CHAPTER III
RESULTS AND DISCUSSION

Four species of Compositae weeds were selected for preliminary screening tests of various activities. The main activity that used to investigate for finding bioactive compounds is plant growth inhibition.

3.1 Biological Activity Results of Preliminary Screening Tests

Air-dried aerial parts of samples were minced to coarse powder and extracted according to the procedure described in Chapter II. Each crude extract was preliminary screened for various activity according to procedures described in Chapter II. Bioassay results are presented in Tables 3.1 – 3.6.

Plant Growth Inhibition (as method 2.5.1(i))

Table 3.1 Inhibitory effect of ethanolic crude extracts of some Compositae on growth of rice (*Oryza sativa* cv. RD23)

Plant	% Inhibition at various concentration			
	Growth of rice part	0.1 (g)	0.5 (g)	1.0 (g)
E. adenophorum	root	96.10	100.00	100.00
	shoot	2.839	100.00	100.00
E. odoratum	root	94.91	100.00	100.00
	shoot	45.83	100.00	100.00
A. conyzoides	root	93.35	100.00	100.00
	shoot	60.43	100.00	100.00
S. africanus	root	95.50	100.00	100.00
	shoot	41.71	100.00	100.00

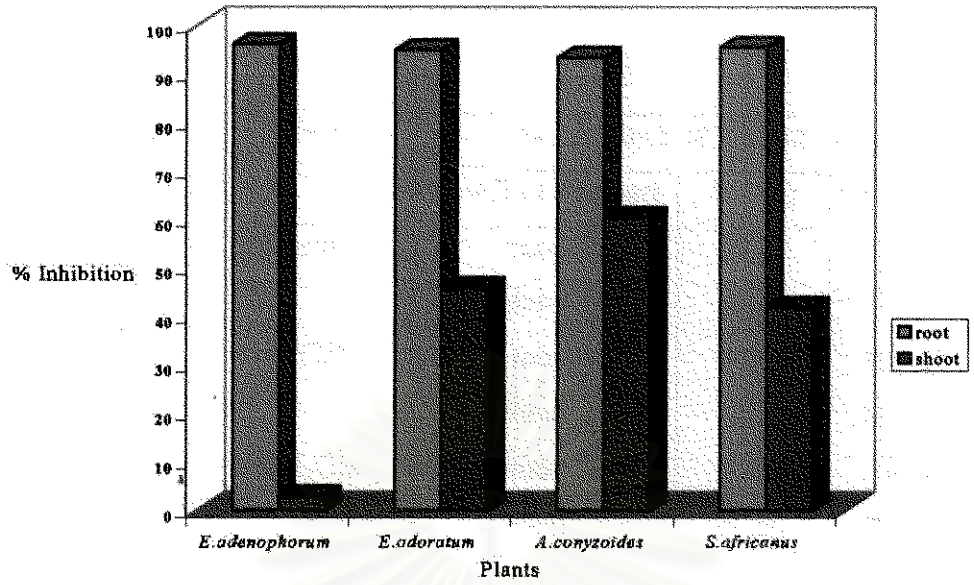


Fig. 3.1 Inhibitory effect of 0.1 g ethanolic crude extract of some Compositae weeds on rice seedling growth

Table 3.2 Inhibitory effect of crude extracts of some Compositae on growth of rice (*Oryza sativa* cv. RD23)

Plant	Solvent	% Inhibition at various concentration			
		Growth of rice part	0.1 (g)	0.5 (g)	1.0 (g)
E. adenophorum	Hexane	root	97.06	100.00	100.00
		shoot	2.70	100.00	100.00
	EtOH	root	96.10	100.00	100.00
		shoot	2.839	100.00	100.00
	CH_2Cl_2	root	95.35	100.00	100.00
		shoot	-2.16	100.00	100.00
	EtOAc	root	90.05	100.00	-
		shoot	21.52	100.00	-
	n-Butanol	root	100.00	100.00	-
		shoot	100.00	100.00	-
	H_2O	root	82.59	100.00	100.00
		shoot	25.55	100.00	100.00

Table 3.2 (cont.)

Plant	Solvent	% Inhibition at various concentration			
		Growth of rice part	0.1 (g)	0.5 (g)	1.0 (g)
E. odoratum	Hexane	root	83.80	93.30	97.72
		shoot	15.12	20.93	38.03
	EtOH	root	94.08	100.00	100.00
		shoot	43.21	100.00	100.00
	CH_2Cl_2	root	94.84	100.00	100.00
		shoot	31.90	28.94	100.00
	EtOAc	root	100.00	-	-
		shoot	100.00	-	-
	n-Butanol	root	100.00	100.00	-
		shoot	100.00	100.00	-
	H_2O	root	96.12	100.00	100.00
		shoot	63.03	100.00	100.00
A. conyzoides	EtOH	root	93.42	100.00	100.00
		shoot	61.79	100.00	100.00
	CH_2Cl_2	root	94.18	100.00	-
		shoot	-9.914	100.00	-
	EtOAc	root	100.00	-	-
		shoot	100.00	-	-
	n-Butanol	root	100.00	100.00	-
		shoot	100.00	100.00	-
	H_2O	root	90.31	100.00	-
		shoot	33.91	100.00	-
S. africanus	EtOH	root	94.36	100.00	100.00
		shoot	42.82	100.00	100.00

By the rice growth inhibitory effect, ethanolic crude extract of every plant species revealed very good activity. The root growth inhibition effect is higher than that of shoot growth. However, the root and shoot growth inhibitory effects were equal, 100% at 0.5 and 1.0 g. The root growth inhibitory effect at 0.1 g of *E. adenophorum* is the highest, 96.10%. That of *S. africanus, E. odoratum* and *A. conyzoides* are 95.50%, 94.91% and 93.35%, respectively. As shown in Table 3.1.

From rice growth inhibition activity, all crude extracts of every plant species showed good activity. Remarkably, the root growth inhibiton effect is higher than the shoot growth in every crude extract. The root and shoot growth inhibitory effects of all crude extracts were 100% at 0.5 and 1.0 g except hexane and dichloromethane crude extracts of *E. odoratum*. The highest root growth inhibitory effect is 100% at 0.1 g of ethyl acetate and butanol crude extracts of *E. odoratum* and *A. conyzoides* and butanol crude extract of *E. adenophorum*. The limitation of crude extracts caused the missing data in some concentration (Table 3.2).

Brine Shrimp Cytotoxicity Test

Table 3.3 Brine shrimp (*Artemia salina*) cytotoxicity test by some Compositae weeds

Plant	Solvent	LC_{50}	Activity
E. adenophorum	Hexane	33.68	medium
	EtOH	12.56	medium
	CH_2Cl_2	11.85	medium
	EtOAc	37.08	medium
	BuOH	13.84	medium
E. odoratum	Hexane	>1000.00	no
	EtOH	8.69	high
	CH_2Cl_2	614.03	low
	BuOH	3.65	high
A. conyzoides	Hexane	32.84	medium
	EtOH	9.15	high

Note : $LC_{50} < 10$ μg / mL High Activity

$LC_{50} < 100$ μg / mL Medium Activity

$LC_{50} < 1000$ μg / mL Low Activity

$LC_{50} > 1000$ μg / mL No activity

For brine shrimp cytotoxicity test, every crude extract from *E. adenophorum* showed medium-high activity. The ethanolic and butanolic crude extracts from *E. odoratum* displayed high activity while hexane and dichloromethane crude extracts exhibited no activity and low activity, respectively. In the case of *A. conyzoides* the ethanolic extract showed high activity whereas the hexane crude extract showed medium activity.

Anticell Lines Cytotoxicity Test

Table 3.4 Cytotoxicity test against various carcinoma cell lines by some Compositae weeds

Plant	Solvent	Cell line				
		BIU	BGC-823	HL-60	K-562	KB
E. adenophorum	Hexane	-	-	+	+	-
	EtOH	*	+	-	-	+
	CH_2Cl_2	*	+	+	*	*
E. odoratum	BuOH	+	-	-	-	-
A. conyzoides	Hexane	+	*	-	-	*
	EtOH	+	+	-	-	+
S. africanus	EtOH	*	-	+	*	-

Note : The results suggested by Beijing Medical University, Beijing, China

+++	very good
++	good
+	fair
-	no activity
*	no data by mis-sending

The data in Table 3.4 showed the results of cytotoxicity tests against various carcinoma cell lines. Crude extracts of *E. adenophorum* were displayed fair activity with the most cell lines except Bladder carcinoma cell line (BIU), while the cytotoxicity of the crude extract of *E. odoratum* was active with Bladder carcinoma cell line (BIU) only. In the case of *A. conyzoides*, the fair activity was found in ethanolic crude extract to Bladder carcinoma cell line (BIU), Gastric carcinoma cell line (BGC-823) and Nasophoryngeal carcinoma cell line (KB). The crude extract of *S. africanus* exhibited fair result in Leukemia carcinoma cell line (HL-60) only.

Other Activities

Table 3.5 Other activities against some Compositae crude extracts

Plant	Solvent	Activity				
		Antifugal		Antioxidant		Larvicidal
		C. c	*C. a*	DPPH	β-carotene	*A.a*
E. adenophorum	Hexane	√	X	X	X	√
	EtOH	√	X	√	X	X
	CH_2Cl_2	√	√	√	X	X
	EtOAc	X	X	√	X	X
	BuOH	X	X	√	X	X
E. odoratum	Hexane	X	X	X	√	X
	EtOH	√	X	X	X	X
	CH_2Cl_2	√	√	√	X	X
	BuOH	X	X	√	X	X
A. conyzoides	Hexane	X	X	X	√	√
	EtOH	X	X	X	X	√

Note : *C. c* *Cladosporium cucumerinum*
C. a *Candida albicans*
A.a *Aedes aegypti*
DPPH = 2,2-diphenyl-1-(2,4,6-trinitrophynyl)hydrazyl radical
√ = activity observed
X = no activity observed

The bioactivity tests of some Compositae crude extracts are reported in Table 3.5. For antifungal activity, the crude extracts *A. conyzoides* were not active, while both of *E. adenophorum* and *E. odoratum* were active. The crude extract of *E. adenophorum* showed higher antifungal activity than those of *E. odoratum* when compared with *Candida albicans*. In case of DPPH antioxidant activity most of the

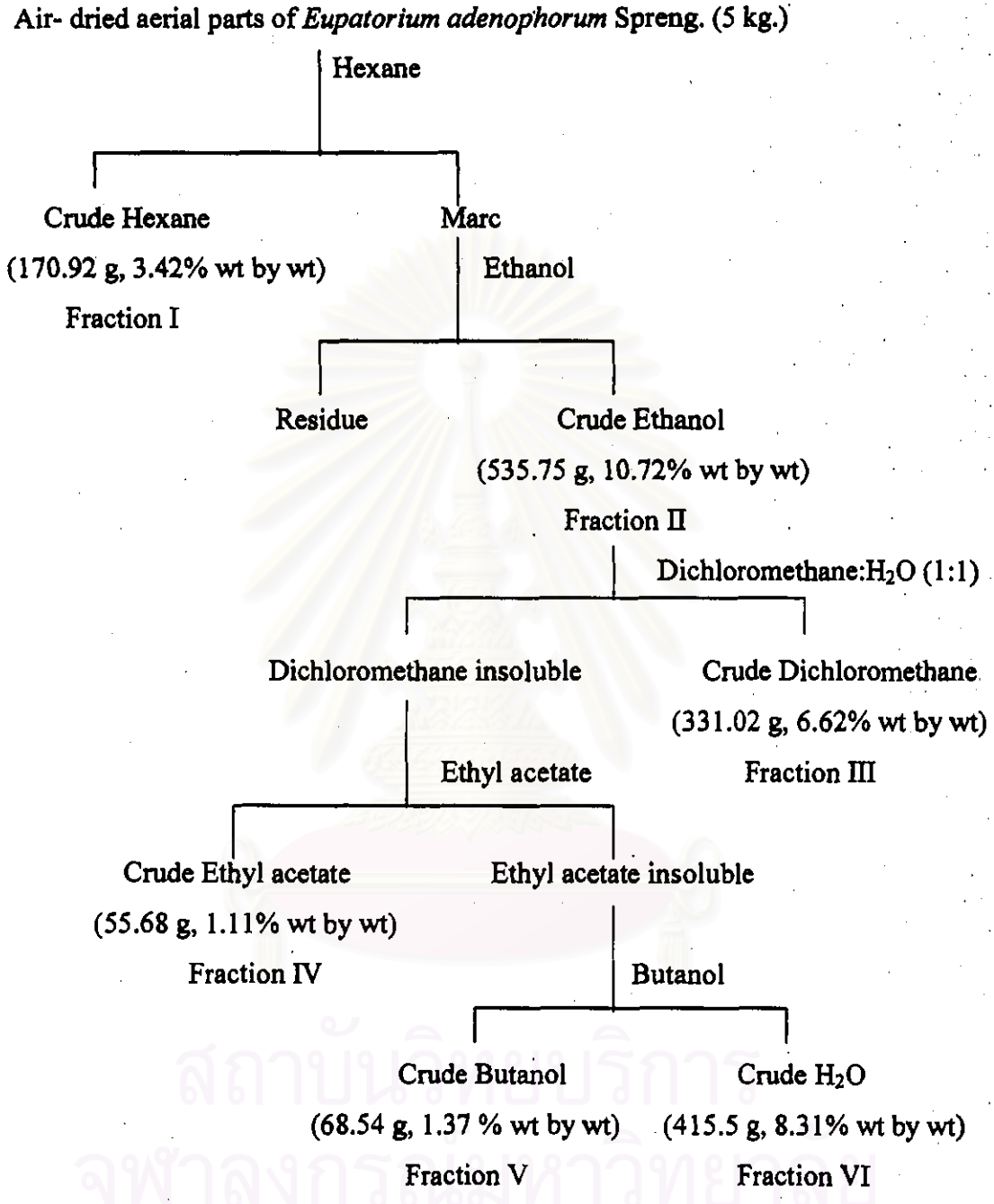
E. adenophorum crude extracts were exhibited higher activity than other plants except for hexane crude extracts of all plants which were not active. The antioxidant activity using β-carotene were observed to be no activity in all crude extracts of all plants except hexane crude extract of *E. odoratum* and *A. conyzoides*. Larvicidal activity were observed to be no activity in all crude extracts of all plants except hexane crude extract of *E. adenophorum* gave the best result at 125 ppm and ethanolic crude extract of *S. africanus* gave the result at 250 ppm.

Crude extracts of *S. africanus* were obtained from Natural Product Research Unit, Department of Chemistry, Chulalongkorn University.

The crude extracts of *E. adenophora, E. odoratum, A. conyzoides* and *S. africanus* showed high activity on plant growth inhibition. Among these plants which gave interesting preliminary results, the aerial parts of *E. adenophora* were selected for further investigation with the aim to search for bioactive compounds.

3.2 The Results of Extraction and Fractionation of *E. adenophorum* Spreng.

Air-dried aerial parts of *E. adenophorum* Spreng. (5 kg) were minced to coarse powder. The plant initially extracted with *n*-hexane by soaking for 4-5 days at room temperature. The residue was re-extracted three times. The solution was filtered and the solvent was evaporated yielding hexane crude extract as viscous yellow-green liquid (Fraction I), 170.92 g (3.42% yield). The plant residue left after hexane extraction was soaked in ethanol and the solution removed. Repeat until the solution was colorless. Filter the solution and evaporate. This yielded ethanol extract as viscous dark-green liquid (Fraction II), 535.75 g (10.72% yield). The methanolic crude extract was further partitioned between dichloromethane and water in ratio 1:1 to yield dichloromethane fraction (Fraction III), 331.02 g (6.62% yield) and water soluble fraction. The water-soluble fraction was extracted with ethyl acetate and yielded ethyl acetate crude extract (Fraction IV), 55.68 g (1.11% yield). The latter was then extracted with *n*-butanol to afford 68.54 g (1.37% yield) of *n*-butanol extract (Fraction V). The extraction procedure of *E. adenophorum* is shown in Scheme 3.1.



Scheme 3.1 The procedure and results of extraction and fractionation of *E. adenophorum* Spreng.

Results of Plant Growth Inhibition Activities

Each crude extract of the aerial parts of *E. adenophorum* Spreng. was preliminary bioassay for plant growth inhibition activities according to the procedure described in Chapter II (2.5.1(i)). All fractions showed the effect on root growth is higher than shoot growth. For the inhibitory effect on root growth of rice, n- butanol crude extract showed strongest inhibitory effect (100%), followed by hexane, ethanol, dichloromethane, ethyl acetate and water crude extract, as shown in Tables 3.6. At higher concentration (0.5 and 1.0 g), the tested plants were completely inhibited 100%.

Table 3.6 Inhibitory effect of crude extracts of *E. adenophorum* on growth of rice (*O. sativa* cv. RD23)

Plant	% Inhibition at various concentration			
	Growth of rice part	0.1 (g)	0.5 (g)	1.0 (g)
Hexane	root	97.06	100.00	100.00
	shoot	2.70	100.00	100.00
EtOH	root	96.10	100.00	100.00
	shoot	2.839	100.00	100.00
CH_2Cl_2	root	95.35	100.00	100.00
	shoot	-2.16	100.00	100.00
EtOAc	root	90.05	100.00	-
	shoot	21.52	100.00	-
n-Butanol	root	100.00	100.00	-
	shoot	100.00	100.00	-
H_2O	root	82.59	100.00	100.00
	shoot	25.55	100.00	100.00

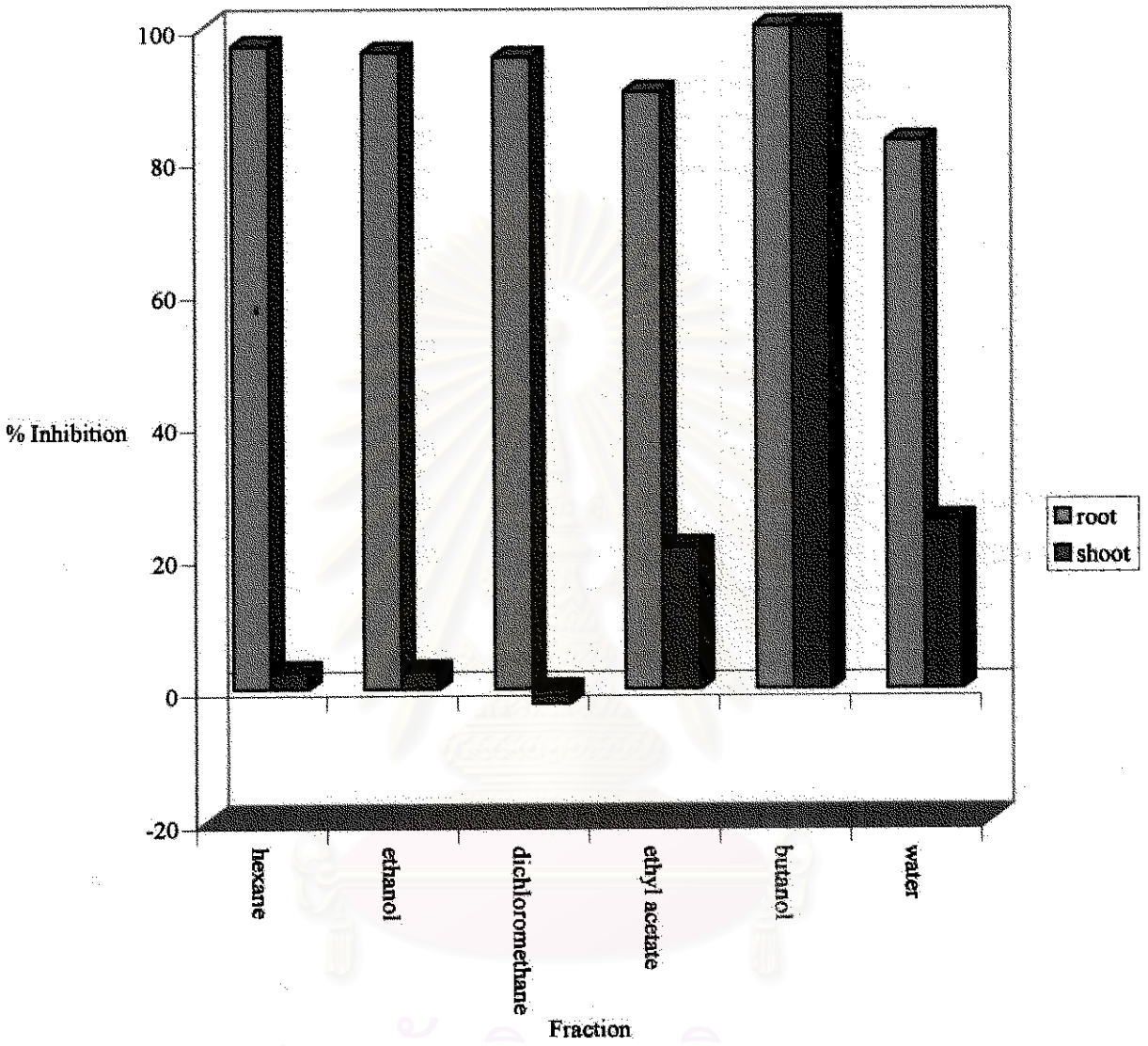
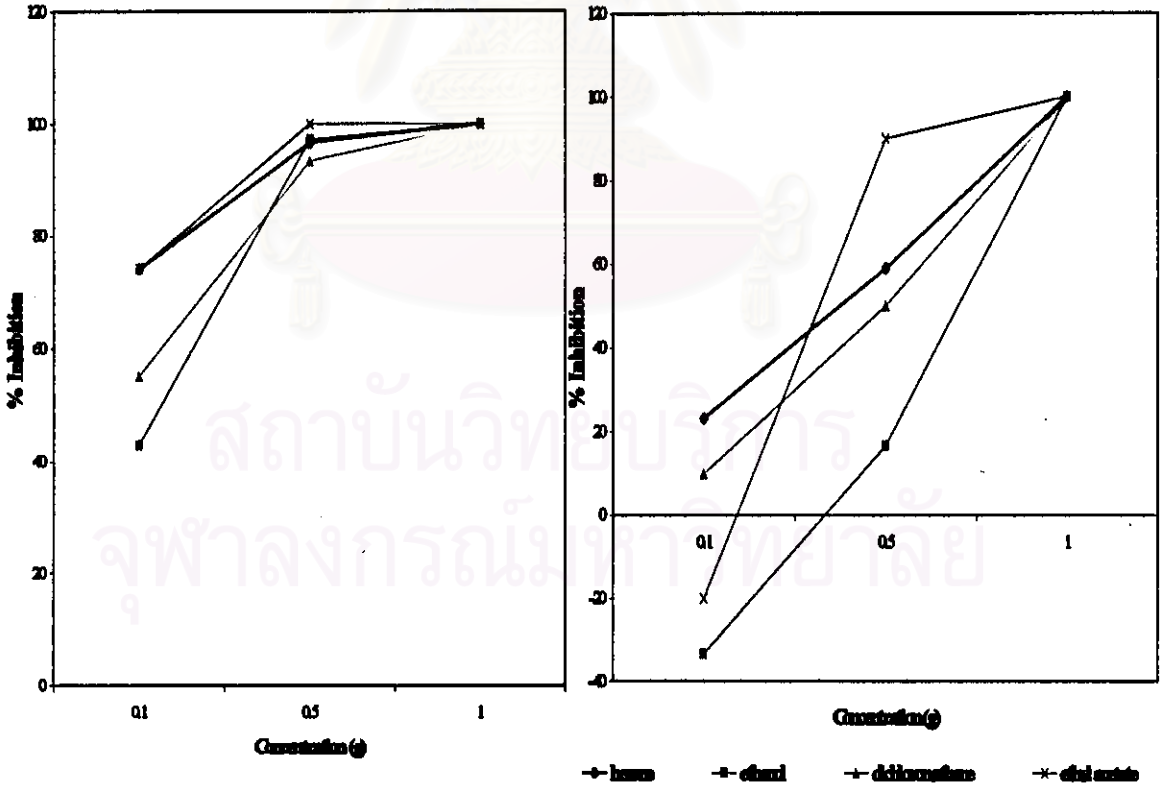


Fig. 3.2 Inhibitory effect of 0.1 g crude extract of *E. adenophorum* on rice seedling growth

Table 3.7 Inhibitory effect of crude extracts of *E. adenophorum* on growth of *Mimosa pigra*

Plant	% Inhibition at various concentration			
	Growth of plant part	0.1 (g)	0.5 (g)	1.0 (g)
Hexane	root	74.11	96.69	100.00
	shoot	23.09	58.89	100.00
EtOH	root	42.87	97.21	100.00
	shoot	-33.33	16.52	100.00
CH_2Cl_2	root	55.06	93.38	100.00
	shoot	9.91	49.85	100.00
EtOAc	root	74.22	100.00	100.00
	shoot	-20.12	90.09	100.00

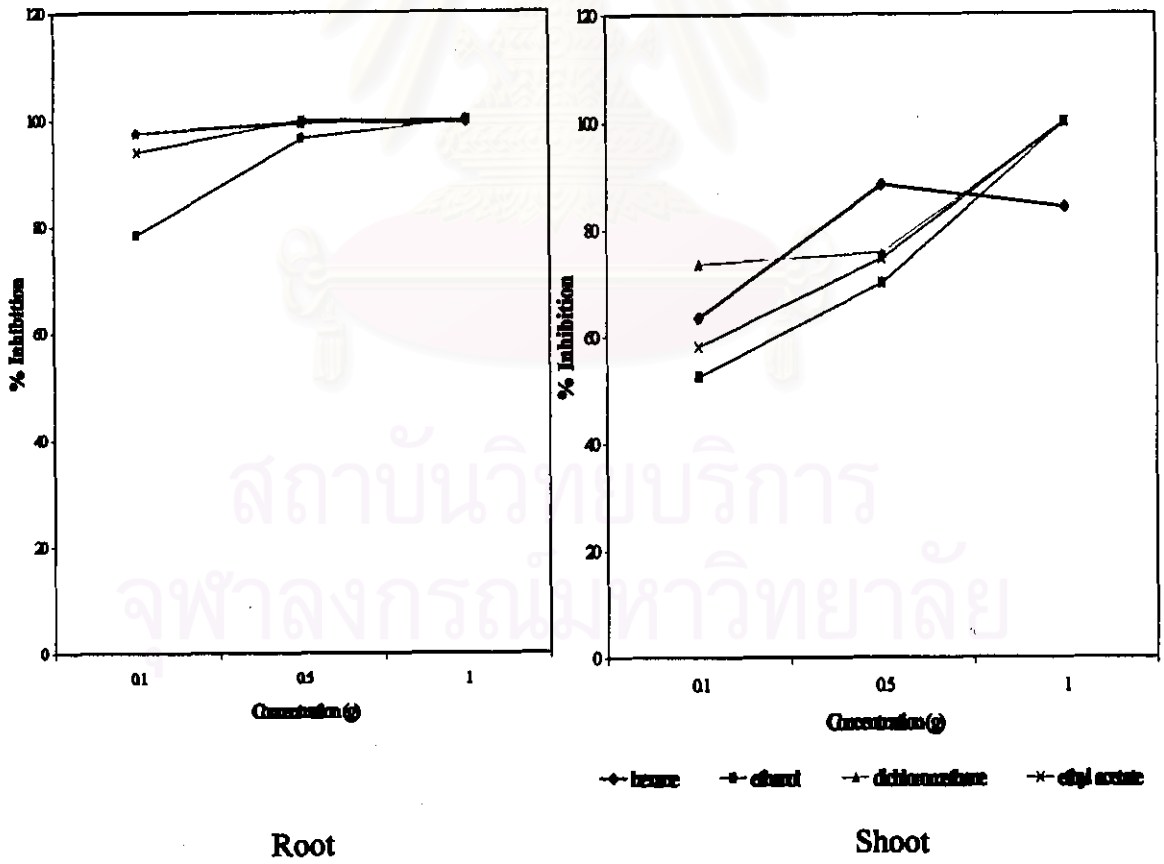


Root Shoot

Fig. 3.3 Inhibitory effect of crude extract of *E. adenophorum* on *M. pigra*

Table 3.8 Inhibitory effect of crude extracts of *E. adenophorum* on growth of *E. crusgalli*

Plant	% Inhibition at various concentration			
	Growth of plant part	0.1 (g)	0.5 (g)	1.0 (g)
Hexane	root	97.41	99.50	99.51
	shoot	63.48	88.33	84.14
EtOH	root	78.43	96.43	100.00
	shoot	52.46	69.92	100.00
CH_2Cl_2	root	97.39	99.30	100.00
	shoot	73.50	75.68	100.00
EtOAc	root	93.91	99.83	100.00
	shoot	58.03	74.59	100.00



Root Shoot

Fig. 3.4 Inhibitory effect of crude extract of *E. adenophorum* on *E. crusgalli*

The inhibitory effect on growth of *M. pigra* is similar to rice, but only the highest concentration (1.0 g) were 100% inhibited both root and shoot growth. The root inhibitory activity at 0.5 g from highest to lowest is ethyl acetate, ethanol, hexane and dichloromethane, but all of the inhibitions are more than 93% (Table 3.7). And all crude extract, the effected on root is higher than shoot.

The inhibitory effect on *E. crusgalli* is similar to rice. All crude extract, the effected on root is higher than shoot. The inhibition on root at 0.5 g from highest to lowest is hexane, dichloromethane, ethyl acetate and ethanol, but all of the inhibitions are more than 96% and are slightly different (Table 3.8). The small amount of crude extract caused of missing data in some concentration.

Results of Brine Shrimp Cytotoxicity Test

Each crude extract of *E. adenophorum* was preliminarily screened for cytotoxicity against brine shrimp (*Artemia salina* Linn.) according to the procedure described in Chapter II. The results are reported in Table 3.9.

Table 3.9 Brine shrimp (*Artemia salina*) cytotoxicity test

Extract	LC_{50}	Activity
Hexane	31.11	medium
CH_2Cl_2	10.43	medium
EtOH	12.56	medium
EtOAc	28.87	medium
BuOH	10.02	medium

Note : $LC_{50} < 10$ μg / mL High Activity

$LC_{50} < 100$ μg / mL Medium Activity

$LC_{50} < 1000$ μg / mL Low Activity

$LC_{50} > 1000$ μg / mL No activity

From Table 3.9, all of crude extracts exhibited medium-high activity on brine shrimp cytotoxicity. Butanol crude extract showed the best LC_{50} followed by dichloromethane, ethanol, ethyl acetate and hexane 10.02, 10.43, 12.56, 28.87 and 31.11, respectively

Results of Anticell Lines Cytotoxicity Test

The crude extracts of *Eupatorium adenophorum* were tested for cytotoxicity against various cell lines, i.e., Nasopharyngeal carcinoma (KB), Bladder carcinoma (BIU), Erythroleukemia carcinoma (K-562), Gastric carcinoma (BGC-823), Leukemia carcinoma (HL-60), Colon carcinoma (HCT-8) and Hepatocellular carcinoma (Bel-7402). The results are reported in Table 3.10.

Table 3.10 Cytotoxicity test against various carcinoma cell lines

Solvent	Cell line						
	Bel-7402	BGC-823	BIU	HCT-8	HL-60	K-562	KB
Hexane	-	-	-	-	+	+	-
EtOH	+	+	++	+	-	-	+
CH_2Cl_2	+	-	-	+	++	-	*
EtOAc	*	+	-	*	+	+	-
BuOH	*	*	+	*	-	-	*

Note : The results suggested by Beijing Medical University, Beijing, China

+++	very good
++	good
+	fair
-	no activity
*	no data by mis-sending

From Table 3.10, the ethanolic crude extract of *E. adenophorum* was showed cytotoxicity activities against Hepatocellular carcinoma (Bel-7402), Gastric carcinoma (BGC-823), Colon carcinoma (HCT-8) and Nasopharyngeal carcinoma (KB) cell lines whereas the dichloromethane crude extract also had cytotoxicity against Hepatocellular carcinoma (Bel-7402) and Colon carcinoma (HCT-8). Other cytotoxicity activity against Gastric carcinoma (BGC-823), Leukemia carcinoma (HL-60) and Erythroleukemia carcinoma (K-562) cell lines was found from the ethyl

acetate crude extract. The last activity against Leukemia carcinoma (HL-60) and Erythroleukemia carcinoma (K-562) cell lines was derived from the hexane crude extract. The data presented in this table showed that the ethanolic crude extract had the activity against Bladder carcinoma (BIU) cell lines and the dichloromethane crude extract revealed the inhibition against Leukemia carcinoma (HL-60) cell lines.

The preliminary screening results of *E. adenophorum* crude extracts on plant growth inhibition, brine shrimp cytotoxicity and anticell lines toxicity, dichloromethane, ethyl acetate and butanol crude extracts displayed the best results (Table 3.6-3.10). From all plant growth inhibition, all crude extracts showed high inhibitory effect, butanol crude extract gave the highest inhibition 100% at 0.1 g. For brine shrimp cytotoxicity all crude extracts displayed medium-high activity, butanol and dichloromethane crude extracts showed the best LC_{50} 10.02 and 10.43 respectively. The separation and fractionation of the three crude extracts was therefore carried out for further investigated of bioactive substances.

3.3 Separation

3.3.1 Separation of Fraction III

According to preliminary plant growth inhibition activities (see Table 3.6-3.8), Fraction III (dichloromethane fraction) gave the most interesting results (95.35% on rice, 55.06% on *M. pigra* and 97.39% on *E. crusgalli*). Thus, 193.8 g of dichloromethane crude extract was further separated by using silica gel quick column chromatography. A mixture of hexane and dichloromethane, dichloromethane and a mixture of methanol and dichloromethane were used as an eluent. 4 L of solution was collected for each fraction and then concentrated to about 10 mL. The results of the separation of Fraction III was tabulated in Table 3.11.

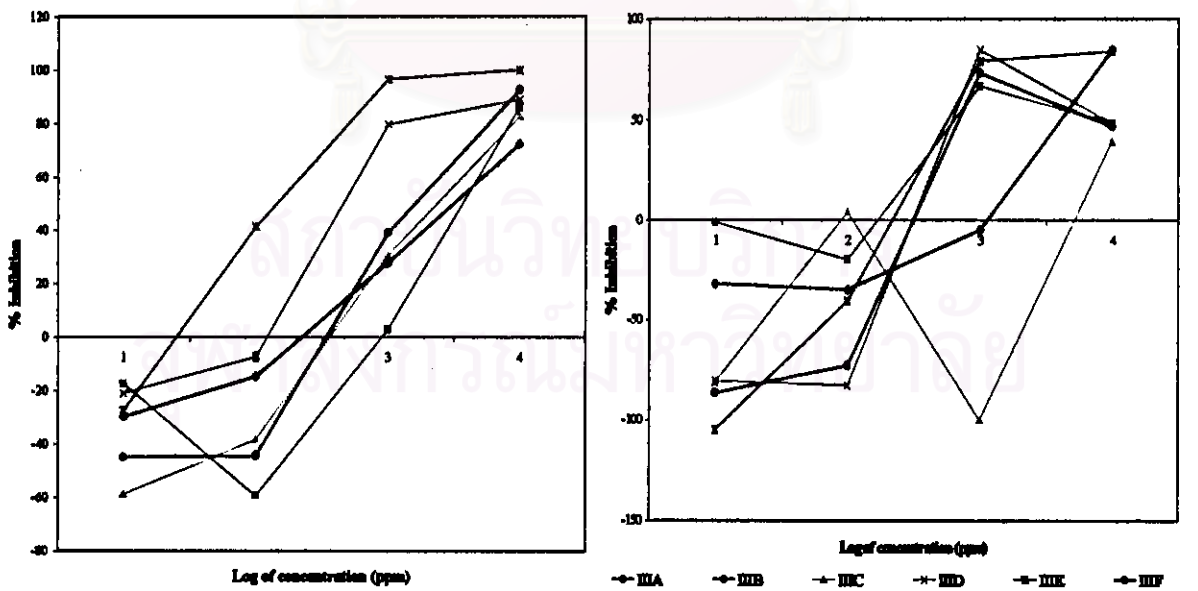
Table 3.11 The results of the separation of Fraction III

Eluents	Fraction No.	Remarks	weight (g)
20% CH_2Cl_2 in Hexane	IIIA (1-8)	dark green viscous liquid	21.39
40% CH_2Cl_2 in Hexane	IIIB (9-16)	dark green viscous liquid	14.69
60% CH_2Cl_2 in Hexane	IIIC (17-24)	dark green liquid	8.42
80% CH_2Cl_2 in Hexane	IIID (25-32)	dark green liquid	6.06
100% CH_2Cl_2	IIIE (33-40)	dark green liquid	3.86
10% MeOH in CH_2Cl_2	IIIF (41-44)	dark green viscous liquid	105.26

Each small fraction derived from the separation of Fraction III was further subjected to plant growth inhibition experiments as method 2.5.1(ii). The rice growth inhibition results are shown in Tables 3.12-3.13 and Fig. 3.5-3.6.

Table 3.12 Inhibitory effect of fractions of dichloromethane crude extract of *E. adenophorum* on growth of rice (*O. sativa* cv. RD23)

Fraction	% Inhibition at various concentration				
	Growth of rice part	10 (ppm)	100 (ppm)	1000 (ppm)	10000 (ppm)
IIIA	root	-29.96	-14.97	27.79	72.46
	shoot	-32.02	-34.97	-5.11	84.74
IIIB	root	-17.52	-59.23	2.82	86.11
	shoot	-1.11	-20.04	66.54	48.00
IIIC	root	-58.70	-38.39	30.61	82.77
	shoot	-81.14	3.99	-100.00	39.29
IIID	root	-21.13	-7.36	79.69	89.05
	shoot	-80.81	-82.97	84.74	48.00
IIIE	root	-27.56	41.32	96.53	100.00
	shoot	-104.78	-40.80	78.91	84.02
IIIF	root	-44.85	-44.34	39.08	92.64
	shoot	-86.61	-72.89	73.22	46.65

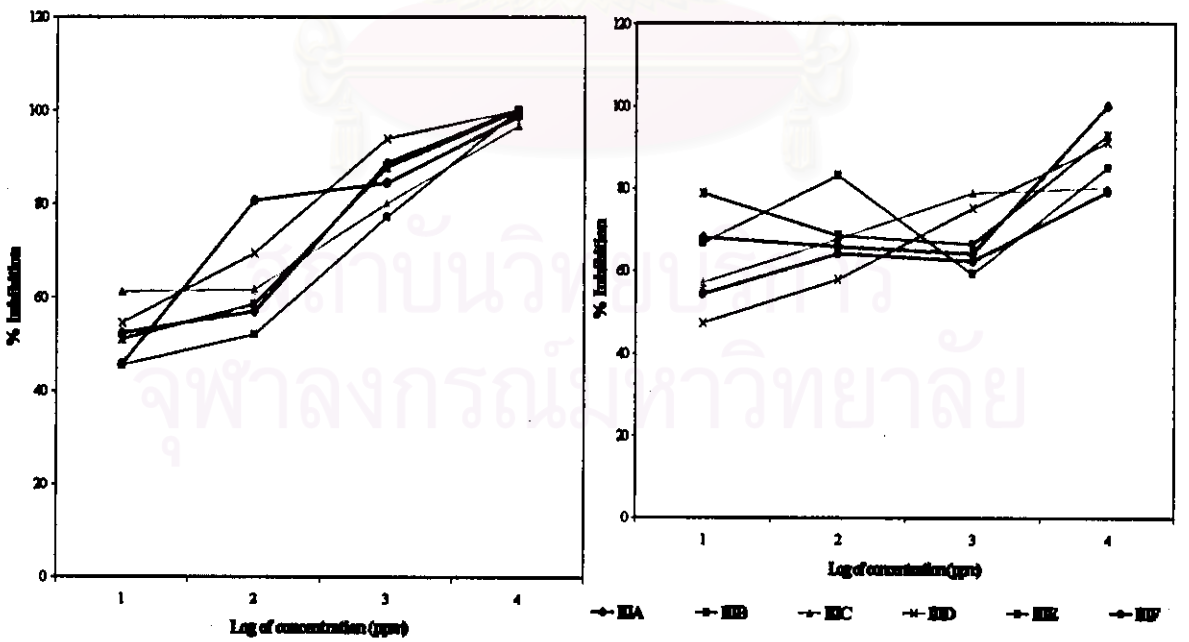


Root Shoot

Fig. 3.5 Inhibitory effect of fractions of dichloromethane crude extract of *E. adenophorum* on rice seedling

Table 3.13 Inhibitory effect of fractions of dichloromethane crude extract of *E. adenophorum* on growth of *E. crusgalli*

Fraction	% Inhibition at various concentration				
	Growth of rice part	10 (ppm)	100 (ppm)	1000 (ppm)	10000 (ppm)
IIIA	root	52.37	56.94	88.58	100.00
	shoot	68.14	65.91	64.16	100.00
IIIB	root	45.52	52.02	77.16	100.00
	shoot	66.83	83.19	59.30	84.95
IIIC	root	61.17	61.69	79.96	96.66
	shoot	57.07	67.70	78.77	80.09
IIID	root	54.48	69.42	93.85	100.00
	shoot	47.35	57.95	75.23	91.16
IIIE	root	50.96	58.53	87.69	100.00
	shoot	78.77	68.58	66.35	92.91
IIIF	root	45.70	80.67	84.36	98.71
	shoot	54.40	64.16	62.41	79.21



Root Shoot

Fig. 3.6 Inhibitory effect of fractions of dichloromethane crude extract of *E. adenophorum* on *E. crusgalli*

By the rice growth inhibitory effect, every fraction revealed good activity. The growth inhibition increased when concentration increasing. At low concentration shoot growth inhibition is higher than root growth, but at high concentration root growth inhibition is higher than shoot growth. Fraction IIIE and IIID is the highest inhibition, followed by IIIF, IIIC, IIIA and IIIB that is 96.53%, 79.69%, 39.08%, 30.61%, 27.79% and 2.82% at 1000 ppm, respectively.

The inhibitory effext on growth of *E. crusgalli* is similar to the rice. Fraction IIID and IIIE is the highest inhibition, 93.85% and 87.69% at 1000 ppm, respectively.

After plant growth inhibition experiments, each fraction was tested against brine shrimp (*A.salina*) cytotoxicity and various anticell lines cytotoxicity. The results of brine shrimp (*A. salina*) were displayed in Table 3.14.

Table 3.14 Brine shrimp (*Artemia salina*) cytotoxicity test

Fraction	LC_{50}	Activity
IIIA	27.94	medium
IIIB	5.26	high
IIIC	31.62	medium
IIID	19.67	medium
IIIE	10.01	medium
IIIF	13.87	medium

Note : $LC_{50} < 10\ \mu g / mL$ High Activity

$LC_{50} < 100\ \mu g / mL$ Medium Activity

$LC_{50} < 1000\ \mu g / mL$ Low Activity

$LC_{50} > 1000\ \mu g / mL$ No activity

Table 3.15 Cytotoxicity test against various carcinoma cell lines

Fraction	Cell line			
	Bel-7402	BGC-823	HCT-8	HL-60
IIIA	+	+	-	-
IIIB	+	+	+	-
IIIC	+	+	+	-
IIIF	+	+	+	+

Note : The results suggested by Beijing Medical University, Beijing, China

+++ very good

++ good

+ fair

- no activity

From Table 3.14 and 3.15, fraction IIIB showed high activity of brine shrimp *(Artemia salina)* test whereas fraction IIIF revealed the cytotoxicity test against Leukemia carcinoma (HL-60) and Colon carcinoma (HCT-8) cell lines. Fractions IIIB and IIIC showed cytotoxicity test against Colon carcinoma (HCT-8) cell line. All fractiond (IIIA-IIIF) exhibited the cytotoxicity test against Hepatocellular carcinoma (Bel-7402) and Gastric carcinoma (BGC-823) cell lines.

After monitored by TLC ; fraction IIID and IIIE showed similar components were combined (fraction IIIDE) then reseparated by column chromatography as the result in Table 3.16.

Table 3.16 The results of the separation of Fraction IIIDE

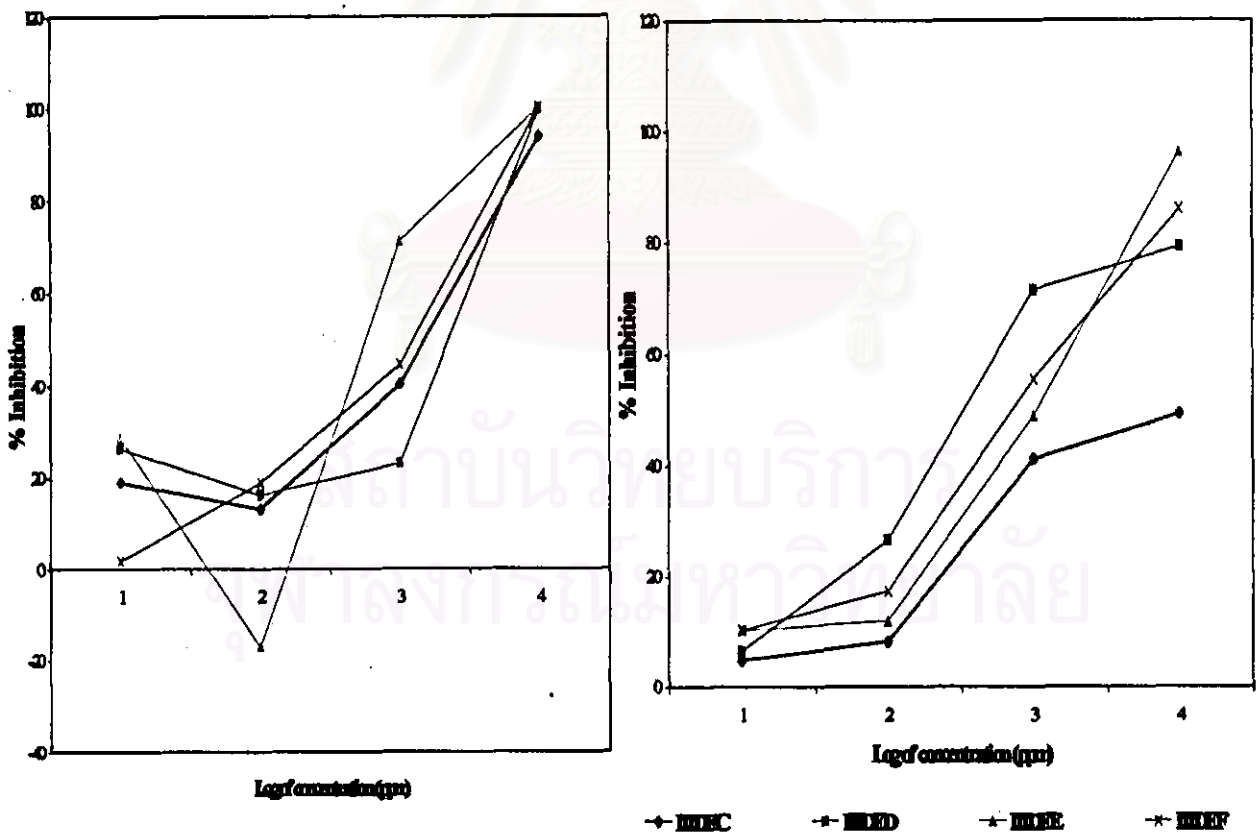
Eluents	Fraction No.	Remarks	Weight (g)
20% CH_2Cl_2 : Hexane	IIIDEA (1-12)	dark green liquid	1.48
40% CH_2Cl_2 : Hexane	IIIDEB (13-45)	dark green liquid (Mixture 3)	3.73
60% CH_2Cl_2 : Hexane	IIIDEC (46-85)	dark green liquid (Mixture 4)	2.77
80% CH_2Cl_2 : Hexane	IIIDED (86-93)	dark green liquid	2.52
100% CH_2Cl_2	IIIDEE (94-135)	dark green liquid	3.02
10% MeOH : CH_2Cl_2	IIIDEF (136-151)	dark green viscous liquid	3.73
20% MeOH : CH_2Cl_2	IIIDEG (152-167)	dark green viscous liquid	2.32

Fraction IIIDEB was first purified by washing with dichloromethane. Then, white amorphous solid was received. This compoud was further purified by recrystallization with hot acetone several times. Mixture 3 was obtained 0.05 g. Fraction IIIDEC was washed with hexane, after recrystallization several times with methanol, white amorphous solid (Mixture 4) was gained 0.035 g.

Then fractions IIIDEC-IIIDEF from dichloromethane crude extract were tested for plant growth activity (as method 2.5.1(ii)). The results of rice growth inhibition of these fractions are tabulated in Table 3.17 and Fig. 3.7.

Table 3.17 Inhibitory effect of fractions of dichloromethane crude extract of *E. adenophorum* on growth of rice (*O. sativa* cv. RD23)

Fraction	% Inhibition at various concentration				
	Growth of rice part	10 (ppm)	100 (ppm)	1000 (ppm)	10000 (ppm)
IIIDEC	root	18.96	13.01	40.29	93.89
	shoot	4.75	8.14	41.15	49.30
IIIDED	root	26.34	16.14	23.04	99.53
	shoot	6.34	26.52	71.40	79.24
IIIDEE	root	28.68	-17.08	71.15	100
	shoot	10.20	11.88	48.96	96.26
IIIDEF	root	1.72	18.80	44.52	100
	shoot	10.20	17.33	55.45	86.04



Root Shoot

Fig. 3.7 Inhibitory effect of fraction IIIDE of dichloromethane crude extract on rice seedling

From the results of rice growth inhibition in Table. 3.17 and Fig. 3.7, fraction IIIDEE of dichloromethane crude extract displayed high growth inhibition on root of rice seedling extremely, 100% at 10000 ppm. So fraction IIIDEE was separated by recolumn chromatography for isolation of active compound in this fraction. The results are showed in Table 3.18.

Table 3.18 The results of separation of Fraction IIIDEE

Eluents	Fraction No.	Remarks	Weight (g)
10% $CHCl_3$: hexane	IIIDEEA (1–18)	dark green viscous liquid	0.03
20-30% $CHCl_3$: hexane	IIIDEEB (19–48)	white crystal in green liquid (Mixture 1)	0.57
30% $CHCl_3$: hexane	IIIDEEC (49–79)	wax in dark green viscous liquid	0.30
40% $CHCl_3$: hexane	IIIDEED (80–85)	dark green viscous liquid	0.20
40-50% $CHCl_3$: hexane	IIIDEEE (86–93)	white crystal in yellow oil (Compound 2)	0.85
60-80% $CHCl_3$: hexane and 100% $CHCl_3$	IIIDEEF (94-122)	dark green viscous liquid	0.02
1-5%MeOH:$CHCl_3$	IIIDEEG (123-137)	dark green liquid	0.08
10-20% MeOH:$CHCl_3$	IIIDEEH (138-160)	dark green liquid	0.25

Fraction IIIDEEB was firstly washed with hexane. After recrystallization several times with hot hexane, the white amorphous solid (Mixture 1) 0.01 g was gained. Fraction IIIDEEE was purified by recrystallization with hexane several times to yield 0.03 g white needle crystal (Compound 2).

3.3.2 Separation of Fraction IV

Based upon the preliminary plant growth inhibition activity (see Table 3.6-3.8), Fraction IV (ethyl acetate fraction) gave the attractive results (90.05% on rice, 74.22% on *M. pigra* and 93.91% on *E. crusgalli*). Thus 49.82 g of crude extract was further separated into small fraction by column chromatography. The column was initially eluted with a mixture of dichloromethane and ethyl acetate, ethyl acetate and a mixture of ethyl acetate and methanol.

The results of the separation of Fraction IV are tabulated in Table 3.19.

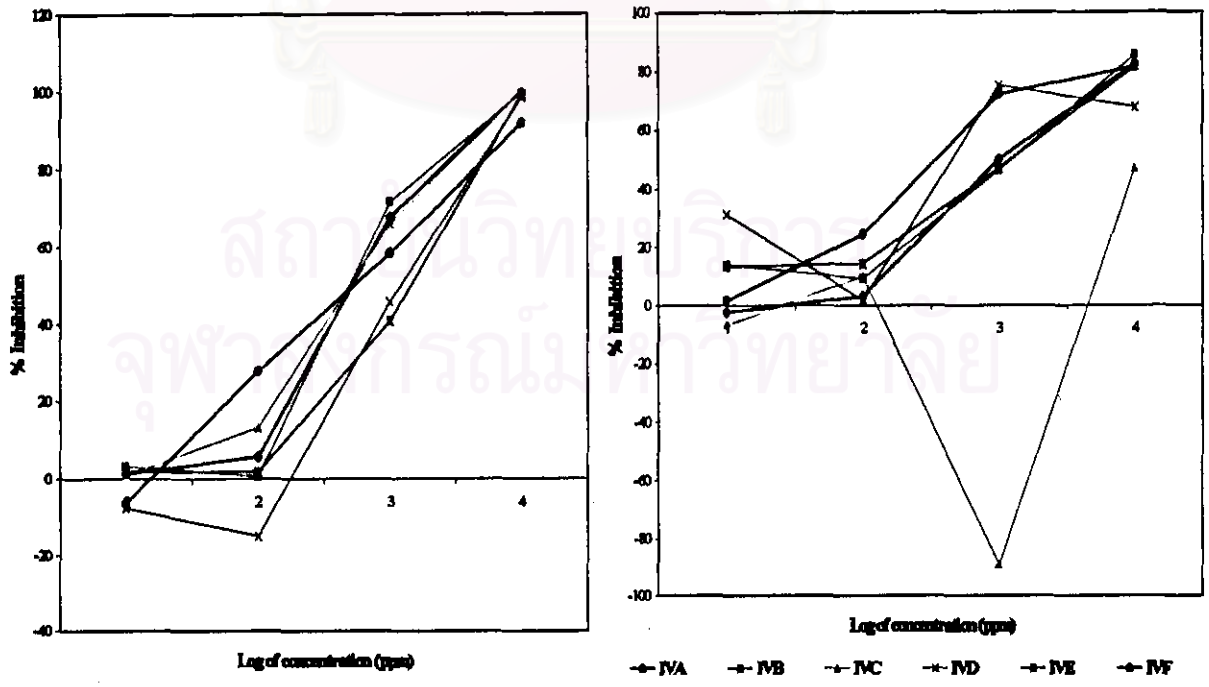
Table 3.19 The results of the separation of Fraction IV

Eluents	Fraction No.	Remarks	Weight (g)
20% EtOAc in CH_2Cl_2	IVA (1-6)	brown liquid	1.27
40% EtOAc in CH_2Cl_2	IVB (7-18)	brown liquid	8.71
60% EtOAc in CH_2Cl_2	IVC (19-26)	brown liquid	3.47
80% EtOAc in CH_2Cl_2	IVD (27-42)	brown liquid	6.31
100% EtOAc	IVE (43-50)	brown liquid	3.11
20% MeOH in EtOAc	IVF (51-74)	dark brown viscous liquid	5.50

Each fraction was subjected to test plant growth inhibition (as method 2.5.1 (ii)). From Table 3.20 and Fig. 3.8 indicated very clear that all fractions showed high activity at 10000 ppm.

Table 3.20 Inhibitory effect of fractions of ethyl acetate crude extract of *E. adenophorum* on growth of rice (*O. sativa* cv. RD23)

Fraction	% Inhibition at various concentration				
	Growth of rice part	10 (ppm)	100 (ppm)	1000 (ppm)	10000 (ppm)
IVA	root	1.10	5.64	67.71	100.00
	shoot	-2.39	3.03	50.0	82.30
IVB	root	3.13	0.48	71.63	99.53
	shoot	13.93	9.15	46.26	85.36
IVC	root	0.95	13.16	65.99	100.00
	shoot	-6.83	9.86	-88.82	46.94
IVD	root	-7.67	-15.05	45.61	98.43
	shoot	31.29	1.68	75.17	67.67
IVE	root	1.98	1.68	40.59	99.16
	shoot	13.29	14.29	46.76	81.50
IVF	root	-6.55	27.75	58.30	91.92
	shoot	1.61	24.34	72.06	81.50



Root Shoot

Fig. 3.8 Inhibitory effect of fractions of ethyl acetate crude extract on rice seedling

The rice growth inhibitory effect, every fraction revealed very good activity. The root growth inhitory effect is higher than shoot growth at 10000 ppm. Fraction IVA, IVB and IVC showed the highest inhibition, 100%, 99.53% and 100%, respectively.

In addition, each fraction was tested against brine shrimp (*A. salina*) cytotoxicity and various anticell lines cytotoxicity.

The six separated fractions were subjected to the brine shrimp *(A. salina)* and carcinoma cell line cytotoxicity tests. The results are displayed in Table 3.21–3.22.

Table 3.21 Brine shrimp cytotoxicity test of ethyl acetate fractions

Fraction	LC_{50}	Activity
IVA	21.12	medium
IVB	27.88	medium
IVC	5.26	high
IVD	34.23	medium
IVE	10.00	high
IVF	25.34	medium

Note : $LC_{50} < 10$ μg / mL High Activity
$LC_{50} < 100$ μg / mL Medium Activity
$LC_{50} < 1000$ μg / mL Low Activity
$LC_{50} > 1000$ μg / mL No activity

From the results of brine shrimp cytotoxicity test, it was found that fractions IVC and IVE revealed high activity.

All six separated fractions from crude ethyl acetate by column chromatography technique were subjected to various cell lines cytotoxicity tests against Leukemia carcinoma (HL-60), Nasopharyngeal carcinoma (KB), Colon carcinoma (HCT-8), Hepatocellular carcinoma (Bel-7402) and Gastric carcinoma (BGC-823). The results are displayed in Table 3.22.

Table 3.22 Cytotoxicity test against various carcinoma cell lines

Extract	Cell line				
	Bel-7402	BGC-823	HCT-8	HL-60	KB
IVA	+	+	+	+	+
IVB	++	-	++	-	-
IVC	+	+	+	+	+
IVD	+	-	+	-	-
IVE	+	-	+	-	-
IVF	+	-	+	-	-

Note : The results suggested by Beijing Medical University, Beijing, China

+++ very good

++ good

+ fair

- no activity

The results displayed that only the two fractions including fractions IVA and IVF, had a satisfied activity to Leukemia carcinoma (HL-60). In addition, for Nasopharyngeal carcinoma (KB) and Gastrict carcinoma (BGC-823) there were only two fractions which displayed this attractive activity including as IVA and IVC while Colon carcinoma (HCT-8) and Hepatocellular carcinoma (Bel-7402) were active to every fractions were active but IVB revealed higher activity than other fractions. It could be concluded fraction IVB showed specific and high activity against Colon carcinoma (HCT-8) and Hepatocellular carcinoma (Bel-7402) but IVA was not exhibit specific activity that was active to every cell lines.

Fractions IVA–IVC gave the best results on plant growth inhibition (Table 3.20) so combined these fractions (Fraction IVAC), then reseparated by column chromatography as the result in Table 3.23.

Table 3.23 The results of separation of fraction IVAC

Eluent	Fraction No.	Remarks	weight (g)
50%EtOAc : hexane	IVACA (1–5)	dark green viscous liquid	4.07
60%EtOAc :hexane	IVACB (6–9)	green viscous liquid	3.06
70%EtOAc : hexane	IVACC (10-14)	green liquid	1.62
80%EtOAc : hexane	IVACD (15–18)	brown-green liquid	0.27
90%EtOAc : hexane	IVACE (19–30)	white crystral in brown-green liquid (Compound 5)	3.22
100% EtOAc	IVACF (31-36)	brown-green liquid	2.91
10%MeOH : EtOAc	IVACG (37-44)	brown liquid	3.82
20-40%MeOH : EtOAc	IVACH (45-64)	brown viscous liquid	4.71

Fraction IVACE was purified by washing with a mixture of methanol and ethyl acetate. The white needle crystal was received (Compound 5). This compound was further purified by recrystallization using a mixture of methanol and dichloromethane several times, then Compound 5 was obtained 0.37 g.

Fractions IVACA gave the attractive components so reseparated by column chromatography as the result in Table 3.24.

Table 3.24 The results of separation of fraction IVACA

Eluent	Fraction No.	Remarks	Weight (g)
30-40%EtOAc : hexane	IVACAA (1 – 5)	brown-green liquid	0.03
60%EtOAc :hexane	IVACAB (6 – 19)	brown-green liquid (Mixture 7, 8)	1.05
70%EtOAc : hexane	IVACAC (20-26)	brown liquid	0.32
80%EtOAc : hexane	IVACAD (27–35)	brown liquid	0.42
90%EtOAc : hexane	IVACAE (36–42)	brown liquid	0.57
100% EtOAc	IVACAF (43-56)	brown-green liquid	0.05
10%MeOH : EtOAc	IVACAG (57-64)	brown-green liquid	0.44
20-40%MeOH : EtOAc	IVACAH (65-74)	brown viscous liquid	0.68

Fraction IVACAB was tried to further purification by recolumn using isocratic elution (80% ethyl acetate : hexane), giving small amount of pale yellow liquid of Mixture 7 and Mixture 8.

3.3.3 Separation of Fraction V

Based upon the preliminary plant growth inhibition activity (see Table 3.6), Fraction V (butanol fraction) gave the best results (100% on rice). Then butanol crude extract, 63.81 g, was separated into eight fractions by column chromatography. The column was eluted with an increasing gradient of methanol in chloroform and saturated water. The separation procedures were the same as those employed in the separation of Fraction III.

Table 3.25 The results of the separation of Fraction V

Eluent	Fraction No.	Remarks	Weight (g)
$CHCl_3$:MeOH:H_2O 88:10:2	VA (1-30)	dark green viscous liquid	16.39
$CHCl_3$:MeOH:H_2O 83:15:2	VB (31-48)	dark green viscous liquid	4.37
$CHCl_3$:MeOH:H_2O 78:20:2	VC (49-60)	green liquid	1.62
$CHCl_3$:MeOH:H_2O 73:25:2	VD (61-81)	green liquid	17.42
$CHCl_3$:MeOH:H_2O 68:30:2	VE (82-93)	yellow liquid	2.43
$CHCl_3$:MeOH:H_2O 60:40:10	VF (94-105)	yellow oil	1.76
$CHCl_3$:MeOH:H_2O 40:60:10	VG (106-129)	brown oil	4.42
$CHCl_3$:MeOH:H_2O 20:80:10	VH (130-139)	pale brown liquid	3.29

Each fraction derived from the separation of Fraction V was further subjected to brine shrimp cytotoxicity test and found that all fractions had low activity. The results of cytotoxicity test are shown in Table 3.26.

Table 3.26 Brine shrimp (*A. salina)* cytotoxicity test

Fraction	LC_{50} (6 h)	Activity
VA	182.41	low
VB	181.79	low
VD	336.46	low
VG	235.47	low

Note : LC_{50} < 10 μg / mL High Activity
LC_{50} < 100 μg / mL Medium Activity
LC_{50} < 1000 μg / mL Low Activity
LC_{50} > 1000 μg / mL No activity

After monitored by TLC, the similar fractions were combined, the VD fraction was resepatated and the results are shown in Table 3.27.

Table 3.27 The results of the separation of fraction VD

Eluent	Fraction No.	Remarks	Weight (g)
10%MeOH : $CHCl_3$	VDA (1-52)	dark brown liquid	2.49
20%MeOH : $CHCl_3$	VDB (53-83)	dark brown liquid (Compound 6)	2.10
30%MeOH : $CHCl_3$	VDC (84-96)	brown liquid	0.78
40%MeOH : $CHCl_3$	VDD (97-107)	brown liquid	2.23
50%MeOH : $CHCl_3$	VDE (108-118)	brown liquid	0.87
60%MeOH : $CHCl_3$	VDF (119-134)	brown liquid	2.21
80%MeOH : $CHCl_3$	VDG (135-139)	brown green liquid	1.57

Fraction VDB was purified by crystallization with hot methanol, then white amorphous solid, Compound 6 was gained (0.44 g).

3.4 Structural Elucidation

Structural Elucidation of Mixture 1 (CH-1)

Mixture 1 is white amorphous solid with melting point 78-80 °C and R_f value 0.67 (Chloroform).

The IR spectrum of this compound (Fig. 3.9) exhibited the characteristic absorption band at 3600-3200 cm^{-1} (O-H stretching of hydroxyl group), 2902 and 2852 (C-H stretching of CH_2, CH_3), 1470 and 1465 (C-H asymmetric bending of CH_2, CH_3), 1060 (C-O stretching of 1° ROH), 730, 720 (C-H rocking of $CH_2 > 4$ groups).

The ^{1}H-NMR spectrum (Fig. 3.10) showed the important triplet signals at δ 3.60 ppm belonging to the signal of the proton on the carbon attaching to oxygen atom. The signal at 1.55 ppm was a hydroxyl proton (CH_2-OH). The high intensity singlet signal at 1.26 ppm revealed that there was several interlinking of methylene group in the molecule of this compound. The signal at 0.87 ppm was corresponded to the methyl group signal.

The ^{13}C-NMR spectrum (Fig. 3.11) showed the carbon signals between δ 14.1-32.8 ppm of methyl and methylene carbons and the carbon signal at 63.1 ppm was belonging to the carbon adjacent to a hydroxyl group (OH).

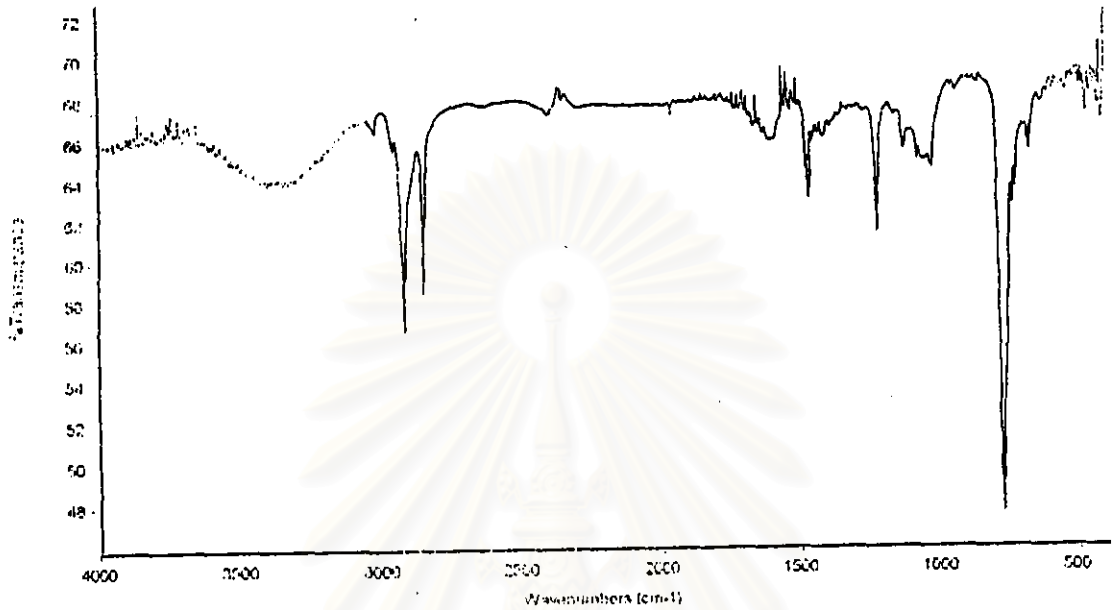


Fig. 3.9 The IR spectrum of Mixture 1

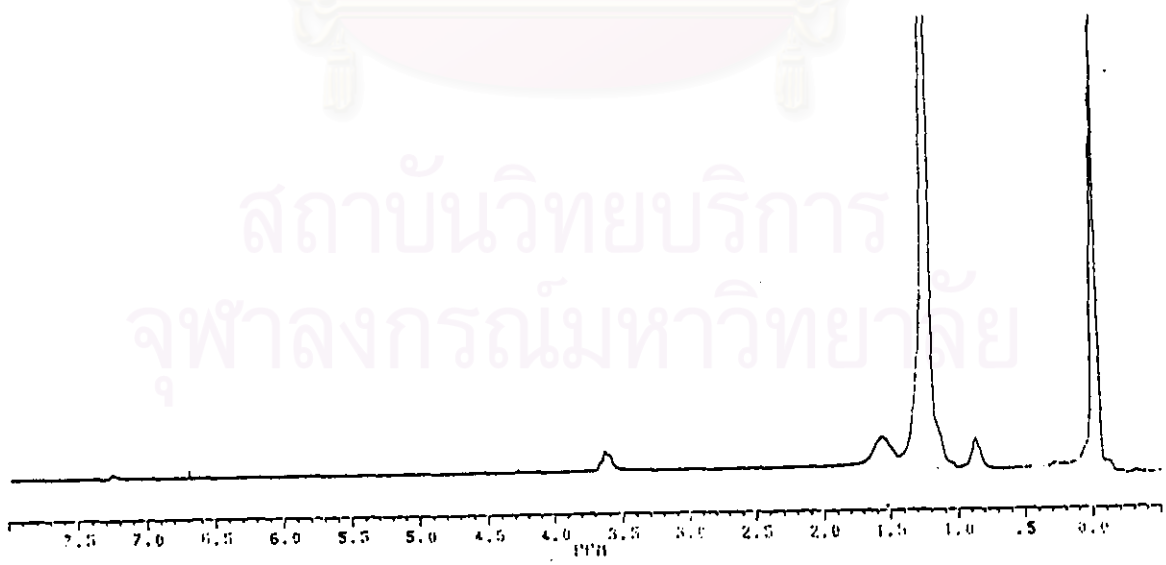


Fig. 3.10 The ^{1}H-NMR spectrum of Mixture 1

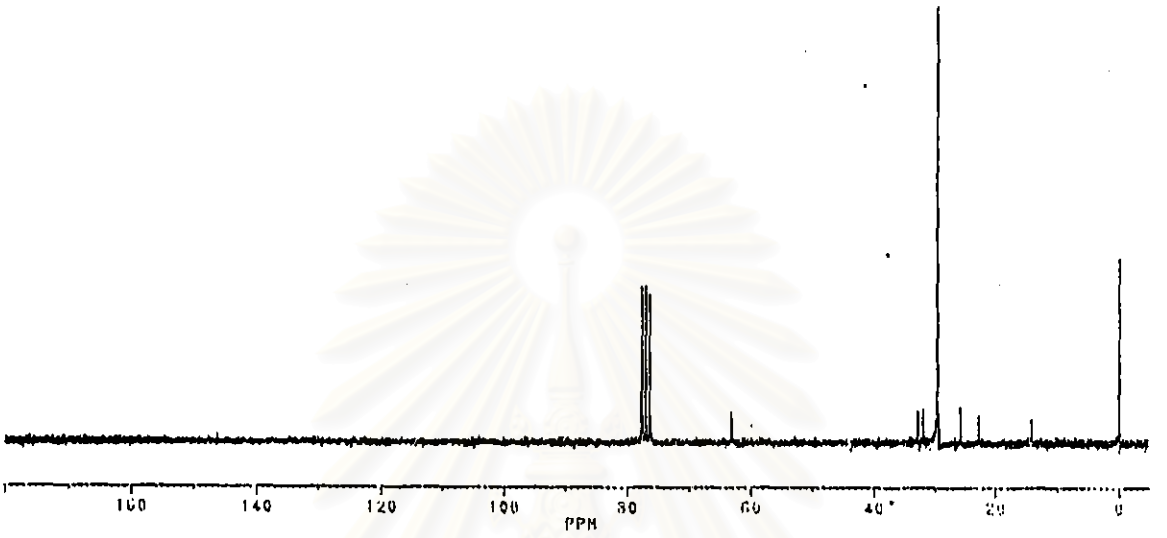


Fig. 3.11 The ^{13}C-NMR spectrum of Mixture 1

From the comparison of physical properties and all spectroscopic data with an authentic sample of a mixture of long chain alcohols, it was found that both of them are corresponded (Cooper, 1980), (Shriner, 1980), (Silverstein, 1981). Thus, Mixture 1 should be a mixture of long chain alcohols.

$$CH_3-(CH_2)_n-CH_2-OH$$

Mixture of long chain alcohols

Mixture 1 (CH-1)

The structural elucidation of compound 2 (CH-2)

Compound 2 is white needle crystal, m.p. 133-135 °C with R_f value 0.45 (50% CH_2Cl_2 : Hexane).

The IR spectrum (Fig. 3.12) showed characteristic absorption peaks at 3500-3300 cm^{-1} of O-H stretching, 2950 and 2850 cm^{-1} of C-H stretching of CH_2 and CH_3, 1650 cm^{-1} of C=C stretching, 1460 and 1380 cm^{-1} of C-H symmetric and asymmetric bending of CH_2 and CH_3, 970 and 960 cm^{-1} of C-H out of plane bending of disubstituted vinyl and 840 and 800 cm^{-1} of C-H out of plane bending of trisubstituted vinyl.

The ^1H NMR spectrum (Fig. 3.13) showed the characteristic signals of steroid at δ 0.51-1.10 ppm which were the signals of angular methyl groups at side chain, C-21, 26, 27 and 29. The proton signals at δ 1.48-2.35 ppm were the signal of methylene groups and methinic groups of steroid. The multiplet signal at δ 3.54 ppm was the signal of a hydroxy proton. The doublet of doublet signal at δ 5.10 ppm was the signal of disubstituted vinyl protons (H-22 and H-23) while the signal at δ 5.40 ppm could be the signal of trisubstituted vinyl proton (H-6).

The ^{13}C-NMR spectrum (Fig. 3.14) showed 26 carbon signals of 29-carbon atom. The characteristic signals of steriod displayed at δ ppm 71.81 (-C-OH), 140.70 and 121.7 (-CH=C), 129.3 and 138.3 (-CH=CH-).

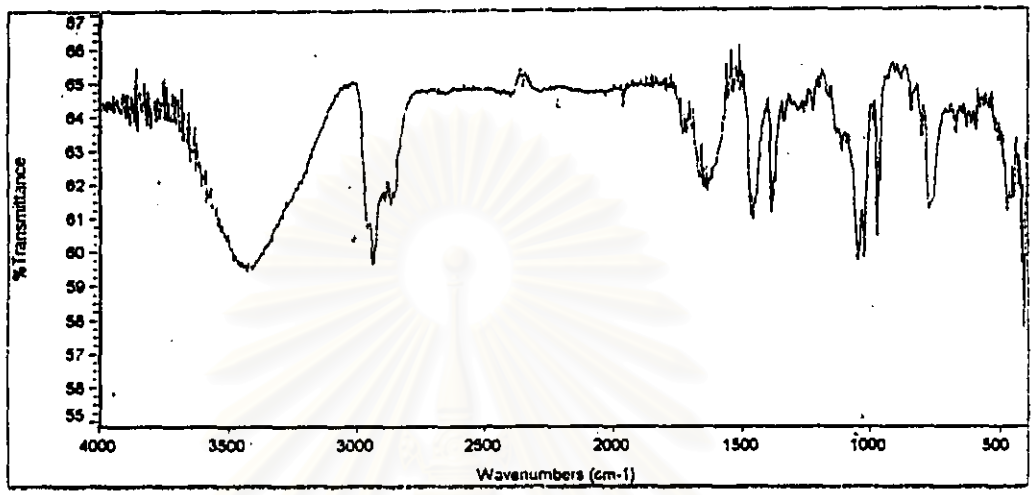


Fig. 3.12 The IR spectrum of Compound 2

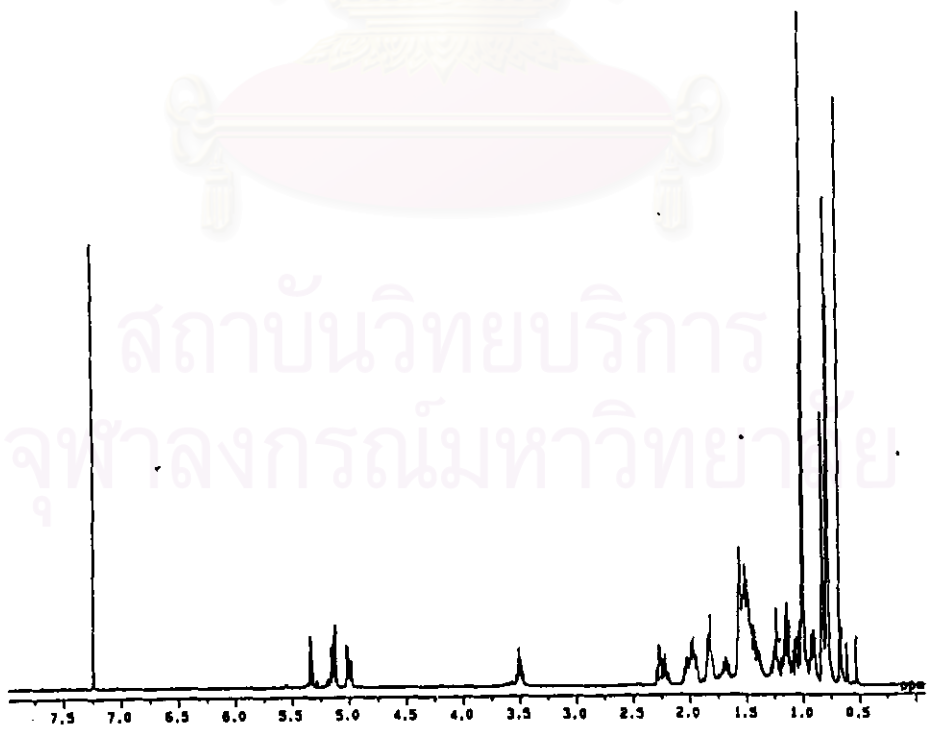


Fig. 3.13 The ^{1}H-NMR spectrum of Compound 2

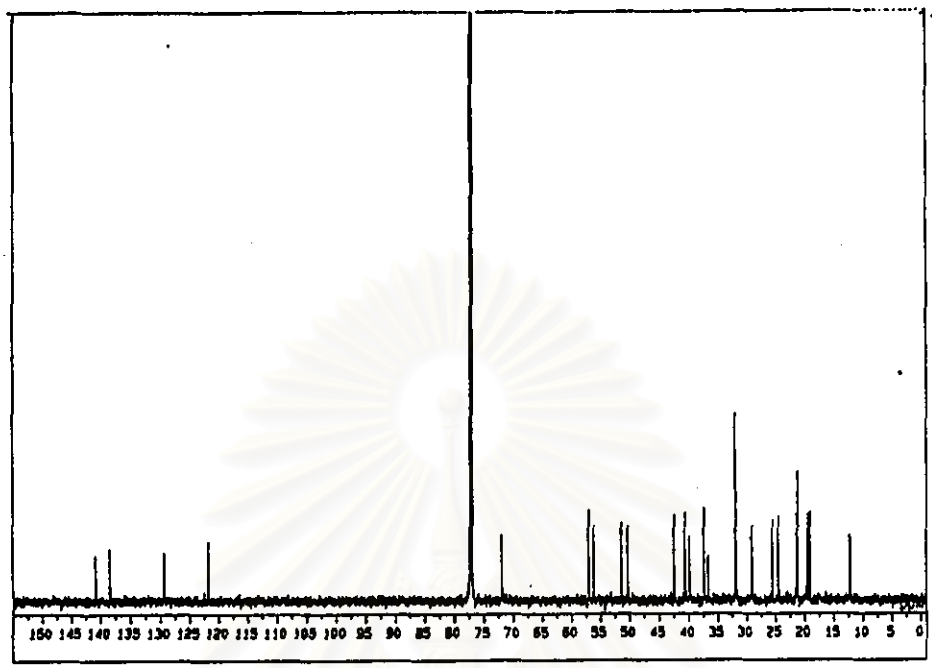


Fig. 3.14 The ^{13}C-NMR spectrum of Compound **2**

According to the information of this compound, it was suggested that this compound be closed to those of steroids (Cooper, 1980), (Shriner, 1980), (Silverstein, 1981).

To confirm the structure, this compound was compared the ^{13}C-NMR spectrum with those of β–sitosterol and stigmasterol (Harborne,1984), (Furniss,1989).

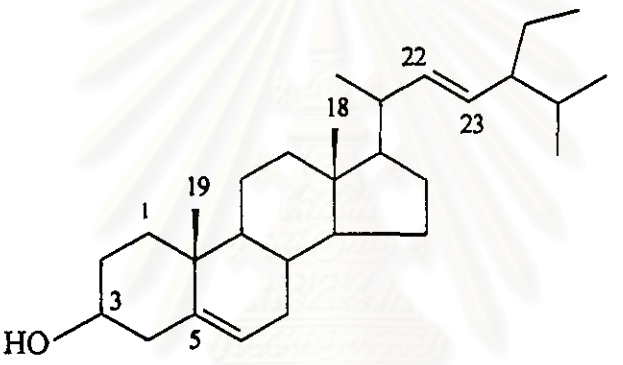
Table 3.28 The ^{13}C-NMR spectrum of β–sitosterol and stigmasterol compared with Compound **2**

Position	Chemical shift (ppm)		
	β–sitosterol	Stigmasterol	Compound **2**
1	37.1	37.4	37.3
2	31.8	31.7	31.7
3	71.9	71.8	71.8
4	42.4	42.4	42.3
5	140.9	140.0	140.7
6	121.8	121.7	121.7
7	32.0	31.9	31.9
8	32.0	31.9	31.9
9	50.3	50.3	50.2
10	36.6	36.6	36.5
11	21.1	21.2	21.2
12	39.9	39.8	39.7
13	42.4	42.4	42.2
14	56.8	57.0	56.9
15	24.3	24.4	24.4
16	28.2	28.9	28.9
17	56.2	56.0	56.0
18	11.9	12.2	12.2
19	19.4	19.4	19.4
20	36.2	40.5	40.5
21	19.1	21.2	21.2
22	34.0	138.4	138.3
23	29.3	129.3	129.3
24	50.3	51.3	51.2
25	26.2	31.9	31.9
26	18.8	21.1	21.1

Table 3.28 (cont.)

Position	Chemical shift (ppm)		
	β-sitosterol	Stigmasterol	Compound **2**
27	19.8	21.1	21.1
28	23.1	25.4	25.4
29	11.9	12.0	12.0

From the comparison of ^{13}C-NMR spectrum, there was no doubt to conclude that this compound is stigmasterol.



Stigmasterol

Compound **2** (CH-2)

The structural elucidation of Mixture 3 (CH-3)

Mixture 3 is white amorphous solid, melting point 73-75 °C and R_f value 0.37 (40% CH_2Cl_2 : Hexane).

The IR spectrum (Fig. 3.15) exhibited characteristic absorption bands at 3500-3000 cm^{-1} (O-H stretching of hydroxyl group, 2950, 2850 of C-H stretching of CH_2 and CH_3, 1720 of C=O stretching of carboxyl group, 720 and 730 of CH_2 rocking of CH_2 >4 groups.

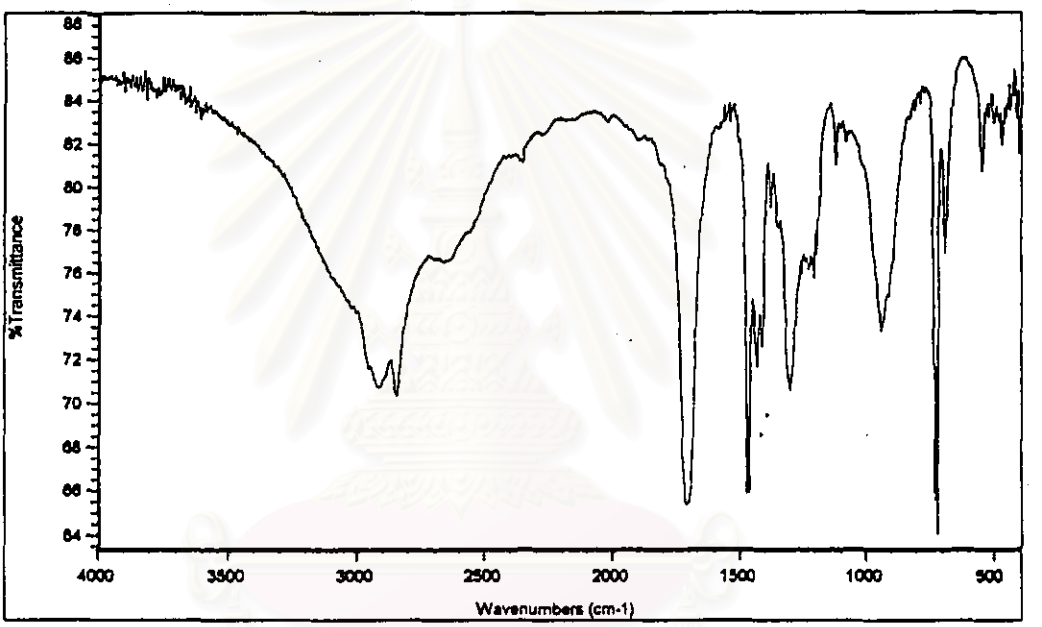


Fig. 3.15 The IR spectrum of Mixture 3

From IR spectrum and physical properties (Cooper, 1980), (Shriner, 1980), (Silverstein, 1981). Mixture 3 can be concluded as a mixture of long chain carboxylic acids.

$$CH_3-(CH_2)_n-CH_2-\overset{O}{\overset{\|}{C}}OH$$

Mixture of long chain carboxylic acids

Mixture 3 (CH-3)

The structural elucidation of Mixture 4 (CH-4)

Mixture 4 is white amorphous solid, melting point 67-69 °C and R_f value 0. 69 (10% CH_2Cl_2 : Hexane).

The IR spectrum (Fig. 3.16) showed characteristic absorption peaks at 2950, 2850 cm^{-1} (C-H stretching of CH_2 , CH_3), 1480 (C-H symmetric bending of CH_2 and asymmetric of CH_3 , 1300 (C-O stretching), 730 and 720 (CH_2 rocking of CH_2 > 4 groups).

The ^{1}H-NMR spectrum (Fig. 3.17) showed two signals belonging to protons of ester. The signal at δ 4.61 ppm should be the signal of α-proton in the alcoholic part and the signal at δ 2.35 ppm was assigned for the signal of α-proton in the acidic portion. Other signals around 1.60 to 0.88 ppm were the signals of methyl and methylene protons.

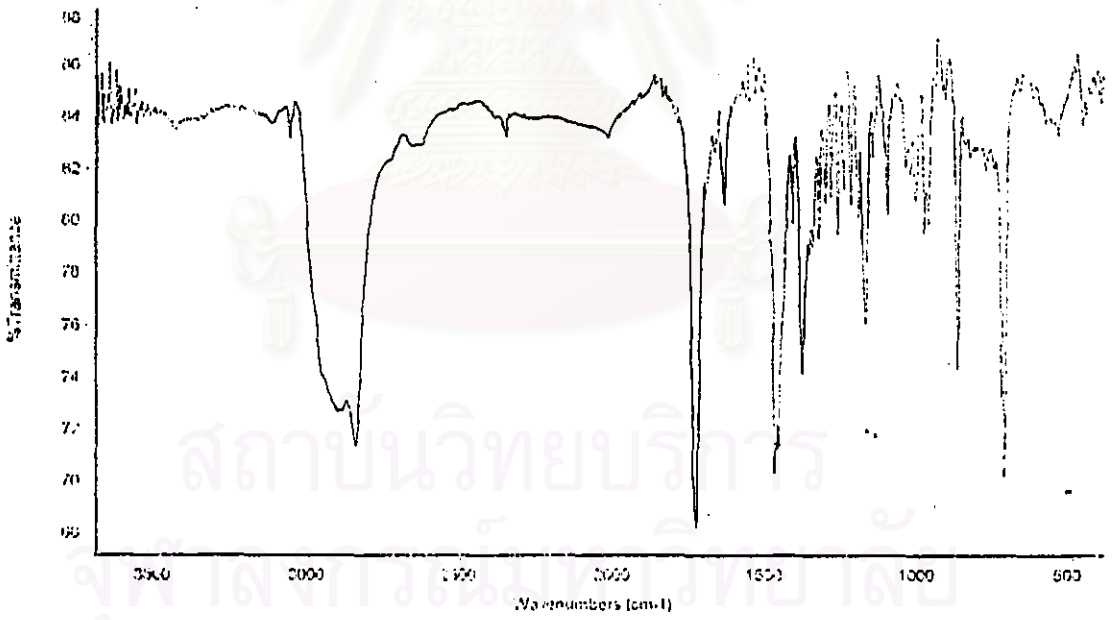


Fig. 3.16 The IR spectrum of Mixture 4

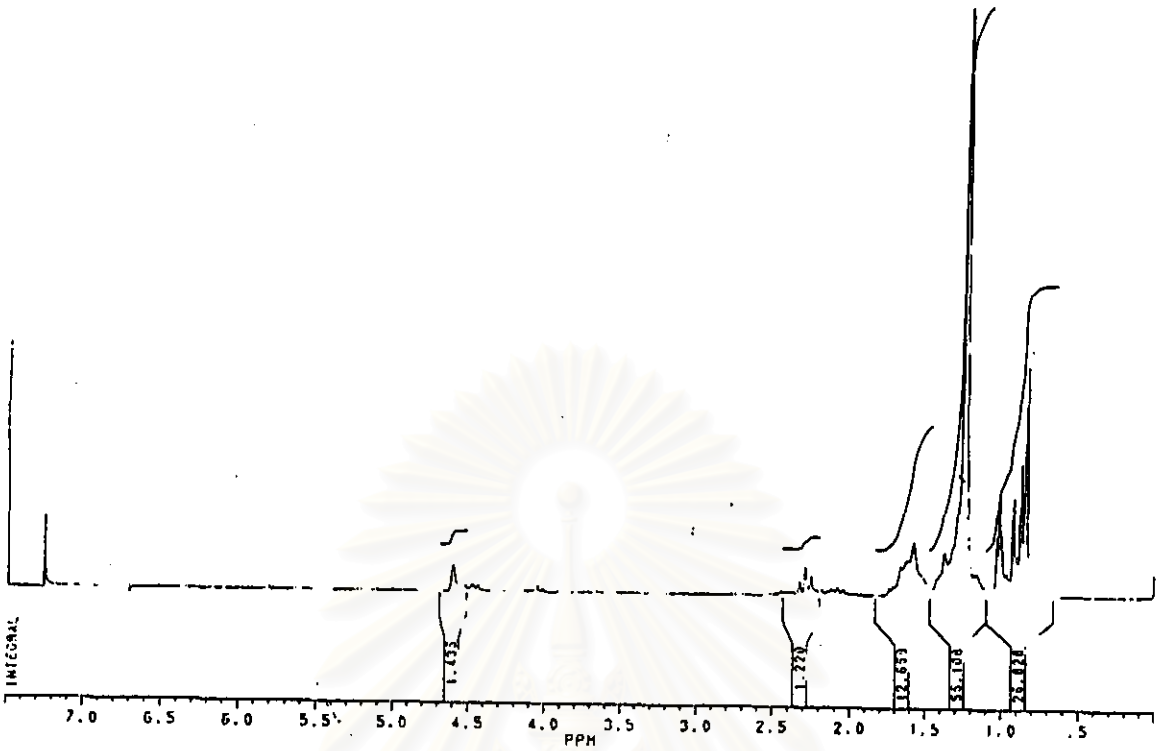
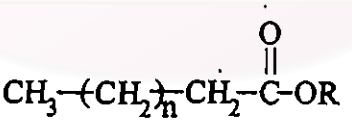


Fig. 3.17 The ^{1}H-NMR spectrum of Mixture **4**

From all of spectroscopic data and physical properties (Cooper, 1980), (Shriner, 1980), (Silverstein, 1981), this mixture was long chain esters.



Mixture of long chain esters

Mixture **4** (CH-4)

The structural elucidation of compound 5 (EtA-11)

Compound **5** as white needle crystal was isolated from ethyl acetate crude extract fractions. After recrystallizations with ethyl acetate:hexane several times, the product with melting point 191-193 °C and R_f 0.67 (ethyl acetate) was obtained.

The IR spectrum (Fig. 3.18) showed characteristic absorption bands at 3600-3300 cm^{-1} of O-H stretching, 1710 cm^{-1} of C=O stretching, 1630, 1595, 1510 and 1460 of C=C stretching of aromatic, 900 and 850 of C-H bending of aromatic.

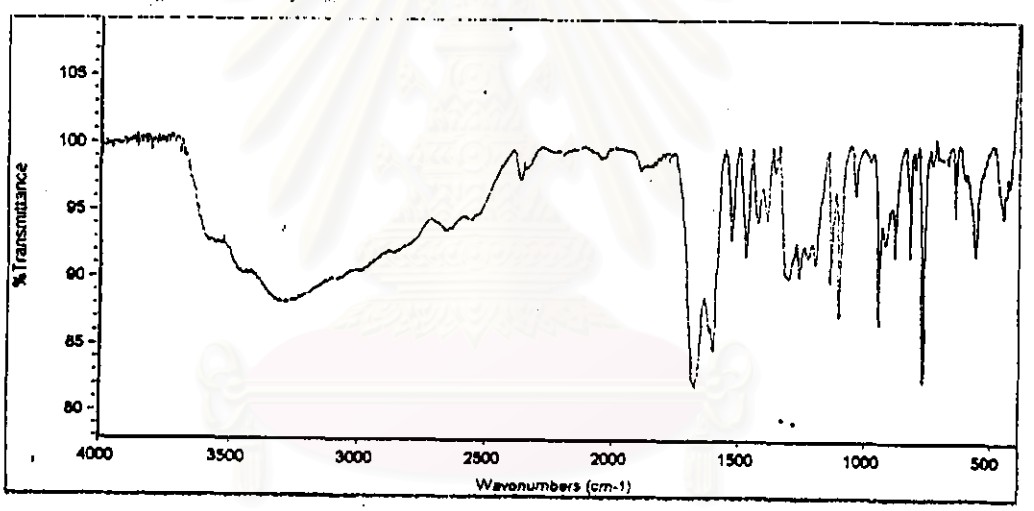


Fig. 3.18 The IR spectrum of Compound **5**

The ^{1}H NMR spectrum (Fig. 3.19) and signal integration indicated important proton signals at δ (ppm) : 7.32 (1H, d, J=1.8 Hz), 7.34 (1H, d, J=2.1 Hz), 7.40 (1H, d, J=2.1 Hz), 6.70 (1H, s) and 6.72 (1H, s).

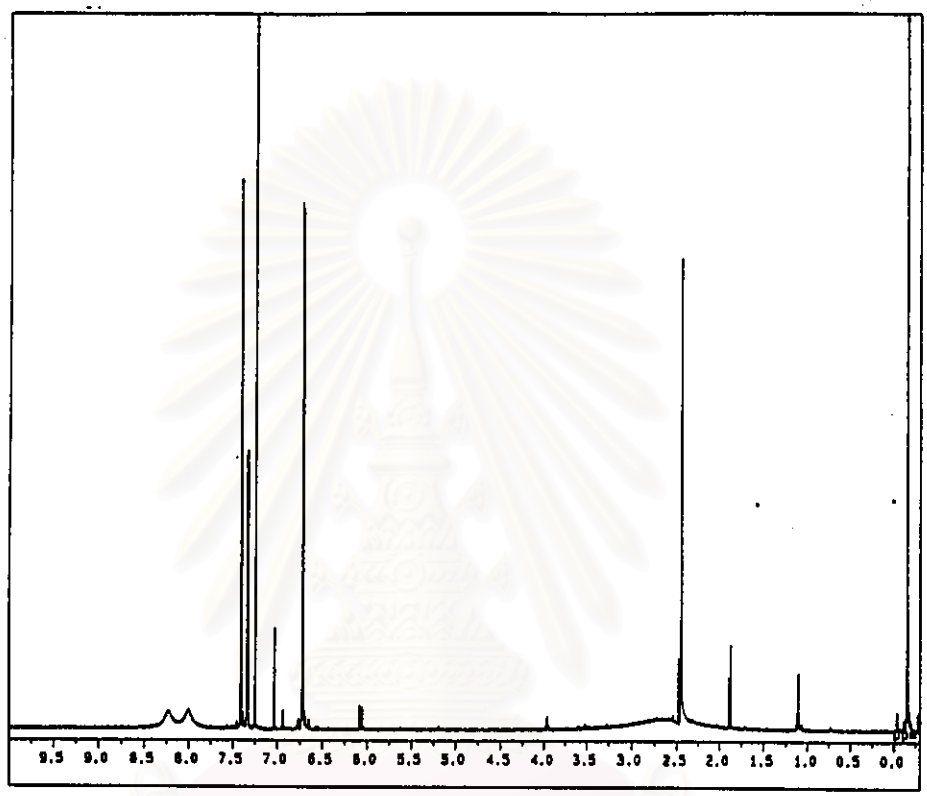


Fig. 3.19 The ^{1}H-NMR spectrum of Compound **5**

The ^{13}C-NMR spectrum (Fig. 3.20) exhibited the carbon signal of carboxylic at δ 168.4 ppm. The other carbon signals of aromatic moiety were observed at δ(ppm) 122.4 (C-1), 116.8 (C-2), 144.2 (C-3), 149.3 (C-4), 114.6 (C-5) and 122.8 (C-6).

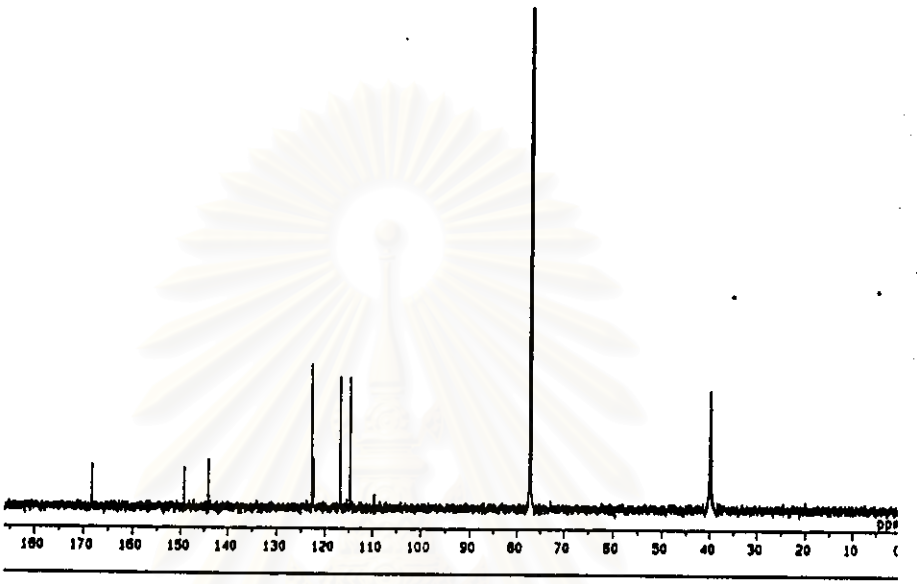


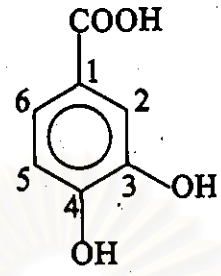
Fig. 3.20 The ^{13}C-NMR spectrum of Compound **5**

From all spectroscopic data (Cooper, 1980), (Shriner, 1980), (Silverstein, 1981), it could be concluded that this compound was benzoic acid derivative. It was closed to reported protocatechuic acid by comparison from literature (Kuntz, 1972). The result was tabulated in Table 3.29.

Table 3.29 The comparison of ^{13}C-NMR of protocatechuic acid

Position	Chemical shift (ppm)	
	Protocatechuic acid	Compound **5**
1	122.4	122.4
2	117.5	116.8
3	145.3	144.2
4	150.8	149.3
5	115.7	114.6
6	123.9	122.8
COOH	169.4	168.4

Therefore, Compound 5 can be assigned as protocatechuic acid (3,4-dihydroxybenzoic acid).



Protocatechuic acid

Compound 5 (EtA-11)

Structural elucidation of compound 6 (Bu-3)

The white amorphous solid of Compound **6** was isolated from butanol crude extract. This compound had melting point 279-280°C and R_f value 0.25 (10% methanol-dichloromethane).

The IR spectrum (Fig. 3.21) showed the presence of a hydroxyl group at 3600-3100 cm^{-1} and glycosidic linkage at 1100 cm^{-1}.

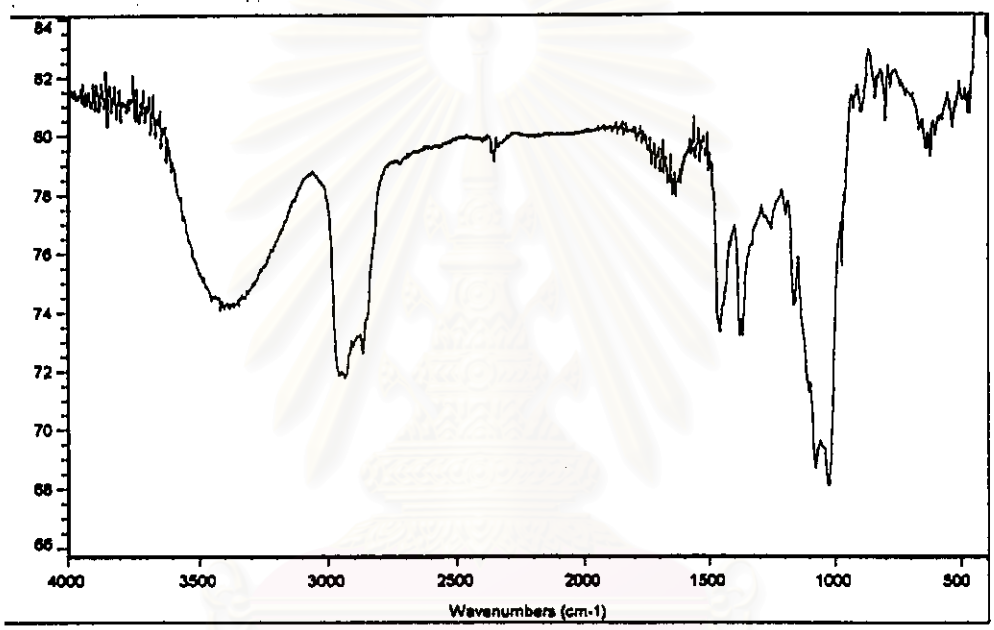


Fig. 3.21 The IR spectrum of Compound **6**

The ^1H NMR spectrum (Fig. 3.22) indicated steroidal proton signals at δ 0.70-2.00 ppm (m) and 4.80 ppm (m). The signals of sugar protons at δ 3.25-3.80 ppm (m) and 4.30 ppm (d, J=6.85 Hz) could be assigned for an anomeric proton.

The ^{13}C-NMR spectrum (Fig. 3.23) showed the methylene carbon signals at 143.2 (C-5) and 126.3 (C-6) ppm and the signals of 6 carbon linking to oxygen of sugar at δ 105.9 (C-1), 75.5 (C-2), 66.3 (C-3), 61.5 (C-4), 55.8 (C-5) and 54.8 (C-6) ppm. The other signals exhibited between δ 47.1-17.1 ppm were belonging to a steriodal moiety.

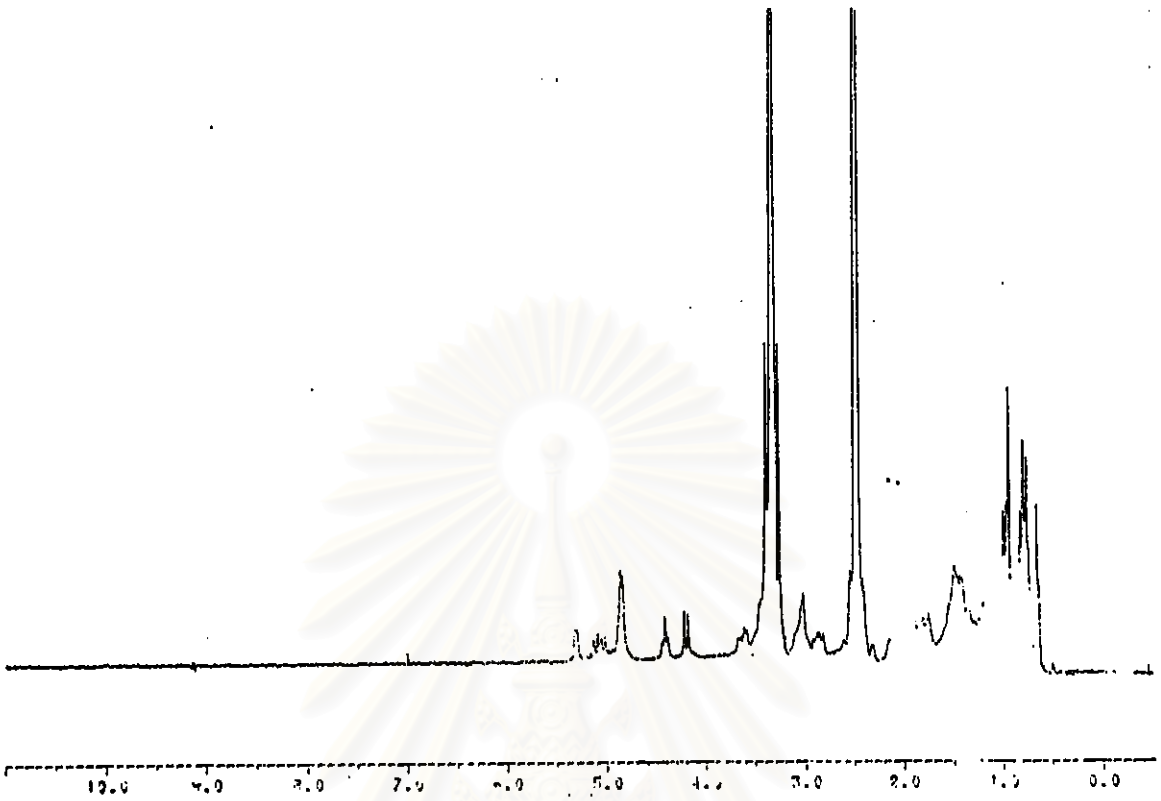


Fig. 3.22 The ^{1}H-NMR spectrum of Compound 6

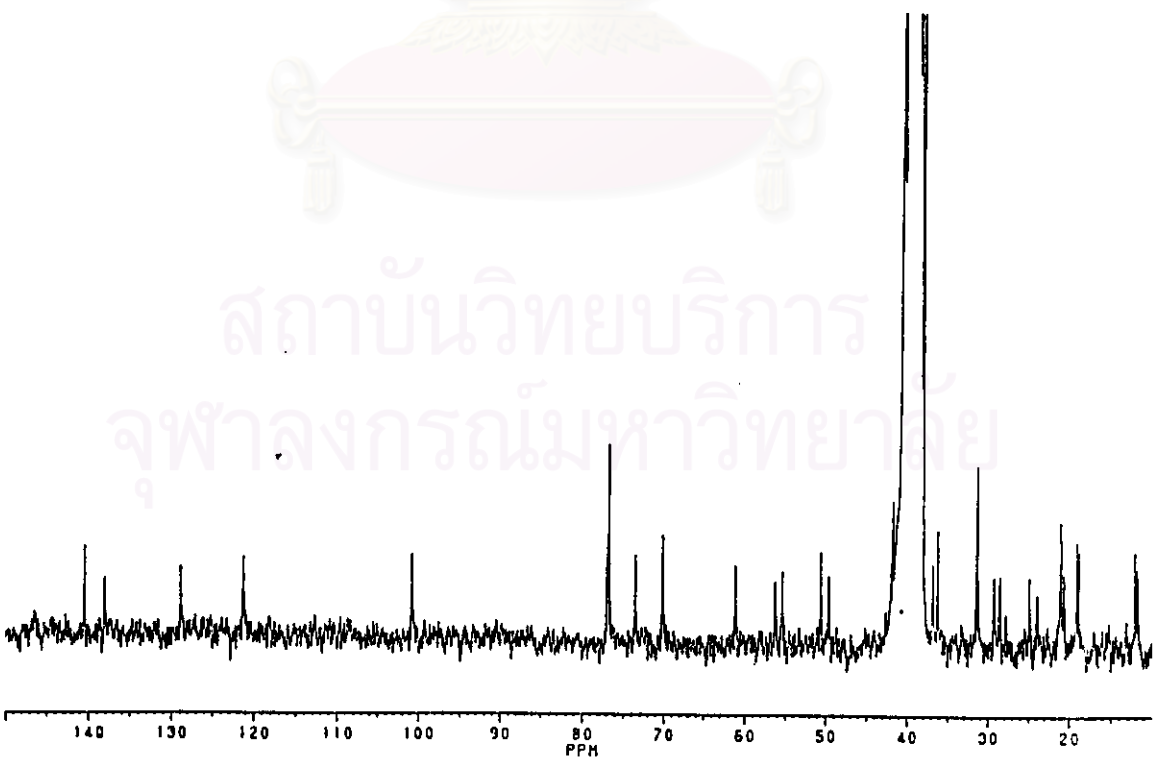


Fig. 3.23 The ^{13}C-NMR spectrum of Compound 6

From NMR spectrum, IR spectrum and physical properties (Cooper, 1980), (Shriner, 1980), (Silverstein, 1981). Compound 6 can be concluded as steroid glycoside.

3.5 Plant Growth Inhibition Activity of Isolated Substances

From the fractionation and purification of *E. adenophorum* crude extracts, six compounds were isolated. Four compounds including a mixture of long chain alcohols, stigmasterol, a mixture of long chain carboxylic acids and a mixture of long chain esters were isolated from dichloromethane crude extract. Protocatechuic acid was isolated from ethyl acetate crude extract and steroid glycoside was isolated from butanol crude extract.

In order to reach the goal of research, the isolated compounds were tested for plant growth inhibitory effect.

Table 3.30 Inhibitory effect of Compound 2 on growth of rice

Compound	% Inhibition at various concentration			
	Growth of rice part	10 (ppm)	100 (ppm)	1000 (ppm)
2	root	-69.15	-87.98	-138.64
	shoot	-74.83	-33.37	22.41

The comparison between root and shoot growth inhibition found that both of them did not show the same trend of activity. In root growth inhibition test, it was found that the more concentration was used, the less activity was observed. On the other hand, the increased concentration of Compound 2 on shoot growth, showed good activity. The results of Compound 2 on rice growth inhibition are shown in Fig. 3.24.

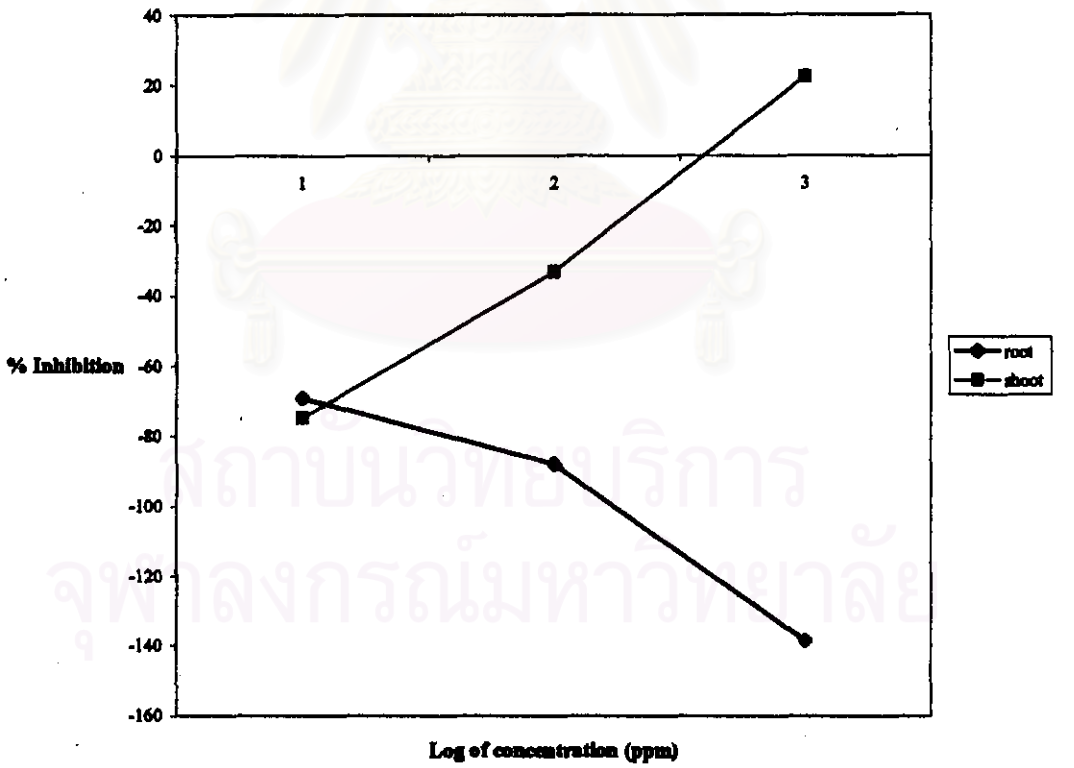


Fig.3.24 Inhibitory effect of Compound 2 on rice seedling growth

Table 3.31 Inhibitory effect of Mixture 3 on growth of rice

Mixture	% Inhibition at various concentration			
	Growth of rice part	10 (ppm)	100 (ppm)	1000 (ppm)
3	root	-4.49	8.82	21.71
	shoot	15.40	5.25	3.92

From this assay it was found that % inhibition of Mixture 3 on root could be active when concentration was increased from 10 to 1000 ppm but this activity could be decreased when tested with shoot. The results of inhibitory effect Mixture 3 are shown in and Fig. 3.25.

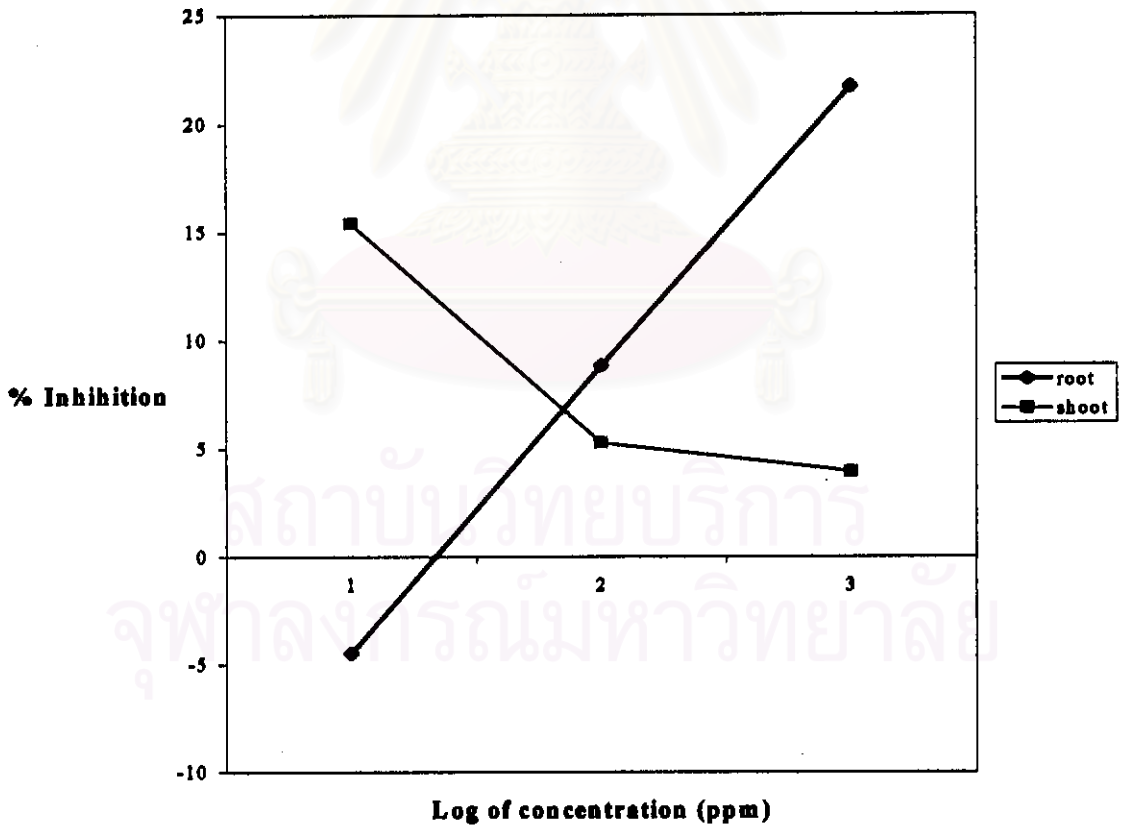


Fig.3.25 Inhibitory effect of Mixture 3 on rice seedling growth

Table 3.32 Inhibitory effect of Mixture 4 on growth of rice

Mixture	% Inhibition at various concentration			
	Growth of rice part	10 (ppm)	100 (ppm)	1000 (ppm)
4	root	2.80	11.62	7.84
	shoot	-0.65	9.5	-0.97

Both root and shoot growth inhibition of Mixture 4 are displayed in Fig. 3.26 and found that at 10 ppm it trended to increase to 100 ppm then % inhibition were decreased.

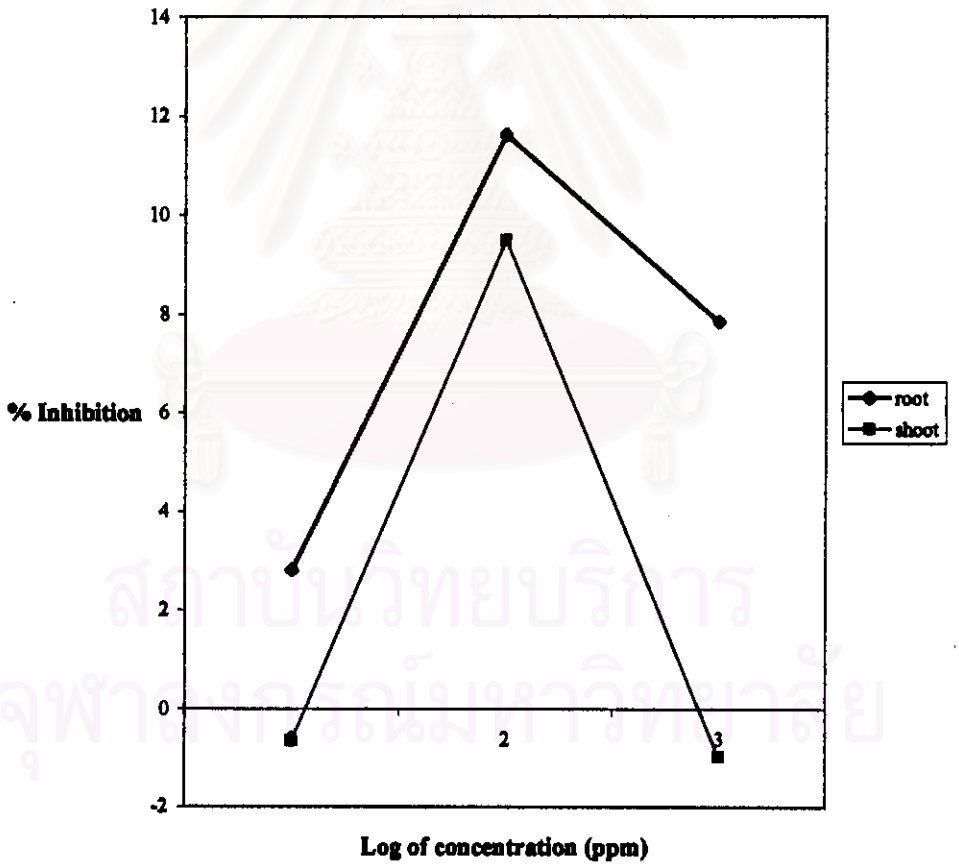


Fig.3.26 Inhibitory effect of Mixture 4 on rice seedling growth

Table 3.33 Inhibitory effect of Compound **5** on growth of rice

Compound	% Inhibition at various concentration			
	Growth of rice part	10 (ppm)	100 (ppm)	1000 (ppm)
5	root	6.29	15.43	92.30
	shoot	-8.20	10.83	84.27

Inhibition on root and shoot of Compound **5** was increased when concentration was increased. The results of Compound **5** on rice growth inhibition are revealed in Fig. 3.27.

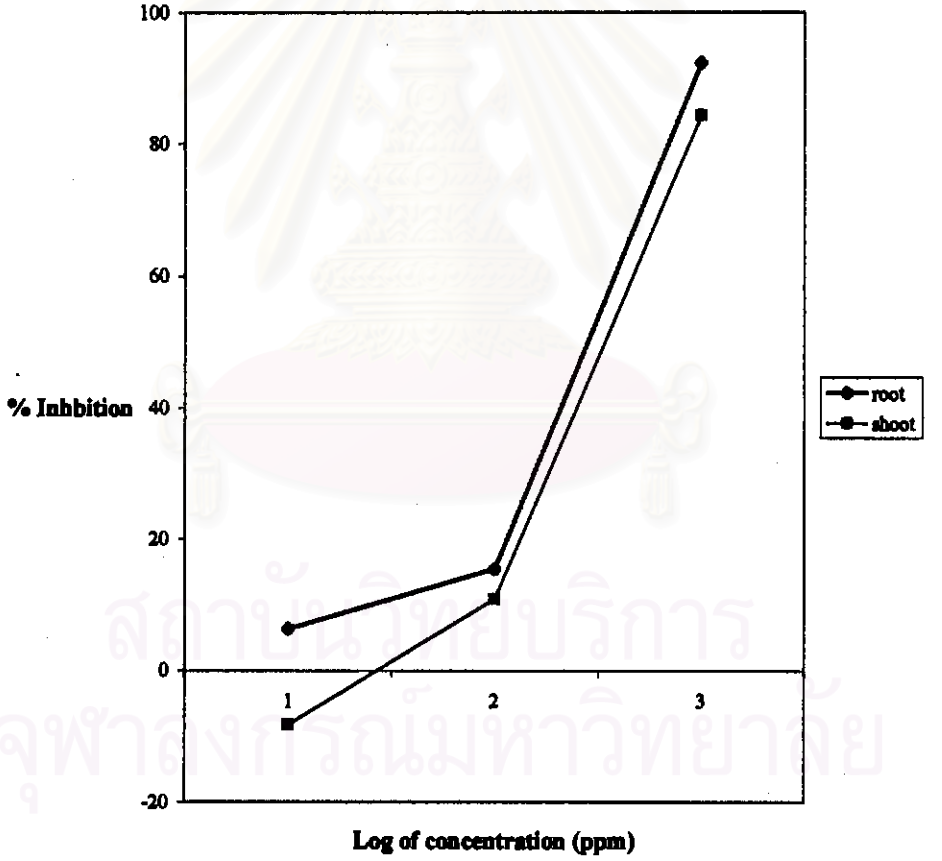


Fig. 3.27 Inhibitory effect of Compound **5** on rice seedling growth

Table 3.34 Inhibitory effect of Compound 6 on growth of rice

Compound	% Inhibition at various concentration			
	Growth of rice part	10 (ppm)	100 (ppm)	1000 (ppm)
6	root	13.00	34.85	43.96
	shoot	8.85	33.43	50.49

The comparison between % inhibition on root and shoot of Compound 6 was observed. Increasing activity in root inhibition less increased than shoot inhibition. And the results of this assay were presented in Fig. 3.28.

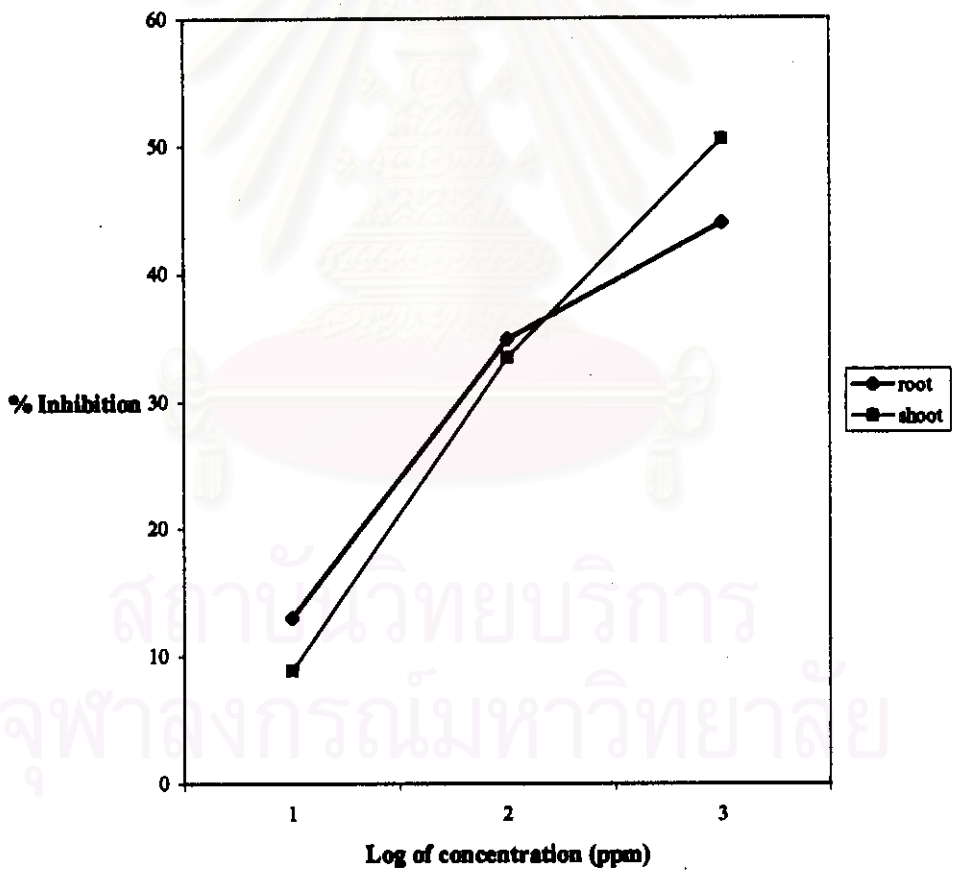


Fig. 3.28 Inhibitory effect of Compound 6 on rice seedling growth

Table 3.35 Inhibitory effect of Mixture 7 on growth of rice

Mixture	% Inhibition at various concentration			
	Growth of rice part	10 (ppm)	100 (ppm)	1000 (ppm)
7	root	-71.22	-49.65	-47.92
	shoot	-77.59	-29.94	-21.80

From the results on rice growth inhibition of Mixture 7 it was found that this mixture displayed root and shoot growth promotion. The comparison between root and shoot growth inhibition is shown in Fig. 3.29.

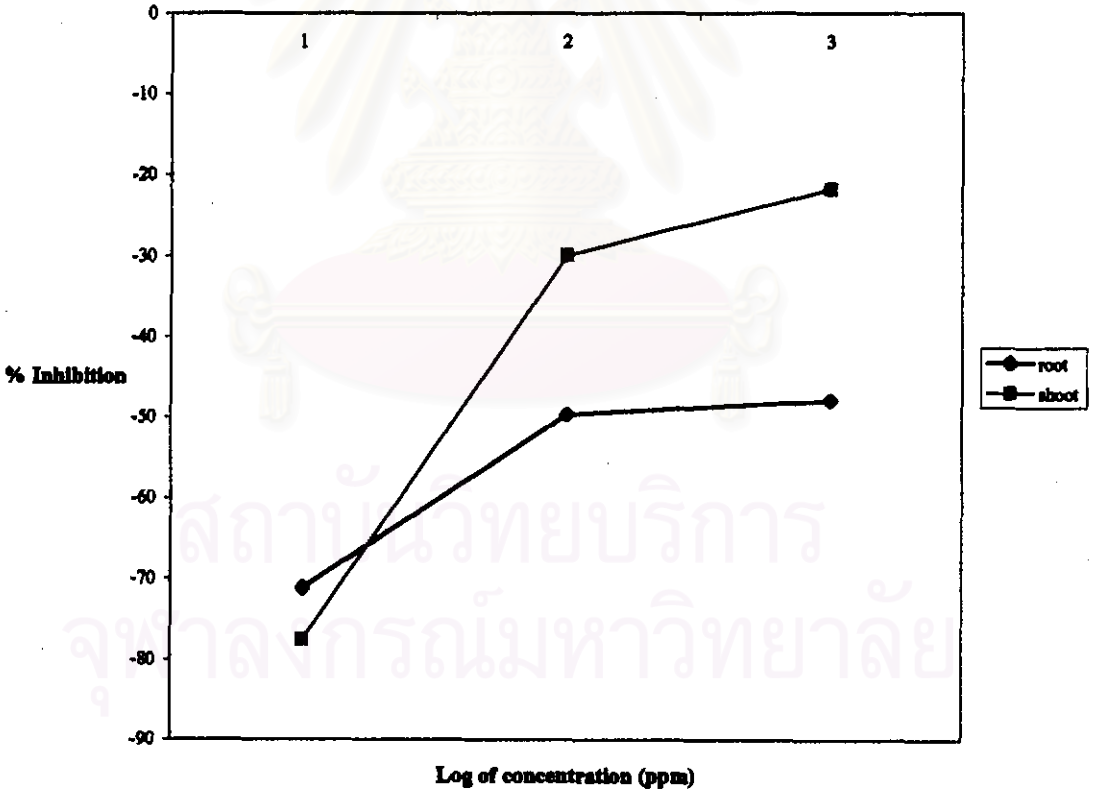


Fig. 3.29 Inhibitory effect of Mixture 7 on rice seedling growth

Table 3.36 Inhibitory effect of Mixture 8 on growth of rice

Mixture	% Inhibition at various concentration			
	Growth of rice part	10 (ppm)	100 (ppm)	1000 (ppm)
8	root	-84.90	-103.39	-82.50
	shoot	-62.58	19.90	-51.75

The results of the Mixture 8 on root and shoot rice growth inhibition were exhibited difference in activity. The trend of shoot growth was increased at low concentration then inhibition was decreased. While, root growth was not inhibited. The graph of rice growth assay is shown in Fig. 3.30.

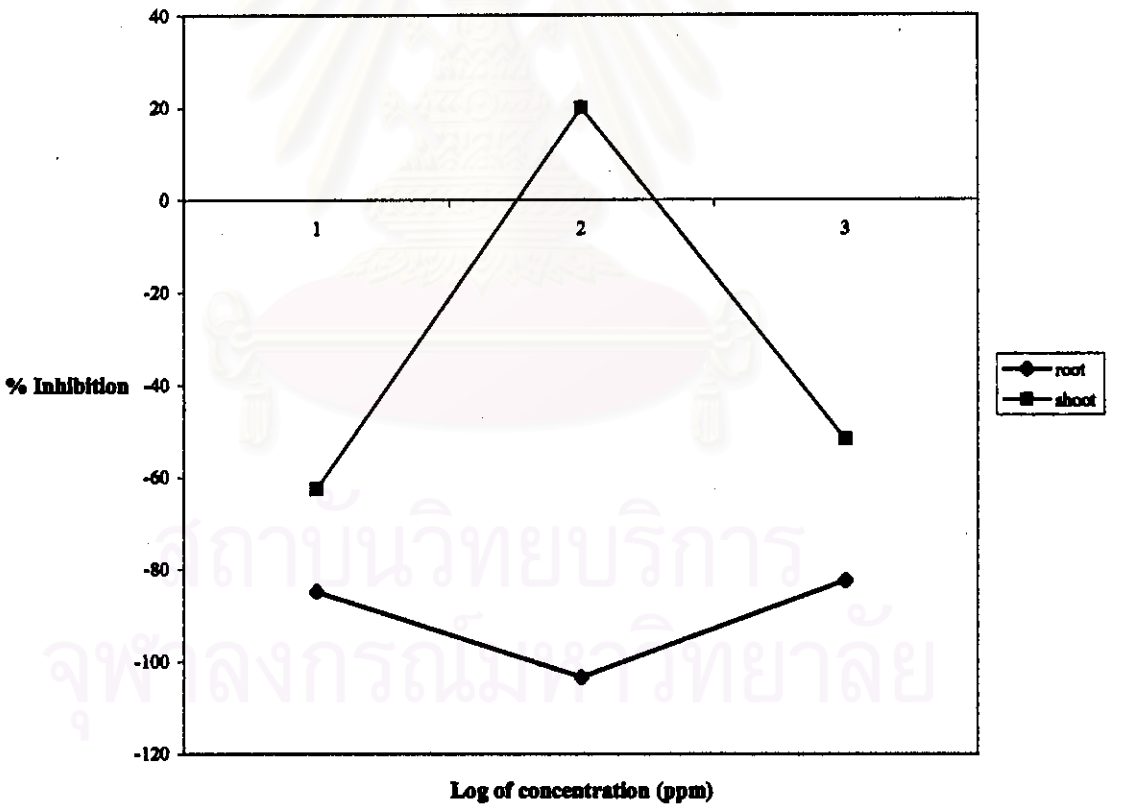


Fig. 3.30 Inhibitory effect of Mixture 8 on rice seedling growth

On the basis of effect on plant growth inhibitory, it can be classified the isolated compounds into two groups, plant growth inhibition and plant growth promotion. The compounds that exhibited as plant growth promotion are stigmasterol, a mixture of long chain ester, Mixture **7** and **8**. The compounds that displayed as plant growth inhibition are a mixture of long chain carboxylic acids, protocatechuic acid and steroid glycoside. Protocatechuic acid showed the highest inhibition 92.30%, followed by steroid glycoside and a mixture of long chain carboxylic acids 43.96% and 21.71%, respectively at 1000 ppm. There was little amount of Compound **1** (a mixture of long chain alcohol), which was not enough to test for the activity and there was reported that it showed no activity on plant growth inhibition (Maneechakr, 1994).