African Journal of Biotechnology Vol. 10(11), pp. 2978-2986, 11 April, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2660
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Evaluation of the antioxidant and antityrosinase activities of three extracts from *Pleurotus nebrodensis* fruiting bodies

Nuhu Alam, Ki Nam Yoon and Tae Soo Lee*

Division of Life Sciences, University of Incheon, Incheon 406-840, Korea.

Accepted 16 February, 2011

Pleurotus nebrodensis **has been widely used for nutritional and medicinal purposes. The aim of the present study was to evaluate the antioxidant activities and tyrosinase inhibitory effects on the fruiting bodies of *P. nebrodensis* extracted with acetone, methanol and hot water. The antioxidant activities were performed on β-carotene-linoleic acid, reducing power, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferrous ions chelating abilities and xanthine oxidase inhibitory activity. In addition to this, phenolic compounds were also detected. The acetonic and methanolic extracts of *P. nebrodensis* showed the strongest β-carotene-linoleic acid inhibition as compare to hot water extract. At 8 mg/ml, the acetonic extract showed a high reducing power of 1.86. The scavenging activity on DPPH radicals, the acetonic and methanolic extracts were effective than that of hot water extract. At lower concentrations, chelating effect of tested mushroom was significantly effective as compare to positive control. Gallic acid, protocatechuic acid, chlorogenic acid, ferulic acid, naringenin, hesperetin, formononetin and biochanin-A were detected from acetonitrile and hydrochloric acid solvent extract. The xanthine oxidase and tyrosinase inhibitory activities of the acetonic, methanolic and hot water extracts increased with increase of concentration. This study suggests that fruiting bodies of *P. nebrodensis* can potentially be used as a readily accessible source of natural antioxidants.**

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

INTRODUCTION

Pleurotus nebrodensis is known as the Bailinggu oyster and white sanctity mushroom. It is one of the popular edible mushrooms in China. Recently, this mushroom was successfully cultivated and commercially available in Korea. The mushroom is cultivated mainly on cotton seed hulls, sawdust or maize cobs (Alam et al., 2009). *P. nebrodensis* is abundant in nutrition including sub-oleic acid, non-saturate fatty acids and many micro-elements such as calcium, zinc and manganese. It is a good source of dietary fiber and other valuable nutrients (Alam et al., 2008). This mushroom also contain a number of of biologically active compounds with therapeutic activities such as modulation of the immune system, inhibition of tumor growth and inflame-mation, hypoglycemic and antithrombotic activities, decreasing blood lipid concentrations, prevention of high blood pressure and atherosclerosis (Choi et al., 2005; Wang and Ng, 2004).

Mushrooms are rich sources of antioxidants such as vitamin A, C, E, carotenoids, polyphenolic compounds and flavonoids (Diplock et al., 1998), which prevent free radical damage and reducing risk of chronic diseases. Thus, the consumption of dietary antioxidants from these sources is beneficial in preventing cardiovascular diseases, especially atherosclerosis (Hu, 2000). Mush-rooms contain various polyphenolic compounds recog-nized as excellent antioxidants due to their ability to scavenge free radicals by single-electron transfer (Hirano et al., 2001). Some common edible mushrooms which are widely consumed in Asia, are currently found to possess antioxidant activity, which is well correlated with

*Corresponding author. E-mail: tslee@incheon.ac.kr. Tel: +82-32-835-8242. Fax: +82-32-835-0763.

Abbreviations: BHT, Butylated hydroxytoluene; **DMSO,** dimethyl sulfoxide; **DPPH,** 1,1-diphenyl-2-picrylhydrazyl; **L-DOPA,** L-phenylalanine; **XO,** xanthine oxidase.

their total phenolic content (Cheung and Cheung, 2005; Lo and Cheung, 2005; Mau et al., 2004). Xanthine oxidase inhibitors have been found in a wide variety of plants and mushrooms used in traditional herbal medicines for the treatment of gout and rheumatism. Flavonoids and polyphenols have been reported to be potent xanthine oxidase inhibitors (Theoduloz et al., 1991).

Tyrosinase, a multifunctional copper-containing enzyme, is widely distributed in fungi, plants and animals (Huang et al., 2006). Tyrosinase is involved in melanin production which might be responsible for some of the histopathological features exclusive to malignant cancer. Therefore, tyrosinase inhibitors may be clinically helpful in dealing with skin cancer (Momtaz et al., 2008). Skin whitening is believed to be partly due to the inhibition of tyrosinase activity (Burdock et al., 2001; Hakozaki et al., 2002; Sugimoto et al., 2004). Irrespective of the medicinal importance or therapeutic potential of *P. nebrodensis*, there have not been many studies on the antioxidant and antityrosinase properties. The objective of this study was to elucidate the antioxidant and antityrosinase activities of acetonic, methanolic and hot water extracts from the fruiting bodies of *P. nebrodensis*. Antioxidant activities were assayed including β-carotene-linoleic acid, reducing power, scavenging effects on radicals, chelating effects on ferrous ions and xanthine oxidase. The contents of potential phenolic and flavonoid components were also determined.

MATERIALS AND METHODS

Chemicals and reagents

β-carotene, linoleic acid, chloroform, polyoxyethylene sorbitan monopalmitate (Tween 40), 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. nebrodensis* 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 accession number IUM-4658 was acquired. Fruiting bodies were dried with hot air at 40°C for 48 h and finely pulverized. Five grams of each powdered sample 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 layers 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 earlier. 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. nebrodensis* were 24.84, 24.44 and 19.04% (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 and 2.5 ml of this reaction mixture was dispensed to test tubes. 0.5 ml of various concentrations (0.5 to 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 the following equation:

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

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:

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

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 to 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 ferricyanide, 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, Munich, 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 milliliters of various concentrations (0.125 to 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 radicals 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 to 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:

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: 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 at 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 chromategraphy 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 to 2 min, 90% A; 2 to 27 min, 70% A; 27 to 50 min, 10% A; 50 to 51 min, 0% A; 51 to 60 min, 0% A; 60 to 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. nebrodensis* 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 to 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 1 N 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 to 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 (Alam et al., 2010). Xanthine oxidase inhibitory activity was expressed as the percentage inhibition of XO in the earlier assay system calculated as:

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 acetonic, methanolic and hot water extracts from the fruiting bodies of *P. nebrodensis* gradually increased with increasing concentration. At 0.5 to 20.0 mg/ml concentration, antioxidant activities of acetonic, methanolic and

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

Solvent and control	Sample concentration (mg/ml)			
	0.5	2.0	8.0	20.0
Acetone	37.47±0.11	78.05±0.72	81.92±0.54	92.62±0.04
Methanol	32.56±0.05	72.11±0.14	88.99±0.16	92.41±0.08
Hot water	37.74±0.14	70.04±0.12	85.80±0.14	91.42±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 *P. nebrodensis.*

Solvent and control	Sample concentration (mg/ml)			
	1.0	2.0	4.0	8.0
Acetone	0.665±0.05	1.220±0.06	1.736±0.03	1.856±0.07
Methanol	0.777±0.22	1.367±0.21	1.721±0.22	1.849±0.19
Hot water	0.447±0.18	0.637±0.17	0.998±0.16	1.817±0.28
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.

hot water extracts of *P. nebrodensis* ranged from 37.47 to 92.62, 32.56 to 92.41 and 37.74 to 91.42%, respectively (Table 1). Results indicate that antioxidant activities of *P. nebrodensis* was lower than the synthetic antioxidant, BHT and TOC at 0.5 mg/ml concentration. However, the acetonic and methanolic extracts showed good activities, while hot water extract showed moderate activities at the concentration tested. The antioxidant activity of carotenoids is based on the radical adducts of carotenoid with free radicles from lenoleic 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 system (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, while antioxidant activity of TBHQ (tertiary butylhydroquinone) standard reached 82.2% at 2 mg/ml. It seems that the antioxidant activity of *P. nebrodensis* fruiting bodies was more effective than those mentioned earlier.

Reducing power

The reducing power of *P. nebrodensis* in the acetonic, methanolic and hot water extracts increased with increase in concentration. At 8 mg/ml, the strongest reducing power inhibition was determined in acetonic extract, a value of 1.86 and the lowest reducing power inhibition (1.82) was exhibited by the hot water extract. However, synthetic antioxidant BHT and TOC exhibited high reducing powers of 3.21 and 2.16, respectively at 1.0 mg/ml (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 (Lee et al., 2007a). Our results indicate that the reducing power of *P. nebrodensis* was higher and significantly effective than those of mentioned mushrooms. 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;

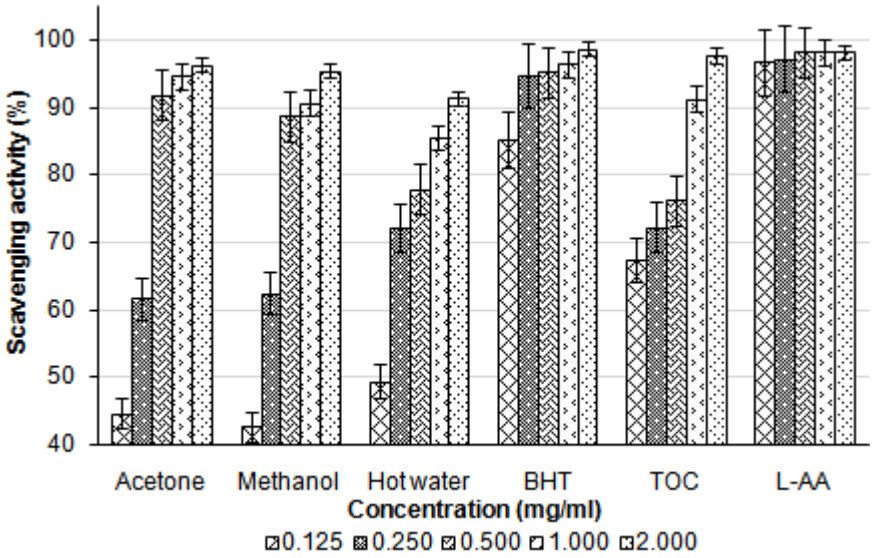

Figure 1. Scavenging activity of various extracts from the fruiting bodies of *P. nebrodensis* against 1,1-diphenyl-2-picrylhydrazyl. Values are expressed as means ± SD (n = 3). BHT, butylated hydroxytoluene; TOC, α-tocopherol; L-AA, L-ascorbic acid.

Barros et al, 2007).

Scavenging effect on DPPH

Scavenging effects of the acetonic, methanolic and hot water extracts from the fruiting bodies of *P. nebrodensis* on DPPH radicals increased with increase in concentration. At 0.125 to 2.0 mg/ml, the scavenging activities of acetonic, methanolic and hot water extracts of *P. nebrodensis* on DPPH radical ranged from 44.54 to 96.34, 42.57 to 95.46 and 49.34 to 91.38%, respectively (Figure 1). However, at 0.125 to 2.0 mg/ml, BHT, TOC and L-ascorbic acid showed excellent scavenging activities of 85.25 to 98.74, 67.37 to 97.78 and 96.74 to 98.23%, respectively.

With regard to ethanolic extracts of *H. marmoreus*, *A. bisporus* and *Pleurotus citrinopileatus* fruiting bodies scavenged DPPH radicals by 46.6 to 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 to 52.3, 37.6 to 48.3 and 19.6 to 23.3%, respectively. It seems that the scavenging activity of *P. nebrodensis* fruiting bodies was more effective than those mentioned earlier. 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 (Frankel, 1991; 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 acetone extract might be due to more hydrogen-donating components contained in the extracts.

Chelating effects on ferrous ions

In the present study, the chelating activity of acetonic, methanolic and hot water extracts at five different concentrations (0.063, 0.125, 0.250, 0.500 and 1.000 mg/ml) from the fruiting bodies of *P. nebrodensis* toward ferrous ions was investigated. BHT and TOC were used as reference standards on ferrous ions. As can be seen from the Figure 2, chelating capacity of the extracts increased with increasing concentration. The strongest chelating effect (82.92%) was obtained from the methanolic extracts at 1.0 mg/ml. At this concentration, the lowest chelating effect was exhibited by hot water extract (75.60%).

With regard to hot water extracts at 20 mg/ml, *Ganoderma tsugae* and *Agrocybe cylindracea* chelated ferrous ions by 42.6 and 45.8%, respectively (Mau et al., 2005; Tsai et al., 2006). At 1 to 5 mg/ml, chelating abilities of *H. marmoreus* and *P. citrinopileatus* were 75.6 to 92.6% (Lee et al, 2007b). It seems that chelating ability of *P. nebrodensis* on ferrous ions was similar to that of *H. marmoreus* and *P. citrinopileatus*, and more effective than those of *G. tsugae* and *A. cylindracea*. Chelating

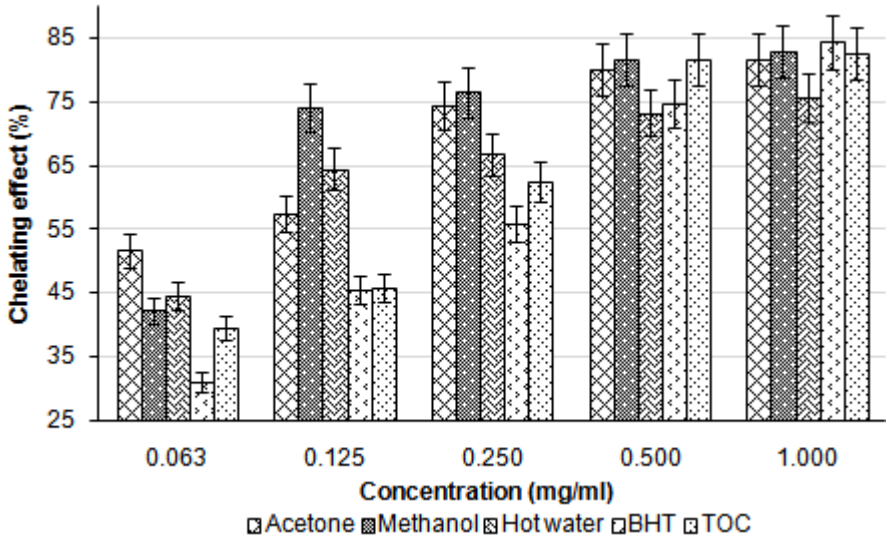

Figure 2. Chelating effect of various extracts from the fruiting bodies of *Pl. nebrodensis*. Values are expressed as means ± SD (n = 3). BHT, butylated hydroxytoluene; TOC, α-tocopherol.

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 (Yamaguchi et al., 1988), the high ferrous-ion chelating abilities of the various extracts from the fruiting bodies of *P. nebrodensis* would be beneficial.

Analysis of phenolic compound

Gallic acid, pyrogallol, homogentisic acid, protocatechuic acid, (+) catechin, chlorogenic acid, caffeic acid, vanillin, ferulic acid, naringin, resveratrol, naringenin, hesperetin, formononetin and biochanin-A were used as standard for the detection of phenolic compounds from the extract of *P. nebrodensis*. Eight phenolic compounds: gallic acid, protocatechuic acid, chlorogenic acid, ferulic acid, naringenin, hesperetin, formononetin and biochanin-A were detected from the extract (Figure 3). The concentration of total phenolic compound was 298 µg/g. The highest and lowest concentration of phenolic compounds were recorded in protocatechuic acid (105 µg/g) and hesperetin and biochanin-A (12 µg/g), respectively. These findings are comparable to the previous studies on edible mushroom (Kim et al., 2008) in which total concentration of phenolic compounds was 174 µg/g. Mushroom species also contained different types of phenolic compound in varying numbers ranging from 3 to 15, and gallic acid was reported to be the most common phenolic compound in 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; Zhao et al., 2006). Mushroom extracts have high levels of phenolic compounds, which are composed of one or more aromatic rings which bear one or more hydroxyl groups, and can exhibit extensive free radical-scavenging activities as hydrogen donors or electron-donating agents and metal ion-chelating properties. The greater number 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. nebrodensis* increased with increasing concentration. At 0.5 to 8.0 mg/ml, the xanthine oxidase inhibition of the acetonic, methanolic and hot water extracts ranged from 2.39 to 61.25, 6.45 to 61.22 and 5.46 to 54.78%, respectively. However, at the same concentrations, allopurinol showed excellent xanthine oxidase inhibitory activity of 92.31 to 94.58% (Figure 4). Results indicate that acetonic and methanolic extracts showed good activities, while hot water extract showed moderate activities at the concentration tested. However, at higher doses of the extraction, xanthine oxidase was 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 could have contributed towards xanthine oxidase inhibition.

Tyrosinase inhibition

Tyrosinase inhibitory activities of the acetonic, methanolic

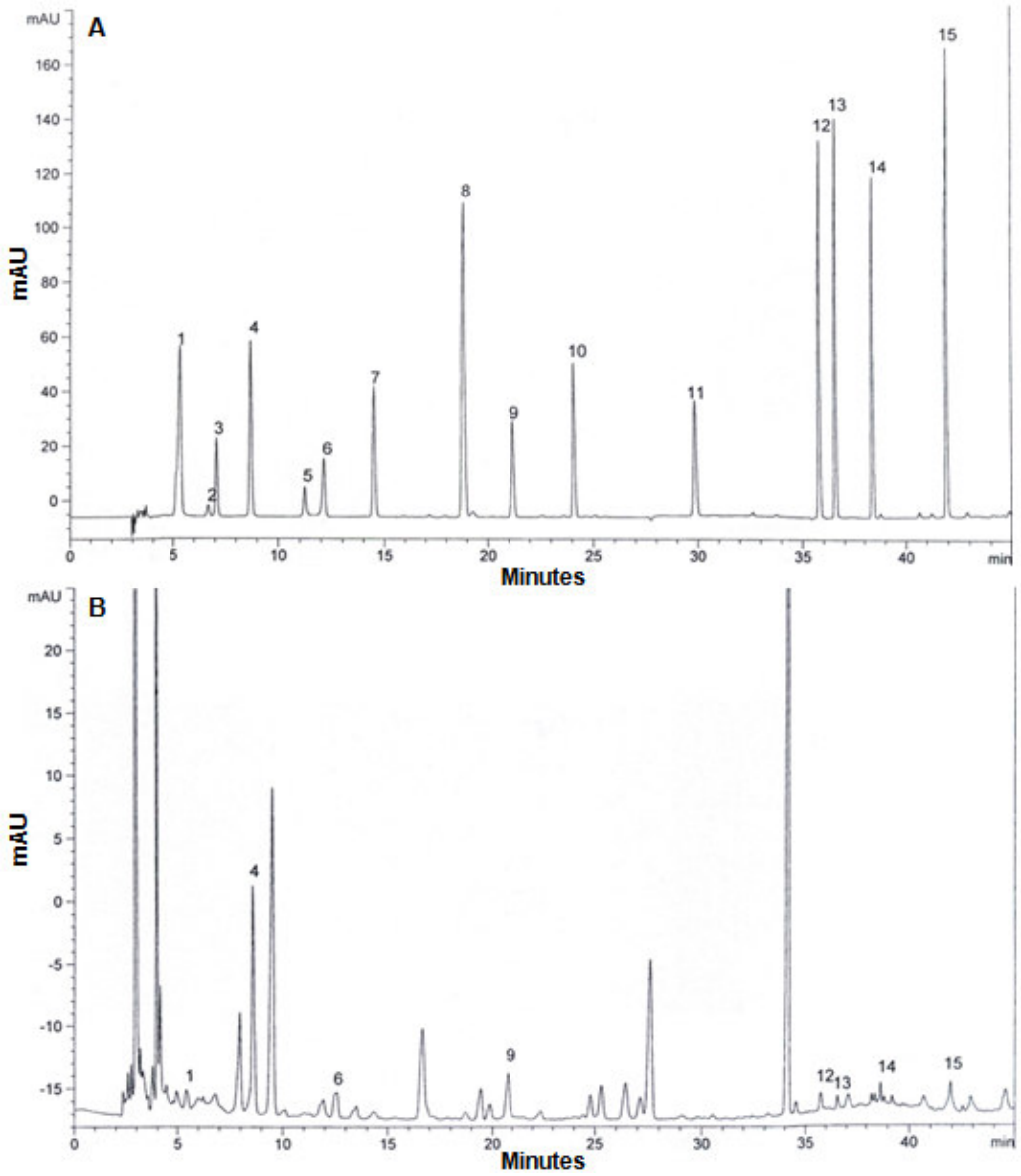

Figure 3. High performance liquid chromatography of phenolic compounds. A, Standard mixture of 15 phenolic compounds; B, *P. nebrodensis* extract. 1, Gallic acid; 2, pyrogallol; 3, homogentisic acid; 4, protocatechuic acid; 5, (+) catechin; 6, chlorogenic acid; 7, caffeic acid; 8, vanillin; 9, ferulic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

and hot water extracts from the fruiting bodies of *P. nebrodensis* increased with increasing concentration. At 0.125 to 1.0 mg/ml, the tyrosinase inhibition of the acetonic, methanolic and hot water extracts ranged from 12.57 to 55.76, 11.54 to 51.54 and 6.52 to 50.96%, respectively (Figure 5). Results indicate that acetonic extract showed good activities, while methanolic and hot water extracts showed moderate activities at the concentration tested. However, at 0.125 to 1.0 mg/ml, L-ascorbic acid and kojic acid showed excellent tyrosinase inhibitory activities of 75.12 to 92.74 and 91.23 to99.00%. The inhibition of tyrosinase activity might depend on the hydroxyl groups of the phenolic compounds of the mushroom extracts that could form a hydrogen bond to the 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 to steric hindrance or changed conformation (Baek et al., 2008). Gallic acid proved to be effective inhibitors of tyrosinase activity as reported (Kubo et al., 2003; Momtaz et al., 2008). The antioxidant activity may also be one of the important mechanisms for tyrosinase inhibitory activity.

Conclusion

This study showed that *P. nebrodensis* had higher chelating effects on ferrous-ions when compared to that of

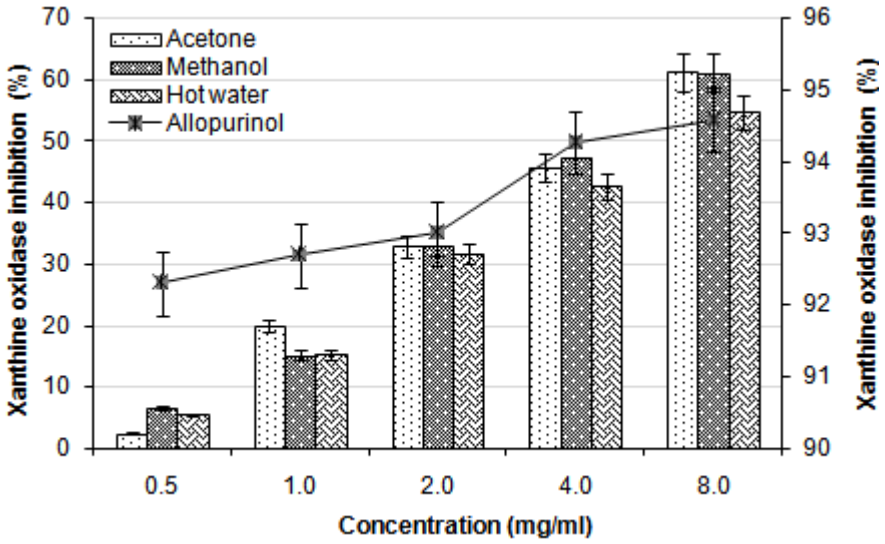

Figure 4. Xanthine oxidase inhibition activity of various extracts from the fruiting bodies of P. nebrodensis. Values are expressed as means ± SD (n = 3).

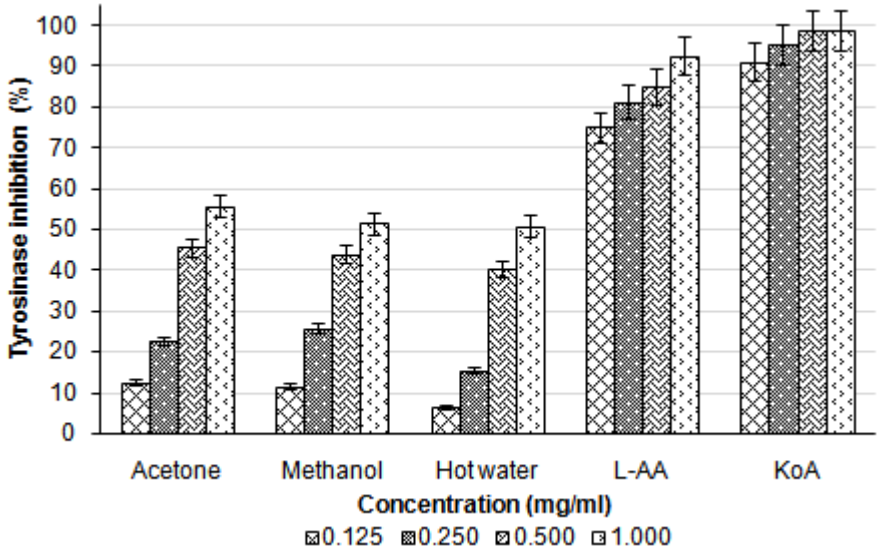

Figure 5. Tyrosinase inhibition activity of various extracts from the fruiting bodies of *P. nebrodensis*. Values are expressed as means ± SD (n = 3). L-AA, L-ascorbic acid; KoA, kojic acid.

BHT and TOC. Eight phenolic compounds were detected from the fruiting bodies of *P. nebrodensis*. The high phenolic content exhibited good antioxidant and antityrosinase activities. On the basis of the results, it is suggested that *P. nebrodensis* can be used as a rich source of natural antioxidants.

ACKNOWLEDGEMENT

This study was supported by a research grant from the Korea National Research Resource Center Program (2011-0000525) through National Research Foundation of Korea (NRF) for Culture Collection and DNA Bank of Mushroom (CCDBM), University of Incheon.

REFERENCES

Alam N, Amin R, Khan A, Ara I, Shim MJ, Lee MW, Lee TS (2008). Nutritional analysis of cultivated mushrooms in Bangladesh: *Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Pleurotus florida* and *Calocybe indica*. Mycobiology, 36: 228-232.

Alam N, Shim MJ, Lee MW, Shin PG, Yoo YB, Lee TS (2009). Phylogenetic relationship in different commercial strains of *Pleurotus*

nebrodensis based on ITS sequence and RAPD. Mycobiology, 37: 183-188.
Alam N, Yoon KN, Lee KR, Shin PG, Cheong JC, Yoo YB, Shim MJ, Lee MW, Lee UY, Lee TS (2010). Antioxidant activities and tyrosinase inhibitory effects of different extracts from *Pleurotus ostreatus* fruiting bodies. Mycobiology, 38: 295-301.
Baek HS, Rho HS, Yoo JW, Ahn SM, Lee JY, Lee J, Kim MK, Kim DH, Chang IS (2008). The inhibitory effect of new hydroxamic acid derivatives on melanogenesis. Bull. Korean Chem. Soc. 29: 43-46.
Barros L, Ferreira MJ, Queiros B, Ferreira IC, Bapista P (2007). Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chem. 103: 413-419.
Burdock GA, Soni MG, Carabin IG (2001). Evaluation of health aspects of kojic acid in food. Regul. Toxicol. Pharmacol. 33: 80-101.
Cheung LM, Cheung PCK (2005). Mushroom extracts with antioxidant activity against lipid peroxidation. Food Chem. 89: 403-409.
Choi D, Kang SH, Song YH, Kwun KH, Jeong KJ, Cha WS (2005). Exopolysaccharide production in liquid culture of *Pleurotus ferulae*. J. Microbiol. Biotechnol. 146: 209-221.
Costantino L, Albasini A, Rastelli G, Benvenuti S (1992). Activity of polyphenolic crude extracts as scavengers of superoxide radicals and inhibitors of xanthine oxidase. Planta Med. 58: 342-344.
Cuendet M, Hostettmann K, Potterat O, Dyatmiko W (1997). Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. Helvetica. Chimica. Acta. 80: 1144-1152.
Dapkevicius A, Venskutonis R, van Beek TA, Linssen JP (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 TC, Madeira VM, Almeida LM (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: 161-169.
Diplock AT, Charleux JL, Crozier-willi G, Kok FJ, Rice-Evan C, Roberfroid M (1998). Functional food science and defence against reactive oxidative species. Br. J. Nutri. 80: 77-112.
Duan X, Wu G, Jiang Y (2007). Evaluation of antioxidant properties of lichi fruit phenolics in relation to pericarp browning prevention. Molecules, 12: 759-771.
Frankel EN (1991). Recent advances in lipid oxidation. J. Sci. Food Agric. 54: 495-511.
Gulcin I, Buyukokuroglu ME, Oktay M, Kufrevioglu OI (2003). Antioxidant and analgesic activities of turpentine of *Pinus nigra* Arn. subsp. *pallsiana* (Lamb.). Holmboe. J. Ethnopharmacol. 86: 51-58.
Hakozaki T, Minwalla L, Zhuang J, Chhoa M, Matsubara A, Miyamoto K, Greatens A, Hillebrand GG, Bissett DL, Boissy RE (2002). The effect of niacinamide on reducing cutaneous pigmentation and suppression of melanosome transfer. Br. J. Dermatol. 47: 20-31.
Herraiz T, Galisteo J, Chamorro C (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.
Hirano R, Sasamoto W, Matsumoto A, Itakura H, Igarashi O, Kondo K (2001). Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. J. Nutr. Sci. Vitaminol. 47: 357-362.
Hu FB (2000). Dietary pattern analysis: a new direction in nutritional epidemiology. Curr. Opin. Lipidol. 13: 3-9.
Huang XH, Chen QX, Wang Q, Song KK, Wang J, Sha L, Guan X (2006). Inhibition of the activity of mushroom tyrosinase by alkylbenzoic acids. Food Chem. 94: 1-6.
Jayaprakasha GK, Singh RP, Sakariah KK (2001). Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. Food Chem. 73: 285-290.
Kim EH, Kim SH, Chung JI, Chi HY, Kim JA, Chung IM (2006). Analysis phenolic compounds and isoflavones in soybean seeds (*Glycine max* (L.) Merill) and sprouts grown under different conditions. Eur. Food Res. Technol. 222: 201-208.
Kim MY, Seguin P, Ahn JK, Kim JJ, Chun SC, Kim EH, Seo SH, Kang EY, Kim SL, Park YJ, Ro HM, Chung IM (2008). Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Korea. J. Agric. Food Chem. 56: 7265-7270.
Kubo I, Chen QX, Nihei KI (2003). Molecular design of antibrowning agents: antioxidative tyrosinase inhibitors. Food Chem. 81: 241-247.
Lee YL, Huang GW, Liang ZC, Mau JL (2007a). Antioxidant properties of three extracts from *Pleurotus citrinopileatus*. LWT-Food Sci. Technol. 40: 823-833.
Lee YL, Yen MT, Mau JL (2007b). Antioxidant properties of various extracts from *Hypsizigus marmoreus*. Food Chem. 104: 1-9.
Lo KM, Cheung PCK (2005). Antioxidant activity of extracts from the fruiting bodies of *Agrocybe aegerita* var. *alba*. Food Chem. 89: 533-539.
Masuda T, Yamashita D, Takeda Y, Yonemori S (2005). Screening for tyrosinase inhibitors among extracts of seashore plants and identification of potent inhibitors from *Garcinia subelliptica*. Biosci. Biotechnol. Biochem. 69: 197-201.
Mau JL, Chang CN, Huang SJ, Chen CC (2004). Antioxidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculenta*, and *Termitomyces albuminosus* mycelia. Food Chem. 87: 111-118.
Mau JL, Tsai SY, Tseng YH, Huang SJ (2005). Antioxidant properties of hot water extracts from *Ganoderma tsugae* Murrill. LWT-Food Sci. Technol. 38: 589-597.
Momtaz S, Mapunya BM, Houghton PJ, Edgerly C, Hussein A, Naidoo S, Lall N (2008). Tyrosinase inhibition by extracts and constituents of *Sideroxylon inerme* L. stem bark, used in South Africa for skin lightening. J. Ethnopharmacol. 119: 507-512.
Owen PL, Johns T (1999). Xanthine oxidase inhibitory activity of North-Eastern North American plant remedies used for gout. J. Ethnopharmacol. 64: 149-160.
Pan Y, Wang K, Huang S, Wang H, Mu X, He C, Ji X, Zhang J, Huang F (2008). Antioxidant activity of microwave-assisted extract of longan (*Dimocarpus longan* Lour.) peel. Food Chem. 106:1264-1270.
Prasad KN, Divakar S, Shivamurthy GR, Aradhya SM (2005). Isolation of a free radical scavenging antioxidant from water spinach (*Ipomoea aquatica* Forsk). J. Sci. Food Agric. 85: 1461-1468.
Rangkadilok N, Sitthimonchai S, Worasuttayangkurn L, Mahidol C, Ruchirawat M, Satayavivad J (2007). Evaluation of free radical scavenging and antityrosinase activities of standardized longan fruit extract. Food Chem. Toxicol. 45: 328-336.
Shahidi F, Wanasundara PK (1992). Phenolic antioxidants. Crit. Rev. Food Sci. Nutr. 32: 67-103.
Shimada K, Fujikawa K, Yahara K, Nakamura T (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem. 40: 945-948.
Sugimoto K, Nishimura T, Nomura K, Sugimoto K, Kuriki T (2004). Inhibitory effects of α-arbutin on melanin synthesis in cultured human melanoma cells and a three-dimensional human skin model. Biol. Pharm. Bull. 27: 510-514.
Theoduloz C, Pacheco P, Schmeda-Hirschmann G (1991). Xanthine oxidase inhibitory activity of Chilean Myrtaceae. J. Ethnopharmacol. 33: 253-255.
Tsai SY, Huang SJ, Mau JL (2006). Antioxidant properties of hot water extracts from *Agrocybe cylindracea*. Food Chem. 98: 670-677.
Wang H, Ng TB (2004). Eryngin, a novel antifungal peptide from fruiting bodies of the edible mushroom *Pleurotus eryngii*. Peptides, 25: 1-5.
Yamaguchi R, Tatsumi Y, Asano M, Kato K, Ueno Y (1988). Effect of metal salts and fructose on the autoxidation of methyl linoleate in emulsions. Agric. Biol. Chem. 52: 849-850.
Zhao M, Yang B, Wang J, Li B, Jiang Y (2006). Identification of the major flavonoids from pericarp tissues of lychee fruit in relation to their antioxidant activities. Food Chem. 98: 539-544.