Acupuncture and Herbal Medicine

Panax notoginseng polysaccharides: a review focusing on the extraction, isolation, structural characterization, biological activities, and applications

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Abstract

Panax notoginseng is a traditional Chinese medicine containing various constituents, including the saponins, polysaccharides, polyacetylenes, amino acids, *etc*. It has beneficial functions, such as the anti-inflammatory, antitumor, hepatoprotective, and anti-aging effects. Among these, *P. notoginseng* polysaccharides (PNPs) have been exploited because of their extensive pharmacological effects, being ranked as one of the current research hotspots, especially for the functional foods and medical practice. In this review, the literature related to PNPs in the past 20 years was surveyed and analyzed using both the China National Knowledge Infrastructure (CNKI) and Web of Science (WOS) databases. The visualization diagram shows that current studies on PNPs mainly focus on the antioxidant and immunomodulatory activities and structural characterization. In addition, the extraction, separation, purification, chemical analysis, structural characteristics, bioactivities, and applications of PNPs are outlined, in detail, aimed to provide valuable information for the further study, development, and utilization regarding PNPs.

Keywords: Biological activity, Extraction, Isolation, *Panax notoginseng* polysaccharides, Structural characterization

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Introduction

Chinese herbal medicine plays a very important role in preventing and treating diseases and promoting health and has been widely used for thousands of years[1]. Polysaccharides, one of the key active ingredients in Chinese herbal medicine, have a wide variety of sources, rich contents, various biological activities, low toxic side effects, and good stability[2]. Polysaccharides play an important biological role in many living organisms by regulating immunity[3], delaying aging, and exerting antiviral, hypoglycemic, and lipid-lowering effects[4]. In addition, functional health beverage products developed with various polysaccharides as raw materials have gradually appeared in the market as well as in cosmetics, which have created huge social and economic benefits. Therefore, in-depth exploration of polysaccharides could not only find useful ways to discover new drugs but also provide a new direction for the development of health care and functional foods[5].

Panax notoginseng (Burk.) F.H. Chen is a perennial upright herb belonging to the Araliaceae family and the ginseng genus[6]. It grows mainly in shady areas of the Yunnan and Guangxi provinces and has been artificially cultivated for over 400 years[7]. *P. notoginseng* possesses the traditional homology of medicine and food and generally takes 4 to 6 years to produce mature roots. *P. notoginseng* is rich in saponins, polysaccharides, flavonoids, and other active substances, among which *P. notoginseng* polysaccharides (PNPs), one of the active components of Chinese medicine, have various biological activities such as immune regulation, antitumor, antiviral, antioxidant[8], anti-inflammation, anti-stress, anti-radiation, and anti-aging effects[9]. Polysaccharides are biological macromolecules with complex structures and complex preparation procedures. This review first presents a statistical analysis of the PNP literature, and then exhaustively summarizes the extraction, purification, chemical analysis, structural characteristics, biological activity, and clinical

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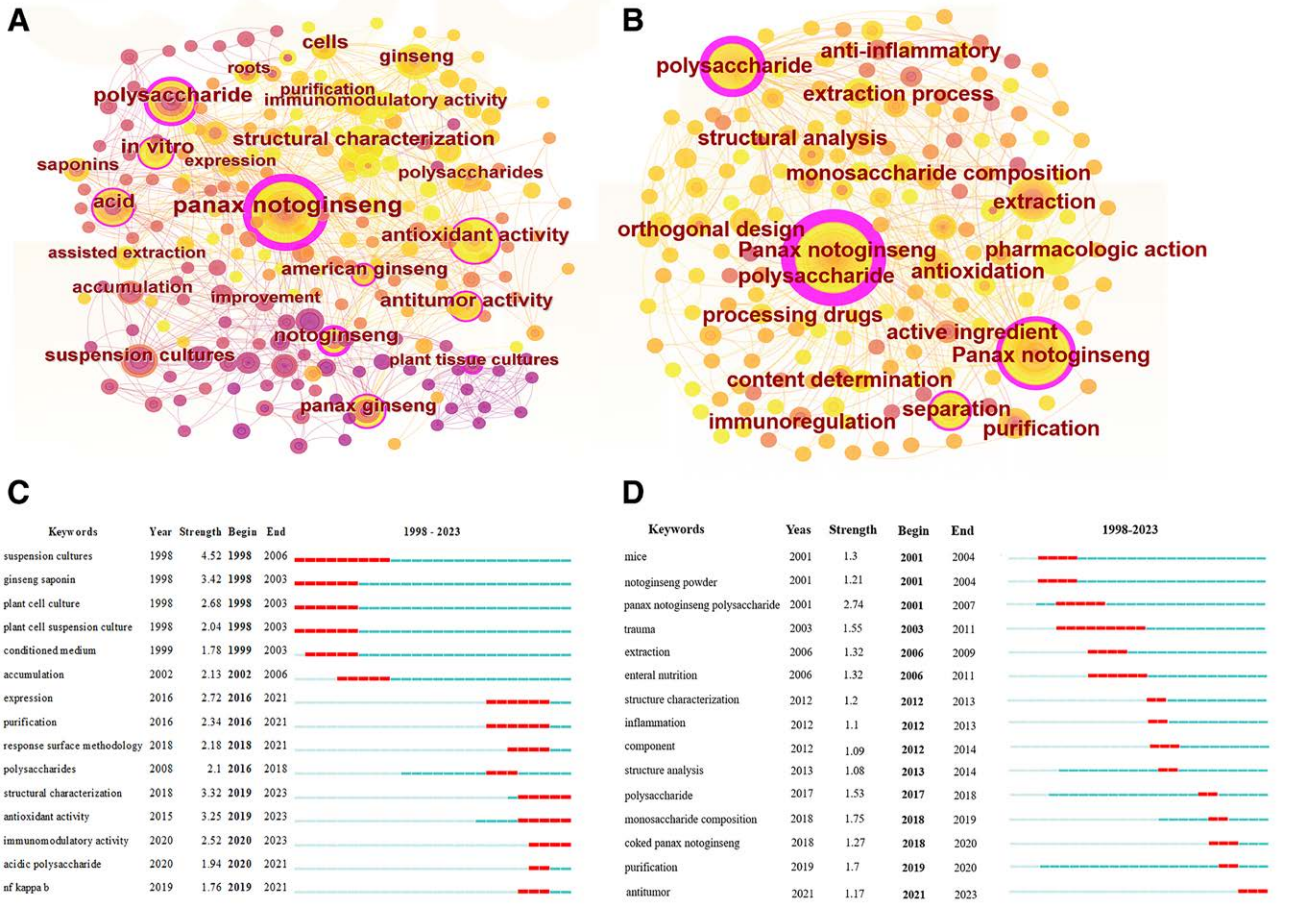

Figure 1. CiteSpace-based visualization analysis atlas of PNPs; (A) network diagram of PNPs-related research hot words in the English literature; (B) network diagram of PNPs-related research hot words in the Chinese literature; (C) keyword burst analysis of PNPs studies in the English literature; (D) keyword burst analysis of PNPs studies in the Chinese literature. PNP: *Panax notoginseng* polysaccharides.

applications of PNPs to provide valuable information for further in-depth studies dedicated to the development and utilization of PNPs.

Bibliometric analysis of PNPs

To evaluate the research status on PNPs, articles with the keyword "*P. notoginseng* polysaccharide" were retrieved mainly in China National Knowledge Infrastructure (CNKI) and Web of Science (WOS) databases, and 227 articles (including 109 Chinese and 118 English articles) were then collected with those irrelevant and repetitive articles eliminated for bibliometric analysis using CiteSpace software. The research keywords and trends of PNPs were visualized to explore the general knowledge, research highlights, and research trends of PNPs (Figure 1A and B), where the visualized nodes show the keywords and the size of the nodes represent the frequency of the keywords. *P. notoginseng*, polysaccharides, antioxidant, and pharmacological effects (Figure 1A) were the keywords of research hotspots in the English literature on PNPs. Polysaccharides, *P. notoginseng*, separation and purification, monosaccharide composition, the extraction process, and content determination (Figure 1B) were the keywords of research hotspots in Chinese literature.

Figure 1C and D shows the top 15 keywords. The blue line represents the time interval, and the red line represents the period in which a keyword appeared to significantly increase its use. The highlighted keywords refer to those used more frequently in a short period, which can predict the research trends of PNPs both domestically and internationally. As shown in Figure 1C, suspension culture for the synthesis of PNPs was mainly studied from 1998 to 2003. Owing to the complexity of the macromolecular structure and the difficulty of analysis, the structural characterization of PNPs has been so difficult that it has only been gradually carried out since 2016. Since 2019, research on the biological activity of PNPs has gradually become popular, and research hotspots involve antitumor, antioxidant, immunoregulatory, and other bioactivities. As shown in Figure 1D, domestic research on PNPs began in 2003, and the research highlights from 2012 to the present are structural characterization and physiological activity. Based on the keyword burst analysis of PNPs assisted by CiteSpace, scientific researchers may conduct structural, biological activity, and mechanism analyses in future study.

Extraction of crude polysaccharides from *P. notoginseng*

The effective extraction of crude polysaccharides from *P. notoginseng* is a prerequisite for the subsequent separation, analysis, and bioactivity screening of PNPs. Extraction methods for PNPs include hot water/aqueous ethanol, enzymatic, ultrasonic, microwave, and supercritical fluid

Table 1

Extraction methods and principles, advantages, and disadvantages

Method	Principle	Advantage	Disadvantage	Reference
Hot water/ aqueous ethanol extraction	Polysaccharides dissolve in water and precipitate in ethanol solutions	Low costs, environmentally friendly and safe	Low yield, low product purity, time-consuming	[10–11]
Enzymatic extraction	Enzymes can hydrolyze starch particles, increasing the dissolution rate of polysaccharides	Simple operation, high extraction rate, time-saving, and low energy consumption	High costs, difficult to scale up to industrial production	[12–13]
Ultrasonic extraction	Ultrasound generates vacuum/small bubbles in the solution, which annihilate and undergo high-temperature and high-pressure cavitation, leading to cell wall rupture	Low temperature, wide applicability, short time, high yield, and low energy consumption	Loud noise, and easy to cause loss and denaturation of active components	[14]
Microwave extraction	Plants absorb microwave energy, leading to an increase of internal temperature and strong molecular thermal motion	Reduce extraction time and costs	Polysaccharides are easily degraded and their biological activity may change	[15]
Inner ebullition method	Ethanol penetrates the plant tissues; hot water is added at a temperature higher than the boiling point of ethanol to evaporate the ethanol in the plant tissues and extract active ingredients	High extraction speed and yield	The desorption volume has a significant impact on extraction efficiency	[16]
Microbial fermentation	Various enzymes secreted by microbial metabolism disrupt the dense structure of plant cells	Generate new compounds, reduce toxic side effects, and improve extraction efficiency	Decompose and transform PNPs	[17]
Foam separation method	Using bubbles as carriers, surfactants is adsorbed onto small bubbles to achieve the separation of different active ingredients	Simplicity, efficiency, and pollution-free	High consumption and difficult to recycle	[18–19]
Supercritical fluid extraction	Supercritical fluids replace ordinary organic solvents	Strong solubility and good permeability	Large equipment investment	[20]

PNP: *Panax notoginseng* polysaccharides.

extraction. Table 1 summarizes the extraction methods, principles, advantages, and disadvantages.

Among these methods, hot water extraction is a conventional method. *P. notoginseng* materials are firstly decocted in water to obtain a crude extract, and then the crude extract is centrifuged and concentrated to afford the supernatant, which is precipitated with ethanol to remove the protein by the Sevage method (a mixture of chloroform and *n*-butanol at a volume ratio of 4:1), and finally, the crude PNPs are prepared for further exploration and application. Before extraction, *P. notoginseng* powder is soaked in ethanol to remove lipophilic substances and improve the purity of the PNPs. In addition, the extraction time and frequency, solid–liquid ratio, and ethanol concentration affect the extraction efficiency[9]. Response surface methodology is used to improve the extraction rate of the polysaccharides. Under the condition of a solid–liquid ratio of 1:60, 75°C, and 2.48 h, the maximum yield of polysaccharides of *P. notoginseng* is 8.31%[10]. By regulating the solid–liquid ratio to 1:20, extracting three times (3 each), and precipitating with 80% ethanol, the extraction rate of crude polysaccharides from *P. notoginseng* reaches 15.96%[21].

Enzyme-assisted extraction is an effective and popular method of polysaccharide extraction. Generally, the powder of *P. notoginseng* is pretreated successively with petroleum ether and ethanol, and extracted twice with 75°C water (3 h for each) after drying. First, the starch is removed by adding α-amylase solution. The protein is removed using Sevage reagent after ethanol precipitation and dialyzed for 48 h to obtain PNPs[12].

For ultrasound extraction, the temperature, ultrasonic power, time, and liquid–solid ratio can be optimized to improve the extraction rate. Under the conditions of 58°C, 320 W, 41 min, and a liquid–solid ratio of 1:50, the yield of PNPs is 19.51%[14].

When mentioning the microwave-assisted extraction of PNPs, experimental parameters, including microwave treatment time, soaking time, liquid–solid ratio, and particle size of powder materials, should be given more attention[15,22]. In a previous report, an orthogonal optimization experimental method was used for microwave-assisted extraction[15].

In addition to the above four common methods, inner ebullition, microbial fermentation, and foam separation have been used for the extraction of PNPs. For example,

with the internal ebullition method, the yield of PNPs is 5.6% under the conditions of a solid–liquid ratio of 15:1 with 80% ethanol solution, extracting 8 min at 95°C[16]. Microbial fermentation is a special extraction method that disrupts the dense structure of plant cells and achieves high extraction rates. The fermentation extraction process for the PNPs was optimized using a single-factor experiment and an orthogonal test. Inoculating 5% edible yellow wine yeast, with a 1:50 solid–liquid ratio, and fermenting at 32°C, gives the extraction rate of PNPs at 50.1%[17]. The foam separation method, which has only been reported once, is rarely used for the extraction of PNPs. When the injection volume is 8.0 mL and the nitrogen flow rate is 15 mL/min, polysaccharides are extracted from the decoction of *P. notoginseng* with a yield of 87.5%[23].

Separation and purification

The PNPs obtained by the extraction methods are usually crude polysaccharides that contain certain impurities, such as proteins and pigments, which need to be further removed to obtain purified PNPs. Purification of PNPs is also important for the further analysis and characterization of their chemical structures and functional activities. Because proteins often form sugar complexes with polysaccharides and their relative molecular weights are similar, protein removal becomes more difficult. Table 2 summarizes the common separation and purification methods. At present, common methods for deproteinization of PNPs include enzymatic, Sevage, resin, and trichloroacetic acid methods[31]. Common PNP decolorization methods include absorbent charcoal, macroporous resin, and H_2O_2 decolorization[20,32]. The Sevage method is the most important for removing proteins from PNPs[20]. A mixed solution of pronase and papain in equal proportions is used to remove proteins by enzymatic hydrolysis using the Sevage method. The obtained protein concentration, protein content, and sugar content are 4.84 μg/mL, 8%, and 83%, respectively, which can remove PN proteins more thoroughly[33]. At present, research on purification methods for PNPs has focused on several conventional methods with few technical innovations. Multiple separation techniques can be used to efficiently obtain purified PNPs. Membrane technology has good prospects for the separation and purification of PNPs[24]. Polysaccharides are purified using Sephadex G-50, DEAE-650 Toyopearl weak-anion resin, preparative gel permeation high-performance liquid chromatography (HPLC), activated charcoal, and membrane separation. In this way, the polysaccharides obtained have the advantages of low protein, heavy metal, and ash contents and stable quality. The general process of PNP extraction and separation is illustrated in Figure 2.

Chemical analysis

Total sugar content

The total sugar content of polysaccharides can be determined using phenol-sulfuric acid, anthrone-sulfuric acid, and 3,5-dinitrosalicylic acid (DNS) colorimetry[34–35].

Different processing methods afford PNPs with different effective components and contents. The polysaccharide contents of raw and cooked *P. notoginseng* are determined using anthrone-sulfuric acid colorimetry, with polysaccharide content in cooked *P. notoginseng* (6.95%) higher than in raw *P. notoginseng* (6.60%)[36]. The Box-Behnken response surface method is also used to optimize the extraction process of cooked PNPs, with the optimum extraction conditions as follows: extraction time 2.5 h, extraction temperature 90°C, solid–liquid ratio of 1:33, 2 times of extraction. The polysaccharide yield is 8.82%[37].

The roots, stems, leaves, and flowers of *P. notoginseng* contain polysaccharides, and a high content of polysaccharides can be extracted from the residues of *P. notoginseng*[1]. For example, polysaccharides have been extracted from the industrial residues of *P. notoginseng* and the crude polysaccharide content was determined to be approximately 2%[38]. Another study determined the polysaccharide content in different parts of *P. notoginseng* and found that the PNP content in the root was 45.0%, whereas that in the stem and leaf was 20.0%, approximately half of that in the root[39]. In addition, a polysaccharide yield of 21.4% is obtained from the leaves of *P. notoginseng*[40].

Content of glucuronic acid

The uronic acid content in polysaccharides is currently determined using spectroscopic and chromatographic methods. Spectroscopic methods include the pyrazole-sulfuric acid, *m*-hydroxybiphenyl, and 3,5-dimethylphenol. Chromatographic methods include gas chromatography (GC), molecular volume-exclusion liquid chromatography, and anion–liquid chromatography. The spectral method is simple to operate, but there are many interfering factors; the determination results deviate greatly from the theoretical value, and the total uronic acid content is measured. Chromatographic methods can accurately determine the contents of different types of uronic acid. Among these, high-performance anion–liquid chromatography does not require derivatization and is commonly used for determining uronic acid content. The byproducts produced in the complex colorimetry process may increase the proportion of uronic acid in the PNPs[41]. In a previous study, the uronic acid content of polysaccharides obtained using different extraction methods was compared. The uronic acid content in the polysaccharides obtained by water extraction and enzymatic precipitation is lower, whereas that obtained by alkali extraction is higher[42].

Structural features

Chemical methods

Monosaccharide composition

Monosaccharide composition analysis is the basis for studying the structure, properties, and structure–activity relationships of plant polysaccharides. Because monosaccharides do not have ultraviolet and fluorescence absorption, they need to be subjected to a series of treatments, such as acid hydrolysis, neutralization, and

Table 2

Comparative analysis of separation methods of PNPs

	Method	Principle	Dominance	Limitation	Application example	Reference
Deproteinization	Sevage method	The free protein was denatured in a certain proportion of chloroform and chloroform, and separated from water-soluble polysaccharide.	Almost all free proteins can be removed.	High demand for solvent, high loss of polysaccharide, chloroform is a toxic reagent, harmful to the human body.	Remove the protein in the crude polysaccharide of *Panax notoginseng*.	[24]
	Resin method	Adsorption of free protein by pores between resins.	Effectively improve the deproteinization rate, and ensure the quality of polysaccharides.	Tedious process and complicated operation.	AB-8 resin and Sevage reagent were used to remove protein from *P. notoginseng*.	[25]
	Trichloroacetic acid method	The conformation of the protein was changed, and many hydrophobic groups were produced, which made it aggregate and precipitate.	Effectively remove the protein in the polysaccharide.	Deproteinization conditions are harsh, and polysaccharides are easily hydrolyzed under strong acid conditions.	PNPs were deproteinized by trichloroacetic acid.	[26]
Decolorize	Absorbent charcoal method	Fading is carried out based on the physical adsorption of activated charcoal.	High fading rate, non-toxic, low cost, reusable.	The conditions are harsh, and the polysaccharide is easily hydrolyzed in an acidic environment, reducing the retention rate.	PNPs were decolorized and refined by activated charcoal.	[27]
	Macroporous resin fading method	Adsorption of pigments by pores between resins.	Little effect on the structure of polysaccharides, large adsorption capacity, simple steps, less usage of organic solvents.	It is only suitable for low viscosity polysaccharides with high removal efficiency, not suitable for large-scale use.	AB-8 resin was used for the decoloration of PNPs.	[28]
Column chromatography	Cellulose column chromatography	The polysaccharide with different molecular weights in the crude polysaccharide have different solubility in eluent (eg, alcohol), which could promote the separation.	The purification effect is better.	Long elution cycle, especially not conducive to the purification of acidic polysaccharides with relatively high viscosity.	The polysaccharide was isolated and purified by DEAE-52 cellulose column and Sephadex G-75 gel column.	[29]
	Anion exchange chromatography	The polysaccharides are separated and purified based on the different charge of the polysaccharides. Under the action of different elution solvents, the polysaccharides are separated by adsorption and desorption.	The distribution of polysaccharides is uniform, and the separation and purification effect are good.	The anion exchanger is more expensive, increasing the cost of separation.	PNPs were separated and purified by DEAE Sepharose Fast Flow anion exchange chromatography.	[30]
	Gel filtration chromatography	Porous gel with three-dimensional network structure is used as stationary phase to separate polysaccharides through differences in molecular size.	The method is mild and do not change the biological activity of the sample.	The resolution is low, and the polysaccharide retention rate is reduced.	The polysaccharides were purified by Sephadex G-200 gel column chromatography.	[25]

DEAE: Diethylaminoethyl dextran cellulose; PNP: *Panax notoginseng* polysaccharides.

derivatization, before being analyzed and determined by instruments. Methods for determining monosaccharide composition include GC, thin-layer chromatography (TLC), HPLC, and high-performance capillary electrophoresis (HPCE). Monosaccharides of *P. notoginseng* are mainly composed of Glc, with less Gal, Ara, GlcA,

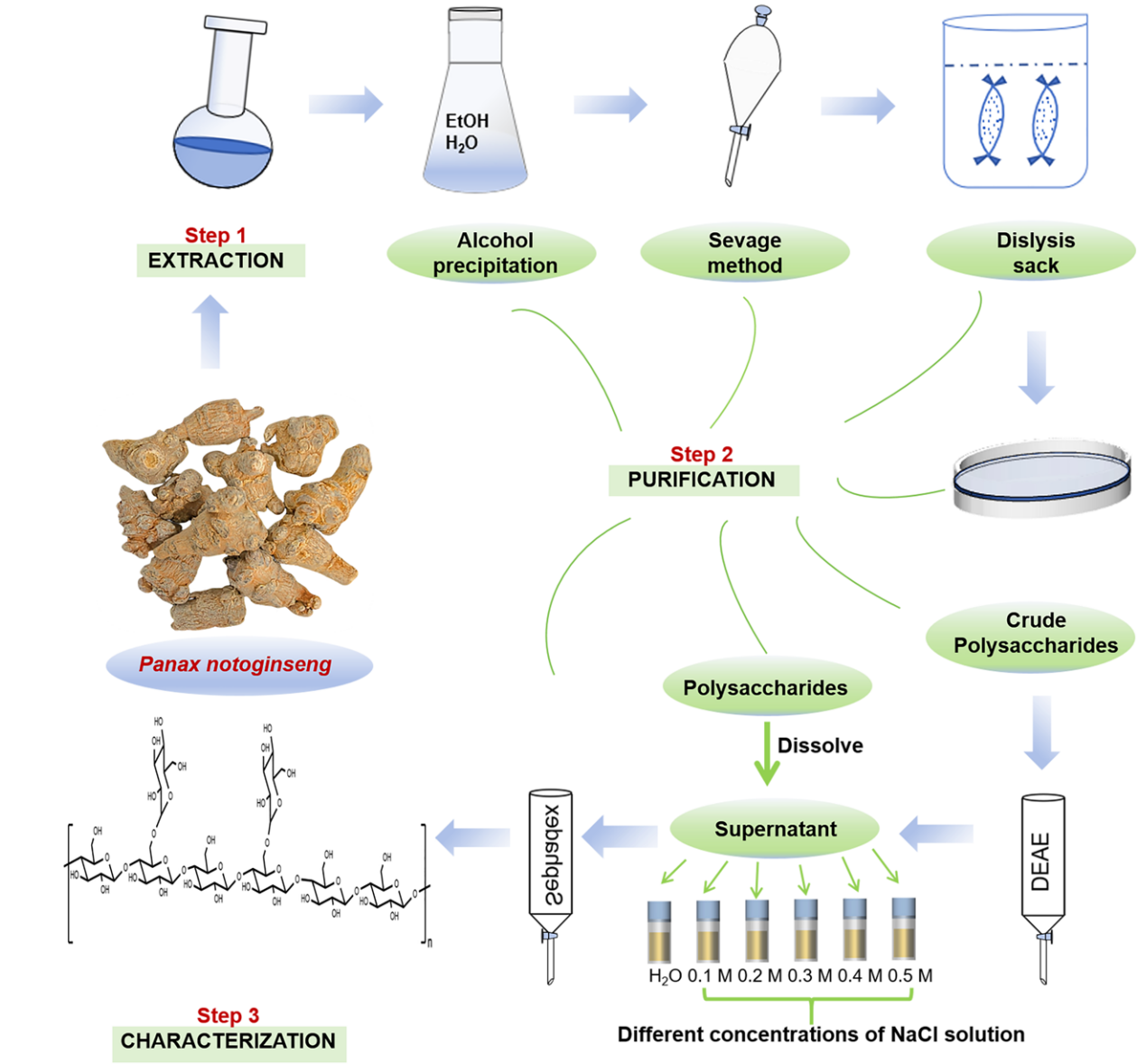

Figure 2. Workflows for the isolation, purification, and structural characterization of PNPs. PNP: *Panax notoginseng* polysaccharides.

and Rha[43]. A novel homogeneous polysaccharide, PNPB1, with a molecular weight of 9.3×10^5 Da, was isolated from *P. notoginseng*, being composed of trace amounts of Glc (88.2%), Gal (9.0%), Ara (2.4%), and GlcA[44]. Gong et al.[45] used GC to explore the monosaccharide composition of refined polysaccharides from *P. notoginseng* flowers, including Glc, Ara, Rha, Xyl, Gal, and Man.

Methylation analysis

Methylation analysis is an important chemical method for studying the glycosidic bonds in polysaccharides. Methylation analysis includes acid hydrolysis and $NaBH_4$ reduction. Periodate oxidation and Smith degradation reactions are not performed for methylation analysis. Combined GC-mass spectrometry (GC-MS) can not only verify the results of monosaccharide composition analysis but also reveal different glycosidic bond connection modes and their proportions corresponding to each monosaccharide residue. GC-MS has the characteristics of high resolution and high sensitivity and is fast and efficient. Therefore, they are widely used to identify polysaccharides. A new polysaccharide MRP5 was isolated from the roots of *P. notoginseng*, and the result of methylation analysis and one-dimensional (1D) and two-dimensional (2D) NNR implicated the presence of repeating units of →3)-*β*-Glc*p*-(1→, →6)-*β*-Glc*p*-(1→, →3,6)-Glc*p*-(1→, *β*-Glc*p*-(1→, →3)-*β*-Gal*p*-(1→, →3,6)-*β*-Gal*p*-(1→, →3)-*β*-Glc*p*-(1→,→6)-*β*-Glc*p*-(1→,→3,6)-Glc*p*-(1→,*β*-Glc*p*-(1→,→3)-*β*-Gal*p*-(1→,→3,6)-*β*-Gal*p*-(1→,→3)-*α*-Rha*p*-(1→, →3)-*α*-Ara*f*-(1→ and *α*-Ara*f*-(1→ residue[46]. Feng[12] analyzed the homogeneous molecular weight polysaccharide purified from the roots of *P. notoginseng* using methylation and nuclear magnetic resonance (NMR). The main chain is mainly composed of →3)-Glc*p*-(1→, →3,6)-Glc*p*-(1→, →6)-Glc*p*-(1→, →3,6)-Gal*p*-(1→, →3)-Gal*p*-(1→ residue.

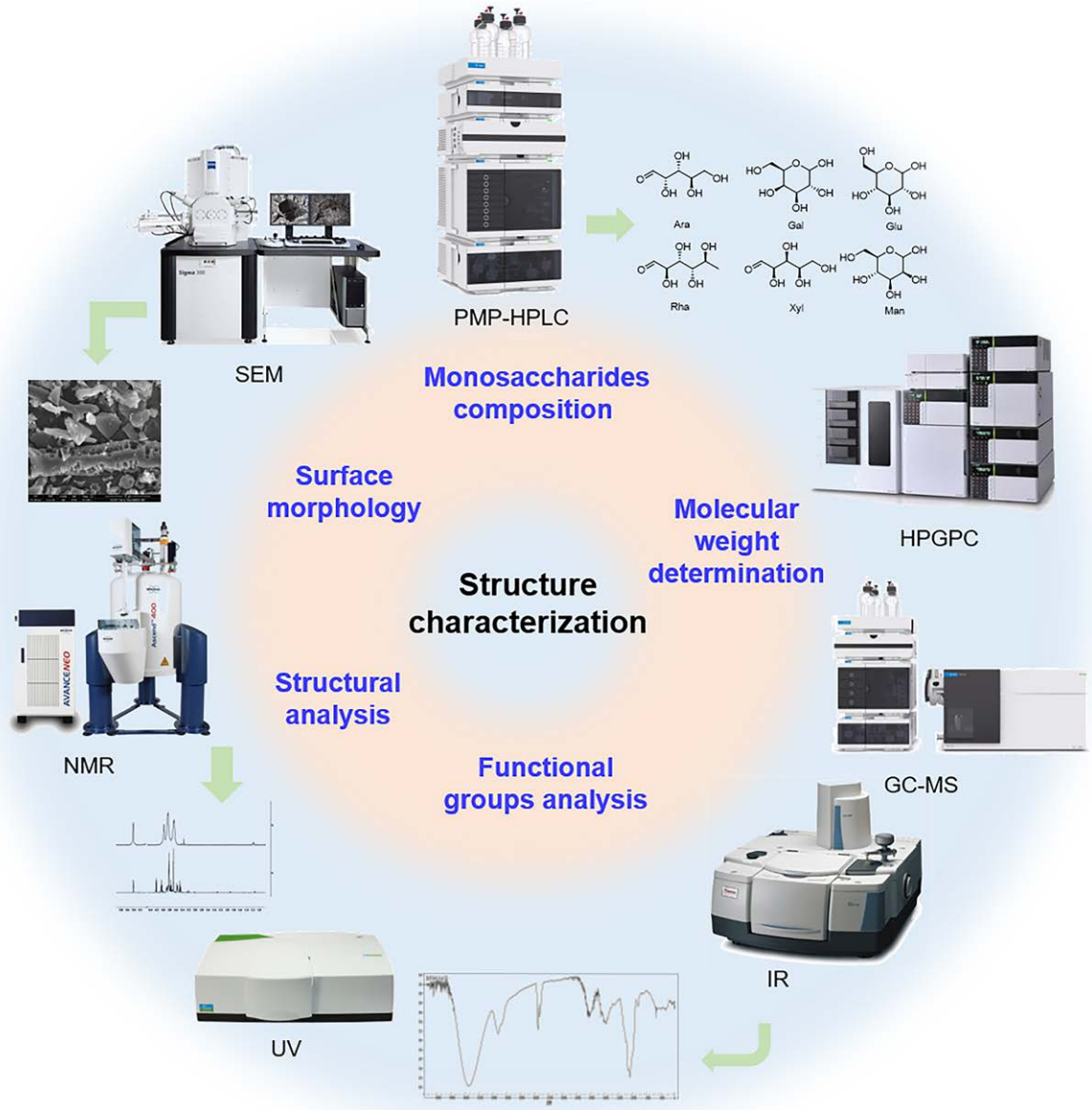

Figure 3. Structural characterization of PNPs. GC-MS: Gas chromatography-mass spectrometry; HPCE: High-performance capillary electrophoresis; HPGCP: High-performance gel permeation chromatography; HPLC: High-performance liquid chromatography; IR: Infrared spectroscopy; NMR: Nuclear magnetic resonance; PMP: 1-phenyl-3-methyl-5-pyrazolinone; PNP: *Panax notoginseng* polysaccharides; SEM: Scanning electron microscopy; TLC: Thin-layer chromatography; UV: Ultraviolet.

Physical methods

The commonly used techniques for the structural characterization of PNPs are summarized in Figure 3.

NMR analysis is a very important means of analyzing the structure of polysaccharides[8,47] as it provides information on monosaccharide composition, sugar chain linkages, links between monosaccharide residues, and the location and configuration of glycosidic bonds. The configuration problem of glycosidic bonds in polysaccharides can be solved using ^{1}H NMR spectroscopy. The α or β configuration of the terminal hydrogen (H1) and terminal carbon (C1) in the sugar residue is recognizable in both ^{1}H and ^{13}C NMR spectra. In most cases, the proton and carbon resonances of an α-configuration H1 and C1 occur at δ_H 5.1 to 5.8 ppm and δ_C 98 to 103 ppm, respectively, whereas those of a β-configuration H1 and C1 are at δ_H 4.3 to 4.8 ppm and δ_C 103 to 106 ppm, respectively[25]. However, although the adjacent protons contain functional groups, such as *O*-acetyl, *O*-alkyl, phosphate, and *O*-sulphate groups, the proton and carbon chemical shifts of H1 and C1 change slightly[48].

2D NMR plays an important role in the assignment of sugar residues and the determination of glycosyl sequences. The homonuclear ^{1}H-^{1}H correlation spectroscopy (^{1}H-^{1}H COSY) experiment with a spin-coupled system reflects the correlation between adjacent protons in sugar residues[49], whereas heteronuclear multi-quantum correlation spectroscopy (HMQC) reveals the ^{13}C-^{1}H direct correlation between protons and the directly connected carbon. To determine the sequence of sugar

residues in polysaccharides, remote ^{1}H and ^{13}C correlation (heteronuclear multiple quantum correlation spectroscopy, HMBC) is preferred to obtain the ^{13}C-^{1}H remote correlation signal[49]. A polysaccharide was extracted from the residue of *P. notoginseng*, and its structural characteristics were revealed through methylation and NMR. The linkage of "→4)-*α*-D-GalA*p*-(1→4-*β*-L-Rha*p*-1→4)-*β*-D-Gal*p*-(1→" is the backbone, and the side chain of "*α*-L-Ara*f*-1→5)-*α*-L-Ara*f*-(1→" is connected to the backbone chain at position *O*-3 of "→4)-*β*-L-Rha*p*-(1→"[3].

More structural information on PNPs can be obtained using infrared spectroscopy (IR)[3]. As exemplified by polysaccharide PNPS-0.3, its IR spectrum shows a strong band at 3,405 cm^{-1}, which is attributed to the stretching vibration of the O-H group. The weak absorption of 2,938 cm^{-1} is caused by the tensile vibration of the C-H bond. At 1,608 cm^{-1}, the stretching of COOH forms a strong absorption band with a peak, indicating the presence of a carboxyl group of PNPS-0.3. The absorption peaks at 1,097 and 952 cm^{-1} were assignable to a pyranoside, and those at 836 and 896 cm^{-1} belonged to the α type and β type glycosides, respectively.

Therefore, with IR, it is possible to determine the α or β type of glycosidic bond, furanose or pyranose, and, at the same time, it is possible to determine whether there is an aldehyde acid. In an IR spectrum of PNPs, a strong band related to O-H group can be observed at 3,427 to 3,334 cm^{-1}. A weak band is observed at 2,926 to 2,850 cm^{-1}, which is attributed to the stretching vibration of C-H and is typically manifested in polysaccharide polymers. The strong peak at 1,610 to 1,644 cm^{-1} results from the characteristic contraction vibration of the carboxyl group. Peaks at 500 to 900 cm^{-1} indicate the presence of pyranose, whereas the peaks of 927 cm^{-1} and 860 to 898 cm^{-1} are attributed to the α-type and β-type glucosidic bonds, respectively.

The average molecular weight of polysaccharides is usually determined using HPLC and high-performance gel permeation chromatography (HPGPC)[50–52]. However, because of their different origins and extraction methods, the molecular weights of the obtained PNPs differ significantly. In one study, PGS-0.5 M polysaccharide was obtained from the stem of *P. notoginseng* by ultrasonic extraction, and the average molecular weight measured by HPGPC was 2,617 kDa. A homogeneous polysaccharide was obtained by boiling water extraction of *P. notoginseng* root powder[53]. The proteins and pigments of the crude polysaccharide were removed using AB-8 resin and Sevage reagent, and the polysaccharide was further purified using a DEAE-52 column and Sephadex G-200 column. As previously reported[46], its molecular weight was determined on an HPGPC system to be 8.27 kDa.

Scanning electron microscopy (SEM) can be used to analyze the sample surface or fracture topography by using an extremely narrow electron beam to scan the sample and through the interaction between the electron beam and the sample to obtain secondary electron signals for imaging observation. SEM offers the advantages of high magnification and clear resolution. Sample preparation is simple and instrumental operation is convenient. SEM is used to observe the surface morphology of the polysaccharides at different concentrations. Different polysaccharides have different morphologies; some have a loose porous structure[54], some have a network cross-linked structure[8], and some have irregular coiled chains and spherical structures[54].

Bioactivities

Antitumor activity

Female mice at 4 to 6 weeks were selected as research subjects. Purified PNPs were fed to the mice, and H22 cells were injected into the axillae of the animals to construct a mouse model. The results showed that, compared with the negative control group, the solid tumors in the axilla of the mice were significantly reduced, and the activity of natural killer (NK) cells and the proliferation of spleen cells was enhanced. The results of hematoxylin and eosin (HE) staining showed an obvious degree of tumor cell rupture in mice fed PNP-1. Cell cycle analysis of mouse solid tumor cells showed that polysaccharides effectively prevented the proliferation of H22 tumor cells and blocked them in the G2/M phase, thereby inhibiting their growth and diffusion[55,56]. *In vivo* animal experiments using a hepatocellular carcinoma model showed that PNP-1 had good antitumor activity: PNP-1 could significantly reduce the tumors of H22 model mice and effectively inhibit the proliferation of tumor cells. The results of tumor cell cycle analysis showed that PNP-1 feeding could induce apoptosis of H22 cells *in vivo* by inhibiting the cell cycle in the S phase[55,56]. After the target rats were treated with PNPs, serum transaminase levels significantly decreased, and the degree of damage to the liver tissue structure was alleviated. PNPs reduce the levels of ALT and AST in the serum of rats with liver ischemia-reperfusion injury, reduce the pathological changes in liver tissue in rats, and exhibit a protective effect against hepatic ischemia-reperfusion injury in rats[57].

In addition, PNPs inhibit the growth of H22 cells, significantly prolong the survival time of tumor-bearing mice, and promote an increase in activated $CD4^{+}$T cells and the improvement of serum IL-2 levels, which may be one of the mechanisms by which PNPs exert antitumor effects. PNPs possess antitumor potential against liver cancer[58], and more specific mechanistic details need to be further elucidated. A functional gene vector (PNP-PEI) was constructed and potential therapeutic nanoparticles (PNP-PEI/shPD-L1) were produced by loading targeted programmed death ligand 1 (PD-L1) clip RNA into the PNP-PEI. *In vivo* studies revealed that PNP-PEI effectively carries therapeutic shPD-L1 into tumor cells, whereas PNP-PEI/shPD-L1 significantly inhibits the growth of B16-F10 cells and the expression of PD-L1. Notably, PNP-PEI treatment reverses macrophages from the M2 to M1 subtype and promotes dendritic cell maturation, which promotes host immunity and enhances therapeutic antitumor effects[59].

Immunomodulatory activity

In recent years, an increasing number of studies have revealed the immune-enhancing efficacy of PNPs, as manifested by their ability to improve specific and

nonspecific cellular immune functions[6]. *P. notoginseng* is used for trauma recovery and PNPs play an important role. In one study, the immune function was significantly decreased after trauma in rats, whereas early enteral nutrition plus PNPs improved the cellular immune function and protected the rat body against trauma by significantly increasing the serum IL-2 level[60]. To study the effects of PNP on immune function in mice, cellular immune function, humoral immune function, mononuclear macrophage count, and NK cell activity were assessed. The results showed that PNP enhanced the cellular and humoral immunity in mice[61].

Antioxidant effect

The *in vitro* antioxidant activity of the PNPs was investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH)-free radical, $ABTS^+$ free radical, and hydroxyl radical scavenging tests. CPPN was isolated and purified to obtain neutral and three acidic polysaccharides. Comparing the components after separation and purification, the crude polysaccharide of *P. notoginseng* showed the strongest scavenging effect on the three types of free radicals with the strongest antioxidant capacity, suggesting that there may be a synergistic effect among the polysaccharides of each component in the crude polysaccharide of *P. notoginseng*. In addition, the antioxidant activities of the three purified acidic polysaccharides were stronger than those of the neutral polysaccharide, further indicating that the antioxidant activity of the PNPs is closely related to their acidic groups[62]. In summary, the antioxidant activity of PNPs is closely related to their structure, providing a new direction for further studies on the biological activity of PNPs. Further studies are needed to deepen our understanding of the antioxidant mechanisms.

Antidiabetic effect

The role of PNPs in lowering blood glucose levels has attracted increasing attention and is considered to have an appreciable therapeutic effect on diabetes. The hypoglycemic mechanism of PNPs involves increasing insulin sensitivity, promoting blood glucose transport and utilization, and inhibiting blood glucose absorption and synthesis.

In a study of PNP intervention in type 2 diabetes model rats, the impact of PNPs on renal pathology, renal function, blood glucose, protein kinase C-η (PKC-η), and protein kinase C-ζ (PKC-ζ) was observed and the results showed that PNPs regulated the blood lipid level of rats, thereby improving the renal function of these animals. This improvement may be related to the decreased expression of PKC-η and PKC-ζ in the kidney[63]. Possible mechanisms include affecting the insulin signaling pathway, regulating the activity of insulin receptors, or affecting blood glucose levels through other pathways; it may also involve biological activities related to insulin secretion and utilization and other effects on the glucose metabolic pathway. However, the specific hypoglycemic mechanism of PNPs needs to be elucidated in future studies. Notably, in a diabetic eye disease rat model injected with streptozotocin (STZ), the effects of PNPs on hypoglycemia and eye diseases were investigated. PNPs were found to exhibit potential efficacies in diabetic retinopathy[64] by lowering blood glucose, increasing the levels of glutathione (GSH) and nitric oxide (NO), and elevating the gene expression levels of vascular endothelial growth factor (VEGF) and nitric oxide synthase (iNOS).

PNPs have the potential to lower blood glucose levels and have a therapeutic effect on diabetes and its complications by regulating the expression levels of multiple genes.

Other activities

In addition to the antitumor, immune regulation, antioxidation, and hypoglycemic biological activities, PNPs also show other functional activities (Figure 4), such as anti-inflammatory, anti-aging, liver protection, and bone marrow protection, with the potential mechanisms reproduced from the literature (Figure 5). The active homogeneous polysaccharide PNP-20 obtained from *P. notoginseng* residue is a novel glucogalactose polysaccharide with an anti-enteritis effect, which also provides a material basis for the role of PNPs in intestinal health[65]. The homogeneous polysaccharides MRP5 and MRP5A obtained from *P. notoginseng* significantly enhance the antioxidative stress ability of *Caenorhabditis elegans* and prolong its lifespan[46]. The hepatoprotective polysaccharides (PNPS-0.5 M) isolated and purified from the waste of *P. notoginseng* prevent the accumulation of peroxides during alcohol metabolism, thereby improving alcohol-induced liver injury[66]. The neutral polysaccharide (NPPN) isolated from *P. notoginseng* shows protective effects against cyclophosphamide-induced bone marrow suppression in mice. NPPN also reduces cell cycle arrest and apoptosis of bone marrow cells[67].

In summary, PNPs exert a variety of physical and chemical activities with favorable application potential for the prevention and treatment of various diseases. However, the mechanism and clinical applications of PNPs require further investigation.

Applications

Numerous Chinese patent prescriptions that contain Notoginseng Radix et Rhizoma (Sanqi), such as the Sanqi tablet, Sanqi Shangyao capsule, Sanqi Xueshangning capsule, Sanqi Tongshu capsule, Yanghuo Sanqi capsule, and Fufang Sanqi Buxue capsule, have been widely used as remedies to help people relax muscles, stimulate blood circulation, and relieve pain, and saponins from *P. notoginseng* have been widely recognized as one of the main active ingredients. However, PNPs are also beneficial to human health and have been applied frequently in the healthcare and industrial fields.

Medical and food fields

PNPs act as prebiotics by accelerating the proliferation of intestinal probiotics[25]. In addition, PNPs with anti-fatigue effects can be used to prepare functional beverages, as revealed in Chinese medicinal patents[68]. Furthermore, PNPs can be prescribed to make beverages with the functions of immunity enhancement, protection

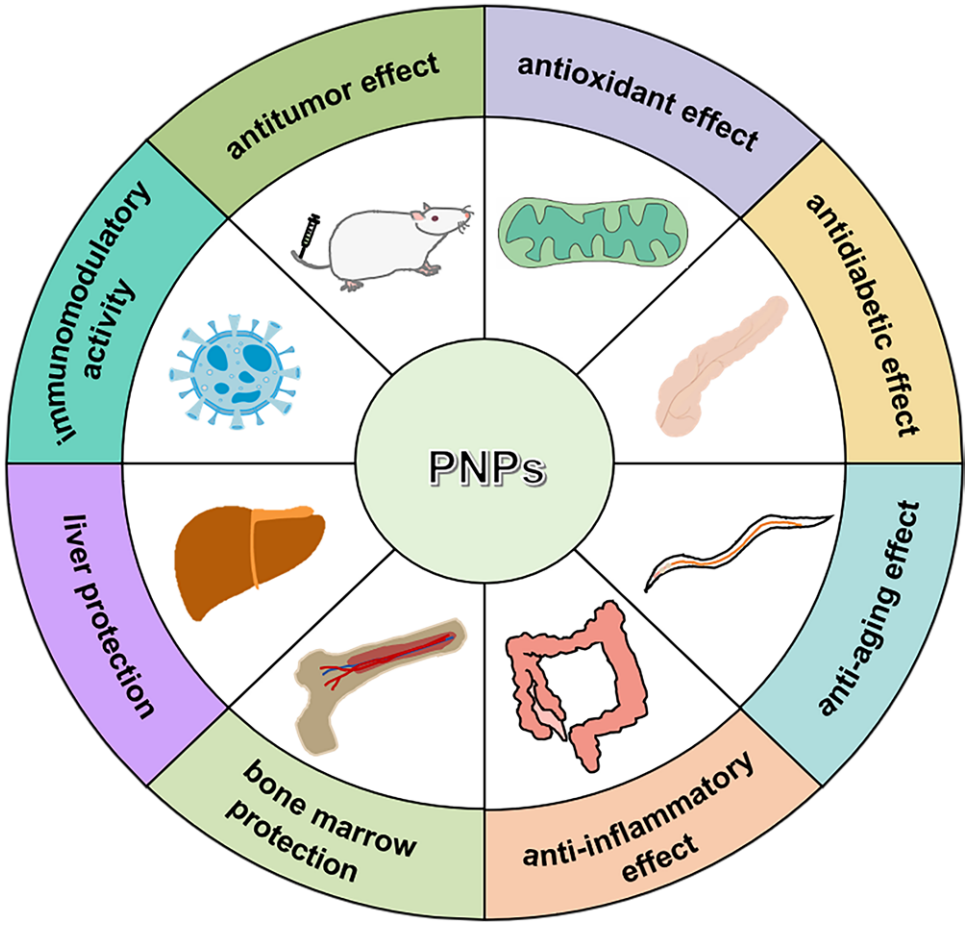

Figure 4. Pharmacological activities of PNPs. PNP: *Panax notoginseng* polysaccharides.

of the cardiovascular system, lowering blood pressure, nourishing the stomach, and protection against aging. This may stimulate the development of PNPs in dual-use products for both medicine and food[69].

Biomaterial field

PNPs are naturally occurring macromolecules with immune activity. It can easily penetrate through the stratum corneum and release the loaded drug into deeper layers of the skin. Thus, satisfactory mechanical strength and skin penetration depth can be achieved when PNPs are used to prepare soluble microneedles[5].

Industry field

A patent showed that PNP granules can be used as a stabilizer to prepare a lotion to fix the problems of time and energy consumption in the synthesis of Pickering granules and reduce hazards to human health and environmental pollution[70]. Furthermore, polysaccharides from the waste residue of *P. notoginseng* were used for fermentation to produce an essence with a special flavor, full and mellow taste, and a unique fruit flavor[71].

Conclusions and future perspective

Visual analysis of the literature on PNPs revealed that current studies focus on the structural characterization and antioxidant, antitumor, and immunomodulatory activities of PNPs. However, these polysaccharides have a large molecular weight and a very complex structure, and their structure needs to be elaborated by combining various technologies, such as NMR, IR, and methylation analysis, and their mechanism of action needs to be further studied in terms of its pharmacological properties. The physicochemical properties, active sites, pharmacodynamic effects *in vivo*, and corresponding structure–activity relationships need to be further investigated in the future. In summary, the extraction, purification, characterization, pharmacological activity, and clinical applications of PNPs are summarized in detail in this study to provide a reference for further research on PNPs dedicated to the development and utilization of *P. notoginseng* resources.

Conflict of interest statement

The authors declare no conflict of interest.

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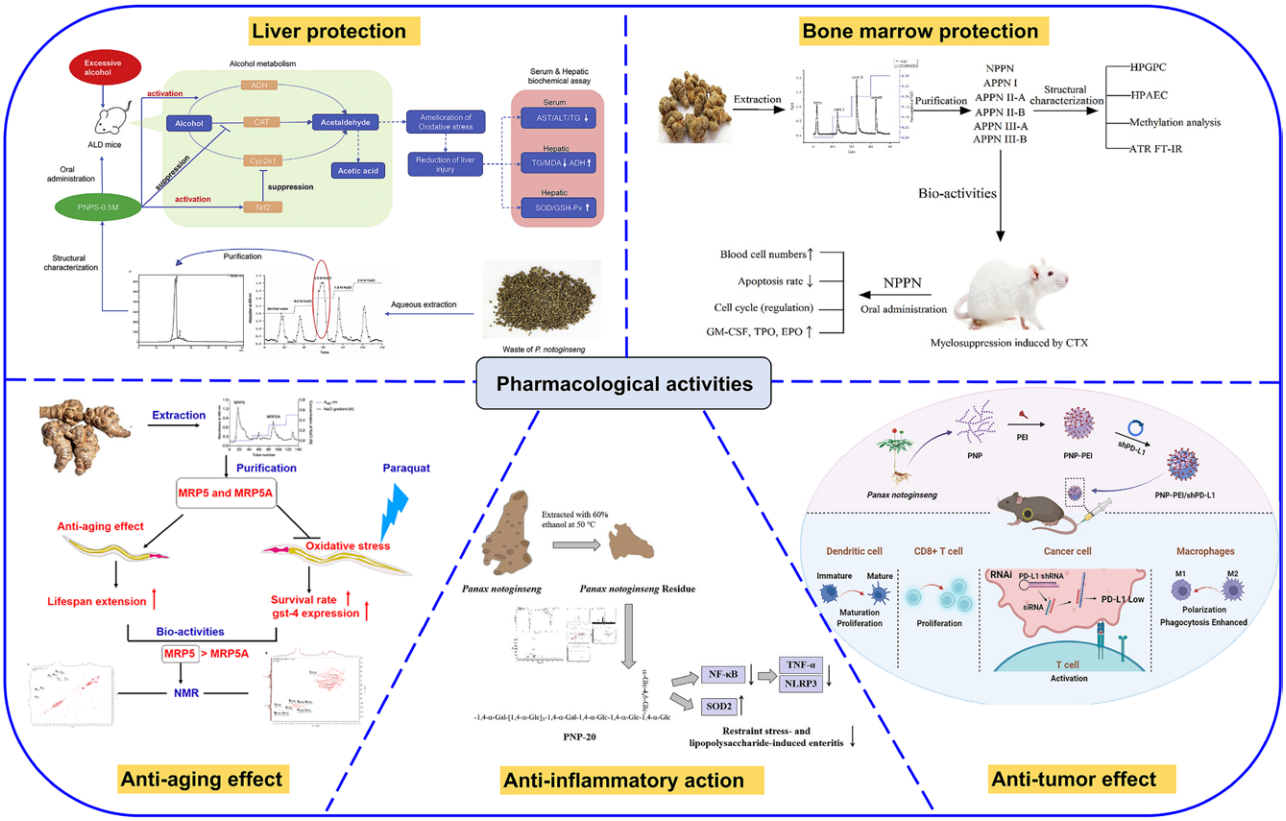

Figure 5. Pharmacological activities of PNPs showing some representative studies (reprint permission from the literatures [46, 59, 65–67]). ADH: Hepatic levels of alcohol dehydrogenase; ALD: Alcoholic liver damage; ALT: The levels of alanine aminotransferase; AST: Hepatic levels of alcohol dehydrogenase; ATR-FTIR: Attenuated total reflectance fourier transform infrared spectroscopy; CTX: Cyclophosphamide; EPO: Erythropoietin; GM-CSF: Granulocyte-macrophage colony-stimulating; GSH-Px: Glutathione peroxidase; HPAEC: High-performance anion exchange chromatography; HPGPC: High-performance gel permeation chromatography; MDA: Hepatic malondialdehyde; MRP5 and MRP5A: Two novel polysaccharides were obtained from the root of *Panax notoginseng*; NF-kB: Nuclear factor-kB; NLRP3: The NOD-like receptor family pyrin domain containing 3; PD-L1: The programmed death-ligand 1; PEI: Positively charged polyethyleneimine; PNP: *Panax notoginseng* polysaccharide; ShPD-L1: Potentially therapeutic nanoparticle; SOD: The antioxidant enzymes superoxide dismutase; TG: Triglyceride; TNF: Tumor necrosis factor; TPO: Thermoplastic polyolefin.

Author contributions

Jiaqi Huang, Yuheng Zhao, and Mengyao Wang conceived and designed this original draft. Xiaojin Tian and Dianxin Cui investigated and analyzed data curation. Qilong Wang, Xue Li, Honghua Wu, and Wenzhi Yang participated in writing, editing, and funding acquisition. All of the authors have read and approved the published version of the manuscript.

Ethical approval of studies and informed consent

Not applicable.

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None.

Data availability

All data generated or analyzed during this study are included in this published article.

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