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Antitumor activities of different fractions of polysaccharide purified from *Ornithogalum caudatum* Ait

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ABSTRACT

Four water-soluble polysaccharide fractions (OCAP-2-1, OCAP-2-2, OCAP-3-1 and OCAP-3-3) extracted from the *Ornithogalum caudatum* Ait, were obtained by DEAE fast flow Sepharose anion-exchange, and Sephadex G-75 gel-permeation chromatography. The monosaccharide components of four polysaccharides were characterized by gas chromatography (GC), and the majority of the monosaccharide components were glucose (30–42.5%) and galactose (23–29%), with low levels of arabinose (6–14%), mannose (2–9%), xylose (4–6%), glucuronic acid (1.0–7.5%), galactose acid (1–6%). The high-performance gel-permeation chromatography (HPGPC) analysis showed that the average molecular weight (M_w) of four polysaccharides were approximately 102.1, 62.3, 46.4 and 22.8 KDa, respectively. The protein contents of four fractions were 2.13%, 2.82%, 3.45% and 3.97%, respectively. All polysaccharide fractions exhibited significantly higher antitumor activity against solid tumor Sarcoma 180 *in vivo* than did a blank control. Fractions OCAP-2-2, OCAP-3-1 and OCAP-3-2 significantly inhibited the growth of K562 cells *in vitro*. Results of these studies demonstrated that the polysaccharide had a potential application as natural antitumor drugs.

1. Introduction

In the last decades, many polysaccharides and polysaccharide–protein complexes extracted from mushrooms, fungi, yeast, algae, lichens, plants and animals have been reported to exhibit a variety of biological activities (Chen, Zhang, Qu, & Xie, 2008; Silipo et al., 2005; Wasser, 2002), including immunostimulation (Yamada, 1994; Zha, Luo, Luo, & Jiang, 2007), antitumor (Liu et al., 2007), antioxidant activities (Wang & Luo, 2007), anti-viral (Talarico et al., 2005), anticoagulant (Athukorala, Jung, Vasanthan, & Jeon, 2006), anti-complementary (Xu, Zhang, Zhang, & Chen, 2007), anti-inflammatory (Wu, Cui, Tang, Wang, & Gu, 2007), anti-ulcer (Ye, So, Liu, Shin, & Cho, 2003), hypoglycemic (Tong, Liang, & Wang, 2008), etc. All these activities are due to their contribution to enhance immune function of the human body (Leung, Liu, Koon, & Fung, 2006; Zhang, Cui, Cheung, & Wang, 2007).

Ornithogalum caudatum Ait (OCA), originally distributed in southern Africa and introduced to ancient China, was known in Chinese folk medicine as exhibiting anticancer, antimicrobial and anti-inflammatory activities and has been used for the treatment of hepatitis, parotitis, and some kinds of tumors in northern China (Liu, Wang, Zhang, Chen, & Chen, 1998; Xu, Xu, Liu, & Xu, 2000). Although the low-molecular-weight chemical constituents from OCA have been investigated by several research groups (Tang, Lou, Wang, & Li, 2001; Tang, Yu, Hu, Wu, & Hui, 2001; Yamada, 1994), the high-molecular-weight components such as polysaccharides and glycoproteins are poorly defined.

In vivo bioassays of the crude polysaccharides isolated from OCA have been reported to have potential immunomodulatory activities (Shi et al., 2004; Toma & Curtis, 1986). However, the chemical components and mechanisms of antitumor activity of a large number of polysaccharides remain unclear.

Therefore, the investigation on basic properties of *Ornithogalum caudatum* Ait polysaccharide (OCAP) was particularly necessary to find their functional properties for the wide applications in pharmaceutical industries. The present experiments were set up to isolate, purify the different polysaccharide fractions from OCA and further investigate their basic physicochemical properties. In addition, the antitumor activities of these polysaccharide fractions were also investigated against murine Sarcoma 180 tumor cells *in vivo* and K562 human leukemic cells *in vitro*, it may provide a basic understanding of the relationship between structural feature of OCAP and its bioactivities.

2. Experimental

2.1. Plant materials and reagents

The *OCA* cultivated in Baishan County, Jilin Province, North China, was provided by Pharmaceutical Co. Ltd. (Tonghua, China). The DEAE fast flow Sepharose and Sephadex G-75 resin for

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chromatography were purchased from Amersham Biosciences. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), lipopolysaccharide (LPS), dimethylsulfoxide (DMSO), methyl iodide, D-glucose (Glc), L-rhamnose (Rha), L-fucose (Fuc), D-arabinose (Ara), D-xylose (Xyl), D-galactose (Gal), D-mannose (Man), D-glucuronic acid (GlcA) and L-galacturonic acid (GalA) were purchased from Sigma Chemical Co. Bovine serum albumin (BSA) were purchased from Kaiyang Biochemistry Co. (Shanghai, China). Trifluoroacetic acid (TFA) was from Merck (Germany). All other chemicals and reagents used were of analytical reagent grade and obtained locally.

2.2. Preparation of crude polysaccharides fraction

The whole dried plant of OCA was soaked with 95% ethanol to remove the pigments, defats and inactivates enzymes, and refluxed by hot distilled water for 4 h at 90 °C. The aqueous extract was concentrated to 30% of the original volume under reduced pressure in a rotary evaporator, and proteins were removed with Sevag method (Vilkas & Radjabi-Nassab, 1986). The obtained solution was precipitated with 40% ethanol. As the solubility, activity of the precipitation obtained from 40% of ethanol was relatively poor, so this article did not further study. The supernatant was added by ethanol up to 60%, and kept at 4 °C overnight. The polysaccharide pellets were obtained by centrifugation at 4000 rpm for 15 min, and completely dissolved in appropriate volume of distilled water followed by intensive dialysis for 2 days against distilled water (cut-off M_w 3500 Da). The retentate portion was then concentrated, and centrifuged to remove insoluble material. Finally the supernatant was lyophilized in the freeze–dry apparatus (ALPHA 2–4/LSC, Marin Christ Co., Germany) to give crude OCAP.

2.3. Isolation and purification of the polysaccharides

The crude OCAP (2 g) was dissolved in 20 ml of 0.2 mol/L tris (hydroxymethyl) aminomethane hydrochloride buffer solution, and filtered through a filter paper (0.45 μm). The solution was passed through a DEAE-Sepharose fast flow anion-exchange chromatography column (10 × 300 mm). The polysaccharides were eluted with Tris–HCl buffer solution, followed with gradient elution of 0.1–0.8 mol/L NaCl at a flow rate of 0.8 ml/min. Fractions of 5 ml were collected and monitored by the phenol–sulfuric acid method at 486 nm (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) were then used to give five polysaccharide conjugate fractions, coded OCAP-1, OCAP-2, OCAP-3, OCAP-4 and OCAP-5 (Fig. 1). The OCAP-2 and OCAP-3 were further purified by gel-permeation chromatography on a column of Sephadex G-75 (16 × 500 mm) into different fractions according to their molecular size, and eluted with 0.1 mol/L NaCl at a flow rate of 1.0 ml/min. The major polysaccharide fractions were collected with a fraction collector, then dialyzed with water, and lyophilized to give four polysaccharides, namely OCAP-2-1, OCAP-2-2, OCAP-3-1 and OCAP-3-2, which were subjected to the subsequent analyses. The isolation and fractionation protocol were shown in Scheme 1.

2.4. Analytical methods

Total neutral sugar content was determined by the reaction with phenol in the presence of sulfuric acid at 486 nm (Dubois et al., 1956) using Glc as standard. Total uronic acid content was determined by photometry with *m*-hydroxybiphenyl at 523 nm (Blumenkrantz & Asboe-Hansen, 1973), using GalA as standard. The protein content of protein-bound polysaccharide was measured (Lowry, Rosebrough, Farr, & Randall, 1951), using BSA as standard. The concentration of column fractions was determined

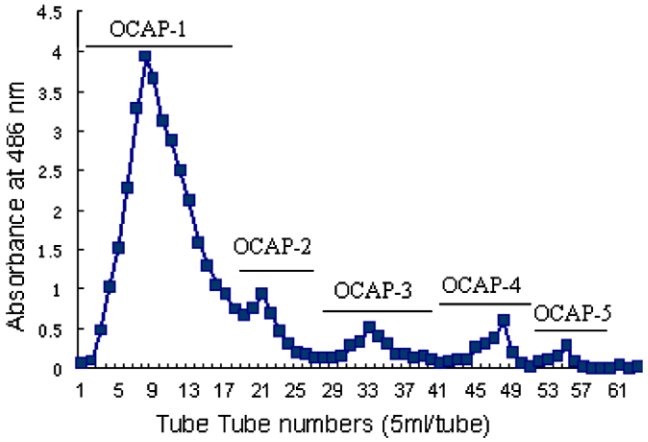


Fig. 1. Elution profiles of crude OCAP on DEAE-Sepharose fast flow anion-exchange chromatography column (50 × 460 mm) with 0–2 M NaCl stepwise elute.

by measuring the absorption at 635 nm. Monosaccharide composition was analyzed according to the following procedure: polysaccharides (20 mg) were hydrolyzed with 4 ml of 2 mol/L TFA at 100 °C for 6 h into monosaccharide under airtight condition, and the TFA was removed through decompression and distillation with methanol reiteration four times at 40 °C. The trimethylsilylated derivatives using the trimethylsilylation reagent (Linda et al., 2001) were loaded onto a HP 5890 capillary gas chromatography (GC–MS) column equipped with a flame-ionization detector (FID). The operation was performed using the following conditions: the rate of N_2 carrier gas was 1.0 ml/min; injection temperature was 210 °C; detector temperature was 300 °C; column temperature was programmed from 100 to 150 °C at 10 °C/min, holding for 2 min at 100 and 150 °C, then increasing to 260 °C at 15 °C/min, holding for 20 min at 260 °C. Monosaccharides identification was done by comparison with reference monosaccharides. The relative molar proportions were calculated by the area normalization method. As references, the following monosaccharides were converted to their silanized derivatives and analyzed: Xyl, Glc, Ara, Rha, Man, Fuc, Gal, GlcA and GalA.

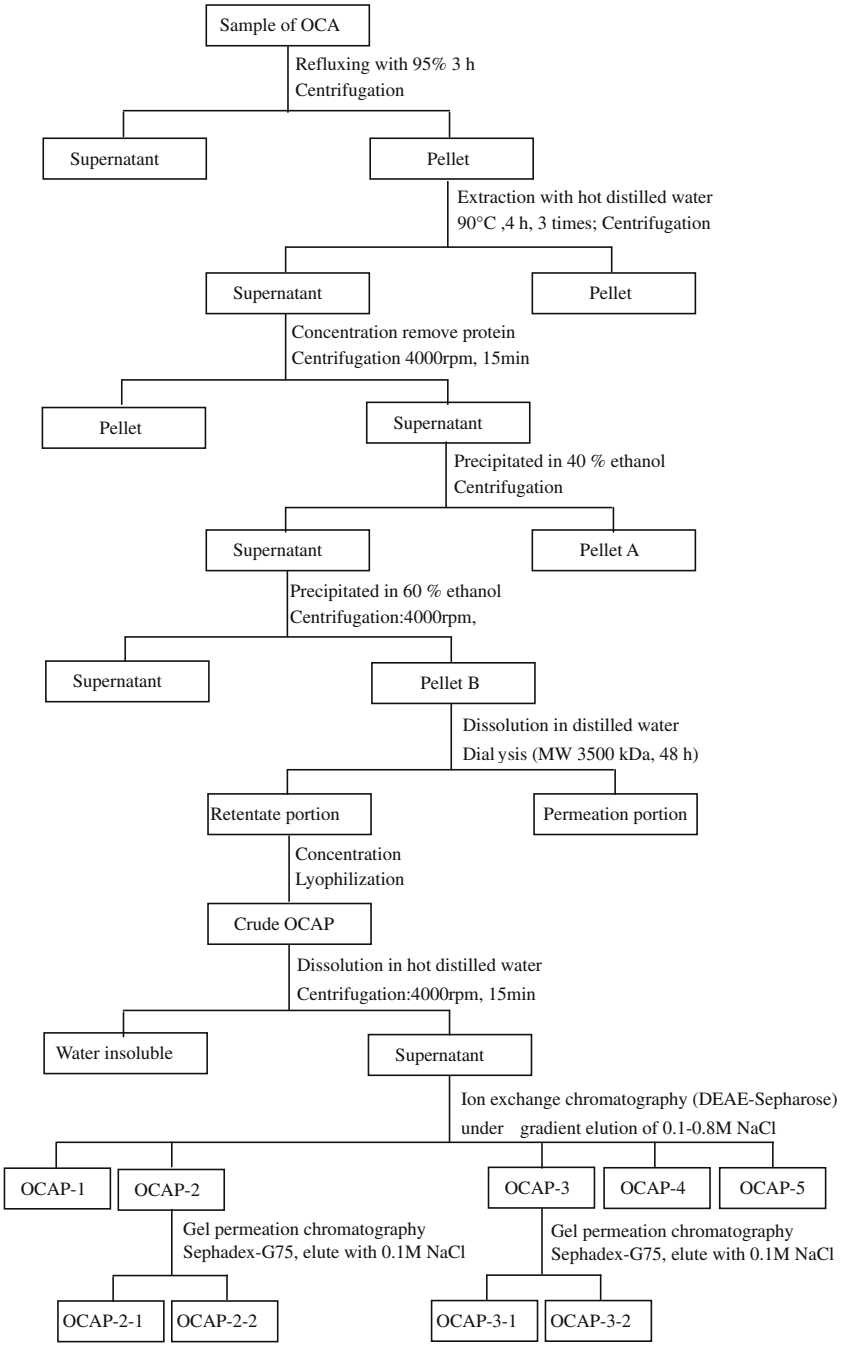
The partially methylated sugars were reduced with $NaBH_4$ at room temperature for 3 h, and acetylated with acetyl anhydride (2 ml) at 100 °C for 2 h. The partially methylated alditol acetates were analyzed by GC–MS.

2.5. Molecular weight determination

The molecular weight of the fractions was determined by gel-permeation chromatography, in combination with a high-performance liquid chromatography instrument (Angilent 1100, USA). The sample (2.0 mg) was dissolved in distilled water (2 ml) and passed through a 0.45 μm filter, applied to a gel-permeation chromatographic column of Ohpak SB-803 HQ (8.0 × 300 mm), maintained at a temperature of 35 °C, eluted with 0.05 mol/L sodium sulfate, at a flow rate of 0.5 ml/min and detected by a refractive index detector. A 20 μl sample was injected in each run. The molecular weight was estimated by reference to the calibration curve made from a Dextran T-series standard of known molecular weight (10,200, 26,290, 40,000, 68,800, 84,000, 158,000 Da).

2.6. Determination of the linkages present in the polysaccharides

The linkages of the polysaccharide fractions were determined (Needs & Selvendran, 1993). The polysaccharide fractions (5 mg) in 5 ml of DMSO were methylated using sodium hydroxide and methyl iodide. The methylated polysaccharide was examined by



Scheme 1. Isolation and fractionation protocol of polysaccharides.

IR spectroscopy. The absence of the absorption peak corresponding to hydroxyl indicated the complete methylation. The fully methylated polysaccharides were then hydrolyzed using 2 mol/L TFA for 6 h at 100 °C, followed by reduction using sodium borodeuteride and finally acetylated with ethyl acetate to give partly acetylated partly methylated alditols. These were analyzed by GC–MS and the original type of linkage of different monosaccharides was determined.

The gas chromatograph was fitted with a split-splitless injector and data were taken at following conditions: the injector temperature was 250 °C; the detector temperature 300 °C; and the column temperature 100 °C when injected, then increased by 20 °C/min to 150 °C, followed by 1.0 °C/min to 200 °C and then 30 °C/min to 300 °C; nitrogen was the carrier gas. The compound at each peak was characterized by the interpretation of the retention times and the characteristic mass spectra. The estimation of the relative amounts of each linkage type was related to the total amount of each monosaccharide type as determined by trimethylsilylation and GC as described above.

2.7. Biological assays

2.7.1. The inhibition ratio of Sarcoma 180 tumor cells

Sarcoma 180 cells (1×10^5 cells/mouse) were subcutaneously inoculated into 8-week-old BALB/c male mice. The tested polysaccharides dissolved in phosphate buffer saline (PBS) (pH 7.0) (at concentrations of 0.01, 0.1, 1.0, 10 and 100 μg/ml in PBS, respectively) were injected intraperitoneally once daily for 7 days, at

24 h after tumor inoculation. The same volume of PBS (pH 7.0) was injected intraperitoneally into the control mice. The tumor was allowed to grow on the mice for 7 days before it was removed from the mice and weighed. The *in vivo* antitumor activity of polysaccharides was expressed as an inhibition ratio (A) calculated as $A = [1 - \frac{W_t}{W_c}] \times 100\%$, where W_c is the average tumor weight of the control group, and W_t is the average tumor weight of the test group.

2.7.2. The inhibition ratio of K562 tumor cells

The inhibition effects of OCAP-2-1, OCAP-2-2, OCAP-3-1 and OCAP-3-2 on the K562 human leukemic cells were evaluated *in vitro* using MTT assay (Chen et al., 2006). The K562 cells were incubated on a 96-well cultivation plate at a concentration of 1×10^6 cells/ml. Each well was inoculated with 100 μl RPMI 1640 media supplemented with 10% fetal bovine serum solution containing the K562 cells and 20 μl samples (at concentrations of 0.01, 0.1, 1.0, 10 and 100 μg/ml in PBS, respectively) under an atmosphere of 5% CO_2 at 37 °C for 24 h. The tumor cells were continuously inoculated for another 4 h after 10 μl MTT (5 mg/ml) had been added. The supernatant was removed by centrifugation, and then 100 μl of DMSO was added to terminate the reaction. Then MTT colorimetric method was used to observe the effect of growth inhibiting of K562 tumor cell induced by OCAP. The sample groups were compared with control groups in the absence of the tested samples. All *in vitro* results were expressed as the inhibition ratio (A) of tumor cell proliferation as $A = [1 - \frac{N_t}{N_c}] \times 100\%$, where N_c and N_t are the average number of viable tumor cells of the control group and test group, respectively.

2.8. Determination of endotoxin contamination

Contaminants of endotoxin possibly associated with the OCAP preparations were removed using Affi-Prep Polymyxin Matrix (Bio-Rad). Briefly, 1 ml of Affi-Prep Polymyxin Matrix was packed in a Bio-spin column, centrifuged at 200 rpm for 2 min, and then 0.5 ml of OCAP-2-1, OCAP-2-2, OCAP-3-1 and OCAP-3-2 (10 μg/ml) and LPS (5 μg/ml) was added. After incubating overnight at 4 °C, the effluent was recovered from the column by centrifugation at the same condition.

3. Results and discussion

3.1. Isolation and purification of polysaccharides composition

The water-soluble crude polysaccharides were obtained from OCA by hot water extraction, ethanol precipitation, deproteinized by Sevag method, dialyzed against water and dried in vacuum, named OCAP. OCAP was fractionated by preparative DEAE fast flow Sepharose anion-exchange chromatography to obtain five main fractions, namely OCAP-1, OCAP-2, OCAP-3, OCAP-4 and OCAP-5, which were selected based on total carbohydrate elution profile (Fig. 1). These four fractions were tested their antitumor effect on Sarcoma 180 mice, that OCAP-2, and OCAP-3 showed higher antitumor activity, so the fraction of OCAP-2 and OCAP-3 were further purified by Sephadex G-75 gel-permeation chromatography into different fractions according to their molecular size (Figs. 2 and 3). The results showed that the OCAP-2 and OCAP-3 were mainly composed of two sub-fractions, namely OCAP-2-1, OCAP-2-2, OCAP-3-1 and OCAP-3-2, respectively. At the same time, OCAP-2-1, OCAP-2-2, OCAP-3-1 and OCAP-3-2 were estimated to have the different average molecular weights (M_w), according to the calibration curve using Dextran T-series as the standards by HPGEC (Table 1). The HPGEC profile (Fig. 4) showed as a single and symmetrically sharp peak, indicating that OCAP-2-1, OCAP-2-2, OCAP-3-1 and OCAP-3-2 were close to homogeneous polysaccharide with different molecular sizes.

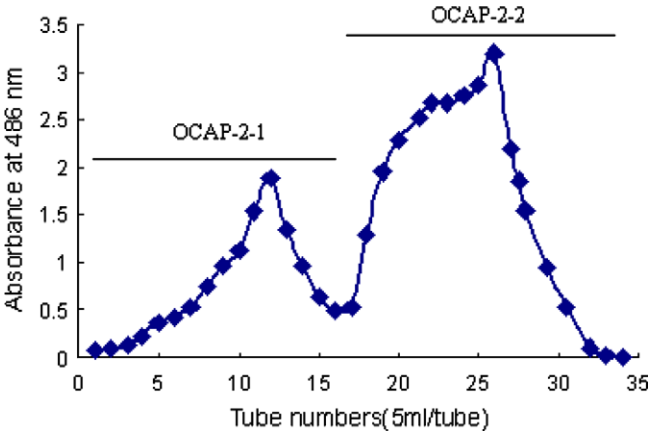


Fig. 2. Elution profiles of crude OCAP-2 on Sephadex G-75 gel-permeation chromatography column (1.6 × 50 cm) with 0.1 M NaCl elute.

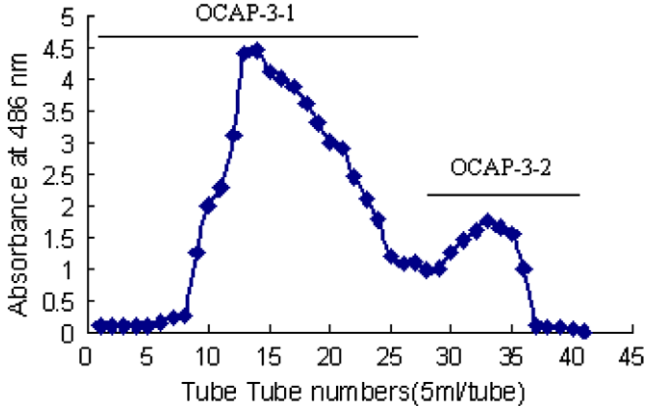


Fig. 3. Elution profiles of crude OCAP-3 on Sephadex G-75 gel-permeation chromatography column (1.6 × 50 cm) with 0.1 M NaCl elute.

3.2. Carbohydrate and protein bound contents of polysaccharides

The total carbohydrate content, total uronic acid content and protein bound content in OCAP-2-1, OCAP-2-3, OCAP-3-1 and OCAP-3-2 were assessed by six calibration solutions of Glc, GlcA, and BSA, respectively. As a consequence, the good linearity was obtained by regression analysis between y (absorbance at 486 nm) and x (standard concentration, μg/ml) and regression equations were as follows: $y = 9.8414x - 0.0488$ ($R^2 = 0.9998$) for Glc at the concentration of 8.0–50.0 μg/ml, $y = 0.0017x - 0.0153$ (y: absorbance at 523 nm, x: μg/ml, $R^2 = 0.9971$) for GlcA at 10.0–50.0 μg/ml, and $y = 1.128123x - 0.6083$ (y: absorbance at 635 nm, x: mg/ml, $R^2 = 0.9985$) for BSA at 1.0–20.0 mg/ml. The average molar weight was estimated by high-performance gel-permeation chromatography (HPGPC), using dextran of known molecular weight as standards, and regression equations were as follows: $\text{Log } Mr = -0.1029\text{TR} + 2.34$ ($R^2 = 0.9856$).

The total carbohydrate content, total uronic acid content and protein bound content and the average molar weight of OCAP-2-1, OCAP-2-3, OCAP-3-1 and OCAP-3-2 were summarized in Table 1. The results showed that these polysaccharides consist primarily of Glc, Gal, Ara, Xyl, Man, GlcA and GalA. Glc was the dominant monosaccharide and represented exceeding 30% (molar ratio) of the total sugars and Gal content is greater than 20%. Other monosaccharides

Table 1
Monosaccharide composition, protein content, uronic acid, and average molecular weight in four major polysaccharide fractions.

Fragments of OCAP	Molecular weights (KDa)	Uronic acid (w/w%)	Protein (w/w%)	Contents of the sugar residues (mol.%)[a]								
				Rha	Ara	Xyl	Man	Fuc	Glc	Gal	GlcA	GalA
OCAP-2-1	102.1	2.2	2.13	nd[c]	12.0	4.0	2.0	nd	30.0	24.0	1.0	1.0
OCAP-2-2	62.3	4.5	2.82	nd	14.0	6.0	3.0	Tr[b]	33.5	24.0	7.5	3.0
OCAP-3-1	46.4	2.6	3.45	nd	13.0	5.0	6.0	Tr[b]	40.0	29.0	2.0	4.0
OCAP-3-2	22.8	3.6	3.97	2.0	6.0	6.0	9.0	3.0	42.5	23.0	3.5	6.0

[a] (mol.%): Quantities of the carboxy-methylated uronic acid are given in mol.%.
[b] Tr = trace, <0.2%.
[c] nd = not detected.

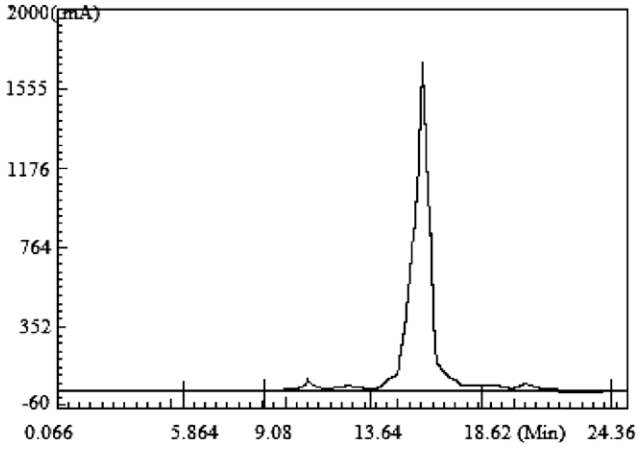


Fig. 4. The profile of the OCAP fraction in HPGPC, with 0.05 M Na_2SO_4 at 0.5 ml/min.

were also present but in minor amounts. Only a small amount of Rha and Fuc were detected in the fractions OCAP-3-2. In comparison to four polysaccharides fractions, the compositions and contents of monosaccharides are quite different, which suggests the difference in polysaccharide structures of each OCAP fractions leading to distinct biological properties. Based on total uronic acid content by photometry, and the molar ratio of GlcA, GalA by GC, the GlcA and GalA contents were also detected in the four polysaccharides fractions. The protein contents of the four fractions were detected and measured by the Bradford assay, and the results were also shown in Table 1. Although the Sevag method has been repeated for many times to remove free proteins, the protein was found in all fractions, and the results were also shown in Table 1. Generally, the pure protein exists as a globular shape in aqueous solution, but protein-bound polysaccharides exhibit a relatively expanded flexible chain, such as proteoglycan monomers (Ghosh & Reed, 1995). At the same time SDS gel electrophoresis and glycosyl composition analysis, preliminary evidence of four-component may contain protein-bound polysaccharide (Peng, Zhang, Zeng, & Kennedy, 2005). Therefore it could be protein-bound polysaccharides.

3.3. Monosaccharide linkage analysis

Through GC, partial hydrolysis with acid, methylation, GC–MS analysis, the results indicate that the polysaccharides exhibit a highly complex nature (Table 2). The ratios of different types of linkages were calculated based on the monosaccharide composition data and the areas of the methylated products. In all fractions, Ara was mostly terminally linked in the furanose form and the inter linkage was mostly 1 → 3 and 1 → 5. Terminally 1 → 4 linked Xyl was also present in all fractions, but only OCAP-2-2 and OCAP-3-1 fractions had a small amount of 2 → 4 linked Xyl. 1 → 2 and 1 → 4 linked Man was present in all fractions, but terminal positions were only identified in OCAP-3-1 and OCAP-3-2. In

Table 2
Linkage analyses of four major polysaccharide fractions.

Sugar	Linkage	OCAP-2-1*	OCAP-2-2*	OCAP-3-1*	OCAP-3-2*
Rha	t-				2
Ara	t-	9	10	10	6
	3-	1	1	1	
	5-	2	3	2	
Xyl	t-	1	2	2	3
	4-	3	3	2	3
	2,4-		1	1	
Man	t-			1	2
	2-	1	1	4	4
	4-	1	2	1	3
Fuc	t-				2
	3,4-		tr	tr	1
Glc	t-	7	9	9	7
	2-	tr			2
	3-		tr	tr	
	4-	28	31.5	29	31.5
	3,4-			tr	2
	4,6-	2	2	1	
	3,4,6-			1	
Gal	t-	4	5	7	12
	3-	3	3	3	1
	4-	2	2	5	4
	6-	4	5	5	2
	3,4-	1	1	1	2
	3,6-	7	8	8	2
GlcA	4-	1	7.5	2	3.5
GalA	4-	1	3	4	6

* Data in % mol.

OCAP-2-1, OCAP-2-2, OCAP-3-1 and in OCAP-3-2 most of the Gal was 1 → 3,6 and terminally linked, and 1 → 3, 1 → 4 and 1 → 6 linked units were also present. OCAP-3-1 and OCAP-3-2 consist of more 1 → 4 linked Gal than those in OCAP-2-1 and OCAP-2-2. GlcA and GalA were only 1 → 4 linked in all fractions. The Glc was mainly terminally and 1 → 4 linked in all fractions, and in OCAP-2-1, OCAP-2-2 and OCAP-3-1 a small amount of 4 → 6 linked Glc was present. Only in OCAP-3-2 there is a small amount of terminal linked Rha (Table 2).

3.4. Antitumor activity

3.4.1. Effect of polysaccharide OCAP on S-180 cells inhibition

Antitumor activities of OCAP-2-1, OCAP-2-2, OCAP-3-1 and OCAP-3-2 were examined in mice inoculated with Sarcoma 180 cells. Antitumor activities of polysaccharides were examined in a system using Sarcoma 180 solid tumors implanted in mice by intraperitoneal injection at concentration of 0.01, 0.1, 1, 10 and 100 μg/ml once daily for 7 days, at 24 h after Sarcoma 180 cells inoculation. The same volume of phosphate buffer (pH 7.0) (PBS) was injected into the peritoneum of the control mice. The tumor was allowed to grow on the mice for 7 days before it was removed from the mice, and weighted. Antitumor activity of OCA-tested

mouse was calculated as the average tumor weight of Sarcoma 180 cells compared with that of the control group. The *in vivo* antitumor activities of the four samples against Sarcoma 180 tumor cells at different concentrations (0.01, 0.1, 1.0, 10 and 100 μg/ml) are summarized in Fig. 5. The *in vivo* antitumor activities of the four fractions against Sarcoma 180 tumor cells were summarized in Fig. 5. The four fractions exhibit relatively strong inhibition ratios at all concentration levels. Especially, the OCAP-3-1 sample had the highest inhibition ratio of 53.9 ± 11.6% at 0.1 μg/ml than the other three fractions, but the *in vivo* inhibition ratios of the four fractions had the lowest inhibition ratio at 100 μg/ml, and no obvious dose–dependency relationship was observed between concentration of the different fractions and growth inhibition of S-180. The *in vivo* antitumor activities against S-180 tumor cells of the different fractions were derived from stimulation of the immunoresponse mechanism, and they do not strictly follow the dose-dependency of chemotherapeutic anticancer agents (Zhang, Cheung, & Zhang, 2001).

3.4.2. Effect of polysaccharide OCAP on K562 cell inhibition

After incubated with OCAP-2-1, OCAP-2-2, OCAP-3-1 and OCAP-3-2 for 24 h at the concentrations from 0.01 to 100 μg/ml, the inhibition ratio of K562 cells was observed and compared with control. The inhibition ratios of the four fractions in different concentration against human leukemic K562 cells were summarized in Fig. 6. Different antitumor activities were observed in the four fractions. At the concentrations from 0.01 to 100 μg/ml, OCAP-2-2, OCAP-3-1 and OCAP-3-2 significantly inhibited the growth of K562 cells, but OCAP-2-1 had not found distinct inhibition activity from 0.1 to 100 μg/ml. OCAP-3-2 exhibit significantly higher inhibition ratios than other fractions at all concentrations. Especially, at the concentration of 0.1 μg/ml, the inhibition activity of OCAP-3-2 was the highest with an inhibition ratio beyond 47.83 ± 6.15%.

From above, OCAP-2-2, OCAP-3-1 and OCAP-3-2 significantly inhibited the growth of human leukemic K562 cells. However, when the concentration was 100 μg/ml, the inhibition ratios of OCAP-3-1 and OCAP-3-2 were 14.69 ± 3.57% and 24.61 ± 12.43%, and less than those at lower concentration.

3.5. The effects of polymyxin B on OCAP-induced K562 cell inhibition

Endotoxin is widely present in many plant materials. Potential contamination of endotoxin is always a concern for the high-molecular-weight components isolated from plants, which may contributes to the OCAP effects of antitumor activity we have observed. To ensure that the effects of OCAP-2-1, OCAP-2-2, OCAP-3-1 and OCAP-3-2 were not due to endotoxin contamination, OCAP-2-1, OCAP-2-2, OCAP-3-1 and OCAP-3-2 were treated with polymyxin B, an inhibitor of LPS activity, and the inhibition ratio of K562 cells was examined. As shown in Fig. 7, passage of LPS solution (5 μg/ml) through polymyxin B-affinity column reduced the inhibition activity almost completely, indicating that polymyxin B-affinity column absorbed LPS almost completely. Passage of OCAP-2-1, OCAP-2-2, OCAP-3-1 and OCAP-3-2 solution (10 μg/ml) with polymyxin B-affinity column, however, only had little effect on the inhibition ratio of K562 cells. These results indicated that the inhibited the growth of human leukemic K562 cells of OCAP was not due to endotoxin contamination.

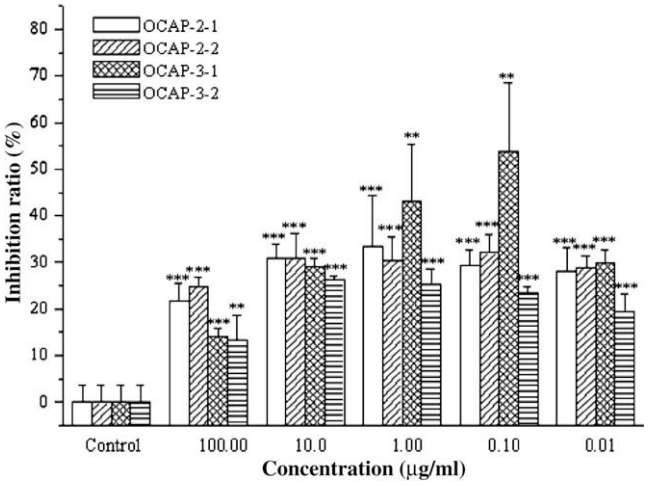


Fig. 5. Inhibition ratio of proliferation of Sarcoma 180 tumor cell (S-180) in vivo by OCAP-2-1, OCAP-2-2, OCAP-3-1, and OCAP-3-2 at different concentration (X ± S, $n = 6$). *Compared with control group $p < 0.05$. **Compared with control group $p < 0.01$. ***Compared with control group $p < 0.001$.

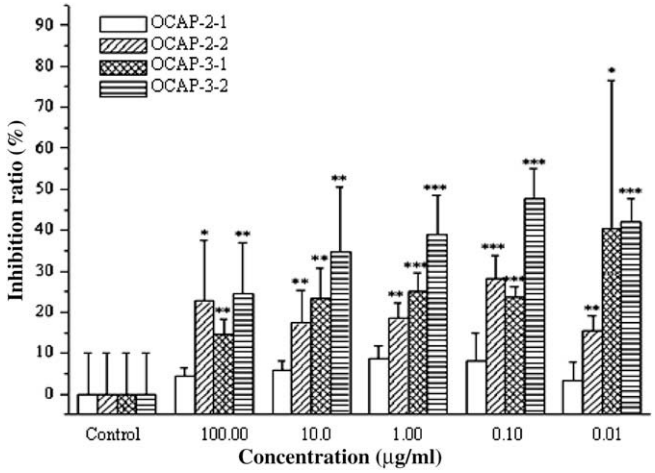


Fig. 6. Inhibition ratio of proliferation of human leukemic K562 cells in vitro by OCAP-2-1, OCAP-2-2, OCAP-3-1, and OCAP-3-2 at different concentration (X ± S, $n = 6$). *Compared with control group $p < 0.05$. **Compared with control group $p < 0.01$. ***Compared with control group $p < 0.001$.

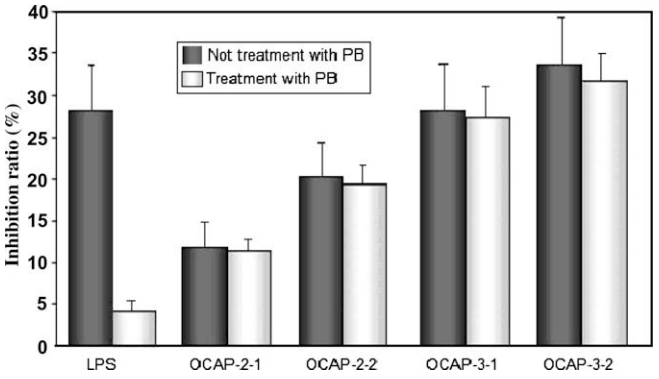


Fig. 7. Effects of polymyxin B treatment on OCAP-2-1 (-2-2, -3-1, -3-2) induced human leukemic K562 cells proliferation. LPS (5 μg/ml), OCAP-2-1 (-2-2, -3-1, -3-2) (10 μg/ml) was added to polymyxin B-affinity column, incubated overnight at 4 °C, eluted from the column by centrifugation, and then added to the cultures of K562 cells for 24 h. The inhibition ratio of proliferation of human leukemic K562 cells in vitro was measured, as described. Values are the mean ± SD ($n = 3$). Statistically significant differences between untreated with PB and treated with PB samples are indicated ($^{*}p < 0.01$).

4. Conclusions

The water-soluble polysaccharides OCAP-1, OCAP-2, OCAP-3, OCAP-4 and OCAP-5 were successfully purified from *Ornithogalum*

caudatum Ait by DEAE-Sepharose fast flow chromatography. OCAP-2 and OCAP-3 were further purified by Sephadex G-75 gel-permeation chromatography into OCAP-2-1, OCAP-2-2, OCAP-3-1, and OCAP-3-2. Four fractions were determined to be a homogeneous heteropolysaccharides composed of Glc, Gal, Ara, Xyl, Man, GlcA and GalA, with the weight-average molecular mass of 102.1, 62.3, 46.4 and 22.8 KDa, respectively. The four fractions all exhibited strong antitumor activities against Sarcoma 180 solid tumor implanted in BALB/c mice *in vivo*. At the same time, OCAP-2-2, OCAP-3-1 and OCAP-3-2 could significantly inhibit the proliferation of K562 cells *in vitro*.

Although *Ornithogalum caudatum* Ait was a popular folk medicine and had attracted great attention due to its role in the antitumor activity against several types of cancer, there is little information available about its polysaccharide component. This is the first report providing *in vivo* evidences of the therapeutical antitumor potential of the bioactive polysaccharides. Our results suggested the antitumor activity of polysaccharide might act through the activation of host immune response to stimulating T-cell subsets and cytokine (TNF-α and IFN-γ) production to participate in the antitumor effects. In addition, it might be achieved by enhancing the immune system functions. However, further investigation about the relationship between immunomodulation and antitumor activity is needed.

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