APPENDIX

Appendix 1. List of chemicals and materials used in the study.
The chemicals used were analytical grade unless specified.

Chemicals/materials	**Source**
Absolute Ethanol	Wako pure chemical industries Ltd., Japan
Azoxymethane	Sigma Chemical Co., U.S.A.
Barium chloride	M&B Ltd., England
Bis-Tris [bis(2-hydroxymethyl) imino-tris(hydroxymethyl)methane]	Sigma Chemical Co., U.S.A.
Bovine serum albumin	Sigma Chemical Co., U.S.A.
tert-butylhydroperoxide	Sigma Chemical Co., U.S.A.
1-Chloro-2,4-dinitrobenzene	Wako pure chemical industries Ltd., Japan
Chloroform	Wako pure chemical industries Ltd., Japan
Cupric sulfate	Nakarai Chemicals Ltd., Japan
2,6-Dichlorophenolindiphenol	Sigma Chemical Co., U.S.A.
Dicoumarol	Sigma Chemical Co., U.S.A.
Diethyl ether	J.T. Baker, U.S.A.
Dimethyl sulfoxide	E. Merck, Germany
Dipotassium hydrogen phosphate	Wako pure chemical industries Ltd., Japan
Disodium hydrogen phosphate anhydrous	Wako pure chemical industries Ltd., Japan
Ethylene diamine-N,N,N',N'-tetra acetic acid	Wako pure chemical industries Ltd., Japan
Flavin adenine dinucleotide	Sigma Chemical Co., U.S.A.
Folin-phenol reagent	Wako pure chemical industries Ltd., Japan

Glucose-6-phosphate	Sigma Chemical Co., U.S.A.
Glucose-6-phosphate dehydrogenase	Sigma Chemical Co., U.S.A.
Glutathione	Sigma Chemical Co., U.S.A.
Glycine (Amino acetic acid)	Wako pure chemical industries Ltd., Japan
Hydrochloric acid	Wako pure chemical industries Ltd., Japan
Isoamylalcohol	Wako pure chemical industries Ltd., Japan
Magnesium chloride	E. Merck, Germany
Methylene blue	Wako pure chemical industries Ltd., Japan
N^7-methylguanine	Sigma Chemical Co., U.S.A.
Nicotinamide adenine dinucleotide reduced form	Sigma Chemical Co., U.S.A.
p-Nitrophenol	Sigma Chemical Co., U.S.A.
p-Nitrophenyl-β-D-glucuronide	Sigma Chemical Co., U.S.A.
O^6-methylguanine	Dr. K. Ishizaki and Dr. M. Ikenaka, Kyoto University, Japan
Phenol crystal	Wako pure chemical industries Ltd., Japan
Potassium chloride	J.T. Baker, U.S.A.
Potassium dihydrogen Phosphate	Wako pure chemical industries Ltd., Japan
Potassium sodium tartrate	Wako pure chemical industries Ltd., Japan
Proteinase K	Worthincton biochemicasl Co., U.S.A.
Ribonuclease A	Worthincton biochemicasl Co., U.S.A.
Ribonuclease T_1	Worthincton biochemicasl Co., U.S.A.
Sodium cacodylate	Wako pure chemical industries Ltd., Japan
Sodium chloride	Wako pure chemical industries Ltd., Japan
Sodium citrate	Wako pure chemical industries Ltd., Japan
Sodium dithionite	Wako pure chemical industries Ltd., Japan
Sodium dodacyl sulfate	Wako pure chemical industries Ltd., Japan
Sodium hydroxide	Wako pure chemical industries Ltd., Japan
2-Thiobarbituric acid	Wako pure chemical industries Ltd., Japan

Trichloroacetic acid	Wako pure chemical industries Ltd., Japan
Tris(hydroxymethane) aminomethane	Sigma Chemical Co., U.S.A.
UDP-glucuronic acid	Sigma Chemical Co., U.S.A.

Appendix 2. List of instrument used in the study

Instrument	**Model**	**Source**
Autoclave	SS-240	Tomy Sciko Co. Ltd., Tokyo, Japan
Fluorescence detector	RF-530	Shimadzu, Japan
High Performance Liquid Chromatography	LC-5A	Shimadzu, Japan
Light microscope		Olympus, Japan
pH meter	701A	Orioh Inc., U.S.A.
Refrigerator	GR-2000TG	Toshiba, Thailand
Refrigerator (-80°C)		Sanyo, Thailand
Spectrophotometer		Shimadzu, Japan
Super speed centrifuge	RC2-B	Ivan Sorval Inc., U.S.A.
water bath	Type 1 No. 7095	Yazama, Japan

Appendix 3 Reagent preparation

10% Formaldehyde in PBS (2 liters)

PBS	1800	ml
formaldehyde	200	ml

Phosphate buffer saline (PBS) pH 7.4 (1 liter)

NaCl	8	g
KCl	0.2	g
Na_2HPO_4	1.44	g
KH_2PO_4	0.24	g
dH_2O	800	ml

adjust pH 7.4 using 1 N HCl then adjust volume to 1 liter

Methylene blue dye 0.2% in saline

Methylene blue	1.0	g
Saline	500	ml

0.1 M Tris-HCl buffer pH 8.0

Tris	12.11	g
dH_2O	800	ml

adjust pH 8.0 using 6N HCl and then adjust volume to 1 liters.

10 mM Sodium cacodylate pH 7.0

Sodium cacodylate	0.214	g
dH_2O	80	ml

adjust pH 7.0 and then adjust volume to 100 ml

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Isoamylalcohol (3-methyl-1-butanol)	1	ml
Chloroform	24	ml

0.05 M EDTA

EDTA.2Na	18.6	g
dH_2O	1000	ml

50 mM Bis-Tris-1 mM $MgCl_2$ pH 6.5

Bis-Tris (50 mM)	1.046	g
$MgCl_2.6H_2O$ (1 mM)	0.020	g
dH_2O	80	ml

adjust pH 6.5 and then adjust volume to 100 ml

10% Sodium dodesyl sulfate-10mM EDTA-20mM Tris-HCl pH 7.4

Tris (20 mM)	1.211	g
EDTA (10 mM)	1.861	g
Sodium dodesyl sulfate	5.0	g
dH_2O	400	ml

adjust pH 7.4 with HCl and then adjust volume to 500 ml

1/100 SSC-0.1 mM EDTA

Sodium chloride (0.15 M)	0.88	g
Sodium citrate (0.015 M)	0.44	g
EDTA (0.1 mM)	0.0037	g
dH_2O	100	ml

4 mM Ammonium Formate pH 3.0

$HCOONH_4$	0.252	g
dH_2O	800	ml

adjust pH 3.0 with HCl and then adjust volume to 1000 ml

6 mM tert-Butylhydroperoxide (t-BHP)

t-BHP	7.72	µl
PBS pH 7.4	10	ml

0.1 M Potassium phosphate buffer pH 7.8

K_2HPO_4	1.742 g in H_2O 100 ml
KH_2PO_4	1.361 g in H_2O 100 ml

adjust pH 7.8 using KH_2PO_4

8.0% Sodium dodesyl sulfate (SDS)

SDS	8	g
dH_2O	100	ml

20% Acetic acid

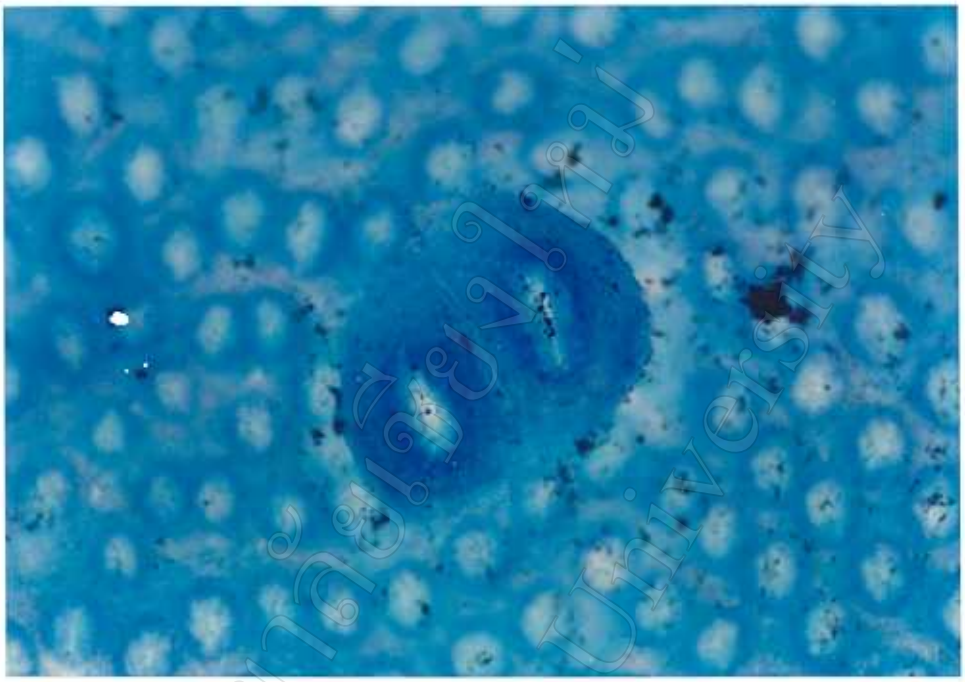
Acetic acid	20	ml
dH_2O	80	ml

0.8% Thiobarbituric acid (TBA)

Thiobarbituric acid	0.8	g
dH_2O	95	ml
6N NaOH	5	ml

Appendix 4 Morphology of Aberrant crypt foci

(a)



(b)

Figure 20. Morphlogy of Aberrant crypt foci (a) 2 crypts/focus and (b) more than 4 crypts/focus

Appendix 5. HPLC profile for DNA adduct determiantion

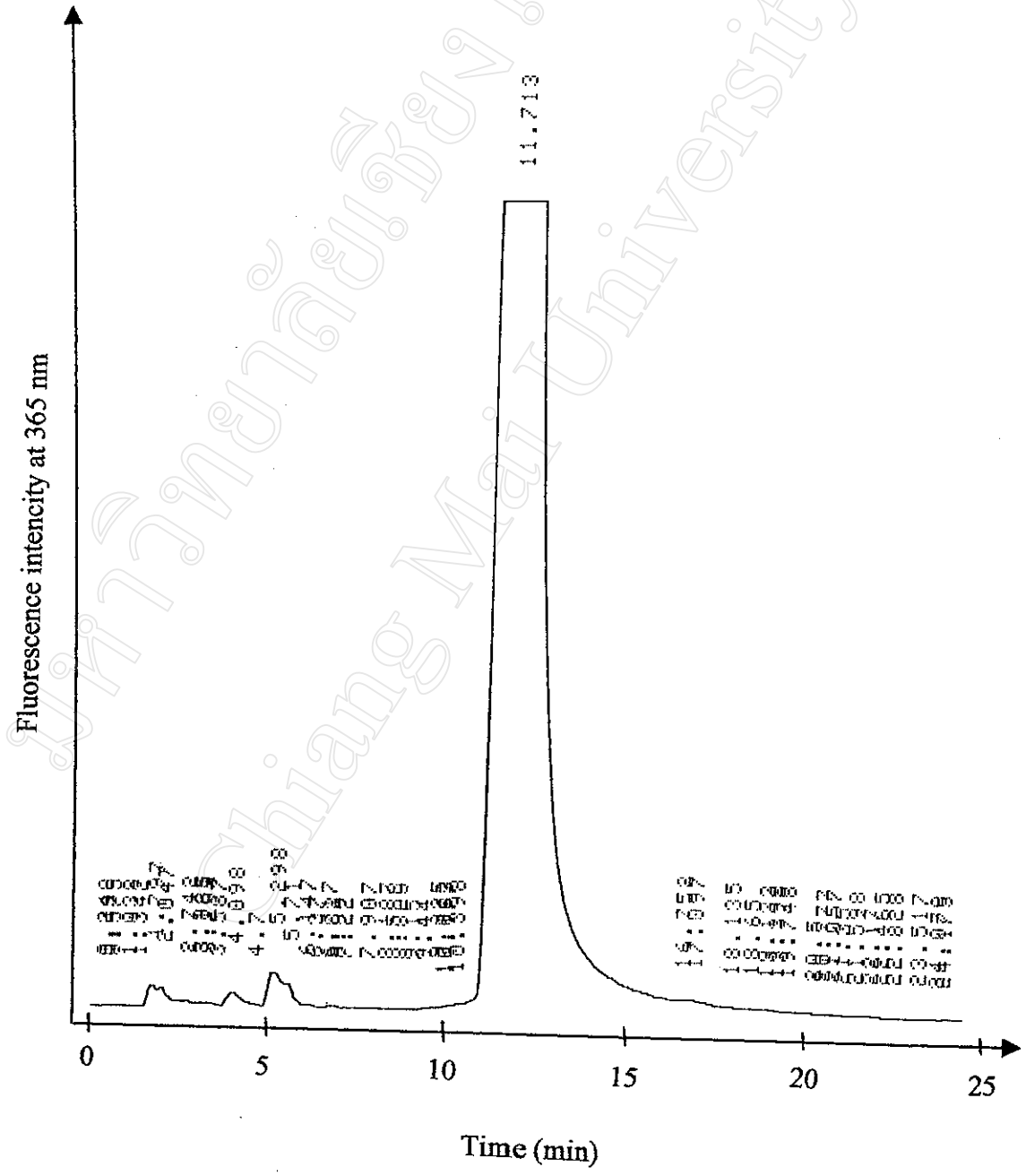


Figure 21. HPLC profile for standard O^6-methylguanine

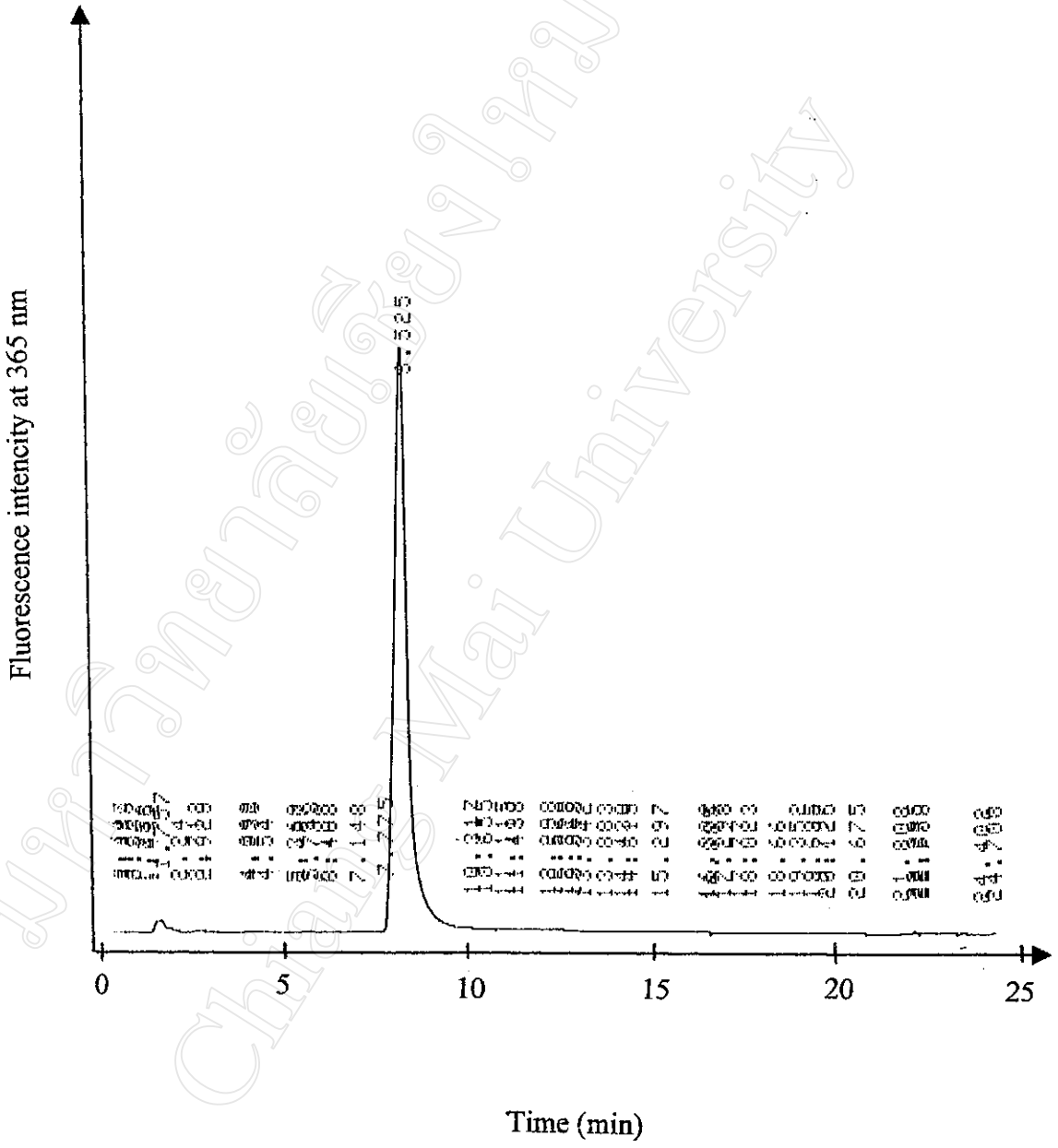


Figure 22. HPLC profile for standard N^7-methylguanine

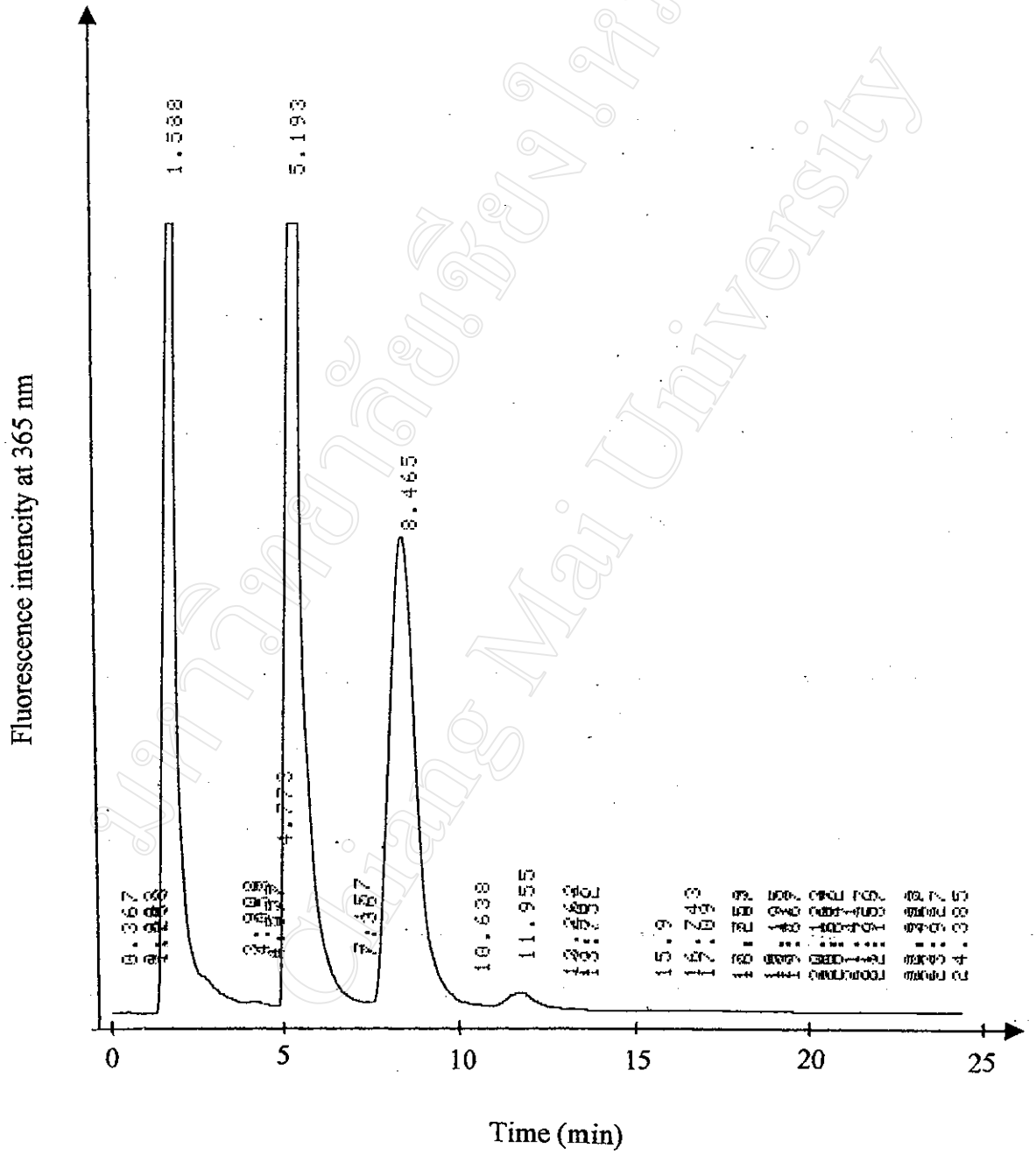


Figure 23. HPLC profile for DNA adduct sample

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Intiyot,Y., Kinouchi, T., Vinitketkumneun, U., Ohnishi, Y.,(1997) Effect of *Murdannia loriformis* extract on cancer formation in rats colon. Presented at the 4th National Cancer Conference, 11-13 November, Bangkok, Thailand

Intiyot,Y., Kinouchi, T., Vinitketkumneun, U., Ohnishi, Y.,(1997) Effect of *Murdannia loriformis* extract on DNA adduct and Aberrent crypt foci formation in rats colon, Chiang Mai. Med. Bull., 36(3)(suppl.):3

Kinouchi, T., Suaeyun, R., Chewonarin, T., Intiyot, Y., Arimochi, H., Kataoka, K., Akimoto, S., Vinitketkumneun, U., Ohnishi, Y., (1997) Chemopreventive effects of Thai medicinal plants of formation of azoxymethane-induced DNA adducts and aberrant crypt foci in the rat colon, The 7th International Conference Evironmental Mutagens, 7-12 September, Toulouse, France.

Ohnishi, Y., Kataoka, K., Kinouchi, T., Arimochi, H., Kuwahara, T., Chewonarin, T., Intiyot, Y., Vinitketkumnuen, U.,(1998) Effect of medicinal plants and antibiotics on formation of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-induced aberrant crypt foci in the rat colon, The 7th N-Aryl : Conference, 4-6 November, Nagoya, Japan.

Intiyot, Y., Kinouchi, T., Vinitketkumnuen, U., Ohnoshi, Y. (1998) Effect of *Murdannia loriformis* extract on xenobiotic metabolizing enzyme induction and AOM-induced colon cancer in rats, The 3rd International Conference on Environmental Mutagens in Human Populations, 29 November-4 December 1998, Thailand.

Intiyot, Y., Suaeyun, R., Lertprasertsuk, N., Vinitketkumnuen, U., (1998) Effect of *M. loriformis* and lemon grass extract on xenobiotic-metabolizing enzymes induction, Chiang Mai Med. Bull., (in press)