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Antioxidant effects and cytotoxicity of three purified polysaccharides from *Ligusticum chuanxiong* Hort.

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ABSTRACT

Water-soluble crude polysaccharide LC was obtained from *Ligusticum chuanxiong* Hort. by boiling-water extraction and ethanol precipitation. The major polysaccharides LCA, LCB, and LCC were isolated and purified from the crude polysaccharide LC, and the monosaccharide components, average molecular weight and the main skeleton were analyzed and determined. The antioxidant properties of LCA, LCB, and LCC were extensively investigated with several biochemical methods, and the antiproliferative activities were also involved. Experimental results showed that all purified polysaccharides exhibited antioxidation and cytotoxicity, and LCB has the highest antioxidant and cytotoxic activity among them. It is possible that LCB is explored as a novel potential antioxidant and cytotoxic natural bioactive macromolecule.

1. Introduction

Oxidation is an essential biological process to many organisms for the production of energy. However, the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, and atherosclerosis as well as in degenerative processes associated with aging (Mau, Lin, & Chen, 2002). Nowadays, most antioxidants are synthesized industrially and are suspected of being responsible for liver damage and carcinogenesis. Thus, it is essential to develop and utilize effective and natural antioxidants so that they can protect the human body from free radicals and retard the progress of many chronic diseases (Luo, 2008; Nandita & Rajini, 2004).

Ligusticum chuanxiong Hort. (*L. chuanxiong*, family Umbelliferae) is also named as Chinese lovage, and has long been employed as a traditional Chinese medicine in folk remedies (Guo, Chen, Qian, Weng, & Qian, 1983). It is widely applied in food preparation as a health protection. Usually it is added to a soup, such as Chuanxiong mutton soup and Chuanxiong fish's head soup. Major chemical components of *L. chuanxiong* are essential oils, alkaloids, phenolic acids, polysaccharides, and proteins (Zhang et al., 2003). Previous investigations mainly focused on tetramethylpyrazine and ferulic acid (Hou, Yang, Zhao, & Yuan, 2004; Tsai, Lai, Huang, Liu, & Liou, 2005). However, existing studies have observed that the quantities of tetramethylpyrazine and ferulic acid in raw *L. chuanxiong* herb were very low (<0.0001% and <1%, respectively) (Li et al., 2003; Yan, Li, Chung, Tam, & Lin, 2005). In contrast, more abundant ingredients are polysaccharides. To date, no investigation has been carried out on polysaccharides of *L. chuanxiong*. The objective of this study is to extract and purify the major polysaccharides from *L. chuanxiong*, and then to investigate the antioxidant and antiproliferative activities.

2. Materials and methods

2.1. Reagents materials

The dry rhizome samples of *L. chuanxiong* were purchased from the Xi'an traditional Chinese medicine market in Shaanxi Province of China. Human liver carcinoma cell line (HepG2) was purchased from the China Centre for Type Culture Collection (CCTCC, Wuhan, China). RPMI 1640 medium and fetal bovine serum (FBS) were obtained from GIBCO-BRL (Grand Island, New York, USA). Dimethyl sulphoxide (DMSO), thiobarbituric acid (TBA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), trichloroacetic acid (TCA), nitro blue tetrazolium salt (NBT), butylated hydroxytoluene (BHT), ascorbic acid (Vc), ferrozine, linoleic acid, and 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) were purchased from Sigma Co. (St. Louis, USA). Ethylenediamine tetraacetic acid (EDTA) was purchased from Fluka Chemie (Buchs, Switzerland). The male ICR mouse was purchased from the experiment animal center of Xi'an Jiaotong University (China). All chemicals used in this study have a purity of 90% or greater.

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2.2. Isolation and purification of the polysaccharides

Ligusticum chuanxiong Hort. was washed, dried at 60 °C and crushed up (50 mesh), then weighed (200 g) and dipped in 20 volumes of double distilled water at 100 °C for 3 h. The suspension was centrifuged (8000*g* for 40 min), and the supernatant was concentrated in a rotary evaporator under reduced pressure at 50 °C, giving a final volume of about 100 mL. The concentrated solution was dialyzed against distilled water at 30 °C for 12 h to exclude most of low molecular weight compounds with a dialysis bag (MWCO 5000, Sigma). Four volumes of anhydrous ethanol were added to this concentrate, and the mixture was stirred at room temperature for 20 min and then left at 4 °C overnight. The precipitated polysaccharides were collected by centrifugation and lyophilized, and 7.6 g crude polysaccharides were obtained.

A DEAE-cellulose anion-exchange chromatography column was used to isolate negatively charged polysaccharides from crude polysaccharides. The sample (1.00 mg) was dissolved in 2 mL distilled water, and the solution was loaded onto a DEAE-cellulose chromatography column (5.0 × 60 cm) equilibrated with distilled water. The column was eluted with stepwise NaCl aqueous solution (0, 0.1, 0.2, 0.3, 0.4, and 0.5 M) for 600 mL, respectively, at 6 mL/10 min/tube. All of these fractions were assayed for carbohydrate content by the phenol-sulfuric acid method (Dubois, 1956). Three sharp peaks were detected at 0, 0.3, and 0.4 M NaCl eluents, respectively. The eluates were gathered for each peak, and further purified on a Sephedex 200 column. Finally, three purified polysaccharides named LCA, LCB, and LCC were obtained by lyophilization.

2.3. Analysis of purified polysaccharides

The protein contents of the purified polysaccharides were measured according to Bradford's method, using bovine serum albumin (BSA) as the standard (Bradford, 1976). The monosaccharide compositions were analyzed by gas chromatography (GC, Agilent USA) with a flame-ionization detector. Ten milligrams purified polysaccharide was hydrolyzed with 10 mL of 2 M trifluoroacetic acid (TFA) at 120 °C for 6 h (Erbing, Jansson, Widmalm, & Nimmich, 1995), then derivatization was carried out using the trimethylsilylation reagent (Guentas et al., 2001). The molecular weight was determined by high-performance gel-permeation chromatography (HPGPC) (Yamamoto, Nunome, Yamauchi, Kato, & Sone, 1995), and dextran standards with different molecular weights (5900, 11800, 22800, 47300, 112000, 212000, 404000, and 788000 Da) were used for preparing calibration curve. The IR spectrum was determined using a Fourier transform infrared spectrophotometer (FTIR, Bruker, Germany). The polysaccharide was ground with KBr powder and then pressed into pellets for FTIR measurement in the frequency range of 4000–500 cm^{-1} (Yamamoto, Nunome, Yamauchi, Kato, & Sone, 2004). The polysaccharide (20 mg) was dissolved in D_2O (0.5 mL), and the ^1H NMR was performed with a Super-conducting Fourier Digital NMR spectrometer (Bruker, 300 MHz, Germany) at 60 or 70 °C. Chemical shifts of the samples are expressed in PPM (δ) relative to external standard of acetone (2.23 for ^1H).

2.4. Antioxidant activity assay

2.4.1. DPPH radical-scavenging activity

The antioxidant activity of polysaccharides was measured on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Braca et al., 2001). One milliliter of sample (0.1, 0.5, 1, 2, 3, 4, and 5 mg/mL) was added to 3 mL of a 0.004% MeOH solution of DPPH. Absorbance at 517 nm was measured after 30 min, and the percent scavenging activity was calculated by the following formula:

$$\text{Scavenging effect } (\%) = (1 - A_{\text{sample}}/A_{\text{control}}) \times 100,$$

where A_{control} is the absorbance of control (DPPH solution without sample), A_{sample} is the test sample (DPPH solution plus test sample or positive control).

2.4.2. Hydroxyl radical-scavenging activity

Hydroxyl radical-scavenging activity of the samples was determined according to the deoxyribose assay (Halliwell, Gutteridge, & Aruoma, 1987). One milliliter phosphate buffer (20 mM pH 7.4, containing 0.1 mM ferric chloride, 0.1 mM EDTA, 2.8 mM deoxyribose), 0.1 mL Vc (1 mM) and 0.5 mL hydrogen peroxide (20 mM) were added to 1 mL of appropriately diluted sample. Following incubation at 37 °C for 90 min, 1.0 mL of 1% (w/v) TCA, and 0.3 mL of 2.8% (w/v) TBA were added, and then the reaction mixture was heated in a boiling-water bath for 15 min. The absorbance of was measured at 532 nm against a blank. A control contained all the reaction reagents except the samples was prepared and measured, BHT was used for comparison, and the hydroxyl radical-scavenging ratio was calculated as Section 2.4.1.

2.4.3. Superoxide anion-scavenging activity

The assay was based on the capacity of the sample to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) in the NADH–NBT–PMS system (Kanatt, Chander, & Sharma, 2007). The reaction mixture consisted 1.0 mL of NBT (78 μM in 20 mM potassium phosphate buffer pH 7.4), 1.0 mL of NADH (468 μM in 20 mM potassium phosphate buffer pH 7.4) and 1.0 mL of an appropriately sample solution. The reaction was initiated by addition of 0.4 mL of PMS (60 μM in 20 mM potassium phosphate buffer pH 7.4) to the mixture. The tube was incubated at surrounding temperature for 5 min and the absorbance was measured at 560 nm against a blank. Decreased absorbance of the reaction mixture indicated increasing superoxide anion-scavenging activity. BHT was used as a positive control in the study, and a control contained all the reaction reagents except the samples or positive control was prepared. The percentage inhibition of superoxide anion generation was calculated using the formula similar to that for DPPH radical-scavenging activity.

2.4.4. Total antioxidant activity in a linoleic acid system

Total antioxidant activity was determined according to the literature (Yu et al., 2006) with some modifications. Five milligrams of β-carotene was dissolved in 10 mL chloroform, and the solution was pipetted into a flask containing 250 μL of linoleic acid and 2 g of Tween 20. The chloroform was removed by rotary vacuum evaporator at 50 °C for 10 min, and 500 mL of oxygenated distilled water was added slowly to the semi-solid residue with vigorous agitation to form an emulsion. A 5 mL aliquot of the emulsion was added to a tube containing 1.0 mL of the sample solution at 0.5 mg/mL, and the absorbance was measured at 470 nm, immediately, against a blank emulsion without β-carotene. The tube was placed in a water bath at 50 °C, and the absorbance was monitored at 30 min intervals until 120 min. BHT was used as a positive control in the study.

2.4.5. Chelating effect on ferrous ion

For each sample, the chelating effect on Fe^{2+} was measured according to the reference method (Dinis, Madeira, & Almeida, 1994) with some modifications. One milliliter sample (0.1–5 mg/mL) was reacted with 0.1 mL $FeCl_2$ (2 mM,) for 30 s, then 0.2 mL of 5 mM ferrozine was added, and the mixed solution was stand for 10 min at room temperature. The absorbance of the mixture was determined at 562 nm. A lower absorbance indicated stronger chelating activity. Ethylenediaminetetraacetic acid (EDTA, 0.1 mg/

mL) was co-assayed as a positive control, and the ability of samples for the ferrous ion was calculated.

2.4.6. Reducing power

The reducing power of polysaccharides was determined referring to the method (Yuan, Carrington, & Walsh, 2005) with some modifications. The different concentrations of samples (0.1, 0.5, 1, 2, 4, and 5 mg/mL) 1 mL were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of TCA (10%) was added to the mixture which was then centrifuged for 10 min at 3000*g*. The upper layer of solution (2.5 mL) was mixed with water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. Vc and BHT were used as positive controls at the same concentration.

2.4.7. Lipid peroxidation assay

A modified thiobarbituric acid-reactive species (TBARS) assay (Banerjee, Dasgupta, & Be, 2005) was used to measure the lipid peroxide formed, using mouse liver homogenates as the lipid rich media (Ruberto, Baratta, Deans, & Dorman, 2000). Malondialdehyde (MDA), a secondary product of the oxidation of polyunsaturated fatty acids, reacts with two molecules of thiobarbituric acid (TBA), yielding a pinkish red chromogen with an absorbance maximum at 532 nm. Mouse liver homogenate (0.5 mL of 10% v/v) and 0.1 mL of samples (0.1, 0.5, 1, 2, 4, and 5 mg/mL) were added to a test tube that was then filled to 1 mL with water. 0.05 mL of $FeSO_4$ (0.07 M) was added to induce lipid peroxidation and incubated for 30 min. Then 1.5 mL of 20% acetic acid (pH adjusted to 3.5 with NaOH), 1.5 mL of 0.8% (w/v) TBA in 1.1% sodium dodecyl sulfate and 0.05 mL 20% TCA were added, and the resulting mixture was vortexed and heated at 95 °C for 60 min. After cooling the mixture, an equal volume of *n*-butanol was added to extract the chromogen in the mixture. The absorbance of the *n*-butanol layer was measured spectrophotometrically at 532 nm. Vc was used as a positive control. The capability to inhibit MDA formation was calculated.

2.5. Cytotoxicity assay

2.5.1. Cell lines and culture

Human liver carcinoma cell line (HepG2) was maintained in RPMI 1640 medium containing 10% (v/v) heat inactivated FBS supplemented with penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37 °C in the air of 5% CO_2.

2.5.2. MTT assay

In this study, HepG2 cell growth inhibition activity was measured by MTT assay (Carmichael, DeGraff, Gazdar, Minna, & Mitchell, 1987; Mossman, 1983). HepG2 cell was seeded in a 96-well plate at the concentration of 5×10^4 cells/mL using RPMI 1640. After 48 h (at 37 °C in a humidified atmosphere of 5% CO_2), then the cells were treated with LCA, LCB, and LCC solutions (solid powder was dissolved in free-serum medium, then the solution was filtered under sterile conditions) at the concentration range from 25 to 125 μg/mL and free-serum medium as a negative control (100% of viability). The wells were then further incubated for a 48 h at 37 °C. MTT stock solution (20 μl; 5 mg/mL in PBS) was then added to each well for a total reaction volume of 220 μl. After incubating for 4 h in a humidified atmosphere of 5% CO_2 at 37 °C, the supernatants were aspirated to remove untransformed MTT. The formazan crystals in each well were dissolved in 150 μl of DMSO. The amount of purple formazan was determined by measuring the absorbance at 540 nm. For treated cells, cytotoxicity was expressed as follow: $(1 - A_{\text{treated}}/A_{\text{negtive control}}) \times 100\%$.

2.6. Statistical treatment of data

All the data were expressed as means ± standard deviation (SD) of three replications, and the one-way analysis of variance (ANOVA) was used for the statistical analysis. Values of $P < .05$ were considered to be a statistically significant finding.

3. Results and discussion

3.1. Polysaccharide compositions and properties

There was no absorption at 280 nm indicating all of LCA, LCB, and LCC were free of any peptide chain. A single symmetric peak at high-performance gel-permeation chromatography showed that each of them was homogenous polysaccharide. The estimated equivalent dextran molecular weights of LCA, LCB, and LCC were around 2.83×10^4 Da, 1.23×10^4 Da, and 6.31×10^4 Da. In comparison to the monosaccharide standards, LCA comprises Ara, Glc, and Man in a ratio of 0.25:1.00:0.05; LCB consists of Ara, Glc, Gal, and Man in a ratio of 1.00:0.03:0.13:0.20; and LCC is composed of Ara, Glc, and Gal in a ratio of 1.00:0.07:0.04. In IR spectrum, LCA displayed the characteristic band at 847.2 cm^{-1} and 1000–1200 cm^{-1}, LCB at 894.8 cm^{-1} and 1000–1200 cm^{-1}, and LCC at 890.2 cm^{-1} and 1000–1200 cm^{-1}. These data showed that main skeleton of LCA was α-glucoside linkaged pyranose, and both LCB and LCC were β-glucoside linkaged pyranose (Guo et al., 2005; Sosnina et al., 1996). In ^1H NMR spectrum, a signal appeared at 5.36 ppm for LCA; some weak signals appeared at 5.12, 5.08, and 5.05 ppm, and strong signals at 4.85 and 4.79 ppm for LCB; some weak signals at 5.10, 5.06, and 5.04 ppm, and strong signals at 4.85 and 4.79 ppm for LCC. These results further demonstrated that LCA had α-glucoside linkaged pyranose, and LCB and LCC had β-glucoside linkaged pyranose, and simultaneously showed that α-glucoside linkaged pyranose was involved in LCB and LCC (Liu & Wang, 2007).

3.2. Antioxidant activities

3.2.1. DPPH radical-scavenging activity

DPPH is a useful reagent for investigating the free radical-scavenging activities of materials. Antioxidants transfer either electrons or hydrogen atoms to DPPH and thus neutralize free radical (Naik et al., 2003). It is noticeable by eye that there is a discolouration from purple to yellow induced by antioxidants. Fig. 1 illus-

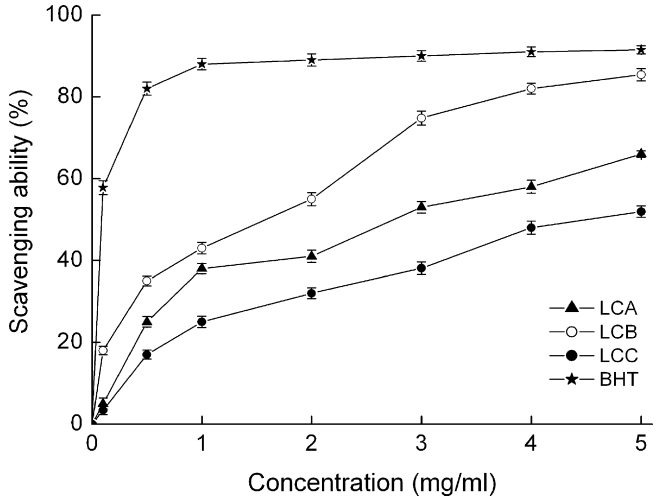

Fig. 1. DPPH radical-scavenging activity of LCA, LCB, and LCC from *L. chuanxiong*.

trates the scavenging activity of the purified polysaccharide samples on the DPPH radical. At 3 mg/mL, the polysaccharides showed inhibition of 38.1–74.8%. At 5 mg/mL, inhibiting abilities increased to 51.9–85.4%. These results indicated that the purified polysaccharides exhibit a significant scavenging activity, especially for LCB. However, the inhibiting ability was lower than that of BHT.

3.2.2. Hydrogen peroxide scavenging activity

Hydroxyl radical (·OH) can easily cross cell membranes, and can readily react with most biomolecules including carbohydrates, proteins, lipids, and DNA in cells, and cause tissue damage or cell death. Thus, removing ·OH is important for the protection of living systems. Fig. 2 shows the percentage ·OH scavenging effects of LCA, LCB, and LCC at the dose of 0.1, 0.5, 1, 5, and 10 mg/mL. At the test concentrations, LCA, LCB, and LCC exhibited scavenging activity on hydroxyl radicals in a concentration-dependent manner. At 10 mg/mL, the inhibiting abilities of LCA, LCB, and LCC were 48.8%, 57.6%, and 37.4%, respectively. However, the activity on scavenging hydroxyl radicals was low. This was similar to the methanolic extract from ear mushrooms and *Ganoderma tsugae* (Mau, Chao, & Wu, 2001; Mau, Tsai, Tseng, & Huang, 2005).

3.2.3. Superoxide anion-scavenging activity

The superoxide radicals were generated in a PMS/NADH system and assayed by the reduction of NBT. Fig. 3 illustrates the superoxide radical-scavenging ability of 0.1, 0.5, 1, 2, 4, and 5 mg/mL of LCA, LCB, and LCC in comparison to the same doses of BHT. At all the concentrations, the polysaccharide samples exhibited varying degrees of antioxidant activity. The percentage inhibitions of LCA, LCB, and LCC were 50.3%, 68.1%, and 42.8% at 2 mg/mL, respectively. Results showed that LCB has a high-level of radical-scavenging effect, and the percentage inhibition is close to that of BHT at a dose of 5 mg/mL.

3.2.4. Total antioxidant activity in a linoleic acid system

The mechanism for the bleaching of β-carotene is a free radical-mediated phenomenon resulting from the hydroperoxides formed from linoleic acid. Fig. 4 shows that all of the polysaccharide samples exhibited definite antioxidant activity compared to the control, although the activity was lower than BHT. However, LCB showed a higher ability to prevent the bleaching of β-carotene, and in the first 60 min the activity closed to BHT. The total antioxidant activity of the samples decreased in the order: LCB > LCA > LCC. The antioxidant activities of the polysaccharides can be attributed to the mechanism, polysaccharides neutralizing the linoleate free radical and other free radicals formed in this model system.

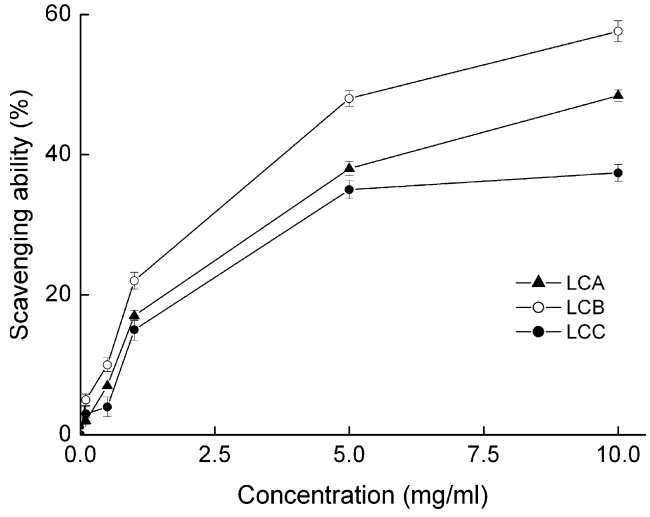

Fig. 2. Hydroxyl radical-scavenging activity of LCA, LCB, and LCC measured by deoxyribose assay.

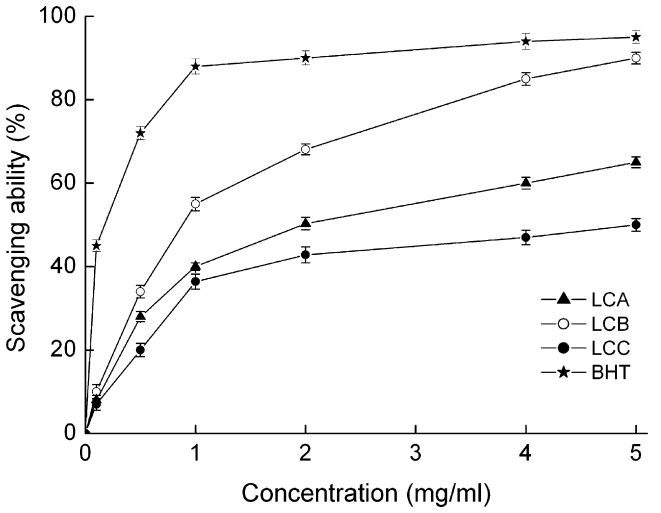

Fig. 3. Superoxide radical-scavenging capacity of LCA, LCB, and LCC determined by the PMS/NADH-NBT method.

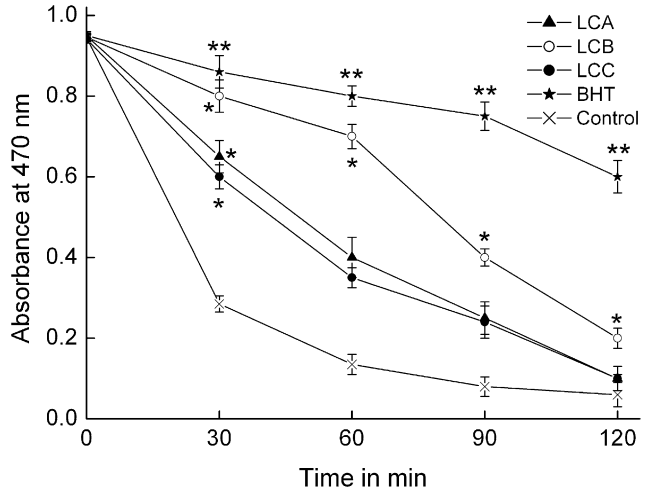

Fig. 4. Total antioxidant activity of LCA, LCB, and LCC in β-carotene–linoleate model system. Each sample was assayed in triplicate for each concentration. $^{*}P < .05$ and $^{**}P < .001$ vs. control.

3.2.5. Metal chelating activity

Fig. 5 shows that LCC exhibited slight chelating effect, and LCA and LCB exhibited a moderate chelating activity to Fe^{2+}. At a concentration of 0.1–5.0 mg/mL, the chelating ability ranged from 3% to 48.3% for LCA, while from 3% to 25.6% for LCC. LCB showed the strongest chelating ability among the three samples, and the chelating ability ranged from 12% to 66.4%. However, compared with EDTA, the chelating ability of the samples, especially LCA and LCC on ferrous ion, was weaker.

3.2.6. Reducing power

In this assay, the reducing power of the tested polysaccharides steadily increased with increasing sample concentration (Fig. 6). At 1 mg/mL, the reducing powers were 0.45, 0.62, and 0.34 for LCA, LCB, and LCC, respectively. At 5 mg/mL, the reducing powers were 1.05, 1.10, and 0.84 for LCA, LCB, and LCC, respectively. LCB showed

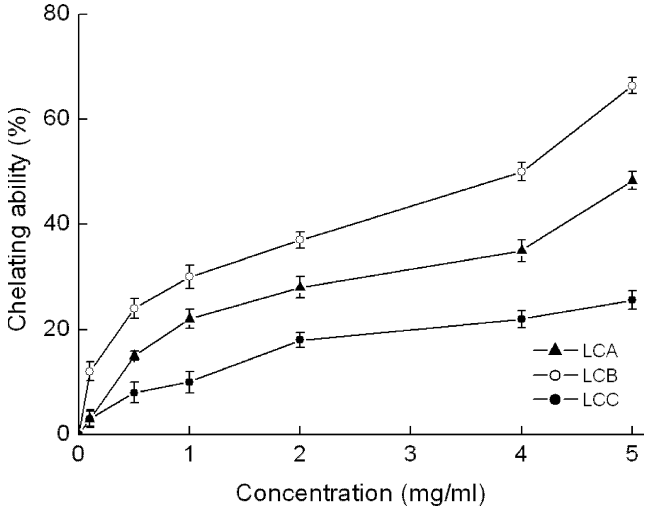

Fig. 5. Metal chelation effect of different concentrations of LCA, LCB, and LCC on ferrous ions.

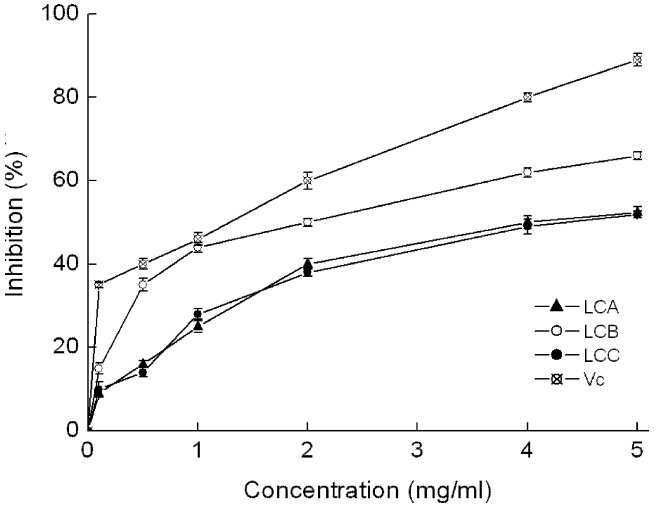

Fig. 7. Inhibition by LCA, LCB, and LCC on $FeSO_4$ induced lipid peroxidation of mouse liver homogenates.

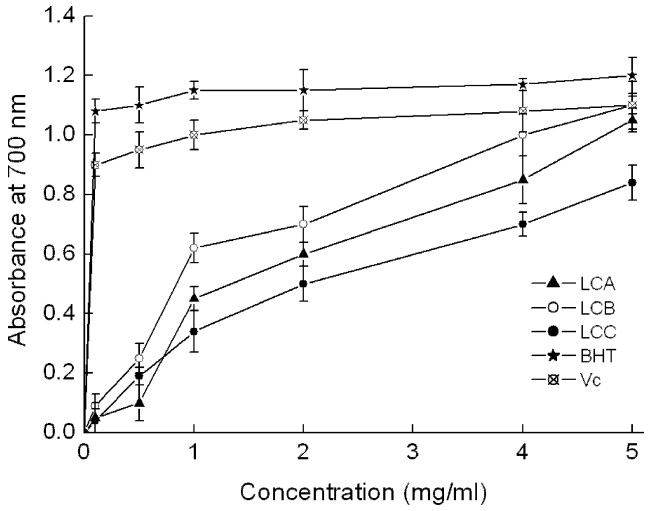

Fig. 6. Reducing power of different concentrations of LCA, LCB, and LCC.

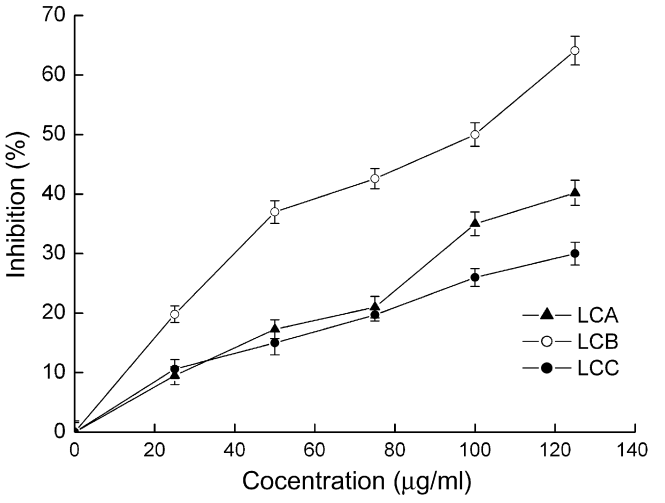

Fig. 8. The MTT assay of HepG2 cells induced with different concentrations of LCA, LCB, and LCC.

slightly higher activity than that of the other two polysaccharides, but less than that of Vc and BHT.

3.2.7. Lipid peroxidation assay

Lipid peroxidation is a typical free radical oxidation and proceeds via a cyclic chain reaction. Fig. 7 shows that the inhibiting effects on the lipid peroxidation of the tested samples were concentration-dependent. The inhibiting effects rose from 9.5% to 51.7% for LCA, 13.8% to 64.1% for LCB and 9.9% to 41.4% for LCC with the concentration increasing from 0.1 to 5 mg/mL. In the iron-induced mouse liver microsomal lipid peroxidation system, LCB showed the highest antioxidant activity, and the inhibiting effect was close to that of Vc at 0.5 and 1 mg/mL.

3.3. Cytotoxic activity on HepG2 cell

The HepG2 cells are observed with microscopy phase contrast, and the cells arrange in typical "cobble-stone" shape with contact inhibition existing among cells. When HepG2 cells treated with different concentrations of LCA, LCB, and LCC, and cultured up to 48 h, there was an increase in the percentage of cells being shrunk. The growth of HepG2 cells could be affected by LCA, LCB, and LCC at a concentration as low as 25 μg/mL, and a dose-dependent manner was observed up to 125 μg/mL (Fig. 8). MTT assay indicated the optimum inhibitory concentration of LCA at 125 μg/mL; LCB showed relatively higher activity (from 13.4% at 25 μg/mL to 64.1% at 125 μg/mL); LCC did not show prominent cytotoxicity at low doses (25 μg/mL). With regard to the therapeutic conditions, LCB can be considered to be optimum for treatment at 125 μg/mL.

4. Conclusion

In the past decades, it has been found that the polysaccharides in plants are not only energy resources but play key biological roles in many life processes as well. The structure and mechanisms of pharmaceutical effects of bioactive polysaccharides on diseases have been extensively studied, and more natural polysaccharides with different curative effects have been tested and even applied in therapies (Wang & Fang, 2004). The bioactivities of polysaccharides can be affected by many factors including chemical components, molecular mass, structure, conformation, even the extraction and isolation methods.

Table 1
IC_{50} values of LCA, LCB, and LCC from *L. chuanxiong*

	IC_{50} value (mg purified polysaccharide/ml)		
	LCA	LCB	LCC
Scavenging ability on DPPH radicals	2.42	0.87	4.46
Scavenging ability on OH radicals	9.25	6.36	13.24
Scavenging ability on superoxide radical	1.74	0.82	2.54
Chelating ability on ferrous ions	6.12	3.19	14.52
Lipid peroxidation inhibition assay	4.44	1.68	4.76
Cytotoxicity assay	0.187	0.085	0.385

Three purified polysaccharides, LCA, LCB, and LCC, were obtained from *Ligusticum chuanxiong* Hort. The estimated weight is around 2.83×10^4 Da, 1.23×10^4 Da, and 6.31×10^4 Da, respectively. LCA is α-glucoside linkaged pyranose, and comprises Ara, Glc, and Man. The main skeleton both of LCB and LCC are α- and β-glucoside linkaged pyranose. LCB consists of Ara, Glc, Gal, and Man, and LCC comprises Ara, Glc, and Gal.

Compared with LCA and LCC, LCB exhibited the highest antioxidant activities and antiproliferation ability. The effective concentrations corresponding to inhibiting effect of 50% (IC_{50} values) of LCB were obviously lower than those of LCA and LCC (Table 1). A relatively low molecular weight and complex chemical components of LCB appeared to increase the antioxidant activity and antiproliferation ability. The results suggested that the molecular weights and chemical components of polysaccharides played an important role on their bioactivity.

Overall, the LCB possessed good antioxidant and antiproliferative properties except for scavenging ability on hydroxyl radicals, and can be developed as a novel potential natural compound for the treatment and prevention of some diseases relating to ROS. We can rationally assume that *L. chuanxiong* has curative effect in traditional medicine partly because of antioxidation and cytotoxicity of polysaccharides in it. So LCB should be explored as investigated further in view of the potential as a therapeatic agent.

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