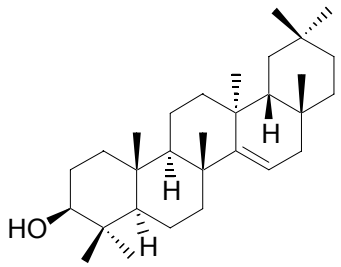
3 RESULTS AND DISCUSSION

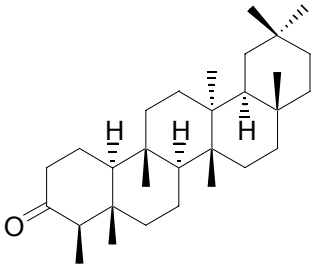
3.1 Structural determination

The twigs of *Mangifera odorata* were dried, chopped and extracted with dichloromethane, acetone and methanol, successively. The extracts were separated by means of chromatography over silica gel. **MF 1 - MF 8**, **MF 9** and **MF 10 - MF 14** were obtained from the dichloromethane, acetone and methanolic extracts, respectively. Their structures were determined by spectroscopic data (1D and 2D NMR spectral data).

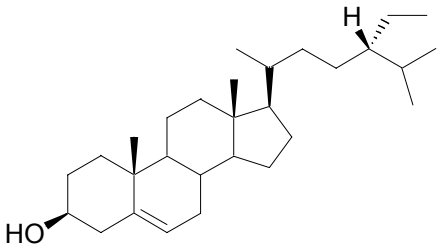
The structures of compounds isolated from the twigs of *Mangifera odorata*



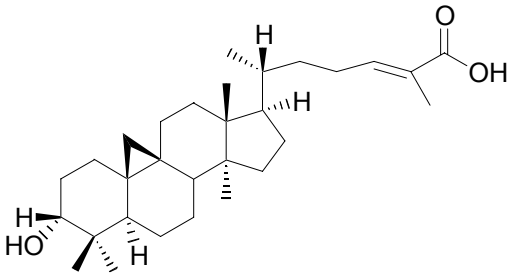
3β-Taraxerol **(MF 1)**



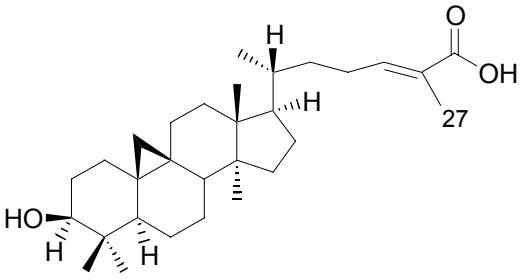
Friedelin (**MF 2**)



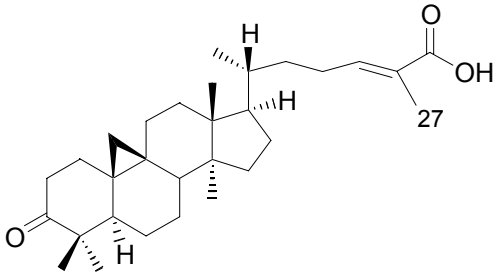
β-Sitosterol (**MF 3**)



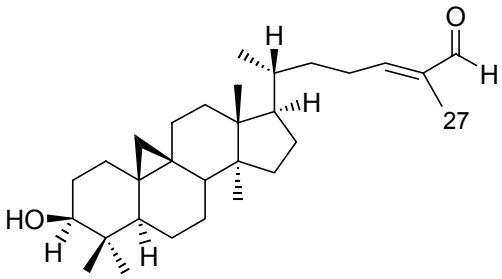
Isomangiferolic acid **(MF 4)**



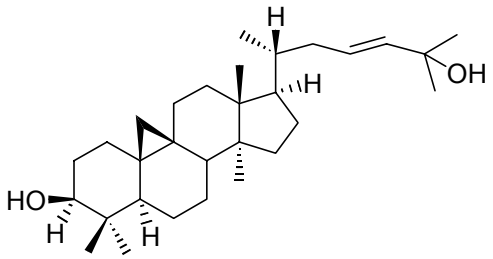
Mangiferolic acid **(MF 5)**



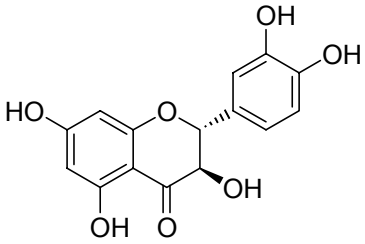
Mangiferonic acid **(MF 6)**



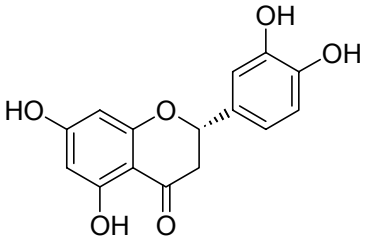
3β-Hydroxy-5α-cycloart-24-en-26-al **(MF 7)**



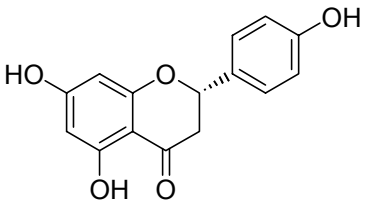
Cycloarta-23-en-3β,25-diol **(MF 8)**



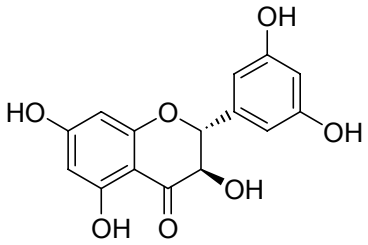
Taxifolin **(MF 9)**



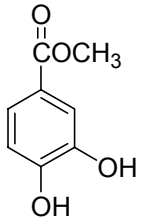
Eriodictyol **(MF 10)**



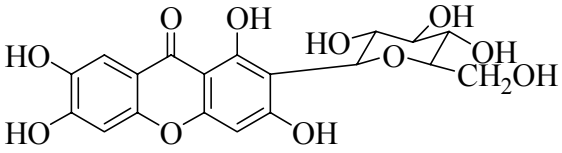
Narigenin **(MF 11)**



3,5,7,3',5'-Pentahydroxyflavanonol (**MF 12**)

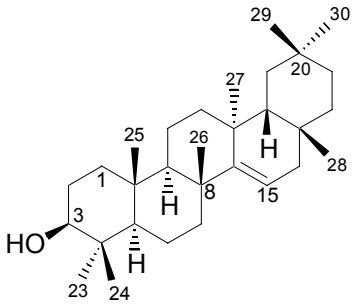


3,4-Dihydroxy benzoic acid methyl ester (**MF 13**)



Mangiferin (**MF 14**)

3.1.1 MF 1: 3β–Taraxerol



MF 1 was isolated as a white solid, m.p. 276-277 °C, $[\alpha]_D^{29}$ + 0.72° (c 1.5×10^{-4} g/cm^3 in MeOH). The IR spectrum showed absorption band of O-H stretching at 3479 cm^{-1}. The ^{1}H NMR spectral data of **MF 1** (**Table 11**) revealed the resonance of a triterpenoid compound including eight methyl groups and a vinylic proton. The resonances of methyl protons appeared as seven singlets at δ 1.09 (CH_3-26), 0.97 (CH_3-23), 0.94 (CH_3-29), 0.92 (CH_3-25), 0.90 (CH_3-27 and 30), 0.82 (CH_3-28) and 0.80 (CH_3-24), corresponding to ^{13}C NMR signals at δ 25.89, 27.97, 33.33, 15.44, 29.80, 21.29, 29.90 and 15.41, respectively. The resonance of vinylic proton (H-15) was shown as a doublet of doublet at δ 5.53 (J = 8.5, 3.5 Hz). A doublet of doublet at δ 3.20 was assigned for oxymethine proton (H-3) with a coupling constants of 11.5 and 5.0 Hz which implied that the H-3 was in the axial α-position. The HMBC correlations of oxymethine proton (H-3) to C-23 (δ 27.97), C-24 (δ 15.41) and correlations of H-15 to C-12 (δ 37.70), C-13 (δ 35.77) and C-17 (δ 38.74) confirmed the assignments of H-3 and H-15. Furthermore, the ^{13}C NMR spectra, DEPT 135° and DEPT 90° spectra indicated that **MF 1** composed of ten methylene carbons (δ 41.28, 37.70, 36.68, 35.08, 33.66, 33.64, 33.06, 27.11, 18.77 and 17.48), five methine carbons (δ 116.86, 79.05, 55.45, 49.24 and 48.70) and seven quaternary carbons (δ 158.05, 38.95, 38.74, 37.96, 37.53, 35.77 and 28.78). Additional informations from HMQC and HMBC correlations (**Table 11)** confirmed the structural assignment. Therefore **MF 1** was assigned to be 27-norolen-14-en-3β-ol which was the same compound as a previously reported 3β–Taraxerol (Sakurai, *et al*., 1987).

Table 11 The ^{13}C, ^{1}H NMR and HMBC spectral data of **MF 1**

position	δ_C (C-Type)*	δ_H (*multiplicity*, J_{Hz})	major HMBC
1	37.68 (CH_2)	1.68 (1H, *m*), 1.60 (1H, *m*)	
2	27.11 (CH_2)	1.59 (2H, *m*)	
3	79.05 (CH)	3.20 (1H , *dd*, 11.5, 5.0 Hz)	C-23, C-24
4	37.96 (C)	-	-
5	55.45 (CH)	0.78 (1H, *m*)	C-3, C-10, C-23, C-24
6	18.77 (CH_2)	1.64 (1H, *m*), 1.45 (1H, *m*)	
7	33.64 (CH_2)		
8	38.95 (C)	-	
9	49.24 (CH)	1.4 (1H, *m*)	C-7, C-25, C-26
10	37.53 (C)	-	-
11	17.48 (CH_2)	1.59 (1H, *m*), 0.95 (1H, *s*)	
12	37.70 (CH_2)	1.90 (2H, *m*)	
13	35.77 (C)	-	-
14	158.05 (C)	-	-
15	116.86 (CH)	5.53 (1H, *dd*, 8.5, 3.5 Hz,)	C-12, C-13, C-17
16	35.08 (CH_2)	1.05 (1H, *m*), 1.34 (1H, *m*)	
17	38.74 (C)	-	-
18	48.70 (CH)	0.96 (1H, *m*)	C-22, C-28
19	41.28 (CH_2)	2.02 (1H, *dt*, 3.0, 13.0), 1.34 (1H, *m*)	
20	28.78 (C)	-	-

Table 11 (continued)

position	δ_C (C-Type)*	δ_H (*multiplicity*, J_{Hz})	major HMBC
21	33.66 (CH_2)	1.32 (1H, *m*), 0.96 (1H, *m*)	
22	33.06 (CH_2)	1.60 (2H, *m*)	
23	27.97 (CH_3)	0.97 (3H, *s*)	C-3, C-5, C-10, C-24
24	15.41 (CH_3)	0.80 (3H, *s*)	C-3, C-5, C-10
25	15.44 (CH_3)	0.92 (3H, *s*)	C-5, C-9
26	25.89 (CH_3)	1.09 (3H, *s*)	C-9, C-10, C-14
27	29.80 (CH_3)	0.90 (3H, *s*)	C-16, C-18
28	29.90 (CH_3)	0.82 (3H, *s*)	C-14, C-21, C-22
29	33.33 (CH_3)	0.94 (3H, *s*)	C-20, C-21, C-22
30	21.29 (CH_3)	0.90 (3H, *s*)	C-21, C-22

* Carbon type was deduced from DEPT experiments.

Table 12 Comparison of the ^{13}C NMR spectral data between **MF 1** and **3β-Taraxerol**

position	**MF 1***	**3β-Taraxerol***
1	37.68	38.1
2	27.11	27.3
3	79.05	79.2
4	37.96	39.1
5	55.45	55.7

Table 12 (continued)

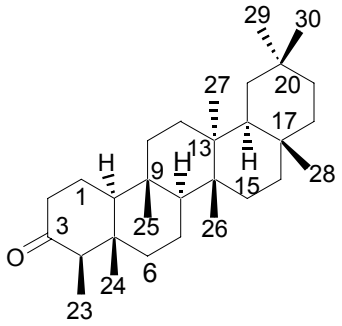
position	**MF 1***	**3β-Taraxerol***
6	18.77	19.0
7	33.64	35.3
8	38.95	38.9
9	49.24	48.9
10	37.53	37.9
11	17.48	17.7
12	37.70	37.9
13	35.77	35.9
14	158.05	158.1
15	116.86	117.0
16	35.08	36.9
17	38.74	38.1
18	48.70	49.4
19	41.28	41.4
20	28.78	29.0
21	33.66	33.9
22	33.06	33.2
23	27.97	28.1
24	15.41	15.6
25	15.44	15.6
26	25.89	30.1
27	29.80	26.0

Table 12 (continued)

position	**MF 1***	**3β-Taraxerol***
28	29.90	30.1
29	33.33	33.5
30	21.29	21.5

***** Recorded in $CDCl_3$

3.1.2 MF 2: Friedelin



MF 2 was obtained as a white solid, m.p. 251.2-252.4 °C and $[\alpha]_D^{29}$ +17.0° (c 9.5×10^{-4} g/cm^3 in MeOH). The IR spectrum showed absorption band at 1702 cm^{-1} for carbonyl group. The ^{1}H NMR spectral data (**Table 13**) indicated that **MF 2** contained seven tertiary methyl groups which resonated as seven singlets at δ 0.72 (CH_3-24), 0.87 (CH_3-25), 1.01 (CH_3-26), 1.05 (CH_3-27), 1.18 (CH_3-28), 0.95 (CH_3-29) and 1.00 (CH_3-30) and a secondary methyl group resonated as a doublet at δ 0.88 (J = 6.5 Hz, CH_3-23). The presence of a carbonyl group was suggested from the carbon resonance at δ 213.32. In ^{13}C NMR spectrum, eight methyl carbons (δ 35.01, 32.07, 31.80, 20.25, 18.66, 17.93, 14.64, 6.82), eleven methylene carbons (δ 41.52, 41.26, 39.23, 35.98, 35.59, 35.32, 32.73, 32.39, 30.49, 22.27, 18.21), four methine carbons (δ 59.44, 58.20, 53.08, 42.75) and six tetrasubstitued carbons (δ 42.14, 39.67, 38.27, 37.41, 29.97, 28.16) were deduced. **MF 2** was then proposed

to be friedelin (Reynolds, *et al*., 1986). The assignment was confirmed by HMBC (**Table 13** and **Figure 2)**.

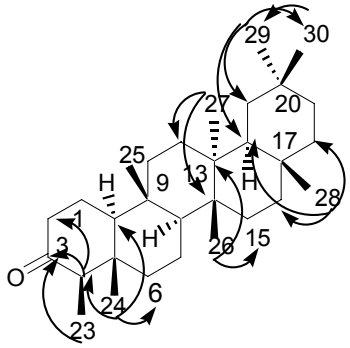


Figure 2 Major HMBC of **MF 2**

Table 13 The ^{13}C, ^{1}H NMR and HMBC spectral data of **MF 2**

position	δ_C (C-Type)*	δ_H (*multiplicity*, J_{Hz})	major HMBC
1	22.27 (CH_2)	1.97 (1H, *m*), 1.68 (1H, *m*)	
2	41.52 (CH_2)	2.39 (1H, *dddd*, 14.0, 7.5, 5.0, 2.5), 1.85 (1H, *dt*, 13.0, 2.5)	
3	213.32 (C=O)	-	-
4	58.20 (CH)	2.25 (1H, *q*, 7.0)	C-2, C-3, C-23, C-24
5	42.14 (C)	-	-
6	41.26 (CH_2)	2.30 (1H, *m*), 1.26 (1H, *m*)	
7	18.21 (CH_2)	-	-
8	53.08 (CH)	1.39 (1H, *m*)	C-9, C-11, C-14, C-25, C-26
9	37.41 (C)	-	-
10	59.44 (CH)	1.53 (1H, *m*)	C-1, C-2, C-5, C-9, C-25
11	35.59 (CH_2)	1.47 (2H, *m*)	

Table 13 (continued)

position	δ_C (C-Type)*	δ_H (*multiplicity*, J Hz)	major HMBC
12	30.49 (CH_2)	1.64 (2H, *m*)	
13	39.67 (C)	-	-
14	38.27 (C)	-	-
15	32.39 (CH_2)	1.56 (2H, *m*)	
16	35.98 (CH_2)	1.59 (2H, *m*)	
17	29.97 (C)	-	-
18	42.75 (CH)	1.55 (1H, *m*)	C-13, C-17, C-19, C-27, C-28
19	35.32 (CH_2)	1.38 (2H, *m*)	
20	28.16 (C)	-	-
21	32.73 (CH_2)	1.28 (2H, *m*)	
22	39.23 (CH_2)	1.48 (1H, *m*), 0.94 (1H, *m*)	
23	6.82 (CH_3)	0.88 (3H, *d*, 6.5)	C-3, C-5
24	14.64 (CH_3)	0.72 (3H, *s*)	C-4 C-6, C-10
25	17.93 (CH_3)	0.87 (3H, *s*)	C-5, C-8, C-10, C-11
26	20.25 (CH_3)	1.01 (3H, *s*)	C-13, C-15
27	18.66 (CH_3)	1.05 (3H, *s*)	C-12, C-14, C-15, C-18
28	32.07 (CH_3)	1.18 (3H, *s*)	C-16 C-18, C-22
29	35.01 (CH_3)	0.95 (3H, *s*)	C-18, C-19, C-30
30	31.80 (CH_3)	1.00 (3H, *s*)	C-29

* Carbon type was deduced from DEPT experiments

Table 14 Comparison of the ^{13}C NMR spectral data between **MF 2** and **Friedelin**

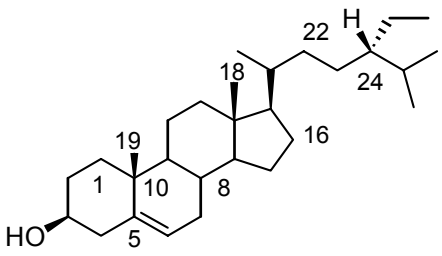
position	**MF 2***	**Friedelin***
1	22.27	22.3
2	41.52	41.5
3	213.32	213.2
4	58.20	58.2
5	42.14	42.1
6	41.26	41.3
7	18.21	18.2
8	53.08	53.1
9	37.41	37.4
10	59.44	59.4
11	35.59	35.6
12	30.49	30.5
13	39.67	39.7
14	38.27	38.3
15	32.39	32.4
16	35.98	36.0
17	29.97	30.0
18	42.75	42.8
19	35.32	35.3
20	28.16	28.1
21	32.73	32.7
22	39.23	39.2
23	6.82	6.8

Table 14 (continued)

position	**MF 2***	**Friedelin***
24	14.64	14.6
25	17.93	17.9
26	20.25	20.2
27	18.66	18.6
28	32.07	32.1
29	35.01	35.0
30	31.80	31.8

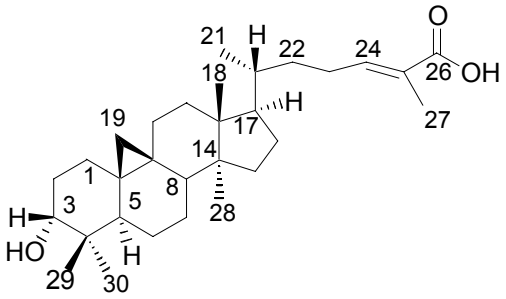
* Recorded in $CDCl_3$

3.1.3 MF 3: *β*-sitosterol



MF 3 was isolated as a white solid, m.p. 128-130 °C, $[\alpha]_D^{29}$ –55.48 ° (c 1.5×10^{-4} g/cm^3 in MeOH). The IR spectrum showed absorption band at 3418 cm^{-1} (O-H stretching). The ^{1}H NMR spectrum showed an oxymethine proton signal at δ 3.53 (*m*), an olefinic proton at δ 5.36 (*dd, J* = 3.0, 2.0 Hz) and six methyl groups at δ 1.25, 1.00, 0.92, 0.85, 0.82 and 0.69. The ^{1}H NMR data, optical rotation value and melting point were corresponded to the previous reported data of *β*-sitosterol. Therefore, **MF 3** was assigned to be *β*-sitosterol.

3.1.4 MF 4: Isomangiferolic acid



MF 4 was isolated as a white solid, m.p. 168-170 °C, $[\alpha]_D^{29}$ +29° (c 1.7×10^{-4} g/cm^3 in MeOH). The maximum absorption band at 215 nm in UV spectrum indicated that **MF 4** possessed the α,β-unsaturated carboxylic acid chromophore. The IR spectrum revealed the presence of a hydroxy group (3421 cm^{-1}) and a carbonyl group (1686 cm^{-1}). The ^{1}H NMR spectral data (**Table 15**) showed the characteristic signals of a cycloartane type triterpene. The signals of cyclopropane methylene protons were shown as two doublets at δ 0.52 and 0.35 (1H each, *d, J* = 4.5 Hz). Four tertiary methyl groups were detected from the singlet resonances at δ0.90 (CH_3-18), 0.97 (CH_3-28), 0.95 (CH_3-29) and 0.89 (CH_3-30). A doublet of a secondary methyl group was observed at δ 0.91, it was assigned for CH_3-21. A downfield shift of a singlet resonance of a methyl group at δ 1.85 suggested a vinylic methyl group (CH_3-27) which was connected to a carbonyl group. A quartet of triplet at δ 6.90 (*J* = 1.5, 8.0 Hz) suggested the presence of a vinylic proton (H-24), the chemical shift of C-24 corresponded to a vinyl carbon. The presence of an oxymethine proton (H-3) was detected at δ 3.48 (*t*) of which the coupling constant of 3.0 Hz implied the equatorial β-position. The remaining protons and carbons were assigned from HMQC, HMBC and DEPT spectral data (**Table 15**). **MF 4** was therefore proposed to be 3α-hydroxy-5α-cycloart-24-en-26-oic acid. The presence of a carboxylic acid carbon was indicated in ^{13}C NMR (δ 172.49). The side chain [-CH(Me)CH_2CH_2CH=C(Me) COOH] was suggested to link to the present structure at C-17 from the correlation of H-21 to C-17 in HMBC. The structure corresponded to 3α-hydroxy-5α-cycloart-24-en-26-oic acid, (isomangiferolic acid) (Anjanneyu, *et al*., 1989).

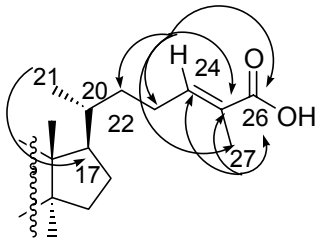


Figure 3 HMBC correlations of the side chain of **MF 4**

Table 15 The ^{13}C, ^{1}H NMR and HMBC spectral data of **MF 4**

position	δ_C (C-Type)*	δ_H (*multiplicity*, J_{Hz})	major HMBC
1	27.46 (CH_2)	1.29 (2H, *m*)	
2	28.12 (CH_2)	1.86 (1H, *m*), 1.01 (1H, *m*)	
3	77.04 (CH)	3.48 (1H, *t*, 3.0)	C-1, C-5
4	39.53 (C)	-	-
5	41.07 (CH)	1.82 (1H, *m*)	C-4, C-6, C-10, C-19
6	21.07 (CH_2)	1.48 (1H, *m*), 0.78 (1H, *m*)	
7	28.55 (CH_2)	1.31 (2H, *m*)	
8	48.03 (CH)	1.52 (1H, *m*)	C-6, C-9, C-11, C-14, C-19
9	19.78 (C)	-	-
10	26.43 (C)	-	-
11	26.24 (CH_2)	2.04 (2H, *m*)	
12	32.86 (CH_2)	1.64 (2H, *m*)	
13	45.27 (C)	-	-
14	48.89 (C)	-	-

Table 15 (continued)

position	δ_C (C-Type)*	δ_H (*multiplicity*, J_{Hz})	major HMBC
15	35.47 (CH_2)	1.32 (1H, *m*), 1.28 (1H, *m*)	
16	25.90 (CH_2)	1.92 (2H, *m*)	
17	52.14 (CH)	1.60 (1H, *m*)	C-13, C-14, C-16, C-21
18	19.27 (CH_3)	0.90 (3H, *s*)	C-13, C-14, C-17, C-20
19	29.80 (CH_2)	α 0.35 (1H, *d*, 4.5)	C-5, C-8, C-9, C-10
		β 0.52 (1H, *d*, 4.5)	C-5, C-8, C-9, C-10
20	35.96 (CH)	1.28 (1H, *m*)	C-13, C-14, C-20, C-22
21	18.03 (CH_3)	0.91 (3H, *d*, 7.0)	C-13, C-17, C-20
22	34.77 (CH_2)	1.61 (2H, *m*)	
23	25.64 (CH_2)	1.12 (2H, *m*)	
24	145.81 (=C-H)	6.90 (1H, *qt*, 1.5, 8.0)	C-23, C-26, C-27
25	126.45 (C)	-	-
26	172.49 (C=O)	-	-
27	11.99 (CH_3)	1.85 (3H, *s*)	C-24, C-25, C-26
28	18.08 (CH_3)	0.97 (3H, *s*)	C-12, C-13, C-14, C-17
29	21.21 (CH_3)	0.95 (3H, *s*)	C-3, C-4, C-5, C-10
30	25.84 (CH_3)	0.89 (3H, *s*)	C-3, C-4, C-5, C-6

***** Carbon type was deduced from DEPT experiments

Table 16 Comparison of the ^{13}C NMR spectral data between **MF 4** and **Isomangiferolic acid**

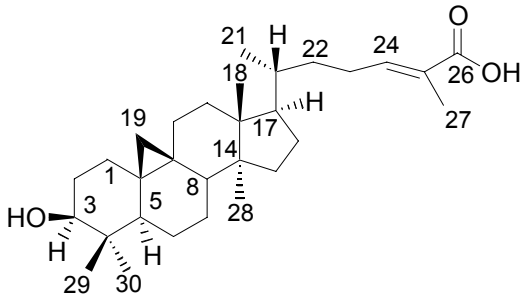
position	**MF 4***	**Isomangiferolic acid***
1	27.46	27.4
2	28.12	28.5
3	77.04	77.1
4	39.53	39.5
5	41.07	41.1
6	21.07	21.0
7	28.55	28.1
8	48.03	48.0
9	19.78	19.8
10	26.43	26.4
11	26.24	26.2
12	32.86	32.9
13	45.27	45.3
14	48.89	48.9
15	35.47	35.5
16	25.90	25.90
17	52.14	52.1
18	19.27	19.3
19	29.80	29.8
20	35.96	35.9
21	18.03	18.0
22	34.77	34.8

Table 16 (continued)

position	**MF 4***	**Isomangiferolic acid***
23	25.64	21.0
24	145.81	145.7
25	126.45	126.7
26	172.49	173.0
27	11.99	11.9
28	18.08	18.10
29	21.21	21.2
30	25.84	25.8

* Recorded in $CDCl_3$

3.1.5 MF 5: Mangiferolic acid



MF 5 was obtained as a white solid. Its melting point was 181-183 °C and the optical rotation was $[\alpha]_D^{29}$ +49° (c 1.6×10^{-4} g/cm^3 in MeOH). The UV (214 nm) and IR (3389 and 1687 cm^{-1}) spectrum indicated the presence of an α,β-unsaturated carboxylic acid. The ^{1}H NMR spectral data (**Table 17**) of **MF 5** are closely related to those of **MF 4**. The difference was shown as a multiplicity and a chemical shift of H-3 which was shown as a triplet (J = 3.0 Hz) at δ 3.48 in **MF 4** but a doublet of doublet (J = 11.5, 4.0 Hz) at δ 3.31 in **MF 5**. The coupling constant of **MF 5** indicated that H-3 was in an axial α-position. Thus **MF 5** was a 3 epimer of **MF 4**. The ^{1}H NMR spectrum suggested the presence of cyclopropane methylene proton CH_2-19 (δ 29.89), four tertiary methyl groups CH_3-18 and CH_3-30 (δ 0.98), CH_3-28 and CH_3-29 (δ 0.83), a secondary methyl group CH_3-21 (δ 0.92, *d, J* = 7.2 Hz), vinylic methyl group CH_3-27 (δ 1.86) and a vinyl proton CH-24 (δ 6.92, *qt*, J = 6.6, 1.2 Hz). The assignments were confirmed by HMBC. The structural assignment, ^{13}C NMR data, the optical rotation value and melting point of **MF 5** corresponded to 3β-hydroxy-5α-cycloart-24-en-26-oic acid, (mangiferolic acid) (Corsano, *et al*., 1965).

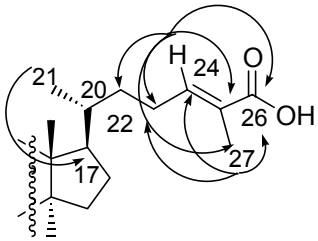


Figure 4 HMBC correlations of the side chain of **MF 5**

Table 17 The ^{13}C, ^{1}H NMR and HMBC spectral data of **MF 5**

position	δ_C (C-type)*	δ_H (*multiplicity*, J_{Hz})	major HMBC
1	31.96 (CH_2)	1.28 (2H, *m*)	
2	30.34(CH_2)	1.77 (1H, *m*),	
		1.73 (1H, *m*)	
3	78.88 (CH)	3.31 (1H, *dd*, 11.5, 4.0)	C-29, C-30
4	40.47 (C)	-	-
5	47.10 (CH)	1.32 (1H, *m*)	C-3, C-4, C-7, C-9, C-10,
			C-19, C-29
6	21.11 (CH_2)	1.61 (2H, *m*)	
7	28.15 (CH_2)	1.30 (2H, *m*)	
8	47.96 (CH)	1.53 (1H, *m*)	C-6, C-9, C-10, C-11,
			C-14, C-15, C-16, C-19
9	19.96 (C)	-	-
10	26.06 (C)	-	-
11	26.44 (CH_2)	1.20 (2H, *m*)	
12	32.89 (CH_2)	1.63 (1H, *m*)	
13	45.34 (C)	-	-
14	48.81 (C)	-	-
15	35.54 (CH_2)	1.35 (2H, *m*)	
16	26.01 (CH_2)	1.10 (2H, *m*)	

Table 17 (continued)

position	δ_C (C-type)*	δ_H (*multiplicity*, J_{Hz})	major HMBC
17	52.20 (CH)	1.64 (1H, *m*)	C-13, C-14, C-16, C-18, C-20, C-21
18	18.11 (CH_3)	0.98 (3H, *s*)	C-8, C-12, C-14, C-17
19	29.89 (CH_2)	α 0.35 (1H, *d*, 4.2)	C-1, C-8, C-9, C-10
		β 0.57 (1H, *d*, 4.2)	C-1, C-8, C-9, C-11
20	35.97 (CH)	1.30 (1H, *m*)	C-21, C-22, C-23
21	19.31 (CH_3)	0.92 (3H, *d*, 7.2)	C-13, C-17, C-22, C-23
22	34.80 (CH_2)	1.60 (2H, *m*)	
23	25.92 (CH_2)	1.37 (2H, *m*)	
24	145.75 (=C-H)	6.92 (1H, *qt*, 6.6, 1.2)	C-22, C-23, C-25, C-26, C-27
25	126.60 (C)	-	-
26	172.94 (C=O)	-	-
27	11.97 (CH_3)	1.86 (3H, *s*)	C-23, C-24, C-26
28	18.06 (CH_3)	0.83 (3H, *s*)	C-8, C-16
29	14.00 (CH_3)	0.83 (3H, *s*)	C-3, C-5, C-30
30	25.43 (CH_3)	0.98 (3H, *s*)	C-3, C-5

***** Carbon type was deduced from DEPT experiments

Table 18 Comparison of the ^{13}C NMR spectral data between **MF 5** and **Mangiferolic acid**

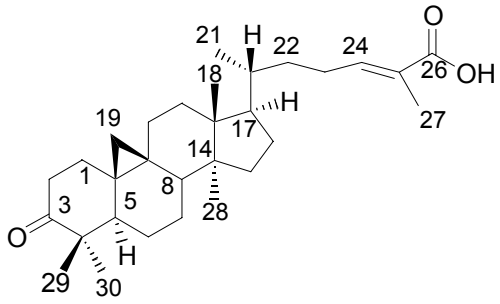
position	**MF 5***	**Mangiferolic acid***
1	31.96	31.9
2	30.34	30.3
3	78.88	78.9
4	40.47	40.5
5	47.10	47.1
6	21.11	21.1
7	28.15	28.15
8	47.96	48.0
9	19.96	19.9
10	26.06	26.1
11	26.44	26.4
12	32.89	32.9
13	45.34	45.3
14	48.81	48.8
15	35.54	35.5
16	26.01	26.0
17	52.20	52.2
18	18.11	18.1
19	29.89	29.9
20	35.97	35.9
21	19.31	19.3

Table 18 (continued)

position	**MF 5***	**Mangiferolic acid***
22	34.80	34.8
23	25.92	25.9
24	145.75	145.7
25	126.60	126.6
26	172.94	172.9
27	11.97	11.9
28	18.06	18.0
29	14.00	14.0
30	25.43	25.4

* Recorded in $CDCl_3$

3.1.4 MF 6: Mangiferonic acid



MF 6 was obtained as a white solid, mp.186-188 °C, $[\alpha]_D^{29}$ +24° (c 1.6×10^{-4} g/cm^3 in MeOH). The IR spectrum showed the presence of a hydroxyl group (3380 cm^{-1}) and carbonyl groups (1705, 1686 cm^{-1}). The maximum absorption band at 216 nm in UV spectrum indicated that **MF 6** possessed the α,β-unsaturated carboxylic acid chromophore. The ^{1}H NMR spectral data (**Table 19**) displayed a similar pattern as **MF 5** except that **MF 6** showed no signal of H-3 at δ 3.31 and ^{13}C NMR spectrum

displayed signal of a carbonyl group at δ 217.50. A characteristic pair of doublet-resonances of cyclopropane methylene protons was at δ 0.58 and 0.78 (1H each, *d*, *J* = 4.2 Hz), and three singlet signals for the four tertiary methyl groups were at δ 1.10 (CH_3-29), 1.05 (CH_3-28 and CH_3-30) and 1.00 (CH_3-18). The proton types suggested that **MF 6** was a normal cycloartanone-type triterpenoid. The lower field shift of the resonance of a vinyl methyl group (CH_3-27) was observed at δ 1.85. A vinylic proton H-24 was shown as a triplet at δ 6.91 (*J* = 5.7 Hz). The signal of carboxyl group in the side chain resonated at δ 172.96. The HMBC correlation of H-21 to C-17 confirmed the position of a side chain at C-17. The remaining signal of protons and carbons were assigned from DEPT, HMQC and HMBC (**Table 19**). **MF 6** was therefore elucidated as 3-oxo-5α-cycloart-24-en-26-oic acid of which its trivial name was mangiferonic acid (Zhang, *et al*., 2003).

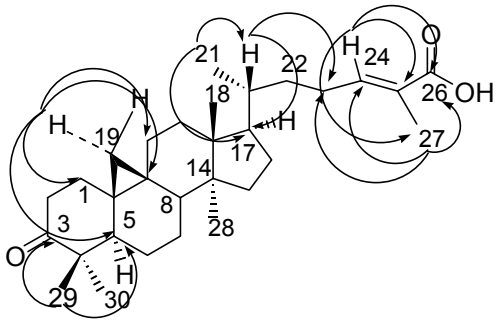


Figure 5 Major HMBC of **MF 6**

Table 19 The ^{13}C, ^{1}H NMR and HMBC spectral data of **MF 6**

position	δ_C (C-Type)*	δ_H (*multiplicity*, J_{Hz})	major HMBC
1	33.43 (CH_2)	1.83 (1H, *m*), 1.52 (1H, *m*)	
2	37.48 (CH_2)	2.72 (1H, *ddd*, 13.8, 6.6, 7.2)	
		2.31 (1H, *dt*, 11.4, 3.9)	
3	217.50 (C)	-	-
4	50.25 (C)	-	-
5	48.44 (CH)	1.69 (1H, *m*)	C-4, C-6, C-29, C-30
6	21.11 (CH_2)	1.53 (2H, *m*)	
7	25.87 (CH_2)	1.40 (2H, *m*)	
8	47.88 (CH)	1.57 (1H, *m*)	C-14, C-15, C-19
9	21.51 (C)	-	-
10	26.01 (C)	-	-
11	26.71 (CH_2)	2.03 (2H, *m*)	
12	32.81 (CH_2)	1.64 (2H, *m*)	
13	45.40 (C)	-	-
14	48.76 (C)	-	-
15	35.55 (CH_2)	1.35 (2H, *m*)	
16	28.16 (CH_2)	1.30 (1H, *m*), 1.88 (1H, *m*)	

Table 19 (continued)

position	δ_C (C-Type)*	δ_H (*multiplicity*, J_{Hz})	major HMBC
17	52.22 (CH)	1.62 (1H, *m*)	C-13, C-14, C-20, C-21
18	18.11 (CH_3)	1.00 (3H, *s*)	C-12, C-14, C-17
19	29.56 (CH_2)	α 0.58 (1H, *d*, 4.2)	C-1, C-5, C-8, C-9, C-10, C-11
		β 0.78 (1H, *d*, 4.2)	C-1, C-5, C-8, C-9, C-10, C-11
20	35.96 (CH)	1.30 (1H, *m*)	C-17, C-21 C-23
21	21.07 (CH_3)	0.92 (3H, *d*, 6.3)	C-13, C-17, C-23
22	25.88 (CH_2)	1.11 (2H, *m*)	
23	34.78 (CH_2)	1.50 (2H, *m*)	
24	145.76 (=C-H)	6.91 (1H, *t*, 5.7)	C-22, C-23, C-25, C-26, C-27
25	126.13 (C)	-	-
26	172.96 (C=O)	-	-
27	11.99 (CH_3)	1.85 (3H, *s*)	C-23, C-24, C-26
28	22.18 (CH_3)	1.05 (3H, *s*)	C-8, C-9, C-15
29	20.78 (CH_3)	1.10 (3H, *s*)	C-3, C-5
30	19.30 (CH_3)	1.05 (3H, *s*)	C-5

***** Carbon type was deduced from DEPT experiments

Table 20 Comparison of the ^{13}C NMR spectral data between **MF 6** and **Mangiferonic acid**

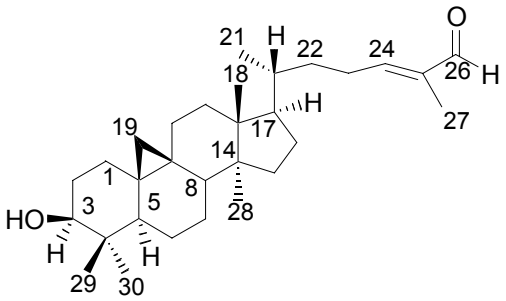
position	**MF 6***	**Mangiferonic acid***
1	33.43	33.44
2	37.48	37.4
3	217.50	216.5
4	50.25	50.2
5	48.44	18.4
6	21.11	21.5
7	25.87	25.8
8	47.88	47.8
9	21.51	21.0
10	26.01	26.0
11	26.71	26.6
12	32.81	32.7
13	45.40	45.3
14	48.76	48.7
15	35.55	35.5
16	28.16	28.1
17	52.22	52.2
18	18.11	18.1
19	29.56	29.5
20	35.96	35.9
21	21.07	18.1

Table 20 (continued)

position	**MF 6***	**Mangiferonic acid***
22	25.88	25.9
23	34.78	34.1
24	145.76	145.8
25	126.13	126.6
26	172.96	173.5
27	11.99	11.9
28	22.18	22.1
29	20.78	20.7
30	19.30	19.3

* Recorded in $CDCl_3$

3.1.5 MF 7: 3β-Hydroxy-5α-cycloart-24-en-26-al



MF 7 was obtained as a white solid. Its melting point was 201-203 °C and the optical rotation was $[\alpha]^{29}_{D}$ +67° (c 1.4×10^{-4} g/cm^3 in MeOH). The UV spectrum showed an absorption band at 228 nm. The IR spectrum indicated the presence of hydroxyl (3430 cm^{-1}) and carbonyl (1686 cm^{-1}) groups. The ^{1}H NMR and ^{13}C NMR spectral data (**Table 21**) indicated that it was a derivative of **MF 5** (3β-hydroxy-5α-cycloart-24-en-26-oic acid). The ^{1}H NMR spectrum showed four tertiary methyl groups at δ 0.97 (CH_3-18 and CH_3-30), 0.93 (CH_3-28), 0.83 (CH_3-29), a secondary methyl group CH_3-21 at δ 0.92 (*d, J* = 6.0 Hz), a vinyl methyl proton CH_3-27 at δ 1.76, a vinyl proton H-24 at δ 6.50 (*t, J* = 7.2 Hz) and a cyclopropyl methylene proton H_2-19 at δ 0.56 and 0.35 (1H each, *d, J* = 4.2 Hz). The difference from **MF 5** was shown as an additional singlet resonance of a formyl proton at δ 9.40 which corresponded to carbon signal at δ 195.45 but no signal at 172.94. The side chain was therefore proposed for [-CH(Me)CH_2CH_2CH=C(Me)CHO]. Consequently, **MF 7** was assigned as 3β-hydroxy-5α-cycloart-24-en-26-al.

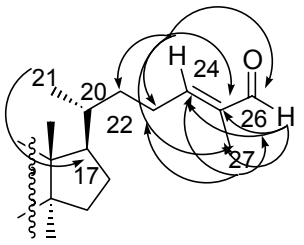


Figure 6 HMBC correlations of the side chain of **MF 7**

Table 21 The ^{13}C, ^{1}H NMR and HMBC spectral data of **MF 7**

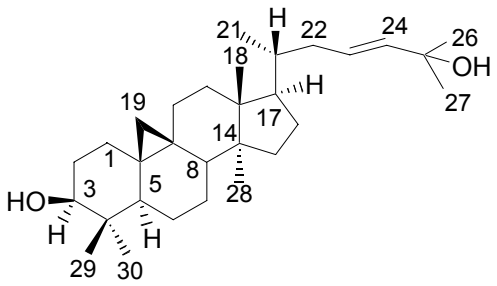
position	δ_C (C-Type)*	δ_H (*multiplicity*, J_{Hz})	major HMBC
1	31.97 (CH_2)	1.28 (2H, *m*)	
2	30.35 (CH_2)	1.77 (1H, *m*), 1.84 (1H, *m*)	
3	78.84 (CH)	3.29 (1H, *dd*, 10.8, 4.5)	C-29, C-30
4	40.48 (C)	-	-
5	47.09 (CH)	1.30 (1H, *m*)	
6	21.10 (CH_2)	1.60 (2H, *m*)	
7	28.20(CH_2)	1.31 (2H, *m*)	
8	47.97 (CH)	1.53 (1H, *m*)	C-7, C-9, C-10, C-14, C-19
9	19.94 (C)	-	-
10	26.43 (C)	-	-
11	26.00 (CH_2)	1.38 (2H, *m*)	
12	32.90 (CH_2)	1.63 (2H, *m*)	
13	45.37 (C)	-	-
14	48.83 (C)	-	-
15	35.52 (CH_2)	1.45 (2H, *m*)	
16	26.06 (CH_2)	1.40 (2H, *m*)	
17	52.19 (CH)	1.64 (1H, *m*)	C-13, C-14, C-16, C-18, C-21
18	18.10 (CH_3)	0.97 (3H, *s*)	C-8, C-12, C-14, C-17

Table 21 (continued)

position	δ_C (C-Type)*	δ_H (*multiplicity*, J_{Hz})	major HMBC
19	29.89 (CH_2)	α 0.35 (1H, *d*, 4.2)	C-1,C-8, C-9, C-10
		β 0.56 (1H, *d*, 4.2)	C-1, C-8, C-9, C-10
20	35.99 (CH)	1.30 (1H, *m*)	C-13, C-17, C-21, C-22
21	19.32 (CH_3)	0.92 (3H, *d*, 6.0)	C-13, C-17, C-22
22	34.78 (CH_2)	1.64 (2H, *m*)	
23	25.92 (CH_2)	1.37 (2H, *m*)	
24	155.64 (=C-H)	6.50 (1H, *t*, 7.2)	C-22, C-23, C-26, C-27
25	139.08 (C)	-	-
26	195.45 (CHO)	9.40 (1H, *s*)	C-24, C-25, C-27
27	9.16 (CH_3)	1.76 (3H, *s*)	C-23, C-24, C-26
28	18.06 (CH_3)	0.93 (3H, *s*)	C-8, C-13, C-15, C-17
29	14.00 (CH_3)	0.83 (3H, *s*)	C-2, C-3, C-5, C-10
30	25.42 (CH_3)	0.97 (3H, *s*)	C-3, C-5, C-29

***** Carbon type was deduced from DEPT experiments

3.1.8 MF 8: Cycloart-23-en-3β,25-diol



MF 8 was isolated as a white solid, m.p. 185-187 °C, $[\alpha]_D^{29}$ +57° (c 1.8×10^{-4} g/cm^3 in MeOH). The IR spectrum revealed the presence of a hydroxy group (3418 cm^{-1}). The ^{1}H NMR spectral data (**Table 22**) showed that **MF 8** was a derivative of 3β-hydroxy-5α-cycloartane. The characteristic signals of cyclopropyl methylene protons (H_2-19) were shown at δ 0.57 (*d, J* = 4.2 Hz) and 0.34 (*d, J* = 4.2 Hz). The signal of six tertiary methyl groups were shown as singlets at δ 1.35 (CH_3-26 and CH_3-27), 0.97 (CH_3-28 and CH_3-30), 0.85 (CH_3-18) and 0.81 (CH_3-29). The CH_3-21 resonated as a doublet (*J* = 8.1 Hz) at δ 0.87. The doublet of doublet resonance of H-3 with coupling constants of 10.8 and 4.5 Hz regarded **MF 8** as a triterpene with 3β-hydroxy group. Chemical shift of C-23 (δ 130.61) and C-24 (δ 134.54) suggested the presence of two vinylic protons (=CH-23 and =CH-24). The large coupling constants (*J* = 15.6 Hz) observed in the ^{1}H NMR spectrum for the double bond signals in **MF 8** indicated the trans nature of this bond. The resonances of two methyls were observed at δ 1.35 (6H). They were assigned for oxymethyl group CH_3-26 and CH_3-27. The side chain was confirmed to be at C-17 from the HMBC correlation of H-21 to C-17. Consequently, cycloart-23-en-3β,25-diol was assigned to **MF 8** (Weber, *et al*., 2001). The data of ^{13}C NMR, DEPT 135°, DEPT 90° and HMBC (**Table 22**) corresponded to the assigned structure.

Table 22 The ^{13}C, ^{1}H NMR and HMBC spectral data of **MF 8**

position	δ_C (C-Type)	δ_H (*multiplicity*, J_{Hz})	major HMBC
1	31.95 (CH_2)	1.28 (2H, *m*)	
2	30.33 (CH_2)	1.74 (1H, *m*), 1.60 (1H, *m*)	
3	78.87 (CH)	3.30 (1H, *dd*, 10.8, 4.5)	C-2, C-29
4	40.47 (C)	-	-
5	47.09 (CH)	1.30 (1H, *m*)	C-3, C-4, C-29
6	21.10 (CH_2)	1.40 (2H, *m*)	
7	25.99 (CH_2)	1.29 (2H, *m*)	
8	47.95 (CH)	1.51 (1H, *m*)	C-9, C-10, C-14, C-15, C-19
9	19.96 (C)	-	-
10	26.10 (C)	-	-
11	26.42 (CH_2)	1.30 (2H, *m*)	
12	32.79 (CH_2)	1.60 (2H, *m*)	
13	45.31 (C)	-	-
14	48.83 (C)	-	-
15	35.56 (CH_2)	1.29 (2H, *m*)	
16	28.08 (CH_2)	1.29 (1H, *m*), 1.9 (1H, *m*)	
17	52.06 (CH)	1.56 (1H, *m*)	C-12, C-13, C-14, C-18, C-21
18	18.08 (CH_3)	0.85 (3H, *s*)	C-8, C-12, C-14, C-17

Table 22 (continued)

position	δ_C (C-Type)*	δ_H (*multiplicity*, J_{Hz})	major HMBC
19	29.87 (CH_2)	α 0.34 (1H, *d*, 4.2)	C-1, C-8, C-9, C-11
		β 0.57 (1H, *d*, 4.2)	C-1, C-8, C-9, C-11
20	36.29 (CH)	1.31 (1H, *m*)	
21	18.34 (CH_3)	0.87 (3H, *d*, 8.1)	C-13, C-17, C-20, C-22
22	39.37 (CH_2)	2.16 (1H, *m*), 1.70 (1H, *m*)	
23	130.61 (=C-H)	5.69 (1H, *ddd*, 15.6, 9.9, 2.4)	C-22, C-24, C-25
24	134.54 (=C-H)	5.54 (1H, *d*, 15.6)	C-22, C-23, C-25
25	70.78 (C)	-	-
26	24.42 (CH_3)	1.35 (3H, *s*)	C-24, C-25, C-27
27	24.40 (CH_3)	1.35 (3H, *s*)	C-24, C-25, C-26
28	25.43 (CH_3)	0.97 (3H, *s*)	C-5, C-13, C-15, C-17
29	14.00 (CH_3)	0.81 (3H, *s*)	C-2, C-3, C-4, C-5
30	19.29 (CH_3)	0.97 (3H, *s*)	C-3, C-4, C-5

* Carbon type was deduced from DEPT experiments

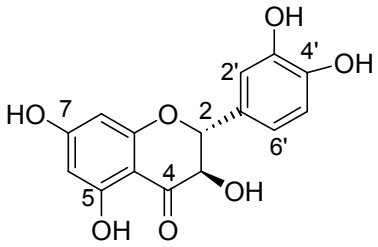
Table 23 Comparison of the ^{13}C NMR spectral data between **MF 8** and **Cycloart-23-en-3β,25-diol**

position	**MF 8***	**Cycloart-23-en-3β,25-diol***
1	31.95	32.0
2	30.33	30.4
3	78.87	78.8
4	40.47	40.5
5	47.09	47.1
6	21.10	21.1
7	25.99	26.0
8	47.95	48.0
9	19.96	20.0
10	26.10	26.1
11	26.42	26.5
12	32.79	32.8
13	45.31	45.3
14	48.83	48.8
15	35.56	35.6
16	28.08	28.1
17	52.06	52.0
18	18.08	18.1
19	29.87	29.9
20	36.29	36.4

Table 23 (continued)

position	**MF 8***	**Cycloart-23-en-3β,25-diol***
21	18.34	18.3
22	39.37	39.0
23	130.61	125.6
24	134.54	139.4
25	70.78	70.7
26	24.42	30.0
27	24.40	29.9
28	25.43	25.4
29	14.00	14.0
30	19.29	19.3

3.1.9 MF 9: Taxifolin



MF 9 was isolated as a yellow solid, m.p. 206-208 °C. The UV spectrum showed the maximum absorptions at 331 and 289 nm. The presence of a carbonyl group (1638 cm^{-1}) and the hydroxy group (3374 cm^{-1}) were suggested in the IR spectrum. The optical rotation was $[\alpha]_D^{29}$ +105.2° (c 1.6×10^{-4} g/cm^3 in MeOH). The ^1H NMR spectral data (**Table 24**) showed a sharp singlet signal of a chelated hydroxyl group (5-OH) at δ 11.70 and three broad singlet signals of three hydroxyl

groups at δ 10.54, δ 8.59 and δ 8.49. A *meta* coupling signal of aromatic protons were present at δ 5.94 and δ 5.99 and were deduced to be signals of H-6 and H-8 from the correlation of H-6 (δ 5.94) to C-4a (δ 100.63), C-5 (δ 162.73), C-7 (δ 167.14), C-8 (δ 96.34) and of H-8 (δ 5.99) to C-4a (δ 100.63), C-6 (δ 95.27), C-7 (δ 167.14) and C-8a (δ 163.66). The ABX pattern of three aromatic protons were resonated at δ 6.84, 6.86 and 7.01 and they were assigned for H-6′, H-5′, and H-2′, respectively. The location of 3′,4′-hydroxyphenyl at C-2 was determined from HMBC correlations of H-2 to C-1′ (δ 128.19), C-2′ (δ 115.34) and C-6′ (δ 119.51). The characteristic resonances of H-2 and H-3 of flavanonol type were shown at δ 4.94 (*d*, J = 12.0 Hz) and 4.49 (*dd,* J = 12.0, 4.5 Hz). The NOE experiment by irradiation at H-2 found no effect on the resonance of H-3. This evidence implied the trans position of H-2 and H-3. The ^{13}C NMR spectrum showed signals of fifteen carbons. Analysis of the DEPT 135° and 90° spectra indicated the presence of a carbonyl carbon (δ 197.58), six methine carbons (δ 119.51, 115.34, 96.34, 95.27, 83.38, and 71.96) and seven quaternary carbons (δ 167.14, 136.66, 162.73, 145.98, 145.15, 128.19 and 100.63). Consequently, **MF 9** was proposed to be 3,5,7,3′,4′-pentahydroxyflavanonol of which (+) isomer was indicated from optical rotation of $[\alpha]_D^{29}$ +105.2° (c 1.6×10^{-4} g/cm^3 in MeOH). This compound was known as taxifolin (Lennart, *et al*, 1988).

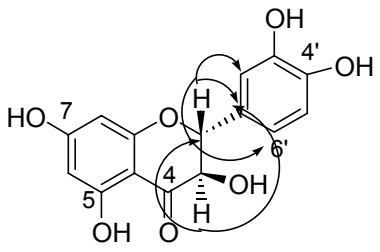


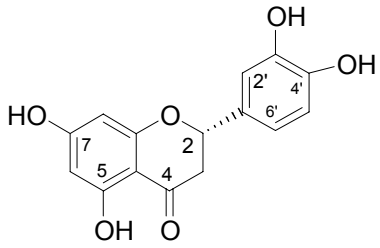
Figure 7 Selected HMBC correlations of **MF 9**

Table 24 The ^{13}C, ^{1}H NMR and HMBC spectral data of **MF 9**

position	δ_C (C-Type)*	δ_H (*multiplicity*, J Hz)	HMBC
2	83.38 (CH)	4.94 (1H, *d*, 12.0)	C-1′, C-2′, C-6′
3α	71.96 (CH)	4.49 (1H, *dd*, 12.0, 4.5)	C-2, C-1′
3-OH		5.24 (1H, *d*, 4.5)	C-2
4	197.58 (C=O)	-	-
4a	100.63 (C)	-	-
5	162.73 (C)	11.70 (*s*, OH)	C-6, C-4a, C-8a
6	95.27 (CH)	5.94 (1H, *d*, 2.0)	C-5, C-7, C-4a, C-8
7	167.14 (C)	10.54 (*br s*, OH)	-
8	96.34 (CH)	5.99 (1H, *d*, 2.0)	C-6, C-7, C-4a, C-8a
8a	163.66 (C)	-	-
1′	128.19 (C)	-	-
2′	115.34 (CH)	7.01 (1H, *s*)	C-2, C-1′, C-2′, C-3′, C-4′, C-6′
3′	145.98 (C)	8.49 (*br s*, OH)**	-
4′	145.15 (C)	8.59 (*br s*, OH)**	-
5′	115.34 (CH)	6.86 (1H, *d*, 8.0)	C-2, C-1′, C-3′, C-4′
6′	119.51 (CH)	6.84 (1H, *d*, 8.0)	C-2, C-1′, C-2′, C-4′

* Carbon type was deduced from DEPT experiments **signals maybe reversed

3.1.10 MF 10: Eriodictyol



MF 10 was obtained as a yellow solid, m.p. 250-252°C. The UV absorption maxima in MeOH (227, 288 and 330 nm) suggested a flavanone structure. The IR absorption bands at 3255 cm^{-1} and 1638 cm^{-1} indicated the presence of hydroxyl and carbonyl groups, respectively. The ^{1}H NMR spectral data (**Table 25**) of **MF 10** showed the signals of a chelated phenolic hydroxyl group (5-OH) at δ 12.08, three non chelated hydroxyl groups at δ 11.70, 8.59, and 8.49. The presence of doublet of doublet at δ 5.28 (1H, *dd, J* = 12.0, 3.0 Hz), 3.07 (1H, *dd, J* = 17.1, 12.0 Hz) and 2.71 (1H, *dd, J* = 17.1, 3.0 Hz) suggested the characteristic resonances of typical H-2, H-3α and H-3β of a flavanone type. The ABX pattern at δ 6.94 (1H, *d*, *J* = 2.1 Hz), 6.83 (1H, *d*, *J* = 8.0 Hz), and 6.75 (1H, *dd J* = 8.0, 2.1 Hz) were assigned to be the resonances of H-2′, H-5′ and H-6′, respectively. The resonances at δ 5.94 (2H, *br s*) were assigned for H-6 and H-8.

The ^{13}C NMR spectrum showed fifteen signals. The DEPT spectra suggested a carbonyl carbon, six methine carbons, a methylene carbon and six quaternary carbons. In the HMBC spectrum the correlation of H-2 (δ 5.28) to C-1′ (δ 134.5) and C-6′ (δ 122.85) suggested that 3′,4′ hydroxy phenyl unit was at C-2 (**Figure 8**). The structure of **MF 10** was then concluded to be 5,7,3′,4′-tetrahydroxyflavanone which corresponded to eriodictyol.

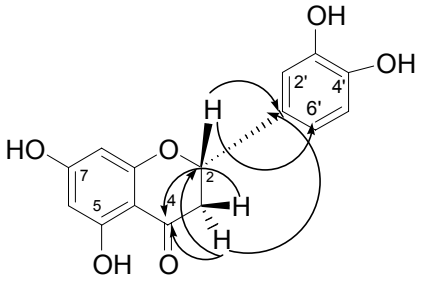


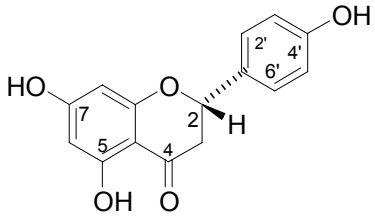
Figure 8 Selected HMBC correlation of **MF 10**

Table 25 The ^{13}C, ^{1}H NMR and HMBC spectral data of **MF 10**

position	δ_C (C-Type)*	δ_H (*multiplicity*, J Hz)	HMBC
2	83.69 (CH)	5.28 (1H, *dd*, 12.0, 3.0)	C-1′, C-6′
3	47.54 (CH_2)	β 2.71 (1H, *dd*, 17.1, 3.0)	C-4
		α 3.07 (1H, *dd*, 17.1, 12.0)	C-2, C-4, C-1′
4	200.76 (C=O)	-	-
4a	107.00 (C)	-	-
5	167.89 (C)	12.08 (*s*, OH)	C-6, C-7, C-4a, C-8a
6	101.10 (CH)	5.94 (1H, *br s*)	C-4, C-5, C-7, C-8, C-4a
7	171.82 (C)	11.70 (*br s*, OH)**	-
8	100.18 (CH)	5.94 (1H, *br s*)	C-4, C-6, C-7, C-4a, C-8a
8a	168.75 (C)	-	-
1′	134.50 (C)	-	-
2′	119.01 (CH)	6.94 (1H, *d*, 2.1)	C-2, C-2′, C-3′, C-4′, C-6′
3′	150.50 (C)	8.49 (*br s*, OH)**	-
4′	150.22 (C)	8.59 (*br s*, OH)**	-
5′	134.00 (CH)	6.83 (1H, *d*, 8.0)	C-1′, C-3′, C-4′
6′	122.85 (CH)	6.75 (1H, *dd*, 8.0, 2.1)	C-2, C-1′, C-2′, C-4′

* Carbon type was deduced from DEPT experiments **signals maybe reversed

3.1.11 MF 11: Naringenin



MF 11 was isolated as a yellow solid, m.p. 240-242 °C. The UV spectrum showed maximum absortions at 225, 286 and 333 nm, which were a typical absorptions of flavanones. The IR spectrum showed the streching of hydroxyl group (3378 cm^{-1}) and carbonyl group (1637 cm^{-1}). The ^{1}H NMR spectral data (**Table 26**) showed the resonances of a chelated hydroxy group at δ 12.08, two non-chelated hydroxy groups at δ 10.40 and 9.30. An AA'BB' type signals which were assigned to be signals of H-2', H-6' and H-3', H-5' at δ 7.31 (2H, *d*, *J* = 8.4 Hz) and δ 6.90 (2H, *d*, *J* = 8.4 Hz), respectively. The resonances of H-2, H-3α and H-3β of flavanone characteristic were observed at δ 5.31 (1H, *dd*, *J* = 13.2, 2.7 Hz), 3.08 (1H, *dd, J* = 17.1, 13.2 Hz) and 2.71 (1H, *dd*, *J* = 17.1, 2.7 Hz). The resonances at δ 5.94 (*br s*) and 5.95 (*br s*) were assigned for H-6 and H-8. The ^{13}C NMR spectrum showed the resonances of six aromatic methine carbons: C-3′, C-5′ (δ 120.43), C-2′, C-6′ (δ 132.64), C-8 (δ 100.26) and C-6 (δ 101.24); six quaternary aromatic carbon at C-7 (δ 171.79), C-5 (δ 167.90), C-8a (δ 168.83), C-4a (δ 107.01), C-1′ (δ 133.76) and C-4′ (δ 162.73); an oxymethine carbon signal C-2 (δ 83.66); a methylene carbon C-3 (δ 47.70) and a carbonyl carbon C-4 (δ 200.54). Consequently, **MF 11** was proposed to be 5,7,4′-trihydroxyflavanone. The results from HMBC (**Table 26**) confirmed the assigned structure. This compound was known as naringenin. It was indicated to be (-) isomer from optical rotation of $[\alpha]_D^{29}$ –18.5 °(c 1.2×10^{-4}.g/cm^3 in MeOH).

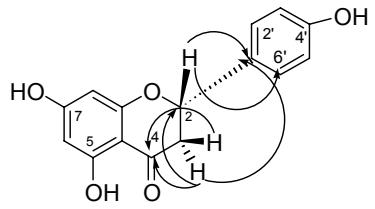


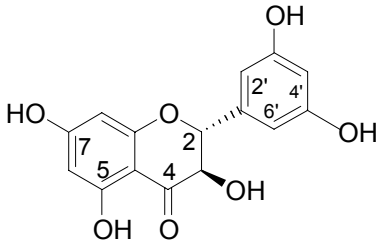
Figure 9 Selected HMBC correlation of **MF 11**

Table 26 The ^{13}C, ^{1}H NMR and HMBC spectral data of **MF 11**

position	δ_C (C-Type)*	δ_H (*multiplicity*, J_{Hz})	HMBC
2	83.66 (CH)	5.31 (1H, *dd*, 13.2, 2.7)	C-4, C-2′, C-1′, C-6′
3	47.70 (CH_2)	β 2.71 (1H, *dd*, 17.1, 2.7)	C-3, C-4, C-4a
		α 3.08 (1H, *dd*, 17.1, 13.2)	C-2, C-3, C-4, C-1′
4	200.54 (C=O)	-	-
4a	107.01 (C)	-	-
5	167.90 (C)	12.08 (*s*, OH)	C-5, C-6, C-4a
6	101.24 (CH)	5.94 (1H, *br s*)	C-4, C-5, C-6, C-7, C-4a, C-8a
7	171.79 (C)	10.40 (*br s*)**	-
8	100.26 (CH)	5.95 (1H, *br s*)	C-4, C-5, C-6, C-7, C-4a, C-8a
8a	168.83 (C)	-	-
1'	133.76 (C)	-	-
2'	132.64 (CH)	7.31 (1H, *d*, 8.4)	C-2, C-1′, C-2′, C-3′, C-4′
3'	120.43 (CH)	6.90 (1H, *d*, 8.4)	C-2′, C-3′, C-4′
4'	162.73 (C)	9.30 (*br s*)**	-
5'	120.43 (CH)	6.90 (1H, *d*, 8.4)	C-2′, C-3′, C-4′
6'	132.64 (CH)	7.31 (1H, *d*, 8.4)	C-2, C-1′, C-2′, C-3′, C-4′

* Carbon type was deduced from DEPT experiments **signals maybe reversed

3.1.12 MF 12: 3,5,7,3′,5′-Pentahydroxyflavanonol



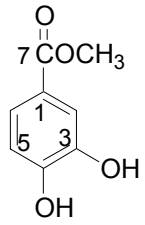
MF 12 was isolated as a yellow solid, m.p. 210-211 °C. The UV spectrum showed the characteristic absorbances for a dihydroflavonol [λ_{max} 289 and 331 nm]. The IR spectrum indicated the presence of hydroxyl (3353 cm^{-1}) and carbonyl (1639 cm^{-1}) groups. The characteristic ^{1}H NMR signal of H-2 and H-3 in axial conformation of 2,3-*trans* dihydroflavonol were observed at δ 4.93 (1H, *d*, *J* = 11.4 Hz) and 4.44 (1H, *d, J* = 11.4 Hz). The presence of a chelated phenolic hydroxy group (5-OH) were suggested from the resonance at δ 11.70. A *meta*-coupled signal of aromatic protons were present at δ 5.91 and 5.87 and were attributed to be the signals of H-6 and H-8. The correlations of H-6 (δ 5.91) to C-4a (δ 105.00), C-5 (δ 167.50), C-8 (δ 100.49) and of H-8 (δ 5.81) to C-4a (δ 105.00), C-4 (δ 201.44), C-6 (δ 101.52) confirmed the assignment. The signals at δ 6.77 (2H, *br s*) and 6.98 (1H, *br s*) were assigned to H-2′, H-6′ and H-4′ of ring B. The location of 3′,5′-hydroxyphenyl at C-2 was determined from HMBC correlations of H-2 to C-1′ and C-2′. The ^{13}C NMR spectrum and DEPT experiments (**Table 27**) showed the resonances of seven quaternary carbons, seven methine carbons and a carbonyl carbon. The NMR structural data proved that **MF 12** was 3,5,7,3′,5′-pentahydroxyflavanonol.

Table 27 The ^{13}C, ^{1}H NMR and HMBC spectral data of **MF 12**

position	δ_C (C-Type)*	δ_H (*multiplicity*, J Hz)	HMBC
2	88.17 (CH)	4.93 (1H, *d*, 11.4)	C-4, C-8a, C-1′, C-6′
3α	77.00 (CH)	4.44 (1H, *d*, 11.4)	C-2, C-4, C-1′, C-2′
3-OH		4.44 (1H, *d*, 11.4)	C-2, C-4, C-1′, C-2′
4	201.44 (C=O)	-	-
4a	105.00 (C)	-	-
5	167.50 (C)	11.70 (*s*, OH)	-
6	101.52 (CH)	5.91 (1H, *d*, 1.5)	C-4, C-5, C-4a, C-8
7	172.21 (C)	-	-
8	100.47 (CH)	5.87 (1H, *d*, 1.5)	C-4, C-6, C-4a
8a	100.47 (C)	-	-
1'	133.01 (C)	-	-
2'	120.13 (CH)	6.77 (1H, *br s*)	C-2, C-1′, C-3′, C-4′, C-6′
3'	150.53 (C)	-	-
4'	119.76 (CH)	6.98 (1H, *br s*)	C-2, C-3′, C-5′, C-6′
5'	149.61 (C)	-	-
6'	124.51 (CH)	6.77 (1H, *br s*)	C-2, C-1′, C-2′, C-4' C-5′

***** Carbon type was deduced from DEPT experiments

3.1.13 MF 13: 3,4-Dihydroxy benzoic acid methyl ester



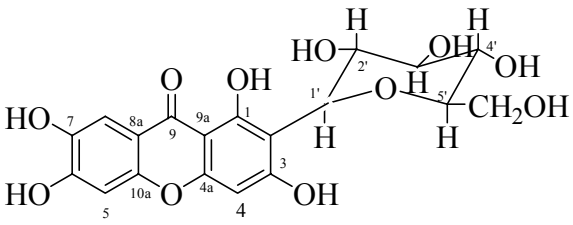
MF 13 was obtained as a pale yellow solid, m.p. 106-107 °C. The UV spectrum showed maximum absorption bands at 215, 257 and 291 nm which revealed the presence of a conjugated system. The IR spectrum showed absorption bands at 3484 cm^{-1} (a hydroxy group) and 1681 cm^{-1} (a carbonyl group). The ^{1}H NMR spectral data (**Table 28**) displayed signals of aromatic protons as ABX pattern at δ 7.63 (*dd*, *J* = 8.0, 2.0 Hz, H-6), δ 7.57 (*d*, *J* = 2.0 Hz, H-2) and δ 6.92 (*d*, *J* = 8.0 Hz, H-5). The presence of a methyl carbonate group was derived from the resonance of $-OCH_3$ at δ 3.93 and the resonance of a ketoester group at δ 168.38 as well as C=O stretching at 1681 cm^{-1}. **MF 13** then was assigned to be tri-substituted benzene derivative with two hydroxyl groups and a methyl carbamate group. The carbon resonances in ^{13}C NMR corresponded to the proposed structure at δ 122.31(C), 112.15 (CH), 146.46 (C), 150.26 (C), 114.24 (CH), 124.09 (CH), 168.38 (C=O) and 55.82 (CH_3). Consequently, **MF 13** was identified as 3,4-dihydroxy benzoic acid methyl ester.

Table 28 The ^{13}C, ^{1}H NMR and HMBC spectral data of **MF 13**

position	δ_C (C-Type)*	δ_H (*multiplicity*, J_{Hz})	HMBC
1	122.31 (C)	-	-
2	112.15 (CH)	7.57 (1H, *d*, 2.0)	C-1, C-3, C-4, C-6, C-7
3	146.46 (C-OH)	-	-
4	150.26 (C-OH)	-	-
5	114.24 (CH)	6.92 (1H, *d*, 8.0)	C-1, C-3, C-4
6	124.09 (CH)	7.63 (1H, *dd*, 8.0, 2.0)	C-2, C-4, C-7
$\underline{CO}OCH_3$	168.38	-	-
$O\underline{CH_3}$	55.82	3.93 (3H, *s*)	-

***** Carbon type was deduced from DEPT experiments

3.1.14 MF 14: Mangiferin



MF 14 is a yellow solid, m.p. 276-278 °C. The UV spectrum showed maximum absorption bands at 366, 315, 257 and 240 nm. The IR spectrum showed the absorption bands of hydroxyl group at 3375 $cm^{-1}$and conjugated carbonyl group at 1629 cm^{-1}. The presence of the carbonyl functionality was confirmed by the signal at δ 179.43 in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum exhibited a sharp singlet signal of a hydroxy proton which formed intramolecular hydrogen bond to a carbonyl group at δ 13.75. Three singlet signals in aromatic region, δ 6.36, 6.86 and 7.37 were observed and assigned to be the signals of aromatic protons H-4, H-5 and H-8, respectively. An anomeric proton H-1' was detected as a doublet at δ 4.58 with a coupling constant of 10.0 Hz implying the β-glucosyl unit which was deduced from the value of coupling constants. The resonances at δ 4.58, 4.04, 3.19, 3.15, 3.41 and 3.67 were assigned to H-1′, H-2′, H-3′, H-4′, H-5′ and H-6′, respectively on sugar moiety. This assignment were confirmed by ^{1}H-^{1}H COSY experiment (**Table 29**) and HMBC experiment (**Table 30**). The ^{13}C NMR spectral data (**Table 30**) showed signals of nineteen carbons. The DEPT spectra indicated the existence of 13 carbon signals of aglycone part; three methine carbons; C-4 (δ 93.64), C-5 (δ 102.92) and C-8 (δ 108.38); nine quaternary carbons; C-1 (δ 162.09), C-2 (δ 107.88), C-3 (δ 164.16), C-4a (δ 156.58), C-6 (δ 154.40), C-7 (δ 144.07), C-8a (δ 112.02), C-9a (δ 101.62) and C-10a (δ 151.14). Six oxygenated carbon signals of sugar moiety were shown; C-6′ (δ 61.80), C-2′ (δ 70.59), C-4′ (δ 70.93), C-1′ (δ 73.40) and C-5′ (δ 81.82). The linkage of a sugar moiety to aglycone was provided from the correlations of anomeric proton (H-1′) to C-1 (δ 62.09), C-2 (δ 107.88) and C-3 (δ 164.16) of aglycone on HMBC spectral data (**Table 30**). This assignment

demonstrated that **MF 14** was 2-*C*-*β*-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone which was known as mangiferin (Pauletti, *et al*., 2003).

Table 29 ^{1}H-^{1}H COSY correlations data of **MF 14**

proton		correlated proton
H-1′	⟷	H-2′
H-2′	⟷	H-1′and H-3′
H-3′	⟷	H-2′
H-4′	⟷	H-5′
H-5′	⟷	H-4′ and H-6′b
H-6′b	⟷	H-6′a

Table 30 The ^{13}C, ^{1}H NMR and HMBC spectral data of **MF 14**

position	δ_C (C-Type)*	δ_H (*multiplicity*, J_{Hz})	HMBC
1	162.09 (C)	13.75 (1H, *s*)	C-2, C-3, C-9, C-9a
2	107.88 (C)	-	-
3	164.16 (C)	-	-
4	93.64 (CH)	6.36 (1H, *s*)	C-4, C-4a, C-9a
4a	156.58 (C)	-	-
5	102.92 (CH)	6.86 (1H, *s*)	C-6, C-7, C-9, C-8a, C-10a
6	154.40 (C)	-	-
7	144.07 (C)	-	-
8	108.38 (CH)	7.37 (1H, *s*)	C-6, C-7, C-9, C-10a

Table 30 (continued)

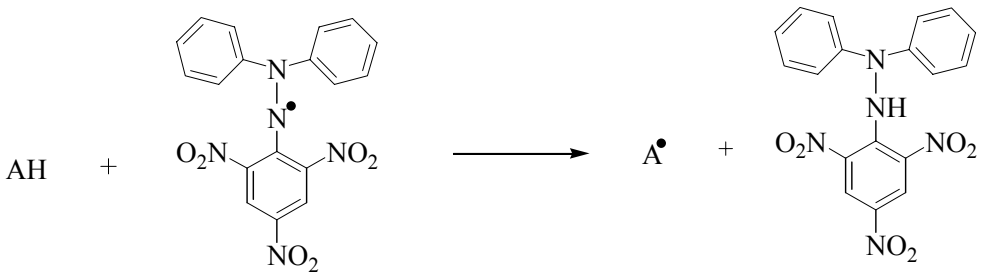
position	δ_C (C-Type)*	δ_H (*multiplicity*, J_{Hz})	HMBC
8a	112.02 (C)	-	-
9	179.43 (C=O)	-	-
9a	101.62 (C)	-	-
10a	151.14 (C)	-	-
1′	73.40(CH)	4.58 (1H, *d*, 10.0)	C-1, C-2, C-3, C-2', C-3', C-5'
2′	70.59 (CH)	4.04 (1H, *t*, 9.0)	C-1', C-3'
3′	79.28 (CH)	3.19 (1H, *dd*, 17.0, 8.0)	C-4'
4′	70.93 (CH)	3.15 (1H, *m*)	C-3′, C-5′, C-6′
5′	81.82 (CH)	3.41 (1H, *m*)	C-5′
6′	61.80 (CH_2)	3.67 (1H, *d*, 10.5) 3.41 (1H, *m*)	C-5′

* Carbon type was deduced from DEPT experiments

3.2 Evalution of Antioxidation Activity

Lipid peroxidation is one of the major factors causing deterioration of foods during the storage and processing. Oxidized polyunsaturated fatty acids may induce aging and carcinogenesis. Although there are some synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are commonly used in processed foods, it has been reported that these compounds have some side effects (Branien, 1975; Ito, *et al*., 1983). Therefore, many researchers have focused on natural antioxidant sources.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is one of the method used for antioxidant testing on free radical terminator. Its odd electron can be used as a convenient tool for the antioxidant assay. DPPH is a stable free radical which shows a purple color and a strong absorption at 517 nm. When DPPH radical accepts hydrogen radical, a more stable compound will form and consequently its characteristic absorption at 517 nm vanishes. The capability of the substances to donate electrons can be estimated from the degree of loss of color (Blois, 1958). Coexistence of an antioxidant compound (AH) and free radical DPPH leads to the disappearance of DPPH free radical and to the appearance the free radical $A^{\bullet}$.



Purple

3.2.1 Screening on the free radical scavenging activity of crude extracts

To determine the scavenging activity, the crude extracts of *M. odorata* were tested for scavenging activity at final concentration of 30 μg/mL in ethanol. The activity was monitored by following the decrease of the absorbance of the solution at 517 nm. The decrease of the absorbance was observed in the solution of acetone and methanolic extracts, whereas the solution of dichloromethane extract showed slightly decolorization. The results indicated that acetone and methanolic extracts were able to scavenge the DPPH radical significantly.

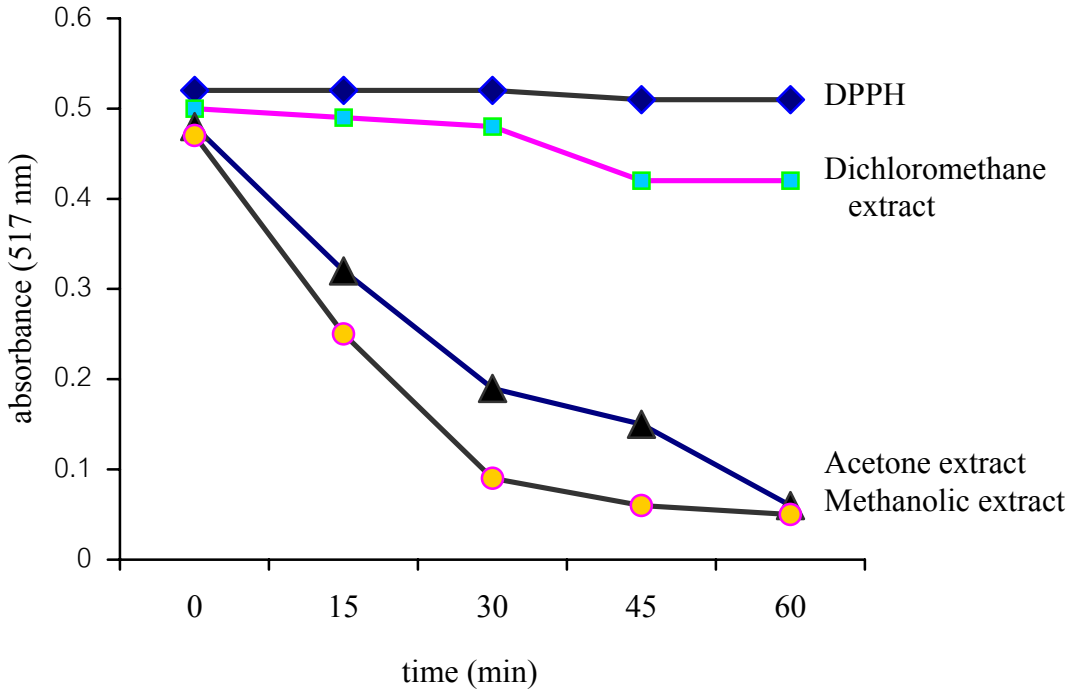


Figure 10 The scavenging activity of crude extracts

The activity was further evaluated in term of the concentration required to scavenge 50 % DPPH free radical (IC_{50}). The results indicated that the acetone and methanolic extracts showed radical scavenging activity with IC_{50} of 24.0 and 14.0 μg/mL, respectively.

Table 31 Inhibitory concentration (IC_{50}) of the crude extracts

sample	IC_{50} (μg/mL, 30 min)
Dichloromethane extract	> 100
Acetone extract	24.0
Methanolic extract	14.0

3.2.2 Free radical scavenging activity of the pure compounds

Pure compounds **MF 9 - MF 14** were tested for radical scavenging activity at the final concentration of 35 μM.

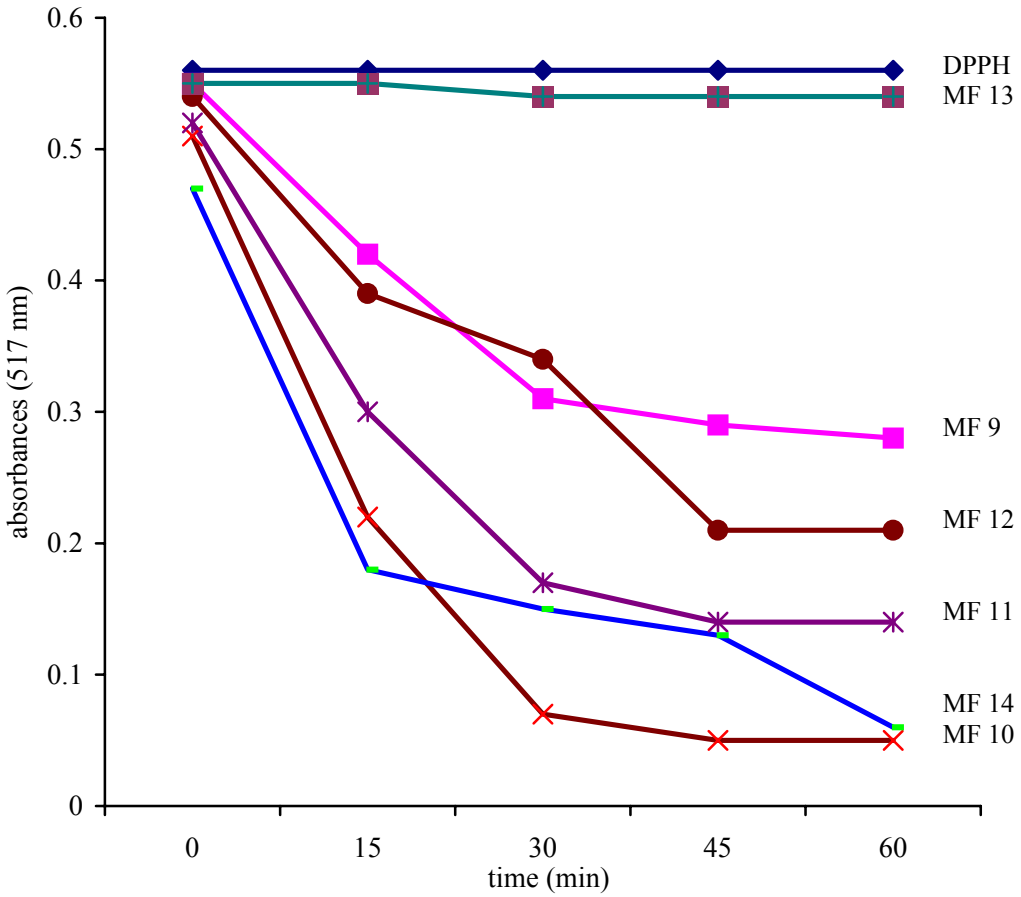


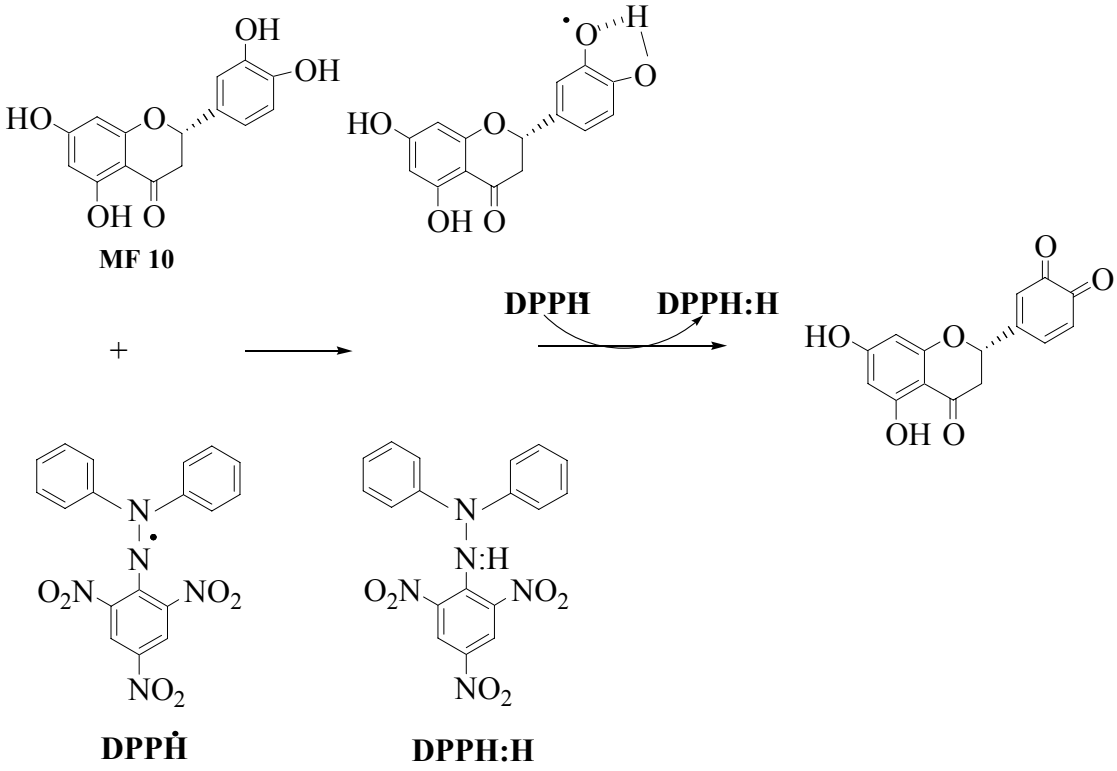
Figure 11 Scavenging activity of compounds from *M. odorata*

The results showed that the tested compounds except **MF 13** exhibited free radical scavenging activity. The inhibitory concentration (IC_{50}) of pure compounds were further evaluated (**Table 32**).

Table 32 Inhibitory concentration (IC_{50}) of pure compounds

compound	IC_{50} (μM, 30 min)
MF 9	40.0
MF 10	18.0
MF 11	25.0
MF 12	55.0
MF 14	13.0

The results in terms of IC_{50} showed that **MF 10**, **MF 11** and **MF 14** showed strong radical scavenging activity. The activity of **MF 10** and **MF 14** possibly exhibited by the catechnolic moiety, ortho dihydroxyl groups (3-OH and 4-OH of B-ring in **MF 10** and 6-OH and 7-OH of A-ring in **MF 14**) (Sata, *et al*., 1992). The mechanism of trapping radical by **MF 10** could be explained. **MF 10** was a phenolic compound, it was therefore capable of donating hydrogen radical. The phenoxy radical was stabilized through an intramolecular hydrogen bonding. A subsequent interaction with a second DPPH radical afforded the dehydro form of **MF 10** as a final product (Shahidi, *et al*., 1992).



In the same means, the activity of **MF 14** was proposed to be due to the catechnolic structure of phenolic hydroxyl groups at 6 and 7 positions. The experiment served as a preliminary indication of the potential of using *Mangifera odorata* as antioxidant.

Conclusion

Investigation of the chemical constituents of dichloromethane, acetone and methanolic extracts of the twigs of *M. odorata* led to the isolation of 14 compounds: 3*β*-taraxerol (**MF 1**), friedelin (**MF 2**), *β*-sitosterol (**MF 3**), isomangiferolic acid (**MF 4**), mangiferolic acid (**MF 5**), mangiferonic acid (**MF 6**), 3*β*-hydroxy-5*α*-cycloart-24-en-26-al (**MF 7**), cycloart-23-en-3*β*,25-diol (**MF 8**), taxifolin (**MF 9**), eriodictyol (**MF 10**), naringenin (**MF 11**), 3,5,7,3′,5′-pentahydroxyflavanonol (**MF 12**), 3,4-dihydroxy benzoic acid methyl ester (**MF 13**) and mangiferin (**MF 14**).

MF 1 - MF 8, MF 9 and **MF 10 - MF 14** were obtained from the dichloromethane, acetone and methanolic extracts, respectively. The crude extracts

and samples of the pure compounds (**MF 9 - MF 14**) were examined for their antioxidative activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The acetone extract, methanolic extract, **MF 10**, **MF 11** and **MF 14** showed strong radical scavenging activity. The other pure compounds showed moderate activity. This work has demonstrated that *Mangifera odorata* are among the potential sources of antioxidation activity. Further exploration should be performed to search for compounds with greater efficacy and specificity for the treatment of many human diseases.

APPENDIX