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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).

The inhibition was shown as the IC_{50} value (unit μg/mL) indicating 50% inhibition of the enzyme activity. Inhibition (%) of AChE was calculated by using the following equation: Inhibition (%) = [1-(A_{samp}/A_{con})/A_{std}] × 100. A_{samp}, A_{con}, and A_{std} are the absorbance measured with a sample, with sample but without enzyme, and without a sample, respectively.

3. Results

Chemical structures of compounds **1**–**5** (Figure 1) were determined by comparison of their spectroscopic data (UV–Vis, IR, ^{1}H- and ^{13}C-NMR) with those reported in the literature. The spectroscopic data of the five compounds are listed as below.

Compound **1**: Tiliroside [kaempferol 3-*O*-(6″-*O*-p-coumaroyl)-β-D-glucopyranoside)].

Yellow powder. UV λ_{max} (MeOH) nm (log ε): 266 (4.18), 267 (4.45); IR (KBr) ν_{max} cm^{-1}: 3 230, 1 684, 1 610, 1 559, 1 508; ^{1}H-NMR (600 MHz, CD_3OD) δ: kaempferol-6.16 (1H, d, *J* = 2.4 Hz, H-6), 6.33 (1H, d, *J* = 2.4 Hz, H-8), 7.32 (2H, d, *J* = 8.4 Hz, H-2′,6′), 6.82 (2H, d, *J* = 8.4 Hz, H-3′,5′); D-glc 5.26 (1H, d, *J* = 7.2 Hz, H-1); *p*-coumaroyl 8.00 (2H, d, *J* = 9.0 Hz, H-2,6), 6.84 (2H, d, *J* = 9.0 Hz, H-3, 5), 7.42 (1H, d, *J* = 16.2 Hz, H-7), 6.09 (1H, d, *J* = 16.2 Hz, H-8); ^{13}C-NMR (150 MHz, CD_3OD) δ: kaempferol 157.0 (C-2), 133.8 (C-3), 178.0 (C-4), 160.0 (C-5), 98.5 (C-6), 164.5 (C-7), 93.5 (C-8), 158.0 (C-9), 104.2 (C-10), 121.3 (C-1′), 129.8 (C-2′,6′), 115.4 (C-3′,5′), 161.5 (C-4′), 115.4 (C-3′), 161.5 (C-4′); D-glc 102.6 (C-1), 74.4 (C-2), 76.6 (C-3), 74.3 (C-4), 62.9 (C-6); *p*-coumaroyl 125.7 (C-1), 130.8 (C-2), 114.7 (C-3), 159.7 (C-4), 114.7 (C-5), 130.8 (C-6), 145.1 (C-7), 113.4 (C-8), 167.4 (C-9). The ^{1}H- and ^{13}C-NMR spectroscopic data of compound **1** were in good agreement with the literature data of tiliroside [19].

Compound **2**: Leonurusoside C (kaempferol 3-O-(3‴-O-syringoyl)-α-L-rhamnopyranosyl(1 → 6)-β-D-glucopyranoside)

Yellow powder. UV λ_{max} (MeOH) nm (log ε): 267 (4.13), 268 (4.41); IR (KBr) ν_{max} cm^{-1}: 3 231, 2 972, 1 699, 1 653, 1 517 (aromatic C=C), 1 056 (glycosidic C—O); ^{1}H-NMR (600 MHz, CD_3OD) δ: Kaempferol – 6.20 (1H, d, *J* = 2.4 Hz, H-6), 6.42 (1H, d, *J* = 2.4 Hz, H-8), 8.10 (2H, d, *J* = 9.0 Hz, H-3′,5′), 6.94 (2H, d, *J* = 9.0 Hz, H-3′,5′); D-glc - 5.15 (1H, d, *J* = 7.8 Hz, H-1); L-rha – 4.59 (1H, brs, H-1), 1.20 (3H, d, *J* = 5.4 Hz, H-6); syringoyl – 7.45 (2H, s, H-2,6), 3.93 (2 × 3H, s, syringoyl OCH_3); ^{13}C-NMR (150 MHz, CD_3OD) δ: kaempferol – 157.2 (C-2), 134.2 (C-3), 178.1 (C-4), 160.0 (C-5), 98.5 (C-6), 164.6 (C-7), 93.6 (C-8), 158.2 (C-9), 104.3 (C-10), 121.5 (C-1′), 131,0 (C-2′,6′), 115.6 (C-3′,5′), 161.6 (C-4′), D-glc 104.6 (C-1), 74.4 (C-2), 76.8 (C-3), 70.1 (C-4), 76.5 (C-5), 67.5 (C-6), syringoyl 120.3 (C-1), 107.3 (C-2,6), 147.4 (C-3,5), 140.7 (C-4), 165.4 (C-7); HR-ESI-MS: *m/z* 797.1907 $[M + Na]^+$ (calcd for $C_{36}H_{38}O_{19}Na$ 797.1900). The ^{1}H- and ^{13}C-NMR spectroscopic data of compound **2** were in good agreement with the literature data of leonurusoside C [14].

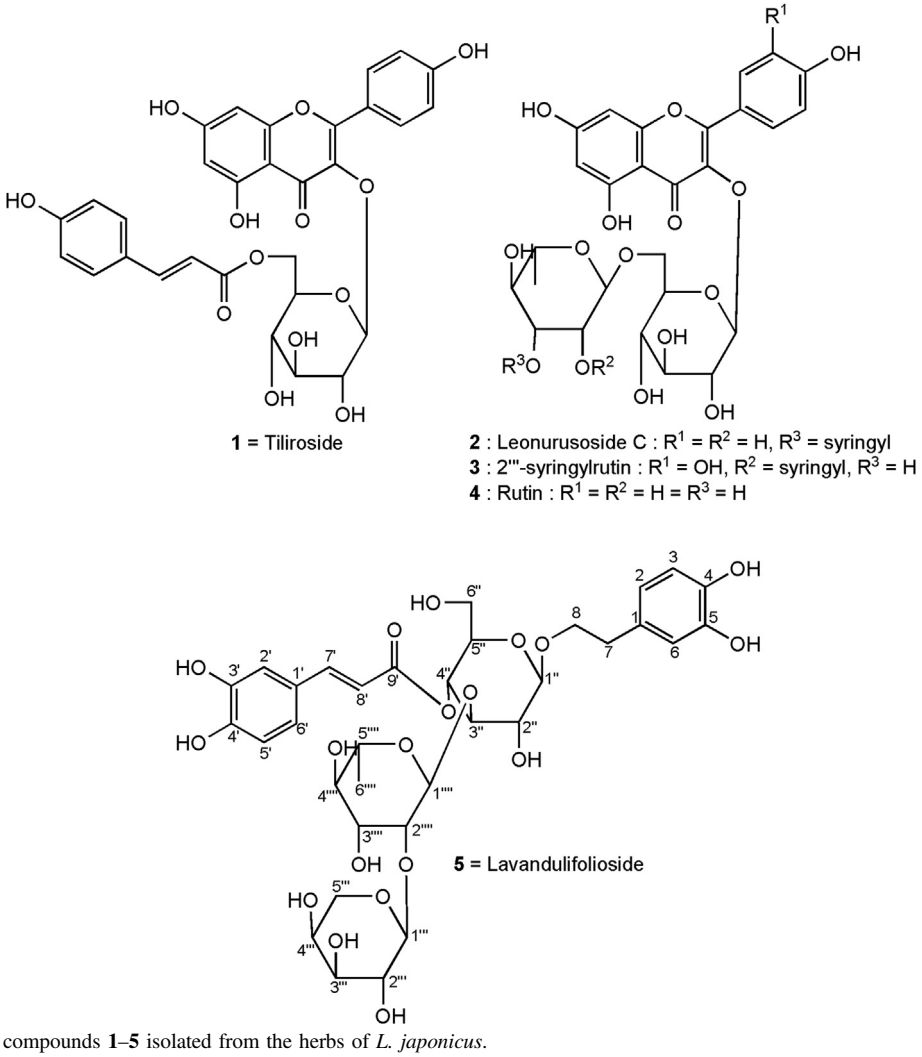

Figure 1. Structure of compounds **1**–**5** isolated from the herbs of *L. japonicus*.

Compound **3**: 2‴-Syringoylrutin (quercetin 3-*O*-(2‴-*O*-syringoyl)-α-L-rhamnopyranosyl(1 → 6)-β-D-glucopyranoside)

Yellow needles. UV λ_{max} (MeOH) nm (log ε): 260 (sh, 4.49), 266 (4.49), 293 (sh, 4.29), 360 (4.30); IR ν_{max} (KBr) cm^{-1}: 3 397 (OH), 1 652 (α,β-unsaturated ketone), 1 608, 1 513 (aromatic C═C), 1 067 (glycoside C—O); ^{1}H-NMR (600 MHz, CD_3OD) δ: quercetin 6.20 (1H, d, *J* = 1.8 Hz, H-6), 6.37 (1H, d, *J* = 1.8 Hz, H-8), 7.69 (1H, d, *J* = 1.8 Hz, H-2), 6.80 (1H, d, *J* = 8.4 Hz, H-5), 7.61 (2H, dd, *J* = 1.8 and 8.4 Hz, H-6); D-glc - 5.13 (1H, d, *J* = 7.8 Hz, H-1); L-rha 4.62 (1H, brs, H-1), 1.20 (3H, d, *J* = 6.0 Hz, rhamnosyl CH_3); syringoyl 7.30 (2H, s, H-2,6), 3.91 (2xCH_3, s, syringoyl OCH_3); ^{13}C-NMR (150 MHz, CD_3OD) δ: quercetin 157.0 (C-2), 134.4 (C-3), 178.0 (C-4), 160.0 (C-5), 98.5 (C-6), 164.6 (C-7), 93.5 (C-8), 157.5 (C-9), 103.1 (C-10), 121.5 (C-1′), 116.5 (C-2′), 144.4 (C-3′), 148.6 (C-4′), 114.7 (C-5′), 122.0 (C-6′), D-glc 104.3 (C-1), 74.4 (C-2), 76.8 (C-3), 70.0 (C-4), 76.2 (C-5), 66.9 (C-6), L-rha 100.5 (C-1), 72.8 (C-2), 72.7 (C-3), 68.7 (C-4), 68.5 (C-5), 16.9 (C-6), syringoyl 119.9 (C-1), 107.2 (C-2,6), 147.4 (C-3,5), 140.7 (C-4), 165.6 (C-7); HR-FAB-MS: 791.2029 ($[M+H]^+$, $C_{26}H_{39}O_{20}$; calc. 791.2035). The ^{1}H- and ^{13}C-NMR spectroscopic data of compound **3** were in good agreement with the literature data of 2‴-syringoylrutin [20].

Compound **4**: Rutin (quercetin 3-*O*-α-L-rhamnopyranosyl (1 → 6)-β-D-glucopyranoside)

Yellow needle. UV λ_{max} (MeOH) nm (log ε): 260, 270 (sh), 298 (sh), 360; IR ν_{max} (KBr) cm^{-1}: 3 400 (OH), 1 650 (C═O); ^{1}H-NMR (600 MHz, CD_3OD) δ: quercetin 6.24 (1H, d, *J* = 1.8 Hz, H-6), 6.42 (1H, d, *J* = 1.8 Hz, H-8), 7.70 (1H, d, *J* = 1.8 Hz, H-2′), 6.90 (1H, d, *J* = 8.4 Hz, H-5′), 7.65 (1H, dd, *J* = 1.8 and 8.4 Hz, H-6″); D-glc 5.13 (1H, d, *J* = 7.8 Hz, H-1); L-rha - 4.52 (1H, brs, H-1), 1.15 (3H, d, *J* = 6.0 Hz, rhamnosyl CH_3); ^{13}C-NMR (150 MHz, CD_3OD) δ: quercetin 157.1 (C-1), 134.4 (C-2), 178.0 (C-4), 161.0 (C-5), 98.6 (C-6), 164.6 (C-7), 93.5 (C-8), 157.9 (C-9), 103.9 (C-10), 121.2 (C-1′), 116.3 (C-2′), 144.4 (C-3′), 148.4 (C-4′), 114.7 (C-5′), 122.4 (C-6′); D-glc 104.3 (C-1), 74.3 (C-2), 75.8 (C-3), 70.7 (C-4), 76.8 (C-5), 67.2 (C-6), 67.2 (C-6); L-Rha 101.0 (C-1), 70.8 (C-2), 71.0 (C-3), 72.6 (C-4), 69.2 (C-5), 16.5 (C-6). The ^{1}H- and ^{13}C-NMR spectroscopic data of compound **4** were in good agreement with the literature data of rutin [21].

Compound **5**: Lavandulifolioside (Hydroxytyrosol 8-*O*-α-L-arabinopyranosy-l(1 → 2)-α-L-rhamnopyranosyl(1 → 2)-(4″-O-caffeoyl)-β-D-glucopyranoside)

Amorphous powder. UV λ_{max} (MeOH) nm (log ε): 220 (4.05), 247 (sh, 3.78), 291 (sh, 3.90), 334 (4.06); IR ν_{max} (KBr) cm^{-1}: 3 429 (broad, OH), 2 926 (C-H), 1 698 (C═O), 1 637, 1 602, 1 525 (aromatic C═C), 1 448, 1 375, 1 274, 1 183, 1 085 (glycosidic C—O); ^{1}H-NMR (600 MHz, CD_3OD) δ: hydroxytyrosol – 6.73 (1H, d, *J* = 1.8 Hz, H-2), 6.70 (1H, d, *J* = 8.4 Hz, H-5), 6.60 (1H, dd, *J* = 1.8, 8.4 Hz, H-6), 2.82 (2H, m, H-7), 3.75 (1H, m, H_a-8), 3.55 (1H, m, H_b-8), caffeoyl – 7.08 (1H, d, *J* = 1.8 Hz, H-2), 6.81 (1H, d, *J* = 8.4 Hz, H-5), 6.98 (1H, dd, *J* = 1.8, 8.4 Hz, H-6), 7.62 (1H, d, *J* = 15.6 Hz, H-7), 6.30 (1H, d, *J* = 15.6 Hz, H-8); D-glc 4.40 (1H, d, *J* = 7.8 Hz, H-1), 3.55 (1H, m, H_a-6), 3.65 (1H, m, H_b-6); L-rha - 5.50 (1H, d, *J* = 1.8 Hz, H-1), 1.09 (3H, d, *J* = 6.0 Hz, H-6); L-ara - 4.34 (1H, d, *J* = 7.2 Hz, H-1), 3.57 (1H, m, H_a-5), 3.87 (1H, m, H_b-5); ^{13}C-NMR (150 MHz, CD_3OD) δ: hydroxytyrosol – 130.1 (C-1), 115.7 (C-2), 144.7 (C-3), 143.3 (C-4), 115.1 (C-5), 119.9 (C-6), 35.2 (C-7), 70.8 (C-8); caffeoyl - 126.3 (C-1), 113.9 (C-2), 145.4 (C-3), 148.4 (C-4), 115.3 (C-5), 114.9 (C-6), 146.6 (C-7), 113.3 (C-8), 166 (C-9); D-glc - 102.8 (C-1), 74.6 (C-2), 81.0 (C-3), 69.2 (C-4), 74.6 (C-5), 60.8 (C-6); L-rha 100.6 (C-1), 81.4 (C-2), 72.9 (C-3), 71.5 (C-4), 69.2 (C-4), 17.0 (C-6); L-ara 106.0, 70.6 (C-2), 72.8 (C-3), 68.4 (C-4), 65.9 (C-5); HR-ESI-MS: 757.2559 ($[M+H]^+$, $C_{34}H_{45}O_{19}$; calc. 757.2555). The ^{1}H- and ^{13}C-NMR spectroscopic data of compound **5** were in good agreement with the literature data of lavandulifolioside [22].

This study was mainly concerned with the phenolic substances with anti-AChE activity derived from the glycosidic substances contained in *L. japonicus*. Therefore, the compounds were extracted with aqueous MeOH, and this extract was further divided into two fractions consisting of $CHCl_3$ fraction with less polar substances and BuOH fraction with more polar glycosides. To confirm the active fraction, those two fractions together with the aqueous MeOH extract were tested using anti-AChE assays. The BuOH fraction inhibited AChE activity more than $CHCl_3$ fraction. IC_{50} values of 80% MeOH extract, $CHCl_3$ fraction and BuOH fraction obtained from *L. japonicus* on AChE activity were 229.88 ± 7.31, 142.67 ± 1.31, and 129.59 ± 10.07 respectively. This suggests that the active substances may be mainly distributed in the BuOH fraction.

In our attempts to demonstrate the anti-AChE activity of Leonuri Herba, the five isolated compounds were tested for their *in vitro* anti-AChE activities. Berberine was used as a positive control. The IC_{50} values of the isolates are shown in Table 1.

Since the most sugar moieties of the glycosides can be hydrolyzed in the human intestinal tract or hepatic tissues. Therefore, the compounds which are producible at the hydrolysis of the isolates were also tested for their anti-AChE activities. The compounds that sugar moieties have been removed from the original glycosides were including *p*-coumaric acid, caffeic acid, hydroxytyrosol, syringic acid, kaempferol, and quercetin. The anti-AChE activities of those compounds together with salidroside (glucoside of hydroxytyrosol) are presented in Table 2. As shown in Figure 2, the peak at 8.25 min has the highest intensity. The rutin content was calculated to be 9.18 mg/g and 66.52 mg/g in the aqueous MeOH and BuOH fractions, respectively, suggesting that it is one of the major substances in *L. japonicus*.

Table 1

IC_{50} values of the compounds isolated from the BuOH fraction on anti-AChE activity.

Compound	IC_{50} (μg/mL)
Tiliroside (**1**)	409.73 ± 12.18
Leonurusoside C (**2**)	352.11 ± 30.18
2‴-syringoylrutin (**3**)	518.65 ± 22.48
Rutin (**4**)	348.27 ± 8.20
Lavandulifolioside (**5**)	227.78 ± 2.84
Berberine	0.40 ± 0.01

Value represents mean ± SEM of triple experiments.

Table 2

IC_{50} values of the aglycones of the isolated glycosides on AChE activity.

Compound	IC_{50} (μg/mL)
p-coumaric acid	18.73 ± 1.07
Caffeic acid	1.05 ± 0.19
Hydroxytyrosol	56.36 ± 0.41
Salidroside	> 100
Syringic acid	> 100
Kaempferol	22.50 ± 0.83
Quercetin	3.58 ± 0.02
Berberine	0.33 ± 0.00

Berberine: Positive control.

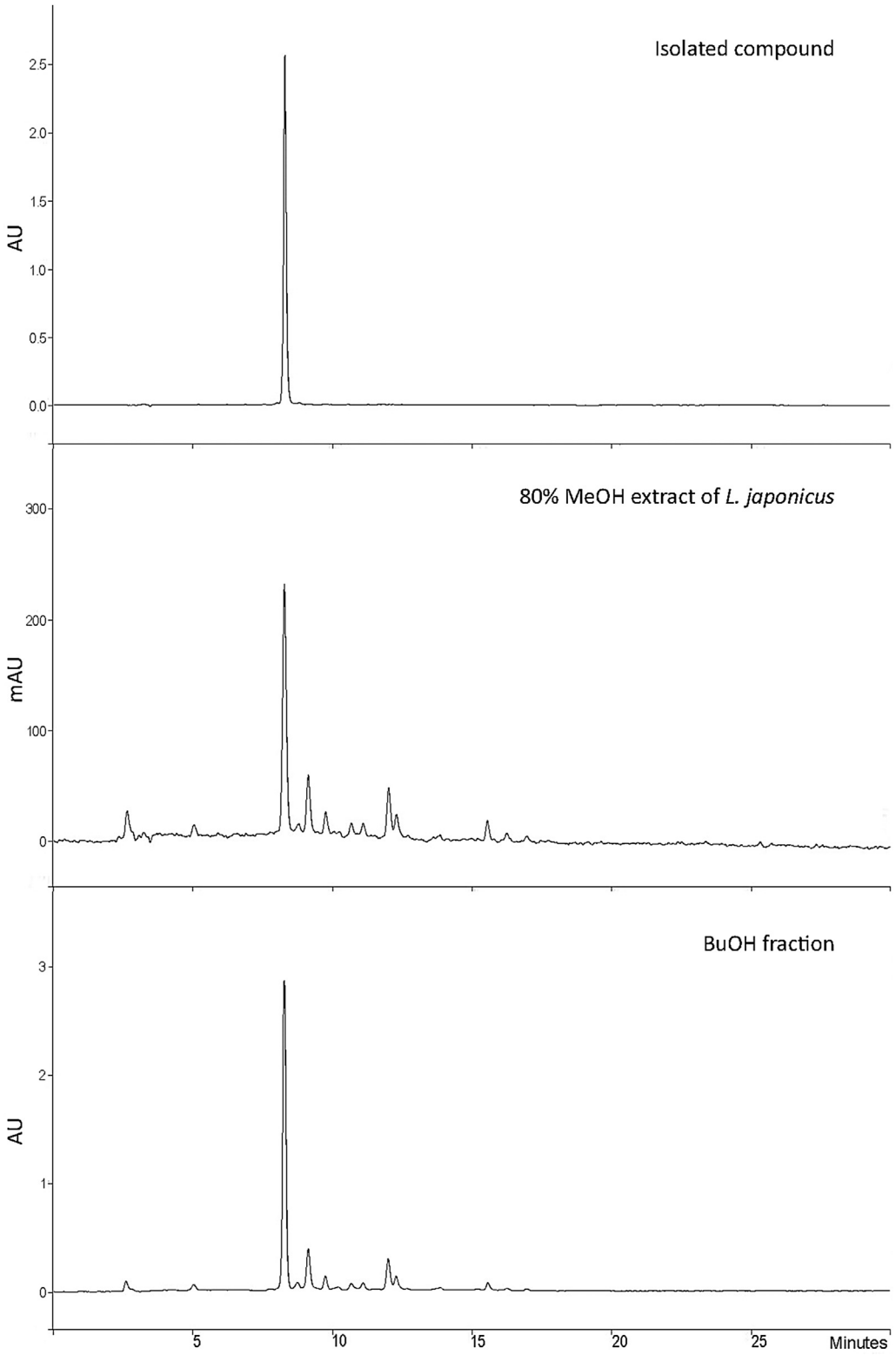

Figure 2. HPLC chromatograms of 80% MeOH extract of *L. japonicus* and its BuOH fraction with the isolated compound (rutin) as a standard.

4. Discussion

Our study showed that, although lavandulifolioside inhibited AChE activity more than other four substances, these glycosides were not so potent. A lot of natural glycosides can be metabolized by human intestinal bacteria to produce various metabolites [4]. Furthermore, the oral administration of acteoside to rats led to the detection of a lot of metabolites from plasma, urine and feces [23]. In spite of no reports of lavandulifolioside with the structure of 2‴-arabinosylacteoside on anti-Alzheimer's activity, anti-amyloid-β peptide aggregation [24] and anti-AChE activities [25] of acteoside have been reported. Moreover, acteoside is known to possess anti-amyloid-β peptide aggregation because of the presence of catechol moiety [26].

In the course of structure identification, we observed that the hydrolysis of compound **2** yielded kaempferol and syringic acid as the non-sugar moiety together with D-glucose and L-rhamnose. In the hydrolysis of compound **5**, two non-sugar moieties

of hydroxytyrosol and caffeic acid, and the three sugar moieties of D-glucose, L-rhamnose, and L-arabinose were observed by TLC. Therefore, *p*-coumaric acid, caffeic acid, hydroxytyrosol, syringic acid, kaempferol, and quercetin, whose sugar moieties have been removed, together with salidroside were used for AChE inhibition assay.

Of the seven non-glycosides, the five compounds, kaempferol, quercetin, *p*-coumaric acid, caffeic acid, and hydroxytyrosol significantly inhibited AChE, whereas the activities of syringic acid and salidroside were very weak (> 100 μg/mL). The activity of hydroxytyrosol was stronger than its glucoside (salidroside) based on their IC_{50}s, suggesting that glycosylation to hydroxytyrosol highly decreases anti-AChE activity. Particularly, quercetin obtainable through the hydrolysis of its glycosides such as rutin or syringoylrutins exhibited an IC_{50} value of 3.58 μg/mL in the anti-AChE assay. Since syringic acid was not active, quercetin moiety may significantly contribute to the enhancement of cognition or memory function rather than its parent glycosides.

The hydrolyates of lavandulifolioside, caffeic acid and hydroxytyrosol, highly inhibited AChE [IC_{50}, caffeic acid: 1.05 μg/mL; hydroxytyrosol: (56.36 ± 0.41) mg/mL], whereas that of salidroside with the structure of hydroxytyrosol 8-*O*-glucoside was very weak. These results suggest that caffeic acid and/or hydroxytyrosol in lavandulifolioside may contribute to anti-AChE activity rather than salidroside. *p*-Coumaric acid that can be obtained from tiliroside also exhibited a potent anti-AChE activity [IC50, (18.73 ± 1.07) mg/mL].

Since we considered that the major substance in the oriental medicinal drugs is usually responsible for the biological activity, we attempted to find any substance present in a large amount and quantify it by HPLC. In conclusion, the substances, caffeic acid, quercetin, *p*-coumaric acid, kaempferol, and hydroxytyrosol that can be produced from their glycosides, exhibited potent anti-AChE activity.

Conflict of interest statement

No conflict of interest associated with this work.

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