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Review

Review of *Astragalus membranaceus* polysaccharides: Extraction process, structural features, bioactivities and applications

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ABSTRACT

Astragalus membranaceus possesses the function of enhancing immunity, protecting the liver, diuretic, anti-aging, anti-stress, anti-hypertensive, and more extensive antibacterial effects. Polysaccharides, one kind of the major active ingredients of *A. membranaceus*, are considered to be responsible for their versatile use. Now, a systematic summary of research progress and prospects of polysaccharides from *A. membranaceus* polysaccharides (AMPs) is necessary to facilitate their further study and application. In this review, the optimal extraction methods, structural features, biological activities, and applications of AMPs were emphasized. The structure–activity relationships are also analyzed and elucidated. Solvent, ultrasonic, microwave, enzyme-assisted, ultra-high pressure, and combined methods have been used to extract AMPs. Among them, solvent extraction is the most commonly used method because it is simple and easy to operate, but the efficiency needs to be improved further. The ultra-high pressure method is the most efficient but has a low economic return. AMPs exhibited various bioactivities, including immunomodulation, antitumor, and antidiabete. The structure–activity relationships revealed that different structure configurations, chain conformations, and physical properties would have different bioactivities. However, the new method for purification of certain polysaccharides, detailed structure–activity relationships (SAR), mechanisms of bioactivities, and quality control of AMPs need to be extensively investigated.

Contents

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1. Introduction

Astragalus membranaceus (Fisch.) Bge. belongs to the Leguminosae family, a perennial herb with upright stems and yellow or yellowish flowers. The medicinal part is the dried root, which is cylindrical and has a pale brownish-yellow or light brown color (Fig. 1). which has been used as both food and medicine and widely used in many countries for more than thousands of years, including China, USA, Japan, Korea, Iran, and Russia (Chen, Liu, Gao, Chen, & Wang, 2020). The USA Dietary Supplement Health and Education Act (1994) as a legal dietary supplement categorized *A. membranaceus*. *A. membranaceus* tea and capsules are being sold as over-the-counter (OTC) health products in the USA dietary supplement market (Zhang et al., 2011). In China, *A. membranaceus* is a well-known traditional Chinese medicine (TCM), which is distributed mainly in Shanxi, Gansu, Heilongjiang provinces, and Inner Mongolia. *A. membranaceus* can be first traced back to an ancient Chinese medical masterpiece *Shennong's Classic of Materia Medica* (*Shen Nong Ben Cao Jing*), and is rated as one of the highest grade TCM drugs (Fan et al., 2011). Remarkably, *A. membranaceus* is included in the edible herbal list created by the Ministry of Health of China, in which more than 100 herbs are identified as health products and medicinal drugs.

The bioactive constituents in the dried roots of *A. membranaceus* are complicated and many active compounds have been isolated from *A. membranaceus*, such as flavonoids, saponins, and polysaccharides (Liu et al., 2020). Scientific investigations in the last two decades have revealed much insight into the pharmacological functions of these components, especially their polysaccharide fractions. Among them, biologically active *A. membranaceus* polysaccharides (AMPs) have attracted much attention. Polysaccharides from *A. membranaceus* possess several pharmacological functions, including the immunomodulatory effect (Qin et al., 2017), anti-viral (Bai, 2014), anti-tumor (Li, Hu, Wang, Jiao, & Song, 2019), anti-aging, anti-oxidation (Chen, 2015) and hyperglycemic (Huang et al., 2017). In addition, AMPs have been produced in many different forms of upplements, including liquid extracts, capsules, powder, and tea, and it has also been widely used in animal husbandry due to their remarkable immunomodulatory effects, i.e., improving the immunity of animals and reducing

Fig. 1. Whole plant (A), roots (B), root slices (C) of *A. membranaceus* and *A. membranaceus* polysaccharide powder (D).

the use of antibiotics (Qin et al., 2017). Therefore, AMPs have great potential for further development as products in pharmaceutical and nutraceutical areas.

The present review aims to summarize previous and current references and give a comprehensive summary regarding the extraction methods, structural characterization, biological activities and application of AMPs to provide new insight for further development (Fig. 2).

2. Extraction of AMPs

Owing to their remarkable biological activities, AMPs show high medicinal potential. Thus, extraction and purification technology are essential for the research and development of AMPs. In recent years, the extraction of AMPs has been studied extensively (Yang, Wang, Ye, & Pan, 2023), and some of the commonly used extraction methods are described below (Table 1).

2.1. Solvent extraction method

Solvent extraction methods are simple and easy to operate, which are the most traditional extraction method for AMPs, mainly involving water extraction, alkali water extraction, etc. Traditionally, prolonged boiling or 'decocting' using water as a solvent is the earliest and most popular method for preparing herbal extracts. Water-extraction and alcohol-precipitation method are the two classical extraction methods of AMPs and has been used generally in industry. They have the advantages of a simple process, convenient operation, and less damage to polysaccharides. The best extractive method of AMPs by water is to perform the extraction twice by heating water at 100 °C for 60 min per extraction. The liquid ratio of the material is 1:10 g/mL, and the rate of extraction is 3.570%. The use of tannic acid is more effective in removing proteins (Yang et al., 2018). Moreover, the purification of AMPs, which is isolated and purified by D101 macroporous absorptive resin, is 65.07% (Liu et al., 2018). Based on an orthogonal array design, the optimal water-extraction and alcohol-precipitation method conditions were found: extraction duration of 90 min, solid–liquid ratio is 1:8, extraction temperature of 100 °C, and extraction three times. Under these conditions, the yield of AMPs was predicted to be 10.35% (Mu, Zhu, Zhao, Zhou, & Jia, 2009). The extraction time, number of extractions, and solid-to-solvent ratio were explored and optimal conditions were determined, at which point the yield of AMPs was expected to be 14.76%. (Li, 2015). The water extraction method will extract other substances, such as saponins and flavonoids, from *A. membranaceus*. These substances are difficult to separate at the later stage, resulting in the low purity of AMPs. Moreover, the energy consumption of this method is high, whereas the economic benefit is relatively low.

Besides, other solutions, such as alcohol-water, aqueous NaOH, and alkaline (NaOH)-alcohol, are also used to extract medical materials (Dai et al., 2022). The extraction ratio of AMPs was significantly improved with the alkali extraction method versus water

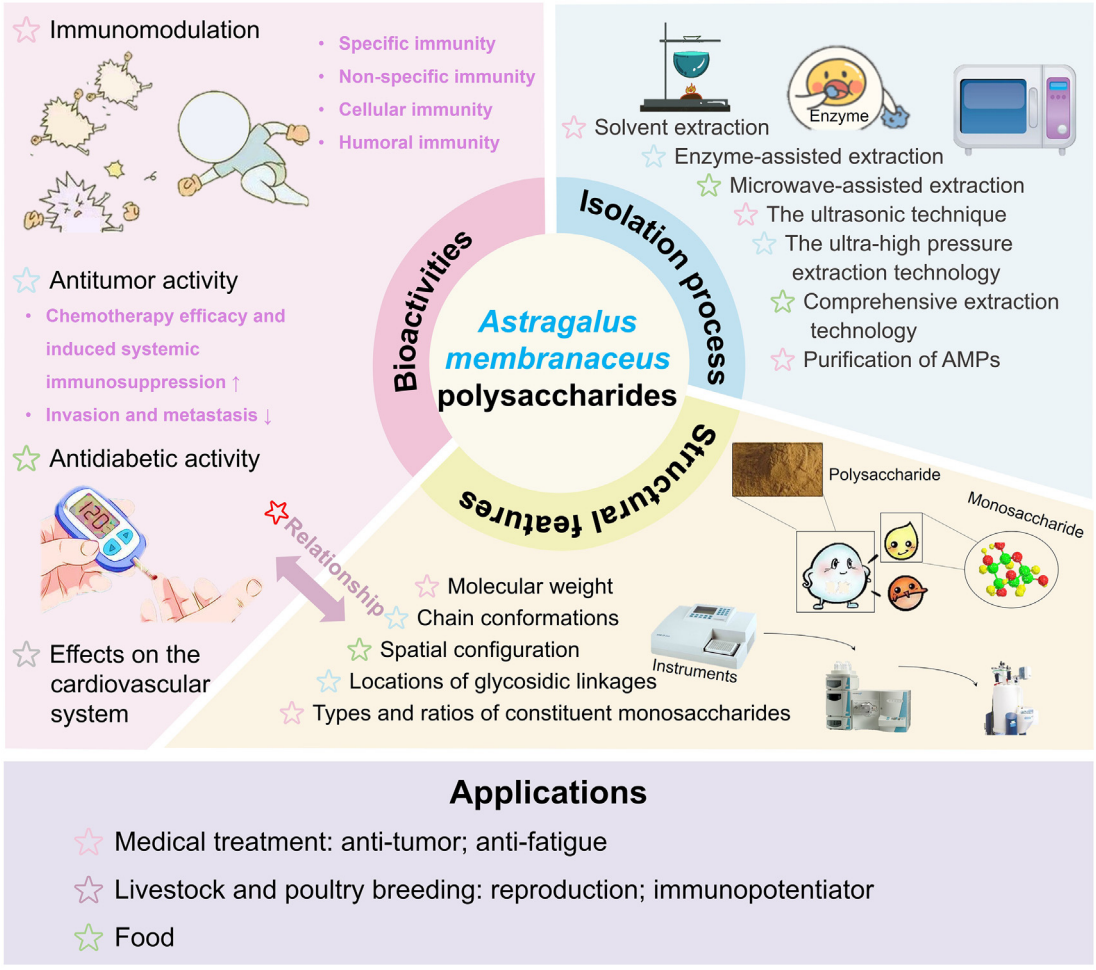

Fig. 2. Bioactivities, isolation methods, structural features and application of AMPs.

Table 1
Extraction methods of *A. membranaceus* polysaccharides.

Extraction methods	Extraction conditions				Extraction rate (%)	Advantages	Disadvantages	References
	Temperatures (°C)	Time (min)	Number of times	Solid-liquid ratio				
Water extraction	95 – 100	45 – 90	1 – 3	1:8 – 1:10	3.57 – 14.76	Simple process, convenient operation, and less damage to polysaccharides	Low efficiency, low yield, and long duration	Yang et al., 2018; Liu et al., 2018; Mu et al., 2009; Li, 2015
Enzyme-assisted extraction	75 – 100	90 – 120	1 – 3	0.4 – 0.8	4.76 – 9.78	Environmental friendliness, high efficiency, and ease of operation	High requirements on environmental conditions, which need to be strictly controlled	Zhang, 2013; Wei et al., 2012; Chen & Ma, 2005; Zheng et al., 2005; Zhang et al., 2013
Microwave-assisted extraction	–	10	1 – 3	1:12	4.50 – 6.55	Time-saving, high-efficiency, and energy saving	Effects on chemical structure and pharmacological activities of polysaccharides are unclear	Li et al., 2001; Li et al., 2017; Chen et al., 2015
Ultrasonic technique	20 – 35	50 – 87	1 – 3	1:22 – 1:30	6.07 – 11.44	A bio-friendly technique with shorter times and fewer energy costs	Cause changes in molecular structure of polysaccharide	Li et al., 2013; Liang et al., 2018
Ultra-high-pressure extraction	–	5	1	1:14 – 1:60	24.28 – 24.36	Short time, low energy consumption, fewer impurities	High requirements on the production equipment	Liu et al., 2009

Note: "–" indicates that there are no restrictions.

extraction. Alkali destroys the fiber of *A. membranaceus*, facilitating the flow of polysaccharides. By comparing the extraction results of four different solutions, it was found that the extraction rate of the alkali-alcohol extraction method was the highest (9.74%) and the water-extraction and alcohol-precipitation method was the lowest (2.81%). The extraction rate of alcohol-water and aqueous NaOH was 3.62% and 7.64%, respectively (Jin, Jin, Yang, Zhang, & Zhou, 2013). The extraction of AMPs using the alcohol alkali method resulted in a 3.53-fold and 2.63-fold higher yield compared with the water extraction and alkali extraction methods, respectively (Chen et al., 2020). In addition, aqueous solutions of CaO and Na_2CO_3 have also been used to prepare AMPs crude extracts, and the results showed that the extraction yield was the highest (11.7%) with a CaO aqueous solution, which was 3.25 and 2.05 times higher than that obtained with water (3.6%) and a Na_2CO_3 aqueous solution (5.7%), respectively (Li, Huang, & Wang, 2000).

In a word, these methods are simple and easy to operate. However, the common problems with this traditional method are low efficiency, low yield, and long duration. To overcome these shortcomings, researchers have made many attempts to explore new technology for AMPs.

2.2. Enzyme-assisted extraction method

Enzyme-assisted extraction is receiving increasing attention because of its environmental friendliness, high efficiency, and ease of operation in recent years, which was considered an alternative method for natural product extraction. The high content of cellulose in *A. membranaceus* raw material is one of the main factors affecting the yield of AMPs. Cellulase can break down the plant cell wall, thereby releasing polysaccharides from cells without destroying the structure of polysaccharides. After being pretreated with cellulase, the extraction rate of AMPs reached 4.76%. Compared with the traditional water extraction method, the extraction rate increased by 54.05% (Zhang, 2013). The reason of the improved extraction rate was that cellulase only destroys the cell wall and reduces the resistance of polysaccharide diffusion in the inner part, thus improving the mass transfer rate and effective diffusion coefficient (Wei, Kang, & Zhang, 2012). For AMPs extraction with cellulase, the optimum enzymatic hydrolysis time was 120 min, the ratio of the enzyme was 0.8%, and the hydrolysis temperature was 75 °C. Under these conditions, the extraction rate of AMPs was 9.78%, and that of total sugar was 50.2% (Chen & Ma, 2005). After pretreatment with three different cellulase concentrations (0.3%, 0.4%, and 0.5%), the yield of AMPs obtained by a water extraction method increased by 314.8%, 392.6%, and 342.6%, respectively, compared with that obtained by the water extraction method alone (Zheng, Wei, & Long, 2005). Based on the orthogonal experiment, the optimal cellulase-assisted extraction method conditions were found: extraction duration of 1.5 h, solid–liquid ratio is 1:12, extraction temperature at 100 °C, and extraction three times (Zhang et al., 2013). In conclusion, enzymatic hydrolysis of plant material can be employed as a pretreatment to improve the yield of AMPs.

2.3. Microwave-assisted extraction method

Microwaves are characterized by strong penetration, high selectivity, and high heating efficiency. The thermal effect of microwaves can break the cell wall and inactivate enzymes in the cell membrane; therefore, polysaccharides can be easily extracted from cells, and the yield can be effectively improved (Dai et al., 2022). Thus, microwaves can be employed in the extraction of AMPs. Microwave-assisted extraction parameters were also explored, including microwave power, extraction time, the ratio of solid to solvent, and microwave duration. For the first time, AMPs were extracted by microwave technology and its content was 6.55% (Li, Liu, & Lu, 2001). The reaction time was shortened by 12 times. The following have been reported as the optimal extraction conditions for microwave-assisted extraction: the water/material ratio, 12:1; pH, regulated by saturated limewater, 9; and two doses of microwave radiation (300 W) for 10 min each. Under these condi-

tions, the yield of the crude AMPs product was 14.6%, and the purity was 88.1% (Li et al., 2017). In another study using a microwave-assisted extraction method, the extraction rate of AMPs was 4.50%, with the AMPs content of 31.25%, indicating that this method was time- and energy-saving. Furthermore, a comparison between microwave-assisted and water-extraction, and alcohol-precipitation methods was made. Results show that the former rate is 4.502% and the latter rate is 4.468% (Chen, Lin, Mo, & Du, 2015). Therefore, microwave-assisted extraction method has the obvious advantages of time saving, high efficiency and energy saving, and is better than the traditional method in improving the yield of AMPs and effectively reducing the extraction time, etc. Although microwave-assisted extraction methods can improve the extraction rate of polysaccharides, the effects of microwaves on the chemical structures and pharmacological activities of polysaccharides are still unclear and require further studies.

2.4. Ultrasonic technique

The ultrasonic technique has been widely used to extract polysaccharides from medicinal plants. Ultrasonic time, temperature, power, and the ratio of solid to solvent all affect the extraction rate of polysaccharides, but ultrasonic time has the greatest effect on the extraction rate of polysaccharides (Li et al., 2013). Based on response surface methodology, the optimal ultrasonic extraction method conditions were found: ultrasonic time for 87 min, the solid–liquid ratio is 1:22, and ultrasonic power is 600 W. Under these conditions, the yield of polysaccharides was 6.07 % (Liang et al., 2018). Ultrasonic treatment can enhance mass transfer between the solids and solvents and improve the extractability of polysaccharides by destroying the cell wall.

However, ultrasonic extraction will cause changes in the monosaccharide molecular structures of AMPs, which will have certain effects on the physicochemical properties and biological activities of polysaccharides. So this method has not been widely applied in practice.

2.5. Ultra-high pressure extraction technology

Ultra-high-pressure extraction technology is a new technology for extracting active ingredients from natural products. The method has the highest extraction rate, 24.28% (Liu, Zhang, Zhang, & Dou, 2009). Compared with the traditional water reflux method, it increased by 1.6%, compared with the ultrasonic extraction technology, it increased by 6.7%. Besides, its extraction time is very short, only 2 min. At the same time, it provides a reliable basis for the comprehensive benefits of AMPsand has practical significance in production and application (Liu et al., 2009).

2.6. Comprehensive extraction technology

With the development of new extraction methods for AMPs extraction, some researchers have also tried to simultaneously use two or more methods. The optimal conditions for AMPs extraction using the combination of microwave and cellulase-assisted methods were: microwave power 480 W, solid–liquid ratio 1:10, microwave time 8 min, cellulase-material ratio 57.6 U/g and zymolytic time 60 min (Dong, Huang, Qi, & Feng, 2011). The results showed that the extraction rate of polysaccharides was up to 16.07% and the polysaccharide mass fraction was up to 88.4%. The method has the advantages of short time, energy saving, high extraction rate, and good product purity. It is an ideal method to extract AMPs. In the practical application of polysaccharide extraction, the ultrasonic method and enzymatic method are often used in combination. The optimal conditions for AMPs extraction using the combination of the ultrasonic and enzymatic methods were (Bi & Wu, 2010) ultrasonic time of 30 min, a temperature of 40 °C, a solid–liquid ratio of 1:20, and the enzyme amount of 10 mg. In addition, the extraction rate of AMPs can be increased by the cooperative extraction of ultrasound and microwave (Du, Cai, Wang, & Dai, 2012).

At present, the extraction process of AMPs is mainly based on traditional water-extraction and alcohol-precipitation methods and enzyme-assisted extraction methods. Although the traditional solvent method has the disadvantages of time consumption, high temperature, and easy inactivation of components, due to the consideration of cost and operability, the traditional extraction method is still the main method in the selection and application of traditional Chinese medicinal materials, and it can be predicted that there will not be a big change in the future for a long time (Liang et al., 2018).

2.7. Purification of AMPs

The purity of the extracts obtained with the above extraction methods is not sufficient for AMPs to be used for chemical composition and structure analyses. The extracted AMPs usually contains oligosaccharides, pigments, proteins, flavonoids, and other impurities. Therefore, purification must be carried out. The common purification methods employed are as follows: enzyme-Sevag, diethyl aminoethyl-Sephadex A-25, and Sephadex G-100; a polyamide column and an AB-8 macroporous resin column (Jiang et al., 2016; Jina, Zhao, Huang, & Shang, 2014); X-5 macroporous resin (Jin et al., 2013); chitosan flocculation; and a type II ZTC1 + 1 natural clarifier (Liang et al., 2018). The purified AMPs obtained by these methods can be used for subsequent chemical composition and structural analyses.

3. Structural features of AMPs

There are a lot of researches on *A. membranaceus* polysaccharides, so it is necessary to summarize and discuss them. Structural features, such as molecular weight, types and ratios of constituent monosaccharides, chain conformations, locations of glycosidic linkages, and spatial configuration of AMPs have been widely studied. Research has shown that molecular weight and methyl ester level are two important parameters in determining the differences in AMPs (Sheng, Liu, & Yang, 2021). Since the first report on PCPs published in 1979 (Shanghai Institute of Materials Medica, 1979), more than 30 kinds of AMPs have been isolated and purified from *A. membranaceus*. Their structures had been roughly characterized by high-performance liquid chromatography (HPLC), gas chromatography (GC), gel permeation chromatography, mass spectrometry (MS), and nuclear magnetic resonance (NMR). The identification methods of polysaccharides have been mentioned, such as the molecular weight, monosaccharide composition, and methyl ester level of polysaccharides were determined using HPLC. The IR and NMR were used to characterize the polysaccharide structures and analyze the bonding patterns. Fundamental structural features of AMPs, such as molecular weight, monosaccharide composition, and primary structure are shown in Table 2. Besides, their bioactivities and corresponding references are also included.

Due to different extraction methods, detection methods, and purification techniques, different results about structural features were given in various reports. The molecular weight of these polysaccharides ranges from 2.10×10^3 to 4.77×10^6 Da. Four polysaccharides were obtained from *Astragali Radix* using different solvents. The molecular weights of the four polysaccharide fractions are 2.58×10^5, 4.01×10^4, 1.53×10^4, and 3.20×10^3, respectively (Jiang et al., 2016).

Table 2
Polysaccharides isolated from *A. Membranaceus*.

No.	Compound names	Monosaccharide composition	Molecular weight	Backbone	Bioactivities	References
1	AMP	Rha, Ara, Gal, and Glc	1.1×10^4	–	Immunomodulation	Wu, 2020
2	WAP	Glc: Gal: Ara: GalA: Rha: Man = 80.9: 6: 4.8: 6.7: 1: 0.6	Four main peaks of > 2.0×10^6, 1.2×10^4, 1.5×10^3, and 800	A mixture of glucan, arabinogalactan, and RG-I regions with the existence of 1,4-Glc,1,3-Gal,1,2-Rha, 1,4-GalA, and 1,3,5-Ara	Intestinal function	Liu et al., 2020
3	AP	Glc	3.6×10^3	An α-(1 → 4)-*D*-glucan with α-(1 → 6)-linked branches attached to the O-6 of branch points	Antitumor activity	Li, Chen, Wang, Tian, & Zhang, 2009
4	APS-I APS-II	Glc: Ara: Xyl = 0.54: 1: 18.14 Glc: Ara: Xyl = 0.23: 1: 29.39	4.77×10^6 8.68×10^3	Major α-(1 → 3)-glucose and a few 1 → 4, 1 → 6 glucose	Anti-tumor	Wang, Ge, Li, Guan, & Li, 2016
5	RAPS	D-ribose: Ara: Rha: Man: Glc: Gal = 1.0: 14.1: 0.3: 19.9: 181.3: 6.3	–	–	Intestinal function	Dan et al., 2018
6	ASP	Ara: Gal: Glc: Man = 1.00: 0.98: 3.01: 1.52	2.1×10^3	With pyranose ring and α-type glycosidic linkages	Anti-tumor activity	Yu, Ji, & Liu, 2018a
7	APS	α-*D*-Glc	2.07×10^4	Repeating (1 → 4)-link backbone with a (1 → 6) – linked branch every 10 residues	Antioxidant	Niu et al., 2011
8	APS	α-(1 → 4)-*D*-Glc	3.60×10^4	A single α-*D*-glucose at C-6 position every nine residues	Renal protective effect	Li & Zhang, 2009
9	I	Glc: Gal: Ara = 1.75: 1.63: 1	3.63×10^4	–	Immunomodulation	Li et al., 2009
	II	D-Glc	1.23×10^4	–	Immunomodulation	
	III	D-Glc	3.46×10^4	–	–	
10	APS-1	Gla: Glc = 1: 49.76	3.84×10^4	α-Glycosidic bonds	Prebiotic activity	Huang et al., 2008
	APS-2	Rha: Gla: Glc = 1: 2.99: 16.26	5.20×10^3	α-Glycosidic bonds and β-glycosidic bonds	Prebiotic activity	
11	APS1	Glc	2.58×10^5	–	No	Jiang et al., 2016
	APS2	Ara	4.01×10^4	–	Immunomodulation	
	APS3	Rha: Glc: Gal: Ara = 1: 10.76: 6.55: 12	1.53×10^4	–	Immunomodulation	
	APS4	Gal: Ara = 3.02: 1	3.20×10^3	–	No	
12	LM_W-ASP	Glc: Gal: Ara: Xyl: GalA = 10.0: 1.3: 1.7: 1.0: 0.9	5.60×10^3	(1 → 4)-Linked Glc	Immunomodulation	Yang, Xiao, Qu, & Wang, 2016
13	APS	Rha: Xyl: Glc: Gal = 1: 4: 5: 1.5	3.01×10^5	1,3-Linked-β-*D*-Gal	ND	Fu, Huang, Zhang, Yang, & Chen, 2013
14	AAP-2A	Rha: Gal: Ara: Glc = 1: 2.13: 3.22: 6.18	2.25×10^6	1,3-Rhap, 1,3-Galp, 1,3-Araf, 1,5-Araf, 1,3,5-Araf, 1,4-Glcp, 1,4,6-Glcp	Antioxidant activity	Pu, Ma, Liu, Ren, & Fan, 2015
15	cAMPs-1A	Fuc: Ara: Gal: Glc: Xyl = 0.01: 0.06: 0.20: 1.00: 0.06	1.23×10^4	α-*D*-Pyranoid Configuration	Anti-tumor and immunomodulatory	Liu et al., 2017
16	APs-1–1	Rha: Ara: Xyl: Glc = 1.00: 5.91: 16.24: 49.56	1.12×10^5	A pyranose unit	Antioxidant activity	Chen et al., 2015
	APs-2–1		9.83×10^4	–	–	
	APs-3–1	Rha: Ara: Xyl: Glc: Gal: GalA = 1.00: 1.74: 4.56: 6.98: 5.23: 2.12. Rha: Ara: Xyl: Glc: Gal: GlcA: GalA = 1.00: 1.82: 11.04: 23.23: 22.51: 1.32: 5.20.	2.04×10^4	–	–	
17	MAPS-5	α-(1 → 4)-D-Glc	1.32×10^4	About two of every 15 sugar residues are replaced by terminal glucose in the C-6 position	Immunomodulation	Gao, Li, & Liu, 2010
18	RAP	Rha: Ara: Glc: Gal: GalA = 0.03: 1.00: 0.27: 0.36: 0.30	1.33×10^6	The backbone consisted of 1,2,4-linked-Rhap, α-1,4-linked-Glcp, α-1,4-linked-GalAp6Me, β-1,3,6-linked-Galp, with branched at O-4 of the 1,2,4-linked Rhap and O-3 or O-4 of β-1,3,6-linked Galp	Immunomodulation	Yin et al., 2012
19	APSI	Glc: Gal: Ara = 1.75: 1.63: 1	3.63×10^7	–	Hypoglycemic effect	Liu et al., 2010
20	APS4	Rha: Ara: Xyl: Man: Glc: Gal = 0.3: 0.6: 1.0: 1.0: 12.1: 1.7	1.61×10^6	(1 → 2,6)-α-*D*-Glcp	Antitumor activity	Yu, Ji, & Liu, 2018b
	APS90	Rha: Ara: Xyl: Man: Glc: Gal = 0.6: 0.8: 0.7: 1.0: 12.3: 2.0	1.22×10^6	(1 → 2,6)-α-*D*-Glcp	Antitumor activity	

(*continued on next page*)

Table 2 (*continued*)

No.	Compound names	Monosaccharide composition	Molecular weight	Backbone	Bioactivities	References
21	AERP1	Man: Rha: GalA: Glc: Gal: Ara = 1.00: 2.59: 12.15: 2.60: 3.07: 4.54	2.01×10^6	Glycosidic bonds of → 3/5-α-araf-(1→, T-α-araf, →4,6-β-manp-(1→, →3/3,6-β-galp-(1→, →2/2,4-α-rha-(1→, →-4/4,6-α-glcp-(1→, →4-α-galpA-(1 → and →4)-6-OMe-α-galpA-(1→	Hypoglycemic effect	Liu et al., 2019
	AERP2	Glc	2.11×10^3	→4/6-α-glcp-(1 → linkage	Hypoglycemic effect	
22	APSID3	Gal: Rha: Glc = 5: 2: 6	5.79×10^5	The main chain is composed of 1,4-linked galacturonic acid and 1,4-linked glucuronic acid and a small amount of 1,3-linked rhamnose.	–	Wang, Shan, Wang, & Hu, 2006
23	ASP	Ara: Gal: Glc: Man = 1.00: 0.98: 3.01: 1.52	2.10×10^3	Pyranose ring and α-type glycosidic linkages	Antitumor activity	Yu et al., 2018a

Note: "–" indicates that there are no restrictions.

Analysis of monosaccharide composition is necessary for structural characterization and activities. The main components of AMPs include heteropolysaccharides, dextran, neutral polysaccharides, and acidic polysaccharides. Heteropolysaccharide is an acidic water-soluble polysaccharide, while dextran is divided into water-soluble and water-insoluble forms. It can be found that AMSs primarily contain 10 monosaccharides, mannose (Man), rhamnose (Rha), galactose (Gal), xylose (Xyl), arabinose (Ara), ribose (Rib), fucose (Fuc), glucose (Glc), glucuronic acid (GlcA) and galacturonic acid (GalA). Fucose is a sort of rare monosaccharide in cAMPs-1A and it might be because it is extracted by using cold water (4 °C) (Liu et al., 2017). Monosaccharide composition analysis indicated that APS1 (*Astragalus* polysaccharides) consisted of glucose only, and APS2 all consisted of arabinose. APS3 and APS4 are heteropolysaccharides, which consist of rhamnose, glucose, galactose, and arabinose in different molar ratios (Jiang et al., 2016). In the study of biological activity, only APS2 and APS3 were found to stimulate the proliferation of lymphocytes. Apart from this, the effect of APS2 also showed a dose-dependent tendency from 6.25 μg/mL to 800 μg/mL. Therefore, AMPs immune activity may not have a close relationship with the molecular weight. However, their monosaccharide composition, combined with the structure, is important for bio-activity. For example, AMPs can significantly improve the solubility and stability of flavonoids and can form complexes with flavonoids in a 1:1 ratio (Liu et al., 2020). Among the purified polysaccharides, AMPs-3–1 shows higher antioxidant activity than that of AMPs-1–1 and AMPs-2–1, but less than that of ascorbic acid. This could be explained that the AMPs-3–1 contained a higher content of uronic acid, and exhibited higher antioxidant activity compared with the other polysaccharides (Chen et al., 2015).

There are eight main glycosidic bond types of AMPs measured through gas-chromatography-mass–mass spectrometry. Of those, 1, 4-glucose linkage is the main, and nuclear magnetic resonance confirmed that anomeric hydrogen is characterized by α configuration.

4. Biological activities of AMPs

Astragali Radix enhances immunity, as well as antioxidation, antiradiation, antitumor, antibacterial, and antiviral effects, and protects the cardiovascular and cerebrovascular systems, as well as the liver, kidneys, and lungs (Gao, Li, & Liu, 2010). AMPs are important chemical components of *Astragali Radix*. Recent studies have indicated that AMPs has immunoregulatory, antitumor, anti-inflammatory, antiviral, antioxidant, antiaging, and other biological activities.

4.1. Immunomodulation

A large number of experimental studies have proved that polysaccharides have extensive effects on specific immunity and non-specific immunity, cellular immunity, and humoral immunity. AMPs have a remarkable immunomodulatory effect. The development of immune organs directly affects the immune ability of the body, which is mainly reflected in the spleen, thymus, lymphatic tissues, bursa, and other immune organs (Li et al., 2017). AMPs can increase the weight of the thymus and spleen in normal mice. AMPs possess a wide variety of immunological effects on mice. It can increase the weight and cell number of mouse spleen, augment the response of mouse spleen against sheep red blood cells, and stimulate the phagocytic activity of peritoneal macrophage (Yin et al., 2012). The effects of AMPs on specific immune cells are mainly reflected in increasing the proliferation and differentiation of B-lymphocytes and T lymphocytes, improving the secretion of plasma cells, increasing the concentration of serum antibodies, and regulating the balance of T lymphocyte subsets, such as CD^{4+} and CD^{8+}. Besides, cytokines and intracellular messengers can participate in regulating the body's immune response and affect the intensity of the immune response. The immunomodulatory activities of two polysaccharides, APS2 and APS3, from *A. membranaceus*, were investigated. APS2 and APS3 can effectively stimulate normal spleen lymphocyte proliferation *in vitro* (Jiang et al., 2016). Moreover, a polysaccharide named cAMPs-1A could significantly inhibit tumor growth and improve body immunity via the promotion of macrophage pinocytosis, the natural killer (NK) cells killing activity, and the percentages of T lymphocyte subsets in peripheral blood of tumor-bearing mice (Liu et al., 2017). Besides, a polysaccharide fraction showed an immunoenhancing ability through heightening the SOD, CAT, GPx, AKP, GOT, and GPT activities in crucian carp. It indicated that oral administration of AMPs effectively improved the growth performance and innate immunity status of crucian carps due to the antioxidant and immunomodulatory activities of AMPs (Wu, 2020). Apart from spleen lymphocytes and macrophages, there are dendritic cells. Dendritic cells (DC) are the main cells that produce interferon (IFN-α), and the DC mainly expresses TLR7 and TLR9. AMPs affect the immune regulation of bone marrow in mice by regulating the function of bone-marrow-derived dendritic cells (Shao, 2006). Some studies have reported that nanoparticles of AMPs can show immune activity *in vitro* and *in vivo* in mice and cause activation of dendritic cells and T cells in human blood (An, Zhang, Kwak, Lee, & Jin, 2022). In summary, AMPs can enhance both humoral and cellular immunity and they can exert their immune effect on the body by regulating immune organs, immune cells, and immune molecules.

Intracellular signal transducers and immune signaling pathways play a key role in the process of immune regulation, as shown in Fig. 3. Studies have shown that AMPs can increase the TLR4/NF-κB and Ca^{2+}-cAMP signaling pathways in RAW264.7 cells (murine mononuclear macrophage leukemia cells) (Fu et al., 2017). The mouse macrophages are activated by triggering the TLR4-mediated signaling pathways, upregulating the expression of phosphorylated-p38 (p-p38), p-extracellular signal-regulated kinase (p-ERK), and p-JNK, inducing inhibitor of IκB-α degradation and NF-κB translocation, and ultimately enhances nitric oxide (NO) and TNF-α (Wei et al., 2016). AMPs can also inhibit the expression of thrombin-induced intercellular cell adhesion molecule-1 by blocking the NF-κB signal transduction in rat bone marrow endothelial progenitor cells and upregulating the expression of vascular endothelial growth factor and its receptor (Zhang, Yao, Ren, Chen, & Yao, 2016). AMPs activated the downstream PI3K/AKT pathway by inducing neuregulin 1 (NRG1), which enhanced the phosphorylation of PI3K and AKT (Chang, Lu, Wang, Lv, & Fu, 2018). The phosphatidylinositol 3-kinase/protein kinase (PI3K/AKT) signaling pathway regulates cell metabolism, growth, migration, and proliferation. Notably, endothelial nitric oxide synthase (eNOS) is a key enzyme in the regulation of endothelial NO production and is regulated by the PI3K/AKT signaling pathway. AMPs can improve muscle atrophy through AKT/mammalian target of rapamycin (AKT/mTOR), autophagy signal transduction, and ubiquitin–proteasome; sodium-dependent neutral amino acid transporter (SNAT2) may be one of the latent targets (Lu et al., 2016). AMP-activated protein kinase (AMPK) activated by AMP is the most important substrate of liver kinase B1 (LKB1); it can sensitively perceive the levels of cellular energy and maintain homeostasis. LKB1/AMPK participates in the regulation of cell growth and cell cycle by regulating mTOR. The mTOR is an important kinase regulating cell growth in eukaryotes, which is also the mechanical target of rapamycin (Lu, Tan, Zhong, & Cheong, 2023). In similar studies, the mTOR inhibitor rapamycin markedly eliminated the protective effect of AMPs on adriamycin-induced cardiac injury; AMPs may play a protective role by regulating LKB1/AMPK to regulate mTOR (Hong, Wen, & Chen, 2016). In pathological conditions, AMPs can downregulate the activity of mTOR and protect cells.

4.2. Antitumor activity

China leads the world in cancer incidence and mortality. At present, radical surgery, traditional chemotherapeutics, radiotherapy, and targeted therapy are the main strategies for the treatment of cancer; however, because of the high chemoresistance of cancer and the severe side effects of current treatment approaches, the therapeutic strategies are still limited in the course of the treatment. Therefore, searching for a novel and efficient anticancer drug from natural materials is of the essence to prevent and treat cancer without or with fewer adverse reactions for cancer patients.

Studies have demonstrated that AMPs possess strong anticancer activities both *in vitro* and *in vivo*. Two novel polysaccharides, APS-I and APS-II, were extracted and purified from the dry roots of *A. membranaceus*. The tumor inhibition ratios of APS-I and APS-II were 55.4% and 47.72%, respectively. Meanwhile, APS-I and APS-II could protect the organs of mice implanted with hepatoma H22 cells, such as the liver, spleen, and thymus (Zhu et al., 2011). One of the anticancer mechanisms of AMPs was believed to be the stimulation of the cell-mediated immune response. In 1983, an anticancer polysaccharide named AMPs was isolated from *A. membranaceus*. AMPs showed a strong inhibitory effect on sarcoma S180. It appeared that AMPs might work by directly acting on tumor cells. AMPs arrested approximately 62% of human lung cancer NCI-H460 cells at the S phase and exhibited a growth inhibitory effect in a concentration-dependent manner, which was related to increased Bax/Bcl-2 ratio through regulating Caspases-3 (Yan et al., 2017). This work did not give the exact anticancer mechanism of AMPs, but it laid a solid foundation for further studies and gave the perspective of developing polysaccharides as anticancer agents. APS4 could arrest human gastric carcinoma MGC-803 cells in the S phase of the cell cycle and significantly suppress the proliferation of MGC-803 cells in a concentration and time-dependent manner. Besides, APS4 treatment could induce the mitochondria-dependent apoptosis, which was closely related to the accumulation of intracellular reactive oxygen species (ROS), the collapse of mitochondrial membrane potential, the increase of the pro-apoptotic/anti-apoptotic (Bax/Bcl-2) ratios, the release of cytochrome *C*, further activating the expression of Caspase-9/-3 and the cleavage of poly-ADP-ribose polymerase (PARP) in MGC-803 cells (Yu, Ji,

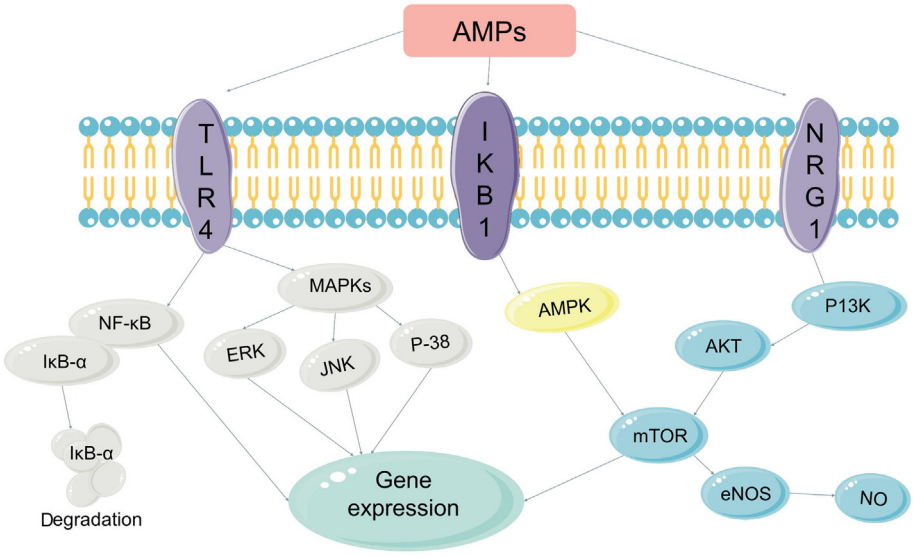

Fig. 3. Effects of AMPs on intracellular signal transducers and immune signaling pathways plays a role in immune regulation.

Dong, Feng, & Liu, 2019). Additionally, the anticancer properties of AMPs have also been investigated *in vivo*. The tumor-bearing male rats were administered with AMPs for two weeks. AMPs improved the activity of intestinal intraepithelial $\gamma\delta$T cells *in vivo*, as cytokines production and cytotoxicity of $\gamma\delta$T cells were all remarkably improved in tumor-bearing mice treated with AMPs. In addition, the levels of TNF-α and IFN-γ were significantly increased, whereas the levels of IL-10 and TGF-β were significantly decreased in tumor-bearing mice treated with AMPs. These results revealed that AMPs possessed strong anticancer potency (Sun et al., 2014).

Besides, AMPs were activating lymphocytes to enhance and restore the body's immunity to play the role of anti-tumor activity. A polysaccharides-rich fraction (AP) from *A. membranaceus* was reported to significantly increase the proliferation of spleen lymphocytes and blood interleukin-2 (IL-2) levels and NK activities (Liu et al., 2017). In addition, the AP treatment dose-dependently significantly increased the blood immune globulin A (LgA), immune globulin G (LgG), and immune globulin M (LgM) levels and CD^{4+} and CD^{4+}/CD^{8+} of rats with gastric cancer. In addition, AMPs have an activation effect on macrophages. AMPs stimulates macrophages to express the *iNOS* gene and induce NO production through the activation of NF-nB/Rel both *in vitro* and *in vivo* (Lee & Jeon, 2005).

In conclusion, AMPs are widely used in anti-tumor therapy and have positive significance for treatment (Fig. 4). However, they are still in the position of the adjuvant drug at present, their anti-tumor mechanisms are not completely clear, and further research is needed.

4.3. Antidiabetic activity

The exploration of novel safe and effective agents with antidiabetic activities has attracted more and more attention. Many studies have been carried out to investigate the antidiabetic effects of AMPs.

Type 1 diabetes mellitus (T1DM) is an organ-specific autoimmune disease mediated by T cells with a certain genetic basis and triggered by a variety of environmental factors. Administration of AMPs can prevent the occurrence of T1DM, improve histological findings of pancreatic islets, and reduce the CD^{4+}/CD^{8+} ratio of T lymphocytes from the spleen and infiltrated islets in non-obese diabetic (NOD) mice. Meanwhile, it decreased the expression of IL-1β, IL-2, IL-6, IL-12, TNF-α, and IFN-γ in the pancreatic tissue of NOD mice (Chen et al., 2015).

Type 2 diabetes mellitus (T2DM) is a type of diabetes mellitus that is characterized by significant insulin resistance and relatively insufficient insulin, insufficient insulin secretion. AMPs treatment can meliorate hyperglycemia and insulin resistance by partially restoring the insulin-induced protein kinase B Ser-473 phosphorylation and glucose transporter 4 translocation in skeletal muscle (Liu, Wu, Mao, Wu, & Ouyang, 2010). AMPs stimulates glucose uptake in L6 myotubes through the AMP-AMPK-AS160 pathway, which may contribute to its hypoglycemic effect (Chang et al., 2018). AMPs are important active components responsible for memory improvement in rats with streptozotocin-induced diabetes. The potential mechanism of action is associated with the effects of AMPs on glucose and lipid metabolism, and antioxidative and insulin resistance. AMPs are potential candidate therapeutic agents for the treatment of memory deficit in diabetes (Gu, Dun, Liu, Qiu, & Zhao, 2016). AMPs enables insulin-sensitizing and hypoglycemic activity at least in part by decreasing the elevated expression and activity of protein tyrosine phosphatase 1B (PTP1B) in the skeletal muscles of T2DM rats (Wu et al., 2005). A study on a polysaccharide of AMP found that its effect of delaying glucose diffusion in diabetic mice *in vitro* was significantly higher than that of the components, and it could show hypoglycemic effects and could regulate the intestinal environment (Liu et al., 2019).

4.4. Effects on cardiovascular system

Chronic myocardial ischemia is one of the common reasons for myocardial damage. During myocardial ischemia, the generation of oxygen radical malondialdehyde (MDA) increases and the expression of SOD decreases, leading to the accumulation of oxygen radicals in the body. Oxygen radicals and their further transformation products, such as hydroxyl radicals, damage myocardial cells, and tissues, resulted in organic damage to the heart. After intervention with AMPs, the expression level of MDA in serum and myocardial tissue decreased, and the expression level of SOD increased, indicating that AMPs can regulate the balance of the antioxidant system and oxidation systems (Wang, 2010). AMPs have an obvious protective effect on experimental myocardial ischemia rats

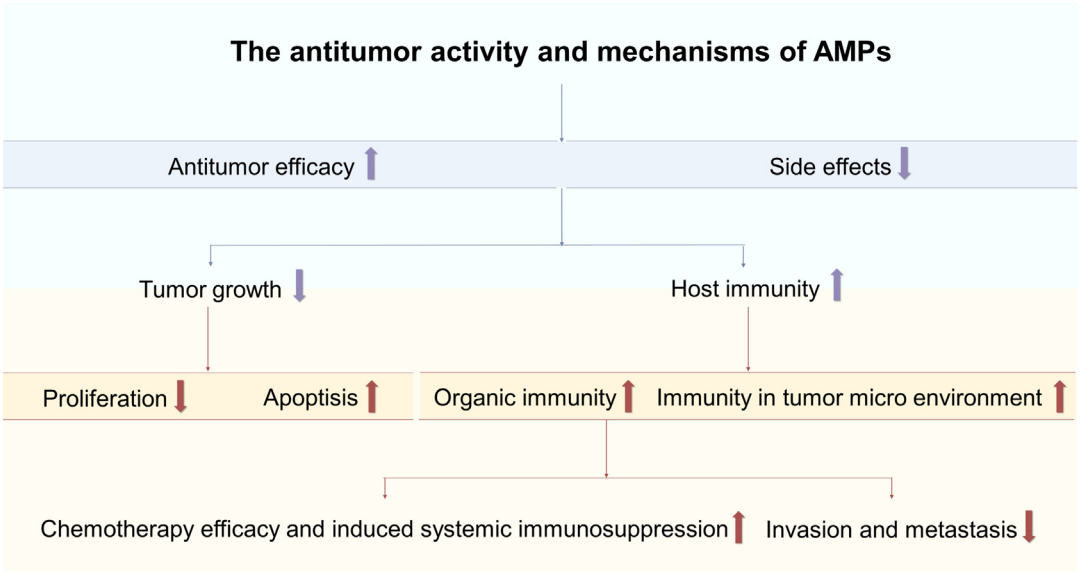

Fig. 4. Antitumor effect mechanism of AMPs.

induced by isoprenaline (Iso), and they can inhibit myocardial cell apoptosis. AMPs play a role by reducing the abnormal elevation of ST segment in myocardial ischemia rats, reducing the activity of plasma LDH and CK, significantly enhancing the activity of myocardial SOD and CAT, and reducing the content of myocardial MDA. The mechanism may be related to scavenging free radicals and inhibiting lipid peroxidation (Zhou, 2012).

In addition, AMPs have a protective effect on acute myocardial ischemia. APS-G has an obvious protective effect on acute myocardial ischemia rats induced by pituitrin. It can resist the arrhythmia induced by $BaCl_2$ in rats and the ventricular fibrillation induced by $CHCl_3$ in mice. Besides, it can improve the platelet adhesion rate and slow the heart rate without affecting the thrombus weight. Hemodynamic studies have shown that it can improve microcirculation (Wu, 2018). Other, AMPs improve cardiac function through the Keap1/Nrf2-ARE signal pathway in an adjuvant arthritis (AA) model (Sun et al., 2016).

AMPs can reduce the ischemia–reperfusion injury caused by radicals and protect the myocardial diastolic function. AMPs preadministrated could inhibit myocardial cell volume increase, while cells of TNF-α were significantly reduced, and ANP mRNA expression decreased in varying degrees (Zhou, Wang, Zhao, Yu, & Zhang, 2012). In addition, AMPs also could inhibit the cardiomyocyte hypertrophy induced by Iso, and decrease the inflammatory response and the expression of TLR4 mRNA. With that manifestation, the cell volume, total protein content, expression of ANP mRNA, TNF-α and IL-6 in extracellular fluid, P65 and TLR4 protein decreased, and IκBα protein increased in a dose-dependent manner (Zhang et al., 2014).

5. Structure-activity relationships (SAR) of AMPs

5.1. Relationship between structure and activity

It was generally regarded that polysaccharides with different bioactivities should have different structure configurations, chain conformations, and physical properties. The bioactivity of polysaccharides strongly depends on their structures. For instance, the triple-helical conformation of (1,3)-*β*-*D*-glucan is essential for explaining its immunomodulatory activity (Chen, Li, Yu, & Shi, 2007).

The effect of monosaccharide composition on polysaccharide activity is much less than that of glycoside linkage and monosaccharide linkage (Nie & Ning, 2003). The branching degree is closely related to the biological activity of polysaccharides. It is too big or too small and cannot make the polysaccharide biological activity reach the ideal state (Nie & Ning, 2003). The activities of the arabinogalactans and pectic arabinogalactans were associated with *β*-*D*-(1 → 3)-galactan moieties branched with *β*-*D*-(11tiegalactooligosaccharide side-chains having degrees of polymerization of 8 or less. Degradation of the *β*-*D*-(1 → 3)-galactan or *β*-*D*-(1 → 6)-galactosyl side-chains in the arabinogalactans significantly decreased immunomodulating activity (Kiyohara et al., 2010). The glucans extracted from *A. membranaceus* are mainly determined as α-(1 → 4)-*D*-glucans. AMPs has a repeating (1 → 4)-linked backbone with a (1 → 6)-linked branch every 10 residues and it has antioxidant activity (Niu et al., 2011). In the same, AP with immune modulating activity was an α-(1 → 4)-*D*-glucan with α-(1 → 6)-linked branches attached to the O-6 (Li et al., 2009).

Chain conformations of polysaccharides also influence their activities. Scanning electronic microscopy (SEM) analysis showed that AMPs has a non-smooth surface and irregular sheet structure (Liu et al., 2020). Fragments of different sizes were loosely grouped with flaky branches at the edges and on the thin sheet.

At present, there are few studies on the specific molecular structure and spatial configuration of AMPs, which makes it difficult to explore the mechanism of the bioactive molecule based on the structure–activity relationship of AMPs. In addition, it is hard to reveal the precise spatial structures of polysaccharides, one of the most important and structurally diverse biomacromolecules, using current methods. Therefore, it is necessary to deeply research AMPs at the level of chemical structure.

5.2. Relationship between structure modification and activity

SAR was evaluated by investigating the bioactivity of chemically modified AMPs with different molecular weights. The SAR of AMPs could be inferred as follows.

The structure of polysaccharides is closely related to biological activity, and the change of the structure can directly affect its pharmacological activity. The existence of substituent groups and the species of substituent groups are closely related to the biological activity of AMPs. The modification of polysaccharide structure refers to the derivation of polysaccharide structure by chemical methods, so that the activity or toxicity of polysaccharides can be optimized to reduce toxicity and improve biological activity. The substitutants can be added or eliminated by chemical methods, such as sulfation, carboxymethylation, phosphorylation, and sulfonylation.

Sulfation of AMPs is a commonly used chemical modification method. There are three common methods of sulfated polysaccharides, that is, sulfuric acid, sulfur trioxide-pyridine, and chlorosulfonic acid-pyridine (CSA-Pyr) methods (Takano et al., 2000). The CSA-Pyr method is the most popular, owing to its convenience, high yield, and high degree of sulfation. The activity of polysaccharide sulfate is closely related to the degree of sulfate substitution: the higher the degree of substitution, the stronger the effect (Alban, Schauerte, & Franz, 2002), The content of sulfate is best when the average of each sugar residue is 1.5 – 2.0 (Wang, 2000). This also was proved by the sulfation modification of AMPs. APS_t, APS_{40}, APS_{50}, and APS_{60} are all modified by CSA-Pyr method. Among the four modified polysaccharides, $sAPS_{60}$ showed the highest degree of sulfuric acid substitution (1.545) among modified polysaccharides, which ranged from 1.5 to 2.0, with the strongest infectious bursal disease virus (IBDV) resistance activity. APS_t is an unpurified polysaccharide with complex components. The modification of APS_t not only fails to achieve the modification effect of pure polysaccharide but may destroy its original structure. $sAPS_t$ has the weakest antiviral effect among modified polysaccharides. Therefore, the antiviral activity of homogenized polysaccharide sulfate was greater than that of heteropolysaccharide sulfate (Huang, Hu, Lu, Zhang, & Guo, 2008). The sulfated APS obtained by the CSA-Pyr method had better anti-inflammatory activity than unmodified APS, *in vitro* and *in vivo* (Wang et al., 2013). Compared with non-sulfated AMPs, sulfated AMPs could significantly increase the antibody titer and promote lymphocyte proliferation with certain dose and time-effect relationships (Huang et al., 2008). Besides, sulfated modification of AMPs can significantly increase the serum antibody titer and promote the proliferation of T lymphocytes, which is stronger than the unmodified polysaccharides (Shi et al., 2009). Therefore, sulfated AMPs can be a candidate for a new-type immunopotentiator (Huang et al., 2008) and a new immune adjuvant (Shi et al., 2009).

Phosphorylation is a covalent modification of hydroxyl groups in side chains of polysaccharides with phosphate groups. Studies have shown that the phosphorylation of polysaccharides can enhance their bioactivities. Phosphorylation of AMPs using the sodium tripolyphosphate-sodium trimetaphosphate method enhanced its antiviral effect against duck viral hepatitis. Reaction with polyphosphoric acid under alkaline conditions resulted in a

good antiviral activity of AMPs against porcine reproductive and respiratory syndrome virus.

Carboxymethyl groups are introduced into polysaccharide chains for complete carboxymethylation of polysaccharides. Carboxymethylation increases the negative charge of polysaccharide chains and their solubility in water. In addition, carboxymethylation has a strong effect on the bioactivity of polysaccharides. The carboxymethyl-modified AMPs was prepared in the reaction with NaOH and $C_2H_3ClO_2$. The optimum reaction conditions were as follows: the reaction temperature, 65 °C; $NaOH/C_2H_3ClO_2$ ratio, 16:1; and reaction time, 3.5 h. The results showed that the carboxymethyl-modified AMPs had the highest growth promotion for immunological activities.

Natural Se-containing polysaccharides have been found in several animals, plants, and microorganisms. As organic Se compounds, polysaccharides modified with Se can exhibit the physiological activities of both Se and polysaccharides. Moreover, the bioavailability of Se and its physiological functions as an essential trace element is effectively improved, while its toxicity and side effects are considerably lower than those of inorganic Se. AMPs with a $SeOCl_2$ reagent and obtained a Se-containing AMPs, with a Se content of 16.820 mg/g (Gong & Zheng, 1998). It has been reported that the inhibitory rate of tumor growth was 51.14% in the Se-AMPs group compared with 23.66% in the water control group, suggesting that combining AMPs with Se might enhance not only the tumor inhibitory effects of AMPs but also the antioxidant effect of Se (Zhang, 1997). It has been reported that a high Se content in Se-modified AMPs increases the antioxidant effect of AMPs.

In summary, most of the SAR studies for AMPs focused on changing their structures by chemical or physical modification. Modification of AMPs with sulfate, phosphate, carboxymethyl, methyl, hydroxyethyl groups, and ultrasound could apparently enhance water-solubility, change the chain conformations and molecular weights, and remarkably enhance the bioactivity of porous coordination polymers (PCPs). Thus, moderate molecular weight, relatively expanded chain stiffness, and good water solubility are critical for the bioactivity of AMPs.

6. Applications

6.1. Medical treatment

AMPs have good immune regulation, anti-tumor, anti-inflammation, and other pharmacological effects. At present, AMPs have made great progress in the treatment of diseases. It has been clinically used to treat cancer, asthma, and diabetes. Treatments for other diseases are still in animal trials (Fig. 5).

6.1.1. Anti-tumor

High mortality rates make cancer a major threat to human health worldwide. Currently, the main cancer treatment is chemotherapy, but chemotherapy is often accompanied by some toxic side effects, and drug resistance may develop. AMPs can not only enhance immunity, inhibit tumor growth, and promote apoptosis, but also reduce the toxic and side effects of drugs. AMPs have developed rapidly in the treatment of cancer in recent years. Studies have shown that AMPs has an inhibitory effect on gastric cancer MGC803 cells, human non-small cell lung cancer A549 cells, and human liver cancer HepG2 cells, and can induce the apoptosis of gastric cancer MGC803 cell (Jin et al., 2013). Using the combination of injection AMPs and radiotherapy for the treatment of gastric cancer, and the treatment rate and tumor volume reduction rate of the combined treatment group were 63.9% and 59%, respectively (Li et al., 2017). At the same time, the immunological,

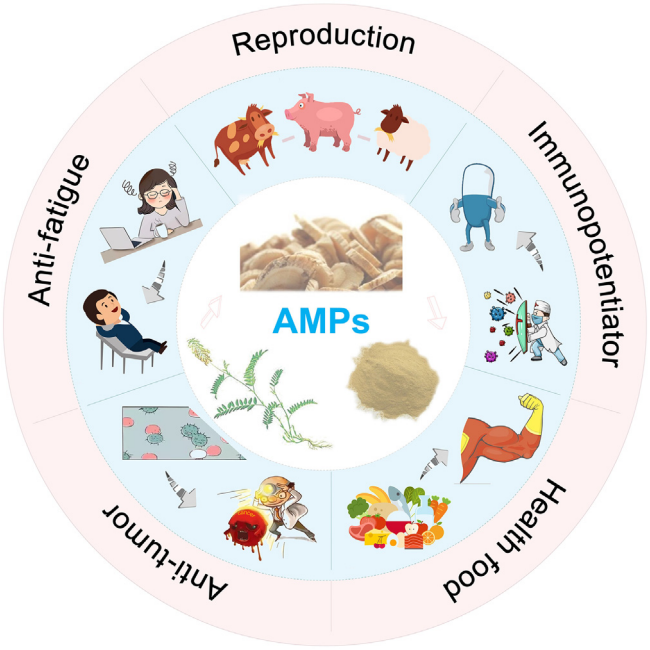

Fig. 5. Applications of AMPs.

hematopoietic, and hepatorenal functional levels of patients in the combined treatment group were significantly higher than those in the control group. Researchers combined AMPs and cytokine-induced killer cells in the treatment of advanced-stage non-small cell lung cancer with *qi*-deficiency (Huang et al., 2008). The disease control rate and Cassidy score were respectively 69.4% and 77.8% in the combined treatment group. The control group was 36.1% and 55.6%. In addition, AMPs can also be combined with gemcitabine for the treatment of pancreatic cancer, and adriamycin lipids for the treatment of liver cancer.

6.1.2. Anti-fatigue

PG2 lyophilized injection, a botanically derived drug, contains a mixture of AMPs extracted, isolated, and purified from the roots of *A. membranaceus*. With high and consistent quality, the preparation of the raw materials, intermediates, and final products complied with GMP requirements (Chao, Wu, Lin, Yang, & Chao, 2017). The molecular weights of PG2 ranged between 2×10^4 and 6×10^4, and the dominant polysaccharides were α-1, 4-linked glucans with varying degrees of branching at the six positions of the backbone residues. Other polysaccharides and glycoproteins in PG2 were arabinogalactans, rhamnogalacturonans, and arabinogalactan proteins (Kuo, Chen, Chuang, Hua, & Lin, 2015). It has been confirmed to be safe and effective and has been approved by the Taiwan Food and Drug Administration (TFDA) as an example of the country's first successfully developed new drug. The product was introduced into the market as a prescription drug. It is indicated for alleviating moderate to severe cancer-related fatigue. The pharmacological mechanism of PG2 Lyophilized Injection involves the enhancement of immune function and stimulation of bone marrow hematopoiesis (Chao et al., 2017).

6.2. Livestock and poultry breeding

6.2.1. Reproduction

Artificial insemination is widely used in modern pig farms. The life of liquid nitrogen-stored semen is short, and the life of cryopreservation can be extended. In the process of freezing and thawing

semen, how to build a powerful antioxidant system for sperm has become an urgent problem. AMPs have good antioxidant function, so AMPs may be a good choice to solve this problem. AMPs can improve the antioxidant capacity of sperm by reducing the content of reactive oxygen species in thawed pig semen, thus improving the efficiency of *in vitro* fertilization and embryo development (Wu, 2018). Furthermore, AMPs could protect boar sperm from oxidative stress and energy deficiency by inhibiting the protein dephosphorylation caused by ROS via the cAMP-PKA signaling pathway (Weng et al., 2018).

6.2.2. Immunopotentiator

AMPs can improve serum environment, stimulate body's immune response, promote secretion of cytokines, and enhance level of antibodies in body to enhance the effect of vaccines. The inactivated *Edwardsiella ictaluri* vaccine was prepared with AMPs as an adjuvant. Then, checking by real-time quantitative RT-PCR assays, in both spleen and head kidney tissues which were the major immune organs, mRNA expressions of inflammatory cytokine IL-1β increased in the early stage of immunity, typical Th1 immune response cytokines IL-2 and IFN-γ2 rose in the whole immune period, and IgM significantly enhanced in the adjuvant supplementation groups. It demonstrated the good efficiency of AMPs as an adjuvant and provided more options for the fish adjuvants (Zhu et al., 2019).

AMPs, as a natural feed additive can not only improve the immunity of the body but also improve the production performance of the body. When the number of AMPs in the feed was 50–400 mg/kg, it could significantly enhance the non-specific immunity and antioxidant capacity of loach. This may be because AMPs increase the level of NO in white blood cells, red blood cells, and serum (Chen et al., 2016). In addition, 1 g/kg of AMPs fed to broilers promoted the growth of young broilers. Compared with the control group, the activities of amylase, lipase and protease in broiler chickens were higher, but the activity of digestive enzymes was decreased when the content of AMPs was too high (Fu et al., 2017).

6.3. Food

The *Manual of Health Food Raw Materials* pointed out that AMPs can be used as health food raw materials. Using AMPs, *Ginkgo biloba* extract, and selenium-rich black tomato as the main functional components, a kind of noodles was developed that can prevent and treat diabetic complications. An energy drink with a comfortable taste and good color was developed, which can enhance immunity and resist fatigue, using AMPs as the raw material (Yeong & Jin, 2005).

7. Conclusion

After several decades of extensive research, great progress has been made in the study of AMPs. The extraction methods and extraction rate of AMPs have been continuously improved. It has been found that water, microwave-assisted, ultrasonic wave, enzymatic hydrolysis, and other extraction methods can be combined to improve the extraction rate of AMPs. Depending on the extraction method and the degree of purification, the chemical composition and structure of AMPs can be confirmed by HPLC, GC, MS, and NMR. The chemical composition of AMPs mainly includes glucose, rhamnose, galactose, arabinose, xylose, mannose, glucuronic acid, and galacturonic acid. However, the monosaccharide composition and the structure of sugar chains of AMPs, obtained by different extraction and purification methods, are different. Therefore, the extraction and purification methods of AMPs need to be improved continuously.

As an important bioactive component of *Astragali Radix*, AMPs show important pharmacological activities, including immunoregulatory, antitumor, anti-inflammatory, antiviral, and other activities. Currently, studies on the modification of AMPs mainly employ chemical methods, including sulfation, phosphorylation, selenation, and carboxymethylation, and suggest that structural modification can change the pharmacological activity of AMPs. However, studies on the pharmacological activities of AMPs usually use crude polysaccharides, which does not allow the establishment of the structure–activity relationship. In addition, the molecular mechanisms of the pharmacological activities of AMPs are still unclear, which also limits their further development and application. Therefore, the separation and purification of AMPs should be improved. Subsequently, the structure–activity relationship of AMPs should be elucidated at the primary and secondary structure levels. Finally, the pharmacological activities and molecular mechanisms of AMPs should be studied with homogeneously purified AMPs. Combining the extraction, isolation, purification, and identification methods of AMPs, and then analyzing the structure–activity relationship from different structural levels, provides effective theoretical support for developing and utilizing AMPs. Specifically, as different extraction methods will lead to discrepancies in structure and activity, it is worthwhile to explore and excavate the optimal preparation structure of polysaccharides, and the relationship between molecular weight and activity is also worth thinking about, as to how to modify the structure of polysaccharides with large molecular weights has also become particularly important.

CRediT authorship contribution statement

Hongyue Tian: Data curation, Formal analysis, Visualization, Writing – original draft. **Lingzhuo An:** Writing – review & editing. **Pengwang Wang:** Conceptualization, Data curation, Project administration, Validation, Writing – original draft, Writing – review & editing. **Xuemin Zhang:** Supervision, Writing – review & editing. **Wenyuan Gao:** Supervision, Writing – review & editing. **Xia Li:** Supervision, Writing – review & editing.

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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