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Chemical analysis of *Astragalus mongholicus* polysaccharides and antioxidant activity of the polysaccharides

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ABSTRACT

Polysaccharide from *Astragalus membranaceus* was investigated for its antioxidant potential. Major functional groups of the polysaccharide were analyzed by Fourier transmission-infrared spectroscopy (FT-IR) and gas chromatography (GC). Analysis of the polysaccharide from *A. membranaceus* revealed four sugars: glucose, galactose, mannose, arabinose. Mannose was the major components, followed by glucose, mannose and arabinose. The presence of aromatic C=C bands in the infrared (1600 cm^{-1}) suggested the presence of polyphenols. The intense bands in the infrared O–H and C=O regions were attributed mainly to the presence of furan ring. Pharmaceutical experiments showed that administration of *Astragalus mongholicus* polysaccharides could significantly increase serum and liver antioxidant enzyme activities in mice and decrease peroxidative lipid levels. In conclusion, *A. mongholicus* polysaccharides may offer good protection against oxidative stress.

1. Introduction

Excessively high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids, and eventually cell death. Various mechanisms have been suggested to contribute to the formation of these reactive oxygen-free radicals. The role of reactive oxygen species (ROS) in normal and abnormal biology is the subject of intense study motivated, in part, by the large and growing number of associations between various pathological conditions and changes in oxidative balance, redox status, and oxidative injury (Obame et al., 2008; Wu, Xu, Shan, & Tan, 2006). The toxicity of ROS in experimental systems is uniequivocal. Despite this research effort and many clues and hypotheses, there exists only circumstantial evidence of a causal role for ROS in nonpulmonary disease. Although it would be expected that developing organisms would be especially vulnerable to oxidative injury, there has been little systematic study of exposures that might generate ROS and of the developmental consequences of such generation (Charles & Huang, 2009).

Astragalus L. (Leguminosae) is a genus widely distributed throughout the temperate regions of the world, located principally in Europe, Asia and North America. *Astragalus membranaceus* (Fisch.) Bge, known by the Chinese name of Huang-qi (Ougi in Japanese), is one of the best-known natural sources of saponins, along with Ginseng and Notoginseng. Modern pharmacological studies have shown that its roots show therapeutic activity as immunostimulant, tonic (adaptogenic), hepatoprotective, diuretic, antidiabetic, analgesic, expectorant, and sedative (Ma, Shi, Duan, Dong, & Tsim, 2002).

The polysaccharides from *A. membranaceus* are the best known, and several research groups have isolated and purified them. The hepatoprotective properties of *A. membranaceus* extracts have been widely studied and, in some cases, the active principles have been described. Research has shown that its antioxidative properties can prevent liver damage. Studies on animals with toxic hepatic injury induced by CCl_4, indicates that *A. membranaceus* root extract prevents a decrease in hepatic glycogen content and raises the levels of total serum protein and albumin (Rios & Waterman, 1998).

In the present study we focused on crude polysaccharide extracted from *A. membranaceus* for its antioxidant properties and to the best of our knowledge, this is the first report of the same.

1.1. Material and method

Astragalus mongholicus polysaccharides were kindly provided by JingLin Biochemistry Company. Its purity was 98%.

1.2. Analysis of chemical composition

Infrared spectra of the polysaccharides were recorded with a Nicolet 170SX FT-IR (Spectrum One, PerkinElmer Co., USA)

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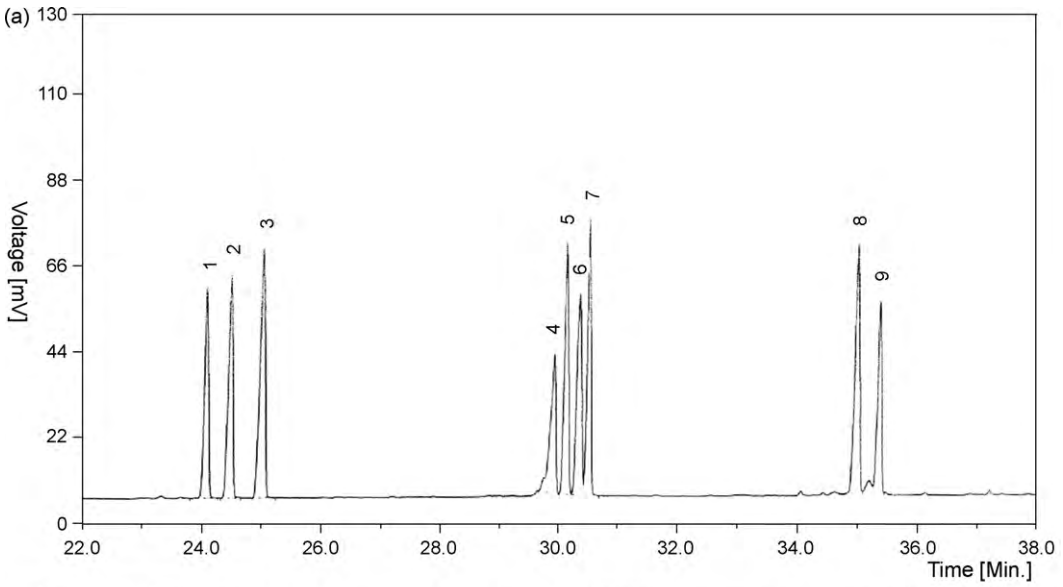
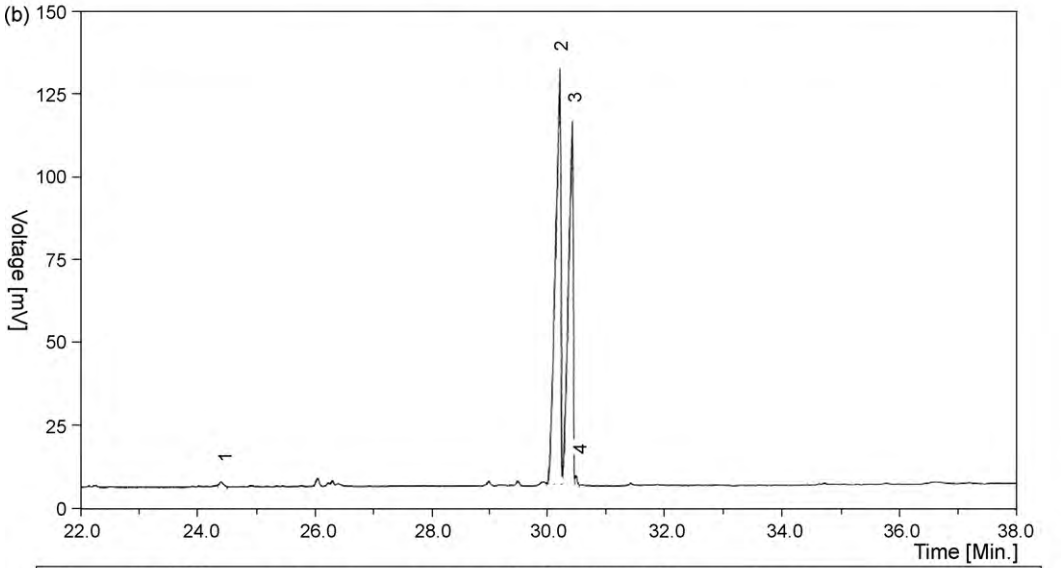


#	RT (min)	Height (mv)	Area (mv.sec)	Area Percentage (%)	content	correction factor
1	24.11	53.98	249.11	9.6538	0.0000	0.00e+000
2	24.52	57.46	296.97	11.5088	0.0000	0.00e+000
3	25.06	63.81	375.26	14.5428	0.0000	0.00e+000
4	29.95	35.55	228.15	8.8415	0.0000	0.00e+000
5	30.16	64.46	301.29	11.6763	0.0000	0.00e+000
6	30.38	51.31	286.70	11.1106	0.0000	0.00e+000
7	30.55	70.73	304.19	11.7884	0.0000	0.00e+000
8	35.02	63.27	328.80	12.7420	0.0000	0.00e+000
9	35.38	48.55	209.93	8.1357	0.0000	0.00e+000
Total		509.11	2580.40	100		



#	RT (min)	Height (mv)	Area (mv.sec)	Area percentage (%)	correction factor	sample content (%)
1	24.40	1.27	5.17	0.3566	0.00e+000	0.0000
2	30.22	124.81	816.30	56.2879	0.00e+000	0.0000
3	30.43	109.13	621.21	42.8357	0.00e+000	0.0000
4	30.50	2.91	7.54	0.5198	0.00e+000	0.0000
Total		238.12	1450.22	100		

Fig. 1. (a) GC analysis of standard sample; (b) GC analysis of *Astragalus mongholicus* polysaccharides.

spectrometer in the range 4000–400 cm^{-1} using the KBr disk method. The elemental compositions of the products were determined by an elemental analyzer (CHN-O-RAPID Heraeus Co., Germany).

Gas chromatography (GC) of the polysaccharides was performed with an HP-6890 gas chromatography (Hewlett Packard, USA) using an Alltech DB-225 capillary column (15 m × 0.25 mm) programmed from 180 to 220 °C at 4 °C/min and held at 220 °C for 30 min. The

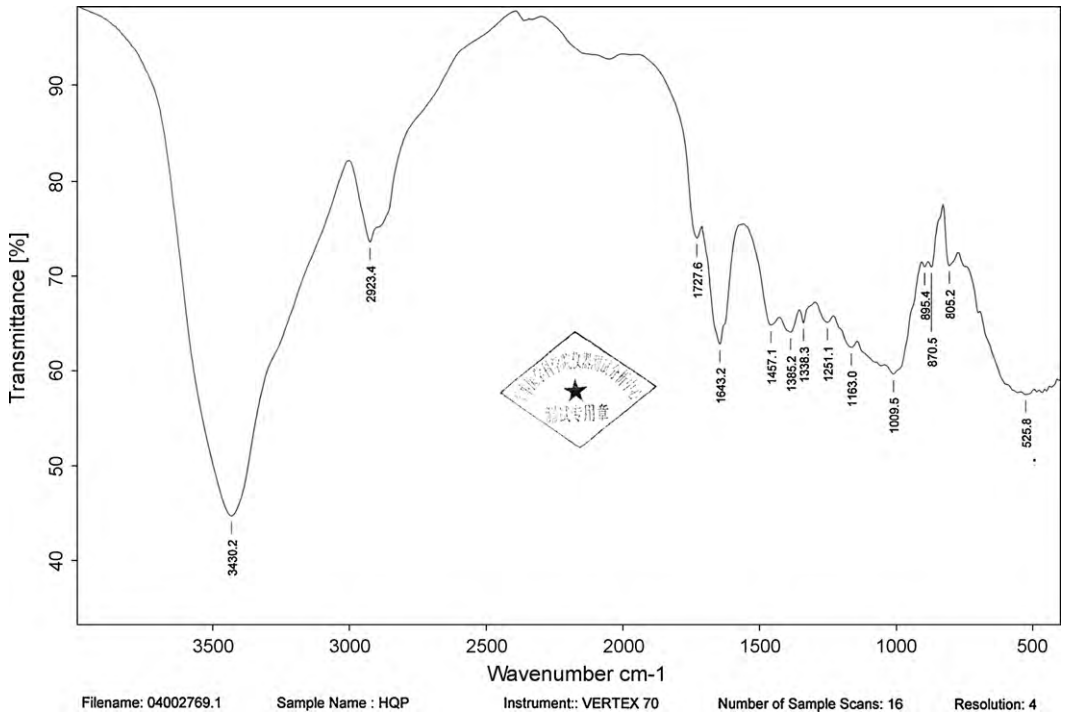


Fig. 2. FT-IR analysis of *Astragalus mongholicus* polysaccharides.

injection sample volume was 2 μL, the carrier gas was high-purity helium, and detection was by flame ionization. For the methylation analysis, the polysaccharides were permethylated twice using CH_3I and solid NaOH in dimethyl sulfoxide (Me_2SO) as a sequential method as described (Needs & Selvendran, 1993a, 1993b). The GC was performed using the following conditions. H_2: 30 ml/min; air: 150 ml/min; N_2: 1 ml/min; injection temperature: 230 °C; detector temperature: 230 °C; column temperatures programmed from 130 to 180 °C at 5 °C/min, holding for 2 min at 180 °C, then increasing to 220 °C at 5 °C/min and finally holding for 3 min at 220 °C.

1.3. Treatment and animals

Male mice (25 ± 1 g) were obtained from the Laboratory Animal Center, and quarantined for 2 weeks. They were housed in an animal room under standardized conditions (i.e. at 20 ± 2 °C, 50 ± 5% relative humidity, with at least ten air changes per hour, and a 12 h light/dark cycle). The animals were housed separately in polycarbonated cages and were provided with food and water ad libitum. The rats were divided into four groups consisting of 10 rats each. Group 1 (normal control) consisted of normal rats that received a standard diet nor any drug. The rats in groups II, III and IV were administered with polysaccharides (40, 80 and 160 mg/kg BW). The rats in groups I were fed the same volume of vehicle. The rats would be treated for 30 days. Blood samples were collected from 12 h fasted rats at 1 h after the last dose administration and analyzed for antioxidant enzymes activities. After the animals were decapitated and exsanguinated, the livers were immediately removed and frozen in liquid nitrogen.

1.4. Biochemical parameters

The quantitative evaluation of the antioxidant capacity of the polysaccharides against lipid peroxidation was determined through the measurement of the inhibition of MDA generation. Results were expressed in mg/L for extracts which were able to inhibit 50% of the generation of MDA (nmol MDA/mg).

Table 1
Effect of *Astragalus mongholicus* polysaccharides on MDA level in blood and liver.

Group	MDA	
	Blood (nmol/ml)	Liver (nmol/mg prot)
Control	6.41 ± 0.57	1.76 ± 0.49
Treatment (40 mg/kg BW)	5.22 ± 0.29**	1.49 ± 0.32
Treatment (80 mg/kg BW)	5.16 ± 0.71**	1.35 ± 0.23
Treatment (160 mg/kg BW)	5.02 ± 0.60**	1.09 ± 0.43*

* $P < 0.05$, compared with control group.
** $P < 0.01$, compared with control group.

The tissues GSH concentration was measured using the method described by Beutler, Dubon, and Kelly (1963). Briefly, 0.2 ml fresh supernatant was added to 1.8 ml distilled water. Three milliliters of the precipitating solution (1.67 g metaphosphoric acid, 0.2 g EDTA and 30 g NaCl in 100 ml distilled water) was mixed with haemolysate. The mixture was allowed to stand for approximately 5 min and then filtered (Whatman No. 42). Two milliliters of filtrate was taken and added into another tube, and then 8 ml of the phosphate solution (0.3 M disodium phosphate) and 1 ml DTNB were added. A blank was prepared with 8 ml of phosphate solution, 2 ml diluted precipitating solution (three parts to two parts distilled water), and 1 ml DTNB reagent. A standard solution of the glutathione was prepared (40 mg/100 ml). The optical density was measured at 412 nm in the spectrophotometer.

Table 2
Effect of *Astragalus mongholicus* polysaccharides on GSH level in blood and liver.

Group	GSH	
	Blood (mg/l)	Liver (mg/g prot)
Control	26.93 ± 9.26	1.95 ± 0.33
Treatment (40 mg/kg BW)	27.95 ± 4.55	2.46 ± 0.21**
Treatment (80 mg/kg BW)	35.37 ± 5.42	2.58 ± 0.63**
Treatment (160 mg/kg BW)	40.87 ± 7.47*	2.91 ± 0.48**

* $P < 0.05$, compared with control group.
** $P < 0.01$, compared with control group.

Table 3
Effect of *Astragalus mongholicus* polysaccharides on SOD, CAT and GPx in blood.

Group	SOD (U/ml)	CAT (U/ml)	GSH-Px (U/0.1 ml)
Control	0.13 ± 0.11	5.69 ± 2.63	550.83 ± 54.39
Treatment (40 mg/kg BW)	0.18 ± 0.12	7.46 ± 1.65	481.67 ± 76.82
Treatment (80 mg/kg BW)	0.25 ± 0.11	9.07 ± 2.21*	633.33 ± 81.88*
Treatment (160 mg/kg BW)	0.34 ± 0.12**	12.43 ± 3.29**	795.83 ± 73.63**

* $P < 0.05$, compared with control group.
** $P < 0.01$, compared with control group.

Table 4
Effect of *Astragalus mongholicus* polysaccharides on SOD, CAT and GSH-Px in liver.

Group	SOD (U/mg prot)	CAT (U/mg prot)	GSH-Px (U/mg prot)
Control	20.66 ± 3.40	0.69 ± 0.25	32.57 ± 5.48
Treatment (40 mg/kg BW)	24.92 ± 9.08	1.00 ± 0.27*	33.88 ± 4.16
Treatment (80 mg/kg BW)	34.71 ± 6.49**	1.29 ± 0.35**	73.68 ± 6.66**
Treatment (160 mg/kg BW)	35.95 ± 6.51**	1.98 ± 0.44**	80.59 ± 5.26**

* $P < 0.05$, compared with control group.
** $P < 0.01$, compared with control group.

After sacrificing the rats, small pieces of liver were immediately washed with ice-cold saline, frozen in liquid nitrogen and stored at −80 °C until biochemical analysis. Superoxide dismutase (SOD) was estimated following the methods of Kakkar, Das, and Viswanathan (1984). CAT and GPx activity were determined following the methods of Clairbone (1986), and of Gunzler, Flohe, and Clairbone (1986), respectively.

1.5. Statistical analysis

Mean ± S.D. were calculated for quantitative data. Significant differences between means were evaluated by one-way analysis of variance (ANOVA) and in the case of significance; frequency data were compared using Ridit procedure. A difference was considered significant at $P < 0.05$.

2. Result

A typical chromatogram is shown in Fig. 1a and b. The peaks for all monosaccharides were sharp and symmetrical. The polysaccharides of *A. mongholicus* contained glucose, galactose, mannose, and arabinose (Fig 1b). Since the area of a peak was proportional to the amount of compound, the ratio of a peak area to the number of moles was constant. Thus the molar ratio of the monosaccharides was calculated from the chromatogram area ratios that were found to be 0.0992:1.26:1.00:0.0115 (arabinose:mannose:glucose:galactose).

Evidence of formation of polysaccharides from *A. mongholicus* can be explained by FT-IR spectroscopy. From the FT-IR spectra, it was obvious that *A. mongholicus* polysaccharides (Fig. 2) showed a broad peak at 3430.2 cm^{-1} for –OH stretching vibrations. The bands at 1163 and 2923.4 cm^{-1} were assigned to C–O stretching and C–H stretching, respectively. One strong band at 1009.5 cm^{-1} was attributed to CH_2–O–CH_2 stretching vibrations. The bands at 1065 and 1068 cm^{-1} (Sun et al., 2009; Zhang et al., 2010) were assigned to arabinose, and therefore the substance having arabinose units would have a negative influence on the polysaccharides values. In addition, the strong band derived from OH group in glucose is reported to appear at 1035 cm^{-1} (Kumar, Joo, Choi, Koo, & Chang, 2004), which is present in sugar standards.

As shown in Table 1, there were significant differences ($P < 0.01$) in MDA level between normal control and polysaccharides-treated animals. Significant decreased MDA level ($P < 0.01$) was observed in blood and liver of polysaccharides-treated rats in comparison to normal rats.

As shown in Table 2, there were significant differences ($P < 0.01$) in GSH level between normal control and polysaccharides-treated animals. Significant increased GSH level ($P < 0.01$) was observed in blood and liver of polysaccharides-treated rats in comparison to normal rats.

Table 3 shows antioxidant related enzyme activities in the blood of normal and polysaccharides-treated rats. It could be found that administrations of *A. mongholicus* polysaccharides was effective in fully recovering enzyme activities as detected in normal groups. As shown in Table 3, administration of *A. mongholicus* polysaccharides effectively ($P < 0.01$) enhanced activities of blood SOD, CAT and GPx in a dose dependent manner.

Table 4 shows antioxidant related enzyme activities in the liver of normal and polysaccharides-treated rats. It could be found that administration of *A. mongholicus* polysaccharides was effective in fully recovering enzyme activities as detected in normal groups. As shown in Table 4, administration of *A. mongholicus* polysaccharides effectively ($P < 0.01$) enhanced activities of liver SOD, CAT and GPx in a dose dependent manner.

3. Discussion

Astragali Radix, the roots of *A. membranaceus* (Huangqi) are amongst the most popular health-promoting herbs in China, their use dated back more than 2000 years, and was recorded in Shen Nong's Materia Medica written in the Han dynasty (Mckenna, Hughes, & Jones, 2002). It is also a main component of Huang-Qi-Gui-Zhi-Wu-Wu-Tang, a traditional herbal medicine that has been used as a therapy for fatigue. Previous research about the immune modulatory effect of *A. membranaceus* was limited to normal or chemopreventive agent-challenged animals (Echeonwu et al., 2008; Huang, Wu, Chen, Yang, & Wang, 2007). So far as we know, this is the first paper that evaluated the effect of *A. membranaceus* on "spleen-qi-deficiency" subjects. The root of Astragalus species is known to be rich in polysaccharides, saponins, and flavonoids. In this study, we focus on studying antioxidant activity of *A. membranaceus* polysaccharides in rats.

The enzymes SOD, CAT and the glutathione system play a key role in the cellular defense against free radical damage (Ravid et al., 1999). Wide body of data indicates that animal tumor cells lack complex enzyme systems, which normally exert protection by scavenging toxic oxygen species such as superoxide radical, hydrogen peroxide and lipid hydroperoxides (Masotti, Casali, & Galeotti, 1988). Our results show a significant increase in the activities of SOD, CAT and GR ($P < 0.01$) in polysaccharides-treated rats.

Blood and liver GSH levels were lowered in polysaccharides-treated rats.

Enhanced peroxidation of lipids in intra- and extracellular membranes results in the damage to the cells, tissues and organs. SOD and CAT are important antioxidant enzymes that protect from this process via elimination of reactive oxygen species (ROS). SOD catalyzes the reaction of superoxide anion radical ($O_2^{\bullet -}$) dismutation to hydrogen peroxide (H_2O_2), whereas CAT degrades H_2O_2 into a molecule of oxygen and a molecule of water (Stohs & Bagchi, 1995). The results obtained regarding the activities of SOD, GPx and CAT, and the concentration of MDA (an indicator of lipid peroxidation) and GSH in the blood and liver clearly indicate that *A. mongholicus* polysaccharides are able to reduce the oxidative stress in rats.

Thus, we predicted that the beneficial effect of *A. mongholicus* polysaccharides on improving some degenerative diseases may in part contribute to its antioxidant activity.

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