

CHAPTER IV

RESULTS

1. Cytotoxicity Test

The maximal concentration of DMSO that did not affect the cytotoxicity to cell culture was 2% (Vimolmas Lipipun et al., 1999). Therefore, in this study, the final concentration of DMSO in every crude extract solution was not more than 2%.

In this study, cytotoxicity of ACV and crude extracts was determined as CD_{50} . All the CD_{50} in this study were determined according to Reed and Muench (1938) (see appendix). The CD_{50} of ACV was 141.59 $\mu\text{g/ml}$. The CD_{50} of all medicinal plant extracts were showed in Table 4. The lowest CD_{50} of crude extract was 11.09 $\mu\text{g/ml}$ (*Bridelia ovata*, F4 and *Cissus quadrangularis*, F4). There were 11 extracts which exhibited high CD_{50} ($>1,000 \mu\text{g/ml}$). Most of them were F5. Generally, any crude extract exhibiting high CD_{50} would show low cytotoxicity to cell culture and vice versa.

2. Antiviral Activity of Medicinal Plant Extracts and ACV Against HSV-2

Antiviral activity of all crude extracts and ACV was determined as ED_{50} (see appendix). The ED_{50} of each crude extract was showed in Table 5. Anti-HSV-2 activity for each medicinal plant extract was evaluated by selectivity index (SI) which was calculated by dividing the CD_{50} by ED_{50} (del Barrio and Parra, 2000; Rajbhandari et al., 2001) (Table 6). The crude extract with $SI > 1$ ($ED_{50} < CD_{50}$) was an active crude extract. Active extracts with $SI > 10$ (28 fractions, 11 plants) were showed in Table 7 and they were considerable as good extracts (Wyde et al., 1993; del Barrio and Parra, 2000; Rajbhandari et al., 2001). In addition, 27 fractions exhibited low ED_{50} (not more than 45 $\mu\text{g/ml}$). Only F5 of *Cissus quadrangularis* [เพชรสังฆาต], F5 showed higher ED_{50} . These 27 extracts are interesting for further study.

Median effective doses of acyclovir of in inactivation, pre-treatment and post-treatment test were 0.48, 0.52 and 0.72 $\mu\text{g/ml}$, respectively and selective indexes were 294.98, 272.29 and 196.65 $\mu\text{g/ml}$, respectively.



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Table 4 *In vitro* median cytotoxic dose (CD₅₀) of medicinal plant extracts.

No.	Plants (Thai name)	Fraction	CD ₅₀ (µg/ml)	
1	<i>Andrographis paniculata</i> (ฟ้าทะลายโจร)	F1	44.67	
		F2	177.83	
		F3	44.67	
		F4	22.39	
		F5	125.00	
2	<i>Bridelia ovata</i> (มะกา)	F1	39.72	
		F2	22.12	
		F3	89.13	
		F4	11.09	
		F5	707.95	
3	<i>Cissus quadrangularis</i> (เพชรสังฆาต)	F1	177.83	
		F2	88.91	
		F3	89.13	
		F4	11.09	
		F5	> 1000	
4	<i>Citrus reticulata</i> (ส้มเขียวหวาน)	F1	89.13	
		F2	44.67	
		F3	89.13	
		F4	44.67	
		F5	707.95	
5	<i>Clinacanthus siamensis</i> (ลิ้นงูเห่า)	F1	707.95	
		F2	89.13	
		F3	354.81	
		F4	354.81	
		F5	707.95	
6	<i>Cocos nucifera</i> (มะพร้าว)	F1	> 1000	
		F2	> 1000	
		F3	> 1000	
		F4	> 1000	
		F5	> 1000	
7	<i>Costus speciosus</i> (เหงือกดิน)	F1	44.67	
		F2	79.43	
		F3	250.00	
		F4	39.81	
		F5	> 1000	
8	<i>Momordica charantia</i> var. <i>maxima</i> (มะระ)	F1	707.95	
		F2	89.13	
		F3	707.95	
		F4	177.83	
		F5	354.81	
9	<i>Momordica charantia</i> var. <i>minima</i> (มะระขี้นก)	Fruits	F1	177.83
			F2	44.57
			F3	354.81
			F4	89.13
			F5	> 1000
	Seeds	F1	177.83	
		F2	177.83	
		F3	177.83	
		F4	177.83	
		F5	177.83	

Table 4 (continued)

No.	Plants (Thai name)	Fraction	CD ₅₀ (µg/ml)
10	<i>Nephelium lappaceum</i> (เงาะ)	F1	88.91
		F2	177.83
		F3	177.41
		F4	44.67
		F5	158.49
11	<i>Orthosiphon aristatus</i> (หญ้าหนวดแมว)	F1	44.67
		F2	44.56
		F3	22.39
		F4	22.18
		F5	354.81
12	<i>Phyllanthus amarus</i> (ลูกใต้ใบ)	F1	89.13
		F2	79.31
		F3	89.13
		F4	44.67
		F5	354.81
13	<i>Schefflera leucantha</i> (หนุมานประดานก)	F1	79.43
		F2	22.12
		F3	316.23
		F4	22.39
		F5	630.96
14	<i>Thunbergia laurifolia</i> (รางจืด)	F1	353.97
		F2	88.49
		F3	177.41
		F4	11.22
		F5	> 1000
15	<i>Vitis vinifera</i> (องุ่น)	F1	707.95
		F2	177.83
		F3	354.81
		F4	177.83
		F5	354.81
16	<i>Barleria lupulina</i> (เสลดพังพอน)	F1	15.63
		F2	88.91
		F3	354.81
		F4	22.39
		F5	707.95
17	<i>Clinacanthus nutans</i> (พญาเสือ)	F1	354.81
		F2	354.81
		F3	> 1000
		F4	354.81
		F5	> 1000

F1: Ethanol extract; F2: Hexane extract; F3: Aqueous-ethanol extract;
F4: Ethyl acetate extract; F5: Aqueous-ethyl acetate extract

Table 5 *In vitro* median effective dose (ED₅₀) of medicinal plant extracts.

No.	Plants (Thai name)	Fraction	ED ₅₀ (µg/ml)			
			Inactivation	Pre-treatment	Post-treatment	
1	<i>Andrographis paniculata</i> (ฟ้าทะลายโจร)	F1	34.18	38.62	39.71	
		F2	146.15	156.45	NA	
		F3	15.19	23.78	36.12	
		F4	12.80	16.33	22.38	
		F5	72.04	108.32	NA	
2	<i>Bridelia ovata</i> (มะกา)	F1	6.83	NA	NA	
		F2	4.11	NA	NA	
		F3	7.18	63.35	NA	
		F4	0.59	8.06	NA	
		F5	44.69	225.64	354.54	
3	<i>Cissus quadrangularis</i> (เพชรสังฆาต)	F1	15.58	28.18	NA	
		F2	10.56	36.29	57.33	
		F3	11.45	83.80	NA	
		F4	2.98	NA	NA	
		F5	93.50	526.86	NA	
4	<i>Citrus reticulata</i> (ส้มเขียวหวาน)	F1	41.75	47.13	41.40	
		F2	22.26	30.03	30.80	
		F3	47.77	64.21	71.29	
		F4	14.62	20.82	18.47	
		F5	326.48	528.73	481.24	
5	<i>Clinacanthus siamensis</i> (ลิ้นงูเห่า)	F1	29.98	213.00	NA	
		F2	NA	NA	NA	
		F3	21.86	132.92	142.02	
		F4	9.90	127.18	112.06	
		F5	76.95	700.02	NA	
6	<i>Cocos nucifera</i> (มะพร้าว)	F1	NA	NA	NA	
		F2	NA	NA	NA	
		F3	915.54	NA	NA	
		F4	NA	NA	NA	
		F5	NA	NA	NA	
7	<i>Costus speciosus</i> (เอื้องดิน)	F1	3.59	32.99	43.37	
		F2	16.76	42.88	61.09	
		F3	18.71	161.89	184.10	
		F4	1.81	29.26	28.75	
		F5	598.34	NA	NA	
8	<i>Momordica charantia</i> var <i>maxima</i> (มะระ)	F1	615.14	669.24	NA	
		F2	11.57	23.67	16.68	
		F3	374.27	614.73	403.65	
		F4	21.16	27.37	29.54	
		F5	NA	NA	NA	
9	<i>Momordica charantia</i> var <i>minima</i> (มะระขี้นก)	Fruit	F1	40.73	50.12	57.19
			F2	5.95	17.51	20.70
			F3	75.80	124.61	116.63
			F4	15.66	30.97	35.26
			F5	960.46	NA	NA
		Seed	F1	168.65	NA	NA
			F2	118.29	164.39	146.85
			F3	NA	NA	NA
			F4	NA	NA	NA
			F5	NA	NA	NA

Table 5 (continued)

No.	Plants (Thai name)	Fraction	ED ₅₀ (μ g/ml)		
			Inactivation	Pre-treatment	Post-treatment
10	<i>Nephelium lappaceum</i> (เงาะ)	F1	14.11	87.83	NA
		F2	NA	NA	NA
		F3	4.14	18.44	14.93
		F4	2.27	30.56	NA
		F5	5.11	56.57	NA
11	<i>Orthosiphon aristatus</i> (หญ้าหนวดแมว)	F1	7.17	27.93	NA
		F2	3.35	23.27	41.68
		F3	2.23	8.97	NA
		F4	5.92	21.62	NA
		F5	15.72	91.44	246.97
12	<i>Phyllanthus amarus</i> (ลูกใต้ใบ)	F1	11.34	25.27	51.96
		F2	20.91	30.82	50.27
		F3	5.89	29.86	24.03
		F4	1.98	11.98	26.85
		F5	15.56	NA	NA
13	<i>Schefflera leucantha</i> (หนุमानประดานกาย)	F1	11.07	44.60	76.89
		F2	7.63	NA	NA
		F3	118.48	249.29	NA
		F4	4.67	16.75	NA
		F5	235.56	537.00	618.48
14	<i>Thunbergia laurifolia</i> (รางจืด)	F1	27.90	248.51	282.82
		F2	12.53	74.98	NA
		F3	43.10	138.64	NA
		F4	1.77	5.45	NA
		F5	32.15	393.86	593.96
15	<i>Vitis vinifera</i> (องุ่น)	F1	31.77	304.16	327.03
		F2	113.74	NA	NA
		F3	27.40	261.29	NA
		F4	20.56	NA	NA
		F5	10.66	332.23	NA
16	<i>Barleria lupulina</i> (เสลดพังพอน)	F1	0.02	8.73	NA
		F2	1.25	22.89	67.57
		F3	40.49	200.46	NA
		F4	6.87	NA	NA
		F5	163.54	NA	NA
17	<i>Clinacanthus nutans</i> (พญาขอ)	F1	61.39	NA	NA
		F2	55.27	NA	NA
		F3	202.72	NA	NA
		F4	28.10	NA	NA
		F5	NA	NA	NA

NA: no activity in subcytotoxic concentration or maximum concentration tested
(1,000 μ g/ml)

F1: Ethanol extract; F2: Hexane extract; F3: Aqueous-ethanol extract;

F4: Ethyl acetate extract; F5: Aqueous-ethyl acetate extract

Table 6 Selectivity index (SI) [CD_{50} / ED_{50}]

No.	Plants (Thai name)	Fraction	SI			
			Inactivation	Pre-treatment	Post-treatment	
1	<i>Andrographis paniculata</i> (ฟ้าทะลายโจร)	F1	1.31	1.16	1.12	
		F2	1.22	1.14	ND	
		F3	2.94	1.88	1.24	
		F4	1.75	1.37	1.00	
		F5	1.74	1.15	ND	
2	<i>Bridelia ovata</i> (มะกา)	F1	5.82	ND	ND	
		F2	5.38	ND	ND	
		F3	12.42	1.41	ND	
		F4	18.95	1.38	ND	
		F5	15.84	3.14	2.00	
3	<i>Cissus quadrangularis</i> (เพชรสังฆาต)	F1	11.41	6.31	ND	
		F2	8.42	2.45	1.55	
		F3	7.78	1.06	ND	
		F4	3.72	ND	ND	
		F5	> 10.70	> 1.90	ND	
4	<i>Citrus reticulata</i> (ส้มเขียวหวาน)	F1	2.13	1.89	2.15	
		F2	2.01	1.49	1.45	
		F3	1.87	1.39	1.25	
		F4	3.06	2.15	2.42	
		F5	2.17	1.34	1.47	
5	<i>Clinacanthus siamensis</i> (ลิ้นงูเห่า)	F1	23.61	3.32	ND	
		F2	ND	ND	ND	
		F3	16.23	2.67	2.50	
		F4	35.84	2.79	3.17	
		F5	9.20	1.01	ND	
6	<i>Cocos nucifera</i> (มะพร้าว)	F1	ND	ND	ND	
		F2	ND	ND	ND	
		F3	> 1.09	ND	ND	
		F4	ND	ND	ND	
		F5	ND	ND	ND	
7	<i>Costus speciosus</i> (เอื้องดิน)	F1	12.45	1.35	1.03	
		F2	4.74	1.85	1.30	
		F3	13.36	1.54	1.36	
		F4	21.96	1.36	1.38	
		F5	>1.67	ND	ND	
8	<i>Momordica charantia</i> var <i>maxima</i> (มะระ)	F1	1.15	1.06	ND	
		F2	7.70	3.77	5.34	
		F3	1.89	1.15	1.75	
		F4	8.40	6.50	6.02	
		F5	ND	ND	ND	
9	<i>Momordica charantia</i> var <i>minima</i> (มะระขี้นก)	Fruit	F1	4.37	3.55	3.11
			F2	7.49	2.55	2.15
			F3	4.68	2.85	3.04
			F4	5.69	2.88	2.53
			F5	> 1.04	ND	ND
		Seed	F1	1.05	ND	ND
			F2	1.50	1.08	1.21
			F3	ND	ND	ND
			F4	ND	ND	ND
			F5	ND	ND	ND

Table 6 (continued)

No.	Plants (Thai name)	Fraction	SI		
			Inactivation	Pre-treatment	Post-treatment
10	<i>Nephelium lappaceum</i> (เงาะ)	F1	6.30	1.01	ND
		F2	ND	ND	ND
		F3	42.85	9.62	11.88
		F4	19.68	1.46	ND
		F5	31.02	2.80	ND
11	<i>Orthosiphon aristatus</i> (หญ้าหนวดแมว)	F1	6.23	1.60	ND
		F2	13.30	1.91	1.07
		F3	10.04	2.50	ND
		F4	3.75	1.03	ND
		F5	22.57	3.88	1.44
12	<i>Phyllanthus amarus</i> (ลูกใต้ใบ)	F1	7.86	3.53	1.72
		F2	3.79	1.58	1.58
		F3	15.13	2.98	3.71
		F4	22.59	3.73	1.66
		F5	22.81	ND	ND
13	<i>Schefflera leucantha</i> (หนุมานประสานกาย)	F1	7.18	1.78	1.03
		F2	2.90	ND	ND
		F3	2.67	1.27	ND
		F4	4.79	1.34	ND
		F5	2.68	1.17	1.02
14	<i>Thunbergia laurifolia</i> (รางจืด)	F1	12.69	1.42	1.25
		F2	7.06	1.18	ND
		F3	4.12	1.28	ND
		F4	6.34	2.06	ND
		F5	>31.10	>2.54	>1.68
15	<i>Vitis vinifera</i> (องุ่น)	F1	22.28	2.33	2.16
		F2	1.56	ND	ND
		F3	12.95	1.36	ND
		F4	8.65	ND	ND
		F5	33.28	1.07	ND
16	<i>Barleria lupulina</i> (เสลดพังพอน)	F1	781.50	1.79	ND
		F2	71.13	3.88	1.32
		F3	8.76	1.77	ND
		F4	3.26	ND	ND
		F5	4.33	ND	ND
17	<i>Clinacanthus nutans</i> (พญาขอ)	F1	5.78	ND	ND
		F2	6.42	ND	ND
		F3	> 4.93	ND	ND
		F4	12.63	ND	ND
		F5	ND	ND	ND

ND: cannot be determined; SI: selective index; F1: Ethanol extract; F2: Hexane extract; F3: Aqueous-ethanol extract; F4: Ethyl acetate extract; F5: Aqueous-ethyl acetate extract

Table 7 Active extracts with SI > 10

Plants	Fraction	SI	
		Inactivation	Post-treatment
<i>Barleria lupulina</i> [เสลดพังพอน]	F1	781.5	
<i>Barleria lupulina</i> [เสลดพังพอน]	F2	71.13	
<i>Nephelium lappaceum</i> [เงาะ]	F3	42.85	11.88
<i>Clinacanthus siamensis</i> [ลิ้นงูเห่า]	F4	35.84	
<i>Vitis vinifera</i> [องุ่น]	F5	33.28	
<i>Thunbergia laurifolia</i> [รางจืด]	F5	>31.10	
<i>Nephelium lappaceum</i> [เงาะ]	F5	31.02	
<i>Clinacanthus siamensis</i> [ลิ้นงูเห่า]	F1	23.61	
<i>Phyllanthus amarus</i> [ลูกใต้ใบ]	F5	22.81	
<i>Phyllanthus amarus</i> [ลูกใต้ใบ]	F4	22.59	
<i>Orthosiphon aristatus</i> [หญ้าหนวดแมว]	F5	22.57	
<i>Vitis vinifera</i> [องุ่น]	F1	22.28	
<i>Costus speciosus</i> [เอื้องดิน]	F4	21.96	
<i>Nephelium lappaceum</i> [เงาะ]	F4	19.68	
<i>Bridelia ovata</i> [มะกา]	F4	18.95	
<i>Clinacanthus siamensis</i> [ลิ้นงูเห่า]	F3	16.23	
<i>Bridelia ovata</i> [มะกา]	F5	15.84	
<i>Phyllanthus amarus</i> [ลูกใต้ใบ]	F3	15.13	
<i>Costus speciosus</i> [เอื้องดิน]	F3	13.36	
<i>Orthosiphon aristatus</i> [หญ้าหนวดแมว]	F2	13.30	
<i>Vitis vinifera</i> [องุ่น]	F3	12.95	
<i>Thunbergia laurifolia</i> [รางจืด]	F1	12.69	
<i>Clinacanthus nutans</i> [พญาสัต]	F4	12.63	
<i>Costus speciosus</i> [เอื้องดิน]	F1	12.45	
<i>Bridelia ovata</i> [มะกา]	F3	12.42	
<i>Cissus quadrangularis</i> [เพชรสังฆาต]	F1	11.41	
<i>Cissus quadrangularis</i> [เพชรสังฆาต]	F5	> 10.70	
<i>Orthosiphon aristatus</i> [หญ้าหนวดแมว]	F3	10.04	

F1: ethanol extract; F2: hexane extract; F3: aqueous-ethanol extract; F4: ethyl acetate extract; F5: aqueous-ethyl acetate extract.

3. Percent Yield of Crude Extracts

Table 8 Percent Yield of crude extracts

No.	Plants [Thai name]	Fresh plant weight (g)	Fraction	Extract weight (g)	Yield (%)
1	<i>Andrographis paniculata</i> [ฟ้าทะลายโจร]	112.14	F1	13.81	12.31
			F2	0.80	0.71
			F3	1.37	1.22
			F4	4.86	4.33
			F5	6.37	5.68
2	<i>Bridelia ovata</i> [มะกา]	534.89	F1	36.94	6.91
			F2	11.42	2.14
			F3	4.50	0.84
			F4	3.21	0.60
			F5	15.70	2.94
3	<i>Cissus quadrangularis</i> [เพชรสังฆาต]	419.13	F1	14.92	3.56
			F2	0.73	0.17
			F3	0.49	0.12
			F4	0.28	0.07
			F5	1.59	0.38
4	<i>Citrus reticulata</i> [ส้มเขียวหวาน]	759.00	F1	70.50	3.56
			F2	3.98	0.17
			F3	5.70	0.12
			F4	8.44	0.07
			F5	40.95	0.38
5	<i>Clinacanthus siamensis</i> [ลิงนู่เห่า]	254.55	F1	18.08	7.10
			F2	0.81	0.32
			F3	2.82	1.11
			F4	3.74	1.47
			F5	6.18	2.43
6	<i>Cocos nucifera</i> [มะพร้าว]	524.21	F1	66.36	12.66
			F2	43.19	8.24
			F3	5.19	0.99
			F4	5.75	1.10
			F5	10.45	1.99
7	<i>Costus speciosus</i> [เอื้องดิน]	733.30	F1	6.14	0.84
			F2	0.91	0.12
			F3	0.91	0.12
			F4	0.75	0.10
			F5	3.32	0.45

Table 8 (continued)

No.	Plants [Thai name]	Fresh plant weight (g)	Fraction	Extract weight (g)	Yield (%)
8	<i>Momordica charantia</i> var. <i>maxima</i> [มะระ]	461.00	F1	9.50	2.06
			F2	0.12	0.03
			F3	0.76	0.16
			F4	0.55	0.12
			F5	7.39	1.60
9	<i>Momordica charantia</i> var. <i>minima</i> [มะระขี้นก] Fruit	516.50	F1	14.10	2.73
			F2	0.33	0.06
			F3	1.85	0.36
			F4	2.10	0.41
			F5	9.11	1.76
	Seed	15.60	F1	2.06	13.21
			F2	0.09	0.58
			F3	0.17	1.09
			F4	0.12	0.77
			F5	1.40	8.97
10	<i>Nephelium lappaceum</i> [เงาะ]	690.50	F1	20.60	2.98
			F2	0.96	0.14
			F3	3.68	0.53
			F4	5.60	0.81
			F5	4.04	0.59
11	<i>Orthosiphon aristatus</i> [หญ้าหนวดแมว]	177.20	F1	9.26	5.23
			F2	1.99	1.12
			F3	1.47	0.83
			F4	1.87	1.06
			F5	2.28	1.29
12	<i>Phyllanthus amarus</i> [ลูกใต้ใบ]	417.00	F1	22.85	5.48
			F2	3.87	0.93
			F3	1.53	0.37
			F4	5.15	1.24
			F5	7.00	1.68
13	<i>Schefflera leucantha</i> [หนุ่ฆานประสาณกาย]	447.90	F1	23.31	5.20
			F2	3.41	0.76
			F3	3.33	0.74
			F4	2.55	0.57
			F5	11.64	2.60
14	<i>Thunbergia laurifolia</i> [รางจืด]	34.30	F1	0.55	1.60
			F2	0.03	0.09
			F3	0.09	0.26
			F4	0.07	0.20
			F5	0.26	0.76

Table 8 (continued)

No.	Plants [Thai name]	Fresh plant weight (g)	Fraction	Extract weight (g)	Yield (%)
15	<i>Vitis vinifera</i> [องุ่น]	28.00	F1	6.11	21.82
			F2	0.07	0.25
			F3	1.68	6.00
			F4	2.08	7.43
			F5	2.28	8.14
16	<i>Barleria lupulina</i> [เสลดพังพอน]	157.57	F1	16.60	10.54
			F2	2.53	1.61
			F3	2.18	1.38
			F4	2.47	1.57
			F5	9.33	5.92
17	<i>Clinacanthus nutans</i> [พญาายอ]	156.62	F1	8.43	5.38
			F2	1.14	0.73
			F3	0.78	0.50
			F4	1.84	1.17
			F5	4.49	2.87

F1: Ethanol extract; F2: Hexane extract; F3: Aqueous-ethanol extract; F4: Ethyl acetate extract; F5: Aqueous-ethyl acetate extract

Percent yield of all crude extracts were 0.03-21.82% of fresh plant weight. Almost fractions that provided high yield were F1 and F5 (higher polarity than others).

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