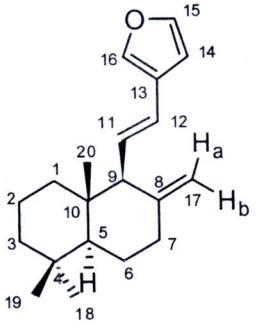
CHAPTER 3

RESULTS AND DISCUSSION

3.1 Structure elucidation of pure compound

Phytochemical studies on the rhizomes of *Amomum uliginosum* resulted in the isolation of eight compounds; coronarin E (**61**) , (*E*)-15,16-bisnorlabda-8(17), 11-dien-13-one (**27**), stigmast-4-en-3-one (**64**), 16-hydroxylabda-8(17),11,13-trien-15,16-olide (**65**), (+)-nerolidol (**22**), villlosin (**66**), 5,6- dehydrokawain (**67**) and a mixture of β-sitosterol (**62**) and stigmasterol (**63**).

Coronarin E (61)



Compound **61** was obtained as a yellow oil with optical rotation $[\alpha]_D^{29}$ + 19.70 (*c* 0.57, $CHCl_3$). The molecular formula was assigned as $C_{20}H_{28}O$ base on the molecular ion peak at *m/z* 284 in the EIMS. The IR spectrum showed the absorption band of exomethylene (2922 and 1156 cm^{-1}) and double bond (1643 cm^{-1}). The ^1H NMR indicated the presence of three methyl groups at δ 0.82 (3H, *s*, H-20), 0.83 (3H, *s*, H-19) and 0.88 (3H, *s*, H-18), as well as exo-methylene protons at δ 4.51 (1H, *d*,

J=1.6 Hz, H-17a) and 4.74 (1H, *d*, *J*=1.6 Hz, H-17b). These characteristic signals suggested that compound **61** has a labdane diterpenoid skeleton. It was further support by peak at *m/z* 137 in the EIMS. The signals of *trans*-olefinic proton signals were observed at δ 5.96 (1H, *dd*, *J*=1 5.7, 9.8 Hz, H-11) and 6.19 (1H, *d*, *J*=15.7 Hz, H-12). The ^{13}C NMR and DEPT spectra of **61** showed 20 signals including three methyl δ 33.56 (C-18), 21.95 (C-19) and 15.00 (C-20), six methylenes at δ 19.12 (C-2), 23.39 (C-6), 36.77 (C-7), 40.78 (C-1), 42.32 (C-3), 107.96 (C-17), seven methines at δ 54.63 (C-5), 61.48 (C-9), 121.75 (C-12), 128.29 (C-11), 107.65 (C-14), 139.59 (C-15) and 143.24 (C-16) and four quarternary carbons at δ 33.56 (C-4), 39.15 (C-10), 124.51 (C-13), 150.22 (C-8). The ^{13}C spectrum also exhibited characteristic signals due to olefinic carbons atδ 128.29 (C-11) and 121.75 (C-12), *exo*-methylene carbon at δ 107.96 (C-17) and three methyl carbons at 33.5 (C-18), 21.9 (C-19) and 15.0 (C-20). ^{1}H-^{1}H COSY and HMBC correlations showed that this olefinic group was directly attached to the decalin nucleus. In addition, the siglet signal at δ 6.52 (1H, *s*, H-14) showed correlations with the signals at δ 121.75 (C-12), 124.51 (C-13), 139.59 (C-16) and 143.24 (C-15) in the HMBC spectrum (Figure 2). On the basis of these observations, it was concluded that the olefinic group is attached directly to the furan ring. From the above evidences, compound **61** was concluded to be coronarin E. This compound has been previously isolated from *Hedychium coronarium*[37] , *Hedychium spicatum* [38], *Hedychium gardnerianum*[39], *Alpinia zerumbe* [40], *Alpinia malaccensis* [41] and *Alpinia chinesis.* [51]

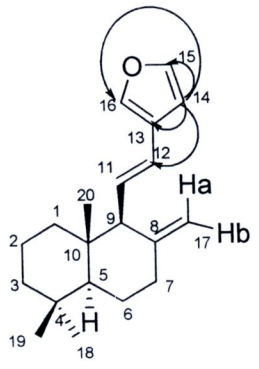
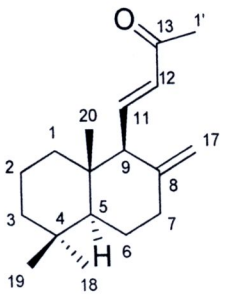


Figure 2 Selected HMBC correlations of coronarin E

Table 10 ^{1}H, ^{13}C-NMR, HMBC data of coronarin E (**61**) in CD_3Cl

Positions	$\delta^{1}H$ (Mult., J in Hz)		$\delta^{13}C$		HMBC
	61	Ref.[51]	**61**	Ref.[51]	
1	α 1.01 (*dt*, 14.4, 4.2)	1.00	40.78	40.20	C-2, 10, 20
	β 1.47 (*m*)	1.40			
2	α 1.42 (*m*)	1.42	19.12	19.10	C-1, 3
	β 1.50 (*m*)	1.52			
3	α 1.18 (*m*)	1.17	42.32	42.30	C-2, 4
	β 1.40 (*m*)	1.40			
4			33.56	33.60	
5	1.11 (*dd*, 12.5, 2.6)	1.09	54.63	54.80	C-4, 6, 10
6	α 1.35 (*m*)	1.53	23.39	23.40	C-5, 7, 8, 10
	β 1.70 (*m*)	1.70			
7	α 2.45 (*ddd*, 13.2, 4.0, 2.1)	2.45	36.77	36.80	
	β 2.10 (*dt*, 13.2, 5.2)	2.07			
8			150.22	150.30	
9	2.37 (*br d*, 9.8)	2.37 (*d*, 9.8)	61.48	61.50	C-8, 10, 11, 20
10			39.25	39.20	
11	5.96 (*dd*, 9.8, 15.7)	5.97 (*dd*, 9.8, 15.6)	128.29	128.30	C-9, 10, 12, 13
12	6.19 (*d*, 9.8)	6.19 (*d*, 9.8)	121.75	121.70	C-9, 11, 13, 14, 16
13			124.51	124.50	
14	6.52 (*s*)	6.55 (*s*)	107.65	107.70	C-13, 15, 16
15	7.33 (*s*)	7.35 (*s*)	139.59	143.30	C-13, 14, 16
16	7.33 (*s*)	7.36 (*s*)	143.24	139.60	C-13, 14, 15
17a	4.51 (*d*, 1.6)	4.52 (*d*, 1.8)	107.96	108.00	C-7, 8, 9
17b	4.74 (*d*, 1.6)	4.75 (*d*, 1.8)			
18	0.88 (*s*)	0.90 (*s*)	33.56	33.60	C-3,4, 5, 19
19	0.83 (*s*)	0.84 (*s*)	21.95	22.00	C-3, 4, 5, 18
20	0.82 (*s*)	0.85 (*s*)	15.00	15.00	C-1, 5, 9, 10

(*E*)-15,16-Bisnorlabda-8(17),11-dien-13-one (27)



Compound **27** was obtained as pale yellow amorphous with optical rotation $[\alpha]_D^{29}$ -8.50 (*c* 0.67, $CHCl_3$). The molecular formula was assigned as $C_{18}H_{28}O$ base on the molecular ion peak at *m/z* 260 in the EIMS. The IR spectrum showed the absorption bands of carbonyl (1664 cm^{-1}), exomethylene (2936 and 898 cm^{-1}) and double bond (1258 cm^{-1}). The ^1H and ^{13}C NMR spectra showed similar patterns to those of compound **61**, suggesting it has the same carbon skeleton. The main difference was the absence of three methine proton signals at δ 6.52 (*s*, H-14), 7.33(*s*, H-15) and 7.33 (*s*, H-16) and the downfield chemical shift of a carbonyl carbon at C-13 at δ 198.1 instead of δ 124.5 in compound **61**. A complete assignment of protons and carbons was assisted by ^1H-^1H COSY, HMQC and HMBC experiments. In ^1H-^1H COSY spectrum, the olefinic proton signal at δ 6.07 (1H, *d*, *J*=15.8 Hz, H-12) correlated with one proton at δ 6.87 (1H, *dd*, *J*=15.8, 10.0 Hz, H-11), which coupled to the signal at δ 2.48 (1H, *br d*, *J*=10.0 Hz, H-9). In the HMBC spectrum, olefinic proton signal at δ 6.87 (1H, *dd*, *J*= 15.8, 10.2 Hz, H-11) and 6.07 (1H, *d*, *J*=15.8 Hz, H-12) showed correlation with the carbonyl at δ 198.18 and long-range correlation between H-12 (δ 6.07) and C-1' (δ 27.21) were also observed (Figure 3). From the above evidences, compound **27** was concluded to be (*E*)-15,16-bisnorlabda-8(17),11-

dien-13-one. This compound has been previously isolated from *Amomum xanthoiodes* [24], *Alpinia zerumbet* [40] and *Alpinia specisa.* [44]

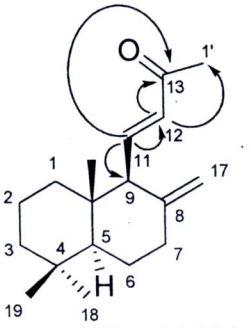
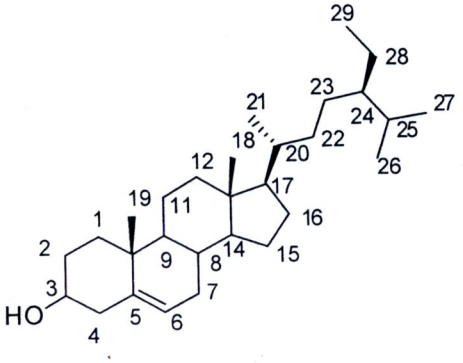


Figure 3 Selected HMBC correlations of (*E*)-15,16-bisnorlabda-8(17),11-dien-13-one

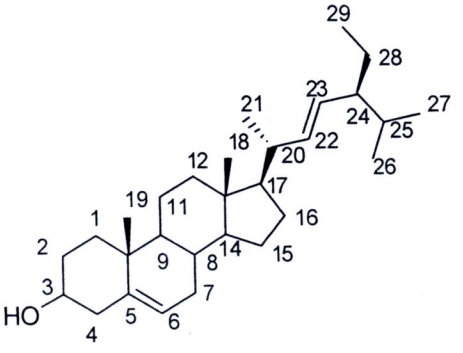
Table 11 ^{1}H and ^{13}C-NMR data of (*E*)-15,16-bisnorlabda-8(17),11-diene-13-one (**27**) in $CDCl_3$.

Positions	δ^{1}H (Mult., *J* in Hz)		δ^{13}C		HMBC
	27	Ref.[24]	**27**	Ref.[24]	
1	α 1.05 (obscured signal)	α 1.05 (*ddd*, 12.5, 12.5, 5.0)	40.86	41.10	C-2, 3,5, 10, 20
	β 1.37 (*m*)	β 1.40 (*m*)			
2	α 1.42 (*m*)	α 1.55 (*m*)	18.99	19.20	C-1, 3, 4
	β 1.54 (*m*)	β 1.55 (*m*)			
3	α 1.20 (*m*)	α 1.21 (*ddd*, 12.5, 12.5, 5.0)	42.09	42.30	C-1,2, 4, 5
	β 1.44 (*m*)	β 1.44 (*m*)			
4			33.54	33.80	
5	1.10 (*dd*, 12.5, 2.6)	1.11 (*dd*, 12.5, 2.5)	54.45	54.60	C-4, 6,9, 10
6	α 1.39 (*m*)	α 1.46 (*m*)	23.23	23.40	C-5, 7, 8, 10
	β 1.71 (*m*)	β 1.74 (*m*)			
7	α 2.09 (*dt*, 12.5, 4.8)	α 2.09 (*dt*, 12.5, 4.8)	36.61	36.80	C-5, 6, 8, 9, 17
	β 2.45 (overlapping signal)	β 2.45 (*ddd*, 12.5, 12.5, 5.0)			
8			146.61	148.80	
9	2.48 (*br d*, 10.0)	2.48 (br d, 10.0)	69.79	61.00	C-8,10,11.12, 17, 20
10			39.33	39.50	
11	6.87 (*dd*, 15.8, 10.0)	6.88 (*dd*, 16.0, 10.0)	146.72	146.90	C-9, 10, 12,13
12	6.07 (*d*, 15.8)	6.08 (*d*, 16.0)	133.57	133.80	C-9, 10, 11, 13, 1'
13			198.18	198.3	
17a	4.40 (*d*, 1.3)	4.42 (*d*, 1.5)	108.61	108.80	
17b	4.79 (*d*, 1.3)	4.79 (*d*, 1.5)			C-7, 8, 9
18	0.89 (*s*)	0.91 (*s*)	33.57	33.70	C-3, 4, 5, 19
19	0.84 (*s*)	0.86 (*s*)	21.92	22.10	C-3,4, 5, 18
20	0.89 (*s*)	0.91 (*s*)	15.11	15.30	C-1, 5, 9, 10
1'	2.27 (*s*)	2.28 (*s*)	27.21	27.40	C-11, 12,13

β-Sitosterol (62) and stigmasterol (63)



62



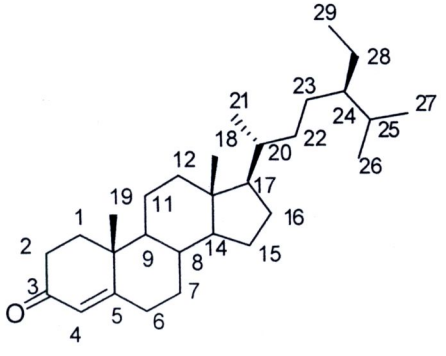
63

A mixture of compounds **62** and **63** was isolated as white crystal. The molecular formulas were assigned as $C_{29}H_{50}O$ and $C_{29}H_{48}O$ base on the molecular ion peak at *m/z* 414 and 412 in the EIMS. The IR spectrum showed the absorption band of hydroxyl (3424 cm^{-1}) group. The ^1H NMR showed the signal of olefinic proton at δ 5.35 (1H, *br s*, H-6) and six methyl proton at δ 0.69 (3H, *s*, H-18), 1.01 (3H,*s*, H-19), 0.93 (3H, *d*, *J*=6.4 Hz, H-21), 0.81 (3H, *d*, *J*=6.5 Hz, H-26), 0.83 (3H, *d*, *J*=6.5 Hz) and 0.85 (3H, *d*, *J*=7.5 Hz, H-29). The ^{13}C NMR spectrum indicated the presence of carbon double bond at δ 140.80 (C-5) and 121.70 (C-6), methine carbon at δ 71.82 (C-3). Six methyl carbon at δ 11.57 (C-18), 19.16 (C-19), 18.53 (C-21), 19.15 (C-26), 19.30 (C-27) and 12.2 (C-29). From the above spectroscopic, data as well as by comparsion of the ^1H and ^{13}C NMR data with those reported in a literature, was the structure of **62** and **63** concluded to be β-sitosterol and stigmasterol.[46] From the ^1H NMR spectrum, integration ratio of proton signals at δ 5.35 (H-6) and 5.15 (H-22,H-23) were 1:0.2, suggesting the presence of β-sitosterol as major component. These compounds have been previously isolated from *Stylochiton lancifolius Pyer* and *Kotchy* (*Araceae*)[46] and *Salvia blepharochaena*.[47]

Table 12 ^{1}H and ^{13}C-NMR data of β-sitosterol (**62**) and stigmasterol (**63**) in $CDCl_3$.

Position	δ ^{1}H (Mult., J in Hz)		δ ^{13}C	
	62	Ref.[47]	**62**	Ref.[47]
1			37.25	37.33
2			31.65	31.63
3	3.48 (*m*)	3.52 (*m*)	71.82	71.73
4			42.29	42.20
5			140.80	140.71
6	5.35 (*br s*)	5.35 (*m*)	121.73	121.63
7			31.92	31.96
8			31.92	31.81
9			51.13	51.13
10			36.15	36.43
11			21.09	21.09
12			39.77	39.79
13			42.29	42.37
14			56.76	56.75
15			24.11	24.15
16			28.26	28.25
17			56.05	56.02
18	0.67 (*s*)	0.69 (*s*)	11.57	11.84
19	1.00 (*s*)	1.01 (*s*)	19.16	19.46
20			36.15	36.07
21	0.93 (*d*, 6.4)	0.92 (*d*, 6.4)	18.53	18.68
22			33.94	33.95
23			26.06	26.10
24			45.83	45.85
25			29.20	29.15
26	0.81 (*d*, 6.5)	0.83 (*d*, 6.8)	19.15	19.77
27	0.83 (*d*, 6.5)	0.81 (*d*, 6.9)	19.30	19.21
28			23.10	23.13
29	0.85 (*t*, 7.5)	0.85 (*t*, 7.8)	12.80	11.04

Stigmast-4-en-3-one (64)

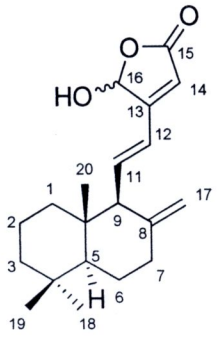


Compound **64** was isolated as white crystal. The molecular formula was assigned as $C_{29}H_{48}O$ base on the molecular ion peak at *m/z* 412 in the EIMS. The IR spectrum showed the absorption band of carbonyl (1683 cm^{-1}) group. The ^{1}H and ^{13}C NMR spectra showed similar patterns to those of compound **62**, suggesting it has the same carbon skeleton. The main difference was the downfield chemical shift of carbon at C-3 and C-4 signals at δ 199.70 and 123.76 instead of δ 71.82 and 42.30 in compound **62**. From the above evidences, compound **64** was concluded to be stigmast-4-en-3-one. This compound has been previously isolated from *Salvia blepharochaena* [47] and *Parkia speciosa.* [48]

Table 13 ^{1}H and ^{13}C-NMR data of stigmast-4-en-3-one (**64**) in $CDCl_3$.

Position	δ ^{1}H (Mult., J in Hz)		δ ^{13}C	
	64	Ref.[47]	**64**	Ref.[47]
1			35.64	35.68
2			33.89	33.89
3			199.70	198.92
4	5.75 (*s*)	5.74 (*d*, 2.2)	123.76	123.64
5			171.75	171.01
6			32.97	32.86
7			32.06	32.07
8			35.70	35.73
9			53.83	53.84
10			38.62	38.58
11			21.04	21.03
12			39.63	39.48
13			42.0	42.35
14			56.89	55.94
15			24.19	24.12
16			28.20	28.10
17			56.02	56.08
18	0.70 (*s*)	0.72 (*s*)	11.98	11.98
19	1.18 (*s*)	1.09 (*s*)	17.40	17.38
20			36.12	36.10
21	0.98 (*d*, 6.5)	0.92 (*d*, 6.4)	18.71	18.72
22			34.01	34.01
23			26.09	25.99
24			45.84	45.80
25			29.16	29.11
26	0.83 (*d*, 7.1)	0.84 (*d*, 6.6)	19.82	19.81
27	0.81 (*d*, 6.9)	0.82 (*d*, 6.8)	19.03	19.18
28			23.08	23.10
29	0.84 (*t*, 7.5)	0.85 (*t*, 7.2)	11.95	11.14

16-Hydroxylabda-8(17),11,13-trien-15,16-olide (65)



Compound **65** was isolated as a pale yellow amorphous optical rotation $[\alpha]_D^{29}$ +24.09 (*c* 0.63, $CHCl_3$). The molecular formula $C_{21}H_{30}O_3$ was determined from the molecular ion peak at *m/z* 339.1938 ($[M+Na]^+$, calcd 339.1936) in HREIMS. The IR spectrum indicated the presence of α, β-unsaturated-γ-lactone (1747 cm^{-1}) and hydroxyl (3373 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra showed similar pattern with those of **61**, with the exception of the difference in the absence of chemical shift of broad one proton signal at C-15 and downfield chemical shift of a carbonyl carbon at δ 172.03 instead of δ 139.59 in compound **61**. In addition, the upfield chemical shift of H-16 (δ6.27/6.29 of **65** and 7.33 of **61**) and carbon (δ98.00/98.02 of **65** and δ 143.24 of **61**) at C-16 were also observed. Compound **65** was isolated as a mixture of two stereoisomers which was indicated by the presence of duplicated ^1H-NMR signals at δ4.38/4.78 (H-17) and of ^{13}C-NMR signals at δ 108.53/108.97 (C-17).

Table 14 ^{1}H, ^{13}C-NMR and HMBC data of 16-hydroxylabda-8(17),11,13-trien-15,16-olide (**65**) in $CDCl_3$.

Positions	$\delta^{1}H$ (Mult., *J* in Hz)		$\delta^{13}C$		HMBC
	65	Ref.[43]	**65**	Ref.[43]	
1	α 1.02 (*m*)	α 1.02 (ddd, 13.2, 13.2, 3.7)	40.84; 40.99	40.90; 41.00	C-2, 3,10, 20
	β 1.38 (*m*)	β 1.38 (*m*)			
2	α 1.41 (*m*)	α 1.40 (*m*)	19.02; 19.07	18.90; 19.00	C-1, 3, 4
	β 1.54 (*m*)	β 1.53 (*m*)			
3	α 1.20 (*m*)	α 1.18 (*m*)	42.14	42.10	C-1, 2 ,4, 18, 19
	β 1.43 (*m*)	β 1.42 (*m*)			
4			33.55	33.5	
5	1.09 (*dd*, 12.6, 2.3)	1.09 (*dd*, 12.5, 2.7)	54.51; 54.53	54.40; 54.50	C-4, 6, 10, 20
6	α 1.40 (*m*)	α 1.39 (*m*)	23.22	23.20	C-5, 7, 8, 10
	β 1.71 (*m*)	β 1.72 (*m*)			
7	α 2.10 (*m*)	α 2.08 (*m*)	36.59; 36.62	36.60	C-5, 6, 8, 9, 17
	β 2.44 (*m*)	β 2.44 (*m*)			
8			148.61; 148.90	148.70; 148.90	
9	2.47 (*br d*, 10.6)	2.47 (*d*, 10.0)	62.16; 62.11	62.10; 62.00	C-8, 10, 11, 12
10			39.50; 39.61	39.50; 39.60	
11	6.58 (*dd*, 16.0, 10.4)	6.58 (*dd*, 16.0, 10.0)	144.05; 144.13	144.00; 144.10	C-8, 9, 10, 13
	6.62 (*dd*, 16.0, 10.4)	6.59 (*dd*, 16.0, 10.0)			
12	6.31 (*d*, 16.0)	6.31 (*d*, 16.0)	122.72; 122.78	122.60; 122.70	C-9, 10, 13, 14, 16
13			161.52	161.00; 161.10	
14	5.85 (*br s*)	5.85 (*s*)	115.33	115.50	C-11, 12, 13, 15, 16
15			172.03	171.20	
16	6.27 (*s*);6.29 (*s*)	6.25 (*s*);6.27 (*s*)	98.00; 98.02	97.50; 97.60	C-14, 15
17a	4.38 (*br s*) /4.48 (*br s*)	4.38 (*d, 1.5*) /4.47 (*d, 1.5*)	108.53; 108.97	108.50; 108.90	C-7, 8, 9
17b	4.78 (*br s*)	4.78 (*br s*)			
18	0.89 (*s*)	0.90 (*s*)	33.58	33.60	C-3, 4, 5, 19
19	0.84 (*s*)	0.85 (*s*)	21.93	21.90	C-3, 4, 5, 18
20	0.86 (s)	0.87 (s)	15.09; 15.16	15.10; 15.20	C-1, 5, 9, 10

The HMBC spectrum showed long-range correlations of olefinic proton signal at δ 5.85 (H-14) with the carbonyl at δ 172.03 (C-15) as well as with the signal at 122.72 (C-12a), 122.78 (C-12b), 161.52 (C-13) and 98.00 (C-16a), 98.02 (C-16b) (Figure 4). From the above evidences, compound **65** was concluded to be an isomeric mixture of 16-hydroxylabda-8(17),11,13-trien-15,16-olide. This compound has been previously isolated from *Etlingera elatior*.[43]

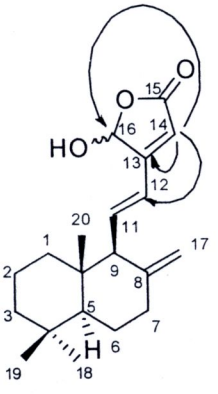
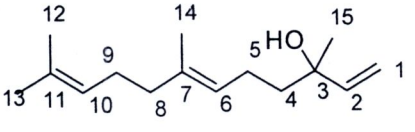


Figure 4 Selected HMBC correlations of 16-hydroxylabda-8(17),11,13-trien-15,16-olide

(+)-Nerolidol (21)



Compound **21** was isolated as a colorless oil with optical rotation $[\alpha]_D^{29}$ +10.43 (*c* 0.41, $CHCl_3$). The molecular formula was assigned as $C_{15}H_{26}O$ base on the molecular ion peak at *m/z* 222 in the EIMS. The IR spectrum showed the absorption band of hydroxyl (1640 cm^{-1}) and exo-methylene (1451 cm^{-1}) groups.

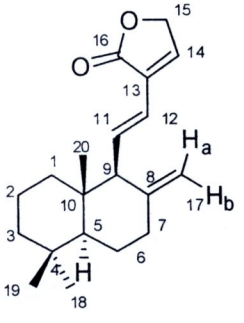
Table 15 ^{1}H- and ^{13}C-NMR data of (+)-nerolidol (**21**) in $CDCl_3$.

Positions	δ^{1}H (Mult., *J* in Hz)		δ^{13}C		HMBC
	21	Ref.[44]	**21**	Ref.[44]	
1a	5.04 (*dd*, 0.7, 10.7)	5.21 (*d*, 11.0)	111.67	111.80	C-2, 3
1b	5.20 (*dd*, 0.7, 17.3)	5.22 (*d*, 18.0)			
2	5.91 (*dd*, 10.7, 17.3)	5.92 (*dd*, 11.0, 18.0)	145.07	145.20	C-3, 4, 15
3			73.50	73.60	
4	1.56 (*m*)		42.06	42.20	C-3, 5, 6
5	1.96 (*m*)		22.78	26.80	C-3, 4
6	5.13 (*t*, 6.8)		124.25	124.40	C-5, 8, 14
7			135.57	135.70	
8	2.01 (*m*)		39.70	39.80	C-6, 7, 9, 14
9	2.05 (*m*)		26.65	28.00	C-8, 10, 11
10	5.09 (*m*)		124.25	124.40	C-9, 13
11			131.43	131.60	
12	1.59 (*s*)	1.60 (*s*)	17.68	16.20	C-11, 13
13	1.59 (*s*)	1.27 (*br s*)	25.69	22.90	C-11, 12
14	1.67 (*s*)	1.59 (*s*)	16.02	17.80	C-6, 7
15	1.28 (*s*)	1.67(*s*)	27.89	25.80	C-2, 3, 4

The ^{1}H NMR showed the signal of four methyl groups at δ 1.28 (3H, *s*, H-15), 1.59 (6H, *s*, H-12, H-13) and 1.67 (3H, *s*, H-14) an methylene at δ 5.04 (1H, *dd*, *J*=0.7, 10.7 Hz, H1a) and 5.20 (1H, *dd*, *J*=0.7, 17.3 Hz, H1b). Three methylene at δ 1.56 (2H, *m*, H-4), 1.96 (2H, *m*, H-5), 2.01 (2H, *m*, H-8) and 2.05 (2H, *m*, H-9), and three olefins at δ 5.09 (1H, *m*, H-10), 5.13 (1H, *t*, *J*=7.0 Hz, H-6) and 5.91 (1H, *dd*, *J*=10.7, 17.3 Hz, H-2). The ^{13}C NMR spectrum indicated the presence of three quaternary carbon at δ 73.5 (C-3), 131.4 (C-11) and 135.6 (C-7). In HMBC spectrum, the proton signal at δ 2.01 (1H, *m*, H-8) showed correlation with the signal δ 124.25 (C-6), 135.57 (C-7) and 16.02 (C-14). The correlation between the carbon signal at

δ 124.25 (C-10) and these aliphatic protons and the methyl signal at δ 1.59 (6H, *s*, H-12, H-13), as well as between the methyl carbons signal at δ 25.69 (C-13) and 16.02 (C-14) and the proton signal at δ 5.13 (1H, *t*, H-6). These data suggested the presence of a double bond between the carbon positions 10 and 11. From the above evidences, compound **21** was concluded to be (+)-nerolidol. This compound has been previously isolated from *Glomerella cingulata.*[44]

Villosin (66)



Compound **66** was obtained as a colorless crystal with optical rotation $[\alpha]_D^{29}$ -1.61 (*c* 1.05, $CHCl_3$). The molecular formula was assigned as $C_{20}H_{28}O_2$ base on the molecular ion peak at *m/z* 284. The IR spectrum showed the absorptions for carbonyl (1640 cm^{-1}) groups. The comparison of ^1H and ^{13}C NMR spectra with these of compound **61** indicated the same skeleton. The main difference was the absence of chemical shift of a broad one proton singlet at C-16 and the downfield chemical shift of a carbonyl carbon at δ 172.4 (C-16) instead of δ 143.2 in compound **61**. In addition, the upfield chemical shift of the proton (δ 4.81) and carbon (δ 69.6) at C-15 were also observed. From HMBC spectrum, the ^1H-^{13}C long-range correlation between H-12 (δ 6.11) and the carbonyl carbon (δ 172.4) indicated that this functional group was located at C-16 rather than at C-15 (Figure 5). From the above evidences, compound **66** was concluded to be villosin. This compound has been previously isolated from

Hedychium coronarium [37] ,*Hedychium gardnerianum* [39], *Alpinia malaccensis* [41] and *Hedychium villosum.* [42]

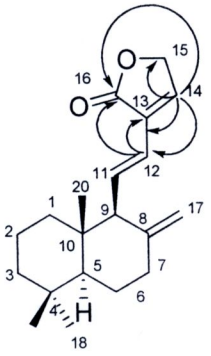
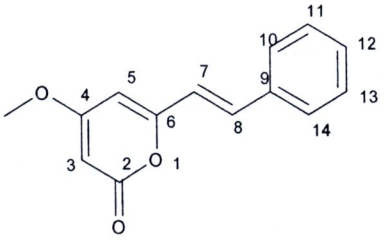


Figure 5 Selected HMBC correlations of villosin

Table 16 ^{1}H, ^{13}C-NMR and HMBC data of villosin (**66**) in $CDCl_3$.

Positions	$\delta^{1}H$ (Mult., *J* in Hz)		$\delta^{13}C$		HMBC
	66	Ref.[42]	**66**	Ref.[42]	
1	α 1.00 (*m*)	α 1.03 (*m*)	40.89	41.05	C-2, 10
	β 1.41 (*m*)	β 1.45 (*m*)			
2	α 1.46 (*m*)	α 1.42 (*m*)	19.19	19.34	C-1, 3
	β 1.51 (*m*)	β 1.60 (*m*)			
3	α 1.17 (*m*)	α 1.21 (*m*)	42.29	42.51	C-2, 4, 18, 19
	β 1.35 (*m*)	β 1.39 (*m*)			
4			33.66	29.98	
5	1.09 (*dd*, 12.5, 2.5)	1.12 (*dd*, 12.5, 2.5)	54.75	54.98	C-4, 6, 7, 10
6	α 1.38 (*m*)	α 1.38 (*m*)	23.45	23.58	C-5, 7, 8, 10
	β 1.73 (*m*)	β 1.70 (*m*)			
7	α 2.10 (*dt*, 12.9, 5.9)	α 2.08 (*dt*, 13.4, 5.1)	36.83	36.75	C-5, 6, 8, 9, 17
	β 2.38 (*ddd*,12.9, 4.3, 2.4)	β 2.44 (*ddd*,13.4, 4.0, 2.0)			
8			149.50	146.69	
9	2.41 (*d*, 10.1)	2.37 (*br d*, 10.1)	62.28	62.44	C-8, 10, 20
10			39.31	39.53	
11	6.92 (*dd*, 15,8, 10.1)	6.90 (*dd*, 15,8, 10.1)	136.92	137.10	C-8, 9, 10, 12
12	6.18 (*d*, 15.8)	6.11 (*d*, 15.8)	120.75	120.62	C-11,13,14, 16
13			129.59	129.62	
14	7.16 (*br s*)	7.15 (*br s*)	142.55	142.21	C-12,13,15, 16
15	4.81 (*br s*)	4.81 (*br s*)	69.69	69.61	C-13, 14
16			172.46	172.31	
17a	4.51 (s)	4.50 (s)	108.49	108.41	C-7, 8, 9
17b	4.76 (s)	4.76 (s)			
18	0.89 (*s*)	0.89 (*s*)	33.66	3.81	C-3,4,5,19
19	0.87 (*s*)	0.87 (*s*)	22.03	22.18	C-3,4,5,18
20	0.84 (s)	0.80 (s)	15.14	15.29	C-1,5,9,10

5,6-Dehydrokawain (67)



Compound **67** was isolated as pale yellow solid. The molecular formula was assigned as $C_{14}H_{12}O_3$ base on the molecular ion peak at *m/z* 228 in the EIMS. The IR spectrum showed the absorption band of carbonyl (1713 cm^{-1}) and aromatic ring (1610-1460 cm^{-1}) group.

Table 17 ^{1}H- and ^{13}C-NMR data of 5,6-dehydrokawain (**67**) in $CDCl_3$.

Positions	δ^{1}H (Mult., *J* in Hz)		δ^{13}C		HMBC
	67	Ref.[45]	**67**	Ref.[45]	
1					
2			164.05	158.60	
3	5.50 (*d*, 1.9)	5.40(*d*,2.5)	88.88	88.90	C-2, 4, 5
4			171.11	171.20	
5	5.95 (*d*, 1.9)	5.85 (*d*, 2.5)	101.33	101.40	C-3, 4, 6, 7
6			158.67	164.00	
7	6.60 (*d*, 16.0)	6.40 (*d*, 16.0)	118.64	118.60	C-5, 8, 6
8	7.53 (*d*, 16.0)	7.40 (*d*, 16.0)	135.85	135.70	C-6, 9, 13
9			135.24	135.10	
10	7.50 (*m*)		127.46	127.40	C-8, 9, 11
11	7.38 (*m*)		128.91	128.90	C-9, 10, 12
12	7.33 (*m*)		129.46	129.40	C-11, 13
13	7.38 (*m*)		128.91	128.90	C-12, 14
14	7.50 (*m*)		127.46	127.40	C-8, 9, 13
4-OMe	3.78 (*s*)	3.75 (*s*)	55.95	55.90	

The ^{1}H NMR showed the signal of methoxy group at δ 3.78 (3H, *s*, 4-OMe), olefinic portons at δ 6.60 (1H, *d*, *J*=16 Hz, H-7) and 7.53 (1H, *d*, *J*= 16 Hz, H-8), two methine proton at δ 5.50 (1H, *d*, *J*=1.9 Hz, H-3), and 5.95 (1H, *d*, *J*=1.9 Hz, H-5), phenyl proton at δ 7.50 (1H, *m*,H-10), 7.38 (2H, *m*, H-11,H-13) and 7.33 (1H, *m*, H-12). The ^{13}C NMR spectrum indicated the presence of carbonyl carbon at δ 164.05 (C-2), methyl carbon at δ 55.95 (4-OMe), and aromatic carbon at δ 135.24 (C-9), 124.76 (C-10, C-14), 128.91 (C-11, C-13) and 129.46 (C-12). The structure of compound **66** was further confirmed by 1D NMR and 2D NMR experiments. In ^{1}H-^{1}H COSY spectrum, the olefinic proton signal at δ 6.60 (1H, *d*, *J*=16.0 Hz, H-7) correlated with one proton at δ 7.53 (1H, *d*, *J*=16.0 Hz, H-8). In the HMBC spectrum, olefinic proton signal at δ 6.60 (1H, *d*, *J*= 16.0, H-7) showed correlation with the signal at δ 101.33 (C-5), 158.67 (C-6), 135.87 (C-8) and 135.24 (C-9) (Figure 6). The proton signal at δ 5.50 (1H, *d*, *J*=1.9 Hz, H-3) showed correlation with the carbonyl carbon at δ 164.05 (C-2), δ 171.11(C-4) and δ 101.33 (C-5). From the above evidences, compound **67** was concluded to be 5,6-dehydrokawain. This compound has been previously isolated from *Alpinia malaccensis* [4]) and *Alpinia rafflesiana.*[45]

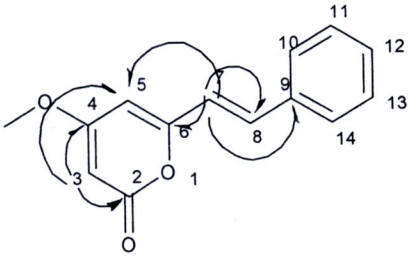


Figure 6 Selected HMBC correlations of 5,6-dehydrokawain

3.2 Biological Activities

The biological activities of bisnorlabdane, (*E*)-15,16-bisnorlabda-8(17),11-dien-13-one (**27**), and labdane-type diterpenes, coronarin E (**61**), 16-hydroxylabda-8(17),11,13-trien-15,16-olide (**65**) and villosin (**65**) were evaluated for their antimycobacterial and cytotoxic activities as the results shown in **Table 8** and **9**.

Compounds **27**, **61**, **65** and **66** were test for their cytotoxicity against the human oral epidermoid carcinoma (KB), human breast cancer (BC), human small cell lung cancer (NCI-H187) and noncancerous Vero cells. The results indicated that compounds **66** and **61** exhibited moderate activity against *Mycobacterial tuberculosis* (MIC 6.25 and 12.50 μg/mL respectively) which compounds **27** and **66** were inactive on antimycobacterial assay.

For cytotoxic activity assay, compounds **65**, **66** and **61** exhibited strong to moderate activity against the KB cells with IC_{50} values of 0.91 and 9.67 μg/mL respectively. Compounds **27** showed weak activity against the KB cells (IC_{50} 36.99 μg/mL). In addition, compounds **65** and **66** showed moderate activity against BC cells with IC_{50} values of 2.89 and 8.50 μg/mL respectively. Whereas compounds **66** showed weak activity against BC cells with IC_{50} values of IC_{50} 49.22 μg/mL. For NCI-H187 cells the results indicated that compounds **66** and **65** showed the highest activities with IC_{50} values of 0.12 and 0.72 μg/mL respectively. Compounds **61** showed weak cytotoxic to Vero cells with IC_{50} values of 25.37 μg/mL. However, compound **65** was very toxic to Vero cells (IC_{50} 5.37 μg/mL) whereas compounds **27** and **66** were non-cytotoxic.

Compounds **27** has been reported to showed moderate cytotoxicity against A549, SK-OV-3, SK-MEL-2 and HCT15 cell with IC_{50} values of 13.9, 15.2, 11.8 and 12.6

μg/mL, respectively.[29] Compound **61** showed cytotoxic activity against HL-60, THP-1, A-375 and A-549 cell with IC_{50} values of 31.21, 33.99, 36.58 and 53.26 μg/mL, respectively. [38] Compounds **61** and **66** isolated from the rhizome of *Hedychium gardnerianum* showed cytotoxicity against NCI-H187 cell line. [39]