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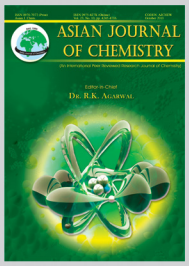

Isolation, Characterization and Antioxidant Activities of Polysaccharides from *Tussilago farfara*

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Tussilago farfara polysaccharides (TPS) were extracted from *Tussilago farfara* using hot water. Two water-soluble polysaccharide fractions (TPS-2-1 and TPS-2-2) were separated. The HPGPC analysis showed that the average molecular weight of two polysaccharides were approximately 456.6 and 5.54 kDa, respectively. The monosaccharide components of two polysaccharides were characterized by GC, among which TPS-2-1 was composed of mannose, glucose and galactose at the molar ratio of 2.467:1.158:1; TPS-2-2 consisted of mannose and glucose at the molar ratio of 1.798:1. TPS-2-1 had higher scavenging effects on DPPH activities.

Key Words: *Tussilago farfara*, Polysaccharides, Isolation, Characterization, Antioxidant.

INTRODUCTION

It is well-known that polysaccharides play an important role in the growth and development of living organisms. In addition, polysaccharides have been demonstrated to possess a variety of medicinal activities, including immunostimulation, antitumor, antioxidant activities, antiviral, anticoagulant, anti-complementary, antiinflammatory, *etc.*[1-9]. Due to the rich variety and availability of polysaccharides from the nature, exploitation of polysaccharides for pharmaceutical application was promising.

Tussilago farfara L. (compositae) grows and is widely cultivated in Shanxi, Shaanxi, Gansu, Qinghai, Sichuan provinces and Inner Mongolia Autonomous Region of China. The flower buds of *T. farfara* had been widely used for the treatment of cough, bronchitis and asthmatic disorders as a traditional Chinese medicine[10,11]. It has been reported to contains triterpenoids, sesquiterpenoids, alkaloids, flavonoids, chlorogenic acids and essential oil[10,12-14]. A crude polysaccharide from *Tussilago farfara* was obtained, which was effective in inducing K562 cell apoptosis *in vitro*[15]. However, there are no references available in the literature to studies performed on the separation and purification of polysaccharides from *Tussilago farfara* to the best of our knowledge. Therefore, the aim of the present research was to isolate and determine the structural features and antioxidant activity of the polysaccharides present in this dried buds of *Tussilago farfara*. In the present paper, a water-soluble polysaccharide from *Tussilago farfara* (TPS) was extracted using hot water. The present paper was concerned with the isolation, chemical characterization and evaluation of the antioxidant activity of *Tussilago farfara* polysaccharides.

EXPERIMENTAL

The species of *Tussilago farfara* was grown in Shaanxi Province of China and supplied by Shaanxi Pharmaceutical Development Center, Shaanxi, China. Dextran standards (Dextran T standards) were purchased from Pharmacia (Sweden). Carbohydrate standards were purchased from Sigma (USA).

***Tussilago farfara* polysaccharides extraction:** *Tussilago farfara* polysaccharides extraction was performed using the method described in previous study[16].

Fractionation: Anion-exchange DEAE52-cellulose chromatography (70 cm × 5.0 cm) was used to fractionate *Tussilago farfara* polysaccharides. 0.1 g crude *Tussilago farfara* polysaccharides was dissolved in distilled water and filtered through a filter paper (0.45 μm). The *Tussilago farfara* polysaccharides was first eluted with distilled water, followed by the elution with NaCl linear gradient (0.1-1.0 M) at a flow rate of 1.0 mL/min. Fractions of 5 mL were collected and analyzed for polysaccharide concentration. Two sub-fractions obtained by separation of DEAE52-cellulose chromatography were coded TPS-1and TPS-2, which was as shown in Fig. 1. The yield of TPS-1 was low, so the TPS-2 was further purified by gel-permeation chromatography on a column of Sephadex G-200 (2.0 cm × 100 cm) into different fractions according to their molecular size and first eluted with distilled water,

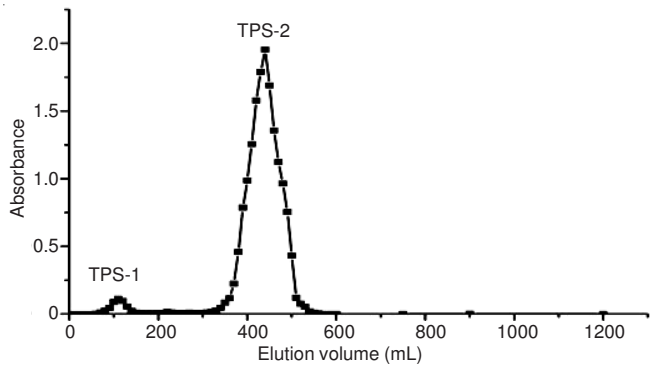

Fig. 1. Chromatography of TPS on DEAE52-cellulose by the purifier

followed by the elution with NaCl linear gradient (0.2-1.0 M) at a flow rate of 1.0 mL/min. The major polysaccharide fractions were collected with a fraction collector, then dialyzed with water and lyophilized to give three polysaccharides, namely TPS-2-1, TPS-2-2 and TPS-2-3 (Fig. 2). The yield of TPS-2-3 was low, so the TPS-2-1 and TPS-2-2 were used in the subsequent studies. The TPS-2-1 and TPS-2-2 were dialyzed and concentrated by rotary evaporation. The obtained precipitate was then purified by adding ethanol and then centrifuged under 4000 rpm for 10 min. The precipitate was dissolved in distilled water for the following HPGPC identification.

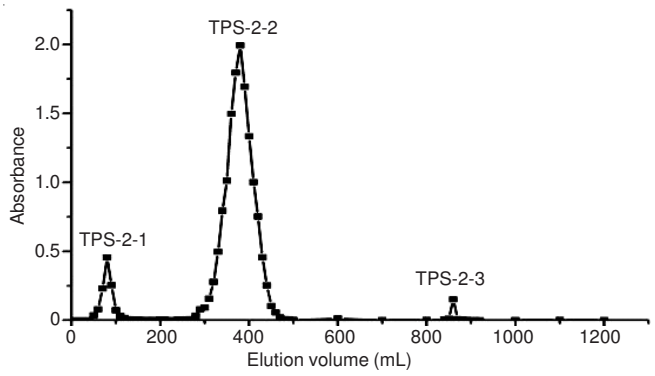

Fig. 2. Chromatography of TPS-2 on G-200 by the purifier

Homogeneity and molecular weight determination: The homogeneity and molecular weight of the TPS-2-1 and TPS-2-2 were determined by HPGPC coupled to refractive index (RI) detection. Each fraction sample was diluted to a concentration of approximately 2 mg/mL by adding 0.001 % NaN_3 solution. 10 µL of sample injected into HPLC system with ShodexOhPakSB_804HQ column (300 mm × 8 mm). The 0.001 % NaN_3 solution was pumped to HPLC system at the flow rate of 0.8 mL/min. The column temperature was constantly kept at 25 ºC. The molecular weight was estimated by reference to the calibration curve made from a Dextran T-series standard of known molecular weight (10,000, 40,000, 70,000, 90,000, 2,000,000 Da). The molecular weight-retention time equation developed by calibration curve was l g Mw = 10.113 – 0.6638 Kav with R^2 = 0.9997 (Mw: the average molecular weight of dextran standards; Kav: partition coefficient of sample/standard in the chromatography column). The mass ratio of TPS-2-1 and TPS-2-2 in the crude polysaccharides were calculated after weighing the corresponding dried fractions.

Monosaccharide composition: Monosaccharide composition was analyzed according to the following procedure: TPS-2-1 and TPS-2-2 (5 mg) were hydrolyzed with 3 mL of 2 mol/L TFA at 110 ºC for 4 h into monosaccharide under airtight condition. The hydrolyzate was repeatedly co-concentrated with methanol, followed by successive reduction with hydroxylamine hydrochloride and acetylation with acetic anhydride-pyridine (1:1, v/v; 2mL) at 90 ºC for 0.5 h. The resulting aldononitrile acetates were analyzed using gas chromatography (GC) with an OV-1701 (30 m × 0.25 mm) capillary gas chromatography column equipped with a flame-ionization detector (FID). As previously mentioned, the following standard sugars were converted to their derivatives and analyzed: D-frulose, D-ribose, L-rhamnose, D-arabinose, D-xylose, D-mannose, D-glucose and D-galactose.

The GC operation was performed using the following conditions:N_2: 10 mL/min; injection temperature: 250 ºC; detector temperature: 260 ºC; column temperature programmed from 150-190 ºC at 7 ºC/min, then increased to 260 ºC at 15 ºC/min.

Infrared spectral analysis of the polysaccharide: The organic groups of *Tussilago farfara* polysaccharides purities were detected with Avatar 360 FTIR spectrophotometer (FTIR Nicolet, USA). The purified polysaccharide was ground with KBr powder (spectroscopic grade) and then pressed into a 1 mm pellet for FTIR measurement in the frequency range 4000-400 cm^{-1}.

Evaluation of DPPH-scavenging activity: The antioxidant activity of polysaccharides was measured on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical test according to the method: described as references with some modifications[17]. Samples (TPS-2-1 and TPS-2-2) were dissolved in distilled water at 0 (control), 1, 2, 3, 4, 5, 6, 7 and 8 mg/mL. One mL test samples were thoroughly mixed with 2 mL of freshly prepared DPPH (0.1 mM) in 50 % ethanol. After shaking vigorously, the mixture was incubated at 25 ºC for 0.5 h in the dark and then the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Ascorbic acid was used as a positive control. The experiment was carried out in triplicate and averaged. The scavenging activity of DPPH radicals was calculated as:

$$\text{Scavenging effect } (\%) = \frac{A_0 - (A - A_b)}{A_0} \times 100 \qquad (1)$$

where A_0 was the absorbance of DPPH solution without sample; A was the absorbance of the test sample mixed with DPPH solution and A_b was the absorbance of the sample without DPPH solution.

RESULTS AND DISCUSSION

Isolation of polysaccharide fractions: *Tussilago farfara* polysaccharides was fractionated by preparative Anion-exchange DEAE52-cellulose chromatography to obtain two fractions, namely TPS-1 and TPS-2, which were selected based on total carbohydrate elution profile. As shown in Fig. 2, the yield of TPS-1 was low, so the TPS-2 was further purified by Sephadex G-200 gel-permeation chromatography into different

fractions according to their molecular size. The results showed that the TPS-2 was mainly composed of three sub-fractions, namely TPS-2-1, TPS-2-2 and TPS-2-3. The yield of TPS-2-3 was low, so the TPS-2-1 and TPS-2-2 were used in the subsequent studies.

Homogeneity and molecular weight determination by HPGPC: As shown in Figs. 3 and 4, the *Tussilago farfara* polysaccharides sub-fractions (TPS-2-1 and TPS-2-2) obtained through cellulose chromatograph separation appeared as a single and symmetrical sharp peak, respectively, which indicates that TPS-2-1 and TPS-2-2 were a homogeneous polysaccharide. The HPGPC retention time of TPS-2-1 and TPS-2-2 was 6.709 and 9.605 min, respectively. Using the developed molecular weight calibration curve, it was determined that the polysaccharide TPS-2-2 had a low average molecular weight value of only 5.54k Da. The TPS-2-1 had comparatively higher average Mw values, which was *ca.* 456.6 kDa.

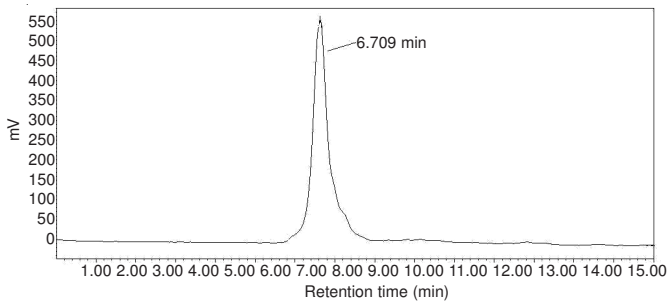

Fig. 3. Profile of the TPS-2-1 fraction in HPGPC

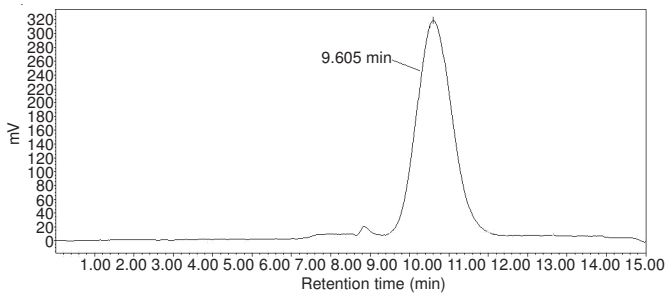

Fig. 4. Profile of the TPS-2-2 fraction in HPGPC

Characterization of the purified polysaccharide

Monosaccharide composition: The monosaccharide composition of TPS-2-1 and TPS-2-2 fraction analyzed by gas chromatograph (GC), was shown in Figs. 5 and 6. Figs. 5 and 6 show all the peaks of eluents when the TPS-2-1 and TPS-2-2 acetate derivatives were analyzed by GC. The peaks of all monosaccharides were sharp and symmetrical. Compared with the monosaccharide standards (D-frulose, D-ribose, L-rhamnose, D-arabinose, D-xylose, D-mannose, D-glucose and D-galactose) (Fig. 7), the TPS-2-1 was mainly consisted of D-mannose, D-glucose and D-galactose with a ratio of 2.467: 1.158:1. The TPS-2-2 was mainly consisted of D-mannose and D-glucose with a ratio of 1.798:1.

IR spectroscopic characterization: As shown in Figs. 8 and 9, the FTIR spectra of two *Tussilago farfara* polysaccharides fractions was found to be similar. The band between 3600 and 3200 cm^{-1} (TPS-2-1: 3407 cm^{-1}; TPS-2-2: 3362 cm^{-1}), represented the stretching vibration of O-H in the constituent

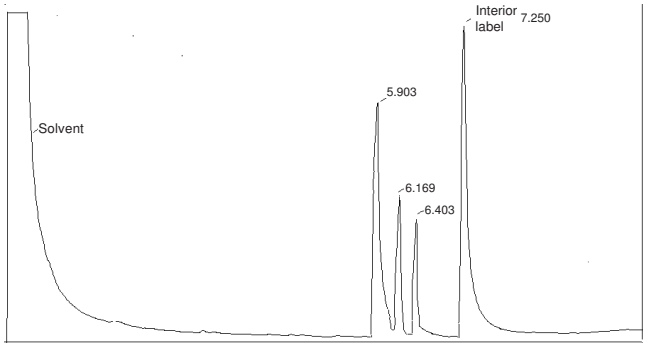

Fig. 5. GC analysis of monosaccharide composition of TPS-2-1

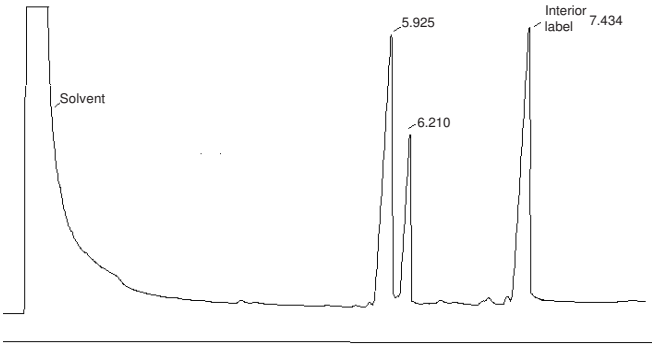

Fig. 6. GC analysis of monosaccharide composition of TPS-2-1

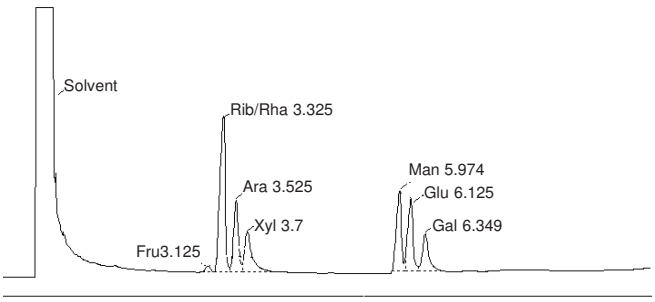

Fig. 7. GC analysis of monosaccharide standard

sugar residues. The small band at around 2928 cm^{-1} was associated with stretching vibration of C-H in the sugar ring. TPS-2-1 and TPS-2-2 all had these bands, which were characteristic of polysaccharides. However, there were some differences among the spectra of two fractions. Two distinct absorbance peaks at 1741 and 1618 cm^{-1} in the IR spectra resulted from the presence of uronic acids in the TPS-2-1. The characteristic absorptions at 1100, 1077 and 1023 cm^{-1} in the IR spectra indicated TPS-2-1 belong to α- and β-type pyran group simultaneously. The characteristic absorptions at 936, 874 and 819 cm^{-1} in the IR spectra indicated TPS-2-2 belong to β-type furan group.

Assay for antioxidant activity of TPS-2-1 and TPS-2-2: Fig. 10 demonstrated DPPH scavenging activity caused by different concentrations of TPS-2-1 and TPS-2-2. The DPPH radical scavenging activity of TPS-2-1 reached 76.02 % at 5 mg/mL and TPS-2-2 reached 68.43 % at 7 mg/mL, respectively. The EC_{50} values of TPS-2-1 and TPS-2-2 for DPPH radicals were 2.15 and 3.0 mg/mL, respectively. Scavenging activity of TPS-2-1 was higher ($p < 0.05$) than that of TPS-2-2 at the concentration rang of 1-8 mg/mL. The scavenging activity

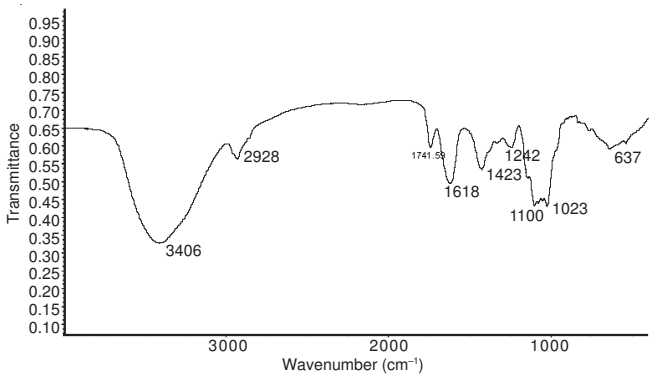

Fig. 8. FTIR spectra of TPS-2-1

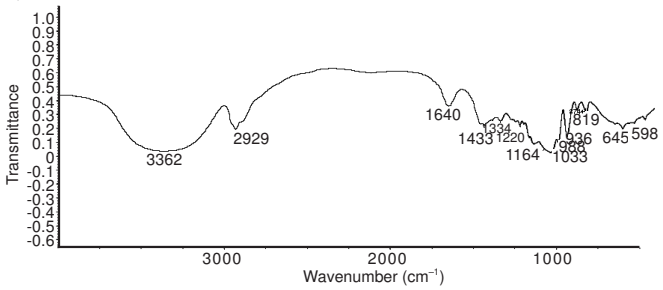

Fig. 9. FTIR spectra of TPS-2-2

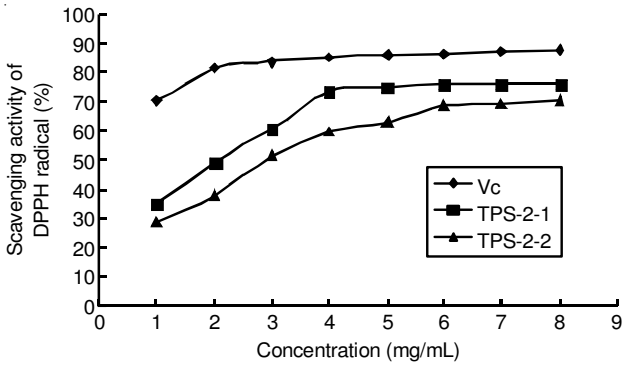

Fig. 10. Scavenging ability of TPS-2-1 and TPS-2-2 against DPPH radical. Results were presented as means ± SD (n = 3)

increased steadily at the concentration rang of 1-8 mg/mL for TPS-2-1 and TPS-2-2, which indicated that the scavenging activity of TPS-2-1 and TPS-2-2 against DPPH radical was less than that of ascorbic acid.

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