Australian Journal of Basic and Applied Sciences, 5(3): 229-239, 2011
ISSN 1991-8178

Antioxidant, Phenolic Compounds Concentration, Xanthine Oxidase and Tyrosinase Inhibitory Activities of *Pleurotus cornucopiae*

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Abstract: *Pleurotus cornucopiae* has been widely used in nutritional and medicinal purposes. This study was initiated to screen the antioxidant activities, tyrosinase inhibitory effects on the fruiting bodies of *P. cornucopiae* extracted with acetone, methanol and hot water. The antioxidant activities were performed on β-carotene-linoleic acid, reducing power, DPPH, ferrous ions chelating abilities, and xanthine oxidase. In addition to this, phenolic compounds were also analyzed. The acetonic, methanolic and hot water extracts showed the similar pattern of β-carotene-linoleic acid inhibition. The acetonic extract (8 mg/ml) showed a significantly high reducing power of 1.86 as compare to other extracts. The scavenging effects on DPPH radicals, the acetonic extract was more effective than other extracts. The strongest chelating effect was obtained from the methanolic extract as compare to tested synthetic antioxidant. Gallic acid, protocatechuic acid, chlorogenic acid, vanillin, ferulic acid, naringin, naringenin, hesperetin, formononetin and biochanin-A were detected in acetonitrile and 0.1N hydrochloric acid (5:1) solvent extract. Xanthine oxidase and tyrosinase inhibitory activities of the acetonic, methanolic, and hot water *P. cornucopiae* extracts increased with increasing concentration. The results suggested that fruiting bodies of *P. cornucopiae* can be used to developed nutraceutical products, which can play significant roles in providing good nutrition and improving human health.

Key words: Antioxidant, phenolic compounds, *Pleurotus cornucopiae*, tyrosinase inhibition, xanthine oxidase.

INTRODUCTION

Pleurotus cornucopiae is an edible mushroom that belongs to the family pleurotaceae and order agaricales. It grows on standing and fallen elm trees on the Siberian Peninsula of Russia and in the Eastern-North parts of Hokkaido, Japan (El-Bohi *et al*., 2005). Currently this mushroom is successfully cultivated and commercially available in Korea and has become increasingly popular due to its delicious taste and unique texture. Angiotensin-I converting enzyme (ACE) is potentially of great importance for controlling blood pressure in the rennin-angiotensin system (Hyoung *et al*., 2004). ACE converts the inactive decapeptide angiotensin-I to the potent vasopressor octapeptide angiotensin-II. Hagiwara *et al*. (2005) reported that D-mannitol, one of the important phytochemicals of *P. cornucopiae* that inhibits ACE activity and decrease the high blood pressure of hypertensive rats.

The scientific community, in searching for new therapeutic alternatives, has studied many kinds of mushrooms and has found variable therapeutic activity such as anticarcinogenic, anti-inflammatory, immunosuppressor and antibiotic (Asfors and Ley, 1993; Longvah and Deosthale, 1998). Oxidation is essential to many living organisms for the production of energy to fuel biological processes. 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 (Halliwell and Gutteridge, 1984). Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherols and glutathione (Mau *et al*., 2002). When the mechanism of antioxidant protection becomes unbalanced by factors such as aging, deterioration of physiological functions may occur resulting in diseases and accelerated aging. However, the antioxidants present in human diet are of great interest as possible protective agents to help the human bodies

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reduce oxidative damage. Flavonoids and polyphenolic crude extracts have been reported to possess xanthine oxidase inhibitory activity (Chang *et al*., 1993). A potential source of such compounds can be obtained from mushrooms (Zhou *et al*., 2001).

Tyrosinase is a copper-containing enzyme is widely distributed in mushrooms, plants and animals, which responsible for melanization. The formation of melanin in the human body influenced or reduced by several mechanisms, including anti-oxidation, direct tyrosinase inhibition, melanin inhibition of migration from cell to cell and hormonal activities etc. (Pawelek and Korner, 1982). Recently tyrosinase inhibitors have been used frequently in cosmetics and depigmenting agents for hyperpigmentation (Funasaka *et al*., 2000). Therefore a concerted effort has been made to search for naturally occurring tyrosinase inhibitors from *P. cornucopiae*. However, the antioxidant properties of this mushroom are not available. Therefore, the aims of this work were to study and compare the antioxidant and tyrosinase inhibitory activities of the acetonic, methanolic, and hot-water extracts from the fruiting bodies of *P. cornucopiae*. Antioxidant activities were assayed including β-carotene-linoleic acid, reducing power, scavenging effects on radicals, chelating effects on ferrous ions, xanthine oxidase. The contents of phenolic compounds were also determined.

MATERIALS AND METHODS

Chemicals and Reagents:

β-carotene, linoleic acid, chloroform, polyoxyethylene sorbitan monopalmitate (Tween40), butylated hydroxytoluene (BHT), α-tocopherol (TOC), 1,1-diphenyl-2-picrylhydrazyl (DPPH), L-ascorbic acid, potassium ferricyanide, trichloroacetic acid, ferrous chloride, ferric chloride, ferrozine, Folin-Ciocalteu reagent, gallic acid, methanol, 3,4-dihydroxy-L-phenylalanine (L-DOPA), xanthine, allopurinol, mushroom tyrosinase, and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All chemicals and solvents were used as HPLC or analytical grade.

Mushroom and Extraction:

Fresh and mature fruiting bodies of *P. cornucopiae* were obtained from Mushroom Research Institute of Gyeonggi Province in Korea. A pure culture was deposited in Culture Collection and DNA Bank of Mushroom (CCDBM), Division of Life Sciences, University of Incheon, Korea and acquired accession number, IUM-4423. Fruiting bodies were dried with hot air at 40°C for 48 h and finely pulverized. Five grams of powdered samples were extracted with 100 ml of 60% acetone and 80% methanol with stirring at 150 rpm for 24 h at 25°C to obtain acetonic and methanolic extracts. The mixture was filtered through two layer of Whatman no. 1 filter paper. The same quantity of sample was boiled at 100°C for 3 h with 100 ml deionized distilled water to obtain a hot water extract. The mixture was cooled to room temperature and filtered through Whatman no. 1 filter paper. The residues were then extracted with two additional 100 ml aliquots of acetone, methanol, and deionized water, as described above. The combined extracts were evaporated with a rotary evaporator (Eyela, Saitama, Japan) at 40°C, and the remaining solvent was removed with a freeze-drier (Optizen, Daejeon, Korea). The yields from the acetonic, methanolic and hot water extracts of *P. cornucopiae* were 23.24, 23.18, and 18.24% (w/w), respectively.

Antioxidant Activity by β-carotene-linoleic Acid:

Antioxidant activity was determined by measuring the inhibition of volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius *et al*., 1998). A stock solution of a β-carotene-linoleic acid mixture was prepared as follows 0.5 mg β-carotene was dissolved in 1 ml of chloroform, and 25 μl of linoleic acid and 200 mg Tween 40 was added. The chloroform was removed completely using a vacuum evaporator. Then, 100 ml of oxygenated distilled water was added with vigorous shaking; 2.5 ml of this reaction mixture was dispensed to test tubes, 0.5 ml of various concentrations (0.5-20.0 mg/ml) of the extracts in methanol was added, and the reaction mixture was incubated for up to 2 h at 50°C. The same procedure was repeated with the positive controls BHT and TOC, and a blank. After the incubation, the absorbance of the mixtures was measured at 490 nm using a spectrophotometer (Optizen POP; Mecasys Co. Ltd., Daejeon, Korea). The absorbance was measured until the β-carotene color disappeared. The β-carotene bleaching rate (R) was calculated according to Eq. (1).

$$R = \ln (a/b)/ t \quad (1)$$

where, ln = natural log, a = absorbance at time t (0), b = absorbance at time t (120 min). The antioxidant

activity (AA) was calculated as the percent inhibition relative to the control using Eq. (2).

$$AA = [(R_{control} - R_{sample}) / R_{control}] \times 100 \quad (2)$$

Antioxidant activities of the extracts were compared with those of BHT and TOC at 0.5 mg/ ml and a blank consisting of 0.5 ml methanol.

Reducing Power:

Reducing power was determined according to the method of Gulcin *et al*. (2003). Each extract (1-8 mg/ml) in methanol (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricynide, and the mixture was incubated at 50ºC for 20 min. Then, 2.5 ml of 10% trichloroacetic acid was added, and the mixture was centrifuged at 200 × g (6K 15; Sigma, Mannchein, Germany) for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. Finally, the absorbance was measured at 700 nm against a blank. BHT and TOC were used as positive controls.

Scavenging Effect on 1,1-diphenyl-2-picrylhydrazyl Radicals:

The hydrogen atoms or electron donation ability of the corresponding extracts and some pure compounds were measured from the bleaching of the purple colored DPPH methanol solution (Cuendet *et al*., 1997). Four ml of various concentrations (0.125-2.0 mg/ml) of the extracts in methanol was added to 1 ml of DPPH radical solution in methanol (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and allowed to stand for 30 min, and the absorbance of the resulting solution was measured at 517 nm using a spectrophotometer. Inhibition of the DPPH free radical in percent (I%) was calculated as:

$$I\ \% = [(A_{control} - A_{sample})/ A_{control}] \times 100$$

where, $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. BHT, TOC, and L-ascorbic acid were used as positive controls.

Chelating Effects on Ferrous Ions:

The chelating effect was determined according to the method of Dinis *et al*. (1994). Briefly, 2 ml of various concentrations (0.063-1.0 mg/ml) of the extracts in methanol was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by adding 5 mM ferrozine (0.2 ml). The total volume was adjusted to 5 ml with methanol, and the mixture was shaken vigorously and left at room temperature for 10 min. The absorbance of the solution was measured spectrophotometrically at 562 nm. The inhibition percentage of the ferrozine-Fe^{2+} complex formation was calculated using the following formula:

$$\text{Metal chelating effect } (\%) = [(A_{Control} - A_{Sample})/ A_{Control}] \times 100$$

where, $A_{Control}$ is the absorbance of the control (control contained $FeCl_2$ and ferrozine; complex formation molecules), and A_{Sample} is the absorbance of the test compound. BHT and TOC were used as positive controls.

Analysis of Phenolic Compounds:

Fifteen standard phenolic compounds, including gallic acid, pyrogallol, homogentisic acid, protocatechuic acid, (+) catechin, chlorogenic acid, caffeic acid, vanillin, ferulic acid, naringin, resveratrol, naringenin, hesperetin, formononetin, and biochanin-A were purchased from Sigma Aldrich and used for calibration curves. The standard stock solutions (50, 100, 250, and 500 ppm) were prepared in DMSO. Sample compounds were identified based on retention times of authentic standards and were quantified by comparing their peak areas with those of the standard curves.

Sample preparation for the phenolic compound analysis followed Kim *et al*. (2006). Two grams of dried mushroom powder were mixed with 10 ml of acetonitrile and 2 ml of 0.1 N hydrochloric acid and stirred 150 rpm for 2 h at room temperature. The suspension was filtered through Whatman no. 42 filter paper. The extract was freeze-dried, and the residues were redissolved in 10 ml of 80% aqueous methanol (HPLC grade) and filtered through a 0.45 µm nylon membrane filter (Titan, Rockwood, TN, USA). The 20 µl filtrate was loaded onto an Agilent-1100 series liquid chromatography HPLC system (Agilent Technologies, Waldbronn, Germany). Separation was achieved on a 250 nm × 4.6 mm i.d., 5 µm, YMC-Pack ODS AM (YMC Co. Ltd., Kyoto,

Japan) column. The mobile phase was distilled water with 0.1% glacial acetic acid (solvent A) and acetonitrile with 0.1% glacial acetic acid (solvent B). The gradient was 0 min, 92% A; 0-2 min, 90% A; 2-27 min, 70% A; 27-50 min, 10% A; 50-51 min, 0% A; 51-60 min, 0% A; 60-63 min, 92% A. The run time was 60 min using a flow rate of 1 ml/min. Detection was performed with a diode array detector at a wavelength of 280 nm.

Xanthine Oxidase Inhibition:

In vitro xanthine oxidase (XO) inhibitory activity of various extracts from the fruiting bodies of *P. cornucopiae* was assayed spectrophotometrically under aerobic conditions, using xanthine as the substrate (Owen and Johns, 1999). The assay mixture consisted of 1 ml extract of the different concentrations (0.5-8.0 mg/ml), 2.9 ml of phosphate buffer (pH 7.5), and 0.1 ml of xanthine oxidase enzyme solution (0.1 units/ml in phosphate buffer, pH 7.5), which was prepared immediately before use. After pre incubation at 25°C for 15 min, the reaction was initiated by the addition of 2 ml of the substrate solution (150 µM xanthine in the same buffer). The assay mixture was incubated at 25°C for 30 min. The reaction was then stopped by the addition of 1 ml of 1N hydrochloric acid and the absorbance was measured at 290 nm using a spectrophotometer. Different concentrations of the extracts were dissolved in DMSO and the final concentration of DMSO was 5%, which did not affect the enzyme assay. Proper controls with DMSO were carried out. Allopurinol (0.5-8.0 mg/ml), a known inhibitor of XO, was used as positive control. One unit of XO is defined as the amount of enzyme required to produce 1 mmol of uric acid/min at 25°C. Xanthine oxidase inhibitory activity was expressed as the percentage inhibition of XO in the above assay system calculated as

$$\text{Inhibition (\%)} = [(A - B) - (C - D)/ (A - B)] \times 100$$

where A is the activity of the enzyme without the extraction, B is the control of A without the extraction and enzyme; C and D are the activities of the extraction with and without XO, respectively.

Tyrosinase Inhibition:

Tyrosinase inhibition activity was determined using the modified dopachrome method with L-DOPA as the substrate (Masuda *et al*., 2005). A 96-well microtiter plate was used to measure absorbance at 475 nm with 700 nm as a reference. Extract fractions were dissolved in 50% DMSO. Each well contained 40 µl of sample with 80 µl of phosphate buffer (0.1 M, pH 6.8), 40 µl of tyrosinase (31 units/ml), and 40 µl of L-DOPA (2.5 mM). The mixture was incubated for 10 min at 37°C, and absorbance was measured at 475 nm using a UVM 340 microplate reader (Asys, Eugendrof, Austria). Each sample was accompanied by a blank containing all components except L-DOPA. L-ascorbic acid and kojic acid were used as positive controls. The results were compared with a control consisting of 50% DMSO in place of the sample. The percentage of tyrosinase inhibition was calculated as follows:

$$[(A_{control} - A_{sample})/ A_{control}] \times 100\%$$

Statistical Analysis:

Data were expressed as means ± standard deviations of three replicate determinations and were analyzed by SPSS V.13 (SPSS Inc., Chicago, IL, USA). A one way analysis of variance and Duncan's new multiple-range test were used to determine the differences among the means.

RESULTS AND DISCUSSION

***Antioxidant Activity on β-carotene-linoleic Acid*:**

The antioxidant activities on β-carotene-linoleic acid of the acetonic, methanolic and hot water extracts from the fruiting bodies of *P. cornucopiae* gradually increased with increasing concentration. At 0.5-20.0 mg/ml antioxidant activity of the acetonic, methanolic, and hot water extracts on β-carotene-linoleic acid inhibition ranged from 69.25-94.62%, 36.71-94.12%, and 66.41-94.52%, respectively (Table 1). Results indicate that β-carotene-linoleic acid inhibition was lower than the synthetic antioxidant, BHT and TOC, respectively at 0.5 mg/ml. The antioxidant activity of carotenoids is based on the radical adducts of carotenoid with free radicals from linoleic acid. The linoleic acid free radical attacks the highly unsaturated β-carotene models. The presence of carotenoid shows, not only a decrease of the free radical concentration, but the reduction of $Fe^{3}+$ to $Fe^{2}+$ by carotenoids. It is probable that the antioxidative components in the mushroom extracts can reduce the extent

of β- carotene destruction by neutralizing the linoleate free radical and other free radicals formed in the reaction process (Jayaprakasha *et al*., 2001). Barros *et al*. (2007) reported that antioxidant activities of *Leucopaxillus giganteus*, *Sarcodon imbricatus* and *Agaricus arvensis* in various extracts increased with increasing concentration. Their antioxidant activities were 61.4%, 54.3% and 46.7% at 5 mg/ml. It seems that the antioxidant activity of *P. cornucopiae* was more effective than those mentioned above.

***Reducing Power*:**

Reducing power of *P. cornucopiae* in the acetonic, methanolic, and hot water extracts and as a function of their concentration is shown in Table 2. The reducing power increased with increasing concentration. At 8 mg/ml, the strongest reducing power inhibition was determined in the acetonic extract a value of 1.86 and the lowest reducing power inhibition (1.36) was exhibited by the methanolic extract. Reducing power of BHT and TOC at 1.0 mg/ml were 3.21and 2.16, respectively (Table 2).

With regard to ethanolic extracts, the reducing power of *Pleurotus citrinopileatus* was 1.03 at 5 mg/ml (Lee *et al*., 2007a) whereas, *Agricus bisporus*, *Pleurotus ferulae* and *Pleurotus ostreatus* showed reducing powers of 0.76, 0.70 and 0.61 at 20 mg/ml, respectively (Lee *et al*., 2007a). It can be seen that the reducing power of *P. cornucopiae* was higher than those of mentioned above. It was reported that the reducing power properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Shimada *et al*., 1992; Barros *et al*., 2007).

Table 1: Antioxidant activity against β-carotene-linoleic acid of different concentrations of various extracts from the fruiting bodies of *Pleurotus cornucopiae*.

Solvent and control	Sample concentration (mg/ml)			
	0.5	2.0	8.0	20.0
Acetone	69.25 ± 0.03	87.09 ± 0.19	91.06 ± 0.16	94.62 ± 0.05
Methanol	36.71 ± 0.05	71.50 ± 0.28	82.18 ± 0.16	94.12 ± 0.17
Hot water	66.41 ± 0.04	87.09 ± 0.07	90.45 ± 0.03	94.52 ± 0.06
BHT	95.21 ± 0.17	-	-	-
TOC	96.02 ± 0.18	-	-	-

Values expressed as means ± SD (n = 3).
-, not analyzed; BHT, butylated hydroxytoluene; TOC, α-tocopherol.

Table 2: Reducing power of different concentrations of various extracts from the fruiting bodies of *Pleurotus cornucopiae*.

Solvent and control	Sample concentration (mg/ml)			
	1.0	2.0	4.0	8.0
Acetone	0.503 ± 0.13	0.817 ± 0.15	1.302 ± 0.17	1.859 ± 0.35
Methanol	0.535 ± 0.07	0.724 ± 0.09	1.012 ± 0.15	1.362 ± 0.16
Hot water	0.490 ± 0.09	0.694 ± 0.12	1.034 ± 0.18	1.454 ± 0.22
BHT	3.212 ± 0.49	-	-	-
TOC	2.162 ± 0.32	-	-	-

Values expressed as means ± SD (n = 3).
-, not analyzed; BHT, butylated hydroxytoluene; TOC, α-tocopherol.

***Scavenging Effect on DPPH*:**

Scavenging effects of the acetonic, methanolic, and hot water extracts from the fruiting bodies of *P. cornucopiae* on DPPH radicals increased with increasing concentration. At 0.125-2.0 mg/ml, the scavenging activities of the acetonic, methanolic, and hot water extracts of *P. cornucopiae* on DPPH radical ranged from 50.65-93.23%, 56.33-92.58%, and 46.28-89.55%, respectively (Fig. 1). Results indicate that the acetonic, methanolic, and hot water extracts, respectively showed good, moderate, and poor activities at the concentration tested. However, at 0.125-2.0 mg/ml, BHT, TOC, and L-ascorbic acid showed the excellent scavenging activities of 85.25-98.74%, 67.37-97.78%, and 96.74-98.23%, respectively.

With regard to the ethanolic extracts of *Hypsizigus marmoreus*, *A. bisporus* and *P. citrinopileatus* fruiting bodies could scavenge DPPH radicals by 46.6-68.4% at 5 mg/ml (Lee et al., 2007a). For cold and hot water extracts, at 20 mg/ml, the scavenging activities of fruiting bodies, mycelia and filtrate were 20.7-52.3%, 37.6-48.3%, and 19.6-23.3%, respectively. It seems that the scavenging activity of *P. cornucopiae* fruiting bodies was more effective than those mentioned above. Various extracts might react with free radicals, particularly the peroxy radicals, which are the major propagators of the autoxidation chain of fat, thereby terminating the chain reaction (Shahidi and Wanasundara, 1992). Antioxidant activity of natural antioxidants has been shown to be involved in termination of free radical reaction (Shimada *et al*., 1992). Furthermore, Herraiz *et al*. (2003)

found that an essential amino acid L-tryptophan could react with phenolic aldehydes in food to form phenolic tetrahydro-β-carboline alkaloids that scavenged 2,2-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid effectively. Therefore, the presence of L-tryptophan in various extracts might most likely account for the scavenging activity on DPPH radicals. However, the better activity of acetonic extract might be due to more hydrogen-donating components contained within the extracts.

***Chelating Effects on Ferrous Ions*:**

The chelating activity of the acetonic, methanolic, and hot water extracts at five different concentrations (0.063, 0.125, 0.25, 0.50, and 1.0 mg/ml) from the fruiting bodies of *P. cornucopiae* toward ferrous ion was investigated. BHT and TOC were used as reference standards on ferrous ions. As can be seen from the Fig. 2, chelating ability of the extracts increased with increasing concentration. The strongest chelating effect (86.52%) obtained from the methanolic extracts at 1.0 mg/ml. At this concentration, the lowest chelating effect was exhibited by hot water extract (81.12%). All of the extracts evaluated here showed significantly higher chelating effects on ferrous ion than those of the standards, BHT and TOC at the concentration of 0.063, 0.125, and 0.25 mg/ ml, respectively.

With regard to hot water extracts at 20 mg/ml, *Ganoderma tsugae* and *Agrocybe cylindracea* chelated ferrous ion by 39.5-42.6% and 45.8%, respectively (Mau *et al*., 2005; Tsai *et al*., 2006). At 1-5 mg/ml, chelating abilities of *H. marmoreus* and *P. citrinopileatus* were 75.6-92.6% (Lee *et al*., 2007b). It seems that chelating ability of *P. cornucopiae* on ferrous ion was similar to that of *H. marmoreus* and *P. citrinopileatus*, while more effective than those of *G. tsugae* and *A. cylindracea*. Chelating agents may serve as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ions. Since ferrous ions were the most effective pro-oxidants on auto oxidation of oil compounds in foods (Yamaguchi *et al*., 1988), the high ferrous-ion chelating abilities of the various extracts from the fruiting bodies of *P. cornucopiae* would be beneficial to the human health.

***Analysis of Phenolic Compounds*:**

Gallic acid, protocatechuic acid, chlorogenic acid, vanillin, ferulic acid, naringenin, hesperetin, formononetin and biochanin-A were detected in acetonitrile and 0.1N hydrochloric acid (5:1) solvent extract (Fig. 3). The total phenolic compounds concentration was 281 μg/g. The highest and lowest phenolic compound concentration were recorded in protocatechuic acid (74 μg/g) and naringenin, hesperetin, and formononetin (11 μg/g), respectively. These findings are comparable to the previous studies on edible mushrooms (Kim *et al*., 2008) in which average total concentration of phenolic compounds was 174 μg/g. Mushroom species also contained varying numbers of phenolic compounds, ranging from 3 to 15, while gallic acid and protocatechuic acid was reported common phenolic compounds found in edible mushrooms. Thus, the content of phenolic compounds could be used as an important indicator of antioxidant capacity. Several reports have convincingly shown a close relationship between antioxidant activity and phenolic content (Duan *et al*., 2007; Pan *et al*., 2008). Mushroom extracts have high levels of phenolic compounds, which are composed of one or more aromatic rings bearing one or more hydroxyl groups, and exhibit extensive free radical-scavenging activities as hydrogen donors or electron-donating agents, as well as metal ion-chelating properties. The greater numbers of hydroxyl groups in the phenolics could exhibit higher antioxidant activity (Prasad *et al*., 2005; Rangkadilok *et al*., 2007).

***Xanthine Oxidase Inhibitory Activity*:**

Xanthine oxidase inhibitory activities of various extracts of *P. cornucopiae* increased with increasing of concentration. At 0.5-8.0 mg/ml, the xanthine oxidase inhibition of the acetonic, methanolic, and hot water extracts ranged from 2.03-59.26%, 2.42-52.87%, and 2.33-46.42%, respectively. However, at the same concentrations, allopurinol showed the excellent xanthine oxidase inhibitory activity of 92.31-94.58% (Fig. 4). Results indicate that the acetonic, methanolic, and hot water extracts showed good, moderate, and poor activities, respectively at the concentration tested. However, at higher doses of *P. cornucopiae* extract, the xanthine oxidase could be significantly inhibited. Flavonoids are a group of polyphenolic compounds, which have been reported to possess xanthine oxidase inhibitory activity (Costantino *et al*., 1992). Hence, the presence of phenolic and flavonoid content in the extract would have contributed towards xanthine oxidase inhibition.

***Tyrosinase Inhibition*:**

Tyrosinase inhibitory activities of the acetonic, methanolic, and hot water extracts from the fruiting bodies of *P. cornucopiae* increased with increasing concentration. At 0.125-1.0 mg/ml, the tyrosinase inhibition of the

acetonic, methanolic, and hot water extracts ranged from 12.11-52.11%, 14.38-55.81%, and 7.54-50.12%, respectively (Fig. 5). Results indicate that the methanolic extract showed good, while the acetonic and hot water extracts showed moderate activities at the concentration tested. However, at 0.125-1.0 mg/ml, L-ascorbic acid and kojic acid showed the excellent tyrosinase inhibitory activities of 75.12-92.74% and 91.23-99.00%. The inhibition of tyrosinase ability might depend on the hydroxyl groups of the phenolic compounds of the mushroom extracts that could form a hydrogen bond to active site of the enzyme, leading to a lower enzymatic activity. Some tyrosinase inhibitors act through hydroxyl groups that bind to the active site on tyrosinase, resulting in steric hindrance or changed conformation (Baek *et al*., 2008). Gallic acid, (-)-epicatechin, procyanidin B2 and (-)-epicatechin-3-gallate, identified in mushrooms, proved to be effective inhibitors of tyrosinase activity, as reported by many researchers (Kubo *et al*., 2003; Momtaz *et al*., 2008). The antioxidant activity may also be one of the important mechanisms for tyrosinase inhibitory activity.

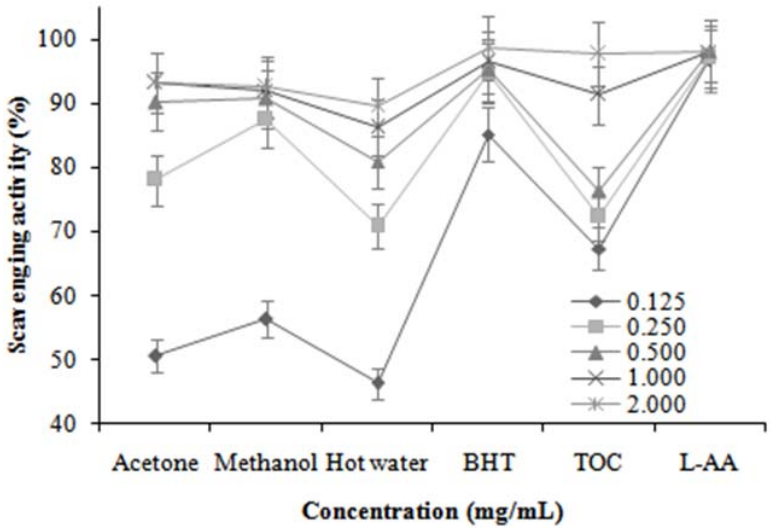

Fig. 1: Scavenging activity of various extracts from the fruiting bodies of *Pleurotus cornucopiae* against 1,1-diphenyl-2-picrylhydrazyl. Values expressed as means ± SE (n = 3). BHT, butylated hydroxytoluene; TOC, α-tocopherol; L-AA, L-ascorbic acid.

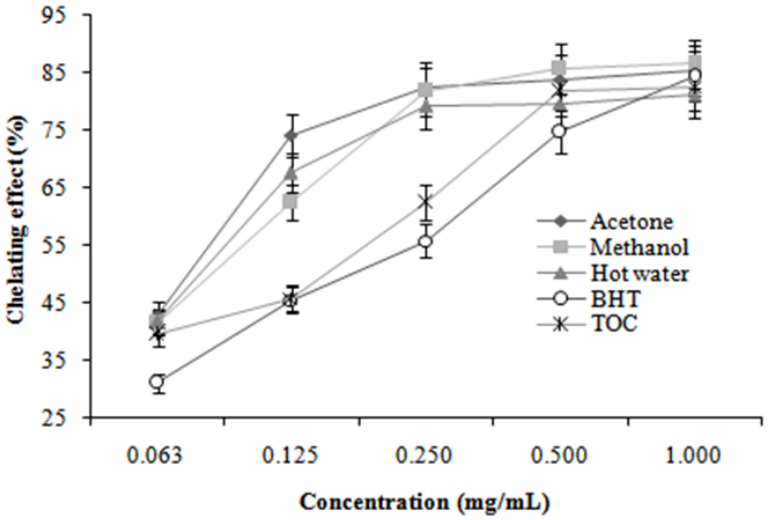

Fig. 2: Chelating effect of various extracts from the fruiting bodies of *Pleurotus cornucopiae*. Values expressed as means ± SE (n = 3). BHT, butylated hydroxytoluene; TOC, α-tocopherol.

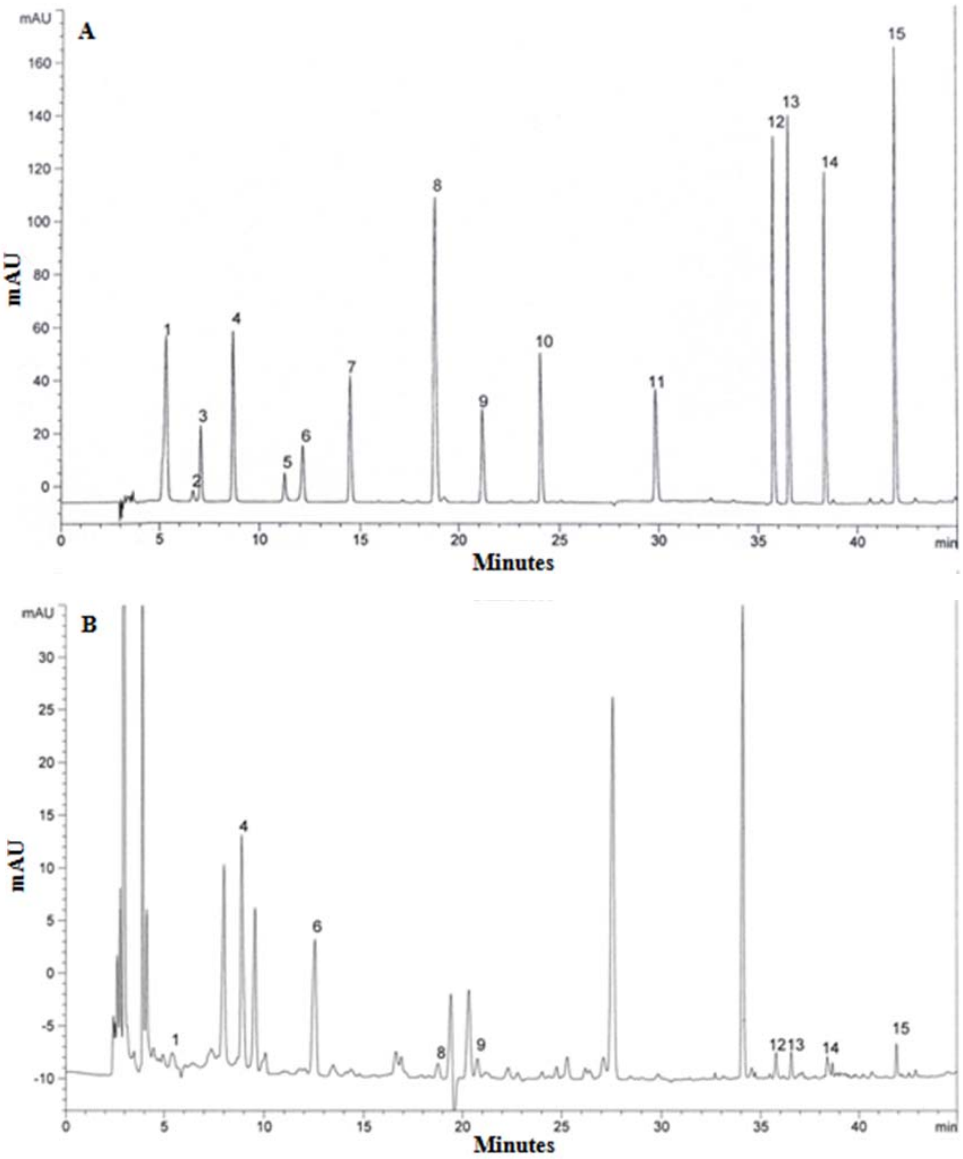

Fig. 3: High performance liquid chromatography of phenolic compounds. A, Standard mixture of 15 phenolic compounds; B, *Pleurotus cornucopiae* extract. 1, gallic acid; 2, pyrogallol; 3, homogentisic acid; 4, protocatechuic acid; 5, (+) catechin; 6, chlorogenic acid; 7, caffeic acid; 8, vanillin; 9, ferulic acid; 10, naringin; 11, resveratrol; 12, naringenin; 13, hesperetin; 14, formononetin; 15, biochanin-A.

***Conclusion*:**

On the basis of the results, it is suggested that three different extracts from the fruiting bodies of *P. cornucopiae* evaluated here could be used as an easily accessible source of natural antioxidants for the nourishment. The study indicated that *P. cornucopiae* contained various phenolic compounds that have shown good potential, which can be used for functional foods and medicinal purposes. The consumption of *P. cornucopiae* might be somewhat beneficial to the antioxidant protection system of the human body against oxidative damage. Overall our report could be useful information for investigating new mushroom materials for functional food additives.

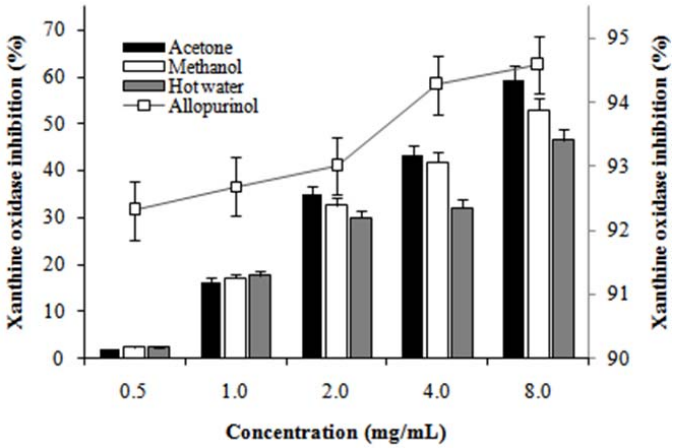

Fig. 4: Xanthine oxidase inhibition activity of various extracts from the fruting bodies of *Pleurotus cornucopiae*. Values expressed as means ± SE (n = 3).

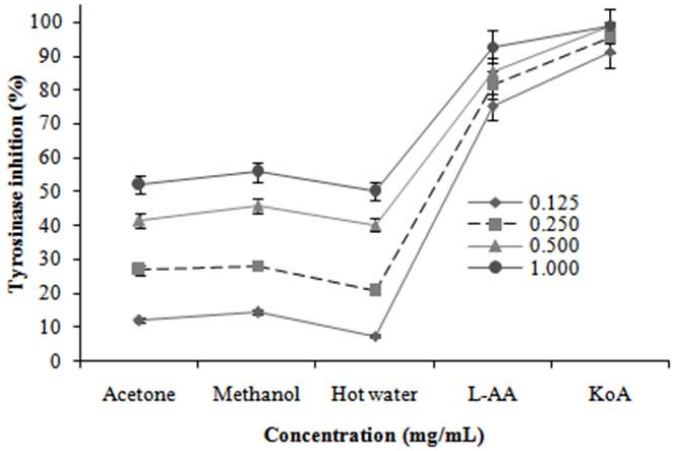

Fig. 5: Tyrosinase inhibition activity of various extracts from the fruiting bodies of *Pleurotus cornucopiae*. Values expressed as means ± SE (n = 3). L-AA, L-ascorbic acid; KoA, kojic acid.

ACKNOWLEDGEMENTS

This research was supported by a mutual research grant from the Rural Development Administration (Agenda 9-27-63; No. 200901OFT092763229).

REFERENCES

Asfors, K.E. and K. Ley, 1993. Sulfated polysaccharides in inflammation. J. Lab. Clin. Med., 121(2): 201-202.

Baek, H.S., H.S. Rho, J.W. Yoo, S.M. Ahn, J.Y. Lee, J. Lee, M.K. Kim, D.H. Kim and I.S. Chang, 2008. The inhibitory effect of new hydroxamic acid derivatives on melanogenesis. Bull. Korean Chemi. Soc., 29(1): 43-46.

Barros, L., M.J. Ferreira, B. Queiros, I.C.F.R. Ferreira and P. Bapista, 2007. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chem., 103(2): 413-419.

El-Bohi, K.M., L. Sabik, K. Muzandu, Z. Shaban, M. Soliman, M. Ishizuka, A. Kazusaka and S. Fujita, 2005. Antigenotoxic effect of *Pleurotus cornucopiae* extracts on the mutagenesis *Salmonella typhimurium* TA98 elicited by benzo[a]pyrene and oxidative DNA lesions in V 79 hamster lung cells. Jpn. J. Vet. Res., 52(4): 163-172.

Chang, W.S., Y.J. Lee, F.J. Lu and H.C. Chaing, 1993. Inhibitory effects of flavonoids on xanthine oxidase. Anticancer Res., 13(6A): 2165-2170.

Costantino, L., A. Albasini, G. Rastelli and S. Benvenuti, 1992. Activity of polyphenolic crude extracts as scavengers of superoxide radicals and inhibitors of xanthine oxidase. Planta Med., 58(4): 342-344.

Cuendet, M., K. Hostettmann, O. Potterat and W. Dyatmiko, 1997. Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. Helvetica Chimica Acta., 80(4): 1144-1152.

Dapkevicius, A., R. Venskutonis, T.A. Van Beek and J.P.H. Linssen, 1998. Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. J. Sci. Food Agric., 77: 140-146.

Dinis, T.C., V.M. Madeira and L.M. Almeida, 1994. Action of phenolic derivatives (acetaminophen, salicylate, and 5-amino salicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys., 315(1): 161-169.

Duan, X., G. Wu and Y. Jiang, 2007. Evaluation of antioxidant properties of lichi fruit phenolics in relation to pericarp browning prevention. Molecules, 12(4): 759-771.

Funasaka, Y., M. Komoto and M. Ichihashi, 2000. Depigmenting effect of alpha-tocophenol ferulate on normal human melanocytes. Pigment Cell Res., 13(8): 170-174.

Gulcin, I., M.E. Buyukokuroglu, M. Oktay and O.I. Kufrevioglu, 2003. Antioxidant and analgesic activities of turpentine of *Pinus nigra* Arn. subsp. *pallsiana* (Lamb.) Holmboe. J. Ethnopharmacol., 86(1): 51-58.

Hagiwara, S.Y., M. Takahashi, Y. Shen, S. Kaihou, T. Tomiyama, M. Yazawa, Y. Tamai, Y. Sin, A. Kazusaka and M. Terazawa, 2005. A phytochemical in the edible Tamogi-take mushroom (*Pleurotus cornucopae*), D-mannitol inhibits ACE activity and lower blood pressure of spontaneously hypertensive rats. Biosci. Biotechnol. Biochem., 69(8): 1603-1605.

Halliwell, B. and J.M. Gutteridge, 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J., 219(1): 1-4.

Herraiz, T., J. Galisteo and C. Chamorro, 2003. L-tryptophan reacts with naturally occurring and food-occurring phenolic aldehydes to give phenolic tetrahydro-β-caroline alkaloids: activity as antioxidants and free radical scavengers. J. Agric. Food Chem., 51: 2168-2173.

Hyoung, L.D., K.J. Ho, P.J. Sik, C.Y. Jun and L.J. Soo, 2004. Isolation and characterization of novel angiotensin-I converting enzyme inhibitory peptide derived from the edible mushroom *Tricholoma giganteum*. Peptides, 25(4): 621-627.

Jayaprakasha, G.K., R.P. Singh and K.K. Sakariah, 2001. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. Food Chem., 73(3): 285-290.

Kim, E.H., S.H. Kim, J.I. Chung, H.Y. Chi, J.A. Kim and I.M. Chung, 2006. Analysis phenolic compounds and isoflavones in soybean seeds (*Glycine max* (L.) Merill) and sprouts grown under different conditions. Eur. Food Res. Technol., 222(1-2): 201-208.

Kim, M.Y., P. Seguin, J.K. Ahn, J.J. Kim, S.C. Chun, E.H. Kim, S.H. Seo, E.Y. Kang, S.L. Kim, Y.J. Park, H.M. Ro and I.M. Chung, 2008. Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Korea. J. Agric. Food Chem., 56(16): 7265-7270.

Kubo, I., Q.X. Chen and K.I. Nihei, 2003. Molecular design of antibrowning agents: antioxidative tyrosinase inhibitors. Food Chem. 81(2): 241-247.

Lee, Y.L., G.W. Huang, Z.C. Liang and J.L. Mau, 2007a. Antioxidant properties of three extracts from *Pleurotus citrinopileatus*. LWT-Food Sci. Technol., 40(5): 823-833.

Lee, Y.L., M.T. Yen and J.L. Mau, 2007b. Antioxidant properties of various extracts from *Hypsizigus marmoreus*. Food Chem., 104(1): 1-9.

Longvah, T. and Y.G. Deosthale, 1998. Compositional and nutritional studies on edible wild mushroom from northeast India. Food Chem., 63(3): 331-334.

Masuda, T., D. Yamashita, Y. Takeda and S. Yonemori, 2005. Screening for tyrosinase inhibitors among extracts of seashore plants and identification of potent inhibitors from *Garcinia subelliptica*. Biosci. Biotechnol. Biochem., 69(1): 197-201.

Mau, J.L., H.C. Lin and S.F. Song, 2002. Antioxidant properties of several speciality mushrooms. Food Res. Int., 35(6): 519-526.

Mau, J.L., S.Y. Tsai, Y.H. Tseng and S.J. Huang, 2005. Antioxidant properties of hot water extracts from *Ganoderma tsugae* Murrill. LWT-Food Sci. Technol., 38(6): 589-597.

Momtaz, S., B.M. Mapunya, P.J. Houghton, C. Edgerly, A. Hussein, S. Naidoo and N. Lall, 2008. Tyrosinase inhibition by extracts and constituents of *Sideroxylon inerme* L. stem bark, used in South Africa for skin lightening. J. Ethnopharmacol., 119(3): 507-512.

Owen, P.L. and T. Johns, 1999. Xanthine oxidase inhibitory activity of North-Eastern North American plant remedies used for gout. J. Ethnopharmacol., 64(2): 149-160.

Pan, Y., K. Wang, S. Huang, H. Wang, X. Mu, C. He, X. Ji, J. Zhang and F. Huang, 2008. Antioxidant activity of microwave-assisted extract of longan (*Dimocarpus longan* Lour.) peel. Food Chem. 106(3): 1264-1270.

Pawelek, J.M. and A.M. Korner, 1982. The biosynthesis of mamlian melanin. Am. Sci., 70(2): 136-145.

Prasad, N.K., S. Divakar, G.R. Shivamurthy and S.M. Aradhya, 2005. Isolation of a free radical scavenging antioxidant from water spinach (*Ipomoea aquatica* Forsk). J. Sci. Food Agric., 85(9): 1461-1468.

Rangkadilok, N., S. Sitthimonchai, L. Worasuttayangkurn, C. Mahidol, M. Ruchirawat and J. Satayavivad, 2007. Evaluation of free radical scavenging and antityrosinase activities of standardized longan fruit extract. Food Chemi. Toxicol., 45(2): 328-336.

Shahidi, F. and P.K. Wanasundara, 1992. Phenolic antioxidants. Crit. Rev. Food Sci. Nutr., 32(1): 67-103.

Shimada, K., K. Fujikawa, K. Yahara and T. Nakamura, 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem., 40(6): 945-948.

Tsai, S.Y., S.J. Huang and J.L. Mau, 2006. Antioxidant properties of hot water extracts from *Agrocybe cylindracea*. Food Chem., 98(4): 670-677.

Yamaguchi, R., Y. Tatsumi, M. Asano, K. Kato and Y. Ueno, 1988. Effect of metal salts and fructose on the autoxidation of methyl linoleate in emulsions. Agric. Biol. Chem., 52: 849-850.

Zhou, C.X., L.D. Kong, W.C. Ye, C.H. Cheng and R.X. Tan, 2001. Inhibition of xanthine and monoamine oxidases by stillbenoids from *Veratrum taliense*. Planta Med., 67(2): 158-161.