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Short communication

Structural analysis of a polysaccharide from *Fructus Mori Albae*

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Abstract

Fructus Mori Albae polysaccharide (FMAP) was first isolated from *Fructus Mori Albae*, and purified by Sephadex G-200 column chromatography. Its average molecular weight ($M_w = 1.30 \times 10^5$) was determined by gel permeation chromatography (GPC). The polysaccharide was found to be composed of D-galactose, D-mannose and D-glucose by TLC and GC–MS. According to IR, methylation and GC–MS, the main chain of the polysaccharide consists of (1 → 3)-linked mannan with branches of galactosyl and glucosyl residues at O-6 on mannosyl residues of the backbone.

Keywords: *Fructus Mori Albae*; *Fructus Mori Albae* polysaccharide; Galactomannan

1. Introduction

The genus mulberry consists of three species: red mulberry (*Morus rubra* L.), white mulberry (*Morus alba* L.) and black mulberry (*Morus nigra* L.) (Doymaz, 2004). *Fructus Mori Albae* is fruit of *Morus alba* L. and has been used as food and fruit in several centuries. This fruit is a traditional medicine in China and other countries. In past decades, many research groups have investigated its chemical constituents and biological activities (Chu, Lin, Tian, & Ye, 2006; Committee of Jiangsu New Medicine College, 1995; Li, Teng, Cheng, & Wu, 2003; Sergio, 1989). It helps to promote the immune system, normalize blood sugar, resist decrepitude, and inhibits blood abnormalities, such as hypoglycemia (Hikino, Konno, Mirin, & Hayashi, 1985; Hikino, Mizuno, Oshima, & Konno, 1985). So *Fructus Mori Albae* is widely used in clinical practice in China (Cao, 1999).

In spite of this extensive exploitation of *Fructus Mori Albae* no information has been reported about the structure of the polysaccharide from it. The purpose of the present study was to contribute further information about isolation, purification and characterization of polysaccharide of *Fructus Mori Albae*. The result of this study introduces *Fructus Mori Albae* as a possible valuable source for galactomannan, which has certain biological activities, such as anti-viral activity (Zhang, Zhang, Shun, & Liang, 1994), anticoagulation, fibrinolysis (Hussen, Helmy, & Salem, 1998). The activities were exhibited by the crude, fractionated and partially degraded galactomannans, and were higher in both the native and enzymatically modified products (Hussen et al., 1998).

2. Materials and methods

2.1. Materials

The plant was purchased from the market in Nanning City. Sephadex G-200(Pharmacia Biotech Limited, Denmark), Dextran standard series T-10, T-40, T-70, T-110, T-500 (Pharmacia Biotech Limited, Denmark); D-arabinose, D-xylose, L-rhamnose, D-mannose, D-glucose, D-galactose (Sigma Chemical, USA).

2.2. Isolation and purification

One-thousand grams of powder of dried *Fructus Mori Albae* were extracted successively with petroleum ether and ethanol 95%, and then extracted with 3000 ml by

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distilled water for 2 h at 80 °C. The extracted solution was filtered and then kept overnight at 4 °C. Then the solution was centrifuged at 4 °C and 8000 rpm for 30 min. Clear solution was separated and then concentrated under vacuum. The residue was precipitated with methanol under stirring at room temperature. This precipitated mixture was kept for 24 h at room temperature and then filtered. The precipitate obtained was dissolved in a small amount of distilled water and then precipitated with methanol again. This precipitate mixture was kept for 24 h at room temperature and then filtered. This process was repeated three times. Then the collected precipitate was washed with methanol and acetone three times. The cake material was dissolved in distilled water and deproteinization was carried out with 100 ml/time Sevag reagent ($CHCl_3$/*n*-BuOH, v/v = 4:1) (Navarini, Gilli, & Gombac, 1999). Then the aqueous solution was concentrated and a residue was obtained. The residue was precipitated with methanol, and then kept at room temperature for 24 h. The precipitate was collected and then washed with methanol and acetone for three times and freeze-dried. In this way 92.8 g of crude polysaccharide was obtained.

Crude polysaccharide 100 mg was applied to a Sephadex G-200 gel column eluted with 0–1 M sodium phosphate buffer (pH 6.0) at a flow rate of 0.5 ml/min. The eluates were collected in 2 ml fractions. These eluates were assayed by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956; Staub, 1980). Only one polysaccharide component was found (see Fig. 1). The eluates were combined, and then dialyzed in distilled water. After salt was removed, this solution was concentrated and then freeze-dried. A purified polysaccharide (FMAP) sample was obtained.

2.3. General analysis method of FMAP

Ultraviolet spectra were recorded with a PC-2501 UV/Vis spectrometer (Shimadzu, Japan), and infrared spectra were obtained using a Nexus 470 infrared spectrometer (Thermo Nicolet, USA) with OMNIC 5.2 software; Gas chromatography–mass spectrometry was performed on a QP-55050A instrument (Shimadzu, Japan) equipped with fused silica capillary OV-225 (0.25 mm × 25 m), a temperature program of 170 °C → 230 °C at 2 °C/min followed by an isothermic phase, and MS of the partially methylated alditol acetates was performed at 70 eV. Dialysis was carried out in Spectrapor 3 tubes (molecular weight cutoff = 6000).

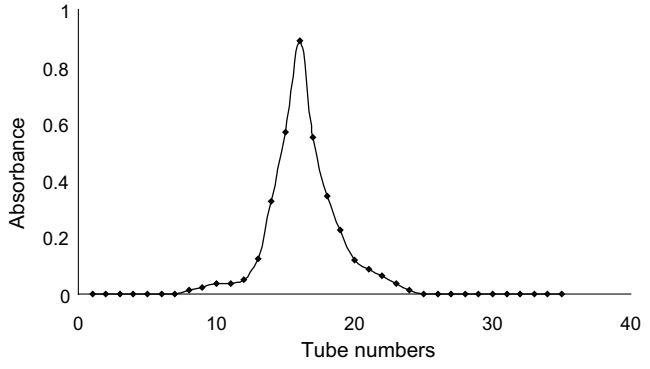

Fig. 1. Elution curve of FMAP by Sephadex G-200 gel filtration.

2.4. Total sugar content

Total sugar content was determined as glucose by the modified phenol–sulfuric acid method (Dubois et al., 1956; Staub, 1980) with D-glucose as a reference.

2.5. Homogeneity and molecular weight

The homogeneity and molecular weight of FMAP were determined by GPC with a Agilent HPLC apparatus (Agilent, USA) equipped with a TSK G-3000 SW column (300 × 7.5 mm), a model 1100 Refractive index detector and a Millennium-32 Workstation (Shen & Perreault, 1998) was used for the calculation of average molecular weights (see Fig. 2). The Dextran standards (T-10, T-40, T-70, T-110, T-500) were used for the calibration curve. The conditions were: column temperature 21 °C; column pressure, 5 Mpa; injection volume, 10 μl; mobile phase, phosphate buffer (pH 6.0); mobile phase flowing rate, 1.0 ml/min; running time, 20 min.

2.6. Total acid hydrolysis

FMAP (10 mg) was hydrolyzed with 2 mol/l H_2SO_4 (3 ml) in a sealed tube for 24 h at boiling water bath. The hydrolyzate was neutralized with $BaCO_3$ and filtered. The filtrate was concentrated, and then analyzed by TLC on silica gel with a solvent system EtOAc/HOAc/*n*-BuOH/H_2O (3:4:5:1, v/v), comparing with monosaccharide standards and stained by aniline/diphenylamine/phosphoric acid at 100 °C.

2.7. Methylation analysis

Eight milligrams of FMAP was methylated by the method of Hakomori (Duenas-Chasco, Rudriguez-Carvajal, & Tejero-Mateo, 1998; Hakomoris, 1964; Harris, Henry, Blakeney, & Stone, 1984).The reaction mixture was dialyzed in moving tap-water and distilled water, then extracted with $CHCl_3$ and the organic solution dried with anhydrous potassium carbonate, then evaporated to dryness. The product showed no band in the region of 3600–3300 cm^{-1} in its IR spectrum. The methylated FMAP was hydrolyzed with 90% formic acid for 6 h at 100 °C and then with 2 mol/l trifluoroacetic acid for 6 h at 100 °C in sealed tubes. The excess acid in the hydrolyzed mixture was evaporated off by co-distillation with methanol. The hydrolysate was then reduced with $NaBH_4$, and the alditolacetate was prepared as usual. The alditol acetate of the methylated sugar was analyzed by GC–MS with a

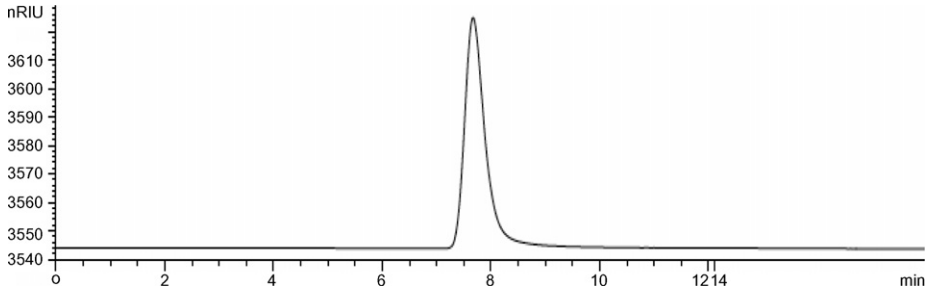

Fig. 2. GPC chromatogram of PMAP on a Angilent HPLC apparatus.

fused silica capillary column. The temperature program started at 150 °C with a 1 min hold, followed by a 3 °C/min gradient to 200 °C, then 5 °C/min gradient to 270 °C and finished with a 15 min hold at 270 °C.

3. Results and discussion

A water-soluble polysaccharide was obtained from *Fructus Mori Albae* by extraction with tap-water three times at 80 °C in 9.28% yield.

FMAP is a brown powder, soluble in water and in Me_2SO. Purification of FAMP was confirmed by sephadex™ G-200 glucan gel column chromatography and gel permeating chromatography, and the results were showed in Figs. 1 and 2. With the Dextran standards, the average molecular weight of the FMAP was obtained as 1.30×10^5. TLC analysis of hydrolizate showed three spots that correspond with D-galactose, D-mannose and D-glucose. So it was concluded that FMAP was consisted of D-galactose, D-mannose and D-glucose. GC–MS data also confirmed it.

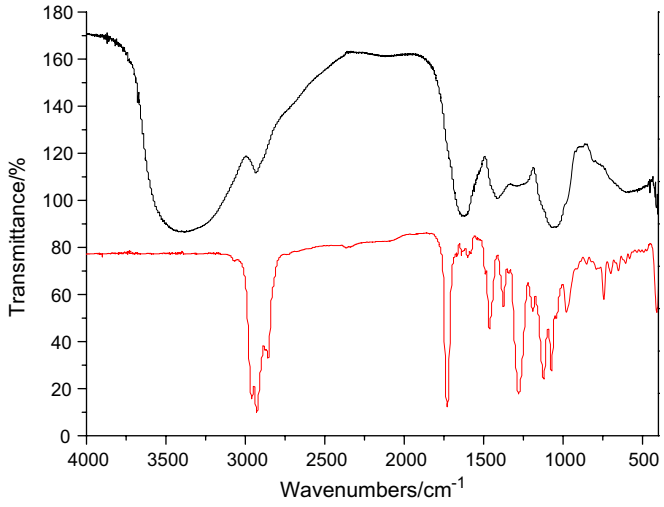

Fig. 3. IR of spectra of FMAP (up) and permethylated FMAP (below).

Absorption at 890 and 1045 cm^{-1} in IR (see Fig. 3) indicated that FMAP had a β-pyranosidic linkages (Miao, Liang, & Zhang, 1993).

The total sugar content was estimated by the phenol–sulfuric acid method (Dubois et al., 1956; Staub, 1980) and was found to be 94.8%.

Methylated FMAP was obtained by repeating the procedure of Hakomori (Duenas-Chasco et al., 1998; Hakomoris, 1964; Harris et al., 1984) three times. Then, the methylated polysaccharide (see Fig. 3) was hydrolyzed with acid, then converted to methylated alditol acetates and analyzed by GC–MS. The results of GC–MS were showed in Table 1. The total content of the galactosyl residues was 28.6% according to ratio of 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-galactitol, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-galactitol in GC–MS analytic results. The content of the mannosyl residues is 65.9% on the base of GC–MS analytic data. The GC–MS results implied that a proportion of monosaccharides in FMAP were in a ratio of D-galactose/D-mannose/D-glucose = 28.6:65.9:5.2. Presence of 1,5-di-acetyl-2,3,4,6-tetra-*O*-methyl-D-mannnitol and 1,5-di-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol indicates that FMAP has 1-linkage of galactosyl and mannosyl terminal residues. The molar ratio of 1,3,5,6,-*O*-acetyl-2,4-di-*O*-methyl-D-mannitol, 1,5,6-tri-acetyl-2,3,4-tri-*O*-methyl-D-galactitol, 1,3,5-tri-acetyl-2,4,6,-tri-*O*-methyl-D-galactitol, and 1,4,5-tri-acetyl-2,3,6-tri-*O*-methyl-D-glucitol were determined to be 39.0:17.6:8.6:5.2

Table 1
The results of methylated analysis of FMAP

No.	Derivatives – partially methylated alditol acetate	Mol %	Retain time	Type of linkage
1	1,3,5-Tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-galactitol	8.6	4.925	1,3-Galp
2	1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-mannitol	26.9	8.258	1-Manp
3	1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol	2.4	8.667	1-Galp
4	1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol	5.2	10.842	1,4-Glcp
5	1,5,6-Tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-galactitol	17.6	11.425	1,6-Galp
6	1,3,5,6-tetra-*O*-Acetyl-2,4-di-*O*-methyl-D-mannitol	39.0	14.325	1,3,6-Manp

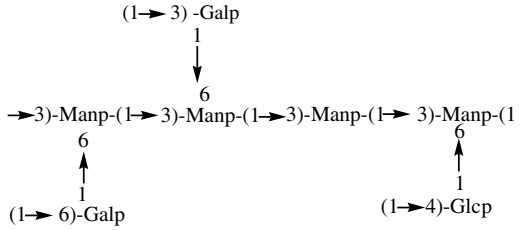

Fig. 4. Linkage of FMAP.

according to the GC data. These results indicated that the polysaccharide has a main chain β-D-(1 → 3)-Manp, and branches β-D-(1 → 6-Galp-β-D-1)-Manp, β-D-(1 → 3-Galp-β-D-1)-Manp and β-D-(1 → 4-Glcp-β-D-1)-Manp, in which Manp is terminal residues, linked to the main chain. The structure of FMAP was shown in Fig. 4.

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