Chapter 3

RESULTS

3.1 Mutagenicity study of lemon grass

It was observed that the lemon grass extract (aqueous, methanol and hexane) did not induce mutation in Salmonella typhimurium either strain TA 98 or TA 100 with and without metabolic activation. (Table 2, and 3).

In order to compare the mutagenicity of lemon grass extracts between different conditions, the raw extract (extract at room temp) and cooked samples (extract at 100 ^{o}C) were assayed by Salmonella mutation and the comparison between the mutagenicity of each condition was made. It was also indicated that no mutagenicity could be detect even the extraction was made at higher temperature.

Table 2 Non-mutagenicity of lemon grass extracts in Salmonella typhimurium strains TA 98 and TA 100 with (+) or without (-) S9 mix. (Extraction at room temperature)

Amount of lemon grass extract (mg)	His$^+$ revertant colonies/plate*			
	TA 98		TA 100	
	- S9	+ S9	- S9	+ S9
Aqueous extract				
0	43 ± 5.0	56 ± 0.94	113 ± 10.2	147 ± 2.05
0.85	32 ± 0.82	71 ± 6.54	126 ± 3.26	147 ± 0.45
2.125	39 ± 0.79	69 ± 10.52	128 ± 0.81	128 ± 4.49
4.25	44 ± 0.43	66 ± 1.24	136 ± 6.69	130 ± 3.09
8.50	35 ± 1.63	67 ± 0.81	158 ± 4.96	145 ± 1.63
Methanol extract				
0	42 ± 2.44	73 ± 4.49	131 ± 5.43	147 ± 3.55
1.5	25 ± 0.81	57 ± 3.26	130 ± 4.08	134 ± 4.71
3.75	23 ± 1.24	59 ± 2.05	124 ± 8.17	123 ± 4.71
7.50	23 ± 0.81	63 ± 1.63	121 ± 5.71	132 ± 1.26
15.00	23 ± 2.05	55 ± 2.44	127 ± 2.44	127 ± 3.43
Hexane extract				
0	42 ± 2.44	73 ± 4.49	131 ± 5.43	147 ± 3.55
3.00	32 ± 1.69	62 ± 1.63	120 ± 2.68	127 ± 2.86
7.50	34 ± 2.86	69 ± 3.55	116 ± 4.49	136 ± 10.52
15.00	27 ± 3.09	50 ± 1.88	108 ± 4.08	126 ± 1.24
30.00	32 ± 2.44	35 ± 3.85	115 ± 6.34	124 ± 6.54

* Results are means ± SD of six plates from two independent experiments

Table 3 Non-mutagenicity of lemon grass extracts Salmonella typhimurium strains TA 98 and TA 100 (B) with (+) or without (-) S9 mix (Extraction at 100° C)

Amount of lemon grass extract (mg)	His$^+$ revertant colonies/plate*			
	TA 98		TA 100	
	- S9	+ S9	- S9	+ S9
Aqueous extract				
0	25 ± 0.47	54 ± 3.29	120 ± 2.44	168 ± 9.39
0.25	20 ± 0.43	70 ± 1.40	118 ± 9.81	171 ± 8.38
0.625	29 ± 5.01	75 ± 2.31	226 ± 15.79	268 ± 16.03
1.25	23 ± 2.11	77 ± 3.03	116 ± 9.42	160 ± 7.33
2.50	25 ± 3.15	65 ± 4.41	228 ± 17.71	165 ± 18.81
Methanol extract				
0	29 ± 4.71	68 ± 5.11	120 ± 8.69	171 ± 7.61
1.50	25 ± 2.63	67 ± 5.42	114 ± 8.89	164 ± 9.37
3.75	23 ± 0.81	63 ± 6.02	112 ± 6.32	163 ± 4.42
7.50	22 ± 2.86	62 ± 3.03	112 ± 7.63	162 ± 7.21
15.00	23 ± 3.82	63 ± 4.40	117 ± 6.71	167 + 6.64
Hexane extract				
0	29 ± 4.71	68 ± 5.11	120 ± 8.69	171 ± 7.61
1.00	23 ± 1.52	64 ± 3.31	112 ± 9.74	167 ± 167
2.50	24 ± 2.16	64 ± 2.21	118 ± 7.52	166 ± 8.81
5.00	27 ± 4.52	67 ± 4.51	116 ± 7.41	166 ± 9.67
10.00	22 ± 2.71	62 ± 6.62	115 ± 6.66	164 ± 7.11

* Results are means ± SD of six plates from two independent experiments

3.2 Antimutagenicity of lemon grass extracts.

The effect of lemon grass extract on the mutagenicity of various known mutagens both required metabolic activation like aflatoxin B_1 (AFB_1), benzopyrene B(a) P, 7,12-dimethylbenzanhtharcene (DMBA) and typical amino acid pylolysates, Trp-P-1, and IQ as well as direct-acting mutagens such as N-metyl-N'-nitro-N-nitrosoguanidine (MNNG), sodium azide (NaN_3), 4-nitroquinolene oxide (4-NQO) and furylfuramide (AF-2) were investigated in the Ames test.

Tables 4 and 5, showed the number of His^+ revertant colonies of S.typhimurium strains TA98 and TA100 which were induced by various known mutagens in the presence and absence of lemon grass extracts. Table 6 and 7, summarized the results for antimutagenicity of lemon grass extract in Salmonella mutation. It was demonstrated that all of lemon grass extracts (aqueous, methanol and hexane extracts) could decrease His^+ revertant colonies of Salmonella typhimurium strains TA 98 and TA 100 which were mutated with the various known mutagens, as follows;

The methanol and hexane extracts could inhibit His^+revertant colonies of S. typhimurium induced with mutagens required metabolic

Table 4.1 His^+ revertant colonies of S. typhimurium strains TA98 and TA 100 induced by various known mutagens in the presence of varying amounts of aqueous extract of lemon grass. (Extraction at room temperature)

Known mutagens	Amounts of extract (mg)	His^+ revertant/plate** TA 98	TA 100	% Inhibition*
[1]B(a)P	0	393 ± 8.93	-	-
(5 ug/plate)	0.85	228 ± 6.54	-	41.9
	2.125	210 ± 14.52	-	46.5
	4.25	207 ± 1.52	-	47.3
	8.50	157 ± 12.49	-	60.0
[1]DMBA	0	138 ± 0.47	-	-
(20 ug/plate)	0.85	128 ± 2.82	-	7.2
	2.125	120 ± 4.71	-	13.0
	4.25	110 ± 2.44	-	20.3
	8.50	148 ± 2.16	-	-
[1]Trp-P-1	0	392 ± 13.93	-	-
(0.02 ug/plate)	0.85	348 ± 30.86	-	11.2
	2.125	359 ± 22.49	-	8.4
	4.25	360 ± 47.76	-	8.2
	8.50	283 ± 30.63	-	27.8
[1]AFB_1	0	892 ± 7.76	-	-
(5 ug/plate)	0.85	693 ± 23.11	-	22.3
	2.125	618 ± 8.21	-	30.7
	4.25	468 ± 5.73	-	47.5
	8.50	438 ± 12.76	-	50.9
[1]IQ	0	-	1332 ± 3.26	-
(0.1 ug/plate)	0.85	-	823 ± 9.79	38.1
	2.125	-	866 ± 27.27	34.9
	4.25	-	899 ± 12.25	32.5
	8.50	-	759 ± 21.35	43.0

Table 4.1 (continued)

Known mutagens	Amounts of extract (mg)	His$^+$ revertant/plate** TA 98	His$^+$ revertant/plate** TA 100	% Inhibition*
[2]AF-2	0	535 ± 21.64	-	-
(0.1 ug/plate)	0.85	541 ± 7.07	-	-
	2.125	523 ± 9.74	-	2.2
	4.25	542 ± 28.17	-	-
	8.50	494 ± 6.48	-	7.6
[2]NaN_3	0	-	313 ± 38.68	-
(0.5 ug/plate)	0.85	-	244 ± 60.02	20.0
	2.125	-	321 ± 8.21	-
	4.25	-	302 ± 53.52	3.5
	8.50	-	277 ± 11.43	11.5
[2]4-NQO	0	159 ± 0.81	-	-
(0.2 ug/plate)	0.85	148 ± 4.08	-	6.92
	2.125	163 ± 4.24	-	-
	4.24	160 ± 6.97	-	-
	8.50	157 ± 4.10	-	1.2
[2]MNNG	0	-	1949 ± 177.99	-
(0.5 ug/plate)	0.85	-	1998 ± 238.28	-
	2.125	-	1734 ± 272.25	11.0
	4.25	-	1602 ± 164.67	17.8
	8.50	-	1271 ± 138.95	34.7
BG(H_2O) +S9	50 ul/plate	63 ± 9.87	151 ± 22.62	
BG(H_2O) -S9	50 ul/plate	43 ± 2.86	131 ± 9.17	

* $\%\ \text{Inhibition} = \frac{(N-P) - (S-P)}{(N-P)} \times 100$

N = Number of revertant colonies induced by mutagen

S = Number of revertant colonies induced by mutagen in the presence of lemon grass extract

P = Number of spontaneous revertant colony (BG)

** Results are means ± SD of six plates from two independent experiments. [1]in the presence of S9 mix, [2]without S9 mix.

Table 4.2 His^+ revertants S. typhimurium strains TA 98 and TA 100 induced by various known mutagens in the presence of varying amounts of methanol extract of lemon grass. (Extraction at room temperature).

Known mutagens	Amounts of extract (mg)	His^+ revertant/plate** TA 98	TA 100	% Inhibition
*B(a)P	0	393 ± 8.98	-	-
(5 ug/plate)	1.50	316 ± 2.86	-	19.5
	3.75	297 ± 2.05	-	24.4
	7.50	262 ± 1.63	-	33.3
	15.00	136 ± 20.12	-	65.3
*DMBA	0	138 ± 0.47	-	-
(20 ug/plate)	1.50	93 ± 1.41	-	32.6
	3.75	65 ± 5.55	-	52.9
	7.50	- ± 2.49	-	100
	15.00	- ± 4.18	-	100
*Trp-P-1	0	392 ± 31.75	-	-
(0.02 ug/plate)	1.50	170 ± 52.01	-	56.6
	3.75	89 ± 5.20	-	77.3
	7.50	70 ± 3.79	-	82.1
	15.00	30 ± 1.74	-	92.3
*AFB_1	0	892 ± 7.76	-	-
(0.05 ug/plate)	1.50	133 ± 14.89	-	85.0
	3.75	87 ± 6.01	-	90.2
	7.50	70 ± 3.17	-	92.3
	15.00	32 ± 9.87	-	96.4
*IQ	0	-	1332 ± 3.26	-
(0.1ug/plate)	1.50	-	208 ± 90.74	84.3
	3.75	-	63 ± 9.76	95.2
	7.50	-	17 ± 6.32	98.7
	15.00	-	-	100

Table 4.2 (continued)

Known mutagens	Amounts of extract (mg)	His$^+$ revertant/plate** TA 98	TA 100	% Inhibition*
[2]AF-2	0	535 ± 21.64	-	-
(0.1 ug/plate)	1.50	561 ± 6.53	-	-
	3.75	508 ± 12.25	-	5.0
	7.50	416 ± 4.54	-	22.2
	15.00	232 ± 8.33	-	56.6
[2]NaN_3	0	-	313 ± 38.68	-
(0.5 ug/plate)	1.50	-	260 ± 25.31	16.9
	3.75	-	244 ± 29.76	22.0
	7.50	-	273 ± 19.35	12.7
	15.00	-	126 ± 21.21	59.7
[2]4-NQO	0	159 ± 0.81	-	-
(0.2 ug/plate)	1.50	130 ± 3.29	-	18.2
	3.75	138 ± 2.79	-	13.2
	7.50	123 ± 5.31	-	22.6
	15.50	107 ± 3.29	-	32.7
[2]MNNG	0	-	1949 ± 177.99	-
(0.5ug/plate)	1.50	-	1412 ± 126.67	22.5
	3.75	-	1064 ± 256.77	45.4
	7.50	-	932 ± 111.05	52.5
	15.00	-	76 ± 3.397	96.1
BG (DMSO) + S9	50 ul/plate	68 ± 9.87	151 ± 22.62	-
BG (DMSO) - S9	50 ul/plate	43 ± 4.89	13 ± 9.17	-

*,**, 1,2 See footnote table 4.1

Table 4.3 His^+ revertant colonies of S. typhimurium strains TA 98 and TA 100 induced by various known mutagens in the presence of varying amounts of hexane extract of lemon grass (Extraction at room temperature).

Known mutagens	Amounts of extract (mg)	His^+ revertant/plate** TA 98	TA 100	% Inhibition*
^{1}B (a)P	0	393 ± 8.98	-	-
(5 ug/plate)	3	433 ± 17.30	-	-
	7.5	194 ± 17.50	-	50.6
	15.00	157 ± 1.88	-	60.0
	30.00	11 ± 16.80	-	97.2
^{1}DMBA	0	138 ± 0.47	-	-
(20 ug/plate)	3	79 ± 0.47	-	42.7
	7.50	75 ± 2.86	-	45.6
	15.00	-	-	100
	30.00	-	-	100
^{1}Trp-P-1	0	392 ± 31.13	-	-
(0.02 ug/plate)	3	248 ± 38.84	-	36.7
	7.50	274 ± 36.77	-	30.1
	15.000	201 ± 8.37	-	48.7
	30.00	131 ± 9.81	-	66.5
^{1}AFB$_1$	0	892 ± 7.76	-	-
(0.05 ug/plate)	3	90 ± 7.78	-	89.9
	7.50	20 ± 2.49	-	97.7
	15.00	8 ± 4.89	-	99.1
	30.00	(K)	-	-
^{1}IQ	0	-	1332 ± 3.26	-
(0.12 ug/plate)	3	-	315 ± 15.57	76.3
	7.50	-	104 ± 12.03	92.1
	15.00	-	86 ± 14.69	93.5
	30.00	-	(K)	-

Table 4.3 (continued)

Known mutagens	Amounts of extract (mg)	His+ revertant/plate** TA 98	TA 100	% Inhibition*
[2]AF-2	0	535 ± 21.64	-	-
(0.1ug/plate)	3.00	456 ± 12.49	-	14.7
	7.50	459 ± 5.55	-	14.2
	15.00	416 ± 4.57	-	22.2
	30.00	44 ± 2.79	-	91.7
[2]NaN_3	0	-	313 ± 38.68	-
(0.5 ug/plate)	3.00	-	163 ± 21.29	47.9
	7.50	-	109 ± 53.30	65.1
	15.00	-	(K)	-
	30.00	-	(K)	-
[2]4-NQO	0	159 ± 0.81	-	-
(0.2 ug/plate)	3.00	116 ± 4.54	-	27.0
	7.50	110 ± 3.29	-	30.8
	15.00	99 ± 6.94	-	37.7
	30.00	26 ± 4.54 (K)	-	-
[2]MNNG	0	-	1949 ± 177.99	-
(0.5 ug/plate)	3.00	-	694 ± 34.72	64.3
	7.50	-	528 ± 28.05	72.9
	15.00	-	(k)	-
	30.00	-	(k)	-
BG (DMSO) +S9	50 ul/plate	68 ± 9.87	151 ± 22.62	
BG (DMSP) -S9	50 ul/plate	43 ± 4.89	131 ± 9.17	

1,2

*,** See footnote table 4.1

K = killing of bacteria

Table 5.1 His^+ revertants colonies of S. typhimurium stains TA 98 and TA 100 induced various known mutagens in the presence of varying amounts of aqueous extract of lemon grass. (Extraction at 100° C).

Known mutagens	Amounts of extract (mg)	His^+ revertant/plate** TA 98	TA 100	% Inhibition*
B(a)P	0	452 ± 14.70	-	-
(5 mg/plate)	0.25	280 ± 15.19	-	38.0
	0.625	253 ± 16.39	-	44.0
	1.25	251 ± 11.57	-	44.6
	2.50	227 ± 17.55		49.7
[1]DMBA	0	143 ± 3.26	-	-
(20 ug/plate)	0.25	136 ± 3.09	-	4.9
	0.625	105 ± 1.24	-	26.5
	1.25	114 ± 2.44	-	20.2
	2.50	143 ± 6.37	-	-
[1]Trp-P-1	0	373 ± 16.39	-	-
0.02 ug/plate)	0.25	267 ± 11.57	-	28.4
	0.625	183 ± 9.71	-	50.9
	1.25	154 ± 6.01	-	58.7
	2.50	137 ± 22.11	-	63.2
[1]AFB_1	0	961 ± 97.48	-	-
(0.05 ug/plate)	0.25	929 ± 36.91	-	3.3
	0.625	822 ± 16.67	-	14.4
	1.25	749 ± 23.31	-	22.0
	2.50	726 ± 102.60	-	24.4
[1]IQ	0	-	1752 ± 18.83	-
(0.05 ug/plate)	0.25	-	1376 ± 124.11	21.4
	0.625	-	1190 ± 20.40	32.0
	1.25	-	1118 ± 25.74	36.1
	2.50	-	808 ± 33.99	53.8

Table 5.1 (continued)

Known mutagens	Amounts of extract (mg)	His$^+$ revertant/plate** TA 98	TA 100	% Inhibition*
[2]AF-2	0	454 ± 19.70	-	-
(0.05 ug/plate)	0.25	507 ± 10.67	-	-
	0.625	483 ± 19.39	-	-
	1.25	501 ± 21.92	-	-
	2.5	424 ± 25.22	-	6.6
[2]NaN_3	0	-	392 ± 31.95	-
(0.05 ug/plate)	0.25	-	384 ± 23.93	2.0
	0.625	-	409 ± 25.74	-
	1.25	-	355 ± 15.54	9.4
	2.5	-	329 ± 30.92	16.0
[2]4-NQO	0	339 ± 8.16	-	-
(0.02 ug/plate)	0.25	237 ± 8.73	-	30.0
	0.625	236 ± 11.89	-	30.3
	1.25	275 ± 4.64	-	18.8
	2.5	296 ± 18.0	-	12.6
[2]MNNG	0	-	1765 ± 151.58	-
(0.05 ug/plate)	0.25	-	1628 ± 193.16	7.7
	0.625	-	1485 ± 12.03	15.8
	1.25	-	1245 ± 89.93	29.4
	2.5	-	997 ± 74.12	43.5
BG (DMSO) +S9	50 ul/plate	68 ± 2.23	171 ± 18.87	-
BG (DMSo) -S9	50 ul/plate	29 ± 6.7	120 ± 16.7	-

1,2 *,** See footnote table 4.1

Table 5.2 His^+ revertants colonies of S. typhimurium strains TA 98 and TA 100 induced by various known mutagens in the presence of varying amounts of methanol extract of lemon grass (Extraction at 100° C).

Known mutagens	Amounts of extract (mg)	His^+ revertant/plate** TA 98	TA 100	% Inhibition*
[1]B(a) P	0	450 ± 14.70	-	-
(5 ug/plate)	1.5	469 ± 14.32	-	-
	3.75	456 ± 23.50	-	-
	7.5	365 ± 4.49	-	18.8
	15	113 ± 14.38	-	74.8
[1]DMBA	0	143 ± 3.26	-	-
(20 ug/plate)	1.4	84 ± 9.10	-	41.2
	3.5	75 ± 4.89	-	47.5
	7	65 ± 1.24	-	54.5
	14	53 ± 2.62	-	62.9
[1]Trp-P-1	0	373 ± 16.39	-	-
(0.01 ug/plate)	1.4	184 ± 13.72	-	19.7
	3.5	115 ± 10.47	-	69.7
	7	79 ± 4.23	-	78.8
	14	53 ± 6.01	-	85.7
[1]AFB_1	0	961 ± 97	-	-
(0.05 ug/plate)	1.5	192 ± 23.32	-	80.02
	3.75	130 ± 17.41	-	86.47
	7.5	61 ± 7.78	-	93.65
	15	46 ± 4.49	-	95.21
[1]IQ	0	-	1752 ± 18.83	-
(0.05 ug/plate)	1.4	-	764 ± 5.09	57.3
	3.5	-	562 ± 14.81	67.9
	7	-	459 ± 6.68	73.8
	14	-	245 ± 13.92	86.0

Table 5.2 (continued)

Known mutagens	Amounts of extract (mg)	His$^+$ revertant/plate** TA 98	TA 100	% Inhibition*
[2]AF-2	0	454 ± 19.70	-	-
(0.05 mg/plate)	1.4	455 ± 4.71	-	-
	3.5	403 ± 16.83	-	11.2
	7	335 ± 16.70	-	26.2
	14	253 ± 11.26	-	44.2
[2]NaN_3	0	-	392 ± 8.98	-
(0.05 ug/plate)	1.4	-	336 ± 28.24	14.29
	3.5	-	336 ± 17.14	14.29
	7	-	327 ± 3.68	16.58
	14	-	299 ± 8.17	23.7
4-NQO	0	339 ± 8.16		-
(0.2 ug/plate)	1.4	298 ± 6.37		12.0
	3.5	286 ± 7.3 4		15.6
	7.0	247 ± 9.27		27.1
	14	213 ± 10.86		37.1
[2]MNNG	0	-	1765 ± 151.43	-
(0.05 ug/plate)	1.4	-	1267 ± 161.17	28.2
	3.5	-	1055 ± 325.32	40.2
	7.0	-	828 ± 113.32	53.0
	14	-	234 ± 27.04	86.7
BG (DMSO) +S9	50 ul/p	78 ± 2.23	163 ± 18.87	
BG (DMSO) -S9	50 ul/p	29 ± 6.7	129 ± 16.7	

1,2 * See footnote table 4.1

Table 5.3 His^+ revertants colonies of S. typhimurium strains TA 98 and TA 100 induced by various known mutagens in the presence of varying amounts of hexane extract of lemon grass (Extraction at 100° C).

Known mutagens	Amounts of extract (mg)	His^+ revertant/plate** TA 98	TA 100	% Inhibition*
[1]B(a) P	0	450 ± 14.70	-	-
(5 ug/plate)	1.0	546 ± 23.33	-	-
	2.5	542 ± 30.00	-	-
	5	404 ± 11.32	-	10.2
	10	384 ± 4.64	-	14.6
[1]DMBA	0	143 ± 3.26	-	-
(20 ug/plate)	1.0	152 ± 4.49	-	-
	2.5	138 ± 13.27	-	3.4
	5	121 ± 0.47	-	9.0
	10	89 ± 4.32	-	37.7
[1]Trp-P-1	0	373 ± 6.39	-	-
(0.02 ug/plate)	1.0	188 ± 20.29	-	49.5
	2.5	178 ± 31.32	-	52.2
	5	128 ± 19.83	-	65.6
	10	115 ± 9.81	-	69.1
[1]AFB_1	0	961 ± 97	-	-
(0.05 ug/plate)	1.0	876 ± 11.17	-	8.8
	2.5	680 ± 15.57	-	29.2
	5	228 ± 13.32	-	76.2
	10	121 ± 4.48	-	84.4
[1]IQ	0	-	1752 ± 18.83	-
(0.1 ug/plate)	1.0	-	1478 ± 35.35	15.6
	2.5	-	1324 ± 17.31	23.4
	5	-	945 ± 21.29	46.0
	10	-	546 ± 8.26	64.8

Table 5.3 (continued)

Known mutagens	Amounts of extract (mg)	His$^+$ revertant/plate** TA 98	TA 100	% Inhibition
[2]AF-2 (0.1)	0	454 ± 19.70	-	-
(0.05 ug/plate)	1.0	435 ± 16.0	-	4.1
	2.5	398 ± 15.23	-	12.3
	5	386 ± 7.80	-	14.7
	10	342 ± 13.32	-	24.6
2 NaN_3	0	-	392 ± 20.48	-
(0.5 ug/plate)	1.0	-	273 ± 25.46	30.3
	2.5	-	271 ± 21.01	30.8
	5	-	257 ± 47.81	34.4
	10	-	218 ± 15.29	44.3
[2] 4-NQO	0	339 ± 6.16	-	-
(0.2 mg/plate)	1.0	324 ± 6.59	-	4.4
	2.5	296 ± 3.09	-	12.6
	5	287 ± 1.88	-	15.3
	10	265 ± 3.24	-	21.8
[2] MNNG	0	-	1765 ± 151.86	-
(0.5 mg/plate)	1.0	-	1152 ± 27.04	34.7
	2.5	-	1030 ± 86.18	41.6
	5	-	980 ± 89.50	44.4
	10	-	875 ± 4.64	50.4
BG (DMSO) +S9	50 ul/plate	68 ± 2.23	171 ± 18.87	-
BG (DMSO) -S9	50 ul/plate	29 ± 6.7	120 ± 16.70	-

1,2 *,** see footnote table 4.1

Table 6 Summary of result for antimutagenicity of lemon grass extract in S. typhimurium. (Extraction at room temperature).

Standard mutagen	Aqueous extract	Methanol extract	Hexane extract
TA 98 -S9			
AF_2	-	+	+
4-NQO	-	+	±
TA 98 +S9			
B(a) P	+	+	+
DMBA	-	+	+
AFB_1	+	+	+
Trp-P-1	-	+	+
TA 100 -S9			
NaN_3	-	-	+
MNNG	+	+	+
TA 100 +S9			
IQ	+	+	+

+ = > 30 % inhibition

- = < 30 % inhibition

± = = 30 % inhibition

Table 7 Summary of result for antimutagenicity of lemon grass extract in S. typhimurium. (Extraction at 100° C)

Standard mutagen	Aqueous extract	Methanol extract	Hexane extract
TA 98-S9			
AF_2	–	+	–
4-NQO	–	±	–
TA 98 +S9			
B(a) P	+	+	–
DMBA	–	+	±
AFB_1	–	+	+
Trp-P-1	+	+	+
TA 100 -S9			
NaN_3	–	–	±
MNNG	+	+	+
TA 100 +S9			
IQ	+	+	+

\+ = > 30 % inhibition

– = < 30 % inhibition

± = = 30 % inhibition

activation like AFB_1, B (a) P, DMBA, Trp-P-1, IQ, and which did not require metabolic activation like MNNG, AF-2 and 4-NQO. The extracts did not have any effect inhibiting on NaN_3. The methanol extract considered to have highest capacity of inhibition. The decrease in mutagens induced reversion was not due to toxic effect of the extracts. The background lawn growth on the plates was observed under a stereomicroscope when the revertant colonies were counted. No obvious toxic effect of the extracts at all doses tested was noted. Furthermore, the extracts caused no obvious decreasing in the frequency of spontaneous reversion. The highest amount of the extracts was mixed with H_2O or DMSO and the spontaneous reversion showed no affecting. While hexane extract at higher amount than 30.0 mg/plate exerted toxicity to bacterial tester strains.

3.2.1 Effect of aqueous extract on the mutagenicity of various known mutagens

As shown in Figure 7 and 8, an aqueous extract of lemon grass reduced revertant colonies in S. typhimurium TA98 induced with IQ, and AFB. Also reduced revertant colonies in TA100 induced with MNNG. The same result obtained when an aqueous extract was made at 100°C.

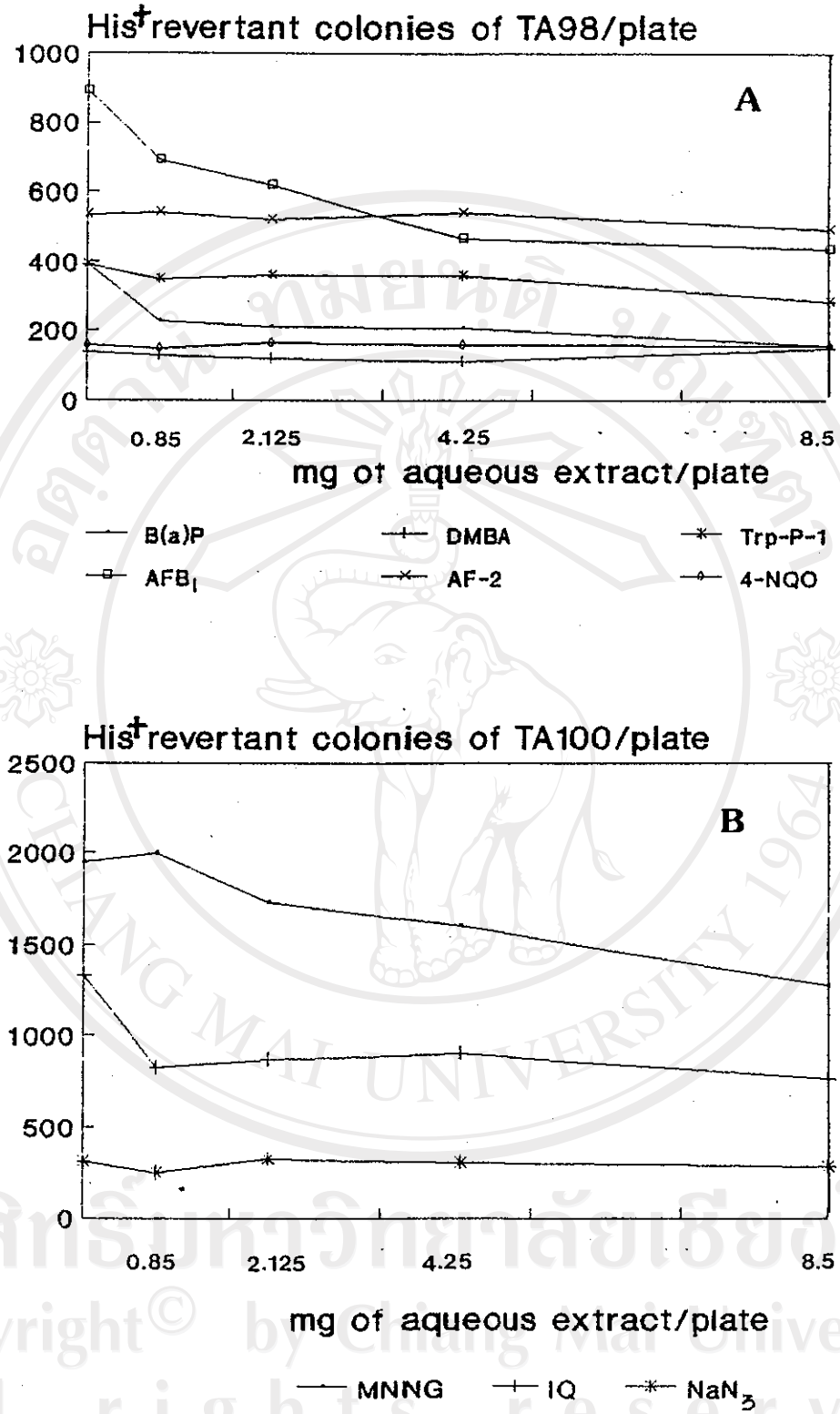


Figure 7 Effect of the aqueous extract (extract at room temperature) on the mutaegnicity of known mutagens which required metabolic activation (S9 mix), [A] and did not [B]

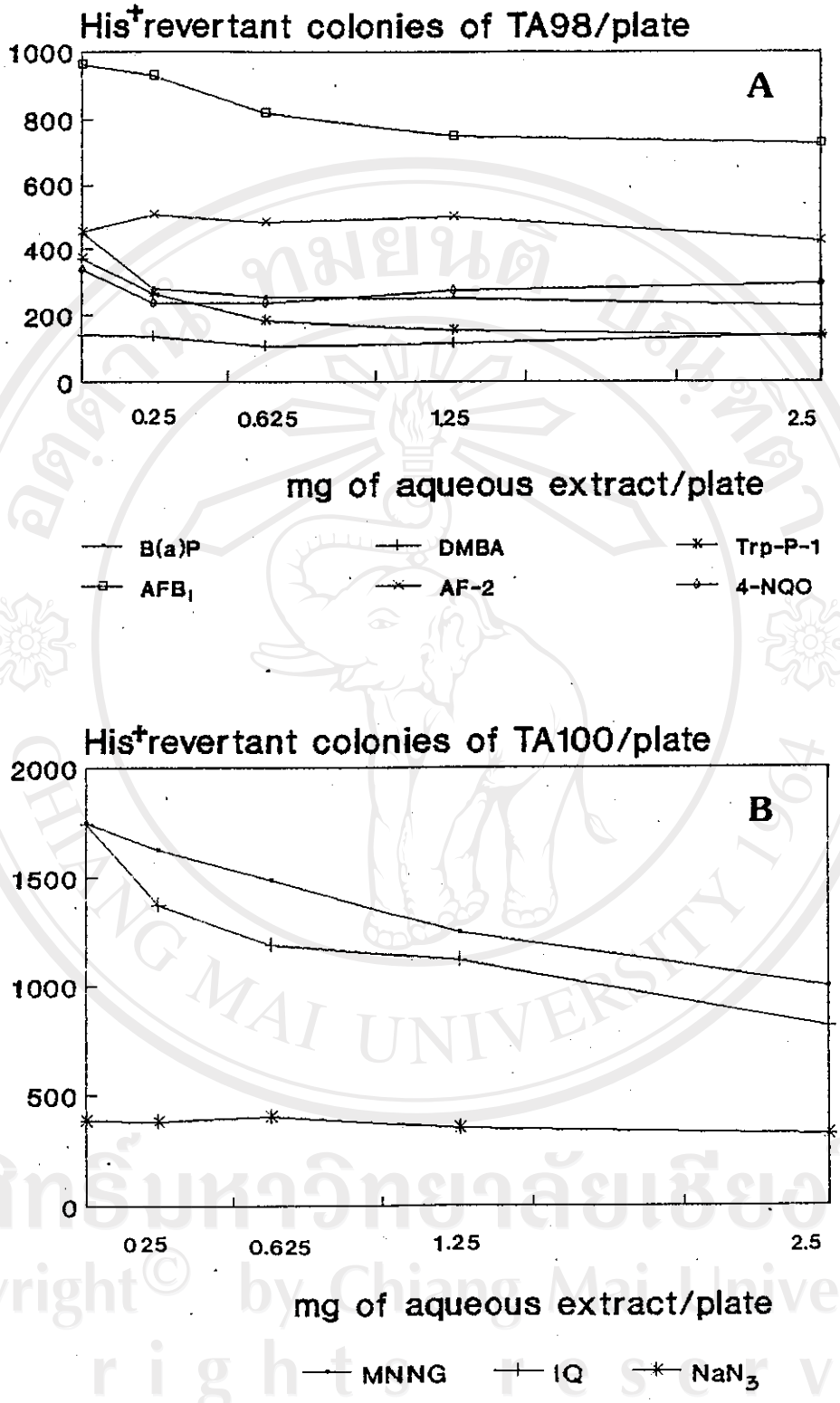


Figure 8 Effect of the aqueous extract (extract at 100° C) on the mutaegnicity of known mutagens which required metabolic activation (S9 mix), [A], and did not [B]

3.2.2 Effect of methanol extract on the mutagenicity of various known mutagens

The methanol extract of lemon grass significantly decreased the revertant colonies in S.typhimurium TA98 and TA100 induced by AFB_1, B (a) P, DMBA, Trp-P-1, IQ, and which did not required metabolic activation like MNNG, AF-2 and 4-NQO. The extracts did not have any effect on NaN_3 as shown in Figures 9 and 10.

3.2.3 Effect of hexane extract on the mutagenicity of various known mutagens

The hexane extract of lemon grass also significantly decreased the revertant colonies in S.typhimurium TA98 and TA100 induced by AFB_1, B (a) P, DMBA, Trp-P-1, IQ, and which did not require metabolic activation like MNNG, AF-2 and 4-NQO. The extracts did not have any effect on NaN_3. The extract also showed killing effect on bacteria when the amount of the extract was increased. The results are shown in Figures 11 and 12.

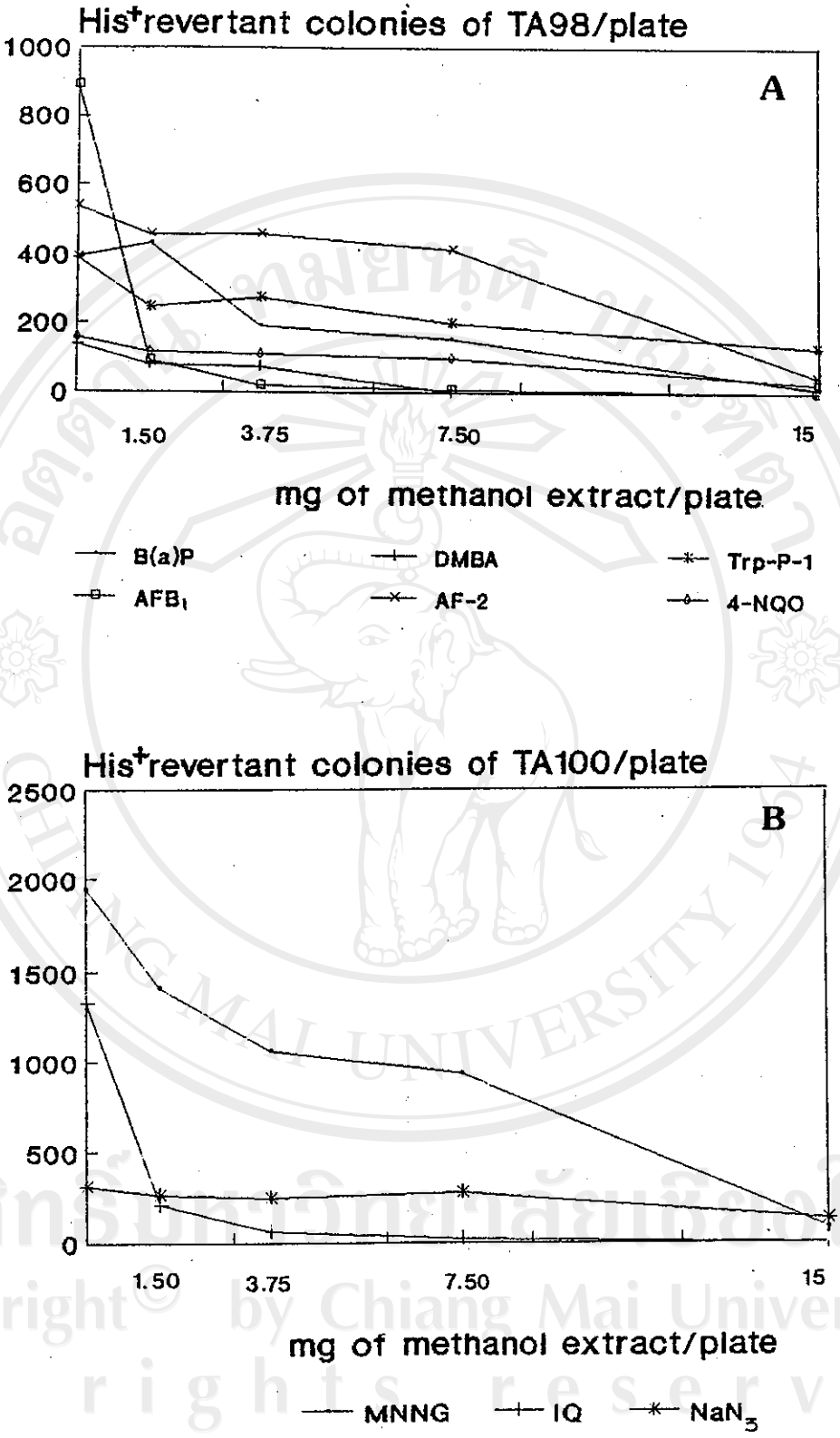


Figure 9 Effect of the methanol extract (extract at room temperature) on the mutagenicity of known mutagens which required metabolic activation (S9 mix), [A] and did not [B]

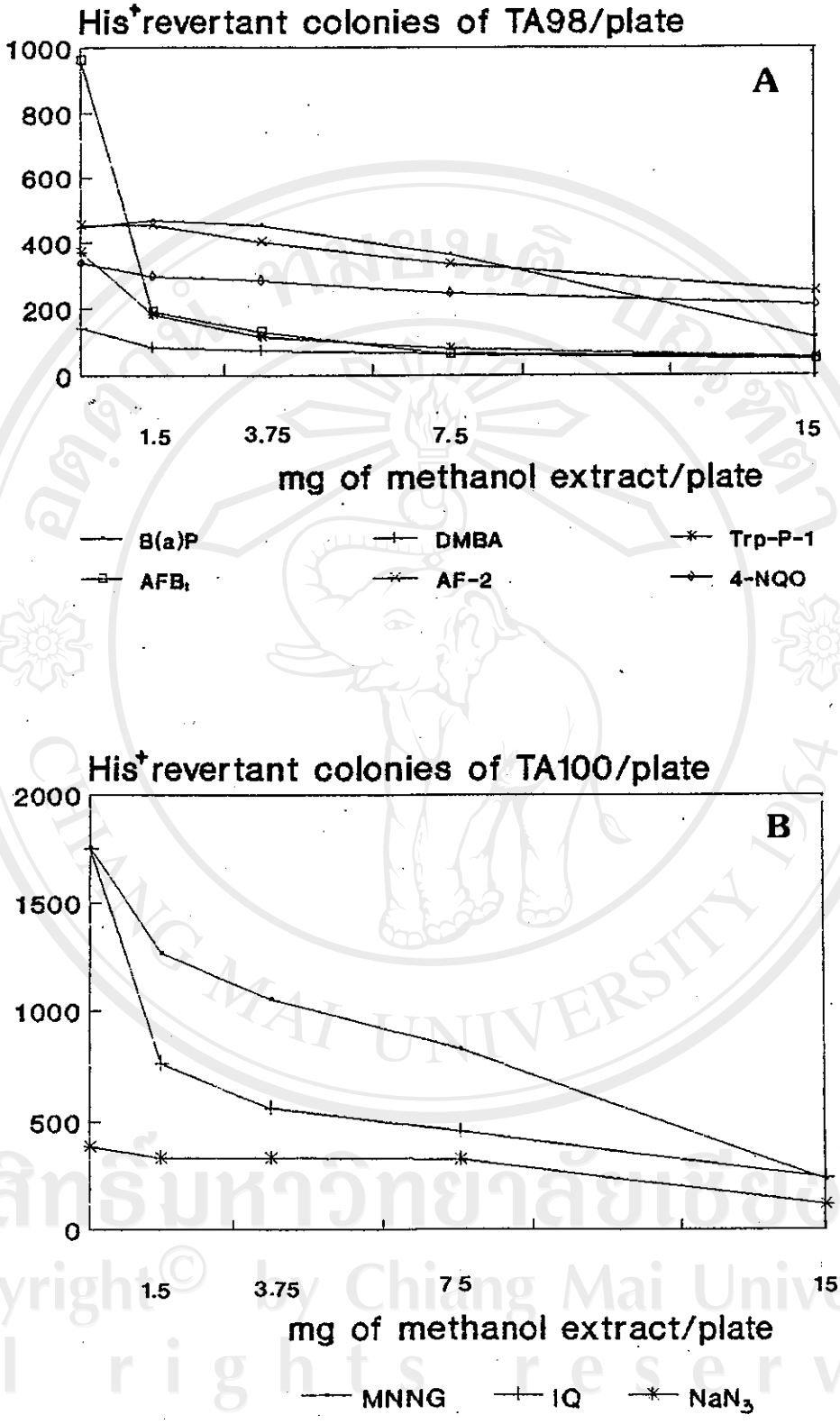


Figure 10 Effect of the methanol extract (extract at 100 ° C) on the mutaegnicity of known mutagens which required metabolic activation (S9 mix), [A] and did not [B]

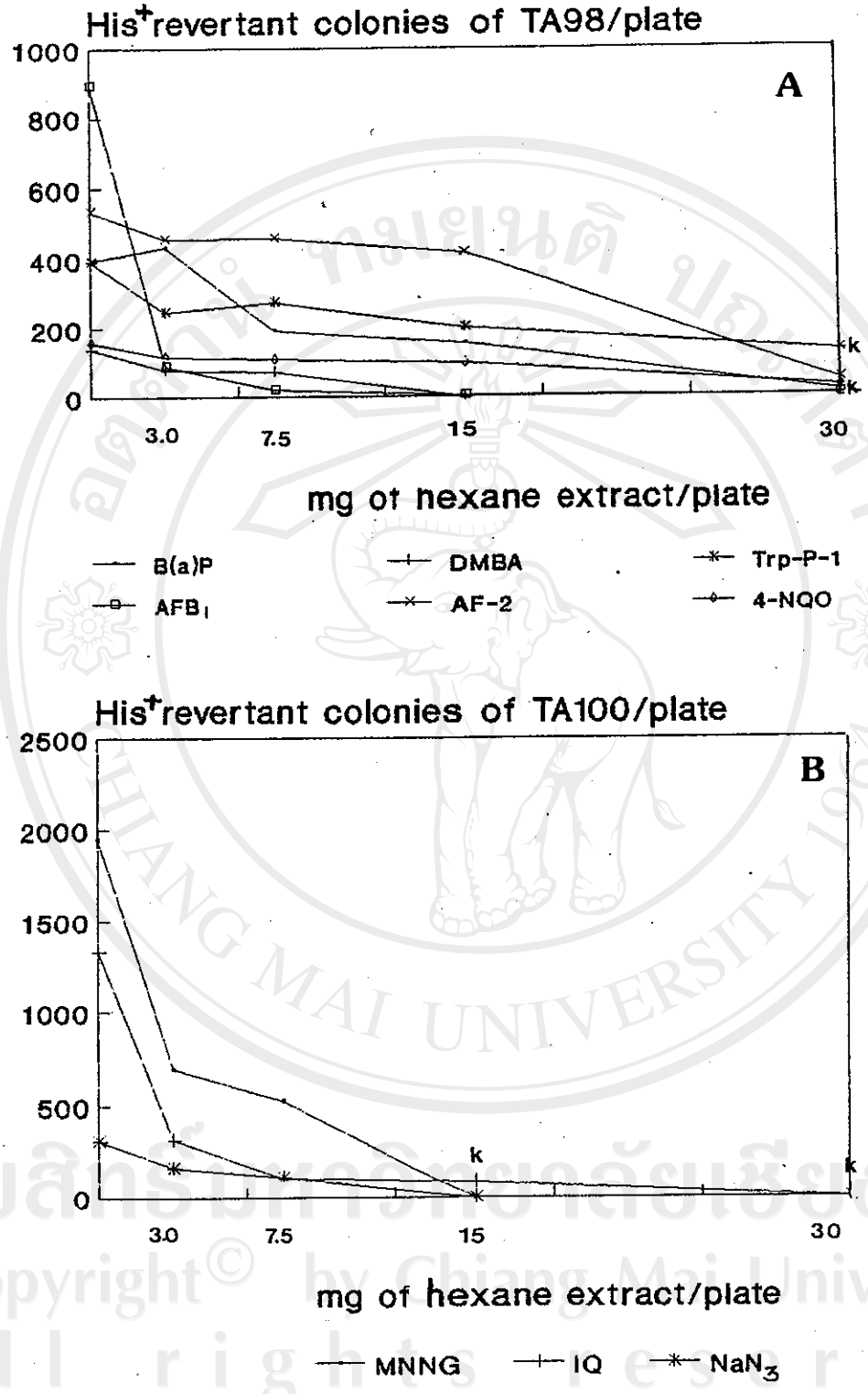


Figure 11 Effect of the hexane extract (extract at room temperature) on the mutagenicity of known mutagens which required metabolic activation (S9 mix) [A], and did not [B]

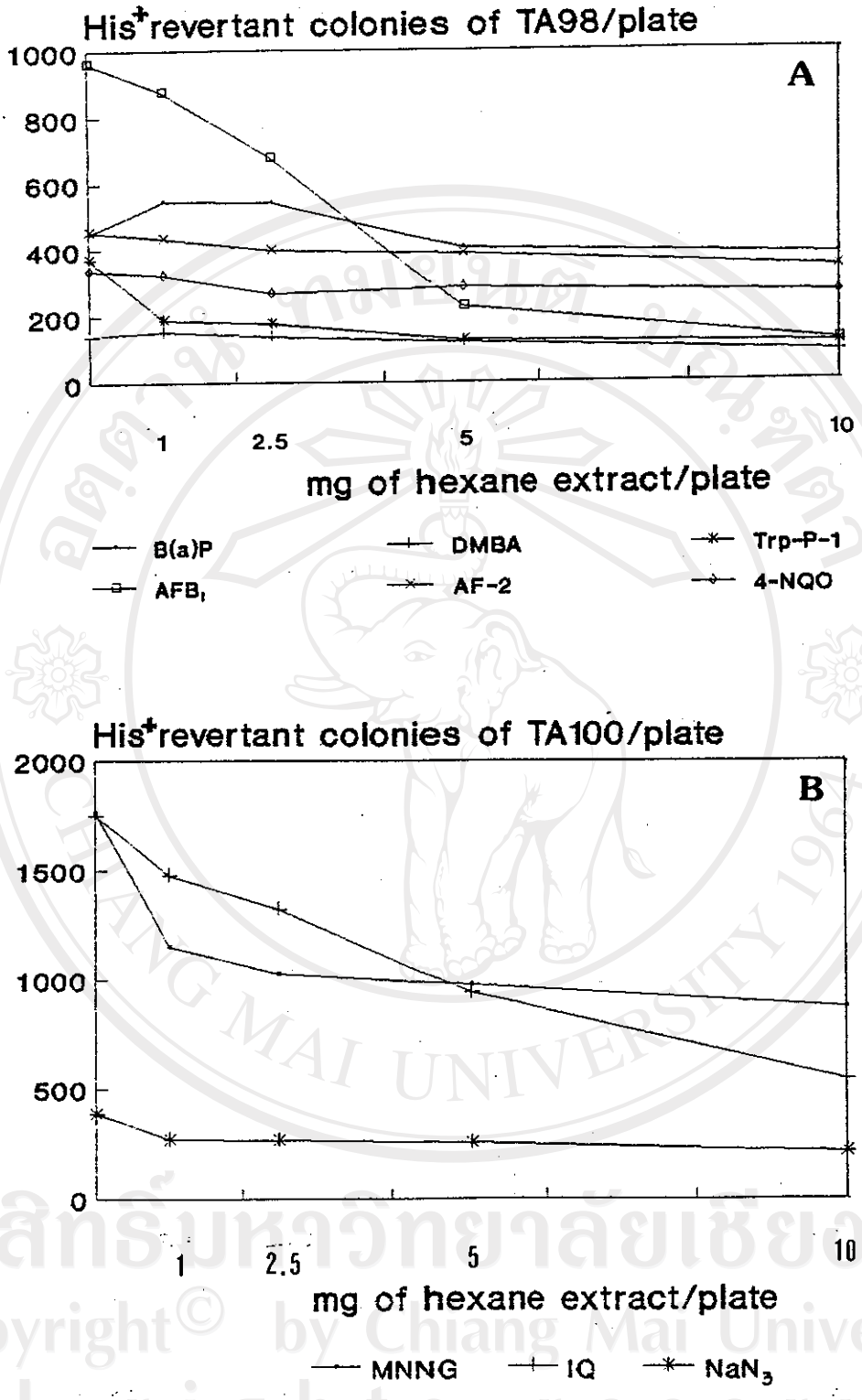


Figure 12 Effect of the hexane extract (extract at 100 °C) on the mutagenicity of knownmutagens which required metabolic activation (S9 mix), [A], and did not [B]

3.3. Possible mechanism of antimutagenicity of methanol extract of lemon grass.

The possible mechanism of methanol extract of lemon grass to inhibit AFB_1 mutagenesis was interesting to be known. In this study, the methanol extract showed highest inhibiting capacity toward AFB_1 induced mutation in S.typhimurium TA 98, with dose-effect relationship. The complication mechanisms may be involved in the inactivation of mutagenicity of AFB_1 by lemon grass extract, including biochemical reactions with enzymes and/or adsorption with some substance and/or chemical reaction with some compounds in lemon grass.

3.3.1 Effect on mutagens

Pretreatment of aflatoxin B_1 with lemon grass extract at 37°C for 30 min, (Figure 5, Procedure 2) could decrease the mutagenicity of AFB-1 (Table 8, Figure 13). The same effect was found when pre-incubation of MNNG with lemon grass, the mutagenicity of MNNG was also decreased (Table 9, Figure 14)

Table 8 Decreased of the number of His^+ revertant colonies when AFB_1 was preincubated with methanol extract of lemon grass at 37° C 30 min

Amount of extract (mg)	His^+ revertant colonies of TA98/plate**	% inhibition*
0	884 ± 25.05	-
0.75	692 ± 4.42	21.7
1.50	556 ± 14.33	37.1
3.00	482 ± 21.07	45.4
7.50	222 ± 12.21	74.8
BG	27 ± 0.81	-

** Results are means ± SD of six plates from two independent experiments

* see footnote table 4.1

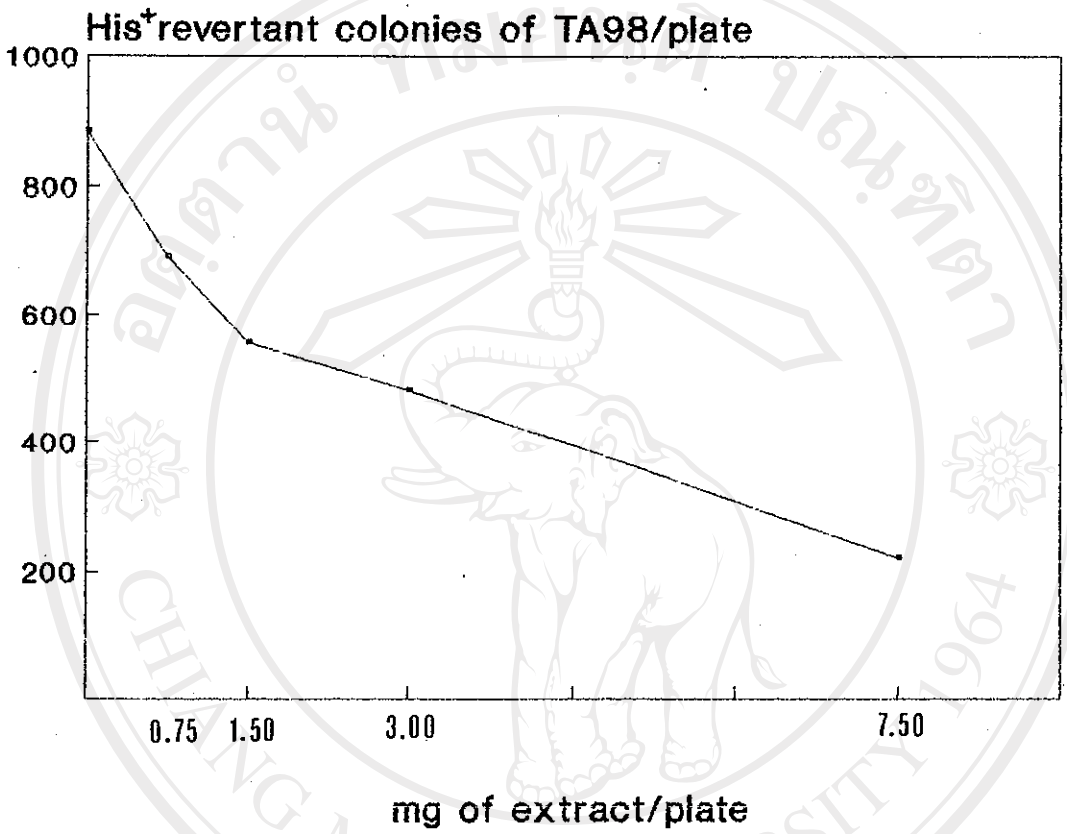


Figure 13 Decrease of the number of His^+ revertant colonies when AFB_1 was preincubated with the methanol extract of lemon grass at 37 °C for 30 min.

Table 9 Preincubation of MNNG with methanol extract lemon grass could alter the mutagenicity of MNNG.

Amount of extract (mg)	His^+ revertant colonies of TA100/plate**	% inhibition*
0	1655 ± 171.85	-
0.75	1584 ± 156.66	4
1.50	1408 ± 142.22	14.9
3.00	1227 ± 93.34	25.8
7.50	1022 ± 53.32	38.2
BG	120 ± 21.88	-

** Results are means ± SD of six plates from two independent experiments

* see footnote table 4.1

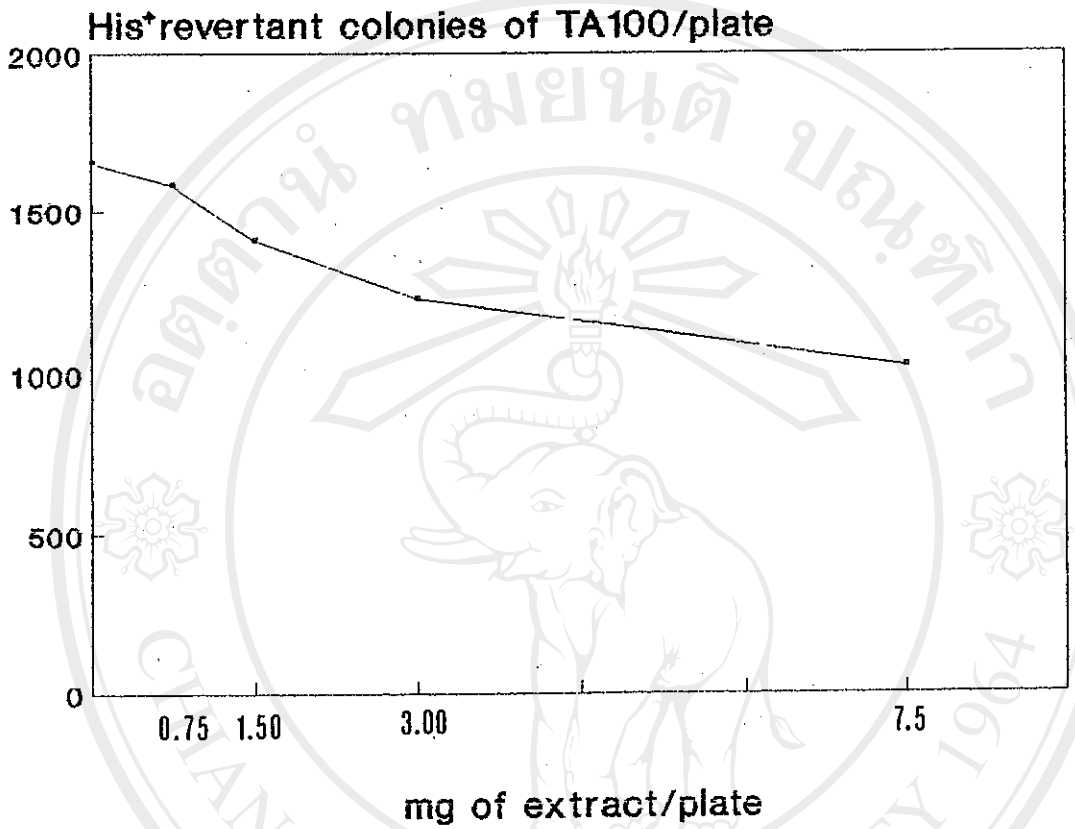


Figure 14 Preincubation of MNNG with the metanol extract of lemon grass could altered the mutagenicity of MNNG

3.3.2 Effect on metabolic activation

Subsequent experiments showed that pre-incubation of lemon grass extract with S9 mix before mixing with AFB-1, (Figure 5, Procedure 1) resulted in inhibition of AFB-1 mutagenesis as shown in Table 10, Figure 15. When the extract was mixed with S9 fraction (without cofactor) (Figure. 5, Procedure 3) it also gave similar effect in the reduction the mutagenicity of aflatoxin B_1 (Table 11, Figure 16).

It was found that the methanol extract of lemon grass had affect on MNNG mutagenesis. (Table 12).

Table 10 Effect of methanol extract of lemon grass of S9 mix. Pre-treatment of S9-mix with the extract at 37° C for 30 min decreased the metabolic activation of AFB_1 metabolism.

Amount of extract (mg)	His^+ revertant colonies of TA98/plate**	% inhibition*
0	1005 ± 212.45	-
0.75	837 ± 67.38	16.7
1.50	574 ± 39.98	42.8
3.00	199 ± 22.31	80.2
7.50	119 ± 11.12	88.1
BG	27 ± 0.81	-

** Results are means ± SD of six plates from two independent experiments

* see footnote table 4.1

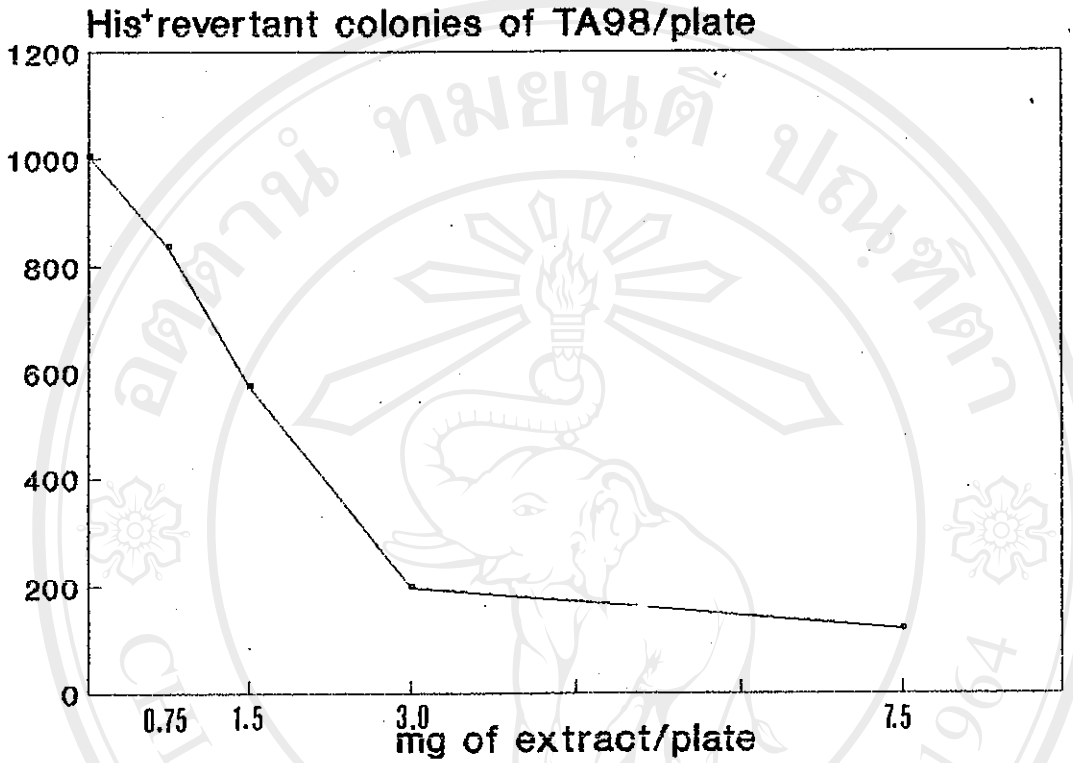


Figure 15 Effect of methanol extract of lemon grass on S9 mix

Table 11 Pre-treatment of buffer with the extract at 37° C for 30 min decreased the mutagenicity of MNNG.

Amount of extract (mg)	His$^+$ revertant colonies of TA100/plate**	% inhibition*
0	1983 ± 115.59	-
0.75	1736 ± 232.24	12.4
1.50	1592 ± 109.87	19.7
3.00	1348 ± 89.92	32.0
7.50	1164 ± 77.76	41.3
BG	100 ± 9.87	-

** Results are means ± SD of six plates from two independent experiments

* see footnot table 4.1

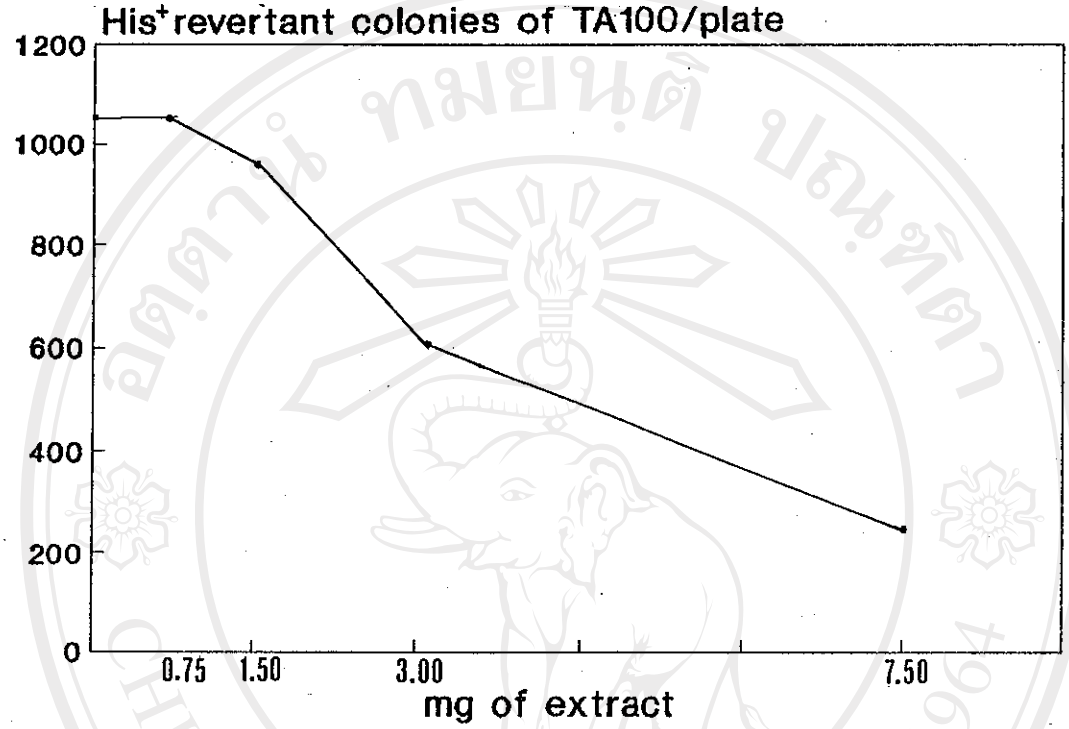


Figure 16 Pretreatment of methanol extract of lemon grss with enzymes in S9 fraction (Without cofactor) resulted in decrease in S9 fraction (Without cofactor) resulted in dectrease in AFB_1-mutagenesis

Table 12 Pretreatment of methanol extract of lemon with enzyme in S9 fraction without cofactor resulted in decrease of AFB_1 - mutagenesis.

Amount of extract (mg)	His^+ revertant colonies of TA98/plate**	% inhibition
0	1050 ± 193.52	-
0.75	1056 ± 187.87	-
1.50	968 ± 210.3	7.8
3.00	604 ± 23.11	42.4
7.50	246 ± 15.19	76.5
BG	32 ± 5.91	-

** Result are mean ± SD of six plates from two independent experiments

* see footnote table 4.1

3.4 Bioantimutagenic activity of lemon grass

As shown in Table 13, and Figure 17, lemon grass extract had slightly repaired the mutation of S. typhimurium strain TA 98 when induced with AFB_1. The number of revertant colonies was not changed too much compared with the number of AFB-1 mutated cells. AFB_1-treated cell might be able to reverse back to Histidine-auxotrophic strain (His^+).

When S. typhimurium strain TA 100 was treated with MNNG and subsequently mixed with lemon grass extract, it was shown that there was slightly decrease of the number of Histidine revertant (Table 14, Figure 18).

3.5 Isolation and partial purification of antimutagenic substance in methanol extract of lemon grass.

Table 6 demonstrated that most of the antimutagenicity of lemon grass was in the methanol extract. Thus the antimutagenic substances in this fraction were subjected to be purified further. The Sephadex LH-20 chromatogram of the extract was shown in Figure. 19. Six peaks (Peak 1, 2, 3, 4, 5 and 6) were separated. All of them showed

Table 13 Decrease of AFB_1-mutated cells (S. typhimurium TA 98) after treatment with methanol extract of lemon grass.

Amount of extract (mg)	His^+ revertant colonies of TA98/plate** (+ S9 mix)	% inhibition*
0	1392 ± 149.27	-
0.75	1376 ± 130.60	1.4
1.50	1149 ± 123.32	17.4
3.00	1076 ± 98.87	22.7
7.50	944 ± 72.22	32.1
15	976 ± 31.8	29.8
BG	59 ± 2.49	-

** Results are means ± SD of six plates from two independent experiments

* see footnot table 4.1

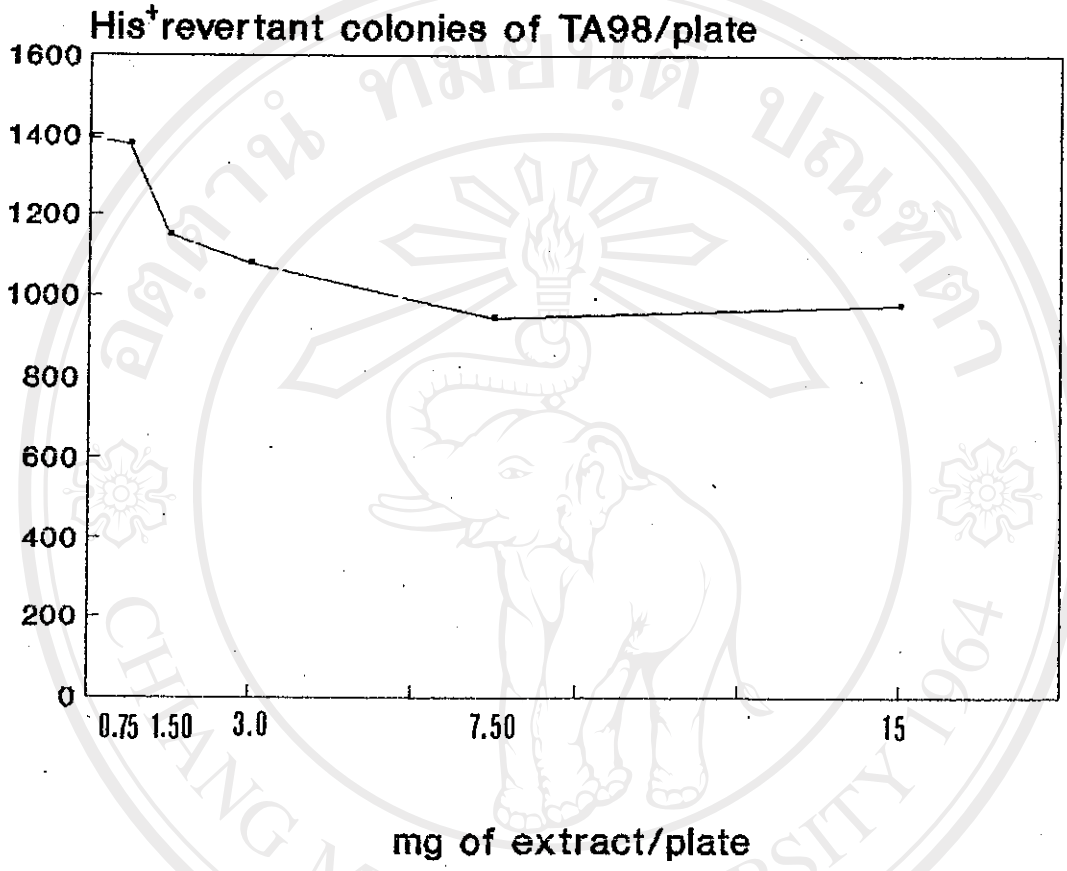


Figure 17 AFB-mutated cells (S.typhimurium strain TA98) after treatment with methanol extract of lemon grass

Table 14 Methanol extract of lemon grass could reverse the MNNG-mutated cells (S. typhimurium TA 100).

Amount of extract (mg)	His^+ revertant colonies of TA100/plate**	% inhibition*
0	1807 ± 139.48	-
0.75	1756 ± 158.37	2.3
1.50	1718 ± 146.38	4.9
3.00	1772 ± 138.77	18.5
7.50	1314 ± 131.0	27.2
15.00	1220 ± 37.42	32.4
BG	125 ± 22.62	-

** Results are means ± SD of six plates from two independent experiments

* see foot note table 4.1

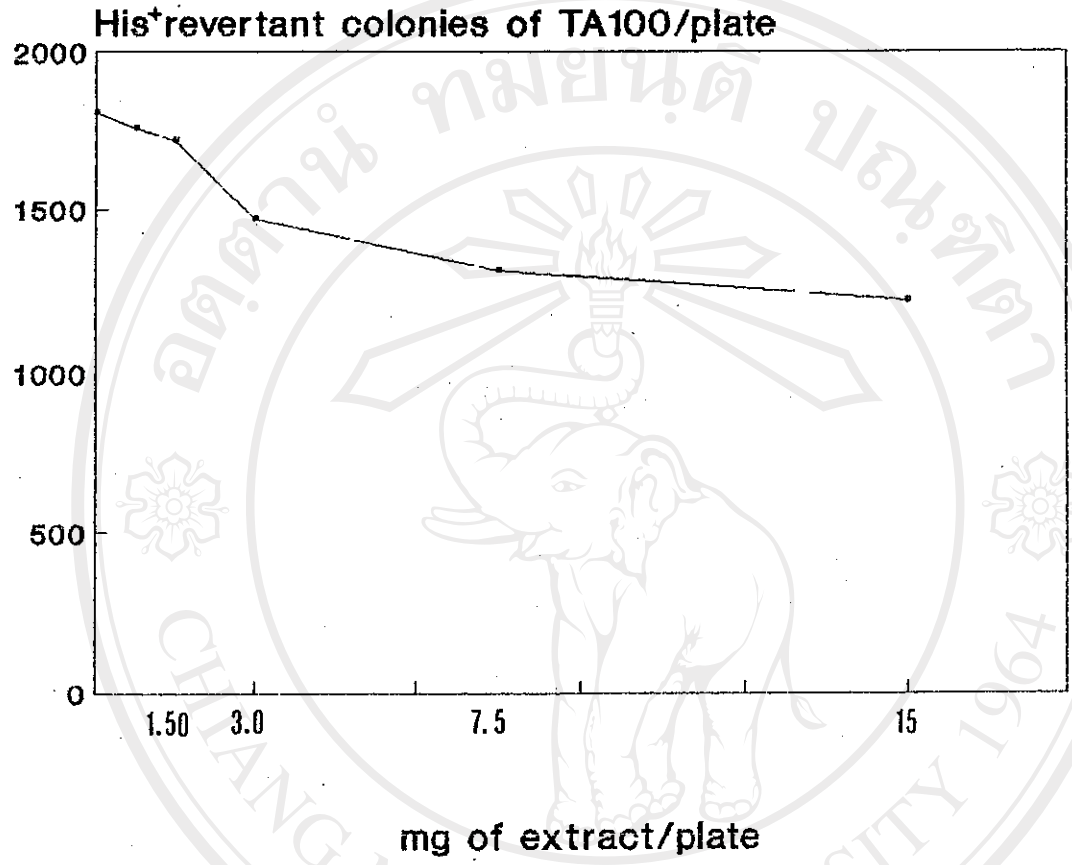
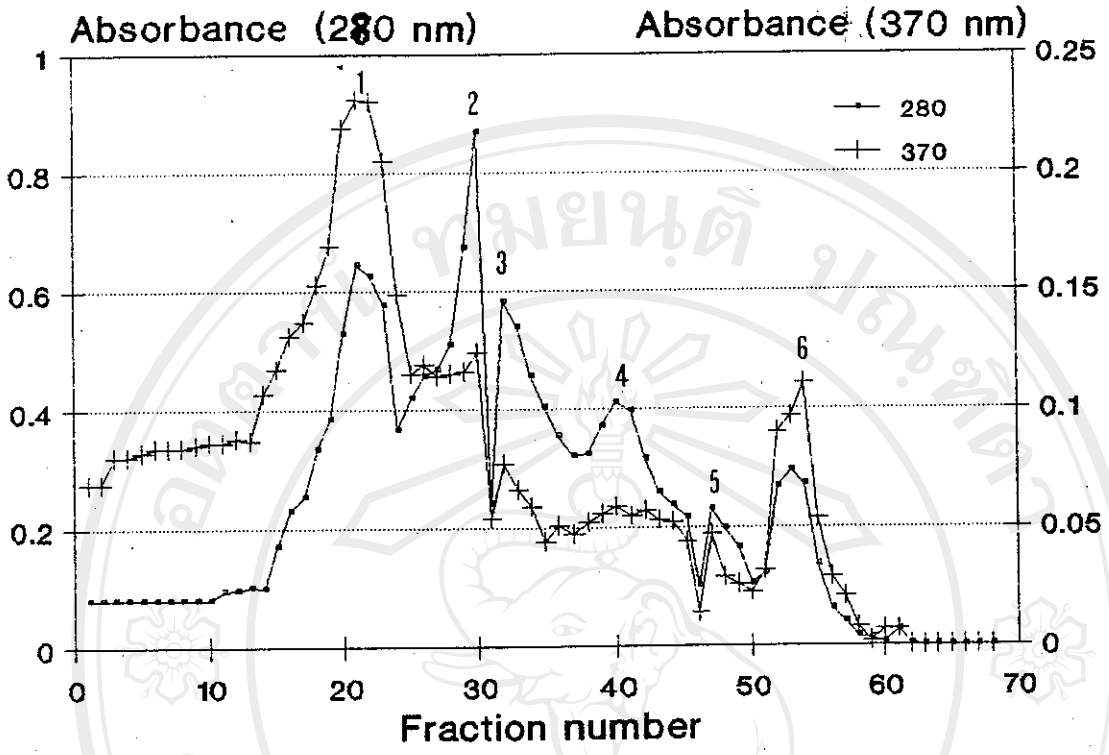


Figure 18 Methanol extract of lemon grass could reverse the MNNG-mutated cells (S. typhimurium strain TA 100)



Peak munber	Fraction number	mg of sample
1	17-23	34.80
2	27-30	51.40
3	32-36	14.80
4	39-45	3.80
5	46-49	9.00
6	52-56	2.60

Figure 19 Gel chromatography of methanol extract of lemon grass on Sephadex LH-20 (40 x 1.5 cm)

antimutagenicity toward AFB_1-induced mutation in S.typhimurium strain TA 98. (Table 15, Figure 20), and to MNNG (Figure 21). The most antimutagenicity was found in the peak 2 which was eluted by methanol at the fraction number 27 - 30 . The residue of this peak showed highest yield among other peaks. Therefore, it was considered worthy to do further purification.

The Peak 2 fraction was further purified by running on the same column and same eluent. The main antimutagenic fraction was eluted in single peak, named peak 2a (Figure22). The antimutagenicity against AFB-1 and MNNG was shown in Figures 23 and 24. It still exerted antimutagenicity toward AFB-1 but reduction of antimutagenicity to MNNG was not shown. This peak was finally purified by HPLC using Bondaclone 10 C_{18} column (Phenomenex) and eluted with methanol. The HPLC chromatogram is shown in Figure 25. Two peaks were separated at retention time 2 - 4 min (peak $2a_1$) and 4.5 - 5.0 min (peak $2a_2$). Both of them could decrease the mutagenicity of AFB-1 in S.typhimurium TA98, but not MNNG (Figures 26 and 27).

Table 15 Antimutagenicity of compound fractionated from methanol extract of lemon grass by Sephadex LH-20 column chromatography (40 x 1.5 cm)

Amount of peak (mg/plate)		His^+ revertant** colonies of TA98/plate (AFB_1)	% inhibition*	His^+ revertant** colonies of TA100/plate (MNNG)	% inhibition*
Peak 1	0	657 ± 23.01	-	1102 ± 116.29	-
	1.25	418 ± 3.35	36.37	1109 ± 34.43	0
	2.50	276 ± 14.98	57.99	915 ± 11.50	16.9
Peak 2	0	657 ± 23.01	-	1102 ± 116.29	-
	1.25	261 ± 12.76	60.27	801 ± 39.30	27.3
	2.50	176 ± 14.99	73.21	640 ± 23.76	41.9
Peak 3	0	657 ± 23.01	-	1102 ± 116.29	-
	1.25	476 ± 29.97	27.54	788 ± 52.98	28.6
	2.50	368 ± 25.48	43.98	850 ± 102.31	22.8
Peak 4	0	657 ± 23.01	-	1102 ± 116.29	-
	1.25	598 ± 16.39	8.98	792 ± 32.77	28.1
	2.50	377 ± 17.65	42.61	804 ± 29.39	27.0
Peak 5	0	657 ± 23.01	-	1102 ± 116.29	-
	1.25	732 ± 14.89	-	1090 ± 34.72	1.0
	2.50	589 ± 19.87	10.35	932 ± 28.21	15.4
Peak 6	0	657 ± 23.01	-	1102 ± 116.29	-
	1.25	521 ± 12.76	20.70	1126 ± 53.30	0
	2.50	403 ± 14.83	38.66	1090 ± 38.68	0.5

** Results are mean ± SD of six plates from two independent experiments

* see footnote table 4.1

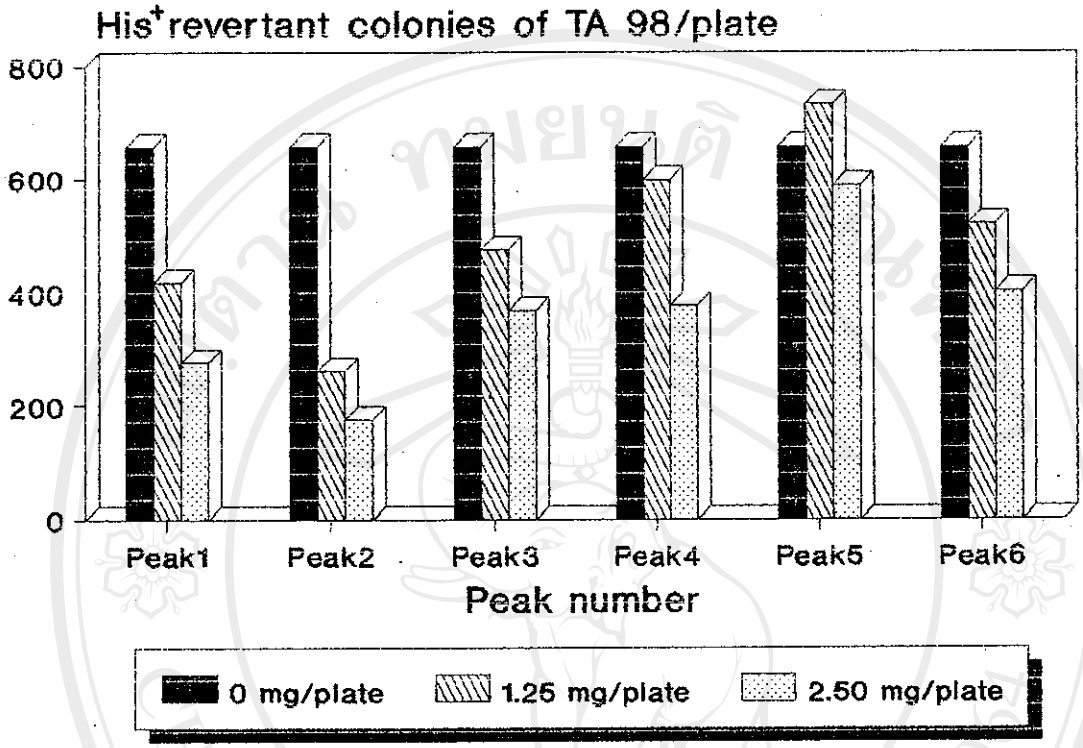


Figure 20 Antimutagenic activity of each peaks separated by Sephadex LH-20 column against AFB-mutagenesis in S.typhimurium Strain TA98

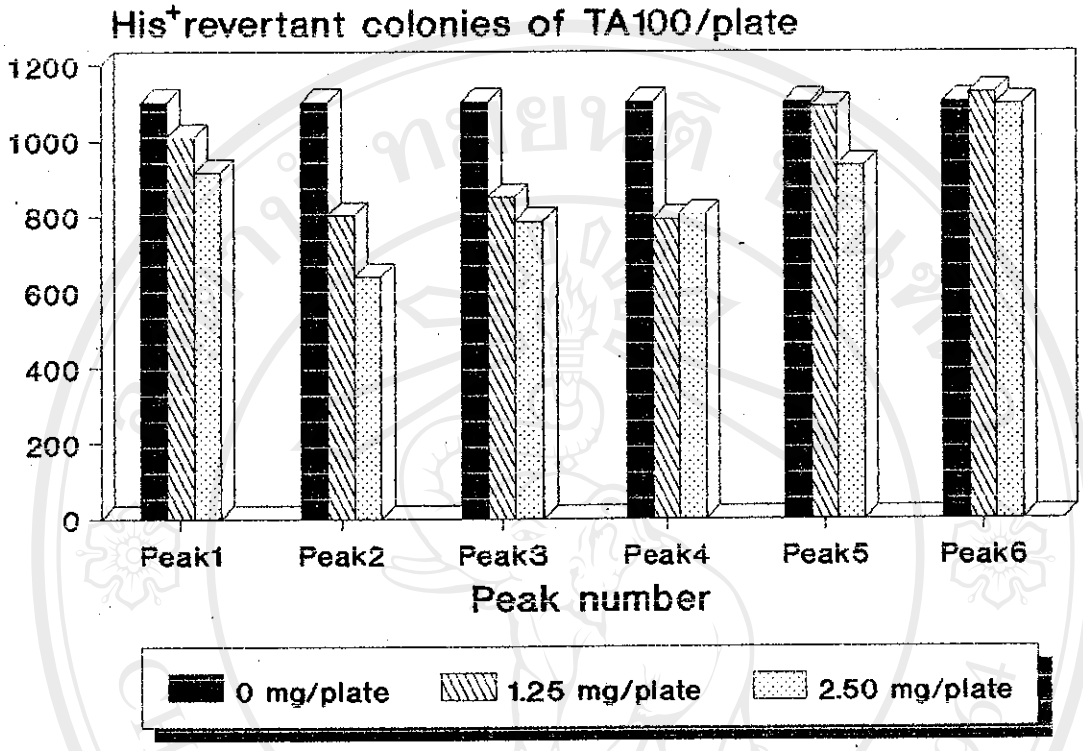


Figure 21 Antimutagenic activity of each peaks separated by Sephadex LH-20 column against MNNG-mutagenesis in S. typhimurium strain TA100

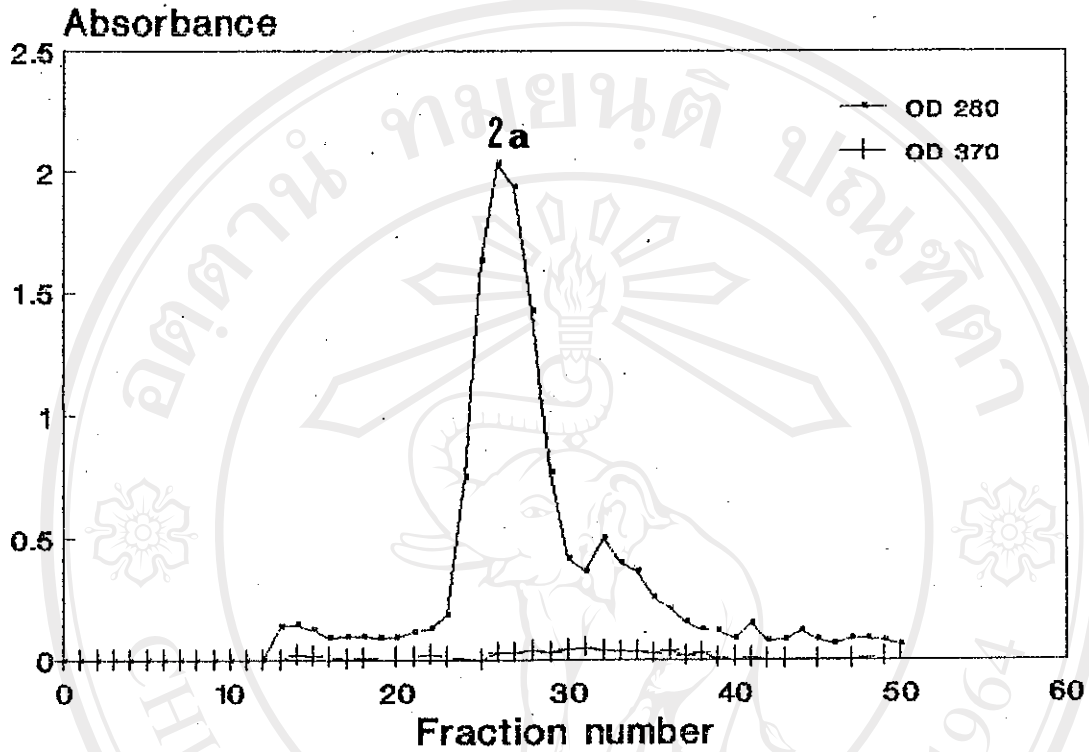


Figure 22 Second purification of compound in peak 2 on Sephadex LH-20 column (40 x 1.5 cm)

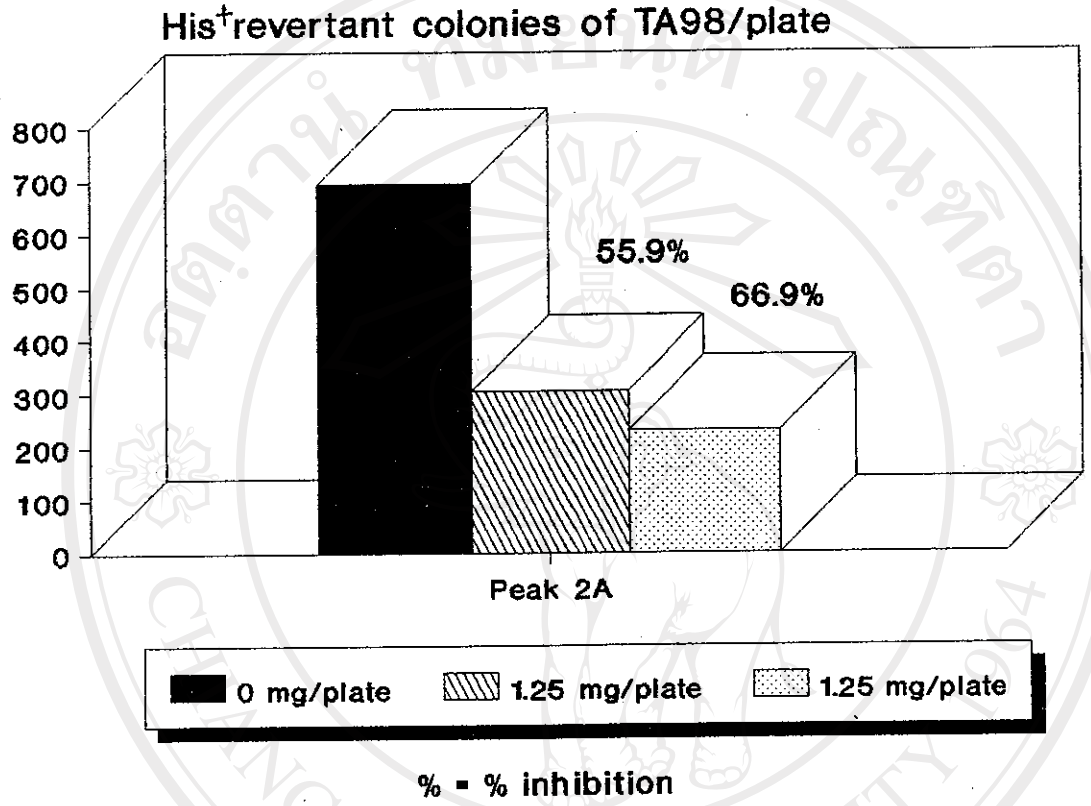


Figure 23 Antimutagenicity of peak 2a after second chromatography toward AFB_1-mutagenesis in S. typhimurium strain TA98

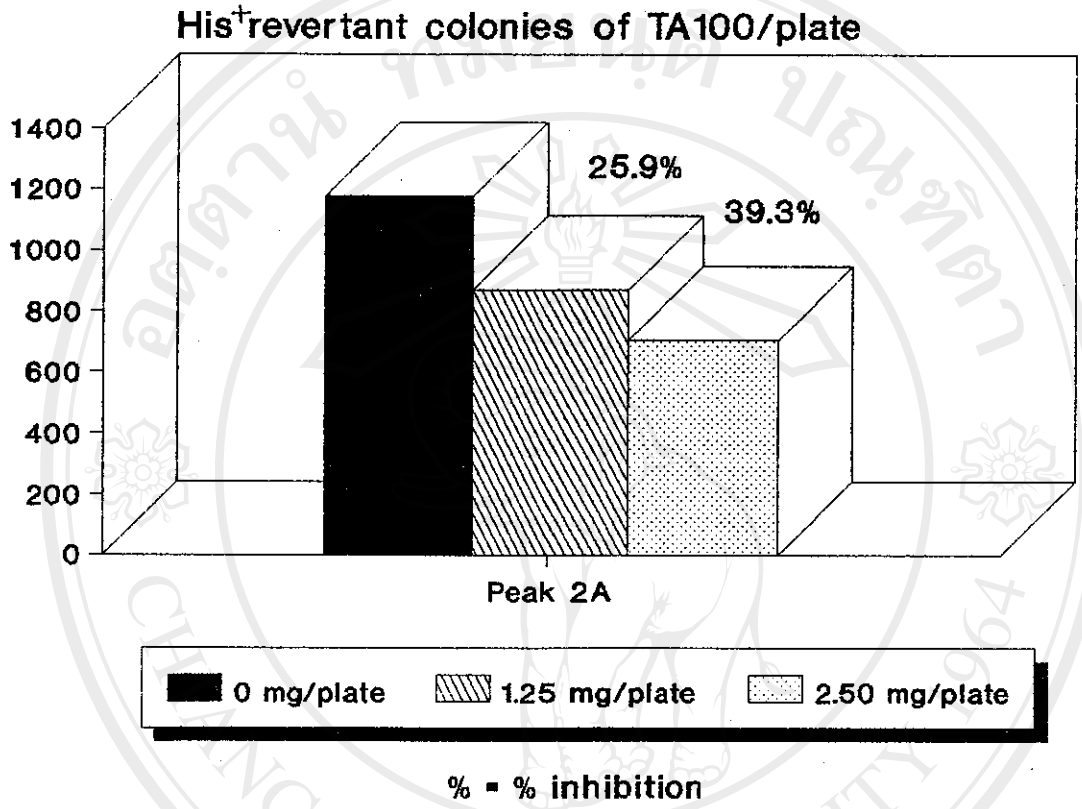


Figure 24 Antimutagenicity of peak 2a after second chromatography toward MNNG-mutagenesis in S. typhimurium strain TA100

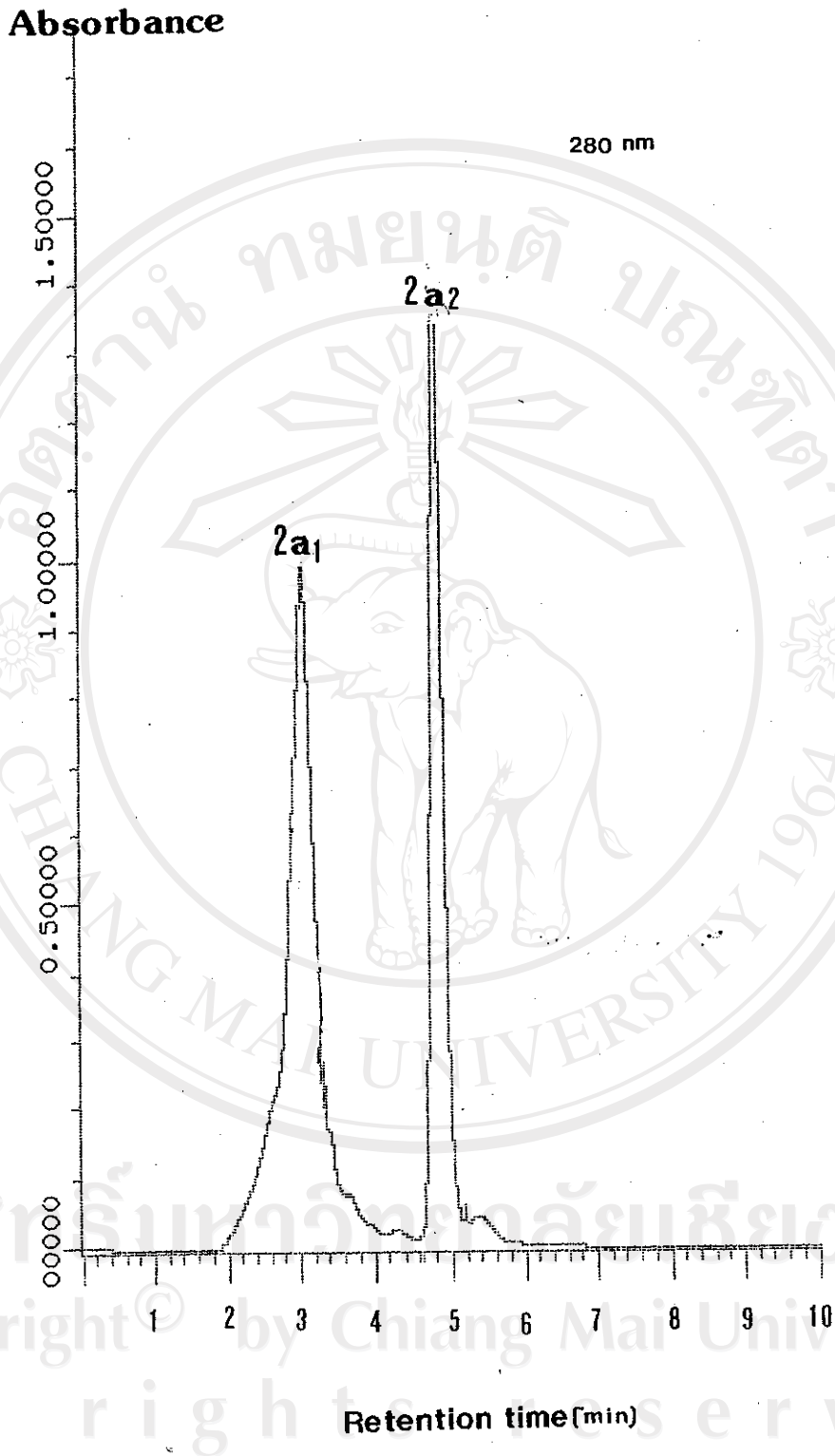


Figure 25 Third purification of compound in peak 2 a on HPLC (Bondclone 10 C_{18} column)

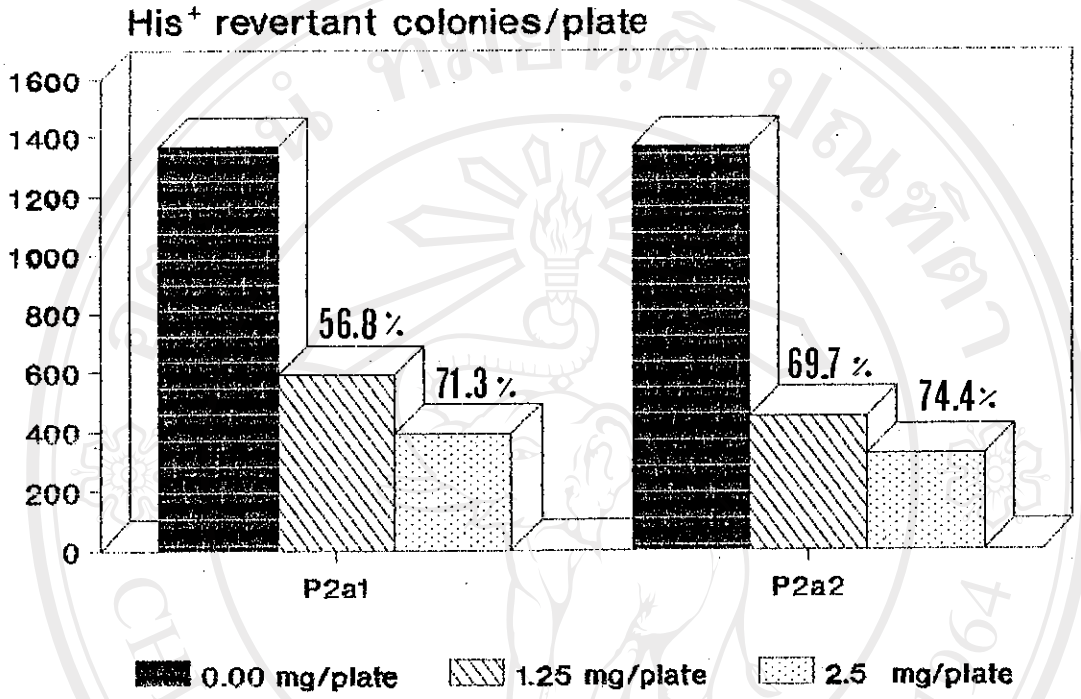


Figure 26 Antimutagenicity of peak $2a_1$ and peak $2a_2$ against AFB_1 mutagenesis in S. typhimurium strain TA98

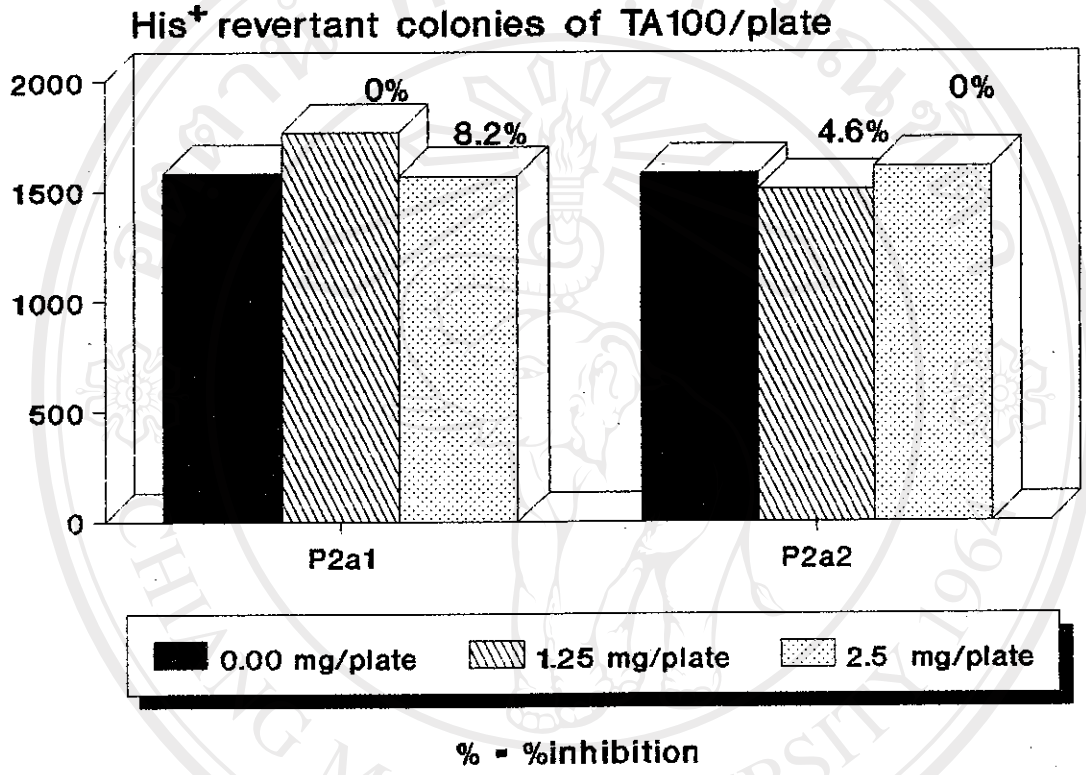


Figure 27 Antimutagenicity of peak $2a_1$ and peak $2a_2$ against MNNG-mutagenesis in S. typhimurium starin TA 100

3.6 Bioantimutagenicity of the partially purified compound (peaks $2a_1$ and $2a_2$) from methanol extract of lemon grass

As shown in Figures 28, and 29., the two peaks,did not have any bioantimutagenic effect on both AFB_1 and MNNG -mutated cells.

3.7 Some characteristic of the partially purified antimutagenic substances.

3.7.1 Rf values of the partial purification of antimutagenic substances in TLC

The Rf value of the two partially purified antimutagenic substances separated from methanol extract of lemon grass was determined by thin layer chromatography using silica gel precoated sheet, after TLC development, the location of the spot was detected by exposure to ultraviolet lamp. The Rf values of the compounds are shown in Table 16. The partially purified substances gave only single spot in the Two different solvent system

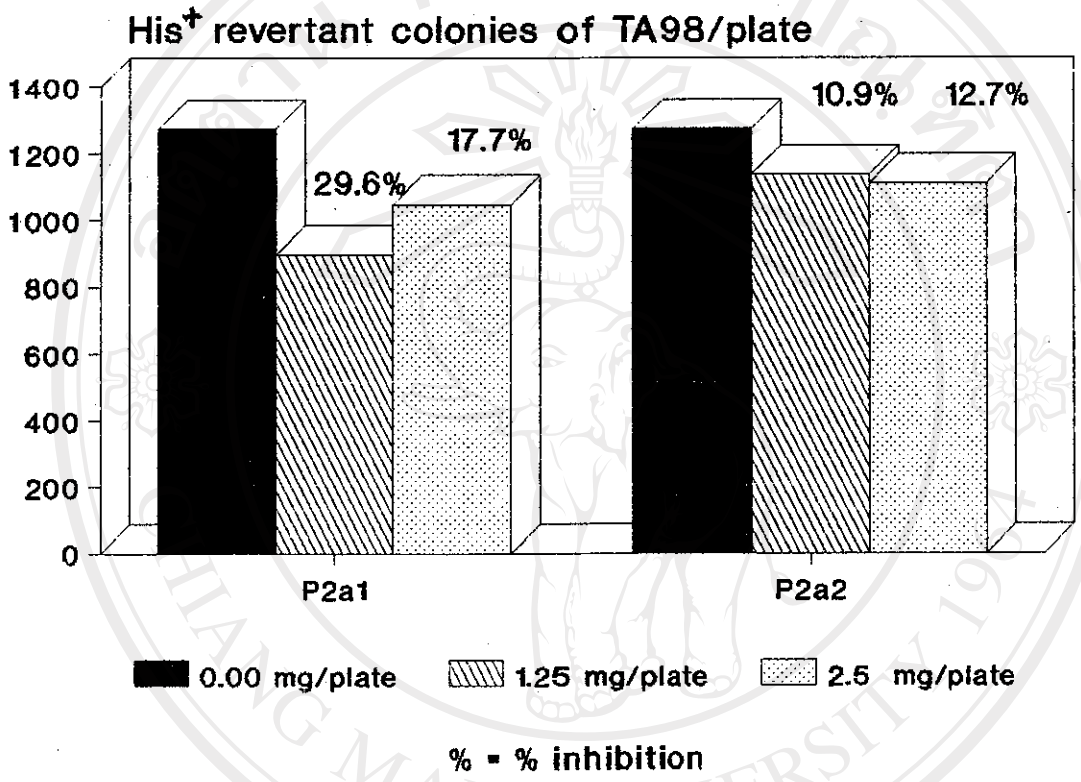


Figure 28 Bioantimutagenicity of peak $2a_1$ and peak $2a_2$ against AFB_1 and MNNG-mutagenesis in S. typhimurium

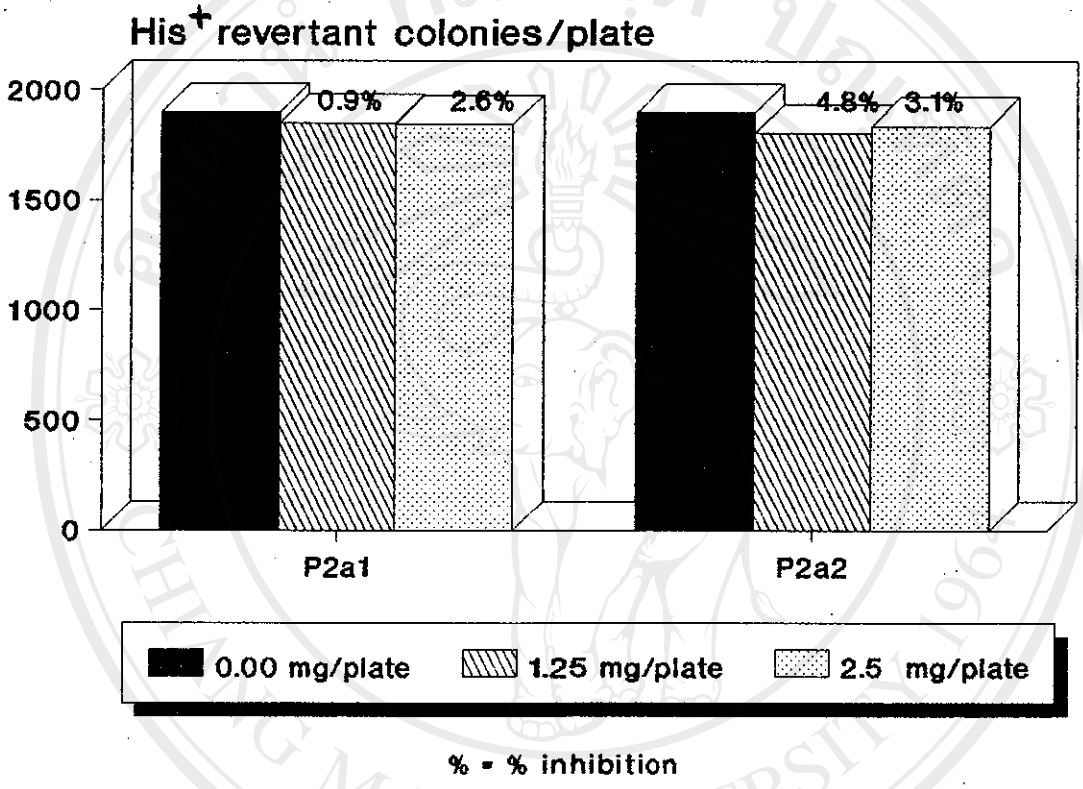


Figure 29 Bioantimutagenicity of peak $2a_1$ on MNNG-mutagenesis

Table. 16 R_f values of partially purified materials of peak 2a from HPLC

Peak	Rf values	
	Solvent system 1	Solvent system 2
$2a_1$	0.87	0.86
$2a_2$	0.93	0.84

Solvent system 1 = acetonitrile : water 7: 3

Solvent system 2 = methanol : water 6: 4

3.7.2 Chemical reaction

The compounds in the two peaks were checked for their colour reaction with ninhydrin, nitroprusside and Benedict reagents. The results are shown in Table 17. The compound in both peaks gave red precipitation with Benedict reagent, and gave negative reaction with Ninhydrin and nitroprusside reagent. There was a possible presence of carbohydrate neither in the partial purified antimutagenic substances, but protein nor sulfhydryl group containing compound.

3.7.3 Ultraviolet absorption spectra of the antimutagenic substances

During HPLC separation, the separated peaks were integrated and scaned by Bio-Rad Chrom-A-Scope detector connected to personal computer which equiped with BDS software. The absorption spectra of the peaks is shown in Figure 30. The peak$2a_1$ gave maximum absorption at 220 nm, and 240 nm, peak $2a_2$ gave maximun absorption at 220 nm and 230 nm.

Table. 17 Chemical reaction properties of partially purified compound from peak 2.

Reaction with	Result	
	peak $2a_1$	peak $2a_2$
Ninhydrin reagent	no purple colour	no purple colour
Benedict reagent	red precipitation	red precipitation
Nitroprusside reagent	no red colour	no red colour

Table .18 Antimutagenicity of compound in peak 2 in each purification

Peak	Weight mg.	Desmutagenicity against		Bio-antimutagenicity against	
		AFB_1	MNNG	AFB_1	MNNG
first isolation by Sephadex LH-20					
2	38	+	±	±	±
2nd Purification by Sephadex LH-20					
2a	26.22	+	±	-	-
3rd Purification by HPLC (Bondclone 10 C_{18} column)					
$2a_1$	15.2	+	-	-	±
$2a_2$	10.06	+	-	-	-

Methanol extract of lemon grass = 100 mg

+ = > 30 % inhibition, ± = 30 % inhibition, and

- = < 30 % inhibition

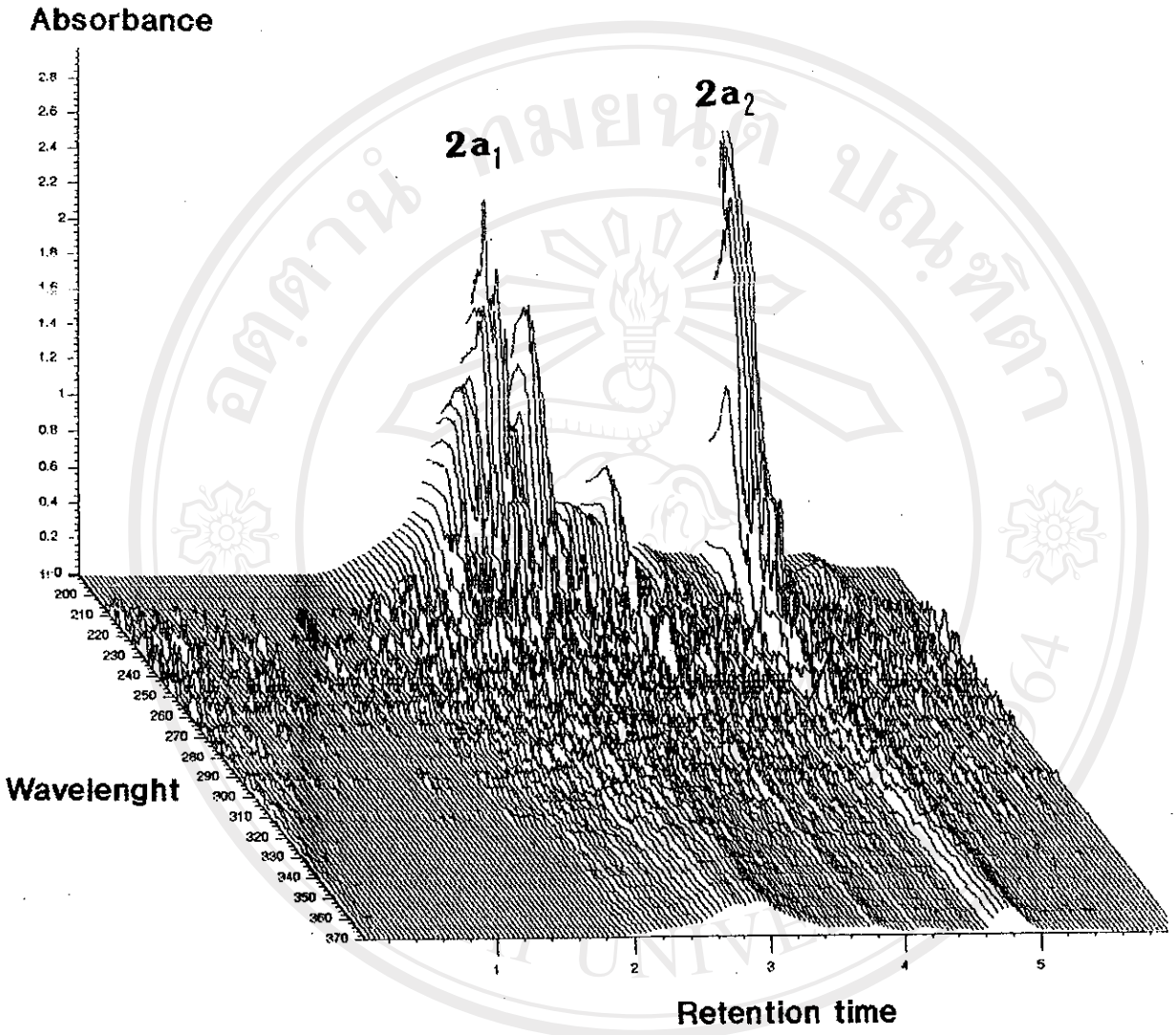


Figure 30 Absorption spectra scan of separated peaks from HPLC

3.7.4 GC-MS spectra

The partially purified antimutagenic substances obtained from methanol extract of lemon grass were analyzed for their molecular weitht by gas chromatography-mass spectra. The GC-MS spectra is shown in Figure 31. It indicated that, the molecular weight of peak $2a_1$, was about 80-90, and peak $2a_2$ was about 387.

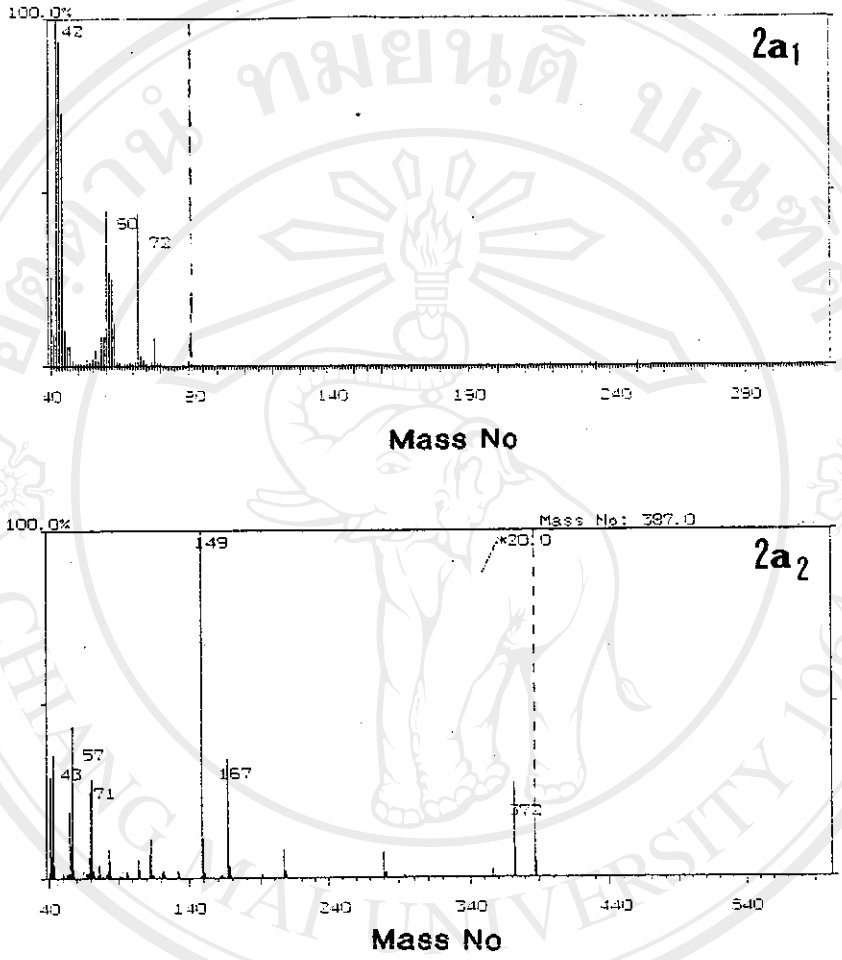


Figure 31 The GC-MS spectrum of separated peak from HPLC.