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1 **Structure and antioxidant activity of galactomannan from**
2 **Gleditsia japonica var. delavayi**

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Abstract

Background: The seeds of Chinese honey locust (*Gleditsia sinensis*) contain galactomannans as one of the main active substances. These polysaccharides are widely used in food, chemical, pharmaceutical and healthcare industries due to their low toxicity, biocompatibility, biodegradability and bioactivity. However, there has been no systematic summary of the isolation, structural characterization, bioactivity, and the various applications of *Gleditsia* species seeds galactomannans (GSGs).

Scope and Approach: This review paper aims to summarize the advances in the recent literature concerning the main methods of extraction, purification, structure elucidation, and the observed bioactivities of the galactomannans. Moreover, the structure–bioactivity relationship of GSGs are critically discussed. Finally, the potential applications of GSGs are analyzed.

Key Findings and Conclusions: This review found that the biological activity of galactomannan depends not only on the source of polysaccharide and the extraction method involved, but also on the chemical structure profile of the extract. hypoglycemic, lipid-lowering and antioxidant activities are the most prominent biological activities of galactomannan, which may be related to the existence of β -(1→6) and α -(1→4) glucosidic bonds. After appropriate preparation, GSGs can be used as ingredients in various industries such as food, pharmaceutical industry and in agriculture. In conclusion, GSGs is a promising polysaccharide with expanding ~~food~~ applications and functions.

Keywords

Gleditsia species, galactomannans, extraction, structural features, biological activities, uses

1. INTRODUCTION

Gleditsia species, a pivotal member of Leguminosae family, is widely distributed in Southeast Asia, and North and South America (Lian and Zhang, 2013; Zhang et al., 2016). The genus *Gleditsia* (*Fabaceae*) comprises 14 species including *Gleditsia aquatica* Marshall, *Gleditsia sinensis* Lam., *Gleditsia caspica* Desf., *Gleditsia triacanthos* L., *Gleditsia japonica* Miq. Etc (Zhang et al., 2016). *Gleditsia* species fruit is a non-cracking pod, long and flattened, 5-13 cm long, 1-1.5 cm wide. Seeds one and more, ovate or elliptic, flat or nearly cylindrical, which are composed of testa, embryo and endosperm (27%: 39%: 34%, w/w) (Adriana et al., 1984). In terms of proximate analysis, the content of moisture, ash, crude fat, total protein, crude fiber, and carbohydrates was found to be $12.00 \pm 0.02\%$, $0.84 \pm 0.04\%$, $0.81 \pm 0.04\%$, $4.20 \pm 0.07\%$, $1.05 \pm 0.03\%$, and 81%, respectively (Sun et al., 2018).

Gleditsia species fruits have been utilized as folk medicine for thousands of years, as documented in several Asian medical classics, such as Shen Nong Ben Cao Jing, Qian Jin Fang and Bo Ji Fang. According to medical records, various parts of *Gleditsia* species, including spines, leaves and fruits, were widely used in many countries to treat various hard to cure diseases, such as measles, indigestion, whooping, smallpox, hematochezia, dysentery, and carbuncle (Zhang et al., 2016). The thorns of *G. sinensis* are still used in China, North and South Korea for treatment of carbuncle, scabies and skin diseases (Bensky et al., 1986). *G. japonica*, which grows in many areas of China, North and South Korea and Japan, has long been used for diuresis and expectoration (Zhao et al., 2006). *G. triacanthos* has also been used for treatment of whooping, ache,

measles, smallpox and difficult labor in the Native American (Cerqueira et al., 2010a). In addition, Gleditsia has also been made into soap ointment, *G. sinensis* pills, "Zaojia Tongluo tablets", "Liuwei Tongluo Yin traditional Chinese medicine prescriptions to treat certain diseases (Lai et al., 2011; Xing and Wu, 1996). With the pursuit of health, the pharmacological and nutritional effects of Gleditsia have received more and more attention. According to previous studies, Gleditsia species are naturally rich in diverse biologically meaningful phytochemicals, including polysaccharides, triterpenes, alkaloids, flavones, and other functional substances (Hamana et al., 1996; Niknam et al., 2021).

Galactomannans are designated as the most abundant cell wall storage polysaccharide in plants, which are extensively occurring in varieties of plant families such as Solanaceae, Convolvulaceae, Iridaceae, Araceae, Orchidaceae, and Palmae (Marcos and Sonia, 1996). In recent years, a large number of studies have revealed a variety range of highly significant pharmacological activities for galactomannans, such as antiviral, anticoagulant, immunomodulatory, and antimicrobial activities (Ammar et al., 2022; Gülsen et al., 2020; Muschin et al., 2016; Yuheng et al., 2022). Moreover, the US Food and Drug Administration (FDA) generally recognized galactomannans are non-toxic, biodegradable polysaccharides during production and application (Siqueira et al., 2015), which could be used as food stabilizer, thickener and additive (Thombare et al., 2017). Therefore, Gleditsia species seeds have great application prospects as a typical functional food.

However, to the best of our knowledge, there have been few systematic reviews of

Gleditsia species seeds' galactomannans until now, thus evidently limiting its further exploitation, utilization, and understanding for the fundamental researchers together with the food and pharmaceutical industries. At the same time, there is body of evidence generated both on the structure and the activity of galactomannans from primary research in the area, as indicated earlier. Therefore, this article reviews the research progress of galactomannans from the aspects of extraction and purification methods, structural characteristics, and biological activities (hypoglycemic, hypolipidemic, antibacterial, etc.). This review article could provide scientific reference for the development and utilization of galactomannans from Gleditsia species (GSGs).

2. PREPARATION OF GSG ISOLATES

The protocol followed by most studies is similar. Briefly, the Gleditsia species seeds are dried and crushed, and then extracted by hot water or acid and base to obtain Gleditsia species water extract. The water extract was further treated by centrifugation, ethanol sink, and deproteinization to remove the inactive substances and proteins, obtaining the crude Gleditsia galactomannans in aqueous solution. The galactomannans aqueous were purified by anion exchange chromatography and gel permeation chromatography. Finally, the purified galactomannans fractions were obtained by a series of processes including concentration, dialysis and lyophilization. These extraction and purification methods of GSGs are summarized in Table. 1.

2.1 Extraction methods

The traditional extraction methodology of GSGs commonly referred to the hot

water extraction attributable to its advantages of readily operation and routine equipment (Xu et al., 2020). As people pay more attention to the medicinal value and healthy value of GSGs, a series of new extraction techniques have been developed to better improve the extraction rate and active substance retention, such as microwave-assisted extraction and ultrasonic-assisted extraction (Niknam et al., 2022). ~~In this review, the methods to extract galactomannan from Gleditsia species has been extensively discussed.~~

Microwave-assisted extraction improves the yield and emulsifying features by generating 2.45 billion times high-frequency oscillations, which migrates the target molecules into the solvent in a relatively short time (REF needed). Niknam et al. used the microwave extraction technique (MET) to extract galactomannan from *G. caspica* seed (Niknam et al., 2022). The experimental conditions were that the powdered seeds were put in an 88 °C water bath for 20 min; then, the power was treated with microwave for 15 min 500 W. Next, the powders were suspended in water with a solid-liquid ratio of 1:10 (w/v), followed by 48 min in a 70 °C water bath to obtain the crude polysaccharides.

Compared to the microwave-assisted extraction, ultrasonic-assisted extraction of polysaccharide from *G. caspica* was used to further improve extraction efficiency and yield (Niknam et al., 2022). The extraction conditions were as follows: ultrasonic power of 500 W and extraction time of 15 min. Some studies found that many factors, such as temperature, time, and power could affect the extraction of polysaccharides from plants (RenJie, 2008; XuJie and Wei, 2008; Yin and Dang, 2008).

Additionally, the extraction of polysaccharides from *G. sinensis* by acid and alkali method have also been studied. As shown in the article by Cerqueira (Cerqueira et al., 2009), the seeds were immersed in 98% sulfuric acid and then placed in a 100 °C water bath for 1.5h, followed by rinse with water and ethanol until the husks and acid are mostly removed. However, this approach may break the glycosidic bonds in the polysaccharide structure and degrade the polysaccharide into low molecular weight fragments. Compared with the water extraction method, the polysaccharide yield of *G. sinensis* after alkali extraction was higher, which might be the reason of the NaOH concentration and residue neutralization with HCl (Sciarini et al., 2009).

2.2. Purification methods

Galactomannans polysaccharide crude extract usually contains a lot of impurities. Thus, a number of purification methods are used to improve the purity. Different separation conditions, such as materials, solvents and concentrations, can produce GSGs with specific structures, thus revealing specific structure-biological functional relationships. The aims of this review on the isolation and purification methodologies tried to be served as guideline for the further development of GSGs.

2.2.1 Ethanol precipitation methods

Ethanol precipitation is the most used method for purification of crude polysaccharides currently. Generally, GSGs supernatant or extract is precipitated with equal proportion of ethanol, filtered with gauze or nylon mesh, and then lyophilized with organic solvent such as acetone or ethyl acetate. According to previous studies, the ethanol concentration used for GSGs precipitation usually are 90%-100%. The

higher the concentration of ethanol, the more ash, pigment and small molecules were removed, and thus the higher the extraction rate. Therefore, Bourbon et al. had adopted the ethanol precipitation method to generate GSGs, which was achieved by adding the supernatant to 99.8% ethanol at a ratio of 1:2 (Bourbon et al., 2010).

Although the ethanol precipitation method is simple to operate, it results in low polysaccharide recovery and a large amount of ethanol consumption. (REF required?) Thus, it is necessary to control the concentration of mixed galactomannan, the rate of adding ethanol and the pH of solution during the operation.

2.2.2 Ion exchange chromatography methods

Ion-exchange chromatography is a chromatographic method that separates the component ions in the mobile phase from the equilibrium ions in the exchange agent based on the binding force difference between the component ions in the mobile phase and the equilibrium ions in the exchange agent (Alois and Rainer, 2009). In the literature, the ion exchange protocols for GSGs usually include DEAE-Sephadex A-2, DEAE-Sephadex A-25, and DEAE Sephadex A-50, which are usually eluted with distilled water, ammonium carbonate, and pyridine acetate. For example, the galactomannan extracted from *G. triacanthos* seed was purified by Sephadex G-100 gel and further purified by DEAE-Sephadex A-25 with pyridine acetate as eluent at a flow rate of 17 mL/h (Mazzini and Cerezo, 1986). The galactomannan from the *G. triacanthos* was extracted by a similar protocol in which a DEAE-Sephadex A-50 cellulose column was used for purification. The column was eluted with a gradient of 0.05M Tris-hydrochloric acid buffer and further purified by the Sephadex G-10

(Adriana et al., 1984).

Although ion-exchange chromatography has a strong ability to remove inorganic ions, it has some disadvantages such as long cycle, large salt consumption and contamination of organic matter (REF required?). In addition, for the presence of a variety of ions in the solution, which also need to choose different resins.

2.2.3 Gel filtration chromatography methods

Gel filtration chromatography is used to separate polysaccharides according to their molecular weight, in which polysaccharides with relatively large molecular weight are eluted out firstly because of the failure to enter the gel sieve pores. Currently, GSGs are often purified by gel filtration chromatography using Sepharose CL-2B, Sephadex G-25, and Sephadex G-100, etc. Mazzini and Cerezo used Sephadex G-100 gel to purify galactomannan-like oligosaccharides from the seed of *G. triacanthos*. The experimental conditions were as follows: deionized water was the eluent, the flow rate was 10 ml/h (Mazzini and Cerezo, 1986). Sun et al. examined the GSGs purified with agarose CL-2B by UV spectrum and found that there was no obvious absorption peak at 260 to 280 nm, which meant that the polysaccharide contained a little protein and has high purity (Sun et al., 2018).

Despite its disadvantages of low resolution, slow operation, large amount of eluent and non-specific adsorption phenomenon, gel-filtration remains a popular method for galactomannan separation due to its versatility, high repeatability, and nearly 100% sample recovery (O'Fagain et al., 2011).

3. STRUCTURAL FEATURES AND PHYSIOCHEMICAL PROPERTIES

3.1. Structural characteristics

The structure of polysaccharide from *Gleditsia* species is characterized by diversity. The structure of polysaccharides recovered was different due to the differences in raw materials, extraction methods and purification methods of different studies (Jixian et al., 2019). Naturally, this difference of polysaccharide profile leads to different biological activities observed in the literature. In general, the chemical structure of polysaccharides includes its average molecular weight (M_w), monosaccharide composition and position of glycosidic bonds (Tao et al., 2018). The sources, M_w , monosaccharide composition, and the ratio of M to G of GSGs are exhibited in Table 1.

3.1.1. Average molecular weight

It is well known that molecular weight (M_w) is closely associated with their biological activities of natural polysaccharides. In general, polysaccharides with higher molecular weight have larger hydrodynamic volume, higher viscosity and more complex structure (Yu et al., 2020; Zhao et al., 2016); With the decrease of molecular weight, the number of polar groups of polysaccharide increases, and the solubility and permeability will be further improved (Xu et al., 2019; Yuan et al., 2019). Nowadays, the molecular weight of polysaccharides is mainly measured by high performance gel permeation (HPGP) chromatography, viscosity method, osmometry and volume rejection chromatograph (Bhadja et al., 2016; Hundschell et al., 2020; Shi et al., 2023; Zhu et al., 2016).

So far, galactomannan has been successfully isolated from different varieties of *G. sinensis*, and their molecular weights are summarized in Table. 1. It could be clearly observed that the molecular weights of GSGs were mostly ranging from 0.652×10^6 Da to 3.310×10^6 Da. For instance, Jian et al. demonstrated that the molecular weights of *G. sinensis* Lam. galactomannans obtained by different extraction methods were 3.310×10^6 Da (Gb), 2.778×10^6 Da (Gw) and 2.603×10^6 Da (Ga), respectively (Jian et al., 2011). In addition, Sun et al. adopted the GPC (gel permeation chromatography) and HPLC chromatography to get the molecular weight of 1.913×10^6 Da (Sun et al., 2018).

On the other hand, compared with the molecular weight of galactomannan extracted from guar seeds ($0.25 \sim 5 \times 10^6$), fenugreek seeds ($3.23 \sim 5.619 \times 10^5$), and tara seed ($0.2 \sim 2 \times 10^6$), the molecular weight of *Gleditsia* species galactomannan was at a medium level (Jiang et al., 2007; Mudgil et al., 2014; Prajapati et al., 2013).

3.1.2. Monosaccharide composition

The monosaccharide structure is normally obtained by hydrolysis and derivatization of galactomannan acid and further analyzed by GC-MS and HPLC (Dan et al., 2021; Hamideh and Kambiz, 2018). Different varieties, extraction methods, and purification techniques had significant effects on the monosaccharide composition of *Gleditsia* species galactomannan. In addition, different growing conditions of the same variety were also different. Therefore, the monosaccharide composition and molar ratio of *Gleditsia* galactomannan are different in different studies.

Sun et al. reported the isolation of galactomannan from endosperm of *Gleditsia japonica* var. *delavayi* seeds (EGSP) by water extraction (Sun et al., 2018). After the

full hydrolysis and derivatization, GC analysis disclosed that the EGSP was constituted of four major monosaccharide composition of glucose, arabinose, mannose, and galactose. Among them, the mannose and galactose monosaccharides were the most abundance ones in purified fraction EGSP. The molar ratio (M/G) of EGSP was calculated as 2.66, which was different from the result of *G. japonica* (M/G ratio of 5.00) (Mirzaeva et al., 2006). This indicated that different growth conditions and extraction methods of the same species had important effects on the preparation of target galactomannans.

M1, M2 and M3 were the three polysaccharides isolated from *G. triacanthos* by Sciarini (Sciarini et al., 2009) in three ways. The results of monosaccharides showed that the three polysaccharides had similar monosaccharide composition, but different component proportions, as shown in Table 1. Among them, M3 has a larger proportion of M/G (3.12) and a higher proportion of mannose, and the values were between 2.7 and 3.2, which were consistent with those found by other authors (Adriana et al., 1984; Xu et al., 2020). Mazzini et al. have purified a novel galactomannan from the crude polysaccharide fraction of *G. triacanthos*, which was consisted of arabinose, xylose, mannose, galactose, and N-acetylglucosamine with a ratio of 28.3%: 15.3%: 19.0%: 10.0%: 13.0% (Mazzini and Cerezo, 1986). According to the above studies, *Gleditsia* galactomannans were mainly constituted of mannose, galactose, fucose, arabinose, xylose, and glucose. In addition, the *Gleditsia* galactomannans mainly contained glucuronic acid and N-acetylglucosamine residuals. Detailed information on the monosaccharide compositions of GSGs is provided in Table 1.

3.1.3. Chemical structure

The biological functions of polysaccharides are not only affected by molecular weight, but also closely related to their complex structure (Li et al., 2022). The galactomannan extracted from leguminous seeds has been extensively studied. Their gross structure consists of a backbone of β -(1 $\rightarrow$ 4)-linked D-man and single α -D-gal (Sharma et al., 2022). Adrian and coworkers conducted methylation analysis of galactomannan obtained from the endosperm of *G. triacanthus* seeds and found that the galactomannan not only had the basic structure of leguminous polysaccharide, but also found that 2,6- and 4, 6-di-O-methyl mannose were located in 2.2% of the mannan chain (Adriana et al., 1984). Therefore, they concluded that small deviations in this structure may be important in the formation of aggregations and gel networks.

It is worth mentioning that Mazzini's (Mazzini and Cerezo, 1986) research also shows that the galactomannan from *G. triacanthos* has different side chain structures. The side chains were connected to the backbone by 1,6-linkages or 1,3-linkages, and contain either 1, 3-1,6-linked or 1,3-1,3-linked with α -D and β -D configurations, respectively. This unusual "unit" may be related to chromium trioxide in the derivatization process, but which had been detected in galactomannan extracted with water in beans seeds (Adriana and Alberto, 1984).

Rakhmanberdyeva obtained crude galactomannan by hot water extraction (Fig. 2A), and confirmed that the galactomannan of *G. delavayi* with a backbone of (1 $\rightarrow$ 6) linked α -Gal and (1 $\rightarrow$ 4) linked β -Man (Rakhmanberdyeva, 2004). In addition, it has been found that galactomannan has traces of di-O-Me-hexose by using methylation

analysis, desulfation and nuclear magnetic resonance (NMR) spectral data. Subsequently, Rakhmanberdyeva reported a water-soluble polysaccharide (WSPS) from *G. macracantha* seeds, which isolated from *G. macracantha* (Fig. 2B). PC and GC identified that the polysaccharide was mainly arabinose, mannose and galactose, and the ratio was 1:3:2:2.8. The structure of the galactomannan was studied by periodate and chromic oxidation and methylation, which results show that galactose and arabinose in the polysaccharide had the α -configuration of the glycoside bond (Rakhmanberdyeva, 2009).

In the study conducted by Sun et al (2018) the glycosyl linkage of galactomannans in *G. japonica* var. *delavayi* (Fig. 2C) was analyzed by GC–MS through Smith degradation (Sun et al., 2018). The results showed that mannose was linked by (1→4) glucosidic bonds, while galactose was linked by (→1) glucosidic bonds. Moreover, the polysaccharides extracted from different developmental stages of *G. sinensis* seeds were analyzed by ^1H NMR. The analyses of result revealed the galactomannans had H-1, 1,4, 6- β -d-Manp and 1, 4- β -d-manp of T- α -d-galp at different developmental time (Yantao et al., 2019).

To date, many experiments have investigated the basic structure of galactomannan in different cultivars of *Gleditsia* species, but further studies on the interior of galactomannan should be conducted using advanced techniques to have a deeper understanding of the structure-bioactivity relationship. And the presumed chemical structures of polysaccharides isolated from *Gleditsia* was shown in Fig. 3.

3.2. Physiochemical properties

3.2.1. Solubility

The solubility of galactomannans is of great significance to promote the application value of *Gleditsia* species (Junchi et al., 2023). Moreover, the solubility is well known to closely relate to the molecular structure (M/G ratio and type) and Mw. Liu et al. reported the solubility of GSGs dramatically decreased with the extension of collection time, which might be related to the higher galactose content as side chain and lower molecular weight (Liu et al., 2019). Liu et al. further found that the solubility of Fenugreek gum (FG) (82.25%) was significantly higher than that of guar gum (GG) (78.75%) and GSG (70.11%) under the same extraction conditions, and these differences might be due to the smallest M/G ratio of FG (1.11 ± 0.01) and the high degree of galactose substitution, thus significantly increasing its affinity with water molecules (Liu et al., 2020). In addition, in the other similar study that the GSGs extracted with hot water have a smaller molar ratio (M/G) ratio and highest solubility, compare with concentration with NaOH and swelling with water treatment (Sciarini et al., 2009).

3.2.2. Rheological properties

The evaluation of rheological properties of polysaccharides plays a great significance to determine the bioactivity and application in food (Abdin et al., 2022). Bourbon and co-workers found that galactomannan from *G. triacanthos* had the longest breaking time and higher viscoelasticity compared with guar gum and locust bean gum, making it an excellent alternative as thickening agents. (Bourbon et al., 2010). In addition, the molecular weight and M/G ratio also have some implications for the

polysaccharide. Jian et al. that the gum obtained by acid pretreatment (Ga) had a characteristic shear thinning behavior and high viscosity due to the lowest Mn value (Jian et al., 2011). Moreover, Liu compared the rheological properties of polysaccharides from *G. sinensis* gum (GSG) and *G. microphylla* gum (GMG). They found the smaller molecular weight and galactomannan content of (GMG) showed higher rheological behavior (Liu et al., 2016).

4. BIOACTIVE PROPERTIES AND STRUCTURE-ACTIVITY RELATIONSHIPS

As a traditional medicine, the genus *Gleditsia* has a wide range of medicinal values. Previous studies have shown that galactomannans are the main bioactive components in *Gleditsia*, and might exhibit beneficial effects on human health and pharmacological activities such as anti-tumor, anti-inflammatory, anti-hyperlipidemic, antiallergic, antibacterial, analgesic, antioxidant activities, and hypoglycemic and hypolipidemic effects.

It has been reported that the biological activities of polysaccharides are greatly influenced by structural features including molecular weight (Mw), monosaccharide composition, and structural characteristics. Unfortunately, there are very few studies on the relationship between polysaccharide structure and biological activity due to the variety and structural differences of *G. sinensis*. Although complicated, an analysis and discussion of structure-activity relationship of *Gleditsia* species reported in the literature is presented below.

Thus, through some literature reports, making some scientific inferences about the, which is of great significance for the development and application of *Gleditsia* species galactomannan.

4.1. Hypoglycemic and hypolipidemic effects

Diabetes mellitus (DM) is a disorder of the endocrine system caused by abnormally high blood sugar, which is a major health issue around the world (Cole and Florez, 2020). Currently, DM is one of the fastest growing diseases in the world, and people living with diabetes are predicted to number as many as 700 million by 2045 (Cho et al., 2018; Craciun et al., 2022). DM patients need to take long-term sulfonylureas, α -glucosidase inhibitors, biguanide, DPP-1 inhibitors and other chemical agents to treat DM and reduce the risk of complications (kidney failure, coronary heart disease, blindness, and ischemic encephalopathy). Moreover, a large number of fundamental studies and clinical investigations have indicated that plant polysaccharides have beneficial hypoglycemic effects (Juan et al., 2018; Monirsadat et al., 2021; Yajun et al., 2017). Side effects and toxicity of chemical agents will cause great damage to patients' health, but plant polysaccharides have few or no side effects (Guang-Jie et al., 2019). Rakhmanberdyeva et al. obtained galactomannans after removal of proteins with hot water extraction method from *G. macracantha* (Rakhmanberdyeva et al., 2005). The animal experiment results revealed that all of the GSGs exhibited in experiments with intact rats a slightly significant down-regulation of fasting blood glucose levels. When combined with alloxouracil, it significantly prevented the development of experimental hyperglycemia and diabetes.

Hyperlipidemia is a chronic metabolic disorder caused by high levels of circulating lipids and lipoproteins in the body (Chen et al., 2017; Yao et al., 2020). The characteristic of this disease is continuous circulation of high triglycerides, phospholipids, fatty acids, fats, cholesterol, and cholesterol esters thus giving rise to the hyperlipidemia, hypercholesterolemia, and atherosclerosis (Pratap et al., 2022). In addition, it was reported that polysaccharides could effectively reduce the levels of Triglyceride (TG), Total cholesterol (TC) and Low-density lipoprotein cholesterol (LDL-C) in plasma, and increase the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione (GSH) in liver tissue (Xu et al., 2022). Some biological investigations towards the hyperlipidemia activity of galactomannans from *Gleditsia* species are also being carried out. Lai et al. extracted galactomannan from the fruit of *G. sinensis* Lam. The biological assays demonstrated that GSGs significantly reduced the lipid levels of serum, aorta and liver in Japanese white rabbits (Lai et al., 2011). Therefore, it has great application potential in the treatment of hyperlipidemia and related complications.

Based on the literature, it is presumed that α -(1→4) in the main chain and (1→6) glycosidic bond have higher hypoglycemic and hyperlipidemia activities because they have good biocompatibility and biodegradability (Wasser, 2002). For example, polysaccharides in Chinese Herbs with (1→3), (1→4), (1→6) glycosidic bonds presented hypoglycemic or a...? (Ai et al., 2021; Wang et al., 2018); Qinghai *L. ruthenicum* fruits with a 1,6- β -d-Galp backbone showed significantly reduce blood glucose level (Dawei et al., 2022). Thus, it could be further demonstrated that (1→6)

linked α -Gal and (1→4) linked β -Man of GSGs had better hypoglycemic and hyperlipidemic activities. Of course, this needs to be also verified and supported by a large amount of experimental data.

4.2. Antibacterial activity

Nowadays, antibiotics are a class of strong bactericidal and bacteriostatic drugs, which have been widely used in the treatment of animals and plants. However, studies have found that the continuous use of antibiotics and human and animal excreta will increase the concentration of residual antibiotics in the living environment, which will further increase the resistance of bacteria (Yin et al., 2022; Yujie et al., 2019). Therefore, it is urgent to find safe and effective antibacterial agents that can reduce the durability of bacteria. As one of the bioactive components, polysaccharides have attracted much attention for their easy access, biocompatibility and safety (Otu Phyllis Naa et al., 2021; Xiao-Li et al., 2018). Many studies have found that plant-derived polysaccharides have strong antimicrobial activity against various Gram-negative and Gram-positive bacteria (Xia et al., 2020). Therefore, it is of great value to explore the antibacterial activity of *Gleditsia* species galactomannans for the development of new antibacterial agents.

Martins et al. have reported a method for use of edible coatings made of galactomannans from *G. triacanthos*. The bacteriostatic tests showed that the edible coatings incorporating nisin not only help in retarding the growth of *Listeria monocytogenes* but also extend its shelf life (Martins et al., 2010). In another similar study, E and coworkers (E et al., 2023) reported a carboxylated galactomannan (CGM) preparation using etherification and radical addition reaction, then combined it with

iron chelates and the enzyme glucose oxidase to form a hydrogel CGM-Fe (III). *In vitro* antibacterial experiments using that preparation shown that CGM-Fe (III) hydrogel has obvious bacteriostatic effect on *Escherichia coli* and *Staphylococcus aureus*.

4.3. Regulation of gut microbiota

The composition of gut microbiota is diverse and complex, which affects the health status of the host (Hye-Bin et al., 2022). Studies have found that beneficial gut microorganisms can play an immunomodulatory role and improve the host's intestinal barrier function; harmful gut microorganisms can produce protein toxins in the gut, causing necrosis of the intestinal mucosa and destroying the host's intestinal environment. (Deng et al., 2018; Samuelson et al., 2015; Simon and Gorbach, 1984). Thus, increasing the number of beneficial bacteria and decreasing the production of harmful bacteria are considered to be important mechanisms for ameliorating host disease.

On the one hand, mannoooligosaccharides (MOS) act as a prebiotic to indirectly regulate DM by promoting the growth of beneficial bacteria and inhibiting pathogenic bacteria (Zheng et al., 2018). For example, Xu et al. prepared *G. microphylla* galactomannans into mannoooligosaccharides (MOS), and shown relieve the symptoms of diabetes (Wei et al., 2022) in mice/humans/in vitro?. This is because MOS can improve the number of beneficial gut microbes, including n-butyric acid, which was reported as active glucose user that helps alleviate complications caused by obesity and diabetes (Zheng et al., 2018). On the other hand, polysaccharides can be metabolized to Short-chain fatty acids (SCFAs) to alter the microbiome and harmful bacteria

population and further promote gut health and relieve inflammation (Jie et al., 2023). Moreover, SCFAs enhance the integrity and compactness of the epithelial barrier by providing energy to intestinal epithelial cells. Qin et al. found that GSGs alleviated DSS-induced ulcerative colitis by enhancing the concentration of propionate, isobutyrate, valerate, and isovalerate and increasing the abundance of *Lactobacillus* (Qin et al., 2023) in mice/humans/in vitro?. In conclusion, the molecular mechanism of GSGs regulating intestinal microbes is not comprehensive enough and needs further efforts that needed to explore the relationship between the molecular structure of GSGs and intestinal flora and anti-diabetic activity.

4.4. Antioxidant activity

Previous studies have found that oxidative stress causes diseases such as arteriosclerosis, diabetes, nephritis and cancer (Forbes et al., 2008; Negre-Salvayre et al., 2008). Scientific research shows that polysaccharides, as biological macromolecules, which has the functions of increasing antioxidant enzyme activity, eliminating free radicals and anti-aging effects (Liu et al., 2018). A number of research studies found that the antioxidant activity of polysaccharides can be exerted through NF- κ B, Nrf2/ ARE signaling pathways. The antioxidant mechanism schematic diagram of polysaccharides was shown in Fig. 5. Under normal physiological conditions, Nrf-2 is present in the cytoplasm along with negative regulatory protein Keap-1. When H₂O₂ cause oxidative stress, Nrf-2 disassociated from Keap-1, and further translocate from the binding site of Keap-1 to the nucleus. Polysaccharide intake can promote the activation of Nrf-2/ARE and reduce oxidative stress in cells, thereby increasing the

activities of T-AOC, CAT, GSH-PX and SOD (Long et al., 2022). NF- κ B is a major regulator of inflammation and immunity, usually coexists with its inhibitor I κ B- α (Afonina et al., 2017). When exogenous or endogenous substances cause oxidative stress, the phosphorylation level of I κ B- α in cells was significantly increased, promoting the translocation of NF- κ B to the nucleus. The intake of polysaccharides can attenuate inflammatory injury via the suppression of the NF- κ B activation (Hu et al., 2019).

Current research studies agree that polysaccharide extracts of *Gleditsia* species seeds exhibit good antioxidant activity in vitro. For example, Rakhmanberdyeva et al. found that the smaller the molecular weight of *Gleditsia* galactomannan, the stronger its antioxidant capacity (Rakhmanberdyeva et al., 2022). Niknam et al. obtained GSGs by hot water extraction of *G. caspica*. The further antioxidant experiments disclosed that GSGs showed DPPH, reducing power, and FRAP were 21.54%~29.85%, 0.045~0.071, and 0.03 to 0.06 mM/L, respectively (Niknam et al., 2021). Cerqueira et al. also obtained the GSGs by three pretreatment methods and ethanol purification of *G. triacanthos*. Subsequent biological evaluation showed that GSGs had considerable antioxidant activity. The GSGs showed DPPH with the IC₅₀ value of 1.40~13.31 mg/mL (Cerqueira et al., 2010b).

Wang et al. thought the bioactivity of plant polysaccharides is also related to monosaccharide composition, and the more complex the monosaccharide composition led to the better of biological functions (Wang et al., 2022). It was reported that the higher the mannose content of polysaccharides, the stronger the immunostimulatory

activity and antioxidant activity (Qin et al., 2023). At present, it has been reported that the monosaccharide composition of *Gleditsia* species galactomannan has a large proportion of mannose (Table.1), which can further support the antioxidant ability of *Gleditsia* species galactomannan. However, the mechanism of GSGs *in vivo* is not completely clear, and further research is needed.

4.5 Anti-inflammatory activity

Inflammation is a very complex pathological process, which is normal protective response of living tissue and vascular system against infection, tissue injury, trauma or harmful irritation (Herold and Mrowka, 2019; Shen et al., 2020). When exposed to external stimuli, some pro-inflammatory cytokines tumor necrosis factor-alpha (TNF- α), interleukin-1 and 6 (IL-1 and IL-6) are induced and released by macrophages (Zhang et al., 2015). The anti-inflammatory effects of polysaccharides may attribute to their potent ability of immune modulation (Yin et al., 2019), which can bind to Toll-like receptors (TLRS) expressed on the surface of macrophages and inhibit the production of proinflammatory cytokines by inhibiting NF- κ B activation, as shown in Fig. 5.

Polysaccharides extracted from the seeds of *Gleditsia sinensis* Lam. (ZJMP) have anti-inflammatory activity. Qin et al. found that dietary ZJMP not only reduced the overproduction of proinflammatory cytokines such as IL-6, IL-1, and TNF- α in colonic mucosa in mice, but also inhibited NO level, MPO and SOD activity, which strongly suggesting that the seeds of *Gleditsia* could lay the foundation as a food with anti-inflammatory properties in the future (Qin et al., 2023). However, current studies on the anti-inflammatory mechanisms from *G. sinensis* seed is still relatively superficial.

Future research should focus on the inhibitory effect of polysaccharide from *G. sinensis* seed on different inflammatory cells.

5. APPLICATIONS

As a non-toxic, degradable, abundant, and low-cost natural biomass, GSGs has potential applications in various fields such as cosmetics, food, drilling, explosives, paper, petroleum, pharmaceuticals and textiles.

5.1. Food industries

Because GSGs has good physicochemical and functional properties, they can be used as thickeners, gelling agents, and stabilizers in the food industry (Débora et al., 2017; Martina et al., 2013; Suthari, 2020). Various factors such as temperature, pH, droplet size in the storage environment can destabilize the emulsion, so emulsifiers are needed to enhance stability. Niknam et al. indicated that the higher the galactomannan concentration, the better the stability and rheological properties of the emulsion (Niknam et al., 2022). In addition, the antioxidant and antibacterial properties of galactomannan can be made into films and coatings, thereby extending the shelf life of food (Cerqueira et al., 2010b; Martins et al., 2010). The addition of GSGs to flour can not only increase the elasticity of the dough, but also reduce the hardness and chewiness of the bread, thereby improving the quality of the bread (Sciarini et al., 2023).

5.2. Pharmaceutical industries

Polysaccharides have attracted much attention in drug delivery systems due to their natural low toxicity and biocompatibility. Since galactomannan provides a

physical barrier for active substances, it protects them from digestion by the stomach and small intestine, thus achieving the purpose of active substances being released under the weakly alkaline conditions of the colon (Zhongyu et al., 2023). Therefore, GSGs have potential applications as release control agents in sustained drug release (Jian et al., 2010). GSGs can be formed with xanthan gum as a slow-release material and encapsulate theophylline in the structure, thereby effectively delaying drug and increasing bioavailability (Honglei et al., 2012). In addition, hydrogels formed from enzyme-induced galactomannan show antibacterial properties and have potential clinical applications as wound adhesives (E et al., 2023).

5.3. Industrial and agricultural applications

In recent years, galactomannan plays an important role in industrial and agricultural fields due to its excellent thickening, stabilizing and gelling abilities. In agriculture, Liu et al. found that the hydrogels prepared with *G. microphylla* galactomannan could significantly improve the water absorption capacity of the sandy soil, which provided reference value for desertification prevention and control strategies (Liu et al., 2022). On the industrial side, the high molecular weight, thermal stability, and filtration properties of GSGs help neutralize reactive clay minerals present in the formation, thus playing an important role in oil and natural cyclone drilling operations (Yurany et al., 2022).

6. CONCLUSIONS AND FUTURE PERSPECTIVES

As the main active components of leguminous seeds, optimizing the extraction

and purification methods, and clarifying their structure and biological activities has aroused scientific interest. Microwave-assisted and ultrasonic-assisted methods have higher yield and emulsification characteristics than hot water extraction alone. Although the extraction rate of polysaccharide from acid and base extraction was higher, the structure of polysaccharide was damaged, and the water solubility was decreased. In addition, different varieties of *G. sinensis* led to the difference in molecular weight of galactomannans. And their glycan backbones are mainly represented by β -(1 $\rightarrow$ 4)-D-Man and α -(1 $\rightarrow$ 6)-Gal. Gleditsia species seeds galactomannans (GSGs) have shown promising biological activities, including antibacterial, antioxidant, immunomodulatory, hypolipidemic and hypoglycemic activities. Previous studies have found that Gleditsia species seeds galactomannans (GSGs) can be used as a thickener of dairy products, slow-release material in colonic administration and texture modifier of starch-based products. The food industry has made use of Gleditsia polysaccharide as films due to their relatively high free radical scavenging effect.

To sum up, the isolation, structure identification, and bioactivities of Gleditsia species seeds galactomannans (GSGs) have been extensively investigated in recent years. However, there are few studies on their advanced structure and pharmacological activity, and the differences of physicochemical properties of galactomannans among different varieties are not clear. At the same time, there is a lack of pharmacologic activity *in vitro*, *in vivo*, studies and clinical application data. Therefore, in order to promote the development and application of Gleditsia species seeds galactomannans (GSGs), it is necessary to further study its physicochemical and functional properties

and conduct in vivo studies and clinical trials.

Credit Author Statement

Jiaqi Sang: Data curation, Writing. Weijuan Huang and Riming Huang: Reviewing and Editing. Xiaoqun Wei: Conceptualization, Methodology. Yujuan Xu: Visualization, Investigation. Anastassiades Tassos: Supervision and Software, Validation. Hongtao Lei: contributed materials and analysis tools.

Conflict of interest

There is no conflict to declare.

Ethics and Availability

Ethics approval was not required for research

Data availability statement

At request made

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Figure captions

Figure 1 (A) Pictures of *Gleditsia japonica* Miq. (B) Pictures of *Gleditsia triacanthos* L. (C) Pictures of *Gleditsia sinensis* Lam. (D) Pictures of *Gleditsia caspica* Desf. (E) Pictures of *Gleditsia aquatica* Marshall. (F) the distribution diagram worldwide of *Gleditsia*.

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Figure 2 Extraction and purification processes of *Gleditsia* species seeds (GSGs).

Figure 3. (A) The presumed chemical structures of galactomannans isolated from *Gleditsia delavayi* (Rakhmanberdyeva, 2004) (B) *Gleditsia macracantha* (Rakhmanberdyeva, 2009) and (C) *Gleditsia japonica* var. *delavayi* (Sun et al., 2018)

Figure 4. Schematic representation of biological properties of GSGs.

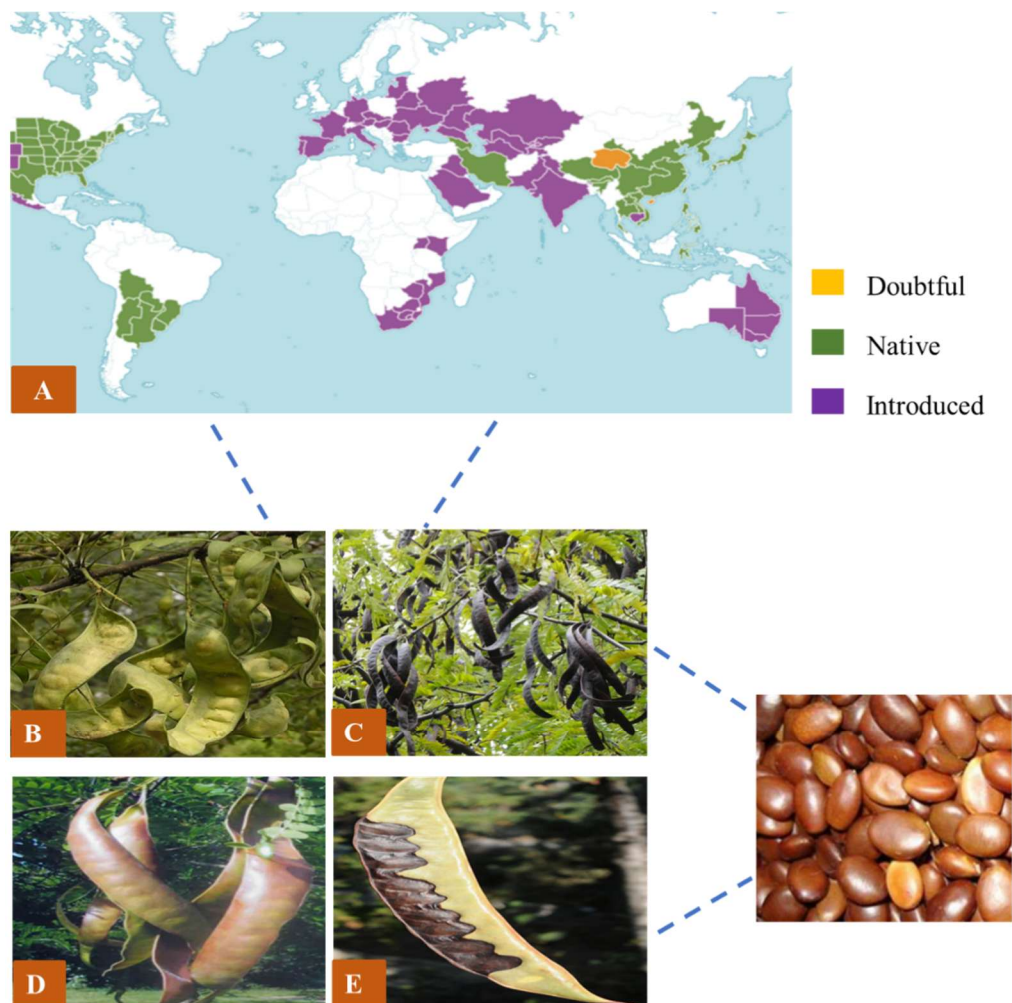
Figure 5. Schematic representation of the underlying molecular mechanism showing antioxidant activity and anti-inflammatory schematic diagram of GSGs.

Table 1. The extraction, purification methods, yields, molecular weight, and monosaccharide composition of GSGs.

Galactomannans source	Origin	Extraction	Purification	Molecular weight (Da)	Monosaccharide compositions	M:G	Biological activity	Reference
G. japonica var. delavayi	China	Hot water	agarose gel filtration or Ion exchange chromatography (Sephacrose CL-2B)	1.913×10^6	ND	2.54~2.66	ND	(Sun et al., 2018)
G. caspica	Soviet or Russia?	Hot water	96% ethanol and acetone	ND	Man:Gal:Glu:Ara=62.1:31.75 :2.63:2.27	ND	antioxidant activity	(Niknam et al., 2021)
		Ultrasound- assisted		ND	ND	1.95	ND	(Niknam et al., 2022)
		Microwave- assisted		ND	ND		ND	
G. triacanthos	N. America	Acidic %?	% ethanol and acetone	1.620×10^6	Man:Gal:Glu:Ara:Fuc=66.9:2 3.7:1.4:4.5:1.3	2.82	Antioxidant activity	(Cerqueira et al., 2009)
		Hot water	ND	ND	Man:Gal=43.1:17.3	2.49	ND	(Sciarini et al., 2009)
	Argentina	Alkaline	ND	ND	Man:Gal=46.8:18.2	2.57	ND	

		Alkaline	agarose gel filtration or Ion exchange chromatography (Sephacrose CL-2B)G- 200 and G-100	ND	Man:Gal:Xyl:Ara:GalA= 62.8:23.5:2.9:4.3:2.8	2.67	ND	(Mazzini and Cerezo, 1986)
	Portugal	Hot water	ethanol	ND	ND	ND	Antibacterial activity	(Martins et al., 2010)
	Portugal	Hot water	ethanol	ND	ND	ND	Antioxidant activity	(Cerqueira et al., 2010b)
	Uzbekis tan	Acidic	ethanol	0.018×10^6	ND	2.1	Antioxidant activity	(Rakhmanberdyeva et al., 2022)
G. sinensis Lam.	China	Hot water	% ethanol and acetone	2.778×10^6	Man:Gal=81.5:18.5	4.40	Antibacterial activity	(E et al., 2023; Jian et al., 2011)
		60% Acidic	% ethanol and acetone	2.603×10^6	Man:Gal=87.4:12.6	6.90	ND	(Egorov et al., 2004)
		water	ethanol	1.282×10^6	ND	3.25	ND	(Jiang et al., 2011)
		Hot water	ethanol water solution	ND	ND	ND	Hypolipidemic	(Lai et al., 2011)

			of HCl				activity	
		Hot water	ethanol	ND	ND	ND	Regulation of gut microbiota and Anti-inflammatory	(Qin et al., 2023)
<i>G. ferox</i> Desf	Russia	ND	ethanol	1.660×10^6	Gal:Man:Glu:Ara:Xyl:Rha=2 7.94:66.41:0.92:1.62:0.27	2.54	ND	(Egorov et al., 2004)
<i>G. caspia</i> Desf.	Russia	Hot water	ethanol	0.685×10^6	ND	2.85	ND	(Shcherbukhin, 1992)
<i>G. microphylla</i>	China	water	ethanol	0.652×10^6	ND	3.31	ND	(Jiang et al., 2011)
<i>G. melanacantha</i>	China	water	ethanol	0.979×10^6	ND	2.30	ND	
<i>G. macracantha</i>	Uzbekistan	water	ethanol	ND	ND	5.00	hypoglycemic	(Rakhmanberdyeva et al., 2005)
<i>G. sinensis</i>	China	Hot water/	ethanol	$2.77^8 \times 10^6$	ND?	3.00	?	?



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Figure 1. (A) the worldwide distribution of Gleditsia species. (B) Pictures of Gleditsia japonica Miq. (C) Pictures of Gleditsia triacanthos L. (D) Pictures of Gleditsia sinensis Lam. (E) Pictures of Gleditsia caspica Desf.

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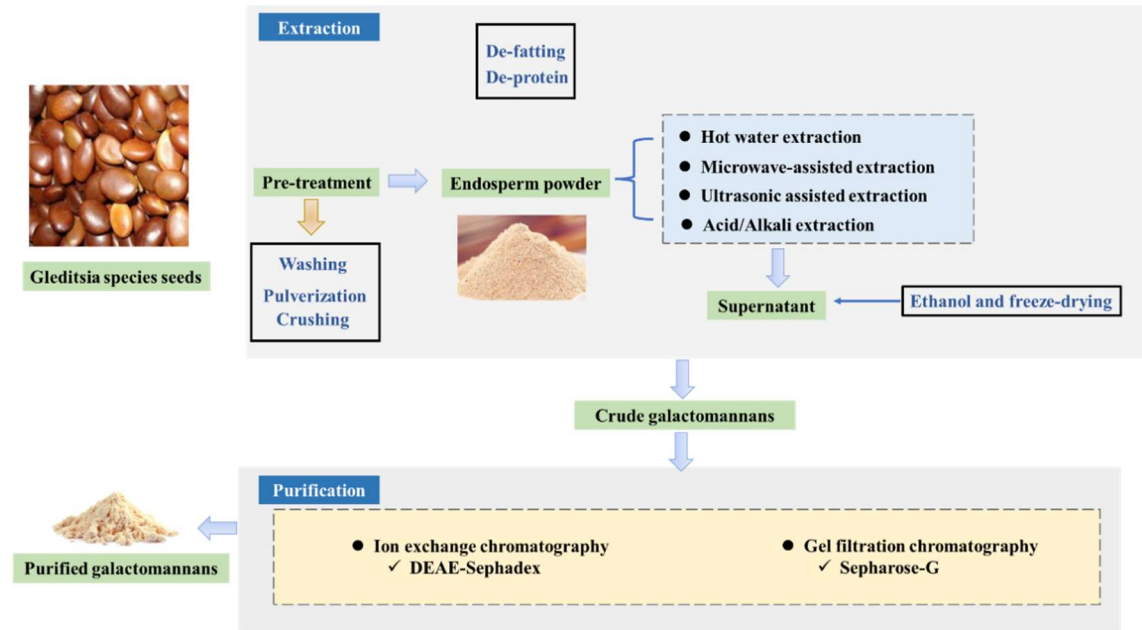


Figure 2. Extraction and purification processes of galactomannans from Gleditsia species seeds (GSGs).

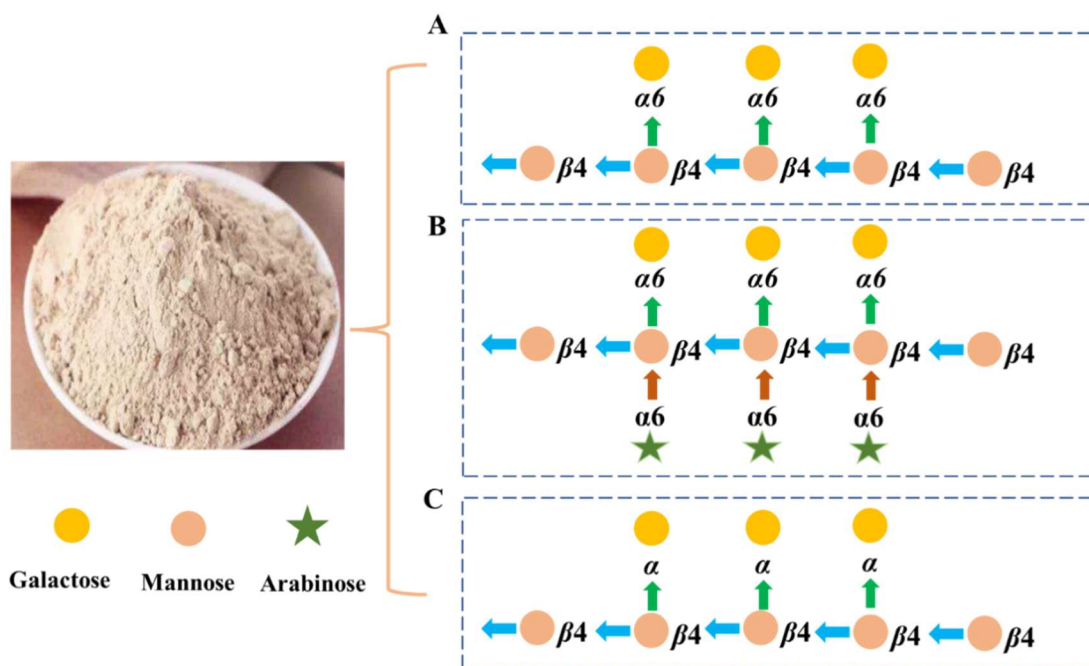


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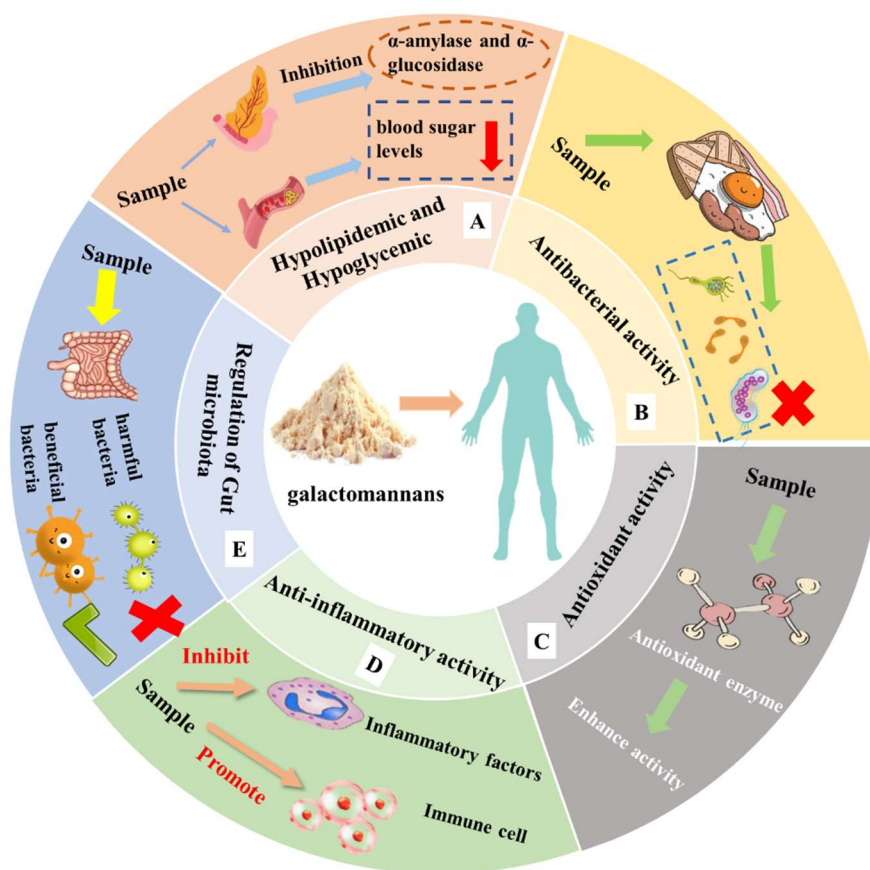


Figure 4 Schematic representation of biological properties of GSGs. The image shows that galactomannans reduced fasting blood glucose levels by inhibiting α -amylase and α -glucosidase. In addition, polysaccharides can effectively reduce the levels of TG, TC and LDL-C in plasma (A). GSGs added to food can inhibit the growth of bacteria, thereby extending the shelf life (B). GSGs can effectively inhibit the production of reactive oxygen species and lipid peroxidation. Remove excess free radicals and improve reducing power (C). GSGs has been reported to inhibit the expression of inflammatory factors, regulate immune function, and induce cytokines secretion (D). GSGs can regulate the composition of intestinal microbiota and enhance the intestinal barrier in diabetes (E). See the text for details.

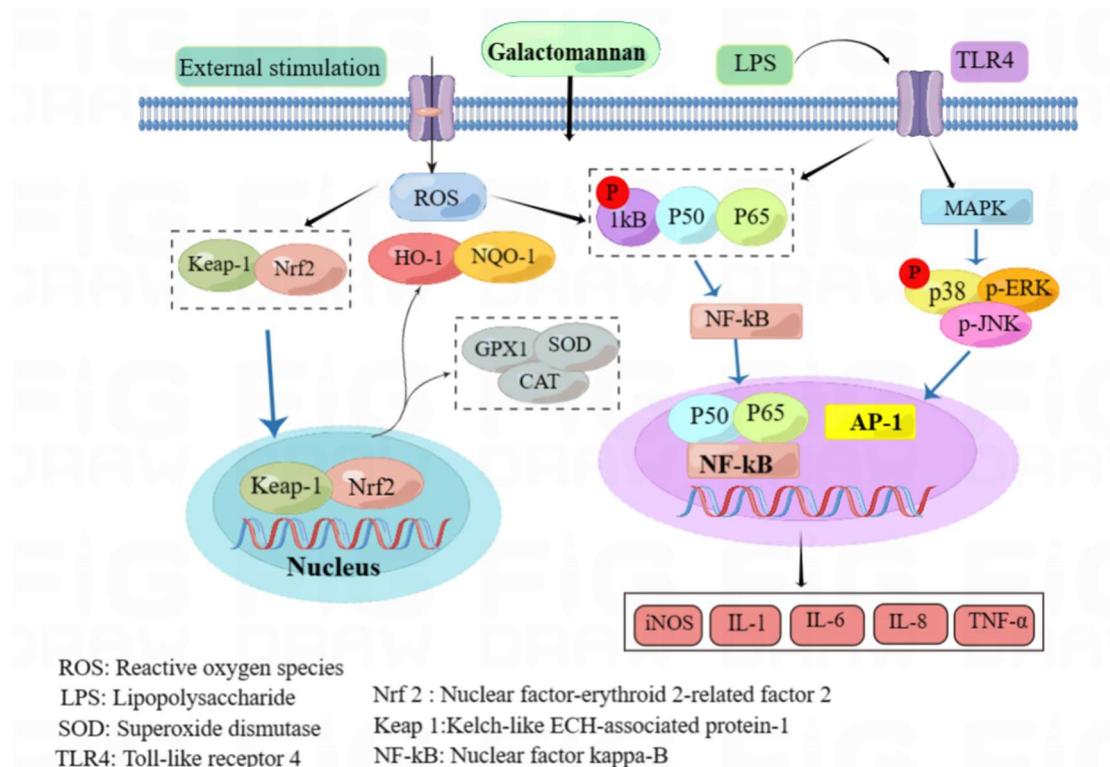


Figure 5. Schematic representation of the underlying molecular mechanism showing antioxidant activity and anti-inflammatory schematic diagram of polysaccharides. GSGs can alleviate oxidative stress by activating Nrf2/ are signaling pathway. In the anti-inflammatory effect, GSGs prevents the activation of NF-κB signaling pathway by reducing p-38 MAPK phosphorylation, i-κB degradation, and nuclear translocation of NF-κB, thereby inhibiting the expression of inflammatory cytokines.