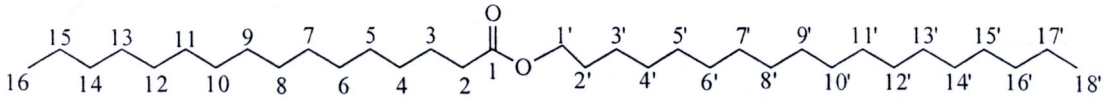
CHAPTER 3

RESULTS AND DISCUSSION

3.1 Structure Elucidation of Pure Compounds

Phytochemical studies of *Globba reflexa* rhizomes afforded seven known compounds namely stearyl palmitate (**26**), methyl palmitate (**27**), (*E*)-15,16-bisnorlabda-8(17),11-dien-13-one (**28**), villosin (**29**), coronarin D (**32**) and mixture of *β*–sitosterol (**13**), stigmasterol (**30**) and campesterol (**31**).

Stearyl palmitate (26)



Compound **26** was obtained as white amorphous solid. Its molecular formula was established $C_{34}H_{68}O_2$ from the molecular ion peak at *m/z* 508 in the EIMS. The mass spectrum show the series of fragmentation at *m/z* 239, 257 and 283 which are the result of eliminate of -OR, McLafferty rearrangement and bond cleavage next to carbonyl group, respectively (Figure **2**). The IR spectrum exhibited the presence of C-H asymmestrical stretching (2924 cm^{-1}), C-H symmestrical stretching (2825 cm^{-1}), carbonyl of aliphatic ester (1729 cm^{-1}), -CH_2 bending (1464), -CH_3 bending (1377) and long chain band of –CH_2 (740) units. The ^1H NMR spectrum of **26** display signals for methylene protons attached to the oxygen in the ester group at δ 4.05 (2H, *t*, *J*= 6.7 Hz, H-1′), *α*-methylene proton attached to carbonyl carbon at δ 2.28 (2H, *m*, H-2), *β*-methylene

proton from carbonyl carbon at δ 1.62 (2H, *m*) and two terminal methyl protons at δ 0.87 (3H, *m*, H-16Me) and δ 0.88 (3H, *m*, H-18′Me). The large signal at δ 1.25 is typically from methylenes in the alkyl and the fatty acid chains. The ^{13}C NMR and DEPT spectra of compound **26** showed 34 carbon signals including two methyls at δ 14.12 (C-16Me) and δ 14.13 (C-18′Me), one α-carbonyl methylene carbon at δ 34.43, one β-carbonyl methylene carbon at δ 25.05 (C-3), methylene carbon attached to oxygen at δ 64.42 (C-1′) and one carbonyl carbon at δ 173.36 (C-1). The structure of compound **26** was further confirmed by COSY, HMQC and HMBC experiments. ^{1}H-^{1}H COSY spectrum showed the presence of methylene proton signal at δ 4.05 (2H, *t*, J= 6.7 Hz, H-1′) correlated with methylene proton at δ 1.60 (2H, *m*, H-2′). In the HMBC spectrum, α-methylene proton at δ 2.28 (2H, *m*, H-2) and methylene protons attached to oxygen showed correlated with carbonyl carbon at δ 173.36 (C-1). From these data, the structure of compound **26** was assigned as stearyl palmitate. This compound has been previously isolated from wheat straw [23] and rice straw. [24]

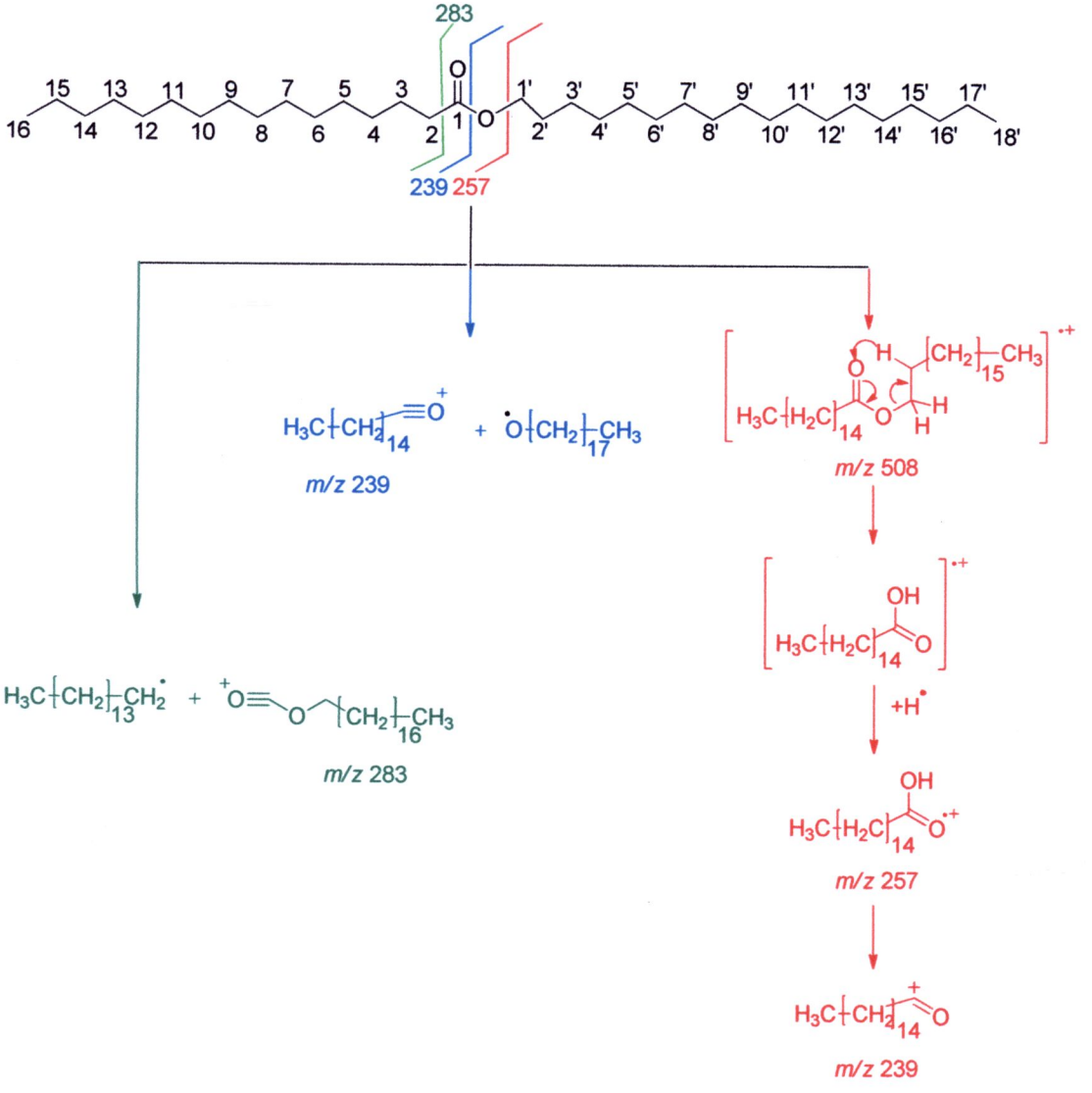
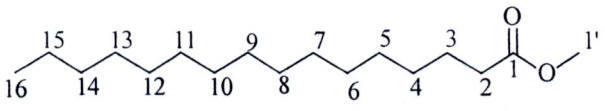


Figure 2 Characteristic fragment of stearyl palmitate (**26**)

Methyl palmitate (27)



Compound **27** was obtained as white amorphous solid. The molecular formula was established as $C_{17}H_{34}O_2$ from the molecular ion peak at *m/z* 270 in the EIMS. The EI spectrum of compound **27** show characteristic fragment ion at *m/z* 74 and 87 which are the result of McLafferty rearrangement and γ-cleavage (Figure **3**). In addition two fragment can result from bond cleavage next to C=O at *m/z* 239. The IR spectrum exhibited the presence of C-H asymmestrical stretching (2924 cm^{-1}), C-H symmestrical stretching (2854 cm^{-1}), carbonyl of aliphatic ester (1742 cm^{-1}), $-CH_2$ bending (1463), $-CH_3$ bending (1385) and long chain band of $-CH_2$ (750) units. The ^1H NMR and ^{13}C NMR spectra showed similar pattern with those of **26**, with the exception of the difference in the upfield shift of one terminal methyl proton and carbon at δ 3.65 and δ 51.9 respectively. In addition, the up field chemical shift of C-1 (δ 173.36 of **26** and δ 120.16 of **27**) were also observed. Thus, compound **27** was identified as methyl palmitate. This compound has been previously isolated from *Helianthus annuus*[25], *Maclura pomifera L.*[26] and *Camellia sinensis.*[27]

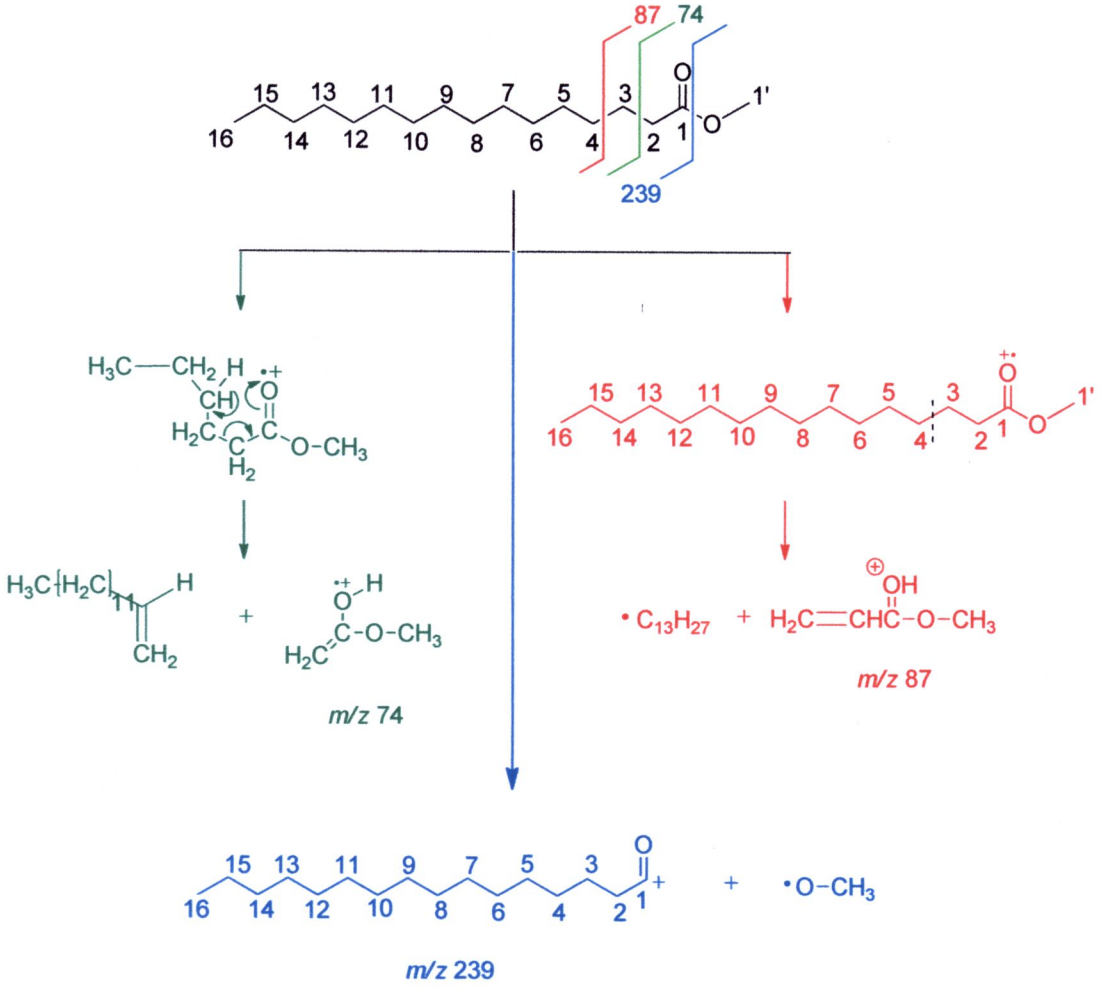
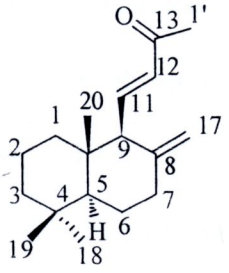


Figure 3 Characteristic fragment of methyl palmitate (**27**)

(*E*)-15,16-bisnorlabda-8(17),11-dien-13-one (28)

(*E*)-15,16-bisnorlabda-8(17),11-dien-13-one (28)



Compound **28** was obtained as colorless amorphous solid with optical rotation $[\alpha]_D^{27}$ -29.24 (*c* 0.2735, $CHCl_3$). The molecular formula was established as $C_{18}H_{28}O$ from the molecular ion peak at *m/z* 260 in the EIMS. The IR spectrum exhibited the presence of carbonyl (1664 cm^{-1}), exo-methylene (2936 and 898 cm^{-1}) and double bond (1664 cm^{-1}) units. The structure of compound **28** was determined from ^1H, ^{13}C, COSY, DEPT 135, HMQC and HMBC experiments. The ^1H NMR spectrum displayed signals for three methyl protons at δ 0.82 (3H, *s*, H-19), δ 0.89 (3H, *s*, H-18) and δ 0.89 (3H, *s*, H-20) as well as *exo*-methylene protons at δ 4.40 (1H, *br d*, *J*=1.3 Hz, H-17a) and 4.78 (1H, *br d*, *J*=1.3 Hz, H-17b) (Table 4), suggesting that compound **28** possesses the bicyclic carbon skeleton of labdane. This was supported by the appearance of a characteristic mass fragment at *m/z* 137(100 %) in the EIMS. The ^1H NMR spectrum showed *trans*-olefinic proton signals at δ 6.87 (1H, *dd*, *J*=15.8, 10.0 Hz, H-11) and δ 6.06 (1H, *d*, *J*=15.8 Hz, H-12). The ^{13}C NMR and DEPT spectra of compound **28** showed 18 carbon signals including four methyls at δ 15.11 (3H, *s*, C-20) 21.92 (3H, *s*, C-19), 27.21 (3H, *s*, C-1′) and 33.57 (3H, *s*, C-18), six methylenes at δ 18.99 (C-2), 23.23 (C-6), 36.61 (C-7), 40.86 (C-1), 42.09 (C-3), 108.61 (C-17), four methines at δ 54.45 (C-5), 60.79 (C-9), 133.57 (C-12) and 146.72

(C-11) and four quarternary carbons at δ 33.54 (C-4), 39.33 (C-10), 148.61 (C-8) and 198.18 (C-13). The carbon signal at δ 198.18 was assigned to a carbonyl group, the presence of which was supported by the absorption band in the IR spectrum. In addition, the signal for methyl proton at δ 2.28 (3H, *s*) indicated the presence of a side chain, α, β-unsaturated ketone. The structure of compound **28** was further confirmed by COSY, HMQC and HMBC experiments. COSY spectrum showed the presence of olefinic proton signal at δ 6.06 (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 2.46 (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, 10.0 Hz, H-11) and 6.06 (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 6.06) and C-1' (δ 27.21) were also observed (Figure **4**). From these data, the structure of compound **28** was assigned as (*E*)-15,16-bisnorlabda-8(17),11-dien-13-one by the above evidences and by comparison of spectroscopic data with those reported values.[28] This compound has been previously isolated from *Alpinia speciosa*[28], *Alpinia calcarata.*[29] and *Curcuma mangga.*[30]

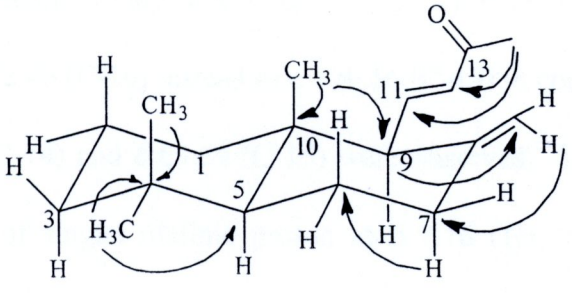
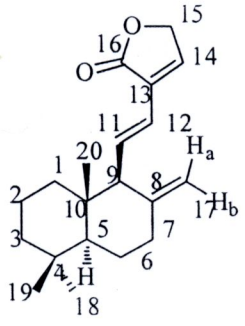


Figure 4 HMBC correlations of (*E*)-15,16-bisnorlabda-8(17),11-dien-13-one (**28**)

Villosin (29)



Compound **29** was isolated as a white amorphous solid with optical rotation $[\alpha]_D^{27}$ -93.00 (*c* 0.043, $CHCl_3$). The molecular formula was established as $C_{20}H_{28}O_2$ from the molecular ion peak at *m/z* 300 in the EIMS. The IR spectrum exhibited the presence of an α,β-unsaturated-γ-lactone (1754 cm^{-1}). The structure of compound **29** was determined from ^1H, ^{13}C, COSY, DEPT 135, HMQC and HMBC experiments. The ^1H NMR and ^{13}C NMR spectra showed similar pattern with these of compound **28**. Comparison of the ^1H NMR spectrum of compound **29** with that of **28** indicated that significant differents were the absence of a singlet signal of acetyl group at δ 2.28, and the presence of two broad singlet signal at δ 7.16 and 4.81. In addition, the ^{13}C NMR spectrum showed the upfield chemical shift of a carbonyl carbon at δ 172.46 enhance of signal at δ 142.55 (C-14), δ 69.69 (C-15) and carbonyl carbon signal at δ 172.46 (C-16) instead of δ 129.56 (C-13) in compound **28**. Another signals at δ 142.5 (C-14) and δ 69.69 (C-15) were observed. The HMBC spectrum showed correlation of singlet olefinic proton at δ 7.16 (1H, *br s*, H-14) with the carbonyl at δ 172.46 as well as with the signals at δ 69.69 (C-15), 120.75 (C-12) and 129.56 (C-13). In addition, long-range correlation between H-12 (δ 6.14) and the

carbonyl carbon (δ 172.46) indicated that this functional group was located at C-16 rather than at C-15 (Figure **5**). By comparison of spectroscopic data with those of the literature values,[31] compound **29** was therefore concluded to be villosin. This compound has also been isolated from *Curcuma Comosa*[31] and *Hydychium coronarium*.[32]

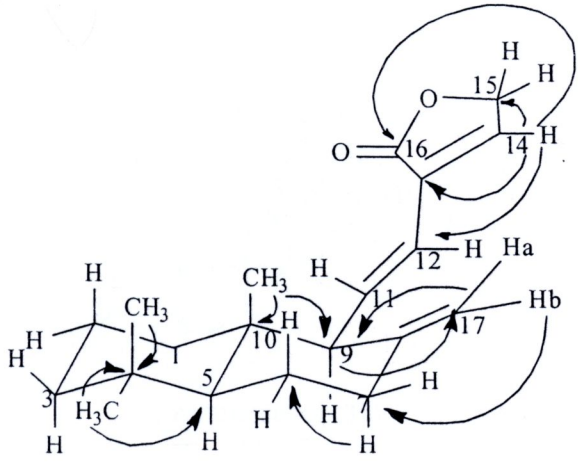
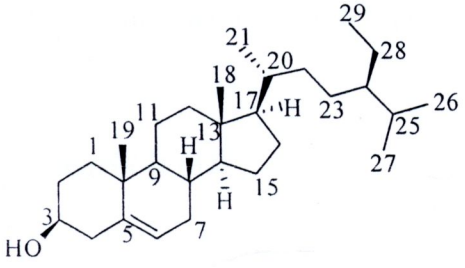
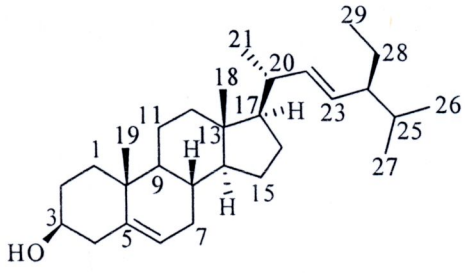


Figure 5 HMBC correlations of villosin (**29**)

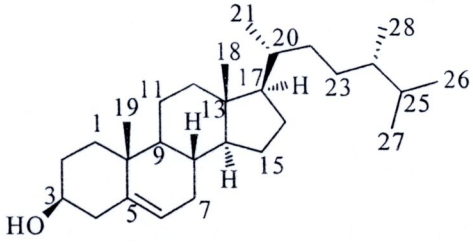
β–Sitosterol (13), Stigmasterol (30) and Campessterol (31)



β–sitosterol (**13**)



stigmasterol (**30**)

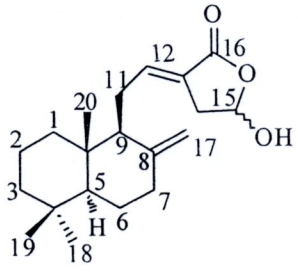


campessterol (**31**)

A mixture of compounds **13**, **30** and **31** was obtained as colorless crystals. EIMS spectra showed a parent molecular ion $[M]^+$ peak at *m/z* 414, 412 and 400 respectively which corresponds to molecular formula $C_{29}H_{50}O$, $C_{29}H_{48}O$ and $C_{28}H_{48}O$, respectively. The IR spectrum showed absorption peaks at 3422 cm^{-1}(O-H str.), 1459 cm^{-1} (-CH_2 bending), 1376 cm^{-1} (-CH_3 bending) and 832 cm^{-1}(out-of-plane bending). In ^1H NMR spectrum showed multiplet signals of carbinolic methine proton at δ 3.53 (1H, *m*, H-3). The methine proton signal at δ 5.35 corresponded to olefinic proton at C-6. The two methine protons signal at δ 5.05 and δ 5.15 were those of the olefinic protons at C-22 and C-23 of stigmasterol. In addition, the signals of methyl protons were found at δ 0.81-1.19. The spectroscopic data was similar to the data in the literature of β–sitosterol[34], stigmasterol[33,34] and campesterol[33]. The structure of

compounds **13**, **30** and **31** were identified as *β*–sitosterol, stigmasterol, and campesterol, which *β*–sitosterol was the major component.

Coronarin D (32)



Compound **32** was isolated as a pale yellow oil with optical rotation $[\alpha]_D^{27}$ +35.25 (*c* 0.033, $CHCl_3$). The molecular formula was established as $C_{20}H_{30}O_3$ from the molecular ion peak at *m/z* 341.2090 ($[M+Na]^+$ calcd 341.2093) in HREIMS. The IR spectrum exhibited the presence of an *α,β*-unsaturated-*γ*-lactone (1737 cm^{-1}). and hydroxyl group (3381 cm^{-1}). The ^1H NMR spectrum of compound **32** displayed signals for three methyl groups at *δ* 0.72 (3H, *s*, H-20), 0.80 (3H, *s*, H-19) and 0.89 (3H, *s*, H-18) as well as *exo*-methylene proton at *δ* 4.82/4.80 (1H, s, H-17b) and 4.38/4.40 (1H, *s*, H-17a) which were characteristics of labdane-type diterpene. The ^1H and ^{13}C NMR spectra of compound **32** were similar with those of compound **29** with the exception of the side chain attached to C-9. The ^1H NMR spectrum of **32** showed olefinic proton signal at *δ* 6.75 (1H, *m*, H-12) and methine proton of hydroxy group signal at *δ* 5.91 (1H, *dd*, H-15). In addition, the downfield chemical shift of C-15 (*δ* 69.69 of **29** and *δ* 95.96 of **32**) were also observed. Compound **32** was isolate as a mixture of two stereoisomers which was indicated by the presence of duplicated

^{1}H NMR signals at δ 107.35/107.63 (C-17). In COSY spectrum, the methine proton signal at δ 5.91 (H-15) and olefinic proton at δ 6.75 (H-12) were coupled to the signal at H-14 (2.70 and 3.01). The HMBC spectrum showed correlation of olefinic proton signal at δ 6.75 (H-12) with the carbonyl carbon at δ 170.50 (C-16) as well as with the signal at δ 56.16 (C-9), 24.54 (C-11) and 33.57 (C-14) (Figure **6**). From these data, the structure of compound **32** was therefore assigned as an isomeric mixture of coronarin D. This compound has also been isolated from *Hydychium coronarium.*[32]

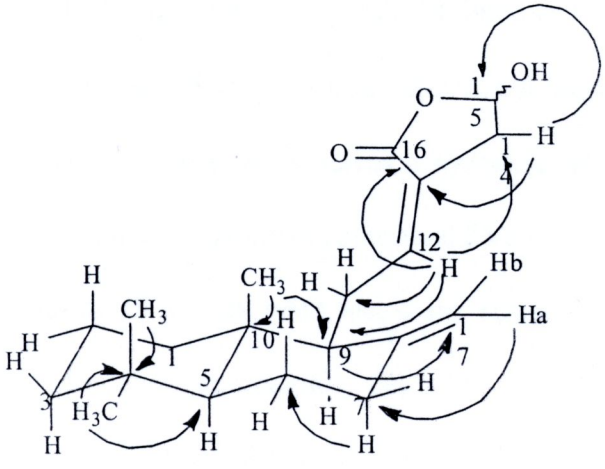


Figure 6 HMBC correlations of coronarin D (**32**)

3.2 Biological Activities

The biological activities of bisnorlabdane, (*E*)-15,16-bisnorlabda-8(17),11-dien-13-one (**28**) and labdane-type diterpenes, villosin (**29**) and coronarin D (**32**) were evaluated for their *in vitro* anticancer activity the cytotoxic represented colorimetric method. The IC_{50} values of these compounds against the human breast cancer (MCF7), human oral epidermoid carcinoma (KB) and human small cell lung cancer (NCI-H187) cell lines are shown in Table **8**. The most active compound was **29** with IC_{50} values of 18.01, 6.85 and 2.12 μg/mL for (MCF7, KB and NCI-H187, respectively. Compounds **28** and **32** showed weak exhibited cell lines with IC_{50} value of 16.25-49.22 μg/mL. These results suggested that a α,β-unsaturated-γ-lactone in compound **29** greatly reduced the effect whereas a hydroxyl substitution at C-15 in compound **29** and α,β-unsaturated ketone in compound **28** showed weak effect against all cell lines. Compounds **28** and **29** were tested for anti-malaria using the pLDH assay (Table **9**). Moreover compounds **28**, **29** and **32** were tested for antimycobacterial using the GFPMA assay (Table **9**). All compounds were inactive to pLDH assay and GFPMA assays.

In previous report; compound **27** showed anti-inflammatory and antifibrotic activities.[35] Compound **30** displayed thyroid inhibitory, antiperoxidative and hypoglycemic effects.[36] Compound **13** showed inhibition to the growth of human prostate cancer PC-3 cells,[37] HT-29 colon cancer cells,[38]