academicJournals

Vol. 7(21), pp. 1543-1549, 3 June, 2013
DOI: 10.5897/JMPR11.1736
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Characterization and antioxidant properties of polysaccharides from flowers of *Sophora japonica* L. (Huaihua)

Lingwen Zhang[1,2], Hongfang Ji[1*], Ailin Du[3], Shuang Li[3] and Mingduo Yang[2]

[1]School of Food Science, Henan Institute of Science and Technology, Xinxiang 453003, P.R. China.
[2]Postdoctoral Research Base of the Chinese Fast Food Research and Development Center, Harbin University of Commerce, Harbin 150076, P.R. China.
[3]Xinxiang Medical University, Xinxiang 453003, P.R. China.

Accepted 19 January, 2012

Sophora japonica L. **flower polysaccharides (SJFP) were extracted with hot water, the crude polysaccharides were de-proteinized by Sevag method and fractionized by DEAE-52 column chromatogram. Five fragments were obtained, namely SJFP-A, SJFP-B1, SJFP-B2, SJFP-B3 and SJFP-B4. SJFP-A and SJFP-B3 were further purified by Superdex G-200 filtration column chromatography. The resulted two fractions SJFP-AA and SJFP-BB were characterized by Fourier transform infrared spectrophotometer (FTIR) and high pressure gel filtration chromatography (HPGFC). Monosaccharide composition was determined by high performance capillary electrophoresis (HPCE). SJFP-AA was composed of rhamnose, xylose, arabinose, and mannose with the molar ratio of 1: 6.14: 4.03: 1.48, while SJFP-BB was made up of rhamnose, arabinose, and mannose with the molar ratio of 6.26: 1: 1.87. Additionally, the crude SJFP exhibited good antioxidant activity in a dose-dependent manner. At the concentration of 4 mg/ml, the hydroxyl radical scavenging effect of crude SJFP was 90.32%, comparable to 92.63% for the positive control ascorbic acid (P > 0.05).**

Key words: Flowers of *Sophora japonica* L., polysaccharides, purification, antioxidant activity.

INTRODUCTION

Huaihua, flowers and buds of *Sophora japonica* L., has been commonly used in traditional Chinese medicine and folk edible material for a long time. Extracts from Huaihua have been reported to have many biological and pharmaceutical properties, for example, anti-oxidation, anti-platelet aggregation, and anti-inflammation (Chen and Hsieh, 2010; Ishida et al., 1989; Kim and Yun-Choi, 2008; López-Revuelta et al., 2006; Wang et al., 2008). Many of its bio-activities were believed to be due to the compounds called flavone, isoflavone, flavonoid-glycosides, isoflavone tetraglycosides, and poly-saccharides (Chen and Hsieh, 2010; Kim and Yun-Choi, 2008; López-Revuelta et al., 2006; Wang et al., 2008). There is a growing interest in extract from Huaihua (Chen et al., 2010; Lo et al., 2009; Paniwnyk et al., 2001; Park et al., 2009; Sun et al., 2007). Unfortunately, through literature retrieval, up to now, there is little information published on the polysaccharides isolated from the flowers of *S. japonica* L. Several synthetic antioxidants such as butyrated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commercially avai-lable and applied in fat and oily foods to prevent oxidative deterioration. Because of their possible toxic properties for human health and environment, these substances may be inappropriate for chronic human consumption (Botterweck et al., 2000; Yuan et al., 2008). Therefore, the development of alternative antioxidants from natural origin has attracted considerable attention and is thought

*Corresponding author. E-mail: jhf300@126.com Tel: +86 373 3040709. Fax: +86 373 3040709.

to be a desirable development.

Many polysaccharides derived from the natural resources were reported to have antioxidant activities (Chen et al., 2012; Song et al., 2010; Sun and Kennedyb, 2010; Thetsrimuang et al., 2011). Therefore, this paper was concerned with the purification and basic structure information of different fractions of *S. japonica* L. flower polysaccharides (SJFP). Simultaneously, the antioxidant properties of the crude SJFP were explored for seeking new biological functional principle used in food and pharmaceutical industry.

MATERIALS AND METHODS

Materials

The flowers of *S. japonica* L., produced in August of 2010, were obtained from Zaozhuang City, Shandong Province, China. The flowers of uniform shape and color were selected and dried at 35°C in a hot air oven until a constant weight was obtained and grounded into powder, sieved to produce samples with particle sizes in the range between 60 and 80 meshes, and refluxed with petroleum ether for 8 h to remove fat, stored in dark at room temperature until used. DEAE-52 and Sephadex G-200 were purchased from Pharmacia (USA).

Chemicals

L-ascorbic acid, 1,1-Diphenyl-1-picrylhydrazyl (DPPH), T-series dextran, ferrozine, standard sugars (rhamnose, xylose, glucose, mannose, arabinose, galactose), xanthine, xanthine oxidase, trichloride ferric ($FeCl_2$), ferrous sulfate ($FeSO_4$) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, USA). All other reagents were of analytical grade.

Extraction and purification of polysaccharide

100 g dried powder of *S. japonica* flowers was extracted with deionized water (2000 ml×3) at 90°C for three times and 4 h for each time. After centrifugation (4000 r/min for 15 min, at 20°C), the supernatants were combined and concentrated to 300 ml, and precipitated with 4 volume of absolute ethanol at 4°C for 24 h. The precipitate was separated by centrifugation on Sigma 3-18 K at 8000 r/min for 30 min (Sigma, German), then washed with absolute ethanol and ether, vacuum dried. The washed precipitate was re-dissolved in deionized water and treated with Sevag reagent (chloroform: n-butanol= 4:1, V/V) to remove proteins (Sevag et al., 1938), repeated this treatment for 4 times. The water-soluble fraction was lyophilized to obtain the crude SJFP.300 mg crude SJFP was dissolved in 30 ml double-distilled water, centrifuged at 10000 r/min for 10 min. The supernatant was loaded on a DEAE-52 column (2.6 cm × 55 cm). The column was eluted with 0, 0.1, 0.2, 0.4 and 0.8 mol/L NaCl, respectively, at a flow rate of 1 ml/min. Eluate was collected every 5 min and monitored with phenol-sulphuric acid method (Dubois et al., 1956) for polysaccharide content. The appropriate fractions were combined, dialyzed against double-distilled water and lyophilized. Five polysaccharides were obtained, that is, SJFP-A, SJFP-B1, SJFP-B2, SJFP-B3, and SJFP-B4. SJFP-A and SJFP-B3 were further purified by gel filtration chromatography on a Sephadex G-200 column (1.6 cm × 80 cm), eluted with 0.15 mol/L NaCl solution at a flow rate of 0.6 ml/min. The polysaccharide content was determined by phenol-sulphuric acid method. The fractions with high polysaccharide content were combined, dialyzed and lyophilized.

Molecular weight determination

The molecular weight of SJFP-AA and SJFP-BB were determined by high pressure gel filtration chromatography (HPGFC), which was performed on Waters 2695 HPLC system (Waters Corporation, MA, USA) with UltrahydrogelTM Linear 300 mm × 7.8 mm i.d. × 2 mm gel filtration column and Waters 2414 refractive index detector. The mobile phase was double-distilled water, and the flow rate was 0.8 ml/min at 40°C. Standard dextrans of T-500 (5×10^5), T-110 (1.1×10^5), T-70 (7×10^4), T-40(4×10^4), and T-10(1×10^4) were also analyzed with this system. Molecular weight distribution of SJFP-AA and SJFP-BB were determined by comparison with the retention time of standard dextrans under the same condition.

Infrared analysis

The infrared spectrum of the polysaccharide was determined using a Fourier transform infrared spectrophotometer (FTIR Tensor 27, Bruker, Germany). 1 mg sample (SJFP-AA and SJFP-BB) was mixed with potassium bromide, thoroughly ground and then was pressed into 1 mm pellet. Spectrums were recorded at the absorbance mode from 4000 to 400 cm^{-1}.

Monosaccharide composition analysis

The total carbohydrate content was determined by the phenol-sulphuric acid method, with glucose as the standard (Dubois et al., 1956). The monosaccharide of SJFP was analyzed by high performance capillary electrophoresis (HPCE) (Ji et al., 2008). Samples were hydrolyzed and derived according to the published method with some modification (Yu et al., 2010). Briefly, 20 mg polysaccharide was admixed with 1 ml 1 mol/L sulfuric acid at 100°C for 8 h. Then the solution was adjusted to pH 7 with 1 mol/L sodium hydroxide solution. The liquid from the above operation was a mixture of monosaccharides from the hydrolysis of polysaccharide. 200 µL polysaccharide hydrolyzate or standard monosaccharide (rhamnose, xylose, glucose, mannose, arabinose, galactose) mixture solution was mixed with 40 µl a-naphthylamine solution in a 2 ml ampoule. Then the ampoules were sealed by fire, and the reaction mixtures were incubated at 80°C for 2 h. The mixture was admixed with 1 ml chloroform and 1 ml deionized water, centrifuged and then the supernatant was drilled to 5 ml and filtered through a 0.45 µm filter for further analysis. The derived products were analyzed by HPCE, identified and estimated with standard monosaccharide as standard. HPCE was performed on P/ACE System MDQ capillary electrophoresis analyzer (Beckman, Duarte, USA). Capillaries with a bore of 50 µm and an effective length of 57 cm were used. Samples were injected in the positive direction, with a pressure of 3.448 kPa, injection time of 10 s, a separation temperature of 25°C, and a detection wavelength of 210 nm. Cleansing liquid was 0.1 mol/L HCl, 0.1 mol/L NaOH, and double-distilled water. And the electrophoresis voltage was 15 KV with the electric field strength of 500 V/cm, buffer was borate buffer with concentration of 75 mmol/L and pH value of 10.5.

Assay for antioxidant activity

DPPH free radical scavenging activity

DPPH radical scavenging assay was done according to the method of Sun and Ho (2005). Briefly, 2 ml sample (0.25, 0.5, 1, 2, or 4 mg/ml) was mixed with 2 ml DPPH solution (0.2 mmol/L in ethanol).

The reaction mixture was shaken and incubated in dark at room temperature for 30 min, and the absorbance was read at 517 nm against ethanol. Controls containing ethanol instead of the crude SJFP solution, and blanks containing ethanol instead of DPPH solution were also made. The scavenging ability of DPPH free radical was calculated according to the following formula:

$$Sd\ (\%)=[1-(A_i-A_j)/A_o]\times 100$$

Where, Sd-DPPH radical scavenging activity; A_i-absorbance of control; A_j-absorbance of sample; A_o-absorbance of blank. Ascorbic acid was used as the positive control.

Hydroxyl radicals scavenging assay

The hydroxyl radical scavenging activity was carried out according to the method of Smirnoff and Cumbes (1989) with minor modification. 1 ml sample (0.25, 0.5, 1, 2, or 4 mg/ml) was mixed with 1 ml 9 mmol/L salicylic acid-ethanol solution and 1 ml 9 mmol/L $FeSO_4$ solution. The reaction was initiated by the addition of 1 ml 8.8 mmol/L H_2O_2. And then, the mixture was shaken vigorously and incubated at 37°C for 30 min, the absorbance was measured at 510 nm against blank. Ascorbic acid was used as the positive standard. The hydroxyl radicals scavenging activity (Sh) was calculated using the following equation:

$$Sh\ (\%)=[A_0-(A_X-A_{X0})]/A_0\times 100$$

Where, A_0 is the absorbance of 1 ml $FeSO_4$ solution + 1 ml salicylic acid-ethanol solution+1 ml distilled water + 1 ml H_2O_2; A_X is the absorbance of 1 ml $FeSO_4$ solution + 1 ml salicylic acid-ethanol solution + 1 ml sample + 1 ml H_2O_2; A_{X0} is the absorbance of 1 ml $FeSO_4$ solution + 1 ml salicylic acid-ethanol solution + 1 ml sample + 1 ml distilled water.

Superoxide radical scavenging activity

The superoxide radical scavenging activity of crude SJFP was evaluated according to the published methods with minor modification (Lu and Foo, 2001; Okamura et al., 1993). 1 ml sample (0.25, 0.5, 1, 2, or 4 mg/ml) was added to a 1 ml mixture of 0.4 mmol/L xanthine and 0.24 mmol/L nitro blue tetrazolium chloride (NBT) in 0.1 mol/L pH 8 phosphate buffer. 1 ml solution of xanthine oxidase (0.049 units/ml), diluted in 0.1 mol/L pH 8 phosphate buffer, was added and the resulting mixture incubated in water bath at 37°C for 40 min. The reaction was terminated by adding 2 ml of an aqueous solution of 69 mmol/L sodium dodecyl sulphate (SDS) and the coloration reaction of superoxide radicals with NBT was determined at 560 nm. The deionized water was used as the blank control and ascorbic acid was used as positive control. The superoxide radical scavenging activity (Ss) was calculated by the equation:

$$Ss\ (\%) = (1-A_1/A_0)\times 100$$

Where, A_0 was the absorbance of the control (deionized water, instead of sample), and A_1 was the absorbance of the test sample mixed with reaction solution.

Reducing power

The reducing power was calculated according to the method of Oyaizu (1986). 2.5 ml sample (0.25, 0.5, 1, 2, or 4 mg/ml) was mixed with 2.5 ml 200 mmol/L pH 6.6 sodium phosphate buffer and 2.5 ml 10 mg/ml potassium ferricyanide, and the mixture was incubated at 50°C for 20 min. After 2.5 ml 100 mg/ml trichloroacetic acid was added, the mixture was centrifuged at 3000 r/min for 10 min. 2.5 ml of the upper layer solution was mixed with 2.5 ml deionized water and 0.5 ml 1 mg/ml ferric chloride, and then the absorbance was measured at 700 nm against a blank. Ascorbic acid was used as the positive standard.

Statistical analysis

One-way analysis of variance (ANOVA) and LSD multiple range test were carried out to determine significant ($P<0.05$) or extremely significant ($P<0.01$) differences between the means by SPSS (Version 11.5 for Windows, SPSS Inc.). All determinations were carried out in triplicates.

RESULTS AND DISCUSSION

Isolation, purification and structure characterization of different polysaccharide fractions

According to the charge difference, five different polysaccharides from the crude polysaccharides were obtained, namely SJFP-A, SJFP-B1, SJFP-B2, SJFP-B3, and SJFP-B4 (Figure 1), account for 26.36, 9.32, 12.36, 32.18, and 4.15% of the total polysaccharides, respectively. This indicated the method was ideal for SJFP separation. After Sephadex G-200 column treatment, two polysaccharides were obtained, namely SJFP-AA and SJFP-BB. The SJFP-AA and SJFP-BB showed a single symmetrically sharp peak, indicating its homogeneity on HPGPC (Figure 2).The total carbohydrate contents, molecular weight and monosaccharide composition of SJFP-AA and SJFP-BB were given in Table 1. The FTIR spectra of SJFP-AA and SJFP-BB showed the characteristic absorption of carbohydrate (Table 2). The stretching vibration of O-H falls into the wave number between 3600 and 3200 cm^{-1} (Silverstein et al., 2005). The bands in the region of 3416.94 and 3432.54 cm^{-1} indicated intermolecular hydrogen bonding of the polysaccharides. The bands in the region of 2915.82 and 2921.84 cm^{-1} were due to C-H stretching vibration, and the bands in the region of 1407.36 and 1388.86 cm^{-1} were due to C-H variable angular vibration. The strong peak at 1068.50 and 1064.02 cm^{-1} was the stretching vibration of C-O. In the anomeric region (950-700 cm–1), the spectrum exhibited the characteristic absorption at 819.16 and 810.82 cm^{-1} due to the presence of mannose.

Antioxidant activity

DPPH radical scavenging activity

Free radicals are harmful by-products generated during normal cellular metabolism, which could initiate oxidative damage to body (Abidi and Ali, 1999). The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants (Cotelle et al., 1996). The DPPH

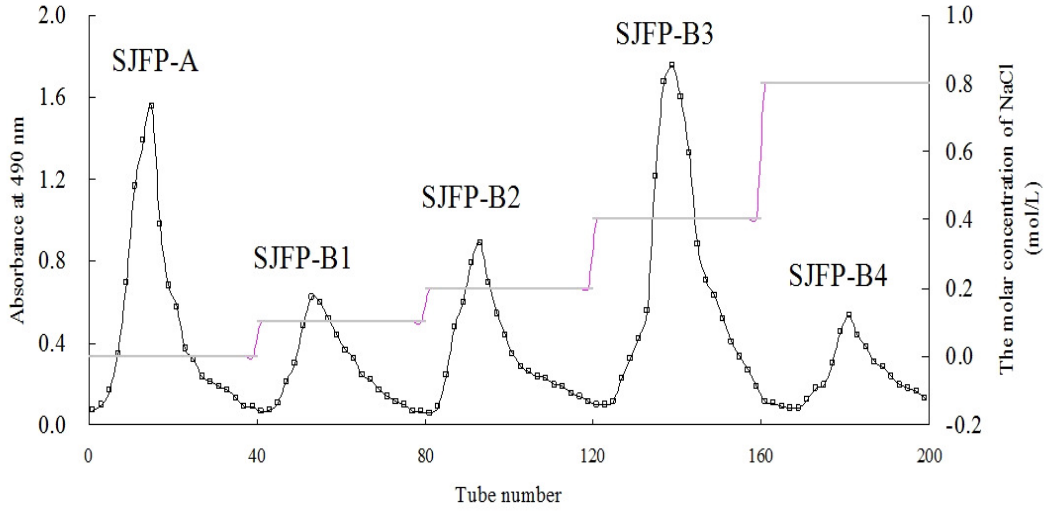

Figure 1. The profile of SJFP isolated from the flowers of *Sophora japonica* L. on a DEAE-52 column eluted with distilled water and stepwise gradient of NaCl aqueous solutions (0, 0.1, 0.2, 0.4, and 0.8 mol/L) at a flow rate of 1 ml/min.

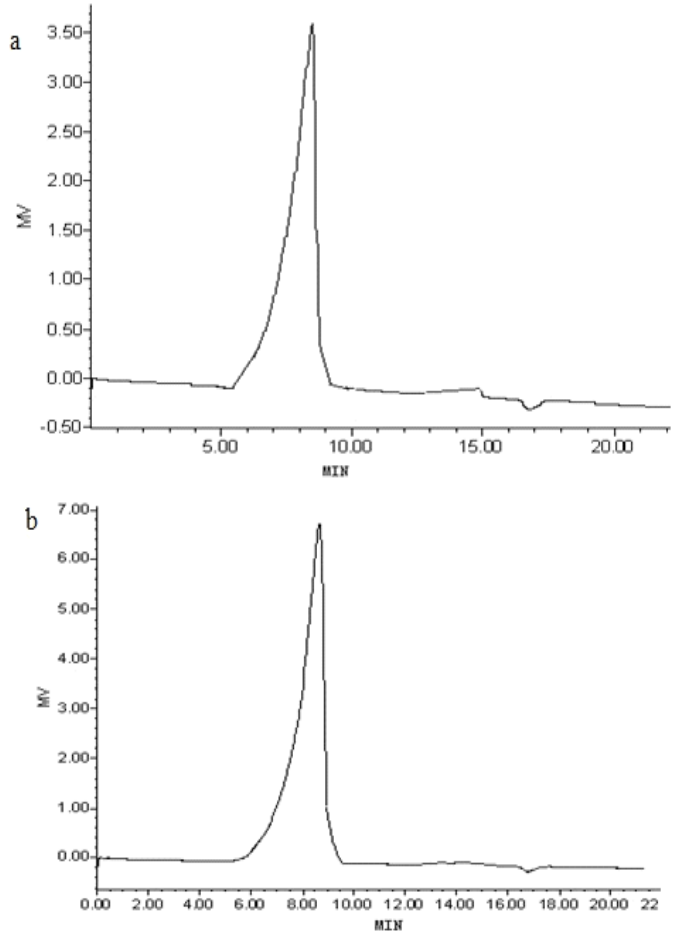

Figure 2. (a) HPGPC profile of SJFP-AA; (b) HPGPC profile of SJFP-BB.

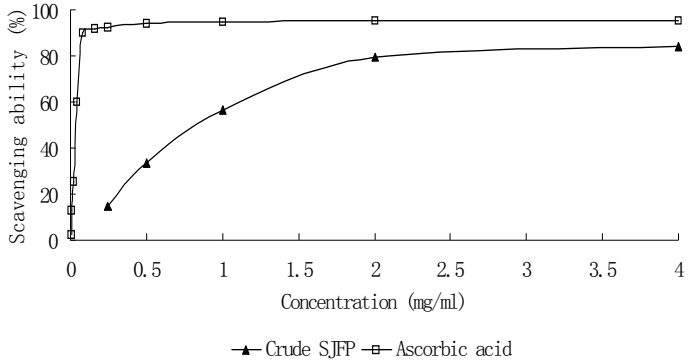

Figure 3. Scavenging ability of crude SJFP on DPPH radicals with ascorbic acid as positive control. Results were presented as mean value (n=3).

radical-scavenging ability of crude SJFP was shown in Figure 3. At the concentration from 0.5 to 2 mg/ml, the DPPH radical scavenging activity increased with the polysaccharide concentration, and this increase became less obvious when the concentration exceeded 2 mg/ml. While, the overall DPPH radical scavenging activity was not as strong as ascorbic acid.

Hydroxyl radical scavenging activity

It is known that the damaging effects of reactive oxygen species are attributed mainly to the more reactive species, such as hydroperoxyl radical (•OH_2), singlet oxygen ($^1O_2$), and especially hydroxyl radical (•OH) (Halliwell, 1978). Fe(III) ions added to H_2O_2 generate •OH

radicals (Fenton system) leading to deoxyribose degradation, which could be inhibited by specific metal chelators, catalase and hydroxyl radical scavengers (Halliwell et al., 1987). As shown in Figure 4, the scavenging capacity of crude SJFP was weak at low concentration, with only 6.26% at the concentration of 0.25 mg/ml. While it increased quickly with higher concentration, and at the concentration of 4 mg/ml, the scavenging capacity of crude SJFP was 90.32%, which was comparable to that of ascorbic acid (92.63%) ($P>0.05$). Results showed that crude SJFP has a high level of hydroxyl radical-scavenging effect.

Superoxide radical scavenging activity

The superoxide radical is a highly toxic species, which could be generated by numerous biological and photochemical reactions. In addition to directly attacking important biological molecules, superoxide radical may also decompose to form singlet oxygen and hydroxyl radicals, which may increase local oxidative stress and initiate cellular damage or lipid peroxidation and pathological incidents (Liu et al., 2010). From Figure 5, it could be seen that the scavenging ability of crude SJFP on superoxide radicals correlated positively well with increasing concentrations (0.5-4 mg/ml). And at 4 mg/ml, the superoxide radical scavenging activity of crude SJFP was 82.3%, while 96.01% for ascorbic acid.

Reducing power

Reducing power assay is often used to evaluate the ability of natural antioxidant to donate electron (Dorman et al., 2003). Many reports have revealed that there is a direct correlation between antioxidant activities and reducing power of certain plant extracts (Yildirim et al., 2001). As shown in Figure 6, the crude SJFP exhibited higher reducing power as its concentration increased. And the reducing power of crude SJFP was 1.035 at 4 mg/ml, this suggested the crude SJFP could donate electron to reactive free radicals, converting them into more stable nonreactive species and terminating the free radical chain reaction. Whereas, the crude SJFP exhibited a weaker reducing power than ascorbic acid.

EC_{50} values in antioxidant properties of crude SJFP

Effectiveness of antioxidant properties inversely correlated with their EC_{50} values. The antioxidant properties of crude SJFP were summarized in Table 3. It can be seen that all of EC_{50} values of crude SJFP were below 1 mg/mL in antioxidant properties assayed except in scavenging effect on superoxide radicals. Additionally, EC_{50} value of crude SJFP in scavenging effect on hydroxyl radicals was 0.942 mg/mL, comparable to 0.678 mg/mL for the positive control ascorbic acid ($P>0.05$). Therefore, crude SJFP might serve as possible protective agents in human diets to help human reduce oxidative damage.

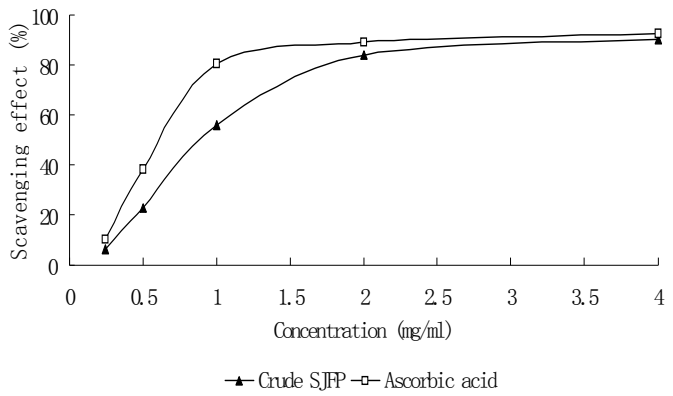

Figure 4. Scavenging effect of crude SJFP on hydroxyl radicals with ascorbic acid as positive control.
Results were presented as mean value (n=3).

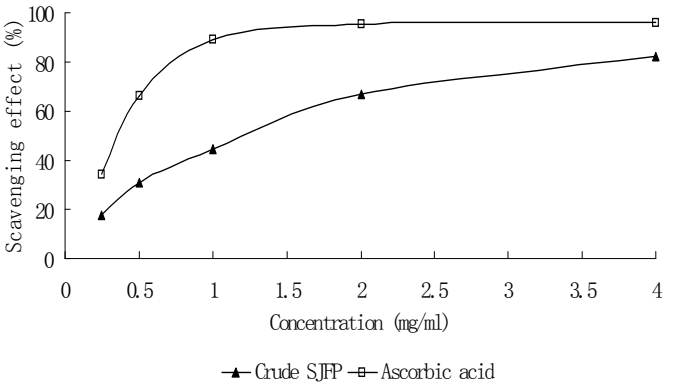

Figure 5. Scavenging effects of crude SJFP on superoxide radicals with ascorbic acid as a positive control.
Results were presented as mean value (n=3).

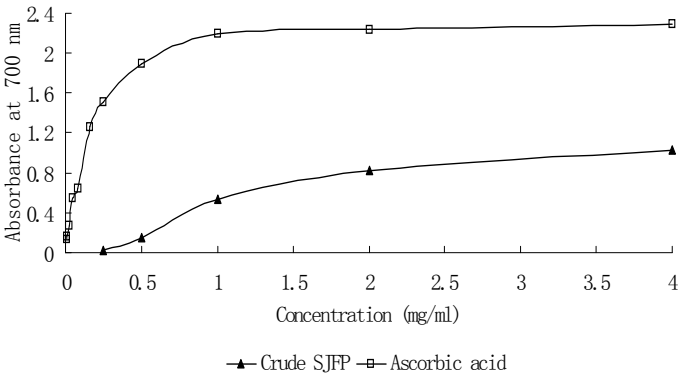

Figure 6. Reducing power of crude SJFP with ascorbic acid as positive control. Results were presented as mean value (n=3).

Table 1. Components of monosaccharide and properties of SJFP.

Sample	SJFP-AA	SJFP-BB
Total sugar (%)	90.4	92.1
Average molecular weights (KDa)	10.86	7.78
Sugar components (mol %)		
Rhamnose	1	6.26
Xylose	6.14	Not detected
Arabinose	4.03	1
Mannose	1.48	1.87

Table 2. The data of IR analysis of SJFP-AA and SJFP-BB.

Sample	Peaks/ signals
SJFP-AA	3416.94 cm^{-1}; 2915.82 cm^{-1}; 1407.36 cm^{-1}; 1068.50 cm^{-1}; 819.16 cm^{-1}
SJFP-BB	3432.54 cm^{-1}; 2921.84 cm^{-1}; 1388.86 cm^{-1}; 1064.02 cm^{-1}; 810.82 cm^{-1}

Table 3. EC_{50} values of the crude SJFP and ascorbic acid for antioxidant properties.

Property	EC_{50} value[A] (mg/mL)	
	Crude SJFP	Ascorbic acid
Scavenging ability on DPPH radicals	0.868 ± 0.115[a, B]	0.041 ± 0.013[b]
Scavenging effect on hydroxyl radicals	0.942 ± 0.114[a]	0.678± 0.180[a]
Scavenging effect on superoxide radicals	1.069± 0.067[a]	0.305± 0.108[b]
Reducing power	0.969± 0.080[a]	0.024± 0.001[b]

[A]EC_{50} value, each of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, hydroxyl radicals, and superoxide radicals was scavenged by 50%; and the absorbance was 0.5 for reducing power. EC_{50} values were obtained by interpolation from logarithm analysis.
[B]Each value was expressed as mean ± standard deviation (n=3). Means with different lowercase letters within a row were significantly different ($P < 0.05$).

Conclusions

In the present study, we successfully obtained two purified homogeneous polysaccharide, termed as SJFP - AA and SJFP-BB. The average molecular weight of SJFP-AA and SJFP-BB was estimated to be 10.86 and 7.78 KDa, respectively. SJFP-AA was composed of rhamnose, xylose, arabinose, and mannose, while SJFP-BB was made up of rhamnose, arabinose, and mannose. The crude SJFP were found to have more potent antioxidant potential by means of the in vitro evaluation of DPPH radical assay, hydroxyl radical assay, superoxide radical assay and reducing power assay in a concentration-dependent manner. Due to the complex mechanism involved in anti-oxidant activity, various oxidative stress and mediated injury models of in vivo experiments should deserve an in-depth research in future studies. Nevertheless, on the basis of the results obtained, consumption of *S. japonica* L. flower might be somewhat beneficial to the antioxidant protection system of the human body against oxidative damage.

ACKNOWLEDGMENTS

This work was financially supported by Henan Institute of Science and Technology (No. 2007041) and the Open Fund of Henan Mental Hospital, Henan Key lab of Biological Psychiatry.

REFERENCES

Abidi S, Ali A (1999). Role of oxygen free radicals in the pathogenesis and etiology of cancer. Cancer Lett. 142:1-9.
Botterweck AA, Verhagen H, Goldbohm RA, Kleinjans J, van den Brandt PA (2000). Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: results from analyses in the Netherlands cohort study. Food Chem. Toxicol. 38:599-605.
Chen HN, Hsieh CL (2010). Effects of *Sophora japonica* flowers (Huaihua) on cerebral infarction. Chinese Med. 5:34.
Chen J, Zhang T, Jiang B, Mu W, Miao M (2012). Characterization and antioxidant activity of *Ginkgo biloba* exocarp polysaccharides. Carbohyd. Polym. 87:40-45.
Chen W, Li P, Wang X (2010). Chemical stability of yellow pigment extracted from the flower bud of *Sophora japonica* L. (Huaimi). Int. J. Food Sci. Technol. 45:1666-1672.

Cotelle N, Bernier JL, Catteau JP, Pommery J, Wallet JC, Gaydou EM (1996). Antioxidant properties of hydroxyl flavones. Free Radic. Bio. Med. 20:35-43.

Dorman HJD, Peltoketo A, Hiltunen R, Tikkanen MJ (2003). Characterisation of the antioxidation properties of de-orourised aqueous extracts form selected Lamiaceae herbs. Food Chem. 83:255-262.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.

Halliwell B (1978). Superoxide-dependent formation of hydroxyl radicals in the presence of iron ions: its role in degradation on hyaluronic acid by a superoxide generating system. FEBS Lett. 96:238-242.

Halliwell B, Gutteridge JMC, Aruoma OI (1987). The deoxyribose method: a simple “test-tube” assay for determination of rate constants for reactions of hydroxyl radicals. Anal. Biochem. 165:215-219.

Ishida H, Umino T, Tsuji K, Kosuge T (1989). Studies on the antihemostatic substances in herbs classified as hemostatics in traditional Chinese medicine. I. On the antihemostatic principles in Sophora japonica L. Chem. Pharm. Bull. 37:1616-1618.

Ji CF, Ji YB, Zou X (2008). Effect of asparagus polysaccharides on migration time of erythrocytes in tumor-bearing organisms using high performance capillary electrophoresis. P-2008 Int. Semin. FBME 199-201.

Kim JM, Yun-Choi HS (2008). Anti-platelet effects of flavonoids and flavonoid-glycosides from *Sophora japonica*. Arch. Pharm. Res. 31:886-890.

Liu W, Wang H, Pang X, Yao W, Gao X (2010). Characterization and antioxidant activity of two low-molecular-weight polysaccharides purified from the fruiting bodies of *Ganoderma lucidum*. Int. J. Biol. Macromol. 46:451-457.

Lo YH, Lin RD, Lin YP, Liu YL, Lee MH (2009). Active constituents from *Sophora japonica* exhibiting cellular tyrosinase inhibition in human epidermal melanocytes. J. Ethnopharmacol. 124:625-629.

López-Revuelta A, Sánchez-Gallego JI, Hernández-Hernández A, Sánchez-Yagüe J, Llanillo M (2006). Membrane cholesterol contents influence the protective effects of quercetin and rutin in erythrocytes damages by oxidative stress. Chemico-Biol. Interact. 161: 79-91.

Lu Y, Foo LY (2001). Antioxidant activities of polyphenols from sage (Salvia officinalis). Food Chem. 75:197-202.

Okamura H, Mimura A, Yakou Y, Niwano M, Takahara Y (1993). Antioxidant activity of tannins and flavonoids in *Eucalyptus rostrata*. Phytochemistry 33:557-561.

Oyaizu M (1986). Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44:307-315.

Paniwnyk L, Beaufoy E, Lorimer JP, Mason TJ (2001). The extraction of rutin from flower buds of *Sophora japonica*. Ultrason. Sonochem. 8:299-301.

Park KW, Lee JE, Park KM (2009). Diets containing Sophora japonica L. prevent weight gain in high-fat diet-induced obese mice. Nutr. Res. 29:819-824.

Sevag MG, Lackman DB, Smolens J (1938). The isolation of the components of streptococcal nucleoproteins in serologically active form. J. Biol. Chem. 124:425-436

Silverstein RM, Webster FX, Kiemle DJ (2005). Spectrometric identification of organic compounds (7th ed.). Hoboken, New Jersey. pp. 127-203.

Smirnoff N, Cumbes Q (1989). Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry 28:1057-1060.

Song H, Zhang Q, Zhang Z, Wang J (2010). In vitro antioxidant activity of polysaccharides extracted from *Bryopsis plumose*. Carbohyd. Polym. 80:1057-1061.

Sun A, Sun Q, Liu R (2007). Preparative isolation and purification of flavone compounds from *Sophora japonica* L. by high-speed counter-current chromatography combined with macroporous resin column separation. J. Sep. Sci. 30: 1013-1018.

Sun T, Ho C (2005). Antioxidant activities of buckwheat extracts. Food Chem. 90:743-749.

Sun YX, Kennedyb JF (2010). Antioxidant activities of different polysaccharide conjugates (CRPs) isolated from the fruiting bodies of *Chroogomphis rutilus* (Schaeff.: Fr.) O. K. Miller. Carbohyd. Polym., 82:510-514.

Thetsrimuang C, Khammuang S, Chiablaem K, Srisomsap C, Sarnthima R (2011). Antioxidant properties and cytotoxicity of crude polysaccharides from *Lentinus polychrous* Lév. Food Chem. 128:634-639.

Wang LH, Duan YF, Ma YL, Ding HJ, Li EC (2008). Studies on extraction and antioxidant function of polysaccharides from *Sophora japonica*. J. Northwest A FU (Nat. Sci. Edit.) 36:213-218.

Yildirim A, Mavi A, Kara AA (2001). Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. J. Agric. Food Chem. 49:4083-4089.

Yu W, Jiang T, Wang Y, Lu Z (2010). Determination of the monosaccharide composition of polysaccharides from *Rhizoma phragminis* and Pollen typhae by capillary zone electrophoresis. Transact. Oceanol. Limnol. 2:162-168.

Yuan JF, Zhang ZQ, Fan ZC, Yang JX (2008). Antioxidant effects and cytotoxicity of three purified polysaccharides from *Ligusticum chuanxiong* Hort. Carbohyd. Polym. 74:822-827.