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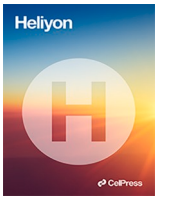

Review article

The traditional use, structure, and immunostimulatory activity of bioactive polysaccharides from traditional Chinese root medicines: A review

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ABSTRACT

As research on traditional Chinese medicine (TCM) has expanded, our understanding of the role it can have in controlling the immune system has increased. Polysaccharides from medicinal plants exhibit numerous beneficial therapeutic properties, presumably owing to their modulation of innate immunity and macrophage function. Numerous studies have demonstrated the multiple ways whereby certain polysaccharides can affect the immune system. In addition to stimulating immune cells, such as T cells, B lymphocytes, macrophages, and natural killer cells, polysaccharides stimulate complements and increase cytokine secretion. The biological functions of polysaccharides are directly correlated with their structures. This paper summarizes the sources, TCM uses, extraction and purification methods, structural characterization, in vitro and in vivo immune activities, and underlying molecular mechanisms of TCM root polysaccharides. Moreover, the structure–activity relationships of TCM root polysaccharides are emphasized and discussed. This review can provide a scientific basis for the research and industrial utilization of TCM root polysaccharides.

1. Introduction

The roots of Chinese and Mongolian herbs are important medicinal components that are richer in carbohydrate compounds than their above-ground parts, such as whole herbs, fruits or seeds. Recently, polysaccharides isolated from roots used in traditional Chinese medicine (TCM) have garnered increasing attention owing to their abundant pharmacological effects, such as antioxidant [1,2], antitumor [3,4], antidiabetic [5,6], antiviral [7], anti-inflammatory [8], and immunomodulatory activities [9,10]. Studies on TCM root tonics have demonstrated the potential in vitro and in vivo antitumor and immune-boosting effects of associated polysaccharides. Root TCM–derived polysaccharides are potent immunomodulatory substances and have been shown to be clinically therapeutic. Various beneficial pharmacological effects of root TCM polysaccharides have been attributed to their ability to modulate macrophage immune function. In general, root TCM polysaccharides can directly activate the immune function of macrophages, T or B lymphocytes, natural killer (NK) cells, and dendritic cells (DCs) as well as complements. Furthermore, these polysaccharides can promote the production of cytokines and achieve multichannel and multilevel regulation of the immune system.

Herein, polysaccharides isolated from 17 kinds of root TCMs are reviewed and mostly sources of highly promising yin-tonifying

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medicinals. Polysaccharides from root TCMs have recently become a hotspot in the field of polysaccharide research owing to their outstanding immunological activities. We review recent literature with a fundamental overview of various natural polysaccharides from root TCMs, including their sources, traditional uses, extraction methodologies, characterization, and applications for immunostimulatory activities in vivo and in vitro.

2. Source and traditional use of root TCMs

We have provided an outline of the 17 studied root herbs for plant classification and traditional medicinal functions in Table 1 and Fig. 1.

This section covers the isolation and purification methods of polysaccharides and determination of their molecular weight as well as Fourier transform infrared spectroscopy (IR), NMR analysis, periodate oxidation, partial acid hydrolysis, methylation, smith degradation, and gas chromatography–mass spectrometry (GC–MS)-based methods for investigating both monosaccharides and glycosidic linkages. A detailed schematic diagram of extraction, purification, structural analysis methods is presented in Fig. 2.

Table 1
Traditional medicinal functions and sources of the summarized root TCMs.

No.	Scientific name	Common name	Plant Sources	Traditional medicial functions	Literatures
1	Ophiopogon japonicus (Thunb.) Ker-Gawl	Mai-dong	Family Liliaceae	Thrombosis, myocardial ischemia, arrhythmias, respiratory disease and hyperglycemia	[11,12]
2	Angelica sinensis (Oliv.) Diels	Dang-gui	Family Umbelliferae	Regulating menstruation, relieving pain, anaemia, and stimulation in blood circulation, etc.	[13]
3	Codonopsis pilosula (Franch.) Nannf.	Dang-shen	Family Campanulaceae	Lower blood pressure and increase white blood cell counts, cure appetite loss, strengthen the immunize system	[14,15, 84]
4	Glehniae radix	Bei-shashen	Family Umbelliferae	Expelling phlegm and arresting coughing , lung inflammation, bronchial asthma, allergic rhinitis and for the effectiveness of immunity boosting post-chemotherapy, etc in clinics	[16]
5	Aconitum kusnezoffii Reichb	Cao-wu	Genus Aconitum	Treat heart failure congestion, neuralgia, rheumatism and gout	[17]
6	Sophora subprosrate or Sophora fla-vescens Ait.	Ku-shen	Family Leguminosae sp.	Relieve pain, reduce swelling, clean away pathogenic heat, remove toxin	[18]
7	Glycyrrhiza uralensis Fisch	Gan-cao	Family Fabaceae (Leguminosae), genus Glycyrrhiza and species Glycyrrhiza glabra, Glycyrrhiza lepidota, and Glycyrrhiza uralensis	Fortifying the spleen and supplementing Qi, and clearing heat and removing toxicity and is widely used in various Chinese medicine formulas	[19]
8	Polygonatum sibiricum	Huang-jing	Family Liliaceae	Treat cough, dizziness and lung trouble	[20]
9	Cistanche deserticola Y. C. Ma	Rou-congrong	Family Orobanchaceae	Treat kidney pain, gynaecological diseases, intestinal infection and constipation	[21,22]
10	Astragalus membranaceus (Fisch) Bge. var. mongolicus (Bge.) Hsiao	Huang-qi	Family Leguminosae sp.	Potential detoxifification, diuresis, antiperspirant, myogenic and antiageing effects	[23]
11	The radix of Platycodon grandiflorum	Jie-geng	Family Campanulaceae	Hepatoprotective, expectorant, and enhanced insulin sensitivity effects	[24,25]
12	Polygonatum odoratum (Mill.) Druce	Yu-zhu	Family Liliaceae	Used as a nutritious tonic and remedy to treat lung disease and upset stomachs, and improve insulin resistance and diabetes	[26,27]
13	Arnebia euchroma (Royle) Johnst	Zi-cao	Family Boraginaceae	Treat lung heat, mtshad and hemostasis	[28]
14	Asparagus cochinchinensis (Lour.) Merr.	Tian-dong	family Liliaceae	treat lung disease and upset stomachs, and improve insulin resistance and diabetes	[29]
15	Cynomorium songaricum Rupr.	Suo-yang	Family Cynomoriaceae	Improve immunity and kidney function and to treat constipation by relaxing the bowels	[30,31]
16	Mirabilis himalaica (Edgew) heim.	Ximalaya-zimoli	Family Nyctaginaceae	kidney warming, kidney nourishment, tissue regeneration, urination, and urinary calculus removal effects	[32]
17	Radix Sophorae Tonkinensis (R. sophorae)	Shan-dou gen	Family Leguminosoae	Used to treat sore throat, swollen gums and viral hepatitis	[33]

Fig. 1. Images of 17 kinds of root TCM.

3. Extraction and purification methods

Polysaccharides from natural sources are characterized by their large molecular weight, complex structure, and difficulty in synthesis and extraction. The higher the purity of the extracted polysaccharides, the higher the efficiency of structural characterization and biological activity. Consequently, the extraction process for polysaccharides has become fundamental to polysaccharide research. In Table 2, we summarized the extraction, isolation method used in root TCM polysaccharides and the crude polysaccharide yield.

Polysaccharides are polar macromolecules and are easily soluble in hot water but are insoluble in organic solvents. In addition to the traditional hot water extraction method, auxiliary methods such as dilute alkali/acid–water extraction [36], cellulose-assisted extraction [57,], ultrasonic extraction[22 , 27 , 37,], and accelerated solvent methods [39] are used.

The principle of ultrasonic-assisted extraction is using ultrasonic waves to disrupt the cell wall and accelerate the release of cell contents, thereby improving extraction efficiency. This has the advantages of simple operation, easy control, short time, and high yield,

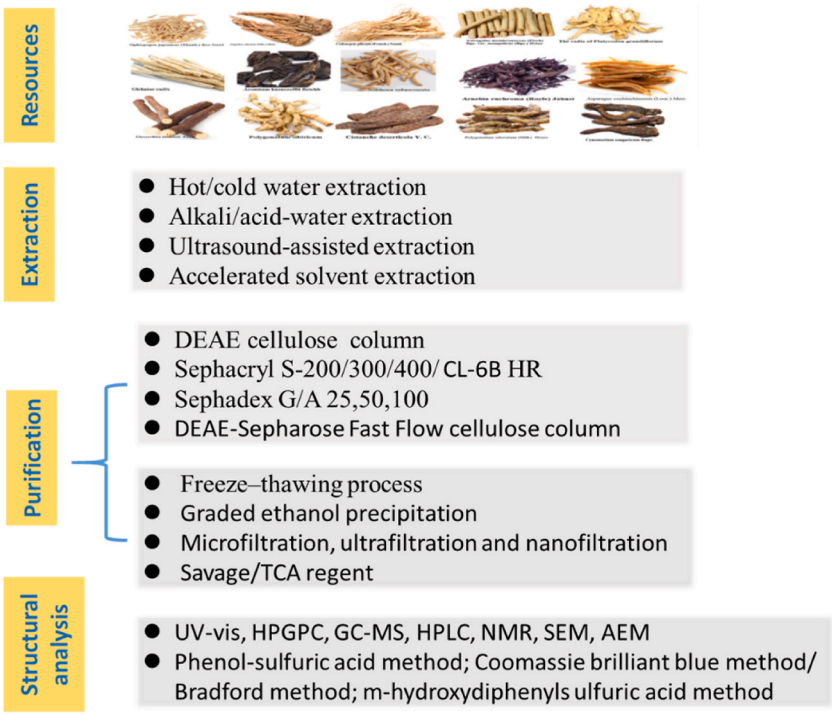

Fig. 2. Summary of sources, extraction, purification and structural analysis methods.

and is often used for extracting polysaccharides [45].

Enzymes have strong specificity and high selectivity. Enzyme extraction has the advantages of mild conditions, high product extraction rates, short extraction times, and easy control, and is often combined with other methods to extract polysaccharides. Zeng et al. found the crude polysaccharide from *Cistanche deserticola* Y. C. Ma has been extracted using hot water combined with enzymes (30 g cellulase, 10 g amylase, and 5 g papain), and the extraction rate was higher than that with hot water alone [57]. The crude polysaccharide from Ophiopogon japonicus (Thunb.) Ker-Gawl was extracted by hot water with 3 % papain and the extraction rate was up to 55.2 ± 1.86 % [54].

From the summarized Table 2, we found hot water extraction is most widely employed and easy to perform; however, it is time-consuming and unsuitable for polysaccharides that do not dissolve easily in hot water. For polysaccharides with poor solubility, the researchers applied ultrasound-assisted extraction [22,27,45,37], microwave-assisted aqueous two-phase extraction [60] and dilute alkali/acid-water extraction method [36,48] for higher efficiency extraction rate.

Preliminary extraction usually produces crude polysaccharides that often contain impurities, such as oils, proteins, and pigments, and have a relatively wide distribution of relative molecular mass. Therefore, additional purification steps of these polysaccharides are required to enable their structure characterization and the subsequent biological assay Commonly used purification methods include graded ethanol precipitation [41,52], different column chromatography, such as macroporous resin column chromatography, ion-exchange chromatography, and gel chromatography, or a combination of multiple methods for polysaccharides purification. Anion exchange chromatography packed with DEAE cellulose [16,61] and its derivatives [17,36,34,35,84] is widely used for polysaccharide decolorization and classification of polysaccharides, after that graded alcohol precipitation [41,52], ultrafiltration [22,42] and gel column chromatography were mainly applied for further separation in root TCMs polysaccharide study. Gel column chromatography has the property of molecular sieve and is mostly used for the fine separation of polysaccharides. The commonly used stationary phase gel packing materials are explored sephadex dextran gels [19,30,36,38,40,55,65], sepharose agarose gels [61,] and sephacryl series polyacrylamide gels [44,49,50]. In the same Table 2, we furthermore resumed deproteinization method and purification methods in detail by different column from 17 studied root herbs.

4. Structural analysis methods

Several techniques have been developed to appraise the primary structure of polysaccharides. Sugar composition, nuclear magnetic resonance, and methylation analysis are currently the most popular methods for investigating glycosidic linkages in polysaccharides. Qualitative GC–MS can be useful for identifying and characterizing polysaccharides. Polysaccharides from root TCMs mainly have been characterized by different spectral methods in Table 3. Polysaccharides are classified based on their composition, function, and origin. Depending on the monosaccharide units, such as glucose, galactose, fructose, and mannose, polysaccharides are classified into two groups: homopolysaccharides, which contain only one kind of polymerized sugar unit, such as starch, xylan, galactan, and fructan,

Table 2
Summary of extraction, separation, and deproteinization methods and crude polysaccharide yield from 17 kinds of tonic root medicines.

No.	Scientific name	Extraction method	Crude polysaccharide yield (%)		purification method	Deproteinization method
1	Glehniae radix	Hot water extraction and ethanol precipitation	14.35	DEAE-52 Cellulose anion exchange , Sephadex G-75 gel filtration column	Sevage method combined with papain	[16 , 41]
2	Aconitum kusnezoffii Reichb	Hot water extraction	6.0 %	Freeze–thawing process (water-insoluble polysaccharide	No	[17]
		Cold water (4 °C, 1:20, w/v) three times (6 h for each) and ethanol precipitation (95 %)	No	DEAE-Sepharose Fast Flow ion-exchange chromatography, Sepharose CL-6B gel filtration chromatography	Sevag reagent	[17]
3	Angelica sinensis (Oliv.) Diels,	Hot water extraction (80 °C) and ethanol precipitation (75 %)	10.8 %	DEAE-Sepharose CL-6B	No	[34]
		Hot water extraction (80 °C) and ethanol precipitation (60 %)	2.2 %	DEAE-Sephadex A-25 column; Sephadex G-100	No	[35]
		Alkali extraction at 100 °C for 1 h and ethanol precipitation (75 %)	2.2 %	DEAE-sephadex A-25 column , Sephadex G-100 column	Sevage method	[36]
		ultrasound-assisted extraction	6.92 ± 0.17 %	No	No	[37]
4	Codonopsis pilosula (Franch.) Nannf.	Water decoction and ethanol precipitation	No	DEAE-52 and Sephadex G-200 columns	Sevage method	[38]
		Accelerated solvent method(ASE)	No	Anion exchange column packed with ANX SepharoseTM 4 Fast Flow	No	[39]
		Hot water extraction (80 °C) and ethanol precipitation (80 %)	9.7 %	DEAE-650 M column chromatography Superdex G-200	No	[40]
		Hot water (90 °C for 2 h)	No	Graded precipitation with 0.5, 1, 3, and 4 vol of ethanol in sequence	Sevag method	[41]
		Hot water extraction (90 °C for 2 h) and ethanol precipitation	9.7 %	Ultrafiltration with 1×10^4 and 3×10^4 Da membrane	Sevage method	[42]
5	Glycyrrhiza uralensis Fisch	Hot water extraction and ethanol precipitation (85 %)	15.1 %	Sephadex G-75 and DEAE-32 column chromatography	No	[19]
		Hot water (1:9, w/v) at 80 °C for 1 h and dehydrated ethanol at 4 °C for 24 h	No	DEAE-52 flow column, Sephadex G-100 column	No	[43]
		90–95 °C and at a water/root mass ratio of 10:1 (v/m) for 3–4 h	No	DEAE- cellulose column, a Sephacry S-400 HR column	15 % (m/m) of trichloroacetic acid	[44]
		The ultrasonic assisted extraction (UAE) associated with central composite design (CCD)	3.86 %	DEAE-52 cellulose chromatography column and sephadex G-100 column	No	[45]
		1:5 petroleum ether twice at 60°Cfor 1 h; 1:5 80 % ethanol twice at 60 °C for1 h. distilled water in water bath at 60°Cfor 2 h	2.73 %	DEAE-52 cellulose chromatography column	No	[46]
6	Polygonatum sibiricum	Hot water extraction and ethanol precipitation (85 %)	4.45 %	No	Savage reagent	[20]
		Hot water extraction and ethanol precipitation (60 %)	5.91 %	DEAE-cellulose Sephadex G-75	TCA reagent	[21]
		Water extraction and alcohol sedimentation	4.45 %	DEAE-Sepharose Fast Flow	Savage reagent	[47]
		0.1 M sodium hydroxide solution with a ratio of 1:10 (w/v) at 100 °C for 3	14.9 %	DE52 column and Superdex-200 column	No	[48]

(*continued on next page*)

Table 2 (*continued*)

No.	Scientific name	Extraction method	Crude polysaccharide yield (%)		purification method	Deproteinization method
		h and precipitation 95 % ethanol				
7	Astragalus membranaceus (Fisch) Bge. var. mongolicus (Bge.) Hsiao ,	Hot water extraction and ethanol precipitation	8.81 %	DEAE-cellulose and Sephacryl S-400 columns	No	[49]
8	The radix of Platycodon grandiflorum,	Hot water extraction and ethanol precipitation	No	DEAE-cellulose column Sephacryl S-300 HR	No	[50]
		Hot water extraction and ethanol precipitation	4.57 %	DEAE-cellulose column Sephacryl S-400/HR column	Sevage reagent	[51]
		Hot water extraction and ethanol precipitation	6.7 %	DEAE cellulose Sephacryl S-300 HR	TCA reagent	[50]
		Hot water extraction and ethanol precipitation	No	Graded precipitation; Sephadex A-25 column	Sevage reagent	[52]
9	Ophiopogon japonicus (Thunb.) Ker-Gawl	Hot water extraction and ethanol precipitation	30.36 % ± 0.87 %	DEAE-Sepharose fast flow column and Sepharose 6 Fast Flow gel chromatographic column	TCA reagent	[53]
		Hot water extraction (100 °C for 30 min 3 times) with 3 % papain and ethanol precipitation	55.2 ± 1.86 %	DEAE-52 cellulose columns	No	[54]
		Hot water extraction (90 °C for 3 h, 3 times) and ethanol precipitation (95 %)	No	DEAE-52 cellulose anion-exchange and Sephadex G-100 gel column	Sevag method	[55]
10	Cistanche deserticola Y. C. Ma	Water extraction at RT	1.63 %	DEAE cellulose column and Sephacryl S-300 column	No	[56]
		Hot water extraction combined with enzymes (30 g cellulase, 10 g amylase and 5 g papain)	2.9 %	DEAE Sepharose™ Fast Flow column (5 cm × 50 cm),	No	[57]
11	Radix Sophorae Tonkinensis (R. sophorae)		NO	DEAE-52 cellulose Sephacryl™ S-200 gel column	Sevag method	[58]
		Hot water extraction (100 °C) and ethanol precipitation (1.5 vol of 95 % EtOH)	No	DEAE-Cellulose column Sepharose CL-6B column	No	[59]
		Microwave-assisted aqueoustwo-phase extraction (MAATPE)	8.52 ± 0.21 % (w/w , top) and 2.18 ± 0.08 % (w/w botton)	H_2O_2 solution (w/v, 30 %)	Sevage	[60]
12	Cynomorium songaricum Rupr.	Hot water extraction and ethanol precipitation	9.1 %	DEAE-52 cellulose column; Sepharose CL-6B column	Sevage method combined with papain	[61]
		Hot water extraction and ethanol precipitation	1.25 %	Sephadex G75 and G50 column	NO	[30]
		Hot water extraction and ethanol precipitation	No	No	Sevage method, combined with papain	[62]
13	Polygonatum odoratum (Mill.) Druce,	Ultrasonically extraction (RSM) method and ethanol precipitation (80 %)	15.76 %	No	No	[27]
		Hot water extraction (100 °C) and ehtonal precipitation (80 %)	11.77 %	No	No	[27]
		Hot water extraction and ethanol precipitation	9.9 %	DEAE-Sepharose Fast Flow cellulose column and Sephadex G100	Sevage method	[63]
14	*Mirabilis himalaica* (Edgew) heim.	Cold water and ethanol precipitation	10 %	DEAE-cellulose and Sephadex G-100 column	No	[11]
	Arnebia euchroma (Royle) Johnst	Hot water extraction and ethanol precipitation	No	No	TCA regent	[28]

(*continued on next page*)

Table 2 (*continued*)

No.	Scientific name	Extraction method	Crude polysaccharide yield (%)		purification method	Deproteinization method
16	Asparagus cochinchinensis (Lour.) Merr.	ultrasonically extraction (RSM) method combined with macroporous resin (HPD 300)	No	microfiltration, ultrafiltration and nanofiltration (nominal molecular weight cut-offs were 300 kDa, 10 kDa and 200 Da	Sevag method	[22]
17	Sophora subprosrate or Sophora fla-vescens Ait.	Hot water extraction and ethanol precipitation (80 %)	7.28 %	DEAE-Sepharose Fast Flow column	Sevage method	[64]
		Hot water extraction and ethanol precipitation (95 %)	No	DEAE-cellulose and Sephadex G-100 column	No	[65]
		Hot water extraction and ethanol precipitation (95 %)	6.58 %	DEAE-52 cellulose column	Sevage method	[66]

and heteropolysaccharides, which have two or more types of sugar units, such as pectin [67].

The study of the monosaccharide composition of polysaccharides is the basis for analyzing the structure of polysaccharides. Usually, after the hydrolysis of polysaccharides into monosaccharides, the detection of monosaccharide composition of polysaccharides is realized by TLC, HPCE, GC, HPAEC and HPLC after derivatization, neutralization and filtration operations. Monosaccharide composition of polysaccharides and their content ratio was determined for further structural analysis.

Monosaccharide composition in 54 of Polysaccharides from 17 root TCMs were determined by the spectral methods GC, GC-MS, HPLC in Table 4. Polysaccharides that mainly comprise galactose and arabinose have been identified in *Codonopsis pilosula* (Franch.) Nannf., *Aconitum kusnezoffii* Reichb., *Arnebia euchroma* (Royle) Johnst., *Angelica sinensis* (Oliv.) Diels, *C. deserticola* Y. C. Ma, *Polygonatum sibiricum*, and the radix of *Sophorae tonkinensis* (R. Sophorae) and *Platycodon grandiflorus*. Polysaccharides that principally comprise glucose have been found in *Astragalus membranaceus* (Fisch) Bge. var. mongolicus (Bge.) Hsiao, *Sophora subprosrate*, *Ophiopogon japonicus* (Thunb.) Ker Gawl., and *Cynomorium songaricum* Rupr., while Glehniae radix. *Polygonatum odoratum* (Mill.) Druce contains a heteropolysaccharide, mainly comprising fructose.

To further obtain more structural information, chemical methods like periodate oxidation, smith degradation and methylation analysis were used and spectral methods such as NMR, IR, UV–vis, HPLC, GC-MS, SEM and AEM for structural analysis of herbal polysaccharides (Table 3). We summarized the primary structure of polysaccharides measured by NMR and methylation method in Table 4.

5. In vitro immunomodulatory activity

The immune system perceives invading pathogenic microorganisms as well as mutated cells and senescent apoptotic cells in the body as “nonself” substances. The immune response is the process whereby the immune system identifies and removes “nonself” substances, and can be divided into two categories: innate immunity (nonspecific immunity) and adaptive immunity (specific immunity). Innate immunity is the first line of defense against pathogens that have evolved over time. The cells involved in innate immunity, such as monocytes, macrophages, DCs, and NK cells, are not as specific as T cells or B cells in recognizing immunogens, but can recognize pathogen-associated molecule patterns (PAMPs) of specific pathogens via pattern recognition receptors (PRRs). We summarize the immune regulatory mechanisms of root TCM polysaccharides in vitro in Fig. 3.

Table 3
Chemical and spectral methods for polysaccharide characterization and measurement.

Chemical and Spectral methods		Characterization and measurement
Fourier transform infrared spectroscopy	FT-IR	Functional group; Sugar ring form (pyran or furan)
High performance liquid gel filtration chromatography, Light scattering method	HPGPC	Homogeneity and Molecular weights
Monosaccharide composition; methylation analysis; Periodate oxidation; Smith degradation; Partial Acid Hydrolysis	GC-MS HPLC	Sequence of constituent Monosaccharide, Anomeric configuration of glycosidic linkages
Monosaccharide composition		Sugar composition
Nuclear magnetic resonance spectroscopy	NMR	Composition, sequence distribution, and substitution pattern
Phenol-sulfuric acid method;	Uv–vis	Sugar/carbohydrate Content
Coomassie brilliant blue method/Bradford method;		Protein content
m-hydroxydiphenyls ulfuric acid method		Uronic acid content
		Specific rotation
Scanning electron microscope	SEM	Morphology and surface Ultrastructure
Atom electron microscope	AEM	Molecular morphology

5.1. T cells

T lymphocytes mature in the thymus and are the most important immune cells, whose main function is to mediate cellular immunity and regulate immune responses. T cells are activated during cellular immune response to proliferate and differentiate by recognizing specific antigens. T cells can then produce various immunological effects through different cell subsets, thus playing important roles in clearing intracellular pathogenic infections, rejecting allografts, and mediating antitumor immune responses. Research has demonstrated that the active ingredients in Chinese herbal compounds and single herbal medicines can enhance T cell–mediated immune responses by influencing their activation and proliferation and their secretion of cytokines and exertion of cytocidal effects. Under normal conditions, the $CD4^+$ subpopulation has helper and inducing functions, whereas the $CD8^+$ subpopulation has suppressive and cytotoxic functions, and a reduced $CD4^+/CD8^+$ ratio is an important indicator of immune deficiency.

Polysaccharides isolated from *S. subprosrate*, *A. sinensis* Diels, *Glycyrrhiza uralensis* Fisch., *O. japonicus*, *C. pilosula* (Franch.) Nannf., and *C. deserticola* Y. C. Ma can have a regulatory effect on the immunity of the body by affecting T cell function.

SSP1, a polysaccharide isolated from *S. subprosrate*, can activate T cells to release NO and secrete interleukin (IL)-2 by modulating the activity of protein kinase C (PKC) and level of intracellular free Ca [84].

An *A. sinensis* polysaccharide (AP) purified from the fresh root of *A. sinensis* Diels promoted the proliferation of total spleen cells, macrophages, and T cells. Treatment with AP increased the production and gene expression of IL-2 and IFN-γ while that of IL-4 was decreased; expression of Th1- and Th2-related cytokines was also regulated [35].

Polysaccharides from *G. uralensis* did not inhibit proliferation of IEC-6 cells even at high concentration. The ED_{50} value was found to be 100 μg/mL. However, these polysaccharides did inhibit the proliferation of cancer cells (CT-26) at a concentration of $\leq$50 μg/mL. Within 72 h of treatment with these polysaccharides, expression of the IL-7 gene was upregulated twofold. IEC-6 cells secrete IL-7 into media when treated with *G. uralensis* polysaccharides, which stimulated proliferation of freshly isolated T lymphocytes within 6 h. The effect of *G. uralensis* polysaccharides was found to depend on molecular weight, with low-molecular-weight polysaccharides having a more profound effect than those of high-molecular-weight and total crude extracts [82].

C. pilosula polysaccharide (CPPS) (Franch.) Nannf. and modified CPPS (selenizing CPPS (sCPPS)) increased the ratio of $CD4^+$ to $CD8^+$ T cells and level of IgG, IgM, IFN-γ, IL-2, and IL-4 in mouse sera [38].

Overall, the polysaccharides extracted from root TCMs can activate the T cell to secrete several related cytokine like NO, IL-2, IFN-γ as on in vitro tests, meanwhile, can increase the ratio of $CD4^+$ to $CD8^+$ T cells and level of IgG, IgM, IFN-γ, IL-2, and IL-4 in vivo.

5.2. B cells

B cells are important immune cells that mediate humoral immunity. Mammalian B cells differentiate and mature in the bone marrow and migrate to the periphery, where they are activated and proliferate upon encountering antigens. Various tonic herbs, such as *Astragalus*, *Ginseng*, Radix *A. sinensis*, *Epimedium*, *Cuscuta*, and *Acanthopanax*, play roles in promoting antibody production.

Niu et al. [49] reported that APS polysaccharide Purified from Astragalus membranaceus (Fisch) Bge. var. mongolicus (Bge.) Hsia were shown to be the main responders to the stimulation of mouse splenic B cells in vitro. This may be valuable for research purposes when B cells are required in a greater quantity than T cells. Zhang et al. [42] demonstrated that a proteoglycan extract from *G. lucidum* also increased the proliferation of mouse splenic B cells in a greater amount than that from T cells. Conversely, Liu et al. [21] showed that polysaccharide extracted from sea urchin eggs stimulated higher proliferation of T cells compared with B cells. Thus, various polysaccharides have different activities on splenic cells, but the mechanism for this stimulation is not yet well understood.

Bioactivity tests of the two major polysaccharide fractions, CDA-1A and CDA-3B, isolated from the cold-water extract of *C. deserticola* Y. C. Ma, showed that CDA-1A was inert for T cell proliferation stimulation but active for B cell proliferation, whereas CDA-3B is potent for the stimulation of both T cell and B cell proliferation [56].

P. sibiricum polysaccharide (PSP) accelerated recovery of spleen and thymus indexes and enhanced T cell and B cell proliferation responses as well as peritoneal macrophage phagocytosis. Additionally, PSP treatment restored the levels of IL-2, tumor necrosis factor (TNF)-α, IL-8, and IL-10 in the serum of the cyclophosphamide (Cy)-treated mice in a dose-dependent manner [87].

5.3. Macrophage cells

Monocyte-macrophages are the main effector cells in anti-infection immunity. After the body is infected, monocyte-macrophages can recognize and engulf pathogens to kill and remove them as well as enhance immune response by secreting cytokines and chemokines to mediate and promote inflammatory response. Furthermore, monocyte-macrophages can remove damaged and senescent cells from the body and play an important role in immune surveillance. Studies have shown that polysaccharides from root tonic medicine, such as Glehniae radix, *Polygonatum sibiricum*, *P. odoratum* (Mill.) Druce, *C. pilosula* (Franch.) Nannf., *O. japonicus* (Thunb.) Ker Gawl., *A. kusnezoffii* Reichb., and *A. sinensis* (Oliv.) Diels, have a promoting effect on the monocyte-macrophage system.

Polysaccharide fractions isolated from *A. kusnezoffii* Reichb. significantly enhanced splenic lymphocyte proliferation and macrophage phagocytosis activity [17]. Similarly, polysaccharides isolated from *P. grandiflorus* (Jacq.) and *P. odoratum* significantly increased phagocytic rates and proliferation of macrophages [27 , 40]. *P. odoratum* and *C. pilosula* polysaccharides markedly enhanced cell viability and RAW264.7 cell and mouse spleen lymphocyte phagocytic activity.

Polysaccharides from *P. grandiflorus* (Jacq.) [52] and *P. odoratum* [27] and two pectin-type polysaccharides from *C. pilosula* [37] stimulated the in vitro release of NO from macrophage-like cells and expression of several immune-related molecules, including TNF-α , IL-1β, and IL-6. Polysaccharides isolated from *P. grandiflorus* (Jacq.) [52]and *P sibiricum* [27,88] stimulated macrophages to

Table 4
Composition of monosaccharides and molecular weights, structures, and bioactivities of polysaccharides isolated from 17 kinds of root TCM.

Origin	Compound name	Molecular weight	Monosaccharide composition	NMR	Methylation	Biological activities	References
1. Platycodon grandiflorum	1. PGP_{80} 2. PGP_{60} 3.PGP_{St}	PGP80 101.0 KDa 4.14 KDa PGP60 93.4 KDa 3.0 KDa PGPSt 166 KDa 1.72 KDa	Glu:Man:Ara:Gal:Xyl:Rha = 54.3:7.3:16.8:15.0:1.0:5.5 62.6:6.1:13.9:10.5:0.7: 6.0 55.3:22.3:10.7:8.1:2.5:0.8	(1 → 3)-d-Glcp-(1 → 6)-d-Glcp residues.	No	Chicken peritoneal Mφ (no obvious AT) Phagocytic rates, proliferation, NO,TNF-α, IL-1β, IL-6↑ Peripheral Lymphocyte T lymphocyte proliferation↑, G0/G1 phase to the S↑,G2/M phases↑, CD^{4+}, CD^{8+} T cells↑	[52] [68]
	PG	ND	inulin-type polysaccharide with a β (2 → 1) linked D-fructose	No	No	DC cell the expression of CD40, CD80, CD86↑, (MHC)-I/II↑, (IL)-12, tumor necrosis factor-a, IL-1β, IL-6, IL-10, and interferon-b↑, phosphorylation of ERK, p38, and JNK, and the nuclear translocation of p-c-Jun, p-CREB, and c-Fos↑,NF-κB signaling↑, IκBα/β, nuclear translocation of p65, p50↓ polyclonal IgM ↑ , B cells↑ iNOS , NO ↑ ,	[69] [24]
	PGAW1	9.2 kDa	Ara: Gal = 1.42:1.0	1,4- and 1,6-linked galactopyranosyl residues, with branches attached to O-3 of 1,6-linked galactose residues.	No	Antiangiogenic Activity Tube formation by human microvascular endothelial cells (HMEC)↓	[70]
	PGA4-3b	8.9 kDa	only GalA	a linear poly-(1 → 4)-a-D-galactopyranosyluronic acid	non-reducing terminal (5 %) and (1 → 4)-linked galactopyranose (Galp) (95 %)	Anti-angiogenesis assay cell tube formation↑	[50]
	ROH05	16.7 kDa	Only Gal	the backbone of 1, 4-linked β-D-galactose and 1, 4, 6-linked β-D-galactose,the branch of terminal-linked β-D-galactose attached at C-6 position of 1, 4-linked β-D-galactose	terminal Galp, 1, 4-linked Galp: 1, 4, 6-linked Galp = 9.5 %:79.0 %:11.5 %	anti-pancreatic cancer activity	[71]
2. Ophiopogon japonicas	OJP2	35.2 kDa	Rha: Ara:xyl: glu: gal = 0.5:5:4:1:10	No	2,5-Me-Xyl (1,3-linked Xyl), 2,3,4-Me-Gal (1,6-linked Gal) and part of Ara were major components of the backbone structure	Antioxidant activity , H_2O_2-Treated HaCaT Cells Glucose-Treated LO_2 Cells : SOD , NO↑ , MDA↓	[77 , 84]
	OJP-1 OJP-2	2735, 124,300,	1. Man, Glu 2. Rha, Xyl, Ara, Gluc	No	No	Antioxidant activity DPPH, •OH radical	[53]

(*continued on next page*)

Table 4 (*continued*)

Origin	Compound name	Molecular weight	Monosaccharide composition	NMR	Methylation	Biological activities	References
	OJP-3 OJP-4	324,652 , 6746 Da	3. Ara, Glu 4. Rha, Glu			scavenging activity↑, Phagocytic capacity, energy metabolism rate, NO and IL-1 ↑	
	POJ-U1a	4.02 × 103 Da	Only Glc	α-configuration polysaccharide with a highly branched structure, and consisted of pyranoside and funanside	1,6-α-D-glucopyranose and 1,3,6-α-D-glucofuranose in the molar ratio of 7:3, while the branched chains were mainly composed of 1,3-α-D-glucopyranose and 1-α-D-glucopyranose in the molar ratio of 1:3.	Antioxidant acitivity	[72]
	ROH05	16.7 kDa	Gal only	1, 4-linked β-D-galactose and 1, 4, 6-linked β-D-galactose, while the branch of terminal-linked β-D-galactose attached at C-6 position of 1, 4-linked β-D-galactose	terminal Galp, 1, 4-linked Galp , 1, 4, 6-linked Galp = 9.5 %:79.0 %:11.5 %	ND	[71]
	OJP	4925Da	Fru:Glu = 29:1	Fru : Glu = 29 : 1	Fruf-(2→, →2)-Fruf-(6→, →6)-Glcp-(1→ and →1, 2)-Fruf-(6→ = 6.8:15.8:1.0:5.8	ND	[54]
	OJP	5000Da	ND	No	No	the splenocyte proliferation, the proportion of $CD4^+$ and $CD8^+$ T cells, f IFN-γ, IL-2, IL-4 and IL-6, antigen-specific antibody titers (IgG, IgG1, IgG2a and IgG2b), immune organ index in the mice immunized	[73]
3. Cistanche deserticola Y. C. Ma	CDA-3B	87 KDa	Rha:Ara:Gala: Glu:=0.23:1.99:1.00:0.47	No	The high proportion of non reducing terminals (1-linked arabinose) and branch points (1,3,5-linked arabinose and 1,3,6-linked galactose) indicate that the polysaccharide possesses a highly branched structure	CDA-3B effective both for T- and B-cell proliferation when the concentration is 10 lg/mL or higher.	[56]
	CDA-1A	10 KDa	Glu	a-(1–4)-D-glucan with a-(1–6)-linked branches attached to the O-6 of branch point	1-, 1,4-, 1,6–1,4,6-linked Glu = 1:5.8:3.5:0.8	CDA-1A inactive for T-cell proliferation but active for B-cell proliferation,	[56]
	CDA-0.05	7.96 kDa	Glu: Gal = 96.4: 3.6	1, 4-linked α-D-Glcp, 1, 4, 6-linked α-D-Glcp and 1, 4-linked β-D-Galp, with branches of T-linked α-D-Glcp attached at C-6 of 1, 4, 6-linked α-D-Glcp residues.	1, 4-linked Glc: 1, 4-linked Gal: 1, 4, 6-linked Glc:Terminal (T)-linked Glc = 64.3 %:3.5 %:16.1 %:16.1 %	intestinal bacteria strains	[57]
	CDP-A CDP-B CDP-C	CDP-A: 4000 kDa and 3946 kDa; CDP-B: 2400 kDa CDP-C: 1300 kDa	Man, Rha, GalUA, Glc, Gal, Ara	No	No	Antioxidant acitivity	[22]

(*continued on next page*)

Table 4 (*continued*)

Origin	Compound name	Molecular weight	Monosaccharide composition	NMR	Methylation	Biological activities	References
4. Glehniae radix	GRP	1.33 × 10^4 Da	Glu only	α-D-glucan containing (1 → 6)-linked and (1 → 3)-linked backbone with a branch of (1 → 6)-linked and terminal glucoses.	(1 → 3), (1 → 6), (1 → 4, 6), terminal Glu = 1:13:1:1	proliferation of splenic lymphocyte, RAW264.7 cells and A549 cell production of nitric oxide (NO) ，A549 cells proliferation ↓	[16]
5. Aconitum kusnezoffii Reichb	WKCP-A WKHP–N(Pectin polysaccharide); WKHP-A, WKCP-N (Neutral polysaccharide)	WKCP-A 160.2 KDa WKHP–N 113.4 KDa WKHP-A 169.6 KDa WKCP-N 9.6 KDa	WKCP-N and WKHP–N： mainly Glu WKCPA： large amounts of Gal and Ara	No	No	WKCP-A had good antioxidant and immunostimulatory activities：DPPH radical, hydroxyl radical, superoxide anion, H2O2 and self-oxidation of 1,2,3-phentriol, ferrous ion-chelating ability and reducing power; Lymphocyte proliferation activity; Macrophage phagocytosis activity	[17]
	AKP	1.4 × 105 Da	Only Glc	a linear -(1 → 3),(1 → 4)-d-glucan, of which the (1 → 3)-linked and (1 → 4)-linked -d-glucopyranosyl residues	(1 → 4)-linked with a number of (1 → 3)-linked Glcp units in a molar ratio of 7:1.	Antioxidant activity	[74]
6. Angelica sinensis (Oliv.) Diels	APFs	ND	Crude; APF1; APF2 Rha,Ara,Man, Glu,Gal = 1.00:2.72:0.72:4.00:2.32; 1.00:5.29:3.66:9.11:5.17; 1.00:4.54:2.98:11.09:7.45 APF1: Rha, Ara, Glu, Gal = 1.00:2.27:7.80:2.69	No	No	macrophages in vitro ，TNF-a ，Cellular lysosomal enzyme activity↑	[75]
	ASP3 (pectic polysaccharides)	3.4 × 10^4 Da	GalA,Rhy,Ara,Man, Glu,Gal = 58.27 1.87 10.50 0.37 0.94 24.93	No	No	ASP3 can protect leucocytes and lymphocytes of mice against radiation induced damage	[34]
	AP	50,000	Rha, Ara, Man, Glu, Gala = 1.00:4.54:2.98:11.09:7.45	No	No	Immunomodulatory activity (免疫调节活性) total spleen cells, macrophages and T cells were promoted IL-2 and IFN-γ ↑, IL-4↓,	[76]
	ASP3	5.1 kDa Pectin polysaccharide	Rha,Ara,Man,Glc,Gal = 1.9:10.5:0.4:0.9:24.9	No	2,3,5-Me3-Ara, 2,3,4,6-Me4-Glcp, 2,3,6-Me3-Glcp, 2,3,4-Me3-Glcp, and 2,3-Me2-Glcp, in the molar ratio of 1:1:7:5:1	ND	[77,78]
	ASP	80 kDa (acidic polysaccharide)	GlcA, Glucose, Arabinose, Gala = 1.00:1.70:1.85:5.02	Yes (1D,2D NMR)	(1 → 3)-linked Galp, (1 → 6)-linked Galp and 2-OMe-(1 → 6)-linked Galp with three branchesattached to O-3 of 2-OMe-(1 → 6)-linked Galp and terminated with GlcpA	HepG2, MCF-7 and A549 cells	[64,79]

(*continued on next page*)

Table 4 (*continued*)

Origin	Compound name	Molecular weight	Monosaccharide composition	NMR	Methylation	Biological activities	References
					and Araf, and all of Araf and the majority of Glcp are distributed in branche		
	APS-1II.	42.1 kDa	Ara:Glc: Fuc = 2.48:1.05:1.00.	Yes (1D,2D NMR)	1,3-α-L-Araf and 1,6-α-D-Glcp with the branches containing 1,5-α-L-Araf, 1,4-β-D-Glcp, T-β-D-Glcp, 1,3-α-L-Fucp and T-α-L-Fucp	In vivo, TNF-α, IFN-γ,IL-2 ↑, splenic lymphocyte proliferation ↑; in vitro, phagocytic activity of peritoneal macrophages↑; cytotoxic activity of NK cell ↑	
7.Codonopsis pilosula (Franch.) Nannf.	RCNP (Neutral polysaccharide) RCAP-1 RCAP-2 (Pectin polysaccharide)	1.14×10^4 5.09×10^4 2.58×10^5	Mainly Gal and Ara	1D NMR	RCNP: arabinan and AG regions RCAP-1, RCAP-2: HG region composed of (1 → 4)-linked GalAp and methyl-esterified GalAp residues and small amounts of RG-I regions.	RAW 264.7 cells NO production ↑	[40]
	CPP1b	1.45×10^5 Da Pectin polysaccharide	Rha, Ara, Gal, GalA = 0.25:0.12:0.13:2.51	Yes (1D,2D NMR)	1,2-linked Rhap, terminal Ara, 1,2,6-linked Galp and 1,4-linked Galp = 0.21:0.08:0.10:1.95	NO	[41]
	50WCP-II-I 50WCP-II-Ia 100WCP-II-I 100WCP-II-Ia 100WCP-II-Ib. pectic polysaccharide	71.6 17.3 53.2 17.3 1.3 KDa	Ara:Rha:Fuc:Xyl:Man:Gal:Glu: GlcA:GalA =	Yes (1D,2D NMR)	Yes	Complement fixation activity	[39]
	CPPS sCPPS	345 Da; 230.6 KD, 73.3 KD, and 5.3KD	Man:GlcN:Rha: GalUA: Glu:Gal: Ara = 1.1:0.4:1.2:1.4:13.8:52.2:29.9 0.4:0.2: : :96.3:2.1::1.0	Yes (1D,2D NMR)	No	lymphocyte proliferation↑, ratio of $CD4^+$ to CD8 + T cells↑, IgG, IgM, IFN-γ, IL-2 and IL-4↑	[38]
	CPPS3	7.4 × 104 Da	Gal, Ara,Rha = 1.13:1.12:1	(1 → 3)-linked-β-GalpNAc, (1 → 3)-linked-α-Rhap and (1 → 2,3)-β-Galp.	Araf(14 %), 2-O-subst.-Rhap(7 %) and 3-O-subst.-Rhap(22 %), 3-O-subst.-Galp (15 %), 4-O-subst.-Galp (12 %) and 2,3-di-O-subst.-Galp units(13 %)	No	[42]
	CPSP-1 pectic polysaccharide	13.1 KDa	Ara:Rha:Gal:GalA:Glc: GlcA:=8.9:9.3:11.0:70.1:Tr:Tr	No	rich in arabinogalactan type II (AG-II) structures	Antioxidant activity	[80]
	LMw-CPP	3.24 × 103Da	Fru: Glc = 2.73:1.00	1D NMR β-fruf and α-Glcp.	No	immune organs index↑, the activities of splenic T/B lymphocytes, NK cells and macrophages↑, IL-2, TNF-α, and IFN-γ↑,in CTX-treated immunosuppression mice	[81]
8. Glycyrrhiza uralensis Fisch	GUPs-1 GUPs-2 GUPs-3	10,160, 11,680 13,360 Da	Glu, Gala,Ara,Rha, Man = 23.4:25.18:8.32:nd:nd 14.25:25.67:17.54:0.61:0.68 1.13:22.04:31.44:3.36:1.95	No	No	Anti-oxidant activity	[43]
	GP	10 kDa	No	No	No	pinocytic activity, the production of nitric oxide (NO), interleukin-1 (IL-1), IL-6 and IL-12	[44]

(*continued on next page*)

Table 4 (*continued*)

Origin	Compound name	Molecular weight	Monosaccharide composition	NMR	Methylation	Biological activities	References
	GPs1 GPs2 GPs3	10,160, 11,680 13,360 Da	Ara: Glu:Gal = Nd: 56.08:23.97 16.6:66.42:19.72 0.48:48.88:19.89	No	No	No	[45]
	Fraction A Fraction B Fraction C	over100 kDa 75 kDa and under 10 kDa 290 kDa and 14 kDa	No	No	No	Proliferation of IEC-6 cells↑,proliferation of CT-26↓,IL-7↑, T lymphocyte proliferation↑	[82]
	GUPS-I GUPS-II GUPS-III	1.06, 29.1, 14.9 kDa	Rha:Xyl:Glc = 2.55:1.9:30.03 Rha:Ara:Man:Glc:Gal = 1:13.87:1.59:16.76:15.72 Ara:Man:Glc:Gal = 1:5.33:1.91:5.97	No	GUPS-II and GUPS-III were flaky with a smooth surface and contained α- and β-glycosidic linkages GUPS-I was granulated and contained only α-glycosidic linkages	the expression of MHC-I, CD40, and CD86 ↑, the secretion of IL-1β, IL-12p40, and TNF-α↑in DC cell in vitro	[46]
9. Polygonatum sibiricum	PSP	No	Gal : Rha : Man : Glu : Xyl = 63.50, 25.14: 8.04:1.75: 1.57 %	No	No	significantly enhanced the phagocytosis of peritoneal macrophages in a dose-dependent manner, IL-2, TNF-α, IL-8 and IL-10	[20 , 72]
	PSP1 PSP2 PSP3 PSP4	4.415 2.236 7.743 6.467 kDa,	Gal:Rha:Man:Glu: Xy = 72.91:nd:14.96:2.13:nd 74.37:20.54:nd:2.06:3.03 37.17:57.69:1.38:2.02:1.74 20.74:72.63:2.00:nd:4.63	Yes	No	Cell viability and phagocytic activity in the macrophages , Spleen lymphocyte proliferation , Splenic NK cytotoxicity , TNF-α, IL-2, IL-4, and IL-10	[47]
	PSPC PSPW	4.01×10^3 1.42×10^4 Da	Gal : Man, Glu, GalA = 29.63 % : 36.10 % : 15.09 % : 10.20 %; 78.77 %: 5.50 %: Nd: 13.84 %	No	No	the cells viability, phagocytic capacity, acid phosphatase activity, and NO production of RAW264.7 cells↑, the immune functions↑, L-2, IL-6, TNF-α, and IFN-γ↓ of imunosuppressive model for spleen deficient mice	[83]
	PSPJWA	141 kDa	Gal: Ara: Rha = 14:4:1	Yes (1D,2D NMR)	an arabinogalactan(The branched residue α-L-Araf-(1 → 5)-α-L-Araf-(1 → 3,5)-α-L-Araf-(1→ attached to the backbone through 1,2,4-linked-α-L-Rhap at C-4 position.)	Antioxidant activity	[48]
10. Astragalus membranaceus		(4.56×10^5 Da)	Man:Ribose: gluA:Glu, Xyl,:Ara = 2.14:3.61:1:2.86:5.98:36.39	No	No	IL-1β, IL-18 and TNF-α,	[35 , 80]
(Fisch) Bge. var. mongolicus (Bge.) Hsiao	APSL	No	No	No	No	the phagocytosis, IL-6, IL-12,NO,iNOS of macrophages the proliferation of DCs, T lymphocytes proliferation, IFN-γ, IL-2, expression of CD80 and CD86	[23]

(*continued on next page*)

Table 4 (*continued*)

Origin	Compound name	Molecular weight	Monosaccharide composition	NMR	Methylation	Biological activities	References
	APS	2.07 × 10^4 Da	Only glucose	repeating (1 → 4)-linked backbone with a (1 → 6)-linked branch every 10 residues.	No	proliferation of mouse B cells without T cell	[49]
11. Polygonatum odoratum	CPP HPP	4.41 × 10^3Da, 8.82 × 10^3 Da 4.52 × 10^3Da	Man, glucosamine, Rha, Glu, Gal, Ara = 7.80:1.08:1.63:65.93:3.58:1.00; 11.22:0.23:0.23:17.59:2.73:9.10	No	No	Raw 264.7 macrophage cells proliferative indexes, pinocytic activity	[27]
	POP-1 (Neutral polysaccharide)	5.1 KDa	Fru: Glc = 30:1	nulin neoserie type of fructose	→1)-β-D-Fruf-(2→, →6)-β-D-Fruf-(2→, →1,6)-β-D-Fruf-(2→, β-D-Fruf-(2→ , →6)-α-D-Glcp-(1→	Cell viability, phagocytosis activity, IL-6 in macrophage cell line RAW264.7	[63]
12. Sophora subprosrate or Sophora fla•vescens Ait.	SSP1	2.24 × 104	Glu only	a (1 → 4) linked -d-glucan to which are attached two glucosyl side chains at 3-O and 6-O of the glucosyl residues in every 12 repeating unite of the main chain	No	proliferation of splenic lymphocytes , IFN-γ , IL-6 and TNF-α	[84]
	SF1 SF2 SF3 SF4	400.9 98.6 99.3 42.7 kDa,	Rha:Ara:Xyl:Man:Gla:Gal (Uronic acid) = 0.3:8.34:0.95:2.49:78.75:16.68 (7.72) 0.94:8.84:8.84:2.72:61.94:16.73 (8.78) 4.22:42.9:1.49:2.45:9.54:39.39 (24.11) 9.06:22.65:1.81:4.62:16.68:45.19 (31.3)	No	No	cell viability, No production, splenocyte proliferation	[64]
13. Cynomorium songaricum Rupr.	CSP-DS1	48.1 × 10^4 Da	Man : Glc : Gal = 5.01: 89.17: 5.82	Yes (1D,2D NMR)	1,6-Manp:1,3-Glcp:t-Glcp: 1,2,3-Glcp: 1,3-Galp = 6.2 %:75.6 %:5.1 %:4.8 %:8.3 %	No	[61]
	CSPA	1.394 × 10^5 Da	Glc : Gal : Ara = 1:4.2:0.34	→3)-α-araf-(1 → 3)-α-d-glcp-(1 → 4)-α-d-galpA6Me-(1→	No	No	[62]
	CSP-1 CSP-2 an acidic polysaccharide	3.7 × 10^4 1.0 × 10^4	Glu : Gal : Man : Ara : Rib = 83.6:2.4:2.9:4.6:2.0 84.4: 1.7:3.6:3.5:2.0	a (1–3)-a-D-glucopyranosidic main chain with a small number of branches	No	No	[30]
14. Arnebia euchroma (Royle) Johnst	ARP	1.23 × 10^4 Da	Gal:Ara: Glu: Man, Rha:Fuc = 53.8:21.3:11.7:6.8:4.3:2.2	Yes (1D NMR, 2D NMR)	Mainly Galp residues of 1 → 6 linkages and Ara residues of 1 → 5 or 1 → 3 linkages	proliferation of splenic lymphocytes , IFN-γ , IL-2 macrophage cell TNF-α, IL-1β, IL-6	[28]
15. Sophorae tonkinensis Radix	STRP1 STRP2	1.30 × 10^4 1.98 × 10^5 Da	Man, Rha, glucuronic acid, Glu, Gala,Ara = 5.1, 2.5,1.8, 25.1, 42.3,23.2; 1.1, 2.2, 5.1, 34.1, 23.5, 34.0	(1 → 3)-linked-α-D-Gal and (1 → 4)-linked-α-D-Glc	1→Ara: 1,2→Rha:1→Glu:1,5 →Ara:1,2→Man:1,3→Gal:1,4→ Glu:1,2,4→Glc: 1,3,6→Glc: 1,3,6→Gal = 5.6:1.0:5.8:2.3:1.4:8.3:1.1:0.8:0.8:3.0 3.9:0.4:1.3:2.8:0.4:2.5:3.4:0.7:1.0:1.3	hepatoprotective activities	[58,59]
	WRSP-A2b WRSP-A3a	13.6 kDa 44.6 kDa	GalA:Rha:Gal:Ara:Glc:Man:Xyl = 41.3:16.0:15.9:22.5:7.8:1.6:1.0 60.4:18.4:9.7:7.3:1.2:2.0:0.9	HG and RG-I domains, with the RG-I domain probably containing α-L-1,5-arabinan, β-D-1,4-galactan and/or AG-I and AG-II as side chains	1,4-linked GalpA: 1,2-linked Rhap: 1,2,4-linked Rhap: Terminal Galp:1,6-linked Galp: 1,3-linked Galp: 1,3,6-linked Galp:1,4-linked Galp: Terminal Araf:1,5-linked Araf: 1,3-linked Araf: 1,3,5-linked	Antioxidant activity	[59]

(*continued on next page*)

Table 4 (*continued*)

Origin	Compound name	Molecular weight	Monosaccharide composition	NMR	Methylation	Biological activities	References
	Top phase polysaccharide Button phase polysaccharide	12,777 Da 7973 Da	Glu: Ara:Gal:Rha: Man:GluA: aminogalactose = 63.70: 18.21: 7.00: 5.10: 3.13: 1.78: 1.07 75.24: 3.79: 3.71: 3.37: 2.51: 1.43: 9.95	No	Araf: 1,2,5-linked Araf : Terminal GlcpTerminal GlcpA:1,4-linked GlcpA = No	No	[60]
16. Mirabilis himalaica (Edgew) heim	MHHP	16.1 KDa	Only Glu	→4) -Glcp-(1→, →4,6)-Glcp-(1→ and Glc-(1 → .	Glc-(1→: →4) -Glcp-(1→: →4,6)-Glcp-(1→ = 0.125 : 0.75 : 0.125	proliferation of splenic lymphocytes , IFN-γ , IL-2 macrophage cell TNF-α, IL-1β, IL-6	[85]
17.Asparagus cochinchinensis (Lour.) Merr.	TD-80	6.8 kDa	Rha, Ara, Gal, Glu, Xyl, Man, GalA = 0.048 : 0.102 : 0.316 : 0.157 : 0.101 : 0.017 : 0.259	No	No	proliferation of splenic lymphocytes , IFN-γ , IL-2, macrophage cell TNF-α, IL-1β, IL-6	[86]

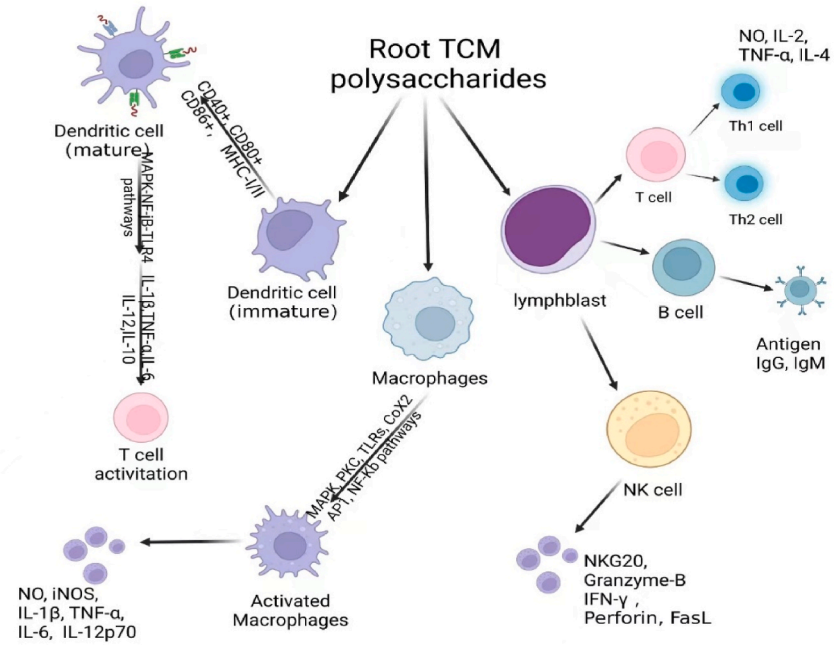

Fig. 3. Immune regulatory mechanisms of root TCM polysaccharides in vitro. Created with BioRender.com.

express the maturation markers CD80 and CD86 in vitro, improved the $CD4^+/CD^{8+}$ lymphocyte ratio in vivo and $CD4^+/CD8^+$ ratio of splenocytes, improved the phagocytic ability of macrophages, and increased the production of NO and secretion of cytokines TNF-α and IL-6 from macrophages.

Although multiple signaling pathways are involved in activating macrophages, the immunomodulatory effects of Toll-like receptors (TLRs)/mitogen-activated protein kinase (MAPK)/nuclear factor (NF)-κB signaling pathway–mediated polysaccharides on macrophages are a popular but challenging area of research. Current studies have shown that polysaccharides activate macrophages by interacting with receptors, and signals can be transmitted to macrophages through the following pathways: MAPK, PKC, TLRs, aberrant cyclooxygenase-2, activator protein-1, and NF-κB.

The immunomodulatory effect of PSP in macrophages is associated with activation of the TLR4-MAPK/NF-κB signaling pathway, particularly through the MyD88-dependent pathway [89].

5.4. Dendritic cells (DCs)

DCs are the most powerful antigen-presenting cells known. Their main function is to ingest, process, and present antigens and stimulate the activation and proliferation of initial T cells to initiate an adaptive immune response. Many active natural polysaccharides can promote DC maturation, mainly by enhancing the expression of the costimulatory molecules CD80 and CD86 on the DC surface and secretion of associated cytokines to promote effective tumor antigen presentation.

P. grandiflorus polysaccharide (PG) induced phenotypic maturation of DCs, as evidenced by the increased expression of cell surface $CD40^+$, $CD80^+$, $CD86^+$, and major histocompatibility complex I/II. PG also induced functional maturation of DCs, as evidenced by the increased production of IL-12, TNF-α, IL-1β, IL-6, IL-10, and interferon (IFN)-α as well as the enhanced ability of PG-treated DCs to stimulate allogeneic T cells and induce DC maturation via activating MAPK and NF-κB signaling downstream of TLR4 [69].

5.5. NK cells

NK cells are the most important immune cells in the body. They are nonspecific immune killer cells that are perpetually present in the body and play a vital role in the intracellular immune process against parasitic infections, tumors, and viruses, and have both immunomodulatory and cytotoxic functions.

Treatment with PSP was used to promote NK cell cytotoxicity and upregulate the expression of genes for IFN-γ, granzyme B, perforin, NKG2D, and FasL [88]. *G. uralensis* Fisch. polysaccharide significantly improved the growth performance of mice (average weight, average daily feed intake, and feed efficiency), immune organ index (spleen and thymus index), and immune function (serum IL-2, $CD4^+/CD8^+$, and NK cell activity) [90].

6. Relationship between structure and immunomodulatory activity

The pharmacological activities of plant polysaccharides are mostly related to their physicochemical properties, glycosyl group compositions, glycosidic bond types, and chain and other advanced structures. Among these, the molecular weight, monosaccharide composition, and chemical modification of polysaccharide structures play a decisive role in their bio pharmacological activity.

Enhanced proliferation of T lymphocytes induced by polysaccharides from *G. uralensis* Fisch. was found to be dependent on molecular weight, with a low-molecular-weight fraction (Fraction B, <10 kDa) having a more profound effect than the high-molecular-weight fractions (Fraction A and C) and total crude extract [82].

sinensis (Oliv.) Diels polysaccharide ASP3 mainly comprise galacturonic acid along with rhamnose, arabinose, and galactose, indicating that these are pectic polysaccharides. ASP3 can protect leucocytes and lymphocytes of mice against radiation-induced damage, and thus has a potential radioprotective effect on acute radiation–injured mice [34].

Immunological activity was afforded by mannose and galactose, which are the key monosaccharide components of the SF3 and SF4 polysaccharides from *Sophora flavescens* Ait. SF3 could combine with the coreceptor on the T cell and significantly promoted T lymphocyte proliferation. SF4 could recognize the coreceptor on the B cell and significantly promoted B lymphocyte proliferation [91].

The effects of A. kusnezoffii Reichb. pectic polysaccharide fractions (WKCP-A and WKHP-A), which mainly comprise galacturonic acid, galactose, and arabinose, were enhanced compared to those of neutral polysaccharide fractions (WKCP–N and WKHP–N), which comprised glucose, in lymphocyte proliferation and macrophage phagocytosis activity assays [17].

Polysaccharides from *O. japonicus* were encapsulated with liposome and could significantly promote the phagocytosis of macrophages, induce the secretion of IL-2 and IL-6 in vitro, and improve the phagocytic index. This promoted lymphocyte proliferation, increased the proportion of T lymphocyte subpopulations $CD4^+$ and $CD8^+$, enhanced antibody titer, and improved the protective rate in vivo. These effects were greater than those induced by *Ophiopogon* polysaccharide alone [92,73].

Five polysaccharides (OJP-1S, OJP-4, OJP-3, OJP-2, and OJP-1) isolated from *O. japonicus* exhibited remarkable macrophage-activating capability by promoting phagocytic capacity, energy metabolism rate, and NO and IL-1 production depending on the content of hexuronic acid and sulfate (OJP-4 > OJP-3 > OJP-2 > OJP-1). These results suggest that hexuronic acid and sulfate were effective indicators of antioxidant and immunomodulation activity of these polysaccharides [53].

Modified *C. pilosula* polysaccharide sCPPS was shown to be more effective than CPPS in promoting lymphocyte proliferation and increasing the ratio of $CD4^+$ to $CD8^+$ T cells. In vivo, sCPPS treatment could increase the content of IgG, IgM, IFN-γ, IL-2, and IL-4 in mouse sera against ovalbumin (OVA) in comparison with that induced by nonselenized CPPS. These results indicate that selenylation can enhance the immune modulation activities of CPPS [38].

Low-molecular-weight PSP from Polygonatum sibiricum derived from degradation via partial hydrolysis upregulated the production of NO while sulfate content affected the toxicity of NK cells and increased the gene expression of IFN-γ, granzyme B, perforin, NKG2D, and FasL [88].

7. In vivo immunoregulatory activity

a) Cyclophosphamide (Cy)-induced immunosuppression in BALB/c mice

The immune regulatory activity of PSP from Polygonatum sibiricum was evaluated using the Cy-induced immunosuppressed in vivo model. PSP could significantly stimulate phagocytosis of RAW264.7 macrophages. Compared with the Cy-treated group, PSP accelerated recovery of spleen and thymus indexes and enhanced T cell and B cell proliferation responses and peritoneal macrophage phagocytosis. Additionally, PSP treatment restored the levels of IL-2, TNF-α, IL-8, and IL-10 in Cy-treated mouse sera in a dose-dependent manner [20].

The effects of the purified PSP3 fraction from Polygonatum sibiricum on the spleen indexes, cytokine secretion (IL-2, TNF-α, IL-8, and IL-10), and Th1/Th2 ratio were significantly higher than those of PSP in Cy-induced immunosuppressed mice [47]. CPPS induced the recovery of spleen, thymus, and liver indexes and restored the levels of IFN-γ, IL-2, IL-10, serum IgG, and secretory IgA (sIgA) in Cy-treated immunosuppressed mice [93–98].

b) Ovalbumin (OVA)-immunized mice

Ophiopogon polysaccharide liposome significantly enhanced splenocyte proliferation and proportion of $CD4^+$ and $CD8^+$ T cells and improved the levels of cytokines and antigen-specific antibody titers and immune organ index in mice immunized with OVA compared with the outcomes induced with *Ophiopogon* polysaccharide treatment [64].

The main component (WCP-Ia) of an acidic polysaccharide from *C. pilosula* Nannf. var. modesta (Nannf.) L. T. Shen (WCP–I) had a better promotion effect than that of WCP-I in vivo, as shown by the increased spleen index and higher concentrations of IL-6, TGF-β, and TNF-α in sera and slight increments in the level of sIgA and $CD4^+/CD8^+$ T lymphocyte ratio. These results suggest that -d-(1 → 4)-galactan-containing chains in WCP-I play an essential role in immunomodulation activity. Combining the results in this and previous studies, the intestinal immune system may be a potential target site of WCP-Ia [39].

c) Dexamethasone-induced immunosuppressed mice

Treatment of *S. subprosrate* polysaccharide (SSP1) in dexamethasone-induced immunosuppressed mice affected splenic lymphocyte

proliferation, cytokine production, and antioxidant capacity. SSP1 increased the levels of IL-6 and TNF-α in mice who received a subcutaneous injection of dexamethasone at 1.25 mg/kg. Administration of SSP1 via intraperitoneal injection significantly increased the spleen index, glutathione level, and glutathione peroxidase and lysozyme activity in the immunosuppressed mice [84].

8. Conclusion

These findings approved that polysaccharides from root TCMs are an important active ingredient and is an attractive immunomodulatory adjuvant in the fields of medicine and functional food supplements.

Although great progress has been made in the development of 17 kinds of root TCMs polysaccharides and related chemical and biological research, there are still some problems, and some suggestions have been made to address these problems. There were mainly the following aspects: first, the extraction and purification methods, sugar composition and glycosidic linkage were mainly described, but three-dimensional structure analysis of natural polysaccharides were rarely few. Second, Studies on the structure-activity relationship have focused on molecular weight, substitution functional groups and monosaccharide composition, but less on the active fragments of polysaccharides.

Overall, existing studies have proposed that polysaccharides and their derivatives may exert immunoregulatory effects directly and indirectly. Further studies are required to better illuminate the exact mechanisms underlying the immunoregulatory effects of root TCM polysaccharide-based products.

9. Research limitations and prospects

Considerable research on polysaccharides has been reported over the past half century. Although knowledge of structural analysis and evaluation of biological activity has increased substantially, understanding of polysaccharides remains far behind that of nucleic acids and proteins.

Research requirements in the field of polysaccharides are large but are balanced by the potential health benefits and considerable size of the market. Research into the quality, safety, mechanism of action, and structure–activity relationships are needed. Furthermore, polysaccharides have been widely accepted as healthcare products for improving immune functions of the body. However, many polysaccharides only remain at the stage of healthcare products and fail to develop into therapeutic drugs. Most of them remain in the laboratory stage, and the mechanism of action is not clear. In order to apply more nature polysaccharide products in clinical practice, the interaction of these polysaccharides with immune pattern recognition and relationship between their structure and bioactivity must be further strengthened.

With the concept of biological pattern receptors, such as PRRs and PAMPs, being put forward, bioactive polysaccharides are now considered a mainstay of biological response modifiers that can interact with hosts by triggering a series of receptors, such as TLRs and Dectin-1. Several intracellular signal transduction pathways have been found to be involved in polysaccharide-induced beneficial activities. However, the interaction of these polysaccharides with immune pattern recognition and relationship between their structure and bioactivity must be further understood.

The research on immunomodulatory mechanism is relatively unclear, though many studies have focused on the immunomodulatory effects of plant polysaccharides, there are still some problems to be solved. Polysaccharides regulates the immune function in multiple links and targets, it is worth noting that the immunomodulatory mechanism of plant polysaccharides involves different receptors on various immune cells and has a certain regulatory effect on messenger molecules between cells, which requires researchers to investigate its mechanism from multiple levels in the process of research.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Surina Bo: Writing – original draft, Project administration, Methodology, Funding acquisition. **Man Zhang:** Writing – review & editing, Investigation. **Mu Dan:** Writing – original draft, Formal analysis, Data curation.

Declaration of competing interest

The authors declare no conflict of interest.

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Abbreviations

DEAE cellulose	Diethylaminoethyl cellulose
PAMPs	Pathogen-associated molecule patterns
PRRs	pattern recognition receptors
IL-2	Interleukin-2
IL-4	Interleukin-6
IL-7	Interleukin-7
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-1β	Interleukin-1β
TNF-α	Tumor necrosis factor-α
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IFN-γ	Interferon-γ
IEC-6 cells	Intestinal crypt epithelial cells
NO	Nitric oxide
TLRs	Toll-like receptors
MAPK	Mitogen-activated protein kinase
(NF)-κB	Nuclear factor
PKC	Protein kinase c
NKG2D	Type II integral membrane protein
FasL	Fas ligand
TGF-β	Transforming growth factor-β
Cy	Cyclophosphamide
OVA	Ovalbumin
sIgA	Secretory immunoglobulin

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