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Antitumor and immunomodulatory activities of a water-soluble polysaccharide from *Chaenomeles speciosa*

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ABSTRACT

In this study, a water-soluble polysaccharide (CSP) was successfully purified from *Chaenomeles speciosa* by DEAE-Sepharose and Sephadex G-100 column chromatography. CSP had a weight-average molecular weight of about 6.3×10^4 Da and was composed of glucose (Glc), galactose (Gal), rhamnose (Rha) and arabinose (Ara) with a relative molar ratio of 4.6:1.3:0.8:0.5. CSP could not only inhibit the growth of S180 tumor transplanted in mice, but also increase the relative spleen index and body weight of tumor bearing mice. Moreover, concanavalin A (ConA) and lipopolysaccharide (LPS) induced splenocyte proliferation and peritoneal macrophage phagocytosis were also enhanced after CSP administration. Furthermore, CSP treatment could improve delayed type hypersensitivity (DTH) and promote the secretion of IL-2, TNF-α and IFN-γ in serum. The overall findings suggest that the antitumor effect of CSP is might be associated with its potent immunostimulatory activity.

1. Introduction

Chaenomeles speciosa (Sweet) Nakai (Rosaceae) is a well-known food in China, and its dried fruit is commonly used in traditional medicine (Jiangsu New Medical College, 1977). Modern studies have shown that *C. speciosa* has a variety of biological and pharmacological activities such as immunomodulatory, antiinflammatory, antitumor, antimicrobial and antioxidant effects (Dai, Wei, Shen, & Zheng, 2003; Xie, Cai, Zhu, & Zou, 2007; Zhang et al., 2010). Evidence has suggest that carbohydrates, amino acids, proteins, tannins and other organic acids are the main ingredients of *C. speciosa* fruits (Chen & Wei, 2003; Chen, Wu, & Dai, 2000; Li & He, 2005).

Cancer is one of the major causes of human death worldwide. The great majority of chemical compounds, which have been identified as cytotoxic to cancer cells, are also toxic to normal cells (Borchers, Stern, Hackman, Keen, & Gershwin, 1999). Recently, research interest has focused on polysaccharides from various plants and herbs with immune-stimulating properties, which may be useful complementary or alternative medicine in helping humans to reduce the risk of cancer as well as infectious disease. Most polysaccharides derived from higher plants are relatively nontoxic and do not cause significant side effects compared with synthetic compounds, and hence they are ideal candidates for modern medicine (Wang, Chen, Jia, Tang, & Ma, 2012; Zhang, Li, Xiong, Jiang, & Lai, 2013). Therefore, discovery and evaluation of polysaccharides with antitumor and immunostimulating properties have emerged as one of the important research fields in chemistry and biology (Fan, Ding, Ai, & Deng, 2012; Li et al., 2012).

Polysaccharides from *C. speciosa* have been found to be involved in antioxidant and antiinflammatory activities (Li & Chen, 2011; Liu, Wang, Lu, Ma, & Zhang, 2011a). However the antitumor and immunoregulatory properties of water-soluble polysaccharides from *C. speciosa* have not previously been investigated, in this regard the objectives of this study are to elucidate the isolation and characterization of the biological polysaccharide from *C. speciosa* and evaluate its antitumor and immunomodulatory activities on the immune response in tumor-bearing mice by using both *in vitro* and *in vivo* assay.

2. Materials and methods

2.1. Materials and reagents

Dried fruits of *C. speciosa* were collected from Changyang in Hubei Province (China). A voucher specimen was deposited in the herbarium of College of Life Sciences, Wuhan University, China.

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Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra zolium bromide (MTT), trifluoroacetic acid (TFA), concanavalin A (ConA), lipopolysaccharide (LPS), 2,4-dinitrofluorobenzene (DNFB), standard sugars and T-series dextran were purchased from Sigma Chemical Co. (St. Louis, MO, USA). RPMI 1640 medium and bovine serum albumin (BSA) were purchased from Gibco (Grand Island, NY, USA). Cyclophosphamide (CTX) was purchased from Jiangsu Hengrui Co. (Lianyungang, China). DEAE-Sepharose and Sephadex G-100 were purchased from Amersham Pharmacia Co. (Sweden). All other chemical reagents used were analytical grade.

2.2. Extraction, isolation and purification of the polysaccharide

Dried powdered fruits (200 g) were extracted by hot water under stirring for 5 h in a boiling-water bath (at the ratio of 1:20, w/v) and repeated twice. The aqueous extract was filtered, and the polysaccharides were precipitated from the filtrates by 95% ethanol (1:4, v/v), then keeping it overnight to ensure the precipitates is complete. After standing out overnight at 4 °C, it was centrifuged for 30 min at 4500 × *g*. The precipitated materials were collected and dissolved in water to sufficiently mix with Sevag reagent for removing the free protein and combined protein (Staub, 1965) in this precipitate. The sample was then dialysed against distilled water and precipitated by four volumes of 95% ethanol. The precipitate was collected by centrifugation, washed successively with ethanol, acetone and petroleum ether and lyophilized to obtain the crude polysaccharide (21 g).

The crude polysaccharide (8 g) was redissolved in distilled water (100 ml) and applied to a DEAE-Sepharose Fast-Flow chromatography column (2.6 × 40 cm), and eluted stepwise with distilled water and a gradient of 0.1–0.5 M NaCl at a flow rate of 1 ml/min. Guided by the phenol–sulfuric acid method, the water eluting fraction with high content of sugar was collected, dialyzed, lyophilized, and purified by Sephadex G-100 (2.6 × 100 cm) gel-permeation chromatography eluted with 0.1 M NaCl at a flow rate of 0.5 ml/min. One purified polysaccharide fraction obtained was named as CSP.

2.3. Analysis of purified polysaccharide

2.3.1. Chemical properties analysis

Total carbohydrate content of polysaccharide was determined by phenol–sulfuric acid colorimetric method using glucose as the standard (Dubois, Gills, Hamilton, Rebers, & Smith, 1956). The protein contents of the purified polysaccharides were measured according to Bradford's method, using BSA as the standard (Bradford, 1976), and the uronic acid content assessed using the Blumenkrantz and Asboe-Hansen method (Blumenkrantz & Asboe-Hansen, 1973).

2.3.2. Homogeneity and average molecular weight

The homogeneity and molecular weight of CSP were determined by high-performance gel-permeation chromatography (HPGPC) on a LC-10A liquid chromatography instrument (Shimadzu, Japan). TSK-Gel G4000 column (7.8 mm ID × 30 cm) was maintained at 35 °C and the mobile phase was 0.1 M Na_2SO_4 (flow rate = 0.5 ml/min), and detected by a RID-10A detector. The sample (2 mg) was dissolved in the mobile phase and was filtered through a 0.45 μm filter. The average molecular weight was estimated by reference to the calibration curve made from a Dextran T-series standard of known molecular weight (T-10, T-40, T-70, T-500 and T-2000).

2.3.3. Monosaccharide composition analysis

The identification and quantification of the monosaccharides of polysaccharide were achieved by gas chromatography (GC) analysis (Liu et al., 2011a; Liu et al., 2011b). CSP (10 mg) were hydrolyzed with 2 M TFA at 100 °C for 2 h, and converted to their alditol acetates as previously described (Honda, Suzuki, Kakehi, Honda, & Takai, 1981). The resulting alditol-acetates was analyzed on an Agilent 6280 instrument fitted with FID and equipped with a HP-5 fused silica capillary (0.25 mm × 30 m × 0.25 μm). The temperature of the column was kept at 150 °C for 10 min and then increased to 250 °C at the rate of 5 °C/min subsequently hold on 5 min. The rate of N_2 carrier gas was 1 ml/min.

2.3.4. FT-IR spectral analysis

The FT-IR spectrum of CSP was recorded with a Nicolet 6700 FT-IR Spectrometer (Thermo Co., USA). The dried sample was ground with potassium bromide powder and pressed into pellet for spectrometric measurement in the frequency range of 4000–400 cm^{-1}.

2.4. Cytotoxicity on Sarcoma 180 cells in vitro

The cytotoxicity of CSP on Sarcoma 180 (S180) cells was evaluated by the colorimetric MTT method (Mosmann, 1983). S180 cells (obtained from China Center for Type Culture Collection (CCTCC), Wuhan University) were prepared from peritoneal cavity of the tumor inoculated mice under aseptic conditions. Then the cells were plated in a 96-well plates at a density of 1×10^4 cells/ml in RPMI-1640 medium and after 24 h incubation at 37 °C they were exposed to various concentrations (50, 100 and 200 μg/ml) of polysaccharide for 24, 48 and 72 h. After then, 10 μl of MTT (0.5 mg/ml) was added to each well and the cells were cultured for another 4 h. The supernatant was removed by centrifuging and 100 μl DMSO was added into each well for the dissolution of formazan crystals. The absorbance was measured at 570 nm on a micro-plate Reader (Bio-rad, USA). The antitumor activity of the tested samples was expressed as an inhibition ratio (percent) calculated as: [1 − (A_{570} of treated cells/A_{570} of untreated cells) × 100%]. All tests were run in triplicate.

2.5. Animal groups and in vivo antitumor test

Male Kunming mice (20 ± 2) g was provided by the Laboratory Animal Center, Wuhan University, China. The animals were housed under normal laboratory conditions (21 ± 2 °C, 12/12 h light/dark cycle). The animals were given a standard laboratory diet and water ad libitum. All animal (used for this experiment) handling procedures were performed in strict compliance with the PR China legislation, with the guidelines established by the Institute for Experimental Animals of Wuhan University, and were approved by the University committee for animal experiments.

Under sterile condition, 0.2 ml of S180 cell suspension was subcutaneously inoculated into Kunming mice (1×10^6 cells/ml). The mice inoculated were divided into five groups (ten mice per group): 50, 100, 200 mg/kg CSP treatment groups, positive controls (20 mg/kg CTX) and negative controls (physiological saline). Daily orally (p. o.) drug administration was begun 24 h after the inoculation and performed once daily for 10 consecutive days. After stopping administration, mice were weighed and sacrificed by cervical dislocation. Tumors and spleens were excised and weighted, respectively. Spleen index was expressed in the spleen weight relative to body weight. The tumor inhibitory rate was calculated by the following formula: the inhibition ratio (%) = $[(A - B)/A] \times 100$, where A is the average tumor weight of the negative control group, and B is that of the treatment group (Sun et al., 2009).

2.6. Spleen lymphocyte proliferation assay

Splenocytes taken from mice of all the groups were suspended in RPMI-1640 medium. Splenocytes was plated in a 96-well culture

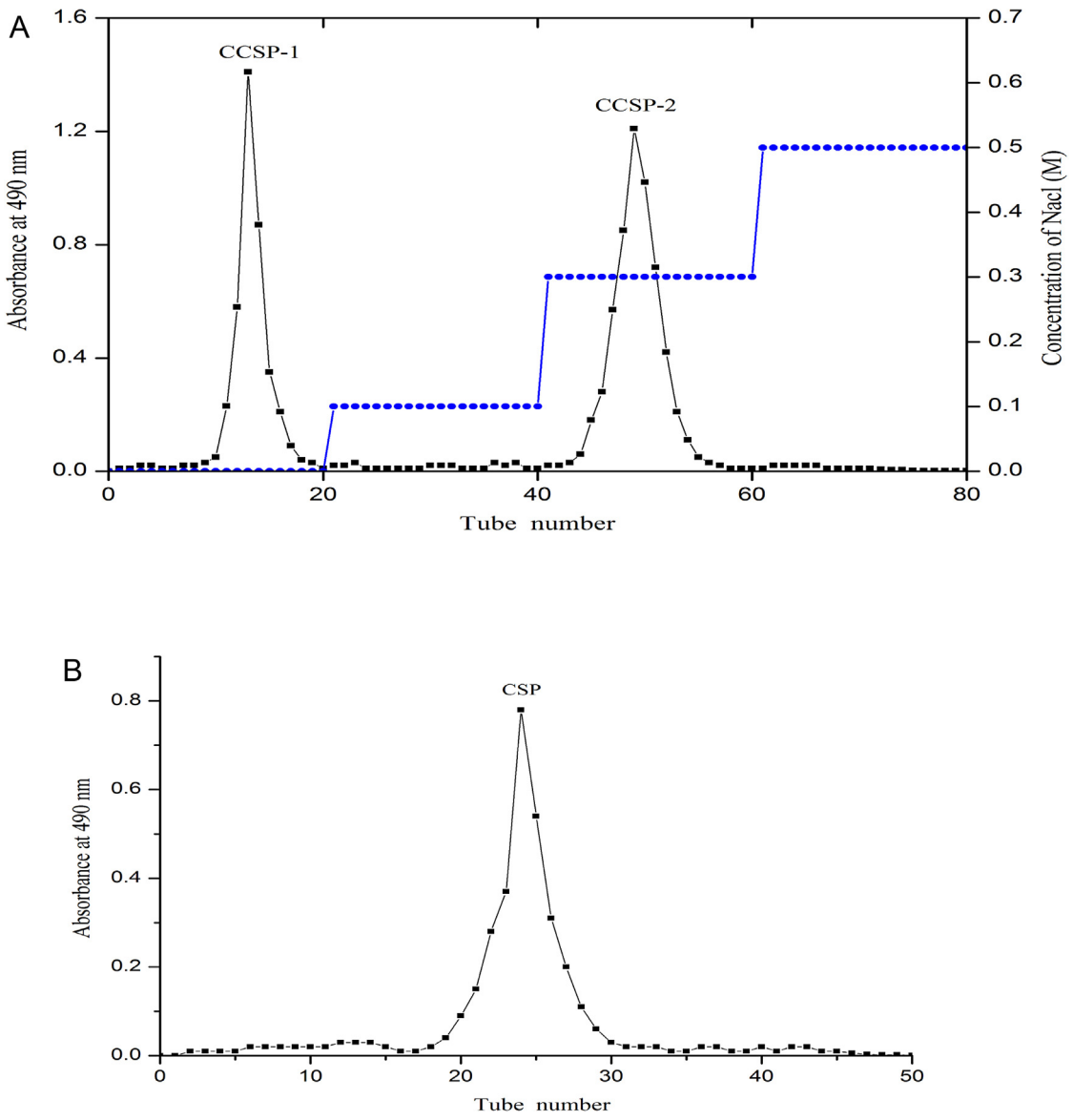

Fig. 1. Elution profiles of crude polysaccharide from the dried fruits of *C. speciosa* on anion-exchange chromatography column of DEAE-Sepharose (A) and gel filtration chromatography column of Sephadex G-100 (B).

plate with or without ConA (5 μg/ml) or LPS (10 μg/ml) at 2×10^6 cells/ml in 200 μl complete medium. The plates were incubated at 37 °C in a humidified atmosphere with 5% CO_2. After 44 h, MTT (20 μl, 2 mg/ml) was added to each well and the plates were incubated for another 4 h. After aspirating the supernatant from the wells, 100 μl of DMSO was added to dissolve formazan crystals. The absorbance of spleen lymphocytes cells in each well were measured at 570 nm by an ELISA reader (Bio-Rad, USA) (Mosmann, 1983).

2.7. Macrophage phagocytosis assay

Phagocytosis of peritoneal macrophages was measured according to the reported method with some modification (Lin, Feng, Pan, Zhang, & Xiao, 1995). The first 10 days of oral administration were the same for Section 2.5, on the last day, all the mice received 0.5 ml of 10% (v/v) chicken red blood cells (CRBC) by intraperitoneally injection, and the mice were euthanized by cervical dislocation 1 h later. 2 ml saline was injected into the abdominal cavity and 1 ml fluid was then collected to make a smear for each mouse. The smears were incubated at 37 °C for 30 min in a wet box, fixed with acetone–methanol (1/1, v/v) solution, and then stained by 4% (v/v) Giemsa-phosphoric acid dye. The number of macrophage ingesting CRBC out of a total of at least 100 cells was calculated by direct visual counting using a light microscope. The phagocytic rate (PR) was calculated using the following formula: PR (%) = number of macrophage ingesting CRBC/total number of macrophages × 100.

2.8. Delayed-type hypersensitivity reaction to DNFB (DTH)

After 5 days of oral administration as Section 2.5, mice were sensitized to DNFB by smearing 50 μl 1% DNFB in acetone–castor oil (v/v = 1:1) on the shaved abdominal of which was unhaired by 3 × 3 cm. Five days later, the DTH reaction was elicited by smearing 10 μl 1% DNFB on both sides of the left ear, while the right unpainted earlap was used as control. Twenty-four hours later, the mice were sacrificed by cervical dislocation; the DTH response to DNFB was evaluated by measuring the weight difference of the right and left ear with an analytical balance (Brodmerkel et al., 2005).

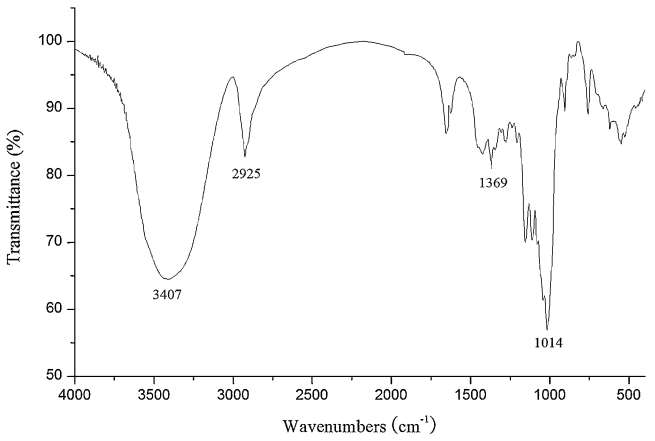

Fig. 2. FT-IR spectrum of CSP.

2.9. Serum IL-2, TNF-α and IFN-γ determination

The serum collected from a different group was detected for interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) level using a commercial ELISA kit according to the instructions of kits (Shanghai Qianchen Biotech Co., China). The absorbance was measured at 450 nm in an ELISA reader (Bio-Rad, USA) (Wang et al., 2007).

2.10. Statistical analysis

Experimental results were expressed as mean ± standard deviation (S.D.). Statistical analysis was performed using Student's *t*-test. The significant difference was set at $p < 0.05$.

3. Results and discussion

3.1. Purification and characteristics of the polysaccharide

The crude polysaccharide was gained from the dried fruit of *C. speciosa*, and the yield was about 5.28% of the dry material. As shown in Fig. 1A, it was separated by using anion-exchange chromatography on DEAE-Sepharose column, which was successively eluted with distilled water and a gradient of 0.1–0.5 M NaCl. Two fractions of polysaccharides were obtained, designated as CCSP-1 and CCSP-2. The CCSP-1 was applied to a Sephadex G-100 gel filtration column for further purification. The column was eluted with 0.1 M NaCl, and the resulting elute was collected. As shown in Fig. 1B, the fraction generated one single elution peak, affording CSP. CSP appeared as a light-yellow powder and contains 95.4% of total sugar as determined by the phenol-sulfuric acid method. It had a negative response to the Bradford method, and no absorption was detected by the UV spectrum at 280 and 260 nm, indicating the absence of protein and nucleic acid. As determined by m-hydroxydiphenyl colorimetric method, the polysaccharide did not contain uronic acid.

A single and symmetrically sharp peak observed on HPGPC indicated that CSP was a homogeneous polysaccharide. According to the calibration curve with standard dextrans, the average molecular weight of it was estimated to be about 6.3×10^4 Da. Analysis by GC indicated that it was composed of glucose (Glc), galactose (Gal), rhamnose (Rha) and arabinose (Ara) with a relative molar ratio of 4.6:1.3:0.8:0.5.

The FT-IR spectrum of CSP was illustrated in Fig. 2. The broad characteristic intense peak around 3407 cm^{-1} was due to the O—H stretching vibration of the polysaccharide. The band in the region of 2925 cm^{-1} was due to C—H stretching vibration and the band at 1369 cm^{-1} was assigned to C—H bending vibration. The absorptions in the range of 1000–1200 cm^{-1}, attributed to the stretching vibrations of C—O—C and C—O—H, were observed.

Table 1
Inhibitory effect of CSP on S180 cells *in vitro*.

Group	Concentration (μg/ml)	Inhibition rate (%)		
		24 h	48 h	72 h
CSP	50	1.86 ± 0.19	2.20 ± 0.19	2.07 ± 0.22
CSP	100	2.73 ± 0.24	3.85 ± 0.37	4.42 ± 0.39
CSP	200	3.13 ± 0.37	3.44 ± 0.35	4.16 ± 0.40

Each value is presented as mean ± S.D. ($n = 3$).

3.2. In vivo and in vitro antitumor activities

The growth inhibition effect of CSP was tested on S180 cells by MTT assay in *vitro*. As shown in Table 1, the inhibition ratio on S180 cells of all CSP groups (50, 100 and 200 μg/ml) at different time was lower than 5%, suggesting that CSP had no direct cytotoxicity against S180 cells.

The antitumor effects of CSP were examined in the S180 tumor-bearing mice *in vivo* at the dose of 50, 100 and 200 mg/kg. As shown in Table 2, after daily p. o. administration of CSP for 10 days, we found that CSP significantly inhibited tumor growth in a dose-dependent manner, with the inhibitory rates of 28.2%, 37.0%, and 44.9%, at the concentrations of 50, 100 and 200 mg/kg respectively and tumor inhibitory rates treated by 20 mg/kg CTX was 59.0%. At the same time, the spleen index was increased obviously in CSP administration ($P < 0.05$), whereas CTX treatment slightly decreased the index compared to the control group. The relative spleen weight was an important index for nonspecific immunity, which suggested that CSP may inhibit tumor growth by strong stimulatory effects to the immunological function of S180 tumor-bearing mice.

3.3. Effects of the polysaccharide on splenocyte proliferation

The effect of CSP on mitogen-stimulated splenocyte proliferation in S180 tumor-bearing mice was presented in Fig. 3. ConA- and LPS-induced splenocyte proliferation in S180 tumor-bearing mice was significantly enhanced by CSP at the doses of 50, 100 and 200 mg/kg in a dose-dependent manner ($P < 0.05$ or $P < 0.01$), but splenocyte proliferations in the CTX-treated group were slightly suppressed than those of the control group.

Lymphocyte proliferation plays a crucial role in the cellular immune responses. Lymphocytes proliferation induced by ConA or LPS may be used as a method to evaluate T or B lymphocyte activity (Han et al., 1998). T and B lymphocytes are two important classes of immunologically active cells. The former is mainly responsible for cellular immunity, and the latter is the only cell capable of producing antibodies (Mosmann & Coffman, 1989). The results indicated

Table 2
Effect of CSP on tumor growth and spleen index in S180 tumor-bearing mice.

Group	Dose (mg/kg)	Tumor weight (g)	Inhibition rate (%)	Spleen index (mg/g)
Control		2.27 ± 0.23		6.76 ± 0.58
CSP	50	1.63 ± 0.16[a]	28.2	8.13 ± 0.67[a]
CSP	100	1.43 ± 0.15[a]	37.0	9.45 ± 0.82[a]
CSP	200	1.25 ± 0.12[b]	44.9	11.12 ± 1.06[b]
CTX	20	0.93 ± 0.09[b]	59.0	5.95 ± 0.49

Each value is presented as mean ± S.D. ($n = 10$).
[a] $P < 0.05$ compared to control group.
[b] $P < 0.01$ compared to control group.

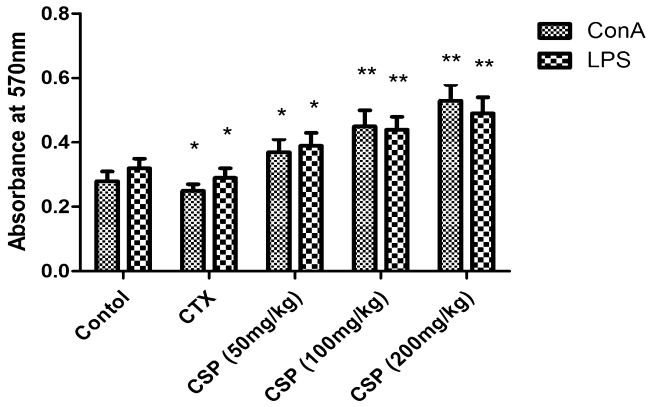

Fig. 3. Effect of CSP on splenocyte proliferation in mice. Each value is presented as mean ± S.D. ($n = 10$). *$P < 0.05$ compared to control group. **$P < 0.01$ compared to control group.

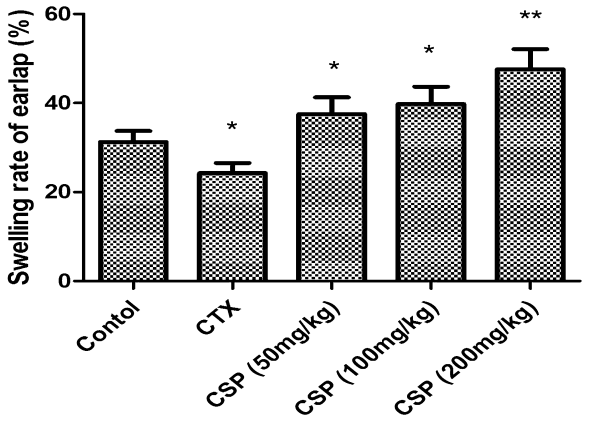

Fig. 5. Effects of CSP on the swelling rate of earlap in mice. Each value is presented as mean ± S.D. ($n = 10$). *$P < 0.05$ compared to control group. **$P < 0.01$ compared to control group.

that CSP could significantly promote the Con A- and LPS-stimulated splenocyte proliferation in tumor-bearing mice, suggesting that immunomodulation might be its anti-tumor mechanism.

3.4. Effects of the polysaccharide on macrophage phagocytosis

The effect of CSP treatment on macrophage phagocytosis in S180 tumor-bearing mice was shown in Fig. 4, a considerable enhancement of the proliferation of peritoneal macrophages was observed in CSP treated groups (50, 100 and 200 mg/kg) when compared to the control group ($P < 0.05$ or $P < 0.01$). In the meantime, CTX apparently inhibited macrophage phagocytosis because of its side effect to the immune system ($P < 0.01$).

As the most important professional phagocyte, macrophage is essential for maintaining homeostasis regardless of varying external conditions and considered to be one of the important components of the host defense against tumor growth (Gamal-Eldeen, Amer, Helmy, Talaat, & Ragab, 2007; Katsiari, Liossis, & Sfikakis, 2010). Phagocytic capacity is one of the most important indicators of the body's non-specific immunity (Schepetkin & Quinn, 2006). It protects the host by phagocytosis, presents antigens to lymphocytes and releases numerous cell factors that regulate the activity of other cells (Jiao et al., 2009). The results of the study indicated that CSP could significantly improve the phagocytic activity of macrophages in S180 tumor-bearing mice, which means the improving of immune state of the host.

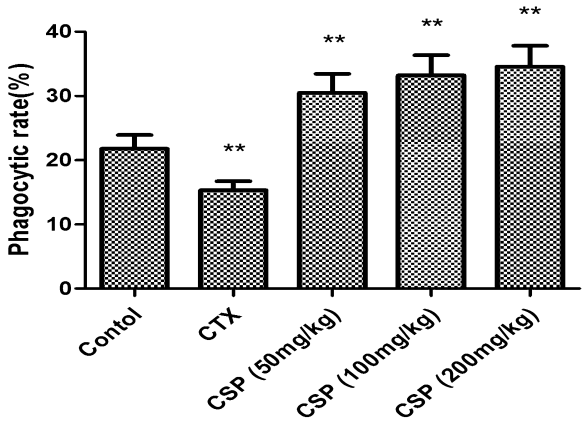

Fig. 4. Effect of CSP on macrophage phagocytosis in mice. Each value is presented as mean ± S.D. ($n = 10$). *$P < 0.05$ compared to control group. **$P < 0.01$ compared to control group.

3.5. Effect of the polysaccharide on DTH response

Fig. 5 showed the effects of CSP on the DNFB-induced DTH of mice. A significant increase in the swelling rate of earlap was observed in CSP-treated group, especially at the dose of 200 mg/kg, compared with control group ($P < 0.01$). However, DTH reaction was obviously suppressed by CTX administration, with respect to the control group ($P < 0.05$).

DTH, is a cell-mediated pathologic response involved in T cell activation and cytokine production (Dai, Zhang, Zhang, & Wang, 2009). The character of DTH reaction is the large aggregation of non-specific inflammatory cells, in which the macrophage is a major participant. It is a type IV hypersensitivity reaction that develops when an antigen activates sensitized T_{DTH} cells (Umesh, Hawna, Paramjit, Dinesh, & Suresh, 2004). Treatment of CSP enhanced DTH reaction, which was reflected from the increased earlap swelling compared to control group suggesting heightening infiltration of macrophages to the inflammatory site. This result may also be supporting a possible role of CSP in assisting the cell-mediated immune response.

3.6. Effect of the polysaccharide on IL-2, TNF-α and IFN-γ production

Cytokines are peptides or proteins with low molecular weights, which affect cell functions and condition their interactions. They are produced by cells that possess regulatory properties and are found very close to or in direct contact with target cells. By binding to specific cell surface receptors, they affect cell proliferation, differentiation and functions. Cytokines exert an effect on hematopoiesis and immune processes including acute phase reaction and antitumor defense. They regulate both cellular and humoral responses (Terlikowski, 2002).

Several polysaccharides derived from plants have been reported to induce production of cytokines including IL-2, TNF-α and IFN-γ (Schepetkin & Quinn, 2006). In this study, the effects of CSP on the secretion of serum IL-2, TNF-α and IFN-γ in S180 tumor-bearing mice were examined by using ELISA kits. As shown in Table 3, CSP significantly augmented the secretion of serum IL-2, TNF-α and IFN-γ as compared with the model control, especially at the doses of 100 mg/kg/day and 200 mg/kg/day ($P < 0.01$). On the contrary, the levels of serum IL-2, TNF-α and IFN-γ in the CTX-treated group were much lower than those of the model control ($P < 0.01$). IL-2 is essential for the growth, proliferation, and differentiation of T cells, and is produced by T cells normally during an immune response (Malek, 2008). TNF-α is a cytokine with tumor necrosis activity that

Table 3
Effect of CSP on serum IL-2, TNF-α and IFN-γ levels in mice.

Group	Dose (mg/kg)	IL-2 (pg/ml)	TNF-α (pg/ml)	IFN-γ (pg/ml)
Control		48.72 ± 4.13	238.44 ± 20.42	172.71 ± 14.52
CSP	50	57.36 ± 6.24[a]	288.16 ± 27.13[b]	233.28 ± 21.75[b]
CSP	100	68.21 ± 6.77[b]	345.79 ± 32.62[b]	287.42 ± 30.14[b]
CSP	200	78.32 ± 8.76[b]	410.23 ± 39.75[b]	345.59 ± 34.82[b]
CTX	20	26.18 ± 2.64[b]	110.32 ± 10.56[b]	92.76 ± 9.89[b]

Each value is presented as mean ± S.D. ($n = 10$).
[a] $P < 0.05$ compared to control group.
[b] $P < 0.01$ compared to control group.

is secreted mainly by macrophages and has been recognized as an important host regulatory molecule (Vilcek & Lee, 1991). IFN-γ is mainly secreted by NK cells and T cells as part of innate immunity and antigen-specific immunity (Boehm, Klamp, Groot, & Howard, 1997). It demonstrated that CSP could enhance the immune function by promoting cytokine levels in S180 tumor-bearing mice.

4. Conclusion

In the present study, a polysaccharide (CSP) from *C. speciosa* was obtained by hot water extraction and then purified by DEAE-Sepharose anion-exchange and Sephadex G-100 gel-permeation chromatography. The CSP was a homogeneous polysaccharide with a molecular weight of 6.3×10^4 Da, as determined by HPGPC. GC analysis showed that CSP was composed of glucose, galactose, rhamnose and arabinose with a relative molar ratio of 4.6:1.3:0.8:0.5. Furthermore, CSP had indirect anti-tumor activity achieved by improving immune response *in vivo*, and it might act as a potential natural anti-tumor agent with immunomodulatory activity. It has been demonstrated that many factors such as monosaccharide composition affected the activities of polysaccharides (Bao, Liu, Fang, & Li, 2001), and polysaccharides composed of glucan have been proved to stimulate the immune system (Kuang, Xia, Yang, Wang, & Wang, 2011). The immunostimulatory activity of CSP might be due to its high contents of glucose. Further researches about elucidating the detailed mechanism of its antitumor actions are in process.

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