Korean J. Chem. Eng., **29**(10), 1393-1402 (2012)
DOI: 10.1007/s11814-012-0010-7

Comparative study on the antioxidant and nitrite scavenging activity of fruiting body and mycelium extract from *Pleurotus ferulae*

Bok Hee Kim*,‡, DuBok Choi,***,‡, Yu Lan Piao****, Sang-Shin Park*****, Myung Koo Lee**, Wol-Suk Cha****, Young-Cheol Chang******, and Hoon Cho****,†**

*Department of Food Science and Nutrition, Chosun University, Gwangju 501-759, Korea
**Department of Pharmacy, College of Pharmacy, Chungbuk National University, Cheongju 361-763, Korea
***Biotechnology Lab., BK Company R&D Center, Jeonbuk 579-879, Korea
****Department of Polymer Science and Engineering, Chosun University, Gwangju 501-759, Korea
*****Department of Biotechnology, Dongguk University, Gyeongju 780-714, Korea
******Division of Applied Sciences, College of Environmental Technology, Graduate School of Engineering, Muroran Institute of Technology, 27-1, Mizumoto, Muroran 050-8585, Hokkaido, Japan
(*Received 15 November 2011 • accepted 27 January 2012*)

Abstract–We investigated the effects of the antioxidant and the nitrite scavenging activities of the extracts from *Pleurotus ferulae* fruiting body grown on the solid state using corn cob and activated bleaching earth (CCABE media) and its mycelium grown in the liquid state. The total phenol and polysaccharide concentrations in hot water extract of fruiting body were approximately 3.6- and 4.3-fold higher than those of the mycelium. Using the hot water extract of fruiting body, the maximum DPPH radical scavenging activity at 9 mg/mL, hydroxyl radical scavenging activity at 12 mg/mL, reducing power at 12 mg/mL, and chelating ability at 12 mg/mL were obtained, 80.5%, 72.4%, 0.99 OD (700 nm), and 77.0%, respectively. However, in the case of hydrogen peroxide scavenging activity, the ethanol extract was the highest, 78.7% at 12 mg/mL. The maximum nitrite scavenging activity was obtained, 89.7% at 6 mg/mL of hot water extract from fruiting body. Hot water extracts were more effective than ethanol extracts in scavenging activity on DPPH radicals and hydroxyl radical scavenging, reducing power, and chelating activity of ferrous, whereas ethanol extracts were more effective in hydrogen peroxide scavenging activity as evidenced by their lower EC_{50} values. These results indicate that the hot water extract of *P. ferulae* fruiting body using CCABE media has good potential to be used as a source of materials or additives for oxidation suppressant in food, cosmetics and drug compositions.

Key words: Antioxidant Activity, *Pleurotus ferulae*, Fruiting Body, Mycelium

INTRODUCTION

Edible mushrooms have long been used in folk medicines and health foods. *Pleurotus ferulae* is a Hymenomycetes fungus belonging to the order Agaricales and family Pleurotaceae. *P. ferulae* originated in the Gobi desert in the Xinjiang autonomous region of China, which makes it suitable for growth in arid climates. The Chinese refer to this mushroom as "western paradise" or "white *Garnoderma*." It is shaped like the traditional Chinese medicine, *Ganoderma*, but it is white, and more importantly, it is rich in nutrients [1]. *P. ferulae* has been reported to inhibit acetylcholinesterase and protect brain cells during Alzheimer disease induction [2], exhibit antimicrobial activity [3], and demonstrate fibrinolytic activity [4]. Recently, we researched the optimal media and growth conditions for *P. ferulae* liquid cultures to effectively produce exopolysaccharide and mycelial growth [5] and determined the viability of human cancer cell lines for use in screening the antitumor substances contained in the *P. ferulae* extract [6].

Various agricultural and industrial by-products have been used as inexpensive growth substrates for the economical production from various mycelial species. Mushroom production is conducted on various substrates: *P. tuber-regium* is grown on cotton waste and crude vegetable oil [7], *P. ostreatus* on sunflower seed hulls and whey permeate [8], *P. pulmonarius* on olive oil mill waste [9], and *P. sajor-caju* and *P. cornucopiae* var. *citrinopileatus* on wheat straw substrates containing olive oil mill waste [10]. Although there have been numerous reports on the cultivation of *Pleurotus* genus using agriculture and industrial wastes, there are no reports on antioxidant properties of *P. ferulae* fruiting bodies cultivated on them. Among these wastes, corn cob generated during wet milling of corn is a promising agricultural resource for mushroom cultivation because of the extensive cultivation of corn and because it contains starch, lignocelluloses, nitrogen, and various minerals. Activated bleaching earth is used in the vegetable oil refining industries. During the oil refining process, the spent activated bleaching earth contains vitamins, protein, carotene, chlorophyll, phosphatides, soaps, minerals, and fatty acids. Traditionally, the generated corn cob waste and spent activated bleaching earth have been sent to landfills. These cause serious environmental problems primarily because of the waste volume and high organic material concentration. To solve these problems, Kim et al. previously attempted to use corn cob waste and activated bleaching earth as the energy source for mushroom cultivation [11].

In this study, to investigate the effects of the antioxidant activities of the extracts of *P. ferulae* fruiting body grown on the solid state

†To whom correspondence should be addressed.
E-mail: hcho@chosun.ac.kr
‡These authors contributed equally to this work.

using the mixture media of corn cob and activated bleaching earth (CCABE media) and its mycelium grown in the liquid state using the solution media, we examined DPPH radical scavenging activity, hydroxyl radical scavenging activity, hydrogen peroxide scavenging activity, reducing power, and chelating activity of ferrous. In addition, the nitrite scavenging activity was studied.

MATERIAL AND METHODS

1. Cultivation for Fruiting Body and Mycelium Production

Pleurotus ferulae was obtained from the culture ground of Kaya-Backsong (Chungnam, Korea). Cultures were maintained on Potato dextrose agar (PDA) plate. Plates were inoculated and incubated at 25 °C for 7 days, and then stored at 4 °C. *P. ferulae* was initially grown on a PDA medium in a petri-dish, and then transferred into the seed medium containing malt extract 10 g/L, yeast extract 4 g/L, and glucose 4 g/L by punching out from the agar plate culture with a sterilized cork borer. The seed was grown in a 300 mL flask containing 100 mL of the seed medium at 25 °C on a rotary shaker at 100 rpm for 5-6 days, and then homogenized at 10,000 rpm for 30 sec. Fruiting body cultures of *P. ferulae* were carried out in propylene bags using the mixture media of corn cob and activated bleaching earth (CCABE media). The CCABE media were prepared as follows: corn cob 40%, activated bleaching earth 31.4%, populous sawdust 15%, rice bran 5%, garlic powder 8%, $NH_4H_2PO_4$ 0.2%, and $CaCO_3$ 0.4%. Five hundred grams of the mixture containing moisture 70% was dispersed into the bag. After autoclave sterilization at 121 °C for 40 min and cooling, a 5% of inoculation was used in each sample. Inoculated blocks were incubated at 25 °C in the dark. After 25 days, the substrates were completed colonized by the mycelium. The blocks were then shocked at 4-5 °C for 48 hr to stimulate production of fruiting bodies. For the mycelium production from of *P. ferulae*, the culture media were used as follows: glucose 50 g/L, CSL 2 g/L, yeast extract 4 g/L, polypeptone 10 g/L, KH_2PO_4 1 g/L, and $MgSO_4$ 0.5 g/L. All media were sterilized at 121 °C for 40 min. The culture medium was inoculated with 5% of the mycelial homogenate and then cultivated at 25 °C in a 50 L air lift bioreactor containing 30 L of working volume under 1.5-2.0 vvm for 10 days.

2. Preparation of Sample Extract

Samples (10 g) of dried fruiting body grown on solid-state using CCABE media and dried mycelium grown in the liquid state were extracted with 500 mL of ethanol, ethyl acetate, petroleum ether, and chloroform using a soxhlet apparatus at room temperature for 3 to 6 hr. Next, the extracts were evaporated. The resulting solid mass was used as the *P. ferulae* extract. In the case of hot water extract, 10 g of fruiting body and mycelium was extracted with 500 mL of hot distilled water using a soxhlet apparatus at 80 °C for 3 hr, centrifuging at 5,000 g for 15 min, and filtering through Whatman No. 1 filter paper. After standing overnight at 4 °C, the mixture was centrifuged and the supernatants were evaporated. The resulting solid mass was used as the hot water extract. The dried extracts were used directly for analyses of antioxidant components and stored at 4 °C for further use.

3. Antioxidant Components

The total phenol concentration was calculated based on the garlic acid calibration curve. One mL of sample was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated Na_2CO_3 (35%) was added to the mixture, which was brought to a volume of 10 mL by adding distilled water. The reaction was maintained in the dark for 90 min, and its absorbance was measured at 750 nm relative to a blank. The total flavonoid concentration was determined in terms of catechin equivalents. One mg of catechin standard was dissolved in 1 mL of ethanol/water (3 : 7, v/v). A 500 μL of sample was collected and mixed with 75 μL of 5% sodium nitrite solution, followed by mixing thoroughly, standing at room temperature for 5 min, adding 150 μL of 10% aluminum chloride solution, standing for an additional 5 min, adding 500 μL of 1 N sodium hydroxide solution, and measuring the absorbance at 510 nm. Beta-carotene concentration was analyzed by high performance liquid chromatograph (HPLC), as described. Each dried extract (100 mg) was extracted with 1 ml of ethanol, 2 mL of n-hexane containing BHA (25 g/mL), and 1 mL of deionized water at 20 g for 45 min at room temperature, and the mixture was centrifuged at 400 g for 10 min. After the removal of the n-hexane layer by N_2 gas, the volume was adjusted to 1 mL using n-hexane and filtered through a syringe-driven filter unit (13 mm, Millipore, Billerica, MA) using 0.45 m PVDF non-sterile filter paper. Immediately after filtration, the filtrate was injected into an HPLC. The HPLC system consisted of a Shimadzu LC-10AT VP pump, a Shimadzu FCV-10AL VP controller, a Rheodyne 7725i injector, a 20-ll-sample loop, a Hitachi D-2500 chromato-integrator, a Shimadzu SPD-10A VP UV-vis detector, and a LiChrospher 100 RP-18 column (4.6×250 mm, Merck). The mobile phase was methanol/toluene, 3 : 1 (v/v) at a flow rate of 1.5 mL/min, and UV detection was performed at 450 nm. Ascorbic acid concentration was calculated on the basis of the calibration curve of authentic L-ascorbic acid. Each extract (20 mg) was extracted with 10 mL of metaphosphoric acid (10 mg/mL) for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The filtrate (1 mL) was mixed with 9 mL of 2, 6-dichloroindophenol, and the absorbance was measured within 15 sec at 515 nm against a blank. Tocopherol concentration was analyzed by HPLC. Each extract (50 mg) was suspended in 6 mL of pyrogallol/ethanol (6 : 94) and 4 mL of potassium hydroxide aqueous solution (600 mg/mL), and the resulting mixture was saponified at 70 °C for 20 min. Deionized water (15 mL) was added and the mixture was extracted with 15 mL of n-hexane. The organic layer was washed with deionized water to neutral, dried over anhydrous sodium sulfate, and rotary evaporated until dry. The residue was redissolved in 5 mL of n-hexane and filtered prior to HPLC. The mobile phase was acetonitrile/methanol (85 : 15) at a flow rate of 1.0 mL/min, and UV detection was performed at 295 nm. The polysaccharide concentration was quantified with a modified phenol-sulfuric acid method. The extract was precipitated with 87.5% ethanol at 4 °C overnight and centrifuged at 7,000 ×g for 30 min. The precipitate was washed twice with absolute ethanol and evaporated in a vacuum to remove residual ethanol. The precipitated polysaccharide was dissolved in distilled water and used for polysaccharide analysis. The color reaction was initiated by mixing 1 mL of polysaccharide solution with 0.5 mL of 5% phenol solution and 2.5 mL of concentrated sulfuric acid, and the reaction mixture was maintained in a 100 °C water bath for 15 min. After cooling at room temperature, the optical density of the mixture was determined at 490 nm, and the polysaccharide concentration was calculated with D-glucose as the standard.

4. DPPH Radical Scavenging Activity

The DPPH (1, 1-diphenyl-2-picry-hydrazil) radical scavenging

effect was determined by spectrophotometer. A sample (1 mg/mL) was added to 1 ml of DPPH (10 mM) in methanol. The mixture was shaken and maintained at room temperature for 10 min. The absorbance was measured at 517 nm.

5. Hydroxyl Radical Scavenging Activity

The hydroxyl radicals reacted with the nitrone spin trap 5, 5-dimethyl pyrroline-N-oxide (DMPO), and the resultant DMPO-OH adducts were detected with an electron paramagnetic resonance (EPR) spectrometer. The EPR spectrum was recorded 2.5 min after mixing each extract (1-10 mg/mL) in deionized water (200 L) with 200 L of 10 mM H_2O_2 (Merck), 200 L of 10 mM Fe^{2+} and 200 L of 10 mM DMPO using a Bruker EMX-10 EPR spectrometer at the following settings: 3480-G magnetic field, 1.0 G modulation amplitude, 0.5 s time constant, and 200 s scan period.

6. Hydrogen Peroxide Scavenging Activity

A 100 μL aliquot of 0.1 M phosphate buffer (pH 5.0) and sample solution were mixed in a 96-well microplate. Next, 20 μL of hydrogen peroxide was added to the mixture, which was incubated at 37 °C for 5 min. Following incubation, 30 μL of 1.25 mM ABTS and 30 μL of peroxidase (1 unit/mL) were added to the mixture, which was subsequently incubated at 37 °C for 10 min. The absorbance at 405 nm was measured with a microplate reader.

7. Reducing Power

One milliliter of the extract was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium hexacyanoferrate ($K_3Fe(CN)_6$, 1%). The mixture was placed in a 50 °C water bath for 30 min. A 2.5 mL of trichloroacetic acid (10%) was added, and the mixture was centrifuged for 10 min at 800 ×g. The supernatant was recovered, and 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1%) were added. The absorbance of the sample was measured at 700 nm.

8. Chelating Activity of Ferrous

The extracts (1-20 mg/mL) in methanol (2 mL) and 200 mL of 1 mmol/L tetramethyl murexide (TMM, Sigma) were added to 2 mL of the 30 mmol/L hexamine, 30 mmol/L potassium chloride and 9 mmol/L ferrous or cupric sulfate mixture. After 3 min at room temperature, the absorbance of the mixture was determined at 485 nm, relative to the absorbance of a blank.

9. Nitrite Scavenging Activity

One milliliter of 1 mM $NaNO_2$ was added to 1 mL of each sample, and pH values of the resulting mixtures were adjusted to 1.2, 4.2, and 6.0. Final volume of each sample was adjusted to 10 mL. The sample was allowed to react at 30 °C for 1 hr, after which 1 mL of each sample was obtained, mixed thoroughly with 5 mL of acetic acid (2%) and 0.4 mL of Griess reagent, and kept at room temperature for 15 min A blank was prepared by adding 0.4 mL of distilled water instead of the Griess reagent. The nitrite scavenging activity was measured at 520 nm.

10. Statistical Analysis

Results presented in tables and figures were expressed as means± standard deviation.

RESULTS

1. Extraction Yield

Various extractants such as ethanol, hot water, ethyl acetate, petroleum ether, and chloroform were used to investigate the extraction yield of fruiting body and mycelium. The results are shown in Fig. 1. The extraction yield depended on the type of extractant. Higher extraction yields were obtained with an increase in solvent polarity, regardless of substrates. When ethyl acetate, petroleum ether, or chloroform was used, the extraction yields of mushroom grown on the solid state and in liquid state were below 10%. However, when hot water was used, the extraction yields of mushroom grown on the solid state and in liquid state were 34.5 and 43.7%, respectively, which was approximately 2-fold higher than that of ethanol. This result was similar to that of hot water extracts from the white mutant of *Hypsizigus marmoreus* [12], *P. citrinopileatus* [13], and *Agricus blazei* [14]. In addition, the yield from hot water extract of *Inonotus obliquus* was approximately 10-fold higher than that of ethanol extract [15]. However, when methanol was used for extraction of *Lentinus edodes* and *Volvariella volvacea*, the yields were approximately 1.5-2 fold higher than those of hot water [16] and approximately 9-14 fold higher than those of petroleum ether or ethylacetate [17]. These results indicate that the extraction yields of *P. ferulae* fruiting body and mycelium, regardless of the culture type, were strongly affected by the choice of extractant. These results also indicate that the use of hot water to extract soluble components from *P. ferulae* can be applied to the preparation of Korean herb medicine and the brewing of herbal tea. Therefore, as compared to other extractants, the information obtained using hot water extract would be more valuable for products used in human diets.

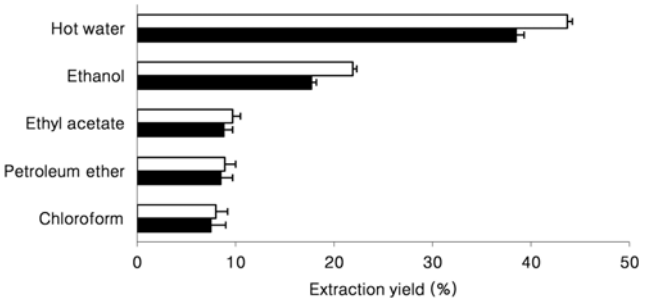

Fig. 1. Effect of various extractants on the extraction yield of fruiting and mycelium. Symbols: fruiting body (□) and mycelium (■).

2. Antioxidant Components

Vegetables and fruits are rich sources of antioxidants that prevent free radical damage and reduce the risk of chronic diseases. Thus, the consumption of dietary antioxidants from these sources is beneficial in preventing cardiovascular diseases such as atherosclerosis. Table 1 lists the total phenol, beta-carotene, ascorbic acid, tocopherol, and polysaccharide concentrations of various extracts of fruiting body and mycelium. The total phenol concentration of hot water extract, regardless of culture type, was higher than that of ethanol extract. Especially, when hot water was used as the extractant, the total phenolic concentration of fruiting body was 22.45 mg/g dry weight; however, for the ethanol extract, the total phenolic concentration was 5.02 mg/g dry weight. Comparatively, the hot water and ethanol extracts of mycelium produced phenolic concentrations of 6.31 and 2.10 mg/g dry weight, respectively. The total flavonoid concentration was 1.5 mg/g dry weight for the hot water extract of fruiting body and, 0.3 mg/g dry weight for ethanol extract. Comparatively, the values for mycelium were 0.4 and 0.2 mg/g dry

Table 1. Effect of various extracts on the total phenol, flavonoid, carotene, ascorbic acid, tocopherol, and polysaccharide concentration

Antioxidant components	Fruiting body		Mycelium	
	Hot water extract	Ethanol extract	Hot water extract	Ethanol extract
Total phenol	22.45±1.10	5.02±0.20	6.31±0.16	2.10±0.30
Total flavonoid	1.50±0.20	0.30±0.01	0.40±0.02	0.20±0.01
Beta-Carotene	ND	0.50±0.02	ND	0.20±0.02
Ascorbic acid	0.01±0.005	0.45±0.02	0.01±0.005	0.12±0.01
Alpha-Tocopherol	0.08±0.003	0.51±0.01	0.09±0.005	0.36±0.03
Gamma-Tocopherol	0.02±0.003	0.46±0.01	0.03±0.001	0.30±0.03
Delta-Tocopherol	0.02±0.001	0.17±0.02	0.03±0.001	0.14±0.02
Polysaccharide	3.90±0.30	ND	0.90±0.02	ND

Unit: mg/g of dry weight

weight, respectively. Beta-carotene was not detected in the hot water extract of fruiting body and mycelium. However, the ethanol extract produced 0.5 mg/g dry weight, which was approximately 2.5 fold-higher than that of mycelium. The ascorbic acid concentration of the ethanol extract of fruiting body was 0.45 mg/mg dry weight, which was approximately 3.8-fold higher than that of mycelium. For the hot water extract, it was 0.08 mg/g dry weight, which was approximately 2-fold higher than that for mycelium. The concentrations of alpha-tocopherol, gamma-tocopherol, and delta-tocopherol of fruiting body were 0.51, 0.46, and 0.17 mg/g in the ethanol extract, respectively, and for hot water extract, they ware 0.08, 0.02, and 0.02 mg/g dry weight, respectively. These results are similar to those for extracts of mycelium. The polysaccharide concentration for the hot water extract of fruiting body was 3.90 mg/g dry weight and 0.9 mg/g dry weight on mycelium. However, it was not detected for ethanol extract, regardless of culture type.

3. Effect of Extracts on the DPPH Radical Scavenging Effect

Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. The method of scavenging DPPH free radicals can be used to evaluate the antioxidant activity of specific compounds or extracts in a short time. Hot water and ethanol were used to investigate the effect of various extractants on the DPPH radical scavenging activity of fruiting body grown on the sold state and mycelium grown in the liquid state. The results are shown in Fig. 2. The hot water and ethanol extracts were shown to scavenge the stable DPPH radical directly to different extents. The DPPH radical scavenging activity sharply increased from 35.7 to 80.5% when the hot water extract concentration of fruiting body was increased from 3 to 9 mg/mL. However, for concentrations greater than 12 mg/mL of hot water extract, the DPPH scavenging activity did not increase. For mycelium, when the hot water extract concentration was increased from 3 to 9 mg/mL, the DPPH scavenging activity increased from 33.8 to 67.4%. When the ethanol extract concentration of fruiting body was increased from 3 to 12 mg/mL, the DPPH radical scavenging activity increased from 22.3 to 58.1%, but it did not increase for ethanol extract concentrations greater than 12 mg/mL. For mycelium grown in the liquid state, when the ethanol extract concentration increased from 3 to 12 mg/mL, DPPH scavenging activity increased from 21.2 to 49.3%. These results showed that the hot water extract of *P. ferulae* contained active substances, including phenolic compounds, which had a high hydrogen-donating capacity to scavenge DPPH radicals as a possible mechanism for their antioxidative activities.

4. Effect of Extracts on the Hydroxyl Radical Scavenging Effect

The abilities of various extracts to scavenge hydroxyl radical were investigated and their results are shown in Fig. 3. The hydroxyl radical scavenging effects of hot water extract of fruiting body were increased with the increase of extracts. Especially, when the hot water extract concentration of fruiting body was increased from 3 to 12 mg/mL, the hydroxyl radical scavenging effect was increased from 20.6 to 72.4%. In the case of hot water extract of mycelium, it was increased from 15.4 to 47.5%. On the other hand, when ethanol ex-

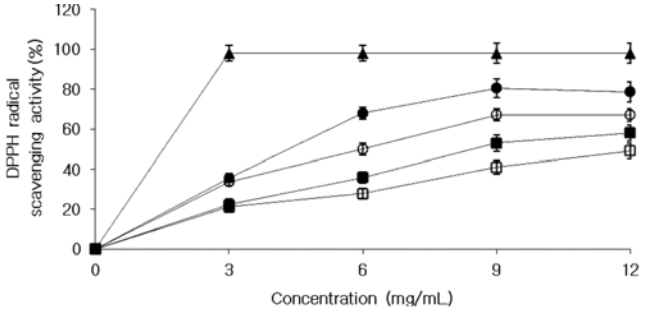

Fig. 2. Effect of various extracts on the DPPH radical scavenging activity. Symbols: hot water extract of fruiting body (●), ethanol extract of fruiting body (■), hot water extract of mycelium (○), ethanol extract of mycelium (□), and BHA (▲).

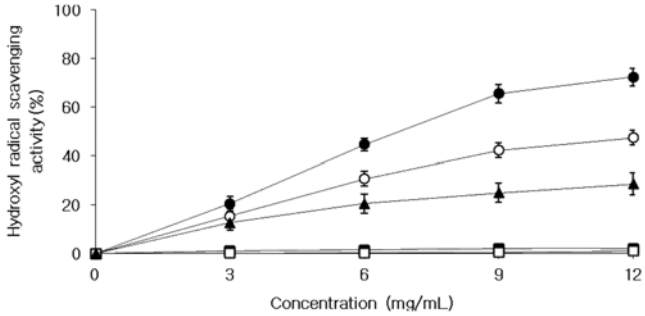

Fig. 3. Effect of various extracts on the hydroxyl radical scavenging activity. Symbols: hot water extract of fruiting body (●), ethanol extract of fruiting body (■), hot water extract of mycelium (○), ethanol extract of mycelium (□), and BHA (▲).

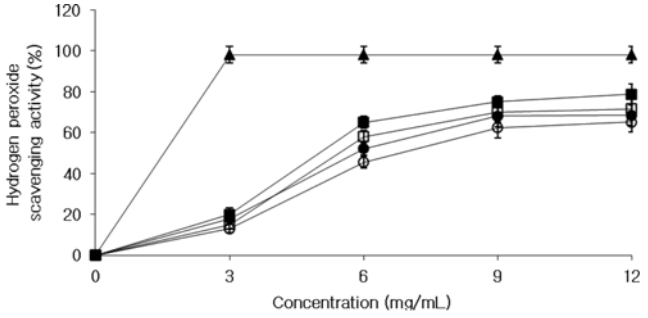

Fig. 4. Effect of various extracts on the hydrogen peroxide scavenging activity. Symbols: hot water extract of fruiting body (●), ethanol extract of fruiting body (■), hot water extract of mycelium (○), ethanol extract of mycelium (□), and BHT (▲).

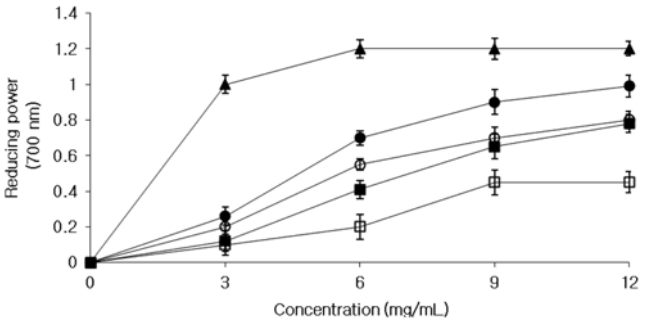

Fig. 5. Effect of various extracts on the reducing power. Symbols: hot water extract of fruiting body (●), ethanol extract of fruiting body (■), hot water extract of mycelium (○), ethanol extract of mycelium (□), and BHA (▲).

tracts of fruiting body and mycelium were used, they were below 2%. The hydroxyl radical scavenging effect of BHA was 28.7% at 12 mg/mL. It is revealed that ethanol extracts from mushrooms including fruit bodies and mycelia are not good scavengers for hydroxyl radicals.

5. Effect of Extracts on the Hydrogen Peroxide Scavenging Effect

Generally, measuring the hydrogen peroxide scavenging activity is one of the most useful methods for determining the ability of an antioxidant to decrease the level of pro-oxidants such as hydrogen peroxide. Scavenging of hydrogen peroxide by antioxidants can be attributed to their electron donating ability. The effects of various extracts on the hydrogen peroxide scavenging activity are shown in Fig. 4. The hydrogen peroxide scavenging activity increased with the concentration of hot water and ethanol extracts. When the concentration of the hot water extract and ethanol extract from mushroom grown on the sold state was at 12 mg/mL, the scavenging activities were 68.4 and 78.7%, respectively. For mushrooms grown in the liquid state, they were 65.2 and 71.5%, respectively. Hydrogen peroxide, which is a reactive non-radical, is very important because it can penetrate biological membranes. Although hydrogen peroxide itself is not very reactive, it is easily converted into more reactive species such as singlet oxygen and hydroxyl radicals, which can then initiate lipid peroxidation or induce toxic effects in cells.

6. Effect of Extracts on the Reducing Power

The reducing power, which provides an estimate of the ability of a compound to reduce ferric iron (III) to ferrous iron (II), was determined by using a redox-linked colorimetric reaction. In addition, the reducing capacity of a compound may serve as a significant indicator of its potential for use as an antioxidant. The effects of various extracts on reducing power are shown in Fig. 5. When the ethanol extract concentration of fruiting body grown on the solid state was increased from 3 to 12 mg/mL, the reducing power increased from 0.25 to 0.78 OD (700 nm). For mycelium grown in the liquid state, it increased from 0.19 to 0.45 OD (700 nm). However, when the hot water extract concentration of fruiting body was increased from 3 to 12 mg/mL, the reducing power increased from 0.41 to 0.99 OD (700 nm). For mycelium, it increased from 0.41 to 0.81 OD (700 nm). According to DPPH activity and reducing power activity, the reducing power of extracts from this mushroom

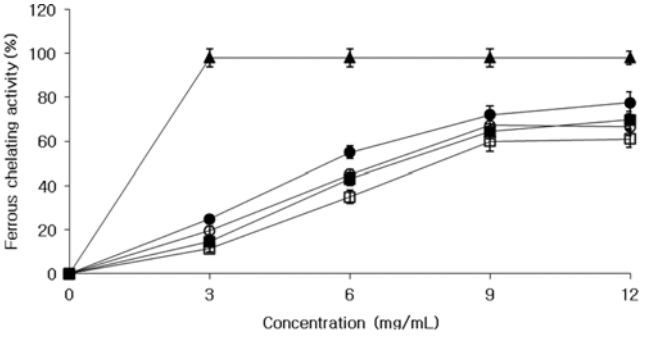

Fig. 6. Effect of various extracts on the ferrous chelating activity. Symbols: hot water extract of fruiting body (●), ethanol extract of fruiting body (■), hot water extract of mycelium (○), ethanol extract of mycelium (□), and EDTA (▲).

correlates with their DPPH scavenging activity, indicating that their reducing power contributes to their antioxidant activity. This result is in agreement with the previously reported result that the reducing power is associated with antioxidant activity.

7. Effect of Extracts on the Chelating Effect

Fig. 6 lists the effects of various extracts on the ferrous chelating ability of extracts from fruiting body grown on the sold state and mycelium grown in the liquid state. When the hot water extract concentration of fruiting body was increased from 3 to 12 mg/mL, the ferrous chelating ability increased from 18.9 to 77.0%. For the ethanol extract, it increased from 14.6 to 69.6%. However, for mycelium grown in the liquid state, when the hot water extract concentration was increased from 3 to 9 mg/ml, the ferrous chelating ability increased from 19.7 to 67.2% and did not increase for concentrations greater than 12 mg/mL. For the ethanol extract, it increased from 11.5 to 61.1% and did not increase for concentrations at 12 mg/mL. These results revealed that the hot water extract demonstrated a marked capacity for iron binding, suggesting that their action as lipid peroxidation and HSA protein protector may be related to its metal binding capacity.

8. Effect of Extracts on the Nitrite Scavenging Effect

Fig. 7 shows the effect of extract concentrations from fruiting body grown on the sold state and mycelium grown in the liquid state on nitrite scavenging activity. The nitrite scavenging activity was increased with the concentrations of extracts by 6 mg/mL, irre-

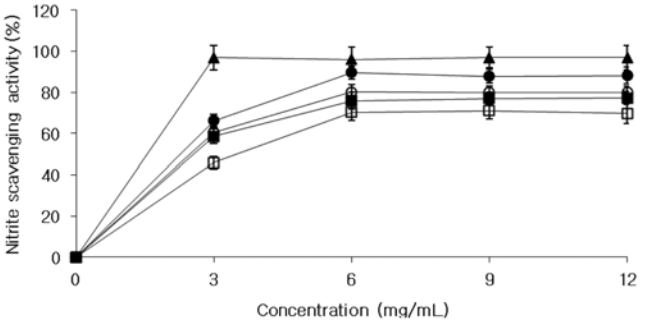

Fig. 7. Effect of various extracts on the nitrite scavenging activity. Symbols: hot water extract of fruiting body (●), ethanol extract of fruiting body (■), hot water extract of mycelium (○), ethanol extract of mycelium (□), and BHT (▲).

spective of culture methods. Especially, when the hot water extract concentration of fruiting body was 6 mg/mL, the nitrite scavenging activity was highest, 89.7%. For the ethanol extract, it was 76.1%. When hot water or ethanol extract concentration was increased above 9 mg/mL, it was not increased. However, in the case of mycelium grown in the liquid state, when the hot water and ethanol extract concentration was 6 mg/mL, they were 80.8 and 70.3%, respectively and did not increase for concentrations greater than 9 mg/mL. These results show that the hot water extract had higher nitrite scavenging activities than ethanol extracts.

DISCUSSION

Reactive oxygen species (ROS), which consist of free radicals such as superoxide anion and hydroxyl radicals and non-free radical species such as hydrogen peroxide and singled oxygen, are different forms of activated oxygen. ROS are produced by all aerobic organisms and can easily react with most biological molecules including proteins, lipids, lipoproteins and DNA. Thus, ample generation of ROS proceeds to a variety of pathophysiological disorders such as arthritis, diabetes, inflammation, cancer and genotoxicity [18]. Therefore, living organisms possess a number of protective mechanisms against the oxidative stress and toxic effects of ROS. Antioxidants regulate various oxidative reactions naturally occurring in tissues and are evaluated as a potential anti-aging agent. Hence, antioxidants can terminate or retard the oxidation process by scavenging free radicals, chelating free catalytic metals and also by acting as electron donors [19]. Therefore, dietary intake of antioxidants is necessary and important.

Currently, many kinds of synthetic antioxidants such as BHT, BHA, TBHQ, and propyl gallate have been used as materials or additives for oxidation suppressant in food, cosmetics and drug compositions. However, the use of these synthetic antioxidants for food or medicine components has been restricted by the toxicity and safety that can lead to problems of the potential health in human. Thus, we have tried to find the more effective oxidation inhibitors that may be used as antioxidants such as *P. nebrodensis* [20], *Fomitopsis pinicola* [21], *Phyllostachys nigra* var. *henonis* [22], *Achyranthis Radix* [23], *Antrodia camphorata* [24], *Astragalus sinicus* L. seed extract [25], and hot water extract of a fish, seaweed, and mushroom mixture [26] for food or medicine composition without the side effects for the past several years.

In this study, we determined antioxidant and nitrite scavenging properties of various extracts from *P. ferulea* fruiting body grown on sold state using CCABE media and mycelium grown in the liquid state by using solution media. The extraction yield was increased in order of hot water>ethanol>ethyl acetate>petroleum ether>chloroform, irrespective of culture type. The extraction yield indicated that the amount of lipophilic substances in the *P. ferulae* was much less than hydrophilic substances. The discrepancy between the yields for hot water and ethanol extracts might be due to the fact that hot water extracts contained polar groups with structures containing -OH and -COOH functional groups that are easily extracted from the samples by the polar solvent. The results suggest that the higher the polarity of that *P. ferulae* extracts, the stronger is the antioxidant and nitrite scavenging activity. This finding also indicates that active components with water soluble characteristics might exist in *P. ferulae* extracts.

Shahidi and Wanasundara reported that the antioxidant activity of plant materials correlates well with the concentration of antioxidant components [27]. Especially, phenols such as BHT and gallate are known to be effective antioxidants. Due to their scavenging abilities on free radicals and chelating abilities on ferrous ions, phenols possess good antioxidant, antimutagenic and anticancer properties. Therefore, it is important to consider the effect of the antioxidant components such as polyphenolic compounds, vitamins, carotenoids, polsacharides, tocopherols, and flavonoids of the extracts from *P. ferulae*. The concentration of total phenol in the extracts was dependent on the extractants used in the extraction. The total phenol concentrations of hot water and ethanol extract of fruiting body were about 3.6- and 2.4-fold higher than those of mycelium. Especially, when hot water was used as the extractant, the total phenolic concentration of fruiting body was approximately 4.5-fold higher than that of ethanol extract. However, for mycelium, it was approximately 3-fold higher than that of ethanol extract. This result is similar to those for the hot water extract of *H. marmoreus*, *P. ostreatus*, and *P. citrinopilratus* [13,28,29]. On the other hand, in the case of *H. marmoreus* white mutant, the total phenolic concentration of hot water extract of mycelium was approximately 1.3-fold higher than that of fruiting body [12]. The total flavonoid concentration of the hot water extract of fruiting body was approximately 5-fold higher than that of ethanol extract. However, for mycelium, it was approximately 2-fold higher than that of ethanol. The total flavonoid concentration of the hot water extract of fruiting body was also increased approximately 3.8-fold compared to that of mycelium. However, for the ethanol extract, the value was similar to that of mycelium grown in the liquid state. When extraction temperature was increased from 80 to 120 °C for 1 hr, the total phenolic and flavonoid concentrations of fruiting body increased approximately 40 and 30%, respectively, compared to those of the cool water extract (data not shown). This suggests that heat treatment might produce changes in their extractability due to the disruption of the plant cell wall; therefore, bound polyphenolic and flavonoid compounds may be released more easily relative to those of raw materials. Beta-carotene was not detected in hot water extract regardless of the culture type. However, for the ethanol extract from fruiting body, it was approximately 2.5-fold higher than that of mycelium. In the case of the ethanol extract from *H. marmoreus* mycelium, beta-carotene

concentration was 2.04 mg/g dry weight [28], and it was 0.05 mg/g dry weight in the ethanol extract of *Clitocybe maxima* fruiting body [14]. However, it was not detected in the hot water and ethanol extracts of the white mutant of *H. marmoreus* fruiting body [12] and *P. citrinopileatus* fruiting body and mycelium [13]. The ascorbic acid concentration of the hot water extract of fruiting body was very low compared to that of the ethanol extract because ascorbic acid is easily degraded by heat. Total phenols were the primary antioxidant component found in hot water extracts, whereas tocopherols were the major antioxidant components found in ethanol extracts due to its fat-soluble nature. Regardless of the culture type, when the ethanol extract was used, the concentrations of alpha-tocopherol, gamma-tocopherol, and delta-tocopherol were higher than those of the hot water extracts. Polysaccharide concentration of hot water extract of fruiting body was approximately 4.3-fold higher than that of mycelium. However, for the ethanol extract, it was not detected, regardless of the culture type. This result is similar to that of the ethanol extract from *I. obliquus* [15].

The DPPH radical scavenging activity increased with the increase of extract concentration of *P. ferulae*, regardless of the culture type. When the hot water extract concentrations of fruiting body and mycelium were at 9 mg/mL, DPPH radical scavenging activity was 78.5 and 67.4%, respectively. In the case of the hot water extract of *Ganoderma tsugae*, *Agrocybe cylindracea*, and *H. marmoreus* fruiting bodies, they were 53.8 [30], 47.3 [31], and 67.2% at 6 mg/mL [28], respectively. It appears that the scavenging activity of the hot water extract from *P. ferulae*, regardless of the culture type, was more effective than those previously reported substances (data not shown). However, the ethanol extracts produced DPPH scavenging activity of 42.7 and 33.7% at 6 mg/mL, respectively. In the case of hot water extract from *H. marmoreus* fruiting body, it was 59.7% at 5 mg/mL [28]. The DPPH radical scavenging activity of hot water extracts from *Agaricus bisporus*, *P. eryngii*, and *P. ostreatus* fruiting bodies was in the range of 46.6-68.4% at 5 mg/mL [27]. The DPPH scavenging activity of BHA was 96.7% at below 125 μg/mL.

Hydroxyl radical is one of the major causes of the damage that free radicals induce in biological systems. It is extremely reactive in biological systems and has been implicated as highly damaging species in free radical pathology, capable of damaging biomolecules of the living cells [19]. The hydroxyl radical scavenging activity of all hot water extracts of *P. ferulae* showed a concentration-dependent increase. In addition, the hydroxyl radical scavenging activities of hot water extracts from *P. ferulae* grown on sold state using CCABE and mycelium grown in the liquid state using solution media showed more effective than those of ethanol extract. The hydroxyl radical scavenging activity of hot water extract of *P. ferulae* fruiting body was about 2.5 fold higher that of BHA. Various extract concentrations of mushrooms using hot water and ethanol extract were investigated. Tsai et al. reported that hydroxyl radical scavenging activity at 10-20 mg/ml of hot water extracts was 36.1-49.6% for *Boletus edulis*, 13.2-61.3% for *Agrocybe cylindracea*, and 53.8-78.6% for *Agaricus blazei*, respectively [14]. The hydroxyl radical scavenging activity of mature and baby Ling chih was 51.7-54.8% to 72.4-78.7% [30]; that of *C. comatus* was 26.2-37.3% [14]; that of *P. citrinopileatus* was 60.7-80.1% [13]; and that of *H. marmoreus* was 33.0-51.8% [1]. Lo found that the hydroxyl radical scavenging activity of hot water extracts from *A. bisporus*, *P. eryngii*, and *P. ostreatus* was in the range of 22.1-33.4 and 38.2-48.1% at 10-20 mg/mL, respectively, [27]. On the other hand, in the case of ethanol extracts, *Agaricus blazei* and *Agrocybe cylindracea* showed none to slight scavenging effect, whereas *B. edulis* exhibited scavenging activity of 8.29-23.3% [14]. The ethanol extracts of fruiting bodies and mycelia from *H. marmoreus* white mutant were 12.2 and 6.35% at 20 mg/mL, respectively [12]. In the case of normal strain of *H. marmoreus*, they showed no activity [28]. These results indicated that many hot water extracts from mushrooms are effective scavengers for hydroxyl free radicals. In addition, Shi et al. [32] reported that the hydroxyl radical scavenging effect of caffeine attributed to the alleged anticarcinogenic properties of caffeine to this effect. Accordingly, it was anticipated that the moderate to high scavenging effect of hot-water extracts might possess some antimutagenic properties.

Addition of hydrogen peroxide to cells in culture can lead to transition metal ion-dependent OH^- mediated oxidative DNA damage. Levels of hydrogen peroxide at or below about 20-50 mg seem to have limited cytotoxicity to many cell types. Since phenolic compounds present in the extract are good electron donors, they may accelerate the conversion of hydrogen peroxide to H_2O [33]. As shown in Fig. 4, the hydrogen peroxide scavenging activity increased with the concentration of hot water and ethanol extracts. Especially, the scavenging activity of ethanol extract was higher than that of hot water extract at 12 mg/mL. Dandamudi and Nageswara [34] investigated the methanol extracts of cap and stipe of commercially obtained mushrooms, *Agaricus bisporus*, *H. ulmarius*, and *C. indica* on hydrogen peroxide scavenging effect. All the extracts showed increasing scavenging effect of hydrogen peroxide with increased concentration. The activity of mushrooms was in the range of 36.8-80.1% at concentration of 2.0 mg/mL. Of the three species, *Agaricus bisporus* cap exhibited excellent scavenging activity of 76.1% followed by *C. indica* cap of 63.6%. Least activity was exhibited by *H. ulmarius* stipe of 36.8%. Yang et al. [35] reported that the strongest antioxidant activity was exhibited by various extracts of *Phellinus* sp. with hydrogen peroxide scavenging activity. Radical scavenging activity and protection levels against H_2O_2-induced damage to PC12 cells were highly correlated with the flavonoid concentration of the extracts. These results indicate that fruiting bodies of *Phellinus* sp. represent a potentially valuable source of natural antioxidants of relevance to both the health and food industries. Shon et al. reported that the methanol and hot water extracts of *Phellinus baumii* exhibited about 80-90% of hydrogen peroxide scavenging activity. These results indicate that the concentration of hydrogen peroxide in water may vary according to the phenolic compounds [36].

The antioxidant activity has been reported to be concomitant with the development of reducing power. Therefore, the antioxidant activity of the extracts might partially be a result of its reducing power. Okuda et al. [37] mentioned that the reducing power of tannins prevented liver injury by inhibiting the formation of lipid peroxides. Further, reductones can react directly with peroxides and with certain precursors, thereby preventing the formation of peroxide. The reducing power of various extracts might be due to their hydrogen-donating ability. Therefore, *P. ferulae* might contain reductones, which could react with free radicals to stabilize and terminate radical chain reactions. When the concentration of the ethanol extract from

P. ferulae fruiting body was 12 mg/ml, the reducing power was 0.78 OD (700 nm). The reducing power of the ethanol extract from *P. citrinopileatus* fruiting body was 1.12 OD (700 nm) at 20 mg/mL [13] and that from *Agaricus bisporus*, *P. eryngii*, *P. ostreatus* , and *H. marmoreus* fruiting body was 0.76, 0.75, 0.70 and 0.61, and 0.74 OD (700 nm), respectively, at 20 mg/mL [27,28]. The reducing power of the ethanol extract from *P. ferulae* fruiting body was better than that of *Agaricus bisporus*, *P. eryngii*, *P. ostreatus*, and *P. citrinopileatus.* The hot water extract demonstrated good ability to reduce ferric iron (III) to ferrous (II). In addition, the reducing power of the hot water extract increased in a dose-dependent manner compared to the other extractants (data not shown). When the hot water extract concentration of *P. ferulae* fruiting body was 12 mg/mL, the reducing power was 0.99 OD (700 nm). Hot water extracts from *G. tsugae* Murrill and *P. citrinopileatus* exhibited reducing powers of 1.08 and 1.10 OD (700 nm), respectively, at 5 mg/mL [13,30]. Comparatively, reducing powers of hot water extracts from *Agrocybe cylindracea* [31] and *H. marmoreus* [28] were 0.99 OD and 1.01 OD (700 nm) at 10 mg/mL, respectively.

Iron can stimulate lipid peroxidation by the fenton reaction, and it also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation [18]. When hot water and ethanol extracts from *P. ferulae* fruiting body were 12 mg/mL, the ferrous chelating activity was 77.0 and 69.7%, respectively, which is more effective than that of the ethanol extract of *P. citrinopileatus* [13]. Lo [27] found that among various extractants, ethanol was the best choice for extracting *Agaricus bisporus*, *P. eryngii*, and *P. ostreatus* fruiting body. Especially, the chelating activities of ethanol extracts from fruiting body were in the range of 41.4-64.0% for *Agaricus bisporus*, 48.2-75.0% for *P. eryngii*, and 73.1-82.3% for *P. ostreatus* at 5-20 mg/mL, respectively. Comparatively, the hot water extracts from *G. tsugae* and *Agrocybe cylindracea* chelated ferrous ions 42.6 and 45.8% at 20 mg/mL, respectively [30,31]. However, the chelating activity of the hot water extract from *H. marmoreus* was 92.6%, which was approximately 40% greater than its ethanol extract [28]. For ethyl acetate, petroleum ether, and chloroform extracts of fruiting body, the values were in the range of 39.7-45.4% (data not shown). However, EDTA showed an excellent chelating activity, 98.9%. Citric acid was not a good chelating agent for ferrous ions; its chelating activity was 21.3% (data not shown). The ferrous chelating activity of the hot water extract from *P. ferulea* fruiting body was more effective than those of *G. tsugae* and *Agrocybe cylindracea.* This chelating effect was attributed to the specific functional groups in its flavanol structure. The adjacent hydroxyl and carbonyl groups in the molecule or hydroxyl groups among molecules could chelate ferrous ion to form a complex [38]. Therefore, the more hydroxyl and carbonyl groups in appropriate positions, the higher the chelating activity the molecule could exhibit. Since ferrous ions are the most effective prooxidants in the food system, the high ferrous-ion chelating activities of hot water extracts from *P. ferulea* would be beneficial if they were formulated into foods or brewed as a drink.

Exposure to carcinogenic *N*-nitroso compounds (NOC) can occur exogenously via consumption of food or endogenously by the reaction of secondary amines with nitrite under acidic conditions. NOCs are potential mutagens and carcinogens in animals and humans, even at low concentrations. Volatile *N*-nitrosamines (NA) are found in various foods, and thiocyanate is known to catalyze their formation, especially under acidic conditions [39]. The formation of these carcinogenic substances may be inhibited by dietary antioxidants such as ascorbic acid, tocopherols, polyphenols, and allyl sulfur compounds [40]. Consumption of whole strawberries, kale juice, garlic juice, Korean green tea, and Maesil (*Prunus mume*), which contain antioxidants such as ascorbic acid, polyphenols, and allyl sulfur compounds, has been reported to be effective in inhibiting the formation of NOC [41,42]. In our study, the nitrite scavenging activities of *P. ferulea* extracts in a range of acidic conditions using hot water and ethanol extracts were investigated. The nitrite scavenging activities of extracts from *P. ferulea* extracts were affected by pH change and extractants, irrespective of culture methods. Maximum nitrite scavenging activity irrespective of extracts occurred at pH 1.2 (data not shown). The fact that the nitrite scavenging activity was high at this pH suggests that nitrosamine production can be inhibited *in vivo*. Especially, when the hot water extract was used, it was higher than that of ethanol extract, irrespective of culture methods. Our results show that the hot water extracts from *P. ferulea* could be useful for preventing nitrosamine formation in foods.

The properties of antioxidant on DPPH radical scavenging activity, hydroxyl radical scavenging activity, hydrogen peroxide scavenging activity, reducing power, and chelating activity of ferrous and nitrite scavenging were normalized and expressed as EC_{50} (mg/mL) values which are the effective concentration of each extract from *P. ferulea* and standard that required to show 50% activity. It is important to note the effectiveness of the edible mushroom in reacting with free radicals under different conditions as a lower EC_{50} value corresponds to higher antioxidant activity of the mushroom's extract. The results are summarized in Table 2. Generally, all EC_{50} values were below 10 mg/mL except for hydroxyl radical scaveng-

Table 2. Effect of various extracts on EC_{50} value

	EC_{50} value (mg extract/mL)	
	Fruiting body	Mycelium
Hot water extract		
DPPH radical scavenging effect	3.3	4.3
Hydroxyl radical scavenging effect	6.9	12.1
Hydrogen peroxide scavenging effect	5.4	6.2
Reducing power	2.6	2.9
Ferrous chelating effect	5.5	6.0
Nitrite scavenging effect	2.3	2.5
Ethanol extract		
DPPH radical scavenging effect	7.3	12.0
Hydroxyl radical scavenging effect	.	.
Hydrogen peroxide scavenging effect	5.2	7.2
Reducing power	3.4	3.8
Ferrous chelating effect	6.7	7.1
Nitrite scavenging effect	2.4	3.5

EC_{50} value, the effective concentration at which the DPPH, hydroxyl radical, and hydrogen peroxide, and nitrite were scavenged by 50%; the absorbance was 0.5 for reducing power; and ferrous ion was chelated by 50%, respectively. EC_{50} value was obtained by interpolation from linear regression analysis

ing effect of hot water extract and DPPH radical scavenging effect of ethanol extract from mycelium. The EC_{50} values of antioxidant and nitrite scavenging activities of hot water extracts from *P. ferulea* extracts irrespective of culture methods were more effective than those of ethanol extracts. In addition, fruiting bodies were more effective than mycelia irrespective of extractant. Especially, the EC_{50} values of DPPH radical scavenging effects were 3.3-4.3 mg/mL of hot water extracts and 7.3-12 mg/mL of ethanol extracts, respectively. In hydroxyl radical scavenging activities, the ethanol extracts were less effective than those of hot water and they were 6.9 mg/mL of how water extracts from fruiting body and 12.1 mg/mL of how water extracts from mycelium, respectively. Based on EC_{50} values in hydrogen peroxide scavenging effect, they were 5.2-7.2 mg/mL of ethanol extracts from fruiting body and 5.4-6 2 mg/mL of hot water extracts from mycelium, respectively. The EC_{50} values of the hot water extracts from fruiting body and mycelium in the reducing power were 2.6 and 2.9 mg/mL, respectively. In the case of ethanol extracts, they were 3.4 and 3.8 mg/mL, respectively. The EC_{50} values of the hot water extracts from fruiting body and mycelium in the chelating activity on ferrous ions were 5.5 and 6.0 mg/mL. In the case of ethanol extracts, they were 6.7 and 7.1 mg/mL. That indicates that the hot water extract of *P. ferulea* irrespective of culture method was good in these antioxidant properties. Effectiveness in nitrite scavenging activity was in a descending order: hot water extract from fruiting body (2.3 mg/mL)>ethanol extract from fruiting body (2.4 mg/mL)>hot water extract from mycelium (2.5 mg/mL)>ethanol extract from mycelium. From EC_{50} values obtained in this study, the fruiting body and mycelium of *P. ferulea* could be used in gram levels as food or a food ingredient and might serve as possible protective agents to help humans reduce oxidative damage in human diets.

In conclusion, the antioxidant and nitrite scavenging activity of extracts from *P. ferulea* is directly dependent on the type of culture. We found that CCABE media can be a suitable substrate for fruiting body production and can be recycled for culture of *P. ferulae*. The hot water extract of fruiting body grown on CCABE media might contribute to its high antioxidant and nitrite scavenging activity. The growth of *P. ferulae* could have multiple effects, such as the degradation of agriculture or industrial wastes and the production of pharmacologically active compounds. However, further studies are necessary to elucidate the relationship between the antioxidant and nitrite scavenging activity and the pharmacological activity of *P. ferulae* extract *in vivo*.

ACKNOWLEDGEMENT

This study was supported by research fund from Chosun University, 2011.

REFERENCES

1. N. L. Huang, *Colored illustrations of mushrooms of China*, China agriculture press, Beijing, 95 (1998).
2. K. H. Hong, B. Y. Kim and H. K. Kim, *J. Kor. Soc. Food Sci. Nutr.*, **33**, 791 (2004).
3. A. Mehmet and K. Sevda, *Eur. J. Biosci.*, **3**, 58 (2009).
4. H. S. Choi, M. K. Kim, H. S. Park, J. S. Kim, M. H. Sim and S. J. Kim, *Kor. J .Food. Sci. Technol.*, **37**, 1039 (2005).
5. D. B. Choi, S. H. Kang, Y. H. Song, K. H. Kwun, K. J. Jeong and W. S. Cha, *J. Microbiol. Biotechnol.*, **15**, 368 (2005).
6. D. B. Choi, W. S. Cha and S. H. Kang, *Biotechnol. Biopro. Eng.*, **9**, 356 (2004).
7. O. O. Kuforiji and I. O. Fasidi, *Bioresour. Technol.*, **12**, 4275 (2008).
8. G B. Bhak, M. K. Song, S. Y. Lee and S. H. Hwang, *Biotechnol. Lett.*, **27**, 1537 (2005).
9. S. R. Cristina, G R. Ana, P. Isabel, J. B. Gemma, R. M. Francisco and H. Wichers, *Inter. Biodeter. Biodegrad.*, **57**, 37 (2006).
10. K. Erbil and S. Sayit, *Inter. Biodeter. Biodegrad.*, **53**, 43 (2004).
11. J. S. Kim, G Y. Seo, I. S. Kang, B. K. Ahn and D. B. Choi, *J. Adv. Eng. Technol.*, **3**, 15 (2010).
12. Y. L. Lee, S. Y. Jian, P. L. Lian and J. L. Mau, *J. Food Compo. Anal.*, **21**, 116 (2008).
13. Y. L. Lee, G W. Huang, Z. C. Liang and J. L. Mau, *Food Sci. Technol.*, **40**, 823 (2007).
14. S. Y. Tsai, H. L. Tsai and J. L. Mau, *Food Sci. Technol.*, **40**, 1392 (2007).
15. H. Hu, Z. Zhang, Z. Lei, Y. Yang and N. Sugiura, *J. Biosci. Bioeng.*, **107**, 42 (2009).
16. L. M. Cheung and P. C. K. Cheung, *Food Chem.*, **89**, 403 (2005).
17. L. M. Cheung, P. C. K. Cheung and V. C. E. Ooi, *Food Chem.*, **81**, 249 (2003).
18. B. Halliwell, *Am. J. Med.*, **91**, 1422 (1991).
19. B. Halliwell, *Ann. Rev. Nutr.*, **16**, 33 (1996).
20. D. B. Choi, H. G Nam and W. S. Cha, *Korean J. Chem. Eng.*, **23**, 241 (2006).
21. D. B Choi, S. S. Park, J. L. Ding and W. S. Cha, *Biotechnol. Biopro. Eng.*, **12**, 516 (2007).
22. D. B Choi, K. A. Cho, M. S. Na, H. S. Choi, Y. O. Kim, D. H. Lim, S. J. Cho and H. Cho, *J. Ind. Eng. Chem.*, **14**, 765 (2008).
23. D. B Choi, H. S. Choi, M. S. Na and M. Y. Lee, *J. Ind. Eng. Chem.*, **15**, 275 (2009).
24. D. B Choi, S. S. Park, J. L. Ding and W. S. Cha, *Biotechnol. Biopro. Eng.*, **14**, 232 (2009).
25. D. H. Lim, D. B Choi, O. Y. Choi, K. A. Cho, R. Kim, H. S. Choi and H. Cho, *J. Ind. Eng. Chem.*, **17**, 510 (2011).
26. D. B Choi, Y. S. Kim, H. G Nam, H. J Shin, M. S. Na, O. Y. Choi, H. D. Lee and W. S. Cha, *Korean J. Chem. Eng.*, **28**, 1266 (2011).
27. S. H. Lo, National Chung-Hsing University, Taichung, Taiwan, Master's Thesis (2005).
28. Y. L. Lee, National Chung- Hsing University, Taichung, Taiwan, Master's Thesis (2003).
29. S. Y. Tsai, S. J. Huang, S. H. Lo, T. O. Wuc, P. Y. Lian and J. L. *Mau*, *Food Chem.*, **113**, 578 (2009).
30. J. L. Mau, S. Y. Tsai, Y. H. Tseng and S. J. Huang, *Food Sci. Technol.*, **38**, 589 (2005).
31. S. Y. Tsai, S. J. Huang and J. L. Mau, *Food Chem.*, **98**, 868 (2006).
32. X. Shi, N. S. Dalal and A. C. Jain, *Food Chem. Toxi.*, **29**, 1 (1991).
33. R. J. Ruch, S. U. Chung and J. E. Klaunig, *Methods in Enzymol.*, **105**, 198 (1984).
34. R. B. Dandamudi and R. Nageswara, *J. Food Sci. Technol.*, DOI 10.1007/s13197-011-0338-8, In press (2012).
35. Y. Yang, J. Hu, Y. Liu, N. Feng, H. Chen, Q. Tang, L. Ye and J. S. Zhang, *Int. J. Med. Mushr.*, **13**, 145 (2011).
36. M. Y. Shon, T. H. Kim and N. J. Sung, *Food Chem.*, **82**, 593 (2003).

37. T. Okuda, Y. Kimura, T. Yoshida, T. Hatano, H. Okuda and S. Arichi, *Chem. Pharma. Bull.*, **31**, 1625 (1983).
38. F. Shahidi and P. K. J. Wanasundara, *Food Sci. Nutr.*, **32**, 67 (1992).
39. K. B. Shapiro, J. H. Hotchkiss and D. A. Roe, *Food Chem. Toxicol.*, **29**, 751 (1991).
40. Y. N. Wu, H. Z. Wang; J. S. Li and C. Han, *Biomed. Environ. Sci.*, **6**, 237 (1993).
41. S. Y. Choi, M. J. Chung and N. J. Sung, *Food Chem. Toxicol.*, **40**, 949 (2002).
42. M. J. Chung, S. H. Lee and N. J. Sung, *Cancer Lett.*, **182**, 1 (2002).