J Food Sci Nutr
Vol 11, p 261-267 (2006)

J Food Science
and Nutrition

Physiological Activities of Different Molecular Weight Fractions of Crude Polysaccharides from *Dŏdŏk* (*Codonopsis lanceolata*)

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Abstract

This study investigated the physiological activities of different molecular weight (MW) fractions of crude polysaccharide from *Dŏdŏk* (*Codonopsis lanceolata*). The crude polysaccharide cut off for each fraction was: <1,000 MW (Fr Ⅰ), 1,000 MW<fraction<3,000 MW (Fr Ⅱ), 3,000 MW<fraction<10,000 MW (Fr Ⅲ), 10,000 MW<fraction<100,000 MW (Fr Ⅳ), 100,000 MW<fraction (Fr Ⅴ) with membrane. Fr Ⅰ exhibited the highest yield of all the fractions. Fr Ⅰ also had the highest total sugar contents, total protein contents, total phenolic compounds content, as well as the greatest growth inhibitory activity against SNU-1 and Hela cells, ACE inhibitory activity, and DPPH radical scavenging effect. Fr Ⅱ exhibited lower physiological activity than Fr Ⅰ, and showed higher activity than those of fraction of above 3,000 MW, but there was no significant ACE inhibitory activity. The Fr Ⅲ exhibited the lowest yield, total sugar contents, total protein contents, and total phenolic contents; as well as the least growth inhibitory activity against SNU-1, Hela, ACE inhibitory activity, DPPH radical scavenging effect.

Key words: physiological activities, polysaccharide, *Codonopsis lanceolata*

INTRODUCTION

Age-related degenerative diseases, such as high blood pressure, kidney disease, and cancers are increasing in Korea; possibly as a result of an aging population, stresses associated with modern life, as well as westernized diets and lifestyles, increased convenience food intake, serious environmental pollution etc. For these reason, there is increased interest in plant-based diets, as opposed to high fat diets containing meat, as part of a healthy lifestyle. Many natural substances have demonstrated anti-cancer activity (1,2), anti-oxidant activity (3), ACE inhibitory activity (4,5), and anti-microbial properties (6). There is considerable research suggesting that bioactive components of plants can reduce the occurrence of many age-related degenerative diseases (7). Therefore, there is increased interest in researches for the functional characteristic of natural materials that display these physiological activities, and for product development to exploit their disease preventing properties. *Codonopsis lanceolata* has been shown to contain effective concentrations of bioactive compounds such as terpenoids, sterols, squalene, cycloartenol, norharman, albigenic acid (8,9) and volatile fragrance ingredients (10), as well as amino acids and inorganic substances. Maeng and Park (11) reported that the ethanol extracts from *Codonopsis lanceolata* exhibited high anti-oxidant activity, and Han et al. (12) reported that the distilled water (DW) extracts from *Codonopsis lanceolata* exhibited anti-oxidant activity that inhibited the formation of lipid peroxides and reduced the accumulation of neutral lipids and cholesterol. Also, Suh (13) reported that the DW extracts from *Codonopsis lanceolata* propagated thymocytes, and increased phagocytic activity of peritoneal macrophages. Suh and Eun (14) postulated that the active material is a polysaccharide. Park et al. (15) and Han et al. (16) reported that polysaccharide from *Schizophyllum commune* and *Phellinus linteus* can be used as anti-cancer and immunotherapy medicines. Kubo et al. (17) reported that a part of polysaccharide from *Ganoderma lucidum* is a component of a compound that controls lipid peroxidation. Also, polysaccharide ingredients such as lentinan and polysaccharide K from mushrooms are efficacious adjuncts in cancer treatment (18). Gao et al. (19) reported that there is anti-complement activity in polysaccharide ginseng DW extracts, and Kwak and Kim (20) reported that crude soluble polysaccharide fractions from Korean ginseng have important biological activities and medicinal properties. Kim et al. (21) reported that DW polysaccharide extracts of *Panax ginseng* can extirpate cancerous cells by increasing Th1 cell activity, cytokine production by macrophages, and activating

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Lymphokine-activated killer cells. *Codonopsis lanceolata* had been widely used as a food from ancient times. In traditional oriental medicine, *Codonopsis lanceolata* has been used for the treatment of respiratory disease with coughing and discharge of phlegm, as well as for promoting milk secretion and during convalescence, because of its usefulness as a ginseng substitute (8). Despite of the reports of various medicinal properties, *Codonopsis lanceolata* has not been well researched and its medicinal potential has not been well explored up to now. Therefore, this research investigated a crude polysaccharide extract from *Codonopsis lanceolata* and characterized physiological activities such as cytotoxicity, ACE inhibitory activity, and DPPH radical-scavenging effects using different molecular weight polysaccharides.

MATERIALS AND METHODS

Material

Codonopsis lanceolata was purchased from a Garack market in Seoul, Korea. The sodium bicarbonate, RPMI 1640, HEPES, penicillin streptomycin, fetal bovine serum (FBS), phosphate buffered saline (PBS), sodium borate, lung acetone powder, 1,1-diphenyl-2-picryhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent, tannic acid, dimethylsulfuroxide (DMSO), hippuryl-his-leu (HHL) were purchased from Sigma Chemical Co., Ltd. Ethanol, hexane, chloroform, ethyl acetate, n-butanol, methanol were purchased from Junsei Chemical Co. Human stomach cancer SNU-1 cells were provided by Korean Cell Line Bank (KCLB, Seoul, Korea). All other chemicals used were of analytical grade.

Preparation of crude polysaccharide from *Dŏdŏk*

The skin of *Dŏdŏk* was carefully separated using knife, cut into small pieces, and then dried in a freeze dryer (Ilshin lab. Co., Ltd). The dried *Dŏdŏk* was ground to a fine powder (80 mesh) and stored in -20°C. One hundred grams of *Dŏdŏk* were extracted three times with DW (1,500 mL) at 100°C for 24 hr, and then, filtered through Toyo No 5C filter paper. The filtrate was concentrated in a vacuum rotary evaporator and the resulting polysaccharide was precipitated by addition of 3 times volumes of absolute EtOH, and recovered by centrifugation at 6,000×*g* for 30 min to remove the supernatant. The precipitate was dissolved in a minimum volume of distilled water, and then, dialyzed with a dialysis tube (MWCO: 12,000 Da) for 3 days. The polysaccharide was finally freeze-dried (Ilshin Lab Co., Ltd, Korea). The dried crude polysaccharide dissolved in distilled water, consecutively partitioned with Millpore filter [MW, cut off; fraction<1,000 (Fr Ⅰ), 1,000<fraction<3,000 (Fr Ⅱ), 3,000<fraction<10,000 (Fr Ⅲ), 10,000<fraction<100,000 (Fr Ⅳ), 100,000<fraction (Fr Ⅴ)].

Yield of extracts, total sugar contents, total protein contents

Yields obtained for the different molecular weights of crude polysaccharides from *Dŏdŏk* were calculated from the dry weights of extract fractions divided by the dry weight of the *Dŏdŏk* sample used. Total sugar contents were determined by the phenol sulfuric method (22). Briefly, 1 mL of sample was mixed in a test tubes with 1 mL of 10% phenol solution, and 5 mL of H_2SO_4 were added. After standing for 30 min at room temperature, the absorbance was measured at 470 nm. Total protein contents were determined by the Lowry method (23), employing bovine serum albumin as the standard.

Total phenolic compounds content

Total phenolic compounds content was determined by using Folin-Ciocalteu's reagent, according to a slightly modified method of Dewanto (24). Briefly, 1 mL of sample was mixed in a test tube with 0.5 mL Folin-Ciocalteu's reagent, and 2 mL of 10% sodium carbonate were added. The mixture was vortexed and held for 30 min at room temperature. The absorbances of all samples were measured at 725 nm. Quantification was done on the basis of a standard curve using tannic acid. Results were expressed as gram of tannic acid equivalent per 100 g of dry weight.

Tumor cell cytotoxicity test

Human cancer cell line : The target cells used in this study were the human stomach cancer SNU-1 cell line, and human cervical cancer Hela cells (KCLB, Seoul, Korea). SNU-1 cells were cultured in a T-flask with Rosewell Park Memorial Institute (RPMI) 1640 containing 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin-streptomycin, HEPES 4.75 g (w/v), sodium bicarbonate 2.0 g (w/v) at 37°C under 5% CO_2 atmosphere incubator (Forma Scientific, Inc., USA).

MTT assay : Cytotoxicity was assayed by the method of Carmichael et al. (25). One hundred thirty μL of SNU-1 cells (1×10^4 cells/mL) and 14.5 μL/well of sample solution were added to a 96 well plate and incubated for 72 hr, after which 14.5 μL of MTT (5 mg/mL, PBS) was added. The supernatant was removed by centrifuging, and after then, 108.5 μL of DMSO was added to terminate the reaction. The survival rate of tumor cells was assayed by measuring the optical density using an ELISA reader (Emax, Molecular Devices, Sunnyvale, USA) at 540 nm. The sample groups were compared with control groups in the absence of the tested samples.

All *in vitro* results were expressed as the inhibition ratio (%) of tumor cell proliferation as follows:

$$\text{Cytotoxicity (\%)} = [1 - (Nt/Ac)] \times 100,$$

where Nc and Nt are the average numbers of viable tumor cells of the control group and test group, respectively.

ACE inhibitory activity

Inhibitory activity against angiotensin-converting enzyme (ACE) was assayed by the method of Cushman and Chung (26) with slight modifications. A mixture consisting of 50 μL of purified ACE and 50 μL of sample was pre-incubated for 5 min at 37°C. For ACE activity determination, the mixture was added to 50 μL of HHL, and incubated for 30 min at 37°C. The reaction was stopped by adding 250 μL of 1 M HCl. The hippuric acid liberated by the ACE reaction was extracted with 1.5 mL ethyl acetate, and the solvent was removed by evaporation in an oven (120°C). The residue was dissolved in 3 mL distilled water, and after standing for 10 min, absorbance was recorded at 228 nm using a UV/VIS spectrophotometer (Jasco TS Science. Co. Ltd., Japan). The extent of inhibition was calculated as follows;

$$\text{Inhibition (\%)} = [1 - \{(Ats - Acs)/Aco - Acb\}] \times 100$$

where Ats was absorbance of the test sample, Acs was the absorbance of control sample, Aco was absorbance of the control, and Acb was the absorbance of the control blank.

DPPH radical scavenging effect

Free radical-scavenging activity was determined by using a stable free radical, DPPH, according to a slightly modified method of Blois (27). DPPH solution was prepared at the concentration of 1×10^{-4} M in methanol. During the assay, 0.15 mL of sample was mixed with 3.5 mL of DPPH solution. The mixture was incubated at room temperature for 30 min. After standing for 30 min, absorbance was recorded at 516 nm. The percentage inhibition was defined by the absorbance at 516 nm in the absence of various solvent extracts to that measured with the sample.

$$\text{DPPH radical scavenging activity (\%)} = [1 - (At/Ac)] \times 100$$

where Ac was the absorbance of the control, At was the absorbance in the presence of the test compound.

Statistical analysis

Data were analyzed using the SPSS package for Windows (2001). All assays were done in triplicate. Values were expressed as mean±standard deviation (SD). The mean values of the tail intensity from each treatment were compared using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. P-value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Yield of fractions

Yields of the different molecular weight fractions of crude polysaccharide from *Dŏdŏk* are shown in Fig. 1. Fr Ⅰ exhibited the highest yield of 5.65%, and Fr Ⅱ and Fr Ⅴ showed yields of 2.77% and 2.32%, respectively. The yield of Fr Ⅳ was 1.37% and Fr Ⅲ showed the lowest yield of 0.28%.

Total sugar and protein contents

Total sugar contents in crude polysaccharide fractions from the different molecular weights of *Dŏdŏk* are shown in Fig. 2. Fr Ⅰ exhibited the highest contents of 6.51 mg/mL, and Fr Ⅱ and Fr Ⅴ showed high contents of 1.74% and 1.67%, respectively. Fr Ⅲ showed the lowest contents of 0.13 mg/mL. Total protein contents of the different molecular weight fractions of crude polysaccharide from *Dŏdŏk* are shown in Fig. 3. Fr Ⅰ exhibited the highest contents of 2.28%, and Fr Ⅱ showed the next highest content at 1.13%. Fr Ⅲ showed the lowest content of 0.07% among the fractions.

Total phenolic compounds content

Total phenolic compounds contents of each different molecular weight fraction of crude polysaccharide from

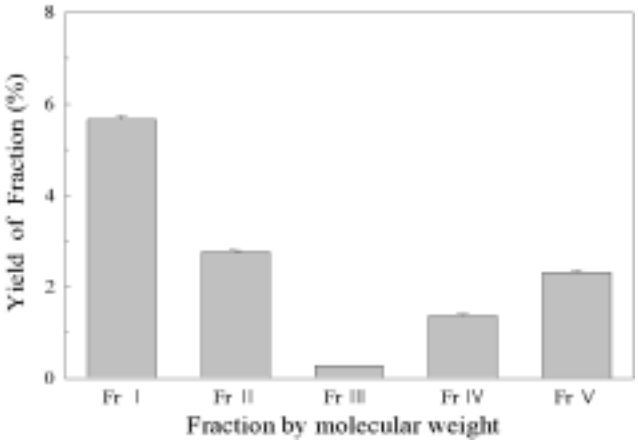

Fig. 1. Yields of different molecular weight fractions of crude polysaccharides from *Dŏdŏk* (*Codonopsis lanceolata*).
Fr Ⅰ, fraction of below 1,000 MW; Fr Ⅱ, fraction of between 1,000 MW and 3,000 MW; Fr Ⅲ, fraction of between 3,000 MW and 10,000 MW; Fr Ⅳ, fraction of between 10,000 MW and 100,000 MW; Fr Ⅴ, fraction of above 100,000 MW.

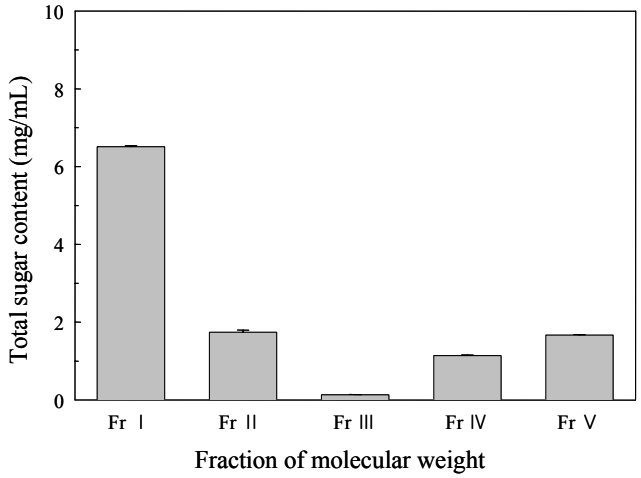

Fig. 2. Total sugar contents of different molecular weight fractions of crude polysaccharides from *Dŏdŏk* (*Codonopsis lanceolata*).
Fractions are the same as in Fig. 1.

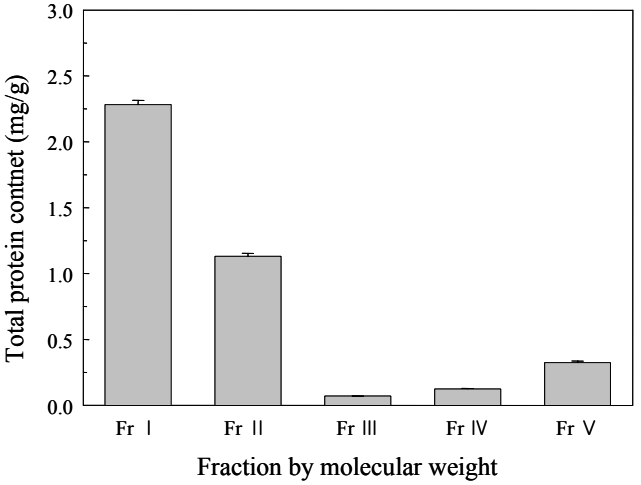

Fig. 3. Total protein contents of different molecular weight fractions of crude polysaccharides from *Dŏdŏk* (*Codonopsis lanceolata*).
Fractions are the same as in Fig. 1.

Dŏdŏk are shown in Fig. 4. Fr Ⅰ exhibited the highest contents of 219.03 ㎍/g among the fractions, and Fr Ⅱ showed next higher contents at 170.33 ㎍/g, higher than those of fractions above 3,000 MW. Fr Ⅲ showed the lowest contents of 12.18 ㎍/g among the fractions, and total phenolic contents of Fr Ⅳ and Fr Ⅴ were 20.75 ㎍/g, 54.03 ㎍/g, respectively. Briefly, total phenolic contents of the fractions below 3,000 MW and the fractions of above 3,000 MW exhibited approximately 4～10 fold differences. Poly-phenolic compounds have DPPH radical-scavenging effects (28). Two studies conducted by Lu and Foo (29) and Kim and Chung (30) reported high correlations between DPPH radical-scavenging ac-

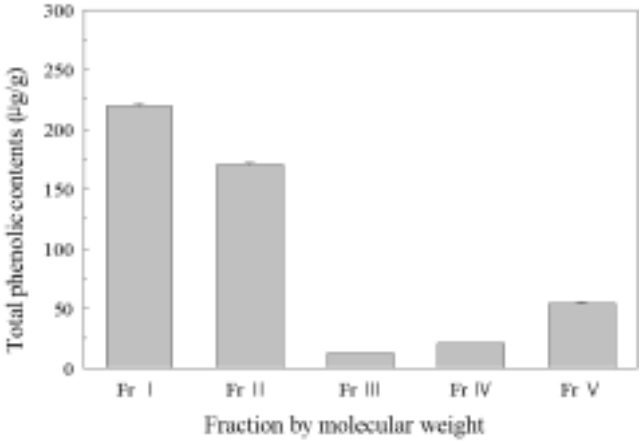

Fig. 4. Total phenolic compounds contents of different molecular weight fractions of crude polysaccharides from *Dŏdŏk* (*Codonopsis lanceolata*).
Fractions are the same as in Fig. 1.

tivities and total poly-phenolics, and Torel et al. (31) reported that total poly-phenolics were highly correlated with ACE inhibitory activity. These results suggest that the differences in total phenolic contents by molecular weight may responsible for the differences in ACE inhibitory activity and growth inhibitory activity against SNU-1, Hela, as well as DPPH radical scavenging effects.

Cytotoxicity

Growth inhibitory activities of the *Dŏdŏk* polysaccharide fractions against SNU-1 human stomach cancer cells are shown in Fig. 5. Fr Ⅱ exhibited the highest growth inhibitory activity of 71.15%, and Fr Ⅰ exhibited the next highest growth inhibition at 17.23%. The fractions of above 3,000 MW exhibited low inhibitory activity of below 10%, and the Fr Ⅳ exhibited no significantly inhibitory activity against SNU-1. Growth inhibitions of the fractions against the human cervical cancer cell, Hela, are shown in Fig. 6. Fr Ⅰ exhibited the strongest inhibitory activity of 67.03%; the fractions of below 3,000 MW, and Fr Ⅱ showed the strongest inhibitory activity among the fractions. However, the fraction of above 3,000 MW exhibited no significantly growth inhibitory activity against Hela. The Fr Ⅰ fraction had the highest total phenolic content, total protein content, total sugar content exhibited strong growth inhibitory activity. Choi et al. (32) reported that a polyphenol fraction extracted from *Panax ginseng* exhibited strong growth inhibitory activity against human colon cancer cell HT-29, and Hwang et al. (33) reported that hot water extracts of *Lentinus edodes* and *Pleurotus eryngii* exhibited strong growth inhibitory activity against

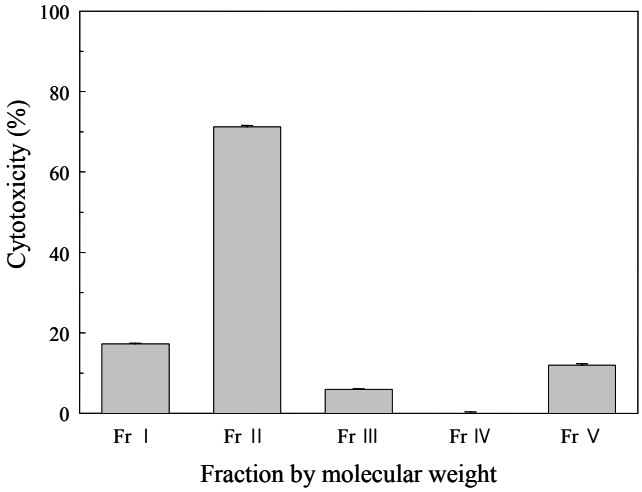

Fig. 5. Growth inhibition activity against human stomach cancer cell, SNU-1 with different molecular weights of crude polysaccharides from *Dŏdŏk* (*Codonopsis lanceolata*).
Fractions are the same as in Fig. 1.

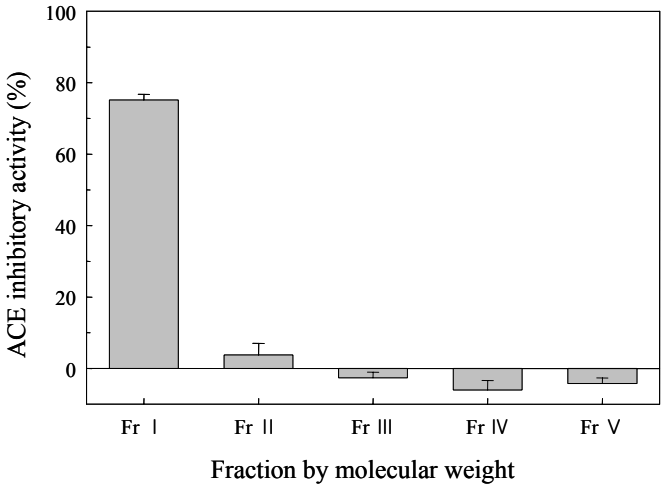

Fig. 7. Angiotensin-I converting enzyme (ACE) inhibitory activity of different molecular weight fractions of crude polysaccharides from *Dŏdŏk* (*Codonopsis lanceolata*).
Fractions are the same as in Fig. 1.

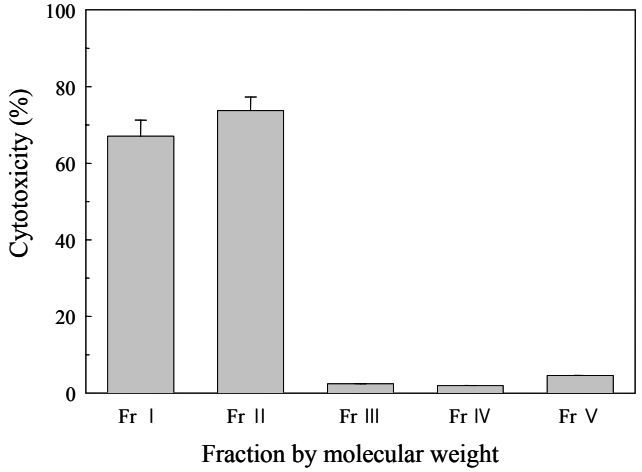

Fig. 6. Growth inhibition activites against human cervical cancer cell, Hela, of different molecular weight fractions of crude polysaccharides from *Dŏdŏk* (*Codonopsis lanceolata*).
Fractions are the same as in Fig. 1.

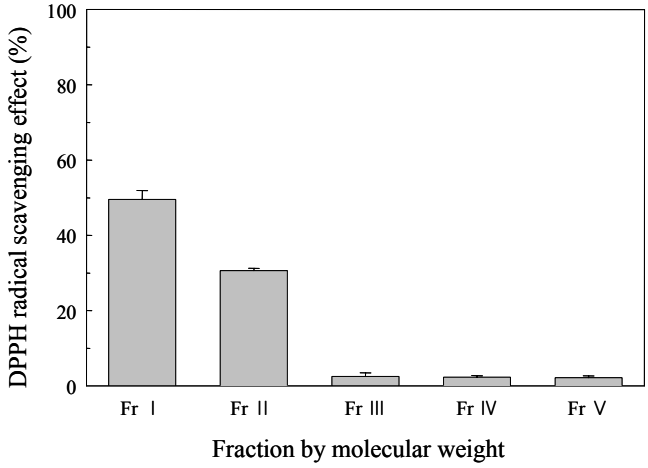

Fig. 8. DPPH radical scavenging effects of different molecular weight fractions of crude polysaccharide from *Dŏdŏk* (*Codonopsis lanceolata*).
Fractions are the same as in Fig. 1.

a human colon cancer cell line.

Kim et al. (21) reported that the polysaccharide, ginsan, separated in DW extracts of *Panax ginseng* can extirpate cancer cells by activating Th1 and LSK cells, and by increasing production of macrophage cytokines. Also, Park et al. (34) and Kim et al. (18) reported that a polysaccharide component of ginseng exhibited high growth inhibitory activity.

ACE inhibitory activity

Angiotensin converting enzyme (ACE) inhibitory activities of the polysaccharide fractions from *Dŏdŏk* are shown in Fig. 7. Fr Ⅰ exhibited the highest inhibitory activity at 75.12%, and Fr Ⅱ exhibited the next highest at 3.77%. The fractions with molecular weights of below 3,000 MW showed strong inhibitory activity, while the fractions of above 3,000 MW exhibited no significant ACE inhibitory activity. Lee et al. (35) reported that the total phenolic compounds content and ACE inhibitory activity are closely related, and An and Lee (36) reported that total phenolic components, such as tannins extracted from *Camellia sinensis* L., showed strong ACE inhibitory activity.

DPPH radical scavenging effect

DPPH radical-scavenging effects of the *Dŏdŏk* polysaccharide fractions are shown in Fig. 8. Fr Ⅰ exhibited the strongest scavenging effect of 49.5% and Fr Ⅱ was

showed the next strong of 30.6%. The higher molecular weight fractions of Fr Ⅲ, Fr Ⅳ, and Fr Ⅴ exhibited scavenging effect of only 2.50%, 2.27%, 2.15%, respectively. In other word, the fractions of below 3,000 MW showed strong scavenging effects, while the fraction of above 3,000 MW exhibited no significant DPPH radical scavenging ability. Lee et al. (35) reported that total phenolic contents and DPPH radical-scavenging effects were closely related with each other.

When considering the results of this study as a whole, it appears to be an interrelationship between total phenolic compounds, total protein content, total sugar content and a physiological activity. A close examination of the physiological activities of the fractions, through more in depth research, is needed to reveal the relationship of the structural characteristics of the polysaccharides and their effects on human metabolism.

ACKNOWLEDGEMENT

This study was supported by grants from Agriculture R&D Promotion Center in 2006.

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(Received September 18, 2006; Accepted November 23, 2006)