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# Isolation and structural characterization of a polysaccharide FCAP1 from the fruit of *Cornus officinalis*

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ABSTRACT

A water-soluble polysaccharide, FCAP1, was isolated from an alkaline extract from the fruits of *Cornus officinalis*. Its molecular weight was 34.5 kDa. Monosaccharide composition analysis revealed that it was composed of fucose, arabinose, xylose, mannose, glucose, and galactose in a molar ratio of 0.29:0.19:1.74:1:3.30:1.10. On the basis of partial acid hydrolysis and methylation analysis, FCAP1 was shown to be a highly branched polysaccharide with a backbone of β-(1→4)-linked-glucose partially substituted at the O-6 position with xylopyranose residues. The branches were composed of (1→3)-linked-Ara, (1→4)-linked-Man, (1→4,6)-linked-Man, (1→4)-linked-Glc, and (1→2)-linked-Gal. Arabinose, fucose, and galactose were located at the terminal of the branches. The structure was further elucidated by a specific enzymatic degradation with an *endo*-β-(1→4)-glucanase and MALDI-TOF-MS analysis. Oligosaccharides generated from FCAP1 indicated that FCAP1 contained XXXG-type and XXG-type xyloglucan fragments.

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## 1. Introduction

*Cornus officinalis* Sieb. et Zucc. belongs to the family Cornaceae. The fruit has been used as a traditional herbal medicine for more than 2000 years in China for its tonic, analgesic, and diuretic effects.[1] It has also been reported to have anti-microbial, anti-inflammatory, anti-shock, cardiotonic, anti-oxidant, anti-aging, and immunoregulatory activities.[2]

Likewise, polysaccharides isolated from *C. officinalis* were shown to have immunostimulatory,[3] anti-oxidant, and anti-aging effects.[4] Significant differences were reported for the monosaccharide composition and the chemical structure of polysaccharides extracted from *C. officinalis* with hot water. For example, cornus polysaccharide SZYP-2 was reported to be composed of rhamnose, arabinose, and glucose.[5] Cornus polysaccharide Co-4 was reported to be composed of glucose, xylose, galactose, and fucose. Linkage analysis showed that Co-4 had a backbone of (1→4)-linked D-glucose residues with some branching at O-6. The branches were mainly composed of (1→2)-linked D-xylose, terminal xylose, terminal fucose, and (1→2)-linked D-galactose.[6]

In contrast, in our previous work,[7] we characterized a polysaccharide FCP5-A extracted with water (30 °C twice and then 80 °C once) from the fruit of *C. officinalis*. FCP5-A was a branched polysaccharide rich in arabinose with a backbone composed of repeating →4)-GalA-(1→2)-Rha-(1→ and side chains attached to O-4 of some rhamnose residues. The side chains contained highly branched arabinan and short linear (1→3)-linked galactans. The polysaccharide can be categorized to rhamnogalacturonan I (RG-I) of pectin.

In this study, we obtained a water-soluble hemicellulosic polysaccharide with XXXG-type and XXG-type xyloglucans by treating the cellular debris with sodium hydroxide (5% and 10%) after water extraction of *C. officinalis*. The glucan backbone is substituted at C-6 of Glc (G) residues by terminal xylopyranose (X) residues, or β-D-Gal*p*-(1→2)-α-D-Xyl*p* (L), or α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→2)-α-D-Xyl*p* (F) side chains leading to XXLG/XLXG, XLLG, XLFG, LGG, XLG, XFG, and LFG xylogulcan fragments. Its isolation, homogeneity, molecular weight, monosaccharide composition, and structural characterization were investigated.

## 2. Experimental

### 2.1. Materials

The dried pitted mature fruit of *C. officinalis* was purchased from Xi'an Medicinal Materials Cooperation, PR China. It was dried at 50 °C and crushed into a powder.

### 2.2. Analytical methods

The carbohydrate content of the powder was determined by the $H_2SO_4$–phenol method using glucose as a standard. Protein content

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was estimated by the method of Bradford using chicken egg white albumin as standard.[8] A Lambda 25 UV/VIS spectrophotometer (Perkin Elmer instrument) was used to record the absorbance. A UV scan in the region of 200–400 nm was performed on the spectrophotometer. The IR spectrum was determined using an EQUINOX 55 Fourier transform infrared spectrophotometer (Bruker, Germany). Matrix-assisted laser desorption ionization-time of flight mass spectrum (MALDI-TOF-MS) was performed on a Shimadzu/Kratos (Columbia, MD) AXIMA CFR mass spectrometer equipped with a nitrogen laser operating at 337 nm in a reflection mode. Profiling of the molecular ions of the sample was achieved in the positive mode using 2,5-dihydroxybenzoic acid (10 mg/mL) as the matrix solution.

### 2.3. Isolation of cornus polysaccharides

The powder of the fruit of *C. officinalis* was extracted with water (30 °C twice and then 80 °C once).[7] The pectic polysaccharide FCP was obtained as reported in our previous work.[7] The remaining cell debris after the water extraction was first extracted with 5% NaOH at 4 °C for 4 h. After neutralization with 2 N HCl and centrifugation, the debris was further extracted with 10% NaOH at 4 °C for another 4 h, then neutralized, and centrifuged. The two combined alkali extracts were pooled and concentrated with a rotary evaporator at a temperature below 40 °C. A fourfold volume of ethanol was added to the supernatant to precipitate the polysaccharides overnight at 4 °C. The precipitate was pelleted by centrifugation and re-dissolved in distilled $H_2O$. The aqueous solution was treated with Sevag reagent to remove protein and then was dialyzed against distilled water for 48 h, concentrated under reduced pressure, and lyophilized. A black crude polysaccharide, CAP, was then obtained.

### 2.4. Decoloring

CAP was dissolved in distilled water at 40 °C with continuous stirring and the solution was adjusted to pH 8.8 with aqueous ammonia. $H_2O_2$ (30%) was then added drop-wise until the color faded. After stirring for another 4 h, the color turned primrose yellow. The solution was neutralized with 1 N HCl, followed by dialysis against distilled water and lyophilization.

### 2.5. Purification of FCAP1

Decolored CAP (373 mg) was dissolved in water and fractionated on DEAE–Cellulose DE-52 anion-exchange column (5.0 cm × 40 cm, $HCO_3^-$ form), eluted first with distilled water, and then with a step gradient (0.1, 0.25, and 0.5 mol/L $NaHCO_3$) at a flow rate of 1 mL/min, monitored spectrophotometrically at 485 nm using the $H_2SO_4$–phenol method. After pooling, dialysis, and lyophilization, the fractions were designated as CAP1 (yields 25.20%), CAP2 (yields 6.43%), CAP3 (yields 11.53%), and CAP4 (yields 14.75%) separately. CAP1 (60 mg) was further purified with gel permeation chromatography on a Bio-Gel P-30 column using 0.1 mol/L NaCl solution as an eluent. One fraction was pooled according to the elution profile. After dialysis and lyophilization, a white fluffy polysaccharide FCAP1 (yields 73.33%) was obtained.

### 2.6. Homogeneity and molecular weight determination

The homogeneity and molecular weight of FCAP1 were determined by high performance gel permeation chromatography (HPGPC) on TSK-Gel G3000SW column (7.5 mm × 300 mm). A Waters 2695 high performance liquid chromatography (HPLC) coupled with Waters Alliance 2414 refractive index detector was used. The temperature of the column and the detector was kept at 30 °C. The sample concentration was 0.2% (w/v), and its injection volume was 20 μL. The eluent was the mixture of 0.1 mol/L phosphate buffer (pH 6.0) and 0.1 mol/L $Na_2SO_4$, passed through Millipore filters (0.45 μm). The flow rate was 0.5 mL/min. The linear regression was calibrated with dextrans 80,000, 48,600, 25,000, 11,600, and 5,2000. $V_t$ and $V_0$ were calibrated with glucose and dextran blue (2,000,000), respectively.

### 2.7. Monosaccharide analysis

The sample (2 mg) was dissolved in 2 mL of 2 mol/L trifluoroacetic acid (TFA) in a screw-capped vial, filled with nitrogen, and hydrolyzed at 120 °C for 2 h. The hydrolyzate was evaporated with a rotary evaporator. Neutral sugars and uronic acids were simultaneously detected by GC using the method described previously.[9] GC was performed by a Shimadzu GC2010 equipped with a capillary column of rtx-50 (30.0 m × 0.25 mm × 0.25 am). The temperature program was: 180 °C for 2 min, then to 210 °C at 6 °C/min, then to 215 °C at 0.3 °C/min, then to 240 °C at 6 °C/min for 30 min. Nitrogen was used as the carrier gas at 0.6 mL/min.

### 2.8. Partial acid hydrolysis

FCAP1 (25 mg) was dissolved in 10 mL of 0.1 mol/L TFA and heated at 100 °C for 2 h. The solution was evaporated to dryness, then re-dissolved in distilled water and dialyzed for 48 h. Both dialyzable and non-dialyzable samples were lyophilized. The non-dialyzable fraction was further purified on a Sephadex G-25 column, giving the partially hydrolyzed polysaccharide FCAP1-I; the dialyzable oligomers were freeze-dried and designated as FCAP1-O. Sugar compositions of FCAP1-I and FCAP1-O were analyzed by GC. The structure of FCAP1-I was further analyzed by methylation.

### 2.9. Methylation analysis

Both the samples of FCAP1 and FCAP1-I were methylated using modified Ciucanu method as described previously.[10] The methylation procedure was repeated three times. Next, the samples were hydrolyzed with 2 mol/L TFA (121 °C, 2 h), then reduced with sodium borohydride, and acetylated to convert into their partially methylated alditol acetates. The resulting alditol acetates were analyzed by GC and GC–MS. GC–MS was performed using a Shimadzu instrument GCMS-QP2010 equipped with an electron impact ion source (ionization energy 70 eV). The capillary column used was rtx-5 ms (30 m × 0.25 mm × 0.25 μm); the temperature program was: 140 °C for 2 min, then to 250 °C at 2 °C/min for 20 min. Helium was used as a carrier gas and the flow rate was 0.6 mL/min. The temperatures of the interface and the ion source were 200 °C and 250 °C, respectively.

### 2.10. Enzymatic hydrolysis

FCAP1 (7 mg) was dissolved in sodium acetate buffer (50 mM, pH 5.0) containing 40 unit of *endo*-β-(1→4)-D-glucanase (EC 3.2.1.4 from *Aspergillus niger*). The solution was incubated at 37 °C for 48 h. The digest was precipitated with four volumes of anhydrous ethanol and centrifuged. The resulting supernatant was concentrated and lyophilized giving a mixture of oligosaccharides P1-XGose. A glucanase-resistant fraction, designated as P1-GRF, was obtained by lyophilization of ethanol-insoluble residues. Monosaccharide composition of P1-XGose and P1-GRF was analyzed by GC. P1-XGose was further analyzed by MALDI-TOF mass spectrum.

## 3. Results and discussion

### 3.1. Isolation and purification of cornus polysaccharides

Extraction of the cellular debris of *C. officinalis* with alkali yielded a crude polysaccharide, CAP. The carbohydrate content of CAP was 85.3% according to the $H_2SO_4$–phenol method. Monosaccharide analysis by GC showed that it was composed of fucose, arabinose, xylose, mannose, glucose, galactose, and trace of rhamnose in a molar ratio of 0.20:0.66:2.12:1:2.37:0.98.

CAP was a black polysaccharide in that the pigment could not be separated by column chromatography. To avoid the influence of pigment during the structure analysis, decoloring was performed with 30% $H_2O_2$. Decolored CAP was purified by anion exchange chromatography, yielding four polysaccharide subfractions CAP1, CAP2, CAP3, and CAP4 (Fig. 1). CAP1 was the dominant sub-fraction accounting for 44.3% of the total polymers recovered from the anion exchanger. CAP1 was further purified by gel permeation chromatography on a Bio-Gel P-30 column. As shown in Figure 2, one sub-fraction, FCAP1, was obtained that accounted for 0.29% of the crude herb.

### 3.2. Monosaccharide composition and character of FACP1

FCAP1 was a water-soluble, white, and fluffy polysaccharide. Its monosaccharide composition is summarized in Table 1. FCAP1 mainly consisted of fucose, arabinose, xylose, mannose, glucose, and galactose in a molar ratio of 0.29:0.19:1.74:1:3.30:1.10, and uronic acid was not detected. As determined by a colorimetric method, the total carbohydrate content was 91.3%, and protein was absent (0%). A UV scan in the region of 200–400 nm showed strong absorbance at about 200 nm but no distinct absorbance at 280 nm, which further indicated that the polysaccharide did not contain protein.

In the FT-IR spectrum, signals in the regions of 3600–3200 $cm^{-1}$, 2926 $cm^{-1}$, 1642 $cm^{-1}$ were due to O–H stretching vibration, C–H stretching vibration, and associated water, respectively. The weak absorbance at 895 $cm^{-1}$ suggested that pyranoses existed in the β-configuration.[11] The IR spectrum showed no specific signature of uronic acid in consistence with the data of GC analysis.

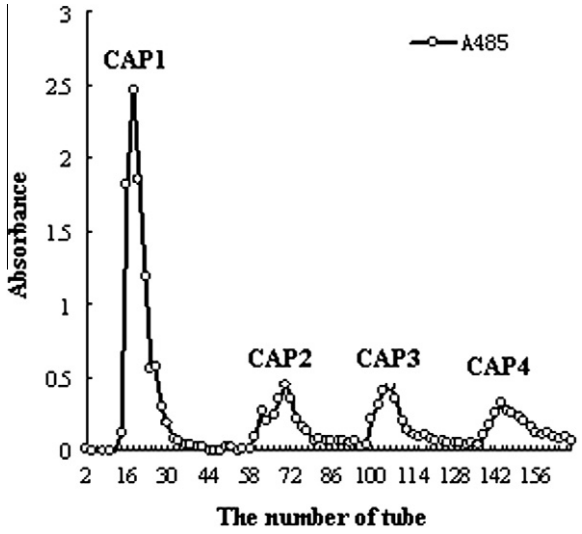


**Figure 1.** Elution profile of the decolored polysaccharide CAP from *Cornus officinalis* on a DEAE–cellulose column ($HCO_3^-$ form). CAP1, CAP2, CAP3, and CAP4 were obtained by eluting with $H_2O$, 0.1, 0.25, and 0.5 mol/L $NaHCO_3$, respectively. $A_{485}$ was determined by phenol–sulfuric acid method.

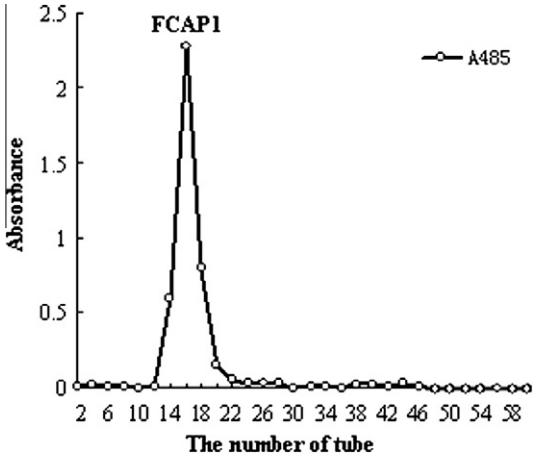


**Figure 2.** Elution profile of CAP1 on a Bio-Gel P-30 column. FCAP1 fraction was obtained by eluting with 0.1 mol/L NaCl solution.

### 3.3. Homogeneity and molecular weight

The homogeneity and molecular weight of FCAP1 were determined by high performance gel permeation chromatography (HPGPC). FCAP1 was eluted with a mixture of 0.1 mol/L phosphate buffer (pH 6.0) and 0.1 mol/L $Na_2SO_4$. The single symmetrical peak indicated that the polymer was homogeneous. Based on the calibration with standard dextrans, the average molecular weight ($M_W$) of the sample was estimated to be 34.5 kDa.

### 3.4. Partial acid hydrolysis

FCAP1 was partially hydrolyzed with 0.1 mol/L TFA. The dialyzable fraction FCAP1-O contained fucose, arabinose, xylose, mannose, glucose, and galactose (Table 1), indicating that they were present on the outer chains. The non-dialyzable fraction was further fractionated on a Sephadex G-25 column and a sub-fraction FCAP1-I was obtained. Based on HPGPC, its molecular weight was 16.6 kDa. FCAP1-I was composed of xylose, glucose, and galactose in a molar ratio of 4.6:9.6:1 (Table 1). The monosaccharide composition of FCAP1-I indicated that xylose, glucose, and galactose were present as the main chain or brink of the main chain. FCAP1-I had no arabinose, fucose, or mannose suggesting that all arabinose, fucose, and mannose were present on the branches of the polysaccharide.

### 3.5. Methylation analysis

Methylation of FCAP1 and FCAP1-I was performed three times to obtain fully methylated polysaccharides. After hydrolysis and alditol acetate derivatization, the partially methylated alditol acetates were analyzed by GC and GC–MS. Gas chromatography of FCAP1 and FCAP1-I is shown in Figure 3. Peaks of methylated sugars were identified by their retention times and mass spectra. Their relative molar ratios were estimated from the peak areas of GC and corresponding response factors.[12] According to their molecular weight and monosaccharide composition, the total molar ratio of FCAP1 was 2.1 times higher than that of FCAP1-I. The results are shown in Table 2.

Data from the methylation analysis indicated that FCAP1 had a highly branched structure. The amount of total terminal sugars was approximately equal to that of branched sugars, indicating the completeness of methylation. The non-reducing termini were composed of arabinose, xylose, fucose, and galactose. Branches were present on glucosyl residues and on a trace of mannosyl res-

**Table 1**
Sugar composition of CAP, FCAP1, and their derivatives from partial hydrolysis with acid and enzymatic hydrolysis

| Polysaccharide fraction | Sugar composition (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Rhamnose | Fucose | Arabinose | Xylose | Mannose | Glucose | Galactose |
| CAP | 0.8 | 2.7 | 8.9 | 28.7 | 13.6 | 32.1 | 13.2 |
| FCAP1 | — | 3.8 | 2.5 | 22.8 | 13.1 | 43.4 | 14.4 |
| FCAP1-I | — | — | — | 30.5 | — | 62.9 | 6.6 |
| FCAP1-O | — | 8.2 | 5.0 | 19.7 | 19.5 | 26.3 | 21.3 |
| P1-XGose | — | 3.7 | 2.1 | 19.4 | 12.4 | 46.1 | 16.3 |
| P1-GRF | — | 1.5 | 4.1 | 27.9 | 17.4 | 36.5 | 12.6 |

—: Not detected.

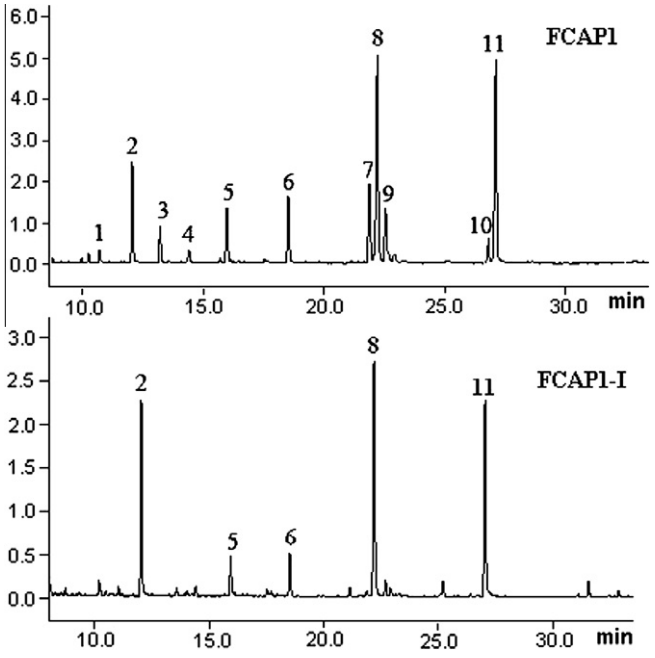


**Figure 3.** Gas chromatography of partially methylated alditol acetates of FCAP1 and FCAP1-I. Column: rtx-5 ms (30 m × 0.25 mm × 0.25 μm); temperature: 140 °C (2 min)–2 °C/min, 250 °C (20 min). (1) 2,3,5-$Me_3$-Ara; (2) 2,3,4-$Me_3$-Xyl; (3) 2,3,4-$Me_3$-Fuc; (4) 2,5-$Me_2$-Ara; (5) 3,4-$Me_2$-Xyl; (6) 2,3,4,6-$Me_4$-Gal; (7) 2,3,6-$Me_3$-Man; (8) 2,3,6-$Me_3$-Glc; (9) 3,4,6-$Me_3$-Gal; (10) 2,3-$Me_2$-Man; (11) 2,3-$Me_2$-Glc.

**Table 2**
The results of methylation analysis of FCAP1 and FCAP1-I

| Peak number | Methylated sugar | Deduced linkage | Molar ratio | |
|---|---|---|---|---|
| | | | FCAP1 | FCAP1-I |
| 1 | 2,3,5-$Me_3$-Ara | Ara-(1- | 2 | — |
| 2 | 2,3,4-$Me_3$-Xyl | Xyl-(1- | 10 | 10 |
| 3 | 2,3,4-$Me_3$-Fuc | Fuc-(1- | 4 | — |
| 4 | 2,5-$Me_2$-Ara | -3)-Ara-(1- | 1 | — |
| 5 | 3,4-$Me_2$-Xyl | -2)-Xyl-(1- | 8 | 3 |
| 6 | 2,3,4,6-$Me_4$-Gal | Gal-(1- | 10 | 3 |
| 7 | 2,3,6-$Me_3$-Man | -4)-Man-(1- | 9 | — |
| 8 | 2,3,6-$Me_3$-Glc | -4)-Glc-(1- | 22 | 17 |
| 9 | 3,4,6-$Me_3$-Gal | -2)-Gal-(1- | 6 | — |
| 10 | 2,3-$Me_2$-Man | -4,6)-Man-(1- | 4 | — |
| 11 | 2,3-$Me_2$-Glc | -4,6)-Glc-(1- | 24 | 14 |

—: Not detected.

idues. 2,3,6-$Me_3$-Glc and 2,3-$Me_2$-Glc accounted for 46% of total methylated sugars suggesting that the backbone was composed of (1→4)-linked-Glc, with substitutions at O-6 of glucose. The xylosyl residues were composed of 2,3,4-$Me_3$-Xyl and 3,4-$Me_2$-Xyl residues, which suggested that xylose was present in the pyranose form. The galactosyl residues were composed of terminal- and (1→2)-linked galactose. The fucosyl residues were present at the non-reducing termini. In addition, the mannosyl residues were composed of (1→4)-linked and (1→4),6-linked mannose, which suggested that mannose was probably existed as (1→4)-linked mannose, with branches at O-6.

FCAP1-I showed only five peaks by GC, which were identified as 2,3,4-$Me_3$-Xyl, 3,4-$Me_2$-Xyl, 2,3,4,6-$Me_4$-Gal, 2,3,6-$Me_3$-Glc, and 2,3-$Me_2$-Glc. Termini were composed of xylose and galactose. 2,3,6-$Me_3$-Glc and 2,3-$Me_2$-Glc accounted for 65% of total methylated sugars of FCAP1-I, which indicated that the backbone of FCAP1-I was composed of (1→4)-linked-Glc, with branches attached to O-6 of some residues. The amount of (1→2)-linked-Xyl was equal to that of terminal galactose. It can be deduced from the results that the structure of FCAP1-I was as follows (Fig. 4).

Compared with FCAP1, FCAP1-I had no terminal Ara, (1→3)-linked-Ara, terminal Fuc, (1→4)-linked-Man, (1→4,6)-linked-Man, and (1→2)-linked-Gal, suggesting that they existed as branches. The number of terminal Xyl did not change, while the number of (1→2)-linked-Xyl, terminal Gal, (1→4)-linked-Glc, and (1→4,6)-linked-Glc was decreased. The decrease of (1→2)-linked-Xyl, terminal Gal, and (1→4,6)-linked-Glc was probably due to Gal-(1→2)-Xyl*p*(1→ substituted at O-6 of glucose was hydrolyzed with 0.1 mol/L TFA, thus (1→4,6)-linked-Glc converted to (1→4)-linked-Glc. The decrease of (1→4)-linked-Glc indicated that some (1→4)-linked-Glc was located at branches.

From the analysis above, it can be concluded that FCAP1 is a branched polysaccharide. The backbone is composed of β-(1→4)-linked-glucose, with some substitution at O-6 position. Xylose is linked directly to the backbone. The branches are composed of (1→3)-linked-Ara, (1→4)-linked-Man, (1→4,6)-linked-Man, (1→4)-linked-Glc, and (1→2)-linked-Gal. Arabinose, fucose, and galactose are at the terminal of the branches.

### 3.6. Enzymatic hydrolysis

It is known that *endo*-β-(1→4)-D-glucanase cleaves (1→4)-β-glucosidic linkages of xyloglucan next to an unbranched glucose residue without damaging the side chains.[13] FCAP1 was treated with an *endo*-β-(1→4)-D-glucanase and oligomers were generated, further indicating that FCAP1 had a backbone of β-(1→4)-linked-glucose. The hydrolysate was precipitated with ethanol and the supernatant, a mixture of oligosaccharides, was designated as P1-XGose, which was a mixture of oligosaccharides generated from the enzymatic hydrolysis. The precipitate fraction, which was the

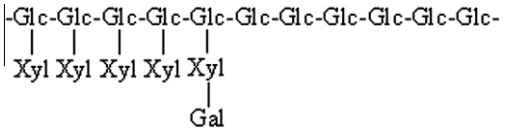


**Figure 4.** Structural unit of FCAP1-I.

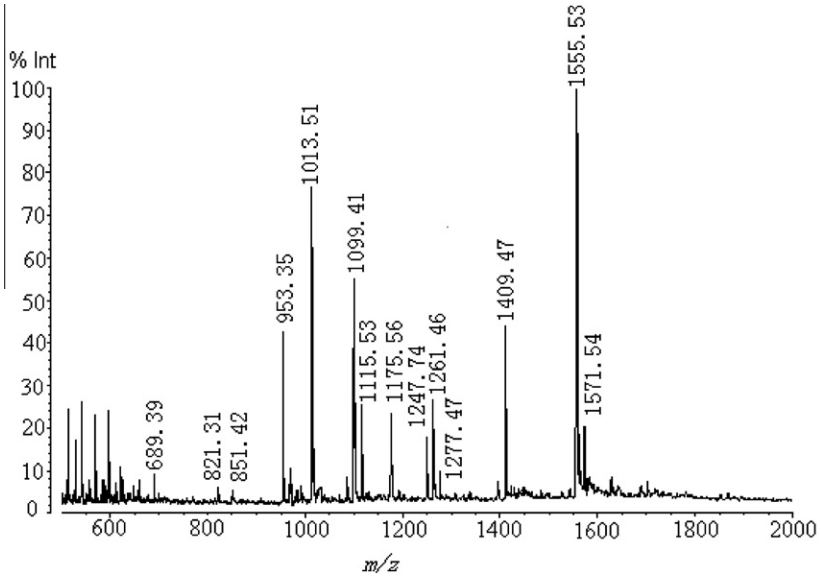


**Figure 5.** MALDI-TOF mass spectrum of oligosaccharides in P1-Xgose.

enzyme-resistant polymeric material, was designated as P1-GRF. Sugar analysis of P1-XGose and P1-GRF is summarized in Table 1.

Xylose and glucose are the major sugars of the P1-XGose in the ratio of 1:2.4. suggesting that FCAP1 is a polysaccharide rich in xyloglucan that could be digested by *endo*-β-(1→4)-D-glucanase. The presence of fucose and galactose in P1-XGose suggested that xyloglucan had fucose and galactose-containing side chains. Mannose was present in P1-XGose. It was likely that the *endo*-β-(1→4)-D-glucanase contains contaminating mannanase activity responsible for mannan hydrolysis.[14] The enzyme-resistant fraction P1-GRF had a similar sugar composition as FCAP1. The presence of xylose and glucose indicated that a part of the xyloglucan was resistant to *endo*-β-(1→4)-D-glucanase.

MALDI-TOF-mass spectrometry is a convenient tool for the structural analysis of highly branched xyloglucan mixtures as it is sensitive in mixture analysis. The MALDI-TOF mass spectrum of P1-XGose is shown in Figure 5. As shown in the figure, the mass spectrum of P1-XGose revealed numerous peaks of oligosaccharide fragments. Structures of the xyloglucan-derived oligomers were proposed, taking into consideration the specificity and mode of action of the *endo*-β-(1→4)-D-glucanase, sugar composition and glycosyl linkage data, and molecular masses of the known xyloglucan oligosaccharides.

Ions at *m/z* 1247.74, 1409.47, and 1555.53, corresponding, respectively, to $[M+Na]^+$ adducts of $Hex_5Pent_3$, $Hex_6Pent_3$, and $Hex_6Pent_3dHex$, can be assigned as XXLG (or XLXG), XLLG, and XLFG fragments of the XXXG-type xyloglucan,[15] respectively, (X: α-D-Xyl*p*-(1→6)-β-D-Glc*p*; L: β-D-Gal*p*-(1→2)-α-D-Xyl*p*-(1→6)-β-D-Glc*p*; G: β-D-Glc*p*; F: α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→2)-α-D-Xyl*p*-(1→6)-β-D-Glc*p*). The high intensity of XLFG indicated that the xyloglucan was fucosylated. Ions at *m/z* 821.31, 953.35, 1099.41, and 1261.46, corresponding, respectively, to $[M+Na]^+$ adducts of $Hex_4Pent$, $Hex_4Pent_2$, $Hex_4Pent_2dHex$, and $Hex_5Pent_2dHex$, can be assigned as LGG, XLG, XFG, and LFG, respectively. These structures can be ascribed to the XXG-type xyloglucan.[16] In addition, sodium adduct ions of hexose polymers ranging from four to seven (*m/z* 689.39, 851.42, 1013.51, and 1175.56) were also observed. P1-XGose contain high amount of hexose residues of mannose, galactose, and glucose, and the branches of FCAP1 contain abundant mannose, galactose, and glucose. Thus these peaks probably originated from oligosaccharides on side chains composed of mannan, galactose, and glucose.

## 4. Conclusion

Based on the methylation analysis, partial acid hydrolysis, enzymatic hydrolysis, GC–MS, and MALDI-TOF-MS analysis, we conclude that FCAP1 is a highly branched xyloglucan with a backbone of β-(1→4)-linked-glucopyranose residues. The backbone was partially substituted at O-6 of glucosyl residues with Xyl*p*-(1→, Gal*p*-(1→2)-Xyl*p*-(1→ and Fuc*p*-(1→2)-Gal*p*-(1→2)-Xyl*p*-(1→. The branches also contained →3)-Ara-(1→4)-Glc-(1→4)-Man(1→4,6)-Man-(1→, Ara-(1→ and Gal-(1→. Herein we also report, for the first time, the degradation of xyloglucan from *C. officinalis* with *endo*-β-(1→4)-D-glucanase.

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## References

1. Hsu, H. Y.; Peacher, W. G. *Chinese Herb Medicine and Therapy*; Oriental Healing Arts Institute of USA, 1982. p 19.
2. Zhou, J. H.; Li, C. S.; Li, D. D. *Chin. J. New Drug* **2001**, *10*, 808–811.
3. Miao, M. S.; Fang, X. Y.; Yang, Y.; Sun, Y. H. *Henan Tradit. Chin. Med.* **2002**, *22*, 24–25.
4. Miao, M. S.; Yang, Y. T. *Henan Tradit. Chin. Med.* **2002**, *22*, 66–67.
5. Chen, R. X.; Xu, G. Y.; Zhang, R.; Xu, H. Z.; Liu, Y. M.; Lu, M. *J. Instrum. Anal.* **2002**, *21*, 68–69.
6. Yang, Y.; Liu, C. P.; Wang, Y. M. *China Acad. J.* **1999**, *24*, 614–616.
7. Yang, L. Y.; Huang, L. J.; Wang, Z. F.; Cao, C. Y.; Sun, W. J. *Chem. J. Chin. Univ.* **2008**, *29*, 936–940.
8. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 48–54.
9. Lehrfeld, J. *Anal. Chem.* **1985**, *57*, 346–348.
10. Needs, P. W.; Swlvendran, R. R. *Carbohydr. Res.* **1993**, *245*, 1–10.
11. Zhang, W. J. *Techniques of Glycoconjugates in Biochemistry*; Zhejiang University Press: Hangzhou, 1994. pp 271–279.
12. Sweet, D. P.; Shapiro, R. H.; Albersheim, P. *Carbohydr. Res.* **1975**, *40*, 217–225.
13. Fry, S. C. *J. Exp. Bot.* **1989**, *40*, 1–11.
14. Mazumder, S.; Lerouge, P.; Loutelier-Bourhis, C.; Driouich, A.; Ray, B. *Carbohydr. Polym.* **2005**, *59*, 231–238.
15. Huisman, M. M. H.; Weel, K. G. C.; Schols, H. A.; Voragen, A. G. J. *Carbohydr. Polym.* **2000**, *42*, 185–191.
16. Hilz, H.; Jone, L. E.; Kabel, M. A.; Verhoef, R.; Schols, H. A.; Voragen, A. G. J. *Carbohydr. Res.* **2007**, *342*, 170–181.