J Appl Biol Chem (2018) 61(1), 39–46
https://doi.org/10.3839/jabc.2018.006

Online ISSN 2234-7941
Print ISSN 1976-0442

Article: Biochemistry/Molecular Biology

Chemical constituents of *Dicentra spectabilis* and their anti-inflammation effect

A Hyeon Kim[1] · Ji Hun Jang[1] · Kyeong Wan Woo[1] · Jong Eel Park[1] · Ki Ho Lee[1] · Ho Kyung Jung[1] · Byeongkwan An[1] · Won Seok Jung[1] · Seong Ho Ham[1] · Hyun Woo Cho[1]

Received: 30 October 2017 / Accepted: 9 January 2018 / Published Online: 31 March 2018

Abstract Column chromatographic separation of the MeOH extract from the roots of *Dicentra spectabilis* yielded fourteen compounds, menisdaurin (**1**), menisdaurilide (**2**), *trans*-N-*p*-coumaroyltyramine (**3**), *trans*-N-*p*-feruloyltyramine (**4**), 4-*O*-feruloylquinicacid (**5**), chlorogenic acid (**6**), 3-*O*-feruloylquinic-acid (**7**), ferulic acid (**8**), protopine (**9**), Kaempferol 3,7-di-*O*-β-D-glucopyranoside (**10**), kaempferol 3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside (**11**), α-rhamnoisorobin (**12**), astragalin (**13**), and nicotiflorin (**14**). Their structures were determined on the basis of NMR spectroscopic data. Among them, compound **1**, **3-8**, and **10-14** isolated from this plant were reported for the first time. The isolated compounds (**1-14**) were tested for nitric oxide (NO) inhibitory activity on lipopolysaccharide-stimulated RAW 264.7 cells. Compound **3**, **4** and **12** significantly inhibited NO production. Moreover, Compound **3** suppressed pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) in a dose- dependent manner. These data suggest that compound **3** possess anti-inflammatory activity and might be useful natural materials for development of anti-inflammatory agent.

Keywords Anti-inflammation effect · *Dicentra spectabilis* · Fumariaceae · Isolation · Structure elucidation

Hyun Woo Cho (✉)
E-mail: johw7@nikom.or.kr

[1]Division of Traditional Korean Medicine Resource, National Development Institute of Korea Medicine, 288, Woodlandgil, Anyangmyeon, Jangheunggun, Jeonnam 59338, Republic of Korea

Introduction

Inflammation is an essential aspect of the host response to infection and injury and is a key factor for the maintenance of good health in response to infections. However, aberrant inflammation underlies many acute and chronic human diseases such as arthritis, and autoimmune disease (Medzhitov and Janeway 1997; Serhan and Savill 2005). Macrophages play an important role in the immune response and inflammation through the release of pro-inflammatory cytokines (tumor necrosis factor-α, interleukin 1β and interleukin 6), and mediators in response to triggers eg, bacterial lipopolysaccharides (LPS), nitric oxide (NO) and prostaglandin E_2 (PGE_2) (Ritchlin et al. 2003).

Dicentra spectabilis are herbaceous perennial plants that are widely distributed in the wet valley areas (Sim et al. 2005). In Korea, only one species is known in *Dicentra* and it is used as an ornamental plant (Lee et al. 2004). The roots of *D. spectabilis* have been used for the treatment of various conditions such as strokes, bruises, and blood circulation (Kim et al. 2017). Previous phytochemical investigations on *D. spectabilis* have reported the isolation of fungitoxic alkaloids (Ma et al. 2000) and compounds with apoptosis-inducing activities (McNulty et al. 2007). However, only a few phytochemical studies and on *D. spectabilis* have been reported. In the course of our continuing search for anti-inflammatory components of Korean medicinal plants (Jang et al. 2016; Woo et al. 2016; Sim et al. 2017), we investigated the active constituents of *D. spectabilis*. Repeated column chromatographic separations of a MeOH extract led to the isolation of fourteen known compounds (**1-14**) (Fig. 1). All compounds were measured for their inhibitory activity of nitric oxide production on a LPS-induced murine macrophage cell line RAW 264.7. Among them, We elucidate, using selective compound **3, 4** and **12** isolated from *D. spectabilis,* anti-inflammatory effects. In the present study, we report the isolation and structural elucidation of compounds **1-14** and study their anti-inflammatory effects possibility.

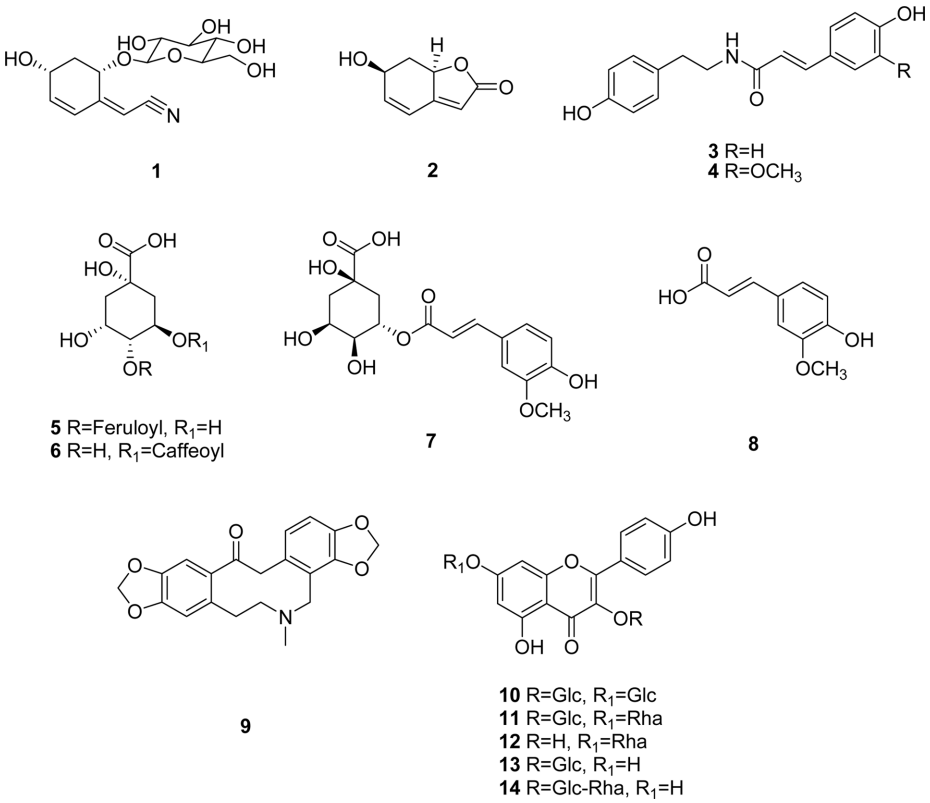

Fig. 1 The structures of **1-14** isolated from *D. spectabilis*

Material and Methods

Plant materials

The roots of *D. spectabilis* were collected in Yeongwol, Gangwon province, Korea in June 2015. The plant was identified by professor Hui Kim (Mokpo National University, Mokpo, Korea). A voucher specimen (TKM-2095) of this plant was deposited in the Herbarium at the National Development Institute of Korea Medicine.

General experimental procedures

TLC was performed using Merck pre-coated silica gel F_{254} plates. Spots were visualized on TLC under UV light or by spraying with 10% H_2SO_4 in EtOH (v/v), and heating. Silica gel 60 (Merck, 230–400 mesh) and RP-C_{18} silica gel (YMC GEL ODS-A, 12 nm, S-75 μm) were used for column chromatography. All the compounds were purified on an Agilent A1200 series HPLC (Agilent Technologies, Santa Clara, CA, USA) using a Phenomenex Luna C_{18}-100A column (25 cm×3 mm, particle size=5 μm). NMR spectra were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C); chemical shifts are given in parts per million (ppm; δ). ESI-mass spectra were obtained on a Schimadzu LCMS-IT-TOF mass spectrometer.

Extraction and isolation

The roots of *D. spectabilis* (1.6 kg) were extracted with 100% MeOH under reflux and filtered. The filtrate was concentrated *in vacuo* to give a MeOH extract (308 g), which was suspended in water (800 mL) and successively partitioned with *n*-hexane and H_2O to give yields of 34 and 260 g, respectively. The H_2O-soluble fraction (100 g) was separated over an RP-C_{18} silica gel column with 0–100% MeCN as the eluent to give seven fractions (D1–D7). Fraction D1 (25 g) was separated over an RP-C_{18} silica gel column with 0-5% MeCN as the eluent to give five fractions (D1-1–D1-5). Sub-fractions D1-3 (6 g) were subjected to column chromatography (CC) on silica gel (230–400 mesh), and eluted with a solvent system of $CHCl_3$/MeOH/ H_2O (20:3:0.3–10:4:0.3) to give eight sub-fractions (D1-3-1–D1-3-8). Sub-fraction D1-3-3 (470 mg) was purified with a RP-C_{18} prep HPLC (7% MeCN, isocratic, 254 nm, 22 mL/min) to yield compound **2** (25 mg). Fraction D3 (10 g) was separated over an RP-C_{18} silica gel column with 0-20% MeCN as the eluent to give sixteen fractions (D3-1–D3-16). Sub-fraction D3-3 (600 mg) was purified with a RP-C_{18} prep HPLC (5% MeCN, isocratic, 282 nm, 25 mL/min) to yield compound **1** (313 mg). Sub-fraction D3-7 (135 mg) was purified with an RP-C_{18} prep HPLC (8% MeCN, isocratic, 254 nm, 25 mL/min) to yield compound **6** (6 mg). Sub-fraction D3-11 (760 mg) was purified with a RP-C_{18} prep HPLC (11% MeCN,

isocratic, 365 nm, 25 mL/min) to yield compound **10** (580 mg). Fraction D3-13 (4.5 g) was separated over an RP-C_{18} silica gel column with 0-30% MeCN as the eluent to give seven fractions (D3-13-1–D3-13-7). Sub-fraction D3-13-3 (800 mg) was subjected to CC on a silica gel (230–400 mesh), eluting with a solvent system of $CHCl_3$/MeOH (25:1–1:1) to give twelve sub-fractions (D3-13-3-1–D3-13-3-12). Sub-fraction D3-13-3-9 (420 mg) was purified with a RP-C_{18} prep HPLC (15–20% MeCN, gradient, 36 min, 237 nm, 20 mL/min) to yield compounds **5** (100 mg) and **7** (30 mg). Sub-fraction D3-14 (2 g) was subjected to CC on silica gel (230-400 mesh), eluting with a solvent system of EtOAc/MeOH/H_2O (15:3:0.3) to give fifteen sub-fractions (D3-14-1–D3-14-15). Sub-fraction D3-14 (495 mg) was recrystallized using methanol to yield compound **11** (61 mg). Sub-fraction D3-9 (61 mg) was recrystallized using methanol to yield compound **9** (12 mg). Sub-fraction D3-16 (1 g) was subjected to CC on silica gel (230-400 mesh), eluting with a solvent system of $CHCl_3$/MeOH/H_2O (25:3.6:0.3–10:3:0.3) to give nine sub-fractions (D3-16-1–D3-16-9). Sub-fraction D3-16-2 (180 mg) was purified with a RP-C_{18} semi-prep HPLC (32% MeCN, 2.7 mL/min) to yield compound **8** (5 mg). Sub-fraction D3-16-5 (50 mg) was purified with a RP-C_{18} semi-prep HPLC (22% MeCN, 2.7 mL/min) to yield compound **13** (13 mg). Sub-fraction D3-16-9 (40 mg) was purified with a RP-C_{18} semi-prep HPLC (22% MeCN, 2.7 mL/min) to yield compound **14** (11 mg). Fraction D4 (7 g) was separated over an RP-C_{18} silica gel column with 0-15% MeCN as the eluent to give six fractions (D4-1–D4-6). Sub-fraction D4-3 (305 mg) was purified with a RP-C_{18} prep HPLC (23–25% MeCN, gradient, 50 min, 279 nm, 27 mL/min) to yield compounds **3** (37 mg) and **4** (28 mg). Sub-fraction D4-4 (215 mg) was purified with an RP-C_{18} prep HPLC (30–40% MeCN, gradient, 35 min, 368 nm, 20 mL/min) to yield compound **12** (20 mg).

Compound **1**. LC-IT TOF MS *m/z* 312 $[M–H]^–$; ^{1}H-NMR (500 MHz, CD_3OD, δ_H) 6.30 (1H, d, *J*=9.5 Hz, H-2), 6.21 (1H, dd, *J*=10.0, 3.0 Hz, H-3), 5.50 (1H, s, H-7), 4.94 (1H, dd, *J*=8.5, 3.5 Hz, H-6), 4.56 (1H, d, *J*=7.5 Hz, H-1'), 4.37 (1H, m, H-4), 3.90 (1H, dd, *J*=12.0, 2.0 Hz, H-6b'), 3.68 (1H, dd, *J*=12.0, 6.0 Hz, H-6b'), 2.28 (1H, ddd, *J*=13.0, 5.0, 3.5 Hz, H-5a), 2.03 (1H, ddd, *J*=13.0, 5.0, 3.5 Hz, H-5b); ^{13}C-NMR (125 MHz, CD_3OD, δ_C) 155.7 (C-1), 139.2 (C-3), 126.3 (C-2), 116.6 (C-8), 100.1 (C-1'), 95.4 (C-7), 76.7 (C-5'), 76.6 (C-3'), 73.1 (C-2'), 71.1 (C-6), 70.3 (C-4'), 64.0 (C-4), 61.7 (C-6'), 34.7 (C-5).

Compound **2**. LC-IT TOF MS *m/z* 151 $[M–H]^–$; ^{1}H-NMR (500 MHz, CD_3OD, δ_H) 6.65 (1H, dd, *J*=10.0, 2.0 Hz, H-5), 6.34 (1H, d, *J*=10.0 Hz, H-4), 5.86 (1H, s, H-3), 5.04 (1H, ddd, *J*=13.0, 5.0, 2.0 Hz, H-8), 4.58 (1H, m, H-6), 2.84 (1H, dt, *J*=10.5, 5.0 Hz, H-7a), 1.55 (1H, dt, *J*=13.5, 5.0 Hz, H-7b); ^{13}C-NMR (125 MHz, CD_3OD, δ_C) 174.5 (C-8), 164.8 (C-1), 144.6 (C-3), 119.0 (C-2), 110.1 (C-7), 78.7 (C-6), 65.9 (C-4), 39.6 (C-5).

Compound **3**. LC-IT TOF MS *m/z* 282 $[M–H]^–$; ^{1}H-NMR (500 MHz, CD_3OD, δ_H) 7.31 (1H, d, *J*=15.5 Hz, H-7), 7.00 (1H, d, *J*=1.5 Hz, H-2), 6.98 (2H, d, *J*=8.5 Hz, H-2', 6'), 6.93 (1H, dd, *J*=8.5, 1.5 Hz, H-6), 6.67 (1H, d, *J*=8.5 Hz, H-5), 6.60 (2H, d, *J*=8.5 Hz, H-3', 5'), 6.28 (1H, d, *J*=15.5 Hz, H-8), 3.36 (2H, t, *J*=7.5 Hz, H-8), 2.64 (2H, t, *J*=7.5 Hz, H-7); ^{13}C-NMR (125 MHz, CD_3OD, δ_C) 167.4 (C-9), 155.8 (C-4'), 147.7 (C-4), 145.7 (C-3), 141.2 (C-7), 131.2 (C-1), 130.9 (C-2', 6'), 127.3 (C-1), 121.2 (C-6), 117.5 (C-8), 116.5 (C-5), 116.2 (C-3', 5'), 114.3 (C-2), 42.2 (C-8'), 34.2 (C-7').

Compound **4**. LC-IT TOF MS *m/z* 312 $[M–H]^–$; ^{1}H-NMR (500 MHz, CD_3OD, δ_H) 7.41 (1H, d, *J*=15.5 Hz, H-7), 7.14 (1H, brs, H-2), 7.04 (2H, d, *J*=8.5 Hz, H-2', 6'), 6.97 (1H, dd, *J*=8.5, 2.0 Hz, H-6), 6.70 (1H, d, *J*=8.5 Hz, H-5), 6.69 (2H, d, *J*=8.5 Hz, H-3', 5'), 6.41 (1H, d, *J*=15.5 Hz, H-8), 3.85 (3H, s, OCH_3), 3.44 (2H, t, *J*=7.5 Hz, H-8'), 2.73 (2H, t, *J*=7.5 Hz, H-7'); ^{13}C-NMR (125 MHz, CD_3OD, δ_C) 167.1 (C-9), 155.2 (C-4'), 147.7 (C-3), 145.0 (C-4), 139.7 (C-7), 129.5 (C-1'), 129.0 (C-2', 6'), 128.1 (C-1), 126.6 (C-6), 118.2 (C-8), 115.2 (C-2), 114.6 (C-3', 5'), 114.5 (C-5), 55.1 (OCH_3), 40.9 (C-8'), 34.1 (C-7').

Compound **5**. LC-IT TOF MS *m/z* 367 $[M–H]^–$; ^{1}H-NMR (500 MHz, CD_3OD, δ_H) 7.71 (1H, d, *J*=16.0 Hz, H-7'), 7.21 (1H, d, *J*=2.0 Hz, H-2'), 7.11 (1H, dd, *J*=8.0, 2.0 Hz, H-6'), 6.83 (1H, d, *J*=8.0 Hz, H-5'), 6.46 (1H, d, *J*=16.0 Hz, H-8'), 4.83 (1H, m, H-4), 4.30 (1H, m, H-3), 4.28 (1H, m, H-5), 3.87 (3H, s, OCH_3), 2.20 (2H, m, H-6), 2.04 (1H, m, H-2); ^{13}C-NMR (125 MHz, CD_3OD, δ_C) 175.0 (C-7), 167.5 (C-9'), 149.2 (C-3'), 148.0 (C-4'), 145.5 (C-7'), 126.4 (C-1'), 122.6 (C-6'), 115.1 (C-5'), 114.3 (C-8'), 110.4 (C-5'), 77.8 (C-4), 74.9 (C-1), 68.2 (C-3), 64.2 (C-5), 55.0 (OCH_3), 41.2 (C-6), 37.1 (C-2).

Compound **6**. LC-IT TOF MS *m/z* 353 $[M–H]^–$; ^{1}H-NMR (500 MHz, CD_3OD, δ_H) 7.55 (1H, d, *J*=16.0 Hz, H-7'), 7.04 (1H, d, *J*=2.0 Hz, H-2'), 6.95 (1H, dd, *J*=8.0, 2.0 Hz, H-6'), 6.77 (1H, d, *J*=8.0 Hz, H-5'), 6.25 (1H, d, *J*=16.0 Hz, H-8'), 5.33 (1H, m, H-3), 4.16 (1H, m, H-5), 3.70 (1H, dd, *J*=8.5, 3.0 Hz, H-4), 2.22 (2H, m, H-6), 2.07 (2H, m, H-2); ^{13}C-NMR (125 MHz, CD_3OD, δ_C) 174.9 (C-7), 165.7 (C-9'), 148.3 (C-3'), 145.5 (C-4'), 144.8 (C-7'), 125.6 (C-1'), 121.2 (C-6'), 115.7 (C-5'), 114.7 (C-2'), 114.3 (C-8'), 73.6 (C-1), 70.9 (C-3), 70.6 (C-4), 68.3 (C-5), 37.2 (C-6), 36.5 (C-2).

Compound **7**. LC-IT TOF MS *m/z* 367 $[M–H]^–$; ^{1}H-NMR (500 MHz, CD_3OD, δ_H) 7.65 (1H, d, *J*=16.0 Hz, H-7'), 7.19 (1H, d, *J*=2.0 Hz, H-2'), 7.07 (1H, dd, *J*=8.0, 2.0 Hz, H-6'), 6.81 (1H, d, *J*=8.0 Hz, H-5'), 6.41 (1H, d, *J*=16.0 Hz, H-8'), 5.37 (1H, m, H-3), 4.15 (1H, m, H-5), 3.86 (3H, s, OCH_3), 3.68 (1H, dd, *J*=8.5, 3.0 Hz, H-4), 2.18 (2H, m, H-6), 2.09 (2H, m, H-2); ^{13}C-NMR (125 MHz, CD_3OD, δ_C) 174.9 (C-7), 167.5 (C-9'), 149.1 (C-3'), 147.9 (C-4'), 145.2 (C-7'), 126.5 (C-1'), 122.6 (C-6'), 115.0 (C-5'), 114.8 (C-2'), 110.2 (C-8'), 74.1 (C-1), 73.2 (C-4), 71.5 (C-3), 67.1 (C-5), 55.0 (OCH_3), 38.9 (C-6), 35.4 (C-2).

Compound **8**. LC-IT TOF MS *m/z* 193 $[M–H]^–$; ^{1}H-NMR (500 MHz, CD_3OD, δ_H) 7.53 (1H, d, *J*=16.0 Hz, H-8), 7.03 (1H, d, *J*=2.0 Hz, H-2), 6.92 (1H, dd, *J*=8.0, 2.0 Hz, H-6), 6.77 (1H, d, *J*=8.0 Hz, H-5), 6.24 (1H, d, *J*=16.5 Hz, H-7), 3.89 (3H, s, OCH_3); ^{13}C-NMR (125 MHz, CD_3OD, δ_C) 168.3 (C-9), 148.1 (C-

3), 147.9 (C-4), 145.4 (C-7), 126.3 (C-1), 121.5 (C-6), 115.1 (C-5), 113.7 (C-8), 110.4 (C-5), 55.6 (OCH_3).

Compound **9**. LC-IT TOF MS *m/z* 352 $[M–H]^-$; ^{1}H-NMR (500 MHz, $CDCl_3$, δ_H) 6.88 (1H, s, H-1), 6.60 (1H, s, H-4), 2.68 (2H, brs, H-5a, 6a), 2.02 (1H, s, H-7), 3.68 (1H, s, H-8a), 6.65 (1H, m, H-11), 6.66 (1H, m, H-12), 3.72 (1H, brs, H-13a), 5.91 (1H, s, H-15), 5.90 (1H, s, H-16); ^{13}C-NMR (125 MHz, $CDCl_3$, δ_C) 183.9 (C-14), 147.9 (C-3), 145.9 (C-2, 10), 145.8 (C-9), 135.0 (C-14a), 131.5 (C-4a), 128.1 (C-12a), 124.5 (C-12), 116.7 (C-8a), 110.0 (C-4), 107.7 (C-1), 106.7 (C-11), 101.1 (C-15), 101.0 (C-16), 57.2 (C-6), 51.1 (C-8), 45.4 (C-13), 41.6 (C-7), 30.7 (C-5).

Compound **10**. LC-IT TOF MS *m/z* 609 $[M–H]^-$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ_H) 8.04 (2H, d, *J*=8.5 Hz, H-2', 6'), 6.87 (2H, d, *J*=8.5 Hz, H-3', 5'), 6.77 (1H, d, *J*=2.0 Hz, H-8), 6.42 (1H, d, *J*=2.0 Hz, H-6), 5.45 (1H, d, *J*=7.5 Hz, H-1"), 5.06 (1H, d, *J*=7.5 Hz, H-1'"); ^{13}C-NMR (125 MHz, DMSO-d_6, δ_C) 178.2 (C-4), 163.3 (C-7), 161.4 (C-5), 160.6 (C-4'), 157.3 (C-2), 156.5 (C-9), 134.0 (C-3), 131.5 (C-2', 6'), 121.3 (C-1'), 115.7 (C-3'), 106.2 (C-10), 101.2 (C-1"), 100.3 (C-1'"), 99.8 (C-6), 95.0 (C-8) 78.0 (C-5") 77.6 (C-5'") 77.0 (C-3"), 76.9 (C-3'"), 74.7 (C-2") 73.6 (C-2'"), 70.4 (C-4"), 70.1 (C-4'"), 61.3 (C-6"), 61.1 (C-6'").

Compound **11**. LC-IT TOF MS *m/z* 593 $[M–H]^-$; ^{1}H-NMR (500 MHz, DMSO-d_6, δ_H) 8.08 (2H, d, *J*=8.5 Hz, H-2', 6'), 6.90 (2H, d, *J*=8.5 Hz, H-3', 5'), 6.81 (1H, d, *J*=2.0 Hz, H-8), 6.44 (1H, d, *J*=2.0 Hz, H-6), 5.54 (1H, brs, H-1"), 5.47 (1H, d, *J*=7.5 Hz, H-1'"), 1.10 (3H, d, *J*=6.0 Hz, H-6"); ^{13}C-NMR (125 MHz, DMSO-d_6, δ_C) 177.5 (C-4), 161.5 (C-7), 160.7 (C-5), 160.0 (C-4'), 156.6 (C-2), 155.9 (C-9), 133.4 (C-3), 130.8 (C-2', 6'), 120.6 (C-1'), 115.0 (C-3', 5'), 105.5 (C-10), 100.5 (C-1'"), 99.2 (C-6), 98.2 (C-1"), 94.3 (C-8), 77.4 (C-5'"), 76.2 (C-3'"), 74.0 (C-2'"), 71.4 (C-4"), 70.2 (C-2", 3", 5", 4'"), 60.7 (C-6'"), 17.7 (C-6").

Compound **12**. LC-IT TOF MS *m/z* 431 $[M–H]^-$; ^{1}H-NMR (500 MHz, CD_3OD, δ_H) 8.07 (2H, d, *J*=8.0 Hz, H-2', 6'), 6.88 (2H, d, *J*=8.0 Hz, H-3', 5'), 6.70 (1H, brs, H-8), 6.38 (1H, brs, H-6), 5.55 (1H, brs, H-1"), 1.26 (3H, d, *J*=6.0 Hz, H-6"); ^{13}C-NMR (125 MHz, CD_3OD, δ_C) 176.2 (C-4), 161.9 (C-7), 161.0 (C-5), 159.4 (C-2, 4'), 156.4 (C-9), 136.3 (C-3), 129.5 (C-2', 6'), 122.2 (C-1'), 115.1 (C-3', 5'), 104.9 (C-10), 98.6 (C-1'), 98.5 (C-6), 94.0 (C-8), 72.3 (C-4'), 70.8 (C-3"), 70.4 (C-2"), 69.9 (C-5"), 16.8 (C-6").

Compound **13**. LC-IT TOF MS *m/z* 447 $[M–H]^-$; ^{1}H-NMR (500 MHz, CD_3OD, δ_H) 8.04 (2H, d, *J*=8.5 Hz, H-2', 6'), 6.86 (2H, d, *J*=8.5 Hz, H-3', 5'), 6.40 (1H, d, *J*=2.0 Hz, H-8), 6.21 (1H, d, *J*=2.0 Hz, H-6), 5.25 (1H, d, *J*=7.5 Hz, H-1"), 3.69 (1H, dd, *J*=12.0, 2.0 Hz, H-6a"), 3.51 (1H, dd, *J*=12.0, 5.0 Hz, H-6b"); ^{13}C-NMR (125 MHz, CD_3OD, δ_C) 178.3 (C-4), 164.8 (C-7), 160.8 (C-5), 160.1 (C-4'), 157.5 (C-9), 157.3 (C-2), 134.2 (C-3), 130.9 (C-2', 6'), 121.3 (C-1'), 114.7 (C-3', 5'), 104.4 (C-10), 102.7 (C-1"), 98.6 (C-6), 93.5 (C-8), 77.2 (C-5"), 76.8 (C-3"), 74.5 (C-2"), 70.2 (C-4"), 61.4 (C-6").

Compound **14**. LC-IT TOF MS *m/z* 593 $[M–H]^-$; ^{1}H-NMR (500 MHz, CD_3OD, δ_H) 8.05 (2H, d, *J*=8.5 Hz, H-2', 6'), 6.87 (2H, d, *J*=8.5 Hz, H-3', 5'), 6.39 (1H, d, *J*=2.0 Hz, H-8), 6.19 (1H, d, *J*=2.0 Hz, H-6), 5.11 (1H, d, *J*=7.5 Hz, H-1"), 4.50 (1H, d, *J*=1.5 Hz, H-1'"), 1.10 (1H, d, *J*=5.5 Hz, H-6'"); ^{13}C-NMR (125 MHz, CD_3OD, δ_C) 178.0 (C-4), 164.9 (C-7), 161.6 (C-5), 160.1 (C-4'), 158.0 (C-9), 157.2 (C-2), 134.1 (C-3), 131.0 (C-2', 6'), 121.4 (C-1'), 114.7 (C-3', 5'), 104.2 (C-10), 103.2 (C-1"), 101.0 (C-1'"), 98.7 (C-6), 93.7 (C-8), 76.8 (C-3"), 75.9 (C-5"), 74.4 (C-2"), 72.5 (C-4'"), 70.9 (C-3'"), 70.7 (C-2'"), 70.1 (C-4"), 68.4 (C-5'"), 67.2 (C-6"), 16.5 (C-6'").

Cell line and cell culture

The Raw 264.7 cells were obtained from the American Type Culture Collection. Raw 264.7 cells were maintained in Dulbecco's modified Eagle's medium. The medium was supplemented with 100 U/mL of penicillin, 100 μg/mL of streptomycin and 10% FBS. The cells were incubated at 37 °C with 5% CO_2 in humidified air.

Cell viability assay

The cytotoxicity of compounds **1-14** of Raw264.7 cells was determined by a MTS assay. Cells were split into 96-well plates at a density of 50,000 cells/well for 24 h and then treated with various concentrations of compounds **1-14** for 24 h. Then, 10 μL of MTS solution was added to each well for 3 h at 37 °C. After, the absorbance was measured with ELISA microplate reader (Infinite 200 pro, Morrisville, NC, USA) at 490 nm.

NO assay

Raw 264.7 cells were plated in 96-well cell plates and stimulated with LPS (500 ng/mL) in the presence or absence of different concentrations of compounds **1-14** for 24 h, Aliquots of cell culture medium (100 μL) were mixed with 50 μL of 1% sulfanilamide (in 5% phosphoric acid) and 50 μL of 0.1% N-(1-Naphthyl)ethylenediamine dihydrochloride at room temperature. After 10 min, the absorbance was determined at 540 nm using an ELISA plate reader (Infinite 200 pro).

Pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) ELISA assay

The suppressive effect of compounds **3, 4** and **12** on the pro-inflammatory cytokines production from LPS (500 ng/mL)-treated RAW 264.7 cells were determined according to the manufacturer's instructions. Raw 264.7 cells were treated with compounds **3, 4** and **12**, respectively, for 1 h, followed by addition of 500 ng/mL LPS for 24 h. Cell culture medium was used for pro-inflammatory cytokines assay using a mouse ELISA kit (R&D system, Minneapolis, MN, USA).

Statistical analysis

All data are presented as the mean ± SD. Statistically significant differences between two group were determined by t-test using SPSS version 21 (Chicago, IL, USA). Statistical significance: $*p<0.05$, $**p<0.01$, $**p<0.001$.

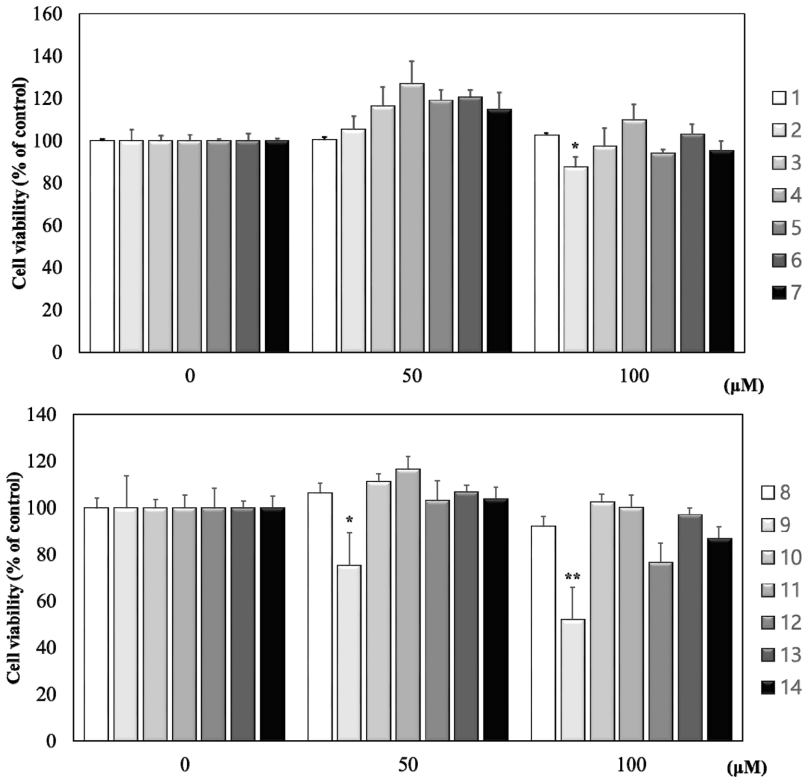

Fig. 2 Effects of compounds **1-7** (A) and **8-14** (B) isolated from *D. spectabilis* on cell viability in RAW 264.7 cells. RAW 264.7 cells were incubated for 24 hr in the presence or absence of compounds **1-14** at indicated dose. Significant differences between treated groups were determined using the Student's t-test. Values shown are the mean ± SD of triplicate determinations from three separate experiments. Statistical significance: *p <0.05, **p <0.01

Result and Discussion

Identification of compound 1-14

Compounds **1-14** were identified by comparing the ^{1}H-, ^{13}C-NMR, and LC-IT TOF MS data with the literature values to be menisdaurin (**1**) (Seigler et al. 2005), menisdaurilide (**2**) (Audran and Mori 1998), *trans*-N-*p*-coumaroyltyramine (**3**) (Kim et al. 2014), *trans*-N-*p*-feruloyltyramine (**4**) (Choi et al. 2016), 4-*O*-feruloylquinicacid (**5**) (Ge et al. 2007), chlorogenic acid (**6**) (Han et al. 2006), 3-*O*-feruloylquinicacid (**7**) (Ge et al. 2007), ferulic acid (**8**) (Han et al. 2006), protopine (**9**) (Seger et al. 2004), Kaempferol 3,7-di-*O*-β-D-glucopyranoside (**10**) (Lee et al. 2009), kaempferol 3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside (**11**) (Lee et al. 2017), α-rhamnoisorobin (**12**) (Cha and Lee 2007), astragalin (**13**) (Seo et al. 2016), and nicotiflorin (**14**) (Tran et al. 2014). Compound **1**, **3-8**, and **10-14** were reported for the first time from this plant.

Effect of isolated compounds on cytotoxicity

First, in order to get knowledge the anti-inflammatory effect in raw 264.7 cells, we investigated cytotoxic levels of compounds **1-14** using an MTS assay. MTS, an indicator of dehydrogenase activity, is commonly used to measure cell viability and proliferation (Dunigan et al. 1995). As a result, compound **9** reduced cell viability in a concentration-dependent manner ranging from 6.25 to 100 μM and was excluded from further testing. Compound **2, 12**, and **14** exhibits toxicity at concentrations over 100 μM in RAW264.7 cells, the other compounds have no cytotoxicity at the concentration (Fig. 2).

Effect of isolated compounds on LPS-induced NO

NO is formed by an arginine deamination reaction. There are three isozymes related to NO production i.e., iNOS, eNOS, and nNOS. Of these isozymes, the iNOS enzyme is closely involved in inflammation and is expressed in monocytes, macrophages and neutrophils (Green et al. 1990; deRojas-Walker et al. 1995). To examine the suppressive effect of compounds on LPS-induced NO production, we measured the NO concentration using a Griess-reagent method and RAW 264.7 cells. As shown in Fig. 3, compounds **3**, **4**, and **12** are effective in reducing NO, but other compounds showed weak or no activities.

Effects of isolated compounds on LPS-induced TNF-α, IL-6, and IL-1β levels

IL-1β, IL-6, and TNF-α are well-known as pro-inflammatory cytokines that are secreted by macrophages as part of the initiation

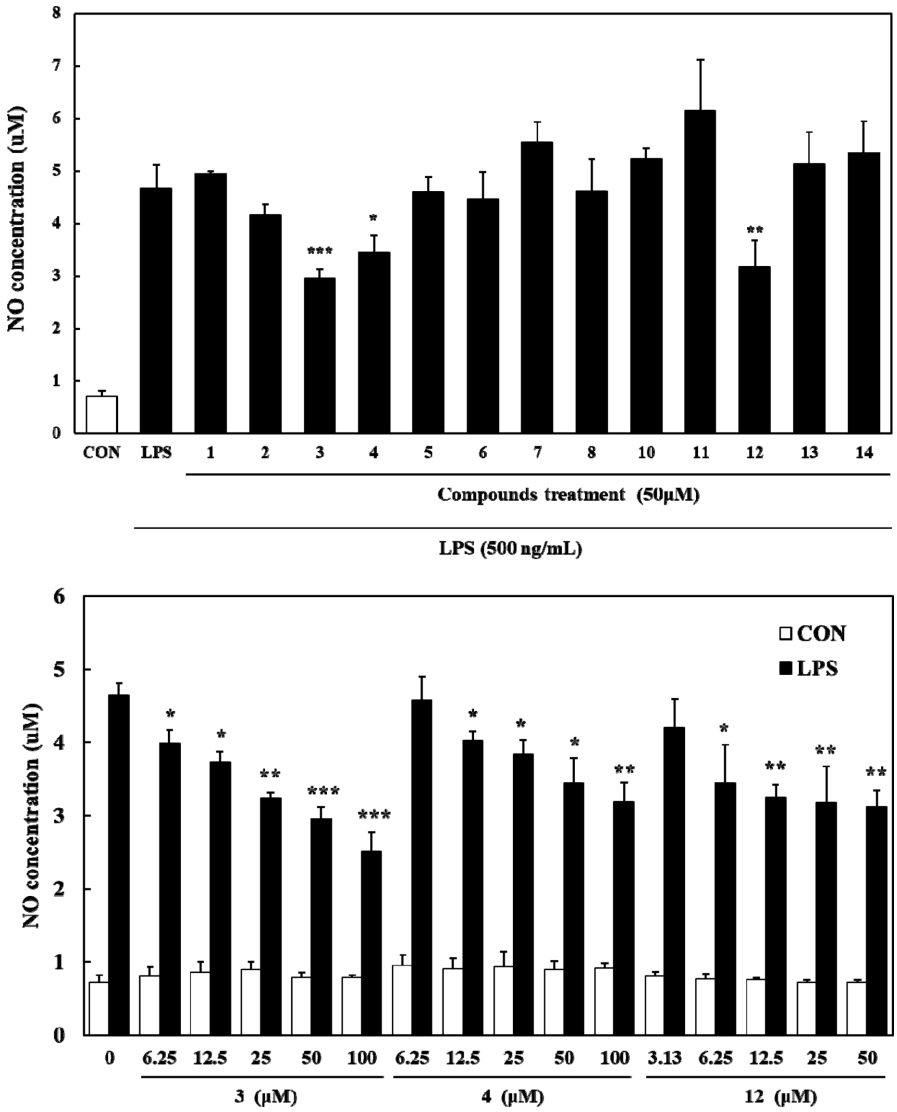

Fig. 3 Inhibitory effects of (A) all compounds (except for 9) at 50 μM concentration, (B) compounds 3, 4 and 12 at various concentration isolated from *D. spectabilis* on LPS-induced NO production in RAW 264.7 cells. After pretreatment with the indicated concentration of compounds for 30 min, RAW 264.7 cells were treated with LPS (500 ng/mL) for 24 hr. Data represent the mean ± SD of triplicate determinations from three separate experiments. Significant differences between treated groups were determined using the Student's t-test. Values shown are the mean ± SD of triplicate determinations from three separate experiments. Statistical significance: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ when compared to the LPS alone treated group

of the inflammatory response (Jang et al. 2016; Zhao et al. 2017). These cytokines play a role in the regulation of cell proliferation, homeostasis, and immune response. But above all, the primary function is to recruit other immune cells to inflammatory sites (Dinarello 1984; Buetler et al. 1985; Bradding et al. 1994). We investigated the effects of compound **3**, **4**, and **12** on the levels of TNF-α, IL-6, and IL-1β secreted levels. IL-1β, IL-6, and TNF-α levels were increased in RAW264.7 cells following addition of LPS. Compound **3** showed a significant inhibitory effect of inflammation via repression of pro-inflammatory cytokines. Compound **4** slightly inhibited cytokines, but compound **12** did not affect cytokine production (Fig. 4).

In the present study, *Dicentra spectabilis*-derived compounds **3**, **4**, and **12** repressed the production of these macrophage mediators in LPS-induced RAW264.7 cells. Compound **3**, in particular, effectively inhibited LPS-induced pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β). Taken together, the results suggest that a Korean indigenous plant *Dicentra spectabilis*-derived compound **3** could be used as anti-inflammatory drugs and substrates.

Acknowledgments This study was conducted as a part of a basic standardization project for herbal medicinal materials of Korean indigenous resources, supported by a grant from the Ministry of Health and Welfare Korea and Jellanamdo, in 2012–2016. We are thankful to the Korea Basic Science Institute (KBSI) for the measurements of NMR and MS spectra.

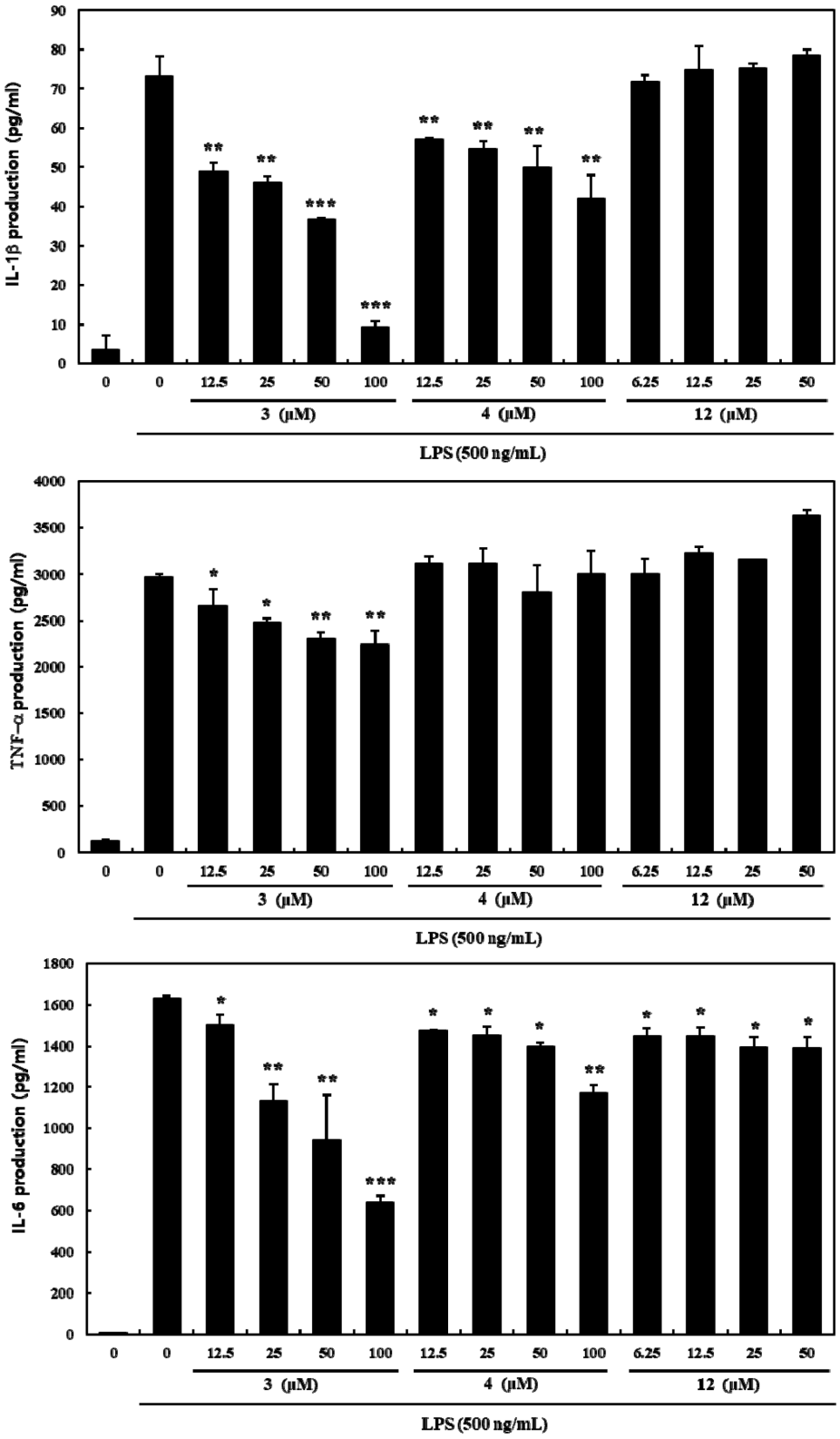

Fig. 4 Effects of compounds **3**, **4**, and **12** isolated from *D. spectabilis* on LPS induced pro-inflammatory cytokines level of (A) TNF-α, (B) IL-6 and (C) IL-1β production in RAW 264.7 cells. Cells were pretreated with compounds for 30 min before being incubated with LPS (500 ng/mL) for 24 hrs. The culture supernatant was collected and analyzed TNF-α, IL-6, and IL-1β using enzyme-linked immunosorbent assay (ELISA). Significant differences between treated groups were determined using the Student's t-test. Values shown are the mean ± SD of triplicate determinations from three separate experiments. Statistical significance: *p <0.05, **p <0.01, ***p <0.001 when compared to the LPS alone treated group

References

Audran G, Mori K (1998) Synthesis of phyllanthurinolactone, the leaf-closing factor of *Phyllanthus urinaria*, and its three stereoisomers. Eur J Org Chem 1998: 57–62

Bradding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Mueller R, Heusser CH, Howarth PH, Holgate ST (1994) Interleukin-4, -5, and -6 and tumor necrosis factor-alpha in normal and asthmatic airways: Evidence for the human mast cell as a source of these cytokines. Am J Respir Cell Mol Biol 10: 471–480

Beutler B, Greenwald D, Hulmes JD, Chang M, Pan YC, Mathison J, Ulevitch R, Cerami A (1985) Identity of tumor necrosis factor and the macrophage-secreted factor cachectin. Nature 316: 552–554

Cha BC, Lee HE (2007) Antioxidant activities of flavonoids from the leaves of *Smilax china* Linne. Kor J Pharmacogn 38: 31–36

Choi HS, Cho JY, Jin MR, Lee YG, Kim SJ, Ham KS, Moon JH (2016) Phenolics, acyl galactopyranosyl glycerol, and lignan amides from *Tetragonia tetragonioides* (Pall.) Kuntze. Food Sci Biotechnol 25: 1275–1281

deRojas-Walker T, Tamir S, Ji H, Wishnok JS, Tannenbaum SR (1995) NO induces oxidative damage in addition to deamination in macrophage DNA. Chem Res Toxicol. 8: 473–477

Dinarello CA (1984) Interleukin-1 and the pathogenesis of acute phase response. N Engl J Med. 311: 1413–1418

Dunigan DD, Waters SB, Own TC (1995) Owen Aqueous Soluble Tetrazolium/Formazan MTS as an Indicator of NADH- and NADPH-Dependent Dehydrogenase Activity. Bio Techniques 19: 640–649

Ge F, Ke C, Tang W, Yang X, Tang C, Qin G, Xu R, Li T, Chen X, Zuo J, Ye Y (2007) Isolation of chlorogenic acids and their derivatives from *Stemona japonica* by preparative HPLC and evaluation of their anti-AIV (H5N1) activity in vitro. Phytochem Anal 18: 213–218

Han T, Li H, Zhang Q, Zheng H, Qin L (2006) New thiazinediones and other components from *Xanthium strumarium*. Chem Nat Comd 42: 567–570

Jang JH, Lee KH, Jung HK, Sim MO, Kim TM, Woo KW, An BK, Cho JH, Cho HW (2016) Anti-inflammatory effects of 6-O-acetyl mangiferin from *Iris rossii* Baker via NF-kb signal blocking in lipopolysaccharide-stimulated RAW 264.7 cells. Chem Biol Interact 257: 54–60

Kim CS, Kim KH, Lee KR (2014) Phytochemical constituents of the leaves of *Hosta longipes*. Nat Prod Sci 20: 86–90

Kim JA, Jung BO, Chung SJ (2017) Antioxidant activity of water-soluble chitosan with *Dicentra spectabilis*. J Chitin Chitosan 22: 28–32

Lee HE, Kim JA, Whang WK (2017) Chemical constituents of *Smilax china* L. stems and their inhibitory activities against glycation, aldose reductase, α-glucosidase, and lipase. Molecules 22: 451/1-451/18

Lee IK, Kim KH, Choi SU, Lee JH, Lee KR (2009) Phytochemical constituents of *Thesuim chinensis* TURCZ and their cytotoxic activities in vitro. Nat Prod Sci 15: 246–249

Lee KS, Sim OK, Shin JS, Choi YE, Kim EY (2004) Mass propagation of *Dicentra spectabilis* L. Lemaire through in vitro suspension culture. Korean J Plant Biotechnol 31: 121–126

Ma WG, Fukushi Y, Tahara S, Osawa T (2000) Fungitoxic alkaloids from Hokkaido Papaveraceae. Fitoterapia 71: 527–534

McNulty J, Poloczek J, Larichev V, Werstiuk NH, Griffin C, Pandey S (2007) Discovery of the apoptosis-inducing activity and high accumulation of the butenolides, menisdaurilide and aquilegiolide, in *Dicentra spectabilis*. Planta Med 73:1543–1547

Medzhitov R, Janeway CA Jr (1997) Innate immunity: impact on the adaptive immune response. Curr Opin Immunol 9: 4–9

Ritchlin CT, Haas-Smith SA, Li P, Hicks DG, Schwarz EM (2003) Mechanisms of TNF-alpha- and RANKL-mediated osteoclastogenesis and bone resorption in psoriatic arthritis. J Clin Invest 111: 821–831

Seger C, Strum S, Strasser EM, Ellmerer E, Stuppner H (2004) ^{1}H and ^{13}C NMR signal assignment of benzylisoquiniline alkaloids from *Fumaria officinalis* L. (Papaveraceae). Magn Reson Chem 42: 882–886

Seigler DS, Pauli GF, Frohlich R, Wegelius E, Nahrstedt A, Glander KE, Ebinger JE (2005) Cyanogenic glycosides and menisdaurin from *Guazuma ulmifolia*, *Ostrya virginiana*, *Tiquilia plicata*, and *Tiquilia canescens*. Phytochemistry 66: 1567–1580

Seo KH, Jung JW, Thi NN, Lee YH, Back NI (2016) Flavonoid glycosides from flowers of *Pulsatilla koreana* Nakai. Nat Prod Sci 22: 41–45

Serhan CN, Savill J (2005) Resolution of inflammation: the beginning programs the end. Nat Immunol 6: 1191–1197

Sim MO, Lee HJ, Jang JH, Lee HE, Jung HK, Kim TM, No JH, Junh JK, Jung DE, Cho HW (2017) Anti-inflammatory and antioxidant effects of *Spiraea prunifolia* Sieb. Et Zucc. var. *simpliciflora* Nakai in RAW 264.7 cells. Korean J. Plant Res 30:335–342

Sim OK, Lee KS, Kim EY, Eun JS (2005) Formation of gametophytes and development of zygotic embryo in *Dicentra spectabilis*. Korean J Plant Res 18: 302–308

Tran TTT, Nguyen TY, Tran MH, Weon KY, Woo MH, Min BS (2014) Compounds from the aerial parts of *Aceriphyllum rossii* and their cytotoxic activity. Nat Prod Sci 20: 146–151

Woo KW, Lee KH, Jang JH, Kim MS, Cho HW, Cho JH, An B (2016) Anti-inflammatory constituents from the aerial parts of *Iris minutiaurea*. Nat Prod Commun 11: 817–819

Zhao JW, Chen DS, Deng CS, Wang Q, Zhu W, Lin L (2017) Evaluation of anti-inflammatory activity of compounds isolated from the rhizome of *Ophiopogon japonicas*. BMC Complement Altern Med 17: 7