Chemical and Biological Technologies in Agriculture

REVIEW

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Recent progress in the research of *Angelica sinensis* (Oliv.) Diels polysaccharides: extraction, purification, structure and bioactivities

Chunyan Hou[1], Mingsong Yin[1], Ping Lan[2], Huiru Wang[1], Hui Nie[3*] and Xiaolong Ji[1*]

Abstract

The root of *Angelica sinensis* (Oliv.) Diels, a well-known Chinese herbal medicine, has been used historically as hematopoietic and anti-inflammatory agents for thousands of years. Recent phytochemistry and pharmacological studies have proved that polysaccharides are one of the major active ingredients in *A. sinensis*. It has been demonstrated that ASPs (*A. sinensis* polysaccharides) had various important biological activities, such as hematopoietic, hepatoprotective, hypoglycemic, anti-inflammatory, antitumor, and antioxidant activities. The purpose of this present review is to appraise previous and current literatures on the extraction, purification, structural characterization and biological activities of ASPs. In addition, the structure–activity relationship will be further explored and discussed. We believe that this review will provide a useful bibliography for the investigation, production, and application of ASPs in functional foods and therapeutic agents. Moreover, this review also highlights the challenges of investigation and future considerations for holistic utilization.

Keywords: Polysaccharide, *Angelica sinensis*, Structure features, Biological activities, Structure–activity relationship

Introduction

The root of *Angelica sinensis* (Oliv.) Diels (*A. sinensis*), belonging to Umbelliferae family, is identified as a multispecies of about 100 and *A. sinensis* is largely cultivated in the temperate regions, particularly in Africa, Asia, parts of South America [1, 2]. The *A. sinensis* has been cultivated for 2000 years in China, mainly distributed in the northwest region (Shaanxi Province, Gansu Province) and southwest region (Yunnan Province, Sichuan Province) [3, 4]. It is also known as "Dang Gui" in China and was first described in the oldest medical material book "Shennong's Classic of Material Medica (300 BCE-200 CE)" [5]. The root was also identified as an excellent herbal medicine in "Compendium of Materia Medica (1552–1578)", and the taste and nature are slightly sweet, neutral, and warm [3, 6]. It has been used as a tonic and hematopoietic agent for the treatment of gynecological diseases, including menstrual disorders, amenorrhea, dysmenorrheal and relaxing bowel [7, 8]. Modern pharmacological studies have shown that *A. sinensis* has hematopoietic, immunomodulatory, antitumor, antioxidant and hepatoprotective activities, and gastrointestinal protective and anti-inflammatory effects [2, 9]. These beneficial effects have been partly attributed to the different and complicated bioactive components of the root of *A. sinensis* which contains over 70 chemical compounds, including carbohydrates, organic acids, essential oils, phenolic compounds, vitamins, protein, amino acids, and other constituents [10, 11].

A. sinensis contains a high amount of carbohydrates, which are a group of reducing sugars and polysaccharides. As we know, plant polysaccharides have become a new research hotpot in recent years for their high bioactivity and low toxicity [12], such as *Ziziphus jujuba* Mill. polysaccharides [13], *Dioscorea* spp. polysaccharides [14],

*Correspondence: 763008174@qq.com; xiaolongjiytu@163.com
[1] College of Food and Bioengineering, Zhengzhou University of Light Industry, Zhengzhou 450001, People's Republic of China
[3] Guangxi Talent Highland of Preservation and Deep Processing Research in Fruit and Vegetables, Hezhou University, Hezhou 542800, People's Republic of China
Full list of author information is available at the end of the article

Lycium barbarum polysaccharides [15], and *Panax ginseng* C. A. Meyer polysaccharides [16], as well as *Angelica sinensis* polysaccharides (ASPs) [4]. Recent phytochemical and pharmacological studies have demonstrated that the diverse activities mentioned above, including hematopoiesis [17, 18], immunomodulation [19, 20], antitumor [21, 22], antioxidant [23, 24], and radioprotection [3, 25], are mainly attributed to the polysaccharides extracted from the root of *A. sinensis.*

To our knowledge, no review concerning ASPs is available. Therefore, we systematically summarized the related research findings of the past two decades and provided comprehensive insights into the physicochemical properties, structural characteristics, pharmacological effects and application prospects of *A. sinensis* polysaccharides, providing detailed reference for the study about their structure–bioactivity relationships, the exploration of commercial applications and potential uses in functional foods as well.

Procedures of extraction and purification

Among the functional components of *A. sinensis*, polysaccharides are the most important active constituents as they exert different biological effects and potential health benefits [4, 26]. Adequate procedures have been designed to obtain pure *A. sinensis* polysaccharides which could be chemically characterized and accurately tested in vitro and in vivo assays [27, 28].

In general, different procedures were usually used in ASPs extraction (Fig. 1). First, the dried roots were ground to a fine powder and defatted by refluxing with a chloroform: methanol (2:1) mixture. Oligosaccharides and small coloring molecules were further removed by refluxing with 80% ethanol at room temperature. The filtered and dried residues were then used to extract the water-soluble polysaccharide mixture using different methods [29]. Basically, moderate extraction conditions were adopted to separate the outer layer of the cell wall from the inner layer, without altering polysaccharide materials [30]. Traditional hot or boiling water extraction is the classical and probably the most convenient method in the laboratory and industrial extraction of plant polysaccharides. It is widely used to extract ASPs. Hot water (80–100 °C for 120–180 min, with a solvent-to-material ratio of 5–10, over two or five extraction rounds) was proved to be the most proper extraction solvent to prepare ASPs. Ai et al. analyzed the influence of different parameters, including extraction time, extraction temperatures, solid/liquid ratio and number of extraction cycles on ASPs yields using an orthogonal array experimental design (OAD) model and found that the maximum polysaccharide yields were obtained in 180 min, 4

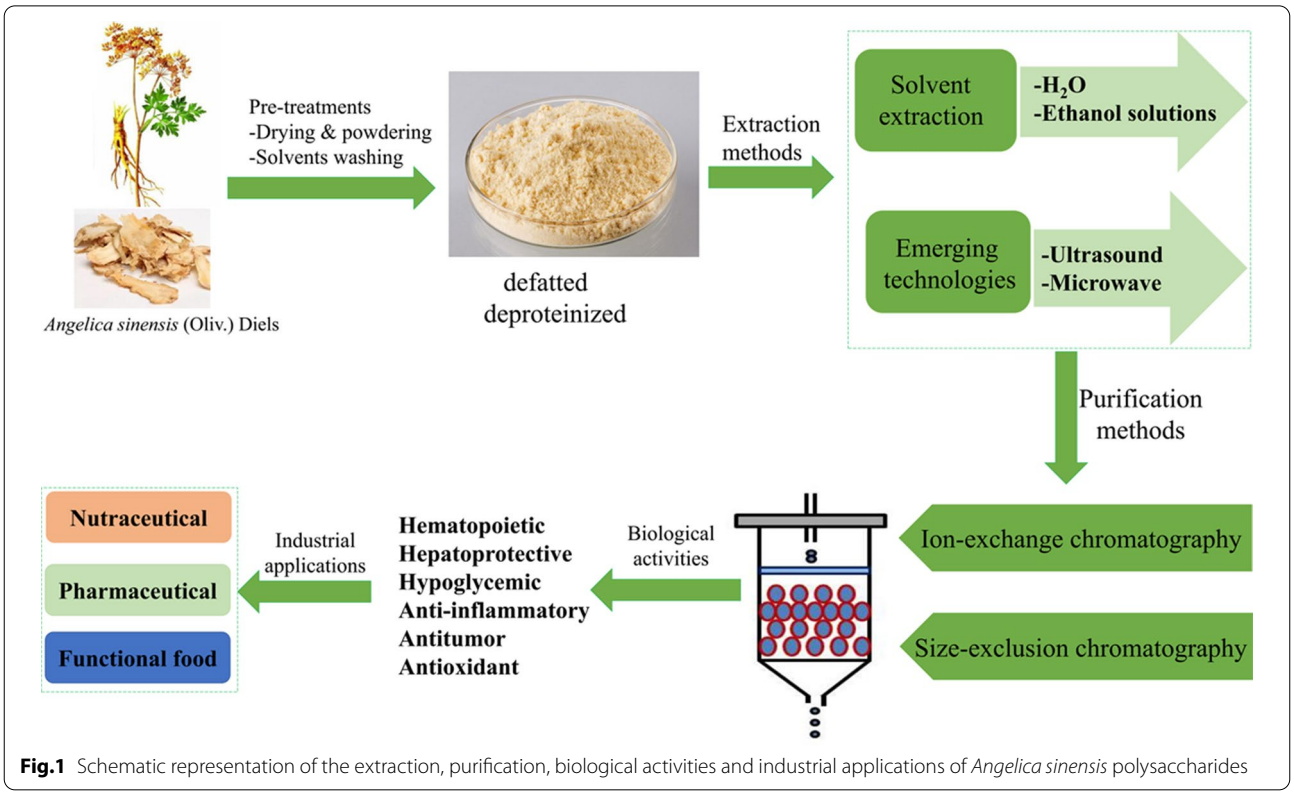

Fig.1 Schematic representation of the extraction, purification, biological activities and industrial applications of *Angelica sinensis* polysaccharides

cycles with a 1:6 solid/liquid ratio mix in boiling water [31]. According to a recent study, the acidic extraction for ASPs after water extraction was also used to obtain different polysaccharide fractions. For instance, Sun et al. (2016) extracted polysaccharides from the *A. sinensis* root using pH 4.50 solutions at 85 °C, allowing the structures of these fractions to be analyzed by methylation, GC–MS and NMR [3].

The *A. sinensis* has rigid cell walls and some polysaccharides are structural components of the cell walls or exist in the cell. Therefore, breaking up cell wall of *A. sinensis* could release polysaccharides easily and improve the extraction yield. Some different technologies could improve the extraction efficiencies, including microwave-assisted treatments and ultrasonic processing [32]. Zhao et al. identified the optimum operational conditions for maximizing polysaccharides yield through response surface methodology as ultrasound power 180 W, ultrasound time 45 min using a water: material ratio (w:w) of 7:1 at 90 °C. Under these conditions, 6.96% of ASPs were achieved [33]. Meanwhile, Tian et al. reported that up to 21.89 ± 0.21% of ASPs were obtained under the optimum extraction conditions as water/raw material ratio 43.31 mL/g, ultrasonic time 28.06 min, power 396.83 W, proving the contribution of ultrasound in polysaccharide extraction [25]. Another extraction method, microwave-assisted extraction, was also frequently used. Li et al. extracted ASPs using distilled water under optimal extraction conditions, including a microwave power of 500 W, microwave time of 20 min, water: material ratio (w:w) of 15:1 at room temperature [34]. In summary, different assisted extraction methods or combined process have been developed to improve the extraction efficiency of ASPs, shorten the processing time and reduce solvent consumption and energy requirements.

The *A. sinensis* polysaccharides are heteropolysaccharides and contain impurities and different fractions as reported [35, 36]. After extraction, purification is essential for the research of chemical structure of plant polysaccharides [37]. As widely used purification techniques, ion-exchange chromatography (DEAE-Sephadex A-25, DEAE-Spharose Fast Flow, and DEAE-Sepharose CL-6B) is used to separates neutral and acidic polysaccharides by gradient salt elution, while gel filtration column chromatography (Superdex 200, Sephacryl S series, and Sepharose CL-6B) is often used to separate ASPs of different molecular weights [27, 38]. For example, Sun et al. (2005) fractionated ASPs through ion-exchange chromatography on a column of DEAE-Sepharose CL-6B (*D* 2.6 cm × 30 cm) eluted with NaCl gradient (0–1 M). And it was found that neutral ASPs were rich in glucose, galactose, arabinose, galacturonic acid in different ratio, while the acidic polysaccharide fractions (ASP1, ASP2, ASP3) eluted with NaCl solution ranging from 0.2 to 0.6 M mainly consisted of galacturonic acid along with rhamnose, arabinose and galactose, indicating the pectic polysaccharide [39]. Good results and higher yield should be based on proper extraction and purification methods [40].

Structure elucidation

The structural features of ASPs, such as molecular weights, monosaccharide compositions, types of glycosyl linkage, and conformational features, have been investigated by chromatography technology, spectrum analysis and other chemical analysis. Some studies on the structures as well as bioactivities of ASPs were summarized and illustrated in Table 1 and Fig. 2.

Molecular weights

HPLC and high-performance gel permeation chromatography (HPGPC) were widely applied to determine the average molecular weights of ASPs [41, 42]. Molecular weights ranging within 10^4–10^5 Da were reported in ASPs extracted from *A. sinensis* roots under different experimental conditions. In addition, different fractions from the same source could be also different. For instance, Zhao et al. demonstrated that the molecular weights of two polysaccharides from the root of *A. sinensis* were 4.90×10^4 (APS-1a) and 6.54×10^4 (APS-3a) Da, respectively [43].

Monosaccharide compositions

Monosaccharide compositions analyses are usually performed with complete acid hydrolysis to cleave glycosidic linkages for derivatization, detection, and quantification with HPLC and GC [44, 45]. Due to the differences in sources, different monosaccharide compositions of ASPs were reported, and the majority of these polysaccharides were composed of mannose, rhamnose, arabinose, glucose, galactose and galacturonic acid with various molar ratios. For example, in a study, Xing et al. (2013) obtained three polysaccharides (ASP1, ASP2 and ASP3) with Sephadex G-50 and analyzed them by GC. Data indicated that the three fractions showed quite notable difference in monosaccharide compositions (Table 1) [46]. Meanwhile, Zhou et al. [47] studied the monosaccharide compositions of crude and neutral polysaccharides (JASP and JASP1A) isolated from *A. sinensis* collected in Gansu Province and found that JASP consisted of glucose, glucuronic acid, galactose and arabinose in a molar ratio of 1.7:1.0:5.02:1.85, while JASP1A only contained rhamnose, galactose and arabinose [47].

Table 1 The polysaccharides isolated from the root of *A. sinensis*

No	Compound name	Molecular weight (Da)	Monosaccharide composition	Structures	Biological activities	Involved mechanism	Ref
1	ASP	3.2×10^3	Man, Rha, GlcA, Gal, Ara, Xyl in the ratio of 0.23: 0.17:14.41:0.39:1.68:0.87	Pyranose form	Lipid-lowering effect	Involvement in lipid metabolism	[41]
2	ASP-24	1.25×10^3	Man, Rha, GalA, Glc, Gal, Ara in the ratio of 0.51: 6.48:22.13:26.99:25.14:18.75	α-pyranose and *β*-pyranose residues	Antioxidant	DPPH, ABTS radical scavenging activity; reducing power activity	[23]
3	APS-1II	4.21×10^4	Ara, Glc, Fuc in the ratio of 2.48:1.05:1.00	Backbone composed of 1,3-α-L-Ara*f* and 1,6-α-D-Glc*p*. Branches composed of 1,5-α-L-Ara*f*, 1,4-β-D-Glc*p*, T-β-D-Glc*p*, 1,3-α- L-Fuc*p* and T-α-L-Fuc*p*	Anti-leukemia activity Immunomodulatory	Promoting splenocytes proliferation, and enhancing macrophages phagocytic activity	[27]
4	ASP	8.0×10^4	GlcA, Glc, Ara, Gal in the ratio of 1.00:1.70:1.85: 5.02	Backbone composed of (1 → 3)-linked Gal*p*, (1 → 6)-linked Gal*p* and 2-OMe-(1 → 6)-linked Gal*p*. Branches composed of *O*-3 of 2-OMe-(1 → 6)-linked Gal*p*, terminated with Glc*p*A and Ara*f*	Hematopoietic activity	Inhibiting NF-κB p65 activation via the IκB kinases-IκBα pathway	[35, 61]
5					Hepatoprotective activity	Alleviating liver injury via an increase in GSH levels	[35, 62]
6	ASP	8.09×10^4	Ara, Glc, Gal in the ratio of 1:1:1.75		Antitumor	Participating in the control of iron metabolism	[22]
7	ASP	7.29×10^4	Ara, Glc, Gal in the ratio of 1:2.5:7.5		Hematopoietic activity	Action of JAK, SMAD and ERK signal pathways	[18]
8					Hypoglycemic Hepatoprotective	Amelioration of insulin resistance	[65]
9	ASP-1a	4.90×10^4	Ara, Glc, Gal in the ratio of 37.67:14.98:57.34	Main structure:1,4-Gal*p*, T-Ara*f*, 1,3,6-Gal*p*,1,3,5-Ara*f*, 1,6-Gal*p*, 1,4-Glc*p*	Hematopoietic activity	Increasing immune cells	[43]
10	ASP-3a	6.54×10^4	Ara, Glc, Gal in the ratio of 6.5:8.96:84.54	Main structure:1,4-Gal*p*, 1,3,6-Gal*p*,1,6-Gal*p*, T-Gal*p*	Hematopoietic activity	Increasing immune cells	
11	ASP3		Rha, Ara, Man, Glc, Gal in the ratio of 1.9:10.5:0.4: 0.9:24.9	Backbone composed of homogalacturonan fragments and rhamnogalacturonan fragments. Side chains mainly composed of β-1,6- and β-1,4-galactopyranan and α-1,5-arabinofuranan			[36]
12	ASDII-3–3	4.4×10^4	Rha, Ara, Xyl, Man, Gal in the ratio of 0.3:1.0: 0.1: 0.2:5.0	Backbone composed of (1 → 2)-linked-Rha and (1 → 4)-linked-Gal. Branches composed of (1 → 5)-linked-Ara terminated with Ara residues, and(1 → 4)-linked-Xyl terminated with Man residues			[50]
13	APS-1cI	1.7×10^5	Glc	Linear α-glucan composed of only (1 → 6)-α-D-Glc			[53]
14	APS-1cII	3.9×10^4	Glc	(1 → 4)-α-D-Glc*p* and (1 → 6)-α-D-Glc*p* in a molar ratio of 4:1			[54]

Table 1 (continued)

No	Compound name	Molecular weight (Da)	Monosaccharide composition	Structures	Biological activities	Involved mechanism	Ref
15	APS-1d	5.1×10^3	Glc, Ara in the ratio of 13.8:1	Backbone composed of 1,4-α-D-Glcp, with Branches attached to *O*-6 of some residues Branches composed of 1,6-α-D-Glc*p* residues, terminated with β-L-Ara*f* residues	Antitumor	Inhibition of tumor cell proliferation	[38]
16	XC-1	1.0×10^5	Glc	α-(1 → 6)-glucan	Immunomodulating		[52]
17	As-IIIa	8.5×10^4	Glc	α-(1 → 3)-glucan			[51]
18	As-IIIb	4.9×10^4	Glc, Man, Ara in the ratio of 10.0:10.0:4.0	Heteropolysaccharide with (1 → 4), (1 → 6) glycosidic bond			
19	W-ASP3	9.48×10^4	Rha, Ara, Man, Glc, Gal in the ratio of 1.87:10.05: 0.37:0.94:24.93	Main structure: α-D-Gal*p*A, *β*-D-Gal*p*, α-L- Ara*f*, α-L-Rha*p*, 1,4-D-Gal*p*A, 1,2,4-L- Ara*f*	Radioprotection	Scavenging free radical	[3]
20	P1	4.12×10^5	Glc, Gal, Xyl in the ratio of 18:1:3	Main structure:1,3,6-Glc*p*,1,3-Glc*p*, 1,3-Xyl*p*, 1,3-Gal*p*	Immunomodulation		[5]
21	P2	2.34×10^5	GlcA, Glc, Gal, Xyl in the ratio of 7.00:7.98: 2.00: 3.01	Main structure: 1,3-GlcA*p*, 1,3,6-Glc*p*,1,3-Glc*p*,1,3-Xyl*p*, 1,3-Gal*p*	Immunomodulation		
22	P3	1.94×10^5	Glc, Xyl in the ratio of 27.94:1.00	Main structure: 1,3,6-Glc*p*,1,6-Glc*p*,1,4-Glc*p*, 1,3-Glc*p*	Immunomodulation		
23	SASP	8.08×10^4	Glc, GlcA, Gal, Ara in the ratio of 1.7:1.0:5.02:1.85	Main structure: (1 → 3)-linked Gal*p*, (1 → 6)-linked Gal*p*, 2-OMe-(1 → 6)-linked Gal*p*	Hematopoietic activity		[47]
24	JASP-1A	8.62×10^5	Rha, Gal, Ara in the ratio of 1:4.55:4.92	Main structure: 1,4-linked Gal*p*, 1,3-linked Rha, 1,4-linked Gal*p*, T-Ara*f*	Hematopoietic activity		
25	ASP1	1.22×10^5	Ara, Rha, Gal, Glc in the ratio of 1:4:10:30	Main structure: 1,4-linked Gal*p*,1,6-linked Gal*p*,1,4-linked Glc*p*,1,6-linked Glc*p*	Antitumor	Inhibition of tumor cell proliferation	[46]
26	ASP2	1.72×10^5	Ara, Rha, Gal, Glc in the ratio of 1:2.5:1:5	Main structure: 1,4-linked Ara*p*,1,6-linked Ara*p*,1,4-linked Glc*p*,1,6-linked Glc*p*	Antitumor	Inhibition of tumor cell proliferation	
27	ASP3	8.09×10^4	Ara, Rha, Gal, Glc in the ratio of 1:2:2:3.5	Main structure: 1,4-linked Gal*p*,1,6-linked Gal*p*,1,4-linked Glc*p*,1,6-linked Glc*p*	Antitumor	Inhibition of tumor cell proliferation	

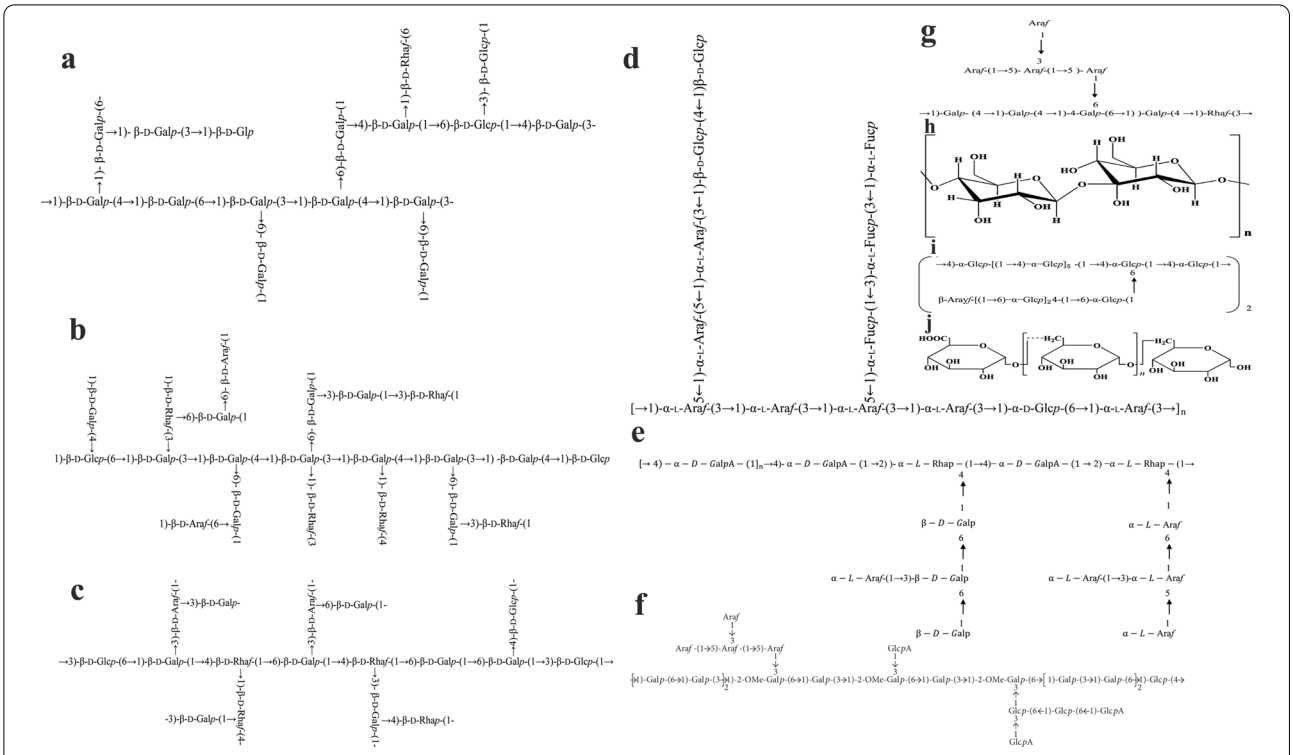

Fig. 2 Schematic structures of *Angelica sinensis* polysaccharides. **a** *A. sinensis* polysaccharides (ASP1), **b** *A. sinensis* polysaccharides (ASP2), **c** *A. sinensis* polysaccharides (ASP2) [46]. **d** A new water-soluble polysaccharide (APS-1II) [27]. **e** An acidic polysaccharide (WASP3) [3]. **f** The predicted chemical structure of ASP [28]. **g** A soluble polysaccharide (JASP-1A) [47]. **h** The chemical structure of ASP [56]. **i** The predicted structure of APS-1d [38]. **j** The structure of ASP [57]

Glycosidic linkage types and positions

To analyze the detailed structure of plant polysaccharide, it is necessary to clarify not only the monosaccharide composition/molecular weights, but also the manner of attachment of various monosaccharides as well as branches [14]. The combination of instrumental techniques, including GC–MS and nuclear magnetic resonance (NMR), with chemical analysis such as periodate oxidation, Smith degradation, partial acid hydrolysis and enzyme digestion was applied to investigate the structures of ASPs [48, 49]. In a previous study, the preliminary structure of APS-1II was successfully identified. According to GC–MS, 1D and 2D NMR results (^{1}H NMR, ^{13}C NMR, TOCSY, NOESY, HMBC), Liu et al. (2019) concluded that it had a glucoarabin backbone, mainly composed of 1,3-Ara*f* and 1,6-Glc*p* with an Ara: Glc ratio of ~ 5:1, and branching occurred from the *C*-5 of 1,3-Ara*f* residues [27]. In another study, Wang et al. (2007) conducted a preliminary separation of *A. sinensis* polysaccharide (ASDII-3–3), and according to the results of infrared spectroscopy, GC–MS and ^{13}C NMR, ASDII-3–3 was proved to have a backbone composed of (1 → 2)-linked-Rha*f* and (1 → 4)-linked Gal*p* with branches composed of (1 → 5)-linked-Ara*f* terminated with Ara residues and (1 → 4)-linked-Xyl*p* terminated with Man*p* residues [50].

An early publication in 1999 of Zhang and Huang first reported that *A. sinensis* polysaccharides As-IIIa was a linear α-glucan composed of only α-(1 → 6)-glucan, and As-IIIb had a repeating unit consisting of (1 → 4) and (1 → 6) glycosidic bonds [51]. In another study mentioned above, the structural features of a water-soluble polysaccharide (ASD-PII) which was purified by DEAE-Sephadex A-25 column were investigated by methylation analysis and GC–MS analysis, and deduced to be a backbone as → 1)Fru*f* (2 → and → 6)Glc*p*(1 → with highly branched structure [55].

Also, it should be noted that the molecular structures of *A. sinensis* polysaccharides are closely related to their inherent local biodiversity (genomic variety, geographic origin, and environmental conditions), which was reflected in the previous reports.

Conformational features

Chain conformation is also an important parameter of plant polysaccharides because of its close relationship with bioactivity [44], so efforts have been made to illuminate this parameter of ASPs. For example, a study by

Wang et al. [23] described the conformation of ASP-24 by SEM 1000-fold amplification as a compact surface structure [23]. In addition, it is reported that the ASP might have a higher value of apparent viscosity due to the intermolecular association of hydrophobic segments in the different size molecules [58].

If there is more detailed information available, it is more well founded for us to build the structure–activity relationship of ASPs. However, unfortunately, reports about the spatial structures of these polysaccharides are quite inadequate and need more extensive investigation by advanced technologies, such as NMR spectroscopy, static and dynamic light scattering, fluorescence spectroscopy, etc.

Biological activities

With the exploitation and application of traditional Chinese medicine (TCM) in recent years, a considerable attention has been paid to ASPs owing to their diverse and potent bioactivities such as hematopoietic, hepatoprotective, hypoglycemic, anti-inflammatory, antitumor, antioxidant and other activities according to the in vitro and in vivo experiments. The multiple bioactivities of ASPs and underlying mechanisms are listed in Table 1.

Hematopoietic activity

ASPs are important bioactive component, and have been used for treating anemia and gynecological disorders. It has been reported that ASP could stimulate hematopoietic cells and muscle tissues and enhance hematopoietic growth factors [59].

Wang et al. [60] found that ASPs could enhance hypoxic induction of erythropoietin (EPO) in Hep3B cell, with a mechanism that involved the stabilization of HIF-2α protein. ASPs rescued the inhibition of EPO induced by TNF-α through blocking GATA binding protein 2 (GATA2) and NF-κB activation. The restoration of EPO production and EPOR mRNA expression with ASPs treatment activated EPOR downstream JAK2/STAT5 and PI3K/Akt signaling, induced their target genes, such as B-cell lymphoma-extralarge (Bcl-xL), Fam132b and TFRc, and increased Bcl-2/Bax ratio in bone marrow-derived mononuclear cells of CKD rats. Meanwhile, it was found that in a rat model of adenine-induced anemia, oral administration of ASPs could correct anemia and alleviate renal damage and inflammation [60] (Fig. 3).

In addition, it is reported that ASPs could inhibit inflammatory hepcidin in both HepG2 cell and ACD (Anemia of chronic disease) rats by blocking the IL-6/STAT3 and BMP/SMAD pathways. Besides, ASP

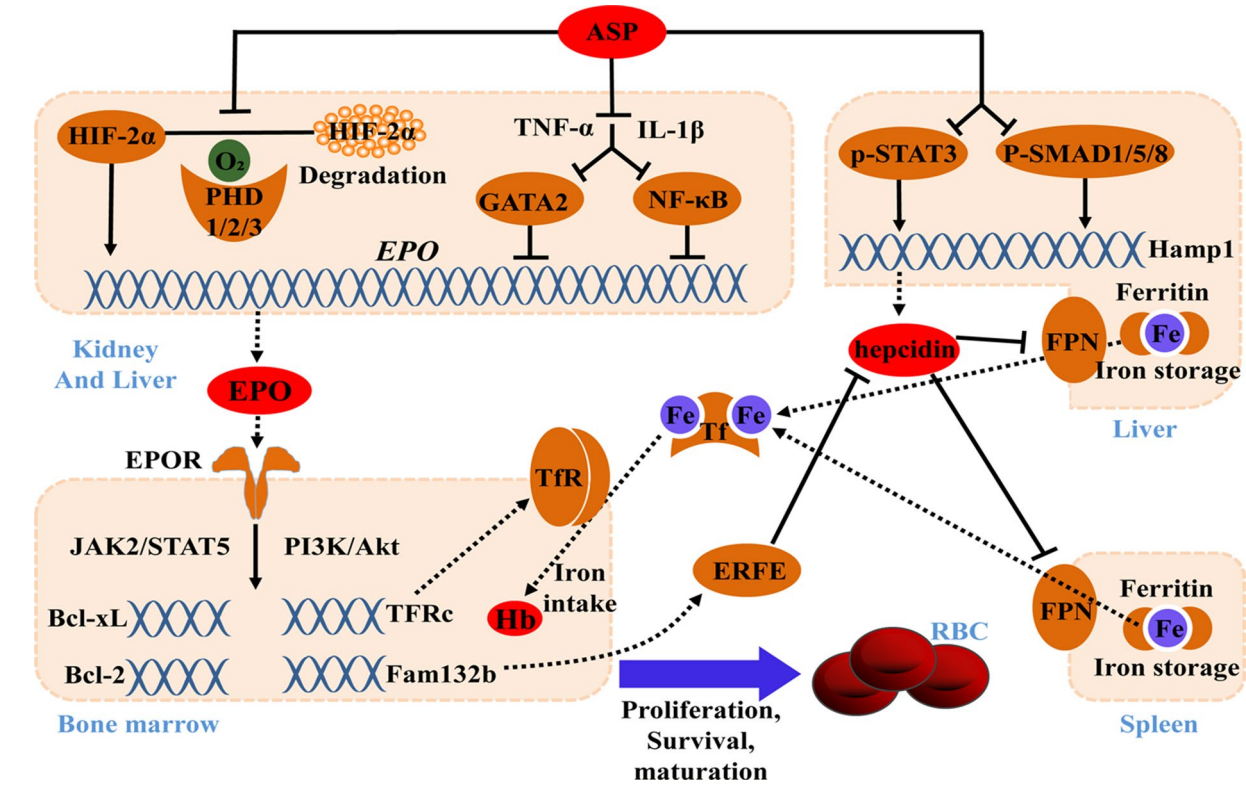

Fig. 3 Graphic representation of the underlying mechanism of ASP in treating anemia

inhibited NF-κB p65 activation via the IκB kinases (IKKs-) IκBα pathway, thereby reducing the secretion of interleukin-6 (IL-6) and TNF-α in ACD rats (Fig. 4a) [61]. Zhang et al. showed that crude polysaccharides extracted from the root of *Angelica sinensis* (Oliv.) were mainly composed of arabinose, glucose, and galactose in a molar ratio of 1:2.5:7.5, with an average molecular weight of 72,900 Da. Studies indicated that ASPs could considerably decrease hepcidin expression via down-regulating the expression of JAK1/2, phospho-JAK1/2, phospho-SMAD1/5/8, phospho-ERK1/2 while promoting the expression of SMAD7 in the liver, thus benefiting the treatments of diseases caused by hepcidin over-expression (Fig. 5) [18]. All these findings mentioned above confirmed the hematopoietic activity of ASPs. So as to improve the hematopoietic activity, polysaccharides through intestinal microbiota fermentation might be a promising research direction in the future.

Hepatoprotective activity

Many investigations have confirmed the direct hepatoprotective effect of ASPs. For example, Wang et al. (2016) explored the mechanisms of ASPs-mediated hepatoprotective effects in type 2 diabetic BALB/c mice and found that ASPs could stimulate insulin secretion, promote hepatic glycogen synthesis, regulate adipokine release, reduce liver fat accumulation, and attenuate liver injury [62]. Moreover, ASPs upregulated the expression of PPARγ and liver insulin signaling proteins, including IRS-2, PI3K, Akt, p-Akt and GLUT2, increased anti-apoptotic protein Bcl-2, decreased

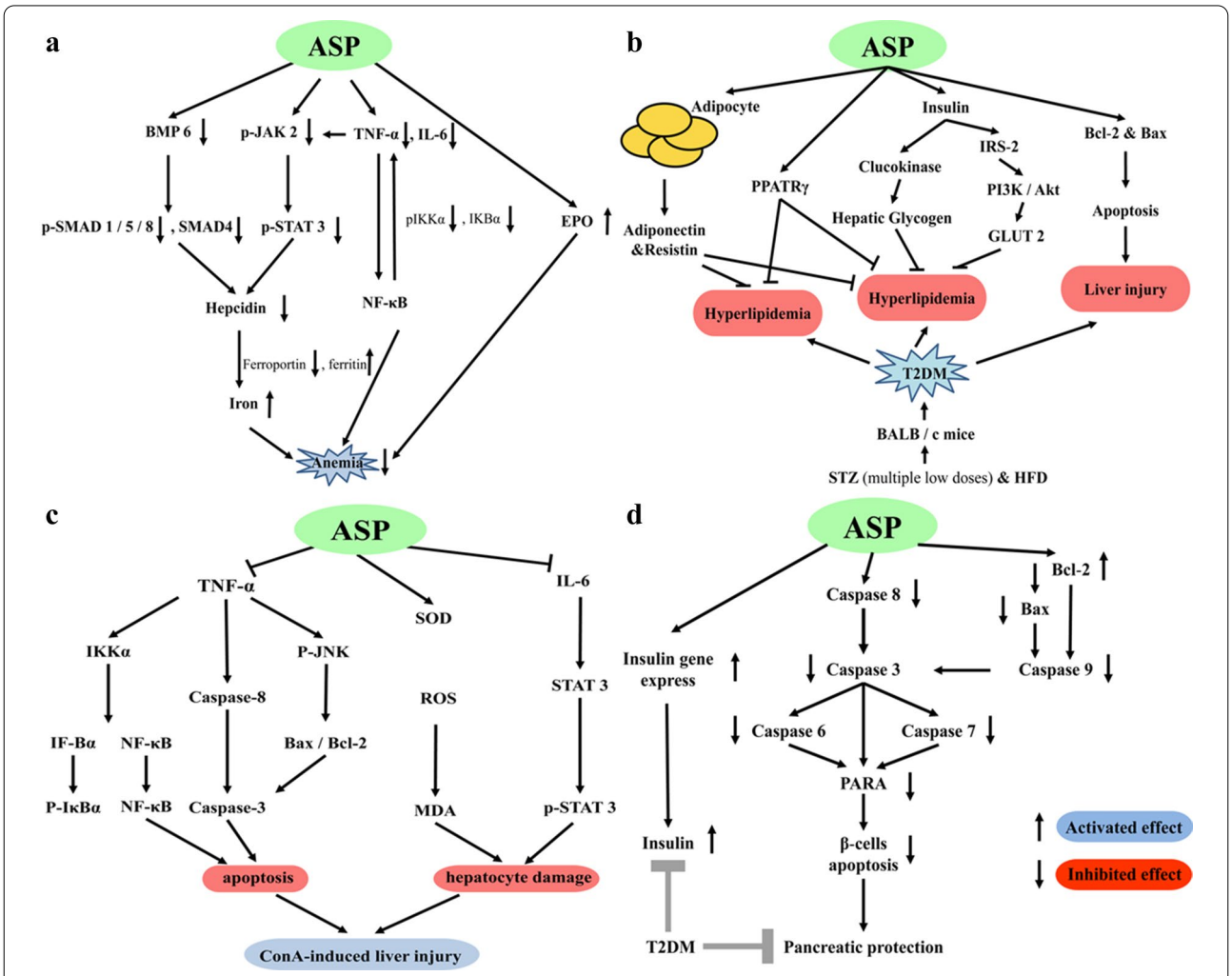

Fig. 4 **a** The mechanism of ASP-reversed anemia of chronic disease. **b** The mechanisms of ASP-mediated hepatoprotective effects in T2DM mice. **c** The mechanism of ASP against ConA-induced liver injury in mice. **d** ASP-mediated therapeutic effects in HFD and STZ-induced diabetic mice

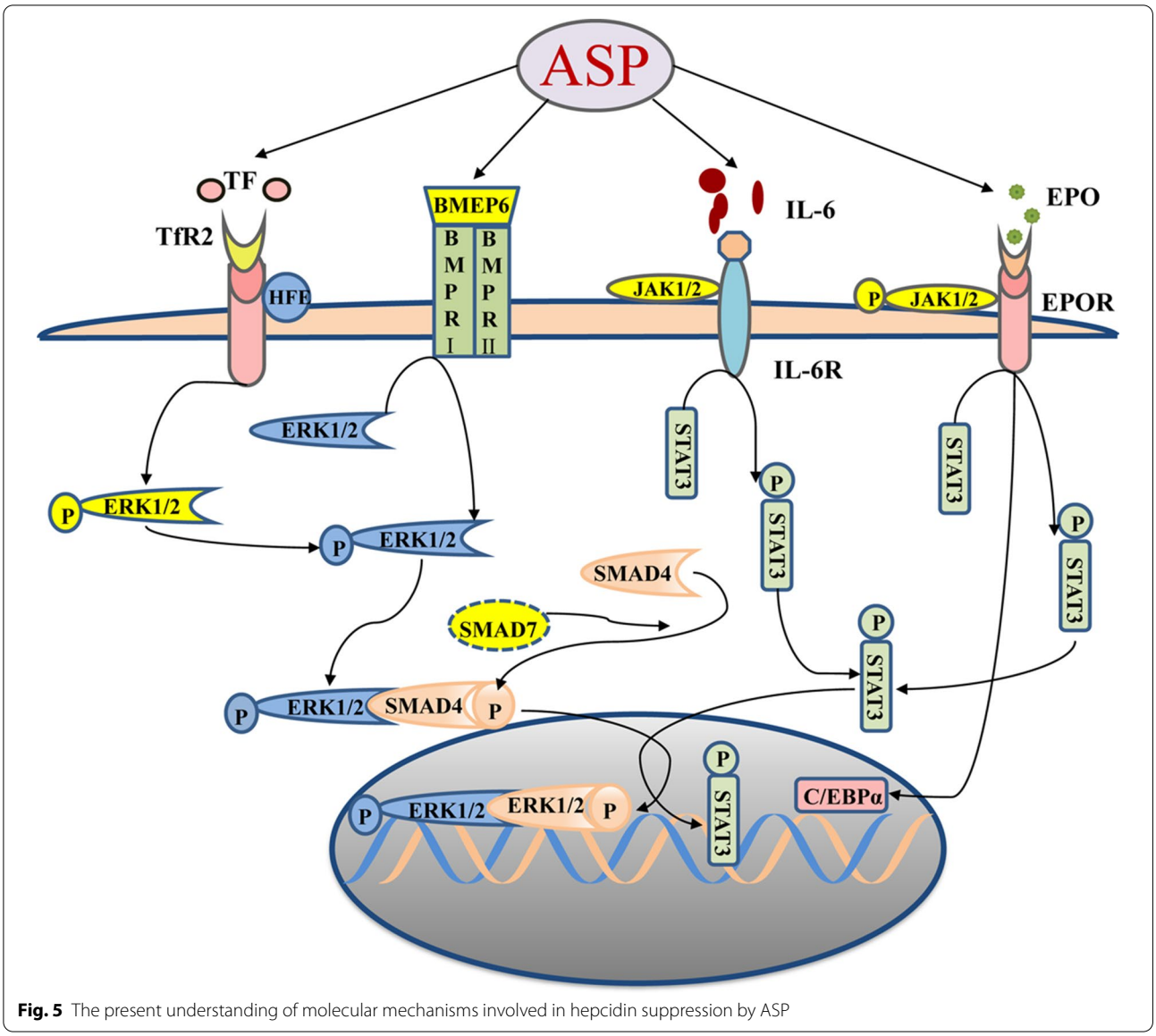

Fig. 5 The present understanding of molecular mechanisms involved in hepcidin suppression by ASP

pro-apoptotic protein Bax expressions, and protected the mice against hepatic damage (Fig. 4b) [62].

In addition, in a previous study, it was shown that 125 μg/mL ASPs pretreatment noticeably decreased pro-inflammatory cytokines (TNF-α, IFN-γ, IL-2 and IL-6), reduced MDA and ROS levels, enhanced SOD activity, and attenuated Caspase-3-dependent apoptosis by Caspase-8 and JNK-mediated pathway, inhibited the activation of IL-6/STAT3 and NF-κB signaling pathways in ConA-induced liver damage in mice (Fig. 4c) [63].

Meanwhile, a novel approach using biochemical parameters coupled with metabolomics based on GC–MS was designed to explain the hepatoprotective effect mechanism of ASP. In a study, pathological change, SOD activity, MDA content, ALT, AST, and γ-GT all had certain degrees of recovery after 120 mg/kg/day ASP intervention. In addition, the regulative effect on nine potential biomarkers in the liver homogenate (malic acid, fumaric acid, glycine, succinic acid, hexadecenoic acid, octadecanoic acid, valine, linoleic acid, arachidonic acid) and ten potential biomarkers in the plasma (fumaric acid, alanine, malic acid, glycine, citric acid, arachidonic acid, octadecanoic acid, hexadecenoic acid, linoleic acid, valine) were found to explain the hepatoprotective mechanism of ASPs [64]. The pharmacokinetics of ASPs including the digestion, absorption, and utilization after oral administration, still needs investigation in the future.

Hypoglycemic activity

Non-starch polysaccharides could decrease levels of blood sugar through different mechanisms of action. In a previous study [65], fasting blood glucose (FBG) levels were reduced after a 4-week oral administration of ASPs in streptozotocin (STZ)-induced diabetic BALB/c mice. The hepatic glycogen (HG) and muscle glycogen (MG) concentrations increased, while insulin resistance (IR)-related inflammatory factors IL-6 and TNF-α in serum reduced in STZ-induced diabetic mice. Meanwhile, researchers found that ASPs could improve the dyslipidemia conditions, and reduce elevated serum total cholesterol (TC), triglyceride (TG) concentrations.

Zhang et al. [66] previously reported the protective effects of ASPs on pancreatic islets of T2DM mice. And they demonstrated that the hypoglycemic effect of ASPs might be closely attached to the promotion of insulin secretion and the inhibition of apoptosis in pancreatic β-cells via blocking both extrinsic and intrinsic pathways (Fig. 4d).

In another study, Chen [67] reported that oral administration of ASPs (20 and 100 mg/kg body weight) for 21 days resulted in a significant reduction in blood glucose levels coupled with the improvement of plasma insulin level in alloxan-induced diabetic rats, which might be associated with the repair and regeneration of the damaged islet B cell [67]. These studies implied that ASPs had hypoglycemic activity therapeutic potential.

Anti-inflammatory activity

Inflammation is subsequent response of the body's immune system to injury, infection and stress. Polysaccharides from plant have attracted increasing attentions of researchers around the world as for their safety and anti-inflammatory activity [68]. For example, Li et al. [69] reported that 4-week oral administration of ASP exhibited significant effect in decreasing pro-inflammatory cytokines (IL-6 and TNF-α) in complete Freund's adjuvant (CFA)-induced arthritic rats besides its activity in improving anemia [69].

Meanwhile, the protective effects of ASP on dextran sulfate sodium-induced ulcerative colitis were investigated by Cheng et al. [70]. It was found that ASP could considerably ameliorate the symptoms of weight loss, disease activity index score, and colon shortening caused by dextran sulfate sodium, and markedly suppress the myeloperoxidase activity in colon tissues. ASP inhibited inflammatory cytokine expressions, decreased apoptosis of intestinal epithelial cells and promoted tight junction protein expression in enteritis mice. Meanwhile, they could protect the intestinal barrier function and accelerate the healing of chronic ulcers [70]. The protective effects of ASP were highly associated with the attenuation of oxidative stress and apoptosis (two important pathogenic factors in the induction of ulcerative colitis) in colonic cells [11]. Although ASPs from various species showed potent anti-inflammatory activity, the effective factors and mechanisms deserves further investigation, especially in intestinal microbiota degradation.

Antitumor activity

The plant polysaccharides could exert antitumor activity through different mechanisms, which included preventing oncogenesis, improving immune response, inducing tumor cells apoptosis, and inhibiting tumor cells proliferation [71]. Fu et al. [72] previous reported that APS could induce apoptosis via regulation of the Janus kinase (JAK)/signal transducers and activators (STAT) of transcription pathway in breast cancer cell [72].

In addition, Cao et al. [73] reported that the total polysaccharide (APS-1d) isolated from *A. sinensis* could decrease HeLa cell proliferation while induce apoptosis in a concentration- and time-dependent manner in vitro, and inhibit tumor growth significantly in athymic nude mice, which primarily involved the activation of the intrinsic mitochondrial pathway [73].

Besides, Ren et al. found that ASPs could inhibit tumor growth in mice xenografted with mouse breast cancer cell line and mouse hepatocellular carcinoma cell line. In vivo experiments showed that ASPs could potently regulate hepcidin expression in liver and serum and decrease iron burden in liver, spleen and grafted tumors in mouse model, and markedly suppress the expression of interleukin-6 (IL-6), JAK2, p-STAT3, and p-SMAD1/5/8 in liver, which provided evidence for the potential use of ASP in cancer treatment in patients with iron overload [74]. ASPs have a good antitumor activity and little toxic side effect, and whether it can directly induce tumor cells is yet to be further verified.

Antioxidant activity

The antioxidant activity of ASPs is frequently carried out through various assay methods in vivo and in vitro. In a study mentioned above, researchers investigated the antioxidant activity of ASPs in vitro and demonstrated that ASPs had certain antioxidant activities in suppressing superoxide anion radicals, hydroxyl radicals, and 2,2-diphenyl-1-picryhydrazyl (DPPH) radicals in a dose-dependent manner [75].

In another study, oral ASPs administration at a dosage levels of 150 mg/kg 3 times a day before meals for 3 months in middle-aged women subjects indicated that the superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione, and lipid peroxidation levels were significantly enhanced while serum vascular cell adhesion molecule-1, interleukin-1 beta, interleukin-6,

and tumor necrosis factor levels were considerably reduced compared to the control group, which was comparable to effects of Tai Chi exercise [76].

In addition, Yang et al. [77] found that APF3 (*A. sinensis* polysaccharide fraction) could effectively inhibit H_2O_2-caused decrease of cell viability, lactate dehydrogenase leakage and malondialdehyde formation, and reduce H_2O_2-caused decline of superoxide dismutase activity and glutathione depletion. Studies revealed that 100 μg/mL APF3 could effectively protect macrophages by inhibiting the release of excess NO and reactive oxygen species induced by high concentrations of H_2O_2 [77].

Other bioactivities

In addition to the above-mentioned ones, other biological activities of ASPs, including anti-aging, immunomodulation, anti-radiation, were also explored and reported in recent years.

For instance, Qian et al. previous reported that ASP treatment could suppress pulmonary fibrosis in rats and fibrogenesis in alveolar type II epithelial (RLE-6TN) cell via a DANCR/AUF1/ FOXO3 regulatory axis, thus benefiting the treatment of idiopathic pulmonary fibrosis (IPF) [78].

Besides, ASPs showed anti-aging activity by attenuating excessive activation of Wnt/β-catenin/TCF-4 signaling in the D-gal-induced senescence of Sca-1^+HSC/HPCs significantly and inhibiting the phosphorylation of GSK-3β which inactivated β-catenin degradation. The antiaging mechanism could be involved in GSK3β interacted with Notch signaling and PI3K/Akt signaling in stem cell aging [79].

In addition, Yang et al. [80] investigated the immunomodulatory activities of an *A. sinensis* polysaccharide (AP) in vitro. It was found that AP promoted the proliferation of total spleen cell, macrophages and T cell. Besides, AP upregulated IL-2 and IFN-γ, while decreased IL-4 on both protein and gene levels. The treatment of AP also remarkably increased the percentage of $CD4^+$ T cell in total spleen cell, while decreased that of $CD8^+$ T cell slightly. Studies indicated that AP had immunomodulatory activity by regulating the expression of Th1- and Th2-related cytokines. Furthermore, the time–effect relation of cytokines response suggested that cells involved in nonspecific immunity, such as macrophages and natural killer cells, were primary activated and helper T cells were secondarily affected after AP treatment [80].

Anti-radiation is another activity attracts extensive attention about ASPs. It is reported that under gamma radiation treatment, compared with the irradiated controls, 7 days pre-treatment of pectic polysaccharide, namely ASP3, exhibited effective protective effect on leucocytes and lymphocytes of mice against radiation-induced damage at a dosage of 200 mg/kg d body weight when mice were exposed to a ^{60}Co source with a dose of 3.0 Gy at a uniform dose rate of 80.0 cGy/min [39]. In another study, researchers found that two homogeneous polysaccharides, APS-1a and APS-3a, could improve the thymus and spleen index, increase the number of red blood cell (RBC), and white blood cell (WBC) in peripheral blood as well as the cellularity of bone marrow cell numbers in irradiated mice, protect mice against radiation-induced micronucleus formation in bone marrow, demonstrating their radio-resistance activity [43]. Because of the complex structure of ASPs, the mechanism of their pharmacological effects and the structure–activity relationship are still unknown.

Structure–activity relationship

The bioactivities of polysaccharides have close relationship with their structures, including their monosaccharide compositions, glycosidic linkages types and positions, molecular weights, chain conformations, etc. ASPs are no exceptions, however, few reports about their structure–activity relationship are available, which needs further investigations. But still some indications can be inferred as follows.

ASPs with radio-protective activity tend to be richer in galacturonic acid, galactose and arabinose in their molecular composition, which could be inferred from those three mentioned above, among which APS-1a was composed of galactose, arabinose and glucose in a relatively molar ratio of 57.34, 27.67 and 14.98%, and APS-3a consisted of galactose, arabinose and glucose in a relatively molar ratio of 84.54, 6.50, and 8.96%, while ASP3 mainly consisted of galacturonic acid (58.27%), galactose (24.93%), arabinose (10.5%) and rhamnose (1.87%) [39, 43].

ASPs with high β-galactoside content seems to possess high antitumor activity as the latter could specifically bind to Galectins (Gal) which play important roles in anti-apoptosis in various cancer cells. For example, Liu et al. [81] reported that a heterogeneous polysaccharide (APS-2I) and its galactosidase digested fraction named G-4 showed higher affinity to Gal-3 with dissociation constant (Kd) of 9.35 ± 0.3 μM and 1.97 ± 0.7 μM, respectively, and exhibited growing apoptosis inducing effect on leukemia cells. The mechanism involved inhibiting the anti-apoptotic effect of Gal-3 and activating the caspase-9 and caspase-3 in leukemia cells by APS-2I and G-4. Structure characterization and comparison indicated that the contents of Gal, GalA and Man as well were important for the anti-leukemia bioactivity of APS-2I, but a high proportion of Ara*f* was not essential. In addition, glycosidic linkages, including T-Gal*p* and 1,

4-Gal*p* could be of great importance in forming active conformation in APS-2I [81].

The influence of molecular weight on the functions of ASPs appears to be controversial. It was reported that high molecular weight might play a crucial role in the formation and maintenance of active spatial conformation of ASPs [4]. Nevertheless, other studies demonstrated that the bioactivity of ASPs have no direct connection with their molecular weights, and sometimes the relationship between them could be quite complex, being related with the object of action and the solubility of ASPs [46, 81].

It is definite that chain conformation means a lot in determining the bioactivities of ASPs, but precise demonstration about this is absent and massive work should be done to uncover the mysterious veil, which will exactly benefit our understanding of the mode of action of ASPs.

Conclusions

Most polysaccharides derived from plants are nontoxic and do not cause any significant side effects. *A. sinensis* polysaccharides could be effectively isolated and purified with various extraction methods. The ASPs have a variety of biological activities including hematopoietic, antitumor, antioxidant, hepatoprotective, hypoglycemic, and anti-inflammatory activities, indicating their potential broad contribution in disease treatment in the future. As for their chemical structure, efforts have been paid to illuminate their monosaccharide composition, molecular weight, primary structure and high order structure. Facing the complexity of ASPs structure, we could use a variety of methods, including computer-aided liquid nuclear magnetic resonance analysis technology, computer molecular docking and molecular dynamics simulation technology, and 3D emerging technologies, to improve the accuracy of polysaccharide structure characterization in the future. However, the relationships of ASPs between their structure and functions have not been well established yet. Further research on the exact structure–bioactivity relationships is required, which would provide guidance for the structural modifications of ASPs to achieve their maximum effects. Meanwhile, to better determine the effects of ASP metabolites on human health, in vivo studies in animals and clinic should be conducted.

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Authors' contributions
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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Not applicable.

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Competing interests
The authors declare that they have no competing interests.

Author details
[1] College of Food and Bioengineering, Zhengzhou University of Light Industry, Zhengzhou 450001, People's Republic of China. [2] School of Chemistry and Chemical Engineering, Guangxi University for Nationalities, Guangxi Key Laboratory of Polysaccharide Materials and Modification, Nanning 530008, People's Republic of China. [3] Guangxi Talent Highland of Preservation and Deep Processing Research in Fruit and Vegetables, Hezhou University, Hezhou 542800, People's Republic of China.

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