

## CHAPTER IV

### RESULTS

#### 4.1 Mutagenicity of the Concentrates from Menstrual Regulatory and Haematinic Traditional Preparations

Tables 2-3 shows the mutagenicity of the concentrates from menstrual regulatory and haematinic traditional preparations in the absence of metabolic activation on *Samonella typhimurium* TA98 and TA100 respectively. None of the sample exhibited direct mutagenicity on both strains of *Samonella typhimurium*. When each sample was interacted with excess amount of sodium nitrite in an acid solution pH 3-3.5, mutagenic activity on both tester strains was expressed according to the criteria of Ames test. Overall results suggested that the extracts from all samples contained some compounds readily to interact with nitrite to produce direct mutagenic causing frame shift and base-pair substitution mutation on the tester strains.

#### 4.2 Mutagenicity of Herbal Drinks

The results exhibited that the hot water extracts from herbs in this study showed no mutagenicity on both strains of *Samonella typhimurium*. (Tables 4 and 5)

**Table 2.** Mutagenicity of the concentrates of the menstrual regulatory and haematinic traditional preparations on *Salmonella typhimurium* TA98 without metabolic activation

Drugs	Amount of drug ( $\mu\text{l}/\text{plate}$ )	No. of revertants/plate <sup>a</sup>		MI <sup>b</sup>	
		without nitrite	with nitrite <sup>c</sup>	without nitrite	with nitrite
1	spontaneous	19 $\pm$ 1	19 $\pm$ 1	1.00	1.00
	4	17 $\pm$ 2	<b>107<math>\pm</math>12</b>	0.89	<b>5.63</b>
	8	18 $\pm$ 4	<b>125<math>\pm</math>8</b>	0.94	<b>6.58</b>
	16	19 $\pm$ 3	<b>139<math>\pm</math>5</b>	1.00	<b>7.32</b>
2	spontaneous	19 $\pm$ 1	19 $\pm$ 1	1.00	1.00
	4	19 $\pm$ 0.8	<b>159<math>\pm</math>7</b>	1.00	<b>8.37</b>
	8	20 $\pm$ 1	<b>206<math>\pm</math>8</b>	1.05	<b>10.84</b>
	16	19 $\pm$ 2	<b>249<math>\pm</math>11</b>	1.00	<b>13.11</b>

<sup>a</sup> Data are expressed as means and standard deviations of four plates from two experiments.

<sup>b</sup> Mutagenicity index is calculated from the average value of number of histidine revertants / plate of drug divided by that of spontaneous.

<sup>c</sup> The reaction with nitrite was done in a mild acid solution containing 250  $\mu\text{l}$  of 0.5 M sodium nitrite and each selected dose of drug.

Bold figures indicate positive mutagenic response

**Table 3.** Mutagenicity of the concentrates of the menstrual regulatory and haematinic traditional preparations on *Salmonella typhimurium* TA100 without metabolic activation

Drugs	Amount of drug ( $\mu$ l/plate)	No. of revertants/plate <sup>a</sup>		MI <sup>b</sup>	
		without nitrite	with nitrite <sup>c</sup>	without nitrite	with nitrite
1	spontaneous	95±7	95±7	1.00	1.00
	4	93±7	<b>531±12</b>	0.98	<b>5.58</b>
	8	99±12	<b>519±15</b>	1.04	<b>5.46</b>
	16	95±6	<b>644±17</b>	1.00	<b>6.78</b>
2	spontaneous	95±7	95±7	1.00	1.00
	4	95±7	<b>569±9</b>	1.00	<b>5.98</b>
	8	96±8	<b>658±11</b>	1.01	<b>6.92</b>
	16	97±9	<b>680±9</b>	1.02	<b>7.15</b>

<sup>a</sup> Data are expressed as means and standard deviations of four plates from two experiments.

<sup>b</sup> Mutagenicity index is calculated from the average value of number of histidine revertants / plate of drug divided by that of spontaneous.

<sup>c</sup> The reaction with nitrite was done in a mild acid solution containing 250  $\mu$ l of 0.5 M sodium nitrite and each selected dose of drug.

Bold figures indicate positive mutagenic response.

**Table 4.** Mutagenicity of the concentrates of herbal drinks on *Salmonella typhimurium* TA98 without metabolic activation

Herbs	Amount of herbal drink ( $\mu\text{l}/\text{plate}$ )	No.of revertants/plate <sup>a</sup>	MI <sup>b</sup>
Roselle			
กระเจี๊ยบ	spontaneous	18 $\pm$ 3	1.00
	4	19 $\pm$ 3	1.06
	8	20 $\pm$ 3	1.11
	16	19 $\pm$ 4	1.06
Chrysanthemum flower			
เก๊กฮวย	spontaneous	20 $\pm$ 6	1.00
	4	28 $\pm$ 1	1.40
	8	30 $\pm$ 6	1.50
	16	24 $\pm$ 6	1.20
Safflower			
คำฝอย	spontaneous	19 $\pm$ 3	1.00
	4	18 $\pm$ 3	0.95
	8	20 $\pm$ 5	1.11
	16	20 $\pm$ 4	1.11
Mulberry leaves			
ใบหม่อน	spontaneous	17 $\pm$ 4	1.00
	4	18 $\pm$ 2	1.06
	8	17 $\pm$ 3	1.00
	16	21 $\pm$ 3	1.24

**Table 4.** (Continued) Mutagenicity of the concentrates of herbal drinks on *Salmonella typhimurium* TA98 without metabolic activation

Herbs	Amount of herbal drink ( $\mu$ l/plate)	No.of revertants/plate <sup>a</sup>	MI <sup>b</sup>
Bael fruit			
มะตูม	spontaneous	15 $\pm$ 1	1.00
	4	21 $\pm$ 6	1.40
	8	25 $\pm$ 7	1.67
	16	19 $\pm$ 4	1.27
Asiatic Pennywort			
บัวบก	spontaneous	19 $\pm$ 3	1.00
	4	19 $\pm$ 3	1.00
	8	21 $\pm$ 5	1.11
	16	25 $\pm$ 5	1.32

<sup>a</sup> Data are expressed as means and standard deviations of four plates from two experiments.

<sup>b</sup> Mutagenicity index is calculated from the average value of number of histidine revertants / plate of the extract of herb divided by that of spontaneous.

Table 5. Mutagenicity of the concentrates of herbal drinks on *Salmonella typhimurium* TA100 without metabolic activation

Herbs	Amount of herbal drink ( $\mu$ l/plate)	No. of revertants/plate <sup>a</sup>	MI <sup>b</sup>
Roselle			
กระเจี๊ยบ	spontaneous	95 $\pm$ 7	1.00
	4	96 $\pm$ 19	1.01
	8	97 $\pm$ 14	1.02
	16	92 $\pm$ 11	0.97
Chrysanthemum flower			
เก๊กฮวย	spontaneous	80 $\pm$ 9	1.00
	4	96 $\pm$ 7	1.20
	8	88 $\pm$ 12	1.10
	16	92 $\pm$ 5	1.15
Safflower			
คำฝอย	spontaneous	81 $\pm$ 6	1.00
	4	95 $\pm$ 10	1.17
	8	95 $\pm$ 15	1.17
	16	91 $\pm$ 12	1.12
Mulberry leaves			
ใบหม่อน	spontaneous	107 $\pm$ 13	1.00
	4	100 $\pm$ 10	0.93
	8	112 $\pm$ 22	1.05
	16	113 $\pm$ 9	1.06

**Table 5.** (Continued) Mutagenicity of the concentrates of herbal drinks on *Salmonella typhimurium* TA100 without metabolic activation

Herbs	Amount of herbal drink ( $\mu$ l/plate)	No. of revertants/plate <sup>a</sup>	MI <sup>b</sup>
Bael fruit			
มะตูม	spontaneous	82 $\pm$ 5	1.00
	4	82 $\pm$ 7	1.00
	8	78 $\pm$ 10	0.95
	16	77 $\pm$ 11	0.94
Asiatic Pennywort			
บัวบก	spontaneous	81 $\pm$ 6	1.00
	4	87 $\pm$ 7	1.07
	8	90 $\pm$ 6	1.11
	16	110 $\pm$ 5	1.36

<sup>a</sup> Data are expressed as means and standard deviations of four plates from two experiments.

<sup>b</sup> Mutagenicity index is calculated from the average value of number of histidine revertants / plate of the extract of herb divided by that of spontaneous.

### 4.3 Effect of Herbal Drinks on the Mutagenicity of Nitrite Treated Menstrual Regulatory and Haematinic Traditional Preparations

The effects of herbal drinks on the mutagenicity of nitrite treated drug number 1 on *Salmonella typhimurium* TA98 in the absence of metabolic activation was shown in Table 6. The alteration on mutagenicity of nitrite treated concentrates of menstrual regulatory and haematinic traditional preparations by herbal drinks were expressed as mutagenicity index (MI). It was found that *Hibiscus sabdariffa* Linn. (roselle, กระเจี๊ยบ) had no effect on the mutagenicity of nitrite treated drug while *Chrysanthemum morifolium* Hemsl. (chrysanthemum flower, เก๊กฮวย), *Carthamus tinctorius* Linn. (safflower, คำฝอย), *Aegle marmelos* (Linn.) Corr. (bael fruit, มะตูม), *Morus alba* Linn. (mulberry leaves, ใบหม่อน), and *Centella asiatica* (Linn.) Urban (asiatic pennywort, บัวบก) showed enhancing effects on the mutagenicity of nitrite treated drug. It was noticed that mutagenicity indices were decreased in the presence of higher amount of mulberry leaves and asiatic pennywort but these figures were still higher than that of drug alone.

It was found that the extracts from roselle and chrysanthemum flower had no effect on the mutagenicity of nitrite treated drug number 1 on *Salmonella typhimurium* TA100 (Table 7). It seemed that the mutagenicity of the nitrite treated drug was enhanced by the extracts from safflower, bael fruit, mulberry leaves, and asiatic pennywort with no clear dose-response relationship. Accordingly, *Salmonella typhimurium* TA100 also showed that MI was decreased when the higher amounts of



mulberry leaves and asiatic pennywort were incorporated into the incubation tube of nitrite treated drug and tester strain but these figures were still higher than that of drug alone.

The effects of herbal drinks on the mutagenicity of nitrite treated drug number 2 on *Salmonella typhimurium* TA98 was shown in Table 8. It was found that roselle showed no effect on the mutagenicity of the treated drug; on the other hand, the mutagenicity was increased in the presence of chrysanthemum flower, safflower, bael fruit, mulberry leaves, and asiatic pennywort during incubation of treated drug and each tester strain.

It was found that the herbal drinks such as roselle, chrysanthemum flower and bael fruit had no effect on the mutagenicity of the treated drug number 2 on *Salmonella typhimurium* TA98 (Table 9). It seemed that the mutagenicity of the nitrite treated drug was enhanced by the extracts from safflower, mulberry leaves, and asiatic pennywort. Although MI was decreased in the presence of higher amount of mulberry leaves, this kind of herbal drink could not diminish the mutagenicity of drug alone.

The percentages of modification of herbal drinks on the mutagenicity of nitrite treated drug number 1 on *Salmonella typhimurium* TA98 and TA100 followed by Calomme *et al.*, 1996 were presented in Table 10. Working on *Salmonella typhimurium* TA98, roselle, bael fruit and mulberry leaves showed no effect on nitrite treated drug number 1 (0-20% enhancement). Chrysanthemum flower showed weak to strong mutagenic activities depending on doses. Safflower showed weak mutagenicity

enhancement (20-40%) while asiatic pennywort exhibited moderate to strong mutagenic activities as a dose dependent manner. However, percent modification was decreased in the presence of higher amount of asiatic pennywort. Working on *Salmonella typhimurium* TA100, roselle, chrysanthemum flower, safflower and mulberry leaves showed no effect (0-20% enhancement). Bael fruit exerted moderate to strong mutagenic activities depending on doses and asiatic pennywort showed moderate mutagenic activities (40-60% enhancement). However, percent modification was decreased in the presence of higher amount of asiatic pennywort.

The percentages of modification from herbal drinks on the mutagenicity of nitrite treated drug number 2 on *Salmonella typhimurium* TA98 and TA100 were shown on Table 11. Working on *Salmonella typhimurium* TA98, roselle, and mulberry leaves showed no effect to nitrite treated drug number 2 (0-20% enhancement). Chrysanthemum flower showed moderate mutagenic activities but percent modification was decreased in the presence of higher amount of chrysanthemum flower. Safflower showed weak to strong mutagenic activities and bael fruit showed weak mutagenic activities (20-40% enhancement). Asiatic pennywort showed moderate to strong mutagenic activities as a dose manner. Working on *Salmonella typhimurium* TA100, roselle, chrysanthemum flower, safflower and mulberry leaves showed no effect (0-20% enhancement) while asiatic pennywort showed weak to moderate mutagenic activities depending on dose.

**Table 6.** Effect of herbal drinks on the mutagenicity of nitrite treated menstrual regulatory and haematinic traditional preparation (drug 1) on *Salmonella typhimurium* TA98 without metabolic activation

Herbs	Amount of herbal drink ( $\mu$ l/plate)	No. of revertants/plate <sup>a</sup>		MI <sup>b</sup>	
		untreated drug	treated drug <sup>c</sup>	untreated drug	treated drug
Roselle (กระเจี๊ยบ)	Spon <sup>d</sup>	19 $\pm$ 1	19 $\pm$ 1	1.00	1.00
	0 <sup>e</sup>	18 $\pm$ 2	134 $\pm$ 15	0.94	7.05
	3.5	19 $\pm$ 3	134 $\pm$ 16	1.00	7.05
	7	20 $\pm$ 4	141 $\pm$ 21	1.05	7.42
	14	21 $\pm$ 4	138 $\pm$ 13	1.11	7.26
<b>Chrysanthemum</b>					
flower (เก๊กฮวย)	Spon <sup>d</sup>	20 $\pm$ 6	20 $\pm$ 6	1.00	1.00
	0 <sup>e</sup>	24 $\pm$ 4	111 $\pm$ 67	1.20	5.55
	3.5	27 $\pm$ 3	139 $\pm$ 17	1.35	6.95
	7	24 $\pm$ 4	145 $\pm$ 16	1.20	7.25
	14	31 $\pm$ 10	172 $\pm$ 20	1.55	8.60
Safflower (คำฝอย)	Spon <sup>d</sup>	19 $\pm$ 3	19 $\pm$ 3	1.00	1.00
	0 <sup>e</sup>	17 $\pm$ 3	80 $\pm$ 9	0.89	4.21
	3.5	20 $\pm$ 4	87 $\pm$ 6	1.05	4.58
	7	21 $\pm$ 6	89 $\pm$ 6	1.11	4.68
	14	19 $\pm$ 5	101 $\pm$ 21	1.00	5.32
<b>Malberry leaves</b>					
(ใบหม่อน)	Spon <sup>d</sup>	17 $\pm$ 4	17 $\pm$ 4	1.00	1.00
	0 <sup>e</sup>	17 $\pm$ 3	107 $\pm$ 7	1.00	6.29
	3.5	16 $\pm$ 4	104 $\pm$ 9	0.94	6.12
	7	20 $\pm$ 2	123 $\pm$ 10	1.18	7.24
	14	17 $\pm$ 3	116 $\pm$ 17	1.00	6.82

**Table 6.** (Continued) Effect of herbal drinks on the mutagenicity of nitrite treated menstrual regulatory and haematinic traditional preparation (drug 1) on *Salmonella typhimurium* TA98 without metabolic activation

Herbs	Amount of herbal drinks ( $\mu$ l/plate)	No. of revertants/plate <sup>a</sup>		MI <sup>b</sup>	
		untreated drug	treated drug <sup>c</sup>	untreated drug	treated drug
Bael fruit (มะตูม)	Spon <sup>d</sup>	15 $\pm$ 1	15 $\pm$ 1	1.00	1.00
	0 <sup>e</sup>	13 $\pm$ 3	90 $\pm$ 3	0.87	6.00
	3.5	18 $\pm$ 4	81 $\pm$ 31	1.20	5.40
	7	20 $\pm$ 4	79 $\pm$ 30	1.33	5.27
	14	17 $\pm$ 5	103 $\pm$ 42	1.13	6.87
Asiatic Pennywort (บัวบก)	spon <sup>d</sup>	19 $\pm$ 3	19 $\pm$ 3	1.00	1.00
	0 <sup>e</sup>	18 $\pm$ 6	111 $\pm$ 23	0.95	5.84
	3.5	20 $\pm$ 3	145 $\pm$ 26	1.05	7.63
	7	22 $\pm$ 4	126 $\pm$ 3	1.16	6.63
	14	17 $\pm$ 3	80 $\pm$ 9	0.89	4.21

<sup>a</sup> Data are expressed as means and standard deviations of four plates from two experiments.

<sup>b</sup> Mutagenicity index is calculated from the average value of number of histidine revertants/plate of drug divided by that of spontaneous.

<sup>c</sup> The reaction with nitrite was done in a mild acid solution containing 250  $\mu$ l of 0.5 M sodium nitrite and 100  $\mu$ l of concentrate of drug.

<sup>d</sup> Spontaneous

<sup>e</sup> An average value of number of histidine revertants per plate of 100  $\mu$ l of concentrate of drug-nitrite mixture incubated for 4 h in the absence of herbal drinks.

Table 7. Effect of herbal drinks on the mutagenicity of nitrite treated menstrual regulatory and haematinic traditional preparation (drug 1) on *Salmonella typhimurium* TA100 without metabolic activation

Herbs	Amount of herbal drink ( $\mu$ l/plate)	No. of revertants/plate <sup>a</sup>		MI <sup>b</sup>	
		untreated drug	treated drug <sup>c</sup>	untreated drug	treated drug
Roselle (กระเจี๊ยบ)	Spon <sup>d</sup>	95 $\pm$ 7	95 $\pm$ 7	1.00	1.00
	0 <sup>e</sup>	89 $\pm$ 5	538 $\pm$ 37	0.94	5.66
	3.5	96 $\pm$ 19	584 $\pm$ 19	1.01	6.15
	7	97 $\pm$ 14	558 $\pm$ 35	1.01	5.87
	14	92 $\pm$ 11	597 $\pm$ 21	0.97	6.28
Chrysanthemum					
flower (เก๊กฮวย)	spon <sup>d</sup>	80 $\pm$ 9	80 $\pm$ 9	1.00	1.00
	0 <sup>e</sup>	80 $\pm$ 15	586 $\pm$ 68	1.00	7.33
	3.5	96 $\pm$ 7	589 $\pm$ 42	1.20	7.36
	7	88 $\pm$ 12	570 $\pm$ 21	1.10	7.12
	14	92 $\pm$ 5	600 $\pm$ 65	1.15	7.50
Safflower(คำฝอย)					
	spon <sup>d</sup>	81 $\pm$ 6	81 $\pm$ 6	1.00	1.00
	0 <sup>e</sup>	94 $\pm$ 15	589 $\pm$ 50	1.16	7.27
	3.5	95 $\pm$ 10	535 $\pm$ 36	1.17	6.60
	7	95 $\pm$ 15	604 $\pm$ 49	1.17	7.46
	14	91 $\pm$ 12	657 $\pm$ 34	1.12	8.11
Malberry leaves					
ใบหม่อน	spon <sup>d</sup>	107 $\pm$ 13	107 $\pm$ 13	1.00	1.00
	0 <sup>e</sup>	104 $\pm$ 9	657 $\pm$ 25	0.97	6.14
	3.5	100 $\pm$ 10	709 $\pm$ 67	0.93	6.63
	7	112 $\pm$ 22	797 $\pm$ 50	1.05	7.45
	14	113 $\pm$ 9	769 $\pm$ 26	1.06	7.19

Table 7. (Continued) Effect of herbal drinks on the mutagenicity of nitrite treated menstrual regulatory and haematinic traditional preparation (drug 1) on *Salmonella typhimurium* TA100 without metabolic activation

Herbs	Amount of herbal drink ( $\mu$ l/plate)	No. of revertants/plate <sup>a</sup>		MI <sup>b</sup>	
		untreated drug	treated drug <sup>c</sup>	untreated drug	treated drug
Bael fruit (มะตูม)	Spon <sup>d</sup>	82 $\pm$ 5	82 $\pm$ 5	1.00	1.00
	0 <sup>e</sup>	89 $\pm$ 11	367 $\pm$ 77	1.09	4.48
	3.5	82 $\pm$ 7	402 $\pm$ 73	1.00	4.90
	7	78 $\pm$ 10	500 $\pm$ 82	0.95	6.10
	14	77 $\pm$ 11	572 $\pm$ 51	0.93	6.98
Asiatic Pennywort (บัวบก)	spon <sup>d</sup>	81 $\pm$ 6	81 $\pm$ 6	1.00	1.00
	0 <sup>e</sup>	94 $\pm$ 15	589 $\pm$ 49	1.16	7.27
	3.5	87 $\pm$ 8	674 $\pm$ 35	1.07	8.32
	7	60 $\pm$ 6	835 $\pm$ 47	0.74	10.31
	14	110 $\pm$ 5	779 $\pm$ 74	1.36	9.62

<sup>a</sup> Data are expressed as means and standard deviations of four plates from two experiments

<sup>b</sup> Mutagenicity index is calculated from the average value of number of histidine revertants / plate of drug divided by that of spontaneous

<sup>c</sup> The reaction with nitrite was done in a mild acid solution containing 250  $\mu$ l of 0.5 M sodium nitrite and 100  $\mu$ l of concentrate of drug.

<sup>d</sup> Spontaneous

<sup>e</sup> An average value of number of histidine revertants per plate of 100  $\mu$ l of concentrate of drug-nitrite mixture incubated for 4 h in the absence of herbal drinks.

**Table 8.** Effect of herbal drinks on the mutagenicity of nitrite treated menstrual regulatory and haematinic traditional preparation (drug 2) on *Salmonella typhimurium* TA98 without metabolic activation

Herbs	Amount of herbal drink ( $\mu$ l/plate)	No. of revertants/plate <sup>a</sup>		MI <sup>b</sup>	
		untreated drug	treated drug <sup>c</sup>	untreated drug	treated drug
Roselle (กระเจี๊ยบ)	Spon <sup>d</sup>	18 $\pm$ 3	18 $\pm$ 3	1.00	1.00
	0 <sup>e</sup>	19 $\pm$ 4	169 $\pm$ 12	1.06	9.39
	3.5	18 $\pm$ 2	159 $\pm$ 5	1.00	8.83
	7	19 $\pm$ 3	167 $\pm$ 8	1.06	9.28
	14	20 $\pm$ 3	168 $\pm$ 17	1.11	9.33
Chrysanthemum					
flower (เก๊กฮวย)	Spon <sup>d</sup>	20 $\pm$ 6	20 $\pm$ 6	1.00	1.00
	0 <sup>e</sup>	27 $\pm$ 4	204 $\pm$ 15	1.35	10.20
	3.5	28 $\pm$ 1	315 $\pm$ 30	1.40	15.75
	7	30 $\pm$ 6	295 $\pm$ 18	1.50	14.75
	14	24 $\pm$ 6	297 $\pm$ 48	1.20	14.85
Safflower (คำฝอย)	Spon <sup>d</sup>	19 $\pm$ 3	19 $\pm$ 3	1.00	1.00
	0 <sup>e</sup>	19 $\pm$ 2	139 $\pm$ 4	1.00	7.32
	3.5	18 $\pm$ 3	165 $\pm$ 17	0.95	8.68
	7	20 $\pm$ 5	177 $\pm$ 8	1.05	9.32
	14	20 $\pm$ 4	223 $\pm$ 30	1.05	11.74
Malberry leaves (ใบหม่อน)	Spon <sup>d</sup>	17 $\pm$ 4	17 $\pm$ 4	1.00	1.00
	0 <sup>e</sup>	21 $\pm$ 4	165 $\pm$ 3	1.24	9.71
	3.5	18 $\pm$ 2	181 $\pm$ 3	1.06	10.65
	7	17 $\pm$ 3	187 $\pm$ 11	1.00	11.00
	14	21 $\pm$ 3	200 $\pm$ 10	1.24	11.76

Table 8. (Continued) Effect of herbal drinks on the mutagenicity of nitrite treated menstrual regulatory and haematinic traditional preparation (drug 2) on *Salmonella typhimurium* TA98 without metabolic activation

Herbs	Amount of herbal drink ( $\mu$ l/plate)	No. of revertants/plate <sup>a</sup>		MI <sup>b</sup>	
		untreated drug	treated drug <sup>c</sup>	untreated drug	treated drug <sup>c</sup>
Bael fruit (มะตูม)	Spon <sup>d</sup>	15 $\pm$ 1	15 $\pm$ 1	1.00	1.00
	0 <sup>e</sup>	22 $\pm$ 3	127 $\pm$ 11	1.47	8.47
	3.5	21 $\pm$ 6	164 $\pm$ 32	1.40	10.93
	7	25 $\pm$ 7	149 $\pm$ 29	1.67	9.93
	14	19 $\pm$ 4	162 $\pm$ 20	1.27	10.80
Asiatic pennywort (บัวบก)	Spon <sup>d</sup>	19 $\pm$ 3	19 $\pm$ 3	1.00	1.00
	0 <sup>e</sup>	19 $\pm$ 4	139 $\pm$ 4	1.00	7.32
	3.5	19 $\pm$ 3	190 $\pm$ 13	1.00	10.00
	7	21 $\pm$ 5	202 $\pm$ 37	1.11	10.63
	14	25 $\pm$ 5	248 $\pm$ 8	1.32	13.05

<sup>a</sup> Data are expressed as means and standard deviations of four plates from two experiments.

<sup>b</sup> Mutagenicity index is calculated from the average value of number of histidine revertants / plate of drug divided by that of spontaneous.

<sup>c</sup> The reaction with nitrite was done in a mild acid solution containing 250  $\mu$ l of 0.5 M sodium nitrite and 100  $\mu$ l of concentrate of drug.

<sup>d</sup> Spontaneous.

<sup>e</sup> An average value of number of histidine revertants per plate of 100  $\mu$ l of concentrate of drug-nitrite mixture incubated for 4 h in the absence of herbal drinks.



**Table 9.** Effect of herbal drinks on the mutagenicity of nitrite treated menstrual regulatory and haematinic traditional preparation (drug 2) on *Salmonella typhimurium* TA100 without metabolic activation

Herbs	Amount of herbal drink ( $\mu$ l/plate)	No. of revertants/plate <sup>a</sup>		MI <sup>b</sup>	
		untreated drug	treated drug <sup>c</sup>	untreated drug	treated drug
Roselle (กระเจี๊ยบ)	Spon <sup>d</sup>	95 $\pm$ 7	95 $\pm$ 7	1.00	1.00
	0 <sup>e</sup>	93 $\pm$ 9	579 $\pm$ 27	0.98	6.09
	3.5	87 $\pm$ 5	596 $\pm$ 18	0.92	6.27
	7	94 $\pm$ 9	576 $\pm$ 30	0.99	6.06
	14	92 $\pm$ 6	596 $\pm$ 31	0.97	6.27
Chrysanthemum					
flower (เก๊กฮวย)	Spon <sup>d</sup>	80 $\pm$ 9	80 $\pm$ 9	1.00	1.00
	0 <sup>e</sup>	77 $\pm$ 10	497 $\pm$ 4	0.96	6.21
	3.5	80 $\pm$ 13	452 $\pm$ 83	1.00	5.65
	7	75 $\pm$ 9	554 $\pm$ 56	0.94	6.93
	14	90 $\pm$ 16	547 $\pm$ 78	1.13	6.84
Safflower (คำฝอย)	Spon <sup>d</sup>	81 $\pm$ 6	81 $\pm$ 6	1.00	1.00
	0 <sup>e</sup>	89 $\pm$ 9	612 $\pm$ 40	1.10	7.56
	3.5	89 $\pm$ 8	671 $\pm$ 67	1.10	8.28
	7	90 $\pm$ 8	565 $\pm$ 56	1.11	6.98
	14	116 $\pm$ 12	752 $\pm$ 51	1.43	9.28
Malberry leaves (ใบหม่อน)	Spon <sup>d</sup>	107 $\pm$ 13	107 $\pm$ 13	1.00	1.00
	0 <sup>e</sup>	109 $\pm$ 10	653 $\pm$ 65	6.11	6.10
	3.5	94 $\pm$ 8	779 $\pm$ 46	0.87	7.28
	7	96 $\pm$ 4	787 $\pm$ 56	0.90	7.36
	14	95 $\pm$ 9	754 $\pm$ 53	0.89	7.05

**Table 9.** (Continued) Effect of herbal drinks on the mutagenicity of nitrite treated menstrual regulatory and haematinic traditional preparation (drug 2) on *Salmonella typhimurium* TA100 without metabolic activation

Herbs	Amount of herbal drink ( $\mu$ l/plate)	No. of His <sup>+</sup> revertants/plate <sup>a</sup>		MI <sup>b</sup>	
		untreated drug	treated drug <sup>c</sup>	untreated drug	treated drug
Bael fruit (มะตูม)	Spon <sup>d</sup>	82 $\pm$ 1	82 $\pm$ 11	1.00	1.00
	0 <sup>e</sup>	74 $\pm$ 13	466 $\pm$ 60	0.90	5.68
	3.5	74 $\pm$ 3	513 $\pm$ 20	0.90	6.26
	7	83 $\pm$ 10	544 $\pm$ 81	1.01	6.63
	14	74 $\pm$ 7	548 $\pm$ 69	0.90	6.68
Asiatic Pennywort (บัวบก)	Spon <sup>d</sup>	81 $\pm$ 6	81 $\pm$ 6	1.00	1.00
	0 <sup>e</sup>	89 $\pm$ 9	612 $\pm$ 40	1.10	7.56
	3.5	90 $\pm$ 5	744 $\pm$ 84	1.11	9.19
	7	90 $\pm$ 8	874 $\pm$ 47	1.11	10.79
	14	116 $\pm$ 12	885 $\pm$ 17	1.43	10.93

<sup>a</sup> Data are expressed as means and standard deviations of four plates from two experiments.

<sup>b</sup> Mutagenicity index is calculated from the average value of number of histidine revertants / plate of drug divided by that of spontaneous.

<sup>c</sup> The reaction with nitrite was done in a mild acid solution containing 250  $\mu$ l of 0.5 M sodium nitrite and 100  $\mu$ l of concentrate of drug.

<sup>d</sup> Spontaneous.

<sup>e</sup> An average value of number of histidine revertants per plate of 100  $\mu$ l of concentrate of drug-nitrite mixture incubated for 4 h in the absence of herbal drinks.

Table 10. Percent modification of herbal drinks on the mutagenicity of nitrite treated menstrual regulatory and haematinic traditional preparation (drug 1) on *Salmonella typhimurium* TA98 and TA100 without metabolic activation

Herbs	Amount of herbal drink ( $\mu$ l/plate)	No.of revertants/plate <sup>a</sup>		MI <sup>b</sup>		Precent modification <sup>c</sup>	
		TA98	TA100	TA98	TA100	TA98	TA100
Roselle (กระเจี๊ยบ)	Spontaneous	19 $\pm$ 1	95 $\pm$ 7	1.00	1.00		
	0 <sup>d</sup>	134 $\pm$ 15	538 $\pm$ 37	7.05	5.66		
	3.5	134 $\pm$ 16	584 $\pm$ 19	7.05	6.15	0	-10.38
	7	141 $\pm$ 21	558 $\pm$ 35	7.42	5.87	-6.09	-4.51
	14	138 $\pm$ 13	597 $\pm$ 21	7.26	6.28	-3.48	-13.32
Chrysanthemum flower (เก๊กฮวย)	Spontaneous	20 $\pm$ 6	80 $\pm$ 9	1.00	1.00		
	0 <sup>d</sup>	111 $\pm$ 67	586 $\pm$ 68	5.55	7.33		
	3.5	139 $\pm$ 17	589 $\pm$ 42	6.95	7.36	-30.77	-0.59
	7	145 $\pm$ 16	570 $\pm$ 21	7.25	7.12	-37.36	+3.16
	14	172 $\pm$ 20	600 $\pm$ 65	8.60	7.50	-67.03	-2.77
Safflower (คำฝอย)	Spontaneous	19 $\pm$ 3	81 $\pm$ 6	1.00	1.00		
	0 <sup>d</sup>	80 $\pm$ 9	589 $\pm$ 50	4.21	7.27		
	3.5	87 $\pm$ 6	535 $\pm$ 36	4.58	6.60	-11.48	+10.63
	7	89 $\pm$ 6	604 $\pm$ 49	4.68	7.46	-14.75	-2.95
	14	101 $\pm$ 21	657 $\pm$ 34	5.32	8.11	-34.43	-13.39
Malberry leaves (ใบหม่อน)	Spontaneous	17 $\pm$ 4	107 $\pm$ 13	1.00	1.00		
	0 <sup>d</sup>	107 $\pm$ 7	657 $\pm$ 25	6.29	6.14		
	3.5	104 $\pm$ 9	709 $\pm$ 67	6.12	6.63	-3.33	-9.45
	7	123 $\pm$ 10	797 $\pm$ 50	7.24	7.45	-17.78	-25.45
	14	116 $\pm$ 17	769 $\pm$ 26	6.82	7.19	-10.00	-20.36

**Table 10.** (Continued) Percent modification of herbal drinks on the mutagenicity of nitrite treated menstrual regulatory and haematinic traditional preparation (drug 1) on *Salmonella typhimurium* TA98 and TA100 without metabolic activation

Herbs	Amount of herbal drink ( $\mu$ l/plate)	No.of revertants/plate <sup>a</sup>		MI <sup>b</sup>		Percent modification <sup>c</sup>	
		TA98	TA100	TA98	TA100	TA98	TA100
Bael fruit (มะตูม)	Spontaneous	15 $\pm$ 1	82 $\pm$ 6	1.00	1.00		
	0	90 $\pm$ 3	367 $\pm$ 77	6.00	4.48		
	3.5	81 $\pm$ 31	402 $\pm$ 73	5.40	4.90	+12.00	-12.28
	7	79 $\pm$ 30	500 $\pm$ 82	5.27	6.10	+14.67	-46.67
	14	103 $\pm$ 42	572 $\pm$ 51	6.87	6.98	-17.33	-71.92
Asiatic Pennywort (บัวบก)	spontaneous	19 $\pm$ 3	81 $\pm$ 6	1.00	1.00		
	0 <sup>d</sup>	80 $\pm$ 9	589 $\pm$ 49	4.21	7.27		
	3.5	111 $\pm$ 23	674 $\pm$ 35	5.84	8.32	-50.81	-16.73
	7	145 $\pm$ 26	835 $\pm$ 47	7.63	0.31	-106.56	-48.46
	14	126 $\pm$ 3	779 $\pm$ 74	6.63	9.62	-75.41	-37.40

<sup>a</sup> Data are expressed as means and standard deviations of four plates from two experiments.

<sup>b</sup> Mutagenicity index is calculated from the average value of number of histidine revertants / plate of sample divided by that of spontaneous.

<sup>c</sup> - or + indicates that the extract increased or decreased the mutagenicity of the model, respectively.

<sup>d</sup> An average value of number of histidine revertants per plate of 100  $\mu$ l of concentrate of drug-nitrite mixture incubated for 4 h in the absence of herbal drinks.

Table 11. Percent modification of herbal drinks on the mutagenicity of nitrite treated menstrual regulatory and haematinic traditional preparation (drug 2) on *Salmonella typhimurium* TA98 and TA100 without metabolic activation

Herbs	Amount of herbal drink ( $\mu$ /plate)	No. of revertants/plate <sup>a</sup>		MI <sup>b</sup>		Percent modification <sup>c</sup>	
		TA98	TA100	TA98	TA100	TA98	TA100
Roselle (กระเจี๊ยบ)	Spontaneous	18 $\pm$ 3	95 $\pm$ 7	1.00	1.00		
	0 <sup>d</sup>	159 $\pm$ 5	579 $\pm$ 27	8.83	6.09		
	3.5	167 $\pm$ 8	596 $\pm$ 18	9.28	6.27	-5.67	-3.51
	7	168 $\pm$ 17	576 $\pm$ 30	9.33	6.06	-6.38	+0.62
	14	169 $\pm$ 12	596 $\pm$ 31	9.39	6.27	-7.09	-3.51
Chrysanthemum flower (เก๊กฮวย)	Spontaneous	20 $\pm$ 6	80 $\pm$ 9	1.00	1.00		
	0 <sup>d</sup>	204 $\pm$ 15	497 $\pm$ 4	10.20	6.21		
	3.5	315 $\pm$ 30	452 $\pm$ 83	15.75	5.65	-60.33	+10.79
	7	295 $\pm$ 18	554 $\pm$ 56	14.75	6.93	-49.46	-13.67
	14	297 $\pm$ 48	547 $\pm$ 78	14.85	6.84	-50.54	-11.99
Safflower (คำฝอย)	Spontaneous	19 $\pm$ 3	81 $\pm$ 6	1.00	1.00		
	0 <sup>d</sup>	139 $\pm$ 4	612 $\pm$ 40	7.32	7.56		
	3.5	165 $\pm$ 17	671 $\pm$ 67	8.68	8.28	-21.67	-11.11
	7	177 $\pm$ 8	565 $\pm$ 56	9.32	6.98	-31.67	+8.85
	14	223 $\pm$ 30	752 $\pm$ 51	11.74	9.28	-70.00	-26.37
Malberry leaves (ใบหม่อน)	Spontaneous	17 $\pm$ 4	107 $\pm$ 13	1.00	1.00		
	0 <sup>d</sup>	165 $\pm$ 3	653 $\pm$ 65	9.71	6.10		
	3.5	181 $\pm$ 3	779 $\pm$ 46	10.65	7.28	-10.81	-23.08
	7	187 $\pm$ 11	787 $\pm$ 56	11.00	7.36	-14.86	-24.54
	14	200 $\pm$ 10	754 $\pm$ 53	11.76	7.05	-23.65	-18.49

**Table 11.** (Continued) Percent modification of herbal drinks on the mutagenicity of nitrite treated menstrual regulatory and haematinic traditional preparation (drug 2) on *Salmonella typhimurium* TA98 and TA100 without metabolic activation

Herbs	Amount of herbal drink ( $\mu$ l/plate)	No. of His <sup>+</sup> revertants/plate <sup>a</sup>		MI <sup>b</sup>		Percent modification <sup>c</sup>	
		TA98	TA100	TA98	TA100	TA98	TA100
Bael fruit (มะตูม)	Spontaneous	15 $\pm$ 1	82 $\pm$ 11	1.00	1.00		
	0 <sup>d</sup>	127 $\pm$ 11	466 $\pm$ 60	8.47	5.68		
	3.5	164 $\pm$ 32	513 $\pm$ 20	10.93	6.26	-33.04	-12.24
	7	149 $\pm$ 29	544 $\pm$ 81	9.93	6.63	-19.64	-20.31
	14	162 $\pm$ 20	548 $\pm$ 69	10.80	6.68	-31.25	-21.35
Asiatic Pennywort (ผักแว่น)	spontaneous	19 $\pm$ 3	81 $\pm$ 6	1.00	1.00		
	0 <sup>d</sup>	139 $\pm$ 4	612 $\pm$ 40	7.32	7.56		
	3.5	190 $\pm$ 13	744 $\pm$ 84	10.00	9.19	-42.50	-24.86
	7	202 $\pm$ 37	874 $\pm$ 17	10.63	10.79	-52.50	-49.34
	14	248 $\pm$ 8	885 $\pm$ 17	13.05	10.93	-90.83	-51.41

<sup>a</sup> Data are expressed as means and standard deviations of four plates from two experiments.

<sup>b</sup> Mutagenicity index is calculated from the average value of number of histidine revertants / plate of sample divided by that of spontaneous.

<sup>c</sup> – or + indicates that the extract increased or decreased the mutagenicity of the model, respectively.

<sup>d</sup> An average value of number of histidine revertants per plate of 100  $\mu$ l of concentrate of drug-nitrite mixture incubated for 4 h in the absence of herbal drinks.