Review of structure and bioactivity of the *Plantago* (Plantaginaceae) polysaccharides

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ABSTRACT

Plantago (Plantaginaceae) is an herbal plant, which is used in folk medicine, functional food, and dietary supplement products. Recent pharmacological and phytochemical studies have shown that polysaccharides isolated from *Plantago* have multiple medicinal and nutritional benefits, including improve intestinal health, hypoglycemic effect, immunomodulatory effect, etc. These health and pharmacological benefits are of great interest to the public, academia, and biotechnology industries. This paper provides an overview of recent advances in the physicochemical, structural features, and biological effects of *Plantago* polysaccharides and highlights the similarities and differences of the polysaccharides from different species and in different parts, including leaves, seeds, and husks. The scientific support for its use as a prebiotic is also addressed. The purpose of this review is to provide background as well as useful and up-to-date information for future research and applications of these polysaccharides.

Introduction

Plantago (Plantaginaceae) is an annual or perennial, stemmed, or stemless herb widely distributed all over the world, which has more than 200 species, such as *Plantago ovata* Forssk., *Plantago asiatica* L., *Plantago major* L., and *Plantago depressa* Willd. and so on (Addoun et al., 2020), and is used in folk medicine, functional food, and dietary supplement products. In China, *P. asiatica*, *P. major*, or *P. depressa* are called Cheqiancao, and their seeds are called Cheqianzi (Akbar, 2020). It was recommended for "disorders of vital energy, pain, diuresis and rheumatism" according to the Shen Nong Ben Cao Jing (Herbal of the Divine Plowman). In Brazilian traditional medicine, this plant was known as *Transagem*, *Tanchagem*, or *Erva-de-orelha* and was used for wound healing; the leaves and seeds were used as antiseptic, anti-inflammatory, and antibacterial. The *P. ovata* (known as psyllium) has been used as a traditional herbal to treat IBD in India and Iran (Akbar, 2020; Belorio & Gómez, 2020). Moreover, psyllium has been widely used in the food industry, such as Metamucil and Mucilin, which are products made from psyllium and have been reported in several clinical studies to increase

satiety, maintain blood glucose levels, lower cholesterol levels, and laxative effects. (Brum, Gibb, Peters, & Mattes, 2016; Lertpipopmetha, Kongkamol, & Sripongpun, 2019).

There are several effective chemical constituents in *Plantago*, such as flavonoids, alkaloids, terpenoids, phenolic acid derivatives, iridoid glycosides, fatty acids, and polysaccharides. Among them, polysaccharides are the most abundant component, and their multiple functions have been widely studied and reported. (Adom et al., 2017; Ji, Hou, & Guo, 2019). Both the leaves and seeds of *Plantago* contain amounts of polysaccharides. The extraction optimization, structural identification, and biological activity of polysaccharides from *Plantago* have been investigated extensively. It is worth mentioning that *P. ovata* seed husk (as known as psyllium husk, ispaghula husk, isabgol, and mucilage) contains more than 85% polysaccharide and has been recognized as a dietary fiber by the FDA (Guo, Cui, Wang, & Young, 2008; Phan et al., 2020). However, the structures and bioactivity of psyllium husk were rarely mentioned in the available reviews related to *Plantago* polysaccharides. In this article, psyllium husk is compared with other polysaccharides from *Plantago* together to give more

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comprehensive information for the present review.

In general, this review provides an overview of the structural information and beneficial effects of *Plantago* polysaccharides and highlights the similarities and differences of the polysaccharides from different species and in different parts, including leaves, seeds, and husks. The scientific support for its use as a prebiotic is also addressed. The purpose of this review is to provide background as well as useful and up-to-date information for future research and applications of these polysaccharides.

Extraction, physicochemical properties, and structural features

The isolation and identification of the polysaccharide from *Plantago* have been started since the 1940 s (Mullan & Percival, 1940). At that time, the scientists had found the polysaccharide extracted from the seed of *P. ovata* Forsk was mainly composed of xylose (80%) and arabinose (14%) (Laidlaw & Percival, 1950). With the rapid development of science and technology, more and more research has been carried out around this field, and the structural features of *Plantago* polysaccharides are more and more detailed. Table 1 shows the physicochemical properties of the polysaccharides obtained from different species of *Plantago*, as well as their extraction parts and extraction methods.

Extraction and purification methods

The extraction of polysaccharides usually includes impurity removal, extraction, concentration, precipitation, dialysis, purification, and freeze-drying. First, monosaccharides, oligosaccharides, and some small coloring molecules in raw materials are extracted by 80% ethanol. Then, the solvent and the materials are extracted 2 to 3 times with an appropriate ratio of 2 to 3 h each. The most common extraction methods are water extraction (Zhou et al., 2020) and alkali extraction (Kumar, Tanna, Mishra, & Jha, 2018), and sometimes auxiliary extraction methods are also used, such as ultrasound (Niknam, Ghanbarzadeh, Ayaseh, & Rezagholi, 2020). Polysaccharides are precipitated by 80% ethanol since they are insoluble in ethanol. After that, protein in polysaccharides is removed by Savage method (Yin, Chen, Lin, Xie, & Nie, 2016) or enzyme method (Huang et al., 2009), and polysaccharide are purified by column chromatography (Yin, Nie, Zhou, Wan, & Xie, 2010). Finally, the purified polysaccharide can be obtained by freeze-drying.

Notably, the choice of solvent, feed-liquid ratio, extraction temperature, and time would affect the yield and even the composition of polysaccharides. Therefore, some studies aimed at optimizing the extraction conditions to seek the most effective way to isolate polysaccharides. Ehsan et al. (2020) optimized the extraction conditions of polysaccharides from the seed of *P. ovata* and found that the temperature of 79 °C, extraction time of 2.5 h, and water to a raw material ratio of 57% was a better method for extraction with a yield of 9 ± 0.25%. Han et al. (2016) found the highest yield (5.68%) of polysaccharides from the leaves of *P. depressa* could be obtained when the raw material was treated with water (w/v, 1:25.34) at 80.44℃ for 1.97 h and 3.28 times.

As shown in Table 1, the polysaccharide content varied among species and extraction parts. The content of polysaccharides in seeds was higher than that in leaves, and the content of polysaccharides in the seeds of *P. ovata* was higher than that in the seeds of other *Plantago* spp.

Table 1
The polysaccharides from *Plantago*: source, extraction method, physical and chemical properties.

Source	Extraction part	Extraction method	Yield	Mw (u)	Monosaccharide composition	Reference
P. asiatica L.	Seeds	Boiled water	6.2%	–	Xyl (72.17%), Ara (20.65%), Rha (2.28%), Gal (3.90%), Glu (0.92%)	(Yin et al., 2012a,b)
P. asiatica L.	Seeds	Alkaline (0.5 M NaOH)	–	3.8×10^6	Xyl (63.95%), Ara (15.41%), Gal (2.58%), Glu (1.29%), Rha (1.00%)	(Yin et al., 2016)
P. ciliata Desf.	Seeds	Hot water (60℃)	18.6%	7×10^6	Xyl (78%), Ara (18%), Rha (3%), GalA (1%)	(Addoun et al., 2020)
P. depressa Willd.	Seeds	Boiled water	12.46%	1.5×10^6 (PDSP-3)	Man (28.1%), Ara (27.9%), Fuc (16.0%), Xyl (10.1%), GalA (8.1%), Gal (4.2%), GluA (3.4%), Glu (1.4%), Rib (0.8%)	(Zhao et al., 2014)
P. major L.	Seeds	Hot water (50℃)	–	–	Xyl (39.7%), Ara (13.1%), GalA (17.2%), GluA (15.5%), Rha (2.1%), Gal (2.5%), Glu (9.9%)	(Berit et al., 1999)
P. notata Lag.	Seeds	Hot water (60℃)	4.66%	2.3×10^6	Xyl (77.4%), Rha (9.20%), Ara (7.58%), Gal (2.58%), GalA (2.21%), Glu (1.00%)	(Benaoun et al., 2017)
P. ovata Forssk.	Seed husks	Hot water (80℃)	18.6%	–	Xyl (68.94%), Ara (15.97%), Rha (9.89%), Gal (2.63%), Man (2.26%)	(Guo et al., 2008)
P. ovata Forssk.	Seed husks	Alkaline (0.5 M NaOH)	61.4%	–	Xyl (71.16%), Ara (24.52%), Man (1.74%), Gal (1.82%), Rha (0.76%),	(Guo et al., 2008)
P. ovata Forssk.	Seed husks	Alkaline (2 M NaOH)	45%	3.64×10^5	Xyl (74.8%), Ara (23.2%), Gal (1.2%), Rha (0.8%)	(Saghir et al., 2008)
P. asiatica L.	Leaves	Hot water	–	3.54×10^4	GalA (36.5%), Gal (34.4%), Ara (10.1%), Rha (8.4%)	(Yin et al., 2019)
P. lanceolata L.	Leaves	Hot water (80℃)	0.64%	–	GalA (70.58%), Ara (29.42%)	(Lukova, Karcheva-bahchevanska, Nikolova, & Mladenov, 2017)
P. major L	Leaves	Hot water (80℃)	1.92%	6.60×10^4	GalA (55.38%), Glu (21.50%), Ara (9.88%), Gal (8.02%), Rha (3.17%), Xyl (2.05%)	(Lukova et al., 2020)
P. media L.	Leaves	Hot water (80℃)	2.79%	–	GalA (64.88%), Ara (35.12%)	(Lukova et al., 2017)
P. notata Lag.	Leaves	Hot water (70℃)	2.0%	–	Gal (43.95%), Rha (20.28%), GalA (12.57%), Glu (11.30%), Ara (9.55%)	(Boual, Chouana, Kemassi, Hamid Oudjana, Daddi Bouhoun, Michaud, et al., 2015)
P. ovata Forssk.	Leaves	Hot water (90℃)	15%	1.07×10^4	Glu (64.3%), Rha (19.5%), Gal (7.5%), Ara (5.1%), Xyl (3.6%)	(Kumar et al., 2018)
P. ovata Forssk.	Leaves	Hot alkaline (90℃)	10%	1.69×10^4	Rha (37.3%), Glu (35.4%), Ara (11.8%), Gal (11.6%), Xyl (3.8%)	(Kumar et al., 2018)
P. palmata Hook. f.	Leaves	Hot water (50℃)	–	1.2×10^6	Gal (39.2%), Ara/Xyl (26.8%, approximately 9:1), GalA (14.2%), Rha (9.6%), Man (5.4%), Glu (4.8%)	(Biringanine et al., 2012)

Abbreviations: Xyl, xylose; Ara, arabinose; Rha, rhamnose; Gal, galactose; Man, mannose; Glu, glucose; Fuc, fucose; Rib, ribose; GalA, galacturonic acid; GluA, glucuronic acid.
"-" represents not studied.

Physicochemical properties and structure features

According to the structural theory of protein, the structure of polysaccharides can also be divided into primary, secondary, and tertiary structures. The primary structure of polysaccharides is centered on the type and order of glycosidic bonds, and some basic physical and chemical properties, including relative molecular mass and monosaccharide composition, which play a crucial role in the identification of polysaccharide structure.

The molecular weight of the polysaccharides from *Plantago* seeds is generally around 1×10^6 u, while leaves-derived polysaccharides are relatively lower. The monosaccharide composition of these polysaccharides is also varied by species. Among them, the seed-derived polysaccharides are mainly arabinoxylan, which is mainly composed of xylose and arabinose, with a small portion of rhamnose, galacturonic acid, etc (Addoun et al., 2020; Nie et al., 2018). The leave-derived polysaccharides are mainly pectin-like polysaccharides, consisting mainly of galacturonic acid, along with some galactose, arabinose, and rhamnose, etc (Biringanine, Ouedraogo, Vray, Samuelsen, & Duez, 2012; Tan & Nie, 2020; Yin et al., 2019).

However, some studies on the structure of polysaccharides from the *Plantago* spp. seed have reported different results. For example, a new type of low molecular weight heteropolysaccharide was found in the seeds of *P. asiatica*, which was mainly composed of rhamnose, galactose, and galacturonic acid (similar to pectin) (Niu et al., 2017), and a new polysaccharide was also found in psyllium husk, which was mainly composed of xylose, glucose, and rhamnose (Patel, Tanna, Gupta, Mishra, & Jha, 2019). It is worth mentioning that in both studies, these polysaccharides were isolated from the ground seeds. It could be inferred that grinding or not has a great influence on the structure of the polysaccharides.

For the chemical structure of polysaccharide, the glycosides forms, such as pyran or furan ring, is often determined by infrared spectrum or NMR. The type of monosaccharide residue and the linkage site of the glycosidic bond can be determined by methylation, periodic acid oxidation, Smith degradation, NMR, etc. The isomeric forms of monosaccharide residues (alpha- and beta-) need to be identified by glycosidic bond hydrolysis, NMR, infrared spectroscopy, and Raman spectroscopy. Besides, the order of linkage among monosaccharide residues can be determined by selective hydrolysis, sequential hydrolysis, and NMR. As early as 1979, Kennedy et al. (1979) studied the polysaccharides from *P. ovata* seed husk and found they are highly branched, acidic arabinoxylan, the xylan backbone having both $(1 + 4)$ and $(1 4 3)$ linkages. The majority of the residues in the xylan backbone are variously substituted at O-2 and O-3 with arabinose, xylose, and an aldobiouronic acid identified as 2–0- (galactopyranosyluronic acid)-rhamnose. About 30 years later, Saghir et al. (2008) also identified the structure of this source of polysaccharide and found that it was also an arabinoxylan which consists of β- (1, 4)-linked D-Xyl*p* residues. A large number of studies on the structure of polysaccharides derived from the seeds of *P. asiatica* have been conducted by Yin et al., and a general structural has been deduced (Yin et al., 2012a,b). This kind of polysaccharide was highly branched heteroxylan which consisted of β-1,4-linked Xyl*p* backbone with side chains attached to *O*-2 or *O*-3. The side chains consisted of β-*T*-linked Xyl*p*, α-*T*-linked Ara*f*, α-*T*-linked GlcA*p*, β-Xyl*p*-$(1 \rightarrow 3)$-α-Ara*f* and α-Ara*f*-$(1 \rightarrow 3)$- βXyl*p*, etc.

Prebiotic activity of *Plantago* polysaccharides

Although limited literature reports that *Plantago* polysaccharides as prebiotics, there is a lot of evidence that they have prebiotic potentials. The criteria for prebiotics include three aspects: (a) resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption; (b) fermentation by intestinal microflora; (c) selective stimulation of the growth and/or activity of intestinal bacteria associated with health and wellbeing (Gibson et al., 2004).

Non-digestible

Dietary polysaccharides often interlink in complex ways by a diverse array of bonds between monosaccharide units, and the enzymes that break those bonds in the digestive tract are limited (although they do produce amylase, amyloglucosidases, etc. to remove α-linked sugar units from starch and some other enzymes to break down sugars such as fructose, sucrose, and lactose). Therefore, polysaccharides are usually resistant to digestion in the gastrointestinal tract and could reach the colon (Wong & Jenkins, 2007, 2007; Oliphant & Allen-vercoe, 2019). Both *in vivo* and *in vitro* studies have demonstrated that polysaccharides from *Plantago* spp. are digestion-resistant (Hu, Nie, Min, & Xie, 2013; Marteau et al., 1994).

Fermentable by gut microbiota

In contrast, the gut microbiota has a large number of glycoside hydrolases in their genome and can make good use of the dietary polysaccharides (Oliphant & Allen-vercoe, 2019). *In vitro* fermentation of polysaccharides by fecal flora or single strains is a common method to study the relationship between polysaccharides and gut microbiota. The change of polysaccharide content can reflect the consumption of polysaccharides by microorganisms. During the fermentation, microorganisms also metabolize some gases and short-chain fatty acids (SCFAs) and reduce the pH of the environment. Therefore, gas production, pH, and SCFAs production are also common indicators for evaluating fermentation.

Marlett and Fischer (2002) studied the fermentation of psyllium husk by rat cecal contents and found that psyllium husk was degraded by intestinal flora and the degree of degradation was 43% after 72 h of fermentation. Similarly, Hu et al. (2013b) studied the fermentation of polysaccharides from the seeds of *P. asiatica* by human fecal microbiota and found that $47.2 \pm 1.6\%$ of total carbohydrate in polysaccharide was consumed. They also found that pH in fecal cultures decreased and the content of acetic, propionic, and *n*-butyric acids all significantly increased when the polysaccharide was fermented, which has been reproduced in their other animal experiments (Hu, Nie, Min, & Xie, 2012). Notably, it has been shown that psyllium seed husk can only be metabolized by *Bifidobacteria* after simulated digestion, indicating the importance of pre-digestion in the fermentation of polysaccharides (Elli, Cattivelli, Soldi, Bonatti, & Morelli, 2008).

It is well recognized that the *Plantago* polysaccharides are difficult or slow to be fermented, either *in vivo* or *in vitro* (Gunn et al., 2020; Marteau et al., 1994), which has been considered as a typical low-fermenting fiber (Gill, Rossi, Bajka, & Whelan, 2020; O'Grady, O'Connor, & Shanahan, 2019). When treated by microwave, ultrasound, ball milling, or enzymatic hydrolysis, the molecular weight and viscosity of those polysaccharides often decrease, and it is easier to be fermented by the flora and produce more SCFAs, reflecting better prebiotic activity (Hu, Nie, Li, Fu, & Xie, 2013; Hu, Nie, Li, Wang, & Xie, 2018; Lukova et al., 2020; Pollet et al., 2012). Therefore, how to improve the fermentation of *Plantago* polysaccharides to improve their prebiotic activity is one of the hot research fields currently.

Stimulation of activity and growth of gut microbiota

The polysaccharides from *Plantago* can be consumed and utilized by gut microbiota, so it has the potential to selectively stimulate the growth and/or activity of gut microbiota. Gamage et al. (2018) studied *in vitro* fermentation of digested psyllium husk with healthy human feces and found the addition of psyllium husk promoted the increase of *Bacteroides*. In a similar study, psyllium husk was found to reduce the total aerobes, *Enterobacteriaceae*, *Enterococcus* spp., and lactic bacterial groups (Tamargo, Cueva, Alvarez, Herranz, Moreno-Arribas, & Laguna, 2019). However, in contrast to seeds-derived polysaccharides, leaves-derived (*P. major*) polysaccharides have been shown to promote the

growth of four *Lactobacillus* strains, including *Lactobacillus acidophilus* N, *L. plantarum* S30, *L. sakei* S16, and *L. brevis* S27 (Lukova et al., 2020).

The effect of polysaccharides on the gut microbiota in animals is similar to that *in vitro*. The polysaccharide from the seeds of *P. asiatica* could promote the abundance of *Bacteroides* sp., *Eubacterium* sp., butyrate-producing bacteria *Butyrivibrio* sp., and probiotics *Bifidobacterium bifidum*, *Lactobacillus fermentum*, and *Lactobacillus reuteri* in normal mice colon (Hu et al., 2014). In type 2 diabetic rats, these polysaccharides significantly increased the diversity and abundance of gut microbiota, including *Bacteroides vulgatus*, *Lactobacillus fermentum*, *Prevotella loescheii,* and *Bacteroides ovatus* and reduced the abundance of *Alistipes obesi*, a newly reported species isolated from the feces of the morbidly obese individual with a genome enriched in amino acids and energy conversion pathways (Nie et al., 2019). DSS-induced colitis mice have more *Enterococcus* after consuming psyllium husk (Lin et al., 2020). In addition, the intake of psyllium husk increased the abundance of *Akkermansia*, a potential bacteria that has been reported to relieve obesity and diabetes (Depommier et al., 2019), in both normal mice or colitis mice (Lin et al., 2020).

Human studies have also shown that the polysaccharides from *Plantago* could stimulate the activity and growth of gut microbiota. It was found that the psyllium husk was able to stimulate the growth of *Bifidobacterium* in the stool of healthy women and bring it to a normal level when the subjects were low in *Bifidobacterium* in their feces (Elli et al., 2008). It implied that the regulatory effect of psyllium husk on gut microbiota depends on the initial gut microbiota of the subjects. In another double-blind experiment, psyllium husk was shown to significantly alter the microbial composition of constipated patients, including an increase in *Lachnospira*, *Faecalibacterium*, *Phascolarctobacterium*, *Veillonella,* and *Sutterella* and a decrease in uncultured *Coriobacteria* and *Christensenella*, and thus increase fecal water content (Jalanka et al., 2019). However, it barely alters the composition of gut microbiota in children with irritable bowel syndrome (Shulman et al., 2017). It can be speculated that the prebiotic effects of the polysaccharides from *Plantago* may be related to the type of disease or age group.

Bioactivities of *Plantago* polysaccharides

Plantago has been used medicinally for thousands of years, mainly for the alleviation of diabetes, obesity, constipation, diarrhea, and sometimes as an anti-inflammatory agent. The function and mechanism of polysaccharides, an important active component of *Plantago*, have been investigated and gradually clarified. In the below sections, we describe and discuss the bioactivities (*in vitro*, animal, human studies) of polysaccharides from different species and extraction parts of *Plantago*.

Regulation of glucose and lipid metabolism

Glucose and lipid are essential nutrients for human beings. They provide energy and building blocks for cells. The metabolisms of glucose and lipid are regulated precisely in healthy individuals and are usually interrelated and regulated. Disorders in the metabolism of glucose and lipid cause many diseases, such as atherosclerosis, diabetes, obesity, nonalcoholic fatty liver disease (NAFLD), etc. (Chen et al., 2019). For example, an adequate supply of cholesterol is required to maintain growth and multiple cellular functions (Espenshade & Hughes, 2007), while excess cholesterol in the circulation is associated with the development of atherosclerosis, coronary heart disease, and stroke (Ou et al., 2018). Another example is fatty acids, which are a source of energy source and can be esterified with glycerol to generate triacylglycerol, which is mainly storage in adipose tissues; however, excessive or ectopic deposition of triacylglycerol in adipose tissue, liver, and muscle would lead to a series of metabolic diseases, such as obesity, NAFLD and insulin resistance (Rasouli, Molavi, Elbein, & Kern, 2007). Besides, insulin resistance and defective glucose absorption are the root cause of hyperglycemia in type 2 diabetes mellitus (T2DM) patients. Therefore,

triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), blood glucose, and insulin levels are usually used to reflect glucose and lipid metabolism, and body weight and BMI are indicators associated with disease characterization.

The polysaccharides from the seed of *P. asiatica* has been found to have hypoglycemic effect in STZ and diet-induced diabetic rats (Nie et al., 2018; Nie et al., 2019) (Table 2), and the inhibition of α-amylase activity and binding to bile acids *in vitro* could explain part of the mechanism (Hu, Nie, Li, & Xie, 2013a). In contrast, the polysaccharide from *P. ovata* has not been extensively studied in animal studies or *in vitro* studies, but many clinical trials have demonstrated that appropriate supplementation of this polysaccharide can improve glucose and lipid metabolism in obese (Pal, Ho, Gahler, & Wood, 2016, 2017), T2DM (Abutair, Naser, & Hamed, 2016), and hypercholesterolemic patients (Ribas, Cunha, Sichieri, & Santana Da Silva, 2015) (Table 2 and Table 3). However, the results of psyllium husk supplementation in clinical studies are not always consistent, so *meta*-analysis is important to validate the effectiveness.

A *meta*-analysis examined whether the administration of psyllium husk was beneficial in patients with T2DM and found triglycerides, low-density lipoprotein, fasting blood sugar, and hemoglobin A1c were significantly reduced by psyllium husk intervention, although there was no significant change in high-density lipoprotein, BMI, cholesterol, and weight (Xiao et al., 2020). The efficacy of psyllium husk in regulating disorders of glucose and lipid metabolism have been demonstrated, but they do not appear to be effective for body weight, waist circumference, and BMI (Darooghegi Mofrad, Mozaffari, Mousavi, Sheikhi, & Milajerdi, 2020); however, this cannot negate their beneficial effects in obese patients. The supplementation of psyllium fiber to a normal diet has been reported to be sufficient to obtain beneficial effects in metabolic syndrome risk factors in overweight and obese individuals, and supplementing a healthy diet with psyllium fiber is more effective (Pal, Khossousi, Binns, Dhaliwal, & Ellis, 2011). Another study suggested that psyllium husk could improve these risk factors when consumed at 10 to 15 g/d and maintained for at least 6 months (Jane, McKay, & Pal, 2019). In summary, in any case, psyllium husk as a dietary supplement produces few adverse effects on individuals and appears to be a safe and effective substance to ameliorate disorders of glucose and lipid metabolism (Wharton, Bonder, Jeffery, & Christensen, 2020).

Improve intestinal health

Constipation is a sign of an unhealthy intestinal tract and can be defined as infrequent and difficult bowel movements accompanied by the production of hard pellet-like stools. It greatly affects the quality of life and leads to decreased work productivity (Dennison Himmelfarb et al., 2005). Traditional treatments for constipation include bulking agents (psyllium, methylcellulose), stool softeners (docusate sodium), stimulant laxatives (senna, bisacodyl), osmotic laxatives (milk of magnesia, lactulose, sorbitol), and polyethylene glycol (PEG) (Attaluri, Donahoe, Valestin, Brown, & Rao, 2011).

It is common to recommend constipated patients to "increase their fiber intake" (Wald, 2016), although the relationship between fiber intake and constipation has not been clinically confirmed (Bharucha, Pemberton, & Locke, 2013). A recent literature review of the laxative effects of psyllium husk and wheat bran found that both psyllium husk and coarse wheat bran increased stool water content, but finely ground wheat bran decreased stool water content which has a stool-hardening effect (McRorie Jr., Fahey Jr., Gibb, & Chey, 2020). The author also pointed out the misconception that all dietary fibers have a laxative effect while recognizing the excellent role of psyllium husk in laxatives (McRorie Jr. et al., 2020). Many studies have reported that supplementation with polysaccharides or dietary fiber from *Plantago* is effective in relieving constipation, such as increasing the number of complete spontaneous bowel movements and decreasing the constipation

Table 2
Bioactivity of polysaccharides from *Plantago* (animal study).

Bioactivity	Plant species	Material	Dosage	Duration	Subjects	Indexes for pathological improvement	References
Improvement of glucose and lipid metabolism	*P. ovata* Forssk.	Hot-water extracts of seed husk	0·5 g/kg, twice a day	4 weeks	High-fat diet and STZ-induced type 1 and type 2 diabetic rats	↓ Glycemic response, TC, TG, NEFA. ↑ Glucose tolerance	(Hannan et al., 2006)
Improvement of glucose and lipid metabolism	*P. asiatica* L.	Polysaccharide from seed	100/200/400 mg/kg	4 weeks	High-fat diet and STZ-induced type 2 diabetic rats	↓ Blood glucose, insulin, total cholesterol, TG, NEFA, and maleic dialdehyde ↑ HDL-C, activities of antioxidant enzymes, SCFA	(Nie et al., 2018, 2019)
Improvement of lipid metabolism	*P. asiatica* L.	Polysaccharide from seed	0.4 g/kg	30 days	Kunming mice/ healthy mice	↓ TC, cholesterol, and atherogenic index in blood serum; total lipid and cholesterol levels in liver	(Hu et al., 2014)
Relieve IBD	*P. ovata* Forssk.	Seed husk	5%/10% Psyllium diet	13 d	DSS-induced colitis mice	↓ Colon damage, pro-inflammatory cytokines ↑ Bodyweight, colon length, tight junction protein expression, 11 extracellular matrix-associated genes, including collagens and fibronectin	(Ogata, Ogita, Tari, Arakawa, & Suzuki, 2017)

Abbreviations: STZ, streptozotocin; TC, total cholesterol; TG, total triglyceride; NEFA, non-esterified fatty acid; HDL-C, high-density lipoprotein cholesterol; SCFAs, short-chain fatty acids

Table 3
Bioactivity of polysaccharides from *Plantago* (human study).

Bioactivity	Plant species	Material	Dosage	Duration	Subjects	Indexes for pathological improvement	Adverse effect	References
Improvement of glucose and lipid metabolism	*P. ovata* Forssk.	Seed husk	10.5 g daily	8 weeks	Diabetes type 2 patients	↓ Body weight, BMI, FBS, HbA1c, insulin level, C-peptide, HOMA, IR, ↑ HOMA-β%	–	(Abutair et al., 2016)
Improvement of glucose and lipid metabolism	*P. ovata* Forssk.	Seed husk	12 g, three times daily	12 weeks	Overweight and obese individuals (18–65 years) Australian adults	↓ Weight, BMI, and % total body fat, TC, LDL-C	Some reports of minor bloating initially, all subjects tolerated the supplements well with no adverse effects reported.	(Pal et al., 2011)
Improvement of glucose and lipid metabolism	*P. ovata* Forssk.	Psyllium product	5 g, three times daily	12 months	Overweight/obese Australian adults	↓ Energy and macronutrient intake, body weight, Insulin, TC (at 3 and 6 months), LDL-C (at 3 months)	There were no adverse events reported.	(Pal et al., 2016, 2017)
Improvement of glucose and lipid metabolism	*P. ovata* Forssk.	Seed husk	5.1 g twice a day	8 weeks	Patients with type II diabetes	↓ FBS, HbA1c, LDL/HDL ratio ↑ HDL-C	Both products were well tolerated. Better gastric tolerance to metformin was recorded in the psyllium group	(Ali et al., 2005)
Improvement of lipid metabolism	*P. ovata* Forssk.	Seed husk	7.0 g daily	8 weeks	Brazilian children and adolescents with mild-to-moderate hypercholesterolemia	↓ Serum TC, LDL-C. No effect on HDL-C or TAG concentrations	None of the participants reported any aversion to the smell, taste, appearance, or texture of psyllium. No serious adverse effects were reported during the study.	(Ribas et al., 2015)
Satiety effects	*P. ovata* Forssk.	Seed husk	3.4–10.2 g before meals	3 days	Healthy volunteers	↓ Hunger ↑ Satiety	Most adverse events were mild gastrointestinal symptoms	(Brum et al., 2016)
Relieve constipation	*P. ovata* Forssk.	Seed husk	5 g, twice a day	4 weeks	Constipated patients	↑ Number of complete spontaneous bowel movements	–	(Erdogan et al., 2016)
Relieve fecal incontinence	*P. ovata* Forssk.	Seed husk	3.4 mg	4 weeks	People with fecal incontinence	↓ Fecal incontinent episodes, symptom severity ↑ Quality of life	Loperamide was associated with more adverse effects, especially constipation.	(Bliss, Weimer, Jung, & Savik, 2013)
Relieve IBS	*P. ovata* Forssk.	Seed husk	6 ~ 12 g	6 weeks	Children (mean age, 13 ± 3 years) with IBS	↓ Number of pain episodes	There were no adverse events reported.	(Shulman et al., 2017)

Abbreviations: BMI, body mass index; FBS, fasting plasma glucose; HbA1c, glycosylated hemoglobin; TC, total plasma cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; HOMA-IR, homeostasis model assessment-insulin resistance; HOMA-β, homeostasis model assessment-β; FPG, fasting plasma glucose.
"-" represents not studied.

symptoms (Table 3). There are two main reasons for its function. One reason is that it can promote the growth and proliferation of probiotics, including *Lachnospira*, *Roseburia*, and *Faecalibacterium* (Jalanka et al., 2019), and increase the production of SCFA, such as butyric acid which has been previously reported to have positive effects on gut health (Cremon, Barbaro, Ventura, & Barbara, 2018). Another reason is its water-holding properties, which traps water in the intestine and increase fecal fluidity and stool frequency, thus facilitating defecation and altering the environment of the colonic (Marteau et al., 1994). In addition to psyllium husk, polysaccharides from the seeds of *P. asiatica* have similar effects, such as lowering intestinal pH, increasing SCFA production, and increasing fecal water content, indicating their potential to alleviate constipation (Hu et al., 2012).

It is amazing that *Plantago* is also used as a traditional medicine to treat diarrhea, IBS, and IBD (Akbar, 2020). It may seem a contradiction that psyllium husk can relieve both constipation and diarrhea (Singh, 2007), but there is indeed some evidence that psyllium husk could increase the number of normal stools and decreases the number of liquid stools in patients with diarrhea (Belknap, Davidson, & Smith, 1997). Although psyllium husk was useful in earlier studies on the effects of diarrhea, such as protease inhibitor-induced diarrhea in HIV patients (Suares & Ford, 2011), more recent studies have shown that it is not very effective on sodium-glucose co-transporter 1 inhibition-associated and tube feeding associated diarrhea (He, Schofield, Mahling, Mendonza, & Hinder, 2020; Lertpipopmetha et al., 2019). Accordingly, the diarrhea-relieving effects of *Plantago* need to be further studied and clarified.

Immunomodulatory effect

Immune stimulation is considered as one of the body's key defense strategies to prevent and fight infections, inflammation, and cancer. Immunomodulation is one of the most important bioactive substances of natural polysaccharides (Yu, Shen, Song, & Xie, 2018) and the main mechanism involves toll-like receptors (TLRs). The mammalian TLR family could trigger immune responses by recognizing microbial pathogen-associated molecular patterns (PAMPs). Among them, TLR2 and TLR4 are well characterized as transmembrane receptors involved in the recognition of ligands containing carbohydrate moieties. Upon sensing the ligands, these TLRs trigger the downstream MyD88/TIRAP-IRAK1-TRAF6-TAK1 signaling cascade, which activates mitogen-activated protein kinases (MAPKs) to further regulate cellular proliferation, survival, and immune responses (Meng, Liang, & Luo, 2016).

Huang et al. (2009) found that the polysaccharides from the seed of *P. asiatica* were effective in inducing DC cell maturation (Table 4). A later in-depth study by their team found that this polysaccharide induced DC cell maturation by triggering TLR4 to mediate the MAPK and NF-κB pathways (Huang, Nie, Jiang, & Xie, 2014; Jiang, Huang, Nie, & Xie, 2018). They also found that carboxymethylation and acetylation modification could improve the immunoregulatory effect of this polysaccharide with higher maturation-stimulating activity on DCs (Jiang,

Nie, Huang, Fu, & Xie, 2018; Jiang, Nie, Zhou, Huang, & Xie, 2014). This polysaccharide could also stimulate macrophage proliferation, which involves TLR2 and TLR4 MAPK signaling pathways (Hu et al., 2016). Moreover, the polysaccharides from the leave of *P. asiatica* were also able to stimulate the production of TNF-α and IL-1β by macrophages (Yin et al., 2019). Polysaccharides from other species of *Plantago* have also been reported to have immunomodulatory activity (Table 4). It has been reported that polysaccharides in the leaves of *P. palmata* and the seeds of *P. depressa* could stimulate the proliferation of macrophages and the production of NO and TNF-α (Biringanine et al., 2005; Zhao et al., 2014). Another study on the polysaccharides from the leaves of *P. major* had been demonstrated that it is a potent complement activator with the activity of the same order of magnitude as human immunoglobulin (Michaelsen, Gilje, Samuelsen, Høgåsen, & Paulsen, 2000) and its immunomodulatory activity has been recognized (Biringanine et al., 2012).

Others

There are also some other bioactivities of *Plantago* polysaccharides. Patel et al. (2019) studied the *in vitro* antiproliferative activity and apoptotic activity of polysaccharides from *P. ovata* seeds and husks and their purified fractions using Huh-7 (hepatocellular carcinoma) and HeLa (human epitheloid cervix carcinoma), and they found that both of them had anti-cancer activity, with the polysaccharides from seeds showing higher activity than the husks. Fu et al. (2019) found the polysaccharides from *P. asiatica* seed had the effect of alleviating blood pressure in phase I spontaneous hypertensive rats, which can effectively regulate blood pressure within 1 h, and daily administration could be able to control the severity of hypertension. Li et al (2019) reported that the polysaccharide from *P. asiatica* seed also could protect mice from lipopolysaccharide-induced liver injury, which may be due to its ability to reduce the production of pro-inflammatory cytokines, increase the activity of glutathione peroxidase, and improve total antioxidant capacity in the mouse liver. Besides, this polysaccharide could alleviate nonylphenol-induced reproductive system injury of male rats via the PI3K/Akt/mTOR pathway (Li et al., 2020).

Conclusion and perspective

Both seeds and leaves of *Plantago* contain large amounts of polysaccharides, which have different characteristics and effects as shown in Fig. 1. The structures of polysaccharides in seeds of different *Plantago* species have similarities in that they are mainly composed of arabinoxylan with a molecular weight greater than 10^6 u. The polysaccharides in leaves are mainly pectin, and their molecular weight is usually lower than that in seeds. Due to their complex structure, the polysaccharides in *Plantago* could resist digestion in the gastrointestinal tract and could be broken down and utilized by the gut microbiota in the colon, thereby selectively promoting the growth of some flora, such as

Table 4
Immunomodulatory effects *in vitro* of *Plantago* polysaccharides.

| Plant species	Material	Dosage	Duration	Subjects	Indexes for pathological improvement	References
P. asiatica L.	Polysaccharide from seed	50–800 μg/mL	24 h	Nonylphenol induced intestinal barrier in Caco-2 cells	↓ Caco-2 cytotoxicity, lactate dehydrogenase release ↑ Transepithelial electrical resistance, paracellular permeability of Caco-2 monolayers	(Li et al., 2020)
P. asiatica L.	Polysaccharide from seed	100 μg/ml	24 h	Dendritic cells, from the bone marrow of BALB/c mice	↓ Endocytosis ↑ The expression of maturation markers major histocompatibility complex II, CD86, CD80, and CD40 on DCs, IL-2, T cell proliferation	(Huang et al., 2014)
P. asiatica L.	Polysaccharide from leaves	20–160 μg/mL	24 h	RAW 264.7 mouse macrophage	↑ TNF-α and IL-1β	(Yin et al., 2019)
P. palmata Hook. f. s.	Polysaccharide from leaves	100 μg/mL	48 h	J774 murine macrophage cell line	↑ NO and TNF-α production	(Biringanine et al., 2005)
P. depressa Willd.	Polysaccharide from seed	5–125 μg/mL	72 h	RAW 264.7 macrophage cells	↑ Splenocyte proliferation, NO and TNF-α production	(Zhao et al., 2014)
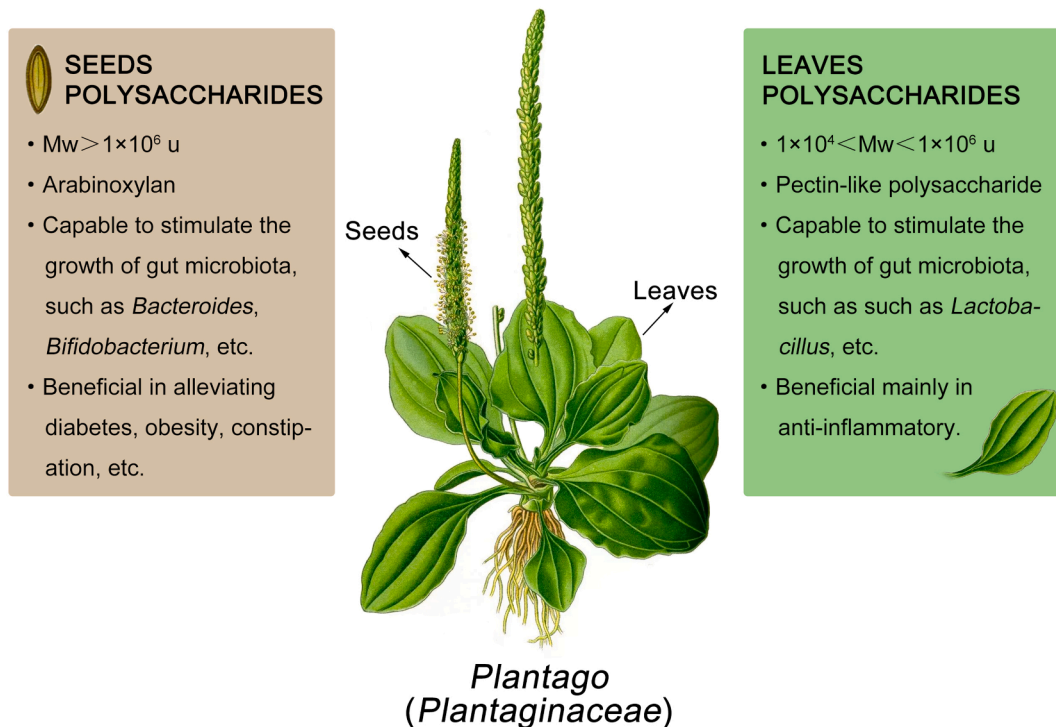
Fig. 1. Characteristics of leaves- and seed-derived polysaccharides from *Plantago.*

Lactobacillus and *Bifidobacterium* (mainly the polysaccharides from seeds). *Plantago* polysaccharide, as a bioactive substance, activity has been widely studied. The polysaccharides in leaves were often used in cell experiments to verify their immunoregulatory activity. In contrast, the polysaccharides in seeds have been studied more extensively and reported to modulate glycolipid metabolism, promote intestinal health, and have immunomodulatory efficacy *in vitro*, animal, and human studies. However, the related human studies all focus on *P. ovata*, and the clinical application of other *Plantago* polysaccharides needs further study. The structures of different sources of polysaccharides are not identical, so more detailed structural information needs to be investigated. Comparing the differences in activities of polysaccharides from different sources of *Plantago* provided a basis for structure–activity relationships and provided a theoretical basis for developing active drugs. These polysaccharides have an important role in regulating the gut microbiota, and their probiotic effects in different initial intestinal environments need to be confirmed in the future to provide a theoretical basis for accurate treatment.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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