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Anti-acetylcholinesterase activity of the aglycones of phenolic glycosides isolated from *Leonurus japonicus*

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ABSTRACT

Objective: To find the genuine structure with anti-acetylcholinesterase (anti-AChE) from the phenolic glycosides abundant in *Leonurus japonicus* (Lamiaceae). The assay for anti-AChE activity is often used to lead anti-Alzheimer's drugs.

Methods: The five phenolic glycosides, tiliroside, leonurusoside C, 2‴-syringoylrutin, rutin, and lavanduliofolioside were isolated from *L. japonicus*. The activities of the glycosides were relatively low. Seven compounds including *p*-coumaric acid, caffeic acid, hydroxytyrosol, salidroside, syringic acid, kaempferol, and quercetin, which are produced by the hydrolysis of the five glycosides, were also assayed for anti-AChE activity.

Results: Of those seven compounds, the five compounds other than salidroside and syringic acid exhibited potent anti-AChE activities. In particular, the IC_{50}s of caffeic acid and quercetin were (1.05 ± 0.19) and (3.58 ± 0.02) μg/mL, respectively. Rutin was the most abundant flavonoid in the extract (9.18 mg/g as measured by HPLC).

Conclusion: The substances with potent anti-AChE were caffeic acid, quercetin, *p*-coumaric acid, kaempferol, and hydroxytyrosol that can be produced from their glycosides.

1. Introduction

Natural glycosides are usually highly contained in crude drugs, though very often they show false negative effects *in vitro* tests. Furthermore, the glycosides are efficiently extracted by water because of their high polarity. A lot of aglycones that are produced through biotransformation from the parent glycosides show higher bioactivities rather than their glycosides [1–3]. It is an example that acteoside, one of phenylethanoid glycosides, can be hydrolyzed by the intestinal bacteria [4].

Acetylcholinesterase (AChE) activity is usually very high in Alzheimer's disease of the most common type of dementia. Memory deficits are caused mainly by the reduction of cerebral acetylcholine which is a neurotransmitter responsible for memory in the brain. Alzheimer's disease has a variety of symptoms including psycho-behavior disturbances, cognitive impairment, memory deficits, and learning disturbance [5,6]. Researchers have studied AChE inhibitors [7] or β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1: β-secretase) inhibitors [8,9] to develop anti-Alzheimer's agents. The three anti-Alzheimer's drugs like donepezil, rivastigmine, and galantamine are belong to the class of AChE inhibitors. Memory-enhancing mechanisms for anti-Alzheimer's activity are usually based on a combined role of anti-inflammatory, antioxidant, and neuroprotective action in signal transduction pathway [10–12]. In this study, anti-AChE activities of the phenolic glycosides isolated in *Leonurus japonicus* (*L. japonicus*) (Lamiaceae) together with their aglycones were investigated.

Leonuri Herba referring to the herb of *L. japonicus* are often used as an oriental herb medicine. *L. japonicus* which is called

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motherwort or Chinese motherwort is a widely distributed biennial plant in Korea, Japan, and China to Cambodia [13]. The constituents known from this herb are flavonoids [14], alkaloids [15], labdane-type diterpenoids [16]. In particular, application of HPLC-MS method to this herb unveiled a variety of phenolic constituents from *Leonurus sibiricus* [17]. Leonuri Herba is known to be effective against hypertension, blood circulation, and menstrual disorder in the Oriental medicinal society [13]. Furthermore, it is also used as a tonic herb to treat sunstroke or anorexia in the summer season in the folkloric society of Korea.

2. Materials and methods

2.1. Instruments and reagents

UV spectra were taken on a UV-160A UV–visible recording spectrophotometer. IR spectra were recorded with KBr disk method on a JASCO 4200 FT-IR spectrometer. ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were taken on a Bruker AM-600 spectrometer using an internal standard tetramethylsilane (TMS). High resolution mass spectra were taken on a Synapt G2 mass spectrometer. The ion exchange resin used for column chromatography was Diaion HP-20 (Mitsubishi Chemical Co.). The Varian HPLC system used for the analysis comprised Prostar 210 pumps, Prostar 325 UV–Vis detector, and a Shiseido Capcell PAK C18 column (5 μm, 4.6 mm × 250.0 mm, Japan). A MetaTherm temperature controller was used to maintain a constant temperature in the HPLC column. Silica gel used for column chromatography was silica gel Art No. 7734 (Merck, Germany). The two mobile phases, H_2O and MeOH, were purchased from J.T.Baker (Phillipsburg, NJ, USA). Standards for the five compounds, *p*-coumaric acid (lot# 65H7705), caffeic acid (lot# 0001416536), syringic acid (lot# BCBR8160V), kaempferol (lot# 075K1574), and quercetin (lot# 14H0957), were purchased from Sigma–Aldrich (NY, USA), and hydroxytyrosol (lot# 11011411) was purchased from Extrasynthese (Genay Cedrex, France). Salidroside that had been isolated from *Acer tegmentosum* was also used.

2.2. Plant material

The aerial parts of *L. japonicus* (Lamiaceae) were collected on the mountain area of Wonju city, Korea in August, 2016. The collected plants were dried in a shaded place, and cut for extraction. This plant was identified by Prof. Byong-Min Song in the Department of Forest Science, Sangji University, Korea. The voucher specimen (natchem# 79) was deposited in the Laboratory of Natural Products Chemistry, Department of Pharmaceutical Engineering, Sangji University, Korea.

2.3. Extraction and fractionation

The plant material (2.0 kg) was extracted thrice with 15 L of 80% MeOH under reflux. The extracted liquid was filtered and concentrated under reduced pressure on a rotatory evaporator to give 285.7 g of aq. MeOH extract. To divide it into two parts, the aq. MeOH extract was fractionated into the two fractions, $CHCl_3$ and BuOH fractions. In brief, 280 g of the aq. MeOH extract was suspended in 2.0 L distilled water and partitioned with 1.6 L $CHCl_3$ four times. The lower $CHCl_3$ soluble part was concentrated *in vacuo* to give a $CHCl_3$ fraction (34.0 g). The residual aqueous part was further fractionated with 1.6 L BuOH four times. The BuOH-soluble part was also concentrated on a rotatory evaporator to give a BuOH fraction (49.3 g).

2.4. Isolation of phenolic glycosides

To isolate phenolic glycosides, the BuOH fraction was further fractionated on a Diaion HP-20 column (Ø 6.0 cm × 40.0 cm) chromatography using MeOH-H_2O solvents with increasing MeOH ratio. The BuOH fractions was washed by eluting with 1.0 L H_2O to remove salt- or sugar-like substances, and successively developed with 30% MeOH. One liter (1 L) of 40% MeOH was added to that column and the eluate was collected, and concentrated to afford LS-40 (0.95 g). Then, this column was further developed using the eluting solvents in the order: each 1.0 L of 50% MeOH, 60% MeOH, and 70% MeOH affording LS-50 (2.87 g), LS-60 (1.91 g), and LS-70 (1.13 g), respectively.

LS-70 (1.0 g) was subjected to silica gel column (Ø 3.0 cm × 30.0 cm, SiO_2, 80 g) chromatography with the eluting solvent $CHCl_3$-MeOH-H_2O (10:3:1, lower phase). The fractions containing the same spot shown on TLC was combined and concentrated to dryness to yield compounds **1** (38 mg) and **2** (360 mg). LS-60 (1.0 g) was chromatographed over silica gel column (Ø 3.0 cm × 30.0 cm, SiO_2, 80 g) using a mobile phase of $CHCl_3$-MeOH-H_2O (10:3:1, lower phase). The fractions showing the same spot on TLC were concentrated to afford compound **3** (683 mg). LS-50 (3.0 g) was subjected to silica gel column chromatography (Ø 4.0 cm × 40.0 cm, SiO_2, 250 g) with the eluting solvent (65:35:10, lower phase). The fractions showing the same spot on TLC was combined, concentrated to dryness to afford compound **4** (530 mg). LS-40 (1.0 g) was chromatographed over silica gel column (Ø 3.0 cm × 30.0 cm, SiO_2, 80 g) using a mobile phase of $CHCl_3$-MeOH-H_2O (10:3:1, lower phase). The fractions showing the same spot on TLC were combined and concentrated to dryness to afford compound **5** (56 mg).

2.5. Hydrolysis of isolated compounds

Hydrolysis of the isolated compounds was performed by dissolving 15 mg of each compound in 5% H_2SO_4 in MeOH-H_2O (1:1) and was heated under reflux for 5 h. The resulting solutions were neutralized with NH_4OH and partitioned with EtOAc. The aqueous- and EtOAc phases were dried *in vacuo*. The non-sugar moieties were identified using standard compounds.

2.6. Anti-AChE assay

The activity of AChE was measured by the modification of Ellman's method [18]. This method measures the activity of AChE serving ACh as the substrate. In brief, 140 μL of 100 mM sodium phosphate buffer (pH 8.0), 20 μL of the sample, and AChE (0.36 U) were added in a 96 well microplate. After incubating at room temperature for 15 min, 200 μL of the reactant filled with 10 μL of DTNB [5,5′-dithiobis(2-nitrobenzoic acid)] and 10 μL of the substrate ACh were put in 96 well plate. After 15 min, the absorbance of yellow 5-thio-2-nitrobenzoate anion produced by the reaction between thiocholine and DTNB were measured at 412 nm using a microplate reader VERSAmax (Molecular Device, CA, USA).