CHAPTER IV

RESULTS AND DISCUSSION

4.1 PRELIMINARY SCREENING 17 HERBS OF THAILAND.

From the searching of Ethnopharmacopoea on medicinal plants of Thailand, it was found that 17 species of herbs were interesting to be screened against breast cancer cell lines (shown in **Table 3.1**). These herbs were extracted with methanol (shown in **Scheme 3.1**) and the crude extract was test for activity against breast cancer cell lines by MTT method. Result of MTT assay by comparing % survival and % growth of breast cancer cell lines revealed that *K. parviflora* had the highest activity of cytotoxic against BT 474 breast cancer cell lines (shown in **Table 4.1**). Therefore, *K. parviflora* was chosen for futher studies.

Table 4.1 Result of cytotoxic activity against BT 474 breast cancer cell lines.

Herbs	Thai common name	% Survival of breast cancer cell line
Hydnophytum formicarum	หัวร้อยรู	84
Cuscuta chinensis	ฝอยทอง	55
Nelambo nucifera	เกสรบัวหลวง	37
Acanthus ebracteatus	เหงือกปลาหมอ	60
Kaempferia parviflora	กระชายดำ	10
Curcuma longa	ขมิ้นชัน	21
Orthosiphon aristatus	หญ้าหนวดแมว	20
Gelonium multiflorum	ขันทองพยาบาท	38
Salacia chinensis	กำแพง 7 ชั้น	82
Rhinacanthus nasutus	ทองพันชั่ง	21
Euphorbia lacel	แก่นสลัดได	20
Garcinia cowa	ใบชมวง	67
Rauvolfia seppentina	รากระย่อม	70
Artemisia pallens	โกศจุฬำ	40

Herbs	Thai common name	% Survival of breast cancer cell line
Zingiber cassumunar	ไพล	15
Curcuma zedoaria	ขมิ้นอ้อย	14
Livisticum officnale	โกศเชียง	15

4.2 PRELIMINARY SCREENING OF CRUDE EXTRACTS OF *K. parviflora*.

The crude extracts of *K. parviflora* with various solvents were tested for cytotoxic activity against BT 474 breast cancer cell lines and the result showed that the crude ethyl acetate was more active than the crude hexane and crude methanol (**Table 4.3**). Therefore, crude ethyl acetate extract was selected for the next experiment.

Table 4.2 The solvent extracts of *K. parviflora*.

Solvent extract	Appearance	Weight (g)	% wt. by wt. of the *K. parviflora* rhizomes
Hexane	Mixer white – yellow solid and yellow oil	10.64	0.2128
Ethyl acetate	Mixer white solid and yellow oil	7.38	0.1476
Methanol	Brown – violet vicous liquid	21.34	0.4268

Table 4.3 Result of cytotoxic activity against BT 474 breast cancer cell lines of crude hexane, ethyl acetate, methanol extracts.

Crude Extract (10 ug/ml)	% survival of BT 474 breast cancer cell line
Crude Hexane	48
Crude EtOAc	20
Crude MeOH	30

4.3 STRUCTURE ELUCIDATION OF THE ISOLATED COMPOUNDS FROM THE FRESH RHIZOMES OF *K. parviflora.*

4.3.1 STRUCTURE ELUCIDATION OF COMPOUND 1

Compound 1 is a colorless needle crystal (535 mg), UV λ_{max} (nm), $CHCl_3$ (log ε): 262, 322 sh (0.0025), $[\alpha]_D^{20}$ + 4.0 ($CHCl_3$, c 0.5).

FT-IR spectrum, (KBr), νmax (cm^{-1}) : 2,993 (w), 2,931 (w), 2,839 (w), 1,635 (s), 1,612 (s), 1,458 (m), 1,427 (m), 1,350 (m), 1,219 (s), 1,165 (m), 1,111 (m), 1,010 (w), 818 (w), 787 (w), 709 (w), 625 (w) **(Fig. A1).**

^{1}H-NMR spectrum ($CDCl_3$, 200 MHz.) δ (PPM) : revealed the significant signals at chemical shift 3.87, 3.88, 3.95 (9H, all S, 3 * OCH_3), 6.34 (1H, d, J=2 Hz, ArH), 6.50 (1H, d, J=2 Hz, ArH), 8.04 (2H, m, 2 * ArH), 7.46 (3H, m, 3 * ArH) **(Fig. A11)**.

^{13}C- NMR spectrum ($CDCl_3$, 200 MHz.) δ (PPM) : 174.1 (s), 163.9 (s), 161.0 (s), 158.9 (s), 152.6 (s), 141.8 (s), 130.8 (s), 130.3 (s), 128.5 (s), 128.1 (s), 109.5 (s), 95.8 (s), 92.4 (s), 60.1 (s), 56.4 (s), 55.8 (s) **(Fig. A21).**

EI-MS spectrum (m/z) 70 ev : 312 (48) [M^+], 311 (100), 293 (36), 281 (13), 251 (8), 181 (8), 152 (9), 105 (16), 77 (20) **(Fig. A32).**

THE ^{1}H-NMR spectrum (**Fig. A11**) of compound 1 had two doublets at δ 6.34 and 6.54, each showed a 5,7-disubstituted in ring A with meta coupling (*J*=2 Hz), and was integrated for proton at C-6 and C-8 in ring A, respectively. Signals at δ 3.87, 3.88 and 3.95 (all singlets) were attributable to three methoxy groups at C-3, 5 and

7,respectively. The aromatic protons of B-ring appeared as two multiplets at δ 7.46 (3H) and δ 8.04 (2H). According to the literature (22), the compound 1 was assigned as 3,5,7-trimethoxyflavone. The ^{1}H-NMR chemical shifts of compound 1 and 3,5,7-trimethoxyflavone were compared as shown in **Table 4.4**, and structure of 3,5,7-trimethoxyflavone shown in **Fig. 4.1**.

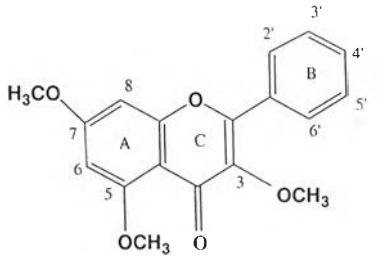


Figure 4.1 Structure of 3,5,7-trimethoxyflavone from *K. parviflora*.

Table 4.4 ^{1}H-NMR spectral data of compound 1 and 3,5,7-trimethoxyflavone.

H	3,5,7-trimethoxyflavone	Compound 1
2	-	-
3	-	-
5	-	-
6	6.27, 1H, (d, J = 2.5)	6.34, 1H, (d, J = 2)
7	-	-
8	6.53, 1H, (d, J = 2.5)	6.50, 1H, (d, J = 2)
2'	8.05, 2H, (m)	8.04, 2H, (m)
3'	7.50, 3H, (m)	7.46, 3H, (m)
4'	7.50, 3H, (m)	7.46, 3H, (m)
5'	7.50, 3H, (m)	7.46, 3H, (m)
6'	8.05, 2H, (m)	8.04, 2H, (m)
3-OCH_3	3.90, 3H, (s)	3.88, 3H, (s)
5-OCH_3	3.97, 3H, (s)	3.95, 3H, (s)
7-OCH_3	3.97, 3H, (s)	3.87, 3H, (s)

The ^{13}C-NMR, DEPT-135, DEPT-90 spectra (**Fig. A21**) showed that compound 1 consists of 18 carbons. One signal of the ketone appeared at 174.1 PPM. Three signals of methoxy groups at 55.8, 56.4 and 60.1 ppm. Result of ^{13}C-NMR, DEPT-135, DEPT-90 spectra confirmed that compound 1 was 3,5,7-trimethoxyflavone. The ^{13}C-NMR chemical shifts of compound 1 are shown in **Table 4.5**.

Table 4.5 ^{13}C-NMR spectral data of compound 1.

^{13}C	Compound 1	^{13}C	Compound 1
2	161.0	1'	141.8
3	130.8	2'	128.1
4	174.1	3'	128.4
5	158.9	4'	130.3
6	95.8	5'	128.4
7	163.9	6'	128.1
8	92.4	3-OCH_3	60.1
9	152.6	5-OCH_3	56.3
10	109.5	7-OCH_3	55.8

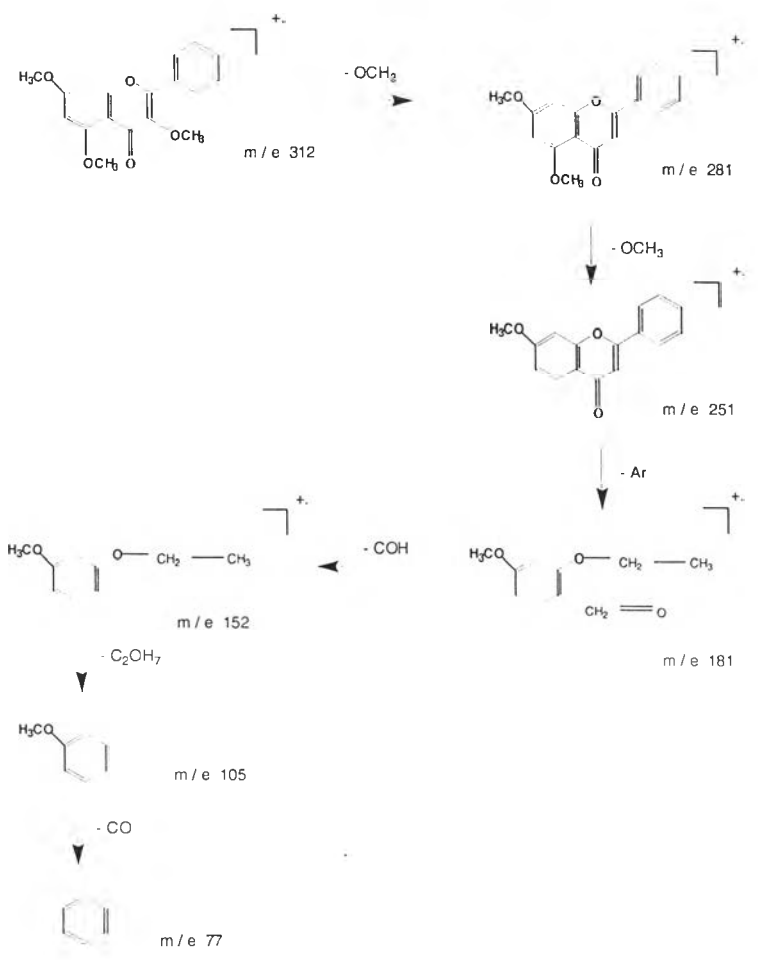
The IR spectrum **(Fig. A1)** of compound 1 showed several characteristic absorption bands which can be assigned as in **Table 4.6**.

Table 4.6 The IR absorption band assignment of Compound 1.

Wave number (cm^{-1})	Intensity	Vibration
2993, 2931, 2839	All weak	C - H stretching vibration of aromatic
1635	Strong	C=O stretching vibration of carbonyl conjugated ketone
1612, 1458, 1427	Strong, medium, medium	C=C stretching vibration of aromatic
1350	Medium	C-H stretching vibration of -CH_3
1219	Strong	C-O unsymmetry stretching vibration of C-O-C
1165, 1111, 1010	Medium, medium, weak	C-O symmetry stretching vibration of C-O-C
818, 787, 709, 625	All weak	.= C-H out of plane bending vibration of aromatic

The mass spectrum **(Fig. A32)** of compound 1 showed the fragmentation as follow, m/z (EIMS) 313 (9), 312 (48) [M^+], 311 (100), 293 (36), 281 (13), 251 (8), 181 (8), 152 (9), 105 (16), 77 (20). Although the molecular ion at m/e 311 was the base peak but molecular weight is 312. The possible fragmentation is shown in **Scheme 4.1.**

The above evidences strongly suggested that compound 1 is 3, 5, 7 - trimethoxyflavone. Moreover, the structure of compound 1 was also confirmed by X-ray diffraction analysis which indicated that compound 1 was 3,5,7 – trimethoxyflavone. Compound 1 was crystallized from ethyl acetate / hexane to yield colorless needles like to colorless needle crystals from hexane / chloroform (22) with m.p. 181-182°C (lit 204-206 °C) (22). The ORTEP drawing of compound 1 is shown in **Fig. 4.2**. The X-ray diffraction is presented in **Table A1, A2, A3, A4** and **A5**, respectively.



Scheme 4.1 The mass fragmentation of the compound 1.

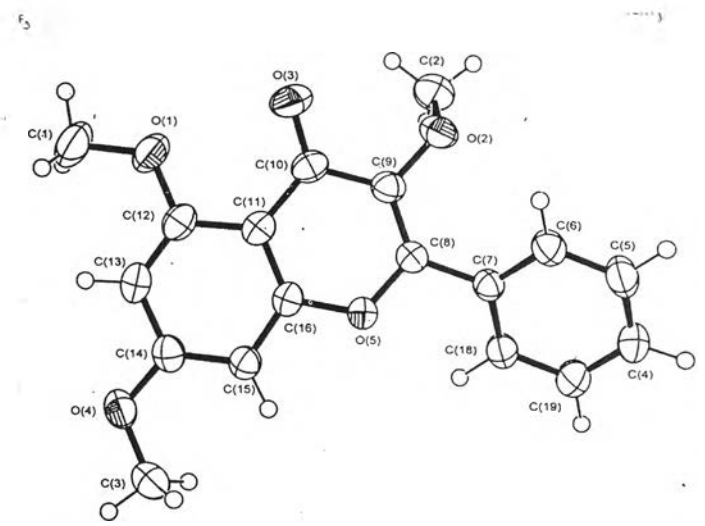


Figure 4.2 The ORTEP drawing of Compound 1.

4.3.2 STRUCTURE ELUCIDATION OF COMPOUND 2

Compound 2 is a colorless needle crystal (2,547 mg), UV λ_{max} (nm), $CHCl_3$ (log ε):262, 302 sh (0.0025), $[\alpha]_D^{20}$ -4.2 ($CHCl_3$, c 0.5).

FT-IR spectrum, (KBr), νmax (cm^{-1}) : 3,425 (m), 3,070 (w), 2,931 (w), 2,862 (w), 1,650 (s), 1,604 (s), 1,458 (m), 1,342 (m), 1,280 (m), 1,203 (m), 1,157 (m), 1,118 (m), 949 (w), 818 (w), 764 (w), 694 (w) **(Fig. A2).**

^{1}H-NMR spectrum ($CDCl_3$, 200 MHz.) δ (PPM) : revealed the significant signals at chemical shift 3.89, 3.94 (6H, all S, 2 * OCH_3), 6.66 (1H, S, ArCOCH = C-Ar), 6.36 (1H, d, J=2 Hz, ArH), 6.55 (1H, d, J=2 Hz, ArH), 7.85 (2H, m, 2*ArH), 7.52 (3H, m, 3*ArH) **(Fig. A12).**

^{13}C- NMR spectrum ($CDCl_3$, 200 MHz.) δ (PPM) : 177.6 (s), 164.1 (s), 160.9 (s), 160.9 (s), 160.6 (s), 159.9 (s), 131.5 (s), 131.2 (s), 128.9 (s), 125.9 (s), 109.0 (s), 96.2 (s), 92.8 (s), 56.4 (s), 55.8 (s) **(Fig. A22).**

EI-MS spectrum (m/z) 70 ev : 283 (14), 282 (100) [M^+], 281 (66), 253 (40), 251 (19), 236 (35), 224 (11), 209 (23) **(Fig. A33).**

The ^{1}H-NMR spectrum (**Fig. A12**) of compound 2 showed two doublets at δ 6.36 and 6.35 with meta coupling (J = 2 Hz) and each doublet integrated for one proton (C-6 and C-8). Two singlets at 3.89 and 3.94 belonged to two methoxy groups at C-7 and C-5, respectively. A singlet at δ 6.66 represented the C-3 aromatic protons. The aromatic protons appeared as two multiplets at δ 7.52 (3H) and 7.85 (2H). From the literature (22), the ^{1}H-NMR data indicated that compound 2 was 5,7 - dimethoxyflavone. The ^{1}H-NMR chemical shifts of compound 2 and 5,7-dimethoxyflavone were compared as shown in **Table 4.7**, and structure of 5,7-dimethoxyflavone is shown in **Fig. 4.3**.

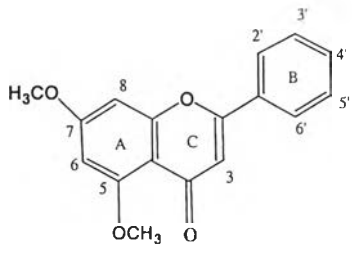


Figure 4.3 Structure of 5,7-dimethoxyflavone from *K. parviflora*.

Table 4.7 ^{1}H-NMR spectral data of compound 2 and 5,7-dimethoxyflavone.

H	5,7-dimethoxyflavone	Compound 2
2	-	-
3	6.65, 1H, (s)	6.66, 1H, (s)
5	-	-
6	6.38, 1H, (d, J = 2.5)	6.36, 1H, (d, J = 2)
7	-	-
8	6.57, 1H, (d, J = 2.5)	6.55, 1H, (d, J = 2)
2'	7.88, 2H, (m)	7.85, 2H, (m)
3'	7.38, 3H, (m)	7.52, 3H, (m)
4'	7.38, 3H, (m)	7.52, 3H, (m)
5'	7.38, 3H, (m)	7.52, 3H, (m)
6'	7.88, 2H, (m)	7.85, 2H, (m)
5-OCH_3	3.92, 3H, (s)	3.94, 3H, (s)
7-OCH_3	3.92, 3H, (s)	3.89, 3H, (s)

The ^{13}C-NMR, DEPT-135, DEPT-90 spectra **(Fig. A22)** showed that compound 2 has 17 carbons which correlated to molecular formular of 5,7-dimethoxyflavone. Moreover the data revealed two signals of methoxy groups at δ 55.8 and 56.4, and one signal of ketone at 177.6 ppm. The ^{13}C-NMR chemical shifts of compound 2 are shown in **Table 4.8**.

Table 4.8 ^{13}C-NMR spectral data of compound 2.

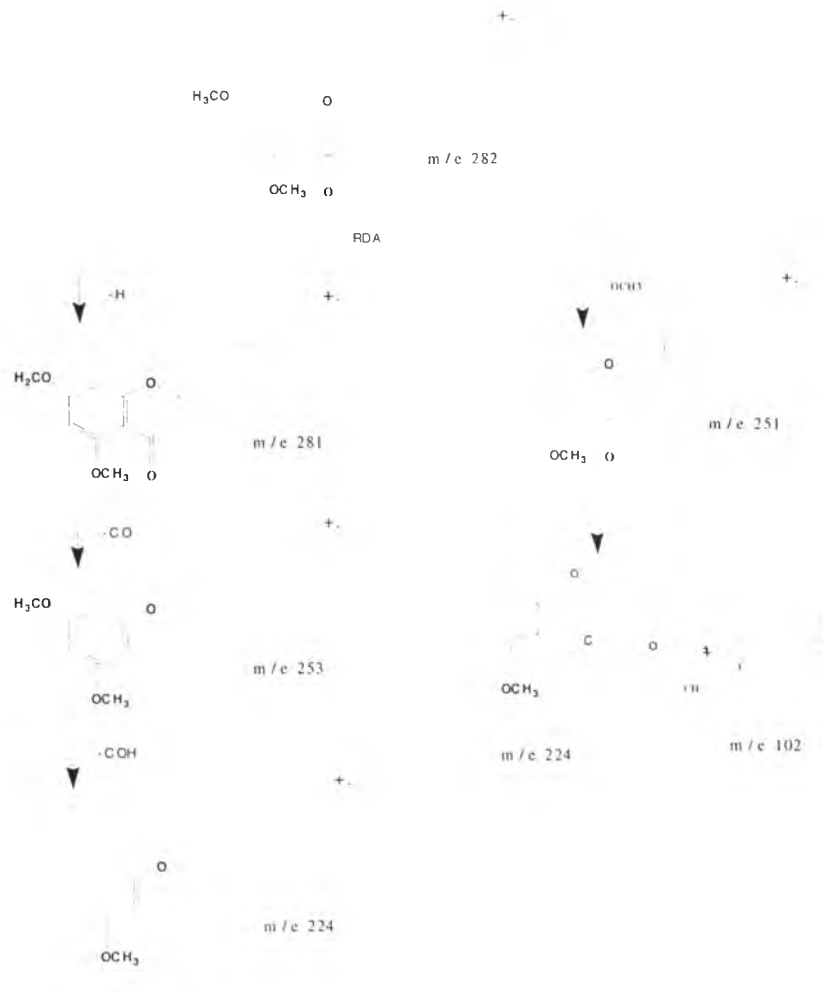
C	Compound 2	C	Compound 2
2	160.9	1'	131.5
3	109	2'	125.9
4	177.6	3'	128.9
5	160.6	4'	131.2
6	96.2	5'	128.9
7	164	6'	125.9
8	92.8	5-OCH_3	56.4
9	159.9	7-OCH_3	55.8
10	109		

The IR spectrum **(Fig. A2)** of compound 2 is summarized in **Table 4.9**.

Table 4.9 The IR absorption band assignment of Compound 2.

Wave number (cm^{-1})	Intensity	Vibration
3,425, 3,070, 2,931, 2,862	All weak	C - H stretching vibration of aromatic
1,650	Strong	C=O stretching vibration of carbonyl conjugated ketone
1,604, 1,458	Strong, medium	C=C stretching vibration of aromatic
1,342	Medium	C-H stretching vibration of -CH_3
1,280, 1,203	Medium, medium	C-O unsymmetry stretching vibration of C-O-C
1,157, 1,118	Medium, medium	C-O symmetry stretching vibration of C-O-C
949, 818, 764, 694	All weak	.= C-H out of plane bending vibration of aromatic

The mass spectrum **(Fig. A33)** of compound 2 showed the fragmentation as follow, m/z (EIMS) 283 (14), 282 (100) [M^+], 281 (66), 253 (40), 251 (19), 236 (35), 224 (11), 209 (23). The mass spectrum of compound 2 showed the molecular ion at m/e 282 which corresponded to the mass of the 5,7-dimethoxyflavone. The possible fragmentations are shown in **Scheme 4.2**.



Scheme 4.2 The mass fragmentation of the compound 2.

Moreover, the structure of compound 2 was also confirmed by X-ray diffraction analysis, which is shown in **Fig. 4.4**. X-ray diffraction data are presented in **Table A6, A7, A8, A9** and **A10**. The colorless needle crystals of 5,7-dimethoxyflavone were obtained from ethyl acetate/hexane and had m.p. 134-135°C colorless needles from chloroform / hexane, m.p. 149-150 °C (22) . The molecular formular and molecular weight were $C_{17}H_{14}O_4$ and 282, respectively.

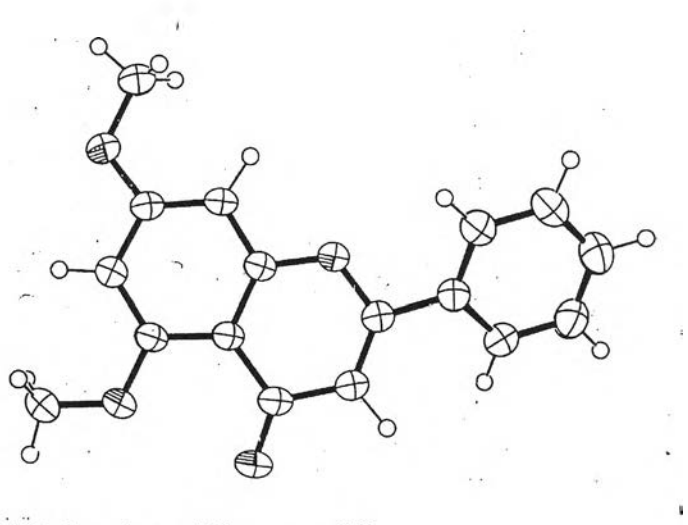


Figure 4.4 The ORTEP drawing of Compound 2.

4.3.3 STRUCTURE ELUCIDATION OF COMPOUND 3

Compound 3 is a white powder (232 mg), UV λ_{max} (nm), $CHCl_3$ (log ε):266, 318 sh (0.05), $[\alpha]_D^{20}$ -3.4 ($CHCl_3$, c 0.5).

FT-IR spectrum, (KBr), νmax (cm^{-1}) : 3,070 (w), 2,939 (w), 1,658 (m), 1,596 (s), 1,504 (m), 1,435 (m), 1,373 (m), 1,342 (m), 1,311 (m), 1,203 (s), 1,165 (m), 995 (w), 833 (w), 702 (w), 633 (w) **(Fig. A3).**

^{1}H-NMR spectrum ($CDCl_3$, 200 MHz.) δ (PPM) : revealed the significant signals at chemical shift 3.85, 3.88, 3.92 (9H, all s, 3 * OCH_3), 6.57 (1H, S, ArCOCH = C-Ar), 6.35 (1H, d, J=2 Hz, ArH), 6.54 (1H, d, J=2 Hz, ArH), 7.80 (2H, d, J=9 Hz, 2 * ArH), 6.98 (2H, d, J=9Hz, 2 * ArH) **(Fig. A13).**

^{13}C- NMR spectrum ($CDCl_3$, 200 MHz.) δ (PPM) : 177.7 (s), 163.9 (s), 162.0 (s), 160.8 (s), 160.7 (s), 159.8 (s), 127.6 (s), 123.7 (s), 114.3 (s), 109.1 (s), 107.5 (s), 96.0 (s), 92.8 (s), 56.4 (s), 55.8 (s), 55.5 (s) **(Fig. A23).**

EI-MS spectrum (m/z) 70 ev : 313 (21), 312 (100) [M^+], 311 (68), 283 (23), 281 (22), 266 (25), 132 (19) **(Fig. A34).**

The ^{1}H-NMR spectrum **(Fig. A13)** of compound 3 showed three singlets at δ 3.85, 3.88, and 3.92 due to nine methoxy protons. Two doublets at δ 6.35 (J = 2 Hz) were assigned to the protons on C-6 and C-8. The singlet at δ 6.57 was due to the C-3 proton. Two doublets at δ 6.98 (J = 9 Hz) and 7.80 (J = 9 Hz) were assigned to the aromatic protons. The ^{1}H-NMR spectrum of compound 3 correlated with the ^{1}H-NMR spectrum of 5,7,4'-trimethoxyflavone (22). Compound 3 was assigned as 5,7,4'-trimethoxyflavone. The ^{1}H-NMR chemical shifts of compound 3 and 5,7,4'-trimethoxyflavone were compared as shown in **Table 4.10**, and structure of 5,7,4'-trimethoxyflavone shown in **Fig. 4.5.**

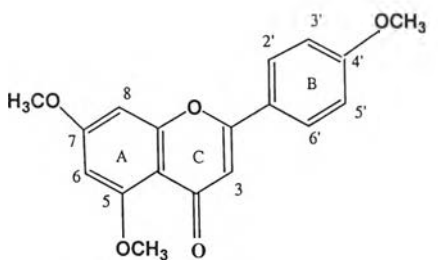


Figure 4.5 Structure of 5,7,4'-trimethoxyflavone from *K. parviflora*.

Table 4.10 ^{1}H-NMR spectral data of compound 3 and 5,7,4'-dimethoxyflavone.

H	5,7,4'-trimethoxyflavone	Compound 3
2	-	-
3	6.55, 1H, (s)	6.57, 1H, (s)
5	-	-
6	6.35, 1H, (d, J = 2.5)	6.35, 1H, (d, J = 2)
7	-	-
8	6.53, 1H, (d, J = 2.5)	6.54, 1H, (d, J = 2)
2'	7.81, 2H, (d, J = 9)	7.80, 2H, (d, J = 9)
3'	6.93, 2H, (d, J = 9)	6.98, 2H, (d, J = 9)
4'	-	-
5'	6.93, 2H, (d, J = 9)	6.98, 2H, (d, J = 9)
6'	7.81, 2H, (d, J = 9)	7.80, 2H, (d, J = 9)
5-OCH_3	3.85, 3H, (s)	3.92, 3H, (s)
7-OCH_3	3.88, 3H, (s)	3.88, 3H, (s)
4'-OCH_3	3.93, 3H, (s)	3.85, 3H, (s)

The ^{13}C-NMR, DEPT-135, DEPT-90 spectra **(Fig. A23)** were in agreement with 5,7,4'-trimethoxyflavone. The signals at δ 55.4, 55.5, 55.7 ppm were signals of the three methoxy groups and δ 177.7 was the signal of a ketone. The ^{13}C-NMR chemical shifts of compound 3 are shown in **Table 4.11**.

Table 4.11 ^{13}C-NMR spectral data of compound 3.

C	Compound 3	C	Compound 3
2	162	1'	123.7
3	107.5	2'	127.5
4	177.7	3'	114.3
5	160.7	4'	160.7
6	96	5'	114.3
7	163.9	6'	127.5
8	92.8	5-OCH_3	56.3
9	159.8	7-OCH_3	55.7
10	109.1	4'-OCH_3	55.4

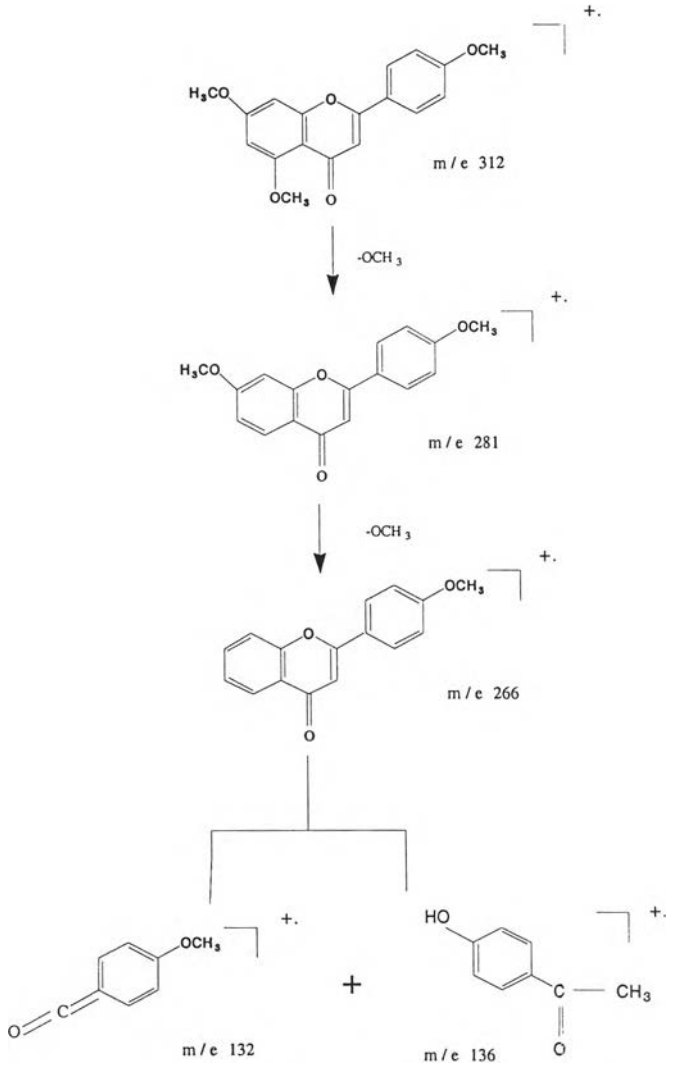
The mass spectrum **(Fig. A34)** of compound 3 showed the fragmentation as follow, m/z (EIMS) 313 (21), 312 (100) [M^+] 311 (68), 283 (23), 281 (22), 266 (25), 132 (19). The mass spectrum of compound 3 showed the molecular ion of m/z 312 as the base peak that confirmed molecular weight of 5,7,4'-trimethoxyflavone and fragmentations are shown in **Scheme 4.3**.

The IR spectrum **(Fig. A3)** of compound 3 is summarized in **Table 4.12**.

Table 4.12 The IR absorption band assignment of Compound 3.

Wave number (cm^{-1})	Intensity	Vibration
3,070, 2,939	Weak, weak	C - H stretching vibration of aromatic
1,658	Medium	C=O stretching vibration of carbonyl conjugated ketone
1,596, 1,504, 1,435	Strong, medium, medium	C=C stretching vibration of aromatic
1,373, 1,342, 1,311	All medium	C-H stretching vibration of $-CH_3$
1,203	Strong	C-O unsymmetry stretching vibration of C-O-C
1,165	Medium	C-O symmetry stretching vibration of C-O-C
995, 833, 702, 633	All weak	.= C-H out of plane bending vibration of aromatic

The above evidence suggested that compound 3 is 5,7,4'-trimethoxyflavone. Moreover compound 3 was obtained from crude ethyl acetate as white powder 139-141° C (lit m.p.149-150) (22). The molecular formular and molecular weight are $C_{18}H_{16}O_5$ and 312, respectively.



Scheme 4.3 The mass fragmentation of the compound 3.

4.3.4 STRUCTURE ELUCIDATION OF COMPOUND 4

Compound 4 is a white powder (96 mg), UV λ_{max} (nm), MeOH (log ε):206, 264, 328 sh (0.05), $[\alpha]_D^{20}$ - 4.7 (MeOH, c 0.5)

FT-IR spectrum, (KBr), νmax (cm^{-1}) : 3,450 - 3,240 (b), 1,639 (s), 1,612 (s), 1,458 (m), 1,358 (m), 1,304 (w), 1,257 (m), 1,211 (m), 1,173 (m), 1,119 (m), 1,057 (w), 833 (m) **(Fig. A4).**

^{1}H-NMR spectrum (DMSO, 200 MHz.) δ (PPM) : revealed the significant signals at chemical shift 3.81, 3.88 (6H, all s, 2 * OCH_3), 6.57 (1H, S, ArCOCH = C-Ar), 6.48 (1H, d, J=2 Hz, ArH), 6.82 (1H, d, J=2 Hz, ArH), 7.87 (2H, d, J=9 Hz, 2 * ArH), 6.90 (2H, d, J=9Hz, 2 * ArH), 10.23 (1H, s, OH) **(Fig. A14).**

^{13}C-NMR spectrum (DMSO, 200 MHz.) δ (PPM) : 175.7 (s), 163.6 (s), 160.5 (s), 160.2 (s), 160.1 (s), 159.1 (s), 127.8 (s), 121.3 (s), 115.8 (s), 108.2 (s), 106.0 (s), 96.1 (s), 93.3 (s), 55.9 (s), 55.8 (s) **(Fig. A24).**

EI-MS spectrum (m/z) 70 ev : 255 (100), 225 (20), 121 (7), 118 (17), 107 (4), 89 (20), 69 (22). **(Fig. A35)**

LC-MS spectrum (m/z) (APCI, MeOH : H_2O, 1:1) : 299 (100) [M^++1] **(Fig. A36)**.

The ^{1}H-NMR spectrum (Fig. A14) of compound 4 showed two singlets at δ 3.81 and 3.88 which were assigned to the methoxy groups. Two doublets at δ 6.48 (J = 2Hz) and 6.82 (J = 2Hz) were due to the protons on C-6, C-8. The singlet at δ 6.57 was assigned to the C-3 proton. The signal at δ 10.23 was belonged to hydroxyl group. Two doublets at δ 7.87 (J = 9 Hz) 6.90 (J = 9 Hz) was assigned to the aromatic protons. From the ^{1}H-NMR data, compound 4 may be assigned as 4'-hydroxy-5,7-dimethoxyflavone (23). The ^{1}H-NMR chemical shifts of compound 4 and 4'-hydroxy-7,4-dimethoxyflavone were compared as shown in **Table 4.13**, and structure of 4'-hydroxy-7,4-dimethoxyflavone is shown in **Fig. 4.6**.

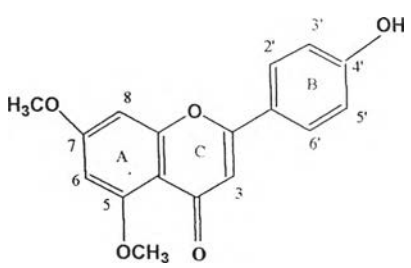


Figure 4.6 Structure of 4'-hydroxy-7,4-dimethoxyflavone from *K. parviflora*.

Table 4.13 ^{1}H-NMR spectral data of compound 4 and 4'-hydroxy-7,4-dimethoxyflavone

H	4'-hydroxy-5,7-dimethoxyflavone	Compound 4
2	-	-
3	6.55, 1H, (s)	6.57, 1H, (s)
5	-	-
6	6.34, 1H, (d, J = 2.5)	6.48, 1H, (d, J = 2)
7	-	-
8	6.81, 1H, (d, J = 2.5)	6.82, 1H, (d, J = 2)
2'	7.85, 2H, (d, J = 8.5)	7.87, 2H, (d, J = 9)
3'	6.89, 2H, (d, J = 8.5)	6.90, 2H, (d, J = 9)
4'	-	-
5'	6.88, 2H, (d, J = 8.5)	6.90, 2H, (d, J = 9)
6'	7.89, 2H, (d, J = 8.5)	7.87, 2H, (d, J = 9)
5-OCH_3	3.88, 3H, (s)	3.88, 3H, (s)
7-OCH_3	3.88, 3H, (s)	3.81, 3H, (s)
4'-OH	12.20, 1H, (s)	10.23, 1H, (s)

The ^{13}C-NMR, DEPT-135, DEPT-90 spectra **(Fig. A24),** showed that compound 4 has 17 carbons, two methoxy groups appeared at δ 55.9 and 55.8 ppm. Ketone group was at δ 175.7 ppm. The ^{13}C-NMR data and ^{1}H-NMR data indicated that compound 4 was 4'-hydroxy-5,7-dimethoxyflavone. The ^{13}C-NMR chemical shifts of compound 4 are shown in **Table 4.14**.

Table 4.14 ^{13}C-NMR spectral data of compound 4.

C	Compound 4	C	Compound 4
2	160.5	1'	121.3
3	106.0	2'	127.8
4	175.7	3'	115.8
5	160.1	4'	160.1
6	96.1	5'	115.8
7	163.6	6'	127.8
8	93.3	5-OCH_3	56.0
9	159.1	7-OCH_3	55.9
10	108.1		

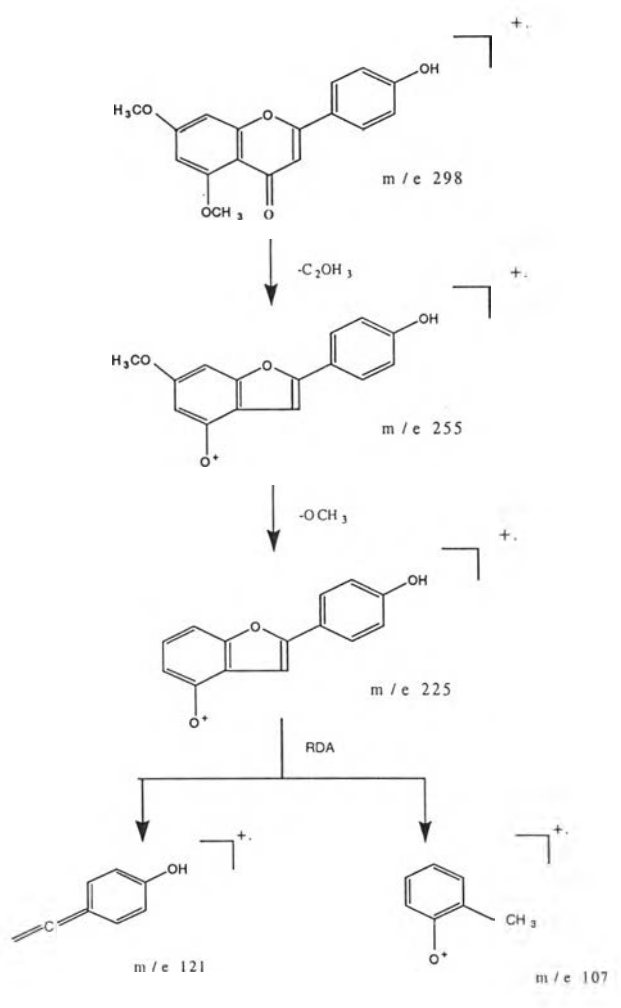
The IR spectrum **(Fig. A4)** of compound 4 showed several characteristic absorption bands which can be assigned as shown in **Table 4.15**.

Table 4.15 The IR absorption band assignment of Compound 4.

Wave number (cm-1)	Intensity	Vibration
3,450 - 3,240	Broad	O-H stretching vibration of hydroxy group
1,636	Strong	C=O stretching vibration of carbonyl Conjugated ketone
1,612, 1,458	Strong, Medium	C=C stretching vibration of aromatic
1,358, 1,304	Medium, Weak	C-H stretching vibration of $-CH_3$
1,257, 1,211	Medium, Medium	C-O unsymmetry stretching Vibration of C-O-C
1,173, 1,119, 1,057	Medium, Medium, Weak	C-O symmetry stretching Vibration of C-O-C
883	Medium	.= C-H out of plane bending vibration Of aromatic

The mass spectrum **(Fig. A35, A36)** of compound 4 showed the fragmentation as follow, m/z (EIMS) 255 (100), 225 (20), 121 (7), 118 (17), 107 (4), 89 (20), 69 (22), The base peak was at m/e 255 which indicated the fragmentation of compound 4 by lossing C=O and CH_3. The fragmentation is shown in **Scheme 4.4**. Moreover the LCMS (APCI, MeOH:H_2O, 1:1) indicated the 299 (100) [M^++1] as the base peak. Therefore, the molecular weight of compound 4 was 298 which corresponds to mass of the 4'-hydroxy-5,7-dimethoxyflavone.

From all spectral data, it suggested that compound 4 was 4'-hydroxy-5,7-dimethoxyflavone. The molecular weight and molecular formular were 298 and $C_{17}H_{14}O_5$, respectively (23). It was white powder, obtained from 80% ethyl acetate / hexane. Melting point of compound 4 was 252-254°C (lit m.p.290-1) (23).



Scheme 4.4 The fragmentation diagram of the compound 4.

4.3.5 STRUCTURE ELUCIDATION OF COMPOUND 5

Compound 5 is a greenish - yellow solid (27 mg) UV λ_{max} (nm), $CHCl_3$ (log ε):243, 287, 393 sh (0.03), $[\alpha]_D^{20}$ -1.1 ($CHCl_3$, c 0.5).

FT-IR spectrum, (KBr), νmax (cm^{-1}) : 3640 - 3250 (b), 2360 (m), 1627 (s), 1504 (m), 1442 (m), 1134 (m), 972 (m), 709 (w), 679 (w). **(Fig. A5)**

^{1}H-NMR spectrum ($CDCl_3$, 200 MHz.) δ (PPM) : revealed the significant signals at chemical shift δ 5.84 (1H, S, 1H), 6.62 (2H, d, J 16 Hz, 3 - H and 3' - H), 7.65 (2H, d, J 16 Hz, 4 - H and 4' - H) and 7.28 - 7.56 (10H, aromatic protons). **(Fig. A15)**

^{13}C- NMR spectrum ($CDCl_3$, 200 MHz.) δ (PPM) : 183.3 (s), 140.7 (s), 134.9 (s), 130.1 (s), 128.9 (s), 128.1 (s), 124.1 (s), 101.8 (s). **(Fig. A25)**

EI-MS spectrum (m/z) 70 ev : 276 [M^+] (8), 169 (14), 144 (13), 132 (13), 131 (22), 116 (34), 115 (50), 103 (50), 102 (45), 91 (29), 78 (42), 77 (100). **(Fig. A37)**

The ^{1}H-NMR spectrum **(Fig. A15)** of compound 5 had a singlet at δ 5.84 for proton at C-1. Two doublets at δ 6.62 (J = 16 Hz) and δ 7.65 (J = 16 Hz) belonged to 4 protons at C-3, 3' and C-4,4'. The 10 aromatic protons of two benzene rings appeared as multiplet at δ 7.28 - 7.56. According to literature (24), the ^{1}H-NMR data of compound 5 indicated that it was dicinnamoylmethane. The ^{1}H-NMR chemical shifts of compound 5 and dicinnamoylmethane were compared as shown in **Table 4.16**, and the structure of dicinnamoylmethane is shown in **Fig. 4.7**.

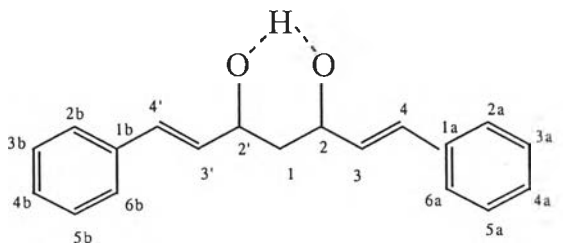


Figure 4.7 The structure of dicinnamoylmethane.

Table 4.16 ^{1}H-NMR spectral data of compound 5 and dicinnamoylmethane.

^{1}H	Dicinnamoylmethane	Compound 5
1	5.86, 1H, (s)	5.84, 1H, (s)
3, 3'	6.64, 2H, (d, J = 16)	6.62, 2H, (d, J = 16)
4, 4'	7.67, 2H, (d, J = 16)	7.65, 2H, (d, J = 16)
10 ArH	7.45, 10H, (m)	7.42, 10H, (m)

The ^{13}C-NMR, DEPT-135, DEPT-90 spectra **(Fig. A25)** showed that compound 5 has 19 carbons. The signal of carbons appeared at δ 101.82 (C-1), δ 183.33 (C-2,2'), δ 124.10 (C-3,3'), δ 140.66 (C-4,4'), δ 134.97 (C-1a,1b), δ 128.13 (C-2a,6a,2b,6b), 128.93 (C-3a,5a,3b,5b), δ 130.13 (C-4a,4b). From the result of ^{13}C-NMR, DEPT-135 and

DEPT-90, it confirmed that compound 5 was dicinnamoylmethane (24). The ^{13}C-NMR chemical shifts of compound 5 and dicinnamoylmethane are shown in **Table 4.17**.

Table 4.17 ^{13}C-NMR spectral of compound 5 and dicinnamoylmethane.

C	Dicinnamoylmethane	Compound 5
1	101.8	101.8
2, 2'	183.3	183.3
3, 3'	124.1	124.1
4, 4'	140.5	140.7
1a, 1b	135	135
2a, 6a, 2b, 6b	128.1	128.1
3a, 5a, 3b, 5b	128.9	128.9
4a, 4b	130.1	130.1

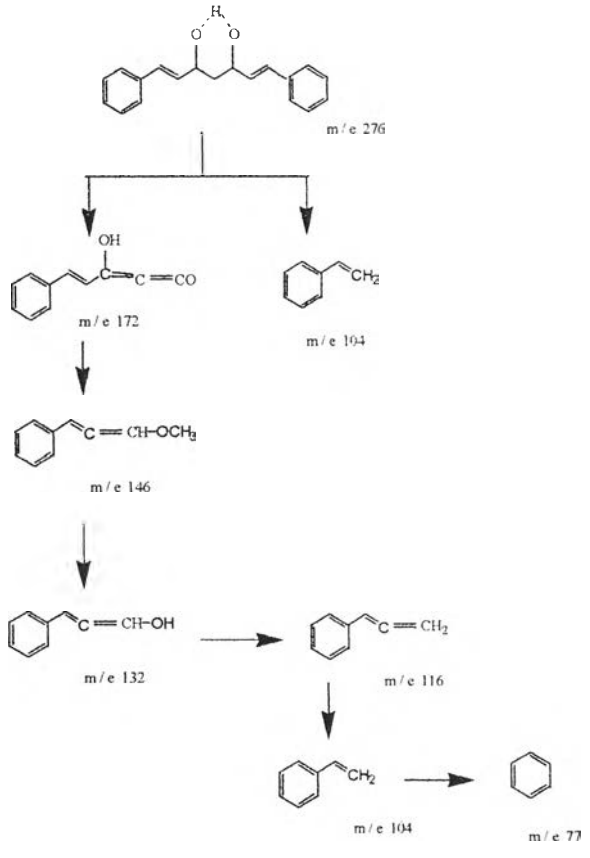
The IR spectrum **(Fig. A5)** of compound 5 showed several characteristic absorption bands in **Table 4.18**.

Table 4.18 The IR absorption band assignment of Compound 5.

Wave number (cm^{-1})	Intensity	Vibration
3640 - 3250	Broad	O-H stretching vibration of hydroxy group
2360	Medium	C - H stretching vibration of aromatic
1627	Strong	C=O stretching vibration of carbonyl Conjugated ketone
1581, 1504, 1442	Medium, Medium, Medium	C=C stretching vibration of aromatic
1134	Medium	C-O symmetry stretching Vibration of C-O-C
972, 709, 679	Medium, Weak, Weak	.= C-H out of plane bending vibration Of aromatic

The mass spectrum (Fig. A37) of compound 5 showed the fragmentation as follow, m/z (EIMS) 276 [M^+] (8), 169 (14), 144 (13), 132 (13), 131 (22), 116 (34), 1 (50), 103 (50), 102 (45), 91 (29), 78 (42), 77 (100). The mass spectrum showed the molecular ion of m/z 276 and the ion of m/z 77 (base peak). The other abundant ions were m/z 115, 103. Mass fragmentation diagram of compound 5 is shown in **Scheme 4.5**.

These data were sufficient to suggest that compound 5 is dicinnamoylmethane. The molecular weight and molecular formular are 276 and $C_{19}O_2H_{15}$, respectively.



Scheme 4.5 The mass fragmentation of the compound 5.

4.3.6 STRUCTURE ELUCIDATION OF COMPOUND 6

Compound 6 is a yellow needle crystal (721 mg) UV λ_{max} (nm), $CHCl_3$ (log ε):268, 330 sh (0.03), $[\alpha]_D^{20}$ -6.1 ($CHCl_3$, c 1).

FT-IR spectrum, (KBr), νmax (cm^{-1}) : 3620 - 3080 (b), 2970 (w), 2820 (w), 1643 (s), 1604 (s), 1500 (w), 1420 (w), 1350 (m), 1257 (m), 1180 (m), 1127 (m), 1034 (m), 833 (m), 625 (m).**(Fig.A6)**

^{1}H-NMR spectrum ($CDCl_3$, 200 MHz.) δ (PPM) : revealed the significant signals at chemical shift 3.85, 3.86 (6H, all s, 2 * OCH_3), 6.35 (1H, d, J=2 Hz, ArH),

6.44 (1H, d, J=2 Hz, ArH), 8.03 (2H, m, 2 * ArH), 7.48 (3H, m, 3 * ArH), 12.56 (1H, S, OH). **(Fig. A16)**

^{13}C- NMR spectrum ($CDCl_3$, 200 MHz.) δ (PPM) : 178.9 (s), 165.6 (s), 161.9 (s), 156.9 (s), 155.9 (s), 139.7 (s), 130.9 (s), 130.4 (s), 128.6 (s), 128.4 (s), 106.2 (s), 97.9 (s), 92.2 (s), 60.4 (s), 55.8 (s). **(Fig. A26)**

EI-MS spectrum (m/z) 70 ev : 298 (75) [M^+], 297 (100), 280 (9), 279 (11), 269 (8), 267 (11), 255 (8), 171 (8), 148 (9), 135 (14), 129 (10), 115 (10), 105 (22), 77 (20). **(Fig. A38)**

The ^{1}H-NMR spectrum **(Fig. A16)** of compound 6 showed two singlets at δ 3.85 and 3.86 due to methoxy protons in C-7 and C-3, respectively. Two doublets at δ 6.35 (d, J = 2 Hz) and 6.44 (d, J = 2 Hz) were due to the protons on C-6 and C-8. The hydroxyl group appeared at δ 12.56. The monosubstituted B-ring appeared as two multiplets at δ 7.48 and 8.03 with integration for 3H and 2H, respectively. From ^{1}H-NMR data compound 6 was interpreted as 5-hydroxy-3,7-dimethoxyflavone (22). The ^{1}H-NMR chemical shifts of compound 6 and 5-hydroxy-3,7-dimethoxyflavone were compared as shown in **Table 4.19**, and the structure of 5-hydroxy-3,7-dimethoxyflavone is shown in **Fig ure 4.8**.

Table 4.19 ^{1}H-NMR spectral data of compound 6 and 5-hydroxy-3,7-dimethoxyflavone.

H	5-hydroxy-3,7-dimethoxyflavone	Compound 6
2	-	-
3	-	-
5	-	-
6	6.37, 1H, (d, J = 2.5)	6.35, 1H, (d, J = 2)
7	-	-
8	6.47, 1H, (d, J = 2.5)	6.44, 1H, (d, J = 2)
2'	8.08, 2H, (m)	8.03, 2H, (m)
3'	7.50, 3H, (m)	7.48, 3H, (m)
4'	7.50, 3H, (m)	7.48, 3H, (m)
5'	7.50, 3H, (m)	7.48, 3H, (m)
6'	8.08, 2H, (m)	8.03, 2H, (m)
3-OCH_3	3.87, 3H, (s)	3.86, 3H, (s)
7-OCH_3	3.87, 3H, (s)	3.85, 3H, (s)
5-OH	12.53, 1H, (s)	12.56, 1H, (s)

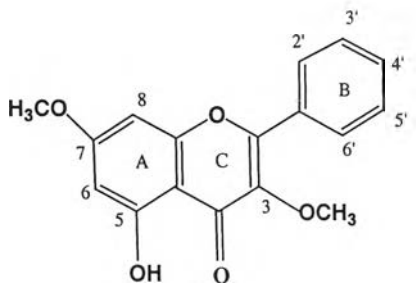


Figure 4.8 The structure of 5-hydroxy-3,7-dimethoxyflavone.

The ^{13}C-NMR, DEPT-135, DEPT-90 spectra **(Fig. A26)** showed that compound 6 has 17 carbons in which two methoxy groups appeared at δ 55.8 and 60.4 PPM and a ketone group appeared at δ 178.9 PPM. Result of the ^{13}C-NMR, DEPT 135, DEPT 90 spectra together with the ^{1}H-NMR data agreed well for 5-hydroxy-3,7-dimethoxyflavone. The ^{13}C-NMR chemical shifts of compound 6 are shown in **Table 4.20**.

Table 4.20 ^{13}C-NMR spectral of compound 6.

C	Compound 6	C	Compound 6
2	161.9	1'	130.4
3	139.6	2'	128.4
4	178.9	3'	128.6
5	156.9	4'	130.9
6	97.9	5'	128.6
7	165.6	6'	128.4
8	92.6	3-OCH_3	60.4
9	155.9	7-OCH_3	55.8
10	106.1		

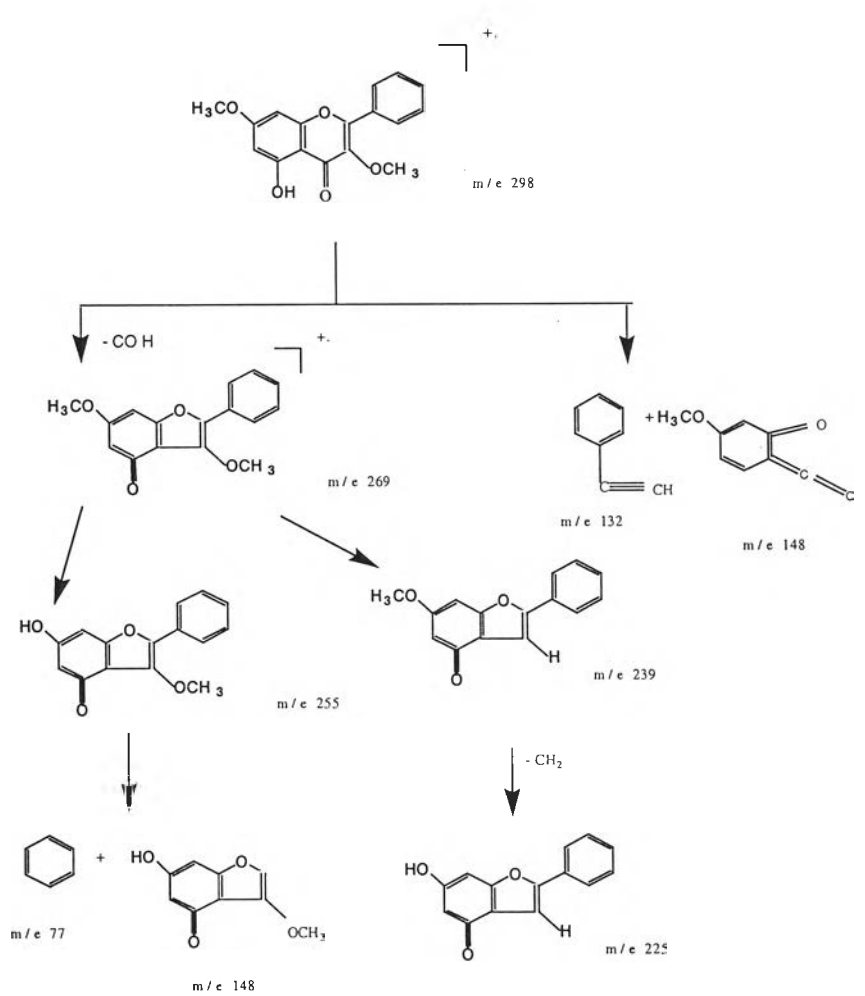
The mass spectrum **(Fig. A38)** of compound 6 showed the fragmentation as follow, m/z (EIMS) 298 (75) [M^+], 297 (100), 280 (9), 279 (11), 269 (8), 267 (11), 255 (8), 171 (8), 148 (9), 135 (14), 129 (10), 115 (10), 105 (22), 77 (20), The molecular weight of 5-hydroxy -3,7-dimethoxyflavone is 298 which the molecular ion at m/e 298 (75%). The fragmentation is shown in **Scheme 4.6**.

The IR spectrum **(Fig. A6)** of compound 6 showed several characteristic absorption bands as shown in **Table 4.21**

Table 4.21 The IR absorption band assignment of Compound 6.

Wave number (cm-1)	Intensity	Vibration
3620 - 3080	Broad	O-H stretching vibration of hydroxy group
2970, 2820	Weak, Weak	C - H stretching vibration of $-CH_3$
1643	Strong	C=O stretching vibration of carbonyl Conjugated ketone
1604, 1500, 1420	Strong, Weak, Weak	C=C stretching vibration of aromatic
1350	Medium	C-H stretching vibration of $-CH_3$
1257	Medium	C-O unsymmetry stretching Vibration of C-O-C
1180, 1127	Medium, Medium	C-O symmetry stretching Vibration of C-O-C
833, 625	Medium, Medium	.= C-H out of plane bending vibration of aromatic

From the X-ray diffraction analysis, it revealed that compound 6 was 5-hydroxy-3,7-dimethoxyflavone. The molecular weight and molecular formular of 5-hydroxy-3,7-dimethoxyflavone are $C_{17}H_{14}O_5$ and 298, respectively. The crystal of 5-hydroxy-3,7-dimethoxyflavone was obtained from ethyl acetate in hexane as yellow needle crystals. The melting point was 129-130 °C (lit 129-130°C) (22). X-ray diffraction data are presented in **Table 11, 12, 13, 14, 15** and **16**. The ORTEP drawing of compound is shown in **Fig. 4.9**.



Scheme 4.6 The mass fragmentation of the compound 6.

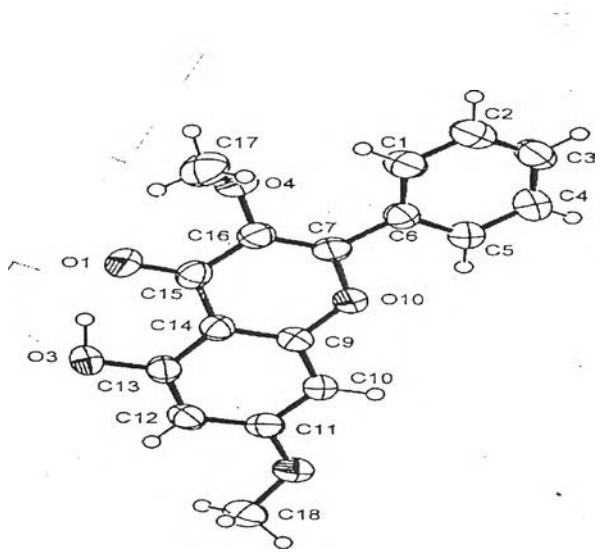


Fig.ure 4.9 The ORTEP drawing of Compound 6.

4.3.7 STRUCTURE ELUCIDATION OF COMPOUND 7

Compound 7 is a yellow plate crystal (328 mg) UV λ_{max} (nm), $CHCl_3$ (logε):270 sh (0.0025), $[\alpha]_D^{20}$ -2.2 ($CHCl_3$, c 1).

FT-IR spectrum, (KBr), νmax (cm^{-1}) : 3600 - 3230 (b), 1666 (s), 1612 (s), 1501 (m), 1450 (m), 1350 (s), 1204 (m), 1157 (m), 1100 (w), 1020 (w), 880 (w), 820 (w), 780 (w), 640 (w), 620 (w) **(Fig. A7).**

^{1}H-NMR spectrum ($CDCl_3$, 200 MHz.) δ (PPM) : revealed the significant signals at chemical shift 3.87 (3H, s, OCH_3), 6.66 (1H, s, ArCOCH = C-Ar), 6.37 (1H, d, J=2 Hz, ArH), 6.49 (1H, d, J=2 Hz, ArH), 7.89 (2H, m, 2 * ArH), 7.53 (3H, m, 3 * ArH), 12.70 (1H, s, OH) **(Fig. A17).**

^{13}C- NMR spectrum ($CDCl_3$, 200 MHz.) δ (PPM) : 182.4 (s), 165.6 (s), 163.9 (s), 162.1 (s), 157.7 (s), 131.8 (s), 131.2 (s), 129.0 (s), 126.2 (s), 105.7 (s), 105.6 (s), 98.2 (s), 92.6 (s), 55.8 (s) **(Fig. A27).**

EI-MS spectrum (m/z) 70 ev : 269 (18), 268 (100) [M^+], 267 (27), 239 (35), 225 (20), 166 (5), 123 (10), 105 (12), 102 (20), 95 (38), 77 (29), 69 (29) **(Fig. A39).**

The ^{1}H-NMR spectrum **(Fig. A17)** of compound 7 showed a singlet at δ 3.87 which was assigned as a methoxy group. Both protons on C-6 and C-8 appeared as two doublets at δ 6.37 (J = 2 Hz) and 6.49 (J = 2 Hz). The singlet at δ 6.66 was assigned to the C-3 proton. The hydroxyl proton appeared at δ 12.70. A monosubstituted B-ring appeared as two multiplets at δ 7.53 and 7.89 with integration for 3H and 2H, respectively. The spectral data indicated that compound 7 was 5-hydroxy-7-methoxyflavone (22). The ^{1}H-NMR chemical shifts of compound 7 and 5-hydroxy-7-methoxyflavone were compared as shown in **Table 4.22**, and the structure of 5-hydroxy-7-methoxyflavone is shown in **Figure 4.10**.

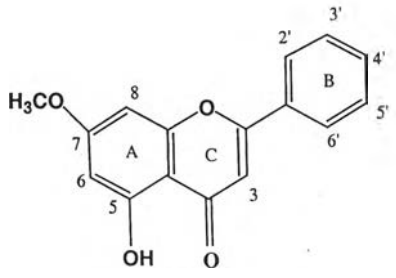


Figure 4.10 The structure of 5-hydroxy-7-methoxyflavone.

Table 4.22 ^{1}H-NMR spectral data of compound 7 and 5-hydroxy-7-methoxyflavone.

H	5-hydroxy-7-methoxyflavone	Compound 7
2	-	-
3	6.67, 1H, (s)	6.66, 1H, (s)
5	-	-
6	6.38, 1H, (d, J = 2.5)	6.37, 1H, (d, J = 2)
7	-	-
8	6.52, 1H, (d, J = 2.5)	6.49, 1H, (d, J = 2)
2'	7.92, 2H, (m)	7.89, 2H, (m)
3'	7.53, 3H, (m)	7.53, 3H, (m)
4'	7.53, 3H, (m)	7.53, 3H, (m)
5'	7.53, 3H, (m)	7.53, 3H, (m)
6'	7.92, 2H, (m)	7.89, 2H, (m)
7-OCH_3	3.87, 3H, (s)	3.87, 3H, (s)
5-OH	12.70, 1H, (s)	12.70, 1H, (s)

The ^{13}C-NMR, DEPT-135, DEPT-90 spectra **(Fig. A27)** showed that compound 7 has 16 carbons in which a methoxy group appeared at δ 55.8 and a ketone group appeared at δ 182.4. The result of ^{13}C-NMR, DEPT-135, DEPT-90 spectra correlated to result of ^{1}H-NMR spectrum which agreed well for 5-hydroxy-7-methoxyflavone. . The ^{13}C-NMR chemical shifts of compound 7 is shown in **Table 4.23**.

Table 4.23 ^{13}C-NMR spectral of compound 7.

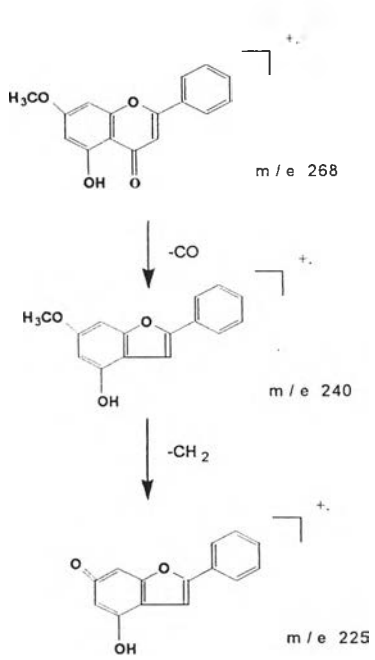
C	Compound 7	C	Compound 7
2	163.9	10	105.6
3	105.7	1'	131.2
4	182.4	2'	126.2
5	162.1	3'	129.0
6	98.2	4'	131.8
7	165.5	5'	129.0
8	92.6	6'	126.2
9	157.7	7-OCH_3	55.8

The IR spectrum **(Fig. A7)** of compound 7 is summarized in **Table 4.24**.

Table 4.24 The IR absorption band assignment of Compound 7.

Wave number (cm-1)	Intensity	Vibration
3600 - 3230	Broad	O-H stretching vibration of hydroxy group
1666	Strong	C=O stretching vibration of carbonyl Conjugated ketone
1612, 1501, 1450	Strong, medium, medium	C=C stretching vibration of aromatic
1350	Strong	C-H stretching vibration of -CH_3
1204	Medium	C-O unsymmetry stretching vibration of C-O-C
1157, 1100, 1020	Medium, weak, weak	C-O symmetry stretching vibration of C-O-C
880, 820, 780, 620	Weak, Weak, Weak, Weak	.= C-H out of plane bending vibration of aromatic

The mass spectrum **(Fig. A39)** of compound 7 showed the fragmentation as follow, m/z (EIMS) 269 (18), 268 (100) [M^+], 267 (27), 239 (35), 225 (20), 166 (5), 123 (10), 105 (12), 102 (20), 95 (38), 77 (29), 69 (29). The molecular ion was at m/e 268 which was molecular weight of 5-hydroxy-7-methoxyflavone. Mass fragmentation of compound 7 is shown in **Scheme 4.7**.



Scheme 4.7 The mass fragmentation of the compound 7.

In addition, the structure of compound 7 was also confirmed by X-ray diffraction analysis. The molecular formular and molecular weight are $C_{16}H_{12}O_4$, 268 respectively. The crystal of 5-hydroxy-7-methoxyflavone was crystallized from ethyl acetate in hexane to yield yellow plates crystal. The melting point was 149-150°C (lit 172-174°C) (22). X-ray diffraction data are presented in **Table 17, 18, 19, 20, 21** and **22,** respectively. The ORTEP drawing of compound 7 is shown in **Fig. 4.11**.

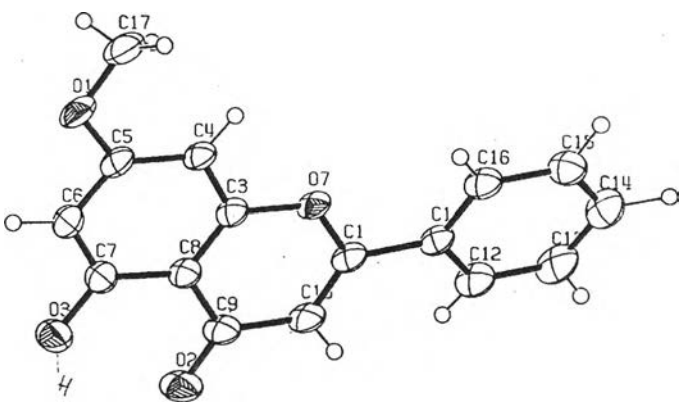


Fig ure 4.11 The ORTEP drawing of Compound 7.

4.3.8 STRUCTURE ELUCIDATION OF COMPOUND 8

Compound 8 is a yellow needles crystal (42 mg) UV λ_{max} (nm), $CHCl_3$ (logε):270, 346 sh (0.05), $[\alpha]_D^{20}$ +3 ($CHCl_3$, c 0.1).

FT-IR spectrum, (KBr), vmax (cm^{-1}) : 3,500 - 3,100 (b), 3,089 (w), 3,000 (w), 2,852 (w), 1,652 (m), 1,597 (s), 1,582 (s), 1,497 (m), 1,375 (m), 1,342 (s), 1,312 (m), 1,257 (s), 1,216 (s), 1,172 (s), 1,090 (m), 1,035 (m), 1,002 (m), 939 (m), 880 (w), 828 (m), 813 (m) **(Fig. A8)**

^{1}H-NMR spectrum ($CDCl_3$, 200 MHz.) δ (PPM) : revealed the significant signals at chemical shift 3.84,3.86, 3.88 (9H, all s, 3*OCH_3), 6.34 (1H, d, J=2 Hz, ArH), 6.43 (1H, d, J=2 Hz, ArH), 8.07 (2H, d, J=9 Hz, 2 * ArH), 7.01 (2H, d, J=9 Hz, 2 * ArH), 12.64 (1H, s, OH) **(Fig. A18)**

^{13}C- NMR spectrum ($CDCl_3$, 200 MHz.) δ (PPM) : 178.8 (s), 165.4 (s), 162.0 (s), 161.7 (s), 159.5, 155.9 (s), 130.2 (s), 130.1 (s), 122.8 (s), 114.1 (s), 106.1 (s), 97.8 (s), 92.2 (s), 60.1 (s), 55.8 (s), 55.4 (s) **(Fig. A28)**

EI-MS spectrum (m/z) 70 ev : 329 (19), 328 (100) [M^+], 327 (95), 309 (14), 297 (11), 285 (39), 269 (5), 255 (5), 150 (42), 135 (48), 119 (24), 107 (8), 92 (16), 77 (16), 69 (10) **(Fig. A40)**

The ^{1}H-NMR spectrum **(Fig. A18)** of compound 8 showed two doublets at δ 6.34 (J = 2 Hz) and 6.43 (J = 2 Hz) and each was integrated for one proton, suggesting a 5,7-disubstitued A-ring. Signals at δ 3.84, 3.86 and 3.88 (all singlets, each 3 H) were attributable to three OMe groups. The pattern of two doublets appeared at δ 7.01 (J = 9 Hz) and 8.07 (J = 9 Hz), indicating the para - substituted B - ring. The singlet at δ 12.64 was assigned to the hydroxyl proton. From ^{1}H-NMR spectrum compound 8 was assigned as 5-hydroxy-3,7,4'-trimethoxyflavone. The ^{1}H-NMR chemical shifts of compound 8 and 5-hydroxy-3,7,4'-trimethoxyflavone were compared as shown in **Table 4.25**, and the structure of 5-hydroxy-3,7,4'-trimethoxyflavone is shown in **Fig. 4.12**.

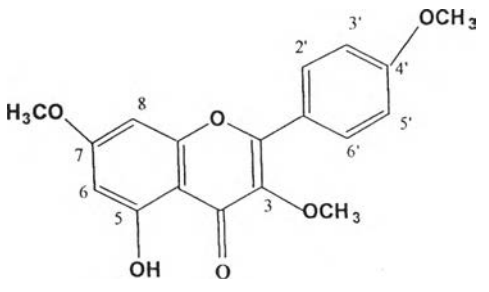


Figure 4.12 The structure of 5-hydroxy-3,7,4'-trimethoxyflavone.

Table 4.25 ^{1}H-NMR spectral data of compound 8 and 5-hydroxy-3,7,4'-methoxyflavone.

H	5-hydroxy-3,7,4'-dimethoxyflavone	Compound 8
2	-	-
3	-	-
5	-	-
6	6.33, 1H, (d, J = 2.5)	6.34, 1H, (d, J = 2)
7	-	-
8	6.42, 1H, (d, J = 2.5)	6.43, 1H, (d, J = 2)
2'	8.04, 2H, (d, J = 9)	8.07, 2H, (d, J = 9)
3'	6.98, 2H, (d, J = 9)	7.01, 2H, (d, J = 9)
4'	-	-
5'	6.98, 2H, (d, J = 9)	7.01, 2H, (d, J = 9)
6'	8.04, 2H, (d, J = 9)	8.07, 2H, (d, J = 9)
3-OCH_3	3.84, 3H, (s)	3.88, 3H, (s)
7-OCH_3	3.84, 3H, (s)	3.86, 3H, (s)
4'-OCH_3	3.87, 3H, (s)	3.84, 3H, (s)
5-OH	10.32, 1H, (s)	12.64, 1H, (s)

The ^{13}C-NMR, DEPT 135, DEPT 90 spectra (Fig. A28) showed that compound 8 has 18 carbons in which the methoxy groups appeared at δ 54.9, 50.6, 50.2 and a ketone group appeared at δ 173.6. Result of ^{13}C-NMR, DEPT 135, DEPT 90 spectra confirmed that compound 8 was 5-hydroxy-3,7,4'-trimethoxyflavone. The ^{13}C-NMR chemical shifts of compound 8 is shown in **Table 4.26**.

Table 4.26 ^{13}C-NMR spectral of compound 8.

C	Compound 8	C	Compound 8
2	159.5	1'	122.8
3	155.9	2'	130.2
4	178.8	3'	114.1
5	160.6	4'	160.6
6	92.2	5'	114.1
7	165.4	6'	130.2
8	97.8	3-OCH_3	60.1
9	161.7	7-OCH_3	55.8
10	106.1	4'-OCH_3	55.4

The IR spectrum **(Fig. A8)** of compound 8 is summarized in **Table 4.27**.

Table 4.27 The IR absorption band assignment of Compound 8.

Wave number (cm-1)	Intensity	Vibration
3500 - 3100	Broad	O-H stretching vibration of hydroxy group
3089, 3000, 2852	All weak	C - H stretching vibration of aromatic
1652	Medium	C=O stretching vibration of carbonyl Conjugated ketone
1597, 1582, 1497	Strong, Strong, Medium	C=C stretching vibration of aromatic
1375, 1342, 1312	Medium, Strong, Medium	C-H stretching vibration of -CH3
1257, 1216	Strong, Strong	C-O unsymmetry stretching vibration of C-O-C
1172, 1090, 1035, 1002	Strong, Medium, Medium Medium	C-O symmetry stretching vibration of C-O-C
939, 880, 828, 813	Medium, Medium, Medium, Medium	.= C-H out of plane bending vibration of aromatic

The mass spectrum **(Fig. A40)** of compound 8 showed the fragmentation as follow, m/z (EIMS) 329 (19), 328 (100) [M^+], 327 (95), 309 (14), 297 (11), 285 (39), 269 (5), 255 (5), 150 (42), 135 (48), 119 (24), 107 (8), 92 (16), 77 (16), 69 (10). The base peak of the molecular ion was at m/e 328 which equal to the molecular weight of 5-hydro-3,7,4'-trimethoxyflavone. The fragmentation diagram is shown in **Scheme 4.8**.

The crystal of 5-hydroxy-3,7,4'-trimethoxyflavone was obtained from ethyl acetate in hexane as yellow needle crystals and melting point 145-147°C (lit 146-148°C) (22). The molecular weight and molecular formular of 5-hydroxy-3,7,4'-trimethoxyflavone are 328 and $C_{18}H_{16}O_6$, respectively (22). The ORTEP drawing is shown in **Fig. 4.13**. X-ray diffraction data are presented in **Table 23, 24, 25, 26, 27** and **28**.

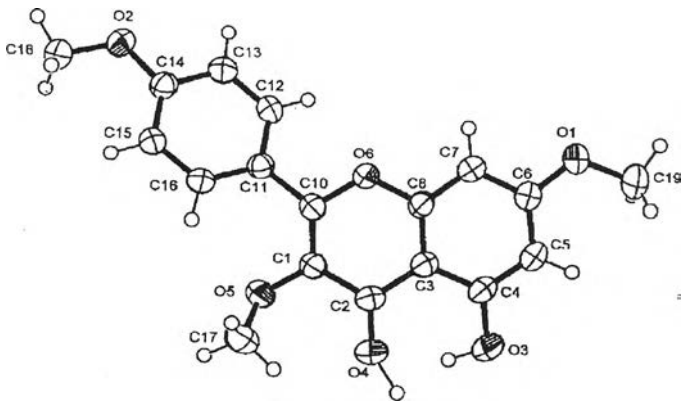
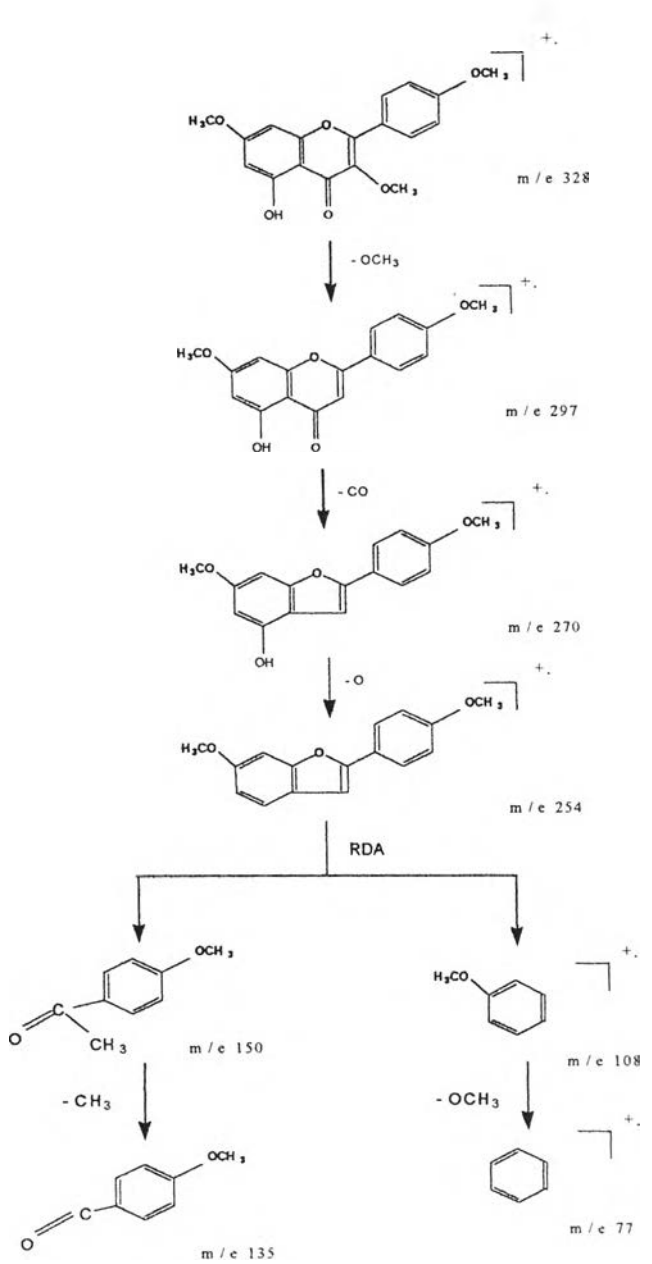


Figure 4.13 The ORTEP drawing of Compound 8.



Scheme 4.8 The mass fragmentation of the compound 8.

4.3.9 STRUCTURE ELUCIDATION OF COMPOUND 9

Compound 9 is a greenish - yellow solid (34 mg), UV λ_{max} (nm), $CHCl_3$ (log ε):250, 322 sh (0.005), $[\alpha]_D^{20}$ +7.8 ($CHCl_3$, c 1).

FT-IR spectrum, (KBr), νmax (cm^{-1}) : 3,560 - 3,250 (b), 1,666 (m), 1,604 (s), 1,512 (m), 1,442 (m), 1,381 (m), 1,273 (m), 1,188 (w), 1,165 (m), 1,026(w), 833 (m) **(Fig. A9)**

^{1}H-NMR spectrum ($CDCl_3$, 200 MHz.) δ (PPM) : revealed the significant signals at chemical shift 3.86, 3.87 (6H, all s, 2*OCH_3), 6.55 (1H, s, ArCOCH=C-Ar), 6.34 (1H, d, J=2 Hz, ArH), 6.46 (1H, d, J=2 Hz, ArH), 7.81 (2H, d, J=7 Hz, 2 * ArH), 6.99 (2H, J=5 Hz, 2 * ArH), 12.49 (1H, s, OH) **(Fig. A19)**

^{13}C- NMR spectrum ($CDCl_3$, 200 MHz.) δ (PPM) : 182.4 (s), 165.4 (s), 164.0 (s), 162.6 (s), 162.1 (s), 157.7 (s), 128.0 (s), 123.5 (s), 114.5 (s), 105.5 (s), 104.3 (s), 98.0 (s), 92.6 (s), 55.8 (s), 55.5 (s) **(Fig. A29)**

EI-MS spectrum (m/z) 70 ev : 299 (24), 298 (69) [M^+], 297 (35), 269 (16), 256 (22), 255 (100), 132 (16), 123 (11), 117 (20), 95 (58), 89 (71), 77 (23), 69 (50), 63 (29). **(Fig. A41)**

The ^{1}H-NMR spectrum **(Fig. A19)** of compound 9 showed two singlet at δ 3.86 and 3.87 which were assigned to the two methoxy groups. Two doublets at δ 6.34 (J = 2 Hz) and 6.46 (J = 2 Hz) were due to the protons on C-6 and C-8. The singlets at δ 6.55 was assigned to the C-3 proton. The hydroxyl group appeared at δ 12.49. Two doublets at δ 6.99 (J = 5 Hz) and 7.81 (J = 7 Hz) indicated the para-disubstitution of the second phenyl ring and were assigned to the protons of C-3', C-5' and C-2', C-6'. From ^{1}H-NMR data, compound 9 was assigned as 5-hydroxy-7,4'-dimethoxyflavone (22). The ^{1}H-NMR chemical shifts of compound 9 and 5-hydroxy-7,4'-trimethoxyflavone were compared as shown in **Table 4.28**, and the structure of 5-hydroxy-7,4'-trimethoxyflavone is shown in **Fig. 4.14**.

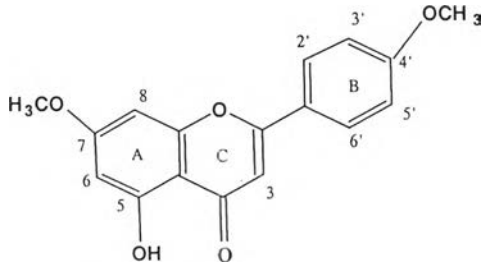


Figure 4.14 The structure of 5-hydroxy-7,4'-dimethoxyflavone.

Table 4.28 ^{1}H-NMR spectral data of compound 9 and 5-hydroxy-7,4'-dimethoxyflavone

H	5-hydroxy-7,4'-dimethoxyflavone	Compound 9
2	-	-
3	6.54, 1H, (s)	6.55, 1H, (s)
5	-	-
6	6.34, 1H, (d, *J* = 2.5)	6.34, 1H, (d, *J* = 2)
7	-	-
8	6.45, 1H, (d, *J* = 2.5)	6.46, 1H, (d, *J* = 2)
2'	7.38, 2H, (d, *J* = 9)	7.81, 2H, (d, *J* = 7)
3'	6.98, 2H, (d, *J* = 9)	6.99, 2H, (d, *J* = 5)
4'	-	-
5'	6.98, 2H, (d, *J* = 9)	6.99, 2H, (d, *J* = 5)
6'	7.38, 2H, (d, *J* = 9)	7.81, 2H, (d, *J* = 7)
7-OCH_3	3.85, 3H, (s)	3.87, 3H, (s)
4'-OCH_3	3.83, 3H, (s)	3.86, 3H, (s)
5-OH	10.28, 1H, (s)	12.49, 1H, (s)

The ^{13}C-NMR, DEPT-135, DEPT-90 spectra **(Fig. A29)** revealed that compound 9 has 17 carbons in which the methoxy groups appeared at δ 55.8 and 55.5. The ketone group was at δ 182.4. Result of the ^{13}C-NMR spectra were in agreement to compound 9. The ^{13}C-NMR chemical shifts of compound 9 are shown in **Table 4.29**.

Table 4.29 ^{13}C-NMR spectral data of compound 9.

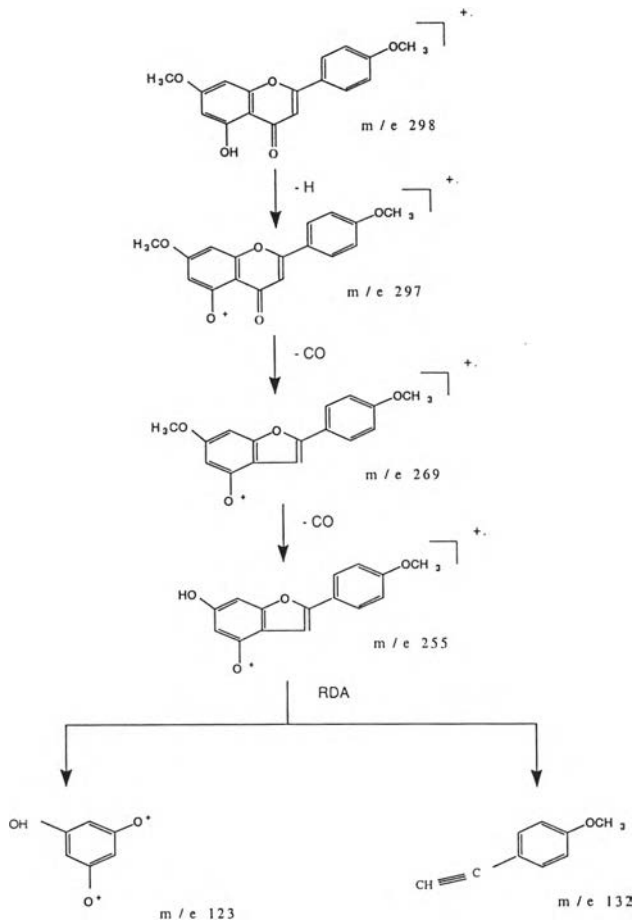
C	Compound 9	C	Compound 9
2	162.6	1'	123.5
3	104.3	2'	128.0
4	182.4	3'	114.5
5	162.1	4'	164.0
6	92.6	5'	114.5
7	165.4	6'	128.0
8	98.2	7-OCH_3	55.8
9	157.7	4'-OCH_3	55.5
10	105.5		

The IR spectrum **(Fig. A9)** of compound 9 is summarized in **Table 4.30**.

Table 4.30 The IR absorption band assignment of Compound 9.

Wave number (cm-1)	Intensity	Vibration
3560 - 3250	Broad	O-H stretching vibration of hydroxy group
1666	Medium	C=O stretching vibration of carbonyl Conjugated ketone
1604, 1512, 1442	Strong, Medium, Medium	C=C stretching vibration of aromatic
1381	Medium	C-H stretching vibration of -CH_3
1273	Medium	C-O unsymmetry stretching Vibration of C-O-C
1188, 1165, 1026	Weak, Medium, Weak	C-O symmetry stretching Vibration of C-O-C
883	Medium	.= C-H out of plane bending vibration of aromatic

The mass spectrum **(Fig. A41)** of compound 9 showed the fragmentation as follow, m/z (EIMS) 299 (24), 298 (69) [M^+], 297 (35), 269 (16), 256 (22), 255 (100), 132 (16), 123 (11), 117 (20), 95 (58), 89 (71), 77 (23), 69 (50), 63 (29). The molecular ion at m/e 255 was the base peak which was due to loss of H, CO, CH_2, Their fragmentations are shown in **Scheme 4.9**.



Scheme 4.9 The mass fragmentation of the compound 9.

The above evidence strongly suggested that compound 9 was 5-hydroxy-7,4'-dimethoxyflavone. The molecular formular is $C_{17}H_{14}O_5$ and molecular weight is 298.

4.3.10 STRUCTURE ELUCIDATION OF COMPOUND 10

Compound 10 is a colorless needle crystal (44 mg) UV λ_{max} (nm), $CHCl_3$ (log ε):284, 316 sh (0.03), $[\alpha]_D^{20}$ -16.3 ($CHCl_3$, c 1).

FT-IR spectrum, (KBr), νmax (cm^{-1}) : 2,978 (w), 2,931 (w), 2,875 (w), 2,856 (w), 1,672 (s), 1,602 (s), 1,569 (s), 1,475 (s), 1,423 (m), 1,376 (m), 1,280 (s), 1,216 (s), 1,207 (s), 1,164 (m), 1,113 (s), 1,066 (m), 819 (m), 766 (m), 702 (m) **(Fig. A10)**

^{1}H-NMR spectrum ($CDCl_3$, 200 MHz.) δ (PPM) revealed the chemical shift at δ 2.78 (1H, dd, J=16,4 Hz), 3.01 (1H, dd, J=16, 14 Hz), 3.89, 3.82 (6H, all s, 2*OCH_3), 5.40 (1H, dd, J=12, 4 Hz), 6.08 (1H, d, J=4 Hz), 6.15 (1H, d, J= 2 Hz), 7.47 (5H, m, 5*ArH) **(Fig. A20)**

^{13}C- NMR spectrum ($CDCl_3$, 200 MHz.) δ (PPM) : 189.2 (s), 165.9 (s), 164.9 (s), 162.3 (s), 138.8 (s), 128.8 (s), 128.7 (s), 126.1 (s), 105.9 (s), 93.5 (s), 93.2 (s), 79.2 (s), 56.2 (s), 55.6 (s), 45.6 (s) **(Fig. A30)**

EI-MS spectrum (m/z) 70 ev : 285 (15), 284 (78), 283 (22), 207 (17), 181 (18), 180 (100), 152 (28), 137 (32), 104 (30), 103 (22), 77 (20) **(Fig. A42)**

The ^{1}H-NMR spectrum **(Fig. A20)** showed a 5,7 - disubstituted A - ring because of the appearance of the two doublets at δ 6.15 and 6.08 with meta coupling (*J* = 2, 4 Hz) and each doublet integrated for one proton (C-8 and C-6). A multiplet at δ 7.47 represented the aromatic protons of ring B. A doublets of doublets centered at δ 5.40 (*J* = *12* Hz, *J* = 4 Hz) was due to the C-2 proton (C-2). The two methoxy groups appeared as two singlets at δ 3.89 and 3.82. The C-3 proton appeared as two doublets of doublets centered at δ 3.01 (*J* = 16 Hz, *J* = 14 Hz) and 2.78 (*J* = 16 Hz, *J* = 4 Hz). From ^{1}H-NMR data, the compound 10 was assigned as 5,7-dimethoxyflavanone (22). The ^{1}H-NMR chemical shifts of compound 10 and 5,7-dimethoxyflavanone were compared as shown in **Table 4.31**, and the structure of 5,7-dimethoxyflavanone shown in **Fig. 4.15**

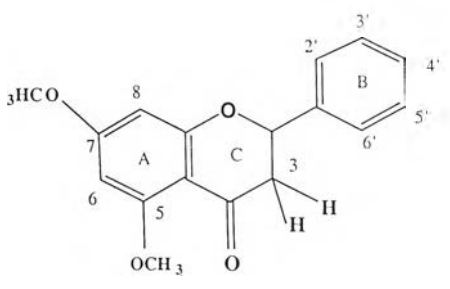


Figure 4.15 The structure of 5,7-dimethoxyflavanone.

Table 4.31 1H-NMR spectral data of compound 10 and 5,7-dimethoxyflavanone.

H	5,7-dimethoxyflavanone	Compound 10
2	5.45, 1H, (q, *J*=11, *J*=5)	5.40, 1H, (dd, *J*=12, *J*=4)
3	2.94, 1H, (d,*J*=11), 2.87, 1H, (d,*J*=5)	3.01, 1H, (dd, *J*= 16, *J*=14), 2.78, 1H, (dd, *J*=16, *J*=4)
5	-	-
6	6.13, 1H, (d, *J*=2.5)	6.08, 1H, (d, *J*=4)
7	-	-
8	6.21, 1H, (d, *J*=2.5)	6.15, 1H, (d, *J*=2)
2'	7.47, 5H, (m)	7.47, 5H, (m)
3'	7.47, 5H, (m)	7.47, 5H, (m)
4'	7.47, 5H, (m)	7.47, 5H, (m)
5'	7.47, 5H, (m)	7.47, 5H, (m)
6'	7.47, 5H, (m)	7.47, 5H, (m)
5-OCH_3	3.89, 3H, (s)	3.89, 3H, (s)
7-OCH_3	3.82, 3H, (s)	3.82, 3H, (s)

The ^{13}C-NMR, DEPT-135, DEPT-90 spectra **(Fig. A30)** revealed that compound 10 has 17 carbons. One signal of the ketone group appeared at 189.20 ppm. Two signals of methoxy groups appeared at δ 56.17, 55.61 ppm. The methylene group was at δ 45.60 ppm. The ^{13}C-NMR chemical shifts of compound 10 is shown in **Table 4.32**.

Table 4.32 ^{13}C-NMR spectral data of compound 10.

C	Compound 10	C	Compound 10
2	79.2	1'	138.8
3	45.6	2'	126.1
4	189.1	3'	128.8
5	164.9	4'	128.7
6	93.5	5'	128.8
7	165.9	6'	126.1
8	93.2	5-OCH_3	56.2
9	162.3	7-OCH_3	55.6
10	105.9		

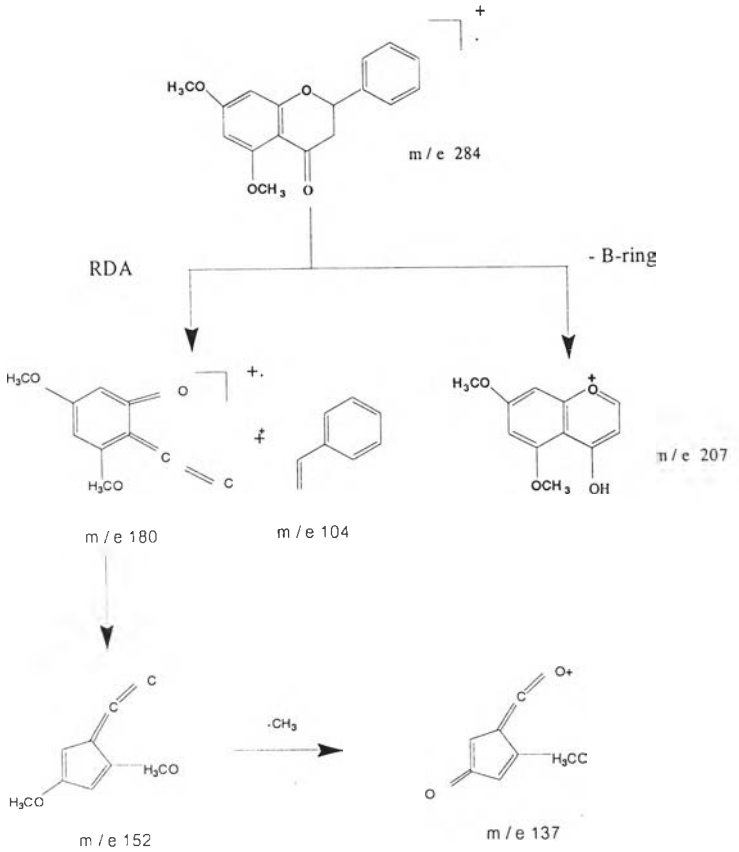
THE IR spectrum (Fig. A10) of compound 10 is summarized in **Table 4.33.**

Table 4.33 The IR absorption band assignment of Compound 10.

Wave number (cm^{-1})	Intensity	Vibration
2978, 2931, 2875, 2856	Weak, Weak, Weak, Weak	C - H stretching vibration of aromatic
1672	Strong	C=O stretching vibration of carbonyl Conjugated ketone
1602, 1569, 1475	Strong, Strong, Strong	C=C stretching vibration of aromatic
1423, 1376	Medium, Medium	C-H stretching vibration of $-CH_3$
1280, 1216, 1207	Strong, Strong, Strong	C-O unsymmetry stretching Vibration of C-O-C
1164, 1113, 1066	Medium, Strong, Medium	C-O symmetry stretching Vibration of C-O-C
819, 766, 702	Medium, Medium, Medium	.= C-H out of plane bending vibration of aromatic

The mass spectrum **(Fig. A42)** of compound10 showed the fragmentation as follow, m/z (EIMS) (Fig) 285 (15), 284 (78), 283 (22), 207 (17), 181 (18), 180 (100), 152 (28), 137 (32), 104 (30), 103 (22), 77 (20). The mass spectrum showed the molecular ion at m/e 284 and the ion at m/e 180 was the base peak. The other abundant ions were m/e 207, 152, 137 etc. The mass fragmentation of compound 10 is shown in **Scheme 4.10**.

Compound 10 was confirmed by X-ray diffraction analysis, which indicated that compound 10 was 5,7-dimethoxyflavanone. The molecular formular is $C_{17}H_{16}O_4$ and molecular weight is 284. The crystal of 5,7-dimethoxyflavanone was crystallized from ethyl acetate in hexane to yield colorless crystal with melting point at 147-149 °C (lit 169 - 170 °C) (22). X-ray diffraction data are presented in **Table A29, A30, A31, A32** and **A33**, respectively. The ORTEP drawing of compound 10 is shown in **Fig. 4.16**.



Scheme 4.10 The mass spectral diagram of the compound 10.

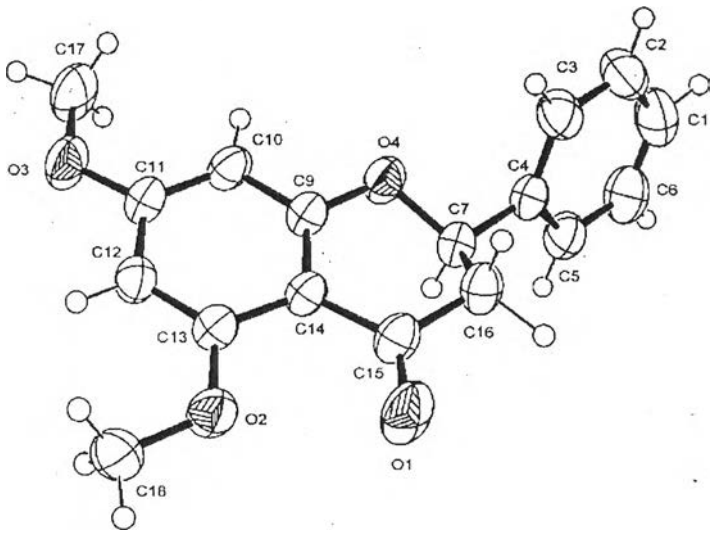


Figure 4.16 The ORTEP drawing of Compound 10.

4.3.11 STRUCTURE ELUCIDATION OF COMPOUND 11

Compound 11 is brown-violet vicous liquid (0.16 g, $3.3*10^{-3}$% wt/wt), UV λ_{max} (nm), MeOH (log ε) : 206, 264, 318 sh (0.125), $[\alpha]_D^{20}$ +60.2 (MeOH, c 0.1).

The ^{13}C-NMR, DEPT-135, DEPT-90 spectra in DMSO **(Fig. A31)** revealed that compound 11 was sucrose. The signal of carbons appeared at δ 103.94 (F_2), δ 91.69 (G_1), δ 82.44 (F_5), δ 76.93 (F_3), δ 74.18 (F_4), δ 72.75 (G_3, G_5), δ 71.55 (G_2), δ 69.74 (G_4), δ 62.90 (F_6), δ 62.07 (F_1), δ 60.39 (G_6). These spectral data are consistent with those of sucrose in D_2O (**Fig. A31a**) (25), where the signal of carbons appeared at δ 142.25 (F_2), δ 93.20 (G_1), δ 83.90 (F_5), δ 77.65 (F_3), δ 75.25 (F_4), δ 74.00 (G_3, G_5), δ 72.50 (G_2), δ 70.60 (G_4), δ 63.90 (F_6), δ 62.80 (F_1), δ 61.50 (G_6). The ^{13}C-NMR chemical shifts of compound 11 and sucrose were shown in **Table 4.34**, and the structure of sucrose shown in **Fig. 4.17**.

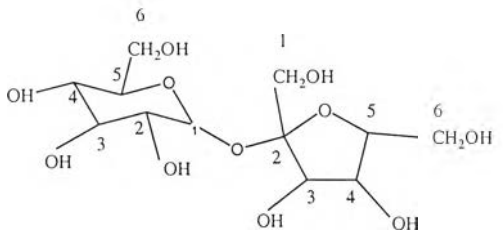


Figure 4.17 Structures of sucrose.

Table 4.34 ^{13}C-NMR spectral data of compound 11 and sucrose.

Carbon	Compound 11	Sucrose
F1	62.1	62.8
F2	103.9	142.25
F3	76.9	77.65
F4	74.2	75.25
F5	82.4	83.9
F6	62.9	63.9
G1	91.7	93.2
G2	71.6	72.5
G3	72.8	74.0
G4	69.7	70.6
G5	72.8	74.0
G6	60.4	61.5

4.4 RESULTS OF BIOLOGICAL ACTIVITY TEST OF THE ISOLATED COMPOUNDS.

4.4.1 CYTOTOXIC ACTIVITY TEST

The *in vitro* activity of isolated compounds (10 μg/ml) from *K. parviflora* were tested against 5 cell lines which composed of BT474 (breast), CHAGO (lung), HEP-G2 (hepatoma), Kato - 3 (gastric) and SW620 (colon) and the results are shown in **Table 4.35**.

Table 4.35 Cytotoxic activity against tumor cell lines of isolated compounds from *K. parviflora*.

Compound	% survival of tumor cell Lines				
(10 ug/ml)	Kato - 3	BT 474	Chago	SW 620	Hep - G2
	(gastric)	(breast)	(lung)	(colon)	(hepatoma)
1	57	99	78	92	83
2	39	120	74	90	79
3	29	75	41	59	26
4	95	126	91	77	76
5	59	117	107	105	84
6	33	84	85	57	47
7	44	82	41	46	50
8	54	59	88	69	51
9	40	90	78	40	80
10	32	58	64	76	87

From **Table 4.35** it indicated that compound 2 and 10 showed cytotoxicity against Kato3 cell lines, compound 3 showed cytotoxicity against Hep-G2, Kato-3, Chago, compound 6 showed cytotoxicity against Hep-G2 and Kato-3, compound 7 showed cytotoxicity against Chago, Kato-3 and SW620, compound 9 showed cytotoxicity against Kato-3 and SW620. The IC_{50} values are reported in **Table 4.36**.

Table 4.36 Cytotoxic activity against tumor cell lines (IC_{50}) of isolated compounds from *K. parviflora*.

Compound	IC_{50} (ug / ml) of tumor cell lines				
	Kato - 3	BT 474	Chago	SW 620	Hep - G2
	(gastric)	(breast)	(lung)	(colon)	(hepatoma)
2	9	> 10	10	> 10	4.4
3	6.1	6.2	7.2	8.0	2.1
6	6.6	5.9	> 10	8.7	4.0
7	2	5.4	9.7	7	1.1
9	2.9	7.0	> 10	4.1	3.0
10	6.1	6.1	> 10	> 10	1.9

IC_{50} was the minimum concentration of 50% inhibitory activity.

4.3.2 Antioxidant activity assay

In order to study and investigate the structure activity relationship, the eleven compounds from *K. parviflora* were tested for antioxidant activity by DPPH method. Vitamin E was used as positive control. The results are summarized in **Table 4.37.**

Table 4.37 Antioxidant activity of 11 compounds from *K. parviflora* by DPPH method.

Compound	Absorbance 517 nm		
	1	2	Average
Standard DPPH solution	1.2	1.2	1.2
Vitamin E [1]	0.19	0.18	0.185
3,5,7-trimethoxyflavone	1.2	1.2	1.2
5,7-dimethoxyflavone	1.2	1.2	1.2
5,7,4'-trimethoxyflavone	1.2	1.2	1.2
4'-hydroxy-5,7-dimethoxyflavone	1.2	1.2	1.2
Dicinnamoylmethane	0.49	0.5	0.495
5-hydroxy-3,7-dimethoxyflavone	1.2	1.2	1.2
5-hydroxy -7-dimethoxyflavone	1.2	1.2	1.2
5-hydroxy-3,7,4'-trimethoxyflavone	1.2	1.2	1.2
5-hydroxy-7,4',-dimethoxyflavone	1.2	1.2	1.2
5,7-dimethoxyflavanone	1.2	1.2	1.2
Sucrose	0.95	0.98	0.965

The results from **Table** 4.37 revealed that dicinnamoylmethane and sucrose showed weak antioxidant activity compare to that of vitamin E. The *in vitro* antioxidant activity of dicinnamoylmethane and sucrose from *K. parviflora* and vitamin E are presented in **Table 4.38**.

Table 4.38 Antioxidant activity of dicinnamoylmethane, sucrose and vitamin E.

Compound	IC_{50} (mM)
Vitamin E	0.5
Dicinnamoylmethane	16
Sucrose	>14

IC_{50} was concentration of samples at 1/2 of absorbance value of DPPH solution.

Amongst the isolated compounds from *K. parviflora*, only dicinnamoylmethane and sucrose showed weak antioxidant activity with IC_{50} 16 mM and >14 mM respectively.

Most of flavonoids are found in nature such as fisetin, (+)-catechin, quercetin etc which are potent antioxidants. However, all 9 flavonoids were found to be inactive in reducing 2,2-diphenylpicryhydrazyl radical. It's possible that flavonoids terminate chain radical reactions by donating hydrogen atoms to the peroxy radical forming a flavonoid radical.

Mechanism of lipid oxidation can be distinguished in three distinct steps: initiation, propagation, termination.

Initiation stage : free radicals abstract hydrogen from polyunsaturated fatty acids to form the lipid radical.

$$RH \longrightarrow R^{\cdot} + H^{\cdot}$$

$$ROOH \longrightarrow RO^{\cdot} + HO^{\cdot}$$

$$2\,ROOH \longrightarrow RO^{\cdot} + ROO^{\cdot} + H_2O$$

Propagation stage : the lipid radical reacts with molecular oxygen to form the lipid peroxy radical which breaks down to generate more free radicals thus maintaining the chain of reactions.

$$R^{\cdot} + {}^3O_2 \longrightarrow ROO^{\cdot}$$

$$ROO^{\cdot} + RH \longrightarrow ROOH + R^{\cdot}$$

Termination stage : the free radical species react together or with antioxidant to form inert products.

$$R^{\cdot} + R^{\cdot} \longrightarrow R - R$$

$$R^{\cdot} + ROO^{\cdot} \longrightarrow ROOR$$

$$ROO^{\cdot} + ROO^{\cdot} \longrightarrow ROOR + O_2$$

Lipid peroxidation (LPO) can be suppressed by enzymatic inactivation of free radicals and antioxidants that inhibit the initiation stage and / or accelerate the termination stage. Flavonoids inhibit LPO in vitro at the initiation stage as scavengers of superoxide anions and hydroxy radicals by donating hydrogen atoms. Thus, the inhibition of LPO is influenced by structural features of flavonoids. It has been proposed that the number of hydroxy groups are the important for antioxidant of flavonoids. It was revealed that the hydroxyl radical scavenging activity of flavonoids increases with the number of hydroxyl groups substituted on the B ring, especially at C-3' and decreases rapidly as the number of hydroxyl groups decreases. The nine flavonoids, eight flavone and one flavanone had only 4'-hydroxy-5,7-dimethoxyflavone that had hydroxy group at C-4' position on the B ring but it didn't show antioxidant activity. In addition, methoxy group may reduce antiperoxidative efficiency of flavonoids in vitro due to steric hindrance. Moreover hydrogenation of 5,7-dimethoxyflavanone at C-3 position of the C ring decreases the antiperoxidative effects.

Dicinnamoylmethane and sucrose showed weak antioxidant activity, due to structure of dicinnamoylmethane has hydroxyl group at C-2, C-2' that can be easy oxidized so it can show weak antioxidant activity. However, it is a poor antioxidant when compare with vitamin E. For sucrose, it is because of the abundant of hydroxyl group even though the structure of sucrose is difficult to oxidize. It can slightly reduce 2,2-diphenylpicryhydrazyl.