World Applied Sciences Journal 12 (10): 1851-1859, 2011
ISSN 1818-4952

Detection of Phenolic Compounds Concentration and Evaluation of Antioxidant and Antityrosinase Activities of Various Extracts from the Fruiting Bodies of *Lentinus edodes*

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Abstract: The purpose of this study was to evaluate the antioxidant activities, tyrosinase inhibitory effects on the fruiting bodies of *Lentinus edodes* extracted with acetone, methanol and hot water. The antioxidant activities were tested against 1,1-diphenyl-2-picrylhydrazyl free radical scavenging, ferrous chelating abilities, β-carotene-linoleic acid, reducing power and xanthine oxidase inhibition. In addition to this, phenolic compounds were also detected. The scavenging effects on 1,1-diphenyl-2-picrylhydrazyl radicals, the acetonic extract was more effective than the methanolic and hot water extracts. The strongest chelating effect (86.45%) was obtained from the acetonic extract at 1.0 mg/ml. The methanolic extract showed the strongest β-carotene-linoleic acid inhibition as compare to the other extracts. The hot water extract (8 mg/ml) showed a significantly high reducing power of 0.96 than the other extracts. A high performance liquid chromatography analysis detected, four phenolic compounds, including naringenin, hesperetin, formononetin and biochanin-A in acetonitrile and 0.1N hydrochloric acid (5:1) solvent extract. Xanthine oxidase and tyrosinase inhibitory activities of the acetonic, methanolic and hot water *L. edodes* extracts increased with increasing of concentration. Results revealed that the acetonic and methanolic extracts showed good, while the hot water extract moderate antioxidant and antityrosinase activities at the concentration tested.

Key words: Antioxidant · *Lentinus edodes* · Phenolic compounds · Tyrosinase inhibition · Xanthine oxidase

INTRODUCTION

Lentinus edodes, widley known as shiitake mushroom. This mushroom has an established history of use in time-honored oriental therapies. Modern clinical practice in Korea, Japan, China and other Asian countries continues to rely on mushroom-derived preparations. Those practices still form the basis of modern scientific studies of mushroom medicinal activities, especially in the field of stomach, prostate and lung cancers [1]. Lentinan, produced from *L. edodes,* is a β (1-3), β (1-6) glucan. There is an immense literature related to the anticancer effect of lentinan on animals and human carcinomas [2].

Reactive oxygen species play a critical role in many diseases, such as cancer, atherosclerosis, gastric ulcer and other conditions. The intake of antioxidants, polyphenols has been effective in the prevention of these diseases [3, 4]. Cells are equipped with several defense systems against free radical damage, including oxidative enzymes, superoxide dismutase and catalase, or chemical compounds such as α-tocopherol, ascorbic acid, carotenoids, polyphenol compounds and glutathione [5]. However, antioxidant supplements or antioxidant-containing foods may be used to help the human body to reduce oxidative damage or to protect food quality by preventing oxidative deterioration [6]. The catalysis of xanthine by the enzyme xanthine oxidase (XO) can lead to the accumulation of uric acid and ultimately cause gout. Allopurinol, a XO inhibitor prescribed for chronic gout, acts as a substrate for competitive inhibitor of the enzyme [7]. A potential source of such compounds can be obtained from edible mushrooms [8]. Flavonoids and polyphenolic crude extracts have been reported to possess xanthine oxidase inhibitory activity [9].

Skin hyperpigmentation can be dependent on either an increased number of melanocytes or activity of melanogenic enzymes. Tyrosinase is a copper-containing enzyme that catalyzes the oxidation of tyrosine into dopa and subsequently dopaquinone [10]. These commercial mushrooms were found to be medically active in several

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therapies such as antitumour, antiviral and immunomodulating treatments [1]. Although research was focused on the therapeutic effects of this mushroom and little information is available about their antioxidant properties. This study was initiated to evaluate and compare the antioxidant and antityrosinase properties of the acetonic, methanolic and hot water extracts from the fruiting bodies of *L. edodes*.

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, mature fruiting bodies of *L. edodes* were purchased in E- mart in Incheon of Korea. A pure culture was deposited in Culture Collection DNA Bank of Mushroom (CCDBM), Division of Life Sciences, University of Incheon, Korea and acquired accession number, IUM-4681. Fruiting bodies were dried with hot air at 40°C for 48 h and finely pulverized. The acetonic, methanolic and hot water extractions were prepared according to the method of Alam *et al*., [11]. 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 *L. edodes* were 29.70, 24.24 and 19.70% (w/w), respectively.

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 [12]. 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*. [13]. 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. 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 [14]. 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*. [15]. 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 50°C 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.

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 Alam *et al*. [11]. 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 hr 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 *L. edodes* was assayed spectrophotometrically under aerobic conditions using xanthine as the substrate [7]. 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 XO 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 [16]. 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). One way analysis of variance and Duncan's new multiple-range test were used to determine the differences among the means.

RESULTS AND DISCUSSION

Scavenging Effect on DPPH: The scavenging effects of the acetonic, methanolic and hot water extracts from the fruiting bodies of *L. edodes* 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 *L. edodes* on DPPH radical ranged from 9.57-77.70, 5.24-72.21 and 6.33-41.26%, respectively (Fig. 1). The results indicated that 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 *H. marmoreus*, *A. bisporus* and *Pleurotus citrinopileatus* fruiting bodies scavenged DPPH radicals by 46.6-68.4% at 5 mg/ml [17]. 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 *L. edodes* fruiting bodies was more effective than those mentioned above. Various extracts might react with free

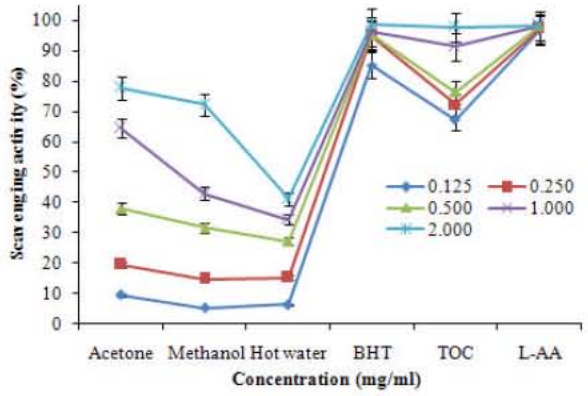

Fig. 1: Scavenging activity against 1,1-diphenyl-2-picrylhydrazyl of different concentrations of various extracts from the fruiting bodies of *Lentinus edodes*. Values expressed as means ± SE (n = 3). BHT, butylated hydroxytoluene; TOC, α-tocopherol; L-AA, L-ascorbic acid.

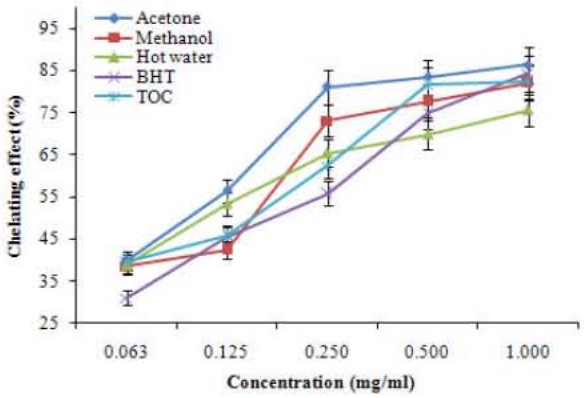

Fig. 2: Chelating effect of various extracts from the fruiting bodies of *Lentinus edodes*. Values expressed as means ± SE (n = 3). BHT, butylated hydroxytoluene; TOC, α-tocopherol.

radicals, particularly the peroxy radicals, which are the major propagators of the autoxidation chain of fat, thereby terminating the chain reaction [18, 19]. Antioxidant activity of natural antioxidants has been shown to be involved in termination of free radical reaction [20]. Furthermore, Herraiz *et al*. [21] 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.

Chelating Effects on Ferrous Ions: The chelating activity of the acetonic, methanolic and hot water extracts at five different concentrations (0.063, 0.125, 0.250, 0.500 and 1.0 mg/ml) from the fruiting bodies of *L. edodes* toward ferrous ions was investigated. BHT and TOC were used as ferrous ion standard. As shown in Fig. 2, the chelating capacity of the extracts increased with increasing concentration. The strongest chelating effect (86.45%) was obtained with the acetonic extract at 1.0 mg/ml. At this concentration, the lowest chelating effect was exhibited by hot water extract (75.60%). All of the extracts evaluated here showed significantly higher chelating effects on ferrous ions than those of the standards, BHT and TOC at the concentration of 0.063, 0.125 and 0.250 mg/ml, respectively.

With regard to the hot water extract at 20 mg/ml, *Ganoderma tsugae* and *Agrocybe cylindracea* chelated ferrous ions by 42.6 and 45.8%, respectively [22, 23]. At 1-5 mg/ ml, the chelating abilities of *H. marmoreus* and *P. citrinopileatus* were 75.6-92.6% [24]. It seems that the chelating ability of *L. edodes* on ferrous ions 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 in food system, [25], the high ferrous-ion chelating abilities of the various extracts from the fruiting bodies of *L. edodes* would be beneficial.

Antioxidant Activity on β-carotene-linoleic Acid: The linoleic acid free radical attacks the highly unsaturated β-carotene models. The presence of different antioxidants in the mushroom can hinder the extent of β-carotene bleaching by neutralizing the linoleate free radical and other free radicals from the system. Using the β-carotene-linoleic acid method, the acetonic, methanolic and hot water *L. edodes* extracts showed different patterns of antioxidant activities. The methanolic extract showed the strongest linoleic acid inhibition capacity (97.52%) at 8 mg/ml, which is higher than the synthetic antioxidant, BHT (95.21%) and TOC (96.02%), respectively at 0.5 mg/ml (Table 1). Barros *et al.* [26] reported that the 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 *L. edodes* fruiting bodies was more effective than the above mentioned mushroom.

Reducing Power: Reducing power of a compound may be serving as a significant indication of its potential antioxidant activity. The reducing power of *L. edodes* 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 hot water extract a value of 0.96 and the lowest reducing power inhibition (0.65) was exhibited by the methanolic extract. The reducing power values of BHT and TOC at 1.0 mg/ml were 3.21and 2.16, respectively (Table 2).

With regard to hot water extracts, the reducing power of *Hypsizygus marmoreus* was 0.99 at 5 mg/ml whereas, *Agricus bisporus*, *Pleurotus eryngii*, *Pleurotus ferulae* and *Pleurotus ostreatus* showed reducing powers of 0.76, 0.75, 0.70 and 0.61 at 20 mg/ml, respectively [17]. It can be seen that the reducing power of *L. edodes* was lower than that of *H. marmoreus* and higher than those of *A. bisporus*, *P. eryngii*, *P. ferulae* and *P. ostreatus*. 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 [20, 26].

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

	Sample concentration (mg/ml)			
Solvent and control	0.5	2.0	8.0	20.0
Acetone	65.89±0.11	89.94±0.09	92.78±0.32	95.63±0.41
Methanol	69.77±0.11	92.52±0.19	93.45±0.55	97.52±0.71
Hot water	55.56±0.45	89.68±0.94	93.04±0.93	96.52±0.15
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 *Lentinus edodes*

	Sample concentration (mg/ml)			
Solvent and control	1.0	2.0	4.0	8.0
Acetone	0.206±0.02	0.271±0.09	0.421±0.12	0.670±0.24
Methanol	0.245±0.02	0.311±0.07	0.437±0.09	0.650±0.14
Hot water	0.169±0.04	0.233±0.09	0.452±0.10	0.960±0.26
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.

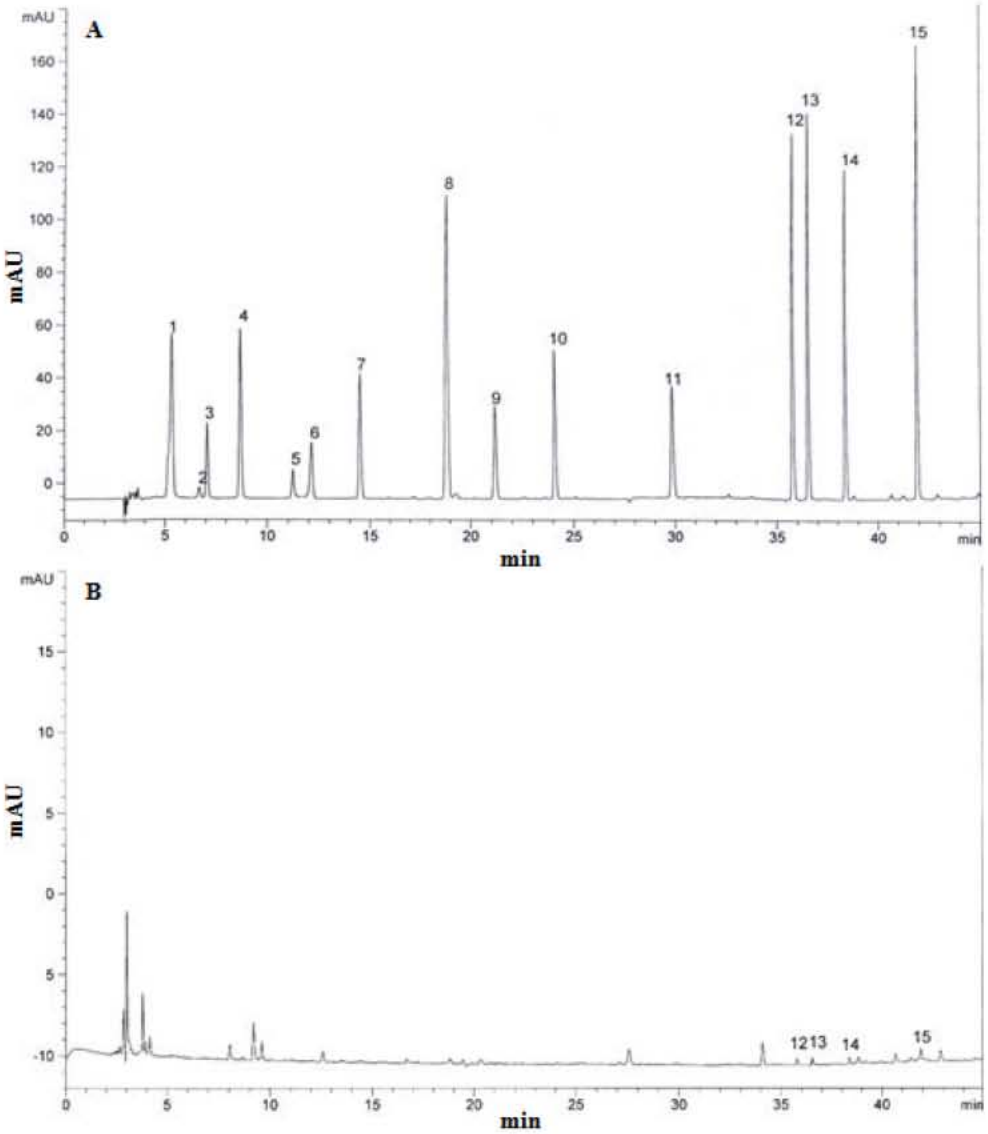

Fig. 3: High performance liquid chromatography of phenolic compounds. A, Standard mixture of 15 phenolic compounds; B, *Lentinus edodes* extract. 1, gallic acid; 2, pyrogallol; 3, homogentisic acid; 4, protocatechuic acid; 5, (+) catechin; 6, chlorogenic acid; 7, caffeic acid; 8, vanillin; 9, ferulic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

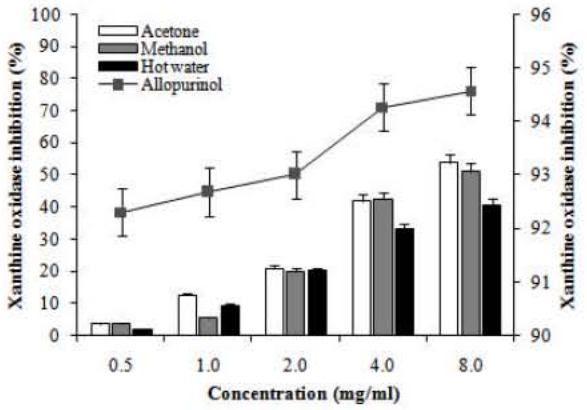

Fig. 4: Xanthine oxidase inhibition activity of various extracts from the fruting bodies of *Lentinus edodes*. Values expressed as means ± SE (n = 3).

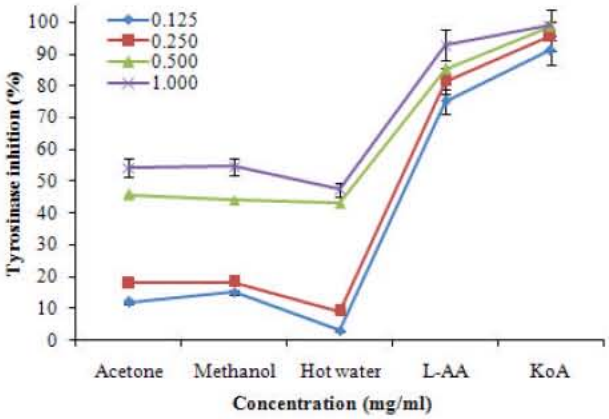

Fig. 5: Tyrosinase inhibition activity of various extracts from the fruiting bodies of *Lentinus edodes*. Values expressed as means ± SE (n = 3). L-AA, L-ascorbic acid; KoA, kojic acid.

Analysis of Phenolic Compound: Four phenolic compounds, naringenin, hesperetin, formononetin and biochanin-A were detected from the fruiting bodies of *L. edodes* in acetonitrile and 0.1N hydrochloric acid (5:1) solvent extract (Fig. 3). The highest phenolic compound concentration was recorded in biochanin-A (15 µg/g) and followed by formononetin (14 µg/g), hesperetin and naringenin (7 µg/g). Edible mushroom species also contained different types of phenolic compounds in varying numbers ranging from 3 to 15 [27]. 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 [28-30]. 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 [31, 32].

Xanthine Oxidase Inhibitory Activity: XO inhibitory activities of various extracts of *L. edodes* increased with increasing concentration. At 0.5-8.0 mg/ ml, the xanthine oxidase inhibition of the acetonic, methanolic and hot water extracts ranged from 3.75-53.94, 3.77-51.21 and 1.95-40.70%, respectively. However, at the same concentrations, allopurinol showed the excellent xanthine oxidase inhibitory activity of 92.31-94.58% (Fig. 4). The results indicated that the acetonic and methanolic extracts showed good, while hot water extract showed poor activities at the concentration tested. However, at higher doses of *L. edodes* extraction, xanthine oxidase would be significantly inhibited. Flavonoids are a group of polyphenolic compounds, which have been reported to possess xanthine oxidase inhibitory activity [33]. 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 *L. edodes* increased with increasing concentration. At 0.125-1.0 mg/ml, the tyrosinase inhibition of the acetonic, methanolic and hot water extracts ranged from 11.94-54.22, 15.12-54.61 and 3.09-47.32%, respectively (Fig. 5). Results indicate that the acetonic and methanolic extracts showed good, while hot water extract showed poor 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%, respectively. 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 [10]. Phenolic acid and flavonoid compounds proved to be effective inhibitors of tyrosinase activity, as reported by many authors [34, 35]. The antioxidant activity may also be one of the important mechanisms for tyrosinase inhibitory

activity. Therefore, high level of phenols and good antioxidant and antityrosinase activities indicated that the *L. edodes* fruiting bodies could be used as a natural food source of antioxidants.

ACKNOWLEDGEMENTS

This research was supported by a research grant from University of Incheon in 2009.

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