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NOTE

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Evaluation of biological activities of extracts from the fruiting body of *Pleurotus citrinopileatus* for skin cosmetics

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Abstract *Pleurotus citrinopileatus* Singer has recently become a popular delicacy in East Asian countries. We prepared a methanol extract, soluble fractions from the methanol extract, and a hot water extract of the fruiting bodies of *P. citrinopileatus*. The biological activities such as melanin biosynthesis inhibition, tyrosinase inhibition, antioxidant activities [1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, oxygen radical absorbance capacity (ORAC), and superoxide dismutase (SOD)-like activity], antibacterial activities, and antihyaluronidase activities of these extracts were evaluated. We found that the *n*-hexane-soluble, diethyl ether-soluble, and ethyl acetate-soluble fractions exhibited melanin biosynthesis inhibition in B16 melanoma cells, as well as antioxidant (ORAC) and antibacterial activities. However, the *n*-butanol-soluble and water-soluble fractions and the methanol and hot water extracts exhibited antioxidant (DPPH radical scavenging, SOD-like activity) and antihyaluronidase activities. These results indicate that the fruiting bodies of *P. citrinopileatus* have the potential to be used as an ingredient in skin cosmetics.

Key words *Pleurotus citrinopileatus* · Melanin inhibition · Antioxidant · Antibacterial · Antihyaluronidase

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Introduction

Mushrooms are a nutritionally functional food and a source of physiologically beneficial medicines. Fruiting bodies of some wild and cultivatable mushrooms contain medicinal compounds that are used in traditional medicines and cosmetics. There are numerous potential medicinal products from mushrooms that could be used in cosmeceuticals (products applied topically, such as creams, lotions, and ointments) or nutricosmetics (products that are ingested orally). There are, however, numerous mushroom species that are untested, undescribed, or not yet cultivatable and that have huge potential for use in the cosmetic industry. Some fungi are also used in biotransformation, and products such as lactic acid and ceramides could potentially be used in cosmetics.[1] The worldwide cosmetic industry is worth tens of billions of US dollars, and the industry is constantly seeking new products, especially natural products. In Asia, mushrooms have been used and acclaimed as health products for thousands of years. In China and Japan, their efficacy was appreciated so much that they were reserved for royal families. Fungi, especially mushrooms and their products, are now finding their way into cosmetics and purportedly as highly active ingredients in cosmetic products.[2]

Pleurotus citrinopileatus is an edible mushroom (Synonymy: *P. cornucopiae*, *P. cornucopiae* var. *citrinopileatus*) belonging to the genus *Pleurotus*, a member of the Pleurotaceae family. The name of this mushroom in English is the golden oyster mushroom, and it is tamogitake in Japanese, yuhuangmo in Chinese, goldenseed in Korean, and weishenga limonaya in Russian. It grows on fallen trees and stumps of broad-leaf tree species such as *Ulmus* sp., *Quercus serrata*, *Acer palmatus*, and *Fraxinus mandshurica*.

Several recent studies have focused on the cultivation of *P. citrinopileatus* with numerous multifunctional biological activities, for example antigenotoxicity,[3] angiotensin-converting enzyme (ACE) inhibition activity,[4] antihyperlipidemic activity,[5] antiatopic dermatitis activity,[6] antioxidant activities,[7] anticancer activity,[7] and HIV-1 reverse transcriptase inhibitory activity.[8] There are a limited number of

previous studies on the chemical composition of *P. citrinopileatus*, and there have been a few reports on lectin, peptides, and proteins identified from water extracts of *P. citrinopileatus*.[4,9]

The purpose of the present study was to evaluate some of the biological activities, such as melanin inhibition, tyrosinase inhibition, antioxidant, antibacterial, and antihyaluronidase activities, of methanol extracts and hot water extracts of *P. citrinopileatus* and crude fractions prepared from the methanol extract in order to assess the potential of *P. citrinopileatus* for use in skin cosmetics.

Materials and methods

Mushroom materials

Fresh fruiting bodies of *P. citrinopileatus* were obtained from Tamogitake Pharmaceutics Co., Ltd. (Nagano, Japan). The fruiting bodies were cleaned to remove any residual materials and then freeze dried. Milled freeze-dried *P. citrinopileatus* samples (10.0 g each) were extracted with methanol (2 × 100 ml) at room temperature with a shaker at 150 rpm for 48 h and then filtered. The methanol extract was concentrated by a rotary evaporator. To prepare the hot water extract, the freeze-dried *P. citrinopileatus* (10.0 g) was treated with 2000 ml of distilled water and boiled (100°C) for 1 h; the hot water extract was then centrifuged at 1.1×10^3 g for 20 min and the supernatant was freeze dried. The yield of the methanol and hot water extracts were 1.0 g (10.0%) and 1.2 g (12.0%), respectively.

The methanol extract (1.0 g) was successively partitioned with a series of organic solvents: *n*-hexane, diethyl ether, ethyl acetate, and *n*-butanol. Each soluble fraction was concentrated by a rotary evaporator and the resultant residue was put in water to form a suspension, and then freeze-dried. The recovery rate for each solvent was *n*-hexane (51.0 mg, 5.1%), diethyl ether (43.0 mg, 4.3%), ethyl acetate (59.0 mg, 5.9%), *n*-butanol (209.0 mg, 20.9%), and water (563.0 mg, 56.3%).

Inhibitory effect on melanogenesis using cultured B16 melanoma cells

Determination of melanin content

This assay was performed as previously described by Arung et al.[10] The melanin content (MC) of cells after treatment was determined as follows. After removing the medium and washing the cells, the cell pellet was dissolved in 1.0 ml of 1 N NaOH. The crude cell extracts were assayed using a microplate reader (Bio-Tek, USA) at 405 nm to determine melanin content. The results from the samples were analyzed as a percentage of the control culture. Arbutin was used as a positive control.

Cell viability

Cell viability (CV) was determined by use of the microculture tetrazolium technique (MTT).[10] Culture was initiated, and after incubation, 50 µl of MTT bromide in phosphate-buffered saline (5 mg/ml) was added to each well. The plates were incubated for 4 h. After removing the medium, formazan crystals were dissolved in 1.0 ml of 0.04 N HCl and the absorbance was measured at 570 nm relative to 630 nm.

Tyrosinase assay

Although tyrosinase catalyzes a reaction between two substrates, a phenolic compound and oxygen, the assay was carried out in an air-saturated solution. Kojic acid[11] was used as a positive control. The assay was performed as previously described.[10] The absorbance at 475 nm was measured using a V530 spectrophotometer (Jasco, Japan) at 3 min for L-DOPA (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and 10 min for L-tyrosine (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Antioxidant assay

Oxygen radical absorbance capacity assay

The oxygen radical absorbance capacity (ORAC) assay was performed as described previously by Prior et al.[12] Data are expressed as milligrams of Trolox equivalent (TE) per milligram of sample (mg TE/mg).

1,1-Diphenyl-2-picrylhydrazyl radical scavenging assay

This assay was performed as described previously by Shimamura et al.,[13] with minor modifications. The sample was first dissolved in ethanol or dimethylsulfoxide (DMSO, Osaka, Japan). The reaction mixture contained 1000 µl of 0.2 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Tokyo Chemical Industry Co., Ltd., Japan) in ethanol, 800 µl of 0.1 M Tris-HCl buffer (pH 7.4), and 200 µl of sample solution in ethanol or DMSO. After the reaction was carried out at room temperature for 30 min, the free radical scavenging activity of the sample was quantified by the decolorization of DPPH at 517 nm.

Superoxide dismutase-like activity

Superoxide dismutase (SOD)-like activity was evaluated by using the SOD Assay Kit-WST (Dojindo Molecular Technologies, Kumamoto, Japan) according to the method described in previous studies.[14] The sample was dissolved in Milli-Q water or ethanol and added to the WST working solutions (200 µl) containing 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2-*H*-tetrazolium in 50 mM carbonate buffer (pH 10.2). The enzyme working solution (20 µl) containing xanthine oxidase in the same buffer was added and then incubated for 10 min. The absorbance of each sample was measured at 450 nm in a Tecan Spectra microplate reader (Tecan Japan, Kanagawa, Japan). One unit of SOD-like activity was defined as the amount of extract in 20 µl of sample solution that inhibits the reduction reaction of WST-1 with superoxide anion

Table 1. Effects of methanol extract, soluble fractions prepared from the methanol extract, and hot water extract of *Pleurotus citrinopileatus* on melanin biosynthesis and cell proliferation of B16 melanoma cells

Samples tested	100 μg/ml			75 μg/ml			50 μg/ml			25 μg/ml		
	MC	CV	Type[a]	MC	CV	Type	MC	CV	Type	MC	CV	Type
Methanol extract	96.1 ± 0.97	87.1 ± 3.95	B	94.5 ± 3.67	95.9 ± 1.57	B	98.5 ± 9.9	93.2 ± 0.49	B	102.8 ± 4.03	97.1 ± 0.96	B
n-Hexane soluble	16.9 ± 1.75	20.1 ± 2.02	C	20.5 ± 1.66	31.5 ± 7.69	A	36.9 ± 6.89	61.3 ± 5.73	A	75.5 ± 7.16	85.3 ± 5.96	B
Diethyl ether soluble	27.7 ± 3.43	53.6 ± 8.42	A	36.0 ± 1.56	65.8 ± 2.48	A	54.6 ± 6.20	65.7 ± 2.40	A	84.5 ± 4.9	95.8 ± 2.69	A
Ethyl acetate soluble	41.2 ± 1.42	70.7 ± 4.28	A	47.3 ± 2.55	75.4 ± 0.87	A	53.8 ± 2.42	76.8 ± 8.61	A	90.2 ± 0.84	97.9 ± 4.04	B
n-Butanol soluble	116.6 ± 2.23	99.7 ± 2.04	B	121.2 ± 4.21	101.2 ± 0.27	B	116.1 ± 1.23	100.5 ± 1.85	B	118.8 ± 4.12	103.4 ± 0.89	B
Aqueous soluble	99.8 ± 3.12	98.4 ± 1.59	B	103.5 ± 4.91	96.1 ± 0.47	B	97.1 ± 3.69	98.2 ± 1.27	B	103.6 ± 1.42	98.3 ± 1.62	B
Hot water extract	116.3 ± 3.31	96.1 ± 3.62	B	104.5 ± 4.11	91.8 ± 6.04	B	116.7 ± 11.24	90.7 ± 1.93	B	106.5 ± 9.73	87.1 ± 4.73	B
Arbutin (positive control)	40.7 ± 1.45	98.4 ± 3.85	A	43.2 ± 2.76	86.4 ± 0.76	A	45.8 ± 2.46	85.2 ± 3.12	A	53.6 ± 0.77	84.7 ± 3.32	A

Data presented as means ± SD ($n = 3$)
MC, melanin content (%); CV, cell viability (%)
[a]Type A, CV-MC ≧ 10%; type B, MC & CV ≧ 75%; type C, MC & CV ≦ 25%

by 50%. The SOD-like activity (U/mg) of each extract was calculated by using the 50% inhibition value (IC_{50}) of the extract.

Antibacterial activity

The *Escherichia coli* IFO 3301 and *Staphylococcus aureus* IFO 13276 bacterial strain was used. The bacterial strains were kept as stock cultures maintained in nutrient broth (NB, Difco, USA) medium supplemented with 50% (v/v) glycerine and stored at –80°C. The stock bacteria were then incubated in nutrient agar (NA, Franklin Lakes, NJ, USA) medium at 37°C for 24 h. A single colony obtained from preculture of 20 ml of NB medium was incubated at 37°C and 160 rpm for 8 h. This antibacterial assay was performed as previously described by Veluri et al.[15] with slight modifications. Eighty milliliters of NB medium and 10 μl of 20% DMSO were added with or without (control) the test samples at various concentrations into 96-well plastic cell culture plates. Precultured bacteria in NB medium (10 μl) at a density of 1.0×10^5 CFU/ml (*E. coli*) or 1×10^6 CFU/ml (*S. aureus*) were then added into each well and incubated for 10 h or 12 h, respectively. The absorbance was measured at 630 nm.

Antihyaluronidase activity

Antihyaluronidase activity was measured by using the previously described method.[16] Test samples were dissolved in DMSO, and each solution was diluted with 0.1 M acetate buffer (pH 4.0) to ten volumes. Hyaluronidase, hyaluronic acid potassium salt, and compound 48/80 were dissolved with the same buffer. A mixture of sample solution (0.20 ml) and 2000 units/ml of hyaluronidase solution (0.10 ml) were preincubated at 37°C for 20 min. Then 0.10 mg/ml of compound 48/80 solution (0.20 ml) was added and each mixture was incubated at 37°C for 20 min. After incubation, the assay was commenced by adding 0.8 mg/ml of hyaluronic acid potassium salt solution (0.50 ml) to each tube and incubating at 37°C for 40 min. Reactions were terminated by the addition of 0.4 N NaOH solution. The absorbance was measured at 585 nm.

Cromolyn interrupts the physiological response to nasal antigens and, when used prophylactically, can prevent the onset of symptoms as well as treat nasal allergy symptoms once they occur. In addition to its effects on mast cells, cromolyn inhibits macrophages, eosinophils, monocytes, and platelets believed to play a role in the inflammatory response.[17] Thus, cromolyn (Sigma Aldrich, USA) was used as a positive control.

Results and discussion

In the present study, a methanol extract and hot water extract of *P. citrinopileatus* and soluble fractions prepared from the methanol extract were examined for biological activities at various concentrations. Several bioassays such as melanin inhibition (Table 1), tyrosinase inhibition

Table 2. The effect of methanol extract, soluble fractions prepared from methanol extract, and hot water extract of *P. citrinopileatus* on tyrosinase

Samples[a]	Tyrosinase inhibition (% *vs* control)	
	L-DOPA[b]	L-Tyrosine[b]
Methanol extract	2.8 ± 2.44	3.0 ± 2.78
n-Hexane soluble	10.5 ± 3.51	28.8 ± 1.22
Diethyl ether soluble	8.3 ± 2.36	2.1 ± 1.82
Ethyl acetate soluble	8.3 ± 2.36	27.4 ± 2.47
n-Butanol soluble	10.3 ± 3.42	41.0 ± 1.35
Aqueous soluble	2.5 ± 3.95	9.1 ± 4.16
Hot water extract	6.5 ± 1.32	2.3 ± 2.15
Kojic acid (positive control)	100.0 ± 0.06	98.6 ± 0.61

Data presented as means ± SD (n = 3)
[a] Sample concentration was 100 μg/ml
[b] Substrate: L-DOPA, L-dihydroxyphenylalanine

Table 3. Antioxidant activity of the methanol extract, soluble fractions prepared from the methanol extract, and the hot water extract of *P. citrinopileatus*

Sample	Antioxidant assay		
	ORAC (mg TE/mg)[a]	DPPH [IC_{50} (mg/ml)]	SOD-like activity (U/mg)
Methanol extract	0.08 ± 0.007	5.4	0.3 ± 0.05
n-Hexane soluble	0.25 ± 0.009	>4.0[b]	0.01[c]
Diethyl ether soluble	0.20 ± 0.008	>4.0[b]	0.01[c]
Ethyl acetate soluble	0.08 ± 0.002	>1.7[b]	0.01[c]
n-Butanol soluble	0.13 ± 0.006	7.9	nd
Aqueous soluble	0.04 ± 0.004	5.2	1.9 ± 0.63
Hot water extract	0.05 ± 0.003	4.2	2.7 ± 0.59

Data presented as means ± SD (n = 3)
[a] ORAC values are expressed as relative Trolox equivalent per microgram
[b] No activity up to each concentration, but not tested at higher concentration because of limited solubility
[c] Hardly any activity
ORAC, oxygen radical absorbance capacity; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical scavenging; SOD, superoxide dismutase; nd, not determined because below measurable limit

(Table 2), antioxidant activity (Table 3), antibacterial activity (Table 4), and antihyaluronidase activity (Table 5) were conducted.

Melanin inhibition activity

After treatment of B16 melanoma cells with various samples for 3 days, the cells were harvested and two different parameters of cellular function were measured by bioassay: cell viability (CV) and melanin content (MC). An important concept when selecting bioactive extracts that modulate skin pigmentation is that, for obvious reasons, they should have minimal effects on cell proliferation and survival. In these experiments, the effects on cell proliferation were assessed using the MTT assay, and the effects on melanin content were measured by absorbance at 405 nm (Table 1).

Table 4. Antibacterial activity of methanol extract, soluble fractions prepared from methanol extract, and hot water extract of *P. citrinopileatus*

Samples tested	Concentration (mg/ml)	Growth inhibition (% *vs* control)	
		S. aureus	*E. coli*
Methanol extract	2.0	–	–
n-Hexane soluble	0.5	79.6 ± 1.81	87.0 ± 4.72
Diethyl ether soluble	1.0	35.5 ± 7.18	25.7 ± 7.42
Ethyl acetate soluble	0.3	75.6 ± 3.79	60.0 ± 7.43
n-Butanol soluble	0.5	–	–
Aqueous soluble	1.0	–	26.4 ± 7.85
Hot water extract	2.0	–	–
Sorbic acid (positive control)	0.2	100 ± 0.68	100 ± 4.12

Data presented as means ± SD (n = 3)
–, no antibacterial activity

Table 5. The effect of methanol extract, soluble fractions prepared from the methanol extract, and hot water extract of *P. citrinopileatus* on hyaluronidase

Samples tested	Concentration (mg/ml)	Hyaluronidase inhibition (% *vs* control)
Methanol extract	4.1	25.4 ± 2.1
n-Hexane soluble	3.7	–
Diethyl ether soluble	2.7	–
Ethyl acetate soluble	2.5	–
n-Butanol soluble	2.0	9.7 ± 2.1
Aqueous soluble	1.1	10.8 ± 1.7
Hot water extract	2.1	–
Cromolyn sodium salt (positive control)	1.0	84.8 ± 1.0

Data presented as means ± SD (n = 3)
–, no hyaluronidase inhibition

Samples which showed a percentage of melanin content of at least 10% less than the cell viability (i.e., CV-MC ≧ 10) were judged as possible active ingredients, and classified as type A. As shown in Table 1, *n*-hexane-soluble, diethyl ether-soluble, and ethyl acetate-soluble fractions were considered as possible candidates based on their effects on B16 melanoma cells. More specifically, the *n*-hexane-soluble (50 μg/ml), diethyl ether-soluble (75 μg/ml), and ethyl acetate-soluble (100 μg/ml) fractions inhibited melanin production of B16 melanoma cells by 63.1%, 64.0%, and 58.8%, with cell viability of 61.3%, 65.8%, and 70.7%, respectively.

This study also found that some extracts showed higher cytotoxicity of more than 75% versus the control at a concentration of 100 μg/ml (e.g., the *n*-hexane-soluble fraction), which prohibited us from evaluating their effects on the melanin biosynthesis of B16 melanoma cells. Therefore, extracts that inhibited the growth of B16 melanoma cells more than 75% versus the control were classified as type C. However, the *n*-hexane-soluble fraction, which showed potent cytotoxicity at 100 μg/ml, exceeded our established standard (type A: CV-MC ≧ 10) at 75 μg/ml and 50 μg/ml.

Tyrosinase inhibition activity

Tyrosinase (EC 1.14.18.1) is a key enzyme in melanin synthesis. We investigated the effects of each soluble fraction and extract of *P. citrinopileatus* on tyrosinase activity. Table 2 shows that all extracts and soluble fractions exhibited poor tyrosinase inhibitory activity compared with kojic acid (a positive control) for substrates (L-DOPA and L-tyrosine) at a concentration of 100 μg/ml. The *n*-hexane-soluble and *n*-butanol-soluble fractions with L-DOPA as a substrate showed tyrosinase inhibition at 10.5% and 10.3%, respectively. The activity of kojic acid used as a positive control was taken as 100.0% inhibition at 100 μg/ml. In addition, at 100 μg/ml of samples with L-tyrosine as substrate, the *n*-hexane-soluble, ethyl acetate-soluble, and *n*-butanol-soluble fractions showed 28.8%, 27.4%, and 41.0% inhibitory activity, respectively. Kojic acid used as a positive control showed 98.6% inhibition at 100 μg/ml. Based on these results, none of the extracts and soluble fractions prepared from *P. citrinopileatus* inhibited more than 50% of tyrosinase activity up to the concentration of 100 μg/ml.

Kojic acid is known as a tyrosinase inhibitor and is used as a skin-whitening cosmetic. Hydroquinone-related compounds have also been used as effective depigmenting agents for skin overpigmentation, but they are strong irritants and exhibit cell toxicity.[18] It has been reported that some growth factors or cytokines such as basic fibroblast growth factor (bFGF), endothelin-1 (ET-1), and α-melanocyte stimulating hormone (α-MSH)[19] are secreted from ultraviolet (UV)-irradiated keratinocytes or melanocytes and stimulate the proliferation or melanogenesis of human melanocytes.

Considering this information, the results shown in Tables 1 and 2 indicate that the inhibitory effects of *n*-hexane-soluble, diethyl ether-soluble, and ethyl acetate-soluble fractions were not caused by the inhibition of tyrosinase activity. The depigmenting mechanism of these extracts involved the suppression of some pigmenting signals in stimulating melanogenesis rather than the direct inhibition of tyrosinase activity.

Antioxidant activity

Skin is a major candidate target of oxidative stress caused by reactive species (RS), including reactive oxygen species and reactive nitrogen species. RS are major and significant contributors to skin hyperpigmentation and skin aging. It is generally believed that agents having antioxidant activity show anti-aging, whitening, and anti-inflammatory activities.[20] If free radicals are inappropriately processed in melanin synthesis, hydrogen peroxide (H_2O_2) is generated, leading to the production of hydroxyl radicals (HO^-) and other reactive oxygen species (ROS).[21] Oxidative stress may be induced by increasing the generation of ROS and other free radicals. UV radiation can induce the formation of ROS in skin such as singlet oxygen and superoxide anions, promoting biological damage in exposed tissues via iron-catalyzed oxidative reactions. These ROS enhance melanin biosynthesis, damage DNA, and may induce proliferation of melanocytes.[22] Yamakoshi et al.[22] found evidence for a role of oxidative stress in the pathogenesis of skin disorders. It is known that ROS scavengers or inhibitors such as antioxidants may reduce hyperpigmentation. Additionally, superoxide dismutase (SOD, EC 1.15.1.1), which catalyzes the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen, is one of the most important antioxidative enzymes.

Table 3 shows that the *n*-hexane-soluble and diethyl ether-soluble fractions contributions to the oxygen radical absorbance capacity (ORAC) were 0.25 and 0.20 mg TE/mg, respectively. However, the ethyl acetate-soluble, *n*-butanol-soluble, and aqueous soluble fractions and the methanol and hot water extracts showed lower oxygen radical absorbance capacities of 0.08, 0.13, 0.04, 0.08, and 0.05 mg TE/mg, respectively.

DPPH is a stable radical that is used in a popular method of screening for free-radical-scavenging ability. Table 3 shows the DPPH radical scavenging activity of the samples. The *n*-butanol-soluble fraction, aqueous-soluble fraction, methanol extract, and hot water extract showed moderate DPPH radical scavenging activity. These extracts caused 50% scavenging (IC_{50}) of the DPPH radical scavenging activity at concentrations of 7.9, 5.2, 5.4, and 4.2 mg/ml, respectively. The aqueous-soluble fraction, methanol extract, and hot water extract exhibited 1.9, 0.3, and 2.7 U/mg of SOD-like activity, respectively.

Ergothioneine (ESH), a potent antioxidant, has been found in certain edible mushrooms.[23] Large variations in total phenolics and ESH content have been found in extracts prepared from the fruiting bodies of different edible mushrooms. High ESH content was found in hydrophilic extracts prepared from the fruiting bodies of four culinary mushrooms: *Flammulina velutipes*, *Lentinula edodes*, *Pleurotus cornucopiae,* and *Pleurotus eryngii*. Among them, the *P. cornucopiae* extract contained the highest amount of ESH at 20.82 mg/ml.[24] Our results suggested that the soluble fractions and extracts from *P. citrinopileatus* are a potential source of antioxidant materials, although their activities were only moderate.

Antibacterial activity

The general category of cosmetic skin care products includes sunscreens to protect the skin from UV damage, antioxidant products to repair or hide skin imperfections (e.g., wrinkles, dark circles), and antibacterial products to treat acne. *S. aureus* and *E. coli* are widespread, potentially pathogenic types of bacteria that cause some skin conditions such as acne, comedo, papules, pustules, and cellulitis. Therefore we evaluated the antibacterial activity of the extracts and soluble fractions prepared from *P. citrinopileatus* against *S. aureus* and *E. coli*. Table 4 shows that the *n*-hexane-soluble (0.5 mg/ml) and ethyl acetate-soluble (0.3 mg/ml) fractions showed high antibacterial activity with growth inhibition of 79.6% and 75.6% against *S. aureus*, and 87.0% and 60.0% against *E. coli*, respectively.

Mushrooms are rich sources of natural antibiotics; in mushrooms, cell wall polysaccharides such as glucans are well known for their immunomodulatory properties, and many of the secreted secondary metabolites (extracellular secretions by the mycelium) combat bacteria and viruses.[25] Several compounds such as lentinic acid and lenthionine extracted from *Lentinula edodes* (shiitake in Japanese, xiang gu in Chinese) revealed antifungal and antibacterial activity against *S. aureus*, *Bacillus subtilis*, and *E. coli*.[26] Several studies have reported on the antimicrobial activity of other edible mushrooms and their bioactive compounds. Antioxidant and antimicrobial activities of an extract of *Laetiporus sulphureus* have been reported and were correlated to its phenol and flavonoid contents; guaiane sesquiterpenoids isolated from the fruit bodies of edible *Lactarius* species proved to have antibacterial activity.[27] In addition, it has already been reported that methyl alcohol extracts of *Pleurotus eryngii* var. *eryngii*, *P. eryngii* var. *ferulae*, *P. ostreatus*, *P. sajor-caju*, *Terfezia boudieri*, and *Agaricus bisporus* inhibit to different degrees the growth of *Bacillus megaterium*, *S. aureus*, *E. coli*, *Klebsiella pneumoniae*, *Candida albicans*, *C. glabrata*, *Trichophyton* spp., and *Epidermophyton* spp.[28] However, study of the antibacterial activity of *P. citrinopileatus* has not been reported. Our results indicated that the hydrophobic components in *n*-hexane-soluble and ethyl acetate-soluble fractions of *P. citrinopileatus* have potential as antimicrobial ingredients.

Antihyaluronidase activity

Hyaluronidase (EC 3.2.1.35) is an endoglycosidase that randomly cleaves internal *β-N*-acetyl-hexosamine glucosidic linkages in hyaluronic acid (HA).[29] Furthermore, hyaluronidase is an enzyme that degrades hyaluronic acid. Mucopolysaccharides are related to histamine release from mast cells in inflammatory reactions. The enzyme is distributed in a wide range of mammalian tissues, such as skin, synovial fluid, serum, alveolar macrophages, brain, kidney, liver, spleen, and lung. Since hyaluronidase is related to histamine release from mast cells, the inhibitory effect of this enzyme is one of the indexes of anti-type I allergy activity.[30] Table 5 shows that the *n*-butanol-soluble fraction, aqueous-soluble fraction, and methanol extract from *P. citrinopileatus* exhibited antihyaluronidase activity of 9.7%, 10.8%, and 25.4%, at concentrations of 2.0, 1.1, and 4.1 mg/ml, respectively.

Conclusions

In this study, the melanin inhibition, tyrosinase inhibition, antioxidant activities, antibacterial activities, and antihyaluronidase activities of methanol and hot water extracts of the fruiting bodies of *P. citrinopileatus* and soluble fractions prepared from the methanol extract were evaluated. We found that some soluble fractions, e.g., those of *n*-hexane (50 μg/ml), diethyl ether (75 μg/ml), and ethyl acetate (100 μg/ml), inhibited melanin production in B16 melanoma cells by 63.1%, 64.0%, and 58.8%, with cell viability of 61.3%, 65.8%, and 70.7%, respectively. The ORAC value of the *n*-hexane-soluble fraction and diethyl ether-soluble fraction were 0.25 and 0.2 mg TE/mg. On the other hand, the *n*-butanol-soluble fraction, aqueous-soluble fraction, methanol extract, and hot water extract exhibited only moderate antioxidant potential; their inhibitory concentration (IC_{50}) that caused 50% scavenging of the DPPH radical scavenging were 7.9, 5.2, 5.4, and 4.2 mg/ml, respectively. The aqueous-soluble fraction, methanol extract, and hot water extract exhibited 1.9, 0.3, and 2.7 U/mg of SOD-like activity, respectively. The *n*-hexane-soluble fraction (0.5 mg/ml) and ethyl acetate-soluble fraction (0.3 mg/ml) showed potential antibacterial activity. The *n*-butanol-soluble fraction (2.0 mg/ml), aqueous-soluble fraction (1.1 mg/ml), and methanol extract (4.1 mg/ml) showed antihyaluronidase activity of 9.7%, 10.8%, and 25.4% respectively. The fruiting bodies of *P. citrinopileatus*, therefore, have potential as a source of ingredients in skin cosmetics because of their antimelanogenesis, antioxidant, antimicrobial, and antihyaluronidase activities.

There are many requirements for cosmetics products, but most importantly they should be safe to use, have no side effects, and have positive effects on the skin.[1] Considering these criteria and the above-described biological activities, the edible mushroom *P. citrinopileatus* should be preferred as a source of ingredients for cosmetics products. Currently, isolation and identification of the biologically active components from the extracts of *P. citrinopileatus* are in progress.

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