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# APPENDIX I

## Chemical agents and Instruments

### A. Chemical agents

- Absolute Methanol GR (E. Merck, Darmstadt., Germany)
- BSA ( Bovine Serum Albumin) (Sigma, MO, U.S.A.)
- Butanol (E. Merck, Darmstadt., Germany)
- Citric acid (E. Merck, Darmstadt., Germany)
- Chloroform (Sigma, MO, U.S.A.)
- Crystal violet (Fluka, Switzerland)
- DMSO (dimethyl sulfoxide ) (Sigma, MO, U.S.A.)
- Formaldehyde 40% w/v AR (Carlo Erba, Milano, Italy)
- Hexane (E. Merck, Darmstadt., Germany)
- Potassium chloride (KCl) (M & B , England)
- Potassium dihydrogen phosphate GR ( $\text{KH}_2\text{PO}_4$ )(E. Merck, Darmstadt., Germany)
- Dipotassium hydrogen phosphate GR ( $\text{K}_2\text{HPO}_4$ ) (M & B , England)
- Sodium bicarbonate ( $\text{Na}_2\text{HCO}_3$ ) AR (E. Merck, Darmstadt., Germany)
- Sodium nitrate ( $\text{NaN}_3$ ) GR (M & B , England)
- Disodium carbonate GR ( $\text{Na}_2\text{CO}_3$ ) (M & B , England)
- Disodium hydrogen carbonate GR ( $\text{Na}_2\text{HCO}_3$ ) (M & B , England)
- Disodium hydrogen phosphate AR ( $\text{Na}_2\text{HPO}_4$  anhydrous) (M & B , England)
- Sulfuric acid (Sigma, MO, U.S.A.)
- Tragacanth ( Pharmaceutical chemicals, Denmark)
- Trypsin - EDTA (10X 0.5/0.2% w/v) (Seromed, Germany)
- Tween 20 (E. Merck, Darmstadt., Germany)

**B. Instruments**

- Analytical balance (Satorious, Germany)
- Automatic pipette, P10-100 / P50-200 (Socorex, Switzerland)
- Centrifuge (Sigma , Germany)
- Hemocytometer (Spenser, U.S.A.)
- Laminar Air Flow (Holten, U.S.A.)
- Mixer Vortex (Scientific, NY, U.S.A.)
- Multichannel automatic pipette (8 channels) P10-100 / P50-200  
(Socorex, Switzerland)
- pH meter (Beckman, U.S.A.)
- Refrigerator 4°C (Sharp, Thailand)
- Refrigerator -20°C (Ariston, U.S.A.)
- Refrigerator -80°C ( Forma Scientific, Ohio, U.S.A.)
- Water bath (Thelco, U.S.A.)

**C. Laboratory supplies**

- Cryotubes 1.8 ml (NUNC, Denmark)
- Glassware (Pyrex, U.S.A.)
- Insulin syringes with needle 1 ml ( Terumo, Tokyo, Japan)
- Microcentrifuge tubes 1.5 ml
- Microtiter plates (NUNC, Denmark)
- Millipore filters (0.2  $\mu\text{m}$ ) (Gelman Sciences, U.S.A.)
- Pipette tips for P10-100 / P50-200 (Treff Lab, Switzerland)
- Tissue culture flasks (NUNC, Denmark)
- Tissue culture plates (NUNC, Denmark)

## APPENDIX II

### MEDIUM AND REAGENTS

#### 1. Vero Cell Growth Medium (Modified Eagle Medium, MEM)

##### MEM

Dissolved 1 packet of MEM with Earle's salt, with L-glutamine, without  $\text{NaHCO}_3$  (Gibco, U.S.A.) in 1,000 ml of sterilized deionized water, mixed well and adjusted pH to 7.2 - 7.4 with 10%  $\text{NaHCO}_3$ , then, sterilized by filtration (0.20  $\mu\text{m}$  millipore)

Preparation before use (with 10%FCS and 1% antibiotic)

MEM	100	ml
Fetal Calf serum	10	ml
Antibiotic (Gibco, U.S.A., containing Penicillin G 10,000 units/ml, Streptomycin 10,000 $\mu\text{g}/\text{ml}$ and Fungizone 1,000 $\mu\text{g}/\text{ml}$ )	1	ml

#### 2. Vero Cell Maintenance Medium

This is similar to growth medium except the amount of fetal calf serum is reduced to 2 %.

#### 3. Freezing Medium

MEM	70	ml
Fetal Calf serum	20	ml
Dimethyl sulfoxide	10	ml

#### 4. Transport Medium

This is similar to growth medium except the amount of antibiotic is added to 2%.

#### 5. Phosphate Buffer Saline Solution (PBS), pH 7.5

NaCl	8.00	gm
KCl	0.20	gm
KH <sub>2</sub> PO <sub>4</sub>	0.20	gm
Na <sub>2</sub> HPO <sub>4</sub>	1.15	gm
Deionized distilled water to	1,000	ml

This solution is sterilized by autoclave at 15 lb/sq inch pressure for 30 minutes.

#### 6. Trypsin - EDTA (1X)

Trypsin - EDTA (10X)	10	ml
PBS	90	ml

#### 7. Plaque Overlay Medium

##### Solution A

2x MEM with 20% FCS and 2% antibiotic

##### Solution B

Tragacanth (Pharmaceutical chemicals, Denmark)	2	g
Deionized distilled water	100	ml

The solution A and solution B are mixed at a ratio of 1 : 1 before use.

## Reagent in ELISA

### 1. Carbonate Buffer, pH 9.6

$\text{Na}_2\text{CO}_3$	1.59	gm
$\text{Na}_2\text{HCO}_3$	2.93	gm
$\text{NaN}_3$	0.20	gm
Distilled water to	1,000	ml

This solution should be stored in the refrigerator for not more than 2 weeks.

### 2. 0.1 M Citric acid- Phosphate Buffer, pH 5.0

Citric acid $\cdot$ $\text{H}_2\text{O}$	7.30	gm
$\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$	23.88	gm
Distilled water to	1,000	ml

### Working Solution

Blocking solution : PBS with 2% bovine serum albumin (BSA)

Washing solution : PBS-T (PBS with 0.05% Tween 20)

Diluent : PBS-T with 0.5%BSA

### 3. Substrate

Ortho-phenylenediamine (OPD)	2	gm
0.1 M Citric acid- Phosphate Buffer, pH 5.0	3	ml

After the OPD was completely dissolved, 5  $\mu\text{l}$  of 30%  $\text{H}_2\text{O}_2$  was added. This solution must be used immediately and protected from light.



## APPENDIX III

ED<sub>50</sub> of medicinal plant extracts and ACV against HSV-2 isolates

NO.	isolate	Carbas odorum (µg/ml)				Clusia roseata (µg/ml)			
		F1	F2	F4	F5	F1	F2	F4	F5
1	A4	7.50	10.75	7.25	3.88	11.25	9.25	23.00	19.80
2	A5	8.50	9.75	7.00	4.50	12.50	8.50	22.00	17.88
3	A6	14.50	12.25	7.25	5.25	15.38	11.00	31.00	20.00
4	A8	14.25	12.75	7.63	6.25	17.50	7.50	29.50	22.00
5	A13	15.75	12.50	9.00	6.63	15.50	8.13	24.00	17.00
6	A17	8.75	10.25	7.75	3.75	13.25	8.88	21.50	22.00
7	A18	8.75	9.75	6.75	3.25	15.50	10.25	22.50	21.25
8	A19	10.50	11.00	6.75	5.25	17.75	10.00	29.88	23.50
9	A23	11.75	11.75	7.63	6.50	12.75	6.00	29.75	26.00
10	A24	10.75	9.63	7.53	5.00	14.25	9.63	25.00	23.38
11	A26	10.50	10.00	7.63	4.50	13.00	8.88	21.50	21.25
12	A29	10.00	9.75	6.75	5.00	14.00	8.88	23.00	24.00
13	A32	9.25	10.25	6.25	6.00	11.13	8.75	23.00	18.63
14	A33	11.25	10.38	7.68	5.63	11.75	9.50	21.50	21.88
15	A34	9.75	10.00	7.00	4.75	15.13	9.25	23.25	24.00
16	A35	9.50	10.75	7.75	5.50	13.75	8.75	21.50	21.00
17	A36	9.50	9.50	8.13	6.63	16.88	7.63	19.25	18.38
18	A43	10.75	10.75	8.25	5.38	12.50	7.83	22.00	20.50
19	A47	12.75	11.25	7.25	5.75	13.00	6.50	19.25	27.50
20	A51	12.00	11.50	9.25	5.88	11.00	6.63	19.75	21.00
21	A59	10.00	8.50	6.63	5.00	12.50	8.25	23.00	24.00
22	A33.1	10.75	10.88	7.75	5.00	11.75	8.50	21.88	20.50
23	B1C8	9.50	10.50	5.88	3.25	13.75	9.13	21.25	21.25
24	TR3	9.00	10.00	7.25	4.25	14.00	8.75	21.00	21.50
25	TR4	9.25	9.50	7.63	3.88	11.00	9.13	19.75	19.00
26	BC2	10.25	10.75	8.00	4.75	13.25	9.13	20.25	20.50
27	BC4	11.25	11.88	8.00	4.75	11.63	8.00	21.50	20.00
28	BC8	9.50	10.25	7.50	3.25	13.50	6.63	22.75	20.75
29	C1	7.75	10.00	8.25	4.25	11.75	8.50	21.88	17.13
30	C3	8.75	10.00	7.50	4.25	13.75	8.75	23.50	16.25
31	C5	9.13	10.75	7.75	5.25	19.25	9.50	23.75	20.13
32	C7	10.25	10.38	8.00	6.00	16.25	8.63	23.75	21.00
33	C8	13.38	11.75	7.00	4.25	13.00	9.25	22.50	17.88
34	C13	11.00	11.75	9.00	5.88	14.25	8.50	20.00	19.50
35	C15	8.00	10.25	5.50	4.75	16.25	10.25	27.50	19.75
36	C17	8.50	6.63	6.25	4.63	11.00	8.25	21.25	17.13
37	C18	9.25	10.50	7.25	6.13	12.75	9.50	22.50	21.25
38	C22	9.50	10.00	7.75	5.88	16.25	8.50	22.88	22.50
sum 1-38		391.25	401.75	282.63	189.63	623.63	340.63	673.00	790.63
mean		10.30	10.57	7.44	4.99	13.78	8.97	22.97	20.61
range		7.50-15.75	8.63-12.75	6.25-9.25	3.25-6.63	11.00-18.25	7.50-11.00	19.25-29.88	17.00-27.50
SD		1.87	0.92	0.75	0.91	2.05	0.78	2.93	2.46

NO.	Inoculae	<i>Colus ambolizus</i> (µg/ml)				<i>Phylo nodifera</i> (µg/ml)			
		F1	F2	F4	F5	F1	F2	F4	F5
1	A4	16.60	2.60	10.00	3.83	18.25	23.38	18.25	18.75
2	A5	17.00	2.75	9.00	4.13	22.50	22.25	16.60	23.75
3	A6	18.00	3.25	11.25	3.50	20.38	30.00	18.13	17.60
4	A9	16.60	4.25	11.25	4.00	24.38	24.00	21.60	16.25
5	A13	19.60	4.60	13.00	3.25	28.13	28.75	19.75	13.25
6	A17	16.68	2.75	12.25	3.89	21.68	23.25	23.75	21.60
7	A18	20.13	4.00	13.00	4.25	25.38	22.75	22.60	23.25
8	A19	21.13	4.00	13.75	3.50	20.75	35.00	18.75	21.25
9	A23	21.25	4.60	12.60	3.25	21.75	35.00	22.00	22.13
10	A24	19.00	4.75	12.25	4.60	26.13	28.75	24.75	21.00
11	A29	17.88	2.75	12.25	3.38	20.38	28.00	28.25	15.60
12	A29	19.25	4.00	13.75	4.00	22.75	24.60	23.25	22.60
13	A32	20.00	4.00	14.00	4.13	23.75	28.00	20.60	23.75
14	A33	17.88	4.60	13.75	3.25	19.75	28.13	24.75	21.25
15	A34	19.60	4.00	16.60	3.38	21.25	22.75	26.25	23.25
16	A35	17.60	3.60	16.25	3.25	19.75	28.75	23.75	20.63
17	A36	17.13	3.38	16.25	3.60	22.68	35.00	22.25	19.00
18	A43	17.63	4.25	14.60	4.60	22.75	23.75	26.25	22.25
19	A47	18.00	3.60	14.25	3.75	20.25	31.25	21.75	22.00
20	A51	20.88	6.00	15.00	3.60	24.38	28.25	27.60	23.75
21	A58	18.25	3.60	12.25	3.63	24.00	23.75	18.60	22.60
22	A33.1	17.00	4.60	10.25	4.13	22.13	28.75	18.75	22.75
23	81C6	19.13	2.60	13.75	2.60	23.00	25.00	16.60	22.38
24	TR3	18.75	4.00	13.75	3.00	18.75	27.60	18.25	16.13
25	TR4	16.60	3.60	15.13	3.25	21.25	21.60	16.68	22.25
26	8C2	17.75	3.00	15.60	3.25	19.60	25.68	17.60	23.60
27	8C4	15.13	2.75	12.25	3.00	19.38	27.60	17.00	16.25
28	8C5	20.75	2.60	13.25	3.60	21.25	24.60	20.25	22.75
29	C1	17.38	2.13	12.00	3.00	20.75	23.60	17.38	21.25
30	C3	17.60	3.60	12.25	2.88	21.25	32.25	17.60	17.60
31	C5	17.75	3.00	12.60	3.75	19.60	32.60	20.75	21.60
32	C7	19.13	3.13	15.13	3.00	18.60	27.60	18.00	19.38
33	C9	20.25	2.75	11.13	3.50	24.38	27.60	23.75	22.60
34	C13	22.00	5.13	11.13	6.25	23.60	34.13	21.00	25.00
35	C15	23.00	3.75	11.60	3.75	23.75	28.25	22.00	22.75
36	C17	20.25	3.00	11.25	4.60	21.75	23.75	22.60	21.60
37	C18	20.60	3.25	14.00	3.75	24.38	25.00	21.00	24.75
38	C22	21.00	3.60	9.75	2.75	23.60	33.60	18.25	27.13
sum 38		706.60	135.60	481.60	136.68	635.68	1035.75	794.13	803.25
mean		18.64	3.67	12.60	3.60	22.00	27.25	20.90	21.14
range		7.60-16.75	2.13-6.13	9.75-16.25	2.50-6.25	16.25-26.13	21.60-35.00	16.25-27.13	13.25-27.13
SD		1.95	0.77	1.86	0.68	2.38	3.90	3.14	3.01

NO.	isolate	<i>Thevitia peruviana</i> (ug/ml)				ACV (ug/ml)
		F1	F2	F4	F5	
1	A4	10.63	2.13	10.75	11.75	0.63
2	A5	10.00	2.75	11.00	11.00	0.60
3	A6	10.25	2.38	12.50	13.25	0.67
4	A8	8.25	2.50	12.25	12.50	0.45
5	A13	12.50	3.50	12.00	12.50	0.63
6	A17	9.00	2.25	13.00	13.00	0.38
7	A18	8.25	3.63	12.00	12.75	0.63
8	A19	9.50	2.50	12.75	13.63	0.46
9	A23	10.63	3.00	13.50	13.50	0.65
10	A24	8.50	3.25	13.50	13.75	0.68
11	A25	11.75	3.00	12.50	13.00	0.72
12	A29	10.75	2.50	10.50	12.25	0.69
13	A32	11.25	3.50	11.50	12.00	0.73
14	A33	12.00	3.50	13.00	13.25	0.53
15	A34	10.50	3.00	12.50	12.50	0.61
16	A35	13.00	4.25	13.50	13.75	0.60
17	A36	13.25	3.75	13.25	13.00	0.47
18	A43	13.00	3.50	13.00	13.75	0.68
19	A47	9.25	2.75	11.50	12.63	0.51
20	A51	8.75	3.00	12.25	12.00	0.60
21	A58	10.50	2.50	12.00	12.50	0.67
22	A33.1	11.75	5.00	12.75	13.00	0.61
23	B1C5	9.50	2.75	12.50	13.00	0.62
24	TR3	9.63	2.25	12.50	13.00	0.49
25	TR4	11.00	3.00	13.00	12.50	0.60
26	BC2	10.00	2.50	11.50	12.50	0.53
27	BC4	10.00	2.75	9.88	12.00	0.43
28	BC5	8.50	2.50	12.50	13.50	0.61
29	C1	8.38	2.25	11.25	11.25	0.40
30	C3	10.75	3.25	12.75	12.50	0.64
31	C5	12.00	3.25	12.00	13.00	0.57
32	C7	13.25	2.50	11.75	12.25	0.55
33	C8	10.00	2.50	12.25	12.50	0.53
34	C13	14.50	4.00	12.75	13.63	0.87
35	C15	9.25	2.50	9.75	10.38	0.55
36	C17	9.25	3.00	9.50	10.00	0.65
37	C18	10.25	3.25	12.50	13.25	0.55
38	C22	10.75	5.00	14.75	13.25	0.75
sum 1-38		404.25	115.38	462.63	479.75	22.23
mean		10.64	3.04	12.17	12.63	0.58
range		8.25-13.25	2.13-5.00	9.5-14.75	10-13.75	0.38-0.87
SD		1.47	0.70	1.10	0.88	0.10

## BIOGRAPHY

Miss Apisda Rasmi was born on June, 1961 in Chacheongsao, Thailand. She graduated with the Bachelor degree of Science in Medical Technology from the Faculty of Associated Medical Science, Khon Kaen University in 1987. Now she works as a medical technologist at the Communicable Disease Control Zone 3, Chonburi, Department of Communicable Disease Control, Ministry of Public Health, Thailand.



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