



## Neolignan Derivatives from the Flower of *Magnolia biondii* Pamp. and their Effects on IL-2 expression in T-cells

Thi Tuyet Mai Nguyen<sup>1</sup>, Thi Thu Nguyen<sup>1</sup>, Hyun-Su Lee<sup>2,3</sup>, Chang-Duk Jun<sup>2</sup>, Byung Sun Min<sup>3</sup>, and Jeong Ah Kim<sup>1,\*</sup>

<sup>1</sup>College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Republic of Korea

<sup>2</sup>School of Life Sciences, Immune Synapse Research Center and Cell Dynamics Research Center, Gwangju Institute of Science and Technology, Gwangju 61005, Republic of Korea

<sup>3</sup>College of Pharmacy, Drug Research and Development Center, Catholic University of Daegu, Gyeongbuk 38430, Republic of Korea

**Abstract** – The isolation of the MeOH extract from the flower bud of *Magnolia biondii* Pamp. using various column chromatographies and HPLC led to eleven neolignan derivatives (**1 - 11**). Their structures were mainly determined by 1D and 2D NMR spectral data analysis and physiological methods. The isolated compounds (**1 - 11**) were tested for anti-allergic effects using IL-2 inhibitory assay in Jurkat T cells.

**Keywords** – *Magnolia biondii* Pamp.; Magnoliaceae; Neolignan; Anti-allergy; IL-2

### Introduction

Magnoliae Flos is a flower bud of *Magnolia biondii* Pamp., commonly known as Sin-yi in Korea. It belongs to the family of Magnoliaceae and is widely distributed in Japan, China, and Korea. The flower buds of *M. biondii* traditionally were used for symptomatic management of allergic rhinitis, sinusitis, and headache in Asia countries<sup>1</sup>.

Extensive studies have been reported that several secondary metabolites such as lignans, neolignans, sesquiterpends, and terpenoids from this plant have various biological activities<sup>2-4</sup>. In particular, several types of neolignans have been studied to have antiperoxidative, antimicrobial, cytotoxic, and inflammatory activities<sup>5-7</sup>.

Mast cell-derived interleukin-2 (IL-2) is an essential cytokine for the T-cell immunity, including differentiation and effector functions. This cytokine is also implicated in the generation and maintenance of Treg cells which antagonize immune responses<sup>8</sup>. Recent study showed evidence that IL-2 of mast cell contributes to the suppression of chronic allergic dermatitis by a regulatory mechanism. The secretion of IL-2 by mast cell enhances the proportion of Treg cells at the site of inflammation

and decreases the fraction of activated T-cells at the inflammatory site<sup>9</sup>.

In our ongoing to find new anti-allergic agents from natural sources, the flower buds of *M. biondii* have been phytochemically investigated to isolate eleven neolignans (**1 - 11**). All the isolates (**1 - 11**) were evaluated for their anti-allergic activity by inhibition of IL-2 in Jurkat T cells.

### Experimental

**General experimental procedures** – The optical rotations were measured using a JASCO DIP-1000 spectropolarimeter (JASCO, Tokyo, Japan). The NMR spectra were recorded using a Bruker Advance Digital 500 MHz NMR spectrometer using TMS as the internal standard. Silica gel 60 (Merck, 60 - 200  $\mu$ m) and reversed phase (RP)-C<sub>18</sub> silica gel (Merck, 40 - 63  $\mu$ m) were used for column chromatography (CC). TLC was performed using Merck precoated silica gel F<sub>254</sub> plates and RP-18 F<sub>254s</sub> plates. HPLC was performed with a HPLC Waters system (Waters, Middleton, USA): 1525 Binary pump; Water 2998 photodiode array detector; YMC Pak ODS column (20  $\times$  250 mm, 5  $\mu$ m);  $t_R$  in min. HPLC solvents were purchased from Burdick & Jackson (USA).

**Plant materials** – The flower buds of *M. biondii* were purchased from Kyungdong traditional market in Seoul (February 2015) and identified by Professor Byung Sun Min at Catholic university of Daegu. It has been deposited

\*Author for correspondence

Jeong Ah Kim, College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Republic of Korea  
Tel: +82-53-950-8574; E-mail: jkim6923@knu.ac.kr

at pharmacognosy laboratory (voucher no. 17AMG40) in the college of pharmacy, Kyungpook national university, Korea.

**Extraction and isolation** – The dried flower buds of *M. biondii* (9.0 kg) were extracted three times refluxing with MeOH (3 h × 4 L) at 60 °C. The filtered MeOH-soluble part was concentrated *in vacuo* to obtain brown residue (1.2 kg) which was suspended in water and partitioned with organic solvents, then afford extracts of chloroform (591.7 g), ethylacetate (EtOAc, 28.2 g), and aqueous extract (580.1 g), successfully. Chloroform-soluble extract was chromatographed on a silica gel vacuum liquid chromatography (VLC) and eluting with a gradient mixture of methylene chloride (MC):hexane (3:7 → 1:0) and EtOAc:MC (1:99 → 1:19) to afford nine fractions (MB-1 to MB-9). Fraction MB4 (78.8 g) was chromatographed on a silica gel VLC and eluting with gradient mixture of 5% to 20% acetone in hexanes to yield six fractions (MB4-1 to MB4-6). Fraction MB4-4 (15.4 g) was fractionated on a silica gel column with a stepwise gradient of 55% to 60% acetone in hexanes to afford six fractions (MB44-1 to MB44-6). Fraction MB44-3 (751.1 mg) was purified using preparative HPLC (6 mL/min, 60 min) with gradient mixture of 60% to 90% MeOH in water to afford compounds **10** (2.0 mg,  $t_R = 38.4$  min) and **8** (3.8 mg,  $t_R = 45.1$  min). Fraction MB4-5 (36.0 g) was chromatographed on silica gel column with gradient of 15% to 55% EtOAc in hexanes to afford seven fractions (MB45-1 to MB45-7). Fraction MB45-6 (2.3 g) was subjected to reversed phase gel column chromatography and eluting with gradient mixture of 45% to 55% acetone in water to afford six fractions (MB456-1 to MB456-6). Fraction MB456-5 (20.6 mg) was purified using preparative HPLC (6 mL/min, 60 min) with gradient elution of 60% to 90% MeOH in water to afford compounds **5** (1.0 mg,  $t_R = 33.5$  min), **6** (7.8 mg,  $t_R = 35.1$  min), and **7** (2.1 mg,  $t_R = 36.8$  min). Fraction MB5 (42.0 g) was chromatographed over silica gel column with a gradient mixture of 5% to 20% acetone in hexanes by using VLC to afford seven fractions (MB5-1 to MB5-7). Fraction MB5-4 (3.2 g) was further subjected to reverse phase gel column chromatography with gradient elution of 40% to 50% MeOH in water to afford seven fractions (MB54-1 to MB54-7). Fraction MB54-3 (1.3 g) was separated by RP-C18 with gradient elution of 50% to 70% MeOH in water to afford nine fractions (MB543-1 to MB543-9). Fraction MB543-4 (10.0 mg) was purified by Waters HPLC (6 ml/min, 60 min) with gradient of 85% to 100% MeOH in water to obtain compound **3** (3.3 mg,  $t_R = 21.3$  min). Fraction MB543-5 (30.0 mg) was purified by using preparative

Waters HPLC (6 ml/min, 60 min) with an isocratic elution of 58% acetonitrile(ACN) in water to afford compound **4** (6.9 mg,  $t_R = 49.8$  min). Compounds **1** (5.7 mg;  $t_R = 35.5$  min) and **2** (2.7 mg,  $t_R = 43.5$  min) were isolated from fraction MB54-7 (100.1 mg) by using Waters HPLC (6 ml/min, 80 min) with gradient mixture of 65% to 70% ACN in water. Fraction MB9 (176.5 g) was fractionated on a silica gel VLC and eluting with gradient mixture of 10% to 40% acetone in hexanes to gain six fractions (MB9-1 to MB9-6). Fraction MB9-5 (60.3 g) was chromatographed on silica gel column and eluting with gradient mixture of 20% to 90% acetone in hexanes to yield nine fractions (MB95-1 to MB95-9). Fraction MB95-5 (8.3 g) was separated on silica gel column using a gradient elution of 1% to 5% MeOH in MC to afford nine fractions (MB955-1 to MB955-9). Fraction MB955-1 (178.6 mg) was isolated by using preparative HPLC (6 mL/min, 60 min) with gradient mixture of 40% to 70% MeOH in water to afford compound **11** (3.5 mg,  $t_R = 49.3$  min). Fraction MB955-6 (2.4 g) was subjected to reversed phase column chromatography and eluting with a stepwise gradient of 40% to 50% MeOH in water to afford compound **9** (613.4 mg).

**Fargesone A (1)** – Colorless oil;  $[\alpha]_D^{20} -106.2$  (*c* 0.1, MeOH);  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 3.

**Fargesone B (2)** – White powder;  $[\alpha]_D^{20} -19.5$  (*c* 0.1, MeOH);  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 3.

**Kadsurin A (3)** – Colorless oil;  $[\alpha]_D^{20} -67.3$  (*c* 0.1, MeOH);  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 3.

**Denudatin B (4)** – White wax;  $[\alpha]_D^{20} +55.2$  (*c* 0.1, MeOH);  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 3.

**Burchellin (5)** – Viscous oil;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 3.

**cis-Burchellin (6)** – Viscous oil;  $[\alpha]_D^{20} -39.0$  (*c* 0.1, MeOH);  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 3.

**1'-epi-Burchellin (7)** – Viscous oil;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 3.

**Acuminatin (8)** – Viscous oil;  $[\alpha]_D^{20} +10.6$  (*c* 0.1, MeOH);  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 2 and 3.

**3',4'-O-Dimethylcedrusin (9)** – Red brown wax;  $[\alpha]_D^{20} +1.9$  (*c* 0.1, MeOH);  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 2 and 3.

**Denudadione A (10)** – Viscous oil;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 2 and 3.

**Magliffonenone (11)** – White powder;  $[\alpha]_D^{20} -70.5$  (*c* 0.1, MeOH);  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 2 and 3.

**Cell culture** – Jurkat T cells (ATCC TIB-152, Manassas, VA) were grown in RPMI medium (Gibco-BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS), penicillin G (100 units/ml), streptomycin (100 µg/

**Table 1.** <sup>1</sup>H NMR spectroscopic data for compounds **1** – **7** ( $\delta$  values)<sup>a</sup>

Position	<b>1</b> <sup>b</sup>	<b>2</b> <sup>b</sup>	<b>3</b> <sup>c</sup>	<b>4</b> <sup>c</sup>	<b>5</b> <sup>c</sup>	<b>6</b> <sup>c</sup>	<b>7</b> <sup>c</sup>
2	6.81, s		6.82, d (1.5)			6.78, m	6.92, m
5	6.77, brs	6.70, m	6.78, d (8.0)	6.96, m	6.85, m	6.84, d (8.4)	6.87, d (7.9)
6			6.75, dd (8.0, 1.5)			6.78, m	6.92, m
7	4.70, d (10.3)	4.24, d (3.6)	4.09, d (10.9)	5.41, d (10.1)	5.42, d (10.0)	6.14, d (5.1)	4.68, dd (17.0, 2.0)
8	2.22, dd (10.3, 6.7)	2.69, m	2.73, m	2.30, dq (9.5, 6.8)	2.30, m	2.83, dd (7.3, 5.1)	2.82, q (7.2)
9	1.07, d (6.7)	1.06, d (7.0)	0.97, d (7.0)	1.15, d (6.8)	1.17, d (6.9)	0.50, d (7.3)	1.21, d (7.2)
1'	2.74, m	2.09, m					
2'	4.43, d (8.8)	4.25, s		6.42, s	5.80, s	5.84, s	5.81, s
5'		5.56, s		5.71, s	5.67, s	5.77, s	5.76, s
7'	2.57, m, 2.74, m	2.37, m, 2.86, m	3.12, ddd (6.7, 2.5, 1.2)	3.11, dd (6.8, 1.2), 3.16, dd (6.8, 1.2)	2.46, dd (13.6, 7.3), 2.68, dd (13.6, 7.3)	2.68, dd (13.5, 7.0), 2.77, dd (13.5, 7.8)	2.23, dd (13.4, 6.8), 2.35, dd (13.4, 8.0)
8'	5.90, m	5.92, m	6.54, d (1.2)	5.92, ddt (16.9, 10.1, 6.9)	5.53, ddt (17.3, 10.1, 7.3)	5.72, m	5.54, dddd (17.0, 10.2, 7.9, 6.8)
9'	5.10, dd (9.9, 1.5), 5.17, dd (17.3, 1.5)	5.05, m, 5.15, dd (17.1, 1.5)	5.14, m	5.13, m	5.04, ddd (18.8, 13.6, 1.9)	5.14, t (12.2)	5.00, dd (10.2, 2.0), 5.47, d (0.96)
-OCH <sub>2</sub> O-	5.95, s	5.92, m	5.95, s		5.97, s	5.96, s	5.99, s
3-OCH <sub>3</sub>				3.82, s			
4-OCH <sub>3</sub>							
3'-OCH <sub>3</sub>	3.21, s	3.32, s	3.19, s	3.14, s	3.69, s	3.67, s	3.67, s
4'-OCH <sub>3</sub>	3.76, s	3.73, s	3.41, s				

<sup>a</sup>  $\delta$  value recorded in ppm (*J* in Hz).<sup>b</sup> <sup>1</sup>H NMR measured at 500 MHz in CDCl<sub>3</sub>.<sup>c</sup> <sup>1</sup>H NMR measured at 500 MHz in CD<sub>3</sub>OD.**Table 2.** <sup>1</sup>H NMR spectroscopic data for compounds **8** – **11** ( $\delta$  values)<sup>a</sup>

Position	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>
2	6.98, d (2.0)	6.95, s	6.58, d (2.0)	6.39, s
5	6.84, d (8.2)	6.82, d (8.7)	6.79, d (8.2)	
6	6.96, dd (8.2, 2.0)	6.95, s	6.66, dd (8.2, 2.1)	6.39, s
7	5.12, d (9.5)	5.55, d (7.4)		2.57, d (6.1)
8	3.46, m	3.60, dd (12.2, 5.8)	2.46, m	2.07, m
9	1.38, d (6.8)	3.90, d (4.6), 3.96, dd (10.9, 5.8)	1.07, d (6.1)	0.62, d (6.5)
2'	6.79, s	6.67, d (2.2)		
3'			3.52, m	5.80, s
6'	6.77, s	6.67, d (2.2)	7.04, s	5.49, s
7'	6.36, dd (15.7, 1.6)	2.65, d (7.9)	3.12, dd (16.4, 8.6)	2.22, d (11.3), 2.39, ddd (11.3, 6.4, 1.4)
8'	6.11, dq (15.7, 6.6)	1.88, m	5.86, ddt (17.6, 10.9, 6.8)	5.05, m
9'	1.87, dd (6.6, 1.6)	3.68, d (6.3)	5.17, d (6.3), 5.20, s	1.76, dd (12.0, 11.9), 2.32, m
3-OCH <sub>3</sub>		3.86, s		3.85, s
4-OCH <sub>3</sub>	3.89, s	3.85, s	3.86, s	3.82, s
5-OCH <sub>3</sub>				3.85, s
3'-OCH <sub>3</sub>	3.89, s	3.88, s		
5'-OCH <sub>3</sub>			3.64, s	3.68, s

<sup>a</sup>  $\delta$  value recorded in ppm (*J* in Hz) and <sup>1</sup>H NMR measured at 500 MHz in CDCl<sub>3</sub>.

**Table 3.**  $^{13}\text{C}$  NMR spectroscopic data for compounds **1** – **11** ( $\delta$  values)<sup>a</sup>

Position	<b>1</b> <sup>b</sup>	<b>2</b> <sup>b</sup>	<b>3</b> <sup>c</sup>	<b>4</b> <sup>c</sup>	<b>5</b> <sup>c</sup>	<b>6</b> <sup>c</sup>	<b>7</b> <sup>c</sup>	<b>8</b> <sup>b</sup>	<b>9</b> <sup>b</sup>	<b>10</b> <sup>b</sup>	<b>11</b> <sup>b</sup>
1	134.4	134.2	135.5	131.3	133.1	133.2	136.4	132.8	133.9	134.0	139.1
2	106.9	106.7	108.8	111.2	107.8	107.3	106.0	109.7	109.5	110.2	104.8
3	148.0	147.9	149.5	151.3	149.8	149.4	149.7	149.3	149.3	148.6	153.4
4	147.6	147.5	149.1	150.8	149.6	148.8	148.6	146.8	149.1	149.6	137.0
5	108.2	108.1	108.1	112.8	109.1	109.2	109.3	110.0	111.1	111.7	153.4
6	120.5	120.1	122.2	121.2	120.2	120.4	120.3	119.4	118.8	119.5	104.8
7	87.2	86.1	86.8	93.3	92.8	89.3	94.9	93.8	87.9	49.0	45.4
8	51.4	48.5	50.6	51.5	37.1	44.2	46.4	45.8	53.9	45.5	37.9
9	8.9	11.2	9.4	6.9	8.3	12.4	18.8	17.8	64.1	13.8	14.6
1'	52.8	54.1	144.5	143.9	53.2	55.8	55.0	132.4	135.5	140.6	50.4
2'	80.0	81.1	140.2	133.6	111.1	112.3	112.1	109.4	112.6	194.5	180.0
3'	84.7	82.3	83.3	79.3	154.3	153.8	153.7	144.3	144.3	70.0	101.5
4'	172.4	173.4	103.4	177.4	185.7	185.5	185.4	149.3	146.7	202.4	183.1
5'	105.7	103.5	44.2	102.9	102.8	102.6	102.7	133.4	127.9	89.5	153.4
6'	196.4	196.9	196.1	189.2	185.3	185.1	185.3	113.5	116.1	147.3	108.9
7'	31.3	29.2	34.5	34.6	50.9	46.5	46.7	131.1	32.1	32.9	60.9
8'	135.4	136.4	136.4	136.6	132.6	132.2	132.9	123.7	34.7	133.9	81.9
9'	117.9	117.0	117.6	117.5	122.1	120.2	118.4	18.5	62.4	118.3	43.7
-OCH <sub>2</sub> O-	101.2	101.2	102.5	–	101.9	102.2	102.7	–	–	–	–
3-OCH <sub>3</sub>	–	–	–	56.5	–	–	–	56.1	56.2	56.1	56.2
4-OCH <sub>3</sub>	–	–	–	56.5	–	–	–	56.1	56.2	56.1	60.9
5-OCH <sub>3</sub>	–	–	–	–	–	–	–	–	–	–	56.2
3'-OCH <sub>3</sub>	51.5	53.4	47.7	51.6	55.8	56.2	55.8	56.8	56.1	–	–
4'-OCH <sub>3</sub>	56.4	56.1	51.4	–	–	–	–	–	–	–	–
5'-OCH <sub>3</sub>	–	–	–	–	–	–	–	–	–	54.1	55.3

<sup>a</sup>  $\delta$  value recorded in ppm.<sup>b</sup>  $^{13}\text{C}$  NMR measured at 125 MHz in CDCl<sub>3</sub>.<sup>c</sup>  $^{13}\text{C}$  NMR measured at 125 MHz in CD<sub>3</sub>OD.

ml), and L-glutamine (2 mM). The cells were cultured at 37 °C in a humidified incubator containing 5% CO<sub>2</sub> and 95% air.

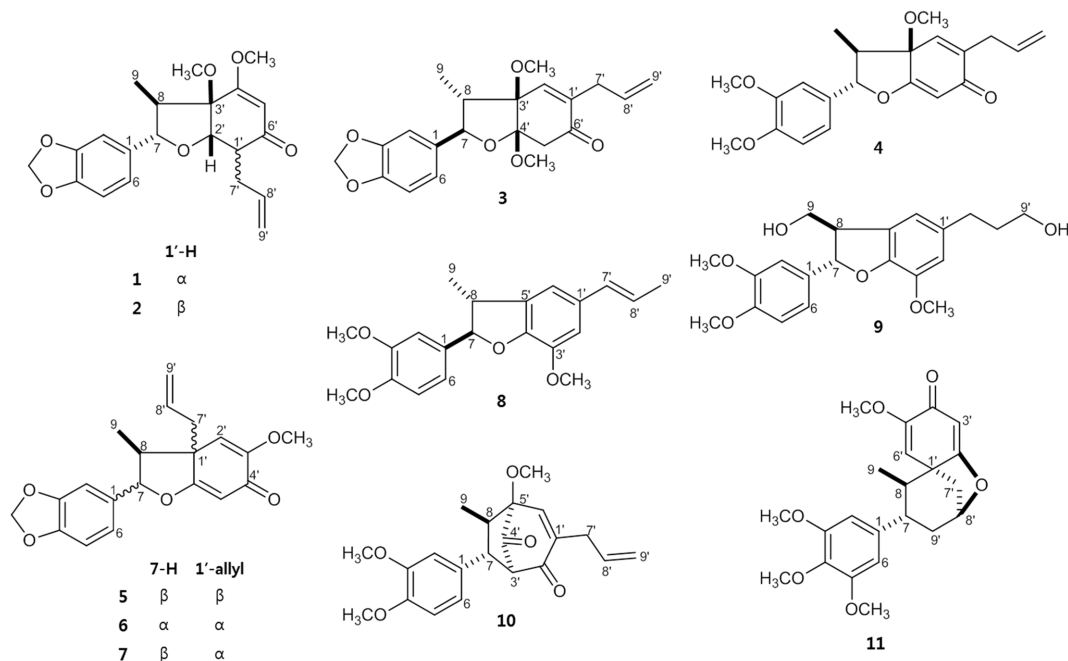
**Reverse transcription PCR and conventional PCR** – Jurkat T cells ( $1 \times 10^6$ ) were incubated with indicated concentrations of compounds for 30 min at 37 °C. Incubated cells were stimulated with PMA (200 nM) and A23187 (1  $\mu\text{M}$ ) for 6 h for PCR. Cells were harvested and total RNAs were isolated with TRIZOL reagent (JBI, Korea). Reverse transcription of the RNA was performed using RT PreMix (enzymomics, Korea). For conventional PCR, the primers and PCR conditions for each gene were used as following: human IL-2, 5'-CAC GTC TTG CAC TTG TCA C-3' and 5'-CCT TCT TGG GCA TGT AAA ACT-3'. Human GAPDH, 5'-CGG AGT CAA CGG ATT TGG TCG TAT-3' and 5'-AGC CTT CTC CAT GGT GGT GAA GAC-3'. The amplification profile was composed of denaturation at 94 °C for 30 s, annealing at 60 °C for 20 s,

and extension at 72 °C for 40 s. The 30 cycles were preceded by denaturation at 72 °C for 7 min. All experiments were performed at least three times unless otherwise indicated.

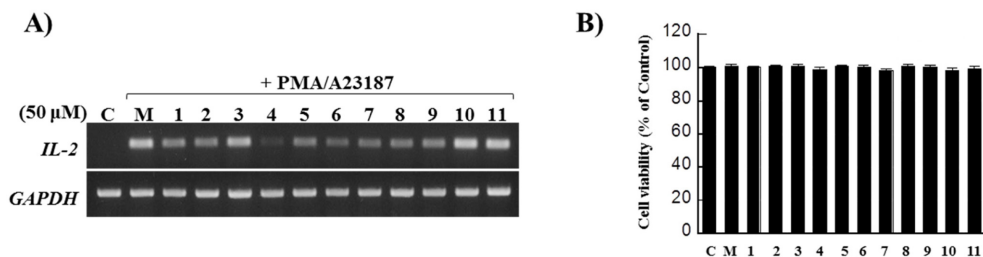
**Cell viability assay** – Jurkat T cells ( $3 \times 10^5$ ) were seeded in a 24 well-plate and incubated with isolates (**1** – **11**) for 24 h. After incubation, cells (180  $\mu\text{l}$ ) were added with MTT solution (20  $\mu\text{l}$ , 5 mg/ml). After 2 h of incubation on 37 °C incubator, cells were centrifuged and supernatants were taken out. 150  $\mu\text{l}$  of DMSO were added and incubated for 15 min on RT. After incubation, absorbance was detected in 590 nm wavelength.

## Result and Discussion

The MeOH extract of dried flower buds of *M. biondii* was partitioned into chloroform, EtOAc-, and water-soluble fractions. Chromatographic purification of chloro-



**Fig. 1.** Chemical structures of neolignans **1-11** isolated from the flower of *Magnolia biondii* Pamp.



**Fig. 2.** Inhibition of IL-2 expression and cell viability by neolignans **1-11** isolated from *M. biondii*.

A) Jurkat T cells ( $1 \times 10^6$ ) were treated with 50  $\mu$ M concentrations of **1-11** for 30 min and stimulated with PMA (100 nM)/A23187 (1  $\mu$ M) for 6 h. After incubation, cells were harvested and total RNA was isolated from harvested cells. Human IL-2 mRNA levels were detected by conventional PCR. B) Jurkat T cells ( $3 \times 10^5$ ) were seeded in a 24 well-plate and incubated with 50  $\mu$ M concentrations of **1-11** for 24 h. After incubation, cell viability was examined by MTT assay. Data was shown as percentages compared to cell viability treated with Mock control.

form-soluble fraction led to the isolation and determination of eleven neolignan derivatives as fargesone A (**1**), fargesone B (**2**)<sup>10</sup>, (+)-kadsurin A (**3**)<sup>11</sup>, denudatin B (**4**)<sup>12</sup>, burchellin (**5**), *cis*-burchellin (**6**)<sup>13,14</sup>, 1'-*epi*-burchellin (**7**)<sup>15</sup>, ( $\pm$ )-acuminatin (**8**)<sup>16</sup>, 3',4'-*O*-dimethylcedrusin (**9**)<sup>17</sup>, denudadione A (**10**)<sup>18</sup>, and maglifloenone (**11**)<sup>19</sup> by comparing their physicochemical and spectroscopic data with those reported in the literature (Fig. 1).

The cytotoxic effects of **1-11** were evaluated using an MTT assay and all isolated compounds were tested to inhibit of IL-2 production in Jurkat T-cells, which were stimulated with stimulated with PMA (200 nM) and A23187 (1  $\mu$ M). Among tested neolignans (**1-11**), denu-

datin B (**4**) showed strong inhibitory effect on production of IL-2 (Fig. 2A). The MTT assay indicated that none of the neolignans showed cytotoxicity in activated Jurkat T-cells at concentrations effective for the inhibition of IL-2 production (Fig. 2B).

Denudatin B is an epimer of 5'-OCH<sub>3</sub> on kadsurenone which has been demonstrated to block platelet-activating factor (PAF) receptor<sup>12</sup>. PAF has been implicated as a mediator of inflammation and anaphylaxis<sup>20</sup>. Furthermore, denudatin B reported to have inhibitory effects on NO production through the decreased expression of the iNOS gene<sup>21</sup> and free radical scavenging activity<sup>22</sup>. Denudatin B from *M. biondii* which were traditionally used for allergy

disease were effective on inhibition of IL-2. The secretion of IL-2 triggers off allergic reaction<sup>9</sup>. The allergy has generally and/or occasionally attended by consequential inflammatory reaction.

Taken together, these findings support denudatin B to be used as a useful natural candidate for inflammation and allergy related activities on the further mechanism. Thus, our study suggests that denudatin B isolated from *M. biondii* may probably be used as an inhibitor of inflammatory and allergic diseases.

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