CHAPTER IV

RESULTS

4.1 Reverse mutation assay (The Ames Salmonella/microsome assay)

4.1.1 Survival Test

The preliminary screening for toxicity dependence dose of plants extracts on *Salmonella* Typhimurium strain TA98 and TA100 was performed. The plant extracts with highest antioxidant activity, (Sutjit,2003), *P. mirifica* from Uthai Thani (2,904.52 ± 33.24 μg/ml), *B. superba* from Loei (653.64 ± 38.83 μg/ml) and *M. collettii* from Chiang Rai (83.06 ± 8.10 μg/ml) province were chosen to test for cytotoxicity dependence dose. The toxicity was found as dose dependent on all plant extracts but the most potency was found in *M. collettii* extract. The LC_{50} of, *P. mirifica*, *B. superba* and *M. collettii* extracted were10.27, 3.64, 0.49 mg/plate or 3,209, 1,137, 153 μg/ml on the growth of *S.* Typhimurium strain TA98; and 17.50, 6.71, 2.44 mg/plate or 5,469, 2,096, 763 μg/ml on the growth of *S.* Typhimurium strain TA100, respectively. Therefore, in this experiment the highest concentration 2.5 mg/plate of each kind of Kwao Krua extract was used in order to maintain the best survival of *S.* Typhimurium TA 98 and TA100 (Table 4.1). Futhermore, the concentration of 0.625, 1.25 and 2.50 mg/plate of Kwoa Krua plant extracts were chosen to perform as for screening mutagenic and antimutagenic activities assay by Ames Test.

Table 4.1 Survival test of the plant extracts on *S.* Typhimurium TA98 and TA100

Plant extracts	Concentration (mg/plate)	Percentage survival (Mean ± S.E.M.)	
		TA98	TA100
P. mirifica	2.5	74.41 ± 5.22[b]	78.28 ± 2.69[b]
	5.0	41.81 ± 2.17[a]	51.04 ± 1.73[a]
	10.0	36.67 ± 3.57[a]	54.23 ± 3.03[a]
	20.0	35.36 ± 1.94[a]	48.54 ± 3.04[a]
B. superba	2.5	60.72 ± 4.17[c]	41.74 ± 1.47[c]
	5.0	24.27 ± 3.20[b]	32.03 ± 1.58[b]
	10.0	6.40 ± 0.05[a]	29.43 ± 1.12[b]
	20.0	4.45 ± 0.31[a]	13.83 ± 3.39[a]
M. collettii	0.5	34.15 ± 3.69[c]	77.93 ± 3.59[c]
	1.25	13.58 ± 3.79[b]	75.63 ± 3.16[c]
	2.5	0.68 ± 0.37[a]	29.42 ± 0.65[b]
	5.0	0.13 ± 0.08[a]	16.17 ± 0.46[a]

Means not sharing a common superscript letter in the same column of each plant extracts are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

4.1.2 Mutagenic activity of the plant extracts toward *S.* Typhimurium TA98 and TA 100 in the absence and presence of metabolic activation

All plant extracts showed no mutagenicity (Appendix A; Table 5.2, 5.3, 5.4 and 5.5) except *B. superba* from Ratchaburi province. Since the highest concentration of the plant extract (2.5 mg/plate) showed significant ($P < 0.05$) higher amount of revertant colonies than those from the negative control and other plant extracts(Figure 4.1).

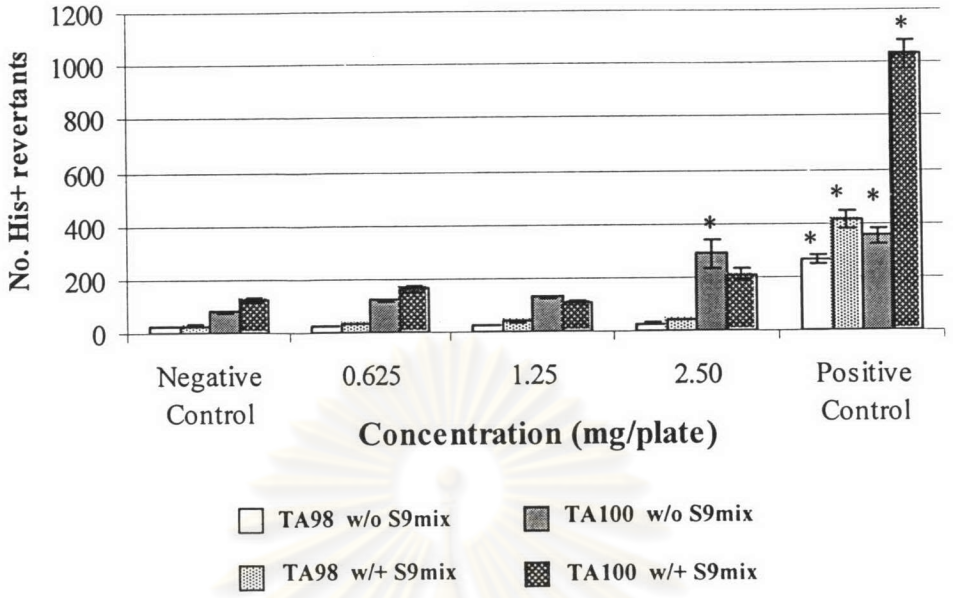


Figure 4.1 *B. superba* extract collected from Ratchaburi province showed mutagenicity on *S.* Typhimurium strain TA100 at the concentration of 2.50 mg/plate under non metabolic active condition. (*$P < 0.05$ as compared with the negative control). The plant extract exhibited 81.25 % of mutagenic effect as compared with the positive control.

4.1.3 Antimutagenic activity of the plant extracts toward *S.* Typhimurium TA98 and TA 100 in the absence and presence of metabolic activation

The potential antimutagenic effect of the plant extracts at the concentration at 0.625, 1.25 and 2.50 mg/plate as compared with the control, set as 100% mutagenicity were tested. The criteria of interpretation was based on the previous report that: the potential of antimutagenic effect was considered strong when the inhibitory effect was higher than 60% inhibition and moderate when the inhibitory effect was in the range 40-60%. Inhibitory effect less than 40% were considered weak, and it was not recognized as a positive result (Ikken, Camberol and Marrrin *et al.*, 1998)

4.1.3.1 Antimutagenic activity of *P. mirifica* extracts

P. mirifica extracts collected from 28 provinces of Thailand were screened for antimutagenic activity. Certain extract significantly decreased the number of revertants induced by AF-2 and B(*a*)P with dose-dependently (Table 4.2, Appendix A; Table 5.2).

At the concentration of 0.625 mg/plate

Using AF-2 (0.1 μg/plate) on *S.* Typhimurium TA98, the extracts of *P. mirifica* from Uttradith exhibited weak inhibition. None of them showed moderate or strong antimutagenic activity. When tested of AF-2 (0.01 μg/plate) on *S.* Typhimurium TA100, the samples from Nakhon Sawan, Lampang, Lop Buri, Nong Bua Lam Phu, Kamphaeng Phet and Sukhothai exhibited weak inhibition. Also, no moderate or strong antimutagenic activity was found in any sample.

Using B(*a*)P (10 μg/plate) on *S.* Typhimurium TA98, the samples from Phetchabun and Chiang Mai exhibited weak inhibition. No sample with moderate or strong inhibition was found. When tested with B(*a*)P (5 μg/plate) on *S.* Typhimurium TA100, the samples from Tak, Sukhothai, Phrachin Buri, Nakhon Ratchasima exhibited weak inhibition activities. Only the sample from Chiang Rai showed moderate inhibition whereas no sample with strong inhibition was detected (Figure 4.2, 4.5).

At the concentration of 1.25 mg/plate

Using AF-2 (0.1 μg/plate) on *S.* Typhimurium TA98, the extracts of *P. mirifica* from Sukhothai, Nan and Uttradith exhibited weak inhibition. None of them showed moderate or strong antimutagenic activity. When tested with AF-2 (0.01 μg/plate) on *S.* Typhimurium TA100, the samples from Nakhon Sawan, Mae Hong Son, Phitsanulok, Chiang Rai, Phetchaburi, Saraburi, Sukhothai, Lop Buri, Payao, Prachuap Kiri Khun, Lumpang and Nan exhibited weak inhibition and only the samples from Nong Bua Lum Phu exhibited moderate inhibition. Also, no strong antimutagenic activity was found in any sample.

Using B(*a*)P (10 μg/plate) on *S.* Typhimurium TA98, the samples from Nan, Lop Buri and Uthai Thani exhibited weak inhibition. There was no sample with moderate or strong inhibition. When tested with B(*a*)P (5 μg/plate) on *S.* Typhimurium TA100, the weak antimutagenic activities were detected in the samples from Chiang Mai, Tak, Payao, Chiang Rai, Prachin Buri and Saraburi and the samples from Sukhothai, Ratchaburi, Phetchabun exhibited moderate inhibition whereas no sample with strong inhibition was detected (Figure 4.3, 4.6).

At the concentration of 2.50 mg/plate

Using AF-2 (0.1 μg/plate) on *S.* Typhimurium TA98, the extracts of *P. mirifica* from Payao exhibited weak inhibition. None of them showed moderate or strong antimutagenic activity. When tested with AF-2 (0.01 μg/plate) on *S.* Typhimurium TA100, the weak antimutagenic activities were found in the samples from Phitsanulok, Saraburi, Nong Bua Lum Phu, Mae Hong Son, Sukhuthai, Uthai Thani and Kanchanaburi and the sample from Uttraradith exhibited moderate inhibition. Also, no strong antimutagenic activity was found in any sample.

Using B(*a*)P (10 μg/plate) on *S.* Typhimurium TA98, the samples from Uthai Thani, Phetchaburi, Chiang Mai, Nan, Nakhon Sawan, Uttraradith and Phetchabun exhibited weak inhibition. None of them showed moderate or strong antimutagenic activity. When tested of B(*a*)P (5 μg/plate) on *S.* Typhimurium TA100, the weak antimutagenic activities were detected in the samples from Chiang Mai, Phetchabun, Phitsanulok, Prachuap Kiri Khun, Ratchaburi, Lumpang, Nong Bua Lum Phu, Chaiyaphum, Lopburi, Kanchanaburi and Nakhon Ratchasima. The samples from Sukhothai, Phrachinburi, Phrae, Chiang Rai and Tak exhibited moderate antimutagenic activities inhibition whereas no sample with strong inhibition was detected (Figure 4.4, 4.7).

Table 4.2 Antimutagenic activities of *P. mirifica* extracts from different provinces of Thailand detected by Ames test using *S.* Typhimurium strain TA98 and TA100 under non metabolic and metabolic activation conditions.

Province	Dose (mg/plate)	PI (%inhibition)			
		TA98		TA100	
		-S9	+S9	-S9	+S9
Chiang Rai	0.625	-33.00 ±11.17^{b*}	-24.76 ±13.82^{a*}	18.43 ± 0.97^{ab}	44.24 ± 3.76^{b*}
	1.25	-4.30 ± 7.75^{a}	0.03 ± 2.47^{ab}	30.24 ± 6.50^{b*}	45.66 ± 4.84^{b*}
	2.50	-32.40 ±11.24^{b*}	5.12 ± 3.83^{b}	4.34 ± 8.04^{ab}	47.75 ± 1.66^{b*}
Chiang Mai	0.625	-28.80 ± 5.82*	20.96 ± 11.36^{b*}	-48.69 ±13.98^{a*}	14.40 ± 5.18^{bc*}
	1.25	-28.53 ± 6.16^{a*}	1.93 ± 7.79^{a}	-32.03 ± 3.78^{a*}	24.88 ± 8.42^{cd*}
	2.50	-34.71 ± 3.48^{a*}	26.53 ± 4.98^{b*}	-56.81 ±19.92^{a*}	36.60 ± 4.01^{d*}
Mae Hong	0.625	11.52 ± 5.49^{b*}	0.81 ± 1.46^{a}	19.23 ± 1.96^{b*}	12.21 ± 4.59^{b*}
Son	1.25	11.96 ± 3.99^{b*}	4.28 ± 9.47^{a}	34.00 ± 6.43^{c*}	19.13 ± 1.77^{b*}
	2.50	13.15 ± 4.03^{b*}	5.54 ± 3.46^{a}	36.13 ± 4.16^{c*}	13.53 ± 3.28^{b*}
Payao	0.625	9.95 ± 0.14^{b*}	5.95 ± 6.15^{a}	7.65 ± 6.49^{ab}	4.57 ± 3.41^{a}
	1.25	7.23 ± 4.25^{a}	6.15 ± 9.52^{a}	24.87 ± 5.90^{c*}	45.79 ± 1.33^{b*}
	2.50	21.52 ± 4.05^{c*}	14.00 ± 4.43^{a}	16.95 ± 8.47^{b*}	39.50 ± 4.11^{b*}
Nan	0.625	2.54 ± 5.22^{a}	15.94 ± 6.37^{b*}	19.40 ± 4.93^{b*}	-10.27 ± 10.57^{a}
	1.25	24.92 ± 7.06^{b*}	36.22 ± 2.37^{c*}	21.83 ± 1.66^{b*}	-1.13 ± 2.76^{a}
	2.50	0.68 ± 4.93^{a}	24.47 ± 6.95^{bc*}	18.95 ± 4.21^{b*}	-4.66 ± 4.88^{a}
Lampang	0.625	0.29 ± 6.14^{a}	8.57 ± 10.84^{ab}	26.05 ± 2.92^{c*}	15.55 ± 8.83^{b*}
	1.25	3.58 ± 3.97^{a}	11.64 ± 7.36^{b}	21.97 ± 7.87^{c*}	15.94 ± 7.81^{b*}
	2.50	13.29 ± 7.32^{b*}	-22.16 ± 6.66^{a*}	14.94 ± 9.86^{bc*}	28.32 ± 2.63^{b*}
Phrae	0.625	-10.34 ± 7.98^{a}	4.86 ± 6.29^{ab}	-7.49 ± 7.09^{a}	-8.35 ± 4.06^{a}
	1.25	-4.32 ± 4.33^{a}	17.38 ± 2.36^{b*}	-7.92 ± 11.69^{a}	6.87 ± 4.59^{b}
	2.50	-0.32 ± 2.70^{a}	16.10 ± 6.47^{b*}	5.62 ± 7.77^{a}	48.79 ± 1.63^{c*}

*$P < 0.05$ as compared with control.

Means not sharing a common superscript letter in the same column of one province are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Results shown were mean ± S.E.M. from triplicate trials.

Table 4.2 Antimutagenic activities of *P. mirifica* extracts from different provinces of Thailand detected by Ames test using *S.* Typhimurium strain TA98 and TA100 under non metabolic and metabolic activation conditions. (continued)

Province	Dose (mg/plate)	PI (%inhibition)			
		TA98		TA100	
		-S9	+S9	-S9	+S9
Lumphun	0.625	$18.14 \pm 7.49^{c*}$	4.25 ± 6.63^{a}	5.17 ± 1.29^{ab}	8.99 ± 3.57^{ab}
	1.25	$16.41 \pm 5.16^{bc*}$	-4.22 ± 7.79^{a}	7.19 ± 3.24^{ab}	$20.21 \pm 4.45^{b*}$
	2.50	2.77 ± 4.03^{ab}	9.54 ± 3.78^{a}	$11.33 \pm 4.21^{b*}$	$21.17 \pm 7.96^{b*}$
Uttraradith	0.625	$26.44 \pm 3.38^{c*}$	-7.56 ± 8.88^{a}	$19.83 \pm 4.00^{b*}$	-4.78 ± 9.26^{a}
	1.25	$23.39 \pm 0.97^{c*}$	1.72 ± 66.58^{a}	$19.81 \pm 10.52^{b*}$	-2.89 ± 3.54^{a}
	2.50	$12.47 \pm 1.81^{b*}$	$21.75 \pm 5.48^{b*}$	$42.89 \pm 2.13^{c*}$	-0.43 ± 5.33^{a}
Sukhothai	0.625	$20.02 \pm 7.26^{b*}$	$-26.55 \pm 5.52^{a*}$	$20.70 \pm 5.43^{b*}$	$26.44 \pm 4.36^{b*}$
	1.25	$32.96 \pm 0.43^{c*}$	$-16.67 \pm 2.79^{ab*}$	$25.08 \pm 1.19^{bc*}$	$34.24 \pm 2.27^{c*}$
	2.50	$-15.14 \pm 2.38^{a*}$	-6.22 ± 5.48^{c}	$33.29 \pm 4.79^{c*}$	$55.95 \pm 1.52^{d*}$
Phitsanulok	0.625	3.27 ± 6.87^{a}	2.13 ± 7.04^{b}	4.75 ± 2.05^{a}	$13.12 \pm 4.63^{b*}$
	1.25	5.50 ± 9.19^{a}	$-18.41 \pm 7.86^{a*}$	$30.72 \pm 2.10^{b*}$	0.07 ± 4.13^{a}
	2.50	-4.28 ± 9.88^{a}	-0.01 ± 4.13^{b}	$38.61 \pm 4.22^{c*}$	$33.85 \pm 8.29^{c*}$
Phetchabun	0.625	$-13.06 \pm 4.62^{a*}$	$28.05 \pm 12.54^{c*}$	$13.57 \pm 7.89^{b*}$	$12.25 \pm 4.80^{b*}$
	1.25	-5.87 ± 4.92^{ab}	$16.48 \pm 6.66^{c*}$	$18.27 \pm 1.46^{c*}$	$27.14 \pm 3.16^{c*}$
	2.50	$17.05 \pm 1.71^{c*}$	$20.85 \pm 0.23^{b*}$	$10.92 \pm 0.87^{b*}$	$34.43 \pm 2.82^{c*}$
Kam	0.625	2.62 ± 4.28^{bc}	-10.16 ± 4.22^{a}	$23.50 \pm 2.81^{c*}$	$-12.63 \pm 2.97^{a*}$
phaeng Phet	1.25	$10.67 \pm 3.53^{c*}$	7.02 ± 6.14^{b}	$17.21 \pm 7.61^{bc*}$	5.98 ± 5.03^{b}
	2.50	$-15.09 \pm 1.48^{a*}$	1.97 ± 4.16^{ab}	7.85 ± 4.50^{ab}	-3.72 ± 5.83^{ab}
Nakhon	0.625	-5.40 ± 5.22^{a}	$14.07 \pm 7.45^{b*}$	$28.57 \pm 6.69^{c*}$	$18.96 \pm 3.44^{c*}$
Sawan	1.25	-7.61 ± 3.81^{a}	$18.33 \pm 2.25^{b*}$	$36.82 \pm 5.19^{c*}$	$12.07 \pm 1.43^{b*}$
	2.50	-2.94 ± 3.57^{a}	$23.58 \pm 1.26^{b*}$	$14.78 \pm 3.55^{b*}$	$21.93 \pm 0.75^{c*}$

*$P < 0.05$ as compared with control.

Means not sharing a common superscript letter in the same column of one province are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Results shown were mean ± S.E.M. from triplicate trials.

Table 4.2 Antimutagenic activities of *P. mirifica* extracts from different provinces of Thailand detected by Ames test using *S.* Typhimurium strain TA98 and TA100 under non metabolic and metabolic activation condition. (continued)

Province	Dose (mg/plate)	PI (%inhibition)			
		TA98		TA100	
		-S9	+S9	-S9	+S9
Uthai Thani	0.625	-55.18 ± 4.81^{a*}	-16.88 ±10.52^{a*}	-2.18 ± 8.51^{a}	-0.43 ± 3.26^{a}
	1.25	-11.02 ± 6.69^{b}	21.73 ± 7.58^{c*}	17.03 ± 7.74^{b*}	21.72 ± 6.71^{b*}
	2.50	-5.37 ± 1.96^{b}	28.28 ± 2.36^{c*}	25.75 ± 4.43^{b*}	20.64 ± 4.18^{b*}
Saraburi	0.625	0.73 ± 6.73^{a}	-9.07 ± 10.01^{a}	13.32 ± 1.71^{b*}	14.57 ± 4.52^{b*}
	1.25	7.00 ± 6.11^{a}	-6.28 ± 9.46^{a}	26.90 ± 7.19^{c*}	42.04 ± 3.61^{c*}
	2.50	0.69 ± 4.50^{a}	-6.94 ± 8.96^{a}	38.21 ± 2.09^{d*}	20.30 ± 2.47^{b*}
Lop Buri	0.625	-1.78 ± 1.07^{a}	3.98 ± 7.77^{a}	24.87 ± 5.55^{b*}	3.42 ± 0.16^{a}
	1.25	-1.77 ± 0.43^{a}	22.03 ± 4.62^{b*}	24.87 ± 3.56^{b*}	16.66 ± 2.21^{b*}
	2.50	7.17 ± 2.94^{b*}	9.66 ± 5.42^{ab}	7.94 ± 13.03^{ab}	25.87 ± 4.32^{c*}
Phrachin Buri	0.625	-30.45 ± 7.58^{a*}	-31.68 ±12.24^{a*}	-1.05 ± 1.87^{a}	21.93 ± 6.63^{b*}
	1.25	-33.94 ± 8.52^{a*}	-8.67 ± 3.44^{b*}	8.22 ± 4.34^{b*}	42.70 ± 9.18^{c*}
	2.50	-18.01 ±11.06^{b*}	-12.11 ± 1.14^{b*}	8.16 ± 2.62^{b*}	54.74 ± 6.86^{c*}
Ratchaburi	0.625	6.36 ± 8.61^{ab}	2.19 ± 2.41^{ab}	2.82 ± 7.38^{a}	4.94 ± 3.43^{a}
	1.25	2.08 ± 2.19^{a}	10.55 ± 7.07^{ab}	1.74 ± 7.07^{a}	27.58 ± 1.96^{b*}
	2.50	13.24 ± 3.47^{b*}	14.63 ± 5.48^{b*}	1.80 ± 5.44^{a}	32.67 ± 4.61^{b*}
Phetcha buri	0.625	13.45 ± 3.72^{b*}	9.15 ± 6.97^{a}	19.48 ± 3.00^{a*}	-2.56 ± 5.14^{a}
	1.25	14.78 ± 3.48^{b*}	12.85 ± 4.89^{a}	27.45 ± 1.18^{b*}	16.06 ± 1.46^{c*}
	2.50	16.10 ± 6.24^{b*}	27.96 ± 3.68^{b*}	19.97 ± 3.82^{a*}	7.99 ± 3.04^{bc}
Sakon Nakhon	0.625	1.57 ± 4.64^{a}	-4.64 ± 1.93^{a}	15.75 ± 3.11^{b*}	12.00 ± 10.57^{ab}
	1.25	6.43 ± 9.03^{a}	-2.57 ± 7.69^{a}	18.49 ± 1.21^{b*}	21.02 ± 9.99^{b*}
	2.50	-1.62 ± 2.18^{a}	0.31 ± 3.73^{a}	11.93 ± 4.42^{b*}	19.98 ± 7.84^{b*}

*$P < 0.05$ as compared with control.

Means not sharing a common superscript letter in the same column of one province are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Results shown were mean ± S.E.M. from triplicate trials.

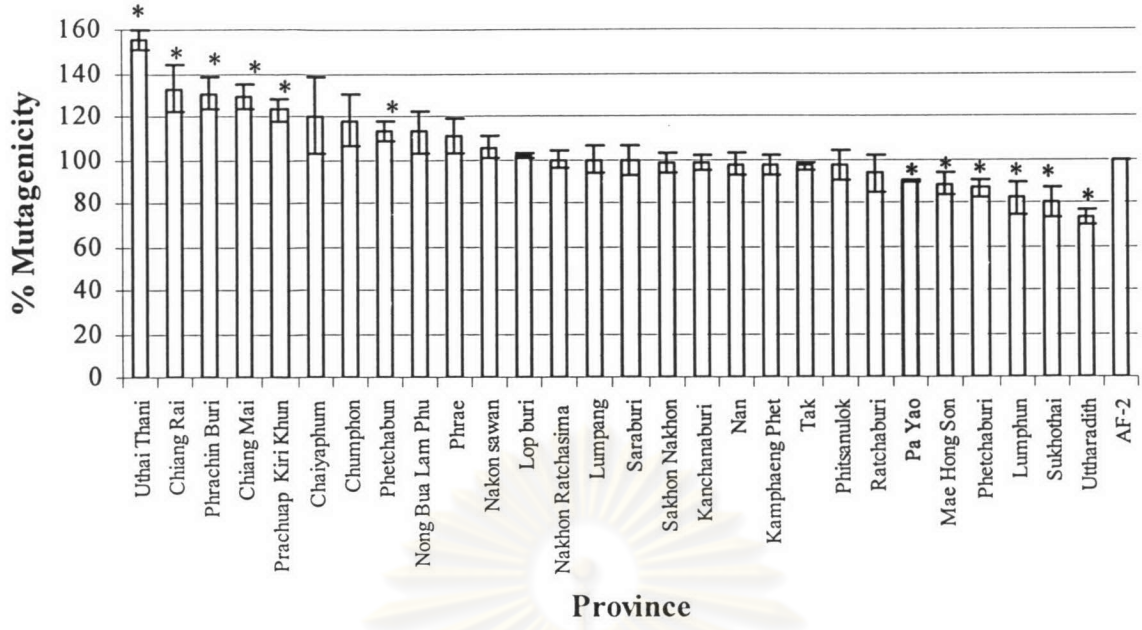
Table 4.2 Antimutagenic activities of *P. mirifica* extracts from different provinces of Thailand detected by Ames test using *S.* Typhimurium strain TA98 and TA100 under non metabolic and metabolic activation condition. (continued)

Province	Dose (mg/plate)	PI (%inhibition)			
		TA98		TA100	
		-S9	+S9	-S9	+S9
Nong Bua	0.625	-12.87 ± 9.46^{a}	-7.32 ± 6.43^{a}	24.15 ± 2.97^{b*}	3.71 ± 1.04^{a}
Lam Phu	1.25	-8.20 ± 9.98^{a}	0.27 ± 2.89^{a}	47.90 ± 5.53^{c*}	18.13 ± 3.09^{b*}
	2.50	-5.88 ± 5.41^{a}	-3.36 ± 1.43^{a}	34.07 ± 6.68^{b*}	27.81 ± 3.21^{c*}
Chaiyaphum	0.625	-20.53 ± 17.58^{a}	11.63 ± 5.85^{a}	-8.24 ± 6.64^{a}	-7.05 ± 9.47^{a}
	1.25	-28.79 ± 8.38^{b*}	2.77 ± 5.49^{a}	3.89 ± 5.97^{ab}	1.20 ± 8.29^{a}
	2.50	-20.18 ± 7.18^{b*}	-2.16 ± 4.75^{a}	12.68 ± 6.69^{c*}	25.98 ± 8.12^{b*}
Nakhon	0.625	0.20 ± 7.97^{a}	7.66 ± 1.99^{a}	3.97 ± 9.30^{ab}	19.47 ± 2.09^{ab*}
Ratchasima	1.25	-3.86 ± 5.44^{a}	4.52 ± 1.47^{a}	15.28 ± 0.61^{b*}	12.61 ± 5.59^{a*}
	2.50	0.27 ± 3.39^{a}	4.18 ± 1.67^{a}	18.41 ± 8.78^{b*}	24.09 ± 1.00^{b*}
Tak	0.625	3.16 ± 1.47^{b}	-1.48 ± 4.95^{a}	-8.42 ± 1.89^{a}	36.69 ± 5.08^{b*}
	1.25	-14.82 ± 3.22^{a*}	-16.71 ± 4.05^{b*}	2.71 ± 3.73^{b}	46.19 ± 4.38^{b*}
	2.50	-2.09 ± 5.38^{b}	-16.81 ± 1.21^{b*}	-5.60 ± 4.29^{ab}	47.54 ± 4.36^{b*}
Kanchana	0.625	1.58 ± 3.78a^{b}	-9.21 ± 9.77^{ab}	17.77 ± 6.09^{b*}	5.46 ± 7.32^{ab}
buri	1.25	4.77 ± 4.66^{b}	-10.10 ± 3.48^{c*}	18.01 ± 7.27^{b*}	12.32 ± 5.94^{b*}
	2.50	-9.36 ± 6.54^{a}	-4.04 ± 1.18^{b}	22.85 ± 3.65^{b*}	25.66 ± 4.44^{b*}
Prachuap	0.625	-22.91 ± 4.80^{a*}	13.10 ± 4.92^{b*}	-23.81 ± 5.05^{ab}	15.93 ± 2.13^{b*}
Kiri Khun	1.25	3.19 ± 4.18^{b}	16.25 ± 4.75^{b*}	23.91 ± 11.19^{c*}	19.28 ± 3.70^{b*}
	2.50	3.31 ± 7.48^{b}	11.07 ± 4.65^{ab}	-35.73 ±14.46^{a*}	32.83 ± 3.83^{c*}
Chumphon	0.625	-17.74 ± 12.00^{a}	3.59 ± 1.34^{ab}	12.84 ± 2.53^{b*}	-4.23 ± 9.81^{a}
	1.25	-27.16 ± 9.32^{b*}	16.53 ± 4.88^{b*}	9.75 ± 4.22^{ab}	-0.96 ± 4.07^{a}
	2.50	-27.49 ±13.03^{b*}	-9.29 ± 1.79^{a}	7.93 ± 13.84^{ab}	0.27 ± 3.86^{a}

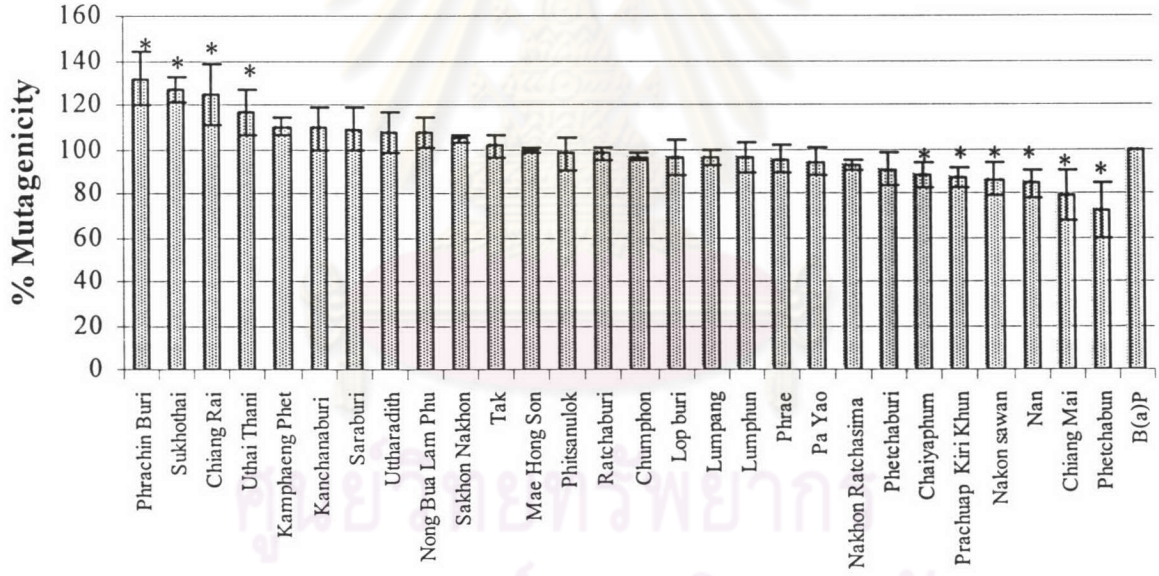
*$P < 0.05$ as compared with control.

Means not sharing a common superscript letter in the same column of one province are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Results shown were mean ± S.E.M. from triplicate trials.

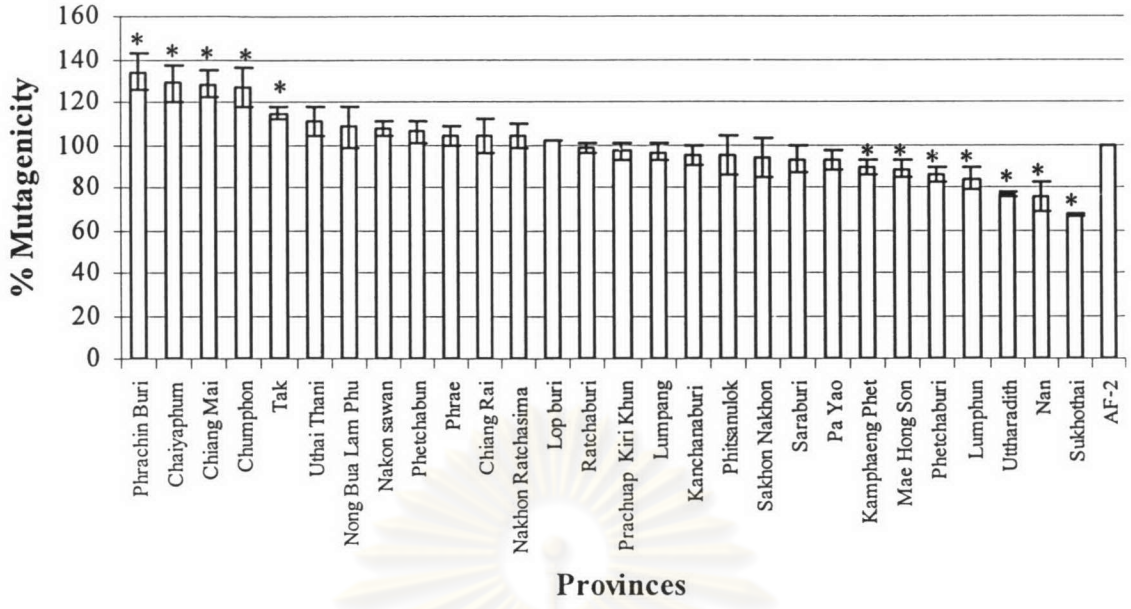


(a)

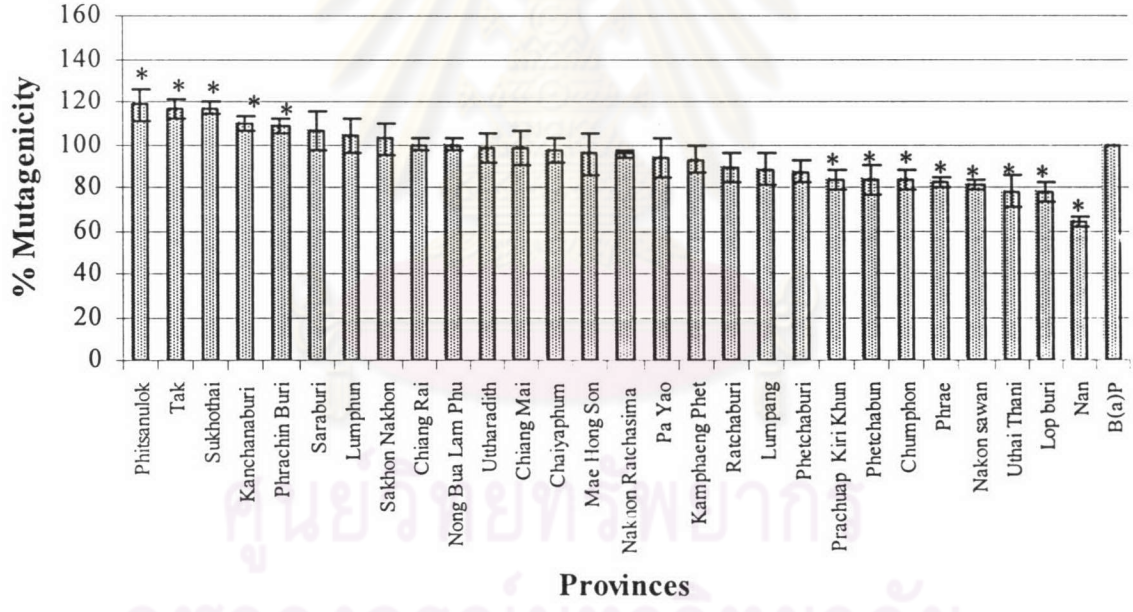


(b)

Figure 4.2 Antimutagenic activities of *P. mirifica* extracts against a) direct-acting of AF-2 0.1 μg/plate and b) indirect-acting of B(*a*)P 10 μg/plate mutagens analyzed by *S.* Typhimurium strain TA98 at concentration 0.625 mg/plate. (*$P < 0.05$ as compared with control, set as 100% mutagenicity)

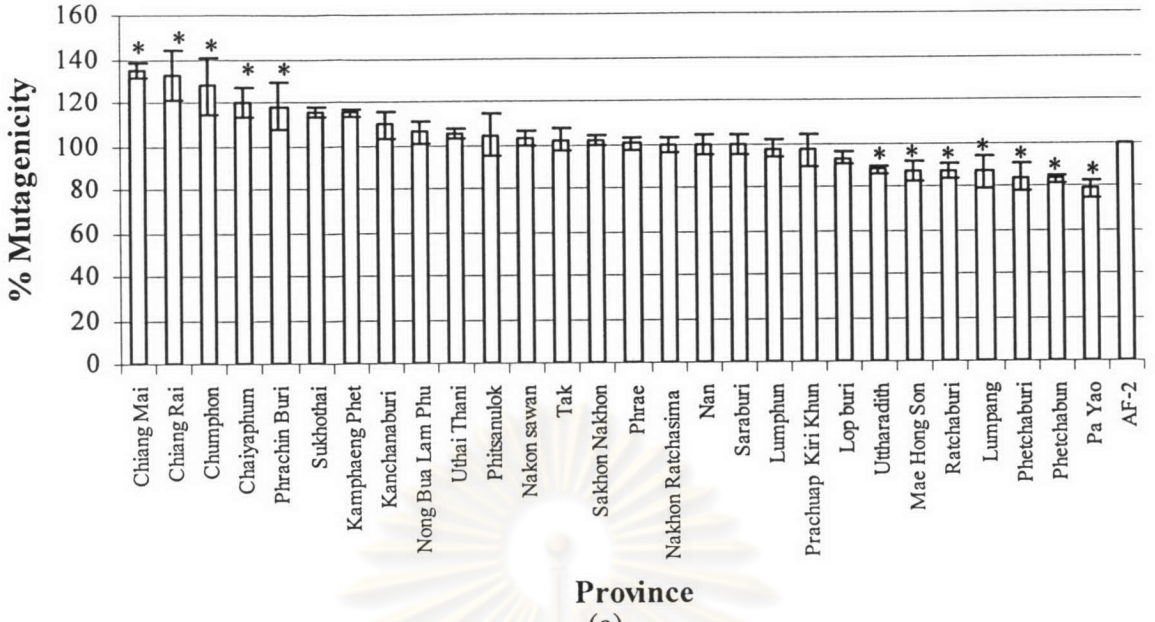


(a)

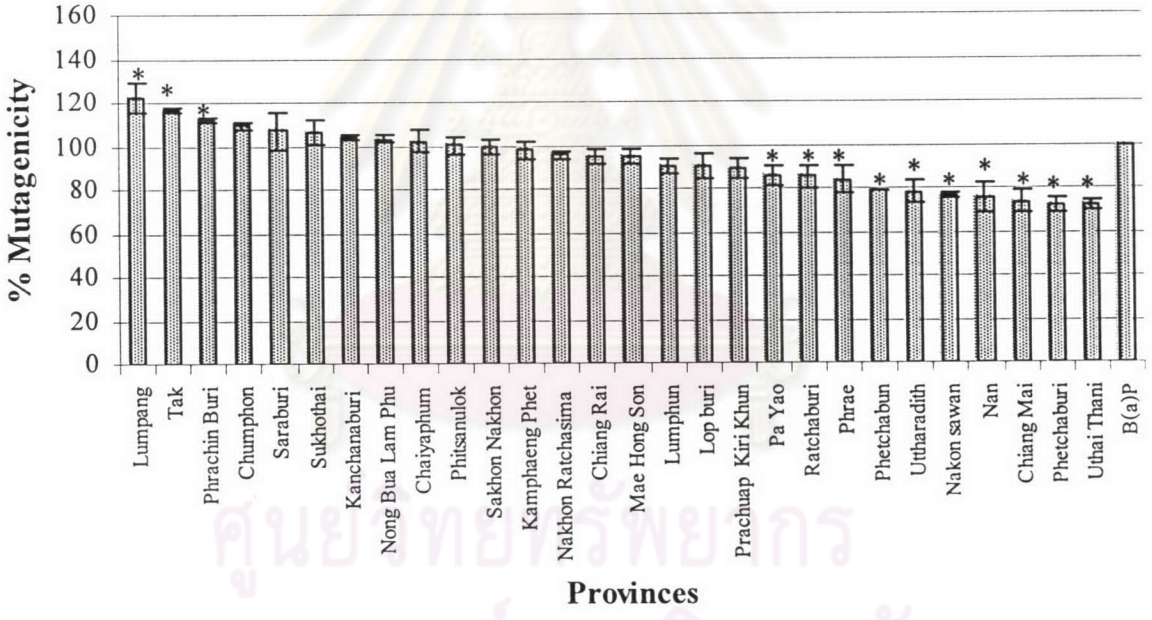


(b)

Figure 4.3 Antimutagenic activities of *P. mirifica* extracts against a) direct-acting of AF-2 0.1 μg/plate and b) indirect-acting of B(*a*)P 10 μg/plate mutagens analyzed by *S.* Typhimurium strain TA98 at concentration 1.25 mg/plate. (*$P < 0.05$ as compared with control, set as 100% mutagenicity)



(a)



(b)

Figure 4.4 Antimutagenic activities of *P. mirifica* extracts against a) direct-acting of AF-2 0.1 μg/plate and b) indirect-acting of B(*a*)P 10 μg/plate mutagens analyzed by *S.* Typhimurium strain TA98 at concentration 2.50 mg/plate. (*$P < 0.05$ as compared with control, set as 100% mutagenicity)

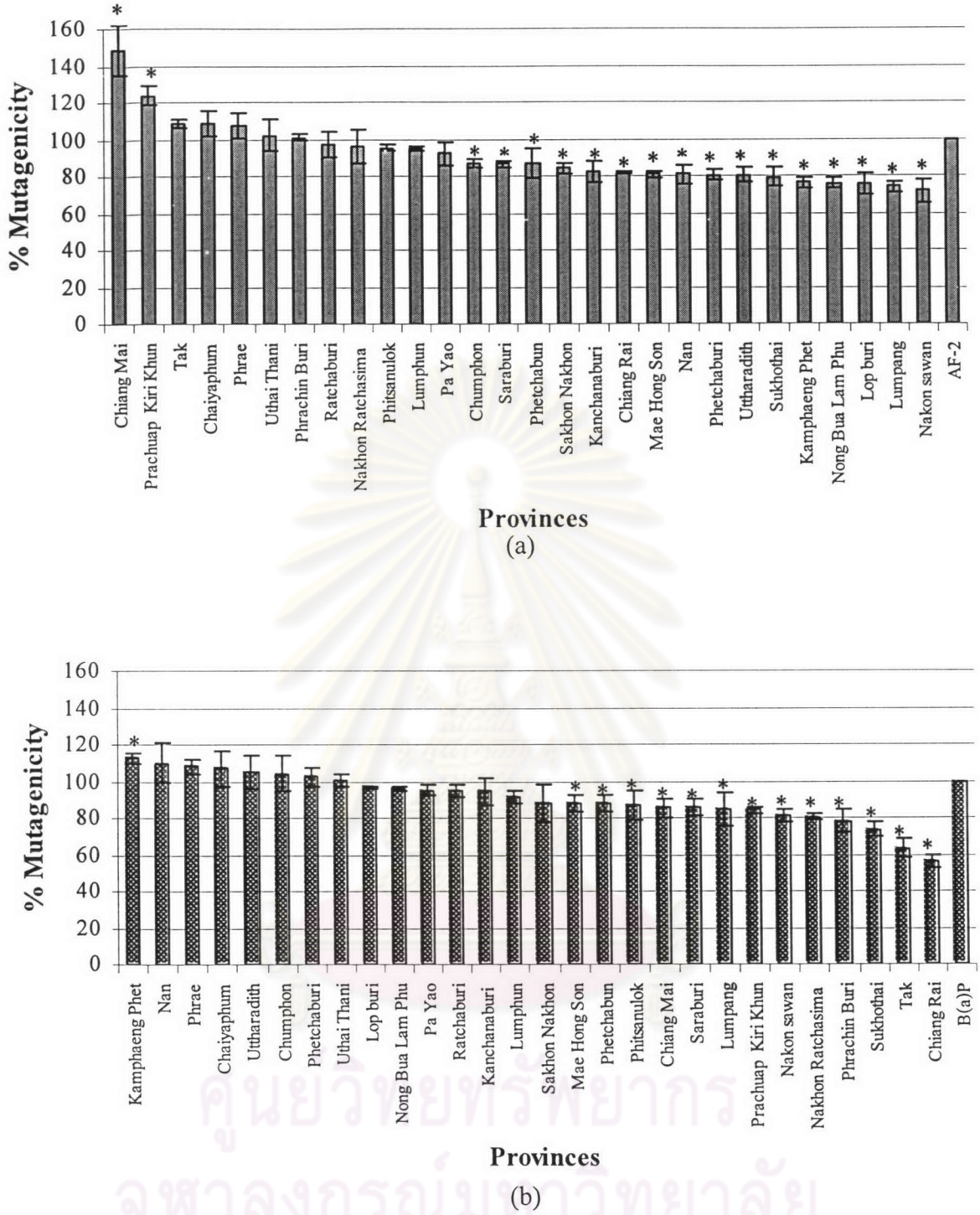


Figure 4.5 Antimutagenic activities of *P. mirifica* extracts against a) direct-acting of AF-2 0.01 μg/plate and b) indirect-acting of B(*a*)P 5 μg/plate mutagens analyzed by *S.* Typhimurium strain TA100 at concentration 0.625 mg/plate. (*$P < 0.05$ as compared with control, set as 100% mutagenicity)

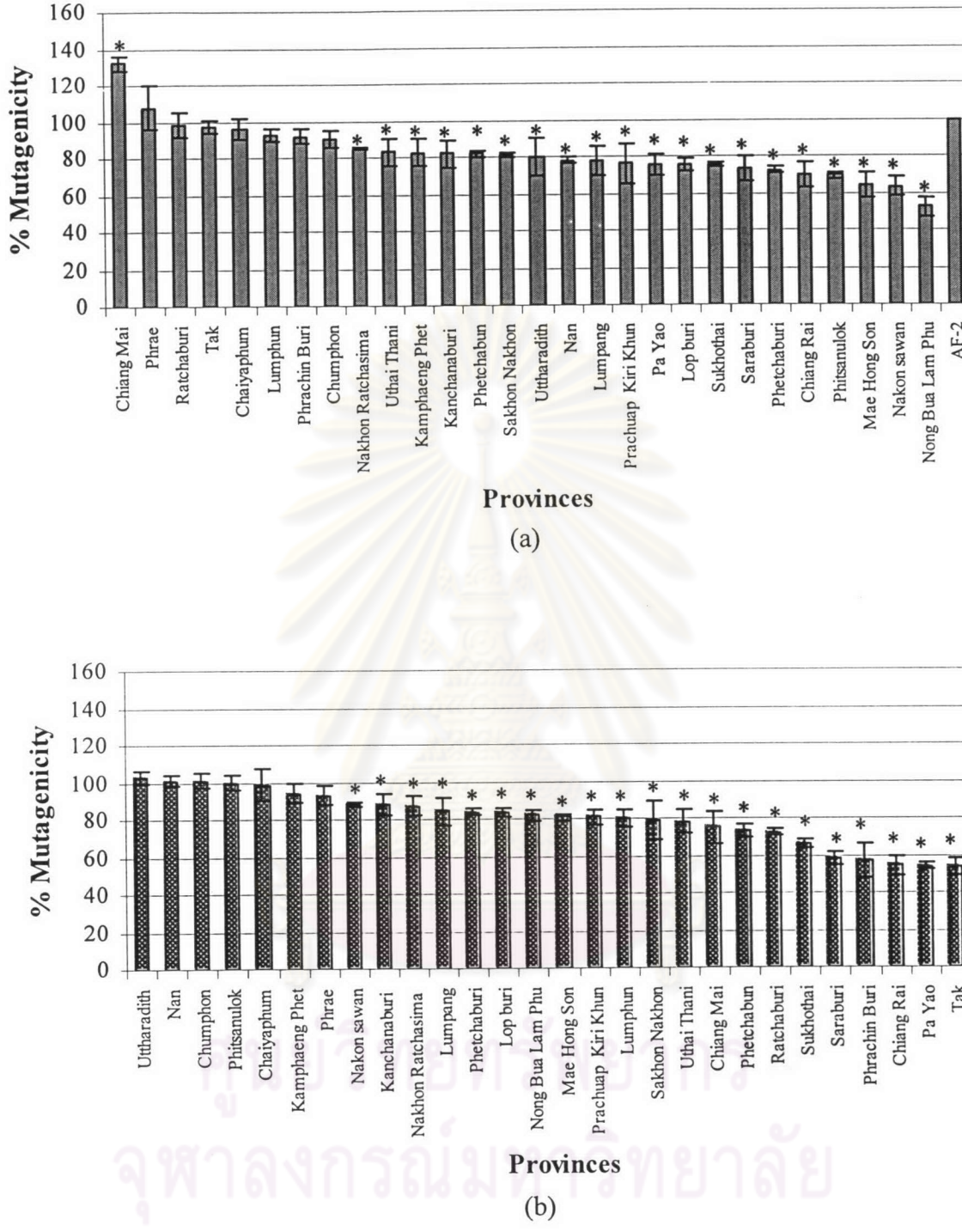


Figure 4.6 Antimutagenic activities of *P. mirifica* extracts against a) direct-acting of AF-2 0.01 μg/plate and b) indirect-acting of B(*a*)P 5 μg/plate mutagens analyzed by *S.* Typhimurium strain TA100 at concentration 1.25 mg/plate. (*$P < 0.05$ as compared with control, set as 100% mutagenicity)

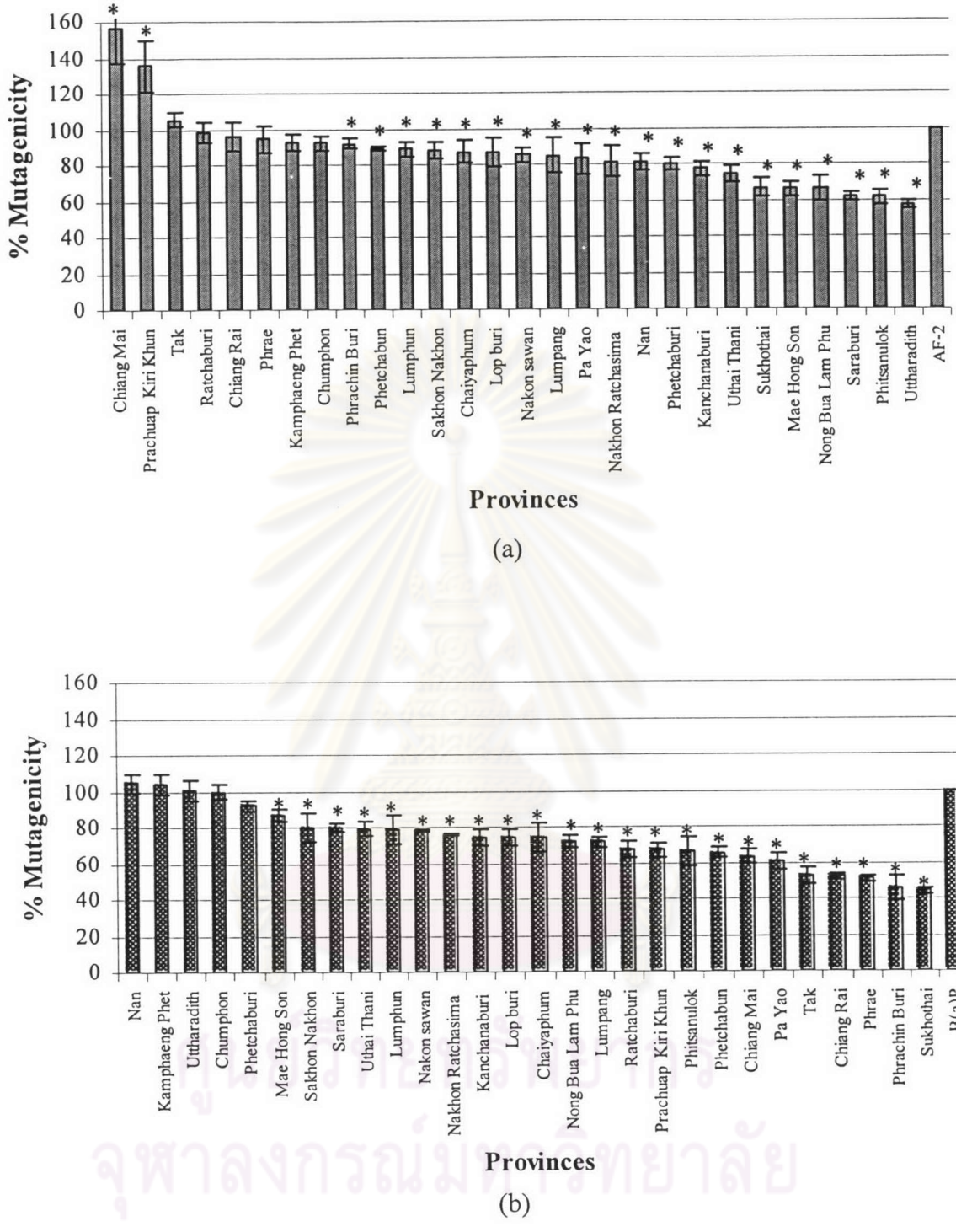


Figure 4.7 Antimutagenic activities of *P. mirifica* extracts against a) direct-acting of AF-2 0.01 μg/plate and b) indirect-acting of B(*a*)P 5 μg/plate mutagens analyzed by *S.* Typhimurium strain TA100 at concentration 2.50 mg/plate. (*$P < 0.05$ as compared with control, set as 100% mutagenicity)

4.1.3.2 Antimutagenic activity of *B. superba* extracts

B. superba extracts collected from 24 provinces of Thailand were screened for antimutagenic activity. Certain extract significantly decreased the number of revertants induced by AF-2 and B(*a*)P with dose-dependently (Table 4.3, Appendix A; Table 5.3).

At the concentration of 0.625 mg/plate

Using AF-2 (0.1 μg/plate) on *S.* Typhimurium TA98, the extracts of *B. superba* from Loei, Kanchanaburi exhibited weak inhibition. None of them showed moderate or strong antimutagenic activity. When tested with AF-2 (0.01 μg/plate) on *S.* Typhimurium TA100, the weak inhibition activities were detected in the samples from Sakhon Nakhon, Khon Kaen and Tak. Only the sample from Kanchanaburi exhibited moderate inhibition. Also, no moderate or strong antimutagenic activity was found in any sample.

Using B(*a*)P (10 μg/plate) on *S.* Typhimurium TA98, the samples from Chiang Rai, Phitsanulok, Chaiyaphum and Phetchaburi exhibited weak inhibition. No sample with moderate or strong inhibition. When tested with B(*a*)P (5 μg/plate) on *S.* Typhimurium TA100, the weak inhibition activities were found in the samples from Loei, Saraburi, Nong Bua Lam Phu, Chachoengsao, Ratchaburi, Chantaburi, Chaiang Mai, Khon Kaen and Chaiyaphum. Only the sample from Phitsanulok exhibited moderate inhibition whereas no sample with strong inhibition was detected (Figure 4.8, 4.11).

At the concentration of 1.25 mg/plate

Using AF-2 (0.1 μg/plate) on *S.* Typhimurium TA98, The extracts of *B. superba* from Prachinburi, Nakhon Ratchasima, Chonburi and Tak exhibited weak inhibition. Only the sample from Loei exhibited moderate inhibition. None of them showed strong antimutagenic activity. When tested with AF-2 (0.01 μg/plate) on *S.* Typhimurium TA100, the samples from Saraburi, Kanchanaburi, Nong Bua Lam Phu, Loei, Nakhon Sawan, Khon Kaen exhibited weak inhibition and the samples from Tak and Sakhon Nakhon exhibited moderate inhibition. Also, no strong antimutagenic activity was found in any sample.

Using B(*a*)P (10 μg/plate) on *S.* Typhimurium TA98, the weak inhibition activities were Nong Bua Lam Phu, Chiang Rai, Loei, Ratchaburi, Chaiyaphum, Phitsanulok and Tak. The samples from Chonburi, Lop buri and Phetchabun exhibited moderate inhibition. No sample with moderate or strong inhibition. When tested of AF-2 B(*a*)P (5 μg/plate) on *S.* Typhimurium TA100, the sample from Chantaburi exhibited weak inhibition and the samples from Phitsanulok, Chachoengsao, Chaiyaphum. Chonburi. Saraburi, Srisaket, Tak, Nakhon Sawan, Ratchaburi, Chiang Mai, Nong Bua Lam Phu, Loei and Chantaburi exhibited moderate inhibition whereas no sample with strong inhibition was detected. (Figure 4.9, 4.12)

At the concentration of 2.50 mg/plate

Using AF-2 (0.1 μg/plate) on *S.* Typhimurium TA98, the extracts of *B. superba* from Nong Bua Lam Phu, Chiang Rai, Chantaburi, Kanchanaburi exhibited weak inhibition. The samples from Chaiyaphum and Chiang Mai exhibited moderate inhibition and only the sample from Loei were strong inhibition. When tested of AF-2 (0.01 μg/plate) on *S.* Typhimurium TA100, the weak inhibition activity was sample from Phrachinburi. Only the samples from Kanchanaburi. Khon Kaen, Loei, Saraburi, Nong Bua Lam Phu, Lop buri, Tak and Chantaburi exhibited moderate inhibition. Also, no strong antimutagenic activity was found in any sample.

Using B(*a*)P (10 μg/plate) on *S.* Typhimurium TA98, the weak inhibition activity was found in the samples from Loei. The samples from Tak, Chaiyaphum, Phitsanulok. Prachinburi, Uttradith, Khon Kaen, Nong Bua Lam Phu, Chantaburi exhibited inhibition acitivity and the samples from Phetchabun and Chonburi exhibited strong inhibition. When tested with B(*a*)P (5 μg/plate) on *S.* Typhimurium TA100, The weak inhibition activity was detected in the samples from Phrachinburi and Chantaburi. The samples from Chiang Mai, Saraburi, Phitsanulok, Loei, Lopburi. Phetchabun, Khon Kaen, Chiang Rai, Chachoengsao, Sakhon Nakhon, Nong Bua Lam Phu, Chaiyaphum, Lumpang, Kanchanaburi exhibited moderate inhibition activities and the samples from Chonburi exhibited strong inhibition (Figure 4.10, 4.13).

Table 4.3 Antimutagenic activities of *B. superba* extracts from different provinces of Thailand detected by Ames test using *S.* Typhimurium strain TA98 and TA100 under non metabolic and metabolic activation conditions.

Province	Dose (mg/plate)	PI (%inhibition)			
		TA98		TA100	
		-S9	+S9	-S9	+S9
Chiang Rai	0.625	-5.55 ± 9.76^{a}	22.55 ± 3.69^{b*}	-20.49 ± 5.49^{a*}	-33.54 ± 6.74^{a*}
	1.25	-2.84 ± 6.07^{a}	24.08 ± 4.35^{b*}	-6.01 ± 6.63^{ab}	13.46 ± 2.64^{c*}
	2.50	27.91 ± 7.58^{b*}	59.75 ± 2.58^{c*}	3.13 ± 5.69^{b}	26.23 ± 4.93^{d*}
Chiang Mai	0.625	9.47 ± 16.18^{a}	10.96 ± 5.79^{ab}	-2.35 ± 9.83^{a}	27.10 ± 6.05^{b*}
	1.25	13.67 ± 9.69^{a}	15.89 ± 3.42^{b*}	-13.45 ± 9.15^{a}	30.13 ± 4.72^{bc*}
	2.50	50.39 ± 3.79^{b*}	11.48 ± 8.69^{ab}	1.62 ± 5.82^{a}	39.72 ± 2.08^{c*}
Mae Hong	0.625	-29.30 ± 6.53^{a*}	3.56 ± 3.85^{a}	-6.93 ± 8.57^{a}	4.68 ± 4.31^{a}
Son	1.25	11.11 ± 13.94^{b}	2.22 ± 7.51^{a}	-4.65 ± 5.12^{a}	16.83 ± 2.63^{b*}
	2.50	-39.27 ± 4.27^{a*}	20.49 ± 9.67^{a}	-3.44 ± 8.71^{a}	14.06 ± 0.36^{b*}
Lampang	0.625	-20.41 ±10.07^{ab}	4.05 ± 3.22^{a}	-1.58 ± 5.19^{a}	8.04 ± 1.31^{a}
	1.25	-34.48 ±14.38^{a*}	9.65 ± 15.31^{ab}	-3.00 ± 2.69^{a}	5.28 ± 9.04^{a}
	2.50	-5.46 ± 7.64^{b}	21.29 ± 9.01^{b*}	-6.83 ± 0.25^{a}	32.41 ± 0.85^{b*}
Uttraradith	0.625	-17.20 ± 1.05^{a*}	17.10 ± 8.45^{bc*}	-1.44 ± 8.68^{a}	1.81 ± 3.33^{a}
	1.25	-7.84 ± 8.95^{ab}	9.38 ± 3.82^{ab}	-8.71 ± 8.88^{a}	3.67 ± 8.29^{a}
	2.50	-27.94 ± 7.81^{a*}	25.32 ± 1.39^{c*}	9.32 ± 5.29^{a}	9.52 ± 2.17^{a}
Phitsanulok	0.625	8.52 ± 5.41^{b}	24.04 ± 11.65^{b*}	0.59 ± 3.69^{a}	51.32 ± 3.23^{b*}
	1.25	-36.04 ± 6.36^{a*}	36.78 ±5.39a^{bc*}	4.92 ± 2.64^{a}	40.13 ± 15.35^{b*}
	2.50	-34.95 ±17.86^{a*}	52.24 ± 7.67b^{c*}	5.08 ± 1.54^{a}	41.53 ± 3.10^{b*}
Phetchabun	0.625	-32.61 ± 6.47^{b*}	38.89 ± 7.24^{a*}	-2.81 ± 11.59^{a}	-10.98 ± 9.89^{a}
	1.25	-1.23 ± 6.18^{c}	58.76 ± 5.81^{b*}	12.97 ± 10.49^{a}	-0.94 ± 8.64^{ab}
	2.50	-52.02 ± 7.31^{a*}	81.79 ± 2.96^{c*}	2.92 ± 8.33^{a}	20.12 ± 8.83^{b}

*$P < 0.05$ as compared with control.

Means not sharing a common superscript letter in the same column of one province are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Results shown were mean ± S.E.M. from triplicate trials.

Table 4.3 Antimutagenic activities of *B. superba* extracts from different provinces of Thailand detected by Ames test using *S.* Typhimurium strain TA98 and TA100 under non metabolic and metabolic activation condition. (continued)

Province	Dose (mg/plate)	PI (%inhibition)			
		TA98		TA100	
		-S9	+S9	-S9	+S9
Nakhon Sawan	0.625	-21.45 ± 7.33^{a*}	6.43 ± 10.28^{a}	-5.87 ± 4.13^{a}	10.13 ± 4.53^{a}
	1.25	5.38 ± 13.66^{b}	9.19 ± 6.29^{a}	31.68 ± 10.03^{c*}	27.34 ± 5.92^{b*}
	2.50	-4.71 ± 2.37^{b}	7.29 ± 10.91^{a}	15.86 ± 9.97^{bc}	9.07 ± 11.96^{a}
Saraburi	0.625	15.82 ± 13.12^{a}	4.05 ± 3.35^{a}	3.85 ± 4.03^{a}	21.39 ± 3.31^{b*}
	1.25	-3.79 ± 7.81^{a}	1.21 ± 7.32^{ab}	21.27 ± 3.60^{b*}	20.15 ± 2.18^{b*}
	2.50	2.28 ± 8.32^{a}	-13.37 ± 6.26^{a}	25.33 ± 0.63^{b*}	40.62 ± 4.49^{c*}
Lop Buri	0.625	-59.12 ±12.79^{a*}	13.44 ± 5.63^{a*}	-14.02 ± 7.66^{a*}	-15.35 ± 3.69^{b*}
	1.25	-33.34 ±19.19^{a*}	46.05 ± 1.65^{b*}	3.16 ± 3.33^{b}	2.39 ± 12.00^{ab}
	2.50	12.89 ± 9.96^{b}	61.12 ± 6.10^{c*}	25.87 ± 3.87^{c*}	56.80 ± 5.07^{c*}
Prachinburi	0.625	1.98 ± 3.83^{a}	7.19 ± 8.08^{a}	9.98 ± 8.07^{a}	5.05 ± 5.12^{ab}
	1.25	21.59 ± 7.36^{b*}	7.72 ± 0.58^{a}	10.27 ± 4.46^{a}	17.93 ± 9.28^{b*}
	2.50	-1.00 ± 4.75^{a}	24.07 ± 4.26^{b*}	38.33 ± 4.49^{b*}	5.51 ± 1.36^{ab}
Ratchaburi	0.625	-20.38 ± 6.87^{a*}	5.73 ± 10.52^{a}	-27.49 ± 5.92^{a*}	24.86 ± 3.67^{b*}
	1.25	-1.72 ± 5.17^{b}	32.38 ± 1.56^{b*}	-11.02 ± 3.00^{b}	28.51 ± 13.52^{b*}
	2.50	-31.19 ± 4.09^{a*}	1.63 ± 9.19^{a}	-12.79 ± 6.45^{b}	35.98 ± 4.23^{b*}
Chachoeng soa	0.625	6.06 ± 12.32^{a}	-28.61 ± 8.15^{a*}	13.67 ± 2.29^{b*}	22.85 ± 14.20^{b*}
	1.25	13.60 ± 8.16^{ab}	-7.34 ± 2.59^{b*}	10.33 ± 4.60^{b*}	44.26 ± 6.88^{c*}
	2.50	14.31 ± 4.59^{b*}	-32.22 ± 6.19^{a*}	2.13 ± 7.69^{ab}	29.69 ± 3.93^{b*}
Sakon Nakhon	0.625	2.09 ± 6.49^{a}	-6.58 ± 12.39^{a}	23.44 ± 1.46^{ab*}	-12.98 ± 3.36^{a*}
	1.25	-6.58 ± 7.76^{a}	12.01 ± 8.03^{a}	43.46 ± 6.73^{b*}	19.01 ±14.19^{bcd}
	2.50	12.99 ± 18.36^{a}	7.38 ± 5.81^{a}	12.85 ± 10.22^{a}	29.71 ± 3.11^{d*}

*$P < 0.05$ as compared with control.

Means not sharing a common superscript letter in the same column of one province are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Results shown were mean ± S.E.M. from triplicate trials.

Table 4.3 Antimutagenic activities of *B. superba* extracts from different provinces of Thailand detected by Ames test using *S.* Typhimurium strain TA98 and TA100 under non metabolic and metabolic activation condition. (continued)

Province	Dose (mg/plate)	PI (%inhibition)			
		TA98		TA100	
		-S9	+S9	-S9	+S9
Loei	0.625	35.74 ± 4.81^{b*}	-18.23 ±11.25^{b*}	12.12 ± 2.76^{b*}	20.28 ± 2.93^{b*}
	1.25	55.88 ± 6.48^{c*}	29.32 ± 9.16^{c*}	23.36 ± 2.88^{c*}	32.37 ± 5.92^{b*}
	2.50	60.82 ± 8.99^{c*}	38.53 ± 1.62^{c*}	23.86 ± 4.41^{c*}	50.22 ± 8.83^{c*}
Nong Bua	0.625	-1.02 ± 6.31^{a}	-9.54 ± 1.10^{a*}	11.86 ± 3.29^{bc*}	22.30 ± 5.14^{b*}
Lam Phu	1.25	2.74 ± 1.67^{a}	20.63 ± 8.09^{c*}	22.34 ± 7.22^{c*}	30.84 ± 6.41^{b*}
	2.50	20.03 ± 0.83^{b*}	30.54 ± 2.44^{c*}	25.39 ± 6.26^{c*}	29.86 ± 14.39^{b*}
Khon Kaen	0.625	-9.25 ± 11.72^{a}	-3.74 ± 4.49^{a}	26.56 ±13.68^{bc*}	29.99 ± 5.69^{b*}
	1.25	-2.71 ± 12.97^{a}	-4.78 ± 2.74^{a}	35.41 ± 9.60^{b*}	28.13 ± 9.17^{b*}
	2.50	-3.79 ± 12.33^{a}	26.00 ± 6.67^{b*}	22.81 ± 9.52^{bc*}	26.12 ± 2.40^{b*}
Chaiyaphum	0.625	-9.19 ± 13.63^{a}	36.15 ± 1.98^{b*}	9.33 ± 6.31^{a}	34.52 ± 4.09^{b*}
	1.25	7.54 ± 10.07^{a}	36.66 ± 5.68^{b*}	17.93 ± 14.18^{a}	47.42 ± 2.26^{c*}
	2.50	40.19 ± 0.87^{b*}	47.08 ± 9.29^{b*}	11.74 ± 10.54^{a}	31.47 ± 3.73^{b*}
Nakhon	0.625	7.93 ± 8.65^{a}	4.79 ± 6.01^{a}	5.82 ± 3.74^{a}	16.74 ± 4.63^{a}
Ratchasima	1.25	21.94 ± 11.57^{b*}	10.30 ± 6.29^{a}	10.55 ± 7.75^{a}	12.99 ± 7.83^{a}
	2.50	17.22 ± 3.39^{b*}	14.58 ± 4.22^{a}	13.89 ± 6.84^{a}	18.07 ± 9.59^{a}
Srisaket	0.625	-1.88 ± 6.04^{a}	-8.53 ± 8.39^{a}	1.95 ± 7.51^{ab}	7.69 ± 3.76^{a}
	1.25	-5.75 ± 5.82^{a}	3.28 ± 8.48^{a}	-12.99 ± 5.88^{a}	20.94 ± 4.44^{b*}
	2.50	-5.33 ± 6.76^{a}	2.98 ± 4.57^{a}	3.98 ± 1.22^{ab}	36.47 ± 6.35^{b*}
Tak	0.625	5.55 ± 4.07^{a}	0.35 ± 9.70^{a}	26.57 ± 5.95^{b*}	7.89 ± 4.55^{a}
	1.25	26.74 ± 7.54^{b*}	37.64 ± 8.59^{b*}	40.18 ± 10.73^{b*}	25.75 ± 2.08^{b*}
	2.50	13.96 ± 5.19^{b*}	41.92 ± 1.57^{b*}	28.26 ± 7.66^{b*}	38.02 ± 2.66^{c*}

*$P < 0.05$ as compared with control.

Means not sharing a common superscript letter in the same column of one province are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Results shown were mean ± S.E.M. from triplicate trials.

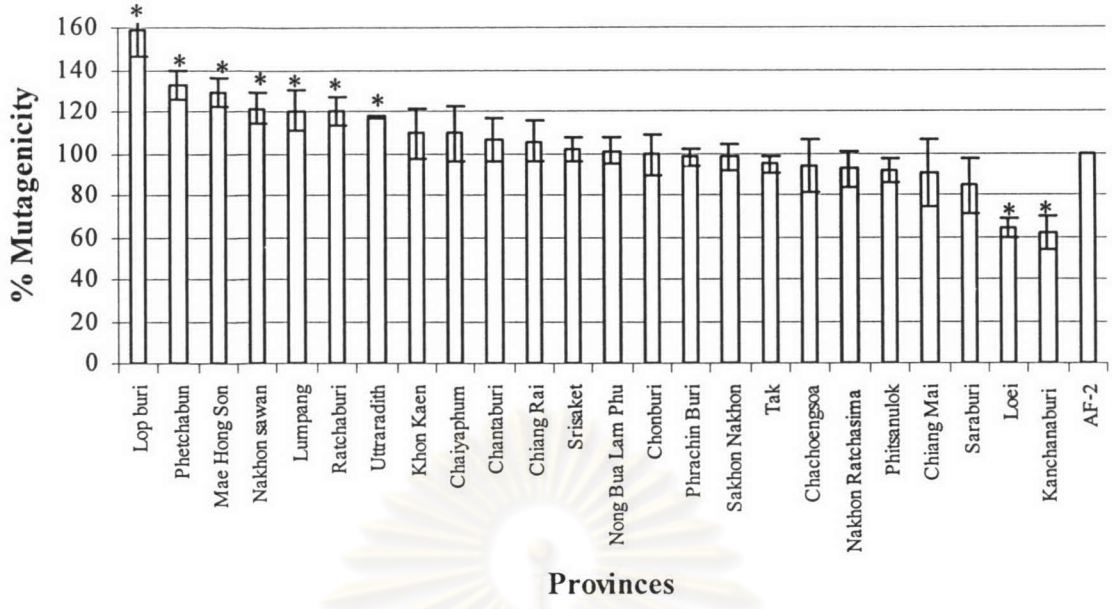
Table 4.3 Antimutagenic activities of *B. superba* extracts from different provinces of Thailand detected by Ames test using *S.* Typhimurium strain TA98 and TA100 under non metabolic and metabolic activation condition. (continued)

Province	Dose (mg/plate)	PI (%inhibition)			
		TA98		TA100	
		-S9	+S9	-S9	+S9
Kanchana buri	0.625	$38.31 \pm 8.27^{b*}$	$-17.12 \pm 5.85^{a*}$	$41.08 \pm 1.37^{c*}$	$-15.24 \pm 3.11^{a*}$
	1.25	5.68 ± 13.17^{a}	-13.15 ± 17.69^{ab}	$21.42 \pm 3.88^{b*}$	-4.83 ± 0.68^{ab}
	2.50	$33.98 \pm 5.14^{b*}$	$-16.58 \pm 8.59^{a*}$	$53.36 \pm 3.55^{d*}$	$33.79 \pm 6.90^{c*}$
Chonburi	0.625	0.89 ± 9.96^{a}	$15.96 \pm 9.12^{a*}$	5.81 ± 1.09^{bc}	-3.02 ± 8.54^{a}
	1.25	$24.27 \pm 1.98^{b*}$	$41.99 \pm 2.25^{b*}$	$12.94 \pm 5.05^{c*}$	$50.28 \pm 6.31^{b*}$
	2.50	9.04 ± 9.27^{ab}	$94.15 \pm 1.42^{c*}$	$-11.86 \pm 3.34^{a*}$	$88.61 \pm 0.24^{c*}$
Chantaburi	0.625	-6.19 ± 10.44^{a}	9.43 ± 5.98^{a}	-0.03 ± 3.42^{a}	$25.55 \pm 5.03^{b*}$
	1.25	5.99 ± 11.72^{ab}	10.94 ± 4.49^{a}	5.58 ± 5.59^{a}	$33.72 \pm 9.12^{b*}$
	2.50	$28.76 \pm 7.61^{b*}$	$34.88 \pm 5.79^{b*}$	$37.54 \pm 13.44^{b*}$	$35.96 \pm 5.94^{b*}$

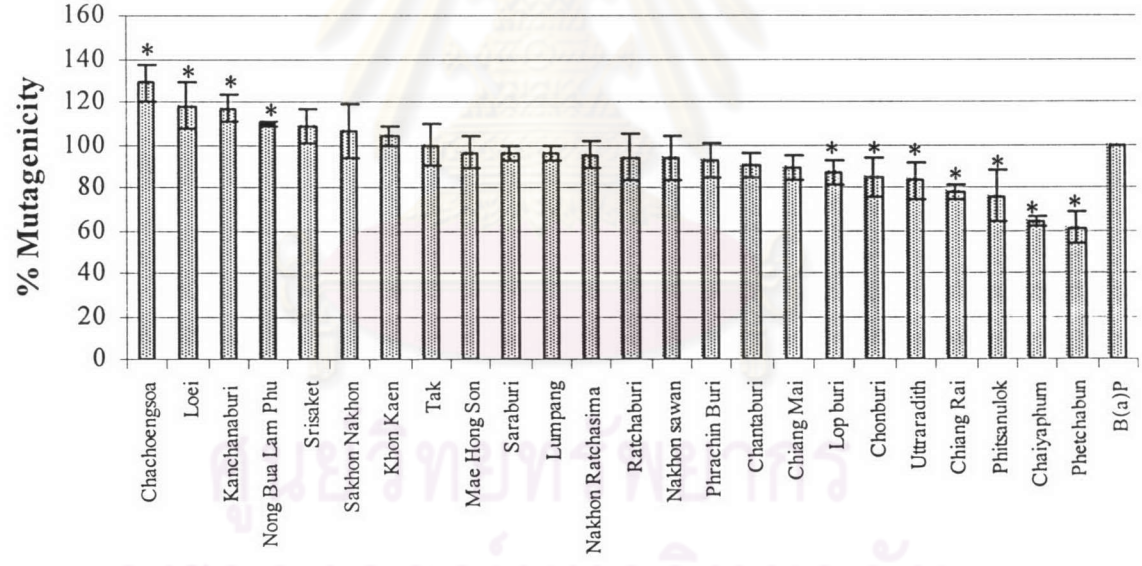
$*P < 0.05$ as compared with control.

Means not sharing a common superscript letter in the same column of one province are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Results shown were mean ± S.E.M. from triplicate trials.

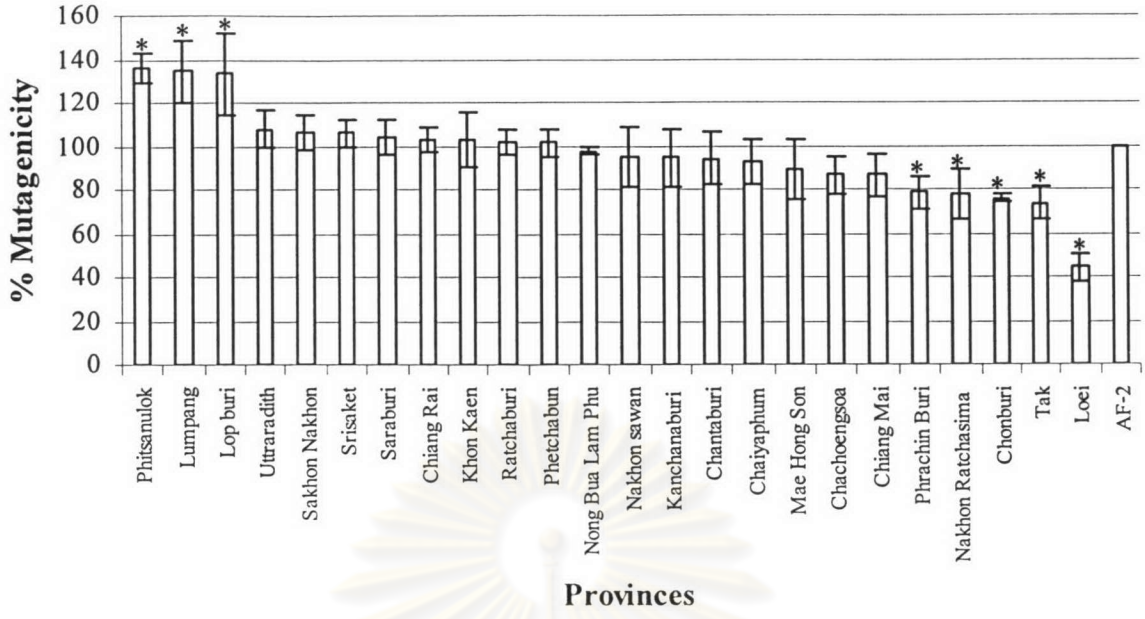


(a)

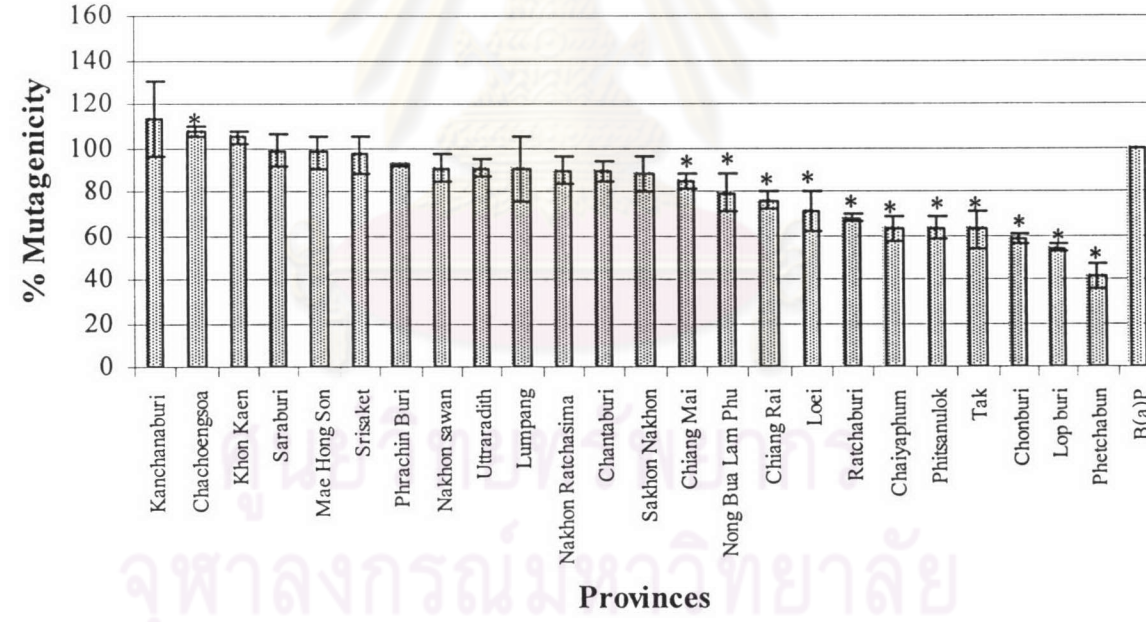


(b)

Figure 4.8 Antimutagenic activities of *B. superba* extracts against a) direct-acting of AF-2 0.1 μg/plate and b) indirect-acting of B(*a*)P 10 μg/plate mutagens analyzed by *S.* Typhimurium strain TA98 at concentration 0.625 mg/plate. (*$P < 0.05$ as compared with control, set as 100% mutagenicity)

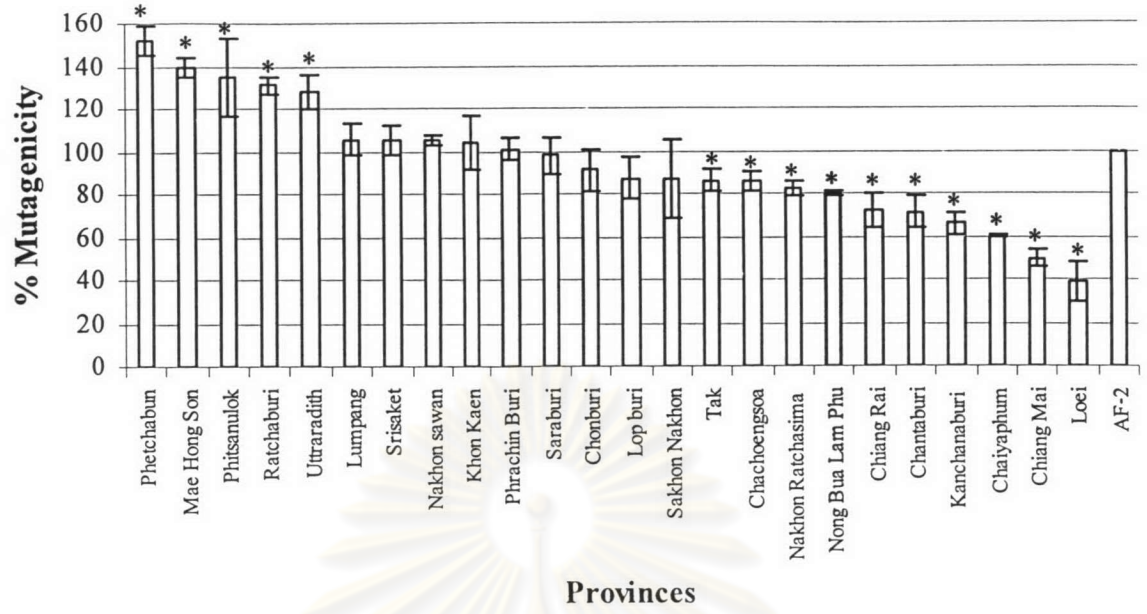


(a)

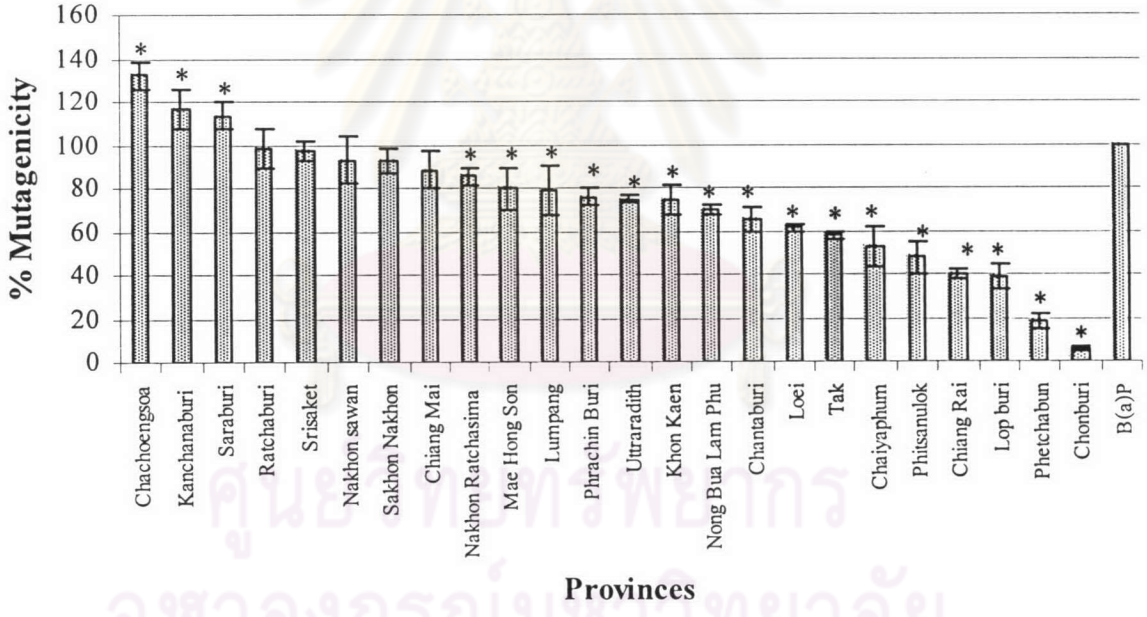


(b)

Figure 4.9 Antimutagenic activities of *B. superba* extracts against a) direct-acting of AF-2 0.1 μg/plate and b) indirect-acting of B(*a*)P 10 μg/plate mutagens analyzed by *S.* Typhimurium strain TA98 at concentration 1.25 mg/plate. (*$P < 0.05$ as compared with control, set as 100% mutagenicity)



(a)



(b)

Figure 4.10 Antimutagenic activities of *B. superba* extracts against a) direct-acting of AF-2 0.1 μg/plate and b) indirect-acting of B(*a*)P 10 μg/plate mutagens analyzed by *S.* Typhimurium strain TA98 at concentration 2.50 mg/plate. (**P* <0.05 as compared with control, set as 100% mutagenicity)

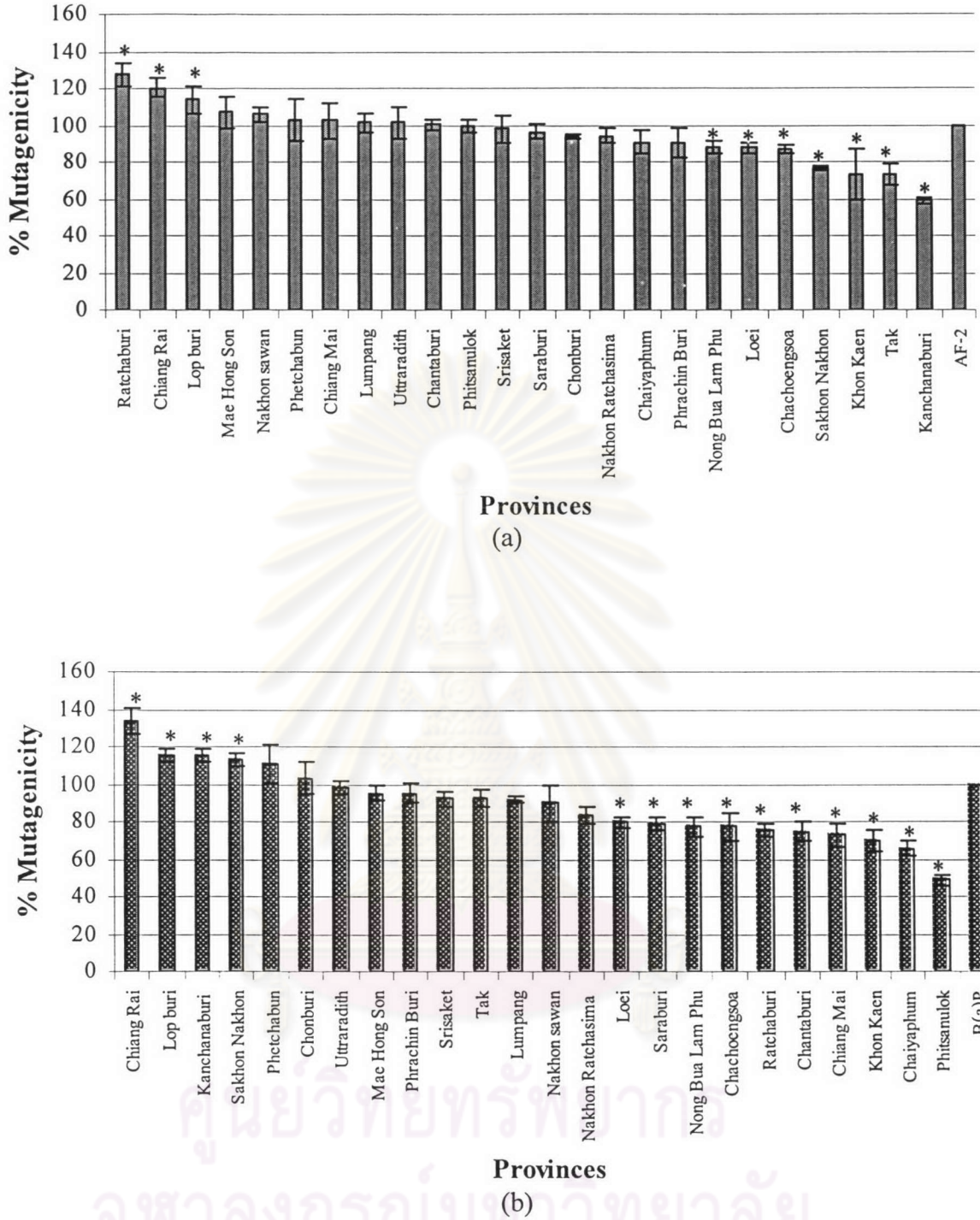
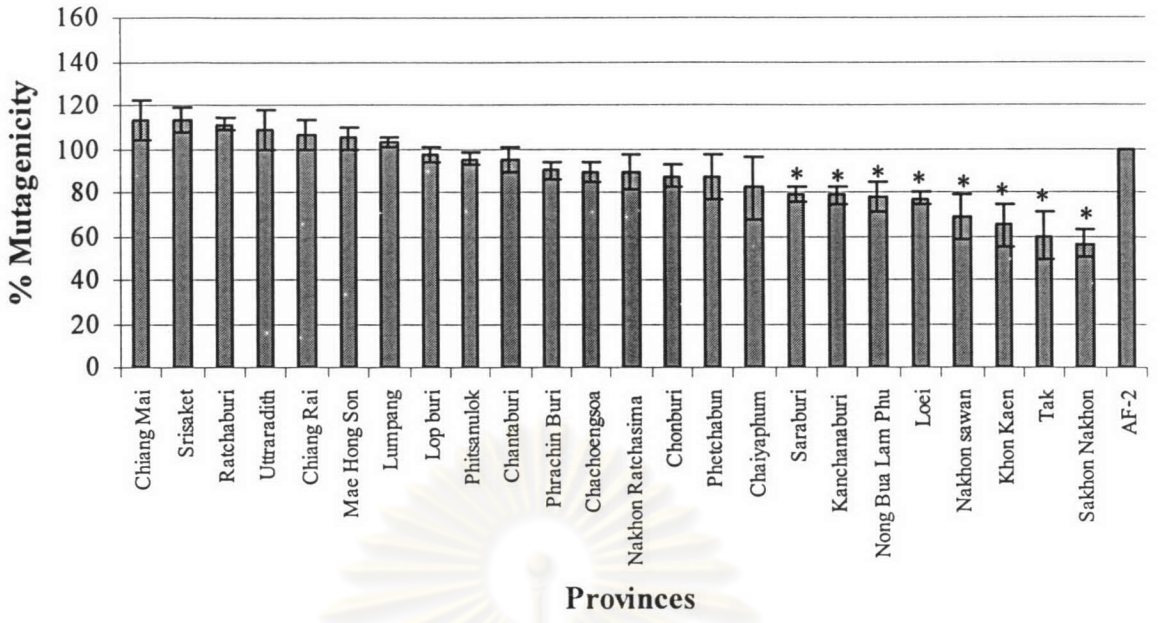
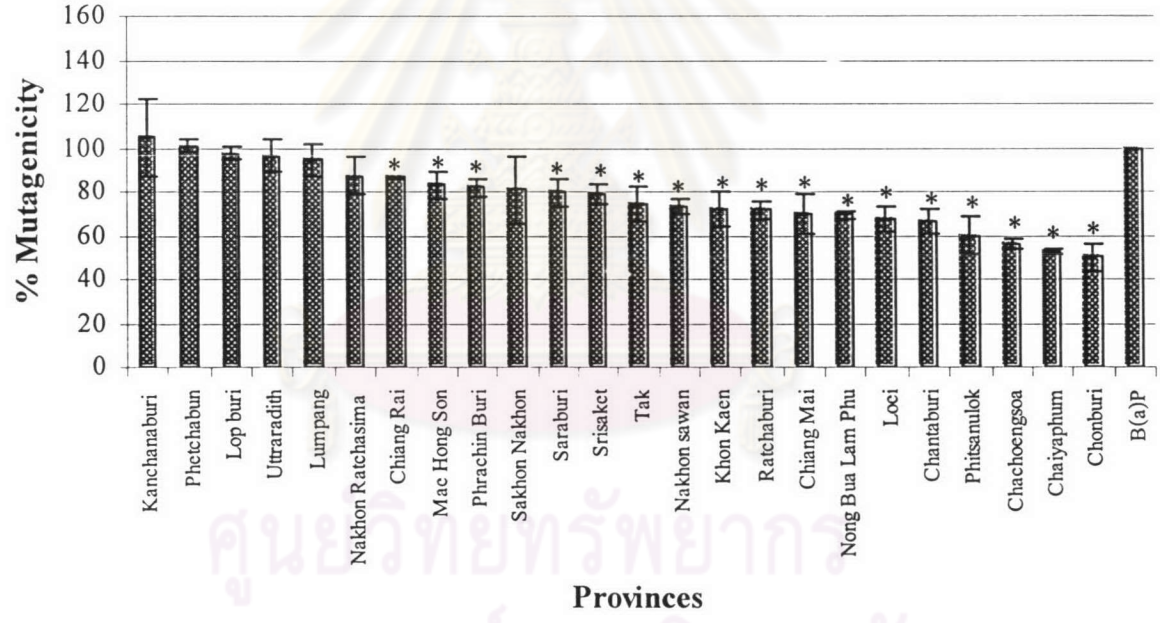


Figure 4.11 Antimutagenic activities of *B. superba* extracts against a) direct-acting of AF-2 0.01 μg/plate and b) indirect-acting of B(*a*)P 5 μg/plate mutagens analyzed by *S.* Typhimurium strain TA100 at concentration 0.625 mg/plate. (*$P < 0.05$ as compared with control, set as 100% mutagenicity)

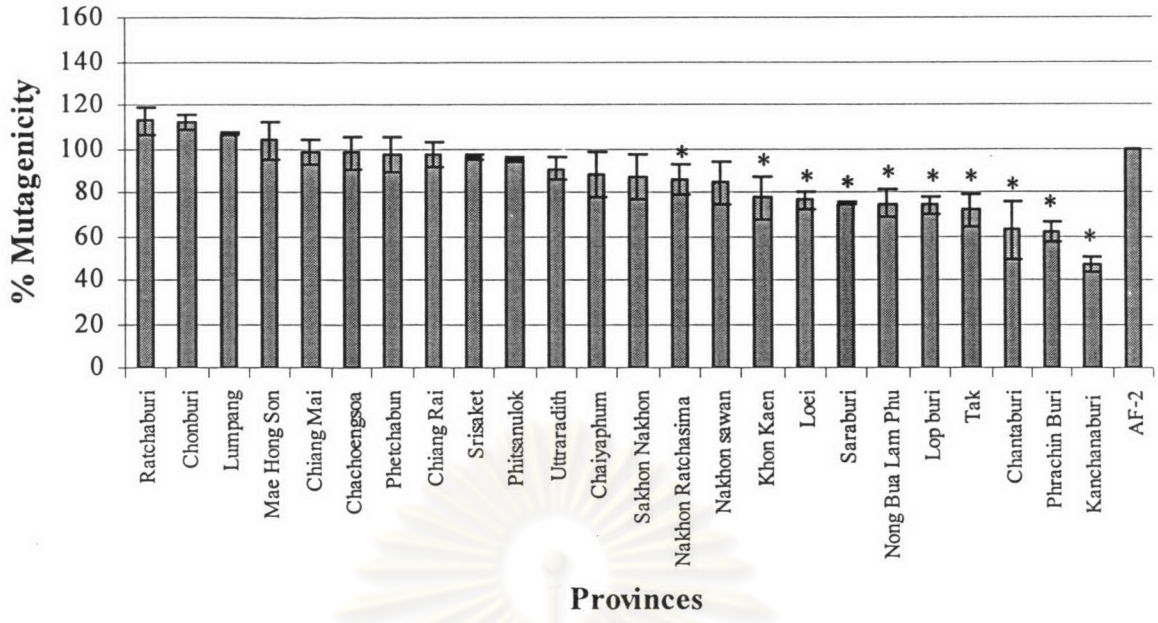


(a)

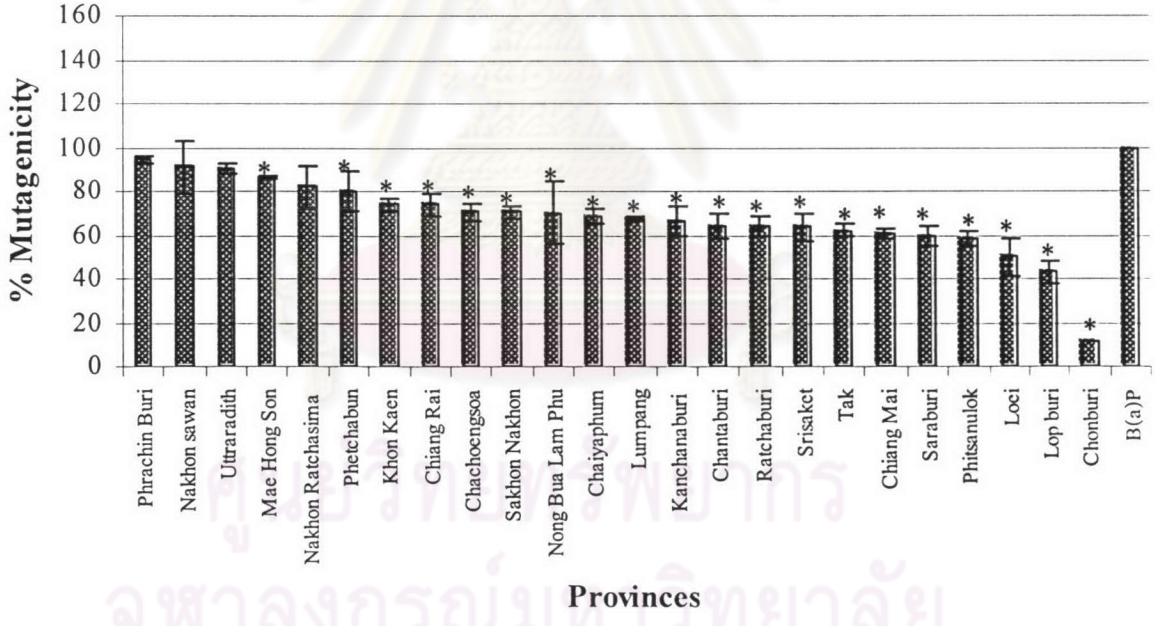


(b)

Figure 4.12 Antimutagenic activities of *B. superba* extracts against a) direct-acting of AF-2 0.01 μg/plate and b) indirect-acting of B(*a*)P 5 μg/plate mutagens analyzed by *S.* Typhimurium strain TA100 at concentration 1.25 mg/plate. (*$P < 0.05$ as compared with control, set as 100% mutagenicity)



(a)



(b)

Figure 4.13 Antimutagenic activities of *B. superba* extracts against a) direct-acting of AF-2 0.01 μg/plate and b) indirect-acting of B(*a*)P 5 μg/plate mutagens analyzed by *S.* Typhimurium strain TA100 at concentration 2.50 mg/plate. (*$P < 0.05$ as compared with control, set as 100% mutagenicity)

4.1.3.3 Antimutagenic activity of the *M. collettii* extracts

M. collettii extracts collected from 4 provinces of Thailand were screened for antimutagenic activity. Certain extract significantly decreased the number of revertants induced by AF-2 and B(*a*)P with dose-dependently (Table 4.4, Appendix A; Table 5.4).

At the concentration of 0.625 mg/plate

Using AF-2 (0.1 μg/plate) on *S.* Typhimurium TA98, the extracts of *M. collettii* from Lumpang exhibited weak inhibition. None of them showed moderate or strong antimutagenic activity. When tested of AF-2 (0.01 μg/plate) on *S.* Typhimurium TA100, only the sample from Chiang Mai exhibited weak inhibition. Also, no moderate or strong antimutagenic activity was found in any sample.

Using with B(*a*)P (10 μg/plate) on *S.* Typhimurium TA98, the weak inhibitory activity was sample from Chiang Mai and Lumpang and the sample from Kanchanaburi exhibited moderate inhibition. No sample with strong inhibition was found. When tested of B(*a*)P (5 μg/plate) on *S.* Typhimurium TA100, the weak inhibitory activity was sample from Chiang Mai and Lumpang. The sample from Kanchanaburi exhibited moderate inhibition whereas no sample with strong inhibition was detected (Figure 4.14 and 4.17).

At the concentration of 1.25 mg/plate

Using AF-2 (0.1 μg/plate) on *S.* Typhimurium TA98, the extracts of *M. collettii* from Chiang Mai exhibited weak inhibitory activity. None of them showed moderate or strong antimutagenic activity whereas no samples exhibited inhibition against AF-2 (0.01 μg/plate) on *S.* Typhimurium TA100.

Using B(*a*)P (10 μg/plate) on *S.* Typhimurium TA98, the sample from Kanchanaburi exhibited strong inhibition. No sample with weak or moderate inhibition was found. When tested with B(*a*)P (5 μg/plate) on *S.* Typhimurium TA100, the moderate inhibitory activity was Chiang Mai and only the sample from Kanchanaburi exhibited strong inhibition. Also, no weak antimutagenic activity was found in any sample (Figure 4.15 and 4.18).

At the concentration of 2.50 mg/plate

No sample exhibited inhibition against AF-2 (0.1 μg/plate) on *S.* Typhimurium TA98. When tested with AF-2 (0.01 μg/plate) on *S.* Typhimurium TA100, only the sample from Chiang Mai exhibited weak inhibition. Also, no moderate or strong antimutagenic activity was found in any sample.

Using B(*a*)P (10 μg/plate) on *S.* Typhimurium TA98, the extracts of *M. collettii* from Lumpang and Chiang Mai exhibited weak inhibition and only the sample from Kanchanaburi exhibited strong inhibition. No sample with moderate inhibition was found. When tested with B(*a*)P (5 μg/plate) on *S.* Typhimurium TA100, the moderate inhibitory activity was Chiang Mai and only the sample from Kanchanaburi exhibited strong inhibition (Figure 4.16 and 4.19).

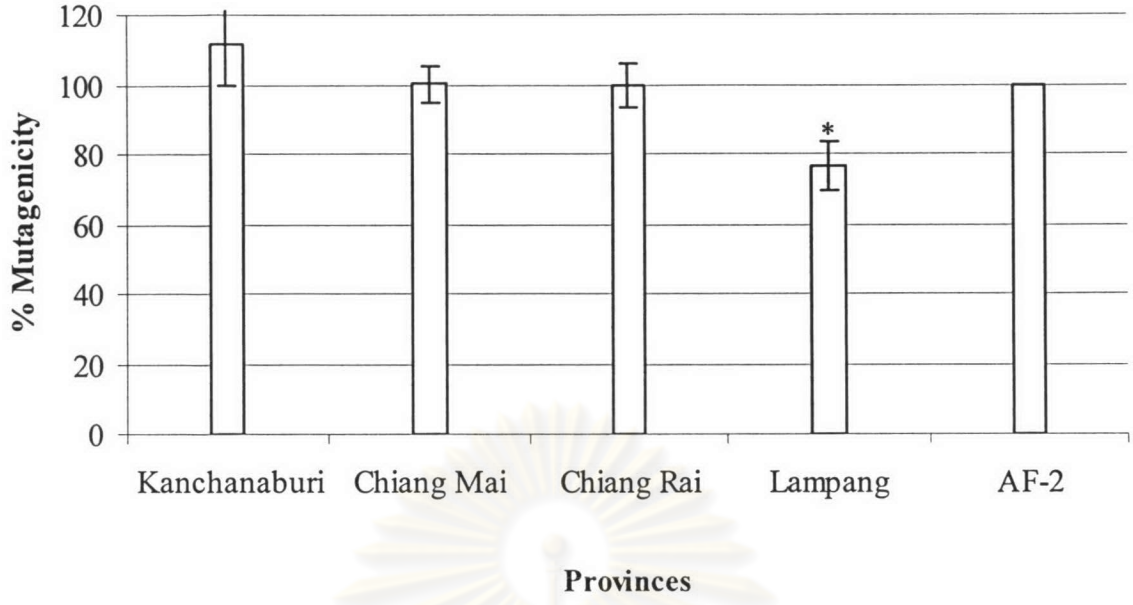
Table 4.4 Antimutagenic activities of *M. collettii* extracts from different provinces of Thailand detected by Ames test using *S.* Typhimurium strain TA98 and TA100 under non metabolic and metabolic activation conditions.

Province	Dose (mg/plate)	PI (%inhibition)			
		TA98		TA100	
		-S9	+S9	-S9	+S9
Chiang Rai	0.625	0.40 ± 6.09^{a}	$20.22 \pm 3.51^{b*}$	2.82 ± 3.31^{b}	5.80 ± 6.48^{a}
	1.25	5.04 ± 5.63^{a}	$26.13 \pm 3.96^{b*}$	-8.77 ± 4.15^{a}	-1.66 ± 1.98^{a}
	2.50	0.95 ± 6.36^{a}	$38.03 \pm 2.29^{c*}$	$16.88 \pm 3.94^{c*}$	$39.46 \pm 4.81^{b*}$
Chiang Mai	0.625	-0.28 ± 5.26^{a}	-5.18 ± 14.22^{ab}	$22.37 \pm 3.27^{b*}$	$24.91 \pm 4.17^{b*}$
	1.25	-6.34 ± 4.93^{a}	$-18.05 \pm 2.09^{b*}$	3.89 ± 3.95^{a}	$45.58 \pm 1.88^{c*}$
	2.50	$14.47 \pm 5.59^{b*}$	2.51 ± 1.26^{ab}	$22.29 \pm 6.83^{b*}$	$50.55 \pm 4.90^{c*}$
Lampang	0.625	$23.42 \pm 7.17^{b*}$	0.87 ± 6.34^{a}	8.64 ± 10.39^{b}	$29.05 \pm 5.31^{c*}$
	1.25	4.13 ± 6.08^{a}	$19.77 \pm 11.78^{b*}$	-8.44 ± 3.71^{b}	8.49 ± 7.98^{ab}
	2.50	-0.44 ± 4.42^{a}	$27.34 \pm 4.55^{b*}$	$-26.64 \pm 3.96^{a*}$	$16.84 \pm 4.49^{bc*}$
Kanchana buri	0.625	-11.84 ± 12.06^{a}	$25.71 \pm 4.89^{b*}$	5.07 ± 8.22^{ab}	$53.57 \pm 2.88^{b*}$
	1.25	3.51 ± 9.40^{a}	$78.21 \pm 1.65^{c*}$	$16.32 \pm 1.37^{b*}$	$87.76 \pm 2.71^{c*}$
	2.50	-7.27 ± 8.12^{a}	$80.97 \pm 1.32^{c*}$	4.39 ± 13.20^{ab}	$92.87 \pm 0.54^{c*}$

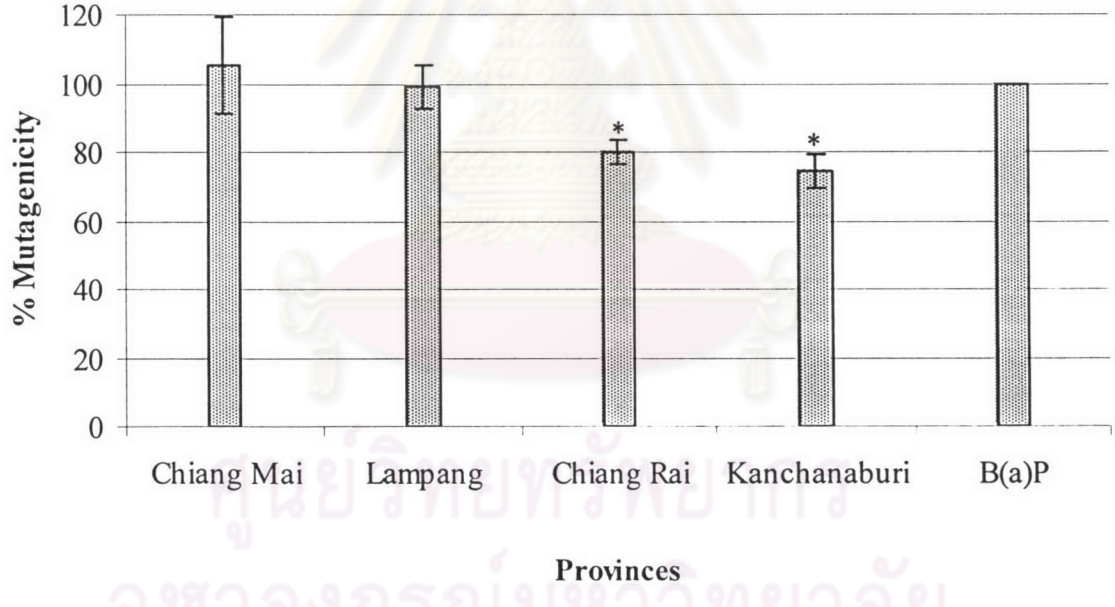
*$P < 0.05$ as compared with control.

Means not sharing a common superscript letter in the same column of one province are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Results shown were mean ± S.E.M. from triplicate trials.

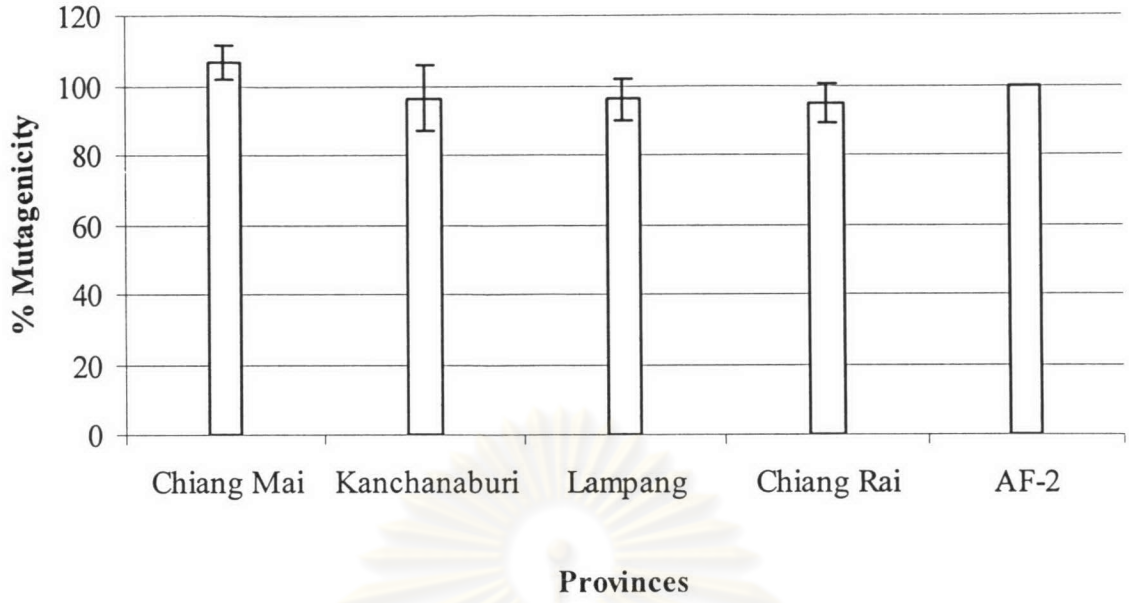


(a)

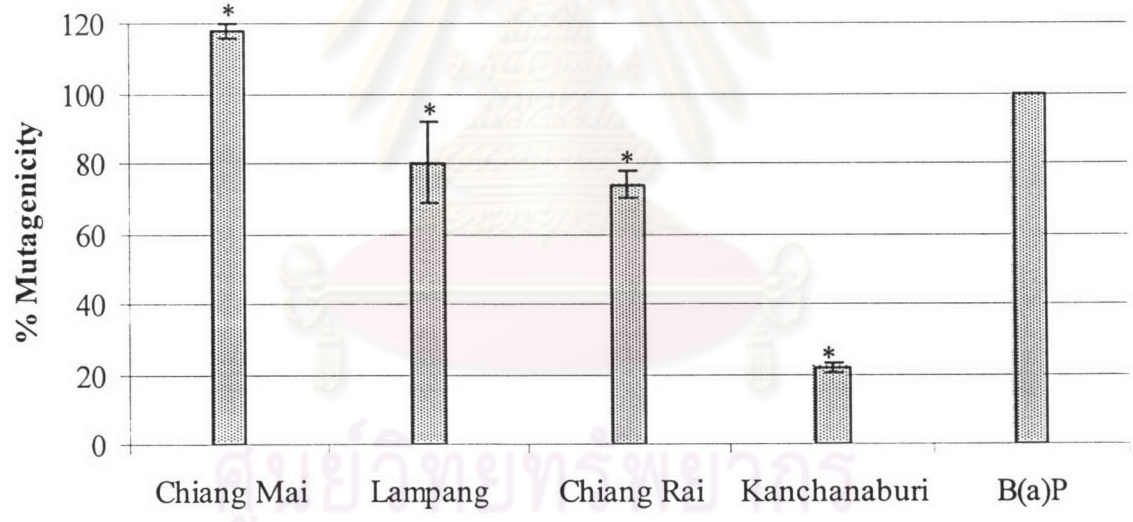


(b)

Figure 4.14 Antimutagenic activities of *M. collettii* extracts against a.) direct-acting of AF-2 0.1 μg/plate and b.) indirect-acting of B(*a*)P 10 μg/plate mutagens analyzed by *S.* Typhimurium strain TA98 at concentration 0.625 mg/plate. (*$P < 0.05$ as compared with control, set as 100% mutagenicity)

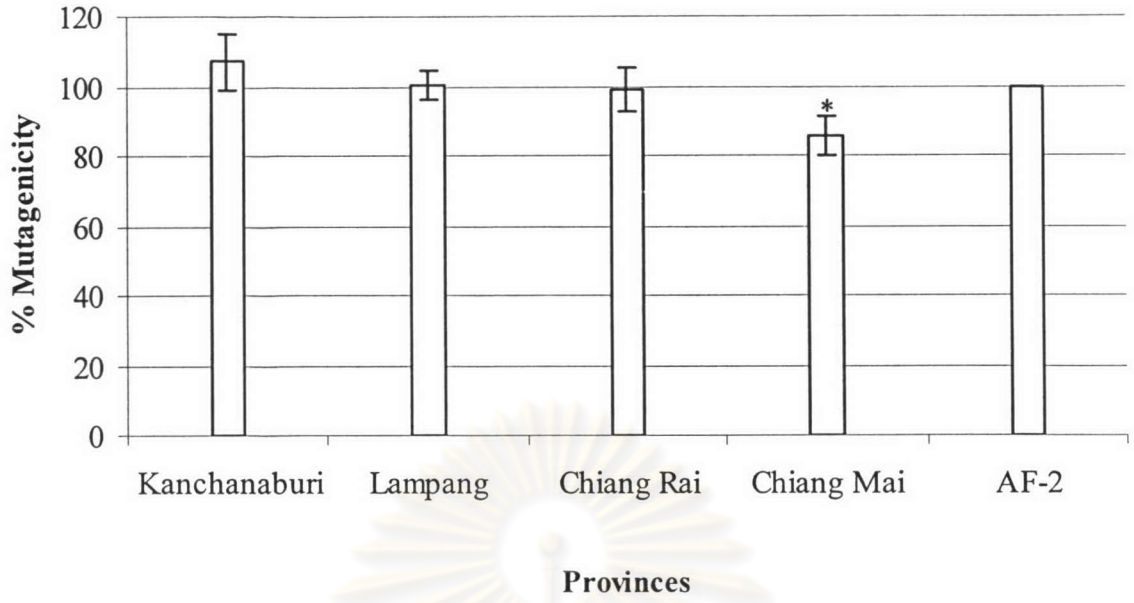


(a)



(b)

Figure 4.15 Antimutagenic activities of *M. collettii* extracts against a) direct-acting of AF-2 0.1 μg/plate and b) indirect-acting of B(*a*)P 10 μg/plate mutagens analyzed by *S.* Typhimurium strain TA98 at concentration 1.25 mg/plate. (*$P < 0.05$ as compared with control, set as 100% mutagenicity)



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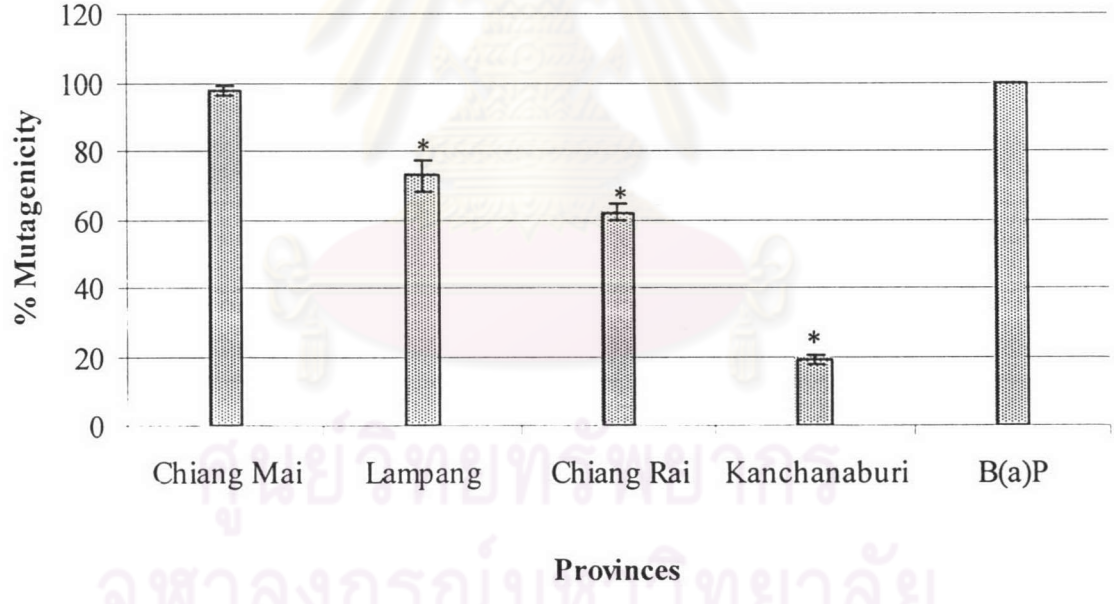
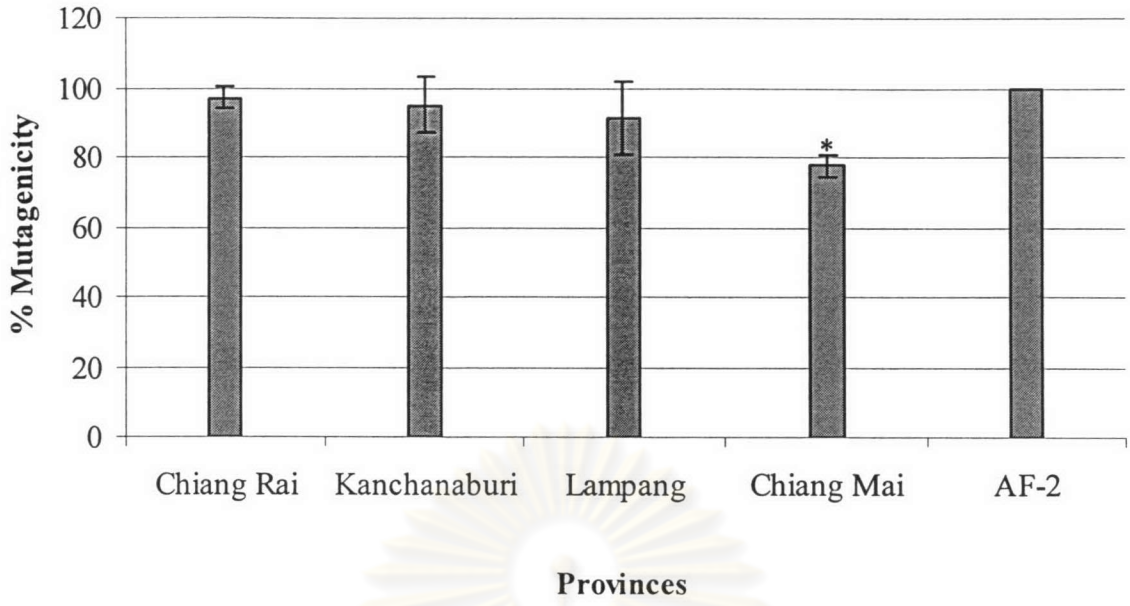


(b)

Figure 4.16 Antimutagenic activities of *M. collettii* extracts against a) direct-acting of AF-2 0.1 μg/plate and b) indirect-acting of B(*a*)P 10 μg/plate mutagens analyzed by *S.* Typhimurium strain TA98 at concentration 2.50 mg/plate. (*$P < 0.05$ as compared with control, set as 100% mutagenicity)



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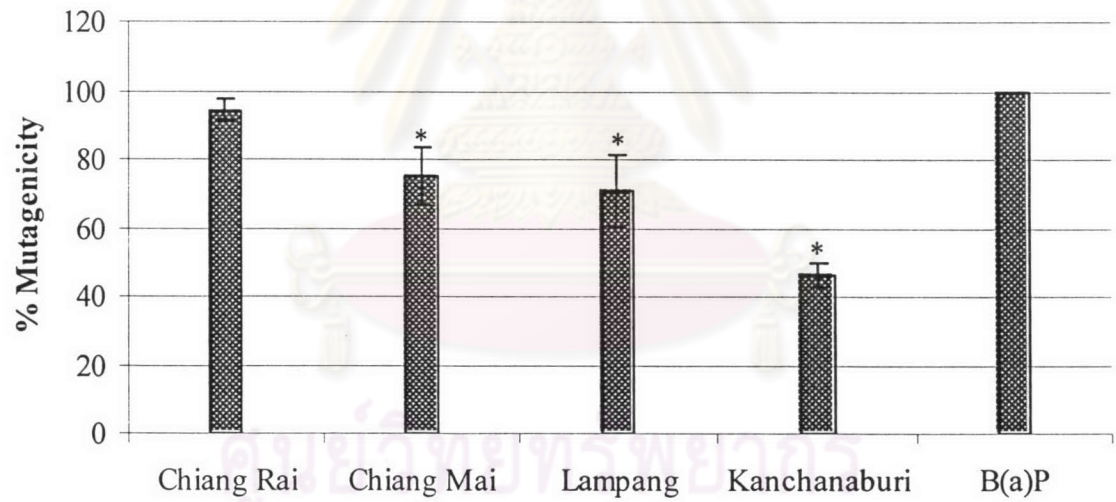
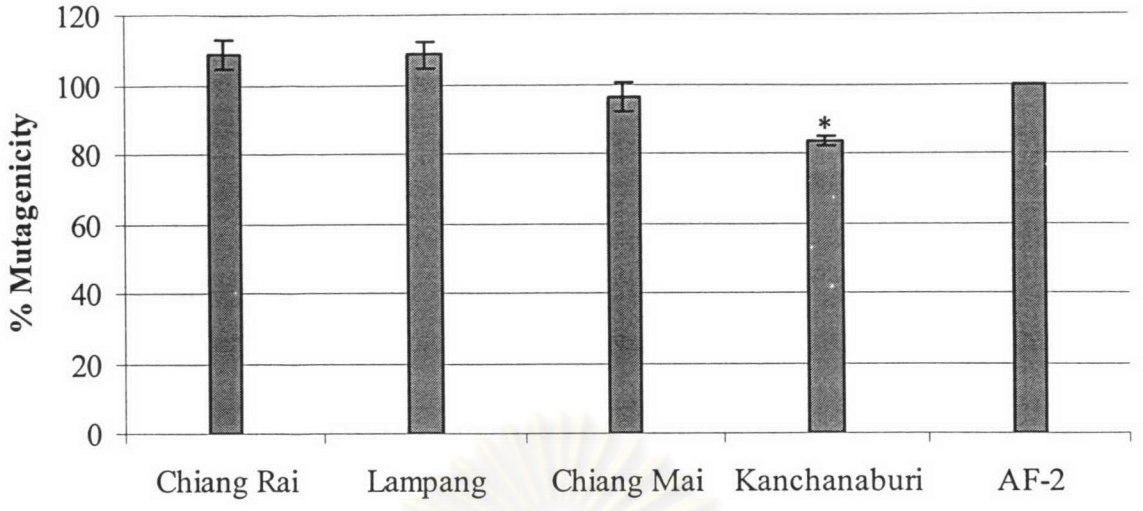
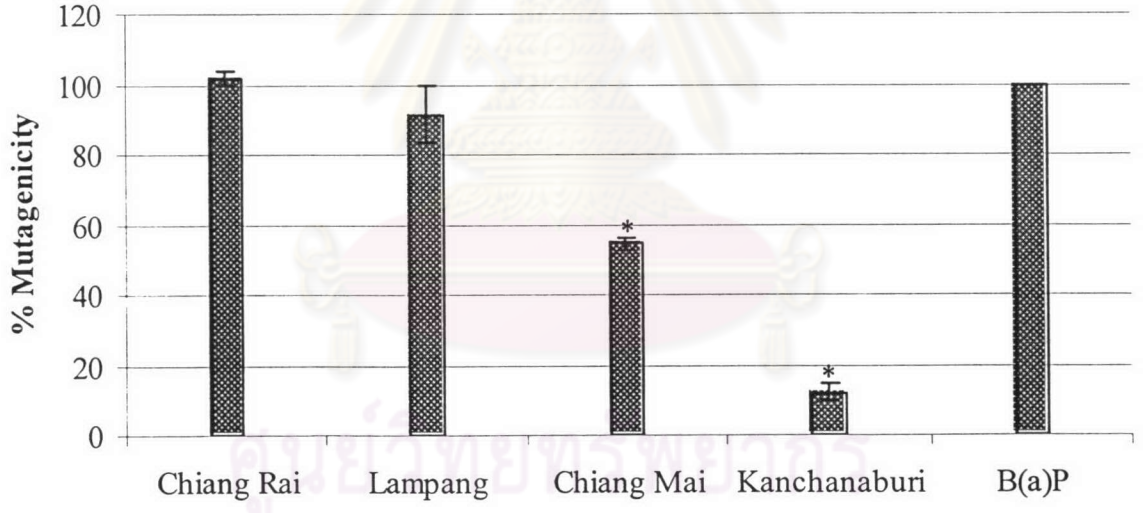


(b)

Figure 4.17 Antimutagenic activities of *M. collettii* extracts against a) direct-acting of AF-2 0.01 μg/plate and b) indirect-acting of B(*a*)P 5 μg/plate mutagens analyzed by *S.* Typhimurium strain TA100 at concentration 0.625 mg/plate. (*$P < 0.05$ as compared with control, set as 100% mutagenicity)

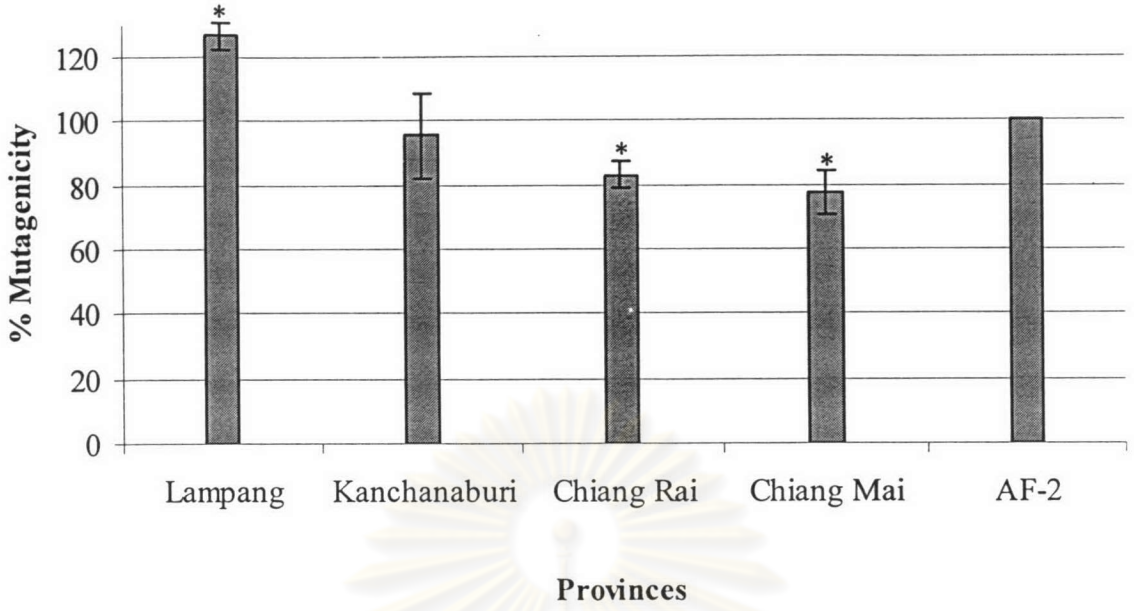


(a)



(b)

Figure 4.18 Antimutagenic activities of *M. collettii* extracts against a) direct-acting of AF-2 0.01 μg/plate and b) indirect-acting of B(*a*)P 5 μg/plate mutagens analyzed by *S.* Typhimurium strain TA100 at concentration 1.25 mg/plate. (*$P < 0.05$ as compared with control, set as 100% mutagenicity)



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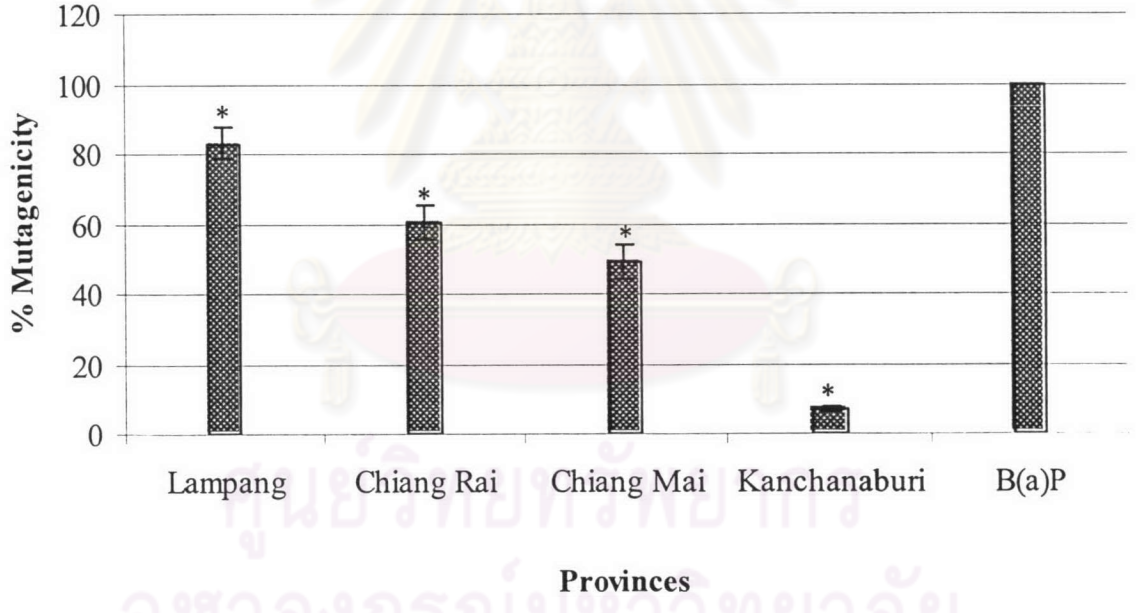

(b)

Figure 4.19 Antimutagenic activities of *M. collettii* extracts against a) direct-acting of AF-2 0.01 μg/plate and b) indirect-acting of B(*a*)P 5 μg/plate mutagens analyzed by *S.* Typhimurium strain TA100 at concentration 2.50 mg/plate. (*$P < 0.05$ as compared with control, set as 100% mutagenicity)

4.1.3.4 Comparison of antimutagenic activity of plant extracts detected by Ames Test

Mean value of antimutagenic activity (PI) of *P. mirifica* population (n=28), *B. superba* population (n=24) and *M. collettii* population (n=4) were shown on Table 4.5 and Figure 4.20. *M. collettii* exhibited the highest against B(*a*)P with moderate inhibition on S. Typhimurium TA98 and TA 100 at concentration 2.50 mg/plate; whereas, *P. mirifica* and *B. superba* population exhibited weak inhibition on S. Typhimurium TA 100 at concentration 2.50 mg/plate. Thus, all Kwao Krua extracts showed significant antimutagenic activity compared with positive control ($P < 0.05$).

Table 4.5 Anti-mutagenic activity of the plant extracts against direct-acting and indirect-acting mutagens detected by *S.* Typhimurium strain TA98 and TA100

Plant extracts	Dose (mg/plate)	PI (%inhibition)			
		TA98		TA100	
		-S9	± S9	-S9	± S9
P. mirifica	0.625	-4.65 ± 3.44^{a}	0.11 ± 2.64^{a}	8.64 ± 3.21^{b*}	9.23 ± 2.57^{b*}
(n = 28)	1.25	-0.19 ± 3.11^{a}	5.18 ± 2.48^{a}	17.73 ± 2.92^{c*}	19.66 ± 2.84^{c*}
	2.50	-2.61 ± 2.81^{a}	6.52 ± 2.66^{a}	13.25 ± 3.95b^{c*}	26.41 ± 3.15^{c*}
B. suberba	0.625	-4.22 ± 4.28^{a}	5.51 ± 3.29^{a}	4.57 ± 3.12^{ab}	10.46 ± 3.93^{b*}
(n = 24)	1.25	3.33 ± 4.16^{a}	17.95 ± 3.77^{b*}	11.17 ±3.40^{bc*}	22.74 ± 3.08^{c*}
	2.50	5.80 ± 5.81^{a}	26.77 ± 6.13^{b*}	13.68 ± 3.39^{c*}	32.43 ± 3.54^{d*}
M. colletti	0.625	2.92 ± 7.39^{a}	10.41 ± 7.44^{a}	9.75 ± 4.38^{a}	28.34 ± 9.82^{b*}
(n = 4)	1.25	1.58 ± 2.66^{a}	26.51 ± 19.79^{ab}	0.75 ± 5.97^{a}	35.04 ±20.29^{b*}
	2.50	1.93 ± 4.55^{a}	37.21 ± 16.37^{b*}	4.23 ± 10.95^{a}	49.93 ±15.94^{c*}

*$P < 0.05$ as compared with control.

Means not sharing a common superscript letter in the same column of each plant extracts are significantly different ($P < 0.05$) as determined by Duncan's multiple range test. Results shown were mean ± S.E.M. from triplicate trials.

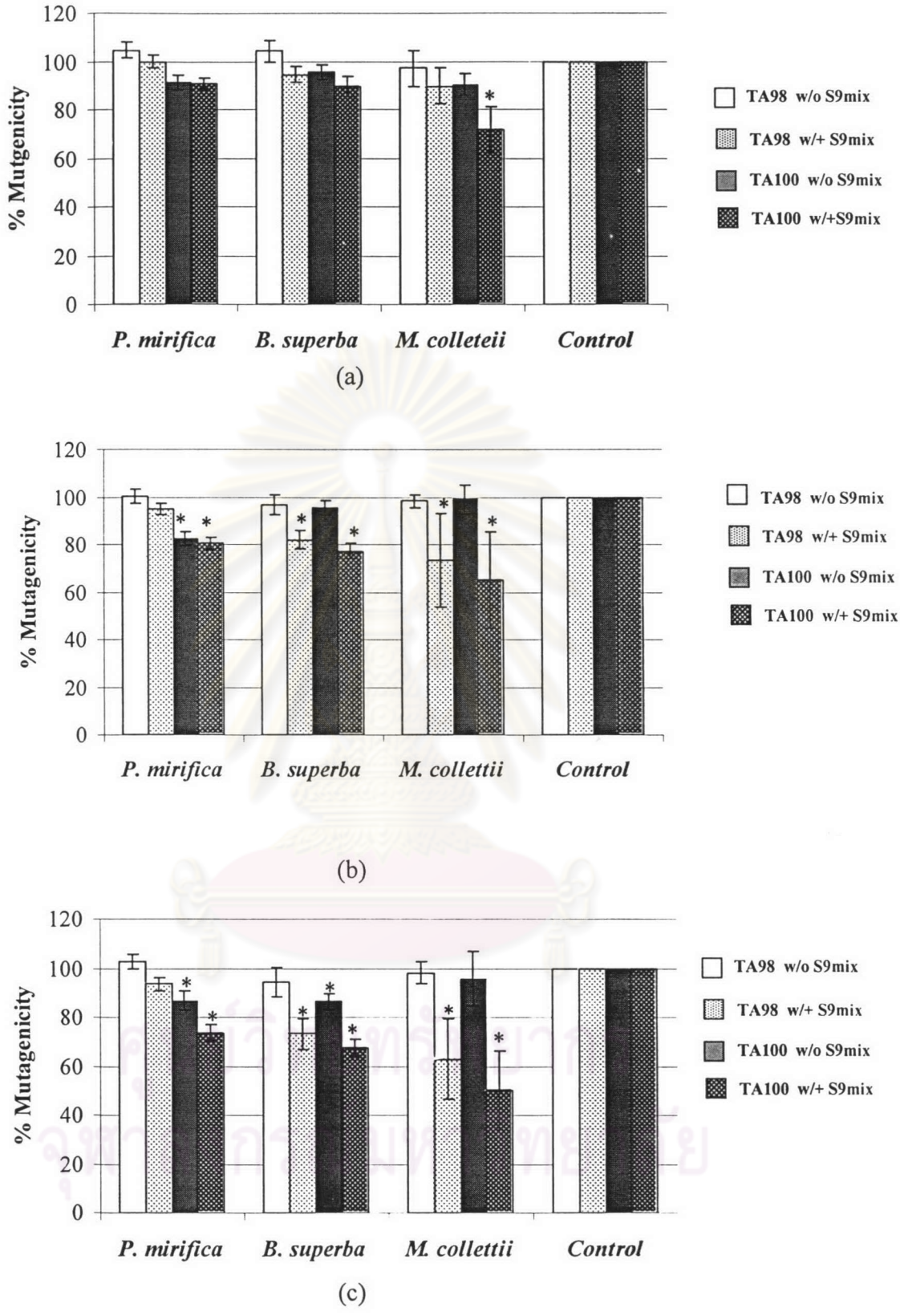

Figure 4.20 Antimutagenic activities of the plant extracts against direct-acting and indirect-acting mutagens detected by Ames test at the concentration (a) 0.625, (b) 1.25 and (c) 2.50 mg/plate. (*$P < 0.05$ as compared with control, set as 100% mutagenicity)

4.2 Forward mutation assay

4.2.1 Mutagenic activity of the plant extracts toward *B. subtilis* H17 (*rec*$^+$) and M45 (*rec*$^-$) in the absence of metabolic activation

The mutagenicity and antimutagenicity assay of plant extracts were confirmed in *rec* assay with *Bacillus subtilis* strain H17 and H45 on non-metabolic activity condition (Table 4.6). All plant extracts at the concentration of 2.5, 5 and 10 mg/well exhibited no mutagenicity. *Mucuna collettii* and *Butea superba* exhibited significantly inhibition against AF-2 at the concentration 2.50 mg/well, and *P. mirifica* exhibited significantly inhibition against AF-2 at the concentration 10 mg/well (Table 4.7).

Table 4.6 Mutagenicity effects of the plant extracts tested by *Rec* assay with *B. subtilis* var. H17 (*Rec*$^+$) and M45 (*Rec*$^-$) on non-metabolic activation condition.

Plant extracts	Concentration (mg/well)	Size of clear zone (mm)[A]		Ratio M45/H17
		H17	M45	
P. mirifica	2.5	8.00 ± 0.00	8.00 ± 0.00*	1.00 ± 0.00*
	5	8.00 ± 0.00	8.00 ± 0.00*	1.00 ± 0.00*
	10	8.00 ± 0.00	8.00 ± 0.00*	1.00 ± 0.00*
B. superba	2.5	8.00 ± 0.00	8.83 ± 0.83*	1.10 ± 0.10*
	5	8.00 ± 0.00	10.53 ± 1.47*	1.32 ± 0.18*
	10	8.00 ± 0.00	11.90 ± 2.48*	1.49 ± 0.31*
M. collettii	2.5	8.00 ± 0.00	8.00 ± 0.00*	1.00 ± 0.00*
	5	8.00 ± 0.00	8.67 ± 0.67*	1.08 ± 0.08*
	10	8.00 ± 0.00	11.00 ± 0.00*	1.38 ± 0.00*
AF-2[B]	**0.1 µg/well**	**8.00 ± 0.00**	**21.36 ± 1.90**	**2.67 ± 0.24**

[A] The results are expressed as a mean value ± S.E.M.

[B] The inhibition zone of Rec$^-$/Rec$^+$ ratio of DMSO were 1.00 ± 0.00 while 0.1 µg/well of AF-2 was 2.67 ± 0.24. Each value represents mean of triplicate plates and the solidified agar was pierced with sterile cork borer of diameter 8 mm.

* $P < 0.05$ as compared with positive control.

4.2.2 Antimutagenic activity of the plant extracts toward *B. subtilis* H17 (*rec*$^+$) and M45 (*rec*$^-$) in the absence of metabolic activation

Table 4.7 Antimutagenicity effects of the plant extracts tested by *Rec* assay with *B. subtilis* var. H17 (*Rec*$^+$) and M45 (*Rec*$^-$) on non-metabolic activation condition.

Plant extracts	Concentration (mg/well)	Size of clear zone (mm)[A]		Ratio
		H17	M45	M45/H17
P. mirifica	2.5	8.00 ± 0.00	18.57 ± 4.19	2.32 ± 0.52
	5	8.00 ± 0.00	17.07 ± 3.15	2.13 ± 0.39
	10	8.00 ± 0.00	8.00 ± 0.00*	1.00 ± 0.00*
B. superba	2.5	11.73 ± 1.88*	21.36 ± 3.08	1.83 ± 0.04*
	5	8.00 ± 0.00	12.06 ± 0.97*	1.51 ± 0.08*
	10	8.00 ± 0.00	8.00 ± 0.00*	1.00 ± 0.00*
M. collettii	2.5	8.00 ± 0.00	8.66 ± 0.67*	1.08 ± 0.08*
	5	8.00 ± 0.00	11.00 ± 0.00*	1.38 ± 0.00*
	10	8.00 ± 0.00	8.00 ± 0.00*	1.00 ± 0.00*
AF-2[B]	**0.1 µg/well**	**8.00 ± 0.00**	**21.36 ± 1.90**	**2.67 ± 0.24**

[A] The results are expressed as a mean value ± S.E.M.

[B] The inhibition zone of Rec$^-$/Rec$^+$ ratio of DMSO were 1.00 ± 0.00 while 0.1 µg/well of AF-2 was 2.67 ± 0.24. Each value represents mean of triplicate plates and the solidified agar was pierced with sterile cork borer of diameter 8 mm.

* $P < 0.05$ as compared with positive control.

4.3 The antimutagenic activity correlation of *P. mirifica*, *B. superba* and *M. collettii* from this study and previous ones

The correlation of antimutagenic activity from *P. mirifica* determined by Ames Test and isoflavone content (Subtang, 2002) was examined. No correlation between antimutagenic activity and isoflavone content was found (Table 4.8).

The correlation of antimutagenic activity from all plant extracts determined by Ames Test, antioxidant (Sutjit, 2003) and anti-proliferation effect on MFC-7 (Trisap, 2003) were tested. It was found that there is no correlation of these factors among all plants (Table 4.9-4.11).

Table 4.8 The correlation between antimutagenic activity at the concentration 2.50 mg/plate of *P. mirifica* samples determined by Ames Test and Isoflavone contents (Subtang, 2002)

Rank of antimutagenic activity	Antimutagenicity (PI)				Isoflavone HPLC fingerprints (mg/100g powder)					
	TA98		TA100		Total Isoflavone	Puerarin	Daidzin	Genistin	Daidzein	Genistein
	-S9	+S9	-S9	+S9						
1st	21.52 ± 4.05 Pa yao	28.28 ± 2.36 Uthai Thani	42.89 ± 2.13 Uttharadith	55.95 ± 1.52 Sukhothai	198.29 ± 4.6 Kanchanaburi	45.25 ± 1.11	50.24 ± 3.23	85.69 ± 1.23	13.92 ± 1.26	3.19 ± 0.29
2nd	17.05 ± 1.71 Phetchabun	27.96 ± 3.68 Phetchaburi	38.61 ± 4.22 Phitsanulok	54.74 ± 6.86 Phrachin Buri	155.00 ± 1.4 Lumphun	33.18 ± 0.92	28.35 ± 0.68	84.13 ± 0.54	8.59 ± 0.09	0.76 ± 0.36
3rd	16.10 ± 6.24 Phetchaburi	26.53 ± 4.98 Chiang Mai	38.21 ± 2.09 Saraburi	48.79 ± 1.63 Phrae	131.25 ± 9.0 Chiang Mai	35.55 ± 3.57	27.39 ± 5.32	58.00 ± 0.71	8.38 ± 0.22	1.93 ± 0.54
26th	-27.49 ± 13.03 Chumphon	-12.11 ± 1.14 Phrachin Buri	5.60 ± 4.29 Tak	-0.43 ± 5.33 Uttharadith	44.83 ± 1.73 Phetchabun	9.40 ± 0.46	10.48 ± 0.67	15.54 ± 1.61	8.11 ± 0.05	1.29 ± 0.02
27th	-28.80 ± 5.82 Chiang Rai	-16.81 ± 1.21 Tak	-35.73 ± 14.46 Prachuap Kiri Khun	-3.72 ± 5.83 Kamphaeng Phet	43.71 ± 4.02 Nakhon Ratchasima	15.44 ± 1.14	7.01 ± 1.10	18.50 ± 4.45	2.31 ± 0.11	0.46 ± 0.08
28th	-32.40 ± 11.24 Chiang Mai	-22.16 ± 6.66 Lumpang	-56.81 ± 19.92 Chiang Mai	-4.66 ± 4.88 Nan	18.85 ± 1.92 Nan	5.32 ± 0.22	7.62 ± 1.36	3.31 ± 0.31	0.24 ± 0.04	18.85 ± 1.92
Correlation	No-Correlation									

Table 4.9 The correlation between antimutagenic activity at the concentration 2.50 mg/plate of *P. mirifica* samples determined by Ames test, and antioxidant activity; and anti-proliferatin effect on MCF-7 cell (Sutjit, 2003; Trisap, 2003)

Rank of antimutagenic activity	Antimutagenicity (PI)				Antioxidant activity (IC_{50}; μg/ml)	Anti-Proliferation effect (10 μg/ml)
	TA98		TA100			
	-S9	+S9	-S9	+S9		
1st	21.52 ± 4.05 Pa yao	28.28 ± 2.36 Uthai Thani	42.89 ± 2.13 Uttharadith	55.95 ± 1.52 Sukhothai	2,470.38 ± 37.81 Uthai Thani	73.08 ± 3.01 Phrae
2nd	17.05 ± 1.71 Phetchabun	27.96 ± 3.68 Phetchaburi	38.61 ± 4.22 Phitsanulok	54.74 ± 6.86 Phrachin Buri	2,489.98 ± 27.62 Nong Bua Lam Phu	73.45 ± 6.18 Phetchabun
3rd	16.10 ± 6.24 Phetchaburi	26.53 ± 4.98 Chiang Mai	38.21 ± 2.09 Saraburi	48.79 ± 1.63 Phrae	2,492.61 ± 83.02 Phetchaburi	73.99 ± 3.64 Phitsanulok
26th	-27.49 ± 13.03 Chumphon	-12.11 ± 1.14 Phrachin Buri	5.60 ± 4.29 Tak	-0.43 ± 5.33 Uttharadith	3,209.30 ± 102.50 Prachin Buri	106.06 ± 6.06 Ratchaburi
27th	-28.80 ± 5.82 Chiang Rai	-16.81 ± 1.21 Tak	-35.73 ± 14.46 Prachuap Kiri Khun	-3.72 ± 5.83 Kamphaeng Phet	3,234.58 ± 141.55 Saraburi	106.38 ± 14.27 Chumphon
28th	-32.40 ± 11.24 Chiang Mai	-22.16 ± 6.66 Lumpang	-56.81 ± 19.92 Chiang Mai	-4.66 ± 4.88 Nan	3,376.97 ± 69.96 Kamphaeng Phet	107.19 ± 4.13 Prachin Buri
Correlation	No-Correlation					

Table 4.10 The correlation of antimutagenic activity at the concentration 2.50 mg/plate of *B. superba* samples determined by Ames test, antioxidant activity and anti-proliferation effect on MCF-7 cell (Sutjit, 2003; Trisap, 2003)

Rank of antimutagenic activity	Antimutagenicity (PI)				Antioxidant activity (IC_{50}; μg/ml)	Anti-Proliferation effect (1 μg/ml)
	TA98		TA100			
	-S9	+S9	-S9	+S9		
1st	60.82 ± 8.99 Loei	94.15 ± 1.42 Chonburi	53.36 ± 3.55 Kanchanaburi	88.61 ± 0.24 Chonburi	227.08 ± 0.38 Loei	61.77 ± 5.98 Ratchaburi
2nd	50.39 ± 3.79 Chiang Mai	81.79 ± 2.96 Phetchabun	38.33 ± 4.49 Phrachin Buri	56.80 ± 5.07 Lop buri	289.82 ± 6.05 Lop Buri	72.84 ± 6.04 Prachinburi
3rd	40.19 ± 0.87 Chaiyaphum	61.12 ± 6.10 Lop buri	34.88 ± 5.79 Chantaburi	50.22 ± 8.83 Loei	323.50 ± 8.43 Saraburi	73.54 ± 3.77 Tak
22nd	-34.95 ± 17.86 Phitsanulok	-13.37 ± 6.26 Saraburi	-6.83 ± 0.25 Lumpang	9.52 ± 2.17 Utharadith	1,129.15 ± 45.44 Nong Bua Lam Phu	103.15 ± 1.00 Chantaburi
23rd	-39.27 ± 4.27 Mae Hong Son	-16.58 ± 8.59 Kanchanaburi	-11.86 ± 3.34 Chonburi	9.07 ± 11.96 Nakhon Sawan	1,185.11 ± 17.69 Khon Kaen	104.85 ± 8.31 Nakhon Sawan
24th	-32.61 ± 6.47 Phetchabun	-32.22 ± 6.19 Chachoengsao	-12.79 ± 6.45 Ratchaburi	5.51 ± 1.36 Phrachin Buri	1,198.04 ± 18.76 NaKhon Sawan	110.7 ± 16.68 Mae Hong Son
Correlation	No-Correlation					

Table 4.11 The correlation of antimutagenic activity at the concentration 2.50 mg/plate of *M. collettii* samples determined by Ames test, antioxidant activity and anti-proliferation effect on MCF-7 cell (Trisap, 2003; Sutjit, 2003)

Rank of antimutagenic activity	Antimutagenicity (PI)				Antioxidant activity (IC_{50}; µg/ml)	Anti-Proliferation effect (1 µg/ml)
	TA98		TA100			
	-S9	+S9	-S9	+S9		
1st	14.47 ± 5.59 Chiang Mai	80.97 ± 1.32 Kanchanaburi	22.29 ± 6.83 Chiang Mai	92.87 ± 0.54 Kanchanaburi	55.53 ± 2.66 Chiang Rai	90.02 ± 12.81 Lampang
2nd	0.95 ± 6.36 Chiang Rai	38.03 ± 2.29 Chiang Rai	16.88 ± 3.94 Chiang Rai	50.55 ± 4.90 Chiang Mai	71.52 ± 0.61 Chiang Mai	97.01 ± 5.11 Chiang Mai
3rd	-0.44 ± 4.42 Lampang	27.34 ± 4.55 Lampang	4.39 ± 13.20 Kanchanaburi	39.46 ± 4.81 Chiang Rai	77.84 ± 0.79 Kanchanaburi	97.63 ± 15.95 Chiang Rai
4th	-7.27 ± 8.12 Kanchanaburi	2.51 ± 1.26 Chiang Mai	-26.64 ± 3.96 Lampang	16.84 ± 4.49 Lampang	127.34 ± 0.47 Lampang	104.25 ± 17.76 Kanchanaburi
Correlation	No-Correlation					