Aldose Reductase Inhibition Effect of Phenolic Compounds Isolated from *Paulownia coreana* Bark^{*1}

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ABSTRACT

Nine compounds, caffeic acid, naringenin, apigenin, luteolin, kaempferol, verbascoside, isoverbascoside, isocampneoside II, and cistanoside F, were isolated from the EtOAc and n-BuOH fractions of *P. coreana* bark. The structures of these compounds (1-9) were elucidated by spectroscopic methods and literature data. All the isolates were subjected to in vitro bioassay to evaluate their inhibitory activity against rat lens aldose reductase. Among these, compounds 6 and 8 indicated the significant inhibitory activity on rat lens aldose reductase with IC₅₀ values of 2.67 and 5.59 μ M, respectively. Especially, The inhibition activity of acteoside was 3.9 times better than that of quercetin as a positive control (10.6 μ M). These results suggested that phenylethanoid glycosides are likely to be the potential compounds for the prevention and/or treatment of diabetic complications.

Keywords : Aldose reductase inhibition, *Paulownia coreana*, phenylpropanoid glycosides, diabetic complications

1. INTRODUCTION

For a long time, many researchers have applied traditional medicinal plants to treat hyperglycemia and diabetes mellitus (Cignarella *et al.*, 1996; Ahmed *et al.*, 2001), and these medicinal plants are known to be effective in treating diabetes (Kesari *et al.*, 2005). These medicinal plants are known to be effective in treating diabetes and expected to achieve a high level of antihyperglycemic effect without adverse re-

actions, unlike conventional antidiabetic drugs. These medicinal plants contain polyphenol in their seeds, fruits, leaves, and barks (Prior and Gu, 2005).

Aldose reductase (EC 1.1.1.21; ALR2) is a NADPH-specific aldo-keto oxidoreductase used to catalyze the conversion of glucose to sorbitol in the polyol pathway (Scheme 1), which is the alternate route of glucose metabolism: a minor part of nonphosphorylism: glucose (Ahmed *et al.*, 2001; Kesari *et al.*, 2005). Because polyols

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Scheme 1. Polyol pathway.

such as sorbitol do not readily move across the cell membranes, they can generate a severe osmotic stress causing swelling and cell damage.

Under hyperglycemia, the increased flux of glucose goes through the polyol pathway because of the saturation of hexokinase with ambient glucose. This leads to overflow of the products of the polyol pathway (Gabbay, 1973) (Scheme 1).

Through polyol pathway, glucose is turned into sorbitol by aldose reductase (AR), the first and rate-limiting enzyme in the pathway, concomitant with conversion of NADPH into NADP⁺. Another enzyme, sorbitol dehydrogenase, then oxidizes sorbitol to fructose. In diabetes melitus, the increased glucose level results in sorbitol being produced at a faster rate than its oxidation to fructose and the accumulation of sorbitol in small blood vessels, nerves, lens, retina, and kidney can produce a hyperosmotic effect, leading to membrane permeability changes and the onset of cellular pathology (Kador *et al.*, 1985). AR inhibitors are thus an attractive pharmacological target for the treatment of diabetic complications.

P. coreana is a fast growing tree and has been used in the traditional medicine from ancient times to treat cough, phlegm, carbuncle, hemorrhoid, gonorrhea, and bronchitis pneumonia (Kim, 1996) and also extensively used in the wood industry for furniture making, musical instruments, and handicrafts.

This study was attempted to determine the possible rat lens aldose reductase-inhibitory effects of 70% acetone extracts from the bark of *P. coreana*, as well as the effects of its organic solvent soluble fractions, including the dichloro-

methane (CH₂Cl₂), ethyl acetate (EtOAc), n-butanol (n-BuOH) and water (H₂O) layers, using DL-glyceraldehyde as a substrate. Via bioassay-guided separations of the above extracts, several flavonoids were isolated as active components.

In the present study, we tried to find out constituents from the barks of *P. coreana* and their inhibitory effects on rat lens aldose reductase (RLAR) was evaluated.

2. MATERIALS and METHODS

2.1. General and Chemical

The ¹H- and ¹³C-NMR spectra were obtained from a Bruker Avance DPX 600 spectrometer at the operating frequency of 600 MHz (¹H) and 100 MHz (¹³C), respectively. Chemical shifts were given in δ values with tetramethylsilane as an internal standard. FAB-MS was performed with a micromass Autospec M363 spectrometer using m-nitro benzyl alcohol as a matrix. DL-glyceraldehyde, the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), and quercetin were purchased from Sigma (St. Louis, MO, USA). Sephadex LH-20 (GE Healthcare Bio-Science AB, Sweden) was used as the column packing material. All other chemicals and reagents were of analytical grade, and commercially available.

2.2. Plant Materials

The barks of *P. coreana* were collected from the experimental forest of Kangwon National University in 2006. It was identified by Prof. Wan-Geun Park of the Department of Forestry. Avoucher specimen (No. WSE0604-2) has been deposited at the herbarium in the Department of Forest biomaterials Engineering, Kangwon National University, Chuncheon, Korea.

2.3. Isolation and Identification

Air-dried barks (4.0 kg) of *P. coreana* were exhaustively extracted with acetone-H₂O (7:3, v/v) for 72 h at room temperature. Combined aqueous acetone solutions were concentrated under reduced pressure and freeze dried. The crude extracts 350 g were suspended in H₂O and fractionated successively with n-hexane, methylenechloride (CH₂Cl₂), ethyl acetate (EtOAc), and butanol (BuOH), respectively. The EtOAc residue (16.7 g) of bark was subjected to column chromatography on SephadexLH-20, eluting with MeOH-H₂O (5:1, v/v) to give three main fractions and labeled PBE-1 (16.4 g), PBE-2 (100 mg) and PBE-3 (131 mg). Fraction PBE-1 was rechromatographed on a column for further purification with MeOH-H₂O (3:1, 1:1, 1:2, 1:3, v/v) and EtOH-Hexane (3:1, v/v) as eluting solvents to isolate compound **1** (caffeic acid, 266 mg) and compound **2** (naringenin, 64 mg), compound **6** (verbascoside, 6.33 g) and compound **7** (isoverbascoside, 872 mg). Compound **5** (kaempferol, 41 mg) and compound **1** (caffeic acid, 44 mg) were obtained from PBE-2 when MeOH-H₂O (3:1, v/v) was used as an eluent. PBE-3 was re-applied on a column for further purification with MeOH-H₂O (3:1, v/v) to get compound **3** (apigenin, 11 mg), compound **4** (luteolin, 39 mg). A portion of BuOH soluble fraction (38 g) was applied on column chromatography on SephadexLH-20, eluting with MeOH-H₂O (3:1, v/v) to give four fractions and labeled PBB-1 (2.4 g), PCSB-2 (33.6 g), PBB-3 (1.8 g) and PBB-4 (149 mg). PBB-4 was identified and numbered as compound **6** (isoverbascoside, 149 mg). PBB-3 was further chromatographed using MeOH-H₂O (1:1, 1:4, v/v) as eluents to give compound **6** (verbascoside, 280 mg), compound **7** (isoverbascoside, 142 mg), compound **8** (isocampneoside II, 35 mg), compound **9** (cistanoside F, 9 mg).

2.4. Assay for Rat Lens Aldose Reductase (RLAR) Inhibitory Activity

Rat lenses were removed from Sprague Dawley rats weighing 250~280 g and frozen until required. The crude aldose reductase was prepared according to the method of Hayman and Kinoshita with some modification (Hayman and Kinoshita, 1965; Lee *et al.*, 2008). A partially purified enzyme with a specific activity of 6.5 U/mg was routinely used to test the enzyme inhibition. The partially purified material was separated into 1.0 ml aliquots and stored at 40°C. RLAR activity was assayed spectrophotometrically by measuring the decrease in the absorption of NADPH at 340 nm over 4 min. period with DL-glyceraldehyde as the substrate. Each 1.0 ml cuvette contained equal units of the enzyme, 0.10 M sodium phosphate buffer (pH 6.2), 0.3 mM NADPH, with or without 10 mM of the substrate and an inhibitor. The concentration of inhibitors giving 50% inhibition of enzyme activity (IC₅₀) was calculated from the least-squares regression line of the logarithmic concentrations plotted against the residual activity.

3. RESULTS and DISCUSSION

It has been acknowledged that plant-derived extracts and phytochemicals are potential alternatives to synthetic inhibitors against AR. Currently, the compounds isolated from plants as ARIs are classified as flavonoids, stilbenes, ellagic acid and its derivatives, and alkaloids (Kawanishi *et al.*, 2003)

The 70% acetone extract of *P. coreana* was shown to exert an inhibitory effect on rat lens aldose reductase. In order to identify the active compounds from the bark of *P. coreana*, the extract was systematically separated into five fractions, which were then tested for inhibitory ac-

Table 1. Inhibitory effects of *P. coreana* bark on rat lens aldose reductas (RLAR)

Extract and Fractions	Inhibition (%) ^{a)}
70% acetone extract	65.39
Hexane fraction	32.88
CH ₂ Cl ₂ fraction	25.59
EtOAc fraction	70.79
BuOH fraction	69.32
H ₂ O fraction	46.13
Quercetin ^{b)}	74.23

Sample concentration was 5 µg/ml.

^{a)} Inhibition rates were calculated as percentages with respect to the control value.

^{b)} Quercetin was used as a positive control.

tivity against aldose reductase. Among them, ethyl acetate fraction exhibited the strongest inhibitory activity against RLAR, where as hexane, methylene chloride, and water fractions were ineffective as shown in Table 1. Further chromatography of active fraction using Sephadex LH-20 gave four phenylethanoid glycosides and six phenolics active against aldose reductase. By comparison with the literature data, their structures were identified as caffeic acid **1** (Kim *et al.*, 2002), naringenin **2** (Shen, 1993), apigenin **3** (Markham and Chari, 1982), luteolin **4** (Watanabe, 1999), kaempferol **5** (Okuyama *et al.*, 1978), verbascoside **6** (Ota *et al.*, 1993), isoverbascoside **7** (Ota *et al.*, 1993), isocampneoside II **8** (Kim *et al.*, 2008), cistanoside F **9** (Kim *et al.*, 2008). The chemical structures of compounds **1-9** isolated from the bark of *P. coreana* are shown in Fig. 1.

Inhibitory activities of compounds **1-9** against rat lens aldose reductase were compared with that of quercetin, a natural aldose reductase inhibitor. As shown in Table 2, compounds **6-9**, phenylethanoid glycosides, were found to be more effective than phenolic compounds in inhibiting rat lens aldose reductase, their IC₅₀ values being 2.7, 9.5, 5.6, and 9.9 µM, respectively.

Table 2. Inhibitory effects of the compounds isolated from *P. coreana* bark on rat lens aldose reductase (RLAR)

Compounds	RLAR IC ₅₀ µM ^{a)}
Quercetin ^{b)}	10.6
1	10.0
2	> 40.0
3	> 40.0
4	9.5
5	14.7
6	2.7
7	9.5
8	5.6
9	9.9

^{a)} Inhibition rates were calculated as percentages with respect to the control value. The concentration of each test sample giving rise to 50% inhibition of activity (IC₅₀) was estimated from the least-squares regression line of the logarithmic concentration plotted against inhibitory activity.

^{b)} Quercetin was used as a positive control.

whereas naringenin (**2**) and apigenin (**3**) (IC₅₀ value of > 40.0 µM) in this bioassay system were showed weak inhibitory activity RLAR.

Phenylethyl glycosides are an interesting group of natural products which are widely distributed in the plant kingdom, most of which are isolated from medicinal plants. They have a common structure, consisting of a hydroxyphenylethyl β-D-glucopyranoside, which is functionalized with a phenylpropanoic acid (e.g., cinnamic acid, *p*-coumaric acid, caffeic acid, and ferulic acid) as an ester. There may also be monosaccharides residue such as rhamnose and glucose attached to the glucose moiety.

Previous study demonstrated the possible relationships of structure to the inhibitory activities of flavonoids; (1) flavones are more active than those with other flavonoid skeleton; (2) flavones and flavonols having a catechol moiety

Fig. 1. Chemical structures of compounds isolated from *P. coreana* bark.

show strainer activity; the 2-3 double bond enhances the activity. Inhibitory activities of two flavones (**3** and **4**), a flavonol (**5**), and a flavanone (**2**) isolated from *P. coreana* against aldose reductase were similar to those previously reported (Shin *et al.*, 1995; Matsuda *et al.*, 2002). Especially, compound **4** possessing the 3',4'-dihydroxy moiety on its B ring exhibits stronger activity than the compound **5** with a 4'-hydroxy group.

4. CONCLUSIONS

In recent years, the possibility of preventing the onset of diabetes using dietary supplements and/or herbal medicines has attracted increasing attention. In this study, aqueous acetone extracts from *P. coreana* bark and their component, most of phenylethanoid compounds, showed inhibitory activity against aldose reductase. Therefore, phenylethanoid glycosides could be offered as leading compounds for further study as

a natural drug for diabetic complications.

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