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CHARACTERIZATION OF A PECTIC POLYSACCHARIDE FROM THE FRUIT OF *Ziziphus jujuba*

Zhihui Zhao,[1] Hui Dai,[3] Xiangmei Wu,[2] Haitao Chang,[2] Xiaoming Gao,[3] Mengjun Liu,[1]* and Pengfei Tu[2]*

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A pectic polysaccharide was isolated from the fruits of Ziziphus jujuba *Mill. cv. jinsixiaozao Hort. and its structure was characterized by acid hydrolysis, methylation, and NMR spectroscopies. The purified fraction Ju-B-7 had a molecular mass of over 2000 kDa. Chemical and spectroscopic analyses indicated that Ju-B-7 is composed mainly of α-1,4-linked D-galactopyranosyluronic acid and 1,2-linked L-rhamnose with a molar ratio of 8.1:1. It can significantly stimulate spleen cell proliferation* in vitro *(P<0.01, 10 μg/mL).*

Key words: *Ziziphus jujuba* Mill. cv. jinsixiaozao Hort., fruit, pectic polysaccharide, immunological activity.

Ziziphus jujuba, as a native plant of China, belongs to the genus *Ziziphus* (Rhamnaceae) and is widely distributed in China and South Korea. Its fruits are used as edible food and traditional Chinese medicine. Yamada et al [1] reported that polysaccharides from the fruits had anti-complementary activities.

We extracted water-soluble polysaccharides (WSPS) from fruits of *Zizizphus jujuba* Mill. cv. jinsixiaozao Hort. and purified several polysaccharides. Within this context, characterization of a pectic polysaccharide Ju-B-7 was made.

Ju-B-7 was determined as a single peak by size exclusive chromatography in an HPLC analysis. The molecular weight of Ju-B-7 was estimated to be over 2000 kDa and it had specific rotation $[\alpha]_D^{20}$ +166° (*c* 1.05, H_2O).

After complete hydrolysis with 2 M TFA, thin-layer chromatography (TLC) showed a large proportion of galacturonic acids. GC analysis of the alditol acetates showed that only rhamnose was detected. The *m*-hydroxydiphenyl method [2] revealed that it contained 90.51% uronic acid, indicating that Ju-B-7 contained galacturonic acid and rhamnose in a molar ratio of 8.1:1. The result of methylation of native and reduced Ju-B-7 showed that Ju-B-7 was composed of 1,4-linked D-Gal*p* A and 1,2-linked L-Rha*p* in a ratio of 8.1:1.

The IR spectrum of Ju-B-7 in the frequency range 400–4000 cm^{-1} showed the characteristics of a pectic polysaccharide with absorption at 1731 cm^{-1} of the carboxyl group and 824 cm^{-1} of the pyranose ring.

In the ^{13}C NMR spectrum, the signal at δ 99.7 was assigned to the anomeric carbon of α-D-GalA*p*, and δ 176.2 was derived from C-6 of α-D-GalA*p*. The signals at δ 68.8, 69.6, 78.6, and 72.0 were attributed to C-2, C-3, C-4, and C-5 of α-D-GalA*p*, respectively.

According to the HMQC spectrum, the signal at δ 5.06 was assigned to the anomeric proton of α-D- GalA*p*. The signals at δ 3.77, 3.99, 4.41, and 4.77 were assigned to H-2, H-3, H-4, and H-5 of α-D-GalA*p*, respectively. The signals at δ 2.1 and 1.21 were assigned to the *O*-acetyl group and H-6 of α-L-Rha*p*. The other signals of rhamnose were not detected because of the small amounts. The content of acetyl group was estimated to be 7.8%.

Using the stimulation effect on spleen cell proliferation, Ju-B-7 revealed significant (P<0.01) immunoenhancing activities in a dose-dependent manner (Fig. 1).

1) Research Center of Chinese Jujube, Agricultural University of Hebei, 071001, Baoding, Hebei, China, Fax: +86 312 7521251, e-mail: kjliu@mail.hebau.edu.cn; 2) Modern Research Center for Traditional Chinese Medicine, School of Pharmaceutical Sciences, Peking University Health Science Center, 100083, Beijing, China, fax: +86 10 82802750, e-mail: pengfeitu@bjmu.edu.cn; 3) School of Basic Medicinal Sciences, Peking University Health Science Center, 100083, Beijing, China. Published in Khimiya Prirodnykh Soedinenii, No. 4, pp. 311-312, July-August, 2007. Original article submitted March 30, 2006.

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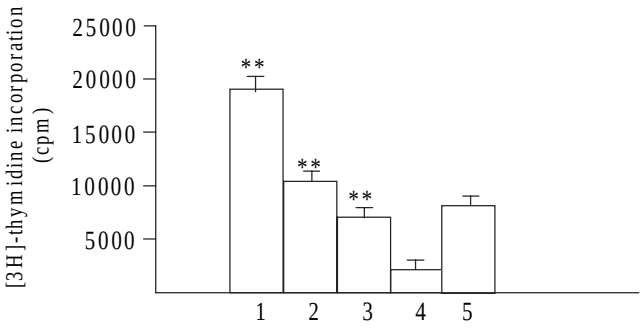

Fig. 1. Immunological activity of Ju-B-7. Each data point represents mean ± SD, n = 6; each has triplicates. Control and LPS indicates absence of sample and positive control, respectively. Sample group *vs* control group is $^{**}p < 0.01$. 1 – 100 μg/mL, 2 – 30 μg/mL, 3 – 10 μg/mL, 4 – control, 5 – LPS

Thus, Ju-B-7 contained galacturonic acid and rhamnose in a molar ratio of 8.1: 1, assumed to be polygalacturonan interspersed with rhamnogalacturonan with reference to literature values [3]. It had significant ($P<0.01$) immunoenhancing effect on spleen cell proliferation.

EXPERIMENTAL

Plant Material. The fruits of *Ziziphus jujuba* Mill. cv. jinsixiaozao Hort. were collected from Cangzhou, Hebei province (China) in October of 2003. The identification of *Ziziphus jujuba* Mill. cv. jinsixiaozao Hort. was done by one of the authors (M-J, Liu). The voucher specimen was deposited in the Research Center of Chinese Jujube (jsxz031003).

Isolation and Purification of Ju-B-7. Dried fruits (1080 g) were soaked in 95% ethanol for 24 h. WSPS (203 g) were obtained by precipitation with alcohol from the aqueous extract of the fruit residue after reflux with ethanol. A portion of this preparation (100 g) was deproteinated using the Sevage method [4]. The aqueous phase resulted in a crude polysaccharide Ju (45 g). A portion of Ju (9 g) was dissolved in water, applied to a DEAE chromatograph (700 × 25 mm), and eluted with 0–2 M gradient NaCl (each eluent in five runs). The 0.2 M NaCl eluate was purified on a Sepharose CL-6B chromatograph (500 × 22 mm) to yield Ju-B-7 (150 mg).

General Analysis. The aqueous extract was deproteinated by the Sevage method. Protein content was analyzed by the Lowry method [5] and there was no protein in Ju-B-7 according to this method.

The homogeneity and molecular weight were evaluated from a calibration curve of the elution volume of standard dextrans (Dextrans T-2000, T-500, T-70, T-40, T-10 and glucose; from Amersham Pharmacia, Sweden) by HPLC performed on an Agilent 1100 series apparatus equipped with a Shodex KS-805 column (Shoko, Japan) and an ELSD detector. Distilled water was used as the solvent and eluent, and the flow rate was kept at 1.0 mL/min. All gel permeation chromatography was eluted with H_2O.

Optical rotations were measured on a Perkin-Elmer 243B polarimeter. IR spectra were determined with an AVATER-360 spectrometer. The ^{1}H, ^{13}C, and 2D NMR spectra were recorded on an INOVA-500 instrument operating at 28°C in D_2O. GC-MS was performed on a Finnigan Trace GC-MS instrument equipped with a DB-5 column and detected by an FID.

Polysaccharide Analyses. The neutral monosaccharide composition was studied by total hydrolysis with 2 M TFA at 110°C for 2 h. Part of the hydrolysates was analyzed using TLC on a silica gel plate containing 5% sodium dihydrogen phosphate and developed with BuOH–EtOAc–isopropyl alcohol–HOAC–H_2O–pyridine (3.5: 10: 6: 3.5: 3: 3). The plate was visualized with 1,3-naphthalenediol reagent, then heated at 110°C for 10 min. The remaining hydrolysates were reduced with $NaBH_4$ (20 mg) for 3 h, then acetylated with Ac_2O (100°C, 1 h). The resulting alditol acetates were analyzed by GC [6]. Uronic acid of the sample was determined by the *m*-hydroxyldiphenyl method with D-galacturonic acid as standard. The sample was reduced by the reported method [7]. The vacuum dried polysaccharides were methylated using the method of Needs and Selvendran [8]. The content of acetyl groups in the polysaccharide was determined from the ^{1}H NMR spectrum [9].

Measurement of Immunomodulating Activity. Mice (6–8 weeks) were sacrificed and their spleens were removed and passed through a sterilized iron sieve to obtain single cell suspensions. The single cell suspension was washed with PBS, and then the red blood cells were lysed with ACK lyse buffer (0.15 M NH_4Cl, 1.0 mM $KHCO_3$, 0.1 mM EDTA) for 3 min. The spleen cells were washed and then cultured in U-bottom well plates (2×10^5/ well) in a volume of 200 μL per well in the presence of 10, 30, and 100 μg/mL of Ju-B-7, negative control, and positive control (LPS, 2.5 μg/mL) groups, respectively. After a three-day drug treatment, DNA synthesis was measured by H^3-thymidine (Du Pont) incorporation (1 μCi / well) in the final 6 h of the cultured period. The data were tested for statistical differences using the *T* test.

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