Food and Chemical Toxicology 55 (2013) 609–616

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Antitumor and immunomodulatory activity of a water-soluble low molecular weight polysaccharide from *Schisandra chinensis* (Turcz.) Baill

Ting Zhao [a], Guanghua Mao [a], Riwen Mao [b], Ye Zou [a], Daheng Zheng [a], Weiwei Feng [a], Yuena Ren [c], Wei Wang [d], Wei Zheng [d], Jia Song [d], Yuanqing Chen [d], Liuqing Yang [c,*], Xiangyang Wu [e,*]

[a] School of Food and Biological Engineering, Jiangsu University, Xuefu Rd. 301, Zhenjiang 212013, Jiangsu, China
[b] Nucell Biotechnology Co. Ltd., Jingsi Rd. 2, Zhenjiang 212009, Jiangsu, China
[c] School of Chemistry and Chemical Engineering, Jiangsu University, Xuefu Rd. 301, Zhenjiang 212013, China
[d] School of Pharmacy, Jiangsu University, Xuefu Rd. 301, Zhenjiang 212013, Jiangsu, China
[e] School of the Environment, Jiangsu University, Xuefu Rd. 301, Zhenjiang 212013, Jiangsu, China

ARTICLE INFO

Article history:
Received 2 November 2012
Accepted 29 January 2013
Available online 12 February 2013

Keywords:
Schisandra chinensis (Turcz.) Baill
Polysaccharide
Low molecular weight
Antitumor
Immunomodulatory

ABSTRACT

A water-soluble low molecular weight polysaccharide (SCPP11) was extracted and purified using DEAE-cellulose and Sephadex G-100 column from *Schisandra chinensis* (Turcz.) Baill. Its *in vivo* and *in vitro* antitumor and immunomodulatory activity were investigated. The results showed that SCPP11 with a molecular weight of 3.4×10^3 Da exhibited indirect cyctotoxic activity against tumor cells *in vitro*, but could significantly inhibit the growth of Heps cells *in vivo* at dose of 50 mg/kg, and its inhibition rate is higher than that in the positive group. Moreover, SCPP11 could ameliorate the hematological and biochemical parameters to almost normal and no significant changes in organ weight, and could increase the body weight. In addition, SCPP11 (at 50 mg/kg) could also increased in thymus indexes as well as IL-2 and TNF-α levels in serum *in vivo* and significantly enhance the phagocytosis activity and the productions of NO of RAW264.7 *in vitro*. The results indicated that antitumor properties of SCPP11 might be achieved by improving immune response. It could be explored as a potential adjuvant against cancer used in the health food and pharmaceutical therapy.

1. Introduction

Cancer, one of the most serious diseases next to cardiovascular disease, effects human health and can even result to death if not treated early. According to GLOBOCAN 2008 statistical reports, about 12.7 million cancer cases and 7.6 million cancer deaths were recorded worldwide in 2008, furthermore, it predicts about almost 21.4 million new cancer cases and over 13.2 million deaths are likely to occur by 2030 (Ahmedin et al., 2011; Ferlay et al., 2010). Chemotherapy is one of the most frequently used therapeutic methods for the treatment of many cancer diseases; however, its continuous use can lead to cumulative toxicity in the host (Chen et al., 2012). Therefore, it is essential to develop high efficiency anti-tumor compounds with low toxicity to help curb this menace. In recent years, there is increasing attention on polysaccharides from natural sources as an efficient herbal medicine to prevent and treat cancer because of their antitumor and immunomodulatory activity (Song and Du, 2012). The antitumor properties are generally related to macrophage activation and complement system modulation (Zhao et al., 2006).

Schisandra chinensis (Turcz.) Baill in the family of Magnoliaceae is a well-known traditional Chinese herbal medicine. It was widely used as health foods and medical products. Current studies have shown that the crude polysaccharide from *S. chinensis* (Turcz.) Baill possesses various effects including antioxidant activities, anti-aging, anti-diabetic, etc. (Yan et al., 2009). However, the effect of anti-tumor activity of a water-soluble homogenous polysaccharide with low molecular weight from *S. chinensis* (Turcz.) Baill on the growth of Heps in mice has not been reported. In this study, a low molecular weight polysaccharide from *S. chinensis* (Turcz.) Baill. was extracted, purified, preliminarily characterized and investigated its antitumor activates *in vivo* and immunomodulatory activities *in vitro*. The study will be helpful to develop novel functional foods and drugs.

2. Materials and methods

2.1. Materials and reagents

The *S. chinensis* (Turcz.) Baill was obtained from Zhongxing Pharmaceutical Co., Ltd., Zhenjiang, Jiangsu Province, China. Assay kits for interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF-α) and Nitric oxide test kits test kits were obtained from

* Corresponding authors. Tel./fax: +86 511 88791800 (L. Yang), tel./fax: +86 511 88791200 (X. Wu).
E-mail addresses: yangliuqing@ujs.edu.cn (L. Yang), wuxy@ujs.edu.cn (X. Wu).

0278-6915/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2013.01.041

Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu Province, China). LPS were from Sigma Chemical Co. (St. Louis, MO, USA). Dextrans of different molecular weights (T-10, T-40, T-70, T-500, and T-2000) were obtained from Pharmacia Co., Ltd. (Uppsala, Sweden). DEAE-52 cellulose and Sephadex G-100 were obtained from Whatman Co., Ltd. (Maidstone, Kent, UK). All other chemicals and solvents used were of analytical reagent grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Animals

ICR mice (female, 20 ± 2 g), were obtained from the Comparative Medicine Center in Yangzhou University, China (the license number SCXK (SU) 2007-0001). The mice were acclimatized for a period of 2–3 days prior to the experiment. During the experiment the mice were fed under controlled environmental conditions and temperature (24 ± 1 °C) with a normal day/night cycle and humidity (55–60%). The mice were provided with a basal diet and free access to drinking water.

All the experimental procedures were conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans,EC Directive 86/609/EEC for animal experiments, Uniform Requirements for manuscripts submitted to Biomedical journals, and approved by the Jiangsu University Committee on Animal Care and Use.

2.3. Cells

Human hepatocellular carcinoma HepG-2 was obtained from Nanjing University (Nanjing, China). Heps tumor cells were obtained from the Jiangsu Cancer Hospital (Nanjing, China). RAW264.7 cells were obtained from Institute of Cell Biology, Chinese Academy Sciences (Shanghai, China). Cells were grown in DMEM medium supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, 100 μg/mL streptomycin, and incubated at 37 °C in a humidified atmosphere with 5% CO_2.

Heps tumor cells were passaged through the ICR mice before initiating the experiment. Seven days after the tumor cells were injected; ascites cells from the mice were harvested and used for the experiments *in vivo*.

2.4. Extraction, isolation and purification of polysaccharides

The fruits of *S. chinensis* were dried at 60 °C for 24 h, then crushed or ground into powder and pre-extracted for 24 h in a Soxhlet system with petroleum ether at 60 °C to remove the lipids. After defatting, the residues were extracted three times with the boiling water (1:10, w/v) for 3 h. The combined extracts were filtered and then concentrated using a rotary evaporator under reduced pressure. 95% (v/v) alcohol was added to the resulting solution slowly, with stirring, until the concentration of the alcohol reached 80% and kept overnight at 4 °C. The precipitates were collected by centrifugation at 4000 rpm for 15 min and freeze-dried. The yield of the crude polysaccharide (SCP) was 19.7%.

The SCP was re-dissolved and subjected to Trichloroacetic acid method to remove the proteins. The obtained polysaccharide was applied to a DEAE-52 (OH^-) cellulose column (1.6 × 50 cm) equilibrated with distilled water. The polysaccharide was fractionated and eluted at 1 mL/min of distilled water and different concentrations of NaCl solutions (0.05, 0.1, 0.15 and 0.2 mol/L), respectively. The main fractions were combined according to the total carbohydrate content quantified by the phenol–sulfuric acid method. Then one fraction of SCPP1 was further fractionated with size-exclusion chromatography on a Sephadex G-100 column (1.6 × 50 cm), and eluted at the flow rate of 0.3 mL/min with 0.1 M NaCl. The fraction was collected, concentrated, dialyzed and lyophilized to obtain a white purified polysaccharide (SCPP11) and the yield of SCPP11 was 44.5%. The SCPP11 was then stored in bottle desiccators at room temperature for further study.

2.5. Characterization of SCPP11

2.5.1. Homogeneity and molecular weight

The homogeneity and molecular weight distribution of SCPP11 were determined with high-performance gel-permeation chromatography (HPGPC) on a LC-10ATvp instrument (Shimadzu, Tokyo, Japan), equipped with a precolumn of TSK-GUARD COLUMN PWH (7.5 × 75 mm, Tosoh corporation, Tokyo, Japan) and a column of TSK-GEL G4000PW (7.5 × 300 mm, Tosoh corporation, Tokyo, Japan) and then eluted with 0.003 mol/L CH_3COONa solution at a flow rate of 0.8 mL/min. The peaks were detected using refractive index detector (Shimadzu RID-10A, Shimadzu). Standard dextran (Pharmacia) including T-10 (molecular mass, 1×10^4), T-40(4×10^4), T-70(7×10^4), T-500(5×10^5), and T-2000 (2×10^6) were used as molecular mass markers.

2.5.2. Monosaccharide composition

The monosaccharide compositions of SCPP11 were determined using the method reported by Yang et al. (2011). Briefly, the SCPP11 was hydrolyzed with 2.0 M H_2SO_4 at 100 °C for 8 h. After neutralization with $BaCO_3$, the supernatants were collected and lyophilized. The hydrolysate was then converted into their corresponding alditol acetates and analyzed with GC using a Shimadzu 2010 instrument, equipped with a HP-5MS column (0.25 mm × 30 m × 0.25 μm) and a flame-ionization detector. The temperature was set as follows: the initial temperature of column was 130 °C, maintained for 5 min and then increased to 240 °C at 4 °C/min. The 240 °C was held for 5 min. The standard monosaccharides were then converted to their acetylated derivative and analyzed using the same procedure.

2.5.3. Infrared spectral analysis

FT-IR analysis of SCPP11 was carried out using the potassium bromide (KBr) pellet method with a NEXUS 670 FT-IR (Thermo Nicolet, USA) spectrophotometer between 400 and 4000 cm^{-1}.

2.6. In vivo anti-tumour activities

2.6.1. Mouse tumor model

A mouse tumor model was established using the tumor inoculation method (Cai et al., 2012) and the grouping design and dose design of all drugs were by reference to the pre-test. Cell suspension was collected from peritoneal lavage of a 7-day-old mouse inoculated with Heps tumor. The survival rate of the Cells was assayed by a hemocytometer to count living cells that excluded the Trypan Blue dye. Cell suspension (2×10^7 cells/mL) was inoculated to the right armpit of the mice subcutaneously for 0.2 mL per mouse at the beginning of the experiment. After the inoculation, the tumor bearing mice were then divided into six groups (ten mice in each group), negative control group (normal saline), positive control group (5-Fu, 25 mg/kg, i.p.), intraperitoneal administration group (SCPP11, 10 or 40 mg/kg) and oral administration group (SCPP11, 50 or 200 mg/kg). Another ten mice were used as normal control group. They were treated with normal saline.

2.6.2. Antitumor activity of SCPP11 in Heps-tumor-bearing mice

After administering the mice with SCPP11 once per day for 10 days, the mice were weighed on the 11th day. Peripheral blood samples from the control and the treated mice were collected through retro-orbital puncture. After the mice were sacrificed by cervical dislocation, the tumors, thymus, spleens, livers, kidneys and hearts of the mice were collected and weighed. The inhibitory rates against the growth of tumors were calculated using the following formula: inhibitory rate (%) = $[(C - T)/C] \times 100$, where C is the average tumor weight of the negative control group while T is the average tumor weight of treated groups. Relative thymus/spleen/liver/kidney/heart weights were expressed as the ratio of the thymus/spleen/liver/kidney/heart to body weight (mg/g). The blood samples were used for hematological and biochemical analyses. The liver, kidney and heart were excised and homogenized in 0.05 g/mL wet weight of ice-cold physiological saline, respectively. The homogenate was centrifuged at 3000 rmp for 10 min, and the supernatant collected for use.

2.6.3. Biochemical assay

The anti-coagulated blood was analyzed for blood morphology and haematology parameters using analytical hematology system Symex XE-2100 (Symex Cooperation, Japan). The serum was separated from the blood without any additives by centrifugation for clinical biochemistry measurements using Olympus AU2700 equipment (Olympus Cooperation, Japan). The concentrations of interleukin-2 (IL-2) and tumor necrosis factor-alpha (TNF-α) in the serum were measured using an enzyme-linked immunosorbent assay (ELISA) (according to the indication of the manufacturer).

2.7. In vitro anti-tumour activities

The MTT assay was used for measuring the proliferation of the tumor cells. Briefly, HepG-2 cells were seeded at a density of 4×10^4 cells/mL in a volume of 0.1 mL in 96-well plates, respectively. After 24 h, the SCPP11 (0–1000 μg/mL) which was dissolved in a medium was added to each well and incubated for 48 h at 37 °C in a CO_2 incubator. 5-Fu (5-fluorouracil) was used as the positive control. After the incubation, MTT solution (100 μL/well, 1 mg/mL) was added to each well and incubated again for 4 h. The culture media were then removed and 100 μL of DMSO was added to each well for 1 h. Absorbance at 570 nm was detected by microplate ELISA reader (Spectra MAX 190, Molecular Devices Corporation, USA). The inhibition ratio of the tumor cells proliferation was determined as follows:

$$\text{Inhibition rate}(\%) = (1 - \text{Abs}_{\text{sample}}/\text{Abs}_{\text{control}}) \times 100$$

2.8. Peritoneal macrophage activation by SCPP11 in vitro

2.8.1. RAW264.7. proliferation assay

The cytotoxic effects of SCPP11 on RAW264.7 cells were measured by MTT method. In brief, RAW264.7 cells in 96-well plates (2×10^5 cells/mL) were cultured with SCPP11 (0–1000 μg/mL) and LPS (10 μg/mL) for 24 h or 48 h, and then 100 μL of MTT (1 mg/mL) was added to each well and plates were incubated for 4 h and the media removed. One hundred microliter of DMSO was later added into each well for 1 h. The absorbance at 570 nm was detected by microplate ELISA reader (Spectra MAX 190, Molecular Devices Corporation, USA).

2.8.2. Neutral red uptake by RAW264.7

RAW264.7 cells were seeded at 2×10^5 cells/mL in a 96-well plate with DMEM medium (10% FBS) and incubated at 37 °C in 5% CO_2 for 24 h. Then cells were cultured with different concentration of SCPP11 (0–1000 μg/mL) for 48 h. DMEM and 10 μg/mL LPS were used as negative and positive controls, respectively. After 48 h, supernatants were removed. One hundred microliters of 0.075% neutral red solution was added to each well and the cells were cultured for a further 4 h. The plate was washed three times with PBS and one hundred microliters of cell lysis buffer (acetic acid/ethanol; 1:1) was added to each well at 4 °C for 2 h. The absorbance at 540 nm was determined using microplate ELISA reader (Spectra MAX 190, Molecular Devices Corporation, USA). All determinations were conducted in triplicate.

2.8.3. NO emission detection in RAW264.7

RAW264.7 cells were cultured with sample in different concentrations as above. LPS (10 μg/mL) was used as positive control. After 48 h, the production of nitric oxide was estimated by measuring nitrite levels in cell supernatant with Nitric oxide test kits (Kim et al., 2012; Zhang and Dai, 2011). Nitrite production was determined by comparing the absorbance at 540 nm against a standard curve. Data were triplicate ± SD.

2.9. Statistical analysis

The statistical values were presented as mean ± standard deviation. One-way analysis of variance (ANOVA) for statistical analysis was used. The Tukey method for the multiple comparisons among the groups was used to determine the significant differences. A value of $p < 0.05$ denoted the presence of a statistically significant difference. Statistical analyses were carried out by SPSS version 16.0 (SPSS Inc, Chicago, USA).

3. Results

3.1. Characterization of SCPP11

The gel filtration chromatograms and HPGFC chromatograms of the SCPP11 is shown in Fig. 1A and B and revealed that the fraction was represented by a single and symmetrical peak which indicates a high purity of the SCPP11. The average molecular weight of the SCPP11 was 3.4×10^3 Da. As can be seen from Fig. 1(C-2), the SCPP11 was composed of mannose, glucose and galactose with molar ratio of 1:11.38:3.55.

The FT-IR spectrum of the SCPP11 is shown in Fig. 1D. The spectrum exhibits the typical signals of a polysaccharide in the range of 4000–400 cm^{-1}. The broad intense characteristic peak around 3395 cm^{-1} was due to the hydroxyl stretching vibration of the polysaccharide. The bands in the region of 2927 cm^{-1} were due to C–H stretching vibration and the band at 1384 cm^{-1} was assigned to C–H bending vibration. The band at 1652 cm^{-1} was due to the presence of bound water (Jin, 2012). The three bands at 1025–1152 cm^{-1} indicated the pyran configurations of polysaccharides. Furthermore, the characteristic absorption bands at 852 cm^{-1} and 893 cm^{-1} indicated that SCPP11 contained both α-glycosidic and β-glycosidic linkages (Jin, 2012). The bands at 855 and 931 cm^{-1} were characteristic of $(1 \rightarrow 3)$ – α-glucan (Zhu et al., 2011).

3.2. Effect of SCPP11 on tumor growth in vivo

A significant reduction in the tumor weight was observed in the SCPP11-treated mice. The inhibition ratio is summarized in Table 1. From the table, the results showed that tumor inhibitory rate of the positive control group (5-Fu) was 61.71%. The SCPP11 has shown different impact on mice transplantable Heps solid tumor at the different concentration and different administration approach. At 50 mg/kg, the oral administration of SCPP11 resulted in the highest tumor inhibition with a ratio of 68.54%, while the oral administration of SCPP11 at 200 mg/kg and the intraperitoneal administration (10 and 40 mg/kg) of SCPP11 recorded the lower inhibition ratio. No obvious dose-dependency relationship was observed between different concentrations and growth inhibition of Heps. The antitumor activities *in vivo* against Heps tumor cells of SCPP11

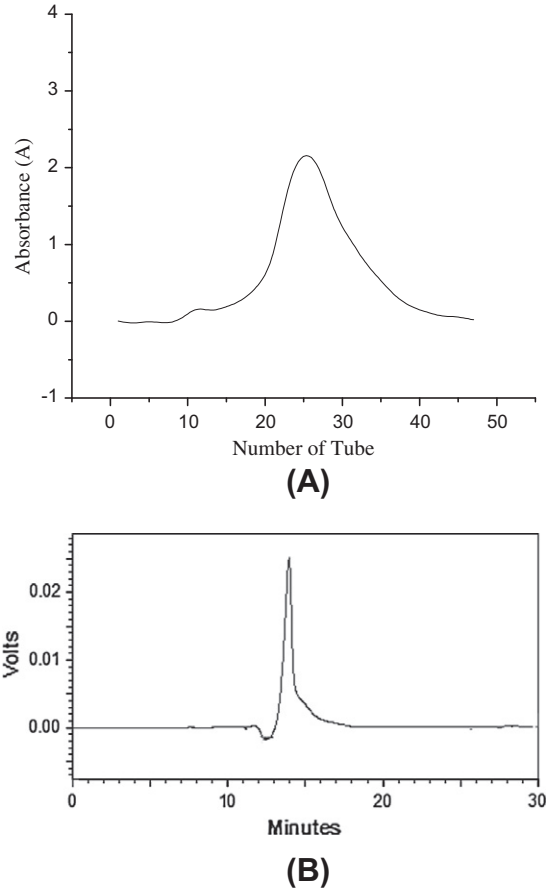


Fig. 1. (A) Gel filtration chromatogram of SCPP11 on Sephadex G-100 column. SCPP1 was obtained from DEAE-52 elution with distilled water, and followed by Sephadex G-100 at the flow rate of 0.3 mL/min with 0.1 M NaCl solution. Total carbohydrate content of the fractions was determined by a phenol–sulfuric acid method (detected at 490 nm). (B) GPC elution profiles of the SCPP11 with refractive index detector. HPGPC was performed at 25 °C. The flow rate was 0.8 mL/min. The elution solvent was 0.003 mol/L sodium acetate solution. (C-1) Gas chromatograms of acetate-derivatized standard monosacharides. (C-2) Gas chromatograms of the monosaccharide compositions of the SCPP11. (a) l-rhamnose (Rha), (b) l-arabinose (Ara), (c) d-xylose (Xyl), (d) d-mannose (Man), (e) d-glucose (Glu), (f) d-galactose (Gal), and (g) acetyl inositol (the internal standard). (D) FT-IR spectrum of the SCPP11 was recorded with a NEXUS 670 FT-IR spectrophotometer between 400 and 4000 cm^{-1} using the KBr-disk method.

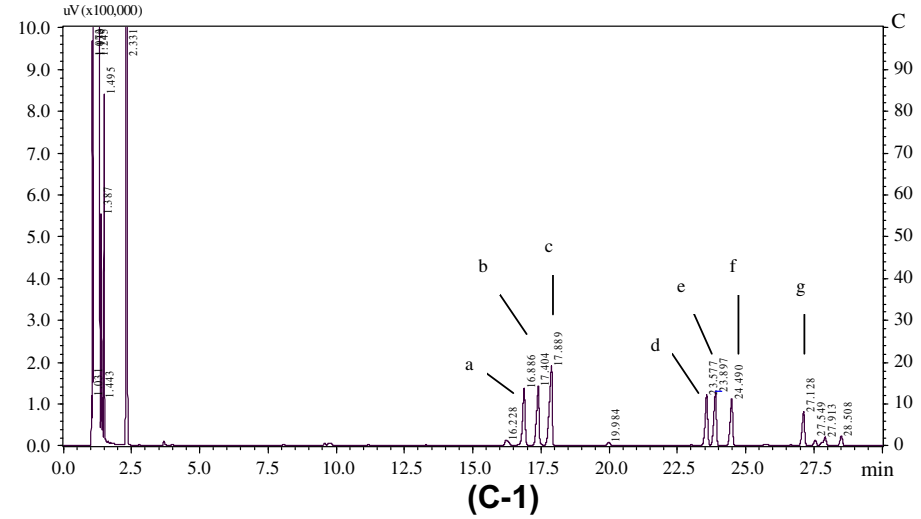
were derived from stimulation of the immunoresponse mechanism, and do not strictly follow the dose-dependency of chemotherapeutic anticancer agents (Chen et al., 2010).

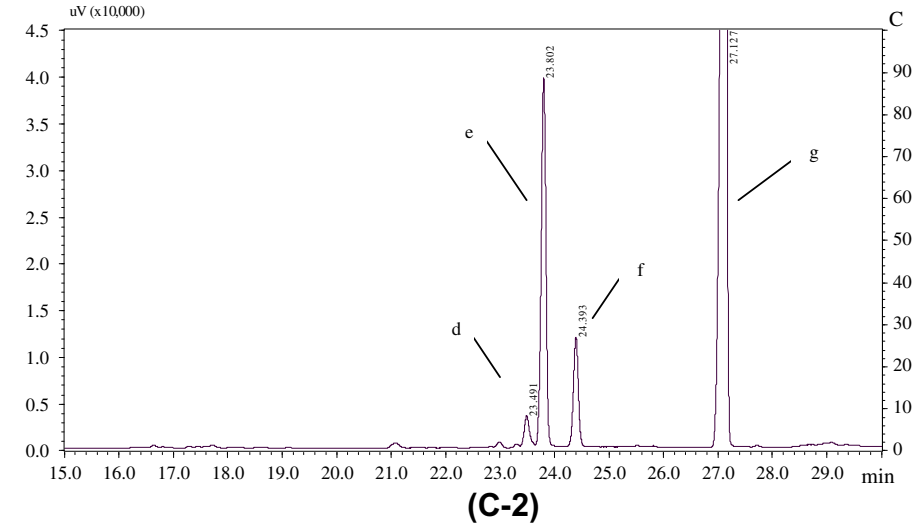
3.3. Effect of SCPP11 on body weight on mice

The body weight gain of the control and experimental mice during the experimental period is shown in Table 1. After 10 days of treatment, the ratios of the body weights gain of the polysaccharide administration groups were significantly higher than those of 5-Fu treated group. The oral administration group of SCPP11 at 50 mg/kg was superior to the 5-Fu treated group in the anti-tumor effect. Furthermore, the ratio of the body weight gain in the oral administration group of SCPP11 at 50 mg/kg was 23.67%, which was significantly higher than that of the 5-Fu treated group (8.52%).

3.4. Effect of SCPP11 on organ weight in mice

The effects of SCPP11 on spleen and thymus weights of Heps-bearing mice are shown in Table 2. A significant decrease in the





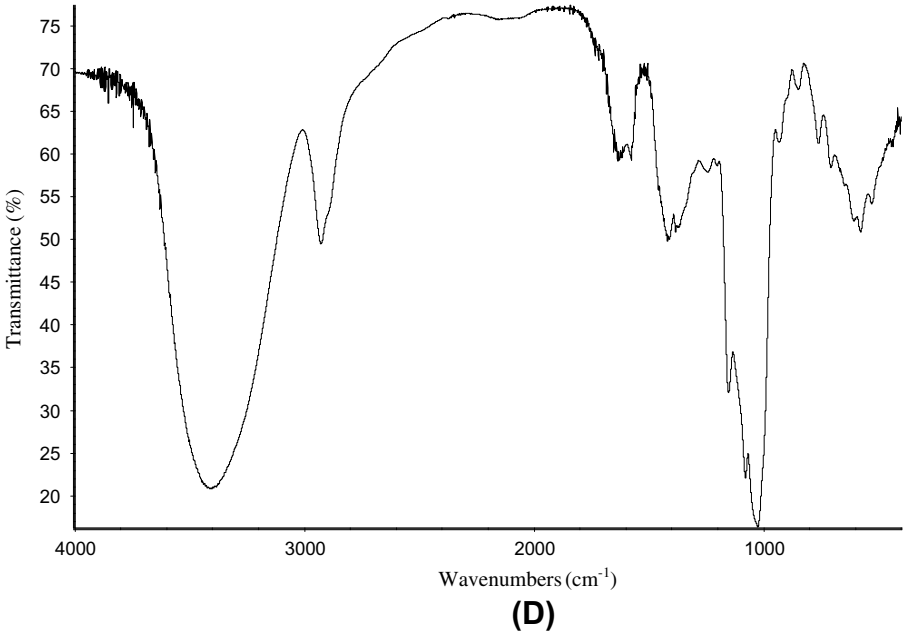


Fig. 1. (*continued*)

thymus and spleen indexes were observed in the 5-Fu group when compared with that in the negative control group, which indicated that 5-Fu would suppress the immunological function in the mice, as described previous studies (Lins et al., 2009; Ni et al., 2010).

Table 1
Antitumor activities of SCPP11 against Heps solid tumor grown in ICR mice.

Groups	Dose (mg/kg)	Ratio of body weight gain (%)	Weight of tumor (g)	Inhibitory rate of tumor (%)
Normal		8.72		
Negative control		29.23	1.82 ± 0.29	
Positive control	25	8.52	0.82 ± 0.23[a]	61.71
SCPP11 (i.g.)	200	35.89	1.36 ± 0.37	23.55
	50	23.67	0.67 ± 0.29[a]	68.54
SCPP11(i.p.)	40	23.41	1.51 ± 0.15	29.35
	10	31.00	1.28 ± 0.38[a]	40.15

Each value is the mean ± SD of 10 separate experiments.
[a] *P* values are shown as $p < 0.05$ vs. positive control group in the same column. A value of $p < 0.05$ denoted the presence of a statistically significant difference according to Tukey method.

Table 2
Effects of SCPP11 on organ weights. Mice were injected with Heps (4.0 × 106 cells/animal). The animals were treated, starting 1 day after tumor implant, for ten consecutive days.

Groups	Dose (mg/kg)	Spleen (mg/g)	Thymus (mg/g)	Live (mg/g)	Kidney (mg/g)	Heart (mg/g)
Normal		4.95 ± 0.63	2.41 ± 0.42	42.50 ± 4.03	10.99 ± 1.22	4.64 ± 0.58
Negative control		5.12 ± 0.37	2.16 ± 0.39	44.93 ± 5.02	10.17 ± 1.55	3.81 ± 0.42
Positive control	25	4.01 ± 0.48 [b]	1.13 ± 0.29[a,b]	50.17 ± 4.35[a]	11.58 ± 1.38	4.32 ± 0.63
SCPP11 (i.g.)	200	5.91 ± 0.36 [c]	2.23 ± 0.17[c]	46.82 ± 4.33	9.80 ± 0.57	3.98 ± 0.71
	50	5.17 ± 0.70 [c]	3.29 ± 0.44[b,cc]	44.56 ± 2.11	10.34 ± 1.72	3.98 ± 0.12
SCPP11(i.p.)	40	5.61 ± 0.67 [c]	3.34 ± 0.62[a,b,cc]	44.97 ± 5.04	10.63 ± 1.02	3.90 ± 0.43
	10	5.44 ± 0.23 [c]	2.59 ± 0.97[c]	44.66 ± 5.65	9.95 ± 0.48	4.25 ± 0.47

Each value is the mean ± SD of 10 separate experiments. *P* values are shown as [a]$p < 0.05$ vs. normal control group, [b]$p < 0.05$ vs. negative control group and [c]$p < 0.05$ or [cc]$p < 0.01$ vs. positive control group in the same column. A value of $p < 0.05$ denoted the presence of a statistically significant difference according to Tukey method.

Moreover, the SCPP11 treatment groups exhibited a stronger effect on the thymus index compared with the negative control group. Especially the oral administration group of the SCPP11 at 50 mg/kg showed significant increase in thymus index compared with the negative control group ($p < 0.05$). Meanwhile the SCPP11 could also increase the spleen indexes to some extent. The results supported the stimulation of the SCPP11 on the immune system.

After the mice were killed at the end of the experiment, the weights of the livers, kidneys and hearts were recorded and their corresponding indexes were recorded and determined. The result is summarized in Table 2. From the table comparing the weights of the livers, kidneys and hearts of the experimented mice with the normal control group, it was realized that no significant changes in the livers, kidneys and hearts occurred in the SCPP11 treatment groups except the livers indexes of the 5-Fu treated group, which supported the toxicity of 5-Fu on the liver.

3.5. Effect of SCPP11 on TNF-α and IL-2 secretion from serum in mice

The effects of SCPP11 on TNF-α and IL-2 level in the serum of Heps tumor-bearing mice were investigated. As can be seen from Fig. 2, the production of TNF-α and IL-2 was significantly decreased in 5-Fu treated mice as compared with negative control ($p < 0.05$), which indicated that 5-Fu could suppress the secretion of two cytokines. However, in the SCPP11-treated mice, the production of TNF-α and IL-2 was restored in a dose-independent manner, especially the serum TNF-α and IL-2 in the oral administration group of the SCPP11 at 50 mg/kg which was enhanced when compared with the negative control, even though these changes were not statistically significant.

3.6. Hematological and biochemical analyses

In order to determine the effect of SCPP11 on hematological changes of the peripheral blood of Heps-bearing mice, comparison was made amongst all the groups. As shown in Table 3, a decrease in total numbers of circulating peripheral leukocytes was observed in the mice treated with 5-Fu when compared with those of the Heps-bearing mice that were treated only with saline. Moreover, the total leukocytes of mice treated with SCPP11 were increased as compared with the mice in negative control group and 5-Fu treated group. In addition, SCPP11 could ameliorate the abnormality of erythrocytes and platelet in peripheral blood of tumor-bearing mice, but 5-Fu had no apparent effect. These results indicated that SCPP11 administration to Heps-bearing mice could reverse the changes in the hematological parameters as compared with 5-Fu administration group.

The AST and ALT activities as biochemical markers for hepatic damage were determined in the plasma of Heps-bearing mice treated with 5-Fu or SCPP11. From Table 4, the mice in the negative group exhibited a significant increase ($p < 0.05$) in the level of AST as compared to those of the normal control group, but there was no differences in relation to the ALT levels. When the tumor bearing mice were given SCPP11 administration, the level of AST was significantly ameliorated to almost normal ($p < 0.05$), but the level in the 5-Fu treated group continued to rise. In addition, 5-Fu treated group also could make tumor-bearing mice abnormal in ALT levels compared with negative group and normal control group.

3.7. Effect of SCPP11 on tumor growth in vitro

The growth inhibitory effects of SCPP11 against HepG-2 cells in *vitro* were examined. As exhibited by other polysaccharides (Silva et al., 2012), at 1000 μg/mL, the inhibition ratio of the SCPP11 was low than 10% *in vitro* (concrete data not shown). It is indicated that the SCPP11 exhibited indirect cyctotoxic activity against tumor cells.

3.8. Effect of SCPP11 on peritoneal macrophages activation in vitro

The cytotoxicity or stimulation of SCPP11 and LPS on RAW264.7 cells are shown in Fig. 3A. After 24 or 48 h incubation with varying concentrations of SCPP11, the OD values did not change significantly. The results indicated that the SCPP11 showed no significant effects on RAW264.7 cells up to 1000 μg/mL.

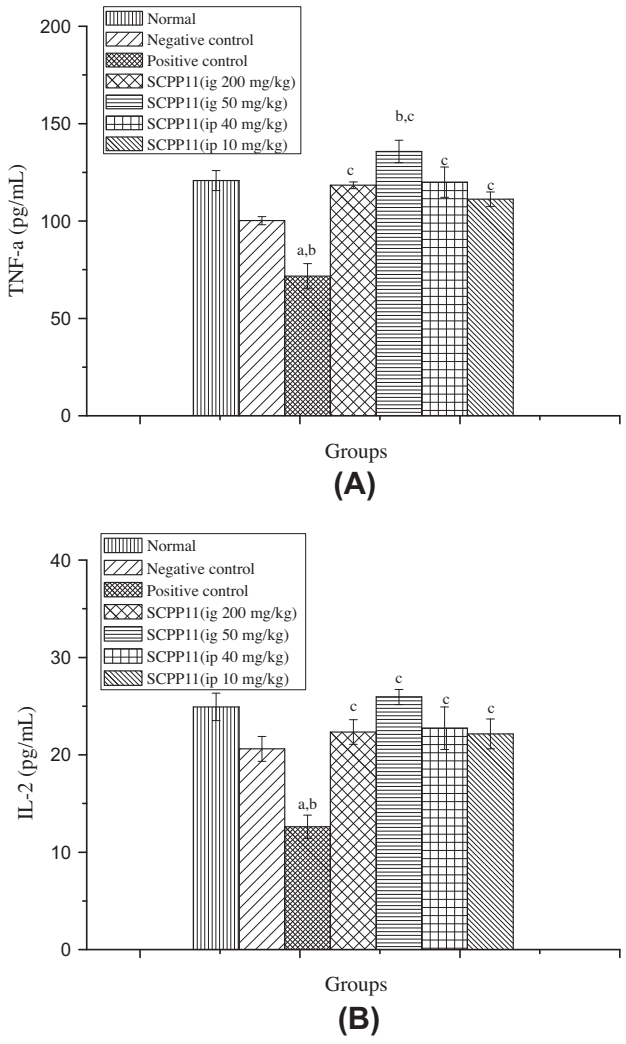


Fig. 2. Effect of SCPP11 on TNF-α and IL-2 secretion from serum in Heps-bearing mice. Mice were injected with Heps (4×10^6 cells per animal, s.c.). After tumor implant, 5-Fu-treated mice were injected i.p. with 5-Fu at a dose of 25 mg/kg for 10 days once every other day. SCPP11-treated mice were administrated orally at the does of 200, 50 mg/kg and (ig) and were injected i.p. at the dose of 40, 10 mg/kg, respectively, for 10 days. On days 11, the blood was collected for the TNF-α and IL-2 levels assays. Each value is presented as mean ± SD. *P* values are shown as $^{b}p < 0.05$ vs. negative control group and $^{c}p < 0.05$ vs. positive control group in the same column. A value of $p < 0.05$ denoted the presence of a statistically significant difference according to Tukey method.

From Fig. 3B, the SCPP11 could enhance the phagocytosis activity of RAW264.7 with a dose-dependent way. Compared with the control group, the higher concentration of SCPP11 significantly stimulated phagocytosis of RAW264.7 cells (250 μg/mL, $^{*}p < 0.05$; 500 μg/mL and 1000 μg/mL $^{**}p < 0.01$). Moreover the changes in positive group (LPS 10 μg/mL) were not significant.

As can be seen from Fig. 3C, SCPP11 induced NO production in dose-dependent manner. The SCPP11 significantly induced production of NO in macrophages at the concentration 125 μg/mL and above compared with the control group ($^{**}p < 0.01$). The results indicated that SCPP11 could stimulate the NO production of RAW264.7.

Table 4
Effect of SCPP11 on the biochemical changes on peripheral blood. Mice were injected with Heps (4.0×10^6 cells/animal). The animals were treated, starting 1 day after tumor implant, for ten consecutive days.

Groups	Dose (mg/kg)	AST	ALT
Normal		213.51 ± 13.44	55.42 ± 2.83
Negative control		643.14 ± 12.73[a]	54.59 ± 3.53
Positive control	25	1020.59 ± 13.44[a,b]	67.95 ± 1.41[a,b]
SCPP11 (i.g.)	200	655.43 ± 8.49[a,c]	58.33 ± 2.83[c]
	50	358.95 ± 41.01[b,c]	61.16 ± 1.41
SCPP11 (i.p.)	40	297.36 ± 39.60[b,c]	52.57 ± 0.71[c]
	10	247.52 ± 58.69[b,c]	53.84 ± 2.82[c]

Each value is the mean ± SD. *P* values are shown as $^{a}p < 0.05$ vs. normal control group, $^{b}p < 0.05$ vs. negative control group and $^{c}p < 0.05$ vs. positive control group in the same column. A value of $p < 0.05$ denoted the presence of a statistically significant difference according to Tukey method.

4. Discussion

Cancer is recognized as one of the most formidable problematic disease on human health. Some chemical compounds have high anti-tumor effect, however, they have also got some side effects. Therefore, it is very important to exploit high efficiency anti-tumor compounds with low toxicity. In recent years, numerous polysaccharides were extracted from plants, fungi, algae and some other animals (Chen et al., 2012). Many kinds of polysaccharides, as natural biological macromolecules, have been proved to exhibit significant antitumor activities with little toxicity to hosts, such as *ganoderma lucidum* (Zhang et al., 2012), *asparagus officinalis* (Zhao et al., 2012b), etc.

The immune system plays an important role in antitumor defense (Chen et al., 2012). Most reports confirmed that polysaccharides exerted their anti-tumor effect because of the activation of the immune system of the host animal (Chen et al., 2012; Han et al., 2011; Wu et al., 2012). As the important immune organs, the spleen indexes and thymus indexes reflect the immune function of the organism. Immunopotentiator could increase the weight of the organism. The IL-2 is one of the important cytokines for regulation of immune responses which can enhance the cytolytic activity of NK cells and T lymphocytes as well as the proliferation of B lymphocytes (Chen et al., 2011). On the other hand, TNF-α is also able to cause apoptosis of tumor cell and plays an important role in immunoregulatory and inflammatory mediators. In

Table 3
Effect of SCPP11 on the hematological changes in peripheral blood. Mice were injected with Heps (4.0×10^6 cells/animal). The animals were treated, starting 1 day after tumor implant, for ten consecutive days.

Groups	Dose (mg/kg)	Platelet (10^5 cells/μL)	Total leukocytes (10^3 cells/μL)	Erythrocytes (10^6 cells/μL)
Normal		11.29 ± 0.89	4.47 ± 0.60	9.83 ± 0.26
Negative control		9.19 ± 2.85	3.31 ± 0.14	8.60 ± 0.46
Positive control	25	8.08 ± 1.53	2.45 ± 0.07	7.98 ± 1.22
SCPP11 (i.g.)	200	9.83 ± 0.59	5.70 ± 0.42[c]	8.57 ± 0.18
	50	9.57 ± 0.17	7.93 ± 1.63[a,b,c]	9.90 ± 0.12
SCPP11(i.p.)	40	9.22 ± 0.82	4.96 ± 1.20	8.25 ± 0.86
	10	10.27 ± 0.38	6.74 ± 1.13[b,c]	8.17 ± 1.01

Each value is the mean ± SD. *P* values are shown as $^{a}p < 0.05$ vs. normal control group, $^{b}p < 0.05$ vs. negative control group and $^{c}p < 0.05$ vs. positive control group in the same column. A value of $p < 0.05$ denoted the presence of a statistically significant difference according to Tukey method.

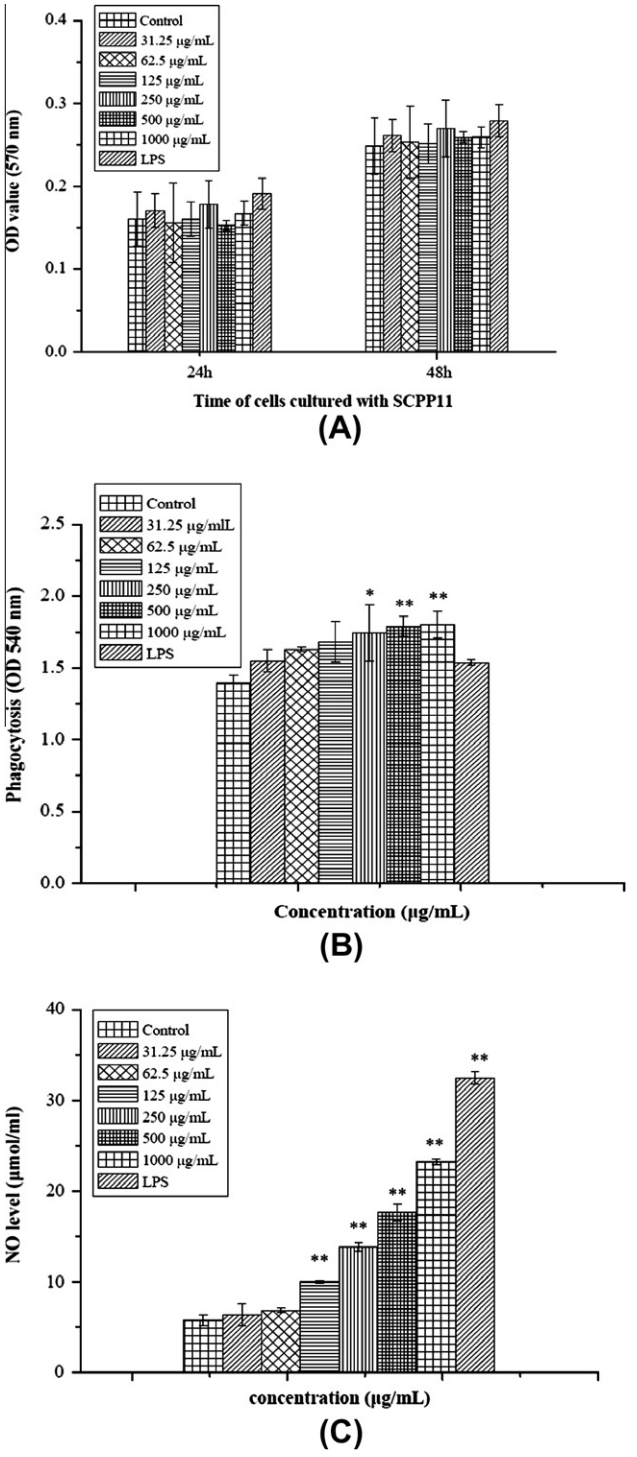


Fig. 3. (A) Effects of SCPP11 on the proliferation of RAW264.7 macrophages. RAW264.7 cells (2×10^5 cells/mL) were stimulated with SCPP11 at concentrations of 0–1000 μg/mL and LPS at 10 μg/mL for 24 h or 48 h. After incubation, the proliferation was measured by MTT assay. Each value is presented as mean ± SD ($n = 5$). (B) Effect of SCPP11 on phagocytosis of RAW264.7 macrophages by neutral red uptake assay. RAW264.7 cells (2×10^5 cells/mL) were stimulated with SCPP11 (0–1000 μg/mL) and LPS (10 μg/mL) for 48 h. The absorbance at 540 nm was determined. Each value is presented as mean ± SD ($n = 5$). P values are shown as $^{*}p < 0.05$, $^{**}p < 0.01$ compared with control group. (C) Effect of the SCPP11 on inducing NO production of RAW264.7 cells. RAW264.7 cells (2×10^5 cells/mL) were stimulated with SCPP11 (0–1000 μg/mL) and LPS (10 μg/mL) for 48 h. The supernatant nitrite levels were determined using Nitric oxide test kits. Each value is presented as mean ± SD ($n = 5$). *P* values are shown as $^{*}p < 0.05$, $^{**}p < 0.01$ compared with control group.

addition, macrophages are one kind of multipotential cells, it participate in both specific and non-specific immune reactions. So macrophages as important immunocytes play an essential role in anti-tumor. In the study, it has revealed that SCPP11 (at 50 mg/kg) could improve the weight of thymus of tumor-bearing mice when compared with negative control group. SCPP11 (at 50 mg/kg) could increase the concentration of IL-2 and TNF-α in serum of Heps-bearing mice simultaneously. Moreover the SCPP11 also could significantly enhance the phagocytosis activity and the productions of NO of RAW264.7 *in vitro*. The result indicated that SCPP11 influences immuno-regulating property, which may be involved in its antitumor activity.

5-Fu is one of the most effective antitumor drugs, but because its toxicity nature, its application is limited. The toxicological profile of SCPP11 was comparatively investigated in this paper. In the preliminary assessment, the toxicity was evaluated based on the body weight and organ weight. Hematological and biochemical analyses were also carried out. The results have showed that, compared with positive control group, the SCPP11 could increase the body weight and reverse the changes in the hematological and biochemical parameters, which indicates that SCPP11 exhibits antitumor effects with no expressive toxicity. It would be an advantage to promote the development of antitumor polysaccharide from *S. chinensis* (Turcz.) Baill.

Previous study indicated that polysaccharide from *S. chinensis* (Turcz.) Baill have many activities including anti-tumor activity, immunostimulatory activity and anti-diabetic activity. Study conducted by Xu et al. (2012a) has shown that polysaccharide from *S. chinensis* leaf with the average molecular weight of 127 kDa could inhibit the proliferation of L5178Y lymphoma *in vivo*. Another polysaccharide fraction from *S. chinensis* (Turcz.) Baill with the average molecular weight of 23 kDa can significantly reduce blood glucose levels *in vivo* (Xv et al., 2008). Those investigations showed that polysaccharides with relatively high molecular weight exhibited stronger activities. Whereas, in this study, a polysaccharide (SCPP11) with a molecular weight of 3.4 kDa could significantly inhibit the growth of Heps transplantable tumor in mice at the dose of 50 mg/kg and exhibit little cytotoxicity to HepG-2 cells *in vitro*. It is important to note that the effect of SCPP11 is greater than that observed with other polysaccharides using the human hepatic tumor model (Liang et al., 2012; Liu et al., 2012; Tao et al., 2006; Xu et al., 2012b; Zhao et al., 2012a; Zhao et al., 2010).

5. Conclusion

A water-soluble low molecular weight homogenous polysaccharide (SCPP11) from *S. chinensis* (Turcz.) Baill. exhibited indirect cyctotoxic activity against tumor cells *in vitro*, but could significantly inhibit the growth of Heps cells *in vivo* at dose of 50 mg/kg, and its inhibition rate is higher than that in the positive group. Moreover, SCPP11 (at 50 mg/kg) could significant elevate the thymus indexes and the IL-2 and TNF-α level in serum. At the same time, SCPP11 could promote the phagocytosis and production of NO of RAW 264.7 compared with control. The results indicated that SCPP11 may indirectly play the role of antitumor activity through improving immunologic function. SCPP11 indicated no toxicity to body weight, the liver, kidney and heart simultaneously, however it could ameliorate the hematological and biochemical parameters to almost normal. This therefore indicates that SCPP11 had potent anti-tumor properties, which would be explored as a potential adjuvant against cancer used in the health food and pharmaceutical therapy. It is also recommended that further study should be carried out to elucidate the structure and possible immune mechanism of SCPP11.

Conflict of Interest

None.

Acknowledgments

This work was supported financially by Natural Science Fund of Jiangu Province (BK2012702) and Graduate Innovative Projects in Jiangu Province (CXZZ11-0589). We also appreciate Dr. Mohanmmed Takase for language polishing.

References

Ahmedin, J., Freddie, B., Center, M.M., Jacques, F., Elizabeth, W., David, F., 2011. Global cancer statistics. CA – Cancer J. Clin. 61, 69–90.

Cai, Z., Li, W., Wang, H., Yan, W., Zhou, Y., Wang, G., Cui, J., Wang, F., 2012. Antitumor and immunomodulating activities of a polysaccharide from the root of *Sanguisorba officinalis* L.. Int. J. Biol. Macromol. 51, 484–488.

Chen, L., Pan, J., Li, X., Zhou, Y., Meng, Q., Wang, Q., 2011. Endo-polysaccharide of *Phellinus igniarius* exhibited anti-tumor effect through enhancement of cell mediated immunity. Int. Immunopharmacol. 11, 255–259.

Chen, R., Meng, F., Liu, Z., Chen, R., Zhang, M., 2010. Antitumor activities of different fractions of polysaccharide purified from Ornithogalum caudatum Ait. Carbohyd. Polym. 80, 845–851.

Chen, X., Nie, W., Yu, G., Li, Y., Hu, Y., Lu, J., Jin, L., 2012. Antitumor and immunomodulatory activity of polysaccharides from *Sargassum fusiforme*. Food Chem. Toxicol. 50, 695–700.

Ferlay, J., Shin, H.R., Bray, F., Forman, D., Mathers, C.D., Parkin, D., 2010. GLOBOCAN 2008, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10.Lyon, France: International Agency for Research on Cancer; Year. <http://globocan.iarc.fr>. (accessed 31.07.11).

Han, X.-Q., Wu, X.-M., Chai, X.-Y., Chen, D., Dai, H., Dong, H.-L., Ma, Z.-Z., Gao, X.-M., Tu, P.-F., 2011. Isolation, characterization and immunological activity of a polysaccharide from the fruit bodies of an edible mushroom, *Sarcodon aspratus* (Berk.) S. Ito. Food Res. Int. 44, 489–493.

Jin, X., 2012. Bioactivities of water-soluble polysaccharides from fruit shell of Camellia oleifera Abel: antitumor and antioxidant activities. Carbohyd. Polym. 87, 2198–2201.

Kim, H.S., Kim, Y.J., Lee, H.K., Ryu, H.S., Kim, J.S., Yoon, M.J., Kang, J.S., Hong, J.T., Kim, Y., Han, S.-B., 2012. Activation of macrophages by polysaccharide isolated from *Paecilomyces cicadae* through toll-like receptor 4. Food Chem. Toxicol. 50, 3190–3197.

Liang, M., Li, S., Shen, B., Cai, J., Li, C., Wang, Z., Li, X., Gao, J., Huang, H., Zhang, X., Li, J., 2012. Anti-hepatocarcinoma effects of Aconitum coreanum polysaccharides. Carbohyd. Polym. 88, 973–976.

Lins, K., Bezerra, D.P., Alves, A., Alencar, N.M.N., Lima, M.W., Torres, V.M., Farias, W.R.L., Pessoa, C., de Moraes, M.O., Costa-Lotufo, L.V., 2009. Antitumor properties of a sulfated polysaccharide from the red seaweed *Champia feldmannii* (Diaz-Pifferer). J. Appl. Toxicol. 29, 20–26.

Liu, Y., Li, Y., Yang, W., Zhang, L., Cao, G., 2012. Anti-hepatoma activity in mice of a polysaccharide from the rhizome of *Anemone raddeana*. Int. J. Biol. Macromol. 50, 632–636.

Ni, W.H., Zhang, X., Wang, B., Chen, Y., Han, H., Fan, Y.Y., Zhou, Y.F., Tai, G.H., 2010. Antitumor activities and immunomodulatory effects of ginseng neutral polysaccharides in combination with 5-fluorouracil. J. Med. Food 13, 270–277.

Silva, D.C., Freitas, A.L.P., Barros, F.C.N., Lins, K., Alves, A., Alencar, N.M.N., de Figueiredo, I.S.T., Pessoa, C., de Moraes, M.O., Costa-Lotufo, L.V., Feitosa, J.P.A., Maciel, J.S., de Paula, R.C.M., 2012. Polysaccharide isolated from *Passiflora edulis*: characterization and antitumor properties. Carbohyd. Polym. 87, 139–145.

Song, G., Du, Q., 2012. Structure characterization and antitumor activity of an α, β-glucan polysaccharide from *Auricularia polytricha*. Food Res. Int. 45, 381–387.

Tao, Y., Zhang, L., Cheung, P.C.K., 2006. Physicochemical properties and antitumor activities of water-soluble native and sulfated hyperbranched mushroom polysaccharides. Carbohyd. Res. 341, 2261–2269.

Wu, X., Mao, G., Fan, Q., Zhao, T., Zhao, J., Li, F., Yang, L., 2012. Isolation, purification, immunological and anti-tumor activities of polysaccharides from *Gymnema sylvestre*. Food Res. Int. 48, 935–939.

Xu, C., Li, Y., Dong, M., Wu, X., Wang, X., Xiao, Y., 2012a. Inhibitory effect of *Schisandra chinensis* leaf polysaccharide against L5178Y lymphoma. Carbohyd. Polym. 88, 21–25.

Xu, C., Liu, Y., Yuan, G., Guan, M., 2012b. The contribution of side chains to antitumor activity of a polysaccharide from *Codonopsis pilosula*. Int. J. Biol. Macromol. 50, 891–894.

Xv, G., Jun, M., Hai, L.J., Bin, T., 2008. Hypoglycemic effects of a water-soluble polysaccharide isolated from *Schisandra chinensis* (Turcz.) Baill in alloxan-induced diabetic mice. J. Biotechnol., 136.

Yan, S., Yang, L.Q., Zhao, T., Zhao, J.L., Zou, Y.M., 2009. Latest research progress on polysaccharide from *Schisandra chinensis* (Turcz.) Baill. J. Jiangsu Univ. (Med. Ed.) 19, 366–368.

Yang, L.Q., Zhao, T., Wei, H., Zhang, M., Zou, Y., Mao, G.H., Wu, X.Y., 2011. Carboxymethylation of polysaccharides from *Auricularia auricula* and their antioxidant activities in vitro. Int. J. Biol. Macromol. 49, 1124–1130.

Zhang, C.-X., Dai, Z.-R., 2011. Immunomodulatory activities on macrophage of a polysaccharide from *Sipunculus nudus* L. Food Chem. Toxicol. 49, 2961–2967.

Zhang, J., Liu, Y.J., Park, H.S., Xia, Y.M., Kim, G.S., 2012. Antitumor activity of sulfated extracellular polysaccharides of *Ganoderma lucidum* from the submerged fermentation broth. Carbohyd. Polym. 87, 1539–1544.

Zhao, H., Li, Y., Wang, Y., Zhang, J., Ouyang, X., Peng, R., Yang, J., 2012a. Antitumor and immunostimulatory activity of a polysaccharide-protein complex from *Scolopendra subspinipes mutilans* L. Koch in tumor-bearing mice. Food Chem. Toxicol. 50, 2648–2655.

Zhao, L., Chen, Y., Ren, S., Han, Y., Cheng, H., 2010. Studies on the chemical structure and antitumor activity of an exopolysaccharide from *Rhizobium* sp. N613. Carbohyd. Res. 345, 637–643.

Zhao, Q., Xie, B., Yan, J., Zhao, F., Xiao, J., Yao, L., Zhao, B., Huang, Y., 2012b. *In vitro* antioxidant and antitumor activities of polysaccharides extracted from *Asparagus officinalis*. Carbohyd. Polym. 87, 392–396.

Zhao, Z., Li, J., Wu, X., Dai, H., Gao, X., Liu, M., Tu, P., 2006. Structures and immunological activities of two pectic polysaccharides from the fruits of *Ziziphus jujuba Mill.* cv. jinsixiaozao Hort. Food Res. Int. 39, 917–923.

Zhu, Z.Y., Liu, R.Q., Si, C.L., Zhou, F., Wang, Y.X., Ding, L.N., Jing, C., Liu, A.J., Zhang, Y.M., 2011. Structural analysis and anti-tumor activity comparison of polysaccharides from Astragalus. Carbohyd. Polym. 85, 895–902.