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**Short Communication**

# Inhibition on LDL-oxidation by Phenolic Compounds from the Fruit Body of *Phellinus linteus*

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**The fruit body of *Phellinus linteus* was extracted with 80% *aqueous* MeOH, and the concentrated extract was successively partitioned using EtOAc, *n*-BuOH and $H_2O$. From the EtOAc and *n*-BuOH fractions, three phenolic compounds were isolated through repeated silica gel and ODS column chromatography. The chemical structures of these compounds were determined as 2-(3',4'-dihydroxyphenyl)-1,3-benzodioxole-5-aldehyde (1), 4-(3,4-dihydroxyphenyl)-3-buten-2-one (2), and protocatechuic acid methyl ester (3) by spectroscopic data including NMR, MS and IR. Compounds 1~3 exhibited low density lipoprotein (LDL) antioxidant activity with $IC_{50}$ values of 1.7, 0.7, and 2.4 μM, respectively.**

**Key words:** *2-(3',4'-dihydroxyphenyl)-1,3-benzodioxole-5-aldehyde, 4-(3,4-dihydroxyphenyl)-3-buten-2-one, LDL-oxidation,* Phellinus linteus, *protocatechuic acid methyl ester, phenolic compound*

*Phellinus linteus* is a fungus belonging to the family Hymenochaetaceae, which is distributed mainly in Asia, tropical America, and North Africa [Dai and Xu, 1998]. Extracts from this fungus have long been used as a traditional oriental medicine in Korea and Japan for the treatment of various diseases including arthritis of the knee, oral ulcers, gastroenteric disorders, lymphatic disease, and various cancers [Heo, 1993; Kang *et al.*, 2004]. It has been reported that *P. linteus* has anti-cancer [Choi *et al.*, 1996; Han *et al.*, 1999], anti-tumor [Ikekawa *et al.*, 1968], anti-mutagenic [Ji *et al.*, 2000], anti-angiogenic [Song *et al.*, 1995], anti-oxidant [Lee *et al.*, 2000], and immune activity [Kim *et al.*, 1996]. A number of researchers have reported various chemical constituents of *P. linteus* such as polysaccharides [Lee *et al.*, 2001], proteoglycans [Lee *et al.*, 2001], sphingolipids [Kang *et al.*, 2004] and hispidine [Park *et al.*, 2004], etc. Among these, β-glucan is especially important as it is known to have anti-tumor activity and immuno-stimulating effects [Han *et al.*, 1999; Lee *et al.*, 2000]. From our previous phytochemical research on the fruit body of this mushroom, we reported the presence of ergosterols [Lyu *et al.*, 2007a; 2007b; 2007c] and phenolic compounds [Lyu *et al.*, 2008]. Except for polysaccharides, however, there has been little research studying various pharmacological activities of lower molecular weight compounds from *P. linteus*. Furthermore, only a few reports have claimed that extracts of the fruit body of *P. linteus* act to prevent atherosclerosis or to inhibit LDL-oxidation.

It has been suggested that the oxidation of LDL cholesterol is an important step in the formation of atherosclerotic lesions [Steinberg *et al.*, 1989; Diaz *et al.*, 1997]. Evidence to support this hypothesis is based in part on observational studies that demonstrate associations between oxidized LDL cholesterol and the presence of both atherosclerotic lesions [Regnstrom *et al.*, 1992] and the progression of carotid artery atherosclerosis [Salonen *et al.*, 1992]. This paper describes three phenolic compounds isolated from *P. linteus* that inhibit LDL oxidation.

**Extraction and isolation.** The dried fruit body of *P. linteus* (1 kg) was extracted two times at room temperature with 80% *aqueous* MeOH (3 L×2). The extracts were successively partitioned with water (1 L), EtOAc (1 L×2) and *n*-BuOH (1 L×2). The EtOAc extract (10 g) was applied to a silica gel cc (6.5×17 cm) and was eluted with a gradient of $CHCl_3$:MeOH (15:1→

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**Abbreviations:** cc, column chromatography; $CHCl_3$, chloroform; EI-MS, electron ionization mass spectrometry; EtOAc, ethylacetate; IR, infra-red; LDL, low density lipoprotein; MeOH, methanol; *n*-BuOH, normal-butanol; ODS, octadecyl silica gel; PLB, *n*-buthanol fraction of *P. lneteus*; PLE, ethyl acetate fraction of *P. lneteus*; TLC, thin layer chromatography; $V_e/V_t$, elution volume/total volume

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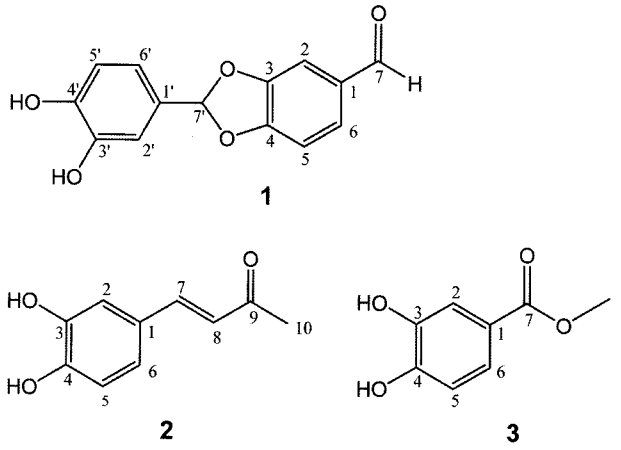


**Fig. 1. Chemical structures of compounds 1~3 isolated from the fruit body of *P. linteus*.**

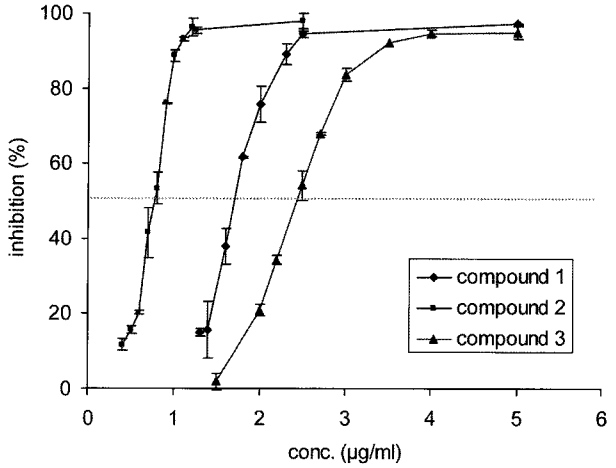


**Fig. 2. Inhibition on LDL-oxidation by compounds 1~3 isolated from the fruit body of *P. linteus*.** *The data are presented as mean±standard deviation of three replications. **Positive control of LDL-oxidation (BHT): 85.9% (±0.5) inhibition at 4 μM

10:1→7:1→5:1→3:1→1:1, 1 L of each) while monitoring by TLC to produce 22 fractions (PLE1 to PLE22). Fraction PLE6 (250 mg, $V_e/V_t$ 0.18-0.20) was applied to an ODS cc (3×8 cm) and was eluted with MeOH:$H_2O$ (1:3→1:1, 700 mL of each) to ultimately produce compound **1** [22.4 mg, $V_e/V_t$ 0-0.03, TLC (ODS $F_{254}$) $R_f$ 0.75, MeOH:$H_2O$=2:1] and compound **2** [17.5 mg, $V_e/V_t$ 0.10-0.13, TLC (ODS $F_{254}$) $R_f$ 0.66, MeOH:$H_2O$= 2:1]. The concentrated *n*-BuOH fraction (PLB, 31 g) was subjected to a silica gel cc (7×23 cm) eluting with a gradient of $CHCl_3$:MeOH:$H_2O$ (6:4:1→5:4:1, 4 L of each), which resulted in 19 fractions (PLB1 to PLB19). Fraction PLB2 (831 mg, $V_e/V_t$ 0.05-0.12) was subjected to a silica gel cc (5×15 cm) and eluting with $CHCl_3$:MeOH (20:1→10:1, 2 L of each) to produce 10 fractions (PLB2-1 to PLB2-10). Fraction PLB2-3 (103 mg, $V_e/V_t$ 0.25-0.40) was applied to an ODS cc (2.5×5 cm) and was eluted with MeOH:$H_2O$ (2:3, 500 mL) yielding compound **3** [6.5 mg, Ve/Vt 0.25-0.35, TLC (ODS $F_{254}$) $R_f$ 0.65, MeOH:$H_2O$ =2:1].

Compound **1**: Pale purple amorphous powder (MeOH); mp 131-137°C; $[\alpha]_D$ ±0° (racemic); IR (KBr, ν) 3329, 3323, 1655, 1647, 1443, 1296, 1167, 878, 754; EI-MS *m/z* 258 $[M]^+$; $^1$H-NMR (400 MHz, $CD_3OD$, δ) 9.67 (1H, s, H-7), 7.30 (1H, dd, *J*=7.2, 2.0, H-6), 7.29 (1H, d, *J*=2.0, H-2), 6.90 (1H, d, *J*=7.2, H-5), 6.84 (1H, d, *J*=1.6, H-2'), 6.74 (1H, d, *J*=6.8, H-5'), 6.74 (1H, d, *J*=6.8, 1.6, H-6'), 5.20 (1H, s, H-7'); $^{13}$C-NMR(100 MHz, $CD_3OD$, $\delta_C$) 192.9 (C-7), 153.5 (C-3), 147.0 (C-4), 146.5 (C-4'), 145.9 (C-3'), 130.9 (C-1'), 130.7 (C-1), 126.3 (C-6), 119.3 (C-6'), 116.1 (C-5), 115.7 (C-5'), 115.2 (C-2), 114.7 (C-2'), 104.7 (C-7').

Compound **2**: Pale purple amorphous powder (MeOH); mp 177-178°C; IR (KBr, ν) 3420, 1651, 1603, 1556, 1286, 1115; EI-MS *m/z* 178 $[M]^+$, 163 $[M-Me]^+$; $^1$H-NMR (400 MHz, $CD_3OD$, δ) 7.51 (1H, d, *J*=16.4 Hz, H-7), 7.07 (1H, d, *J*=2.4 Hz, H-2), 6.98 (1H, dd, *J*=8.4, 2.4, H-6), 6.78 (1H, d, *J*=8.4 Hz, H-5), 6.54 (1H, d, *J*=16.4, H-8), 2.32 (3H, s, H-10); $^{13}$C-NMR (100 MHz, $CD_3OD$, $\delta_C$) 201.39 (C-9), 149.83 (C-4), 146.74 (C-3), 146.74 (C-7), 127.56 (C-1), 124.55 (C-8), 123.44 (C-6), 116.42 (C-5), 115.14 (C-2), 27.02 (C-10).

Compound **3**: Pale purple amorphous powder (MeOH); mp 136°C; IR (KBr, ν) 3338, 1685, 1604, 1521, 1438, 1301; EI-MS *m/z* 168 $[M]^+$, 137, 109, 81, 63, 53; $^1$H-NMR (400 MHz, $CD_3OD$, δ) 7.38 (1H, d, *J*=8.4, 2.0, H-6), 7.39 (1H, d, *J*=2.0, H-2), 6.77 (1H, d, *J*=8.4, H-5), 3.80 (3H, s, H-7-OMe); $^{13}$C-NMR (100 MHz, $CD_3OD$, $\delta_C$) 168.9 (C-7), 151.7 (C-4), 146.2 (C-3), 123.6 (C-6), 122.5 (C-1), 117.4 (C-2), 115.8 (C-5), 52.7 (C-7-OMe).

**Low-density lipoprotein isolation and oxidation assay.** Plasma was obtained from fasted, healthy normolipidemic volunteers. The LDL was isolated by a standard procedure with a slight modification [Havel *et al.*, 1955]. The TBARS assay of Buege and Aust [Buege and Aust, 1978] was used with a slight modification. Thus, an LDL solution (250 μL, 50-100 μg of protein) in 10 mM PBS (pH 7.4) was supplemented with 10 μM $CuSO_4$. The oxidation was performed in a screw-capped 5 mL glass vial at 37°C in a shaking water bath. After 4 h incubation, the reaction was terminated by the addition of 1 mL of 20% trichloroacetic acid. Following precipitation, 1 mL of 0.67% thiobarbituric acid (TBA) in 0.05 N NaOH was added and vortexed, and the final mixture was heated for 5 min at 95 μ, cooled on ice, and then centrifuged for 2 min at 1,000×g. The optical density of the produced malondialdehyde (MDA) was measured at 532 nm. Calibration was completed with an MDA standard prepared from tetramethoxypropane.

When the MeOH extract of the fruit body of *P. linteus* was developed on a silica gel TLC, visualization by spraying with a 10% $H_2SO_4$ solution and then heating the TLC plate gave purple spots, indicating the presence of phenolic compounds in the

extract. The MeOH extracts were partitioned into EtOAc, *n*-BuOH, and $H_2O$ layers through solvent fractionation. Three phenolic compounds (compounds **1~3**) were isolated through repeated silica gel and ODS cc of the EtOAc and *n*-BuOH fractions, and their structures were determined to be 2-(3',4'-dihydroxyphenyl)-1,3-benzodioxole-5-aldehyde (**1**), 4-(3,4-dihydroxyphenyl)-3-buten-2-one (**2**), and protocatechuic acid methyl ester (**3**) by interpretation of extensive spectroscopic data and comparisons with literature data [Tagashira *et al.*, 1998; Lyu *et al.*, 2008]. Especially, the stereostructure of the double bond in compound **2** was determined as *trans*-configuration from the coupling constant ($J$=16.4 Hz) observed between H-7 and H-8 in the $^1$H-NMR spectrum.

In order to determine whether these compounds might be effective in the development of hypercholesterolemic or antiatherogenic agents, their potential for inhibiting LDL oxidation was evaluated. Compounds **1~3** demonstrated LDL antioxidant activity with $IC_{50}$ values of 1.7, 0.7, and 2.4 μM, respectively, and were more potent than the positive control, BHT, which had an $IC_{50}$ value of 3.0±0.4 mM [Hung *et al.*, 2006]. In this study, we have demonstrated that compounds **1**, **2**, and **3** isolated from the fruit body of *P. linteus* inhibited LDL oxidation activity.

LDL oxidation inhibitors from natural sources have been rarely reported. Because compounds **1-3** not only exhibit activity similar to the positive control used in this study but are derived from natural sources which have been used as an oriental medicine in Korea, they may prove useful for treating hypercholesterolemia and atherosclerosis.

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