Research progress on polysaccharide components of Cistanche deserticola as potential pharmaceutical agents

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Abstract

Cistanche deserticola is a traditional and precious Chinese herbal medicine, known as "desert ginseng", with anti-inflammatory, anti-oxidant, improving immunity, nourishing the kidneys and other pharmacological effects. Its chemical components mainly include phenylethanol glycosides, iridoids, polysaccharides and volatile components, among which polysaccharides have received extensive attention due to their biological activities such as regulating immune activity, anti-aging, anti-spleen deficiency and antitumor. In recent years, a large number of research have been carried out on the extraction and isolation, chemical structure analysis and biological activity of *Cistanche deserticola* polysaccharides. The methods of polysaccharide extraction mainly include traditional extraction method, ultrasonic assisted method, microwave assisted method and enzyme assisted method, etc. The extracted polysaccharides were analyzed by chemical methods including methylation, acid hydrolysis and Smith degradation and spectroscopy methods such as NMR and IR. A variety of polysaccharides with new structures were obtained, and some polysaccharides with known structures were also investigated for their biological activities and their structure-activity relationships. However, the relationship between polysaccharides structure and their biological activities is still unclear due to the large number of polysaccharide components, their complex structures and the lack of systematic research and analysis on them. It is expected that the subsequent study of polysaccharide structure and active conformational relationship will be highly valuable for the application of *Cistanche deserticola* in pharmaceutical sciences and health food.

Keywords: *Cistanche Deserticola;* Polysaccharides; Extraction and Isolation; Chemical Structure; Biological Activity; Research Progress; Pharmaceutical agents.

1. Introduction

Cistanche Hoffmg. Et Link is a perennial herbaceous parasitic plant of the Orobanchaceae family. There are about 27 species in the world, mainly distributed in the arid lands and deserts in the northern hemisphere and mainly concentrated in Inner Mongolia, Ningxia, Gansu, Xinjiang and Qinghai provinces in China [1]. A further study confirmed that four species and one variation of *Cistanche* distributed in China [2], i.e. *Cistanche deserticola* Y.C. Ma, *Cistanche tubulosa* (Schenk) R. Wight, *Cistanche salsa* (C.A. Mey.) G. Beck, *Cistanche sinensis* G. Beck and *Cistanche salsa* (C.A. Mey.) G. Beck *var. albiflora* P.F. Tu et Z.C. Lou (one variation of *Cistanche*).

The traditional Chinese medicine *Cistanche* consists of the dried succulent stems of *Cistanche* species of this genus, also known as Cunyun, Dayun, Goblin, etc. It is attached underground to the roots of the dicotyledonous plant Haloxylon ammodendron Bunge and developes by obtaining nutrients from the host plants that it parasitize. *Cistanche deserticola* is a rare and valuable Chinese herb in China [3] and honored as "Desert ginseng" attributing to its promising medical functions and nourishing value. It is proved that *Cistanche deserticola* Y.C. Ma which is mainly produced in the western part of Inner Mongolia are the authentic Chinese medicine *Cistanche* and documented in Chinese Pharmacopoeia [4]. Modern pharmacological research have disclosed that *Cistanche* possesses wide-reaching biological activities including anti-inflammatory, anti-oxidative, immunity-enhancing, anti-aging, nerve protection, memory-improving, prevention and treatment of osteoporosis, and anti-fatigue, etc [5-6]. Modern research has shown that the chemical components of *Cistanche deserticola* mainly include phenylethanoid glycosides, iridoid glycosides, lignans, polysaccharides, amino acid and volatile compounds, etc [7]. The active component that attracted more attention is reported to be phenylethanoid glycosides, which have significant effect in treating kidney deficiency [8] and protecting muscle cells [9] and so on.

In recent years, polysaccharide which obtained from natural herbs has been proved to possess multiple biological activities and lower cytotoxicity. Thus, polysaccharide as one of the main active ingredients of *Cistanche deserticola* has also received extensive attention, and it has been studied that polysaccharide has various pharmacological effects such as lowering blood sugar, blood lipids [10], regulating immunity [11] and anti-aging [12]. The biological activities of polysaccharides are closely related to their physicochemical properties, chemical composition and content of polysaccharides, etc. However, the relationship between the chemical structure of polysaccharides and their biological activities of *Cistanche deserticola* is unclear due to the complexity of the composition and structure of polysaccharides. Therefore, it is of great significance to study the structure-activity relationship of polysaccharides for the safety and efficacy of the use of *Cistanche deserticola*. The extraction and isolation process of polysaccharides is the first prerequisite for its in-depth study as it may affect their yield, chemical structure, and biological activities. Although these aspects have been reported in the literature [13], it is not sufficiently detailed and comprehensive, especially in terms of extraction, chemical structure and pharmaceutical application. The aim of this review is to introduce recent developments in the study of the extraction and isolation, chemical structure and biological activities of polysaccharides from *Cistanche deserticola*.

2. Extraction and Isolation

In the past, people mainly relied on traditional water extraction methods to obtain polysaccharides from *Cistanche deserticola*, which is not only time-consuming and labor-intensive, but the yield and purity of polysaccharides are not very advanced. With the increasing research interest in the polysaccharides of *Cistanche deserticola*, the extraction process of polysaccharides has been greatly developed. Various extraction methods including ultrasonic-assisted, microwave-assisted and enzyme-assisted have been continuously developed based on aqueous extraction process, with the aim of achieving high yield, energy-saving and less time consuming. Each extraction method has advantages and disadvantages, and the impact of these methods on the chemical structure and biological activity of polysaccharides should be considered comprehensively. These methods can be summarized in Table 1.

Table 1 *Cistanche deserticola* polysaccharide extraction method

Extraction Methods	Principle	Advantages	Disadvantages
Traditional Water Extraction	"Water extraction and alcohol precipitation"	Not require special operations, low cost	Time-consuming, labor-intensive, and there are more factors affecting the yield
Ultrasonic-Assisted Extraction	Ultrasonic waves generate strong cavitation and stirring effects, making polysaccharides easily diffuse and dissolve	Less solvent usage, shorter working time of extraction and lower extraction temperature	Prolonged ultrasonication may lead to the destruction of the polysaccharides structure
Microwave-Assisted Extraction	The microwave can generate strong pressure to break through cell membranes and cell walls, and promote the dissolution of polysaccharides	High efficiency, rapidity and energy saving	Use of microwave instruments is dangerous
Enzyme-Assisted Extraction	Biological enzymes effectively dissolve polysaccharides through the destruction of plant cell walls	The extraction conditions are mild and simple, less destructive to polysaccharide structure, and the extraction effect is significantly enhanced	Enzyme activity is unstable, high cost
Ultrasonic-Microwave Synergistic Extraction	-	Shorten the extraction time, obtain high-yield polysaccharides	Multiple methods are complicated to operate
Ultrasonic-Enzyme-Assisted Extraction	-	A fast and inexpensive method	Multiple methods are complicated to operate

2.1 Traditional Water Extraction Methods

The traditional extraction method utilizes the characteristics of traditional Chinese medicine

polysaccharides that are easily soluble in water but insoluble in organic solvents such as ethanol, petroleum ether, ether, etc., which is often referred to as "water extraction and alcohol precipitation" method. This method does not require special operations, however, it is time-consuming and labor-intensive, and there are more factors affecting the yield, such as extraction temperature, time, and solvent dosage. Naran and co-workers reported the subterraneous parts of the holoparasite, *Cistanche deserticola*, was extracted with methanol and subsequently methanol-insoluble extracts was extracted using in succession cold water, hot water, 0.5 M NaOH and 0.01 M EDTA [14]. Yield analysis of the isolated polysaccharide fractions revealed that the yield of 0.5 M NaOH and 0.01 M EDTA extract was superior to that of cold and hot water. Naran and co-workers then described starch fractions have been isolated from the underground part of *Cistanche deserticola* Y. C. Ma using hot water and alkali treatments, respectively [15]. Chemical, enzymic and spectroscopic analyses revealed that the hot water and alkali extracts contained different proportions of amylose and amylopectin components. The yield of polysaccharides from the hot water extract was 1.3 %, while the yield of polysaccharides from the alkali extract was 2.9 %.

2.2 Ultrasonic-Assisted Extraction Methods

Ultrasonic-assisted extraction methods (UEM) utilizes ultrasonic waves to generate strong cavitation and stirring effects, making polysaccharides easily diffuse and dissolve, thereby increasing its extraction efficiency. But prolonged ultrasonication may lead to the destruction of the polysaccharides structure. With less solvent usage, shorter working time of extraction and lower extraction temperature, UEM is a fast and inexpensive method compared to traditional methods [16]. Lian and co-workers reported extraction of *Cistanche herba* polysaccharides by ultrasonic-assisted extraction and studied the main factors influencing the yield of polysaccharides by single factor test method [17]. The optimal extraction process is solid-liquid ratio was 1:25 with 75 min ultrasonic-assisted extraction under 60 ^{o}C, the yield of polysaccharides from *Cistanche herba* was 18.90 %.

2.3 Microwave-Assisted Extraction Methods

Microwave-assisted extraction method is based on the difference in the ability of substances to absorb microwaves, certain compounds are extracted by selective heating. The microwave has strong penetrating ability, high selectivity, and rapid heating. It can generate strong pressure to break through cell membranes and cell walls, and promote the dissolution of polysaccharides. The advantages of this method being widely used are high efficiency, rapidity and energy saving [18]. Teng and co-workers described four different methods for extracting polysaccharides, including microwave-assisted extraction, ultrasound-microwave synergistic extraction, ultrasonic-assisted extraction, etc [19]. The results demonstrated that the finest extraction method was ultrasound-microwave synergistic extraction, followed by microwave-assisted extraction. It can be observed that the penetrating and heating ability of microwaves play essential roles in the extraction process of polysaccharides.

2.4 Enzyme-Assisted Extraction Methods

The enzyme-assisted extraction method is to effectively dissolve polysaccharides through the destruction of plant cell walls by biological enzymes. The extraction conditions are mild and simple, less destructive to polysaccharide structure, and the extraction effect is significantly enhanced [20]. Gao and co-workers reported employed enzyme-assisted extraction to extract the polysaccharides [21]. Taking the enzyme dosage, enzymolysis temperature and enzymolysis time as the referebce factors, the polysaccharides extraction yield was the indicator, and the optimal process of enzyme-assisted extraction was determined through orthogonal experiments. The results showed that the actual yield of polysaccharides increased from 4.61 % to 8.35 % when the enzyme dosage was 0.2 %, enzymatic hydrolysis time was 1.5 h, and hydrolysis temperature was 50 ^{o}C.

In addition, scientists have developed a combination of multiple extraction methods to achieve the effect of maximizing the strengths and avoiding weaknesses of a single extraction method, such as ultrasonic-microwave synergistic extraction, ultrasonic-enzyme-assisted extraction, etc. These methods not only shorten the extraction time, simplify the extraction process and reduce the operational difficulty, but obtain high-yield polysaccharides. Teng and co-workers firstly employed ultrasonic-microwave synergistic extraction method to extract polysaccharides from *Cistanche tubulosa*, which have the best extraction effect as it can exert stronger and more consistent force on the sample, and accelerate the process of dissolving polysaccharides into the solvent [18]. Zhang and co-workers reported the effective extraction of *Cistanche tubulosa* polysaccharides utilizing ultrasound-cellulase-assisted [22]. Single factoris was employed to further optimize ultrasound-cellulase-assisted extraction conditions and the maximum yield of polysaccharides was 22 % under the condition of pH of 5.2 for 31.5 min at 54.1 ^{o}C.

2.5 Other Extraction Methods

As the pharmacological effects of traditional Chinese medicine polysaccharides become more and more significant, their extraction process has also received extensive attention as the primary work of research. For the extraction research of *Cistanche deserticola* polysaccharides, scholars have tried other different extraction methods. Zhang and co-workers firstly used flash extraction method to extract polysaccharides from *Cistanche deserticola*, and studied the effects of solid-liquid ratio, voltage and extraction time on the yield of polysaccharides [23]. Under the optimal extraction conditions, the polysaccharides yield was 12.35 %. This method has the advantages of time-saving, efficient and easy operation in the extraction of polysaccharides.

3. Chemical Structure

Cistanche deserticola is rich in chemical components, and scholars at home and abroad have isolated and structurally identified more than one hundred compounds, including phenylethanol glycosides, lignans, iridoids, polysaccharides, amino acids, etc [24-25]. Among them, phenylethanol glycosides are considered to be the main active ingredients for the medicinal effects of *Cistanche deserticola*. However, as the research on the pharmacological effects of *Cistanche deserticola* has

intensified, it has been found that the polysaccharides also plays a crucial role in the pharmacological activity. The chemical structure of polysaccharides is the material basis for their biological activities and polysaccharides with different chemical structures have different pharmacological effect. Therefore, more and more attention has been paid to the structure research of polysaccharides from *Cistanche deserticola*. In the 90s, studies on the polysaccharides of *Cistanche deserticola* focused on the isolation , purification and compositional analysis of monosaccharides [26-27], while the precise structure of polysaccharides was not elucidated until 1997 [28]. Recent developments in modern chromatography and spectroscopy have also facilitated the characterization of the chemical structures of polysaccharides.

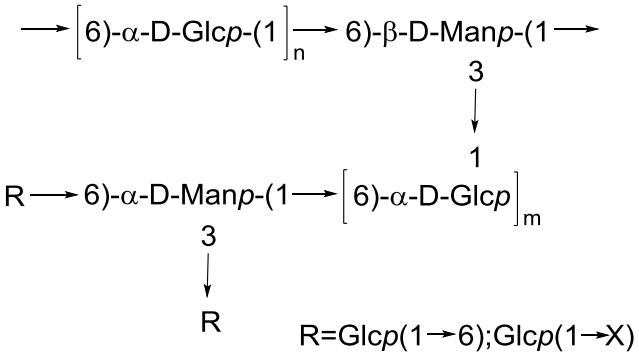
Ebringerová A and co-workers employed dilute alkali to extract three pectic polysaccharides (P1、P2 and P3) from *Cistanche deserticola* and structurally characterized them by chemistry, enzymology and NMR spectroscopy [28]. The results indicated that the main relative molecular mass peak of P1, P2 and P3 at 1.87×10^5、1.25×10^5、0.8×10^4, respectively, as well as arabinose and galactose are prevailing neutral sugar fractions. The mainly differences between these pectins are proportions of homogalacturonan and rhamnogalacturonan RG-I sequences, contents of galacturonic acid, degree of methyl esterification and acetylation, etc. All pectins showed remarkable immunomodulating activities in both mitogenic and comitogenic tests. In addition the authors suggested that immunomodulatory properties of *Cistanche* pectins may be related to rhamnogalacturonan and α-3,5-arabinan moieties [29]. The acidic pectic polysaccharide, bupleuran 2IIb, from *Bupleurum falcatum* L. showed a potent enhancing immune activity [30]. The experimental result suggested that the ramified region which consisted of a rhamnogalacturonan core with neutral carbohydrate chains is important for this enhancing activity. These validated the relationship between the chemical structure of rhamnogalacturonan and immunomodulatory activity. This article not only examined chemical structure of the pectic polysaccharides in more detail, but specifically studied the structure in relation to its biological activity.

Subsequently Ebringerová A et al reported the crude polysaccharide extracted by cold water was purified by column chromatography to yield Cistan A with relative mean molecular mass was 2.01×10^5 [31]. Analysis of sugar composition revealed that Cistan A consisted of L-arabinose, D-galactose, L-rhamnose and D-galacturonic acid in a molar ratio of 6.3:10.0:1.0:0.8, in addition to very low amounts of D-xylose and D-glucose. NMR spectroscopy and methylation analysis demonstrated that Cistan A contains mainly a complex of pectic arabino-3,6-galactan type II with lowly-branched 3,5-α-L-arabinan. In immunological activities test, Cistan A showed the highest activities compared to commercial immunomodulator Zymosan and other extracted polysaccharides. Moreover the ramified regions of the galacturonan core and both the neutral side chains consisting of 3,6-β-D-galactan and 3,5-α-L-arabinan were considered to be engaged in the bioactive expression [29].

Tu and co-workers reported the structural characteristics of polysaccharide named CDP-4 isolated from the immunologically active polysaccharides of *Cistanche deserticola* [32]. Chemical and spectroscopic analyses measured a relative molecular mass of 1.4×10^4, and TLC analysis of

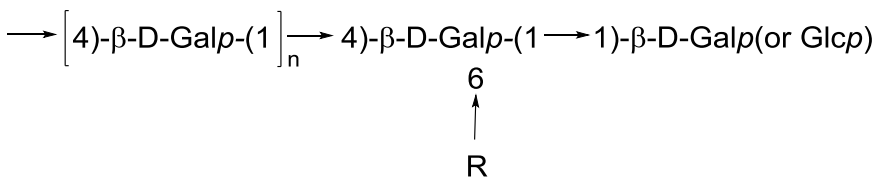
the complete acid hydrolysis product of CDP-4 indicated that the polysaccharide contained only glucose. The methylation analysis showed that CDP-4 was composed of 1,4-linked glucopyranose and 1,6-linked glucopyranose, and molar ratio was 3:1. The absence of branching chain residues indicated that it was a straight-chain glucan. Furthermore, the aqueous solution of this polysaccharide was reacted with iodine as a colorless solution, and there was no absorption between 200-700 nm indicating that its spatial structure is completely different from that of straight-chain starch, which is a new kind of glucan.

In the same year, Tu et al extracted the polysaccharide of *Cistanche deserticola* by water extraction and alcohol precipitation, and yielded CDP-6 through purification processes such as deproteinization and column chromatography [33]. The polysaccharide was composed of 89.2 % glucose and 10.4 % mannose, and relative molecular mass was 6.8×10^4. Methylation analysis demonstrated that CDP-6 has a backbone composed of 1,6-linked glucosyl residues and 1,6-linked mannosyl residues, and branches at O-3 of the mannose that consists of terminal, 1,6-linked glucosyl residues and 1,3,6-linked mannosyl residues (Scheme 1). Pharmacological investigations have indicated that CDP-6 has an active effect on the T and B cell systems, and can mildly stimulate the proliferation of T and B lymphocytes.



Scheme 1. Chemical structure of CDP-6

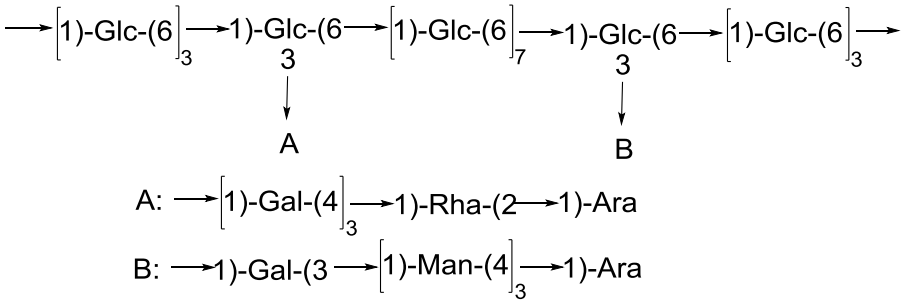
Wu and co-workers extracted and purified the homogeneous polysaccharide ACDP-2 with a relative molecular mass of 5.6×10^5 from the dried flesh stems of *Cistanche deserticola* [34]. After completed acid hydrolysis, analysis of sugar composition showed that ACDP-2 contains arabinose, galactose, and glucose in a molar ratio of 3.5:1.8:0.9, in addition to trace amounts of rhamnose and mannose. ACDP-2 was structurally analogous to immunoreactive arabinogalactan, that is the backbone is composed of 1,4-linked D-galactopyranose and 1,4-linked D-glucopyranose, and there is a branch chain at the 6-carbon which consists of terminal, 1,5-linked and 1,3,5-linked arabinofuranosyl residues (Scheme 2). ACDP-2 displayed a stimulatory effect on the immune response and induces cell proliferation when applied to cultured mouse lymphocytes. However authors indicated that ACDP-2 did not obey those common structure-function relationships of arabinogalactan. Structure was different from pectic arabinogalactans derived from *Centilla asiatica* [35], and biological activity was similar to the pulps of *Melocactus depressus* [36]. Same as *Melocactus*, ACDP-2 consists of a side chain of neutrally charged arabinofuranoside, which may participate in the expression of mitogenic activity.



Scheme 2. Chemical structure of ACDP-2

Afterwards, Wu et al extracted polysaccharides from *Cistanche deserticola* with 0.5 M NaOH and yielded three polysaccharides with relative molecular mass of >2×10^6, 1.5×10^5 and 3.3×10^4, respectively [37]. Complete acid hydrolysis and GLC analysis revealed that these polysaccharides were only consisted of glucose, in addition, methylation analysis and NMR spectroscopy suggested that they were linear α-D-(1→6)-glucans. The results of reaction between the three polysaccharides and KI-I_2 revealed that they have different structures from linear starches. Through comparison, the authors found that the glucans of *Cistanche deserticola* in Mongolica have similar structures to starch and contain a (1→4)-α-D-glucan backbone, not a (1→6)-α-D-glucan [38], suggesting that climate has a significant effect on the large molecular weight compounds of *Cistanche deserticola*.

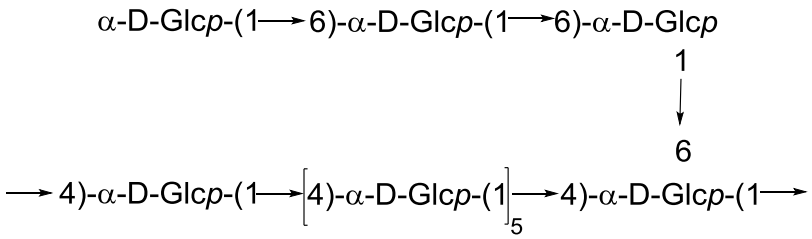
Zhao and co-workers analyzed the structure of the purified polysaccharide SPA of *Cistanche deserticola*, employing chemical methods such as partial acid hydrolysis, periodic acid oxidation, Smith degradation and methylation analysis, and various spectral methods such as gas chromatography, infrared and NMR spectroscopy [39]. SPA was composed of arabinose, rhamnose, mannose, galactose, and glucose in the molar ratio of 1.83:1.00:3.06:4.56:14.74, with relative molecular mass of 7.6×10^3 [40]. The structure of SPA is mainly β-configuration glycosidic bond, with (1→6) glucose forming the backbone and (1→4) galactose, (1→4) mannose, (1→3) galactose and (1→2) rhamnose forming the branches. One repeat unit for every 24 sugar residues, with a backbone of 14 sugar residues and an average of one branch at 3-O for every seven (1→6) glucose (Scheme 3).



Scheme 3. Chemical structure of SPA

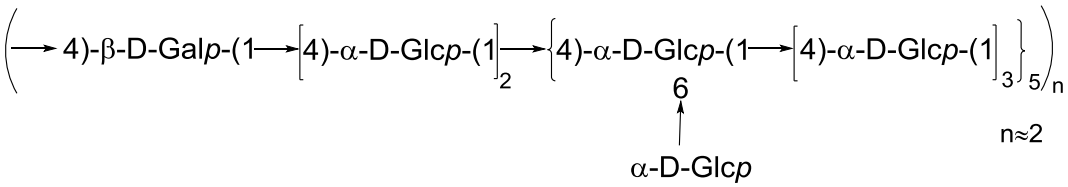
Ding and co-workers purified the crude polysaccharide of *Cistanche deserticola* isolated by water extraction to yield two polysaccharides, namely CDA-1A and CDA-3B [41]. Composition and methylation analysis demonstrated CDA-1A contains only glucose and 1-, 1,4-, 1,6-1,4,6-linked glucose, in the ratio of 1:5.8:3.5:0.8, in addition contains a backbone of α-(1→4)-D-glucan with α-(1→6) branches (Scheme 4). The relative molecular masses of CDA-1A and CDA-3B are 1×10^4 and 8.7×10^5. CDA-3B was composed of rhamnose, arabinose, galactose, glucose, and galacturonic acid in a ratio of 0.31:1.99:1.00:0.22:0.28 and the galacturonic acid was 1,4-linked, as previously reported for other pectic polysaccharides. Mild and

stronger acid hydrolysis of CDA-3B showed it possesses a rhamnogalacturonan backbone, to which is attached arabinogalactan or arabinan branches. Biological activity test revealed that CDA-1A is inert to T cell proliferation stimulation, but active on B cell proliferation; while CDA-3B can stimulate both T cell and B cell proliferation.



Scheme 4. Chemical structure of CDA-1A

Subsequently Ding et al extracted neutral polysaccharide CDA-0.05 from *Cistanche deserticola* by water extraction with the assistance of enzymes with an average molecular mass of 7.96×10^3 [42]. Composition and methylation analysis indicated that it consisted of glucose and galactose in a molar ratio of 96.4:3.6, and contained 1,4-linked Glc (64.3 %), 1,4-linked Gal (3.5 %), 1,4,6-linked Glc (16.1 %) and Terminal linked Glc (16.1 %). Combined with various analytical methods, the backbone of CDA-0.05 included 1,4-linked α-D-glucopyranosyl, 1,4,6-linked α-D-glucopyranosyl and 1,4-linked β-D-galactopyranosyl, with branches contain T-linked α-D-glucopyranosyl (Scheme 5). Bioactivity experiments showed that CDA-0.05 can effectively promote the growth of three kinds of *Bacteroides* and some probiotics, and these results indicated that it may contribute to the maintenance of homeostasis in the intestinal tract and may be beneficial as part of a fiber or drug candidate by modulating intestinal bacteria.



Scheme 5. Chemical structure of CDA-0.05

Although the relationship between chemical structure and biological activity of *Cistanche deserticola* polysaccharides has been studied and some new components and structures of polysaccharides have been obtained, the chemical components and structures have not been summarized systematically, and structure-activity relationships of polysaccharides have not been clearly and systematically delineated. Based on the above study, it can be seen that different kinds of polysaccharides are often obtained from different extraction and purification processes from different sources of *Cistanche deserticola*, which also confirms the complexity of the composition and structure of *Cistanche deserticola* polysaccharides. Polysaccharides have various pharmacological effects such as lowering blood sugar, blood lipids, regulating immunity and anti-aging, but most of the above structure-activity relationship studies only focus on their biological activities to improve immunity, and there is a lack of extensive and systematic research. Therefore, the chemical composition, structure and biological activity of polysaccharides and their correlations need to be further explored. In-depth study of the biological activity, mechanism and structure-activity relationship of *Cistanche deserticola* polysaccharide will promote the further

development and industrial application of *Cistanche deserticola*.

4. Biological Activities

Polysaccharide, as one of the main active ingredients of *Cistanche deserticola*, has many pharmacological effects such as regulating immune activity, anti-aging, anti-liver damage, anti-virus, anti-tumor, and affecting intestinal flora, and is widely used in clinical and health food applications.

4.1 Regulation of immune activity

Cistanche deserticola polysaccharide (CDPS) is an important active component of *Cistanche deserticola* that exerts immunomodulatory effects. It promotes the proliferation of lymphocytes and improves immune function. And also has an activating effect on macrophages and enhances phagocytic activity, thus increasing the synthesis and secretion of cytokines that regulate immune response and playing an immunomodulatory role.

Zeng et al investigated immunomodulatory effects on mouse T cells of CDPS [43]. CDPS was found to not only increase the proliferative response of mouse spleen and thymus lymphocytes alone, but also stimulate the proliferation of mouse thymus lymphocytes in concert with ConA and PHA. In addition, the polysaccharide significantly increased secretion of interleukin 2 by lymphocytes, so it is speculated that the promotional effect of CDPS on IL-2 secretion may be one of the mechanisms by which *Cistanche deserticola* can enhance immune function and anti-aging. Subsequently Zeng et al also investigated the mechanism of the immunomodulatory effects of CDPS [44]. They determined the immunomodulatory function of CDPS on the proliferation of mouse thymic lymphocytes in vitro and studied the cell cycle and its intracellular Ca^{2+} concentration. Finally, it is speculated that the mechanism may be that CDPS promotes the release of calcium from mouse thymic lymphocytes, and the elevated calcium ion concentration promotes IL-2 gene expression, leading to T cell proliferation. Wang et al observed the effect of CDPS on lymphocyte proliferation in vivo and in vitro, and also found that this effect is closely related to CDPS promoting the release of IL-2 from lymphocytes [45].

Wang et al. determined the activation effect of CDPS on macrophages [46]. The results showed that CDPS significantly enhanced the phagocytosis of macrophages and the release of NO, TNF-α and IL-1 from RAW264.7 cells, a mouse mononuclear macrophage commonly used to test immune-related experiments, as well as the release of NO from immunosuppressed RAW264.7 cells, which demonstrated that the immune enhancing effect of CDPS was correlated with its activation of macrophages. Zhang et al also discovered that CDPS may exert immunomodulatory functions by enhancing phagocytosis of THP-1 cells and promoting the release of cytokines [47].

4.2 Anti-aging

Research has demonstrated that CDPS has significant anti-aging activity, mainly through anti-oxidation, anti-fatigue, improved learning and memory, and enhanced telomerase activity.

Sun et al used D-galactose to cause experimental aging model in mice and observed the antioxidant function of CDPS on aging mice [48]. The results showed that CDPS inhibited lung collagen increase and elastin decrease and altered the imbalance of oxidative/antioxidative function of the body. It is hypothesized that it may eliminate the increase in free radical production caused by D-galactose and avoid lung tissue damage due to depletion of antioxidant substances, thus achieving a slowing effect on aging. Subsequently the group studied the effect of ozone-injured experimental lung aging mice administered with CDPS [49]. It was found that after injection of CDPS, the hypoxia tolerance of aging mice was significantly improved, the activity of superoxide dismutase for scavenging free radicals was enhanced, and the content of lipid peroxidation metabolites was reduced, indicating that CDPS can enhance the antioxidant function of aging mice.

Reduced fatigue resistance is one of the main features of aging. Yan et al examined the effect and mechanism of CDPS on anti-fatigue in D-galactose-induced aging mice [50]. It was revealed that both high and low doses (400 mg/kg, 100 mg/kg) of CDPS had anti-fatigue effects on aging mice. The mechanism may be to reduce serum urea nitrogen and lactate levels, increase content of liver glycogen and muscle glycogen, and enhance the ability of mice to fight free radical damage and antioxidant.

Recently one of the causes of Alzheimer's disease is thought to be a decrease in the number of neurons in brain tissue caused by apoptosis, and CDPS has anti-aging effects, so scholars have studied their related effects. Yin et al observed the effect of CDPS on learning and memory ability of model rats with Alzheimer’s disease [51]. It was found that CDPS inhibited NO production and release through the inhibition of ROS activity, and improved the decrease of learning and memory ability in rats. The mechanism may be to reduce the damage of oxygen free radicals and accelerating the scavenging of free radicals in vivo. Ma et al discovered that CDPS was effective in improving learning and memory ability in D-galactose-induced ageing mice [52]. It is speculated that CDPS enhances learning memory function by upregulating cAMP response element binding protein expression in neurons, promoting neuronal survival and neuronal morphological and functional recovery, and reducing apoptosis. Wu et al examined the effect of CDPS on the improvement of learning memory ability in aging mice and revealed the mechanism was that CDPS modestly increased the release of excitatory neurotransmitter through upregulation of the cAMP/PKA/CREB/BDNF signaling pathway [53].

Zhang et al employed a D-galactose aging mouse model to observe the effect of CDPS on it [54]. It was observed that CDPS significantly reduced malondialdehyde content in tissues and increased telomerase activity in heart and brain tissues, lymphocyte proliferative response and

phagocytosis of macrophages. Therefore, it is inferred that CDPS has good effects in antagonizing free radical damage, enhancing telomerase activity and immune function, and delaying the effect on aging.

4.3 Gut Protection and laxative effect

Jia et al revealed that polysaccharide-rich aqueous extracts of *Cistanche deserticola* can effectively prevent colorectal cancer and intestinal inflammation in a mouse model [55]. The mechanism may be to stimulate the immune active system, reducing inflammatory mucosal hyperplasia and intestinal helicobacter infection. This study demonstrated that CDPS may have clinical potential in the prevention and treatment of colorectal cancer. Fu et al investigated the effect of CDPS on gut microbiota and discovered that all species of polysaccharides could regulate the diversity of intestinal microbiota, increase beneficial bacteria, promote the absorption of echinacoside, and improve the disordered gut microbiota [56]. In addition, low molecular weight polysaccharides may treat diseases related to the gut microbiota. Gao et al investigated the effects of CDPS on mice with cognitive decline and dysbiosis of gut microflora due to D-galactose [57]. The results indicate that CDPS improving low cognitive performance in mice by restoring gut microbial homeostasis.

Liu et al demonstrated the regulatory effects of CDPS on the constipation of aged rats and CDPS markedly increased the content of beneficial bacteria while dramatically decreasing the content of harmful bacteria [58]. In addition, CDPS enhances immunity and prevents pathogenic bacterial infections. The mechanism of the laxative effect of CDPS mainly included metabolic energy and amino acid synthesis.

4.4 Anti-spleen deficiency and anti-liver damage

Xu et al investigated the regulatory effect of CDPS on CCl_4-induced liver qi stagnation and spleen deficiency in mice [59]. The results revealed that CDPS alleviated weight loss in mice with hepatic depression and spleen deficiency, and also modulated immune deficiency. Wang et al investigated the protective effects of CDPS on CCl_4-induced acute liver injury in mice and showed that CDPS could reduce malondialdehyde content and increase superoxide dismutase levels in mouse liver [60]. CDPS significantly improved the hepatic redox status in mice, and the mechanism may be that CDPS counteracts lipid peroxide damage to hepatocytes, improves their antioxidant capacity, and performs normal defense and compensatory functions. Guo et al extracted three polysaccharides from *Cistanche deserticola*, of which the polysaccharide named CDP-C had higher anti-liver damage and antioxidant activity [61]. Bioactivity experiments suggest that CDP-C may exert protective effects against alcohol-induced chronic liver injury by reducing malondialdehyde and triglyceride levels and modulating the activity of related enzymes. The

biological activity correlates with the fact that CDP-C contains a higher proportion of galacturonic acid.

4.5 Prevent and treat osteoporosis

Song et al investigated the function and mechanism of CDPS on osteoporosis. The result showed CDPS inhibited osteoclastogenesis and hydroxyapatite absorption, also restrained the expression of osteoclast marker genes. Consequently, CDPS is expected to be applied clinically for the treatment of osteoporosis [62]. Wang et al explored the types of active ingredients and mechanisms involved in the treatment of osteoporosis in *Cistanche deserticola*. Senescence accelerated mice were administrated *Cistanche deserticola* extract total glycosides and polysaccharides. The result demonstrated that these extracts can promote osteoblastogenic bone formation and improve bone microstructure injury by modulating Wnt/β-catenin signaling pathway [63]. Xiao et al investigated the effect of extracted and purified CDPS on the generation and function of osteoclasts and the treatment of osteoporosis. The results exhibited that CDPS treatment prevented OVX-induced osteoporosis and improved bone loss by inhibiting osteoclast activity and function [64].

4.6 Other biological activities

In terms of biological activities, CDPS not only exhibits common effects such as regulation of immune function, anti-aging, anti-splenial deficiency, anti-hepatic injury and intestinal protection. But many scholars have also studied the pharmacological activities in other aspects, including anti-tumor, wound healing, promotion of bone marrow hematopoietic function and improvement of menopause, in order to provide reference for *Cistanche deserticola* in clinical applications and health care drugs.

Zhang et al revealed that CDPS significantly inhibited Lewis lung tumor and solid tumor S_{180} in mice and prompted natural killer cell and interleukin-2 activity [65]. Therefore, it is speculated that the tumor suppressive effect of CDPS is related to its ability to increase the activity of natural killer cells and interleukin-2, which alleviates the depressed immune function of mice. These effects increase with increasing doses of CDPS, which may be used clinically as an adjuvant to tumor treatment. Gao et al investigated the effects of various concentrations of CDPS on cultured human fibroblasts in vitro and found that the drug significantly promoted the growth of fibroblasts in vitro [66]. The concentration of CDPS has an impact on its effect, and the appropriate concentration should be adopted to promote the healing of wounds, providing a theoretical basis for its clinical application. Chen et al observed the effect of CDPS on hematopoietic function in myelosuppressed anemic mice and confirmed that CDPS may promote the recovery of bone marrow hematopoietic function through promoting the transformation of the bone marrow cell cycle in myelosuppressed anemic mice [67]. Zhang et al explored the effect of CDPS on the regulation of serum endocrine function in rats with menopausal hypertension and established an animal model by removing the ovaries of rats and giving them dry chow [68]. CDPS was identified to enhance HPOA function to achieve regulation of neurotransmitters and endocrine hormones, improve neurological function, stabilize the body environment, and accelerate metabolism, thus

improving menopausal symptoms.

5. Conclusion

Cistanche deserticola has been used in China for more than 2,000 years. Its taste is sweet and slightly salty, and it has many health functions including nourishing the kidneys, laxative effect, delaying aging and enhancing memory. Polysaccharide is one of the important active ingredients extracted from *Cistanche deserticola*, which has received wide attention for its pharmacological activities such as immunomodulation, anti-aging, anti-tumor and anti-liver damage. Therefore, the extraction and isolation of polysaccharides, chemical structure analysis and the study of its biological activity have important research significance. At present, the extraction methods of *Cistanche deserticola* polysaccharides mainly include traditional extraction method, ultrasonic-assisted method, microwave-assisted method and enzyme-assisted method, etc. Analysis of the chemical structure of polysaccharides is the primary prerequisite for studying its pharmacological activity and efficiently utilizing the transformation. The extracted polysaccharides were analyzed by chemical methods such as methylation, periodate oxidation, acid hydrolysis and Smith degradation, and various spectroscopy methods including NMR, IR and HPLC to obtain a variety of polysaccharides with new structures. The relationship between the chemical structure and biological activity of polysaccharides has been studied by a small number of scholars, but the relationship remains unclear due to the large number of components, complex structure and lack of systematic studies on polysaccharides. In addition, there are few studies on the effects of *Cistanche deserticola* polysaccharide extraction methods on its chemical structure and biological activity. It is expected that the subsequent research on polysaccharide structure and active conformational relationship will be of high value to realize the precise transformation of big health products, with a view to achieving the modernization of *Cistanche deserticola* in Chinese medicine and making major breakthroughs and innovations in pharmaceutical sciences, clinical medicine, food and health products, functional cosmetics, etc.

Declaration of competing interest

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

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