APPENDIX

Appendix A

HPLC Chromatograms of bitter melon extracts

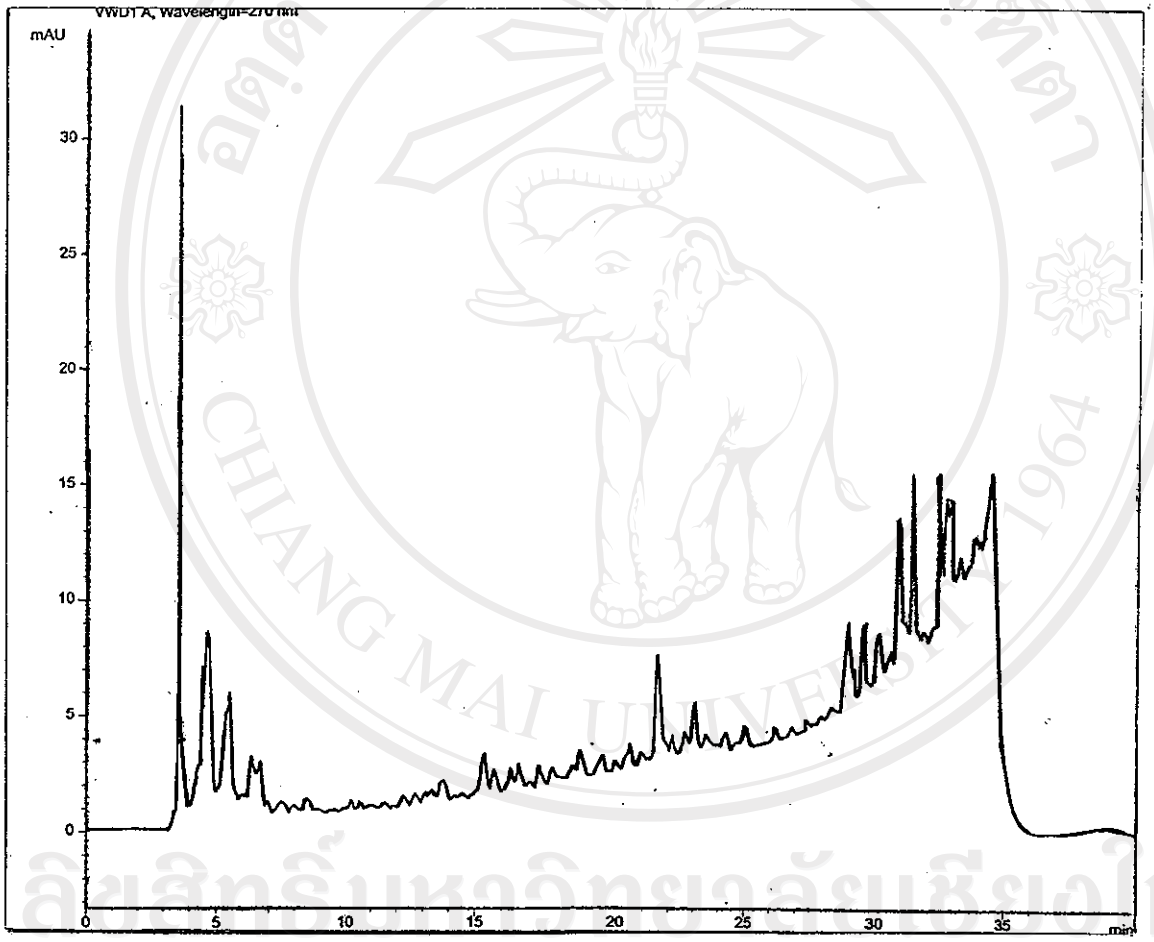

Figure 23. HPLC chromatogram of the whole plant extract at wavelength 270 nm

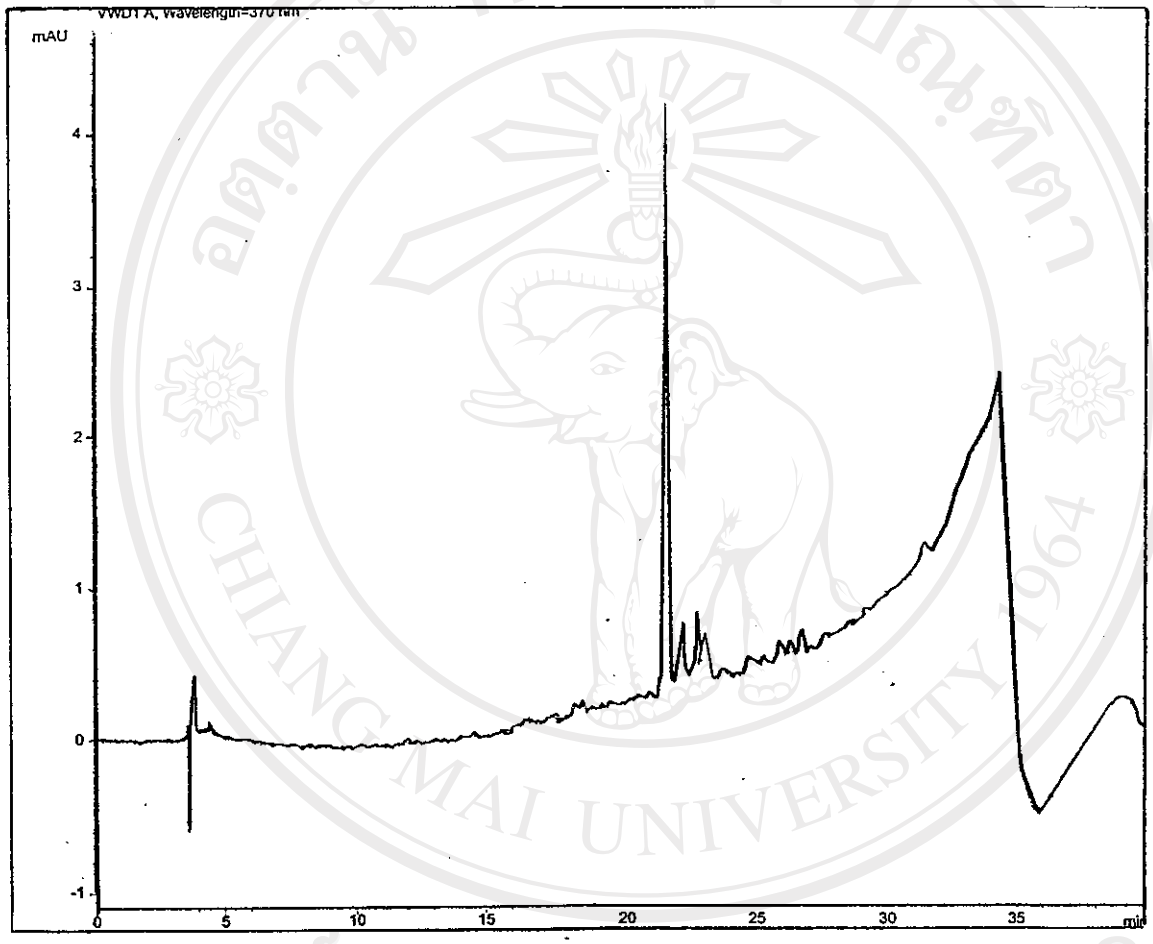

Figure 24. HPLC chromatogram of the whole plant extract at wavelength 370 nm

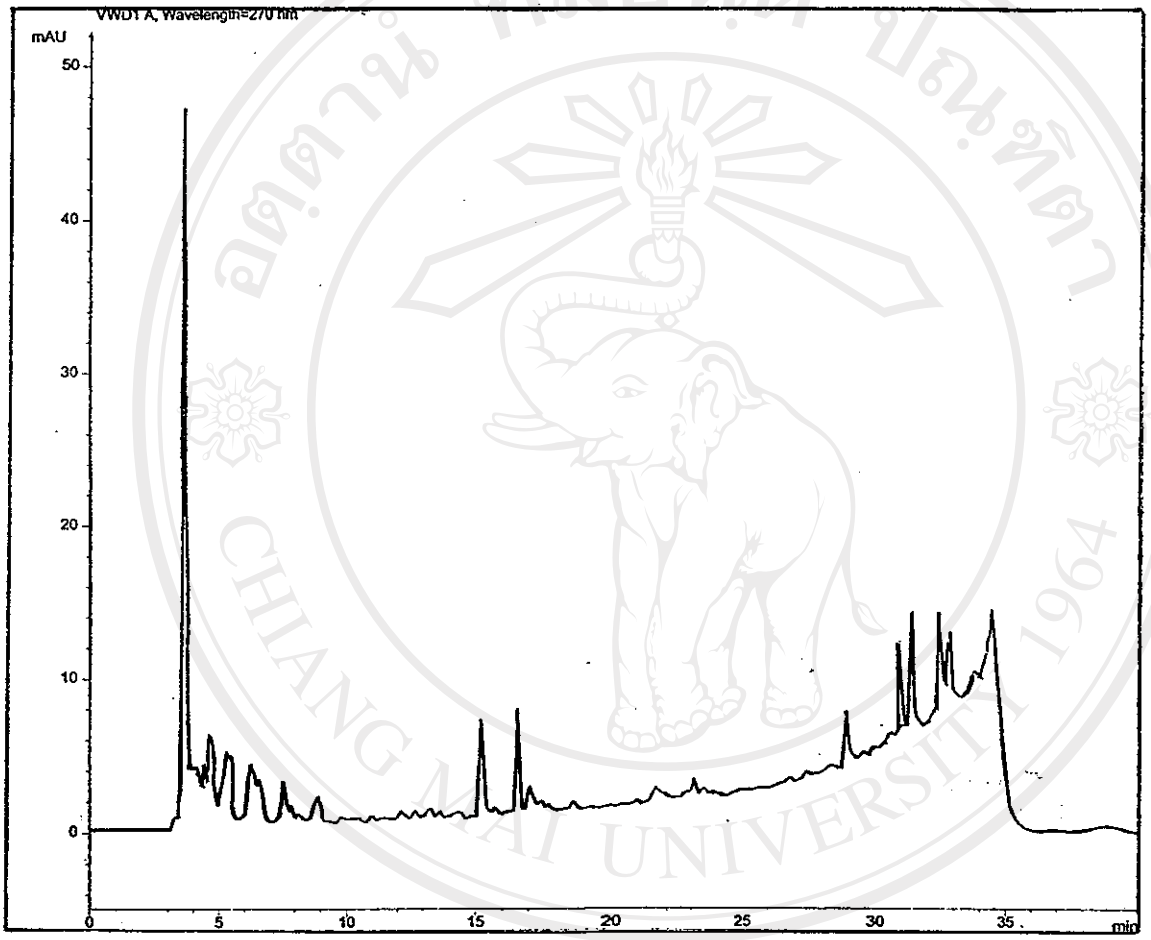

Figure 25. HPLC chromatogram of the leaf extract at wavelength 270 nm

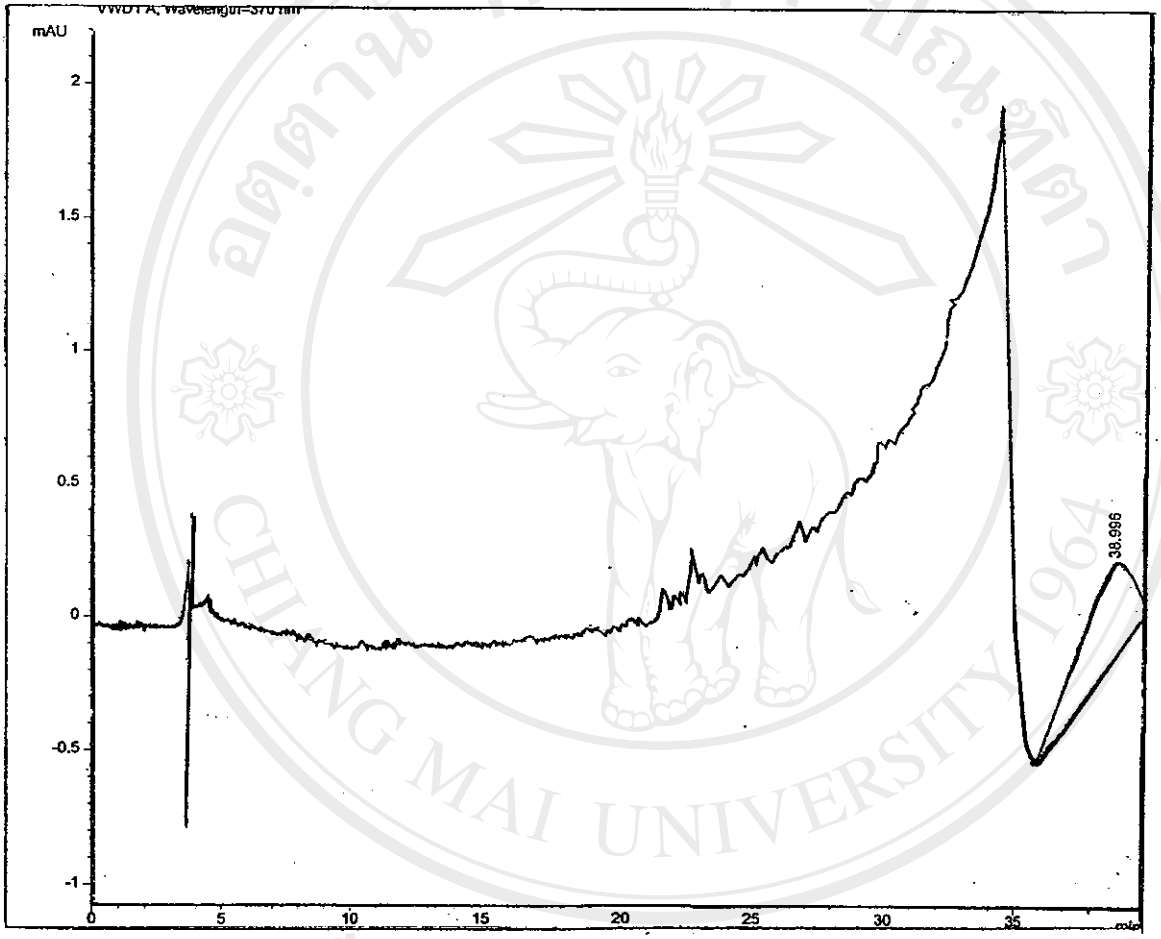

Figure 26. HPLC chromatogram of the leaf extract at wavelength 370 nm

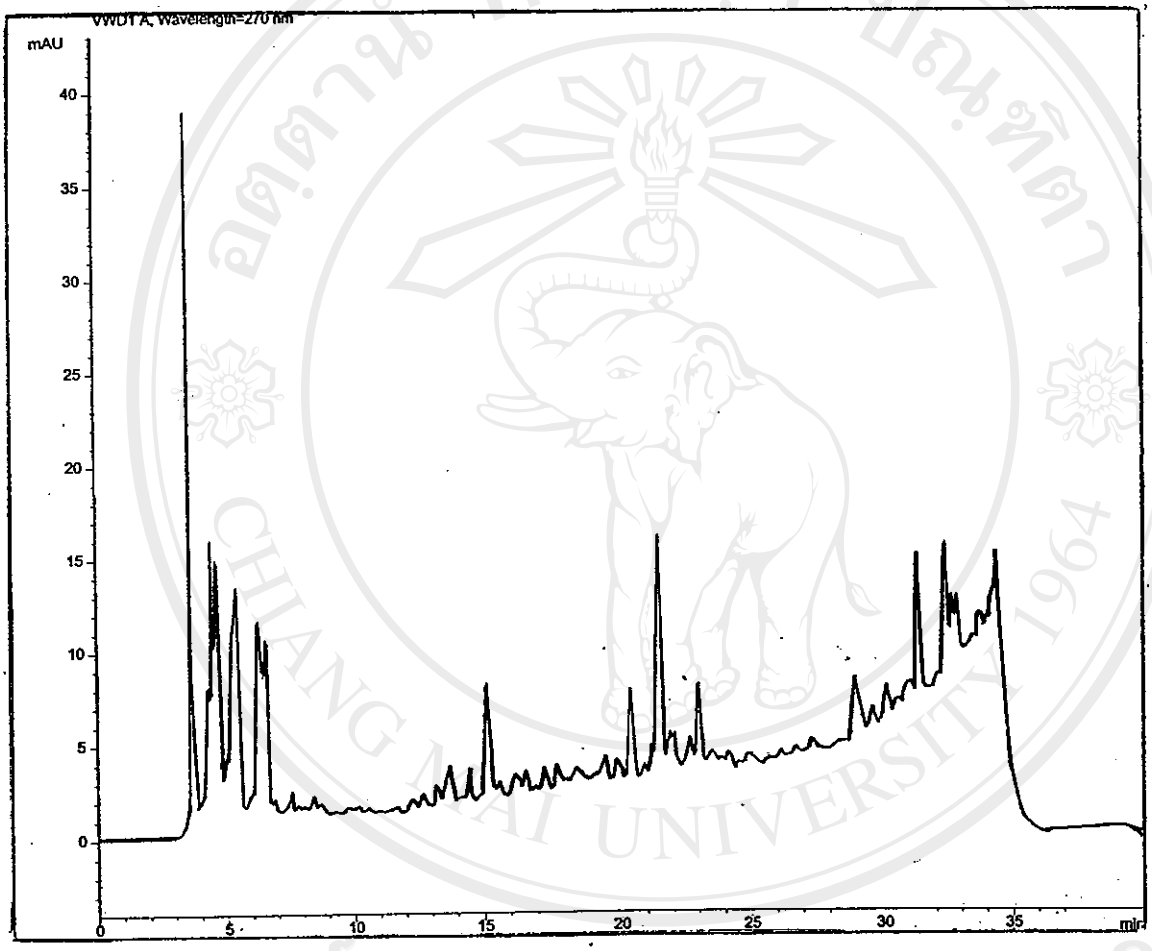

Figure 27. HPLC chromatogram of the fruit extract at wavelength 270 nm

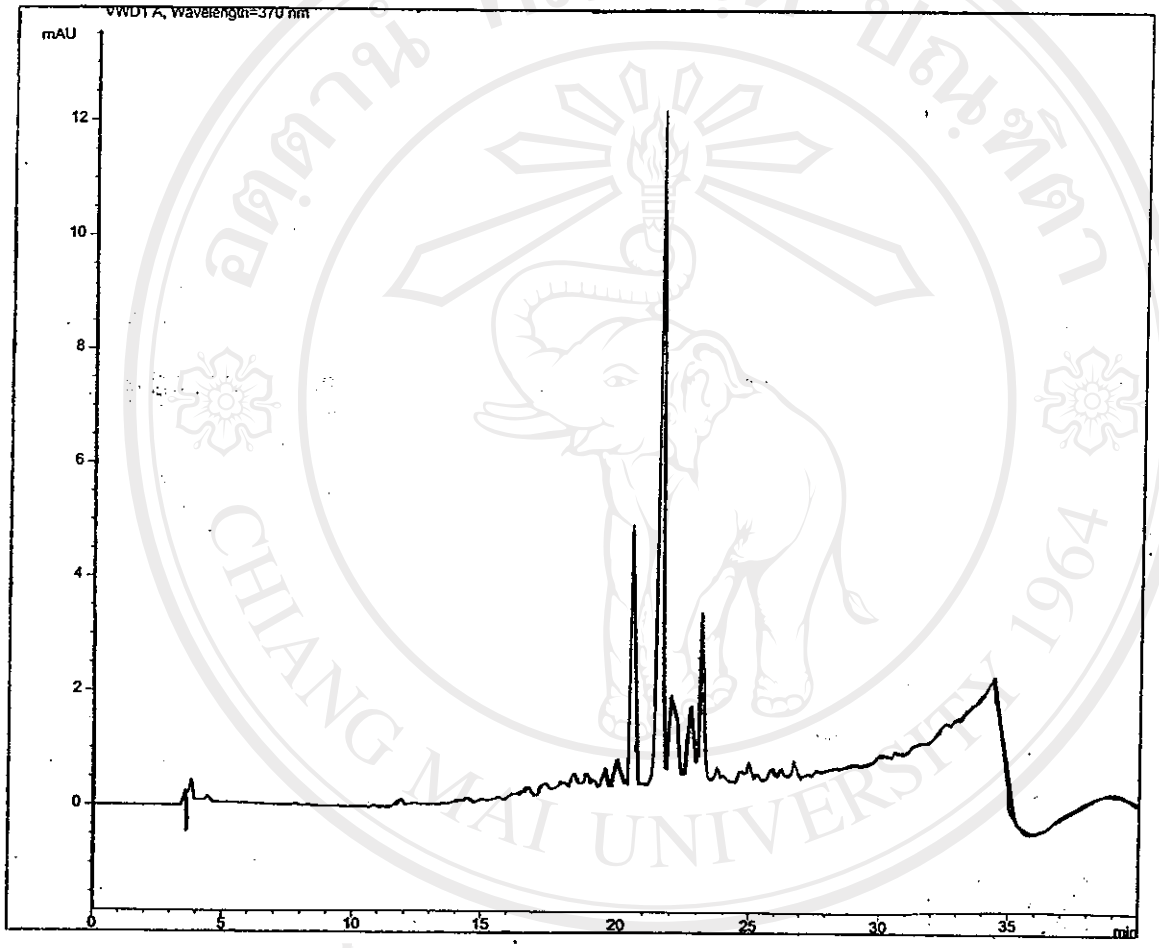

Figure 28. HPLC chromatogram of the fruit extract at wavelength 370 nm

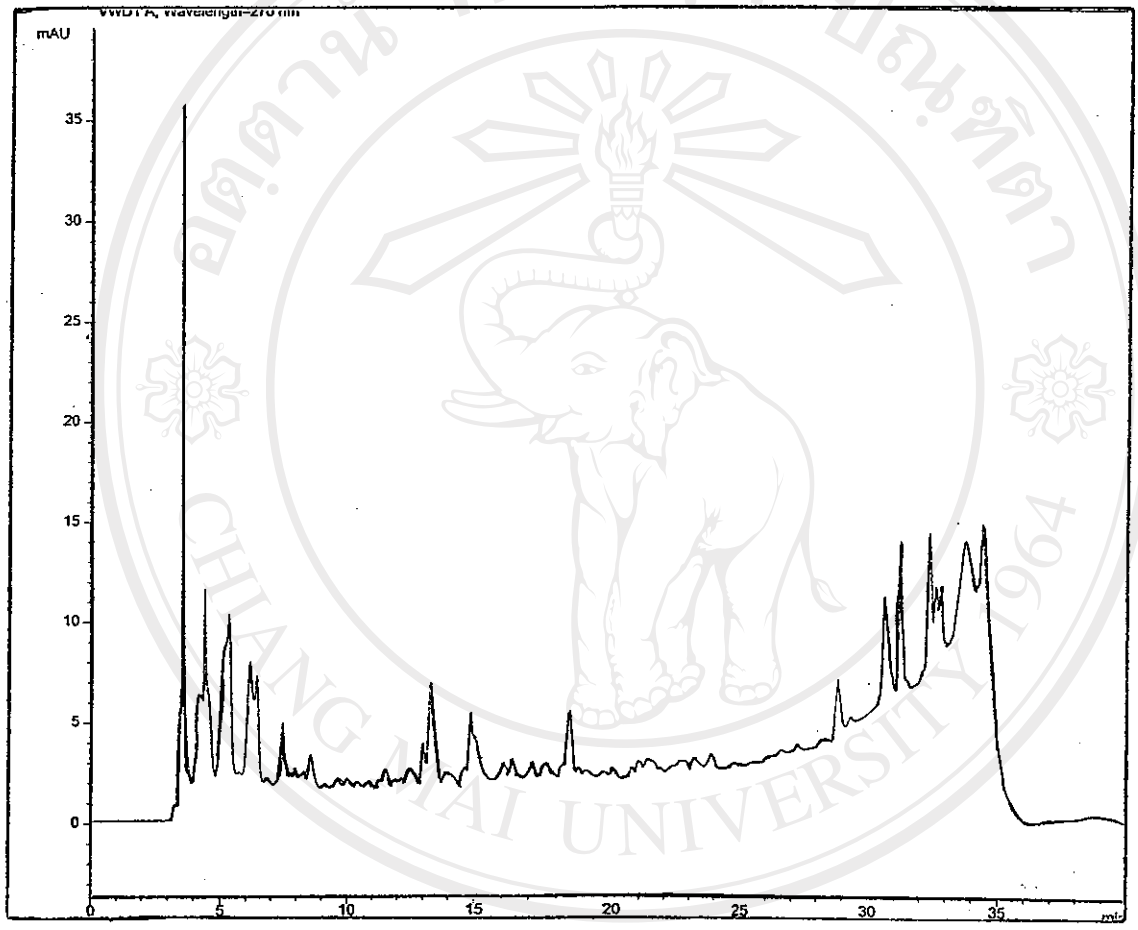

Figure 29. HPLC chromatogram of the tendril extract at wavelength 270 nm

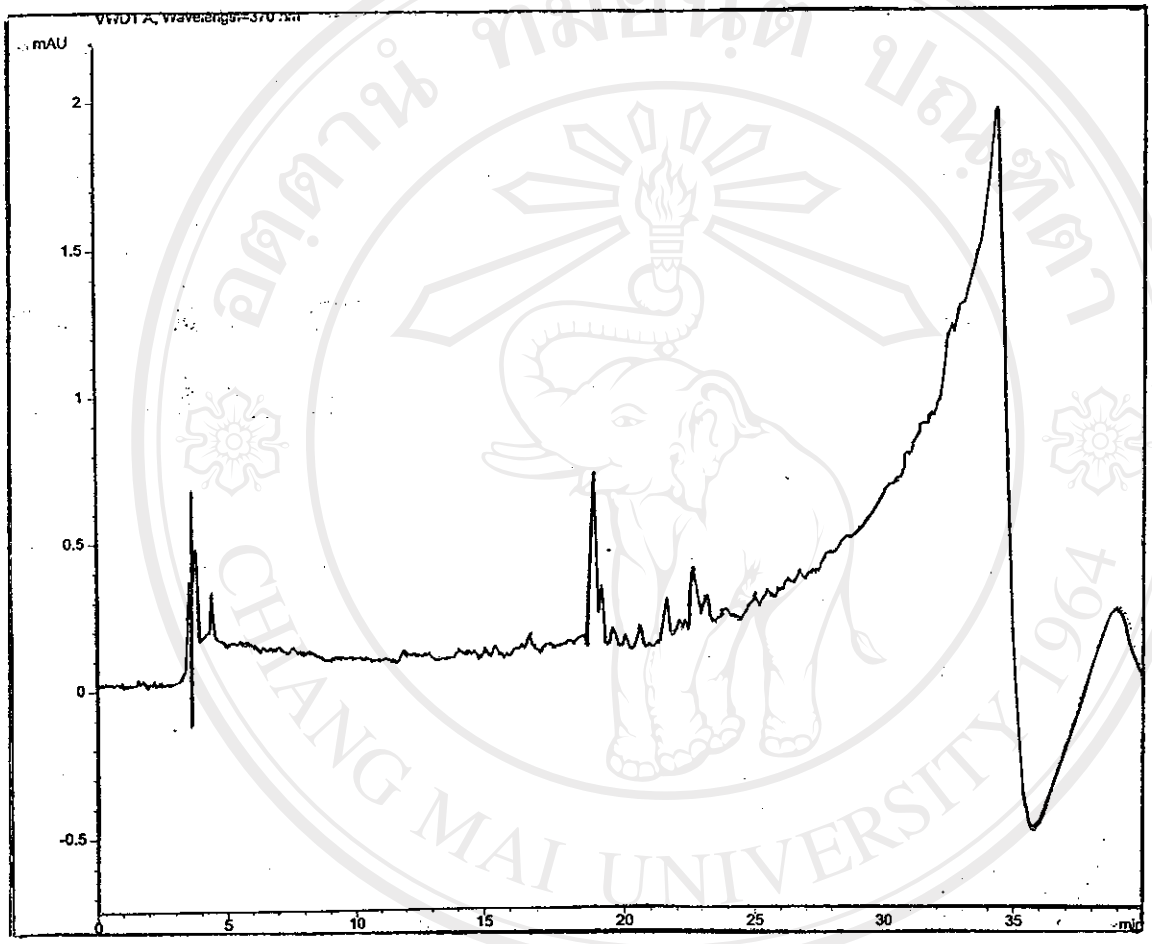

Figure 30. HPLC chromatogram of the tendril extract at wavelength 370 nm

Appendix B

List of chemicals and reagents used in this study

Name of Chemicals	Company
Acetic acid	E.Merck, Germany
Acrylamide	Sigma-Aldrich, USA
Acrylamide (Eastman)	Sigma-Aldrich, USA
Amido black B	Sigma-Aldrich, USA
Ammonium persulfate	Sigma-Aldrich, USA
Bis (Estaman)	Sigma-Aldrich, USA
Bovine serum albumin	PIERCE, USA
Bromphenol blue	Sigma-Aldrich, USA
Coomassie brillant blue R-250	Sigma-Aldrich, USA
Coomasie® Plus Protein Assay Reagent	PIERCE, USA
Copper sulfate	E.Merck, Germany
Dibasic sodium phosphate	Sigma-Aldrich, USA
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich, USA
Dulbecco's modified eagle medium	GIBCO, USA
Ethanol	E.Merck, Germany
Fetal calf serum	Seromed, USA
Folin&clocalteu's phenol reagent	E.Merck, Germany
Glycerol	Sigma-Aldrich, USA
Glycine	Sigma-Aldrich, USA
HEPES	Sigma-Aldrich, USA
High range molecular weight marker	BIO-RAD, USA
HRP-conjugated goat anti-mouse IgG	Sigma-Aldrich, USA
Hydrochloric acid	E.Merck, Germany
Magnesium chloride	Sigma-Aldrich, USA
Mercaptoethanol	Sigma-Aldrich, USA
Methanol	E.Merck, Germany

Mouse monoclonal anti P-glycoprotein clone F4	Sigma-Aldrich, USA
Monobasic sodium phosphate	Sigma-Aldrich, USA
MTT Thiazolyl blue	Sigma-Aldrich, USA
Penicillin-streptomycin	GIBCO, USA
Phenylmethyl sulfonyl fluoride (PMSF)	Sigma-Aldrich, USA
Potassium chloride	Sigma-Aldrich, USA
Potassium phosphate	Sigma-Aldrich, USA
POPOP	Sigma-Aldrich, USA
PPO	Sigma-Aldrich, USA
2-propanol	E.Merck, Germany
SDS-PAGE standard broad range	Sigma-Aldrich, USA
Skim milk	Difco, USA
Sodium carbonate	Sigma-Aldrich, USA
Sodium chloride	Sigma-Aldrich, USA
Sodium dodecyl sulfate	Sigma-Aldrich, USA
Sodium potassium tartrate	Sigma-Aldrich, USA
Sodium hydroxide	Sigma-Aldrich, USA
SuperSignal®West Pico Chemiluminescent substrate	PIERCE, USA
Tris (hydroxymetry) aminomethane	Sigma-Aldrich, USA
Tween 20	Sigma-Aldrich, USA
Verapamil	Sigma-Aldrich, USA
Vinblastine sulphate salt	Sigma-Aldrich, USA
[G- ^{3}H] vinblastine sulphate	Amersham, UK

Appendix C

List of instruments used in this study

Instrument	Company
Analytical balance AC 100	Satorious
Autoclave	Tomy autoclave SS-240
Automatic pipette	GIBCO
β counter (liquid scintillation counter)	Pharmacia
Carbondioxide incubator	Forma Scientific
Deionized water machine	Barnstead
Distilled water machine	Hamilton
ECL-hyper film	Amersham
Evapolator	Buchi
Freezer (-80 °C)	Forma scientific
Freezer (-20 °C)	Sanyo
Glassware	Pyrex
Hood	British Klocker Switchgear
Hot air oven	Haraeus
Inverted microscope	Nikon
Light microscope	Olympia Tokyo
Liquid nitrogen tank	Taylor-wharton
Lyophilizer	Christ Alpha1-4
Magnetic stirrer	Sybron / Thermolyne
Microcentrifuge, bench-topped	Clay
Mini protein II slab gel	BIO-RAD
Pasture pipette	Pyrex
pH meter	Hanna Instruments 8417
Power supply	E-C Appraratus corporation
Refrigerator	Sanyo, Hitachi
Serological pipette	Pyrex
Shaker bath	Unitronic 320 OR

Slab gel dryer	Savant
Spectrophotometer	MILTON ROY spectonic 1001 plus
25 or 75 cm^3 T-flask	Nunc
Trans-blot® electrophoretic tranfer cell	BIO-RAD
Ultracentrifuge	Ivan Sorval Inc.,USA
Vertical gel electrophoresis apparatus	Life Technologies TM
Vortex	Scientific industries
Water bath	GFL 1083

Appendix D

Preparation of some reagents and buffers

Human cervical carcinoma cell culture

1. Incomplete DMEM medium with phenol red

DMEM	1	package (13.5 g)
HEPES	2.603	g
$NaHCO_3$	3.7	g
0.34% 2-mercaptoethanol	1.0	ml
Deionize distilled water	800	ml

Adjust pH to 7.2-7.4 then adjust volume to 1,000 ml and steriled by suction filter (membrane pore size 0.2 μM)

2. Completed DMEM medium with phenol red

Incomplete DMEM medium	89.5	ml
Fetal calf serum	10	ml
Pen/strep	0.5	ml

Stored at 4°C.

3. Freezing solution

Fetal calf serum	9.2	ml
DMSO	0.8	ml

Stored at 4°C.

Cell survival measurement

1. MTT stock dye solution

MTT	1.0	g
PBS pH 7.4	200	ml

After dissolved MTT dye, filtrated any nonsoluble powder by filtration with membrane filter pore size 0.2 μm, collected in dark container.

2. Phosphate buffer saline (PBS) pH 7.4

KH_2PO_4	0.24	g
Na_2HPO_4	1.44	g
NaCl	8.0	g
KCl	0.2	g

Dissolved in 800 ml deionize distilled water, adjusted pH to 7.4 then top up to 1,000 ml. Sterilized by autoclave.

Radiolabeled drug accumulation and efflux

1. 3N Sodium hydroxide

NaOH	12	g
Deionize distilled water	100	ml

2. 6N Hydrocholic acid

12N HCl was diluted in deionize distilled water to 6 N.

3. Tripop scintillation cocktail

PPO	10	g
POPOP	0.25	g
Toluene	2.5	l

Plasma membrane preparation

1. Hypotonic buffer

1 M Tris-HCl pH 7.4	0.5	ml
4.2 M $MgCl_2$	18	µl
KCl	0.0373	g
100 mM PMSF	1	ml
Deionize distilled water up to	50	ml

2. Laemmli buffer

1.0 M Tris-HCl, pH 6.8	0.5	ml
Glycerol	1.0	ml
10% SDS	2.0	ml
Deionize distilled water	6.25	ml

Protein determination

1. **Reagent A**

2% (w/v) Na_2CO_3 in 0.1 N NaOH

NaOH	2	g
Na_2CO_3	10	g
Deionize distilled water	500	ml

2. **Reagent B**

Part A; $CuSO_4$	0.5	g
Distilled water	50	ml
Part B; Na-K Tatrate	1	g
Deionize distilled water	50	ml

Before using 0.5 ml of part A and B were mixed with the final concentration 0.5%(w/v) $CuSO_4.5H_2O$ and 1%$NaKC_4H_4O_6.2H_2O$ (Na-K Tatrate).

3. **Reagent C**

Working solution was freshly prepared by mixing reagent A 50 ml and reagent B ratio 50:1.

4. **Folin-ciocalteau phenol reagent 1N**

Folin-ciocalteau phenol reagent 2N was diluted in deionize distilled water to 1 N.

SDS-PAGE analysis

1. **Stock solution A : separating gel buffer 1.5 mM Tris HCl, pH 8.8**

Tris base	18.15	g
Deionize distilled water	80	ml

Adjusted pH to 8.8 then adjusted volume to 100 ml and filtrated any nonsoluble powder by filtration with membrane filter pore size 0.2 μm, collected in dark container.

2. **Stock solution C: stock acrylamide solution (30% T, 2.7%)**

Acrylamide	29.2	g
Bis (Estaman)	0.8	g
Deionize distilled water	70	ml

Adjusted volume to 100 ml and and filtrated any nonsoluble powder by filtration with membrane filter pore size 0.2 μm, collected in dark container.

3. **Stock solution D : stacking gel buffer 0.5 mM Tris HCl pH 6.8**

Tris base	6.05	g
Deionize distilled water	70	ml

Adjusted pH to 6.8 then adjusted volume to 100 ml and and filtrated any nonsoluble powder by filtration with membrane filter pore size 0.2 μm, collected in dark container.

4. **Stock ammonium persulfate solution** (10% w/v APS in deionized water)

Ammonium persulfate	0.1	g
Deionize distilled water	1	ml

5. **Electrode buffer**

Tris-base	3.0	g
Glycine	14.4	g
SDS	1.0	g

Dissolved in deionized water 1,000 ml then filtrated by suction filter and stored at 4 °C.

6. 5X nonreducing buffer

1.0 M Tris-HCl pH6.8	0.625	ml
Glycerol	1.0	ml
1% Bromphenol blue	0.125	ml

Adjusted volume to 10 ml with distilled water.

7. 5X reducing buffer

5X nonreducing buffer	475	µl
2-mercaptoethanol	25	µl

8. High range molecular weigh marker

Marker	1	µl
5X reducing buffer	19	µl

9. Coomassie blue

Coomassie blue	0.25	g
Methanol	20	ml
Acetic acid	10	ml
Deionized water was top up to	100 ml	

10. Coomassie blue destaining solution

Methanol	100	ml
Acetic acid	50	ml
Deionized water was top up to	500	ml

11. Stock 10% SDS solution

SDS	0.2	ml
Deionize distilled water	1	ml

12. Separating gel 7.5%

Deionize distilled water	2.425	ml
Tris-HCl, pH 8.8 (solution A)	1.25	ml
10% SDS	50	µl
Acrylamide/Bis (solution C)	1.25	ml
10% APS	25	µl
TEMED	2.5	µl

13. Stacking gel 4%

Deionize distilled water	3.05	ml
Tris-HCl, pH 6.8 (solution D)	1.25	ml
10% SDS	50	µl
Acrylamide/Bis (solution C)	0.65	ml
10% APS	25	µl
TEMED	5	µl

Proetin Western blot analysis

1. Bloting buffer

Tris-base	3.03	g
Glycine	14.4	g
Methanol	200	ml

Dissolved in deionize distilled water 1,000 ml then filtrated by filtration and stored at 4 °C.

2. Amido black

Amido black	0.25	g
Isopropanol	62.5	ml
Acetic acid	25.0	ml

Top up with deionize distilled water to 250 ml

3. PBS, pH7.4

Na_2HPO_4	1.3	g
NaH_2PO_4	0.204	g
NaCl	7.28	g

Adjusted pH to 7.4 then adjusted volume to 1,000 ml and steriled by filtration.

4. Amido black destaining solution

Isopropanol	125	ml
Acetic acid	50	ml

Top up with deionized water to 250 ml.

5. Blocking reagent

Skim milk	5	g
Anti foam	20	µl

Dissolved in PBS, pH 7.4.

6. Washing buffer

PBS pH 7.4	500	ml
Tween 20	500	µl

7. Film developer (Kodak)

Part A	2.99	g
Part B	21.8	g
Part C	0.7246	g

Top up with deionized water to 250 ml. Stored at 4 °C in dark.

8. Film fixer (Kodak)

Part A	50	ml
Part B	10	ml

Top up with deionized water to 250 ml. Stored at 4 °C in dark.

VITA

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