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Review Article

Research progress on extraction, structure, bioactivity, structure-activity relationship and product applications of polysaccharides from Mori Fructus

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Abstract: Mori Fructus (MF) is the dried fruit of *Morus alba* L., which is a dual-purpose resource for medicine and food. As one of the main active components of MF, polysaccharides exhibit many biological activities such as immunomodulation, hypoglycaemia effects, liver protection, and regulation of the intestinal flora. In recent years, the extraction and purification methods, structure, biological activity, and structure-activity relationship of Mori Fructus polysaccharides (MFP) have attracted extensive attention. MFP composed of repetitive disaccharide fragments of 1,4-linked *α*-Gal*p*A and 1,2-linked *α*-Rha*p*. Structural information such as extraction yield, molecular weight (*M*w), and monosaccharide composition of MFP differs based on the extraction process used. In addition, the *M*w, monosaccharide composition, and chemical modification of MFP are closely related to its biological activity. Currently, the research of MFP is still in the primary stage, and further exploration is required to fully reveal their structure and biological functions. This paper systematically reviews the research progress of extraction, purification, structural characterization, and biological activities of MFP, deeply analyzes their structure-activity relationship and product application status, and discusses some existing problems in order to provide support for the further development and utilization of MFP.

Keywords: mori fructus; polysaccharides; structural characteristics; bioactivities; structure-activity relationship; application

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1 Introduction

Morus alba L. is a short-lived, fast-growing, and medium-sized tree species. The most common tree species are white mulberry (*M. alba* L.), black mulberry (*M. nigra* L.) and red mulberry (*M. rubra* L.) (Fig. 1)[1, 2]. Mulberry trees are mostly distributed in temperate and subtropical regions in the northern hemisphere and tropical regions in the southern hemisphere. They have extensive requirements for climate, topography, and soil conditions[3]. MF is the dried fruit of *Morus alba* L., and it is a medicinal and edible resource[4]. MF has various functions, such as immunomodulation, antioxidation, hypoglycemic, hypolipidemic, neuroprotective, anti-tumor, as well asliver, kidney, and eye protection[5-8]. Currently, MF is used in products like oral liquid, health yogurt, and is widely applied in functional foods, dietary fiber supplements,etc[9]. As one of the main active components of MF, MFPs has many biological activities, such as immunomodulation, anti-inflammation, liver protection and regulating intestinal flora activity[10-13]. In recent years, MFP has attracted extensive attention because of its good biological activity. At present, the widely used extraction methods of MFP include hot water extraction (HWE), enzyme-assisted extraction (EAE) and ultrasonic-assisted extraction (UAE)[14-16]. Studies have shown that the structure of polysaccharide is closely related to its biological activity[3, 13]. Currently, certain chemical structural characteristics of MFP have been identified, and its various biological activities have been extensively evaluated. However, the structural features and biological properties of multifunctional peptides obtained from different sources and extraction methods exhibit significant variations, thereby greatly impeding the development and utilization of MFPs[17-19].

Therefore, this paper systematically summarizes the extraction and purification methods, structural characteristics, biological activities, structure-activity relationships and applications in medicine and health foods, which provided a reference for further research and practical application of MFP. The research idea of this paper is shown in Fig. 2.

2 Extraction of MFP

Polysaccharides are widely distributed in nature, but there are few active polysaccharides in the organisms[20]. Selecting an efficient extraction method is crucial in polysaccharide research. The extraction rate, chemical composition, and structural

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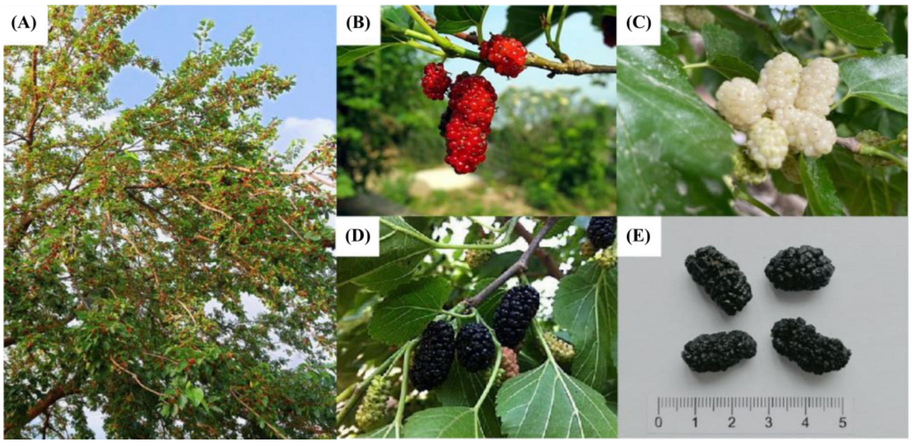

Figure 1 Tree of *Morus alba* L. (A), Fresh fruits of red mulberry (*M. rubra* L.) (B), Fresh fruits of white mulberry (*M. alba* L.) (C), Fresh fruits of black mulberry (*M. nigra* L.) (D), Mori Fructus (E).

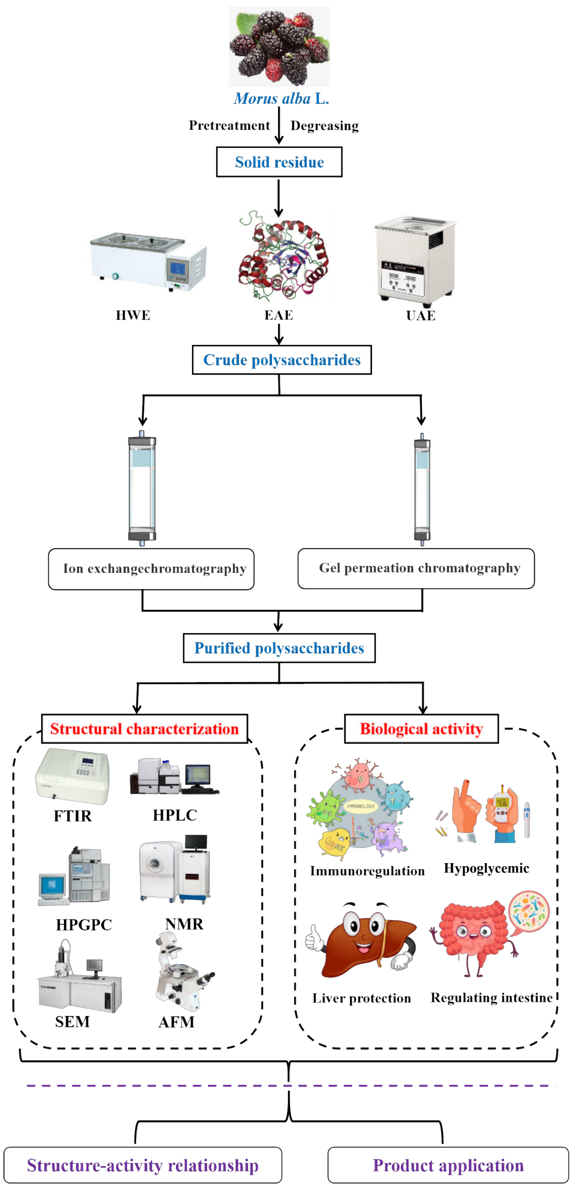

Figure 2 Overall research idea chart of MFPs.

morphology of polysaccharides prepared by different extraction processes will be different[21]. HWE, EAE, and UAE are the primary extraction methods for MFP. Future research should focus on selecting appropriate extraction methods to obtain high-quality MFP with strong biological activity[22]. The extraction methods, conditions, and yields of MFP are shown in Table 1.

2.1 HWE method

HWE is a simple and efficient method to extract plant polysaccharides, which includes two steps: HWE and ethanol precipitation[23]. Based on the difference in solubility of polysaccharides in water and ethanol, the extraction solution is precipitated in ethanol overnight at a specific ratio. The polysaccharides are obtained after precipitation and drying[24]. Wei *et al.* extracted three times under the conditions of solid-liquid ratio of 1: 3 (g/mL), extraction temperature of 80 °C, extraction time of 2 h, and the extraction rate of *Fructus Mori Albae* polysaccharide (FMAP) was 9.28%[25]. Huang *et al.*[26] added defatted mulberry fruit to distilled water at a ratio of 1: 15 (mg/mL) and extracted twice in hot water at 80 °C for 3 h, and the extraction rate was 8.45 ± 0.85%. Similarly, Chen *et al.*[27] extracted with hot water (85-90 °C) at the ratio of 1: 5 kg/L for three times, each time for 2 h, and the extraction rate of MFPs was 4.45%. In conclusion, the solid-liquid ratio, extraction temperature, extraction duration, and extraction periods all affect the HWE method's extraction rate. Besides, HWE has the advantages of low equipment requirements, high recovery rate, no subsequent reagents and suitability for industrial mass production. It does, however, have certain drawbacks, including a high solvent consumption rate for ethanol, a lengthy extraction process, and limited extraction efficiency.

2.2 EAE method

Since the majority of the active ingredients in plants are found in their cellulose-based cell walls, adding enzymes to the extraction process helps the polysaccharides dissolve[28,29]. Ai *et al.*[30] used cellulase to obtain a water-soluble polysaccharide-CE-BMP from black mulberry with an extraction rate of 25.1%, and compared with the polysaccharide extracted without enzyme or with other enzymes, the addition of cellulase improved the extraction rate of polysaccharide. Similarly, Deng *et al.*[31] compared the extraction rates of MFP with 0.15% glucose oxidase, 0.15% pectinase, 0.15% β-dextranase, 0.15% cellulase and without enzyme at 14.23%, 14.01%, 13.08%, 11.69% and 9.21%, respectively. Li *et al.*[32] used cellulase, amylase, and papain to extract MFP, achieving an extraction rate of 15.20%. The aforementioned findings demonstrate how adding specific amounts of enzymes, such as

Table 1 Different extraction methods of MFPs

Source	Name	Method of extraction	Time (min)	Temperature/Pressure/ Power (°C/W)	Water/Materia l ratio (g/mL)	Yield (%)	Ref.
Mulberry fruits	MFP	HWE	2 h	90 °C	1:40 (*w/v*)	5.86%	[18]
Fructus *Mori Albae*	FMAP	HWE	2 h	80 °C	--	9.28%	[25]
Mori Fructus	MFP	HWE	2 h	85-90 °C and extract 3 times	1:5 (kg/L)	4.45%	[27]
Morus alba fruit	MFP50	HWE	3 h	80 °C and extract 3 times	1:10 (*w/v*)	40.90%	[39]
	MFP90	HWE	3 h	80 °C and extract 3 times	1:10 (*w/v*)	3.75%	
Mori Fructus	MFPs	EAE	38 min	glucose oxidase (0.033%) at 58 °C	1:11 (*w/v*)	3.25%	[15]
Black mulberry	CE-BMP	EAE	4 h	45 °C, pH 4.5 and enzyme amount of 0.3%	1:6 (*w/v*)	25.1%	[30]
Fructus Mori	CFMP	EAE	2 h	glucose oxidase and 50 °C	1:10 (*w/v*)	14.23%	[31]
Fructus Mori	MFP	EAE	1 h	30 g cellulase, 10 g amylase and 5 g papain at 55-60 °C	--	15.2%	[32]
Mulberry fruits	MFP	UAE	75 min	69 °C and 190 W	1:40.25 (*w/v*)	3.13 ± 0.07%	[16]
Fructus Mori	MFPh	HWE	2 h	90 °C	1:40 (g/mL)	54.13 ± 5.88%	[17]
	MFPc	CAE	2 h	90 °C	1:40 (g/mL) HCl solution (0.1 mol/L)	73.31 ± 5.01%	
	MFPa	AAE	2 h	90 °C	1:40 (g/mL) NaOH solution (0.1 mol/L)	68.42 ± 6.13%	
	MFPu	UAE	2 h	90 °C and 200 W	1:40 (g/mL)	61.17 ± 5.57%	
Mori Fructus	MFP-I	CWE	12 h	room temperature and extract 2 times	1:15 (g/mL)	11.06 ± 0.61%	[26]
	MFP-II	BWE	3 h	96-98 °C and extract 2 times	1:15 (g/mL)	6.08 ± 0.54%	
	MFP-III	UAE	30 min	50% (*v/v*) anhydrous ethanol and extract 2 times	1:15 (g/mL)	13.32 ± 0.98%	
	MFP-IV	UAE	30 min	25% (*v/v*) anhydrous ethanol and extract 2 times	1:15 (g/mL)	4.00 ± 0.59%	
	MFP-V	UAE	30 min	extract 2 times	1:15 (g/mL)	2.10 ± 0.50%	
	MFP-VI.	HWE	3 h	80 °C and extract 2 times	1:15 (g/mL)	8.45 ± 0.85%	
Mulberry fruits	MFP-HWE 100	HWE	2 h	90 °C	1:40 (*w/v*)	4.66%	[37]
	MFP-EAE 100	EAE	2 h	1%(w/v) cellulase at 50 °C and pH 5.0	1:40 (*w/v*)	5.64%	
	MFP-UAE 100	UAE	75 min	69 °C and 150 W	1:40 (*w/v*)	5.50%	
	MFP-HSEHE 100	HSEHE	1 h	rate of shear 12000 g, shearing time 2 min extraction temperature 60 °C	1:40 (*w/v*)	7.52%	

Notes: -- not mentioned. Acid-assisted extraction (CAE), Alkali-assisted extraction (AAE), Cold water extraction (CWE), Boiling water extraction (BWE), High-speed shear homogenization-assisted hot water extraction (HSEHE)

cellulase, glucose oxidase, and pectinase, can greatly increase the yield at which polysaccharides are extracted. The advantages of EAE are mild reaction conditions, fast reaction speed, and high repeatability; however, enzymes are expensive and easily inactivated by external factors, making reaction conditions challenging to control.

2.3 UAE method

The principle of UAE is to use the cavitation effect of ultrasonic waves to increase the movement speed of the medium molecules, allowing the polysaccharides to dissolve more quickly[33-34]. Chen *et al.*[16] optimized the UAE of mulberry polysaccharide-MFP using single factor experiment. The optimal extraction conditions was as follows: solid-liquid ratio of 1: 40.25 (g/mL), extraction temperature 69 °C, ultrasonic power 190 W, extraction time 75 min, and the yield of MFP was 3.13 ± 0.07%. Similarly, Zhang[35] obtained the optimum extraction conditions of sMFPs through the orthogonal test: extraction time 30 min, solid-liquid ratio 1: 30 (g/mL), ultrasonic power 160 W, ultrasonic time 12.5 min, extraction temperature 30 °C, and extraction rate of MFP 6.63 ± 0.07%. In addition, the order of influencing factors of MFP extraction rate is ultrasonic power > extraction temperature > ultrasonic time > solid-liquid ratio. Generally speaking, UAE has low requirements on temperature, which can effectively prevent the influence of high temperature on the chemical and physical characteristics and biological activity of MFP. However, if the ultrasonic time is too long, the spatial structure of polysaccharide will also be destroyed.

2.4 Other methods

In addition to the above extraction methods, some scholars have also used microwave extraction technology, alcohol extraction and new instruments to assist in the extraction of MFP. Zhang *et al.*[36] used microwave-assisted technology to extract polysaccharides from mulberry. The results of an orthogonal test showed that the ideal technological parameters of mulberry polysaccharide were as follows: the ratio of material to liquid was 1: 25 g/mL, and the microwave irradiation time was 150 seconds. The yield of polysaccharide was 11.31% after three times extraction. Compared with other technologies (such as HWE and UWE), microwave extraction has several advantages, including high efficiency, rapidity, energy saving and high yield. Lee *et al.*[10] extracted polysaccharides (JS-MP) from Korean mulberry fruit *Oddi* with 75% ethanol using alcohol extraction based on similar dissolution principles. Li *et al.*[37] used high-speed shear homogenization-assisted HWE (HSEHE) and the above several common extraction methods to extract MFP and found that HSEHE100 extracted by HSEHE had the highest extraction rate of

7.52%.

In summary, the extraction rate of MFP is related to the source of raw materials, extraction conditions and extraction methods. HWE is still the most commonly used extraction method because of its convenient operation. Compared to the other two methods, EAE has the highest extraction rate, despite its somewhat complex operation. The UAE has the lowest extraction rate, which may be caused by variations in temperature, duration, and other factors. The extraction method of MFP is relatively simple, so researchers can combine two or more methods, or use some emerging extraction methods (such as supercritical fluid extraction and ultrafiltration extraction) in the extraction process of MFP in order to achieve higher extraction rate.

3 Isolation and purification

The components of MFP extracted by the above extraction method are relatively complex and have lower purity, and further separation and purification are required to obtain components with high purity, which is convenient for structural and biological activity research.

Currently, the separation and purification methods of polysaccharides are mainly membrane separation, precipitation separation, and column chromatography[38-40]. The principle of membrane separation technology is to apply driving force on both sides of the membrane, so that polysaccharide liquid can selectively pass through the membrane according to different molecules[41]. Yuan *et al.*[42] applied membrane technology to separate MFP in stages. After HWE, 300, 50, 5 and 1 kDa ultrafiltration membranes were used for the fractional separation of MFP, which were recorded as MFP300, MFP50, MFP5 and MFP1, respectively. Results showed the main components and biological activities of the four polysaccharides showed certain differences. MFP300 exhibited the best antioxidant, antiallergic, and *in vitro* ethanol dehydrogenase (ADH) activity, while MFP1 exhibited the best hypoglycemic activity.

Precipitation separation was according to the different Mw of polysaccharide solubility in different solvents, by gradually increasing the concentration of ethanol, polysaccharides were precipitated according to the Mw[43]. Hu *et al.*[44] obtained three different components of mulberry fruit by fractional alcohol precipitation with 30%, 60%, and 90% ethanol. Different amounts of sugar, galacturonic acid (GalA), and protein were present in the MFP. MFP is also frequently separated and purified using gel column chromatography and ion exchange column chromatography. The ion exchange column method is used to separate polysaccharides according to their different polarity and charge properties, while the gel column chromatography method is used to separate polysaccharides according to their different spacings between gel molecules and their different Mw[45,46]. To achieve a better purification effect, it was necessary to comprehensively utilize the combination of two purification methods, which was the most common combination method for purifying MFP. Wang *et al.*[19] used a DEAE- agarose fast flow column and G-100 gel column to purify MFP-4P, and the polysaccharide content was 95.4%. The difference is that BMP-1-1 and BMP-2-1 were obtained by purifying MCP with DEAE-52 cellulose and a G-100 column, and the sugar content was 83.01% and 88.15% respectively[14]. Deng *et al.*[47] used the combination of cellule DEAE-52 column and Sephadex G-100 column to separate and purify three components (MFP-1, MFP-2, and MFP-3) from the Mori Fructus polysaccharide and explore their effects on the activity of alcohol dehydrogenase. At present, the separation and purification methods of MFP are mostly concentrated on ion exchange column chromatography and gel column chromatography, while other purification methods are less common and require further research.

4 Structural characterizations

As the structural characteristics of MFP are closely related to its biological activity, a thorough understanding of MFP's structure can facilitate its application and optimize its bioactivity. Therefore, the MFPs reported in the past few years are summarized, and the related literatures including molecular weight, monosaccharide composition, glycosidic bond type and spatial conformation are summarized in Table 2.

4.1 Molecular weight

Currently, the commonly employed methods for determining Mw are high performance gel permeation chromatography (HPGPC), high-performance liquid chromatography (HPLC), and size exclusion chromatography with multiangle laser light scattering (SEC-MALLS)[14,17,48]. Chen *et al.*[18] isolated and purified MFP3P from MFP, and separated and purified to obtain MFP3P, with an average Mw of 136.6 kDa determined by HPGPC system equipped with TSK-GEL G-5000 PWXL and TSK-GEL G-3000 PWXL columns. Zhou *et al.*[15] extracted MFP from fresh fruits of *Morus alba* L. using HWE and determined their average Mw to be 112.2 kDa to 181.9 kDa using HPLC equipped with Ultrahydrogel 250 column and refractive index detection. Dong *et al.*[48] determined that the Mw of Fruits Mori polysaccharide (MFP1P) was 56.78 kDa using SEC-MALLS technology. In comparison with HPGPC, SEC-MALLS is a more efficient method for measuring the Mw of polysaccharides. It can assess the weight-average Mw, number average Mw and coefficient of MFP[49,50]. According to Table 2, the Mw distribution spans of polysaccharides isolated from mulberry is very large, ranging from 0.7 kDa to 1639 kDa. This may be related to different sources of MF and different extraction and purification methods.

4.2 Monosaccharide composition

In the determination of monosaccharides, polysaccharides are usually hydrolyzed by HLPC and high performance anion exchange chromatography with pulsed current detector[51-53]. Liu *et al.*[54] determined mulberry polysaccharide (PMF-3) by HPLC method, and found that it was composed of glucose (Glc), arabinose (Ara), GalA, Glucuronic acid (GlcA), mannose (Man), rhamnose (Rha), galactose (Gal) and fucose (Fuc), among which the proportion of Glc was the largest. Zhang *et al.*[55] isolated three kinds of acidic *Fructus Mori* L. polysaccharides (MFP1, MFP2 and MFP3), and analyzed their monosaccharide composition by ion exchange chromatography. The results indicated that all of them were composed of Fuc, Ara, Gal, Glc, Xyl, Man, GalA and GlcA. Among them, GalA was the acidic monosaccharide with the largest proportion in MFPs. Huang *et al.*[26] determined the MFP obtained by six extraction methods by HPLC, which were mainly composed of Glc, followed by Man, Rha, Xyl and Ara. Different extraction methods have little effect on the types of monosaccharides, but the molar ratio is different. In summarize, Glc is the main neutral monosaccharide of MFP, and GalA is the main acidic monosaccharide of MFP. Additionally, different extraction methods have a negligible impact on the monosaccharide composition of polysaccharides; they merely affect the proportion of the monosaccharide composition.

Table 2 Structural characteristics of MFPs

Source	Name	*Mw* (kDa)	Monosaccharide composition	Architectural feature	Ref.
White mulberry fruits	JS-MP-1	1639	Gal: Ara: Rha: Glc: Xyl: Man: Fuc = 37.6: 36.3: 18.4: 3.1: 1.7: 1.6: 1.3	T-Araf: 1,5-Araf: 1,2-Rhap: 1,2,4-Rhap: T-Glcp: T-Galp: 1,3-Galp: 1,4-Galp: 1,6-Galp: T-Galp: 1,3,6-Galp = 27.7: 11.0: 16.3: 3.2: 2.2: 0.2: 4.6: 12.4: 3.8: 28.6	[10]
Black mulberry fruit	BMP-1-1	615	Man: Rha: GlcA: Gal: Ara = 1.46: 2.14: 2.16: 48.27: 45.97	--	[14]
	BMP-2-1	405	Rha: GlcA: GalA: Gal: Ara = 23.55: 2.04: 27.5: 22.38: 24.54		
Mori Fructus	MFPs	112.2-181.9	Glc: GalA: Rha: Gal = 39.79: 17.15: 15.16: 14.48, along with a small quantity of GlcA, Xyl, Ara and Man	--	[15]
Mulberry fruits	MFP3P	136.6	Rha, Ara, Gal, Glc and GalA = 25.98, 21.51, 23.1, 13.06 16.35%	--	[18]
Mulberry fruit	MFP4P	198.2	Gal: Ara: Rha: Glc: GalA = 3.29: 9.86: 13.60: 67.85: 5.40	1,6-Glc: T-Ara: 1,2-Rha: 1,3-Rha: 1,3-Gal = 10.34: 1.81: 1.77: 0.83: 0.58	[19]
Fructus *Mori Albae*	FMAP	130	--	1,3-Galp: 1-Manp: 1-Galp: 1 4-Glcp: 1,6-Galp: 1,3,6-Manp = 8.6: 26.9: 2.4: 5.2: 17.6: 39.0	[25]
Fructus Mori	FMP-6-S2	86.83	Rha: GalA: Gal: Ara = 30.91: 24.78: 28.70: 15.61	T-Ara: 1,5-Ara: 1,2-Rha: T-Gal: 1,2,4-Rha: 1,4-Gal: 1,3,6-Gal = 12.31: 10.53: 16.10: 17.38: 22.15: 11.70: 9.83	[32]
Mulberry fruit	MFP- Ⅱ	115	GalA: Glc: Gal: Man: Rha: Xyl: Ara = 3.6: 23.1: 10.9: 2.9: 30.5: 2.7: 26.3	Rhap-(1→: →2)-Rhap-(1→: →4)-Galp-(1→: →6)-Galp-(1→: Araf-(1→= 6.9: 20.1: 38.8: 6.4: 27.8	[44]
Fructus Mori	MFP1P	56.78	--	→3)-α-D-Gal(1→, β-DMan-(1→, and →6)-α-D-Glc(1→	[48]
Mulberries	PFM-3	96.17	Glc: Ara: GalA: Glu: Man: Rha: Gal: Fuc = 33: 2.57: 1: 7.43: 8.57: 5.29: 5.57: 7.43	--	[52]
Mori Fructus	P1-MFPs	316	Man: Rha: GlcA: Glc: Xyl = 11.0: 5.4: 4.7: 33.5: 45.5	--	[27]
	P1-MFPs	398	Man: Rha: Glc: Xyl = 7.2: 1.4: 28.6: 62.8		
Mulberry fruits	MFP-HWE100	363.30	Man: Rha: GalA: Glc: Gal: Ara: Fuc = 1.03: 0.95: 0.13: 2.08: 1.00: 0.55: 0.76	1,4-Glcp: 1,4-Rhap: 1-Araf: 1-Fucp: 1,6-Galp: 1,4,6-Glcp: 1,6-Manp: 1,3,6-Manp = 23.48: 12.91: 8.63: 10.94: 13.56: 10.26: 12.17: 7.05	[37]
	MFP-EAHE100	309.07	Man: Rha: GalA: Glc: Gal: Ara: Fuc = 2.57: 1.24: 0.19: 5.24: 1.00: 0.51: 1.06	1,4-Glcp: 1,4-Rhap: 1-Araf: 1-Fucp: 1,6-Galp: 1,4,6-Glcp: 1,6-Manp: 1,3,6-Manp = 30.69: 10.67 : 5.87: 9.44: 8.17: 14.40: 13.21: 7.55	
	MFP-UAHE100	269.08	Man: Rha: GalA: Glc: Gal: Ara: Fuc = 3.47: 1.45: 0.21: 6.96: 1.00: 0.47: 1.55	1,4-Glcp: 1,4-Rhap: 1-Araf: 1-Fucp: 1,6-Galp: 1,4,6-Glcp: 1,6-Manp: 1,3,6-Manp = 31.19: 9.69: 3.15: 8.47: 7.38: 17.52: 13.16: 9.44	
	MFP-HSEHE100	167.59	Man: Rha: GalA: Glc: Gal: Ara: Fuc = 2.49: 1.44: 0.35: 6.11: 1.00: 0.34: 1.49	1,4-Glcp: 1,4-Rhap: 1-Araf: 1-Fucp: 1,6-Galp: 1,4,6-Glcp: 1,6-Manp: 1,3,6-Manp = 30.03: 8.35: 3.14: 9.16: 8.03: 18.90: 12.35: 10.03	
Mori Fructus	MFP-1	20.93	Gal: Glc: Ara: Rha: Man = 26.80: 20.40: 8.70: 5.00: 1.00	--	[47]
	MFP-2	56.23	Ara: Gal: Rha: Glc: GalA = 34.2: 38.2: 8: 17.5: 15.1		
	MFP-3	118.76	Glc: Gal: Rha: GalA: Ara: GlcA = 47.4: 34.9: 36.1: 33.1: 19.9: 4.1		
Black mulberry fruit	MFP-1	144.22 and 35.66	Fuc: Ara: Gal: Glc: Xyl: Man: GalA: GlcA = 2.08: 20.84: 17.55: 2.99: 0.61: 2.84: 50.69: 2.41	--	[53]
	MFP-2	62.59 and 21.46	Fuc: Ara: Gal: Glc: Xyl: Man: GalA: GlcA = 0.56: 15.01: 8.36: 2.11: 0.44: 0.20: 71.55: 1.76		
	MFP-3	351.28	Fuc: Ara: Gal: Glc: Xyl: Man: GalA: GlcA = 1.44: 25.30: 12.90: 3.87: 0.82: 1.43: 49.12: 5.12		
Black mulberry	MFP-1	9.5,7.9,1.0 and 0.7	Ara: Gal: Glc: Xyl: Man: GalA = 19.19:31.40: 26.31: 5.98: 7.12: 1.57	--	[61]
	MFP-2	149.4, 9.3, 2.6 and 1.5	Ara: Gal: GalA = 44.90: 55.20: 2.11		
	MFP-3	166.7, 5.0 and 1.5	Ara: Gal: GalA = 55.30: 44.70: 6.18		
	MFP-4	184.7, 64.4 and 1.5	Ara: Gal:Glc: GalA = 59.86: 27.15: 12.99: 31.90		
Fructus Mori fruit	FPA-1	10.6-49.1	Rha: GalA: Gal: Ara = 1.0: 2.4: 2.7: 4.9	--	[66]
	FPA-2	22.9-122.7	GalA: Gal: Ara = 36.93: 11.19: 19.69		
	FPA-4	57.1-105.4	Rha: GalA: Ara = 14.6: 38.04: 15.8		
	FPA-5	14.8-19.6	Rha: GalA: Ara = 5.81: 44.38: 21.66		

Notes: - not mentioned.

4.3 Glycosidic bond type

Polysaccharides are formed by connecting more than 10 monosaccharides through glycosidic bonds. The type of glycosidic bonds is an important part of determining the primary structure of polysaccharides[56]. Currently, the principal approaches for determining the composition of polysaccharide residues and the connection positions of glycosidic bonds are mainly methylation-gas chromatography-mass spectrometry (GC-MS)[57]. Fourier transform infrared (FT-IR) spectroscopy and nuclear magnetic resonance (NMR) spectrum are mainly used to determine the types of polysaccharide rings[58]. MFP4P was obtained by Wang *et al.*[19] through DEAE-agarose gel rapid flow and Sephadex G-100 column purification, and MFP4P was confirmed to have *β*-glycosidic bond by FT-IR spectrum. NMR spectrum showed that the linkage type of MFP4P was (1→6)-linked *α*-D-Glc*p*, (1→2)-linked *α*-L-Rha*p*, (1→3)-linked *α*-D-Gal*p*, (1→3)-linked *β*-L-Rha*p* and (→1)-linked *α*-L-Ara*f*, and the FT-IR spectrum was also confirmed using NMR spectrum. Li *et al.*[32] purified mulberry polysaccharide (FMP-6-S2) by DEAE-Sepharose fast flow column and Sephacryl S-300 HR gel column, and its NMR results were shown in Fig. 3A. The main chain of the polysaccharide was a repeating unit of 1,4-linked *α*-GalpA and 1,2-linked *α*-Rhap, which was a typical feature of RG I, and the branch was connected at C-4 of Rhap. The branches included 1,4-linked *β*-Gal*p*, 1,3,6- and T-linked *β*-Gal*p*, and T- and 1,5-linked *α*-Ara*f*. It was hypothesized that the main chain of FMP-6-S2 could be rhamnogalacturonan acid polysaccharide (RG-I) pectin polysaccharide, and the branch chain was arabinose gum polysaccharide. Similarly, JS-MP-1 (Fig. 3B) was isolated and studied by Lee *et al.*[10] and NMR results showed that the main chain was 1,4-*α*-D-Gal*p*A, 1, 2-*α*-Rha*p* and the side chains were (1→5)-*α*-L-Ara*f* and arabinose galactan type II (AG II), so it was deduced that JS-MP-1 was a pectin polysaccharide of RG I with Ara*f* and AG II side chains. In addition, Wei *et al.*[25] analyzed the structure of FMAP (Fig. 3C), in which the main chain was composed of (1→3) linked Man*p*, and the branches of Gal*p* residue and Glc*p* residue were attached to *O*-6. A possible reason for the diverse structures might be that the sources, extraction, and purification methods of polysaccharides vary, thus they may also possess different biological activities.

4.4 Microtopography and conformation

The biological activity of polysaccharides is influenced by their micro-morphology and spatial conformation[59]. Currently, techniques like scanning electron microscopy (SEM), atomic force microscopy (AFM), dynamic light scattering (DLS), and electronic circular dichroism (ECD) have been utilized to investigate the micro-morphology and chain conformation of MFP[60-62]. Zhang *et al.*[55] and Chen *et al.*[63] used the HWE method to extract mulberry fruit polysaccharides MFP with different components after separation and purification. The SEM results demonstrated that all the several purified components of MFPs presented flaky structures with smooth and dense surfaces, suggesting that separation and purification exerted little influence on the surface morphology of MFPs. However, some studies showed that the surface morphology of MFP may be related to the extraction method. Huang *et al.*[26] used CWE to obtain the MFP-I with a wrinkled surface, BWE obtained the MFP-II (the sugar content was 64.33%) with irregular and rough surfaces with small particles of different shapes, while the MFP-IV (the sugar content was 81.28%) extracted by UAE had a relatively smooth surface, compact structure and stable sheet structure. This might be because the polysaccharides extracted by different methods have

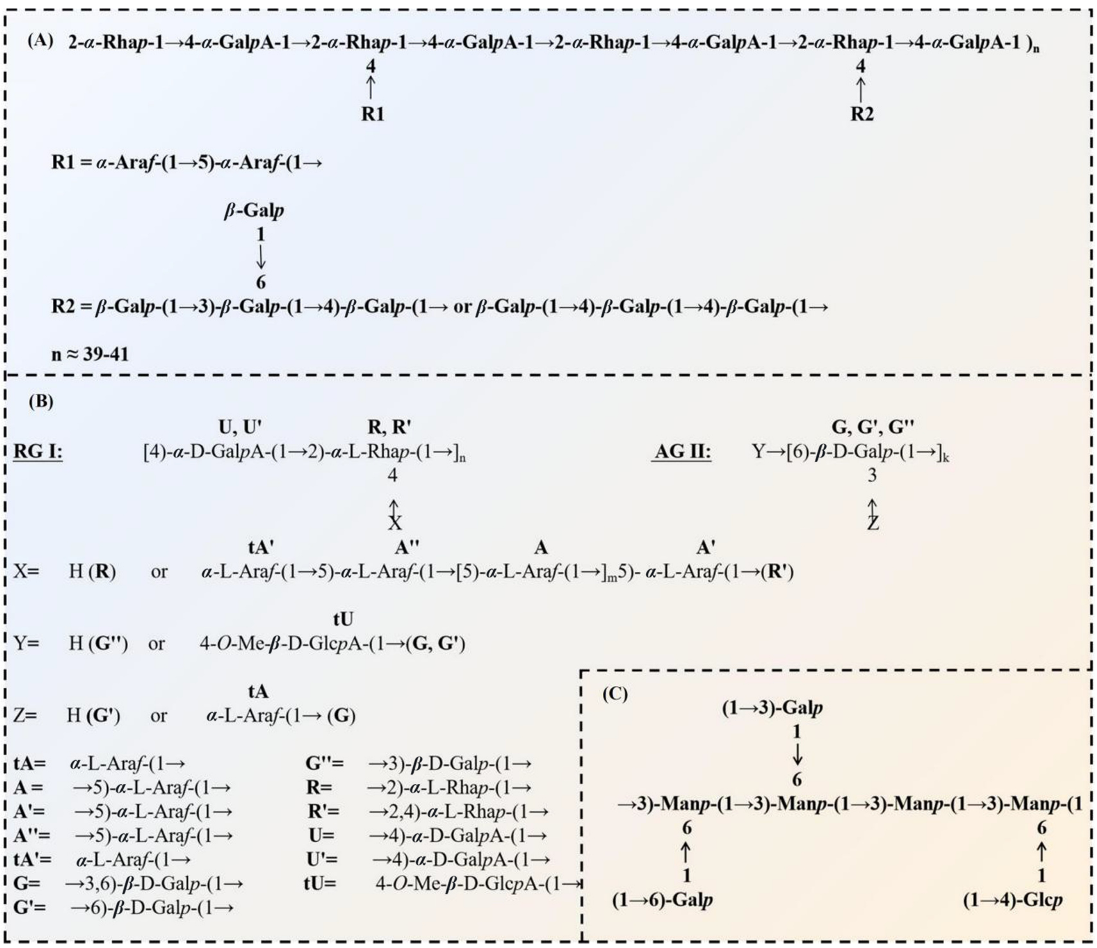

Figure 3 Structural schematic diagram of FMP-6-S2 (A), JS-MP-1 (B), FMAP (C)[32, 10, 25].

different polysaccharide content and viscosity. Similarly, surface of BMP (the sugar content was 37.6-56.0%) obtained by Ai *et al.*[30] by enzymatic extraction had more voids and cracks. In addition, the structural modification will also cause the appearance change of MFP. Wang *et al.*[19] modified the structure of MFP, and the SEM results showed that the surface of the MFP4P sample was coral-like with closely arranged gaps, while the MFP4P-Zn modified by Zn was curly and flaky, with rough surface. According to AFM analysis of MFP3P by Chen *et al.*[18], MFP3P is monodisperse spherical and irregular particles in dilute solution. Dong *et al.*[48] combined with AFM, DLS and SEC-MALLS technology, it was further confirmed that MFP presented a uniform spherical chain conformation in solution.

Additionally, some researchers also studied the spatial form of MFP. The ECD spectra of MFPs obtained by Chen *et al.*[17] by different extraction methods showed a positive Cotton effect around 191 nm-211 nm, and a negative cotton effect in different degrees in the range of 205 nm-240 nm, which indicated that MFPs all had asymmetric structures. The Congo red test is employed to determine the triple helix structure within polysaccharides. Chen *et al.*[63] conducted the Congo red test and discovered that the maximum absorption peak position of all MFP components did not change along with the increase in NaOH concentration, suggesting that they did not possess a triple helix conformation. This was similar to the research results of Dong *et al.*[48]

In summary, the Mw of MFP is widely distributed, mainly in the range of 5-405 kDa, and the monosaccharide composition and proportion are different, which are related to the source of raw materials, extraction methods and separation methods. And MFP is mainly RGI pectin-type polysaccharides consisting of 1,4-linked *α*-Gal*p*A and 1,2-linked *α*-Rha*p* repeated disaccharide fragments, with the branches of arabinoxylan and AG-II at the same time, as well as a small part of galactomannan structure. In addition, the surface of MFP is flaky and closely arranged, presenting a uniform spherical chain conformation in solution and having a certain asymmetric structure. However, there are several issues in the study of advanced structures such as the stereo morphology and spatial conformation of MFP. To better clarify the relationship between the structure and spatial conformation of MFP and its biological activity, researchers can further analyze the structure of MFP by using emerging technologies.

5 Bioactivities of MFP

In recent years, polysaccharides has been used as medicine to treat many diseases due to their excellent biological activity, low toxicity and high bioavailability, and they also hold great potential in health food supplements. Therefore, this paper systematically summarizes and analyzes the biological activities and mechanism of MFP, providing a theoretical basis for the future development and application of MFP in numerous fields such as medicine and food. The biological activities of MFP and their mechanism of action are summarized in Table 3.

5.1 Immunomodulatory activity

The immunomodulatory activity of polysaccharides is one of the most significant activities, and the immunomodulatory effect of polysaccharides is closely associated with natural killer cells, T cells, B cells, macrophages, and dendritic cells[64]. Macrophages, an important member of the mononuclear phagocyte system, are the first step in resisting the invasion of pathogens into the human body, and dendritic cells, as the strongest antigen-presenting cells, can cause most of the immune response[65,66]. Polysaccharides can activate macrophages to release inflammatory cytokines and chemokines and induce nitric oxide synthase (iNOS) to produce nitric oxide (NO)[67]. Wang *et al.*[68] measured the immune factors of mouse peritoneal macrophages and discovered that the acidic component of *Morus alba* L. polysaccharide (FPA-1) led to a greater stimulation in the production of NO, tumor necrosis factor-α (TNF-α), and reactive oxygen species (ROS). Liu *et al.*[54] confirmed that PFM-3 could promote the decrease of TNF-α and the secretion of interleukin-10 (IL-10) in RAW264.7 cells, regulate the level of inflammation, and had good immunomodulatory activity. Similarly, JS-MP-1 also could stimulate mouse macrophage RAW264.7 cells to release chemokines (RANTES and MIP-1α), TNF-α and interleukin-6 (IL-6), and induce the expression of iNOS, thus playing an immunomodulatory role[10]. Choi *et al.*[69] carried out further research and found that JS-MP-1 decreased the activity of 3T3-L1 preadipocytes in a dose-dependent manner, significantly stimulated the division of caspases 9, caspases 3 and poly (ADP-ribose) polymerase (PARP) in cysteamine, and reduced the ratio of the expression level of anti-apoptosis protein (Bcl-2) to pro protein (Bax), resulting in mitochondrial dysfunction and apoptosis of preadipocytes. At the same time, Liu and Lin[11] also found that mulberry fruit polysaccharides (MFP) could regulate the levels of Bak and Bcl-2 proteins in LPS-stimulated macrophages and significantly protect the primary immune cells from apoptosis. Besides, MFP can make dendritic cells secrete factors and regulate dendritic cells through signaling pathways[70]. Shin *et al.*[71] found that *Mori Fructus* polysaccharide MFP could induce dendritic cells to mature by increasing phosphorylation of mitogen-activated protein kinase, and nuclear transport of nuclear factor-kappa B (NF-κB) p65 subunit.

The immunomodulatory mechanism of MFP is shown in Fig. 4. On the one hand, MFP can activate macrophages, induce mature dendritic cells to release inflammatory cytokines, and attack pathogens or abnormal cells. At the same time, macrophages secrete cluster of differentiation 40 (CD40), cluster of differentiation 80 (CD80), and major histocompatibility complex class II (MHC II) molecules to facilitate the proliferation and differentiation of T cells. On the other hand, MFP binds to mature dendritic cells through toll-like receptors (TLR), thus up-regulating the expression levels of IKKb, p-p65, total p65 and NF-κB, and activating the NF-κB pathway to participate in the immune regulation of mature dendritic cells. Based on the above analysis, MFP can be used as an effective immunostimulant. However, the immunomodulatory mechanism of MFP is mainly centered on *in vitro* activity experiments, and there is a shortage of research on the *in vivo* activity mechanism, which deserves the attention of researchers.

5.2 Hypoglycemic activity

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia caused by many reasons. Diabetic patients need long-term medication, so natural polysaccharide with hypoglycemic effect has attracted wide attention because of their safety and low toxicity and side effects[72]. Chen *et al.*[63] found that MFP had good *α*-amylase and *α*-glucosidase inhibition ability. The inhibitory effect may be due to the large Mw and complex spatial structure of polysaccharides, which can encapsulate starch and enzyme, thus delaying the action of *α*-amylase and *α*-glucosidase. At the same time, Chen *et al.*[73] also found *in vitro*

Table 3 Biological activities of MFPs

Activity	*In vitro/ vivo*	Name	Animal model or evaluation methodology	Modeofaction	Ref.
Immunomodulatory activity	*In vitro*	JS-MP-1	The murine RAW264.7 macrophage cell	RANTES, MIP-1α, TNF-α, IL-6, NO, PGE2↑ Induce iNOS and COX-2	[10]
		MP	Mouse primary splenocytes and peritoneal macrophages	Bak and Bcl-2↑	[11]
		PFM-3	HepG2 cells and RAW 264.7 cells	The G2 phase cell block↓, TNF-α, IL-10↑	[54]
		FPA-1	Mouse macrophageRAW264.7	Phagocytosis↑, NO, TNF-α, ROS↑	[58]
		MFP	Female C57BL/6, BALB/c, C3H/HeN and C3H/HeJ dendrites cells, Spleen response T cells in BALB/c mice	IL-12, IL-1b, TNF-a, IFN-β↑ Phosphorylation of mitogen-activated protein kinase↑ The nuclear transport of NF-κB p65 subunit↑	[71]
Hypoglycemic activity	*In vitro*	MFP-2	Colorimetric analysis	Had strong inhibitory effect on α -glucosidase and glucose diffusion	[16]
		MFP3P-Se	HepG2 cells, L02 cells and RIN-m5F cell	β cell proliferation↑, Insulin secretion↑, Glucose utilization↑	[18]
		MFPs	Colorimetric analysis	The IC_{50} value of MFP-III inhibition of *α*-glucosidase and α-amylase were 3.094 mg/mL and 10.583 mg/mL, respectively	[26]
		MFPs	Colorimetric analysis	The IC_{50} values of MFP1 ability to inhibit *α*-glucosidase and *α*-amylase were 0.7944 mg/mL and 4.6419 mg/mL, respectively	[42]
		FM	Male KM mice HepG2 cells	Had a significant effect on PI3K/Akt and AMPK signaling pathways and protein expression of their effectors	[78]
	In vivo	MFP4P MFP4P-Zn	L02 cell, RIN -m5f cell and db/db male diabetic mice	liver cell: cell multiplication↑, Insulin secretion↑, Insulin resistance↓ Mice: blood sugar level↓, glucose tolerance↓	[19]
		MFP50 MFP90	Diabetic rats	FBG, OGTT-AUC, FINS, HOMA-IR, TG↓ Repair pancreatic tissue injury InsR, IRS-2, Akt, GLUT4↑	[28]
		MFP	db/db obese diabetic mice	HDL-C↑, TC, TG, LDL-C, MDA, FFA↓ Repair liver, kidney and pancreas tissue damage Improve the abundance level of Bacteroidetes and Firmicutes.	[74]
Hepatoprotective activity	*In vitro*	MFPs	Lipotoxicity of hepatocytes induced by palmitic acid	CAT, GSH-Px↑ Phosphorylation and nuclear translocation of Nrf2↑	[44]
		TMP	HepG2 cells	Activation of PI3K/AKT mediated Nrf2 pathway, ROS↓	[83]
		MFP	KM male mice	SOD↑, ALT, AST, TG, MDA↓ Improve linoleic acid metabolism, *α*-linolenic acid metabolism and glycerol phospholipid metabolism.	[12]
		MFPs	Male KM mice	Repair liver tissue damage ALT, AST, MDA↓GSH, SOD, GSH-Px↑	[15]
		MFPs	Male KM mice	ALT, AST, TG, MDA↓; SOD, GSH-Px↑	[27]
		MFP	Pathogen-free male Sprague-Dawley rats	AST, ALT, ALP, GGT, total bilirubin, albumin↓ AFP, CEA, CA19.9↓, caspase3, caspase9↑ IL-1β, TNF-α, NF-κB, MTH1↓	[84]
Regulate the activity of intestinal flora	*In vitro*	BMPs	Fecal fermentation	Bacteroidetes, Firmicutes, SCFAs↑ Fusobacteria, Proteobacteria↓	[30]
		FMP-6-S2	Bacteroides thetaiotaomicron plate culture	B. thetaiotaomicron↑	[32]
		EMPS	Healthy human feces	propionic acid, butyric acid, acetic↑ Bacteroidetes↑, Firmicutes↓	[87]
		MFP	Healthy human feces	Lactobacillus rhamnosuswas↑ The ratio of Bacteroidetes and Firmicutes↑	[89]
	In vivo	MFP	Male C57BL/6J mice	Muribaculum, Lachnospiraceae_NK4A136_group↑ Prevotella_2, Bacteroides, Faecalibacterium, Fusobacterium↓	[90]
Hypolipidemic activity	*In vitro*	JS-MP-1	3T3-L1 pre-adipocyte cells	Adipocyte proliferation↓	[69]
		MFS	Simulated *in vivo* digestion	Rate and degree of lipid digestion↓	[91]
Antifatigue activity	*In vivo*	MA	Kunming mice	Contents of hepatic glycogen and BUN↑	[93]
Antihypertensive activity	*In vivo*	WMFPs	Sprague-Dawley rats	Endothelial relaxation of mesenteric artery↑, NO↑	[94]

Notes: ↑ Increased, ↓ reduced; free fatty acid (FFA), prostaglandin E2 (PGE2), rat islet tumor cell (RIN -m5F), mutT homologue gene 1 (MTH1), recombinant regulated on activation in normal T-cell expressed and secreted (RANTES), macrophage inflammatory protein 1α (MIP-1α), Alpha-FetoProtein (AFP), carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19.9), Adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK)

that mulberry fruit polysaccharide MFP had significant insulin sensitizing activity by increasing insulin secretion and promoting pancreatic β cell proliferation. In addition, MFP also could prevent tissue damage and control the development of diabetes by

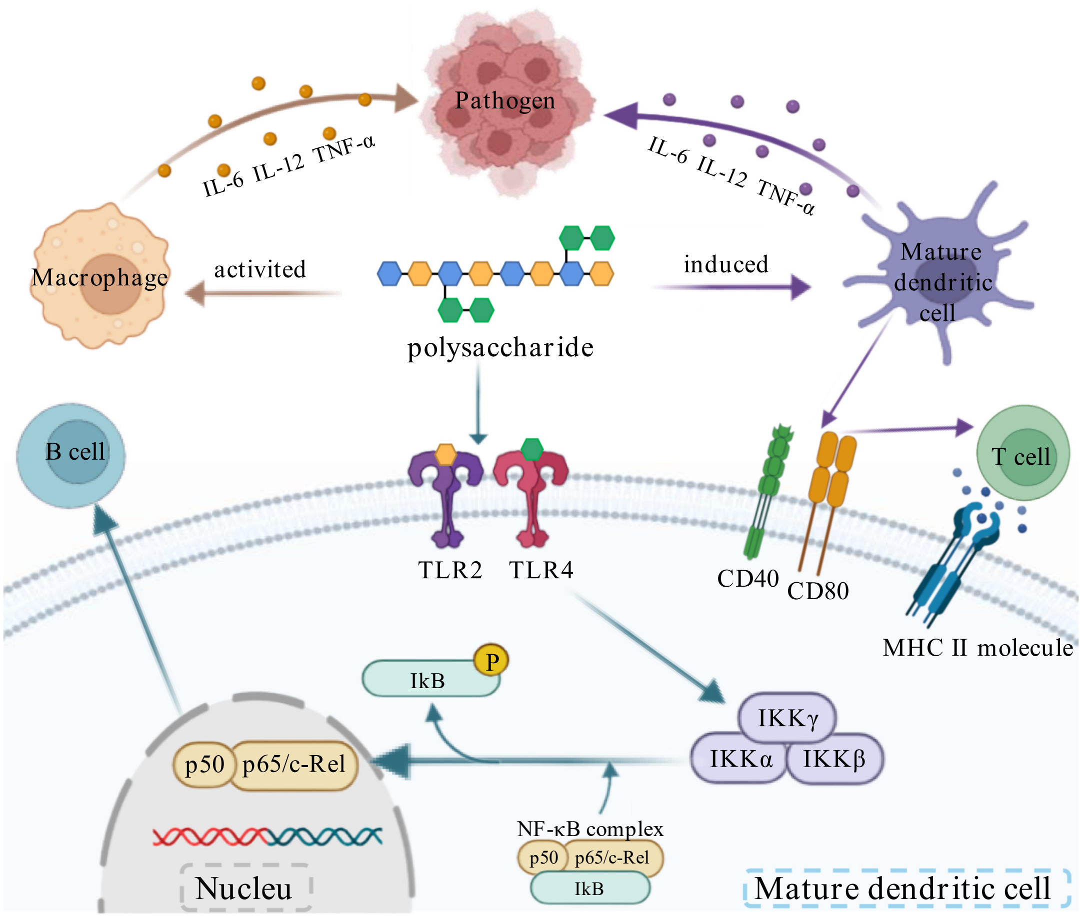

Figure 4 Immune regulation mechanism diagram of MFPs.

blocking oxidative factors. Chen *et al.*[74] discovered that mulberry fruit polysaccharide MFP therapy could not only raise the levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) in liver tissue, but also effectively decrease the content of malondialdehyde (MDA), reduce the oxidative stress damage of the pancreas, and play a role in lowering blood sugar. The related signal pathway of blood sugar regulation was also an important way for MFP to play its hypoglycemic activity[75]. The research by Wei *et al.*[76] showed that polysaccharides could activate protein kinase B (Akt) transferred glucose transporter (GLUTs) to the cell membrane, enhanced glucose uptake, promoted the phosphorylation of glycogen synthase kinase 3β (GSK-3β), and enhanced glycogen synthesis, thereby reducing liver glucose production. Nie *et al.*[77] proved that MFP could inactivate transcription factors by phosphorylating Foxo1, thus promoting glycogen synthesis and lipid metabolism. Long *et al.*[78] found that fermented mulberry was capable of regulating the expression of key signals such as insulin receptor (IR) and phosphorylated Akt (p-Akt) in the phosphatidylinositol 3 kinase (PI3K)/protein kinase (PI3K-Akt) signaling pathway. The expression was significantly reduced, while the expression of phosphorylated glycogen synthase kinase-3β (p-GSK-3β) and phosphoenolpyruvate carboxykinase (PEPCK) signals significantly increased. Jiao *et al.*[39] showed that MFP significantly increased the expression levels of insulin receptor (InsR), insulin receptor substrate 2 (IRS-2), Akt and GLUT4 in rats, but MFP failed to increase insulin sensitivity contradicted the conclusions of other researchers.

To sum up, MFP can reduce blood sugar by adjusting the oxidation factor, improving glucose intake and insulin sensitivity, and its mechanism diagram is shown in Fig. 5. On the one hand, after being stimulated by oxidative stress, c-Junn-terminal kinase (JNK) signal activates IRS, further activates PI3K signaling pathway and activates Akt. Activated Akt can promote the phosphorylation and glycogen synthesis of GSK-3β, or inactivate Foxo1 transcription factor by phosphorylation, thus affecting glucose and lipid metabolism. On the other hand, activated Akt can transfer glucose transport receptors (especially GLUT4) to the cell membrane, promote glucose uptake, and thus play a role in lowering blood sugar.

5.3 Hepatoprotective activity

Liver injury is a normal liver dysfunction caused by many factors. Natural polysaccharides had significant liver protection activity and could avoid the secondary damage caused by liver-protectivedrugs[79]. The hepatoprotective activity of polysaccharides might be associated with numerous mechanisms, such as activating ADH, eliminating free radicals and inhibiting the generation of oxidative factors[80]. Chen *et al.*[27] measured the ADH activities of P1-MFPs and P2-MFPs. When the concentrations of P1-MFPs and P2-MFPs were 5 mg/mL, the activation rates of ADH were 58.2 1.3% and 58.5 1.5%, respectively. Zhou *et al.*[15] found that MFP could reduce the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum and MDA in the liver, and increase the levels of glutathione (GSH), SOD and GSH-Px in the liver of rats with alcoholic liver injury. Tan[81] confirmed that MFPs could effectively enhance the activities of SOD and GSH-Px in the liver of mice

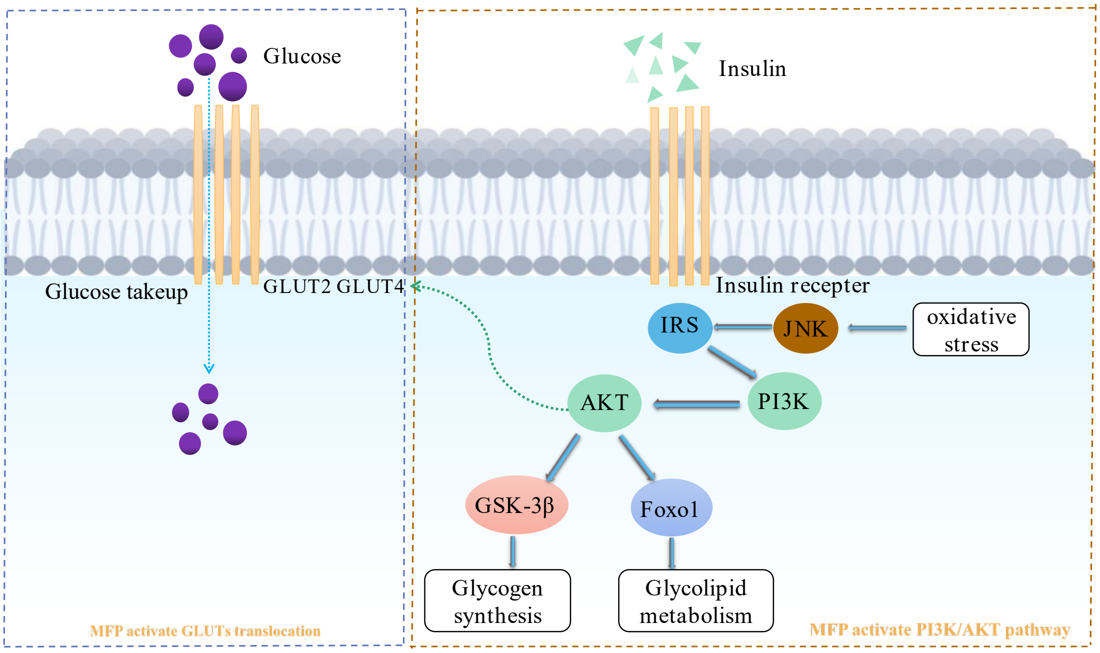

Figure 5 Mechanism of MFP in regulate glucose uptake and increasing insulin sensitivity.

with acute alcoholic liver injury and effectively decrease the MDA content in the liver of mice with acute alcoholic liver injury. Similarly, another study showed that mulberry polysaccharide (MP) could reduce serum ALT and AST levels of CCl_4-induced acute liver injury model mice and recover liver SOD and GSH-Px content[82]. To further clarify the protective mechanism of MFP through oxidative stress, Li *et al.*[83] showed that *Moris nigra* L. (TMP) could alleviate H_2O_2-induced liver injury in HepG2 cells by up-regulating SOD and GSH-Px contents and activating PI3K/Akt mediated nuclear factor E2-related factor 2 (Nrf2) pathway. Another study indicated that MFP could further enhance the activities of CAT and GSH-Px by augmenting the phosphorylation of Nrf2 and its nuclear translocation, thereby activating the Nrf2/ARE signaling pathway[37]. Inflammation was also an important way for MFP to achieve liver protection, Li *et al.*[84] found that MFP could increase the expressions of Bax, Caspase-3 and Caspase-9, decrease the expression of Bcl-2, and inhibit the expressions of IL-1 beta (IL-1β) and TNF-α in the liver, and provide chemoprevention for liver cancer induced by Diethylnitrosamine/Phenobarbital by inhibiting inflammation and inducing apoptosis. In addition, MFP could also protect the liver by regulating liver metabolism. Bian *et al.*[12] conducted an analysis of liver lipids using ultra performance liquid chromatography y-Quadrupole-Orbitrap-high resolution mass spectroscopy, and proposed that the liver protection effects of MFP may be linked to linoleic acid metabolism, linolenic acid metabolism, and glycerophosphate metabolism.

To sum up, MFP has good protective activity against alcoholic liver injury and drug-induced liver injury. *In vitro*, it can significantly improve the activity of ADH; *In vivo,* it canplay a protective role in the liver by regulating oxidative cytokines, inflammatory factors, metabolic pathways and inducing apoptosis. At present, researchers have conducted in-depth research on the protection mechanism of MFP, but the relationship between the structure of MFP and its liver-protecting activity is still unclear, and more experiments are needed to further clarify the relationship between molecular structure and liver-protecting activity.

5.4 Regulate the activity of intestinal flora

Intestinal flora plays an important role in maintaining the stability of the internal environment of the body[85]. As a prebiotic, polysaccharides can improve health by regulating the composition of intestinal flora[86]. The final product form of degradation fermentation of MFP in the intestine, mainly short-chain fatty acids (SCFAs). Chen *et al.*[87] investigated the *in vitro* fermentation behavior of MFP in human feces, revealing a gradual decrease in pH value with increasing fermentation time, as well as a significant increase in the output of short-chain fatty acids (SCFAs), particularly acetic and butyric acids. This increase in SCFAs may be attributed to the fermentation of galactose (Gal), galactose-N-acetylglucosamine (GalA), arabinose, and glucose present in MFP. The interaction between polysaccharides and intestinal microorganisms changed the composition of the intestinal microflora, which greatly affected the metabolism of the host[88]. Chen *et al.*[74] investigated the impact of MFP on diabetic mice and found that it significantly elevated the relative abundance of *Lactobacillus* and *Allobaculum*, enhanced the content of *Akkermansia*, and lowered insulin resistance, consequently diminishing the blood sugar levels of diabetic mice. Similarly, the experimental results of *Lactobacillus rhamnosus* cultured with mulberry oligosaccharides (EMPS) prepared by Li *et al.*[89] *in vitro* simulating the gastrointestinal environment also confirmed the same conclusion. Wan *et al.*[90] confirmed that *Morus alba* L. fruit polysaccharide MFP could reduce harmful bacteria such as *Prevotella_2, Bacteroides, Faecalibacterium* and *Fuso Bacterium* by increasing beneficial bacteria groups *Muribaculum* and *Lachnospiraceae_NK4A136_group*, thus alleviating metabolic syndrome induced by high-fat diet.

To sum up, because the structure of MFP is very complicated, MFP can almost completely reach the large intestine and be degraded by various flora in the large intestine, thereby increasing the content of SCFAs. In addition, MFP can maintain homeostasis by adjusting the ratio of *Firmicutes* to *Bacteroides*. However, there are few reports regarding the regulation of intestinal flora by MFP. In order to gain a deeper comprehension of the biological mechanisms underlying MFP's activity, it is essential that future

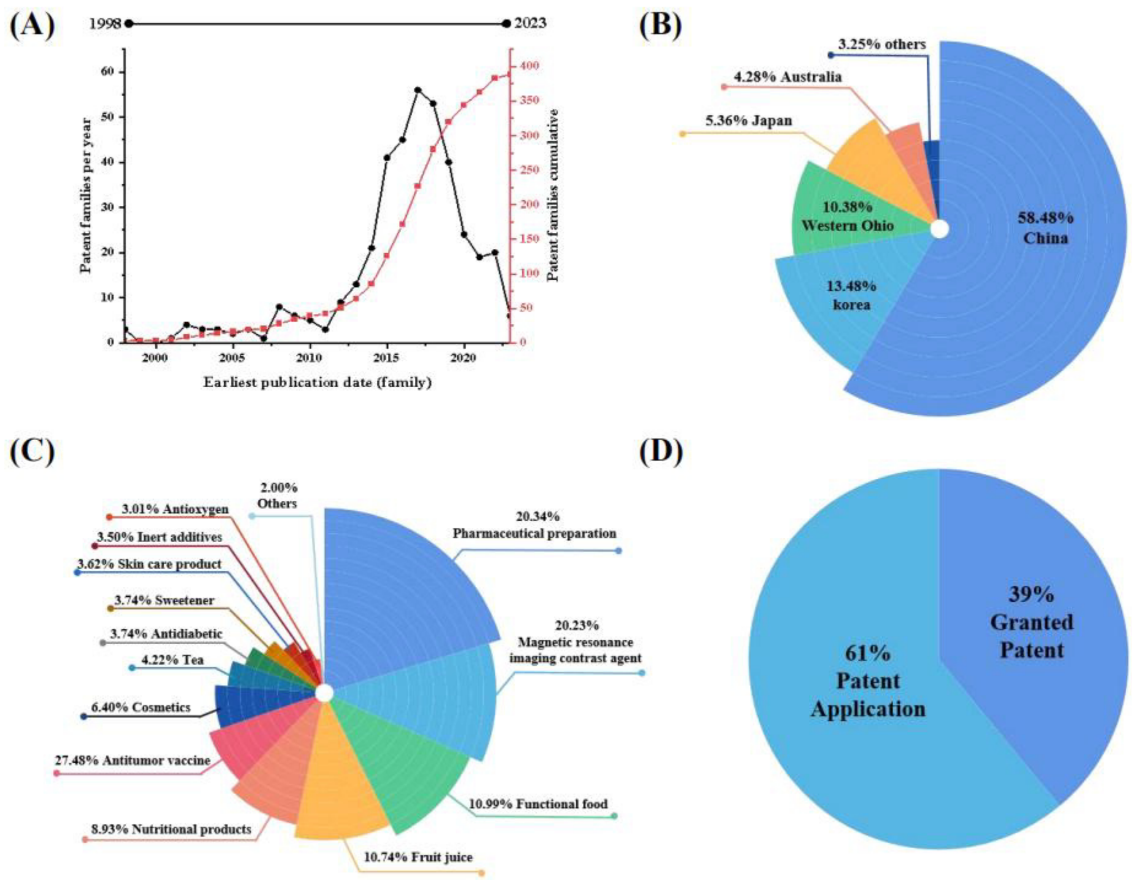

Figure 6 Analysis of 389 patents browsed in https://www.epo.org/ using the search terms "Mori Fructus polysaccharide": (A) Patent applications per years, (B) Jurisdictions, (C) Central product classification, (D) Document type.

research incorporates both *in vivo* and *in vitro* experiments to explore the interplay between intestinal flora and signaling pathways. This approach will enable a more thorough examination of the relationship between these factors and MFP's activity.

5.5 Other activities

MFP can be incorporated as an essential functional component in health food supplements, playing a significant role in both the prevention and treatment of obesity. Scientific studies have demonstrated that MFP is capable of breaking down fat by reducing the rate and intensity of lipid digestion[91]. And JS-MP-1 could inhibit the proliferation of preadipocytes effectively, and the *in vitro* experiments on mouse adipose cells (3T3-L1 cells) showed that the potential mechanism of JS-MP-1 against the proliferation of adipocytes was related to the induction of DNA fragments and apoptosis[69]. MFP exhibits a range of beneficial biological activities, including antioxidant, lipid-lowering, blood pressure-lowering, and anti-fatigue properties. It's *in vitro* antioxidant efficacy is particularly notable for its ability to neutralize 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) radicals, along with its commendable iron-reducing capability. These attributes underscore MFP's potential as a multifaceted therapeutic agent with a robust antioxidant profile[37]. Cell experiments showed that MFP had reducing capacity and protective effect on H_2O_2-induced oxidative damage in PC12 cells, and scavenging effects on DPPH, ABTS and hydroxyl free radicals[14]. In addition, it has been reported that MFP could improve the contents of liver glycogen and serum blood urea nitrogen (BUN) to relieve fatigue[92, 93]. Wang *et al.*[94] showed that White mulberry fruit polysaccharides (WMFPs) could induce endothelium-dependent relaxation of the mesenteric artery in rats to regulate blood pressure, suggesting that WMFPs could be used as a new antihypertensive drug.

6 Structure-activity relationship

The biological activity of polysaccharides is closely related to molecular size, composition and chemical modification, so this paper summarizes the effects of *M*w, monosaccharide composition and structural modification on MFP activity.

6.1 *M*w and biological activity

The *M*w plays a pivotal role in defining the relationship between the structural attributes and the biological efficacy of polysaccharides. Discernible differences in *M*w are known to exert a profound impact on the spectrum of biological activities displayed by these intricate carbohydrate chains[95]. Studies have found that polysaccharides with smaller *M*w tend to show stronger antioxidant activity[96]. Chen *et al.*[63] separated four components (*M*w = 7.93, 149, 167 and 1.47 kDa) from MFP, among which MFP-4 (*M*w = 1.47 kDa) with the smallest *M*w has stronger free radical scavenging ability and reducing ability *in vitro*, showing stronger antioxidant activity. This may be because low *M*w polysaccharides have better water solubility, which makes it easier to contact with free radicals. The lower *M*w polysaccharide PFM-3 (*M*w = 96.17 kDa) isolated and purified by Liu *et al.*,[54] could restore nuclear morphometry and mitochondrial membrane potential (MMP) of oxidative stress cells and enhance the activity of GSH-Px. At the same time, some related research results showed that the moderate *M*w polysaccharide had good immunomodulatory activity. Hu *et al.*[44] obtained three components (*M*w=641.8 kDa, 115.0 and 9.0 kDa) by ethanol fractionation, and found that MFP-II with medium *M*w (115.0 kDa) had the strongest protective effect on palmitic acid-induced lipotoxicity of HepG2 cells. Similarly, Wang *et al.*[68] isolated 5 components from FPA and discovered that FPA-1 with a moderate molecular weight (10.6-49.1 kDa) could significantly enhance the phagocytosis of macrophages, boost the secretion of NO and TNF-α, and increase the production of ROS. However, there is no research on the relationship between *M*w and the immunomodulatory activity of MFP, so further research is needed in the future.

6.2 Monosaccharide composition and biological activity

The composition and proportion of monosaccharides have the ability to directly affect the physical and chemical properties of polysaccharides, subsequently exerting an important influence on their biological activities[97]. GalA, as the main acidic monosaccharide of MFP, and its content can directly affect the biological activity of polysaccharides. Zhang *et al.*[98] explored different components of mulberry fruits polysaccharides (MFP). Compared with other components, MFP-90-40 with 31.02% GalA and 2.45% GlcA components have higher uronic acid content, better inhibitory effect on *α*-amylase and *α*-glucosidase and stronger bile acid binding ability. Similarly, Huang *et al.*[26] discovered that MFP-IV exhibited good antioxidant activity due to its high uronic acid content. This might be because MFP with a high uronic acid content possess more hydroxyl and carboxyl groups, which were more prone to combine with free radicals and demonstrate antioxidant activity. In addition, Zhou *et al.*[15] showed that MFPs with uronic acid content of 31.03% could restore the activity of antioxidant enzymes, and had a significant liver protection effect on acute and subacute alcoholic liver injury. Glc was the main neutral sugar in MFP, and MFP components with higher Glc content often had better antioxidant activity. Chen *et al.*[16] reported that MFP-1, which contained a significantly higher proportion of (Glc) at 86.48%, demonstrated enhanced antioxidant capabilities compared to MFP-2, where Glc constituted only 29.87%. Specifically, MFP-1 exhibited superior DPPH and hydroxyl radical scavenging abilities, as well as a higher oxygen radical absorbance capacity (ORAC), indicating its stronger free radical neutralization and oxidative stress mitigation potential. The content of Glc in MFP1P prepared by Dong *et al.*[48] was 66.62%, the ORAC value was 291.63 ± 6.81 μmol TE/g, and the IC_{50} value of MDA was 0.289 ± 0.022 mg/mL, which showed good antioxidant activity.These results show that monosaccharide composition has certain influence on biological activity, but it is necessary to further explore which composition is more beneficial to biological activity.

6.3 Structural modification and biological activity

Structural modification is an important method to improve the biological activity of MFP. Currently, the structural modification methods used in the field of mulberry polysaccharide mainly include zinc modification, selenium modification, phosphorylation modification, carboxymethylation modification and sulfation modification. The research results of Wang *et al.*[19] showed that the Zn-converted product MFP4P-Zn not only exhibited hypoglycemic activity by promoting islet cell proliferation, increasing glucose metabolism and insulin secretion, but also exhibited significantly increased antioxidant activity compared with MFP4P. Chen *et al.*[18] demonstrated that MFP3P-Se significantly reduced cytotoxicity and increased glucose consumption compared with MFP3P, possibly due to conformational changes of polysaccharides after selenization with better antioxidant and hypoglycemic activities. Hu *et al.*[99] studied the antioxidant activity of phosphorylated mulberry polysaccharide (PMP). PMP showed better reducing force, Fe^{2+} chelating ability and hydroxyl radical scavenging ability. The observed effects might be attributed to the phosphorylation modification of MFP, which potentially alters its molecular structure. This alteration could lead to an increased exposure of hydroxyl groups, facilitating the formation of a greater number of glycoprotein complexes, thereby enhancing the immunomodulatory properties of MFP. Compared with other modification methods, the MFP modified by carboxymethyl showed stronger liver protection activity. Deng *et al.*[47] prepared 9 kinds of MFPs derivatives by oxidative degradation, sulfation and carboxymethylation. The results showed that compared with MFP, all the modified polysaccharides could improve ADH activity, and carboxymethyl polysaccharide (C-MFP-1) showed the best ADH activity. Nevertheless, certain studies have indicated that, in contrast to their natural counterparts, modified forms of MFP do not necessarily exhibit an increase in biological activity. This suggests that the process of modification may not always result in the desired enhancement of the polysaccharide's efficacy. Tan *et al.*[100] found that compared with sulfated polysaccharide (S-MFPAs), unmodified MFPA1 showed stronger antioxidant activity *in vivo* and had the strongest anti-acute alcoholic liver injury activity, which might be due to the change in the composition and structure of MFP after sulfation modification. Therefore, it is necessary to further explore the molecular mechanism of structural modification and its influence on the physical and chemical properties and biological activity of polysaccharides.

To sum up, *M*w, monosaccharide composition, and structural modification of MFP are closely related to its biological activity. Currently, the research on the structure-activity relationship of MFP mainly centers on the connection between the primary structure and its immunomodulation, hypoglycemic, and antioxidant activities. However, the relationship between the spatial structure of MFP and other biological activities remains unclear. Therefore, further scientific research is urgently needed to clarify the relationship between the advanced structure and biological activity of MFP.

7 Patents and application

7.1 Related patents

MF was used to make healthy yogurt, oral liquid and meal replacement powder because of its antioxidant, hypoglycemic and hypolipidemic activities[101-103]. In China market, 101 types of MF-related drugs and functional foods have been approved by the State Administration of Market Supervision. As one of the main components of MF, MFP has excellent physical and chemical properties and biological activity, and has a wide range of application values[104-106]. In the Chinese market, the State Administration of Market Supervision has granted approval to a total of 101 MF-related pharmaceuticals and functional foods, reflecting the significant presence and regulatory acceptance of these products in the industry. At present, polysaccharides have been widely used in health food, biomedicine and cosmetics. MFP has great potential in product development because of its green and natural source, low toxic and side effects and good biological activity. By 2023, there are 389 patents related to mulberry polysaccharides in the world (Fig. 6). Since 1998, MFP has been put into practical application. In the past 20 years, the overall number of patents has shown an increasing trend. The proportion of patent applications is the largest at 61%, while the proportion of patent authorization is the smallest at 39%. Among them, China has the highest proportion of patents, accounting for 58.48% of the total number of patents. Korea, Western Ohio in the United States, Japan, Australia, and other countries account for 41.52%. The patent application shows that MFP is mainly used in medicine, functional food, fruit juice, cosmetics and additives, but there are still some problems such as small quantity, incomplete application and immature market development. In future research, researchers need to further strengthen and broaden the research of MFP in product development and application.

7.2 Application in the field of food

In the field of food, MFP is an excellent regulator of physical and chemical properties of food. Li *et al.*[37] found that the MFP prepared by HWE and ultra-fine grinding pretreatment was pseudoplastic fluid behavior, showing shear thinning phenomenon. Simultaneously, the intersection of the frequency points of G' (elastic modulus) and G" (viscous modulus) was observed, indicating the material's viscoelastic nature and its favorable gel properties. This characteristic suggests its potential for development as a thickener, emulsifier, and gelling agent within the food industry. Additionally, whey protein is extensively utilized in the food sector due to its rich nutritional content, broad applicability, and ease of digestion and absorption. Wang *et al.*[105] found that glycation occurred between MFP and whey protein, which improved whey protein's emulsifying properties and antioxidant activity, which is of great significance for the development of emulsifiers in the food field. In addition, Ai *et al.*[30] found that MFP can promote the growth of probiotics, inhibit the growth of harmful bacteria, and show good fermentation characteristics, which was expected to become an excellent starter and prebiotic in the food field. At present, the application of MFP in food field mainly focuses on improving the physical and chemical properties of food, thickening agent, starter, and regulating the function of prebiotics, but there are few reports on related health foods of MFP, which need further research by researchers.

7.3 Application in the field of medicine

Mulberry is one of the homologous varieties of medicine and food, and there should be no strict boundary between medicine and food[107]. That is, it can be utilized as food, and simultaneously, it can have a beneficial impact on the human body after being consumed. Thus, in the medical field, MFP has a wide application prospect in the development of new drugs due to its outstanding biological activity. Lee *et al.*[10] found that JS-MP-1 could stimulate mouse macrophage RAW264.7 cells to release RANTES and MIP-1α, TNF-α and IL-6, and induce the expression of iNOS, which was expected to be developed into an immunomodulatory drug. Tan *et al.*[38] found that MFP showed excellent activity of activating alcohol dehydrogenase *in vitro*, and it can also protect acute liver injury by regulating oxidative stress and lipid metabolism *in vivo*, so it is expected to be developed as a natural therapeutic agent for liver injury. Chen *et al.*[74] discovered that MFP can assume an anti-diabetic role by regulating oxidative stress, lipid metabolism disorder and other related biochemical indicators, as well as by regulating the abundance and species composition of intestinal bacteria. This is expected to provide a basis for the development of natural diabetes treatment drugs.

To sum up, the application of MFP in the medical field mainly focuses on the research and development of immunomodulatory drugs, liver-protecting drugs and hypoglycemic drugs. But at present, it is mainly in the laboratory research stage, and there is no report on MFP drugs, which still have broad development potential.

8 Conclusions and opinions

MF, as a medicinal and edible resource, is a common functional food in life. As the main active ingredient of MF, MFP has the functions of immunomodulation, blood sugar reduction, protecting the liver and regulating intestinal flora. MFP is mainly an RGI-type pectin polysaccharide composed of repetitive disaccharide fragments of 1,4-linked *α*-Gal*p*A and 1,2-linked *α*-Rha*p*. There are disparities in structural information such as the extraction rate, *M*w, and monosaccharide composition of MFP obtained through different extraction processes. In addition, the *M*w, monosaccharide composition and chemical modification of MFP are closely related to its biological activity. Some progress has been made in the research on extraction techniques, structural characteristics and biological activity of MFP. However, many problems remain to be solved. For example: (1) The extraction methods of MFP mainly focus on HWE, EAE and UAE, and lack the application of new methods and new technologies, which still need to be further studied. (2) The biological activity research of MFP is mainly focused on the *in vitro* experiment, and the research on the *in vivo* action mechanism is still insufficient. (3) Advanced structure and spatial conformation are the key factors affecting the biological activity of polysaccharides. However, the current studies on the structure of MFP mostly focus on primary structures, and the interpretation of the spatial structure and structure-activity relationship is not clear enough. (4) The development and application of MFP in practical use are relatively few, and subsequent studies can be mostly focused on such fields as dietary supplements, immune enhancers and auxiliary drugs. In summary, the research of MFP is still in its early stage, and the comprehensive disclosure of its structure and biological activity needs exploration by scholars. This paper systematically summarizes the research advancements of MFP in extraction, purification, structural characteristics, biological activity, structure-activity relationship, and product development and application. It discusses the shortcomings in certain aspects of MFP at present and puts forward several suggestions, which can provide a foundation for further studies on MFP.

CRediT authorship contribution statement:

Yongshuai Jing: Conceptualization, Writing-original draft, Funding acquisition. Jinying Hu: Writing-original draft, Formal analysis. Ziying Wang: Investigation, Methodology, Writing-original draft. Cheng Tao: Investigation, Data curation. Shilin Zhang: Formal analysis, Methodology. Beibei Hu: Software, Validation. Zhiwei Li: Writing-review & editing, Project administration.

Conflicts of interest

The authors declare that there are no conflicts of interest in this work.

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