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Full Length Research Paper

The different antioxidant and anticancer activities depending on the color of oyster mushrooms

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Oyster mushroom is a popular edible mushroom which has various colorful fruit bodies. The objective of this study was to determine the antioxidant and the anticancer activities of oyster mushrooms (OM) with different colors such as dark-grey strain (*Pleurotus ostreatus*), yellow strain (*Pleurotus cornucopiae)*, and pink strain (*Pleurotus salmoneostramineus*). The methanolic extracts from OMs were prepared for this study. Among these OMs, the extract from the yellow strain showed the highest radical scavenging activity, reducing power, ferrous chelating ability, and total phenolic contents. Radical scavenging activity of yellow strain was about 3 times higher than that of dark-grey strain. On the other hand, the extracts of dark-grey and pink strains showed higher suppressive effect against growth of human colon cancer cell line HT-29 with survival rates of 39.9 and 40.7%, respectively, than that of yellow strain. These results showed that the antioxidant and the anticancer activities of OMs varied by the colors of fruit bodies.

Key words: *Pleurotus* species, oyster mushroom, antioxidant, anticancer.

INTRODUCTION

Mushrooms have been not only used as food materials with their unique flavor and texture, but also recognized as an important source of biologically active compound of medicinal value (Breene, 1990). Mushrooms have a variety of accumulated secondary metabolites such as phenolic compounds, polypetides, terpenes, and steroids. Mushrooms also have lectins, polysaccharides, polysaccharide-peptides, and polysaccharide-protein complexes which are known to have immunomodulatory and anticancer activities (Sun and Liu, 2009).

Oyster mushroom (OM) is an edible white-rot fungus and is classified into *Pleurotus* species comprising about 40 species (Jose and Janardhanan, 2000). OM is mainly found in northern temperate zones and grows on wood in clusters where the weather condition is warm and wet. OM is one of the most widely cultivated mushrooms including South Korea, and its annual commercial production in South Korea was about 45,957 M/T in 2007.

The pigments in colorful fruits and vegetables are recently attracted in view points of reducing or preventing a few diseases such as obesity, atherosclerosis, hyper-piesia, and cancer (Stintzing and Carle, 2004). Some kinds of OM contain colored fruit bodies, and one of which is yellow (*Pleurotus cornucopiae*) (El Bohi et al., 2005), pink (*Pleurotus salmoneostramineus*) (Shibata et al., 1997), or white (*Pleurotus florida*) (Li et al., 2008). Shibata et al. (1997) reported that the pink color of *P. salmoneostramineus* was chromoprotein, which plays a photosynthetic function. Although there are many reports about medicinal effects of mushrooms, little information is now available on the physiological effects of OM with different colors. To determine the antioxidant and the anticancer properties of the methanolic extracts from three OMs with different colors (dark gray, pink, and yellow), radical scavenging activity, reducing power, ferrous chelating activity, total phenolic contents, and total flavonoid content were analyzed, and 3-(4, 5-dimethyl- thiazol-2-yl)-2,5-

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diphenyl-tetrazolium bromide (MTT) assay was performed.

MATERIALS AND METHODS

Materials

Three kinds of OM (dark-gray, *Pleurotus ostreatus*; yellow, *P. cornucopiae*; and pink, *P. salmoneostramineus*) were harvested at Gyeonggido Mushroom Research Station (Gwangju city, Korea). The fruit bodies were cleaned to remove any residual and then freeze-dried. The freeze-dried mushrooms were ground using a mill, passed through a 0.5-mm sieve and then stored in the nitrogen gas-added plastic bags. A 100 g of the dried mushroom sample was extracted using 2 L methanol overnight at room temperature, and the extract was filtered using a Whatman No.2 filter paper (Advantec, Tokyo, Japan). The residue was then extracted with two additional portion of methanol under the same condition. The methanolic extracts of OM were combined and evaporated using a rotary evaporator (Eyela NE1001, Tokyo, Japan) at 40°C for dryness. The final dried methanolic extracts were used to determine for further analyses. For analyzing samples, the dried extract was resolubilized in methanol and then three different concentrations of OM methanolic extracts (0.5, 1, and 2 mg/mL) were prepared.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The scavenging activity was determined by the DPPH method (Blois, 1958). A 1-mL of methanolic extract was mixed with 1 mL of ethanol solution containing DPPH radicals (Sigma Chemical Co., St. Louis, MI, USA), resulting in 0.041 mM of the final concentration of DPPH. The mixture was shaken vigorously and left to stand for 10 minutes. The absorbance was measured at 517 nm using a spectrophotometer (Shimadzu UV-2550, Tokyo, Japan).

Reducing power

Reducing power of OM was determined by the method of Oyaizu (1986). Each concentration of the methanolic extracts (1.0 mL) was added to 1 mL of potassium ferricyanide (10 mg/mL), and the mixture was incubated at 50°C for 20 min. After incubation, a 1-ml of trichloroacetic acid (100 mg/mL) was added to the mixture and then the mixture was centrifuged at 13,400 × g for 5 min. The supernatant (1.0 mL) was mixed with 1.0 ml of distilled water and 0.1 mL of ferric chloride (1.0 mg/mL), and then its absorbance was measured at 700 nm.

Chelating effects on ferrous ions

Chelating ability was determined according to the method of Shimada et al. (1992). Each extract (1 mL) was mixed with 0.1 mL of 2 mM $FeCl_2$. The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine (Sigma Chemical Co.). After 10 minutes at room temperature, the absorbance of the mixture was determined at 562 nm.

Total phenolic contents (TPC)

TPC in the methanolic extracts of OM were measured according to the method of Gutfinger (1981). Each extract (1.0 mL) was mixed with 1.0 mL of 2% Na_2CO_3 and 0.2 mL of 50% Folin-Ciocalteau reagent added into the mixture. After incubation for 30 minutes at room temperature, the mixture was centrifuged at 13,400 × g for 5 min. The absorbance was measured at a 750 nm. TPC were expressed as gallic acid equivalents.

Total flavonoid contents (TFC)

Flavonoid contents in the methanolic extracts of OM were determined by Choi et al. (2006), and results were expressed as mg (+)-catechin equivalents per g of mushrooms. Standard solution or mushroom extract (250 µl) was mixed with 1.25 mL of distilled water and 75 µl of 5% $NaNO_2$ solution. After incubation for 5 minutes, 150 µl of 10% $AlCl_3 \cdot H_2O$ was added. After 6 min, 500 µl of 1 M NaOH and 275 µl of distilled water were added to the mixture. The solution was mixed well and the intensity of pink color was measured at 510 nm.

MTT reduction assay

Cell viability was measured with blue formazan formed by the reduction of MTT by mitochondrial dehydrogenase, which is activated only in live cells. HT-29 cells were incubated in 96-well plates at a density of 1.0×10^5 cells per well for 24 h. Cells were treated with various concentrations of extracts. After incubation for 24 h, a 1-ml of MTT reagent (5 mg/mL) was added to each well, and the plate was incubated for an additional 1 hour at 37 °C. The media were then removed and the intracellular formazan product was dissolved in 100 µl of DMSO. The absorbance of each well was then measured at a wavelength of 540 nm using a ELISA reader (model 680, Bio-Rad, Hercules, CA, USA). Optical density values from untreated control cells were designated as 100% for the standard.

Statistical analysis

All the analyses were performed in triplicate, and these results were reported as means ± standard derivation (SD). The significance of differences among treatment means were determined by one-way analysis of variance (ANOVA) using SAS Program (Enterprise Guide 3.0 version, SAS Institute, Cary, NC, USA) with a significant level of 0.05.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

The DPPH radical scavenging activity of the methanolic OM extracts increased with increasing the concentrations at all OMs. Among three OMs, yellow strain showed the highest radical scavenging ability under the same concentration (Figure 1). At 2 mg/mL concentration, the radical scavenging activities of OMs were 84.4, 54.5 and 29.0% for yellow, pink and dark-grey strain, respectively, revealing that yellow strain had higher radical scavenging activity by about 3 times than dark-grey strain had.

Mau et al. (2001) and Lo (2005) reported that DPPH radical scavenging activities of dark-grey OM were 81.8% at 6.4 mg/mL and 68.4% at 5 mg/mL. Our results showed that all OMs had higher radical scavenging activity values than those of previous studies. On the other hand, Yang et al. (2002) also reported that yellow winter mushroom (*Flammulina velutipes*) was more effective in scavenging radicals than white strain. Thus, it can be concluded that the radical scavenging activities of OMs varied by fruit body colors.

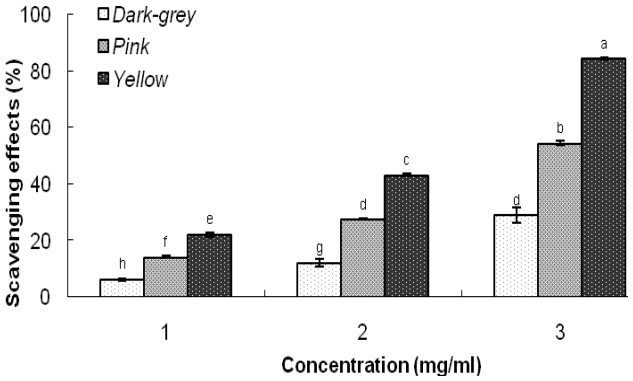

Figure 1. Scavenging effects of DPPH radical of the methanolic extracts from colored oyster mushrooms at different concentrations. [a-h] Different letters within a row are significantly different (P<0.05), n=3

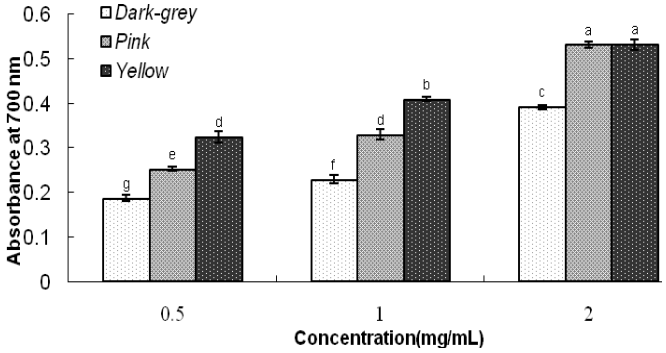

Figure 2. Reducing power of methanolic extracts from colored oyster mushrooms. [a-g] Different letters within a row are significantly different (P<0.05), n=3

Reducing power

Reducing power of a compound may serve as a signify-cant indicator of its potential antioxidant activity (Oyaizu, 1986). The presence of reducers (i.e. antioxidants) causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. In the present study, yellow strain OM had an excellent reducing power (0.32 at 0.5 mg/mL and 0.41 at 1.0 mg/mL), showing that its reducing ability was more effective than those of pink or dark-grey strains (Figure 2). Overall, reducing power of methanolic extracts from three different colored OMs was at the order: yellow > pink > dark-grey strains. The odor of reducing power of OMs was coincident with that of the radical scavenging activity. Reducing power of colored OMs might be due to their hydrogen-donating ability (Shimada et al., 1992). Therefore, yellow OM might contain higher amount of reductants than other colored OMs, which could react with free radicals to stabilize and terminate radical chain reaction.

Chelating effects on ferrous ion

Chelating effects of methanolic extracts from colored OMs on ferrous ions increased as the concentration increased from 1.0 to 1.5 mg/mL (Figure 3). At 1.0 mg/mL, yellow strain OM had an outstanding chelating ability (79.7%), which was similar to that of dark-grey (77.5%) or pink (80.7%) strain at 1.5 mg/mL. At 1.5 mg/mL, chelating effects among the three colored OMs was not different. The results showed that yellow strain was a primary ferrous chelator at 1mg/mL.

Fruit bodies of *Pleurotus citrinopileatus* have been reported to chelate ferrous ions by 82.1% at 5 mg/mL (Lee et al., 2007), showing that it was more effective than its mycelia. Lo (2005) reported that chelating abilities of *Pleurotus eryngii*, *Pleurotus ferulae* and *Pleurotus ostreatus* at 5 mg/mL were 41.4-64%. OMs used in this study

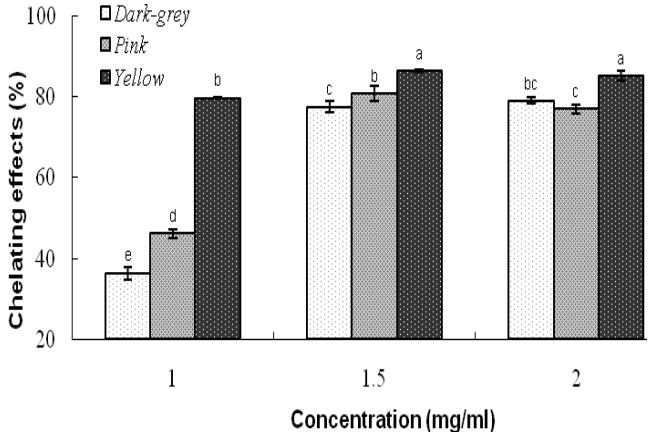

Figure 3. Chelating effects of methanolic extracts from colored oyster mushrooms on ferrous ion. [a-e] Different letters within a row are significantly different (P<0.05), n=3.

exhibited higher chelating abilities (78-80%) at lower concentration (1.5 mg/mL) than those previously studied strains. Since ferrous ions are the most effective pro-oxidants in food system (Yamaguchi et al., 1988), high chelating effect of methanolic extracts from colored OMs might be beneficial.

Total phenolic contents (TPC)

Phenolic compounds such as flavonoids, anthocyanins, and carotenoids are the major naturally occurring antioxi-dant components, which are free radical scavengers not only because of their ability to donate hydrogen atoms or electrons but also because of their stable radical interme-diates (Shahidi and Wanasundara, 1992). The TPC of the colored OMs were in the order of yellow (39.3 mg/g) > pink (30.1 mg/g) > dark-grey (21.2 mg/g) (Table 1). These TPC results could show the differences of the antioxidant activities of the three colored OM in terms of

Table 1. Total phenols and flavonoids contents of methanolic extracts from colored oyster mushrooms (mg/g)

Content	Dark-grey	Pink	Yellow
Total phenols	21.2±0.1[c]	30.1±0.2[b]	39.3±0.1[a]
Total flavonoids	2.16 ±0.05[a]	1.21±0.09[c]	1.96±0.02[b]

Each value is expressed as mean±standard deviation (n=3). Means with different letters within a row are significantly different (P<0.05)

radical scavenging, reducing power, and chelating effects. Since mushrooms also possess phenolic compounds such as asiaticusin A and B (in *B. asiaticus*), p-terphenyls (in *Paxillus panuoides*), p-hydroxybenzoic acid (in *Amanita rubescenes*, *Russula cyanoxantha*, and *Tricholoma equestre*), and quercetin (in *Suillus luteus* and *Suillus granulatus*) (Ribeiro et al., 2006; Wada et al., 1996), it is interesting to investigate the antioxidant activity of mushroom in relation to their TPC (Cheung et al., 2003; Sarikurkcu et al., 2008).

Total flavonoid contents (TFC)

Flavonoids are usually glycosylated and can be classified as anthocyanidins, flavanols (catechins), flavones, flavanones, and flavonols, which responsible for the orange, red and blue color in fruits and vegetables. Generally, deep-colored fruits, vegetables or foods are recognized as more healthy to human body (Lin and Tang, 2007). There has been a growing interest in pigment components of natural food, which may promote human health or lower the risk for disease. As shown in Table 1, TFC in colored OMs were in the order of dark-grey (2.16 mg/g) > yellow (1.96 mg/g) > pink (1.21 mg/g). This result was slightly difference of what was expected in that the highest flavonoid contents were observed in dark-grey strain but not yellow strain. Carotenoids such as lutein, lycopene, β-carotene and zeaxanthin in colored OMs were not detected (data not shown). Therefore, these results indicate that the pigment of mushrooms should be water-soluble rather than fat-soluble.

Cytotoxic activity against HT-29 cells

The inhibitory effects of OMs on the growth of HT-29 human colon carcinoma cells were determined by MTT reduction assay. HT-29 cells were originally derived from a human colon carcinoma and were chosen because they represent a hypovascular tumor. These cells show strong tolerance to anticancer agents *in vitro* and *in vivo*. HT-29 cells were exposed for 24 hours at various concentrations of three OMs (5-500 µg/mL). As shown in Figure 4, dark-grey and pink strain at 500 µg/mL had an effective inhibition with 60.1 and 59.3%, respectively, whereas the viability of HT-29 cell exposed to yellow strain was relatively high. This result indicates that the anticancer activities depending on the color of OM were different from the antioxidant activities.

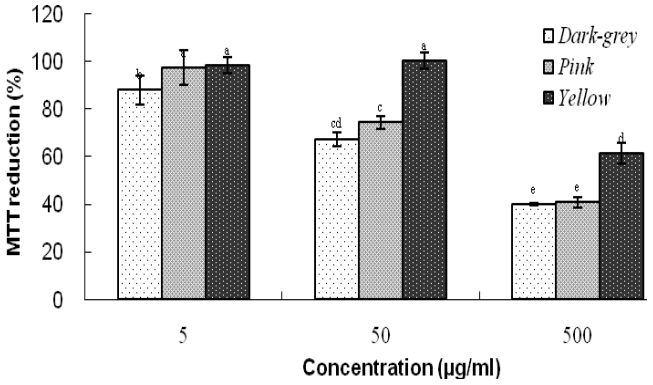

Figure 4. Effects of the methanolic extracts from colored oyster mushrooms on the viability of HT-29 cells. The viability was measured by MTT reduction assay. [a-e] Different letters within a row are significantly different (P<0.05), n=3

Some polysaccharides or polysaccharide-protein complexes from mushrooms are able to exert antitumor activity and antigenotoxicity ability through the stimulation of the non-specific immune system (Wasser and Weis, 1999). Mushroom β-glucans (especially, lentinan from *Lentinula edodes*, shizophyllan from *Schizophyllum commune*, MD-fraction from *Grifola frondosa*, and krestin from *Trametes versicolor*) exert a high antitumor activity, which are recently in clinical use for the adjuvant tumor therapy (Amarowicz and Shahidi, 1997). Cancer protective effects of *P. ostreatus* fruit bodies were demonstrated in rats (Zusman et al., 1997). Besides, *P. ostreatus* also diminishes the toxicity of cyclophosphamide in mice (Gerasimenya et al., 2002). Accordingly, the active substance of anticancer of colored OMs might be polysaccharides.

Conclusion

This study showed that the methanolic extracts from different colored OMs had considerable antioxidant and anticancer properties. Especially, yellow strain exhibited the strongest antioxidant activities including scavenging ability, reducing power, and chelating ability. Furthermore, antioxidant properties of three different colored OMs were intimately linked with phenolic compounds content. Contrarily, dark-grey and pink strains were more effective in inhibiting HT-29 cancer cells than yellow strain. Therefore, these results indicate that colored OMs could be very beneficial for defending radical mediated toxicity and inhibiting human colon carcinoma cells.

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