Natural Product Sciences
14(1) : 1-11 (2008)

Peroxynitrite scavengers from *Phellinus linteus*

Da Mi Jeong, Hyun Ah Jung, Hye Sook Kang, and Jae Sue Choi*

Division of Food Science and Biotechnology, Pukyong National University, Busan 608-737, Korea

Abstract – Peroxynitrite ($ONOO^-$) is a cytotoxic species formed from nitric oxide and superoxide anion, which are highly implicated in the pathogenesis of oxidative stress-mediated diseases. The aim of this study was to investigate the scavenging effects of *Phellinus linteus* on authentic $ONOO^-$, and further phytochemical studies are planned that will attempt to identify the active principles. From the active EtOAc fraction, a mixture of fungisterol and 5-dihydroergosterol (**1**), a mixture of betulin and 1,2-benzenedicarboxylic acid bis (2-methyl heptyl) ester (**2**), protocatechualdehyde (**3**), protocatechuic acid (**4**), cirsiumaldehyde (**5**), hispidin (**6**), caffeic acid (**7**), phelligridin D (**8**), uracil (**9**), gallic acid (**10**), 2,5-dihydroxybenzoic acid (**11**), ferulic acid (**12**), 2,3-dihydroxybenzaldehyde (**13**), arbutin (**14**), isoferulic acid (**15**), guanosine (**16**), and ellagic acid (**17**) were isolated, and their structures were characterized based on spectroscopic data. All compounds except **3**, **6**, **7** and **16** were isolated for the first time from *P. linteus*. Compounds **3**, **4**, **6-8**, **10-15**, and **17** showed potent scavenging activity on $ONOO^-$, with IC_{50} values of 2.06 ± 0.10, 3.45 ± 0.57, 0.71 ± 0.05, 2.78 ± 0.36, 5.42 ± 0.26, 1.13 ± 0.02, 1.82 ± 0.17, 0.91 ± 0.19, 1.59 ± 0.09, 1.88 ± 0.07, 1.22 ± 0.37, and 2.01 ± 0.02 μM, respectively, as compared to the positive control, DL-penicillamine, with an IC_{50} value of 5.04 ± 0.06 μM.
Keywords – *Phellinus linteus*, peroxynitrite scavengers

Introduction

Peroxynitrite ($ONOO^-$), formed from the reaction of nitric oxide and superoxide anion, can produce cytotoxic reaction products (Beckman *et al.*, 1990; Pryor *et al.*, 1996; Szabo, 1996). $ONOO^-$ possesses paradoxical properties in biological systems: one being that it is a strong oxidizing and nitrating species that damages cellular compositions, including protein, DNA, and lipids; the other is its beneficial characteristics in inflammatory reactions in terms of the oxidative decomposition of intruding microorganisms (Klotz *et al.*, 2003). Despite its non-radical nature, $ONOO^-$ is highly reactive as compared to its parent molecules. $ONOO^-$ initiates lipid peroxidation, causes DNA breakage, and reacts with thiol (-SH) groups on proteins, including the nitration of tyrosine and nitrosation (e.g. the formation of *S*-nitrosoglutathione) (Virag *et al.*, 2003). Many researches have provided evidence for the *in vivo* formation of $ONOO^-$ in human atherosclerosis, acute lung injury, and chronic inflammation (Ho *et al.*, 2007; Darley-Usmar *et al.*, 1995; Kooy *et al.*, 1995). Due to the lack of endogenous enzymes responsible for $ONOO^-$ inactivation, and the pathological relevance to several degenerative diseases, the development of effective $ONOO^-$ scavengers may be an important strategy to treat and prevent $ONOO^-$ related disorders. Various methods have been utilized to develop powerful $ONOO^-$ scavengers, and synthetic $ONOO^-$ scavengers such as selenomethionine, selenocystine, and ebselen (Sies and Masumoto, 1997) are predominant examples. Currently, there has been focus on naturally-occurring compounds, including ascorbic acid (Sandoval *et al.*, 1997), flavonoids (Pollard *et al.*, 2006), isoflavonoids (Bouersma *et al.*, 1999), ergothioneine (Aruoma *et al.*, 1999), and polyhydroxyphenols (Tipoe *et al.*, 2007), acquired from natural sources.

Phellinus linteus (Berk & Curt) Aoshima, an orange colored mushroom that grows well on mulberry trees, is a well known fungus of the *Phellinus* genus in the family Hymenochaetaceae, and is called 'Sang-Hwang' mushroom in Korea. This mushroom has long been used in traditional oriental medicine to cure stomachache and arthritis of the knee (Huh *et al.*, 1981). Since the anti-tumor effect of *P. linteus* was first reported by Ikekawa *et al.* (1968), this mushroom has attracted considerable attention due to its various bioactive properties. Several biological activities of *P. linteus* have been reported, including anti-tumor (Guo *et al.*, 2007; Nakamura *et al.*, 2004; Cho *et al.*, 2002; Rhee *et al.*, 2000; Park *et al.*,

*Author for correspondence
Fax: +82-51-629-5842; E-mail: choijs@pknu.ac.kr.

2004a), anti-mutagenic (Shon and Nam, 2001), anti-angiogenic, xanthine oxidase inhibitory (Song *et al.*, 2003), antioxidant (Song *et al.*, 2003; Park *et al.*, 2004b), anti-inflammatory (Kim *et al.*, 2007), and antibacterial activities (Kim *et al.*, 1998; Hur *et al.*, 2004); as well as inhibitory effects on rat intestinal α-glucosidase (Kim *et al.*, 1998) and on tumor growth and metastasis (Han *et al.*, 1999). However, there is limited information on its bioactive principles, with the exception of the polysaccharides from *P. linteus* (Han *et al.*, 2006; Shin *et al.*, 2007). Previously, we reported on the isolation and structural elucidation of five compounds from *P. linteus*: cerebroside B, protocatechualdehyde, 5-hydroxymethyl-2-furaldehyde (HMF), succinic acid, and fumaric acid, as well as on the tyrosinase inhibitory activity of those compounds using L-tyrosine as a substrate (Kang *et al.*, 2004). Also, two novel furan derivatives, phellinusfurans A and B, from this mushroom were reported to possess anti-complement activity (Min *et al.*, 2006).

In this paper, we report on the isolation of a mixture of fungisterol and 5-dihydroergosterol (**1**), a mixture of betulin and 1,2-benzenedicarboxylic acid bis (2S-methyl heptyl) ester (**2**), protocatechualdehyde (**3**), protocatechuic acid (**4**), cirsiumaldehyde (**5**), hispidin (**6**), caffeic acid (**7**), phelligridin D (**8**), uracil (**9**), gallic acid (**10**), 2,5-dihydroxybenzoic acid (**11**), ferulic acid (**12**), 2,3-dihydroxybenzaldehyde (**13**), arbutin (**14**), isoferulic acid (**15**), guanosine (**16**), and ellagic acid (**17**) from *P. linteus*, as well as their $ONOO^-$ scavenging activities. Although there are numerous studies on phenolic compounds as $ONOO^-$ scavengers (Choi *et al.*, 2007; Kerry and Rice-Evans, 1999; Pannala *et al.*, 1998; Salah *et al.*, 1995), the $ONOO^-$ scavenging activity of *P. linteus*, and its bioactive principles **6**, **8**, **11**, **14**, and **15** is reported here for the first time.

Experimental

General experimental procedures – NMR experiments were performed on a JEOL JNM-ECP 400 (^{1}H-NMR 400 MHz; ^{13}C-NMR 100 MHz) spectrometer. The 2D NMR (DEPT, HMBC, HMQC) spectra were recorded on a JEOL JNM-EPC 400 using pulsed field gradients. The chemical shifts were referenced to the respective residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD, δ_H 2.50 and δ_C 39.5 for DMSO-d_6, and δ_H 7.26 and δ_C 77.2 for $CDCl_3$), recorded in values and expressed in ppm. IR spectra were performed on a Perkin-Elmer 2000 FT-IR spectrophotometer using KBr disc methods. UV spectrophotometer was performed using a VARIAN UV-visible spectrophotometer. The EI-MS data were recorded using a QP-5050A (Shimadzu, Japan) spectrometer. The spots of thin layer chromatography (TLC) were detected under the wavelengths of 365 nm and 254 nm, using UV lamp (Model ENF-240C, Spectroline, U.S.A.). The fluorescence intensity was measured with a microplate fluorescence reader FLx 800 (Bio-Tek Instruments Inc.).

Plant materials – The *P. linteus* used in this experiment was kindly provided by Cho-A Pharmaceutical Co., Ltd., and a specimen was deposited in the author's laboratory (No. 20020304).

Chemicals – Column chromatography was carried out using silica gel 60 (Si gel, 70 - 230 mesh ASTM, Merck, Art. 7734) and sephadex LH-20 (bead size 25 - 100 μm, Sigma), and RP-18 (Lichroprep® RP-18, 40 - 63 μm, Merck). TLC was performed on a Kieselgel 60 F_{254} (0.25 mm, precoated, Merck, Art. 5715) and RP 18 F_{254s} (Merck, Art. 5685), 50% H_2SO_4 was used as spray reagent. All solvent used in the extraction and column chromatography were a first class reagent. As performed NMR experiments, samples dissolved in DMSO-d_6 (Cambridge Isotope Laboratories, deuterium degree 99.9%), $CDCl_3$ (Aldrich Chemicals, deuterium degree 99.8%), and CD_3OD (Cambridge Isotope Laboratories, deuterium degree 99.8%). DCFH-DA (2',7'-Dichlorodihydrofluorescein diacetate), DL-penicillamine (DL-2-amino-3-mercapto-3-methylbutanoic acid), and DHR 123 (dihydrorhodamine 123) were purchased from Molecular Probes (Eugene, OR, U.S.A.), and $ONOO^-$ was purchased from Cayman Chemicals Company (Ann Arbor, MI, U.S.A.).

Extraction, fractionation, and isolation – The lyophilized fruiting body of *P. linteus* (4.5 kg) was powdered and refluxed with MeOH (3 × 9 L) for 3 h. The extract (838 g) was suspended in water and partitioned with CH_2Cl_2 (101.5 g), EtOAc (36.7 g), *n*-BuOH (366 g), and H_2O (244 g), in sequence. Since the EtOAc fraction showed the strongest $ONOO^-$ scavenging activity, this fraction (36.0 g) was chromatographed over a Si gel column (8 × 80, 1.2 kg), eluting with CH_2Cl_2 : MeOH (9 : 1) to yield 12 subfractions (F1 ~ F12). The combined fractions (264 mg) of F1 and F2 were chromatographed over an Si gel column with CH_2Cl_2 : MeOH (1 : 0 to 0 : 1, gradient) to yield compounds **1** (12 mg) and **2** (11 mg). F3 (2.8 g) was further chromatographed over an Si gel column eluting with hexane : EtOAc (3 : 2) to give compounds **3** (377 mg) and **4** (45 mg). F4 (87 mg) was chromatographed over Sephadex LH-20 and eluted with 100% MeOH, followed by RP-18 gel column chromatography and eluting with aqueous MeOH (30 ~ 100%, gradient) to afford compounds **5** (10 mg), **6** (8 mg), and **7** (13 mg). F7 (4.3 g) was chromatographed over Sephadex

LH-20 eluting with 100% MeOH, and then repeated column chromatography on Sephadex LH-20 eluting with aqueous MeOH (50 ~ 100%, gradient) to afford compounds **8** (49 mg), **9** (26 mg), and **10** (9 mg). For F8 (1.6 g), Si gel column chromatography was carried out, eluting with hexane : EtOAc (3 : 2), followed by column chromatography on Sephadex LH-20 eluting with 30% aqueous MeOH to afford compounds **11** (8 mg) and **12** (9 mg). F9 (2.1 g) underwent RP-18 column chromatography, eluting with 20% aqueous MeOH to yield compounds **13** (14 mg) and **14** (10 mg). The combined fractions (7 g) of F10, F11, and F12 were chromatographed over Si gel with EtOAc : MeOH (10 : 1), followed by column chromatography on RP-18 eluting with 20% aqueous MeOH to afford compounds **15** (9 mg), **16** (17 mg), and **17** (16 mg). The structures of the isolated compounds (**1** ~ **17**) were elucidated by NMR and MS analyses, and identified as follows: a mixture of fungisterol and 5-dihydroergosterol (**1**), a mixture of betulin and 1,2-benzenedicarboxylic acid bis (2-methyl heptyl) ester (**2**), protocatechualdehyde (**3**), protocatechuic acid (**4**), cirsiumaldehyde (**5**), hispidin (**6**), caffeic acid (**7**), phelligridin D (**8**), uracil (**9**), gallic acid (**10**), 2,5-dihydroxybenzoic acid (**11**), ferulic acid (**12**), 2,3-dihydroxybenzaldehyde (**13**), arbutin (**14**), isoferulic acid (**15**), guanosine (**16**), and ellagic acid (**17**).

A mixture of fungisterol (1a) and 5-dihydroergosterol (1b) (1) – EI-MS *m/z*: 400 $[C_{28}H_{48}O]^+$ (46) (**1a**) and 398 $[C_{28}H_{46}O]^+$ (19) (**1b**), 383 $[C_{28}H_{46}O\text{-}CH_3]^+$ (9), 355 $[C_{28}H_{46}O\text{-}C_3H_7]^+$ (3), 300 (10), 285 $[300\text{-}CH_3]^+$ (3), 273 $[C_{28}H_{46}O\text{-}C_9H_{17}]^+$ (30), 271 $[C_{28}H_{46}O\text{-}(C_9H_{17}+2H)]^+$, 255 $[C_{28}H_{46}O\text{-}(C_9H_{17}+H_2O)]^+$ (53), 229 (19), 213 (24), 55 (100); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.19 (1H, m, H-7), 3.60 (1H, m, H-3), 1.02 (3H, d, J = 6.6 Hz, 21-CH_3), 0.86 (3H, d, J = 6.9 Hz, 26-CH_3), 0.81 (3H. s, 19-CH_3), 0.80 (3H, d, J = 5.9 Hz, 27-CH_3), 0.78 (3H, d, J = 4.4 Hz, 28-CH_3), 0.53 (3H, s, 18-CH_3) (**1a**) and δ: 5.19 (2H, m, H-22,23), 5.17 (1H, s, H-7), 3.60 (1H, m, H-3), 1.02 (3H, d, J = 6.6 Hz, 21-CH_3), 0.91 (3H, d, J = 4.8 Hz, 28-CH_3), 0.86 (3H, d, J = 6.9 Hz, 27-CH_3), 0.82 (3H, d, J = 6.6 Hz, 26-CH_3), 0.80 (3H. s, 19-CH_3), 0.54 (3H, s, 18-CH_3) (**1b**); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 139.5 (C-8), 117.4 (C-7), 71.1 (C-3), 56.0 (C-17), 55.0 (C-14), 49.4 (C-9), 43.4 (C-13), 40.2 (C-5), 39.5 (C-12), 39.0 (C-24), 38.0 (C-1), 37.1 (C-4), 36.6 (C-20), 34.3 (C-10), 33.6 (C-22), 31.5 (C-25), 30.7 (C-23), 29.6 (C-6), 28.1 (C-16), 27.9 (C-2), 22.9 (C-15), 21.5 (C-11), 20.5 (C-27), 19.0 (C-21), 17.6 (C-26), 15.4 (C-28), 13.0 (C-19), 11.9 (C-18) (**1a**) (Rosecke *et al.*, 2000) and δ: 139.6 (C-8), 135.7 (C-22), 131.8 (C-23), 117.4 (C-7), 71.1 (C-3), 55.9 (C-17), 55.1 (C-14), 49.4 (C-9), 42.8 (C-24), 43.3 (C-13), 40.5 (C-20), 40.2 (C-5), 39.4 (C-12), 37.1 (C-1), 34.3 (C-10), 33.1 (C-4), 31.5 (C-25), 29.6 (C-6), 28.1 (C-16), 27.9 (C-2), 22.9 (C-15), 21.5 (C-11), 21.1 (C-21), 19.9 (C-26), 19.6 (C-27), 17.6 (C-28), 13.0 (C-19), 12.1 (C-18) (**1b**) (Lee *et al.*, 2006a).

A mixture of betulin (2a) and 1,2-benzenedicarboxylic acid bis (2-methyl heptyl) ester (2b) (2) – EI-MS *m/z*: 442 $[C_{30}H_{50}O_2]^+$ (18) (**2a**) and 390 $[C_{24}H_{38}O_4]^+$ (0.1) (**2b**), 279 (17), 167 (52), 149 (100), 132 (3), 113 (17), 104 (9), 83 (16), 71 (45), 57 (67); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.68 and 4.58 (each 1H, m, H-29), 3.79 and 3.33 (1H, d, J = 10.6 Hz, H-28), 3.20 (1H, dd, J = 4.7, 10.6 Hz, H-3), 1.68 (3H, s, H-30), 1.02 (3H, s, H-27), 0.98 (3H, s, H-26), 0.97 (3H, s, H-25), 0.82 (3H, s, H-24), 0.76 (3H, s, H-23) (**2a**) and δ: 0.90 (6H, m, H-7' and H-7"), 0.97 (6H, d, J = 4.4 Hz, 2' and 2"-CH_3), 1.32 (16H, m, H-3', 3", 4', 4", 5', 5", 6', 6"), 1.80 (2H, m, H-2' and 2"), 4.20 (4H, m, H-1' and H-1"), 7.52 (2H, dd, J = 3.3, 5.5 Hz, H-3 and H-6), 7.70 (2H, dd, J = 3.3, 5.5 Hz, H-4 and H-5) (**2b**); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 150.5 (C-20), 109.7 (C-29), 79.0 (C-3), 60.5 (C-28), 55.3 (C-5), 50.4 (C-9), 48.7 (C-18), 47.8 (C-17), 47.8 (C-19), 42.7 (C-14), 40.9 (C-8), 38.9 (C-4), 38.7 (C-1), 37.3 (C-13), 37.1 (C-10), 34.2 (C-7), 33.9 (C-22), 29.7 (C-21), 29.1 (C-16), 28.9 (C-23), 27.4 (C-2), 27.0 (C-15), 25.2 (C-12), 20.8 (C-11), 19.1 (C-30), 18.3 (C-6), 16.1 (C-25), 16.0 (C-26), 15.4 (C-24), 14.8 (C-27) (**2a**) (Im *et al.*, 2006) and δ: 167.8 (C = O), 132.4 (C-1 and C-2), 130.87 (C-3 and C-6), 128.8 (C-4 and C-5), 68.2 (C-1' and C-1"), 38.7 (C-2' and C-2"), 30.3 (C-4' and C-4"), 29.3 (C-5' and C-5"), 23.7 (C-6' and C-6"), 23.0 (C-3' and C-3"), 14.0 (C-7' and C-7"), 10.9 (2'-CH_3 and 2"-CH_3) (**2b**) (Singh *et al.*, 2006).

Protocatechualdehyde (3) – yellowish needle, EI-MS *m/z*: 138 (92) $[C_7H_6O_3]^+$, 137 (100) $[C_7H_6O_3\text{-}H]^+$, 109 (73), 81 (65), 63 (33), 53 (34); IR ν_{max} (KBr) cm^{-1}: 3222, 3329, 1652, 1596, 1535, 1445, 1297, 1165; ^{1}H-NMR (400 MHz, CD_3OD) δ: 9.68 (1H, s, CHO); 7.30 (1H, dd, J = 1.7, 9.5 Hz, H-6), 7.28 (1H, d, J = 1.4 Hz, H-2), 6.90 (1H, d, J = 8.1 Hz, H-5); ^{13}C-NMR (100 MHz, CD_3OD) δ: 193.8 (CHO), 154.5 (C-4), 148.0 (C-3), 131.6 (C-1), 127.2 (C-6), 117.0 (C-5), 116.2 (C-2) (Kang *et al.*, 2004).

Protocatechuic acid (4) – yellowish powder, EI-MS *m/z*: 154 $[C_7H_6O_4]^+$; ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.43 (1H, dd, J = 1.9, 8.1 Hz, H-6), 6.79 (1H, d, J = 8.1 Hz, H-5), 7.42 (1H, d, J = 1.9 Hz, H-2); ^{13}C-NMR (100 MHz, CD_3OD) δ: 171.2 (COOH), 152.2 (C-4), 146.8 (C-3), 124.7 (C-1), 124.1 (C-6), 118.5 (C-5), 116.5 (C-2) (Sun *et al.*, 2006).

Cirsiumaldehyde (5) – white powder, EI-MS *m/z*: 234 $[C_{12}H_5O_{10}]^+$; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.58 (1H, s, 2 × CHO), 7.51 (1H, d, J = 3.52 Hz, H-3 and H-

3'), 6.76 (1H, d, J = 3.52 Hz, H-4 and H-4'), 4.62 (2H, s, 2 × CH_2); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 178.4 (2 × CHO), 157.3 (C-5 and C-5'), 152.3 (C-2 and C-2'), 123.8 (C-3 and C-3'), 112.3 (C-4 and C-4'), 63.7 (2 × CH_2) (Yun-Choi *et al.*, 1997).

Hispidin (6) – yellow powder, ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.29 (1H, d, J = 16.1 Hz, H-8), 7.01 (1H, s, H-10), 6.95 (1H, d, J = 2.1 Hz, H-14), 6.76 (H, d, J = 8.0 Hz, H-8), 6.59 (1H, d, J = 15.7 Hz, H-7), 6.10 (1H, s, H-5), 5.28 (1H, s, H-3); ^{13}C-NMR (100 MHz, CD_3OD) δ: 173.8 (C-2), 167.7 (C-4), 162.1 (C-6), 148.7 (C-11), 146.8 (C-12), 137.3 (C-8), 128.8 (C-9), 121.9 (C-14), 116.8 (C-7), 116.5 (C-13), 114.8 (C-10), 101.8 (C-5), 90.2 (C-3) (Park *et al.*, 2004b).

Caffeic acid (7) – Yellow powder, ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.39 (1H, d, J = 15.8 Hz, H-7), 7.01 (1H, d, J = 1.7 Hz, H-2), 6.94 (1H, dd, J = 1.5, 8.1 Hz, H-6), 6.75 (1H, d, J = 8.3, H-5), 6.16 (1H, d, J = 15.8 Hz, H-8); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 170.6 (COOH), 152.4 (C-4), 145.5 (C-3), 144.4 (C-7), 125.7 (C-1), 121.0 (C-6), 115.7 (C-5), 114.5 (C-2) (Lim *et al.*, 2003).

Phelligridin D (8) – Yellow powder, EIMS *m/z*: 380 $[C_{20}H_{12}O_8]^+$; UV (MeOH) λ_{max} (log ε) 255 (4.43), 413 (4.54) nm; IR (KBr) ν_{max} 3414, 1671, 1608, 1524, 1406, 1293, 1016 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 10.77 (1H, s, H-9), 10.10 (1H, s, H-8), 9.58 (1H, s, H-6'), 9.17 (1H, s, H-5'), 8.34 (1H, s, H-10), 7.52 (1H, s, H-7), 7.29 (1H, d, J = 15.8 Hz, H-2'), 7.08 (1H, d, J = 1.9 Hz, H-4'), 7.00 (1H, dd, J = 1.9, 8.3 Hz, H-8'), 6.79 (1H, d, J = 15.8 Hz, H-1'), 6.78 (1H, d, J = 8.3 Hz, H-7'), 6.73 (1H, s, H-4); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 160.8 (C-4a), 159.5 (C-1), 158.7 (C-6), 158.4 (C-3), 153.6 (C-9), 147.9 (C-6'), 146.9 (C-8), 145.7 (C-5'), 135.9 (C-2'), 127.0 (C-10a), 126.6 (C-3'), 120.9 (C-8'), 115.8 (C-7'), 115.4 (C-1'), 114.5 (C-7), 114.0 (C-4'), 111.4 (C-6a), 110.4 (C-10), 99.0 (C-10b), 98.8 (C-4) (Mo *et al.* 2004).

Uracil (9) – ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 11.00 (1H, s, 2-NH), 10.81 (1H, s, 4-NH), 7.39 (1H, d, J = 7.7 Hz, H-5), 5.44 (1H, d, J = 7.7 Hz, H-6); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 164.3 (C-1), 151.5 (C-3), 142.1 (C-5), 100.2 (C-6) (Lee *et al.*, 2004).

Gallic acid (10) – white powder, ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.91 (2H, s, H-2, H-6); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 167.5 (C-7), 145.4 (C-3 and C-5), 138.0 (C-4), 120.4 (C-1), 108.7 (C-2 and C-6) (Park *et al.*, 1993).

2,5-Dihydroxybenzoic acid (11) – white powder, ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.15 (1H, d, J = 2.93 Hz, H-6), 6.96 (1H, dd, J = 2.93, 8.06 Hz, H-4), 6.78 (1H, d, J = 8.79 Hz, H-3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 171.8 (COOH), 154.1 (C-2), 149.4 (C-5), 123.7 (C-4), 117.8 (C-3), 114.5 (C-6), 112.6 (C-2) (Park *et al.*, 2004c).

Ferulic acid (12) – colorless needles, ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.59 (1H, d, J = 16.1 Hz, H-8), 7.17 (1H, d, J = 1.8 Hz, H-2), 7.05 (1H, dd, J = 8.0, 1.83 Hz, H-6), 6.80 (1H, d, J = 8.42 Hz, H-5), 6.30 (1H, d, J = 15.7 Hz, H-7), 3.88 (1H, s, 3-OCH_3); ^{13}C-NMR (100 MHz, CD_3OD) δ: 171.0 (C-9), 150.5 (C-4), 149.3 (C-3), 146.9 (C-7), 127.8 (C-1), 124.0 (C-6), 116.4 (C-8), 115.9 (C-5), 111.6 (C-2), 56.4 (OCH_3) (Aoki *et al.*, 1982).

2,3-Dihydroxybenzaldehyde (13) – yellowish needles, ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.80 (1H, s, CHO), 7.13 (1H, dd, J = 1.46, 8.06 Hz, H-4), 7.06 (1H, dd, J = 1.46, 7.69 Hz, H-6), 6.79 (1H, dd, J = 6.04, 9.78 Hz, H-5); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 192.8 (CHO), 149.7 (C-2), 146.1 (C-3), 122.7 (C-1), 121.2 (C-6), 119.6 (C-5), 119.3 (C-4) (Nishida *et al.*, 2006).

Arbutin (14) – yellow powder, ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 6.86 (2H, d, J = 7.0 Hz, H-2 and H-6), 6.65 (2H, d, J = 7.0 Hz, H-3 and H-5), 4.63 (1H, d, J = 7.3 Hz, H-1'), 3.68 (1H, m, H-6'a), 3.45 (1H, m, H-6'b), 3.23 (4H, m, H-2', H-3', H-4', H-5'); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 152.2 (C-1), 150.4 (C-4), 117.7 (C-2 and C-6), 115.5 (C-3 and C-5), 101.7 (C-1'), 77.0 (C-3' and C-5'), 76.6 (C-2'), 73.3 (C-4"), 60.8 (C-6') (Hisatomi *et al.*, 2000).

Isoferulic acid (15) – colorless needles, ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.54 (1H, d, J = 16.1 Hz, H-8), 7.05 (1H, d, J = 4.03 Hz, H-2), 7.04 (1H, dd, J = 11.1, 1.8 Hz, H-6), 6.93 (1H, d, J = 8.1 Hz, H-5), 6.26 (1H, d, J = 16.1 Hz, H-7), 3.88 (1H, s, 3-OCH_3); ^{13}C-NMR (100 MHz, CD_3OD) δ: 170.8 (C-9), 151.4 (C-4), 148.0 (C-3), 146.6 (C-7), 128.9 (C-1), 122.7 (C-6), 116.6 (C-8), 114.7 (C-5), 112.5 (C-2), 56.4 (OCH_3) (Aoki *et al.*, 1982).

Guanosine (16) – ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.34 (1H, s, H-1), 8.13 (1H, s, H-8), 7.34 (2H, s, H-2), 5.87 (1H, d, J = 6.2 Hz, H-1'), 5.76 (1H, s, H-2'), 5.19 (1H, s, H-3'), 4.60 (1H, s, 5'-OH), 4.02 (1H, dd, J = 7.0, 12.0 Hz, H-2'), 3.96 (1H, dd, J = 3.4, 8.0 Hz, H-3'), 3.55 (1H, s, H-4'), 3.16 (2H, s, H-5'); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 156.1 (C-6), 152.3(C-2), 148.8 (C-4), 139.8 (C-8), 118.6 (C-5), 87.8 (C-1'), 85.8 (C-2'), 73.4 (C-3'), 70.6 (C-4'), 61.6 (C-5') (Teijeira *et al.*, 1997).

Ellagic acid (17) – white powder, EIMS *m/z* 302 $[C_{14}H_6O_8]^+$, ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.46 (1H, s, H-5 and H-5'); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 159.1 (H-7 and H-7'), 148.1 (H-4 and H-4'), 139.6 (H-3 and H-3'), 136.4 (H-2 and H-2'), 112.3 (H-1 and H-1'), 110.2 (H-5 and H-5'), 107.6 (H-6 and H-6') (Cha *et al.*, 2003).

Measurement of the $ONOO^-$ scavenging activity – $ONOO^-$ scavenging activity was measured by monitoring

Table 1. $ONOO^-$ scavenging activity of various fractions obtained from the MeOH extract of *P. linteus*

Samples	IC_{50} [a] (μg/mL)	
MeOH ext.	48.86	± 1.61
CH_2Cl_2 fr.	41.30	± 0.08
EtOAc fr.	8.64	± 0.05
BuOH fr.	86.82	± 3.71
H_2O fr.	180.47	± 5.04
DL-Penicillamine	1.72	± 0.05

[a] The values of peroxynitrite scavenging activity are expressed as the mean ± S.D.M. of 50% inhibitory concentrations of three experiments obtained by the interpolation of concentration-inhibition curves.

the oxidation of dihydrorhodamine 123 by modifying the method of Kooy *et al.* (1994). A stock solution of DHR 123 (5 mM) in dimethylformamide was purged with nitrogen and stored at −80°C. A working solution of DHR 123 (f.c. 5 μM) diluted from the stock solution was placed on ice and was not exposed to light prior to the study. The buffer of 90 mM sodium chloride, 50 mM sodium phosphate (pH 7.4), and 5 mM potassium chloride with 100 μM (f.c.) diethylenetriaminepentaacetic acid (DTPA) was purged with nitrogen and placed on ice before use. The $ONOO^-$ scavenging ability by the oxidation of DHR 123 was determined at room temperature on a microplate fluorescence reader FLx 800 (Bio-Tek instruments, USA) at excitation and emission wavelengths of 485 nm and 528 nm respectively. The background and final fluorescent intensities were measured 5 min after treatment with or without the addition of authentic $ONOO^-$ (f.c. 10 μM) in 0.3 M sodium hydroxide, and its final fluorescent intensity remained unchanged over time. The results were expressed as the mean ± S.D.M. ($n = 3$) of the final fluorescence intensity minus the background fluorescence. The effects were expressed as the percentage of inhibition of the DHR 123 oxidation. Penicillamine was used as a positive control.

Statistical Analysis – All values were expressed as the mean ± S.D.M. of triplicate experiments.

Results and Discussion

To identify the bioactive principles, we evaluated the solvent soluble fractions, including the CH_2Cl_2, EtOAc, and *n*-BuOH fraction, as well as the H_2O layer derived from the MeOH extract of *P. linteus*. As summarized in Table 1, the EtOAc soluble fraction of the MeOH extract from *P. linteus* showed the strongest $ONOO^-$ scavenging activity, with an IC_{50} (50% inhibitory concentration) value of 8.64 ± 0.05 μg/mL. Bioassay-guided fractionation of

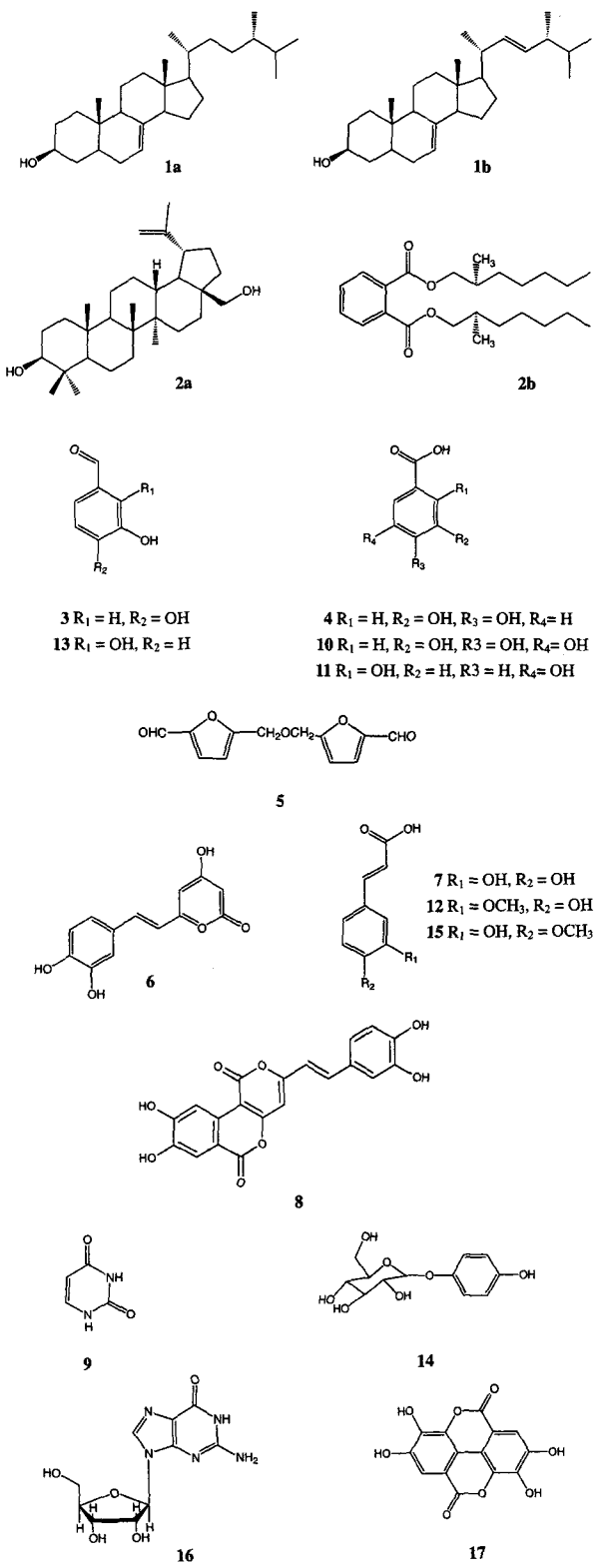

Fig. 1. Structures of compounds **1** - **17**.

the EtOAc soluble fraction led to the isolation of compounds **1** ~ **17** (Fig. 1). All compounds except **3**, **6**, **7**

and **16** were isolated for the first time from *P. linteus*.

The EI-MS spectrum of **1** showed molecular ion peaks at *m/z* 400 and 398, indicating a mixture of two sterols (**1a** & **1b**). A molecular ion peak for **1a** at *m/z* 400 was established as $C_{28}H_{48}O$, corresponding to fungisterol, and an additional molecular ion peak for **1b** at *m/z* 398 was recognized to be $C_{28}H_{46}O$, and determined as 5-dihydroergosterol. Another peak of *m/z* 271 was due to $[C_{28}H_{46}O\text{-}(C_9H_{17}+2H)]^+$. To confirm the structures of the two mixed components of **1**, NMR analyses were performed. The ^{1}H-NMR spectrum of **1a** showed singlet signals of angular methyl groups at δ 0.81 (3H, s, 19-CH_3) and δ 0.53 (3H, s, 18-CH_3); doublet signals of methyl groups at δ 1.02 (3H, d, J = 6.6 Hz, 21-CH_3), δ 0.86 (3H, d, J = 6.9 Hz, 26-CH_3), δ 0.80 (3H, d, J = 5.9 Hz, 27-CH_3), and δ 0.78 (3H, d, J = 4.4 Hz, 28-CH_3); and a signal of δ 3.60 (1H, m, H-3) and a olefinic proton signal at δ 5.19 (1H, m, H-7), indicating fungisterol. Moreover, additional peaks that included singlet signals of angular methyl groups at δ 0.80 (3H, s, 19-CH_3) and δ 0.54 (3H, s, 18-CH_3); doublet signals of methyl groups at δ 1.02 (3H, d, J = 6.6 Hz, 21-CH_3), δ 0.91 (3H, d, J = 4.8 Hz, 28-CH_3), δ 0.86 (3H, d, J = 6.9 Hz, 27-CH_3), and δ 0.82 (3H, d, J = 6.6 Hz, 26-CH_3); and then, a signal of δ 3.60 (1H, m, H-3) and olefinic proton signals at δ 5.19 (2H, m, H-22, 23) and δ 5.17 (1H, s, H-7), indicating 5-dihydroergosterol, which has two double bonds among a sterol skeleton. The DEPT of **1** showed 56 carbon atoms that were classified as twelve methyls, eighteen methylenes, twenty methines, and six quaternary carbon atoms. The ^{13}C-NMR spectrum showed methyl carbon signals at δ 20.5 (C-27), δ 19.0 (C-21), δ 17.6 (C-26), δ 15.4 (C-28), δ 13.0 (C-19), and δ 11.9 (C-18); and an olefinic proton signal at δ 5.19 (1H, m, H-7) connected with one carbon signal at δ 117.4 (C-7), and correlated with a carbon due to the double bond at δ 139.5 (C-8), thus demonstrating the presence of fungisterol, which has one double bond of a sterol. Furthermore, the observed methyl carbon signals of δ 21.1 (C-21), δ 19.9 (C-26), δ 19.6 (C-27), δ 17.6 (C-28), δ 13.0 (C-19), and δ 12.1 (C-18); an olefinic proton signal at δ 5.17 (1H, s, H-7) connected with one carbon at δ 117.4 (C-7); and an additional olefinic proton signal at δ 5.19 (2H, m, H-22, 23) connected with two carbons due to *exo* double bonds at δ 135.7 (C-22) and δ 131.8 (C-23), indicated 5-dihydroergosterol. Based on the above spectral data and by comparison with published spectral data, compound **1** was confirmed to be a mixture (1 : 1) of fungisterol (**1a**) (Rosecke *et al.*, 2000) and 5-dihydroergosterol (**1b**) (Lee *et al.*, 2006a).

The EI-MS spectrum of **2** showed molecular ion peaks at *m/z* 442 and 390. One molecular ion peak at *m/z* 442 was established as $C_{30}H_{50}O_2$, corresponding to the molecular formula of betulin (**2a**), and the other molecular ion peak at *m/z* 390 was determined to be $C_{24}H_{38}O_4$, corresponding to the molecular formula of 1,2-benzenedicarboxylic acid bis (2-methyl heptyl) ester (**2b**). The base ion peak at *m/z* 189 was a characteristic of betulin (**2a**), due to the opening of C-8, C-11, and C-12 bonds. An additional base ion peak at *m/z* 149 was formed due to the loss of water from the prominent peak at *m/z* 167, which is characteristic of a long chain phthalic acid ester. The other prominent peak at *m/z* 113 indicated a C_8H_{17} side chain. To confirm the structures of the two combined components of **2**, NMR analyses were performed. The ^{1}H-NMR spectrum of **2a** indicated signals assignable to *exo* methylene protons at δ 4.68 and δ 4.58 (each 1H, m, H-29); two oxygenated methylene protons at δ 3.79 and δ 3.33 (each 1H, s, J = 10.6 Hz, H-28); an oxygenated methene proton at δ 3.20 (1H, dd, J = 4.7, 10.6 Hz, H-3); and six methyl protons at δ 1.68 (3H, s, H-30), δ 1.02 (3H, s, H-27), δ 0.98 (3H, s, H-26), δ 0.97 (3H, s, H-25), δ 0.82 (3H, s, H-24), and δ 0.76 (3H, s, H-23). Moreover, the ^{1}H-NMR spectrum of **2b** exhibited two terminal methyls of a side chain at δ 0.90 (6H, m, H-7' and H-7"), and two branched chain methyls at δ 0.97 (6H, d, J = 4.4 Hz, 2' and 2"-CH_3). Also, the signals of eight methylenes of a side chain at δ 1.32 (16H, m, H-3', 3"', 4', 4"', 5', 5", 6', 6"), and two oxymethylene groups of a side chain at δ 4.20 (4H, m, H-1' and H-1") appeared. In the ^{13}C-NMR spectrum of **2**, a total of 30 carbon signals were observed, including olefinic carbons at δ 150.5 (C-20) and δ 109.7 (C-29), an oxygenated methine carbon at δ 79.0 (C-3), and an oxygenated methylene carbon at δ 60.5 (C-28). Based on the above results and by comparison with published spectral data, compound **2** was determined to be a mixture (1 : 1) of betulin (**2a**) (Im *et al.*, 2006) and 1,2-benzenedicarboxylic acid bis(2-methyl heptyl) ester (**2b**) (Singh *et al.*, 2006).

Compound **3** was obtained as a yellowish needle. The EI-MS spectrum of **3** showed a molecular ion peak at *m/z* 138, and its molecular formula was established as $C_7H_6O_3$. The IR spectrum of **3** showed absorption bands for hydroxyl groups (3222 and 3329 cm^{-1}), a conjugated carbonyl group (1652 cm^{-1}), and aromatic rings (1596, 1535, and 1445cm^{-1}). The ^{1}H-NMR spectrum indicated signals assignable to a 1,3,4-trisubsituted benzene ring at δ 7.30 (1H, dd, J = 1.7, 9.5 Hz, H-6), δ 7.28 (1H, d, J = 1.4 Hz, H-2), and δ 6.90 (1H, d, J = 8.1 Hz, H-5), and showed a singlet signal of aldehyde at δ 9.68 (1H, s, CHO). This possibility was further confirmed by the ^{13}C-

NMR spectrum, and compound **3** was determined as protocatechualdehyde (Kang *et al.*, 2004).

Compound **4** was obtained as a yellowish powder. The EI-MS spectrum of **4** showed a molecular ion peak at *m/z* 154, and its molecular formula was established as $C_7H_6O_3$. The ^{1}H-NMR spectrum showed an ABX coupled system attributable to *meta/ortho*, *meta*, and *ortho* couplings at δ 7.43 (1H, dd, J = 1.9, 8.1 Hz, H-6), δ 7.41 (1H, d, J = 1.9 Hz, H-2), and δ 6.80 (1H, d, J = 8.1 Hz, H-5), respectively. The ^{13}C-NMR spectrum displayed an oxygen-bearing aromatic ring at δ 151.5 (C-4) and δ 146.0 (C-3), and a carbonyl carbon at δ 170.2 (COOH). Based on the above results, compound **4** was determined as protocatechuic acid (Sun *et al.*, 2006).

Compound **5** was obtained as a white powder. The EI-MS spectrum of **5** showed a molecular ion peak at *m/z* 234, and its molecular formula was established to be $C_{12}H_5O_{10}$. The ^{1}H-NMR spectrum exhibited one aldehyde peak at δ 9.58 (1H, s, 2 × CHO), two aromatic doublets at δ 7.51 (1H, d, J =3.52 Hz, H-3 and H-3') and δ 6.76 (1H, d, J = 3.52 Hz, H-4 and H-4'), and one oxygenated methylene singlet at δ 4.62 (2H, s, 2 × CH_2). The ^{13}C-NMR spectrum showed only six peaks, one was a carboxylic carbon at δ 178.4 (2 × CHO); four aromatic carbons at δ 157.3 (C-5 and C-5'), δ 152.3 (C-2 and C-2'), δ 123.8 (C-3 and C-3'), and δ 112.3 (C-4 and C-4'); and one oxygenated methylene carbon at δ 63.7 (2 × CH_2). Therefore, compound **5** was determined as cirsiumaldehyde (Yun-Choi *et al.*, 1997).

Compound **6** was obtained as a yellow powder. In the ^{1}H-NMR spectra, two doublets at δ 7.29 (1H, d, J = 16.1 Hz, H-8) and δ 6.59 (1H, d, J = 15.7 Hz, H-7) indicated the typical resonances of *trans*-olefinic protons. Three aromatic protons at δ 7.01 (1H, s, H-10), δ 6.95 (1H, d, J = 2.1 Hz, H-14), and δ 6.76 (1H, d, J = 8.0 Hz, H-8) could be assigned to the protons of a 1,3,4-trisubstituted benzene. In the ^{13}C-NMR spectra, six signals corresponding to sp^2 methine carbons at δ 121.9 (C-14), δ 116.8 (C-7), δ 116.5 (C-13), δ 114.8 (C-10), δ 101.8 (C-5), and δ 90.2 (C-3); and seven quaternary carbons, which included carbonyl carbon at δ 173.8 (C-2), and then signals at δ 167.7 (C-4), δ 162.1 (C-6), δ 148.7 (C-11), δ 146.8 (C-12), δ 137.3 (C-8), and δ 128.8 (C-9) were observed. Based on the above results, compound **6** was determined as hispidin (Park *et al.*, 2004b).

Compound **7** was obtained as a yellowish powder. Its ^{1}H-NMR spectrum showed a typical ABX system attributable to *meta*, *meta/ortho*, and *ortho* coupling at δ 7.01 (1H, d, J = 1.7 Hz, H-2), δ 6.94 (1H, dd, J = 1.5, 8.1 Hz, H-6), and δ 6.75 (1H, d, J = 8.3, H-5), respectively. Moreover, *trans* coupling peaks were observed at δ 7.39 (1H, d, J = 15.8 Hz, H-7) and 6.16 (1H, d, J = 15.8 Hz, H-8). The ^{13}C-NMR spectrum exhibited an oxygen-bearing aromatic ring at δ 148.2 (C-4) and δ 145.5 (C-3), and a carboxyl carbon at δ 168.0 (COOH). According to these results, compound **7** was determined as caffeic acid (Lim *et al.*, 2003).

Compound **8** was isolated as a yellow powder. The EI-MS spectrum of **8** showed a molecular ion peak at *m/z* 380, indicating the molecular formula of $C_{20}H_{12}O_8$. The IR spectrum of **8** showed absorption bands for a hydroxyl group (3414 cm^{-1}), a conjugated carbonyl group (1671 cm^{-1}), and aromatic rings (1608, 1524, and 1406 cm^{-1}). The ^{1}H-NMR spectrum of **8** showed signals attributable to 1,3,4-trisubstituted benzene ring moieties at δ 6.78 (1H, d, J = 8.3 Hz, H-7'), δ 7.00 (1H, dd, J = 1.9, 8.3 Hz, H-8'), and δ 7.08 (1H, d, J = 1.9 Hz, H-4'); a *trans* disubstituted double bond at δ 6.79 (1H, d, J = 15.8 Hz, H-1') and δ 7.29 (1H, d, J = 15.8 Hz, H-2'); four phenolic hydroxyl protons at δ 10.77, δ 10.10, δ 9.58, and δ 9.17 (each 1H); and three singlets at δ 8.34 (1H, s, H-10), δ 7.52 (1H, s, H-7), and δ 6.73 (1H, s, H-4). The ^{13}C and DEPT spectrum of **8** showed twenty sp^2 carbon signals, including eight methines and twelve quaternary carbons (eight of which were oxygenated, δ > 145 ppm). The protonated carbons and their corresponding protons for **8** were unambiguously assigned by the HMQC experiment, and the connectivity of the carbons in **8** was established by the HMBC experiment. Based on the above results, compound **8** was determined as phelligridin D (Mo *et al.* 2004).

Compound **9** was isolated as a white powder. The ^{1}H-NMR spectrum of **9** showed the signals of two NH moieties at δ 11.00 (1H, s, 2-NH) and δ 10.81 (1H, s, 4-NH). The ^{13}C-NMR spectrum exhibited two carbonyl units at δ 164.3 (C-1) and δ 151.5 (C-3). Thus, compound **9** was determined as uracil (Lee *et al.*, 2004).

Compound **10** was obtained as a white powder. In the ^{1}H-NMR spectrum, a galloyl moiety was exhibited at δ 6.91 (2H, s, H-2, H-6). The ^{13}C-NMR spectrum exhibited a carboxyl moiety at δ 167.5 (COOH). Therefore, compound **10** was determined as gallic acid (Park *et al.*, 1993).

Compound **11** was obtained as a white powder. In the ^{1}H-NMR spectrum, three signals appeared at δ 7.15 (1H, d, J = 2.93 Hz, H-6), δ 6.96 (1H, dd, J = 2.93, 8.06 Hz, H-4), and δ 6.78 (1H, d, J = 8.79 Hz, H-3). In the ^{13}C-NMR spectrum, a carboxyl moiety at δ 171.8 (COOH), and six aromatic carbons at δ 154.1 (C-2), δ 149.4 (C-5), δ 123.7 (C-4), δ 117.8 (C-3), δ 114.5 (C-6), and δ 112.6 (C-2) were observed. Based on these results, compound **11** was determined as 2,5-dihydroxybenzoic acid (Park *et*

al., 2004c).

Compound **12** was obtained as a white needle. The ^{1}H-NMR spectrum exhibited the signals of olefinic protons of *trans* form at δ 7.54 (1H, d, J = 16.1 Hz, H-8) and δ 6.26 (1H, d, J = 16.1 Hz, H-7), and a methoxyl moiety at δ 3.88 (3H, s, 3-OCH_3). The ^{13}C-NMR spectrum exhibited a signal for a carboxyl moiety at δ 170.8 (COOH). Based on these results, compound **12** was determined as *trans*-ferulic acid (Aoki *et al.*, 1982).

Compound **13** was obtained as a yellowish needle. The ^{1}H-NMR spectrum of **13** indicated signals assignable to a 1,2,3-trisubsituted benzene ring at δ 7.13 (1H, dd, J = 1.46, 8.06 Hz, H-4), δ 7.06 (1H, dd, J = 1.46, 7.69 Hz, H-6), and δ 6.79 (1H, dd, J = 6.04, 9.78 Hz, H-5), and then an observed singlet signal of aldehyde at δ 9.80 (1H, s, CHO). This possibility was further confirmed by the ^{13}C-NMR spectral data, including an aldehyde signal at 192.8 (CHO) and six aromatic carbons at δ 149.7 (C-2), δ 146.1 (C-3), δ 122.7 (C-1), δ 121.2 (C-6), δ 119.6 (C-5), and δ 119.3 (C-4). Therefore, compound **13** was determined as 2,3-dihydroxybenzaldehyde (Nishida *et al.*, 2006).

Compound **14** was isolated as a yellow powder. The ^{1}H-NMR spectrum of **14** showed a signal at δ 3.23 (4H, m, H-2', H-3', H-4', H-5'), and the ^{13}C-NMR spectrum of **14** exhibited signals at δ 101.7 (C-1'), δ 77.0 (C-3' and C-5'), δ 76.6 (C-2'), δ 73.3 (C-4'), and δ 60.8 (C-6'), indicating a glucose moiety. An anomeric proton appeared as a doublet at δ 4.63 (1H, d, J = 7.3 Hz, H-1'), indicative of a sugar β-configuration. The ^{1}H-NMR signals of δ 6.86 (2H, d, J = 7.0 Hz, H-2 and H-6) and δ 6.65 (2H, d, J = 7.0 Hz, H-3 and H-5), and ^{13}C-NMR signals of δ 152.2 (C-1), δ 150.4 (C-4), δ 117.7 (C-2 and C-6), and δ 115.5 (C-3 and C-5), indicated the presence of a benzene ring substituted at the C-1 and C-4 positions. Therefore, compound **14** was determined as arbutin (Hisatomi *et al.*, 2000).

Compound **15** was obtained as a white needle. The ^{1}H-NMR spectrum exhibited the signals of olefinic protons of *trans* form at δ 7.59 (1H, d, J = 16.1 Hz, H-8) and δ 6.30 (1H, d, J = 15.7 Hz, H-7), and a methoxyl moiety at δ 3.88 (3H, s, 4-OCH_3). The ^{13}C-NMR spectrum exhibited signal of a carboxylated carbon at δ 170.8 (COOH). Therefore, compound **15** was determined as *trans*-isoferulic acid (Aoki *et al.*, 1982).

Compound **16** was isolated as a white powder. The ^{1}H-NMR spectrum showed the signal of a NH moiety at δ 8.34 (1H, s, 1-NH); an anomeric methine at δ 5.87 (1H, d, J = 6.18 Hz, H-1); and three oxygenated methines at δ 5.19 (1H, s, H-3), δ 3.96 (1H, dd, J = 3.36, 8 Hz, H-3), and δ 3.55 (1H, s, H-4). There were also hydroxylated methylenes attributed to a ribose moiety at δ 4.60 (1H, s, 5-OH) and δ 3.16 (2H, s, H-5). The ^{13}C-NMR spectrum allowed for the identification of the sugar moiety as ribose. According to the above results, compound **16** was determined as guanosine (Teijeira *et al.*, 1997).

Compound **17** was obtained as a white powder. The EI-MS spectrum of **17** showed a molecular ion peak at *m/z* 302, indicating the molecular formula of $C_{14}H_6O_8$. The ^{1}H-NMR spectrum of **17** indicated only a singlet signal at δ 7.46 (1H, s, H-5 and H-5'). Therefore, compound **17** was determined as ellagic acid by comparison with published ^{13}C-NMR signals (Cha *et al.*, 2003). Among the isolated compounds, **1**, **2**, **4**, **5**, and **8** - **15** and **17** were isolated in *P. linteus* for the first time.

As part of searching for $ONOO^-$ scavengers from natural sources, the $ONOO^-$ scavenging activities of compounds **1** - **17**, isolated from *P. linteus*, were evaluated. Although the antioxidant activities of hispidin (Park *et al.*, 2004b), phelligridin D (Lee *et al.*, 2006b), 2,5-dihydroxybenzoic acid (Okai *et al.*, 2006), arbutin (Pozharitskaya *et al.*, 2007), and isoferulic acid (Hamauzu *et al.*, 2006), were previously reported, their $ONOO^-$ scavenging activities were not yet evaluated. To determine the scavenging of authentic $ONOO^-$ by the bioactive principles, the oxidation of DHR 123 was detected using fluorescence. The $ONOO^-$ scavenging activities of all the isolated compounds (**1** - **17**) are shown in Table 2. With the exception of compounds **1**, **2**, **5**, **9**, and **16**, the other isolated compounds, **3**, **4**, **6** - **8**, **10** - **15**, and **17**, showed more potent $ONOO^-$ scavenging activities than the positive control, DL-penicillamine. The IC_{50} values of **3**, **4**, **7**, **8**, **13**, and **17** were 2.06 ± 0.10, 3.45 ± 0.57, 2.78 ± 0.36, 5.42 ± 0.26, 1.59 ± 0.09, and 2.01 ± 0.02 μM, respectively, indicating that the catechol groups may function as a pharmacophore. Also, the $ONOO^-$ scavenging activities of **12** and **15** were potent, with IC_{50} values of 0.91 ± 0.19 and 1.22 ± 0.37 μM, respectively. Their effects may in part depend on their hydroxyl and methoxyl groups. Moreover, the IC_{50} values of **6**, **10**, **11**, and **14**, exhibiting 0.71 ± 0.05, 1.13 ± 0.02, 1.82 ± 0.17, and 1.88 ± 0.07 μM, respectively, suggested that their effects might depend on the catechol and monohydroxyl groups of **6**, the galloyl moiety of **10**, the dihydroxyl group of **11**, and the monohydroxyl group of **14**, as pharmacophores. In particular, the $ONOO^-$ scavenging activities of **3**, **6**, **10** - **15**, and **17** were two-fold greater than DL-penicillamine, with an IC_{50} value of 5.04 ± 0.06 μM. The results indicate that the positions and numbers of the hydroxyl and methoxyl groups in the phenolic compounds contributed to their potent $ONOO^-$ scavenging activity.

Table 2. $ONOO^-$ scavenging activities of compounds **1** - **17** isolated from the EtOAc soluble fraction of the methanolic extract of *P. linteus*

Compounds	Dose (μg/mL)	Inhibition % (triplet)			Average (%)	IC_{50}[a] (μg/mL)	IC_{50}[a] (μM)
1	20	1.21	0.83	0.91	0.98 ± 0.12	> 50	> 200
2	20	0.64	0.23	0.24	0.37 ± 0.14	> 50	> 200
3	1	84.84	86.76	82.93	84.84 ± 1.11	0.28 ± 0.01	2.06 ± 0.10
	0.2	46.86	44.05	46.54	45.82 ± 0.89		
4	1	77.04	78.96	75.73	77.24 ± 0.94	0.53 ± 0.05	3.45 ± 0.57
	0.2	35.16	34.58	31.58	33.77 ± 1.11		
5	20	66.45	54.64	57.55	59.55 ± 3.55	4.96 ± 0.29	19.53 ± 0.75
	4	47.64	46.74	44.84	46.41 ± 0.83		
6	0.4	61.81	52.93	55.83	56.85 ± 2.61	0.18 ± 0.02	0.71 ± 0.05
	0.08	48.82	37.45	36.72	41.00 ± 3.92		
7	1	79.22	84.14	86.19	83.18 ± 2.07	0.50 ± 0.04	2.78 ± 0.36
	0.2	24.61	34.84	30.17	29.87 ± 2.96		
8	4	89.02	88.55	81.80	86.46 ± 2.33	2.06 ± 0.10	5.42 ± 0.26
	0.8	29.83	32.64	38.30	33.59 ± 2.49		
9	20	2.34	2.78	1.92	2.35 ± 0.25	> 50	> 200
10	0.2	54.09	53.77	51.91	53.26 ± 0.68	0.19 ± 0.00	1.13 ± 0.02
	0.04	11.33	15.35	12.21	12.96 ± 1.22		
11	0.8	69.87	69.72	65.60	68.40 ± 1.40	0.28 ± 0.04	1.82 ± 0.17
	0.16	49.95	50.01	48.64	49.53 ± 0.44		
12	0.2	59.42	59.91	64.57	61.30 ± 1.64	0.18 ± 0.02	0.91 ± 0.19
	0.04	20.84	15.40	25.31	20.51 ± 2.87		
13	0.8	80.91	83.70	76.20	80.27 ± 2.19	0.22 ± 0.02	1.59 ± 0.09
	0.16	48.86	50.93	49.37	49.72 ± 0.62		
14	0.8	66.09	62.44	65.08	64.54 ± 1.09	0.51 ± 0.03	1.88 ± 0.07
	0.16	42.47	46.37	43.49	44.11 ± 1.17		
15	1	82.03	85.23	76.98	81.41 ± 2.40	0.24 ± 0.04	1.22 ± 0.37
	0.2	46.67	43.86	51.47	47.33 ± 2.22		
16	20	0.87	0.21	0.43	0.50 ± 0.19	> 50	> 200
17	0.8	76.32	82.04	76.93	78.43 ± 1.81	0.61 ± 0.01	2.01 ± 0.02
	0.16	32.85	31.61	33.19	32.55 ± 0.48		
DL-Penicillamine	0.8	63.69	69.01	61.03	64.58 ± 2.35	0.75 ± 0.01	5.04 ± 0.06
	0.16	24.49	25.87	27.17	25.84 ± 0.77		

[a] The values of peroxynitrite scavenging activity are expressed as the mean ± S.D.M. of 50% inhibitory concentrations of three experiments obtained by the interpolation of concentration-inhibition curves.

Pannala *et al.* (1998) suggested that there are two possible mechanisms to scavenge $ONOO^-$, i.e., nitration and electron donation. Phenolic compounds can inhibit $ONOO^-$-mediated nitration of tyrosine either by acting as alternative substrates for nitration, as seen with monohydroxylated structures such as *p*-coumaric acid and ferulic acid, or by reducing reactive nitrogen species, as demonstrated for catechol structures such as caffeic acid (Pannala *et al.*, 1997, 1998; Kerry and Rice-Evans, 1999). Hydroxycinnamates and related phenolic acids are reported to function as potent antioxidants by virtue of their hydrogen-donating properties (Rice-Evans *et al.*, 1996) and metal-chelating properties (Salah *et al.*, 1995). It was reported that aromatic feruloyl groups play an important role in scavenging $ONOO^-$. In particular, curcumin I, with two feruloyl groups, showed more potent scavenging activity that was superior to curcumin II with one feruloyl group (Kim *et al.*, 2003). Therefore, the $ONOO^-$ scavenging activities of **3**, **6**, **10** - **15**, and **17** agree well with the proposed $ONOO^-$-scavenging mechanisms described above.

In conclusion, the MeOH extract and various fractions, as well as the active biological principles of *P. linteus*, may serve to alleviate undesirable accumulations of

$ONOO^-$, thus resulting in their possible application as drugs for $ONOO^-$-related diseases, including Alzheimer's disease, rheumatoid arthritis, cancer, ischemic/reperfusion injury, inflammation, and atherosclerosis.

Acknowledgement

This work was supported by the Pukyong National University Research Fund in 2006 (PK-2006-031).

References

Aoki, T., Takagi, K., Hirata, T., and Suga, T., Two naturally ocuuurring acyclic diterpene and norditerpene aldhydes from *Tetragonia tetragonoides*. *Phytochemistry* **21**, 1361-1363 (1982).

Aruoma, O.I., Spencer, J.P., and Mahmood, N., Protection against oxidative damage and cell death by the natural antioxidant ergothioneine. *Food Chem. Toxicol.* **37**, 1043-1053 (1999).

Beckman, J.S., Beckman, T.W., Chen, J., Marshall, P.A., and Freeman, B.A., Apparent hydroxyl radical production by peroxynitrite: Implication for endothelial injury from nitric oxide and superoxide. *Proc. Nat. Acad. Sci.* **87**, 1620-1624 (1990).

Boersma, B.J., Patel, R.P., Kirk, M., Jackson, P.L., Muccio, D., Darley-Usmar, V.M., and Barnes, S., Chlorination and nitration of soy isoflavones. *Arch. Biochem. Biophys.* **368**, 265-275 (1999).

Cha, B.C., Lee, H.W., and Rhim, T.J., Antioxidnat constituents and activities of acorn hull and chestnut hull. *Yakhak Hoeji* **47**, 212-217 (2003).

Cho, J.H., Cho, S.D., Hu, H., Kim, S.H., Lee, S.K., Lee, Y.S., and Kang, K.S., The roles of ERK 1/2 and p38 MAP kinases in the preventive mechanisms of mushroom *Phellinus linteus* against the inhibition of gap junctional intercellular communication by hydrogen peroxide. *Carcinogenesis* **23**, 1163-1169 (2002).

Choi, C.W., Jung, H.A., Kang, S.S., and Choi, J.S., Antioxidant constituents and a new triterpenoid glycoside from *Flos lonicerae*. *Arch. Pharm. Res.* **30**, 1-7 (2007).

Darley-Usmar, V., Wiseman, H., and Halliwell, B., Nitric oxide and oxygen radicals: a question of balance. *FEBS Lett.* **369**, 131-135 (1995).

Guo, J., Zhu, T., Collins, L., Xiao, Z.X., Kim, S.H., and Chen, C.Y., Modulation of lung cancer growth arrest and apoptosis by *Phellinus linteus*. *Mol. Carcinog.* **46**, 144-154 (2007).

Han, S.B., Lee, C.W., Jeon, Y.J., Hong, N.D., Yoo, I.D., Yang, K.H., and Kim, H.M., The inhibitory effect of polysaccharides isolated from *Phellinus linteus* on tumor growth and metastasis. *Immunopharmacol.* **41**, 157-164 (1999).

Han, S.B., Lee, C.W., Kang, J.S., Yoon, Y.D., Lee, K.H., Lee, K., Park, S. K., and Kim, H.M., Acidic polysaccharide from *Phellinus linteus* inhibits melanoma cell metastasis by blocking cell adhesion and invasion. *Int. Immunopharmacol.* **6**, 697-702 (2006).

Hamauzu, Y., Inno, T., Kume, C., Irie, M., and Hiramatsu, K., Antioxidant and antiulcerative properties of phenolics from Chinese quince, quince, and apple fruits. *J. Agric. Food Chem.* **54**, 765-772 (2006).

Hisatomi, E., Matsui, M., Kubota, K., and Kobayashi, A., Antioxidative activity in the pericarp and seed of Japanese pepper (*Xanthoxylum piperitum* DC). *J. Agric. Food Chem.* **48**, 4924-4928 (2000).

Ho, S.C., Tsai, T.H., Tsai, P.J., and Lin, C.C., Protective capacities of certain spices against peroxynitrite-mediated biomolecular damage. *Food Chem. Toxicol.* **30**, Epub ahead of print (2007).

Hur, J.M., Yang, C.H., Han, S.H., Lee, S.H., You, Y.O., Park, J.C., and Kim, K.J., Antibacterial effect of *Phellinus linteus* against methicillin-resistant *Staphylococcus aureus*. *Fitoterapia* **75**, 603-605 (2004).

Huh, J., *Dong-Eui-Bo-Gam*, Namsandang Press, Seoul, 1981, p. 26.

Ikekawa, T., Nakanish, M., Uehara, N., Chihara, G., and Fukuoka, F., Antitumor action of some Basidiomycetes, especially *Phellinus linteus*. *Gann.* **59**, 155-157 (1968).

Im, K.R., Jeong, T.S., Kwon, B.M., Baek, N.I., Kim, S.H., and Kim, D.K., Acyl-CoA: Cholesterol acyltransferase inhibitors from *Llex macropoda*. *Arch. Pharm. Res.* **29**, 191-194 (2006).

Kang, H.S., Choi, J.H., Cho, W.K., Park, J.C., and Choi, J.S., A sphingolipid and tyrosinase inhibitors from the fruiting body of *Phellinus linteus*. *Arch. Pharm. Res.* **27**, 742-750 (2004).

Kerry, N. and Rice-Evans, C.A., Inhibition of peroxynitrite-mediated oxidation of dopamine by flavonoid and phenolic antioxidants and their structural relationships. *J. Neruochem.* **73**, 247-253 (1999)

Kim, D.H., Choi, H.J., and Bae, E.A., Effect of artificially cultured *Phellinus linteus* on harmful intestinal bacterial enzymes and rat intestinal a-glucosidases. *J. Food Hyg. Safety* **13**, 20-23 (1998).

Kim, J.E., Kim, A.R., Chung, H.Y., Han, S.Y., Kim, B.S., and Choi, J.S., *In vitro* peroxynitrite scavenging activity of diarylheptanoids from *Curcuma longa*. *Phytother. Res.* **17**, 481-484 (2003).

Kim, H.G., Yoon, D.H., Lee, W.H., Han, S.K., Shrestha, B., Kim, C.H., Lim, M.H., Chang, W., Lim, S., Choi, S., Song, W.O., Sung, J.M., Hwang, K.C., and Kim, T.W., *Phellinus linteus* inhibits inflammatory mediators by suppressing redox-based NF-kappaB and MAPKs activation in lipopolysaccharide-induced RAW 264.7 macrophage. *J. Ethnopharmacol.* **114**, 307-315 (2007).

Klotz, L.O. and Sies, H., Defenses against peroxynitrite: selenocompounds and flavonoids. *Toxicol. Lett.* **140**, 125-132 (2003).

Kooy, N.W., Royall, J.A., Ischiropoulos, H., and Beckman, J.S., Peroxynitrite-mediated oxidation of dihydrorhodamine 123. *Free Radic. Biol. Med.* **16**, 149-156 (1994).

Kooy, N.W., Royall, J.A., Ye, Y.Z., Kelly, D.R., and Beckman, J.S., Evidence for *in vivo* peroxynitrite production in human acute lung injury. *Am. J. Respir. Crit. Care Med.* **151**, 1250-1254 (1995).

Lee, S.H., Shim, S.H., Kim, J.S., and Kang, S.S., Constituents from the fruiting bodies of *Ganoderma applanatum* and their aldose reductase inhibitory activity. *Arch. Pharm. Res.* **29**, 479-483 (2006a).

Lee, I.K., Jung, J.Y., Seok, S.J., Kim, W.G., and Yun, B.S., Free radical scavengers from the medicinal mushroom *Inonotus xeranticus* and their proposed biogenesis. *Bioorg. Med. Chem. Lett.* **16**, 5621-5624 (2006b).

Lee, Y.S., Lee, H.S., Shin, K.H., Kim, B.K., and Lee, S.H., Constituents of the Halophyte *Salicornia herbacea*. *Arch. Pharm. Res.* **27**, 1034-1036 (2004).

Lim, E.K., Higgins, G.S., Li, Y., and Bowles, J., Regioselectivity of glucosylation of caffeic acid by a UDP-glucose:glucosyltransferase is maintained in planta. *Biochem. J.* **373**, 987-992 (2003).

Min, B.S., Yun, B.S., Lee, H.K., Jung, H.J., Jung, H.A., and Choi, J.S., Two novel furan derivatives from *Phellinus linteus* with anti-complement activity. *Bioorg. Med. Chem. Lett.* **16**, 3255-3257 (2006).

Mo, S., Wang, S., Zhou, G., Yang, Y., Li, Y., Chen, X., and Shi, J., Phelligridins D-F: cytotoxic pyrano[4,3-c][2]benzopyran-1,6-dione and furo[3,2-c]pyran-4-one derivatives from the fungus *Phellinus igniarius*. *J. Nat. Prod.* **67**, 823-828 (2004).

Nakamura, T., Matsugo, S., Uzuka, Y., Matsuo, S., and Kawagishi, H., Fractionation and anti-tumor activity of the mycelia of liquid-cultured *Phellinus linteus*. *Biosci. Biotechnol. Biochem.* **68**, 868-872 (2004).

Nishida, J. and Kawabata, J., DPPH radical scavenging reaction of hydroxyl- and methoxychalcones. *Biosci. Biotechnol. Biochem.* **70**, 193-202 (2006).

Okai, Y. and Higashi-Okai, K., Radical-scavenging activity of hot water

extract of Japanese rice bran-association with phenolic acids. *J. UOEH.* **28**, 1-12 (2006).

Pannala, A., Rice-Evans, C.A., Halliwell, B., and Singh, S., Inhibition of peroxynitrite mediated tyrosine nitration by catechin polyphenols. *Biochem. Biophy. Res. Commun.* **232**, 164-168 (1997).

Pannala, A., Razaq, R., Halliwell, B., Singh S, and Rice-Evans, C.A., Inhibition of peroxynitrite dependent tyrosine nitration by hydroxy-cinnamates: Nitration or electron donation? *Free Radic. Biol. Med.* **24**, 594-606 (1998).

Park, I.H., Jeon, S.Y., Lee, H.J., Kim, S.I., and Song, K.S., A beta-secretase (BACE1) inhibitor hispidin from the mycelial cultures of *Phellinus linteus. Planta Med.* **70**, 143-146 (2004a).

Park, I.H., Chung, S.K., Lee, K.B., Yoo, Y.C., Kim, S.K., Kim, G.S., and Song, K.S., An antioxidant hispidin from the mycelial cultures of *Phellinus linteus. Arch. Pharm. Res.* **27**, 615-618 (2004b).

Park, H.W., Baek, N.I., Kim, S.H., Kwon, B.M., Chung, I.S., Park, M.H., Kim, S.H., and Kim, D.K., Phytochemical components from the whole plants of *Arabis glabra* (L.) Bernh. *Kor. J. Pharmacogn.* **35**, 320-323 (2004c).

Park, W.Y., Lee, S.C., Ahn, B.T., Lee, S.H., Ro, J.S., and Lee, K.S., Phenolic compounds from *Acaloha australis* L. *Kor. J. Pharmacogn.* **24**, 20-24 (1993).

Pollard, S.E., Kuhnle, G.G., Vauzour, D., Vafeiadou, K., Tzounis, X., Whiteman, M., Rice-Evans, C., and Spencer, J.P., The reaction of flavonoid metabolites with peroxynitrite. *Biochem. Biophys. Res. Commun.* **350**, 960-968 (2006).

Pozharitskaya, O.N., Ivanova, S.A., Shikov, A.N., Makarov, V.G., and Galambosi, B., Separation and evaluation of free radical-scavenging activity of phenol components of green, brown, and black leaves of *Bergenia crassifolia* by using HPTLC-DPPH method. *J. Sep. Sci.* **30**, 2447-2452 (2007)

Pryor, W. and Squadrito, G., The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. *Am. J. Physiol.* **268**, 699-722 (1996).

Rhee, Y.K., Han, M.J., Park, S.Y., and Kim, D.H., *In vitro* and *In vivo* antitumor activity of the fruit body of *Phellinus linteus. Korean J. Food Sci. Technol.* **32**, 477-480 (2000).

Rice-Evans, C.A., Miller, N.J., and Paganga, G., Structure-antioxidant relationships of flavonoids and phenolic acids. *Free Radic. Biol. Med.* **20**, 933-956 (1996).

Rosecke, J. and Konig, W.A., Constituents of various wood-rotting basidiomycetes. *Phytochemistry* **54**, 603-610 (2000).

Salah, N., Miller, N.J., Paganga, G., Tijburg, L., Bolwell, G.P., and Rice-Evans, C., Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Arch. Biochem. Biophys.* **322**, 339-346 (1995).

Sandoval, M., Zhang, X.J., Liu, X., Mannick, E.E., Clark, D.A., and Miller, M.J., Peroxynitrite-induced apoptosis in T84 and RAW 264.7 cells: attenuation by L-ascorbic acid. *Free Radic. Biol. Med.* **22**, 489-495 (1997).

Shin, J.Y., Lee, S., Bae, I.Y., Yoo, S.H., and Lee, H.G., Structural and biological study of c arboxymethylated *Phellinus linteus* polysaccharides. *J. Agric. Food Chem.* **55**, 3368-3372 (2007).

Shon, Y.H. and Nam, K.S., Antimutagenicity and induction of anticarcinogenic phase II enzymes by basidiomycetes. *J. Ethnopharmacol.* **77**, 103-109 (2001).

Sies, H. and Masumoto, H., Ebselen as a glutathione peroxidase mimic and as a scavenger of peroxynitrite. *Adv. Pharmacol.* **38**, 229-246 (1997).

Singh, N., Mahmood, U., Kaul, V.K., and Jirovetz, L., A new phthalic acid ester from *Ajuga bracteosa. Nat. Prod. Res.* **20**, 593-597 (2006).

Song, S.S., Kim, S.H., Sa, J.H., Jin, C., Lim, C.J., and Park, E.H., Anti-angiogenic, antioxidant and xanthine oxidase inhibition activities of the mushroom *Phellinus linteus. J. Ethnopharmocol.* **88**, 113-116 (2003).

Sun, L.X., Fu, W.W., Ren, J., Xu, L., Bi, K.S., and Wang, M.W., Cytotoxic constituents from *Solanum lyratum. Arch. Pharm. Res.* **29**, 135-139 (2006).

Szabo, C., The role of peroxynitrite in the pathophysiology of shock, inflammation and ischemia-reperfusion injury. *Shock* **6**, 79-88 (1996).

Teijeira, M., Santana, L., and Uriarte, E., Assignment of the ^{13}C-NMR spectra of some adenine, hypoxanthine and guanine carbonucleosides. *Magn. Reson. Chem.* **35**, 806-807 (1997).

Tipoe, G.L., Leung, T. M., Hung, M. W., and Fung, M. L., Green tea polyphenols as an anti-oxidant and anti-inflammatory agent for cardiovascular protection. *Cardiovasc. Hematol. Disord. Drug Targets.* **7**, 135-144 (2007).

Virag, L., Szabo, E., Gergely, P., and Szabo, C., Peroxynitrite-induced cytotoxicity: mechanism and opportunities for intervention. *Toxicol. Lett.* **140**, 113-124 (2003).

Yun-Choi, H.S., Pyo, M.K., and Park, K.M., Cirsiumaldehyde from *Gastrodia elata, Nat. Prod. Sci.* **3**, 104-105 (1997).

(Accepted February 3, 2008)