APPENDIX

APPENDIX A

List of the chemicals and materials were used in the study

Chemicals/materials	Source
Absolute ethanol	E. Merck, Germany
Absolute methanol (HPLC grade)	E. Merck, Germany
Acetic acid	J.T. Baker Inc., USA
Aflatoxin B_1	Sigma-Aldrich Chemical Co., USA
AFB_1-ovalbumin conjugate	IARC, Lyon, France
Albumin from chicken egg (OVA)	Sigma-Aldrich Chemical Co., USA
Alkaline Phosphatase	Sigma-Aldrich Chemical Co., USA
Ammonium sulfate	May & Baker Ltd., England
2-amino-2-methyl-1,3-propanediol	Sigma-Aldrich Chemical Co., USA
Anti-AFB_1-lysine antibody	IARC, Lyon, France
Boric acid	E. Merck, Germany
Bovine serum albumin	Sigma-Aldrich Chemical Co., USA
Citric acid trisodium salt	Fluka A.G., Buchs, Switzerland
Coomasie Brilliant Blue G250	Fluka A.G., Buchs, Switzerland
2 -deoxyguanosine	Sigma-Aldrich Chemical Co., USA
Desferoxamin (Desferol)	Novartis Pharma AG, Basle, Switzerland
Diethyl ether	BDH, England
Dimethyl sulfoxide	E. Merck, Germany
Disodium hydrogen phosphate	E. Merck, Germany
Disodium tetraborate	Sigma-Aldrich Chemical Co., USA
Fetal calf serum	Seromed, Germany

Chemicals/materials	Source
Goat anti-rabbit IgG peroxidase conjugate	Sigma-Aldrich Chemical Co., USA
L-γ-glutamyl-p-nitroanilide	Sigma-Aldrich Chemical Co., USA
Hydrochloric acid	Fluka A.G., Buchs, Switzerland
Hydrogen peroxide	Fluka A.G., Buchs, Switzerland
Isopropanol	E. Merck, Germany
Magnesium chloride	May & BAKER, England
Nuclease P_1 (From *P. citinum*)	Sigma-Aldrich Chemical Co., USA
Orthophosphoric acid 85%	E. Merck, Germany
2-oxo-glutaric acid	Fluka A.G., Buchs, Switzerland
Potassium chloride	J.T. Baker Inc., USA
Potassium dihydrogen phosphate	Fluka A.G., Buchs, Switzerland
Protease	QIAGEN, GmbH, Germany
Proteinase K	Sigma-Aldrich Chemical Co., USA
3,3 ,5 ,5 -tetramethylbenzidine	Sigma-Aldrich Chemical Co., USA
Tween 20	Sigma-Aldrich Chemical Co., USA
Triton X-100	Sigma-Aldrich Chemical Co., USA
Sodium chloride	E. Merck, Germany
Sodium dihydrogen phosphate	Fluka A.G., Buchs, Switzerland
Sodium iodide	APS Chemical, Ltd. Australia
Sodium pyruvate	Sigma-Aldrich Chemical Co., USA
Sucrose	Fisher, U.K
Zinc chloride	Sigma-Aldrich Chemical Co., USA

APPENDIX B

List of the instruments used in the study

Instrument	Model	Source
Analytical balance	AC100	Metter Instrument A.G, Switzerland
Autoclave	SS-245	Tomy Seiko Co.Ltd., Japan
Capillary electrophoresis	P/ACE MDQ	Beckman Instruments Inc., Fullerton, CA, USA
Centrifuge	CR3i	JOUAN S.A., France
ELISA plate reader	MCC/340 Titertek multiscan	ICN, Flow, USA
ELISA plate shaker	DESAGA, TPM-2	Germany
Freeze dryer	ALPHA 1-2	MARTIN CHRIST, Germany
Minishaker	VIBRAX-VXR	IKA-WORK, INC., USA
pH meter		Eutech Cybernetics, Singapore
Refrigerated centrifuge	H-103N	Kokusan, Japan
Refrigerator (-20°C)		Sanyo, Thailand
Refrigerator (-80°C)		Forma Scientific
Semi-automatic photometer	Screen master 3000	Biochemical Systems, ITALY
Sep-Pak C18 cartridge		Sigma Chemical Co. Ltd.
Shaking water bath	Grant OLS 200	Grant Instruments Cambridge Ltd.
Speed Vac Concentrator	UNIVAPO 100H	UniEquip, Martinsried, Germany
UV-VIS spectrophotometer	UV1200	Shimadzu Co., Japan
Water bath	Yamaha, Type 1	Japan

APPENDIX C

Reagents preparation

1 Preparation of reagents for Albumin extraction

1.1 Saturated ammonium sulfate

Ammonium sulfate was dissolved in 200 ml of distilled water until the powder could not be dissolved.

1.2 Phosphate buffer saline (PBS) pH 7.4 (2 liters)

KCl	0.4 g
KH_2PO_4	0.4 g
$Na_2HPO_4 \cdot 12H_2O$	4.6 g
NaCl	16.0 g

Ingradients were dissolved in 2 liters of distilled water. After completely dissolved, pH of solution was adjusted to 7.4 by 1 N HCl.

2 Preparation of reagents for protein quantitation

2.1 Bradford's reagent

Coomassies Brilliant Blue G 250	500 mg
95% (v/v) ethanol	250 ml
85% (w/v) phosphoric acid	500 ml

Adjust to a volume of 5 liters with double-distilled water and store in dark at room temperature for up 6 months. Filter the solution prior to use.

2.2 Albumin standard (Sigma), 500 μg/ml

5 mg of BSA was dissolved in 10 ml of distilled water

3 Preparation of reagents for albumin hydrolyzation

3.1 Proteinase K, 10 mg/ml

10 mg of proteinase K was dissolved in 10 ml of distilled water

3.2 Bovine serum albumin , 100 mg/ml

100 mg of BSA was dissolved in 10 ml of PBS, pH 7.4

4 Preparation of reagents for ELISA

4.1 PBS-Tween (10 liters)

KCl	2.0 g
KH_2PO_4	2.0 g
$Na_2HPO_4 \cdot 12H_2O$	23.0 g
NaCl	80.0 g

After ingredients were completely dissolved in 10 liters of distilled water, pH of solution was adjusted to 7.4 by 1 N HCl. Then 5.0 ml of PBS solution was replaced by Tween 20 and mixed well.

4.2 Citrate buffer pH 5.0

Citric acid trisodium salt	7.35 g
Distilled water	500 ml

After completely dissolved, pH of solution was adjusted to 5.0 by 1 NHCl and then stored in -20°C.

4.3 TMB solution

3,3',5,5'-tetramethylbenzidine	5 g
Dimethylsulfoxide	500 µl

Note; TMB was fleshly dissolved in DMSO prior to use.

4.4 TMB substrate

Citrate buffer	10.0 ml
TMB solution	100 µl

30% H_2O_2	2 µl

Note; Ingredients was mixed well before use.

5 Preparation of reagents for determination of GGT activity

5.1 GGT buffer

Dissolve 1.26 g 2-amino-2-methyl-1,3-propanediol in about 80 ml of distilled water and pH was adjusted to 8.2 by 1 N HCl. Then solution was made up to 100 ml with distilled water.

5.2 GGT reagents

L-γ-glutamyl-p-nitroanilide	0.1122 g
Glycylglycine	1.3210 g

Ingredients were dissolved in 100 ml of GGT buffer

6 Preparation of reagents for DNA extraction

This protocol is followed by ESCODD. Deionized water is used for all solution.

6.1 Buffer A

Tris	0.12 g
Sucrose	10.95 g
$MgCl_2$	0.10 g
desferoxamine mesylate	0.0065 g

After ingredients were completely dissolved in 80 ml of deionized water, pH of solution was adjusted to 7.5 by 1 N HCl and made up to 100 ml with deionized water.

6.2 Buffer A (containing Triton X-100)

Tris	0.12 g
Sucrose	10.95 g
$MgCl_2$	0.10 g
desferoxamine mesylate	0.0065 g

After ingredients were completely dissolved in 80 ml of deionized water, pH of solution was adjusted to 7.5 by 1 N HCl and make up to 100 ml with deionized water. Then 1% Triton X-100 was added. Make up buffer to 200 ml. Store at -20°C.

6.3 Buffer B

Tris	0.12 g
Na_2EDTA	0.19 g
Desferoxamine mesylate	0.0098 g

After ingredients were completely dissolved in 80 ml of deionized water, pH of solution was adjusted to 8.0 by 1 N HCl and made up to 100 ml with deionized. Store at -20°C.

6.4 NaI solution

Tris	0.48 g
Na_2EDTA	0.74 g
desferoxamine mesylate	0.0197 g

After ingredients were completely dissolved in 70 ml of deionized water, with vigorous stirring, 20 g of NaI was added. When this has dissolved, more NaI and continue until all NaI has been added. When the ingredients have nearly dissolved, pH of solution was adjusted to 8.0 by 1 N HCl. and make up to 100 ml with deionized water. Store at 4°C.

Note; This solution should not turn yellow.

6.5 Protease, 20 mg/ml

20 mg of protease was dissolved in 1 ml of deionized water. Store at 4°C.

6.6 RNAase A, 100 mg/ml

100 mg of RNAase A was dissolved in 1 ml of 10 mM Tris, pH 8.0.

6.7 deferoxamine mesylate, 0.1 mM

0.0065 mg of desferoxamine mesylate was dissolved in 100 ml of 10 mM Tris-HCl, pH 7.4.

7 Preparation of reagents for DNA hydrolyzation

7.1 Sodium acetate, pH 4.8

$CH_3COONa \cdot 3H_2O$	0.2722 g
$ZnCl_2$	0.0136 g

After ingredients were completely dissolved in 80 ml of deionized water, pH of solution was adjusted to 4.8 by 1 N Acetic acid and made up to 100 ml with deionized.

7.2 Nuclease P_1

Stock nuclease P_1 (4 U/µl) was prepared by dissolve 1 mg of nuclease P_1 (Sigma N8630) in 500 µl 20 mM sodium acetate, pH 4.8. Store at -20°C.

Before use, 20 µl of Stock nuclease P_1 was diluted in 180 µl of 20 mM sodium acetate, containing 1 mM $ZnCl_2$, pH 4.8 to obtain the working enzyme containing 0.4 U/µl nuclease P_1.

7.3 Alkaline phosphatase, 5U/10 µl

Working enzyme was prepared as follow:

Alkaline phospatase	5 µl
10 ×Tris-HCl	10 µl
Deionized water (sterile)	85 µl

The ingredients were mixed to obtain the working enzyme containing 0.5 U/µl alkaline phosphatase.

8 Preparation of reagents for CE

8.1 Boric acid, 1 M

0.6183 g of boric acid was dissolved in 100 ml of deionized water.

8.2 Borate buffer, 10 mM

0.3814 g of Sodium tetraborate was dissolved in 80 ml of deionized water. Then pH of solution was adjusted to 7.4 by 1M boric acid and made up to 100 ml with deionized water.

APPENDIX D

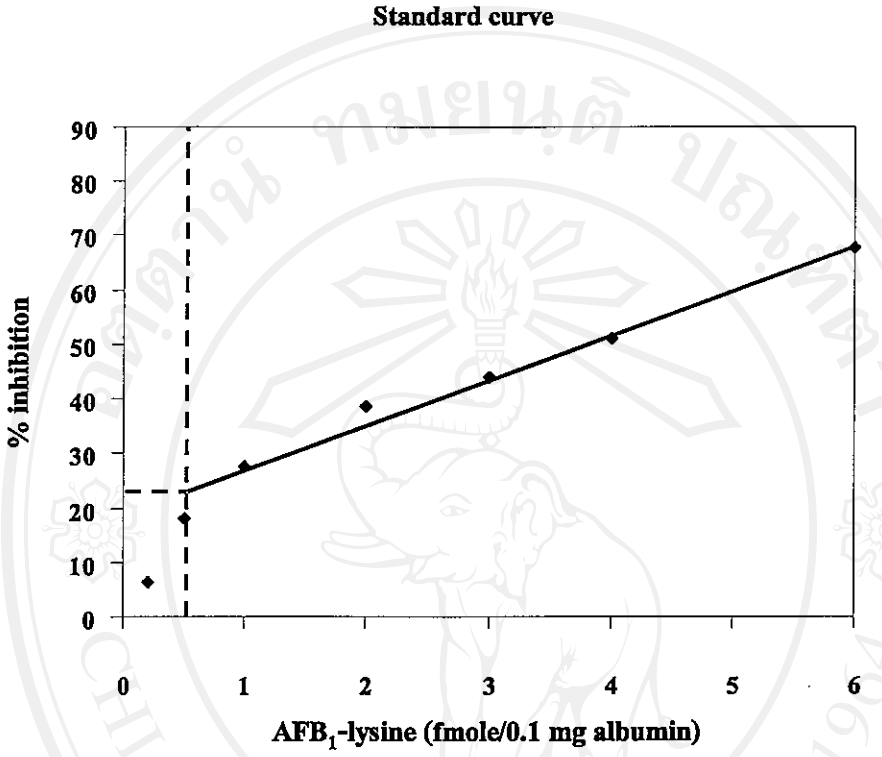

Figure 28 Standard inhibition curve in ELISA for AFB_1-lysine

The detection limit are shown by vertical dash line at 23% inhibition represent about 0.5 fmole/0.1 mg albumin (or 2.4 pg/mg albumin)

APPENDIX E

Fingerprints of *Centella asiatica* extract used in the study

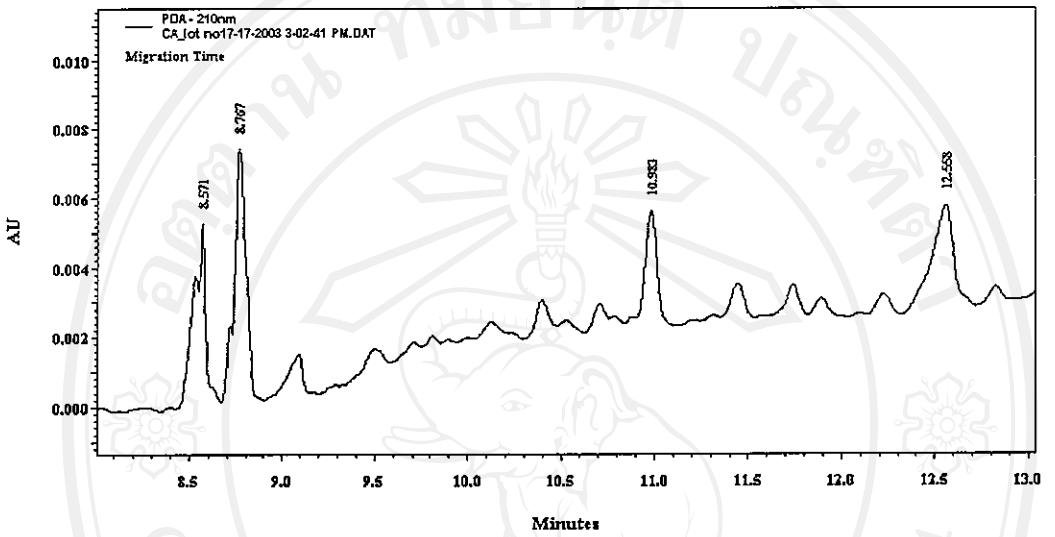

Figure 29 Electropherogram (fingerprint) of the water extract of *C. asiatica* extract (lot no. 1 : March)

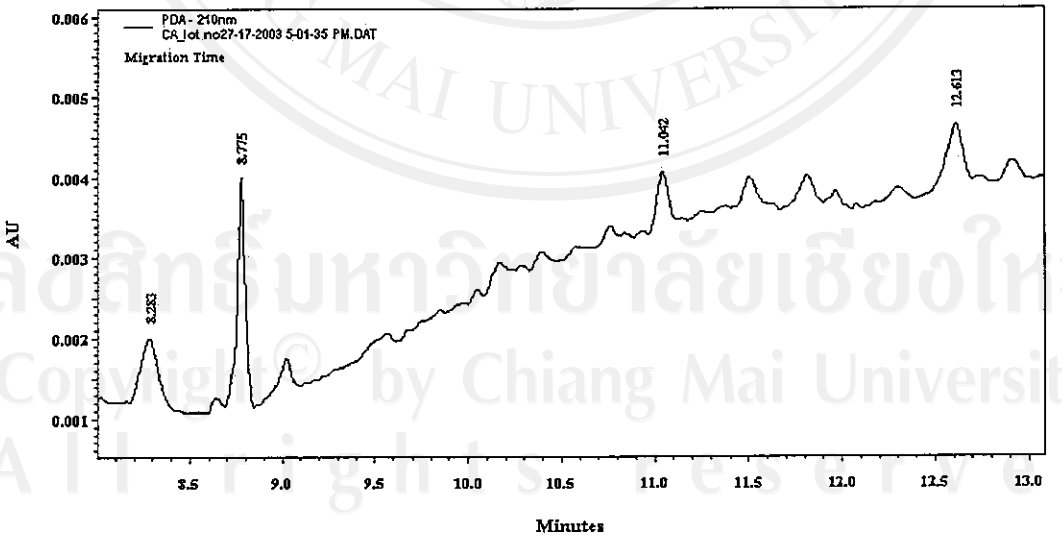

Figure 30 Electropherogram (fingerprint) of the water extract of *C. asiatica* extract (lot no. 2 : March)

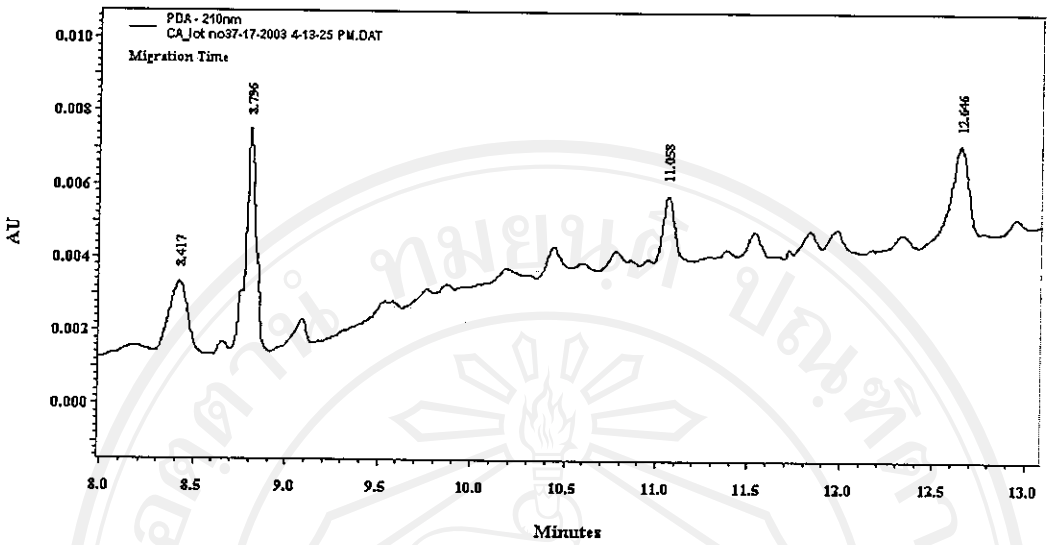

Figure 31 Electropherogram (fingerprint) of the water extract of *C. asiatica* extract (lot no. 3 : March)

CE condition : for determination the fingerprint of *C. asiatica* extract

Capillary:	Uncoated fused silica (diameter 75 μm, Length to detector 21 cm, total capillary length 35 cm)
Capillary temperature:	25 °C
Separation buffer:	30 mM borate buffer pH 9.0
Injection time:	0.5 psi, 5 sec
Separation voltage:	15 kV
Detection:	PDA 210 nm

VITA

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Publication	1. Wongyao, N. and Vinitketkumnuen, U., 2004, "Effect of *Centella asiatica* Extract on Aflatoxin B_1-Albumin Adduct Formation in Wistar Rat", The 4th National Symposium on Graduate Research. Chiang Mai, p. 251. 2. Wongyao, N. and Vinitketkumnuen, U., 2004, "Effect of *Centella asiatica* Extract on Aflatoxin B_1-Albumin Adduct Formation in Wistar Rat", Annual Biochemical Research Meeting. 4th Annual meeting, Department of Biochemistry, Faculty of medicine, Chiang Mai University, p. 41-42.