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# Isolation, purification and structure of a new water-soluble polysaccharide from *Zizyphus jujuba cv. Jinsixiaozao*

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ABSTRACT

A novel water-soluble polysaccharide (ZSP3c) was isolated from *Zizyphus jujuba cv. Jinsixiaozao* and its structure was investigated. ZSP3c was obtained by extraction with hot water followed by purification using DEAE-SepharoseCL-6B anion-exchange, SepharoseCL-6B and Sephadex G-200 column chromatography. It was eluted as a single symmetrical narrow peak on high-performance gel-permeation chromatography (HPGPC) and the average molecular weight (Mw) was estimated to be $1.4 \times 10^5$ Da. The ZSP3c was composed of L-rhamnose, D-arabinose and D-galactose in a molar ratio of 1:2:8. The structural features of ZSP3c were investigated by partial hydrolysis with acid, methylation analysis and NMR spectroscopy analysis. The results revealed that the main backbone chain of ZSP3c was (1 → 4)-D-galacturonopyranosyl residues interspersed with (1 → 2)-L-rhamnopyranosyl residues and (1 → 2,4)-L-rhamnopyranosyl residues. The neutral side chains of arabinofuranosyl residues and galactopyranosyl residues were attached to the backbone at the *O*-4 position of rhamnopyranosyl residues.



## 1. Introduction

In recent years, increasing attention is being placed on polysaccharides extracted from plants and fungus by biochemical and nutritional researchers, due to their various potential biological activities that could be applied to healthcare foods or medicine, especially anti-oxidant, immunostimulatory, anti-oxidative and anti-tumor effects (Li et al., 2003; Li, Chen, Wang, Tian, & Zhang, 2009; Qiao et al., 2009; Sun & Liu, 2008; Yuan, Zhang, Fan, & Yang, 2008). Therefore, discovery and evaluation of polysaccharides extracted from plants and fungus as new safe compounds for functional foods or medicine has become a hot research spot.

Chinese jujube (*Zizyphus jujuba Miller*), which is mainly distributed in the subtropical regions of Asia, has been cultivated for over 3000 years and used as food, food additives, flavors, and pharmaceuticals for a long time. It has been widely planted in re-forested areas within the Yellow River valley, and chosen as a variety compatible with the present ecology and economy (Yan & Gao, 2002). The fruit of Chinese jujube is a kind of favorable and profitable fruit, and is much admired for its high nutritional value. It has been commonly used as a crude drug in traditional Chinese medicine for the purpose of analeptic, palliative, antibechic and also commonly used as food, food additives and flavors for thousands of years. The Chinese share of the world jujube production is about 90%, and its production has increased in the last 10 years due to demands for the food and pharmaceutical (Yan & Gao, 2002.). A steady rise in consumer demand has been reflected by an expansion of Chinese production that was 1.4 million tons in 2001, reaching 3.0 million tons in 2007. Much of the annual Chinese jujube production has been consumed in fresh and drying forms, so there are numerous studies, experimental works as well as simulations, dealing with preservation of Chinese jujube and drying to enhance quality (Lu, Li, & Lu, 1992; Wan, Tian, & Qin, 2003; Wang, Li, & Dan, 2003). However, there is relatively little information pertaining to isolation, purification, and activity determination of the water-soluble polysaccharide from Chinese jujube, especially detailed studies of the structure. Since structure and functions are intimately related, an in-depth study of the structure of the polysaccharides would be of interest. Nowadays, *ZiZyphus Jujuba cv. Jinsixiaozao* is cultivar of Chinese jujube that was commonly planted in China. Therefore, the aim of this work was mainly to report on the extraction and purification of a water-soluble polysaccharide from *Zizyphus jujuba cv. Jinsixiaozao*, by ion exchange chromatography and gel-filtration chromatography, and elucidate its structural characterization by a combination of chemical and instrumental analysis.

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## 2. Materials and methods

### 2.1. Material

DEAE-SepharoseCL-6B, SepharoseCL-6B and Sephadex G-200 were purchased from Pharmacia Chemical Co. T-series dextran, dimethyl sulfoxide (DMSO) and standard sugars were purchased from Sigma Chemical Co. All of other reagents were analytical grade from Shanghai Chemical Co.

*Zizyphus jujuba cv. Jinsixiaozao* was obtained from the Research Institute of Jujube (Shandong, China). It was dried at 60 °C in an oven for 48 h after removal of seeds, and then it was ground to pass through a 1 mm screen and stored in a refrigerator.

### 2.2. Proximate composition analysis

Total carbohydrate was determined by the phenol–sulphuric acid colorimetric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) with D-glucose as standard at 490 nm. Uronic acid contents were determined according to Blumenkrantz and Asboe-Hansen's method by measuring the absorbance at 525 nm using the m-hydroxybiphenyl colorimetric procedure and with D-galacturonic acid as the standard (Blumenkrantz & Asbose-Hansen, 1973).

### 2.3. Extraction and purification of polysaccharide

Ground *Zizyphus jujuba cv. Jinsixiaozao* was refluxed with 95% ethanol at 70 °C in a water bath for 3 h. Subsequently, the dried ethanol-extracted residue was extracted with distilled water at 80 °C for 3 h, filtered through gauze and centrifuged to remove water-insoluble materials. The aqueous extract was concentrated, and then mixed with four volumes of cold 95% ethanol for isolation of the polysaccharides. The precipitate was recovered by centrifugation, and finally lyophilized. Brown water-soluble polysaccharide (ZSP) was obtained.

### 2.4. Isolation and purification of polysaccharide

ZSP was dissolved in distilled water, centrifuged, and then the supernatant was applied to a DEAE-SepharoseCL-6B column (2.6 cm × 37 cm) equilibrated with 0.1 M NaAc-buffer (pH 5.0). After loading with ZSP, the column was eluted with 0.1 M NaAc-buffer (pH 5.0) at a flow rate of 1.25 mL/min and then a linear gradient (1500 mL) from 0 to 1.5 M NaCl in 0.1 M NaAc-buffer (pH 5.0) was applied to the column. Test tubes were collected using an automated step-by-step fraction collector. Total carbohydrate content of each tube was measured at 490 nm by the phenol–sulphuric acid colorimetric method, and protein absorption at 280 nm was measured for each fraction. Main fraction containing carbohydrate from the elution step was lyophilised. The four collected fractions were denoted as ZSP1, ZSP2, ZSP3 and ZSP4. The fraction ZSP3 was further applied on a gel-filtration column (2.6 cm × 160 cm) of SepharoseCL-6B, and eluted with 0.02 M NaCl at a flow rate of 0.5 mL/min and fractionated into three fractions (denoted as ZSP3a, ZSP3b and ZSP3c). To eliminate any remaining contaminants, ZSP3c was further applied on a gel-filtration column (2.6 cm × 160 cm) of Sephadex G-200, the fractions containing carbohydrate were combined and concentrated, and then used as the purified polysaccharide (ZSP3c).

### 2.5. Homogeneity and molecular weight determination of ZSP3c

The homogeneity and molecular weight of ZSP3c were identified by high-performance gel-permeation chromatography (HPGPC) with a Waters HPLC apparatus equipped with two serially linked UltrahydrogelTM Linear (Ø7.8 mm × 300 mm ID) columns, a Waters 2410 interferometric refractometer detector and UV detector connected in series with a Millennium32 workstation. Detailed experimental conditions were as follows: a 20 μL sample (20 mg/mL) was injected in each run at room temperature and was eluted at a flow rate of 0.9 mL/min and detected with a Waters 2410 interferometric refractometer detector. The mobile phase was 0.1 M $NaNO_3$. The molecular weight was estimated by reference to the calibration curve made under the conditions described above from Dextran T-series standards of known molecular weight.

### 2.6. Monosaccharide identification and quantification of ZSP3c

Gas chromatography (GC) was used for identification and quantification of the monosaccharides of ZSP3c. The composition of neutral monosaccharides was analyzed by gas chromatography. ZSP3c was dissolved in 2 M trifluoroacetic acid (TFA) and hydrolysed at 121 °C for 3 h in a sealed glass tube. The solution was evaporated to dryness and then a mixture of methanol–water (1:1) was added to give a clear solution, which was evaporated again to dryness. Acetylation was carried out with 10 mg hydroxylamine hydrochloride and 0.5 mL pyridine for 30 min at 90 °C. 0.5 mL of acetic anhydride was then added with continuously heating and the alditol acetate derivative (1–2 mL) was analyzed by using a gas chromatograph. The percentages of monosaccharides in the sample were calculated from the peak areas using response factors.

### 2.7. FT-IR spectra analysis of ZSP3c

FT-IR spectra of the materials were obtained at a resolution of 8 $cm^{-1}$. The samples were incorporated into KBr and pressed into a 1 mm pellet. Spectra were recorded at the absorbance mode from 4000 $cm^{-1}$ to 400 $cm^{-1}$ on a Nicolet Nexus FT-IR spectrometer.

### 2.8. Partial acid hydrolysis of ZSP3c

ZSP3c (100 mg) was hydrolyzed with 0.05 mol/L TFA (3 mL) for 1 h at 121 °C, TFA was removed by evaporation, and the hydrolysate was dialyzed against distilled water for 48 h in a dialysis bag (molecular weight 3500 Da cut off). The solution outside of the dialysis bag was collected for GC analysis, and the solution inside of the dialysis bag was concentrated and lyophilized for further hydrolysis. The solution outside of the dialysis bag was hydrolyzed in turn 0.1 mol/L, 0.5 mol/L and 2 mol/L TFA (10 mL) for 1 h at 121 °C (Fig. 1). The fraction outside of the dialysis bag and all other fractions were analyzed by GC in order to determine monosaccharides.

### 2.9. Methylation analysis of ZSP3c

Prior to methylation, ZSP3c was reduced to the corresponding neutral sugars (York, Darvill, Neill, Stevenson, & Albersheim, 1985). Reduced ZSP3c was methylated separately using the method of Ciucanu and Kerek (1984). The methylated polysaccharides were extracted with methylene chloride, passed through a sodium sulphate column to remove water and dried under a stream of nitrogen. The product was then hydrolyzed in 4 mol/L TFA (0.5 mL) at 100 °C for 6 h and dried under a stream of nitrogen. The acid hydrolysate was reduced with deuterated sodium borohydride before it was acetylated with acetic anhydride (0.5 mL). The resulting product was subjected to linkage analysis by GC–MS system (ThermoQuest Finnigan, San Diego, CA) fitted with a SP-2330 column (Supelco, Bellefonte, PA) (30 m × 0.25 mm, 0.2 mm film thickness, 160–210 °C at 2 °C/min, then 210–240 °C at 5 °C/min) and equipped with an ion trap MS detector. The components were identified by a combination of the main fragments in their mass spectra

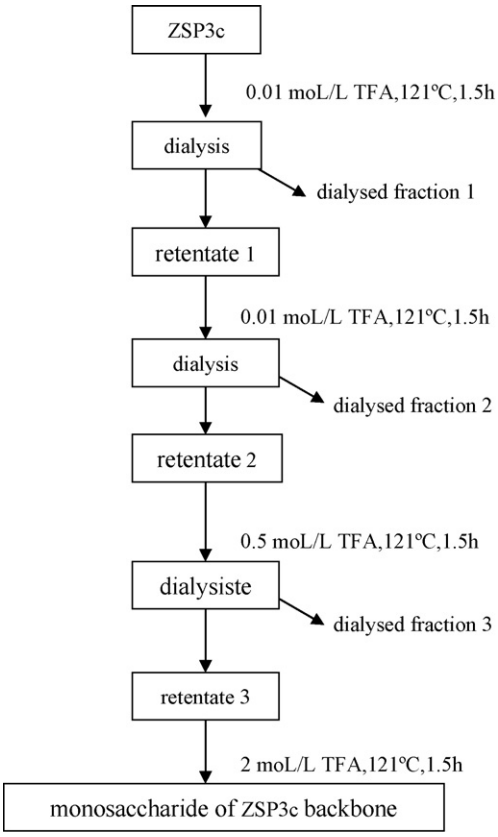


**Fig. 1.** Scheme for partial acid hydrolysis of ZSP3c.

and relative GC retention times; the molar ratios for each sugar were calibrated using the peak areas and response factors.

### 2.10. NMR spectroscopy analysis of ZSP3c

ZSP3c was dried in a vacuum over $P_2O_5$ for several days, and then exchanged with deuterium by lyophilizing with $D_2O$ for several times (Duenas-Chasco et al., 1997). The deuterium-exchanged ZSP3c (50 mg) was put in a 5-mm NMR tube and dissolved in 0.7 mL of 99.9% $D_2O$. NMR-spectra were recorded with a Brucker AM-400 NMR spectrometer using standard pulse sequences with 5 mm tubes at 60 °C.

## 3. Results and discussion

### 3.1. Isolation and purification of ZSP3c

Ground *Zizyphus jujuba cv. Jinsixiaozao* was refluxed with ethanol to deactivate the endogenous enzymes and remove some soluble materials, including free sugars, amino acids and some phenols. Then the dried ethanol-extracted residue was extracted with distilled water at 80 °C. After dialysis and precipitation with ethanol, a crude water-soluble polysaccharide (ZSP) was obtained as a brownish powder. The ZSP was separated and purified by anion-exchange chromatography (DEAE-SepharoseCL-6B). Four fractions of ZSP1, ZSP2, ZSP3 and ZSP4 were eluted. ZSP1 were recovered from the NaAc-buffer eluate, and ZSP2, ZSP3 and ZSP4 recovered from the NaCl eluate. ZSP1, ZSP3 and ZSP4 are soluble in hot water with characteristic absorption of polysaccharide at 190 nm and protein at 280 nm, and no absorption at 260 nm for nucleic acid. ZSP2 is soluble in hot water with characteristic absorption of polysaccharide at 190 nm, and no absorption at 280 and 260 nm for protein and nucleic acid. ZSP3 was further

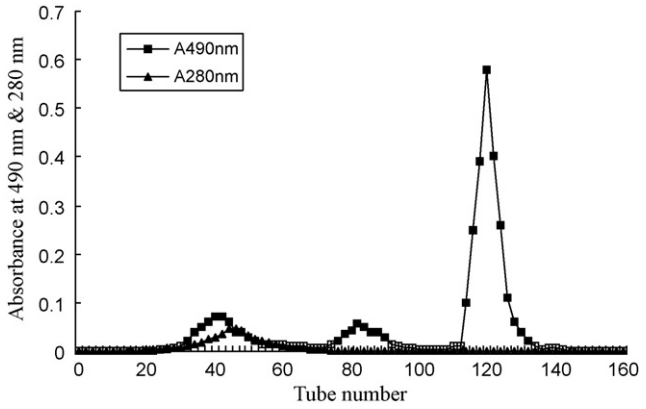


**Fig. 2.** Chromatogram of ZSP3 on the SepharoseCL-6B column.

purified through SepharoseCL-6B column (gel-permeation chromatography), leading to the isolation ZSP3a, ZSP3b and ZSP3c of three water-soluble purified polysaccharides (Fig. 2). ZSP3c was selected and chromatographed on a Sephadex G-200 column and a single peak was obtained. ZSP3c was also eluted as a single symmetrical narrow peak on high-performance gel-permeation chromatography (HPGPC), indicating that ZSP3c is a homogeneous polysaccharide (Fig. 3), and the average molecular weight was estimated to be $1.4 \times 10^5$ Da in reference to Dextran T-series standard samples of known molecular weights. As determined by meta-hydroxydiphenyl colorimetric method, ZSP3c contains uronic acid.

### 3.2. Monosaccharide identification and quantification of ZSP3c

GC analysis could give the accurate content of sugars in the polysaccharides. On hydrolysis by 2 mol/L TFA, ZSP3c was detected by GC analysis. The results showed that ZSP3c is composed of L-rhamnose, D-arabinose and D-galactose in a molar ratio of 1:2:8. The big magnitude $[\alpha]_D^{25} = +131^\circ$ (c 0.1 mg/mL; $H_2O$) of its positive specific rotation suggested the main glycosidic bond was a-configuration (Liu, Lin, Gao, Ye, Xing & Xi, 2007; Mathlouthi & Koenig, 1986). This result was in agreement with IR spectrum observation, that the characteristic absorptions at 830 $cm^{-1}$ in the IR spectra were α-configuration (Zhang, 1999).

### 3.3. Partial acid hydrolysis of ZSP3c

Partial degradation of polysaccharide by acid hydrolysis is based on the fact that some glycosidic linkages are more labile to acid than

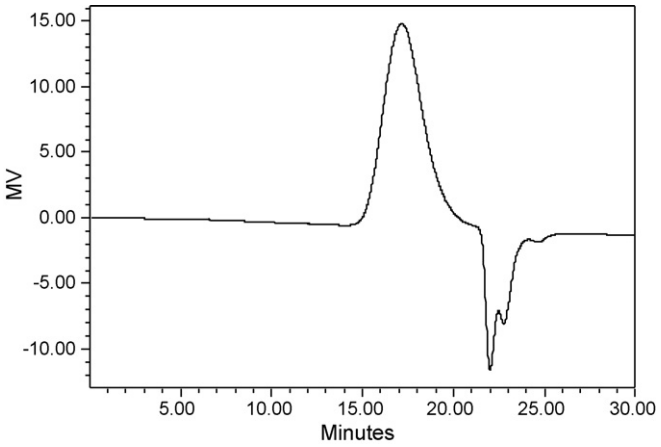


**Fig. 3.** HPGPC profile of ZSP3c.

**Table 1**
Chemical component content of the hydrolysates.

| Fractions | Hydrolysates | Neutral sugars | Galacturonic acid |
|---|---|---|---|
| ZSP3c-1 | 0.05 mol/L dialysed fraction | 87.5 | n.d.[a] |
| ZSP3c-2 | 0.1 mol/L dialysed fraction | 81.3 | 2.1 |
| ZSP3c-3 | 0.5 mol/L dialysed fraction | 75.6 | 9.8 |
| ZSP3c-4 | 2 mol/L hydrolysates | 8.2 | 81.4 |

[a] Content was given in w/w, %, and n.d. means not determined.

**Table 2**
Monosaccharide composition of ZSP3c after partial acid hydrolysis (mol.%).

| Fractions | Hydrolysates | Rhamnose | Arabinose | Galactose |
|---|---|---|---|---|
| ZSP3c-1 | 0.05 mol/L dialysed fraction | n.d.[a] | 100 | n.d.[a] |
| ZSP3c-2 | 0.1 mol/L dialysed fraction | 2.2 | 50.3 | 47.5 |
| ZSP3c-3 | 0.5 mol/L dialysed fraction | 4.5 | 5.1 | 90.4 |
| ZSP3c-4 | 2 mol/L hydrolysates | 98.9 | n.d.[a] | 1.1 |

[a] Not determined.

others. After partial acid hydrolysis of ZSP3c, four fractions ZSP3c-1 (fraction out of dialysis sack after 0.05 mol/L TFA hydrolysis), ZSP3c-2 (fraction out of dialysis sack after 0.1 mol/L TFA hydrolysis), ZSP3c-3 (fraction out of dialysis sack after 0.5 mol/L TFA hydrolysis), ZSP3c-4 (hydrolysates after 2 mol/L TFA hydrolysis) were obtained, and all the fractions were subjected to GC analysis. Chemical component content and monosaccharide composition of the hydrolysates are given in Tables 1 and 2.

Most arabinoses were hydrolyzed by 0.05 mol/L TFA. Arabinose and galactose were completely hydrolyzed while only a small portion of rhamnose and galacturonic acid were hydrolyzed by 0.1 mol/L TFA. The results indicated glucose and arabinose residues could be on the branch of ZSP3c. With the continual increase in the extent of TFA hydrolysis, the content of rhamnose and galacturonic acid increased steadily while no arabinose in the dialysates were found in the 2 mol/L TFA hydrolysates. The results indicated rhamnose and galacturonic acid could exist in the backbone of ZSP3c.

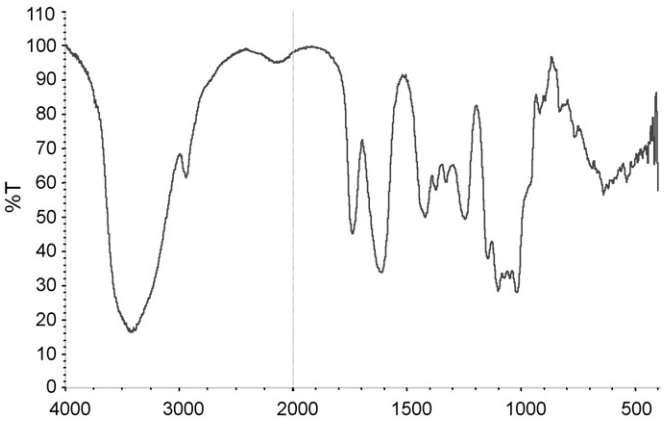


**Fig. 4.** Fourier transform infrared spectra of ZSP3c.

### 3.4. FT-IR spectra analysis of ZSP3c

The FT-IR spectrum of ZSP3c is presented in Fig. 4. The attributions of the main absorptions are characteristic of glycosidic structures and are related to CO stretching (1102 $cm^{-1}$, 1015 $cm^{-1}$, and 1050 $cm^{-1}$) and anomeric $C_1H$ group vibration (830 $cm^{-1}$ and 919 $cm^{-1}$). Moreover, the characteristic absorptions at 830 $cm^{-1}$ and 919 $cm^{-1}$ in the IR spectra indicated that α-

**Table 3**
Methylation analysis ofZSP3c (mol.%).

| 保留时间(min) | 甲基化碎片 | 糖苷键的类型 | 摩尔百分比(mol.%) |
|---|---|---|---|
| 18.06 | 2,3,6-$Me_3$-D-Gal*p*A | →4)–D-Gal*p*A (1→ | 38.16 |
| Total | | | 38.16 |
| 8.77 | 2,3,4-$Me_3$-L-Rha*p* | T-L-Rha*p* (1→ | 1.01 |
| 16.34 | 3-Me-L-Rha*p* | →2,4)-L-Rha*p* (1→ | 1.09 |
| 22.02 | 3,4-$Me_2$-Rha*p* | →2)-L-Rha*p* (1→ | 3.61 |
| Total | | | 5.71 |
| 8.05 | 2,3,5-$Me_3$-L-Ara*f* | T-L-Ara*f*(1→ | 2.16 |
| 11.59 | 2,5–$Me_2$-L-Ara*f* | →3)-L-Ara*f* (1→ | 2.73 |
| 12.77 | 2,3-$Me_2$-L-Ara*f* | →5)-L-Ara*f* (1→ | 2.79 |
| 16.15 | 2-Me-L-Ara*f* | →3,5)-L-Ara*f* (1→ | 3.66 |
| Total | | | 11.34 |
| 14.30 | 2,3,4,6-$Me_4$-D-Gal*p* | T-D-Gal*p* (1→ | 7.74 |
| 18.48 | 2,3,6-$Me_3$-D-Gal*p* | →4)-D-Gal*p* (1→ | 1.83 |
| 20.89 | 2,3,4-$Me_3$-D-Gal*p* | →6)-D-Gal*p* (1→ | 8.49 |
| 22.98 | 2,4-$Me_2$-D-Gal*p* | →3,6)-D-Gal*p* (1→ | 17.04 |
| 23.75 | 2,3-$Me_2$-D-Gal*p* | →4,6)-D-Gal*p* (1→ | 9.69 |
| Total | | | 44.79 |

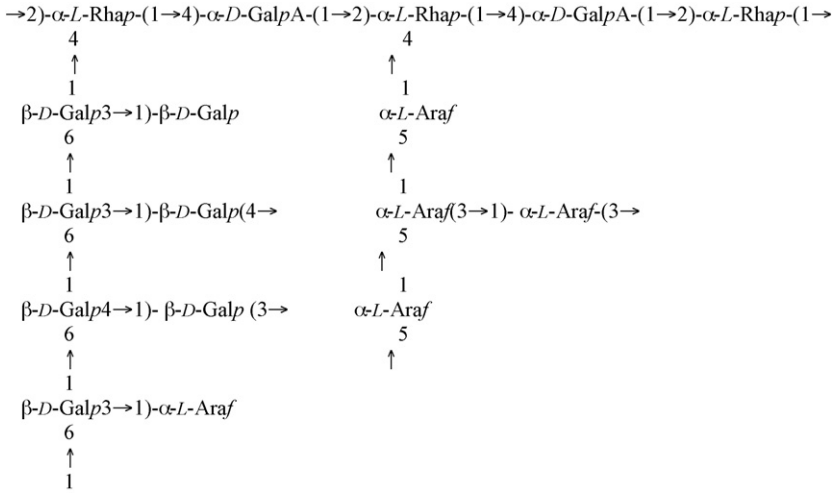


**Fig. 5.** Schematic structure of ZSP3c.

and β-configurations were simultaneously. Stronger bands occurring between 1742 $cm^{-1}$ and 1616 $cm^{-1}$ are derived from the ester carbonyl (–COOR) groups and carboxylate ion stretching band (–COO–), respectively. Further analysis of the FT-IR spectra revealed that the broader band of absorption between 3600 $cm^{-1}$ and 2500 $cm^{-1}$ was due to O–H stretching. The absorption at 3413 $cm^{-1}$ is assigned to stretching of –OH groups and that one at 2934 $cm^{-1}$ to the C–H stretching.

### 3.5. Methylation analysis of ZSP3c

Methylation analysis, the most widely used method, is used to determine the structure of carbohydrate for over a century and it is still the most powerful method in determining the sugar linkage of polysaccharides (Cui, 2005). To measure the linked types of uronic acid, GalA was reduced to Gal before melylation analysis. The reduced ZSP3c was hydrolyzed with acid, converted into alditol acetates, and analyzed by GC–MS. As summarized in Table 3, the reduced ZSP3c showed the presence of 13 derivatives, namely 2,3,5-$Me_3$-Araf; 2,5-$Me_2$-Araf; 2,3-$Me_2$-Araf; 2-Me-Araf; 2,3,4-$Me_3$-L-Rhap; 3-Me-Rhap; 3,4-$Me_2$-L-Rhap; 2,3,6-$Me_3$-GalpA; 2,3,4,6-$Me_4$-Galp; 2,3,6-$Me_3$-Galp; 2,3,4-$Me_3$-Galp and 2,4-$Me_2$-Galpin molar ratios of 2.16:2.73:2.79:3.66:1.01:1.09:3.61:38.16:7.74:1.83:8.49:17.04:9.69. These molar ratios agree with the overall monosaccharide composition described above.

The methylation results of ZSP3c indicated that ZSP3c consisted of (1 → 4)-D-galacturonopyranosyl residues, non-reducing terminal (1→)-L-rhamnopyranosyl residues, (1 → 2)-L-rhamnopyranosyl residues, (1 → 2,4)-L-rhamnopyranosyl residues, non-reducing terminal (1→)-L-arabinofuranosyl residues (1 → 3)-L-arabinofuranosyl residues, (1 → 5)-L-arabinofuranosyl residues (1 → 3,5)-L-arabinofuranosyl residues, non-reducing terminal (1→)-D-galactopyranosyl residues, (1 → 4)-D-galactopyranosyl residues, (1 → 6)-D-galactopyranosyl residues, (1 → 3,6)-D-galactopyranosyl residues, (1 → 4,6)-D-galactopyranosyl residues. (1 → 4)-D-Galacturonopyranosyl residue was the main residue of ZSP3c. Rhamnopyranosyl residues were mainly 1,2-linked in which more than 23% was substituted at *O*-4 position. Galactopyranosyl residues were positioned at non-reducing terminals, 1,3-, 1,4-, 1,3,6- and 1,4,6- linked. According to the peak areas, five types of galactopyranosyl residues are in the ratio of 17:4:19:38:22. Therefore, the methylation data of ZSP3c suggest the main backbone chain of ZSP3c was (1 → 4)-D-galacturonopyranosyl residues interspersed with (1 → 2)-L-rhamnopyranosyl residues and (1 → 2,4)-L-rhamnopyranosyl residues. The neutral side chains of arabinofuranosyl residues and galactopyranosyl residues were attached to the backbone at the *O*-4 position of rhamnopyranosyl residues.

### 3.6. NMR spectroscopy anlysis of ZSP3c

NMR spectroscopy has simplified the procedure for the structural analysis of carbohydrates and become the most powerful technique for the structure analysis of complex polysaccharides. NMR can provide detailed structural information of carbohydrates, including identification of monosaccharide composition, elucidation of α- or β-anomeric configurations, establishment of linkage patterns and sequences of the sugar units in the polysaccharides (Cui, 2005). The anomeric protons from each monosaccharide give recognizable signals depending on their α- or β-configurations. Of the two types of anomeric protons, signals derived from α-anomeric protons appear in the 5–6 ppm region while most of the β-anomeric protons will appear between 4 and 5 ppm (Cui, 2005). In the anomeric region of the $^1H$ NMR spectrum of ZSP3c, thirteen signals occurred at d5.01, d5.21, d5.23, d5.24, d5.19, d5.21, d5.04, d5.09, d4.47, d4.63, d4.52, d4.66 and d4.47 ppm, which were designated as (1 → 4)-D-galacturonopyranosyl residues, non-reducing terminal (1 → )-L-rhamnopyranosyl residues, (1 → 2)-L-rhamnopyranosyl residues, (1 → 2,4)-L-rhamnopyranosyl residues, non-reducing terminal, (1 → )-L-arabinofuranosyl residues, (1 → 3)-L-arabinofuranosyl residues, (1 → 5)-L-arabinofuranosyl residues, (1 → 3,5)-L-arabinofuranosyl residues, non-reducing terminal (1 → )-D-galactopyranosyl residues, (1 → 4)-D-galactopyranosyl residues, (1 → 6)-D-galactopyranosyl residues, (1 → 3,6)-D-galactopyranosyl residues, (1 → 4,6)-D-galactopyranosyl residues, respectively. All the results confirmed the presence of three sugar residues and their configurations: galacturonopyranosyl residues, rhamnopyranosyl residues and arabinofuranosyl residues were α-configurations and galactopyranosyl residues was β-configurations, consistent with GC and FT-IR data. Accordingly, in the anomeric region of the DEPT-135° NMR spectrum of ZSP3c, 13 carbon resonances appeared at d98.6, d99.8, d99.4, d98.8, d107.2, d108.5, d108.5, d107.8, d106.0, d106.0, d106.0, d106.5 and d106.0 ppm. All the NMR chemical shifts were compared with the literature values (Fan, Zhang, Tang, Liu, Zhang & Pan, 2006; Habibi, Heyraud, Mahrouz, & Vignon, 2004; Habibi, Mahrouz, & Vignon, 2005; Li, Chen, & Xu, 2005; Sun et al., 2008; Wang, Liu, & Fang, 2005; Zhang et al., 2006).

On the basis of above-mentioned results, it can be concluded the suggested repeat unit of ZSP3c (Fig. 5). In the current work, a water-soluble polysaccharide was purified from Chinese jujube and identified its structure as follows: the main backbone chain of ZSP3c was (1 → 4)-D-galacturonopyranosyl residues interspersed with (1 → 2)-L-rhamnopyranosyl residues and (1 → 2,4)-L-rhamnopyranosyl residues. The neutral side chains of arabinofuranosyl residues and galactopyranosyl residues were attached to the backbone at the *O*-4 position of rhamnopyranosyl residues.

## 4. Conclusions

This study demonstrated that ZSP3c isolated from Chinese jujube, which is a heteropolysaccharide consisting of L-rhamnose, D-arabinose and D-galactose in a molar ratio of 1:2:8. The repeating unit of structure were proposed that the main backbone chain of ZSP3c was (1 → 4)-D-galacturonopyranosyl residues interspersed with (1 → 2)-L-rhamnopyranosyl residues and (1 → 2,4)-L-rhamnopyranosyl residues. The neutral side chains of arabinofuranosyl residues and galactopyranosyl residues were attached to the backbone at the *O*-4 position of rhamnopyranosyl residues.

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