



Prospects of sea buckthorn (*Hippophae rhamnoides* L.) polysaccharides: preparation, structural characterization, and bioactivities diversity

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ABSTRACT

Sea buckthorn (*Hippophae rhamnoides* L.) is a natural homologous substance of medicine and food. Polysaccharide, as one of its primary active components, has very superior biological activity and can be used as a dietary supplement for functional foods, with good commercial prospects. Although initial progress has been made in the study of sea buckthorn polysaccharides, related studies have been fragmented and lacked systematic and generalization. This manuscript presents a critical analysis and systematic summary of the extraction and purification methods, structural characterization and physicochemical properties, biological activity and potential mechanisms, and structure-activity relationships of sea buckthorn polysaccharides. Accumulating evidence has indicated that sea buckthorn polysaccharides, which were widely prepared by water extraction and column chromatography purifications, exhibited exhibit superior biological activities *in vitro* and *in vivo*, including antioxidant, immunomodulatory, anti-inflammatory, hepatorenal protective, antibacterial, antiviral, and prebiotic activities. After analysis, it was concluded that there is a correlation between the relevant activities of sea buckthorn polysaccharides and that the structure of sea buckthorn polysaccharides has a great influence on their biological activity. We reviewed the challenges and limitations of sea buckthorn polysaccharides, summarized the critical aspects, and provided suggestions for potential breakthroughs in the research and application of sea buckthorn polysaccharide.

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1. Introduction

Sea buckthorn (*Hippophae rhamnoides* L.) is a deciduous shrub or small tree species in the family Elaeagnaceae^[1]. It is the collective name of the plant and its fruit. Sea buckthorn has numerous thorns, old branches are rough and grey-black, and young branches are brown-green. The leaves are narrowly lanceolate or oblong-lanceolate, 30–80 mm long and 4–10 mm wide. The fruit is round,

4–6 mm in diameter, and orange-yellow or orange red (Fig. 1)^[2–3]. Sea buckthorn grows mainly in temperate biomes and has a natural global distribution in Europe to southern Siberia and southern central China and has been artificially introduced to certain areas of cities in Russia, Canada and Albania^[4–5].

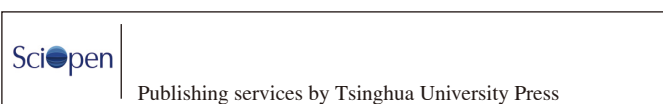


Fig. 1 Plant morphology of sea buckthorn.

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Sea buckthorn is a unique plant with significant overall value. It is one of the few plants that contains all 4 omega fatty acids, and is abundant in essential vitamins, as well as polysaccharides, flavonoids, carotenoids, and essential minerals^[6-8]. From a medicinal standpoint, sea buckthorn has been a prominent part of traditional medicine for centuries due to its anti-inflammatory, antibacterial, antioxidant, and immunomodulatory properties. Studies have shown that it may aid in lowering cholesterol levels, improving skin health, and promoting wound healing, among other things^[9-10]. Sea buckthorn is classified as an adaptogen, indicating its potential ability to assist the body in adapting to stress and enhancing its resistance to stressors^[11]. From an environmental standpoint, sea buckthorn is extremely resilient and able to thrive in harsh conditions such as high altitudes, arid and nutrient-poor soils, and extreme temperatures^[12]. This is due to its deep roots, which absorb water and nutrients from the soil, as well as its ability to fix nitrogen, which enhances soil fertility^[13]. It is extensively cultivated in Asia and Europe for ecological restoration purposes, such as soil and water conservation and desert greening. From an application point of view, sea buckthorn has a diverse range of uses. It can be employed as a natural fertilizer, animal feed, and biofuel. Moreover, its versatility and medicinal properties allow it to be processed into various foods, cosmetics, and more^[14-17]. As research into its health benefits continues, the popularity and value of sea buckthorn are expected to grow and become a valuable resource for multiple industries.

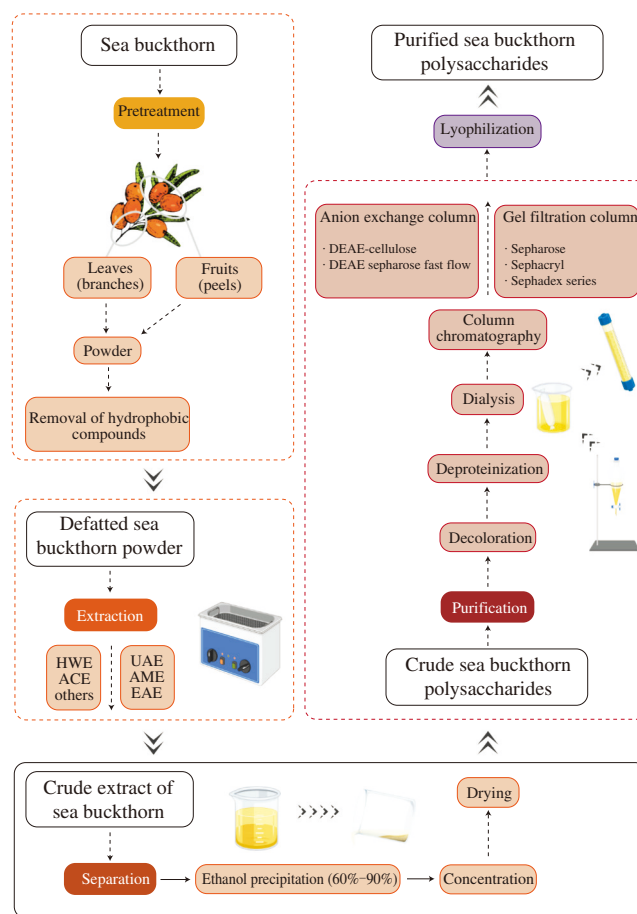
As sea buckthorn is widely promoted, its active substance, polysaccharide, has only begun to attract attention in recent years^[18]. One of the most unique components of sea buckthorn is its polysaccharides, which are complex carbohydrates made up of long chains of simple sugar molecules^[19]. Compared with other polysaccharides sea buckthorn has great advantages and prospects. Research shows that sea buckthorn is full of treasures, and the polysaccharides of both leaves and fruits are medicinal polysaccharides, which have inherent advantages both in clinical medicine and as dietary supplements. As the raw material is easy to grow and cultivate, its sea buckthorn polysaccharide is also quite easy to obtain. Not only that, sea buckthorn polysaccharides are structurally diverse, which provides more possibilities for the subsequent structural modification of sea buckthorn polysaccharides. The superior physical properties of sea buckthorn polysaccharides also lay the foundation for its application in food processing. Most notably, sea buckthorn polysaccharides have a very wide range of pharmacological activities, including antioxidant, immune, anti-inflammatory, antibacterial, antiviral, antitumor and prebiotic activities^[6]. It can not only play a preventive role, but also achieve the effect of one medicine for multiple diseases. Sea buckthorn polysaccharides have many potential benefits, such as being a natural, sustainable source that is biocompatible and has positive effects on health^[20]. These advantages make it an attractive area of research for the development of new natural products with potential applications in medicine, nutrition, and food processing.

However, there is no dedicated review summarizing the progress of research on sea buckthorn polysaccharides. This review provides a comprehensive summary of the results and progress related to sea buckthorn polysaccharides over the past decades, covering extraction and purification methods, structural features, bioactivities and

mechanisms, and structure-activity relationship of polysaccharides. It also provides a comprehensive insight and outlook in relation to the existing knowledge and problems, aiming to attract more scholars to invest in the basic research of sea buckthorn and provide useful reference values for advancing the development and application of sea buckthorn extracts in the domains of functional foods and pharmaceuticals.

2. Preparation procedures

The general preparation process of sea buckthorn polysaccharides, and the whole extraction process can be divided into 4 parts: pretreatment, extraction, separation and purification (Fig. 2)^[21-22].



- (1) Pretreatment: Before extraction, sea buckthorn is usually dried and soaked in ethanol or petroleum ether to remove hydrophobic compounds.
- (2) Extraction: The pretreated sea buckthorn powder or marc must be dried before extraction. The methods of extracting sea buckthorn polysaccharides vary, but they are typically aqueous solution extractions combined with various methods of auxiliary extraction.
- (3) Separation: Sea buckthorn polysaccharides are typically obtained through graded precipitation of ethanol, which separates polysaccharides of different molecular weights (M_w). The concentration range for alcohol precipitation is usually between 60% and 90%. As the specific ethanol concentration increases, the solubility of larger M_w polysaccharides decreases, allowing smaller M_w polysaccharides to precipitate out.
- (4) Purification: First, proteins, pigments, and small molecule impurities contained in crude sea buckthorn polysaccharides are removed. Then, the sea buckthorn polysaccharides are further separated and purified by anion exchange columns. Finally, they are freeze-dried and collected.

Fig. 2 Preparation process diagram of sea buckthorn polysaccharides.

There are many methods for extracting, purifying, and separating polysaccharides from sea buckthorn. Table 1 shows the different source regions, parts, extraction methods, extraction conditions, purification means, and crude polysaccharide yields of sea buckthorn.

2.1 Extraction methods

There are various extraction methods for sea buckthorn polysaccharides, among which hot water extraction (HWE) is the simplest, lowest cost, and most widely used method^[23-24]. However, its extraction process is time-consuming and low in yield^[25]. In contrast, physical-assisted extraction methods, such as pressurized hot water extraction (PHWE), ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE), can extract polysaccharides more efficiently and increase the yield of sea buckthorn crude polysaccharides while ensuring environmental protection^[26-31]. Nevertheless, care should be taken to control a reasonable temperature range, as high temperature may lead to degradation of polysaccharides, leading to a lower extraction yield. According to research, the method of polysaccharide extraction has a direct impact on its antioxidant activity, with the highest activity observed at a microwave power of 600 W^[32]. Besides, it has been found that the extraction of sea buckthorn polysaccharide using flash extraction (FE) can effectively protect the active components of sea buckthorn polysaccharide from thermal damage, and has the advantages of small solvent dosage, short extraction time and high efficiency^[33]. In addition to physical-assisted extraction methods, some scholars try to use acidic-water extraction (ACE) or alkaline water extraction (ALE) to increase the yield of polysaccharides. These two methods are cost-effective but have certain limitations^[31,34-35]. When using these two methods, the extraction conditions must be carefully optimized, and the pH adjustment range must be controlled to prevent potential degradation of polysaccharides. Additionally, enzyme-assisted extraction (EAE) is a gentle, safe, and environmentally friendly method used for extracting sea buckthorn polysaccharides. Papain is a commonly used enzyme in EAE for this purpose^[36]. In a study aimed at comparing the yields of sea buckthorn polysaccharides using different extraction methods, Xu et al.^[37] found that the joint extraction of sea buckthorn polysaccharides by UAE-EAE had the highest yield. Similarly, Wang et al.^[38] used various extraction methods to extract sea buckthorn polysaccharides and discovered that the extraction rate of polysaccharides was significantly higher than that of other single extraction methods. Consequently, it can be inferred that collaborative extraction and joint action methods for extracting sea buckthorn polysaccharides will be the general trend in future extraction processes.

The extraction method and specific conditions closely related to it are crucial factors affecting the yield of sea buckthorn polysaccharides. Therefore, it is vital to investigate the optimal extraction conditions. It is commonly analyzed using single-factor, orthogonal, optimization design (Box-Behnken design and response surface), and so on^[39-40]. Wei et al.^[32] obtained the optimal extraction conditions for MAE by combining single factor test and optimization design. Zhu et al.^[33] obtained the optimal extraction process for FE of sea buckthorn polysaccharides using single factor and response surface optimization design and found that the influence was: liquid to material ratio > extraction temperature > extraction time > rotation rate. Wang et al.^[38] employed both single factor and orthogonal tests to determine the optimal parameters of UAE-MAE synergetic extraction for the extraction of polysaccharide from sea buckthorn.

The efficacy of polysaccharide extraction is influenced not only by the extraction technique and parameters but also by the cultivation environment of the source material. Despite the absence of yield comparisons of sea buckthorn polysaccharides across different growing regions, previous research has demonstrated that the accumulation of sea buckthorn polysaccharides, derived from both the fruit and leaf, is influenced by harvesting time and meteorological conditions. Specifically, it has been found that the optimal harvesting time for sea buckthorn is between October and November, while precipitation is negatively associated with polysaccharide content^[41].

To achieve industrial production of sea buckthorn polysaccharides, a multifaceted research base is necessary. Sea buckthorn extraction requires a focus on appropriate growing regions, harvesting environments, efficient extraction techniques, optimized extraction conditions, and energy efficiency and cleanliness. However, current research on the extraction of sea buckthorn polysaccharides from regions other than China is insufficient. Additionally, relatively new extraction techniques such as supercritical fluid and subcritical water extraction have yet to be widely applied^[42]. There has been much evidence that the extraction method of polysaccharides affects their bioactivity. Therefore, further studies are necessary to investigate the effects of various extraction methods as well as conditions on bioactivities of sea buckthorn polysaccharides.

2.2 Purification methods

Crude sea buckthorn polysaccharides are obtained by alcoholic precipitation following extraction. To obtain pure sea buckthorn polysaccharides for subsequent use, a series of measures are required to remove impurities. Contaminants in crude sea buckthorn polysaccharides typically include pigments, proteins, and small molecule impurities. While different crude sea buckthorn polysaccharides may require targeted measures, the general impurity removal process involves decolorization, deproteinization, and dialysis^[43-44].

In general, organic reagents or activated carbon and macroporous resins are used to decolorize polysaccharides. According to method by Zhu et al.^[34], a solution of crude sea buckthorn polysaccharide was decolorized by adding sodium metabisulfite at a volume concentration of 0.000 4%. While Ma et al.^[24] used 0.5% (*m/m*) activated carbon. Among these methods, the use of macroporous resin for pigment adsorption is the most efficient and conducive to protecting the polysaccharide structure. However, this method has not been applied to decolorize sea buckthorn polysaccharides^[45]. Since the pigment impurities in sea buckthorn polysaccharides are minimal and subsequent purification steps can remove small pigment molecules, many researchers skip the decolorization step.

Deproteinization of sea buckthorn crude polysaccharides is typically achieved using the Sevag method, which is a well-established and commonly used method for eliminating protein impurities from polysaccharides^[46]. Despite its effectiveness, the Sevag method has some limitations, including high residual levels of toxic reagents and a lengthy process to attain near-complete protein removal, which necessitates multiple repetitions. In the future, alternative techniques

Table 1

Extraction methods, conditions, and total yield of sea buckthorn polysaccharides.

Region	Part	Extraction methods	Condition				Purification methods	Yield (%)	References
			Liquid-to-material ratio (mL/g)	Temperature (°C)	Time (min)	Power/Enzyme			
Heilongjiang	Fruits	HWE	20	70	120	N/A	N/A	5.01	[37]
Tibet	Fruits	HWE	20	90	360	N/A	DEAE-cellulose anion-exchange column, dialysis with M_w cut-off 3 500 Da and Sepharose CL-6B gel filtration column	5.50	[23]
Qinghai	Fruits	HWE	20	90	360	N/A	DEAE-cellulose anion-exchange column, Sephadex G-75 gel filtration column and DEAE-Sepharose Fast Flow ion-exchange column	4.78	[25]
Shanxi	Fruits	HWE	20	80	240	N/A	Dialysis with M_w cut-off 3 500 Da, Q Sepharose Fast Flow anion-exchange column and Sephacryl S-200 column	6.1	[63]
Shanxi	Fruits	HWE	25	90	180	N/A	Dialysis with M_w cut-off 1 000 Da, DEAE-cellulose 52 anion-exchange column, dialysis with M_w cut-off 3 500 Da and Sephadex G-75 gel filtration column	N/A	[24]
Shanxi	Fruits	HWE	20	90	240	N/A	dialysis with M_w cut-off 3 500 Da	4.74	[31]
Jilin	Fruits	HWE	60	90	240	N/A	Dialysis; DEAE-cellulose 52 anion-exchange column	12.06	[66]
Shanxi	Fruits	PHWE	20	115	60	N/A	Dialysis with M_w cut-off 3 500 Da	4.54	[31]
Heilongjiang	Fruits	UAE	20	25	55	480 W	N/A	48.63	[37]
Xinjiang	Fruits	UAE	20	70	60	N/A	DEAE-cellulose 52 anion-exchange column	N/A	[27]
Tibet	Fruits/Leaves	UAE	30	30	30	600 W	N/A	N/A	[27]
Shanxi	Fruits	UAE	20	8	60	500 W	Dialysis with M_w cut-off 3 500 Da	6.05	[31]
Qinghai	Leaves	UAE	8	70	60	N/A	N/A	6.42	[26]
Heilongjiang	Fruits	EAE	20	45	20	2% papain and pH 5.5	N/A	44.28	[37]
Jilin	Fruits	MAE	10	85	6	600 W	DEAE-cellulose anion-exchange column and dialysis	0.264	[32]
Azerbaijan	Fruits	ACE	5	65	20	Cavitation index: 0.6	N/A	N/A	[35]
Shanxi	Fruits	ACE	20	60	180	0.1 mol/L HCl	Dialysis with M_w cut-off 3 500 Da	3.75	[31]
Qinghai	Peels	ACE	10	80	60	pH 2	N/A	8	[34]
Shanxi	Fruits	ALE	20	60	180	0.1 mol/L NaOH	Dialysis with M_w cut-off 3 500 Da	6.59	[31]
Jilin	Fruits	FE	38	90	0.25	Rotation rate: 5 000 r/min	Dialysis; DEAE-32-cellulose column Sephadex G-150 column	15.28	[117]
Heilongjiang	Fruits	UAE-EAE	20	UAE: 25 EAE: 45	UAE: 55 EAE: 20	2% papain and 480 W	DEAE-cellulose anion-exchange column and Sepharose CL-4B gel filtration column	54.68	[37]
Xinjiang	Fruits	UAE-MAE	30	N/A	3	450 W	N/A	22.50	[38]
Inner Mongolia	Fruits	UAE-EAE	25	UA: 80 EA: 65	UAE: 40 EAE: 360	0.4% papain	Dialysis with M_w cut-off 8 000 Da, Sephadex G-150 column and Sephadex G-100 column	8.00	[36]

Note: N/A: not applicable. The same below.

such as enzymatic hydrolysis and freeze-thawing could be employed to eliminate protein impurities from sea buckthorn polysaccharides. These methods are characterized by high rates of removal, gentle effects, and a high retention of polysaccharides^[47].

Small molecule impurities, such as pigments and inorganic salts, found in crude sea buckthorn polysaccharides are typically removed via dialysis^[48]. Dialysis is a simple, efficient and clean purification method that uses semi-permeable membranes of different pore sizes to separate and remove small molecule impurities^[24,36].

After removing impurities, a mixture of polysaccharides with varying components was obtained. Further purification is necessary to obtain a single piece of pure sea buckthorn polysaccharide. Column chromatography is a common and effective purification method for the separation and purification of natural components^[49]. Among them, the column chromatographic methods used for sea buckthorn polysaccharides mainly include anion-exchange column chromatography and gel column chromatography. Anion-exchange column chromatography, as the primary stage of polysaccharide purification, is mainly suitable for the separation of acidic sugars, neutral sugars and mucopolysaccharides^[50]. The more commonly used anion exchange columns for the purification of sea buckthorn

polysaccharides are DEAE-cellulose column and DEAE-agarose column. Gel column chromatography is to further purify the polysaccharides according to the characteristics of molecular sieves specific to different gels^[51]. The commonly used gel chromatographic columns for purification of sea buckthorn polysaccharides are agarose gel column, dextran gel column and acryl dextran gel column. The specific column types are listed in Table 1.

Subsequently, the eluate obtained by elution is measured for its sugar content using the phenol-H₂SO₄ method with glucose as a standard^[52]. Finally, the desired eluate is lyophilized to yield pure sea buckthorn polysaccharide powder.

3. Structural characterization

Understanding the structure of sea buckthorn polysaccharides is crucial for their further development and utilization. This entails quantitative determination, primary structure characterization, and advanced structure characterization of these polysaccharides. The methods for characterizing and analyzing sea buckthorn polysaccharides are summarized in Table 2.

Table 2
Physiochemical, structural features and analytical techniques of sea buckthorn polysaccharides.

Region	Part	Name	M_w (Da)	Monosaccharide composition										Structural features	Physicochemical properties	Analytical techniques	References
				Ara	Fuc	Gal	Glc	Man	Rha	Rib	Xyl	GalA	GlcA				
Tibet	Fruits	HRWP	194 200	3.50	N/A	9.7	20.10	4.20	1.30	N/A	1.10	59.40	0.70	N/A	The endotoxin level 0.5 EU/mL	UV, limulus amoebocyte lysate (LAL) assays, 3-methyl-1-phenyl-1 <i>H</i> -pyrazol- 5(4 <i>H</i>)-one (PMP) labeling and HPLC	[23]
Inner Mongolia	Fruits	HRP	N/A	1.02	N/A	1.00	4.24	2.02	9.22	N/A	N/A	N/A	N/A	N/A	No acute toxicity (oral administration by 10–1 000 mg/kg)	UV, FT-IR, PMP labeling and HPLC	[36]
Qinghai	Fruits	HRWP-N	1 000–50 000	5.00	N/A	15.03	38.73	6.24	0.77	N/A	0.81	3.42	N/A	N/A	The endotoxin level $\leq$ 0.005 EU/mg	N/A	[25]
Qinghai	Fruits	HRWP-A	4 992	0.83	N/A	1.22	0.49	N/A	N/A	N/A	N/A	97.46	N/A	It has repeated units of (1→4)- β -D- galactopyranosyluronic residues	The endotoxin level $\leq$ 0.005 EU/mg. A natural high-methoxyl HG-type pectin (DE: 85.16%).	HPGPC, HPLC, FT-IR, NMR (^1H , ^{13}C)	[25]
Shanxi	Fruits	HRP-W	281.52, 28.04	34.24	N/A	5.33	5.83	1.34	0.94	N/A	3.28	47.9	1.14	Triple helix structure	Semi-crystalline substances. A mixture of rough fragments and irregular filaments with some small pores	HPGPC, PMP labeling and HPLC, particle size, FT-IR, Congo red, XRD, SEM, thermogravimetric-differential thermal analysis (TG/DTA), and rheometer	[31]
Shanxi	Fruits	HRP-H	117.55	25.53	N/A	5.34	8.05	1.47	1	N/A	6.31	53.19	1.11	N/A	Semi-crystalline substances. A mixture of rough fragments and irregular filaments with some small pores	HPGPC, PMP labeling and HPLC, particle size, FT-IR, Congo red, XRD, SEM, TG/DTA, and rheometer	[31]
Shanxi	Fruits	HRP-U	244.63, 28.04	34.21	N/A	3.27	5.86	1.19	0.6	N/A	1.08	52.56	1.24	N/A	Semi-crystalline substances. A long filamentous network with loose structure. Good thermal stability	HPGPC, PMP labeling and HPLC, particle size, FT-IR, Congo red, XRD, SEM, TG/DTA, rheometer	[31]
Shanxi	Fruits	HRP-C	289.77, 17.26, 7.67	22.3	N/A	5.17	1.6	0.94	0.88	N/A	3.17	63.05	2.89	N/A	Semi-crystalline substances. A large sheet-like structure. A pronounced sticky character	HPGPC, PMP labeling and HPLC, particle size, FT-IR, Congo red, XRD, SEM, TG/DTA, rheometer	[31]
Shanxi	Fruits	HRP-A	116.27, 6.41	38.07	N/A	11.01	3.1	0.29	5.32	N/A	5.3	29.24	7.67	N/A	Semi-crystalline substances. A relatively small, smooth and uniform fragment	HPGPC, PMP labeling and HPLC, particle size, FT-IR, Congo red, XRD, SEM, TG/DTA, rheometer	[31]
Jilin	Fruits	HRP-1	380 590	45.18	N/A	10.55	15.18	4.55	5.46	1.00	2.36	97.64	1.27	N/A	N/A	UV, FT-IR, PMP labeling and HPLC, SEM, particle size, Zeta potential, and Congo red	[33]
Jilin	Fruits	HRP-2	288 240	31.80	N/A	13.00	15.00	5.80	10.20	1.00	2.60	26.00	2.20	N/A	N/A	UV, FT-IR, PMP labeling and HPLC, SEM, particle size, Zeta potential, and Congo red	[33]
Jilin	Fruits	SBLP-S-1	338 659	25.60	N/A	18.90	26.97	5.28	6.37	N/A	5.45	2.97	3.725	N/A	N/A	UV, FT-IR, PMP labeling and HPLC, HPSEC, Congo red	[66]
Jilin	Fruits	SBLP-S-2	401 305	12.6	N/A	14.49	44.09	5.70	10.99	N/A	2.44	1.42	1.64	N/A	N/A	UV, FT-IR, PMP labeling and HPLC, HPSEC, Congo red	[66]
Jilin	Fruits	SBLP-S-3	599 849	3.97	N/A	5.36	43.99	5.76	14.01	N/A	2.79	2.40	3.28	N/A	N/A	UV, FT-IR, PMP labeling and HPLC, HPSEC, Congo red	[66]
Jilin	Fruits	SBLP-S-4	393 904	9.45	N/A	23.27	25.27	5.17	16.80	N/A	3.60	8.99	N/A	N/A	N/A	UV, FT-IR, PMP labeling and HPLC, HPSEC, Congo red	[66]

Table 2 (Continued)

Region	Part	Name	M_w (Da)	Monosaccharide composition										Structural features	Physicochemical properties	Analytical techniques	References
				Ara	Fuc	Gal	Glc	Man	Rha	Rib	Xyl	GalA	GlcA				
Jilin	Fruits	SBLP-S-5	626 895	3.95	N/A	5.89	29.8	6.04	15.24	N/A	2.88	3.77	3.07	N/A	N/A	UV, FT-IR, PMP labeling and HPLC, HPSEC, Congo red	[66]
Jilin	Fruits	SBLP-S-6	176 862	4.46	N/A	6.72	46.70	6.80	17.25	N/A	3.16	3.37	3.41	N/A	N/A	UV, FT-IR, PMP labeling and HPLC, HPSEC, Congo red	[66]
N/A	Fruits	SP	275 420	1.02	N/A	1.00	4.24	2.02	9.22	N/A	N/A	N/A	N/A	N/A	N/A	PMP labeling and HPLC	[58]
Jilin	Fruits	PSB	33 601	N/A	N/A	13.52	1.62	6.89	1.00	N/A	N/A	N/A	N/A	N/A	N/A	HPSEC, GC, UV, FT-IR, SEM	[32]
The backbone consisting of $\rightarrow 4$)-α-D-Glcp-(1 $\rightarrow$ and $\rightarrow 4$)-α-D-Manp-(1 $\rightarrow$ residues. The side chains consisted of 1,5-linked-α-L-Araf. Single α-Araf' was linked to O-3 position of the 1,5-linked-α-L-Araf. The Ara residues were linked to the main chain through 1,4-linked-β-D-Galp residues to O-6 position of the $\rightarrow 4$)-α-D-Glcp-(1 $\rightarrow$ (Fig. 3B)																	
Shanxi	Fruits	SP0.1-1	26 200	11.20	N/A	1.90	2.30	1.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A	HPGPC, PMP labeling and HPLC, Methylation, NMR (^1H , ^{13}C , ^1H - ^1H COSY, HSQC, NOESY)	[63]
Xinjiang	Fruits	SBP-I	N/A	1.18	N/A	1.45	32.17	2.20	N/A	N/A	1.00	N/A	N/A	N/A	N/A	FT-IR, GC	[27]
Xinjiang	Fruits	SBP-II	N/A	N/A	N/A	0.20	1.02	0.28	N/A	N/A	1.00	N/A	N/A	N/A	N/A	FT-IR, GC	[27]
Xinjiang	Fruits	SBP-III	N/A	N/A	N/A	0.28	2.15	N/A	N/A	N/A	1.00	N/A	N/A	N/A	N/A	FT-IR, GC	[27]
It consists of a mixture of linear high-molecular polymers α-D-galacturonane and other polysaccharides, whose macromolecules include galacturonic acid residues and neutral sugars It has a backbone composed of $\rightarrow 3,4$)-β-L-Rhap-(1 $\rightarrow$ 4)-α-D-GalAp-(1 $\rightarrow$ 4)-α-D-GalAp-(1 $\rightarrow$ with side chains comprised of α-L-Araf, β-D-Galp, β-D-Glcp and α-D-Glcp (Fig. 3A)																	
Azerbaijan	Fruits	sea buckthorn pectin	42 000	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	A low-esterified pectin. A high antidote potential in relation to heavy metals and radionuclides	[35]
Shanxi	Fruits	SBP-1-A	9 944	44.60	N/A	19.70	28.20	N/A	2.10	N/A	N/A	5.30	N/A	N/A	N/A	HPGPC, IC, FT-IR, Methylation, GC-MS, NMR (^1H , ^{13}C , DEPT-135, ^1H - ^1H COSY, HSQC, HMBC, NOESY)	[24]
A typical high methoxyl pectin (DE: 57.75%). A sheet and layered stacked structure with a smooth and dense surface. It can form a jelly-like gel with an acid and high sucrose condition, exhibiting a shear thinning behavior																	
Qinghai	Peels	SBHMP	N/A	15.35	1.07	5.73	2.31	3.18	4.50	N/A	0.73	66.49	0.64	N/A	N/A	PMP labeling and HPLC, FT-IR, SEM, DSC, rheometer	[34]

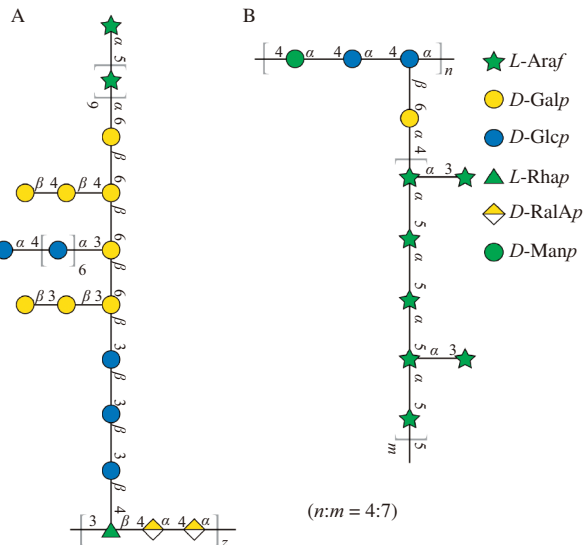


Fig. 3 (A) The molecular structures of SBP-1-A^[3]; (B) the molecular structures of SP0.1-1^[63].

3.1 Quantitative analysis

Quantification of sea buckthorn polysaccharides is a prerequisite for their structural characterization. Initially, Protein content was determined using the method of Sedmak and Grossberg^[53] or BCA analysis^[54]. Based on this, absorption peaks at 260 and 280 nm were detected using UV spectroscopy to determine whether the polysaccharides were related to nucleic acids or proteins^[32]. The sugar content is determined using the phenol-H₂SO₄ method or the 3,5-dinitrosalicylic acid method, with purity considered acceptable at 85% or higher^[55]. The *m*-hydroxybiphenyl method is primarily used to quantify the prevalence of glyoxylates in sea buckthorn polysaccharides, while endotoxin levels are analyzed using a gel-clot *Limulus* amebocyte lysate assay^[23].

3.2 Primary structural characterization

The primary structure of polysaccharides includes various features, such as M_w , monosaccharide composition, configuration of glycosidic linkages, types of glycosidic linkages, and positions of glycosidic linkages, among others^[56]. M_w is a crucial characteristic of sea buckthorn polysaccharide structure. The M_w of sea buckthorn polysaccharides have been determined using various techniques, including high-performance liquid chromatography (HPLC), high-performance gel permeation chromatography (HPGPC), and high-performance size-exclusion chromatography^[57]. The M_w distribution of sea buckthorn polysaccharides is presented in Table 2, showing a range from 1 Da to 626 895 Da^[25,58]. These polysaccharides consist of neutral monosaccharides and glyoxylates and are commonly analyzed using gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), ion chromatography (IC), and HPLC techniques to determine their monosaccharide fractions^[59-60]. The sea buckthorn polysaccharides in Table 2 are heteropolysaccharides composed of various monosaccharides, including Ara, Rha, Xyl, Man, Glc, and Gal, and contain substantial quantities of the glyoxalates GlcA and GalA^[34].

Based on available literature, limited information is available regarding the structural composition of sea buckthorn polysaccharides beyond their M_w and monosaccharide composition. Various analytical techniques, such as Fourier transform-infrared (FT-IR), methylation, GC-MS, and nuclear magnetic resonance (NMR), have been employed to analyze the glycosidic linkages of these polysaccharides^[61-62]. Specifically, it is possible to identify the poly containing functional groups, i.e., to determine the type of glycosidic linkages, by means of the characteristic absorption peaks of the infrared spectra. By glyoxalate reduction, methylation reaction, hydrolysis, reduction, acetylation, and mass spectra were obtained by GC-MS in order to analyze to obtain the linkage sites of glycosidic bonds, the mode of glycosidic bonding, the molar percentage, etc. However, the appearance of NMR simplifies the steps of analyzing polysaccharides, and many structural information of polysaccharides can be presented through the diagram of NMR, including the determination of the number of monosaccharide residues, α or β isomers, sugar ring configuration, glycosidic bond and its configuration, the way and order of glycosidic residue linkage, substitution of functional groups and substitution position, and the attribution of chemical shifts. Wang et al.^[25] have reported that HRWP-A is a high methoxy homogalacturonan pectin (degree of esterification (DE) = 85.16%) comprising repeating units of (1 $\rightarrow$ 4)- β -D-galactopyranosyluronic residues, as determined by FT-IR and NMR (¹H and ¹³C) analysis. Ma et al.^[24] have characterized SBP-1-A (Fig. 3A) by FT-IR, methylation, GC-MS, and NMR (¹H, ¹³C, DEPT-135, HSQC, ¹H-¹H COSY, HMBC, NOSY). The arabinose, glucose, and galactose residues are determined as the main monosaccharide compositions with a proportion exceeding 92%. Shen et al.^[63] have identified that SP0.1-1 is a heteropolysaccharide, based on GC-MS analysis of partially *O*-methylated alditol acetates, the predicted chemical structure is shown in Fig. 3B. Farzaliyev et al.^[35] have isolated low-esterified pectin (DE: 28.8%), consisting of a mixture of linear high-molecular polymers α -D-galacturonic and other polysaccharides with macromolecules including galacturonic acid residues and neutral sugars. Zhu et al.^[34] have extracted a polysaccharide named SBHMP from sea buckthorn peel and have found it to be a complex pectin with high glyoxylate content (DE: 57.75%), which can be classified as a complex consisting of homogalacturonans (HG) and rhamnogalacturonan I (RG-I) type pectin polysaccharides.

The extant scientific literature on sea buckthorn polysaccharides has been chiefly preoccupied with examining those derived from Chinese sea buckthorn, thus offering scant attention to comparative analyses of sea buckthorn sourced from disparate regions. The perplexing fact remains that we are bereft of a comprehensive understanding of how different extraction methodologies impinge upon the intricate structure of sea buckthorn polysaccharides. Furthermore, we are confronted with a gaping void in our knowledge regarding the intricate structures of polysaccharides present in other facets of the sea buckthorn plant, such as branches and leaves. It is therefore an exigent requirement that we undertake further efforts towards characterizing the structure of sea buckthorn polysaccharides.

3.3 High-level structural characterization

After obtaining the primary structure information of sea buckthorn polysaccharides, it is necessary to solve the high-level structure of sea buckthorn polysaccharides in order to further explore the diversity of sea buckthorn polysaccharides. However, the conformations of sea buckthorn polysaccharides are very complex, so it is very difficult to characterize their senior structures. Currently, researchers have attempted to analyze the physicochemical properties as well as the conformation of sea buckthorn polysaccharides using various analytical techniques in order to explore the high-level structure of sea buckthorn polysaccharides. The particle size represents the aggregation degree of polysaccharide molecules in solution, which can affect the metabolism of polysaccharides in organisms. Li et al.^[31] determined the particle size of polysaccharides and found that the particle size of sea buckthorn polysaccharides corresponded to the M_w size. The range was about 188.4–854.6 nm. Similarly, Zhao et al.^[65] found that the particle size of sea buckthorn polysaccharides increased with the increase of their M_w . The particle size of HRP-1, which has the highest M_w , was 1 031.7 nm. Some scholars observed the microscopic morphology of sea buckthorn polysaccharides by scanning electron microscopy (SEM). SEM is a kind of using high-energy electron beam and polysaccharide interaction, to stimulate a variety of signals, the reception of this information, transformation, amplification, and then image to achieve the purpose of the characterization of sea buckthorn polysaccharides microscopic morphology. Wei et al.^[32] compared the microstructure of sea buckthorn polysaccharides extracted by HWE and MAE using SEM and found that the MAE-extracted samples had disorganized parenchyma, indicating that MAE accelerated cell damage and promoted polysaccharide release from sea buckthorn better than HWE. A study identified several properties of the sea buckthorn pectin polysaccharide named SBHMP, including its apparent sheet-like and layered stacked structure with a smooth and dense surface as observed by SEM^[34]. Zhao et al.^[65] observed sea buckthorn polysaccharide (HRPI) and found that its microscopic morphology was a blocky structure with rough surface, small interstices and loose arrangement. Zhu et al.^[33] isolated two fractions from sea buckthorn and found that their microscopic morphologies were not similar. HRP-1 had a loose surface, agglomerated flocculent structure and high density, while HRP-2 had a smooth surface, many voids in a banded structure, and a lower density. Li et al.^[31] conducted SEM analysis on sea buckthorn polysaccharides obtained by different extraction methods. The sea buckthorn polysaccharides extracted by hot water shown a mixture of rough fragments and irregular filaments with some small pores. After pressure-assisted extraction, the size of fragments and the length of filaments became smaller. After ultrasonic extraction, it shown a long filamentous network with loose structure. After acid extraction, it shown a dense, rough, and irregular porous surface with a large sheet-like structure, and after alkaline extraction, it shown a mixture of rough fragments and irregular filaments with some small pores. X-ray diffraction (XRD) is a technique for analyzing the structure of a substance using the diffraction effect of X-rays in crystalline substances. The amorphous or crystalline structure of sea buckthorn polysaccharides can be further characterized by XRD analysis. Li et al.^[31] found that the HRPs of sea buckthorn polysaccharides obtained by various extraction

methods were semi-crystalline. In contrast, Zhao et al.^[65] found that the HRPIs of sea buckthorn berry polysaccharides belonged to amorphous structure. Whether the polysaccharide contains a triple-helix structure can be initially determined by whether the maximum absorption wavelength (λ_{max}) of Congo red-polysaccharide complex will produce red-shift when the polysaccharide had a triple-helix structure in the Congo red experiment. Zhu et al.^[33] found that neither of their two sea buckthorn polysaccharide fractions isolated from whole sea buckthorn plants had a triple helical structure. Li et al.^[31] found that the hot water extracted sea buckthorn fruit polysaccharide HRP-W had a weak triple helical structure. Liu et al.^[66] found that of the six sea buckthorn polysaccharides isolated from sea buckthorn fruits, BLP-S-1 and SBLP-S-3 did not have a triple helical structure, and SBLP-S-4, SBLP-S-5, and SBLP-S-6 had a weak triple helix conformation, while SBLP-S-2 had a strong triple helix structure.

We found that the advanced structures of sea buckthorn polysaccharides exhibited similar diversity. In addition to the above methods, there are many other methods for characterizing the high-level structure of polysaccharides^[67]. For example, the atomic force microscopy (AFM), which uses a sharp probe to sense the interaction force between the tip of the needle and the atoms on the surface of the sample, reflects the undulation of the surface of the sea buckthorn polysaccharide aggregates through the change of the atomic force, and then characterizes its apparent morphology. The crystal structure of polysaccharides can be analyzed using transmission electron microscopy (TEM), which is similar in principle to X-rays, but its electron beam has the advantages of a shorter wavelength and simple scattering by matter. It has been shown that circular dichroism (CD) can analyze the conformational information of polysaccharides by using the difference in the absorption of left- and right-polarized light by asymmetric molecules. In addition, the spatial conformation of polysaccharides can be analyzed by combining computer simulation techniques. Researchers need to further analyze the advanced structure of sea buckthorn polysaccharides by understanding its detailed structure and performing derivatization, through conformational analysis, to better develop sea buckthorn polysaccharides.

4. Biological activities

Sea buckthorn has been used medicinally in folk medicine worldwide for the traditional treatment of various diseases, with various parts of the plant, including the fruit, branches, leaves, and seeds being utilized^[68]. Research conducted both *in vitro* and *in vivo* has demonstrated the potential bioactivities of sea buckthorn polysaccharides, which may significantly contribute to human health and pharmacological advancements. Fig. 4 provides a summary of the bioactivities of sea buckthorn polysaccharides and the modulation of related active factors.

4.1 Antioxidant activity

With the advancement of research, it is increasingly acknowledged that various diseases are associated with oxidative stress, including but not limited to aging, inflammation, diabetes, cancer, and others^[69–70]. Sea buckthorn polysaccharides exhibit potent antioxidant activity *in vivo* and *in vitro* by scavenging free radicals, enhancing antioxidant enzyme activity, and regulating antioxidant signaling pathways^[69].

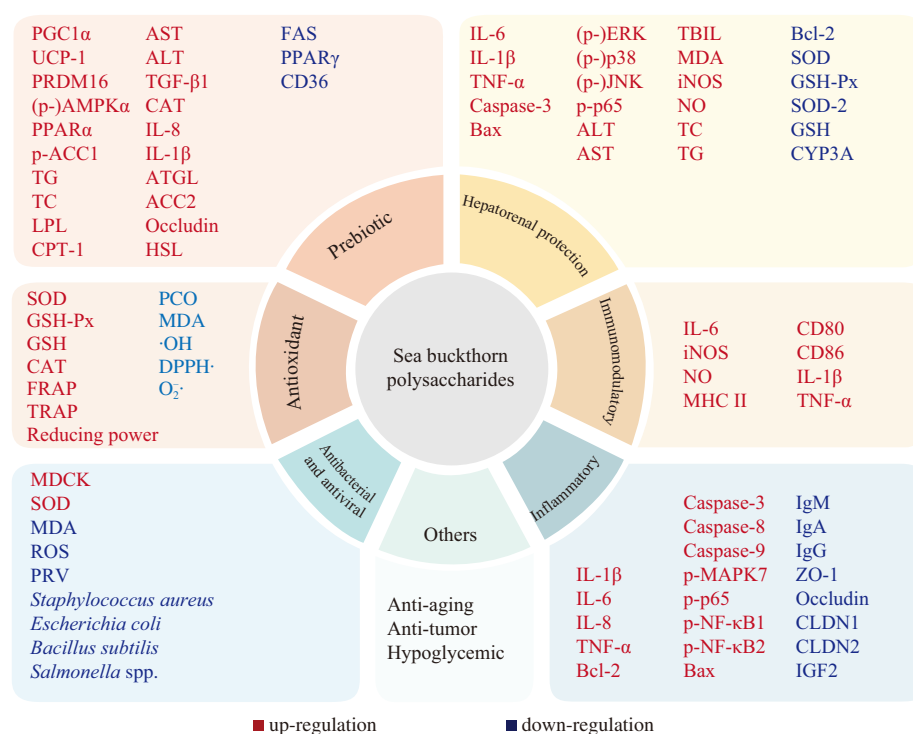


Fig. 4 Biological activities of sea buckthorn polysaccharides and their modulation of related active factors. LPL: lipoprotein lipase, CPT-1: carnitine palmitoyltransferase-I, CAT: catalase, ATGL: adipose triglyceride lipase, ACC2: acetyl CoA carboxylase, HSL: hormone-sensitive lipase, FAS: fatty acid synthase, FRAP: ferric ion reducing antioxidant power; TRAP: total peroxyl radical-trapping antioxidant parameter, TBIL: total bilirubin, CLDN1: Claudin 1, CLDN2: Claudin 2, IGF2: insulin-like growth factor 2.

The researchers investigated the *in vitro* antioxidant activity of sea buckthorn polysaccharides, mainly including the DPPH radical-scavenging capacity, scavenging activity of ABTS radical, hydroxyl radical-scavenging capacity, oxygen radical absorption capacity, and reducing capacity assay of sea buckthorn polysaccharides oxygen radical absorption capacity and reducing capacity. The results suggested that sea buckthorn polysaccharides had significant *in vitro* antioxidant activity^[31-33,66,71-72]. Researchers explored the antioxidant activity of sea buckthorn polysaccharides in response to oxidative stress-induced diseases associated *in vivo*. *In vivo* experiment of ethanol-induced oxidative stress model mouse shown that under the influence of PSB, malondialdehyde (MDA) content decreased, superoxide dismutase (SOD) activity increased, protein carbonylation (PCO) content decreased, and glutathione (GSH) level increased significantly when the PSB dose was 100–200 mg/kg. Zhang et al.^[58] evaluated the antioxidant activity of SP in CCl $_4$ -induced mice and found that it increased SOD activity in serum and liver tissues, increased glutathione peroxidase (GSH-Px) and GSH levels in the liver and decreased MDA levels to inhibit oxidative stress. Shen et al.^[63] investigated the impact of incorporating SP0.1-1 into the diet of *Drosophila melanogaster* exposed to H $_2$ O $_2$ -induced oxidative stress. They observed that the inclusion of SP0.1-1 led to increased activity levels of antioxidant enzymes such as SOD, GSH-Px, and catalase, while also decreasing the amount of MDA present in the *D. melanogaster*. Zhao et al.^[65] found that sea buckthorn polysaccharides exerted anti-oxidative effects by regulating Nrf2/Keap1 pathway in Alzheimer's disease mice, and significantly enhanced the activities of SOD and GSH-Px and reduced oxidative stress. Yang et al.^[41] found that sea buckthorn polysaccharides from

the southeastern Tibet plateau region had significant antioxidant activity. Their leaf polysaccharide antioxidant activity was higher than fruit polysaccharides in all months by measuring the DPPH free radical scavenging capacity, ferric ion reducing antioxidant power, and total radical trapping antioxidant parameter.

In summary, both sea buckthorn fruit and leaf polysaccharides are excellent natural antioxidants. Although sea buckthorn leaf polysaccharides exhibit superior antioxidant activity compared to sea buckthorn fruit polysaccharides, there is insufficient research on the *in vivo* antioxidant properties of sea buckthorn leaf polysaccharides, indicating a need for further investigation. Similarly, studies evaluating the effects of different extraction methods and parameters on the antioxidant capacity of sea buckthorn polysaccharides will help to understand their function and stability and provide ideas for the development of antioxidant cosmetics and antioxidant foods.

4.2 Immunomodulatory activity

Immunomodulation is essential for maintaining the body's health, and many different polysaccharides, such as mushroom polysaccharides, sea cucumber polysaccharides, and astragalus polysaccharides, have been proven to have immunomodulatory activity and are natural immunomodulators^[73-74]. Sea buckthorn polysaccharides have also been shown to possess immunomodulatory effects by mediating cellular signaling pathways with immunomodulatory cells, such as T and B lymphocytes, macrophages, and natural killer (NK) cells, and by promoting the release of various cytokines, facilitating antibody production, or initiating the complement system (Fig. 5). Zhu et al.^[33] have shown

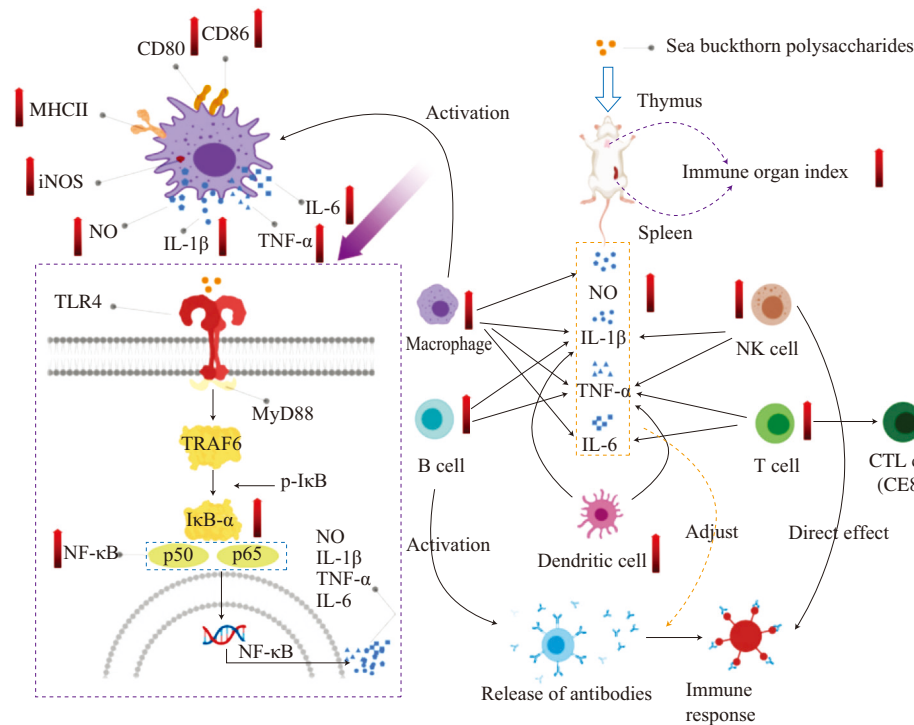


Fig. 5 Potential immunomodulatory mechanisms of sea buckthorn polysaccharides. TRAF6: TNF receptor-associated factor 6, IκB-α: NF-kappa-B inhibitor alpha, p-IκB: phospho- NF-kappa-B inhibitor alpha.

in vitro studies on macrophages (RAW264.7) that sea buckthorn polysaccharide (HRP-2) in the concentration range of 25–800 µg/mL can enter macrophages via endocytosis, enhance macrophage proliferation and boost macrophage phagocytic activity to enhance immunomodulatory activity. Liu et al.^[66] isolated 6 different fractions of sea buckthorn leaf polysaccharides from sea buckthorn leaves to investigate *in vitro* immunomodulatory activities. It was found that all 6 polysaccharides increased the proliferation ability of RAW264.7 cells compared with the blank control group. The phagocytosis of RAW264.7 cells was promoted by all six polysaccharides at different concentrations, and the phagocytosis activity of SBLP-S-2 was the strongest at a concentration of 50 µg/mL. On this basis, the release of NO was measured to reflect the activating effect of polysaccharides on macrophages. At all concentrations, all 6 polysaccharides significantly accelerated the release of NO from macrophages, and the peak amount of NO was reached at a concentration of 800 µg/mL. Wang et al.^[25] analyzed the immunomodulatory activity of sea buckthorn polysaccharide HRWP-A in mouse LEWIS lung cancer cells (LLC) model mice. The results shown that it could enhance lymphocyte proliferation, promote NO and tumor necrosis factor-alpha (TNF-α) levels, enhance NK cell activity, cytotoxic T lymphocyte (CTL) cytotoxicity, and augment macrophage activity. Wang et al.^[75] established a cyclophosphamide (CTX)-induced immunosuppression mouse model to investigate the immunomodulatory mechanism of sea buckthorn polysaccharide (HRWP-A). HRWP-A promoted the expression of major histocompatibility complex class II (MHCII), cluster of differentiation 80 (CD80), and cluster of differentiation 86 (CD86) on activated macrophages and significantly upregulated and restored the CTX-suppressed NO, interleukin-1 beta (IL-1β), TNF-α, and interleukin-6 (IL-6) levels. Exploration of its mechanisms revealed that HRWP-A promoted the secretion of pro-inflammatory

factors and macrophages mainly by activating the myeloid differentiation factor 88/nuclear factor kappa-B (MyD88/NF-κB) signaling pathway by toll-like receptor 4 (TLR4).

However, the mechanisms related to the immunomodulatory activity of sea buckthorn polysaccharides require further refinement. Nevertheless, it has good prospects for development as a natural active ingredient to regulate and enhance the immune function of the body.

4.3 Anti-inflammatory activity

Inflammation is a process that elicits a systemic response to a specific state, which can arise from different conditions such as infection, injury, or altered states of the body^[75]. The biology of inflammation is relevant to all human diseases. Zhao et al.^[65] found that treatment with sea buckthorn polysaccharide reversed the increase in IL-6 and TNF-α levels and reduced TLR4 and MyD88 protein expression in the hippocampus of AD mice. The results suggest that sea buckthorn polysaccharide can regulate the inflammatory response in AD mice by inhibiting the TLR4/MyD88 pathway. Lan et al.^[76] found that dietary addition of seabuckthorn berry polysaccharide (SBP) effectively reversed NF-κB phosphorylation, dramatically up-regulated the expression of inducible nitric oxide synthase (iNOS), ionized calcium binding adaptor molecule-1 (IBA-1) and decreased the expression of inflammation-associated factors IL-1β, IL-6, and COX-2 in the brains of mice. It was demonstrated that dietary addition of SBP could alleviate the HFD-induced inflammatory response in the mouse brain. Moreover, the addition of SBP inhibited the up-regulation of NF-κB phosphorylation, iNOS and COX-2 expression in the intestinal tissues of HFD mice. It also reduced the expression of inflammatory factors such as TNF-α, IL-1β and IL-6 in the intestinal tissues of

HFD mice. It was demonstrated that dietary supplementation with SBP could alleviate the inflammatory response in the intestinal tract of mice. In addition, serum LPS levels and inflammatory factors were significantly downregulated after SBP supplementation. The multiple anti-inflammatory effects of SBP were demonstrated. Zhao et al.^[77] conducted an *in vitro* study to examine the impact of HPR on inflammatory damage to porcine small intestinal epithelial cell line (IPEC-J2) cells induced by lipopolysaccharide (LPS) mixed culture. Treatment with varying concentrations of HPR (0–600 µg/mL) was found to be associated with significant reductions in inflammatory factors including IL-1β, IL-6, IL-8, and TNF-α. Moreover, HPR treatment led to a decrease in apoptosis, as evidenced by decreased expression of B-cell lymphoma-2 protein (Bcl-2), Bcl-2-associated X protein (Bax), cysteine-requiring aspartate protease-3 (caspase-3), cysteine-requiring aspartate protease-8 (caspase-8), and cysteine-requiring aspartate protease-9 (caspase-9). Conversely, HPR treatment resulted in increased levels of key immunoglobulins, including immunoglobulin M (IgM), immunoglobulin A (IgA), and immunoglobulin G (IgG), as well as enhanced expression of tight junction proteins, including Zonula occludens protein 1 (ZO-1), occludin, and claudin 1. Further exploration of the mechanism of anti-inflammatory and immunomodulatory activity found that the 600 µg/mL HRP treatment significantly decreased the expression of TLR4, MyD88, and phosphorylated NF-κB protein in IPEC-J2 cells induced by LPS. Zhao et al.^[78], and Li et al.^[79] employed transcriptomics, bioinformatics, and proteomics to investigate the anti-inflammatory mechanism of HRP. They found that pre-treatment with HRP increased the levels of claudin 2 and insulin-like growth factor two and reduced the expression of phosphorylated mitogen-activated protein kinase (p-MAPK7), phosphorylated transcription factor p65 (p65), phosphorylated NF-κB1, and phosphorylated NF-κB2 in IPEC-J2 cells, as confirmed by Western blotting analyses. Bioinformatics analysis (Gene Ontology, Kyoto Encyclopedia of Genes and Genomes, and Series Cluster) identified critical regulatory genes, including IL-8 and NF-κB2, among others. Additionally, transcriptome sequencing revealed that HRP treatment downregulated the expression of differentially expressed genes and suppressed the MAPK/NF-κB signaling pathway.

Current research has demonstrated the anti-inflammatory properties of sea buckthorn polysaccharides and their underlying mechanisms *in vitro*, using omics techniques. However, further investigations are necessary to establish the efficacy of sea buckthorn polysaccharides *in vivo*.

4.4 Hepatorenal protection activity

In traditional Chinese medicine, the liver and kidneys are believed to have a complementary relationship referred to as “liver-kidney homology”^[80]. The proper functioning of both organs is essential for maintaining normal metabolism and the stability of the body’s internal environment. While kidney injury can result from pathological factors such as tumors, stones, inflammation, or hydronephrosis, liver injury can be caused by various factors such as drug-related, alcoholic or viral liver injury^[81]. To date, numerous studies have shown that sea buckthorn polysaccharides possess significant protective effects on the liver and kidneys. Cen et al.^[82] injected sodium oxalate into the biliopancreatic duct of rats to establish an *in vivo* rat model of severe

acute pancreatitis (SAP), inducing SAP and causing kidney damage in the rats. Treatment with sea buckthorn polysaccharide attenuated sodium taurocholate-induced increase of IL-6, IL-1β and TNF-α levels in kidney tissue, and attenuated sodium taurocholate-induced decrease of Bcl-2 and increase of cleaved caspase-3 and Bax. The mechanism involved was that sea buckthorn polysaccharide inhibited activation of the MAPK/NF-κB pathway in SAP rats by down-regulating the expression of phospho-extracellular regulated protein kinases (p-ERK), phospho-p38 mitogen activated protein kinases (p-p38), phospho-c-Jun N-terminal kinase (p-JNK), and p-p65 in the kidney. Liu et al.^[36] and Wang et al.^[83] found that sea buckthorn polysaccharides significantly inhibited LPS/*D*-galactosamine 2-sulfate sodium salt (LPS/*D*-GalN) -induced increase in alanine transaminase (ALT) and aspartate aminotransferase (AST) levels and decreased the production of TNF-α and IL-1β. In addition, it also reduced MDA content, restored the activity of SOD and GSH-Px, and effectively inhibited the expression of TLR4, p-ERK, p-JNK and p-p38 MAPK in the liver of LPS/*D*-GalN mice. It was also verified that sea buckthorn polysaccharide protected the liver by inhibiting the TLR4-activated NF-κB signaling pathway, independent of the regulation of suppressor of cytokine signaling three expressions. Liu et al.^[84] used the same animal model. In addition to the above results, the study also found that the protective effect of SP on LPS/*D*-GalN-induced acute liver injury in mice was related to the up-regulation of SOD-2 and inhibition of Bax expression. Zhang et al.^[58] explored the hepatoprotective mechanism of SP against CCl₄-induced hepatotoxicity and found it synergistic in protecting the liver through two active manners, anti-inflammatory and antioxidant. Antioxidant: SP enhanced SOD and GSH-Px activity, increased GSH levels and reduced MDA content. Anti-inflammatory: SP reduces the expression of TNF-α and IL-1β at mRNA and protein levels, the expression of *iNOS* at mRNA level and the production of NO. Overall, SP treatment significantly reduced ALT, AST and total bilirubin, and the key mechanism critical to reducing the expression of TRL4 and inhibited the activation of p38 MAPK, JNK, and the phosphorylation of NF-κB in CCl₄-challenged mice. Liu et al.^[85] established a model of immune liver injury induced by intravenous injection of *Bacillus Calmette-Guérin* (BCG) in rats to investigate its hepatoprotective mechanism. After HRP intervention, it not only reversed the up-regulation of NF-κB, inflammatory cytokines (IL-1β, TNF-α) and activation of *iNOS* by BCG treatment but also inhibited the downregulation of cytochrome P450 3A (CYP3A) protein expression. Briefly, HRP reversed the downregulation of CYP3A and protected liver function by inhibiting NF-κB signaling, which involved transcriptional, translational, and post-translational regulation of the enzyme. Liu et al.^[86] found that liver disease was closely related to intestinal flora and that the combination of sea buckthorn polysaccharide and astragalus polysaccharide significantly modulated the distribution of intestinal flora microflora, which in turn affected a significant reduction in serum AST, ALT, total cholesterol (TC) and triglyceride (TG) levels in alcoholic fatty liver disease (AFLD) mice.

In summary, the protective mechanism of sea buckthorn polysaccharides on the liver and kidney is multifaceted and involves synergistic effects such as inhibition of oxidative stress, anti-inflammatory activity, apoptosis inhibition, and regulation of intestinal flora (Fig. 6). Therefore, sea buckthorn polysaccharides

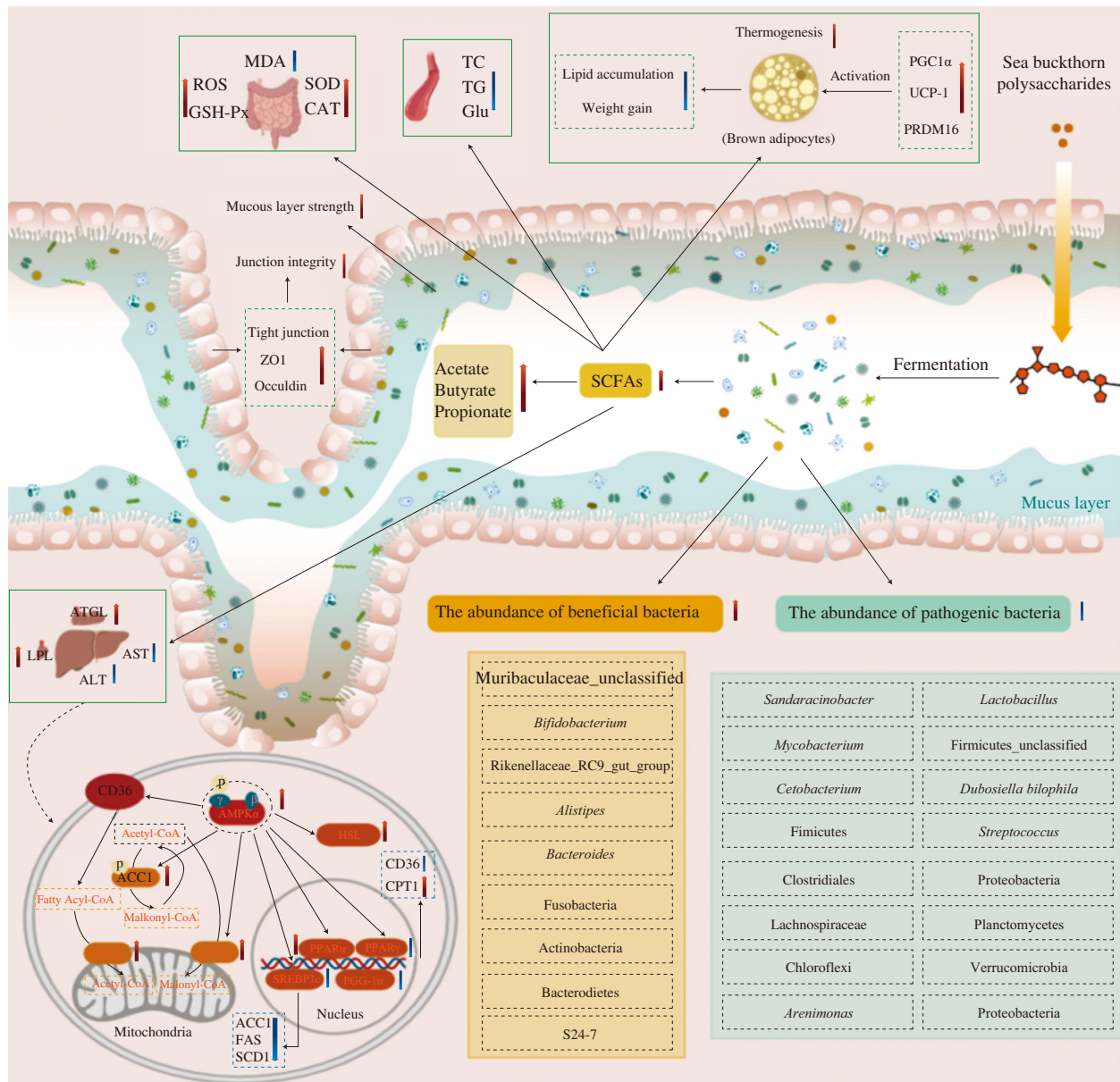


Fig. 7 Potential mechanisms underlying the prebiotic activity of sea buckthorn polysaccharides. SREBP1c: sterol regulatory element binding protein-1c, PGC-1: peroxisome proliferator-activated receptor-gamma coactivator 1 alpha, SCD1: stearoyl coenzyme A desaturase-1.

proliferation of *Mycobacterium* and downregulating the proliferation of *Cetobacterium*. This positively influenced the fish's ability to alleviate HFD-induced oxidative stress by increasing NF-E2-related factor 2 (Nrf2) and Cu/Zn-SOD expression and attenuating HFD-induced inflammatory response by increasing transforming growth factor beta-1 (TGF-β1) expression and inhibiting P38MAPK, IL-8, and IL-1β expression. SP significantly improved the survival rate of HFD zebrafish and reduced serum TG, TC, AST, and ALT levels, as well as attenuated lipid accumulation. In a study conducted by Ma et al.^[24], SBP-1 was found to have several beneficial effects on HFD mice. The expression of three proteins associated with brown adipocyte activation, namely peroxisome proliferator-activated receptor-gamma coactivator-alpha (PGC1α), uncoupling protein 1 (UCP-1) and positive regulatory domain-containing 16 (PRDM16), was promoted by modulating intestinal flora to enhance thermogenesis and prevent lipid accumulation and weight

gain. According to research, gut epithelial cells that encounter gut microbiota generate ROS upon exposure to microbial signals^[94]. However, sea buckthorn polysaccharides can inhibit oxidative stress caused by harmful intestinal bacteria by inhibiting them. It could inhibit the H₂O₂-induced increase in ROS levels in the *D. melanogaster* intestine and apoptosis of *D. melanogaster* intestinal epithelial cells to maintain intestinal homeostasis and alleviate oxidative stress-induced damage^[63]. Lan et al.^[95] found that SP, as a dietary supplement, modulated the intestinal flora of hyperlipidemic mice, upregulating the proliferation of Muribaculaceae_unclassified, *Bifidobacterium*, Rikenellaceae_RC9_gut_group, *Alistipes*, and *Bacteroides*, while downregulating the proliferation of *Lactobacillus*, Firmicutes_unclassified, *Dubosiella*, *Bilophila*, and *Streptococcus*. This led to an increase in the metabolism of short-chain fatty acids (SCFAs) in feces. As a result, there was increased phospho-adenosine 5'-monophosphate-activated protein kinase-alpha (p-AMPKα) and

peroxisome proliferator-activated receptor- α (PPAR α) protein expression in mouse liver, stimulated phosphorylation of acetyl coenzyme A carboxylase 1 (ACC1), and inhibited protein expression of fatty acid synthase, PPAR γ , and CD36 in mouse liver. In turn, HFD mice exhibited reduced blood lipid levels and liver TG levels as well as decreased body weight. According to research conducted by Liu et al.^[86], the combination of sea buckthorn polysaccharide and astragalus polysaccharide led to a significant reduction in the ratio of Firmicutes and Bacteroides, ultimately increasing the abundance of beneficial bacteria (S24-7) and decreasing the abundance of pathogenic bacteria (Lachnospiraceae). As a result, the liver function of AFLD mice improved, which led to the reduction of AST, ALT, TC, and TG levels, contributing to liver protection. Lan et al.^[76] found that dietary supplementation with SBP was effective in suppressing the dramatic decrease in gut microbial abundance in HFD mice. The addition of SBP not only corrected the significant increase in the relative abundance of the Firmicutes in the feces of HFD mice, but also reversed the decreases in the abundance of Bacteroidia, Clostridiales and Muribaculaceae. In addition, it also reduced the relative abundance of *Lactobacillus*, *Dubosiella*, *Olsenella*, *Helicobacter*, and *Ruminiclostridium*_9 in the gut on the HFD diet. Improved intestinal inflammation by upregulating the expression of intestinal tight junction proteins (occludin, claudin 1 and ZO-1) and Mucin-2 (MUC-2) mediated by the gut microbiota.

In conclusion, sea buckthorn polysaccharides are expected to be a new type of prebiotic with many health benefits. Not only can it function as a carbon source to promote the proliferation of beneficial intestinal microorganisms, but it can also produce healthy SCFAs that regulate the intestinal microenvironment and influence other biological activities. However, the relationship between sea buckthorn polysaccharides and intestinal flora still needs to be further explored in order to better exploit it as a functional food.

4.7 Other activities

In addition to the aforementioned bioactivities, sea buckthorn polysaccharides have demonstrated significant performance in various other biological activities, such as anti-aging, improving cognitive impairment, anti-tumor, hypoglycemic and hypolipidemic activities. For instance, sea buckthorn polysaccharide HRWP was found to have anti-fatigue and antioxidant capacity by inhibiting the increase in blood urea nitrogen (BUN), TG, creatine phosphokinase (CK), lactic dehydrogenase (LDH), and MDA levels, and preventing the decrease in Glc, SOD, and GSH-Px levels induced by forced swim test^[23]. Zhao et al.^[65] extracted novel polysaccharides (HRPI) from fresh sea buckthorn berries and reduced the accumulation of amyloid beta-protein (A β) in the brain through the positive modulation of inflammatory factors and oxidative stress interactions by sea buckthorn polysaccharides in Alzheimer disease (AD) mice, which led to the improvement of the level of cognitive functioning and the pathological morphology of the hippocampus. Additionally, sea buckthorn polysaccharide HRWP-A inhibited the proliferation of cancer cells by activating the immune system, increasing lymphocyte accumulation, activating macrophage action and cytotoxicity, promoting NO and TNF- α levels, and eliminating tumor cells mediated by NK cells and CTL cytotoxicity^[25,96]. Moreover, the sea buckthorn polysaccharide SPO reduced glucose levels and lowered

serum TC, TG, and LDL-C levels in diabetic mice^[97]. Polysaccharide HRP from the branches and leaves of sea buckthorn has also been found to exert hypoglycemic effects by inhibiting α -glucosidase in the intestinal tract^[26]. Li et al.^[31] found that HRP-C, a sea buckthorn berry polysaccharide extracted by acid extraction, has strong bile acid binding capacity. It was proved to have potential hypolipidemic activity.

It is worth noting that most of the sea buckthorn polysaccharides used in studies are unpurified crude polysaccharides, with only a few pure polysaccharides. Despite the extensive research on the biological activity of sea buckthorn polysaccharides, the mechanisms underlying their effects remain unexplored. To date, no studies have been conducted to compare the biological activity of sea buckthorn polysaccharides derived from various sources. The investigation of the bioactivities and mechanism of sea buckthorn leaf polysaccharides is much less than that of sea buckthorn fruit polysaccharides, despite the former showing no less biological activity than the latter. Hence, more attention is needed to be paid to the development of the activity and mechanism of sea buckthorn leaf polysaccharides.

5. Structure-activity relationship

The relationship between the structure and activity of polysaccharides has long puzzled researchers, who have struggled to definitively explain this phenomenon. Polysaccharides exhibit a high degree of complexity, with variations in M_w , monosaccharide composition, types of glycosidic bonds, conformation, and structural modifications, all of which make it challenging to understand the relationship between their structure and activity^[98-99]. As such, the complete clarification of the structure and the intricate relationship between structure and activity in the present study is a formidable undertaking. Although the relationship between the frame and activity of sea buckthorn polysaccharides is not yet fully explicated, inferences can be made based on extant research.

5.1 Molecular weight

M_w is a crucial factor affecting the biological activity of sea buckthorn polysaccharides. Studies have shown that the Mw of polysaccharides needs to be within a suitable range in order to exert the best activity^[100]. The larger the M_w , the larger the polysaccharide molecule volume, and its transmembrane resistance will also increase, so it is not conducive to absorption and utilization, thus affecting the activity. However, if the M_w is too low, the polysaccharide will not be able to form an active structure, thus reducing its biological activity^[101]. Zhu et al.^[33] isolated two fractions, HRP-1 and HRP-2, from sea buckthorn polysaccharides, but the smaller M_w HRP-2 exhibited more significant antioxidant and immune-enhancing effects than the larger M_w HRP-1. Wei et al.^[32] isolated an antioxidant polysaccharide from sea buckthorn berries, and its main antioxidant role was played by the lowest M_w subunit (33 601 Da) in PSB. In addition, Liu et al.^[66] isolated six fractions from sea buckthorn leaves, of which the lowest M_w polysaccharide fraction was SBLP-6, and the ability of SBLP-S-6 to activate NO secretion from macrophages was more significant than other high M_w polysaccharides. Ma et al.^[24] found excellent *in vivo* anti-obesity activity of the small M_w sea buckthorn pectin polysaccharide SBP-1 (9 944 Da). It is mainly because the higher molecular mass of pectin has fewer exonuclease

sites for galacturonase, while the viscosity of intestinal contents increases, leading to a decrease in the frequency of enzyme-substrate contact, which reduces the enzymatic properties of intestinal microorganisms, whereas smaller molecular mass pectin produces more SCFA with better probiotic efficacy when it is fermented in the large intestine^[102]. Not only that, the low fraction of sea buckthorn pectin polysaccharides can also show its superior anticancer efficacy^[25]. Pectin is broken down into smaller fragments with lower M_w during degradation, which are more easily absorbed by cancer cells and thus show better antitumor activity^[103]. It has also been reported that polysaccharides with M_w in the range of 10–1 000 kDa are more likely to interact with TLR4, and polysaccharides with too high or too low M_w are less likely to bind to TLR4^[104]. Appropriately, Zhang et al.^[58] found that sea buckthorn polysaccharide with a M_w of 275 420 prevented CCl₄-induced hepatotoxicity precisely by affecting the expression of TLR4, and consequently its downstream genes.

5.2 Monosaccharide composition

Several studies have demonstrated that the monosaccharide composition of sea buckthorn polysaccharides is closely related to their biological activity^[105]. The types of monosaccharide composition of TLR4-associated polysaccharides have been reported to be very diverse^[104]. The top 5 TLR4-associated polysaccharides were Glu, Gal, Man, Ara, and Rha, and these TLR4-associated polysaccharides shared the same characteristics of common monosaccharide composition as those of sea buckthorn polysaccharides as outlined by Liu et al.^[56] and Zhang et al.^[58]. It was verified that they exert their anti-inflammatory and antioxidant effects through the down-regulation of TLR4 expression, which in turn affects multiple signaling pathways downstream of them, thereby protecting the liver. Notably, adding polysaccharides containing Ara, Xyl, arabinoxylan, and β -glucan has been found to decrease oxidative stress and apoptotic cell count in the border zone^[106]. Moreover, some residues attached to the backbone, such as arabinose, can be released from the polysaccharide by hydrolysis in the gut^[107]. Coincidentally, this observation is consistent with the results of a recent study by Shen et al.^[63], who reported that a sea buckthorn polysaccharide SP0.1-1, rich in Ara monosaccharides extracted from sea buckthorn fruit, has exhibited intestinal protective activity by inhibiting oxidative stress. It has also been noted that galactose is closely related to antitumor effects. The retention and proportion of galactose side chains is critical to the anti-tumor effects of pectin. Not only that, a new study on the metabolism of cancer cells shown that when cancer cells carrying a common oncogene were exposed to galactose, these cancer cells died. This is consistent with the results obtained by Wang et al.^[108], that sea buckthorn pectin polysaccharides with repeating units of (1→4)- β -D-galactopyranosyluronic residues have a significant anticancer ability against lung cancer.

5.3 Content of uronic acid

The sea buckthorn polysaccharides can be categorized into neutral and acidic polysaccharides due to the content of uronic acid^[109]. Wei et al.^[27] isolated the crude polysaccharide of sea buckthorn to obtain three polysaccharide fractions, including two acidic polysaccharides and one neutral polysaccharide. It was found that

the antioxidant properties of the acidic polysaccharides SBP-III and SBP-II were significantly higher than those of the neutral polysaccharide SBP-I. This indicated that the antioxidant activity of acidic polysaccharides was better than that of neutral sugars. In addition, the physicochemical properties of sea buckthorn polysaccharides also have an effect on the biological activity, and some sea buckthorn polysaccharides were identified as pectic polysaccharides because of the type of glucuronic acid and glycosidic bonding. Farzaliyev et al.^[35] obtained a low methoxyl pectin rich in galacturonic acid and found that it had a high complexation capacity with lead ions, which is also consistent with the report that low methoxyl pectin has the ability to adsorb heavy metals^[110].

5.4 Conformation properties

The advanced structure of polysaccharides also has a great influence on their biological activities^[111]. The specific spatial conformation of polysaccharides is necessary for their biological activity, such as some polysaccharides with immunomodulatory activity have a triple helical structure^[112]. Liu et al.^[66] found that SBLP-2 with triple helical conformation has more significant immunomodulatory activity than other polysaccharides in different fractions of polysaccharides isolated from the same raw material. Besides, a study has been reported that the modified polysaccharide could enhance its biological activity or reflect new activity. Tang et al.^[113] extracted SLP from sea buckthorn leaves and subsequent preparation of sulfated sea buckthorn leaf polysaccharide (SSLP) via chlorosulfonic acid-pyridine revealed that both SSLP and SLP could increase the activity of alcohol dehydrogenase (ADH), accelerate the oxidative metabolism of alcohol, and alleviate the oxidative stress induced by alcohol metabolism by reducing ROS levels. The ability of SSLP to mitigate the effects of alcohol was significantly better than that of SLP. This was mainly since after sulfation modification of the polysaccharide, the hydroxyl group on the saccharide ring and the sulfate group form hydrogen bonds, which, in turn, prolong the glycoside chain to form a helical structure while reducing the viscosity of the polysaccharide^[114].

5.5 Conclusions and future perspectives

Sea buckthorn polysaccharides are a promising natural resource that has attracted considerable attention from researchers due to their high content, non-toxicity, and high bioactivity. This paper reviews and provides insights into relevant research reports on sea buckthorn polysaccharides, including preparation, structural features, bioactivities, and mechanisms, as well as the relationship between structure and activity of sea buckthorn polysaccharides. The aim of this review is to promote the further development and utilization of sea buckthorn polysaccharides in food, pharmaceutical, and cosmetic applications. Despite extensive research on sea buckthorn polysaccharides, several areas still require improvement. Research around sea buckthorn polysaccharides needs to focus on exploring more efficient, clean, and energy-saving technologies for their extraction, achieving standardization and high quality, and promoting their industrialization. Additionally, most of the sea buckthorn polysaccharides involved in the study were crude, and only a few of the purified fractions were characterized, therefore,

structural characterization needs to be advanced. The study of the structure-activity relationship of polysaccharides is both a priority and a challenge, and researchers need to analyze the structure of polysaccharides accurately, comprehensively study their biological activities, and establish a database of polysaccharide conformational relationships.

Furthermore, molecular modifications of sea buckthorn polysaccharides, such as acetylation, phosphorylation, sulphation, carboxylation modifications, and so on, need to be investigated to understand their effects on the physicochemical properties and to further improve the biological activity of the modified sea buckthorn polysaccharides^[115-116]. The powerful prebiotic activity of sea buckthorn polysaccharides is now known to be closely related to many other biological activities, and further exploration of its bioavailability is necessary. Finally, modern technologies such as genomics, proteomics, metabolomics, transcriptomics, and bioinformatics can be utilized to unravel the bioactivity mechanisms of sea buckthorn polysaccharides.

Conflict of interest

Nan Shang is a associate editor for *Food Science and Human Wellness* and was not involved in the editorial review on the decision to publish this article. None of the authors have any conflict of interest.

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