CHAPTER IV

RESULTS

4.1 Antioxidant activity of plant extracts

4.1.1 *P. mirifica*

4.1.1.1 Antioxidant activity of *P. mirifica*

P. mirifica collected from 28 provinces in Thailand showed significant variation of IC_{50} (Table 4.1, Figure 4.1). *P. mirifica* from Uthai Thani showed highest antioxidant activity with IC_{50} of 2,470.38 μg/ml. The sample from Kamphaeng Phet showed lowest antioxidant activity with IC_{50} of 3,376.97 μg/ml. The Mean value of antioxidant activity of *P. mirifica* population (2,904. 52 μg/ml) was compared with individual sample. The results revealed that 6 sample exhibited significant higher antioxidant activity and 8 samples showed significant lower antioxidant activity than the Mean value of *P. mirifica* population ($p < 0.05$). Antioxidant activity of *P. lobata* (2,482.00 μg/ml) showed significant higher than the Mean value of *P. mirifica* population ($p < 0.05$). Furthermore, antioxidant activity of *P.lobata* was compared with each of *P. mirifica* sample. The results revealed that 18 samples exhibited significant lower antioxidant activity than *P. lobata* while the other provinces showed no significant different ($p<0.05$). Nevertheless, *P. lobata* showed obviously significant lower antioxidant activity than α-tocopherol.

Table 4.1 Ranked antioxidant activity of *P. mirifica* express in term of IC_{50} (μg/ml) in comparison with *P. lobata* and α-tocopherol

No.	Sources (Provinces)	IC_{50} (μg/ml)
1	Uthai Thani	2,470.38±37.81*
2	Nong Bua Lam Phu	2,489.98±27.62*
3	Phetchaburi	2,492.61± 83.02*
4	Phitsanulok	2,529.47±46.92*
5	Phetchabun	2,563.71+86.35*
6	Ratchaburi	2,612.14± 34.75*
7	Chiang Mai	2,648.18±22.01
8	Nakhon Sawan	2,656.94±94.96
9	Lamphun	2,680.74± 83.70
10	Nan	2,726.04 ± 81.03
11	Chiang Rai	2,817.63± 40.06[a]
12	Sukhothai	2,889.48±70.73[a]
13	Kanchanaburi	2,893.51±159.05[a]
14	Lop Buri	2,900.25±23.03[a]
15	Phrae	2,915.64±186.91[a]
16	Chumphon	2,928.93±93.89[a]
17	Nakhon Ratchasima	3,042.79±31.20[a]
18	Sakon Nakhon	3,070.63±157.95[a]
19	Phayoa	3,076.20±113.01[a]
20	Uttharadith	3,117.09±143.44[a]
21	Lampang	3,182.16±88.66**[a]
22	Tak	3,192.05±23.17**[a]
23	Chaiyaphum	3,197.86±119.83**[a]
24	Mae Hong Son	3,205.37±51.14**[a]
25	Prachuap Khiri Khan	3205.84±114.92**[a]
26	Prachin Buri	3,209.30±102.50**[a]
27	Saraburi	3,234.58 ±141.55**[a]
28	Kamphaeng Phet	3,376.97±69.96**[a]
Mean ± S.E.		2,904.52±33.24
P. lobata		2,482.00 ± 66.11***
α-tocopherol		68.02 ± 5.52**** [b]

IC_{50} value; the concentration of sample required to scavenge 50% DPPH free radicals s under experimental conditions, Mean±S.E.

*, ***, ****; IC_{50} value was less than the Mean value of *P. mirifica* population.

**; IC_{50} value was more than the Mean value of *P. mirifica* population.

a; IC_{50} value was more than *P. lobata* .

b; IC_{50} value was less than *P. lobata.*

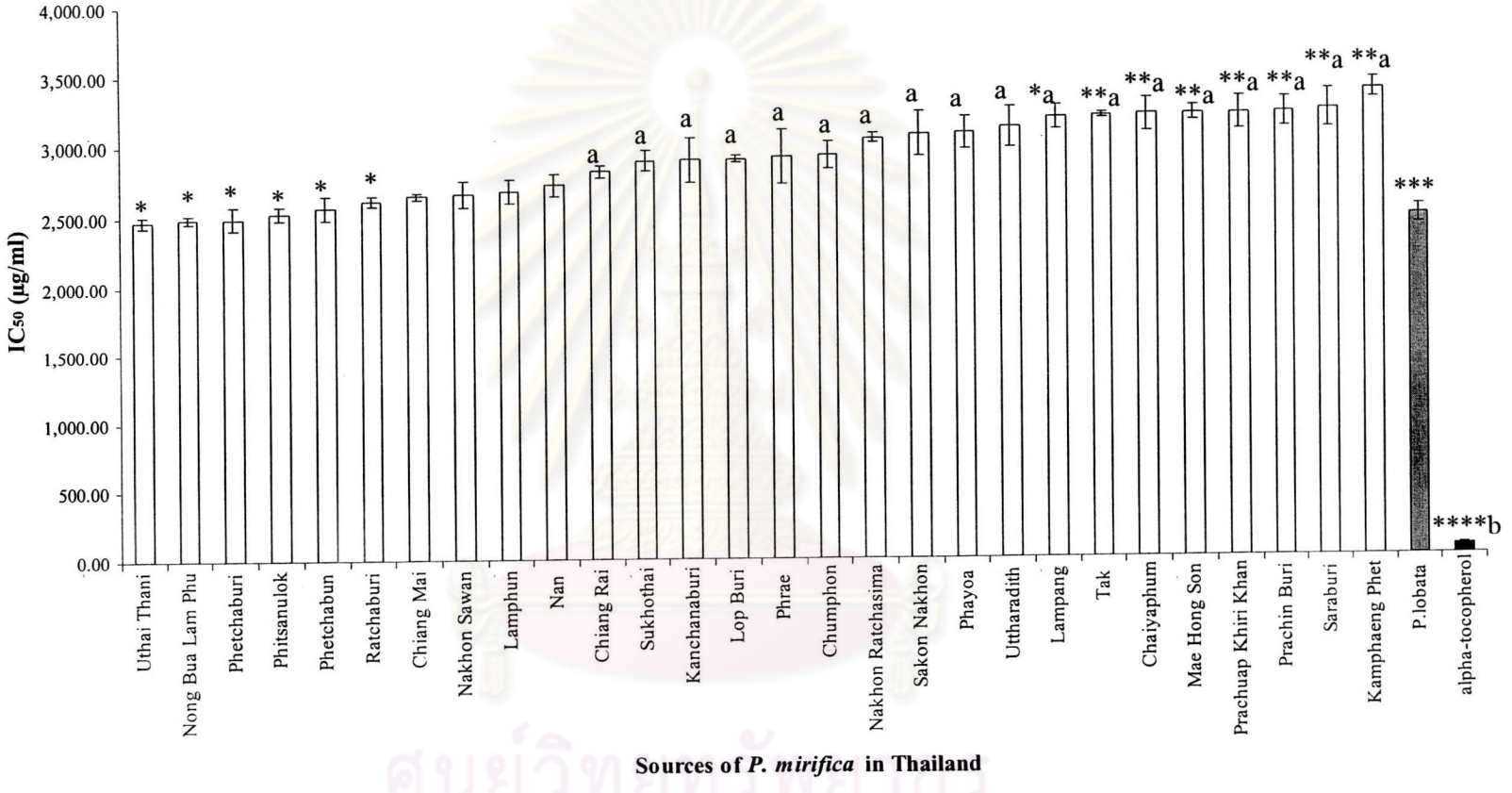

Figure 4.1 Antioxidant activity of *P. mirifica* from 28 provinces in Thailand, Significant difference from control groups ($p<0.05$) (Mean±S.E.). *, ***, ****; IC_{50} value was less than the Mean value of *P. mirifica* population. **; IC_{50} value was more than the Mean value of *P. mirifica* population.a; IC_{50} value was more than *P. lobata*,. b; IC_{50} value was less than *P. lobata.*

4.1.1.2 Percent inhibition of the free radicals of *P.mirifica*

The percent inhibition (PI) was calculated for inhibiting the free radicals of *P. mirifica* collected from 28 provinces (Table 4.2). The effective concentrations were in the range of 75-300 μg/ml. The results revealed that *P. mirifica* was able to inhibit DPPH free radicals. The dose of 300 μg/ml were evaluated for the highest and lowest PI. *P. mirifica* extract from Uthai Thani showed the highest PI value and the sample from Kamphaeng Phet showed the lowest PI value. Seven provinces of samples showed significant higher PI value whereas 5 samples showed significant lower PI value than the Mean PI value of *P.mirifica* population. *P. lobata* showed significant less PI value than *P .mirifica* population aswell as *P. mirifica* colleted from 21 provincs.

Table 4.2 Percent inhibition (PI) of α-tocopherol

Sources	PI value					
	Concentration of the extract (μg/ml)					
	12.5	**25**	**50**	**100**	**150**	**200**
α-tocopherol	16.66±0.48	26.11±1.2	44.51±0.45	79.99±0.82	85.96±0.25	88.40±0.37^{****b}

****, PI value was less than the Mean value of *P. mirifica* population in Table 4.3

Table 4.3 Percent inhibition (PI) of *P. mirifica* in the concentration range of 75-300 μg/ml

No.	Sources (Provinces)	PI value					
		Concentration of the extracts (μg/ml)					
		75	112.5	150	187.5	225	300
1	Uthai Thani	10.23±0.85	10.88±0.15	12.33±0.19	13.36±0.21	14.78±0.18	16.99±0.36*
2	Nong Bua Lam Phu	10.90±0.64	10.41±0.32	12.44±0.06	13.03±0.25	14.36±0.35	16.41±0.32*
3	Phetchaburi	10.26±0.38	10.66±0.47	11.82±0.29	13.56±0.21	14.01±0.58	16.15±0.53*
4	Phitsanulok	10.01±0.55	9.57±0.43	11.12±0.37	12.26±0.73	13.87±0.24	15.52±0.25*
5	Phetchabun	10.50±0.24	10.84±0.17	12.13±0.34	12.60±0.19	13.84±0.14	15.93+0.65*
6	Ratchaburi	10.12±0.37	9.70±0.38	11.40±0.67	12.65±0.45	13.27±0.29	14.99±0.07*
7	Chiang Mai	11.06±0.49	11.38±0.36	12.55±0.27	13.70±0.21	14.58±0.15	16.66±0.31*
8	Nakhon Sawan	12.19±0.52	11.34±0.25	12.71±0.40	13.39±0.34	14.09±0.23	15.89±0.34*
9	Lamphun	9.41±0.47	8.57±0.64	9.38±0.58	10.23±0.29	9.80±0.57	14.00±0.44[a]
10	Nan	9.31±1.07	10.97±0.42	10.68±0.58	11.94±0.23	12.77±0.02	14.26±0.41[a]
11	Chiang Rai	9.79±0.18	8.81±0.49	9.93±0.64	11.33±0.73	11.76±0.29	12.91±0.48[a]
12	Sukhothai	8.02±0.51	9.33±0.09	9.07±0.36	10.10±0.46	11.08±0.59	12.57±0.51[a]
13	Kanchanaburi	12.22±1.20	12.18±0.34	14.01±0.48	13.96±0.21	14.32±0.91	13.94±1.50[a]
14	Lop Buri	8.38±1.90	8.32±0.45	9.31±0.56	10.45±0.46	10.99±0.33	12.39±0.55[a]
15	Phrae	7.35±0.86	6.47±1.30	7.07±1.50	9.33±0.85	9.31±1.40	11.83±1.6[a]
16	Chumphon	10.39±0.29	9.44±0.34	10.40±0.07	11.90±0.67	12.58±0.67	12.95±0.27[a]
17	Nakhon Ratchasima	7.31±0.23	6.12±0.17	7.42±0.53	8.81±0.58	9.33±0.22	10.83±0.30**[a]
18	Sakon Nakhon	7.49+0.80	7.97+0.26	8.17+0.22	8.71+0.19	9.50±0.61	11.17±1.20[a]
19	Phayoa	8.92±0.07	9.02±0.36	9.58±0.14	9.44±0.40	10.62±0.15	12.38±0.38[a]
20	Uttharadith	10.57±0.16	9.37±0. 33	9.86±0.65	11.34±0.30	11.22±0.08	12.67±0.66[a]
21	Lampang	8.10±0.03	9.45±0.30	8.53±0.98	10.35±0.12	10.63±0.72	10.33±1.20**[a]
22	Tak	8.78±0.20	8.96±1.56	9.86±1.19	8.69±0.61	9.94±0.34	11.00±0.30[b]
23	Chaiyaphum	8.12±1.01	7.90±0.64	8.65±0.75	9.72±0.71	9.88±0.52	10.88±0.04**[a]
24	Mae Hong Son	8.88±0.27	8.61±0.27	8.34±0.75	9.54±0.52	10.37±0.22	11.40±0.36[a]
25	Prachuap Khiri Khan	7.27±0.78	6.42±0.53	7.21±0.71	8.10±0.64	9.38±0.53	11.02±1.50**[a]
26	Prachin Buri	9.30±0.34	8.27±0.50	8.14±0.52	8.85±0.73	10.24±0.83	10.90±0.10[a]
27	Saraburi	9.15±1.20	8.58±0.53	9.60±0.62	10.63±0.11	10.08±0.46	11.94±0.52[a]
28	Kamphaeng Phet	6.66±0.89	5.55±0.12	7.90±1.20	7.57±0.55	7.26±1.23	6.36±1.49**[a]
Mean ± S.E.		**9.29±0.18[A]**	**9.17±.018[A]**	**10.01±0.21[B]**	**10.90±0.21[C]**	**11.61±0.23[D]**	**12.97±0.28[Eb]**
P. lobata		9.30±0.34	12.18±0.15	13.49±0.18	13.93±0.24	15.77±0.29	16.95±0.21***

*,***,****, PI value was less than the Mean value of *P. mirifica* population

**, PI value was more than the Mean value of *P. mirifica* population

a; the PI value was more than *P. lobata*

b; the PI value was less than *P. lobata*

A, B, C, D, E; PI value was significant different ($p<0.05$)

4.1.1.3 Correlation of antioxidant activity and isoflavone content of *P. mirifica*

The chemical isoflavone contents of *P. mirifica* tuber consist of puerarin, daidzin, genistin, daidzein and genistein analysis by HPLC (Subtang, 2002) (Table 4.4-4.5). To establish indirect correlation between antioxidant activity and isoflavone content, the Mean value of the amount of isoflavone of the first 6 highest antioxidant activities, namely Uthai Thani, Nong Bua Lam Phu, Phetchaburi, Phitsanulok, Phetchabun and Ratchaburi was compared with the left 22 samples. It was found that only daidzein and genistein content were significant between the test groups (Table 4.6).

Table 4.4 IC_{50} value of the first 6 highest antioxidant activity in correlation with isoflavone content (%) (Subtang, 2002)

No.	Sources (Provinces)	IC_{50} (µg/ml)	Puerarin (%)	Daidzin (%)	Genistin (%)	Daidzein (%)	Genistein (%)
1	Uthai Thani	2,470.38±37.81	10.53±0.55	21.17±0.66	48.73±0.77	15.99±0.49	3.57±0.06
2	Nong Bua Lam Phu	2,489.98±27.62	21.74±3.30	20.25±4.31	41.51±4.95	13.10±1.96	3.38±0.60
3	Phetchaburi	2,492.61±83.02	16.76±0.13	26.37±1.32	47.78±1.44	7.64±0.36	1.44±0.02
4	Phitsanulok	2,526.47±46.92	41.94±0.34	14.60±0.21	31.58±0.23	9.95±0.06	1.94±0.02
5	Phetchabun	2,563.71±86.35	20.96±0.42	23.46±1.78	34.52±2.44	18.13±0.72	2.90±0.11
6	Ratchaburi	2,612.14±34.75	10.43±0.10	18.14±0.44	60.32±0.27	8.08±0.66	3.01±0.28
Mean ± S.E.		**2,525.88±21.86***	**20.39±2.61**	**20.66±1.13**	**44.07±2.46**	**12.15±1.00**	**2.70±0.20**

Table 4.5 Isoflavone content (%) in 22 *P. mirifica* samples with lower antioxidant activity than the first 6 highest antioxidant activity (Subtang, 2002)

No.	Sources (Provinces)	IC_{50} (µg/ml)	Puerarin (%)	Daidzin (%)	Genistin (%)	Daidzein (%)	Genistein (%)
7	Chiang Mai	2,648.18±22.01	21.51±1.26	22.77±1.40	45.17±3.60	8.06±1.21	2.48±11.26
8	Nakhon Sawan	2,656.94+94.96	21.15±1.55	25.81±1.37	44.41±2.76	7.46±0.27	1.15±0.50
9	Lamphun	2,680.74±83.70	21.40±0.59	18.28±0.27	54.28±0.37	5.54±0.02	0.49±0.22
10	Nan	2,726.04±81.03	28.37±1.54	12.61±0.92	40.04±4.17	17.69±1.47	1.31±0.25
11	Chiang Rai	2,817.63±40.06	32.89±1.42	14.19±1.97	48.58±3.06	3.55±0.07	0.79±0.44
12	Sukhothai	2,889.48±70.73	13.76±0.51	24.45±0.22	50.20±0.71	10.86±0.30	0.72±0.25
13	Kanchanaburi	2,893.51±15.05	9.14±0.54	21.26±0.89	58.79±1.24	8.61±0.33	2.19±0.09
14	Lop Buri	2,900.25±23.03	28.13±0.78	9.94±0.56	57.09±0.73	3.41±1.01	1.41±0.06
15	Phrae	2,915.64±18.91	38.46±1.59	11.57±0.67	42.99±3.02	5.24±1.24	1.73±0.18
16	Chumphon	2,928.93±93.89	16.15±1.09	14.13±1.09	63.93±2.43	4.98±0.03	0.80±0.28
17	Nakhon Ratchasima	3,042.79±31.20	29.72±2.67	12.69±0.22	54.46±1.68	2.66±0.79	0.46±0.42
18	Sakon Nakhon	3,070.63±15.95	72.80±0.29	9.56±0.07	12.40±0.16	4.00±0.29	1.19±0.10
19	Phayoa	3,076.20±13.01	22.41±1.14	14.68±0.57	56.41±1.11	5.25±0.45	1.25±0.49
20	Uttharadith	3,117.09±14.34	48.71±0.09	22.04±0.03	16.54±0.04	12.69±0.08	0.00±0.00
21	Lampang	3,182.16±88.66	37.71±0.77	18.09±0.26	36.29±0.60	6.23±0.01	1.67±0.10
22	Tak	3,192.05±23.17	20.35±0.97	13.89±0.30	48.18±1.23	15.63±0.69	1.93±0.13
23	Chaiyaphum	3,197.86±19.83	23.32±1.97	19.15±0.69	43.95±1.31	10.64±1.83	2.92±0.85
24	Mae Hong Son	3,205.37±51.14	26..80±0.90	20.15±0.81	33.52±1.53	16.27±0.68	3.23±0.10
25	Prachuap Khiri Khan	3,205.84±114.92	23.57±0.76	20.97±1.64	44.48±0.59	9.05±0.39	1.92±0.31
26	Prachin Buri	3,209.30±102.50	26.48±0.79	27.80±1.30	35.51±0.86	9.12±1.21	1.07±0.18
27	Saraburi	3,234.58±141.55	14.80±4.42	35.76±12.58	42.95±8.44	4.56±0.42	1.89±0.42
28	Kamphaeng Phet	3,376.97±69.96	35.66±2.98	16.65±4.03	41.26±7.47	5.33±0.26	1.08±0.30
Mean±S.E.		**3,007.00±64.00**	**27.80±1.66**	**18.47±0.93**	**44.15±1.60**	**8.04±0.54**	**1.44±0.11**

Table 4.6 Mean of isoflavone content (%) in first 6 higest antioxidant compared with 22 *P. mirifica* samples

Groups	IC_{50} (µg/ml)	Puerarin (%)	Daidzin (%)	Genistin (%)	Daidzein (%)	Genistein (%)
Mean±S.E. of 6 *P. mirifica*	2,525.88±21.86*	20.39±2.61	20.66±1.13	44.07±2.46	12.15±1.00*	2.70±0.20*
Mean±S.E. of 22 *P. mirifica*	3,007.00±64.00	27.80±1.66	18.47+0.93	44.15±1.60	8.04±0.54	1.44±0.11

6 *P. mirifica*; the first 6 highest antioxidant activity *P. mirifica*
22 *P. mirifica*; the left 22 *P. mirifica* with lower antioxidant activity

4.1.1.4 Correlation of antioxidant activity and isoflavone glycoside and aglycoside contents of *P.mirifica*

The percentage of the amount of isoflavone glycoside, with glucose binding (daidzin plus genistin) and isoflavone aglycoside, without glucose binding (daidzein plus genistein) of the first 6 highest antioxidant activities, including Uthai Thani, Nong Bua Lam Phu, Phetchaburi, Phitsanulok, Phetchabun and Ratchaburi was compared with the left 22 samples (Table 4.7). It was found that isoflavone aglycoside content were significant between the test groups. The isoflavone content of *P. lobata* showed no significant different when compared with isoflavone content of *P. mirifica*.

Table 4.7 Isoflavone glycoside and aglycoside content (%) of the first 6 highest antioxidant activity *P. mirifica* (Subtang, 2002)

No.	Sources (Provinces)	IC_{50} (µg/ml)	Isoflavone glycoside (%)	Isoflavone aglycoside (%)
1	Uthai Thani	2,470.38±37.81	78.12±0.65	21.87±0.65
2	Nong Bua Lam Phu	2,489.98±27.62	79.14±2.46	20.86±2.46
3	Phetchaburi	2,492.61±83.02	89.08±0.48	10.91±0.48
4	Phitsanulok	2,526.47±46.92	79.53±0.48	20.46±0.26
5	Phetchbun	2,563.71±86.35	73.38±0.97	26.61±0.97
6	Ratchaburi	2,612.14±34.75	87.61±0.84	12.39±0.84
Mean±S.E.		**2,525.88±21.86***	**81.14±1.39**	**18.85±1.39***
P. lobata		2,482.00±66.11	81.06±0.65	18.93±0.65

*; Significant difference as compared with the Mean of 22 samples of *P. mirifica*

Table 4.8 Isoflavone glycoside and aglycoside content (%) in 22 *P. mirifica* samples with lower antioxidant activity than the first 6 highest antioxidant activity (Subtang, 2002)

No.	Sources (Provinces)	IC_{50} (µg/ml)	Isoflavone glycoside (%)	Isoflavone aglycoside (%)
7	Chiang Mai	2,648.18±22.01	86.51±2.06	13.48±2.06
8	Nakhon Sawan	2,656.94±94.96	89.06±0.39	10.93±0.39
9	Lamphun	2,680.74±83.70	92.33±0.26	7.67±0.26
10	Nan	2,726.04±81.03	73.34±3.02	26.65±3.02
11	Chiang Rai	2,817.63±40.06	93.54±0.47	6.46±0.47
12	Sukhothai	2,889.48±70.73	86.56±0.14	13.43±0.14
13	Kanchanaburi	2,893.51±15.05	88.11±0.50	11.89±0.50
14	Lop Buri	2,900.25±23.03	93.29±1.42	6.71±1.42
15	Phrae	2,915.64±18.91	88.54±2.36	11.46±2.36
16	Chumphon	2,928.93±93.89	93.09±0.43	6.91±0.43
17	Nakhon Ratchasima	3,042.79±31.20	95.69±1.62	4.31±1.62
18	Sakon Nakhon	3,070.63±15.95	80.95±1.19	19.04±1.19
19	Phayoa	3,076.20±13.01	91.64±1.13	8.35±1.13
20	Uttharadith	3,117.09±14.34	75.25±0.12	24.75±0.12
21	Lampang	3,182.16±88.66	87.30±0.30	12.69±0.30
22	Tak	3,192.05±23.17	77.93±0.81	22.06±0.81
23	Chaiyaphum	3,197.86±19.83	82.45±2.95	17.54±2.95
24	Mae Hong Son	3,205.37±51.14	73.34±0.89	26.65±0.89
25	Prachuap Khiri Khan	3,205.84±114.92	85.62±1.04	14.37±1.04
26	Prachin Buri	3,209.30±102.50	86.09±1.61	13.90±1.61
27	Saraburi	3,234.58±141.55	92.38±0.53	7.61±0.53
28	Kamphaeng Phet	3,376.97±69.96	89.89±1.37	10.10±1.37
Mean±S.E.		**3,007±0.64**	**86.49±0.83**	**13.50±0.84**
P. lobata		2,482.00±66.11	81.06±0.65	18.93±0.78

*; Significant difference as compared with the Mean of 6 samples of *P. mirifica*

Table 4.9 Isoflavone contents of *P. mirifica* from 28 provinces in comparision with *P. lobata* (Subtang, 2002)

No.	Sources (Provinces)	IC_{50} (µg/ml)	Puerarin (Mean+S.E)	Daidzin (Mean+S.E)	Genistin (Mean+SE)	Daidzein (Mean+SE)	Genistein (Mean+ SE.)	Total Isoflavone (Mean+SE.)
1	Uthai Thani	2,470.38±37.81*	10.85±1.01^{**b}	21.70±0.84*	50.17±3.57^{*a}	16.48±1.35^{**a}	3.66±0.16^{*a}	102.86±6.53^{*a}
2	Nong Bua Lam Phu	2,489.98±27.62*	12.65±2.42^{**b}	11.91±3.02^{b}	23.65±2.14	7.46±0.96^{*b}	1.91±0.25^{*a}	57.58±3.61
3	Phetchaburi	2,492.61± 83.02*	13.19±0.45^{**b}	20.82±1.78*	37.56±1.33^{*a}	6.00±0.24^{b}	1.13±0.04	78.71±3.15^{*a}
4	Phitsanulok	2,529.47±46.92*	32.24±1.06*	12.26±0.13^{b}	26.53±0.57	8.36±0.23^{*b}	1.63±0.05^{*a}	84.02±1.91^{*a}
5	Phetchabun	2,563.71±86.35*	9.40±0.45^{**a}	10.48±0.67^{b}	15.53±1.60^{**b}	8.10±0.04^{*b}	1.29±0.01	35.42±1.31^{**b}
6	Ratchaburi	2,612.14± 34.75*	8.85±0.36^{**b}	15.39±0.79^{**b}	51.15±1.75^{*a}	6.84±0.53^{b}	2.54±0.15^{*a}	84.77±2.67^{*a}
7	Chiang Mai	2,648.18±22.01	11.66±0.63^{**b}	12.37±0.58^{b}	24.87±3.25	4.33±0.41^{b}	1.34±0.11^{a}	54.58±3.08**
8	Nakhon Sawan	2,656.94±94.96	13.34±1.46^{**b}	16.28±1.64^{*a}	27.71±0.75	4.70±0.37^{b}	0.72±0.32	62.75±3.24
9	Lamphun	2,680.74± 83.70	33.18±0.92*	28.35±0.68^{*a}	84.13±0.54^{*a}	8.59±0.09^{*b}	0.76±0.36	155.00±1.42^{*a}
10	Nan	2,726.04 ± 81.03	5.32±0.44^{**b}	2.36±0.22^{**b}	7.62±1.36^{**b}	3.31±0.31^{**b}	0.24±0.04$^{**}_{b}$	18.85±1.92^{**b}
11	Chiang Rai	2,817.63± 40.06^{a}	20.02±1.42^{b}	8.61±1.12^{**b}	29.58±2.43	2.16±0.08^{**b}	0.50±0.28**	60.87±3.30
12	Sukhothai	2,889.48±70.73^{a}	14.12±0.92^{**b}	25.09±1.50^{*a}	51.43±2.40^{*a}	11.16±0.85*	0.73±0.23	102.52±5.35^{*a}
13	Kanchanaburi	2,893.51±159.05^{a}	7.15±0.51^{**b}	16.61±0.70^{*b}	45.93±0.75^{*a}	6.73±0.32^{b}	1.71±0.98^{*a}	78.13±0.94^{**a}
14	Lop Buri	2,900.25±23.03^{a}	19.50±1.44^{b}	6.84±0.09^{**b}	39.47±1.65^{*a}	2.42±0.79^{**b}	0.98±0.09	69.21±3.77^{a}
15	Phrae	2,915.64±186.91^{a}	17.47±2.21^{b}	5.34±1.00^{**b}	19.62±2.87**	2.30±0.39^{**b}	0.81±0.19	45.55±5.89^{**b}
16	Chumphon	2,928.93±93.89^{a}	8.45±0.22^{**b}	7.38±1.11^{**b}	34.17±4.81^{a}	2.64±0.26^{**b}	0.40±0.17**	53.04±5.30**
17	Nakhon Ratchasima	3,042.79±31.20^{a}	13.09±0.77^{**b}	5.61±0.07^{**b}	24.15±1.42	1.20±0.37^{**b}	0.21±0.2^{**b}	44.27±1.27^{**b}
18	Sakon Nakhon	3,070.63±157.95^{a}	87.05±0.79^{*a}	11.48±0.21^{b}	14.83±0.22^{**b}	4.78±0.37^{b}	1.42±0.14^{a}	119.57±1.39^{*a}
19	Phayoa	3,076.20±113.01^{a}	12.91±0.99^{**b}	8.46±0.62^{**b}	32.43±1.35	3.03±0.36^{**b}	0.73±0.30	57.56±3.02
20	Uttharadith	3,117.09±143.44^{a}	30.25±0.44**	13.69±0.21^{b}	10.27±0.19^{**b}	7.88±0.18^{*b}	0.00±0.0^{**b}	62.08±1.03
21	Lampang	3,182.16±88.66^{**a}	34.65±0.72*	16.59±0.08^{*b}	33.30±0.08^{a}	5.72±0.09^{b}	1.53±0.12^{a}	91.79±1.72^{*a}
22	Tak	3,192.05±23.17^{**a}	12.58±1.45^{**b}	8.55±0.73^{**b}	29.48±0.67	9.60±0.81*	1.20±0.15	61.42±4.41
23	Chaiyaphum	3,197.86±119.83^{**a}	15.83±2.43^{b}	12.91±1.44^{b}	29.48±2.33	7.02±0.89^{b}	1.89±0.42^{*a}	67.13±5.47
24	Mae Hong Son	3,205.37±51.14^{**a}	10.40±1.03^{**b}	7.82±0.81^{**b}	12.90±0.58^{**b}	6.26±0.23^{b}	1.25±0.92	38.65±2.53^{**b}
25	Prachuap Khiri Khan	3205.84±114.92^{**a}	9.29±0.61^{**b}	8.21±0.38^{**b}	17.50±2.66^{**b}	3.57±0.27^{**b}	0.76±0.14	39.33±1.36^{**b}
26	Prachin Buri	3,209.30±102.50^{**a}	12.42±0.26^{**b}	13.05±0.65^{b}	16.69±0.78^{**b}	4.28±0.56^{b}	0.51±0.09**	46.94±1.12^{**b}
27	Saraburi	3,234.58±141.55^{**a}	7.82±3.31^{**b}	14.50±1.39^{b}	21.82±7.20**	2.21±0.59^{**b}	0.81±0.33	47.18±9.50^{**b}
28	Kamphaeng Phet	3,376.97±69.96^{**a}	15.44±1.14^{b}	7.01±1.10^{**b}	18.50±4.45^{**b}	2.31±0.11^{**b}	0.46±0.08**	43.71±4.02^{**b}
Mean ± SE.		**2,904.52±33.24**	**17.93±1.72**	**12.48±0.67**	**29.42±1.80**	**5.69±0.37**	**1.11±0.09**	**66.55±3.15**
P. lobata		2,482.0 ± 66.11***	32.85±0.72***	21.94±0.74***	25.63±0.86***	10.34±0.79***	0.81±0.08	91.98±1.20***

**, the value was less than the Mean value of *P. mirifica* population

*;***, the value was greater than the Mean value of *P. mirifica* population

a; the value was greater than *P. lobata*

b; the value was less than *P. lobata*

Table 4.10 The ranked *P. mirifica* sample according to the amount of isoflavone; puerarin, daidzin, genistin, daidzein and genistein contents

Antioxidant activity	Puerarin (%)	Daidzin (%)	Genistin (%)	Daidzein (%)	Genistein (%)	Total isoflavone
1st Uthai Thani	20th Uthai Thani 10.55	4th Uthai Thani 21.11	4th Uthai Thani 48.78	1st Uthai Thani 16.02	1st Uthai Thani 3.56	3rd Uthai Thani
2nd Nong Bua Lam Phu	16th Nong Bua Lam Phu 21.97	15th Nong Bua Lam Phu 20.69	18th Nong Bua Lam Phu 41.07	8th Nong Bua Lam Phu 12.96	3rd Nong Bua Lam Phu 3.32	16th Nong Bua Lam Phu
3rd Phetchaburi	13th Phetchaburi 16.75	4th Phetchaburi 26.46	7th Phetchaburi 47.72	13th Phetchaburi 7.62	13th Phetchaburi 1.44	8th Phetchaburi
4th Phitsanulok	2nd Phitsanulok 41.95	14th Phitsanulok 14.60	15th Phitsanulok 31.57	5th Phitsanulok 9.95	6th Phitsanulok 1.94	7th Phitsanulok
5th Phetchabun	22nd Phetchabun 20.98	17th Phetchabun 23.39	23rd Phetchabun 34.66	6th Phetchabun 18.08	10th Phetchabun 2.89	27th Phetchabun
6th Ratchaburi	24th Ratchaburi 10.44	8th Ratchaburi 18.16	3th Ratchaburi 60.33	10th Ratchaburi 8.07	2th Ratchaburi 2.99	6th Ratchaburi
Mapping frequency	1/6	2/6	2/6	3/6	4/6	2/6

4.1.1.5 The antioxidant activity of isoflavone

Puerarin, daidzin, genistin, daidzein and genistein, were investigated for antioxidant activity by DPPH assay (Table 4.11) for direct correlation assay. It was found that puerarin express the highest antioxidant activity. Puerarin, daidzin and daidzein showed no significant different as compared with α-tocopherol.

Table 4.11 The antioxidant activity of isoflavone as compared with α-tocopherol

No.	Chemicals	IC_{50} (μg/ml)
1	Puerarin	93.26 ± 9.14^{a}
2	Daidzin	108.26 ± 9.94^{ab}
3	Genistin	362.10 ± 25.46^{c}
4	Daidzein	82.58 ± 4.53^{a}
5	Genistein	155.75 ± 25.19^{b}
6	α-tocopherol	72.33 ± 6.48^{a}

a, b, c, d; Significant difference ($p<0.05$) as compared with α-tocopherol

4.1.2 *B. superba*

4.1.2.1 Antioxidant activity of *B. superba*

B. superba collected from 23 provinces in Thailand was able to scavenge DPPH radicals by 50% (IC_{50}). The antioxidant activity value of *B. superba* showed significant variation (Table 4.12, Figure 4.2). *B. superba* from Loei showed highest antioxidant activity with IC_{50} of 227.08 μg/ml. The sample from Nakhon Sawan showed lowest antioxidant activity with IC_{50} of 1,198.04 μg/ml. The Mean value of antioxidant activity of *B. superba* population (653.64 μg/ml) was compared with individual sample. It was found that 13 samples exhibited significant higher antioxidant activity value and 9 samples exhibited significant lower antioxidant activity value than the Mean value of *B. superba* population ($p<0.05$). The antioxidant activity of α-tocopherol (68.02 μg/ml) showed obviously significant higher level than the Mean value of *B. superba* population.

Table 4.12 Antioxidant activity of *B.superba*

No.	Sources (Provinces)	IC_{50} (μg/ml)
1	Loei	227.08±0.38*
2	Lop Buri	289.82±6.05*
3	Saraburi	323.50±8.43*
4	Lampang	358.39±4.76*
5	Uttharadith	371.58±0.95*
6	Phetchabun	372.07±3.00*
7	Chonburi	377.89±8.10*
8	Sakon Nakhon	401.84±12.42*
9	Phitsanulok	413.34±4.34*
10	Chiang Mai	435.85±5.20*
11	Tak	470.10±3.75*
12	Ratchaburi	535.14±5.92*
13	Chiang Rai	579.01±2.77*
14	Chantaburi	685.07±17.45
15	Kanchanaburi	808.09±5.29**
16	Chaiyaphum	839.74±9.70**
17	Chachoengsoa	966.74±30.06**
18	Srisaket	970.56±21.33**
19	Phrachinburi	1,006.96±12.56**
20	Mae Hong Son	1,055.36±34.70**
21	Nong Bua Lam Phu	1,129.15±45.44**
22	Khon Kaen	1,185.11±17.69**
23	Nakhon Sawan	1,198.04±18.76**
Mean ± S.E.		**653.64±38.83**
α-tocopherol		68.02±5.52***

IC_{50} value; the concentration of sample required to scavenge 50% DPPH free radicals s under experimental conditions, Mean±S.E.

*, ***, IC_{50} value was greater than the Mean value of *B. superba* population

**; IC_{50} value was less than the Mean value of *B. superba* population

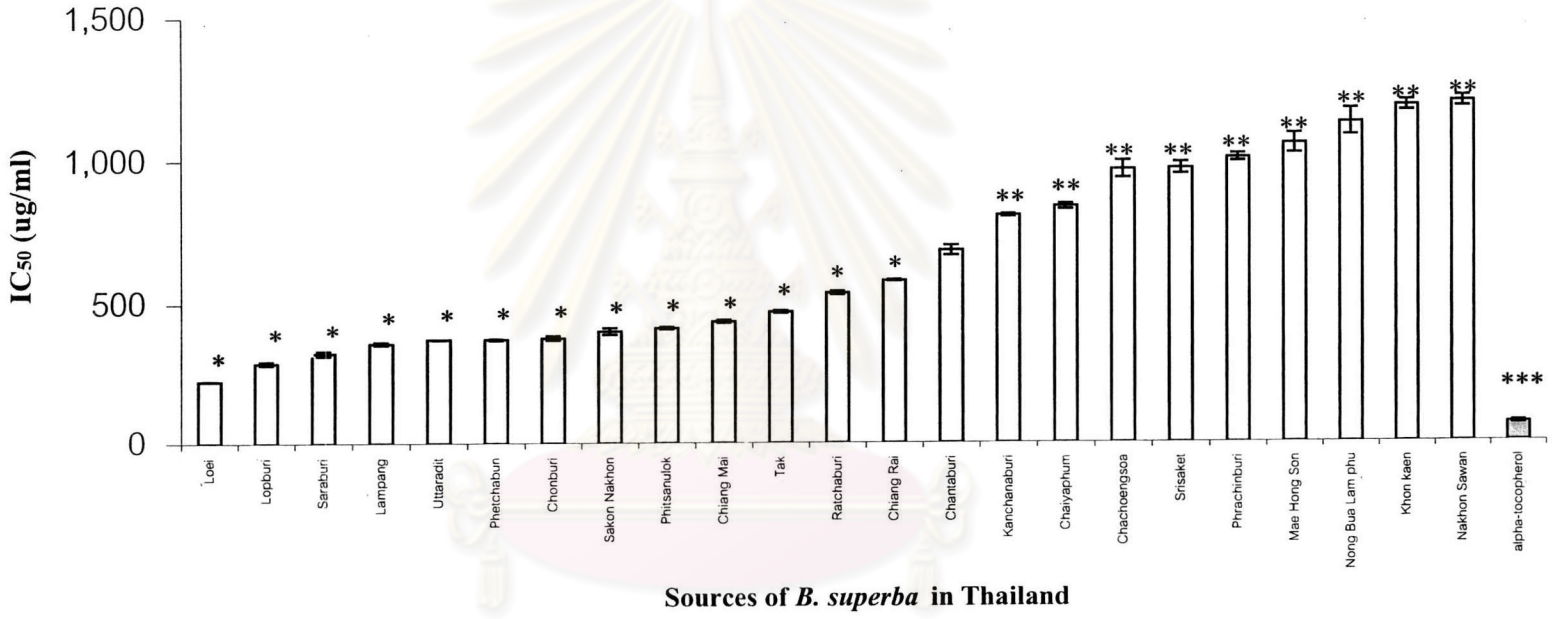

Figure 4.2 Antioxidant activity of *B. superba* from 23 provinces in Thailand. Significant difference from control ($p<0.05$) (Mean±SE). *, ***, IC_{50} value was greater than the Mean value of *B. superba* population.**; IC_{50} value was less than the Mean value of *B. superba* population.

4.1.2.2 Percent inhibition of the free radicals of *B. superba*

The percent inhibition (PI) was calculated for inhibiting the free radicals of *B. superba* collected from 23 provinces (Table 4.13). The most effective concentrations were in the range of 56.25-150 μg/ml and there exhibited significant inhibition of the DPPH free radicals. *B. superba* extract at the concentration of 150 μg/ml from Loei (91.11%) showed the highest PI value and the sample from Nakon Sawan (15.52%) showed the lowest PI value as well as IC_{50} value. The PI value was concentration-dependent and showed significant different ($p<0.05$). Thus, the Mean value of PI of individual *B. superba* at highest dose (150 μg/ml) was selected for comparision with the Mean value of *B. superba* population. Twelveth provinces of sample showed significant higher PI value and 11 samples showed PI value lower than the Mean value of *B. superba* population. In contrary, the PI value of α-tocopherol was significant higher than the Mean value of all *B. superba* samples.

Table 4.13 Percent inhibition (PI) of *B. superba*

No.	Sources (Provinces)	PI value					
		Concentration of the extract (μg/ml)					
		56.25	**75**	**93.75**	**112.5**	**131.25**	**150**
1	Loei	60.78±072	73.42±0.46	84.91±0.15	89.30±0.30	90.98±0.05	91.11±0.12*
2	Lop Buri	48.37±1.11	58.31±3.21	69.18±0.63	77.57±0.50	84.17±0.95	87.14±0.34*
3	Saraburi	39.78±0.91	52.07±0.82	62.33±0.96	71.66±1.56	80.40±0.50	86.60±1.33*
4	Lampang	34.29±1.30	45.83±0.36	53.85±0.82	63.70±0.34	72.17±0.14	81.43±1.38*
5	Uttharadith	37.11±0.42	45.03±0.36	53.41±0.71	61.36±0.30	69.04±0.72	77.44±0.60*
6	Phetchabun	36.58±1.19	44.90±0.38	52.21±0.98	61.26±0.57	70.23±0.75	77.64±1.52*
7	Chonburi	32.24±1.81	43.95±2.52	52.38±2.39	57.11±0.87	70.76±2.08	76.12±3.16*
8	Sakon Nakhon	31.91±2.75	39.98±2.29	49.37±2.61	55.34±2.04	65.36±4.10	69.80±2.30*
9	Phitsanulok	31.63±1.09	41.65±1.10	48.32±0.45	55.10±0.52	62.33±0.19	70.68±0.60*
10	Chiang Mai	27.38±1.42	33.57±1.87	42.34±2.39	50.39±1.91	57.06±2.10	61.62±2.49*
11	Tak	27.35±1.00	33.26±1.03	40.88±0.62	47.69±0.17	54.14±0.36	62.32±1.01*
12	Ratchaburi	25.81±0.48	30.40±1.02	34.58±0.34	40.80±0.80	45.91±0.33	52.46±0.90
13	Chiang Rai	23.56±0.22	25.09±1.92	33.13±0.58	37.79±0.30	41.78±0.62	46.38±0.24**
14	Chantaburi	21.46±1.07	23.96±0.85	27.37±0.69	30.14±1.05	33.68±1.39	37.00±1.39**
15	Kanchanaburi	17.37±0.54	18.69±0.63	21.57±0.31	24.29±0.67	25.97±0.44	29.87±1.20**
16	Chaiyaphum	17.95±0.84	20.46±0.28	20.95±0.34	22.20±1.10	25.32±0.33	27.79±0.35**
17	Chachoengsoa	14.11±0.25	14.47±0.94	16.53±1.00	19.17±1.37	19.59±0.75	22.05±1.03**
18	Srisaket	14.92±0.44	16.08±0.16	17.20±0.65	19.95±0.71	20.44±0.60	21.76±0.78**
19	Phrachinburi	12.63±0.10	12.34±0.52	14.78±0.47	16.64±0.45	18.14±0.66	20.38±0.48**
20	Mae Hong Son	12.97±0.21	12.44±0.78	14.37±0.33	15.52±1.11	17.54±0.92	19.30±0.74**
21	Nong Bua Lam Phu	11.28±0.24	11.23±0.43	12.88±0.36	13.39±0.38	15.12±0.25	17.76±2.09**
22	Khon kaen	10.08±0.41	10.67±0.27	11.56±0.54	12.49±0.19	13.89±0.25	15.28±0.29**
23	Nakhon Sawan	10.21±0.20	10.80±0.27	11.42±0.13	12.68±0.15	13.40±0.38	15.52±0.29**
Mean ± S.E.		**26.09±1.56^{A}**	**31.24±2.0^{AB}**	**36.76±2.48^{BC}**	**41.56±2.78^{CD}**	**46.41±3.09^{DE}**	**50.76±3.26^{E}**

*,***; amount of isoflavone was greater than Mean value of *B. superba* population

**; amount of isoflavone was less than the value of *B. superba* population

A, B, C, D, E; PI value was significant different ($p<0.05$)

4.1.2.3 The antioxidant activity of flavonoid

Flavonoid and sterol fraction antioxidant activity were investigated by DPPH assay (Table 4.14). It was found that flavonoid fraction express certain level of antioxidant activity, while sterol fraction showed no activity. Flavonoid fraction showed significant different as compared with α-tocopherol.

Table 4.14 The antioxidant activity of flavonoid as compared with α-tocopherol

No.	Chemicals	IC_{50} (μg/ml)
1	Flavonoid fraction	$1,354.29 \pm 46.75^{b}$
2	Sterol fraction	-
3	α-tocopherol	72.33 ± 6.48^{a}

a,b; Significant different as compared with α-tocopherol

4.1.3 *M. collettii*

4.1.3.1 Antioxidant activity of *M. collettii*

The great difference between the sample with the highest and lowest antioxidant activity, IC_{50} of 55.53 μg/ml versus IC_{50} of 127.34 μg/ml of the samples from Chiang Rai and Lampang, respectively. There were 2 samples, from Chiang Rai and Chiang Mai that showed higher antioxidant activity as well as α-tocopherol and 1 sample, from Lampang showed lower antioxidant activity than the Mean value of *M. collettii* population (83.06±8.10 μg/ml) (Table 4.15, Figure 4.3)

Table 4.15 Antioxidant activity of *M. collettii*

No.	Sources (Provinces)	IC_{50} (μg/ml)
1	Chiang Rai	55.53 ± 2.66*
2	Chiang Mai	71.52 ±0.61*
3	Kanchanaburi	77.84 ± 0.79
4	Lampang	127.34 ±0.47**
Mean ± S.E.		**83.06±8.10**
α-tocopherol		68.02 ± 5.52***

IC_{50} value; the concentration of sample required to scavenge 50% DPPH free radicals s under experimental conditions, Mean±S.E.

,*; amount of isoflavone was greater than Mean value of *M. collettii* population

*; amount of isoflavone was less than the value of *M. collettii* population

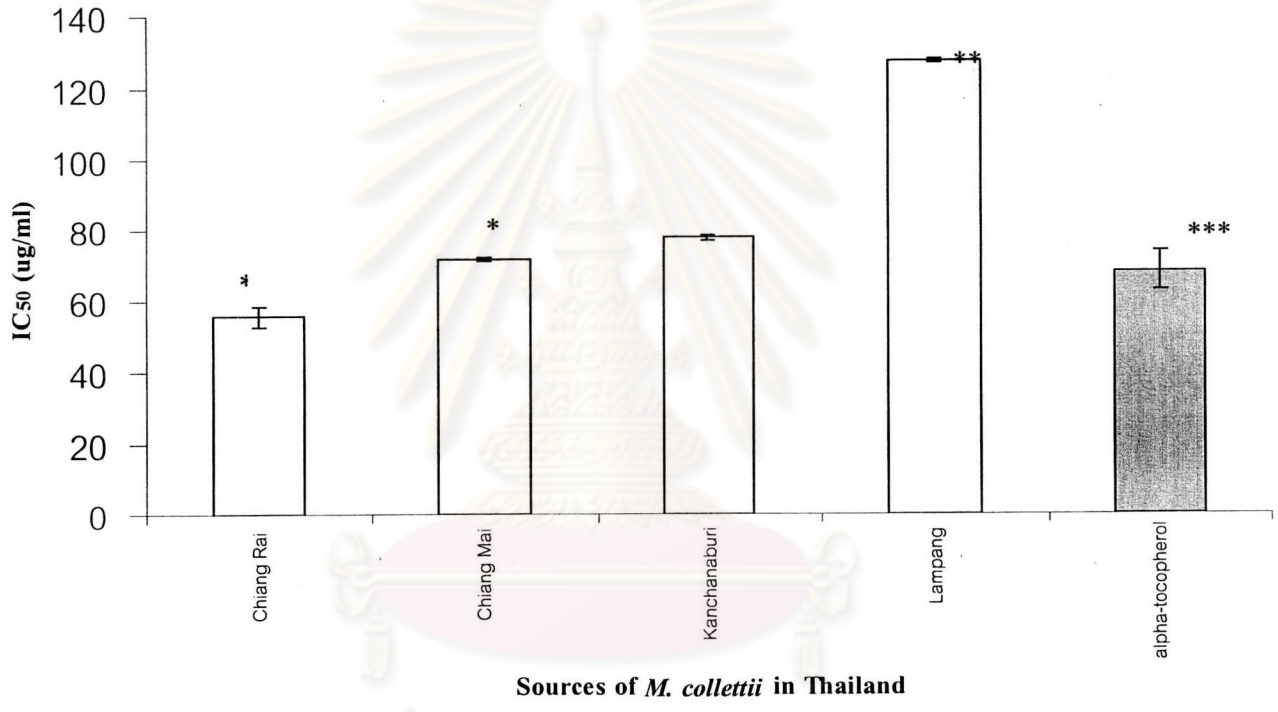

Figure 4.3 Antioxidant activity of *M. collettii* from 4 provinces in Thailand. Significant difference from control ($p<0.05$) (Mean±S.E.) **,***; amount of isoflavone was greater than Mean value of *M. collettii* population *; amount of isoflavone was less than the value of *M. collettii* population.

4.1.3.2 Percent inhibition of the free radicals of *M. collettii*

The PI value was calculated at each of concentration of *M. collettii* collected from 4 provinces in Thailand (Table 4.16). The concentration of 20-200 μg/ml showed significant different. The PI value of *M. collettii* was compared at the higest does (200 μg/ml). The sample from Chiang Rai showed the highest PI value and the sample from Lampang showed the lowest PI value as well as IC_{50} value, whereas three samples exhibited significant higher and 1 sample exhibited significant lower PI value than Mean value of *M. collettii* population.

Table 4.16 Percent inhibition (PI) of *M. collettii*

No.	Sources (Provinces)	PI value					
		Concentration of the extracts (μg/ml)					
		20	**40**	**60**	**80**	**100**	**200**
1	Chiang Rai	26.97±0.15	44.29±1.28	62.15±2.02	76.38±0.73	85.05±1.81	90.89±0.45*
2	Chiang Mai	22.90±0.43	36.87±0.64	48.97±0.31	62.72±0.745	74.44±0.82	90.17±0.17*
3	Kanchanaburi	22.35±0.79	32.23±0.12	46.50±1.20	57.85±0.30	68.78±0.77	89.75±0.21*
4	Lampang	16.22±1.14	23.43±0.72	29.49±0.18	36.38±0.27	42.85±0.98	70.83±0.28**
Mean ± S.E.		**22.12±1.19a**	**34.21±2.30b**	**46.78±3.53c**	**58.33±4.34d**	**67.78±4.71e**	**85.41±2.54f**

*; ***, PI value was greater than the Mean value of *M. collettii* population

**, PI value was less than Mean value of *M. collettii* population

4.1.4 Ranking for the antioxidant activity of *P. mirifica*, *P. lobata*, *B. superba* and *M. collettii*

The Mean value of antioxidant activity (IC_{50}) of *P. mirifica* population, *P. lobata* population, *B. superba* population and *M. collettii* population (Table 4.17) showed variation. The results reveal that *M. collettii* population exhibited the highest antioxidant activity value (83.06μg/ml), *B. superba* population showed the second order (653.64 μg/ml), and *P. lobata* showed the third order (2,482.00 μg/ml) and *P. mirifica* population showed the fourth order (2,904.52 μg/ml). In comparison with individual plant extracts, there showed significant lower antioxidant value than the Mean value of antioxidant activity of α-tocopherol. The direct relation between the antioxidant activity effectiveness of *M. collettii*, *B. superba*, *P. mirifica*, *P. lobata* and α-tocopherol for the DPPH radicals scavenging assay (Figure 4.4).

Table 4.17 The Mean value of antioxidant activities (IC_{50}) of *P. mirifica* population, *B. superba* population and *M. collettii* population, *P. lobata* and α-tocopherol

Plant extracts	**IC_{50} (μg/ml)**
P. mirifica population	$2,904.52 \pm 33.24^{D}$
P. lobata	$2,482.00 \pm 66.11^{C}$
B. superba population	653.64 ± 38.83^{B}
M. collettii population	83.06 ± 8.10^{A}
α-tocopherol	68.02 ± 5.52^{E}

IC_{50} value; the concentration of sample required to scavenge 50% DPPH free radicals under experimental conditions, Mean±S.E.

A, B, C, D, E; significant different between test samples ($p<0.05$)

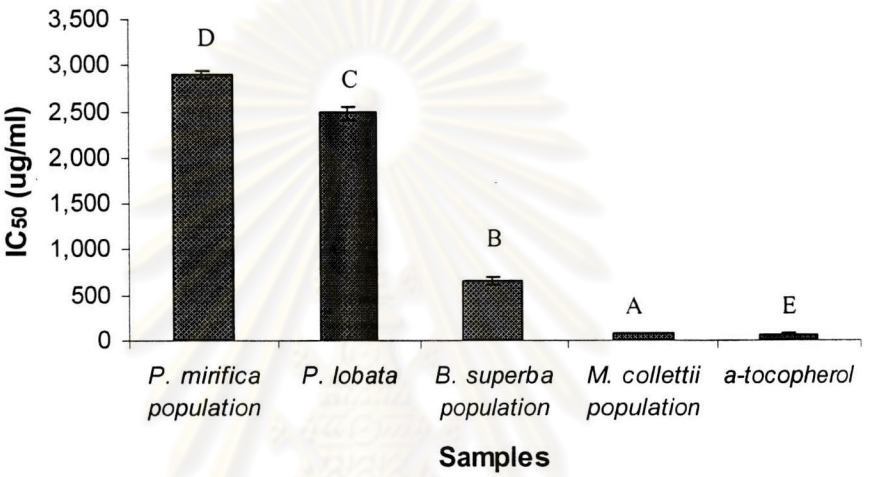

Figure 4.4 The Mean value of antioxidant activities (IC_{50}) of *P. mirifica* population, *B. superba* population and *M. collettii* population, *P. lobata* and α-tocopherol. A, B, C, D, E; significant different between test samples ($p<0.05$)

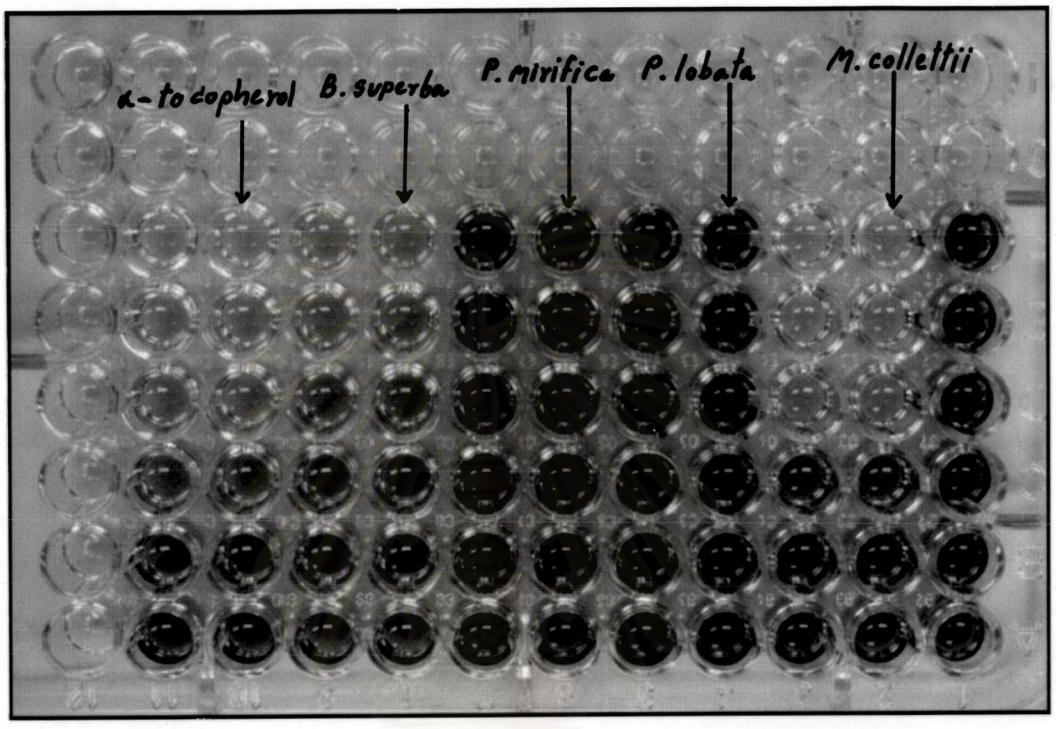

Figure 4.5 Color of DPPH free radicals s (purple color) were against with the plant extracts for 30 min compared with α-tocopherol (yellow color)

4.2 Mutagenicity and antimutagenicity by Ames' test (preincubate technique)

Plant extracts with highest antioxidant activity, *P. mirifica* from Uthai Thani (2,904.52±33.24 µg/ml), *B. superba* from Loei (653.64±38.83 µg/ml), *M. collettii* from Chiang Rai (83.06±8.10 µg/ml) and *P. lobata* (2,482.00±66.11µg/ml) were chosen for mutagenicity (Table 4.18) and antimutagenicity (Table 4.19).assay by Ames' test. The potential mutagenicity of plant extracts were investigated at the concentration; 2.5, 5, 10 and 20 mg/plate of *P. mirifica* as well as *P. lobata*, 0.5, 1.0, 2.5 and 5 mg/plate of *B. superba*, 0.5, 1.0, 2.5, 5, 10 and 20 mg/plate of *M. collettii* extract. Positive control, which used diagnostic mutagens to confirm the reversion properties of S. Typhimurium, AF_2 (not required the metabolic activation) for TA 98 strain (0.1 µg/plate) and TA100 strain (0.01 µg/plate), respectively, B(a)P mutagen (required the metabolic activation) for TA 98 (5 µg/plate) and TA100 (10 µg/plate), respectively (Figure 4.6-4.7).

4.2.1 Mutagenicity of plant extracts

4.2.1.1 Mutagenicity of *P. mirifica*

P. mirifca showed no mutagenicity effect to TA98 and TA100 strain both with and without enzymatic activation. *P. mirifica* (2.5 mg/plate) showed significant revertant colonies lower value than the negative control, but partial killing effect was not found. *P. mirifica* (10 mg/plate) which showed significant higher value of revertant colonies than negative control and *P. lobata* under the absence of enzymatic activation of TA98, however, cytotoxic effect was found in TA100 strain as partial killing was presence at the highest dose (20 mg/plate) in both TA98 and TA100. It was also found in TA98 under activation with enzyme.

4.2.2 Mutagenicity of *P. lobata*

P. lobata showed neither mutagenic nor toxic effect to TA98 and TA100 strain which absence and presence the metabolic activation, except at highest dose of TA100 strain as activate with enzyme showed partial killing effect. *P. lobata* showed no significantly different as compared with the negative control.

4.2.3 Mutagenicity of *B. superba*

B. superba showed neither mutagenic nor toxic effect to TA98 and TA100 strain which absence the metabolic activation. *B. superba* showed significant the revertant colonies hiher value than the negative control, but not more than 2 times. *B. superba* showed significant the revertant colonies lower and higher value than *P. lobata* at same dose both absence and presence the activation of enzyme.

4.2.4 Mutagenicity of *M.collettii*

M. collettii showed no mutagenic effect to TA98 and TA100 strain both in the absence and presence of the metabolic activation. Partial killing effect was found at the concentration of 10 and 20 mg/plate both in the absence and presence of the activation with enzyme in TA98 strain, while in TA100 was found at the concentration of 5 mg/plate under the absence of the enzyme. When activation with enzymatic, partial killing was presence at highest dose. *M. collettii* showed significant higher revertant colonies than the negative control, but not double as well as *P. lobata*.

Table 4.18 Mutagenicity of the plant extracts analyzed by S. Typhimurium TA 98 and TA100 on non-metabolic and metabolic activation

Plant extracts	Concentration (mg/plate)	Average of his$^+$ revertant colonies (Mean±SE)			
		TA 98		TA 100	
		-S9 mix	+S9 mix	-S9 mix	+S9 mix
P. mirifica (Uthai Thani)	0	27.31±4.10	49.32±3.70	136.02±7.70	165.04±18.00
	2.5	27.00±1.51	47.61±1.31	134.31±1.55	137.60±5.03*
	5	27.41±3.22	49.00±2.01	137.34±3.10	142.33±3.21
	10	38.61±0.83*a	52.32±1.20	PK	161.00±9.52
	20	PK	53.06±1.12	PK	PK
P .lobata	0	28.71±2.30	54.51±3.90	150.62±5.20	168.66±3.71
	2.5	27.33±3.90	46.62±2.72	142.64±1.40	151.62±1.20
	5	28.73±0.90	51.61±6.50	143.01±1.52	160.01±2.52
	10	33.01±1.52	51.02±2.00	155.77±4.10	164.74±2.90
	20	34.02±3.14	50.01±5.22	162.32±3.90	PK
B .superba (Loei)	0	26.60±3.10	33.31±0.80	123.66±4.92	168.62±16.72
	0.5	29.35±1.41	40.09±2.00	102.61±2.32	186.00±4.70
	1.0	31.36±1.80	44.37±2.31*	110.00±5.53	192.02±4.51
	2.5	35.67±2.40*	45.00±4.02*	109.13±3.72a	200.63±6.70*a
	5.0	34.69±2.42*	54.39±2.11*	108.60±1.40a	201.04±2.00*a
M. collettii (Chiang Rai)	0	31.08±1.50	46.66+2.00	109.63+4.71	151.64±6.30
	2.5	33.67±3.51	63.31±5.01*a	150.30±6.12*a	149.01±4.90
	5	33.32±2.60	67.34±8.70*a	PK	153.72±5.01a
	10	PK	PK	PK	168.80±0.70
	20	PK	PK	PK	PK
Positive control (µg/plate)					
AF_2	0.1	490.62±7.60	ND	ND	ND
	0.01	ND	ND	700.60±5.91	ND
B(a)P	5	ND	693.71±3.32	ND	ND
	10	ND	ND	ND	883.00±5.42

ND = Not determined

* ; significant compared with negative control ($p<0.05$), but non-mutagenic (the number of revertant colony less than 2 times of control)

a ; significant compared with *P. lobata* at same dose ($p<0.05$)

PK ; partial killing effect

4.2.2 Antimutagenicity of plant extracts

4.2.2.1 Antimutagenicity of *P. mirifica*

P. mirifica (10 mg/plate) showed weak antimutagenicity in TA98 strain (-S9 mix) and there showed positive antimutagenicity in TA100 strain (+S9 mix)

4.2.2.2 Antimutagenicity of *P. lobata*

P. lobata (10 and 20 mg/plate) showed weak antimutagenicity (-S9 mix) as well as at the concentration of 2.5, 5 and 10 mg/plate under enzymatic activation (+S9 mix) while at highest dose (20 mg/plate) showed positive antimutagenicity in strain TA98. *P. lobata* (10 and 20 mg/plate) showed weak antimutagenicity as well as at dose 5 and 10 mg/plate under enzymatic activation (+S9 mix) in TA100 strain.

4.2.2.3 Antimutagenicity of *B. superba*

B. superba (0.5 mg/plate) showed weak antimutagenicity in TA100 (-S9 mix) while other concentration showed negative result in either TA98 or TA100 strain.

4.2.2.4 Antimutagenicity of *M. collettii*

M. collettii (10 and 20 mg/plate) showed positive and strong antimutagenicity, respectively under the absence of the metabolic activation (-S9 mix) but showed weak and positive antimutagenicity in the presence of the metabolic activation (+S9 mix) in TA98 strain. *M. collettii* (10 mg/plate) showed strong antimutagenicity in the presence of the metabolic activation (+S9 mix) in TA100 strain.

Table 4.19 Antimutagenicity of *P. mirifica*, *B. superba* and *M. collettii* in comparison with *P. lobata* in S. Typhimurium TA98 and TA100 strains, in the absence and presence of the metabolic activation system

Treatment	Dose (mg/plate)	Antimutagenicity (PI value)			
		TA98		TA100	
		-S9	+S9	-S9	+S9
P. mirifica (Uthai Thani)	2.5	8.21±5.92*	0*	3.45±2.21*	0*
	5	18.32±2.74*	0*	2.47±1.80*	7.34±2.82*
	10	27.58±1.91**	0*	ND	42.09±7.41***
	20	ND	0*	ND	ND
P. lobata	2.5	6.83±1.90*	27.03±0.97**	7.97±1.53*	18.50±7.62*
	5	16.15±2.01*	28.52±1.94**	16.02±1.10*	26.81±4.40**
	10	29.74±3.44**	29.09±2.26**	21.29±7.12**	35.53±3.01**
	20	30.02±4.71**	45.85±3.30***	22.73±8.26**	ND
B. superba (Loei)	0.5	7.51±2.27*	0*	24.83±4.03**	0*
	1	13.19±4.93*	0*	2.41±0.40*	0*
	2.5	4.63±1.30*	0*	0*	0*
	5	0*	11.44±2.13*	0*	0*
M. collettii (Chiang Rai)	0.5	0*	0*	2.24±1.28*	0*
	1	5.40±1.10*	0*	0.57±1.63*	0*
	2.5	11.37±1.30*	0*	2.30±1.70*	0*
	5	56.91±0.41***	30.74±1.42**	ND	13.42±0.57*
	10	63.79±1.24****	52.16±4.84***	ND	85.10±4.71****
	20	ND	ND	ND	ND
Spontaneous		30.60±1.20	40.00±1.10	114.32±6.61	119.00±6.21
AF_2	0.1	505.66±7.60	ND	ND	ND
	0.01	ND	ND	694.32±8.80	ND
B(a)P	10	ND	742.01±22.52	ND	ND
	5	ND		ND	705.00±29.11

*, Negative antimutagenicity effect

**, Weak antimutagenicity effect

***, Positive antimutagenicity effect

****, Strong antimutagenicity effect

ND = Not determined

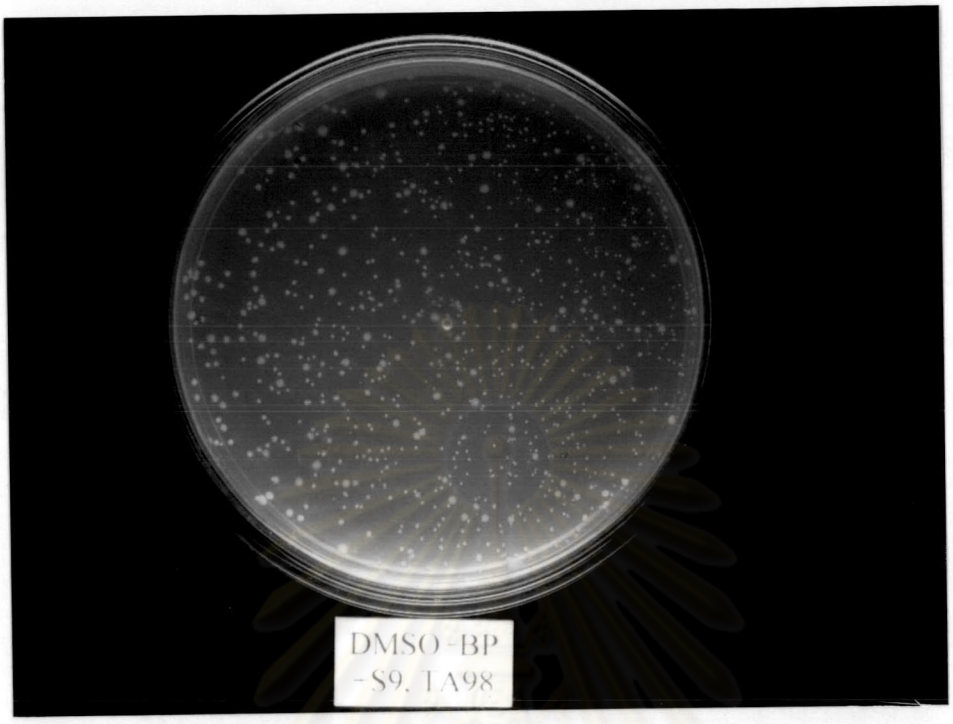

Figure 4.6 The revertant colonies of mutagen in S. Typhimurium TA98

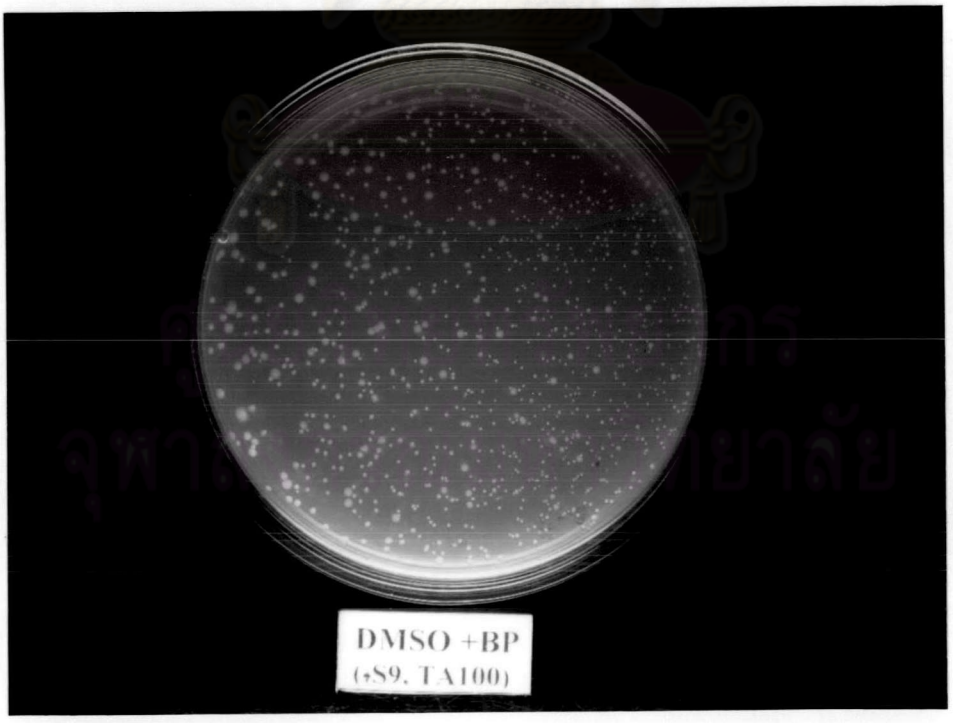

Figure 4.7 The revertant colonies of mutagen in S. Typhimurium TA100

4.3 Genotoxicity by micronucleus test

The PCEs and NCEs of erythrocytes, which stain differentially, the combination of the May-Gruenwald and Giemsa stains PCEs bluish and NCEs relatively red dish in color and micronucleus in PCEs (Figure 4.8). An increase in the frequency of micronucleated PCE (MNPCEs) in test agent-treated animals is an indication of induced chromosome damage

4.3.1 Dose variation study

The preliminary on micronucleus formation of only *M. collettii* crude extract at the concentration approximately 1, 10 and 20 g powder/kg B. W. in male rats at 30 hours is shown in Table 4.20. *M. collettii* at the highest dose showed significant diffece as compared with the negative control and no treat group (Table 4.20).

Table 4.20 Micronucleus test in rats' bone marrow after oral administration of *M. collettii* extracts at 30 hours

Samples	**Dose (g/kg B.W.)**	**Time (hours)**	**No. of MN/1000PCEs** (Mean±S.E.)	**PCEs/NCEs** (Mean±S.E.)
			Male	**Male**
No treat	0	30	2.66±0.80	0.57±0.11
1% absolute ethanol	0		2.33±0.88	0.53±0.05
M. collettii	1		2.33±0.33	0.53±0.71
	10		4.00±0.57	0.67±0.09*
	20		9.01±0.68*	0.47±0.54
Cyclophosphamide (i.p)	0.08		18.33±0.89**	0.33±0.50**

*, **; Significant diffence ($p<0.05$)

4.4.2 Time variation study of plant extracts

The frequency of micronucleated polychromatic erythrocytes (MNPCEs) in 1,000 PCEs and the ratio of PCE to NCE after 24, 48 and 72 hours exposure to *P. mirifica*, *P. lobata*, *B. superba* and *M. collettii* extract crude extract (Table 4.21).

4.4.2.1 Micronucleus test of *P. mirifica*

P. mirifica extract showed non-mutagenic and non-toxicity effect to bone marrow cells of male rats. *P. mirifica* showed no significant difference as compare with the negative control groups ($p<0.01$). Micronucleus frequencies and PCEs/NCEs of *P. mirifica* exhibited no significant different as compared with *P. lobata*.

4.4.2.2 Micronucleus test of *P. lobata*

P. lobata extract showed non-mutagenic and non-toxicity effect to bone marrow cells of male rats. *P. lobata* showed no significant difference as compared with the negative control groups ($p<0.01$).

4.4.2.3 Micronucleus test of *B. superba*

B. superba extract showed weakly mutagenic activity and non-toxicity effect to bone marrow cells of male rats at tested dose. *B. superba* could induce significantly increase in the frequency of MNPCEs after 24 hours as compared with the negative control ($p<0.01$). Micronucleus frequencies of *B. superba* exhibited significant different as compared with *P. lobata*.

4.4.2.4 Micronucleus test of *M. collettii*

M. collettii showed weak mutagenic activity but non-toxicity effect to bone marrow cells of male rats. *M. collettii* exhibited significant increase of the MNPCE frequencies after treated sample in comparison to the negative control. The micronucleus frequencies of *M. collettii* exhibited significant different ($p<0.01$) as compared with *P. lobata*.

Table 4.21 Micronucleus test in rats' bone marrow after oral administration of plant extracts at 24, 48 and 72 hours

Compound	Dose (g/kg B.W.)	Time (hours)	No. of MN/1000 PCEs (Mean±S.E.)	PCEs/NCEs (Mean±S.E.)
			Male	Male
1%EtoH	0	24	1.49±0.22	0.62±0.03
		48	1.97±0.35	0.65±0.04
		72	2.31±0.33	0.70±0.03
P. mirifica (Uthai Thani)	16	24	1.74±0.69	0.59±0.06
		48	1.14±0.29	0.74±0.05
		72	0.98±0.36	0.63±0.04
P. lobata	16	24	0.33±0.21	0.63±0.03
		48	0.78±0.27	0.65±0.03
		72	1.13±0.29	0.70±0.05
B. superba (Loei)	16	24	5.49±1.23** a	0.56±0.06
		48	4.12±0.29a	0.70±0.04
		72	4.92±0.72a	0.60±0.07
M collettii (Chiang Rai)	16	24	4.43±1.24a	0.52±0.06
		48	5.54±1.04**a	0.70±0.04
		72	4.94±0.75**a	0.68±0.06
Cyclophosphamide (i.p)	0.08	24	15.61±0.89** a	0.39±0.03**a
		48	16.12±0.67**a	0.40±0.05**a
		72	14.43±1.16**a	0.34±0.04**a

Mean±S.E., n = 6, 2000 PCEs scored per animal, Mean±S.E., n = 6, 1,000 erythrocytes (PCEs+NCEs) scored per animal.

* ; Significant difference from control ($p<0.01$)

**; Ratio of PCEs/NCEs in 1,000 erythrocytes/slides (≤ 0.1) as no toxic effect

a ; Significant difference from *P. lobata* ($p<0.01$)

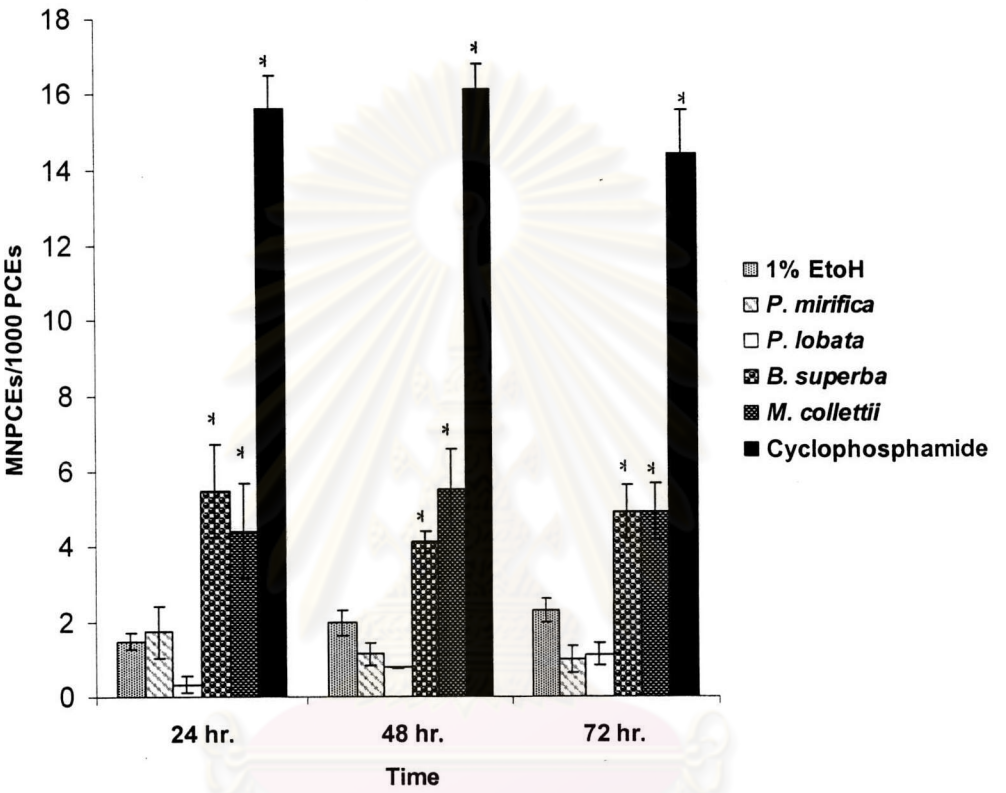

Figure 4.8 The micronucleus per 1,000 PCEs in bone marrow of male rats after oral administration at 24, 48 and 72 hours of *P. mirifica*, *P. lobata*, *B. superba* and *M. collettii* extracts

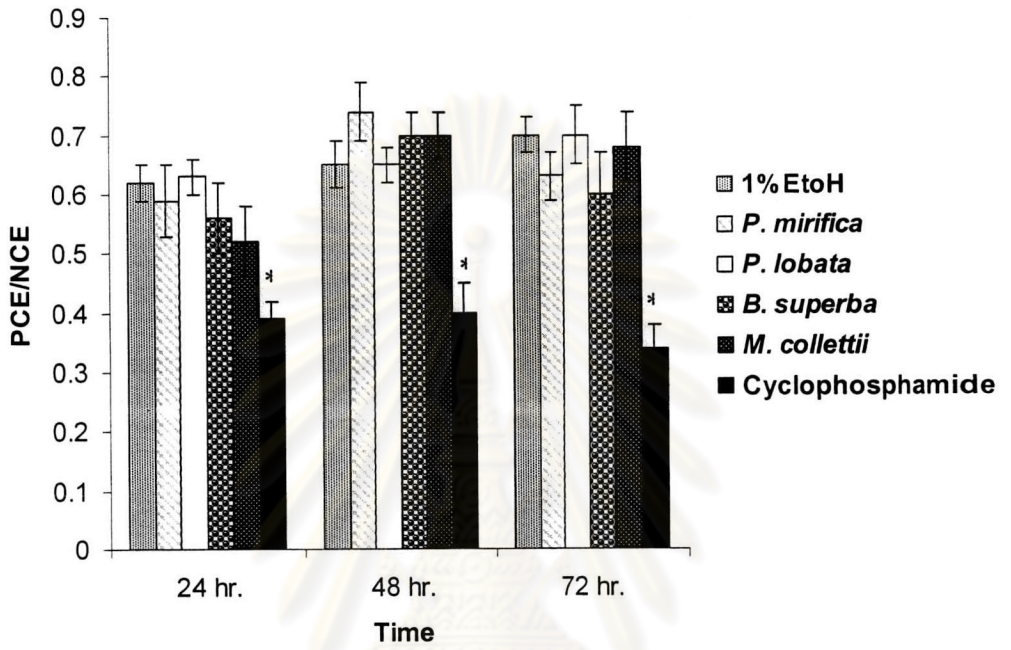

Figure 4.9 The polycromatic erythrocytes (PCEs) per normochromatic erythrocytes (NCEs) in bone marrow of male rats after oral administration at 24, 48 and 72 hours of *P. mirifica*, *P. lobata*, *B. superba*, *M. collettii* extracts

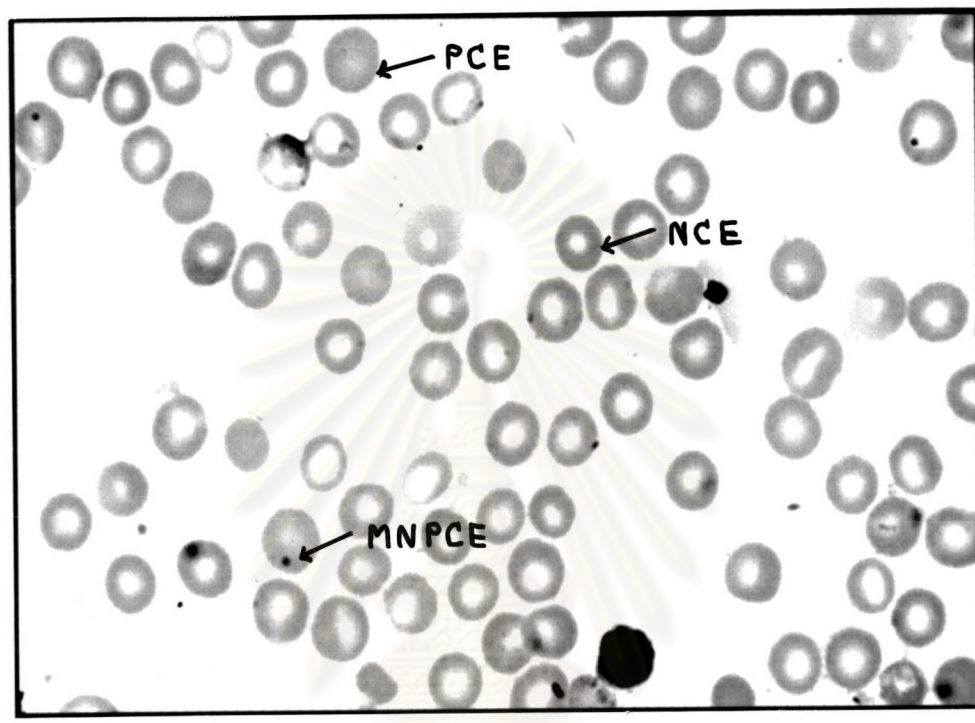

Figure 4.10 A photograph of rat whole bone marrow smear showing the microscopic observation of micronucleus in polychromatic erythrocyte of cyclophosphamide