



Review Article

Extraction, purification, structural features, and pharmacological properties of polysaccharides from *Glycyrrhiza uralensis*: a review

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Received: 3 September 2024 / Revised: 18 September 2024 / Accepted: 19 September 2024

Abstract: *Glycyrrhiza uralensis*, known as “Gan-Cao” in Chinese, is one of the most widely used herbs in the world. As a long-established and widely used traditional Chinese medicine, *G. uralensis* has spleen and qi promoting, heat clearing and detoxifying effects and is listed in the pharmacopoeias of several countries. In recent years, the extraction, purification, biological activity, and applications of *G. uralensis* polysaccharides (GUPs) have attracted the attention of researchers. Studies have shown that GUPs possess various biological functions, including anti-inflammatory, immune regulation, antioxidant, anti-tumor, anti-diabetic, and antibacterial properties. Therefore, a comprehensive systematic review is needed to summarize the latest progress on GUPs and to promote a deeper understanding of their biological functions. This paper reviews the research progress of GUPs in terms of extraction and purification methods, chemical structure, biological activity, possible mechanisms of action and potential applications, providing valuable insights and the latest information for the further development and application of GUPs in fields such as medicine, feed additives, vaccination and food additives.

Keywords: *Glycyrrhiza uralensis* polysaccharides; Extraction; Structural characteristics; Biological activities; Potential applications

Citation: Liu J., Wang R., Zhai S. C., et al. Extraction, purification, structural features, and pharmacological properties of polysaccharides from *Glycyrrhiza uralensis*: a review. *Food & Medicine Homology*, 2027, 4: 9420175. <https://doi.org/10.26599/FMH.2027.9420175>

1 Introduction

Glycyrrhiza uralensis, known as “Gan-Cao” in Chinese, is one of the most widely used herbs in the world and is derived from the legume family. It is the dried root and rhizome of *G. uralensis* Fisch^[1,2] (Fig. 1). According to classical Chinese medicine theory, about 90% of herbal formulations contain *G. uralensis*^[3]. *G. uralensis* was listed as a national second-class protected plant in the National Key Protected Wild Plants Directory in 2021^[4]. As a long-established and widely used traditional Chinese medicine, *G. uralensis* has the efficacy of strengthening the spleen, benefiting the qi, clearing heat and detoxifying the toxin^[5], and has been recorded in several national pharmacopoeias^[6]. Ethnopharmacological studies have demonstrated its ability to uplift the spirit, remove heat and toxins, moisten the lungs and reduce blood congestion, relieve spasms and discomfort, and minimize adverse drug reactions^[7,8]. Globally, *G. uralensis* is widely distributed in China, Mongolia, Siberia in Russia, Kazakhstan, Pakistan, and other Eurasian countries and regions. In China, it is mainly distributed in provinces in the Northeast, North, and Northwest regions^[9]. In addition, more than 300 different chemicals have been identified in *G. uralensis*, and its

main chemical constituents include triterpenoid saponins^[10], flavonoids^[11], alkaloids^[12], coumarins^[13], polysaccharides^[14], and proteins^[15]. Some of them have biological activities such as antibacterial, antiviral, antitumor, anti-inflammatory, antidiabetic and hepatoprotective^[16-19].

In recent years, *G. uralensis* polysaccharides (GUPs) have been of great interest to researchers due to their unique properties such as non-toxicity and non-specific immune system stimulation^[20,21]. Their extraction, isolation, structural characterization, pharmacological activities and applications have received considerable attention. The monosaccharide components of GUPs include rhamnose, arabinose, xylose, mannose, fucose, ribose, glucose, galactose, glucuronic acid and galacturonic acid^[22-24]. Current studies have demonstrated that GUPs exhibit a variety of remarkable pharmacological activities, including anti-inflammatory^[25], immunomodulatory^[26], antioxidant^[27], antitumor^[28], antidiabetic^[29], antimicrobial^[30], as well as other biological activities^[31]. Since GUPs exhibit a wide range of remarkable pharmacological activities, they are widely used in animal feed additives^[32], vaccination^[33] and food additives^[34].

Currently, the research on GUPs in aspects such as extraction, structure, biological activity, and applications is increasing and is

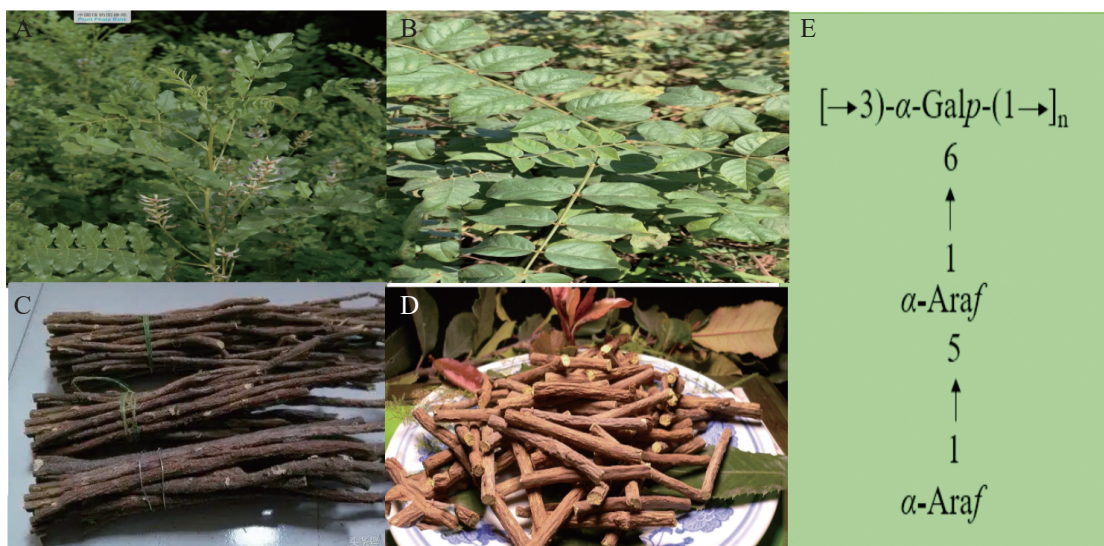


Figure 1 *G. uralensis* (A) the whole grass, (B) the leaves, (C) the rhizomes, (D) Chinese patent medicine, and (E) structure (GiP-3)^[35].

of great importance. However, existing studies lack a systematic and comprehensive review of GUPs, which limits the depth of understanding in their development and application within the pharmaceutical industry. This article provides a comprehensive analysis of the research progress on GUPs, focusing on detailed summaries from several aspects, including extraction methods, structural features, biological activities, conformational relationships, and potential applications. This summary not only helps to clarify the biological functions and application potential of GUPs but also provides a solid theoretical basis for the effective utilization of their resources. Through a systematic review, we hope to promote in-depth research on GUPs in drug development and other related fields, thereby advancing their application process in new drug development.

2 Extraction, isolation, and purification

To obtain GUPs with a high yield and significant bioactivity, we summaries the existing literature on extraction, isolation, and purification. Table 1 summarizes the sources, extraction processes, monosaccharide compositions, and structural characterization methods of GUPs. Figure 2 represents a network visualization plot of all keywords co-occurring in GUPs publications. A schematic representation of the extraction and purification of GUPs is shown in Fig. 3.

2.1 Extraction

The extraction methods of GUPs include hot water extraction^[36], acid extraction^[34], ultrasonic-assisted extraction^[37], microwave-assisted extraction^[38], supercritical carbon dioxide extraction^[39], enzyme extraction^[40], ultrasonic-assisted deep eutectic solvent extraction^[41]. Before extraction, organic solvents such as ethyl acetate, ethanol, petroleum ether or a combination of ethanol and petroleum were used for pretreatment to remove surface lipids^[42].

GUPs are neutral or acidic^[43], so water, acid and other solvents are commonly used for extraction. Guo *et al.*^[44] obtained crude polysaccharides from *G. uralensis* (GP)-HA and GP-LA by hot acid extraction (HA) and low temperature acid extraction (LA), respectively, with yield of 3.76% and 2.80%. Gao *et al.*^[24] employed acidic solutions with varying pH values (1.3, 5.0 mol/L) to extract GUPs from *Glycyrrhiza* residue. The results demonstrated that

the yield of P1 was the highest (3.12%), followed by P3 (2.08%) and P5 (1.83%). The pH value exhibited a pronounced impact on the yield of GUPs from acidic solutions. This phenomenon may be attributed to the fact that as the pH value decreases, the damage to the cell wall increases, leading to a greater degree of polysaccharide precipitation. A water-soluble polysaccharide GP was isolated from *G. uralensis* Fisch by Cheng *et al.*^[45] by hot water extraction, and the crude polysaccharide yield was about 4.25%. Aipire *et al.*^[46] separated GUPS-C by hot water extraction-ethanol precipitation method, and the yield was about 2.73%. Polysaccharides were extracted from the desiccated seeds of *G. uralensis* using a range of organic solvents, including *n*-hexane, chloroform, acetone and 95% ethanol. Following the drying of the extract, water (1:150) was extracted at 100 °C for 2 h in order to extract the water-soluble polysaccharides. The polysaccharide yields of GGu-1 and GGu-2 were 2.690 and 2.836 g, respectively, representing 5.38% and 5.67% of the seed weight, respectively^[23]. Guo *et al.*^[31] used the hot water extraction method to extract GUP. Using the yield of GUP as the index, the extraction process of *Glycyrrhiza* crude polysaccharide was optimized by single factor and orthogonal experiment. The optimum extraction process was as follows: extraction time 3.5 h, extraction temperature 90 °C, solid-liquid ratio 1:20, extraction 2 times. The yield of GUP under this extraction process was 5.67%. The optimal extraction process increased the yield of GUP and saved the extraction cost. Lian *et al.*^[47] decocted 3 times with 9 times the amount of distilled water, respectively, decocted for 4, 2, 1 h, and then purified by Sephadex G, freeze-dried to obtain purified GUP, the yield was 16%. Furthermore, the extraction conditions exert a certain influence on the yield of GUPs. The extraction process of GUPs can be optimized through the utilization of an orthogonal experiment and a response surface method, thereby enhancing the yield of GUPs. Chen *et al.*^[43] conducted a process optimization of the GUP extraction procedure. The response surface method and Box-Behnken design (BBD) were employed to optimize the extraction temperature, material-water ratio and extraction time in order to maximize the yield of GUP. The findings indicated that the highest yield of GUP-II (16.41%) was achieved when the extraction temperature was set at 99 °C, the material-water ratio was 15:1, and the extraction time was 2 h. Solvent extraction methods such as water and acid have the advantages of simple operation and low cost, but there are some problems such as long

Table 1 Structural characteristics of the purified polysaccharide from *G. uralensis*.

No.	Polysaccharides fraction	Extraction, isolation, and purification procedure	Source	Molecular weight (Mw) (kDa)	Monosaccharide composition	Structural features	Structural characterization method	Reference
1	GP-HA	HA (85 °C, 2 h), DEAE-cellulose Sephacryl S-200	Whole herb	1.291×10^2	Fuc:Rha:Ara:Gal:Glc: Man:Xyl:GalA = 5.27:5.21:5.15:5.10:4.89:4.85: 4.54:4.39	$\rightarrow 4$)- β -D-Glcp-(1 $\rightarrow$, $\rightarrow 6$)- β -D-Galp-(1 $\rightarrow$, - α -D-Glcp (1 $\rightarrow$, $\rightarrow 3,4$)- α -D-Glcp-(1 $\rightarrow$, $\rightarrow 2,4$)- α -D-Glcp-(1 $\rightarrow$, $\rightarrow 4,6$)-D-Glcp-(1 $\rightarrow$	MS, UV, FT-IR, NMR, SEM, AFM, XRD	[44]
2	GP-LA	LA (28 °C, 8 h), DEAE-cellulose Sephacryl S-200	Whole herb	3.023×10^2	Fuc:Rha:Ara:Gal:Glc: Man:Xyl:GalA = 5.29:5.14:5.12:5.05:4.99:4.87: 4.54:4.30	β -L-Araf-(1 $\rightarrow$, $\rightarrow 5$)- β -L-Araf-(1 $\rightarrow$, $\rightarrow 4$)- β -D-Xylp-(1 $\rightarrow$, β -D-Glcp-(1 $\rightarrow$, $\rightarrow 3,4$)- α -D-Glcp-(1 $\rightarrow$, $\rightarrow 4,6$)- α -D-Glcp-(1 $\rightarrow$	MS, UV, FT-IR, NMR, SEM, AFM, XRD	[44]
3	GUPS	Petroleum ether, ethanol and hot water extraction (4 h), DEAE-cellulose Petroleum ether,	Roots	29.1	NA	NA	GC-MS	[67]
4	GUPS-I	ethanol and hot water extraction (4 h), DEAE-cellulose Petroleum ether,	Roots	1.06	Rha:Xyl:Glc = 2.55:1.9:30.03	NA	GC-MS, HPGPC, SEM, FT-IR, NMR	[46]
5	GUPS-II	ethanol and hot water extraction (4 h), DEAE-cellulose Petroleum ether,	Roots	29.1	Rha:Ara:Man:Glc:Gal = 1:13.87:1.59:16.76:15.72	α -L-Araf-(1-, 3)- α -L-Rha-(1-, 3)- α -D-Galp-(1-, α -D-Xylp-(1-, and -4)- α -D-Glcp-(1- residues	GC-MS, HPGPC, SEM, FT-IR, NMR	[46]
6	GUPS-III	ethanol and hot water extraction (4 h), DEAE-cellulose Ultrasonic assisted extraction (343.15 K, 600 W, m/V = 1:15, 60 min),	Roots	14.9	Ara:Man:Glc:Gal = 1:5.33:1.91:5.97	α -L-Araf-(1-, 3)- α -L-Rha-(1- residues	GC-MS, HPGPC, SEM, FT-IR, NMR	[46]
7	GP	DEAE-cellulose Hot water extraction (99 °C, m/V = 15:1, 2 h), DEAE-cellulose Hot water extraction (3 times, 4 h),	Whole herb	NA	NA	NA	NA	[49]
8	GUP-II	Sephadex G Hot water extraction (m/V = 1:9, 80 °C, for 1 h), DEAE-52,	Roots	NA	Rha, Ara, Gal, and Glu	NA	HPAEC	[43]
9	GUP	Sephadex G Hot water extraction (m/V = 1:9, 80 °C, for 1 h), DEAE-52,	Whole herb	38.032	NA	NA	NA	[47]
10	GUPS-1	Sephadex G Hot water extraction (m/V = 1:9, 80 °C, for 1 h), DEAE-52,	Roots	10.160×10^{-3}	Glu:Gal:Ara = 23.4:25.18:8.32	NA	FT-IR, GC-MS, HPGPC	[22]
11	GUPS-2	Sephadex G Hot water extraction (m/V = 1:9, 80 °C, for 1 h), DEAE-52,	Roots	11.680×10^{-3}	Glu:Gal:Ara:Rha:Man = 14:25.67:17.54:0.61:0.68	NA	FT-IR, GC-MS, HPGPC	[22]
12	GUPS-3	Sephadex G Hot water extraction (m/V = 1:9, 80 °C, for 1 h), DEAE-52,	Roots	13.360×10^{-3}	Glu:Gal:Ara:Rha:Man = 1.13:22.04:31.44:3.36:1.95	NA	FT-IR, GC-MS, HPGPC	[22]
13	GGu-1	Hot water extraction (m/V = 1:150, 100 °C, for 2 h), KU-2-8 cation	Seeds	1,379	Gal:Man = 1:1.52	The major backbone is comprised of (1 $\rightarrow$ 4) bound β -D mannopyranose residues substituted at C-6 with single α -D-galactopyranose residues	HPTLC, IR, NMR, GC-MS	[23]
14	GGu-2	Hot water extraction (m/V = 1:150, 100 °C, for 2 h), KU-2-8 cation	Seeds	877	Gal:Man = 1:1.50	The major backbone is comprised of (1 $\rightarrow$ 4) bound β -D mannopyranose residues substituted at C-6 with single α -D-galactopyranose residues	HPTLC, IR, NMR, GC-MS	[23]
15	GP	Hot water extraction (m/V = 1:10, 90–95 °C, for 3–4 h), DEAE-52, Sephacryl S	Roots	11	NA	α -(1–4) linked D-glucana	FT-IR, GC-MS	[45]
16	GP	Hot water extraction (m/V = 1:10, 90–95 °C, for 3–4 h), DEAE-52, Sephacryl S	Roots	10	NA	The main chain of GP was composed of β -1,3-linked D-Gal residues and/or α -1,4-linked D-	FT-IR, GC-MS	[63]

(Continued)

No.	Polysaccharides fraction	Extraction, isolation, and purification procedure	Source	Molecular weight (Mw) (kDa)	Monosaccharide composition	Structural features	Structural characterization method	Reference
17	P1	Acid extraction (pH: 1 mol/L, 55 °C, for 3 h), high performance size exclusion chromatography	Whole herb	413	Fuc:Rha:Ara:Gal:Glc:GalA = 1.77:6.93:17.54:8.58:62.19:3.00	→4)-β-D-Glcp-(1→	SEC-MALLS, UV, FT-IR, NMR	[24]
18	P3	Acid extraction (pH: 3 mol/L, 55 °C, for 3 h), high performance size exclusion chromatography	Whole herb	682	Fuc:Rha:Ara:Gal:Glc:GalA = 1.31:7.10:10.7:35.69:78.4:4.45	→4)-β-D-Glcp-(1→	SEC-MALLS, UV, FT-IR, NMR	[24]
19	P5	Acid extraction (pH: 5 mol/L, 55 °C, for 3 h), high performance size exclusion chromatography	Whole herb	572	Fuc:Rha:Ara:Gal:Glc:GalA = 1.66:7.52:11.85:8.98:65.59:4.39	→4)-β-D-Glcp-(1→	SEC-MALLS, UV, FT-IR, NMR	[24]
20	A-GUPs	Ultrasonic assisted extraction (500 W, m/V = 20:1, 60 min, 60 °C), Sepharose CL-6B	Whole herb	212	Ara:Rib:Xyl:Gal:Glu: Man:GalA = 11.30:11.71:9.51:13.55:16.56:23.91:13.47	NA	GC-MS	[50]
21	W-GUPs	Ultrasonic assisted extraction (500 W, m/V = 20:1, 60 min, 60 °C), Sepharose CL-6B	Whole herb	34.1	Ara:Rib:Xyl:GluA:Gal:Glu: Man:GalA = 7.93:9.13:2.12:2.33:12.83:27.28:15.10:23.28	NA	GC-MS	[20]
22	GUP-1	Hot water extraction (m/V = 1:20, 90 °C, for 3.5 h), DEAE-52, Sephacryl G	Whole herb	294.373	Man:Rha:GalA:Glc:Gal:Ara = 3.3:2.3:17.0:1.3:19.0:37.8	α-Glucopyranose, triple helical structure, containing 1→6 or 1→, 1→3, 1→2 or 1→4 glycosidic bonds	UV, FT-IR, NMR, HPGFC	[31]
23	GUP-2	Hot water extraction (m/V = 1:20, 90 °C, for 3.5 h), DEAE-52, Sephacryl G	Whole herb	17.4	Man:Rha:Glc:Gal:Ara = 0.7:6.0:213.5:4.8:15.1	α-Glucopyranose, with 1→6 or 1→, 1→3, 1→2 or 1→4 glycosidic bonds	UV, FT-IR, NMR, HPGFC	[68]
24	GiP-3	Hot water extraction (m/V = 1:20, 90 °C, for 3.5 h), Sepharose CL	Whole herb	21	Rha:Ara:Gal = 0.1:1.0:18.0	(Main chain →3)-α-Galp-(1→, branched chain →5)-α-Araf-(1→ and to a lesser extent 2,4)-α-Rhap-(1→; the branched chain is located at the O-6 position of the α-Galp sugar residue)	HPGPC, IR, NMR	[35]
24	GiP-B1	Hot water extraction (m/V = 1:20, 90 °C, for 3.5 h), Sepharose CL, Sephadex G	Whole herb	> 200	Rha:Ara:Gal:GalA = 1.0:7.4:2.6:2.5	(Main chain →4)-GalpA-(1→and→2)-Rhap-(1→, branched→5)-Araf-(1→and→3)-Galp-(1→, branched at the O-4 position of the Rhap residue)	HPGPC, IR, GC, GC-MS, NMR	[62]
25	GiP-C1	Hot water extraction (m/V = 1:20, 90 °C, for 3.5 h), Sepharose CL, Sephadex G	Whole herb	> 200	Rha:Ara:Gal:GalA = 1.0:2.0:0.9:1.7	(Main chain →4)-GalpA-(1→and→2)-Rhap-(1→, branched →5)-Araf-(1→ and →3)-Galp-(1→, branched at the O-4 position of the Rhap residue)	HPGPC, IR, GC, GC-MS, NMR	[69]
26	GiP-D1	Hot water extraction (m/V = 1:20, 90 °C, for 3.5 h), Sepharose CL, Sephadex G	Whole herb	> 200	Rha:Ara:Gal:GalA = 1.0:2.1:2.9:4.4	(Main chain →4)-GalpA-(1→and→2)-Rhap-(1→, branched →5)-Araf-(1→ and →3)-Galp-(1→, branched at the O-4 position of the Rhap residue)	HPGPC, IR, GC, GC-MS, NMR	[61]

Note: NA, not mentioned; GiP-3, *Glycyrrhiza inflata* polysaccharides; GiP-B1, GiP-C1, GiP-D1, *Glycyrrhiza inflata* polysaccharides; UV, Ultraviolet; HPGPC, High performance gel permeation chromatography; UPLC, Ultra performance liquid chromatography; SEC-MALLS, Size exclusion chromatography with multiple angle laser light scattering.

extraction time, high extraction temperature, large amount of solvent and low yield.

The ultrasound-assisted extraction method primarily utilises the mechanical effect and cavitation of ultrasound to disrupt biological

tissues, including the cell wall and cell membrane. This process enhances mass transfer efficiency within cells, facilitating the release and extraction of plant polysaccharides^[48]. It has the advantages of high efficiency, energy saving and fast operation^[49].

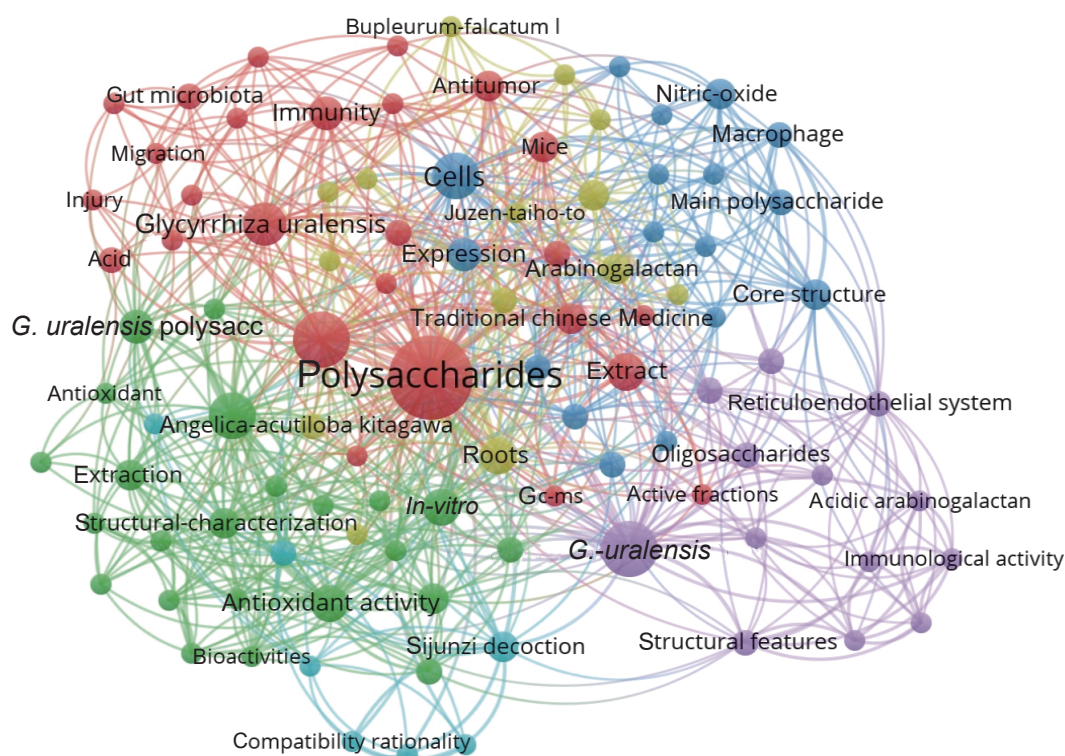


Figure 2 Network visualization map of all keywords co-occurrence for GUPs publications.

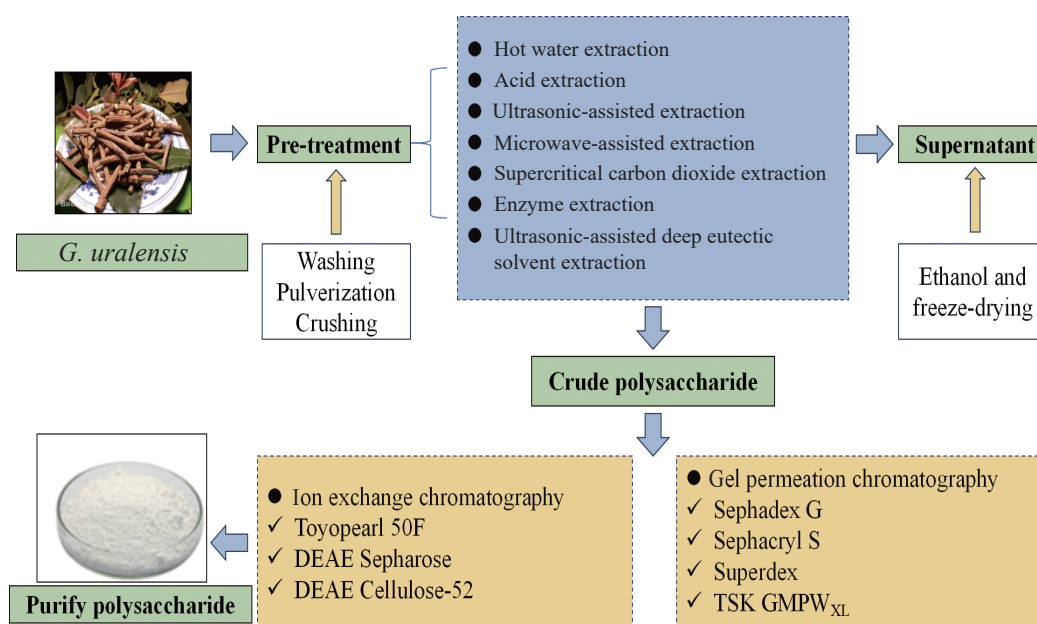


Figure 3 Schematic diagram of extraction, and purification of polysaccharides isolated from *G. uralensis*.

Compared with the traditional solvent extraction method, ultrasonic extraction can shorten the extraction time and reduce the extraction temperature. He *et al.*^[50] optimized the conditions of ultrasound-assisted extraction of GUPs. The optimal extraction conditions were determined by single factor experiments: when the ultrasonic power was set at 500 W, the liquid-solid ratio was 20:1 (mL/g), the ultrasonic time was 60 min, the temperature was 60 °C, and the yield was 2.26%. Wang *et al.*^[49] found that the highest yield of GP was 3.53% under the conditions of 343.15 K, ultrasonic power 600 W and solid-liquid ratio 1:15 for 60 min. In their study, Sun *et al.*^[41] employed the ultrasound-assisted deep

eutectic solvent method to investigate the impact of various factors, including deep eutectic solvent type, molar ratio, water content, extraction temperature, solid-liquid ratio, ultrasonic time, and ultrasonic power, on the yield of GUPs. They also optimized the extraction of GUPs through the use of a response surface experimental design. The results demonstrated that the optimal conditions for polysaccharide extraction were a water content of 40%, a molar ratio of 1:3 for the choline chloride-isopropanol system, an extraction temperature of 39 °C, a ratio of 1:50 (g/mL) between the material and liquid, an ultrasonic time of 30 min, and an ultrasonic power of 250 W. The resulting yield of

polysaccharide was 8.31%.

Microwave-assisted extraction can shorten the extraction time, improve the yield and save the solvent^[51]. Zhang *et al.*^[51] used microwave method to extract GUPs. On the basis of single factor test, orthogonal test was carried out on microwave power, microwave treatment time and solid-liquid ratio. The results showed that the order of factors affecting the yield of GUPs was microwave treatment time > microwave power > solid-liquid ratio. The optimum conditions for microwave extraction of GUPs were as follows: microwave power 500 W, microwave treatment time 4 min, solid-liquid ratio 1:30 (g/mL). Microwave extraction of GUPs plays an important role in shortening the extraction time, improving the yield and reducing the cost. In the past decade, supercritical carbon dioxide (CO₂) extraction technology has emerged as a novel extraction technology. The process is known as "green separation" due to its numerous advantages, including non-toxicity, the absence of solvent residue, low treatment temperature, strong selectivity, non-flammability and safety. A quadratic polynomial mathematical model for the yield of GUPS by supercritical CO₂ extraction was established by Li *et al.*^[52] using response surface analysis. The impact of extraction temperature (60–70 °C), pressure (30–50 MPa) and time (70–90 min) on the yield of GUPS was examined. The model enabled the optimization of the process parameters. The yield of GUPS was identified as the index, and the optimum conditions for supercritical CO₂ extraction were determined to be as follows: temperature 62.6 °C, pressure 37.7 MPa, 82.9 min. In accordance with the aforementioned conditions, the yield of GUPS reached a maximum of 7.34%. As an efficient and green extraction method, enzymatic extraction has attracted more and more attention^[40]. The process employs enzymes as catalysts to facilitate the release of active ingredients within cells by disrupting the plant cell walls. The specificity and selectivity of enzymes enable the reaction to occur under mild conditions, thereby maintaining the biological potential of bioactive substances. In their study, Li *et al.*^[40] selected cellulase and pectinase for the extraction of licorice residue polysaccharides (LRPs) under the conditions of the experiment that had been optimized. They then compared the yields of LRPs extracted by the two enzymes. The results demonstrated that the yield of LRPs extracted by pectinase and cellulase was 8.43% and 10.71%, respectively, indicating that the yield of cellulase-extracted polysaccharides was higher.

Various auxiliary extraction methods increase the polysaccharide yield while saving time and cost. Hot water extraction is simpler to operate and has greater process applicability compared to enzymatic extraction, acid extraction, and microwave extraction. However, there is currently a lack of effective, energy-saving, safe, and environmentally friendly preparation methods for large-scale industrial production. Therefore, it is of paramount importance to establish a standardized extraction process and reliable quality control methods for the subsequent research and process production of GUPs through an in-depth study of the extraction, and purification methods for GUPs.

2.2 Isolation and purification

Regardless of the method used to extract polysaccharides, crude polysaccharides usually contain a large number of impurities, such as pigments, proteins and inorganic salts, which may have an impact on subsequent structural characterization and activity studies^[53]. Consequently, supplementary separation procedures are necessary to obtain a well-structured purified polysaccharide. The

conventional approach involves the extraction of polysaccharides through ethanol precipitation, followed by additional processes such as degreasing, decolorization, and deproteinization, which facilitate the separation of diverse polysaccharide components^[54]. Next, the purified polysaccharide was purified by column chromatography, and finally purified by concentration, dialysis and freeze-drying^[55]. At present, GUPs are mainly deproteinized by Sevage method and trichloroacetic acid method^[56]. The main techniques of column chromatography include gel permeation chromatography (such as Sephadex G, Sephacryl S, Superdex and TSK GMPW_{XL}) and ion exchange chromatography (such as DEAE Sepharose, DEAE Cellulose-52 and Toyopearl 50F)^[57]. These methods can effectively remove impurities, improve the purity of polysaccharides, and lay the foundation for subsequent research.

Guo *et al.*^[44] employed DEAE-52 column chromatography and Sephacryl S-200 gel filtration column chromatography to isolate and purify polysaccharides GP-HA and GP-LA. The resulting purified polysaccharides were found to be devoid of protein. Aipire *et al.*^[46] further separated GUPS-C by DEAE-52 anion exchange column and eluted with different concentrations of sodium chloride. GUPS-I, GUPS-II and GUPS-III were obtained. After purification, GUPS-I and GUPS-II contained no protein, while GUPS-C and GUPS-III contained 4% and 21% proteins, respectively. Zhang *et al.*^[22] successfully isolated and purified three water-soluble polysaccharides (GUPS-1, GUPS-2 and GUPS-3) from the root of *G. uralensis* by employing DEAE-52 and Sephadex G-100 column chromatography. The protein content of the crude CGUPs was found to be 6.1%, whereas no protein was detected in GUPS-1, GUPS-2 and GUPS-3. This suggests that the CGUPs proteins isolated by DEAE-52 and Sephadex G-100 column chromatography have been entirely removed. Guo *et al.*^[31] used the Sevage method to remove proteins in GUPs and the dialysis method to remove small molecular impurities. The protein content in GUP obtained by hot water extraction was 4.8%, and the protein content was 0.58% after protein removal, indicating that this method can efficiently remove the protein in the polysaccharide. Guo *et al.*^[31] purified the crude polysaccharide of GUP by DEAE-52 cellulose column chromatography and Sephadex G-200 gel column chromatography, thereby obtaining two polysaccharide components, GUP-1 and GUP-2, which were subsequently determined by high-performance gel filtration chromatography. The results of the elemental analysis demonstrated that GUP-1 and GUP-2 were devoid of the S element and exhibited a minimal N element proportion, indicating that the two components were largely devoid of protein. The total sugar content of GUP-1 was found to be 98.58%, while that of GUP-2 was 98.39%. The protein content of both components was determined to be 0.13% and 0.09%, respectively.

3 Structural characteristics

The biological activity of polysaccharides mainly depends on their chemical structure, making the characterization of polysaccharide structures particularly important^[58]. The chemical structure of GUPs determines their biological activity characteristics in multiple aspects. An in-depth study of the relationship between these structures and their activity will help optimize the application of GUPs in drug development and nutritional health products^[59]. GUPs have been the subject of considerable interest due to the intricate structures that they exhibit, which may be closely associated with the processes

involved in their sourcing, extraction, separation and purification. The structural attributes of polysaccharides are contingent upon a number of factors, including their monosaccharide composition, molecular weight, the types of glycosidic bonds present and the structural conformations that they adopt^[60]. The analysis of polysaccharide structures can be broadly classified into two main categories: chemical analysis and instrumental analysis. Each of these approaches has its own set of advantages and disadvantages. Chemical analysis is relatively straightforward in determining the structural characteristics of polysaccharides, allowing for rapid assessments based on observations of colour changes, precipitates, and other phenomena. Additionally, it is a cost-effective and relatively simple process. However, the results may be influenced by the operator's experience and skill level, which could introduce a degree of subjectivity into the analysis. In contrast, instrumental analysis offers a number of advantages, including high sensitivity, high accuracy, and a high degree of automation. The resulting quantitative data is particularly useful for more accurately analysing polysaccharide structures^[61]. However, the cost of equipment and maintenance for instrumental analysis is relatively high, and the operation process is also more complex.

Due to the complexity of polysaccharide structures and technical limitations, current structural analysis of GUPs is largely restricted to primary structures, which include determining the monosaccharide composition and molar ratio, average molecular weight, glycosidic bond configuration, and the positions of glycosidic bond connections. Detailed information on the structure is summarized in Table 1.

3.1 Monosaccharide composition, and linkages

As shown in Table 1, GUPs are a type of polysaccharide, and the monosaccharide components include rhamnose, arabinose, xylose, mannose, fucose, ribose, glucose, galactose, glucuronic acid, and galacturonic acid. Although there are certain variations in the monosaccharide compositions from different sources, mannose, rhamnose, glucose, galactose, and arabinose are predominant in all cases. However, the molar ratio of the monosaccharides obtained varies because of different analytical methods.

The results of MS and nuclear magnetic resonance (NMR) analyses indicate that both GP-HA and GP-LA are heteropolysaccharides comprising Fuc, Rha, Ara, Gal, Glc, Man, Xyl, and Gala. Glc is identified as the predominant monosaccharide in GUPs. The molar ratios of GP-HA are 5.27:5.21:5.15:5.10:4.89:4.85:4.54:4.39, while the molar ratios of GP-LA are 5.29:5.14:5.12:5.05:4.99. The molar ratios of GP-HA are 4.87:4.54:4.30^[44]. The monosaccharide compositions of GUPS-I, GUPS-II, and GUPS-III were analyzed using GC-MS, revealing that GUPS-II and GUPS-III have similar monosaccharide compositions, while GUPS-I is distinctly different. GUPS-I consists of Rha, Xyl, and Glc in a molar ratio of 2.55:1.9:30.03; GUPS-II comprises Rha, Ara, Man, Glc, and Gal with a molar ratio of 1:13.87:1.59:16.76:15.72; GUPS-III is made up of Ara, Man, Glc, and Gal in a molar ratio of 1:5.33:1.91:5.97^[46]. CGUPS, GUPS-1, GUPS-2, and GUPS-3 are all heteropolysaccharides, comprising the monosaccharides Glc, Gal, and Ara. Rha and Man are present in CGUPS, GUPS-2, and GUPS-3, whereas they are undetected in GUPS-1. The molar ratio of monosaccharide composition in GUPS-1 is Glu:Gal:Ara = 23.4:25.18:8.32. In GUPS-2, the molar ratio of monosaccharide composition is Glu:Gal:Ara:Rha:Man = 14:25.67:17.54:0.61:0.68. For GUPS-3, the molar ratio is Glu:Gal:Ara:Rha:Man = 1.13:22.04:31.44:3.36:1.95.

Analysis of the monosaccharide compositions of GGU-1 and GGU-2 using GC-MS indicates that the ratios of Gal to Man are 1:1.52 and 1:1.50, respectively^[23]. The monosaccharide compositions of P1, P3, and P5 were determined using ion chromatography, and the results indicated that the monosaccharide compositions of P1, P3, and P5 are similar. The monosaccharide composition and molar ratio of P1 are Fuc:Rha:Ara:Gal:Glc:GalA = 1.77:6.93:17.54:8.58:62.19:3.00; the monosaccharide composition and molar ratio of P2 are Fuc:Rha:Ara:Gal:Glc:GalA = 1.31:7.10:10.7:35.69:78.4:4.45; the monosaccharide composition and molar ratio of P3 are Fuc:Rha:Ara:Gal:Glc:GalA = 1.66:7.52:11.85:8.98:65.59:4.39^[24]. The monosaccharide component analysis of artificial *Glycyrrhiza* polysaccharide A-GUPs and wild *Glycyrrhiza* polysaccharide W-GUPs was conducted using GC-MS. The results revealed that the main monosaccharide components of A-GUPs and W-GUPs are Man and Glc, with additional presence of Ara, ribose, Xyl, Gal, GlcA. This indicates that both A-GUPs and W-GUPs are acidic polysaccharides^[50]. Pre-column derivatization was employed to determine the monosaccharide compositions of GUP-1 and GUP-2, showing that the compositions and molar ratios of GUP-1 are: GlcA:::Ara = 3.32:2.26:17.03:1.26:18.96:37.83. GUP-2 consists of Man, Rha, Glc, Gal, and Ara, with molar ratios of ::::Ara = 0.75:5.98:213.54:4.76:15.51. GUP-1 mainly comprises GlcA, Gal, and Ara, whereas GUP-2 is primarily composed of Glc, indicating a difference in monosaccharide composition between GUP-1 and GUP-2^[31]. GiP-3 consists of Ara, Rha, and Gal in a 1:0.12:18 ratios, forming a neutral arabinogalactan^[35]. GiP-B1, GiP-C1, and GiP-D1 are acidic heteropolysaccharides composed of Ara, Rha, Gal, and GlcA in varying molar ratios. The molar ratio of GiP-B1 is Rha:Ara:Gal:GalA = 1.0:7.4:2.6:2.5. The molar ratio of GiP-C1 is Rha:Ara:Gal:GalA = 1.0:2.0:0.9:1.7. The molar ratio of GiP-D1 is Rha:Ara:Gal:GalA = 1.0:2.1:2.9:4.4^[62].

3.2 Average molecular weights

A total of more than 40 polysaccharides have been isolated from *G. uralensis*, with molecular weights ranging from 1.06 to 1 379 kDa. The relative molecular weights of GP-LA and GP-HA are 3.02×10^2 and 1.29×10^2 kDa, respectively^[44]. The relative molecular weights of GUPS-I, GUPS-II, and GUPS-III are 1.06, 29.1, and 14.9 kDa, respectively^[46]. The relative molecular weights of GUPS-1, GUPS-2, and GUPS-3 are 10.160, 11.680, and 13.360 Da, with Glc contents of 23.4%, 14%, and 1.13%, respectively^[22]. The relative molecular weights of GGU-1 and GGU-2 are 1 379 and 877 kDa, respectively^[23]. The relative molecular weight of GP is approximately 11 kDa^[45], and another measurement indicates it to be about 10 kDa^[63]. The molecular weights of monosaccharides were determined using high-performance size exclusion chromatography. The molecular weight of P1 was found to be 413 kDa, while that of P3 was 682 kDa and that of P5 was 572 kDa. P1 exhibited the smallest molecular weight, which is likely due to severe hydrolysis of glycosidic bonds at low pH, resulting in a smaller molecular weight^[24]. The relative molecular weights of the artificial *Glycyrrhiza* polysaccharide A-GUPs and the wild *Glycyrrhiza* polysaccharide W-GUPs are 212 kDa and 34.1 kDa, respectively^[50]. Guo *et al.*^[31] calculated the molecular weights of GUP-1 and GUP-2 using standard curves, finding that GUP-1 has a larger molecular weight of 294.373 kDa, indicating it has polymerized more monosaccharides. In contrast, GUP-2 has a smaller molecular weight of 17.416 kDa. Palida *et al.*^[35] obtained a homogeneous polysaccharide component GiP-3 with an average relative molecular weight of 21 kDa.

3.3 Chemical structures

A review of the existing literature reveals that the structural characterisation of GUPs typically involves Fourier transform infrared spectroscopy (FT-IR) and methylation analysis, which are combined with NMR analysis. This is summarised in detail in Table 1. FT-IR and NMR analysis results indicate that the main chain of GP-HA and GP-LA were $\rightarrow 4$)- β -D-Glcp-(1 $\rightarrow$. For GP-HA, the side chain was mainly composed of $\rightarrow 4$)- β -D-Glcp-(1 $\rightarrow$, $\rightarrow 6$)- β -D-Galp-(1 $\rightarrow$, α -D-Glcp-(1 $\rightarrow$, $\rightarrow 3,4$)- α -D-Glcp-(1 $\rightarrow$, $\rightarrow 2,4$)- α -D-Glcp-(1 $\rightarrow$, $\rightarrow 4,6$)-D-Glcp-(1 $\rightarrow$, and for GP-LA, the side chain was mainly composed of β -L-Araf-(1 $\rightarrow$, $\rightarrow 5$)- β -L-Araf-(1 $\rightarrow$, $\rightarrow 4$)- β -D-Xylp-(1 $\rightarrow$, β -D-Glcp-(1 $\rightarrow$, $\rightarrow 3,4$)- α -D-Glcp-(1 $\rightarrow$, $\rightarrow 4,6$)- α -D-Glcp-(1 $\rightarrow$, linked units, respectively^[44]. The FT-IR and NMR results indicate that GUPS-II and GUPS-III are sheet-like in structure, with a smooth surface, and contain both α - and β -glycosidic bonds. In contrast, GUPS-I is granular in nature and contains only α -glycosidic bonds^[46]. According to infrared spectroscopy (IR) and NMR spectroscopic data and methylation analysis, the polymeric chains of GGu-1 and GGu-2 are the major backbone is comprised of (1 $\rightarrow$ 4) bound β -D mannopyranose residues substituted at C-6 with single α -D-galactopyranose residues^[23]. IR and NMR validated those three polysaccharides (P1, P2, P3) may primarily consist of the linking unit $\rightarrow 4$)- β -D-Glcp-(1 $\rightarrow$ ^[24]. Both GUP-1 and GUP-2 contain a combination of 1 $\rightarrow$ 6 and 1 $\rightarrow$ glycosidic bonds, in addition to 1 $\rightarrow$ 3 glycosidic bonds, and 1 $\rightarrow$ 2 and 1 $\rightarrow$ 4 glycosidic bonds. However, the proportions of these bonds differ between the two. GUP-2 has a higher proportion of 1 $\rightarrow$ 3 glycosidic bonds. GUP-1 is characterized by a triple helix structure, which is not present in GUP-2^[31]. The backbone structure of GiP-3 consists of $\rightarrow 3$) α -Galp (1 $\rightarrow$ residues, with $\rightarrow 5$) α -Araf (1 $\rightarrow$ branches and a small amount of $\rightarrow 2,4$) α -Rhap (1 $\rightarrow$ branches located at the O-6 position of the α -Galp residue^[35]. Structural analysis showed that the backbones of GiP-B1, GiP-C1, and GiP-D1 are composed of 1,4-GalpA and 1,2-Rhap residues, with branches formed by 1,5-linked Araf and 1,3-linked Galp, and the branching points located at the O-4 position of the Rhap residues^[62].

3.4 Conformational features

Polysaccharides display a range of chain conformations, including random coils, single helices, double helices, worm-like structures, triple helices, aggregates, and spherical conformations. These variations are attributed to differences in linkage types, substituents, branching structures, branching degrees, and molecular interactions. Of these, triple-helical polysaccharides have attracted considerable attention^[64,65], they possess various biological activities, excellent safety, and biodegradability, and are considered potential health products or natural drugs^[66].

The Congo red experiments provided confirmation of the existence of GP-HA and GP-LA triple helical structures. GP-HA was observed to exhibit a single branch structure, while GP-LA displayed a cross-linked network structure. Scanning electron microscopy (SEM) revealed the presence of a considerable number of irregularly shaped, spherical particles with smooth surfaces in both types of GUPs. X-ray diffraction (XRD) analysis indicated that GP-LA has both crystalline and amorphous structures, whereas GP-HA lacks a crystalline structure^[44]. Observations of GUPS-I, GUPS-II, and GUPS-III under SEM at 2,000 $\times$ and 10,000 $\times$ magnification showed that GUPS-I is granular, while GUPS-II and GUPS-III display a flaky structure without a porous configuration. Furthermore, at 10,000 $\times$ magnification, the surfaces of GUPS-II and GUPS-III are smooth,

suggesting the potential formation of amorphous structures^[46]. SEM images of GP particles at varying magnifications illustrate a flake-like morphology with the presence of micropores. At 1,000 $\times$ magnification, the examination of tiny bubble-like pores and thin fragments of GP in aqueous solutions reveals the existence of two-dimensional and three-dimensional structures, indicating a nearly uniform surface with some protrusions. The high concentration of glycoproteins results in enhanced hydrogen bonding interactions between glycoprotein molecules and hydroxyl groups, which in turn leads to the formation of a tighter polysaccharide aggregation of glycoprotein molecules, thereby creating a membrane-like structure^[49]. The Congo red experiment suggests that P1 and P5 may possess triple helical conformations; SEM showed that P3 and P5 have similar morphological structures, characterized by numerous spherical particles aggregating into a rough net-like structure, whereas P1 exhibits a flaky structure with an uneven surface and porous structure. Atomic force microscopy (AFM) reveals that the polysaccharides extracted by three different methods from *G. uralensis* present mutually branched and entangled structures, with microscopic structures consisting of a small number of smaller spherical aggregates. XRD analysis indicates that P1 possesses both crystalline and amorphous structures, while P3 and P5 lack crystalline structures^[24].

As a result of the gradual and in-depth study of the conformational characteristics of GUPs, we have gained a deeper understanding of their complex diversity. Nevertheless, the detailed structural analysis of GUPs remains incomplete, particularly with regard to the identification of key structural features and the characterization of spatial conformations. It is recommended that future research concentrate on these two areas, with the objective of elucidating the structural and functional changes that occur following polysaccharide modification. This will facilitate the identification of potential biological functions and applications. In order to facilitate the application and development of GUPs in fields such as food and medicine, it is essential to combine the strengths of related disciplines.

4 Pharmacological properties

Recent studies indicate that GUPs possess various significant pharmacological activities, including anti-inflammatory^[20], immunomodulatory^[69], antioxidant^[43], anti-tumor^[68], anti-diabetic^[29], antibacterial, and other biological activities^[31]. Many studies have systematically investigated the biological activity of GUPs, and the specific studies are summarized below. A detailed summary is presented in Table 2, and the schematic representation of main bioactivities of GUPs are also presented in Figs. 4 and 5, respectively.

4.1 Anti-inflammatory activity

Inflammation is the body's defense response to local injury, mainly triggered by inflammatory factors. This process involves a series of complex pathological mechanisms^[70,71]. *In vitro* and *in vivo* studies have demonstrated that GUPs display substantial anti-inflammatory activity, which has significant potential for application in the treatment of diseases such as sepsis and ulcerative colitis^[72].

The objective of the study conducted by Hui *et al.*^[20] was to investigate the anti-inflammatory effects and underlying mechanisms of GUP. In the study, GUP was administered to mice at a dose of 400 mg/(kg-day) for 5 consecutive days. It was found that GUP could effectively inhibit the activation of the

Table 2 Bioactivities of polysaccharides purified from *G. uralensis*.

Name	Types	Testing subject	Doses/Duration	Effects/Mechanism	References
Anti-inflammatory activity					
GUP	<i>In vivo</i>	STING agonist (DMXAA) and CLP mice model	400 mg/kg, i.g., for 21 days	cGAS-STING pathway ↓; the expression of IFN-β, IL-6 and TNF-α ↓.	[20]
GUP	<i>In vitro</i>	BMDMs cells	0, 0.5, 1, and 2 mg/mL	cGAS-STING pathway ↓; the phosphorylation of IRF3 and typical STING stimulators (HT-DNA, 2'3'-cGAMP, diABZI and DMXAA ↓; the mRNA expression of <i>IFN-β</i> , <i>IL-6</i> , <i>TNF-α</i> and <i>CXCL10</i> ↓; IRF3 transcriptional activity and the expression of type I IFNs ↓; the interaction of STING with TBK1 and TBK1 with IRF3 ↓.	[20]
Se-GUPS	<i>In vivo</i>	LPS-induced mouse model Mouse peritoneal capillary permeability model, mouse ethanol-induced gastric ulcer model, Rauwolfia serpentina gastric ulcer model	100, 200, 300 mg/kg, i.g., for 10 days	The secretion of TNF-α and IL-1β ↓.	[73]
GUPS	<i>In vivo</i>	mouse ethanol-induced gastric ulcer model, Rauwolfia serpentina gastric ulcer model	3, 10 mg/g, i.g., for 7 days	The high doses have inflammation suppression rates of 38.95%, 61.39%, and 13.39% respectively.	[74]
GUPS	<i>In vivo</i>	COPD mouse model	50 mg/kg, i.g., for 10 days	The expression of IL-17, IL-6 and TNF-α ↓; The expression of IL-10 ↑.	[75]
GUPS	<i>In vivo</i>	Epileptic rat model	50 mg/kg, i.p., for 30 days	The purinergic receptor P2X ion channel 7 in the hippocampus, the expression of NF-κB protein, and the levels of IL-18 and TNF-α ↓.	[76]
GP	<i>In vivo</i>	CTX-induced immunosuppressed and intestinal mucosal injury models	100, 200 mg/kg, i.g., for 3 days	Muribaculaceae_unclassified, <i>Ali stipes</i> , Lachnospiraceae_NK4A136_group, <i>Ligilactobacillus</i> , and Clostridia_vadinBB60_group ↑; the abundance of Clostridiales unclassified, <i>Candidatus Arthromitus</i> , Firmicutes unclassified, and <i>Clostridium</i> ↓.	[77]
Immunomodulatory activity					
GUPS-1	<i>In vitro</i>	RAW264.7 cells	100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000 μmol/L	The proliferation of immune cells ↑; NO, IL-2, IL-1β, IFN-γ, TNF-α, and IL-12p70 ↑; the proliferation immune cells and elevate their cytokine secretion ↑.	[69]
GUPS-1	<i>In vivo</i>	Lohmann Brown chickens	1, 2, and 4 mg/mL, i.m., for 3 days	Chickens' body weight and ND serum antibody titer ↑; IL-2, IFN-γ, and immune organs ↑.	[69]
GUPS	<i>In vitro</i>	Human monocyte-derived DCs	1, 5, 10, 20 and 50 μg/mL, for 12 h	The level of IL-12 ↑; Th1 skewing cytokine profile ↑.	[67]
GUPS	<i>In vivo</i>	Immunosuppressive mouse model	20 mg/kg, i.g., for 7 days	IL-12 production ↑; the expression of surface markers including CD40, CD80, CD86, MHC-I/-II and CCR7 ↑.	[67]
GUPS-I, GUPS-II, GUPS-III	<i>In vitro</i>	DCs	10, 50 μg/mL, for 12 h	The expression of MHC-I, CD40, and CD86 ↑; the secretion of IL-1β, IL-12p40, and TNF-α ↑.	[46]
GUPS-I, GUPS-II, GUPS-III	<i>In vivo</i>	C57BL/6 mice	100 μg, i.m., for 12 h	The expression of MHC-I, CD40, and CD86 ↑; the secretion of IL-1β, IL-12p40, and TNF-α ↑.	[46]
GUPS	<i>In vivo</i>	SPF Kunming mice	1, 20, 50 and 100 mg/mL, i.g., for 28 days	The serum IL-2, CD4 ⁺ /CD8 ⁺ and the activity of NK cells ↑; the spleen index and thymus index ↑.	[82]
GUPS	<i>In vitro</i>	Peritoneal macrophages	0, 10, 50, 100, 200, and 400 μg/mL, for 48 h	NO production and NO synthase (iNOS) transcription ↑; iNOS mRNA expression ↑; NO and iNOS production ↑.	[45]
GUPS	<i>In vitro</i>	Macrophages	10, 100, 200, and 400 μg/mL, for 72 h	The pinocytic activity, the production of NO, IL-1, IL-6 and IL-12 in a dose-dependent manner ↑; O ²⁻ production ↑.	[63]
GUPS	<i>In vivo</i>	Arbor Acres broiler chickens	150 mg/kg, p.o., for 3 days	the abundance of Bacteroidetes, <i>Bacteroides</i> , <i>Faecalibacterium</i> , <i>Desulfovibrio</i> , and <i>Butyrivibrio</i> ↓; the abundance of Firmicutes, <i>Prevotella</i> , <i>Parabacteroides</i> , <i>Ruminococcus</i> , and <i>Alistipes</i> ↑.	[83]
Anti-oxidant activity					
GUPS-1	<i>In vitro</i>	Vitamin C, RAW264.7 cells	2.44, 4.88, 9.77, 19.53, 39.06, 78.12, 156.5, 312.5, 625, and 1,259 μg/mL.	At the concentration between 1 and 5 mg/mL, scavenging rates rose from 13.47% to 45.29%. at the concentration ranging from 1 to 5 mg/mL, ABTS ^{•+} scavenging radicals at 93.53%–99.42%.	[69]
GUP-II	<i>In vitro</i>	BHT, DPPH [•] , •OH	0.05, 0.1, 0.2, 0.5, and 1.0 mg/mL	DPPH [•] , •OH ↓; exhibited significant radical-scavenging activities, scavenging rates of 21.7%–48.33%, 31.81%–60.94%, respectively.	[43]
GUP	<i>In vitro</i>	Superoxide anion radical, •OH, DPPH [•]	3.2 mg/mL	The superoxide anion free radicals, •OH, DPPH [•] ↓; at 3.2 mg/mL, exhibited significant radical-scavenging activities, scavenging rates of 56.17%, 23.44%, 26.5% respectively.	[47]
GUP	<i>In vivo</i>	Kunming mice	100, 150, 228, 347, 527, and 800 mg/kg, i.g., for 30 days	MDA levels in the serum and livers ↓.	[47]
GUPS-1, GUPS-2, GUPS-3	<i>In vitro</i>	DPPH [•] , O ₂ ^{•-} , •OH, Fe ²⁺ -chelating	0.25, 0.5, 1, 2 and 4 mg/mL	The efficiently active in scavenging DPPH radical, O ₂ ^{•-} , •OH and chelating Fe ²⁺ .	[22]

(Continued)

Name	Types	Testing subject	Doses/Duration	Effects/Mechanism	References
GUPS	<i>In vitro</i>	•OH, O ₂ ^{•-} and DPPH•	3, 6, 9, 12, 15, 18 mg/mL	Exhibited significant radical-scavenging activities, IC ₅₀ of 9.40, 9.03 and 8.04 mg/mL, respectively.	[39]
LRPs	<i>In vitro</i>	DPPH•, •OH, ABTS•	0.02, 0.04, 0.06, and 0.08 mg/mL	The IC ₅₀ values of DPPH•, •OH, ABTS•. Scavenging activity by pectinase and cellulase were 0.002, 43, 0.305, 0.040, 3 mg/mL and 0.226, 0.023, 8, 0.040, 1 mg/mL, respectively.	[40]
Anti-tumor activity					
LP	<i>In vivo</i>	CT-26 tumor bearing BALB/c mice	500 mg/kg, i.g., for 14 days	The tumor suppression rate ↑; CD4 ⁺ and CD8 ⁺ immune cells population ↑; IL-2, IL-6, IL-7 levels ↑; TNF-α levels ↓.	[68]
GCP	<i>In vivo</i>	The tumor-bearing mouse model	0.1 mL, i.g., for 14 days	The spleen index ↑; tumor growth and metastasis ↓; <i>Parasutterella</i> , <i>Clostridium_sensu_s-</i> and <i>Blautia</i> ↓; lymphocyte recruitment.	[94]
GUPS	<i>In vitro</i>	Mouse intestinal epithelial cell	0.001, 0.01, 0.1, 1, 10, 100 μg/mL, for 3 days	At a concentration of ≤ 50 μg/mL, the proliferation of cancer cells (CT-26) ↓; proliferation of T lymphocytes ↑.	[88]
GP	<i>In vitro</i>	Treg cells and Th1/Th2 cytokines in H22 hepatocarcinoma tumor-bearing mice	250 mg/kg day, i.g., for 7 days	The tumor progression ↓; the population of Treg cells ↓; lymph node <i>Foxp3</i> and <i>IL-10</i> mRNA expression ↓; IL-10 and TGF-β level in serum ↓; IL-2 and IL-12p70 level in serum ↑.	[89]
GUPS	<i>In vivo</i>	CT-26 cell tumor-bearing mouse animal model	500 mg/kg, i.g., for 14 days	The tumor suppression rate is 21.56%, mouse body weight and thymus index ↑; the expression of <i>IL-7</i> mRNA in small intestinal mucosal epithelial cells and IL-7 levels in serum ↑.	[90]
GUPS	<i>In vivo</i>	Tumor mouse model	25, 50, and 75 mg/kg, i.g., for 7 days	The number of apoptotic cells ↑.	[92]
GUPS	<i>In vitro</i>	H22 cells and BEL7402 cells	2, 10, 50, 250, 1,250 μg/mL, for 48 h	In hepatocellular carcinoma cell proliferation ↓.	[92]
GIP1	<i>In vitro</i>	Human oral cancer cell line SCC-25 cells	50, 100, and 200 μg/mL, for 24–72 h	Anti-apoptotic protein Bcl-2 ↓; pro-apoptotic protein Bax ↑; the initiator caspases-9, and the caspases-3 ↑.	[93]
Anti-diabetic activity					
GUP	<i>In vivo</i>	T2D mice model	100, 200, and 400 mg/kg, i.g., for 4 weeks	The integrity of the intestinal barrier ↑; the levels of pro-inflammatory cytokines and serum LPS levels ↓; serum creatinine and urea nitrogen levels ↓.	[29]
GUPS	<i>In vivo</i>	T1DM mice model	200, 400 mg/kg, i.g., for 8 weeks	The Glc tolerance (oral Glc tolerance test (OGTT)) level ↑; the levels of SOD, CAT, and GSH ↑; the content of MDA ↓.	[95]
Anti-bacterial activity					
GCP2	<i>In vitro</i>	Gram-positive bacteria and Gram-negative bacteria	3.0, 4.0 mg/mL	Best effect on <i>E. coli</i> and <i>K. pneumoniae</i> , with an inhibition zone diameter of 11 mm.	[97]
GUPS	<i>In vitro</i>	<i>Bacillus subtilis</i> , <i>E. coli</i>	6.25, 12.5 μg/mL	The MIC values are 6.25 μg/mL for <i>B. subtilis</i> and 12.5 μg/mL for <i>E. coli</i> .	[98]
Other activities					
GUP-1, GUP-2	<i>In vivo</i>	Acute hyperuricemias model	150, 300 mg/kg, i.g., for 1 day	Blood uric acid values ↓; the XOD activity in serum and liver ↓; kidney disease ↓; uric acid synthesis ↓.	[31]

Note: Increase, ↑; Decrease, ↓.

cGAS-STING signaling pathway by upregulating the expression of type I interferon-related genes and inflammatory factors in BMDMs, THP-1 cells, and human PBMCs. It is noteworthy that GUP demonstrated considerable therapeutic efficacy in both the 5,6-dimethylxanthone-4-acetic acid (DMXAA)-induced *in vivo* agonist experiment and the mouse cecal ligation and puncture (CLP) sepsis model. In light of these findings, the researchers concluded that GUP is an effective inhibitor of the cGAS-STING pathway and may have potential as a therapeutic agent for inflammatory diseases mediated by the cGAS-STING pathway. Zhu *et al.*^[73] investigated the anti-inflammatory activity of Se-GUPS, using dexamethasone as a positive control. In the experiment, mice received different doses (100, 200, 300 mg/kg) of Se-GUPS and glycyrrhizin for 10 consecutive days. Additionally, a mouse inflammation model was established using lipopolysaccharides (LPS) to measure changes in serum tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). The results showed that Se-GUPS effectively inhibited ear swelling and capillary permeability, with its anti-inflammatory activity significantly superior to that of GUPS, especially at the high dose of Se-GUPS. Zhao *et al.*^[74] assessed the anti-inflammatory effects of GUP using three mouse models (intraperitoneal capillary permeability model, ethanol gastric ulcer model, and reserpine

gastric ulcer model), revealing that the high-dose group of GUP (1 g/kg) achieved inflammatory inhibition rates of 38.95%, 61.39%, and 13.39% for the three models, respectively. Wu *et al.*^[75] studied the repair effects of GUPS on lung tissue inflammation damage in COPD mice. The results indicated that GUPS treatment significantly improved the mice's mental state, increased food intake and activity; the expressions of pro-inflammatory factors IL-17, IL-6, and TNF-α were significantly decreased, while IL-10 expression was significantly increased. A dosage of 50 mg/kg showed good reparative effects on lung tissue and bronchial inflammatory damage in COPD mice. Wu *et al.*^[75] found that GUPS inhibited the expression of pro-inflammatory cytokines in COPD mice and promoted the expression of IL-10. Furthermore, GUPS reduced the inflammatory response in pentylenetetrazol-induced epileptic rats by downregulating the expression of purinergic receptor P2X purinergic receptor (P2X)ligand-gated ion channel 7 and nuclear factor-κB (NF-κB) proteins, resulting in decreased levels of IL-18 and TNF-α^[76]. Research has found that GP reverses cyclophosphamide-induced intestinal damage by regulating the Th1/Th2 balance, increasing Goblet cells and CD4⁺/CD8⁺ T lymphocytes, and enhancing mucin content. Meanwhile, GP promotes the secretion of short-chain fatty acids and cytokines by regulating the CEBP/p38/JNK pathway.

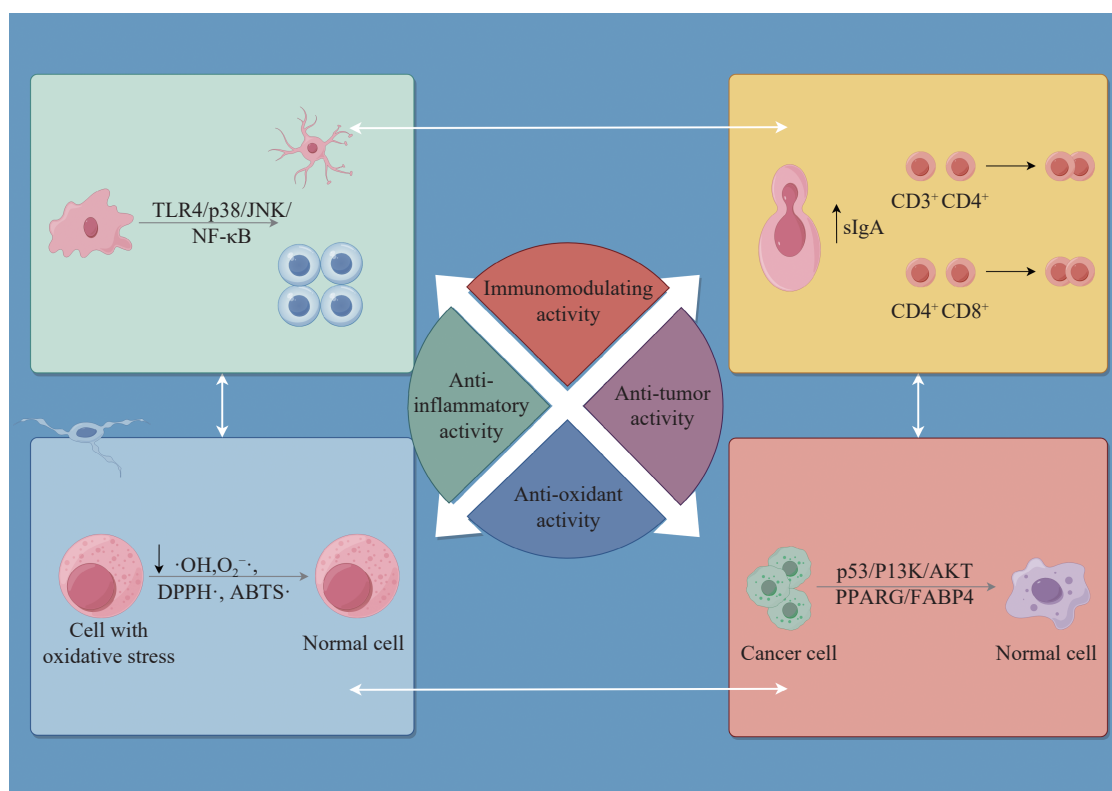


Figure 4 Schematic representation of underlying molecular mechanism displaying the primary biological activity of polysaccharides isolated from *G. uralensis*.

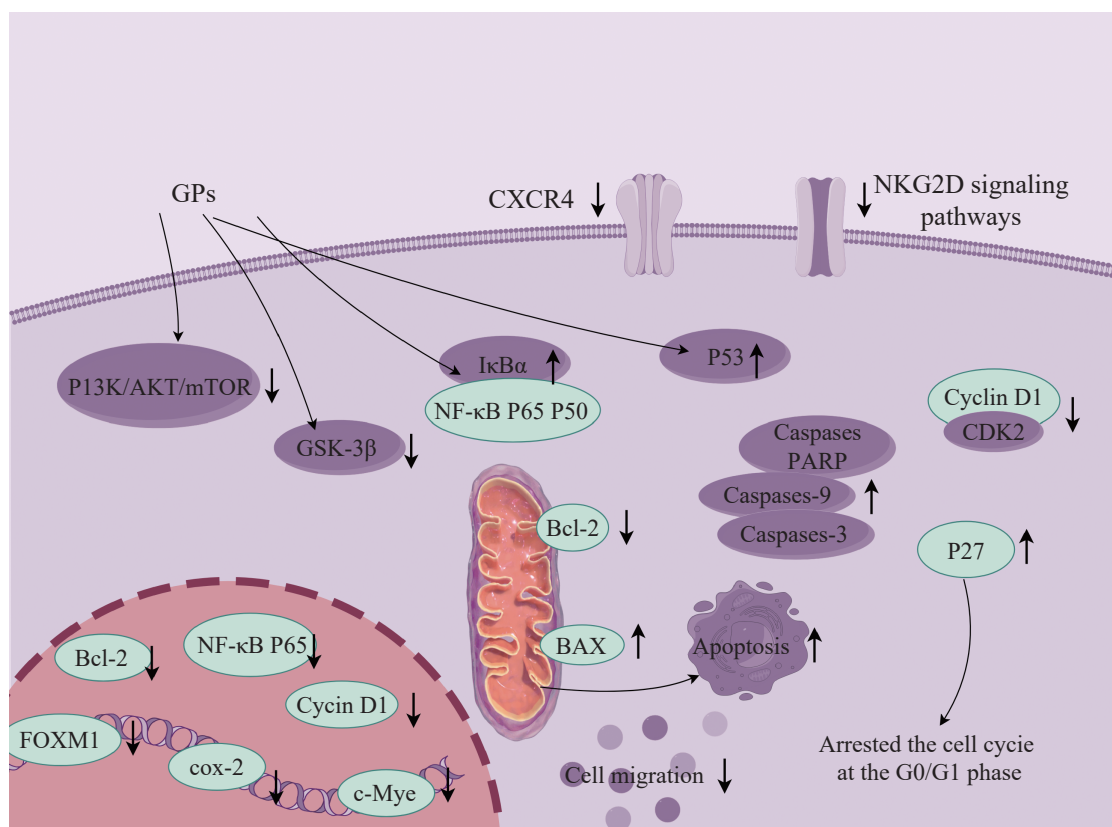


Figure 5 Schematic representation of underlying molecular mechanism displaying the anti-tumor activity of polysaccharides isolated from *G. uralensis*.

Microbiome analysis showed that GP promotes the proliferation of probiotics and inhibits the growth of *Proteus* and other pathogenic bacteria. The study confirms the important role of intestinal microbes in the immune regulation mediated by GP,

indicating the potential of GP in the treatment of inflammatory bowel diseases^[77].

In summary, studies conducted both *in vitro* and *in vivo* indicate that GUPs have significant anti-inflammatory activity.

Subsequent experiments could consider their combination with other anti-inflammatory drugs or therapies to enhance their anti-inflammatory effects. Future research may delve deeper into the anti-inflammatory mechanisms of GUPs to provide targets for drug development. Additionally, the changes in specific inflammatory factors can be studied to confirm their role in the therapeutic process.

4.2 Immunomodulating activity

Natural plant polysaccharides have been demonstrated to possess immune-regulating activity, which can stimulate the proliferation of immune cells and enhance overall immunity. This provides a basis for plants to exert additional physiological and pharmacological functions^[78,79]. Numerous studies have shown that GUPs possess significant immune-regulating activity, playing an important role in enhancing the body's immune function and regulating immune responses^[80]. By promoting the proliferation and activation of immune cells, GUPs may effectively improve the body's ability to resist diseases, thus holding broad application prospects in health care and disease prevention^[81].

The research conducted by Zhou *et al.*^[69] demonstrated, through *in vitro* experiments, that GUPS-1 has the capacity to significantly promote the proliferation of immune cells and is closely associated with the secretion of IL-12, interferon- γ , and TNF- α by lymphocytes. Furthermore, the levels of NO, IL-2, IL-1 β , interferon- γ , TNF- α , and IL-12p70 were markedly elevated. *In vivo* experiments were conducted by administering Lohmann Brown chicks with GUPS-1 at doses of 19.53, 9.77, and 4.88 $\mu\text{g/mL}$ for three consecutive days. The findings demonstrated that GUPS-1 markedly elevated the body weight of the chicks, facilitated the secretion of IL-2 and interferon- γ in the chickens, and facilitated the development of immune organs. In conclusion, the results indicate that GUPS-1 can not only promote the proliferation of immune cells and cytokine secretion *in vitro* but also enhance the immune response of chickens *in vivo*. Research by Aipire *et al.*^[67] showed that GUPS has a significant immunostimulating effect, significantly promoting the maturation and cytokine secretion of human monocyte-derived and mouse bone marrow-derived dendritic cells (DCs) through the Toll-like receptor 4 (TLR4) and its downstream p38, c-Jun N-terminal kinase (JNK), and NF- κB signaling pathways. In addition, GUPS enhances the migration ability of DCs to lymph nodes. In mouse models, GUPS increased the production of IL-12 in serum but did not increase TNF- α . GUPS also mitigated the side effects of cyclophosphamide and improved the immunity of immunosuppressed mice, demonstrating its potential in tumor therapy. Furthermore, Aipire *et al.*^[46] utilized *In vitro* cultured DC and mouse models to further investigate the immunostimulatory activity of three GUPS components with undetectable levels of endotoxin. The findings indicated that *in vitro*, GUPS-II and GUPS-III exhibited heightened potency in inducing dendritic cell maturation and cytokine production relative to GUPS-I. In comparison to GUPS-I and GUPS-III, GUPS-II was observed to significantly promote DC maturation *in vivo*, indicating its potential utility as an immunostimulant in conjunction with vaccines or cancer immunotherapy. Chen *et al.*^[82] studied the effects of GUPs on growth performance and immune function in mice. The results showed that after gavaging 0.5 mL of GUPs at concentrations of 1, 20, 50, and 100 mg/mL, the average body weight, feed conversion ratio, serum IL-2, CD4⁺/CD8⁺ ratio, and natural killer (NK) cell activity in mice reached the highest levels 28 days post-administration, significantly increasing with dosage,

with the 100 mg/mL group showing the most prominent effects, where the spleen and thymus indices were 1.51 and 1.43 times that of the control group, respectively. In summary, GUPs can significantly enhance the growth performance and immune function of mice, providing a research foundation for the clinical application of GUPs. Cheng *et al.*^[45] demonstrated that GP can markedly stimulate macrophages to produce NO and induce the transcription of inducible nitric oxide synthase (iNOS). Furthermore, they observed that concentrations ranging from 10 to 400 $\mu\text{g/mL}$ can enhance the generation of NO and iNOS. In comparison to the use of GP alone, the combined action of GP with lipopolysaccharide/interferon was observed to significantly promote the production of NO and iNOS. The RT-PCR results indicate that GP may serve as a secondary trigger for iNOS mRNA expression, suggesting that iNOS-mediated NO synthesis may represent a key mechanism of GP-mediated therapeutic action. This finding highlights the potential of GP as an immune enhancer and provides a foundation for further optimization of its applications. Cheng's research^[45] showed that purified polysaccharide GP has an immunomodulatory effect on macrophages, dose-dependently increasing the phagocytic activity and cytokine production in mice. Specifically, GP at 10 $\mu\text{g/mL}$ can induce IL-1, while 100 $\mu\text{g/mL}$ significantly promotes the production of NO, IL-6, and IL-12. Within 6 h after treatment, IL-1, NO, and IL-12 in macrophages significantly increased, and the generation of superoxide anion (O_2^-) was also notably elevated. The study results indicate that GP exerts therapeutic effects by modulating the immune functions of macrophages, providing important data and theoretical foundation for understanding its immunomodulatory mechanism^[63]. Qiao's research^[83] indicates that the addition of 150 mg/kg of GUPS in the diet significantly improves the growth performance, intestinal health, and microbial flora of Arbor Acres roosters. In the experiment, the average daily weight gain of the GUPS group increased, and the feed-to-gain ratio decreased. At 42 days, the levels of immunoglobulin A (IgA), IgM, and IgG significantly increased, while the levels of TNF- α , IL-1 β , IL-6, and diamine oxidase (DAO) activity significantly decreased. Moreover, GUPS increased the height of intestinal villi (VH) and the VH/crypt depth (CD) ratio, upregulated the mRNA expression of tight junction protein, *claudin-1*, and *MUC2*, and adjusted the intestinal microbial flora to promote the proliferation of probiotics.

The above research suggests that GUPs can exert immunomodulatory effects either directly or indirectly, which not only offers great potential for the treatment and prevention of various diseases but also lays the foundation for their widespread application as immunomodulators. Future studies should explore the immunomodulatory mechanisms of GUPs in depth, utilizing advanced technologies such as transcriptomics, proteomics, and metabolomics to systematically analyze their pathways and targets, and clarify the specific effects of GUPs on immune cells, thereby providing a more solid theoretical basis for their clinical application.

4.3 Anti-oxidant activity

Oxidative stress is a term proposed by Helmut Sies, referring to the imbalance between the production of oxidants and the antioxidant defense in the body. This imbalance can cause damage to biological systems, leading to various diseases^[84]. The majority of natural plant polysaccharides have been demonstrated to scavenge reactive oxygen species, inhibit the production of lipid oxidation products and enhance the activity of antioxidant

enzymes. This has been shown to help prevent a number of chronic diseases that are triggered by oxidative stress^[85,86]. A large number of studies have shown that GUPs have significant antioxidant activity, which presents a broad application prospect in the prevention and treatment of diseases related to oxidative stress.

GUPS-1 exhibits significant antioxidant performance *in vitro*, effectively eliminating various free radicals, including DPPH, hydrogen peroxide, and ABTS, showing concentration dependence. When the concentration ranges from 1 to 5 mg/mL, the scavenging rate of hydroxyl radicals by GUPS-1 increases from 13.47% to 45.29%. At 1 mg/mL, the scavenging rate for DPPH radicals is 15.06%, and it increases to 86.60% at 5 mg/mL (half maximal inhibitory concentration (IC₅₀) is 3.07 mg/mL). Although the effect is significant, the free radical scavenging ability of GUPS-1 is still lower than that of ascorbic acid^[69]. Chen *et al.*^[43] found that GUP-II exhibits effective and concentration-dependent DPPH radical and OH radical scavenging activities, but these activities are lower than those of butylated hydroxytoluene (BHT). The DPPH radical scavenging activity of GUP-II ranges from 21.7% to 48.33%, and the scavenging rate for OH radicals range from 31% to 60% within the concentration range of 0.05–1.0 mg/mL. In their study, Lian *et al.*^[47] demonstrated that Se-GUP demonstrated greater antioxidant activity than GUP in both *in vitro* and *in vivo* models. These findings suggest that selenium-based modification markedly enhances the antioxidant activity of Se-GUP, thereby increasing its potential for application as an antioxidant. Zhang *et al.*^[22] evaluated the *in vitro* antioxidant activity of GUPS-1, GUPS-2, and GUPS-3 through DPPH radical scavenging assay, superoxide anion radical scavenging assay, hydroxyl radical scavenging assay, and Fe²⁺ chelation assay. The results indicated that there were significant differences in antioxidant capacities among these polysaccharides, and they exhibited synergistic effects with increasing concentrations. Among them, GUPS-1 exhibited robust scavenging capabilities against DPPH, superoxide anion, and hydroxyl radicals, and displayed notable Fe²⁺ chelation activity, which may be associated with its lower relative molecular weight and monosaccharide composition ratio. This indicates that the optimization of these polysaccharides may result in an enhancement of their antioxidant properties. Li *et al.*^[39] found that under optimal extraction conditions, GUPS has a certain reducing ability and showed strong scavenging capability against •OH, O₂^{•−}, and DPPH•, with IC₅₀ values of 9.40, 9.03, and 8.04 mg/mL, respectively. The results of the antioxidant experiments indicate that the polysaccharides extracted by 0.226, 0.023, 8, and 0.040, 1 mg/mL against DPPH•, •OH, and ABTS•, respectively. This suggests that the polysaccharides extracted by pectinase demonstrate better scavenging ability against DPPH free radicals, while the polysaccharides extracted by cellulase have superior scavenging ability against hydroxyl free radicals, with both showing nearly identical scavenging ability against ABTS free radicals^[40].

The considerable antioxidant efficacy of GUPs has been substantiated in both *in vitro* and *in vivo* investigations. In the future, the antioxidant activity of natural plant polysaccharides can be enhanced through a number of avenues, including an in-depth analysis of polysaccharide composition, a comprehensive investigation into the mechanisms by which they act as antioxidants, *in vivo* experiments, the optimisation of extraction methods and structural modification, and the development of functional foods with the aim of expanding their potential applications.

4.4 Anti-tumor activity

Tumors are one of the common diseases threatening human health, with an increasing incidence rate in recent years^[87]. There are many reports on the anti-tumor activity of GUPs. GUPs mainly exert anti-tumor activity by inhibiting tumor growth and promoting tumor cell apoptosis.

In their study, Ayeka *et al.*^[68] investigated the immunomodulatory effects of licorice polysaccharide (LP) *in vivo* and evaluated its anti-cancer properties. The findings demonstrated that LP exerts immunomodulatory effects on BALB/c mice, markedly inhibiting tumour growth and enhancing the immune organ index. Furthermore, it has been demonstrated that LP can activate CD4⁺ and CD8⁺ immune cell populations, influencing the production of various cytokines. This is evidenced by the increased levels of IL-2, IL-6, and IL-7 observed while the level of TNF-α was reduced. These findings suggest that LP has anti-tumour potential, as it can inhibit tumour production and circulation, thereby affecting and suppressing tumour growth. According to the study by Ayeka *et al.*^[88], different molecular weight components of GUPs play an important role in immune regulation and anti-tumor effects. In IEC-6 cells, GUPs do not inhibit cell proliferation even at high concentrations, while they effectively inhibit the proliferation of colonic cancer cells CT-26 at ≤ 50 µg/mL, with a significant upregulation of IL-7 gene expression. Furthermore, GUPs stimulate IEC-6 cells to secrete IL-7, promoting the proliferation of T lymphocytes, and the effect is molecular weight-dependent. These findings suggest that low molecular weight glycan polysaccharides have potential anti-cancer effects, with immune regulation possibly being one of the mechanisms of action, providing important references for tumor treatment. The effects of GP on Treg cells and Th1/Th2 cytokines in H22 tumour-bearing mice were investigated by He *et al.*^[89]. The findings indicated that GP markedly reduced the proportion of Treg cells and the mRNA expression of forkhead box P3 (*Foxp3*) and *IL-10* in tumor-bearing mice, while elevating the levels of IL-2 and IL-12 p70. This suggests that GP may impede tumor growth by regulating the equilibrium of Treg cells and Th1/Th2 cytokines. Furthermore, GP was observed to markedly diminish the serum levels of IL-10 and transforming growth factor-β (TGF-β). These findings suggest that GP may exert a partial inhibitory effect on tumor growth by downregulating Treg cells and promoting Th1 cytokine production. Moreover, Wang *et al.*^[90] explored the anti-tumor effects of GUPs and their mechanisms. By establishing a CT-26 tumor-bearing mouse model and randomly dividing it into different groups, they administered the treatment continuously for 14 days. The results demonstrated that GUPs markedly diminished the tumor mass in mice, with an inhibition rate of 21.56%. Additionally, there was a notable increase in the body mass and thymus index of the mice, which led to an improvement in their quality of life. Additionally, GUPs were observed to enhance the expression of IL-7 mRNA in small intestinal mucosal epithelial cells and the levels of IL-7 in serum. Following the blockade of IL-7 antibodies, a notable decline was observed in the anti-tumor effect and thymus index, suggesting that the anti-tumor mechanism is linked to the enhanced secretion of IL-7.

Apoptosis is a natural phenomenon in the growth, development, and death processes of multicellular organisms. It plays a role in the timely removal of damaged and excess cells in the body to maintain the homeostasis of tissues, organs, and internal environment^[87]. GUP has a significant pro-apoptotic effect on mouse S-180 sarcoma cells, with apoptosis rates of 24.7% (1.0 g/kg), 23.2% (0.75 g/kg), and 44.6% (0.5 g/kg), with the lowest

dosage group (0.5 g/kg) showing the highest level of apoptosis^[91]. Chen *et al.*^[92] found that GUPS induces apoptosis in mouse liver cancer H22 cells by blocking the PI3K/AKT pathway, while inhibiting the proliferation of human liver cancer BEL7402 cells in a dose-dependent manner. After 48 h of glycyrrhiza inflata polysaccharide (GIP) treatment, the percentage of annexin V-positive SCC-25 cells increased in a dose-dependent manner, with apoptosis rates rising from 4.7% in the control group to 32.7% in the 50 g/mL group, and 41.4% in the 100 g/mL group^[93].

Moreover, Zhang and colleagues^[94] investigated the anti-tumor mechanism of glycyrrhiza polysaccharide (GCP) from the perspective of the gut microbiota. Following a 14-day course of GCP treatment in a CT-26 colon cancer mouse model, a significant reduction in tumor weight was observed, accompanied by effective suppression of tumor metastasis as evidenced by tissue sections. High-throughput 16S rRNA sequencing analysis revealed no significant alterations in the gut microbiota between the model group and the cyclophosphamide group. Experimental results indicated that the anti-tumor effect of GCP is closely related to the gut microbiota; GCP lost its anti-tumor ability after the depletion of gut microbiota, while the transplantation of feces treated with GCP could inhibit tumor growth. These findings provide important references for optimizing the relationship between gut microbiota and tumor treatment.

In summary, GUPs primarily exert their anticancer activity by inhibiting tumor growth and promoting apoptosis in tumor cells. Future research suggests combining modern technologies, such as nanotechnology and gene editing, to enhance the bioavailability and targeting of GUPs. Additionally, attention should be paid to the combined application of GUPs with other anticancer treatments to assess their synergistic effects and promote their clinical application.

4.5 Anti-diabetic activity

Diabetes is a metabolic disease characterized primarily by insufficient insulin secretion or inadequate insulin action, leading to abnormally elevated blood sugar levels^[29]. Recent studies have demonstrated that GUPs exhibit promising potential for the treatment of diabetes. The research conducted by Ye *et al.*^[29] indicates that GUP has a beneficial effect on rats with type 2 diabetes (T2D) mellitus induced by a high-fat diet and streptozotocin, including a reduction in hyperglycaemia, insulin resistance and oxidative stress. Moreover, GUP has been observed to enhance the integrity of the intestinal barrier in T2D mice, a process that appears to be mediated by a reduction in pro-inflammatory cytokines and serum endotoxin levels. The study also showed that GUP can increase the diversity of the gut microbial community in T2D mice, enhancing beneficial bacteria such as *Akkermansia*, *Lactobacillus*, *Dorea*, and *Bacteroides* while reducing the diversity of harmful bacteria such as *Bacteroides*, *Shigella*, and *Clostridium*. The research highlights the potential of GUP in alleviating complications in T2D mice and promoting gut health, providing valuable insights for dietary strategies aimed at diabetes control and overall health improvement. Wang and colleagues^[95] investigated the impact of GUPs on blood Glc, lipid metabolism, and oxidative stress-related biomarkers in a type 1 diabetes mellitus (T1DM) murine model. The baseline indicators and variations in relevant indicators of the mice were quantified. The findings indicated that the fasting blood Glc level in the high-dose GUPs group was 15.9 mmol/L, which was not statistically different from the positive control group but demonstrated a notable enhancement in Glc tolerance. The levels

of total cholesterol, triglycerides, high-density lipoprotein, and low-density lipoprotein in the high-dose GUPs group were significantly different from those in the model group, and the levels of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) were significantly increased, while malondialdehyde (MDA) levels were significantly decreased. In summary, GUPs have good antioxidant properties and lower blood Glc levels by improving lipid metabolism and oxidative stress levels in T1DM mice.

The future research directions for GUPs in diabetes management can focus on exploring their mechanisms, optimizing extraction and modification to improve bioavailability, assessing their synergistic effects with other drugs, and developing new functional foods and pharmaceutical formulations to enhance the effectiveness of diabetes treatment.

4.6 Anti-bacterial activity

Bacteria are one of the main threats to human safety, especially certain pathogenic bacteria that can cause various infections and diseases^[96]. GUPs have shown antibacterial effects in suppressing both Gram-positive and Gram-negative bacteria. The inhibition effect of different concentrations of GCP2 on several bacteria was determined using the filter paper method. GUP exhibited a strong inhibitory effect on *Escherichia coli* and *Klebsiella pneumoniae*, with an inhibition zone diameter of up to 11 mm at a concentration of 4.0 mg/mL^[97]. The research conducted by Zhang *et al.*^[98] revealed that GUPs exert a pronounced inhibitory impact on bacterial growth, with minimum inhibitory concentration (MIC) values of 6.25 µg/mL for *Bacillus megaterium* and 12.5 µg/mL for *E. coli*. Furthermore, a notable inhibitory impact was observed on *Saccharomyces cerevisiae*, with an MIC value of 12.5 µg/mL. Conversely, the inhibitory effect on *Penicillium* was relatively limited.

Future research can investigate the differences in antibacterial activity of GUPs from different sources and structures, exploring how chemical modifications can enhance their antibacterial effects. At the same time, in-depth studies on the mechanisms of action of GUPs against different types of bacteria will help increase understanding of the antibacterial effects of GUPs.

4.7 Other bioactivity

GUPs also have uric acid-lowering activity. Guo *et al.*^[31] established a rat model of acute hyperuricemia for the purpose of investigating the uric acid-lowering effects of GUP, GUP-1, and GUP-2 components. The findings suggest that the diverse polysaccharide constituents within the acute hyperuricemia rat model can reduce serum uric acid levels and mitigate pathological damage to the liver and kidneys. Among them, GUP at doses of 300 and 150 mg/kg significantly reduced blood uric acid levels; the GUP-1 group at the same doses also showed significant uric acid-lowering effects; while all doses of the GUP-2 group significantly lowered blood uric acid levels. Moreover, each component can dose-dependently inhibit the activity of xanthine oxidase (XOD) in serum and liver. Renal tissue sections showed that the GUP-2 group performed better in reducing renal lesions, while the GUP-1 group showed considerable effects in this regard. Liver tissue sections indicated that the GUP-2 group also had a significant effect in reducing liver lesions. In conclusion, the GUP-2 component is superior to GUP-1 in inhibiting XOD activity, lowering uric acid concentration, and alleviating liver and kidney pathological damage. This study lays the foundation for the

development of natural uric acid-lowering drugs.

In summary, GUPs have the ability to lower uric acid levels. Although there is currently limited research on the uric acid-lowering activity of GUPs, this may be a promising area for future studies.

5 Potential applications

The multiplicity of pharmacological activities exhibited by GUPs has led to their extensive utilization in a multitude of contemporary fields, including animal feed additives, vaccination, and food additives. As animal feed additives, GUPs have been demonstrated to effectively enhance animal immunity, promote growth and development, while improving the nutritional value and safety of the feed^[83]. Additionally, in vaccination, GUPs are used as immunoadjuvants to enhance the immunogenicity of vaccines, promoting animal health^[69]. In the field of food additives, GUPs are also incorporated to enhance the functionality of food^[99]. In summary, the extensive application of GUPs not only helps improve the overall health level of animals but also contributes to the sustainable development of agriculture and food safety.

5.1 Animal feed additives

Zhang *et al.*^[100] conducted a study on GCP's application in poultry feed using 400 broiler chickens, divided into four groups, including a control. The experimental groups received 0.5%, 1.0%, and 1.5% GCP in their diets for 42 days. Results indicated that GCP significantly improved growth performance, with the 1% group achieving the best outcomes. This group showed a notable increase in virus antibody titer and phagocytic index, along with a higher number of lactobacilli and bifidobacteria in cecal contents, suggesting that GCP enhances growth, immune response, and intestinal microbial balance. In conclusion, GCP demonstrates strong potential as a feed additive for promoting poultry growth and immunity, particularly at a 1% inclusion level. Qiao *et al.*^[83] found that adding 150 mg/kg of GUPS to feed significantly impacts chickens' growth performance, gut health, and gut microbiota. In their study with 480 Arbor Acres roosters, GUPS resulted in increased average daily weight gain and reduced feed conversion ratio. After 42 days, levels of IgA, IgM, and IgG were significantly elevated in the GUPS group, while TNF- α , IL-1 β , IL-6, and DAO activity were notably reduced. GUPS also enhanced VH and the VH/CD in the duodenum, jejunum, and ileum, upregulating mRNA expression of tight junction proteins, *claudin-1*, and *MUC2*. In summary, GUPS significantly improved broiler growth by promoting gut health and regulating gut microbial balance. When the addition amount increases to 300, 600, or 900 mg/kg, it can further improve the final weight, average daily gain, and body length of broilers from 1 to 42 days old, and significantly reduce the feed-to-weight ratio^[101]. Adding 500, 1,000, and 1,500 mg/kg of GUPs to quail feed can significantly improve average daily gain in a dose-dependent manner, reduce feed-to-weight ratio and mortality rate; on day 20, the relative weights of the thymus and bursa of Fabricius increased linearly with the dosage of polysaccharides; from day 60 to day 80, average egg production rate and average egg weight increased linearly, while the feed-to-egg ratio decreased^[1].

GUPs show a wide range of application potential in animal feed additives. They not only effectively enhance the immune system of animals, improve disease resistance, and promote overall health, but also facilitate growth and development and improve feed

conversion rates, allowing animals to absorb nutrients more efficiently. In addition, GUPs also play a significant role in enhancing the nutritional value and safety of feed, reducing the content of harmful substances, and ensuring the healthy growth of animals. Therefore, the multiple applications of GUPs not only improve farming efficiency but also provide innovative solutions for food safety and the development of sustainable agriculture.

5.2 Vaccination

GUPS-1 has been confirmed to act as an immunoadjuvant in conjunction with Newcastle disease (ND). In *in vivo* experiments, researchers conducted vaccination trials on 120 Lohmann Brown chickens, administering GUPS-1 *via* intramuscular injection at doses of 19.53, 9.77, and 4.88 $\mu\text{g/mL}$ for 3 consecutive days, in addition to a vaccine control group (VC). The experimental results showed that the combination of GUPS-1 and ND significantly increased the body weight of chicks and the serum antibody titers against ND, enhanced the secretion of IL-2 and interferon- γ in the chickens, and promoted the development of their immune organs. Furthermore, the study indicated that GUPS-1 can promote the proliferation of immune cells and the secretion of cytokines *in vitro*, thereby enhancing the body's immune response. This demonstrates the potential of GUPS-1 as an immunoadjuvant for ND^[69].

In recent years, multiple patents have confirmed that GUPs can effectively inhibit animal diseases caused by viral infections, further supporting their potential applications in animal health and providing a scientific basis for the development of new prevention and treatment strategies. In 2022, Xuan *et al.*^[102] proposed the application of GUPs in formulations against porcine pseudorabies virus (PRV) infections, aimed at inhibiting the adsorption and entry of the PRV. Cell experiments showed that GUPs could effectively inhibit PRV infection in PK-15 cells. The antiviral mechanism primarily involves preventing the virus from binding to the cells and the entry process, offering a safe and effective drug for clinical prevention or treatment of PRV infections. Subsequently, in 2024, Li *et al.*^[103] disclosed the application of GUPs in the preparation of drugs against feline infectious peritonitis virus (FIPV). This research revealed for the first time the significant suppressive effect of GUPs on FIPV, along with low cytotoxicity. Further studies found that GUPs not only have a direct virucidal effect but can also inhibit viral replication and adsorption within cells without affecting the internalization process. This demonstrates high safety and low toxic side effects of GUPs in the preparation of drugs against FIPV. Additionally, GUPs can enhance the immune function of the body, promoting activation and proliferation of immune cells, thereby increasing resistance to viral infections.

5.3 Food additives

Natural polysaccharides are a popular new type of health food ingredient, widely used in various functional foods. Research by Guo *et al.*^[99] showed that GP has a significant positive impact on the quality and sensory characteristics of steamed buns. Specifically, after adding GP, the specific volume of the steamed buns increased to 2.55 mL/g, enhancing the hardness and chew strength of fresh steamed buns while maintaining the integrity of the protein network within the buns. Additionally, the sensory evaluation indicators remained relatively balanced. More importantly, the addition of GP altered the digestibility characteristics of starch, increasing the resistant starch (RS)

content from 8.62% to 43.46%, and lowering the expected glycemic index (EGI) from 97.50 to 73.8, making it a medium GI (MGI) food. Furthermore, analysis by XRD and differential scanning calorimetry showed that the inclusion of GP delayed the aging of steamed buns during storage. In conclusion, an appropriate amount of GP can significantly improve the overall quality of steamed buns and has the potential as a functional ingredient to help reduce postprandial blood sugar levels.

6 Conclusion and future prospects

GUPs, as one of the main active components of *G. uralensis*, have significant potential application value in anti-inflammatory, immune regulation, antioxidant, antitumor, intestinal flora regulation, antidiabetic, and antibacterial effects. Given the importance of GUPs, this article reviews the extraction and purification methods, structural characterization, pharmacological properties, and applications of GUPs. In addition, the applications of GUPs in animal feed additives, vaccination, and food additives are systematically elaborated.

Despite significant advances in the research of GUPs, there are indeed a series of challenges that need to be addressed: 1) In the future, the separation of GUPs will benefit from new technologies such as membrane separation technology, ultrasonic-assisted extraction, and microwave-assisted extraction. These technologies significantly improve extraction efficiency and purity while reducing time and resource consumption. Membrane separation technology achieves efficient separation through selective permeation, while ultrasonic and microwave-assisted extraction enhance the permeability of solvents. Moreover, separation technologies based on nanomaterials also demonstrate good application potential. These new technologies will open up broader prospects for the extraction and application of GUPs. 2) The bioactivity of polysaccharides is closely related to their extraction, purification methods, and structural characteristics. However, existing literature on the relationship between the conformation of GUPs and their bioactivity is relatively limited, making it difficult to clarify the connection between extraction and purification methods and their bioactivity. Therefore, there is an urgent need for in-depth studies on the effects of different extraction and purification methods on bioactivity, and active exploration of the structure-activity relationship of GUPs. This will help to more comprehensively analyze the bioactivity mechanisms of GUPs and provide scientific evidence for further application research. 3) GUPs demonstrate rich pharmacological activity, and its specific mechanisms of action still require in-depth exploration. By employing techniques such as molecular biology, cell biology, and bioinformatics, a deeper study of the molecular mechanisms by which GUP regulates biological processes can be conducted, thereby providing more precise targets for drug development based on GUP. 4) GUPs should strengthen patent protection in animal feed, vaccines, and other applications. In animal feed, GUPs serve as natural additives that can enhance animal immunity and promote growth; in the vaccine field, they can improve immune efficacy. By applying for relevant patents, we can protect technological innovations, prevent imitation behavior in market competition, and promote the commercialization and industrialization of these applications. This not only helps companies safeguard their interests but also drives technological advancement and sustainable development in related fields.

Overall, GUPs exhibit significant development potential in various fields such as modern medicine, animal feed additives, vaccination, and food additives. Despite facing a situation of both

opportunities and challenges, GUPs have opened an effective pathway for the internationalization of traditional Chinese medicine. We firmly believe that GUPs have broad market prospects and application potential in drug development and functional foods. With continuous advancements in related research and technology, GUPs are expected to play an increasingly important role in enhancing human health and meeting market demands.

Data availability

Not applicable.

Conflicts of interest

The authors declared that they have no conflicts of interest.

Acknowledgements

Some cartoon elements were obtained from <https://www.figdraw.com> for mechanism drawing.

Funding

This work was financially supported by the Scientific Research Project Program of Shaanxi Provincial Education Department (No. 25JR059), the Natural Science Basic Research Program of Shaanxi Province (No. 2026JC-YBMS-0878), the Shaanxi Province Postdoctoral Research Funding Project (No. 2025BSHSDZZ390), the Science and Technology Innovative Talent Program of Shaanxi University of Chinese Medicine (No. 2024-CXTD-03), and the Natural Science Basic Research Program of Shaanxi Province (No. 2024JC-YBQN-0280).

Author contribution statement

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 Jing Sun: Investigation; Methodology; Project administration
 Fei Luan: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation
 Junbo Zou: Project administration; Resources; Software; Supervision; Funding acquisition

Informed consent

Not applicable

Ethics statement

Not applicable

Use of AI statement

None.

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