



*Original Article*

## Anti-allergic and anti-inflammatory compounds from *Aglaia andamanica* leaves

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### Abstract

The leaves from *Aglaia andamanica* were determined for their anti-allergic and anti-inflammatory effects using RBL-2H3 and RAW264.7 cells, respectively. Among the isolated compounds, 24-epi-piscidinol A (5) exhibited the highest anti-allergic activity against  $\beta$ -hexosaminidase release with an  $IC_{50}$  value of 19.8  $\mu$ M, followed by (-)-yangambin (3,  $IC_{50}$  = 33.8  $\mu$ M), pyramidaglaine A (8,  $IC_{50}$  = 37.1  $\mu$ M), pachypodol (2,  $IC_{50}$  = 38.3  $\mu$ M) and pyramidaglaine B (9,  $IC_{50}$  = 44.8  $\mu$ M), respectively; whereas other compounds possessed moderate to mild effects ( $IC_{50}$  = 67.5->100  $\mu$ M). For anti-inflammatory activity, 24-epi-piscidinol A (5) possessed potent activity with an  $IC_{50}$  value of 24.0  $\mu$ M, followed by pyramidaglaine B (9,  $IC_{50}$  = 25.6  $\mu$ M), pachypodol (2,  $IC_{50}$  = 34.5  $\mu$ M) and (-)-yangambin (3,  $IC_{50}$  = 37.4  $\mu$ M), respectively; whereas other compounds exhibited moderate to mild activities ( $IC_{50}$  = 54.2->100  $\mu$ M). These active compounds could be developed as anti-allergic and anti-inflammatory agents in the future.

**Keywords:** RBL-2H3 cells, RAW264.7 cells, *Aglaia andamanica*, Meliaceae

### 1. Introduction

*Aglaia andamanica* is a plant belonging to the Meliaceae family. This plant grows at a height of 30 m, and is distributed in South and Southeast Asia. Its leaves are used for treatment of headache (Mabberley *et al.*, 1995). Some of benzofuran derivatives from *Aglaia* genus show interesting biological effects including anti-viral (Joshi *et al.*, 1987), anti-leukemic (Hayashi *et al.*, 1982) and insecticidal activities (Ishibashi *et al.*, 1993).

The allergy is an immune dysfunction, which is a serious health problem. Substances that cause allergic reaction are called allergens including pollen, dust mites, food, cosmetics and animal hairs. Hypersensitivity type I,

an allergic reaction, is an IgE-mediated immune response, resulting in histamine secretion from mast cells and blood basophils. The histamine causes smooth muscle contraction, increased vascular permeability and vasodilation (Goldsby *et al.*, 2002). Since,  $\beta$ -hexosaminidase is usually released along with histamine from mast cells or basophils, this enzyme is used as the marker for mast cell degranulation in RBL-2H3 cell line (Cheong *et al.*, 1998).

Nitric oxide (NO) is one of the inflammatory mediators that causes inflammation in several organs. This inorganic free radical has been implicated in physiological and pathological processes, such as vasodilation, non-specific host defense and acute or chronic inflammation. NO acts as a host defense by damaging pathogenic DNA and as a regulatory molecule with homeostatic activities (Kou & Schroder, 1995). However, excessive production of this free radical is pathogenic to the host tissue, because NO can bind with superoxide radicals and turn to be a reactive radical which directly

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damages the function of normal cells (Moncada *et al.*, 1991). Since the extract of *Aglaia andamanica* possessed potent anti-allergic and anti-inflammatory effects with  $IC_{50}$  values of 19.3 and 22.1  $\mu$ g/mL, respectively, the compounds isolated from this plant were then tested for anti-allergic and anti-inflammatory activities.

## 2. Materials and Methods

### 2.1 Reagents

Minimum Essential Medium Eagle (MEM) and anti-DNP-IgE (Monoclonal anti-DNP) were purchased from Sigma; fetal calf serum (FCS) was from Gibco; dinitrophenylated bovine serum albumin was prepared as described previously (Tada & Okumura, 1971). Other chemicals were from Sigma. 24-well and 96-well plates were from Nunc.

Lipopolysaccharide (LPS, from *Salmonella enteritidis*), RPMI-1640 medium, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), L-nitroarginine (L-NA), caffeic acid phenethyl ester (CAPE), indomethacin and phosphate buffer saline (PBS) were purchased from Sigma. FCS was from Gibco. Penicillin-streptomycin was from Invitrogen. 96-well microplates were bought from Nunc. Other chemicals were from Sigma.

### 2.2 Plant material

Leaves of *Aglaia andamanica* Hiern were collected in Thon-Nga-Chang National Park, Hat-Yai, Songkhla, Thailand, in September 1995. A voucher specimen (JP9001) was identified by Dr. S. Vajrodaya, Department of Botany, Kasetsart University, Bangkok, Thailand, and has been deposited in the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

### 2.3 Extraction and isolation of *Aglaia andamanica* leaves

The compounds (**1-10**) were isolated and identified by comparison of their physical and spectral data with those reported in the literatures (Puripattanavong *et al.*, 2000; Greca *et al.*, 1994; Vidari *et al.*, 1971; Malan & Roux, 1979; Picker *et al.*, 1973; Agrawal 1989; McChesney *et al.*, 1997; Anjaneyulu *et al.*, 1981; Marrien and Polonsky, 1971; Saifah *et al.*, 1993; Figliuolo *et al.*, 1987).

### 2.4 Anti-allergic activity assay

#### 1) Inhibitory effects on antigen-induced $\beta$ -hexosaminidase release from RBL-2H3 cells

Inhibitory effects on the release of  $\beta$ -hexosaminidase from RBL-2H3 cells (purchased from ATCC) were evaluated by the following modified method (Matsuda *et al.*, 2004). Briefly, RBL-2H3 cells were dispensed in 24-well plates at a

concentration of  $2 \times 10^5$  cells/well using MEM containing 10% FCS, penicillin (100 units/mL), streptomycin (100 unit/mL) and anti-DNP IgE (0.45  $\mu$ g/mL), then incubated overnight at 37°C in 5% CO<sub>2</sub> for sensitization of the cells. The cells were washed twice with 500  $\mu$ L of Siraganian buffer [119 mM NaCl, 5 mM KCl, 5.6 mM glucose, 0.4 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 25 mM piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES), 0.1 % bovine serum albumin (BSA) and 40 mM NaOH, pH 7.2] and then incubated in 160  $\mu$ L of Siraganian buffer for an additional 10 min at 37 °C. After that, 20  $\mu$ L of test sample solution was added to each well and incubated for 10 min, followed by addition of 20  $\mu$ L of antigen (DNP-BSA, final concentration is 10  $\mu$ g/mL) at 37°C for 20 min to stimulate the cells to degranulate. The supernatant was transferred into a 96-well plate and incubated with 50  $\mu$ L of substrate (1mM *p*-nitrophenyl-N-acetyl- $\beta$ -D-glucosaminide) in 0.1 M citrate buffer (pH 4.5) at 37°C for 1 h. The reaction was stopped by adding 200  $\mu$ L of stop solution (0.1 M Na<sub>2</sub>CO<sub>3</sub>/NaHCO<sub>3</sub>, pH 10.0). The absorbance was measured with a microplate reader at 405 nm. The test sample was dissolved in dimethylsulfoxide (DMSO), and the solution was added to Siraganian buffer (final DMSO concentration was 0.1 %). The inhibition (%) of the release of  $\beta$ -hexosaminidase by the test samples was calculated by the following equation, and  $IC_{50}$  values were determined graphically:

$$\text{Inhibition \%} = [1 - (T-B-N)/(C-N)] \times 100$$

Control (C): DNP-BSA (+), Test sample (-); Test (T) : DNP-BSA (+), Test sample (+); Blank (B) : DNP-BSA (-), Test sample (+); Normal (N) : DNP-BSA (-), Test sample (-)

#### 2.5 Assay for NO inhibitory effect from RAW264.7 cells

Inhibitory effect on NO production by murine macrophage-like RAW264.7 cells was evaluated using a modified method from that previously reported (Banskota *et al.*, 2003). Briefly, the RAW264.7 cell line was cultured in RPMI medium supplemented with 0.1% sodium bicarbonate and 2 mM glutamine, penicillin G (100 units/mL), streptomycin (100  $\mu$ g/mL) and 10% FCS. The cells were harvested with trypsin-EDTA and diluted to a suspension in a fresh medium. The cells were seeded in 96-well plates and allowed to adhere for 1 h at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. After that the medium was replaced with a fresh medium containing 100  $\mu$ g/mL of LPS together with the test samples at various concentrations and was then incubated for 48 h. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess reagent. Cytotoxicity was determined using the MTT colorimetric method. Briefly, after 48 h incubation with the test samples, MTT solution was added to the wells. After 4 h incubation, the medium was removed, and isopropanol containing 0.04 M HCl was then added to dissolve the formazan production in the cells. The optical density of the formazan solution was measured with a microplate reader at 570 nm.

L-NA, CAPE and indomethacin were used as positive controls. The stock solution of each test sample was dissolved in DMSO, and the solution was added to the medium RPMI (final DMSO is 1%). Inhibition (%) was calculated using the following equation and  $IC_{50}$  values were determined graphically (n=4):

$$\text{Inhibition (\%)} = \frac{(A - C) - (B - C)}{(A - C)} \times 100$$

A-C :  $\text{NO}_2^-$  concentration ( $\mu\text{M}$ ) [A : LPS (+), sample (-); B : LPS (+), sample (+); C: LPS (-), sample (-)].

## 2.6 Statistical analysis

The results were expressed as mean  $\pm$  S.E.M of four determinations at each concentration for each sample. The  $IC_{50}$  values were calculated using the Microsoft Excel program. Statistical significance was calculated by one-way analysis of variance (ANOVA), followed by Dunnett's test.

## 3. Results and Discussion

The leaves of *Aglaia andamanica* were determined for their anti-allergic and anti-inflammatory effects using RBL-2H3 and RAW264.7 cells, respectively. Among the isolated compounds (Figure 1), 24-epi-piscidinol A (**5**) exhibited the highest anti-allergic activity against  $\beta$ -hexosaminidase release with an  $IC_{50}$  value of 19.8  $\mu\text{M}$ , followed by (-)-yangambin (**3**,  $IC_{50} = 33.8 \mu\text{M}$ ), pyramidaglain A (**8**,  $IC_{50} = 37.1 \mu\text{M}$ ), pachypodol (**2**,  $IC_{50} = 38.3 \mu\text{M}$ ) and pyramidaglain B (**9**,  $IC_{50} = 44.8 \mu\text{M}$ ), respectively; whereas other compounds possessed moderate to mild effects ( $IC_{50} = 67.5\text{--}100 \mu\text{M}$ ) (Table 1). Compounds **5**, **3**, **8**, **2** and **9** exhibited higher anti-allergic effect than that of ketotifen fumarate, an anti-histamine drug ( $IC_{50} = 47.5 \mu\text{M}$ ).

For anti-inflammatory activity, 24-epi-piscidinol A (**5**) possessed potent activity with an  $IC_{50}$  value of 24.0  $\mu\text{M}$ , followed by pyramidaglain B (**9**,  $IC_{50} = 25.6 \mu\text{M}$ ), pachypodol (**2**,  $IC_{50} = 34.5 \mu\text{M}$ ) and (-)-yangambin (**3**,  $IC_{50} = 37.4 \mu\text{M}$ ), respectively; whereas other compounds exhibited moderate to mild effects ( $IC_{50} = 54.2\text{--}100 \mu\text{M}$ ) (Table 2). However, pachypodol (**2**) and (-)-yangambin (**3**) exhibited cytotoxicity (25-30%) at high concentration (100  $\mu\text{M}$ ). Compounds **5**, **9**, **2** and **3** showed higher activity against NO production when compared to that of the positive controls, L-NA (NO synthase inhibitor,  $IC_{50} = 61.8 \mu\text{M}$ ) and indomethacin (non-steroidal anti-inflammatory drug,  $IC_{50} = 46.5 \mu\text{M}$ ) but less effective than CAPE, an NF- $\kappa$ B inhibitor ( $IC_{50} = 5.2 \mu\text{M}$ ).

Methoxyflavones isolated from the rhizomes of *Kaempferia parviflora* have been reported to have anti-allergic effect (Tewtrakul *et al.*, 2008). This result is consistent with our study that methoxyflavone, pachypodol (**2**), showed marked anti-allergic activity. Epimagnolin B isolated from *Magnolia fargesii*, whose structure is similar to (-)-yangambin, has been reported to have anti-inflammatory effect against NO and PGE<sub>2</sub> production (Kim *et al.*, 2009).

*Aglaia odorata* which contains diterpenoids and triterpenoids has shown anti-inflammatory activity toward NO and PGE<sub>2</sub> release (Yodsoue *et al.*, 2012). It has been reported that compound isolated from *Kaempferia parviflora*, 5-hydroxy-3, 7, 3', 4'-tetramethoxyflavone, exhibited marked activity against NO production (Tewtrakul *et al.*, 2008). These results are accompanied with the present study that flavonoids, triterpenes and lignan derivatives possess anti-inflammatory effect.

In conclusion, the present study shows that *Aglaia andamanica* possesses appreciable anti-allergic and anti-inflammatory activities. Pachypodol (**2**), (-)-yangambin (**3**), 24-epi-piscidinol A (**5**), pyramidaglain A (**8**) and pyramidaglain B (**9**) are responsible for both anti-allergic and anti-inflammatory activities of this plant. The mechanisms for anti-allergic

Table 1. Anti-allergic activity of compounds **1**–**10** isolated from *Aglaia andamanica* leaves

Compound	$IC_{50}$ ( $\mu\text{M}$ ) <sup>a</sup>
Retusin ( <b>1</b> )	67.5 $\pm$ 1.4
Pachypodol ( <b>2</b> )	38.3 $\pm$ 1.1
(-)-Yangambin ( <b>3</b> )	33.8 $\pm$ 1.5
Pyramidatine ( <b>4</b> )	>100
24-epi-Piscidinol A ( <b>5</b> )	19.8 $\pm$ 1.5
Aglaiodiol ( <b>6</b> )	93.6 $\pm$ 1.8
Cycloart-23E-ene-3 $\beta$ -25 diol ( <b>7</b> )	>100
Pyramidaglain A ( <b>8</b> )	37.1 $\pm$ 1.0
Pyramidaglain B ( <b>9</b> )	44.8 $\pm$ 1.8
N-Methyl-trans-4-hydroxy-L-proline ( <b>10</b> )	>100
Ketotifen fumarate	47.5 $\pm$ 0.8

<sup>a</sup>Each value represents mean  $\pm$  S.E.M. of four determinations.

Table 2. Anti-NO production of compounds **1**–**10** isolated from *Aglaia andamanica* leaves

Compound	$IC_{50}$ ( $\mu\text{M}$ ) <sup>a</sup>
Retusin ( <b>1</b> )	>100
Pachypodol ( <b>2</b> )	34.5 $\pm$ 1.5
(-)-Yangambin ( <b>3</b> )	37.4 $\pm$ 1.4
Pyramidatine ( <b>4</b> )	>100
24-epi-Piscidinol A ( <b>5</b> )	24.0 $\pm$ 1.1
Aglaiodiol ( <b>6</b> )	>100
Cycloart-23E-ene-3 $\beta$ -25 diol ( <b>7</b> )	54.5 $\pm$ 1.1
Pyramidaglain A ( <b>8</b> )	54.2 $\pm$ 1.3
Pyramidaglain B ( <b>9</b> )	25.6 $\pm$ 1.6
N-Methyl-trans-4-hydroxy-L-proline ( <b>10</b> )	>100
L-Nitroarginine (L-NA)	61.8 $\pm$ 1.9
Caffeic acid phenethyl ester (CAPE)	5.2 $\pm$ 1.2
Indomethacin	46.5 $\pm$ 0.6

<sup>a</sup>Each value represents mean  $\pm$  S.E.M. of four determinations.

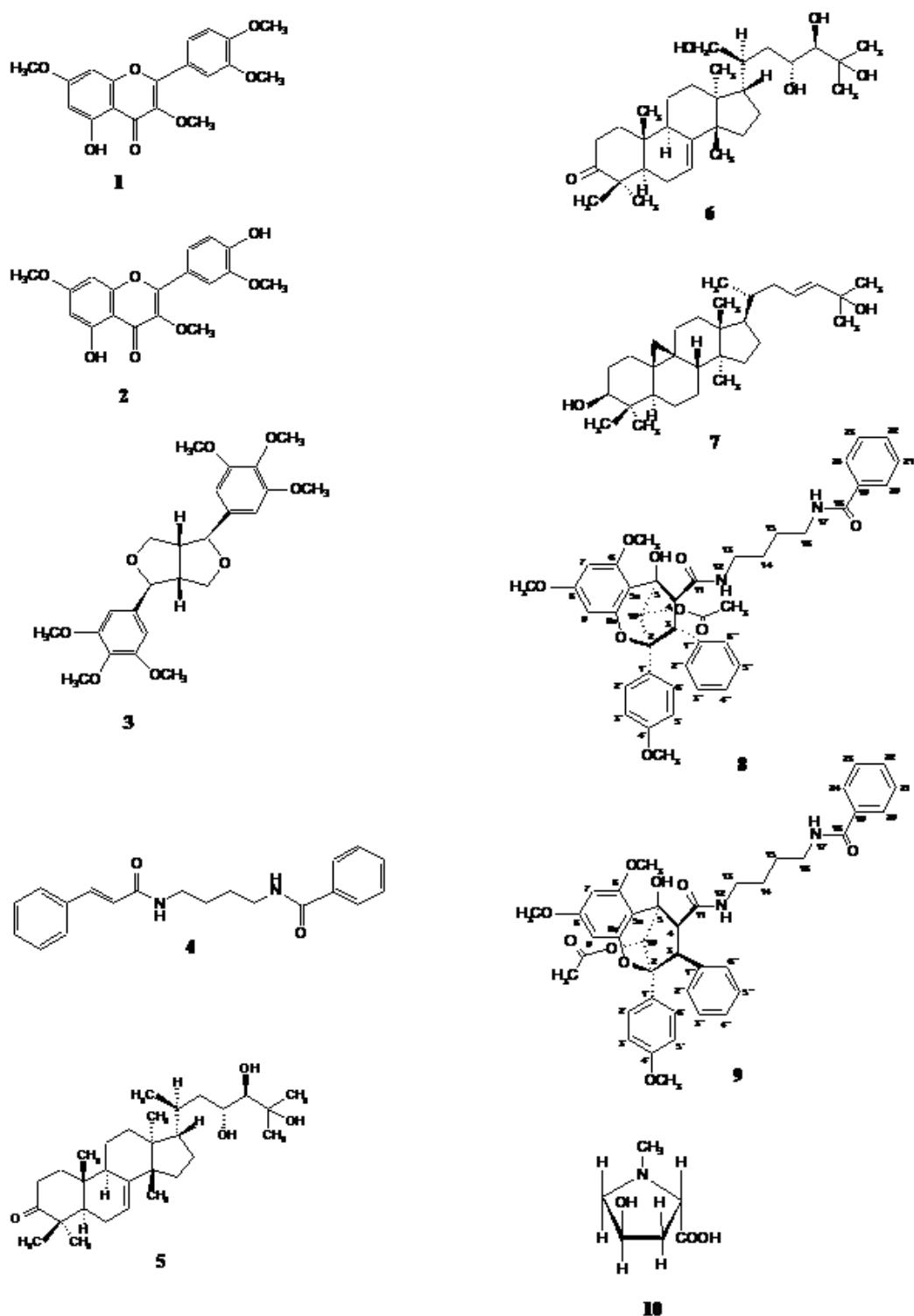


Figure 1. Structure of compounds from *Aglaia andamanica* leaves

and anti-inflammatory effects of these compounds will be further investigated. This is the first report of *Aglaia andamanica* and its compounds for anti-allergic and anti-inflammatory activities.

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