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Bioactive Compounds in Three Edible Lentinus Mushrooms

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Abstract

The generation of free radicals is a major issue in the development of carcinogenesis-induced mutations and tumor promotion. Bioactive compounds which are able to scavenge free radicals and provide protections against cancers caused by them are called antioxidants. We aimed to show that bioactive compounds from mushrooms will be valuable for health promotion. Catechin is a major group of phenolic compounds found in 3 species of mushroom, *Lentinus squarrosulus*, *Lentinus polychrous* and *Lentinus edodes*, especially in *L. squarrosulus* by LC-MS. The percentage of inhibition of free radical scavenging activity by DPPH assay was higher than 70 % in ethanolic extract of *L. squarrosulus* and *L. polychrous*, while in *L. edodes* it was lower as 30 %. Using FRAP to investigate the micromolar trolox equivalent of *L. squarrosulus* also showed it to be higher than the other 2 species. The highest amount of total phenolic compound was displayed in *L. squarrosulus* extract**,** which corresponds with the DPPH and FRAP assays. An anticancer protein, polysaccharide peptide (PSP), was also detected at a level of approximately 70 kDa via western immunoblotting. The amounts of PSP from protein extracts of *L. squarrosulus*, *L. polychrous* and *L. edodes* were 9.87 ± 0.03, 0.41 ± 0.03 and 0.17 ± 0.04 mg/g protein, respectively, by using ELISA method. The cell viability of human colorectal adenocarcinoma, HT-29, diminished significantly in the treatment group with protein extract from *L. squarrosulus.* This *L. squarrosulus* extract may be an effective natural product for clinical trials in patients, because these edible mushrooms are nontoxic and are also a consumable food for human health.

Keywords: Anticancer, mushroom, Lentinus mushroom, antioxidant, bioactive proteins

Introduction

Oxidative stress is one of the main causes of disease and shorter lifespan. Oxidative stress, or reactive oxygen species (ROS), is generated during normal biochemical processes in the body, inflammation, smoking, and after ingestion of certain drugs and pollutants. A fundamental relationship between the presence of oxidants and the generation of lipid hydroperoxides may be an important etiological mechanism of many diseases, such as several kinds of cancers, diabetes, cardiovascular disease, and cirrhosis [1]. Especially, about 7 million people die from cancer-related cases per year, and it is estimated that there will be more than 16 million new cancer cases every year by 2020 [2,3]. In recent years, the use of some synthetic antioxidants has been restricted because of their possible toxic and carcinogenic effects [4]. Therefore, there is growing interest in natural and safer antioxidants from natural sources. Mushrooms have been known as a gourmet cuisine and functional food across the world because of their unique taste and soluble flavor. Many mushrooms are also known to be biologically-active or nutraceutical compounds in Japan, China and Korea, and other Asian countries [5]. Many compounds with antioxidant or antitumor potential identified in mushrooms, thus far, include low molecular weight

(LMW, e.g. quinones, cerebrosides, isoflanones, catechols, amines, sesquiterpenes and selenium) and high molecular weight compounds (HMW, e.g. homo and heteroglycans, glycan, glycoproteins, glycopeptides, proteoglycans, proteins and RNA-protein complexes). Bioactive proteins isolated from more than 30 mushroom species have shown antitumor, antiviral, antimicrobial, antioxidant and immunomodulatory action, such as lectins, fungal immunomodulatory proteins (FIP), laccases, protease, ribonuclease, proteoglycans, PSP (polysaccharopeptide) and other proteins [6,7]. PSP extracted from turkey tail (known as *Coriolus versicolor* or Yun-zhi) has a molecular mass of approximately 100 kDa [8]. PSP contains α-1,4 and β-1,3 glucosidic linkages in their polysaccharide moieties. Of the *C. versicolor*-derived therapeutic extracts, polysaccharopeptides are commercially the best established. The polysaccharopeptide obtained from *C. versicolor*, known as *C. versicolor* polysaccharide, is a complicated protein-bound polysaccharide extracted from its mycelium, or fruiting body. PSP significantly improved the quality of life, provided substantial pain relief, and enhanced immune status in 70 - 97 % of patients with cancers of the stomach, esophagus, liver, lung, ovaries, and cervix [9]. Presently, PSP is considered as a potential candidate for drug development in the treatment and prevention of human cancer because of its immunological properties, as well as its ability to distinguish cancerous cells from normal cells. Besides bioactive proteins, mushrooms have become rich sources of natural antioxidant compounds, such as phenolic compounds, tocopherols, ascorbic acid, and carotenoids. There are many phenolic compounds found in mushrooms, such as benzoic acid, gallic acid, catechin, tannic acid, caffeic acid, and resveratrol [10]. Many phenolic compounds have been reported to process potent antioxidant activity and to have anticancer or anticarcinogenic/antimutagenic, antiatherosclerotic, antibacterial, antiviral, and anti-inflammatory activities, to a greater or lesser extent. The aim of this study is to quantitate PSP and phenolic compounds in local cultivated edible mushrooms and then verify the antioxidative activity *in vitro* and the anticancer properties in cell culture. *L. squarrosulus* was the outstanding mushroom species in terms of antioxidant and anticancer properties.

Materials and methods

Chemicals

All chemicals are from Sigma (Sigma Chemical Co., St. Louis, MO), unless otherwise noted.

Preparation of protein extract in mushrooms

Fruiting bodies of edible mushrooms, *Lentinus polychrous*, *Lentinus squarrosulus*, and *Lentinus edodes*, were collected from the Chiang Mai and Phitsanulok Provinces. Those fruiting bodies were dried at 50 °C, ground into powder and stored at −50 °C until used for protein preparation and the phenolic compounds assay. Approximately 10 g of the mushroom powder was prepared by homogenizing in hot water and 0.15 N NaCl. The homogenized samples were then precipitated with 40 - 80 % $(NH_4)_2SO_4$ saturation. Fractions extracted were resuspended in 50 mM Tris-HCl, pH 7.5, and dialyzed overnight against the same buffer. The resulting solution had an addition of protease inhibitor cocktail (AMRESCO LLC, OH 44139, USA) and was collected at −20 °C in order to quantitate protein concentration and PSP levels, including treat cell lines. The protein concentration of the extract was determined by Bradford assay [11] with BSA (Bovine serum albumin) as standard.

Western blot analysis

Twenty micrograms of protein extract from *L. squarrosulus* was boiled in Laemmli buffer [12] and resolved by 10 % SDS-PAGE. Then, proteins were transferred to a PVDF membrane and subjected to Western blotting using an anti-PSP antibody specific to commercial PSP (JHS Natural Products, OR 97402, USA) as a primary antibody at a dilution of 1:500. The blots were developed using a horseradish peroxidase-conjugated secondary antibody (Jackson Immuno Research Laboratories INC.) at a dilution of 1:5,000.

Development of ELISA to quantify PSP concentration in edible mushrooms

To quantify PSP in fruiting bodies, ELISA was performed following the procedure described previously in the quantitation of vitellin and vitellogenin in the ovaries and hemolymph of prawns [13]. Briefly, PSP and protein extracted from fruiting bodies were coated on 96 well plates at room temperature. An anti-PSP antibody specific to commercial PSP was used as a primary antibody at a dilution of 1:500. The enzyme reaction was carried out by *o*-phenylenediamine after an addition of anti-rabbit IgG-HRP (dilution 1:5,000). PSP concentrations in the samples were calculated on the basis of PSP concentrations according to a standard curve. The sensitivity of the assay for immunoreactive PSP was from 1 - 100 ng per assay.

Cell culture and cell viability assay

The Human colorectal adenocarcinoma, HT-29 cell line, was used in this study. Cells were routinely cultured in RPMI 1640 medium containing 2.05 mM L-glutamine, 100 U/ml penicillin, and 100 units/ml streptomycin. The cells were cultured as a monolayer in a humidified atmosphere containing 5 % CO_2 at 37 °C. The medium was supplemented with 10 % FCS and changed every 24 h. These HT-29 cells were plated at 5×10^3 cells/ well in a 96-well culture plate at for 24 h. Protein extracts from mushroom, commercial PSP, and PBS (vehicle, negative control) were added to a final volume of 100 µl per well. The plates were incubated at 37 °C and 5 % CO_2 for 72 h, and then cell viability of control and treatments were determined by MTT assay. Thirty microliters was added to each well and incubated at 37 °C for 4 h. The medium was removed and 100 µl Me_2SO was added into each well after the plate was shaken for 15 min. The absorbance of the samples was measured at 570 nm.

Quantitation of phenolic compounds

Ten grams of dried mushrooms were incubated in 100 ml of solvent (absolute ethanol and HCl acid media (1 %,v/v)) at room temperature on a shaker at 150 rpm for 24 h. and then centrifuged at 5,000 rpm for 10 min. The solution was filtered through a 0.45 µm membrane filter, and then the residue was re-extracted. An Agilent 1100 HPLC system equipped with a quaternary pump, refrigerated autosampler, and column heater, and with DAD, FLD and MS ion trap detectors was used. For phenolic analysis, a 150×4.6 mm Zorbax SB C18 column was used at a flow rate of 1 ml/min and a gradient elution of 0 - 5 min B 100 % constant, 5 - 10 min A 0 - 20 %, 10 - 20 min A 20 % constant, 20 - 60 min A 20 - 40 % (Buffer A: 10 mM formic acid pH 3.5 with NH_4OH; Buffer B: 100 % methanol with 5 mM ammonium formate). The column temperature was 40 °C and UV detection was at 270, 330, 350 and 370 nm. Compound identification was confirmed by injection of authentic standard. Compounds were quantitated by the external standard method using authentic standards, gallic, tannic, catechin, rutin, isoquercetin, eriodictyol, quercetin, hydroguinin, kaempferol, and apiginin.

Preparation of ethanolic mushroom extract

Ten grams of dried mushrooms were extracted by stirring with 100 ml of ethanol at 25 °C for 24 h and filtering through Whatman No.4 filter paper. The residue was then extracted with the addition of 100 ml of ethanol. The combined ethanolic extracts were then rotary evaporated at 40 °C to dryness. The dried extract was resuspended in ethanol and stored at 4 °C for further use in DPPH, FRAP and total phenolic compound assays.

Total phenolic compound

Total phenolic compounds (TPC) were determined using a modified version of the Folin-Ciocalteu method [14], and 0.1 ml of the extract was added to 1 ml deionized water and 1 ml of Folin-Ciocalteu phenol reagent (Merck-Schuchardt, Hohenbrun, Germany). The mixture was then allowed to stand for 5 min and 2 ml of sodium carbonate was added to the mixture. The resulting blue complex was then measured at 760 nm. Gallic was used as a standard phenolic compound. The amount of total phenolic compound in the ethanol extract of mushrooms was determined as a milligram (mg) of gallic acid equivalent (GAE) using a calibration curve of gallic acid graph.

DPPH assay

The DPPH (2,2-diphenylpicrylhydrazyl) assay [15] was used to determine the free radical scavenging activity of mushroom extracts. Each mushroom extract in ethanol was mixed with 1 ml of methanolic solution containing DPPH radicals, resulting in a final concentration of 0.2 mM DPPH. The mixture was shaken vigorously and left to stand for 30 min in dark conditions, and the absorbance was then measured at 517 nm.

FRAP assay

The FRAP (Ferric reducing-antioxidant power assay) was done according to Benzie and Strain [16] with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3$.$6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml $FeCl_3.6H_2O$ solution, and then warmed at 37 °C before using. Mushroom extracts were allowed to react with FRAP solution for 30 min in dark conditions. Readings of the colored product (ferrous tripyridyltriazine complex) were then taken at 593 nm absorbance. The standard curve was linear between 25 and 800 mM Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a water-soluble analog of vitamin E). Results were expressed in µM TE (Trolox equivalent antioxidant capacity) per gram of dried mushroom. Additional dilutions were needed if the FRAP value measured was over the linear range of the standard curve.

Results and discussion

Anticancer PSP in *Lentinus squarrosulus*, *Lentinus polychrous* and *Lentinus edodes*

Figure 1a outlines the SDS-PAGE profiles of protein extracts from dried fruiting body mushroom. Protein bands ranging from 35 - 100 kDa were present in the 3 edible mushrooms. The stained bands of *Lentinus squarrosulus*, *Lentinus polychrous* and *Lentinus edodes* proteins may be anticancer mushroom proteins, as reported in previous studies, such as lectin (16 - 51 kDa), ribonuclease (45 kDa) [17], laccase (58 - 62 kDa), fungal immuomodulatory proteins (FIPs) (27 kDa) and Se-containing protein (36 kDa) [7]. At first, xylose-specific lectin was isolated from the fruiting bodies of the wild mushroom, *Xylaria hypoxylon* [13]. Lectin exhibited highly potent antiproliferative activity toward tumor cell lines. Mushroom RIPs (ribosome inactivating proteins) have been purified from several species of mushrooms, like *Calvatia caelata*, *Falmmulina velutipes*, *Hypsizigus marmoreus* and *Pleurotus tuber-regium*. RIPs have also inhibited proliferation of hepatoma Hep G2 cells and breast cancer MCF-7 cells. One of the laccases was purified from *Tricoloma mongolicum*, and it also could inhibit proliferation of hepatoma HepG2 cells and breast cancer MCF7 cells [14]. A protein of FIPs from *Ganoderma microsporum* was investigated for its activity in suppressing tumor invasion and metastasis [15]. However, only having data from molecular weight in SDS-PAGE is not sufficient to identify those bioactive proteins, so we are now extracting and characterizing them. One of the prominent anticancer proteins was polysaccharide peptide (PSP) which is now being used to treat patients [8]. In this study, we used Western blotting and ELISA assays to identify PSP protein in mushrooms. An immunoreactive band approximately 70 kDa in size was shown in protein extract from *L. squarrosulus*, as displayed in **Figure 1b**.

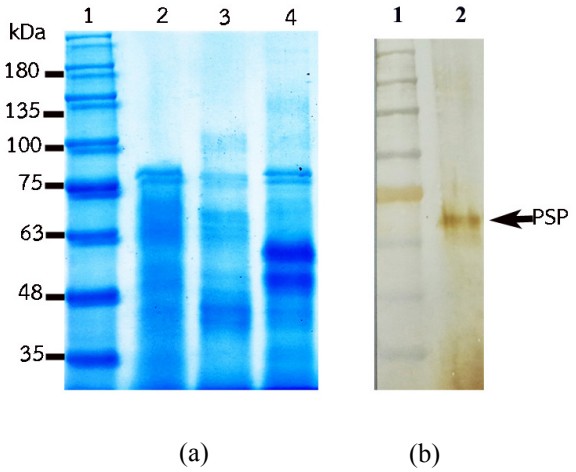

Figure 1 Detection of PSP in protein extracts (a) 4 - 20 % SDS-PAGE profiles of mushroom proteins, 15 µg each 1: molecular weight standards, 2: *L. squarrosulus* proteins, 3: *L. polychrous* proteins, and 4: *L. edodes* proteins. (b) Western blot analysis, 1: molecular weight standards and 2: *L. squarrosulus*.

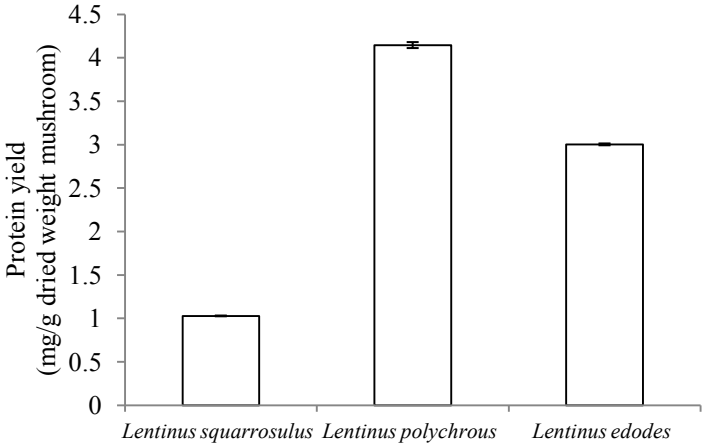

Figure 2 Protein yields of 3 mushrooms, *L. squarrosulus*, *L. polychrous* and *L.edodes*.

The protein yield from the 3 mushrooms is shown in **Figure 2**. The yield of protein was 1.02 ± 0.004, 4.14 ± 0.034 and 3 ± 0.010 mg/g dried weight mushroom in *L. squarrosulus*, *L. polychrous* and *L. edodes*, respectively. Mean ± SD (n = 3).

Overall, mushrooms hold little fat or digestible carbohydrates, yet they have higher protein contents than most vegetables. After protein extraction from 3 Lentinus mushrooms, the highest protein yield was 4.14 mg/g in *L. polychrous*, as shown in **Figure 2**. In **Figure 3**, PSP level was quantitated in different edible Lentinus mushrooms using indirect ELISA. In one gram of protein concentration in 3 mushrooms, PSP level in *L. squarrosulus* was significantly higher than *L. polychrous* and *L. edodes*. We report firstly about PSP level in Lentinus mushrooms. As previously reported, PSP was produced from *Coriolus versicolor* mushrooms harvested in the wild or cultivated commercially, or from mycelial growth of *C.*

versicolor in submerged fermentation [15,16]. PSP also enhanced the cytotoxicity of certain S-phase targeted-drugs, such as doxorubicin, etoposide, camptothecin and cyclophosphamide, on human cancer [17]. In addition, PSP has shown a chemopreventive effect on prostate cancer *via* the targeting of prostate cancer stem cell-like populations [18]. The immunoreactive protein of an anti-commercial PSP antibody using ELISA and Western blot may be PSP, as reported in *C. versicolor*. To prove the immunoreactive protein characteristic, the purification and characterization will be investigated in the next report.

Anticancer activity of protein extracts from *Lentinus squarrosulus*

Crude protein extracts from *L. squarrosulus* were designated to test the antiproliferative activity in human colorectal adenocarcinoma, HT-29 cells, because of their highest PSP levels. The protein extracts inhibited proliferation of HT-29 cells in a dose dependent manner when added to HT-29 cell lines for 72 h, as shown in **Figure 4**. This result corresponds with treatment of the cell lines with commercial PSP. The IC_{50} value of *L. squarrosulus* extracts and PSP were about 0.05 and 0.01 mg/ml, respectively. We report here for the first time about potent cytotoxicity of crude protein extracts from *L. squarrosulus* with HT-29. Methanolic extract of oyster mushrooms showed IC_{50} at 0.5 mg/ml [24] and recently, ethanolic extract of *Phellinus linteus* presented IC_{50} at 0.15 mg/ml with HT-29 cell line [25]. In our previous study, ethanolic extract of *L. polychrous* and *L. squarrosulus* also inhibited cholangiocarcinoma cell line, M213and HepG2 (human hepatocellular liver carcinoma cell line) (data not shown). PSP significantly suppressed the proliferation of human breast cancer cell lines (T-47D) through upregulation of the p53 protein expression and down regulation of Bcl-2 protein expression, but in MCF-7 cells it was by means of down-regulation Bcl-2 protein expression only [26]. The mechanism of protein extraction from *L. squarrosulus* that inhibited cell proliferation in cell lines is still not clear, because many proteins were present in this study.

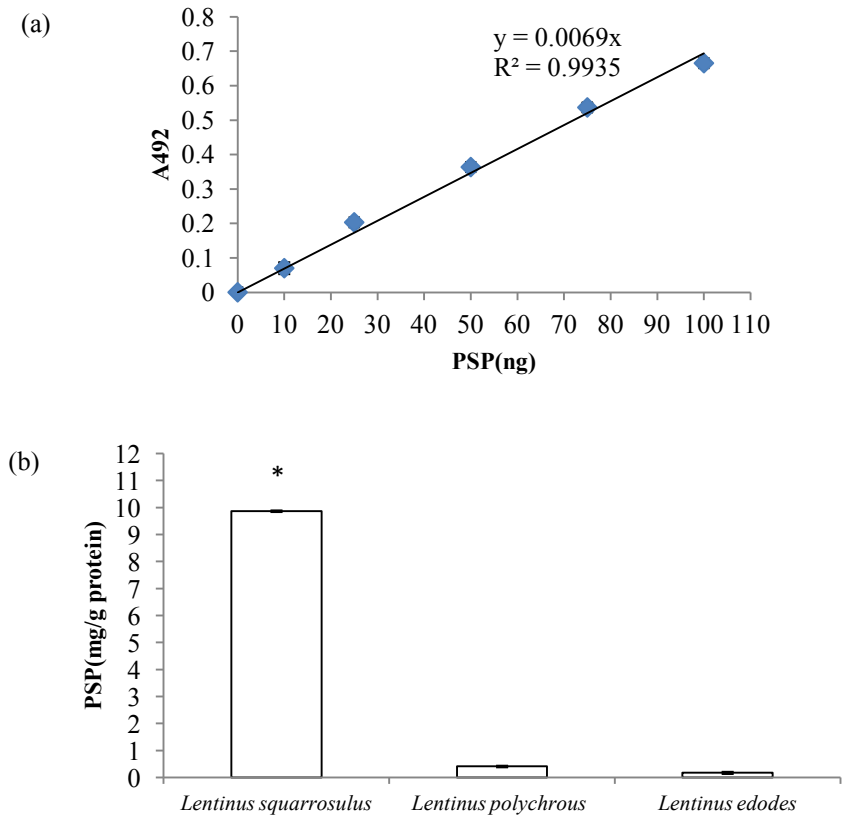

Figure 3 Detection of PSP concentration with indirect ELISA in 3 mushrooms species, *L. squarrosulus*, *L. polychrous* and *L. edodes*. (a) Calibration curve of PSP standard. (b) Concentration of PSP (mg/g of protein) in 3 mushroom species. Data represent mean±standard deviation (n = 3).
*, $p < 0.05$ vs. *L. polychrous* and *L. edodes*.

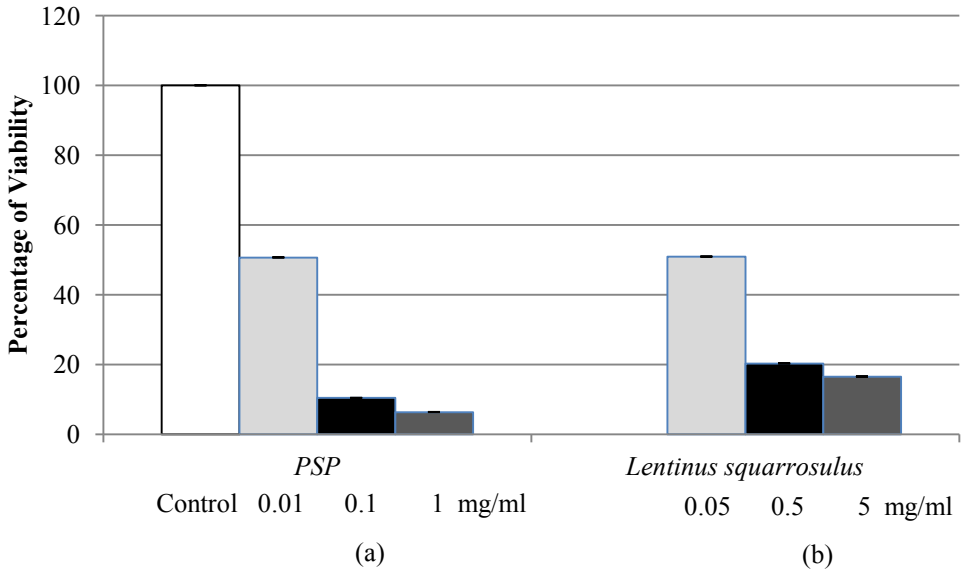

Figure 4 *In vitro* inhibitory effect of PSP and *L. squarrosulus* on proliferation of HT-29 cell line. HT-29 cells were treated with protein extract from *L. squarrosulus* at concentrations of 0.05, 0.5 and 5 mg/ml (a) and PSP at concentrations of 0.01, 0.1 and 1 mg/ml (b) Cells were tested for antiproliferative activity after treatment via PSP and protein extract from *L. squarrosulus* for 72 h. Data represent mean±standard deviation (n = 3).

Characterization of the polyphenol content in mushrooms

Catechin was the major phenolic compound in 3 Lentinus mushrooms; the highest concentration was existent in *Lentinus polychrous*. From the results in **Figure 5**, the amount of catechin comprised over 200 mg/kg dried weight of the mushroom. We also detected that the major catechin derivative in *Lentinus polychrous* and *Lentinus squarrosulus* was epigallocatechin (data not shown). Epigallocatechin (EGC) of green tea induced apoptosis of human breast cancer cells, but not of their normal counterparts [27]. The second and third highest amount of phenolic compound in ethanol extract of mushrooms were tannic and quercetin, respectively. Gallic, isoquercetin, kaempferol and eriodictyol levels were less than 50 mg/kg dried weight of the mushroom, and these phenolic contents were detected at the highest amounts in *Lentinus squarrosulus*. Rutin and hydroquinin were not detected in any ethanolic extracts of mushrooms in this study. Catechin is a group polyphenol of condensed tannins. Normally, cathechins are the main compounds in green tea [28]. Catechin serves as a powerful antioxidant against lipid peroxidation when phospholipid bilayers are exposed to aqueous oxygen radicals. Catechin prevents cancer of the liver, lung, breast and colon, and also suppresses cancer promotion. Besides anticancer activity, antibacterial, antiviral agent, and anti-hyperglycemic action of catechin have also been reported [29]. A systematic review revealed that tannic acid, or the commercial product of tannin, have also been reported to exert many physiological effects, such as accelerated blood clotting, reduction of blood pressure, decrease of serum lipid level, production of liver necrosis, and modulation of immunoresponses [30].

Antioxidant activities of edible 3 lentinus mushrooms

Phenolic compounds have been reported to be the major antioxidant components found in mushroom species, whereas other potential antioxidants, such as ascorbic acid, β-carotene, and tocopherol have only been found in very small amounts [31]. Fu *et al*. informed that several cultivated edible mushrooms, such as *Agaricus bisporus*, *Hericium erinaceus*, *Flammulina velutipes*, *Lentinus*

edodes, *Pleurotus eryngii*, and *Pleurotus ostrreatus*, have antioxidant and free radical scavenging activities [32]. Several medicinal mushrooms have also been the subjects of reports about their antioxidant activity, such as *Agaricus blazei*, *Sparassis crispa*, *Phellinus linteus*, *Ganoderma lucidum* and *Inonotus obliquus.* Free radical scavenging is one of the mechanisms in inhibiting lipid oxidation commonly used to estimate antioxidant activity [33]. A wide variety of antioxidant assays are used when determining the antioxidant activity of bioactive compound extracts. Here, we used 2 common assays, based on the scavenging of the DPPH assay and FRAP assay. Total phenolic content (TPC) of the extracts was also used as an indirect measure of antioxidant capacity in this study [34]. The total phenolic contents in ethanolic extract of *L. squarrosulus*, *L. polychrous* and *L. edodes* were 30.98 ± 0.003, 8.74 ± 0.007, and 19.53 ± 0.009 mg of Gallic acid equivalents (GAEs)/g extract, respectively. Previous data of TPC in *L. squarrosulus* and *L. edodes* was reported at 15 and 6.27 - 9.11 mg of Gallic acid equivalents (GAEs)/g extract, respectively [35,36]. The different amounts of TPC in mushroom extracts might be dependent on the solvent and method used in extraction. The antioxidant activities of mushroom extracts were estimated by means of Trolox Equivalent Antioxidant Capacity (TEAC), calculated from the DPPH and FRAP assay. At a concentration of 0.5 mg/ml, the scavenging effects of *L. squarrosulus*, *L. polychrous* and *L. edodes* were about 80, 73, and 30 %, respectively, while the scavenging activity of trolox by DPPH assay at 0.15 mg/ml was 88 %. The scavenging effects of 0.5 mg/ml *L. squarrosulus* extract showed its highest value at 0.5 mM TE/g dried mushroom in the FRAP assay; this data was correlated with the TPC and DPPH assays. The FRAP value of *L. squarrosulus* extract was similar to the *Cynomorium coccineum* extract [37]. The scavenging activity of Lentinus mushrooms in the FRAP assay were dependable with TPC; a higher TPC content in the ethanol extract displayed a higher trolox equivalent, as shown in **Figures 6** and **8**. From the DPPH assay in **Figure 7**, the *L. polychrous* extract showed a percentage of inhibition, or scavenging effect, higher than *L. edodes*; this result was contrastable via the FRAP and TPC assays. Normally, the results of the DPPH and FRAP assays for plant extracts were highly correlated in previous reports, because the 2 assays used a similar mechanistic basis [34]. The principle of both assays is the transferal of electrons from the antioxidant in order to reduce an oxidant. This conflicting data of *L. polychrous* and *L. edodes* in the FRAP and DPPH assays might be the effect of noteworthy chemical and methodological differences between the 2 assays. Through conferring, for the full evaluation of the antioxidant capacity of any extracts, the use of a wider variety of assays is usually suggested.

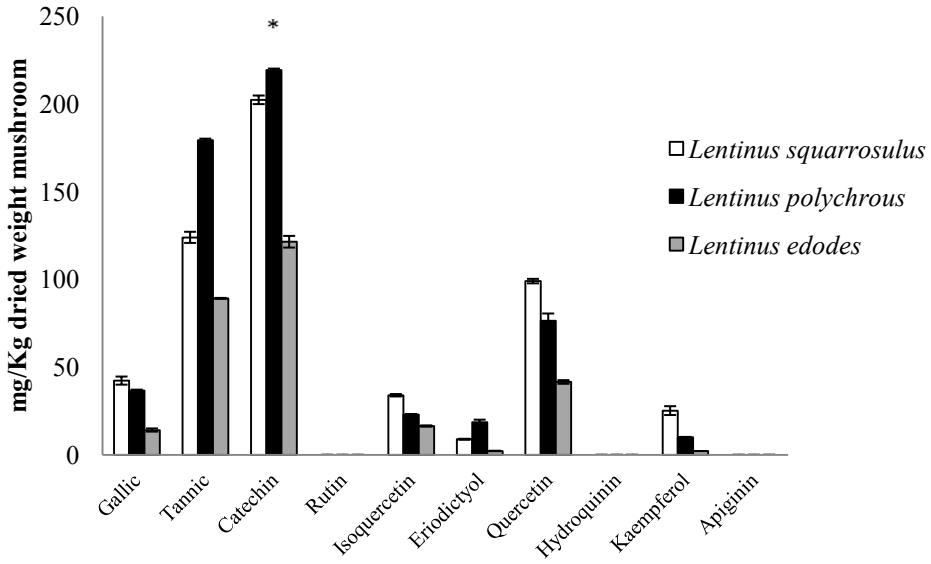

Figure 5 Concentration of phenolic compounds in 3 mushrooms, *L.squarrosulus*, *L. polychrous* and *L. edodes*. Catechin and tannic were the major phenolic compound in 3 lentinus mushrooms. Data represent mean±standard deviation (n = 3).
*, $p < 0.05$ vs. other phenolic compounds.

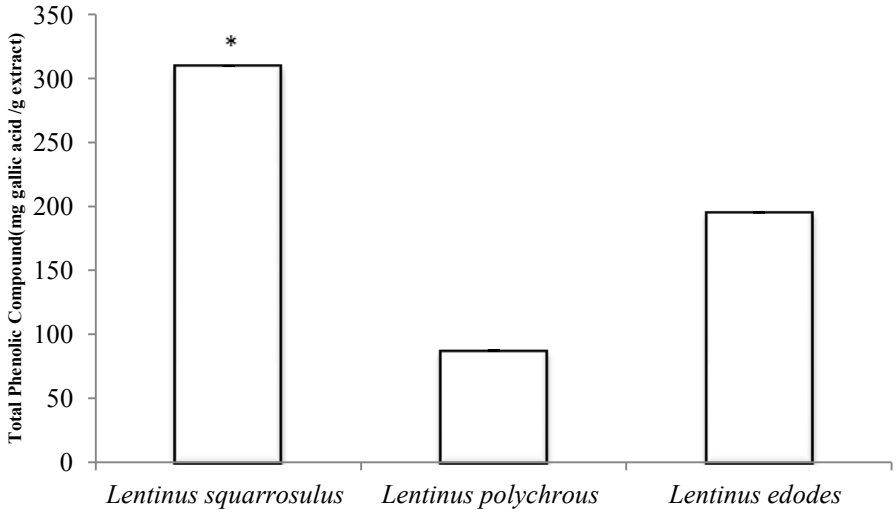

Figure 6 Total phenolic compound in 3 mushrooms, *L. squarrosulus*, *L. polychrous* and *L. edodes*. The highest total phenolic compound was shown in *Lentinus squarrosulus*. Data represent mean±standard deviation (n = 3).
*, $p < 0.05$ vs. *L. polychrous* and *L. edodes*.

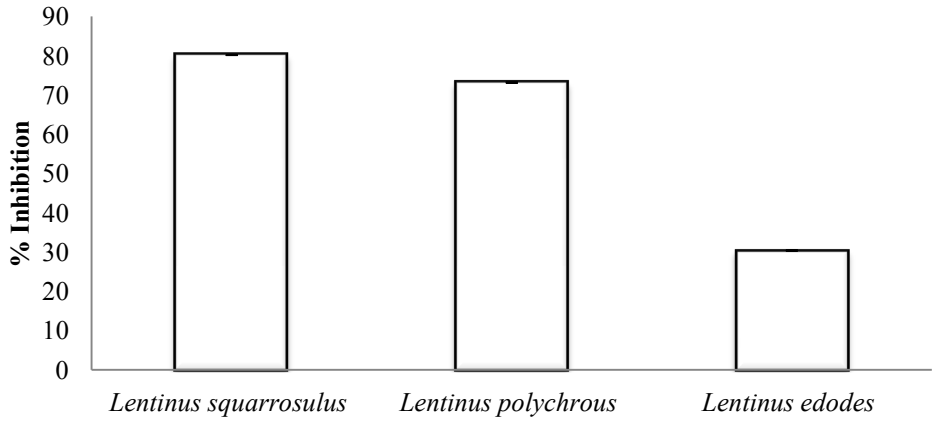

Figure 7 Percent inhibition of the DPPH radical of 3 mushrooms, *L. squarrosulus*, *L. polychrous* and *L. edodes*. Extract from *L. squarrosulus* and *L. polychrous* which showed percent inhibition greater than 50 %. Data represent mean±standard deviation (n = 3).

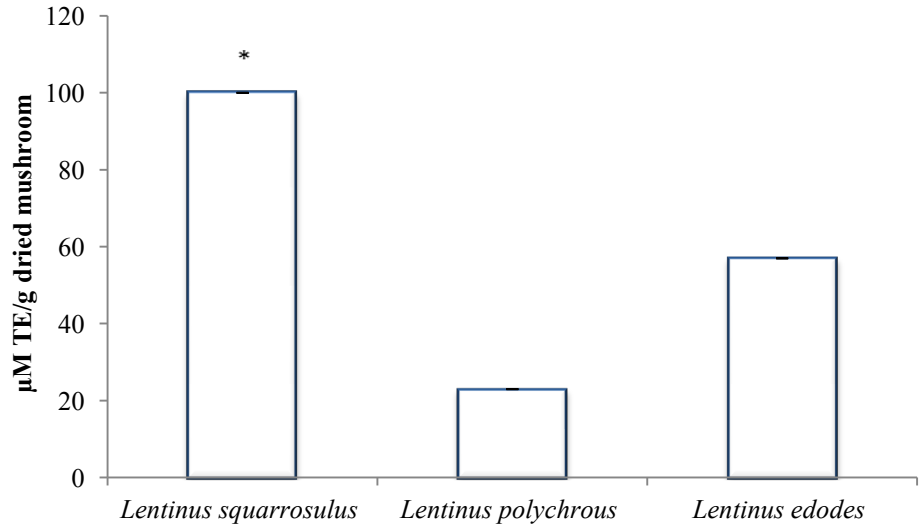

Figure 8 FRAP assay, comparison of micromolar of trolox equivalent (µM TE) per gram dried mushroom. *L. squarrosulus*, *L. polychrous* and *L. edodes*. *L. squarrosulus* extracts showed the most potential in antioxidant activity. Data represent indicate mean±standard deviation (n = 3).
*, $p < 0.05$ vs. *L. polychrous* and *L. edodes*.

Conclusions

Today's mushrooms are not only intended to satisfy hunger and provide essential nutrients, but also to prevent disease and improve the physical and mental well-being of consumers. Mushrooms do well to grow in darkness and dampness in highly competitive environments, and protect themselves from the masses of attacking microbes by using natural protective substances. Some advantages of using mushrooms over plants as sources of bioactive compounds are that, often, the fruiting body can be produced in much less time, and can be manipulated to produce optimal quantities of active compounds. This study is the first time PSP in fruiting bodies of *L. squarrosulus* and *L. polychrous* as detected by ELISA and Western blot analysis. In addition, other bioactive proteins apart from PSP may also exist in *L. squarrosulus*. However, further studies are required to confirm that this is the same PSP as characterized in *C. versicolor*. Purification and characterization of the PSP of *L. squarrosulus* has been studied, and this data will be reported soon in a different manuscript. Good antioxidant activity of *L. squarrosulus* extract and high phenolic content in *L. polycrous* will challenge us to prove its mechanism in cancer cell lines. Moreover, the mushroom species can be used as a simple and available source of natural antioxidants and as a safe supplement for the food or pharmaceutical industries.

Acknowledgements

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