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ARTICLE

Phenylpropanoids from *Lilium* Asiatic hybrid flowers and their anti-inflammatory activities

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Abstract Three phenylpropanoids were isolated from the flowers of *Lilium* Asiatic hybrids through repeated silica gel or octadecyl silica gel column chromatographies. The chemical structures were determined to be 1-*O*-*trans*-caffeoyl-*β*-D-glucopyranoside (**1**), regaloside A (**2**), and regaloside B (**3**), based on spectroscopic data gathered from nuclear magnetic resonance (NMR) spectroscopy, electron ionization mass spectrometry (EI/MS), polarimetry, and infrared spectroscopy (IR) experiments. Compounds **1** and **2** showed significant DPPH radical scavenging activity of 60.1 and 58.0% at 160 ppm, respectively, compared with the 62.0% activity of the positive control, α-tocopherol. At a concentration of 50 μg/mL, compounds 1–3 inhibited the expression of iNOS to 4.1 ± 0.01, 70.3 ± 4.07, and 26.2 ± 0.63, respectively, and decreasing COX-2 expression to 67.8 ± 4.86, 131.6 ± 8.19, and 98.9 ± 4.99. Also, at the same concentration, compounds **1**–**3** decreased the ratio of p-p65/p-65 to 43.8 ± 1.67, 40.7 ± 1.30, and 43.2 ± 1.60, respectively, and the expression of VCAM-1 to 42.1 ± 2.31, 48.6 ± 2.65, and 33.8 ± 1.74, respectively.

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Introduction

The *Lilium* genus is comprised of 110 accepted species of flowering plants with a wide geographical distribution. Many *Lilium* species, including ornamental cultivars and hybrids, are cultivated for their esthetic value, as well as for food and medicinal use [1]. Among hybrid lilies, the Lilium Asiatic hybrids (*Lilium* spp.) are one of the main hybrid groups. They are derived from interspecific crosses with species from the section *Sinomartagon*, which are mainly distributed in East Asia [2]. The plants grow easily, with stem heights reaching 30–100 cm. Flowers are star or bowl shaped, with or without spots. Even though these flowers are not fragrant, they still attract butterflies with their wide variety of colors. The large variation in hue is a result of the accumulation of anthocyanins and carotenoids, which results especially in pink, yellow/orange, or red coloration [3–5]. Plants from Asiatic hybrid—*Sinomartagon* section crossing have been used traditionally in China and Japan as a sedative, an anti-inflammatory, an antitussive, and as a general tonic [6, 7]. There have been several phytochemical studies that have isolated diverse secondary metabolites, such as steroidal saponin glycosides, phenolic glycosides, and flavonoid glycosides, from the parental generation of *Lilium callosum, L. lancifolium, L. pumilum,* and so on [8–10]. However, no study has yet been reported on the secondary metabolites of Asiatic hybrid lilies. Therefore, this study focused on the isolation, identification, and investigation of the biological activities of secondary metabolites from *Lilium* Asiatic hybrids. This

paper describes the isolation and structural analysis of three phenylpropanoids from the flowers of *Lilium* Asiatic hybrids, as well as the evaluation of the anti-inflammatory activities of the isolated compounds.

Experimental

General methods

Column chromatography (c.c.): silica gel (SiO_2, Kieselgel 60, Merck, Darmstadt, Germany), octadecyl silica gel (ODS, LiChroprep RP-18, 40–60 μm, Merck). TLC: Kieselgel 60 F_{254S} (Merck) plates; visualization was performed with a Spectroline Model ENF-240 C/F UV lamp (Spectronics Corporation, Westbury, NY, USA), and plates were sprayed with 10% H_2SO_4 solution in water and heated. Optical rotations: JASCO P-1010 digital polarimeter (Tokyo, Japan). IR spectra: Perkin Elmer Spectrum One FT-IR spectrometer (Buckinghamshire, England). FAB/MS and EI/MS: JEOL JMSAX-700 mass spectrometer (Tokyo, Japan). NMR spectra: Varian Unity Inova AS-400 FT-NMR spectrometer (Palo Alto, CA, USA). Antibodies against iNOS (sc-8310), COX-2 (sc-1747), and VCAM-1 (sc-8304) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). p-p65 (#3031), p65 (#3034), and β-actin (#3700) were purchased from Cell Signaling Technology (Beverly, MA, USA).

Plant materials

The flowers of *Lilium* Asiatic hybrids were provided by VWS Export–Import of Flowerbulbs B.V. Company, Broek op Langedijk, The Netherlands in May 2015, and were identified by Professor Song Cheon Young, Department of Floriculture, Korea National College of Agriculture and Fisheries, Jeollabuk-do, Republic of Korea.

Extraction and isolation

The dried flowers of *Lilium* Asiatic hybrids (180 g) were extracted with 80% aqueous methanol (MeOH) (9.5 L × 4) at room temperature for 24 h. The extracts were filtered using filter paper and concentrated in a rotary vacuum evaporator to yield a residue (57 g). The concentrated residue (57 g) was then suspended in 0.5 L water and successively extracted with ethyl acetate (EtOAc) (0.5 L × 3) and *n*-butanol (*n*-BuOH) (0.5 L × 2). The organic and aqueous layers were concentrated to yield EtOAc (LDE, 3 g), *n*-BuOH (LDB, 16 g), and H_2O (LDH, 37 g) fractions. The *n*-BuOH fraction (LDB, 16 g) was further fractionated by silica gel (SiO_2) c.c. (12 × 15 cm) and eluted with $CHCl_3$-MeOH-H_2O (13:3:1 → 9:3:1 → 6:4:1; 16.3 L total volume for each). The eluting solutions produced 16 fractions (LDB-1–LDB-16). Fraction LDB-7 (350 mg, elution volume/total volume (V_e/V_t): 0.297/0.347) was subjected to SiO_2 c.c. (3.5 × 15 cm) and eluted with EtOAc-*n*-BuOH-H_2O (35:3:1 → 10:3:1 → 6:3:1; 3.0 L total volume for each) to produce 16 fractions (LDB-7-1–LDB-7-16). Fraction LDB-7-8 (67.7 mg, V_e/V_t: 0.227/0.313) was subjected to octadecyl SiO_2 (ODS) c.c. (2 × 16 cm) and eluted with MeOH-H_2O (1:3 → 1:1; 0.4 L total volume for each) to produce nine fractions (LDB-7-8-1–LDB-7-8-9) along with a purified compound **2** [LDB-7-8-3; 48.0 mg; V_e/V_t: 0.187/0.563; TLC (SiO_2 F_{254}) R_f: 0.50; EtOAc-*n*-BuOH-H_2O = 6:3:1]. Fraction LDB-12 (1.87 g, V_e/V_t 0.678–0.709) was subjected to ODS c.c. (3 × 5 cm) and eluted with MeOH-H_2O (1:3 → 1:2 → 1:1, 2.5 L total volume for each) to produce 21 fractions (LDB-12-1–LDB-12-21) along with two purified compounds: compound **1** [LDB-12-3; 49.7 mg; V_e/V_t: 0.0095/0.0111; TLC (SiO_2 F_{254}) R_f: 0.48; $CHCl_3$-MeOH-H_2O = 6:4:1] and compound **3** [LDB-12-5; 37.2 mg; V_e/V_t: 0.0222/0.0286; TLC (SiO_2 F_{254}) R_f: 0.46; $CHCl_3$-MeOH-H_2O = 6:4:1].

Compound **1** (1-*O*-*trans*-caffeoyl-*β*-D-glucopyranoside): Black-colored syrup; IR ν_{max}^{KBr} cm^{-1}: 3400, 1690, 1635, 1605, 1515; negative FAB/MS *m/z* 341 $[M–H]^-$; ^{1}H-NMR (400 MHz, CD_3OD, δ_H): 7.65 (1H, d, J = 16.0 Hz, H-7), 7.09 (1H, br.s, H-2), 6.92 (1H, br.d, J = 8.0 Hz, H-6), 6.75 (1H, d, J = 8.0 Hz, H-5), 6.30 (1H, d, J = 16.0 Hz, H-8), 5.58 (1H, d, J = 7.2 Hz, H-1′), 3.86 (1H, dd, J = 11.2, 4.6 Hz, H-6′a), 3.68 (1H, dd, J = 11.2, 2.5 Hz, H-6′b), 3.30–3.37 (4H, m, H-2′ ∼ H-5′); ^{13}C-NMR (100 MHz, CD_3OD, δ_C): 167.7 (C-9), 149.9 (C-4), 148.1 (C-7), 146.8 (C-3), 127.5 (C-1), 123.2 (C-6), 116.5 (C-5), 115.3 (C-2), 114.3 (C-8), 95.7 (C-1′), 78.7 (C-5′), 78.0 (C-3′), 74.0 (C-2′), 71.2 (C-4′), 62.3 (C-6′).

Compound **2** (regaloside A): Pale-yellow amorphous powder; $[\alpha]_D^{25}$-16.3° (*c* 1.00, MeOH); UV λ_{max}^{MeOH} nm log (ε): 228 (4.22), 301 sh (4.43), 312 (4.47); IR ν_{max}^{KBr} cm^{-1}: 3300, 1700, 1640, 1610, 1510; EI/MS *m/z* 400 $[M]^+$; ^{1}H-NMR (400 MHz, CD_3OD, δ_H): 7.95 (2H, d, J = 8.0 Hz, H-2′,6′), 7.65 (1H, d, J = 15.6 Hz, H-7′), 6.80 (2H, d, J = 8.0 Hz, H-3′,5′), 6.35 (1H, d, J = 15.6 Hz, H-8′), 4.32 (1H, d, J = 7.6 Hz, H-1″), 4.25 (1H, overlapped, H-1a), 4.05 (1H, dd, J = 5.2, 5.2 Hz, H-2), 4.02 (1H, overlapped, H-1b), 3.95 (1H, dd, J = 10.4, 4.8 Hz, H-3a), 3.87 (1H, overlapped, H-6″a), 3.63 (2H, overlapped, H-3b, H-6″b), 3.20–3.35 (4H, m, H-2″–H-5″); ^{13}C-NMR (100 MHz, CD_3OD, δ_C): 169.2 (C-9′), 161.7 (C-4′), 146.9 (C-7′), 131.2 (×2, C-2′, 6′), 126.9 (C-1′), 116.3 (× 2, C-3′,5′), 114.7 (C-8′), 104.7 (C-1″), 77.79 (C-5″), 77.9 (C-3″), 75.0 (C-2″), 71.9 (C-3), 71.5 (C-4″), 69.7 (C-2), 66.6 (C-1), 62.7 (C-6″).

Compound **3** (regaloside B): Yellow amorphous powder; $[\alpha]_D^{25}$-11.5° (*c* 0.62, MeOH); UV λ_{max}^{MeOH} nm log (ε):

241 sh (4.02), 300 sh (4.02), 300 sh (4.12), 328 (4.32); IR ν_{max}^{KBr} cm^{-1} 3400, 1680, 1620, 1595, 1510. EI/MS *m/z* 416 $[M]^+$; ^{1}H-NMR (400 MHz, CD_3OD, δ_H): 7.60 (1H, d, $J = 16.0$ Hz, H-7′), 7.07 (1H, d, $J = 2.0$ Hz, H-2′), 6.95 (1H, dd, $J = 8.0$, 2.0 Hz, H-6′), 6.78 (1H, d, $J = 8.0$ Hz, H-5′), 6.29 (1H, d, $J = 16.0$ Hz, H-8′), 4.31 (1H, d, $J = 8.0$ Hz, H-1″), 4.23 (2H, overlapped, H-1), 4.18 (1H, m, H-2), 3.92 (1H, dd, $J = 10.4$, 5.2 Hz, H-3a), 3.71 (1H, overlapped, H-3b), 3.87 (1H, overlapped, H-6′a), 3.69 (1H, overlapped, H-6′b), 3.20–3.90 (4H, m, H-2″–H-5″); ^{13}C-NMR (100 MHz, CD_3OD, δ_C): 169.2 (C-9′), 149.6 (C-4′), 147.2 (C-7′), 146.8 (C-3′), 127.7 (C-1′), 123.0, (C-6′), 116.5 (C-5′), 115.2 (C-2′), 114.9 (C-8′), 104.7 (C-1″), 78.0 (C-5″), 77.7 (C-3″), 75.1 (C-2″), 71.9 (C-3), 71.5 (C-4″), 69.7 (C-2), 66.7 (C-1), 62.7 (C-6″).

Antioxidant and anti-inflammatory activity assays

DPPH radical scavenging assay

The antioxidant activity of each compound was measured by a DPPH radical scavenging assay. Briefly, 0.9 mL of each sample in 500 ppm was added to 1.9 mL of 100% methanol containing 0.1 mM DPPH. After incubation at 37 °C in a dark chamber for 30 min, the absorbance (OD) was measured at 517 nm using a spectrophotometer. α-Tocopherol was used as a positive control under the same conditions. DPPH scavenging activity (%) was calculated using this formula: DPPH scavenging activity (%) = [(Control OD − Sample OD)/Control OD] × 100.

Anti-inflammatory activity assay based on nuclear translocation of NF-κB

Cell culture Human aortic smooth muscle cells (HASMCs) and Raw264.7 cells were cultured in smooth muscle cell medium (SMCM) and Dulbecco's modified essential medium (DMEM), respectively. SMCM was supplemented with 2% fetal bovine serum (FBS), 1% smooth muscle cell growth supplement, and 1% penicillin–streptomycin. DMEM was supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. Cells were incubated at 37 °C in a 5% CO_2 humidified atmosphere.

Cell viability assay To investigate cell viability, an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] assay was performed. RAW264.7 cells were seeded at a density of 1×10^5 cells/ml in 96-well plates. Cells were treated with each sample for 24 h and then were incubated with 10 μL of 5 mg/ml MTT reagent for 4 h. After the supernatant was removed, 100 μL of DMSO was added to each well, and the resulting formazan crystals were dissolved using a shaker for 10 min. The optical density (OD) was measured at a 570 nm wavelength with a multi-reader.

Western blot Cells were washed with PBS and dissolved with PRO-PREP™ protein extraction solution for 1 h. After the lysate was collected, equal amounts of total protein were boiled for 5 min, and proteins were separated by 12% SDS-PAGE and transferred to a nitrocellulose membrane. The membranes were incubated with iNOS (1:500), COX-2 (1:500), VCAM-1 (1:500), p-p65 (1:1000), or p65 (1:1000) overnight at 4 °C. Subsequently, membranes were incubated with either horseradish-peroxidase-conjugated anti-mouse or anti rabbit secondary antibody for 1 h at room temperature. Bands were visualized with the EZ-Western Lumi Pico reagents according to the manufacturer's instructions.

Statistical analysis

Data are expressed as mean ± SEM. Results were subjected to an analysis of the variance (ANOVA), using Tukey's test as a post hoc test. Significant values are indicated by a superscript ($^{\#}P < 0.05$ compared with the NC group, $^{*}P < 0.05$ compared with the LPS or TNF-α group).

Results and discussion

The dried flowers of *Lilium* Asiatic hybrids were extracted with aqueous MeOH, and the concentrated extract was successively fractionated into EtOAc, *n*-BuOH, and H_2O fractions. Repeated SiO_2 and ODS c.c. of the *n*-BuOH fractions afforded three phenylpropanoids (**1–3**). The chemical structures of the phenylpropanoids were determined based on spectroscopic data, including nuclear magnetic resonance (NMR) spectroscopy, fast atom bombardment mass spectrometry (FAB-MS), electron ionization mass spectrometry (EI/MS), polarimetry, and infrared spectroscopy (IR).

Structural analysis of the phenylpropanoids

Compound **1** was isolated as a black-colored syrup. The molecular weight was determined to be 342 from the molecular ion peak *m/z* 341 $[M–H]^-$ on the negative FAB/MS spectrum. The IR spectrum showed absorbance bands for a hydroxyl group (3400 cm^{-1}), a conjugated ester group (1690 cm^{-1}), a double bond (1635 cm^{-1}), and aromatic rings (1605, 1515 cm^{-1}). ^{1}H-NMR data showed five olefin methine proton signals in the low magnetic field due to a 1,2,4 trisubstituted benzene ring at δ_H 7.09 (1H, br.s,

H-2), 6.92 (1H, br.d, $J = 8.0$ Hz, H-6), and 6.75 (1H, d, $J = 8.0$ Hz, H-5) and a double bond with a *trans*-configuration at δ_H 7.65 (1H, d, $J = 16.0$ Hz, H-7) [11] and δ_H 6.30 (1H, d, $J = 16.0$ Hz, H-8) as the signals of an aglycone moiety. The proton signals of the sugar moieties showed one hemiacetal proton signal at δ_{H-} 5.58 (1H, d. $J = 7.2$ Hz, H-1′), one oxygenated methylene proton signal at δ_H 3.86 (1H, dd, $J = 11.2$, 4.6 Hz, H-6′a) and δ_H 3.68 (1H, dd, $J = 11.2$, 2.5 Hz, H-6′b), and four oxygenated methine proton signals at δ_H 3.30–3.37 (4H, m, H-2′ ~ H-5′). The coupling constant of the anomeric proton signal ($J = 7.2$ Hz) indicated that the anomeric hydroxyl of compound **1** has a β-configuration [12]. Therefore, compound **1** was suggested to be a caffeoyl monoglycoside. The ^{13}C-NMR spectrum showed 15 carbon signals, which we attributed to a phenylpropanoid and a hexose. In the low magnetic field, the carbon signals of one ester at δ_C 167.7 (C-9), two oxygenated olefin quaternaries at δ_C 149.9 (C-4) and 146.8 (C-3), one olefin quaternary at 127.5 (C-1), and five olefin methines at δ_C 148.1 (C-7), 123.2 (C-6), 116.5 (C-5), 115.3 (C-2), and 114.3 (C-8) suggested that the aglycone of compound **1** is a caffeic acid. The sugar was identified as a β-glucopyranose based on the chemical shift of one hemiacetal carbon signal at δ_C 95.7 (C-1′), four oxygenated methine carbon signals at δ_C 78.7 (C-5′), 78.0 (C-3′), 74.0 (C-2′), and 71.2 (C-4′), and one oxygenated methylene carbon signal at δ_C 62.3 (C-6′). In the gHMBC spectrum, the anomeric proton signal at δ_H 5.58 (C-1′) showed a cross-peak with the ester carbon signal at δ_C 167.7 (C-9), indicating that a glucopyranose was located at the C-9 position. Taken together, these results indicate that compound **1** was 1-*O*-*trans*-caffeoyl-β-D-glucopyranoside, which was confirmed by a comparison of the spectroscopic data obtained in this study with those found in the literature [13].

Compound **2** was isolated as a pale-yellow amorphous powder. The molecular weight was determined to be 400 from the molecular ion peak *m/z* 400 $[M]^+$ in the EI/MS spectrum. In the IR spectrum, the absorbance bands of a hydroxyl group (3300 cm^{-1}), conjugated ester group (1700 cm^{-1}), double bond (1640 cm^{-1}), and aromatic rings (1610, 1510 cm^{-1}) were detected. ^{1}H-NMR spectrum exhibited four olefin methine proton signals at δ_H 7.95 (2H, d, $J = 8.0$ Hz, H-2′, 6′) and δ_H 6.8 (2H, d, $J = 8.0$ Hz, H-3′, 5′) due to a *para*-disubstituted benzene ring, and two olefin methine proton signals at δ_H 7.65 (1H, d, $J = 15.6$ Hz, H-7′) and δ_H 6.35 (1H, d, $J = 15.6$ Hz, H-8′) derived from a double bond with a *trans*-configuration. ^{1}H-NMR data also exhibited the signals of a glycerol group, i.e., one oxygenated methine proton signal at δ_H 4.05 (1H, dd, $J = 5.2$, 5.2 Hz, H-2) and two oxygenated methylene proton signals at δ_H 4.25 (1H, overlapped, H-1a), δ_H 4.02 (1H, overlapped, H-1b), δ_H 3.95 (1H, dd, $J = 10.4$, 4.8 Hz, H-3a), and δ_H 3.63 (1H, overlapped, H-3b). Also, the signals of a sugar moiety included one hemiacetal proton signal at δ_H 4.32 (1H, d, $J = 7.6$ Hz, H-1″), one oxygenated methylene proton signal at δ_H 3.87 (1H, dd, overlapped, H-6″a) and δ_H 3.63 (1H, overlapped, H-6″b), and four oxygenated methine proton signals at δ_H 3.20–3.35 (4H, m, H-2″–H-5″). Therefore, compound **2** was identified as a monoglycoside with a *p*-coumaroyl, with a glyceryl as the aglycone moiety. The ^{13}C-NMR spectrum showed 18 carbon signals, including a glyceryl, a phenylpropanoid, and a hexose sugar moiety. In the low magnetic field, one ester carbon signal at δ_C 169.2 (C-9′), one oxygenated olefin quaternary carbon signal at δ_C 161.7 (C-4′), one olefin quaternary carbon signal at δ_C 126.9 (C-1′), and six olefin methine carbon signals at δ_C 146.9 (C-7′), 131.2 (C-2′, 6′), 116.3 (C-3′, 5′), and 114.7 (C-8′) were observed. In the oxygenated region, two oxygenated methylene carbon signals at δ_C 71.9 (C-3) and 66.6 (C-1) and one oxygenated methine carbon signal at δ_C 69.7 (C-2) due to a glyceryl group were observed. The carbon chemical shifts of the sugar moiety were observed as one hemiacetal at δ_C 104.7 (C-1″), four oxygenated methines at δ_C 77.9 (C-5″, 3″), 75.0 (C-2″), and 71.5 (C-4″), and one oxygenated methylene at δ_C 62.7 (C-6″), which together represented a β-glucopyranose. Also, the large coupling constant ($J = 7.6$ Hz) of the anomeric proton signal confirmed that the sugar was a β-D-glucopyranose. The gHMBC spectrum showed a correlation between the oxygenated methylene proton signal of the glyceryl group at δ_{H-} 4.25 (H-1) and the ester carbon signal of the coumaroyl group at δ_C 169.2 (C-9′), as well as a correlation between the oxygenated methylene proton signal of the glyceryl group at δ_{H-} 4.95 (H-3) and the anomeric carbon signal at δ_C 104.7 (C-1″). These data indicate that the coumaroyl group is linked to the C-1 of glyceryl group, and the glucopyranosyl is linked to the C-3 of the glyceryl group. These findings were confirmed by the observed downfield shift of the oxygenated methylene proton signal H-1 (δ_{H-} 4.25) due to the esterification effect [14] and the downfield shift of the oxygenated methylene carbon signal C-3 (δ_C 71.9) due to the glycosidation effect [14]. Taken together, these results identified compound **2** as 2*S*-1-*O*-*p*-coumaroyl-3-*O*-β-D-glucopyranosylglycerol, regaloside A. This was confirmed by comparison of the spectroscopic data gathered in this study with previously published literature values [15].

Compound **3** was isolated as a yellow amorphous powder. The molecular weight was determined to be 416 from the molecular ion peak 416 $[M]^+$ in the EI/MS spectrum. The IR spectrum revealed the presence of a hydroxyl group (3400 cm^{-1}), a conjugated ester group (1680 cm^{-1}), a double bond (1620 cm^{-1}), and aromatic

rings (1595, 1510 cm^{-1}). NMR data of compound **3** were similar to those of compound **2**, with the exception of the addition of a phenolic hydroxyl group. This was confirmed with the measurement of three olefin methine proton signals in the ^{1}H-NMR data at δ_H 7.07 (1H, d, $J = 2.0$ Hz, H-2′), 6.95 (1H, dd, $J = 8.0$, 2.0 Hz, H-6′), and 6.78 (1H, d, $J = 8.0$ Hz, H-5′), which we attributed to a 1,2,4 trisubstituted benzene ring instead of the para-disubstituted benzene ring observed in compound **2**. The ^{13}C-NMR spectrum also showed the signals of a 1,2,4 trisubstituted benzene ring: two oxygenated olefin quaternary carbon signals at δ_C 149.6 (C-4′) and 146.8 (C-3′), one olefin quaternary carbon signal at δ_C 127.7 (C-1), and three oxygenated olefin methine carbon signals at δ_C 115.2 (C-2′), 116.5 (C-5′), and 123.0 (C-6′). Therefore, compound **3** was identified as 2*S*-1-*O*-caffeoyl-3-*O*-*β*-D-glucopyranosylglycerol, regaloside C, and was confirmed by comparison of the obtained spectroscopic data with those in the reported literature [16].

DPPH radical scavenging activity

In order to evaluate the antioxidant potential of isolated phenylpropanoids **1**–**3**, a DPPH radical scavenging assay was carried out. α-Tocopherol was used as a positive control at 500 ppm [17]. As shown in Fig. 2, compounds **1** and **3** showed significant activity of 60.1% and 58.0%, respectively, compared with the positive control α-tocopherol, which showed 62% DPPH scavenging activity.

Effect of phenylpropanoids on expression of iNOS and COX-2 in Raw 264.7 cells

A variety of immune cells are involved in the control of the inflammatory response in the body [18]. Among them, macrophages are known to be involved in homeostasis by participating in a variety of host responses, such as acquired immunity and innate immunity. Macrophages produce inflammatory-promoting factors, such as nitric oxide (NO) and prostaglandin E_2 (PGE_2), which are created through interactions of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) [19].

To identify whether the samples had an effect on inflammation, the expression of iNOS and COX-2 was investigated. Raw 264.7 cells were co-treated with either 1-*O*-caffeoyl-β-D-glucopyranose (**1**), regaloside A (**2**), or regaloside B (**3**) (50 μg/ml) and LPS (1 μg/ml). Figure 1 shows that phenylpropanoids **1**-**3** all significantly inhibited iNOS expression by 4.1 ± 0.01, 70.3 ± 4.07, and 26.2 ± 0.6, respectively, in comparison with the LPS group. In addition, COX-2 expression was also, respectively,

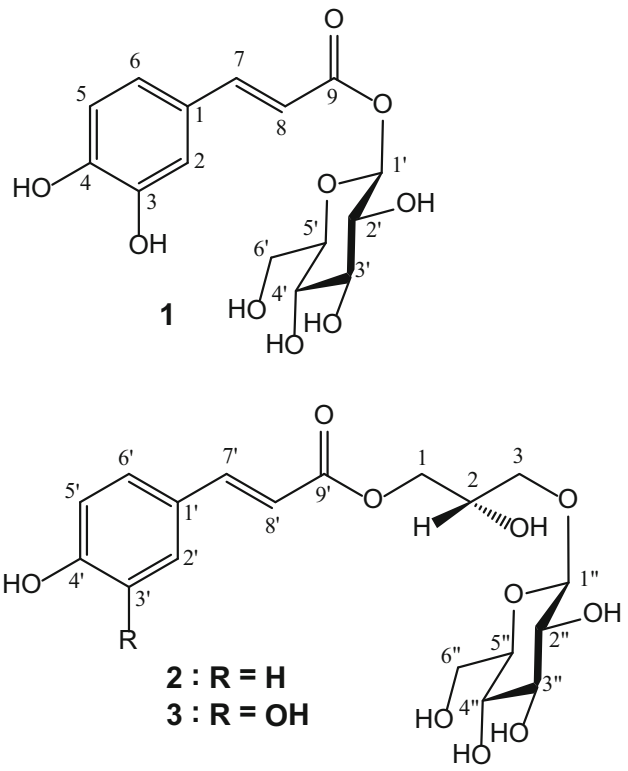

Fig. 1 Chemical structures of the phenylpropanoids isolated from the flowers of *Lilium* Asiatic hybrids

decreased by 67.8 ± 4.86, 131.6 ± 8.19, and 98.9 ± 4.99 compared with the LPS group (Fig. 2).

Assessment of a potential inhibitory effect on inflammation of the newly identified phenylpropanoids by investigating nuclear translocation of NF-kB

Inhibition of the inflammatory response was measured by investigating the nuclear translocation of NF-kB. NF-kB regulates the transcription of an exceptionally large number of genes, particularly those involved in immune and inflammatory responses [20]. It is readily activated by LPS, which frequently acts as an inflammatory cytokine. When activated by phosphorylation, the NF-kB structure contains p-p65 as a major subunit. To measure the ability of the isolated phenylpropanoids to inhibit inflammation, p-p65 protein levels in cells treated with the compounds were measured. RAW264.7 cells were co-treated with LPS (1 μg/mL) and the phenylpropanoids at a concentration of 50 μg/mL for 2 h. Then, p-p65 and p65 levels were determined by Western blot. As shown in Fig. 3, compounds **1**–**3** significantly decreased the ratio of p-p65 to p65 by 43.8 ± 1.67, 40.7 ± 1.30, and 43.2 ± 1.60 at 50 μg/mL, respectively, compared with the ratio found in LPS-treated cells.

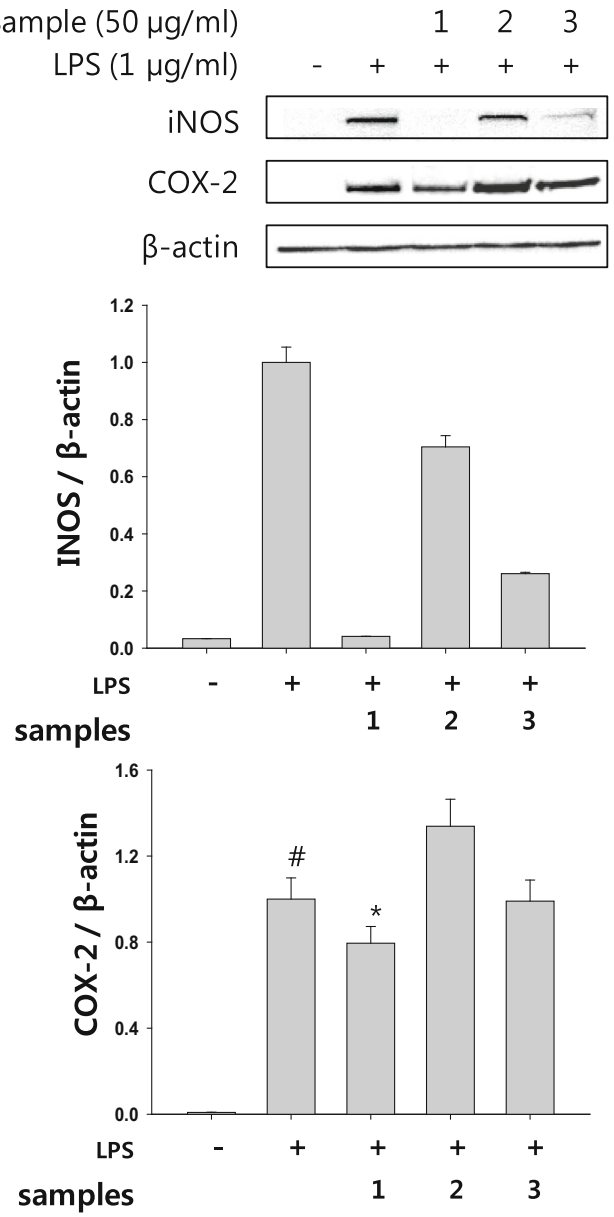

Fig. 2 Effect of phenylpropanoids isolated from the flowers of *Lilium* Asiatic hybrids on expression of iNOS and COX-2 in Raw 264.7 cells. Raw 264.7 cells were co-treated with LPS (1 μg/ml) and the compounds (50 μg/ml) for 24 h. 1. 1-*O*-caffeoyl-*β*-D-glucopyranose (**1**); 2. Regaloside A (**2**); 3. Regaloside B (**3**). Data shown are the mean ± SEM ($n = 3$), $^{\#}P < 0.05$ compared with the NC group; $^{*}P < 0.05$ compared with the LPS group

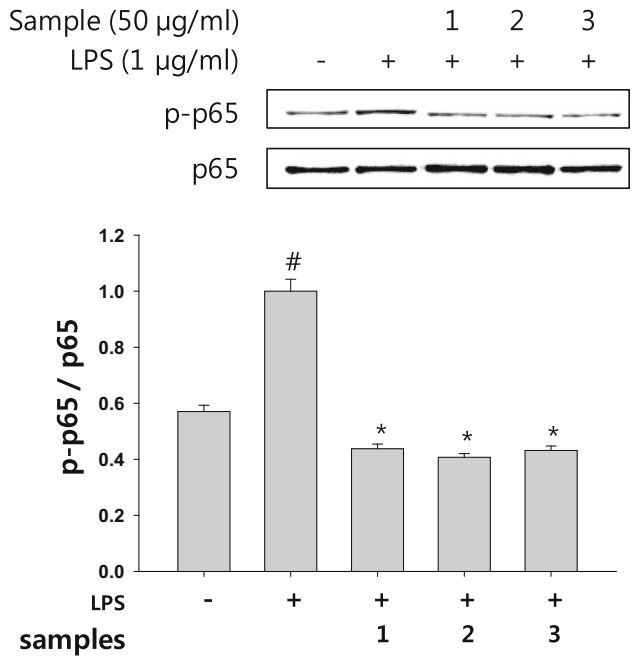

Fig. 3 Effects of the phenylpropanoids from the flowers of *Lilium* Asiatic hybrids on the inflammatory response, as assessed by nuclear translocation of NF-kB. Raw 264.7 cells were treated with LPS (1 μg/ml) for 15 min and then were incubated with the compounds (50 μg/ml) for 2 h. 1. 1-*O*-caffeoyl-*β*-D-glucopyranose (**1**); 2. Regaloside A (**2**); 3. Regaloside B (**3**). Data shown are the mean ± SEM ($n = 3$), $^{\#}P < 0.05$ compared with the NC group; $^{*}P < 0.05$ compared with the LPS group

Effect of treatment with the novel phenylpropanoids on the expression of VCAM-1 in HASMCs

During vascular inflammation, lipoprotein particles secrete inflammatory cytokines such as TNF-α and induce VCAM-1 activity through translocation of NF-Kb (p65) [21]. Therefore, to induce vascular inflammation, HASMCs were treated with TNF-α. The effects of 1-*O*-caffeoyl-*β*-D-glucopyranose (**1**), regaloside A (**2**), and regaloside B (**3**) on the inhibition of adhesion molecules were investigated. HASMCs were first pre-treated with the phenylpropanoids (50 μg/ml) for 2 h and then incubated with TNF-α (10 ng/ml) for 12 h. Compounds 1–3 markedly inhibited the expression of VCAM-1 by 42.1 ± 2.31, 48.6 ± 2.65, and 33.8 ± 1.74, respectively, compared with cells treated with TNF-α only (Fig. 4).

The inflammatory response is the primary immune response that occurs to protect the body from pathogenic infections and chemical and physical damage. However, when the inflammatory response becomes excessive, it can cause arteriosclerosis, as well as rheumatoid arthritis and multiple sclerosis. Therefore, development of anti-inflammatory drugs is important for prevention and treatment of various chronic diseases [22]. Macrophages play an important role in controlling the inflammatory response. Stimulation with LPS induces the translocation of NF-kB [20] and increases NO and PG2 production by increasing levels of iNOS and COX-2, ultimately resulting in inflammation [18]. Our data show that compounds **1** and **3** had an effect on the inflammatory response, markedly inhibiting the expression of p-p65, iNOS, and COX-2. The antioxidative effects of compounds **1** and **3** were

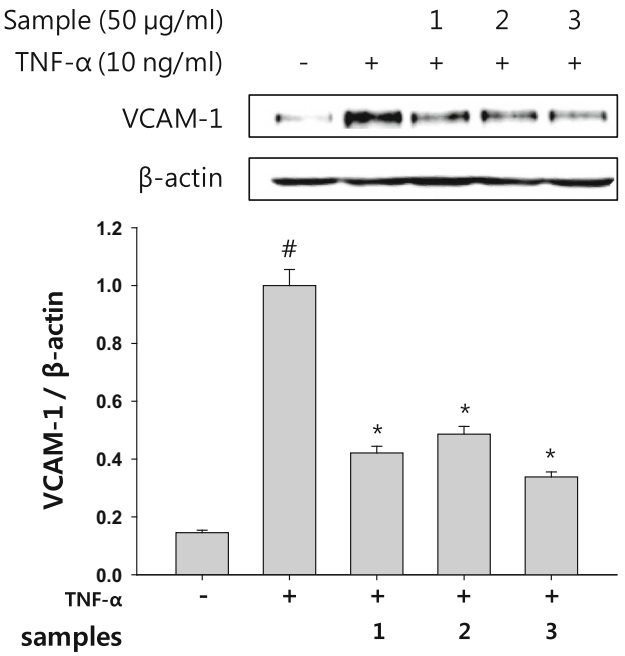

Fig. 4 Effects of phenylpropanoids from the flowers of *Lilium* Asiatic hybrids on the expression of VCAM-1 in HASMCs. HASMCs were treated with the compounds (50 μg/ml) for 2 h and then were incubated with TNF-α (10 ng/ml) for 12 h. 1. 1-*O*-caffeoyl-*β*-D-glucopyranose (**1**); 2. Regaloside A (**2**); 3. Regaloside B (**3**). Data shown are the mean ± SEM ($n = 3$), $^{\#}P < 0.05$ compared with the NC group; $^{*}P < 0.05$ compared with the TNF-α group

also confirmed with a DPPH assay. TNF-α is a crucial cytokine for the development of atherosclerotic lesions by inducing expression of VCAM-1 in endothelial cells. As compounds **1–3** decreased the expression of VCAM-1 induced by TNF-α, they might help to treat vascular inflammation.

In conclusion, three phenylpropanoids were isolated from the flowers of *Lilium* Asiatic hybrids through c.c. and were subsequently identified based on several spectroscopic analyses. To evaluate the isolated compounds for their potential use as functional materials, the antioxidant and anti-inflammatory activities of the compounds were measured. Compounds **1** and **3** displayed DPPH radical scavenging activities equal to that of a well-known antioxidant, α-tocopherol. Moreover, compounds **1–3** significantly inhibited the expression of a variety of inflammatory factors, including iNOS, COX-2, p-p65, and VCAM-1, in RAW264.7 cells. This study suggests that the phenylpropanoid compounds isolated from the flowers of *Lilium* Asiatic hybrids are promising whitening materials.

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