CHAPTER IV

RESULTS AND DISCUSSION

In this study, the dried and diced whole plant of *Dendrobium ellipsophyllum* (4.8 kg) was macerated with methanol. The crude methanol extract was concentrated under reduced pressure to give 400 g of a crude extract. The methanol extract was separated by Vaccum Liquid Chromatography (VLC) give to 5 fractions (A-E). Fractions C and D were found to possess cytotoxic against human oral cavity cancer cell with 45% inhibition at a concentration of 50 µg/ml. Fraction D was subjected to silica gel and sephadex LH-20 separations to give ten pure compounds [DE1-DE10] including, four flavonoids, three bibenzyls, one dihydrophenanthrene, one chromone and one phenylpropionic acid. The structures of these compounds were determined by spectroscopic analysis, including UV, IR, MS, and NMR. In addition, they were evaluated for cytotoxicity against KB and MCF-7 cancer cells, anti-metastatic activity and anti-herpes simplex virus (HSV-1, HSV-2) effects.

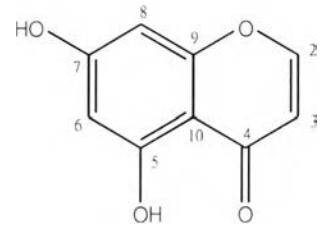
1. Structure characterization of isolated compounds

1.1 Structure determination of compound DE1

Compound DE1 was obtained as colorless needles. The ESI mass spectrum (Figure 5) show a sodium adduct molecular ion $[M+Na]^+$ at *m/z* 201.0153 (calcd for $C_9H_6O_4Na$ 201.0163), suggesting the molecular formula $C_9H_6O_4$. The IR spectrum (Figure 6) showed absorption bands for hydroxyl (3434 cm^{-1}), aromatic (3083, 1616, 1463 cm^{-1}) and carbonyl (1644 cm^{-1}) groups. The UV spectrum (Figure 7) showed absorption bands at 295, 256 and 216 nm, indicative of a chromone structure (Kuo, Lee, and Wein, 2002), and this was supported by the ^1H NMR (Figure 8) and the ^1H-^1H COSY spectra (Figure 10) which showed signals at δ_H 6.21 (1H, d, *J* = 6.0 Hz, H-3) and 8.05 (1H, d, *J* = 6.0 Hz, H-2). The ^1H NMR spectrum (Figure 8 and Table 2) also revealed the presence of two *meta*-coupled protons at δ_H 6.26 (1H, d, *J* = 2.1 Hz, H-6) and 6.39 (1H, d, *J* = 2.1 Hz, H-8), indicating two substitutions on the chromone

nucleus. Moreover, a chelated hydroxyl proton at δ_H 12.76 of 5-OH was observed. The ^{13}C NMR and DEPT 135 spectra (Figures 9, 11 and Table 2) displayed nine carbon atoms which had four methine carbons. The ketone group appears at δ_C 182.5 (C-4) of ^{13}C NMR spectrum.

Based on the above spectral evidence and through the comparision of its previous reported data (Du, Jerz, and Winterhalter, 2005), compound DE1 was identified as 5,7-dihydroxy-chromen-4-one [**271**]. This compound has been isolated from *Dendrobium* plants for the first time in this study.



5,7-Dihydroxy-chromen-4-one [**271**]

Table 2 NMR Spectral data of compound DE1 (acetone-d_6) and 5,7-dihydroxy-chromen-4-one (MeOH-d_4)

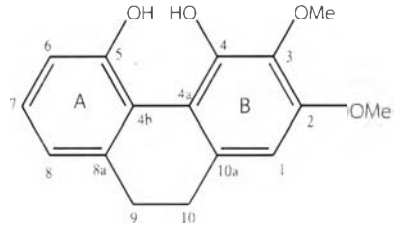
Position	Compound DE1		5,7-Dihydroxy-chromen-4-one*	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
2	8.05 (d, 6.0)	157.6	7.95 (d, 6.0)	158.0
3	6.21 (d, 6.0)	111.5	6.18 (d, 6.0)	111.7
4	-	182.5	-	183.6
5	-	163.4	-	163.5
6	6.26 (d, 2.1)	99.8	6.21 (d, 2.0)	100.3
7	-	165.1	-	166.2
8	6.39 (d, 2.1)	94.7	6.33 (d, 2.0)	95.1
9	-	159.2	-	159.9
10	-	106.4	-	106.8
5-OH	12.76 (s)	-	-	-

* From: Du *et al.*, 2005.

1.2 Structure determination of compound DE2

Compound DE2 was obtained as a brown amorphous solid. The ESI mass spectrum (Figure 12) show a pseudomolecular ion $[M+H]^+$ at *m/z* 273, suggesting the molecular formula $C_{16}H_{16}O_4$. The IR spectrum (Figure 13) showed absorption bands for hydroxyl (3434 cm^{-1}) and aromatic (3025, 1615, 1458 cm^{-1}) groups. The UV spectrum (Figure 14) showed absorption bands at 275 and 221 nm, indicative of a 9,10-dihydrophenanthrene (Bai *et al.*, 1998). This was supported by the presence of a multiplet of methylene protons at δ_H 2.72 (4H, m, H_2-9 and H_2-10) which correlated to the carbon atoms at δ_C 30.9 in the HSQC spectrum (Figure 18). The presence of an ABC spin system at δ_H 6.98 (1H, d, *J* = 8.0 Hz, H-6), 7.16 (1H, t, *J* = 8.0 Hz, H-7) and 6.87 (1H, d, *J* = 8.0 Hz, H-8) suggested a monosubstitution for ring A. The assignment of H-8 was based on its HMBC (Figure 17) correlation with C-9. The appearance of a sharp proton singlet signal at δ_H 6.56, assignable to H-1 from its HMBC correlation with C-10, suggested a 2, 3, 4-trisubstituted of ring B. The ^{1}H NMR spectrum also exhibited two methoxyl groups at δ_H 3.93 (3H, s, MeO-2) and 3.99 (3H, s, MeO-3). The first methoxyl (δ_H 3.93) was located at C-2, as shown by its NOESY (Figure 19) interaction with H-1. The HMBC correlation of C-3 with methoxyl (δ_H 3.99) and H-1, placed the second methoxyl at C-3.

From the above observation including the ^{1}H NMR, ^{13}C NMR, UV, IR and mass spectra, compound DE2 was identified as 4,5-dihydroxy-2,3-dimethoxy-9,10-dihydrophenanthrene [**150**]. This compound was previously isolated from *D. sinense*. (Chen *et al.*, 2013).



4,5-Dihydroxy-2,3-dimethoxy-9,10-dihydrophenanthrene [**150**]

Table 3 NMR Spectral data of compound DE2 ($CDCl_3$)

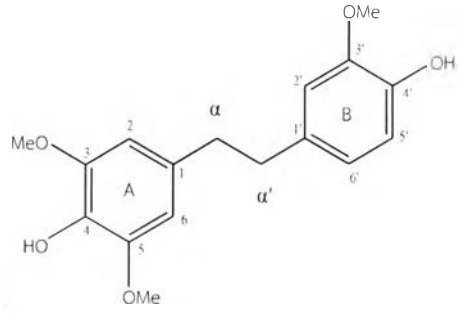
Position	Compound DE2		HMBC	NOESY
	δ_H	δ_C		
	(mult., *J* in Hz)			
1	6.56 (s)	105.0	C-2, C-3, C-4a, C-10, C-10a	H_2-10, 2-OMe
2	-	150.4	-	-
3	-	134.0	-	-
4	-	143.7	-	-
4a	-	113.1	-	-
4b	-	120.4	-	-
5	-	153.2	-	-
6	6.98 (d, 8.0)	118.0	C-4b, C-5, C-8	-
7	7.16 (t, 8.0)	128.0	C-5, C-6, C-8a	-
8	6.87 (d, 8.0)	120.0	C-4b, C-6, C-7, C-9	H_2-9
8a	-	140.2	-	-
9	2.72 (m)	30.9	C-4b, C-8, C-8a, C-10a	H-8
10	2.72 (m)	30.9	C-1, C-4a, C-8a, C-10a	H-1
10a	-	136.7	-	-
2-OMe	3.93 (s)	55.9	C-2	H-1
3-OMe	3.99 (s)	61.2	C-3	-

1.3 Structure determination of compound DE3

Compound DE3 was obtained as a brown amorphous solid. The ESI mass spectrum (Figure 20) show a pseudomolecular ion $[M+H]^+$ at *m/z* 305, suggesting the molecular formula $C_{17}H_{20}O_5$. The IR spectrum (Figure 21) showed absorption bands for hydroxyl (3437 cm^{-1}) and aromatic (3022, 1611, 1455 cm^{-1}) groups. The UV spectrum (Figure 22) showed absorption bands at 286 and 228 nm, indicative of a bibenzyl derivative (Liu *et al.*, 2004). This was supported by the presence of methylene protons at δ_H 2.78 (4H, m, H_2-α, H_2-α') in the ^{1}H NMR spectrum (Figures 23-24 and Table 4) and two methylene carbon signals at δ_C 38.3 (C-α') and 38.8 (C-α) in the ^{13}C NMR spectrum (Figures 26-27 and Table 4). The ^{1}H NMR spectrum (Figure 24) also showed signals for three methoxyl groups at δ_H 3.75 (6H, s, MeO-3, MeO-5) and 3.76 (3H, s, MeO-3′). On ring A, the ^{1}H NMR spectrum (Figure 25) exhibited *meta* coupled signals at δ_H 6.48 (2H, s, H-2, H-6), indicating the presence of tetrasubstituted phenyl group. On ring B, the ^{1}H NMR spectrum (Figure 25) showed signals at δ_H 6.78 (1H, d, *J* = 2.0 Hz, H-2′), 6.75 (1H, d, *J* = 8.0 Hz, H-5′) and 6.64 (1H, dd, *J* = 8.0, 2.0 Hz, H-6′). The ^{13}C NMR spectrum (Figures 26-28) showed seventeen carbon signals.

From the above observations and through comparison of previous reported data (Majumder and Sen, 1987), compound DE3 was identified as moscatilin [**58**].

Moscatilin was a bibenzyl derivative firstly isolated from *D. moscatum* (Majumder and Sen, 1987). Besides, this compound was also found in *D. amoenum*, *D. aurantiacum var.denneanum*, *D. capillipes*, *D. chrysanthum*, *D. densiflorum*, *D. gratiosissimum*, *D. loddigesii*, *D. longicornu* and *D. secundum* (Majumder *et al.*, 1999; Yang *et al.*, 2006a; Phechrmeekha *et al.*, 2012; Yang *et al.*, 2006b; Fan *et al.*, 2001; Zhang *et al.*, 2008a; Chen *et al.*, 1994; Ito *et al.*, 2010; Hu *et al.*, 2008a; Sritularak *et al.*, 2011b).



Moscatilin [**58**]

Table 4 NMR Spectral data of compound DE3 (acetone-d_6) and moscatilin ($CDCl_3$)

Position	Compound DE3		Moscatilin*	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	-	133.1a	-	132.8a
2	6.48(s)	106.7	6.36 (s)	105.2
3	-	148.3b	-	146.8b
4	-	134.8a	-	133.5a
5	-	148.3b	-	146.8b
6	6.48 (s)	106.7	6.36 (s)	105.2
1′	-	134.1a	-	132.8a
2′	6.78 (d, 2.0)	112.9	6.65 (d, 2.0)	111.2
3′	-	147.9b	-	146.1b
4′	-	145.3	-	143.7
5′	6.75 (d, 8.0)	115.4	6.94 (d, 8.0)	114.1
6′	6.64 (dd, 8.0, 2.0)	121.6	6.75 (dd, 8.0, 2.0)	121.0
α	2.78 (m)	38.8c	2.89 (s)	38.3c
α′	2.78 (m)	38.3c	2.89 (s)	37.8c
3-OMe	3.75 (s)	56.5d	3.77 (s)	56.2d
3′-OMe	3.76 (s)	56.1d	3.77 (s)	55.8d
5-OMe	3.75 (s)	56.5d	3.77 (s)	56.2d

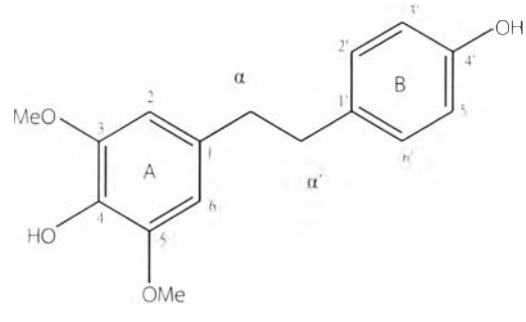
* From: Majumder & Sen, 1987.

a-d values are interchangeable.

1.4 Structure determination of compound DE4

Compound DE4 was obtained as a brown amorphous solid. The ESI mass spectrum (Figure 29) show a pseudomolecular ion $[M+H]^+$ at *m/z* 275, suggesting the molecular formula $C_{16}H_{18}O_4$. The IR spectrum (Figure 30) showed absorption bands for hydroxyl (3399 cm^{-1}) and aromatic (3016, 1614, 1458 cm^{-1}) groups. The UV spectrum (Figure 31) showed absorption bands at 279 and 228 .nm, indicative of a bibenzyl derivative (Liu *et al.*, 2004). The ^{1}H NMR spectrum (Figures 32-34 and Table 5) showed a multiplet signal of protons H_2-α and H_2-α' (4H, δ_H 2.76), confirming the bibenzyl skeleton. On ring A, two equivalent aromatic protons appeared at δ_H 6.46 (2H, s, H-2, H-6). The ^{1}H NMR spectrum also showed two methoxyl proton signals at δ_H 3.76 (6H, s, MeO-3, MeO-5). The presence of two methoxyls at C-3 and C-5 was confirmed by HMBC correlations (Figures 36-39) of C-3 (C-5) with 3-OMe (5-OMe) and H-2 (H-6). The appearance of AA′BB′ spin system at δ_H 7.00 (2H, d, *J* = 8.5, H-2′, H-6′) and 6.72 (2H, d, *J* = 8.5, H-3′, H-5′) indicated a *para*-substitued B ring. The ^{13}C NMR spectrum (Figure 35 and Table 5) presented the sixteen carbon peaks, in which the methylene carbon signals appeared at δ_C 39.0 (C-α) and 38.0 (C-α').

Based on the above spectral evidence and through comparison with previously reported data (Katerere *et al.*, 2012), compound DE4 was identified as 4,4′-dihydroxy-3,5-dimethoxybibenzyl [**45**]. This compound was previously isolated from *D. candidum* (Li *et al.*, 2008).



4,4′-Dihydroxy-3,5-dimethoxybibenzyl [**45**]

Table 5 NMR Spectral data of compound DE4 (acetone-d_6) and 4,4′-dihydroxy-3,5-dimethoxybibenzyl (CD_3OD)

Position	Compound DE4			4,4′-Dihydroxy-3,5-dimethoxybibenzyl*	
	δ_H (mult., *J* in Hz)	δ_C	HMBC	δ_H (mult., *J* in Hz)	δ_C
1	-	133.1	-	-	138.3
2	6.46 (s)	106.7	C-1, C-3, C-4, C-6, C-α	6.35 (s)	105.8
3	-	148.4	-	-	147.9
4	-	134.9	-	-	133.0
5	-	148.4	-	-	147.9
6	6.46 (s)	106.7	C-1, C-2, C-5, C-4, C-α	6.35 (s)	105.8
1′	-	133.5	-	-	133.5
2′	7.00 (d, 8.5)	130.1	C-3′, C-4′, C-6′	6.93 (d, 8.5)	129.3
3′	6.72 (d, 8.5)	115.8	C-1′, C-4′, C-5′	6.66 (d, 8.5)	114.9
4′	-	156.3	-	-	155.3
5′	6.72 (d, 8.5)	115.8	C-1′, C-3′, C-4′	6.66 (d, 8.5)	114.9
6′	7.00 (d, 8.5)	130.1	C-2′, C-4′, C-5′	6.93 (d, 8.5)	129.5
α	2.76 (m)	39.0	C-1, C-2, C-6, C-α′	2.75 (s)	38.4
α′	2.76 (m)	38.0	C-1′, C-2′, C-6′, C-α	2.75 (s)	37.3
3-OMe	3.76 (s)	56.4	C-3	3.74 (s)	59.9
5-OMe	3.76 (s)	56.4	C-5	3.74 (s)	59.9

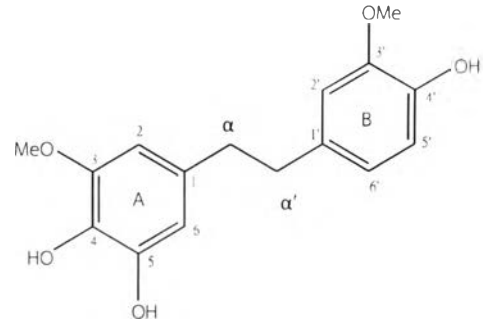
* From: Katerere *et al.*, 2012.

1.5 Structure determination of compound DE5

Compound DE5 was obtained as a brown amorphous solid. The ESI mass spectrum (Figure 40) showed a pseudomolecular ion $[M+H]^+$ at *m/z* 291, suggesting the molecular formula $C_{16}H_{18}O_5$. The IR spectrum (Figure 41) showed absorption peaks for hydroxyl (3434 cm^{-1}) and aromatic (2923, 1615, 1464 cm^{-1}) groups. The UV spectrum (Figure 42) showed maximal absorptions at 281,256 and 227 nm, characteristic of a bibenzyl skeleton (Liu *et al.*, 2004).

The NMR data (Figures 43-45 and Table 6) indicated characteristic proton signals for a bibenzyl structure at δ_H 2.75 (4H, m, H_2-α, H_2-α') which were correlated to the carbon signals at δ_C 38.7 (C-α) and 38.3 (C-α'). Moreover, the ^{1}H NMR spectrum showed five signals (5H) in aromatic region at δ_H 6.73 (1H, d, *J* = 8.0 Hz, H-5′), 6.64 (1H, dd, *J* = 8.0, 2.0 Hz, H-6′), 6.78 (1H, d, *J* = 2.0 Hz, H-2′), 6.39 (1H, d, *J* = 2.0 Hz , H-6) and 6.34 (1H, d , *J* = 2.0 Hz, H-2). These spectral features suggested the presence of five substitutents on the bibenzyl nucleus. The ^{1}H NMR spectrum (Figure 44) showed two methoxyl groups at δ_H 3.74 (3H, s, MeO-3) and 3.77 (3H, s, MeO-3′) which were correlated to the carbon signals at δ_C 56.3 and 56.1, respectively. The structure was supported by the ^{13}C NMR spectrum (Figures 46-48) which exhibited sixteen peaks, including twelve peaks for twelve aromatic carbons, two peaks for methylene carbons and two peaks for two methoxyl groups. The NOESY spectrum (Figure 49) exhibited correlations of H_2-α with H-2 (δ_H 6.34) and H-6 (δ_H 6.39), and H_2-α' with H-2′ (δ_H 6.78) and H-6′ (δ_H 6.64). Besides, correlation peaks were observed for the two methoxyl protons, comprising MeO-3 and MeO-3′ with H-2 (δ_H 6.34) and H-2′ (δ_H 6.78), respectively.

From the above data and through comparison with previously reported values (Sritularak *et al.*, 2011b), compound DE5 was identified as 4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl [**68**]. This compound was a bibenzyl derivative firstly isolated from *D. secundum*, and showed appreciable DPPH free radical scavenging potential (Sritularak *et al.*, 2011b).



4,5,4′ –Trihydroxy-3,3′-dimethoxybibenzyl [**68**]

Table 6 NMR Spectral data of compound DE5 (acetone-d_6) and 4,5,4′–trihydroxy-3,3′-dimethoxybibenzyl ($CDCl_3$)

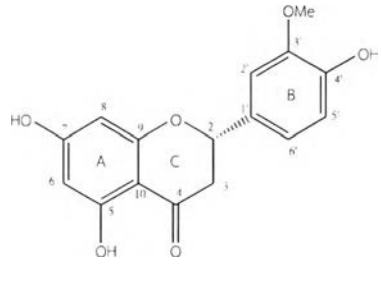
Position	Compound DE5			4,5,4′–Trihydroxy-3,3′-dimethoxybibenzyl*	
	δ_H (mult., *J* in Hz)	δ_C	NOESY	δ_H (mult., *J* in Hz)	δ_C
1	-	132.6	-	-	130.4
2	6.34 (d, 2.0)	104.5	-	6.21 (d, 2.0)	103.5
3	-	148.6	-	-	146.6
4	-	134. 2	-	-	133.7
5	-	145.3	-	-	143.7
6	6.39 (d, 2.0)	109.6	-	6.42 (d, 2.0)	108.6
1′	-	133.7	-	-	133.8
2′	6.78 (d, 2.0)	112.8	-	6.60 (d, 2.0)	111.2
3′	-	147.9	-	-	146.2
4′	-	145.9	-	-	143.7
5′	6.73 (d, 8.0)	115.4	-	6.80 (d, 8.0)	114.1
6′	6.64 (dd, 8.0, 2.0)	121.5	-	6.65 (dd, 8.0, 2.0)	121.0
α	2.75 (m)	38.7	H-2, H-6	2.75 (m)	38.2
α′	2.75 (m)	38.3	H-2′, H-6′	2.78 (m)	37.7
3-OMe	3.74 (s)	56.3	H-2	3.80 (s)	56.1
3′-OMe	3.77 (s)	56.1	H-2′	3.83 (s)	55.9

* From: Sritularak *et al.*, 2011b.

1.6 Structure determination of compound DE6

Compound DE6 was obtained as colorless needles. The ESI mass spectrum (Figure 50) showed a pseudomolecular ion $[M+H]^+$ at *m/z* 303, suggesting the molecular formula $C_{16}H_{14}O_6$. The IR spectrum (Figure 51) showed absorption bands for hydroxyl (3464 cm^{-1}), aromatic (3046, 1614, 1468 cm^{-1}) and carbonyl (1635 cm^{-1}) groups. The UV spectrum (Figure 52) showed maximal absorptions at 280 and 214 nm, indicative of a flavanone skeleton (Liu, Ho, and Cassady, 1992). The ^{1}H NMR signals at δ_H 2.72 (H-3_{cis}), 3.19 (H-3_{trans}) and 5.41 (H-2) and the ^{13}C resonances at δ_C 80.1 (C-2), 43.5 (C-3) and 197.2 (C-4) confirmed a flavanone nucleus. The ^{1}H NMR spectrum (Figures 53-57 and Table 7) showed signals for A ring protons at δ_H 5.94 (1H, d, *J* = 2.5 Hz, H-6) and 5.96 (1H, d, *J* = 2.5 Hz, H-8). On the B ring, three protons appeared at δ_H 6.86 (1H, d, *J* = 8.0 Hz, H-5′), 6.98 (1H, dd, *J* = 8.0, 2.0 Hz, H-6′) and 7.17 (1H, d, *J* = 2.0, H-2′). A methoxyl group at ring B was indicated by the ^{1}H NMR signal at δ_H 3.87 (3H, s, MeO-3′) which correlated with H-2′ (δ_H 7.17) in the NOESY spectrum (Figure 59). The ^{13}C NMR spectrum (Figure 58 and Table 7) showed sixteen carbon atoms, including one methoxyl carbon, one methylene carbon, one conjugated carbonyl carbon, six methine carbons, and seven quartenary carbons. In addition, this spectrum showed a conjugated carbonyl carbon peak at δ_C 197.2 (C-4). The absolute stereochemistry at C-2 was assigned as *S* based its levorotatory specific rotation ($[\alpha]^{25}_D$ -18.67 (*c* 0.1, MeOH)) (Slade, Ferreira, and Marais, 2005).

Through comparison of these data with reported values (Liu *et al.*, 1992), compound DE6 was identified as (2*S*)-homoeriodictyol [**88**]. This compound was previously isolated from *D. densiflorum* and exhibited anti-platelet aggregation activity in preliminary pharmacological tests (Fan *et al.*, 2001).



(2*S*)-Homoeriodictyol [**88**]

Table 7 NMR Spectral data of compound DE6 (acetone-d_6) and (2*S*)-homoeriodictyol [^{1}H NMR (acetone-d_6) and ^{13}C NMR (DMSO-d_6)]

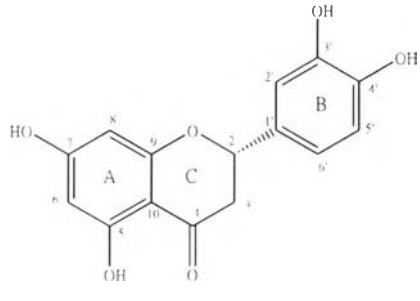
Position	Compound DE6			(2*S*)-Homoeriodictyol*	
	δ_H (mult., *J* in Hz)	δ_C	NOESY	δ_H (mult., *J* in Hz)	δ_C
2	5.41 (dd, 13.0, 3.0)	80.1	H-2′, H-3_{cis}, H-3_{trans}, H-6′	5.43 (dd, 12.8, 2.7)	78.6
3_{cis}	2.72 (dd, 17.0, 3.0)	43.5	H-2	2.73 (dd, 17.1, 2.7)	42.1
3_{trans}	3.19 (dd, 17.0, 13.0)	43.5	H-2	3.21 (dd, 17.1, 12.8)	42.1
4	-	197.2	-	-	196.3
5	-	165.2	-	-	163.5
6	5.94 (d, 2.5)	96.7	-	5.96 (br s)	95.8
7	-	167.3	-	-	166.6
8	5.96 (d, 2.5)	95.8	-	5.96 (br s)	95.0
9	-	164.3	-	-	162.9
10	-	103.1	-	-	101.8
1′	-	131.2	-	-	129.5
2′	7.17 (d, 2.0)	111.1	3′-OMe	7.18 (d, 1.8)	111.3
3′	-	148.3	-	-	147.6
4′	-	147.8	-	-	147.0
5′	6.86 (d, 8.0)	115.6	-	6.88 (d, 8.1)	115.2
6′	6.98 (dd, 8.0, 2.0)	120.4	-	6.98 (dd, 8.1, 1.8)	119.6
3′-OMe	3.87 (s)	56.2	H-2	3.88 (s)	55.8
5-OH	12.17 (s)	-	-	12.18 (s)	-

* From: Liu *et al.*, 1992

1.7 Structure determination of compound DE7

Compound DE7 was obtained as colorless needles. The ESI mass spectrum (Figure 60) showed a sodium adduct molecular ion $[M+Na]^+$ at *m/z* 311.0505 (calcd for $C_{15}H_{12}O_6Na$ 311.0531), suggesting the molecular formula $C_{15}H_{12}O_6$. The IR spectrum (Figure 61) showed absorption bands for hydroxyl (3366 cm^{-1}), aromatic (2922, 1604, 1451 cm^{-1}) and carbonyl (1636 cm^{-1}) groups. The UV spectrum (Figure 62) showed maximal absorptions at 288 and 227 nm, indicative of a flavanone structure (Liu *et al.*, 1992). The ^1H NMR spectrum (Figure 63 and Table 8) exhibited signals for A ring protons at δ_H 5.93 (2H, d, *J*= 2.0 Hz, H-6, H-8). On ring B, the proton signals appeared at δ_H 7.02 (1H, s, H-2′) and 6.85 (2H, s, H-5′, H-6′), and ring C showed proton signals at δ_H 5.37 (1H, dd, *J* = 12.5, 3.0 Hz, H-2), 2.72 (1H, dd, *J* = 17.0, 3.0 Hz, H-3_{cis}) and 3.11 (1H, dd, *J* = 17.0, 12.5 Hz, H-3_{trans}). The ^{13}C NMR spectrum (Figure 64 and Table 8) displayed fifteen carbon atoms, which had one methylene carbon as identified from the DEPT 135 spectrum (Figure 65). A conjugated carbonyl carbon peak showed at δ_C 197.1 (C-4). This structure was confirmed by 2D-NMR analysis. The HSQC spectrum (Figure 66) showed 1-bond correlation between carbons and protons. The NOESY spectrum (Figure 67) showed that H-2 (δ_H 5.37) correlated with H-3_{cis} and H-3_{trans}. Furthermore, H-2 (δ_H 5.37) exhibited HMBC correlations (Figures 68-69) with C-2′ (δ_C 114.6), C-6′ (δ_C 119.2), C-4 (δ_C 197.1) and C-1′ (δ_C 131.5). The absolute stereochemistry at C-2 was also assigned as *S* based on its levorotatory specific rotation ($[\alpha]^{25}_D$ -18.66 (*c* 0.1, MeOH)) (Slade, Ferreira, and Marais, 2005).

Based on the above spectral evidence and through comparison with previously reported data (Liu *et al.*, 1992), compound DE7 was identified as (2*S*)-eriodictyol [**272**]. This compound has been found in *Dendrobium* for the first time in this study.



(2*S*)-Eriodictyol [**272**]

Table 8 NMR Spectral data of compound DE7 (acetone-d_6) and (2*S*)-eriodictyol (DMSO-d_6)

Position	Compound DE7				(2*S*)-Eriodictyol*	
	δ_H (mult., *J* in Hz)	δ_C	NOESY	HMBC	δ_H (mult., *J* in Hz)	δ_C
2	5.37 (dd, 12.5, 3.0)	79.9	-	C-1′, C-2′, C-6′	5.36 (dd, 13.4, 3.1)	78.3
3_{cis}	2.72 (dd, 17.0, 3.0)	43.4	H-2	C-4, C-10	2.66 (dd, 18.4, 3.1)	42.0
3_{trans}	3.11 (dd, 17.0, 12.5)	43.4	H-2	C-2, C-4, C-1′	3.17 (dd, 18.4, 13.4)	42.0
4	-	197.1	-	-	-	196.0
5	-	164.2	-	-	-	163.4
6	5.93 (d, 2.0)	96.7	-	C-5, C-8, C-10	5.87 (s)	95.6
7	-	167.2	-	-	-	166.5
8	5.93 (d, 2.0)	95.8	-	C-6, C-9, C-10	5.86 (s)	94.9
9	-	165.2	-	-	-	162.8
10	-	103.1	-	-	-	101.7
1′	-	131.5	-	-	-	129.4
2′	7.02 (s)	114.6	-	C-2, C-4′, C-6′	6.86 (s)	114.2
3′	-	145.9	-	-	-	145.0
4′	-	146.3	-	-	-	145.5
5′	6.85 (s)	115.9	-	C-1′, C-3′	6.73 (s)	115.3
6′	6.85 (s)	119.2	-	C-2, C-2′, C-4′	6.73 (s)	117.8
5-OH	12.16 (s)	-	-	-	12.13 (s)	-

* From: Liu *et al.*, 1992.

1.8 Structure determination of compound DE8

Compound DE8 was obtained as a yellow powder. The ESI mass spectrum (Figure 70) showed a pseudomolecular ion $[M+H]^+$ at *m/z* 301, suggesting the molecular formula $C_{16}H_{12}O_6$. The IR spectrum (Figure 71) showed absorption bands for hydroxyl (3411 cm^{-1}), aromatic (3087, 1599, 1435 cm^{-1}) and carbonyl (1652 cm^{-1}) groups. The UV spectrum (Figure 72) showed maximal absorptions at 286 and 226 nm, indicative of a flavone skeleton (Liu *et al.*, 1992).

The ^1H NMR spectrum (Figure 74) showed a singlet proton signal of H-3 (δ_H 6.69), confirming the flavone skeleton. The ^1H NMR spectrum (Figures 73-74 and Table 9) showed A ring protons at δ_H 6.24 (1H, d, *J*=2.0 Hz, H-6) and 6.54 (1H, d, *J* = 2.0 Hz, H-8). Ring B showed aromatic proton signals at δ_H 7.63 (1H, d, *J* = 2.0 Hz, H-2′), 7.00 (1H, d, *J* = 8.4 Hz, H-5′) and 7.59 (1H, dd, *J* = 8.4, 2.0 Hz, H-6′). The methoxyl proton appearing at δ_H 3.98 (3H, s, MeO-3′) was located at C-3′ based on its correlation with H-2′ (δ_H 7.63) in the NOESY spectrum (Figures 77-78). Moreover, H-2′ (δ_H 7.63) showed NOESY correlation (Figure 77) to H-3 (δ_H 6.69), indicating that the aromatic B ring was substituted at C-2. The ^{13}C NMR (Figure 75 and Table 9) and DEPT 135 (Figure 76) spectra showed sixteen carbons atoms, including nine quaternary carbons, six methine carbons and one methoxyl carbon. A conjugated carbonyl signal appeared at δ_C 183.1 (C-4).

From the above data and through comparison with previously reported data (Liu *et al.*, 1992), compound DE8 was identified as chrysoeriol [**273**]. This compound has been identified in *Dendrobium* for the first time in this investigation.

Chrysoeriol [**273**]

Table 9 NMR Spectral data of compound DE8 (acetone-d_6) and chrysoeriol (DMSO-d_6)

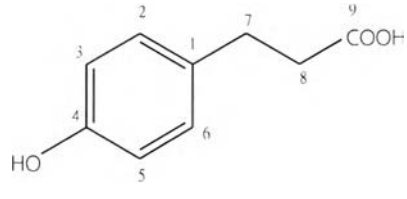
Position	Compound DE8			Chrysoeriol*	
	δ_H (mult., *J* in Hz)	δ_C	NOESY	δ_H (mult., *J* in Hz)	δ_C
2	-	163.3	-	-	163.4
3	6.69 (s)	104.4	-	6.87 (s)	103.1
4	-	183.1	-	-	181.5
5	-	159.0	-	-	161.5
6	6.24 (d, 2.0)	99.7	-	6.17 (br s)	98.8
7	-	164.9	-	-	164.4
8	6.54 (d, 2.0)	94.7	-	6.48 (br s)	93.9
9	-	157.8	-	-	157.3
10	-	105.1	-	-	103.2
1′	-	123.6	-	-	121.4
2′	7.63 (d, 2.0)	110.5	3′-OMe	7.53 (s)	110.3
3′	-	148.8	-	-	147.9
4′	-	151.4	-	-	150.7
5′	7.00 (d, 8.4)	116.3	H-6′	6.92 (d, 9.6)	115.7
6′	7.59 (dd, 8.4, 2.0)	121.3	H-5′	7.55 (d, 9.6)	120.2
3′-OMe	3.98 (s)	56.5	H-2′	3.88 (s)	55.9
5-OH	13.01 (s)	-	-	12.95s)	-

* From: Liu *et al.*, 1992.

1.9 Structure determination of compound DE9

Compound DE9 was obtained as a brown amorphous solid. The ESI mass spectrum (Figure 79) showed a sodium adduct molecular ion $[M+Na]^+$ at *m/z* 189.0442 (calcd for $C_9H_{10}O_3Na$ 189.0527), suggesting the molecular formula $C_9H_{10}O_3$. The IR spectrum (Figure 80) showed absorption bands for hydroxyl (3421 cm^{-1}), carbonyl (1713 cm^{-1}) and aromatic (3015, 1602, 1456 cm^{-1}) groups. The UV spectrum (Figure 81) showed maximal absorptions at 265 and 223 nm, indicative of a phenylpropanoid (Owen *et al.*, 2003). The ^{1}H NMR spectrum (Figure 82 and Table 10) indicated the presence of two substituents on aromatic ring, with symmetrical substitution, showing four aromatic protons at δ_H 7.05 (2H, d, *J*=8.1 Hz, H-2, H-6) and 6.74 (2H, d, *J*=8.1 Hz, H-3, H-5). The ^{13}C NMR (Figure 83 and Table 10) and DEPT 135 (Figure 84) spectra showed nine carbons atoms, including two methylene, four methine and three quaternary carbons. A carboxylic carbon appeared at δ_C 173.1 (C-9).

From the above data and through comparison with previously reported data (Owen *et al.*, 2003), compound DE9 was identified as phloretic acid [**214**]. This compound was previously isolated from *D. candidum* (Li *et al.*, 2010).



Phloretic acid [**214**]

Table 10 NMR Spectral data of compound DE9 (acetone-d_6) and phloretic acid (CD_3OD)

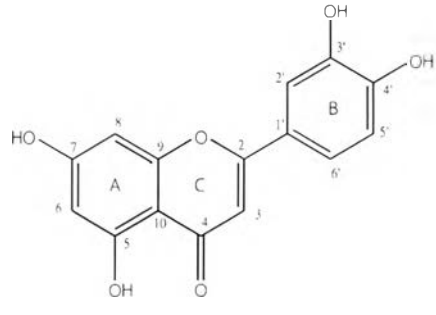
Position	Compound DE9		Phloretic acid*	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	-	132.5	-	133.6
2	7.05 (d, 8.1)	131.7	7.03 (d, 8.3)	130.2
3	6.74 (d, 8.1)	115.0	6.70 (d, 8.3)	116.2
4	-	155.4	-	156.1
5	6.74 (d, 8.1)	115.0	6.70 (d, 8.3)	116.2
6	7.05 (d, 8.1)	131.7	7.03 (d, 8.3)	130.2
7	2.79 (t, 7.8)	29.8	2.79 (t, 7.8)	31.7
8	2.53 (t, 7.8)	35.4	2.48 (t, 7.8)	38.6
9	-	173.1	-	179.1

* From: Owen *et al.*, 2003.

1.10 Structure determination of compound DE10

Compound DE10 was obtained as a yellow powder. The ESI mass spectrum (Figure 85) showed a pseudomolecular ion $[M+H]^+$ at *m/z* 287, suggesting the molecular formula $C_{15}H_{10}O_6$. The IR spectrum (Figure 86) showed absorption bands for hydroxyl (3425 cm^{-1}), aromatic (2919, 1616, 1447 cm^{-1}) and carbonyl (1653 cm^{-1}) groups. The UV spectrum (Figure 87) showed maximal absorptions at 349 and 223 nm, indicative of a flavone skeleton (Owen *et al.*, 2003). The ^{1}H NMR spectrum (Figures 88-89 and Table 10) showed a pair of meta coupled protons at δ_H 6.24 (1H, d, *J*=2.0 Hz, H-6) and 6.51 (1H, d, *J*=2. Hz, H-8) for ring A, and an ABM spin system at δ_H 7.49 (1H, d, *J*=2.1 Hz, H-2′), 7.00 (1H, d, *J*=8.4 Hz, H-5′) and 7.47 (1H, dd, *J*=8.4, 2.1 Hz, H-6′) for ring B. The C ring proton appeared as a singlet at δ_H 6.57 (H-3). The ^{13}C NMR (Figures 90-91 and Table 11) and DEPT 135 (Figure 92) spectra showed fifteen carbons atoms, including six methine and nine quarternary carbons.

Based on the above spectral evidence and through comparison with previously reported data (Park *et al.*, 2007), compound DE10 was identified as luteolin [**92**]. This compound was previously isolated from *D. aurantiacum var.denneanum* (Liu *et al.*, 2009a).



Luteolin [**92**]

Table 11 NMR Spectral data of compound DE10 (acetone-d_6) and luteolin (DMSO-d_6)

Position	Compound DE10		Luteolin*	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
2	-	164.8	-	163.9
3	6.57 (s)	104.2	6.65 (s)	102.9
4	-	183.0	-	181.7
5	-	163.4	-	161.5
6	6.24 (d, 2.0)	99.7	6.18 (d, 2.1)	98.9
7	-	165.1	-	164.1
8	6.51 (d, 2.0)	94.6	6.43 (d, 2.1)	93.9
9	-	158.8	-	157.3
10	-	105.3	-	103.8
1′	-	123.8	-	121.6
2′	7.49 (d, 2.1)	114.1	7.39 (d, 2.2)	113.4
3′	-	146.4	-	145.8
4′	-	150.0	-	149.7
5′	7.00 (d, 8.4)	116.6	6.89 (d, 9.0)	116.1
6′	7.47 (dd, 8.4, 2.1)	120.1	7.40 (dd, 9.0, 2.2)	119.0

* From: Park *et al.*, 2007.

2. Cytotoxic activity on KB oral cavity and MCF-7 breast cancer cells

The results of cytotoxicity evaluation are summarized in **Table 12**. It can be seen that only two isolated compounds 4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl [**DE5**] and luteolin [**DE10**] were active, whereas the other compounds were devoid of activity. 4,5,4′-Trihydroxy-3,3′-dimethoxybibenzyl [**DE5**] and luteolin [**DE10**] exhibited moderate effects against KB cells with IC_{50} values of 61.93 and 56.22 μM, respectively, as compared with the positive controls ellipticine (IC_{50} 4.99 μM) and doxorubicin (IC_{50} 2.19 μM). In addition, 4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl [**DE5**] and luteolin [**DE10**] showed cytotoxicity against MCF-7 cells with IC_{50} values of 135.48 and 68.01 μM, respectively, in comparision with the positive controls tamoxifen (IC_{50} 20.46 μM) and doxorubicin (IC_{50} 26.29 μM). In this study, 0.5% DMSO was used as negative control.

Table 12 IC_{50} Values (μM) for cytotoxicity on KB and MCF-7 cells

Compounds	KB cells (μg/mL, μM)	MCF-7 cells (μg/mL, μM)
5,7-Dihydroxy-chromen-4-one [**DE1**]	NA	NA
4,5-Dihydroxy-2,3-dimethoxy-9,10-dihydrophenanthrene [**DE2**]	NA	NA
Moscatilin [**DE3**]	NA	NA
4,4′-Dihydroxy-3,5-dimethoxybibenzyl [**DE4**]	NA	NA
4,5,4′–Trihydroxy-3,3′-dimethoxybibenzyl [**DE5**]	17.96, 61.93	39.29, 135.48
(2*S*)-Homoeriodictyol [**DE6**]	NA	NA
(2*S*)-Eriodictyol [**DE7**]	NA	NA
Chrysoeriol [**DE8**]	NA	NA
Phloretic acid [**DE9**]	NA	NA
Luteolin [**DE10**]	16.08, 56.22	19.45, 68.01
Ellipticine	1.23, 4.99	NA
Doxorubicin	1.19, 2.19	14.29, 26.29
Tamoxifen	NA	7.60, 20.46

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

3. Cytotoxicity on H292 lung cancer cells

The compounds (DE1-DE10) were further investigated for cytotoxicity on human lung cancer cells. Subconfluent (80-90%) monolayer H292 cells were treated with these compounds at the concentrations of 100 μM for 24 h, and the cell viability was evaluated by XTT assay. The IC_{50} values of all compounds were determined, and shown in **Table 13**. Two bibenzyls, namely, 4,4′-dihydroxy-3,5-dimethoxybibenzyl [**DE4**] and 4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl [**DE5**], and two flavonoids, namely, chrysoeriol [**DE8**] and luteolin [**DE10**] exhibited appreciable cytotoxic effect against H292 cells. It is worth noting that among the isolates, 4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl [**DE5**] exhibited the strongest cytotoxicity with an IC_{50} value of 96.59 μM. The compounds possessing potent cytotoxicity with IC_{50} value less than 220 μM were selected for further investigation in terms of anti-metastatic effects.

Table 13 IC_{50} Values (μM) for cytotoxicity on H292 cells

Compounds	H292 cells (μM)
5,7-Dihydroxy-chromen-4-one [**DE1**]	>250
4,5-Dihydroxy-2,3-dimethoxy-9,10-dihydrophenanthrene [**DE2**]	>250
Moscatilin [**DE3**]	226.09
4,4′-Dihydroxy-3,5-dimethoxybibenzyl [**DE4**]	197.74
4,5,4′–Trihydroxy-3,3′-dimethoxybibenzyl [**DE5**]	96.56
(2*S*)-Homoeriodictyol [**DE6**]	>250
(2*S*)-Eriodictyol [**DE7**]	>250
Chrysoeriol [**DE8**]	217.74
Phloretic acid [**DE9**]	>250
Luteolin [**DE10**]	202.57

3.1 Apoptosis induction effect of the compounds

Lung cancer cells were exposed to compounds 4,4′-dihydroxy-3,5-dimethoxybibenzyl [**DE4**], 4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl [**DE5**], chrysoeriol [**DE8**] and luteolin [**DE10**] for 24 hours, and apoptosis as well as necrosis cell death were determined by Hoechst33342 and propidium iodide (PI) co-staining assay. Hoechst33342 is a cell-permeable DNA stain that is excited by ultraviolet light and emiss blue fluorescence at 460 to 490 nm. Hoechst33342 binds preferentially to adenine-thymine (A-T) regions of DNA. This stain binds into the minor groove of DNA. The red fluorescing dye PI is only permeable to dead cells and can not enter the intact plasma membrane of living cells.

The bar graph from **Figure 3 (A)** showes that all compounds at the concentrations of 100 and 200 μM significantly induced the apoptosis of the cells, as indicated by an increase in cell possessing condensed and/or fragmented nuclei. Moreover, the morphology of apoptotic nuclei stained with Hoechst 33342 and propidium iodide, following on **Figure 3 (B)** showes that blue-fluorescent Hoechst33342 dye, which stains the condensed chromatin of apoptotic cells more brightly than the chromatin of nonapoptotic cells, and red-fluorescent PI dye, which stains dead cells. These dyes make it possible to distinguish normal, apoptotic and dead cell populations by fluorescence microscopy. The results are consistent with the above finding that all compounds induced apoptosis effect which compound 4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl [**DE5**] has the highest apoptosis activity.

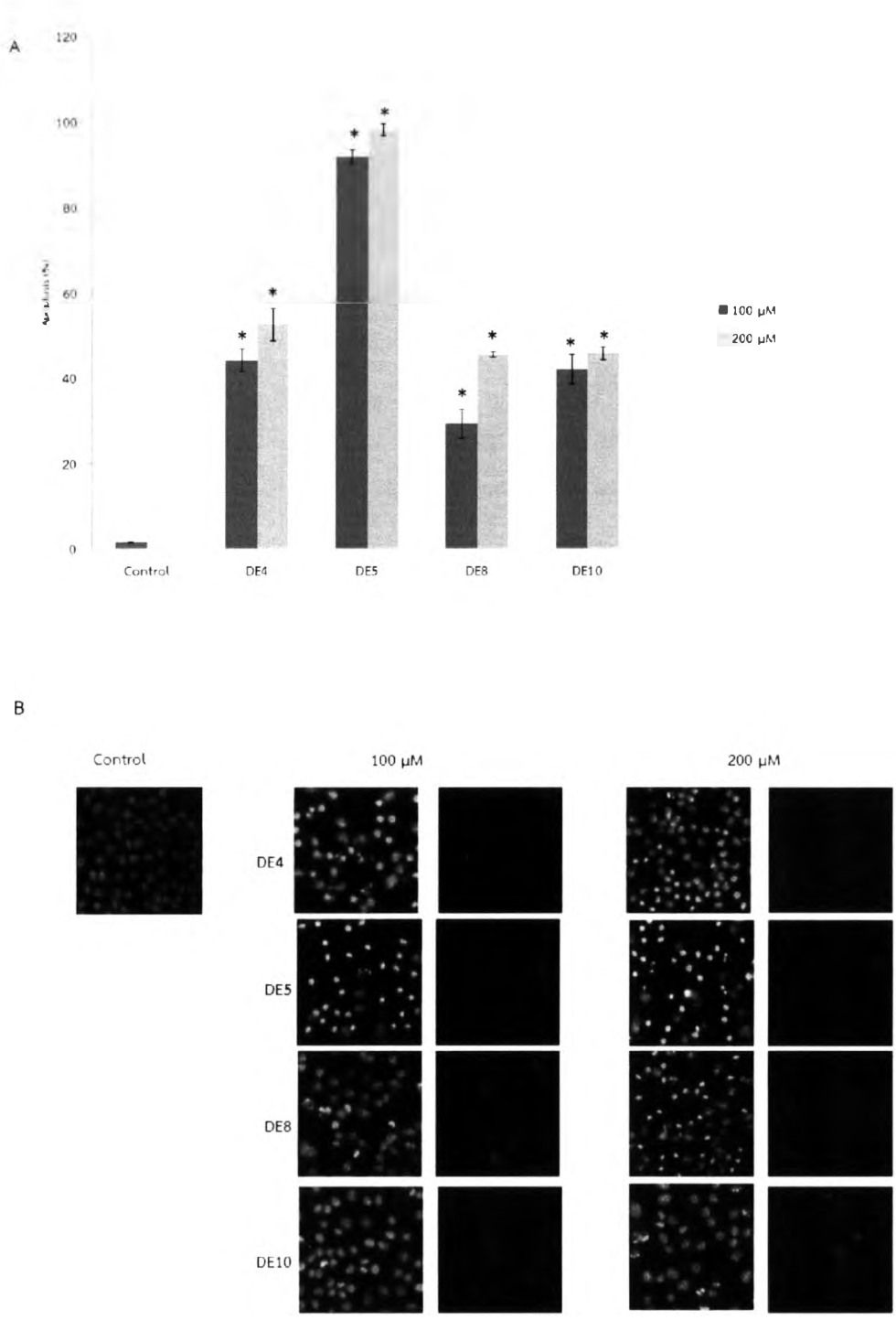


Figure 3 (A) Percentage of cell apoptosis of compounds DE4, DE5, DE8 and DE10 obtained from Hoechst 33342/propidium iodide (PI) assays. Data represent the mean ± SD (n = 3). * $P < 0.05$ versus untreated control cells. **(B)** Morphology of apoptotic nuclei stained with Hoechst 33342 and propidium iodide.

3.2 Anoikis sensitizing activity

Prior to this study, the anoikis sensitizing activity and anti-metastasic potential of compounds 4,4′-dihydroxy-3,5-dimethoxybibenzyl [**DE4**], 4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl [**DE5**] and chrysoeriol [**DE8**] were not investigated. This study attempted to examine their ability in enhancing anoikis response of the metastatic lung cancer cells. Although anti-metastatic effect of luteolin [**DE10**] has been reported, there is no record on its anoikis sensitizing activity. The anti-metastatic property of luteolin [**DE10**] was due to its ability to inhibit Raf and phosphatidylinositol 3-kinase (PI3K) activities (Kim *et al.*, 2013). We first investigated the compounds at non-cytotoxic concentrations. The results indicated that all compounds at the concentration of 1-5 μM caused no effect on cell viability of the adhered H292 cells (data not shown). The cells were then treated with 0-5 μM in detached condition and cell viability over time was evaluated as described in the section of materials and methods. Interestingly, our results (**Figure 4**) indicated that the metastatic lung cancer cells had high anoikis resistance as indicated by approximately 60 % of the cells remaining survive after 24-h detachment. Importantly, the treatment of the cells with compounds at these non-toxic concentrations significantly enhanced cell anoikis. These results suggested the possible anoikis sensitizing effect of the compounds. It is interesting to us that compound 4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl [**DE5**], which possesed highest cytotoxic activity, also had the fastest action in sensitizing the cells to anoikis. The significant effect could be detected as early as 6 hours after exposure to the cells.

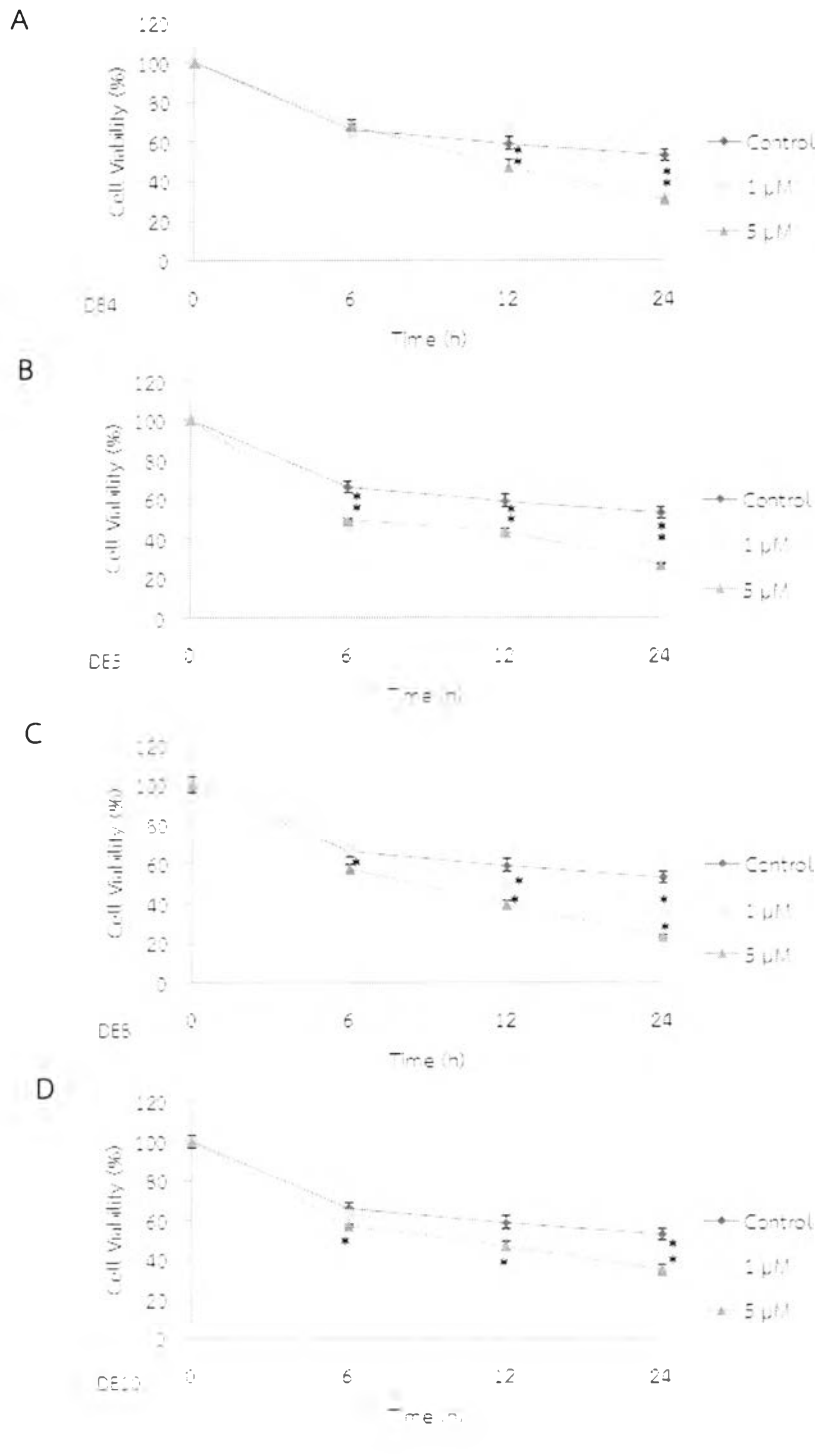


Figure 4 Anoikis sensitizing activity of compounds DE4 (**A**), DE5 (**B**), DE8 (**C**) and DE10 (**D**) as assessed by anoikis assay. The cells were exposed with various concentrations of each compound (0-5 μM) and cell viability was determined by XTT assay at the indicated time. Data represent the mean ± SD (n = 3). * $P < 0.05$ versus untreated control cells.

4. Anti-Herpes Simplex activity

All of the isolated compounds were evaluated for anti-HSV activity using a plaque reduction assay (inactivation). The results showed that only 4,4′-dihydroxy-3,5-dimethoxybibenzyl [**DE4**] had weak anti-herpes simplex virus activity against HSV1 and HSV2 with IC_{50} 313.61 ± 40.40 μM (85.93 ± 11.07 μg/mL) and 334.56 ± 52.66 μM (91.67 ± 14.43 μg/mL), respectively. Acyclovir was used as a positive control for HSV-1 (IC_{50} 0.9 ± 0.17 μM or 0.2 ± 0.04 μg/mL) and HSV-2 (IC_{50} 1.8 ± 0.02 μM or 0.41 ± 0.0045 μg/mL).