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NOTE

A glucosylceramide with antimicrobial activity from the edible mushroom *Pleurotus citrinopileatus*

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Abstract The golden oyster mushroom *Pleurotus citrinopileatus* is a popular edible mushroom with multifunctional biological activities, but there are a limited number of previous studies on its chemical composition. This is the first report of the isolation of glucosylceramide with antimicrobial activity from the fruiting body of this mushroom. This compound was identified as 1-*O*-*β*-D-glucopyranosyl-(2*S*, 3*R*,4*E*,8*E*)-2-[(2*R*)-2-hydroxyhexadecanoylamino]-9-methyl-4,8-octadecadiene-1,3-diol. The IC_{50} value of this compound for the growth of *Escherichia coli* and *Staphylococcus aureus* was 275.1 μM (200 μg/ml) and 323.2 μM (235 μg/ml), respectively.

Keywords *Pleurotus citrinopileatus* · Glucosylceramide · Antimicrobial activity · Tamogitake

Introduction

Mushrooms are a nutritionally functional food and a source of physiologically beneficial medicines. *Pleurotus citrinopileatus* is an edible mushroom (Synonymy: *P. cornucopiae*, *P. cornucopiae* var. *citrinopileatus*) belonging to the genus *Pleurotus*, Pleurotaceae family. The name of this mushroom in English is golden oyster mushroom, tamogitake in Japanese, yuhuangmo in Chinese, goldenseed in Korean, and weishenga limonaya in Russian. A half-dozen recent studies have focused on the cultivation of *P. citrinopileatus* for its numerous multifunctional biological activities, including antigenotoxicity [1], angiotensin-converting enzyme (ACE) inhibition activity [2], antihyperlipidemic activity [3], antiatopic dermatitis activity [4], antioxidant activities [5], anticancer activity [5], and HIV-1 reverse transcriptase inhibition activity [6]. There are a limited number of previous studies on the chemical composition, and there have been few reports identifying the lectin, peptide, and protein from water extracts of *P. citrinopileatus* [2, 7].

Our previous study showed that the fruiting body of *P. citrinopileatus* had melanin biosynthesis inhibition, antioxidant, antibacterial, and antihyaluronidase activities [8]. Thus, the investigation of these biologically active constituents in the fruiting bodies of *P. citrinopileatus* led us to isolate an antimicrobial compound, glucosylceramide.

Materials and methods

General experimental procedure

The nuclear magnetic resonance (NMR) spectra were obtained on a JNM-AL400 FT-NMR spectrometer (JEOL, 400 MHz) in CD_3OD. The structure was assigned by means of ^1H and ^{13}C NMR, distortionless enhancement by polarization transfer (DEPT), heteronuclear multiple quantum correlation (HMQC), heteronuclear multiple bond correlation (HMBC), and ^1H–^1H correlation spectroscopy (COSY) experiments, in combination with liquid chromatography electrospray ionization-time of flight mass spectrometry (LC-ESI-IT-TOF–MS) (Shimadzu Corporation)

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and fast atom bombardment mass spectrometry (FAB-MS) (JEOL, JMS-700 mass spectrometer). Column chromatography was performed by silica-gel (Wakogel C-200 particle size 75–150 μm, Wako). Thin-layer chromatography (TLC) was carried out using Merck pre-coated silica-gel 60 F_{254} plates (0.25 mm) and spots were detected with I_2 detection and under UV light. The compound was isolated by preparative high-performance liquid chromatography (HPLC) using a Waters™ 600 Controller, Waters™ 486 Tunable Absorbance Detector and Waters 600 Multi-solvent Delivery System. Optical rotation was measured on a JASCO DIP-370 digital polarimeter. The absorbance was measured by Tecan Spectra microplate reader (Tecan Japan, Kanagawa, Japan) and UV/vis Spectrometer V-530 (JASCO Corporation). Fluorescence was recorded on FP-6500 Research Fluorescence Spectrometer (JASCO Corporation).

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. The milled freeze-dried *P. citrinopileatus* (900.0 g) were extracted with methanol (2 × 9.0 l) at room temperature for 1 week and then filtered. The methanol extract was concentrated by a rotary evaporator. The yield of the methanol extract was 114.0 g (12.7%).

Extraction and isolation

A portion of the methanol extract (100.0 g) was applied to a silica gel column [Wakogel C-200 (4.0 kg), 19 cm i.d. × 50 cm] and eluted with *n*-hexane/chloroform (7:3, 5:5, 3:7, 0:10), ethyl acetate, acetone, ethanol, and methanol (each 8.0 l), followed by methanol/water (50:1, 6.0 l; 11:1, 2.0 l; 8:1, 3.0 l), affording eight fractions (Fr 1 to Fr 8). Fr 4 (1.0 mg/ml) and Fr 5 (1.4 mg/ml) showed antimicrobial activity with growth inhibition of 98.5 and 100.0% against *Escherichia coli* IFO3301 for 8 h, respectively. Based on TLC analysis, using hexane/chloroform/methanol (1:9:1), Fr 4 (R_f = 0.34, 0.42, 0.60, 0.72, 0.84) and Fr 5 (R_f = 0.34, 0.47, 0.60, 0.72, 0.84) were combined to yield Fr 4′ (6.9 g) (R_f = 0.34, 0.42, 0.47, 0.60, 0.72, 0.84). A portion of the Fr 4′ (4.5 g) was then applied to a silica gel column [Wakogel C-200 (750 g), 5.5 cm i.d. × 120 cm] and eluted with *n*-hexane/chloroform (9:1, 0:10), chloroform/ethyl acetate (5:5), chloroform/ethyl acetate/ethanol (4:8:2), ethyl acetate/ethanol (5:5), *n*-butanol/ethanol (5:5), dichloromethane/*n*-butanol/ethanol (2:2:10), ethanol, ethanol/methanol (5:5), and methanol (each 1.4 l) to give eight fractions (Fr 4′-1 to Fr 4′-8). The Fr 4′-6 (303.5 mg, R_f = 0.06, 0.16, 0.30, 0.82; chloroform/ethyl acetate = 5/5) was purified by preparative HPLC and eluted with an Inertsil Prep-ODS column (20 mm i.d. × 250 mm) at a flow rate of 8.0 ml/min of methanol and a retention time (t_R) was 25.5 min to give compound **1**, which was identified by NMR and MS analysis as a glucosylceramide (38.4 mg) as follows.

1-*O*-*β*-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*)-2-[(2*R*)-2-hydroxyhexadecanoylamino]-9-methyl-4,8-octadecadiene-1,3-diol. (**1**): White amorphous powder. $[\alpha]_D^{23}$ + 5.0° (*c* = 0.3, CH_3OH). FAB-MS (positive ion mode; matrix: nitrobenzyl alcohol) *m/z*: 710 $[M+H-H_2O]^+$, 548 $[M+H-H_2O-Glu]^+$; LCMS-IT-TOF *m/z*: 750.5488 $[M+Na]^+$, 726.5535$[M-H]^-$ (calcd for $C_{41}H_{76}NO_9$: 726.5520).

^{1}H-NMR (in CD_3OD): δ 3.25 (m, 1H, H-1a), 3.78 (m, 1H, H-1b), 3.63 (m, 1H, H-2), 3.88 (m, 1H, H-3), 5.39 (dd, *J* = 15.3, 7.3 Hz, 1H, H-4), 5.64 (m, 1H, H-5), 1.88 (m, 2H, H-6), 1.97 (m, 2H, H-7), 4.95 (m, 1H, H-8), 1.97 (m, 2H, H-10), 1.19–1.31 (m, 14H, H-11/17), 0.80 (t, *J* = 6.9 Hz, 3H, H-18), 1.50 (s, 3H, H-19), 7.25 (1H, d, *J* = 8.7 Hz, NH), 4.04 (m, 1H, H-2′), 1.62 (m, 1H, H-3′a), 1.45 (m, 1H, H-3′b), 1.19/1.31 (m, 24H, H-4′/15′), 0.80 (t, *J* = 6.9 Hz, 3H, H-16′), 4.17 (d, *J* = 7.7, 1H, H-1″), 3.10 (dd, *J* = 9.1, 7.8 Hz, 1H, H-2″), 3.17/3.19 (m, 3H, H-3″/5″), 3.60 (m, 2H, H-6″).

^{13}C NMR (in CD_3OD): δ 69.73 (C-1), 54.67 (C-2), 72.94 (C-3), 134.60 (C-4), 131.10 (C-5), 33.05 (C-6), 33.76 (C-7), 124.82 (C-8), 136.79 (C-9), 40.75 (C-10), 23.70–30.79 (C-11/C-17), 14.41 (C-18, CH_3), 16.15 (C-19), 177.18 (C-1′), 73.15 (C-2′), 35.88 (C-3′), 23.70–30.79 (C-4′/15′), 14.41 (C-16′), 104.72 (C-1″), 75.01 (C-2″), 77.99 (C-3″), 71.60 (C-4″), 77.90 (C-5″), 62.73 (C-6″).

Antibacterial activity

The *E. coli* IFO 3301 or *Staphylococcus aureus* IFO 13276 bacterial strain was used. The antibacterial assay was performed as previously described by Meng et al. [8]. Eighty microliters 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. Pre-cultured bacteria in the NB medium (10 μl) at a density of 1.0 × 10^5 CFU/ml were then added into each well, and incubated for 8 h. The absorbance was measured at 630 nm.

Inhibitory effect on melanogenesis using cultured B16 melanoma cells

Determination of melanin content

This assay was performed as previously described by Arung et al. [9]. The melanin content 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 M NaOH. The crude cell extracts were assayed using a micro plate reader (Bio-Tek, USA) at 405 nm to determine melanin content. The results from the samples were analyzed as a percent of the control culture. Arbutin was used as a positive control.

Cell viability

Cell viability was determined by use of the microculture tetrazolium technique (MTT assay). A culture was initiated, and after incubation, 50 µl of MTT [(3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium 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 M HCl and the absorbance was measured at 570 nm relative to 630 nm.

Antioxidant assay

Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay was performed as described previously by Shimamura et al. [10]. Data are expressed as milligram of Trolox equivalents (TE) per milligram of sample (mg TE/mg).

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

This assay was performed as described previously by Meng et al. [8]. The free radical scavenging activity of the sample was quantified by the decolorization of DPPH at 517 nm.

SOD-like activity

SOD-like activity was evaluated using the SOD assay kit-WST (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) according to the method described in previous studies [11]. The absorbance of each sample was measured at 450 nm in a Tecan Spectra microplate reader (Tecan Japan Co., Ltd., Kanagawa, Japan).

Antihyaluronidase activity

Antihyaluronidase activity was measured using the previously described method [8]. 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 [12]. Thus, cromolyn (Sigma Aldrich, USA) was used as a positive control.

Results and discussion

Our previous study has reported that the extract of the fruiting body of *P. citrinopileatus* have melanin biosynthesis inhibition, antioxidant, antibacterial, and antihyaluronidase activities [8]. In addition, 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 pathogenic bacteria. For example, *S. aureus* is widespread, potentially pathogenic types of bacteria that cause some skin conditions such as acne, comedo, papules, pustules, and cellulitis. These led us to investigate the biologically active components in the fruiting bodies of *P. citrinopileatus,* with a special focus here on the antimicrobial activity. The methanol extract of *P. citrinopileatus* was successfully chromatographed on a silica gel column (Wakogel C-200, 19 cm i.d. × 50 cm and 5.5 cm i.d. × 120 cm), preparative HPLC [Inertsil Prep-ODS column (20 mm i.d. × 250 mm)] to lead to the isolation of compound **1** with antimicrobial activity. The structure was established based on the NMR and mass spectral data, as well as by comparison with the data reported in the literature [13, 14].

Compound **1** was isolated as an optically active white amorphous solid ($[\alpha]_D^{23}$ + 5.0°; c = 0.3, CH_3OH). Positive FABMS (positive ion mode; matrix: nitrobenzyl alcohol) exhibited a molecular ion peak at *m/z* 710 $[M+H–H_2O]^+$ and the characteristic ion peak at *m/z* 548 $[M+H–H_2O–Glc]^+$ with the loss of a hexose residue from the quasi-molecular ion. The molecular formula of $C_{41}H_{77}NO_9$ for **1** was determined by the negative ion high-resolution LCMS-IT-TOF spectrum at *m/z* 726.5535 $[M-H]^-$ ($C_{41}H_{76}NO_9$, calcd. 726.5520). In its ^{13}C NMR spectrum, derived through DEPT, a D-glucopyranose moiety was indicated by the signals at δ 104.72 (C-1″, CH), 75.01 (C-2″, CH), 77.99 (C-3″, CH), 71.60 (C-4″, CH), 77.90 (C-5″, CH), and 62.73 (C-6″, CH_2). The sugar part of the molecular was indicated by the cross-peak between H-1″ (δ 4.17, 1H, d, J = 7.7 Hz) and C-1 (δ 69.73) exhibited in the HMBC spectrum. The ^{13}C NMR spectrum displayed the signal of an anomeric carbon atom at δ 104.72 (C-1″) and anomeric proton at δ 4.17 (d, J = 7.7, 1H, H-1″), indicative of a β-configuration of C-1″. [15].

Its ^1H NMR spectrum presented an amide NH doublet at δ 7.25 (1H, d, J = 8.7 Hz). The ^{13}C NMR and DEPT spectrum of compound **1** showed the signals at δ 177.18 (C-1′) of a carbonyl carbon and δ 54.67 (C-2) of a methine

carbon; both carbons were connected to amide nitrogen. Correlation peaks between NH and H-2 (δ 3.63, m, 1H) in ^{1}H–^{1}H COSY, and crosspeaks between NH and C-1′ and H-2 and C-1′ in HMBC were observed. The existence of a methyl side chain and two long aliphatic chains in the molecule was shown by the presence of signals of three methyl groups [δ_C 14.41 (C-18), 14.41 (C-16′), 16.15 (CH_3-19); δ_H 0.80 (6H, t, CH_3-18, 16′), 1.50 (3H, s, CH_3-19)] and a complex region with overlapping signals characteristic of methylenes of the long alkyl chain in the NMR spectra that were essentially identical with those of ceramide, strongly suggesting the glycosphingolipid nature of this molecule [16, 17].

Detailed analysis of the ^{1}H–^{1}H COSY spectrum was conducted, and the correlation peaks between H-4 and H-3, H-4 and H-5, H-5 and H-6, H-6 and H-7, and H-7 and H-8 were observed. The above correlation analysis unambiguously assigned the positions of the two double bonds at C-4 and C-8, respectively. This was further supported by the HMBC spectrum of compound **1**, which displayed the correlation between H-6 and C-4, H-3 and C-5, H-7 and C-9, and H-8 and C-10. On the other hand, the presence of a C-19 allylic methyl group (δ_C 16.15) in the branched long-chain base was also confirmed by the HMBC spectrum, in which the correlation between H-8 and C-19 was observed. The geometry of the C-4/C-5 alkene bond was determined to be *E* by the large vicinal coupling constant ($J = 15.3$ Hz) displayed between H-4 and H-5, as also evidenced by the ^{13}C NMR chemical shift of the methylene carbon C-6 (δ_C 33.05) next to the olefinic carbon [18], and the signals of olefinic protons (H-4 and H-5) appeared in the range of δ_H 5.39–5.64 as a multiplet [19]. Furthermore, the ^{13}C NMR chemical shift of the C-19 methyl group (δ_C 16.15) in turn supported the assignment of the Δ8-*trans* isomer, as demonstrated by comparison of the chemical shifts of the C-3 methyl groups in the *E* (δ_C 15.4) and *Z* (δ_C 22.7) isomers of 3-methyl-3-hexene [20]. It is thus clear that compound **1** possesses a branched sphingoid moiety with (4*E*,8*E*) geometry, 2-amino-1,3-dihydroxyl-9-methyl-4,8-octadecanediene. Moreover, the optical rotations of compound **1** ($[\alpha]_D^{23}$ + 5.0°, $c = 0.3$, CH_3OH) is also in accordance with that of glucosyl-(2*S*,3*R*)-sphingadienine ($[\alpha]_D^{23}$ + 5.4°) [21]. On the basis of the combined evidence presented above, the structure of compound **1** was established as 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*)-2-[(2*R*)-2-hydroxyhexadecanoylamino]-9-methyl-4,8-octadecadiene-1,3-diol (Fig. 1) and was confirmed by the comparison with the published NMR data [13–16].

Our previous study has reported that some soluble fractions prepared from the methanol extract of *P. citrinopileatus*, for example, *n*-hexane-soluble (50 μg/ml), diethylether-soluble (75 μg/ml), and ethyl acetate-soluble (100 μg/ml) fractions inhibited the melanin production in B16 melanoma cells at 63.1, 64.0, and 58.8%, with cell viability at 61.3, 65.8, and 70.7%, respectively. The ORAC value of *n*-hexane-soluble and diethyl ether-soluble fractions were 0.25 and 0.2 mg TE/mg. On the other hand, the *n*-butanol-soluble, aqueous-soluble fractions, and methanol extract exhibited moderate antioxidant potential; their inhibitory concentration (IC_{50}) that caused 50% scavenging of the DPPH radical scavenging were 7.9, 5.2, and 5.4 mg/ml, respectively. The aqueous-soluble fraction and methanol extract exhibited 1.9 and 0.3 of SOD-like activity (U/mg), respectively. 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. The *n*-butanol-soluble (2.0 mg/ml), aqueous-soluble (1.1 mg/ml) fractions, and methanol extract (4.1 mg/ml) showed antihyaluronidase activity of 9.7, 10.8, and 25.4%, respectively [8].

Although the extract of *P. citrinopileatus* has the multifunctional biological activities mentioned above, we first focused on its antimicrobial activity. Antimicrobial-activity-guided fractionation led to isolation of glucosylceramide (**1**). This compound showed growth inhibitory activity against *S. aureus* and *E. coli* with IC_{50} at 323.2 μM (235 μg/ml) and 275.1 μM (200 μg/ml) for 8 h, respectively (Table 1). It should be noted that the IC_{50} of sorbic acid known as an antimicrobial compound against *S. aureus* and *E. coil* was 1560.0 μM (175 μg/ml) and 1516.1 μM (170 μg/ml), respectively.

Also, we evaluated the effect of compound **1** in several bioassays. The DPPH radical scavenging activity was 0.7% at concentrations of 724.8 μM, 9.4 U/mg of SOD-like activity (852.8 μM) and antihyaluronidase activity of 4.7%

Fig. 1 Structure of compound 1

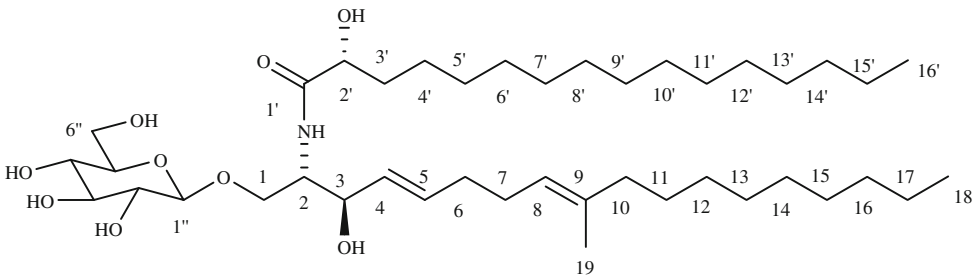

Table 1 Biological activity of compound **1** from methanol extract, the fruiting bodies of *P. citrinopileatus*

Test samples	Antimicrobial activity [IC_{50} (μM)]		Antioxidant activity (%)			Effect in B16 melanoma cells		Hyaluronidase inhibition (%)
	S. aureus	*E. coli*	ORAC	DPPH	SOD	MC (%)	CV (%)	
Compound **1**	323.2	275.1	No activity	0.7 ± 0.5	9.4 ± 4.4	100.1 ± 5.9	98.2 ± 6.5	4.7 ± 1.7[a]
Sorbic acid[b]	1560.0	1516.1						
Arbutin[c]						70.3 ± 4.1	99.0 ± 5.3	
Cromolyn sodium salt[d]								44.2 ± 5.7

Data presented as means ± SD ($n = 3$)

S. aureus, *Staphylococcus aureus*; *E. coli*, *Escherichia coli*; ORAC, oxygen radical absorbance capacity at 1595.6 μM; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical scavenging at 724.8 μM; SOD, superoxide dismutase at 852.8 μM; MC, melanin content (%); CV, cell viability (%)

[a] Compound 1 at 433.3 (μM)

[b] Positive control of antibacterial activity

[c] Positive control of melanin inhibition activity at 367.3 μM

[d] Positive control of hyaluronidase activity at 936.9 μM

(433.3 μM), respectively. Our results suggested that compound **1** has no distinguishing activities other than antimicrobial activity (Table 1). It is not known what other active ingredients besides those responsible for antimicrobial activity are present in *P. citrinopileatus*, and that this needs to be investigated in the future.

Glucosylceramide is a sphingolipid. Sphingolipids are members of membrane lipids and are widely distributed in the animal, plant, and fungi kingdoms [22, 23]. Sphingolipids, e.g., ceramides, cerebrosides, sphingomyelin, and gangliosides, are important building blocks of the plasma membrane of eukaryotic cells. Their function is to anchor lipid-bound carbohydrates to cell surfaces and to create an epidermal water permeability barrier, as well as to participate in antigen–antibody reactions and transmission of biological information. [24] Some have shown antiulcerogenic, antihepatotoxic, antitumor, and immunostimulatory activities [25–27].

A large number of studies have revealed that sphingolipids exert a significant effect on the structure of biological membranes and lipoproteins [28]. The biological activities of ceramide vary with the chemical structure: the carbon-chain structure of the sphingoid base and/or composition of amide-linked fatty acids. It has been reported that glycosphingolipids exert their biological activities by interacting with the receptor proteins on the cell membrane, with consequences to the modulation of the signal transduction [29].

In conclusion, glucosylceramide (**1**) isolated from *P. citrinopileatus* showed antimicrobial activity against *E. coli* and *S. aureus*. This is the first report of the isolation of glucosylceramide from *P. citrinopileatus*, and the first to show its antimicrobial activity. An efficacious dose of this compound has potential health benefits, including controlling infection, and the compound can also be used as a food additive to prevent decay.

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References

1. Khlood M, Laila S, Kaampwe M, Zein S, Mohamed S, Mayumi I, Akio K, Shoichi F (2005) Antigenotoxic effect of *Pleurotus cornucopiae* extracts on the mutagenesis of *Salmonella typhimurium* TA 98 elicited by benzo [a] pyrene and oxidative DNA lesions in V79 hamster lung cells. Jpn J Vet Res 52:163–172
2. Janga JH, Jeonga SC, Kimb JH, Leeb YH, Jub YC, Leea JS (2011) Characterization of a new antihypertensive angiotensin I-converting enzyme inhibitory peptide from *Pleurotus cornucopiae*. Food Chem 127:412–418
3. Shu HH, Zeng CL, Yi CC (2006) Antihyperlipidemic and antioxidant effects of extracts from *Pleurotus citrinopileatus*. J Agric Food Chem 54:2103–2110
4. Tomiyama T, Kaihou S, Ishida M, Nishikawa H, Yamazaki N, Tsuji K, Mitsutake S, Igarashi Y (2008) The water retention effects and action for atopic dermatitis-like symptoms of ethyl alcohol extract (from tamogi-take mushroom) on animal model of atopic dermatitis. J Jpn Soc Nutr Food Sci 61:21–26
5. Kim JH, Kim SJ, Park HR, Choi JI, Ju YC, Nam KC, Kim SJ, Lee SC (2009) The different antioxidant and anticancer activities depending on the color of oyster mushrooms. J Med Plants Res 3:1016–1020
6. Wong JH, Ng TB, Jiang Y, Liu F, Sze SC, Zhang KY (2010) Purification and characterization of a laccase with inhibitory activity toward HIV-1 reverse transcriptase and tumor cells from an edible mushroom (*Pleurotus cornucopiae*). Protein Peptide Lett 17:1040–1047
7. Takakura Y, Oka N, Kajiwara H, Tsunashima M, Usami S, Tsukamoto H, Ishida Y, Yamamoto T (2010) Tamavidin, a versatile affinity tag for protein purification and immobilization. J Biotechnol 145:317–322

8. Meng TX, Furuta S, Fukamizu S, Yamamoto R, Ishikawa H, Arung ET, Shimizu K, Ohga S, Kondo R (2011) Evaluation of biological activities of extracts from the fruiting body of *Pleurotus citrinopileatus* for skin cosmetics. J Wood Sci 57:452–458
9. Arung ET, Shimizu K, Kondo R (2006) Inhibitory effect of artocarpanone from *Artocarpus heterophyllus* on melanin biosynthesis. Biol Pharm Bull 29:1966–1969
10. Shimamura T, Matsuura R, Tokuda T, Sugimoto N, Yamasaki T, Matsufuji H, Matsui T, Matsumoto K, Ukeda H (2007) Comparison of conventional antioxidant assays for evaluating potencies of natural antioxidants as food additives by collaborative study. J Jpn Soc Food Sci 54:482–487
11. Ukeda H, Kawana D, Maeda S, Sawamura M (1999) Spectrophotometric assay for superoxide dismutase based on the reduction of highly water-soluble tetrazolium salts by xanthine–xanthine oxidase. Biosci Biotechnol Biochem 63:485–488
12. Holgate ST (1989) Reflections on the mechanism(s) of action of sodium cromoglycate (Intal) and the role of mast cells in asthma. Resp Med 83:25–31
13. Gao JM, Zhu WM, Zhang SQ, Zhang X, Zhang AL, Chen H, Sun YY, Tang M (2004) Sphingolipids from the edible fungus *Tuber indicum*. Eur J Lipid Sci Technol 106:815–821
14. Thushara D, Vanisree M, Gary M, David L, Muraleedharan G (2008) Health-beneficial qualities of the edible mushroom, *Agrocybe aegerita*. Food Chem 108:97–102
15. Liu JK, Hu L, Dong ZJ (2003) A glucosylceramide with a novel ceramide and three novel ceramides from the basidiomycete *Cortinarius umidicola*. Lipids 38:669–675
16. Gao JM, Hu L, Dong ZJ, Liu JK (2001) New glycosphingolipid containing an unusual sphingoid base from the basidiomycete *Polyporus ellisii*. Lipids 36:521–527
17. Gao JM, Shen J, Zhang AL, Zhu W, Zhang X, Liu JK (2003) Chemical constituents of the fungus *Leccinum extremiorientale*. Chin J Org Chem 23:853–857
18. Sang S, Kikuzaki H, Lapsley K, Rosen RT, Nakatani N, Ho CT (2002) Sphingolipid and other constituents from almond nuts (*Prunus amygdalus* Batsch). J Agric Food Chem 50:4709–4712
19. Higuchi R, Inagaki M, Togawa K, Miyamoto T, Komori T (1994) Isolation and structure of cerebrosides from the sea cucumber *Pentacta australis*. Liebigs Ann Chem 7:653–658
20. Sitrin RD, Chan G, Dingerdissen J, De BC, Mehta R, Roberts G, Rottschaefer S, Staiger D, Valenta J, Snader KM, Stedman RJ, Hoover JRE (1988) Isolation and structure determination of pachybasium cerebrosides which potentiate the antifungal activity of aculeacin. J Antibiot 41:469–480
21. Murakami T, Shimizu T, Taguchi K (2000) Synthesis of sphingadienine-type glucocerebrosides. Tetrahedron 56:533–545
22. Schmelz EM, Merrill AJ (1998) Ceramides and ceramide metabolites in cell regulation: evidence for dietary sphingolipids as inhibitors of colon carcinogenesis. Nutrition 14:717–719
23. Sullards MC, Lynch DV, Merrill AJ, Adams J (2000) Structure determination of soybean and wheat glucosylceramides by tandem mass spectrometry. J Mass Spectrom 35:347–353
24. Kolter T, Sandhoff K (1999) Sphingolipids, their metabolic pathways and the pathobiochemistry of neurodegenerative disease. Angew Chem Int Ed 38:1532–1568
25. Okuyama E, Yamazaki M (1983) The principles of tetragonia tetragonoides having anti-ulcerogenic activity. II. Isolation and structure of cerebrosides. Chem Pharm Bull 31:2209–2219
26. Kim SY, Choi YH, Huh H, Kim J, Kim YC, Lee HS (1997) New antihepatotoxic cerebroside from *Lycium chinense* fruits. J Nat Prod 60:274–276
27. Natori T, Morida M, Akimoto K, Koezuka Y (1994) Agelasphins, novel antitumor and immunostimulatory cerebrosides from the marine sponge *Agelas mauritianus*. Tetrahedron 50:2771–2784
28. Barenholz Y, Thompson TE (1980) Sphingomyelins in bilayers and biological membranes. Biochim Biophys Acta 604:129–158
29. Zhou D, Mattner J, Cantu C, Schrantz N, Yin N, Gao Y, Sagiv Y, Hudspeth K, Wu YP, Yamashita T, Teneberg S, Wang D, Proia RL, Levery SB, Savage PB, Teyton L, Bendelac A (2004) Lysosomal glycosphingolipid recognition by NKT cells. Science 306:1786–1789