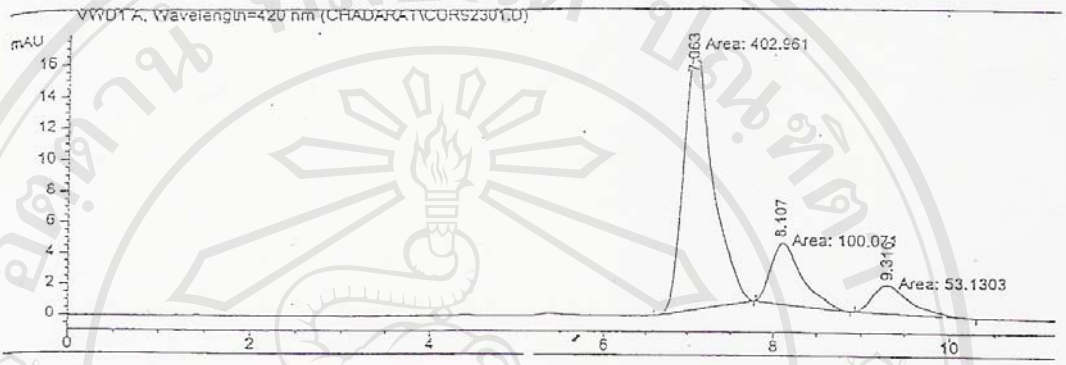
APPENDIX

Appendix A



Area Percent Report

rted By	: Signal
ltiplier	: 1.0000
lution	: 1.0000

Signal 1: VWD1 A, Wavelength=420 nm
Results obtained with standard integrator!

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	7.063	MM	0.4018	402.96057	16.71300	72.4538
2	8.107	MM	0.4240	100.07101	3.93403	17.9931
3	9.316	MM	0.4881	53.13030	1.81405	9.5530
Totals :				556.16188	22.46108	

Figure 72. HPLC chromatograms of curcumin mixture from Sigma

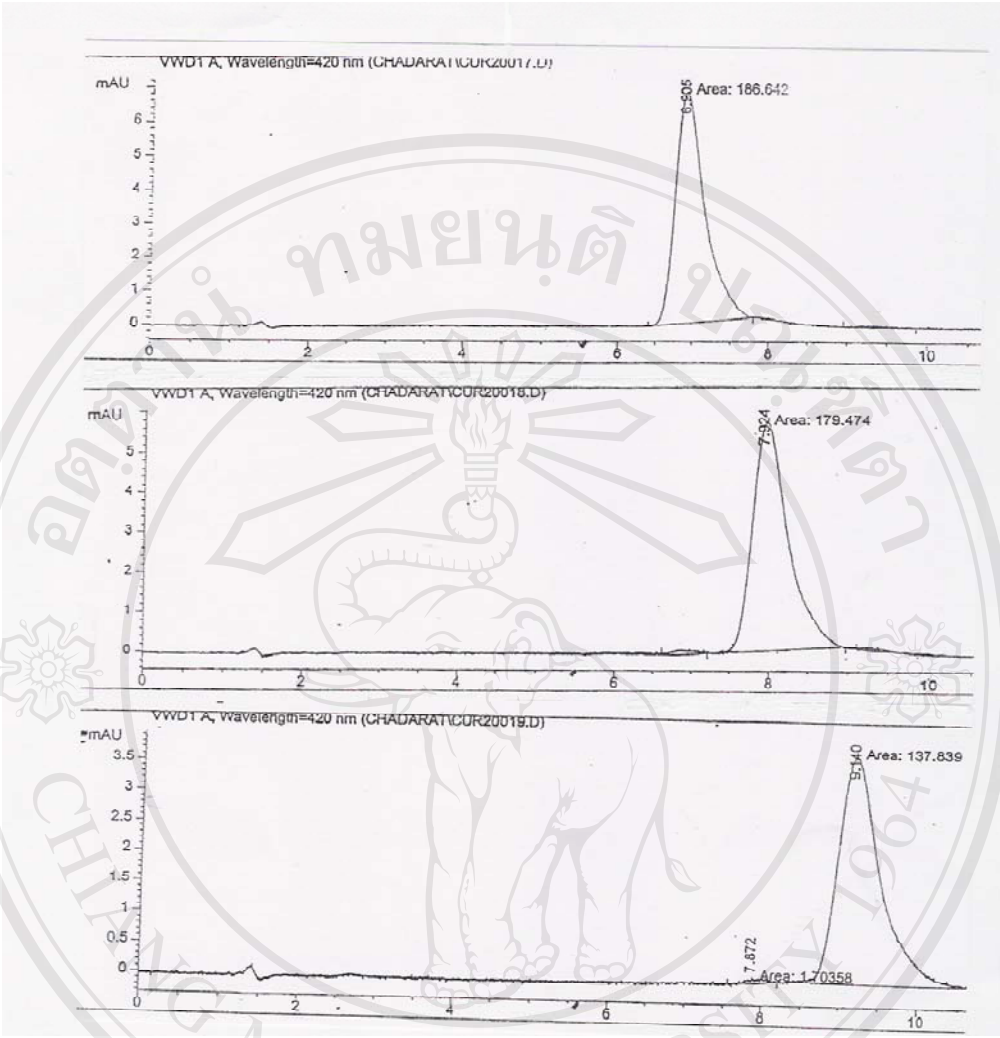


Figure 73. HPLC chromatograms of curcumin I, II and III

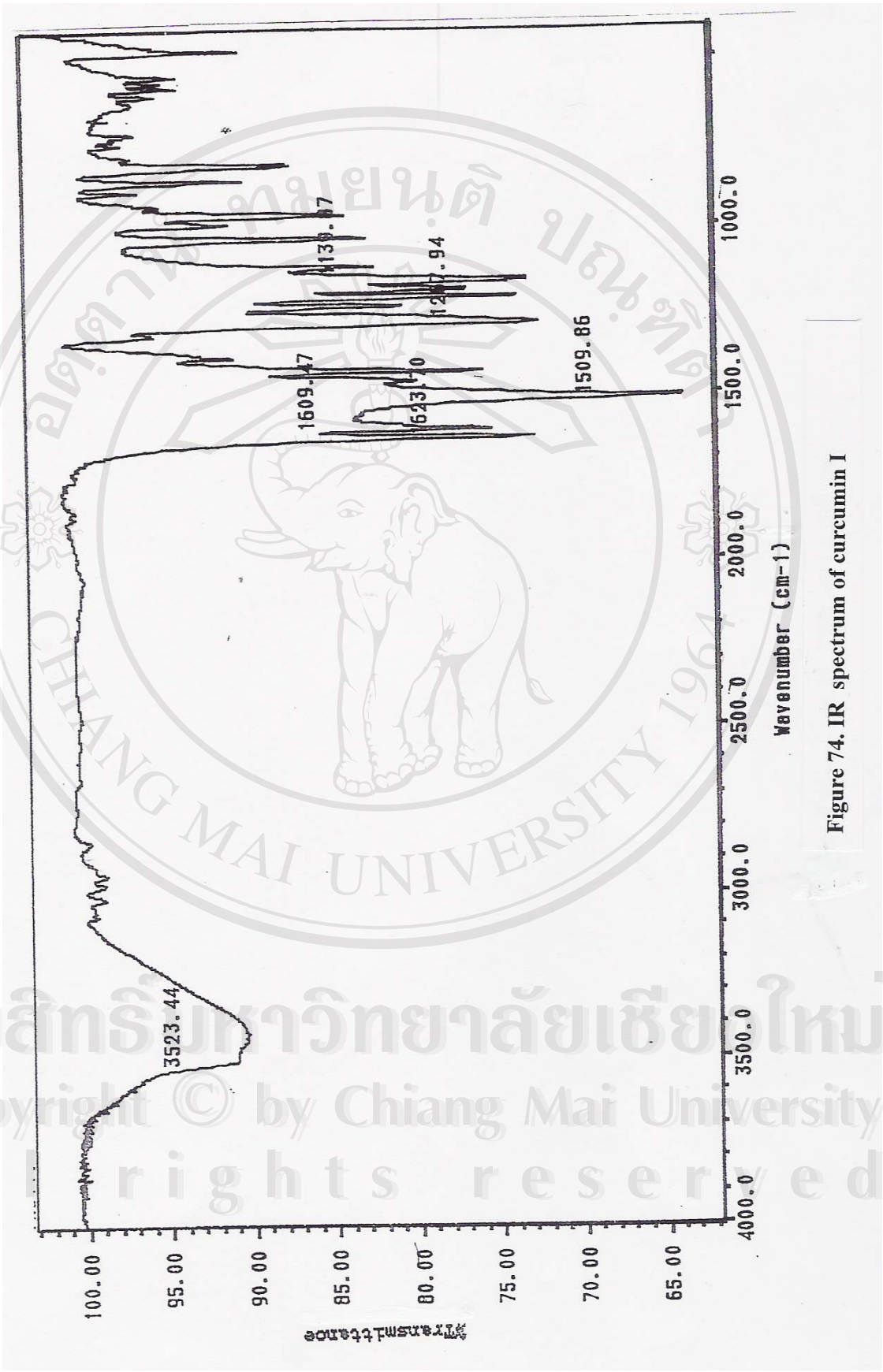


Figure 74. IR spectrum of curcumin I

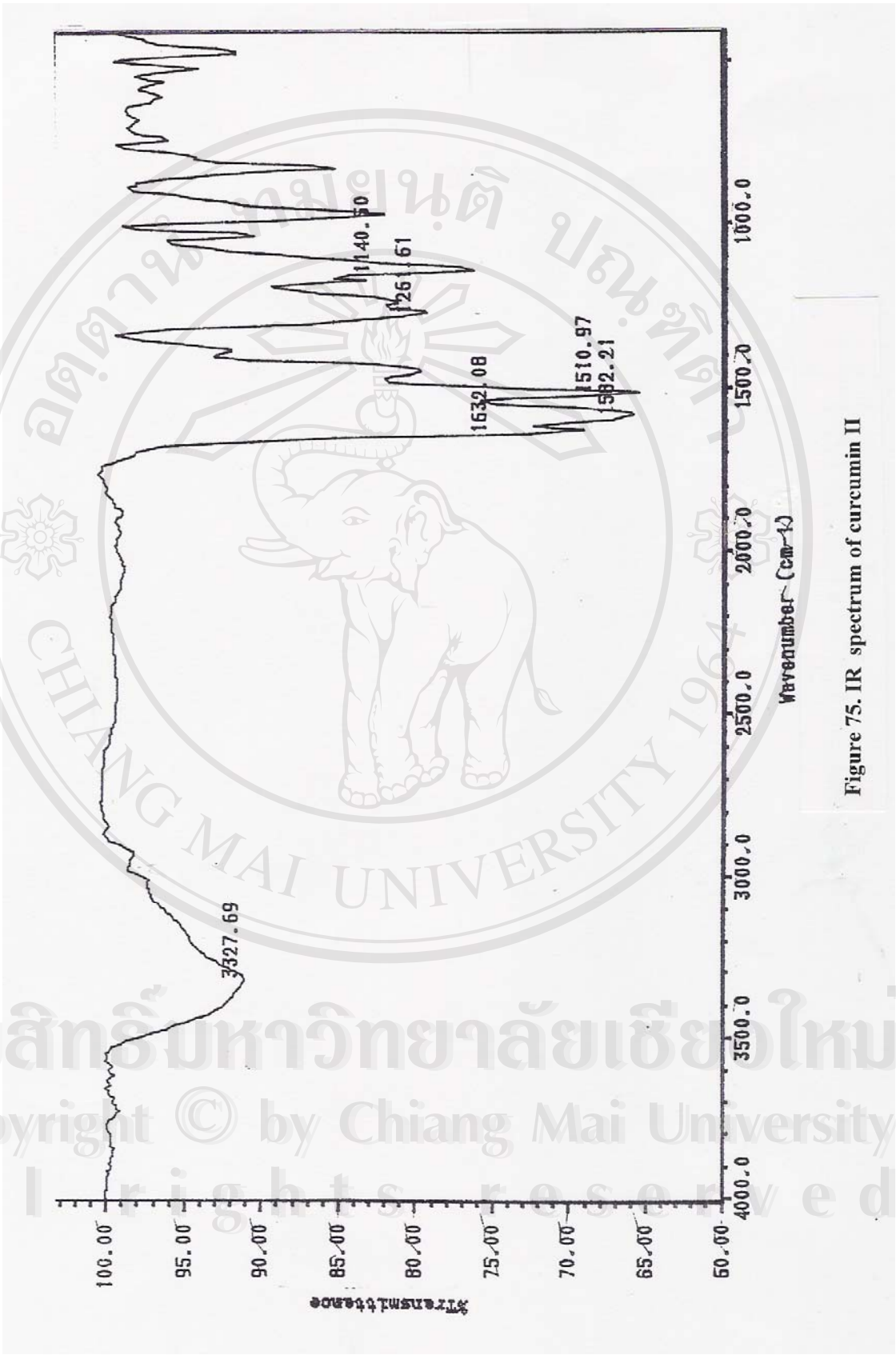


Figure 75. IR spectrum of curcumin II

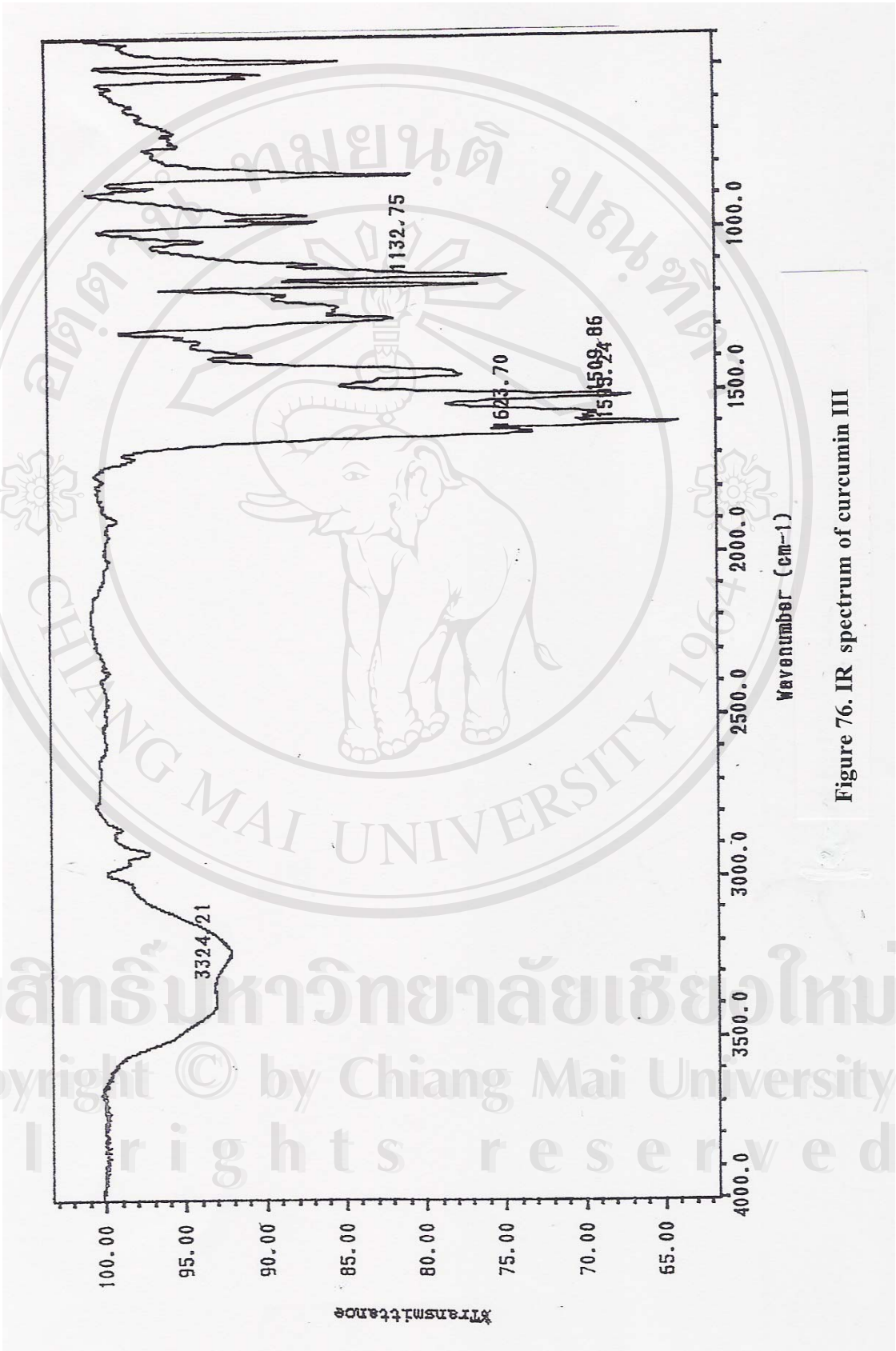


Figure 76. IR spectrum of curcumin III

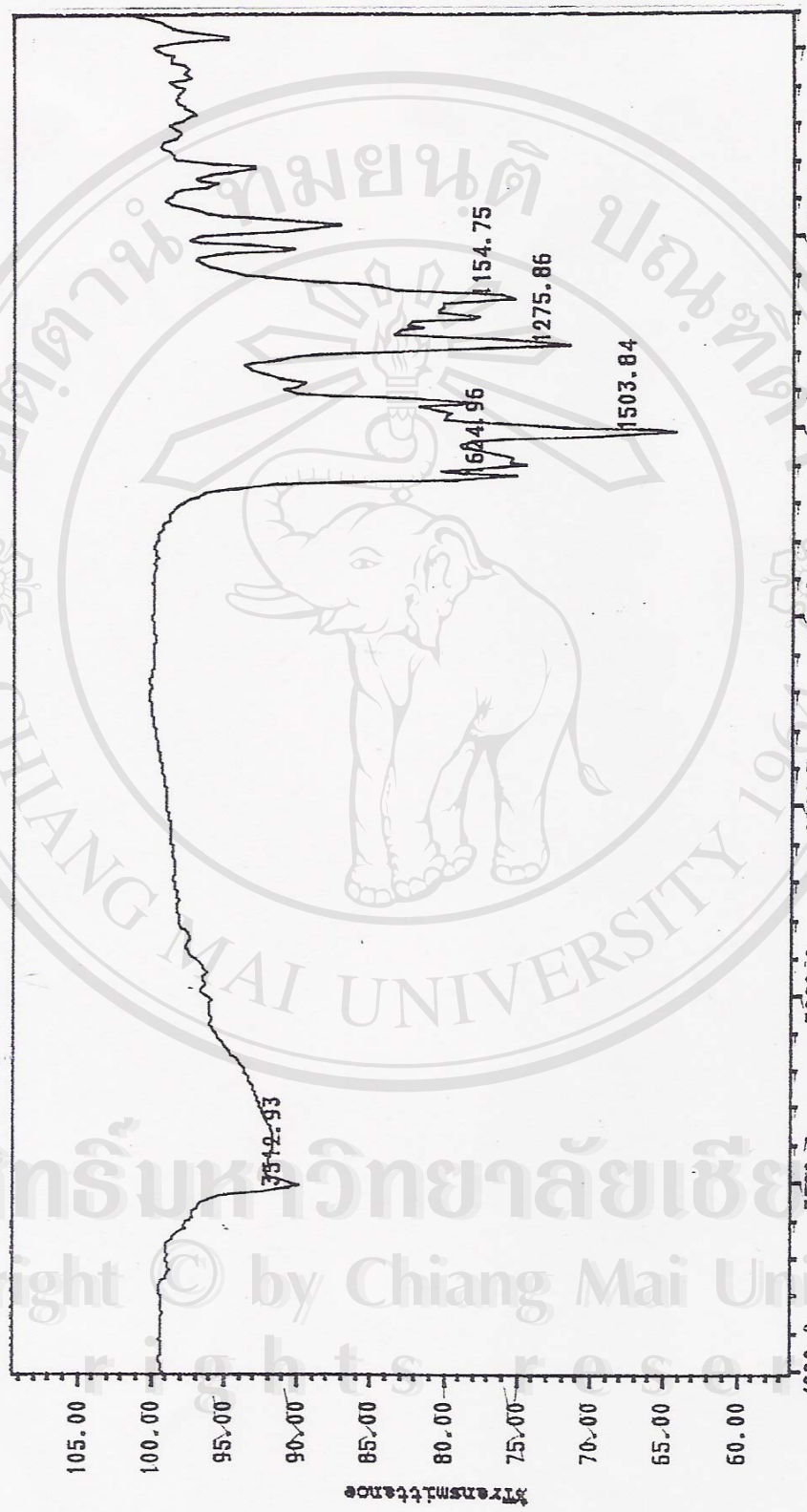


Figure 77. IR spectrum of curcumin standard

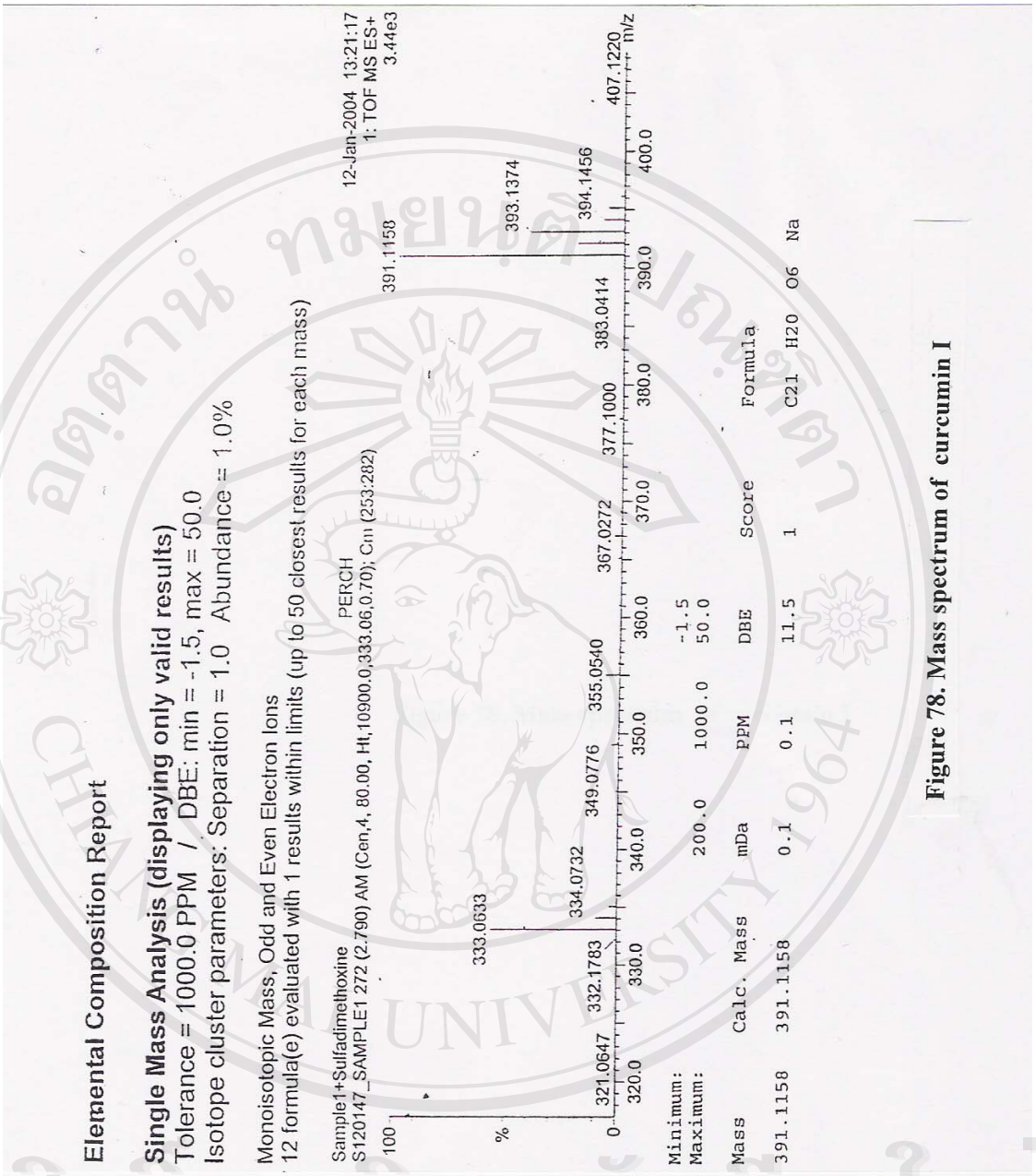


Figure 78. Mass spectrum of curcumin I

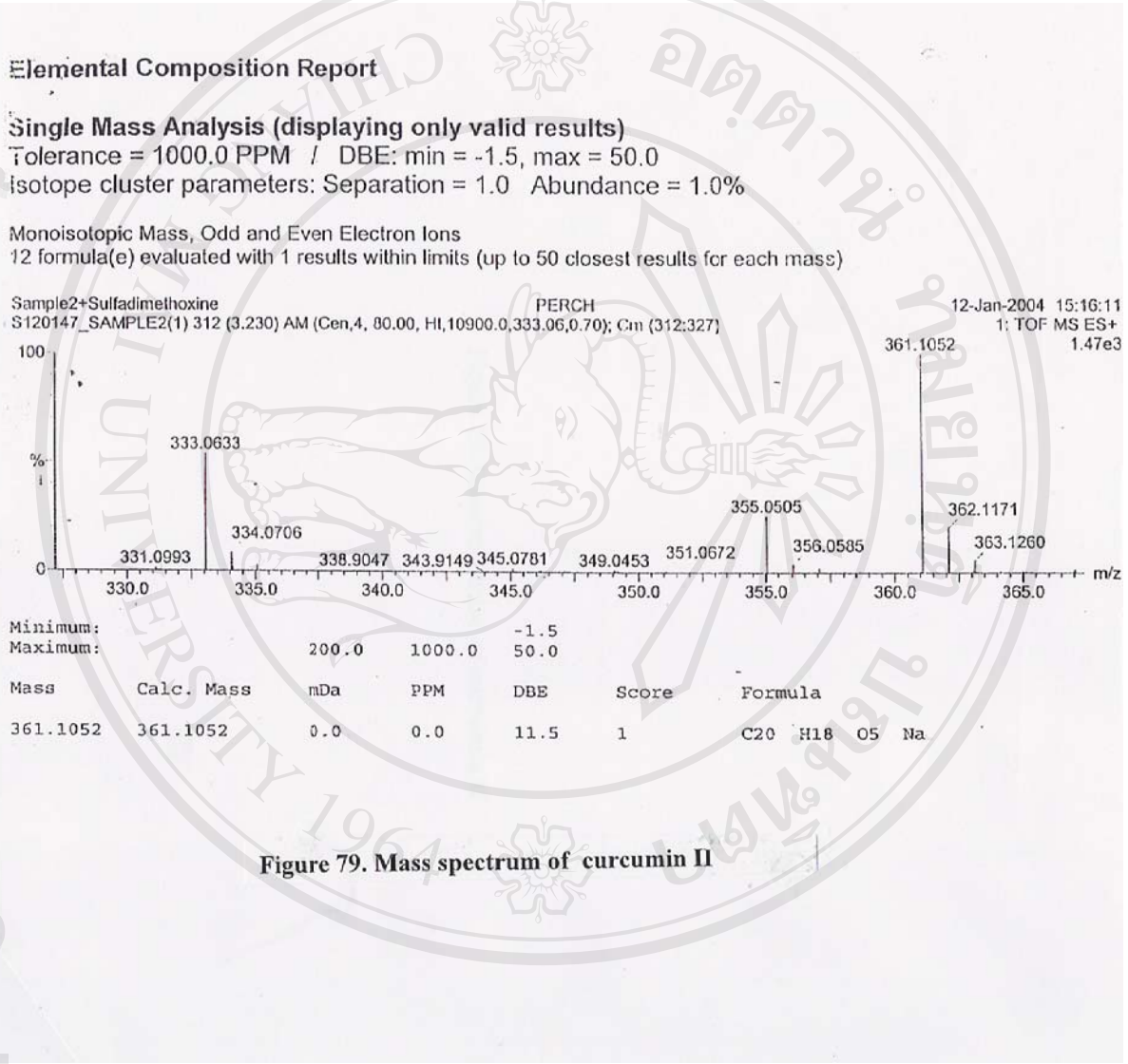


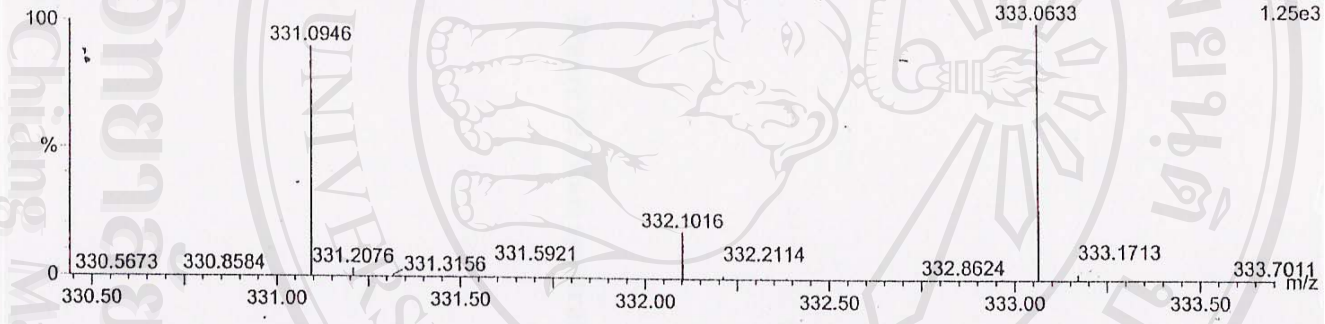
Figure 79. Mass spectrum of curcumin II

Elemental Composition Report

Single Mass Analysis (displaying only valid results)
Tolerance = 1000.0 PPM / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
11 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Sample3+Sulfadimethoxine PERCH 12-Jan-2004 14:10:27
S120147_SAMPLE3 423 (4.357) AM (Cen,4, 80.00, Ht,10900.0,333.06,0.70); Cm (393:429) 1: TOF MS ES+
1.25e3



Minimum:				-1.5		
Maximum:		200.0	1000.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
331.0946	331.0946	0.0	-0.1	11.5	1	C19 H16 O4 Na

Figure 80. Mass spectrum of curcumin III

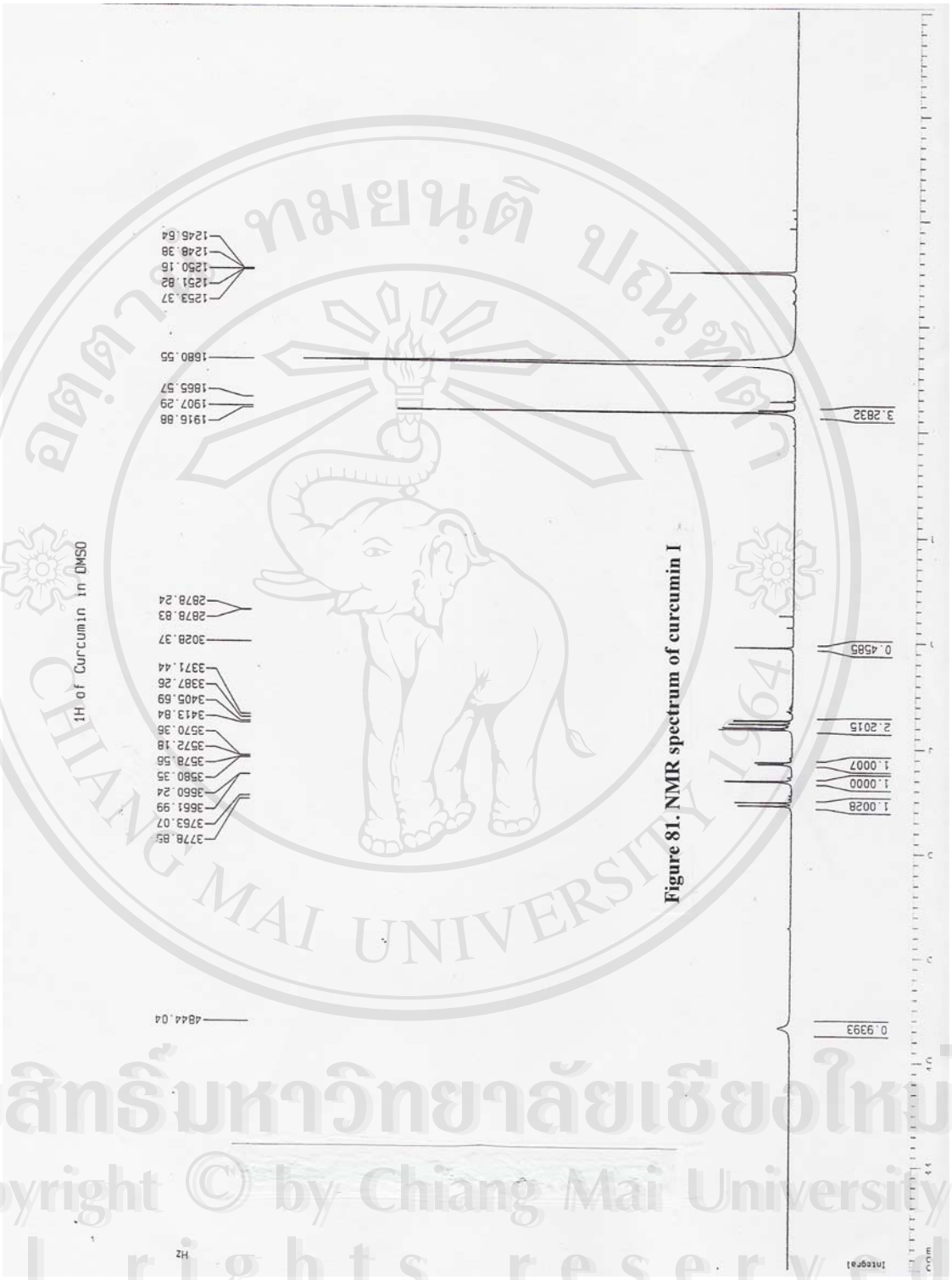


Figure 81. NMR spectrum of curcumin I

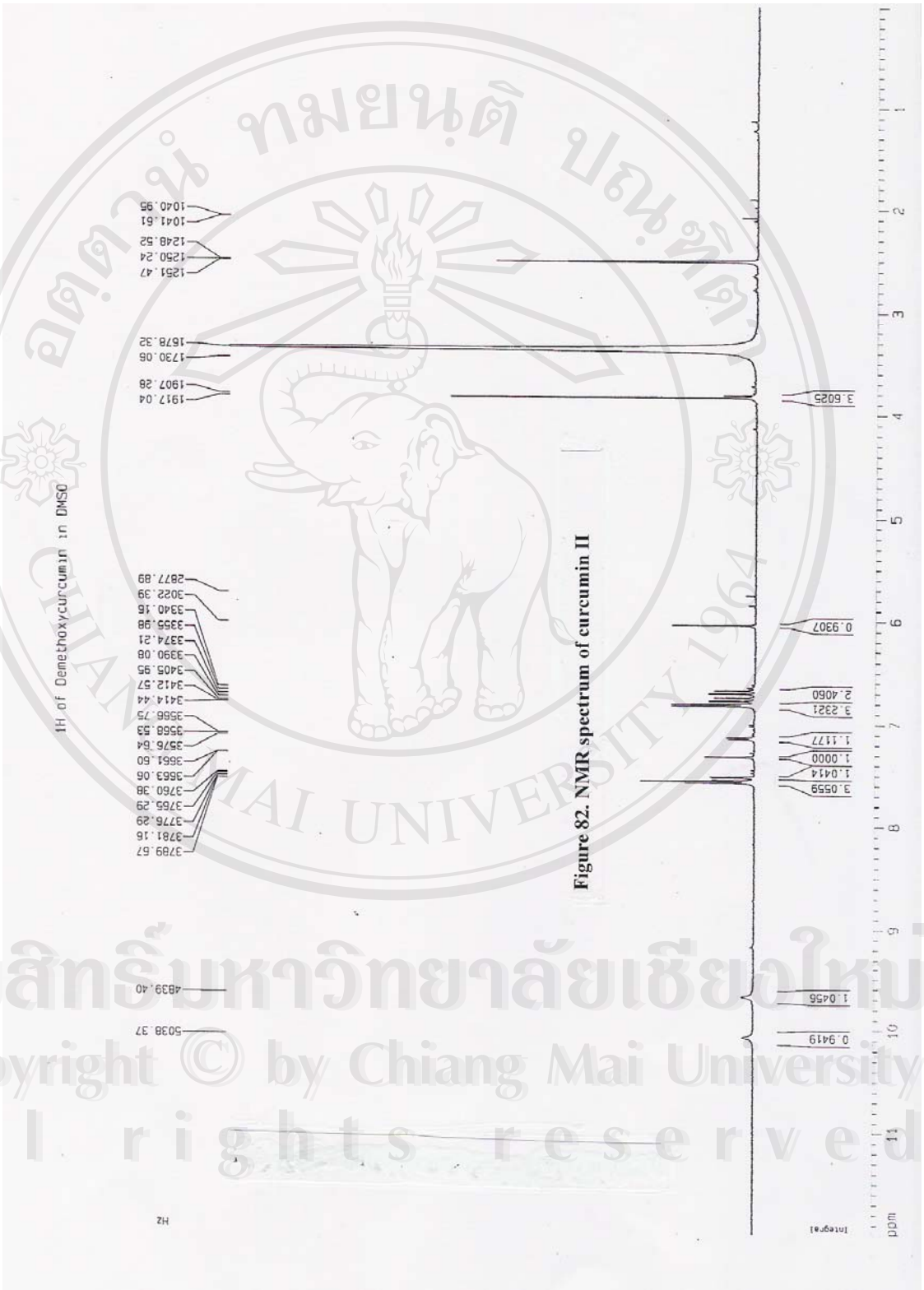


Figure 82. NMR spectrum of curcumin II

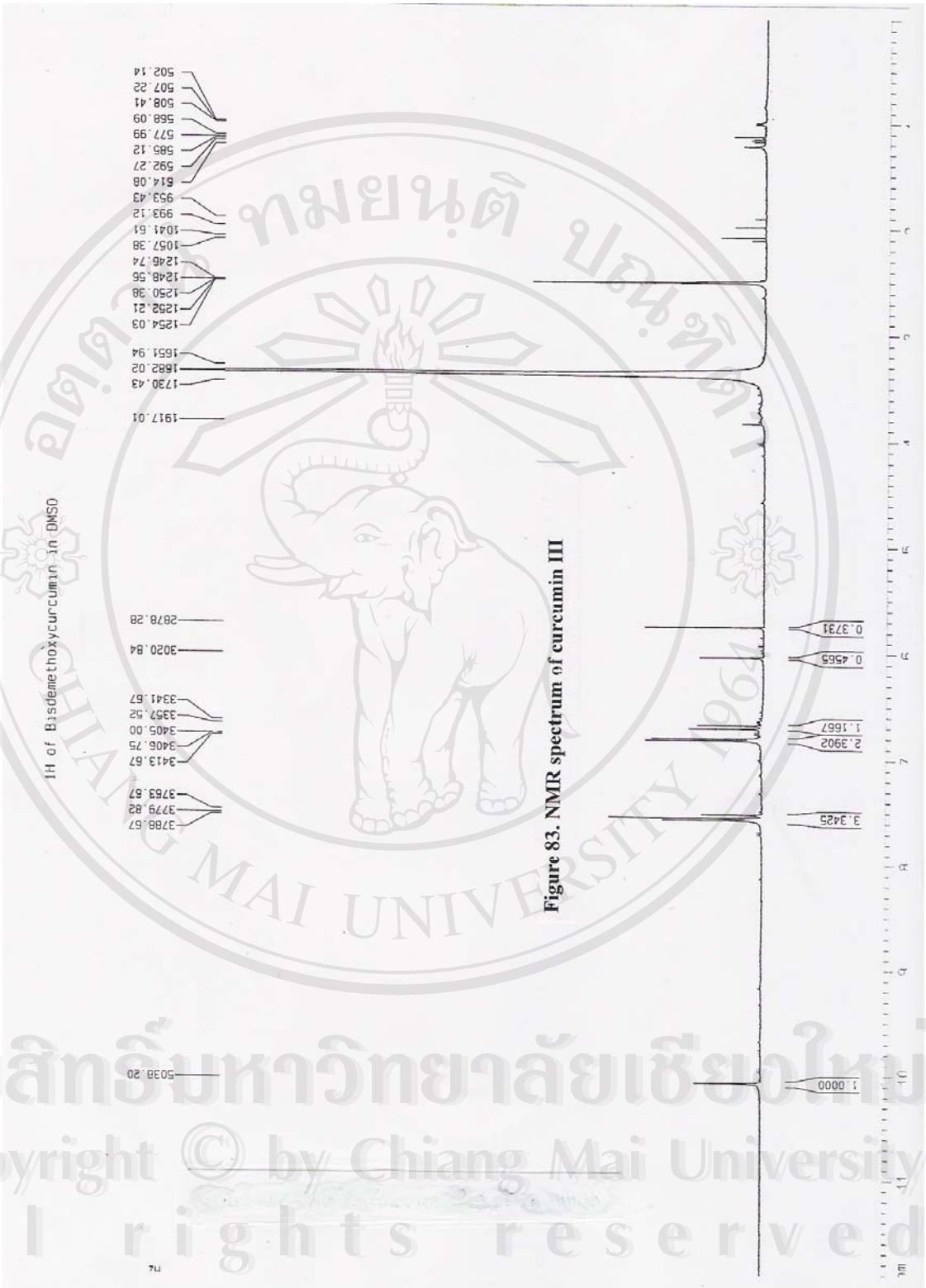


Figure 83. NMR spectrum of curcumin III

Table 30. NMR, IR and MS data of curcumin I [129].

^{1}H NMR[a] Assignments				
Chemical Shift (ppm)	Multiplicity	Integration Ratio	Assignment	Coupling Constant (Hz)
9.69	s	2	3,17–OH	
7.54	d	2	7,13–H	$J_{7\text{-}8} = J_{12\text{-}13} = 16$
7.32	d	2	1,15–H	$J_{1\text{-}5} = J_{15\text{-}19} = 2$
7.15	dd	2	5,19–H	$J_{4\text{-}5} = J_{18\text{-}19} = 8$
6.82	d	2	4,18–H	$J_{4\text{-}5} = J_{18\text{-}19} = 8$
6.76	d	2	8,12–H	$J_{7\text{-}8} = J_{12\text{-}13} = 16$
6.06	s	1	10–H, enol form	
3.83	s	6	2,16–OCH_3	
^{13}C Chemical Shifts[b] (ppm): 183.7, 149.8, 148.5, 141.2, 126.8, 123.6, 121.6, 116.2, 111.8, 101.3, 56.2				
IR Absorption Bands (cm^{-1}): 3523 (O–H str); 1624 (conjugated C=C str); 1609 (α,β-unsaturated C=O str); 1510 (aromatic ring str); 1268 and 1050 (=C–O–CH_3 str); 1140 (C–OH str)				
MS (*m/z*, rel int): 391.12 (100%, M^+ + Na); 333.06 (56%)				

[a] ^{1}H NMR spectra were collected in DMSO-d_6 at 500 MHz.

[b] ^{13}C NMR spectra were collected in DMSO-d_6 at 125.8 MHz.

Table 31. NMR, IR and MS data of curcumin II [129].

^{1}H NMR[a] Assignments				
Chemical Shift (ppm)	Multiplicity	Integration Ratio	Assignment	Coupling Constant (Hz)
10.07	s	1	17–OH	
9.68	s	1	3–OH	
7.58	d	2	15,19–H	$J_{15\text{-}16} = J_{18\text{-}19} = 9$
7.54	d	1	7–H	$J_{7\text{-}8} = 16$
7.53	d	1	13–H	$J_{12\text{-}13} = 16$
7.32	d	1	1–H	$J_{1\text{-}5} = 2$
7.14	dd	1	5–H	$J_{4\text{-}5} = 9$, $J_{1\text{-}5} = 2$
6.82	m	3	4,16,18–H	$J_{4\text{-}5} = J_{15\text{-}16} = J_{18\text{-}19} = 9$
6.76	d	1	12–H	$J_{12\text{-}13} = 16$
6.69	d	1	8–H	$J_{7\text{-}8} = 16$
6.04	s	1	10–H, enol form	
3.83	s	3	2–OCH_3	
^{13}C Chemical Shifts[b] (ppm): 183.7, 183.6, 160.3, 149.8, 148.5, 141.2, 140.8, 130.8, 126.8, 126.3, 123.7, 121.5, 121.3, 116.4, 116.1, 111.7, 101.4, 56.2				
IR Absorption Bands (cm^{-1}): 3328 (O–H str); 1632 (conjugated C=C str); 1582 (α,β-unsaturated C=O str); 1511 (aromatic ring str); 1262 and 1060 (=C–O–CH_3 str); 1140 (C–OH str)				
MS (*m/z*, rel int): 361.11 (100%, M^+ + Na); 355.05 (25%); 333.06 (54%)				

[a] ^{1}H NMR spectra were collected in DMSO-d_6 at 500 MHz.

[b] ^{13}C NMR spectra were collected in DMSO-d_6 at 125.8 MHz.

Table 32. NMR, IR, and MS Data for Curcumin III [129].

^{1}H NMR[a] Assignments				
Chemical Shift (ppm)	Multiplicity	Integration Ratio	Assignment	Coupling Constant (Hz)
10.07	s	2	3,17–OH	
7.58	d	4	1,5,15,19–H	$J_{1\text{-}2} = J_{4\text{-}5} = J_{15\text{-}16} = J_{18\text{-}19} = 9$
7.54	d	2	7,13–H	$J_{7\text{-}8} = J_{12\text{-}13} = 16$
6.82	d	4	2,4,16,18–H	$J_{1\text{-}2} = J_{4\text{-}5} = J_{15\text{-}16} = J_{18\text{-}19} = 9$
6.70	d	2	8,12–H	$J_{7\text{-}8} = J_{12\text{-}13} = 16$
6.04	s	1	10–H, enol form	
^{13}C Chemical Shifts[b] (ppm): 183.7, 160.3, 140.8, 130.8, 126.3, 121.3, 116.4, 101.4				
IR Absorption Bands (cm^{-1}): 3324 (O–H str); 1624 (conjugated C=C str); 1595 (α,β-unsaturated C=O str); 1510 (aromatic ring str); 1133 (C–OH str)				
MS (*m/z*, rel int): 333.06 (100%, M^+ + Na); 332.10 (19%); 331.09 (90%)				

[a] ^{1}H NMR spectra were collected in DMSO-d_6 at 500 MHz.

[b] ^{13}C NMR spectra were collected in DMSO-d_6 at 125.8 MHz.

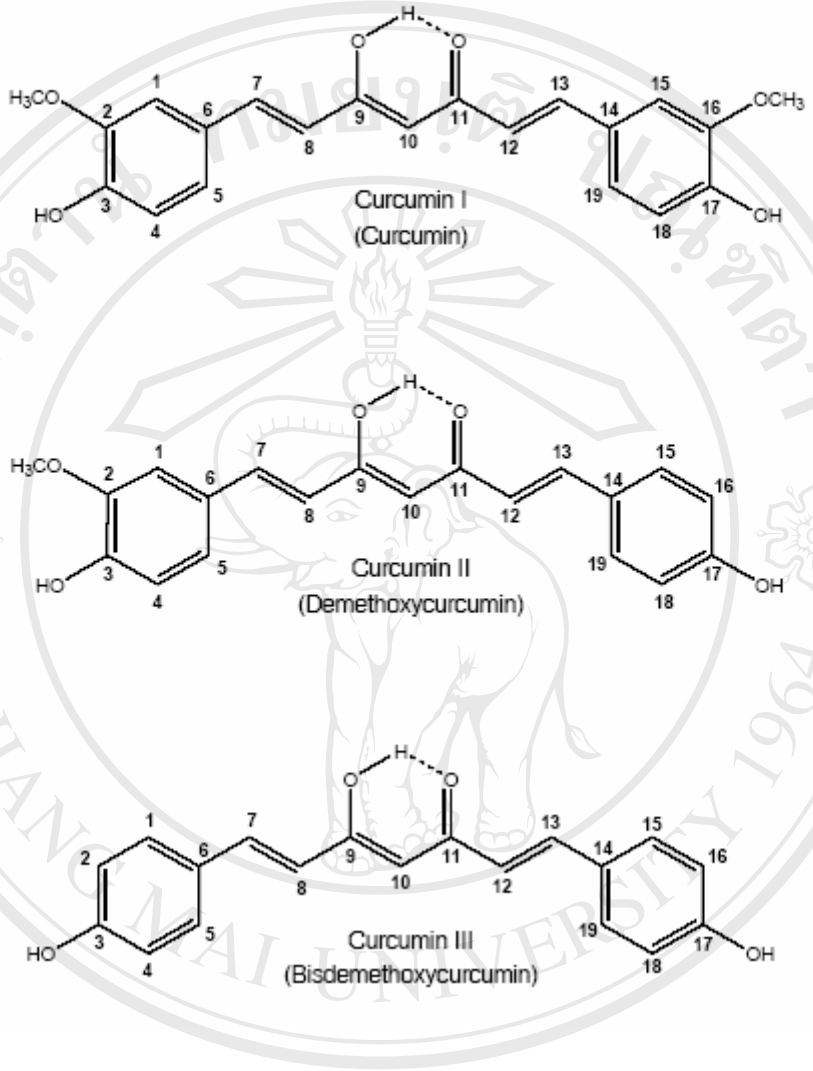


Figure 84. Chemical structure of curcuminoids indicated by NMR data. The three major curcuminoid purified from the turmeric powder are curcumin I (curcumin or diferuloyl methane), curcumin II (demethoxycurcumin or p-hydroxy-cinnamoyl-feruoyl methane) and curcumin III (bisdemethoxycurcumin or pp'-dihydroxy-dicinnamoyl-methane).

Appendix B

Name of Chemicals	Company
Absolute ethanol	E.Merck, Germany
Acetic acid	E.Merck, Germany
Acrylamide	Sigma-Aldrich, USA
Acrylamide (Eastman)	Sigma-Aldrich, USA
Amido black	Sigma-Aldrich, USA
Ammonium persulfate	Sigma-Aldrich, USA
8-AzidoATP	Affinity Labelling Technologies, USA
Bodipy-FL-vinblastine	Molecular Probes, USA
Bodipy-prazosin	Molecular Probes, USA
Bovine serum albumin	PIERCE, USA
Bis (Eastman)	Sigma-Aldrich, USA
C219 antibody	Centocor Dianostics, USA
Calcein-AM	Molecular Probes
Colchicine	Sigma-Aldrich, USA
Coomassie Brilliant blue R-250	Sigma-Aldrich, USA
Coomassie® Plus Protein Assay Reagent	PIERCE, USA
Copper sulfate	E.Merck, Germany
Cyclosporin A	Calbiochem, USA
Dibasic sodium phosphate	Sigma-Aldrich, USA
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich, USA
Dulbecco's modified eagle medium	GIBCO, USA
Dulbecco's phosphate buffered saline (PBS)	GIBCO, USA
ECL reagents	Amersham Pharmacia Biotech, USA
Ethanol	E.Merck, Germany
Etoposide	Sigma-Aldrich, USA
Fetal calf serum	Seromed, USA

Fetal bovine serum	HyClone, USA
FITC conjugated anti-mouse IgG2a 2nd Antibody	Pharmingen, USA
Fluo-4 AM	Molecular Probes, USA
Geneticin	GIBCO, USA
Glycerol	Sigma-Aldrich, USA
Glycine	Sigma-Aldrich, USA
Hank balance salt solution (HBSS)	GIBCO, USA
HEPES	Sigma-Aldrich, USA
High Five insect cells	Invitrogen, USA
High range molecular weight marker	BIO-RAD, USA
Hydrochloric acid	E.Merck, Germany
[^{125}I]-Iodoarylazidoprazosin (IAAP)	Perkin Elmer Life Sciences, USA
Iscove's modified Dulbecco's medium (IMDM)	GIBCO, USA
Indomethacine	Sigma-Aldrich, USA
L-glutamine	Life Technologies, USA
Magnesium chloride	Sigma-Aldrich, USA
Mercaptoethanol	Sigma-Aldrich, USA
Methanol	E-Merck, Germany
MK-571	Alexis Corp, USA
2-(N-morpholino) ethanesulfonic acid (NES)	Molecular Probes
Monobasic sodium phosphate	Sigma-Aldrich, USA
Mouse IgG2a Kappa	Pharmingen
MRPr1antibody	Chemicon, USA
MRPm6 antibody	Chemicon, USA
MTT thiazolyl blue	Sigma-Aldrich, USA
[α-^{32}P]8-azidoATP	Affinity Labelling Technologies, USA
Paclitaxel	Sigma-Aldrich, USA
Penicillin-streptomycin	GIBCO, USA
Potassium chloride	Sigma-Aldrich, USA
Potassium phosphate	Sigma-Aldrich, USA
POPOP	Sigma-Aldrich, USA

PPO	Sigma-Aldrich, USA
Rhodamine 123	Molecular Probes, USA
Low range molecular weight marker	BIO-RAD, USA
Skim milk	Difco, USA
Sodium carbonate	Sigma-Aldrich, USA
Sodium chloride	Sigma-Aldrich, USA
Sodium hydroxide	Sigma-Aldrich, USA
Sodium potassium tartrate	Sigma-Aldrich, USA
Silica gel 60	E.Merck, Germany
SuperSignal ® West Pico Chemiluminescenct substrate	PIERCE, USA
Tris (hydroxymetry) aminomethane	Sigma-Aldrich, USA
Trypsin-EDTA	GIBCO, USA
Tween20	Sigma-Aldrich, USA
UV lamp assembly	PGC scientifics, USA
Verapamil	Sigma-Aldrich, USA
Vinblastine sulphate salt	Sigma-Aldrich,USA
[G- ^{3}H] vinblastine sulphate	Amersham, UK

Appendix C

Instrument	Company
Analytical balance AC 100	Satorious
Autoclave	Tomy autoclave SS-240
Automatic pipette	GIBCO
Bio-Max MR film	Eastman, Kodak, USA
β counter (liquid scintillation counter)	Pharmacia
Carbondioxide incubator	Forma Scientific
Deionized water machine	Barnstead
Distilled water machine	Hamilton
ECL-hyper film	Amersham
Freezer (-80 °C)	Forma scientific
Freezer (-20 °C)	Sanyo
Glassware	Pyrex
Gel doc	Bio-Rad
Hood	British Klocker Switchgear
Hot air oven	Haraeus
Inverted microscope	Nikon
Laminar flow biological cabinet	NUAIR2000 Fembrook Lane Plymouth, MN55447
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
Polystyrene FACS tube (10x75 mm)	Falcon
Power supply	E-CApparatus corporation

Refrigerator	Sanyo, Hitachi
Serological pipette	Pyrex
Shaker bath	Unitronic 320 OR
Slab gel dryer	Savant
Sonicator	Sci Med
Spectrophotometer	MILTON ROY spectronic 1001
25 or 75 cm^3 T-flask	Nunc
96 or 24 or 6 well plate	Nunc
STORM 860 phosphorimager system	Molecular dynamics, USA
Trans-blot 9® electrophoretic transfer cell	BIIO-RAD
Untracentrifuge	Ivan Sorval Inc., USA
Vortex	Scientific industries
Water bath	GFL 1083

Appendix D

Preparation of some reagents and buffers

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

Adjusted pH to 7.2-7.4 then topped up volume with deionized water 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. Completed DMEM without phenol red

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

Stored at 4°C.

4. Freezing solution

Fetal calf serum	9.2	ml
DMSO	0.8	ml

Stored at 4°C.

5. Eagle's minimum essential medium (MEM)

MEM	440	ml
Fetal calf serum	50	ml
Pen/strep (stock 5000 unit/ml)	5	ml
Glutamine (stock 200 mM)	5	ml

Stored at 4°C.

6. Geneticin, G418 (100 mg/ml)

Geneticin	5	g
Incomplete medium	50	ml

Adjusted pH to 7.2-7.4 with $NaHCO_3$, steriled by filtration with membrane pore size 0.2 μm.

Measurement of cell survival

1. MTT stock dye solution

MTT	1.0	g
PBS pH 7.4	200	ml

Filtrated 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.

Measurement of fluorescence drug accumulation

1. Rhodamine123 (1 mg/ml)

Rhodamine123	0.001	g
DMSO	1	ml

Aliquot and stored at -20^{o}C

2. Calcein AM (Molecular probe, $C_{46}H_{46}N_2O_{23}$ Mw 994.87), 1 mM

Calcein AM	1	mg
DMSO	1	ml

Stored at -20^{o}C, in dark condition (avoid prolonged or repeated exposure)

3. Fluo-4, AM (Molecular probe, $C_{51}H_{50}F_2N_2O_{23}$ Mw 1096.95), 1 mM

Fluo-4, AM	1	mg
DMSO	912	μl

Stored at -20^{o}C, in dark condition (avoid prolonged or repeated exposure)

4. Bodipy® FL vinblastine (Molecular probe, $C_{58}H_{69}BF_2N_6O_9$ Mw 1043.02) (100 µM)

Bodipy® FL vinblastine	100	µg
DMSO	960	µl

Stored at -20°C, in dark condition (avoid prolonged or repeated exposure)

5. Bodipy® FL prazosin (Molecular probe, $C_{28}H_{32}BF_2N_7O_3$ Mw 563.41), 100 µM

Bodipy® FL prazosin	1	mg
DMSO	1775	µl

Stored at -20°C, in dark condition (avoid prolonged or repeated exposure)

6. Verapamil stock (50 mg/ml)

Verapamil	0.05	g
DMSO	1	ml

Aliquot and stored at -20°C

7. Cyclosporin A (Calbiochem, $C_{62}H_{111}N_{11}O_{12}$ Mw 1202.6), 10 mM

Cyclosporin A	100	mg
DMSO	8.32	ml

Aliquot and stored at -20°C

8. MK-571.sodium salt (Alexis Biochemicals Corporation, $C_{26}H_{26}CIN_2O_3S_2$.Na Mw 514.1), 20 mM

MK-571	5	mg
DMSO	486	µl

Aliquot and stored at -20°C

9. Fumitremorgin C (FTC, gift from Dr. Susan Bates,NCI/NIH, $C_{22}H_{25}N_3O_3$ Mw 379), 10 mM

FTC	2	mg
DMSO	530	µl

Aliquot and stored at -20°C

10. Hank's balance salt solution (HBSS) without phenol red and sodium bicarbonet

HBSS powder	9.7	g/package
NaHCO3	0.35	g
HEPES	2.603	ml

0.34% 2-mecaptoethanol	1.0	ml
Deionized distilled water	800	ml

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

11. Complete HBSS without phenol red

Incomplete HBSS	90	ml
Fetal calf serum	10	ml

Store at 4 ^{o}C

12. Complete Iscove's Modified Dulbecco's Medium (IMDM)

IMDM	500	ml
Fetal calf serum	25	ml

Store at 4 ^{o}C

13. Phosphate buffer saline (PBS) containing 0.1% of Bovine Serum Albumin (BSA)

PBS	500	ml
BSA (powder)	0.5	g

Store at 4 ^{o}C

Measurement of radiolabeled drug accumulation and efflux

1. Sodium hydroxide (3N)

NaOH	12	g
Deionize distilled water	100	ml

2. Hydrocholic acid (6N)

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

Crude membrane preparation from Baculovirus-infected HF cells

1. PBS : Dulbecco's phosphate buffered saline without Ca^{2+} and Mg^{2+}

2. Homogenization buffer for 500 ml

1 M Tris HCl, pH 7.5 (final 50 mM)	25	ml
Mannitol (final 50 mM)	4.55	g
125 mM EGTA (final 2 mM)	8	ml
Aprotinin (final 1%)	5	ml
1.0 M DTT(final 2 mM)	1	ml
300 mM AEBSF (final 1 mM)	1.7	ml
Deionize distilled water up to	500	ml

3. Resuspension buffer for 500 ml

1 M Tris HCl, pH 7.5 (final 50 mM)	25	ml
Mannitol(final 300 mM)	27.5	g
125 mM EGTA(final 2 mM)	4	ml
Aprotinin (final 1%)	5	ml
1.0 M DTT(final 1 mM)	0.5	ml
300 mM AEBSF(final 1 mM)	1.7	ml
Deionize distilled water up to	500	ml

4. Final resuspension buffer, pH 8.0 for 250 ml

1 M Tris HCl, pH 8.0 (final 20 mM)	5	ml
Mannitol(final 300 mM)	13.75	g
125 mM EGTA(final 1 mM)	2	ml
Glycerol (final 10%)	25	ml
Aprotinin (final 1%)	2.5	ml
1.0 M DTT(final 1 mM)	0.25	ml
300 mM AEBSF(final 1 mM)	840	µl
Deionize distilled water up to	250	ml

Crude membrane preparation from HEK 293 cells

1. Lysis buffer for 500 ml

1 M Tris HCl, pH 7.5 (final 10 mM)	5	ml
NaCl (final 10 mM)	0.3	g
2 M $MgCl_2$ (final 1 mM)	250	µl
Aprotinin (final 1%)	5	ml

300 mM AEBSF(final 1 mM)	1.7	ml
Deionize distilled water up to	500	ml

2. Resuspension buffer (TSNa buffer) for 500 ml

1 M Tris HCl, pH 7.5 (final 20 mM)	10	ml
NaCl (final 50 mM)	1.5	g
Sucrose (final 250 mM)	42.8	g
Aprotinin (final 1%)	5	ml
300 mM AEBSF(final 1 mM)	1.7	ml
Deionize distilled water up to	500	ml

3. Storage buffer, pH 8.0 for 250 ml

1 M Tris HCl, pH 7.5 (final 20 mM)	5	ml
NaCl (final 50 mM)	0.75	mg
Sucrose (final 250 mM)	21.4	g
Glycerol (final 10%)	25	ml
Aprotinin (final 1%)	2.5	ml
300 mM AEBSF(final 1 mM)	840	µl
Deionize distilled water up to	250	ml

Protein determination

Amido black protein assay

1. Stained solution for 100 ml

Amidoschwartz 10 B (Napthol blue black)	0.1	g
Methanol	45	ml
Glacial acetic acid	10	ml
Deionized water	45	ml

2. Destained solution for 100 ml

Methanol	90	ml
Acetic acid	2	ml
Deionized water	8	ml

3. Elution buffer for 100 ml

NaOH (final 25 mM)	0.1	g
EDTA (final 0.05 mM)		

Ethanol (50%)	50	ml

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 topped up volume with deionized water 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 with deionized water to 100 ml 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 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 or low 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 up to	100	ml

10. Coomassie blue destaining solution

Methanol	100	ml
Acetic acid	50	ml
Deionized water 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

Western blot analysis

1. Bloting buffer

Tris-base	3.03	g
Glycine	14.4	g
Methanol	200	ml

Dissolved in deionized 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

Adjusted volume to 250 ml with deionized distilled water.

3. PBS, pH7.4

Na_2HPO_4	1.3	g
NaH_2PO_4	0.204	g
NaCl	7.28	g
Distilled deionized water	700	ml

Adjusted pH to 7.4 then topped up volume with deionized water to 1,000 ml and steriled by filtration.

4. Amido black destaining solution

Isopropanol	125	ml
Acetic acid	50	ml

Adjusted volume to 250 ml with deionized water.

5. Blocking reagent (20%)

Skim milk	5	g
Anti foam	20	µl

Dissolved in 25 ml of PBS , pH 7.4.

6. Washing buffer

PBS pH 7.4	500	ml
Tween 20	250	µl

7. Film developer (Kodak)

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

Adjusted volume with deionized water to 250 ml. Stored at 4 °C in dark.

8. Film fixer (Kodak)

Part A	50	ml
Part B	10	ml

Adjusted volume with deionized water to 250 ml. Stored at 4 °C in dark.

ATPase assay

1. ATPase buffer (MES-Tris-HCl)

100 mM MES	20	ml
1 M KCl	2.5	ml
0.25 M NaN_3	1	ml
0.125 M EGTA	400	µl
Ouabain	29.2	mg
1 M DTT	50	µl
2 M $MgCl_2$	250	µl

2. 2-(N-Morpholino) ethanesulfonic acid,MES pH 6.8, 100 mM, 100 ml

MES	1.95	g
Deionized water	50	ml

Adjusted pH to 6.8 with 2 M Tris-HCl solution

3. Tris-HCl, 2 N for 25 ml, Mw 121.14

Tris-HCl	6.01	g
Deionized water up to	25	ml

4. Sodium orthovanadate, freshly prepared, 10 mM

Sodium orthovanadate	1.8	mg
Deionized water	1	ml

Incubated at 100^{o}C for 3 min then measured the absorbance at 268 nm (orthovanate at 268 nm OD = 3.6 corresponds to 1 mM stock), adjusted the concentration to 10 mM with deinoized water

5. Beryllium sulfate terahydrate (Fluka, $BeO_4S.4H_2O$ Mw 177.4), 1 M

$BeSO_4$	0.887	g
Deionized water	5	ml

6. Sodium fluoride, NaF , 1 M , Mw 42, 1 M

NaF	0.21	g
Deionized water	5	ml

7. ATP solution

ATP	650	mg
N-methyl-D-glutamine	625	mg
Deionized water	7	ml

Adjusted pH with 6 N HCl to pH 7.0, then top up volume to 10 ml. Diluted 1:10,000 then measured the absorbance at 259 nm, calculated the concentration as following; extinction coefficient = 15400

$$\text{ATP concentration (mM)} = \frac{\text{Absorbance value at OD 259 nm}}{15400}$$

Aliquot and stored at -80^{o}C

8. SDS, 5%

10% SDS	25	ml
Deionized water	25	ml

9. Pi reagent for 500 ml

Ammonium molybdate (final 1%)	5	g
Antimony potassium tartrate (final 0.014%)	70	mg
36.2 N Sulfuric acid (final 2.5 N)	34.5	ml

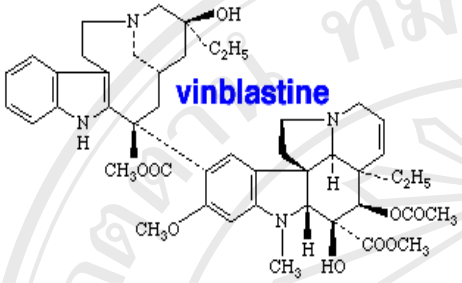
Adjusted volume with deionized water to 500 ml. Stored at room temperature in dark container to protect from light

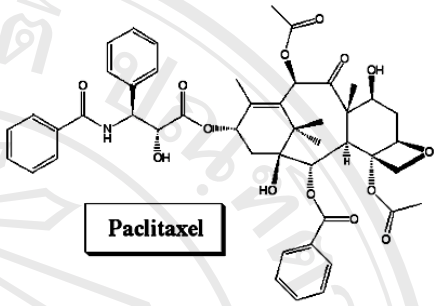
10. Ascorbic acid, 1% (freshly prepared)

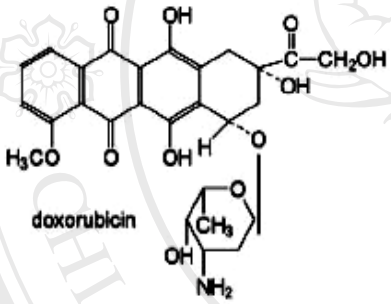
Ascorbic acid	100	mg
Deionized water	10	ml

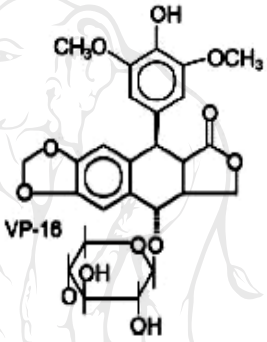
Appendix E

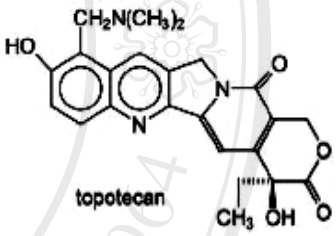
Structures of the compounds used in the present study

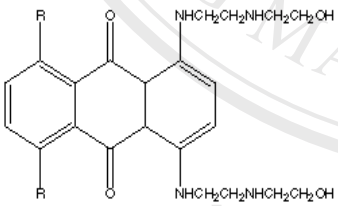


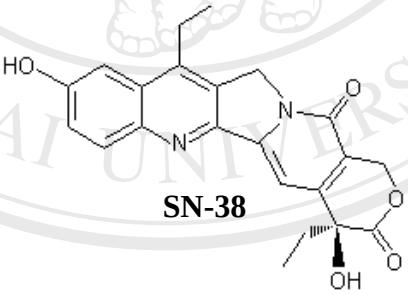




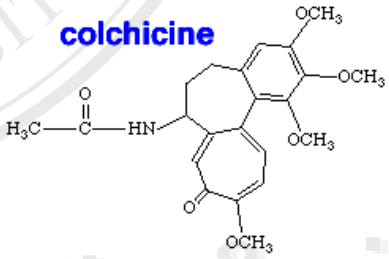




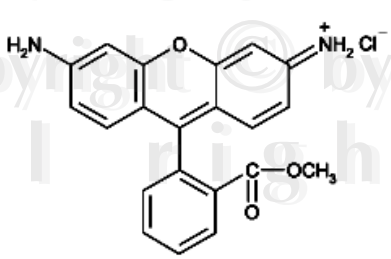


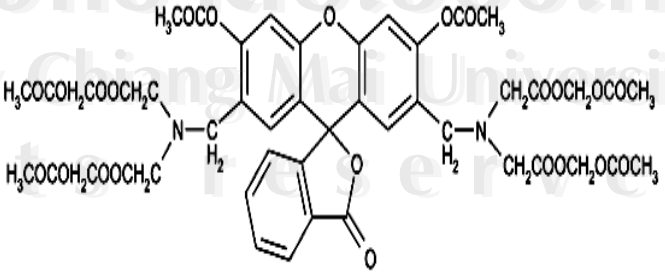


SN-38

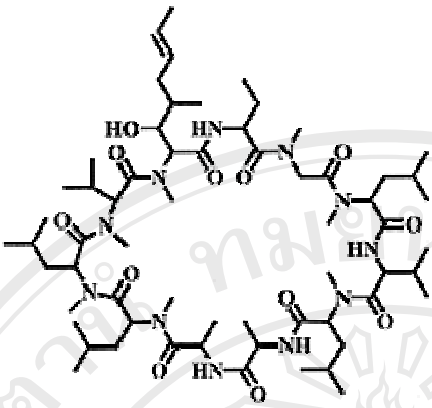


mitoxantron



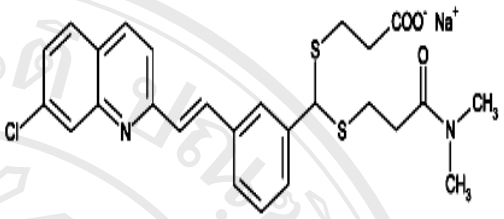


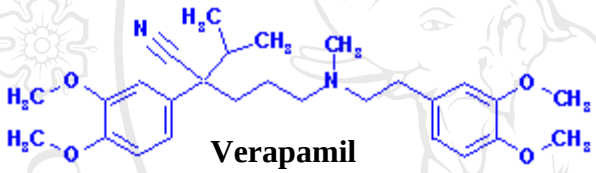
Rhodamine123 **Calcein AM**



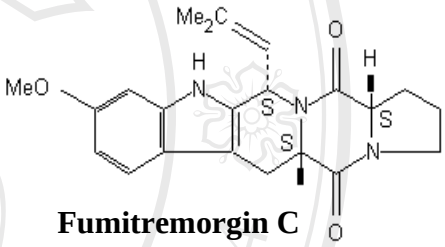
Cyclosporin A

MK-571

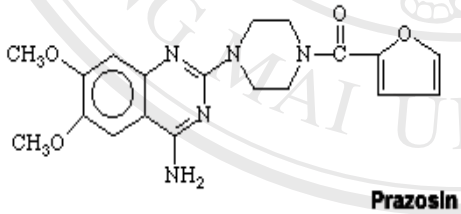




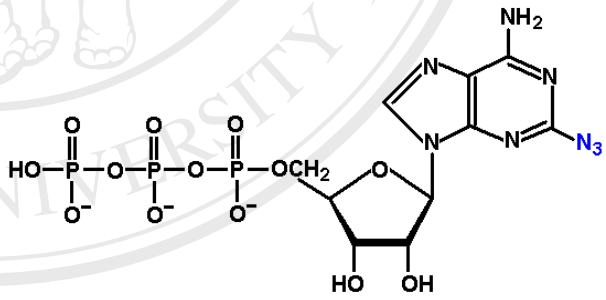
Verapamil



Fumitremorgin C

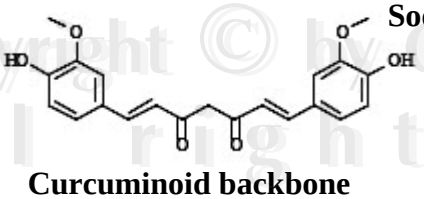


Prazosin

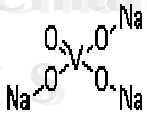


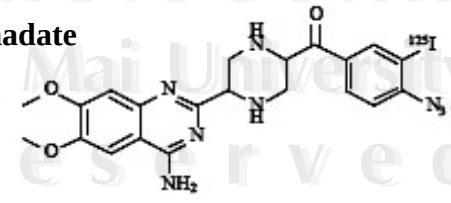
2-Azidoadenosine 5'-triphosphate ($2N_3ATP$)

Sodium orthovanadate



Curcuminoid backbone





Iodoaryl azidoprazosin(IAAP)

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Publications

1. Chearwae W, Anuchapreeda S, Nandigama K, Ambudkar S and Limtrakul P, *Biochemical mechanism of modulation of human P-glycoprotein (ABCB1) by curcumin I, II and III purified from Turmeric powder.* Biochem Pharmacol, 2004. **68**: p. 2043-2052
2. Chearwae W, Wu CP, Chu HY, Lee TR, Ambudkar SV and Limtrakul P, *Curcuminoids purifed from turmeric powder modulate the function of human multidrug resistance protein 1 (ABCC1).* Cancer Chemother Pharmocol, 2005. **14**: p. 1-13
3. Limtrakul P, Chearwae W and Anuchapreeda S, *The effect of curcumin on the proliferation of cancer cell lines*. Chiang Mai Med Bull, 1999. **38**(3-4): p. 55-61
4. Limtrakul P, Chearwae W and Leukamharn S, *Inhibitory effect of curcumin on TPA (12-O-tetrahydecanoylphorbol-13-acetate)-induced activation of protein kinase isoenzyme epsilon and c-fos protein level in human keratinocytes*. Sceince Asia, 2005. **31**:p. 113-120
5. Chearwae W and Limtrakul P, *Turmeric: Multidrug resistance modulator*. The Medicine Journal, 2005. **82**: p. 59-60

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1. Limtrakul P and Chearwae W. Effect of curcumin on level and distribution of TPA-induced PKC-epsilon in human keratinocytes. Proceeding of the FASEB Journal, June 3rd-8th 2000
2. Chearwae W, Sakkhachornphop S, Chumpookhod A, Chumpookhod S, Palanan P, Tejafong K, Kitisri J and Chanbancherd P. Application of dried blood spots in molecular epidemiological study of HIV-1. The 19th Annual Health Science Meeting, August 24th 2001, Chiang Mai, Thailand
3. Chearwae W, Ambudkar S and Limtrakul P. Modulation of P-glycoprotein function by curcumin, demethoxycucumin and bisdemethoxycurcumin in multidrug resistant human KB cells. NIH Research Festival, October 4th -17th 2003, Maryland USA
4. Chearwae W, Nandigama K, Ambudkar S. MDR reversing activity of curcuminoids isolated from turmeric: Mechanistic study. The fourth Annual Fellows and Young Investigators Retreat, March 9th -11th 2004, Virginia USA
5. Chearwae W, Anuchapreeda S, Nandigama K, Ambudkar S, and Limtrakul P. Biochemical mechanism of modulation of human P-glycoprotein (ABCB1) by curcumin I, II and III purified from turmeric powder. 30th Congress on Science and Technology of Thailand, October 19th – 21st 2004 , Bangkok, Thailand

Oral presentations

In English

1. Chearwae W. Modulation of MRP1 functions by curcuminoids. MDR meeting, February 19th 2004, NCI, NIH, Maryland USA
2. Chearwae W, Ambudkar S, Limtrakul P. Curcumin I, II and III: novel modulators reversing multidrug resistance in HEK transfected with ABCG2. The Forth National Symposium on Graduate Research, August 10th – 11th 2004, Lotus Pang Suan Kaew Hotel, Chiang Mai, Thailand
3. Chearwae W, Anuchapreeda S, Nandigama K, Ambudkar S, and Limtrakul P. Biochemical mechanism of modulation of human P-glycoprotein (ABCB1) by curcumin I, II and III purified from turmeric powder. August 19th , Faculty of Medicine, Chiang Mai, Thailand

4. Chearwae W, Ambudkar S, Limtrakul P. Inhibition of ABCG2 activity and reversal of multidrug resistance in vitro by curcuminoids. The Forth Joint Seminar on Biomedical Science among Chiang Mai University, Kunming Medical College, Prince of Songkhla University and Toho University, October 11th – 12th 2004, Rydges Tapae Hotel, Chiang Mai, Thailand

5. Chearwae W, Wu C.P, Chu H.Y, Lee R, Ambudkar S, Limtrakul P. Curcuminoids purified from turmeric powder modulate the function of human multidrug resistance protein 1 (ABCC1). RGJ-Ph.D Congress VI, April 28th -30th 2005, Jom Tean Hotel, Chon Buri, Thailand

In Thai

1. Chearwae W, Ambudkar S, Limtrakul P. Modulation of multidrug resistance related protein 1 (MRP1) function by curcuminoids purified from turmeric powder. Annual Biochemical Research Meeting, October 21st – 22nd 2004, Department of Biochemistry, Chiang Mai University, Chiang Mai, Thailand

2. Chearwae W, Ambudkar S, Limtrakul P.Biochemical mechanism of modulation of ABC transporters: P-glycoprotein (P-gp, ABCB1), Multidrug resistance related protein 1 (MRP1) and Mitoxantrone resistance protein (MXR, ABCG2) by curcuminoids. RGJ-Ph.D Congress VI, April 28th -30th 2005, Jom Tean Hotel, Chon Buri, Thailand