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Note

Water-soluble polysaccharides from finger citron fruits (*Citrus medica* L. var. *sarcodactylis*)

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ABSTRACT

Four water-soluble polysaccharides, **FCp-1**, **FCp-2**, **FCp-3**, and **FCp-4** were obtained from finger citron fruits (*Citrus medica* L. var. *sarcodactylis*) by hot-water extraction and ethanol precipitation, followed by routine separation procedure. Based on the calibration curve, molecular weights of them were estimated to be 113.9, 32.6, 140.3, and 177.1 kDa respectively. The acid hydrolysis, methylation, IR, GC–MS, and NMR experiments were used for composition analysis. **FCp-1** was a heteropolysaccharide composed of arabinose, galactose, glucose, rhamnose, and xylose, with a molar ratio of 3.0:7.0:4.1:1.0:1.5. **FCp-2** and **FCp-4** were →4)-α-D-Gal*p*A(1→ linking galacturonan differ in molecular weights. **FCp-3** was a →6)-α-D-Glc*p*(1→ linking glucan. According to the results of in vitro assays, **FCp-3** showed significantly and moderately enhancing capacities toward the proliferation of splenocytes and thymocytes respectively. Thus, **FCp-3** or analogs may have further use as immunomodulatory agents.

The finger citron (*Citrus medica* L. var. *sarcodactylis* Hort) (FC) belongs to the family *Rutaceae*, and is widely cultivated in Oriental countries. FC fruit is used in traditional Chinese medicine as adjuvants in the treatment of a variety of chronic diseases, including hypertension and respiratory tract infections,[1,2] and are also consumed as functional foods, being considered beneficial to liver, pancreatic, and stomach function.[3] Previous studies have shown FC fruit contains significant quantities of essential oil, with this material possessing antioxidant activity that could possibly be commercially exploited,[3,4] and which have also demonstrated insulin secretagogue and anti-inflammatory activities.[5] However, a detailed analysis of the structural and biological properties of polysaccharides derived from FC fruit has yet to be reported, except for research on extraction methods of crude polysaccharide.[3] Given the presence and diverse biological activities of polysaccharides throughout nature,[6–11] and their use in many healthcare products, we decided to investigate the polysaccharide profile of FC fruit. In this paper, we report the fractionation (Fig. 1), compositional analysis, structural characterization, and immunological activity of the polysaccharides (Fig. 2) from the FC fruit.

1. Experimental

1.1. General procedures

Ultraviolet–visible (UV–vis) spectra were recorded on a SHIMADZU UV-1800 UV–vis spectrophotometer. IR spectra were recorded from KBr pellets in the 4000–400 cm^{-1} range on a Bruker Alpha-T FT-IR instrument. ^{1}H and ^{13}C NMR spectra of 50 mg compound samples were recorded at 25 °C in D_2O on a Bruker 500 MHz spectrometer. Chemical shifts (δ) are reported in ppm relative to Me_4Si (δ_H 0.0). Gas chromatography–mass spectrometry (GC–MS) analyses were performed on a Thermo Fisher Scientific TRACE DSQ Single Quadrupole GC–MS instrument, using an Agilent HP-5 column (30 m × 0.25 mm i.d). The ionization potential was 70 eV, and the temperature of the ion source was 200 °C.

1.2. Plant material

Fresh FC fruits (*Citrus medica* L. var. *sarcodactylis* Hort) were collected from Jinhua, Zhejiang Province, China, in March 2012, and identified by Dr. Ende Liu (Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan Province, China).

1.3. Extraction and isolation of polysaccharides FCp-1, FCp-2, FCp-3, and FCp-4

Whole FC fruit (1.2 kg) was ground to a pulp, and the resulting material was divided into three portions. Each portion was treated with deionized water (2.0 L), and the resulting mixtures were boiled at 100 °C for 3 h and then filtered. The combined filtrates were concentrated to dryness under reduced pressure at 50 °C. The residue (210 g) was suspended in deionized water (800 mL) and washed successively with petroleum ether, EtOAc and finally *n*-BuOH. The remaining aqueous layer was concentrated to a volume of 200 mL under reduced pressure at 50 °C, and the resulting

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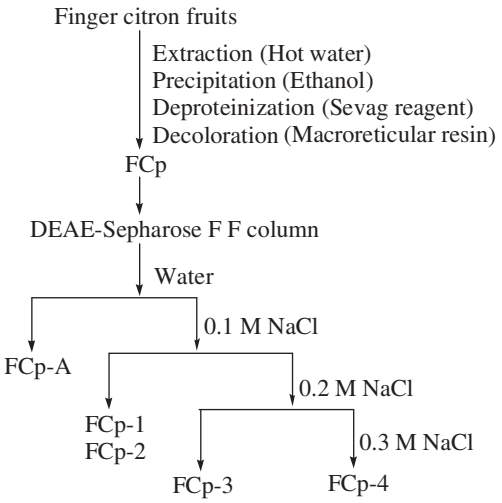

Figure 1. Fractionation procedure of the polysaccharides.

solution was added to 95% aqueous EtOH (3.0 L). This mixture was left to stand for 12 h, and the resulting crude polysaccharide precipitate was collected by filtration. This precipitate was freeze-dried to yield a crude polysaccharide mixture (18.5 g), a portion (10 g) of which was then re-dissolved in deionized water (300 mL). This solution was deproteinated by treatment with Sevag reagent (chloroform/*n*-butanol at a ratio of 4:1, v/v), according to literature methods.[12] The deproteinated solution was decolorized by passing through a macroreticular anion-exchange resin column (D315) and washed with double column volume deionized water, and the resulting solution was concentrated to a volume of 100 mL.[13] This solution was dialyzed in 3500 Da MWCO (molecular weight cut-off) tubing at 37 °C for 48 h, and the solution remaining within the dialysis membrane was freeze-dried to yield polysaccharide (**FCp**; 5.5 g).

A sample of **FCp** (1 g) was dissolved in deionized water (5 mL), and the mixture was centrifuged. The supernatant was separated, and then loaded onto a DEAE-Sepharose Fast Flow (Pharmacia) chromatography column (2.6 × 40 cm), and eluted successively with deionized water, 0.1 M NaCl, 0.2 M NaCl, and 0.3 M NaCl (each 300 mL). The proportion of polysaccharide in each of these eluents was determined by the phenol–sulfuric acid colorimetric method.[14] This analysis revealed that the desired polysaccharide resided in the three NaCl eluents, and dialyzed in 3500 Da MWCO tubing in deionized water at 37 °C for 48 h, respectively. The solution remaining within the dialysis membrane was then concentrated to dryness under reduced pressure, and the residue freeze-dried to give three crude solid. These materials were further purified on a Sephadex G-100 (Pharmacia) gel-filtration chromatography column (2.6 × 40 cm) using 0.01 M NaCl as an eluent. Appropriate fractions were combined, dialyzed, and freeze-dried as before, to yield four homogeneous polysaccharides, denoted **FCp-1** (56 mg), **FCp-2** (84 mg), **FCp-3** (64 mg), and **FCp-4** (220 mg) (Fig. 2).

1.4. Determination of purity and molecular weight of FCp-1, FCp-2, FCp-3, and FCp-4 by high-performance gel-permeation chromatography (HPGPC)

HPGPC was used to determine the purity of **FCp-1**, **FCp-2**, **FCp-3**, and **FCp-4**. Analyses were conducted on a Waters 515 instrument (Milford, PA, USA) equipped with a Waters 2410 refractive index detector and a TSKgel G4000SWxl (Tosoh Biosep, Japan) size-exclusion analytical column (7.8 × 300 mm), using deionized water as the mobile phase and with a sample concentration of 3 mg/mL. T-series dextran standards (Sigma–Aldrich) with defined molecular masses ranging from 10 to 300 kDa were used to calibrate the HPGPC system. All data obtained were collected and analyzed using the Waters Millennium®32 software package.

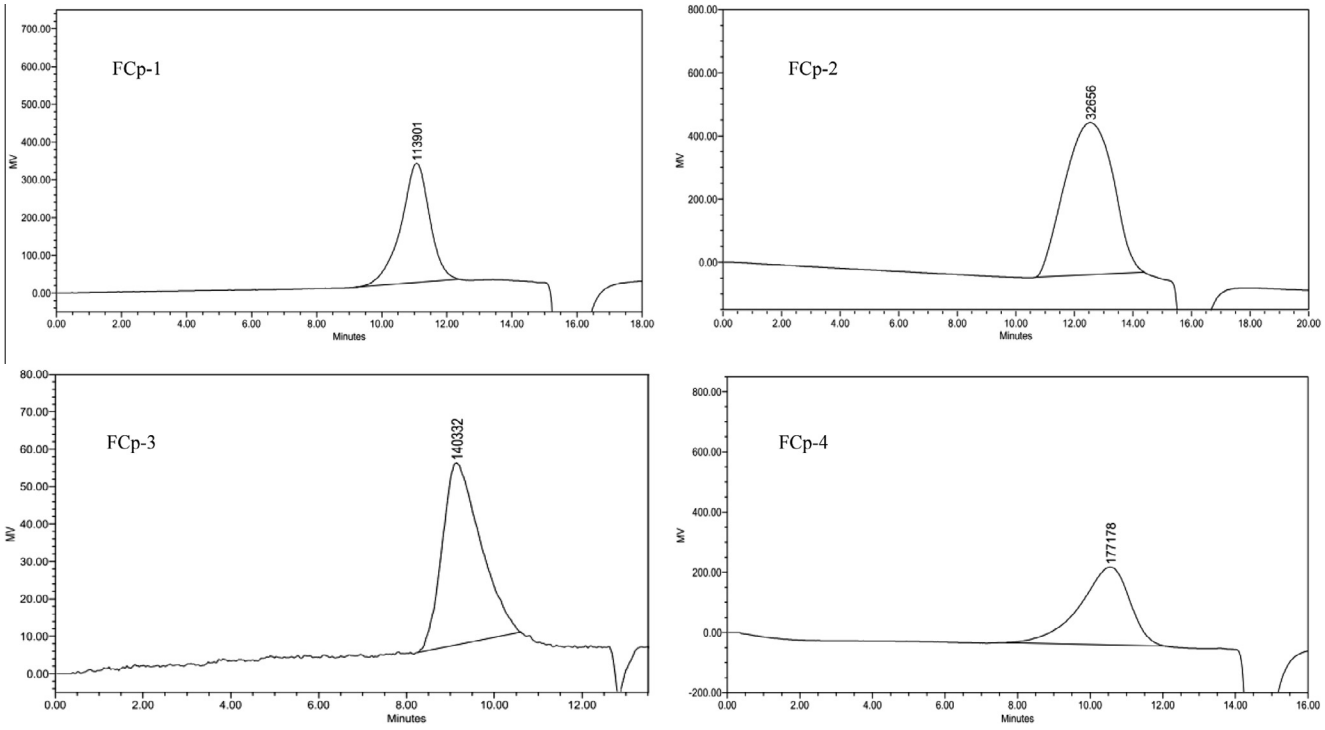

Figure 2. HPGPC profiles for obtained polysaccharides **FCp-1** (PD = 1.16), **FCp-2** (PD = 1.06), **FCp-3** (PD = 1.22), and **FCp-4** (PD = 1.41). (PD = Mw/Mn; PD, polydispersity; Mw, weight-average molecular mass; Mn, number-average molecular mass).