

RESEARCH NOTE

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

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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 *Clitocybe*, 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

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

Phylum	Class	Order	Family	Genus	Species
Ascomycotina	Discomycetes	Pezizales	Pezizaceae	<i>Peziza</i>	<i>Vesiculosa</i>
	Pyrenomycetes	Xylariales	Xylariaceae	<i>Entonaema</i>	<i>Splendens</i>
Basidiomycotina	Heterobasidiomycetes	Tremellales	Tremellaceae	<i>Tremella</i>	<i>Foliacea</i>
	Eubasidiomycetes	Agaricales	Agaricaceae	<i>Agaricus</i>	<i>subrutescens</i>
			Amanitaceae	<i>Amanita</i>	<i>longistriata</i>
					<i>rubescens</i>
					<i>spissacea</i>
					<i>vaginata</i>
					<i>volvata</i>
			Boletaceae	<i>Boletus</i>	<i>subvelutipes</i>
					<i>fraternus</i>
					sp.
				<i>Xerocomus</i>	sp.
			Cortinariaceae	<i>Cortinarius</i>	sp.
				<i>Gymnopilus</i>	<i>liquiritiae</i>
			Russulaceae	<i>Russula</i>	<i>compacta</i>
					<i>japonica</i>
					<i>laurocerasi</i>
					<i>sanguinea</i>
			Strophariaceae	<i>Pholiota</i>	sp.
			Tricholomataceae	<i>Laccaria</i>	<i>laccata</i>
					<i>vinaceoavellanea</i>
				<i>Marasmius</i>	sp.
				<i>Panellus</i>	<i>stypiticus</i>
				<i>Oudemansiella</i>	<i>platyphylla</i>
				<i>Clitocybe</i>	<i>gibba</i>
					sp.
	Hymenomycetidae	Aphyllophorales	Polyporaceae	<i>Roseofomes</i>	<i>subflexibilis</i>
				<i>Coriolus</i>	<i>consors</i>
				<i>Bjerkandera</i>	<i>fumosa</i>
				<i>Laetiporus</i>	<i>sulphureus</i> var. <i>miniatus</i>
				<i>Tyromyces</i>	<i>sambuceus</i>
			Stereaceae	<i>Stereum</i>	<i>ostrea</i>

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 *Laetiporus sulphureus* var. *miniatus*, *Stereum ostrea*, and *Tyromyces sambuceus* exhibited relatively potent activity, with DPPH radical scavenging effects of 40~60% (Fig. 2). *Stereum ostrea*, *Tyromyces sambuceus*, *Boletus subvelutipes*, *Xerocomus*

sp., and *Laetiporus sulphureus* var. *miniatus* exhibited significant scavenging activity against both ABTS and DPPH radicals.

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

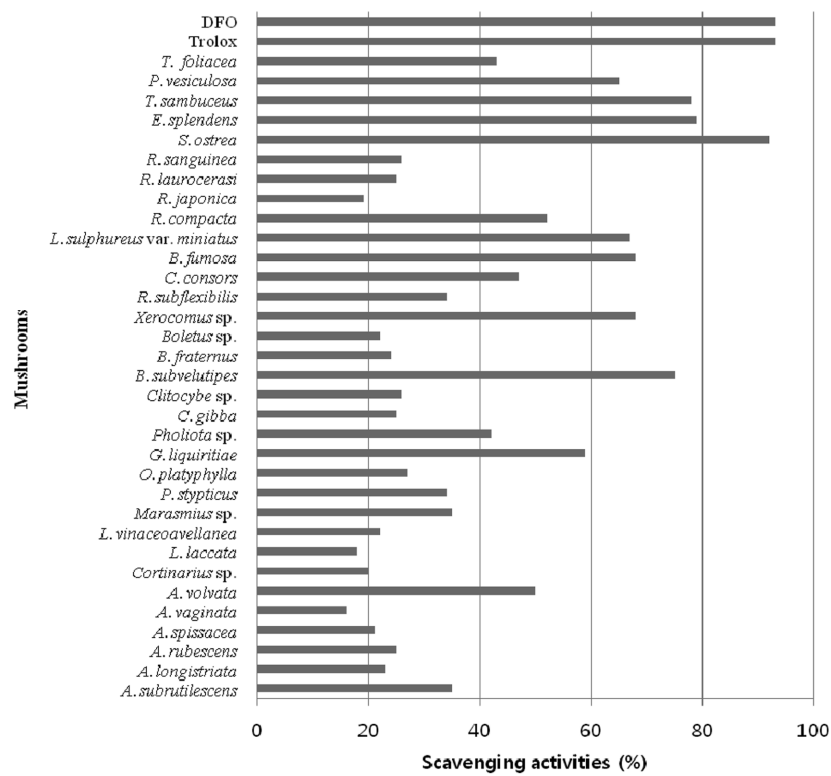


Fig. 1. 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical scavenging activities of mushroom methanolic extracts. Methanol was used for extraction of mushrooms, and 10 μ L of the methanol extracts (10 mg/mL) was added to 190 μ L of ABTS radical cation solution for measurement of ABTS radical scavenging activity.

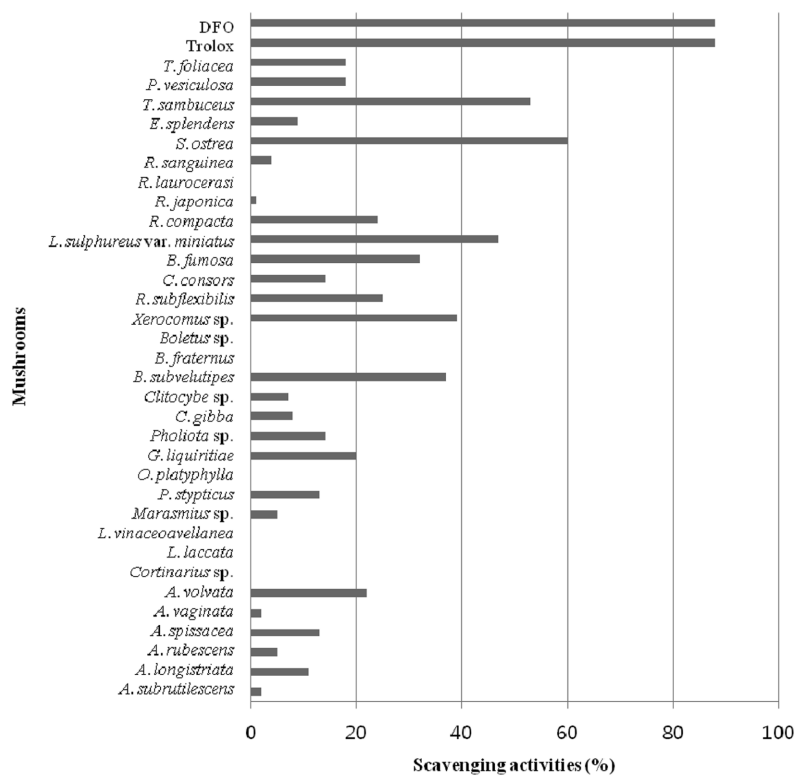


Fig. 2. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities of mushroom methanolic extracts. Methanol was used for extraction of mushrooms, and 10 μ L of the methanol extracts (10 mg/mL) was added to 90 μ L of 150 μ M methanolic DPPH for measurement of DPPH radical scavenging activity.

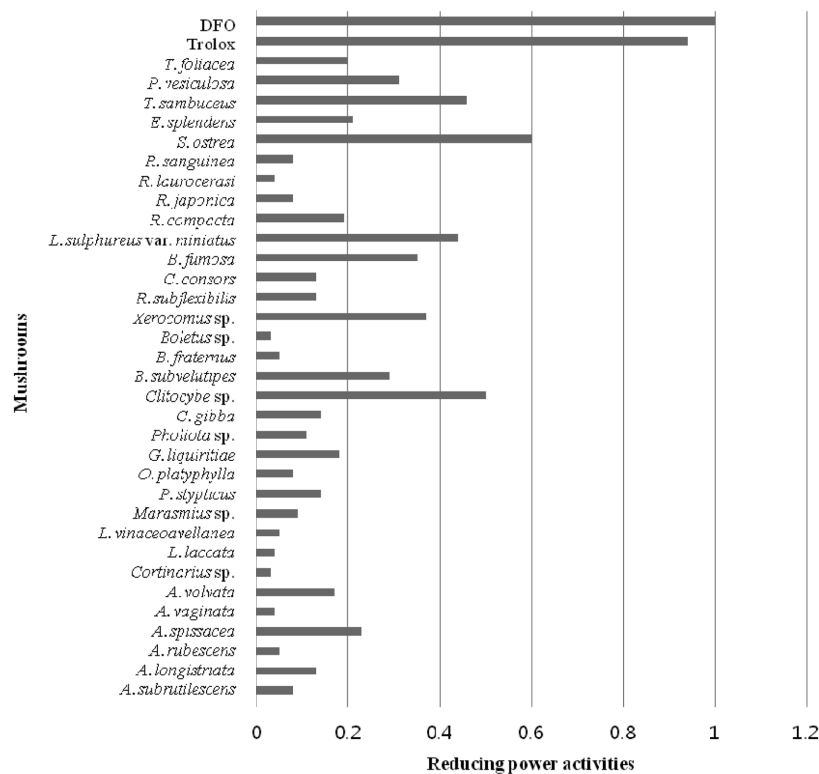


Fig. 3. Reducing power activities of mushroom methanolic extracts. Methanol was used for extraction of mushrooms, and 20 μ L of the methanol extracts (10 mg/mL) was used for measurement of reducing power activity, as described in the text.

their activity was less than that of positive controls, as shown in Fig. 3.

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