Inhibition of Low Density Lipoprotein-oxidation, ACAT-1, and ACAT-2 by Lignans from the Bark of *Machilus thunbergii*

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The bark of *Machilus thunbergii* was extracted with 80% aqueous methanol (MeOH), and the concentrated extract was partitioned using ethyl acetate (EtOAc), butanol (n-BuOH), and H₂O, successively. From the EtOAc fraction, five lignans were isolated through the repeated silica gel, octadecyl silica gel (ODS), and Sephadex LH-20 column chromatography. Based on nuclear magnetic resonance (NMR), mass spectroscopy (MS), and infrared spectroscopy (IR) spectroscopic data, the chemical structures of the compounds were determined to be machilin A (1), machilin F (2), licarin A (3), nectandrin A (4), and nectandrin B (5). This study presents a comparative account of five lignans from *M. thunbergii* bark contributing inhibition of low density lipoprotein (LDL), ACAT-1, and ACAT-2. Compounds 2-5 showed varied degree of antioxidant activity on LDL with IC₅₀ values of 2.1, 11.8, 15.3, and 4.1 µM. Compounds 1, 2, and 3 showed inhibition activity on ACAT-1 with values 63.4±6.9% (IC₅₀=66.8 µM), 53.7±0.9% (IC₅₀=109.2 µM), and 78.7±0.2% (IC₅₀=40.6 µM), respectively, at a concentration of 50 mg/mL, and on ACAT-2 with values 47.3±1.5% (IC₅₀=149.7 µM), 39.2±0.2% (IC₅₀=165.2 µM), and 52.1±1.0% (IC₅₀=131.0 µM), respectively, at a concentration of 50 mg/mL.

Key words: ACAT-1, ACAT-2, LDL-oxidation, licarin A, machilin A, machilin F, *Machilus thunbergii*, nectandrin A, nectandrin B

*Machilus thunbergii* Siebold & Zuccarinii (Lauraceae) is a widely distributed tree used in Korean traditional medicine [Kim, 1984]. The cortex of the plant is used for treatment of leg edema, abdominal distension, and pain [Chung and Shin, 2000]. Several lignans and neolignans have been reported from the bark of this plant [Shimomura et al., 1987; 1988]. Previous studies on the bark of *M. thunbergii* reported nitric oxide synthesis inhibitory butanolides [Kim and Ryu, 2003]. Some lignans from the bark have been shown to be antioxidant [Yu et al., 2000], melanin biosynthesis inhibitory [Li et al., 2003], caspase-3 activating [Park et al., 2004], and neuroprotective [Ma et al., 2004; 2009]. In this study we isolated 5 lignans and investigated on their inhibition effect on LDL-oxidation and ACAT-1 and ACAT-2 activities.

Oxidation of low-density lipoprotein (LDL) is considered as an early event in the development of atherosclerosis [Glass and Witztum, 2001]. Antioxidants such as probucol, N,N-diphenylnaphthalenediamine, and butylated hydroxy-toluene (BHT) have been shown to decrease the degree of oxidation and the extent of atheromatous lesions in animal models of atherosclerosis, but have side effects [Jialal and Devaraj, 1996]. Thus antioxidants from natural source are attractive alternatives. Cholesterol acyltransferase (ACAT) catalyses the acylation of cholesterol to cholesteryl ester and it exists in two isoforms, ACAT-1 and ACAT-2. ACAT-1 is in charge of foam cell formation in macrophages, whereas ACAT-2 controls the cholesterol absorption in intestinal mucosal cells [Rudel et al., 2001]. Therefore, ACAT inhibition is a useful strategy for treating hypercholesterolemia and atherosclerosis by the effect of lowering plasma cholesterol in humans [Lawrence and Gregory, 2000].

The dried bark of *M. thunbergii* was received from Green Flower Cosmetics Co., in 2009 and was identified by Prof. Dae-Keun Kim, College of Pharmacy, Woosuk University, Jeonju, Korea. A voucher specimen (KHU-090826) was deposited at the laboratory of Natural Products Chemistry, Kyung Hee University, Yongin, Korea. The dried and powdered bark of *M. thunbergii* (500 g) was extracted two times with 80% aqueous methanol (MeOH) [4 L] at room temperature. The MeOH extract was successively partitioned with water (2 L), ethyl...
acetate (EtOAc) [2 L×2], and n-butanol (1 L×2). The concentrated EtOAc extract (MTE, 16 g) was applied to a silica gel (Merck 60A, 70-230 mesh ASTM, Darmstadt, Germany) column chromatography (c.c.) (φ 6×14 cm) and was eluted with CHCl3-MeOH-H2O (20:3:1→17:3:1→9:3:1→6:4:1, 6 L of each) with monitoring by thin layer chromatography (TLC) to provide 20 fractions (MTE-1 to MTE-20).

Fraction MTE-2 [1.79 g, elution volume/total volume (V/V) 0.006-0.009] was subjected to the silica gel c.c. (φ 4×12 cm) eluted with n-hexane-EtOAc (10:1→5:1, 2.5 L of each), yielding 22 fractions (MTE-2-1 to MTE-2-22) and an isolated compound 1 [33 mg, V/V 0.019-0.020, TLC (SiO2 Rf 0.20, n-hexane-EtOAc=10:1).

Subfraction MTE-2-17 (100 mg, V/V 0.218-0.356) was subjected to an octadecyl silica gel (ODS) c.c. (φ 2.5×2 cm) and eluted with n-hexane-EtOAc-MeOH-H2O (4:2:4:2), yielding 5 fractions (MTE-2-17-1 to MTE-2-17-5). Then, MTE-2-17-3 (66 mg) subjected to a Sephadex LH-20 c.c. (φ 2×35 cm) eluted with n-hexane-EtOAc-MeOH-H2O (4:2:4:2), yielding 5 fractions (MTE-2-17-3-1 to MTE-2-17-3-5). Further, MTE-2-17-3-3 (52 mg, V/V 0.40-0.56) was applied to the ODS c.c. (φ 2×25 cm) eluted with MeOH-H2O (5:4), yielding 7 fractions (MTE-2-17-3-1 to MTE-2-17-3-7) and ultimately isolated compound 2 [18 mg, V/V 0.110-0.115, TLC (ODS F254S Rf 0.47, MeOH-H2O=6:1) and compound 3 [19.5 mg, V/V 0.27-0.65, TLC (ODS F254S Rf 0.42, MeOH-H2O=6:1).

Subfraction MTE-2-21 (107.5 mg, V/V 0.66-0.89) was applied to the ODS c.c. (φ 2.5×4 cm) eluted with MeOH-H2O (4:1), yielding 18 fractions (MTE-2-21-2 to MTE-2-21-18) and ultimately isolated compound 4 [25.5 mg, V/V 0.14-0.18, TLC (ODS F254S Rf 0.55, MeOH-H2O=6:1) and compound 5 [21.5 mg, V/V 0.08-0.10, TLC (ODS F254S Rf 0.65, MeOH-H2O=6:1).
Inhibitory activity of lignans from the bark of *Machilus thunbergii* on LDL-oxidation, ACAT-1, and ACAT-2

In a search of biologically active materials in *M. thunbergii*, the barks were extracted with MeOH and partitioned into EtOAc, n-BuOH, and H$_2$O layers through solvent fractionation. Successive repeated silica gel, ODS, and Sephadel LH-20 c.c. of the obtained fractions led to isolation of five lignans. Structural identifications of these lignans were determined to be machilin A (1), machilin F (2), licarin A (3), nectandrin A (4), and nectandrin B (5) by interpretation of extensive spectroscopic data and comparison of data with those described in the literature [Shimomura *et al.*, 1987; 1988; Lee *et al.*, 2009] (Fig. 1).

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 observation that demonstrate associations between oxidized LDL cholesterol and both the presence of atherosclerotic lesions [Regnstrom *et al.*, 1992] and the progression of carotid artery atherosclerosis [Salonen *et al.*, 1992]. In order to determine whether the compounds might be effective in the development of hypercholesterolemic or antiatherogenic agents, their potential for inhibiting LDL oxidation was evaluated. Compounds 2-5 showed varied degree of antioxidant activity with IC$_{50}$ values of 2.1, 11.8, 15.3, and 4.1 µM, respectively. The key factor governing the antioxidant activity is attributed to phenolic hydroxyl. Among these values compounds 2 and 5 showed significant inhibition in comparison to the positive control, BHT, which had an IC$_{50}$ value 2.1 µM. Compounds 2 and 5 showed more potent activity than saururin (IC$_{50}$ 8.5 µM) and virolin (IC$_{50}$ 4.3 µM) from *Saururus chinensis*, the compound 2 was also more effective than machilin D (IC$_{50}$ 2.9 µM), [Ahn *et al.*, 2001]. This is first report of LDL oxidation activity for compounds 2-5.

Compounds 1, 2, and 3 showed significant inhibitory activity on ACAT-1 with values of 63.4±6.9, 53.7±0.9, and 78.7±0.2%, respectively, and on ACAT-2 with values 47.3±1.5, 39.2±0.2, and 52.1±1.0%, respectively, at a concentration 50 mg/mL (Table 1). Compounds 1 and 3 expressed as mean±SD of three replicated experiments.

Table 1. Inhibition activity of lignans from the bark of *M. thunbergii* on LDL-oxidation, ACAT-1, and ACAT-2

<table>
<thead>
<tr>
<th>compounds</th>
<th>LDL-oxidation Inhibition (%)</th>
<th>ACAT-1 Inhibition (%)</th>
<th>ACAT-2 Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 µg/mL 5 µg/mL 2.5 µg/mL</td>
<td>50 µg/mL IC$_{50}$ (µM)</td>
<td>50 µg/mL IC$_{50}$ (µM)</td>
</tr>
<tr>
<td>1</td>
<td>-16.9±2.6 N.D N.D</td>
<td>63.4±6.9 66.8</td>
<td>47.3±1.5 149.7</td>
</tr>
<tr>
<td>2</td>
<td>97.6±0.1 94.9 70.7±0.1</td>
<td>53.7±0.9 109.2</td>
<td>39.2±0.2 165.2</td>
</tr>
<tr>
<td>3</td>
<td>89.8±0.0 70.0 60.3±1.2</td>
<td>78.7±0.2 40.6</td>
<td>52.1±1.0 131.0</td>
</tr>
<tr>
<td>4</td>
<td>96.1±0.5 90.1 61.4±1.1</td>
<td>16.7±1.7 -</td>
<td>14.5±0.6 -</td>
</tr>
<tr>
<td>5</td>
<td>84.4±0.5 40.1 11.0±1.9</td>
<td>20.3±2.6 -</td>
<td>13.0±0.7 -</td>
</tr>
</tbody>
</table>

Data are means±SD (n=3).

1* Positive control of LDL-Oxidation, BHT, showed 85.0±0.3% inhibition at 3.0 µM with IC$_{50}$ value of 2.1 µM.

2* Positive control of ACAT-1, OAA, showed 33.6±1.5% inhibition at 0.1 µM with IC$_{50}$ value of 0.126 µM.

3* Positive control of ACAT-2, OAA, showed 36.0±0.6% inhibition at 0.1 µM with IC$_{50}$ value of 0.138 µM.

Fig. 1. Chemical structures of compounds 1-5 isolated from the bark of *M. thunbergii*.

![Chemical structures of compounds 1-5](image-url)
showed more potent activity than meso-dihydroguaiaretic acid from *Myristica fragrans* with ACAT 60.0±1.2% at concentration 100 mg/mL and compound 2 showed more potent activity than syringing methyl ether from *M. fragrans* with ACAT 27.2±0.9% at concentration 100 mg/mL [Song *et al.*, 2004] and oleanolic acid from *Albizia julibrissin* with ACAT-1 52.5±0.7% and ACAT-2 22.0±2.6% at concentration 50 mg/mL [Baek *et al.*, 2010]. This is first report of ACAT-1 and ACAT-2 inhibitory sources. The compounds worth further investigation. There are few reports of LDL-oxidation inhibitors, ACAT-1, and ACAT-2 from the natural resources. The compounds 2, 3, 4, and 5 exhibited similar activity for LDL-oxidation in comparison with the positive control. Compounds 1, 2, and 3 showed significant inhibitory activity on ACAT-1, and on ACAT-2. Therefore, the bark of *M. thunbergii* is used in oriental medicine in Korea can be useful source for treating hypercholesterolemia and atherosclerosis, and dementia. Among the active compounds, compound 2 appeared as the most potent inhibitor of LDL-oxidation with an IC₅₀ value of 2.1 μM and compound 3 as the most potent inhibitor of ACAT-1 and ACAT-2 with IC₅₀ value of 40.6 μM and 131.0 μM, respectively.

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References


