สารประกอบฟีแนนทรีนและไบเบนซิลจากลำต้นของกล้วยไม้สกุลหวายพันธุ์โจแดง และฤทธิ์ต้านการอักเสบ

PHENANTHRENES AND BIBENZYLS FROM THE STEMS OF *DENDROBIUM* SONIA 'RED JO' AND THEIR ANTI-INFLAMMATORY ACTIVITY

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บทคัดย่อ

กล้วยไม้สกุลหวาย จัดเป็นพืชเศรษฐกิจที่สำคัญของไทย ซึ่งส่วนใหญ่ถูกนำมาใช้เป็นไม้ตัดดอกหรือไม้ประดับ กล้วยไม้สกุลหวายหลายชนิดถูกนำมาใช้เป็นยาแผนโบราณอย่างยาวนานในประเทศทางแถบเอเชีย เพื่อใช้รักษาโรคที่เกิดจากความผิดปกติของกระเพาะอาหาร รักษาอาการไข้ และใช้เป็นยาบำรุง อย่างไรก็ตามไม่มีรายงานการใช้ประโยชน์ทางด้านเภสัชวิทยาของกล้วยไม้สกุลหวายของไทยมาก่อน งานวิจัยนี้มีจุดประสงค์เพื่อศึกษาองค์ประกอบทางเคมีจากกล้วยไม้สกุลหวายลูกผสมของไทยพันธุ์โจแดง รวมทั้งศึกษาฤทธิ์ต้านการอักเสบของสารผลิตภัณฑ์ธรรมชาติที่แยกได้ ทั้งนี้เพื่อเพิ่มมูลค่ากล้วยไม้สกุลหวายของไทยในด้านการใช้ประโยชน์เชิงการแพทย์ จากผลการทดลองพบว่า การแยกส่วนสกัดเอทิลอะซิเตตของลำต้นกล้วยไม้สกุลหวายพันธุ์โจแดง โดยใช้เทคนิคทางโครมาโตกราฟี พบสารบริสุทธิ์ที่เป็นอนุพันธ์ของสติลบินอยด์ทั้งหมด 5 สาร แบ่งเป็นสารประกอบฟีแนนทรีน 3 สาร ได้แก่ nudol (**1**) confusarin (**2**) และ lusianthridin (**3**) สารประกอบไบเบนซิล 2 สาร ได้แก่ gigantol (**4**) และ tristin (**5**) โครงสร้างทางเคมีของสารบริสุทธิ์ที่แยกได้ ถูกพิสูจน์เอกลักษณ์โดยใช้เทคนิคนิวเคลียร์แมกเนติกเรโซแนนซ์สเปกโตรสโคปี และแมสสเปกโตรเมตรี สารทั้งหมด (สาร **1**-**5**) ถูกนำมาทดสอบฤทธิ์ต้านการอักเสบ โดยการออกฤทธิ์ยับยั้งการหลั่งไนตริกออกไซด์ (NO) ในเซลล์ RAW264.7 ที่ถูกกระตุ้นด้วยลิโปโพลีแซคคาไรด์ จากผลการทดลองพบว่า confusarin (**2**) และ gigantol (**4**) แสดงฤทธิ์การยับยั้งการหลั่งไนตริก ออกไซด์ในระดับที่ดี ที่ความเข้มข้น 5 ไมโครกรัม/มิลลิลิตร โดยมีเปอร์เซ็นต์การยับยั้ง 79.42%

และ 71.57% ตามลำดับ และไม่แสดงความเป็นพิษต่อเซลล์ RAW264.7 ดังนั้นการศึกษานี้ได้สนับสนุนความเป็นไปได้ในการนำเอากล้วยไม้สกุลหวายพันธุ์โจแดงมาใช้เป็นแหล่งของสารเคมีที่มีฤทธิ์ในการต้านการอักเสบ

คำสำคัญ: กล้วยไม้สกุลหวายพันธุ์โจแดง วงศ์กล้วยไม้ สารประกอบฟีแนนทรีน สารประกอบไบเบนซิล ฤทธิ์ต้านการอักเสบ

Abstract

Dendrobium orchids are one of the important economic crops in Thailand and most of them are used as cut flowers or ornamental plants. Several *Dendrobium* species have been utilized as folk medicines since ancient times in Asian countries for treatments of gastric disorders, fever, diabetes and for tonic purposes. However, the pharmacological applications of Thai *Dendrobium* orchids have not been reported. The aims of this study are to investigate the chemical constituents from *Dendrobium* Sonia 'Jo Daeng' (Orchidaceae) and to evaluate the anti-inflammatory properties of the isolated compounds in order to promote Thai *Dendrobium* orchids with valued-added medical features. As a result, the isolation of the ethyl acetate fraction from the stems of *D.* Sonia 'Jo Daeng' by using repeated chromatography techniques provided five stilbenoid derivatives including three phenanthrenes: nudol (**1**), confusarin (**2**) and lusianthridin (**3**), and two bibenzyls: gigantol (**4**) and tristin (**5**). Their structures were determined on the basis of MS and NMR spectroscopic data. The isolated compounds (**1**-**5**) were evaluated for their anti-inflammatory effects by inhibiting nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophage cells. Among these, confusarin (**2**) and gigantol (**4**) were found to exhibit promising anti-inflammatory activity with 79.42% and 71.57% inhibition, respectively, at 5 μg/mL without cytotoxicity. Therefore, this study supports the possibility of using *D.* Sonia 'Jo Daeng' as an anti-inflammatory inhibitor source.

Keywords: *Dendrobium* Sonia 'Jo Daeng' orchid, Orchidaceae, Phenanthrenes, Bibenzyls, Anti-inflammatory activity

Introduction

Dendrobium, one of the largest genera in the Orchidaceae family, contains nearing 2,000 species which can be found throughout Asia, Australia and Europe [1-3]. More than 150 *Dendrobium* species are found in Thailand and most of them are cultivated as cut flowers and potted ornamental plants. Besides their precious flowers, the *Dendrobium* orchids have been found to be medicinally valuable. Several members of *Dendrobium* species have been used as traditional medicine in several countries for treatments of diabetes, fever, pain, arthritis, hepatitis and stomach disorder and for tonic purposes [4-5]. Especially in China, more than 50 species of *Dendrobium* have been used as crude drugs [6]. For example, the stems

and leaves of *D. nobile*, *D. chrysotoxum* and *D. fimbriatum*, major ingredients in traditional Chinese medicine named “Shi-Hu”, are used for treatment of gastric disorder, reducing fever and curing hyperglycemia [7]. Dried stems of *D. officinale*, called as Tie-Pi Shi-Su, are considered to be the best *Dendrobium* species for the improvement of the immune system [8]. Previous reports revealed that the major phytoconstituents of *Dendrobium* orchids were bibenzyls [9], phenanthrenes [10-11], sesquiterpenes [12-13], fluorenones, alkaloids, flavonoids, coumarins [14] and polysaccharides [8]. Several isolated compounds obtained from *Dendrobium* orchids showed promising antitumor, immunomodulatory, neuroprotective, antiangiogenetic, antidiabetic, hepatoprotective, antibacterial, antiviral, anti-platelet aggregation and anti-inflammatory activities [15-19].

Dendrobium Sonia ‘Jo Daeng’ is one of the important Thai *Dendrobium* hybrid orchids grown for cutting flower. After 4-5 years of flower production, the flower productivity drops to such a low level that it is no longer economically sustainable. Re-plantation is therefore necessary and the old stems are removed and sold at a very low price. Based on previous literature, there are only two studies on the phytochemistry and pharmacological activity of *D.* Sonia ‘Jo Daeng’. The first report was on the analyses of the total phenolics, anthocyanins and antioxidant activity from the different parts of *D*. Sonia ‘Jo Daeng’. The highest total phenolic and anthocyanin contents were found in the leaf and flower methanolic extracts, respectively whereas the flower and root extracts showed the highest antioxidant activities [20]. The second report was on the evaluations of the flavonoid contents and anti-tyrosinase activity of various parts of *D*. Sonia ‘Jo Daeng’. The experiment revealed that the leaf extract had the highest total flavonoid contents and tyrosinase inhibitory effect [21]. However, the investigation of the chemical constituents and other biological activities of this species has so far not been reported. Herein, we report on the first in depth phytochemical investigation including isolation and structure identification of the *D.* Sonia ‘Jo Daeng’ stems as well as anti-inflammatory evaluation of the isolated compounds. Our aims were to find bioactive natural products and to get further information on the potential inflammatory inhibitory applications of the *D.* Sonia ‘Jo Daeng’.

Objectives

The aims of this present study are to investigate the chemical compositions from the stems of *D.* Sonia ‘Jo Daeng’ and to determine the anti-inflammatory activity of the isolated compounds.

Methods

General experimental procedures

Mass spectrometry experiments were recorded using a Micro TOF Brüker Daltonic mass spectrometer. ^{1}H and ^{13}C NMR data were obtained using a 300 MHz Brüker AVANCE spectrometer and TMS was used as internal reference. Column chromatography-based separations were performed on silica gel (230-400 mesh, Merck) and LiChroprep RP-C18 silica gel (40-63 mesh, Merck). Preparative thin layer

chromatography was performed on precoated RP-C18 WF254s Merck or silica gel 60F254 Merck. Compounds were detected under UV light at 254 nm or by spraying with vanillin in H_2SO_4 followed by heating. The Griess nitrite assay kit was purchased from Promega.

Plant material

The stems of *Dendrobium* Sonia 'Jo Daeng' (Figure 1) were collected from Mana Orchid Farm in Sam Pran district, Nakhon Pathom province, Thailand in October 2017 and identified by Asst. Prof. Dr. Kullanart Obsuwan. A voucher specimen (SU20171001) was deposited at the Department of Chemistry, Faculty of Science, Silpakorn University.

Figure 1. Flowers and stems of *D.* Sonia 'Jo Daeng'

Extraction and isolation

The dried stems of *D.* Sonia 'Jo Daeng' (1 kg) were powdered and extracted with 95% EtOH at room temperature (3 times for 3 days each). A dark brown residue (52.2 g) was obtained from the crude ethanol extract after concentration under reduced pressure at 40 ^{o}C. The residue was suspended in water (1.0 L), followed by successive partitioning with hexane, ethyl acetate and butanol (500 mL x 3). Respective solvents then were evaporated to give the hexane (4.1 g), ethyl acetate (7.8 g), butanol (12.3 g) and water (23.8 g) extracts. The ethyl acetate soluble fraction displayed 70.63% inhibitory effect on the nitric oxide production at 10 µg/mL concentration and was selected for further separation. The ethyl acetate fraction (7.8 g) was subjected to silica gel flash column chromatography (CC) and eluted with a gradient system of hexane-EtOAc (100:0 to 0:100) to provide 5 fractions. Fraction 2 (2.5 g) was further separated by silica gel CC, eluting with 5-20% EtOAc in hexane to give fractions 2.1-2.7. Subsequently, fraction 2.3 (350 mg) was separated by a silica gel column eluted with 10% EtOAc in hexane:benzene (1:1) to give compounds **1** (28.4 mg, 0.36%) and **2** (35.4 mg, 0.45%). Fraction 3 (2.1 g) was applied to a silica gel column, eluted with EtOAc in hexane gradient mixtures (10-40%) to afford 5 fractions (fractions 3.1-3.5). Fraction 3.3 (350 mg) was purified by column chromatography (silica gel, hexane: ethyl acetate 4:1) to afford 2 subfractions. The second subfraction (159.5 mg) was subjected to column chromatography over RP-C18 using MeOH:H_2O (1:1) as the eluent to afford compounds **3** (20.5 mg, 0.26%) and **4** (100.6 mg, 1.29%). Fraction 3.4 (70 mg) was purified by RP-C18 column chromatography using CH_3CN:H_2O (1:1.5) as the eluent to give compound **5** (17.3 mg, 0.22%).

Preparation of RAW264.7 cell line

Mouse macrophage cell line (RAW264.7) was cultured in Dulbecco's modified Eagles' medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% L-glutamine. The cells were maintained at 37 °C with humidified 5% CO_2 atmosphere.

Anti-inflammatory evaluation

The production of nitric oxide (NO) was determined by using the Griess nitrite assay kit (Promega), according to the manufacturer's instructions. RAW264.7 cells (2.5 x 10^4 cells) were seeded into 24-well plates overnight. Then the cells were stimulated with lipopolysaccharide (LPS, 100 ng/mL) for two hrs before being treated with compounds **1**-**5** and indomethacin (positive control) at various concentrations. Then all treated cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 hrs before measuring the accumulation of nitrite in the supernatant using the Griess reaction by adding 50 µL of 1% sulphanilamide and 50 µL of 0.1% *N*-1-naphthylethylenediamine dihydrochloride (NED) in 5% phosphoric acid to 50 µL of culture supernatant in each well and incubated at room temperature for 10 min in the dark. Subsequently, the absorbance was measured at 540 nm with a microplate reader. Standard curves were prepared using sodium nitrite ($NaNO_2$) at various concentrations in the same manner. Percentages of inhibition were calculated using the following equation:

$$\% \text{ Inhibition} = \frac{(A-B)}{(A-C)} x100$$

A: RAW264.7 with LPS (+), compound (-); B: RAW264.7 with LPS (+), compound (+); C: RAW264.7 with LPS (-), compound (-)

Cell viability assay

Cell viability was assayed by the MTT method according to the previous report [22]. RAW264.7 cells were seeded into 96-well plates at the density between 5,000-8,000 cells per well in DMEM supplemented with 10% FBS overnight. Then the cells were pretreated with LPS (100 ng/mL) for two hrs before being treated with compounds **1**-**5** at the determined concentrations. The treated cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 hrs before the MTT assay. After 24 hrs, the media containing tested molecules were removed and replaced with 100 µL fresh media containing 0.5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution. The plate was incubated for two hrs before dissolving the cells with 100 µL dimethylsulfoxide (DMSO). The absorbance of the solubilized cells was analyzed with a microplate reader at 570 nm.

Results

Structural identification

The phytochemical investigation of the ethyl acetate soluble fraction from the stems of *D.* Sonia 'Jo Daeng' resulted in the isolation of five stilbenoids, three phenanthrenes and two bibenzyls. The identification and structural elucidation of these compounds were based on spectral data (^1H and ^{13}C NMR and MS data, see supporting information) and by comparison with reports in the literature. Compounds

1–**5** were identified as nudol (**1**) [23], confusarin (**2**) [24], lusianthridin (**3**) [25], gigantol (**4**) [26] and tristin (**5**) [27], respectively. The chemical structures of compounds **1**–**5** are demonstrated in Figure 2. The spectral data of the isolated compounds are shown as follows.

Nudol (**1**): white amorphous powder. ^{1}H-NMR (300 MHz, acetone-d_6) δ ppm: 3.97 (3H, s, OCH_3-4), 4.00 (3H, s, OCH_3-3), 7.15 (s, H-1), 7.19 (1H, dd, *J* = 9.2, 2.8 Hz, H-6), 7.24 (1H, d, *J* = 2.8 Hz, H-8), 7.50 (1H, d, *J* = 8.9 Hz, H-10), 7.53 (1H, *J* = 8.9 Hz, H-9), 9.32 (1H, d, *J* = 9.2 Hz, H-5); ^{13}C-NMR (75 MHz, acetone-d_6) δ ppm: 59.2 (OCH_3-4), 60.4 (OCH_3-3), 108.9 (C-1), 111.6 (C-8), 116.7 (C-6), 118.3 (C-4a), 123.5 (C-4b), 126.2 (C-9), 126.8 (C-10), 128.0 (C-5), 129.4 (C-10a), 133.7 (C-8a), 142.0 (C-3), 149.0 (C-2), 151.3 (C-4), 154.9 (C-7); HR-ESI-MS *m/z* 293.0785 $[M+Na]^+$ (calcd. for $C_{16}H_{14}O_4Na$, 293.0790).

Confusarin (**2**): white amorphous powder. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm: 3.97 (6H, s, OCH_3-4, OCH_3-8), 4.11 (3H, s, OCH_3-3), 5.79 (1H, brs, OH), 5.99 (1H, brs, OH), 7.19 (1H, s, H-1), 7.29 (1H, d, *J* = 9.3 Hz, H-6), 7.60 (1H, d, *J* = 9.1 Hz, H-10), 7.85 (1H, d, *J* = 9.1 Hz, H-9), 9.20 (1H, d, *J* = 9.3 Hz, H-5); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm: 59.8 (OCH_3-4), 61.3 (OCH_3-3), 61.9 (OCH_3-8), 108.2 (C-1), 116.1 (C-6), 119.0 (C-4a), 119.4 (C-9), 123.9 (C-5), 124.8 (C-4b), 126.3 (C-8a), 127.4 (C-10), 129.3 (C-10a), 141.0 (C-3, C-8), 145.5 (C-7), 147.7 (C-2), 150.7 (C-4); HR-ESI-MS *m/z* 323.0892 $[M+Na]^+$ (calcd. for $C_{17}H_{16}O_5Na$, 323.0895).

Lusianthridin (**3**): white amorphous powder. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm: 2.71 (4H, s, H-9, H-10), 3.79 (3H, s, OCH_3-2), 6.34 (1H, d, *J* = 2.4 Hz, H-3), 6.42 (1H, d, *J* = 2.4 Hz, H-1), 6.74 (2H, overlapped, H-6, H-8), 7.94 (1H, d, *J* = 9.2 Hz, H-5); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm: 29.9, 30.5 (C-9, C-10) , 55.3 (OCH_3), 100.9 (C-3), 106.5 (C-1), 113.2 (C-6), 114.8 (C-4a), 115.1 (C-8), 125.5 (C-4b), 127.3 (C-5), 140.2 (10a), 141.1 (C-8a), 153.3 (C-4), 153.7 (C-7), 158.7 (C-2); HR-ESI-MS *m/z* 241.0861 $[M-H]^-$ (calcd. for $C_{15}H_{13}O_3$, 241.0865).

Gigantol (**4**): pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm: 2.80 (4H, m, H-1, H-2), 3.74 (3H, s, OCH_3-5'), 3.83 (3H, s, OCH_3-3''), 6.25 (1H, brd, *J* = 2.0 Hz, H-2'), 6.25 (1H, brd, *J* = 2.0 Hz, H-4'), 6.31 (1H, brd, *J* = 2.0 Hz, H-6'), 6.62 (1H, d, *J* = 1.8 Hz, H-2''), 6.67 (1H, dd, *J* = 8.0, 1.8 Hz, H-6''), 6.83 (1H, d, *J* = 8.0 Hz, H-5''); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm: 37.2 (C-1), 38.3 (C-2), 55.3 (OCH_3-5'), 55.9 (OCH_3-3''), 99.0 (C-4'), 106.8 (C-6'), 108.1 (C-2'), 111.2 (C-5''), 114.2 (C-2''), 121.0 (C-6''), 133.7 (C-1''), 143.7 (C-4''), 144.5 (C-1'), 146.3 (C-3''), 156.6 (C-3'), 160.8 (C-5'); HR-ESI-MS *m/z* 297.1096 $[M+Na]^+$ (calcd. for $C_{16}H_{18}O_4Na$, 297.1103).

Tristin (**5**): pale yellow oil. ^{1}H-NMR (300 MHz, acetone-d_6) δ ppm: 2.74 (4H, m, H-1, H-2), 3.80 (3H, s, OCH_3-3''), 6.18 (1H, d, *J* = 2.0 Hz, H-4'), 6.21 (1H, d, *J* = 2.0 Hz, H-2'), 6.21 (1H, d, *J* = 2.0 Hz, H-6'), 6.65 (1H, dd, *J* = 8.0, 1.7 Hz, H-6''), 6.72 (1H, d, *J* = 8.0 Hz, H-5''), 6.80 (1H, d, *J* = 1.7 Hz, H-2''); ^{13}C-NMR (75 MHz, acetone-d_6) δ ppm: 38.1 (C-2), 39.1 (C-1), 56.3 (OCH_3-3''), 101.1 (C-4'), 107.9 (C-2'),

107.9 (C-6'), 113.0 (C-2''), 115.6 (C-5''), 121.7 (C-6''), 134.3 (C-1''), 145.3 (C-1'), 145.6 (C-4''), 148.2 (C-3''), 159.4 (C-3'), 159.4 (C-5'); HR-ESI-MS *m/z* 283.0943 $[M+Na]^+$ (calcd. for $C_{15}H_{16}O_4Na$, 283.0946).

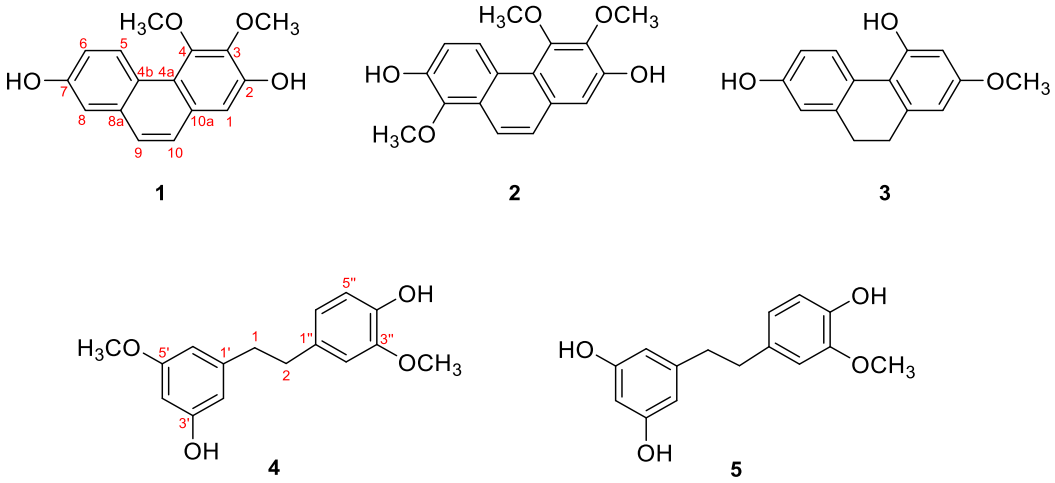

Figure 2. Chemical structures of compounds **1**-**5** obtained from *D.* Sonia 'Jo Daeng'.

Effects of the isolated compounds on nitric oxide (NO) production and cell viability

All isolated compounds were evaluated for their anti-inflammatory effects by determining the level of NO production in LPS-induced RAW264.7 cells according to the Griess assay with indomethacin as a positive control. The results demonstrated that compounds **1**-**5** can reduce the NO production compared with untreated cells (Table 1 and Figure 3). However, based on the cytotoxic effects of compounds **1**-**5** as shown in Table 2 and Figure 4, compounds **1** and **3** are toxic toward macrophage RAW264.7 cells after treatment for 24 hrs (% viable cell < 50), whereas compounds **2**, **4** and **5** are slightly toxic in a dose-dependent manner. Since confusarin (**2)** and gigantol (**4)** at the lowest concentration (5 µg/mL) can inhibit the NO production by 79.42% and 71.57%, respectively from LPS stimulation, they might be potential promising targets for the anti-inflammatory investigation in the future.

Table 1 Percentages of NO inhibition in RAW264.7 cells stimulated with LPS (100 ng/mL) in the presence and absence of compounds **1**-**5** at the different concentrations.

Compounds	% NO inhibition		
	5 µg/mL	10 µg/mL	20 µg/mL
1	98.52 ± 1.23	106.86 ± 0.63	94.13 ± 0.83
2	79.42 ± 0.56	100.00 ± 0.13	107.35 ± 0.11
3	102.47 ± 0.21	102.47 ± 0.25	104.39 ± 0.21
4	71.57 ± 1.08	72.56 ± 1.14	68.61 ± 0.65
5	39.74 ± 1.34	84.30 ± 1.26	99.01 ± 0.11
Indomethacin	95.61 ± 0.45	95.61 ± 0.87	96.54 ± 0.32

Table 2 Effects of compounds **1**-**5** on cell viability in RAW264.7 cells.

Compounds	% Viable cells		
	5 μg/mL	10 μg/mL	20 μg/mL
1	53.01 ± 0.02	32.45 ± 0.02	28.61 ± 0.04
2	93.23 ± 0.05	66.55 ± 0.01	24.26 ± 0.14
3	3.21 ± 0.04	3.46 ± 0.05	1.25 ± 0.00
4	96.29 ± 0.09	87.03 ± 0.18	46.10 ± 0.37
5	85.36 ± 0.14	69.83 ± 0.10	61.31 ± 0.19

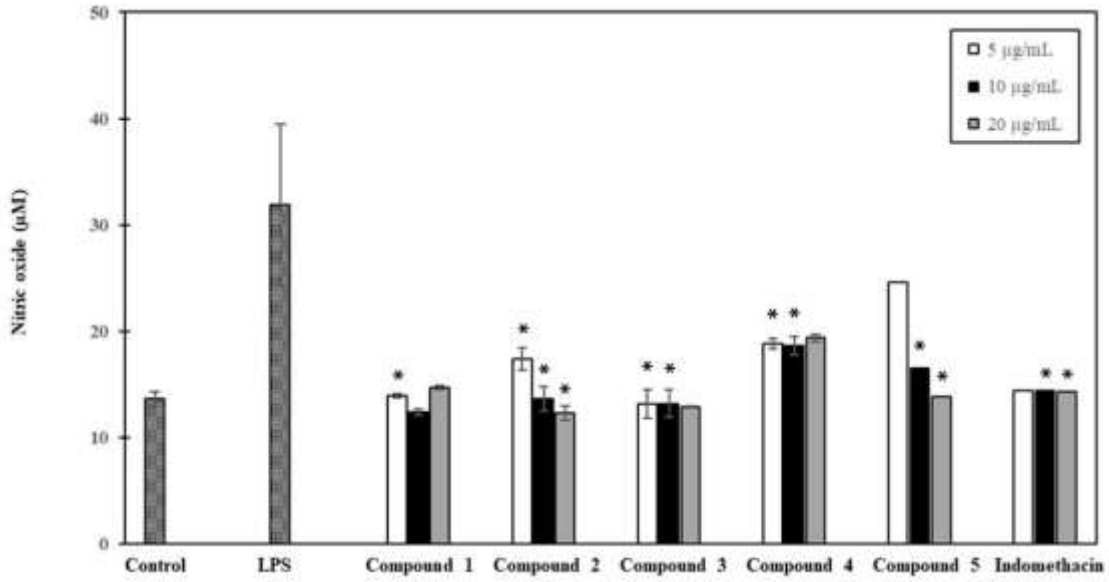

Figure 3. Level of NO production in RAW264.7 stimulated with LPS (100 ng/mL) in the presence and absence of compounds **1**-**5**. Indomethacin was used as a positive control. Compounds **1**-**5** and indomethacin were treated at 5 μg/mL (white), 10 μg/mL (black) and 20 μg/mL (gray). Control was untreated RAW264.7. *$p < 0.05$ compared with LPS group.

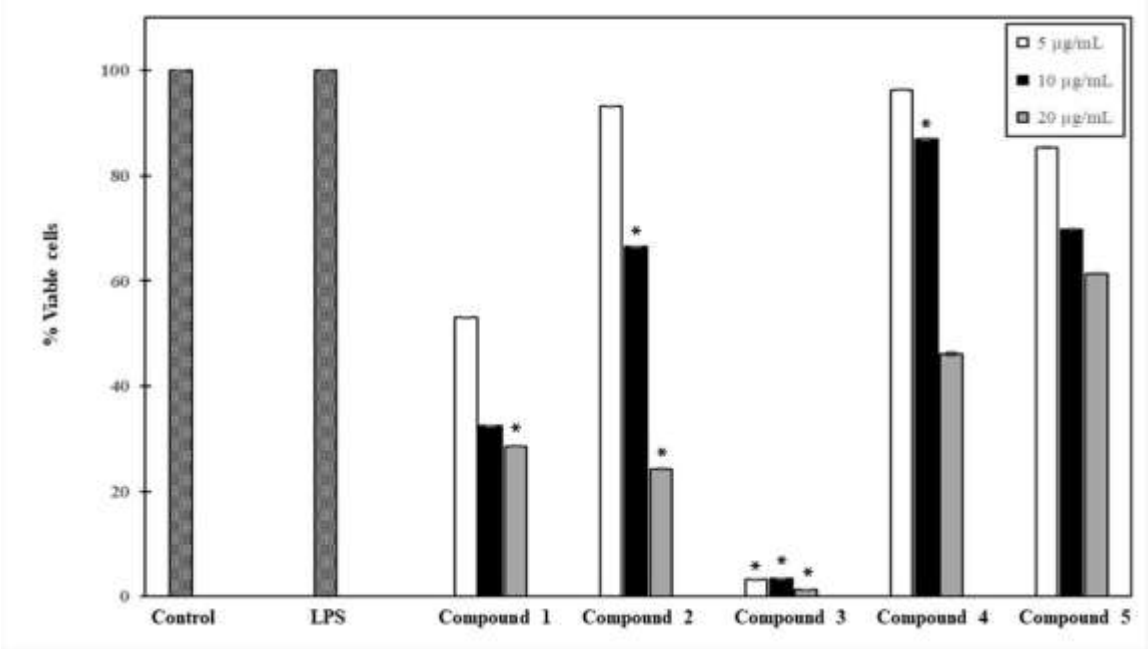

Figure 4. RAW264.7 cell viability stimulated with LPS (100 ng/mL) and maintained in the presence and absence of compounds **1**-**5** for 24 hrs. Compounds **1**-**5** were treated at 5 µg/mL (white), 10 µg/mL (black) and 20 µg/mL (gray). Control was untreated RAW264.7.

Conclusions and Discussion

The first phytochemical investigation of the Thai hybrid *Dendrobium* orchid *D.* Sonia 'Jo Daeng' resulted in the isolation of five stilbenoids including nudol (**1**), confusarin (**2**), lusianthridin (**3**), gigantol (**4**) and tristin (**5**). The bibenzyl gigantol (**4**) was isolated as the major constituent (1.29%) while confusarin (**2**) is the major component of phenanthrene isolates. These results are consistent with the previous reports which indicated that stilbenoids are the major secondary metabolites occurring mainly in the *Dendrobium* orchids. Compounds **1**-**5** have been previously isolated from *D. nobile* [11, 28]. Confusarin (**3**), gigantol (**4**) and tristin (**5**) are the main components in *D. officinale* [6]. In addition, gigantol (**4**) was also found in *D. chrysotoxum* and *D. fimbriatum* [16]. Our findings revealed that the chemical constituents in the *D.* Sonia 'Jo Daeng' stems are similar to those of compounds found in the *Dendrobium* Chinese herbs.

All isolated compounds (**1**-**5**) were examined *in vitro* for their inhibition effects on LPS-induced NO production in RAW264.7 cells. Of these, confusarin (**2**) and gigantol (**4**) showed better NO inhibitory activities with percentage inhibitions of 79.42% and 71.57%, respectively (observed at 5 µg/mL). Both compounds displayed no cytotoxicity against RAW264.7 cells at the effective concentration. This finding supported the use of *D.* Sonia 'Jo Daeng' as a new natural source for anti-inflammatory agents. Moreover, the isolated compounds obtained from this species have been found to possess a broad spectrum of pharmaceutical activities. For example, nudol (**1**) has significant anti-proliferative activity against osteosarcoma [29] and HeLa cell lines [30]; gigantol (**4**) has anti-cancer, anti-inflammatory, anti-diabetes,

antibacterial, antiviral and antimalarial effects [16, 28] and tristin (**5**) has inhibitory ability against the growth of human stomach cancer SGC-7901 [27]. Therefore, this report promotes the potential of *D.* Sonia 'Jo Daeng' in medicinal applications and also encourages the further phytochemical investigation as well as biological activity evaluation of different Thai *Dendrobium* orchids.

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