Efficient polysaccharides from *Crinum asiaticum* L.'s structural characterization and anti-tumor effect

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**Abstract**

In this study, an efficient polysaccharide, named CAL-n (*Crinum asiaticum* L.-n) was isolated and purified from *Crinum asiaticum* L for the first time, Mw(molecular weight) of 730,000 Da. CAL-n comprised Rha (rhamnose), Sor(sorbose), Gal(galactose) and Glu(glucosein) the molar ratio of 1:61.6:1.66:4.74. The chemical structure of CAL-n was studied by Infrared spectrum and GC–MS(Gas Chromatography–Mass Spectrometer) analysis. Experimental results reflected, that the backbone of CAL-n comprised (1→2), (1→6), (1→3) beta-pyran glycoside bond, without (1→4) beta-pyran glycoside bond. In addition, an MTT assay indicated that the growth of HepG2 cells was affected by CAL-n, with a concentration dependent ration. The results indicated that CAL-n should be explored as anti-tumor activities in vivo.

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**2. Materials and methods**

**2.1. Materials and reagents**

The crude CAL-n was extracted from the dried fresh seeds *Crinum asiaticum* L. which was purchased from Hainan Province, China. HepG2 cells were provided by Cell Bank of Institute (Shanghai, China). MTT, gluan, monosaccharide (glucose, sorbose and so on) were bought from Sigma Chemical Co. (St. Louis, MO, USA).
Fetal bovine serum, RPMI-1640 media were bought from Gibco/Invitrogen (Grand Island, NY, USA). Others chemicals were analytical grade (see Fig. 4).

2.2. CAL-n’s primary structural characterization

2.2.1. Determination of relative molecular mass

1 ml of double distilled water to dissolve CAL-n 2 mg purified polysaccharide, formulated as a 2 mg/ml concentration of the polysaccharide solution was analyzed by liquid chromatography under the same conditions, record retention time, to obtain samples of the elution volume polysaccharides (Cetindag et al., 2019; Alnaim and Almaz, 2017), by formula obtained Kav, then Kav polysaccharide sample values into a standard curve obtained l g Mw, finally calculated molecular weight polysaccharide test sample (see Fig. 5).

2.2.2. Ultraviolet spectrometry

Dissolve CAL-n purified polysaccharide in double distilled water to prepare a concentration of 1 mg/ml solution, continuous scanning in the UV spectral range of 700–190 nm, detection at 260 nm and 280 nm whether absorption of proteins and nucleic acids (see Fig. 6).

2.2.3. IR (Infrared absorption spectrum) analysis

Weigh CAL-n 2 mg, a polysaccharide mixed with 400 mg of dry KBr, placed in an agate mortar for 5–10 min after tableting, polysaccharide was measured infrared spectrum 4000–400 cm⁻¹ in the infrared spectrometer (see Fig. 7).

2.2.4. Monosaccharide composition analysis

Monosaccharide composition was analyzed by Yang, Zhang, Tang, and Pan (2005). GPC (Gel permeation chromatography) to determine average Mw of CAL-n (see Fig. 8).

2.2.5. Periodate oxidation, smith degradation and GC analysis

CAL-n was analyzed by periodate oxidation, gas chromatography (GC) and Smith degradation and modified according to actual conditions. Firstly, prepare a 0.015 M sodium metaperiodate and sodium iodate solution, and mix the two in proportions of 5:0, 4:1, 3:2, 2:3, 1:4 and 0:5. 0.1 ml of sodium metaperiodate and sodium iodate solution mixed in different ratios and, then dilute, determination of absorbance (Abs). Draw standard curve with NaIO₄ concentration and Abs as the X and Y axes (Li et al., 2019; Wu et al., 2019). Then, 40 mg of 0.015 M NaIO₄ solution was used to dissolve 20 mg of polysaccharide to determine the consumption of NaIO₄ during the reaction, reaction conditions: 4 °C protected from light, 2 h. Then, 0.1 ml sodium metaperiodate and sodium iodate solution mixed in different ratios were diluted to 25 ml, and reaction mixture's Abs was measured at 223 nm every 24 h until it remains constant. At the same time, 1 ml of ethylene glycol was added and the reaction was allowed to break down the excess NaIO₄ for 30 min. Calculate the consumption of NaIO₄. Determina-
tion of formic acid’s yield by titration. Add 1 ml of product to 0.5%, 50\(^{-1}\) phenolphthalein and mix well, then add 0.5 mM NaOH until the solution turns purple, then calculate formic acid’s yield (see Fig. 9).

2.3. CAL-n’s advance structural characterization

2.3.1. Analysis of CAL-n

Weigh refined polysaccharide milled and sieved (100 mesh), X-ray diffraction experiments using powder samples made after weighing evidence. Using XRD/MAX 2200 VPC diffractometer at 20 of 3–90\(^{\circ}\) continuous scanning, taking the number of intervals 0.02\(^{\circ}\), scanning speed of 5\(^{\circ}/\text{min}\); voltage of 40 kV, current 40 mA. Under this condition, crinum refined polysaccharide measured XRD pattern (see Fig. 10).

2.3.2. X-ray diffraction measurement

Weigh crinum refined polysaccharide dissolved in 100 ml of distilled water 2.0 mg dubbed dilute solution, using a laser particle size distribution analyzer observations polysaccharide molecule size distribution. All data are presented 90 plus particle size distribution analysis software (see Table 1).

2.4. Anti-tumor effect

In vitro anti-tumor effect of CAL-n was determined by MTT method. After incubation 72 h, each well was aspirated liquid, in the dark environment, each well was added 200 \(\mu\)l 0.5 mM of MTT solution, and then placed in the incubator 96, culture was continued 4 h. 4 h after removing the same 96-well plate was added to each well in the dark environment dimethyl sulfoxide 200 \(\mu\)l, and shaking and mixing using a micro-oscillator, and then determine its optical density (OD) by an ELISA plate reader, reference wavelength for 490 nm, detection wavelength was 570 nm. Calculation of drug on tumor cell inhibition rate using the following equation, and calculates the IC50 (see Table 2).

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Fig. 4. GC chromatogram of mixed standard monosaccharide. The order and the time of standard monosaccharide peak. 1 (Rha): 13.69 2. (Ara): 14.31 3. (Xyl): 14.88 4. (Man): 23.75 5. (Glu): 24.23 6. (Gal): 25.09 7. (Sor): 29.21.

Fig. 5. GC chromatogram of Crinum polysaccharides.

Fig. 6. The AFM diagram of Crinum polysaccharide (50 \(\mu\)g/ml) (a: 20 \(\mu\)m \(\times\) 20 \(\mu\)m, b: 1.5 \(\mu\)m \(\times\) 1.5 \(\mu\)m).
3. Experimental result

3.1. Preliminary properties and chemical composition of CAL-n

Crude polysaccharide (CAL-n) were scanned by UV spectroscopy at 700–190 nm range, spectral figure crinum polysaccharide at 260 nm and no absorption peak at 280 nm, can explain crinum refined polysaccharide didn't contain nucleic acid and protein. Determination of heavy refined Crinum average molecular mass polysaccharide gel using high performance liquid chromatography, the conclusion that the weight average molecular weight of 7.3 × 10^5 Da. Infrared spectroscopic analysis of polysaccharide
structure, Crinum purified polysaccharide 3422 cm\(^{-1}\) and 2882 cm\(^{-1}\) carbohydrate part had a characteristic absorption peak, the absorption peak of 1618 cm\(^{-1}\), 1420 cm\(^{-1}\) and 1332 cm\(^{-1}\), respectively at the C=COOH, CO stretching vibration of O asymmetric stretching vibration, −COOH, −COOH of the symmetric stretching vibration of C=O absorption peak, three peaks described crinum polysaccharide containing uronic acid; 1239 cm\(^{-1}\) is one carbohydrate CC telescopic vibration peak, indicating that contain β - type glycosidic bonds, thus can show crinum polysaccharide β-pyranoid glycoside bond. Gas chromatography analysis of the monosaccharide composition of refined polysaccharide crinum, crinum monosaccharide composition which mainly rhamnose, glucose, galactose, sorbitol, and its ratio of rhamnose: glucose: galactose: sorbitol = 1:4.74:1.66:61.61. Periodate oxidation and Smith degradation results further confirmed Crinum polysaccharide containing (1 → 2), (1 → 3), (1 → 6) glucosidic key is connected, containing uronic acid groups, and side chains having more triple helix structure. Crinum refined polysaccharide has the effect of inhibiting the growth of tumor cells (HepG2).

### 3.3. Antitumor activity of CAL-n

The results showed that the crinum polysaccharides had effect on HepG2 growth inhibition, the IC50 value was 128.07 µg/ml.

### 4. Conclusions

In summary, an effective purified polysaccharide (CAL-n) with a Mw of 7.3 × 105 Da was obtained from cultured Crinum asiaticum L. Through a series of chemical and instrument method for primary and advanced structural crinum refined polysaccharide conducted a preliminary analysis, detection crinum refined polysaccharide on tumor cell proliferation by MTT assay, the following conclusions. The CAL-n mainly composed of rhamnose, glucose, galactose, sorbose to β-pyranose (1 → 2), (1 → 3), (1 → 6) glucosidic key is connected, containing uronic acid groups, and side chains having more triple helix structure. Crinum refined polysaccharide had the effect of inhibiting the growth of tumor cells (HepG2).

### Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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