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Inhibitory Activity of IL-6 Production by Flavonoids and Phenolic Compounds from *Geranium thunbergii*

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Abstract – Three flavonoids (**1** - **3**) and three phenolic compounds (**4** - **6**) were isolated from the whole plant of *Geranium thunbergii* Sieb. et Zucc (Geraniaceae). Their structures were determined by chemical and spectral analysis. These compounds were examined for the inhibitory activity of IL-6 production in TNF-α stimulated MG-63 cell. Among the isolated compounds, gallic acid (**4**) and gallic acid methyl ester (**6**) showed potent inhibitory activity.
Keywords – *Geranium thunbergii* Sieb. et Zucc., Geraniaceae, gallic acid, IL-6 inhibitory activity

Introduction

Geranium thunbergii Sieb. et Zucc. (Geraniaceae) is a perennial plant that is distributed throughout Korea, China, and Japan. The whole plant is used in oriental medicine as an antihemorrhage, sterilization, diarrhea, and astringent (Bae, 2000). Previous phytochemical studies on this species reported the extraction of tannins and flavonoids, such as geraniin, corilagin, ellagic acid, gallic acid, quercetin, kaempferol, and kaempferol-7-rhamnoside (Ito *et al.*, 1999; Okuda *et al.*, 1986). In our previous studies of the whole plant of *G. thunbergii*, we reported a new furofuran, 4-hydroxykobusin, together with known lignans, kobusin, and 7,7'-dihydroxybursherenin from the methylene chloride soluble fraction of the MeOH extract (Liu *et al.*, 2006), and studied their effects on the expression of inducible nitric oxide synthase (*iNOS*) gene in RAW264.7 cells (Yuba *et al.*, 2007). During our search for the phytochemical constituents of the ethyl acetate soluble fraction of this plant, three flavonoids and three phenolic compounds were isolated. For the isolated compounds, the inhibitory activity of IL-6 production in TNF-α stimulated MG-63 cell was examined. This paper reports the isolation and the inhibitory activity of IL-6 production of the constituents from the ethyl acetate fraction of *G. thunbergii*.

Materials and Methods

General procedure – The melting point was obtained with a Fisher Scientific melting point apparatus and was uncorrected. The UV spectra were obtained on a Shimadzu UV/Visible Spectrophotometer. The IR spectra were recorded on an IMS 85 (Bruker). The NMR spectra were recorded on a Varian Unity Inova 500 (500 MHz) spectrometer. The ^{1}H-^{1}H COSY, DEPT, HMQC, and HMBC NMR spectra were obtained using the usual pulse sequences. The HR-EIMS was determined on a JMS 700 (JEOL). TLC and column chromatography were carried out on precoated Si Gel F_{254} plates (Merck, art. 5715), RP-18 F_{254} plates (Merck, art. 15423), and Si gel 60 (Merck, 230 - 400 mesh).

Plant material – The whole plant of *Geranium thunbergii* Sieb. et Zucc. (Geraniaceae) was collected from the Herbarium of College of Pharmacy, Chosun University, Korea, in May 2003. A voucher specimen was deposited in the Herbarium of College of Pharmacy, Chosun University (CSU-1019-17).

Extraction and isolation – The air-dried whole plant of *Geranium thunbergii* (0.46 kg) was cut and extracted with MeOH (3 L × 3) at 60 °C for 4 hrs (× 3). The MeOH extract (82.92 g) was suspended in water (1.0 L), and then partitioned sequentially with equal volumes of dichloromethane, ethyl acetate, and *n*-butanol. Each fraction was evaporated *in vacuum* to yield the residues of the CH_2Cl_2 (12.18 g), EtOAc (20.97 g), *n*-BuOH (12.43 g), and water (22.49 g) fractions. A portion of the EtOAc soluble fraction (2.0 g) was subjected to column chromatography over a HP-20 eluting with a MeOH-H_2O = 0 : 10 → 10 : 30 → 10 : 10 → 30 : 10 → 10 : 0 in a gradient system. The fractions were combined based on their TLC pattern to yield the subfractions designated E1-E5. Subfraction E1 (317 mg) was further purified by column chromato-

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graphy over a RP-18 eluting with a 10% aq. MeOH afford compound **4** (gallic acid, 9.6 mg). Subfraction E2 (150 mg) was purified by repeated Sephadex LH-20 column chromatography (MeOH-H_2O = 3 : 2 → 1 : 0) and RP-18 column chromatography (MeOH-H_2O = 1 : 1) to give compounds **5** (protocatechuic acid, 10.4 mg) and **6** (gallic acid methyl ester, 26.8 mg), respectively. Subfraction E4 (389.4 mg) was purified by RP-18 column chromatography (MeOH-H_2O = 6 : 4) and silica gel column chromatography ($CHCl_3$-MeOH = 5 : 1) to give compound **1** (kaempferol-3-*O*-rhamnoside, 8.3 mg), **2** (quercetin-3-*O*-rhamnoside, 70.1 mg), and **3** (kaempferol-3-*O*-rutinoside, 10.6 mg), respectively.

Kaempferol 3-*O*-α-L-rhamnopyranoside (1) – Yellow amorphous solid; mp : 172 - 174 °C; $[\alpha]^{24}_D$ −73.5° (*c* 0.5, MeOH); UV (MeOH) λ_{max} nm: 342, 265; IR ν_{max} (KBr) cm^{-1} : 3747, 3370, 2361, 1651, 1607, 1176; ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.71 (2H, d, *J* = 8.5 Hz, H-2'/H-6'), 6.88 (2H, d, *J* = 8.5 Hz, H-3'/H-5'), 6.11 (1H, d, *J* = 2.5 Hz, H-8), 6.00 (1H, d, *J* = 2.5 Hz, H-6), 5.33 (1H, d, *J* = 1.5 Hz, Rha-H-1"), 4.21 (1H, dd, *J* = 1.5, 3.0 Hz, Rha-H-2"), 3.73 (1H, dd, *J* = 3.5, 9.0 Hz, Rha-H-3"), 3.35 (2H, m, Rha-H-4"/H-5"), 0.92 (3H, d, *J* = 6.0 Hz, Rha-H-6"); ^{13}C-NMR (125 MHz, CD_3OD) δ: 178.63 (s, C-4), 163.09 (s, C-7), 162.75 (s, C-5/C-4'), 159.48 (s, C-9), 158.29 (s, C-2), 135.53 (s, C-3), 131.84 (d, C-2'/6'), 122.39 (s, C-1'), 117.13 (d,C-3'/C-5'), 103.62 (s, C-10), 103.35 (d, Rha-1"), 97.32 (d, C-8), 73.46 (d, Rha-4"), 72.32 (d, Rha-2"), 72.12 (d, Rha-3"), 72.08 (d, Rha-5"), 17.80 (q, Rha-6").

Quercetin 3-*O*-α-L-rhamnopyranoside (2) – Yellow amorphous solid; mp : 178 - 180 °C; $[\alpha]_D$ −178° (*c* 0.1, MeOH); UV (MeOH) λ_{max} nm: 254, 350; IR ν_{max} (KBr) cm^{-1}: 3320, 1660, 1610, 1500, 1450, 1360, 1140; ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.33 (1H, d, *J* = 2.0 Hz, H-2'), 7.30 (1H, dd, *J* = 2.0 8.5 Hz, H-5'), 6.90 (1H, d, *J* = 8.5 Hz, H-6'), 6.33 (1H, d, *J* = 2.0 Hz, H-8), 6.17 (1H, d, *J* = 2.0 Hz, H-6), 5.34 (1H, d, *J* = 1.5 Hz, Rha-H-1"), 4.20 (1H, dd, *J* = 1.5 3.5 Hz, Rha-H-2"), 3.75 (1H, dd, *J* = 3.5 9.5 Hz, Rha-H-3"), 3.43 (2H, m, Rha-H-4"/H-5"), 0.92 (3H, d, *J* = 6.0 Hz, Rha-H-6"); ^{13}C-NMR (125 MHz, CD_3OD) δ: 179.62 (s, C-4), 163.28 (s, C-7), 161.77 (s, C-2), 159.26 (s, C-5), 158.81 (s, C-9), 150.05 (s, C-4'), 146.64 (s, C-3'), 136.26 (s, C-3), 123.12 (d, C-6'), 122.98 (s, C-1'), 117.03 (d, C-2'), 116.26 (d, C-5'), 105.58 (s, C-10), 103.69 (d, Rha-1"), 100.52 (d, C-6), 95.25 (d, C-8), 73.43 (d, Rha-4"), 72.28 (d, Rha-3"), 72.17 (d, Rha-5"), 72.07 (d, Rha-2"), 17.80 (d, Rha-6").

Kaempferol 3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside, Kaempferol-3-*O*-rutinoside (3) – Yellow amorphous solid; mp: 190 - 192 °C; $[\alpha]_D$ −9.5° (*c* 0.31, MeOH); UV (MeOH) λ_{max} nm: 266, 349; ^{1}H-NMR (500 MHz, CD_3OD) δ: 8.07 (2H, d, *J* = 9.0 Hz, H-2'/H-6'), 6.86 (2H, d, *J* = 9.0 Hz, H-3'/H-5'), 6.25 (1H, d, *J* = 1.5 Hz, H-8), 6.09 (1H, d, *J* = 2.0 Hz, H-6), 4.93 (1H, d, *J* = 8.0 Hz, Glc-1"), 4.51 (1H, s, Rha-1'"), 3.81-3.23 (9H, m, Glc-H-2", H-3", H-4", H-5", H-6"; Rha-H-2'", H-3'", H-4'", H-5'"), 0.94 (3H, d, *J* = 6.5 Hz, Rha-H-6'"); ^{13}C-NMR (125 MHz, CD_3OD) δ: 178.94 (s, C-4), 162.78 (s, C-7), 161.91 (s, C-5), 159.16 (s, C-9/4'), 158.63 (s, C-2), 135.69 (s, C-3), 132.49 (d, C-3'/5'), 122.85 (s, C-1'), 116.32 (d, C-2'/6'), 106.52 (d, C-6), 96.48 (d, C-8), 102.61 (d, Glc-1"), 74.03 (d, Glc-2"), 75.49 (d, Glc-3"), 69.87 (d, Glc-4"), 75.35 (d, Glc-5"), 67.59 (d, Glc-6"), 102.08 (d, Rha-1'"), 72.43 (d, Rha-2'"/4'"), 72.21 (d, Rha-3'"), 73.15 (d, Rha-5'"), 18.12 (q, Rha-6'").

Gallic acid (4) – White crystal; mp 215 - 217 °C; UV (MeOH) λ_{max} nm: 218, 275; IR ν_{max} (KBr) cm^{-1} :3400, 1700, 1620, 1330, 1250; ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.06 (2H, s, H-2, 6); ^{13}C-NMR (125 MHz, CD_3OD) δ: 122.22 (s, C-1), 110.46 (d, C-2, 6), 146.53 (s, C-3, 5), 139.70 (s, C-4), 170.60 (s, C-7).

Protocatechuic acid (5) – Brown powder; mp: 199 - 200 °C; UV (MeOH) λ_{max} nm: 218, 273; IR ν_{max} (KBr) cm^{-1}: 3444, 1635; ^{1}H-NMR (300 MHz, CD_3OD) δ: 7.42 (1H, d, *J* = 7.2 Hz, H-5), 7.41 (1H, d, *J* = 1.8 Hz, H-2), 6.79 (1H, dd, *J* = 1.8, 7.2 Hz, H-6); ^{13}C-NMR (75 MHz, CD_3OD) δ: 170.42 (s, -COOH), 151.50 (s, C-4), 145.05 (s, C-3), 123.86 (d, C-6), 123.21 (s, C-1), 117.69 (d, C-2), 115.73 (d, C-5).

Gallic acid methyl ester (6) – Bright yellow powder; UV(MeOH) λ_{max} nm : 276; IR ν_{max} (KBr) cm^{-1}: 3360, 1690, 1620, 1370, 1329, 1260; ^{1}H-NMR (300 MHz, CD_3CD) δ: 7.04 (2H, s, H-2/6), 3.81 (3H, s, $COOCH_3$); ^{13}C-NMR (75 MHz, CD_3OD) δ: 168.99 (s, $COOCH_3$), 146.47 (s, C-3/5), 139.72 (s, C-4), 121.40 (s, C-1), 109.99 (d, C-2/6), 52.27 (q, $COOCH_3$).

Bioassay of interleukin (IL)-6 – The IL-6 bioassay was carried out using a slight modification of an established method (Cai *et al.*, 2003; Kim *et al.*, 2003). Briefly, 500 μL of the MG-63 cells (3×10^4 cells/mL) in DMEM containing 10% FBS were dispensed into a 24 well plate. The culture was incubated for 24 h at 37 °C. 5 μL of TNF-α (15 ng/mL) and 5 μL of the DMSO with or without the compounds were then added. The medium was incubated at 37 °C in an atmosphere containing 5% CO_2 for 24 h, and stored at −20 °C until needed. The medium was used to determine the IL-6 content using an ELISA procedure. 96 well plates were coated with 100 μL of the purified rat anti-human IL-6 monoclonal antibody in 0. 1M $NaHCO_3$ (pH 9.6) by an overnight

incubation at 4 ℃. The wells were blocked with 200 μL of 3% BSA in PBS for 2 h at RT, and incubated with 100 μL of the specific antibody for 2 h at RT. 100 μL of HRP conjugated rabbit anti-goat IgG (1 : 1000 dilution) was added to the well and incubated for 2 h at RT. 100 μL of a TMB (3,3',5,5'-tetramethyl-benzidine) substrate solution was added and incubated for 10 min at RT. The color reaction was quenched with 50 μL of 0.4N HCl, and the optical density was read at 450 nm using a Microplate Reader. (Molecular Devices Co., Ltd., U.S.A.) The inhibition percentages of IL-6 was calculated using the following equation: Inhibition (%) = (A – B)/A × 100 [Where A is the IL-6 concentration when the TNF-α only was treated, and B is the IL-6 concentration when the compounds were treated] A and B values were obtained by subtraction of the none-treated value from the TNF-α or compounds-treated value. Results were expressed as the mean ± S.E. from three separate experiments.

Results and Discussion

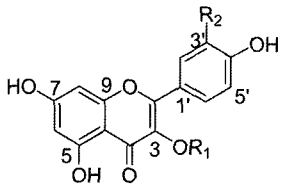
The EtOAc soluble fraction of the MeOH extract of *G. thunbergii* was chromatographed on silica gel, Sephadex LH-20, reversed phase C-18 columns afforded six compounds (**1 - 6**) (Fig. 1).

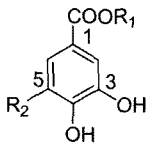
Compound **1** was obtained as an amorphous powder. The ^{1}H-NMR data for **1** showed signals for two *meta*-coupled protons at δ 6.11 (1H, d, J = 2.5 Hz, H-8) and δ 6.00 (1H, d, J = 2.5 Hz, H-6), together with two *ortho*-coupled protons at δ 7.71 (2H, d, J = 8.5 Hz, H-2'/H-6') and δ 6.88 (2H, d, J = 8.5 Hz, H-3'/H-5'), indicating a kaempferol derivatives (Agrawal, 1992). A doublet at δ 0.92 (3H, d, J = 6.0 Hz, H-6") and a doublet at δ 5.33 (1H, d, J = 1.5 Hz, H-1") in the ^{1}H-NMR spectrum suggested the presence of rhamnose in its structure (Markham, 1994). Two carbon signals at δ 158.29 (C-2) and δ 135.53 (C-3) in the ^{13}C-NMR spectrum suggested that the sugar moiety was attached to the *O*-atom at C-3 of the aglycone. The spectrum of **1** also revealed the rhamnosyl moiety to be α-linked ($J_{H\text{-}1\text{-}H\text{-}2}$ = 1.5 Hz) and in the pyranose form (Harbone, 1994). The chemical shifts observed for **1** were in good agreement with those measured with other flavonol 3-rhamnoside. Based on the spectral data of **1** and the comparison with literature, **1** was identified as kaempferol 3-*O*-α-L-rhamnopyranoside (Kim *et al.*, 2004; Rao *et al.*, 2002; Chung *et al.*, 2004; Fossen *et al.*, 1999).

Compound **2** was obtained as an amorphous powder. The ^{1}H-NMR data for **2** showed signals for three *meta*-coupled protons at δ 7.33 (1H, d, J = 2.0 Hz, H-2'), 6.33 (1H, d, J = 2.0 Hz, H-8), 6.17 (1H, d, J = 2.0 Hz, H-6), one *ortho-meta* coupled doublet at δ 7.30 (1H, dd, J = 2.0 8.5 Hz, H-5') and one *ortho*-coupled doublet at δ 6.90 (1H, d, J = 8.5 Hz, H-6'). The spectral data of **2** were quite similar to those of **1**, except for one more hydroxyl substitutuent on B-ring of a flavonol skeleton. These observations suggested that **2** was the quercetin derivatives and identified as quercetin 3-*O*-α-L-rhamnopyranoside (quercitrin) (Zhong *et al.*, 1997; *Peng et al.*, 2003).

Compound **3** was obtained as an amorphous powder. The ^{1}H-NMR data for **3** showed signals for two *meta*-coupled protons at δ 6.25 (1H, d, J = 1.5 Hz, H-8) and δ 6.09 (1H, d, J = 2.0 Hz, H-6), together with two *ortho*-coupled protons at δ 8.07 (2H, d, J = 9.0 Hz, H-2'/H-6') and δ 6.86 (2H, d, J = 9.0 Hz, H-3'/H-5'). From the analysis of ^{1}H-NMR and coupling constants of **3**, there were characteristic signals of rhamnopyranoside and glucopyranoside at δ 4.51 (1H, s, H-1''') and δ 4.93 (1H, d, J = 8.0 Hz, H-1"), respectively (Pauli, 2000). On the basis of the above spectral data, **3** was identified as kaempferol 3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (Ly *et al.*, 2006; Jung *et al.*, 2003; Sawada et al., 1993; Vermes *et al.*, 1976; Farkas *et al.*, 1976).

Compounds **4 - 6** were identified as a gallic acid (Nawar





R_1=Rha, R_2=OH : Kaempferol-3-rhamnoside (**1**)
R_1=Rha, R_2=OH : Quercetin-3-rhamnoside (**2**)
R_1=Rha-(1→6)-Glc, R_2=H : Kaempferol-3-rutinoside (**3**)

R_1= H, R_2=OH : Gallic acid (**4**)
R_1= H, R_2=H : Protocatechuic acid (**5**)
R_1= CH_3, R_2=OH : Gallic acid methyl ester (**6**)

Fig. 1. Chemical structures of compounds **1-6** isolated from *G. thunbergii*.

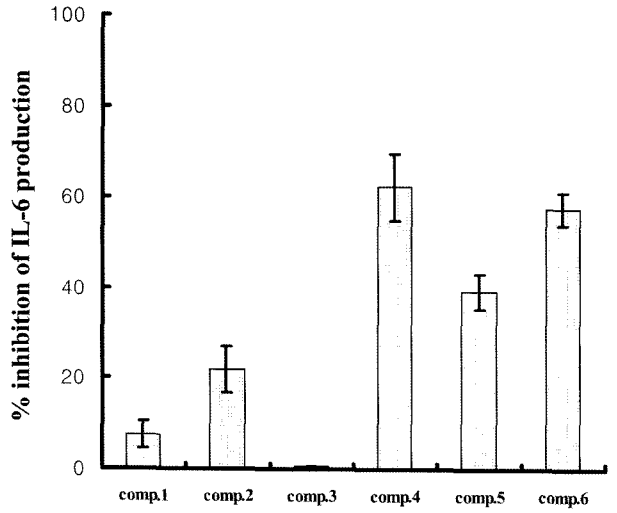


Fig. 2. Inhibitory activity of compounds **1 - 6** on IL-6 production from TNF-α stimulated MG-63 cell.
MG-63 (3×10^4 cells/well) was incubated for 24 h. Cultures were incubated with TNF-α and DMSO with or without compounds for 24 h. IL-6 in the supernatant was measured by ELISA as described in Materials and Methods. Results are expressed as the mean ± S.E. from three separate experiments.

et al., 1982; Park *et al.*, 1993), protocatechic acid (Lee *et al.*, 2002; Park *et al.*, 1993), and gallic acid methyl ester (Kang *et al.*, 1993; Kang *et al.*, 2003) by comparing the NMR spectral data with those reported in the literature, respectively.

Interleukin-6 (IL-6) is a cytokine originally identified as a T-cell-derived factor regulating B-cell growth and differentiation (Hirano *et al.*, 1986). Human IL-6 is an important component of the inflammatory cascade. In particular, the dysregulation of IL-6 production has been implicated in a variety of inflammatory/autoimmune diseases including rheumatoid arthritis, cardiac myxoma, Castleman's disease, and mesangial proliferative glomerulonephritis (Hirano *et al.*, 1990). The proinflammatory cytokines IL-1 and tumor necrosis factor-α (TNF-α) markedly stimulate IL-6 production (Van Damme *et al.*, 1987).

The inhibitory activity of IL-6 production in TNF-α stimulated MG-63 cell was examined (Fig. 2). Among the isolated compounds, flavonoids derivatives **1 - 3** showed negligible activity against IL-6 production, while gallic acid **(4)** and gallic acid methyl ester **(6)** showed potent inhibitory activity. In our results, the number of hydroxyl groups of aromatic ring assumes a crucial role in IL-6 inhibitory activities. The IL-6 inhibitory activity of gallic acid which carries three hydroxyl groups of aromatic ring is more potent than protocatechic acid which carries two hydroxyl groups of aromatic ring. In addition, the masking of free carboxylic group of gallic acid doesn't affect on the expression of IL-6 inhibitory activity. The inhibitory activity of compounds **1 - 6** against IL-6 production in TNF-α stimulated MG-63 cell was 7.6 ± 3.0%, 21.9 ± 5.02%, 0.05 ± 0.01%, 62.4 ± 7.4%, 39.2 ± 3.9%, 57.6 ± 3.6%, respectively, at a concentration of 100 μg/mL.

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References

Agrawal, P.K., NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. *Phytochemistry* **31**, 3307-3330 (1992).

Bae, K.W., The medicinal plants of Korea. Kyo-Hak Publishing Co., Seoul, pp (2000).

Cai, X.F., Shen, G, Dat, N.T., Kang, O.H., Kim, J.A., Lee, Y.M., Lee, K.J., and Kim, Y.H. Inhibitory effect of TNF-α and IL-8 secretion by pimarane-type diterpenoids from *Acanthopanax koreanum*. *Chem. Pharm. Bull.* **51**, 605-607 (2003).

Chung, S.-K., Kim, Y.-C., Takaya, Y., Terashima, K., and Niwa, M., Novel flavonol glycoside, 7-*O*-methyl mearnsitrin, from *Sageretia theezans* and its antioxidant effect. *J. Agric. Food. Chem.* **52**, 4664-4668 (2004).

Farkas, L., Vermes, B., Nógrádi, M., and Kálmán, A., The final structure of robinin and biorobin and their total synthesis. *Phytochemistry* **15**, 215-218 (1976).

Fossen, T., Larsen, A., Kiremire, B.T., and Andersen, M., Flavonoids from blue flowers of *Nymphaea caerulea*. *Phytochemistry* **51**, 1133-1137 (1999).

Hirano, T., Yasukawa, K., Harada, H., Taga, T., Watanabe, Y., Matsuda, T., Kashiwamura, S., Nakajima, K., Koyama, K., Iwamatsu, A., Tsunsawa, S., Sakiyama, F., Matsui, H., Takahara, Y., Taniguchi, T., and Kishimoto, T., Complementary DNA for a novel human interleukin (BSF-2) that induced lymphocytes to produce immunoglobulin. *Nature* **324**, 73-76 (1986).

Hirano, T., Akira, S., Taga, T., and Kishimoto, T., Biological and clinical aspects of interleukin 6. *Immunol. Today* **11**, 443-449 (1990).

Harbone, J.B., The flavonoids: advances in research since 1986. Champman & Hall, London, pp. 442-473 (1994).

Ito, H., Hatano, T., Namba, O., Shirono, T., Okuda, T., and Yoshida, T., Constituents of *Geranium thunbergii* Sieb. Et Zucc. XV. Modified dehydroellagitannins, geraniinic acids B and C, and phyllanthusiin F. *Chem. Pharm. Bull.* **47**, 1148-1151 (1999).

Jung, H.A., Kim, J.E., Chung, H.Y., and Choi, J.S., Antioxidant principles of *Nelumbo nucifera* stamens. *Arch. Pharm. Res.* **26**, 279-285 (2003).

Kang, S.S., Kim, J.S., Choi, S.Y., and Han, B.H., Phytochemical studies on *Peoniae Radix*. *Kor. J. Pharmacogn.* **24**, 247-250 (1993).

Kang, S.J., Hong, S.S., Hwang, B.Y., Ro, J.S., Lee, K.S., and Towers, G.H.N., Phenolic compounds from the stems of *Sapium japonicum*. *Kor. J. Pharmacogn.* **12**, 125-128 (2006).

Kim, B.H., Chung, E.Y., Ryu, J.-C., Jung, S.-H., Min, K.R., and Kim, Y., Anti-inflammatory mode of isoflavone glycosides sophoricoside by inhibition of interleukin-6 and cyclooxygenase-2 in inflammatory response. *Arch. Pharm. Res.* **26**, 306-311 (2003).

Kim, Y.-K., Kim, Y.S., Choi, S.U., and Ryu, S.Y., Isolation of flavonol rhamnosides from Loranthus tanakae and cytotoxic effect of them on human tumor cell lines. *Arch. Pharm. Res.* **27**, 44-47 (2004).

Lee, S.H., Kim, B.K., Cho, S.H., and Shin, K.H., Phytochemical constituents from the fruits of *Acanthopanax sessiliflorus*. *Arch. Pharm. Res.* **25**, 280-284 (2002).

Liu, Q.H., Jeong, J.-E., Choi, E.J., Moon, Y.H., and Woo, E.-R. A new furofuran lignan from *Geranium thunbergii*. *Arch. Pharm. Res.* **29**, 1109-1113 (2006).

Ly, T.N., Shimoyamada, M., and Yamauchi, R., Isolation and characterization of rosmarinic acid oligomers in *Celastrus hindsii* Benth leaves and their antioxidative activity. *J. Agric. Food. Chem.* **54**, 3786-3793 (2006).

Markham, K.R., Techniques of flavonoid identification, Academic Press, London, New York, pp. 75-78 (1994).

Nawar, M.A., Buddrus, J., and Bauer, H., Dimeric phenolic constituents from the roots of *Tamarix nilotica*. *Phytochemistry* **21**, 1755-1758 (1982).

Okuda, T., Yoshida, T., Hatano, T., Ikeda, Y., Shingu, T., and Inoue, T., Constituents of *Geranium thunbergii* Sieb. Et Zucc. XIII. Isolation of water-soluble tannins by centrifugal partition chromatography, a biomimetic synthesis of elaeocarpusin. *Chem. Pharm. Bull.* **34**, 4075-4082 (1986).

Park, W.Y., Lee, S.C., Ahn, B.T., and Lee, S.H., Phenolic compounds from *Acalypha australis* L. *Kor. J. Pharmacogn.* **24**, 20-25 (1993).

Pauli, G.F., Higher order and substituent chemical shift effects in the NMR glycosides. *J. Nat. Prod.* **63**, 834-838 (2000).

Peng, Z.F., Strack, D., Baumert, A., Subramaniam, R., Goh, N.K., Chia, T.F., Tan, S.N., and Chia, L.S., Antioxidant flavonoids from leaves of *Polygonum hydropiper* L. *Phytochemistry* **62**, 219-228 (2003).

Rao, L.M., Yada, H., Ono, H., and Yoshida, M., Acylated and non-acylated flavonol monoglycosides from the indian minor spice Nagkesar (*Mammea longifolia*). *J. Agric. Food. Chem.* **50**, 3143-3146 (2002).

Sawada, H., Miyakoshi M., Isoda, S., Ida, Y., and Shoh, J., Saponins from leaves of *Acanthopanax sieboldianus*. *Phytochemistry* **34**, 1117-1121 (1993).

Van Damme, J., Opdenakker, G., Simpson, R. J., Rubira, M. R., Cayphas, S., Vink, A., Billiau, A., and Van Snick, J., Identification of the human 26-kD protein, interferon beta 2 (IFN-beta 2), as a B cell hybridoma/plasmacytoma growth factor induced in interleukin 1 and tumor necrosis factor. *J. Exp. Med.* **165**, 914-919 (1987).

Vermes, B., Farkas, L., Nógrádi, M., Wagner, H., and Dirscherl, R., The synthesis of afzelin, paeonoside and kaempferol 3-*O*-β-rutinoside. *Phytochemistry* **15**, 1320-1321 (1976).

Yuba, P., Liu, Q.H., Oh, J.W., Woo, E.-R., Kang, K.W. 4-Hydroxykobusin inhibits the induction of nitric oxide synthase by inhibiting NF-k B and AP-1 activation. *Biol. Pharm. Bull.* **30**, 1097-1101 (2007).

Zhong, X.N., Otsuka, H., Ide, T., Hirata, E., Takushi, A., and Takeda, Y., Three flavonol glycosides from leaves of *Myrsine Seguinii*. *Phytochemistry* **46**, 943-946 (1997).

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