Mushrooms Collected from Deogyu Mountain, Muju, Korea and Their Antioxidant Activity

Seong-Eun Kim1, In-Kyoung Lee1, Yun-A Jung2, Ji-Hee Yeon1, Dae-Won Ki1, Myeong-Seok Lee1, Ja-Gyeong Song1, Yong-Ju Jin2, Soon-Ja Seok2 and Bong-Sik Yun1*

1Division of Biotechnology, Chonbuk National University, Iksan 570-752, Korea
2Agricultural Microbiology Division, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-707, Korea

(Received June 18, 2012. Revised June 19, 2012. Accepted June 19, 2012)

Mushrooms collected from Deogyu mountain, Korea, in 2011, were identified as four classes, four orders, 13 families, 22 genera, and 33 species. In particular, agaricales was most abundant and comprised more than 70%. Their antioxidant activities were estimated using three different bioassay methods, the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical scavenging assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, and reducing power assay. As a result, the methanol extracts of *Stereum ostrea*, *Laetiporus sulphureus* var. *miniatus*, and *Tyromyces sambuceus* exhibited potent antioxidant activity in all bioassays tested.

KEYWORDS : Antioxidant activity, Free radical scavenging activity, Mushrooms, Reducing power activity

Mushrooms are a rich source of secondary metabolites with unprecedented structural features and remarkable bioactivities. Therefore, they exhibit a very broad spectrum of pharmacological activities, including antifungal, anti-inflammatory, antitumor, antiviral, antibacterial, antiparasitic, immunomodulating, and hepatoprotective activities [1-5].

Free radicals have been implicated in the pathogenesis of various diseases, including ischemia, arteriosclerosis, diabetes, arthritis, inflammation, and cancer, as well as in the aging process [6, 7]. According to considerable evidence, due to their capacity to quench free radicals, antioxidants could aid in prevention of these diseases [8]. Thus, the demand for antioxidants has shown a gradual increase. As part of a continuous search for new natural antioxidants [9, 10], we have assessed the antioxidant activity of mushrooms collected from Deogyu-mountain, Muju, Korea, in 2011. In this study, mushrooms collected from Deogyu-mountain and their antioxidant activity are described.

As shown in Table 1, mushrooms collected from Deogyu mountain, Muju, Korea, in 2011, were identified as 33 taxa of macrofungi belonging to 22 genera in 13 families. Among them, agaricales was most abundant and comprised more than 70%. Included are many edible species, which belong to the Genus *Peziza*, *Tremella*, *Boletus*, *Laccaria*, and *Citocybe*, whereas genus *Amanita* is known to contain a poison that is fatal to humans. Fruiting bodies of 33 species were cut into small pieces and extraction was performed using 80% aqueous MeOH at room temperature for one day. The methanolic extract was filtered and concentrated under reduced pressure. The concentrates were dissolved in dimethyl sulfoxide at a concentration of 10 mg/mL, and their antioxidant activity was estimated. Three different bioassay methods, the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical scavenging assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, and reducing power assay were used for evaluation of the antioxidant capacity of the methanolic extracts of the collected mushrooms.

The ability to scavenge free radicals is the primary characteristic of an antioxidant. The ABTS radical cation and DPPH radical scavenging assay methods were used for evaluation of the free radical scavenging efficacies of the methanolic extracts. A method described in the literature was used for measurement of radical scavenging activity of ABTS [11]. In brief, ABTS was dissolved in water to a concentration of 7 mM. The ABTS cation radical was produced by reaction of the ABTS stock solution with 2.45 mM potassium persulfate and by allowing the mixture to stand in the dark for 12 hr. Following addition of 190 µL of ABTS radical cation solution to 10 µL of mushroom extracts, absorbance was measured using a microplate reader at 734 nm after mixing for up to 7 min. As shown

*Corresponding author <E-mail : bsyun@jbnu.ac.kr>

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
in Fig. 1, among the methanol extracts tested, Stereum ostrea exhibited the most potent ABTS radical scavenging activity, with a scavenging effect of above 90%, which was comparable to that of butylated hydroxyanisole (BHA) and trolox, which were used as positive controls. Although their activity was less than that of positive controls, the methanol extracts of Boletus subvelutipes, Entonaema Splendens, and Tyromyces sambuceus exhibited potent ABTS radical scavenging activity above 70%.

DPPH radical scavenging activity was measured using a method described in the literature [12]. Ten µL of each extract was combined with 90 µL of 150 µM methanolic DPPH. Following incubation at room temperature for 10 min, absorbance was read at 517 nm using a Molecular Devices Spectromax microplate reader (Molecular Devices Corp., Sunnyvale, CA, USA). According to the results, the methanolic extracts of Boletus subvelutipes, Entonaema Splendens, and Tyromyces sambuceus exhibited potent ABTS radical scavenging activity above 70%.

Table 1. Mushrooms collected at Deogyu mountain, Muju, Korea, in July 2011

| Phylum                  | Class          | Order     | Family      | Genus   | Species                        |
|-------------------------|----------------|-----------|-------------|---------|--------------------------------|
| Ascomycotina            | Discomycetes   | Pezizales | Pezizaceae  | Peziza  | Vesiculosa                     |
|                         | Pyrenomycetes  | Xylariales| Xylariaceae | Enontaema| Splendens                      |
| Basidiomycotina         | Heterobasidiomycetes | Tremellales | Tremella | Foliacea |
|                         | Eubasidiomycetes | Agaricales | Agaricaceae| Agaricus | subrutilescens                |
|                         |                |           | Amanitaceae| Amanita  | longistriata rubescens         |
|                         |                |           |            |          | spissacea vagina               |
|                         |                |           |            |          | volvata                        |
|                         | Boletaceae     | Boletus   |             |          | subvelatipes fraternus         |
|                         |                |           |             |          | sp.                            |
|                         | Cortinariaceae | Xerocomus |             |         | sp.                            |
|                         |                | Cortiniarius |             |         | sp.                            |
|                         |                | Gymnopilias |             |         | liqiritiae                     |
|                         | Russulaceae    | Russula   |             |         | compacta                       |
|                         |                |           |             |          | japonica                       |
|                         |                |           |             |          | laurocerasi sanguinea                  |
|                         | Strophariaceae | Pholiota  |             |         | sp.                            |
|                         | Tricholomataceae | Laccaria  |             |         | laccata vinaceavellanea       |
| Hymenomycetidae         | Aphyllorhales  | Polyporaceae | Roseofomes | subflexibilis |
|                         |                |             | Coriolus  | consors                  |
|                         |                |             | Bjerkandera | fumosa                  |
|                         |                |             | Laetiporus | sulphureus var. miniatus        |
|                         |                |             | Tyromyces | sambuceus                  |
|                         | Stereaceae     | Stereum    |             |         | ostrea                          |

The potassium ferricyanide reduction method was used with minor modification for evaluation of reducing power activity [13]. In brief, sample (20 µL) was mixed with 50 µL of 200 mM potassium phosphate buffer (pH 6.6) and 50 µL of 1% potassium ferricyanide, followed by incubation at 50°C for 20 min. After addition of 50 µL of 10% trichloroacetic acid (w/v), the mixture was centrifuged at 650 rpm for 10 min. The upper layer (100 µL) was mixed with 100 µL distilled water and 20 µL of 0.1% ferric chloride, followed by measurement of absorbance at 700 nm. Assays were performed in triplicate, and antioxidants BHA and trolox were used as positive controls. Results are expressed as relative activity against trolox (absorbance value of sample/absorbance value of 10,000 ppm trolox). In reducing power activity, among the mushroom extracts tested, Stereum ostrea showed the highest activity, and Clitocybe sp., Laetiporus sulphureus var. miniatus, and Tyromyces sambuceus exhibited moderate activity, although
Fig. 1. 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical scavenging activities of mushroom methanolic extracts. Methanol was used for extraction of mushrooms, and 10 µL of the methanol extracts (10 mg/mL) was added to 190 µL of ABTS radical cation solution for measurement of ABTS radical scavenging activity.

Fig. 2. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities of mushroom methanolic extracts. Methanol was used for extraction of mushrooms, and 10 µL of the methanol extracts (10 mg/mL) was added to 90 µL of 150 µM methanolic DPPH for measurement of DPPH radical scavenging activity.
their activity was less than that of positive controls, as shown in Fig. 3.

Acknowledgements

This work was supported by a grant from the Korea Forest Service and, in part, by the National Research Foundation of Korea Grant funded by the Korean Government (NRF-2009-353-F00020), Republic of Korea.

References

1. Hobbs C. Medicinal mushrooms: an exploration of tradition, healing, and culture. Portland: Botanica Press; 1986.
2. Ferreira IC, Barros L, Abreu RM. Antioxidants in wild mushrooms. Curr Med Chem 2009;16:1543-60.
3. Zjawiony JK. Biologically active compounds from Aphyllorales (polypore) fungi. J Nat Prod 2004;67:300-10.
4. Lee IK, Yun BS. Styrlypyrone-class compounds from medicinal fungi Phellinus and Inonotus spp., and their medicinal importance. J Antibiot (Tokyo) 2011;64:349-59.
5. Quang DN, Hashimoto T, Asakawa Y. Inedible mushrooms: a good source of biologically active substances. Chem Rec 2006;6:79-99.
6. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44-84.
7. Gutteridge JM. Free radicals in disease process: a compilation of cause and consequence. Free Radic Res Commun 1993;19:141-58.
8. Halliwell B. Free radicals and antioxidants: updating a personal view. Nutr Rev 2012;70:257-65.
9. Lee IK, Cho SM, Seok SJ, Yun BS. Chemical constituents of Gymnopilus spectabilis and their antioxidant activity. Mycobiology 2008;36:55-9.
10. Lee IK, Han MS, Lee MS, Kim YS, Yun BS. Styrlypyrones from the medicinal fungus Phellinus baumii and their antioxidant properties. Bioorg Med Chem Lett 2010;20:5459-61.
11. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26:1231-7.
12. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958;181:1199-200.
13. Ferreira IC, Baptista P, Vilas-Boas M, Barros L. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: individual cap and stipe activity. Food Chem 2007;100:1511-6.