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ISOLATION AND PHYSICOCHEMICAL CHARACTERIZATION OF POLYSACCHARIDE FRACTIONS ISOLATED FROM *Schisandra chinensis*

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In the past decades, it was believed that polysaccharides and their conjugates in plant are not only energy resources but also play crucial biological roles in many life processes [1]. The structure and mechanisms of pharmaceutical effects of bioactive polysaccharides on various diseases have been extensively studied, and many natural polysaccharides with different curative effects have been examined and even applied in therapies [2].

Schisandra chinensis, distributed abundantly in the northeast region of China, has been used in traditional Chinese medicine for thousands of years [3]. It is officially listed in the Chinese Pharmacopoeia and indexed as a tonic and sedative. It is also listed in the "Shen Nong Ben Cao Jing" book, year 1596 (2697 BC) as a superior drug that helps in coughs and prevents asthma [4, 5].

Unexpectedly, at present there are no specific studies on polysaccharides from *S. chinensis*. Therefore, the present studies were carried out to isolate and purify the polysaccharide fractions from *S. chinensis* and to further investigate their basic physicochemical properties in order to most effectively acquire high-performance polysaccharide products and exploit the applied potential of *S. chinensis*.

In this study, the AKTA purification system was employed to purify *S. chinensis* polysaccharide fractions with Hiload 16/30 DEAE-cellulose column and Hiload 26/100 Sephacryl S-200 column.

The yield of the crude water-soluble polysaccharide extracted from *S. chinensis* was 7.8% of dried material. After the freeze–thaw process and deproteination by a combination of proteinase and the Sevag method, the crude polysaccharide sample (cSCPS) was loaded onto the DEAE-cellulose column and eluted with de-ionized water and 0→1 M gradient of NaCl solution at a flow rate of 1 mL/min. The main fraction eluted by de-ionized water was collected, lyophilized, and further fractionated onto a Sephacryl S-200 column and eluted with 0.15 M NaCl solution at a flow rate of 0.5 mL/min. Three main fractions (SCPS-a, SCPS-b, and SCPS-c) were separated for further analysis of physicochemical properties.

The total sugar, protein, uronic acid contents, molecular weight, and monosaccharide compositions of the polysaccharide fractions are summarized in Table 1. The polysaccharide fractions SCPS-b and SCPS-c had a higher total carbohydrate content (93.6% and 95.8%, respectively) than SCPS-a (79.3%). According to the Bradford method, the protein content of SCPS-a was 19.6%; it was not detected in SCPS-b and SCPS-c.

Furthermore, as measured by the *m*-hydroxydiphenyl method, SCPS-a had the highest uronic acid content (2.2%) in comparison with PPS-1 (0.9%) and PPS-3 (not detected). The average molecular weights of SCPS-a, SCPS-b, and SCPS-c calculated by HPGPC were 76.100, 20.400, and 5.300, respectively.

According to GC analysis, SCPS-a was composed of rhamnose, fucose, arabinose, mannose, galactose, glucose, and trace galacturonic acid with molar ratios of 1.2:0.5:0.9:1.6:1:3.3:0.3, and SCPS-b was composed of rhamnose, fucose, mannose, galactose, glucose and galacturonic acid with molar ratios of 1.6:1.4:0.4:1:2.8:0.1. SCPS-c was composed only of four monosaccharides: rhamnose, fucose, galactose, and glucose with molar ratios of 1.8:0.6:1:4.2.

Plant Material and Reagents. *S. chinensis* was purchased from a medicine market in Jilin City. Sephacryl S-200 was purchased from Amersham Pharmacia Co. (Sweden). T-series dextrans and trifluoroacetic acid (TFA) were purchased from Fluka. DEAE-cellulose, standard monosaccharides, and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemical reagents used were of analytical grade.

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TABLE 1. Molecular Weight and Composition of Different Polysaccharide Fractions Isolated from *S. chinensis*

Physicochemical properties	SCPS-a	SCPS-b	SCPS-c	Physicochemical properties	SCPS-a	SCPS-b	SCPS-c
Sugar components, mol%				Molecular weight	76.100	20.400	5.300
Rhamnose	1.2	1.6	1.8	Total sugar, %	79.3	93.6	95.8
Fucose	0.5	1.4	0.6	Protein, %	19.6	Nd.	Nd.
Arabinose	0.9	Nd.	Nd.	Uronic acid, %	2.2	0.9	Nd.
Mannose	1.6	0.4	Nd.				
Galactose	1	1	1				
Glucose	3.3	2.8	4.2				
Galacturonic acid	0.3	0.1	Nd.				

Nd.: not detected.

Isolation and Purification of Polysaccharide Fractions. The dried *S. chinensis* were defatted with 85% alcohol for 24 h. After filtration, the residues were extracted with distilled water. The aqueous extracts were filtered and concentrated. The supernatant was treated with alcohol for precipitation. The precipitate was deproteinated by a combination of proteinase and the Sevag method [6]. Finally, the supernatant was lyophilized to give crude *S. chinensis* polysaccharides (cSCPS).

The cSCPS was dissolved in distilled water, applied onto DEAE-cellulose column, and eluted successively with distilled water and a gradient of 0→1 M NaCl. Fractions was collected and monitored using the phenol–sulfuric acid method at 490 nm absorbance for polysaccharides, and at 280 nm absorbance for proteins. The eluate was further fractionated on Sephacryl S-200 column and eluted with 0.15 M NaCl to yield three main fractions, SCPS-a, SCPS-b, and SCPS-c.

Analysis of Monosaccharide Composition and Carbohydrate and Protein Contents. Gas chromatography (GC) was used for identification and quantification of the monosaccharide compositions as described [7]. Total carbohydrate contents were determined by the phenol–sulfuric acid colorimetric method [8]. Total uronic acid contents were measured by the *m*-hydroxydiphenyl method [9]. In addition, proteins were quantified according to the Bradford method [10].

Homogeneity and Molecular Weight. The homogeneity and molecular weight of polysaccharide fractions was evaluated and determined by HPGPC as described [11].

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