Inhibition of Interleukin-4 and β-Hexosaminidase Release in RBL-2H3 Cells by Compounds Isolated from *Lobelia chinensis*

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Abstract − *Lobelia chinensis* Lour. has commonly been used in traditional Chinese medicine for the treatment of antidote, diuretic, diarrhea, and inflammation. This study aimed to identify the active compounds in an aqueous extract of *L. chinensis* responsible for its anti-atopic effect *in vitro* using RBL-2H3 cells. A chemical investigation of secondary metabolites in an aqueous extract of *L. chinensis* led to the isolation of nine chemical constituents, which included the four marker compounds, and these were evaluated for their inhibitory effects on IL-4 mRNA expression and the release of β-hexosaminidase in propidium iodide-induced RBL-2H3 cells. We found diosmetin and fraxidin inhibited cellular IL-4 mRNA expression, and that diosmetin and 6,8-dimethoxycoumarin inhibited DNP-specific IgE-induced degranulation in these cells. Our study suggests that diosmetin, fraxidin, and 6,8-dimethoxycoumarin are potential candidates for the treatment of atopic diseases.
Keywords − *Lobelia chinensis*, Atopic dermatitis, IL-4, β-hexosaminidase

Introduction

Atopic dermatitis is a chronically relapsing inflammatory skin disease accompanied by dryness, lichenification, papules, and intense pruritis.[1] AD affects all ages from infants to adults and has a worldwide prevalence of 1 to 20%.[2] Although the pathological mechanism is unknown, AD is believed to be caused by interactions between genetic and environmental factors and immunomodulatory disorders.[3] Immunologically, increased levels of Th2 cytokines (IL-4, IL-13) are known to play important roles in the pathogenesis and exacerbation of atopic dermatitis and to induce Th1/Th2 immune imbalance and promote serum IgE production.[4,5] Furthermore, serum IgE can exacerbate inflammation, as it induces the activations of mast cells and basophils and the release and degranulation of inflammatory mediators (histamine, TNF, prostaglandin D2).[6,7] Currently, atopic dermatitis is treated using topical corticosteroids and calcineurin inhibitors.[8] However, although these treatments can prevent inflammation by inhibiting T cell activation, their long-term use can cause topical and systemic side effects.[8,9]

Lobelia chinensis Lour. is a perennial herb that belongs to the Campanulaceae family and it is distributed widely in East Asia such as China, Korea, and Japan. The plant is used for the treatment of antidote, diuretic, edema, diarrhea, and jaundice in traditional Chinese medicine.[10] Previous phytochemical studies have reported the isolations of various flavonoids, coumarins, alkaloids, and terpenoids from *L. chinensis*[11-13], and natural flavonoids and coumarins are known to have anti-inflammatory and anti-atopic activities.[14,15] Furthermore, *in vivo* and *in vitro* studies on *L. chinensis* extracts have described anti-inflammatory activity that inhibits inflammatory cytokines.[16] Nevertheless, the bioactive compounds responsible for the activities of *L. chinensis* extracts have not been identified, and the properties, compositions, and contents of active compounds may depend on the extraction methods used. In this study, we isolated nine compounds from an aqueous extract of *L. chinensis*, elucidated their structures, and investigated their anti-atopic effects on IgE-stimulated RBL-2H3 cells.

Experimental

General experimental procedures – The NMR spectra (^{1}H and ^{13}C) were obtained using Bruker 400 MHz (Agilent Technologies, Santa Clara, CA, USA) and Bruker 500 MHz (Bruker, Billerica, MA, USA) instruments. δ values (relative to TMS) and J values are given in Hz. HRESIMS (High-Resolution ESIMS) was performed using an Agilent 6530 Accurate-Mass Q-TOF LC/MS unit (Agilent Technologies, Santa Clara, CA, USA). Preparative HPLC (High-Performance Liquid Chromatography) was conducted using the Shimadzu system (Shimadzu Corporation, Kyoto, Japan), a UV/VIS detector (SPD-20A), two pumps (LC-20AT), and a system controller (CBM-20A). MPLC (Middle-Pressure Liquid Chromatography) separations were conducted using the CombiFlash RETRIEVE system (Teledyne ISCO, Lincoln, NE, USA) and a RediSep® Rf C18 column. Silica gel (230–400 mesh, Merck, Germany), Sephadex LH-20 (25–100 mM mesh, Pharmacia, Sweden) were used for column chromatography, and TLC (thin-layer chromatography) was performed on Merck precoated silica gel 60 F254 plates (1.05554.0001, Merck, Germany).

Plant materials – The whole parts of *L. chinensis* were collected from medicinal plants institute in Kolmar BNH (Jecheon, Chungbuk province, South Korea), during November 2020, and identified by Hyuk Joon Kwon (Ph. D. in Agriculture, the Institute of Medicinal Plants at Kolmar BNH Co. Ltd.). The voucher specimens (PNU-0036) were deposited in the Medicinal Herb Garden, Pusan National University.

Extraction and isolation – Air-dried, whole *L. chinensis* L. (6 kg) plants were ground and reflux extracted six times in water (6 L) for 180 minutes at 90 ± 5 °C. The *L. chinensis* extract (2.43 kg) obtained was suspended in water (H$_2$O, 3 L) and then partitioned sequentially with the same volume (3 L) of *n*-hexane (457.3 g), chloroform (34.2 g), ethyl acetate (181.6 g), and *n*-butanol (105.3 g), and aqueous (10.3 g). The remaining aqueous fraction (10.3 g) was subjected to MPLC flash chromatography and eluted with H$_2$O:MeOH (9:1 → 100% MeOH, v/v) to give 8 fractions (LCW1 to LCW8). Compound **1** (luteolin-7-O-β-D-glucouronopyranosyl (1→2)-O-β-D-glucuronopyranoside, 10.5 mg) was isolated from subfraction LCW1 (83.4 mg) by preparative HPLC using 0.1% formic acid in ACN: 0.1% formic acid in H$_2$O (17:83, v/v) as eluent. Compound **2** (clerodendrin, 12.4 mg) was isolated from subfraction LCW2 (266.4 mg) by prep HPLC using the same eluent. Compound **3** (chrysoeriol-7-O-diglucuronide, 5.2 mg) was isolated from subfraction LCW3 (64.7 mg) by prep HPLC

also using the same eluent. The chloroform fraction (34.2 g) was subjected to open silica gel column chromatography and eluted with CH$_2$Cl$_2$:MeOH (30:1 → 100% MeOH, v/v) to give 15 subfractions (LCC1 to LCC15). Subfraction LCC4 (67.6 mg) was recrystallized from MeOH to afford compound **5** (tomentin, 35.4 mg) and further purified by preparative HPLC (0.1 % formic acid in ACN:0.1% formic acid in H$_2$O = 30:70 → 70:30, v/v) to afford compound **7** (citropten, 15.1 mg). Subfraction LCC6 (1.2 g) was subjected to silica gel column chromatography using a *n*-hexane:EtOAc (2:1 → 1:1 → 1:3 → 100% MeOH, v/v) to give 13 subfractions (LCC6-1 to LCC6-13). Compound **4** (diosmetin, 3.6 mg) and compound **6** (fraxidin, 4.0 mg) were isolated from subfraction LCC6-5 (62.8 mg) by prep HPLC by gradient elution using 0.1% formic acid in ACN:0.1% formic acid in H$_2$O (20:80 → 90:10, v/v). Compound **9** (methyl caffeate, 7.2 mg) was isolated from subfraction LCC12 (229.4 mg) by prep HPLC by gradient elution using 0.1% formic acid in ACN: 0.1% formic acid in H$_2$O (30:70 → 90:10, v/v). The *n*-hexane fraction (20.4 g) was subjected to open silica gel column chromatography and eluted with CH$_2$Cl$_2$:MeOH (20:1 → 100% MeOH, v/v) to give 6 fractions (LCH1 to LCH6). Compound **8** (6,8-dimethoxycoumarin, 2.4 mg) was afforded from LCH1 (12.6 mg) by Sephadex LH-20 (100% MeOH) column chromatography using isocratic elution.

Luteolin-7-O-β-D-glucuronopyranosyl (1→2)-O-β-D-glucuronopyranoside (1) – Pale yellow powder; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 12,97 (1H, s, OH-5), 7.46 (1H, s, H-2′), 7.44 (1H, d, J = 8.29 Hz, H-6′), 6.90 (1H, d, J = 8.31 Hz, H-5′), 6.77 (1H, s, H-3), 6.74 (1H, s, H-8), 6.41 (1H, s, H-6), 5.40 (1H, d, J = 6.60 Hz, H-1″), 4.54 (1H, d, J = 7.88 Hz, H-1‴); ^{13}C-NMR (DMSO-d_6, 150 MHz): δ 181.97 (C-4), 170.38 (C-6‴), 170,14 (C-6″), 164.47 (C-2), 162.29 (C-7), 161.14 (C-5), 156.90 (C-9), 149.88 (C-4′), 145.81 (C-3′), 121,42 (C-1′), 119,12 (C-6′), 115.99 (C-5′), 113.64 (C-2′), 105.47 (C-10), 104.37 (C-1‴), 103.13 (C-3), 99.42 (C-6), 97.67 (C-1″), 94.68 (C-8), 82.43 (C-2″), 75.64 (C-5″), 75.58 (C-5‴), 75.06 (C-3‴), 74.69 (C-3″), 74.18 (C-2‴), 71.67 (C-4‴), 70.85 (C-4″).

Clerodendrin (2) – Pale yellow powder; ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 12.97 (1H, s, 5-OH), 7.96 (2H, d, J = 8.69 Hz, H-2′, 6′), 6.95 (2H, d, J = 8.48 Hz, H-3′,5′), 6.86 (1H, s, H-3), 6.78 (1H, d, J = 2.17 Hz, H-8), 6.43 (1H, d, J = 2.15 Hz, H-6), 5.35 (1H, d, J = 6.89 Hz, H-1″), 4.54 (1H, d, J = 7.88 Hz, H-1‴), 3.98 (1H, d, J = 9.75 Hz, H-5″), 3.64 (1H, d, J = 9.08 Hz, H-5‴); ^{13}C-NMR (DMSO-d_6, 150 MHz): δ 182.09 (C-4), 170.37 (C-6″), 170.15 (C-6‴), 164.28 (C-2), 162.39 (C-7), 161.35 (C-5), 161.18 (C-4′), 156.87 (C-9), 128.64 (C-2′, 6′),

121.09 (C-1′), 116.02 (C-3′, 5′), 105.46 (C-10), 104.43 (C-3), 103.11 (C-1″), 99.31 (C-6), 97.71 (C-1‴), 94.87 (C-8), 82.47 (C-2″), 75.72 (C-5‴), 75.65 (C-3‴), 75.07 (C-3″), 74.67 (C-5″), 74.21 (C-2‴), 71.67 (C-4‴), 70.95 (C-4″)

Chrysoeriol-7-O-diglucuronide (3) – Amorphous yellow powder; ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 7.57(1H, d, $J = 8.37$ Hz, H-2′, 6′), 6.99 (2H, d, $J = 8.03$ Hz, H-3′, 5′), 6.90 (1H, s, H-8), 6.62 (1H, d, $J = 8.03$ Hz, H-3), 6.58 (1H, d, $J = 2.12$ Hz, H-6), 5.40 (1H, d, $J = 7.39$ Hz, H-1″), 4.72 (1H, d, $J = 7.82$ Hz H-1‴), 4.00 (1H, d, $J = 9.00$ Hz, H-5″), 3.89 (s, OCH$_3$-4′), 3.80 (1H, d, $J = 8.9$ Hz, H-5″); ^{13}C-NMR (DMSO-d_6, 150 MHz): δ 182.61 (C-4), 171.96 (C-6″), 171.63 (C-6‴), 164.73 (C-2), 163.74 (C-7), 161.21 (C-5), 157.21 (C-4′), 148.55 (C-9), 129.53 (C-2′, 6′), 121.78 (C-′), 116.40 (C-3′, 5′), 105.93 (C-10), 104.09 (C-3), 102.22 (C-1″), 100.37 (C-6), 99.06 (C-1‴), 95.91 (C-8), 82.31 (C-2″), 76.95 (C-3‴), 76.34 (C-3″), 76.02 (C-5″), 74.84 (C-2‴), 73.91 (C-3‴); HRMS *m/z:* 653.13 [M+H]$^+$.

Diosmetin (4) – Pale yellow powder; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 12.92 (1H, s, OH-5), 9.54 (1H, s, OH-3), 7.55 (1H, d, $J = 8.95$ Hz, H-6′), 7.46 (1H, d, $J = 2.71$ Hz, H-2′), 7.08 (1H, d, $J = 8.72$ Hz, H-5′), 6.73 (1H, d, $J = 9.5$ Hz, H-3), 6.44 (1H, d, $J = 6.06$ Hz, H-8), 6.17 (1H, d, $J = 6.00$ Hz, H-6), 3.85 (3H, s, OCH$_3$-4′); ^{13}C-NMR (DMSO-d_6, 150 MHz): δ 181.51 (C-4), 163.31 (C-2), 161.44 (C-9),157.41 (C-5), 151.08 (C-4′), 146.91 (C-3′), 123.09 (C-6′), 121.16 (C-5′), 119.65 ((C-1′), 112.911 (C-2′), 103.45 (C-3), 103.36 (C-10), 99.139 (C-6), 94.06 (C-8), 55.77 (OCH$_3$-4′).

Tomentin (5) – Pale yellow powder; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 10.25 (1H, s, OH-5), 8.03 (1H, d, $J = 9.64$ Hz, H-4), 6.61 (1H, s, H-8), 6.17 (1H, d, $J = 9.5$ Hz, H-3), 3.87 (3H, s, OCH$_3$-6), 3.67 (3H, s, C-7-OCH$_3$); ^{13}C-NMR (DMSO-d_6, 101 MHz): δ 160.50 (C-2), 156.50 (C-7), 151.45 (C-9), 147.07 (C-5), 139.51 (C-4), 132.42 (C-6), 110.41 (C-3), 103.14 (C-10), 91.53 (C-8), 60.50 (OCH$_3$-6), 56.26 (OCH$_3$-7).

Fraxidin (6) – Pale Yellow powder; ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 8.84 (1H, s, HO-7), 8.00 (1H, d, $J = 9.37$ Hz, H-4), 6.66 (1H, s, H-5), 6.14 (1H, d, $J = 9.55$ Hz, H-3), 3.92(3H, s, OCH$_3$-7), 3.89 (3H, s, OCH$_3$-6); ^{13}C-NMR (DMSO-d_6, 150 MHz): δ 160.22 (C-2), 151.82 (C-6), 149.80 (C-4), 143.46 (C-7), 139.14 (C-9), 127.13 (C-8), 110.502 (C-3), 102.97 (C-10), 92.58 (C-5), 56.43 (OCH$_3$-7), 56.38 (OCH$_3$-6).

Citropten (7) – Amorphous orange powder; ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 7.95 (1H, d, $J = 9.15$ Hz, H-4), 7.25 (1H, d, $J = 2.64$ Hz, H-6), 7.06 (1H, d, $J = 2.65$ Hz,

H-8), 6.29 (1H, d, $J = 9.5$ Hz, H-3), 3.86 (3H, s, OCH$_3$-5), 3.80 (3H, s, OCH$_3$-7); ^{13}C-NMR (DMSO-d_6, 150 MHz): δ 160.72 (C-7), 152.64 (C-2), 149.50 (C-9), 145.97 (C-5), 144.48 (C-4), 112.72 (C-3), 111.30 (C-10), 109.03 (C-6), 100.10 (C-8), 56.19 (C-5), 55.91 (C-7).

6,8-Dimethoxycoumarin (8) – Amorphous white powder; ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 8.00 (1H, d, $J = 9.68$ Hz, H-4), 6.60 (1H, s, H-7), 6.52 (1H, s, H-5), 6.18 (1H, d, $J = 9.64$ Hz, H-3), 3.90 (3H, s, OCH$_3$-8), 3.85 (3H, s, OCH$_3$-6); ^{13}C-NMR (DMSO-d_6, 150 MHz): δ 164.08 (C-8), 160.85 (C-2), 157.23 (C-6), 156.70 (C-9), 139.33 (C-4), 111.13 (C-3), 103.57 (C-10), 95.48 (C-7), 93.59 (C-5), 56.50 (OCH$_3$-8), 56.80 (OCH$_3$-6).

Methyl caffeate (9) – Amorphous white powder; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.46 (1H, d, $J = 15.86$ Hz, H-3), 7.26 (1H, s, H-5), 7.07 (1H, d, $J = 7.90$ Hz, H-8), 6.78 (1H, d, $J = 8.12$ Hz, H-9), 6.36 (1H, d, $J = 15.87$ Hz, H-2), 3.81 (3H, s, OCH$_3$-1); ^{13}C-NMR (DMSO-d_6, 150 MHz): δ 168.13 (C-1), 149.02 (C-7), 147.90 (C-6), 146.82 (C-3), 144.18, 125.84 (C-4), 122.75 (C-9), 116.05 (C-8), 115.50 (C-2), 114.85 (C-5), 114.13, 111.08, 55.67 (C-1-OCH$_3$).

Cell culture – The RBL-2H3 (Rat basophilic leukemia) cells were purchased from the Korean Cell Line Bank (Seoul, Korea). RBL-2H3 cells were cultured in DMEM medium supplemented with 10% FBS (HyClone Laboratories Inc., Logan, UT, USA) and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin; Invitrogen, Carlsbad, CA, USA) at 37ºC in a humidified incubator with a 5% CO$_2$.

Determination of IL-4 mRNA expression – RBL-2H3 cells were seeded in 6-well plates, cultured overnight, treated with nine compounds (10 μM), then 1 h later treated with propidium iodide (PI) to induce inflammation. Total RNA was then extracted using RNeasy mini kit (Qiagen, Hilden, Germany), cDNA was synthesized using RevertAid First Strand cDNA Synthesis Kit (Invitrogen), and IL-4 mRNA levels were assessed by RT-PCR. The sequences of the primers used were as follows: IL-4 forward: 5′-ACC TTG CTG TCA CCC TGT TC-3′; IL-4 reverse: 5′-TTG TGA GCG TGG ACTCAT TC-3′; β-actin forward: 5′- TCA TCA CCA TCG GCA ACG-3′, β-actin reverse:5′-TTC CT GAT GTC CAC GTC GC-3′. Band intensities were measured using the image program. Transcript quantities were versus β-actin.

Measurement of β-hexosaminidase release – RBL-2H3 cells were treated with anti-DNP IgE (anti-dinitrophenyl immunoglobulin E) and incubated for 24 h. After washing with Siraganian buffer (Biosolution, Suwon, Korea), cells were incubated in the buffer for 15 min, treated with

nine compounds (10 μM) for 10 min, and then with DNP-BSA antigen (10 μg/mL) for 20 min to stimulate degranulation. After stimulation, supernatants were transferred to a 96-well plate and incubated with substrate (1 mM 4-nitrophenyl-N-acetyl-β-D-glucosaminide in 0.1 M citrate buffer) at 37ºC for 3 h. Absorbances were measured using a microplate reader (Infinite M1000 Pro, Tecan, Männedorf, Switzerland) at 405 nm.

Result and Discussion

Mast cells are the primary immune response cells in allergic diseases.[17] When mast cells are activated, cytokines such as IL-4 and IL-13, which increase Th2 cell response, are synthesized and secreted. These mediators are involved in both acute and chronic inflammatory reactions.[18-21] In this study, RT-PCR was used to determine whether the nine compounds isolated from *L. chinensis* inhibited the expressions of allergy-related inflammatory cytokines in RBL-2H3 cells (a rat basophil leukemia and histamine-releasing cell line). RBL-2H3 cells were activated using CsA or PI, and then RNA was isolated, cDNA prepared, and mRNA levels were

assessed by RT-PCR. The mRNA expression of IL-4 was higher in PI-treated cells than in non-treated controls, and CsA pretreatment suppressed PI-induced IL-4 expression increases to 81.80%. Eight of the nine compounds, except luteolin-7-O-β-D-glucuronopyranosyl (1→2)-O-β-D-glucuronopyranoside (**1**), inhibited IL-4 the PI-induced expression of IL-4. In particular, diosmetin (**4**) significantly inhibited IL-4 expression by 37.86% compared to non-treated controls (100%), citropten (**7**) significantly inhibited its expression by 32.70%, and methyl caffeate (**9**) significantly inhibited it by 20.65%. Therefore, eight of the nine compounds isolated from *L. chinensis* inhibited IL-4 expression. In particular, diosmetin (**4**), citropten (**7**), and methyl caffeate (**9**) had the greatest inhibitory effects (Fig. 2).

Mast cells exist in endothelial cells of blood vessels, and histamine is secreted by degranulation reaction or activation of mast cells.[22] Histamine is involved in itching in a variety of skin conditions, including hives and various allergic reactions.[23] In addition, β-hexosaminidase is present in the secretory granules of mast cells or basophils and is secreted out of the cell together with histamine due to antigen stimulation.[24,25] The concen-
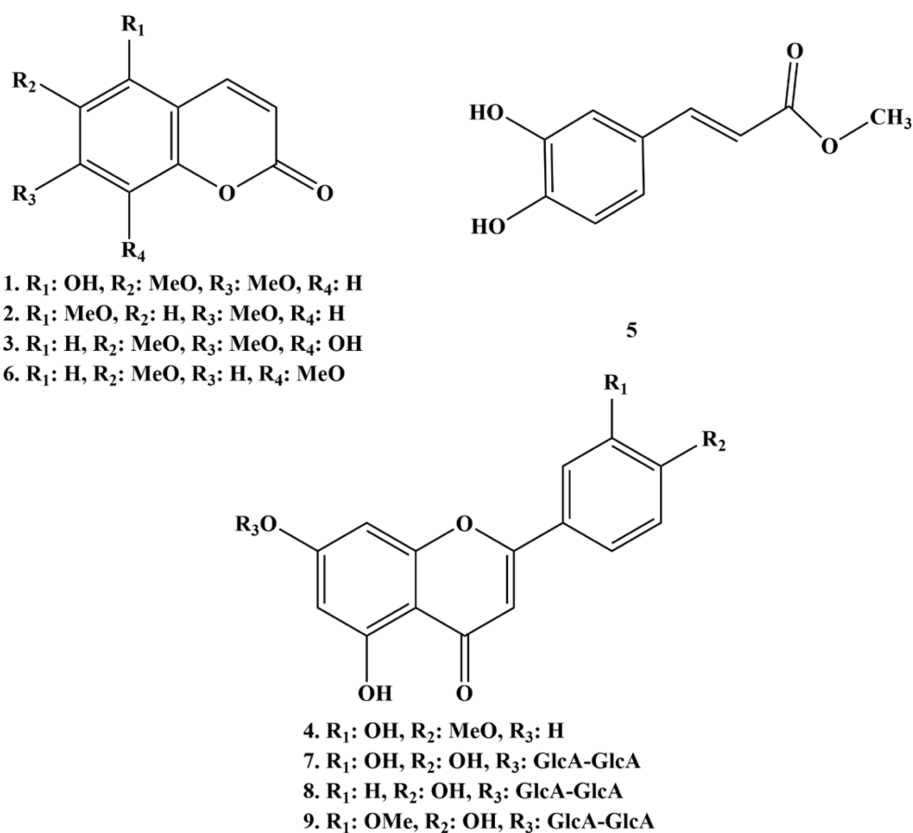

1. R₁: OH, R₂: MeO, R₃: MeO, R₄: H
2. R₁: MeO, R₂: H, R₃: MeO, R₄: H
3. R₁: H, R₂: MeO, R₃: MeO, R₄: OH
6. R₁: H, R₂: MeO, R₃: H, R₄: MeO

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4. R₁: OH, R₂: MeO, R₃: H
7. R₁: OH, R₂: OH, R₃: GlcA-GlcA
8. R₁: H, R₂: OH, R₃: GlcA-GlcA
9. R₁: OMe, R₂: OH, R₃: GlcA-GlcA

Fig. 1. The structures of compounds **1 - 9**.

tration of histamine in the process of degranulation is very low, the degree of degranulation can be evaluated by measuring β-hexosaminidase, which is contained in a large amount in the cell and secreted together with histamine. Therefore, inhibition of histamine and β-hexosaminidase release from mast cells is an indicator of cellular degranulation and a useful indicator for the treatment of allergic diseases.[26,27] In this study, to evaluate the anti-allergic effects of the nine compounds isolated from *L. chinensis*, we investigated the effects of the compounds on mast cell degranulation. RBL-2H3 cells were sensitized using IgE antibody and then treated with DNP-BSA antigen to induce the secretion of β-hexosaminidase by mast cells. After IgE sensitization, DNP-BSA treat-
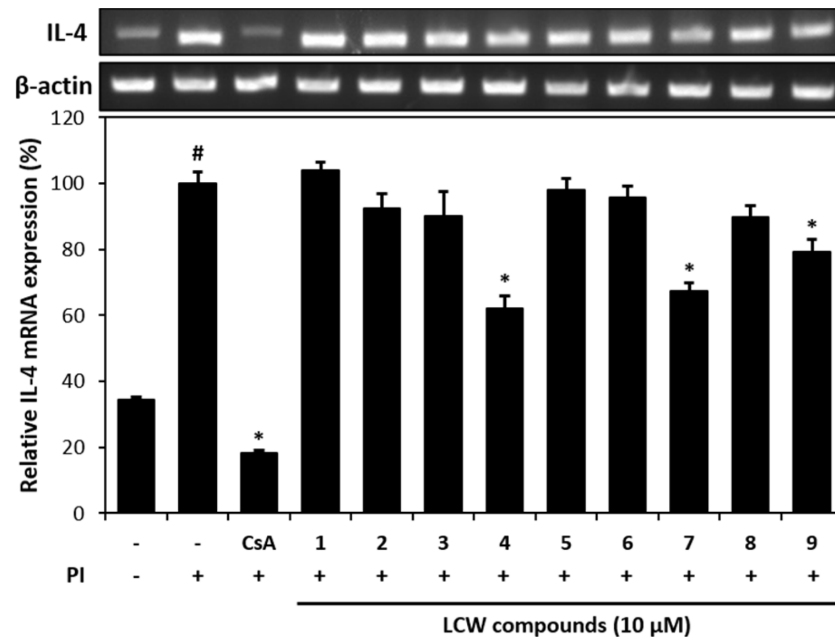

Fig. 2. Inhibitory effect of compounds **1 - 9** on PI-induced IL-4 mRNA expression in RBL-2H3 cells. Results are presented as the means ± SDs of two independent experiments. Error bars represent the standard deviation. # $p < 0.05$ versus. non-treated cells; * $p < 0.05$. versus. PI-treated cells. Abbreviations: PI, propidium iodide; CsA, cyclosporin; IL-4, interleukin-4.
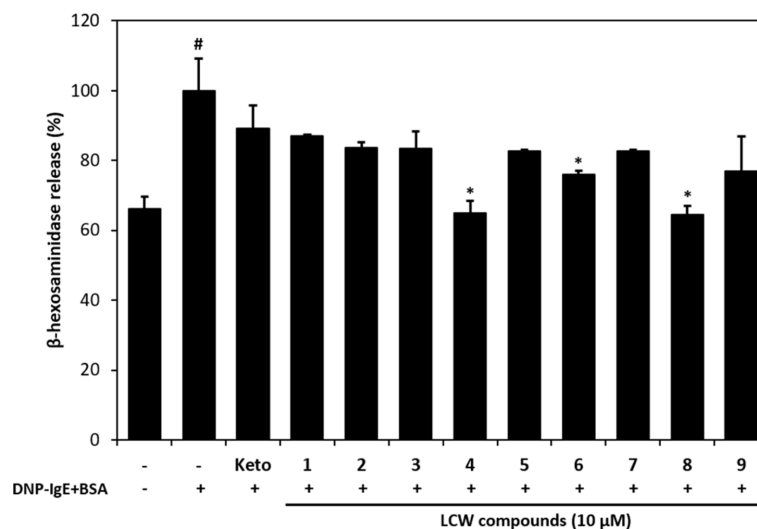

Fig. 3. Inhibitory effect of compounds **1 - 9** against β-hexosaminidase release in DNP-IgE + BSA induced RBL-2H3 cell. Results are presented as the means ± SDs of two independent experiments. Error bars represent the standard deviation. # $p < 0.05$ versus. non-treated cells; * $p < 0.05$. versus. IgE + DNP-BSA treated cells. Abbreviations: IgE, Immunoglobulin E; DNP + BSA, Bovine Serum Albumin; keto; ketotifen.

ment increased β-hexosaminidase secretion 1.5-fold, which meant cell degranulation had increased and indicated an allergic reaction. However, pretreatments with the nine compounds significantly inhibited this increase in β-hexosaminidase release. In particular, in RBL-2H3 cells treated with diosmetin (**4**), fraxidin (**6**), or 6,8-dimethoxycoumarin (**8**), β-hexosaminidase release was reduced to 33.67%, 26.33%, and 12.11% versus DNP-BSA treated controls (100%). Diosmetin (**4**), fraxidin (**6**), and 6,8-dimethoxycoumarin (**8**) were found to most inhibit β-hexosaminidase release (Fig. 3).

In conclusion, we studied the anti-allergic effects of nine compounds isolated from *L. chinensis* in RBL-2H3 cells. Eight of the nine compounds inhibited PI-induced IL-4 expression in RBL-2H3 cells, and all nine inhibited DNP-BSA-induced β-hexosaminidase release. In addition, compounds **4**, **7**, and **9** significantly PI-induced IL-4 expression, and compounds **6** and **8** significantly inhibited DNP-BSA-induced β-hexosaminidase release. In particular, compound **4** significantly inhibited both PI and DNP-BSA-induced IL-4 expression and β-hexosaminidase release, which is an excellent result as regards anti-inflammatory and anti-allergic effects. These results indicate that it would be useful for those developing preventive or therapeutic agents for inflammatory and allergic diseases.

Acknowledgments

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