

## 4.0 CHAPTER FOUR: RESULTS AND DISCUSSIONS

### 4.1 Phytochemical analysis

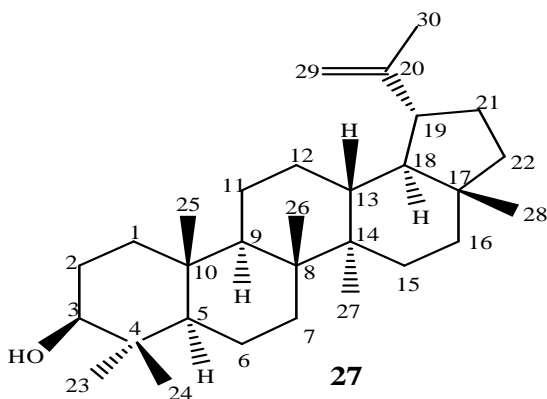
#### 4.1.1 Structural elucidation of compounds from *Lonchocarpus eriocalyx* (Harms)

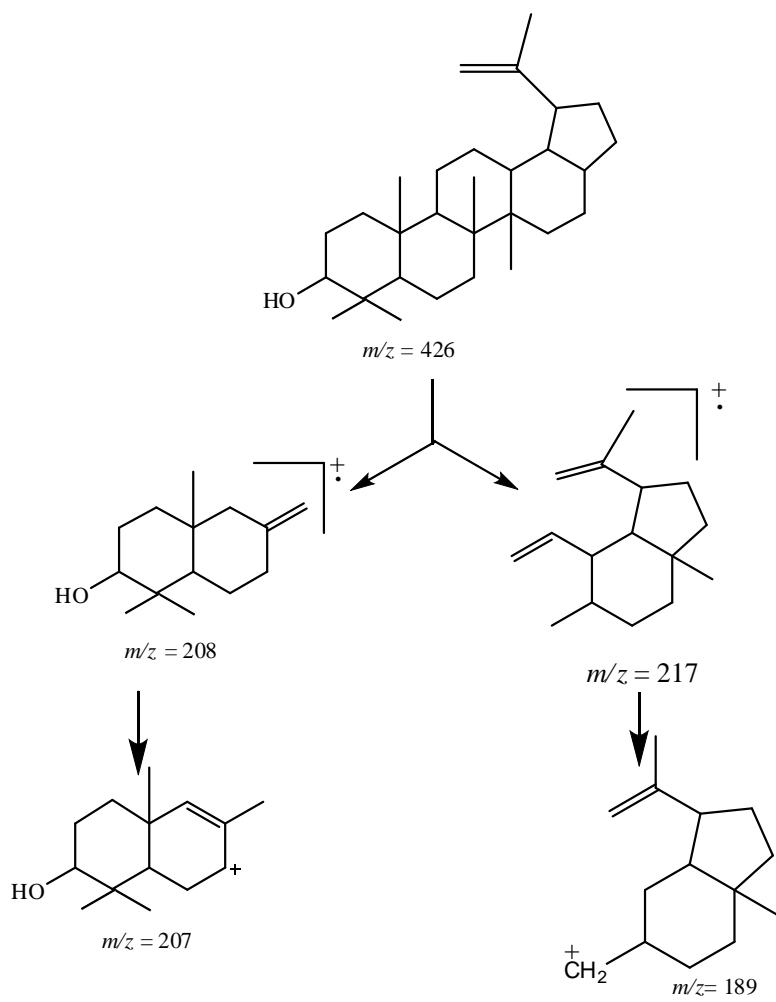
Preliminary validation of the extracts for bioactivity revealed that the *n*-hexane, DCM and MeOH extracts of the stem bark of *L. eriocalyx* were active against *P. falciparum*, *A. gambiae* larvae, *B. anthracis*, and *A. niger*. This formed the the basis of fractionation of these crude extracts whereby lupeol (**27**), quercetin (**65**), apigenin (**68**) friedelin (**133**),  $\beta$ -sitosterol (**134**), lupenone (**135**),  $\beta$ -sitosterol-3-*O*-glucoside (**136**), chrysin (**137**) morinhydrate (**138**), quercetin-3-*O*-glucoside (**139**), 4',5-dihydroxystilbene 3-*O*-glucoside (**140**) and rutin (**141**) were isolated. These compounds were obtained after successive silica gel column chromatography and their structures elucidated using physical and spectroscopic (NMR and MS) methods as well as comparison with literature data.

##### 4.1.1.1 Lupeol (**27**)

Compound **27** was isolated as white needle-like crystals; m.p. 214-216°C (Lit. 215-216°C; Saratha *et al.*, 2011). It gave a positive Libermann-Burchard test indicating a triterpenoid or steroid skeleton (Attarde *et al.*, 2010). The IR spectrum  $\nu_{\max}$  (KBr) had significant peaks appearing at 3315 and 1650  $\text{cm}^{-1}$  typical of OH and olefinic functional groups. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (**Table 7**) had signals consistent with a pentacyclic lupane-type triterpene with olefinic protons appearing at  $\delta$  4.57 and 4.67 (1H each, d,  $H_{\alpha}$ -29 and  $H_{\beta}$ -29) (Pavia *et al.*, 2009). The latter signals were confirmed by the appearance on the  $^{13}\text{C}$  NMR spectrum of olefinic carbons at  $\delta$  149 for C-20 which was downfield typical of a quaternary  $\text{Sp}^2$  carbon. Another upfield signal at  $\delta$  108.1 was due to the

olefinic carbon at C-29 (Pavia *et al.*, 1979). Both the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (**Appendix 1**) showed a signal typical of hydroxymethine group (H-3) at  $\delta_{\text{H}}$  3.20 (dd,  $J = 11.4, 4.8$  Hz) with corresponding carbon (C-3)  $\delta_{\text{C}}$  77.7 attributed to oxygenated carbon atom (Thanakijcharoenpath and Theanphong, 2007). Seven singlets for tertiary methyl protons at  $\delta_{\text{H}}$  0.76 (2 x  $\text{CH}_3$ , 23, 28-Me), 0.79 (24-Me), 0.83 (25-Me), 0.94 (26-Me), 1.02 (27-Me) and 1.20 (30-Me) (integrating for 3H each) with corresponding  $^{13}\text{C}$  NMR signals at  $\delta_{\text{C}}$  27.3 (C-23), 14.9 (C-24), 15.9 (C-25), 16.0 (C-26), 15.0 (C-27), 18.0 (C-28) and 19.5 (C-30) respectively as deduced from the HMQC which were in agreement with the structure of lupeol previously isolated from *Lonchocarpus sericeus* and *Holarrhena floribunda* (Abdullahi *et al.*, 2013; Correa *et al.*, 2009). The proposed structure was further supported by the EI-MS which had a molecular peak at  $426 [\text{M}]^+$  consistent with formula  $\text{C}_{30}\text{H}_{50}\text{O}$ . Confirmation of lupeol structure was further substantiated by extensive analysis of the HMBC and significant EI-MS (70 eV fragments which appeared at  $m/z$  217 ( $\text{C}_{16}\text{H}_{25}$ ) and 207 ( $\text{C}_{14}\text{H}_{21}\text{O}$ ) (**Figure 5**). Thus, on the basis of physical and spectroscopic data as well as comparison with literature data, compound **27** was confirmed identified as lupeol.





**Figure 4:** Possible fragmentation pattern of compound **27** in EI-MS (70 eV)

**Table 7:  $^1\text{H}$  (300 MHz) and  $^{13}\text{C}$  (75 MHz) NMR ( $\text{CDCl}_3$ ) data for Lupeol (27)**

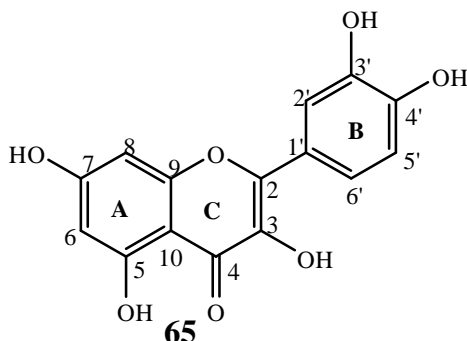
C #	$^1\text{H}$ Multiplicity, ( $J$ in Hz)	$^1\text{H}$ (Multiplicity, ( $J$ in Hz))	$^{13}\text{C}$	$^{13}\text{C}^*$
1	-	-	38.7	38.7
2	-	-	27.2	27.4
3	3.20 (1H, dd, $J = 11.4, 4.8$ )	3.23 (1H, dd, $J = 11.5, 4.7$ )	77.7	79.0
4	-	-	38.7	38.9
5	-	-	54.0	55.5
6	-	-	18.6	18.5
7	-	-	34.3	34.2
8	-	-	41.7	40.9
9	-	-	49.2	50.5
10	-	-	37.5	37.2
11	-	-	21.3	21.0
12	-	-	37.9	38.1
13	-	-	38.0	38.1
14	-	-	41.6	42.9
15	-	-	28.6	27.1
16	-	-	35.9	35.5
17	-	-	42.9	43.0
18	3.10 (1H, dd, $J = 11.0, 5.0$ )	3.15 (1H, dd, $J = 11.5, 5.3$ )	49.2	48.3
19	-	-	48.0	48.0
20	-	-	149.7	151.0
21	-	-	30.0	29.9
22	-	-	39.6	40.0
23	0.76 (3H,s)	0.77, s	27.3	28.0
24	0.79 (3H s)	0.80, s	14.9	15.5
25	0.83 (3H,s)	0.82, s	15.9	16.1
26	0.94 (3H,s)	0.95, s	16.0	16.0
27	1.02 (3H,s)	1.10, s	15.0	14.8
28	0.76 (3H,s)	0.75, s	18.0	18.0
29	4.57 (1H,d, $J = 0.4, \text{H}_\alpha\text{-29}$ ) 4.67 (1H,d, $J = 0.5, \text{H}_\beta\text{-29}$ )	4.60 (d, $J = 0.5, \text{H}_\alpha\text{-29}$ ) 4.70 (d, $J = 0.6, \text{H}_\beta\text{-29}$ )	108.1	109.0
30	1.20 (3H, s)		19.5	19.7

$^1\text{H}^*$  and  $^{13}\text{C}^*$  NMR data (Abdullahi *et al.*, 2013)

#### 4.1.1.2 Quercetin (65)

Compound **65** was isolated as a pale yellow amorphous powder with  $R_f$  value of 0.5 (eluent,  $\text{CH}_2\text{Cl}_2$ -MeOH, 98:2) and m.p. 314-316°C (Lit. 316-318°C; Esra, *et al.*, 2015). The yellow colour intensified on exposure to conc. ammonia vapour and also turned dark brown when sprayed with ferric chloride solution suggesting that it could be a flavonoid derivative. The UV spectrum of this compound run in MeOH solution exhibited two major absorption bands at  $\lambda_{\text{max}}$  258 (band I) and 358 (band II) nm for ring A and B typical of flavonols (Sathyadevi, 2015). The IR spectrum showed the presence of hydroxyl ( $3500\text{-}2500\text{ cm}^{-1}$ ), and  $\alpha,\beta$ -unsaturated carbonyl ( $1610\text{ cm}^{-1}$ ) and aromatic ring ( $1600\text{ cm}^{-1}$ ) (Heneczowski *et al.*, 2001). The  $^1\text{H}$  NMR spectral data (**Table 8**) revealed two sets of aromatic system; AX system at  $\delta_{\text{H}}$  6.19, (d,  $J = 1.8$  Hz) for H-6 and 6.41 (d,  $J = 1.8$  Hz) for H-8 and an ABX system at  $\delta_{\text{H}}$  7.68 (d,  $J = 1.8$  Hz) for H-2', 7.33 (dd,  $J = 8.4, 2.4$  Hz) for H-6' and 6.89 (d,  $J = 8.4$  Hz) for H-5' which suggested a flavonol pattern similar to quercetin (Sathyadevi, 2015). In addition, there was a signal at  $\delta$  12.48 representing a strongly hydrogen bonded C-5 hydroxyl group (Batterham and Highet, 1963). The  $^{13}\text{C}$  NMR spectroscopic data (**Table 8**) of compound **65** revealed the presence of a flavonol skeleton of 15 carbons, including five aromatic CH and ten quaternary carbons (one carbonyl and six C-O-bearing carbons) as evidenced by DEPT 135 NMR spectrum (**Appendix 2**). This further suggested that compound **65** is 3,5,7,3',4'-pentahydroxyflavone, commonly known as quercetin (Sathyadevi, 2015). The molecular ion peak at  $m/z$  301 which is consistent with a molecular formula  $\text{C}_{15}\text{H}_{10}\text{O}_7$  together with daughter ion at  $m/z$  153 and 137 (**Figure 6**) confirmed that the compound is quercetin

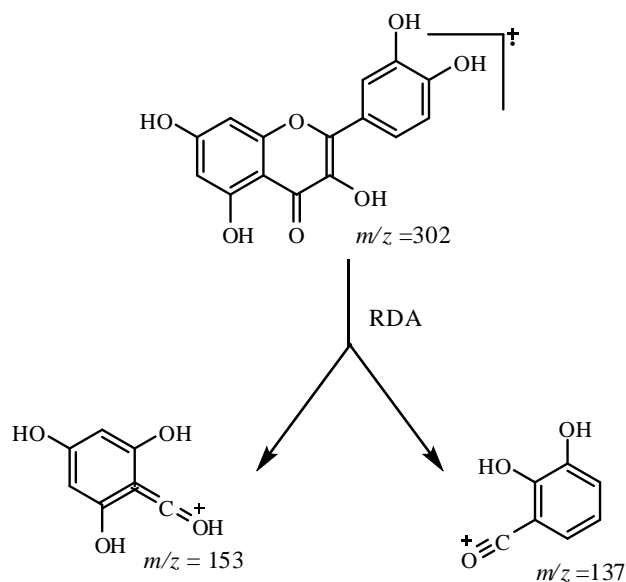
Thus, on the basis of spectroscopic and physical data; this compound was identified as quercetin.



**Table 8:**  $^1\text{H}$  NMR data (400 MHz,  $\text{DMSO-d}_6$ ) and  $^{13}\text{C}$  NMR data (100 MHz,  $\text{DMSO-d}_6$ ) for Quercetin (65)

C#	$^1\text{H}$ Multiplicity, ( <i>J</i> in Hz)	$^1\text{H}^*$ Multiplic ( <i>J</i> in Hz)	$^{13}\text{C}$	$^{13}\text{C}^*$
2	-	-	147.7	148.0
3	-	-	135.6	137.0
4	-	-	175.8	177.0
5	-	-	160.7	162.0
6	6.19 (d, <i>J</i> = 1.8)	6.20 (d, <i>J</i> = 2)	98.1	99.0
7	-	-	163.8	165.0
8	6.41 (d, <i>J</i> = 1.8)	6.39 (d, <i>J</i> = 2)	93.3	94.0
9	-	-	156.1	161.0
10	-	-	103.0	104.0
1'	-	-	122.0	124.0
2'	7.68 (d, <i>J</i> = 1.8)	7.7 (d, <i>J</i> = 2)	115.0	115.0
3'	-	-	146.8	148.0
4'	-	-	145.0	146.0
5'	6.89 (d, <i>J</i> = 8.4)	6.86 (d, <i>J</i> = 8.3)	120.0	116.0
6'	7.53 (dd, <i>J</i> = 8.4, 2.4)	7.58 (dd, <i>J</i> = 8.3)	122.0	121.0
3-OH	10.81, s	11.00	-	-
5-OH	12.48, s	12.50	-	-
7-OH	9.60, s	9.80	-	-
3'-OH	9.40, s	9.60	-	-
4'-OH	-	-	-	-

$^1\text{H}^*$  and  $^{13}\text{C}$  NMR data (Sathyadevi, 2015)

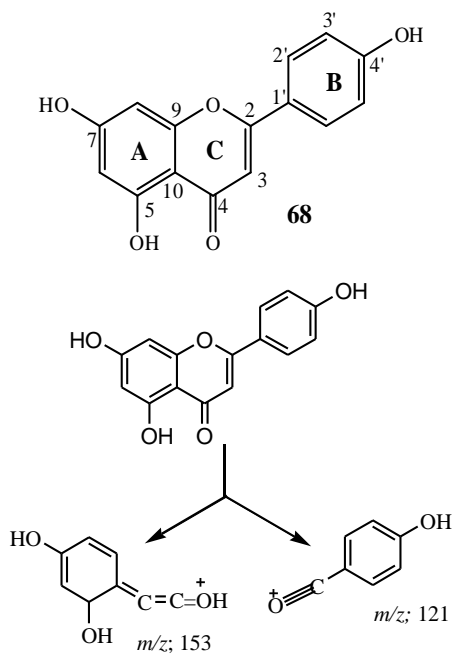


**Figure 5:** RDA fragmentation of compound **65**: EI-MS (70 eV) (Sathyadevi, 2015)

#### 4.1.1.3 Apigenin (68)

Compound **68** was isolated as a yellow amorphous powder m.p. 346-348°C (Lit. 345-350°C; Chaturvedula and Prakash, 2013). The yellow colour intensified on exposure to conc. ammonia vapour. It also turned dark brown upon spraying with ferric chloride solution suggesting that it was a flavonoid (Batterham and Highet, 1963). The UV spectrum of compound **68** in MeOH showed absorption maxima at  $\lambda_{\max}$  268 (band I) and 336 (band II) nm suggesting that **68** is flavone derivative with no hydroxyl at C-3 (Chaturvedula and Prakash, 2013; Dordevic *et al.*, 2001; Mabry *et al.*, 1970). This was supported by a singlet at  $\delta_{\text{H}}$  6.60 in the  $^1\text{H}$  NMR spectrum representing the H-3 signal confirming a flavone molecule. The  $^1\text{H}$  NMR spectral data (**Table 9, Appendix 3**) of compound **68** showed the presence of two *meta*-coupled aromatic doublets at  $\delta_{\text{H}}$  6.70 (1H,  $J = 2.2$  Hz) and  $\delta$  6.46 (1H,  $J = 2.2$  Hz) corresponding to H-8 and H-6 respectively (Mabry *et al.*, 1970). In ring A, an AA'XX' system at  $\delta_{\text{H}}$  7.86 (d,  $J = 9.0$  Hz) and 6.94 (d,

$J = 9.0$  Hz) were assigned to H-3'/H-5' and H-2'/H-6,' respectively. Therefore the  $^1\text{H}$  NMR spectral data (**Table 9, Appendix 3**) is consistent with a 5,7,4'-trisubstituted flavone (Chaturvedula and Prakash, 2013, Batterham and Highet, 1964). In agreement with this, the  $^{13}\text{C}$  NMR data (**Table 9**) showed the presence of fifteen carbon signals sorted out into six aromatic CH, CH of double bond and eight quaternary carbons including a conjugated carbonyl carbon of a flavone. The ESI-MS showed a molecular ion peak at  $m/z$  270 which is 32 amu less than that of compound **68** corresponded to  $\text{C}_{15}\text{H}_{10}\text{O}_5$ . This together with typical RDA fragmentation (**Figure 7**) appearing at  $m/z$ ; 153 and 121 confirmed the presence of 5,7,4'-trihydroxyflavone (Chaturvedula and Prakash, 2013). Thus basing on physical and spectroscopic data as well as comparison with data already reported in various literatures, this compound was identified as apigenin (**68**). This compound is widely reported in this genus, similar taxa and related plants (Oyedeji *et al.*, 2015).



**Figure 6:** RDA fragmentation of compound **68** in EI-MS (70 eV) (Chaturvedula and Prakash, 2013)



**Table 9: <sup>1</sup>H (300 MHz) and <sup>13</sup>C (75 MHz (CD<sub>3</sub>OD) NMR data for Apigenin (68)**

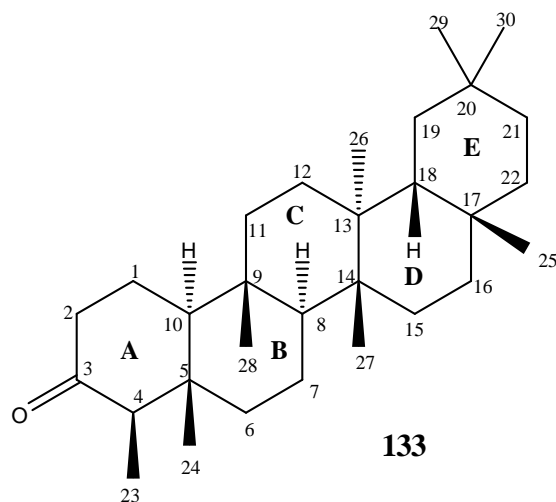
ATOM	<sup>1</sup> H Multiplicity, (J in Hz)	<sup>1</sup> H* Multiplicity, (J in Hz)	<sup>13</sup> C	<sup>13</sup> C*
2	-	-	164.0	164.2
3	6.60 s	6.62 s	103.9	106.5
4	-	-	180.7	180.8
5	-	-	164.4	164.5
6	6.70 (d, J = 2.2)	6.88 (d, J = 2.1)	104.8	104.8
7	-	-	160.0	160.0
8	6.46 (d, J = 1.8)	6.68 (d, J = 2.1)	95.1	98.8
9	-	-	160.7	160.7
10	-	-	109.5	109.6
1'	-	-	123.2	123.3
2'	7.86 (d, J = 9.0)	7.81 (d, J = 8.8)	129.2	129.3
3'	6.94 (d, J = 9.0)	6.95 (d, J = 8.8)	117.1	117.0
4'	-	-	162.5	162.6
5'	6.94 (d, J = 9.0)	6.95 (d, J = 8.8)	116.0	117.0
6'	7.86 (d, J = 9.0)	7.81 (d, J = 8.8)	129.5	129.3

<sup>1</sup>H\* and \*<sup>13</sup>C NMR data (Chaturvedula and Prakash, 2013)

#### 4.1.1.4 Freidelin (133)

Compound **133** was isolated as white powder, m.p. 254-256°C (Lit. 260-262°C; Majidul *et al.*, 2015). It gave a positive Libermann-Burchard test suggesting that it could be a terpene/sterol (Attarde *et al.*, 2010). The IR spectrum (**Appendix 4**) of this compound showed an intense band at 1702 cm<sup>-1</sup> suggesting the presence of a carbonyl moiety in the compound (Grasiely *et al.*, 2012). In the <sup>1</sup>H NMR spectrum (**Table 10, Appendix 4**), a doublet at δ<sub>H</sub> 1.20 (J = 6.8 Hz, 23-Me), integrating for three protons together with resonances for seven tertiary methyl groups observed at δ<sub>H</sub> 0.74 (25-Me), 0.88 (29-Me), 0.89 (30-Me), 0.91 (26-Me), 1.02 (24-Me), 1.03 (27-Me) 1.20 (23-Me) and 1.07 (28-Me) with corresponding <sup>13</sup>C NMR signals (**Appendix 4**) at δ<sub>C</sub> 20.5 (C-25), 32.4 (C-29), 32.7 (C-30), 18.9 (C-26), 14.9 (C-24), 18.5 (C-27), 7.1 (C-23), 18.9 (C-26) and 32.1 (C-28),

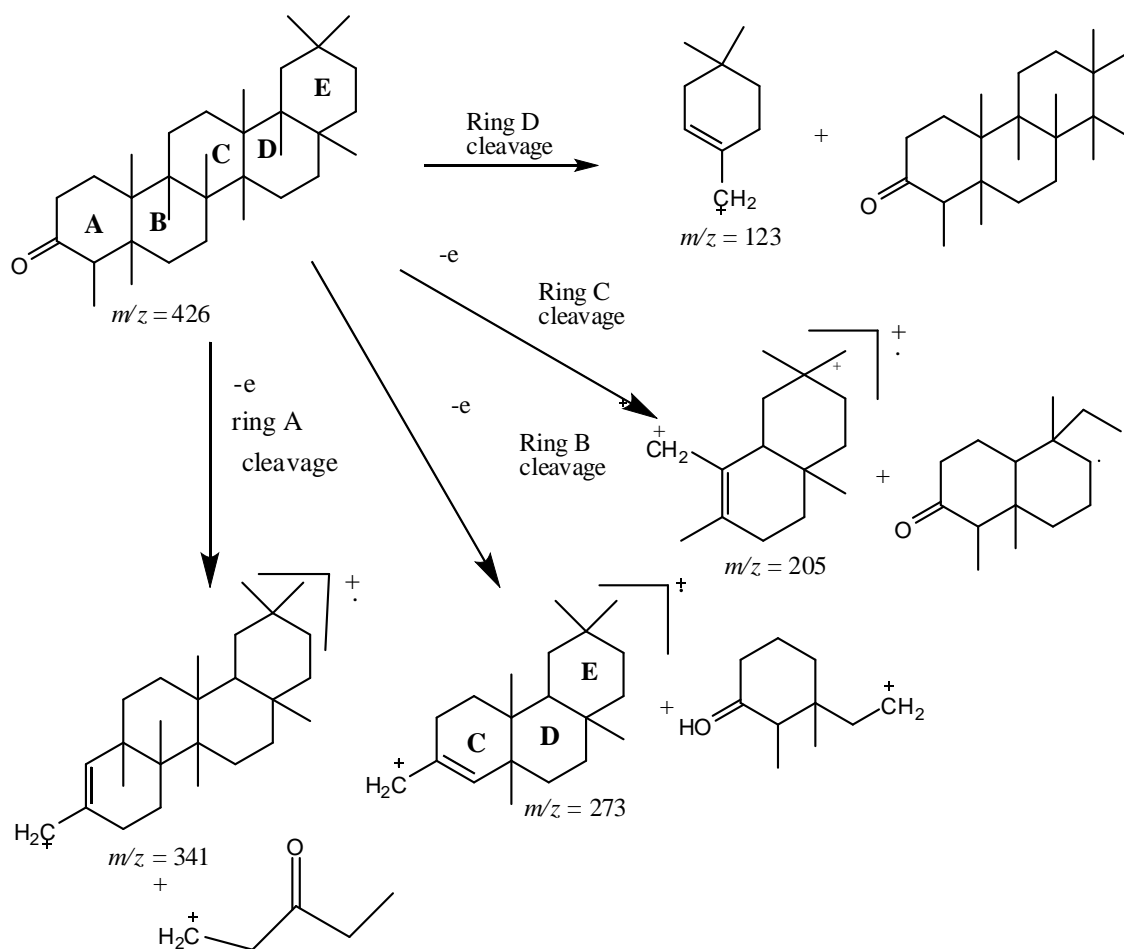
suggested that the compound was a friedelone-type triterpene (Jong *et al.*, 2012). The fore-going evidence was further supported by the appearance of a quartet signal integrating for one proton at  $\delta$  2.27 which was assigned to H-4. The  $^{13}\text{C}$  NMR spectral data (**Table 10**) showed the presence of 30 carbon signals resolved into eight  $\text{sp}^3$  methyls, eleven  $\text{sp}^3$  methylenes, four  $\text{sp}^3$  methines, six  $\text{sp}^3$  quaternary carbons and one  $\text{sp}^2$  quaternary carbon as evidenced by 135 DEPT experiment. The presence of a  $\text{C}=\text{O}$  carbon was supported by a signal at  $\delta_{\text{C}}$  213.4 typical of a ketonic carbon (Majidul *et al.*, 2015). The EI-MS (70 eV) afforded a molecular ion peak at  $m/z$  426 which suggested the molecular formula  $\text{C}_{30}\text{H}_{30}\text{O}$ . This together with the fragmentation pattern in the EI-MS spectrum (**Figure 8**) strongly suggested that the compound was friedelin. In fact, the presence of signals due to one secondary and six quaternary methyls in the  $^{13}\text{C}$  NMR spectrum together with cross correlation between a doublet at methyl signal at  $\delta$  1.20 and a quartet of methine at  $\delta$  2.27 in the  $^1\text{H}$ - $^1\text{H}$  COSY spectrum strongly supported the structure of the compound as friedelin (Majidul *et al.*, 2015; Grasiely *et al.*, 2012). Thus on the basis of spectroscopic evidence, compound **133** was identified as friedelin.



**Table 10:  $^1\text{H}$  (300 MHz) and  $^{13}\text{C}$  (75 MHz) NMR ( $\text{CDCl}_3$ ) data for Friedelin (133)**

<b>C #</b>	<b><math>^1\text{H}</math> Multiplicity, (J in Hz)]</b>	<b><math>^1\text{H}^*</math> Multiplicity, (J in Hz)]</b>	<b><math>^{13}\text{C}</math></b>	<b><math>^{13}\text{C}^*</math></b>
<b>1</b>	1.76 (dd, $J = 13.0, 7.5$ )	1.82 (dd, $J = 12.8, 8.0$ )	22.5	22.3
<b>2</b>	2.25 ( $\text{H-}\alpha$ , d, $J = 6.8$ ) 2.72 ( $\text{H-}\beta$ , dd, $J = 13, 7.5$ )	2.54 (d, $J = 7.0$ ) 2.76 (dd, $J = 13.5, 8.0$ )	41.6	41.5
<b>3</b>	-	-	213.4	213.2
<b>4</b>	2.27, (q, $J = 5.4$ )	2.37, m	58.5	58.2
<b>5</b>	-	-	42.4	42.2
<b>6</b>	1.62 (dd, $J = 11.4, 5.2$ )	1.56 (dd, $J = 11.5, 5.6$ )	41.4	41.3
<b>7</b>	1.31, m	1.31	36.3	35.8
<b>8</b>	1.39, m	1.39	53.4	53.1
<b>9</b>	-	-	37.7	37.5
<b>10</b>	1.39, m	1.30	59.8	59.5
<b>11</b>	1.56, m	1.56	33.5	33.2
<b>12</b>	1.56, m	1.56	30.8	30.5
<b>13</b>	-	-	41.8	41.2
<b>14</b>	-	-	41.6	41.0
<b>15</b>	1.31, m	1.31	30.3	30.2
<b>16</b>	1.31, m	1.31	35.9	36.8
<b>17</b>	-	-	30.8	30.0
<b>18</b>	1.39, m	1.39	43.1	42.8
<b>19</b>	1.45, m	1.45	35.7	35.5
<b>20</b>	-	-	29.9	29.4
<b>21</b>	1.31, m	1.29	33.1	32.8
<b>22</b>	1.31, m	1.31	39.6	39.3
<b>23-Me</b>	1.20, (d, $J = 6.8$ )	1.11	7.1	6.8
<b>24-Me</b>	1.02, m	1.04	14.9	14.7
<b>25-Me</b>	0.74, s	1.04	20.5	21.0
<b>26-Me</b>	0.91, s	1.04	18.9	18.8
<b>27-Me</b>	1.03, s	2.04	18.5	18.8
<b>28-Me</b>	1.07, s	1.04	32.1	32.1
<b>29-Me</b>	0.88, s	0.99	32.4	31.7
<b>30-Me</b>	0.89, s	0.99	32.7	31.8

$^1\text{H}^*$  and  $^{13}\text{C}^*$  NMR data (Majidul *et al.*, 2015)



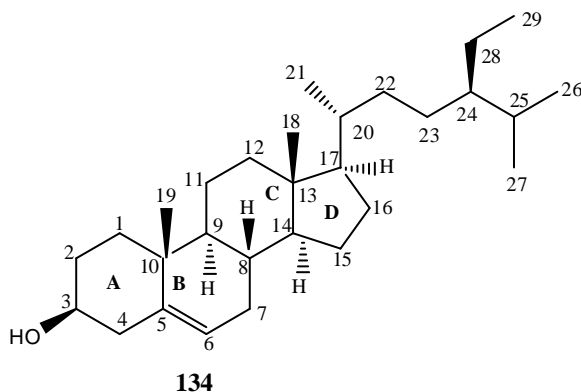
**Figure 7:** Fragmentation pattern of compound **133** in EI-MS (70 eV)  
(Grasiely *et al*, 2012)

#### 4.1.1.5 $\beta$ -sitosterol (134)

Both the *n*-hexane and the  $\text{CH}_2\text{Cl}_2$  extracts of *L. eriocalyx* after repeated chromatographic separation afforded white needle-like crystals of  $R_f$  0.63 (eluent *n*-hexane- $\text{CH}_2\text{Cl}_2$ , 2:3), m.p 132-134°C [Lit. 136-138°C; Orabi, 2011]. It afforded a bluish-purple colour when sprayed with concentrated sulphuric acid on TLC, after heating at 100 °C for a minute. The  $^1\text{H}$  NMR spectral data (**Table 11, Appendix 5**) displayed two tertiary methyl groups as singlets at  $\delta$  1.03 (18-Me) and 0.78 (19-Me). Three secondary methyls appearing as doublets at  $\delta$  1.12 ( $J = 6.5$  Hz, 21-Me), 0.83 ( $J = 6.8$  Hz, Me-27) and 0.84 (d,  $J = 6.8$ ,

Me-26) together with a primary methyl triplet at  $\delta$  0.86 ( $J = 7.1$  Hz, 29-Me) suggested the presence of C-29 sterol skeleton (Alam *et al.*, 1996). A characteristic signal for  $\Delta^5$ -sterol appeared at  $\delta$  5.35 (t,  $J = 5.2$  Hz, H-6). The  $^{13}\text{C}$  NMR spectrum (**Table 11, Appendix 5**) showed the presence of 29 carbon atoms which were resolved into six methyl, eleven methylenes, nine methines and three non-protonated carbon as evidenced by 135 DEPT spectrum which accounted for 49 protons of the molecule. The remaining proton was part of the hydroxyl functionality evidenced by the  $^1\text{H}$  NMR peak at  $\delta$  3.52. The presence of an exocyclic double bond at C-5 was substantiated by peaks appearing at  $\delta_{\text{C}}$  140.8 (C-5 and 121.7 (C-6). (Reginatto *et al.*, 2001; Alam *et al.*, 1996). The ESI-MS of this compound gave a molecular ion peak at  $m/z$  414 suggesting a molecular formula of  $\text{C}_{29}\text{H}_{50}\text{O}$  with fragmentation pattern typical of sterol derivative (Luhata and Munkombwe, 2015). The daughter ions observed at  $m/z$  396 was due to loss of  $\text{H}_2\text{O}$  from the molecular ion while the peak at  $m/z$  273 was due to cleavage of  $\text{C}_{17}\text{-C}_{20}$ . (Reginatto *et al.*, 2001). On the other hand, a fragment ion at  $m/z$  303 was due to  $\text{C}_{23}\text{-C}_{24}$  bond scission while the ion at  $m/z$  381 was attributed to cleavage of  $\text{C}_{23}\text{-C}_{24}$  from  $\text{M}^+$ . Other significant ions observed in the EI-MS spectrum (**Appendix 5**) at  $m/z$  354 represented  $\text{M}^+$ -isopropyl- $\text{H}_2\text{O}$  ion while at  $m/z$  329 represented cleavage at  $\text{C}_{23}\text{-C}_{24}$  which further confirmed the presence of a sterol derivative (Chaturverdula and Prakash, 2012). Additional evidence in favor of structure **134** as  $\beta$ -sitosterol was provided by comparison of physical and spectroscopic data with those reported in the literature for this compound (Chaturverdula and Prakash, 2012, Alam *et al.*, 1996). Thus, on the basis of spectroscopic data as well as comparison with literature, compound **134** was concluded to be  $\beta$ -sitosterol. Since there

was enough evidence confirming the structure of this compound, no further spectral analysis was done.



**Table 11:  $^1\text{H}$  (300 MHz) and  $^{13}\text{C}$  (75 MHz) NMR ( $\text{CDCl}_3$ ) data for  $\beta$ -sitosterol (134)**

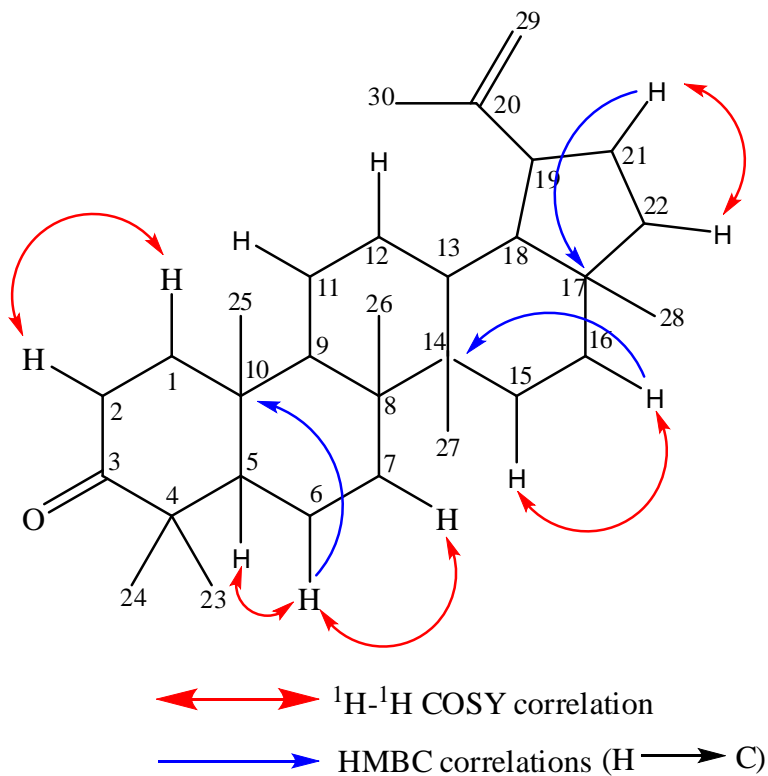
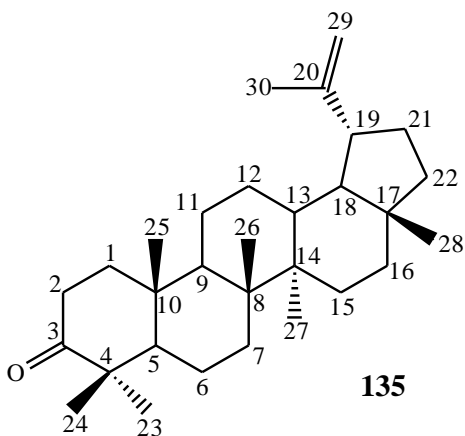
C#	$^1\text{H}$ Multiplicity, ( <i>J</i> in Hz)	$^{13}\text{C}$	$^{13}\text{C}^*$	C#	$^1\text{H}$ Multiplicity, ( <i>J</i> in Hz)	$^{13}\text{C}$	$^{13}\text{C}^*$
<b>1</b>	1.43 (d, <i>J</i> = 3.2)	37.3	37.3	<b>16</b>	-	28.3	28.6
<b>2</b>	1.65 (d, <i>J</i> = 5.6)	31.7	31.7	<b>17</b>	-	56.1	56.1
<b>3</b>	3.52 m	71.8	71.8	<b>18</b>	1.03 s	12.0	12.1
<b>4</b>	2.19 (d, <i>J</i> = 9.8)	39.8	42.4	<b>19</b>	0.78, s	19.4	20.6
<b>5</b>	-	140.8	140.8	<b>20</b>	1.33 m	11.7	12.2
<b>6</b>	5.35 (d, <i>J</i> = 5.2)	121.7	121.9	<b>21</b>	1.12 (d, <i>J</i> = 6.5)	19.3	19.4
<b>7</b>	1.52 (d, <i>J</i> = 12.4)	31.9	31.9	<b>22</b>	1.20, m	46.8	46.1
<b>8</b>		31.7	31.9	<b>23</b>	1.20, m	23.1	23.1
<b>9</b>		50.1	50.2	<b>24</b>	1.26, m	45.9	46.0
<b>10</b>		36.5	36.6	<b>25</b>	1.67, m	23.1	30.0
<b>11</b>		21.1	21.0	<b>26</b>	0.84 (d, <i>J</i> = 6.8)	19.0	19.6
<b>12</b>		37.2	39.8	<b>27</b>	0.83 (d, <i>J</i> = 6.8)	19.6	20.0
<b>13</b>		42.3	42.3	<b>28</b>	1.20, m	23.1	22.7
<b>14</b>		56.8	56.8	<b>29</b>	0.86 (t, <i>J</i> = 7.1)	11.9	11.4
<b>15</b>		24.3	24.4				
<b>3-OH</b>	3.52 (m)						

$^1\text{H}^*$  and  $^{13}\text{C}^*$  NMR data (Chaturvedula and Prakash, 2012)

#### 4.1.1.6 Lupenone (135)

Along with compound **27** was isolated another white powder; compound **135** with  $R_f$  0.48 (eluent: *n*-hexane-CH<sub>2</sub>Cl<sub>2</sub>, 2:3) and m.p. 254-256°C (Lit. 260-262°C, Aher *et al.*, 2010). Like in the case of compound **27**, it gave a positive Liebermann-Burchard test (Attarde *et al.*, 2010), suggesting that it could be a terpene or sterol derivative. The EI-MS of this compound gave the M<sup>+</sup> ion at  $m/z$  424 which is 2 amu less than that of **27** corresponding to C<sub>30</sub>H<sub>48</sub>O formula. In addition to the molecular formula, both <sup>1</sup>H and <sup>13</sup>C NMR data (**Table 12, Appendix 6**) of this compound were in agreement with compound **27** except for the absence of oxymenthine proton which appeared at  $\delta$  3.20 in the latter compound. Apparently, in compound **135**, the oxymethine proton was replaced by a keto moiety as evidenced by a peak at  $\delta_C$  216 in the <sup>13</sup>C NMR spectrum. Furthermore, the <sup>13</sup>C NMR spectral data showed 30 distinct resonances attributed to 7 methyl, 11 methylenes, 5 methines and 7 quaternary carbons. The <sup>1</sup>H NMR data (**Table 12, Appendix 6**) supported the <sup>13</sup>C NMR data by displaying 7 tertiary methyl groups at  $\delta$  1.11, 1.01, 0.92, 0.90, 0.83, 0.78 and 0.68 which corresponded to <sup>13</sup>C NMR signals at  $\delta$  18.0, 32.4, 33.3, 21.3, 14.8, 19.3 and 21.2, respectively. This together with characteristic peaks resonating at  $\delta$  4.68 (d,  $J = 6.6$  Hz) and 4.46 (d,  $J = 6.6$  Hz) which were assigned to the exocyclic double bond protons further supported that compound **135** is a derivative of lupeol (**25**) in which C-3 has a keto-group (Rajavel *et al.*, 2012; Anandhi, 2012). H-H COSY spectrum showed significant connectivities; [H-1 and H-2, H-5 and H-6, H-15 and H-16, H-21 and H-22] (**Figure 9**) which supported the suggested structure for compound **135**. HMBC spectrum (**Figure 9, Appendix 6**) which shows association of protons with corresponding carbons and long range correlation [C-10 and H-6, C-14 and H-16, C-17

and H-21] led to a complete assignment of the signals and to the conclusion that this compound was lupenone.



**Figure 8:** Significant HMBC and COSY correlations for compound **135** (Anandhi, 2012)



**Table 12: <sup>1</sup>H (500 MHz) and <sup>13</sup>C (125 MHz) NMR (CDCl<sub>3</sub>) data for Lupenone (135)**

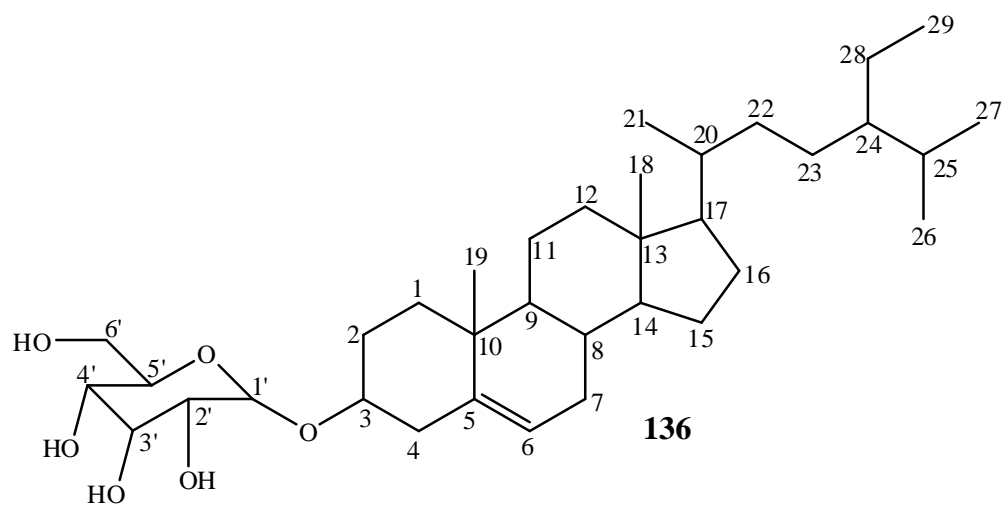
C #	<sup>1</sup> H Multiplicity, (J in Hz)	<sup>13</sup> C	<sup>13</sup> C*	C#	<sup>1</sup> H Multiplicity, (J in Hz)	<sup>13</sup> C	<sup>13</sup> C*
1	1.66, (d, J = 12.5)	39.9	39.7	16	1.38, m	35.5	35.1
2	1.69, s	27.5	27.4	17	-	42.2	42.2
3	-	216.7	212.8	18	1.44, m	51.4	53.1
4	-	42.3	41.6	19	1.43, m	42.2	42.6
5	1.61, s	56.6	59.3	20	-	150.8	150.4
6	1.49, m	17.7	18.5	21	1.65, m	29.8	29.7
7	1.32, m	34.5	34.7	22	-	40.0	40.4
8	-	42.11	41.4	23	1.01, s	32.4	32.0
9	1.39, m	50.7	58.0	24	0.92, s	33.3	32.4
10	-	36.8	36.9	25	0.78, s	19.3	18.9
11	1.49, m	21.3	22.5	26	1.11, s	18.0	18.0
12	1.47, m	29.2	29.4	27	0.83, s	14.8	15.1
13	1.41, m	37.6	37.9	28	0.68, s	21.2	20.2
14	-	40.0	41.4	29	4.46 (H <sub>α</sub> , d, J = 6.6) 4.68 (H <sub>β</sub> , d, J = 6.6)	109.5	108.8
15	1.40, m	26.2	26.4	30	1.90, br s	21.3	21.0

<sup>1</sup>H\* and <sup>13</sup>C\* NMR data (Anandhi, 2012)

#### 4.1.1.7 β-sitosterol-3-O-glucoside (136)

Chromatographic separation of both the CH<sub>2</sub>Cl<sub>2</sub> and the MeOH extracts of *L. eriocalyx* stem bark as described in experimental section paragraph 3.3.5 afforded compound **136** with R<sub>f</sub> value of 0.32 (eluent: CH<sub>2</sub>Cl<sub>2</sub>-MeOH; 99:1) as colourless needles; m.p 270-272°C (Lit. 272-274°C; Mahbuba *et al.*, 2012). It showed a positive Liebermann-Burchard test (Attarde *et al.*, 2010), whereas with acidified vanillin, it gave bluish-purple colour on TLC after heating, which signified either a sterol or a terpene derivative. The <sup>1</sup>H NMR spectrum (Table 13, Appendix 7) determined in CD<sub>3</sub>OD showed a characteristic signal for anomeric proton as a doublet at δ 4.90 (d, J = 7.9 Hz). The coupling constant ascertained the β-configuration of the sugar residue. The <sup>1</sup>H NMR spectrum further showed signals for two tertiary methyl groups as singlets at δ 0.67 (19-

Me) and 0.89 (18-Me), respectively. The signals of further three secondary methyl groups observed as doublets were centred at  $\delta$  1.00 (21-Me), 0.80 (d,  $J = 6.7$  Hz, 26-Me) and 0.78 (d,  $J = 6.8$  Hz, 27-Me). A triplet at  $\delta$  0.67 (t,  $J = 7.2$  Hz, 29-Me) was due to a primary methyl group while a proton resonance appearing at  $\delta$  5.34 (H-6) as a doublet was characteristic of  $\Delta^5$ -sterol (Ahmad *et al.*, 2012). The attachment of the sugar residue at C-3 was confirmed by a shift of the H-3 resonance to higher  $\delta$  value of 3.63. The  $^{13}\text{C}$  NMR spectrum of compound **136** (Table 13, Appendix 7) showed the presence of 35 carbon signals of which six were in the glycosidic region corresponding to a hexose moiety. The remaining 29 carbons were due to the aglycone. The olefinic carbon signals at  $\delta$  124.3 and 137.5 corresponded to the double bond between C-5 and C-6, respectively, which are characteristic of  $\Delta^5$  type of sterol C (Mahbuba *et al.*, 2012). Comparison of the chemical shift of the sugar carbons with reported data were in agreement those of glucose (Orabi, 2011). The downfield shift signal of the aglycone C-3 further supported linkage of sugar moiety at this particular carbon (Pandey *et al.*, 2006). The EI-MS (70 eV) showed a molecular peak at  $m/z$  576 corresponding to  $[\text{M}]^+$  analysed for  $\text{C}_{35}\text{H}_{60}\text{O}_6$  formula. A peak appearing at  $m/z$  414 indicated loss of a hexose moiety from the molecule. This was supported by acid hydrolysis (2% HCl) which yielded a free sugar identified as  $\beta$ -D-glucose by comparison with authentic samples on silica gel TLC as well as paper chromatography. Conclusive evidence for the structure of compound **136** was further provided by the extensive interpretation of  $^1\text{H}$  and  $^{13}\text{C}$  chemical shift correlations experiment which further supported both  $^1\text{H}$  and  $^{13}\text{C}$  NMR results (Ahmad *et al.*, 2012). Therefore, on the basis of the above accumulated evidence, the structure of **136** was established as  $\beta$ -sitosterol 3-*O*- $\beta$ -glucoside.



**Table 13: <sup>1</sup>H (400 MHz) and <sup>13</sup>C (125 MHz) NMR (DMSO-d<sub>6</sub>) data for β-sitosterol -3 O-Glucoside (136)**

C#	<sup>1</sup> H (Multiplicity, <i>J</i> in Hz)	<sup>13</sup> C	<sup>13</sup> C*	C#	<sup>1</sup> H (Multiplicity, <i>J</i> in Hz)	<sup>13</sup> C	<sup>13</sup> C*
<b>1</b>	1.46 (d, <i>J</i> = 7.5)	36.7	36.7	<b>19</b>	0.67, s	-	19.1
<b>2</b>	1.50, m	-	29.1	<b>20</b>	1.26, m	35.7	35.7
<b>3</b>	3.63, m	78.2	78.6	<b>21</b>	1.00, d, <i>J</i> = 6.4)	-	18.7
<b>4</b>	2.35, m	40.0	42.1	<b>22</b>	1.76, m		33.5
<b>5</b>	-	137.5	140.0	<b>23</b>	1.76, m	25.2	25.6
<b>6</b>	5.34 (dd, <i>J</i> = 7.4, 1.7)	124.3	121.5	<b>24</b>	1.13, m	45.6	45.5
<b>7</b>	2.86 (H-7 <sub>α</sub> , dd, <i>J</i> = 12.3, 2.6) 2.50 (H-7 <sub>β</sub> , dd, <i>J</i> = 11.5, 11.3)	-	31.4	<b>25</b>	2.12, m	-	28.7
<b>8</b>	2.10, m	-	31.5	<b>26</b>	0.80 (d, <i>J</i> = 6.7)	-	18.7
<b>9</b>	1.94, m	50.6	49.8	<b>27</b>	0.78 (d, <i>J</i> = 1.6)	-	18.4
<b>10</b>		36.7	36.3	<b>28</b>	0.78, m	-	22.6
<b>11</b>		-	20.2	<b>29</b>	0.67 (t, <i>J</i> = 7.2)	-	12.3
<b>12</b>		39.9	38.2	<b>1'</b>	4.90 (d, <i>J</i> = 7.9)	101.7	100.7
<b>13</b>		42.6	41.9	<b>2'</b>	4.88, m	74.0	73.2
<b>14</b>		56.6	56.4	<b>3'</b>	4.87, m	76.8	76.2
<b>15</b>		23.2	23.8	<b>4'</b>	4.46, m	71.0	70.0
<b>16</b>		28.4	27.8	<b>5'</b>	2.89, m	76.2	75.2
<b>17</b>		56.4	55.7	<b>6'</b>	4.88 (H-6' <sub>α</sub> , dd, <i>J</i> = 11.7, 2.4) 4.92 (H-6' <sub>β</sub> , dd, <i>J</i> = 11.7, 5.3)	63.1	61.4
<b>18</b>	0.89 (s)	-	11.3				

<sup>1</sup>H\* and <sup>13</sup>C\* NMR data (Mahbuba *et al.*, 2012)

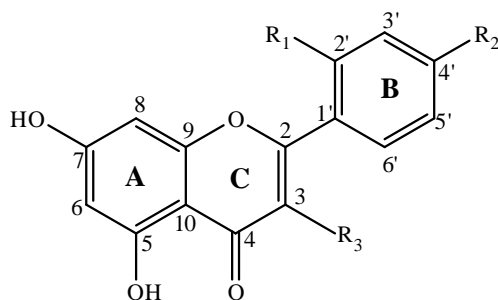
#### 4.1.1.8 Chrysin (137) and morinhydrate (138)

Compound **137** was isolated as yellow powder, m.p. 291-292°C (Lit. 285-286°C; Kikuchi *et al.*, 1991). The UV spectrum of compound **137** in MeOH showed absorption maxima at λ<sub>max</sub> 268 (band I) and 336 (band II) nm suggesting a flavone derivative. (Chaturvedula and Prakash, 2013; Mabry *et al.*, 1970). The <sup>1</sup>H NMR spectrum (**Table 14, Appendix 8**) showed the presence of two *meta*-coupled protons consisting of an AX system typical of

ring A of a flavone derivative at  $\delta$  6.48 for H-8 (d,  $J = 1.5$  Hz) and 6.23 for H-6 (d,  $J = 1.5$  Hz). A characteristic  $^1\text{H}$  NMR singlet resonance at  $\delta$  6.73 was assigned to H-3. Furthermore, another set of five aromatic signals of ring B involving *ortho*-coupled doublets at  $\delta$  7.99 (d,  $J = 6.5$  Hz) for H-2' and H-6' (AX system) and a multiplet at  $\delta$  7.58 (3H) assigned to H-3', H-4' and H-5' (ABX system) were observed together with  $\delta_{\text{C}}$  resonances for C-2 and C-3 at 162.8 and 133.4 suggesting that compound **137** is a flavone derivative (Charturvedula and Prakash, 2011; Vijay *et al.*, 2011). On the other hand, the  $^{13}\text{C}$  NMR spectrum (**Table 15, Appendix 8**) revealed the presence of 15 carbon signals out of which 7 were aromatic CH, one olefinic CH ( $\delta_{\text{C}}$ , 106.1) and 7 quaternary carbons including  $\alpha,\beta$ -unsaturated carbonyl at  $\delta_{\text{C}}$  183.9 (C-4) as evidenced by DEPT spectrum. The EI-MS showed a protonated molecular ion peak at  $m/z$  255  $[\text{M}+1]^+$  which was consistent with the formula  $\text{C}_{15}\text{H}_{10}\text{O}_4$  confirming that compound **137** was chrysin (Miyachi *et al.*, 2006) Thus on the basis of spectroscopic data, compound **137** was identified to be chrysin.

Similarly, compound **138** was isolated as light yellow powder, m.p. 301-303°C (Lit. 300-301°C; Liu *et al.*, 2012). Just like for chrysin (**137**), the  $^1\text{H}$  NMR spectrum (**Table 14, Appendix 9**) showed the presence of two *meta*-coupled protons of ring A appearing at  $\delta$  6.44 (1H, d,  $J = 1.5$  Hz) and 6.17 (1H, d,  $J = 1.5$  Hz) which was assigned to H-8 and H-6, respectively. This observation was further evidenced by EI-MS fragment appearing at  $m/z$  153 indicating that ring A had no other substituents other than the two hydroxyl groups at C-5 and C-7. Careful examination of the  $^1\text{H}$  NMR spectrum revealed the absence of H-3 of flavones implying that C-3 was substituted, a fact further supported by the  $^{13}\text{C}$  NMR peak at  $\delta$  133.4 for C-3. The  $^1\text{H}$  NMR also exhibited an AMX spin system at  $\delta$  7.43 ( $J =$

7.0 Hz, H-6), 6.50 ( $J = 2.0$  Hz, H-5), 6.33 ( $J = 1.8$  Hz, H-3 for ring B which is oxygenated at C-2' and C-4' (Miyaichi *et al.*, 2006). The  $^{13}\text{C}$  NMR spectrum (Table 15, Appendix 9) showed the presence of 15 carbon signals, five aromatic CH and 10 quaternary carbons including  $\alpha,\beta$ -unsaturated carbonyl at  $\delta$  177.7 evidenced by  $^{13}\text{C}$  spectrum (Appendix 9). The compound displayed a molecular ion peak at  $m/z$  302 which is consistent with a molecular formula  $\text{C}_{15}\text{H}_{10}\text{O}_7$ , suggesting that it is a derivative of quercetin (65). Changes of diagnostic values were observed in  $^{13}\text{C}$  NMR spectrum of compound 138 in comparison with quercetin (65) whereby C-3' shifted upfield at  $\delta$  104.6 due to the absence of OH group at this position while C-2' shifted downfield at  $\delta$  157.6 arising from OH substitution (Liu *et al.*, 2012). This was confirmed by HMBC correlation between H-5' ( $\delta$  6.50) and C-3' ( $\delta$  104.6) Thus, on the basis of spectroscopic data, compound 138 was structurally elucidated as morinhydrate (Miyaichi *et al.*, 2006).



**137:**  $\text{R}_1 = \text{R}_2 = \text{R}_3 = \text{H}$

**138:**  $\text{R}_1 = \text{R}_2 = \text{R}_3 = \text{OH}$

**Table 14: <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD) data for Chrysin (137) and morinhydrate (138)**

C #	137 <sup>1</sup> H Multiplicity (J in Hz)	138 <sup>1</sup> H* Multiplicity (J in Hz)	138 <sup>1</sup> H Multiplicity (J in Hz)	138 <sup>1</sup> H* Multiplicity (J in Hz)
2	-	-	-	-
3	6.73, s	6.93, s	-	-
4	-	-	-	-
6	6.23 (d, J = 1.5)	6.22 (d, J = 2.2)	6.17 (d, J = 1.5)	6.20 (d, J = 2.0)
8	6.48 (d, J = 1.5)	6.52 (d, J = 2.2)	6.43 (d, J = 1.5)	6.51
9	-	-	-	-
10	-	-	-	-
1'	-	-	-	-
2'	7.99 (d, J = 6.5)	8.30, m	-	11.85
3'	7.58, m	7.58, m	6.33 (d, J = 1.8)	6.14
4'	7.58, m	7.58, m	-	9.90
5'	7.58, m	7.58, m	6.50 (d, J = 2.0)	6.25, m
6'	7.99 (d, J = 1.5)	8.30, m	7.43 (d, J = 7.0)	7.04
5-OH	-	12.83, m	-	11.90
7-OH	-	10.90, s	-	10.30

<sup>1</sup>H\* NMR data (Miyaichi *et al.*, 2006)

**Table 15: <sup>13</sup>C (125 Hz, CD<sub>3</sub>OD) NMR data for Chrysin (137) and morinhydrate (138)**

ATOM	<sup>13</sup> C 108	<sup>13</sup> C* 108	<sup>13</sup> C 109	<sup>13</sup> C*109
2	163.3	163.1	162.8	161.4
3	106.1	108.4	133.4	134.2
4	183.9	182.4	177.7	182.1
5	165.7	151.5	162.5	161.8
6	105.6	105.7	99.3	98.3
7	159.6	158.1	165.6	166.4
8	95.2	97.1	94.7	94.0
9	165.6	163.5	149.9	158.8
10	106.1	104.7	105.4	104.4
1'	127.5	126.5	109.4	110.4
2'	130.3	110.9	157.6	157.8
3'	-	149.1	104.6	103.5
4'	-	149.3	159.0	159.1
5'	-	110.0	111.6	108.4
6'	132.6	131.2	132.7	131.3

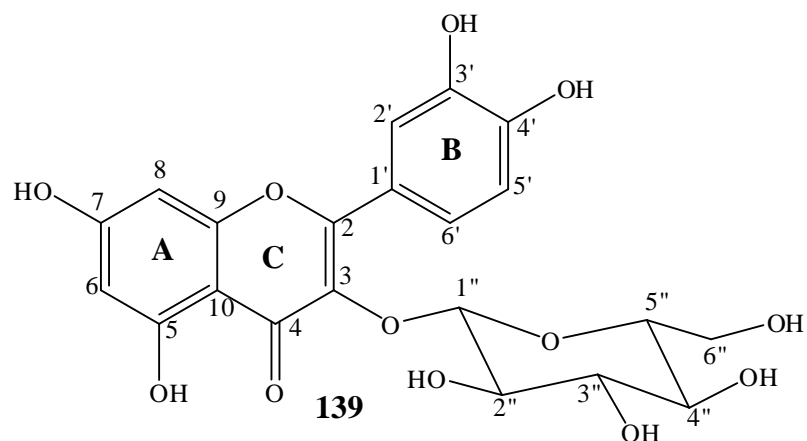
<sup>13</sup>C\* NMR data (Miyaichi *et al.*, 2006)

#### 4.1.1.9 Quercetin-3-*O*-glucoside (139)

Compound **139** was obtained as a yellow powder, m.p. 227-230°C (Lit. 225-227°C; Ahmad *et al.*, 2012). It gave a yellow colour upon exposure to ammonia vapour and also upon spraying with sulphuric acid on the TLC suggesting that the compound contained a sugar moiety (Tatke *et al.*, 2014). Its  $R_f$  value (0.81 in 15% HOAc) was relatively high on TLC (cellulose) suggesting the presence of a glycone moiety. However, a relatively low value (0.21) was obtained with Butanol-HOAc-H<sub>2</sub>O (4:1:5) (Markham, 1982). The quercetin structure was also confirmed by the <sup>1</sup>H and <sup>13</sup>C NMR spectral data (**Table 16**, **Appendix 10**), and further supported by mass spectrum (70 eV) which showed a molecular ion peak at  $m/z$  302 consistent with quercetin structure. The downfield part of the <sup>1</sup>H NMR spectrum (**Table 16**) showed the characteristic pattern of quercetin derivative depicted by the AX at  $\delta$  6.34 and 6.20 representing for H-8 and H-6 protons (d,  $J = 2.1$  Hz) and ABX spin system appearing at  $\delta$  7.71 (d,  $J = 2.0$  Hz), 7.55 (dd,  $J = 7.6$ , 2.5 Hz), and 6.68 (d,  $J = 7.7$  Hz) attributed to H-2', H-6' and H-5', respectively. The anomeric proton signal in the <sup>1</sup>H NMR spectrum (**Appendix 10**) of compound **139** appeared downfield at  $\delta$  5.22 (d,  $J = 7.4$  Hz) indicating the 1,2-diaxial coupling between the protons on C-1'' and C-2'' in a  $\beta$ -linked D-glucopyranose (Markham 1982). The other proton signal of the glucose moiety appeared at  $\delta$  3.51 (H-2''), 3.30 (H-3''), 3.38 (H-4''), 3.41 (H-5''), and 3.50, 3.74 for the methylene protons at C-6''. The presence of the 3-*O*-glucoside was corroborated further by  $\delta_C$  signals at 100.4 (C-1''), 75.2 (C-2''), 78.5 (C-3''), 71.6 (C-4'') (Sathyadevi, 2015). 78.7 (C-5'') and 63.0 (C-6''). The position of the sugar moiety was confirmed by the HMBC correlation (**Appendix 10**) between the anomeric proton H-1'' and C-3 (Ana *et al.*, 2009). Complete hydrolysis (2 % HCl)



yielded quercetin (**65**) and glucose (confirmed by co-spotting on TLC with authentic samples). In the mass spectrum (**Appendix 10**), a peak at  $m/z$  302 corresponded to the aglycone that had lost sugar moiety. Extensive analysis of the spectroscopic data of this compound led to its unequivocal identity as quercetin-3-*O*-glucoside.



**Table 16: <sup>1</sup>H NMR (600 MHz, DMSO-d<sub>6</sub>) and <sup>13</sup>C NMR data (100 MHz, DMSO-d<sub>6</sub>) data of Quercetin 3-*O*-glucoside (139)**

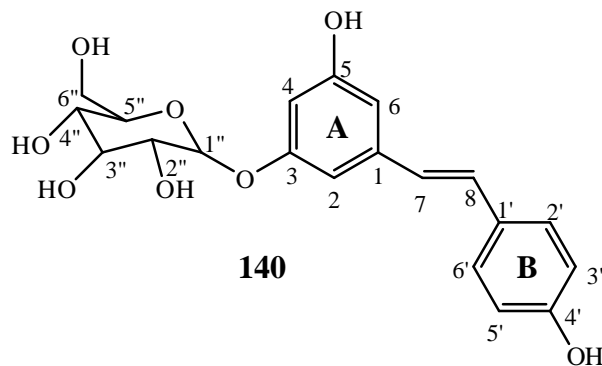
C#	Multiplicity ( <i>J</i> in Hz)	Multiplicity ( <i>J</i> in Hz)	<sup>13</sup> C	<sup>13</sup> C*
2	-	-	158.8	158.0
3	-	-	136.1	135.0
4	-	-	179.9	179.0
5	-	-	163.4	163.0
6	6.20 (d, <i>J</i> = 2.1)	6.27 (d, <i>J</i> = 2.4)	95.2	99.0
7	-	-	166.4	165.0
8	6.34 (d, <i>J</i> = 2.1)	6.36 (d, <i>J</i> = 2.4)	95.4	94.0
9	-	-	159.5	159.0
10	-	-	104.9	105.0
1'	-	-	123.5	123.4
2'	7.71 (d, <i>J</i> = 2.0)	7.80 (d, <i>J</i> = 2.2)	116.4	115.0
3'	-	-	150.3	149.0
4'	-	-	146.3	145.0
5'	6.68 (d, <i>J</i> = 7.7)	6.64 (d, <i>J</i> = 7.6)	118.1	117.0
6'	7.55 (dd, <i>J</i> = 7.6, 2.5)	7.54 (dd, <i>J</i> = 7.6, 2.5)	123.7	123.9
1''	5.22 (d, <i>J</i> = 7.4)	5.22 (d, <i>J</i> = 7.6)	100.4	100.0
2''	3.51, m	3.53 m, overlap	75.2	74.0
3''	3.30, m	3.28 m, overlap	78.5	76.0
4''	3.38, m	3.38 (bt, <i>J</i> = 9.4)	71.6	70.0
5''	3.41 (dd, <i>J</i> = 9.4, 4.9)	3.44 (dd, <i>J</i> = 5.3, 9.4)	78.7	77.0
6''	3.74 (dd, <i>J</i> = 12.0, 6.0)	3.75 (dd, <i>J</i> = 11.8, 5.5)	63.0	61.0
	3.50 (dd, <i>J</i> = 12.0, 5.6)	3.52 (dd, <i>J</i> = 11.8, 5.3)		

<sup>1</sup>H\* and <sup>13</sup>C \*NMR data (Sathyadevi, 2015)

#### 4.1.1.10 4', 5-Dihydroxystilbene-3-*O*-glucoside (140)

Compound **140** was obtained as reddish powder; m.p. 220-222°C [Lit 223-226°C, Liu *et al.*, 2012]. The <sup>1</sup>H NMR spectral (Table 17, Appendix 11) showed characteristic aglycone pattern of resveratrol derivative namely: three *meta*-coupled aromatic protons of ring A appearing at δ 6.62 (H-6, 1H, s), 6.45 (H-2, d, *J* = 1.2 Hz), and 6.77 (t, *J* = 2.2, 1.2 Hz) (Ana *et al.*, 2009). The other set of aromatic protons signifying AA'XX' spin system at δ 7.36 (d, *J* = 9.0 Hz, H-2'/H-6') and 6.79 (d, *J* = 10.2, 6.6 Hz, H-3'/H-5'), respectively. These were supported by δ<sub>C</sub> signals at 128.9 (C-2'), 116.5 (C-3'), 116.2 (C-

5') and 128.6 (C-6'). These together with an isolated olefinic protons observed at  $\delta$  7.00 (d,  $J = 16.8$  Hz, H-8) and 6.83 (d,  $J = 15.7$  Hz, H-7) suggested that the aglycone is resveratrol (Fulvia *et al.*, 1997; Xiao-Hua *et al.*, 2013; Liu *et al.*, 2012). The anomeric proton signal in the  $^1\text{H}$ NMR spectrum (Table 17, Appendix 11) appeared at  $\delta$  4.83 (H-1'', d,  $J = 7.2$  Hz) was in accordance with the axial-axial coupling between protons on C-1'' and C-2'' in a  $\beta$ -linked hexose (Markham, 1982). The other signals in the relatively upfield region of the spectrum at  $\delta$  3.47, 3.37, 3.31, 3.39 and 3.54, 3.70 corresponding to one proton each accounted for H-2'', H-3'', H-4'', H-5'' and H-6''. Accordingly, the  $^{13}\text{C}$  NMR DEPT (Table 17, Appendix 11) displayed 20 signals which were resolved into 1 methylene  $\delta$  62.6 (C-6''), 14 methines and five quaternary carbons at  $\delta$  141.6 (C-1), 159.6 (C-5), 126.7 (C-1') and 158.5 (C-4'). The position of the hydroxyl groups as well as attachment of the sugar to the aglycone was established from HMBC experiments which indicated a three H-C correlations: H-1'' correlated with C-3 ( $\delta_{\text{C}}$  160.5) whereas H-2' and H-6' showed cross peaks with C-4' ( $\delta_{\text{C}}$  158.5) indicating that the sugar was attached at C-3, while a hydroxyl group was placed at C-4'. In the mass spectrum (Appendix 11), fragment ion at  $m/z$  228 corresponding to the aglycone was due to loss of a hexose moiety. Acid hydrolysis of compound **140** gave resveratrol and glucose, confirmed by the TLC and paper chromatography with authentic samples. Thus, the above data for compound **140** are consistent with the structure of 4',5-dihydroxystilbene-3-*O*-glucoside.



**Table 17:  $^1\text{H}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ ) and  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ ) data of 4',5-dihydroxystilbene 3-O-glucoside (140)**

C#	$^1\text{H}$ NMR, Multiplicity ( $J$ in Hz)	$^1\text{H}^*$ NMR, Multiplicity ( $J$ in Hz)	$^{13}\text{C}$	$^{13}\text{C}^*$
1	-	-	141.6	140.1
2	6.45, (d, $J = 1.2$ )	6.46, s	102.4	102.7
3	-	-	160.5	159.1
4	6.77, (t, $J = 2.2, 1.2$ )	6.46 (t, $J = 2.3, 1.7$ )	104.1	105.6
5	-	-	159.6	158.3
6	6.62, bs	6.59, s	107.1	107.0
7	6.83 (d, $J = 16.8$ )	6.45 (d, $J = 16.6$ )	130.0	128.6
8	7.00 (d, $J = 16.8$ )	6.62 (d, $J = 16.6$ )	126.7	125.3
1'	-	-	130.4	129.0
2'	7.35 (d, $J = 9.0$ )	7.35 (d, $J = 8.6$ )	128.9	127.6
3'	6.79 (d, $J = 10.2, 6.6$ )	6.75 (t, $J = 10.1, 8.6$ )	116.5	115.1
4'	-	-	158.5	156.9
5'	6.79 (d, $J = 10.2, 6.6$ )	6.75 (t, $J = 10.1, 8.6$ )	116.2	115.1
6'	7.35 (d, $J = 9.0$ )	7.35 (d, $J = 8.6$ )	128.6	127.6
1''	4.83 (d, $J = 7.2$ )	3.91 (dd, $J = 7.1, 1.7$ )	102.4	101.1
2''	3.47, dd, $J = 9.4, 6.8$ )	3.41, s	75.0	73.6
3''	3.37 (dd, $J = 9.0, 4.2$ )	3.36, s	78.1	76.7
4''	3.31 (bt, $J = 9.8$ )	3.35, s	71.5	70.1
5''	3.39 (dd, $J = 9.8, 6.0$ )	3.38, s	78.3	76.9
6''	3.54 ( $\text{H}_\alpha$ , dd, $J = 13.2, 6.6$ ) 3.70 ( $\text{H}_\beta$ , dd, $J = 12.0, 6.6$ )	3.44 ( $\text{H}_\alpha$ , t, $J = 12.4, 6.3$ ) 3.69 ( $\text{H}_\beta$ , dd, $J = 10.7, 6.0$ )	62.6	61.2

$^1\text{H}^*$  and  $^{13}\text{C}^*$  NMR data (Xiao-Hua *et al.*, 2013)

#### 4.1.1.11 Rutin (141)

This compound was obtained as pale yellow powder solid, m.p. 234-236°C [(Lit. 238-240°C; Okoth, 2013). The  $^1\text{H}$  NMR spectrum (Table 18, Appendix 12) of this compound consisted of signals typical of hydrogen bonded OH at  $\delta$  12.55, (5-OH, s) (Batterham and Hight, 1964, 1963); aromatic proton signals  $\delta$  6.19 (1H, d,  $J = 2.0$  Hz, H-6), 6.39 (1H, d,  $J = 2.5$  Hz, 8-H), 7.54 (1H, d,  $J = 2.0$  Hz, H-2'), 6.84 (1H, d,  $J = 8.0$  Hz, H-3'), 7.52 (1H, d,  $J = 2.0$  Hz, H-6'). Six proton resonances for the  $\beta$ -glucopyranose were observed at  $\delta_{\text{H}}$  5.32 (H-1'', 1H, d,  $J = 7.5$  Hz), 3.47 (H-2'', 1H, d,  $J = 13.00$  Hz), (3.25 (H-3'', d,  $J = 3.0$  Hz), 3.29 (H-4'', H-5'' d,  $J = 9.9$  Hz), 3.38 (6''-H $_{\alpha}$ , d,  $J = 9.5$  Hz), 3.83 (6''-H $_{\beta}$ , d,  $J = 9.5$  Hz) typical of  $\beta$ -linkage (Satterfield and Brodbelt, 2001). An additional six proton resonances for the second sugar moiety were observed  $\delta_{\text{H}}$  4.37 (1'''-H, d,  $J = 1.0$  Hz), 3.28 (dd,  $J = 3.5, 1.5$  Hz), 3.25 (3'''-H, dd,  $J = 9.5, 3.9$  Hz), 3.29 (d,  $J = 9.5$  Hz), 3.25 (d,  $J = 9.5$ ) and 0.97 (3H, d,  $J = 6.0$  Hz H-6' which were typical of  $\alpha$ -rhamnose (Okoth, 2013; Okoth *et al.*, 2013; Satterfield and Brodbelt, 2001).

The  $^{13}\text{C}$  NMR spectra of this compound correlated with that of a quercetin moiety with rutinose. There were ten signals for the flavones moiety, six signals for the aromatic ring and an additional six signals for each of the glucose and rhamnose moieties. The glucose was found to be attached at C-3 ( $\delta_{\text{C}}$  134.0) which was based on HMBC correlation between H-1'' ( $\delta$  5.32) and C-3. The rhamnose group was then the terminal sugar which was linked to glucose through C-6'' as evidenced by the HMBC correlation observed between the anomeric proton of the D-rhamnose ( $\delta_{\text{H}}$  4.37) and the C-6'' of glucose ( $\delta_{\text{C}}$  70.3). The ESI-MS of this compound showed a sodiated molecular ion,

$[M+Na]^+$  at  $m/z$  633; and  $m/z$  of 611 which was consistent with the molecular formula of  $C_{27}H_{30}O_{16}$ . The  $^1H$  and  $^{13}C$  NMR spectral data (**Table 18, Appendix 12**) was comparable to that of rutin previously isolated from *Lanena shweinfurthii* (Okoth *et al.*, 2013; Markham, 1982). Based on spectroscopic data as well as comparison with literature data compound **141** was structurally elucidated as rutin.

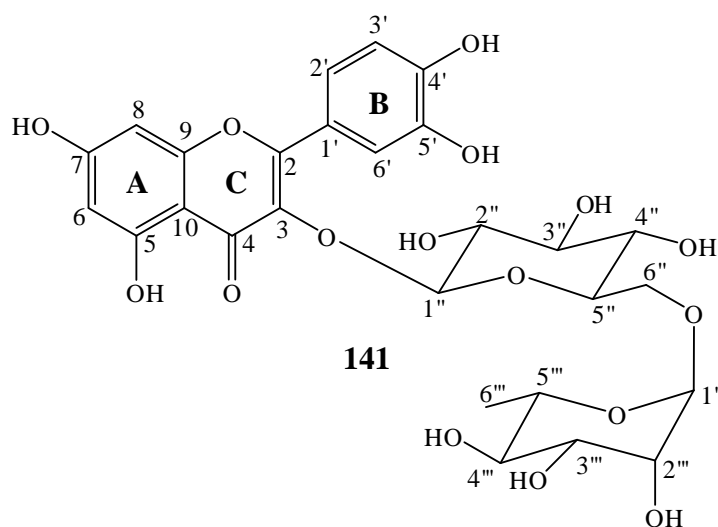


Table 18:  $^1\text{H}$  (500 MHz, DMSO- $d_6$ ) and  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ) of Rutin (141)

C#	$^1\text{H}$ NMR, Multiplicity ( $J$ in Hz)	$^1\text{H}^*$ NMR, Multiplicity ( $J$ in Hz)	$^{13}\text{C}$	$^{13}\text{C}^*$
2	-	-	156.8	158.5
3	-	-	134.0	135.7
4	-	-	177.7	179.4
5	-	-	157.0	-
6	6.19 (d, $J = 2.0$ )	6.19 (d, $J = 2.0$ )	101.0	100.0
7	-	-	164.3	-
8	6.39 (d, $J = 2.5$ )	6.37 (d, $J = 2.1$ )	94.0	94.9
9	-	-	161.5	162.9
10	-	-	101.4	105.6
1'	-	-	122.0	123.6
2'	7.54 (d, $J = 2.0$ )	7.67 (d, $J = 2.16$ )	115.5	116.1
3'	6.84 (d, $J = 8.0$ )	6.87 (d, $J = 8.48$ )	116.6	117.7
4'	-	-	148.7	-
5'	-	-	145.0	-
6'	7.52 (dd, $J = 2.0$ )	7.62 (dd, $J = 2.16$ )	121.5	123.1
1''	5.32 (d, $J = 7.5$ )	5.10 (d, $J = 7.48$ )	104.3	104.8
2''	3.47 (d, $J = 13.00$ )	3.49 (d, $J = 13.77$ )	72.1	72.1
3''	3.28 (d, $J = 3.0$ )	3.35 (d, $J = 2.8$ )	70.8	72.3
4''	3.29 (d, $J = 9.0$ )	3.28 (d, $J = 15.01$ )	74.4	74.0
5''	3.29 (d, $J = 9.0$ )	3.28 (d, $J = 15.01$ )	68.5	69.7
6''	3.38 ( $\text{H}_\alpha$ , d, $J = 9.5$ ) 3.83 ( $\text{H}_\beta$ , d, $J = 9.5$ )	3.38 (d, $\text{H}_\alpha$ , d, $J = 10.13$ ) 3.82 (d, $\text{H}_\beta$ , d, $J = 2.8$ )	70.3	70.9
1'''	4.37 (d, $J = 1.0$ )	4.53 (d, $J = 1.36$ )	101.0	102.4
2'''	3.28, (dd, $J = 3.5, 1.5$ )	3.66 (dd, $J = 3.34, 1.58$ )	70.6	72.1
3'''	3.25 (d, $J = 9.5, 3.9$ )	3.54 (dd, $J = 9.4, 3.4$ )	70.2	72.3
4'''	3.29 (d, $J = 14.9$ )	3.29 (d, $J = 15.09$ )	76.4	74.0
5'''	3.25 (d, $J = 9.5$ )	3.45 (d, $J = 8.40$ )	70.0	69.7
6'''	0.97 (3H, d, $J = 6.0$ )	1.13 (d, $J = 6.20$ )	17.9	17.9
5-OH	12.55, s	12.30, s	-	159.3
7-OH	10.8 (OH, s)	-	-	166.0
4'-OH	9.17 (OH, s)	-	-	149.8
5'-OH	9.60 (OH, s)	-	-	145.8

$^1\text{H}^*$  and  $^{13}\text{C}^*$  NMR data, (Okoth *et al.*, 2013)

#### 4.1.2 Structural elucidation of compounds from *Alysicarpus ovalifolius*

##### (Schumach)

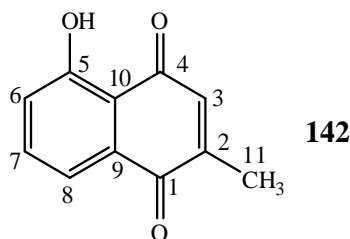
Phytochemical evaluation of the root bark of *A. ovalifolius* led to the isolation of quercetin (**65**), apigenin (**68**),  $\beta$ -sitosterol (**134**),  $\beta$ -sitosterol glucoside (**136**), quercetin-3-*O*-glucoside (**139**), plumbagin (**142**), orientin (**143**), mohanimbine (**144**), koenimbine (**145**) and koenidine (**146**). The structures of these compounds were elucidated using physical and spectroscopic methods as well as comparison with the literature data.

##### 4.1.2.1 Plumbagin (**142**)

Compound **142** was isolated as an orange substance with mp 74-76°C (Lit. 76-78°C; Singh *et al.*, 2012; Tangmouo *et al.*, 2005). The compound on exposure to conc. ammonia solution turned purple suggesting that it was a quinone derivative and this was supported by its UV spectrum which exhibited absorptions at  $\lambda_{\max}$  254 and 420 nm. The  $^1\text{H}$  NMR spectrum (**Table 19, Appendix 13**) showed a singlet at  $\delta$  6.80 for H-3, a doublet of doublets at  $\delta$  7.26 for H-6, a multiplet at  $\delta$  7.60 for H-7 and another multiplet which resonated at  $\delta$  7.58 was attributable to H-8 proton. A singlet at  $\delta$  1.25 was assigned to the three hydrogen of methyl group attached to C-2. Another singlet which resonated at  $\delta$  11.96 was assigned the OH at C-5. Similarly,  $^{13}\text{C}$  NMR spectrum (**Table 19, Appendix 13**) showed six quaternary carbons; two of which were carbonyl carbons at  $\delta$  184.8 and 190.3 assigned to C-1 and C-4 respectively. C-4 appeared downfield due to the anisotropic effect of the neighboring OH-group at C-5 coupled by field and resonance inductive effects (Batterham and Highet, 1964). Four methine carbons were observed at  $\delta$  135.5, 124.2, 136.1 and 119.3 attributable to C-3, C-6, C-7 and C-8, respectively while



the methyl carbon appeared at  $\delta$  16.5. The attachment of CH<sub>3</sub> at C-2 was evidenced by a strong correlation on the HMBC spectrum C-2 carbon and the 11-Me protons (**Appendix 13**). Consequently, based on physical, chemical, spectral data and comparison with literature, the compound was concluded to be plumbagin which has been isolated before from *Diospyros kaki* root bark (Singh *et al.*, 2012, Dubey *et al.*, 2009).



**Table 19: <sup>1</sup>H (300 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C (75 MHz, CDCl<sub>3</sub>) NMR data for Plumbagin (142)**

ATOM	<sup>1</sup> H Multiplicity (J in Hz)	<sup>1</sup> H*, Multiplicity (J in Hz)	<sup>13</sup> C	<sup>13</sup> C*
<b>1</b>	-	-	184.8	184.7
<b>2</b>	-	-	149.6	150.3
<b>3</b>	6.80, s	7.00, s	135.5	135.6
<b>4</b>	-	-	190.3	191.4
<b>5</b>	-	-	161.2	161.4
<b>6</b>	7.26 (dd, 2.0, 7.0)	7.30, dd (1.5, 8.0)	124.2	124.4
<b>7</b>	7.60 m	7.45, m	136.1	136.3
<b>8</b>	7.58 m	7.45, m	119.3	119.4
<b>9</b>	-	-	132.1	132.3
<b>10</b>	-	-	115.2	115.0
<b>5-OH</b>	11.96, s	11.96, s		
<b>11-Me</b>	1.25, m	1.25, s	16.5	16.7

<sup>1</sup>H\* and <sup>13</sup>C\* NMR data (Singh *et al.*, 2012)

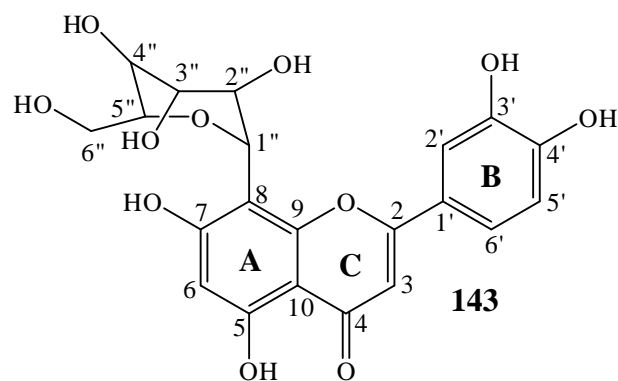
#### 4.1.2.2 Orientin (143)

The compound was isolated as a pale yellow amorphous powder (MeOH-H<sub>2</sub>O mixture) with m.p. 255-257°C (Lit. 260-262°C; Wen *et al.*, 2007). It showed an intense yellow colour on TLC when exposed to conc. ammonia vapour suggesting that it could be a

flavonoid derivative (Wen *et al.*, 2007). Its IR spectrum revealed the presence of hydroxyl ( $3400\text{ cm}^{-1}$ ), carbonyl ( $1655\text{ cm}^{-1}$ ) and aromatic ( $1613$  and  $1415\text{ cm}^{-1}$ ) functionalities. The UV spectrum of compound **143** in MeOH showed absorption maxima at  $\lambda_{\text{max}}$  274 (band I) and 346 (band II) nm suggesting that **143** is flavone derivative (Chaturvedula and Prakash, 2013; Dordevic *et al.*, 2001; Mabry *et al.*, 1970). The  $^1\text{H}$  NMR spectrum (**Table 20, Appendix 14**) of this compound did not show a resonance peak for hydrogen bonded hydroxyl group at  $\delta$  12.50 assignable to OH-5 (Markham, 1982). This could be attributed to the solvent (MeOD) used during spectral analysis. Similarly, the spectrum showed a singlet at  $\delta_{\text{H}}$  6.40 in ring A attributed to either H-6 or H-8. Another singlet at  $\delta_{\text{H}}$  6.60 was assigned to H-3 of ring C. These together with B ring signals with a ABX spin system suggested a C-glycosidated luteolin derivative with sugar moiety attached at either C-8 or C-6 (Sharma *et al.*, 2014; Yang, *et al.*, 2015).

The  $^{13}\text{C}$  NMR (**Table 20, Appendix 14**) and DEPT showed 21 distinct carbon signals accounting for ten methines, one methylene and ten quaternary carbons of which fifteen of the peaks were similar to those of 5,7,3',4'-tetrahydroxyflavone 8-C-glycoside (Sharma *et al.*, 2014) while the remaining 6 carbon signals could be assignable to either  $\beta$ -glucose or  $\beta$ -galactose moiety (Sharma *et al.*, 2014; Drozd, 1972). Changes of diagnostic value were observed in the  $^{13}\text{C}$  NMR spectrum of compound **143** in comparison with luteolin (Kim, *et al.*, 2000) whereby C-8 shifted downfield at  $\delta$  104.1 due to C-glycosylation (Drozd, 1972), a fact further confirmed by HMBC correlation between H-6 (6.40) and C-8. Similarly, peaks observed at  $\delta$  162.9 and 158.9 were assigned to C-7 and C-9, respectively on the basis that C-8 glycosylation should affect C-7 and C-9 shifts (Kim, *et al.*, 2000). Unequivocal information on the type of sugar moiety attached to the aglycone

was substantiated by the couplings of the C-4'' hydroxyl group based on the axial-equatorial coupling between H-1'' and H-2''; H-4'' and H-5'' and 1,2 diaxial coupling between H-4'' and H-3'', an interpretation that was facilitated by the  $^1\text{H}$ - $^1\text{H}$  proximity between H-4'' and H-3'', an interpretation that was facilitated by the  $^1\text{H}$ - $^1\text{H}$  proximity between H-4'' and H-5'' defining the sugar moiety to be  $\beta$ -glucose. Based on extensive spectroscopic analysis as well as comparison with literature, compound **143** was structurally confirmed as 5, 7, 3', 4'-tetrahydroxyflavone 8-C- $\beta$ -glucoside.



**Table 20: <sup>1</sup>H (400 MHz, MeOD) and <sup>13</sup>C (100 MHz, MeOD)  
NMR data for Orientin (143)**

<b>ATOM</b>	<b><sup>1</sup>H</b>	<b><sup>1</sup>H*</b>	<b><sup>13</sup>C</b>	<b><sup>13</sup>C*</b>
	<b>Multiplicity</b>	<b>Multiplicity</b>		
	<b>(<i>J</i> in Hz)</b>	<b>(<i>J</i> in Hz)</b>		
<b>2</b>	-	-	164.3	164.1
<b>3</b>	6.60, s	6.27, s	102.6	102.3
<b>4</b>	-	-	184.0	182.0
<b>5</b>	-	-	164.3	160.4
<b>6</b>	6.40, s	6.60, s	99.8	98.1
<b>7</b>	-	-	162.9	162.8
<b>8</b>	-	-	104.8	104.5
<b>9</b>	-	-	158.9	156.0
<b>10</b>	-	-	104.1	103.9
<b>1'</b>	-	-	123.0	121.9
<b>2'</b>	7.82 (d, <i>J</i> = 9.0)	7.48, br s	116.0	114.0
<b>3'</b>	-	-	138.8	165.8
<b>4'</b>	-	-	129.6	129.7
<b>5'</b>	6.88 (d, <i>J</i> = 9.0)	6.86 (d, <i>J</i> = 8.4)	117.1	119.3
<b>6'</b>	7.80 (d, <i>J</i> = 9.0)	7.52 (br d, <i>J</i> = 8.4)	115.9	115.7
<b>1''</b>	4.84 (d, <i>J</i> = 7.7)	4.68 (d, <i>J</i> = 8.8)	74.0	73.4
<b>2''</b>	3.48 (d, <i>J</i> = 7.7)	3.50 (d, <i>J</i> = 7.8)	71.4	70.8
<b>3''</b>	3.42 (d, <i>J</i> = 7.8)	3.40 (d, <i>J</i> = 8.0)	78.3	78.8
<b>4''</b>	3.28 d, <i>J</i> = 9.1)	3.30 (d, <i>J</i> = 8.4)	70.1	70.7
<b>5''</b>	3.33 (d, <i>J</i> = 9.6)	3.34 (d, <i>J</i> = 9.0)	79.1	82.0
<b>6''</b>	3.54 m	3.60, m	62.5	61.6

<sup>1</sup>H\* and <sup>13</sup>C\* NMR data (Drozd, 1972)

#### 4.1.2.3 Mohanimbine (144)

This compound was isolated as colourless powder with m.p. 92-94°C (Lit. 88-90°C; Abu Bakar *et al.*, 2007). It showed a positive Dragendorff's test suggesting it could be an alkaloid compound (Khatun *et al.*, 2014). The <sup>1</sup>H NMR spectrum (**Table 21, Appendix 15**) of compound **144** showed two methyl singlets at δ 1.77 and 1.70 (3H each, vinyl methyls), 2.38 (3H, an aromatic methyl) and 1.49 (3H) besides a vinyl proton on a trisubstituted double bond at δ 5.13 (br t, *J* = 6.6 Hz). The latter peak correlated with the vinyl methyls in the HMBC spectrum suggesting the presence of a terminal-CH<sub>2</sub>CH<sub>2</sub>CH=C(CH<sub>3</sub>)<sub>2</sub> group in the compound (Mohammad *et al.*, 2013, Abu Bakar *et al.*, 2007). Furthermore, in the <sup>1</sup>H NMR spectrum a pair of *meta*-coupled olefinic protons at C-3' and C-4' appeared as doublets at δ 6.64 (*J* = 9.6 Hz) and 5.67 (*J* = 9.6 Hz) which together with a <sup>13</sup>C NMR spectrum (**Table 22, Appendix 15**) diagnostic peak at δ 78.2 signified the presence of C-O-C bond in a pyran ring typical of carbazole alkaloids (Dheeref *et al.*, 2014; Mohammad *et al.*, 2013; Abu Bakar *et al.*, 2007). Comparison of <sup>1</sup>H and <sup>13</sup>C NMR spectra of compound **144** with those of mohanimbine previously isolated from *Murraya koenigii* revealed close similarities (Dheeref *et al.*, 2014; Mohammad *et al.*, 2013; Abu Bakar *et al.*, 2007; Chakraborty *et al.* 1978; Feibig *et al.*, 1985). Analyses of both <sup>1</sup>H and <sup>13</sup>C NMR data of compound **144** taking into consideration the fragmentation pattern *m/z* 332 [M]<sup>+</sup> (100), 331.3 (10), 276.2 (7), 250 (30), 248 (10), 210 (10) in the EI-MS was consistent with the structure of mohanimbine. In addition, the mass spectrum (**Appendix 15**) showed a molecular ion peak at *m/z* 332 [M+1]<sup>+</sup> which was consistent with the molecular formula C<sub>23</sub>H<sub>25</sub>NO further confirming that the compound was mohanimbine.

#### 4.1.2.4 Koenimbine (145)

Compound **145** was obtained as colourless crystals and also afforded a positive Drangedorff's test for alkaloids (Khatun *et al.*, 2014), m.p. 196-197°C (Lit. 194-195°C; Nayak *et al.*, 2010). The <sup>1</sup>H NMR spectrum (**Table 21, Appendix 15**) of this compound showed a set of olefinic signals at δ 6.78 (d, *J* = 11.0 Hz) and 5.68 (d, *J* = 14.7 Hz) which together with a broad singlet at δ 7.61 typical of -NH resonance suggested that compound **145** is a carbazole alkaloid (Dheeref *et al.*, 2014). The <sup>13</sup>C NMR data (**Table 22, Appendix 15**) in combination with DEPT (90° and 135°) (**Appendix 15**) had 19 distinct C-signals; DEPT, <sup>13</sup>C NMR (90°: 6CH all SP<sup>2</sup>); Θ = 135°: 6CH and 4CH<sub>3</sub>) including a methoxy group at δ 56.7. The DEPT spectrum (**Appendix 15**) of this compound comprised of 6CH and 4CH<sub>3</sub> which totalled to C<sub>10</sub>H<sub>9</sub> suggested the presence of either a hydroxyl or a -NH group in the compound. Comparative analysis of both <sup>1</sup>H and <sup>13</sup>C NMR data of the compound taking into consideration of the molecular ion *m/z* 294.3 and the fragmentation pattern in the ESI-MS (rel. int): *m/z* 294.3 [M]<sup>+</sup> (100), 296 (10), 293.3 (24) revealed the compound to be koenimbine previously isolated from *M. koenigii* (Ito *et al.*, 2006; Mohammed *et al.*, 2013). On the basis of physical and spectroscopic data compound **145** was identified as koenimbine.

#### 4.1.2.5 Koenidine (146)

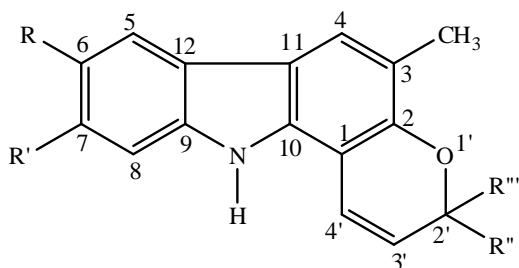
Koenidine was obtained as pale yellow needles with m.p. 225-226°C (Lit. 224-225°C; Mohammad *et al.*, 2013). The ESI-MS indicated a molecular ion peak at *m/z* 324.3 [M]<sup>+</sup> (100) (**Appendix 15**) suggesting a molecular formula of C<sub>20</sub>H<sub>21</sub>NO<sub>3</sub> which is 30 a.m.u. (OCH<sub>2</sub> moiety) higher than that for compound **145**, thus suggesting that compound **146** is

a methoxy derivative of **145**. Comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR (**Table 21, 22, Appendix 15**) spectra of **146** with those of **145** showed close similarities with notable difference between the two compounds being the presence of additional methoxy group in **146** as evidenced by a  $^1\text{H}$  NMR peak at  $\delta$  3.97 (s, 3H) with corresponding  $\delta_{\text{C}}$  54.8. Further confirmation of the structure of **146** was accomplished using HSQC and HMBC data which aided unequivocal assignment of all the signals associated with the compound. Thus, based on spectroscopic data (**Tables 21 and 22, Appendix 15**) coupled with reported literature data, compound **146** was established to be koenidine also previously isolated from *M. koenigii* (Ito *et al.*, 2006; Mohammed *et al.*, 2013).

**Table 21:  $^1\text{H}$  NMR Spectral Data ( $\text{CDCl}_3$ , 300MHz) for Compounds 144, 145 and 146**

C#	$^1\text{H}$ Multiplicity (J in Hz)		
	144	145	146
4	7.69, s	7.63, s	7.40, s
5	7.96 (d, J = 7.5)	7.41 (d, J = 7.6)	7.53, s
6	7.19 (d, J = 7.5)	7.25 (d, J = 7.2)	-
7	7.28 (d, J = 5.0)	-	-
8	7.36 (d, J = 7.2)	7.42 (d, J = 7.6)	6.95, s
3'	6.64 (d, J = 9.6)	6.78 (d, J=11.0)	6.64 (d, J = 9.6)
4'	5.67 (d, J = 9.6)	5.68 (d, J = 14.7)	5.69 (d, J = 9.6)
5'	1.62 (t, J= 8.1)	-	-
6'	1.83, m	-	-
7'	5.13 (br t, J = 6.6)	-	-
8'	-	-	-
9'-Me	1.77, s	-	-
10'-Me	1.70, s	-	-
2'-Me	1.49, s	1.47, 2.28, s	1.28,1.50, s
3-Me	2.38, s	2.29, s	2.35, s
7'-Me	-	-	-
OMe (6)	-	-	3.51, s
OMe (7)	-	3.87, s	3.97, s
N-H	7.41	7.61, s	7.30

$^1\text{H}^*$  NMR data (Mohammad *et al.*, 2013)



**144:** R = R' = H, R'' = CH<sub>3</sub>, R''' = <sup>5'</sup>CH<sub>2</sub>-<sup>6'</sup>CH<sub>2</sub>-<sup>7'</sup>CH=C(CH<sub>3</sub>)<sub>2</sub><sup>8'</sup> [9',10']

**145:** R = H, R' = OCH<sub>3</sub>, R'' = R''' = CH<sub>3</sub>

**146:** R = R' = OCH<sub>3</sub>, R'' = R''' = CH<sub>3</sub>

**Table 22:** <sup>13</sup>C NMR Spectral Data (CDCl<sub>3</sub>, 75 MHz) for Compounds **144**, **145** and **146**

	<b>144</b> <sup>13</sup> C	<b>144</b> <sup>13</sup> C*	<b>145</b> <sup>13</sup> C	<b>145</b> <sup>13</sup> C*	<b>146</b> <sup>13</sup> C	<b>146</b> <sup>13</sup> C*
<b>1</b>	104.2	104.0	103.7	103.1	103.4	102.9
<b>2</b>	149.9	148.4	150.9	151.0	146.7	147.2
<b>3</b>	131.7	130.7	125.3	125.9	133.9	134.1
<b>4</b>	117.5	116.9	122.1	123.0	115.7	116.3
<b>5</b>	119.5	120.0	103.4	103.0	101.1	100.9
<b>6</b>	119.3	118.9	114.2	115.2	142.5	143.1
<b>7</b>	121.2	121.0	155.2	155.6	146.9	117.3
<b>8</b>	110.4	109.8	112.3	112.1	93.6	94.2
<b>9</b>	134.9	133.4	136.6	137.0	133.9	141.1
<b>10</b>	139.5	140.1	132.9	133.1	117.1	118.0
<b>11</b>	118.4	118.0	118.5	119.1	-	-
<b>12</b>	116.7	117.0	116.0	117.2	117.1	117.6
<b>2'</b>	78.2	79.1	77.1	76.9	74.2	75.1
<b>3'</b>	128.5	129.0	130.1	131.2	127.4	126.9
<b>4'</b>	124.2	123.9	118.1	119.2	118.7	118.9
<b>5'</b>	40.8	41.0	-	-	-	-
<b>6'</b>	22.7	23.0	-	-	-	-
<b>7'</b>	116.7	115.9	-	-	-	-
<b>8'</b>	131.7	131.1	-	-	-	-
<b>9'</b>	25.9	25.5	-	-	-	-
<b>10'</b>	25.7	24.2	-	-	-	-
<b>3-Me</b>	16.1	17.0	28.2	28.9	26.3	26.5
<b>2'-Me</b>	17.6	18.1	16.5	17.1	14.8	15.0
<b>OMe (6)</b>	-	-	-	-	55.3	56.1
<b>OMe (7)</b>	-	-	56.7	57.1	54.8	55.3

<sup>13</sup>C\* NMR data (Mohammad *et al.*, 2013)



### 4.1.3 Structural elucidation of compounds from the leaves of *Erythrina abyssinica* (DC)

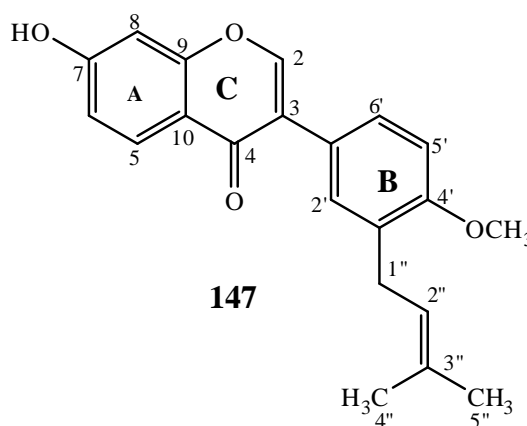
Phytochemical investigation of the methanolic extract of the leaves of *E. abyssinica* led to the isolation of 7-hydroxy-4'-methoxy-3-prenylisoflavone (**147**) and erythrinasinatate A (**148**). These compounds were obtained after successive silica gel column chromatography and their structures elucidated using spectroscopic (NMR and MS) methods as well as comparison with literature data.

#### 4.1.3.1 7-hydroxy-4'-methoxy-3-prenylisoflavone (**147**)

Compound **147** was obtained an amorphous yellow powder, m.p. 194-196°C (Lit. 192-193°C; Yenesew *et al.*, 2009). The  $^{13}\text{C}$  NMR spectrum showed  $\delta_{\text{C}}$  signals at 154.0 and 125.9 which were typical of C-2 and C-3 of flavones (Yenesew *et al.*, 2006). The  $^1\text{H}$  NMR spectrum had a signal at  $\delta_{\text{H}}$  3.88 typical of  $\text{OCH}_3$  protons suggesting the presence a methoxy group in the flavone molecule (Batterham and Highet 1964). In addition, the  $^1\text{H}$  NMR spectrum (**Table 23, Appendix 16**) exhibited an AXY spin system for ring-A at  $\delta$  8.06 (1H, d,  $J = 8.7$  Hz), 6.96 (1H, dd,  $J = 8.7, 2.1$ Hz) and 6.90 (d,  $J = 2.1$ ) attributed to H-5, H-6 and H-8, respectively. Likewise, an ABX spin system was also observed at  $\delta$  7.40 (1H, d,  $J = 2.1$ ), 7.43 (1H, dd,  $J = 8.4, 2.1$ , Hz) and 6.99 (1H, d,  $J = 8.4$ ) assignable to H-2', H-6' and H-5' respectively. An olefinic proton which appeared at  $\delta$  5.31 was assigned to H-2''. This together with vinylic methyl groups at  $\delta$  1.72 and 1.70 (both singlets), and methylene group appearing at  $\delta$  3.34 suggested that the flavone could be prenylated (Yenesew *et al.*, 2006), The signals at  $\delta$  8.14 in the  $^1\text{H}$  NMR was to assigned to H-2 with corresponding  $^{13}\text{C}$  NMR resonances at  $\delta_{\text{C}}$  154.0. Also observed on the  $^{13}\text{C}$

NMR spectrum were resonances appearing at  $\delta_C$  125.9 and 176.4 for C-3 and C-4 suggested an isoflavone skeleton (Batterham and Highet 1964). The HMBC spectrum (**Appendix 16**) revealed a correlation of the methylene protons at  $\delta$  3.34 with C-4' ( $\delta$  158.7) and C-6' ( $\delta$  129.3) confirming the substitution pattern of ring B.

The EI-MS showed a molecular ion peak at  $m/z$  336 corresponding to the molecular formula  $C_{21}H_{20}O_4$ . Similarly, in the EI-MS spectrum,  $m/z$  at 137 resulted from retro-Diels-Alder fragmentation implying that ring-A had one -OH group at C-7 hence the methoxy and the prenyl moieties could only be on ring B (Saxena and Bhadoria, 1990, Batterham and Highet 1964). The resonances of this compound were similar to a flavonoid isolated from *Erythrina sacleuxii* and complete assignment was achieved through comparison with those contained in literature (Yenesew *et al.*, 2006). This compound was first reported as a synthetic derivative of 3'-prenyl-4-methoxyisoflavone-7-O- $\beta$ -D-(2''-O-p-coumaroyl) glucopyranoside (Saxena and Bhadoria, 1990) and after extensive structure elucidation, it was identified as 7-hydroxy-4'-methoxy-3-prenylisoflavone.



**Table 23:  $^1\text{H}$  (CDCl<sub>3</sub>, 400 MHz) and  $^{13}\text{C}$  NMR (CDCl<sub>3</sub>, 100 MHz) data for 7-hydroxy-4'-methoxy-3-prenylisoflavone (147)**

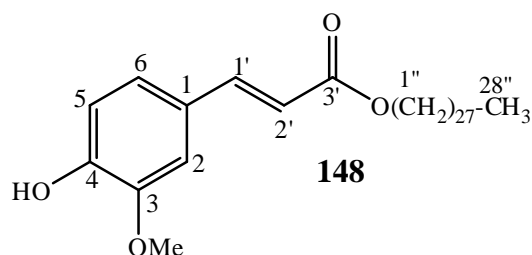
C #	$^1\text{H}$ Multiplicity, ( <i>J</i> in Hz)	$^1\text{H}^*$ ( $\delta\text{H}$ , Multiplicity, ( <i>J</i> in Hz)	$^{13}\text{C}$	$^{13}\text{C}^*$
2	8.14, s	8.20, s	154.0	156.0
3	-	-	125.9	124.9
4	-	-	176.4	178.4
5	8.06 (d, <i>J</i> = 8.7)	8.10 (d, <i>J</i> = 7.7)	129.1	130.1
6	6.96 (d, <i>J</i> = 8.7, 2.1)	7.00 (d, <i>J</i> = 2.4)	111.6	113.6
7	-	-	159.5	160.5
8	6.90 (d, <i>J</i> = 2.1)	7.00 (d, <i>J</i> = 2.4)	103.8	104.8
9	-	-	164.2	166.2
10	-	-	130.9	131.9
1'	-	-	126.0	127.0
2'	7.40 (d, <i>J</i> = 2.1)	7.70 9d, <i>J</i> = 2.3)	131.6	132.6
3'	-	-	130.9	1311.9
4'	-	-	158.7	159.7
5'	6.99 (d, <i>J</i> = 8.4)	7.10 (d, <i>J</i> = 8.8)	119.1	120.1
6'	7.43 (d, <i>J</i> = 2.1, 8.4)	7.52(d, <i>J</i> = 8.7 ,2.5)	129.3	128.3
1''	3.34 (d, <i>J</i> = 7.5)	3.50 (d, <i>J</i> = 7.9)	31.8	32.0
2''	5.31, m	5.40, m	124.3	125.3
3''	-	-	133.2	134.2
4''-Me	1.72, s	1.77, s	26.6	27.6
5''-Me	1.70, s	1.80, s	18.5	19.5
4'-OMe	3.88, s	4.00, s	56.5	56.0

$^1\text{H}^*$  and  $^{13}\text{C}^*$  NMR data (Yenesew *et al.*, 2006)

#### 4.1.3.2 Erythrasinate A (148)

Compound **148** was obtained as a white powder, m.p. 112-114°C (Lit. 114-116°C; Nkengfack, *et al.*, 1997; Yenesew *et al.*, 2006) with a molecular ion at *m/z* of 587.5 corresponding to the molecular formula of C<sub>38</sub>H<sub>67</sub>O<sub>4</sub>. The  $^1\text{H}$  NMR spectrum (**Table 24, Appendix 17**) exhibited the presence of an ABX spin system at  $\delta_{\text{H}}$  7.04 (1H, d, *J* = 1.6 Hz, H-2), 6.92 (1H, d, *J* = 8.0 Hz, H-5) and 7.10 (1H, dd, *J* = 8.0, 1.6 Hz, H-6,) of a trisubstituted benzene ring. Also observed were signals typical of olefinic protons which were *trans* in orientation (Pavia *et al.*, 2009) at  $\delta$  7.62 (1H, d, *J* = 16.2 Hz, H-1') and 6.30

(H-2', 1H, d,  $J = 15.8$  Hz) which together with peaks at  $\delta$  4.19 (H-1'', 2H, t,  $J = 7.0$  Hz), 1.66 (H-2'', m) and a broad singlet at  $\delta$  1.25 (4''-28-CH<sub>2</sub>)<sub>n</sub> and  $\delta$  0.88 (28-Me, t,  $J = 7.0$  Hz) suggested that this compound was a cinnamyl ester derivative substituted at C-3 and C-4 (Nkengfack *et al.*, 1997, Pavia *et al.*, 2009). The presence of hydroxyl group at C-4 was evidenced by a broad singlet signal at  $\delta$  5.89 while the methoxy group at C-3 appeared as a singlet at  $\delta$  3.93. The <sup>1</sup>H NMR signals were corroborated by corresponding <sup>13</sup>C NMR signals at  $\delta$  109.9 (C-2), 114.6 (C-5), 123.1 (C-6) typical of aromatic carbons, 115.6 (C-1'), 167.0 (C-2') characteristic of olefinic carbons and  $\delta$  29.7 which was assigned to 4''-25''. The spectral data of this compound was consistent with that of erythrisinate **A** previously isolated from other *Erythrina* species (Nkengfack *et al.*, 1997, Yenesew *et al.*, 2006; Wandji *et al.*, 1990).



**Table 24:  $^1\text{H}$  ( $\text{CDCl}_3$ , 400 MHz) and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz) data for Erythrasinate A (148)**

C #	$^1\text{H}$ Multiplicity, ( <i>J</i> in Hz)	$^1\text{H}^*$ Multiplicity, ( <i>J</i> in Hz)	$^{13}\text{C}$	$^{13}\text{C}^*$
1		-	127.0	127.3
2	7.04 (d, <i>J</i> = 1.6)	7.04 (d, <i>J</i> = 1.1)	109.9	109.5
3	-	-	147.8	148.1
4	-	-	146.7	147.0
5	6.92 (dd, <i>J</i> = 8.0, 1.6)	6.93 (dd, <i>J</i> = 8.1, 1.1)	114.6	114.9
6	7.10 (d, <i>J</i> = 8.0, 1.6)	7.07 (d, <i>J</i> = 8.4)	123.1	123.3
1'	7.62 (d, <i>J</i> = 16.2)	7.61 (d, <i>J</i> = 16.0)	115.6	144.8
2'	6.30 (d, <i>J</i> = 15.8)	6.28 (d, <i>J</i> = 16.0)	167.0	115.9
3'	-	-	167.5	167.6
1''	4.91 (t, <i>J</i> = 7.0)	4.20	64.6	64.9
2''	1.66, m	1.70	29.4	29.0
3''	-	-	26.0	26.2
4''-25''	1.25, br s	1.27, br s	29.7	29.9
26''	-	-	31.9	32.1
27''	-	-	22.7	22.9
28''	0.88 (t, 7.0)	0.93 (t, 6.7)	14.1	14.3
3-OMe	3.93, s	3.90, s	55.9	56.2
4-OH	5.89, s	5.86, s	-	-

$^1\text{H}^*$  and  $^{13}\text{C}^*$  NMR data (Yenesew *et al.*, 2006)

## **4.2 Biological Activity Studies**

### **4.2.1 Preliminary bioassay analysis of crude extracts**

The crude extracts of the three plants were initially investigated for bioactivity against *Plasmodium falciparum*, *Anopheles gambiae* larvae and mature adults, some fungi: *Candida albicans*, *Aspergillus fumigatus*, *Aspergillus niger* and some bacteria: *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Streptococcus faecalis*, *Bacillus anthracis*, *Escherichia coli* and *Pseudomonas aeruginosa in vitro*. The plant parts which showed significant activities against any of the test organisms were subjected to chromatographic separation. The results are highlighted in **section 4.2.1.1**.

#### **4.2.1.1 *In vitro* Antiplasmodial activities of crude extracts from *Lonchocarpus eriocalyx*, *Alysicarpus ovalifolius* and *Erythrina abyssinica***

From *L. eriocalyx*, the MeOH extract showed high antiplasmodial activity ( $p \leq 0.05$ ) with  $IC_{50}$  values of **423.0** and **365.2  $\mu\text{g/mL}$**  against W2 and D6 strains of *P. falciparum*. Likewise, the activity of the DCM extract of *A. ovalifolius* was also high ( $p \leq 0.05$ ) with  $IC_{50}$  values of **234.0** and **482.0  $\mu\text{g/mL}$** . Similarly, the DCM extract of *E. abyssinica* showed the highest activity ( $p \leq 0.05$ ) with  $IC_{50}$  values of **165.1** and **215.1  $\mu\text{g/mL}$** . However, the activity of mefloquine which used as positive control was superior with  $IC_{50}$  values **16.1** and **22.30  $\mu\text{g/mL}$**  against the same clones, respectively. In the previous investigations, the MeOH extract of the root bark of *L. eriocalyx* showed antiplasmodial activity against these strains of *P. falciparum* (Tuwei, 2006). In another study, the EtOAc extract of the stem bark of *E. abyssinica* also showed antiplasmodial activity against the W2 and D6 strains of *P. falciparum* with  $IC_{50}$  values of 490 and 640  $\mu\text{g/mL}$  (Yeneses *et al.*, 2006, 2004). This study therefore concurs with what is reported for *L. eriocalyx* and

*E. abyssinica* with respect to antiplasmodial activities. However, activity of *A. ovalifolius* is being reported for the first time.

**Table 25: *In vitro* antiplasmodial activity (IC<sub>50</sub>) of crude extracts against D6 and W2 strains of *P. falciparum***

Plant	Test Sample	Clone		Mean Test Sample	CV%
		W2	D6		
		IC <sub>50</sub> (µg/mL)	IC <sub>50</sub> (µg/mL)		
<i>L. Eriocalyx</i> (Stem)	<i>n</i> -hexane	0.0	0.0	0.0	0.96
	EtOc	645.7	578.9	612.3	
	DCM	575.5	478.8	527.2	
	MeOH	<b>423.0</b>	<b>365.2</b>	394.1	
	Mean Clone	411.1	355.7		
	<b>LSD (p≤0.05)</b>	<b>0.06</b>		<b>0.02</b>	
<i>A. ovalifolius</i> (Root)	<i>n</i> -hexane	323.0	546.0	434.5	
	EtOc	320.0	496.0	408.0	
	DCM	<b>234.0</b>	482.0	358.0	
	MeOH	265.0	579.0	422.0	
	Mean Clone	285.5	525.8		
	<b>LSD (p≤0.05)</b>	<b>0.06</b>		<b>0.02</b>	
<i>E. abyssinica</i> (Leaves)	<i>n</i> -hexane	0.0	0.0	0.0	
	EtOc	427.5	468.8	448.2	
	DCM	<b>165.1</b>	<b>215.1</b>	190.1	
	MeOH	327.5	368.8	348.2	
	Mean Clone	230.0	263.2		
	<b>LSD (p≤0.05)</b>	<b>0.06</b>		<b>0.02</b>	
Mefloquine (1 µg/mL)		<b>16.1</b>	<b>22.3</b>		

#### 4.2.1.2 Larvicidal and Mosquitocidal activities of crude extracts from

##### *Lonchocarpus eriocalyx*, *Alysicarpus ovalifolius* and *Erythrina abyssinica*

The DCM extract of the stem bark of *L. eriocalyx* showed moderate larvicidal activity of **53.0±0.02%** mortality with LC<sub>50</sub> value of 423.56 µg/mL. MeOH extract was also moderately active against the adult mosquito with **59.4±0.06%** and a corresponding LC<sub>50</sub> value of **745.09 µg/mL**. This study concurs with a previous investigation in which the stem bark of this plant showed larvicidal and mosquitocidal activities against *Aedes aegypti* (Yeneses *et al.*, 2003a). The observed activity confirms the use of this plant by the Embu-Mbeere people as a mosquito repellent (Kareru *et al.*, 2007, Coastes, 2002). From *A. ovalifolius*, the DCM and MeOH extracts of the root bark were highly active against *A. gambiae* larvae with **87.70±0.01** and **77.80±0.01%** mortalities and corresponding IC<sub>50</sub> values of **9.86** and **45.64 µg/mL**, respectively. MeOH extract also showed high adulticidal activity with **88.5±0.01%** mortality and IC<sub>50</sub> value of **17.83 µg/mL**. The MeOH extract of the leaves of *E. abyssinica* showed moderate larvicidal and mosquitocidal activities with **65.1±0.40** and **65.5±0.14%** mortality and corresponding IC<sub>50</sub> values of **218.90** and **231.90 µg/mL** respectively. These results are in agreement with an earlier study in which the crude extracts of *E. abyssinica* showed larvicidal and mosquitocidal activities against *Aedes aegypti* (Irungu, 2012, Yenesew *et al.*, 2006, Yenesew *et al.*, 2003b). However, these activities were far much low compared to that of temephos (**99.9±0.01%**) which acted as a positive control. Details are contained in **Table 26**.



**Table 26: Larvicidal and mosquitocidal activities of crude extracts as % mortality and LC<sub>50</sub> values**

Plant	Test Samples	Larvicidal activity		Mosquitocidal activity	
		Mortality *(%)	LC <sub>50</sub> (µg/mL)	Mortality *(%)	LC <sub>50</sub> (µg/mL)
<i>L. eriocalyx</i> (Stem)	<i>n</i> -hexane	39.9±0.05	754.98	39.4±0.06	945.09
	DCM	<b>53.0±0.02</b>	423.56	23.2±0.02	788.59
	MeOH	29.9±0.05	654.98	<b>59.4±0.06</b>	745.09
<i>A. ovalifolius</i> (Root)	<i>n</i> -hexane	25.9±0.01	120.61	5.1±0.01	500.56
	EtOAc	57.8±0.01	95.64	05.1±0.01	435.17
	DCM	<b>87.7±0.01</b>	<b>9.86</b>	<b>88.5±0.01</b>	<b>17.83</b>
	MeOH	<b>77.8±0.01</b>	<b>45.64</b>	15.1±0.01	495.17
<i>E. abyssinica</i> (Leaves)	<i>n</i> -hexane	37.5±0.2	664.87	37.4±0.52	805.43
	DCM	47.5±0.20	564.87	47.4±0.52	605.43
	MeOH	<b>65.1±0.40</b>	<b>218.90</b>	<b>65.5±0.14</b>	<b>231.90</b>
	1% acetone	<b>1.0±0.34</b>	-	-	-
	Temephos	<b>99.9±0.01</b>	<b>0.5</b>	-	-
	Lambdacyhalothrin (1 µg/mL)	-	-	<b>98.02±0.1</b>	<b>0.01</b>

\* Values are means ± SD of three replicates recorded at a concentration of 125 µg/mL

(>75%: highly active; 50-74%: moderate; 25-49%: weak; <25%: inactive (Globade, *et al.*, 2002)

#### 4.2.1.3 *In vitro* Antifungal and Antibacterial tests

The *n*-hexane and DCM extracts of the stem bark of *L. eriocalyx* were inactive against *C. albicans*, *A. fumigatus* and *A. niger*, but, the MeOH extract showed mild activity against *A. niger* with inhibition zone of **8.5±0.4 mm**. The antifungal activity of the crude extracts of this plant is reported and this has been confirmed by this study (Abu Bakar *et al.*, 2007). The *n*-hexane, EtOAc and DCM extracts of the root bark of *A. ovalifolius* were also inactive against *C. albicans*, *A. fumigatus* and *A. niger*, however, the MeOH extract showed an intermediate activity with **11.2±0.1 mm** inhibition zone against *C. albicans*.

The *n*-hexane and MeOH extracts of *E. abyssinica* also showed intermediate activity against *C. albicans* with inhibition zone of **10.4±0.2** and **13.2±0.1 mm**, respectively.

The crude samples were also investigated for antibacterial activity, whereby the DCM extract of *L. eriocalyx* was weak against *B. anthracis* and *E. coli* with inhibition zones of **7.4±0.2** **7.9±0.1 mm**, respectively. From *A. ovalifolius*, the DCM and MeOH extracts showed intermediate activity against *S. aureus*, with inhibition zones of **15.2±0.1** **12.3±0.1 mm**. Similarly, the DCM extract also showed weak activity against *E. coli* and *K. pneumoniae* with inhibition zones of **10.8±0.1** **10.3±0.1 mm**, respectively. The activity of MeOH extract was also intermediate against *S. typhimurium* with inhibition zone of **13.3±0.1 mm**. The antimicrobial assay of *A. ovalifolius* is being reported for the first time. The DCM extract of *E. abyssinica* showed intermediate activity against *S. aureus*, *E. coli* and *K. pneumoniae* with inhibition zones of **15.3±0.1**, **10.8±0.1** and **10.3±0.1 mm**, respectively. Likewise, the MeOH extract also showed intermediate activity against *S. aureus* with inhibition zones of **11.3±0.1 mm**. In a previous study, the DCM extract of the stem bark of *E. abyssinica* was active against *S. aureus* and *Trichophyton mentagrophytes* with inhibition zones of **16** and **23 mm**, respectively (Irungu, 2012). It is also reported that the MeOH extract of this plant showed intermediate antifungal activity against *C. albicans* and *Cryptococcus neoformans* (Tapiwa *et al.*, 2016). Likewise, in a research study done on several *Erythrina* species in Uganda, the antibacterial activity was confirmed by positive results on tests against *S.aureus* and *B. subtilis* (Olila *et al.*, 2007).

The antimicrobial activity tests in this study were quite comparable to what is contained in literature about *E. abyssinica* (Irungu, 2012; Yenesew, 2006). Detailed information of these activities are captured in **Table 27** and **28**.

**Table 27: *In vitro* antifungal activities of crude extracts**

Test Samples		Diameter/zones of inhibition (mm)		
		<i>C. albicans</i>	<i>A. fumigatus</i>	<i>A. niger</i>
<i>L. eriocalyx</i> (Stem)	<i>n</i> -hexane	ND	ND	ND
	DCM	5.0±0.2	5.5±0.2	5.1±0.3
	MeOH	5.4±0.2	6.3±0.3	<b>8.5±0.4</b>
<i>A. ovalifolius</i> (Root)	<i>n</i> -hexane	5.3±0.1	ND	ND
	EtOAc	5.3±0.01	5.2±0.01	ND
	DCM	ND	5.3±0.01	5.2±0.01
	MeOH	<b>11.2±0.1</b>	7.4±0.1	5.8±0.2
<i>E. abyssinica</i> (Leaves)	<i>n</i> -Hexane	<b>10.4±0.2</b>	7.3±0.3	8.5±0.4
	EtOAc	5.3±0.01	5.2±0.01	ND
	DCM	5.3±0.01	5.2±0.01	ND
	MeOH	<b>13.2±0.1</b>	5.4±0.1	5.8±0.2
	<b>Flu (20 µg/mL)</b>	<b>17.3±0.2</b>	<b>19.5±0.1</b>	<b>15.8±0.3</b>

\* Values are means ± SD of three replicates recorded at a concentration of 1000 µg/mL

**Key:** *C. albicans* (HG 392), *A. fumigatus* (HG 420), *A. niger* (ATCC 90028),

Flu = Fluconazole, ND = Not Detected

**Activity scale: (> 17: Highly active; 11-16: intermediate; 7-10: weak; <6: resistant)**  
(Singh *et al.*, 2002, McChesney *et al.* 1991).

**Table 28: *In vitro* antibacterial activities of crude extracts**

Test Samples		Diameter/zones of inhibition (mm)						
		<i>S. aureus</i>	<i>S. faecalis</i>	<i>B. anthracis</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. typhimurium</i>	<i>P. aeruginosa</i>
<i>L. eriocalyx</i> (Stem)	<i>n</i> -hexane	ND						
	DCM	6.2±0.1	6.1±0.1	<b>7.4±0.2</b>	<b>7.9±0.1</b>	6.2±0.2	3.4±0.1	5.8±0.1
	MeOH	5.3±0.2	5.0±0.4	5.3±0.4	5.1±0.2	6.3±0.3	8.4±0.3	5.4±0.4
<i>A. ovalifolius</i> (Root)	<i>n</i> -hexane	7.2±0.01	5.5±0.01	ND	ND	5.6±0.1	ND	ND
	EtOAc	5.0±0.4	5.2±0.04	5.5±0.06	5.6±0.01	5.6±0.51	5.0±0.08	ND
	DCM	<b>15.2±0.1</b>	6.0±0.1	4.6±0.1	<b>10.8±0.1</b>	<b>10.3±0.1</b>	5.0±0.1	5.1±0.1
	MeOH	<b>12.3±0.1</b>	6.2±0.1	5.6±0.1	8.1±0.1	8.0±0.2	<b>13.3±0.1</b>	6.1±0.2
<i>E. abyssinica</i> (Leaves)	<i>n</i> -Hexane	5.2±0.01	5.5±0.01	5.6±0.01	5.2±0.0	5.6±0.01	ND	ND
	EtOAc	5.2±0.07	5.5±0.01	5.6±0.01	ND	5.0±0.01	ND	ND
	DCM	<b>15.3±0.1</b>	7.0±0.1	4.6±0.1	<b>10.8±0.1</b>	<b>10.3±0.1</b>	5.0±0.1	4.1±0.1
	MeOH	<b>11.3±0.1</b>	6.2±0.1	5.6±0.1	<b>8.1±0.1</b>	5.0±0.2	5.3±0.1	5.1±0.2
	Am (20 µg/mL)	<b>19.5±0.1</b>	<b>19.3±0.3</b>	<b>16.7±0.1</b>	<b>18.5±0.1</b>	<b>19.9±0.2</b>	<b>19.6±0.0</b>	<b>17.7±0.3</b>

**Key:** *S. aureus*, (ATCC 25922), *S. faecalis* (ATCC 25925), *K. pneumoniae* (ATCC 90028), *S. typhimurium* (ATCC 25927), *E. coli* (K 12), *P. aeruginosa* (ATCC 25923), *B. anthracis* (QST 713), Am = Amoxycillin, ND = Not Detected

\* Values are means ± SD of three replicates recorded at a concentration of 1000 µg/mL

**Activity scale: (> 17: Highly active; 11-16: intermediate; 7-10: weak; <6: resistant)**

**(Singh *et al.*, 2002, McChesney *et al.* 1991).**

## 4.2.2 Bioassay analysis of pure isolates

### 4.2.2.1 *In vitro* Antiplasmodial activities of pure isolates from *Lonchocarpus*

#### *ericalyx*, *Alysicarpus ovalifolius* and *Erythrina abyssinica*

The pure isolates obtained from the three plants were also investigated for bioactivity against *Plasmodium falciparum*, *Anopheles gambiae* larvae and mature adults, some fungi: *Candida albicans*, *Aspergillus fumigatus* and *Aspergillus niger* and some bacteria: *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Streptococcus faecalis*, *Bacillus anthracis*, *Escherichia coli* and *Pseudomonas aeruginosa in vitro*. These results are presented in the sections below;

From *L. ericalyx*, lupeol (**27**) showed the highest activity of IC<sub>50</sub> values of **104.4±0.4** and **109.9±0.4** µg/mL against W2 and D6 strains of *P. falciparum*, respectively. However, the activity of mefloquine which was used as the positive control was superior with IC<sub>50</sub> values **16.1±0.02** and **22.30±0.01** µg/mL against the same clones, respectively. This result are in agreement with a previous investigations in which lupeol (**27**) showed good antiplasmodial activity (Yenesew *et al.*, 2003a). The activity of β-sitosterol (**134**) against *P. falciparum* concurred with results from a previous study in which the IC<sub>50</sub> values of 424.21±3.8 and 564.31±0.2 µg/mL were obtained against W2 and D6 strains respectively (Tuwei, 2006). Koenidine (**146**) from *A. ovalifolius* was the most active with IC<sub>50</sub> values of **63.0±0.01** and **54.2±0.04** µg/mL against W2 and D6 clones. The activity of Erythrinasin A (**148**) from *E. abyssinica* was high and quite comparable to a previous study (Yenesew *et al.*, 1997). The activity of mefloquine was however much higher than that of the isolates. Details are as shown in **Table 29**.

**Table 29: *In vitro* antiplasmodial activity (IC<sub>50</sub>) of pure isolates against D6 and W2 strains of *P. falciparum***

Plant	Test Samples	IC <sub>50</sub> values* (µg/mL)	
		W2	D6
<i>L. eriocalyx</i>	Lupeol (27)	<b>104.4±0.4</b>	<b>109.9±0.4</b>
	Quercetin (65)	308.1±0.04	294.7±0.6
	Apigenin (68)	972.1±0.05	606.1±0.05
	Friedelin (133)	250.7±0.6	231.4±0.5
	β-sitosterol (134)	187.6±0.04	206.6±0.03
	Lupenone (135)	208.1±0.04	394.7±0.6
	β-sitosterol-3- <i>O</i> -glucoside (136)	579.3±0.3	559.9±0.5
	Chrysin (137)	998.1±0.05	606.1±0.05
	Morinhydrate (138)	369.9±0.04	481.9±0.8
	Quercetin 3 <i>O</i> -glucoside (139)	665.8±0033	453.1±0.05
	4', 5-dihydroxystilbene 3- <i>O</i> -glucoside (140)	909.1±0.04	546.1±0.15
	Rutin (141)	123.4±0.08	122.9±0.9
	<i>A. ovalifolius</i>	Plumbagin (142)	248.5±0.04
Orientin (143)		ND	ND
Mohanimbine (144)		130.0±0.01	279.0±0.01
Koenimbine (145)		161.4±0.02	176.7±0.01
Koenidine (146)		63.0±0.01	54.20±0.04
<i>E. abyssinica</i>	7-Hydroxy-4'-methoxy-3-prenylisoflavone (147)	148.2±0.30	126.3±0.50
	Erythinasinate A (148)	104.5±0.70	109.1±0.50
		161.08±0.10 <sup>b</sup>	
	<b>Mefloquine (1 µg/mL)</b>	<b>16.1±0.02</b>	<b>22.3±0.01</b>

<sup>b</sup>(Yenesew *et al.*, 1997)

\* Values are means ± SD of three replicates recorded at a concentration of 100 µg/mL

#### 4.2.2.2 Larvicidal and Mosquitocidal activities of isolates from *Lonchocarpus eriocalyx*, *Alysicarpus ovalifolius* and *Erythrina abyssinica*

The larvicidal activity of lupeol (**27**) and  $\beta$ -sitosterol (**134**) from *L. eriocalyx* were moderate with  $60.7 \pm 0.50$  and  $57.6 \pm 0.06$  % mortality and corresponding LC<sub>50</sub> values of 157.88 and 212.33  $\mu\text{g/mL}$ , respectively. Compound **27** also gave  $50.3 \pm 0.53\%$  knock down to the adult mosquito, while the larvicidal activity of  $\beta$ -sitosterol-3-*O*-glucoside (**136**) was also moderate. Mohaninbine (**144**) from *A. ovalifolius* showed the highest activity of  $82.3 \pm 0.01\%$  mortality with the lowest IC<sub>50</sub> value of  $5.56 \mu\text{g/mL}$ . The activity of compound **144** explains the observed activity of the DCM extract which was notably high. Larvicidal assay of this plant is being reported for the first time. Pure isolates from *E. abyssinica* were weak/inactive against larvicidal as well as mosquitocidal activities. The observed activities were quite low relative to the positive controls used in the study. Details are contained in **Table 30**.

**Table 30: Larvicidal and mosquitocidal activities of pure isolates as % mortality and LC<sub>50</sub> values**

Plant	Test Samples	Larvicidal activity		Mosquitocidal activity	
		Mortality *(%)	LC <sub>50</sub> (µg/mL)	Mortality *(%)	LC <sub>50</sub> (µg/mL)
<i>L. eriocalyx</i>	Lupeol (27)	<b>60.7±0.50</b>	<b>157.88</b>	<b>50.3±0.53</b>	<b>171.20</b>
	Quercetin (65)	24.4±0.06	876.22		788.06
				29.3±0.05	
	Apigenin (68)	ND	-	ND	-
	Friedelin (133)	47.6±0.50	456.99	17.8±0.54	891.23
	β-sitosterol (134)	<b>57.6±0.06</b>	<b>212.33</b>	26.1±0.03	587.43
	Lupenone (135)	35.8±0.44	512.56	05.5±0.32	734.09
	β-sitosterol-3- <i>O</i> -glucoside (136)	<b>55.5±0.43</b>	998.77	15.1±0.14	923.40
	Chrysin (137)	10.9±0.12	621.90	14.1±0.21	341.45
	Morinhydrate (138)	ND	436.99	17.8±0.50	991.23
	Quercetin 3 <i>O</i> -glucoside (139)	3.6±0.08	582.33	16.1±0.03	587.43
	4', 5-dihydroxystilbene 3- <i>O</i> -glucoside (140)	30.9±0.32	211.70	34.1±0.61	331.55
	Rutin (141)	55.8±0.34	312.56	25.5±0.32	734.09
<i>A. ovalifolius</i>	Plumbagin (142)				
	Orientin (143)	31.1±0.03	223.14	22.3±0.01	230.04
	Mohanimbine (144)	<b>82.3±0.01</b>	<b>5.56</b>	44.3±0.03	213.90
	Koenimbine (145)	53.6±0.01	41.76	42.6±0.02	260.46
	Koenidine (146)	34.7±0.04	87.54	30.7±0.03	289.07
<i>E. abyssinica</i>	7-Hydroxy-4'-methoxy-3-prenylisoflavone (147)	18.2±0.60	544.60	18.7±0.64	532.50
	Erythrinasinat A (148)	14.5±0.9	234.95	4.3±0.19	623.45
	1% acetone	<b>1.0±0.34</b>	-	-	-
	Temephos	<b>99.9±0.01</b>	<b>0.5</b>	-	-
	Lambdacyhalothrin (1 µg/mL)	-	-	<b>98.02±0.1</b>	<b>0.01</b>

\* Values are means ± SD of three replicates recorded at a concentration of 100 µg/mL (>75%: highly active; 50-74%: moderate; 25-49%: weak; <25%: inactive (Globade, *et al.*, 2002))



#### 4.2.2.3 *In vitro* Antimicrobial tests for pure isolates from *L. eriocalyx*,

##### *A. ovalifolius* and *E. abyssinica*

Lupeol (**27**) apigenin (**68**) and morinhydrate (**138**) isolated from *L. eriocalyx* showed mild activity against *C. albicans* with inhibition zones of **9.60±0.10**, **7.0±0.20** and **8.4±0.2 mm** respectively. From *A. ovalifolius*, koenimbine (**145**) showed intermediate activity against *C. albicans* with inhibition zone of **13.5±0.1 mm**. Plumbagin (**142**), orientin (**143**) and koenidine (**146**) showed a weak activity against the same fungus with inhibition zones of **9.1±0.1**, **8.4±0.2** and **8.0±0.2**, respectively. 7-hydroxy-4'-methoxy-3-prenylisoflavone (**147**) from *E. abyssinica* exhibited intermediate antifungal activity against *C. albicans* with a zone of inhibition of **14.5±0.1 mm**. The activity of compound **147** has confirmed the reported enhanced efficacy of prenylated flavonoids (Yenesew *et al.*, 2004). Fluconazole was used as the standard drug and had inhibition zone of **17.3±0.2**, **19.5±0.1** and **15.8±0.3** against *C. albicans*, *A. fumigatus* and *A.niger*, respectively.

For antibacterial activity tests,  $\beta$ -sitosterol (**134**) from *L. eriocalyx* showed intermediate activity against *S. typhimurium* with inhibition zone of **9.9±0.1 mm**. Rutin (**141**) also showed intermediate activity against all the Gram-negative bacteria used as test microorganisms with inhibition zones of **11.3±0.3**, **9.8±0.3** and **9.1±0.2 mm** against *S. typhimurium*, *P. aeuriginosa* and *K. pneumoniae*, respectively. The activities of other compounds obtained from this plant were low relative to the standard drug used. The antimicrobial activity of *L. eriocalyx* is reported hence this study confirms what is documented in literature (Abu Bakar *et al.*, 2007). From *A. ovalifolius*, only mohanimbine (**144**) exhibited intermediate activity against *C. albicans* with an inhibition zone of **13.8±0.1 mm**. The two compounds obtained from *E. abyssinica*, showed weak activity against the

test bacteria used in this study. This study is in agreement with what is contained in literature about the antibacterial activities of the genus *Erythrina* (Tuwei, 2006). Moreover, flavonoids from *Erythrina stricta* showed activity against *Mycobacterium tuberculosis* with an MIC value of 12.5 µg/mL (Thitima *et al.*, 2007). In a previous study, the root bark of *E. abyssinica* produced several pterocarpenes and flavonoids which exhibited antimicrobial activities (Yenesew *et al.*, 2003a). However, the activity of amoxyllin which was used as the positive control was much higher than the isolates.

**Table 31: *In vitro* antifungal activities of pure isolates**

Test Samples		Diameter/zones of inhibition (mm)		
		<i>C. albicans</i>	<i>A. fumigatus</i>	<i>A. niger</i>
<i>L. eriocalyx</i>	Lupeol (27)	<b>9.6±0.1</b>	4.4±0.2	5.1±0.2
	Quercetin (65)	5.0±0.2	<b>6.0±0.1</b>	5.1±0.2
	Apigenin (68)	<b>7.0±0.2</b>	6.5±0.2	6.1±0.3
	Friedelin (133)	5.0 ±0.3	5.2±0.2	5.3±0.7
	β-sitosterol (134)	5.2 ±0.1	ND	ND
	Lupenone (135)	5.0±0.2	5.5±0.2	5.1±0.3
	β-sitosterol-3- <i>O</i> -glucoside (136)	5.1±0.2	5.1±0.4	5.1±0.1
	Chrysin (137)	6.6±0.1	5.4±0.2	5.1±0.2
	Morin hydrate (138)	<b>8.4±0.2</b>	5.3±0.3	5.5±0.4
	Quercetin 3- <i>O</i> -glucoside (139)	ND	5.1±0.2	5.3±0.3
	4', 5-dihydroxystilbene 3- <i>O</i> -glucoside (140)	6.6±0.1	5.4±0.2	5.1±0.2
Rutin (141)	ND	ND	ND	
<i>A. ovalifolius</i>	Plumbagin (142)	<b>9.1±0.1</b>	<b>7.2±0.2</b>	6.5±0.1
	Orientin (143)	<b>8.4±0.2</b>	6.3±0.3	<b>7.5±0.4</b>
	Mohanimbine (144)	5.3±0.5	5.1±0.6	5.1±0.5
	Koenimbine (145)	<b>13.5±0.1</b>	6.7±0.1	6.5±0.1
	Koenidine (146)	<b>8.0±0.2</b>	6.5±0.2	7.1±0.3
<i>E. abyssinica</i>	7-Hydroxy-4'-methoxy-3-prenylisoflavone (147)	<b>14.5±0.1</b>	6.7±0.1	6.5±0.1
	Erythrasinate A (148)	5.0±0.2	5.5±0.2	6.1±0.3
	<b>Flu (20 µg/mL)</b>	<b>17.3±0.2</b>	<b>19.5±0.1</b>	<b>15.8±0.3</b>

**Key:** *C. albicans* (HG 392), *A. fumigatus* (HG 420), *A. niger* (ATCC 90028),  
Flu = Fluconazol, ND = Not Detected

\* Values are means ± SD of three replicates recorded at a concentration of 100 µg/mL

**Activity scale: (> 17: Highly active; 11-16: intermediate; 7-10: weak; <6: resistant)**  
(Singh *et al.*, 2002, McChesney *et al.* 1991).

**Table 32: *In vitro* antibacterial activities of pure isolates**

Test Samples		Diameter/zones of inhibition (mm)						
		<i>S. aureus</i>	<i>S. faecalis</i>	<i>B. anthracis</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. typhimurium</i>	<i>P. aeruginosa</i>
<i>L. eriocalyx</i>	Lupeol (27)	5.4±0.0	6.3±0.1	5.1±0.1	6.7±0.2	5.2±0.1	ND	5.6±0.2
	Quercetin (65)	5.4±0.0	5.3±0.1	5.1±0.1	6.8±0.1	5.3±0.1	ND	5.6±0.2
	Apigenin (68)	5.3±0.2	5.3±0.2	5.2±0.4	5.0±0.2	5.1±0.2	5.3±0.3	ND
	Friedelin (133)	5.2±0.2	5.6±0.1	5.2±0.1	5.1±0.2	5.7±0.2	5.0±0.1	5.2±0.2
	β-sitosterol (134)	5.1±0.1	5.1±0.2	5.0±0.2	5.0±0.3	5.6±0.3	<b>9.9±0.1</b>	5.1±0.3
	Lupenone (135)	5.3±0.2	5.1±0.4	5.3±0.4	5.1±0.2	5.3±0.3	7.4±0.3	5.4±0.4
	β-sitosterol-3- <i>O</i> -glucoside (136)	5.4±0.0	5.3±0.1	5.1±0.1	6.3±0.1	5.3±0.1	5.0±0.2	5.6±0.2
	Chrysin (137)	7.2±0.1	5.1±0.1	5.4±0.2	5.0±0.1	5.2±0.2	5.4±0.1	4.8±0.1
	Morin hydrate (138)	5.3±0.2	5.1±0.4	5.3±0.4	5.1±0.2	5.3±0.3	7.4±0.3	5.4±0.4
	Quercetin 3- <i>O</i> glucoside (139)	5.3±0.2	5.1±0.4	5.3±0.4	5.1±0.2	5.3±0.3	7.4±0.3	5.4±0.4
	4', 5-dihydroxystilbene 3- <i>O</i> -glucoside (140)	ND	ND	ND	ND	5.3±0.1	5.8±0.3	5.3±0.2
Rutin (141)	5.3±0.2	5.3±0.2	5.2±0.4	5.1±0.2	<b>9.1±0.2</b>	<b>11.3±0.3</b>	<b>9.8±0.3</b>	
<i>A. ovalifolius</i>	Plumbagin (142)	5.2±0.1	5.1±0.1	5.4±0.2	5.3±0.1	5.2±0.2	5.2±0.1	5.8±0.1
	Orientin (143)	5.1±0.1	5.7±0.1	5.2±0.2	ND	ND	5.3±0.3	ND
	Mohanimbine (144)	<b>13.8±0.1</b>	5.5±0.3	4.4±0.3	5.6±0.3	7.2±0.2	8.1±0.2	5.3±0.2
	Koenimbine (145)	6.4±0.0	6.3±0.1	5.1±0.1	5.8±0.1	5.3±0.1	5.0±0.2	6.6±0.2
	Koenidine (146)	5.2±0.1	6.1±0.1	7.4±0.2	5.9±0.1	6.2±0.2	9.4±0.1	8.8±0.1
<i>E. abyssinica</i>	7-Hydroxy-4'-methoxy-3-prenylisoflavone (147)	5.4±0.01	5.3±0.1	5.1±0.1	5.8±0.1	5.3±0.1	5.0±0.2	5.6±0.2
	Erythrasinate A (148)	5.3±0.2	5.3±0.2	5.2±0.4	5.1±0.2	5.7±0.2	5.6±0.3	ND
	Am (20 µg/mL)	<b>19.5±0.1</b>	<b>19.3±0.3</b>	<b>16.7±0.1</b>	<b>18.5±0.1</b>	<b>19.9±0.</b>	<b>19.6±0.0</b>	<b>17.7±0.3</b>

**Key:** *S. aureus*, (ATCC 25922), *S. faecalis* (ATCC 25925), *K. pneumoniae* (ATCC 90028), *S. typhimurium* (ATCC 25927), *E. coli* (K 12), *P. aeruginosa* (ATCC 25923), *B. anthracis* (QST 713), Am = Amoxycillin, ND = Not Detected

\* Values are means ± SD of three replicates recorded at a concentration of 100 µg/mL

**Activity scale: (> 17: Highly active; 11-16: intermediate; 7-10: weak; <6: resistant)**

**(Singh *et al.*, 2002, McChesney *et al.* 1991).**