Saudi Journal of Biological Sciences 26 (2019) 2085–2090

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

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

Miao Yu [a,c], Yingxiang Chen [a], Yingjie Liu [a,c], Miao Yu [a], Ying Xu [a], Bing Wang [b,*]

[a] Research Center on Life Science and Environmental Science, Harbin University of Commerce, Harbin 150076, China
[b] School of Food Engineering, Harbin University of Commerce, Harbin 150076, China
[c] Postdoctoral Scientific Research Workstation, Harbin University of Commerce, Harbin 150076, China

ARTICLE INFO

Article history:
Received 29 August 2019
Revised 12 September 2019
Accepted 13 September 2019
Available online 14 September 2019

Keywords:
Crinum asiaticum L
Polysaccharides
Structure
Antitumor activity

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 $HepG_2$ cells was affected by CAL-n, with a concentration dependant ration. The results indicated that CAL-*n* should by exploration as anti-tumor activities in vivo.

1. Introduction

Crinum asiaticum L. var. sinicum (Roxb. ex Herb.) Baker is an herbaceous perennial plant distributed in extratropical and subtropical zone. The whole plant was applied in clinic for the treatment of anti-inflammatory, activating blood, especially purpura, rheumatic arthralgia, traumatic injury and also used to treat cancer nowadays (see Fig. 1).

Polysaccharide was a kind of polymer, comprised more than 10 monosaccharide of glycoside bond polymerization which primarily distributed in the higher plants, animals, the cell membrane and microbial cell walls (Gu et al., 2006; Jiang et al., 2013; Li et al., 2014; Mosmann, 1983). Recently, more and more people are working on polysaccharides and related derivatives' biological properties. Investigations on the bioactive constituents indicated that immune function (Qiao et al., 2010), anti-tumor, anti-virus (Rout et al., 2008), anti-aging, anti-coagulation (Zhang et al., 2000; Zou et al., 2010), lipid-lowering, *etc*., so the research was very active in recent years about the polysaccharide (see Fig. 2).

As everyone knows, the biological characteristics of natural plants are determined by their chain conformation and unique chemical structure. But, the chemical structure and conformation of CAL-n are not clear. So, firstly, further fractionation of CAL-n, followed by structural characterization to better clarify the relationship between structure and biological activity. Herein, distillate the neutral component from CAL (*Crinum* asiaticum L.) and name it CAL-n, then the chemical structure of CAL-*n* was studied by Infrared spectrum and GC–MS spectroscopy and study on the chain conformation of CAL-n by dynamic and static light scattering, finally establish the structural parameters of CAL-n (Gao et al., 2017). In addition, $HepG_2$ cells were used for in vitro experiments to more fully evaluate CAL-n. This experimental study can help us to learn more about bioactive extracts of natural plant resources (see Fig. 3).

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. $HepG_2$ cells were provided by Cell Bank of Institute (Shanghai, China). MTT, glucan, monosaccharide (glucose, sorbose and so on) were bought from Sigma Chemical Co. (St. Louis, MO, USA).

* Corresponding author.
E-mail address: yumiao913@163.com (B. Wang).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2019.09.017
1319-562X/

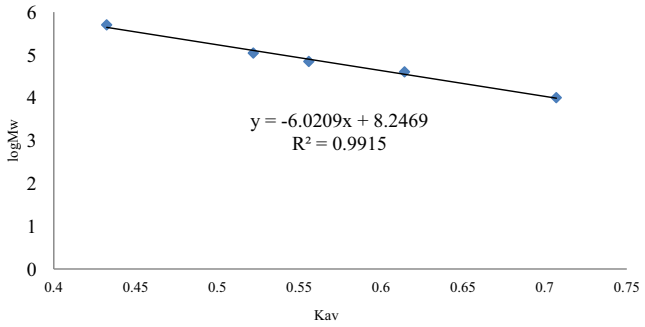


Fig. 1. The standard curve of molecular weights.

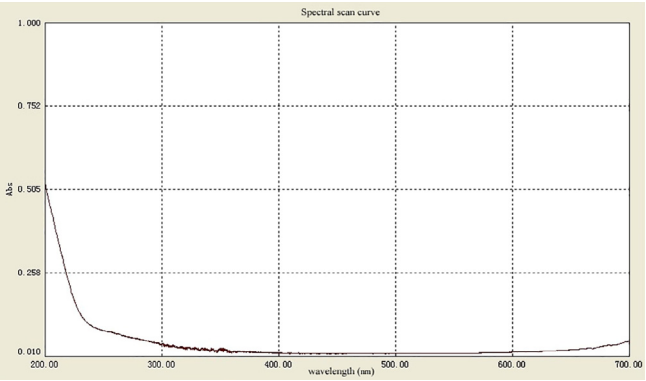


Fig. 2. UV spectra of the purified Crinum polysaccharide.

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 determinate 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 $NaIO_4$ concentration and Abs as the X and Y axes (Li et al., 2019; Wu et al., 2019). Then, 40 mg of 0.015 M $NaIO_4$ solution was used to dissolve 20 mg of polysaccharide to determine the consumption of $NaIO_4$ 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 $NaIO_4$ for 30 min. Calculate the consumption of $NaIO_4$. Determina-

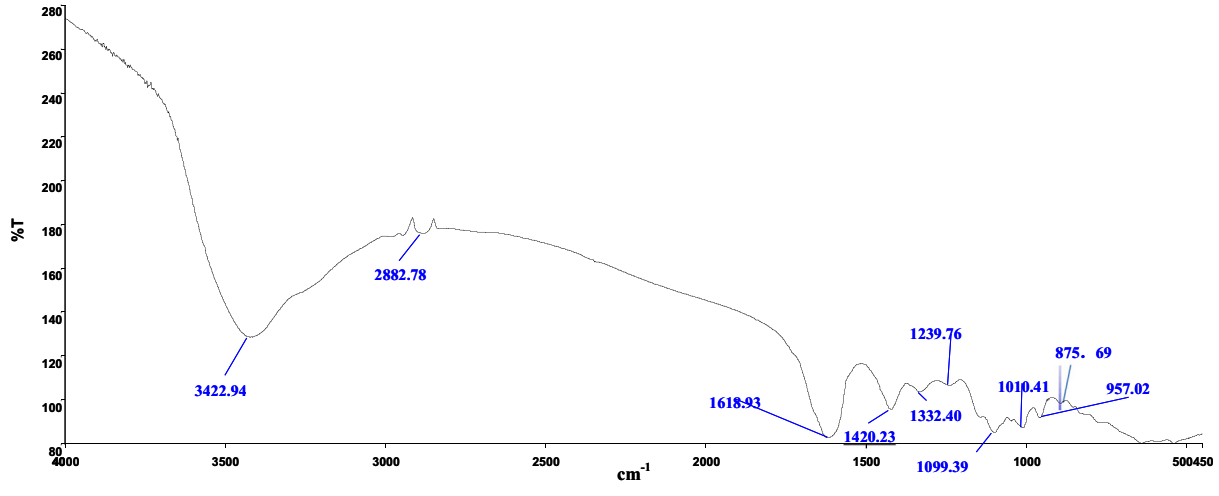


Fig. 3. IR spectrum of the purified Crinum polysaccharides.

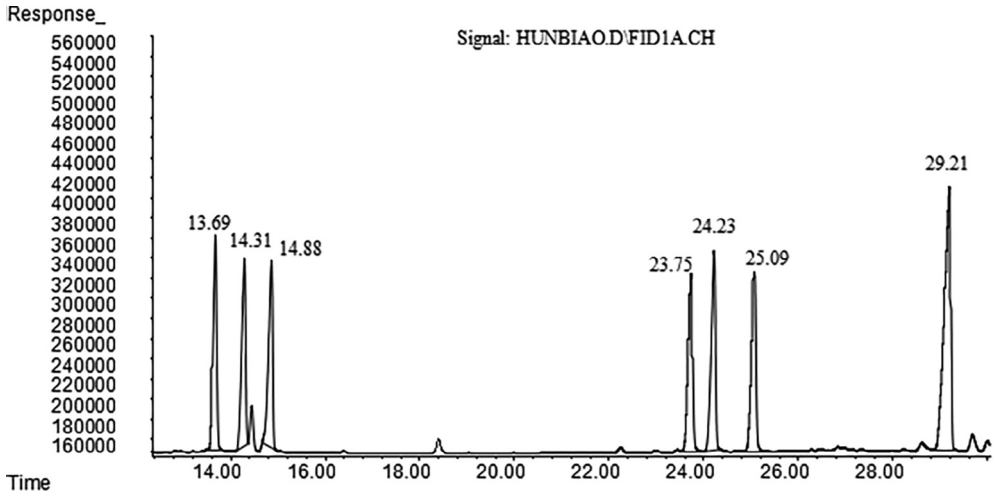


Fig. 4. GC chromatogram of mixed standard monosaccharide. The order and the time of standard monosaccharide peak. 1 (Rha):13.69 2. (Ara):14.31 3. (Xyl):14.88 4. (Man):23.75 5. (Glu):24.23 6. (Gal):25.09 7. (Sor):29.21.

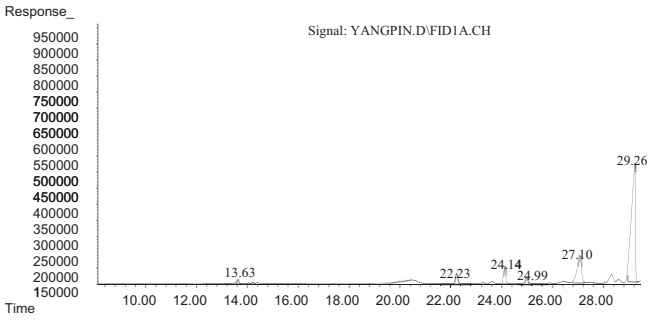


Fig. 5. GC chromatogram of Crinum polysaccharides.

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

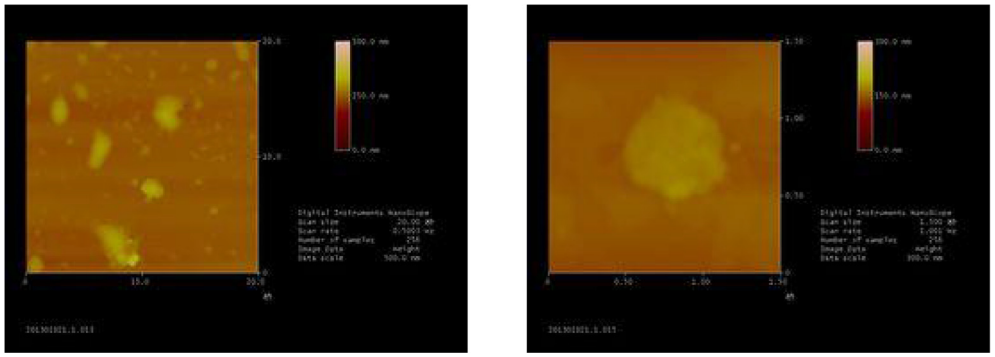
Weigh refined polysaccharide milled and sieved (100 mesh), X-ray diffraction experiments using powder samples made after weighing evidence. Using XRD/MAX 2200 VPC diffractometer at 2θ of 3–90° continuous scanning, taking the number of intervals 0.02°, scanning speed of 5°/min; voltage of 40 kV, current 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).

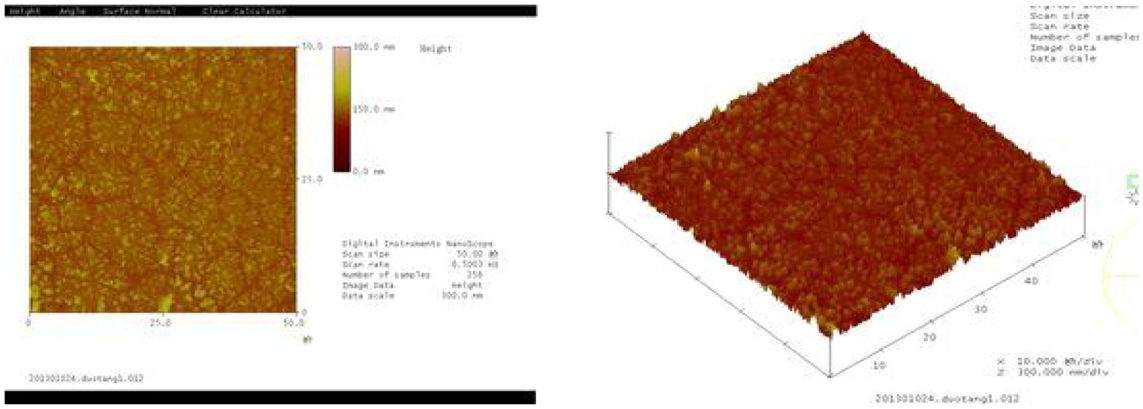
2.4. Anti-tumor effect

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 μl 0.5 m/ml 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 μl, 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).



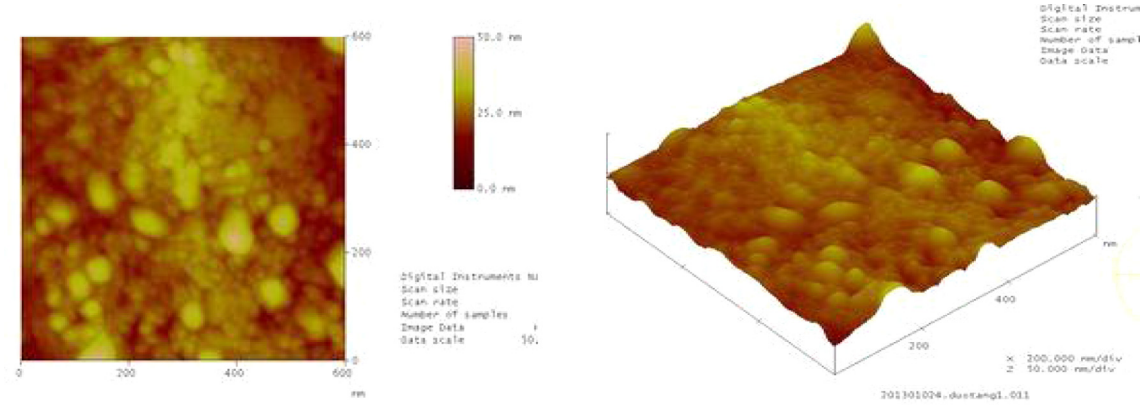
a b

Fig. 6. The AFM diagram of Crinum polysaccharide (50 μg/ml) (a: 20 μm × 20 μm, b: 1.5 μm × 1.5 μm).



a b

Fig. 7. The AFM diagram of Crinum polysaccharide (10 μg/ml), scanning range: 20 μm × 20 μm; a is original, b is the three-dimensional map.



a b

Fig. 8. The AFM diagram of Crinum polysaccharide (10 μg/ml), scanning range: 600 nm × 600 nm; a is original, b is the three-dimensional map.

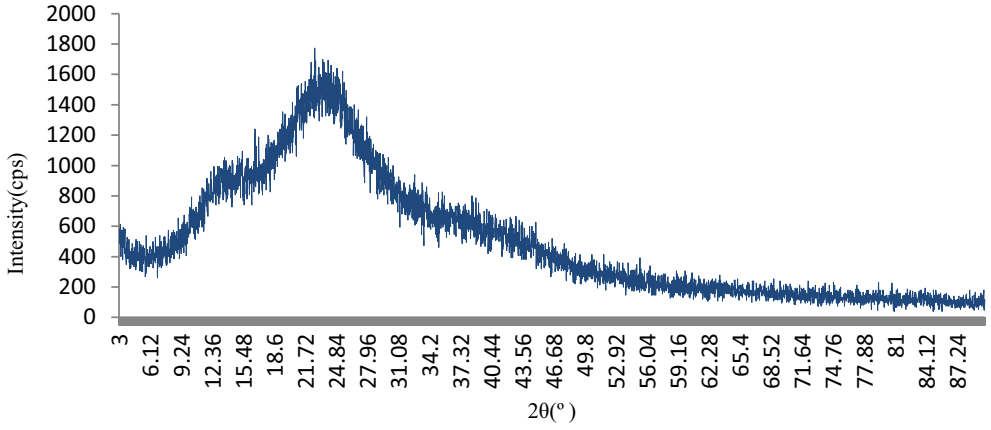


Fig. 9. XRD patterns of Crinum polysaccharide powder.

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 × 105 Da. Infrared spectroscopic analysis of polysaccharide

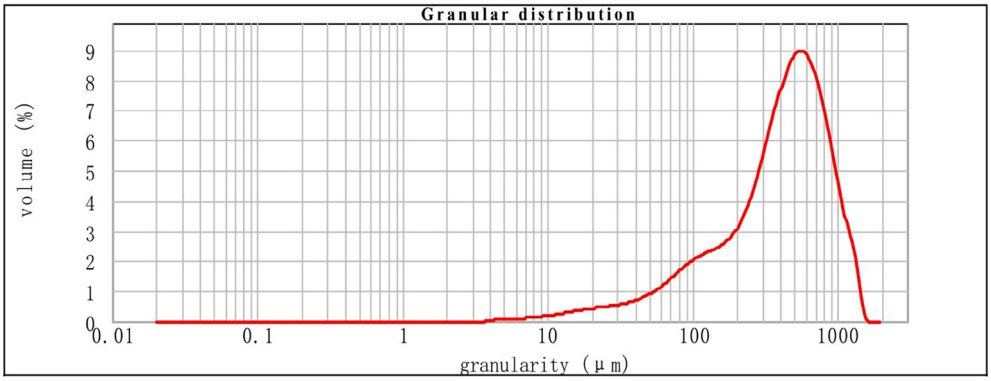


Fig. 10. Particle size distribution of polysaccharides Crinum.

Table 1
Retention time and molecular weight of the standard polysaccharides.

Name	Retain time	K_{av}	lgM_w	MW (KDa)
Blue dextran	5.984	–	6.301	2000
Dextran T500	8.976	0.432	5.699	500
Dextran T110	9.585	0.522	5.041	110
Dextran T70	9.819	0.556	4.845	70
Dextran T40	10.223	0.614	4.602	40
Dextran T10	10.862	0.707	4.000	10

Table 2
IR analysis of functional group in Crinum polysaccharides.

Structural characteristics	Functional group	Peak intensity	Absorption peak (cm^{-1})
—O—H (stretching vibration)	O—H	S	3422
—C—H (stretching vibration)	$—CH_2—$	W	2882
—C═O (Asymmetric stretching vibration)	—COOH	S	1618
—C—O (stretching vibration)	—COOH	M	1420
—C═O (Symmetric stretching vibration)	—COOH	W	1332
C—C (stretching vibration)	C—C	W	1239

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, 957 cm^{-1} for rhamnose characteristic absorption peak wave number 1099 cm^{-1}, 1010 cm^{-1}, 957 cm^{-1} Department has an absorption peak for pyranoid sugar ring feature, 875 cm^{-1} Department has an absorption 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 → 6), (13) glycosidic bond, do not contain (1 → 4) glycosidic bonds.

3.2. Advance properties and chemical composition of CAL-n

Experimental results show that Congo red crinum refined polysaccharide having a helical structure and formation Congo red complexes and has characteristic reactions occur Congo red triple helix structure. Iodine-potassium iodide reaction results may indicate the presence of a long side chain branching structure and more refined polysaccharide crinum. AFM results show that Crinum refined polysaccharide molecule having a plurality of side chains, and chain to chain entanglement between molecules and to generate a mesh, rod-like or branched structure with side chains by way of the link between different saccharide units; an image display crinum single strand diameter 200–300 nm, length of 600–900 nm, not a single sugar chain per instructions, but by a number of sugar chains wrapped into shares; at the same time from the high-resolution image can be clearly seen in these mesh and there are gaps between the rod-like structures appear polysaccharide particles diameter of about 42.56 nm. X-ray diffraction analysis showed that the powder polysaccharide crinum refined polysaccharide or polysaccharide complexes containing crystalline very small, very low degree of crystallinity, basically in the form of amorphous, crystalline poor; particle size distribution results show that the average particle size crinum polysaccharide 470.12 μm.

3.3. Antitumor activity of CAL-n

The results showed that the crinum polysaccharides had effect on HepG2 growth inhibition, the IC50 value was128.07 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.

Acknowledgments

We would like to express sincere thanks to National Natural Science Foundation of China (41702368); Project funded by China

Postdoctoral Science Foundation (2015M581467); Postdoctoral foundation of Heilongjiang Province (LBH-Z15107); Heilongjiang Postdoctoral special Foundation (LBH-TZ1613); Harbin Special Foundation for Young Technological Innovative Talented Person (2013RFQXJ150); The Scientific Research Program of Harbin University of Commerce (17XN061); The Excellent Youth Project of Heilongjiang Natural Science Foundation (YQ2019B004); Research Projects of Harbin Commercial University (18NX066), The Graduate Students Innovative Scientific Research Project of Harbin University of Commerce (YJSCX2018- 484HSCD); Heilongjiang Natural Science Foundation (LH2019H066); Project funded by China Postdoctoral Science Foundation (2019M651296); The Training Program for Young Innovative Talented Person of Heilongjiang Province (Grant No. UNPYSCT-2018140).

References

Alnaim, L., Almaz, S., 2017. A study of barriers and facilitators of clinical practice guidelines implementation among physicians. Indian J. Pharm. Sci. 79 (6), 923–929.

Cetindag, M.F., Ozsavran, A.Y., Yalcin, B., Cetindag, I., Ercan, K., Turkolmez, S., Yegen, D., 2019. The results of nasopharyngeal cancer patients treated by simultaneous integrated boost technique and concomitant chemotherapy. Turk. J. Med. Sci. 49 (2), 558–565.

Gao, W., Wang, Y., Wang, W., Shi, L., 2017. The first multiplication atom-bond connectivity index of molecular structures in drugs. Saudi Pharm. J. 25 (4), 548–555.

Gu, R., Yu, Y., Cai, Y., 2006. Analysis and evaluation of the nutritive composition of Cyclina sinensis. Chinese J. Zool. 41, 70–74.

Jiang, C., Xiong, Q., Gan, D., Jiao, Y., Liu, J., Ma, L., et al., 2013. Antioxidant activityand potential hepatoprotective effect of polysaccharides from Cyclina sinensis. Carbohyd. Polym. 91, 262–268.

Li, W., Jia, M., Wang, J., Lu, J., Deng, J., Tang, J., 2019. Association of MMP9-1562C/T and MMP13-77A/G polymorphisms with non-small cell lung cancer in Southern Chinese population. Biomolecules 9 (3), 107.

Li, X., Wang, D., Xiao, J., Wang, X., Zha, X., Pan, L., et al., 2014. Structural identification and sulfated modification of an antiglycation Dendrobium huoshanense polysaccharide. Carbohyd. Polym. 106, 247–254.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Qiao, D., Liu, J., Ke, C., Sun, Y., Ye, H., Zeng, X., 2010. Structural characterization of polysaccharides from Hyriopsis cumingii. Carbohyd. Polym. 82, 1184–1190.

Rout, D., Mondal, S., Chakraborty, I., Islam, S.S., 2008. The structure and conformation of a water-insoluble (1→3)-, (1→6)-d-glucan from the fruiting bodies of Pleurotus florida. Carbohyd. Res. 343, 982–987.

Wu, J., Wei, W., Zhang, L., Wang, J., Ius, R.D.E., Li, J., Wang, H., Wang, G., Zhang, X., Yuan, J., Niak, M.W., 2019. Risk assessment of hypertension in steel workers based on LVQ and fisher-SVM deep excavation. IEEE Access 7 (1), 23109–23119.

Zhang, L., Zhang, M., Zhou, Q., Chen, J., Zeng, F., 2000. Solution properties of antitumor sulfated derivative of-(1→3)-d-glucan from Ganoderma lucidum. Biosci. Biotech. Bioch. 64, 2172–2178.

Zou, C., Du, Y., Li, Y., Yang, J., Zhang, L., 2010. Preparation and in vitro antioxidant activity of lacquer polysaccharides with low molecular weights and their sulfated derivatives. Int. J. Biol. Macromol. 46, 140–144.