## **CHAPTER 3**

### **Results and Discussions**

### 3.1 Isolated Compounds from Leaves of G. mckeaniana

The leaves of *G. mckeaniana* (0.7 kg) were extracted with acetone to provide acetone extract (95.6 g) as a dark brown gum. The extract was subjected to column chromatography to led the isolation of three new xanthones, mckeanianones A-C (**GML1, GML4** and **GML5**) and a new biflavone, mckeaniabiflavone (**GML6**) together with four known compounds, bannaxanthones I and E (**GML2** and **GML3**) (Na *et al.*, 2010 and Han *et al.*, 2008), amentoflavone (**GML7**) (Carbonezi *et al.*, 2007) and vomifoliol (**GML8**) (Hammani *et al.*, 2004). All compounds were identified using spectroscopic data and compared those data with previously publications.



#### 3.2 Isolated Compounds from the Stem Bark of G. mckeaniana

The air dried stem bark (2.50 kg) was extracted with acetone and then removal of solvent by reduced pressure afforded acetone extract (56.6 g) as a dark brown gum. An acetone extract was purified by chromatographic techniques to provide 11 known compounds, R-(-)-mellien (GMB1) (Efdi et al., 2007), cotoin (GMB2) (Gottlieb et al., 1958 and Mitasev et al., 2009), 2,3',4,6-tetrahydroxybenzophenone (GMB3) (Jamila et al., 2014 and Peters et al., 1998), pancixanthone A (GMB4) (Ito et al., 1996), assiguxanthones A and B (GMB5 and GMB7) (Ito et al., 1997), 1,3,7-trihydroxy-2-(3methylbut-2-enyl)-xanthone (GMB6) (Garcia Cortez et al., 1998), 1,3,5trihydroxyxanthone (GMB8) (Iinuma et al., 1997 and Helesbeux et al., 2004), 1,3,7trihydroxyxanthone (GMB9) (Zuo et al., 2014), norathyriol (GMB10) (Locksley et al., 1966 and Fromentin et al., 2012) and montixanthone (GMB11) (Ngouela et al., 2005). The isolated compounds were characterized by spectroscopic methods including UV, IR, 1D and 2D NMR and MS spectra. The known compounds were compared the <sup>1</sup>H/and/or <sup>13</sup>C NMR with literature reports.



#### 3.3 Structure Elucidation of Isolated Compounds from Leaves of G. mckeaniana

#### 3.3.1 Compound GML1

Compound **GML1** was obtained as a yellow solid, mp 187-189 °C. The molecular formula of  $C_{28}H_{30}O_7$  was deduced from its HRESI-TOFMS [found m/z

479.2063 (calcd for 479.2070) [M+H]<sup>+</sup>]. The UV spectrum displayed absorption bands at  $\lambda_{max}$  217, 295, 337 and 384 nm, suggesting the presence of a xanthone chromophore (Ito et al., 2003). The IR spectrum showed hydroxy and carbonyl stretching bands at 3418 and 1649 cm<sup>-1</sup>, respectively. The <sup>1</sup>H NMR spectrum (Table 2) showed resonances for a chelated hydroxy proton at  $\delta_{\rm H}$  13.97 (1H, s), a dimethylchromene unit [ $\delta_{\rm H}$  6.69  $(1H, d, J = 10.0 \text{ Hz}, \text{H}-11), 5.69 (1H, d, J = 10.0 \text{ Hz}, \text{H}-12) \text{ and } 1.47 (6H, s, CH_3-14),$ CH<sub>3</sub>-15)] and two sets of prenyl units [ $\delta_{\rm H}$  5.31 (1H, br t, J = 6.2 Hz, H-22), 4.17 (2H, d, J = 6.2 Hz, H-21), 1.86 (3H, s, CH<sub>3</sub>-25) and 1.64 (3H, s, CH<sub>3</sub>-24); and 5.21 (1H, br t, J = 6.8, H-17), 3.42 (2H, d, J = 6.8 Hz, H-16), 1.83 (3H, s, CH<sub>3</sub>-20) and 1.63 (3H, s, CH<sub>3</sub>-19)]. The chelated hydroxy group ( $\delta_{\rm H}$  13.97) was assigned as 1-OH because this signal showed <sup>2</sup>J and <sup>3</sup>J HMBC correlations with C-1 ( $\delta_{C}$  156.7) and C-9a ( $\delta_{C}$  104.1). The dimethylchromene ring was located at C-2/C-3 linkage due to H-11 ( $\delta_{\rm H}$  6.69) showed HMBC correlations to C-2 ( $\delta_{\rm C}$  104.7) and C-3 ( $\delta_{\rm C}$  157.7); and ether linkage at C-3. The first group of the prenyl units ( $\delta_{\rm H}$  5.21, 3.42, 1.83 and 1.63) was placed at C-4 ( $\delta_{\rm C}$ 106.9), according to the HMBC correlations of the methylene protons, H-16 ( $\delta_{\rm H}$  3.42), of this prenyl unit with C-3, C-4 ( $\delta_{\rm C}$  106.9) and C-4a ( $\delta_{\rm C}$  154.3). The other prenyl unit was located at C-8 ( $\delta_{\rm C}$  129.0) because of the HMBC correlations between methylene protons, H-21 ( $\delta_{\rm H}$  4.17) with C-7 ( $\delta_{\rm C}$  141.8), C-8 and C-8a ( $\delta_{\rm C}$  111.8). Thus, compound GML1 was named as mckeanianone A which was new compound.



Position	$\delta_{\rm C}$ , type	¢	$\delta_{\mathrm{H}}$ , ( <i>mult.</i> , J in H	Iz)	HMBC ( $^{1}H\rightarrow^{13}C$ )	Position	$\delta_{\rm C}$ , typ	be	$\delta_{\rm H}$ , ( <i>mult.</i> , J in Hz)	HMBC ( $^{1}H\rightarrow^{13}C$ )
1	156.7	С			1000	13	78.5	С		
2	104.7	С			\$/0	14	28.4	CH <sub>3</sub>	1.47 (s)	12, 13
3	157.7	С		11	§./	15	30.6	CH <sub>3</sub>	1.47 (s)	12, 13, 14
4	106.9	С		11	a Land	16	21.9	$CH_2$	3.42 ( <i>d</i> , 6.8)	3, 4, 4a, 17, 18
4a	154.3	С		1-8	24 3	17	123.3	CH	5.21 (br t, 6.8)	4, 16
5	$152.5^{*}$	С		改		18	131.4	С		
6	152.6*	С				19	25.9	CH <sub>3</sub>	1.63( <i>s</i> )	17, 18, 20
7	141.8	С			21	20	18.1	CH <sub>3</sub>	1.83 (s)	17, 18, 19
8	129.0	С			21	21	26.3	$CH_2$	4.17 ( <i>d</i> , 6.2)	7, 8, 8a, 22
8a	111.8	С			14	22	124.4	СН	5.31 ( <i>br t</i> , 6.2)	8, 21
9	183.5	С			C'AL.	23	131.5	С		
9a	104.1	С			AII	24	26.0	CH <sub>3</sub>	1.64 (s)	22, 23, 25
10a	153.6	С				25	18.3	CH <sub>3</sub>	1.86 ( <i>s</i> )	22, 23, 24
11	116.5	CH	6.69 ( <i>d</i> , 10.0)		2, 3, 13, 14	1-OH			13.97 (s)	1, 9a

Table 2 NMR (400 MHz) Data of GML1 in Acetone-d<sub>6</sub>

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28

#### 3.3.2 Compound GML2

Compound **GML2** was isolated as a yellow solid, mp 212-214 °C. The UV and IR spectra similar to that of **GML1** indicating the xanthone core skeleton. The <sup>1</sup>H NMR spectrum data of **GML2** (Table 3) also showed signal similar to **GML1** except for the losing of CH<sub>3</sub>-25 and additional of signals of an acetoxy group  $\delta_{\rm H}$  2.16 (3H, *s*), one oxymethylene group  $\delta_{\rm H}$  4.82 (2H, *s*) and one singlet aromatic proton  $\delta_{\rm H}$  6.87 (1H, *s*) were observed. The aromatic proton at  $\delta_{\rm H}$  6.87 was attributed at C-5 (& 101.3) due to this signal showed <sup>2</sup>J and <sup>3</sup>J HMBC correlation with C-4a (& 153.9), C-6 (& 152.5), C-8a (& 110.9) and C-9 (& 182.8). The oxymethylene group ( $\delta_{\rm H}$  4.82) was located at C-25 (& 64.6) which showed HMBC correlation with carbonyl of acetoxy group ( $\delta_{\rm C}$ 172.8), C-22 (& 128.8) and C-24 (& 20.8). Therefore, the acetoxyl group ( $\delta_{\rm H}$  2.16) was joined with methylene group ( $\delta_{\rm H}$  4.82) of the prenyl unit at C-25. The identification of **GML2** was bannaxanthone I (Na *et al.*, 2010).



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Position	$\delta_{\rm C}$ , type	e	$\delta_{\rm H}$ , ( <i>mult.</i> , J in Hz)	HMBC ( $^{1}H\rightarrow^{13}C$ )	Position	δ <sub>C</sub> , ty <sub>l</sub>	be	$\delta_{\rm H}$ , ( <i>mult.</i> , J in Hz)	HMBC ( $^{1}H\rightarrow^{13}C$ )
1	157.2	С			14	28.2	CH <sub>3</sub>	1.46 ( <i>s</i> )	12, 13
2	103.7	С			15	28.2	CH <sub>3</sub>	1.46 (s)	12, 13
3	155.8	С			16	21.3	$CH_2$	3.43 ( <i>d</i> , 7.2)	4, 17
4	106.5	С		- Com	17	122.4	СН	5.21 ( <i>t</i> , 7.2)	18, 19
4a	153.9	С	\$		18	129.7	С		
5	101.3	CH	6.87 (s)	4a, 6, 8a, 9	19	25.7	CH <sub>3</sub>	1.67 (s)	4, 17, 18, 20
6	152.2	С		$\alpha$	20	18.0	CH <sub>3</sub>	1.74 (s)	4, 17, 18, 19
7	139.1	С		H L	21	25.8	CH <sub>2</sub>	4.32 ( <i>d</i> , 6.4)	7, 8, 8a, 22
8	126.4	С		E.	22	128.8	CH	5.52 ( <i>t</i> , 6.4)	8, 24, 25
8a	110.9	С		120	23	131.3	С		
9	182.8	С		MAT	24 25	20.8	CH <sub>3</sub>	1.73 (s)	22, 25
9a	104.2	С		In	25	64.6	$CH_2$	4.82 (s)	22, 24, CO(Ac)
10a	153.7	С			COCH <sub>3</sub>	172.8	С		
11	116.1	CH	6.71 ( <i>d</i> , 10.0)	1, 3, 9a, 13, 14, 15	CO <u>CH</u> <sub>3</sub>	21.1	$CH_3$	2.16 (s)	25, CO(Ac)
12	127.0	CH	5.58 ( <i>d</i> , 10.0)	2, 13	1-OH	00	υu	13.58 (s)	
13	77.7	С	Copy	right <sup>©</sup> by C	hiang Mai	Uni	vers	sity	

 Table 3 NMR (400 MHz) Data of GML2 in CDCl3

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30

# 3.3.3 Compound GML3

Compound **GML3** was obtained as a yellow viscous oil. Its UV, IR and <sup>1</sup>H NMR spectrum data of **GML3** (Table 4) were closely to those of **GMB2** except for the missing acetoxy group as well as the high field shift of oxymethylene at H<sub>2</sub>-25 were observed in the <sup>1</sup>H NMR spectrum. This information indicated that acetoxy group at C-25 were replaced by hygroxy group. Therefore, compound **GML3** was identified as bannaxanthone E (Han *et al.*, 2008).



**Table 4** <sup>1</sup>H NMR (400 MHz) data of **GML3** in CDCl<sub>3</sub> and Bannaxanthone E in Acetone-*d*<sub>6</sub>

Desition	GML3	Bannaxanthone E		
Position	$\delta_{\rm H}$ , ( <i>mult.</i> , J in Hz)	(z) $\delta_{\rm H}$ , ( <i>mult.</i> , J in Hz		
5	6.77 ( <i>s</i> )	6.88 (s)		
11	6.72 ( <i>d</i> , 10.0)	6.69 ( <i>d</i> , 10.0)		
12 <b>8</b> 0	5.56 ( <i>d</i> , 10.0)	5.69 ( <i>d</i> , 10.0)		
14	1.44 (s)	1.48 (s)		
15	1.44 (s)	1.48 (s)		
16	3.39 ( <i>d</i> , 7.4)	3.42 ( <i>d</i> , 7.4)		
17	5.18 ( <i>t</i> , 7.4)	5.22 ( <i>t</i> , 7.4)		
19	1.64 (s)	1.65 (s)		
20	1.83 (s)	1.87 (s)		
21	4.19 ( <i>d</i> , 7.6)	4.25 ( <i>d</i> , 7.6)		
22	5.62 ( <i>d</i> , 7.6)	5.56 ( <i>d</i> , 7.6)		
24	1.76 ( <i>s</i> )	1.74 (s)		
25	4.28 (s)	4.34 (s)		
1-OH	13.73 (s)	13.87 (s)		

#### 3.3.4 Compound GML4

Compound GML4 was obtained as a yellow viscous oil. The molecular formula was determined as  $C_{28}H_{30}O_8$  by HRESI-TOFMS [found m/z 517.1839 (calcd for 517.1838)  $[M+Na]^+$ ]. The UV and IR spectra showed absorption bands similar to those of compound GML3. The main difference were found that GML4 showed an additional oxygenated prenyl unit [ $\delta_{\rm H}$  5.40 (1H, t, J = 8.0 Hz, H-17), 4.30 (2H, s, H-20), 3.60 (2H, d, J = 8.0 Hz, H-16), 1.73 (3H, s, CH<sub>3</sub>-19)] instead of prenyl group and the single aromatic signal ( $\delta_{\rm H}$  6.25) was shift to higher field in <sup>1</sup>H NMR spectrum. The <sup>1</sup>H NMR spectrum (Table 5) displayed resonances for a chelated hydroxy proton ( $\delta_{\rm H}$  13.78, br s), a dimethylchromene ring [ $\delta_{\rm H}$  6.69 (1H, d, J = 10.0 Hz, H-11), 5.54 (1H, d, J = 10.0 Hz, H-12) and 1.44 (6H, s, CH<sub>3</sub>-14, CH<sub>3</sub>-15)] and a set of oxygenated prenyl units and 5.59 (1H, t, J = 7.8 Hz, H-22), 4.26 (2H, s, H-25), 4.13 (2H, d, J = 7.8 Hz, H-21) and 1.73 (3H, s, CH<sub>3</sub>-24)]. The aromatic proton resonating at  $\delta_{\rm H}$  6.25 was assigned to H-4 due to its correlations with the <sup>13</sup>C NMR resonances for C-2 ( $\delta_{\rm C}$  104.3), C-3( $\delta_{\rm C}$  159.7), C-4a ( $\delta_{\rm C}$  156.3) and C-9a ( $\delta_{\rm C}$  103.8), in the HMBC spectrum. The oxygenated prenyl unit was placed on C-5 due to H-16 ( $\delta_{\rm H}$  3.60) showed cross peaks with the resonances of the aromatic carbons C-5 (& 112.2), C-6 (& 151.0) and C-10a (& 149.7). The geometry of two isoprenyl moieties were assigned by NOESY experiment. The H-16  $(\delta_{\rm H} 3.60)$  showed a cross peak with H-20  $(\delta_{\rm H} 4.30)$  and H-22  $(\delta_{\rm H} 5.59)$  with H-24  $(\delta_{\rm H}$ 1.73) indicating the geometry as  $Z-\Delta^{17,18}$  and  $Z-\Delta^{22,23}$ . Thus, compound **GML4** was identified as mckeanianone B.



Position	$\delta_{\rm C}$ , type		$\delta_{\rm H}$ , ( <i>mult.</i> , <i>J</i> in Hz)	HMBC ( $^{1}H\rightarrow^{13}C$ )
1	157.7	С		
2	104.3	С		
3	159.7	С		
4	94.1	CH	6.25 (s)	2, 3, 4a, 9, 9a
4a	156.3	С		
5	112.2	С		
6	151.0	Co	181816	
7	139.1	С	00	UD.
8	124.2	С	Dille -	3.21
8a	111.3	C		13
9	182.8	С	$\langle 0 \rangle$	1131
9a	103.8	С	Contraction of the second seco	~   .
10a 🗳	149.7	С	1 = m	-582
11	115.8	СН	6.69 ( <i>d</i> , 10.0)	1, 2, 3, 13, 14, 15
12	127.2	СН	5.54 ( <i>d</i> , 10.0)	2, 13, 14, 15
13	78.0	С	MACA	8
14	28.4	CH <sub>3</sub>	1.44 (s)	12, 13, 15
15	28.4	CH <sub>3</sub>	1.44 (s)	12, 13, 14
16	22.4	CH <sub>2</sub>	3.60 ( <i>d</i> , 8.0)	5, 6, 10a
17	125.4	CH	5.40 ( <i>t</i> , 8.0)	19, 20
18	134.7	С		
19	22.6	CH <sub>3</sub>	1.73 (s)	17, 18, 20
20	62.1	CH <sub>2</sub>	4.30 (s)	17, 18, 19
21.00	26.1	CH <sub>2</sub>	4.13 ( <i>d</i> , 7.8)	7, 8, 8a, 22, 23
22	127.5	CH	5.59 ( <i>t</i> , 7.8)	8, 24, 25
23	133.7	С		sciveu
24	22.9	CH <sub>3</sub>	1.73 (s)	22, 23, 25
25	62.3	$\mathrm{CH}_2$	4.26 (s)	22, 23, 24
1-OH			13.78 ( <i>br s</i> )	

Table 5 NMR (400 MHz) Data of GML4 in CDCl<sub>3</sub>

#### 3.3.5 Compound GML5

Compound **GML5**, a yellow viscous oil, gave a  $[M+Na]^+$  ion at m/z 559.1945 in the HRESI-TOFMS which corresponded to the molecular formula  $C_{30}H_{32}O_9$  (calcd for  $C_{30}H_{32}O_9Na$ , m/z 559.1944). The UV, IR, <sup>1</sup>H and <sup>13</sup>C NMR spectra were very similar to those of compound **GML4**, except the IR spectrum of compound **GML5** showed an additional band at 1736 cm<sup>-1</sup> for an ester carbonyl functional group in IR spectrum, while the <sup>1</sup>H NMR spectrum showed additional resonance of an acetoxy group at  $\delta_H 2.12$  (3H, s) (Table 6). The location of the acetoxy group at C-20 ( $\delta_C$  63.6) was confirmed by <sup>3</sup>J HMBC correlation of H<sub>2</sub>-20 ( $\delta_H$  4.87) and an acetoxy carbonyl ( $\delta_C$  171.7). Therefore, compound **GML5** was identified as mckeanianone C.



Table 6 NMR (400 MHz) Data of GML5 in CDCl<sub>3</sub>

Position	$\delta_{\rm C}$ , type		$\delta_{\rm H}$ , ( <i>mult.</i> , J in Hz) HMBC ( <sup>1</sup> H $\rightarrow$ <sup>13</sup> C)		
1	158.0	С			
2	104.5	С	อธิกลอดัสเซียงใหม		
300	159.7	С	าวและเสอเอออเมท		
4 opvi	94.2	CH	6.31 ( <i>s</i> ) 2, 3, 4a, 9a		
4a	156.3	С	hts rosorvod		
5	112.2	С	ints reserved		
6	151.3	С			
7	139.4	С			
8	124.0	С			
8a	111.3	С			
9	182.9	С			

Table 6 (Continued)

P	osition	$\delta_{\rm C}$ , typ	e	$\delta_{\rm H}$ , ( <i>mult.</i> , J in Hz)	HMBC ( $^{1}H\rightarrow^{13}C$ )
98	a	103.9	С		
10	Da	150.0	С		
1	1	115.9	CH	6.73 ( <i>d</i> , 10.0)	1, 2, 3, 13
12	2	127.2	CH	5.56 ( <i>d</i> , 10.0)	2, 13, 14, 15
13	3	78.0	С		
14	4	28.5	CH <sub>3</sub>	1.47 (s)	12, 13, 15
1:	5	28.5	CH <sub>3</sub>	1.47 (s)	12, 13, 14
10	5	22.4	$CH_2$	3.67 ( <i>d</i> , 7.4)	5, 6, 10a, 17, 18
1′	7	127.5	СН	5.52 ( <i>t</i> , 7.4)	19, 20
18	8 // 8	130.8	С		131
19	9/6	21.5	$CH_3$	1.73 (s)	17, 18, 20
20	C	63.6	$CH_2$	4.87 (s)	17, 18, 19, <u>CO</u> CH <sub>3</sub>
2	1	26.5	CH <sub>2</sub>	4.21 ( <i>d</i> , 8.0)	7, 8, 8a, 22, 23
22	2 500	128.5	CH	5.73 ( <i>t</i> , 8.0)	24, 25
23	3	132.9	С	N & J	A
24	4	23.4	$CH_3$	1.79 (s)	22, 23, 25
2	5	63.2	$CH_2$	4.37 (s)	22, 23, 24
<u>C</u>	<u>O</u> CH₃	171.7	С	6620	511
С	О <u>СН</u> 3	21.2	$CH_3$	2.12 (s)	COCH <sub>3</sub>
1.	-OH			13.80 (br s)	

# 3.3.6 Compound GML6 by Chiang Mai University

Compound **GML6** was obtained as a yellow solid (237-239 °C). The molecular formula,  $C_{31}H_{20}O_{10}$ , was deduced from the HRESI-TOFMS which show pseudomolecular ion at m/z 553.1155 [M+H]<sup>+</sup> (calcd for 553.1135). The UV spectrum of compound **GML6** showed maximum absorption bands at  $\lambda_{max}$  218, 269 and 331 nm while the IR spectrum showed absorption bands for hydroxy and conjugated carbonyl groups at 3444 and 1650 cm<sup>-1</sup>, respectively. The <sup>1</sup>H and <sup>13</sup>C NMR spectra data (Table 7) as well as the assistant of HMBC correlations, this compound was established as biflavone skeleton. The first set of biflavone contained a chelated hydroxyl proton at  $\delta_{H}$  12.97 (1H, *br s*, 5-OH), *meta*-couple aromatic proton on ring A at  $\delta_{H}$  6.57 and 6.33

(each 1H, *br s*, H-8 and H-6) and set of 1,4-disubstituent aromatic ring at  $\delta_{\rm H}$  7.49 and 6.76 (each 2H, *d*, *J* = 8.4 Hz, H-2', H-6' and H-3', H-5'). The other unit showed signals a hydroxyl chelated proton at  $\delta_{\rm H}$  13.09 (1H, *br s*, 5"-OH), 1,4-disubstituent aromatic ring at  $\delta_{\rm H}$  7.74 and 6.97 (each 2H, *d*, *J* = 8.4 Hz, H-2''', H-6''' and H-3''', H-5'''), an olefinic proton  $\delta_{\rm H}$  6.63 (1H, *s*, H-3"), a singlet aromatic proton at  $\delta_{\rm H}$  6.33 (1H, *br s*, H-6") and a methoxy proton at  $\delta_{\rm H}$  3.82 (3H, *s*, 7"-OCH<sub>3</sub>). The HMBC correlations of H-6" with C-3 ( $\delta_{\rm C}$  111.2) and C-8" ( $\delta_{\rm C}$  100.5) indicating the linkage of two flavone units at C-3 and C-8". The identification of **GML6** was mckeaniabiflavone, which was first report here.



Table 7 NMR (400 MHz) Data of GML6 in Acetone-d<sub>6</sub>

	1 1 2			
Position	δ <sub>C</sub> , typ	e	$\delta_{\rm H}$ , ( <i>mult.</i> , <i>J</i> in Hz)	HMBC ( $^{1}H\rightarrow^{13}C$ )
2	165.1	C	1336	
3	111.2	C	Mr.	-oSV
4	182.1	С	AI UNIV	FU
5	163.1	С		
6	99.8	CH	6.33 ( <i>br</i> s)	7, 8
7 80	165.2	С	ทาวทยาล	สยเชยงเทม
8 Cor	94.7	CH	6.57 ( <i>br s</i> )	4, 6, 7, 9, 10
9	158.9	С		
10	104.7	С	gnts r	eservea
1′	124.8	С		
2'	130.9	CH	7.49 ( <i>d</i> , 8.4)	2, 3', 4'
3'	116.1	СН	6.76 ( <i>d</i> , 8.4)	2, 1', 2', 4'
4′	160.8	С		
5'	116.1	СН	6.76 ( <i>d</i> , 8.4)	2, 1', 2', 4'
6′	130.9	CH	7.49 ( <i>d</i> , 8.4)	2, 3', 4'

Table 7 (Continued)

Position	δ <sub>C</sub> , typ	e	$\delta_{\rm H}$ , ( <i>mult.</i> , J in Hz)	HMBC ( <sup>1</sup> H $\rightarrow$ <sup>13</sup> C)
2"	164.7	С		
3″	104.5	CH	6.63 ( <i>s</i> )	2", 4", 10", 1'''
4″	183.3	С		
5″	163.0	С		
6″	99.9	CH	6.33 ( <i>br s</i> )	3, 4", 5", 7", 8", 10"
7″	163.5	С		
8″	100.5	C	กมยนลิ	
9″	156.4	C	dimerich	2/5
10″	105.6	С	000	- 4 an
1'''	124.2	C		1.51
2'''	128.8	СН	7.74 ( <i>d</i> , 8.4)	3''', 4'''
3'''	115.4	CH	6.97 ( <i>d</i> , 8.4)	1''', 4'''
4'''	163.6	С	4-20	225
5'''	115.4	СН	6.97 ( <i>d</i> , 8.4)	1''', 4'''
6'''	128.8	СН	7.74 ( <i>d</i> , 8.4)	4''', 5'''
7"-OCH <sub>3</sub>	55.9	$CH_3$	3.82 (s)	7"
5-OH	12	$\langle \cdot \rangle$	12.97 (br s)	5, 6, 10
5″-OH	11.	12	13.09 ( <i>br s</i> )	5", 6", 10"
		6	MAI UNIVE	SI'

3.4.7 Compound GML7

Compound **GML7** was obtained as yellow solid, mp. 273-275 °C. Its UV data exhibited absorption bands at  $\lambda_{max}$  217, 269 and 334 nm. IR spectrum showed absorption band at 3442 and 1650 cm<sup>-1</sup> for the vibration of hydroxy and carbonyl functionalities. The <sup>1</sup>H and <sup>13</sup>C NMR spectra data of **GML7** (Table 8) showed the assistant of HMBC correlations, this compound was established as biflavone skeleton. The first set of biflavone contained a chelated hydroxyl proton at  $\delta_{\rm H}$  13.17 (1H, *s*, 5-OH), *meta*-couple aromatic proton on ring A at  $\delta_{\rm H}$  6.52 and 6.24 (each 1H, *br s*, H-8 and H-6), an olefinic proton at  $\delta_{\rm H}$  6.73 (1H, *s*, H-3) and set of 1,2,4-trisubstituent aromatic ring at  $\delta_{\rm H}$  8.13 (1H, *br d*, *J* = 8.4 Hz, H-2'), 8.03 (1H, *br d*, *J* = 8.4 Hz, H-6') and 7.25 (1H, *d*, *J* = 8.4 Hz, H-5'). The other unit showed signals a hydroxyl chelated proton at  $\delta_{\rm H}$  13.00 (1H, *s*, 5"-OH), 1,4-disubstituent aromatic ring at  $\delta_{\rm H}$  7.66 and 6.83 (each 2H,

*d*, J = 8.2 Hz, H-2<sup>'''</sup>, H-6<sup>'''</sup> and H-3<sup>'''</sup>, H-5<sup>'''</sup>), an olefinic proton  $\delta_{\rm H} 6.66$  (1H, *s*, H-3<sup>''</sup>) and a singlet aromatic proton at  $\delta_{\rm H} 6.45$  (1H, *s*, H-6<sup>''</sup>). The HMBC correlations of H-6<sup>''</sup> with C-3' ( $\delta_{\rm C} 120.9$ ) and C-8<sup>''</sup> ( $\delta_{\rm C} 104.5$ ) indicating the linkage of two flavone units at C-3' and C-8<sup>''</sup>. Thus, compound **GML7** was assigned as amentoflavone (Carbonezi *et al.*, 2007).



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Position	$\delta_{\rm C}$ , type	<b>)</b>	$\delta_{\rm H}$ , ( <i>mult.</i> , J in Hz)	HMBC $(^{1}\text{H} \rightarrow ^{13}\text{C})$	Position	$\delta_{\rm C}$ , type	$\delta_{\rm H}$ , ( <i>mult.</i> , J in Hz)	HMBC $(^{1}\text{H}\rightarrow^{13}\text{C})$
2	165.2	С	1/2	11/0	2"	165.1	С	
3	104.4	СН	6.73 ( <i>s</i> )	2, 4, 1'	3"	103.9	CH 6.66 ( <i>s</i> )	2", 4", 1'''
4	183.0	С	12.	1/	4"	183.4	C	
5	163.1	С	1 10/	1 Com	5″	162.6	C	
6	99.7	CH	6.24 ( <i>br s</i> )	5, 7, 8, 10	6"	99.6	CH 6.45 (s)	3', 5", 6", 7", 10"
7	164.8	С	了。	S.	7"	156.2	C C	
8	94.8	CH	6.52 ( <i>br s</i> )	6, 7, 9, 10	8″	104.5	C	
9	158.9	С	1 E		9"	162.2	C	
10	105.5	С			10″	105.5	C	
1′	123.4	С		h	1'''	123.3	C	
2'	132.6	СН	8.13 ( <i>br s</i> )	2, 3, 4', 6'	2'''	129.2	CH 7.66 ( <i>d</i> , 8.2)	2", 4''', 6'''
3'	120.9	С		AII	3'''	116.7	CH 6.83 ( <i>d</i> , 8.2)	1''', 4''', 5'''
4′	160.3	С			4'''	161.8	С	
5'	117.5	СН	7.25 ( <i>d</i> , 8.4)	1', 3', 4'	5'''	116.7	CH 6.83 ( <i>d</i> , 8.2)	1''', 3''', 4'''
6′	128.8	CH	8.03 (br d, 8.4)	2, 2', 4'	6'''	129.2	CH 7.66 ( <i>d</i> , 8.2)	2", 2"', 4""
5-OH			13.17 (s)	5, 6, 10	hiang	5″-OH	13.00( <i>s</i> )	5", 6", 10"

**Table 8** NMR (400 MHz) data of **GML7** in acetone-d6

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#### 3.4.8 Compound GML8

Compound GML8 was isolated as yellow viscous oil. The UV spectrum showed the maximum absorption bands at 214 and 238 nm indicating for  $\alpha,\beta$ unsaturated carbonyl in the molecule. Its IR spectrum exhibited absorption bands for hydroxy and conjugated carbonyl group at 3444 and 1650 cm<sup>-1</sup>, respectively. The <sup>1</sup>H NMR spectrum of **GML8** (Table 9) displayed an olefinic proton  $\delta_{\rm H}$  5.86 (1H, br s, H-5), a (*E*)-but-3-en-2-ol unit [ $\delta_{\rm H}$  5.83 (1H, br d, J = 15.6 Hz, H-8, 5.79 (1H, d, J = 15.6 Hz, H-7), 4.39 (1H, m, H-9) and 1.28 (3H, d, J = 6.4 Hz, CH<sub>3</sub>-10)], one methylene group [( $\delta_{\rm H}$  2.44 and 2.22 (each 1H, d, J = 17.0 Hz, H-3)] and three methyl groups [ $\delta_{\rm H}$ 1.89 (3H, br s, CH<sub>3</sub>-13), 1.06 and 0.99 (each 3H, s, CH<sub>3</sub>-11 and 12). The <sup>13</sup>C, DPET135 and DEPT90 spectra showed 13 signals corresponding to an  $\alpha,\beta$ -unsaturated carbonyl carbon ( $\delta_{\rm C}$  198.3), an oxygenated quaternary carbon ( $\delta_{\rm C}$  79.2), an aliphatic quaternary carbon ( $\delta_{\rm C}$  41.3), a methine carbon ( $\delta_{\rm C}$  68.0), a disubstituent double bond ( $\delta_{\rm C}$  135.9 and 129.1), a trisubstituent double bond (& 163.2 and 126.9), a methylene carbon (& 50.1), and four methyl ( $\delta_c$  24.2, 23.9, 23.0 and 19.0). The methylene group ( $\delta_H$  2.44 and 2.22) at C-3 ( $\delta_{\rm C}$  50.1) connected with carbonyl group by showed <sup>2</sup>J and <sup>3</sup>J HMBC correlation with C-1 ( $\delta_{\rm C}$  79.2), C-2 ( $\delta_{\rm C}$  41.3) and C-4 ( $\delta_{\rm C}$  198.3). An olefinic proton ( $\delta_{\rm H}$  5.86) at C-5 ( $\delta_c$  126.9) was linked with other side of carbonyl group due to HMBC correlations with C-3 and methyl group ( $\delta_{\rm C}$  19.1) and this methyl group also located at C-6 ( $\delta_{\rm C}$ 163.2) by HMBC data. The unit of (E)-but-3-en-2-ol was confirmed by the <sup>1</sup>H-<sup>1</sup>H COSY spectrum data. This unit was placed at quaternary C-1 ( $\delta_c$  79.1) from HMBC correlation and connected with hydroxyl group by the chemical shift of carbon. For absolute configuration of **GML8** found specific rotation value of at +62 and similar to that of vomifoliol Thus, the identification of GML8 was vomifoliol (Hammami et al., 2004).



Table 9 NMR	(400 MHz)	data of	GML8 i	n CDCl3
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Position	$\delta_{\rm C}$ , type	;	$\delta_{\rm H}, (mult., J \text{ in Hz})$	HMBC ( $^{1}H \rightarrow ^{13}C$ )
1	79.2	С		
2	41.3	С		
3	50.1	CH <sub>2</sub>	2.44 ( <i>d</i> , 17.0) 2.22 ( <i>d</i> , 17.0)	1, 2, 4, 5
4	198.3	С		
5	126.9	CH	5.86 ( <i>br s</i> )	3, 13
6	163.2	C	<b>ง</b> มยนต	~ /
7	129.1	CH	5.79 ( <i>d</i> , 15.6)	1, 6, 8, 9
8	135.9	CH	5.83 (br d, 15.6)	1, 7, 9, 10
9	68.0	СН	4.39 ( <i>m</i> )	7, 8, 10
10	23.9	CH <sub>3</sub>	1.28 ( <i>d</i> , 6.4)	8,9
11	23.0	CH <sub>3</sub>	1.06 (s)	1, 2, 3, 12
12	24.2	CH <sub>3</sub>	0.99 (s)	1, 2, 3, 4, 11
13	19.0	CH <sub>3</sub>	1.89 (s)	1, 5, 6

3.4 Structure Elucidation of Isolated Compounds from the Stem Bark of

G. mckeaniana

#### 3.4.1 Compound GMB1

Compound **GMB1** was obtained as a yellow powder. The UV spectrum showed maximum absorption bands at  $\lambda_{max}$  217, 246 and 313 nm. The IR spectrum displayed the absorption of hydroxy and carbonyl ester at 3387 and 1675 cm<sup>-1</sup>. The <sup>1</sup>H NMR data of **GMB1** (Table 10) showed resonating for chelated hydroxy proton at  $\delta_{\rm H}$ 11.03 (1H, *s*, 8-OH), a set of 1,2,3-trisubstituent aromatic protons [ $\delta_{\rm H}$  7.41 (1H, *t*, *J* = 8.0 Hz, H-6), 6.89 (1H, *d*, *J* = 8.0 Hz, H-5) and 6.69 (1H, *d*, *J* = 8.0 Hz, H-7)], one methine ( $\delta_{\rm H}$  4.73, 1H, *m*, H-3), one methylene ( $\delta_{\rm H}$  2.93, 2H, *d*, *J* = 7.2 Hz, H-4) and one methyl group ( $\delta_{\rm H}$  1.53, 3H, *d*, *J* = 6.3 Hz, CH<sub>3</sub>-11). The hydroxy group at  $\delta_{\rm H}$  11.03 has hydrogen bonding by chelated with carbonyl ester group. A triplet aromatic proton ( $\delta_{\rm H}$ 7.41) was attributed to position H-6 of 1,2,3-trisubstituent aromatic ring which couple with H-5 and H-7, respectively. The rest protons ( $\delta_{\rm H}$  4.73, 2.93 and 1.53) showed characteristic of lactone ring according to the oxymethine proton ( $\delta_{\rm H}$  4.73) displayed the lowest field chemical shift due to the inductive effect of ester group. This proton was assigned to position H-3 and coupled either methylene and methyl groups by its splitting pattern and coupling constant values. The aromatic ring was fused with lactone ring between methylene and carbonyl ester group. Compound **GMB1** showed specific rotation value at -29 similar to that of *R*-(-)-mellien indicating that relative configuration at C-3 of **GMB1** assigned as *R*-configuration. The compound **GMB1** was identified as *R*-(-)-mellien. (Efdi *et al.*, 2007)

OH O

Table 10<sup>1</sup>H NMR (400 MHz) Data of GMB1 and *R*-mellien in CDCl<sub>3</sub> *R*-mellien<sup>\*</sup> GMB1 Position  $\delta_{\rm H}$ , (*mult.*, J in Hz)  $\delta_{\rm H}$ , (mult., J in Hz) 4.73 (m) 3 4.73 (m) 2.93(d, 7.4)4 2.93(d, 7.2)5 6.89 (d, 8.0) 6.89(d, 8.0)6 7.41 (t, 8.0) 7.41 (t, 8.0) 6.69 (*d*, 8.0) 6.69 (*d*, 8.0) 1.53(d, 6.3)1.53(d, 6.3)11.03(s)11.00(s)recorded at 500 MHz

#### 3.4.2 Compound GMB2

Compound **GMB2** was isolated as a yellow solid, mp 127-128 °C. The UV spectrum showed maximum absorption bands at  $\lambda_{max}$  217, 251 and 302 nm and IR spectrum showed absorption bands at 3449 and 1631 cm<sup>-1</sup> for hydroxy and carbonyl functional groups, respectively. The molecular formula, C<sub>14</sub>H<sub>12</sub>O<sub>4</sub>, was determined by HRESI-TOFMS and found pseudomolecular peak at *m/z* 245.0810 [M+H]<sup>+</sup> (calcd. for

245.0814). The <sup>1</sup>H NMR data of **GMB2** (Table 11) displayed signal for monosubstituent aromatic ring [ $\delta_{\rm H}$  7.65 (2H, *d*, *J* = 7.6 Hz, H-9 and H-13), 7.59 (1H, *t*, *J* = 7.6 Hz, H-11), 7.53 (2H, *t*, *J* = 7.6 Hz, H-10 and H-12)], two broad singlet aromatic protons ( $\delta_{\rm H}$  6.03, 2H, *br s*, H-2 and H-4) and one methoxy group ( $\delta_{\rm H}$  3.83, 3H, *s*, 3-OCH<sub>3</sub>). The <sup>13</sup>C NMR spectrum showed 11 signals for total of 14 carbons; six quaternary carbons ( $\delta_{\rm C}$  197.5, 167.2, 162.1 (2C), 139.9 and 104.6), seven methine carbons ( $\delta_{\rm C}$  132.6, 129.4 (2C), 128.0 (2C), 95.2 (2C)) and one methyl carbon ( $\delta_{\rm C}$  55.8). From these information, compound GMB2 was a simple benzophenone which previously reported by Mitasev. Detail assignments of <sup>1</sup>H, <sup>13</sup>C as well as HMBC are summarized in Table 11. This compound was identified as cotoin (Gottlieb *et al.*, 1958 and Mitasev *et al.*, 2009)

12

OH

HO

OCH<sub>3</sub>

Table 11 NMR (400 MHz) Data of GMB2 in CDCl<sub>3</sub>

Position	$\delta_{\rm C}$ , type		$\delta_{\rm H}$ , ( <i>mult.</i> , <i>J</i> in Hz)	HMBC ( $^{1}H\rightarrow ^{13}C$ )
1	162.1	C	Good	S*//
2	95.2	CH	6.03 ( <i>br</i> s)	1, 3, 6, 7
3	167.2	C	UNIVE	
3-OCH <sub>3</sub>	55.8	CH <sub>3</sub>	3.83 (s)	3
4 21	95.2	CH	6.03 ( <i>br</i> s)	3, 5, 6, 7
5	162.1	С		000this
6 <b>Co</b> p	104.6	C	by Chiang Mai	University
7 A I	197.5	C	hts res	erved
8	139.9	C		
9	128.0	CH	7.65 ( <i>d</i> , 7.6)	7, 11
10	129.4	CH	7.53 ( <i>t</i> , 7.6)	8, 12
11	132.6	CH	7.59 ( <i>t</i> , 7.6)	9, 13
12	129.4	CH	7.53 ( <i>t</i> , 7.6)	8, 10
13	128.0	CH	7.65 ( <i>d</i> , 7.6)	7, 11

#### 3.4.3 Compound GMB3

Compound **GMB3** was obtained as a yellow solid, mp. 248-250 °C. The UV and IR data of **GMB3** were closely related to that of **GMB2**. The main difference was found that compound **GMB2** appeared the methoxy group at  $\delta_{\rm H}$  3.83 while compound GMB3 was a hydroxyl group. This result was confirmed by the HMBC correlation of H-2 ( $\delta_{\rm H}$  5.83) with C-1/C5 ( $\delta_{\rm C}$  158.7), C-3 ( $\delta_{\rm C}$  161.2), C-6 ( $\delta_{\rm C}$  106.2) and C-7 ( $\delta_{\rm C}$  196.3) (Table 12). Therefore, compound **GMB3** was identified as 2,3',4,6-tetrahydroxy benzophenone (Jamila *et al.*, 2014).

HO OH

Table 12 NMR (400 MHz) Data of GMB3 in DMSO-d<sub>6</sub>

			102	-382
Position	$\delta_{\rm C}$ , type		$\delta_{\rm H}$ , ( <i>mult.</i> , J in Hz)	HMBC ( $^{1}H\rightarrow^{13}C$ )
1	158.7	C	N ZI	12
2	94.4	СН	5.83 (br s)	1, 3, 6, 7
3	161.2	С	ABU/	All
4	94.4	CH	5.83 (br s)	3, 5, 6, 7
5	158.7	C	AI INIVER	
6	106.2	C	UNIT	
7	196.3	С	0	a ? '
668	141.0	C	หาวทยาลยเ	ชยงเหม
9	119.4	CH	7.07 ( <i>d</i> , 7.8)	7, 11, 13
10	129.2	CH	7.23 ( <i>t</i> , 7.8)	12
11 A I	119.0	СН	6.90 ( <i>dd</i> , 7.8, 1.8)	9, 12, 13 C
12	157.1			
13	115.2	CH	7.04 (br s)	7, 12

#### 3.4.4 Compound GMB4

Compound GMB4 was obtained as a yellow viscous oil. The UV spectrum displayed absorption bands at  $\lambda_{max}$  212, 245, 319 and 359 nm indicating a xanthone structure (Ito et al., 2003). The IR spectrum showed absorption bands for hydroxy (3429 cm<sup>-1</sup>), carbonyl (1647 cm<sup>-1</sup>) and aromatic (1579 and 1500 cm<sup>-1</sup>) functionalities. The <sup>1</sup>H NMR spectrum (Table 13) showed a chelated hydroxy proton  $\delta_{\rm H}$  13.19 (1H, s), 1,2,3-trisubstitued aromatic protons [ $\delta_{\rm H}$  7.71 (1H, br d, J = 7.6 Hz, H-8), 7.27 (1H, d, J = 7.6 Hz, H-6 and 7.22 (1H, t, J = 7.6 Hz, H-7)], one single aromatic proton at  $\delta_{\rm H}$  6.28 (1H, s) and a set of 1,1-dimethylallyl group [ $\delta_{\rm H}$  6.63 (1H, dd, J = 17.7, 10.6 Hz, H-14), 5.35 (1H, d, J = 17.7 Hz, H-15), 5.22 (1H, d, J = 10.6 Hz, H-15) and 1.71 (6H, s, CH<sub>3</sub>-11 and 12)]. The hydroxy group ( $\delta_{\rm H}$  13.19) attributed at C-1 ( $\delta_{\rm C}$  161.1) according to chelate with carbonyl group. An aromatic proton at  $\delta_{\rm H}$  7.71 located at C-8 ( $\delta_{\rm C}$  115.9) due to the low field chemical shift from anisotropic effect of carbonyl group while two aromatic protons ( $\delta_{\rm H}$  7.27 and 7.22) located at C-6 ( $\delta_{\rm C}$  119.7) and C-7 ( $\delta_{\rm C}$  124.1) by splitting pattern of <sup>1</sup>H NMR data. The aromatic H-7 ( $\delta_{\rm H}$  7.22) correlated with oxygenated aromatic carbon C-5 ( $\delta_{\rm C}$  145.5) by HMBC data. A singlet aromatic proton resonating at  $\delta_{\rm H}$  6.28 was assigned to H-2 due to the <sup>2</sup>J and <sup>3</sup>J HMBC correlations to C-1 ( $\delta_{c}$  161.1), C-3 ( $\delta_{c}$  164.4), C-4 ( $\delta_{c}$  112.1), C-9 ( $\delta_{c}$  181.4) and C-9a ( $\delta_{c}$  103.4). The 1,1-dimethyl allyl group was placed at C-4 due to H-12 ( $\delta_{\rm H}$  6.63) showed HMBC correlation to C-4 ( $\delta_{\rm C}$  112.1). Therefore, compound GMB4 was determined as pancixanthone A (Ito et al., 1996).

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Position	$\delta_{\rm C}$ , type	è	$\delta_{\rm H}$ , ( <i>mult.</i> , <i>J</i> in Hz)	HMBC ( $^{1}H\rightarrow^{13}C$ )
1	161.1	С		
2	99.7	CH	6.28 (s)	1, 3, 4, 9, 9a
3	164.4	С		
4	112.1	С		
4a	155.1	С		
5	145.5	С		
6	119.7	CH	7.27 ( <i>d</i> , 7.6)	8, 10a
7	124.1	CH	7.22 ( <i>t</i> , 7.6)	5, 8, 8a
8	115.9	СН	7.71 ( <i>br d</i> , 7.6)	6, 9, 10a
8a	120.6	C	Jer I	131
9	181.4	С		121
9a	103.4	С		
10a	144.5	С	La ma	-582
11	41.4	С	THE D	202
12	27.7	CH <sub>3</sub>	1.71 (s)	4, 11, 14, 13
13	27.7	CH <sub>3</sub>	MARA	4, 11, 14, 12
14	154.9	CH	6.63 ( <i>dd</i> , 17.7, 10.6)	4, 11, 12, 13
15	105.6	CH <sub>2</sub>	5.35 ( <i>d</i> , 17.7)	11, 14
			5.22 ( <i>d</i> , 10.6)	
1-OH			13.19 (s)	

Table 13 NMR (400 MHz) Data of GMB4 in CDCl<sub>3</sub>

# 3.4.5 Compound GMB5 by Chiang Mai University

Compound **GMB5** was isolated as a yellow amorphous. The UV, IR and NMR spectra were similar to that of **GMB4** indicated the skeleton of xanthone (Table 14). The main difference was found that the splitting pattern of A ring was difference. For <sup>1</sup>H NMR data compound **GMB4** was ABC system while **GMB5** was AB system [ $\delta_{\rm H}$  7.58 (1H, d, J = 8.7 Hz, H-8) and 6.97 (1H, d, J = 8.7 Hz, H-7)]. Thus, compound **GMB5** was identified as assiguxanthone A (Ito *et al.*, 1997).



Table 14 <sup>1</sup>H NMR (400 MHz) Data of GMB5 and Assiguxanthone A in Acetone- $d_6$ 

GMB5	Assiguxanthone A
$\delta_{\rm H}$ , ( <i>mult.</i> , J in Hz)	$\delta_{\rm H}$ , ( <i>mult.</i> , J in Hz)
13.45 (s)	13.44 (s)
6.28 (s)	6.29 (s)
6.97 ( <i>d</i> , 8.7)	6.97 ( <i>d</i> , 8.4)
7.58 ( <i>d</i> , 8.7)	7.56 ( <i>d</i> , 8.4)
1.75 ( <i>s</i> )	1.73 (s)
1.75 (s)	1.73 (s)
6.57 ( <i>dd</i> , 17.5, 10.6)	6.55 ( <i>dd</i> , 17.2, 10.6)
5.08 ( <i>d</i> , 17.5)	5.05 ( <i>br d</i> , 17.2)
4.92 ( <i>d</i> , 10.6)	4.89 (br <i>d</i> , 10.6)
	GMB5 $\delta_{\rm H}$ , (mult., J in Hz) 13.45 (s) 6.28 (s) 6.97 (d, 8.7) 7.58 (d, 8.7) 1.75 (s) 1.75 (s) 6.57 (dd, 17.5, 10.6) 5.08 (d, 17.5) 4.92 (d, 10.6)

# 3.4.6 Compound GMB6

Compound **GMB6** was the xanthone cores structure, (yellow solid, mp 226-228 °C) which showed the UV and IR spectra similar to that of **GMB2-GMB5**. The <sup>1</sup>H NMR spectrum (Table 15) of **GMB6** showed a chelated hydroxy proton at  $\delta_{\rm H}$  13.26 (1H, *s*, 1-OH), a set of ABX system of aromatic protons [ $\delta_{\rm H}$  7.57 (1H, *d*, *J* = 3.0 Hz, H-8), 7.42 (1H, *d*, *J* = 9.0 Hz, H-5) and 7.33 (1H, *dd*, *J* = 9.0, 3.0 Hz, H-6)], one singlet aromatic proton at  $\delta_{\rm H}$  6.49 (1H, *s*, H-4) and one isoprenyl unit [ $\delta_{\rm H}$  5.28 (1H, *t*, *J* = 7.2 Hz, H-12), 3.36 (2H, *d*, *J* = 7.2 Hz, H-11), 1.78 (3H, *s*, CH<sub>3</sub>-15) and 1.65 (3H, *s*, CH<sub>3</sub>-14)]. Comparison the <sup>1</sup>H NMR data of **GMB6** with 1,3,7-trihydroxy-2-(3-methylbut-2enyl)-xanthone. That prenyl unit and aromatic proton were placed at C-2 and C-4, respectively. Therefore, **GMB6** was 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone (Garcia Cotez *et al.*, 1998).



Table 15 <sup>1</sup>H NMR (400 MHz) Data of GMB6 and 1,3,7-trihydroxy-2-(3-methylbut-2-

Position	GMB6	1,3,7-trihydroxy-2-(3-methylbut- 2-enyl)-xanthone		
	$\delta_{\rm H}$ , ( <i>mult.</i> , J in Hz)	$\delta_{\rm H}$ , ( <i>mult.</i> , J in Hz)		
1-OH	13.26 (s)	13.26 (s)		
4	6.49 (s)	6.49 ( <i>s</i> )		
5	7.42 ( <i>d</i> , 9.0)	7.42 ( <i>d</i> , 8.8)		
6	7.33 ( <i>dd</i> , 9.0, 3.0)	7.32 ( <i>dd</i> , 9.0, 2.8)		
8	7.57 ( <i>d</i> , 3.0)	7.57 ( <i>d</i> , 2.8)		
11	3.36 ( <i>d</i> , 7.2)	3.48 ( <i>d</i> , 7.4)		
12	5.28 ( <i>t</i> , 7.2)	5.28 ( <i>t</i> , 7.4)		
14	1.65 (s)	1.65 (s)		
15	1.78 (s)	1.79 (s)		

enyl)-xanthone in Acetone- $d_6$ 

#### 3.4.7 Compound GMB7

Compound **GMB7** was isolated as yellow solid, mp 258-260 °C. The <sup>1</sup>H NMR data (Table 16) of **GMB7** was similar to that of **GMB6** except that **GMB7** showed two aromatic protons 7.54 (1H, *br s*, H-8) and 6.89 (1H, *br s*, H-5) instead of ABX system in **GMB6**. Thus, compound **GMB7** was determined as assiguxanthone B (Ito *et al.*, 1997).

S.



GMB7	Assiguxanthone B	
$\delta_{\rm H}$ , ( <i>mult.</i> , J in Hz)	$\delta_{\rm H}$ , ( <i>mult.</i> , J in Hz)	
13.48 (s)	13.52 (s)	
6.46 ( <i>s</i> )	6.43 (s)	
6.89 ( <i>br s</i> )	6.87 (s)	
7.54 ( <i>br s</i> )	7.52 (s)	
3.34 ( <i>d</i> , 7.1)	3.34 ( <i>d</i> , 7.4)	
5.27 (br t, 7.1)	5.27 (m)	
1.64 (s)	1.63 (s)	
1.77 (s)	1.77 (s)	
	GMB7 δ <sub>H</sub> , ( <i>mult.</i> , J in Hz) 13.48 (s) 6.46 (s) 6.89 (br s) 7.54 (br s) 3.34 (d, 7.1) 5.27 (br t, 7.1) 1.64 (s) 1.77 (s)	

Table 16 <sup>1</sup>H NMR (400 MHz) Data of GMB7 and Assiguranthone B in Acetone- $d_6$ 

#### 3.4.8 Compounds GMB8-GMB11

Compound **GMB8**, a simplest xanthone, was isolated as yellow solid (mp 230-232 °C) and was 1,3,5-trihydroxyxanthone. Its <sup>1</sup>H NMRspectrum data (Table 17) displayed a chelated hydroxy proton at  $\delta_{\rm H}$  12.96 (1H, *s*, 1-OH), 1,2,3-trisubstituent aromatic protons [ $\delta_{\rm H}$  7.66 (1H, *dd*, *J* = 7.8, 1.4 Hz, H-8), 7.35 (1H, *dd*, *J* = 7.8, 1.4 Hz, H-6), 7.28 (1H, *t*, *J* = 7.8 Hz, H-7)] and two *meta* coupled aromatic protons [ $\delta_{\rm H}$  6.48 (1H, *d*, *J* = 2.0 Hz, H-4), 6.27 (1H, *d*, *J* = 2.0 Hz, H-2)] (Helesbeux *et al.*, 2004).

Compound **GMB9** (yellow solid, mp decomposed >300 °C) was also identified as a simple xanthone. **GMB9** was a structural isomer of **GMB8** which difference on the splitting pattern of proton on ring A. Compound **GMB9** displayed signal for 1,2,4-trisubstituent aromatic protons [ $\delta_{\rm H}$  7.56 (1H, d, J = 3.0 Hz, H-8), 7.44 (1H, d, J = 9.0 Hz, H-5) and 7.34 (1H, dd, J = 9.0, 3.0 Hz, H-6)] instead of 1,2,3-trisubstituent aromatic protons of **GMB8**. Therefore, compound **GMB9** was identified as 1,3,7-trihydroxyxanthone (Zuo *et al.*, 2014).

Compound **GMB10** was isolated as yellow powder. A simplest xanthone **GMB10** was identified to norathyriol base on its <sup>1</sup>H NMR spectral data (Table 17): two singlet aromatic protons [ $\delta_{\rm H}$  7.36 (1H, *br s*, H-8) and 6.68 (1H, *br s*, H-5)] and two *meta* 

coupled aromatic protons [ $\delta_{\rm H}$  6.25 (1H, *br s*, H-4) and 6.11 (1H, *br s*, H-2) that were suggested to H-8 and H-5; and H-4 and H-2, respectively.

Compound **GMB11** was obtained as a yellow solid, mp. decomposed >270 °C. This xanthone was a methoxy derivative of **GMB10**. The <sup>1</sup>H NMR spectrum (Table 17) of **GMB11** was closely to **GMB10** except for an additional methoxy group ( $\delta_{\rm H}$  3.93 3H, *s*). The methoxy group was located at position C-1 by comparison <sup>1</sup>H NMR data with montixanthone (Ngouela *et al.*, 2005).



Table 17<sup>1</sup>H NMR (400 MHz) Data of GMB8-GMB11

	1306	17 2 6		2024	
Position -	$\delta_{\rm H}$ , ( <i>mult.</i> , J in Hz)			1995	
	GMB8 <sup>a</sup>	GMB9 <sup><i>a</i></sup>	GMB10 <sup>b</sup>	GMB11 <sup>b</sup>	
2	6.27 ( <i>d</i> , 2.0)	6.25 ( <i>d</i> , 1.8)	6.11 ( <i>br s</i> )	6.13 ( <i>d</i> , 2.1)	
4	6.48 ( <i>d</i> , 2.0)	6.40 ( <i>d</i> , 1.8)	6.25 (br s)	6.27 ( <i>d</i> , 2.1)	
5	J. C	7.44 ( <i>d</i> , 9.0)	6.68 (br s)	6.73 (br s)	
6	7.35 ( <i>dd</i> , 7.8, 1.4)	7.34 ( <i>dd</i> , 9.0, 3.0)	VERO		
7	7.28 ( <i>t</i> , 7.8)				
8 8	7.66 ( <i>dd</i> , 7.8, 1.4)	7.56 ( <i>d</i> , 3.0)	7.36 (br s)	7.46 ( <i>br s</i> )	
1-OH	12.96 (s)	12.98 (s)	g Mai Ur	niversity	
1-OCH <sub>3</sub>			0	3.93 (s)	

<sup>*a*</sup> recorded in Acetone-*d*<sub>6</sub>, <sup>*b*</sup> recorded in CD<sub>3</sub>OD

#### 3.5 Biological Assays

All isolated compounds were evaluated for antimalarial activity against *Plasmodium falciparum* (TM4 and K1, multidrug resistant strains) as shown in Table 18. Tetracyclic xanthones with two isoprenyl units, compounds **GML1-GML5** exhibited strong activity with IC<sub>50</sub> values ranging  $6.0\pm1.1 - 8.5\pm1.2$  and  $3.6\pm1.7$  -

7.3±1.2  $\mu$ M. Tricyclic xanthones with one prenyl unit, compounds **GMB 4**, **GMB6** and **GMB7** showed moderate activity with IC<sub>50</sub> values in the range of 10.4±1.9 - 56.0 and 11.5±2.0 - 38.4±5.2  $\mu$ M (TM4 and K1, multidrug resistant strains). The cytotoxic against cancer cell lines (oral cavity cancer cells, KB) and normal cell lines (African green monkey kidney, Vero cells), compounds **GML1-GML4** also exhibited significant activity against KB and Vero cells with the IC<sub>50</sub> values in the range of 2.3 - 26.9±3.6 and 5.1 - 13.2±4.6  $\mu$ M, respectively while **GMB4** showed weak activity against the KB and Vero cells with the IC<sub>50</sub> values of 53.8 and 44.8±10.6  $\mu$ M, respectively. The compounds **GMB7** and **GML5** were toxic to normal cell line with the IC<sub>50</sub> values at 22.9±1.0 and 29.5±3.9  $\mu$ M. The other compounds were inactive. The selected compounds **GMB3-GMB4**, **GMB6**, **GMB8**, **GMB10** and **GML1-GML4** and **GML6-GML8** were inactive to against antibacterial activity against *B.cereus*, *E. col*, *M. luteus*, *P. aeruginosa*, *S. typhimurium*, *S. aureus* and *S. Epidermidis*.

Compounds	Antimalarial activity (IC <sub>50</sub> , $\mu$ M)		Cytotoxicity (IC50, µM)		
Compounds	TM4	K1	KB	Vero cells	
Crude GMB	1.2.	5.16*	-1	48.13 <sup>*</sup>	
<b>Crude GML</b>	Nº6	4.17*	8° //	6.31 <sup>*</sup>	
GMB4	56.0	38.4±5.2	53.8	44.8±10.6	
GMB6	14.9±2.0	14.9±3.3	_	-	
GMB7	10.4±1.9	11.5±2.0	_	22.9±1.0	
GML1	$6.2 \pm 0.4$	5.2±0.4	2.3	5.1	
GML2	8.5±1.2	3.6±1.7	7.6	6.9	
GML3	8.3±0.9	7.3±1.2	17.5	13.2±4.6	
GML4 CODV	6.7±0.6	6.4±0.5	26.9±3.6	12.6±0.9	
GML5	$6.0 \pm 1.1$	6.6±0.7	-	29.5±3.9	
Chloroquine <sup>a</sup>	0.03	0.3±0.04	erv	e a	
Cycloguanil <sup>a</sup>	$0.04 \pm 0.01$	3.2±0.8			
Pyrimethamine <sup>a</sup>	$0.08 \pm 0.01$	31.0±8.4			
Doxorubicin <sup>b</sup>			1.0	-	
Ellipticine <sup>b</sup>			-	0.4	

Table 18 Antimalarial Activity and Cytotoxicity of the Isolated Compounds

\*Concentration in µg/mL, <sup>*a*</sup> Standard compound for antimalarial activity, *b* Standard compound for cytotoxicity