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Original Article

Extraction of water-soluble polysaccharide and the antioxidant activity from *Semen cassiae*

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ABSTRACT

Water-soluble polysaccharide was isolated from *Semen cassiae* using water for extraction and ethanol for deposition. The optimized conditions for polysaccharide isolation by orthogonal experiments were a sample to liquid ratio of 1:30 at 80°C for 3.5 hours; the yield of polysaccharide from *Semen cassiae* under these conditions was 5.46%. Different polysaccharides (SCPW-1, SCPW-2, SCPW-3, SCPW-4, SCPW-5, SCPS-1, SCPS-2) were obtained from the extract (i.e., crude polysaccharide) by DEAE-cellulose column chromatography. The polysaccharides obtained showed different structures by Fourier transform infrared therein the five elected from the seven kinds separated. The antioxidant activities of the extract were evaluated. The scavenging rates of the present extract on hydroxyl and superoxide were 43.32% and 64.97%, respectively, at a concentration of polysaccharide of 94.03 μg/mL, which was better than vitamin C at the same concentration. The scavenging rate of the present extract on 1,1-diphenyl-2-picrylhydrazyl was 13.33% at a polysaccharide concentration of 94.03 μg/mL, which was less than vitamin C at the same concentration.

1. Introduction

Reactive oxygen species (ROS) including superoxide anion, hydrogen peroxide (H_2O_2), and hydroxyl radicals have been implicated in the cytotoxicity and carcinogenicity of certain chemical carcinogens, as well as in the etiology of some human diseases such as inflammatory diseases, cancer, aging, and other many diseases [1–7]. However, superoxide and hydroxyl radicals are inevitably formed in almost all aerobic organisms. Under normal metabolic conditions, the DNA in each cell of our body is exposed to a good number of oxidative hits, leading to the formation of various oxidative DNA lesions. *In vivo*, some of these ROS play a positive role such as energy production, phagocytosis, regulation of cell growth, and intracellular signaling. There is a balance between generation of ROS and the antioxidant system in organisms. In pathological conditions, ROS are overproduced and result in lipid peroxidation and oxidative stress. The imbalance between ROS and antioxidant defense mechanisms leads to oxidative modification in cellular membranes or intracellular molecules [4,8].

In almost all organisms a variety of complex defense and repair systems have evolved to protect them against oxidative

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damage; however, these systems are insufficient to entirely prevent the damage. Fortunately, antioxidant supplements containing antioxidants can help the human body prevent or reduce the oxidative damage caused by toxic radicals (Zhao et al). Therefore, inhibition of free radical-induced oxidative damage by supplementation of antioxidants has become an attractive therapeutic strategy for reducing the risk of these diseases [4,6].

Many antioxidant compounds, naturally occurring in several traditional Chinese medicines, have been identified as free radical or active oxygen scavengers [9–11]. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials, to replace synthetic antioxidants, which are being restricted due to side effects such as carcinogenicity [1,8,12–14].

Semen cassiae, a well-known traditional Chinese medicine, is the dry and mature seed of *Cassia obtusifolia* L. or *Cassia tora* L. belonging to the *Cassia* genus of Leguminosae. *Semen cassiae* was used for preventing and curing diseases for centenaries; it was recorded in the Compendium of Materia Medica that *Semen cassiae* had the functions of clearing the liver, improving vision, and relaxing the bowel. It could cure conjunctival congestion, pain in the eye, photophobia, hyperdacryosis, headache, dizziness, constipation, etc. [15,16]. Recent research indicated that *Semen cassiae* had the function of strengthening immunity and had antiaging properties. Research on *Semen cassiae* was focused on the chrysophanol, rheum emodin, and anthraquinone components [16–23].

In this study, the aim was to obtain the optimized conditions for isolation of water-soluble polysaccharides from *Semen cassiae*, and to determine and compare their chemical structures and antioxidant activities, including scavenging effects on the hydroxyl radical, superoxide anion radical, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.

2. Materials and methods

All reagents were of analytical-reagent grade, unless otherwise stated. DPPH was purchased from Johnson Matthey Company. Diethylaminoethyl-celluose (DEAE-cellulose) was from Sigma. The IRprestige-21 Fourier transform infrared (FTIR) spectrophotometer was from Shimadzu, Japan. The instrument parameter was resolution 4.0, range 400–4000 cm^{-1}.

2.1. Water-soluble polysaccharide extraction

Semen cassiae, which was purchased from a drugstore in China Dalian City, was dried at 70°C and comminuted into powder, then sieved through a sieve of 80 mesh size.

About 2 g of the prepared sample powder was disposed with water for extracting the water soluble polysaccharide, filtered, and about a five-fold volume of ethanol was added into the filtrate for polysaccharide precipitation; the solution was then placed in a refrigerator at 4°C for 24 hours, and the polysaccharide precipitate was obtained.

The orthogonal matrix method was used for optimizing the extracting conditions [24]. The polysaccharide concentration was determined with the phenol-sulfuric acid method at 481 nm [24]. The process route is as shown below.

Polysaccharide content in the extract (%, w/w) = (concentration × diluted times × sample volume)/(the extract sample weight) × 100%.

Polysaccharide yield (%, w/w) = (polysaccharide content × the total weight of the extract)/(*Semen cassiae* sample weight) × 100%.

2.2. Purification of crude polysaccharide

The crude polysaccharide sample was purified by gel column chromatography. DEAE-cellulose (60 g) was soaked in deionized water and dumped the clarity supernatant liquid. The cellulose was soaked in 1000 mL of 0.5 mol/L NaOH for 30 minutes, bathing the cellulose with water until neutral; it was then soaked in another 1000 mL of 0.5 mol/L HCl for 30 minute, bathing the cellulose with water until neutral, and the process repeated a third time; now the DEAE-cellulose was ready for use.

The crude polysaccharide sample was dissolved in deionized water and centrifuged, and the supernatant was loaded onto a DEAE-cellulose column (50 × 2.5 cm, internal diameter), which was eluted with deionized water and 0.1 M NaCl solution in order. The elution (3 mL) was collected and carbohydrate content determined based on the phenol-sulfuric acid method at 481 nm absorbance [24]. The polysaccharides were divided into seven fractions, which were then freeze-dried; they were named as SCPW-1, SCPW-2, SCPW-3, SCPW-4, SCPW-5, SCPS-1, and SCPS-2, respectively.

2.3. FTIR analysis

FTIR spectra of the polysaccharide fractions (in KBr pellets) were measured using an FTIR spectrophotometer operating from 400 cm^{-1} to 4000 cm^{-1} at 4 cm^{-1} resolution. Because SCPW-1, SCPW-2, SCPW-3, and SCPS-1 were the main fractions, they and SCPS-2 were selected for infrared spectrum analysis.

2.4. Determination of antioxidant activities of the extract

The scavenging capacities of the extract on hydroxyl radical, superoxide radical, and DPPH radical were evaluated and compared with those of vitamin C. The concentration of the extract was signified by the concentration of the polysaccharide in the extract in the experiment; all the experiments were performed in triplicate and the results were averaged. The scavenging activity of the hydroxyl radical was generated by using Fenton's reaction [24]. The reaction contained 1.5 mL of sodium phosphate buffer (0.15 mol, pH 7.4), 0.2 mL of safranin T (260 μg/mL), 0.7 mL of EDTA-Na_2-Fe^{2+} (2 mmol), 1 mL of the sample and 0.8 mL of 3% H_2O_2 (v/v). The solution was incubated at 37°C for 30 minutes. In the essential control, sample and EDTA-Na_2-Fe^{2+} solutions were replaced by 3% H_2O_2 (v/v), and in blanks, deionized water was used as a substitute for the sample. The antioxidant activity of the sample was evaluated with clearance rate, E% = $[(A-A_0)/(A_e-A_0)] \times 100\%$, where A is the absorbance of the sample, A_0 is the absorbance of the blank, and A_e is the absorbance of the essential control.

Table 1 – Factor and level of the orthogonal experiment.

Level	A Sample-to-liquid ratio (w/v)	B Temperature (°C)	C Time (h)
1	1 (1:20)	1 (70)	1 (2.5)
2	2 (1:30)	2 (80)	2 (3.0)
3	3 (1:40)	3 (90)	3 (3.5)

Note: A, B and C were factors.

The scavenging activity of the extract on DPPH was determined [24]. Two milliliters of the sample was placed in a cuvette, and 2 mL of ethanol solution of 0.16 mmol DPPH was added. The solution was incubated at 25°C for 15 minutes, and determined at 525 nm. The antioxidant activity of the sample was evaluated with clearance rate, $E\% = [(1-A/A_0)] \times 100\%$, where A is the absorbance of the sample and A_0 is the absorbance of the DPPH solution.

The scavenging activity of the extract on superoxide radicals was determined by the pyrogallic acid method [24]. Volumes of 4.5 mL Tris-HCl buffer (0.05 mol, pH 8.2), 1 mL of sample solution, and 0.4 mL pyrogallic acid (3.0 mmol) were added together; the solution was incubated at 25°C for 15 minutes, 0.5 mL of thick hydrochloric acid was added for termination the reaction, and it was determined at 525 nm. The antioxidant activity of the sample was evaluated with clearance rate, $E\% = [(1-A/A_0)] \times 100\%$, where A is the absorbance of the sample and A_0 the absorbance of the blank.

3. Results and discussion

3.1. Extraction

The design of three levels orthogonal experiment was employed for electing the optimum conditions of polysaccharide. Sample-to-liquid ratio, extracting temperature, and extracting hours were chosen as the primary factors for the orthogonal experiment (Table 1). The results of the yield of the polysaccharide from *Cassia torae* seeds are shown in Table 2.

Table 2 – Results of the orthogonal experiment $L_9(3^3)$.

Test no.	Factor			Polysaccharide yield %
	A	B	C	
1	1	1	1	3.04
2	2	1	2	3.93
3	3	1	3	4.77
4	3	2	1	4.51
5	2	2	3	5.46
6	1	2	2	1.92
7	1	3	3	4.14
8	2	3	1	4.17
9	3	3	2	3.56
K_1	9.10	11.74	11.72	
K_2	13.56	11.89	9.41	
K_3	12.84	11.87	14.27	
k_1	3.03	3.91	3.91	
k_2	4.52	3.96	3.13	
k_3	4.28	3.95	4.76	
R	1.49	0.05	0.85	
R order	A > C > B			
Best level	A_2	B_2	C_3	

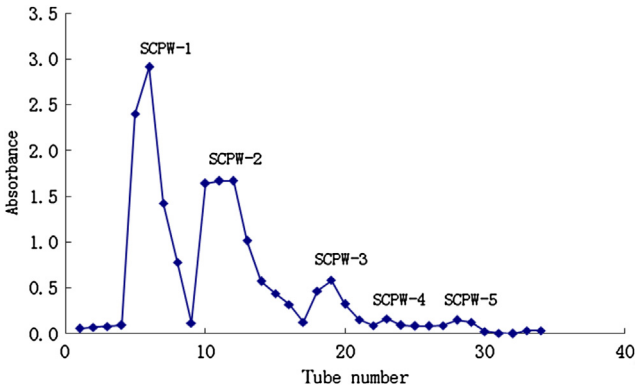

Fig. 1 – Elution curve of the extract by DEAE-cellulose column chromatography eluted by water.

In Table 2, the K_1 value represented three mixed values of polysaccharide yield under the first level, with the same for K_2 and K_3. The k_1 value represented the dividing value of K_1 by test times under the same level, with the same for k_2 and k_3. The R value represented the extreme difference of extraction yield of the three factors of the k value. The results indicated that the largest R value was factor C, then factor A; the smallest was factor B, i.e., the main factor was sample to liquid ratio, then time, and then temperature. The optimum condition was $A_2B_2C_3$, which was a sample to liquid ratio of 1:30, at 80°C for 3.5 hours; the yield of polysaccharide from *Semen cassiae* under these condition was 5.46%.

3.2. Polysaccharide content determination

Glucose was used as the standard for polysaccharide determination; the linear equation was $y = 0.0904x - 0.019$ with a correlation coefficient of 0.9986.

3.3. Purification of crude polysaccharide

The crude polysaccharide (the extract) sample isolated from *Semen cassiae* was fractionated on a DEAE-cellulose column.

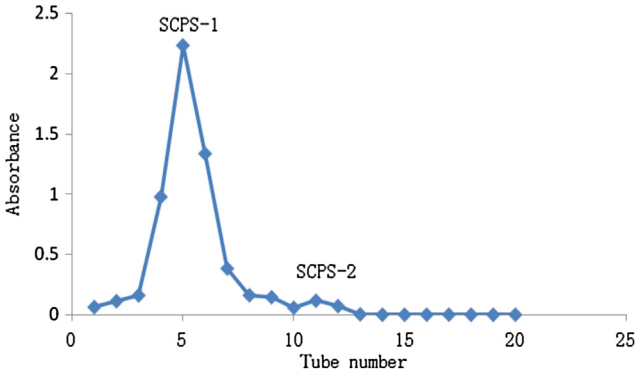

Fig. 2 – Elution curve of the extract by DEAE-cellulose column chromatography eluted by 0.1 M NaCl.

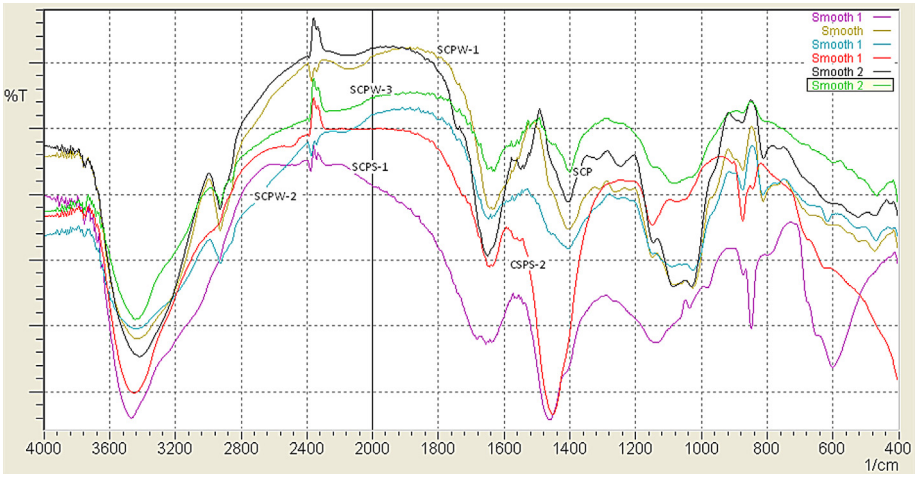

Fig. 3 – Fourier transform infrared (FTIR) spectra of SCPW-1, SCPW-2, SCPW-3, and SCPS-1, SCPS-2.

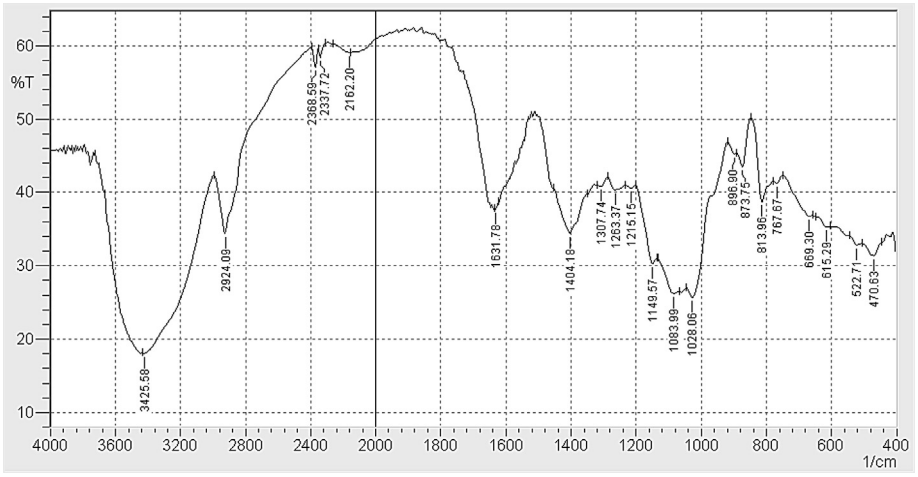

Fig. 4 – Fourier transform infrared (FTIR) spectra of SCPW-1 with wave number.

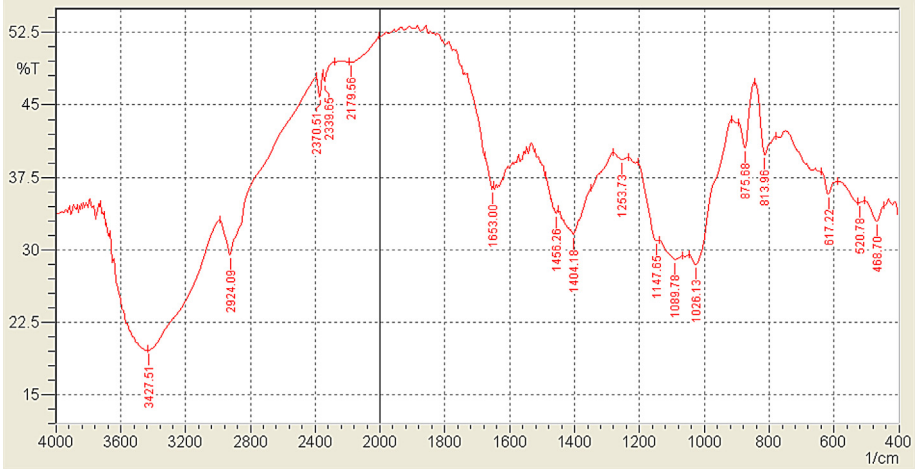

Fig. 5 – Fourier transform infrared (FTIR) spectra of SCPW-2 with wave number.

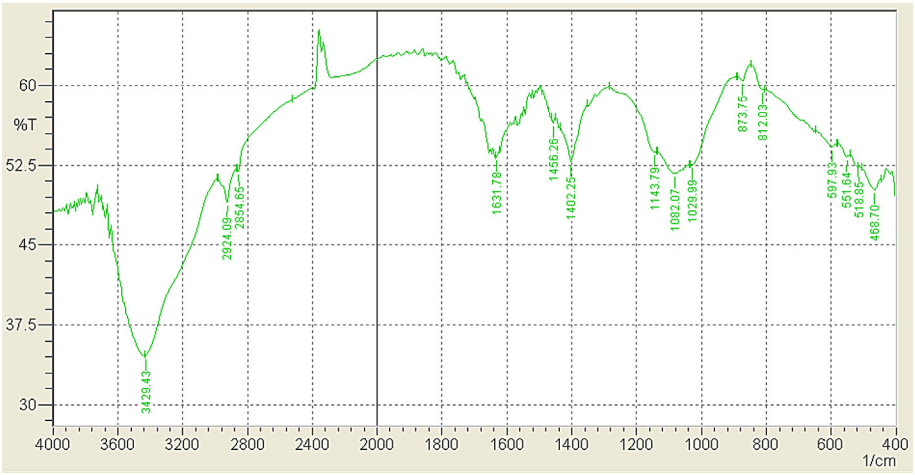

Fig. 6 – Fourier transform infrared (FTIR) spectra of SCPW-3 with wave number.

Seven fractions were then obtained and named SCPW-1, SCPW-2, SCPW-3, SCPW-4, SCPW-5, SCPS-1, and SCPS-2. SCPW (SCPW-1, SCPW-2, SCPW-3, SCPW-4, and SCPW-5) was from the water elute fraction (Fig. 1), SCPS (SCPS-1 and SCPS-2) was from the 0.1 M NaCl elute fraction (Fig. 2).

3.4. *FTIR analysis*

FTIR spectroscopy is typically used for the qualitative analysis of organic functional groups. The FTIR spectra of SCPW-1, SCPW-2, SCPW-3, SCPS-1, SCPS-2 and the crude polysaccharides (SCP) are illustrated in Fig. 3 for comparisons. There were certain differences in the FTIR of the five polysaccharides. The FTIR spectra of SCPW-1, SCPW-2, and SCPW-3 with wave numbers are illustrated in Figs. 4–6, respectively. The wave numbers and the peaks of the FTIR spectra of the polysaccharides are shown in Table 3. The FTIR spectra of SCPS-1 and SCPS-2 with wave numbers are illustrated in Figs. 7 and 8, respectively. The wave numbers and the peaks belonging to the FTIR spectra of the polysaccharides are shown in Table 4. Thus, carbohydrates were preliminarily identified from the infrared spectra, and significant

Table 3 – Wave numbers and peaks belonging to the Fourier transform infrared (FTIR) spectra of the polysaccharides.

Wave number (cm^{-1})			The peak belonging [25]	
SCPW-1	SCPW-2	SCPW-3		
3425.58	3427.51	3429.43	Stretching vibration of saccharides O–H and N–H	
2924.09	2924.08	2924.09	$-CH_2$, C–H asymmetric stretching vibration	
—	—	2854.65	$-CH_2$, C–H symmetrical stretching vibration	
2162.20	2179.56	—	N–H stretching vibration	
—	1653.60		Aldehyde, C=O stretching vibration	
1631.78	—	1631.78	β-diketone, C=O stretching vibration	
—	1456.26	—	CH_3–O–, symmetrical deformation vibration	
1404.18	1404.18	1402.25	O–H, deformation vibration in plane	
1307.74	—	—	Aldehyde, C–CHO skeleton vibration	
1263.37	1253.73	—	C–O–C, stretching vibration, ring formation	
1215.15	—	—	C–N stretching vibration	
1149.57	1147.65	1143.79	C–O, stretching vibration; O–H, deformation vibration	Pyranose configurations
1083.99	1089.78	1082.07	C–O–C, unsymmetrical stretching vibration	
1028.06	1026.12	1029.99	Aldehyde, C–CHO skeleton vibration	
896.90	—	—	β-anomerism, C–H transfer angular vibrations	β-D-staphylo-pyranose
873.75	875.68	873.75	C–O–C skeleton vibration	Asymmetric stretching vibration of hydroxylfuran ring
813.96	813.96	812.03	α-D-galactopyranose	Symmetric stretching vibration of hydroxylfuran ring
767.67	—	—	α-D-xylopyranose	Symmetric stretching vibration of pyranoid ring
669.30	—	—	O–H, out-of-plane deformation vibration	
615.29	617.22	—	Region of 1500–400 cm^{-1} is the region of X–Y stretching	
—	—	597.93	vibration and X–H deformation vibration. Any tiny	
—	—	551.64	changes in chemical construction can be perceived in this region.	
522.71	520.78	—		
470.63	468.70	468.70		

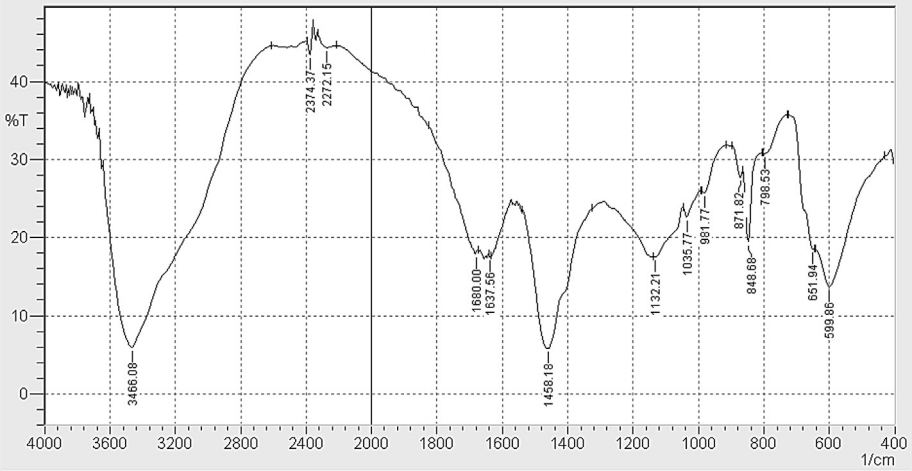

Fig. 7 – Fourier transform infrared (FTIR) spectra of SCPS-1 with wave number.

differences were seen in the FTIR of the five polysaccharides (Tables 3 and 4).

3.5. *Antioxidant activity*

3.5.1. *Antioxidant activity on hydroxyl radical*

The results of scavenging activity of the extract on the hydroxyl radical are shown in Fig. 9 with five polysaccharide concentrations of the extract; the clearance effect on the hydroxyl radical was better than vitamin C at the same concentration with polysaccharide. The extract gave a clearance rate of 9.41% at a concentration of polysaccharide of 18.81 μg/mL in the extract. At a higher concentration, the clearance rate was much better and reached 43.32% at a concentration of polysaccharide of 94.03 μg/mL, better than vitamin C (Fig. 9). This may be better than extracts containing polysaccharide from other plants, except *Ginkgo biloba* leaves. Herba houttuynia, *Sarcandra glabra*, and fructus lycii, which are kinds of traditional Chinese medicine, were able to scavenge the hydroxyl radical [24,26]. The scavenging rate of the polysaccharide from herba houttuynia was 15.9% at a concentration of polysaccharide of 2 mg/mL. The scavenging rate of the polysaccharide from *Sarcandra glabra* was 60% at 2 mg/mL. The scavenging rate of the polysaccharide from fructus lycii was 48.1% at 229.5 μg/mL. However, the scavenging effect of polysaccharide from *Semen cassiae* on the hydroxyl radical was less than that from *Ginkgo biloba* leaves.

3.5.2. *Antioxidant activity on DPPH*

Fig. 10 shows the results of the scavenging activity of the extract on the DPPH radical; the clearance effect on DPPH was less than that of vitamin C.

3.5.3. *Antioxidant activity on superoxide radical*

Fig. 11 shows the results of scavenging activity of the extract on superoxide radical; the clearance effect on superoxide radical was better than that of vitamin C. The clearance rate was 57.91% at a concentration of polysaccharide of 18.81 μg/mL in the extract; the clearance rate reached 64.97% at a concentration of polysaccharide of 94.03 μg/mL, better than vitamin C (Fig. 11). Previous studies indicated that the scavenging rate of the polysaccharide from plantain, a type of traditional Chinese medicine, was 50% at a concentration of polysaccharide of 172.85 mg/L [27]. The scavenging rate of

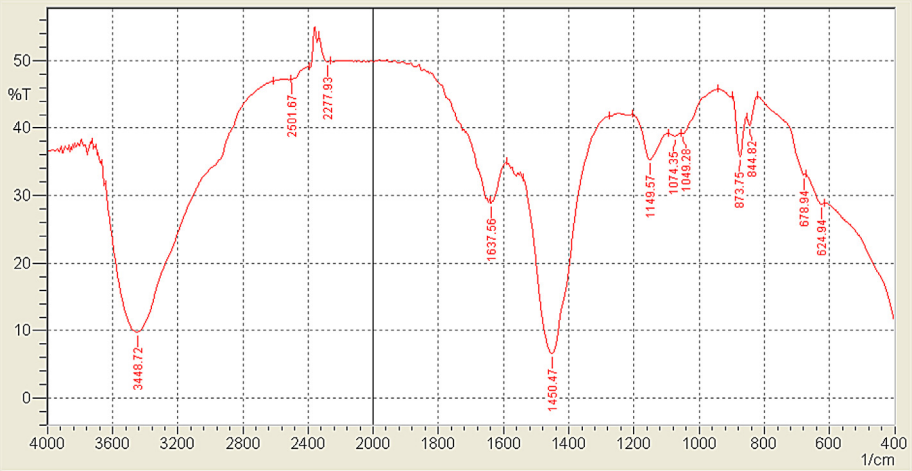

Fig. 8 – Fourier transform infrared (FTIR) spectra of SCPS-2 with wave number.

Table 4 – Wave numbers and peaks belonging of the Fourier transform infrared (FTIR) spectra of the polysaccharides.

Wave number, cm^{-1}		The peak belonging [25]
SCPS-1	SCPS-2	
3466.08	3448.72	Stretching vibration of saccharides O–H and N–H
2272.15	2277.93	–N=C=O, asymmetric stretching vibration
1680.00	—	C=O stretching vibration
1637.56	1637.56	β-diketone, C=O stretching vibration
1458.18	1450.47	CH_3-N<
1132.21	1149.57	C–O, stretching vibration; O–H, deformation vibration
—	1074.35	C–O–C, unsymmetrical stretching vibration
—	1049.28	Aldehyde, C–CHO skeleton vibration
1035.77	—	C–N stretching vibration and –NH deformation vibration of secondary amide
981.77	—	C–O stretching vibration
871.82	873.75	Epoxide ethers
848.68	844.82	α-Anomerism saccharous pyranoid ring
798.53	—	C–H, deformation vibration
—	678.94	O–H, out-of-plane deformation vibration
651.94	—	OCN deformation vibration of amide
—	624.94	
599.86	—	

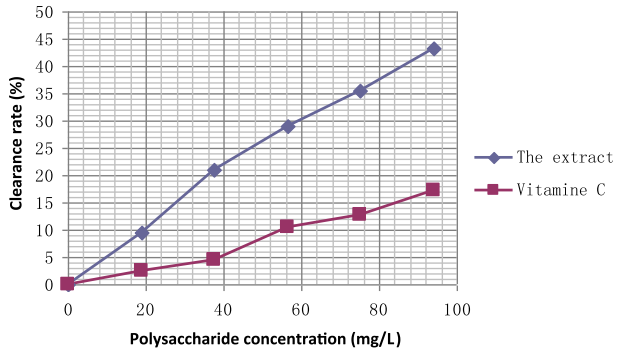

Fig. 9 – Scavenging effects of the extract and vitamin C on hydroxyl radical.

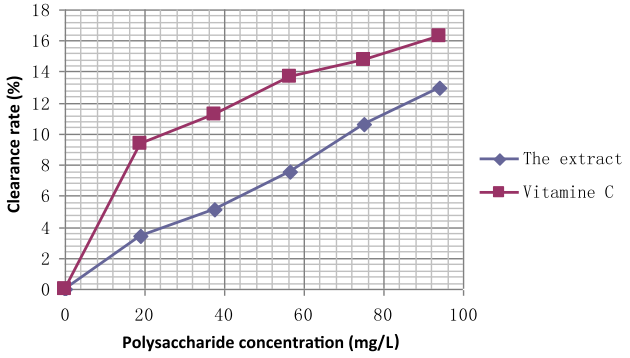

Fig. 10 – Scavenging effects of the extract and vitamin C on 1,1-diphenyl-2-picrylhydrazyl (DPPH).

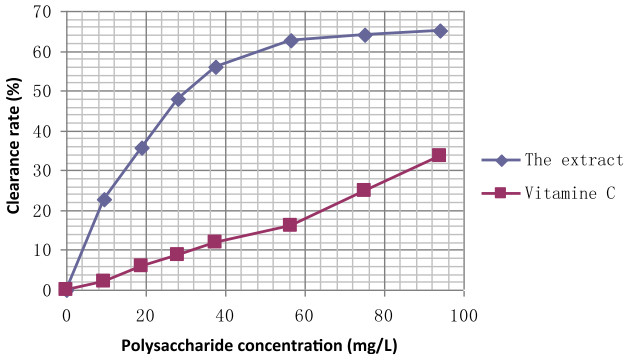

Fig. 11 – Scavenging effects of the extract and vitamin C on superoxide radical.

the polysaccharide from fructus lycii was 13.5% at 229.5 μg/mL [26].

4. Conclusion

The optimum extraction condition of polysaccharide from *Semen cassiae* was performed through an orthogonal experiment. The optimum conditions were sample to liquid ratio of 1:30 at 80°C for 3.5 hours; the yield of polysaccharide from *Semen cassiae* under these conditions was 5.46%. Polysaccharides obtained from the extract by DEAE-cellulose column chromatography showed different structures by FTIR. The results of scavenging activity showed that the extract from *Semen cassiae* was a better inhibitor against hydroxyl and superoxide radicals than vitamin C, except on DPPH, and the antioxidant activity on the superoxide radical was better than on the hydroxyl radical. Our results indicated that the present extract seemed to be a good antioxidant and could probably prevent the oxidative deterioration of food or provide a basic reference for pharmacology research on traditional Chinese medicine.

Conflicts of interest

All authors declare no conflicts of interest.

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