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Yijun Fan
Department of Landscape Plants, Sichuan Agriculture University, Chengdu 611130, China

Heqin Li
Department of Landscape Plants, Sichuan Agriculture University, Chengdu 611130, China

Aoxue Luo
Department of Landscape Plants, Sichuan Agriculture University, Chengdu 611130, China

Correspondence:
Aoxue Luo
Department of Landscape Plants, Sichuan Agriculture University, Chengdu 611130, China
E-mail: aoxueluo@sina.com

Extraction and scavenging activities on free radicals *in-vitro* of a polysaccharide from *Semen coicis*

*Yijun Fan, Heqin Li, Aoxue Luo**

Abstract

The water-soluble polysaccharide (SCP) was isolated from *Semen Coicis* through hot water extraction followed by ethanol precipitation. The polysaccharide was deproteinized by using the Sevag reagent, and the polysaccharide was free of proteins as scan by UV Spectra in 260 nm and 280 nm. In order to evaluate its antioxidant activities, Free radicals scavenging activities on superoxide radicals, hydroxyl radicals, DPPH and ABTS in vitro were carried out. The results indicated that SCP has significant radicals scavenging abilities on superoxide radicals and hydroxyl radicals and strong reducing capacity. The scavenging effects were powerful, which closed to the positive control. Therefore, the polysaccharide SCP should be explored as novel potential antioxidants.

Keywords: Hydroxyl radicals, Superoxide radicals, *Semen coicis*, Polysaccharide, Extraction.

Introduction

Semen coicis, is a precious herbal plant highly valued in Traditional Chinese Medicine and archived in the Pharmacopoeia of the People's Republic of China. Sections of the semen from coicis have been used for the treatment of cancer, antivirus, hypoglycemic, hypotensive and immunoregulation.[1] As *for Semen coicis* phytochemicals, much research has been carried out on the low molecular compounds, such as aliphatic acid, sterol and triterpene.[2] But, based on previous studies, polysaccharides exist widely in numerous plants and are identified as essential biomacromolecules in plant life, playing important roles in cell-cell communication, cell adhesion, and molecular recognition in the immune system.[3-6] In recent years, some bioactive polysaccharides isolated from natural sources have attracted much attention in the field of biochemistry and pharmacology.[7-8] However, the polysaccharides from *Semen coicis* have been little reported. Therefore, the purpose of the present investigation was to elucidate the isolation of water-soluble polysaccharide from the *Semen coicis*, as well as to evaluate its antioxidant activities in vitro.

Materials and methods

Vitamin C and BHT were purchased from Sigma Chemical Co. 1,1-diphenyl-2-picryl-hydrazyl (DPPH), potassium ferricyanide [$K_3Fe(CN)_6$], trichloroacetic acid (TCA), polyoxyethylenesorbitan monolaurate (Tween-20), and Berberine hydrochloride were purchased from Sigma (Sigma- Aldrich GmbH, Sternheim, Germany). Thiobarbituric acid (TBA), sodium dodecyl sulphate, nitroblue tetrazolium (NBT), nicotinamide

adenine dinucleotide (NADH), and phenazine methosulphate (PMS) were purchased from Applichem. ABTS radical was purchased from Merck. And all other chemicals were analytical grade and were made in China.

Extraction of *Semen coicis* polysaccharide

Extraction of the polysaccharide was carried out according to the method of Luo et al. [9] with some modifications. The *Semen coicis* were thoroughly washed with water, dried at 60 ℃, and then powdered with a pulverizer. The powder was extracted successively with petroleum ether and ethanol, at first. After filtered, the residue was further extracted with double-distilled water at 100℃ for 3 h three times. Then all extracts were combined, concentrated and filtrated. The extract was deproteinized 4 times using the Sevag reagent[10], and the polysaccharide was free of proteins as scan by UV Spectra in 260 nm and 280 nm. After removal of the Sevag reagent, the extract was precipitated by adding ethanol (4 times the volume of aqueous extract), and the mixture was kept overnight at 4℃ for the polysaccharide. The precipitate was collected by centrifugation at 4000 rpm for 20 min, washed successively with petroleum ether, acetone and ethanol, the procedure of precipitation was perform iteration, and then dissolved in water and dialyzed against deionized water for 72 h, freeze-drying to yield the polysaccharide, which named SCP.

Superoxide anion scavenging assay

Measurement of superoxide anion scavenging activity of *Semen coicis* polysaccharide was based on the method described by Wang et al. [11], with slight modification. 4.5 ml Tris–HCl buffer (50 mmol/L,pH 8.2) and 1.0 ml tested samples with various concentrations (3,10,30,100,300,1000,3000, 4000 μg/mL) were mixed in tubes with lids. Then the mixture was incubated for 20 min in the water bath at 25 ℃. Meanwhile, 0.4 ml of 25 mmol/L pyrogallol preheated at 25℃ was added immediately. After 4 min, the reaction was terminated by 0.1 ml HCl solution (8mol/L) and the mixture was centrifuged at 4000 rpm for 15min. The absorbance of sample and control were determined by UV spectrophotometer at 325 nm. The curve was made based on the absorbance value. Vc was used as the positive control compounds. Scavenging activity was calculated using the following equation:

$$\text{Superoxide anion scavenging effect (\%)} = (Ao\text{-}A_s)/Ao \times 100$$

where Ao is the absorbance without sample, and As is absorbance with sample.

DPPH radicals scavenging assay

In the present test, DPPH scavenging activities of the SCP was measured according to the method of Shimada et al [12], with some modifications. Vitamin C was used as reference material. Briefly, 0.1 mM solution of DPPH in methanol was prepared and 1.0 mL of this solution was added with 3.0 mL of the samples of various concentrations (3,10,30,100,300,1000,3000 μg/mL). The solution was kept at room temperature for 30 min, and the absorbance at 517 nm (A517) was measured. The DPPH scavenging effect was calculated as follows:

$$\text{DPPH scavenging effect (\%)} = [Ao\text{-}(A\text{-}Ab)]/Ao \times 100$$

Where Ao: A517 of DPPH without sample, A: A517 of sample and DPPH, and Ab: A517 of sample without DPPH.

Hydroxyl radical scavenging assay

The hydroxyl radicals scavenging activity of the polysaccharide was measured according to the method of Wang et al.[13], with some modifications. Different concentrations (3, 10, 30, 100, 300, 1000, 3000, 4000 μg/mL) samples were incubated with 2.0 mmol/L EDTA-Fe (0.5 ml), 3% H_2O_2(1.0 ml) and 0.36 mg/ml crocus in 4.5 ml sodium phosphate buffer (150 mM, pH 7.4) for 30 min at 37 ℃ and hydroxyl radical was detected by monitoring absorbance at 520 nm. The hydroxyl radical scavenging effect was calculated as follows:

$$\text{Hydroxyl radical scavenging effect (\%)} = [(Ac\text{-}As) / Ac] \times 100$$

Where As is the A520 of sample and Ac is the A520 of control in the control, sample was substituted with distilled water, and sodium phosphate buffer replaced H_2O_2.

ABTS radicals scavenging assay

The radicals scavenging activity of SCP against radical cation (ABTS+) was measured using the methods of Luo et al.[14], with some modifications. ABTS+ was produced by reacting 7 mmol/L of ABTS+ solution with 2.45 mmol/L of potassium persulphate, and the mixture would be kept in the dark at room temperature for 16 h. In the moment of use, the ABTS+ solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. The sample (0.2 ml) with various concentrations (10, 30, 100, 300, 1000, 3000 μg/mL) were added to 2 ml of ABTS+ solution and mixed

vigorously. After reaction at room temperature for 6 min, the absorbance at 734 nm was measured. The ABTS+ scavenging effect was calculated by the following formula:

ABTS scavenging effect (%) = [Ao-(A-Ab)]/Ao ×100

Where Ao: A734 of ABTS without sample, A: A734 of sample and ABTS, and Ab: A734 of sample without ABTS.

Reducing power

The reducing power of SCP was quantified by the method described earlier by Raza et al. [15], with some modifications. SCP was used as reference material. Briefly, SCP and BHT were used at differing concentrations (10-4000 µg/mL). 1mL of sample was mixed with phosphate buffer (2.5 ml, 0.2 mol/l, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50 ℃ for 20 min. Then the reaction was terminated by 2.5 ml TCA solution (0.1%) and the mixture was centrifuged at 3000 rpm for 10min. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5ml, 6 mmoll/l), and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Statistical analysis

The data were presented as mean ± standard deviation. Statistical analysis was conducted with the SPSS 16.0 software package.

Results and discussion

Effect of scavenging superoxide radicals

Superoxide radical is known to be a very harmful species and plays an important role in the formation of other reactive oxygen-species such as hydroxyl radical, hydrogen peroxide, or singlet oxygen in living systems. Fig. 1 shows the superoxide scavenging activity of SCP was increased with the increase of concentration. At the high doses of 1000-4000µg/ml, the polysaccharide exhibited high effect, when got to 4000 µg/ml, the scavenging rates of vitamin C and SCP reached 82% and 64%, respectively. The results indicated that SCP exhibited very good superoxide radical scavenging activity.

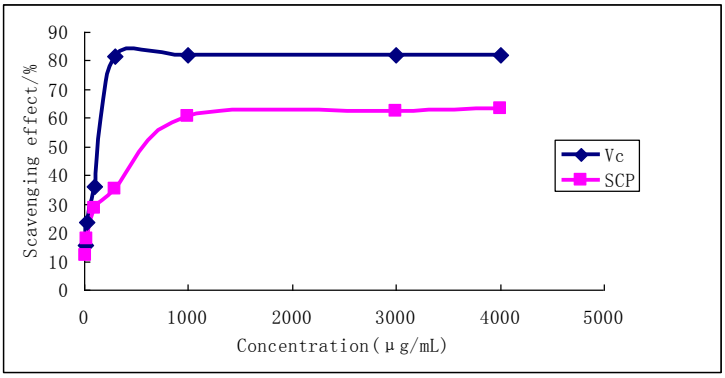

Figure 1: The superoxide radicals scavenging activities of SCP and Vc

Effect of scavenging DPPH radicals

In this experiment, the scavenging ability of SCP on DPPH free radical were examined in the concentration range of 3-3000 µg/mL using the DPPH colorimetric assay. And the results were given in Table 1. As is illustrated in the table, all the samples obvious scavenge activity in a concentration dependent manner. while it was not significantly active at different concentrations. At the concentration of 3000 µg/ml, the scavenging rates of vitamin C and SCP reached 91.05% and 6.72%, respectively. So, the results indicated that the polysaccharide SCP exhibited very low superoxide radical scavenging activity.

Table 1: The DPPH scavenging activity of SCP and the references

Concentration (µg/ml)	3	10	30	100	300	1000	3000
Scavenging effect of VC (%)	2.503	15.84	46.8	90.21	90.45	90.69	91.05
Scavenging effect of SCP (%)	1.59	2.73	3.53	3.99	5.125	6.15	6.72

Effect of scavenging hydroxyl radicals

The scavenging ability of SCP compared to those of vitamin C was shown in Fig.2. The samples exhibited obvious scavenging activities on hydroxyl radical in a concentration-dependent manner. The scavenging activities of SCP increased very significantly with increasing concentrations (1000-4000μg/ml). Especially in the high doses 4000μg/ml), SCP exhibited very high radical scavenging, which was close to that of Vitamin C ($p<0.05$). So, it was obvious that SCP has significant effects on hydroxyl radicals scavenging.

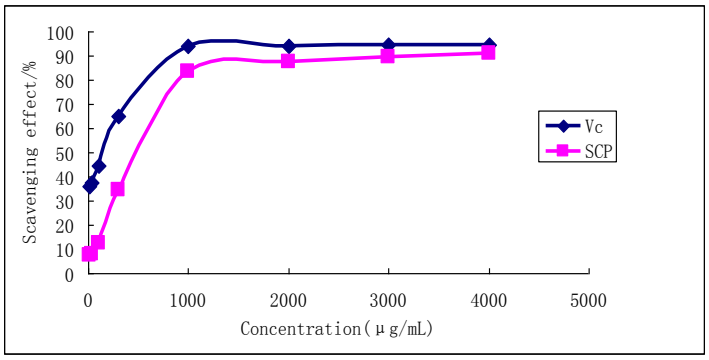

Figure 2: The Hydroxyl radicals scavenging activity of SCP and the references

Scavenging effects of polysaccharide on ABTS

The scavenging ability of SCP on ABTS free radical was shown in Fig.3. The scavenging powers of SC and Vc correlated well with increasing concentrations. The reference material exhibited an excellent scavenging effect in high doses (from 100 to 3000μg/ml). At the same time, the polysaccharide SCP showed low scavenging effect on ABTS. Even at the high dose 3000μg/ml, the effect was 23.25%. Therefore, the results indicated that SCP has no significant effect on scavenging power for ABTS radicals.

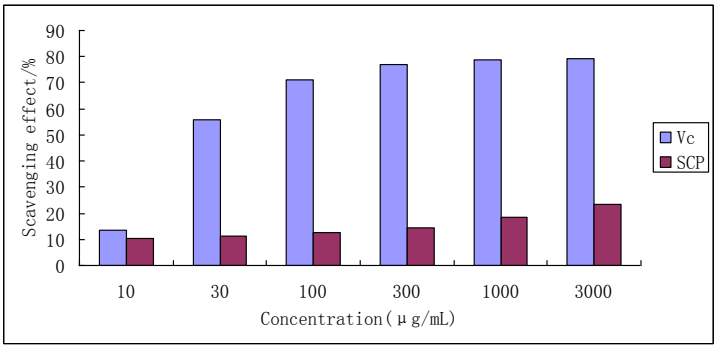

Figure 3: The ABTS scavenging activity of SCP and the references

Effect of reducing power

To measure reductive power of SCP, we investigated the Fe^{3+}-Fe^{2+} transformation in the presence of different concentrations sample, Vc were used as reference material. The reductive capabilities of SCP and reference material were exhibited as Fig.4. From the result, reducing powers of all samples were in a concentration-dependent manner. The reference exhibited strong reducing power. The reducing power of SCP was also strong, at the high concentrations (from 2000 to 4000 μg/ml), which was close to BHT ($P < 0.05$).

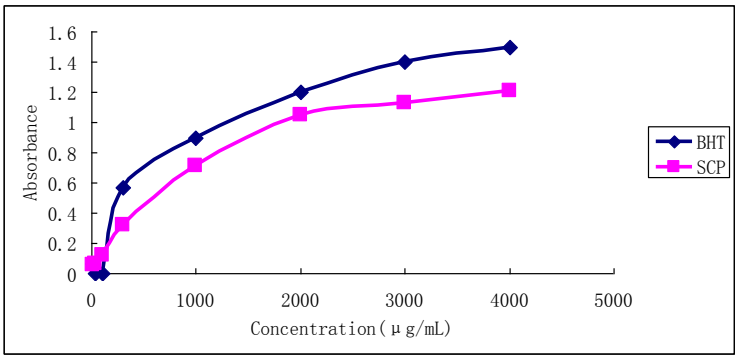

Figure 4: Reductive abilities of SCP and the reference

Conclusion

In the present study, the polysaccharide (SCP) was isolated from *Semen coicis* by water extraction and ethanol precipitation. Free radicals scavenging activities in vitro indicated that SCP has significant radicals scavenging abilities on superoxide radicals and Hydroxyl radicals and strong reducing capacity. The scavenging effects were powerful, which closed to the positive control. Therefore, the polysaccharide SCP should be explored as novel potential antioxidants. On the other hand, SCP exhibited a weak scavenging effect on ABTS and DPPH radical compared to the reference. Therefore, further investigation of its antioxidant activities *in vivo* elucidate and the mechanism of action relevant to its anti-oxidative activity will be carried out in our later work.

References

1. Hu S.H., Xiao X.N. Study Progress of Coix Seed. lishizhen medicine and materia medica research.2009,20(5):1059-1060.

2. Wen X.R. Progress in Study of Chemical Constituents and Anti-tumor Activities of *Semen coicis*. Journal of Liaoning University of Traditional Chinese Medicine.2008; 10(3):5-8.

3. Tong H.B.et al. Structural characterization and in vitro antitumor activity of a novel polysaccharide isolated from the fruiting bodies of *Pleurotus ostreatus*. Bioresource Technol. 2009; 100:1682-1686.

4. Cao W. et al. A novel polysaccharide, isolated from Angelica sinensis (Oliv.) Diels induces the apoptosis of cervical cancer HeLa cells through an intrinsic apoptotic pathway. Phytomedicine 2010; 17: 598-600.

5. Kiyohara H.et al. Intestinal immune system modulating polysaccharides in a Japanese herbal (Kampo) medicine, Juzen-Taiho-To. Phytomedicine 2002; 9: 614-624.

6. Sun Y.X. et al.. Structural elucidation and immunological activity of a polysaccharide from the fruiting body of *Armillaria mellea*. Bioresource Technol. 2009; 100:1860-1863.

7. Dwek R.A. Glycobiology: Toward understanding the function of sugars. Chem. Rev. 1996; 96: 683-720.

8. Rout S. Banerjee R. Free radical scavenging, anti-glycation and tyrosinase inhibition properties of a polysaccharide fraction isolated from the rind from *Punica granatum*. Bioresource Technol. 2007; 98: 3159-3163.

9. Luo A.X. et al. Chun, Z. In vitro antioxidant activities of a water-soluble polysaccharide derived from *Dendrobium nobile* Lindl. Extracts. Int. J. Biol. Macromol. 2009; 45:359-363.

10. Navarini L. et al. Polysaccharides from hot water extracts of roasted *Coffea arabica* beans: isolation and characterization. Carbohyd. Polym.1999; 40:71-81.

11. Wang B.S.et al. Antioxidant and free radical scavenging activities of pigments extracted from molasses alcohol wastewater. Food Chem. 2008, 107: 1198-1204.

12. Shimada K.et al. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agr. Food Chem. 1992; 40:945-948

13. Wang J.et al. Antioxidant activity of sulfated polysaccharide fractions extracted from *Laminaria japonica*. Int J Bio Macromol. 2008;42: 127-132.

14. Luo A,X.et al. *In Vitro* and *In Vivo* Antioxidant Activity of a Water-Soluble Polysaccharide from *Dendrobium denneanum.* Molecules. 2010;16:1579-1592.

15. Raza F.et al. Antioxidant activity of various extracts of old tea leaves and black tea wastes (*Camellia sinensis* L.). Food Chem.2007; 100:231-236.