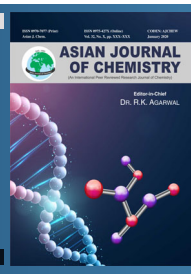
Asian Journal of Chemistry; Vol. 32, No. 7 (2020), 1788-1792

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2020.22690



Potential Antifungal Activity against *Pythium insidiosum* of Isoflavonoids from the Stems of *Dalbergia cultrata*

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Received: 21 February 2020; *Accepted*: 30 April 2020; *Published online*: 27 June 2020; AJC-19949

Chemical investigation of *Dalbergia cultrata* Grah. stems growing in Thailand resulted in the isolation and characterization of 14 known compounds, including tectorigenin (**1**), calycosin (**2**), pratensein (**3**), afromosin (**4**), irilin D (**5**), biochanin A (**6**), daidzein (**7**), formononetin (**8**), 8-O-methylretusin (**9**), 7,3′-dihydroxy-5′-methoxyisoflavone (**10**), (3*S*)-sativanone (**11**), (3*S*)-violanone (**12**), erycibenin D (**13**) and isoliquiritigenin (**14**). Their structures were established mainly based on NMR spectroscopic techniques and physical properties. The isolated compounds were evaluated for antifungal activity against *Pythium insidiosum* using disc diffusion assay. Antifungal drugs available now are not effective to treat this microorganism. It was found that compound **1** exhibited stronger antifungal activity than amphotericin-B standard with an MIC value of 10 µg/disc.

Keywords: *Dalbergia cultrata*, *Pythium insidiosum*, Antifungal activity, Isoflavonoids, Chalcones.

INTRODUCTION

The *Dalbergia* genus belongs to the family Fabaceae (or Leguminosae) and contains more than 300 recognized species that are widely distributed in tropical and subtropical regions [1]. The genus is a good source of flavonoids, rotenoids and terpenoids [2]. Members of the *Dalbergia* genus have previously been found to possess a wide spectrum of biological activities, such as antibacterial and α-glucoside inhibition, antioxidant, anticancer, antimicrobial, antimalarial, anti-HIV, anticoagulant, anti-inflammatory, as well as anti-osteoporotic activities [3-6]. *Dalbergia cultrata* Grah. locally known in Thai as Kra Phi Khao Khwai, is a moderate-sized deciduous tree. Previous studies of the stem bark and heartwood of this plant have revealed the presence of cinnamylphenols and neoflavanoids [7]. In previous works, we reported antifungal activity against *Pythium insidiosum* of carbazole alkaloids from the roots of *Clausena harmandiana* [8], lignans from the stems of *Alyxia schlechteri* [9] and coumarins from the fruits of *Scaevola taccada* [10]. In the continuation of our work on antifungal activity against *P. insidiosum*, the chemical constituents from *D. cultrata* have been evaluated against this microorganism.

Pythium insidiosum is a fungus-like organism that can cause pythiosis in humans, mammals and birds [11]. The first reports of what is believed to be pythiosis were published in the mid late 1800s associated with lesions in horses [11,12]. The first human infection case was found in Thailand and since then other cases have been reported in tropical, subtropical and temperate regions [8]. This microorganism shows some different morphological and biochemical characteristics from true fungi, and this may partially account for difficulties and failures in the therapies employed for disease treatment [13]. Because of unsuccessful drug and vaccine treatments, along with high rates of morbidity and mortality due to infection, study to find new antifungal agents from medicinal plants for the treatment of this infection is also necessary. Herein, we report the phytochemical studies of *D. cultrata* and antifungal activity against *Pythium insidiosum.*

EXPERIMENTAL

Melting points were acquired using a SANYO Gallenkamp melting point apparatus. The UV-visible absorbances were measured on an Agilent 8453 UV-visible spectrophoto-

meter. IR spectra were taken using a Perkin-Elmer Spectrum One FT-IR spectrophotometer. The NMR spectra were collected at 400 MHz (^{1}H) and at 100 MHz (^{13}C) using a Varian Mercury Plus spectrometer and the chemical shifts are expressed as δ values. A Micromass Q-TOF 2 hybrid quadrupole time-of-flight (Q-TOF) mass spectrometer was used to determine the mass spectra. Column chromatography was performed using Si gel less than 0.063 mm, 0.063-0.200 mm or RP-18. Thin layer chromatography was carried out on Merck silica gel 60 F_{254} TLC aluminium sheet. Preparative TLC was run on silica gel 60 F_{254} (Merck). UV light at 254 and 365 nm was applied to detect compounds and acidic anisaldehyde solution was used as the spraying agent. The organic solvents were distilled before use in the separation process.

Plant material: The stems of *Dalbergia cultrata* Grah. (Voucher specimen KKU032018) were collected from Phu Wiang District, Khon Kaen Province, Thailand, in August 2018. The title plant was characterized by Assoc. Prof. Suppachai Tiyaworanant, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

Extraction and isolation: The stems (7.5 kg) of *D. cultrata* were air-dried, pulverized and sequentially extracted with *n*-hexane, EtOAc and MeOH at room temperature. Solvents were evaporated under reduced pressure to produce *n*-hexane, EtOAc and MeOH extracts. The EtOAc crude extract (200 g) was subjected to column chromatography (CC) on silica gel eluted with gradients of *n*-hexane:acetone and acetone:MeOH to afford ten fractions (EF1-EF10) analyzed by TLC pattern. Subfraction EF6 was rechromatographed on silica gel FCC with EtOAc:*n*-hexane (20:80 to 80:20, v/v) and monitored by TLC to afford five subfractions, EF6.1-EF6.5. Subfraction EF6.3 was further separated by silica gel FCC (MeOH:CH_2Cl_2, 1:99 to 5:95) and gave four subfractions, EF6.3.1-6.3.4. Compound **11** (24.2 mg) was isolated from subfraction EF6.3.2 (silica gel CC; CH_2Cl_2:*n*-hexane). Subfraction EF6.3.4 was passed over silica gel FCC using a 2:98 mixture of MeOH and CH_2Cl_2 as the solvent system and monitored by TLC to obtain compounds **1** (71.4 mg), **3** (5.1 mg), and **9** (4.5 mg). Subfraction EF6.3.4 was separated over silica gel FCC eluted with MeOH:CH_2Cl_2 mixture (2:98) to yield compounds **8** (81.1 mg), and **12** (7.6 mg). Six subfractions, EF6.4.1-EF6.4.6, were obtained from the isolation of subfraction EF6.4 eluted with MeOH:CH_2Cl_2 gradient (from 30:70 to 100:0). Crystallization of subfraction EF6.4.2 using EtOAc:*n*-hexane gave compound **4** (59.9 mg). Further purification of EF6.4.5 eluted with a solvent system of MeOH:CH_2Cl_2 (2:98) provided compound **14** (6.3 mg). Subfraction EF7, was further separated by RP-18 CC using a gradient of H_2O-MeOH (from 30:70 to 0:100) to obtain compounds **2** (60.2 mg), **10** (6.2 mg) and **13** (5.4 mg). The MeOH extract (183 g) was separated over silica gel CC (EtOAc:MeOH), affording six subfractions, MF1-MF6. Subfraction MF2 was subjected to silica gel FCC using a step-gradient of EtOAc: *n*-hexane (from 20:80 to 100:0) to provide compounds **5** (12.3 mg), **6** (7.3 mg), and **7** (28.7 mg).

Fungal isolates: *Pythium insidiosum* strain SIMI6666, was clinically isolated from the cornea pus of a patient with ocular pythiosis who lives in Kampang Phet province, Thailand [14]. The isolated fungus was identified by its phenotypic (macroscopic and microscopic morphology, zoospore production) and genotypic profiles (PCR-based technique). The fungal strain was subcultured on Sabouraud dextrose agar (Oxoid, UK) slant and stored at 25 ºC.

Antifungal susceptibility: The antifungal susceptibility test of the isolated compounds was performed with *Pythium insidiosum* strains by the disc diffusion method and applied following CLSI M-51P guideline. The entire surface of each 100 mm diameter non-supplemented Sabouraud dextrose agar (SDA) (Oxoid, UK) plate was inoculated with the 1 × 1 cm hyphal block of *Pythium insidiosum*.

To test the minimum inhibitory concentration of isolated compounds, 10-fold serial dilutions of the compound in the range from 1 mg to 1 ng/100 µL were prepared. Then, 20 mL of each dilution of the tested compound were impregnated on sterilized discs (6.0 mm) (Whatman, England) and placed on a Sabouraud dextrose agar (SDA) plate (Oxoid, UK). Amphotericin-B (20 mg/disc) (Himedia, India) and a disc with either EtOAc or MeOH only was used as control disc. Plates were kept at room temperature for 2 h in a laminar flow cabinet, and incubated at 25 ºC for 3, 6 and 9 days. The MIC of each compound was determined by visual observation and represents the inhibition of 100% of mycelium growth of *Pythium insidiosum*.

RESULTS AND DISCUSSION

Extraction, isolation and structure elucidation: Chemical investigation of the stems of *Dalbergia cultrata* using chromatographic methods led to the isolation of 14 known compounds, including 10 isoflavones (**1**-**10**), 2 isoflavanones (**11**-**12**), a flavanonol (**13**) and a chalcone (**14**) (Fig. 1). By comparing their NMR spectra and CD data with those reported in literature, the known compounds were identified as tectorigenin (**1**) [15], calycosin (**2**) [16], pratensein (**3**), afromosin (**4**), irilin D (**5**) [17], biochanin A (**6**), daidzein (**7**) [18], formononetin (**8**), 8-O-methylretusin (**9**) [19], 7,32-dihydroxy-52-methoxyisoflavone (**10**), (3*S*)-sativanone (**11**) [20], (3*S*)-violanone (**12**) [21], erycibenin D (**13**) [22], and isoliquiritigenin (**14**) [23].

Tectorigenin (1): Yellow amorphous solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 8.34 (1H, s, H-2), 6.51 (1H, s, H-8), 7.38 (2H, d, *J* = 8.6 Hz, H-2′,6′), 6.82 (1H, d, *J* = 8.6 Hz, H-3′,5′), 13.06 (1H, s, OH), 3.75 (3H, s, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 155.1 (C-2), 123.0 (C-3), 181.8 (C-4), 153.9 (C-5), 132.3 (C-6), 157.8 (C-7), 95.4 (C-8), 154.1 (C-9), 106.1 (C-10), 122.8 (C-1′), 131.5 (C-2′,6′), 116.5 (C-3′,5′), 155.1 (C-4′), 61.4 (OCH_3).

Calycosin (2): White solid; m.p.: 250-252 ºC; ^{1}H NMR (400 MHz, acetone-d_6) δ_H: 8.16 (1H, s, H-2), 8.06 (1H, d, *J* = 8.3 Hz, H-5), 6.98 (1H, dd, *J* = 8.3, 2.3 Hz, H-6), 6.90 (1H, d, *J* = 2.3 Hz, H-8), 7.17 (1H, d, *J* = 2.1 Hz, H-2′), 7.00 (1H, d, *J* = 8.3 Hz, H-5′), 7.07 (1H, dd, *J* = 8.3, 2.1 Hz, H-6′), 3.88 (3H, s, OCH_3). ^{13}C NMR (100 MHz, acetone-d_6) δ_C: 152.6 (C-2), 124.2 (C-3), 174.8 (C-4), 127.6 (C-5), 114.8 (C-6), 162.5 (C-7), 102.2 (C-8), 157.9 (C-9), 117.6 (C-10), 125.4 (C-1′), 116.1 (C-2′), 146.2 (C-3′), 147.4 (C-4′), 111.3 (C-5′), 120.2 (C-6′), 55.4 (OCH_3).

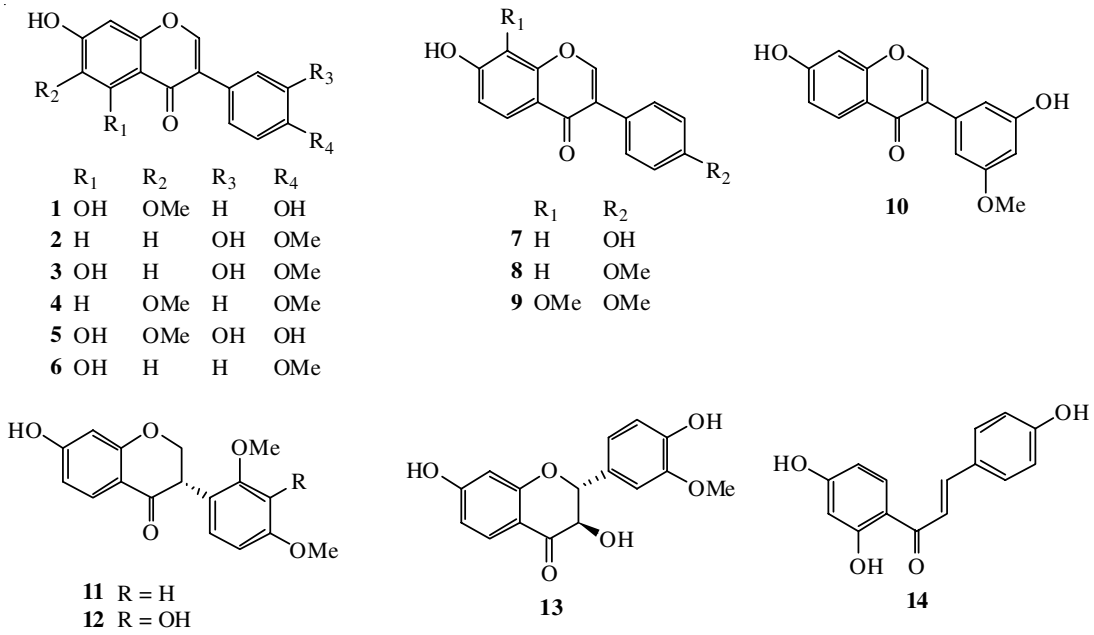


Fig. 1. Chemical structures of isolated compounds **1**-**14** from the stems of *D. cultrata*

Pratensein (3): Colorless crystals; m.p.: 269–271 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 8.30 (1H, s, H-2), 6.35 (1H, d, J = 1.5 Hz, H-8), 6.19 (1H, d, J = 1.5 Hz, H-6), 7.03 (1H, d, J = 1.9 Hz, H-2′), 6.94 (1H, m, H-5′,6′), 12.95 (1H, s, OH), 9.07 (1H, s, OH), 3.80 (3H, s, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 154.2 (C-2), 122.2 (C-3), 180.3 (C-4), 161.4 (C-5), 99.2 (C-6), 164.0 (C-7), 94.2 (C-8), 157.7 (C-9), 104.5 (C-10), 123.1 (C-1′), 115.9 (C-2′), 145.2 (C-3′), 147.6 (C-4′), 112.0 (C-5′), 120.6 (C-6′), 55.5 (OCH_3).

Afromosin (4): Needles; m.p.: 225-228 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 8.32 (1H, s, H-2), 7.43 (1H, s, H-5), 6.95 (1H, s, H-8), 7.51 (2H, d, J = 8.7 Hz, H-22, 62), 6.98 (2H, d, J = 8.7 Hz, H-32, 52), 3.88 (3H, s, OCH_3). 3.78 (3H, s, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 153.0 (C-2), 122.8 (C-3), 174.5 (C-4), 104.8 (C-5), 147.1 (C-6), 153.1 (C-7), 103.0 (C-8), 151.9 (C-9), 116.4 (C-10), 124.6 (C-1′), 130.3 (C-22, 6′), 113.8 (C-32, 5′), 159.1 (C-4′), 56.0 (OCH_3), 55.3 (OCH_3).

Irilin D (5): Amorphous powder; ^{1}H NMR (400 MHz, acetone-d_6) δ_H: 8.17 (1H, s, H-2), 6.50 (1H, s, H-8), 7.15 (1H, d, J = 1.8 Hz, H-2′), 6.88 (1H, d, J = 8.2 Hz, H-5′), 6.94 (1H, dd, J = 8.2, 1.8 Hz, H-6′), 13.27 (1H, s, OH), 3.87 (3H, s, OCH_3). ^{13}C NMR (100 MHz, acetone-d_6) δ_C: 153.7 (C-2), 122.8 (C-3), 181.2 (C-4), 153.6 (C-5), 131.3 (C-6), 156.9 (C-7), 93.5 (C-8), 153.3 (C-9), 105.7 (C-10), 122.7 (C-1′), 116.4 (C-2′), 144.8 (C-3′), 145.4 (C-4′), 115.1 (C-5′), 120.6 (C-6′) , 59.8 (OCH_3).

Biochanin A (6): Brown amorphous gum; ^{1}H NMR (400 MHz, $CDCl_3$:CD_3OD, 6:1) δ_H: 7.79 (1H, s, H-2), 6.30 (1H, d, J = 2.0 Hz, H-8), 6.23 (1H, d, J = 2.0 Hz, H-6), 7.38 (2H, d, J = 8.8 Hz, H-2′,6′), 6.92 (2H, d, J = 8.8 Hz, H-5′,3′), 12.88 (1H, s, OH), 3.84 (3H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$: CD_3OD, 6:1) δ_C: 152.6 (C-2), 123.4 (C-3), 180.7 (C-4), 164.0 (C-5), 99.3 (C-6), 162.1 (C-7), 94.1 (C-8), 158.1 (C-9), 105.4 (C-10), 123.0 (C-1′), 130.1 (C-2′,6′), 114.0 (C-3′,5′), 159.6 (C-4′), 55.3 (OCH_3).

Daidzein (7): White powder; m.p.: 320-322 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 8.27 (1H, s, H-2), 7.96 (1H, d, J = 8.8 Hz, H-5), 6.94 (1H, dd, J = 8.8, 1.4 Hz, H-6), 6.85 (1H, d, J = 1.4 Hz, H-8), 7.38 (2H, d, J = 8.3 Hz, H-2′,6′), 6.80 (2H, d, J = 8.3 Hz, H-3′,5′). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 152.8 (C-2), 122.5 (C-3), 174.7 (C-4), 127.3 (C-5), 115.1 (C-6), 162.6 (C-7), 102.1 (C-8), 157.4 (C-9), 116.6 (C-10), 123.5 (C-1′), 130.1 (C-2′,6′), 114.9 (C-3′,5′), 157.2 (C-4′).

Formononetin (8): Needles; m.p.: 256-258 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 8.31 (1H, s, H-2), 7.97 (1H, d, J = 8.7 Hz, H-5), 6.94 (1H, d, J = 8.7, 2.0 Hz, H-6), 6.86 (1H, d, J = 2.0 Hz, H-8), 7.50 (2H, d, J = 8.8 Hz, H-2′,6′), 6.97 (2H, d, J = 8.8 Hz, H-3′,5′), 3.77 (3H, s, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 153.2 (C-2), 123.5 (C-3), 174.6 (C-4), 127.3 (C-5), 115.2 (C-6), 162.6 (C-7), 102.1 (C-8), 157.5 (C-9), 116.6 (C-10), 124.2 (C-1′), 130.1 (C-2′,6′), 113.6 (C-3′,5′), 159.0 (C-4′), 55.2 (OCH_3).

8-O-Methylretusin (9): Needles; m.p.: 231-232 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 7.98 (1H, d, J = 8.8 Hz, H-5), 7.97 (1H, s, H-2), 7.49 (2H, d, J = 8.6 Hz, H-2′, 6′), 7.05 (1H, d, J = 8.8 Hz, H-6), 6.98 (2H, d, J = 8.6 Hz, H-3′, 5′), 6.28 (1H, s, OH), 3.85 (3H, s, OCH_3), 4.08 (3H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 151.4 (C-2), 124.8 (C-3), 175.7 (C-4), 122.2 (C-5), 113.7 (C-6), 152.9 (C-7), 133.9 (C-8), 149.9 (C-9), 119.0 (C-10), 123.9 (C-1′), 130.2 (C-2′,6′), 114.0 (C-3′, 52), 159.7 (C-4′), 61.9 (OCH_3), 55.3 (OCH_3).

7,3′-Dihydroxy-5′-methoxyisoflavone (10): Pale yellow needles; m.p. 210-211 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 8.24 (1H, s, H-2), 7.95 (1H, d, J = 8.8 Hz, H-5), 6.91 (1H, m, H-6), 6.84 (1H, d, J = 2.1 Hz, H-8), 7.01 (1H, s, H-4′), 6.93 (2H, s, H-2′,6′), 3.76 (3H, s, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 153.5 (C-2), 124.9 (C-3), 175.2 (C-4), 127.7 (C-5), 115.6 (C-6), 162.9 (C-7), 102.5 (C-8), 157.8 (C-9), 116.9 (C-10), 123.7 (C-1′), 112.3 (C-2′), 146.3 (C-3′), 116.7 (C-4′), 147.9 (C-5′), 120.2 (C-6′) , 56.0 (OCH_3).

(3S)-Sativanone (11): White amorphous solid; $[\alpha]_D^{26.5}$ +42.0 (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$:CD_3OD, 3:1) δ_H: 7.78 (1H, d, *J* = 8.7 Hz, H-5), 6.33 (1H, d, *J* = 2.2 Hz, H-8), 6.49 (1H, dd, *J* = 8.7, 2.2 Hz, H-6), 4.51 (1H, dd, *J* = 11.0, 11.2 Hz, H-2a), 4.39 (1H, dd, *J* = 11.0, 5.4 Hz, H-2b), 4.16 (1H, dd, *J* = 11.2, 5.4 Hz, H-3), 6.94 (1H, d, *J* = 8.4 Hz, H-6′), 6.42 (1H, dd, *J* = 8.4, 2.4 Hz, H-5′), 6.46 (1H, d, *J* = 2.4 Hz, H-3′), 3.74 (3H, s, OCH_3), 3.72 (3H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD, 3:1) δ_C: 70.9 (C-2), 47.4 (C-3), 193.0 (C-4), 129.4 (C-5), 110.7 (C-6), 164.7 (C-7), 102.6 (C-8), 164.1 (C-9), 114.5 (C-10), 116.0 (C-1′), 158.4 (C-2′), 98.9 (C-3′), 160.5 (C-4′), 104.7 (C-5′), 130.6 (C-6′), 55.2 (OCH_3), 55.1 (OCH_3).

(3*S*)-Violanone (**12**): White needles; m.p.: 200-202 ºC; ^{1}H NMR (400 MHz, $CDCl_3$:CD_3OD, 3:1) δ_H: 7.76 (1H, d, *J* = 8.7 Hz, H-5), 6.45 (1H, dd, *J* = 8.7, 2.1 Hz, H-6), 6.31 (1H, d, *J* = 2.1 Hz, H-8), 4.49 (1H, dd, *J* = 11.5, 11.0 Hz, H-2a), 4.37 (1H, dd, *J* = 11.0, 5.4 Hz, H-2b), 4.09 (1H, dd, *J* = 11.5, 5.4 Hz, H-3), 6.54 (1H, d, *J* = 8.5 Hz, H-6′), 6.50 (1H, d, *J* = 8.5 Hz, H-5′), 3.75 (3H, s, OCH_3), 3.79 (3H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD, 3:1) δ_C: 71.3 (C-2), 47.8 (C-3), 192.8 (C-4), 129.4 (C-5), 110.7 (C-6), 164.7 (C-7), 102.7 (C-8), 164.1 (C-9), 114.5 (C-10), 121.6 (C-1′), 148.2 (C-2′), 139.1 (C-3′), 145.9 (C-4′), 106.7 (C-5′), 119.5 (C-6′), 60.1 (OCH_3), 55.9 (OCH_3).

Erycibenin D (13): Pale yellow powder; $[\alpha]_D^{26.5}$ -35.0 (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$:CD_3OD, 1:1) δ_H: 7.65 (1H, d, *J* = 8.7 Hz, H-5), 6.46 (1H, dd, *J* = 8.7, 2.2 Hz, H-6), 6.28 (1H, d, *J* = 2.2 Hz, H-8), 4.94 (1H, d, *J* = 11.9 Hz, H-2), 4.45 (1H, d, *J* = 11.9 Hz, H-3), 7.00 (1H, d, *J* = 1.9 Hz, H-2′), 6.91 (1H, dd, *J* = 8.2, 1.9 Hz, H-6′), 6.79 (1H, d, *J* = 8.2 Hz, H-5′), 3.83 (3H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$:CD_3OD, 1:1) δ_C: 84.2 (C-2), 73.2 (C-3), 193.1 (C-4), 128.9 (C-5), 111.3 (C-6), 166.0 (C-7), 102.8 (C-8), 163.7 (C-9), 111.8 (C-10), 128.4 (C-1′), 110.9 (C-2′), 147.5 (C-3′), 146.9 (C-4′), 114.9 (C-5′), 120.8 (C-6′), 55.4 (OCH_3).

Isoliquiritigenin (14): Pale yellow solid; 203-205 ºC; ^{1}H NMR (400 MHz, $CDCl_3$:CD_3OD, 3:1) δ_H: 7.48 (2H, d, *J* = 8.6 Hz, H-2, 6), 6.81 (2H, d, *J* = 8.6 Hz, H-3, 5), 7.74 (1H, d, *J* = 8.8 Hz, H-6′), 6.37 (1H, dd, *J* = 8.8, 2.4 Hz, H-5′), 6.30 (1H, d, *J* = 2.4 Hz, H-3′), 7.75 (1H, d, *J* = 15.0 Hz, H-β), 7.38 (1H, d, *J* = 15.0 Hz, H-α).

Antifungal activity: The isolated compounds were evaluated for antifungal activity against *P. insidiosum* using disc diffusion assay. Four of the isolated compounds, including tectorigenin (**1**), (3*S*)-sativanone (**11**), (3*S*)-violanone (**12**) and isoliquiritigenin (**14**) exhibited antifungal activity which was compared with the antifungal agent, amphotericin B standard and the results are shown in Table-1. Among the tested compounds, compound **1** showed the highest antifungal activity against *P. insidiosum* with an MIC value of 10 mg/disc, which is more potent than the standard (conc. 20 mg/disc) (Fig. 2). Comparing the activity of isoflavones **1** and **5**, it was suggested that the hydroxy group at the C-3′ position is detrimental to the activity. In the cases of compounds **1**, **3** and **6**, it seems that the methoxy group at the C-6 position is necessary for the activity, and in addition, the methoxy group at the C-4′ position led to a reduction in potency. Comparing between compounds **1** and **4**, the results show that the presence of hydroxy groups at C-5 and C-4′ was important for the activity. It was found that 7,4′-dihydroxyisoflavone (**7**), which does not contain the hydroxy group at C-5 and methoxy group at C-6, showed no activity, indicating that these two groups were important for the inhibition of the mycelial growth of this microorganism. In our previous report, an isoflavanone (vestitol) from *Dalbergia stipulacea* gave a strong inhibition activity against *Pythium insidiosum* (MIC = 1 mg/disc) [24] while isoflavanones **11** and **12** showed an MIC value of 100 mg/disc. Thus, the position of hydroxy group at C-2′ on ring B may play an important role in this activity. All these data indicate that compound **1** is likely to be useful as a lead compound for the development of an antifungal agent to *Pythium insidiosum*.

Conclusion

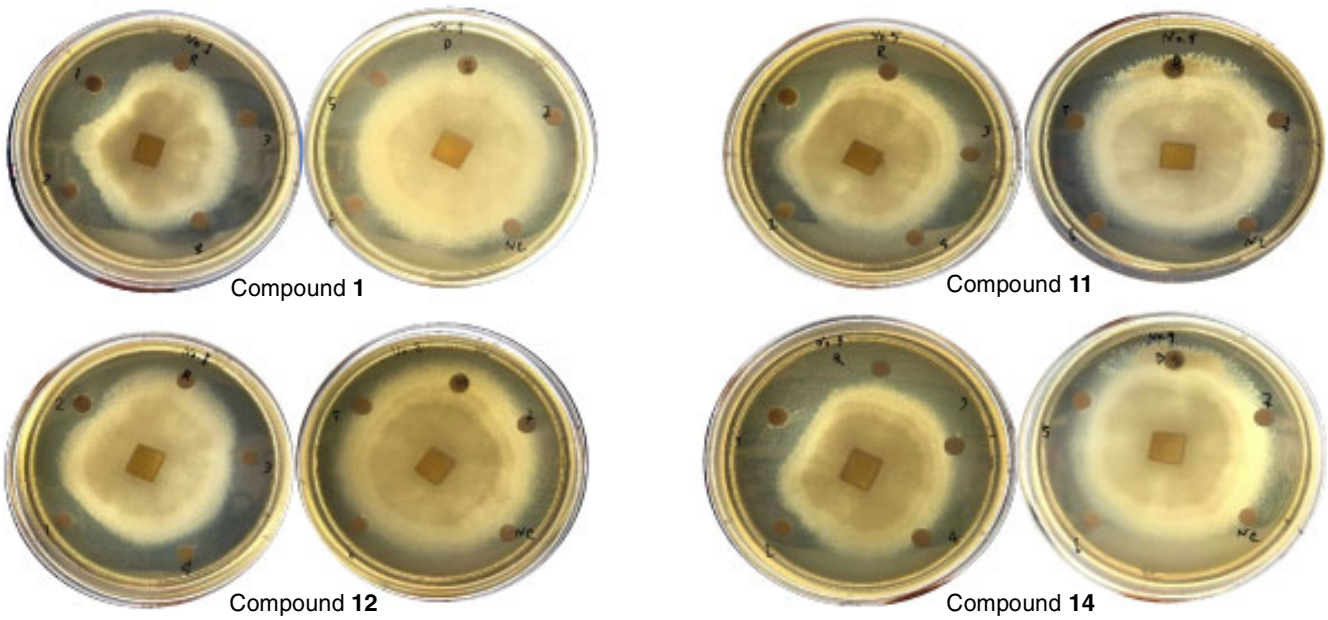
In this study, 14 compounds (**1**-**14**), including 10 isoflavones (**1**-**10**), 2 isoflavanones (**11**-**12**), a flavanonol (**13**) and a chalcone (**14**) were isolated from the stems of *Dalbergia cultrata*. The antifungal activity of the isolated compounds against *P. insidiosum* shows that compounds **1**, **11**, **12,** and **14** can inhibit the mycelial growth of *Pythium insidiosum* with MIC values of 10 to 100 mg/disc. Moreover, compound **1** exhibits stronger activity than the amphotericin-B standard, with an MIC value of 10 µg/disc.

ACKNOWLEDGEMENTS

The Post-Doctoral Training Program from the Research Affairs and Graduate School, Khon Kaen University for Thurdpong Sribuhom is acknowledged (Grant no. 591471). This work was supported by the Thailand Research Fund and Khon Kaen University (RSA6280050). The Center of Excellence for Innovation in Chemistry (PERCH-CIC), Ministry of Higher Education, Science, Research and Innovation (Implementation Unit, Khon Kaen), Thailand is acknowledged.

TABLE-1
ANTIFUNGAL ACTIVITY OF COMPOUNDS AGAINST *P. insidiosum*

Compound	Inhibition zone (mm)				
	1000 (µg/disc)	100 (µg/disc)	10 (µg/disc)	1 (µg/disc)	MIC (µg/disc)
1	7.09	4.93	1.71	Inactive	10
11	4.76	2.01	Inactive	Inactive	100
12	5.47	2.06	Inactive	Inactive	100
14	3.77	2.79	Inactive	Inactive	100
The others	Inactive	Inactive	Inactive	Inactive	Inactive
Amphotericin-B	Inactive at 20 µg/disc				



D = Drug – Amphotericin-B; NC = Sterile distilled water; R = Solvent
1 = Conc. 1 mg/disc; 2 = Conc. 0.1 mg/disc; 3 = Conc. 0.01 mg/disc; 4 = Conc. 1 µg/disc;
5 = Conc. 0.1 µg/disc; 6 = Conc. 0.01 µg/disc; 7 = Conc. 0.001 µg/disc

Fig. 2. Disc diffusion of antifungal activity against *P. insidiosum*

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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