

Phenolic Constituents of the Rhizomes of the Thai Medicinal Plant *Belamcanda chinensis* with Proliferative Activity for Two Breast Cancer Cell Lines

Orawan Monthakantirat,[†] Wanchai De-Eknamkul,[†] Kaoru Umehara,^{*,‡} Yohko Yoshinaga,[‡] Toshio Miyase,[‡] Tsutomu Warashina,[§] and Hiroshi Noguchi[‡]

Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan, and Institute for Environmental Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan

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From the rhizomes of *Belamcanda chinensis*, three new compounds, belalloside A (**1**), belalloside B (**2**), and belamphenone (**3**), along with 13 known compounds, resveratrol (**4**), iriflophenone (**5**), irisfloreantin (**6**), tectorigenin (**7**), irilin D (**8**), tectoridin (**9**), iristectorin A (**10**), iristectorin B (**11**), hispiduloside, androsin, irigenin, iridin, and jaceoside, have been isolated and characterized. Isolates were evaluated for their cell proliferation stimulatory activity against the MCF-7 and T-47D human breast cancer cell lines. Along with **4**, **5**, **7**, and **9**, **3** was shown to stimulate not only MCF-7 but also T-47D human breast cancer cell proliferation.

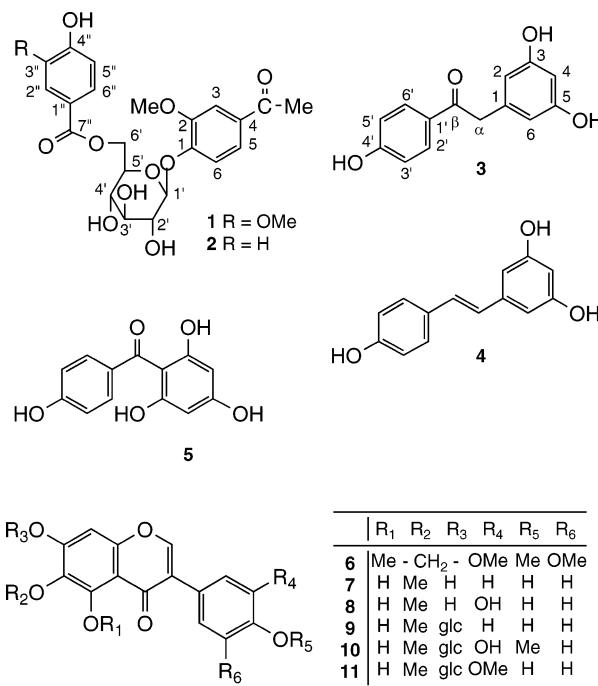
Estrogenic activity has been reported for many compounds produced by animals, plants, and microorganisms, in addition to industrially manufactured chemicals.¹ Phytoestrogens, phytochemicals that exhibit estrogen-like activities, include certain flavonoids, isoflavonoids, stilbenes, and lignans.² The most common phytoestrogens are isoflavonoids.^{3,4}

A methanol extract of the rhizomes of *Belamcanda chinensis* L. (Iridaceae) was found to have a stimulatory activity against estrogen-responsive MCF-7 human breast cancer cells. *Belamcanda chinensis*, commonly known as blackberry lily, is a perennial herbaceous plant having fan-shaped leaves that reach 2 to 3 feet in length on branching stems. The dried rhizomes are used in Chinese traditional medicine for treatment of inflammation and asthma as well as throat disorders, such as cough, tonsillitis, and pharyngitis.⁵ In Thai folk medicine, the rhizomes are used for the regulation of menstrual disorders.⁶ Isoflavonoids^{7–11} and iridal-type triterpenoids^{12–14} have been isolated from the rhizomes, and two major isoflavones, tectorigenin and its glucoside, tectoridin, were reported to have selective estrogen receptor modulating (SERM) properties.¹⁵

In searching for new estrogenic compounds from the plant kingdom, we investigated the extract of the rhizomes of *B. chinensis*, and three new phenolic compounds (**1–3**), together with 13 known compounds, were isolated. This paper deals with the isolation of these compounds, their characterization by spectroscopic methods, and the assessment of their estrogenic activity by measuring the proliferation of cells and stimulatory potency of the substances in estrogen-dependent cell lines.¹⁶

Results and Discussion

A part of the methanolic extract of the rhizomes of *B. chinensis* was absorbed on silica gel and then eluted sequentially with *n*-hexane, CHCl₃, EtOAc, and MeOH. Proliferation of MCF-7 cells was stimulated following treatment with the EtOAc-soluble extract. Purification of the EtOAc extract using silica gel column chromatography



and HPLC gave 16 pure phenolic compounds (**1–16**), including three new compounds (**1–3**). Thirteen known compounds were identified as resveratrol (**4**),¹⁷ iriflophenone (**5**),¹⁸ irisfloreantin (**6**),⁸ tectorigenin (**7**),¹⁹ irilin D (**8**),²⁰ tectoridin (**9**),²¹ iristectorin A (**10**),²² iristectorin B (**11**),²³ hispiduloside,²⁴ androsin,²⁵ irigenin,²¹ iridin,²⁶ and jaceoside,²⁷ respectively, by comparison of their spectroscopic data with reported values in the literature.

Belalloside A (**1**) and B (**2**) were assigned the molecular formulas C₂₃H₂₆O₁₁ and C₂₂H₂₄O₁₀, respectively, as determined from their molecular ion [M + Na]⁺ peaks at *m/z* 501.1357 and 471.1247, in their HRFABMS. The ¹H and ¹³C NMR spectra of these compounds were very similar to those of androsin except for one more aromatic ester moiety and suggested their structures as androsin vanillic acid ester (**1**) and androsin 4-hydroxybenzoic acid ester (**2**), respectively. The ester and aglycon moieties of **1** and **2** were confirmed by direct comparison with authentic samples

* To whom correspondence should be addressed. Phone and Fax: +81-54-264-5661. E-mail: umehara@ys7.u-shizuoka-ken.ac.jp.

[†] Chulalongkorn University.

[‡] School of Pharmaceutical Sciences, University of Shizuoka.

[§] Institute for Environmental Sciences, University of Shizuoka.

Table 1. Cell Proliferation Stimulatory Activities of Test Compounds against MCF-7 and T47D Cells^a

compound	MCF-7		T47D	
	EqE ₁₀ (μ M)	EqE ₁₀₀ (μ M)	EqE ₁₀ (μ M)	EqE ₁₀₀ (μ M)
genistein	0.003	0.013	0.001	0.009
3	0.8	12.8	0.09	37.1
4	1.6	ND	0.03	ND
5	0.7	6.8	4.9	ND
6	50.3	ND	ND	ND
7	0.3	1.0	0.04	0.5
8	5.6	12.7	ND	ND
9	0.02	0.08	0.2	ND
10	26.1	ND	ND	ND
11	77.3	ND	ND	ND

^a EqE₁₀ and EqE₁₀₀ represent the concentration of the compound that stimulated the cell proliferations equivalent to 10 and 100 pM estradiol, respectively. These values were determined by linear regression analysis using at least five different concentrations in quadruplicate. Compounds **1**, **2**, androsterin, irigenin, iridin, hispiduloside, and jaceoside were not determined (ND).

after acid hydrolysis. The sugar component of **1** and **2** was identified as D-glucose by GC analysis after conversion to a thiazolidine derivative.²⁸ From the HMBC spectrum of **1**, H–C long-range coupling was observed between H-6' [δ 4.20 (dd, J = 12, 8 Hz)] of the glucose and the carbonyl carbon (δ 165.2) of vanillic acid and between the anomeric proton [δ 5.10 (d, J = 8 Hz)] of the glucose and an oxygenated aromatic carbon (δ 150.2) of the aglycon, acetovanillone. In turn, the HMBC spectrum of **2** showed H–C long-range couplings between H-6' [δ 4.19 (dd, J = 12, 8 Hz)] of the glucose and the carbonyl carbon (δ 165.2) of 4-hydroxybenzoic acid and between the anomeric proton [δ 5.10 (d, J = 8 Hz)] of the glucose and the oxygenated aromatic carbon (δ 150.2) of acetovanillone. Accordingly, the structures of belallosides A (**1**) and B (**2**) were assigned as acetovanillone 1-O- β -D-(6-O-vanilloyl)glucopyranoside and acetovanillone 1-O- β -D-(6-O-4-hydroxybenzoyl)glucopyranoside, respectively.

Belamphenone (**3**) was assigned a molecular formula of C₁₄H₁₂O₄, as determined from the molecular ion [M + H]⁺ peak at m/z 245.0820 in its HRFABMS. Its spectral features suggested that **3** had a similar structure to resveratrol (**4**). The ¹H NMR spectrum of **3** showed the presence of two aromatic rings from 1,4-substituted aromatic proton signals [δ 6.82 (2H, d, J = 9 Hz), 7.86 (2H, d, J = 9 Hz)] and 1,3,5-trisubstituted aromatic proton signals [δ 6.03 (1H, t, J = 2 Hz), 6.09 (2H, d, J = 2 Hz)]. From the HMBC spectrum of **3**, H–C long-range couplings were observed between the H-2 [δ 6.09 (2H, d, J = 2 Hz)] and the methylene carbon (δ 44.5) and between H-2',6' [δ 7.86 (d, J = 9 Hz)] and the conjugated carbonyl carbon (δ 195.7). Accordingly, the structure of belamphenone (**3**) was defined as 1-(4-hydroxyphenyl)-2-(3,5-dihydroxyphenyl)ethanone. This is the first report on the isolation of this compound as a natural product, but it is known as a synthetic product.²⁹

Effects on MCF-7 cell proliferation were tested with increasing concentrations of isoflavones ranging from 10 nM to 100 μ M, and their EqE₁₀ and EqE₁₀₀ values were determined for the required concentrations against cell proliferation equivalent to 10 and 100 pM of estradiol(E2) treatment, respectively (Table 1). Compounds **7**, **8**, and **9** were found to have stimulatory activities (EqE₁₀: 0.3 μ M for **7**, 5.6 μ M for **8**, and 0.02 μ M for **9**) against the cell proliferation at low concentrations and showed high potencies, as effective as 100 pM of E2 (EqE₁₀₀: 1.0 μ M for **7**, 12.7 μ M for **8**, and 0.08 μ M for **9**). Compound **9** showed

higher activity compared to genistein at a concentration of 1 μ M, whereas cytotoxicity of this compound was observed at a concentration of 100 μ M. Although **6**, **10**, and **11** were observed to enhance cell proliferation (EqE₁₀: 50.3 μ M for **6**, 26.1 μ M for **10**, and 77.3 μ M for **11**), their EqE₁₀₀ values could not be assessed since their stimulatory activities were not high enough, even at a concentration of 100 μ M. The remaining compounds did not show any significant activities against the cell line at these concentrations, and their EqE₁₀ values could not be evaluated.

Two subtypes of the ER are known to date; the ER α ³⁰ and ER β ³¹ and both receptors have a distinct distribution and play a distinct role in physiology.³² Genistein, the major phytoestrogen in soy, is a better ligand for ER β than ER α .³³ These offer a theoretical possibility to explain differences in the cell proliferation between the two cell types: MCF-7 (ER α positive) and T-47D (ER α and - β positive).³⁴ Both cell systems for detection of the biological activities of the constituents of the rhizomes of *B. chinensis* were compared. The effect of isoflavones on breast cancer cell proliferation was tested in T-47D cells (Table 1). Almost all compounds showed cytotoxicities at concentrations of 10 μ M or above, whereas they exhibited maximum effects on MCF-7 cell proliferation at those concentrations. Compounds **7** and **9** stimulated T47D cell proliferation (EqE₁₀: 0.04 μ M for **7** and 0.2 μ M for **9**). Compounds **10** and **11** showed significant activity against this cell line at concentrations of less than 1 μ M, but their EqE₁₀ values could not be evaluated as a result of their low activity. Other compounds had no effect on cell proliferation over a concentration range of 1 nM to 10 μ M.

The stimulatory effects of **1**–**5** on MCF-7 and T-47D cell proliferation were examined. Treatment of these cells with **3** and **5** resulted in an increase of cell proliferation in a concentration-dependent manner (EqE₁₀ against MCF-7: 0.8 μ M for **3** and 0.7 μ M for **5**, EqE₁₀ against T47D: 0.09 μ M for **3** and 4.9 μ M for **5**). Compound **3** showed the highest activity, and it was the only compound of the six to afford a EqE₁₀₀ value against both cell lines (EqE₁₀₀ against MCF-7: 12.8 μ M, EqE₁₀₀ against T47D: 37.1 μ M). However, cytotoxicity of this compound was observed at a concentration of 100 μ M against T-47D cells. Compound **4** showed activity against these two cell lines (EqE₁₀ against MCF-7: 1.6 μ M, EqE₁₀₀ against T47D: 0.03 μ M), whereas cytotoxicity of this compound was observed at, or above, concentrations of 10 μ M. Acetovanillone glucosides (**1** and **2**) had no effect on both cell proliferations over a concentration range of 1 nM to 100 μ M.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-360 digital polarimeter. UV spectra were recorded on a Hitachi U3410 spectrometer. ¹H and ¹³C NMR spectra were recorded on a JEOL JNM- α 400 instrument, and chemical shifts are given in δ (ppm) with tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained using a JEOL JMS-SX 102 mass spectrometer. HPLC was carried out with JASCO model 887-PU pump and an 875-UV variable-wavelength detector with a reversed-phase column (Capcell Pak ODS, 5 μ m, 2 \times 25 cm, Shiseido Fine Chemicals Co. Ltd., at 6 mL/min with detection at 205 nm, and Develosil-Lop-ODS, 10–20 μ m, 5 \times 100 cm, Nomura Chemical Co., Ltd, at 45 mL/min with detection at 205 nm).

Chemicals. Eagle's MEM and RPMI media were purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). Fetal bovine serum (FBS) was purchased from Gibco (Grand Island, NY). Antibiotics were purchased from Meiji Seika Kaisha Ltd. (Tokyo, Japan). L-Glutamine was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). 17 β -Estradiol and

dextran-coated charcoal (DCC) were purchased from Sigma Chemicals (St. Louis, MO).

Plant Material. Commercially available rhizomes of *Belamcanda chinensis* were purchased from Thai medicinal herb store in Bangkok, in September 2003. The plant was identified by Prof. A. Ueno of School of Pharmaceutical Sciences, University of Shizuoka. A voucher specimen is deposited at the herbarium of School of Pharmaceutical Sciences, University of Shizuoka (C003101).

Extraction and Isolation. The dried rhizomes of *B. chinensis* (1 kg) were extracted three times with MeOH (3 × 20 L) at room temperature. The extracts were combined and concentrated under reduced pressure at 60 °C to yield 280 g of a viscous mass. A part of this concentrated extract (140 g) was adsorbed on silica gel (400 g) and then eluted successively with *n*-hexane (5 L), CHCl₃ (5 L), EtOAc (5 L), and MeOH (5 L), to yield *n*-hexane- (9.5 g), CHCl₃- (25.5 g), EtOAc- (9 g), and MeOH-soluble (70.5 g) extracts. The EtOAc-soluble extract was chromatographed on a silica gel column (3.5 × 20 cm) and fractionated using chloroform–MeOH (85:15, 15 L). Fractions of 300 mL were collected and pooled by TLC analysis to afford a total of 12 combined fractions. Purification of fraction 1 (85 mg) using HPLC on Capcell Pak ODS (2 × 25 cm, flow rate 6 mL/min with detection at 205 nm) with MeCN–H₂O (45:55) as eluent gave **7** (59 mg) (*t*_R, 41 min). Purification of fraction 2 (850 mg) using HPLC on Develosil-Lop-ODS (5 × 100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN–H₂O (30:70) as eluent gave **7** (90 mg) and irigein (115 mg) (*t*_R = 228 and 272 min, respectively). Purification of fraction 3 (520 mg) using HPLC on Develosil-Lop-ODS (5 × 100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN–H₂O (27:73) as eluent gave **8** (4 mg) (*t*_R = 208 min). Purification of fraction 4 (1.28 g) using HPLC on Develosil-Lop-ODS (5 × 100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN–H₂O (18:82) as eluent gave **1** (1.5 mg), **2** (1.5 mg), **3** (3.6 mg), **4** (385 mg), **5** (3.9 mg), and androsin (9.7 mg) (*t*_R = 304, 312, 188, 476, 168, and 164 min, respectively). Purification of fraction 5 (550 mg) using HPLC on Develosil-Lop-ODS (5 × 100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN–H₂O (18:82) as eluent gave **10** (30 mg) (*t*_R = 432 min). Purification of fraction 7 (680 mg) using HPLC on Develosil-Lop-ODS (5 × 100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN–H₂O (15:85) as eluent gave **9** (96.1 mg), **11** (155.5 mg), iridin (283.3 mg), hispiduloside (20.9 mg), and jaceoside (11.2 mg) (*t*_R = 372, 452, 692, 800, and 864 min, respectively). Purification of fraction 11 (2.8 g) using HPLC on Develosil-Lop-ODS (5 × 100 cm, flow rate 45 mL/min with detection at 205 nm) with MeCN–H₂O (18:82) as eluent gave **9** (1.1 g) (*t*_R = 212 min). Compounds **4**–**11**, hispiduloside, androsin, irigein, iridin, and jaceoside were identified by comparison of their spectral data with published data.^{17–27}

Belalloside A (1): amorphous powder; [α]_D²⁵ –37.7° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 221 (sh) (4.27), 264 (3.78), 294 (3.90) nm; ¹H NMR (DMSO-*d*₆, 400 MHz) δ 2.48 (3H, s, H-8), 3.76 (3H, s, MeO-3''), 3.80 (3H, s, MeO-3), 5.10 (1H, d, *J* = 8 Hz, H-1'), 6.87 (1H, d, *J* = 9 Hz, H-5''), 7.11 (1H, d, *J* = 9 Hz, H-5), 7.26 (1H, dd, *J* = 9, 2 Hz, H-6), 7.40 (1H, d, *J* = 2 Hz, H-2''), 7.42 (1H, d, *J* = 2 Hz, H-2), 7.46 (1H, dd, *J* = 9, 2 Hz, H-6''); ¹³C NMR data, see Table 1; HRFABMS *m/z* [M + Na]⁺ 501.1357 (calcd for C₂₃H₂₆O₁₁Na, 501.1373).

Belalloside B (2): amorphous powder; [α]_D²⁵ +9.5° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 224 (sh) (4.14), 260 (3.86), 299 (3.64) nm; ¹H NMR (DMSO-*d*₆, 400 MHz) δ 2.50 (3H, s, H-8), 3.80 (3H, s, MeO-3), 5.10 (1H, d, *J* = 8 Hz, H-1'), 6.84 (2H, d, *J* = 9 Hz, H-3'', 5''), 7.13 (1H, d, *J* = 9 Hz, H-5), 7.32 (1H, dd, *J* = 9, 2 Hz, H-6), 7.43 (1H, d, *J* = 2 Hz, H-2), 7.78 (1H, d, *J* = 9 Hz, H-2''); ¹³C NMR data, see Table 1; HRFABMS *m/z* [M + Na]⁺ 471.1247 (calcd for C₂₂H₂₄O₁₀Na, 471.1267).

Belamphenone (3): amorphous powder; UV (MeOH) λ_{max} (log ε) 219 (sh) (4.00), 278 (3.87) nm; ¹H NMR (DMSO-*d*₆, 400 MHz) δ 3.98 (2H, s, H-α), 6.03 (1H, t, *J* = 2 Hz, H-4), 6.09 (2H, d, *J* = 2 Hz, H-2, 6), 6.82 (2H, d, *J* = 9 Hz, H-3', 5'), 7.86 (2H, d, *J* = 9 Hz, H-2', 6'); ¹³C NMR data, see Table 1;

Table 2. ¹³C NMR Data of Compounds **1**–**4** in DMSO-*d*₆

position	1	2	position	3	4
aglycon moiety					
1	130.8	130.8	1	137.2	139.2
2	110.9	111.0	2	107.3	104.2
3	148.6	148.6	3	158.2	158.4
4	150.2	150.2	4	100.7	101.7
5	114.1	114.2	5	158.2	158.4
6	122.1	122.1	6	107.3	104.2
7	196.0	196.1			
8	26.1	26.2	α	44.5	125.6
			β	195.7	127.8
sugar moiety					
1'	99.1	99.1	1'	127.9	128.0
2'	72.9	72.9	2'	131.0	127.7
3'	76.6	76.6	3'	115.1	115.4
4'	70.1	70.1	4'	161.9	157.1
5'	73.9	73.9	5'	115.1	115.4
6'	63.7	63.5	6'	131.0	127.7
ester moiety					
1''	120.4	120.1			
2''	112.9	131.4			
3''	147.3	115.2			
4''	151.6	162.0			
5''	115.0	115.2			
6''	123.5	131.4			
7''	165.2	165.2			
OMe-3	55.5	55.5			
OMe-3''	55.6				

HRFABMS *m/z* [M + H]⁺ 245.0820 (calcd for C₁₄H₁₃O₄, 245.0814).

Acid Hydrolysis of 1 and 2. Compound **1** (1 mg) was dissolved in 10% H₂SO₄ (1 mL) and heated at 95 °C for 1 h. After cooling, the reaction mixture was diluted with H₂O (2 mL) and extracted with ethyl acetate (2 mL × 3). The ethyl acetate phases were evaporated, and acetovanillone and vanillic acid were identified by direct comparison with authentic samples. The water layer was passed through an Amberlite IRA-60E column (6 × 60 mm), and the eluate was concentrated. The residue was dissolved in pyridine (50 μL) and stirred with *D*-cysteine methyl ester (3 mg) for 1.5 h at 60 °C. To the reaction mixture, hexamethyldisilazane (15 μL) and trimethylsilyl chloride (15 μL) were added, and the mixture was stirred for 30 min at 60 °C. The supernatant was then analyzed by GC [column: GL Sciences TC-1, 0.25 mm × 30 m; column temperature: 235 °C; carrier gas: N₂; retention time: *D*-Glc (21.4 min), *L*-Glc (20.4 min)²⁹], and from compound **1**, *D*-Glc was detected. Acid hydrolysis of **2** was performed in the same manner, and *D*-Glc was detected.

Cell Culture. MCF-7 and T47D human breast cancer cells were purchased from the American Type Culture Collection (Manassas, VA). The MCF-7 cells were grown in MEM supplemented with 6 ng/mL insulin, 1 mM sodium pyruvate, 1 mM nonessential amino acids, 2 mM glutamine, 10% FBS, and antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin), under a 5% CO₂ humidified atmosphere at 37 °C. The T47D cells were grown in RPMI-1640 supplemented with 1 mM sodium pyruvate, 1 mM nonessential amino acids, 2 mM glutamine, 10% FBS, and antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin), under a 5% CO₂ humidified atmosphere at 37 °C.

Cell Proliferation Assay. Cells were seeded into 96-well tissue culture plates in 5% DCC-treated, FBS-supplemented RPMI phenol red-free medium at a density of 1 × 10⁴ cells/well. Test compounds were added in DMSO solution (control contained 1% DMSO) and incubated at 37 °C with 5% CO₂ for 96 h. In all experiments, serial dilutions of estradiol were added as a positive control.¹³ To evaluate relative cell concentrations, Alamar Blue reagent was used. After 3 h, fluorescence was measured at 590 nm with excitation at 530 nm using a FL500 spectrophotometer (Bio-Tek Instruments Inc, Winooski, VT).³⁵

Data and Statistical Analysis. Statistical differences were determined by analysis of variance followed by Dunnett's

multiple comparison test. Statistical significance was established at the $p < 0.05$ level.

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References and Notes

- (1) Zava, D. T.; Blen, M.; Duwe, G. *Environ. Health Perspect.* **1997**, *105*, 637–645.
- (2) Cos, P.; Bruyne, T. D.; Apers, S.; Berghe, D. V.; Pieters, L.; Vlietinck, A. J. *Planta Med.* **2003**, *69*, 589–599.
- (3) Cornwell, T.; Cohick, W.; Raskin, I. *Phytochemistry* **2004**, *65*, 995–1016.
- (4) Magee, P. J.; Rowland, I. R. *Br. J. Nutr.* **2004**, *91*, 513–531.
- (5) Chang, S., C. A. *Dictionary of Chinese Crude Drugs*; New Medical College, Shanghai Scientific Technological Publisher: Shanghai, 1997; p 1883.
- (6) Pongboonrod, S. *Folkloric Uses of Thai and Foreign Medicinal Plants*; Chaiwat Press: Bangkok, 1976.
- (7) Ito, H.; Onoue, S.; Yoshida, T. *Chem. Pharm. Bull.* **2001**, *49*, 1229–1231.
- (8) Morita, N.; Arisawa, M.; Kondo, Y.; Takemoto, T. *Chem. Pharm. Bull.* **1973**, *21*, 600–603.
- (9) Yamaki, M.; Kato, T.; Kashima, M.; Takagi, S. *Planta Med.* **1990**, *56*, 335.
- (10) Zhou, L. X.; Lin, M. *Chin. Chem. Lett.* **1997**, *8*, 133–134.
- (11) Eu, G. H.; Woo, W. S.; Chung, H. S.; Woo E. H. *Saengyak Hakhoehi* **1991**, *22*, 13–17.
- (12) Takahashi, K.; Hoshino, Y.; Suzuki, S.; Hano, Y.; Nomura, T. *Phytochemistry* **2000**, *53*, 925–929.
- (13) Ito, H.; Onoue, S.; Miyake, Y.; Yamada, T. *J. Nat. Prod.* **1999**, *62*, 89–93.
- (14) Abe, F.; Chen, R.; Yamauchi, T. *Phytochemistry* **1991**, *30*, 3379–3382.
- (15) Seidlova-Wuttke, D.; Heiden, L.; Jarry, H.; Christoffel, V.; Spengler, B.; Wuttke, W. *J. Menopause* **2000**, *2*, 28–31.
- (16) Soto, A. M.; Sonnenschein, C.; Chung, K. L.; Fernandez, M. F.; Olea, N.; Serrano, F. O. *Environ. Health Perspect.* **1995**, *103*, 113–122.
- (17) Anjaneyulu, A. S. R.; Reddy, R. A. V.; Reddy, D. S. K.; Ward, R. S.; Adhikesavalu, D.; Cameron, S. T. *Tetrahedron* **1984**, *40*, 4245–4252.
- (18) Arisawa, M.; Morita, N.; Kondo, Y.; Takemoto, T. *Chem. Pharm. Bull.* **1973**, *21*, 2323–2328.
- (19) Oh, K.; Kang, H.; Matsuoka, H. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 939–942.
- (20) Choudhary, M. I.; Nur-e-Alam, M.; Baig, I.; Akhtar, F.; Khan, A. M.; Ndoghii, P. O.; Badarchiin, T.; Purevsren, G.; Nahar, N.; Atta-ur-Rahman. *J. Nat. Prod.* **2001**, *64*, 857–860.
- (21) Park, H.; Park, J.; Moon, J.; Lee, K.; Jung, W.; Oh, S. *Phytochemistry* **1999**, *51*, 147–151.
- (22) Morita, N.; Shimokoriyama, M.; Shimizu, M.; Arisawa, M. *Chem. Pharm. Bull.* **1972**, *20*, 730–733.
- (23) Morita, N.; Arisawa, M.; Kondo, Y.; Takemoto, T. *Chem. Pharm. Bull.* **1973**, *21*, 600–603.
- (24) Ayhan, U.; Eser, A.; Dennis, C. W.; Brown, G. K.; Mabry, T. J. *J. Nat. Prod.* **1982**, *45*, 363–364.
- (25) Rosa, S. D.; Giulio, A. D.; Tommonaro, G. *Phytochemistry* **1996**, *42*, 1031–1033.
- (26) Ali, A. A.; El-Emary, N. A.; El-Moghazi, M. A.; Darwish, F. M.; Frahm, A. W. *Phytochemistry* **1983**, *22*, 2061–2063.
- (27) Kamataki, T.; Kitada, M.; Kitakawa, H. *Chem. Pharm. Bull.* **1973**, *21*, 2329–2331.
- (28) Hara, S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1986**, *34*, 1843–1845.
- (29) Eddarir, S.; Abdelhadi, Z.; Rolando, C. *Tetrahedron Lett.* **2001**, *42*, 9127–9130.
- (30) Green, S.; Walter, P.; Kumar, V.; Krust, A.; Bornert, J. M.; Argos, P.; Chambon, P. *Nature* **1986**, *320*, 134–139.
- (31) Kuiper, G. G.; Enmark, E.; Peltto-Huikko, M.; Nilsson, S.; Gustafsson, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5925–5930.
- (32) Couse, J. F.; Korach, K. S. *Endocr. Rev.* **1999**, *20*, 358–417.
- (33) Kuiper, G. G.; Carlsson, B.; Grandien, K.; Enmark, E.; Haggblad, J.; Nilsson, S.; Gustafsson, J. A. *Endocrinology* **1997**, *138*, 863–870.
- (34) Vladusic, E. A.; Hornby, A. E.; Guerra-Vladusic, F. K.; Lakins, J.; Lupu, R. *Oncol. Rep.* **2000**, *7*, 157–167.
- (35) Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004–1009.

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