ABSTRACT

Objective: Isolation of compounds from *Dendrobium williamsonii* and evaluation of each isolates for its free radical scavenging, antitherpetic and cytotoxic activities. Results: Six phenolic compounds were isolated including tetratriacontanyl-trans-p-coumarate (1), trans-docosanoylferulate (2), 3,3′-dihydroxy-4,5-dimethoxybibenzyl (3), moscatilin (4), apigenin (5) and vanillic acid (6). Among these isolates, compounds 3, 4 and 5 exhibited recognizable DPPH free radical scavenging potential. Only 3 exhibited weak activity against *Herpes simplex* virus, whereas 3 and 4 showed cytotoxicity against KB and MCF-7 cancer cells. Conclusion: This study is the first report on the chemical and biological properties of *D. williamsonii*. Compounds 3, 4 and 5 are responsible for free radical scavenging activity of this plant. Compound 4 showed the strongest cytotoxic effect on KB cancer cells.

Keywords: *Dendrobium williamsonii*, Orchidaceae, Anti-herpetic Cytotoxicity, Free radical scavenging activity

INTRODUCTION

The genus *Dendrobium* (Orchidaceae) is represented by more than 1,100 species widely distributed throughout Asia and Australia, and in Thailand about 150 species of *Dendrobium* have been identified.[1] The stems of several *Dendrobium* species have been used in traditional Chinese medicine as tonics to reduce fever and promote the production of body fluid.[2] They are also used to treat kidney and lung disorders, stomach diseases, red tongue, swelling, dry mouth, hyperglycemia and diabetes.[3] Plants of the genus *Dendrobium* (Orchidaceae) have been known to produce a wide variety of chemical compounds, including alkaloids, bibenzyls, phenanthrenes, fluorenones, steroids, sesquiterpenes, coumarins and polysaccharides.[4,5] Recent biological studies have shown that some *Dendrobium* species possess anti-platelet aggregation, anti-fibrotic, free radical scavenging, immunomodulatory and cytotoxic activities.[5–8]

*Dendrobium williamsonii* Day &Rchb. f. is known in Thai as “Ueangngoen sad”. [9] It is also known as Williamson’s *Dendrobium*. This plant species is found in Thailand, India, Vietnam, China and Mianmar.[9] In Yunnan Province of China, the decoction of stems or whole plant from *D. williamsonii* has been used as poultice to treat adynamia, dyspepsia, numbness of limbs, and injuries from falls and fracture.[10] In Thailand, this plant is an ornamental plant without recorded traditional medicinal uses. Prior to this investigation, there were no reports on the chemical and biological properties of this plant. As a part of our continuing studies on bioactive phenolics from *Dendrobium* plants,[11–14] an extract prepared from the whole part of this plant was evaluated and found to possess DPPH radical scavenging, cytotoxic, and anti-herpetic activities. In this communication, we report the chemical components of this plant, as well as our studies on their DPPH free radical scavenging, cytotoxic and anti-herpetic properties.
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MATERIALS AND METHODS

General experimental procedures

Mass spectra were recorded on a Micromass LCT mass spectrometer (ESI-MS). NMR spectra were recorded on a Bruker Advance DPX-300 FT-NMR spectrometer or a Varian Unity INOVA-500 NMR spectrometer. Microtiter plate reading was performed on a Perkin-Elmer Victor™ 1420 multilabel counter. Vacuum-liquid chromatography (VLC) and column chromatography (CC) were performed by CC over silica gel (CH2Cl2-hexane-EtOAc, gradient) and then further purified on Sephadex LH-20 (790 mg) was separated by CC (silica gel; CH2Cl2-MeOH, gradient) to give 6 (3 mg). Trans-Docosanoylferulate (2)

White powder, C32H54O8, ESI-MS m/z 525 [M+Na]+.1H NMR (500 MHz, CDCl3) δ 8.05 (3H, t, J = 7.0 Hz, CH3), 1.23 (38H, br s, CH2-n), 1.67 (2H, m, CH2-2′), 3.90 (3H, s, MeO-3), 4.16 (2H, t, J = 7.6 Hz, CH2-O-1′), 6.26 (1H, d, J = 16.0 Hz, H-b′), 6.89 (1H, d, J = 8.0 Hz, H-5), 7.01 (1H, d, J = 1.5 Hz, H-2), 7.05 (1H, dd, J = 8.0, 1.5 Hz, H-6), 7.58 (1H, d, J = 16.0 Hz, H-6). 13C NMR (125 MHz, CDCl3) δ 14.1 (CH3), 22.7-31.9 (long chain CH2), 55.9 (MeO-3), 64.6 (CH2-O-1′), 109.3 (C-2), 114.7 (C-5), 115.6 (C-b′), 123.0 (C-6), 127.0 (C-1), 144.6 (C-α), 146.7 (C-3), 147.9 (C-4), 167.4 (OC=O).

Dihydroxy-4,5-dimethoxybibenzyl (3)

Brown amorphous solid, C18H18O6, ESI-MS m/z 275 [M+H]+. 1H NMR (300 MHz, CDCl3) δ: 2.83 (4H, br s, H2-α, H2-α′), 3.83 (3H, s, MeO-2), 3.89 (3H, s, MeO-4), 6.27 (1H, brs, H-6), 6.49 (1H, brs, H-2′), 6.68 (1H, br, H-2), 6.76 (1H, brd, J = 7.6, H-6′), 7.16 (1H, brd, J = 7.5, H-4′), 7.16 (1H, dd, J = 7.6, 7.5 Hz, H-5′). 13C NMR (75 MHz, CDCl3) δ: 37.5 (C-α), 37.6 (C-α′), 55.8, 60.9 (MeO-3, MeO-4), 104.6 (C-6), 108.0 (C-2), 112.9 (C-4′), 115.4 (C-2′-2′), 120.7 (C-6′), 129.4 (C-5′), 133 (C-1′), 138.1 (C-4), 143.5 (C-1), 148.9 (C-3), 152.1 (C-5), 155.7 (C-3′).

Moscatilin (4)

Brown amorphous solid, C15H18O5, ESI-MS m/z 305 [M+H]+. 1H NMR (300 MHz, CDCl3) δ: 2.80 (4H, br s, H2-α, H2-α′), 3.83 (9H, s, MeO-3, MeO-3′, MeO-5′), 6.34 (2H, s, H-2), 6.60 (1H, brs, H-2′), 6.66 (1H, brd, J = 8.1 Hz, H-6′), 6.82 (1H, d, J = 8.1 Hz, H-5′). 13C NMR (75 MHz, CDCl3) δ: 37.8 (C-α), 38.4 (C-α′), 55.2 (MeO-3′), 56.0 (MeO-3), 60.9 (MeO-4), 104.6 (C-6), 108.0 (C-2), 112.9 (C-4), 115.4 (C-2′-2′), 120.7 (C-6′), 129.4 (C-5′), 133 (C-1′), 138.1 (C-4), 143.5 (C-1), 148.9 (C-3), 152.1 (C-5), 155.7 (C-3′).
55.8 (MeO-3, MeO-5) 105.2 (C-2,6), 111.2 (C-2), 114.1 (C-5), 121.0(C-6), 132.8 (C-1), 132.9 (C-1), 133. (C-4), 143.7 (C-4'), 146.2 (C-3'), 146.8(C-3,5).

**Apigenin (5)**

Yellow powder, C_{15}H_{10}O_{5} ESI-MSm/z 271 [M+H]+. \(^1\)H NMR (300 MHz, Acetone -d\(_6\)) \(\delta\): 6.24 (1H,d, J = 1.7 Hz, H-6), 6.54 (1H, d, J = 1.7 Hz, H-8), 6.63 (1H, s, H-3), 7.01 (2H, d, J = 8.7 Hz, H-3',5'), 7.95 (2H, d, J = 8.7 Hz, H-2',6'), 13.01 (1H,br s, HO-5). \(^1^3\)C NMR (75 MHz, Acetone -d\(_6\))\(\delta\): 94.7 (C-8), 99.7 (C-6), 104.1(C-3), 105.3 (C-10), 116.9 (C-3',5'), 123.3 (C-1'), 129.2 (C-2',6'), 158.8 (C-9), 161.9 (C-4'), 163.4 (C-5), 164.9 (C-7), 165.1 (C-2), 183.1 (C-4).

**Vanillic acid (6)**

Colorless powder, C_{9}H_{5}O_{3}ESI-MS m/z 169 [M+H]+. \(^1\)H NMR (500 MHz, Acetone -d\(_6\)) \(\delta\): 3.89 (3H, s, MeO-3), 6.89 (1H, d, J = 8.5 Hz, H-5), 7.75 (1H, d, J = 2.0 Hz, H-2), 7.58 (1H, dd, J = 8.5, 2.0 Hz, H-6). \(^1^3\)C NMR (125 MHz, Acetone-d\(_6\))\(\delta\): 56.3 (MeO-3), 113.4 (C-2), 115.5 (C-5), 122.9 (C-1), 124.8 (C-6), 148.0 (C-4), 152.0 (C-3), 167.4 (COOH).

**DPPH radical scavenging method**

The free radical scavenging effect of the samples was assessed by measuring their ability to decolor a methanolic solution of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH, Sigma).\(^{[21]}\) Briefly, test samples were initially prepared as a solution in EtOH (1000 µg/ml). Each compound was first tested at the concentration of 100 µg/ml. An \(IC_{50}\) value was determined if the compound showed more than 50% inhibition. For \(IC_{50}\) analysis, two fold serial dilutions were performed to give seven concentrations. The test was done by addition of the sample solution (20 µl) to the solution of 50 µM DPPH in EtOH (180 µl) in a 96-well microtiter plate. The reaction mixture was incubated at room temperature for 30 min, and then its absorbance at 510 nm was measured with a microplate reader. Quercetin (Sigma) and vitamin C were used as positive control.

**Assay of Anti-HSV activity**

Antiviral activity against HSV-1 (Strain KOS) and HSV-2 (Strain 186) was determined using plaque reduction method, as previously described.\(^{[19]}\) Briefly, virus (30 PFU/25 ml) was mixed with 25 ml of complete medium containing various concentrations of test compound and then incubated at 37°C for 1h. After incubation, the mixtures were added to Vero cells (6 × 10\(^5\) cells/well) in 96-well microtiter plates and incubated at 37°C for 2 h. The overlay medium containing the various concentrations of test compound was added to the Vero cells and incubated at 37°C in humidified CO\(_2\) incubator for 2 days. Then, virus growth inhibition was evaluated by counting the virus plaque forming on Vero cells compared with the controls. The cells also were stained with 1% crystal violet in 10% formalin for 1 h. The percent plaque inhibition was determined. Acyclovir was used as positive control.

**Cytotoxic activity assay**

Evaluations of cytotoxic activity against KB (epidermoid carcinoma of oral cavity) and MCF-7 (breast cancer) cancer cells were performed by using resazurinmicroplate assay (REMA).\(^{[13]}\) Ellipticine, doxorubicin and tamoxifen were used as positive controls, and 0.5% DMSO was used as a negative control. Briefly, cells at a logarithmic growth phase were harvested and diluted to \(7 \times 10^4\) cells/ml for KB and \(9 \times 10^4\) cells/ml for MCF-7 in fresh medium. Consecutively, 5 ml of test sample diluted in 5% DMSO, and 45 ml of cell suspension were added to 384-well plates then incubated at 37°C in 5% CO\(_2\) incubator. After incubation for 3 days, 12.5 ml of 62.5 mg/ml resazurin solution was added to each well, then incubated at 37°C for 4 hours. After that the fluorescent signal at the excitation and emission wavelengths of 530 nm and 590 nm, respectively, were measured with a SpectraMax M5 multi-detection microplate reader (Molecular Devices, USA). The percent inhibition of cell growth was calculated by using the following equation.

\[
\% \text{Inhibition} = \left[1-\left(\text{FU}_{\text{t}}/\text{FU}_{\text{c}}\right)\right] \times 100
\]

Whereas, \(\text{FU}_{\text{t}}\) and \(\text{FU}_{\text{c}}\) are the mean fluorescent unit from treated and untreated conditions, respectively. The dose response curves were plotted from 6 concentrations of 2-fold serially diluted test compounds and the sample concentrations that inhibit cell growth by 50% (\(IC_{50}\)) can be acquired by using the SOFTMax Pro software (Molecular Devices, USA).

**RESULTS AND DISCUSSION**

Phytochemical investigation of the MeOH extract of the whole plant of *D. williamsonii* led to the isolation of six phenolic compounds (Figure 1). The structures of the isolates were determined through analysis of their spectroscopic data in comparison with reported values, and were identified as tetraatricontanyl-trans-p-coumarate(1)\(^{[16,17]}\), trans-docosanolylferulate(2)\(^{[18]}\), 3,3'-dihydroxy-4,5-dimethoxybenzyl (3)\(^{[19]}\), moscatilin (4)\(^{[20]}\), apigenin (5)\(^{[21]}\) and vanillic acid (6)\(^{[22]}\).
To the best of our knowledge, this is the first report on the chemical constituents and biological activities of *D. williamsonii*. Among the isolated compounds (1–6), tetraatriacantyl-trans-p-coumarate (1) and 3, 3′-dihydroxy-4, 5-dimethoxybibenzyl (3) were identified from *Dendrobium* and Orchidaceous plants for the first time. Compounds 1 and 3 were the first isolated from the root sprouts of *Agrimoniapilosa* (Rosaceae) and the leaves of crowberry (*Empetrumnigrum*, Ericaceae), respectively, and there are no records of biological activity of both compounds.

These isolates (1–6) were then evaluated for DPPH free radical scavenging, cytotoxic and anti-HSV activities and the results are shown in Table 1. For the DPPH radical scavenging activity, moscatilin (4) showed the strongest activity with IC₅₀ 8.5 µM, whereas 3,3′-dihydroxy-4, 5-dimethoxybibenzyl (3) and apigenin (5) exhibited appreciable activity (IC₅₀ 19.5 and 19.3 µM, respectively), as compared with the positive controls quercetin (IC₅₀ 8.3 and 42.4 µM, respectively). Apigenin, a flavonoid found in many plants such as Chinese cabbage, garlic, guava, and celery, was found to possess strong radical scavenging against several reactive oxygen species. Additionally, 3, 3′-dihydroxy-4, 5-dimethoxybibenzyl (3) exhibited weak cytotoxicity against KB cells (IC₅₀ 195.0 µM) and MCF-7 cells (IC₅₀ 187.7 µM). Previously, cytotoxicity of moscatilin against several cancer cell lines has been reported. Moscatilin was able to induce apoptosis in colorectal cancer cells through tubulin depolymerization and DNA damage stress. This compound also exhibited anti-angiogenic activity by inhibition of angiogenic factor signaling pathway.

Moreover, this compound can inhibit lipid peroxidation in isolated rat hepatocytes. Free radical scavenging of moscatilin also have been report. Recent study demonstrated that moscatilin was able to inhibit human lung cancer cell migration and invasion via an attenuation of endogenous reactive oxygen species especially suppression of hydroxyl radical. For cytotoxic activity, as expected, moscatilin (4), a compound previously identified from several other *Dendrobium* species, showed strong cytotoxic activity against KB cell line with an IC₅₀ value of 2.6 µM but exhibited weak activity against MCF-7 cell line (IC₅₀ 112.4 µM), in comparison with the positive controls ellipticine (IC₅₀ 1.8 µM) and doxorubicin (IC₅₀ 1.0 µM). Therefore, it should be noted that moscatilin (4) has a high selectivity to KB cell line. In addition, 3, 3′-dihydroxy-4, 5-dimethoxybibenzyl (3) exhibited weak cytotoxicity against KB cells (IC₅₀ 195.0 µM) and MCF-7 cells (IC₅₀ 187.7 µM). Previously, cytotoxicity of moscatilin against several cancer cell lines has been reported. Moscatilin was able to induce apoptosis in colorectal cancer cells through tubulin depolymerization and DNA damage stress. This compound also exhibited anti-angiogenic activity by inhibition of angiogenic factor signaling pathway.

As above data, moscatilin has been shown to be a potential anticancer agent, and *D. williamsonii* can be a source for this compound. For anti-HSV activity, only compound 3 exhibited antiviral effect against HSV-1 and HSV-2 (IC₅₀ 304.1 µM and 334.5 µM, respectively), when compared with acyclovir (IC₅₀ 1.5 µM and 2.9 µM, respectively), whereas, other compounds were devoid of anti-herpetic activity.

Figure 1. Compounds 1-6 isolated from *D. williamsonii*. 
Table 1. IC₅₀ values (µM) for DPPH free radical scavenging, cytotoxic and anti-herpetic activities

<table>
<thead>
<tr>
<th>Compound</th>
<th>DPPH</th>
<th>Cytotoxicity (IC₅₀, µM)</th>
<th>Anti-herpetic (IC₅₀, µM)</th>
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<tr>
<td></td>
<td>IC₅₀ (µM)</td>
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<td>2</td>
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<td>3</td>
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<tr>
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</tr>
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NA = less than 50% inhibition at 100 µg/mL.

Conflicts of interest

All authors have none to declare.

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