Chemical Constituents of *Dendrobium williamsonii*

Pathrapa Rungwichaniwat, Boonchoo Sritularak* and Kittisak Likhitwitayawuid

Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

Submission Date: 30-1-2014

Accepted Date: 24-3-2014

ABSTRACT

Objective: Isolation of compounds from *Dendrobium williamsonii* and evaluation of each isolates for its free radical scavenging, antiherpetic 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.

***Corresponding author.**

Tel: +662 218 8356. Fax: +662 218 8357.

E-mail: boonchoo.sr@chula.ac.th. (B. Sritularak)

DOI: 10.5530/pj.2014.3.6

MATERIALS AND METHODS

General experimental procedures

Mass spectra were recorded on a Micromass LCT mass spectrometer (ESI-MS). NMR spectra were recorded on a BrukerAvance 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.Vaccuum-liquid chromatography (VLC) and column chromatography (CC) were performed on silica gel 60 (Merck, Kieselgel 60, 70-320 mesh) and silica gel 60 (Merck, Kieselgel 60, 230-400 mesh), respectively. Size-exclusion chromatography was conducted on Sephadex LH-20 (25-100 μm, Pharmacia Fine Chemical Co. Ltd.).

Plant material

The whole plant of *D. williamsonii* Day &Rchb. f. was purchased from Jatujak market, in July 2010. Plant identification was done by Prof. ThatreePhadungcharoen (Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand). A voucher specimen (BS-DW-072553) is on deposit at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

Extraction and Isolation

Dried and powdered whole plant of *D. williamsonii* (1.8 kg) was extracted with MeOH(3 × 10 L) at room temperature to give a viscous mass of dried extract (165 g) after evaporation of the solvent. This material was initially subjected to vacuum-liquid chromatography (VLC) on silica gel (*n*-hexane-EtOAc, gradient) to give 6 fractions (A-F). Fraction D (9.1 g) was further separated by column chromatography (CC) over silica gel, eluted with *n*-hexane-EtOAc (gradient) to give 10 fractions (D1-D10). Fraction D3 (650 mg) was subjected to CC (silica gel; *n*-hexane-$CHCl_2$, gradient) to give 33 fractions. Compound 1(3 mg) was obtained from fraction 26 after recrystallization from *n*-hexane. Fraction 16 was purified on Sephadex LH-20 (CH_2Cl_2-MeOH, 1:1) to furnish 2 (38 mg). Fraction D5 (790 mg) was separated by CC (silica gel; *n*-hexane-EtOAc, gradient) and then further purified on Sephadex LH-20 (CH_2Cl_2-MeOH, 1:1) to furnish 3 (20 mg). Fraction D6 (1.7 g) was subjected to repeated CC (silica gel; *n*-hexane-EtOAc, gradient) to give 7 fractions (D6.1-D6.7). Fraction D6.5 (30 mg) was separated on Sephadex LH-20 (CH_2Cl_2-MeOH, 1:1) to yield 4 (5 mg). Compound 5 (5 mg) was obtained from fraction D6.6 after purification on Sephadex LH-20 (CH_2Cl_2-MeOH, 1:1). Fraction D-7 (863 mg) was separated by CC (silica gel; CH_2Cl_2-acetone, gradient) to give 13 fractions. Separation of fraction 3 (50 mg) was performed by CC over silica gel (CH_2Cl_2-MeOH, gradient) to give 6 (3 mg).

Tetratriacontanyl-*trans-p*-coumarate (1)

White powder, $C_{43}H_{76}O_3$, ESI-MS *m/z* 663 $[M+Na]^+$.^{1}H NMR (500 MHz, $CDCl_3$) δ:0.85(3H, t, *J* = 7.0 Hz, CH_3), 1.23 (62H, br s, CH_2-n), 1.67 (2H, m, CH_2-2′), 4.16 (2H, t, *J* = 7.0 Hz, CH_2O-1′), 6.27 (1H, d, *J* = 16.0 Hz, H-*β*), 6.82 (2H, d, *J* = 8.5 Hz, H-3, H-5), 7.40 (2H, d, *J* = 8.5 Hz, H-2, H-6), 7.60 (1H, d, *J* = 16.0 Hz, H-α). ^{13}C NMR (125 MHz, $CDCl_3$) δ:14.1 (CH_3), 22.6-31.9 (long chain CH_2), 64.7 (CH_2O-1′), 115.5 (C-*β*), 115.8 (C-3, C-5), 127.1 (C-1), 129.9 (C-2, C-6), 144.4 (C-α), 157.8 (C-4), 167.7 (OC=O).

trans-Docosanoylferulate (2)

White powder, $C_{32}H_{54}O_4$, ESI-MS *m/z* 525 $[M+Na]^+$.^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.85 (3H, t, *J* = 7.0 Hz, CH_3), 1.23 (38H, br s, CH_2-n), 1.67 (2H, m, CH_2-2′), 3.90 (3H, s, MeO-3), 4.16 (2H, t, *J* = 7.0 Hz, CH_2O-1′), 6.26 (1H, d, *J* = 16.0 Hz, H-*β*), 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-α). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.1 (CH_3), 22.7-31.9 (long chain CH_2), 55.9 (MeO-3), 64.6 (CH_2O-1′), 109.3 (C-2), 114.7 (C-5), 115.6 (C-*β*), 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.$C_{16}H_{18}O_4$, ESI-MS*m/z* 275 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.83 (4H, br s, H_2-α ,H_2- 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, brs, H-2′), 6.76 (1H, brd, *J* = 7.6, H-6′), 7.15 (1H, brd, *J* = 7.5, H-4′), 7.16 (1H, dd, *J* = 7.6 ,7.5 Hz, H-5′).^{13}C NMR (75 MHz, $CDCl_3$) 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.$C_{17}H_{20}O_5$ ESI-MS*m/z* 305 $[M+H]^+$.^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.80 (4H, br s, H_2-α ,H_2-α′), 3.83 (9H, s, MeO-3, MeO-3′, MeO-5′), 6.34 (2H,s, H-2,6), 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′).^{13}C NMR (75 MHz, $CDCl_3$)δ: 37.8(C-α), 38.4 (C- α′), 55.2 (MeO-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-MS*m/z*271 $[M+H]^+$. ^1H NMR (300 MHz, Acetone -d_6) δ: 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). ^{13}C NMR (75 MHz, Acetone -d_6)δ: 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, $C_8H_8O_4$,ESI-MS *m/z* 169 $[M+H]^+$.^1H NMR (500 MHz, Acetone -d_6) δ: 3.89 (3H, s, MeO-3), 6.89 (1H, d, *J* = 8.5 Hz, H-5), 7.55 (1H, d, *J* = 2.0 Hz, H-2), 7.58 (1H, dd, *J* = 8.5, 2.0 Hz, H-6). ^{13}C NMR (125 MHz, Acetone-d_6) δ: 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).[11] 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.[15] Briefly, virus (30 PFU/25 ml) was mixed with 25 ml of complete medium containing various concentrations of test compound and then incubated at 37ºC for 1h. After incubation, the mixtures were added to Vero cells (6 × 10^5 cells/well) in 96-well microtiter plates and incubated at 37ºC for 2 h. The overlay medium containing the various concentrations of test compound was added to the Vero cells and incubated at 37ºC 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 × 10^4 cells/ml for KB and 9 × 10^4cells/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 37ºC 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 37ºC 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 M5multi-detection microplate reader (Molecular Devices, USA). The percent inhibition of cell growth was calculated by using the following equation.

$$\% \text{ Inhibition} = [1-(FU_T/FU_C)] \times 100$$

Whereas, FU_T and FU_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 tetratria contanyl-*trans*-*p*-coumarate(1)[16,17], *trans*-docosanoylferulate(2)[18], 3,3′-dihydroxy-4,5-dimethoxybibenzyl (3)[19],moscatilin (4)[20], apigenin (5)[21] and vanillic acid (6)[22].

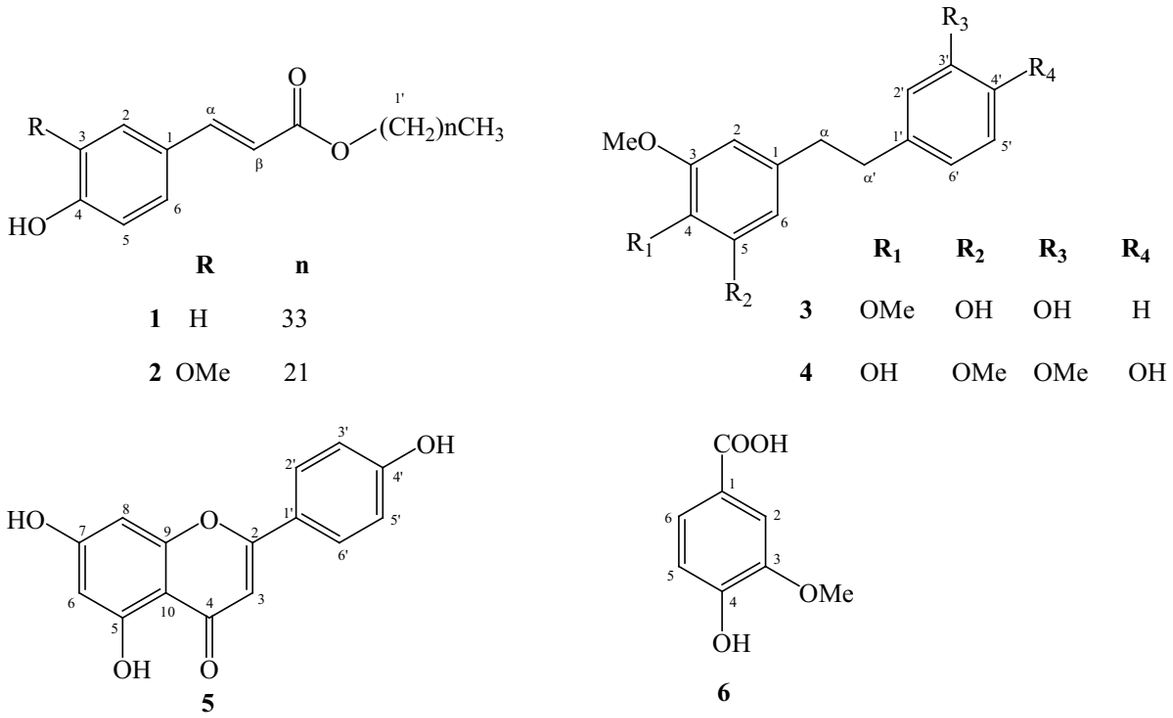

Figure 1. Compounds 1-6 isolated from *D. williamsonii*.

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), tetratriacontanyl-*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)[16] and the leaves of crowberry (*Empetrumnigrum*, Ericaceae)[19], 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 IC_{50} 8.5 μM, whereas 3,3′-dihydroxy-4, 5-dimethoxybibenzyl (3) and apigenin (5) exhibited appreciable activity (IC_{50} 19.5 and 19.3 μM, respectively), as compared with the positive controls quercetin and vitamin C (IC_{50} 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.[23,24] Moreover, this compound can inhibit lipid peroxidation in isolated rat hepatocytes.[25] Free radical scavenging of moscatilin also have been report.[4] 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.[26] For cytotoxic activity, as expected, moscatilin (4), a compound previously identified from several other *Dendrobium* species[27], showed strong cytotoxic activity against KB cell line with an IC_{50} value of 2.6 μM but exhibited weak activity against MCF-7 cell line (IC_{50}112.4 μM), in comparison with the positive controls ellipticine (IC_{50} 1.8 μM) and doxorubicin (IC_{50} 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(IC_{50} 195.0 μM) and MCF-7 cells (IC_{50} 187.7μM). Previously, cytotoxicity of moscatilin against several cancer cell lines has been reported.[14] Moscatilin was able to induce apoptosis in colorectal cancer cells through tubulin depolymerization and DNA damage stress.[28] This compound also exhibited anti-angiogenic activity by inhibition of angiogenic factor signaling pathway.[29] As above data, moscatilin has been shown to be a potential anticancer agent, and *D. williamsonii* can be asource for this compound. For anti-HSV activity, only compound 3 exhibited antiviral effect against HSV-1 and HSV-2 (IC_{50} 304.1 μM and 334.5 μM, respectively), when compared with acyclovir (IC_{50} 1.5 μM and 2.9 μM, respectively), whereas, other compounds were devoid of anti-herpetic activity.

Table 1. IC_{50} values (μM) for DPPH free radical scavenging, cytotoxic and anti-herpetic activities

Compound	DPPH	Cytotoxicity (IC_{50}, μM)		IC_{50} (μM)	
	IC_{50} (μM)	KB	MCF-7	HSV-1	HSV-2
1	NA	NA	NA	NA	NA
2	NA	NA	NA	NA	NA
3	19.5	195.0	187.7	304.1	334.5
4	8.5	2.6	112.4	NA	NA
5	19.3	NA	NA	NA	NA
6	NA	NA	NA	NA	NA
Ellipticine	NA	1.8	NA	NA	NA
Doxorubicin	NA	1.0	15.1	NA	NA
Tamoxifen	NA	NA	24.9	NA	NA
Quercetin	8.3	NA	NA	NA	NA
Vitamin C	42.4	NA	NA	NA	NA
Acyclovir	NA	NA	NA	1.5	2.9

NA = less than 50 % inhibition at 100 μg/mL.

Conflicts of interest

All authors have none to declare.

ACKNOWLEDGMENTS

This work was supported by research grants from Ratchadaphiseksomphot Endowment Fund of Chulalongkorn University, and from The Thailand Research Fund (BRG 5580004). We thank Prof. Thatree Phadungcharoen and Prof. Vimolmas Lipipun (Chulalongkorn University) for the plant identification, and the assays of antiherpetic activity, respectively. Thanks are also due to The Research Instrument Center of the Faculty of Pharmaceutical Sciences, for providing research facilities.

REFERENCES

1. Seidenfaden G, Orchid genera in Thailand XII. *Dendrobium* Sw.*Opera Bot*. 1985; 83:1–295.
2. Hu J, Fan W, Dong F, Miao Z, Zhou J, Chemical components of *Dendrobium chrysotoxum*.*Chin. J. Chem*. 2012; 30:1327–1330.
3. Hossain MM, Therapeutic orchids: traditional uses and recent advances-an overview. *Fitoterapia* 2011; 82:102–140.
4. Zhang X, Xu JK, Wang J, Wang NL, Kurihara H, Kitanaka S, Yao XS, Bioactive bibenzyl derivatives and fluorenones from *Dendrobium nobile*.*J. Nat. Prod*. 2007; 70:24–28.
5. Ito M, Matsuzaki K, Wang J, Daikonya A, Wang NL, Yao XS, Kitanaka S, New phenanthrenes and stilbenes from *Dendrobium loddigesii*.*Chem. Pharm. Bull*. 2010; 58:628–633.
6. Hu JM, Chen JJ, Yu H, Zhao YX, Zhou J, Two novel bibenzyls from *Dendrobium trigonopus*.*J. Asian Nat. Prod.Res*. 2008;10:647–651.
7. Yang H, Sung SH, Kim YC, Antifibrotic phenanthrenes of *Dendrobium nobile* stems. *J. Nat. Prod*. 2007; 70:1925–1929.
8. Lo SF, Mulabagal V, Chen CL, Kuo CL, Tsay HS, Bioguided fractionation and isolation of free radical scavenging component from in vitro propagated Chinese medicinal plants *Dendrobium tosaense* Makino and *Dendrobium moniliforme* SW.*J. Agric. Food Chem.* 2004; 52:6916–6919.
9. Vaddhanaphuti N, *A field guide to the wild orchids of Thailand Forth and expanded edition*. Silkworm Books, Chiang Mai, 2005, p. 132.
10. Long CL, Li R, Ethnobotanical studies on medicinal plants used by the Red-headed Yao people in Jinping, Yunnan Province, China. *J. Ethnopharmacol*. 2004; 90:389–395.
11. Sritularak B, Anuwat M, Likhitwitayawuid K, A new phenanthrene quinone from *Dendrobium draconis*.*J. Asian Nat. Prod. Res.* 2011; 13:251–255.
12. Sritularak B, Likitwitayawuid K, New bisbibenzyls from *Dendrobium falconeri*.*Helv.Chim.Acta* 2009; 92:740–744.
13. Phechrmeekha T, Sritularak B, Likhitwitayawuid K, New phenolic compounds from *Dendrobium capillipes* and *Dendrobium secundum*.*J. Asian Nat. Prod. Res.* 2010; 14:748—754.
14. Chanvorachote P, Kowitdamrong A, Ruanghirun T, Sritularak B, Mungmee C, Likhitwitayawuid K, Anti-metastatic activities of bibenzyls from *Dendrobium pulchellum*. *Nat. Prod. Commun*. 2013; 8:115–118.
15. Sritularak B, Tantrakarnsakul K, Lipipun V, Likhitwitayawuid K, Flavonoids with anti-HSV activity from the root bark of *Artocarpus lakoocha*. *Nat. Prod. Commun*. 2013; 8:1079–1080.
16. Pei YH, Li X, Zhu TR, Studies on the structure of a new isocoumarin glucoside of the root sprouts of *Agrimoniapilosa* Ledeb.*Yaoxue Xuebao* 1989; 24:837–840.
17. Mahmood U, Kaul VK, Acharya R, Jirovetz L. *p*-Coumaric acid esters from *Tanacetum longifolium*. *Phytochemistry* 2003; 64:851–853.
18. Ulubelen A, Topcu G, Olçal S, Rearranged abietane diterpenes from *Teucrium divaricatum* subsp. *Villosum*. *Phytochemistry* 1994; 37:1371–1375.
19. Arriaga-Giner FJ, Wollenweber E, Dörr M, Bibenzyls from crowberry leaves, *Phytochemistry.*1993; 33:725–726.
20. Majumder PL, Sen RC, Moscatilin, a bibenzyl derivative from the orchid *Dendrobium moscatum*. *Phytochemistry.*1987; 26: 2121–2124.
21. Han XH, Hong SS, Hwang JS, Lee MK, Hwang BY, Ro JS, Monoamine oxidase inhibitory components from *Cayratia japonica*.*Arch. Pharm. Res*.2007; 30:13–17.
22. Sakushima A, Coşkun M, Maoka T, Hydroxybenzoic acids from *Boreavaorientalis*. *Phytochemistry* 1995; 40:257–261.

23. Miean KH, Mohamed S, Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *J. Agric. Food Chem.* 2001; 49:3106–3112.
24. Ellnain-Wojtaszek M, Kruczyński Z, Kasprzak J, Investigation of the free radical scavenging activity of *Ginkgo biloba* L. leaves. *Fitoterapia* 2003; 74:1–6.
25. Nuutila AM, Puupponen-Pimiä R, Aarni M, Oksman-Caldentey KM, Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. *Food Chem.* 2003; 81:485–-493.
26. Kowitdamrong A, Chanvorachote P, Sritularak B, Pongrakhananon V, Moscatilin inhibits lung cancer cell motility and invasion via suppression of endogenous reactive oxygen species. *BioMed Res. Inter.* 2013;Article ID 765894:1–11.
27. Ho CK, Chen CC, Moscatilin from the orchid *Dendrobium loddigesii* is a potent anticancer agent, *Cancer Invest*. 2003; 21:729–736.
28. Chen TH, Pan SL, Guh JH, Liao CH, Huang DY, Chen CC, Teng CM, Moscatilin induces apoptosis in human colorectal cancer cells: a crucial role of c-Jun NH_2-terminal protein kinase activation causes by tubulin depolymerization and DNA damage. *Clin. Cancer Res*. 2008; 14:4250–4257.
29. Tsai AC, Pan SL, Liao CH, Guh JH, Wang SW, Sun HL, Liu YN, Chen CC, Shen CC, Chang YL, Teng CM, Moscatilin, a bibenzyl derivative from the India orchid *Dendrobium loddigesii*, suppress tumor angiogenesis and growth *in vitro* and *in vivo*. *Cancer Lett.* 2010; 292:163–170.