8-2017

# Preparation-related structural diversity and medical potential in the treatment of diabetes mellitus with ginseng pectins

Qilei Chen
*Hong Kong Baptist University*

Lin Zhu
*The Chinese University of Hong Kong*

Yina Tang
*Sichuan Academy of Chinese Medical Sciences*

Zhongzhen Zhao
*Hong Kong Baptist University*

Tao Yi
*Hong Kong Baptist University*, yitao@hkbu.edu.hk

*See next page for additional authors*



Link to published article: http://dx.doi.org/10.1111/nyas.13424

# Preparation-related structural diversity and medical potential in the treatment of diabetes mellitus of ginseng pectins

Qi-Lei Chen[a†], Lin Zhu[b†], Yi-Na Tang[c], Zhong-Zhen Zhao[a], Tao Yi[a]*, Hu-Biao Chen[a]*

[a] *School of Chinese Medicine, Hong Kong Baptist University, Hong Kong Special Administrative Region, P.R. China*

[b] *Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen, P.R. China*

[c] *Sichuan Acadamy of Chinese Medical Sciences, Sichuan, P.R. China*

Addresses for correspondence:

Tao Yi, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong Special Administrative Region, Hong Kong 999077, P.R. China. yitao@hkbu.edu.hk;

Hubiao Chen, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong Special Administrative Region, Hong Kong 999077, P.R. China. hbchen@hkbu.edu.hk

† These authors contributed equally to this work.

**Abstract**

Pectins isolated from *Panax ginseng* C.A. Meyer are potential therapeutic agents for the treatment of diabetes mellitus, a global health challenge. Soil-to-bench procedures of ginseng pectins preparation significantly affect the polysaccharide structures. Various forms of ginseng pectins rich in homogalacturonan, rhamnogalacturonan-I, rhamnogalacturonan-II, and arabinogalactan have been found with independent or collaborative effects on hyperglycemia, oxidative stress, immunological dysfunction, and neoplasms. Monosaccharide compositions, peptide contents, degrees of esterification and methylation, and inter- and intra-molecular linkages all influence pectin bioactivity. Understanding the preparation–structure and structure–function relationships of ginseng pectins can lead to safer and more pertinent treatment of diabetes with efficacy-oriented modifications of the pectins. To reach this goal, standardization of preparation procedures, understanding of intricate structures, and exploration of complex interactions with receptors are crucial steps to be taken in taking full advantages of the medical potential of ginseng pectins.

**Keywords:** Ginseng pectin; diabetes mellitus; homogalacturonan; rhamnogalacturonan; arabinogalactan.

## 1. Introduction

The number of people in the world with diabetes mellitus (diabetes) has quadrupled since 1980. In 2012, the disease directly caused 1.5 million deaths worldwide.[1] "Diabetes" is actually a spectrum of chronic metabolic disorders characterized by hyperglycemia.[2] There are two major categories: type 1 diabetes, which is classified as an autoimmune disease with insulin deficiency; and type 2 diabetes, which is mainly caused by insulin ineffectiveness.[3] Medications that can continuously control blood glucose levels and other diabetic symptoms are still being sought.

Asian ginseng (ginseng), derived from the rhizome and root of *Panax ginseng* C.A. Meyer, has promise as an antidiabetic therapeutic. According to *Bencao Gangmu* (Compendium of *Materia Medica*, written in 16$^{th}$ century A.D.), ginseng has long been used to treat hyperphagia (excessive food intake) and weight loss, which are two classic symptoms of diabetes in modern medicine.[4] Ginseng pectins have been extensively studied since the mid-twentieth century.[5] It has been revealed that ginseng pectins possesses potent hypoglycemic[6], anti-oxidative[7], immunomodulative[8], and anticancer[9] activities, alleviating diabetic conditions and complications. Therefore, ginseng pectins have potential to be developed as antidiabetic medicines.

Pectins is a family of galacturonic acid (GalA)-rich polysaccharides which are mostly found in primary cell walls.[10] Three types of ginseng pectins have been isolated and investigated: homogalacturonan (HG), rhamnogalacturonan (RG-I), and rhamnogalacturonan II (RG-II) (Fig. 1). HG is a linear chain of 1,4-linked α-D-galactopyranosyluronic acid (Gal*p*A) with partly methyl-esterified carboxyl groups.[11] RG-I has a backbone of disaccharides composed of α-D-Gal*p*A and rhamnosyl (Rha) bound; the most frequent side chains are arabinogalactans (AG), linear or branched, containing α-L-arabinofuranosyl (Ara*f*) and galactopyranosyl (Gal*p*). RG-II has a poly-GalA backbone[12], decorated with rarely observed monosaccharides such as apiose (Api), and special glycosyl linkages, including 3,4-linked fucose (Fuc) and 2-linked glucuronic acid (GlcA). It is often found existing as a dimer with a borate diester cross-link.[13]

Bioactivities of polymers vary greatly according to their structures. For competent management of and therapy for diabetes with few adverse effects, structurally-tailored pectins

are anticipated.[14] Yet current studies of ginseng pectins have not been systematized. Researchers from different laboratories applied respective methods of sample preparation. The resulting pectins were investigated on various cell and animal models. The therapeutic efficacies of such pectins can hardly be compared because they are of distinct structures in the first place. Sorting out and analyzing literature regarding ginseng pectins is inevitable for in-depth study of the carbohydrates.

Here, we summarize how ginseng pectin structures can be affected by preparation methods and how the different forms thus produced act against multiple targets in treating diabetes. Preparation–structure and structure–function relationships are defined, as far as possible, based on existing information. Strategies for using this knowledge to tailor ginseng pectins specifically for treating diabetes are proposed.

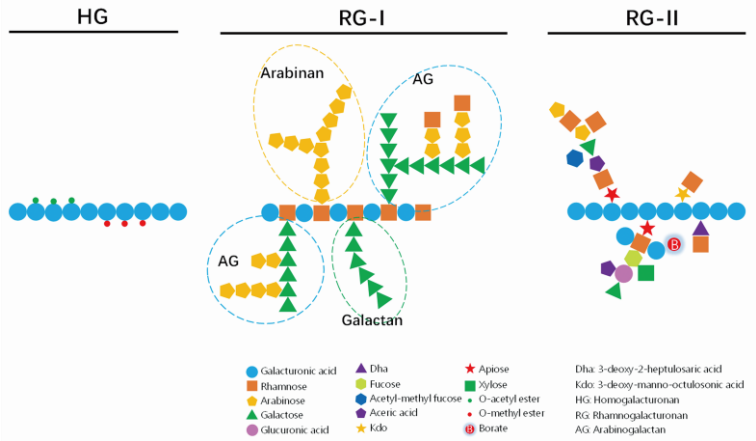


**Figure 1.** Schematic illustration of representative ginseng pectins.

## 2. Preparation-related structural diversity of ginseng pectins

### *2.1. Sample collection and extraction*

Growing area and botanical parts determine pectins content and properties, even before the samples enter a laboratory. Purified polysaccharides of ginseng collected from Korea, China, and Japan were compared by NMR spectrometry and sugar content analysis, and the results greatly varied among the samples.[15–19] It has been shown that ginseng leaves contained more acidic polysaccharides than the roots do, and that the ginseng pectins isolated from the leaves has different pharmacological efficacies from that derived from the roots.[20] Up till now, most of the RG-II ginseng pectins used in experiments has come from the leaves instead of other plant parts.[21] In comparison, root-derived ginseng pectins consist of multiple domains

including HG, RG-I, and AG.

A series of studies have been carried out in Yifa Zhou's laboratory on how extraction methods affect the structure and consequent pharmacological activities of ginseng pectins. Hot water, α-amylase, and EDTA were used to extract ginseng root, yielding carbohydrates with diverse characteristics, correspondingly named WGP[22], WGPE[23], and EGP[24]. Lymphocyte proliferation potency *in vivo* was determined in decreasing sequence as EGP > WGPE >WGP. Structures of the pectic fractions have not yet been elucidated in detail. The studies jointly demonstrated that EDTA and α-amylase, compared to hot water, can extract some novel and active polysaccharides from ginseng.

### *2.2. Chemical modification: acid hydrolysis and heat processing*

The degree of acid hydrolysis affects the structures of highly-branched carbohydrates. For example, when being hydrolyzed, the molecular weight of an RG-I type pectin isolated from ginseng root was observed steadily decreasing; the Ara residues dramatically reduced; while Rha and GalA were not hydrolyzed.[25] The results indicated that the RG-I backbone consisting of Rha and GalA remained intact during hydrolysis while the AG side chains were gradually cleaved. Ara was located near the surface of the pectin molecule while Gal resided closer to its core. The conclusion is consistent with findings from work on other herbal pectin that the acid-affected structural variations of pectins are mainly related to their RG-I contents and integrity.[26]

It has been traditionally agreed that steaming helps to improve the therapeutic potency of ginseng. There are three common ginseng preparations on the market, referring to the medicine not steamed at all (white ginseng); or steamed for a short period of time (red ginseng), or for a long period of time (black ginseng).[27] Corresponding to the traditional usage, heat processing has been confirmed to increase acidic polysaccharides[27], and to enhance anti-hyperglycemic and antioxidant activities of ginseng pectins[28]. The pharmacological changes can be attributed to the temperature-induced changes in the structures of the extract. Steaming affects pectic structures by slightly increasing molecular weight and reducing Gal content, and by un-esterifying GalA residues. Moreover, polysaccharide domains respond differently to the steaming process: HG is the most sensitive

to high temperatures, AG less sensitive, and neutral carbohydrates insensitive to the treatment.[9]

### *2.3. Isolation and purification*

Multiple approaches have been developed to isolate pectins from other substances (Table 1): 1) adding ethanol of high concentrations (80%~95%) to remove small molecules; 2) applying Sevag method to remove protein; and 3) using cetrimonium bromide (CTAB) and boric acid to eliminate DNA from the samples. The three methods have been wildly confirmed as effective to purify polysaccharides, however, the procedures involve physical and chemical disturbance to polysaccharides, which may affect the carbohydrate structural properties (e.g. polymerization degree, the types of sugar chain and glucosidic linkage) to various extents. The influence of the above techniques to pectic structures needs to be further elucidated.

DEAE-Sepharose and DEAE-Cellulose are two representative ion-exchange column (IEC) types used to separate pectins per ionic interaction between the polar carbohydrates and the stationary phase. Increasing salt concentration in the elution buffer leads to displacement of the bound molecules with the lowest isoelectric points (p*I*).[29] Therefore, raising the concentration of the eluent (e.g. NaCl) releases pectin fractions with more uronic acid contents, especially GalA.[22,30,31] The more acidic pectins are often found with more potent pharmacological effects.[30] Following IEC, size-exclusion chromatography (SEC) has usually been applied to further separate pectins mainly according to molecular weight. It has been observed that the content of uronic acid also affects the elution time.[22,28]

**Table 1.** Selected pectins isolated from *P. ginseng* of respective preparation methods and representative structural features.

| Pectin | Sample source | Extraction (reagent/temp./duration/ No. of times) | Purification | Hydrolysis or steaming (reagent/temp./duration) | Column chromatography | MW (Da) | Sugar (%) | Peptide (%) | Monosaccharide and uronic acid composition (%) | | | | | | | | | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Rha | Ara | Gal | Glc | Xyl | Man | Fuc | Gal A | Glc A | |
| WGP | Chinese white ginseng roots | Water/100°C/4 h/thrice | 95% EtOH (pptn.); Sevag | N[a] | N | --[b] | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 22 |
| WGPE | WGP residue | Water/100°C/0.5 h/once; 4000 U α-amylase/50°C/24 h/once | N | ” | ” | -- | 72.5 | 0.7 | 1.0 | 4.8 | 4.5 | 83.4 | 0.0 | 0.0 | 0.0 | 6.3 | 0.0 | 23 |
| EGP | WGPE residue | 50 mM EDTA[c]/60°C/3 h/once | ” | ” | ” | -- | 72.1 | 1.1 | 3.2 | 11.6 | 10.5 | 50.4 | 0.0 | 0.0 | 0.0 | 24.2 | 0.0 | 24 |
| WGPA | WGP | N | ” | ” | IEC(0.5 M)[d] | -- | -- | -- | 2.5 | 15.5 | 18.0 | 18.5 | 0.0 | 0.0 | 0.0 | 42.2 | 1.3 | 22 |
| WGPA-1-RG | WGPA | ” | ” | ” | IEC(0.1 M)-GFC(A)[e] | $1.0\times10^5$ | -- | -- | 0.2 | 34.0 | 56.2 | 3.5 | 0.0 | 2.5 | 0.0 | 1.8 | 1.9 | 22 |
| WGPA-1-HG | ”[f] | ” | ” | ” | IEC(0.1 M)-GFC(B) | $3.5\times10^3$ | -- | -- | 1.6 | 7.1 | 15.2 | 7.6 | 0.0 | 3.6 | 0.0 | 62.4 | 2.6 | 22 |
| WGPA-2-RG | ” | ” | ” | ” | IEC(0.2 M)-GFC(A) | $1.1\times10^5$ | -- | -- | 4.1 | 40.9 | 44.4 | 2.9 | 0.0 | 0.4 | 0.0 | 5.3 | 2.0 | 22 |
| WGPA-2-HG | ” | ” | ” | ” | IEC(0.2 M)-GFC(B) | $6.5\times10^3$ | -- | -- | 3.0 | 4.6 | 5.1 | 1.9 | 0.0 | 0.2 | 0.0 | 83.6 | 1.6 | 22 |
| WGPA-3-RG | ” | ” | ” | ” | IEC(0.3 M)-GFC(A) | -- | -- | -- | 7.3 | 38.0 | 29.0 | 3.2 | 0.0 | 0.0 | 0.0 | 20.2 | 0.0 | 30 |
| WGPA-3-HG | ” | ” | ” | ” | IEC(0.3 M)-GFC(B) | $1.6\times10^4$ | -- | -- | 1.5 | 2.2 | 3.5 | 1.3 | 0.0 | 0.0 | 0.0 | 90.9 | 0.5 | 22 |
| WGPA-4-RG | ” | ” | ” | ” | IEC(0.5 M)-GFC(A) | -- | -- | -- | 11.4 | 26.1 | 13.5 | 4.4 | 0.0 | 0.0 | 0.0 | 38.4 | 0.0 | 30 |
| WGPA-4-HG | ” | ” | ” | ” | IEC(0.5 M)-GFC(B) | $4.5\times10^4$ | -- | -- | 0 | 0 | 5.9 | 2.0 | 0.0 | 0.0 | 0.0 | 92.1 | 0.0 | 22 |
| RG-0.5H-I | WGPA-2-RG | ” | ” | 0.1 M TFA[g]/80°C/0.5 h | N | $9.9\times10^4$ | -- | -- | 5.7 | 30.8 | 52.8 | 2.7 | 0.0 | 0.0 | 0.0 | 5.5 | 2.4 | 25 |
| RG-1H-I | RG-0.5H-I | ” | ” | 0.1 M TFA/80°C/0.5 h | N | $8.7\times10^4$ | -- | -- | 6.9 | 21.2 | 59.9 | 2.0 | 0.0 | 0.0 | 0.0 | 6.7 | 3.4 | 25 |
| RG-6H-I | RG-1H-I | ” | ” | 0.1 M TFA/80°C/5 h | N | $4.0\times10^4$ | -- | -- | 10.1 | 0.0 | 76.1 | 0.6 | 0.0 | 0.0 | 0.0 | 8.6 | 4.6 | 25 |
| PGP2a | Chinese white ginseng roots | 95% EtOH/40°C/2 h/once; residue: water/100°C/3 h/once | 95% EtOH (pptn.); Sevag | N | IEC(0.15 M)-IEC(0.3 M)-GFC | $3.2\times10^4$ | 75.8 | 9.9 | 0.0 | 1.6 | 3.7 | 0.5 | 0.0 | 0.0 | 0.0 | 5.4 | 0.0 | 59 |
| GPW | Chinese white ginseng roots | 95% EtOH/40°C/once; residue: water/100°C/4 h/thrice | 95% EtOH (pptn.) | ” | IEC(0.5 M)-GFC | -- | 58.3 | 1.0 | 8.7 | 26.1 | 34.4 | 8.7 | 0.0 | 0.0 | 0.0 | 17.3 | 0 | 28 |
| GPR | ” | ” | ” | Steaming: 100°C/3 h | ” | -- | 57.4 | 3.9 | 4.7 | 18.5 | 28.7 | 10.1 | 0.0 | 0.0 | 0.0 | 28.0 | 0 | 28 |
| GPS | ” | ” | ” | Steaming: 120°C/3 h | ” | -- | 57.2 | 4.9 | 9.9 | 28.7 | 11.2 | 12.3 | 0.0 | 0.0 | 0.0 | 35.5 | 0 | 28 |
| GPW-1 | GPW | N | N | N | GFC(A) | $8.51\times10^5$ | -- | -- | 1.4 | 61.8 | 26.3 | 3.6 | 0.0 | 0.0 | 0.0 | 6.4 | 0 | 28 |
| GPW-2 | ” | ” | ” | ” | GFC(B) | $2.95\times10^5$ | -- | -- | 3.6 | 32.2 | 22.3 | 6.2 | 0.0 | 0.0 | 0.0 | 29.1 | 0 | 28 |
| GPR-1 | GPR | ” | ” | ” | GFC(A) | $8.86\times10^5$ | -- | -- | 1.9 | 54.6 | 20.0 | 12.3 | 0.0 | 0.0 | 0.0 | 10.6 | 0 | 28 |
| GPR-2 | ” | ” | ” | ” | GFC(B) | $2.58\times10^5$ | -- | -- | 6.9 | 8.6 | 18.8 | 1.5 | 0.0 | 0.0 | 0.0 | 61.6 | 0 | 28 |
| GPS-1 | GPS | ” | ” | ” | GFC(A) | $9.61\times10^5$ | -- | -- | 2.1 | 30.7 | 39.9 | 13.3 | 0.0 | 0.0 | 0.0 | 11.3 | 0 | 28 |
| GPS-2 | ” | ” | ” | ” | GFC(B) | $3.39\times10^5$ | -- | -- | 6.7 | 3.7 | 6.6 | 9.7 | 0.0 | 0.0 | 0.0 | 68.1 | 0 | 28 |
| PA | Korean white ginseng roots | Hot water/0.5 h/twice; 0.2 M NaCl/0.5 h/twice | 1% $Na_2SO_4$ and 5% CTAB[h] (pptn.) | ” | GFC-IEC(0.2 M)-GFC | $1.6\times10^5$ | -- | -- | 2.0 | 21.3 | 53.4 | 0.0 | 0.0 | 0.0 | 0.0 | 16.0 | 2.7 | 45 |
| PB | ” | ” | ” | ” | GFC-IEC(0.3 M)-GFC | $5.5\times10^5$ | -- | -- | 8.1 | 11.0 | 32.2 | 0.0 | 0.0 | 0.0 | 0.0 | 39.9 | 5.0 | 45 |
| S-IA | ” | Hot water/0.5 h/twice | ”; supernatant: 80% EtOH (pptn.) | ” | IEC(0.2 M acetate buffer)-GFC-AC[i]-GFC | $5.6\times10^4$ | -- | -- | 0.0 | 42.3 | 50.8 | 0.0 | 0.0 | 0.0 | 0.0 | 6.9 | 0.0 | 46 |
| S-IIA | ” | ” | ” | ” | IEC(0.3 M acetate buffer)-GFC-AC-GFC | $1.0\times10^5$ | -- | -- | 0.0 | 42.0 | 32.6 | 6.2 | 0.0 | 0.0 | 0.0 | 19.2 | 0.0 | 46 |
| Ginsan | ” | Water/rm. temp./ overnight/once | MeOH (pptn.); 95% EtOH (pptn.) | ” | N | $2.0\times10^6$ | 90.2 | 3.7 | 0.0 | 0.0 | Gal+Glc: 47.1 | | 0.0 | 0.0 | 0.0 | 43.1 | 0.0 | 42,54 |
| PGAP | Korean red | 85% EtOH/once; | N | ” | ” | -- | -- | -- | 0.0 | trace | trace | 26.1 | 0.0 | 0.0 | 0.0 | 5.1 | 51.8 | 52 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GL-4IIb2 | ginseng Chinese ginseng leaves | residue: water/once 30% EtOH/rm. temp./once; residue: water/100°C/3 h/thrice; MeOH/1 h/thrice, residue was used | 95% EtOH (pptn.); 8% CTAB (pptn.); 1% boric acid and 2 M NaOH (pptn.) | ” | IEC(0.2 M) | $1.1\times10^4$ | 93.1 | 6.9 | 4.0 | 1.6 | 2.1 | 4.2 | 5.1 | 3.6 | 5.8 | 27.8 | 5.0 | 62 |
| GL-AIa | ” | ” | ”; supernatant was used | ” | IEC(2→100 mM)-AC-GFC | $6.6\times10^4$ | -- | -- | 11.5 | 38.5 | 38.5 | 0.0 | 0.0 | 0.0 | 0.0 | 7.7 | 3.8 | 20,60 |

[a] No treatment; [b] no data available; [c] ethylenediaminetetraacetic acid; [d] ion-exchange chromatography (molar of NaCl as the eluent unless specified); [e] gel filtration chromatography (elution order of fractions); [f] same as above; [g] trifluoroacetic acid; [h] cetrimonium bromide; [g] Concanavalin A-Sepharose affinity chromatography; (for S-IA and S-IIA, the previous fraction was dissolved in 1/15 M phosphate buffer, pH 7.0. containing 0.15 M NaCl, 1 mM $MgCl_2$, and 1 mM $CaCl_2$, equilibrated and eluted with the same buffer; for GL-AIa, the previous fraction was dissolved in Tris-HCl buffer, pH 7.4, containing 1mM $CaCl_2$, 1 mM $MgCl_2$, 1mM $MnCl_2$, and 0.15 M NaCl, equilibrated and eluted with the same buffer; for the three pectins, unbound fraction was used).

## 3. Structure-associated antidiabetic activities of ginseng pectins

### *3.1. Hypoglycemic effects*

Insulin secretion deficiency and insensitivity to insulin action can both result in glucose metabolism disorders and cause hyperglycemia.[32] In the 1980s, a group of polysaccharides named panaxans A~U were isolated from ginseng, with respective anti-hyperglycemic effects on normal mice and/or alloxan-induced diabetic mice.[15–19] However, not all of them were determined as pectin molecules, and their hypoglycemic mechanisms were unclear. Two glucans, panaxans A and B, reduced blood glucose probably by affecting hepatic enzymes[33], and by increasing insulin levels in blood plasma and by increasing cellular sensitivity to insulin.[34]

Since then, information from investigations of hypoglycemic activities of ginseng pectins has gradually accumulated (Table 2). One of the glucose-lowering metabolic effects of ginseng pectins may be stimulating insulin secretion to promote glycogen synthesis. A pectin fraction of ginseng polysaccharide, WGPA, has been proved effective to reduce fasting blood glucose in streptozocin (STZ)-induced diabetic mice. The fraction significantly increased serum insulin and hepatic glycogen after ten days of *i.g.* administration at 10 mg/kg to the animals.[35] The hypoglycemic effects of ginseng pectins increased with the steaming temperature.[28] The heat processing transformed the esterified GalA into un-esterified form, which may explain the increased potency of enhancing serum insulin level, reducing blood glucose and regaining body weight in diabetic mice.

It has been claimed that ginseng glycopeptides (GGP) exhibit different effects on glycogenesis, also reducing blood sugar levels, probably by inhibiting insulin resistance.[36] GGP is a fraction of ginseng pectins containing 5.6% polypeptides that link with the polysaccharide moiety via covalent bonds.[6] Unlike insulin, which increases liver glycogen, GGP significantly decreases hepatin but still reduce blood glucose in both normal and diabetic animals, including rats, mice and rabbits. The hypoglycemic activity may be attributed to the enhancement of aerobic glycolysis through stimulating β-adrenoceptor and the activation of rate-limiting enzymes related to the tricarboxylic acid cycle.[36] The varied effect of GGP on liver glycogen level from other ginseng pectins might be due to its higher peptide percentage and characteristic molecular structure.

Ginseng pectins has been found to have other regulatory effects on blood glucose, including affecting the metabolism of purine, tryptophan, and fatty acids, and energy production.[37] In a study of ginseng pectins on type 2 diabetic rats, a clear separation of urinary excretion profiles was observed between the disease model group and the pectin-treated group. Eight urinary biomarkers have been investigated before and after pectin consumption. A two-week pectin administration evidently increased the urinary concentrations of inosine, serotonin, phenylpropionylglycine, dodecanedioic acid, and tetrahydrocortisal. Simultaneously, 1-methyladenine, 4-deoxyerythronic acid, and 5-hydroxyhexanoic acid were down-regulated, indicating that ginseng pectins can regulate DNA metabolism, organic acid metabolism, and steroid hormone metabolism, all of which contribute to the complicated metabolic network of diabetes.

### *3.2. Anti-oxidative effects*

Insulin resistance and excess glucose in the internal environment is responsible for the increase of oxidative stress.[38] Apart from anti-hypoglycemic effects, WGPA showed notable anti-oxidative activities in both diabetic and normal mice.[7,35] When used to treat STZ-induced diabetic mice, WGPA restored the levels of maleic dialdehyde (MDA) and superoxide dismutase (SOD) to the baseline.[35] In a forced swim test, the immobility times were compared among normal mice orally administrated with WGPA, ginseng neutral polysaccharides, and ginseng crude polysaccharides.[7] WGPA was demonstrated as the most dose-efficient among the three fractions. Compared to the forced swim rats treated with saline, rats that swam after taking WGPA were found with increased SOD and glutathione peroxidase (GSH-Px), and reduced triglyceride (TG), creatine phosphokinase (CK), lactic dehydrogenase (LDH), and MDA. The combined inhibition of oxidative stress by WGPA was probably due to protection of corpuscular membranes by preventing lipid oxidation via modifying several enzyme activities.

Steaming is positively correlated with the reductive ability of ginseng pectins.[28] Alloxan-induced diabetic mice orally treated with steamed ginseng pectins were found to have increased SOD, GSH-Px, vitamin C (VC), and vitamin E (VE), and decreased MDA, in both serum and liver, compare to the model control group. The blood TG and total cholesterol (TCH) levels were also down-regulated in the pectin-treated animals. Un-esterified GalA

induced by heat may contribute to prevent cellular damage by scavenging free radicals generated by reactive oxygen species.

**Table 2.** Selected ginseng pectin of respective structural features, hyperglycemic and oxidative activities, and possible mechanisms.

| Pectin | Key domain | Diabetogen | Subject | Dose | Bioactivity | Possible mechanism | Ref |
|---|---|---|---|---|---|---|---|
| WGP | RG-I, HG, AG | STZ | Wistar rats | 1000 mg/kg; *i.g.*; 2 wk | Urine: 1-Methyladenine↓, 4-deoxyerythronic acid↓, 5-hydroxyhexanoic acid↓, inosine↑, serotonin↑, phenylpropionylglycine↑, dodecanedioic acid, ↑ tetrahydrocortisal↑ | Hypoglycemic effects: regulation of glucose metabolism involving synthesis of proteins, fatty acids, and hormones | 37 |
| WGPA | RG-I, HG, AG | Streptozocin (STZ) | ICR mice | 10 mg/kg; *i.p.*; 10 d | Fasting blood glucose (FBG) (-)[a] | Hypoglycemic effects: insulin-like stimulation of glycogenesis | 35 |
| | | | | 10 mg/kg; *i.g.*; 10 d | FBG↓↓[b]; serum: insulin↑, maleic dialdehyde (MDA)↓, superoxide dismutase (SOD)↑; liver glycogen↑ | | 35 |
| | | None | ICR mice | 200 mg/kg; *p.o.*; 15 d | Immobility time in forced swim test↑↑↑; serum: triglyceride (TG)↓↓, creatine phosphokinase (CK)↓↓, lactic dehydrogenase (LDH)↓↓, MDA↓↓↓, SOD↑, glutathione peroxidase (GSH-Px)↑ | Anti-oxidative effects: enzymatic prevention of lipid oxidation | 7 |
| GPW | RG-I, HG, AG | Alloxan | ICR mice | 100 mg/kg; *p.o.*; once/wk; 4 wk | Body weight↑↑, body glucose↓↓; serum: insulin↑; TG↓; total cholesterol (TCH)↓, MDA↓, SOD↑,GSH-Px↑,vitamin C (VC)↑, vitamin E (VE)↑; liver: glycogen↑, MDA↓, SOD↑, GSH-Px↑↑, VC↑, VE↑ | Steaming process enhanced hypoglycemic and anti-oxidative effects of GPW, GPR, and GPS | 28 |
| GPR | RG-I, HG, AG | Alloxan | ICR mice | 100 mg/kg; *p.o.*; once/wk; 4 wk | Body weight↑↑, body glucose↓↓; serum: insulin↑, TG↓, TCH↓↓, MDA↓↓, SOD↑↑, GSH-Px↑↑, VE↑; liver: glycogen↑, MDA↓, SOD↑↑, GSH-Px↑↑, VC↑↑; VE↑ | | 28 |
| GPS | RG-I, HG, AG | Alloxan | ICR mice | 100 mg/kg; *p.o.*; once/wk; 4 wk | Body weight↑↑, body glucose↓↓; serum: insulin↑, TG↓, TCH↓↓, MDA↓↓, SOD↑↑, GSH-Px↑↑, VE↑; liver: glycogen↑, MDA↓, SOD↑↑, GSH-Px↑↑, VC↑↑, VE↑ | | 28 |
| GGP | RG-I, HG, AG, peptides | None | Mice | 200 mg/kg; *i.p.*; 3 d | Blood glucose ↓; liver glycogen ↓↓ | Hypoglycemic effects: inhibition of insulin resistance | 6 |
| | | | | 200 mg/kg; *s.c.*; 3 d | Blood glucose ↓; liver glycogen ↓ | | 6 |
| | | None | Rabbits | 60 mg/kg; *i.m.*; 3 d | Blood glucose ↓; liver glycogen ↓ | | 6 |
| | | Alloxan | Rats | 100 mg/kg; *s.c.*; 4 d | Blood glucose ↓↓; liver glycogen ↓↓ | | 6 |
| | | STZ | Mice | 100 mg/kg; *s.c.*; 6 d | Blood glucose ↓↓ | | 6 |

[a] Down-regulation; [b] compared to the model control group (with diabetogen) or control group (without diabetogen): single arrow, $p < 0.05$; double arrows, $p < 0.01$; triple arrows, $p < 0.001$.

### *3.3. Immunoregulatory effects*

Diabetes, especially type 1 diabetes, includes symptoms of autoimmunity; the increased susceptibility to infection is led by primary immunodeficiency and immune dysfunction.[2] An HG-rich pectin extracted from Korean red ginseng, PGAP, can alleviate abnormalities in the immune system. It increased spleen weight and spleen cells in normal mice, mainly by increasing macrophages (Table 3).[39,40] Splenic hyperplasia could be either immunopotentiating or immunosuppressive when the pectins were administrated in different dose ranges. Within 10~30 mg/kg *i.p.* to mice, PGAP significantly promoted generation of $CD11b^+$ cells and antibody-forming cell (AFC) response[12]; while in the range of 100~300 mg/kg *i.p.* to mice, PGAP reduced the $CD11b^+$ cells and the AFC response[13]. It is believed that macrophage-derived nitric oxide (NO) was the mediator of the immunosuppression effect of RGAP at higher doses.[40,41]

Ginsan, an HG-rich pectin from Korean white ginseng, exerted immunomodulatory effects both *in vitro* and *in vivo*. When added to mice peritoneal macrophages (PM), ginsan promoted mRNA expression of inducible nitric oxide synthase (iNOS) and a group of correlated cytokines produced by Th1 cells and macrophages, including interleukin (IL)-1β, IL-6, IL-12, and tumor necrosis factor (TNF)-α.[42] However, when injected to *Staphylococcus aureus*-infected mice, the HG pectins notably down-regulated the cytokines.[8] The pharmacokinetics of pectins are different in cell and animal models, thus leading to varied immune responses. The decrease of pro-inflammatory cytokines *in vivo* was possibly caused by ginsan inhibiting Toll-like receptor (TLR) 2. Inhibition of TLR2 expression down-regulates the activity of adaptor molecule MyD88, decreasing the expression of phospho-c-Jun N-terminal kinase (JNK) 1/2, phos-p38 mitogen-induced protein kinase (MAPK), and nuclear factor-κB (NF-κB).

Contradicting to HG countering TLR2 *in vivo*, RG-II-rich pectins can function as a TLR4 agonist *in vitro*. RG-II matured bone marrow-derived dendritic cells (BMDC), activating MAPK families in a TLR-4-dependent manner.[43] Stimulated BMDC boosted generation of cytokines IL-1β, IL-12, TNF-α, and interferon (IFN)-γ, and proliferation of $CD8^+$ T cells. Besides, RG-II ginseng pectins interact with other targets including the Fc receptor (FcR). In a mouse model, an RG-II pectin increased FcR expression to activate mononuclear phagocytosis, in turn enhancing immune complex clearance.[13] It also induced IL-6

production in mice peritoneal macrophages (PM).[44]

AG side chains also exhibit significant immunoregulatory activities. WGPA-2-RG is a ginseng pectin fraction with a small RG-I backbone and long AG side chains.[22] Partial acid hydrolysis of WGPA-2-RG gave a new pectin named RG-6H-I, keeping the RG backbone but completely eliminating the AG branches.[25] Both pectin fragments enhanced phagocytic activities of mice PM, while only WGPA-2-RG increased lymphocyte proliferation and stimulated macrophage NO production. AG side chains and Ara residues are therefore essential for lymphocyte proliferation and NO secretion; however, they are less involved than RG-I in macrophage phagocytosis. More specifically, α-1,5-linked arabino-β-3,6-branched galactan in AG can exert remarkable reticuloendothelial system (RES)-potentiating activities *in vivo*.[20,45–48] Activated RES triggers the complementary system and intensified immune response through the alternative pathway.[20] Moreover, an AG-rich pectin S-IIA has been found inducing mRNA expression and cytokine production of IL-8 in Th1 cells and human blood monocytes, showing *in vitro* immunomodulating activities.[47]

### *3.4. Tumoricidal effects*

Due to many overlapped pathogenic factors, diabetes (mainly type 2) is interlinked with cancer epidemiologically and biologically, increasing risk of tumor generation and progression in the liver, pancreas, colorectum, stomach, lung, bladder, and breast.[49,50] Convincing evidence indicates that cancer is a major complication of diabetes, and that some types of cancer, e.g. carcinoma of pancreas, can destroy the insulin-making cells and cause diabetes.[35] Therefore, therapeutic agents that fight against tumors can both manage diabetic complications and prevent diabetes (Table 4).

HG-rich pectins have been found working synergistically with other cancer therapeutic agents. Paclitaxel is a tetracyclic diterpenoid that blocks mitosis, which is extensively applied in treatment of various cancers, but with clinical toxicities.[51] When PGAP pectin and paclitaxel were administrated simultaneously to tumor-bearing mice, tumor weight decreased and animal survival rate was notably elevated, compared to monotherapy with either agent. Moreover, PGAP restored spleen cell number and NK cell activity suppressed by paclitaxel. Therefore, PGAP allied with paclitaxel to enhance antitumor potencies and to reduce

toxicities.[52] Recombinant-derived lymphokine interleukin-2 (rIL-2) is an immunotherapeutic agent with serious dose-limiting toxicity, which is seldom used alone.[53] Lymphokine-activated killer (LAK) cells are innately oriented to kill tumor cells.[53] In collaboration with rIL-2, pectin ginsan generated LAK cells from both NK and T cells through endogenously produced cytokines, presenting effectiveness in the immunotherapy of cancer.[54]

The tumoricidal effects of PGAP and ginsan can be possibly explained by their bioactive HG domains. A study on colon cancer cells demonstrated that HG-rich ginseng pectin potently inhibited tumor proliferation.[9] Steaming further enhanced the antitumor activities, probably by generating or enriching specific functional structures. Steamed HG pectins followed two dose-related routes of HT-29 inhibition: inducing cell cycle arrest at lower concentrations (0.6~1.25 mg/ml, 72 h), and inducing apoptosis at higher concentrations (1.25~2.5 mg/ml, 72 h). The pectins may induce apoptosis by activating caspase-3-mediated apoptosis pathway. Other studies found that HG pectins enhanced production of cytokines from Th1 cells[54] and macrophages[42] and thus stimulated the production of cytotoxic cells.

Fibroblasts have a prominent role in the progression, growth, and spreading of cancers, involving angiogenesis and injury response.[55] It has been discovered that HG and RG-I pectins can inhibit cell migration of fibroblasts, including L-929 and HT-1080 cells, and that GalA and Rha contents are positively correlated to the reduction of cell adhesion and cell spreading, two mediators of cell migration.[30] Such inhibition may be related to the galectin-3-binding property of ginseng pectins. Galectin-3 is a chimeric protein characterized by a conserved carbohydrate recognition domain (CRD) with specific binding to β-galactosides.[56] The protein contributes to tumor progression; galectin-3 antagonists are promising cancer therapeutic agents.[57] To test whether ginseng pectins bind to galectin-3 to inhibit tumor growth, an RG-I pectin and its modified pectic fragments were assayed *in vitro*, disclosing that both RG-I backbones and AG side chains were important structural elements for anti-galactin-3 activity.[58] The RG-I backbones maintained structural confirmation to preserve high binding affinity of the molecule. The β-1,4-galactan side chains are essential for the binding. Ara residues either promoted (terminal Ara) or impaired (α-1,5-arabinan side chains) the affinity. The side chains cooperated; the activity of a galactan chain was proportional to the length of up to 4 Gal residues and remained unchanged thereafter.

The AG domain has also been discovered to have apoptotic functions. PGP2a, an AG-rich ginseng pectin, arrested HGC-27 gastric tumor cells at G2/M phase and dose-dependently induced apoptosis, via both mitochondrial-mediated intrinsic and death receptor (DR)-mediated extrinsic apoptotic pathways.[59] The pectin-induced apoptosis might be mediated by modulation of Twist and its downstream gene expression.

**Table 3.** Selected ginseng pectin of respective structural features, immunoregulatory and tumoricidal effects, and possible mechanisms

| Pectin | Key domain | Subject/Model | Dose | Bioactivity | Possible mechanism | Ref |
|---|---|---|---|---|---|---|
| WGPE | RG-I, HG, AG | ICR mice | 50 mg/kg; *i.p.*; 14 d | Lymphocyte proliferation↑↑ [a] | Immunoregulatory effects: AG is | 23 |
| EGP | RG-I, HG, AG | ICR mice | 10~50 mg/kg; *i.p.*; 14 d | Lymphocyte proliferation↑↑ | essential for lymphocyte | 24 |
| WGPA | RG-I, HG, AG | ICR mice | 50 mg/kg; *i.p.*; 14 d | Proliferation of T cells↑↑↑ and B cells↑↑ | proliferation and NO production | 22 |
| GL-AIa | RG-I, AG | Normal human serum | 100~1000 μg/ml; 37°C; 30 min | Inhibition of $TCH_{50}$ and $ACH_{50}$ (+)[b] | | 48,60 |
| WGPA-2-RG | RG-I, AG | Normal mice splenocytes | 100~200 μg/ml; 37°C; 4 h | Lymphocyte proliferation↑↑ | | 25 |
| | | Normal mice PM | 50~200 μg/ml; 37°C; 24 h | Phagocytosis↑↑ | | 25 |
| | | | 50~200 μg/ml; 37°C; 48 h | NO production↑↑ | | 25 |
| | | Normal human serum | 100~1000 μg/ml; 37°C; 30 min | Inhibition of $TCH_{50}$ and $ACH_{50}$ (+) | | 45 |
| PA, PB | RG-I, AG | ICR mice | 20 mg/kg; *i.p* | Phagocytic index↑ | Immunoregulatory effects: arabino-3,6-galactan neutral side chains are essential for activating RES and the compliment system | 45 |
| Purified GL-4 | RG-II | C57BL/6 mice bone marrow dendritic cells (BMDC) | 0.25~1 mg/ml; 37°C; 24 h | Production of IL-12p70↑, IL-1β↑↑, and TNF-α↑↑↑ | Immunoregulatory effects: activation of TLR4 on BMDC | 43 |
| | | | 0.5~1 mg/ml; 37°C; 24 h | mRNA expression of CCR7↑↑ and CCR1↓↓ | | 43 |
| GL-4IIb2 | RG-II | ICR mice | 25~30 mg/kg; *i.p.*; 7 d | Immune complex clearance from circulation↓ | Immunoregulatory effects: | 13 |
| | | ICR mice PM | 2~10 ug/ml; 37°C; 15 h | Glucose oxidase-anti-glucose oxidase complexes (GAG) binding to Fc receptor (FcR) (+)<br>Phagocytosis against IgG-sensitized sheep erythrocytes (+) | activation of FcR and the mononuclear phagocytic system | 13 |
| WGPA-3-RG | RG-I, HG | L-929 cells | 0.5 mg/ml; 3 h | Cell adhesion↓↓↓; cell spreading↓↓↓ | Tumoricidal effects: inhibition of cell migration by preventing cell adhesion and spreading | 30 |
| RG-I-4 | RG-I, AG-I | Galectin-3-mediated hemagglutination assay | MIC: 0.25±0.02 μg/ml | | Tumoricidal effects: binding and | 58 |
| | | Galectin-3-mediated HT-29 cell adhesion | $IC_{50}$: 0.02 μg/ml; 37°C; 1 h | | inhibition of galectin-3 | 58 |
| RG-6H-I | RG-I | Normal mice PM | 50~200 μg/ml; 37°C; 24 h | Phagocytosis↑↑ | | 25 |
| | | C3H/HeJ mice PM | 10 ug/ml; 37°C; 24 h | IL-6 production (+) | Tumoricidal effects: cytokine-stimulated generation of cytotoxic cells | 44 |
| | | EG7 lymphoma-bearing C57BL/6 mice | 0.5~1 mg/kg, *i.p.*; 42 d | Tumor growing speed↓↓ | | 44 |
| PGP2a | AG | HGC-27 cells | $IC_{50}$: 115.71μg/ml; 48h | Cell proliferation (-)[c] | Tumoricidal effects: induction of apoptosis via intrinsic and extrinsic pathways, involving Twist/AKR1C2/NF-1 expression | 59 |
| S-IIA | AG | ICR mice | 20 mg/kg; i.p | Phagocytic index↑ by activating RES | | 46 |
| | | Normal human serum | 100~1000 μg/ml; 37°C; 30 min | Inhibition of $TCH_{50}$ and $ACH_{50}$ (+) | | 46 |
| | | Th1 cells | 10μg/ml; 37°C; 24 h | IL-8 production↑↑ | | 47 |
| | | | 100~400 μg/ml; 48 h | Cell cycle arrest at G2/M phase (+) | | 47 |
| PGAP | HG | BALB/c mice | 10~30 mg/kg; *i.p.*; 7 d | Spleen weight↑[a]; splenocytes number↑; antibody-forming cell (AFC) response↑; $CD11b^+$ cells percentile and number↑ | Immunoregulatory effects: splenic hyperplasia by increasing | 39 |
| | | | 100~300 mg/kg; *i.p.*; 7 d | Spleen weight and cells per spleen↑↑; AFC response↓↓; $CD11b^+$ cells percentile↓↓; nitric oxide (NO) accumulation in macrophages↑↑ | macrophages; NO might mediate immunosuppressive activities | 40 |
| | | BALB/c mice splenocytes | 10~500 μg/ml; 37°C; 72 h | Lymphocyte proliferation↑ | | 39 |
| | | | 50~1000 μg/ml; 37°C; 24 h | IL-6 production↑↑ | | 52 |
| | | Sarcoma 180 tumor-bearing ICR mice | 25 mg/mg; *i.p.*; 7 d; paclitaxel 15 | Survival rate (+) | | 52 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | mg/kg; *i.p.*; days 1, 3, and 5 | | | |
| | | B16-F10 melanoma-bearing C57BL/6 mice | 100 mg/mg; i.p; 7 d; paclitaxel 10 mg/kg; *i.p.*; days 1, 3, and 5 | Tumor weight↓↓ | Tumoricidal effects: induction of cell cycle arrest and apoptosis; | 52 |
| | | C57BL/6 mice splenocytes | 100 mg/mg; *i.p.*; 7 d; paclitaxel 10~20 mg/kg; *i.p.*; days 1, 3, and 5 | NK cell cytotoxicity against Yac-1 cells↑ | synergistic activities with paclitaxel | 52 |
| | | BALB/c mice peritoneal macrophages (PM) | 50~500 μg/ml; 37°C; 24 h | NO accumulation↑↑ | | 40 |
| | | | 100 μg/ml + paclitaxel 30nM; 37°C; 24 h | Cytotoxicity against P815 mastocytoma cells↑↑ | | 52 |
| Ginsan | HG | C3H/HeN mice PM | 50 μg/ml; 37°C; 6~24 h | mRNA expression of IL-1β, IL-6, IL-12, TNF-α, and iNOS (+)<br>Cytotoxicity against Yac-1 tumor cells (+) | Immunoregulatory effects: poduction of pro-inflammatory cytokines from Th1 cells and macrophages; | 42 |
| | | *Staphylococcus aureus*-infected BALB/c mice | 25 μg/kg; *i.p.*; 11 h | Production of IL-1β, TNF-α, IFN-γ, and IL-10↓↓↓; IL-6↓↓; IL-12↓ | inhibition of Toll-like receptor (TLR) 2 on macrophages | 8 |
| | | B16-F10 melanoma-bearing C57BL/6 mice | 200 mg/kg; 3 times/wk; 2 wk | Lung tumor↓ | | 54 |
| | | | 200 mg/kg; *i.p.*; 3 times/wk; 2 wk; rIL-2[c]: 20,000 U/injection; *i.p.*; every 8 h from day 3~8 | Lung tumor↓↓ | Tumoricidal effects: cytokine-stimulated generation of cytotoxic cells; synergistic activities with rIL-2 | 54 |
| | | C57BL/6 mice splenocytes | 10~250 μg/ml; 37°C; 6 h | mRNA expression of cytokines IL-1α, GM-CSF, IFN-γ, and IL-2 (+) | | 54 |
| | | | 50 μg/ml + rIL-2: 1, 3, and 10 U/ml; 37°C; 5 d | LAK cells↑↑ | | 54 |
| WGPA-3-HG | HG | L-929 cells | 0.5 mg/ml; 3 h | Cell adhesion↓↓↓; cell spreading↓↓↓ | Tumoricidal effects: induction of apoptosis by activating caspase-3 | 30 |
| | | HT-29 cells | 2.5, 5 mg/ml; 72, 120 h | Cell proliferation↓↓ | | 30 |
| | | | 1.25, 5 mg/ml | Cell cycle arrest at G2/M phase (+); apoptosis by activating caspase-3 | | 30 |
| MWGPA-3-HG | HG | HT-29 cells | 2.5~5 mg/ml; 72h, 120 h | Cell proliferation↓↓ | | 9 |
| | | | 0.6, 1.25 mg/ml | Cell cycle arrest at S and G2/M phase (+) | | 9 |
| | | | 1.25, 2.5 mg/ml | Cell cycle arrest at sub-G1 phase (+) | | 9 |

[a] Compared to the control group: single arrow, $p < 0.05$; double arrows, $p < 0.01$; triple arrows, $p < 0.001$; [b] up-regulation; [c] down-regulation; [d] recombinant-derived lymphokine interleukin-2.

## 4. Well-defined pectin preparation is envisaged

Different treatments of pectins yield macromolecules with diverse structures (Table 1). Preparation methods vary between laboratories, making inter-lab comparison of polysaccharide fractions difficult. Several groups of scientists have dedicated to ginseng pectin research for decades, each concentrating on different bioactive polymers. A group of Japanese researchers isolated and purified polysaccharides from ginseng root grown in Asian countries with diversified chemical and pharmacological properties.[15–19] Another Japanese team led by Haruki Yamada focused on ginseng leaf polysaccharides, and has shown variation from those isolated from roots.[20,44,48,60–62] Two South Korean research groups investigated pectin from Korean red[39,40] and white[8,54,63] ginseng. A Chinese group from Jilin province has focused their work on ginseng glycopeptides, which are pharmacologically unique.[6,36] Yifa Zhou and his colleagues fractioned a series of pectin fragments by different methods for further investigation.[22,24,25,31,64]

Such a diversity of methods used in the preparation of ginseng pectins largely hinders its quality control and evaluation. An optimized treatment template remains to be defined, with which researchers can obtain a standard, or at least an authenticated range, of the polymers. Key preparation procedures of ginseng pectins include 1) sample collection, 2) extraction, 3) purification, 4) chemical modification, and 5) isolation, all heavily affects the pectic chemical and pharmacological properties (Fig. 3). 1) During sample collection, selecting the appropriate origin of growth and botanical part of the herbal material is necessary. 2) Extraction with long time, high temperature, large solvent volume, and multiple extraction steps favors high yield of polysaccharides. Hot water, enzymes, and different concentrations of organic solvents are choices of extractants according to target products. 3) Ethanol precipitation is usually applied after extraction, in order to remove small molecules; Sevag method is a common deproteinizing choice to purify the samples; CTAB and boric acid can be further used to eliminate DNAs from pectins. 4) Steaming is an optional step to modify ginseng pectin structures, which may increase the acidity and alter biological efficacies of pectins. 5) Ion-exchange chromatography and gel filtration chromatography are major techniques for isolating polysaccharides, according to their charges and molecular weights, respectively.

To establish an entire preparation process for designated ginseng pectins, it is essential to optimize the parameters for each step in the abovementioned treatment template. There is room for improvement of particular approaches. For example, the current column separation methods are tedious and lack accuracy, thus a replacement for such long-time fractionation is sorely needed.

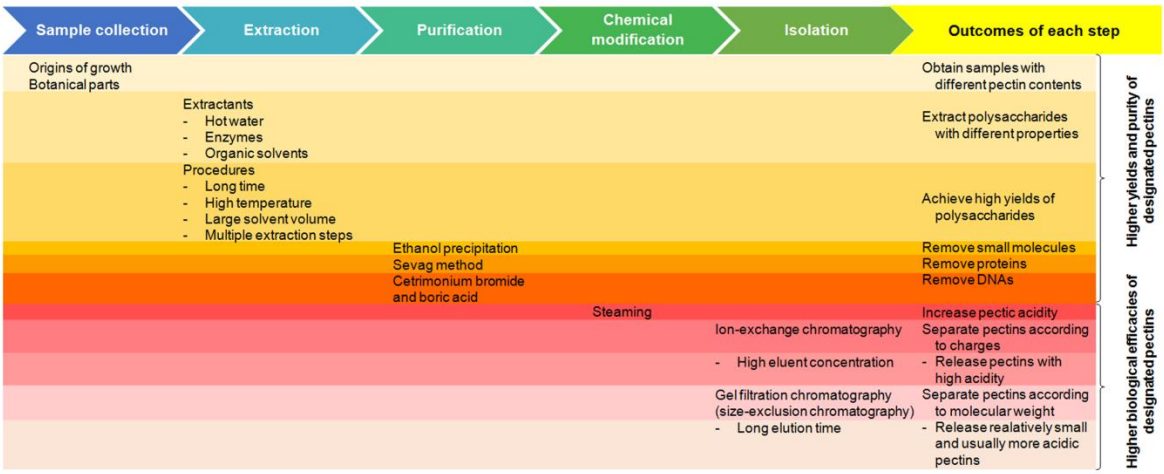


**Figure 2.** Proposed treatment template of ginseng pectins.

## 5. Toward multidimensional determination of pectin structure

At present, the criteria for judging whether a polysaccharide is homogeneous is whether it gives a single peak in ion-exchange or size-exclusion chromatography.[22,48,59] The criteria is misleading in that the purity of a polysaccharide sample does not solely rely on charge or molecular weight.[65] Low purification and high heterogeneity of pectin structures hinders establishment of structure-function relationships.[66] Hence when assessing the purity of a polysaccharide, influencing factors other than a symmetrical peak should also be considered, such as the branching degree of the carbohydrates, as well as contents of other polymeric components including nucleic acids and peptides.

Multiple polysaccharide structure determination approaches have been developed, including monosaccharide composition[30], GC-MS[60], FT-IR[65], and $^{1}H$[15] and $^{13}C$[28] NMR spectra. The techniques are informative of pectic primary structure, such as polysaccharide category, molecular weight, branching degree, glycosyl linkage, functional groups, and degrees of esterification and methylation. These structural features are all related to bioactivities to

varied extents.[66] However, more delicate structures are to be discovered. It is worth noting that not only the existence but also the distribution of the structural elements contributes to the overall properties of a pectin.

In addition to the elementary structural units, advanced constructions of polysaccharides also remain to be explored. Inter- and intra-molecular interactions significantly affect the 3-D structure of polysaccharides. A future challenge lies in computer-assisted modulation of the 3-D edible macromolecules, calculating their binding affinities to receptors, and designing the optimal polysaccharides with health-promoting potencies.

## 6. Pectin-target interactions in diabetes therapy

Polysaccharides rich in HG, RG-II, RG-I, and AG domains have structurally-specific mechanisms to alleviate abnormalities in the metabolic system, consequently managing diabetes (Fig. 2). Modification of GalA, the most abundant monosaccharide in pectins, will largely alter therapeutic potency of pectin. Un-esterified GalA plays an inevitable role in anti-hyperglycemic and anti-oxidative activities. Low pH and high temperature facilitates de-esterification and thereby promotes pectin bioactivitties.[25,28] On RG-I backbones, unbranched GalA residues contribute to stimulate the immune system, while the branched ones, usually at O-2 or O-3, significantly suppress the anti-complementary activity of the pectin.[48]

HG pectin has both antitumor and immunoregulatory functions. Reasons of HG pectins fighting against tumor may include 1) induction of cell cycle arrest and apoptosis[9]; 2) inhibition of cell migration and thus preventing cancer generation and progression[30]; and 3) production of pro-inflammatory cytokines stimulated generation of tumor killing cytotoxic cells[42]. As for the immunregulatory effects, HG consists of a linear chain of Gal*p*A with a high degree of esterification that competes with lipopolysaccharides (LPS), thus the carbohydrate moiety can block TLR2, preventing inflammatory cell activation.[67]

The poly-GalA backbone of both HG and RG-II pectin enables their binding to TLR proteins with high affinity. However, unlike HG, which is a linear chain, RG-II possess unique monosaccharide composition, side chain decoration, and borate dimerization, making the

moiety an agonist of TLR4, instead of blocking the protein.[43] Detailed mechanisms of specific pectin structures activating and inhibiting TLR awaits further study.

The RG-I backbone usually serves as a support for its side chains which have bioactivities. The AG moiety can not only affect enzymes related in the apoptosis pathways[59] but also modulate immune responses[25]. Particularly, in AG chains, the occurrence of β-1,4-galactans, α-1,5-arabinans, and terminal Ara residues, as well as the length of the galactan chains all have different effects on protein binding, potentially preventing cancer growth.[58] α-1,5-Linked arabino-β-3,6-branched galactans have special contributions to complement activation.[20,45–48] AG chains are greatly diversified in pectin, and they frequently interact with other structural elements, contributing to the overall carbohydrate therapeutic potency.

Because pectins are polymers with multiple functional structural units, its pharmacokinetics and signal transduction pathways are extremely complicated. Studies on cell models of ginseng pectin have provided valuable information on its potential in treating diabetes, yet the experimental results should be critically evaluated to avoid over-interpretation of phenomenological observations. Compared to *in vitro* tests, animal models and clinical studies can better reflect ginseng pectin performance in complex body systems. Since diabetes is derived from a group of metabolic disorder, the biomarkers chosen to characterize the model and treatment groups should be both practical and representative in the complicated environment. Moreover, when taken orally, the carbohydrates will interact with or be degraded by gut microbiota.[68] The resulting metabolites with possibly modified structures and bioactivities will then function as antidiabetic agents acting on different receptors. To further explore structure–function relationship of ginseng pectin, it is essential to reveal the interplay between structurally defined samples and complicated and as yet unspecified targets.

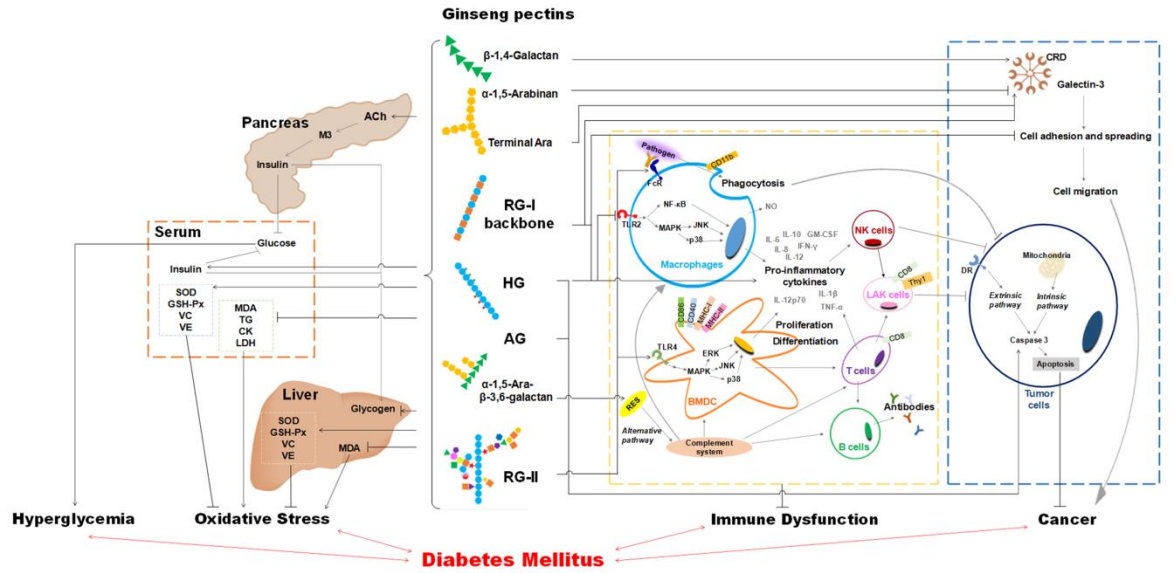


**Figure 3.** Illustration of antidiabetic potential of different domains of ginseng pectins. Ara, arabinose; RG, rhamnogalacturonan; HG, homogalacturonan; AG, arabinogalactan; ACh, acetylcholine; M3, muscarinic acetylcholine receptor M3; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; VC, vitamin C; VE, vitamin E;MDA, malondialdehyde; TG, triglyceride; CK, creatine phosphokinase; LDH, lactic dehydrogenase; FcR, Fc receptor; TLR, Toll-like receptor; NF-κB, nuclear factor κB; MAPK, mitogenactivated protein kinase; JNK, c-Jun N-terminal kinase; ERK, extracellular signal–regulated kinase;NO, nitric oxide; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF-_, tumor necrosis factor-_; NK cells, natural killer cells; LAK cells, lymphokine-activated killer cells; CD, cluster of differentiation; Thy1, thymus cell antigen 1; MHC, major histocompatibility complex; BMDC, bone marrow–derived dendritic cell; DR, death receptor; CRD, carbohydrate recognition domain.

## 7. Conclusion

The exact structures of ginseng pectins purified from the herbal material are strongly dependent on preparation procedures, including sample collection, extraction, chemical modification, isolation, and purification. Single or combined domains of HG, RG-I, RG-II, and AG, can be applied as antidiabetic agents due to prominent effects of lowering blood glucose, reducing oxidative stress, alleviating immunological disorders, and inhibiting cancers. Both backbones and side chains of the polysaccharides are essential pharmacologically. Domain-specific functions are associated with but not limited to monosaccharide composition, peptide content, degrees of esterification and methylation, and inter- and intra-pectin linkages. To standardize the pectin preparation process, to disclose detailed and advanced pectin structures, and to involve complex models with targeting networks, are among key strategies to further elucidate the relationship between structural diversity and antidiabetic therapeutic potency of ginseng pectins. The present review

systematizes the available information regarding preparation–structure and structure–function relationships of ginseng pectin with antidiabetic potential, shedding light on safer and more pertinent treatment of diabetes with efficacy-oriented modified polysaccharides.

## 8. Conflict of interest

The authors declare no conflicts of interest.

## 9. Acknowledgments

The work was financially supported by the National Natural Science Foundation of China (81673691). The authors thank Dr. Martha Dahlen for polishing the manuscript.

## 10. References

1. Roglic G. 2016. WHO Global report on diabetes: A summary. *Int. J. Noncommunicable Dis.* **1:** 3–8.
2. Skyler J.S. 2004. Diabetes mellitus: Pathogenesis and treatment strategies. *J. Med. Chem.* 4113–4117.
3. Kleinberger J.W. & Pollin T.I. 2015. Personalized medicine in diabetes mellitus: Current opportunities and future prospects. *Ann. N. Y. Acad. Sci.* **1346:** 45–56.
4. Yuan H.D., Kim J.T., Kim S.H., *et al.* 2012. Ginseng and diabetes: The evidences from in vitro, animal and human studies. *J. Ginseng Res.* **36:** 27–39.
5. Ovodov Y.S. & Solov'eva T.F. 1966. Polysaccharides of *Panax ginseng*. *Chem. Nat. Compd.* **2:** 243–245.
6. Wang B.X., Zhou Q.L., Yang M., *et al.* 2003. Hypoglycemic activity of ginseng glycopeptide. *Acta Pharmacol Sin* **24:** 50–54.
7. Wang J., Li S., Fan Y., *et al.* 2010. Anti-fatigue activity of the water-soluble polysaccharides isolated from *Panax ginseng* C. A. Meyer. *J. Ethnopharmacol.* **130:** 421–423.
8. Ahn J.Y., Choi I.S., Shim J.Y., *et al.* 2006. The immunomodulator ginsan induces resistance to experimental sepsis by inhibiting Toll-like receptor-mediated

inflammatory signals. *Eur. J. Immunol.* **36:** 37–45.

9. Cheng H., Li S., Fan Y., *et al.* 2011. Comparative studies of the antiproliferative effects of ginseng polysaccharides on HT-29 human colon cancer cells. *Med. Oncol.* **28:** 175–181.
10. Willats W.G.T., McCartney L., Mackie W., *et al.* 2001. Pectin: cell biology and prospects for functional analysis. *Plant Mol. Biol.* **47:** 9–27.
11. Ridley B.L., O'Neill M.A. & Mohnen D. 2001. Pectins: Structure, biosynthesis, and oligogalacturonide-related signaling. *Phytochemistry* 929–967.
12. Pérez S., Rodríguez-Carvajal M.A. & Doco T. 2003. A complex plant cell wall polysaccharide: rhamnogalacturonan II. A structure in quest of a function. *Biochimie* 109–121.
13. Sun X.B. & Yamada H. 1994. Purification of immune complexes clearance enhancing polysaccharide from the leaves of *Panax ginseng*, and its biological activities. *Phytomedicine* **1:** 225–231.
14. Geshi N., Petersen B.L. & Scheller H.V. 2010. Toward tailored synthesis of functional polysaccharides in plants. *Ann. N. Y. Acad. Sci.* **1190:** 50–57.
15. Konno C., Sugiyama K., Kano M., *et al.* 1984. Isolation and hypoglycaemic activity of pPanaxans A, B, C, D and E, glycans of *Panax ginseng* roots. *Planta Med.* **50:** 434–436.
16. Hikino H., Oshima Y., Suzuki Y., *et al.* 1985. Isolation and hypoglycemic activity of panaxans F, G, and H, glycans of *Panax ginseng* roots. *Jpn J Pharmacog* **39:** 331–333.
17. Oshima Y., Konno C. & Hikino H. 1985. Isolation and hypoglycemic activity of panaxans I, J, K and L, glycans of *Panax ginseng* roots. *J. Ethnopharmacol.* **14:** 255–259.
18. Konno C. & Hikino H. 1987. Isolation and hypoglycemic activity of panaxans M, N, O and P, glycans of *Panax ginseng* roots. *Int. J. Crude Drug Res* **25:** 53–56.
19. Chohachi K., Miki M., Yoshiteru O., *et al.* 1985. Isolation and hypoglycemic activity of panaxans Q, R, S, T and U, glycans of *Panax ginseng* roots. *J. Ethnopharmacol.* **14:** 69–74.
20. Gao Q., Kiyohara H., Cyong J., *et al.* 1989. Chemical properties and anti-complementary activities of polysaccharide fractions from roots and leaves of *Panax ginseng*. *Planta Med.* **55:** 9–12.
21. Shin K.-S., Kiyohara H., Matsumoto T., *et al.* 1997. Rhamnogalacturonan II from the leaves of *Panax ginseng* C.A. Meyer as a macrophage Fc receptor

expression-enhancing polysaccharide. *Carbohydr. Res.* **300:** 239–249.

22. Zhang X., Yu L., Bi H., *et al.* 2009. Total fractionation and characterization of the water-soluble polysaccharides isolated from *Panax ginseng* C. A. Meyer. *Carbohydr. Polym.* **77:** 544–552.
23. Sun L., Wu D., Ning X., *et al.* 2015. α-Amylase-assisted extraction of polysaccharides from *Panax ginseng*. *Int. J. Biol. Macromol.* **75:** 152–157.
24. Zheng Y., Yang G., Zhao Z., *et al.* 2016. Structural analysis of ginseng polysaccharides extracted by EDTA solution. *RSC Adv.* **6:** 2724–2730.
25. Zhang X., Li S., Sun L., *et al.* 2012. Further analysis of the structure and immunological activity of an RG-I type pectin from *Panax ginseng*. *Carbohydr. Polym.* **89:** 519–525.
26. Kaya M., Sousa A.G., Crépeau M.J., *et al.* 2014. Characterization of citrus pectin samples extracted under different conditions: Influence of acid type and pH of extraction. *Ann. Bot.* **114:** 1319–1326.
27. Jin Y., Kim Y.J., Jeon J.N., *et al.* 2015. Effect of white, red and black ginseng on physicochemical properties and ginsenosides. *Plant Foods Hum. Nutr.* **70:** 141–145.
28. Jiao L., Zhang X., Wang M., *et al.* 2014. Chemical and antihyperglycemic activity changes of ginseng pectin induced by heat processing. *Carbohydr. Polym.* **114:** 567–573.
29. Tishchenko G.A., Bleha M., Škvor J., *et al.* 1998. Effect of salt concentration gradient on separation of different types of specific immunoglobulins by ion-exchange chromatography on DEAE cellulose. *J. Chromatogr. B Biomed. Sci. Appl.* **706:** 157–166.
30. Fan Y., Cheng H., Li S., *et al.* 2010. Relationship of the inhibition of cell migration with the structure of ginseng pectic polysaccharides. *Carbohydr. Polym.* **81:** 340–347.
31. Yu L., Zhang X., Li S., *et al.* 2009. Rhamnogalacturonan I domains from ginseng pectin. *Carbohydr. Polym.* **79:** 811–817.
32. Ohtsubo K., Chen M.Z., Olefsky J.M., *et al.* 2011. Pathway to diabetes through attenuation of pancreatic beta cell glycosylation and glucose transport. *Nat. Med.* **17:** 1067–1076.
33. Suzuki Y. & Hikino H. 1989. Mechanisms of hypoglycemic activity of panaxans A and B, glycans of *Panax ginseng* roots: Effects on the key enzymes of glucose metabolism in the liver of mice. *Phyther. Res.* **3:** 15–19.
34. Suzuki Y. & Hikino H. 1989. Mechanisms of hypoglycemic activity of panaxans A

and B, glycans of *Panax ginseng* roots: Effects on plasma level, secretion, sensitivity and binding of insulin in mice. *Phyther. Res.* **3:** 20–24.

35. Sun C., Chen Y., Li X., *et al.* 2014. Anti-hyperglycemic and anti-oxidative activities of ginseng polysaccharides in STZ-induced diabetic mice. *Food Funct.* **5:** 845–848.
36. Wang B.X., Zhou Q.L., Yang M., *et al.* 2003. Hypoglycemic mechanism of ginseng glycopeptide. *Acta Pharmacol. Sin.* **24:** 61–66.
37. Niu J., Pi Z., Yue H., *et al.* 2012. Effect of ginseng polysaccharide on the urinary excretion of type 2 diabetic rats studied by liquid chromatography-mass spectrometry. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **907:** 7–12.
38. Maritim A.C., Sanders R.A. & Watkins J.B. 2003. Diabetes, oxidative stress, and antioxidants: A review. *J. Biochem. Mol. Toxicol.* 24–38.
39. Park K.M., Jeong T.C., Kim Y.S., *et al.* 2000. Immunomodulatory effect of acidic polysaccharide fraction from Korean red ginseng (*Panax ginseng*). *Nat. Prod. Sci.* **6:** 31–35.
40. Park K.M., Kim Y.S., Jeong T.C., *et al.* 2001. Nitric oxide is involved in the immunomodulating activities of acidic polysaccharide from *Panax ginseng*. *Planta Med.* **67:** 122–126.
41. Eisenstein T.K., Huang D., Meissler J.J.J., *et al.* 1994. Macrophage nitric oxide mediates immunosuppression in infectious inflammation. *Immunobiology* **191:** 493–502.
42. Song J.Y., Han S.K., Son E.H., *et al.* 2002. Induction of secretory and tumoricidal activities in peritoneal macrophages by ginsan. *Int. Immunopharmacol.* **2:** 857–865.
43. Park S.N., Noh K.T., Jeong Y.-I., *et al.* 2013. Rhamnogalacturonan II is a Toll-like receptor 4 agonist that inhibits tumor growth by activating dendritic cell-mediated $CD8^+$ T cells. *Exp. Mol. Med.* **45:** 1–10.
44. Shin K.S., Kiyohara H., Matsumoto T., *et al.* 1998. Rhamnogalacturonan II dimers cross-linked by borate diesters from the leaves of *Panax ginseng* C.A. Meyer are responsible for expression of their IL-6 production enhancing activities. *Carbohydr. Res.*
45. Tomoda M., Takeda K., Shimizu N., *et al.* 1993. Characterization of two acidic polysaccharides having immunological activities from the root of *Panax ginseng*. *Biol. Pharm. Bull.* **16:** 22–25.
46. Tomoda M., Hirabayashi K., Shimizu N., *et al.* 1993. Characterization of two novel polysaccharides having immunological activities from the root of *Panax ginseng*. *Biol.*

*Pharm. Bull.* **16:** 1087–1090.

47. Sonoda Y., Kasahara T., Mukaida N., *et al.* 1998. Stimulation of interleukin-8 production by acidic polysaccharides from the root of *Panax ginseng*. *Immunopharmacology* **38:** 287–294.
48. Gao Q.-P., Kiyohara H., Cyong J.-C., *et al.* 1988. Characterisation of anti-complementary acidic heteroglycans from the leaves of *Panax ginseng* C.A. Meyer. *Carbohydr. Res.* **181:** 175–187.
49. Shi Y. & Hu F.B. 2014. The global implications of diabetes and cancer. *Lancet* 1947–1948.
50. Tseng C.-H. 2011. Diabetes conveys a higher risk of gastric cancer mortality despite an age-standardised decreasing trend in the general population in Taiwan. *Gut* **60:** 774–779.
51. Rowinsky E.K. & Donehower R.C. 1991. The clinical pharmacology and use of antimicrotubule agents in cancer chemotherapeutics. *Pharmacol. Ther.* **52:** 35–84.
52. Shin M.J., Kim Y.S., Kwak Y.S., *et al.* 2004. Enhancement of antitumor effects of paclitaxel (taxol) in combination with red ginseng acidic polysaccharide (RGAP). *Planta Med.* **70:** 1033–1038.
53. Rosenberg S.A., Lotze M.T., Muul L.M., *et al.* 1985. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. *N. Engl. J. Med.* **313:** 1485–1492.
54. Kim K.-H., Lee Y.-S., Jung I.-S., *et al.* 1998. Acidic polysaccharide from *Panax ginseng*, ginsan, induces Th1 cell and macrophage cytokines and generates LAK Cells in synergy with rIL-2. *Planta Med.* **64:** 110–115.
55. Kalluri R. & Zeisberg M. 2006. Fibroblasts in cancer. *Nat. Rev. Cancer* **6:** 392–401.
56. Demetriou M., Granovsky M., Quaggin S., *et al.* 2001. Negative regulation of T-cell activation and autoimmunity by Mgat5 N-glycosylation. *Nature* **409:** 733–739.
57. Zou J., Glinsky V. V, Landon L.A., *et al.* 2005. Peptides specific to the galectin-3 carbohydrate recognition domain inhibit metastasis-associated cancer cell adhesion. *Carcinogenesis* **26:** 309–318.
58. Gao X., Zhi Y., Sun L., *et al.* 2013. The inhibitory effects of a rhamnogalacturonan I (RG-I) domain from ginseng Pectin on galectin-3 and its structure-activity relationship. *J. Biol. Chem.* **288:** 33953–33965.
59. Li C., Tian Z.N., Cai J.P., *et al.* 2014. *Panax ginseng* polysaccharide induces apoptosis by targeting Twist/AKR1C2/NF-1 pathway in human gastric cancer. *Carbohydr.*

*Polym.* **12:** 103–109.

60. Gao Q.-P., Kiyohara H., Cyong J.-C., *et al.* 1991. Chemical properties and anti-complementary activities of heteroglycans from the leaves of *Panax ginseng*. *Planta Med.* **57:** 132–136.
61. Sun Y. 2011. Structure and biological activities of the polysaccharides from the leaves, roots and fruits of *Panax ginseng* C.A. Meyer: An overview. *Carbohydr. Polym.* **85:** 490–499.
62. Sun X.B., Matsumoto T. & Yamada H. 1994. Purification of immune complexes clearance enhancing polysaccharide from the leaves of *Panax ginseng*, and its biological activities. *Phytomedicine* **1:** 225–231.
63. Shin J.-Y., Song J.-Y., Yun Y.-S., *et al.* 2002. Immunostimulating effects of acidic polysaccharides extract of *Panax ginseng* on macrophage function. *Immunopharmacol. Immunotoxicol.* **2415:** 892–3973.
64. Sun L., Wu D., Ning X., *et al.* 2015. α-Amylase-assisted extraction of polysaccharides from *Panax ginseng*. *Int. J. Biol. Macromol.* **75:** 152–157.
65. Xing X., Cui S.W., Nie S., *et al.* 2013. A review of isolation process, structural characteristics, and bioactivities of water-soluble polysaccharides from *Dendrobium* plants. *Bioact. Carbohydrates Diet. Fibre*.
66. Ferreira S.S., Passos C.P., Madureira P., *et al.* 2015. Structure–function relationships of immunostimulatory polysaccharides: A review. *Carbohydr. Polym.* **132:** 378–396.
67. Leclere L., Cutsem P. Van & Michiels C. 2013. Anti-cancer activities of pH- or heat-modified pectin. *Front. Pharmacol.* **4:** 1–8.
68. Xu J., Chen H.-B. & Li S.-L. 2017. Understanding the molecular mechanisms of the interplay between herbal medicines and gut microbiota. *Med. Res. Rev.* 1–46.