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APPENDICES

APPENDIX A

A. Recipes for reagents and media

Vogel–Bonner (VB salts) medium E (50X)

Use: salts for the GM agar plates

Ingredients	**Per liter**
Warm distilled water (about 50^0C)	650 ml
Magnesium sulfate ($MgSO_4.H_2O$)	10 g
Citric acid monohydrate	100 g
Potassium phosphate, dibasic, anhydrous (K_2HPO_4)	500 g
Sodium ammonium phosphate ($Na_2NH_2PO_4.4H_2O$)	175 g

Add the above ingredients in the order into a 2-L flask containing warm water, making sure that each salt is dissolved thoroughly by stirring on a magnetic stirrer before adding the next salt. It takes about 1 hour to dissolve. Adjust volume to 1 L. Distribute as 20-ml aliquots into vial and autoclave, loosely capped, for 30 min at 121°C. When the solutions have cooled, tighten the caps and store at room temperature in the dark.

Glucose solution (10% v/v)

Use: as carbon source for the MGA agar plates

Ingredients	**Per liter**
Distilled water	700 ml
Glucose	100 g

Add the glucose to the water in a 3-l flask. Stir on a magnetic stirrer until mixture is clear. Add additional water to bring the final volume to 1000 ml. Dispense 50-ml aliquots into 250 ml screw-cap bottles. Label as 10% glucose with date. Autoclave 121^0C for 20 min making sure the caps are on loosely. When cooled, tighten the caps and store at 4^0C.

Minimal glucose agar plates

Use: bottom agar for mutagenicity assay

Ingredients	**Per liter**
Distilled water	900 ml
Agar	15 g
VB salt solution (50x)	20 ml
Glucose solution (10% v/v)	50 ml

Add the agar to the water in a 3-L flask. Autoclave for 30 min at 121 ^{0}C. Let cool for about 45 min to about 65^{0}C. Add 20 ml of sterile VB salts and mix thoroughly, then add the 50 ml of a sterile glucose (10% v/v) solution; again swirl thoroughly. Dispense the agar medium in 100x15 mm petri dishes (approximately 25 ml/plate). When solidified, the plates can be stored at 4 ^{0}C for several weeks when placed in sealed plastic bags. Before use, the plates should be warmed up to room temperature and examined for excess moisture. If too much moisture is present, incubate the plates overnight at 37 ^{0}C prior to use. It takes about 12 L of agar medium to prepare one case of Petri plates (500 plates).
Note: A precipitate may form when the VB salts are added. However, thorough mixing will solubilize the salts. The agar should never be autoclaved together with the VB salts and glucose. The GM plates prepared this way will not fully support the growth of the *Salmonella* tester strains.

Histidine/biotin solution (0.5 mM)

Use: to supplement top agar with excess biotin and a trace amount of histidine

Ingredients	**Per liter**
Distilled water	1000 ml
d-biotin (F.W 247)	124 mg
L-Histidine.HCl (F.W 191.7)	96 mg

Bring water to a boil and add the biotin and histidine. It may take the biotin a little while to completely dissolved; histidine is readily dissolved. If used immediately, follow the procedure for making top agar. If not used immediately sterilize the solution either by filtration through a 0.45 μm membrane filter or by autoclaving for 20 min at 121^{0}C. Store at 4^{0}C in a glass bottle.

Top agar supplemented with histidine/biotin

Use: to deliver the bacteria, chemical and buffer or S-9 mix to the bottom agar

Ingredients	**Per liter**
Distilled water	900 ml
Agar	6 g
Sodium chloride	6 g
Histidine/biotin solution (0.5 mM)	100 ml

Add the agar and sodium chloride to a 3-L flask containing 900 ml of distilled water. Heat for 10 min in an autoclave, liquid cycle, to melt the agar. Then, add 100 ml of limited histidine and biotin solution (0.5 mM). Dispense 200 ml aliquots in 500 ml screw-cap bottles. Label as TA with date with the date of preparation. Autoclave for 30 °C and store at room temperature in the dark. When ready to use, melt the top agar in a microwave oven or in boiling water.

Nutrient broth

Use: to grow the tester strains overnight

Ingredients	**Per liter**
Distilled water	1000 ml
Oxoid nutrient broth No.2	25 g

Add the nutrient broth powder to the water and stir to dissolve. Dispense 50 ml in 125 ml Erlenmeyer flasks or 5 ml in 100x16 mm test tubes. Autoclave for 20 min. When cooled, store in the dark at room temperature.

Note: Difco nutrient broth can also be used but the overnight cultures are usually less dense compared to when Oxoid nutrient broth is used. Follow the manufacturer's directions for preparing the Difco nutrient broth.

Sodium phosphate buffer, 0.1 mM, pH 7.4

Use: for testing chemicals in the absence of metabolic activation

Ingredients	**Per liter**
Sodium phosphate, monobasic (0.1 M)	120 ml

To a 1 l water add 13.8 g $NaH_2PO_4.H_2O$

Sodium phosphate, dibasic (0.1 M) 880 ml

To a 1 l water add 14.2 g $Na_2HPO_4.H_2O$

After mixing the two ingredients, mix well. Adjust pH to 7.4 using 0.1 M dibasic sodium phosphate solution. Dispense 100 ml aliquots in 250 ml screw-cap bottles and label as "Buffer, 0.1 M, pH 7.4" with date. Autoclave for 30 min at 121°C. Make sure the caps fit loosely during autoclaving. When cooled, tighten the caps and store the bottles at room temperature in the dark.

Co-factors for S9 mix

Use: to provide the NADH regenerating system

Ingredients	**Per liter**
Distilled water	900 ml
d-Glucose-6-phosphate	1.6 g
Nicotinamide adenine dinucleotide phosphate (NADP)	3.5 g
Magnesium chloride ($MgCl_2$)	1.8 g
Potassium chloride (KCl)	2.7 g
Sodium phosphate, dibasic ($Na_2HPO_4.H_2O$)	12.8 g
Sodium phosphate, monobasic ($NaH_2PO_4.H_2O$)	2.8 g

To 900 ml of water, add each ingredient sequentially making sure that each ingredient is dissolved before adding the next one. This process may take up to 1 h. When all ingredients are dissolved, filter sterilize the cofactors (0.45 μm filter). Dispense in sterile glass bottles in aliquots of 7, 9 and 9.5 ml, or multiples of these volumes, for convenient use when in need of 30%, 10% or 5% S9, respectively, in the final S9 mix (10 ml volumes). Label "Cofactors" with date and store at -20°C.

Note: Prior to each experiment thaw sufficient co-factors and S9 fraction and keep on ice as soon as the S9 mix is prepared. A volume of 0.5 ml of the S9 mix is usually added per plate.

Enriched MGA agar plates

Use: to provide medium supplemented with essential nutrients and antibiotics for the strain check or propagation of the strains and preparation of stock culture master plates.

Prior to preparing the 1-L MGA agar plates (see recipe above) add the following item(s) as required to the flasks containing the MGA agar medium; mix well before dispensing.

- Biotin (B) plates: 8 ml of 0.01% solution.
- Histidine (H) plates (excess histidine): 8 ml of 0.5% solution.
- Biotin/Histidine (BH) plates: 8 ml of 0.01% biotin and 0.5% histidine solution.
- Biotin/Histidine/Ampicillin (BHA) plates: same as BH plates but add 3 ml of ampicillin solution (8 mg/ml) to give a final concentration of ampicillin of 24 mg/ml.
- Biotin/Histidine/Tetracycline (BHT) plates: same as BH plates but add 0.25 ml of tetracycline solution (8 mg/ml) which will give a final concentration of 2 mg/ml.
- Biotin/Histidine/Ampicillin/Tetracycline (BHAT) plates: same as BH plates but add 3 ml of ampicillin solution (8 mg/ml) and 0.25 ml of tetracycline (8 mg/ml), to give a final concentration of 24 and 2 mg/ml, respectively.

Biotin solution (0.01%, w/v)

Use: to enrich MGA agar plates for strain check, reisolation of strain and stock culture master plate.

Ingredients	**Per 100 ml**
Distilled water	100 ml
d-biotin	10 mg

Heat water to a boil and add the biotin; stir until dissolved. Filter sterilize using a 0.45 μm filter. Store at 4 °C.

Histidine solution (0.5%, w/v)

Use: to enrich MGA agar plates for strain check, reisolation of strain and stock culture master plate.

Ingredients	**Per 100 ml**
Distilled water	100 ml
L-histidine	500 mg

Dissolve the histidine in the water. Autoclave for 15 min at 121 °C. Store at 4 °C.

Ampicillin solution (0.8%, w/v)

Use: to confirm the presence of plasmid pKM101 in strain TA97, TA98, TA100 and TA102 and preparation of master plates for plasmid-carrying strains.

Ingredients	**Per 100 ml**
Distilled water	100 ml
Ampicillin	8 mg

Dissolve the ampicillin in warm (65 °C) water. Filter sterilize using a 0.45 μm filter. Store at 4 °C.

Tetracycline solution (0.8%, w/v)

Use: to confirm the presence of plasmid pAQ1 in strain TA102 and preparing master plate for strain TA102.

Note: the stock culture master plate for strain TA102 should contain both ampicillin and tetracycline.

Ingredients	**Per 100 ml**
Hydrochloric acid (0.02 N)	100 ml
Tetracycline	8 mg

Dissolve the tetracycline in the 0.02 N HCl. Filter sterilize using a 0.45 μm filter. Dispense in 10 ml aliquots in sterile test tubes. Store at 4 °C in the dark to protect against light (tetracycline is light sensitive).

Crystal violet solution (0.1%, w/v)

Use: to confirm the presence of the *rfa* mutation in all the tester strains

Ingredients	**Per 100 ml**
Distilled water	100 ml
Crystal violet	100 mg

Dissolve the crystal violet in the 100 ml of water. Mix well and store at 4°C in a brown glass bottle to protect against light.

Nutrient agar plates

Use: (1) to streak newly received cultures for single colonies, (2) to test for crystal violet sensitivity (*rfa*) in lieu of using MGA agar plates supplemented with excess histidine and biotin, (3) to test for viability of bacteria

Ingredients	**Per liter**
Distilled water	1000 ml
Agar	15 g
Oxoid Nutrient Broth No.2	25 g

Add the agar to the water in a 2-L flask and heat to dissolve. Add the nutrient broth powder and stir until dissolved. Autoclave for 20 min at 121 °C. Let the agar cool to about 65 °C. Dispense 20 to 25 ml in sterile petri plates. Store upside down in sealed plastic bags at 4 °C.

Table 5.1 Spontaneous revertant control values

Strain	**Number of revertants**	
	Without S9	**With S9**
TA97	75-200	100-200
TA98	20-50	20-50
TA100	75-200	75-200
TA102	100-300	200-400
TA104	200-300	300-400
TA1535	5-20	5-20
TA1537	5-20	5-20
TA1538	5-20	5-20

Ref; Mortlemans and Zeiger, 2000

B. Procedure for isolation and bacterial culture

B.1) ***Reisolation of tester strains***

- Take one frozen permanent of the standard tester strains, *S.*Typhimurium TA98 and TA100 from freezer, thaw at room temperature.
- Apply of small aliquot (0.05 ml) thawed culture into 12 ml of Oxoid Nutrient Broth No.2
- Incubate the plates at 37 °C for 14 hours.
- The cultures are reisolated by streaking on Ampicilin plates.
- Incubate the plate at 37 °C for 48 hours, 5 single colonies of each strain are picked up and grown in Oxoid Nutrient Broth No.2 on a shaker water bath at 37 °C for 14 hours.
- The cultures are used in preparation of master plate and confirming of the genotypes of the tester strains.

B.2) ***Preparation of master plates***

Master plates are used as the source of starting bacteria for inoculating the overnight culture of the tester strains. Using of these plates should avoid the problems that arise when the frozen permanents are opened frequently.

- Each of the overnight culture 0.3 ml of the single colony isolation spread on Ampicillin plates.
- Incubate the plate at 37 °C for 24 hours.
- The master plates are then stored at 4 °C and discarded after 2 months or sooner if the number of spontaneous revertant colonies per plate falls out of the range specified for a strain.

B.3) ***Procedure for growing cultures***

- Taking a single sweep from the master plates with a sterile wire loop, and grow in a 50 ml flask containing 12 ml Oxoid Nutrient Broth No.2
- Incubate the flask at 37 °C with shake 120 rpm in shaking water bath for 14-16 hours.

- The culture will grow to density of 1-2x10^9 CFU/ml ($O.D._{540}$ between 0.1 to 0.2)
- Remove the culture to an ice bath and dark place until it is required for the assay.

B.4) ***Confirming genotypes of tester strains***

The genotypes of bacterial strains used in mutation assay should be determined from time to time. Checks are necessary when strains are first received or the results of positive control assays fail to meet the standards accepted by the particular laboratory. A good compromise would be to determined genotypes at regular intervals such as two month. Confirming genotype used with five recommendation; histidine requirement, R-factor, *rfa* mutation, *uvrB* mutation, procedure for growing cultures, spontaneous reversion and the response to standard carcinogen.

B.4.1) Histidine requirement

The several single colonies of tester strains can be tested on the same plate, which can be determined as followed;

- Histidine dependence *(his^-);* streak a loopful of the culture across a GM agar plate supplemented with an excess of biotin.
- Biotin dependence *(bio^-);* streak a loopful of the culture across a GM agar plate supplemented with an excess of histidine.
- Histidine and biotin dependence *(his^-, bio^-);* streak a loopful of the culture across a MGA agar plate supplemented with an excess of histidine and biotin.
- Incubate at 37 °C for 24 hours.
- All *Salmonella* strain should be growth on the histidine-plus plate and no growth on histidine-minus plate. (Figure 5.1)

B.4.2) R-factor

Tester strain TA98 and TA100 contain R-factor plasmid (pKM101) should be test for the presence of the amplicilin resistance factor because the plasmid is some what unstable and can be lost from the bacteria which can be determined as follow;

- Streak a loopful of the culture across a MGA agar plate supplemented with an excess of biotin and histidine.

- Place a strile 6 mm. filter paper disc containing 10 µl/ml ampicillin in the center of the streak .
- Incubate the plates at 37 °C for 24 hours.
- The *Salmonella* strain should show a zone of growth inhibition surrounding the disc only with strain carrying pKM101. (Figure 5.2)

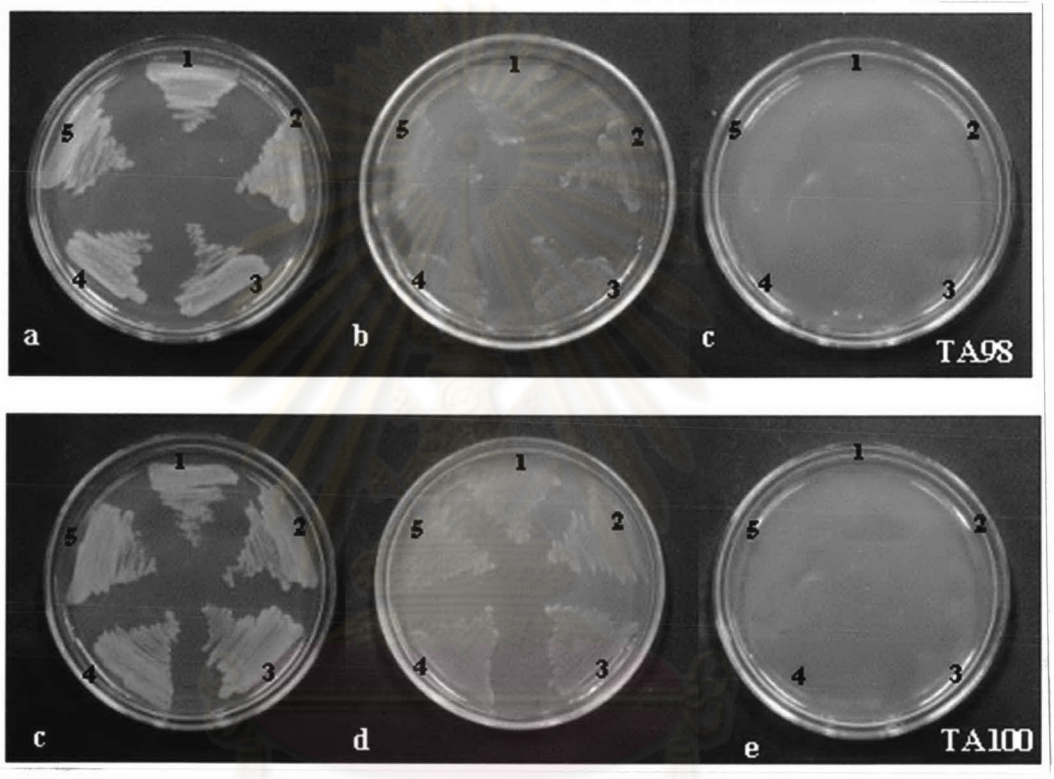

Figure 5.1 Confirmation the genotypes (*his* ⁻ , *bio* ⁻) of S. Typhimurium strains TA98 and TA100. Single revertant colony from MGA plate with Amp^r was picked and streaked on (a and c) MGA plate with histidine and biotin , then (b and d) MGA plate with biotin , and (c and e) MGA plate.

B.4.3) *rfa* mutation

Tester strain TA98 and TA100 as carrying the *rfa* mutation are more permeable to large molecules. Thus, this characteristic is confirmed by testing their sensitivity to the lethal effect of a high molecular weight which the crystal violet dye was used which can be determined as followed;

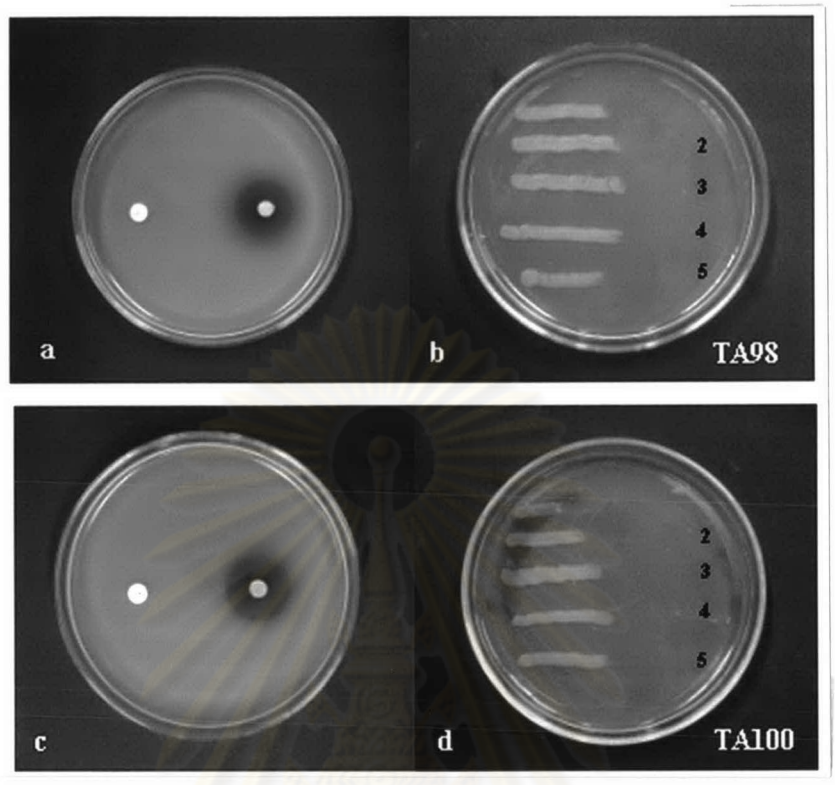

Figure 5.2 Confirmation the genotypes (pKM101^{+}, *rfa*$^{+}$, *uvrB* $^{-}$) of S. Typhimurium strains TA98 and TA100. Single revertant colony from MGA plate with Amp^{r} including histidine and biotin was cultured on MGA plate containing histidine and biotin (a and c) by pour plate technique; and was also streaked on the same kind of plate (b and d). No inhibition zone surrounding Amp^{r} disc (on the left of a and c) was detected. But clear zones surrounding crystal violet disc (on the right of a and c) were observed. No growth of bacteria after exposure to UV light as shown on the right- streaked lines (1-5) of plate b and d was found.

- Add 0.1 ml of fresh overnight culture of each tester strain TA98 and TA100 into a tube containing 2 ml of molten top agar and held at 45 °C and vortexed for 3 sec at low speed and poured on a nutrient agar plate. Rotate the plate to distribute the top agar evenly, place it on a level surface.
- Allow several minutes for the agar to become firm.

- Place a sterile filter paper disc in the center of the streak and apply 10 µl of sterile 0.1 % crystal violet solution.
- Incubate the plate at 37°C for 12 hours.
- All *Salmonella* strain should grow on overlay, except around the disc, and showed a clear zone of inhibition (approximately 14 mm). Therefore, the result indicated the presence of *rfa* mutation as permitting large molecules permeate into the cells, leading to cell death (Figure 5.2).

B.4.4) *uvrB* mutation

Tester strain TA98 and TA100 contain the *uvrB* mutation that make them sensitive to the ultraviolet light which can be determined as follow;

- The cultures were streaked across the nutrient agar plate in the parallel stripe.
- Place a piece of card board over the uncovered plate so that the half of each bacterial streak is covered.
- Plates were irradiation with a 15-Watts germicidal lamp at a distance of 33 cm for 8 sec.
- Incubate the plates at 37°C for 24 hours
- The salmonella strain should be no growth on the uncovered slide of the plate (Figure 5.2)

B.4.5) Spontaneous reversion

The condition of spontaneous reversion of the tester strains to histidine independence as well as in mutagenicity test by using solvent (DMSO) instead of the test chemicals was used. Each tester strain reverts spontaneously at different frequency as shown in Table 5.1, the numbers might slightly be different on plates which could be determined as follow;

- Taking the solvent (DMSO) 0.1 ml to sterile capped culture tubes as placed in ice bath.
- 0.5 ml the S9 mix or $NaPO_4$-KCl buffer pH 7.4; in case of without S9 activation
- 0.1 ml of fresh overnight culture 14-16 hours was added.

- 2 ml of molten top agar was added, vortex the tube gently and pour on GM agar plates.
- Rotate the plates then left it to become harden.
- Incubate at 37°C for 48 hours.
- The histidine revertant colonies of each strain were counted as shown in Figure 5.1

B.4.6) The response to standard carcinogens

The standard carcinogens were used to confirm the sensitivity and specificity of each strain and the efficacy of the S-9 mix. These experiment are B(*a*)P for TA98 and TA100 strains in the presence of S-9 mix. AF_2 for TA98 and TA100 strains in the absence of S9 mix. Testing as well as the method in spontaneous reversion but 0.1 ml of B(*a*)P for TA98 (10 μg/plate) and TA100 (5 μg/plate) in the presence of S9 mix and AF_2 for TA98 (10 μg/plate) and TA100 (5 μg/plate) in the absence of S9 mix are used instead of 0.1 ml of DMSO (Figure 5.3)

C. Preparation of mammalian liver enzymes

C.1) ***Induction of mammalian liver enzyme.***

Liver homogenates from rat was treated with sodium phenobabital and 5,6-benzoflavone. The induction procedure was prepared as described as follows (Massushima *et al.,* 1976);

- A single (i.p) ingection of 10 mg/ml of sodium phenobarbital in saline solution, at a dosage of 30 mg/kg B.W. in the morning of the first day.
- A single (i.p) injection of 20 mg/ml of sodium phenobabital in saline solution, at a dosage of 60 mg/kg B.W. in the morning of the second day.
- A single (i.p) injection of 20 mg/ml of sodium phenobabital in saline solution, at a dosage of 60 mg/kg B.W. in the morning as second day and a single (i.p) injection of 10 mg/ml of 5,6-benzoflavone in the corn oil at dosage of 80 mg/kg B.W. in the afternoon of the third day.

- A single (i.p) injection of 20 mg/ml of sodium phenobabital in saline solution, at a dosage of 60 mg/kg B.W. in the morning of the fourth day as second day, and the animal are given drinking water adlibitum until 12 hours before sacrificed.

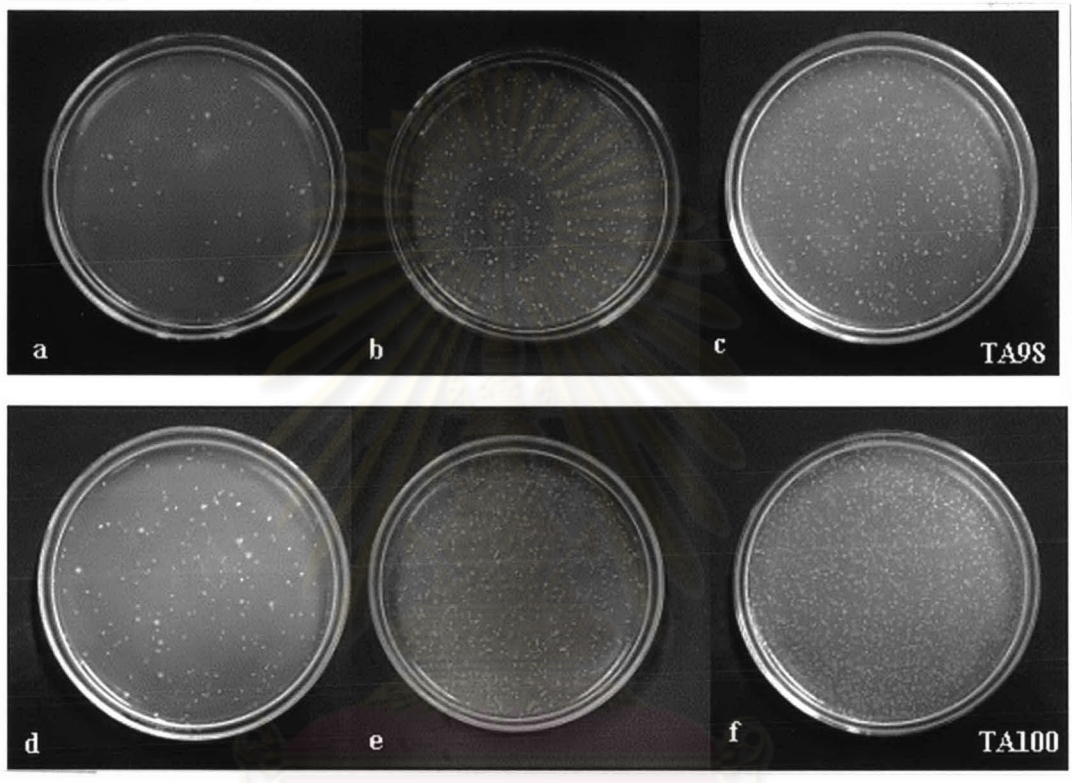

Figure 5.3 The spontaneous revertant colonies on MGA plate (a,d) and on MGA added with standard mutagen AF-2, 0.1 μg/plate (b), 0.01 μg/plate (e); B(*a*)P 10 μg/plate(c), 5 μg/plate (f) of S. Typhimurium strains TA98 and TA100.

C.2) ***Remove of liver***

All surgical instruments, glass and solution were sterilized ones. The rats were killed by cervical dislocation on the fifth day. The livers must be removed with sterile technique, as followed;

- Flooded the for around the abdominal with 70% ethanol and the cut through skin with sterile scissor.
- Swabbed the muscle layer with 70% ethanol and then cut through it with a fresh pair of sterile scissor.

- The livers were removed by cutting through the blood vessels and connective tissue.

C.3) ***Liver homogenate S9 fraction*** (Garder *et al.*,1972)

All steps of the procedure were carried out at 0-4 °C using cold, sterile solutions and glasswares as followed;

- The freshly excised livers were placed in the preweighed beaker containing approximately 1 ml 0f chilled 0.15 M KCl. After weighing, the liver will be washed several times in the fresh chilled KCl.
- Transferred the washed livers to the beaker containing three volume of 0.15 M KCl (3 ml/g of wet liver).
- Chop the livers with a sterile scissor and homogenized in the Potter-Elvehjem apparatus with a teflon pastle.
- The homogenate was centrifuged for 20 minutes at 9,000 x g at 4°C.
- The supernatant (S9 fraction) were decanted immediately and distributed in 1-2 ml portions into cryogenic tube, stored at -80 °C.

The sterility of S9 preparation was determined by plating 0.1 ml on minimal glucose agar plate containing histidine and biotin. There should have no growth of bacteria on the plates.

C.4) ***Determine protein of S9 fraction*** (Lowry *et al.*,1951)

The stock of S9 fraction for Ames assay, must be determined for protein content in S9 prepared from mammalian liver. The protein concentration was approximately 40 mg/ml and cytochrome P450 was 5.63 nmol/mg as efficient for mutagenicity assay.

Reagents for determine protein by Lowry method.

Reagent A

Ingredients	**Per liter**
Distilled water	1000 ml
Na_2CO_3 (Sodium carbonate anhydrous)	20 g
NaOH (Sodium hydroxide)	4 g
$NaKC_4H_4O.4H_20$ (Sodium potassium tartrate)	0.2 g

Reagent B

Ingredients	**Per 100 ml**
Distilled water	100 ml
$CuSO_4.5H_20$ (Copper sulfate pentahygrate)	0.5 g

Reagent C (Phenol reagent)

Ingredients	**Per 100 ml**
Distilled water	50 ml
Folin-Ciocateu's phenol reagent	50 ml

Reagent D

Ingredients	
Reagent A	50 ml
Reagent B	1 ml

- Add sample 1 ml from diluted at 100, 250 and 500 fold of S-9 fraction sample.
- Add reagent D 3.0 ml, mix well and wait 10 minutes before adding 0.3 ml of reagent C.
- After 30 minutes, measured the absorbance at 650 nm and calculated protein content from calibration curve by using bovine serum albumin as standard protein concentrate at 0 to 200 μg/ml (Figure 5.4)

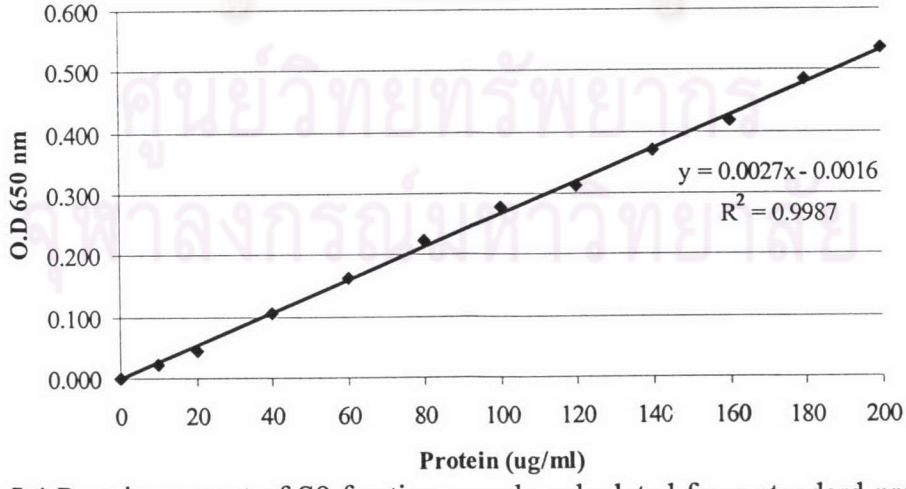

Figure 5.4 Protein content of S9 fraction sample calculated from standard protein curve, the S9 fraction sample contains 41.86 mg/ml protein.

Table 5.2 Mutagenic and 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)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Chiang Rai	Negative	26 ± 0^a	34 ± 1^a	77 ± 2^a	123 ± 6^a	26 ± 0^a	34 ± 1^a	77 ± 2^a	123 ± 6^a
	0.625	30 ± 2^a	38 ± 3^a	83 ± 3^a	108 ± 9^a	381 ± 4^d	504 ± 35^c	354 ± 14^{bc}	534 ± 57^b
	1.25	25 ± 2^a	41 ± 3^a	83 ± 2^a	103 ± 2^a	303 ± 35b^c	409 ± 51^b	301 ± 17^b	513 ± 11^b
	2.50	26 ± 0^a	35 ± 2^a	74 ± 1^a	108 ± 2^a	374 ± 24^c	385 ± 4^b	410 ± 67^c	498 ± 31^b
	Positive	289 ± 25^b	424 ± 16^b	484 ± 9^b	1157 ± 70^b	291 ± 27^b	407 ± 15^b	434 ± 15^c	957 ± 72^c
Chiang Mai	Negative	24 ± 1^a	29 ± 0^a	87 ± 4^a	96 ± 2^a	24 ± 1^a	29 ± 0^a	87 ± 4^a	96 ± 4^a
	0.625	28 ± 1^a	30 ± 2^a	95 ± 3^{ab}	124 ± 13^a	294 ± 10^c	281 ± 24^b	533 ± 32^c	889 ± 32^c
	1.25	30 ± 4^a	34 ± 2^a	95 ± 1^{ab}	122 ± 4^a	293 ± 4^c	352 ± 14^c	476 ± 14^c	775 ± 14^{bc}
	2.50	27 ± 2^a	32 ± 2^a	103 ± 6^b	106 ± 6^a	308 ± 6^c	264 ± 8^b	561 ± 51^c	656 ± 51^b
	Positive	257 ± 3^b	387 ± 48^b	445 ± 3^c	1037 ± 30^b	229 ± 8^b	364 ± 37^c	361 ± 14^b	1039 ± 14^d

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.2 Mutagenic and 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)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mae Hong Son	Negative	29 ± 1^{a}	35 ± 1^{a}	99 ± 3^{a}	114 ± 4^{a}	29 ± 1^{a}	35 ± 1^{a}	99 ± 3^{a}	114 ± 4^{a}
	0.625	31 ± 2^{a}	39 ± 8^{a}	114 ± 8^{a}	123 ± 9^{a}	208 ± 11^{bc}	333 ± 14^{b}	327 ± 10^{c}	787 ± 23^{b}
	1.25	32 ± 4^{a}	50 ± 5^{a}	111 ± 2^{a}	127 ± 19^{a}	207 ± 11^{bc}	319 ± 17^{b}	269 ± 34^{b}	728 ± 32^{b}
	2.50	32 ± 4^{a}	46 ± 1^{a}	112 ± 6^{a}	131 ± 3^{a}	204 ± 9^{b}	318 ± 21^{b}	258 ± 10^{b}	776 ± 12^{b}
	Positive	256 ± 21^{b}	388 ± 27^{b}	331 ± 11^{b}	874 ± 39^{b}	235 ± 5^{c}	337 ± 20^{b}	406 ± 14^{d}	899 ± 20^{c}
Payao	Negative	23 ± 1^{a}	34 ± 4^{a}	89 ± 1^{a}	97 ± 4^{a}	23 ± 1^{a}	34 ± 4^{a}	89 ± 1^{a}	97 ± 4^{a}
	0.625	28 ± 2^{a}	47 ± 4^{ab}	96 ± 2^{a}	107 ± 5^{a}	251 ± 11^{bc}	244 ± 5^{b}	434 ± 23^{cd}	923 ± 84^{c}
	1.25	29 ± 2^{a}	32 ± 5^{a}	90 ± 2^{a}	102 ± 7^{a}	255 ± 27^{bc}	242 ± 14^{b}	355 ± 12^{bc}	521 ± 25^{b}
	2.50	25 ± 1^{a}	55 ± 4^{b}	111 ± 4^{a}	100 ± 9^{a}	219 ± 17^{b}	224 ± 14^{b}	392 ± 22^{b}	578 ± 17^{b}
	Positive	298 ± 6^{b}	317 ± 8^{c}	317 ± 17^{b}	938 ± 45^{b}	279 ± 16^{c}	261 ± 12^{b}	477 ± 32^{d}	963 ± 57^{c}

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 µg/plate or B*(a)*P 10 and 5 µg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.2 Mutagenic and 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)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Nan	Negative	25 ± 0^{a}	34 ± 0^{a}	76 ± 2^{a}	117 ± 5^{a}	25 ± 0^{a}	34 ± 0^{a}	76 ± 2^{a}	117 ± 5^{a}
	0.625	28 ± 1^{a}	33 ± 2^{a}	86 ± 7^{a}	112 ± 5^{a}	293 ± 7^{c}	341 ± 28^{c}	349 ± 11^{b}	901 ± 60^{b}
	1.25	28 ± 0^{a}	37 ± 1^{a}	93 ± 2^{a}	108 ± 2^{a}	225 ± 15^{b}	259 ± 18^{b}	340 ± 15^{b}	826 ± 61^{b}
	2.50	37 ± 3^{a}	37 ± 1^{a}	89 ± 7^{a}	115 ± 3^{a}	299 ± 11^{c}	305 ± 20^{bc}	351 ± 10^{b}	860 ± 46^{b}
	Positive	289 ± 10^{b}	362 ± 2^{b}	386 ± 11^{b}	904 ± 38^{b}	301 ± 9^{c}	405 ± 14^{d}	434 ± 13^{c}	822 ± 24^{b}
Lumpang	Negative	36 ± 4^{a}	57 ± 2^{a}	101 ± 14^{a}	120 ± 6^{a}	36 ± 4^{a}	57 ± 2^{a}	101 ± 14^{a}	120 ± 6^{a}
	0.625	28 ± 1^{a}	43 ± 5^{a}	91 ± 1^{a}	124 ± 3^{a}	244 ± 11^{c}	304 ± 45^{b}	281 ± 16^{b}	1053 ± 68^{b}
	1.25	30 ± 1^{a}	43 ± 5^{a}	85 ± 8^{a}	80 ± 5^{a}	236 ± 4^{bc}	296 ± 70^{b}	293 ± 8^{b}	1053 ± 88^{b}
	2.50	28 ± 1^{a}	61 ± 8^{a}	89 ± 6^{a}	94 ± 8^{a}	211 ± 12^{b}	403 ± 24^{b}	319 ± 13^{b}	900 ± 36^{b}
	Positive	241 ± 10^{b}	328 ± 12^{b}	415 ± 40^{b}	1424 ± 85^{b}	245 ± 7^{c}	330 ± 12^{b}	381 ± 27^{c}	1257 ± 47^{c}

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.2 Mutagenic and 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)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Phare	Negative	26 ± 0^{a}	27 ± 1^{a}	80 ± 1^{a}	117 ± 2^{a}	26 ± 0^{a}	27 ± 1^{a}	80 ± 1^{a}	117 ± 2^{a}
	0.625	23 ± 2^{a}	30 ± 3^{a}	79 ± 10^{a}	129 ± 2^{a}	289 ± 7^{b}	358 ± 16^{cd}	472 ± 5^{c}	889 ± 22^{d}
	1.25	28 ± 2^{a}	28 ± 0^{a}	79 ± 9^{a}	128 ± 6^{a}	278 ± 16^{b}	311 ± 2^{b}	472 ± 23^{c}	765 ± 41^{c}
	2.50	28 ± 1^{a}	33 ± 4^{a}	91 ± 10^{a}	113 ± 7^{a}	264 ± 5^{b}	316 ± 23^{bc}	414 ± 11^{b}	420 ± 11^{b}
	Positive	276 ± 18^{b}	386 ± 19^{b}	419 ± 25^{b}	811 ± 26^{b}	269 ± 26^{b}	377 ± 7^{d}	443 ± 25^{bc}	821 ± 14^{cd}
Lumphun	Negative	26 ± 2^{a}	36 ± 2^{a}	113 ± 6^{a}	121 ± 3^{a}	26 ± 2^{a}	36 ± 2^{a}	113 ± 6^{a}	121 ± 3^{a}
	0.625	27 ± 1^{a}	35 ± 0^{a}	107 ± 8^{a}	122 ± 8^{a}	209 ± 13^{b}	319 ± 30^{b}	414 ± 14^{b}	1099 ± 32^{c}
	1.25	27 ± 1^{a}	33 ± 2^{a}	114 ± 7^{a}	127 ± 10^{a}	214 ± 10^{b}	345 ± 22^{b}	404 ± 6^{bc}	961 ± 16^{b}
	2.50	30 ± 2^{a}	29 ± 1^{a}	113 ± 4^{a}	117 ± 4^{a}	249 ± 5^{c}	299 ± 30^{b}	386 ± 14^{bc}	947 ± 62^{b}
	Positive	220 ± 10^{b}	322 ± 14^{b}	403 ± 12^{b}	1046 ± 38^{b}	256 ± 7^{c}	332 ± 8^{b}	436 ± 9^{c}	1211 ± 57^{c}

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.2 Mutagenic and 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)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Uttraradith	Negative	28 ± 4^{a}	40 ± 3^{a}	96 ± 1^{a}	102 ± 8^{a}	28 ± 4^{a}	40 ± 3^{a}	96 ± 1^{a}	102 ± 8^{a}
	0.625	28 ± 0^{a}	40 ± 4^{a}	112 ± 9^{a}	101 ± 5^{a}	172 ± 10^{b}	316 ± 26^{c}	312 ± 24^{c}	792 ± 51^{b}
	1.25	27 ± 2^{a}	41 ± 3^{a}	107 ± 5^{a}	128 ± 17^{a}	179 ± 3^{b}	288 ± 13^{c}	308 ± 30^{c}	780 ± 18^{b}
	2.50	26 ± 1^{a}	41 ± 4^{a}	113 ± 10^{a}	139 ± 8^{a}	205 ± 6^{c}	229 ± 9^{b}	221 ± 1^{b}	764 ± 57^{b}
	Positive	226 ± 13^{b}	311 ± 7^{b}	405 ± 27^{b}	854 ± 39^{b}	234 ± 7^{d}	294 ± 8^{c}	388 ± 14^{d}	759 ± 24^{b}
Sukhothai	Negative	30 ± 2^{a}	37 ± 1^{a}	111 ± 3^{a}	131 ± 1^{a}	30 ± 2^{a}	37 ± 1^{a}	111 ± 3^{a}	131 ± 1^{a}
	0.625	29 ± 2^{a}	39 ± 3^{a}	105 ± 7^{a}	131 ± 4^{a}	215 ± 16^{c}	400 ± 12^{d}	318 ± 8^{c}	936 ± 33^{c}
	1.25	26 ± 3^{a}	33 ± 3^{a}	118 ± 2^{a}	133 ± 5^{a}	181 ± 3^{b}	369 ± 11^{cd}	302 ± 11^{bc}	841 ± 57^{c}
	2.50	26 ± 2^{a}	40 ± 3^{a}	97 ± 12^{a}	135 ± 1^{a}	310 ± 1^{e}	335 ± 6^{bc}	268 ± 15^{b}	562 ± 31^{b}
	Positive	284 ± 14^{b}	335 ± 6^{b}	402 ± 33^{b}	929 ± 11^{b}	270 ± 4^{d}	317 ± 16^{b}	403 ± 16^{d}	1285 ± 111^{d}

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.2 Mutagenic and 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)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Phitsanu lok	Negative	32 ± 1^{a}	42 ± 5^{a}	112 ± 3^{a}	118 ± 5^{a}	32 ± 1^{a}	42 ± 5^{a}	112 ± 3^{a}	118 ± 5^{a}
	0.625	37 ± 3^{a}	34 ± 1^{a}	124 ± 5^{a}	128 ± 10^{a}	223 ± 6^{b}	337 ± 32^{b}	307 ± 7^{c}	896 ± 22^{bc}
	1.25	29 ± 0^{a}	39 ± 5^{a}	121 ± 12^{a}	113 ± 1^{a}	217 ± 7^{b}	405 ± 17^{c}	223 ± 9^{b}	1040 ± 137^{c}
	2.50	30 ± 1^{a}	38 ± 4^{a}	124 ± 4^{a}	126 ± 4^{a}	241 ± 18^{b}	342 ± 10^{b}	198 ± 13^{b}	677 ± 98^{b}
	Positive	284 ± 14^{b}	309 ± 4^{b}	337 ± 13^{b}	1168 ± 114^{b}	233 ± 16^{b}	343 ± 11^{b}	322 ± 3^{c}	1034 ± 32^{c}
Phetcha bun	Negative	23 ± 0^{a}	44 ± 1^{a}	96 ± 2^{a}	134 ± 4^{a}	23 ± 0^{a}	44 ± 1^{a}	96 ± 2^{a}	134 ± 4^{a}
	0.625	27 ± 1^{a}	40 ± 4^{a}	109 ± 8^{a}	155 ± 1^{a}	255 ± 13^{d}	233 ± 29^{b}	273 ± 25^{b}	784 ± 46^{c}
	1.25	24 ± 0^{a}	41 ± 1^{a}	94 ± 2^{a}	131 ± 4^{a}	238 ± 8^{cd}	273 ± 9^{b}	260 ± 3^{b}	650 ± 27^{b}
	2.50	31 ± 2^{a}	62 ± 3^{a}	113 ± 4^{a}	145 ± 3^{a}	187 ± 6^{b}	260 ± 12^{b}	283 ± 8^{bc}	585 ± 22^{b}
	Positive	234 ± 6^{b}	311 ± 18^{b}	331 ± 18^{b}	990 ± 69^{b}	225 ± 3^{c}	329 ± 15^{c}	$318 \pm {}^{7c}$	893 ± 17^{d}

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.2 Mutagenic and 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)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Kam phaeng Phet	Negative	27 ± 2^a	35 ± 2^a	99 ± 5^a	116 ± 5^a	27 ± 2^a	35 ± 2^a	99 ± 5^a	116 ± 5^a
	0.625	28 ± 4^a	28 ± 1^a	98 ± 3^a	118 ± 4^a	216 ± 12^{bc}	310 ± 17^b	350 ± 11^b	1028 ± 2^d
	1.25	27 ± 4^a	34 ± 1^a	91 ± 4^a	117 ± 4^a	198 ± 8^b	261 ± 16^b	377 ± 17^{bc}	857 ± 24^b
	2.50	30 ± 4^a	32 ± 2^a	100 ± 1^a	118 ± 2^a	255 ± 2^d	275 ± 19^b	422 ± 17^{cd}	946 ± 40^c
	Positive	253 ± 14^b	313 ± 5^b	429 ± 8^b	951 ± 40^b	221 ± 4^c	282 ± 14^b	459 ± 23^d	914 ± 24^{bc}
Nakhon Sawan	Negative	28 ± 1^a	33 ± 1^a	88 ± 2^a	122 ± 4^a	28 ± 1^a	33 ± 1^a	88 ± 2^a	122 ± 4^a
	0.625	28 ± 1^a	31 ± 2^a	102 ± 7^a	122 ± 2^a	223 ± 6^b	247 ± 18^b	309 ± 22^b	1040 ± 33^b
	1.25	23 ± 1^a	32 ± 3^a	90 ± 3^a	116 ± 4^a	228 ± 7^b	235 ± 6^b	274 ± 24^b	1129 ± 24^c
	2.50	25 ± 1^a	30 ± 2^a	93 ± 11^a	125 ± 7^a	218 ± 10^b	220 ± 7^b	369 ± 9^c	1002 ± 15^b
	Positive	241 ± 4^b	324 ± 16^b	411 ± 23^b	1212 ± 11^b	212 ± 4^b	288 ± 5^c	434 ± 13^d	1284 ± 14^d

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.2 Mutagenic and 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)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Uthai Thani	Negative	29 ± 2^a	38 ± 2^a	110 ± 7^a	113 ± 5^a	29 ± 2^a	38 ± 2^a	110 ± 7^a	113 ± 5^a
	0.625	29 ± 2^a	38 ± 2^a	115 ± 4^a	135 ± 6^a	399 ± 13^c	395 ± 54^c	513 ± 32^c	759 ± 9^c
	1.25	24 ± 2^a	41 ± 3^a	115 ± 5^a	120 ± 8^a	284 ± 2^b	268 ± 52^b	419 ± 8^b	595 ± 63^b
	2.50	24 ± 1^a	40 ± 2^a	118 ± 4^a	121 ± 3^a	272 ± 16^b	244 ± 0^b	377 ± 7^b	600 ± 25^b
	Positive	227 ± 7^b	341 ± 33^b	478 ± 12^b	827 ± 38^b	258 ± 16^b	341 ± 9^{bc}	512 ± 39^c	757 ± 21^c
Saraburi	Negative	24 ± 1^a	28 ± 2^a	84 ± 6^a	121 ± 3^a	24 ± 1^a	28 ± 2^a	84 ± 6^a	121 ± 3^a
	0.625	27 ± 1^a	34 ± 2^a	93 ± 1^a	115 ± 2^a	213 ± 8^b	314 ± 8^b	276 ± 3^d	774 ± 15^c
	1.25	28 ± 2^a	29 ± 2^a	88 ± 6^a	107 ± 6^a	200 ± 9^b	309 ± 25^b	232 ± 20^c	525 ± 21^b
	2.50	26 ± 2^a	30 ± 2^a	87 ± 2^a	124 ± 3^a	214 ± 4^b	311 ± 26^b	196 ± 3^b	724 ± 32^c
	Positive	298 ± 12^b	299 ± 23^b	323 ± 17^b	868 ± 13^b	216 ± 5^b	292 ± 21^b	318 ± 6^e	909 ± 31^d

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.2 Mutagenic and 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)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Lop Buri	Negative	26 ± 1^{a}	41 ± 2^{a}	132 ± 6^{b}	124 ± 4^{a}	26 ± 1^{a}	41 ± 2^{a}	132 ± 6^{a}	124 ± 4^{a}
	0.625	31 ± 0^{a}	39 ± 3^{a}	99 ± 7^{a}	138 ± 3^{ab}	212 ± 1^{c}	322 ± 23^{c}	261 ± 12^{b}	1054 ± 18^{d}
	1.25	31 ± 0^{a}	60 ± 1^{b}	98 ± 5^{a}	166 ± 16^{b}	212 ± 1^{c}	261 ± 8^{b}	261 ± 4^{b}	910 ± 36^{c}
	2.50	41 ± 3^{a}	51 ± 6^{ab}	96 ± 8^{a}	141 ± 11^{ab}	193 ± 5^{b}	303 ± 12^{bc}	276 ± 34^{b}	808 ± 35^{b}
	Positive	264 ± 15^{b}	320 ± 5^{c}	347 ± 12^{c}	1084 ± 15^{c}	208 ± 1^{c}	336 ± 9^{c}	349 ± 16^{c}	1092 ± 18^{d}
Phrachin Buri	Negative	27 ± 2^{a}	45 ± 1^{a}	101 ± 5^{a}	119 ± 1^{a}	27 ± 2^{a}	45 ± 1^{a}	101 ± 5^{a}	119 ± 34^{a}
	0.625	28 ± 2^{a}	44 ± 3^{a}	102 ± 5^{a}	122 ± 2^{a}	335 ± 16^{c}	407 ± 26^{c}	310 ± 10^{c}	704 ± 47^{c}
	1.25	28 ± 0^{a}	41 ± 4^{a}	100 ± 5^{a}	117 ± 0^{a}	344 ± 26^{c}	338 ± 15^{b}	281 ± 9^{b}	508 ± 35^{b}
	2.50	27 ± 3^{a}	39 ± 2^{a}	100 ± 4^{a}	118 ± 10^{a}	$303 \pm 3b^{c}$	349 ± 18^{b}	281 ± 3^{b}	404 ± 76^{b}
	Positive	269 ± 25^{b}	307 ± 1^{b}	363 ± 18^{b}	902 ± 45^{b}	257 ± 31^{b}	311 ± 13^{b}	307 ± 5^{c}	912 ± 73^{d}

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.2 Mutagenic and 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)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Ratchaburi	Negative	26 ± 1^{a}	36 ± 1^{a}	108 ± 0^{a}	115 ± 5^{a}	26 ± 1^{a}	36 ± 1^{a}	108 ± 0^{a}	115 ± 5^{a}
	0.625	24 ± 2^{a}	37 ± 5^{a}	112 ± 8^{a}	113 ± 10^{a}	231 ± 8^{bc}	357 ± 5^{cd}	367 ± 6^{b}	1134 ± 99^{c}
	1.25	27 ± 2^{a}	35 ± 2^{a}	111 ± 8^{a}	133 ± 3^{a}	243 ± 4^{bc}	325 ± 19^{bc}	372 ± 10^{b}	859 ± 30^{b}
	2.50	22 ± 1^{a}	38 ± 5^{a}	115 ± 10^{a}	125 ± 2^{a}	216 ± 10^{b}	311 ± 13^{b}	373 ± 18^{b}	800 ± 62^{b}
	Positive	262 ± 13^{b}	340 ± 15^{b}	342 ± 5^{b}	1353 ± 118^{b}	250 ± 14^{c}	365 ± 8^{d}	382 ± 27^{b}	1190 ± 75^{c}
Phetchaburi	Negative	28 ± 2^{a}	44 ± 4^{a}	104 ± 1^{a}	124 ± 3^{a}	28 ± 2^{a}	44 ± 4^{a}	104 ± 1^{a}	124 ± 3^{a}
	0.625	26 ± 3^{a}	42 ± 1^{a}	108 ± 7^{a}	123 ± 4^{a}	220 ± 6^{b}	334 ± 17^{cd}	331 ± 7^{c}	882 ± 31^{d}
	1.25	25 ± 1^{a}	45 ± 2^{a}	117 ± 4^{a}	115 ± 6^{a}	217 ± 10^{b}	320 ± 9^{c}	299 ± 0^{b}	724 ± 26^{b}
	2.50	26 ± 4^{a}	47 ± 0^{a}	124 ± 0^{a}	121 ± 9^{a}	214 ± 14^{b}	265 ± 10^{b}	329 ± 12^{c}	792 ± 11^{bc}
	Positive	267 ± 15^{b}	358 ± 16^{b}	363 ± 10^{b}	992 ± 22^{b}	255 ± 5^{c}	369 ± 10^{d}	412 ± 6^{d}	862 ± 30^{cd}

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 trail.

DMSO was negative control, AF_2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF_2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.2 Mutagenic and 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)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Prachuap	Negative	24 ± 1^a	38 ± 1^a	112 ± 3^a	133 ± 12^a	24 ± 1^a	38 ± 1^a	112 ± 3^a	133 ± 12^a
Kiri Khun	0.625	23 ± 2^a	48 ± 6^a	112 ± 7^a	132 ± 4^a	312 ± 6^c	275 ± 13^b	472 ± 10^d	1089 ± 29^c
	1.25	23 ± 1^a	38 ± 1^a	114 ± 6^a	128 ± 3^a	246 ± 8^b	265 ± 13^b	288 ± 34^b	1044 ± 16^c
	2.50	23 ± 1^a	37 ± 3^a	111 ± 4^a	120 ± 9^a	248 ± 28^b	281 ± 15^{bc}	515 ± 40^d	868 ± 18^b
	Positive	271 ± 4^b	315 ± 17^b	350 ± 17^b	1274 ± 38^b	255 ± 9^b	316 ± 3^c	382 ± 19^c	1297 ± 45^d
Chumphon	Negative	21 ± 2^a	29 ± 1^a	73 ± 1^a	106 ± 2^a	21 ± 2^a	29 ± 1^a	73 ± 1^a	106 ± 2^a
	0.625	24 ± 0^a	32 ± 1^a	74 ± 1^a	107 ± 3^a	270 ± 10^{bc}	324 ± 15^{bc}	404 ± 17^b	858 ± 23^b
	1.25	28 ± 1^a	35 ± 2^a	80 ± 3^a	106 ± 5^a	289 ± 28^c	280 ± 20^b	419 ± 28^b	829 ± 72^b
	2.50	28 ± 2^a	32 ± 1^a	82 ± 4^a	107 ± 5^a	292 ± 7^c	370 ± 17^c	421 ± 40^b	822 ± 36^b
	Positive	236 ± 20^b	328 ± 10^b	349 ± 16^b	873 ± 37^b	232 ± 18^b	339 ± 16^c	465 ± 30^b	834 ± 60^b

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.2 Mutagenic and 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)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Sakon	Negative	23 ± 1^a	51 ± 8^a	86 ± 4^a	119 ± 4^a	23 ± 1^a	51 ± 8^a	86 ± 4^a	119 ± 4^a
Nakhon	0.625	27 ± 2^a	37 ± 3^a	82 ± 4^a	114 ± 5^a	266 ± 18^b	375 ± 9^b	372 ± 8^b	776 ± 20b^c
	1.25	27 ± 2^a	46 ± 4^a	81 ± 4^a	108 ± 6^a	251 ± 13^b	366 ± 14^b	360 ± 8^b	696 ± 25^b
	2.50	28 ± 1^a	39 ± 4^a	90 ± 1^a	111 ± 6^a	273 ± 16^b	357 ± 3^b	388 ± 13^b	704 ± 52^b
	Positive	253 ± 12^b	339 ± 5^b	443 ± 5^b	910 ± 25^b	271 ± 15^b	359 ± 12^b	442 ± 7^c	900 ± 76^c
Nong Bua	Negative	28 ± 2^a	39 ± 0^a	102 ± 2^a	123 ± 1^a	28 ± 2^a	39 ± 0^a	102 ± 2^a	123 ± 1^a
Lam Phu	0.625	28 ± 3^a	38 ± 3^a	106 ± 3^a	133 ± 2^a	279 ± 19^b	356 ± 20^b	415 ± 7^c	892 ± 5^d
	1.25	37 ± 3^a	46 ± 2^a	111 ± 5^a	121 ± 4^a	267 ± 19^b	330 ± 15^b	283 ± 14^b	758 ± 23^c
	2.50	32 ± 1^a	39 ± 2^a	104 ± 5^a	137 ± 2^a	263 ± 19^b	341 ± 28^b	359 ± 23^c	668 ± 21^b
	Positive	257 ± 10^b	349 ± 18^b	510 ± 25^b	923 ± 46^b	249 ± 17^b	332 ± 9^b	549 ± 29^d	927 ± 14^d

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.2 Mutagenic and 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)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Chaiya	Negative	26 ± 1^{a}	34 ± 1^{a}	92 ± 3^{a}	119 ± 0^{a}	26 ± 1^{a}	34 ± 1^{a}	92 ± 3^{a}	119 ± 0^{a}
phum	0.625	28 ± 0^{a}	32 ± 1^{a}	88 ± 3^{a}	116 ± 2^{a}	334 ± 35^{bc}	305 ± 22^{b}	480 ± 14^{c}	904 ± 18^{c}
	1.25	29 ± 2^{a}	32 ± 1^{a}	89 ± 4^{a}	128 ± 4^{a}	359 ± 8^{c}	336 ± 24^{b}	428 ± 27^{bc}	836 ± 30^{c}
	2.50	30 ± 1^{a}	32 ± 1^{a}	93 ± 1^{a}	120 ± 4^{a}	335 ± 10^{bc}	352 ± 16^{b}	387 ± 18^{b}	622 ± 25^{b}
	Positive	292 ± 7^{b}	350 ± 13^{b}	424 ± 17^{b}	940 ± 22^{b}	280 ± 12^{b}	345 ± 6^{b}	446 ± 16^{bc}	856 ± 67^{c}
Nakhon	Negative	27 ± 1^{a}	42 ± 1^{ab}	90 ± 2^{a}	103 ± 13^{a}	27 ± 1^{a}	42 ± 1^{a}	90 ± 2^{a}	103 ± 13^{a}
Ratchasima	0.625	24 ± 1^{a}	45 ± 2^{ab}	87 ± 2^{a}	131 ± 2^{a}	284 ± 21^{b}	343 ± 6^{b}	411 ± 15^{bc}	$980 \pm 24b^{c}$
	1.25	27 ± 2^{a}	40 ± 1^{a}	93 ± 9^{a}	111 ± 1^{a}	296 ± 17^{b}	355 ± 10^{b}	368 ± 25^{bc}	1062 ± 61^{c}
	2.50	25 ± 2^{a}	57 ± 2^{b}	92 ± 3^{a}	126 ± 2^{a}	284 ± 2^{b}	356 ± 42^{b}	349 ± 17^{b}	924 ± 20^{b}
	Positive	270 ± 4^{b}	333 ± 10^{c}	449 ± 15^{b}	1258 ± 87^{b}	285 ± 8^{b}	372 ± 6^{b}	434 ± 29^{c}	1217 ± 21^{d}

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.2 Mutagenic and 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)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Tak	Negative	24 ± 2^{a}	45 ± 1^{a}	74 ± 8^{a}	127 ± 2^{a}	24 ± 2^{a}	45 ± 1^{a}	74 ± 8^{a}	127 ± 2^{a}
	0.625	22 ± 1^{a}	42 ± 2^{a}	70 ± 3^{a}	132 ± 4^{a}	253 ± 2^{b}	267 ± 26^{bc}	362 ± 12^{b}	561 ± 29^{c}
	1.25	24 ± 1^{a}	50 ± 4^{a}	70 ± 0^{a}	127 ± 2^{a}	300 ± 5^{c}	307 ± 9^{c}	324 ± 9^{b}	478 ± 31^{b}
	2.50	27 ± 2^{a}	43 ± 1^{a}	75 ± 2^{a}	123 ± 5^{a}	267 ± 12^{b}	308 ± 4^{c}	353 ± 18^{b}	465 ± 23^{b}
	Positive	273 ± 13^{b}	304 ± 12^{b}	328 ± 19^{b}	916 ± 38^{b}	262 ± 2^{b}	263 ± 1^{b}	334 ± 5^{b}	891 ± 31^{d}
Kanchana buri	Negative	22 ± 2^{a}	26 ± 2^{a}	89 ± 1^{a}	109 ± 2^{a}	22 ± 2^{a}	26 ± 2^{a}	89 ± 1^{a}	109 ± 2^{a}
	0.625	24 ± 1^{a}	27 ± 1^{a}	79 ± 2^{a}	121 ± 1^{a}	251 ± 2^{b}	323 ± 23^{b}	297 ± 12^{b}	831 ± 15^{cd}
	1.25	20 ± 1^{a}	25 ± 2^{a}	81 ± 3^{a}	120 ± 3^{a}	243 ± 3^{b}	327 ± 16^{b}	296 ± 16^{b}	771 ± 6^{c}
	2.50	18 ± 1^{a}	31 ± 0^{a}	80 ± 5^{a}	119 ± 5^{a}	279 ± 9^{c}	308 ± 7^{b}	279 ± 9^{b}	655 ± 5^{b}
	Positive	232 ± 11^{b}	277 ± 22^{b}	315 ± 13^{b}	920 ± 55^{b}	$256 \pm 12b^{c}$	296 ± 6^{b}	363 ± 17^{c}	887 ± 55^{d}

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.3 Mutagenic and 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)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Chiang Rai	Negative	27 ± 0^{a}	28 ± 0^{a}	101 ± 3^{a}	138 ± 5^{a}	27 ± 0^{a}	28 ± 0^{a}	101 ± 3^{a}	138 ± 5^{a}
	0.625	25 ± 1^{a}	34 ± 2^{a}	133 ± 5^{c}	128 ± 7^{a}	278 ± 24^{c}	258 ± 13^{c}	448 ± 17^{c}	1180 ± 12^{e}
	1.25	30 ± 3^{a}	35 ± 0^{a}	114 ± 1^{ab}	129 ± 4^{a}	273 ± 25^{c}	253 ± 15^{c}	395 ± 27^{bc}	766 ± 20^{c}
	2.50	30 ± 1^{a}	30 ± 2^{a}	121 ± 7^{bc}	139 ± 1^{a}	189 ± 11^{b}	134 ± 8^{b}	361 ± 24^{b}	650 ± 13^{b}
	Positive	245 ± 23^{b}	320 ± 21^{b}	320 ± 8^{d}	856 ± 74^{b}	265 ± 15^{c}	333 ± 15^{d}	372 ± 4^{b}	888 ± 44^{d}
Chiang Mai	Negative	25 ± 1^{a}	31 ± 2^{a}	97 ± 10^{a}	109 ± 5^{a}	25 ± 1^{a}	31 ± 2^{a}	97 ± 10^{a}	109 ± 5^{a}
	0.625	32 ± 3^{a}	38 ± 2^{a}	103 ± 5^{a}	104 ± 17^{a}	232 ± 28^{c}	303 ± 16^{bc}	400 ± 48^{b}	756 ± 46^{c}
	1.25	26 ± 1^{a}	34 ± 0^{a}	95 ± 5^{a}	123 ± 13^{a}	224 ± 20^{c}	287 ± 7^{b}	429 ± 32^{b}	726 ± 48^{bc}
	2.50	31 ± 1^{a}	34 ± 1^{a}	84 ± 1^{a}	106 ± 13^{a}	129 ± 4^{b}	302 ± 29^{bc}	364 ± 68^{b}	626 ± 15^{b}
	Positive	286 ± 26^{b}	346 ± 8^{b}	390 ± 22^{b}	1006 ± 21^{b}	262 ± 15^{c}	341 ± 7^{c}	393 ± 43^{b}	1040 ± 22^{d}

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.3 Mutagenic and 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. (continued)

Province	Dose (mg/plate)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mae Hong Son	Negative	32 ± 4^{a}	26 ± 2^{a}	122 ± 3^{a}	115 ± 1^{a}	32 ± 4^{a}	26 ± 2^{a}	122 ± 3^{a}	115 ± 1^{a}
	0.625	34 ± 1^{a}	31 ± 4^{a}	96 ± 2^{a}	106 ± 4^{a}	314 ± 13^{cd}	303 ± 4^{c}	441 ± 35^{b}	848 ± 12^{c}
	1.25	24 ± 1^{a}	47 ± 5^{a}	106 ± 8^{a}	121 ± 5^{a}	212 ± 47^{b}	308 ± 11^{c}	432 ± 19^{b}	740 ± 11^{b}
	2.50	27 ± 3^{a}	47 ± 14^{a}	123 ± 15^{a}	130 ± 5^{a}	339 ± 20^{d}	245 ± 25^{b}	427 ± 34^{b}	766 ± 24^{b}
	Positive	242 ± 7^{b}	339 ± 22^{b}	390 ± 13^{b}	790 ± 95^{b}	244 ± 18^{bc}	319 ± 27^{c}	413 ± 2^{b}	892 ± 26^{c}
Lampang	Negative	25 ± 2^{a}	44 ± 5^{a}	110 ± 4^{a}	134 ± 14^{a}	25 ± 2^{a}	44 ± 5^{a}	110 ± 4^{a}	134 ± 14^{a}
	0.625	25 ± 2^{a}	39 ± 1^{a}	125 ± 10^{a}	180 ± 9^{a}	300 ± 12^{cd}	320 ± 29^{b}	412 ± 23^{b}	1060 ± 12^{c}
	1.25	22 ± 0^{a}	43 ± 4^{a}	117 ± 6^{a}	151 ± 9^{a}	334 ± 22^{d}	302 ± 28^{b}	417 ± 7^{b}	1094 ± 116^{c}
	2.50	22 ± 0^{a}	42 ± 2^{a}	129 ± 3^{a}	152 ± 15^{a}	263 ± 8^{bc}	265 ± 29^{b}	433 ± 2^{b}	780 ± 27^{b}
	Positive	280 ± 12^{b}	319 ± 17^{b}	374 ± 10^{b}	1230 ± 41^{b}	251 ± 11^{b}	342 ± 23^{b}	405 ± 2^{b}	1153 ± 25^{c}

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.3 Mutagenic and 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. (continued)

Province	Dose (mg/plate)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Uttraradith	Negative	26 ± 1^{a}	26 ± 2^{a}	80 ± 4^{a}	108 ± 8^{a}	26 ± 1^{a}	26 ± 2^{a}	80 ± 4^{a}	108 ± 8^{a}
	0.625	23 ± 1^{a}	24 ± 1^{a}	81 ± 5^{a}	102 ± 4^{a}	255 ± 7^{cd}	279 ± 31^{bc}	486 ± 47^{b}	1001 ± 40^{b}
	1.25	21 ± 1^{a}	27 ± 1^{a}	72 ± 1^{a}	123 ± 8^{a}	233 ± 10^{bc}	304 ± 7^{cd}	519 ± 36^{b}	981 ± 84^{b}
	2.50	22 ± 2^{a}	25 ± 2^{a}	98 ± 1^{a}	113 ± 15^{a}	277 ± 6^{d}	251 ± 6^{b}	434 ± 25^{b}	922 ± 23^{b}
	Positive	251 ± 26^{b}	390 ± 3^{b}	424 ± 13^{b}	949 ± 13^{b}	218 ± 8^{b}	336 ± 29^{d}	478 ± 6^{b}	1019 ± 7^{b}
Phitsanulok	Negative	34 ± 2^{a}	43 ± 6^{a}	112 ± 3^{a}	136 ± 10^{a}	34 ± 24^{a}	43 ± 6^{a}	112 ± 3^{a}	136 ± 10^{a}
	0.625	32 ± 1^{a}	46 ± 2^{a}	146 ± 8^{a}	121 ± 16^{a}	236 ± 40^{b}	254 ± 30^{c}	449 ± 22^{b}	433 ± 24^{b}
	1.25	34 ± 3^{a}	47 ± 11^{a}	136 ± 4^{a}	149 ± 10^{a}	353 ± 26^{c}	215 ± 24^{bc}	429 ± 11^{b}	506 ± 86^{b}
	2.50	23 ± 0^{a}	48 ± 2^{a}	168 ± 19^{a}	165 ± 9^{a}	342 ± 20^{c}	160 ± 20^{b}	428 ± 8^{b}	529 ± 74^{b}
	Positive	223 ± 20^{b}	363 ± 11^{b}	380 ± 23^{b}	916 ± 66^{b}	258 ± 32^{b}	339 ± 13^{d}	451 ± 7^{b}	903 ± 112^{c}

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 µg/plate or B*(a)*P 10 and 5 µg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.3 Mutagenic and 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. (continued)

Province	Dose (mg/plate)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Phetchabun	Negative	36 ± 4^{a}	44 ± 9^{a}	121 ± 15^{a}	124 ± 4^{a}	36 ± 4^{a}	44 ± 9^{a}	121 ± 15^{a}	124 ± 4^{a}
	0.625	33 ± 2^{a}	49 ± 2^{b}	111 ± 5^{a}	127 ± 2^{a}	295 ± 14^{c}	205 ± 19^{c}	449 ± 14^{b}	1002 ± 4^{c}
	1.25	43 ± 3^{a}	77 ± 9^{c}	101 ± 8^{a}	158 ± 6^{a}	225 ± 13^{b}	139 ± 18^{b}	379 ± 10^{b}	914 ± 33^{bc}
	2.50	39 ± 1^{a}	38 ± 3^{b}	120 ± 4^{a}	187 ± 6^{a}	339 ± 19^{d}	61 ± 8^{a}	426 ± 6^{b}	740 ± 124^{b}
	Positive	246 ± 8^{b}	350 ± 8^{d}	447 ± 17^{b}	1046 ± 58^{b}	223 ± 2^{b}	338 ± 9^{d}	447 ± 49^{b}	920 ± 93^{bc}
Nakhon	Negative	28 ± 3^{a}	31 ± 4^{a}	95 ± 0^{a}	112 ± 12^{a}	28 ± 3^{a}	31 ± 4^{a}	95 ± 16^{a}	112 ± 12^{a}
Sawan	0.625	31 ± 6^{a}	28 ± 2^{a}	99 ± 7^{a}	143 ± 7^{a}	358 ± 13^{b}	327 ± 44^{b}	446 ± 41^{d}	1072 ± 65^{bc}
	1.25	31 ± 2^{a}	25 ± 2^{a}	143 ± 5^{a}	130 ± 11^{a}	281 ± 47^{b}	313 ± 5^{b}	287 ± 42^{b}	860 ± 80^{b}
	2.50	30 ± 1^{a}	29 ± 3^{a}	112 ± 7^{a}	131 ± 9^{a}	309 ± 11^{b}	328 ± 60^{b}	354 ± 1^{bc}	1076 ± 59^{bc}
	Positive	245 ± 17^{b}	323 ± 20^{b}	462 ± 37^{b}	1156 ± 35^{b}	295 ± 7^{b}	348 ± 24^{b}	421 ± 35^{cd}	1236 ± 205^{c}

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.3 Mutagenic and 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. (continued)

Province	Dose (mg/plate)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Saraburi	Negative	34 ± 2^{a}	29 ± 2^{a}	98 ± 4^{a}	97 ± 5^{ab}	34 ± 2^{a}	29 ± 2^{a}	98 ± 4^{a}	97 ± 5^{a}
	0.625	27 ± 2^{a}	29 ± 4^{a}	92 ± 3^{a}	85 ± 1^{a}	246 ± 15^{b}	322 ± 17^{b}	455 ± 7^{c}	792 ± 18^{c}
	1.25	25 ± 2^{a}	32 ± 1^{a}	87 ± 2^{a}	93 ± 3^{ab}	303 ± 24^{b}	330 ± 16^{b}	372 ± 4^{b}	806 ± 29^{c}
	2.50	28 ± 2^{a}	36 ± 2^{a}	86 ± 2^{a}	113 ± 9^{b}	290 ± 5^{b}	379 ± 11^{c}	354 ± 13^{b}	600 ± 55^{b}
	Positive	292 ± 5^{b}	332 ± 12^{b}	464 ± 16^{b}	1026 ± 12^{c}	303 ± 32^{b}	335 ± 10^{b}	475 ± 22^{c}	1009 ± 22^{d}
Lop Buri	Negative	26 ± 1^{a}	32 ± 1^{a}	101 ± 4^{a}	115 ± 7^{a}	26 ± 1^{a}	32 ± 1^{a}	101 ± 4^{a}	115 ± 7^{a}
	0.625	27 ± 3^{a}	35 ± 2^{a}	96 ± 4^{a}	138 ± 4^{a}	455 ± 27^{c}	295 ± 36^{c}	457 ± 17^{d}	894 ± 20^{c}
	1.25	29 ± 2^{a}	44 ± 5^{a}	93 ± 1^{a}	109 ± 5^{a}	379 ± 41^{c}	182 ± 11^{b}	389 ± 8^{c}	757 ± 92^{c}
	2.50	26 ± 2^{a}	36 ± 0^{a}	88 ± 7^{a}	108 ± 5^{a}	248 ± 20^{b}	133 ± 27^{b}	299 ± 23^{b}	335 ± 41^{b}
	Positive	265 ± 24^{b}	322 ± 18^{b}	394 ± 41^{b}	742 ± 80^{b}	287 ± 12^{b}	338 ± 19^{c}	402 ± 11^{c}	776 ± 6^{c}

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.3 Mutagenic and 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. (continued)

Province	Dose (mg/plate)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Prachinburi	Negative	25 ± 0^a	27 ± 1^a	104 ± 0^a	120 ± 1^a	25 ± 0^a	27 ± 1^a	104 ± 0^a	120 ± 4^a
	0.625	31 ± 3^a	32 ± 1^a	103 ± 3^a	115 ± 1^a	363 ± 5^c	373 ± 21^c	421 ± 4^c	902 ± 29^{bc}
	1.25	29 ± 1^a	42 ± 4^a	122 ± 1^a	117 ± 4^a	293 ± 2^b	373 ± 13^c	418 ± 8^c	778 ± 70^b
	2.50	31 ± 4^a	37 ± 3^a	115 ± 4^a	123 ± 3^a	372 ± 5^c	305 ± 5^b	289 ± 8^b	901 ± 36^{bc}
	Positive	321 ± 4^b	444 ± 19^b	465 ± 4^b	925 ± 19^b	370 ± 42^c	404 ± 15^c	476 ± 45^c	953 ± 28^c
Ratchaburi	Negative	24 ± 1^a	28 ± 1^a	81 ± 3^a	125 ± 7^{ab}	24 ± 1^a	28 ± 1^a	81 ± 3^a	125 ± 7^a
	0.€25	24 ± 0^a	31 ± 0^a	124 ± 4^a	162 ± 10^a	276 ± 1^c	371 ± 15^c	472 ± 7^d	870 ± 79^b
	1.25	24 ± 1^a	40 ± 4^a	130 ± 6^a	109 ± 5^{ab}	233 ± 2^b	271 ± 21^b	412 ± 17^c	805 ± 91^b
	2.50	28 ± 1^a	40 ± 0^a	286 ± 51^b	209 ± 21^b	302 ± 7^d	380 ± 49^c	417 ± 10^c	744 ± 84^b
	Positive	268 ± 17^b	413 ± 32^b	352 ± 26^b	1033 ± 54^c	231 ± 12^b	402 ± 40^c	371 ± 16^b	1157 ± 85^c

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.3 Mutagenic and 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. (continued)

Province	Dose (mg/plate)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Chachoengsoa	Negative	25 ± 0^a	33 ± 0^a	103 ± 3^a	119 ± 5^a	25 ± 0^a	33 ± 0^a	103 ± 3^a	119 ± 5^a
	0.625	26 ± 1^a	30 ± 0^a	94 ± 0^a	115 ± 3^a	273 ± 26^b	430 ± 24^c	443 ± 23^b	660 ± 90^c
	1.25	25 ± 2^a	44 ± 4^a	122 ± 6^a	127 ± 5^{ab}	252 ± 20^b	359 ± 11^b	461 ± 51^b	480 ± 35^b
	2.50	28 ± 0^a	38 ± 5^a	113 ± 10^a	141 ± 9^b	250 ± 4^b	442 ± 19^c	500 ± 24^b	610 ± 10^{bc}
	Positive	280 ± 29^b	370 ± 38^b	568 ± 18^b	821 ± 7^c	293 ± 11^b	334 ± 4^b	514 ± 24^b	872 ± 46^d
Sakon	Negative	38 ± 1^a	36 ± 4^a	118 ± 4^a	115 ± 11^{ab}	38 ± 1^a	36 ± 4^a	118 ± 4^a	115 ± 11^a
Nakhon	0.625	39 ± 3^a	35 ± 3^a	135 ± 11^a	138 ± 5^b	244 ± 12^b	306 ± 12^b	328 ± 9^c	953 ± 33^d
	1.25	36 ± 1^a	36 ± 1^a	106 ± 7^a	103 ± 4^a	261 ± 15^b	254 ± 11^b	242 ± 30^b	685 ± 52^b
	2.50	36 ± 4^a	46 ± 1^a	123 ± 5^a	90 ± 9^a	210 ± 19^b	262 ± 7^b	374 ± 47^{cd}	592 ± 12^b
	Positive	252 ± 14^b	338 ± 21^b	477 ± 11^b	780 ± 14^c	253 ± 26^b	295 ± 36^b	428 ± 5^d	844 ± 21^c

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.3 Mutagenic and 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. (continued)

Province	Dose (mg/plate)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Loei	Negative	31 ± 2^a	32 ± 5^a	102 ± 24^a	87 ± 5^a	31 ± 3^a	32 ± 2^a	102 ± 5^a	87 ± 20^a
	0.625	40 ± 7^a	35 ± 4^a	87 ± 36^a	89 ± 9^a	173 ± 4^c	347 ± 4^d	362 ± 20^c	739 ± 57^c
	1.25	41 ± 3^a	62 ± 7^a	118 ± 5^a	122 ± 4^a	119 ± 3^b	207 ± 13^b	314 ± 4^b	628 ± 85^c
	2.50	33 ± 1^a	36 ± 4^a	117 ± 9^a	150 ± 14^a	103 ± 10^b	182 ± 17^b	312 ± 10^b	463 ± 8^b
	Positive	325 ± 5^b	380 ± 24^b	458 ± 4^b	1026 ± 28^b	274 ± 49^d	296 ± 12^c	411 ± 9^d	928 ± 78^d
Nong Bua	Negative	30 ± 1^a	40 ± 1^a	119 ± 8^a	122 ± 4^a	30 ± 1^a	40 ± 1^a	119 ± 8^a	122 ± 4^a
Lam Phu	0.625	30 ± 3^a	33 ± 2^a	96 ± 5^a	122 ± 1^a	247 ± 7^c	412 ± 8^c	323 ± 18^{cd}	796 ± 71^b
	1.25	24 ± 2^a	35 ± 1^a	113 ± 11^a	144 ± 6^a	238 ± 6^c	299 ± 33^b	282 ± 2^b	701 ± 32^b
	2.50	34 ± 3^a	35 ± 0^a	110 ± 5^a	219 ± 21^b	196 ± 6^b	261 ± 8^b	273 ± 23^b	697 ± 104^b
	Positive	243 ± 3^b	376 ± 7^b	387 ± 12^b	915 ± 2^c	245 ± 10^c	376 ± 4^c	369 ± 33^d	1026 ± 79^d

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.3 Mutagenic and 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. (continued)

Province	Dose (mg/plate)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Khon Kaen	Negative	22 ± 2^a	33 ± 0^a	88 ± 4^a	107 ± 2^a	22 ± 2^a	33 ± 0^a	88 ± 4^a	107 ± 2^a
	0.625	24 ± 0^a	29 ± 2^a	101 ± 4^a	102 ± 2^a	245 ± 6^b	362 ± 22^c	389 ± 38^b	833 ± 49^b
	1.25	27 ± 1^a	45 ± 4^a	100 ± 7^a	114 ± 6^a	230 ± 14^b	376 ± 39^c	345 ± 26^b	848 ± 66^b
	2.50	26 ± 0^a	28 ± 3^a	105 ± 8^a	113 ± 1^a	232 ± 13^b	260 ± 2^b	416 ± 28^b	882 ± 23^b
	Positive	272 ± 15^b	384 ± 40^b	539 ± 8^b	1068 ± 63^b	228 ± 20^b	358 ± 33^c	549 ± 49^c	1198 ± 72^c
Chaiyaphum	Negative	32 ± 2^a	36 ± 1^a	89 ± 7^a	128 ± 9^a	32 ± 2^a	36 ± 1^a	89 ± 7^a	128 ± 9^a
	0.625	34 ± 2^a	47 ± 2^a	74 ± 5^a	150 ± 12^a	310 ± 10^c	282 ± 6^b	427 ± 12^b	548 ± 33^c
	1.25	23 ± 2^a	32 ± 3^a	95 ± 13^a	133 ± 15^a	263 ± 5^c	278 ± 17^b	382 ± 47^b	440 ± 17^b
	2.50	23 ± 0^a	31 ± 2^a	89 ± 5^a	119 ± 6^a	174 ± 17^b	232 ± 39^b	414 ± 35^b	574 ± 31^c
	Positive	271 ± 29^b	382 ± 29^b	477 ± 30^b	888 ± 54^b	290 ± 25^c	442 ± 18^c	476 ± 41^b	837 ± 3^d

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.3 Mutagenic and 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. (continued)

Province	Dose (mg/plate)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Nakhon	Negative	26 ± 3^a	26 ± 0^a	124 ± 12^a	142 ± 20^a	26 ± 86^a	26 ± 1^a	124 ± 7^a	142 ± 9^a
Ratchasima	0.625	27 ± 2^a	32 ± 3^a	133 ± 10^{ab}	171 ± 8^a	223 ± 2b^c	291 ± 8^{cd}	325 ± 16^b	775 ± 16^b
	1.25	28 ± 2^a	37 ± 6^a	169 ± 12^b	159 ± 10^a	187 ± 7^b	274 ± 10^{bc}	306 ± 3^b	807 ± 28^b
	2.50	24 ± 2^a	33 ± 0^a	135 ± 9^{ab}	153 ± 25^a	204 ± 17^{bc}	261 ± 4^b	297 ± 23^b	758 ± 57^b
	Positive	256 ± 11^b	367 ± 15^b	332 ± 15^c	962 ± 30^b	246 ± 24^c	307 ± 11^d	347 ± 28^b	938 ± 59^c
Srisaket	Negative	21 ± 5^a	30 ± 1^a	78 ± 3^a	120 ± 3^a	21 ± 5^a	30 ± 1^a	78 ± 3^a	120 ± 3^a
	0.625	24 ± 0^a	38 ± 5^a	77 ± 3^a	116 ± 4^a	232 ± 14^b	372 ± 36^b	404 ± 19^b	753 ± 47^{cd}
	1.25	20 ± 3^a	25 ± 4^a	93 ± 7^a	115 ± 2^a	238 ± 25^b	328 ± 6^b	467 ± 21^c	632 ± 50^{bc}
	2.50	19 ± 1^a	32 ± 2^a	85 ± 6^a	112 ± 2^a	239 ± 7^b	335 ± 35^b	397 ± 10^b	522 ± 3^{6b}
	Positive	241 ± 12^b	380 ± 10^b	398 ± 25^b	821 ± 28^b	228 ± 13^b	343 ± 22^b	414 ± 13^b	840 ± 110^d

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.3 Mutagenic and 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. (continued)

Province	Dose (mg/plate)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Tak	Negative	32 ± 5^{a}	35 ± 3^{a}	72 ± 2^{a}	119 ± 5^{a}	32 ± 5^{a}	35 ± 3^{a}	72 ± 2^{a}	119 ± 5^{a}
	0.625	28 ± 0^{a}	34 ± 1^{a}	98 ± 14^{a}	139 ± 13^{a}	252 ± 15^{c}	360 ± 69^{c}	372 ± 7^{c}	1021 ± 95^{c}
	1.25	24 ± 2^{a}	49 ± 5^{a}	91 ± 13^{a}	121 ± 4^{a}	195 ± 16^{b}	223 ± 21^{b}	298 ± 28^{b}	818 ± 25^{b}
	2.50	30 ± 4^{a}	40 ± 3^{a}	97 ± 0^{a}	129 ± 6^{b}	230 ± 18^{bc}	211 ± 16^{b}	362 ± 11^{bc}	682 ± 7^{b}
	Positive	230 ± 2^{b}	354 ± 10^{b}	483 ± 8^{b}	1060 ± 46^{b}	268 ± 18^{c}	362 ± 16^{c}	512 ± 37^{d}	1105 ± 54^{c}
Kanchana buri	Negative	26 ± 1^{a}	39 ± 1^{a}	101 ± 3^{a}	120 ± 2^{a}	26 ± 1^{a}	39 ± 1^{a}	101 ± 3^{a}	120 ± 2^{a}
	0.625	29 ± 3^{a}	34 ± 2^{a}	93 ± 2^{a}	122 ± 5^{a}	172 ± 20^{b}	346 ± 17^{b}	232 ± 10^{b}	864 ± 18^{d}
	1.25	26 ± 2^{a}	34 ± 2^{a}	99 ± 7^{a}	137 ± 24^{a}	261 ± 20^{c}	332 ± 44^{b}	310 ± 23^{c}	786 ± 19^{c}
	2.50	26 ± 1^{a}	42 ± 2^{a}	109 ± 3^{a}	115 ± 24^{a}	186 ± 16^{b}	343 ± 10^{b}	185 ± 21^{b}	494 ± 40^{b}
	Positive	264 ± 12^{b}	386 ± 3^{b}	431 ± 22^{b}	780 ± 29^{b}	282 ± 19^{c}	297 ± 22^{b}	394 ± 17^{d}	750 ± 23^{c}

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.3 Mutagenic and 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. (continued)

Province	Dose (mg/plate)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Chonburi	Negative	30 ± 0^a	30 ± 2^a	75 ± 2^a	107 ± 2^a	30 ± 0^a	30 ± 2^a	75 ± 2^a	107 ± 2^a
	0.625	25 ± 2^a	32 ± 1^a	78 ± 2^a	114 ± 4^a	256 ± 10^c	325 ± 72^{bc}	482 ± 6^c	877 ± 56^c
	1.25	27 ± 0^a	26 ± 1^a	84 ± 3^a	120 ± 4^a	198 ± 13^b	244 ± 26^b	445 ± 18^b	422 ± 47^b
	2.50	31 ± 1^a	31 ± 1^a	82 ± 2^a	112 ± 2^a	235 ± 15^{bc}	24 ± 5^a	572 ± 3^d	97 ± 2^a
	Positive	266 ± 12^b	416 ± 14^b	541 ± 19^b	828 ± 15^b	261 ± 17^c	423 ± 52^c	512 ± 12^c	856 ± 37^c
Chantaburi	Negative	32 ± 2^a	31 ± 0^a	95 ± 4^a	100 ± 6^a	32 ± 2^a	31 ± 0^a	95 ± 4^a	100 ± 6^a
	0.625	26 ± 2^a	41 ± 2^a	102 ± 4^{ab}	114 ± 5^a	263 ± 23^c	389 ± 17^{cd}	482 ± 8^c	616 ± 32^b
	1.25	26 ± 1^a	40 ± 4^a	118 ± 4^b	115 ± 5^a	231 ± 13^c	382 ± 10^c	454 ± 8^c	548 ± 71^b
	2.50	30 ± 4^a	87 ± 4^b	119 ± 5^b	122 ± 3^a	176 ± 10^b	279 ± 21^b	304 ± 69^b	531 ± 49^b
	Positive	265 ± 11^b	418 ± 26^a	500 ± 12^c	895 ± 50^b	249 ± 23^c	430 ± 9^d	484 ± 25^c	830 ± 13^c

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.4 Mutagenic and 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)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Chiang Rai	Negative	21 ± 1^a	56 ± 4^a	74 ± 3^a	110 ± 3^a	21 ± 1^a	56 ± 4^a	74 ± 3^a	110 ± 3^a
	0.625	21 ± 1^a	53 ± 5^a	78 ± 5^a	113 ± 4^a	256 ± 18^b	271 ± 10^c	409 ± 7^c	862 ± 47^c
	1.25	30 ± 4^a	45 ± 1^a	81 ± 8^a	105 ± 1^a	244 ± 12^b	250 ± 9^c	458 ± 14^d	932 ± 7^c
	2.50	27 ± 1^a	57 ± 5^a	73 ± 1^a	110 ± 7^a	254 ± 7^b	210 ± 3^b	350 ± 12^b	554 ± 14^b
	Positive	232 ± 7^b	322 ± 13^b	409 ± 10^b	930 ± 16^b	257 ± 10^b	340 ± 13^d	422 ± 6^c	917 ± 84^c
Chiang Mai	Negative	20 ± 0^a	29 ± 0^a	80 ± 4^a	124 ± 5^a	20 ± 0^a	29 ± 0^a	80 ± 4^a	124 ± 5^a
	0.625	20 ± 0^a	29 ± 2^a	85 ± 1^a	111 ± 1^a	301 ± 7^c	401 ± 42^{bc}	281 ± 25^b	543 ± 32^c
	1.25	18 ± 1^a	26 ± 3^a	80 ± 0^a	114 ± 0^a	319 ± 6^c	454 ± 16^c	346 ± 20^c	394 ± 20^b
	2.50	20 ± 1^a	30 ± 3^a	77 ± 2^a	128 ± 2^a	257 ± 12^b	375 ± 18^b	277 ± 5^b	356 ± 26^b
	Positive	287 ± 7^b	411 ± 6^b	394 ± 21^b	780 ± 20^b	301 ± 8^c	385 ± 14^{bc}	362 ± 29^c	723 ± 17^d

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

Table 5.4 Mutagenic and 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. (continued)

Province	Dose (mg/plate)	Average of *His*$^+$ revertant colonies (Mean ± S.E.M.)							
		Mutagenicity				Anti-Mutagenicity			
		TA98		TA100		TA98		TA100	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Lampang	Negative	24 ± 1^{a}	38 ± 1^{a}	64 ± 2^{a}	106 ± 4^{a}	24 ± 1^{a}	38 ± 1^{a}	64 ± 2^{a}	106 ± 4^{a}
	0.625	28 ± 2^{a}	46 ± 3^{a}	66 ± 2^{a}	103 ± 1^{a}	209 ± 24^{b}	363 ± 14^{c}	464 ± 16^{b}	874 ± 29^{b}
	1.25	26 ± 0^{a}	39 ± 0^{a}	73 ± 3^{a}	114 ± 6^{a}	260 ± 10^{c}	291 ± 28^{b}	$560 \pm 44b^{c}$	1130 ± 88^{cd}
	2.50	30 ± 1^{a}	44 ± 2^{a}	69 ± 1^{a}	121 ± 2^{a}	273 ± 6^{c}	266 ± 14^{b}	653 ± 45^{c}	1026 ± 8^{bc}
	Positive	262 ± 30^{b}	415 ± 24^{b}	592 ± 39^{b}	1220 ± 47^{b}	272 ± 6^{c}	368 ± 21^{c}	517 ± 41^{b}	1241 ± 62^{d}
Kanchana buri	Negative	25 ± 0^{a}	41 ± 1^{a}	85 ± 2^{a}	114 ± 5^{a}	25 ± 0^{a}	41 ± 1^{a}	85 ± 2^{a}	114 ± 5^{a}
	0.625	26 ± 1^{a}	44 ± 0^{a}	78 ± 2^{a}	108 ± 4^{a}	265 ± 14^{b}	301 ± 13^{c}	465 ± 21^{b}	456 ± 6^{b}
	1.25	31 ± 0^{a}	47 ± 2^{a}	85 ± 3^{a}	118 ± 4^{a}	231 ± 23^{b}	88 ± 4^{b}	413 ± 23^{b}	119 ± 21^{a}
	2.50	37 ± 2^{a}	37 ± 3^{a}	85 ± 28^{a}	115 ± 3^{a}	255 ± 8^{b}	77 ± 5^{b}	469 ± 60^{b}	70 ± 3^{a}
	Positive	301 ± 41^{b}	390 ± 25^{b}	554 ± 50^{b}	965 ± 17^{b}	240 ± 11^{b}	406 ± 8^{d}	493 ± 20^{b}	988 ± 48^{b}

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.

DMSO was negative control, AF-2 and B*(a)*P were positive control which as standard mutagens when tested in the absence and the present of S9 mix. The revertant colonies induced by AF-2 0.1 and 0.01 μg/plate or B*(a)*P 10 and 5 μg/plate for *S.* Typhimurium TA98 and TA100, respectively.

APPENDIX B

A. Recipes for reagents and media

Tryptic Soy Agar (TSA)

Use: medium agar for mutagenicity and anti-mutagenicity assay

Ingredients	**Per liter**
Distilled water	1000 ml
Agar	15 g
Tryptic Soy Broth	30 g

Add the agar to the water in a 2-L flask and heat to dissolve. Add the nutrient broth powder and stir until dissolved. Autoclave for 20 min at 121°C. Let the agar cool to about 65°C. Dispense 25 ml in sterile petri plates. Store upside down in sealed plastic bags at 4°C and keep incubator at 37 °C prior use.

Mc.Farland No. 0.5

Use: calibrate density of culture bacteria

Ingredients	**Per 10 ml**
1% Barium chloride ($Bacl_2$)	0.5 ml
1% Sulfuric acid (H_2SO_4)	9.5 ml

Dissolve 1% Barium chloride and 1% Sulfuric acid. Mix well and store at 4 °C in the dark.

BIOGRAPHY

Mr. Kade Pulcharoen was born on January 10, 1980 in Naratiwat province, Thailand. He received his Bachelor of Science in Applied radiation and isotope, Faculty of Science, Kasetsart University in 2002. He has studied for Master's Degree in Biotechnology program at Chulalongkorn University since 2003.

Academic presentation:

1. Pulcharoen, K., Sutjit, W., Rengpipat, S. and Cherdshewasart, W. Mutagenic and antimutagenic activities of extracts from white kwao krua *Pueraria mirifica*, red krua *Butea superba*, black kwao krua *Mucuna collecttii* and Kudzu *Pueraria lobata* by Ames test. The 13th Congress on Science, 16-17 March 2005, Faculty of Science, Chulalongkorn University, Bangkok, Thailand. p. 111.
2. Pulcharoen, K., Rengpipat, S. and Cherdshewasart, W. Mutagenic and antimutagenic activities of extracts from white kwao krua *Pueraria mirifica*, red krua *Butea superba*, black kwao krua *Mucuna collecttii* and Kudzu *Pueraria lobata* by Ames test. *In* Proceedings of the 17 th Annual Meeting of the Thai Society for Biotechnology, Bangkok, Thailand, during November 2-3, 2006. p. 129-135 (CD)

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