CHAPTER IV

RESULTS

Ten selected flowers namely, red hibiscus (*Hibiscus rosa-sinensis* Linn.) (ชบา), Mexican creeper (*Antigonon leptopus* Hook. & Arn.) (พวงชมพู), ixora (*Ixora coccinea* Linn.) (เข็ม), white frangipani (*Plumeria obtusa* Linn. (ลั่นทม), malay apple (*Syzygium malaccense* (Linn.) Merr.& Perry) (เกสรชมพู่), kra chiew (*Curcuma sessilis* Gage.) (กระเจียว), sacred lotus (*Nelumbo nucifera* Gaertn.) (บัวหลวง), indian cork tree (*Millingtonia hortensis* Linn.) (ปีป), thong pun chang (*Rhinacanthus nasutus* ((Linn.) Kurz.) (ทองพันชั่ง), and pomegranate (*Punica granatum* Linn.) (ทับทิม) are sometime used as food ingredients and/or medicine for centuries. In the present investigation they were differentially extracted with dichloromethane, methanol and water, respectively; then, they were elucidated for their safeness and antimutagenic effect against 1-aminopyrene in Ames test and against urethane in somatic mutation and recombination test (SMART) in order to reveal their possible antimutagenic activity.

The physical characteristic of each extract in terms of color is described in Table 6. Extracting with dichloromethane, it was found that red hibiscus, ixora, Malay apple, sacred lotus, Indian cork tree, thong pun chang, and pomegranate had color different from methanol or water. Dichloromethane extracts of flowers showed yellow color except for Mexican creeper, kra chiew and thong pun chang. The percent yields of each dried extract of flowers are shown in Table 7. It was found that percent yield of dichloromethane extracts exhibited lower than others.

Table 6. The color of the flower extracts.

Flower	Dichloromethane extract	Methanol extract	Water extract
Hibiscus rosa-sinensis (Red hibiscus, ชบา)	Yellow	Red	Red
Antigonon leptopus (Mexican creeper,พวงชมพู))	Brown	Brown	Brown
Ixora coccinea (Ixora, เข็ม)	Yellow	Red	Red
Plumeria obtuse (White frangipani, ลั่นทม)	Yellow	Yellow	Brown
Syzygium malaccense (Malay apple,เกสรชมพู่)	Yellow	Red	Red
Curcuma sessilis (Kra chiew,กระเจียว)	Brown	Brown	Brown
Nelumbo nucifera (Sacred lotus, บัวหลวง)	Yellow	Brown	Brown
Millingtonia hortensis (Indian cork tree, ปีป)	Yellow	Brown	Brown
Rhinacanthus nasutus (Thong pun chang, ทองพันชั่ง)	Green	Brown	Red brown
Punica granatum (Pomegranate, ทับทิม)	Yellow	Red brown	Red brown

Table 7. Percent yield of the flower extracts from dry flower 20 g.

sample	Percentage of yield (%)		
	Dichloromethane extract	Methanol extract	Water extract
Red hibiscus	5.0	28.0	39.5
Mexican creeper	5.0	31.2	16.7
Ixora	2.9	21.5	14.7
White frangipani	4.9	24.0	15.0
Malay apple	2.5	37.5	10.5
Kra chiew	4.0	12.5	21.5
Sacred lotus	4.5	27.0	35.8
Indian cork tree	5.5	40.2	13.2
Thong pun chang	4.5	36.5	14.2
Pomegranate	4.5	38.5	33.5

4.1 Brine Shrimp Bioassay

The shrimp nauplii (brine shrimp larvae) have been used for a number of bioassay systems in which flower extracts were tested at concentration of 1000 µg/ml, 100 µg/ml and 10 µg/ml in vials containing 5 ml of brine and ten nauplii in each of the five replicates. The significant lethality of flower extracts to the brine shrimp was indicative of the presence of potent cytotoxicity components. The LC_{50} values of the brine shrimp obtained from dichloromethane, methanol and water extracts of flower are given in Table 8. Dichloromethane extract of malay apple showed most prominent activity with LC_{50} 516 µg/ml. Methanol extract and water extract of ixora exhibited brine shrimp lethality with LC_{50} values 686 and 997 µg/ml respectively. In addition, methanol extract of sacred lotus exhibited brine shrimp lethality with LC_{50} value 964 µg/ml. The degree of lethality was found to be directly propotional to the concentration of the extract. Dichloromethane, methanol or water extract of red hibiscus, Mexican creeper, white frangipani, kra chiew, Indian cork tree, thong pun chang and pomegranate showed brine shrimp lethality with LC_{50} values more than 1000 µg/ml.

Table 8. Brine shrimp bioassay results of the flower extracts

Flower	Percent deaths at 24 h			LC_{50}
	10 µg/ml	100 µg/ml	1000 µg/ml	µg/ml,24h
Red hibiscus				
-dichloromethane	4	6	10	>1000
-methanol	0	0	2	>1000
-water	2	4	7	>1000
Mexican creeper				
-dichloromethane	0	0	0	>1000
-methanol	2	6	19	>1000
-water	0	2	13	>1000
Ixora				
-dichloromethane	6	33	38	>1000
-methanol	10	55	57	686
-water	7	13	50	997
White frangipani				
-dichloromethane	0	0	8	>1000
-methanol	0	0	10	>1000
-water	0	0	0	>1000
Malay apple				
-dichloromethane	0	45	78	516
-methanol	0	0	2	>1000
-water	0	2	2	>1000
Kra chiew				
-dichloromethane	2	8	10	>1000
-methanol	0	0	0	>1000
-water	0	14	20	>1000
Sacred lotus				
-dichloromethane	0	2	4	>1000
-methanol	0	2	52	964
-water	6	15	40	>1000

Table 8. Brine shrimp bioassay results of the flower extracts

Flower	Percent deaths at 24 h			LC_{50}
	10 µg/ml	100 µg/ml	1000 µg/ml	µg/ml,24h
Indian cork tree				
-dichloromethane	7	7	11	>1000
-methanol	0	2	6	>1000
-water	4	7	9	>1000
Thong pun chang				
-dichloromethane	0	4	10	>1000
-methanol	0	0	0	>1000
-water	0	0	9	>1000
Pomegranate				
-dichloromethane	2	10	13	>1000
-methanol	4	10	17	>1000
-water	2	9	9	>1000

4.2 Antioxidant Activity

The antioxidant capacities were influenced by many factors, which cannot be fully described by a single method. Therefore, it was necessary to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of antioxidant action. The DPPH radical scavenging activity and FRAP were used in this study. In addition, the total phenolic contents of flower extracts were measured using the Folin-Ciocalteu method. Antioxidant activity and total phenolic content of extract of flowers are shown in Table 9.

The reduction of DPPH by antioxidant in the samples expressed as mg of Trolox equivalent antioxidant capacities (TEAC)/g dry extract of flower ranged from 0.010 to 0.291 mg TEAC/g dry extract and the difference of antioxidant capacities was very large, up to 29 fold. Water extract of Malay apple possessed the highest antioxidant capacity (0.291 mg TEAC/g dry extract) followed by methanol extract of kra chiew (0.221 mg TEAC/g dry extract) and water extract of ixora (0.207 mg TEAC/g dry extract). Dichloromethane extract of kra chiew showed the lowest antioxidant capacity (0.010 mg TEAC/g dry extract)

The antioxidant capacities obtained from FRAP (Ferric Reducing Antioxidant Power) assay was ranged from 51.37 to 610.92 mg Fe (II)/g dry extract and the difference of antioxidant capacities was very large, up to 12 fold. Water extract of ixora possessed the highest antioxidant capacity (610.92 mg Fe (II)/g dry extract) followed by water extract of Malay apple (535.48 mg Fe (II)/g dry extract) and methanol extract of sacred lotus (437.93 mg Fe (II)/g dry extract). Water extract of red hibiscus possessed the lowest antioxidant capacity (51.37 mg Fe (II)/g dry extract).

The amounts of total phenolic contents of flower extracts were varied from 3.70 to 145.13 mg GAE/g dry extract. Water extract of ixora had the highest phenolic content (145.13 mg GAE/g dry extract), followed by water extract of pomegranate (98.60 mg GAE/g dry extract). Water extract of red hibiscus had the lowest phenolic content (3.70 mg GAE/g dry extract).

According to the result shown in Table 9, the water extract of ixora exhibited the strongest antioxidant activity (FRAP assay) and showed the highest total phenolic content.

Table 9. Antioxidant activity and total phenolic content of the flower extracts

Sample	DPPH assay	FRAP assay	Total phenolic content
	TEAC value[a]	FRAP value [b]	GAE value [c]
Red hibiscus			
-dichloromethane	0.011±0.001	53.75±0.25	6.26±0.35
-methanol	0.105±0.002	240.38±4.36	25.96±1.80
-water	0.064±0.001	51.37±0.91	3.70±0.57
Mexican creeper			
-dichloromethane	0.120±0.006	372.28±9.07	30.06±2.22
-methanol	0.099±0.000	384.90±13.11	63.49±7.13
-water	0.183±0.003	337.49±11.48	33.34±2.77
Ixora			
-dichloromethane	0.046±0.004	123.71±3.74	9.94±1.81
-methanol	0.143±0.002	422.24±2.40	78.23±1.95
-water	0.207±0.001	610.92±3.69	145.13±5.65
White frangipani			
-dichloromethane	0.027±0.003	58.69±1.88	5.25±0.26
-methanol	0.065±0.001	78.93±1.17	10.28±1.83
-water	0.161±0.002	188.77±5.38	27.36±1.68
Malay apple			
-dichloromethane	0.062±0.003	152.06±7.02	29.11±3.68
-methanol	0.051±0.003	80.31±1.53	13.80±2.17
-water	0.291±0.001	535.48±8.41	38.51±2.15
Kra chiew			
-dichloromethane	0.010±0.002	79.70±7.53	10.60±0.05
-methanol	0.221±0.021	394.38±7.45	54.19±2.35
-water	0.092±0.002	144.01±11.51	15.05±1.68
Sacred lotus			
-dichloromethane	0.014±0.003	134.94±2.73	10.28±1.29
-methanol	0.113±0.000	437.93±9.38	48.17±4.02
-water	0.085±0.001	259.57±9.10	20.31±1.47

Table 9. Antioxidant activity and total phenolic content of the flower extracts (continued).

Sample	DPPH assay	FRAP assay	Total phenolic content
	TEAC value[a]	FRAP value [b]	GAE [c]
Indian cork tree			
-dichloromethane	0.023±0.001	117.53±1.60	8.62±0.79
-methanol	0.039±0.000	56.12±6.29	6.47±0.89
-water	0.161±0.002	269.48±2.69	25.69±1.14
Thong pun chang			
-dichloromethane	0.086±0.007	388.38±1.96	22.12±2.26
-methanol	0.073±0.010	116.19±8.23	12.88±2.47
-water	0.082±0.004	167.17±7.02	9.68±0.69
Pomegranate			
-dichloromethane	0.088±0.009	169.22±4.42	23.44±3.63
-methanol	0.079±0.002	316.99±16.55	63.13±1.23
-water	0.092±0.001	323.21±3.09	98.60±2.20

Results were the mean±standard deviations of six parallel measurements.

[a] TEAC=Trolox equivalent antioxidant capacity of extracts were determined using calibration curve of trolox standard solutions (mg TEAC/g dry extract).

[b] FRAP value= FRAP value of extracts were determined using calibration curve of $FeSO_4.7H_2O$ standard solutions (mg Fe(II)/g dry extract).

[c] GAE= Gallic acid equivalents of extracts were determined using calibration curve of gallic acid standard solutions (mg GAE/g dry extract).

4.3 Mutagenicity of the Flower Extracts in Ames Test

Tables 10 and 11 show the number of revertant colonies obtained from each concentration of the extract toward *salmonella* TA 98 and TA 100 respectively. None of the extracts was toxic to the bacteria at the concentrations tested since the numbers of revertants were similar to the spontaneous mutation. It can be seen that there was not increase of revertants due to the treatment with the extract. It was indicated that the samples tested did not induce frame shift mutation and base pair substitution towards *salmonella* TA 98 and TA 100 respectively. However, all nitrite treated flower extracts, except of those of dichloromethane extracts of red hibiscus, white frangipani, malay apple, methanol extract of malay apple and water extract of red hibiscus, were mutagenic on *S. typhimurium* TA 98 and TA 100. Methanol extract of sacred lotus exhibited the highest mutagenicity on both strains. It induced 1194 revertants of 3.2 mg of sample per plate on TA 98 and 992 revertants of 1.6 mg of sample per plate on TA 100.

Table 10. Mutagenicity of the flower extracts towards *Salmonella typhimurium* TA 98 without metabolic activation

Sample	Amount of extract (mg/plate)	No of revertants/plate[a]					
		Dichloromethane extract		Methanol extract		Water extract	
		w/o nitrite	With nitrite	w/o nitrite	With nitrite	w/o nitrite	With nitrite
Red hibiscus	Positive[b]	-	1611±192	-	1905±100	-	2115±158
	Spontaneous[c]	26±4	26±4	19±3	19±3	11±3	11±3
	0.16	20±4	29±6	18±3	29±7	17±7	18±6
	0.8	20±2	26±4	24±5	28±9	14±3	18±5
	1.6	21±4	35±5	21±4	33±7	15±5	18±4
	3.2	25±3	34±7	24±4	35±8	16±4	19±6
Mexican creeper	Positive[b]	-	2150±101	-	1905±100	-	2115±158
	Spontaneous[c]	17±5	17±5	19±3	19±3	13±4	13±4
	0.16	29±4	29±4	28±6	**53±9**	13±1	19±6
	0.8	20±3	33±2	25±8	**54±13**	12±1	24±5
	1.6	20±1	**97±10**	27±8	**61±16**	12±2	23±6
	3.2	17±3	**155±24**	30±5	**61±7**	14±3	**58±11**

Table 10. Mutagenicity of the flower extracts towards *Salmonella typhimurium* TA 98 without metabolic activation (continued)

Sample	Amount of extract (mg/plate)	No of revertants/plate[a]					
		Dichloromethane extract		Methanol extract		Water extract	
		w/o nitrite	With nitrite	w/o nitrite	With nitrite	w/o nitrite	With nitrite
Ixora	Positive[b]	-	1115±69	-	1931±83	-	1160±21
	Spontaneous[c]	12±2	12±2	15±2	15±2	11±3	11±3
	0.16	12±2	21±1	13±1	**61±14**	12±3	**23±3**
	0.8	12±2	**35±5**	11±2	**66±15**	14±4	**26±4**
	1.6	13±1	**40±6**	11±4	**52±18**	12±2	**30±5**
	3.2	16±2	**65±10**	18±2	**44±9**	9±1	**26±6**
White frangipani	Positive[b]	-	1063±61	-	1393±11	-	1063±61
	Spontaneous[c]	38±3	38±3	31±5	31±5	13±4	13±4
	0.16	37±5	45±7	27±5	46±6	14±3	19±2
	0.8	39±2	58±9	26±5	**111±18**	14±4	**90±5**
	1.6	42±5	60±5	33±2	**179±23**	16±3	**124±20**
	3.2	40±2	58±5	33±4	**203±37**	19±2	**94±9**

Table 10. Mutagenicity of the flower extracts towards *Salmonella typhimurium* TA 98 without metabolic activation (continued)

Sample	Amount of extract (mg/plate)	No of revertants/plate[a]					
		Dichloromethane extract		Methanol extract		Water extract	
		w/o nitrite	With nitrite	w/o nitrite	With nitrite	w/o nitrite	With nitrite
Malay apple	Positive[b]	-	1022±33	-	1201±76	-	1563±66
	Spontaneous[c]	27±1	27±1	34±10	34±10	20±2	20±2
	0.16	26±2	38±5	29±3	23±7	19±2	27±3
	0.8	27±6	43±10	34±10	29±5	25±3	27±5
	1.6	23±2	35±8	33±7	26±6	29±8	30±8
	3.2	23±4	33±7	31±5	36±8	31±3	**41±8**
Kra chiew	Positive[b]	-	836±22	-	1195±63	-	1563±66
	Spontaneous[c]	14±1	14±1	13±3	13±3	19±2	19±2
	0.16	16±2	17±4	17±4	**97±7**	22±3	26±4
	0.8	17±2	21±5	16±3	**105±8**	23±7	25±3
	1.6	16±4	**32±4**	15±3	**38±7**	25±4	**46±11**
	3.2	15±4	**30±6**	15±2	PK	31±2	**56±8**

Table 10. Mutagenicity of the flower extracts towards *Salmonella typhimurium* TA 98 without metabolic activation (continued)

Sample	Amount of extract (mg/plate)	No of revertants/plate[a]					
		Dichloromethane extract		Methanol extract		Water extract	
		w/o nitrite	With nitrite	w/o nitrite	With nitrite	w/o nitrite	With nitrite
Sacred lotus	Positive[b]	-	2150±101	-	1931±83	-	1563±66
	Spontaneous[c]	17±5	17±5	22±4	22±4	19±2	19±2
	0.16	17±4	**113±21**	22±2	**229±31**	18±3	**89±4**
	0.8	22±6	**249±43**	19±4	**714±51**	23±9	**203±17**
	1.6	21±5	**358±63**	22±3	**1055±109**	22±3	**406±15**
	3.2	24±6	**653±97**	28±7	**1194±71**	25±2	**241±29**
Indian cork tree	Positive[b]	-	1426±85	-	1201±76	-	1886±68
	Spontaneous[c]	12±1	12±1	34±10	34±10	21±3	21±3
	0.16	12±3	19±2	26±11	49±8	20±3	**104±8**
	0.8	18±3	**35±6**	27±8	48±6	21±3	**163±17**
	1.6	18±3	**46±5**	25±8	**119±22**	21±2	**192±43**
	3.2	18±2	**61±5**	28±9	60±21	20±3	**149±9**

Table 10. Mutagenicity of the flower extracts towards *Salmonella typhimurium* TA 98 without metabolic activation (continued)

Sample	Amount of extract (mg/plate)	No of revertants/plate[a]					
		Dichloromethane extract		Methanol extract		Water extract	
		w/o nitrite	With nitrite	w/o nitrite	With nitrite	w/o nitrite	With nitrite
Thong pun chang	Positive[b]	-	1611±192	-	1248±96	-	1617±95
	Spontaneous[c]	23±4	23±4	40±2	40±2	11±3	11±3
	0.16	15±3	20±3	30±3	51±7	10±2	**104±8**
	0.8	21±4	27±5	31±2	58±10	15±2	**129±16**
	1.6	22±3	**52±10**	37±2	72±11	11±1	**119±17**
	3.2	22±4	**94±9**	45±12	**80±25**	11±2	**103±14**
Pomegranate	Positive[b]	-	1115±69	-	836±22	-	1063±61
	Spontaneous[c]	12±2	12±2	14±1	14±1	38±3	38±3
	0.16	11±2	**27±3**	14±1	26±6	36±5	47±9
	0.8	11±2	**59±6**	17±2	**35±6**	45±2	**82±7**
	1.6	13±3	**65±9**	15±1	**30±4**	32±2	72±8
	3.2	13±2	**108±14**	15±4	**32±7**	37±4	51±10

[a] mean±SD of His^+ revertants per plate of two independent experiments (N=6) of each concentration of sample.

b= positive control (1-aminopyrene treated with nitrite) , c= spontaneous (blank solvent)

Bold figures indicate positive mutagenic response, PK: Partial killing effect

Table 11. Mutagenicity of the flower extracts towards *Salmonella typhimurium* TA 100 without metabolic activation

Sample	Amount of extract (mg/plate)	No of revertants/plate[a]					
		Dichloromethane extract		Methanol extract		Water extract	
		w/o nitrite	With nitrite	w/o nitrite	With nitrite	w/o nitrite	With nitrite
Red hibiscus	Positive[b]	-	596±44	-	932±114	-	645±46
	Spontaneous[c]	98±15	98±15	81±12	81±12	107±24	107±24
	0.16	88±10	106±10	89±4	**205±12**	95±5	121±9
	0.8	85±13	131±21	88±16	**244±23**	84±20	133±19
	1.6	78±14	134±13	69±4	**276±64**	112±28	175±8
	3.2	92±6	183±16	78±14	**341±32**	109±10	180±25
Mexican creeper	Positive[b]	-	826±31	-	932±114	-	645±46
	Spontaneous[c]	105±14	105±14	81±12	81±12	107±24	107±24
	0.16	99±14	157±8	86±10	**302±16**	115±13	125±14
	0.8	107±6	173±23	84±20	**390±30**	111±16	**239±22**
	1.6	105±25	**448±44**	92±9	**348±18**	111±15	**333±41**
	3.2	119±26	**641±90**	102±12	**306±42**	113±12	**421±77**

Table 11. Mutagenicity of the flower extracts towards *Salmonella typhimurium* TA 100 without metabolic activation (continued)

Sample	Amount of extract (mg/plate)	No of revertants/plate[a]					
		Dichloromethane extract		Methanol extract		Water extract	
		w/o nitrite	With nitrite	w/o nitrite	With nitrite	w/o nitrite	With nitrite
Ixora	Positive[b]	-	535±52	-	559±43	-	723±64
	Spontaneous[c]	89±19	89±19	107±11	107±11	115±15	115±15
	0.16	76±17	88±8	116±14	**311±51**	113±9	139±10
	0.8	90±8	121±17	113±16	**415±52**	118±6	**314±32**
	1.6	87±10	140±20	119±16	**407±65**	116±14	**333±40**
	3.2	88±11	**218±24**	117±20	**333±59**	115±12	**394±31**
White frangipani	Positive[b]	-	459±41	-	559±23	-	459±41
	Spontaneous[c]	99±11	99±11	137±9	137±9	82±8	82±8
	0.16	96±9	120±8	129±6	212±20	79±9	107±22
	0.8	101±4	130±14	145±12	**406±68**	85±14	**330±30**
	1.6	98±12	150±16	155±12	**392±57**	69±1	**489±40**
	3.2	105±8	180±12	151±24	**353±62**	91±7	**372±7**

Table 11. Mutagenicity of the flower extracts towards *Salmonella typhimurium* TA 100 without metabolic activation (continued)

Sample	Amount of extract (mg/plate)	No of revertants/plate[a]					
		Dichloromethane extract		Methanol extract		Water extract	
		w/o nitrite	With nitrite	w/o nitrite	With nitrite	w/o nitrite	With nitrite
Malay apple	Positive[b]	-	650±54	-	505±54	-	609±48
	Spontaneous[c]	88±7	88±7	82±8	82±8	102±8	102±8
	0.16	83±11	92±6	86±14	105±10	101±8	125±33
	0.8	76±6	85±6	75±6	109±16	101±14	154±16
	1.6	88±14	104±18	81±5	158±16	120±13	**207±13**
	3.2	76±9	100±12	93±6	153±19	120±18	**277±40**
Kra chiew	Positive[b]	-	327±15	-	457±24	-	609±48
	Spontaneous[c]	91±7	91±7	100±16	100±16	102±8	102±8
	0.16	101±11	106±9	91±7	**395±20**	96±7	141±10
	0.8	92±5	127±20	84±8	**495±29**	95±6	**218±20**
	1.6	94±5	139±21	91±6	**524±62**	92±6	**277±15**
	3.2	80±7	165±19	100±10	PK	104±7	**331±27**

Table 11. Mutagenicity of the flower extracts towards *Salmonella typhimurium* TA 100 without metabolic activation (continued)

Sample	Amount of extract (mg/plate)	No of revertants/plate[a]					
		Dichloromethane extract		Methanol extract		Water extract	
		w/o nitrite	With nitrite	w/o nitrite	With nitrite	w/o nitrite	With nitrite
Sacred lotus	Positive[b]	-	826±31	-	559±43	-	609±48
	Spontaneous[c]	105±14	105±14	123±2	123±2	102±8	102±8
	0.16	108±14	127±19	128±3	**244±26**	105±8	120±19
	0.8	88±5	199±30	135±4	**843±75**	85±5	**234±11**
	1.6	95±14	**326±23**	135±5	**992±103**	91±6	**428±8**
	3.2	93±8	**524±24**	143±4	**952±98**	98±7	**388±34**
Indian cork tree	Positive[b]	-	700±17	-	505±54	-	687±27
	Spontaneous[c]	83±8	83±8	82±8	82±8	133±6	133±6
	0.16	83±7	81±13	91±3	**282±11**	114±6	**511±23**
	0.8	74±12	154±8	93±2	**375±30**	114±9	**622±40**
	1.6	80±12	**279±32**	92±11	**388±25**	121±9	**644±26**
	3.2	89±12	**429±32**	93±17	**434±71**	127±6	**735±61**

Table 11. Mutagenicity of the flower extracts towards *Salmonella typhimurium* TA 100 without metabolic activation (continued)

Sample	Amount of extract (mg/plate)	No of revertants/plate[a]					
		Dichloromethane extract		Methanol extract		Water extract	
		w/o nitrite	With nitrite	w/o nitrite	With nitrite	w/o nitrite	With nitrite
Thong pun chang	Positive[b]	-	596±44	-	679±30	-	843±11
	Spontaneous[c]	98±15	98±15	90±11	90±11	110±5	110±5
	0.16	91±9	83±8	109±7	**250±15**	124±9	**389±23**
	0.8	108±15	123±22	110±12	**276±29**	94±8	**405±42**
	1.6	108±11	**207±16**	114±13	**352±22**	121±20	**510±87**
	3.2	102±20	**394±22**	115±8	**450±47**	127±6	**517±41**
Pomegranate	Positive[b]	-	535±52	-	327±15	-	459±41
	Spontaneous[c]	89±19	89±19	91±7	91±7	99±11	99±11
	0.16	76±18	93±16	85±6	137±5	104±11	151±18
	0.8	72±3	130±15	90±7	**221±25**	92±5	**326±31**
	1.6	83±7	**218±20**	98±5	**336±47**	92±9	**346±27**
	3.2	95±6	**310±16**	99±13	**482±63**	101±5	**402±28**

[a] mean±SD of His^+ revertants per plate of two independent experiments (N=6) of each concentration of sample.

b= positive control (1-aminopyrene treated with nitrite) , c= spontaneous (blank solvent)

Bold figures indicate positive mutagenic response, PK: Partial killing effect.

4.4 Modulating Effect of the Flower Extracts in Ames Test

The inhibitory effect of different extracts of flowers on the mutagenicity of positive mutagens using the plate incorporation assay is shown in Tables 12 (transformed to be Figures 25, 26 and 27) and 13 (transformed to be Figures 28, 29 and 30) on *S. typhimurium* TA 98 and TA 100, respectively. Doses of 20 µl and 40 µl of 1-aminopyrene-nitrite model were chosen for Ames test. The doses of the extract of each sample were 5, 10 and 15 mg/plate and they presented no killing effect.

All the dichloromethane extracts of flowers inhibited the mutagenicity of the reaction mixture of 1-aminopyrene nitrite model in the absence of metabolic activation on both tester strains. They are moderately (40-60% inhibition) to strongly (>60% inhibition) active on both strains. All concentrations of dichloromethane extract of flowers showed strong antimutagenicity on both strains except for red hibiscus. At dose 15 mg/plate, malay apple and kra chiew exhibited strong inhibition activity against 1-aminopyrene nitrite model on *S. typhimurium* TA 98 (97%) and TA 100 (99%).

Methanol extracts of all kinds of flowers inhibited the mutagenicity of the product of the reaction mixture of 1-aminopyrene nitrite model in the absence of metabolic activation on *S. typhimurium* TA 98. They ranged from negligible (0-20% inhibition) to strongly active (>60% inhibition). In addition, it was found that methanol extracts of flowers inhibited the mutagenicity of the product of the reaction mixture of 1-aminopyrene nitrite model in the absence of metabolic activation on *S. typhimurium* TA 100 except for sacred lotus and Indian cork tree. However, sacred lotus and Indian cork tree were negligible enhancement (0-20%). All concentrations of methanol extract of Mexican creeper, kra chiew and pomegranate exhibited strong antimutagenicity on both strains. At dose 15 mg/plate, the methanol extract of kra chiew exhibited strong antimutagenicity on *S. typhimurium* TA 98 (98%) and pomegranate exhibited strong antimutagenicity on *S. typhimurium* TA 100 (100%).

Water extracts of almost all kinds of flowers showed the negligible to strongly inhibition effects on both tester strains. However, at 10 mg/plate water extract of malay apple and water extract of Mexican creeper were negligible enhancement on *S. typhimurium* TA 98 and TA 100, respectively. The highest antimutagenic activity, observed at the dose 15 mg/plate, was the water extracts of white frangipani (TA 98) and pomegranate (TA 100). All concentrations of water extract of white frangipani showed strong inhibition on both strains. However, the water extract of kra chiew

exhibited weak and negligible antimutagenic on *S. typhimurium* TA 98 and TA 100 respectively.

Table 12. Modification effect of the flower extracts on the mutagenicity of sodium nitrite treated 1-aminopyrene (0.06µg/plate) expressed as percent modification of number of revertants of *Salmonella typhimurium* strains TA 98 without metabolic activation

Sample	Amount of extract (mg/plate)	Dichloromethane extract			Methanol extract			Water extract		
		No.of revertants per plate[a]	%Modification		No.of revertants per plate[a]	%Modification		No.of revertants per plate[a]	%Modification	
			Inh.	Enh.		Inh.	Enh		Inh.	Enh
Red hibiscus										
-Negative control	0	22±4			16±5			19±2		
-Positive control	0	1316±36			1618±36			1563±66		
	5	672±51	50(m)	-	1352±29	17(n)	-	833±50	47(m)	-
	10	524±30	61(s)	-	1273± 91	22(w)	-	567±65	65(s)	-
	15	291±20	79(s)	-	1257±194	23(w)	-	491±41	69(s)	-
Mexican creeper										
-Negative control	0	16±4			28±2			26±3		
-Positive control	0	1151±90			1103±61			2094±36		
	5	401±42	66(s)	-	183±49	86(s)	-	1347±225	36(w)	-
	10	328±37	73(s)	-	108±11	93(s)	-	1698±127	19(n)	-
	15	102±26	92(s)	-	114±23	92(s)	-	1544±51	27(w)	-

Table 12. Modification effect of the flower extracts on the mutagenicity of sodium nitrite treated 1-aminopyrene (0.06μg/plate) expressed as percent modification of number of revertants of *Salmonella typhimurium* strains TA 98 without metabolic activation (continued)

Sample	Amount of extract (mg/plate)	Dichloromethane extract			Methanol extract			Water extract		
		No.of revertants per plate[a]	%Modification		No.of revertants per plate[a]	%Modification		No.of revertants per plate[a]	%Modification	
			Inh.	Enh.		Inh.	Enh		Inh.	Enh
Ixora										
-Negative control	0	32±3			21±3			40±1		
-Positive control	0	1391±83			1903±55			1418±64		
	5	437±24	79(s)	-	884±17	54(m)	-	762±48	48(m)	-
	10	353±66	83(s)	-	596±64	69(s)	-	942±38	35(w)	-
	15	281±37	87(s)	-	586±96	70(s)	-	592±18	60(s)	-
White frangipani										
-Negative control	0	21±3			31±5			19±2		
-Positive control	0	1903±55			809±47			1563±66		
	5	530±59	73(s)	-	453±52	46(m)	-	470±8	71(s)	-
	10	259±34	87(s)	-	286±16	67(s)	-	244±9	85(s)	-
	15	171±16	92(s)	-	213±40	77(s)	-	151±11	91(s)	-

Table 12. Modification effect of the flower extracts on the mutagenicity of sodium nitrite treated 1-aminopyrene (0.06µg/plate) expressed as percent modification of number of revertants of *Salmonella typhimurium* strains TA 98 without metabolic activation (continued)

Sample	Amount of extract (mg/plate)	Dichloromethane extract			Methanol extract			Water extract		
		No.of revertants per plate[a]	%Modification		No.of revertants per plate[a]	%Modification		No.of revertants per plate[a]	%Modification	
			Inh.	Enh.		Inh.	Enh		Inh.	Enh
Malay apple										
-Negative control	0	21±3			13±4			19±2		
-Positive control	0	1903±83			2115±158			1563±66		
	5	111±6	95(s)	-	1383±94	35(w)	-	1370±99	13(n)	-
	10	71±11	97(s)	-	825±71	61(s)	-	1577±41	-	1(n)
	15	73±6	97(s)	-	781±69	63(s)	-	1492±21	5(n)	-
Kra chiew										
-Negative control	0	22±4			28±2			21±3		
-Positive control	0	1316±36			1903±61			1903±55		
	5	111±15	93(s)	-	91±27	94(s)	-	1319±59	31(w)	-
	10	130±36	92(s)	-	49±19	98(s)	-	1324±94	31(w)	-
	15	62±13	97(s)	-	50±16	98(s)	-	1287±68	33(w)	-

Table 12. Modification effect of the flower extracts on the mutagenicity of sodium nitrite treated 1-aminopyrene (0.06µg/plate) expressed as percent modification of number of revertants of *Salmonella typhimurium* strains TA 98 without metabolic activation (continued)

Sample	Amount of extract (mg/plate)	Dichloromethane extract			Methanol extract			Water extract		
		No.of revertants per plate[a]	%Modification		No.of revertants per plate[a]	%Modification		No.of revertants per plate[a]	%Modification	
			Inh.	Enh.		Inh.	Enh		Inh.	Enh
Sacred lotus										
-Negative control	0	32±3			16±5			40±1		
-Positive control	0	1931±83			1618±95			1507±111		
	5	242±33	89(s)	-	1011±54	38(w)	-	1501±80	0.4(n)	-
	10	145±11	94(s)	-	883±86	46(m)	-	1268±69	16(n)	-
	15	129±18	95(s)	-	498±48	70(s)	-	1148±65	24(w)	-
Indian cork tree										
-Negative control	0	21±3			28±2			21±3		
-Positive control	0	1903±55			1103±61			1563±66		
	5	379±37	81(s)	-	1103±164	0(n)	-	974±91	38(w)	-
	10	259±62	87(s)	-	854±95	23(w)	-	524±11	67(s)	-
	15	173±41	92(s)	-	676±81	40(w)	-	398±72	76(s)	-

Table 12. Modification effect of the flower extracts on the mutagenicity of sodium nitrite treated 1-aminopyrene (0.06µg/plate) expressed as percent modification of number of revertants of *Salmonella typhimurium* strains TA 98 without metabolic activation (continued)

Sample	Amount of extract (mg/plate)	Dichloromethane extract			Methanol extract			Water extract		
		No.of revertants per plate[a]	%Modification		No.of revertants per plate[a]	%Modification		No.of revertants per plate[a]	%Modification	
			Inh.	Enh.		Inh.	Enh		Inh.	Enh
Thong pun chang										
-Negative control	0	16±4			13±4			40±1		
-Positive control	0	1151±90			2115±158			1507±111		
	5	292±75	76(s)	-	1078±62	49(m)	-	1445±128	4(n)	-
	10	240±14	80(s)	-	841±99	61(s)	-	1397±31	7(n)	-
	15	111±20	92(s)	-	572±48	73(s)	-	1174±116	23(n)	-
Pomegranate										
-Negative control	0	38±3			31±5			21±3		
-Positive control	0	1063±61			809±147			1903±55		
	5	184±24	86(s)	-	251±33	72(s)	-	776±79	60(s)	-
	10	110±24	93(s)	-	158±22	84(s)	-	519±42	74(s)	-
	15	97±2	94(s)	-	99±20	91(s)	-	455±18	77(s)	-

[a] mean±SD of His^+ revertants per plate of two independent experiments (n=6)Antimutagenic potential: (n) = negligible, (w) = weak, (m) = moderate, (s) = strong
Inh = Inhibition; Enh. = Enhancement

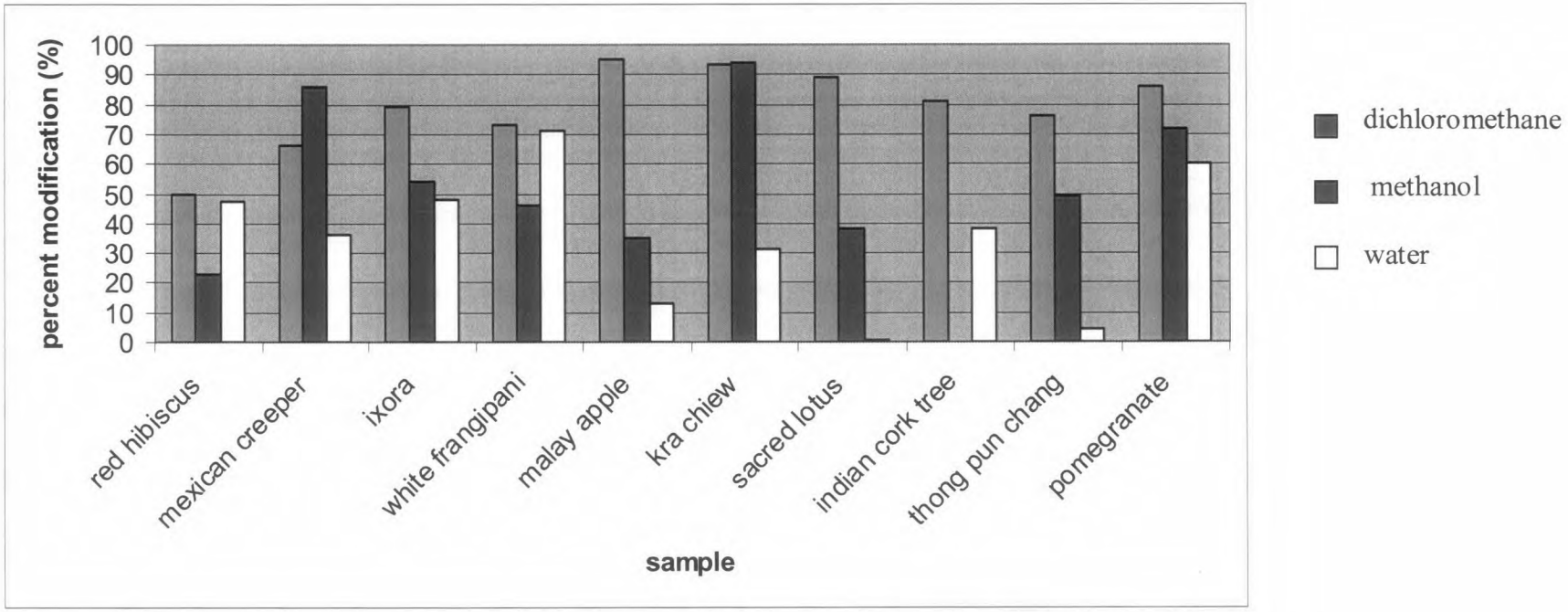

Figure 25. Modification effect of flower extracts (5 mg/plate) on the mutagenicity of sodium nitrite treated 1-aminopyrene on *Salmonella typhimurium* strains TA 98 without metabolic activation.

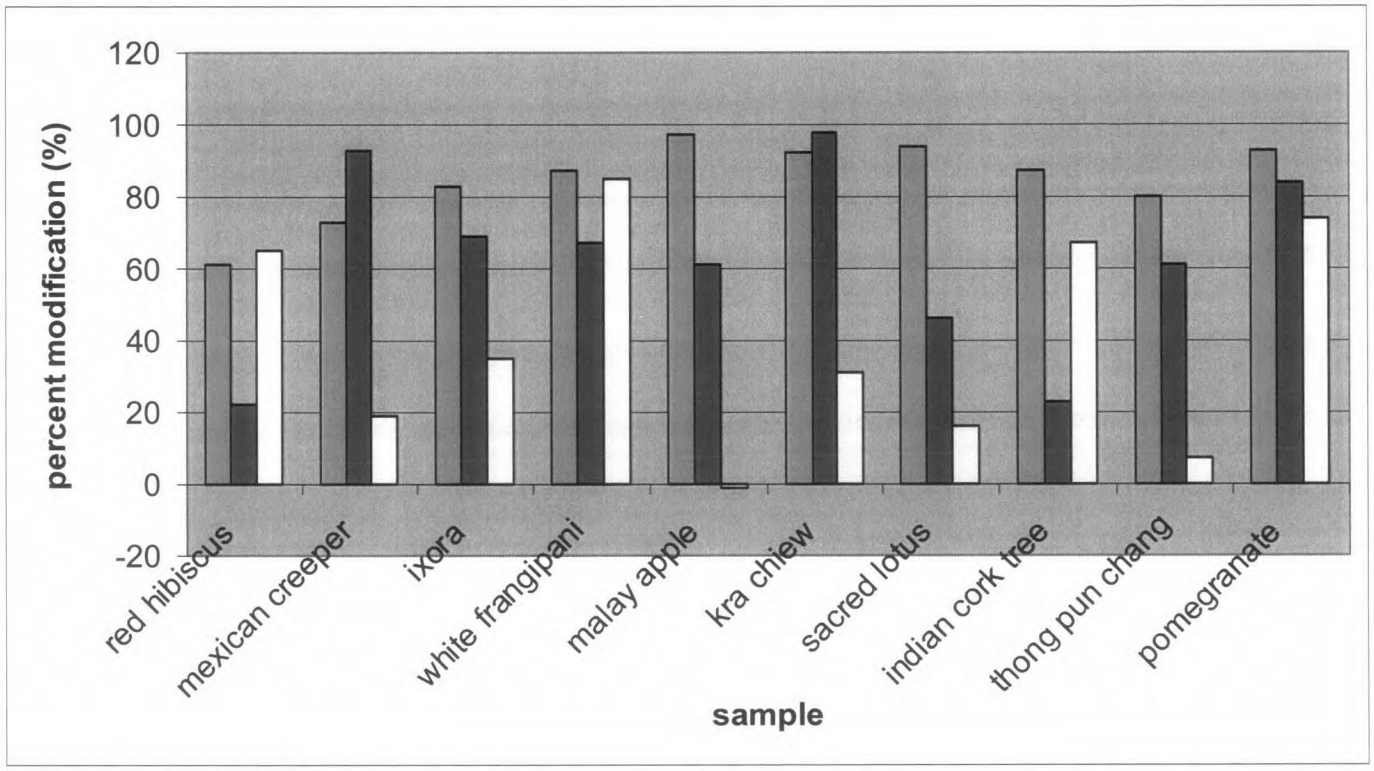

Figure26. Modification effect of flower extracts (10mg/plate) on the mutagenicity of sodium nitrite treated 1-aminopyrene on *Salmonella typhimurium* strains TA 98 without metabolic activation.

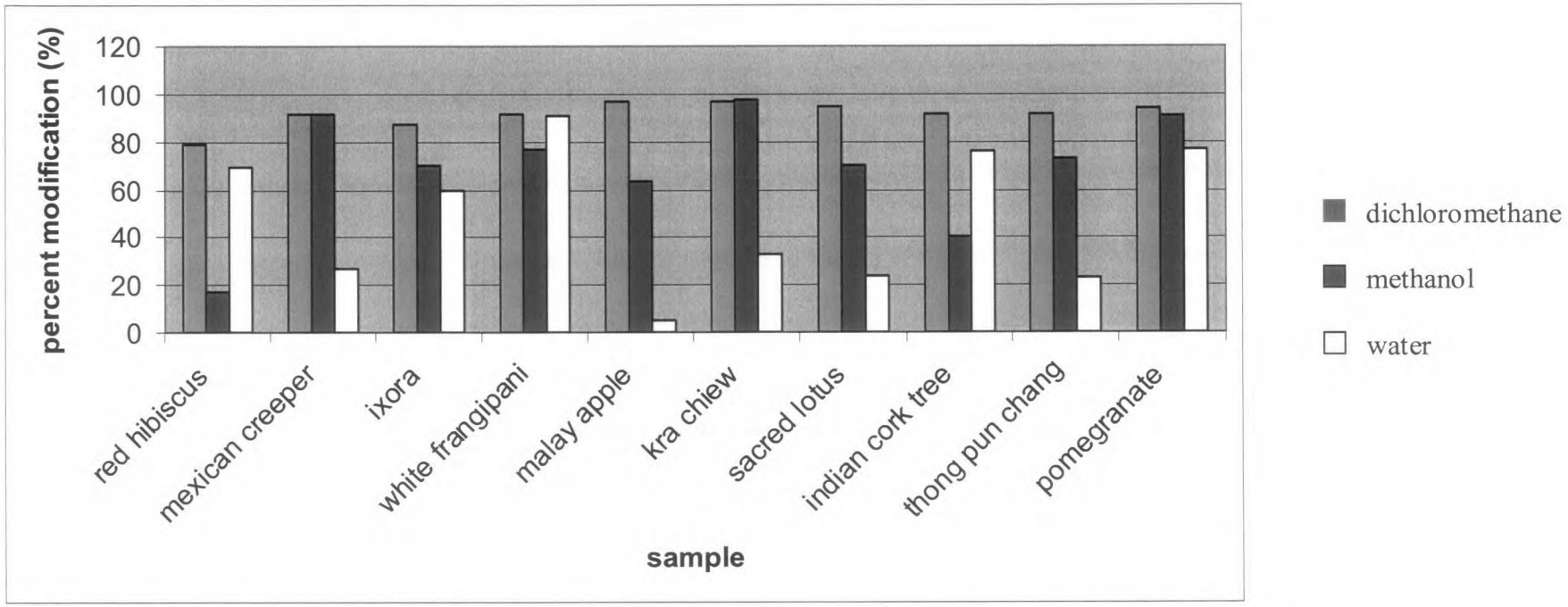

Figure 27. Modification effect of flower extracts (15 mg/plate) on the mutagenicity of sodium nitrite treated 1-aminopyrene on *Salmonella typhimurium* strains TA 98 without metabolic activation.

Table 13. Modification effect of the flower extracts on the mutagenicity of sodium nitrite treated 1-aminopyrene (0.12 µg/plate) expressed as percent modification of number of revertants of *Salmonella typhimurium* strains TA 100 without metabolic activation

Sample	Amount of extract (mg/plate)	Dichloromethane extract			Methanol extract			Water extract		
		No.of revertants per plate[a]	%Modification		No.of revertants per plate[a]	%Modification		No.of revertants per plate[a]	%Modification	
			Inh.	Enh.		Inh.	Enh		Inh.	Enh
Red hibiscus										
-Negative control	0	123±2			76±9			102±8		
-Positive control	0	575±43			514±55			609±48		
	5	391±15	41(m)	-	510±29	1(n)	-	346±17	52(m)	-
	10	313±18	58(m)	-	438±56	17(n)	-	307±16	60(s)	-
	15	258±18	70(s)	-	471±32	10(n)	-	287±37	64(s)	-
Mexican creeper										
-Negative control	0	100±4			92±7			95±8		
-Positive control	0	438±18			531±60			759±45		
	5	208±19	68(s)	-	236±54	67(s)	-	617±19	21(w)	-
	10	152±42	84(s)	-	183±32	79(s)	-	772±4	-	2(n)
	15	136±33	89(s)	-	222±31	70(s)	-	613±25	22(w)	-

Table 13. Modification effect of the flower extracts on the mutagenicity of sodium nitrite treated 1-aminopyrene (0.12 μg/plate) expressed as percent modification of number of revertants of *Salmonella typhimurium* strains TA 100 without metabolic activation (continued)

Sample	Amount of extract (mg/plate)	Dichloromethane extract			Methanol extract			Water extract		
		No.of revertants per plate[a]	% Modification		No.of revertants per plate[a]	%Modification		No.of revertants per plate[a]	%Modification	
			Inh.	Enh.		Inh.	Enh		Inh.	Enh
Ixora										
-Negative control	0	98±6			133±6			83±5		
-Positive control	0	559±43			687±27			571±17		
	5	240±50	69(s)	-	445±20	44(m)	-	423±56	30(w)	-
	10	217±24	74(s)	-	478±54	38(w)	-	271±49	61(s)	-
	15	171±4	84(s)	-	410±39	50(m)	-	295±14	57(m)	-
White frangipani										
-Negative control	0	133±6			137±9			102±8		
-Positive control	0	687±27			314±21			609±48		
	5	333±39	64(s)	-	309±2	3(n)	-	276±13	66(s)	-
	10	293±3	71(s)	-	275±40	22(w)	-	230±4	75(s)	-
	15	201±95	88(s)	-	185±21	73(s)	-	227±31	75(s)	-

Table 13. Modification effect of the flower extracts on the mutagenicity of sodium nitrite treated 1-aminopyrene (0.12 µg/plate) expressed as percent modification of number of revertants of *Salmonella typhimurium* strains TA 100 without metabolic activation (continued)

Sample	Amount of extract (mg/plate)	Dichloromethane extract			Methanol extract			Water extract		
		No.of revertants per plate[a]	%Modification		No.of revertants per plate[a]	% Modification		No.of revertants per plate[a]	% Modification	
			Inh.	Enh.		Inh.	Enh		Inh.	Enh
Malay apple										
-Negative control	0	133±6			107±24			102±8		
-Positive control	0	687±27			645±46			609±48		
	5	193±20	89(s)	-	500±76	27(w)	-	344±16	52(m)	-
	10	182±7	91(s)	-	424±43	41(m)	-	488±12	24(w)	-
	15	135±21	99(s)	-	463±48	34(w)	-	451±39	31(w)	
Kra chiew										
-Negative control	0	123±2			92±7			133±6		
-Positive control	0	575±43			531±60			687±27		
	5	202±10	83(s)	-	189±15	78(s)	-	769±40	-	15(n)
	10	159±23	92(s)	-	126±13	92(s)	-	652±27	6(n)	-
	15	128±6	99(s)	-	127±22	92(s)	-	650±35	7(n)	-

Table 13. Modification effect of the flower extracts on the mutagenicity of sodium nitrite treated 1-aminopyrene (0.12 μg/plate) expressed as percent modification of number of revertants of *Salmonella typhimurium* strains TA 100 without metabolic activation (continued)

Sample	Amount of extract (mg/plate)	Dichloromethane extract			Methanol extract			Water extract		
		No.of revertants per plate[a]	%Modification		No.of revertants per plate[a]	%Modification		No.of revertants per plate[a]	%Modification	
			Inh.	Enh.		Inh.	Enh		Inh.	Enh
Sacred lotus										
-Negative control	0	98±6			76±9			83±5		
-Positive control	0	559±43			543±31			570±14		
	5	238±25	70(s)	-	599±20	-	12(n)	514±98	11(n)	-
	10	232±34	71(s)	-	642±89	-	21(w)	536±98	7(n)	-
	15	206±14	77(s)	-	581±8	-	8(n)	529±107	8(n)	-
Indian cork tree										
-Negative control	0	133±6			92±7			133±6		
-Positive control	0	687±27			565±50			609±48		
	5	202±19	88(s)	-	622±103	-	12(n)	341±37	56(m)	-
	10	179±6	92(s)	-	567±60	-	0.4(n)	321±23	61(s)	-
	15	176±9	92(s)	-	437±52	27(w)	-	276±37	70(s)	-

Table 13. Modification effect of the flower extracts on the mutagenicity of sodium nitrite treated 1-aminopyrene (0.12 μg/plate) expressed as percent modification of number of revertants of *Salmonella typhimurium* strains TA 100 without metabolic activation (continued)

Sample	Amount of extract (mg/plate)	Dichloromethane extract			Methanol extract			Water extract		
		No.of revertants per plate[a]	%Modification		No.of revertants per plate[a]	%Modification		No.of revertants per plate[a]	%Modification	
			Inh.	Enh.		Inh.	Enh		Inh.	Enh
Thong pun chang										
-Negative control	0	100±4			107±24			83±5		
-Positive control	0	438±18			645±46			570±14		
	5	218±36	65(s)	-	494±16	28(w)	-	505±102	13(n)	-
	10	240±51	59(s)	-	351±43	55(m)	-	457±49	23(w)	-
	15	207±7	68(s)	-	237±35	76(s)	-	468±36	21(w)	-
Pomegranate										
-Negative control	0	99±11			137±9			133±6		
-Positive control	0	459±41			314±21			687±27		
	5	171±23	80(s)	-	202±25	63(s)	-	440±32	45(m)	-
	10	148±13	86(s)	-	184±16	73(s)	-	321±17	66(s)	-
	15	165±13	82(s)	-	133±15	100(s)	-	268±16	76(s)	-

[a] mean±SD of His^+ revertants per plate of two independent experiments (n=6)Antimutagenic potential: (n) = negligible, (w) = weak, (m) = moderate, (s) = strong
Inh = Inhibition; Enh. = Enhancement

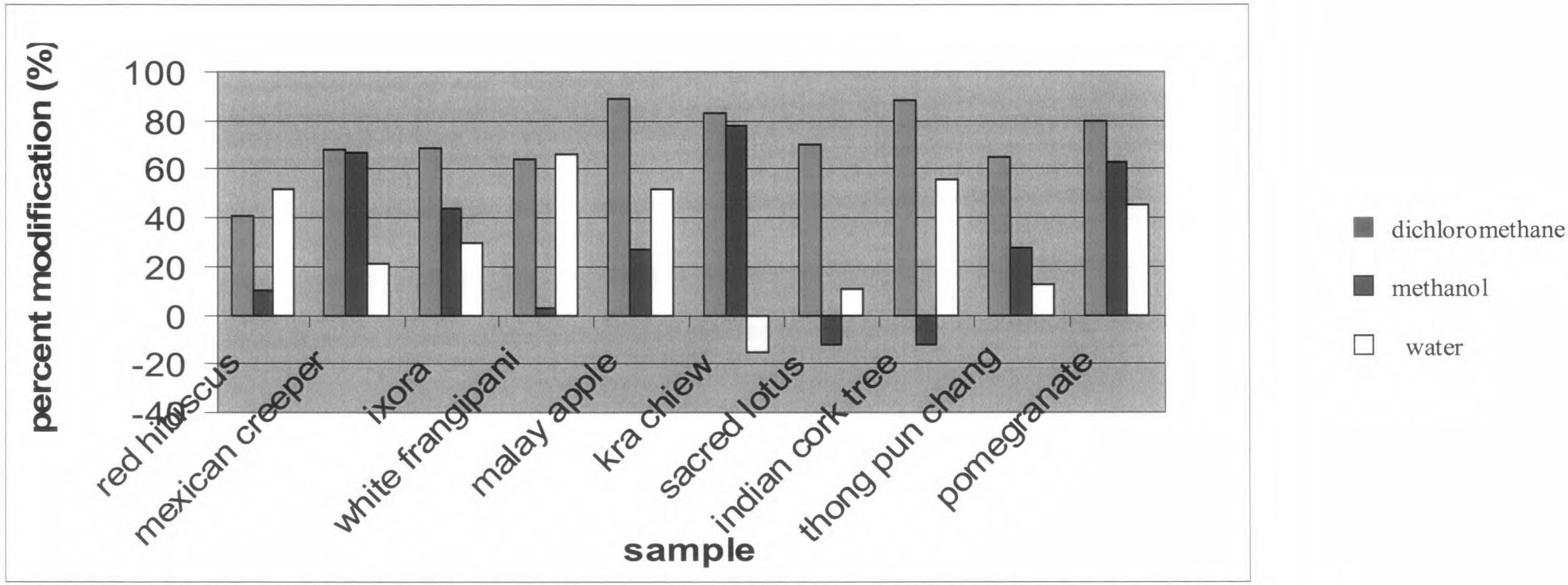

Figure 28. Modification effect of flower extracts (5 mg/plate) on the mutagenicity of sodium nitrite treated 1-aminopyrene on *Salmonella typhimurium* strains TA 100 without metabolic activation.

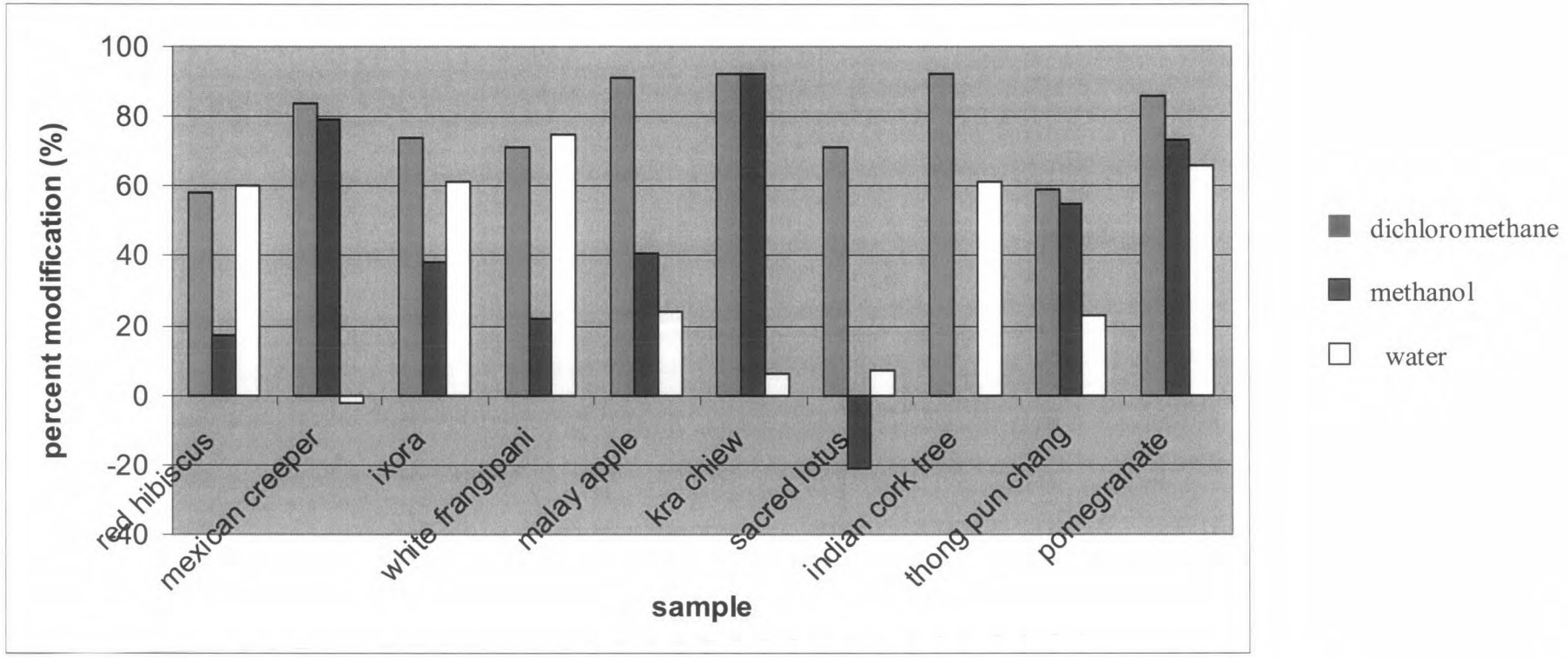

Figure 29. Modification effect of flower extracts (10 mg/plate) on the mutagenicity of sodium nitrite treated 1-aminopyrene on *Salmonella typhimurium* strains TA 100 without metabolic activation.

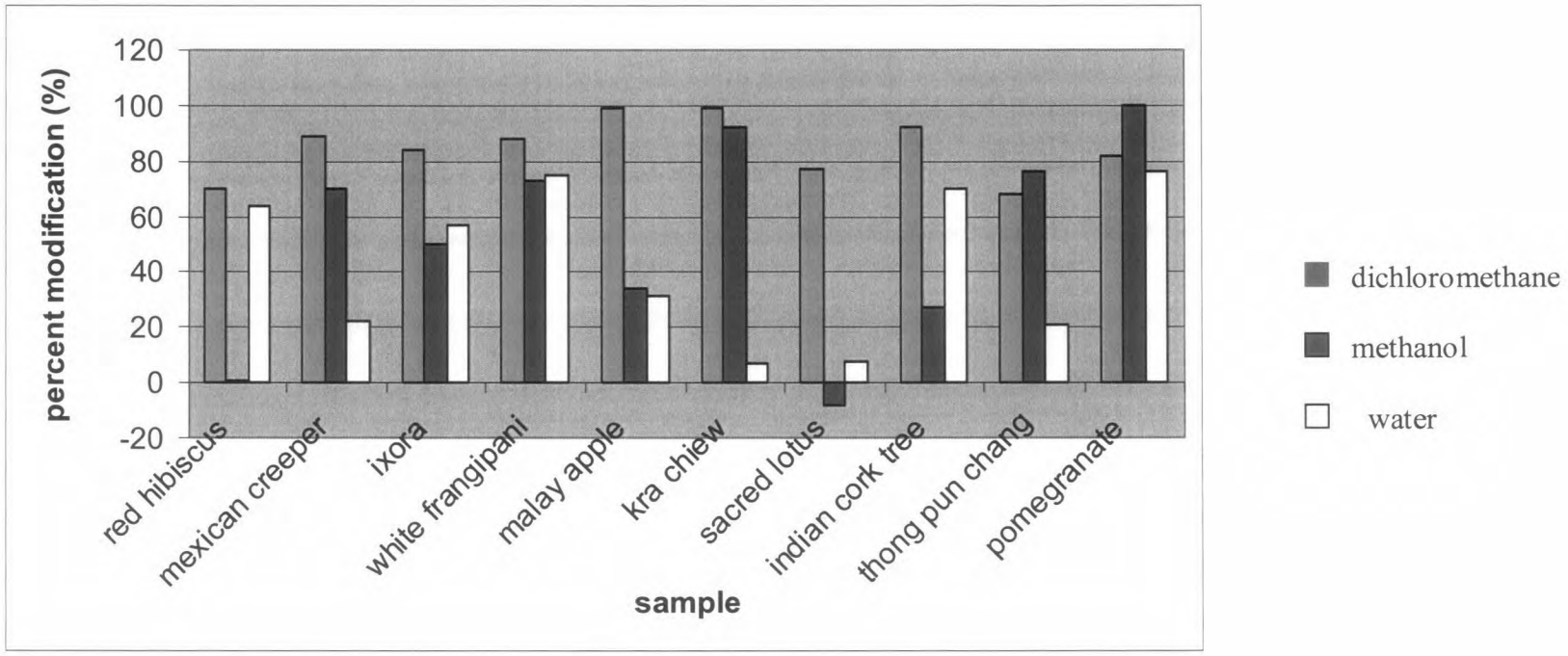

Figure 30. Modification effect of flower extracts (15 mg/plate) on the mutagenicity of sodium nitrite treated 1-aminopyrene on *Salmonella typhimurium* strains TA 100 without metabolic activation.

4.5 Survival Rate of Adult Flies and Mutagenicity of Samples in SMART

Table 14 shows the number of surviving flies obtained from the larvae brought up on each sample medium containing of dichloromethane extracts, methanol extracts and water extracts of flowers, negative control medium and positive control medium. The percentages of surviving adult flies brought up on all experimental media are higher than 50%. Overall results indicated that none was too toxic for further testing.

The mutagenicity expressed as total spots/wing obtained from the larvae brought up on each sample medium of dichloromethane, methanol and water extracts of flowers are shown in Tables 15, 16 and 17, respectively. The data indicated that all samples were not mutagenic since they did not significantly induce the frequencies of mutant spots, at any testing concentrations, to be higher than that of the negative control.

Table 14. The percentage of survival adult flies fed on control and sample medium containing flower extracts (400 mg per tube).

Sample	Percent of surviving flies (%)					
	Dichloromethane extract		Methanol extract		Water extract	
	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
Water	98	98	88	80	84	99
Urethane	74	74	68	72	62	79
Red hibiscus	95	98	81	79	94	89
Mexican creeper	76	82	76	78	89	98
Ixora	95	82	81	88	81	88
White frangipani	90	87	78	89	95	89
Malay apple	94	96	81	70	92	81
Kra chiew	95	82	67	59	67	71
Sacred lotus	85	70	80	73	76	95
Indian cork tree	76	82	77	71	84	97
Thong pun chang	67	71	66	62	97	77
Pomegranate	94	83	77	65	61	65

Table 15. Mutagenicity of dichloromethane extracts of flowers reported as wing spot induction on *Drosophila melanogaster* from 100 *trans* heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross.

Trial	Sample	Type of media[a]	Amount of extract (mg/tube)	No. of wings	Spots per wing[b]			
					Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)
1	Water	Negative	-	40	0.125 (5)	0.025(1)	0	0.150(6)
	Urethane	Positive	-	40	11.325 (453)+	6.475 (259)+	0.900(36)+	18.700(748)+
	Red hibiscus	Sample	400	40	0.100 (4)i	0.050(2)i	0	0.150(6)i
	Mexican creeper	Sample	400	30	0.075(3)i	0	0	0.075(3)-
	Ixora	Sample	400	40	0.125(5)i	0.025(1)i	0	0.150(6)i
	White frangipani	Sample	400	40	0.075(3)i	0.025(1)i	0	0.100(4)i
	Kra chiew	Sample	400	40	0.150(6)i	0.050(2)i	0	0.200(8)i
	Sacred lotus	Sample	400	40	0.175(7)i	0.050(2)i	0	0.225(9)i
	Thong pun chang	Sample	400	38	0.105(4)i	0.025(1)i	0	0.131(5)i
	Pomegranate	Sample	400	40	0.200(8)i	0	0	0.200(8)i
	Water	Negative	-	40	0.200(8)	0	0.025(1)	0.225(9)
	Urethane	Positive	-	40	9.475(379)+	4.45(178)+	1.975(79)+	15.900(636)+
	Malay apple	Sample	400	40	0.350(14)i	0	0.025(1)i	0.375(15)i
	Indian cork tree	Sample	400	40	0.250(10)i	0	0	0.250(10)i

Table 15. Mutagenicity of dichloromethane extract of flowers reported as wing spot induction on *Drosophila melanogaster* from 100 *trans* heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross (continued).

Trial	Sample	Type of media[a]	Amount of extract (mg/tube)	No. of wings	Spots per wing[b]			
					Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)
2	Water	Negative	-	40	0.150(6)	0.025(1)	0	0.175(7)
	Urethane	Positive	-	40	10.400(416)+	5.475(219)+	1.450(58)+	17.325(693)+
	Red hibiscus	Sample	400	40	0.075(3)-	0.050(2)i	0	0.125(5)i
	Mexican creeper	Sample	400	30	0.100(4)i	0.025(1)i	0	0.125(5)i
	Ixora	Sample	400	40	0.175(7)i	0	0	0.175(7)i
	White frangipani	Sample	400	40	0.100(4)i	0.050(2)i	0	0.150(6)i
	Kra chiew	Sample	400	40	0.225(9)i	0	0	0.225(9)i
	Sacred lotus	Sample	400	36	0.166(6)i	0.027(1)i	0	0.175 (7)i
	Thong pun chang	Sample	400	40	0.150(6)i	0.050(2)i	0	0.200(8)i
	Pomegranate	Sample	400	40	0.125(5)i	0.025(1)i	0	0.150(6)i

Table 15. Mutagenicity of dichloromethane extract of flowers reported as wing spot induction on *Drosophila melanogaster* from 100 *trans* heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross (continued).

Trial	Sample	Type of media[a]	Amount of extract (mg/tube)	No. of wings	Spots per wing[b]			
					Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)
2	Water	Negative	-	40	0.225(9)	0	0.025(1)	0.250(10)
	Urethane	Positive	-	40	9.375(375)+	4.750(190)+	2.175(87)+	16.300(652)+
	Malay apple	Sample	400	40	0.300(12)i	0	0	0.300(12)i
	Indian cork tree	Sample	400	40	0.200(8)i	0	0	0.200(8)i

[a] Type of media: Negative control = water ; Positive control= urethane

[b]statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Würgler (1988) for comparison with negative control:+ = positive; - = negative; i= inconclusive; Propability level $\alpha=\beta=0.05$. One side statistical tests.

Table 16. Mutagenicity of methanol extract of flowers reported as wing spot induction on *Drosophila melanogaster* from 100 *trans* heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross.

Trial	Sample	Type of media[a]	Amount of extract (mg/tube)	No. of wings	Spots per wing[b]			
					Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)
1	Water	Negative	-	40	0.150(6)	0	0	0.150(6)
	Urethane	Positive	-	40	9.600(384)+	3.150(126)+	0.575(23)+	13.325(533)+
	Red hibiscus	Sample	400	40	0.325(13)i	0.025(1)i	0	0.350(14)i
	Sacred lotus	Sample	400	30	0.250(10)i	0	0	0.250(10)i
	Indian cork tree	Sample	400	40	0.150(6)i	0	0.025(1)i	0.175(7)i
	Thong pun chang	Sample	400	40	0.175 (7)i	0.150(6)i	0.025(1)i	0.350(14)i
	Water	Negative	-	40	0.225(9)	0	0	0.225(9)
	Urethane	Positive	-	40	8.125(325)+	3.750(150)+	1.650(66)+	13.525(541)+
	White frangipani	Sample	400	40	0.200(8)i	0	0.025(1)i	0.225(9)i
	Mexican creeper	Sample	400	36	0.225(9)	0.025(1)	0	0.250 (10)
	Malay apple	Sample	400	40	0.100(4)-	0	0.025(1)i	0.125(5)-
	pomegranate	Sample	400	40	0.200(8)i	0	0	0.200(8)i

Table 16. Mutagenicity of methanol extract of flowers reported as wing spot induction on *Drosophila melanogaster* from 100 *trans* heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross (continued).

Trial	Sample	Type of media[a]	Amount of extract (mg/tube)	No. of wings	Spots per wing[b]			
					Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)
1	Water	Negative	-	40	0.150(6)	0	0	0.150(6)
	Urethane	Positive	-	40	9.600(384)+	3.150(126)+	0.575(23)+	13.325(533)+
	Ixora	Sample	400	40	0.125(5)i	0.025(1)i	0	0.150(6)-
	Kra chiew	Sample	400	40	0.275(11)i	0	0	0.275(11)i
2	Water	Negative	-	40	0.175(7)	0.025(1)	0.025(1)	0.225(9)
	Urethane	Positive	-	40	9.250(370)+	5.075(203)	2.350(94)+	16.675(667)+
	Red hibiscus	Sample	400	40	0.175(7)i	0.025(1)i	0	0.200(8)i
	Sacred lotus	Sample	400	30	0.225(9)i	0.025(1)i	0	0.250(10)i
	Indian cork tree	Sample	400	40	0.200(8)i	0.050(2)	0.050(2)i	0.300(12)i
	Thong pun chang	Sample	400	30	0.166 (5)i	0	0	0.166 (5)-
	Water	Negative	-	40	0.175(7)	0.025(1)	0.025(1)	0.225(9)
	Urethane	Positive	-	40	9.250(370)+	5.075(203)	2.350(94)+	16.675(667)+
	White frangipani	Sample	400	40	0.225(9)i	0	0.025(1)i	0.250(10)i

Table 16. Mutagenicity of methanol extract of flowers reported as wing spot induction on *Drosophila melanogaster* from 100 *trans* heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross (continued).

Trial	Sample	Type of media[a]	Amount of extract (mg/tube)	No. of wings	Spots per wing[b]			
					Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)
2	Mexican creeper	Sample	400	40	0.150(6)i	0.025(1)i	0	0.175(7)i
	Malay apple	Sample	400	40	0.150(6)i	0	0	0.150(6)-
	Pomegranate	Sample	400	40	0.225(9)i	0	0.025(1)i	0.250(10)i
	Water	Negative	-	40	0.175(7)	0.025(1)	0.025(1)	0.225(9)
	Urethane	Positive	-	40	9.250(370)+	5.075(203)+	2.350(94)+	16.675(667)+
	Ixora	Sample	400	40	0.275(11)i	0.025(1)i	0	0.300(12)i
	Kra chiew	Sample	400	40	0.250(10)i	0.025(1)i	0	0.275(11)i

[a] [a] Type of media: Negative control = water ; Positive control= urethane

[b]statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Würgler (1988) for comparison with negative control:+ = positive; - = negative; i= inconclusive; Propability level $\alpha=\beta=0.05$. One side statistical tests.

Table 17. Mutagenicity of water extract of flowers reported as wing spot induction on *Drosophila melanogaster* from 100 *trans* heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross.

Trial	Sample	Type of media[a]	Amount of extract (mg/tube)	No. of wings	Spots per wing[b]			
					Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)
1	Water	Negative	-	40	0.225(9)	0.025(1)	0	0.250(10)
	Urethane	Positive	-	40	9.175(367)+	3.275(131)+	0.450(18)+	12.900(516)+
	Red hibiscus	Sample	400	40	0.200(8)i	0.075(3)i	0	0.275(11)i
	Malay apple	Sample	400	40	0.250(10)i	0.025(1)i	0	0.275(11)i
	Kra chiew	Sample	400	40	0.075(3)-	0.025(1)i	0	0.100(4)-
	Sacred lotus	Sample	400	40	0.100(4)-	0	0	0.100(4)-
	Indian cork tree	Sample	400	40	0.150(6)-	0	0	0.150(6)-
	Pomegranate	Sample	400	36	0.277(10)i	0.027(1)i	0	0.305(11)i
	Water	Negative	-	40	0.175(7)	0.025(1)	0.025(1)	0.225(9)
	Urethane	Positive	-	40	10.875(435)+	4.875(195)+	2.600(104)+	18.350(734)+
	ixora	Sample	400	40	0.125(5)i	0.025(1)i	0	0.150(6)i
	Mexican creeper	Sample	400	36	0.166(6)i	0.027(1)i	0	0.194(7)i
	White frangipani	Sample	400	40	0.350(14)i	0	0.025(1)i	0.375(15)i
	Thong pun chang	Sample	400	40	0.275(11)i	0	0	0.275(11)i

Table 17. Mutagenicity of water extract of flowers reported as wing spot induction on *Drosophila melanogaster* from 100 *trans* heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross (continued).

Trial	Sample	Type of media[a]	Amount of extract (mg/tube)	No. of wings	Spots per wing[b]			
					Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)
2	Water	Negative	-	40	0.200(8)	0	0.025(1)	0.225(9)
	Urethane	Positive	-	40	9.475(379)+	4.450(178)+	1.975(79)+	15.900(636)+
	Red hibiscus	Sample	400	40	0.225(9)i	0.025(1)i	0	0.250(10)i
	Mexican creeper	Sample	400	40	0.275(11)i	0.025(1)i	0.025(1)i	0.325(13)i
	ixora	Sample	400	40	0.300(12)i	0	0.025(1)i	0.325(13)i
	White frangipani	Sample	400	40	0.275(11)i	0.025(1)i	0.025(1)i	0.325(13)i
	Malay apple	Sample	400	40	0.325(13)i	0.025(1)i	0	0.350(14)i
	Kra chiew	Sample	400	40	0.150(6)i	0.025(1)i	0	0.175(7)i
	Sacred lotus	Sample	400	40	0.300(12)i	0.050(2)	0.025(1)i	0.375(15)i
	Indian cork tree	Sample	400	40	0.225(9)i	0.025(1)i	0	0.250(10)i
	Thong pun chang	Sample	400	40	0.300(12)i	0.025(1)i	0	0.325(13)i
	Pomegranate	Sample	400	34	0.235(8)i	0.029(1)i	0	0.264(9)i

[aa] Type of media: Negative control = water ; Positive control= urethane

[b]statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Würgler (1988) for comparison with negative control:+ = positive; - = negative; i= inconclusive; Propability level $\alpha=\beta=0.05$. One side statistical tests.

4.6 Antimutagenicity of samples in SMART

The percent inhibition on urethane mutagenicity was calculated to show the mutagenic modification effects of flower extracts. Table 18, 19 and 20 show the effects antimutagenicity of the dichloromethane extracts, methanol extracts and water extracts of selected flowers on mutagenicity induced by urethane in somatic mutation and recombination test. They were transformed to be Figures 31 and 32. The antimutagenicity of most dichloromethane extracts of flowers ranged from weak to moderate activities (30.95-58.91%) in trial 1 and 2. In trial 1, it was found that sacred lotus showed the highest antimutagenicity (58.67%), followed by thong pun chang (58.36%). In trial 2, Mexican creeper exhibited the highest antimutagenicity (58.91%), followed by thong pun chang (56.12%). Malay apple possessed the lowest antimutagenicity in trial 1 (34.01%) and pomegranate showed the lowest antimutagenicity in trial 2 (30.95%).

The antimutagenicity of methanol extracts of flowers ranged from negligible to strong (11.69-76.91%) in trial 1 and 2. In trial 1, sacred lotus exhibited the highest antimutagenicity (60.58%), followed by kra chiew (59.06%). In trial 2, sacred lotus showed the same manner (76.91%), followed by Indian cork tree (63.01%). White frangipani possessed the lowest antimutagenicity in trial 1 (11.69%) and trial 2 (14.84%).

The antimutagenicity of water extracts of flowers ranged from negligible to strong (0.10-87.59%) in trial 1 and 2. In trial 1, ixora possessed the highest antimutagenicity (86.51%), followed by pomegranate (64.29%). In trial 2, ixora showed the same manner (87.59%), followed by Mexican creeper (59.35%). White frangipani showed the lowest antimutagenicity in trial 1 (4.76%) and trial 2 (0.10%).

The percent inhibition on mutagenicity of urethane by each sample (Table 18) suggested that the water extract of ixora in trial I (86.51%) or trial II (87.59%) was the strongest antimutagenicity in this study. Interestingly, all three types of extracts of Mexican creeper, thong pun chang and sacred lotus showed moderate to strong antimutagenic activity in trial 1and 2; such result might be had some correlation with their high antioxidant activity. Dichloromethane extract of white frangipani showed moderate antimutagenicity, however, methanol and water extract exhibited negligible antimutagenicity in trial 1 and 2.

Table 18. Antimutagenicity of dichloromethane extracts of flowers on urethane induced wing spots of *Drosophila melanogaster* derived from 100 *trans*-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross in the co-administration study.

Trial	Sample	Type of media[a]	No. of wings	Spots per wing[b]				% Inhibition
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
1	Water	Negative	40	0.200 (8)	0.050(2)	0.025(1)	0.275(11)	
	Urethane	Positive	40	6.775(271) +	4.475(179) +	1.175(47) +	12.425(497) +	
	Red hibiscus	experiment	40	2.550(102) +	2.700(108) +	0.825(33) +	6.075(243) +	51.10(m)
	Water	Negative	40	0.200(8)	0.050(2)	0.025(1)	0.275(11)	
	Urethane	Positive	40	6.775(271)+	4.475(179) +	1.175(47) +	12.425(497) +	
	Mexican creeper	experiment	40	2.975(119) +	2.525(101) +	0.875(35) +	6.375(255) +	48.69(m)
	Water	Negative	40	0.200(8)	0.050(2)	0.025(1)	0.275(11)	
	Urethane	Positive	40	6.775(271) +	4.475(179) +	1.175(47)	12.425(497) +	
	Ixora	experiment	40	3.425(137) +	3.075(123) +	0.551(22)	7.050(282) +	43.25(m)
	Water	Negative	40	0.200(8)	0.050(2)	0.025(1)	0.275(11)	
	Urethane	Positive	40	6.775(271) +	4.475(179) +	1.175(47) +	12.425(497) +	
	White frangipani	experiment	36	2.916(105) +	2.805(101) +	1.027(37) +	6.750(243) +	45.67(m)

Table 18. Antimutagenicity of dichloromethane extracts of flowers on urethane induced wing spots of Drosophila melanogaster derived from 100 trans-heterozygous (mwh+/+flr3) larvae of improved high bioactivation cross in the co-administration study (continued).

Trial	Sample	Type of media[a]	No. of wings	Spots per wing[b]				% Inhibition
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
1	Water	Negative	40	0.125 (5)	0.075(3)	0	0.200 (8)	
	Urethane	Positive	40	5.950(238) +	4.875(195) +	1.450(58) +	12.275(491) +	
	Malay apple	experiment	20	4.400(88) +	2.300(46) +	1.400(28) +	8.100(162) +	34.01(w)
	Water	Negative	40	0.125 (5)	0.075(3)	0(0)	0.200 (8)	
	Urethane	Positive	40	5.95(238) +	4.875(195) +	1.45(58) +	12.275(491) +	
	Kra chiew	experiment	40	4.350(174) +	2.275(91) +	1.050(42) +	7.675(307) +	37.47(w)
	Water	Negative	40	0.125 (5)	0.075(3)	0(0)	0.200 (8)	
	Urethane	Positive	40	9.025(361) +	5.4(216) +	3.05(122) +	17.475(699)+	
	Sacred lotus	experiment	36	2.770(100) +	3.500(126) +	0.944(34) +	7.222(260) +	58.67(m)
	Water	Negative	40	0.125 (5)	0.075(3)	0(0)	0.200(8)	
	Urethane	Positive	40	5.95(238) +	4.875(195) +	1.450(58) +	12.275(491) +	
	Indian cork tree	experiment	40	3.750(150) +	1.725(69) +	0.700(28) +	6.175(247) +	49.69(m)

Table 18. Antimutagenicity of dichloromethane extracts of flowers on urethane (experimental medium) induced wing spots of *Drosophila melanogaster* derived from 100 *trans*-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross in the co-administration study (continued).

Trial	Sample	Type of media[a]	No. of wings	Spots per wing[b]				% Inhibition
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
1	Water	Negative	40	0.125 (5)	0.075(3)	0(0)	0.200 (8)	
	Urethane	Positive	40	9.025(361) +	5.400(216) +	3.050(122) +	17.475(699)+	
	Thong pun chang	experiment	40	3.500(140) +	2.625(105) +	1.150(46) +	7.275(291) +	58.36(m)
	Water	Negative	40	0.125 (5)	0.075(3)	0(0)	0.200(8)	
	Urethane	Positive	40	5.950(238) +	4.875(195) +	1.450(58) +	12.275(491) +	
	Pomegranate	experiment	40	3.875(155) +	2.325(93) +	0.875(35) +	7.075(283) +	42.36(m)
2	Water	Negative	40	0.200(8)	0.050(2)	0.025(1)	0.275(11)	
	Urethane	Positive	40	6.775(271) +	4.475(179) +	1.175(47) +	12.425(497) +	
	Red hibiscus	experiment	40	2.475(99) +	2.650(106) +	0.825(33) +	5.950(238) +	52.11(m)
	Water	Negative	40	0.200(8)	0.050(2)	0.025(1)	0.275(11)	
	Urethane	Positive	40	6.775(271)+	4.475(179) +	1.175(47) +	12.425(497) +	
	Mexican creeper	experiment	38	2.552(97) +	1.789(68) +	0.763(29) +	5.105(194) +	58.91(m)

Table 18. Antimutagenicity of dichloromethane extracts of flowers on urethane (experimental medium) induced wing spots of *Drosophila melanogaster* derived from 100 *trans*-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross in the co-administration study. (continued).

Trial	Sample	Type of media[a]	No. of wings	Spots per wing[b]				% Inhibition
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
2	Water	Negative	40	0.200(8)	0.050(2)	0.025(1)	0.275(11)	
	Urethane	Positive	40	6.775(271) +	4.475(179) +	1.175(47) +	12.425(497) +	
	Ixora	experiment	32	4.312(138) +	2.531(81) +	1.218(39) +	8.062(258) +	35.11(w)
	Water	Negative	40	0.200(8)	0.050(2)	0.025(1)	0.275(11)	
	Urethane	Positive	40	6.775(271) +	4.475(179) +	1.175(47) +	12.425(497) +	
	White frangipani	experiment	28	2.500(70) +	2.857(80) +	0.642(18) +	6.000(168) +	51.71(m)
	Water	Negative	40	0.125 (5)	0.075(3)	0(0)	0.2 (8)	
	Urethane	Positive	40	5.950(238) +	4.875(195) +	1.450(58) +	12.275(491) +	
	Malay apple	experiment	40	3.350(134) +	2.275(91) +	0.700(28) +	6.325(253) +	48.47(m)
	Water	Negative	40	0.125 (5)	0.075(3)	0(0)	0.2 (8)	
	Urethane	Positive	40	5.950(238) +	4.875(195) +	1.450(58) +	12.275(491) +	
	Kra chiew	experiment	40	3.975(159) +	1.875(75)	0.775(31)	6.625(265) +	46.02(m)

Table 18. Antimutagenicity of dichloromethane extracts of flowers on urethane (experimental medium) induced wing spots of *Drosophila melanogaster* derived from 100 *trans*-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross in the co-administration study (continued).

Trial	Sample	Type of media[a]	No. of wings	Spots per wing[b]				% Inhibition
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
2	Water	Negative	40	0.125 (5)	0.075(3)	0(0)	0.2 (8)	
	Urethane	Positive	40	9.025(361) +	5.400(216) +	3.050(122) +	17.475(699) +	
	Sacred lotus	experiment	40	3.850(154) +	3.450(138) +	1.775(71) +	9.075(363) +	48.06(m)
	Water	Negative	40	0.125(5)	0.075(3)	0(0)	0.200(8)	
	Urethane	Positive	40	5.950(238) +	4.875(195) +	1.450(58) +	12.275(491) +	
	Indian cork tree	experiment	40	3.375(135) +	1.850(74) +	0.725(29) +	5.950(238) +	51.53(m)
	Water	Negative	40	0.125(5)	0.075(3)	0(0)	0.200(8)	
	Urethane	Positive	40	9.025(361) +	5.400(216) +	3.050(122) +	17.475(699) +	
	Thong pun chang	experiment	30	3.667(110) +	3.067(92) +	0.933(28) +	7.667(230) +	56.12(m)

[a]**Table 18.** Antimutagenicity of dichloromethane extracts of flowers on urethane (experimental medium) induced wing spots of *Drosophila melanogaster* derived from 100 *trans*-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross in the co-administration study (continued).

Trial	Sample	Type of media[a]	No. of wings	Spots per wing[b]				% Inhibition
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
2	Water	Negative	40	0.125(5)	0.075(3)	0(0)	0.200(8)	
	Urethane	Positive	40	5.950(238) +	4.875(195) +	1.450(58) +	12.275(491) +	
	Pomegranate	experiment	40	4.650(186) +	2.700(108) +	1.125(45) +	8.475(339) +	30.95(w)

[a] Type of media: Negative control = water ; Positive control= urethane

[b]statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Würgler (1988) for comparison with negative control:+ = positive; - = negative; i= inconclusive; Propability level $\alpha=\beta=0.05$. One side statistical tests.

Antimutagenic potential: (n)=negligible, (w)=weak, (m)=moderate, (s)=strong

Table 19. Antimutagenicity of methanol extracts of flowers on urethane induced wing spots of *Drosophila melanogaster* derived from 100 *trans*-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross in the co-administration study.

Trial	Sample	Type of media[a]	No. of wings	Spots per wing[b]				% Inhibition
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
1	Water	Negative	40	0.075(3)	0(0)	0.050(2)	0.125(5)	
	Urethane	Positive	40	4.875(195)+	3.575(143) +	1.825(73) +	10.275(411) +	
	Red hibiscus	experiment	40	4.625(185) +	2.525(101) +	1.100(44) +	8.250(330) +	19.70(n)
	Water	Negative	40	0.075(3)	0(0)	0.050(2)	0.125 (5)	
	Urethane	Positive	40	4.875(195)+	3.575(143) +	1.825(73) +	10.275(411) +	
	Mexican creeper	experiment	40	4.175(167) +	1.000(40) +	0.575(23) +	5.750(230) +	44.03(m)
	Water	Negative	40	0.075(3)	0(0)	0(0)	0.075(3)	
	Urethane	Positive	40	9.675(387) +	5.375(215) +	2.150(86)+	17.200(688) +	
	Ixora	experiment	40	9.575(383) +	1.175(47) +	0.800(32)+	11.550(462) +	32.84(w)
	Water	Negative	40	0.525(21)	0.025(1)	0.050(2)	0.600(24)	
	Urethane	Positive	40	9.375(375) +	4.075(163) +	1.775(71)	15.225(609) +	
	White frangipani	experiment	18	9.055(163) +	2.888(52)	1.500(27)+	13.444(242)+	11.69(n)

Table 19. Antimutagenicity of methanol extracts of flowers on urethane induced wing spots of *Drosophila melanogaster* derived from 100 *trans*-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross in the co-administration study (continued).

Trial	Sample	Type of media[a]	No. of wings	Spots per wing[b]				% Inhibition
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
1	Water	Negative	40	0.075(3)	0(0)	0.050(2)	0.125(5)	
	Urethane	Positive	36	4.875(195)+	3.575(143) +	1.825(73) +	10.275(411) +	
	Malay apple	experiment	40	6.325(253) +	1.500(60) +	0.750(30) +	8.575(343) +	16.54(n)
	Water	Negative	40	0.125(5)	0.025(1)	0(0)	0.150(6)	
	Urethane	Positive	40	9.025(361) +	5.400(216) +	2.100(84)	16.525(661) +	
	Kra chiew	experiment	34	4.058(138) +	1.764(60)	0.941(32)+	6.764(230)+	59.06(m)
	Water	Negative	40	0.075 (3)	0(0)	0.05(2)	0.125 (5)	
	Urethane	Positive	40	4.875(195) +	3.575(143) +	1.825(73) +	10.275(411) +	
	Sacred lotus	experiment	20	2.800(56)+	0.850(17) +	0.400(8) +	4.050(81) +	60.58(s)
	Water	Negative	40	0.075 (3)	0(0)	0.050(2)	0.125 (5)	
	Urethane	Positive	40	4.875(195)+	3.575(143) +	1.825(73) +	10.275(411) +	
	Indian cork tree	experiment	40	3.900(156) +	0.725(29) +	0.450(18) +	5.075(203) +	50.60(m)

Table 19. Antimutagenicity of methanol extracts of flowers on urethane induced wing spots of *Drosophila melanogaster* derived from 100 *trans*-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross in the co-administration study (continued).

Trial	Sample	Type of media[a]	No. of wings	Spots per wing[b]				% Inhibition
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
1	Water	Negative	40	0.075 (3)	0(0)	0.050(2)	0.125 (5)	
	Urethane	Positive	40	4.875(195)+	3.575(143) +	1.825(73) +	10.275(411) +	
	Thong pun chang	experiment	40	3.900(156) +	0.775(31) +	0.525(21) +	5.200(208) +	49.39(m)
	Water	Negative	40	0.175 (7)	0(0)	0(0)	0.175 (7)	
	Urethane	Positive	40	8.025(321) +	3.450(138) +	2.175(87) +	13.650(546) +	
	Pomegranate	experiment	20	6.800(136) +	1.200(24) +	0.450(9) +	8.450(169) +	38.09(w)
2	Water	Negative	40	0.175(7)	0(0)	0(0)	0.175(7)	
	Urethane	Positive	40	8.025(321) +	3.450(138) +	2.175(87) +	13.650(546) +	
	Red hibiscus	experiment	40	7.475(299) +	2.425(97) +	0.825(33) +	10.725(429) +	21.42(w)
	Water	Negative	40	0.075 (3)	0(0)	0.050(2)	0.125 (5)	
	Urethane	Positive	40	4.875(195)+	3.575(143) +	1.825(73) +	10.275(411) +	
	Mexican creeper	experiment	40	3.525(141) +	1.500(60)+	0.425(17)+	5.450(218) +	46.95(m)

Table 19. Antimutagenicity of methanol extracts of flowers on urethane induced wing spots of *Drosophila melanogaster* derived from 100 *trans*-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross in the co-administration study.

Trial	Sample	Type of media[a]	No. of wings	Spots per wing[b]				% Inhibition
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
2	Water	Negative	40	0.075(3)	0(0)	0(0)	0.075(3)	
	Urethane	Positive	40	9.675(387) +	5.375(215) +	2.150(86) +	17.200(688) +	
	Ixora	experiment	20	11.900(238) +	1.000(20) +	0.700(14) +	13.600(272) +	20.93(w)
	Water	Negative	40	0.175(7)	0.025(1)	0.025(1)	0.225(9)	
	Urethane	Positive	40	9.250(370)	5.075(203) +	2.350(94) +	16.675(667) +	
	White frangipani	experiment	40	8.225(329)	3.875(155)	2.100(84) +	14.200(568) +	14.84(n)
	Water	Negative	40	0.075 (3)	0(0)	0.050(2)	0.125(5)	
	Urethane	Positive	40	4.875(195)+	3.575(143) +	1.825(73) +	10.275(411) +	
	Malay apple	experiment	40	5.950(238) +	1.750(70) +	0.775 (31) +	8.475(339) +	17.52(n)
	Water	Negative	40	0.125(5)	0.025(1)	0(0)	0.150(6)	
	Urethane	Positive	40	9.025(361) +	5.400(216) +	2.100(84)	16.525(661) +	
	Kra chiew	experiment	34	3.764(128) +	2.058(70)+	1.058(36)+	6.882(234)+	58.35(m)

Table 19. Antimutagenicity of methanol extracts of flowers on urethane induced wing spots of *Drosophila melanogaster* derived from 100 *trans*-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross in the co-administration study (continued).

Trial	Sample	Type of media[a]	No. of wings	Spots per wing[b]				% Inhibition
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
2	Water	Negative	40	0.125(5)	0.075(3)	0(0)	0.2 (8)	
	Urethane	Positive	40	5.950(238)	4.875(195) +	1.450(58) +	12.275(491) +	
	Sacred lotus	experiment	18	1.111(20)	1.277(23) +	0.444(8) +	2.833(51) +	76.91(s)
	Water	Negative	40	0.075 (3)	0(0)	0.05(2)	0.125(5)	
	Urethane	Positive	40	4.875(195)+	3.575(143) +	1.825(73) +	10.275(411) +	
	Indian cork tree	experiment	40	3.275(131) +	0.3(12) +	0.225(9) +	3.800(152) +	63.01(s)
	Water	Negative	40	0.075(3)	0(0)	0.050(2)	0.125(5)	
	Urethane	Positive	40	4.875(195)+	3.575(143) +	1.825(73) +	10.275(411) +	
	Thong pun chang	experiment	26	3.346(87)+	0.538(14)	0.192(5)	4.076(106)	60.33(s)

Table 19. Antimutagenicity of methanol extracts of flowers on urethane induced wing spots of *Drosophila melanogaster* derived from 100 *trans*-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross in the co-administration study (continued).

Trial	Sample	Type of media[a]	No. of wings	Spots per wing[b]				% Inhibition
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
	Water	Negative	40	0.175(7)	0(0)	0(0)	0.175(7)	
	Urethane	Positive	40	8.025(321) +	3.450(138) +	2.175(87) +	13.650(546) +	
	Pomegranate	experiment	20	6.500(130) +	1.450(29) +	0.950(19) +	8.900(178) +	34.79(w)

[a] Type of media: Negative control = water ; Positive control= urethane

[b]statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Würgler (1988) for comparison with negative control:+ = positive; - = negative; i= inconclusive; Propability level $\alpha=\beta=0.05$. One side statistical tests.

Antimutagenic potential: (n)=negligible, (w)=weak, (m)=moderate, (s)=strong

Table 20. Antimutagenicity of water extracts of flowers on urethane induced wing spots of *Drosophila melanogaster* derived from 100 *trans*-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross in the co-administration study.

Trial	Sample	Type of media[a]	No. of wings	Spots per wing[b]				% Inhibition
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
1	Water	Negative	40	0.075(3)	0	0	0.075(3)	
	Urethane	Positive	40	9.675(387)+	5.375(215)+	2.150(86)+	17.200(688)+	
	Red hibiscus	experiment	38	9.473(360) +	2.236(85) +	1.368(52) +	13.078(497) +	23.95(w)
	Water	Negative	40	0.200(8)	0.050(2)	0.025(1)	0.275(11)	
	Urethane	Positive	40	6.775(271)+	4.475(179) +	1.175(47) +	12.425(497) +	
	Mexican creeper	experiment	40	2.525(101) +	1.975(79) +	0.725(29) +	5.225(209) +	57.94(m)
	Water	Negative	40	0.200(8)	0.050(2)	0.025(1)	0.275(11)	
	Urethane	Positive	40	6.775(271)+	4.475(179) +	1.175(47) +	12.425(497) +	
	Ixora	experiment	40	1.350(54) +	0.300(12)+	0.025(1) +	1.675(67)	86.51(s)
	Water	Negative	40	0.525 (21)	0.025(1)	0.050(2)	0.600(24)	
	Urethane	Positive	40	9.375(375) +	4.075(163) +	1.775(71) +	15.225(609) +	
	White frangipani	experiment	36	9.500(342)+	2.916(105) +	2.083(75) +	14.500(522) +	4.76(n)

Table 20. Antimutagenicity of water extracts of flowers on urethane induced wing spots of *Drosophila melanogaster* derived from 100 *trans*-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross in the co-administration study (continued).

Trial	Sample	Type of media[a]	No. of wings	Spots per wing[b]				% Inhibition
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
1	Water	Negative	40	0.175 (7)	0	0	0.175(7)	
	Urethane	Positive	40	8.025(321) +	3.450(138) +	2.175(87) +	13.650(546) +	
	Malay apple	experiment	40	6.025(241) +	1.800(72)+	1.425(57)+	9.250(370)+	32.23(w)
	Water	Negative	40	0.525 (21)	0.025(1)	0.050(2)	0.600(24)	
	Urethane	Positive	40	9.375(375) +	4.075(163) +	1.775(71) +	15.225(609) +	
	Kra chiew	experiment	40	8.875(355) +	2.250(90) +	1.450(58) +	12.575(503) +	17.40(n)
	Water	Negative	40	0.175 (7)	0.025(1)	0.025(1)	0.225(9)	
	Urethane	Positive	40	9.250(370) +	5.075(203) +	2.500(94)	16.675(667)	
	Sacred lotus	experiment	40	5.350(214)+	1.700(68)+	0.950(30)+	7.800(312)+	53.22(m)
	Water	Negative	40	0.175(7)	0.025(1)	0.025(1)	0.225(9)	
	Urethane	Positive	40	10.875(435)+	4.875(195)+	2.600(104)+	18.350(734)+	
	Indian cork tree	experiment	40	9.300(372)+	3.275(131)+	1.650(66)+	14.225(569)+	22.47(w)

Table 20. Antimutagenicity of water extracts of flowers on urethane induced wing spots of *Drosophila melanogaster* derived from 100 *trans*-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross in the co-administration study (continued).

Trial	Sample	Type of media[a]	No. of wings	Spots per wing[b]				% Inhibition
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
1	Water	Negative	40	0.075(3)	0(0)	0(0)	0.075 (3)	
	Urethane	Positive	40	9.675(387)+	5.375(215) +	2.150(86) +	17.200(688) +	
	Thong pun chang	experiment	20	4.700(94)+	2.050(41) +	0.900(18) +	7.650(153) +	55.52(m)
	Water	Negative	40	0.075(3)	0(0)	0(0)	0.075 (3)	
	Urethane	Positive	40	9.675(387)+	5.375(215) +	2.150(86) +	17.200(688) +	
	Pomegranate	experiment	28	5.178(145) +	0.392(11) +	0.571(16) +	6.142(172) +	64.29(s)
2	Water	Negative	40	0.175 (7)	0.025(1)	0.025(1)	0.225 (9)	
	Urethane	Positive	40	9.250(370)+	5.075(203) +	2.350(94) +	16.675(667) +	
	Red hibiscus	experiment	40	7.300(292) +	3.900(156) +	2.025(81)	13.225(529) +	20.68(w)
	Water	Negative	40	0.200(8)	0.050(2)	0.025(1)	0.275(11)	
	Urethane	Positive	40	6.775(271)+	4.475(179) +	1.175(47) +	12.425(497) +	
	Mexican creeper	experiment	40	2.675(107) +	1.850(74) +	0.525(21) +	5.050(202) +	59.35(m)

Table 20. Antimutagenicity of water extracts of flowers on urethane induced wing spots of *Drosophila melanogaster* derived from 100 *trans*-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross in the co-administration study.

Trial	Sample	Type of media[a]	No. of wings	Spots per wing[b]				% Inhibition
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
2	Water	Negative	40	0.075(3)	0	0.050(2)	0.125(5)	
	Urethane	Positive	40	4.875(195)+	3.575(143) +	1.825(73) +	10.275(411) +	
	Ixora	experiment	40	0.675(27) +	0.525(21)+	0.075(3)+	1.275(51)+	87.59(s)
	Water	Negative	40	0.525 (21)	0.025(1)	0.050(2)	0.600(24)	
	Urethane	Positive	40	9.375(375) +	4.075(163) +	1.775(71) +	15.225(609) +	
	White frangipani	experiment	24	11.041(265)+	2.833(68) +	1.333(32) +	15.208(365) +	0.10(n)
	Water	Negative	40	0.200(8)	0	0.025(1)	0.225(9)	
	Urethane	Positive	40	9.475(379) +	4.450(178) +	1.975(79) +	15.900(636) +	
	Malay apple	experiment	40	5.725(229)+	4.350(174)+	1.175(47)+	11.250(450)+	29.24(w)
	Water	Negative	40	0.525(21)	0.025(1)	0.050(2)	0.600(24)	
	Urethane	Positive	40	9.375(375) +	4.075(163) +	1.775(71) +	15.225(609) +	
	Kra chiew	experiment	40	9.125(365) +	2.400(96) +	1.175(47) +	12.700(508) +	16.58(n)

Table 20. Antimutagenicity of water extracts of flowers on urethane induced wing spots of *Drosophila melanogaster* derived from 100 *trans*-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross in the co-administration study (continued).

Trial	Sample	Type of media[a]	No. of wings	Spots per wing[b]				% Inhibition
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
2	Water	Negative	40	0.175(7)	0.025(1)	0.025(1)	0.225(9)	
	Urethane	Positive	40	9.250(370)+	5.075(203) +	2.350(94) +	16.6 75(667) +	
	Sacred lotus	experiment	40	5.050(202)+	2.225(89) +	1.200(48)+	8.475(339)+	49.17(m)
	Water	Negative	40	0.175(7)	0.025(1)	0.025(1)	0.225(9)	
	Urethane	Positive	40	9.250(370)+	5.075(203) +	2.350(94) +	16.6 75(667) +	
	Indian cork tree	experiment	34	5.382(183) +	1.794(61)+	1.147(39)+	8.323(283)+	50.08(m)
	Water	Negative	40	0.175(7)	0.025(1)	0.025(1)	0.225(9)	
	Urethane	Positive	40	9.475(379) +	4.450(178) +	1.975(79) +	15.900(636) +	
	Thong pun chang	experiment	36	4.333(156)+	1.888(68)+	0.861(31)+	7.083(255)+	55.45(m)

Table 20. Antimutagenicity of water extracts of flowers on urethane induced wing spots of *Drosophila melanogaster* derived from 100 *trans*-heterozygous (*mwh+/+flr*^{3}) larvae of improved high bioactivation cross in the co-administration study (continued).

Trial	Sample	Type of media[a]	No. of wings	Spots per wing[b]				% Inhibition
				Small single (m=2)	Large single (m=5)	Twin (m=5)	Total (m=2)	
2	Water	Negative	40	0.175(7)	0.025(1)	0.025(1)	0.225 (9)	
	Urethane	Positive	40	9.250(370)+	5.075(203)+	2.350(94)+	16.675(667)+	
	Pomegranate	experiment	34	5.029(171)+	1.088(37) +	0.882(30) +	7.000(238)+	58.02(m)

[a] Type of media: Negative control = water ; Positive control= urethane

[b] statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Würgler (1988) for comparison with negative control:+ = positive; - = negative; i= inconclusive; Propability level $\alpha=\beta=0.05$. One side statistical tests.

Antimutagenic potential: (n)=negligible, (w)=weak, (m)=moderate, (s)=strong

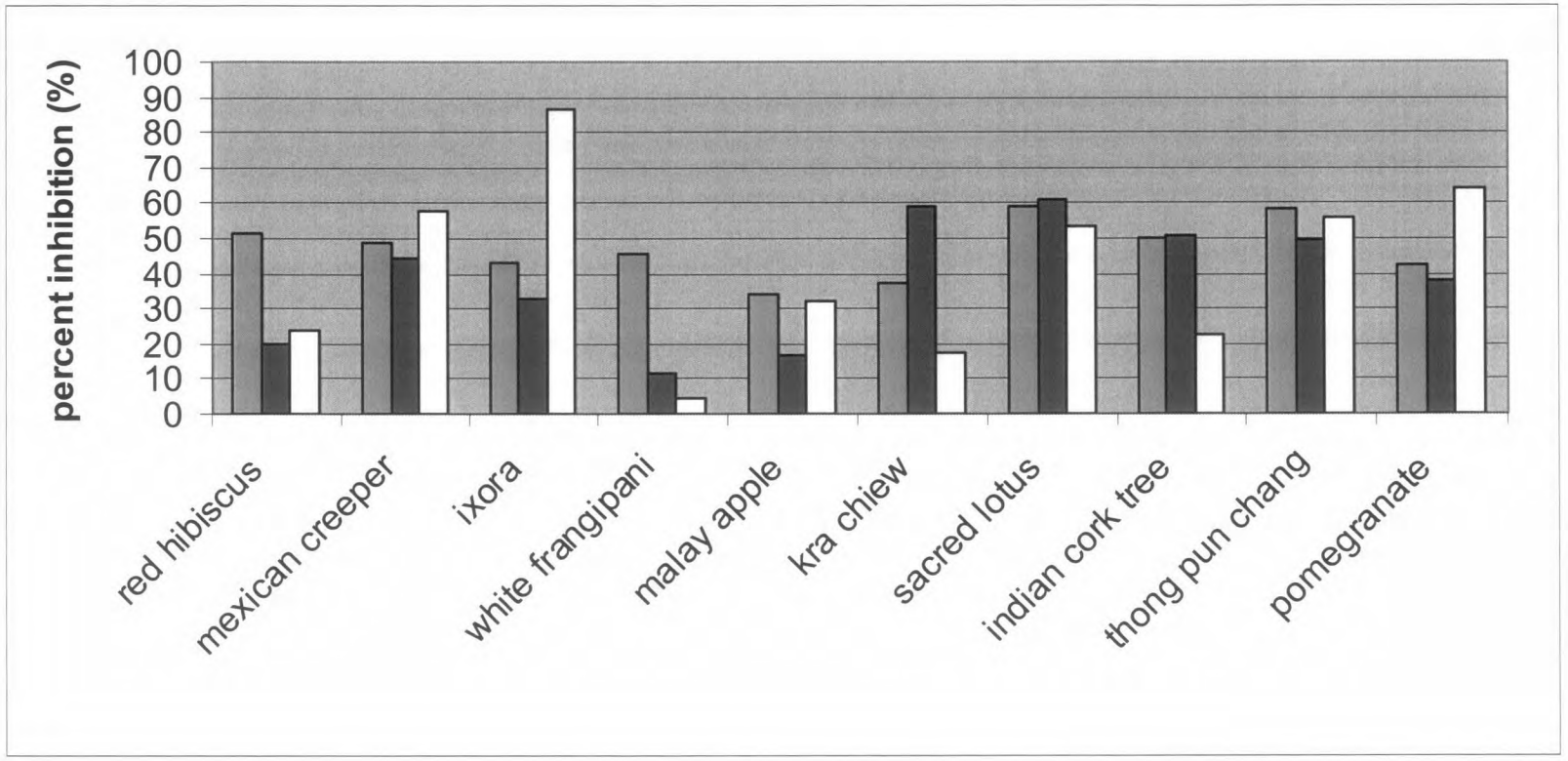

Figure 31 Antimutagenicity of dichloromethane, methanol and water extract of flower in co-administration in trial I

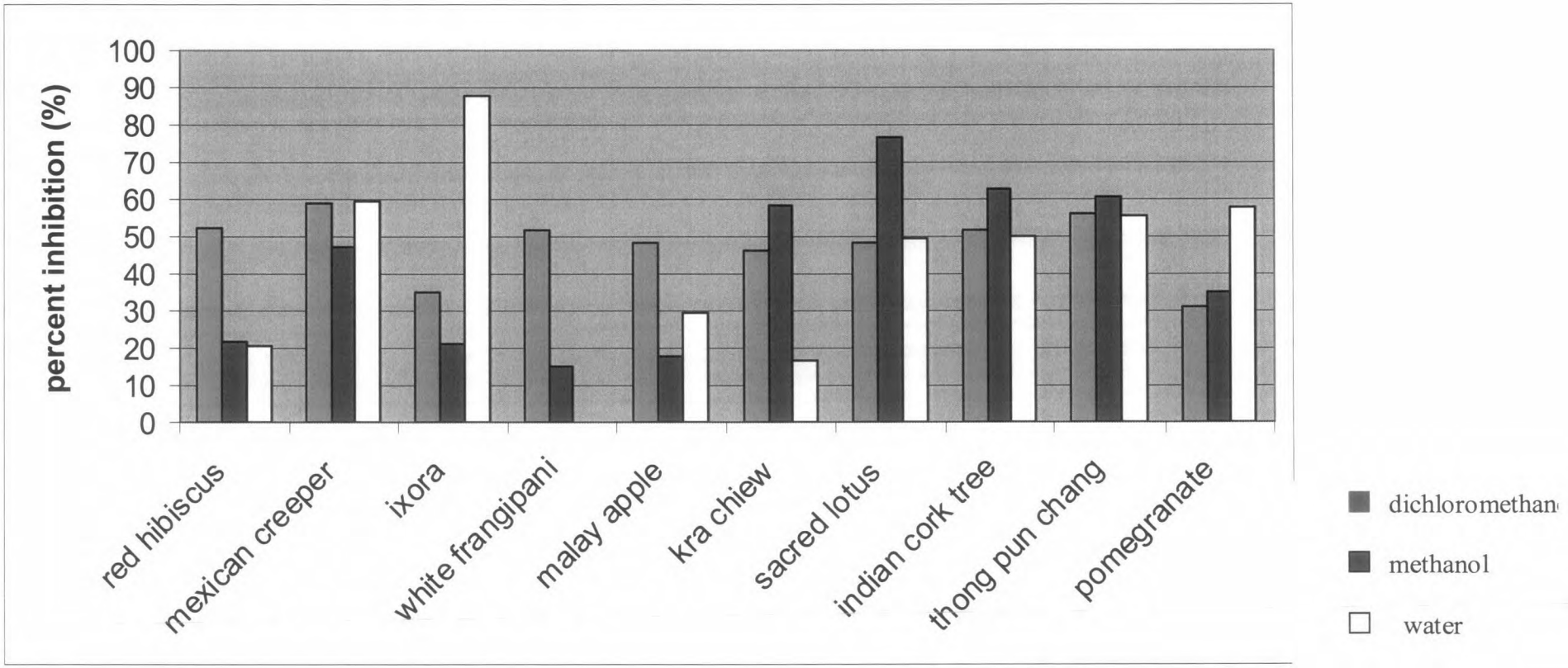

Figure 32 Antimutagenicity of dichloromethane, methanol and water extract of flower in co-administration in trial II