CHAPTER IV

RESULTS AND DISCUSSION

4.1 Results of separation of *B. superba* Roxb. crude extract

The methanol crude extract (40.01 g) was separated flash column chromatographic technique by using dichloromethane - methanol gradient in a stepwise fashion. The results from separation of methanol crude extract are summarized in Table 2.

Table 2 Results form separation of methanol crude extract

Fraction Number	Eluent	Appearance	Weight (g)
1	100% CH_2Cl_2	Greenish oil	2.9782
2	100% CH_2Cl_2	White solid in yellowish oil	2.0504
3	1% MeOH in CH_2Cl_2	Yellowish oil	3.5614
4	1% MeOH in CH_2Cl_2	Solid in brown oil	2.1260
5	1.5% MeOH in CH_2Cl_2	Solid in Brown oil	2.4894
6	2% MeOH in CH_2Cl_2	Solid in Brown oil	2.4069
7	2.5% MeOH in CH_2Cl_2	Brown oil	1.6611
8	3% MeOH in CH_2Cl_2	Brown oil	0.7784

Table 2 Continued

Fraction Number	Eluent	Appearance	Weight (g)
9	3.5% MeOH in CH_2Cl_2	Brown oil	0.6784
10	4% MeOH in CH_2Cl_2	Brown oil	0.9597
11	4.5% MeOH in CH_2Cl_2	Brown oil	0.2604
12	5% MeOH in CH_2Cl_2	Brown oil	0.4650
13	5.5% MeOH in CH_2Cl_2	White solid in brown oil	0.6650
14	6% MeOH in CH_2Cl_2	Brown oil	1.2012
15	6.5% MeOH in CH_2Cl_2	Solid in brown oil	0.3192
16	7% MeOH in CH_2Cl_2	Solid in brown oil	0.6713
17	7.5% MeOH in CH_2Cl_2	Solid in brown oil	0.4736
18	8% MeOH in CH_2Cl_2	Brown oil	0.3662
19	8.5% MeOH in CH_2Cl_2	Brown oil	0.2769
20	9% MeOH in CH_2Cl_2	Brown oil	0.8299
21	9.5% MeOH in CH_2Cl_2	Brown oil	0.4261
22	10% MeOH in CH_2Cl_2	Solid in brown oil	1.8076
23	10.5% MeOH in CH_2Cl_2	Brown oil	0.9455
24	11% MeOH in CH_2Cl_2	Solid in brown oil	0.1907
25	11.5% MeOH in CH_2Cl_2	Brown gummy	0.2246

Table 2 Continued

Fraction Number	Eluent	Appearance	Weight (g)
26	12% MeOH in CH_2Cl_2	Brown gummy	0.2888
27	12.5% MeOH in CH_2Cl_2	Brown gummy	0.2788
28	13% MeOH in CH_2Cl_2	Brown gummy	0.1810
29	13.5% MeOH in CH_2Cl_2	Brown gummy	0.3597
30	14% MeOH in CH_2Cl_2	Brown gummy	0.1276
31	14.5% MeOH in CH_2Cl_2	Brown gummy	0.2976
32	15% MeOH in CH_2Cl_2	Brown gummy	0.3195
33	15.5% MeOH in CH_2Cl_2	Brown gummy	0.2701
34	16.5% MeOH in CH_2Cl_2	Brown gummy	0.1426
35	20% MeOH in CH_2Cl_2	Dark brown gummy	1.2116
36	25% MeOH in CH_2Cl_2	Dark brown gummy	0.4852
37	30% MeOH in CH_2Cl_2	Dark brown gummy	0.2611
38	35% MeOH in CH_2Cl_2	Dark brown gummy	0.0852
39	40% MeOH in CH_2Cl_2	Dark brown gummy	0.1251
40	45% MeOH in CH_2Cl_2	Dark brown gummy	0.2542
41	50% MeOH in CH_2Cl_2	Dark brown gummy	0.3887
42	60% MeOH in CH_2Cl_2	Brown gummy	0.3806

Table 2 Continued

Fraction Number	Eluent	Appearance	Weight (g)
43	70% MeOH in CH_2Cl_2	Brown gummy	0.2872
44	80% MeOH in CH_2Cl_2	Brown gummy	0.1681
45	90% MeOH in CH_2Cl_2	Brown gummy	0.1528
46	100% MeOH	Brown gummy	0.1432

The fractions that separated by flash column chromatography were purified by crystallization and preparative TLC. The results of purified compounds are summarized in Table 3.

Table 3 Results from purified compounds of *B. superba* Roxb. crude extract

Item	Eluent	Physical appearance	Weight (mg)	% Yields from dried powder
1	100% CH_2Cl_2- 2.5% MeOH in CH_2Cl_2	White crystal	762.3	0.014
2	1% MeOH in CH_2Cl_2	Pale brown crystal	6.0	0.00013
3	1% MeOH in CH_2Cl_2	White crystal	5.0	0.00009
4	1.5% MeOH in CH_2Cl_2	Yellow crystal	4.0	0.00007
5	1.5% MeOH in CH_2Cl_2	Pale yellow crystal	1.2	0.000009
6	2% MeOH in CH_2Cl_2	Pale orange crystal	6.0	0.00013
7	6.5% MeOH in CH_2Cl_2	White crystal	6.0	0.00013

4.2 Structure elucidation of mixture 1

Mixture 1 was obtained by flash column chromatography using 100% CH_2Cl_2 to 2.5% MeOH in CH_2Cl_2 and re-crystallization technique. The structure of mixture 1 was elucidated by ^{1}H-NMR and ^{13}C-NMR data as follows.

The ^{1}H-NMR spectrum (Figure 26 and Table 4) of mixture 1 possessed chemical shifts of methyl, methylene and methine protons at 0.68-2.31 ppm, of a tertiary alcohol proton at 3.51 ppm, of an aliphatic methine proton at 5.11 ppm and of a vinyl proton at 5.37 ppm.

The ^{13}C-NMR spectrum (Figure 27 and Table 5) of mixture 1 showed 29 signals, which the sp^2-carbon signals of alkene corresponded to the signal, 140.77 and 121.74 ppm. There were 27 sp^3-carbon signals between 11.89-71.81 ppm.

From data analysis, it could be concluded that the mixtures 1 exhibited the both ^{1}H-NMR and ^{13}C-NMR chemical shifts similar to that of the mixture steroids **[17]**, including campesterol, stigmasterol and β-sitosterol in Figure 6. Because of, the spectroscopic and physical data of mixture 1 was identical to those reported for mixture steroids obtained from *B. superba Roxb.* **[17]**.

The ^{1}H-NMR and ^{13}C-NMR chemical shifts of mixture 1 and mixture steroids were compared as showed in Table 4 and 5, respectively.

Table 4 ^{1}H-NMR chemical shifts of mixture 1 and mixture steroids

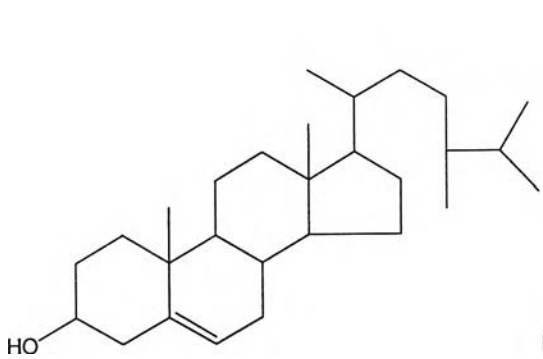
^{1}H-NMR Chemical shifts (ppm)	
Mixture 1 ($CDCl_3$ 400 MHz)	**Mixture steroids ($CDCl_3$) [17]**
0.68-2.31	0.68-2.28
3.51	3.50
5.11	5.10
5.37	5.35

Table 5 ^{13}C-NMR chemical shifts of mixture 1 and mixture of steroids

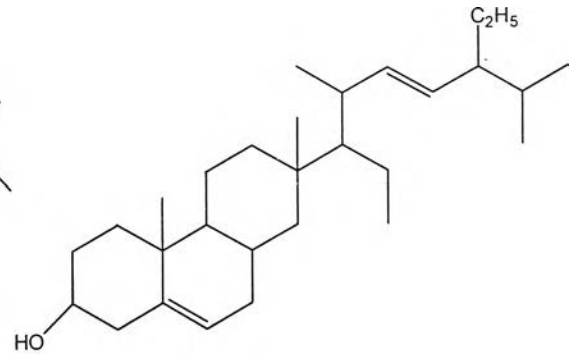
Carbon position	**^{13}C-NMR Chemical shifts (ppm)**	
	Mixture 1 ($CDCl_3$ 100 MHz)	**Mixture steroids ($CDCl_3$) [17]**
1	37.27	37.23
2	31.66	31.62
3	71.81	71.80
4	42.31	42.30
5	140.77	140.72
6	121.74	121.70
7	31.92	31.90
8	31.92	31.90
9	50.14	50.12
10	36.52	36.49
11	21.10	21.06
12	39.79	39.75
13	42.31	42.26

Table 5 Continued

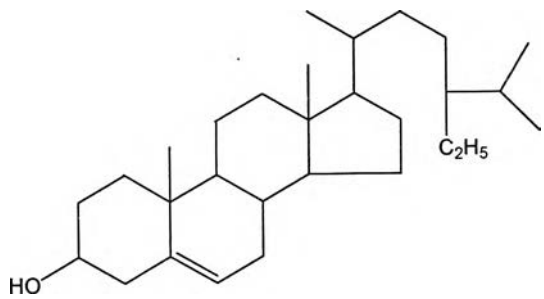
Carbon position	^{13}C-NMR Chemical shifts (ppm)	
	Mixture 1 ($CDCl_3$ 100 MHz)	**Mixture steroids** ($CDCl_3$) [17]
14	56.78	56.84
15	24.33	24.34
16	28.28, 28.90	28.22, 28.89
17	56.06	56.03
18	11.96, 12.23	11.95, 12.22
19	19.43	19.37
20	36.52, 40.49	36.49, 40.46
21	19.06, 21.20	19.01, 21.18
22	36.52, 138.31	36.49, 138.28
23	29.15, 129.27	29.13, 129.25
24	51.26	51.21
25	25.39, 31.92	25.37, 31.90
26	19.06	19.01
27	19.82, 21.20	19.79, 21.18
28	23.08, 25.39	23.04, 25.37
29	11.89, 12.06	11.83, 12.02



Campesterol



Stigmasterol



β-sitosterol

Figure 6 Structure of mixture 1

4.3 Structure elucidation of compound 1

Compound 1 was obtained by flash column chromatography using 1% MeOH in CH_2Cl_2 and re-crystallization technique. The structure of compound 1 was elucidated using high resolution mass spectrometry, 1D and 2D NMR spectroscopic data as follows.

The ^{1}H-NMR spectrum (Figure 28 and Table 6) of compound 1 possessed chemical shifts of methyl proton at 3.81 ppm, of four aromatic protons at 6.35, 6.40, 6.89 and 7.33 ppm, respectively and of an alkene proton at δ_H 7.80 ppm.

The ^{13}C-NMR spectrum (Figure 29 and Table 6) of compound 1 showed 14 signals, which the sp^2-carbon signals of aromatic corresponded to the signal, 165.50, 162.75, 158.00, 157.19, 130.15, 121.72, 115.53, 106.18, 98.18 and 92.43 ppm. There were two olefinic carbon signals at 152.81 and 124.05 ppm and a methoxy carbon signal at 55.78 ppm. A carbonyl group of carboxylic acid - carbon corresponded to a signal at 181.02 ppm.

The TOF-MS (High resolution mass) (Figure 34) showed the $[M + Na]^+$ ion peak at *m/z* of 307.0583. It seems reasonable to assume that the molecular weight of compound 1 was 284.0583 and the molecular formula was $C_{16}H_{12}O_5$.

From these data indicated that compound 1 should be an isoflavone group. According to the comparison with the 1D-NMR spectrum of isoflavone, compound 1 should have same the structure of 5,4′-dihydroxy-7-methoxyisoflavone (Prunetin) which was reported in the literature **[29]**. The ^{1}H-NMR and ^{13}C-NMR chemical shifts of compound 1 and Prunetin are showed in Table **6**.

Table 6 ^{1}H-NMR and ^{13}C-NMR chemical shifts of compound 1 and Prunetin

Carbon position	^{1}H-NMR Chemical shifts (ppm)		^{13}C-NMR Chemical shifts (ppm)	
	Compound 1 ($CDCl_3$: CD_3OD (5:1), 400 MHz)	Prunetin [29] ($DMSO-d_6$)	Compound 1 ($CDCl_3$: CD_3OD (5:1), 100 MHz)	Prunetin [29] ($DMSO-d_6$)
2	7.80 (1H, s)	8.35 (s)	152.81(d)	154.27
3	-	-	124.05 (s)	122.90
4	-	-	181.02 (s)	180.08
4a	-	-	106.18 (s)	104.42
5	-	-	162.75 (s)	164.36
6	6.32 (2H, d; *J*= 2.4 Hz)	6.22 (d; *J*= 2.0 Hz)	98.18 (d)	99.01
7	-	-	165.50 (s)	161.97
8	6.35 (2H, d; *J*= 2.0 Hz)	6.38 (d; *J*= 2.0 Hz)	92.43 (d)	93.70
8a	-	-	158.00 (s)	157.57
1′	-	-	121.72 (s)	121.92
2′	7.33 (2H, d; *J*=8.0 Hz)	7.49 (d; *J*=8.7 Hz)	130.15 (d)	130.14
3′	6.89 (2H, d; *J*=8.8 Hz)	6.99 (d; *J*=8.7 Hz)	115.53 (d)	113.68
4′	-	-	157.19 (s)	159.13
5′	6.89 (2H , d; *J*=8.8 Hz)	6.99 (d; *J*=8.7 Hz)	115.53 (d)	113.68
6′	7.33 (2H , d; *J*=8.0 Hz)	7.49 (d; *J*=8.7 Hz)	130.15 (d)	130.14
7-OCH_3	3.81 (3H, s)	3.78 (s)	55.78 (q)	55.14

Moreover, the information from 2D-NMR spectroscopic techniques of compound 1 including gHSQC (Figure 30), gHMBC (Figure 31), gCOSY (Figure 32) and NOESY (Figure 33) spectral data were used to assist the interpretation of compound 1 structure. All of the data showed in Table 7, Figure 8, 9 and, 10, respectively.

Table 7 ^{1}H-NMR, ^{13}C-NMR, gHSQC, gHMBC, gCOSY and NOESY spectral data of compound 1

Carbon position	δ_C	δ_H	gHMBC	gCOSY	NOESY
2	152.81(d)	7.80 (1H, s)	C-3, C-4, C-8a, C-1′	-	H-2′ , H-6′
3	124.05 (s)	-	-	-	-
4	181.02 (s)	-	-	-	-
4a	106.18 (s)	-	-	-	-
5	162.75 (s)	-	-	-	-
6	98.18 (d)	6.32 (2H, d; *J*= 2.4 Hz)	C-4a, C-5, C-7, C-8	-	H-7-OCH_3
7	165.50 (s)	-	-	-	-
8	92.43 (d)	6.35 (2H, d; *J*= 2.0 Hz)	C-4a, C-6, C-7, C-8a	-	H-7-OCH_3
8a	158.00 (s)	-	-	-	-
1′	121.72 (s)	-	-	-	-
2′	130.15 (d)	7.33 (2H, d; *J*=8.0 Hz)	C-3, C-4′, C-6′	H-3′	H-2, H-5′
3′	115.53 (d)	6.89 (2H, d; *J*=8.8 Hz)	C-4′, C-1′, C-5′	H-2′	H-6′
4′	157.19 (s)	-	-	-	-
5′	115.53 (d)	6.89 (2H , d; *J*=8.8 Hz)	C-4′, C-1′, C-3′	H-6′	H-2′
6′	130.15 (d)	7.33 (2H , d; *J*=8.0 Hz)	C-3, C-2′, C-4′	H-5′	H-2
7-OCH_3	55.78 (q)	3.81 (3H, s)	C-7	-	H-6, H-8

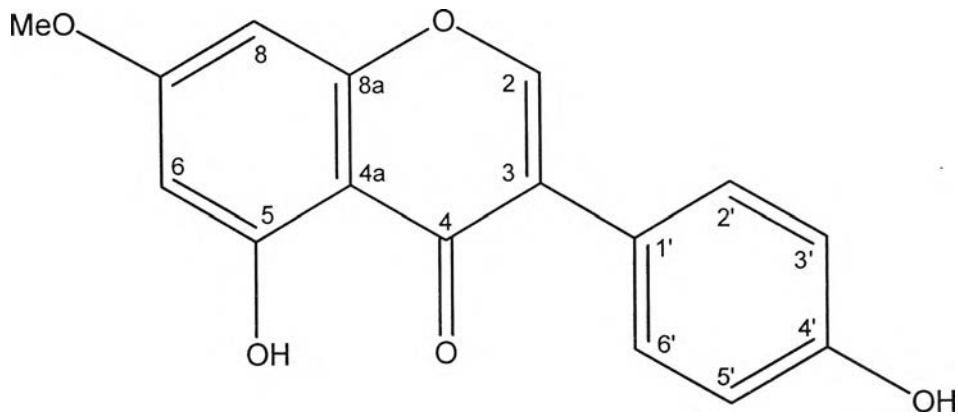
From the Table 7, the H-C connections (HSQC) of compound 1 showed that the C-2 was connected to proton at δ_H 7.80 (1H, s) ppm, the C-6 was connected to proton at δ_H 6.32 (2H, d; *J*= 2.4 Hz) ppm, the C-8 was connected to proton at δ_H 6.35 (2H, d; *J*= 2.0 Hz) ppm, the C-2′ and C-6′ were connected to proton at δ_H 7.33 (2H, d; *J*= 8.0 Hz) ppm, the C-3′ and C-5′ were connected to proton at δ_H 6.89 (2H, d; *J*= 8.8 Hz) ppm, and the C-7-OCH_3 was connected to proton at δ_H 3.81 (3H, s) ppm, respectively.

The ^{1}H-^{13}C long range connections (HMBC) of compound 1 (Figure 8 and Figure 31 in appendix), which shows the correlations between the H-2 signal and the C-3, C-4, C-8a and C-1′ signals, the H-6 signal and the C-4a, C-5, C-7 and C-8 signals, the H-8 signal and the C-4a, C-6, C-7 and C-8a signals, the H-2′ signal and the C-3, C-4′ and C-6′ signals, the H-3′ signal and the C-4′, C-1′ and C-5′ signals, the H-5′ signal and the C-4′, C-1′ and C-3′ signals, the H-6′ signal and the C-3, C-2′ and C-4′ signals, and the H-7-OCH_3 signal and the C-7 signal, respectively.

The 400 MHz ^{1}H-^{1}H COSY of compound 1 (Figure 9 and Figure 32 in appendix) showed that the H-2′ signal shows coupling with the H-3′ signal, and the H-5′ signal shows coupling with the H-6′ signal, respectively.

The 400 MHz ^{1}H-^{1}H NOESY of compound 1 (Figure 10 and Figure 33 in appendix) showed that the H-2 signal shows coupling with the H-2′ and H-6′ signals, the H-6 and H-8 signals shows coupling with the H-7-OCH_3 signal, the H-2′ signal shows coupling with the H-5 signal, and the H-3′ signal shows coupling with the H-6′ signal, respectively.

After elucidation of compound 1 by 1D and 2D-NMR spectroscopic technique, the chemical shift on ^{1}H-NMR and ^{13}C-NMR spectrum of compound 1 and 5,4′-dihydroxy-7-metoxy-isoflavone (Prunetin) was compared. These results indicated that the structure of compound 1 is identical to prunetin. Thus, it could be concluded that compound 1 was prunetin. The structure is presented in Figure 7. The prunetin founded firstly from hardwoods of *Pterocarpus* species in 1953 **[30]** and founded in other plant such as the heartwood of *Myristica malabarica* **[31]** and the stem of *Sophora secundiflora* **[32]**. It showed only moderate antiplasmodial activity against *Plasmodium falciparum* of poW and Dd2 strains at IC_{50} 27.8 μg/ml and >50 μg/ml, respectively **[33]**. Besides, it showed cAMP PDE inhibitory activity at IC_{50} values >100 μm/l **[34]**.



5,4′-dihydroxy-7-metoxy-isoflavone (Prunetin)

Figure 7 Structure of compound 1

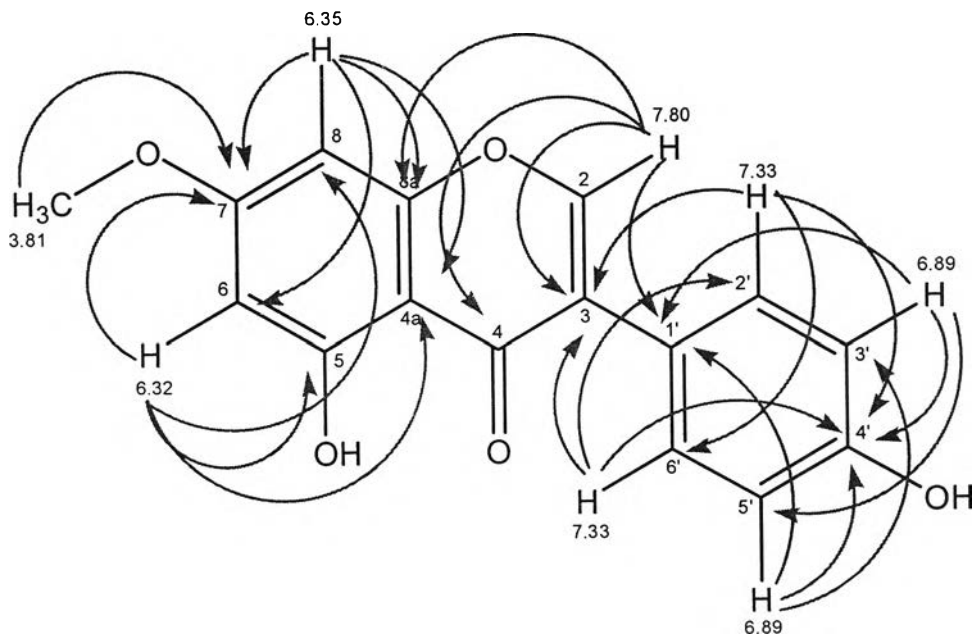


Figure 8 HMBC of compound 1

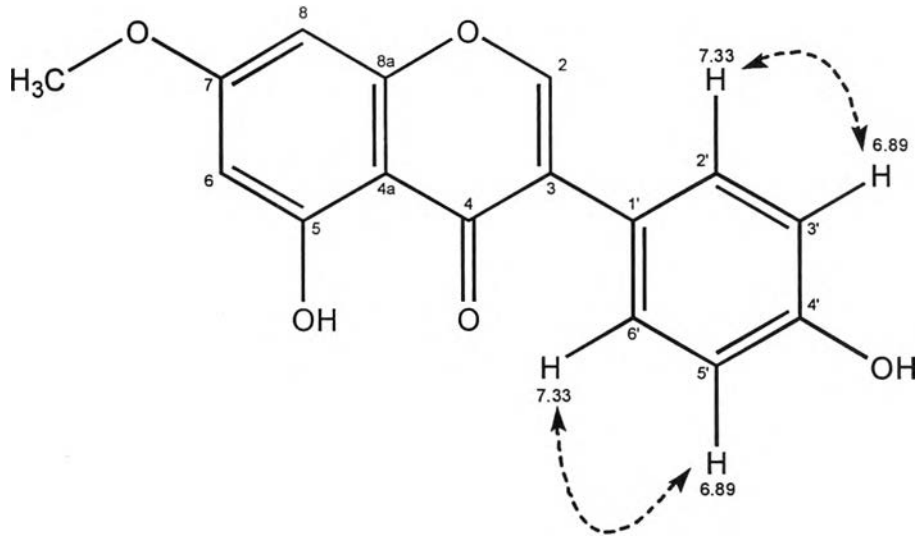


Figure 9 COSY of compound 1

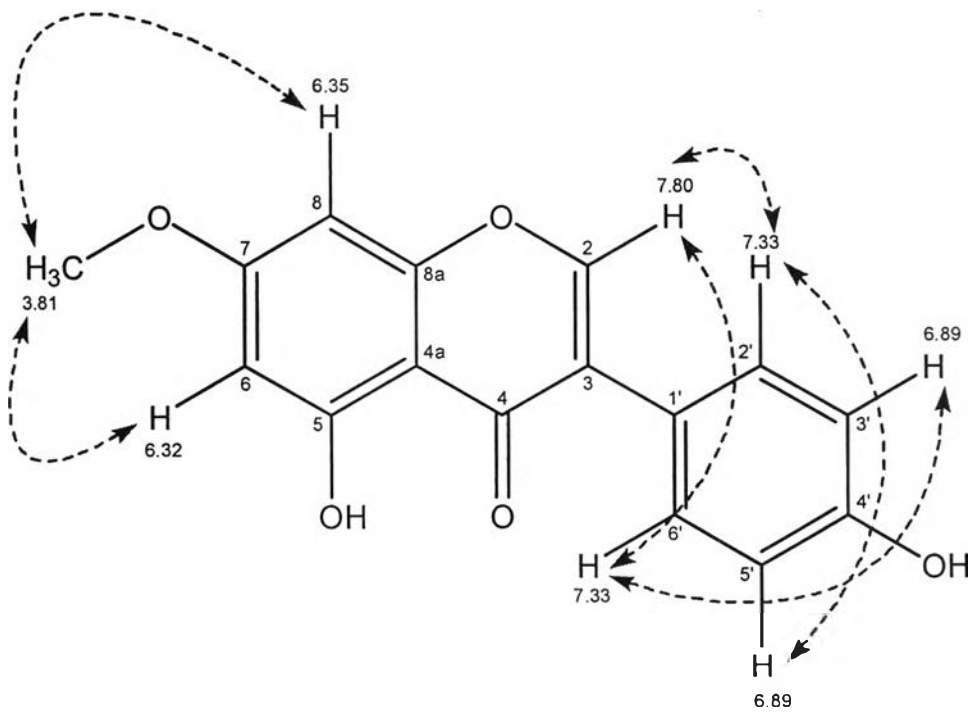


Figure 10 NOESY of compound 1

4.4 Structure elucidation of compound 2

Compound 2 was obtained by flash column chromatography using 1% methanol in dichloromethane and preparative TLC technique of 1% methanol in dichloromethane and 100% dichloromethane. The structure of compound 2 was elucidated using high resolution mass spectrometry, 1D and 2D NMR spectroscopic data as follows.

The ^{1}H-NMR spectrum (Figure 36 and Table 8) of compound 2 possessed chemical shifts of methyl proton at 3.77 ppm, of six aromatic protons at 6.43, 6.45, 6.46, 6.56, 7.13 and 7.39 ppm, respectively, of two methine protons at 3.53 and 5.49 ppm, and of two methylene proton at 3.62 and 4.24 ppm.

The ^{13}C-NMR spectrum (Figure 37 and Table 8) of compound 2 showed 15 signals, which the sp^2-carbon signals of aromatic corresponded to the signal, 96.91 (d), 103.65 (d), 106.37 (d), 109.74 (d), 112.50 (s), 119.15(s), 124.76 (d), 132.20 (d), 157.21 (s), 156.65 (s), 160.40 (s) and 161.27 (s) ppm. There were three sp^3-carbon signals at 39.46 (d), 66.53 (t) and 78.51(d) ppm, and a methoxy carbon signal at 55.51 ppm.

The TOF-MS (High resolution mass (Figure 42) showed the $[M + Na]^+$ ion peak at *m/z* of 293.0782. It seems reasonable to assume that the molecular weight of compound 2 was 270.0782 and the molecular formula was $C_{16}H_{14}O_4$.

From these data indicated that compound 2 should be a 3-hydroxy-9-methoxypterocarpan (Medicarpin), which was reported in the literature **[35, 36]**. According to the comparison with the 1D-NMR spectrum of compound 2 and medicarpin should have same the structure. The ^{1}H-NMR and ^{13}C-NMR chemical shifts of these compounds are showed in Table 8.

Table 8 ^{1}H-NMR and ^{13}C-NMR chemical shifts of compound 2 and Medicarpin

Carbon position	^{1}H-NMR Chemical shifts (ppm)		^{13}C-NMR Chemical shifts (ppm)	
	Compound 2 ($CDCl_3$, 400 MHz)	Medicarpin [35] ($CDCl_3$)	Compound 2 ($CDCl_3$, 100 Hz)	Medicarpin [35] ($CDCl_3$)
1	7.39 (1H, d; *J*= 8.4 Hz)	7.41 (1H, d; *J*= 8.4 Hz)	132.20(d)	132.9(d)
2	6.56 (1H, d; *J*= 8.4 Hz)	6.58 (1H, dd; *J*= 2.4,8.4 Hz)	109.74 (d)	112.0 (d)
3	-	-	157.21 (s)	160..6 (s)
4	6.45 (1H, s)	6.44 (1H, d; *J*= 2.4 Hz)	103.65 (d)	104.3 (d)
4a	-	-	156.65 (s)	157.5 (s)
6	3.62 (1H, t)	4.26 (1H, dd; *J*= 5.0, 10.0 Hz)	66.53 (t)	66.8 (t)
	4.24 (1H, dd; *J*= 4.4 and 10.4 Hz)	3.55 (1H, ddd; *J*= 11.0,5.1,6.7 Hz)		
6a	3.53 (1H, m)	3.64 (1H, dd; *J*= 11.0, 10.9 Hz)	39.46 (d)	40.1 (d)
6b	-	-	119.15 (s)	120.1 (s)
7	7.13 (1H, d; *J*= 8.8 Hz)	7.15 (1H, d; *J*= 8.8 Hz)	124.76 (d)	125.4 (d)
8	6.46 (2H, s)	6.48 (2H, m)	96.91 (d)	106.6 (d)
9	-	-	161.27 (s)	161.7 (s)
10	6.43 (1H, d; *J*= 8.8 Hz)	6.48 (2H, m)	106.37 (d)	97.2 (d)
10a	-	-	160.40 (s)	161.5 (s)
11a	5.49 (1H , d; *J*= 6.4 Hz)	5.52 (1H , d; *J*= 6.8 Hz)	78.51 (d)	79.3 (d)
11b	-	-	112.50 (s)	112..0 (s)
9-OCH_3	3.77 (3H, s)	3.79 (3H, s)	55.51(q)	55.4(q)

Moreover, the information from 2D-NMR spectroscopic techniques of compound 2 including gHSQC (Figure 38), gHMBC (Figure 39), gCOSY (Figure 40) and NOESY (Figure 41) spectral data were used to assist the interpretation of compound 2 structure. All of the data showed in Table 9, Figure 12, 13 and 14, respectively.

Table 9 ^{1}H-NMR, ^{13}C-NMR, gHSQC, gHMBC, gCOSY and NOESY spectral data of compound 2

Carbon position	δ_C	δ_H	gHMBC	gCOSY	NOESY
1	132.20(d)	7.39 (1H, d; *J*= 8.4 Hz)	C-3, C-4a, C-11a	H-2	-
2	109.74 (d)	6.56 (1H, d; *J*= 8.4 Hz)	C-4, C-11b	H-1	-
3	157.21 (s)	-	-	-	-
4	103.65 (d)	6.45 (1H, s)	C-2, C-3, C-4a, C-11b	-	-
4a	156.65 (s)	-	-	-	-
6	66.53 (t)	3.62 (1H, t)	C-4a, C-11a	H-6(δ_H 4.24)	H-6(δ_H 4.24)
		4.24 (1H, dd; *J*= 4.4 and 10.4 Hz)	C-4a, C-6a	H-6(δ_H 3.62)	H-6(δ_H 3.62)
6a	39.46 (d)	3.53 (1H, m)	C-6b, C-11a	-	-
6b	119.15 (s)	-	-	-	-
7	124.76 (d)	7.13 (1H, d; *J*= 8.8 Hz)	C-6a, C-9, C-10a	H-8	-
8	96.91 (d)	6.46 (2H, s)	C-10	H-7	H-9-OCH_3
9	161.27 (s)	-	-	-	-
10	106.37 (d)	6.43 (2H, d; *J*= 8.8 Hz)	C-6b, C-8	-	H-9-OCH_3
10a	160.40 (s)	-	-	-	-
11a	78.51 (d)	5.49 (1H , d; *J*= 6.4 Hz)	C-1, C-4a, C-6, C-11b	-	-
11b	112.50 (s)	-	-	-	-
9-OCH_3	55.51(q)	3.77 (3H, s)	C-9	-	H-8, H-10

From the Table 9, the H-C connections (HSQC) of compound 2 showed that the C-1 was connected to proton at δ_H 7.39 (1H, d; *J*= 8.4 Hz) ppm, the C-2 was connected to proton at δ_H 6.56 (1H, d; *J*= 8.4 Hz) ppm, the C-4 was connected to proton at δ_H 6.45 (1H, s) ppm, the C-6 was connected to proton at δ_H 3.62 (1H, t) and 4.24 (1H, dd; *J*= 4.4 and 10.4 Hz) ppm, the C-6a was connected to proton at δ_H 3.53 (1H, m) ppm, the C-7 was connected to proton at δ_H 7.13 (1H, d; *J*= 8.8 Hz), the C-8 was connected to proton at δ_H 6.46 (2H, s), the C-10 was connected to proton at δ_H 6.43 (2H, d; *J*= 8.8 Hz) ppm, the C-11a was connected to proton at δ_H 5.49 (1H , d; *J*= 6.4 Hz) and C-9-OCH_3 was connected to proton at δ_H 3.77 (3H, s) ppm, respectively.

The ^{1}H-^{13}C long range connections (HMBC) of compound 2 (Figure 12 and Figure 39 in appendix), which shows the correlations between the H-1 signal and the C-3, C-4a and C-11a signals, the H-2 signal and the C-4 and C-11b signals, the H-4 signal and the C-2, C-3, C-4a and C-11b signals, the H-6 (δ_{II} 3.62 ppm) signal and the C-4a, and C-11a signals, the H-6 (δ_{II} 4.24 ppm) signal and the C-4a and C-6a signals, the H-6a signal and the C-6b and C-11a signals, the H-7 signal and the C-6a, C-9 and C-10a signals, the H-8 signal and the C-10 signal, the H-10 signal and the C-6b and C-8 signals, the H-11a signal and the C-1, C-4a, C-6 and C-11b signals, and the H-9-OCH_3 signal and the C-9 signal, respectively.

The 400 MHz ^{1}H-^{1}H COSY of compound 2 (Figure 13 and Figure 40 in appendix) showed that the signal at H-1 signal shows coupling with the H-2 signal, H-6 (δ_{II} 3.62 ppm) signal shows coupling with the H-6 (δ_{II} 4.24 ppm) signal, and H-7 shows coupling with the H-8 signal, respectively.

The 400 MHz ^{1}H-^{1}H NOESY of compound 2 (Figure 14 and Figure 41 in appendix) showed that the signal at the H-6 (δ_{II} 3.62 ppm) signal shows coupling with the H-6 (δ_{II} 4.24 ppm) and H-9-OCH_3 signal shows coupling with the H-8 and H-10 signals, respectively.

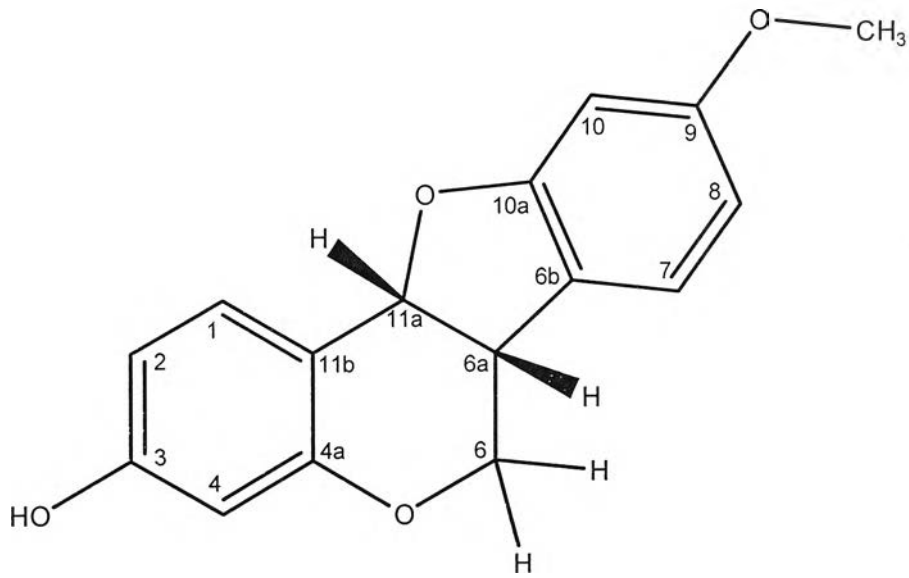
After elucidation of compound 2 by 1D and 2D-NMR spectroscopic technique, the chemical shift on ^{1}H-NMR and ^{13}C-NMR spectrum of compound 2 and 3-hydroxy-9-methoxypterocarpan (Medicarpin) was compared. These results indicated that the structure of compound is identical to medicarpin. Thus, it could be concluded that compound 2 was medicarpin. The structure is presented in Figure 11.

The medicapin was founded in the root of *Taverniera abyssinica* (A. Richi, Leguminosae) in 1994. It showed antimicrobial activity against *Paecilomyces variotii, Penicillium notatum, Bacillus brevis, Bacillus subtilis, Mircrococcus luteus, Proteus vulgaris, Staphylococcus aureus, Candida albicans, Nadsonia fulvescens, Mucor miehei, Nematospora coryli, Saccharomyces cerevisiae, Rhodotorula glutinis* **[37]**, and founded other plant such as *Dalbergia odorifera* in 1998 **[38]**

In 1997, medicarpin was isolated from *Tephrosia purpurea* that showed quinone reductase (QR) activity with cultured Hepa 1c1c7 mouse hepatoma cells, IC_{50} >74.0 μM, CD = 13.7 μM and CI >5.4 μM **[39]**. In the same years, medicarpin was isolated from *Wistaria brachybot*rys that showed anti-tumer promoting activity more than 80% inhibition of activation at 100 mol ratio/TPA **[40]**.

In 2000, medicarpin was isolated from *Glycyrrhiza glabra* that inhibited lymphocyte blastogenesis in dose-dependent fashion with IC_{50} values ranging from 3.0-7.7 μg/ml, which corresponded to 20-100 times that of prednisolone IC_{50} 0.08 μg/ml **[41]**.

In 2002, Zhen-Li and co-workers found that medicarpin (10 μg/ml) showed significant apoptosis-inducing effects on human PBMCs. In human lung fibroblasts, medicarpin exhibited a higher activity to induce apoptosis compared to the control **[42]**.



3-hydroxy-9-methoxypterocarpan (Medicarpin)

Figure 11 Structure of compound 2

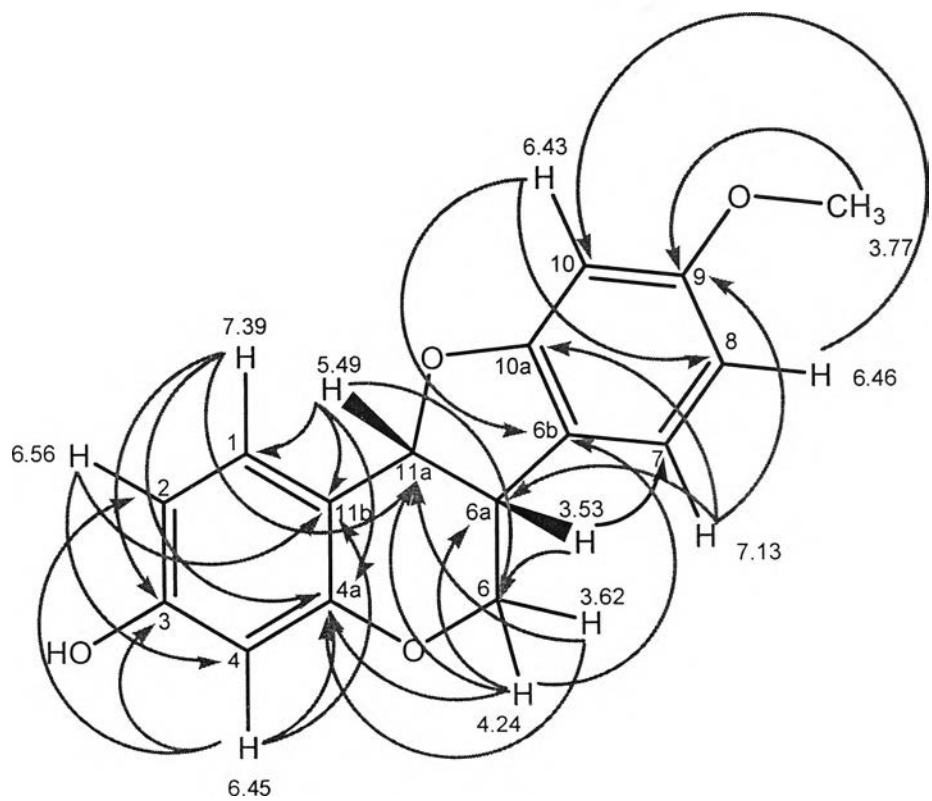


Figure 12 HMBC of compound 2

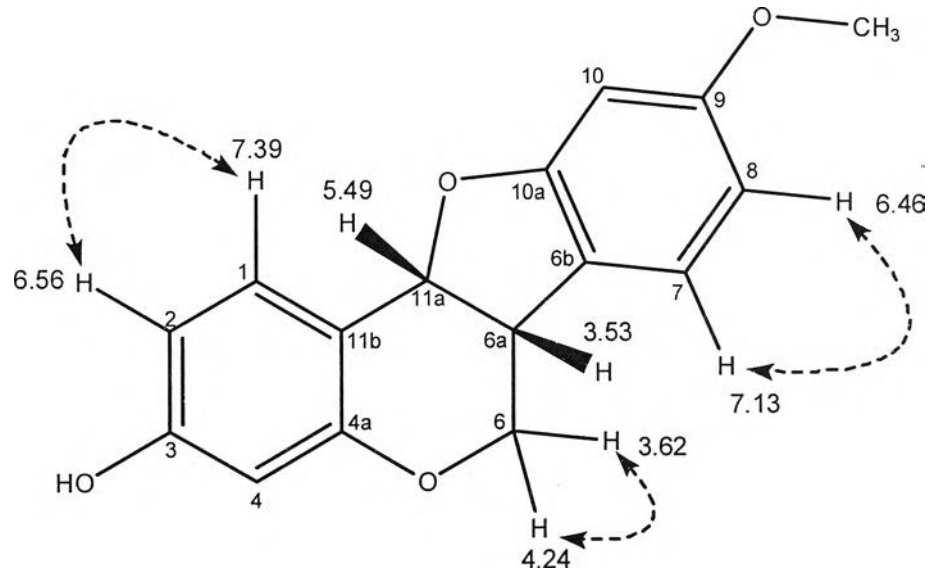


Figure 13 COSY of compound 2

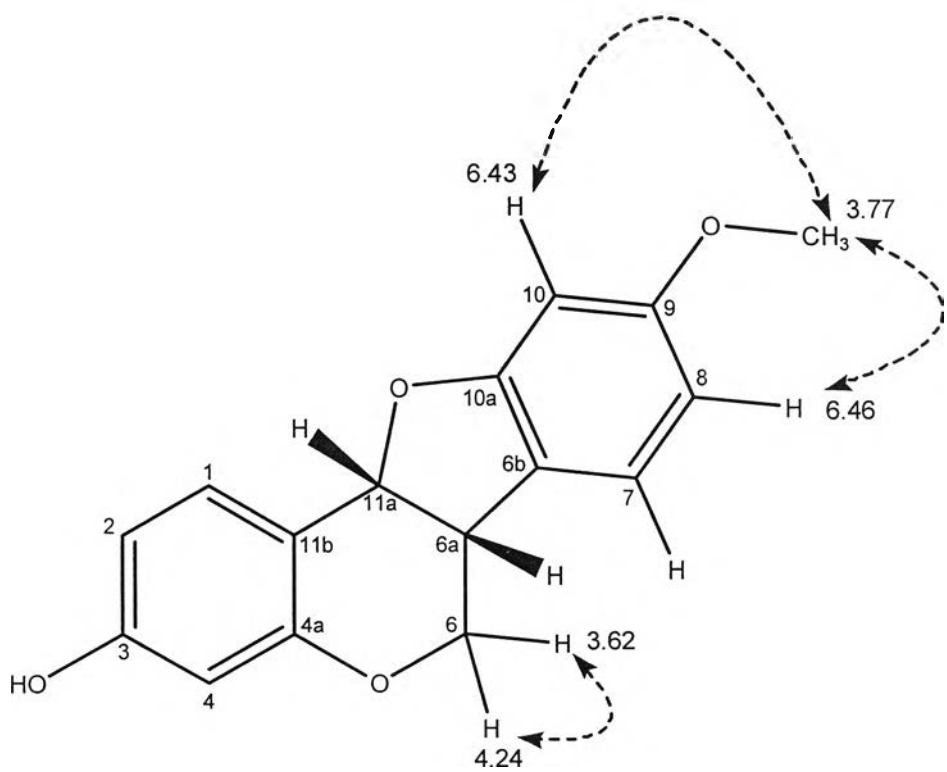


Figure 14 NOESY of compound 2

4.5 Structure elucidation of compound 3

Compound 3 was obtained by flash column chromatography using 2% methanol in dichloromethane, the crystallization of 1% methanol in dichloromethane and the preparative TLC technique of 2% methanol in dichloromethane. The structure of compound 3 was elucidated using high resolution mass spectrometry, 1D and 2D NMR spectroscopic data as follows.

The ^{1}H-NMR spectrum (Figure 44 and Table 10) of compound 3 possessed chemical shifts of methyl proton at 3.81 ppm, of seven aromatic protons at 6.79 (1H, d; *J*= 2.4 Hz), 6.93 (2H, d; *J*= 9.2 Hz), 6.88 (1H, dd; *J*=2.0 and 9.2 Hz), 7.43 (2H, d; *J*=9.2 Hz), 7.87 (1H, s) ppm, respectively, and of an alkene proton at 8.09 ppm.

The ^{13}C-NMR spectrum (Figure 45 and Table 10) of compound 3 showed 13 signals, which the sp^2-carbon signals of aromatic corresponded to the signal, 102.41 (d), 113.88 (d), 115.09 (d), 117.44 (s), 124.13 (s), 124.52 (s), 127.81 (d), 130.15 (d), 152.33(d), 158.21 (d), 159.60 (s), 162.43 (s) and 176.50 (s) ppm, and a methoxy carbon signal at 55.30 ppm.

The TOF-MS (High resolution mass) (Figure 50) showed the $[M + Na]^+$ ion peak at *m/z* of 291.0627. It seems reasonable to assume that the molecular weight of compound 3 was 268.0627 and the molecular formula was $C_{16}H_{12}O_4$.

From these data indicated that compound 3 should be a 7-hydroxy-4′-methoxy-isoflavone (Formononetin), which was reported in the literature **[43, 44]**. According to the comparison with the 1D-NMR spectrum of compound 3 and 7-hydroxy-4′-methoxy-isoflavone should have same the structure. The ^{1}H-NMR and ^{13}C-NMR chemical shifts of these compounds are showed in Table 10.

Table 10 ^{1}H-NMR and ^{13}C-NMR chemical shifts of compound 3 and Formononetin

Carbon position	^{1}H-NMR Chemical shifts (ppm)		^{13}C-NMR Chemical shifts (ppm)	
	Compound 3 ($CDCl_3$: CD_3OD (3:1), 400 MHz)	Formononetin [43] (DMSO-d_6+D_2O)	Compound 3 ($CDCl_3$: CD_3OD (3:1), 100 MHz)	Formononetin [43] (DMSO-d_6 + D_2O)
2	7.87 (1H, s)	8.21 (1H, s)	152.33 (d)	153.0
3	-	-	124.52 (s)	124.2
4	-	-	176.50 (s)	174.6
5	8.09 (1H, d; *J*= 9.2 Hz)	7.98 (1H, d; *J*= 8 Hz)	127.81 (d)	127.2
6	6.88 (1H, dd; *J*= 2.0 and 9.2 Hz)	6.93 (1H, dd; *J*= 8 and 12 Hz	115.09 (d)	115.1
7	-	-	162.43 (s)	162.6
8	6.79 (1H, d; *J*= 2.4 Hz)	6.87 (1H, d; *J*= 2 Hz)	102.41 (d)	102.1
8a	-	-	158.21 (s)	157.4
4a	-	-	117.44 (s)	116.1
1′	-	-	124.13 (s)	123.2
2′	7.43 (2H, d; *J*= 9.2 Hz)	7.51 (2H, d; *J*= 9 Hz)	130.15 (d)	130.0
3′	6.93 (2H , d; *J*= 9.2 Hz)	6.97 (2H , d; *J*= 9 Hz)	113.88 (d)	113.5
4′	-	-	159.60 (s)	158.9
5′	6.93 (2H , d; *J*= 9.2 Hz)	6.97 (2H , d; *J*= 9 Hz)	113.88 (d)	113.5
6′	7.43 (2H, d; *J*= 9.2 Hz)	7.51 (2H, d; *J*= 9 Hz)	130.15 (d)	130.0
4′-OCH_3	3.81 (3H, s)	3.80 (3H, s)	55.30 (q)	55.1

Moreover, the information from 2D-NMR spectroscopic techniques of compound 3 including gHSQC (Figure 46), gHMBC (Figure 47), gCOSY (Figure 48) and NOESY (Figure 49) spectral data were used to assist the interpretation of compound 3 structure. All of the data showed in Table 11, Figure 16, 17 and 18, respectively.

Table 11 ^{1}H-NMR, ^{13}C-NMR, gHSQC, gHMBC, gCOSY and NOESY spectral data of compound 3

Carbon position	δ_C	δ_H	gHMBC	gCOSY	NOESY
2	152.33 (d)	7.87 (1H, s)	C-8a, C-4	-	-
3	124.52 (s)	-	-	-	-
4	176.50 (s)	-	-	-	-
5	127.81 (d)	8.09 (1H, d; *J*= 9.2 Hz)	C-8a, C-4	H-6	
6	115.09 (d)	6.88 (1H, dd; *J*= 2.0 and 9.2 Hz)	C-8	H-5	
7	162.43 (s)	-	-	-	-
8	102.41 (d)	6.79 (1H, d; *J*= 2.4 Hz)	C-8a	-	-
8a	158.21 (s)	-	-	-	-
4a	117.44 (s)	-	-	-	-
1′	124.13 (s)	-	-	-	-
2′	130.15 (d)	7.43 (2H, d; *J*= 9.2 Hz)	C-3, C-4′, C-6′	H-3′	H-4′-OCH_3
3′	113.88 (d)	6.93 (2H , d; *J*= 9.2 Hz)	C-1′, C-4′, C-5′	H-2′	-
4′	159.60 (s)	-	-	-	-
5′	113.88 (d)	6.93 (2H , d; *J*= 9.2 Hz)	C-1′, C-3′, C-4′	H-6′	H-6′
6′	130.15 (d)	7.43 (2H, d; *J*= 9.2 Hz)	C-3, C-2′, C-4′	H-5′	H-5′
4′-OCH_3	55.30 (q)	3.81 (3H, s)	C-4′	-	H-2′

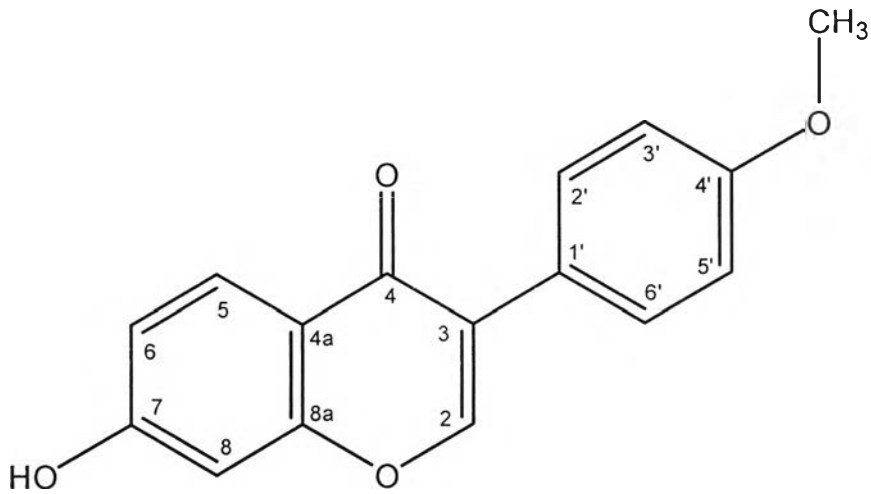
From the Table 11, the H-C connections (HSQC) of compound 2 showed that the C-2 was connected to proton at δ_H 7.87 (1H, s) ppm, the C-5 was connected to proton at δ_H 8.09 (1H, d; *J*= 9.2 Hz) ppm, the C-6 was connected to proton at δ_H 6.88 (1H, dd; *J*= 2.0 and 9.2 Hz) ppm, the C-8 was connected to proton at δ_H 6.79 (1H, d; *J*= 2.4 Hz) ppm, the C-2′ and C-6′ were connected to proton at δ_H 7.43 (2H, d; *J*= 9.2 Hz) ppm, the C-3′ and C-5′ were connected to proton at δ_H 6.93 (2H , d; *J*= 9.2 Hz) ppm, and the C-4′-OCH_3 was connected to proton at δ_H 3.81 (3H, s) ppm, respectively.

The ^{1}H-^{13}C long range connections (HMBC) of compound 3 (Figure 16 and Figure 47 in appendix), which shows the correlations between the H-2 signal and the C-8a and C-4 signals, the H-5 signal and the C-8a and C-4 signals, the H-6 signal and the C-8 signal, the H-3 signal and the C-8a signal, the H-2′ signal and the C-3, C-4′ and C-6′ signals, the H-3′ signal and the C-1′, C-4′ and C-5′ signals, the H-5′ signal and the C-1′, C-3′ and C-4′ signals, the H-6′ signal and the C-3, C-2′ and C-6′ signals, and the H-4′-OCH_3 signal and the C-4′ signal, respectively.

The 400 MHz ^{1}H-^{1}H COSY of compound 3 (Figure 17 and Figure 48 in appendix) showed that the signal at H-5 signal shows coupling with the H-6 signal, H-2′ signal shows coupling with the H-3′ signal, and the H-5′ signal shows coupling with the H-6′ signal, respectively.

The 400 MHz ^{1}H-^{1}H NOESY of compound 3 (Figure 18 and Figure 49 in appendix) showed that the signal at the H-2′ signal shows coupling with the H-4′-OCH_3 signal, and the H-5′ signal shows coupling with the H-6′ signal, respectively.

After elucidation of compound 3 by 1D and 2D-NMR spectroscopic technique, the chemical shift on ^{1}H-NMR and ^{13}C-NMR spectrum of compound 3 and 7-hydroxy-4'-methoxy-isoflavone (Formononetin) was compared. These results indicated that the structure of compound is identical to 7-hydroxy-4′-methoxy-isoflavone. Thus, it could be concluded that compound 3 was 7-hydroxy-4′-methoxy-isoflavone. The structure is presented in Figure 15. In 1989, Sakamoto and co-workers founded that formononetin showed cAMP PDE inhibitory activity, IC_{50}= 57.4 ($x10^{-5}$ m/l) **[45]**. The formononetin was isolated from *Virola surinamensis* that showed antifungal activity against *Cladosporium cladosporioides* of 10-fold higher than the positive control Nystatin **[46]**. Besides, in 2000 it was isolated from *Dalbergia frutescens* Bark. that showed antigiardial activity at IC_{50} 0.03±0.01 µg/ml **[47]** and showed antimicrobial activity at higher concentration (>25 µg/ml) in 2002 **[48]**.



7-hydroxy-4′-methoxy-isoflavone (Formononetin)

Figure 15 Structure of compound 3

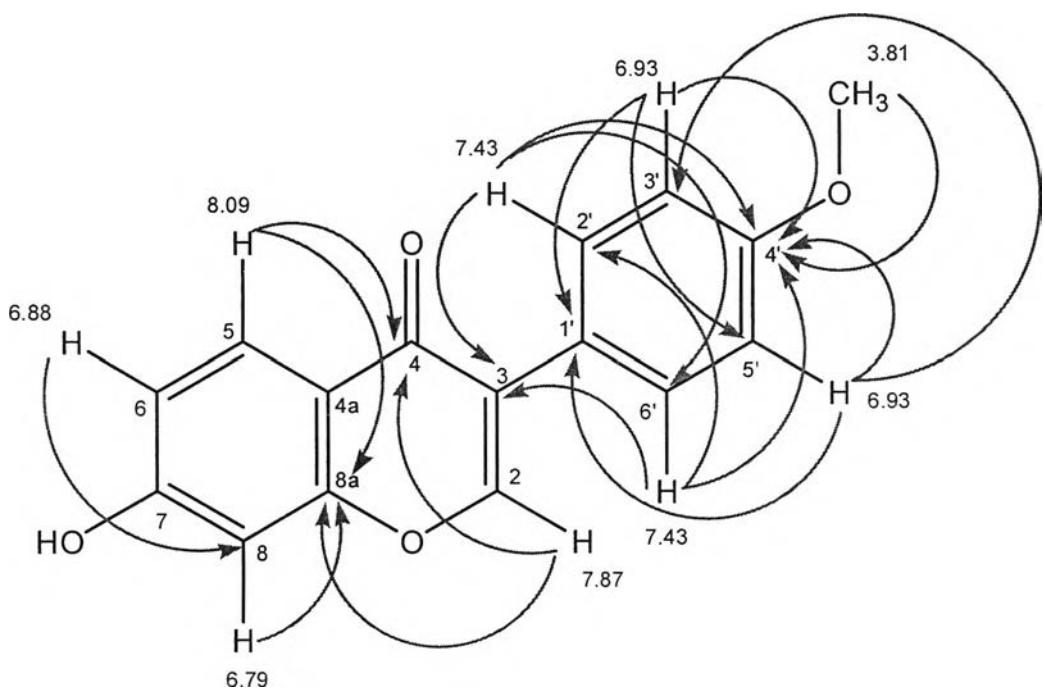


Figure 16 HMBC of compound 3

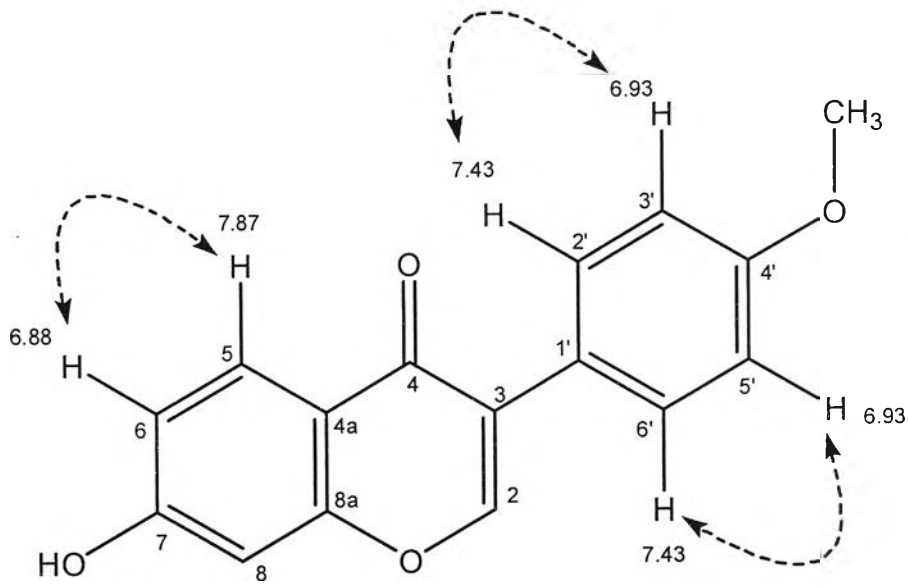


Figure 17 COSY of compound 3

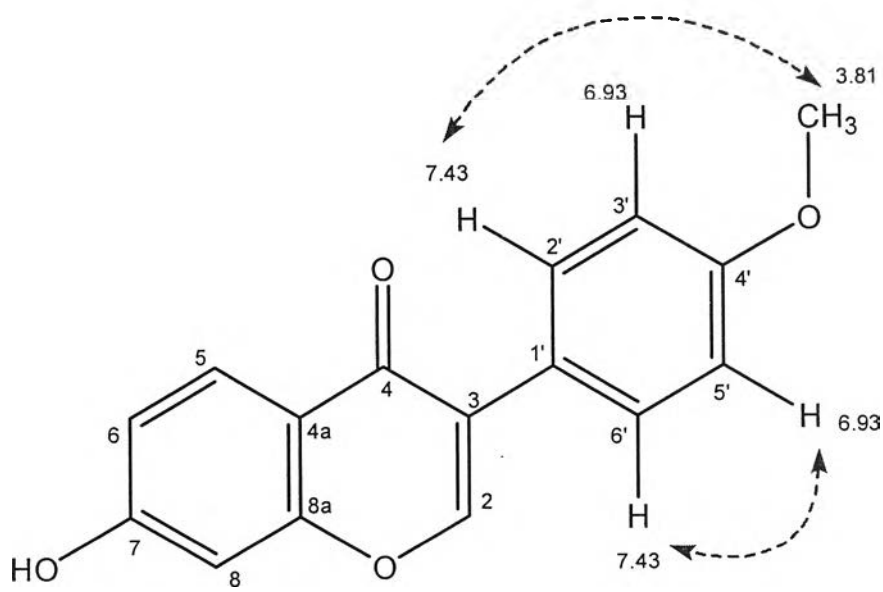


Figure 18 NOESY of compound 3

4.6 Structure elucidation of compound 4

Compound 4 was obtained by flash column chromatography using 1.5% methanol in dichloromethane and preparative TLC technique of 70% ethyl acetate in hexane, 2% methanol in dichloromethane and 1% methanol in dichloromethane, respectively. The structure of compound 4 was elucidated using high resolution mass spectrometry, 1D and 2D NMR spectroscopic data as follows.

The ^{1}H-NMR spectrum (Figure 52 and Table 12) of compound 4 possessed chemical shifts of methyl proton at 3.95 and 3.78 ppm, of six aromatic protons at 6.91, 6.92, 7.43 and 7.59 ppm, respectively and of an alkene proton at 7.86 ppm.

The ^{13}C-NMR spectrum (Figure 53 and Table 12) of compound 4 showed 16 signals, which the sp^2-carbon signals of aromatic corresponded to the signal, 102.66 (d), 104.79(d), 113.98 (d), 117.92 (s), 122.51 (s), 124.44 (s), 130.19 (d), 145.39 (s), 151.30 (s), 152.10 (d), 152.58 (s), 159.82 (s) and 175.75 (s) ppm. There were two methoxy carbon signals at 55.38 (q) and 56.54 (q) ppm.

The TOF-MS (High resolution mass) (Figure 58) showed the $[M+ H]^+$ ion peak at *m/z* of 299.0930. It seems reasonable to assume that the molecular weight of compound 4 was 298.0930 and the molecular formula was $C_{17}H_{14}O_5$.

From these data indicated that compound 4 should be a 7-hydroxy-6-4′-dimethoxyisoflavone, which was reported in the literature **[35]**. According to the comparison with the 1D-NMR spectrum of compound 4 and 7-hydroxy-6-4′-dimethoxyisoflavone should have same the structure. The ^{1}H-NMR and ^{13}C-NMR chemical shifts of these compounds are showed in Table 12.

Table 12 ^{1}H-NMR and ^{13}C-NMR chemical shifts of compound 4 and 7-hydroxy-6-4′-dimethoxyisoflavone

Carbon position	^{1}H-NMR Chemical shifts (ppm)		^{13}C-NMR Chemical shifts (ppm)	
	Compound 4 ($CDCl_3$: CD_3OD (5:1), 400 MHz)	7-hydroxy-6-4′-dimethoxyisoflavone [35] (DMSO-d_6)	Compound 4 ($CDCl_3$: CD_3OD (5:1), 400 MHz)	7-hydroxy-6-4′-dimethoxyisoflavone [35] (DMSO-d_6)
2	7.86 (1H, s)	8.32 (1H, s)	152.10(d)	152.8
3	-	-	124.44 (s)	124.4
4	-	-	175.75 (s)	174.2
4a	-	-	117.92 (s)	116.2
5	7.59 (1H, s)	7.42 (1H, s)	104.79 (d)	104.6
6	-	-	145.39 (s)	147.0
7	-	-	152.58 (s)	153.0
8	6.92 (1H, s)	6.94 (1H, s)	102.66 (d)	102.8
8a	-	-	151.30 (s)	151.7
1′	-	-	122.51 (s)	122.6
2′	7.43 (2H, d; *J*= 8.8 Hz)	7.50 (2H, d; *J*= 8.7 Hz)	130.19(d)	130.0
3′	6.91 (2H, d; *J*= 8.8 Hz)	6.98 (2H, d; *J*= 8.7 Hz)	113.98 (d)	113.6
4′	-	-	159.82 (s)	158.9
5′	6.91 (2H, d; *J*= 8.8 Hz)	6.98 (2H, d; *J*= 8.7 Hz)	113.98 (d)	113.6
6′	7.43 (2H, d; *J*= 8.8 Hz)	7.50 (2H, d; *J*= 8.7 Hz)	130.19(d)	130.0
6-OCH_3	3.95 (3H, s)	3.87 (3H, s)	56.54(q)	55.8
4′-OCH_3	3.78 (3H, s)	3.77 (3H, s)	55.38(q)	55.1

Moreover, the information from 2D-NMR spectroscopic techniques of compound 4 including gHSQC (Figure 54), gHMBC (Figure 55), gCOSY (Figure 56) and NOESY (Figure 57) spectral data were used to assist the interpretation of compound 4 structure. All of the data showed in Table 13, Figure 20, 21 and 22, respectively.

Table 13 ^{1}H-NMR, ^{13}C-NMR, gHSQC, gHMBC, gCOSY and NOESY spectral data of compound 4

Carbon position	δ_C	δ_H	gHMBC	gCOSY	NOESY
2	152.10(d)	7.86 (1H, s)	C-3, C-4	-	-
3	124.44 (s)	-	-	-	-
4	175.75 (s)	-	-	-	-
4a	117.92 (s)	-	-	-	-
5	104.79 (d)	7.59 (1H, s)	C-4, C-4a, C-6, C-8a	-	H-6-OCH_3
6	145.39 (s)	-	-	-	-
7	152.58 (s)	-	-	-	-
8	102.66 (d)	6.92 (1H, s)	-	-	-
8a	151.30 (s)	-	-	-	-
1′	122.51 (s)	-	-	-	-
2′	130.19(d)	7.43 (2H, d; *J*= 8.8 Hz)	C-4′, C-3, C-6′	H-3′	-
3′	113.98 (d)	6.91 (2H, d; *J*= 8.8 Hz)	-	H-2′	H-4′-OCH_3
4′	159.82 (s)	-	-	-	-
5′	113.98 (d)	6.91 (2H, d; *J*= 8.8 Hz)	-	H-6′	H-4′-OCH_3
6′	130.19(d)	7.43 (2H, d; *J*= 8.8 Hz)	C-4′, C-3, C-2′	H-5′	-
6-OCH_3	56.54(q)	3.95 (1H, s)	C-6	-	H-5
4′-OCH_3	55.38(q)	3.78 (1H, s)	C-4′	-	H-3′, H-5′

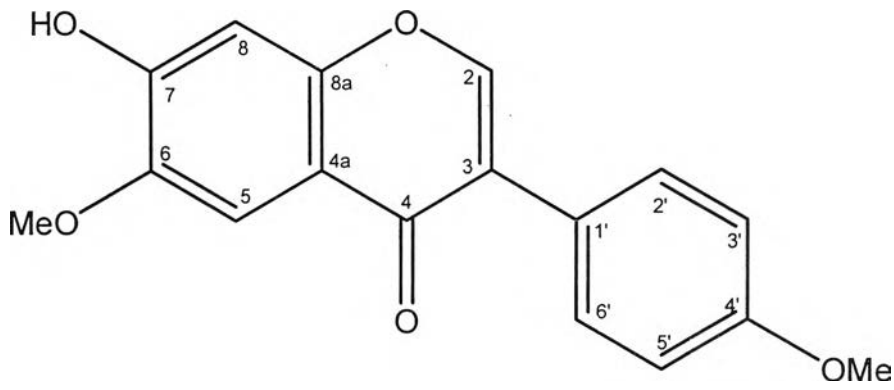
From the Table 13, the H-C connections (HSQC) of compound 4 showed that the C-2 was connected to proton at δ_H 7.86 (1H, s) ppm, the C-5 was connected to proton at δ_H 7.59 (1H, s) ppm, the C-8 was connected to proton at δ_H 6.92 (1H, s) ppm, the C-2′and C-6′ was connected to proton at δ_H 7.43 (2H, d; *J*= 8.8 Hz) ppm, the C-3′and C-5′ was connected to proton at δ_H 6.91 (2H, d; *J*= 8.8 Hz) ppm, the C-6-OCH_3 was connected to proton at δ_H 3.95 (1H, s) ppm, and the C-4′-OCH_3 was connected to proton at δ_H 3.78 (1H, s) ppm., respectively.

The ^{1}H-^{13}C long range connections (HMBC) of compound 4 (Figure 20 and Figure 55 in appendix), which shows the correlations between the H-2 signal and the C-3 and C-4 and signals, the H-5 signal and the C-4, C-4a, C-6 and C-8a signals, the H-2′ signal and the C-4′, C-3 and C-6′ signals, the H-6′ signal and the C-4′, C-3 and C-2′ signals, the H-6-OCH_3 signal and the C-6 signal, and the H-4′-OCH_3 signal and the C-4′ signal, respectively.

The 400 MHz ^{1}H-^{1}H COSY of compound 4 (Figure 21 and Figure 56 in appendix) showed that the signal at the H-2′ signal shows coupling with the H-3′ signal, and the H-6′ signal shows coupling with the H-5′ signal, respectively.

The 400 MHz ^{1}H-^{1}H NOESY of compound 4 (Figure 22 and Figure 57 in appendix) showed that the signal at the H-5 signal shows coupling with the H-6-OCH_3 signal, and the H-4′-OCH_3 signal shows coupling with the H-3′ and H-5′ signals, respectively.

After elucidation of compound 4 by 1D and 2D-NMR spectroscopic technique, the chemical shift on ^{1}H-NMR and ^{13}C-NMR spectrum of compound 4 and 7-hydroxy-6-4′-dimethoxyisoflavone was compared. These results indicated that the structure of compound is identical to 7-hydroxy-6-4′-dimethoxyisoflavone. Thus, it could be concluded that compound 4 was 7-hydroxy-6-4′-dimethoxyisoflavone. The structure is presented in Figure 19. The 7-hydroxy-6-4′-dimethoxyisoflavone was isolated from *Afromosia elata* Harms. in 1960 **[49]** and isolated from other plant such as *Gliricidia sepium* **[35]**, *Andira inermis* that showed antiplasmodial activity against *Plasmodium falciparum* of Pow and Dd2 stains at IC_{50} 36.6±3.3 and 38.5±7.3 μg/ml **[50]**.



7-hydroxy-6-4′-dimethoxyisoflavone

Figure 19 Structure of compound 4

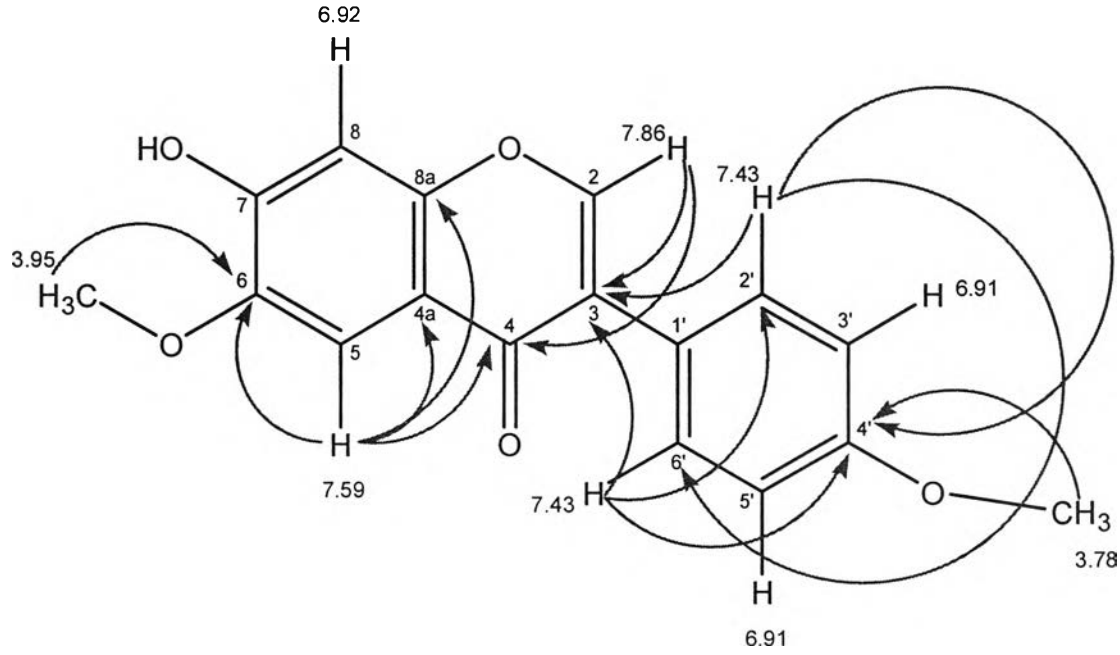


Figure 20 HMBC of compound 4

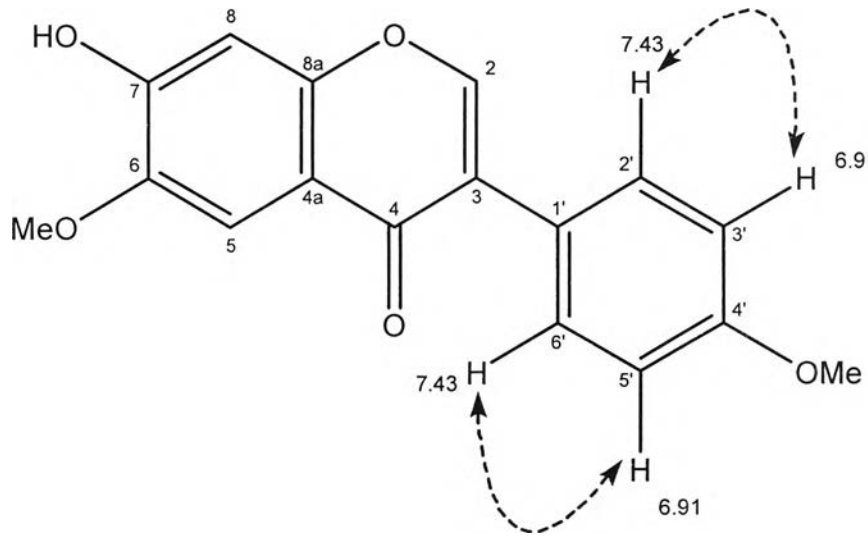


Figure 21 COSY of compound 4

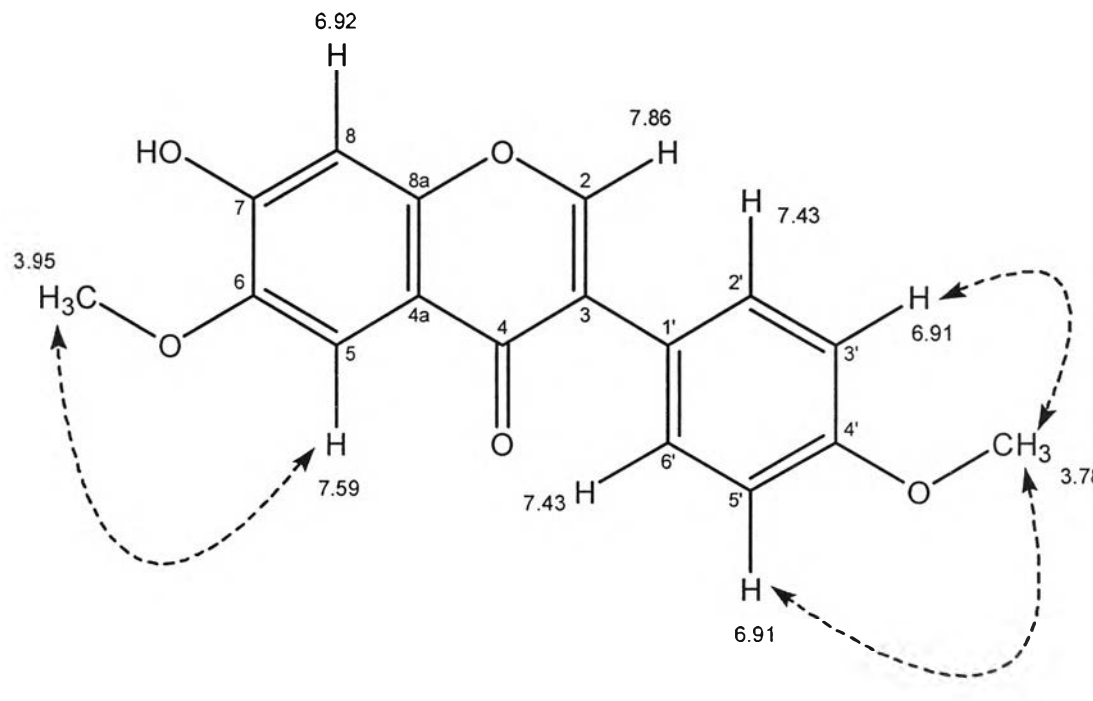


Figure 22 NOESY of compound 4

4.7 Structure elucidation of compound 5

Compound 5 was obtained by flash column chromatography using 1.5% methanol in dichloromethane and preparative TLC technique of 70% ethyl acetate in hexane, 1% methanol in dichloromethane and 100% dichloromethane, respectively. The structure of compound 5 was elucidated using high resolution mass spectrometry, 1D and 2D NMR spectroscopic data as follows.

The ^{1}H-NMR spectrum (Figure 60 and Table 14) of compound 5 possessed chemical shifts of methyl proton at 3.84 and 3.92 ppm, of seven aromatic protons at 6.85 (1H, d; *J*= 2.2 Hz), 6.97 (1H, d; *J*= 8.8 Hz), 6.99 (1H, dd; *J*=8.8 and 2.2 Hz), 7.50 (2H, d; *J*=8.4 Hz) and 8.21 (1H, s) ppm, respectively and of an alkene proton at 7.92 ppm.

The ^{13}C-NMR spectrum (Figure 61 and Table 14) of compound 5 showed 17 signals, which the sp^2-carbon signals of aromatic corresponded to the signal, 100.10 (d), 113.95 (d), 114.56 (d), 118.58 (s), 124.32 (s), 124.88 (s), 127.81 (s), 130.14 (d), 152.08 (d), 158.01 (s), 159.65 (s), 163.96 (s) and 175.95 (s) ppm. There were two methoxy carbon signals at 55.35 (q) and 55.84 (q) ppm.

The TOF-MS (High resolution mass) (Figure 65) showed the $[M+ Na]^+$ ion peak at *m/z* of 305.0790. It seems reasonable to assume that the molecular weight of compound 5 was 282.0790 and the molecular formula was $C_{17}H_{14}O_4$.

From these data indicated that compound 5 should be a 7,4′-dimethoxy-isoflavone, which was reported in the literature **[44, 51].** According to the comparison with the 1D-NMR spectrum of compound 5 and 7,4′-dimethoxyisoflavone should have same the structure. The ^{1}H-NMR and ^{13}C-NMR chemical shifts of these compounds are showed in Table 14.

Table 14 ^{1}H-NMR and ^{13}C-NMR chemical shifts of compound 5 and 7,4′-dimethoxyisoflavone

Carbon position	^{1}H-NMR Chemical shifts (ppm)		^{13}C-NMR Chemical shifts (ppm)	
	Compound 5 ($CDCl_3$, 400 MHz)	7,4′-dimethoxy-isoflavone [44] ($CDCl_3$)	Compound 5 ($CDCl_3$, 100 MHz)	7,4′-dimethoxy-isoflavone [44] ($CDCl_3$)
2	7.92 (1H, s)	7.92 (1H, s)	152.08(d)	152.0
3	-	-	124.88 (s)	125.0
4	-	-	175.92 (s)	175.9
5	8.21(1H, d; *J*= 8.4 Hz)	8.21(1H, d; *J*= 8.9 Hz)	127.81 (s)	127.9
6	6.99(1H, dd; *J*= 8.8 and 2.2 Hz)	6.99(1H, dd; *J*= 8.9 and 2.3 Hz)	114.56 (d)	114.5
7	-	-	163.96 (s)	164.0
8	6.85 (1H, d; *J*= 2.2 Hz)	6.85 (1H, d; *J*= 2.3 Hz)	100.10 (d)	100.2
9	-	-	158.01 (s)	158.0
10	-	-	118.58 (s)	118.5
1′	-	-	124.32 (s)	124.3
2′	7.50 (2H, d; *J*= 8.4 Hz)	7.50 (2H, d; *J*= 8.9 Hz)	130.14 (d)	130.2
3′	6.97 (1H, d; *J*= 8.8 Hz)	6.97 (1H, d; *J*= 8.9 Hz)	113.95 (d)	114.0
4′	-	-	159.65 (s)	159.7
5′	6.97 (1H, d; *J*= 8.8 Hz)	6.97 (1H, d; *J*= 8.9 Hz)	113.95 (d)	114.0
6′	7.50 (2H, d; *J*= 8.4 Hz)	7.50 (2H, d; *J*= 8.9 Hz)	130.14 (d)	130.2
7-OCH_3	3.92 (3H, s)	3.92 (3H, s)	55.84 (q)	55.8
4′-OCH_3	3.84 (3H, s)	3.84 (3H, s)	55.35 (q)	55.4

Moreover, the information from 2D-NMR spectroscopic techniques of compound 5 including gHSQC (Figure 62), gHMBC (Figure 63) and gCOSY (Figure 64) spectral data were used to assist the interpretation of compound 5 structure. All of the data showed in Table 15, Figure 24 and 25, respectively.

Table 15 ^{1}H-NMR, ^{13}C-NMR, gHSQC, gHMBC, and gCOSY spectral data of compound 5

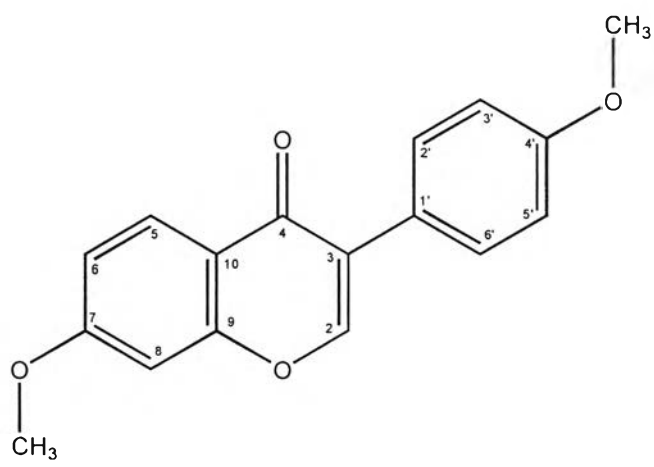
Carbon position	δ_C	δ_H	gHMBC	gCOSY
2	152.08(d)	7.92 (1H, s)	C-3, C-4, C-9, C-1′	-
3	124.88 (s)	-	-	-
4	175.92 (s)	-	-	-
5	127.81 (s)	8.21 (1H, d; *J*= 8.4 Hz)	C-7, C-9	-
6	114.56 (d)	6.99 (1H, dd; *J*= 8.8 and 2.2 Hz)	C-10	H-6
7	163.96 (s)	-	-	H-5
8	100.10 (d)	6.85 (1H, d; *J*= 2.2 Hz)	C-9, C-6	-
9	158.01 (s)	-	-	-
10	118.58 (s)	-	-	-
1′	124.32 (s)	-	-	-
2′	130.14 (d)	7.50 (2H, d; *J*= 8.4 Hz)	C-3, C-1′,C-4′, C-6′	H-3′
3′	113.95 (d)	6.97 (1H, d; *J*= 8.8 Hz)	C-1′, C-4′	H-2′
4′	159.65 (s)	-	-	-
5′	113.95 (d)	6.97 (1H, d; *J*= 8.8 Hz)	C-1′, C-4′	H-6′
6′	130.14 (d)	7.50 (2H, d; *J*= 8.4 Hz)	C-3, C-1′, C-4′, C-6′	H-5′
7-OCH_3	55.84 (q)	3.92 (3H, s)	C-7	-
4′-OCH_3	55.35 (q)	3.84 (3H, s)	C-4′	-

From the Table 15, the H-C connections (HSQC) of compound 5 showed that the C-2 was connected to proton at δ_H 7.92 (1H, s) ppm, the C-5 was connected to proton at δ_H 8.21 (1H, d; *J*= 8.4 Hz) ppm, the C-6 was connected to proton at δ_H 6.99 (1H, dd; *J*= 8.8 and 2.2 Hz) ppm, the C-8 was connected to proton at δ_H 6.85 (1H, d; *J*= 2.2 Hz), the C-2′ and C-6′ was connected to proton at δ_H 7.50 (2H, d; *J*= 8.4 Hz) ppm, the C-3′ and C-5′ was connected to proton at δ_H 6.97 (1H, d; *J*= 8.8 Hz) ppm, the C-7-OCH_3 was connected to proton at δ_H 3.92 (3H, s) ppm, and the C-4′-OCH_3 was connected to proton at δ_H 3.84 (3H, s) ppm, respectively.

The ^{1}H-^{13}C long range connections (HMBC) of compound 5 (Figure 24 and Figure 63 in appendix), which shows the correlations between the H-2 signal and the C-3, C-4, C-9 and C-1′ signals, the H-5 signal and the C-7 and C-9 signals, the H-6 signal and the C-10 signal, the H-8 signal and the C-9 and C-6 signals, the H-2′ and H-6′ signals and the C-3, C-1′, C-4′ and C-6′ signals, the H-3′ and H-5′ signals and the C-1′ and C-4′ signals, the H-7-OCH_3 signal and the C-7 signal, and the H-4′-OCH_3 signal and the C-4′ signal, respectively.

The 400 MHz ^{1}H-^{1}H COSY of compound 5 (Figure 25 and Figure 64 in appendix) showed that the signal at the H-2′ signal shows coupling with the H-3′ signal, the H-6′ signal shows coupling with the H-5′ signal and the H-5 signal show coupling with the H-6 signal, respectively.

After elucidation of compound 5 by 1D and 2D-NMR spectroscopic technique, the chemical shift on ^{1}H-NMR and ^{13}C-NMR spectrum of compound 5 and 7,4′-dimethoxyisoflavone was compared. These results indicated that the structure of compound is identical to 7,4′-dimethoxyisoflavone. Thus, it could be concluded that compound 5 was 7,4′-dimethoxyisoflavone. The structure is presented in Figure 23. The 7,4′-dimethoxyisoflavone was isolated from the leaves of soybean in 1981 **[51]** and the leaves of *Ateleia Herbert- smithii* in 2003 **[44]**.



7,4′-dimethoxyisoflavone

Figure 23 Structure of compound 5

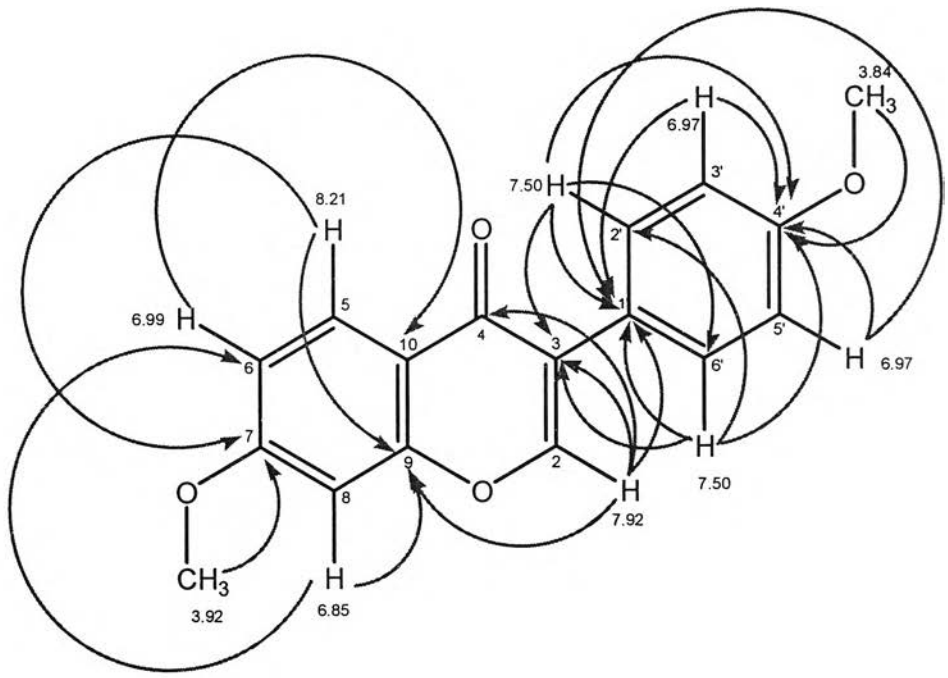


Figure 24 HMBC of compound 5

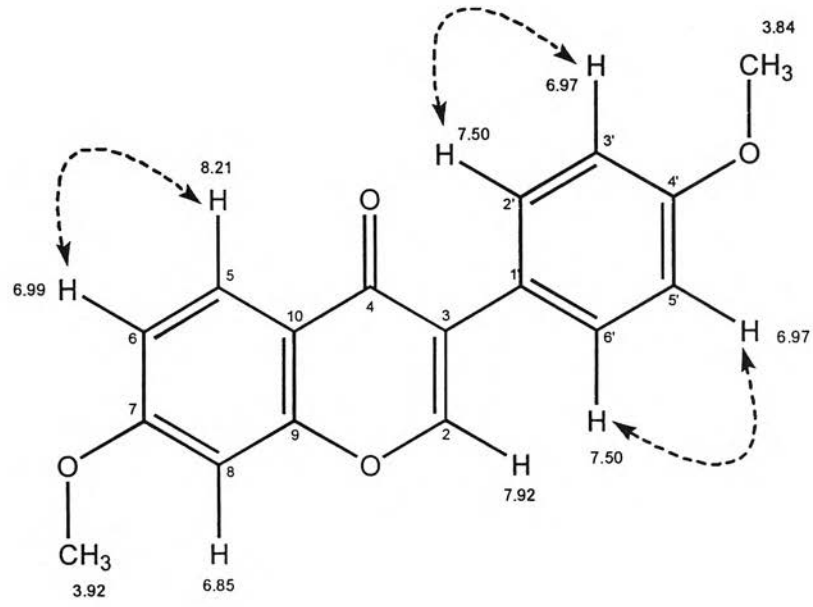


Figure 25 COSY of compound 5

4.8 Structure elucidation of compound 6

Compound 6 was obtained by flash column chromatography using 6.5% methanol in dichloromethane, re-crystallizing of 6% methanol in dichloromethane and washing of methanol, respectively. The structure of compound 6 was elucidated using low resolution mass spectrometry, ^{1}H-NMR and ^{13}C-NMR spectroscopic data as follows.

The ^{1}H-NMR spectrum (Figure 67 and Table 16) of compound 6 possessed chemical shifts of methyl(-CH_3) proton at 0.81 ppm, of the methylene(-CH_2-) protons at 1.20, 1.55 and 2.27 ppm, of a hydroxyl (-OH) proton at 2.00 ppm, of the -CH_2-OH protons at 3.50 (dd; *J*=6.0 and 11.2 Hz) and 3.60 (dd; *J*= 3.2 and 11.6 Hz) ppm, of the -CH-OH proton at 3.83 ppm and of the -CH_2-O- proton at 4.07 ppm, respectively.

^{13}C-NMR spectrum (Figure 68 and Table 16) of compound 6 showed 10 signals, which the sp^2-carbon signals of ketone corresponded to the signal, 174.51(s) and the sp^3-carbon signals of methine (-CH-), methylene (-CH_2-) and methyl (-CH_3-) carbons at 14.12-70.08 ppm.

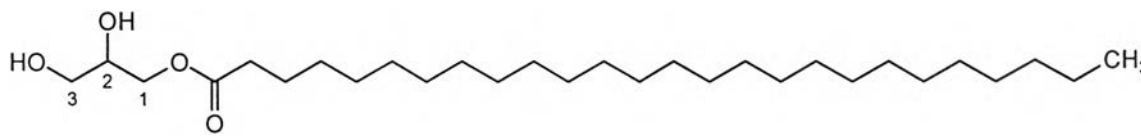
The TOF-MS (Low resolution mass) (Figure 69) showed the $[M+ Na]^+$ ion peak at *m/z* of 493.79. It seems reasonable to assume that the molecular weight of compound 6 was 470.79 and the molecular formula was $C_{29}H_{58}O_4$.

From these data indicated that compound 6 should be a hexacosanoic acid 2,3-dihydroxy-propyl ester, which founded from the root of *Caragana chamlagu* (Leguminosae) in 1990 **[52]** and the twigs of *Carapa guianensis* Aubl.(Meliaceae) in 2004 **[53]**. The ^{1}H-NMR and ^{13}C-NMR chemical shifts of this compound were showed in Table 16.

Table 16 ^{1}H-NMR and ^{13}C-NMR chemical shifts of compound 6

The chemical shifts of compound 6	
400 MHz ^{1}H-NMR (ppm)($CDCl_3$)	**100 MHz ^{13}C-NMR (ppm) ($CDCl_3$)**
0.81 ($-CH_3$)	14.12 ($-CH_3$)
1.20 and 1.55 ($-CH_2-$)	22.70, 24.89 and 31.93 ($-CH_2-$)
2.27 ($-CH_2-\underset{\vert}{C}=O$)	34.18 ($-CH_2-\underset{\vert}{C}=O$)
3.50 and 3.60 ($-CH_2-OH$)	63.27 ($-CH_2-OH$)
3.83 (-CH-OH)	70.08 (-CH-OH)
4.07 ($-CH_2-O-$)	65.12 ($-CH_2-O-$)
1.55 $(-CH_2-)_{20}$	29.70 $(-CH_2-)_{20}$
2.00 (-OH)	174.51 (C=O)

After elucidation of compound 6 by ^{1}H-NMR, ^{13}C-NMR spectroscopic data and low resolution mass spectrometry technique. These results indicated that the structure of compound 6 is identical as hexacosanoic acid 2,3-dihydroxy-propyl ester. Thus, it could be concluded that compound 6 was hexacosanoic acid 2,3-dihydroxy-propyl ester. The structure is presented in Figure 26.



hexacosanoic acid 2,3-dihydroxy-propyl ester

Figure 26 Structure of compound 6

4.9 Results of biological assay

4.9.1 Results of Anti-cancer

The in *vitro* activity of isolated compounds (20 μg/ml) of *Butea superba* Roxb. from Lumpang Province against three cell lines are reported in Table 17

Table 17 Anti-cancer activity against cancer cell lines of isolated compounds of *Butea superba* Roxb. from Lumpang Province

Compound	Activity (μg/ml)		
	KB (Epidermoid carcinoma)	**BC (Breast cancer)**	**NCI-H 187 (Small cell lung)**
	IC_{50}	**IC_{50}**	**IC_{50}**
1	>20	>20	>20
2	19.2±0.8	12.9±0.3	>20
3	10.0±2.5	8.8±1.5	>20
4	>20	>20	>20
5	>20	>20	>20
6	>20	>20	>20

**** Positive control;**

Ellipticine : IC_{50} = 0.55 μg/ml

Doxorubicine : IC_{50} = 0.18 μg/ml

In Table 17 founded that the compound 2 was anti-cancer activity against KB cell line, BC cell line and NCI-H 187 cell line at IC_{50} 19.2±0.8, 12.9±0.3 and >20 μg/ml, respectively. The compound 3 was anti-cancer activity against KB cell line, BC cell line and NCI-H 187 cell line at IC_{50} 10.0±2.5, 8.8±1.5 and >20 μg/ml, respectively. The compound 1, 4, 5 and 6 were anti-cancer activity against KB cell line, BC cell line and NCI-H 187 cell line at IC_{50} >20 μg/ml, respectively.