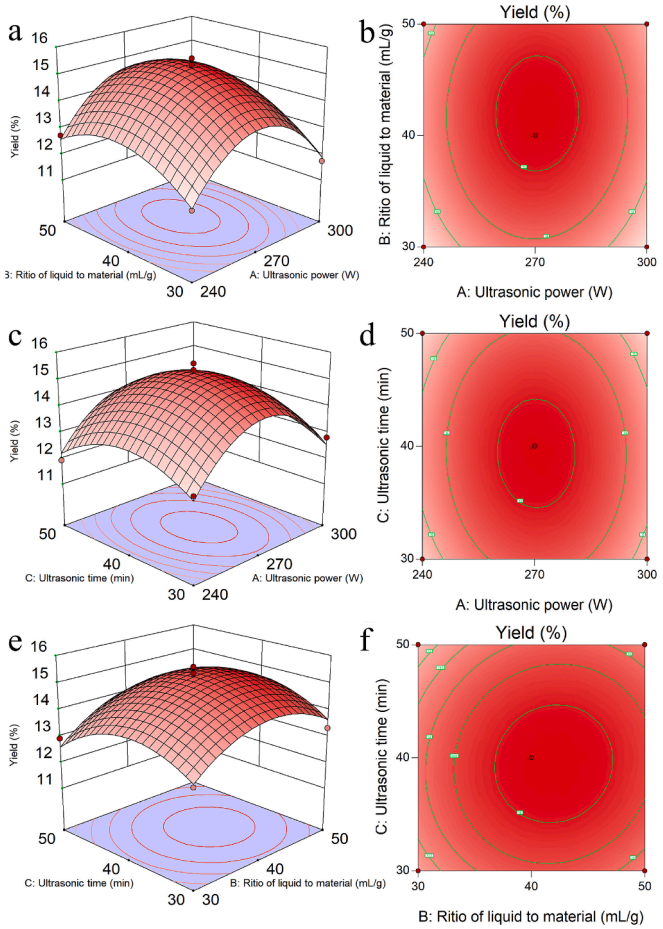

Fig. 1. The effects of various conditions on the extraction rate of SOPs (%).

3.4. Structural characteristics

3.4.1. Purity and molecular weight

Molecular weight is an important indicator related to biological activity. The HPGPC result showed a major peak of SOUP-1, which indicated that SOUP-1 is a homogeneous polysaccharide, as illustrated in Fig. 2b. Meanwhile, the molecular weight of SOUP-1 is approximately 17.1 kDa. Additionally, UV spectroscopy analysis of SOUP-1 showed almost no absorption in the range (260–280 nm), as seen in Fig. 2c. These findings confirm that impurities such as proteins and nucleic acids have been effectively removed from the SOUP-1, making it suitable for subsequent experiments. In Fig. S4, a peak is observed at 3385.1 cm^{-1}, which was associated with ν(O–H). The 2933.0 cm^{-1} was attributed to the ν(C–H). The 1664.8 cm^{-1} was attributed to the scissoring vibration of bound water [9]. Furthermore, the 1370.2 cm^{-1} was attributed to the ν(C–O) and the O–H angular vibration. Additionally, the 1152.7 and 1025.4 cm^{-1} were associated with the C–O, C–O–C, and pyranose rings. The 834.2 cm^{-1} suggested the presence of an α-glycosidic linkage.

3.4.2. Monosaccharide composition

Analyzing the monosaccharide composition of polysaccharides is essential for structural characterization, as different monosaccharide compositions and proportions can significantly influence polysaccharide activity. The analysis results of the standards and SOUP-1 sample (Fig. 3a and b) indicate that SOUP-1 is primarily composed of Glc (86.70 %), with a small portion of Man (2.37 %), Gal (5.29 %), and Ara (5.64 %).

3.4.3. Methylation analysis

SOUP-1 was derivatized using the methylation method, and analyzed by GC–MS to study the glycosidic bonds of SOUP-1, providing further insights into its structure. The main linkages identified were shown in Table 1.

3.4.4. NMR analysis

NMR techniques were used to analyze the molecular structure and conformation of SOUP-1. Fig. 4 showed the 1D and 2D spectra of SOUP-1. In the region of 3.0–5.6 ppm region correspond to terminal hydrogens (H-1), a significant concentration of proton resonance signals was observed, which are typical of polysaccharide signals. Specifically, signals in the 4.5–5.6 ppm, several distinct hydrogens (H-1) were observed (Fig. 4a), and the signal summits exhibited overlap, posing challenges in signal assignment. Considering the intricate composition of SOUP-1, it was deemed essential to employ 2D NMR analysis for the comprehensive determination of its chemical shifts. After completing the attribution of proton resonance signals within residues, the attribution of anomeric carbon chemical shifts can be completed based on HSQC spectra. The residue sequence can be determined from COSY and HMBC spectra.

The carbon NMR signals of SOUP-1 are predominantly distributed in the δ 60–105 ppm range. Major anomeric carbon peaks appeared at δ 101.93, 99.84, 99.82, and 97.79 ppm. The anomeric carbon region is primarily between δ 95–105 ppm, which is coincidence with the typical chemical shifts observed in polysaccharides. The major signal peaks at δ 71.66, 73.46, 76.93, 71.32, 60.55, 71.35, 73.23, 73.32, 70.24, 60.35, 71.33, 71.93, 77.30, 69.47, 65.68, 71.22, 73.27, 73.17, 71.46, and 68.96 ppm are distributed in the 60–80 ppm region, which correspond to sugar ring carbons. The signal peak around δ 4.79 ppm corresponds to the D_2O signal. Anomeric hydrogen with chemical shifts >5.0 ppm usually indicates an α-glycosidic bond configuration, whereas with chemical shifts <5.0 ppm indicated a β-glycosidic bond configuration. The main terminal hydrogen signals at δ 5.52, 5.48, 5.21, and 5.10 ppm are concentrated in the 5.0–5.6 ppm region, indicating that SOUP-1 is composed of α-type monosaccharides (Fig. 4a and b). Signals in the δ 3.0–4.3 ppm range are proton signals from the sugar ring. Based on the obtained results, further structural analysis was conducted.

The HSQC spectrum shows signal at δ 99.84 ppm corresponds to the signal at δ 5.52 ppm. Additionally, correlations were observed at δ 99.82, 101.93, and 97.79 ppm correspond to anomeric hydrogen signals at δ 5.48, 5.21, and 5.10 ppm, respectively (Fig. 4c). Through ^1H–^1H COSY spectrum analysis, the Fig. 4d indicated that H1-H6 signals correlations. Based on these correlations, we can deduce that H1-H6 are at δ 5.52, 3.77, 4.08, 3.78, 3.75, and 3.99 ppm, respectively. Combining this data with the HSQC spectrum, it is evident that H1-H6 correspond to C1-C6 as δ 99.84, 71.66, 73.46, 76.93, 71.32, and 60.55 ppm, respectively. These signals suggest that this linkage belongs to the glycosidic bond → 4)-α-Glcp-(1→ (residues A). By applying similar analytical rules and

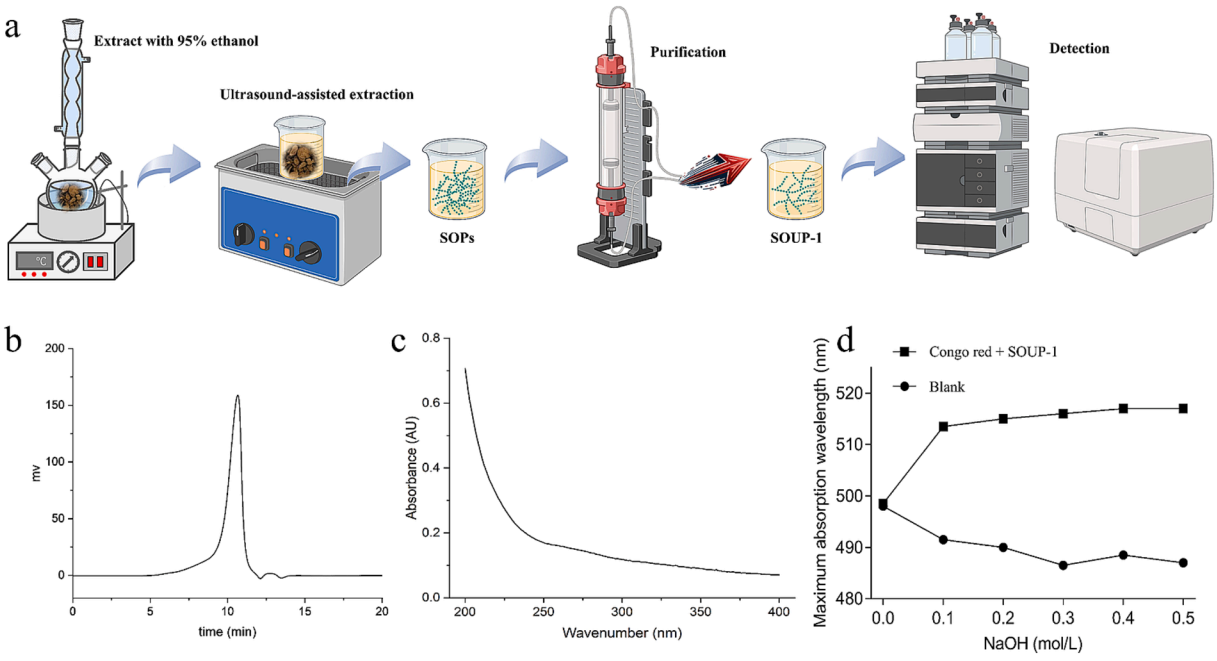

Fig. 2. (a) Schematic diagram of extraction, separation, and detection of the polysaccharide. (b) HPGPC chromatogram of SOUP-1. (c) The UV spectrum of SOUP-1 (200 - 400 nm). (d) The Congo red analysis of SOUP-1.

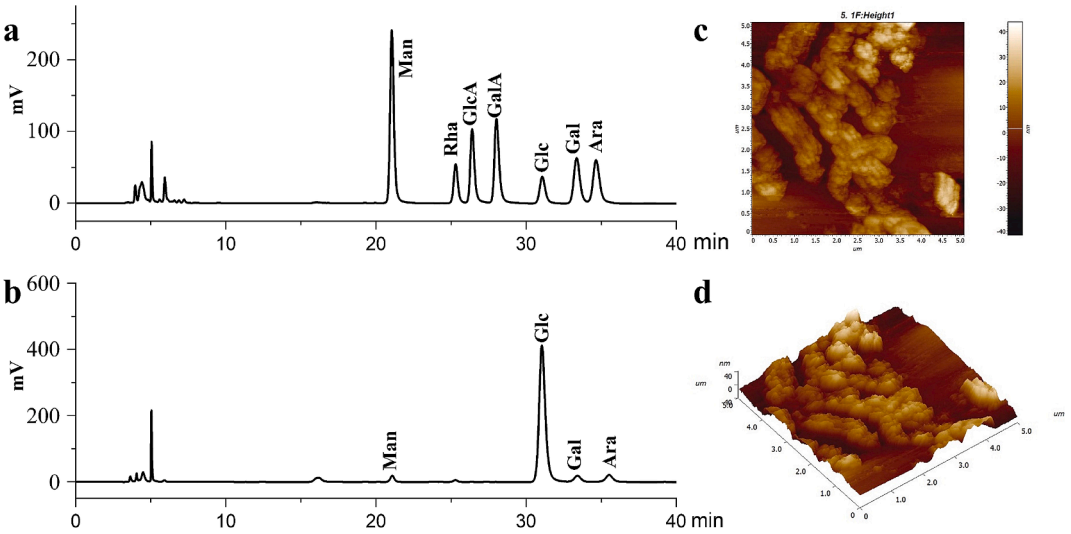

Fig. 3. Monosaccharide composition analysis of SOUP-1. (a and b) Standards and SOUP-1 analysis. (c and d) AFM analysis of SOUP-1.

Table 1
Methylation analysis of SOUP-1.

Peak	Methylated sugar	Mass fragments (*m/z*)	Linkage types	Mol %
1	2,3,4,6-Me4-Glcp	58, 71, 87, 101, 117, 129, 145, 161, 205	Glcp-(1→	7.89
2	2,3,6-Me3-Glcp	58, 87, 99, 101, 113, 117, 129, 131, 161, 173, 233	→4)-Glcp-(1→	69.42
3	2,3,4-Me3-Glcp	58, 87, 99, 101, 117, 129, 161, 189, 233	→6)-Glcp-(1→	13.79
4	2,3-Me2-Glcp	58, 71, 85, 87, 99, 101, 117, 127, 159, 161, 201	→4,6)-Glcp-(1→	8.9

Note: Calculated on the basis of the peak area as a relative percentage of all derivatives.

considering methylation results, chemical shift information, and relevant literature [27–29], the δ 5.48/99.82, 5.21/101.93, and 5.10/97.79 ppm pairs can be attributed to α-D-Glcp-(1→ (residues B), →4,6)-α-D-Glcp-(1→ (residues C), and → 6)-α-D-Glcp-(1→ (residues D), respectively. The results of the chemical shift assignments for SOUP-1 can be found in Table 2.

The Fig. 4e (HMBC spectrum), along with the information obtained from the HSQC and COSY spectra, was used to further study the glycosidic linkages in SOUP-1. For instance, the δ 5.52/76.93 ppm signal (residue A) confirmed the presence of →4)-α-D-Glcp-(1 → 4)-α-D-Glcp-(1→. Furthermore, the δ 5.52 ppm (residue A) shows a correlated peak with the C4 at δ 77.30 ppm (residue C), proving the occurrence of the linkage →4)-α-D-Glcp-(1 → 4,6)-α-D-Glcp-(1→. The δ 5.48 ppm (residue B) shows a correlated peak with the C6 at δ 68.96 ppm (residue D), indicating the existence of α-D-Glcp-(1 → 6)-α-D-Glcp-(1→. Similarly, the presence signal peak of C1 δ 5.21 (residue C) and H6 δ 68.96 ppm (residue D) indicated the existence of the linkage 4,6)-α-D-Glcp-(1 → 6)-α-D-Glcp-(1→. Furthermore, the signal of H1 δ 5.10 ppm (residue D) and C6 (residue D) and C4 (residue A), confirming the presence of linkage → 6)-α-D-Glcp-(1 → 6)-α-D-Glcp-(1→ and →6)-α-D-Glcp-(1 → 4)-α-D-Glcp-(1→.

In conclusion, the structure of SOUP-1 was deduced based on the above analysis. The SOUP-1 has a main chain of 4)-α-D-Glcp-(1 → 4,6)-α-D-Glcp-(1→ with branch chains substituted at C6 with →6)-α-D-Glcp-(1→. The primary structure was shown in Fig. 4f.

3.4.5. AFM analysis

The molecular conformation of polysaccharides is intimately associated with the functionality and biological activities [30]. AFM is commonly used to analyze the surface and the microstructure of the polysaccharide. Fig. 3c and d showed the AFM planar and three-dimensional images of SOUP-1. These images reveal that the surface of SOUP-1 is relatively smooth. The Congo red test was employed for the purpose of evaluating the triple helix structure of SOUP-1. Fig. 2d showed the Congo red result of SOUP-1. The maximum absorption wavelength is observed to undergo a change in accordance with increasing in NaOH concentration, which illustrate the presence of a triple-helix conformation of SOUP-1.

It has been reported that the immunostimulatory activity of alpha glucan with a triple helix conformation is closely related to its triple helix conformation and exhibits significant immune activity [10,31]. Polysaccharides with triple helix structures have been demonstrated to possess excellent antioxidant and immunomodulatory activities [32].

3.5. Antioxidant activity analysis

The association between oxidative stress and disease has been demonstrated in numerous studies [33]. The study of oxidation and antioxidant processes is of great significance in the field of medicine, as they play a pivotal role in various physiological and pathological processes [34].

An imbalance in free radicals can lead to oxidative stress, resulting in significant cellular damage and contributing to various diseases [35]. The DPPH method is commonly employed to quantitatively assess the free radical scavenging ability. Following treatment with SOUP-1, the color of the DPPH solution faded, indicating effective radical scavenging activity. The SOUP-1 achieved 51.7 % scavenging rate at 1.0 mg/mL (Fig. 5a). Hydroxyl radicals (OH) are highly reactive and can cause extensive damage to biomolecules [36]. The OH scavenging activity of SOUP-1 was evaluated, showing that as the concentration of SOUP-1 increased, the ability to scavenge hydroxyl radicals also increased, demonstrating a clear dose-dependent effect. At 1.0 mg/mL, SOUP-1 reached 43.4 % scavenging rate (Fig. 5b).

Therefore, SOUP-1 can serve as a natural source of antioxidants. Low molecular weight polysaccharides typically have superior antioxidant activity compared to their high molecular weight counterparts due to their reducing groups (hydroxyl and amino) that can come into contact with reactive free radicals and oxidants. Furthermore, polysaccharides comprising high levels of Glc, Gal, and Ara may exhibit enhanced antioxidant activity, which aligns with the findings of our research [35].

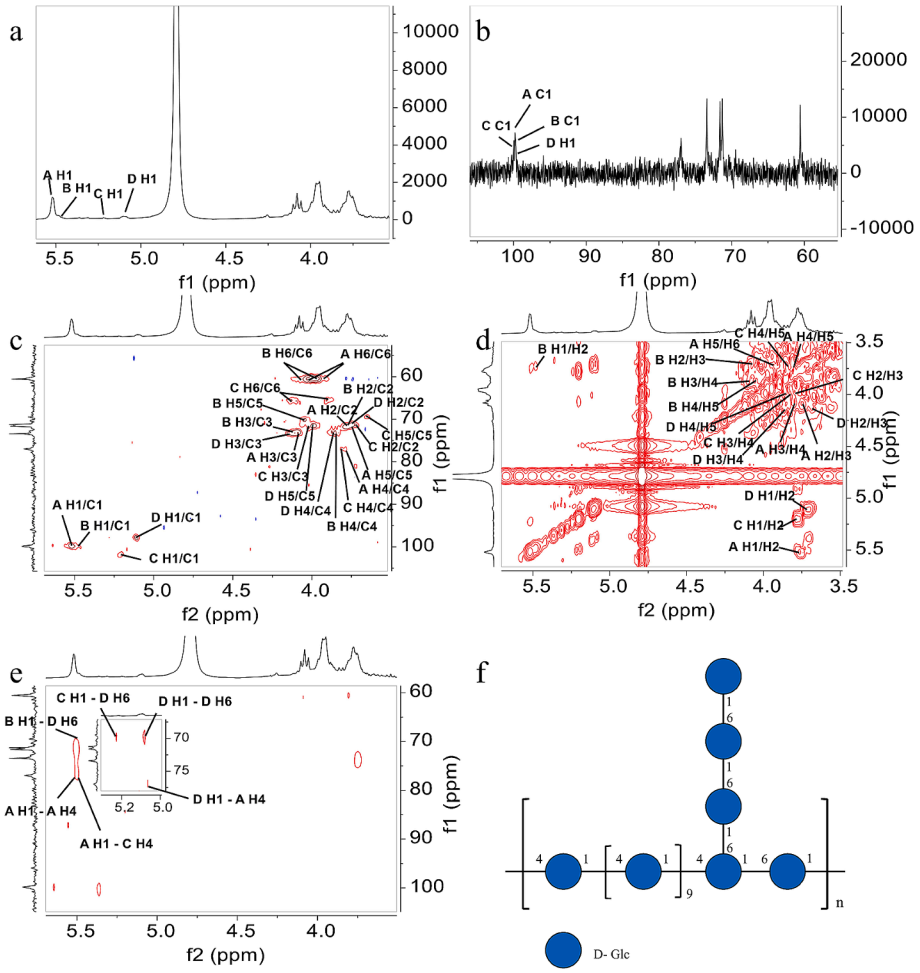

Fig. 4. NMR spectra of SOUP-1. (a) ^{1}H spectrum of SOUP-1, (b) ^{13}C spectrum of SOUP-1, (c) HSQC spectrum of SOUP-1, (d) COSY spectrum of SOUP-1, (e) HMBC spectrum of SOUP-1, and (f) Putative structure of SOUP-1.

Table 2
NMR chemical shift assignments for SOUP-1 according to the NMR spectra of SOUP-1.

Glycosyl residues		Chemical shifts (δ, ppm)					
		H1/C1	H2/C2	H3/C3	H4/C4	H5/C5	H6/C6
A	→4)-α-D-Glcp-(1→	5.52	3.77	4.08	3.78	3.75	3.99/3.92
		99.84	71.66	73.46	76.93	71.32	60.55
B	α-D-Glcp-(1→	5.48	3.75	4.10	3.86	4.04	4.03/3.96
		99.82	71.35	73.23	73.32	70.24	60.35
C	→4,6)-α-D-Glcp-(1→	5.21	3.72	4.01	3.81	3.65	4.10/3.88
		101.93	71.33	71.93	77.30	69.47	65.68
D	→6)-α-D-Glcp-(1→	5.10	3.74	4.12	3.84	3.99	4.01/3.64
		97.79	71.22	73.27	73.17	71.46	68.96

3.6. Hemostatic activity analysis

The coagulation cascade is a complex process that involves three primary pathways: the external pathway, the intrinsic pathway and the common pathway. The extrinsic pathway is initiated by tissue factor, which is highly expressed in the subendothelial and epidermis cells around blood vessels and is released by tissue injury [37]. It binds to and activates factor VII, which ultimately leads to the formation of a fibrin clot [38]. On the other hand, when a negatively charged surface comes into contact with factor XII, the intrinsic pathway, also referred to as the contact pathway, is triggered. Factor XII is converted to an active conformation, which activates factor XI and then activates factor IX, which then binds to factor VIII [39,40]. Both the inner and outer pathways converge on a common pathway. This pathway activates FX to Xa, converting prothrombin into thrombin, hydrolyzing fibrinogen to form a fibrin network/clot to prevent bleeding. Finally, fibrinolysis assists in the tissue remodeling of clots/thrombi, clearing the way for blood to flow in arteries and veins [41].

To explore the biological activity of SOUP-1, we conducted an in vitro analysis of its hemostatic activity. APTT assesses endogenous coagulation activity, focusing on factors IX, XI, and XII. TT measures the duration required for clot formation and the conversion of fibrinogen to fibrin, which is crucial in diagnosing coagulation disorders [42]. PT tests the extrinsic and common coagulation pathways, aiding in the detection of deficiencies in coagulation factors II, V, VII, and X [43]. In the APTT test, which evaluates the intrinsic pathway of the coagulation system, SOUP-1 significantly shortened the coagulation time compared to the saline group (blank control group) (25.8 ± 1.0 s) (Fig. 6b). Similarly, in the PT test, which assesses the extrinsic pathway, SOUP-1 significantly reduced coagulation time compared to the saline group (13.4 ± 0.1 s) (Fig. 6c). The TT test results showed that SOUP-1 could shorten coagulation time, especially at 100 μg/ml, the coagulation time was significantly shorter compared to the saline control (Fig. 6d). Moreover, SOUP-1 exhibited a dose-dependent hemostatic effect in APTT, PT, and TT assays at concentrations of 20 μg/mL, 50 μg/mL, and 100 μg/mL. In

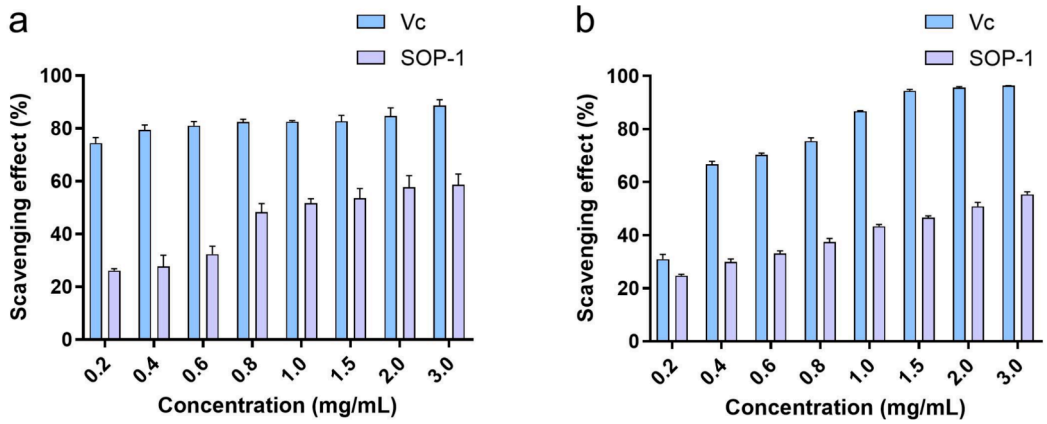

Fig. 5. Antioxidant activities of SOUP-1. (a) DPPH radical scavenging activitiy. (b) Hydroxyl radicals scavenging activity.

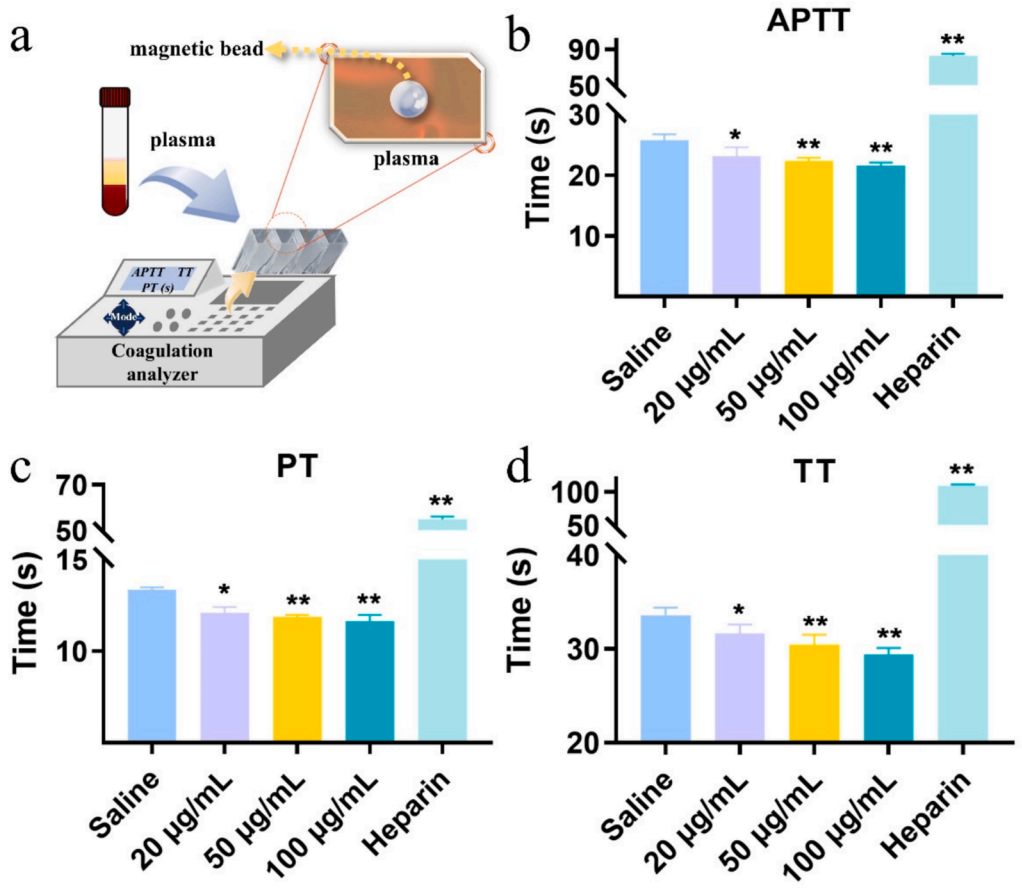

Fig. 6. In vitro hemostatic activity of SOUP-1 (20, 50, and 100 μg/mL). (a) Process of hemostatic activity test. (b) APTT. (c) PT. (d) TT. $^{*}P < 0.05$, $^{**}P < 0.01$, vs. the Saline group.

summary, SOUP-1 exerts its hemostatic effects by activating both the intrinsic and extrinsic coagulation pathways, as well as enhancing the conversion of fibrinogen to fibrin. Hemostasis is a well-known activity of the *S. officinalis*. Identifying novel natural products with anticoagulant or hemostatic activity from natural products remains a crucial area of research. Previous studies have shown that saponins in *S. officinalis* contribute to its hemostatic activity [20,44]. This study demonstrates for the first time that *S. officinalis* polysaccharides also possess hemostatic activity.

3.7. Immunoactivity analysis

Natural polysaccharides are increasingly recognized as ideal immunomodulators, owing to their potent efficacy and low toxicity profiles [21]. We conducted in vitro experiments using RAW264.7 cells to analyze the immunoactivity of SOUP-1. As the concentration of SOUP-1 increased, the proliferation of RAW264.7 cells was inhibited at 500 μg/mL to some extent (Fig. 7a). Based on these findings, SOUP-1 concentrations of 62.5, 125, and 250 μg/mL were selected for subsequent experiments.

Phagocytic activity is a crucial factor in activating the immune

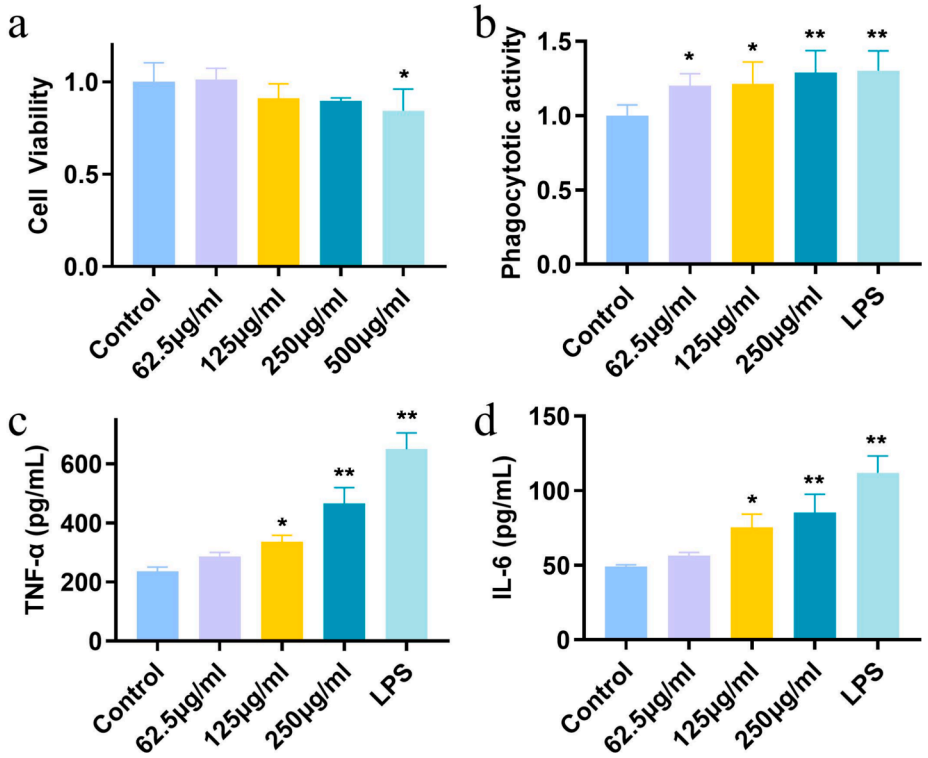

Fig. 7. Effects of SOUP-1 (62.5, 125, and 250 μg/mL) on RAW264.7 cells. (a) Cell viability by MTT; (b) Phagocytotic activity; (c and d) Expression of TNF-α and IL-6. $^{*}P < 0.05$, $^{**}P < 0.01$, vs. the control group.

response of macrophages. As SOUP-1 concentrations increased, RAW264.7 cell phagocytic activity was enhanced to varying degrees, exhibiting a dose-dependent effect, as illustrated in Fig. 7b. TNF-α is a multifunctional cytokine involved in a broad array of biological processes, including immune regulation. TNF-α plays a pivotal role in the immune system by activating and regulating the functions of immune cells. Additionally, TNF-α can stimulate the production of other inflammatory factors, such as IL-6. The production of TNF-α and IL-6 promotes the proliferation and activation of T cells and B cells. We employed ELISA assay to measure the effects of SOUP-1 on TNF-α and IL-6 activity in RAW264.7 cells, as shown in Fig. 7c and d. The results indicate that varying concentrations of SOUP-1 significantly influence the activity levels of TNF-α and IL-6, demonstrating that SOUP-1 possesses immunomodulatory activity by regulating the inflammatory microenvironment.

Many studies have suggested that the immunological activity of polysaccharides is closely related to the molecular weight [45]. Polysaccharides typically showed well immune activity with molecular weights in the range of 10–1000 kDa. Polysaccharides less than 10 kDa cannot exhibit good immunological activity due to their inability to maintain a stable chain conformation [46]. The effect of polysaccharides on immune activity can also be influenced by their monosaccharide domain [46]. Currently, studies have shown that high glucose content has good immunomodulatory activity [9,29]. Moreover, polysaccharide with glucans rich in α-1,4-D-glucosyl and α-1,6-D-glucosyl have active immune regulatory functions and are excellent immunomodulators [10,47,48]. In addition, highly branched D-glucan tends to contribute to triple helix formation, which is positively correlated with immunological activity [49].

In this study, SOUP-1 derived from plants demonstrated outstanding immunomodulatory activity, which mainly related to the structural characterization mentioned above. SOUP-1 (17.1kDa) is mainly composed of glucose, which is composed of →4)-α-D-Glcp-(1→, α-D-Glcp-(1→, →4,6)-α-D-Glcp-(1→, →6)-α-D-Glcp-(1→, and SOUP-1 also has a triple helix structure. The above results structurally demonstrate the feasibility of immunological activity of SOUP-1, and further contribute to the understanding of the structure–activity relationship of polysaccharides on immunological activity.

4. Conclusion

In this study, we identified the optimal extraction process for *S. officinalis* polysaccharides via ultrasonic-assisted extraction. We obtained a novel neutral polysaccharide, SOUP-1 (17.1 kDa) is predominantly consisted of glucose with trace amounts of galactose, mannose, and arabinose. The main chain is structured as →4)-α-D-Glcp-(1 → 4,6)-α-D-Glcp-(1→ substituted at C6 with branch chain →6)-α-D-Glcp-(1→. The study demonstrated the antioxidant activities of SOUP-1. And it also had the immunological activity through increasing the expression level of cytokines (TNF-α and IL-6). Additionally, this study validated the hemostatic activities of SOUP-1 for the first time. This study expands the scope of research on *S. officinalis*, offering theoretical support for the application of SOPs. It enhances the research value of *S. officinalis* polysaccharide, offering a solid foundation for the potential application as novel natural immunomodulators in both the food and pharmaceutical industries.

CRediT authorship contribution statement

Shuang Jiang: Writing – original draft, Methodology, Data curation, Conceptualization. **Xiaotian Wu:** Writing – original draft, Validation, Data curation. **Xuepeng Shi:** Writing – original draft, Formal analysis, Data curation. **Yuanqiu Mu:** Visualization, Data curation. **Li Zhang:** Investigation, Data curation. **Shulu Zhang:** Validation, Methodology, Formal analysis. **Lin Wei:** Validation, Methodology, Formal analysis. **Zheng Feng:** Methodology, Investigation, Data curation. **Yinze Zhong:** Validation, Data curation. **Xinhui Huang:** Validation, Data curation. **Yeqing Xu:** Visualization, Data curation. **Shah Syed Faizan Ali:** Data curation. **Zhaonan Xu:** Investigation, Data curation. **Xiaotong Wang:** Writing – review & editing. **Chunli Gan:** Writing – review & editing.

Zhibin Wang: Writing – review & editing, Funding acquisition, Conceptualization. **Yanan Sun:** Formal analysis, Data curation, Conceptualization. **Chunjuan Yang:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2025.107292.

References

[1] P. Zhou, J. Li, Q. Chen, L. Wang, J. Yang, A. Wu, N. Jiang, Y. Liu, J. Chen, W. Zou, J. Zeng, J. Wu, A comprehensive review of genus Sanguisorba: traditional uses, chemical constituents and medical applications, Front. Pharmacol. 12 (2021) 750165.
[2] E. Jang, K.-S. Inn, Y.P. Jang, K.-T. Lee, J.-H. Lee, Phytotherapeutic activities of Sanguisorba officinalis and its chemical constituents: a review, Am. J. Chin. Med. 46 (2018) 299–318.
[3] A.-C. Tocai, F. Ranga, A.G. Teodorescu, A. Pallag, A.M. Vlad, L. Bandici, S.I. Vicas, Evaluation of polyphenolic composition and antimicrobial properties of Sanguisorba officinalis L. and Sanguisorba minor Scop, Plants 11 (2022) 3561.
[4] T. Yu, L. Wu, T. Zhang, H. Hao, J. Dong, Y. Xu, H. Yang, H. Liu, L. Xie, G. Wang, Y. Liang, Insights into Q-markers and molecular mechanism of Sanguisorba saponins in treating ulcerative colitis based on lipid metabolism regulation, Phytomedicine 116 (2023) 154870.
[5] X. Chen, F. Shang, Y. Meng, L. Li, Y. Cui, M. Zhang, K. Qi, T., Xue, Ethanol extract of Sanguisorba officinalis L. inhibits biofilm formation of methicillin-resistant Staphylococcus aureus in an ica-dependent manner, J. Dairy Sci. 98 (2015) 8486–8491.
[6] W. Zhang, C. Peng, J. Yan, P. Chen, C. Jiang, S. Sang, Y. Yuan, Y. Hong, M. Yao, Sanguisorba officinalis L. suppresses 5-fluorouracil-sensitive and-resistant colorectal cancer growth and metastasis via inhibition of the Wnt/β-catenin pathway, Phytomedicine 94 (2022) 153844.
[7] R.A. Dwek, Glycobiology: toward understanding the function of sugars, Chem. Rev. 96 (1996) 683–720.
[8] G. Borjigin, F. Wei, S. Jiang, Q. Li, C. Yang, Extraction, purification, structural characterization and biological activity of polysaccharides from Fritillaria: a review, Int. J. Biol. Macromol. 242 (2023) 124817.
[9] S. Jiang, Q. Wang, Z. Wang, G. Borjigin, J. Sun, Y. Zhao, Q. Li, X. Shi, S. Faizan Ali Shah, X. Wang, C. Gan, Y. Wu, X. Song, Q. Li, C. Yang, Ultrasound-assisted polysaccharide extraction from Fritillaria ussuriensis Maxim. and its structural characterization, antioxidant and immunological activity, Ultrason. Sonochem. 103 (2024) 106800.
[10] X. Yang, S. Wei, X. Lu, X. Qiao, J. Simal-Gandara, E. Capanoglu, Ł. Woźniak, L. Zou, H. Cao, J. Xiao, X. Tang, N. Li, A neutral polysaccharide with a triple helix structure from ginger: characterization and immunomodulatory activity, Food Chem. 350 (2021) 129261.
[11] G. Cai, C. Wu, T. Zhu, S. Peng, S. Xu, Y. Hu, Z. Liu, Y. Yang, D. Wang, Structure of a Pueraria root polysaccharide and its immunoregulatory activity on T and B lymphocytes, macrophages, and immunosuppressive mice, Int. J. Biol. Macromol. 230 (2023) 123386.
[12] Y. Zhu, Y. Li, X. Li, T. Chen, H. Zhao, H. Zhou, Activities of polysaccharide fractions from corn silk: hemostatic, immune, and anti-lung cancer potentials, Int. J. Biol. Macromol. 262 (2024) 130156.
[13] H. Zhang, J. Chen, Y. Cen, Burn wound healing potential of a polysaccharide from Sanguisorba officinalis L. in mice, Int. J. Biol. Macromol. 112 (2018) 862–867.
[14] W. Zhao, X. Zeng, F. Meng, X. Bi, D. Xu, X. Chen, Q. Li, Y. Han, Structural characterization and in vitro–in vivo evaluation of effect of a polysaccharide from Sanguisorba officinalis on acute kidney injury, Food Funct. 10 (2019) 7142–7151.
[15] J. Hu, Y. Liu, L. Cheng, R. Shi, A. Qayum, A. Bilawal, M.-A. Gantumur, M. A. Hussain, Z. Jiang, B. Tian, Comparison in bioactivity and characteristics of Ginkgo biloba seed polysaccharides from four extract pathways, Int. J. Biol. Macromol. 159 (2020) 1156–1164.
[16] C. Wang, J. Li, Y. Cao, J. Huang, H. Lin, T. Zhao, L. Liu, P. Shen, D. Julian McClements, J. Chen, C. Liu, J. Liu, Q. Li, Extraction and characterization of pectic polysaccharides from Choerospondias axillaris peels: comparison of hot water and ultrasound-assisted extraction methods, Food Chem. 401 (2023) 134156.
[17] L. Guo, N. Kong, X. Zhang, H. Ma, Multimode ultrasonic extraction of polysaccharides from maca (Lepidium meyenii): optimization, purification, and in vitro immunoregulatory activity, Ultrason. Sonochem. 88 (2022) 106062.
[18] E.W. Davie, O.D. Ratnoff, Waterfall sequence for intrinsic blood clotting, Science 145 (1964) 1310–1312.
[19] R.G. Macfarlane, An enzyme cascade in the blood clotting mechanism, and its function as a biochemical amplifier, Nature 202 (1964) 498–499.
[20] W. Sun, Z.-L. Zhang, X. Liu, S. Zhang, L. He, Z. Wang, G.-S. Wang, Terpene glycosides from the roots of Sanguisorba officinalis L. and their hemostatic activities, Molecules 17 (2012) 7629–7636.
[21] Q. Li, S. Jiang, Q. Wang, J. Sun, Z. Wang, X. Wang, X. Shi, Y. Mu, L. Wei, C. Yang, Structural characterisation and anti-colon cancer activity of an arabinogalactan RSA-1 from Raphani semen, Carbohydr. Polym. 342 (2024) 122417.
[22] I. Ciucanu, F. Kerek, A simple and rapid method for the permethylation of carbohydrates, Carbohyd Res. 131 (1984) 209–217.
[23] L. Wang, L. Wang, C. Yan, Y. Fu, J.-F. Yang, J. Ma, S. Song, Structural characterization of a fucoidan from Ascophyllum nodosum and comparison of its protective effect against cellular oxidative stress with its analogues, Int. J. Biol. Macromol. 239 (2023) 124295.
[24] C. Nie, P. Zhu, S. Ma, M. Wang, Y. Hu, Purification, characterization and immunomodulatory activity of polysaccharides from stem lettuce, Carbohydr. Polym. 188 (2018) 236–242.
[25] W.-B. Li, J. Lei, M.-M. Qu Mo, J. Li, J. Wei, Y. Liu, S. Wang, Y.-C. Hu, L. Zou, D.-T. Wu, Impacts of ultrasound-assisted Fenton degradation and alkaline de-esterification on structural properties and biological effects of pectic polysaccharides from Tartary buckwheat leaves, Ultrason. Sonochem. 106 (2024) 106895.
[26] Y. Wang, Y. Liu, Y. Hu, Optimization of polysaccharides extraction from Trametes robiniophila and its antioxidant activities, Carbohydr. Polym. 111 (2014) 324–332.
[27] X. Guan, Q. Wang, B. Lin, M. Sun, Q. Zheng, J. Huang, G. Lai, Structural characterization of a soluble polysaccharide SSPS1 from soy whey and its immunoregulatory activity in macrophages, Int. J. Biol. Macromol. 217 (2022) 131–141.
[28] Y. Wu, C. Liu, Y. Jiang, B. Bai, X. He, H. Wang, J. Wu, C. Zheng, Structural characterization and hepatoprotective effects of polysaccharides from Anoectochilus zhejiangensis, Int. J. Biol. Macromol. 198 (2022) 111–118.
[29] L. Huang, J. Zhao, Y. Wei, G. Yu, Q. Li, Characterization of a neutral polysaccharide from pumpkin (Cucurbita moschata Duch) with potential immunomodulatory activity, Int. J. Biol. Macromol. 188 (2021) 729–739.
[30] X. Guo, J. Kang, Z. Xu, Q. Guo, L. Zhang, H. Ning, S.W. Cui, Triple-helix polysaccharides: formation mechanisms and analytical methods, Carbohydr. Polym. 262 (2021) 117962.
[31] B.H. Falch, T. Espevik, L. Ryan, B.T. Stokke, The cytokine stimulating activity of (1→3)-β-d-glucans is dependent on the triple helix conformation, Carbohydr. Res. 329 (2000) 587–596.
[32] J. Miao, J.M. Regenstein, J. Qiu, J. Zhang, X. Zhang, H. Li, H. Zhang, Z. Wang, Isolation, structural characterization and bioactivities of polysaccharides and its derivatives from Auricularia – a review, Int. J. Biol. Macromol. 150 (2020) 102–113.
[33] H.J. Forman, H. Zhang, Targeting oxidative stress in disease: promise and limitations of antioxidant therapy, Nat. Rev. Drug Discov. 20 (2021) 689–709.
[34] K. Neha, M.R. Haider, A. Pathak, M.S. Yar, Medicinal prospects of antioxidants: a review, Eur. J. Med. Chem. 178 (2019) 687–704.
[35] N. Chen, T. Jiang, J. Xu, W. Xi, E. Shang, P. Xiao, J.-A. Duan, The relationship between polysaccharide structure and its antioxidant activity needs to be systematically elucidated, Int. J. Biol. Macromol. 270 (2024) 132391.
[36] Y. Peng, Z. Zhang, W. Chen, S. Zhao, Y. Pi, X. Yue, Structural characterization, α-glucosidase inhibitory activity and antioxidant activity of neutral polysaccharide from apricot (Armeniaca Sibirica L. Lam) kernels, Int. J. Biol. Macromol. 238 (2023) 124109.
[37] S.A. Smith, R.J. Travers, J.H. Morrissey, How it all starts: Initiation of the clotting cascade, Crit. Rev. Biochem. Mol. Biol. 50 (2015) 326–336.
[38] D. Lasne, B. Jude, S. Susen, De l'hémostase normale à l'hémostase pathologique, Can. J. Anaesth. 53 (2006) S2–S11.
[39] F. Carvalhal, R.R. Cristelo, D.I.S.P. Resende, M.M.M. Pinto, E. Sousa, M. Correia-da-Silva, Antithrombotics from the sea: polysaccharides and beyond, Mar. Drugs (2019).
[40] S. He, H. Cao, C. Thålin, J. Svensson, M. Blombäck, H. Wallén, The clotting trigger is an important determinant for the coagulation pathway in vivo or in vitro-inference from data review, Semin. Thromb. Hemost. 47 (2021) 63–73.
[41] A. Malik, F.U. Rehman, K.U. Shah, S.S. Naz, S. Qaisar, Hemostatic strategies for uncontrolled bleeding: a comprehensive update, J. Biomed. Mater. Res. B Appl. Biomater. 109 (2021) 1465–1477.
[42] A. Undas, Determination of fibrinogen and thrombin time (TT), Methods Mol. Biol. 1646 (2017) 105–110.
[43] J.H. Levy, F. Szlam, A.S. Wolberg, A. Winkler, Clinical use of the activated partial thromboplastin time and prothrombin time for screening: a review of the literature and current guidelines for testing, Clin. Lab. Med. 34 (2014) 453–477.

[44] W. Gu, H. Wang, M. Su, Y. Wang, F. Xu, Q. Hu, X. Cai, J. Song, H. Tong, Y. Qian, H. Zhao, J. Chen, Investigation of tannins transformation in sanguisorbae radix over carbonizing by stir-frying, Front. Mol. Biosci. 9 (2022) 762224.
[45] Y. Deng, J. Xie, Z. Luo, S.-P. Li, J. Zhao, Synergistic immunomodulatory effect of complex polysaccharides from seven herbs and their major active fractions, Int. J. Biol. Macromol. 165 (2020) 530–541.
[46] R. Chen, J. Xu, W. Wu, Y. Wen, S. Lu, H.R. El-Seedi, C. Zhao, Structure–immunomodulatory activity relationships of dietary polysaccharides, Curr. Res. Food Sci. 5 (2022) 1330–1341.
[47] X. Zhang, Z. Liu, C. Zhong, Y. Pu, Z. Yang, Y. Bao, Structure characteristics and immunomodulatory activities of a polysaccharide RGRP-1b from radix ginseng Rubra, Int. J. Biol. Macromol. 189 (2021) 980–992.
[48] J. Yang, J. Tu, H. Liu, L. Wen, Y. Jiang, B. Yang, Identification of an immunostimulatory polysaccharide in banana, Food Chem. 277 (2019) 46–53.
[49] Z. Zhang, F. Wang, M. Wang, L. Ma, H. Ye, X. Zeng, A comparative study of the neutral and acidic polysaccharides from Allium macrostemon Bunge, Carbohydr. Polym. 117 (2015) 980–987.