

CHAPTER IV

RESULTS

1. Determination of 50% Cytotoxic concentration and 50% inhibition concentration

The 50 % cytotoxic concentration (IC_{50}) and inhibition concentration (CC_{50}) of all medicinal plant extracts were shown in table 6 and table 7, respectively. The lowest IC_{50} of crude extract was found in *Saussurea lappa* (42.18 $\mu\text{g/ml}$), followed by *Phyllanthus amarus*, *Derris scandens*, *Rhinacanthus nasutus*, *Santalum album*, *Duranta repens*, *Houttuynia cordata*, *Gossypium herbaceum*, *Caesalpinia sappan*, *Homalomena aromatica*, *Loranthus pentandrus*, and *Phyllanthus emblica*, respectively. The IC_{50} of *Litchi chinensis* was over 2000 $\mu\text{g/ml}$. The CC_{50} of *Litchi chinensis*, *Loranthus pentandrus*, and *Phyllanthus emblica* was over 2000 $\mu\text{g/ml}$. *Saussurea lappa* and *Rhinacanthus nasutus* had an CC_{50} of 557.31 and 541 $\mu\text{g/ml}$, respectively. The lowest CC_{50} was found in *Duranta repens* (166.38 $\mu\text{g/ml}$). All of crude extract exhibited selectivity index more than 1 (table 8.). The highest SI was found in *Saussurea lappa* (13.21).

Table 6. *In vitro* IC_{50} of medicinal plant extracts

No.	Plants	IC_{50} ($\mu\text{g/ml}$)
1.	<i>Saussurea lappa</i> (โกฐกระดูก)	42.18
2.	<i>Phyllanthus amarus</i> (ลูกใต้ใบ)	46.72
3.	<i>Derris scandens</i> (เถาว์วัลย์เปรียง)	138.32
4.	<i>Rhinacanthus nasutus</i> (ทองพันชั่ง)	140.48
5.	<i>Santalum album</i> (แก่นจันทน์)	155.88
6.	<i>Duranta repens</i> (เทียนหยด)	159.07
7.	<i>Houttuynia cordata</i> (พลูคาว)	202.44
8.	<i>Gossypium herbaceum</i> (ฝ้าย)	221.31
9.	<i>Caesalpinia sappan</i> (ฝาง)	225.06

Table 6. (continued)

No.	Plants	IC ₅₀ (µg/ml)
10.	<i>Homalomena aromatica</i> (เต่าเกียด)	335.97
11.	<i>Loranthus pentandrus</i> (กาฝากมะม่วง)	486.74
12.	<i>Phyllanthus emblica</i> (มะขามป้อม)	555.26
13.	<i>Litchi chinensis</i> (ลิ้นจี่)	>2000

Table 7. *In vitro* CC₅₀ of medicinal plant extracts.

No.	Plants	CC ₅₀ (µg/ml)
1.	<i>Litchi chinensis</i> (ลิ้นจี่)	>2000
2.	<i>Loranthus pentandrus</i> (กาฝากมะม่วง)	>2000
3.	<i>Phyllanthus emblica</i> (มะขามป้อม)	>2000
4.	<i>Saussurea lappa</i> (โกฐกระดูก)	557.31
5.	<i>Rhinacanthus nasutus</i> (ทองพันชั่ง)	541.00
6.	<i>Homalomena aromatica</i> (เต่าเกียด)	379.75
7.	<i>Caesalpinia sappan</i> (ฝาง)	325.99
8.	<i>Gossypium herbaceum</i> (ฝ้าย)	281.00
9.	<i>Houttuynia cordata</i> (พลูคาว)	238.95
10.	<i>Phyllanthus amarus</i> (ลูกใต้ใบ)	218.27
11.	<i>Derris scandens</i> (เถาวัลย์เปรียง)	186.72
12.	<i>Santalum album</i> (แก่นจันทร์)	172.15
13.	<i>Duranta repens</i> (เทียนหยด)	166.38

Table 8. Selectivity index (SI) [CC₅₀ / IC₅₀]

No.	Plants	SI
1.	<i>Saussurea lappa</i> (โกฐกระดูก)	13.21
2.	<i>Phyllanthus amarus</i> (ลูกใต้ใบ)	4.67
3.	<i>Loranthus pentandrus</i> (กาฝากมะม่วง)	>4.11

Table 8. (continued)

No.	Plants	SI
4.	<i>Rhinacanthus nasutus</i> (ทองพันชั่ง)	3.85
5.	<i>Phyllanthus emblica</i> (มะขามป้อม)	>3.60
6.	<i>Caesalpinia sappan</i> (ฝาง)	1.45
7.	<i>Derris scandens</i> (เถาวัลย์เปรียง)	1.35
8.	<i>Gossypium herbaceum</i> (ฝ้าย)	1.27
9.	<i>Houttuynia cordata</i> (พญาคาว)	1.18
10.	<i>Duranta repens</i> (เทียนหยด)	1.14
11.	<i>Homalomena aromatica</i> (เต่าเกียด)	1.13
12.	<i>Santalum album</i> (แก่นจันทน์)	1.10
13.	<i>Litchi chinensis</i> (ลิ้นจี่)	UD

Three out of thirteen crude extracts which exhibited the lowest IC_{50} were selected for further study. Although the crude extracts from *Phyllanthus amarus* and *Saussurea lappa* had high selectivity index in our study but their active ingredients which exhibited anti-HBsAg activity were already reported by Huang et al. (Huang et al., 2003), and Chen et al. (Chen et al., 1995), respectively. Thus, we continued studying on *Derris scandens*, *Rhinacanthus nasutus*, and *Santalum album*.

These crude extracts were partitioned with hexane, chloroform, ethyl acetate, and butanol, respectively. The CC_{50} and IC_{50} were shown in table 9, and the SI was shown in table 10. The lowest IC_{50} of *Derris scandens*, *Rhinacanthus nasutus*, and *Santalum album* were found in butanol fraction, hexane fraction, and hexane fraction, respectively. In *Derris scandens*, ethyl acetate and butanol fraction had the highest SI. The highest SI of *Rhinacanthus nasutus* and *Santalum album* was found in hexane and chloroform fraction, respectively.

Table 9. *In vitro* CC₅₀ and IC₅₀ of solvent extracts

No.	Plants	Fraction	CC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)
1.	<i>Derris scandens</i> เถาวัลย์เปรียง	Hexane	163.61	119.67
		Chloroform	674.99	498.88
		Ethyl acetate	158.34	68.68
		Butanol	126.53	54.82
2.	<i>Rhinacanthus nasutus</i> ทองพันชั่ง	Hexane	257.87	126.12
		Chloroform	215.58	134.25
		Ethyl acetate	456.25	465.80
		Butanol	>1000	>1000
3.	<i>Santabulum album</i> แก่นจันทน์	Hexane	124.05	74.46
		Chloroform	360.41	194.18
		Ethyl acetate	>500	>1000
		Butanol	>500	>1000

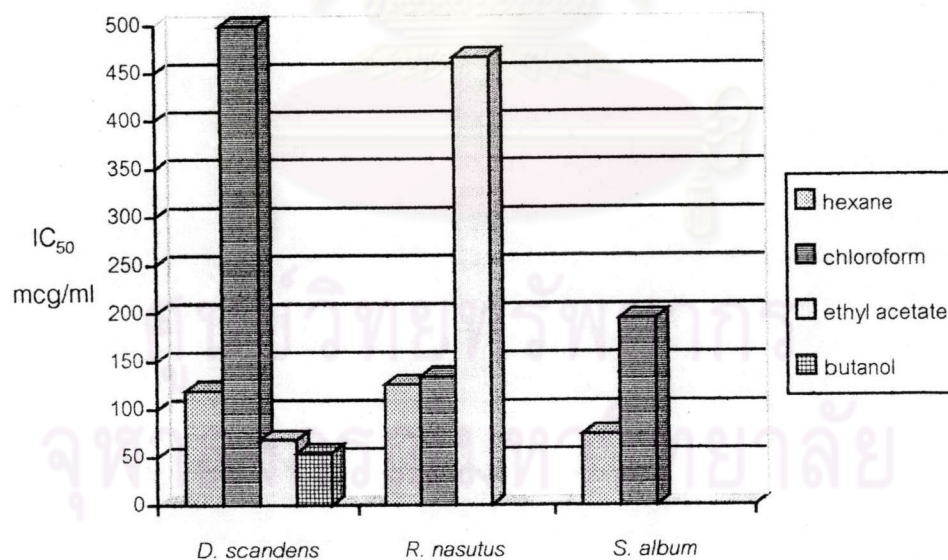
Figure 8. *In vitro* IC₅₀ of solvent extracts

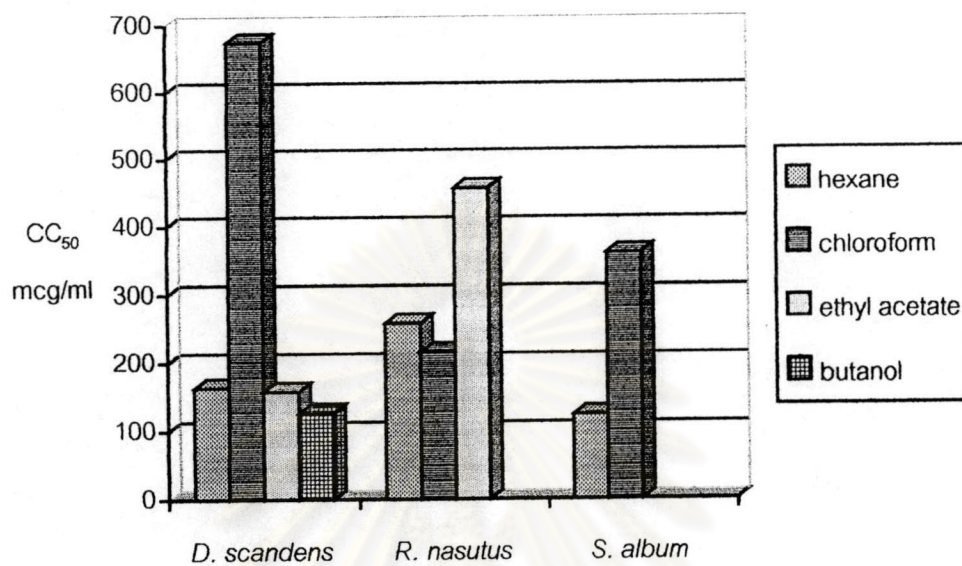
Figure 9. *In vitro* CC_{50} of solvent extracts

Figure 10. SI of solvent extracts

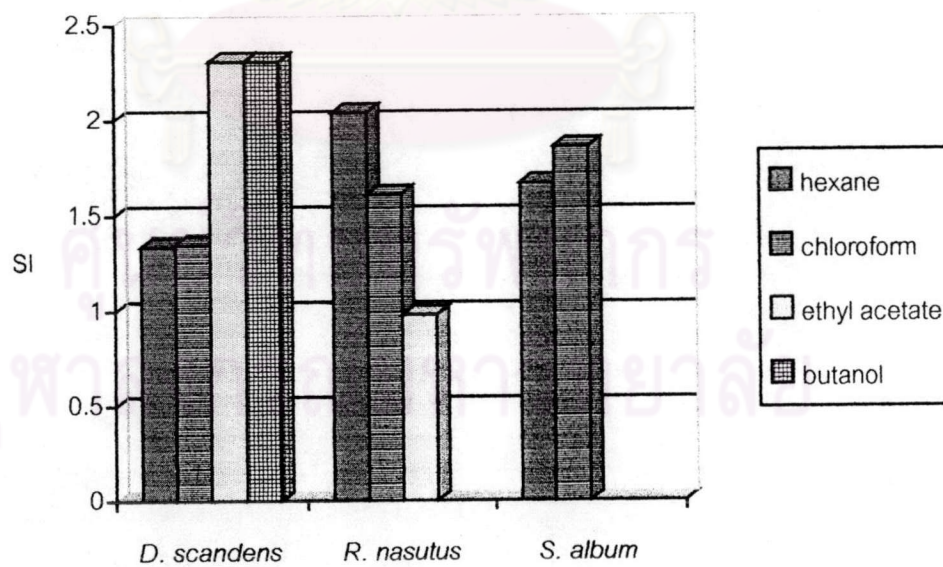


Table 10. SI of solvent extracts

No.	Plants	Fraction	SI
1.	<i>Derris scandens</i> เถาวัลย์เปรียง	Hexane	1.34
		Chloroform	1.35
		Ethyl acetate	2.31
		Butanol	2.31
2.	<i>Rhinacanthus nasutus</i> ทองพันชั่ง	Hexane	2.04
		Chloroform	1.61
		Ethyl acetate	0.98
		Butanol	UD
3.	<i>Santalum album</i> แก่นจันทร์	Hexane	1.67
		Chloroform	1.86
		Ethyl acetate	UD
		Butanol	UD

The fraction that had lowest IC_{50} , from *Derris scandens* and *Rhinacanthus nasutus*, were partially purified with column chromatography. Sixty-five fractions from column chromatography of butanol fraction of *Derris scandens* were received. Each fraction of fraction 1 to fraction 64 was eluted with 20 ml hexane : ethyl acetate (93 : 7). After that, fraction Me (methanol) was eluted with methanol. Eighty-three fractions from column chromatography of hexane fraction of *Rhinacanthus nasutus* were received. Each of fraction 1 to fraction 75 was eluted with 20 ml hexane : ethyl acetate (94 : 6). After that, each of fraction Me1 to fraction Me8 was eluted with 60 ml methanol.

The CC_{50} , IC_{50} , and SI were shown in table 11, 11, and 12, respectively. Fraction 56-64 of *Derris scandens* had CC_{50} lower than IC_{50} . Fraction Me had the highest SI (2.72). In case of *Rhinacanthus nasutus*, fraction 5-7, 8-11, 12-19, 20-29, 30-38, 39-49, and 50-56 had CC_{50} and IC_{50} over 500 $\mu\text{g/ml}$. Fraction 57-Me2 had CC_{50} lower than IC_{50} . The lowest IC_{50} was found in fraction Me5-Me8 and the highest SI was found in fraction Me3-Me4.

Table 11. *In vitro* CC_{50} and IC_{50} of Chromatographic extracts

No.	Plants	Fraction	CC_{50} ($\mu\text{g/ml}$)	IC_{50} ($\mu\text{g/ml}$)
1.	<i>Derris scandens</i> เถาวัลย์เปรียง	28	UD	23.71
		29-35	90.99	48.36
		36-55	118.63	84.53
		56-64	4.59	6.13
		Me	148.49	54.51
2.	<i>Rhinacanthus nasutus</i> ทองพันชั่ง	5-7	>500	>500
		8-11	>500	>500
		12-19	>500	>500
		20-29	>500	>500
		30-38	>500	>500
		39-49	>500	>500
		50-56	>500	>500
		57-Me2	157.36	295.32
		Me3-Me4	408.79	249.80
Me5-Me8	373.77	238.51		

Me, Me1, Me2, etc. = Methanol fraction, Methanol fraction 1, Methanol fraction 2, etc.

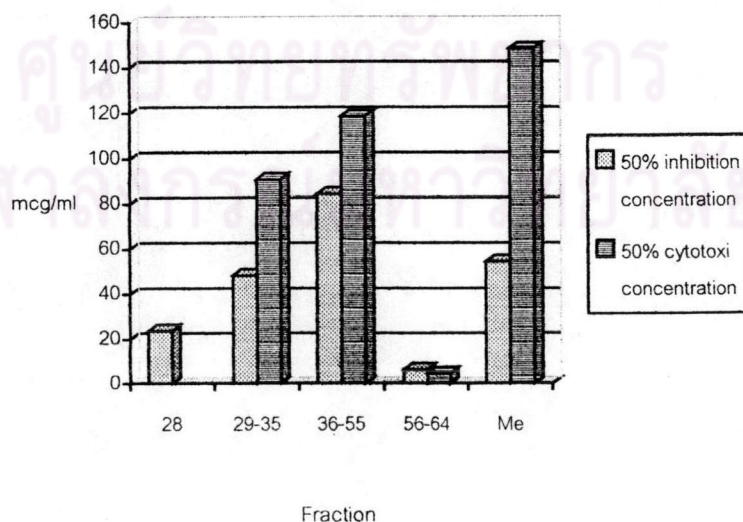
Figure 11. *In vitro* IC_{50} and CC_{50} of Chromatographic extracts of *Derris scandens*

Figure 12. SI of Chromatographic extracts of *Derris scandens*

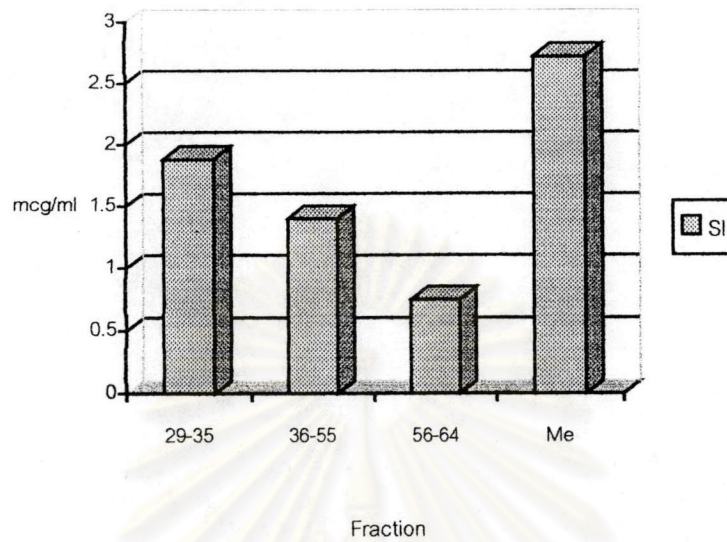


Figure 13. *In vitro* IC₅₀ and CC₅₀ of Chromatographic extracts of *Rhinacanthus nasutus*

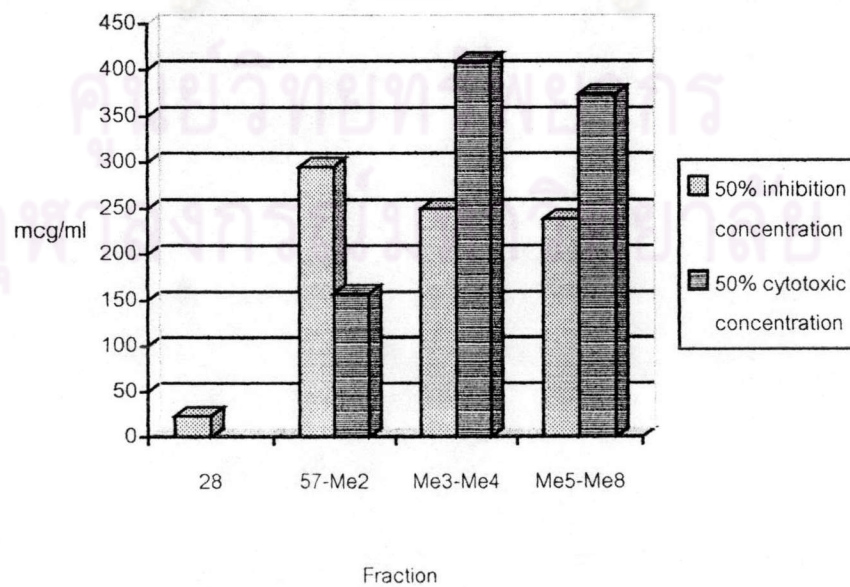


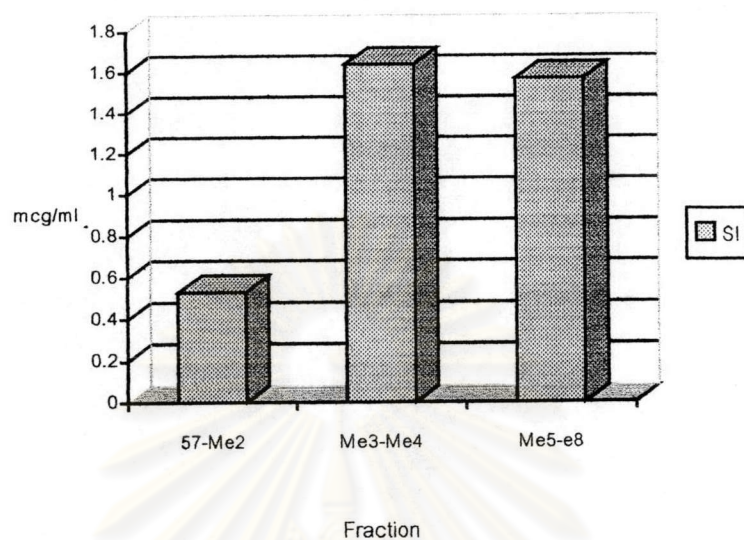
Figure 14. SI of Chromatographic extracts of *Rhinacanthus nasutus*

Table 12. SI of chromatographic extracts

No.	Plants	Fraction	SI
1.	<i>Derris scandens</i> เถาวัลย์เปรียง	28	UD
		29-35	1.88
		36-55	1.40
		56-64	0.75
		Me	2.72
2.	<i>Rhinacanthus nasutus</i> ทองพันชั่ง	5-7	UD
		8-11	UD
		12-19	UD
		20-29	UD
		30-38	UD
		39-49	UD
		50-56	UD
		57-me2	0.53
		Me3-Me4	1.64
		Me5-Me8	1.57

In case of *Rhinacanthus nasutus*, fraction Me5-Me8 was performed with gel filtration chromatography and twenty-five fractions were received by eluted with 15 ml methanol in each fraction.

The CC_{50} and IC_{50} were shown in table 13, and the SI was shown in table 14. Fraction 13-16 had SI less than 1. Fraction 17-18 had IC_{50} of 3.13 $\mu\text{g/ml}$ and SI of 4.51, respectively.

Table 13. *In vitro* CC_{50} and IC_{50} of gel filtration chromatographic extracts

No.	Plants	Fraction	CC_{50} ($\mu\text{g/ml}$)	IC_{50} ($\mu\text{g/ml}$)
1.	<i>Rhinacanthus nasutus</i> ทองพันชั่ง	4 – 12	757.01	>500
		13 – 16	31.29	67.76
		17 – 18	14.11	3.13

Table 14. SI of gel filtration chromatographic extracts

No.	Plants	Fraction	SI
1.	<i>Rhinacanthus nasutus</i> ทองพันชั่ง	4 – 12	UD
		13 – 16	0.46
		17 – 18	4.51

2. Percent yield of extracts

Percent yield of crude extracts were shown in table 15, solvent extracts in table 16, chromatographic extracts in table 17, and gel filtration chromatographic extracts in table 18.

Table 15. Percent yield of crude extracts

No.	Plants	Weight (g)	Extract weight (g)	Yield (%)
1.	<i>Caesalpinia sappan</i> ฝาง	19 (wet)	1.6608	8.74
2.	<i>Derris scandens</i> เถาว์ลยเป็รียง	19.5 (wet)	0.9466	4.85
3.	<i>Duranta repens</i> เทียนหยด	78	3.6472	4.68
4.	<i>Homalomena aromatica</i> เต่าเกียด	10 (dry)	2.0448	20.45
5.	<i>Gossypium herbaceum</i> ฝ้าย	70 (fresh)	2.2935	3.28
6.	<i>Houttuynia cordata</i> พลูคาว	46 (fresh)	0.877	1.93
7.	<i>Litchi chinensis</i> ลิ้นจี่	40 (dry)	4.7442	11.86
8.	<i>Loranthus pentandrus</i> กาฝากมะม่วง	136 (wet)	3.072	2.26
9.	<i>Santalum album</i> แก่นจันทน์	10 (dry)	0.7082	7.08
10.	<i>Phyllanthus amarus</i> ลูกใต้ใบ	65 (wet)	1.9691	3.03
11.	<i>Phyllanthus emblica</i> มะขามป้อม	10 (dry)	3.4652	34.65
12.	<i>Rhinacanthus nasutus</i> ทองพันชั่ง	76 (fresh)	1.6271	2.14
13.	<i>Saussurea lappa</i> โกฐกระดูก	20 (dry)	0.9961	4.98

Table 16. Percent yield of solvent extracts

No.	Plants	Weight (g)	Fraction	Extract weight (g)	Yield (%)
1.	<i>Derris scandens</i> เถาวัลย์เปรียง	1188 (dry)	Hexane	10.7975	0.91
			Chloroform	13.2230	1.11
			Ethyl acetate	2.93222	0.25
			Butanol	1.0741	0.09
2.	<i>Santalum album</i> แก่นจันทร์	894 (dry)	Hexane	3.4336	0.38
			Chloroform	11.3876	1.27
			Ethyl acetate	1.0568	0.12
			Butanol	0.5271	0.06
3.	<i>Rhinacanthus nasutus</i> ทองพันชั่ง	2000 (dry)	Hexane	33.8651	1.69
			Chloroform	3.4284	0.17
			Ethyl acetate	2.5288	0.13
			Butanol	3.5412	0.18

Table 17. Percent yield of chromatographic extracts

No.	Plants	Weight (g)	Fraction	Extract weight (g)	Yield (%)
1.	<i>Derris scandens</i> เถาวัลย์เปรียง	0.6712	24-25	0.0028	0.42
			26-27	0.0076	11.32
			28	0.0224	3.34
			29-35	0.1087	16.19
			36-55	0.0621	9.25
			56-64	0.0810	12.07
			Me	0.1866	27.80

Table 17. (continued)

No.	Plants	Weight (g)	Fraction	Extract weight (g)	Yield (%)
2.	<i>Rhinacanthus nasutus</i> ทองพันชั่ง	15	5-7	0.047	0.31
			8-11	0.1726	1.15
			12-19	0.4274	2.85
			20-29	0.2205	1.47
			30-38	0.1279	0.85
			39-49	0.1313	0.88
			50-56	0.0760	0.51
			57-Me2	0.7169	0.48
			Me3-Me4	1.5464	10.31
	Me5-Me8	1.2876	8.58		

Table 18. Percent yield of gel filtration chromatographic extracts

No.	Plants	Weight (g)	Fraction	Extract weight (g)	Yield (%)
1.	<i>Rhinacanthus nasutus</i> ทองพันชั่ง	0.9415	4-12	0.2930	31.12
			13-16	0.3494	37.11
			17-18	0.0341	3.62

3. Thin layer chromatography

3.1 *Derris scandens*

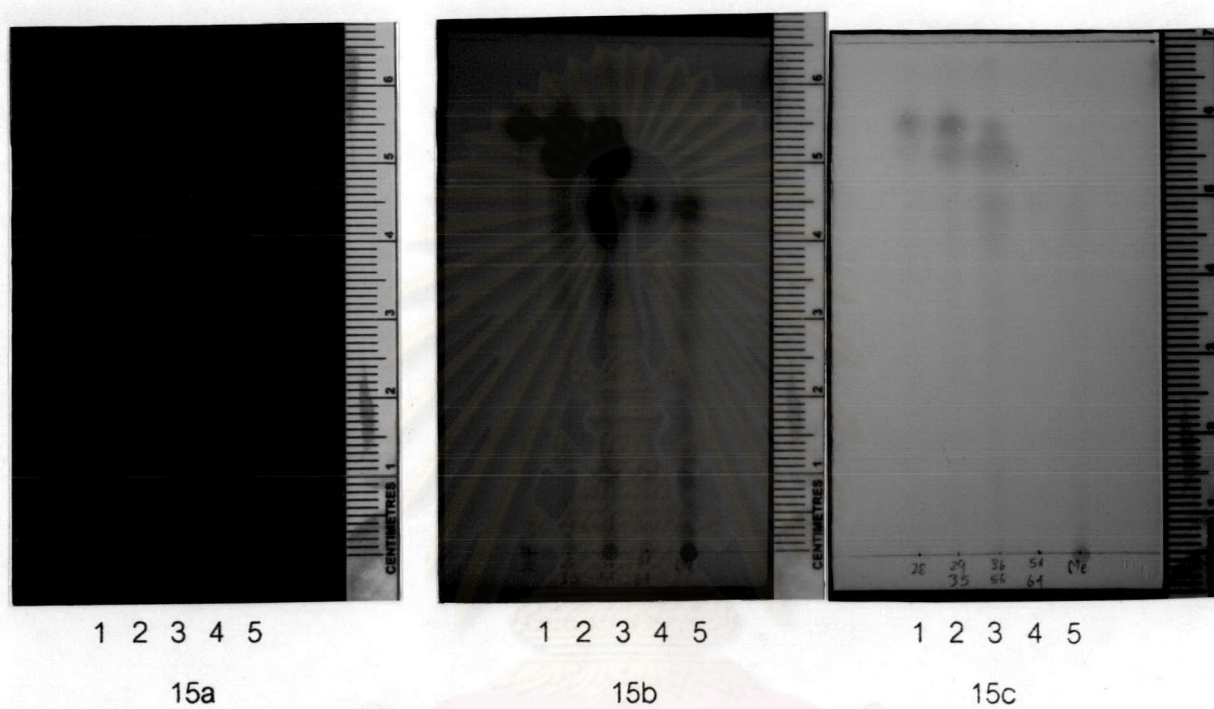


Figure 15. TLC of *Derris scandens* chromatographic extracts (hexane:ethyl acetate, 1:1)

15a = detected with 365 nm ultraviolet

15b = detected with 254 nm ultraviolet

15c = detected with 10% H_2SO_4 in ethanol

1 = fraction 28

2 = fraction 29-35

3 = fraction 36-55

4 = fraction 56-64

5 = fraction methanol

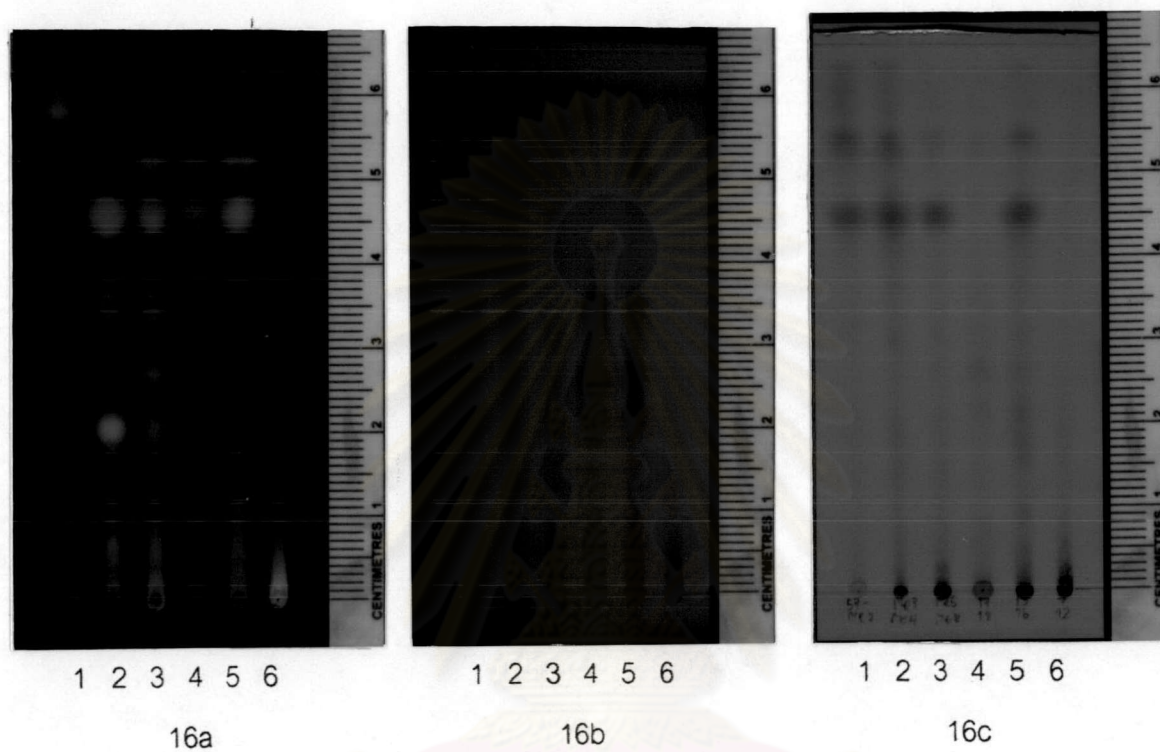
3.2 *Rhinacanthus nasutus*

Figure 16. TLC of *Rhinacanthus nasutus* extracts (hexane:ethyl acetate, 2:1)

16a = detected with 365 nm ultraviolet

16b = detected with 254 nm ultraviolet

16c = detected with 10% H_2SO_4 in ethanol

1 = fraction 57-Me2 of chromatographic extract

2 = fraction Me3-Me4 of chromatographic extract

3 = fraction Me5-Me8 of chromatographic extract

4 = fraction 17-18 of gel filtration extract

5 = fraction 13-16 of gel filtration extract

6 = fraction 4-12 of gel filtration extract

Fractions 28, 29-35, 36-55, 56-64 of *Derris scandens* chromatographic extracts were investigated by TLC (Fig. 15). Spots on this TLC plate were detected with 365 nm ultraviolet, 254 nm ultraviolet, and 10% H₂SO₄ in ethanol. Fraction 28 had obvious spot at Rf of 0.82. Fraction 29-35 had obvious spots at Rf of 0.82, and 0.75. Fraction 36-55 had obvious spots at Rf of 0.82, 0.75, and 0.66. Fractions 56-64 and Me had obvious spot at Rf of 0.66. Fraction Me also had non-movement substances at starting line.

Figure 16 showed TLC of *Rhinacanthus nasutus* chromatographic and gel filtration extracts. Many spots were observed in each fraction.



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