

CHAPTER IV

RESULTS

1. Setting up the isoflavone analysis

The analytical system was started with 100 : 0 to 0 : 100 of 1.5 % (v/v) acetic acid : acetonitrile gradient system, 100 min. by running 5 reference standard isoflavones including puerarin, daidzin, genistin, daidzein and genistein. The last reference standard (genistein) was eluted at retention time 40 min. approximately. The analytical isoflavone form *P. mirifica* clone Chaing Dao was performed at the wavelength of 254 nm and 280 nm for comparison of the peak separation of isoflavone. The absorbance at 254 nm exhibited the higher significant different isoflavone than 280 nm. The absorbance at 254 nm and 280 nm showed isoflavone content of 81.65 mg/100g powder powder and 40.66 mg/100g powder powder, respectively (Table 4, Figure 13).

Five reference standard isoflavones were injected to generate calibration curve. Calibration curve of puerarin, daidzin, genistin, daidzein and gnistein were plotted between peak area (y) and microgram injected (x) and obtained r^2 of 0.9999, 0.9995, 0.9995, 0.9999 and 0.9995, respectively. (Figure 14-18)

Table 4 Isoflavone content in mg/100g powder of *P. mirifica* clone Chiang Dao analyzed at wavelength

Wavelength (nm)	Puerarin (Mean±S.E.)	Daidzin (Mean±S.E.)	Genistin (Mean±S.E.)	Daidzein (Mean±S.E.)	Genistein (Mean±S.E.)	Total (Mean±S.E.)
254	17.56± 0.09	19.42± 0.87	37.84± 4.88	6.82 ± 0.62	2.01 ± 0.17	81.65 ± 4.64
280	9.53 ± 0.06	14.86 ± 0.19	11.93 ± 0.06	4.34 ± 0.03	1.23 ± 0.01	40.66 ± 0.16

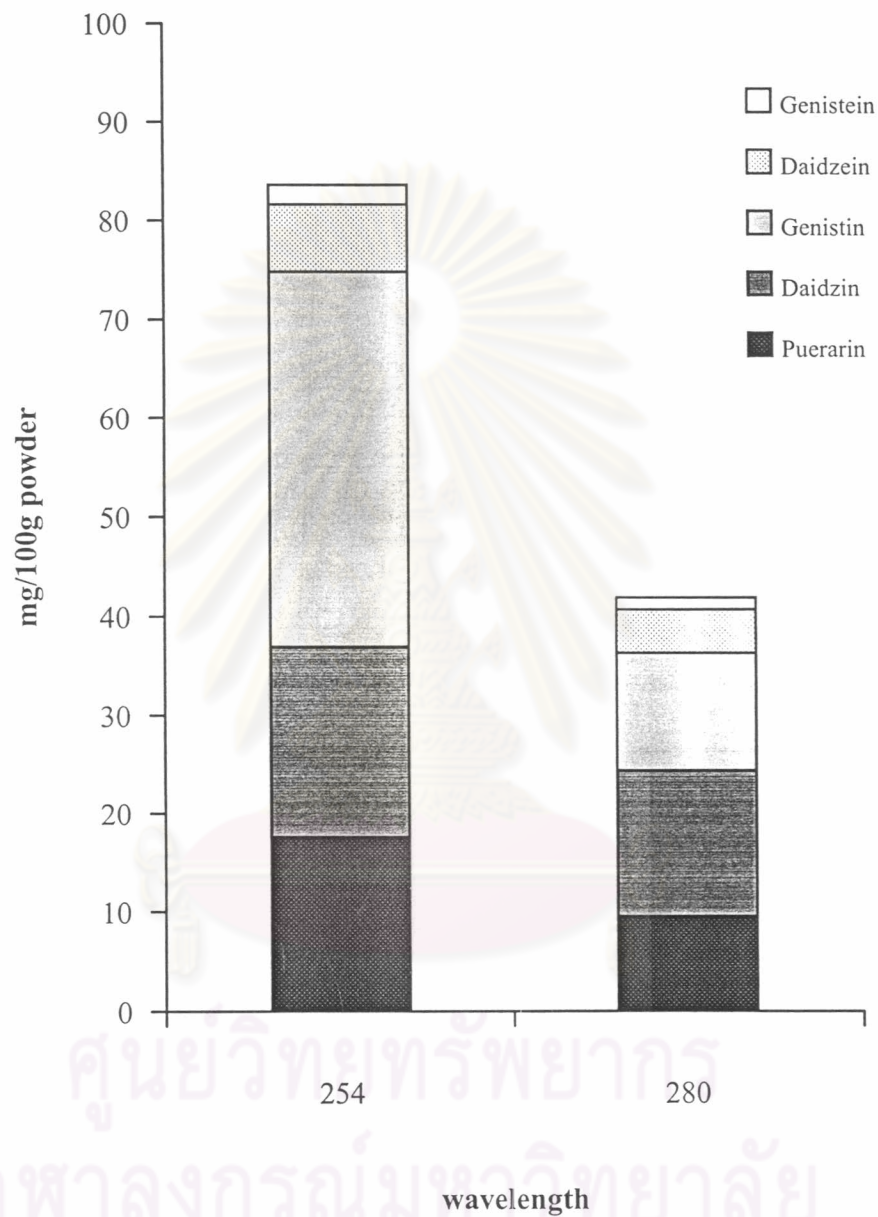


Figure 13 Isoflavone content in mg/100g powder of wild *P. mirifica* clone Chiang Dao in comparison with wavelength

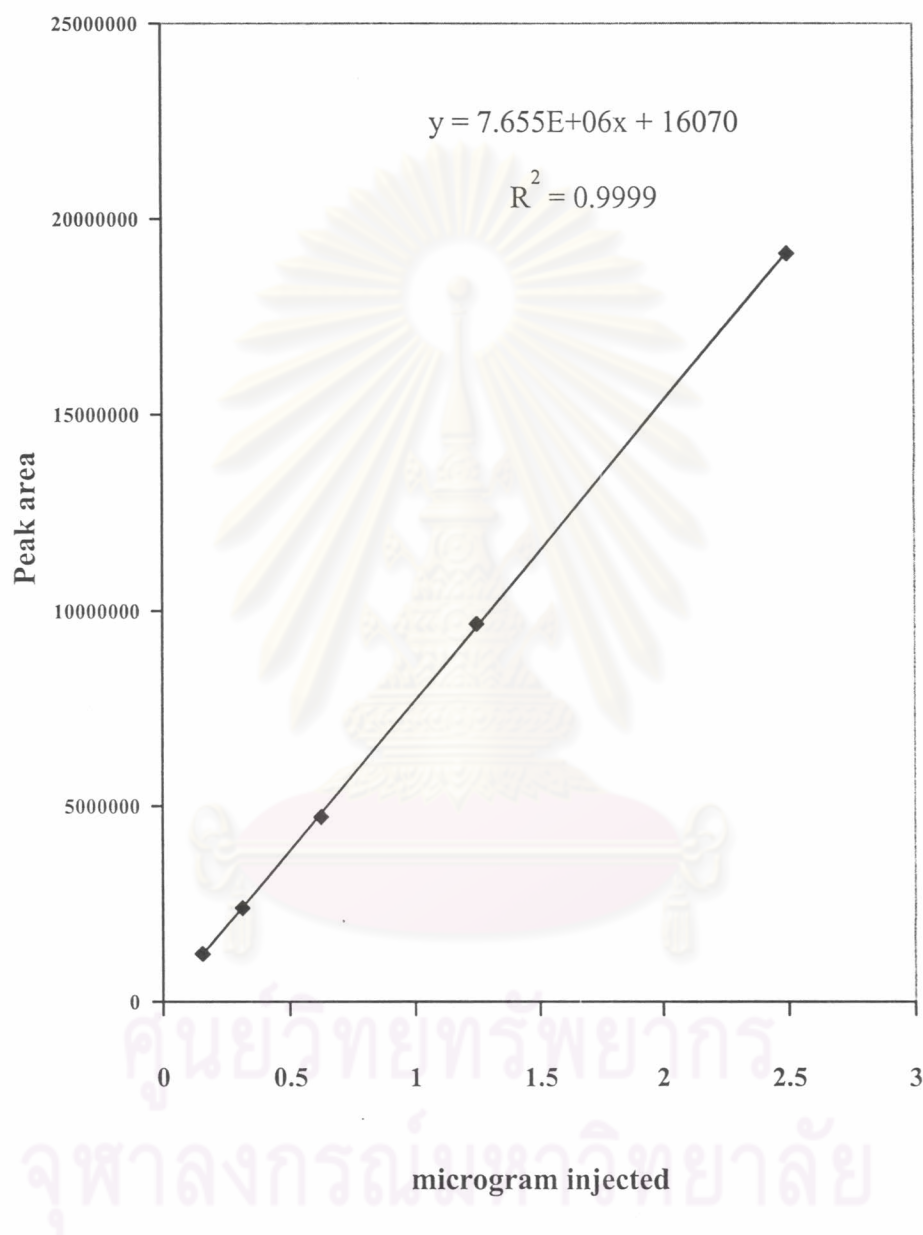


Figure 14 Calibration curve of Puerarin

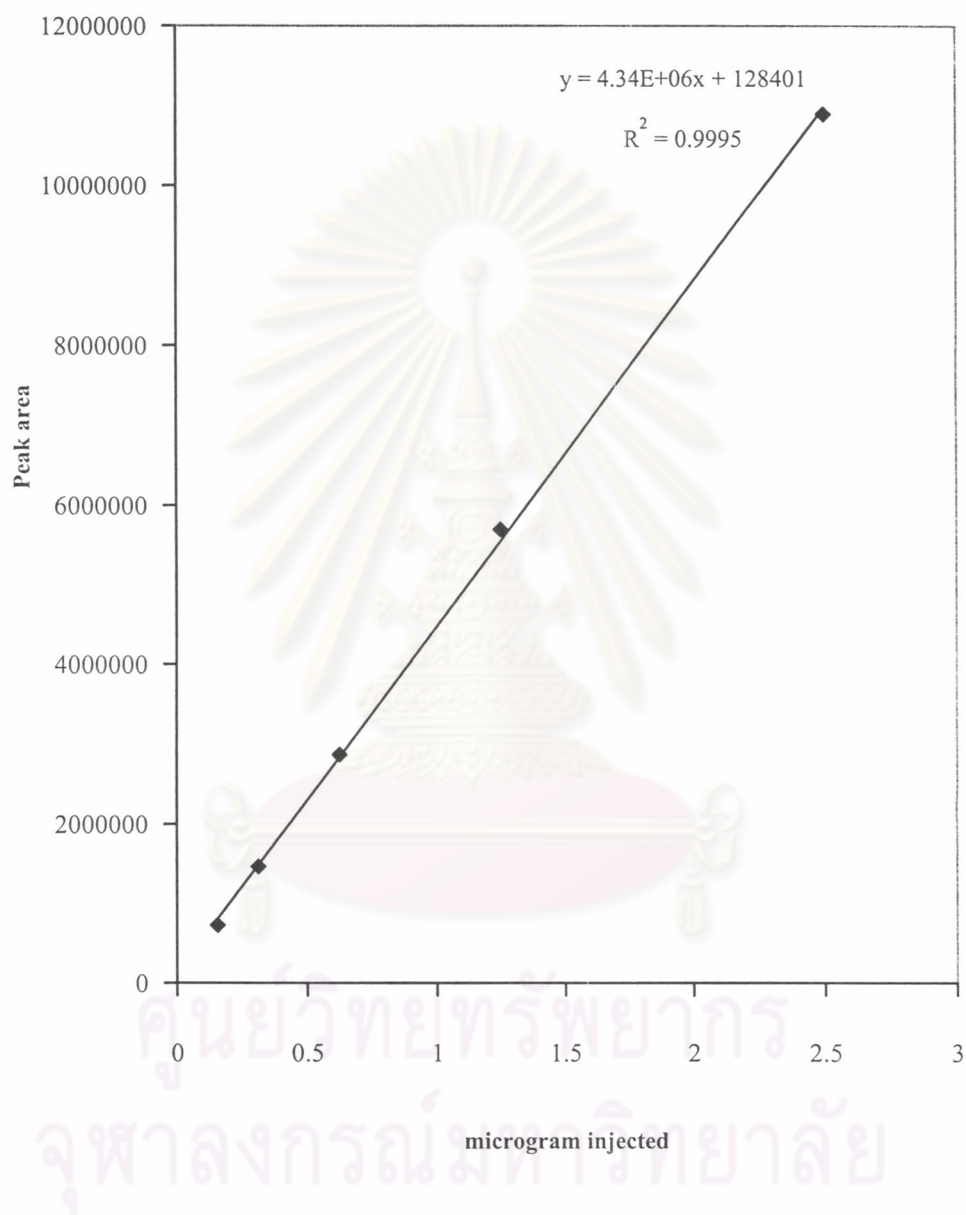


Figure 15 Calibration curve of Daidzin

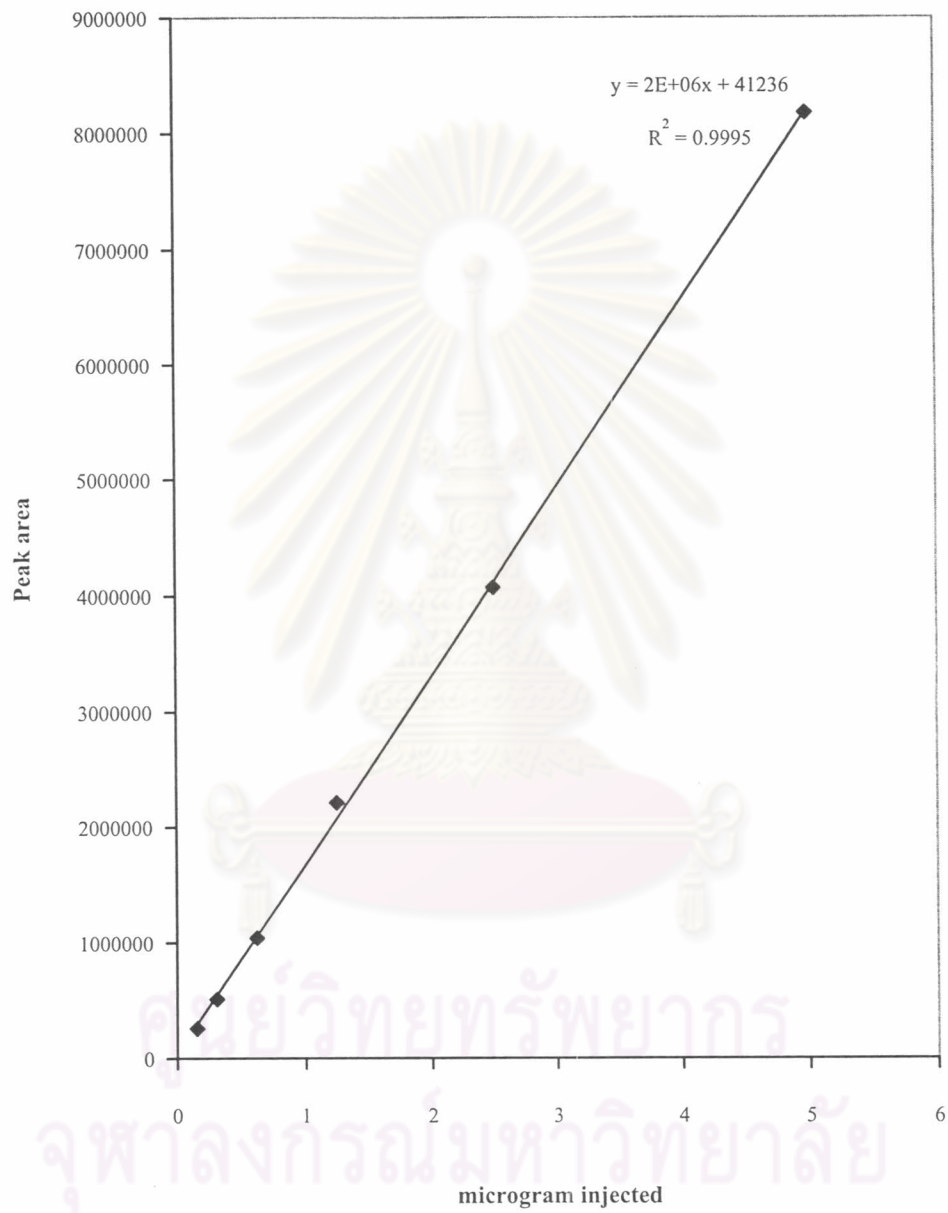


Figure 16 Calibration curve of Genistin

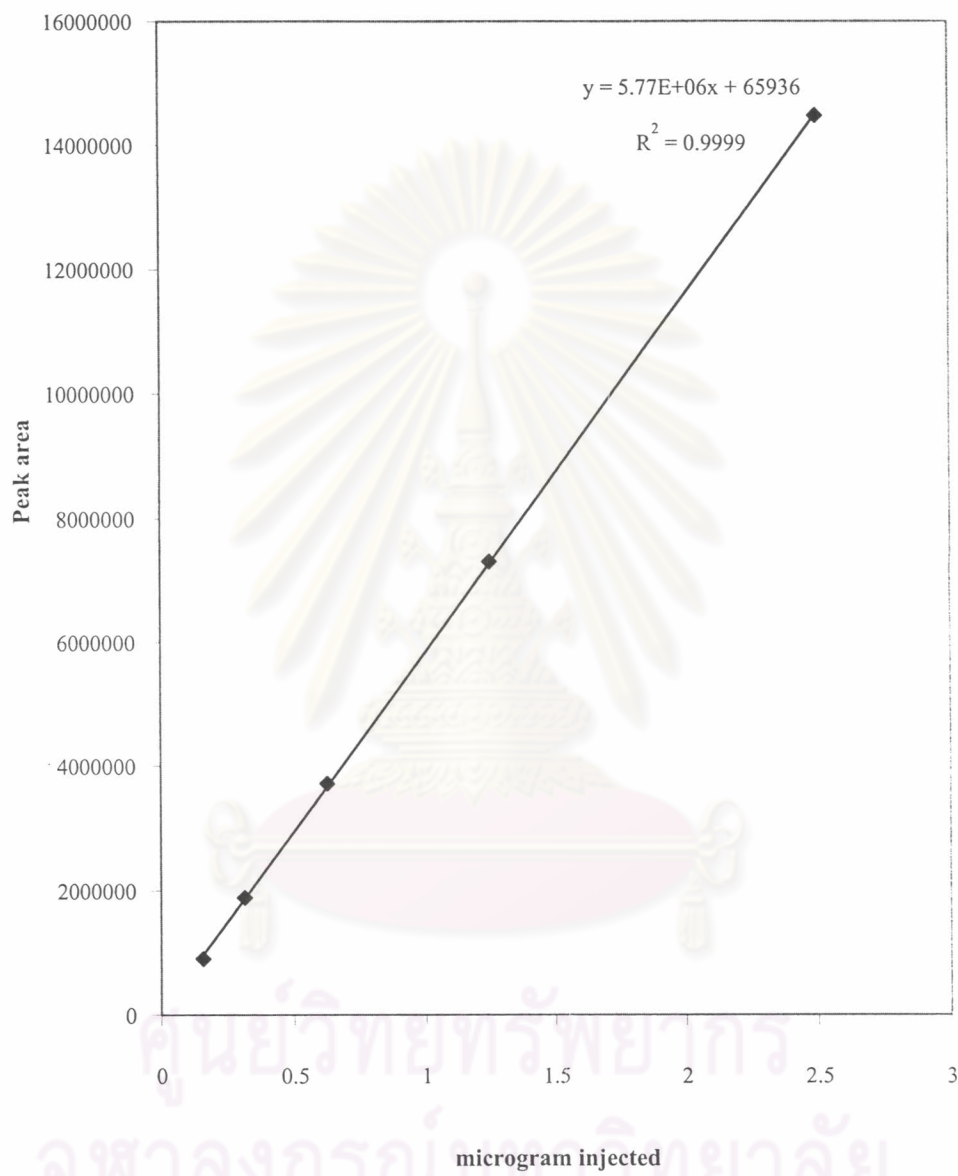


Figure 17 Calibration curve of Daidzein

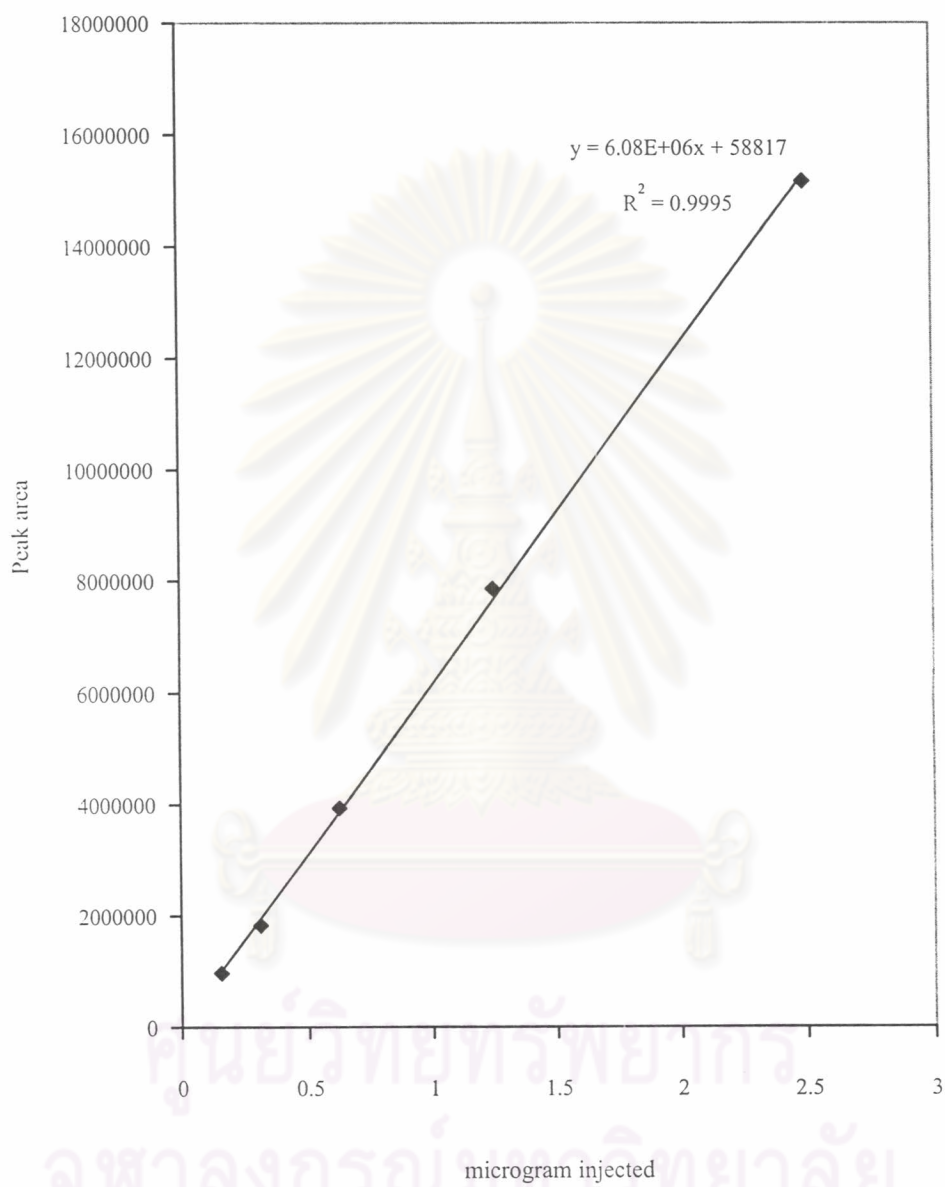


Figure 18 Calibration curve of Genistein

2. Qualitative analysis of the varied sample preparation

Comparative isoflavone analysis from samples derived from 4 different preparative method namely methanol, ethanol and water extract of the tuber powder as well as spray dry of the tuber juice revealed that spray dry exhibited the highest amount of isoflavone (109.66 mg/100g powder). Methanol extract showed the second order (54.58 mg/100g powder). Water extract showed the third order (26.09 mg/100g powder). Ethanol extract showed the least amount (6.90 mg/100g powder)(Table 5, Figure 19).

Table 5 Isoflavone content in mg/100g powder of *P. mirifica* clone Chiang Dao in comparison with preparation

Preparation	Puerarin (Mean±S.E.)	Daidzin (Mean±S.E.)	Genistin (Mean±S.E.)	Daidzein (Mean±S.E.)	Genistein (Mean±S.E.)	Total (Mean±S.E.)	% relative amount
Spray Dry	41.503 ± 0.22	32.87 ± 0.82	19.72 ± 0.51	13.14 ± 0.20	2.42 ± 0.09	109.66 ± 0.57	31.38
Methanol	11.67 ± 0.06	12.37 ± 0.58	24.88 ± 3.25	4.33 ± 0.41	1.34 ± 0.11	54.58 ± 3.08	30.83
Water	8.92 ± 0.12	4.75 ± 0.13	10.48 ± 0.81	1.74 ± 0.41	0.20 ± 0.09	26.09 ± 1.56	8.08
Ethanol	1.63 ± 0.01	1.27 ± 0.02	2.33 ± 0.06	1.19 ± 0.12	0.49 ± 0.08	6.90 ± 0.12	27.81

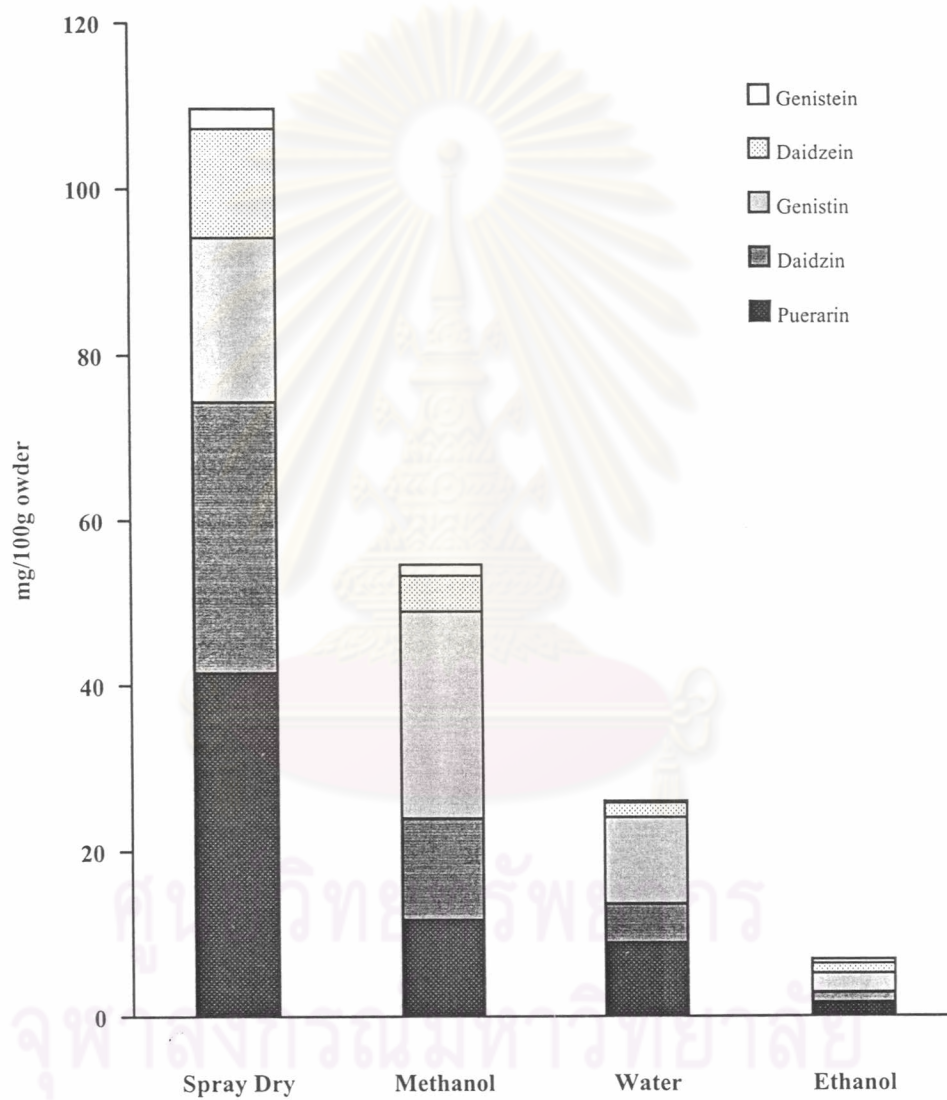


Figure 19 Isoflavone content in mg / 100g powder of *P. mirifica* clone Chiang Dao in comparison with preparation

3. Isoflavone HPLC fingerprint analysis of wild *P. mirifica* collected from 29 provinces.

The isoflavone content by HPLC analysis from wild *P. mirifica* collected from 29 provinces in Thailand showed a significant variation with the maximum amount of 198.29 mg/100g powder from Kanchanaburi province and the minimum amount of 18.85 mg/100g powder from Nan province (Table 6, Figure 20). The great difference was clearly demonstrated in the comparison of the minimum and maximum content samples. The sample from Nan province contained isoflavone only 9.51 % of the sample from Kanchanaburi province. The sample from Nan province contained isoflavone 43.12 % of the sample from the second least collected site namely Kamphaeng Phet province. The sample from the second highest amount namely Lamphoon province contained isoflavone 78.17 % of Kanchanaburi province. The different in isoflavone content were also observed among the other samples.

The mean values of isoflavone content of the total 29 collected samples were compared. There were 5 samples, including Mae Hong Son, Sakon Nakhon, Chiang Mai, Lamphun and Kanchanaburi province exhibited significant higher level and 4 samples, including samples collected from Nan, Kamphaeng Phet, Petchabun and Nakhon Ratchasima province exhibited significant lower level than mean value of isoflavone content of the total collected samples (80.79 mg/100g powder).

The isoflavone content of *P. lobata* was found to be 46.18 % of the maximum amount sample from Kanchanaburi province and 485.83 % of the minimum amount sample from Nan province. It was found that there were 5 samples, including Mae Hong Son, Sakon Nakhon, Chiang Mai, Lamphun and Kanchanaburi province exhibited significant higher level and 16 samples, including samples collected from Phetchaburi, Phrae, Lop Buri, Loei, Chaiyaphum, Uttharadith, Nakhon Sawan, Chiang Rai, Nong Bua Lam Phu, Phayao, Prachuap Khiri Khan, Chumphon, Phrachin Buri, Petchabun, Nakhon Ratchasima, Kamphaeng Phet and Nan province exhibited significant lower level than amount of isoflavone in *P. lobata*.

In comparison with the percentage of individual isoflavone, sample collected from Phetchabun, Mae Hong Son, Uthai Thani, Sakon Nakhon and Chumphon province exhibited the highest percentage of puerarin (72.81 %), daidzin (27.80 %), genistin (64.82 %), daidzein (18.08 %) and genistein (3.56 %), respectively whereas sample from Phetchaburi, Phetchabun, Phetchabun, Chiang Mai and Kamphaeng Phet province exhibited the lowest percentage of individual isoflavone (Table 7, Figure 21).

The percentage of individual isoflavone of *P. lobata* was found to be puerarin 35.87 %, daidzin 23.96 %, genistin 27.99 %, daidzein 11.29 % and genistein 0.88 %. There were 3 samples including Phetchabun, Chaiyaphum and Phayao province exhibited higher percentage of puerarin, 6 samples including samples collected from Mae Hong Son, Loei, Uttharadith, Nan and Prachuap Khiri Khan exhibited higher percentage of daidzin, 28 samples except Phetchabun province exhibited higher percentage of genistin, 4 samples including samples collected from Sakon Nakhon, Kanchanaburi, Chumphon and Tak province exhibited higher percentage of daidzein and 24 samples including samples collected from Chumphon, Tak, Nakhon Sawan, Sakon Nakhon, Phitsanulok, Chaiyaphum, Phetchaburi, Lop Buri, Phayao, Nan, Nakhon Ratchasima, Loei, Phrae, Nong Bua Lam Phu, Phrachin Buri, Kanchanaburi, Lampang, Phetchabun, Uttharadith, Ratchaburi, Sukhothai, Mae Hong Son, Lamphun and Chiang Rai province exhibited higher percentage of genistein as compared with *P. lobata*. (Table 7, Figure 21).

The content of individual isoflavone showed a great variation as well. The puerarin content of *P. mirifica* collected from 29 provinces in Thailand showed a significant variation with the maximum amount of 87.05 mg/100g powder from Sakon Nakhon province and the minimum amount of 5.32 mg/100g powder from Nan province (Table 8, Figure 22). In comparison with the mean isoflavone content of the total 29 collected samples, there were 3 samples, including samples collected from Sakon Nakhon, Kanchanaburi and Mae Hong Son province exhibited significant higher level and 1 sample from Nan province exhibited significant lower level than mean value of the total collected samples (22.01 mg/100g powder).

There were 3 samples, including samples collected from Sakon Nakhon, Kanchanaburi, Mae Hong Son province exhibited significant higher level and 20 samples, including samples collected from Phrae, Saraburi, Chiang Rai, Lop Buri, Chaiyaphum, Kamphaeng Phet, Sukhothai, Nakhon Sawan, Phetchaburi, Nakhon Ratchasima, Phayao, Nong Bua Lam Phu, Phrachin Buri, Uthai Thani, Prachuap Khiri Khan, Petchabun, Ratchaburi, Chumphon, Loei and Nan province exhibited significant lower level than amount of isoflavone as compared with *P. lobata* (32.85 mg/100g powder, Table 8, Figure 22).

The analysis of daidzin content revealed that sample collected from Kanchanaburi province showed the maximum amount (50.24 mg/100g powder) and the minimum amount was found in sample from Nan province (2.36 mg/100g powder, Table 9, Figure 23). In comparison with the mean isoflavone content of the total 29 collected samples it was found that there were 4 samples, including samples collected from Kanchanaburi, Lamphun, Chiang Mai and Sukhothai province exhibited significant higher level and 2 samples, including Nakhon Ratchasima and Nan province exhibited significant lower level than mean value of the total collected samples (14.96 mg / 100 g powder).

There were 3 samples, including samples collected from Kanchanaburi, Lamphun and Chiang Mai province exhibited significant higher level and 21 samples, including samples collected from Lampang, Nakhon Sawan, Ratchaburi, Loei, Uttharadith, Phrachin Buri, Chaiyaphum, Phitsanulok, Nong Bua Lam Phu, Sakon Nakhon, Phrae, Phetchabun, Prachuap Khiri Khan, Tak, Chiang Rai, Phayao, Chumphon, Kamphaeng Phet, Lop Buri, Nakhon Ratchasima and Nan province exhibited significant lower level than amount of isoflavone as compared with *P. lobata* (21.94 mg/100g powder, Table 9, Figure 23).

The analysis of genistin content revealed that the sample collect from Kanchanaburi province exhibited the maximum amount (85.69 mg/100g powder) and the minimum amount was found in sample from Nan province (7.62 mg/100g powder, Table 10, Figure 24). In comparison with the mean isoflavone content of the total 29 collected samples it was found that there were 4 samples, including samples collected

from Kanchanaburi, Lamphun, Chiang Mai and Mae Hong Son exhibiting significant higher level and 5 samples, including samples collected from Kamphaeng Phet, Phrachin Buri, Phetchabun, Sakon Nakhon, Uttharadith and Nan exhibited significant lower level than mean value of the total collected samples (35.84 mg / 100 g powder).

There were 15 samples, including samples collected from Kanchanaburi, Lamphun, Chiang Mai, Mae Hong Son, Sukhothai, Ratchaburi, Uthai Thani, Tak, Lop Buri, Loei, Saraburi, Phetchaburi, Chumphon, Lampang and Phayao exhibiting significant higher level and 5 samples, including samples collected from Kamphaeng Phet, Phrachin Buri, Phetchabun, Sakon Nakhon, Uttharadith and Nan exhibited significant lower level than amount of isoflavone as compared with *P. lobata* (25.63 mg/100g powder, Table 10, Figure 24).

The analysis of daidzein content revealed that the sample collected from Uthai Thani province exhibited maximum amount (16.48 mg/100g powder) and the minimum amount was found in Nakorn Ratchasima province (1.20 mg/100g powder, Table 11, Figure 25).

In comparison with the mean isoflavone content of the total 29 collected samples, there were 3 samples, including samples collected from Uthai Thani, Kanchanaburi and Sukhothai exhibited significant higher level and 5 samples, including samples collected from Lop Buri, Kamphaeng Phet, Chiang Rai, Prachuap Khiri Khan and Nakhon Ratchasima exhibited significant lower level than mean value of the total collected samples (6.13 mg/100g powder).

There were 2 samples, including samples collected from Kanchanaburi and Uthai Thani exhibited significant higher level and 25 samples, including samples collected from Lamphun, Chiang Mai, Phitsanulok, Phetchabun, Mae Hong Son, Nong Bua Lam Phu, Uttharadith, Chaiyaphum, Ratchaburi, Loei, Phetchaburi, Lampang, Phrae, Saraburi, Sakon Nakhon, Nakhon Sawan, Tak, Phrachin Buri, Nan Phayao, Chumphon, Lop Buri, Kamphaeng Phet, Chiang Rai, Prachuap Khiri Khan and Nakhon Ratchasima exhibited significant lower level than amount of isoflavone as compared with *P. lobata* (10.34 mg/100g powder, Table 11, Figure 25).

The analysis of genistein content revealed that the samples collected from Uthai Thani exhibited maximum amount (3.66 mg/100g powder) and the minimum amount was found in sample collected from Chumphon province (0.07 mg/100g powder, Table 12, Figure 26).

In comparison with the mean isoflavone content of the total 29 collected samples, there were 3 samples, including samples collected from Uthai Thani, Kanchanaburi and Ratchaburi exhibited significant higher level and 3 samples, including samples collected from Nan, Nakhon Ratchasima and Chumphon exhibited significant lower level than mean value of the total collected samples (1.24 mg/100 g powder).

There were 9 samples, including samples collected from Uthai Thani, Kanchanaburi, Ratchaburi, Chiang Mai, Nong Bua Lam Phu, Chaiyaphum, Phitsanulok, Lampang and Mae Hong Son exhibits significant higher level and no samples exhibited significant lower level than amount of isoflavone as compared with *P. lobata* (0.81 mg/100g powder, Table 12, Figure 26).



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Table 6 Isoflavone content in mg/100g powder of wild *P. mirifica* from 29 provinces in comparison with *P. lobata*

No.	Province	Puerarin (Mean±S.E.)	Daidzin (Mean±S.E.)	Genistin (Mean±S.E.)	Daidzein (Mean±S.E.)	Genistein (Mean±S.E.)	Total (Mean±S.E.)	Relative amount
1	Kanchanaburi	45.25±1.11	50.24±3.23	85.69±1.23	13.92±1.26	3.19±0.29	198.29±4.6	100
2	Lamphun	33.18±0.92	28.35±0.68	84.13±0.54	8.59±0.09	0.76±0.36	155.00±1.4	78.17
3	Chiang Mai	35.55±3.57	27.39±5.32	58.00±0.71	8.38±0.22	1.93±0.54	131.25±9.0	66.19
4	Sakon Nakhon	87.05±0.79	11.48±0.21	14.83±0.22	4.78±0.37	1.42±0.14	119.57±1.3	60.30
5	Mae Hong Son	36.99±2.07	17.63±1.74	55.44±3.43	7.52±1.27	1.54±0.08	119.12±6.5	60.07
6	Uthai Thani	10.85±1.01	21.70±0.84	50.17±3.57	16.48±1.35	3.66±0.16	102.86±6.5	51.87
7	Sukhothai	14.12±0.94	25.09±1.50	51.43±2.40	11.16±0.85	0.73±0.23	102.52±5.3	51.70
8	Lampang	34.65±1.34	16.59±0.08	33.30±0.08	5.72±0.09	1.54±0.12	91.80±1.72	46.29
9	Tak	29.06±2.07	8.97±0.99	43.86±1.91	4.56±0.32	1.15±0.19	87.60±4.87	44.18
10	Saraburi	23.42±1.21	17.92±0.59	37.94±3.42	4.86±0.95	0.87±0.46	85.01±4.04	42.87
11	Ratchaburi	8.85±0.36	15.39±0.79	51.15±1.75	6.84±0.53	2.54±0.15	84.77±2.67	42.37
12	Phitsanulok	35.24±1.06	12.26±0.13	26.53±0.57	8.36±0.23	1.63±0.05	84.02±1.91	42.75
13	Phetchaburi	13.19±0.45	20.82±1.78	37.56±1.33	6.00±0.24	1.13±0.04	78.71±3.15	39.69
14	Phrae	25.20±1.54	10.55±1.18	30.61±0.81	5.45±0.56	1.34±0.14	73.16±4.21	36.89
15	Lop Buri	19.50±1.44	6.84±0.09	39.47±1.65	2.42±0.79	0.98±0.09	69.21±3.77	34.90
16	Loei	6.54±0.66	15.19±2.24	39.11±1.09	6.59±0.41	1.30±0.15	68.72±4.36	34.66
17	Chaiyaphum	15.83±2.43	12.91±1.44	29.48±2.33	7.02±0.89	1.89±0.42	67.13±5.47	33.85
18	Uttharadith	30.25±0.44	13.69±0.21	10.27±0.19	7.88±0.18	0.87±0.01	62.96±1.03	31.75
19	Nakhon Sawan	13.34±1.46	16.28±1.64	27.71±0.75	4.70±0.37	0.72±0.32	62.75±3.24	31.64
20	Chiang Rai	20.02±1.42	8.61±1.12	29.58±2.43	2.16±0.08	0.50±0.28	60.87±3.30	30.70
21	Nong Bua Lam Phu	12.65±2.42	11.91±3.02	23.65±2.14	7.46±0.96	1.91±0.25	57.58±3.61	29.03
22	Phayao	12.91±0.99	8.46±0.62	32.43±1.35	3.03±0.36	0.73±0.30	57.56±3.02	29.04
23	Prachuap Khiri Khan	10.42±1.03	9.62±0.44	30.31±1.17	2.11±0.36	0.59±0.07	53.05±2.57	26.75
24	Chumphon	8.45±0.22	7.38±1.11	34.17±4.81	2.64±0.26	0.07±0.06	52.70±5.46	26.58
25	Phrachin Buri	12.42±0.26	13.05±0.65	16.69±0.78	4.28±0.56	0.51±0.09	46.94±1.12	23.67
26	Phetchabun	9.40±0.46	10.48±0.67	15.54±1.61	8.11±0.05	1.29±0.02	44.83±1.73	22.32
27	Nakhon Ratchasima	13.09±0.77	5.61±0.07	24.15±1.42	1.20±0.37	0.21±0.19	44.27±1.27	22.61
28	Kamphaeng Phet	15.44±1.14	7.01±1.10	18.50±4.45	2.31±0.11	0.46±0.08	43.71±4.02	22.04
29	Nan	5.32±0.44	2.36±0.22	7.62±1.36	3.31±0.31	0.24±0.04	18.85±1.92	9.51
	Mean	22.01±1.77	14.96±1.02	35.84±2.04	6.13±0.38	1.24±0.09	80.79±4.13	-
	<i>P.lobata</i>	32.85±0.72	21.94±0.74	25.63±0.86	10.34±0.79	0.81±0.08	91.58±3.18	59.08

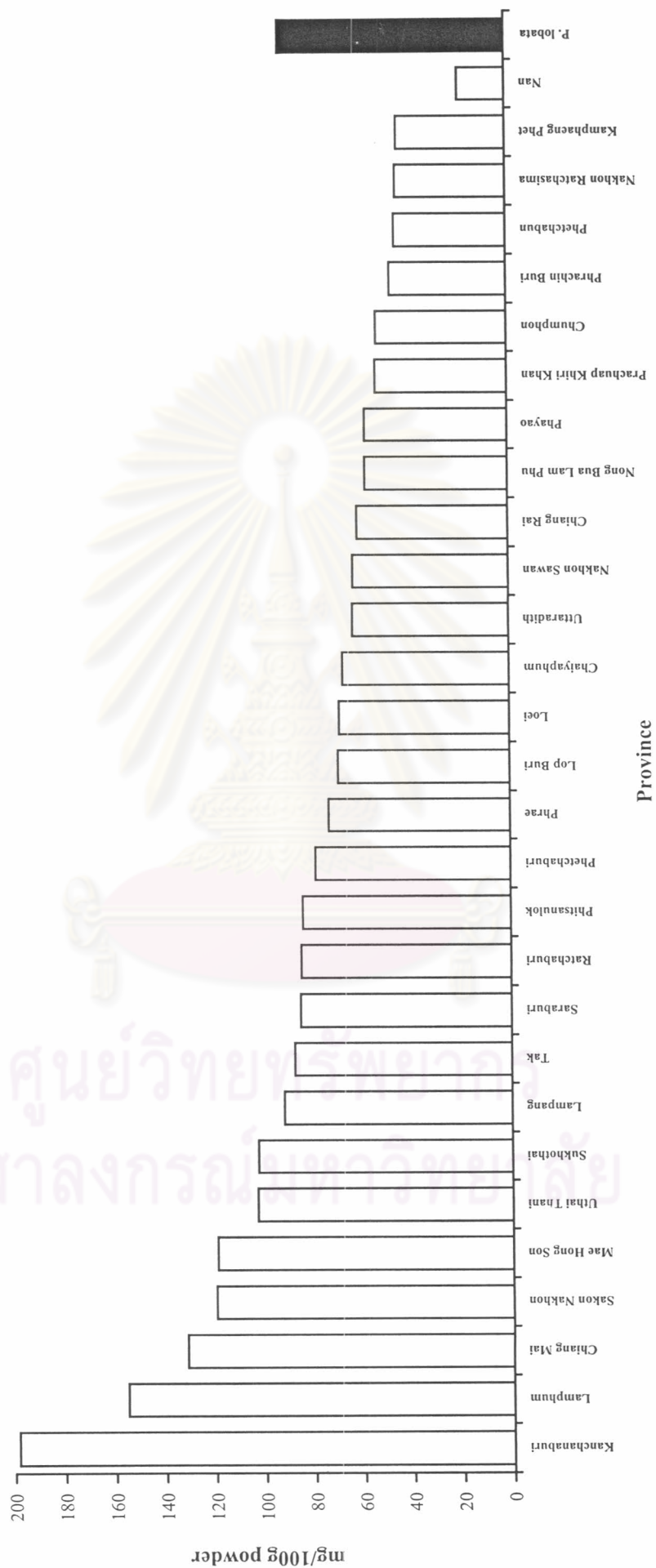


Figure 20 Isoflavone content in mg/100g powder of wild *P. mirifica* from 29 provinces in comparison with *P. lobata*

Table 7 The percentage of puerarin, daidzin, genistin, daidzein and genistein of wild *P. mirifica* from 29 provinces in comparison with *P. lobata* and ranked according to Table 6

No.	Province	Puerarin	Daidzin	Genistin	Daidzein	Genistein
1	Kanchanaburi	28.21	12.52	40.42	17.57	1.28
2	Lamphun	35.33	16.03	42.33	5.28	1.04
3	Chiang Mai	29.57	12.68	54.55	2.70	0.49
4	Sakon Nakhon	20.97	23.39	34.66	18.08	2.89
5	Mae Hong Son	26.46	27.80	35.55	9.12	1.08
6	Uthai Thani	16.03	14.00	64.82	5.02	0.12
7	Sukhothai	19.63	18.14	57.14	3.98	1.10
8	Lampang	22.42	14.70	56.33	5.27	1.27
9	Tak	21.97	20.69	41.07	12.95	3.32
10	Saraburi	32.90	14.14	48.60	3.54	0.82
11	Ratchaburi	21.26	25.94	44.16	7.49	1.15
12	Phitsanulok	23.58	19.23	43.91	10.46	2.81
13	Phetchaburi	9.52	22.10	56.91	9.58	1.89
14	Phrae	28.18	9.88	57.03	3.50	1.41
15	Lop Buri	34.45	14.42	41.85	7.45	1.83
16	Loei	16.75	26.46	47.72	7.62	1.44
17	Chaiyaphum	41.95	14.60	31.57	9.95	1.94
18	Uttharadith	21.26	25.94	44.16	7.49	1.15
19	Nakhon Sawan	10.44	18.16	60.34	8.07	2.99
20	Chiang Rai	27.55	21.08	44.63	5.72	1.02
21	Nong Bua Lam Phu	33.17	10.24	50.07	5.20	1.32
22	Phayao	37.74	18.08	36.27	6.24	1.68
23	Prachuap Khiri Khan	13.77	24.47	50.16	10.88	0.71
24	Chumphon	10.55	21.10	48.78	16.02	3.56
25	Phrachin Buri	31.05	14.80	46.54	6.32	1.29
26	Phetchabun	72.81	9.60	12.40	4.00	1.19
27	Nakhon Ratchasima	27.08	20.87	44.19	6.39	1.47
28	Kamphaeng Phet	21.41	18.29	54.27	5.54	0.49
29	Nan	22.82	25.34	43.21	7.02	1.61
	<i>P. lobata</i>	35.87	23.96	27.99	11.29	0.88

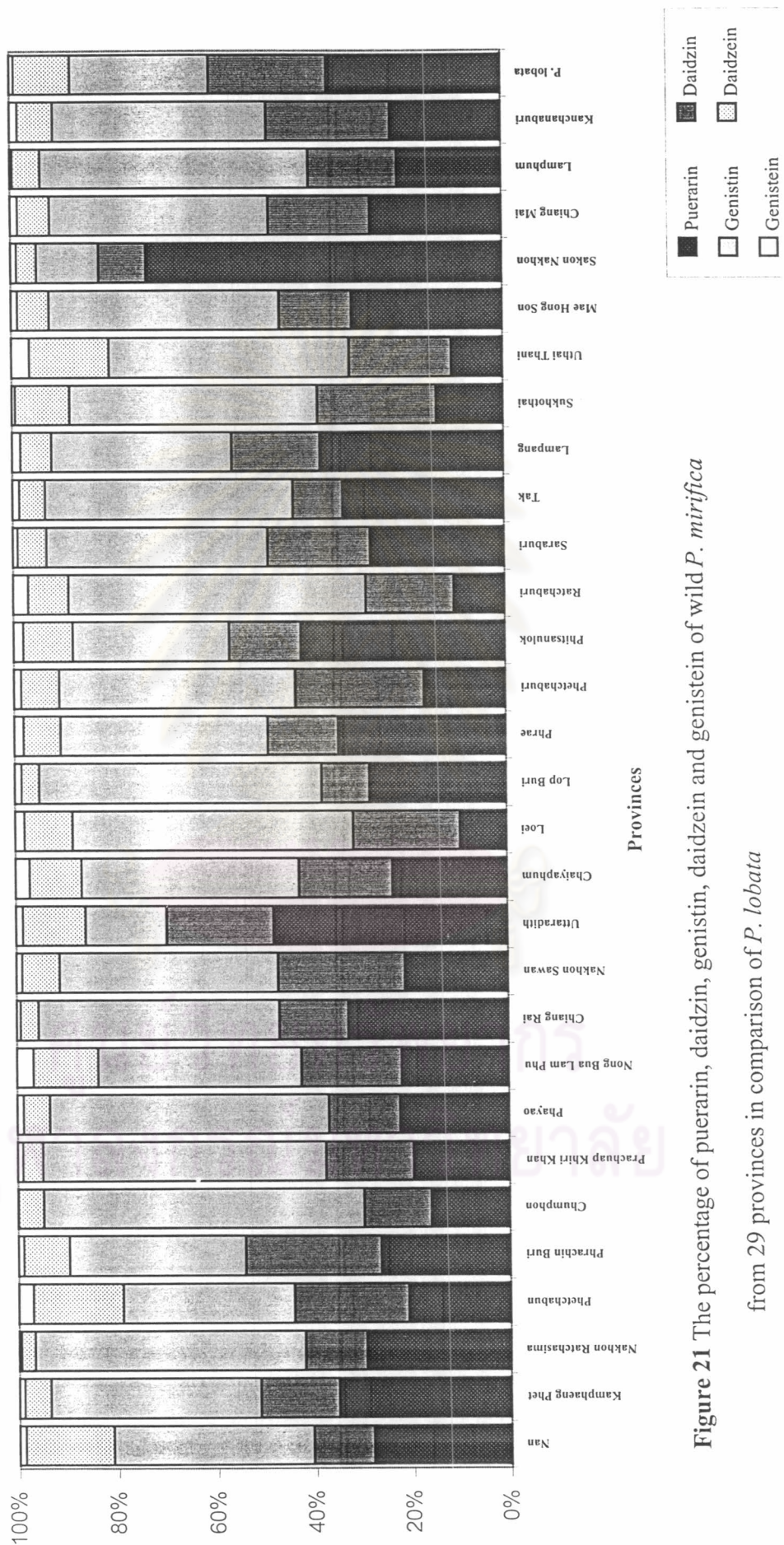


Figure 21 The percentage of puerarin, daidzin, genistin, daidzein and genistein of wild *P. mirifica* from 29 provinces in comparison of *P. lobata*

Table 8 Puerarin content in mg/100g powder of wild *P. mirifica* from 29 provinces in comparison with *P. lobata*

No.	Province	Puerarin (Mean±S.E.)
1	Sakon Nakhon	87.05 ± 0.79
2	Kanchanaburi	45.25 ± 1.11
3	Mae Hong Son	36.99 ± 2.07
4	Chiang Mai	35.55 ± 3.57
5	Phitsanulok	35.24 ± 1.06
6	Lampang	34.65 ± 1.34
7	Lamphun	33.18 ± 0.92
8	Uttharadith	30.25±0.44
9	Tak	29.06 ± 2.07
10	Phrae	25.20 ± 1.54
11	Saraburi	23.42 ± 1.21
12	Chiang Rai	20.02 ± 1.42
13	Lop Buri	19.50 ± 1.44
14	Chaiyaphum	15.83 ± 2.43
15	Kamphaeng Phet	15.44 ± 1.14
16	Sukhothai	14.12 ± 0.94
17	Nakhon Sawan	13.34 ± 1.46
18	Phetchaburi	13.19 ± 0.45
19	Nakhon Ratchasima	13.09 ± 0.77
20	Phayao	12.91 ± 0.99
21	Nong Bua Lam Phu	12.65 ± 2.42
22	Phrachin Buri	12.42 ± 0.26
23	Uthai Thani	10.85 ± 1.01
24	Prachuap Khiri Khan	10.42 ± 1.03
25	Phetchabun	9.40 ± 0.46
26	Ratchaburi	8.85 ± 0.36
27	Chumphon	8.45 ± 0.22
28	Loei	6.54 ± 0.66
29	Nan	5.32 ± 0.44
Mean		22.01±1.77
<i>P. lobata</i>		32.85 ± 0.72

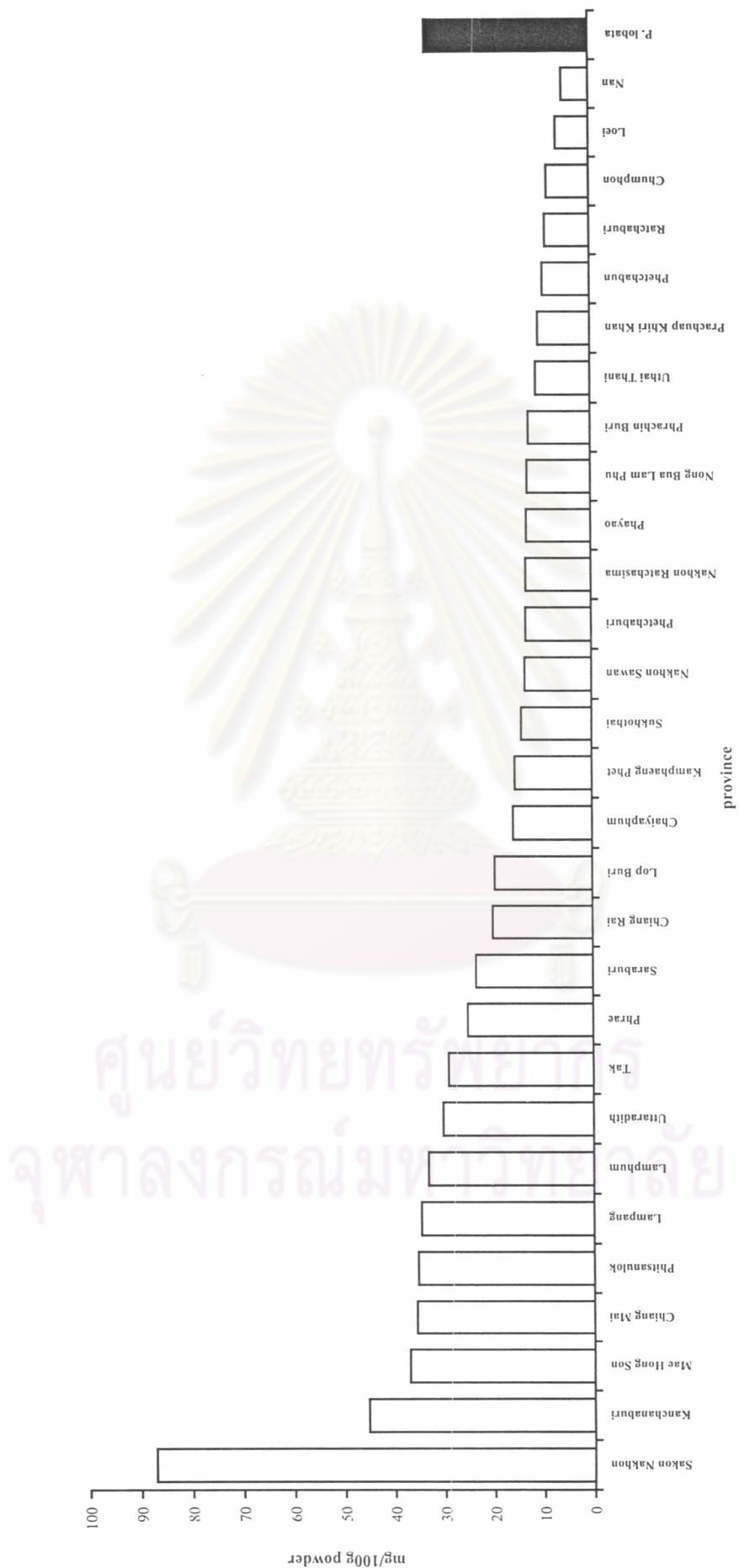


Figure 22 Puerarin content in mg/100g powder of wild *P. mirifica* from 29 provinces in comparison with *P. lobata*

Table 9 Daidzin content in mg/100g powder of wild *P. mirifica* from 29 provinces in comparison with *P. lobata*

No.	Province	Daidzin (Mean±S.E.)
1	Kanchanaburi	50.24 ± 3.23
2	Lamphun	28.35 ± 0.68
3	Chiang Mai	27.39 ± 5.32
4	Sukhothai	25.09 ± 1.50
5	Uthai Thani	21.70 ± 0.84
6	Phetchaburi	20.82 ± 1.78
7	Saraburi	17.92 ± 0.59
8	Mae Hong Son	17.63 ± 1.74
9	Lampang	16.59 ± 0.08
10	Nakhon Sawan	16.28 ± 1.64
11	Ratchaburi	15.39 ± 0.79
12	Loei	15.19 ± 2.24
13	Uttharadith	13.69±0.21
14	Phrachin Buri	13.05 ± 0.65
15	Chaiyaphum	12.91 ± 1.44
16	Phitsanulok	12.26 ± 0.13
17	Nong Bua Lam Phu	11.91 ± 3.02
18	Sakon Nakhon	11.48 ± 0.21
19	Phrae	10.55 ± 1.18
20	Phetchabun	10.48 ± 0.67
21	Prachuap Khiri Khan	9.62 ± 0.44
22	Tak	8.97 ± 0.99
23	Chiang Rai	8.61 ± 1.12
24	Phayoa	8.46 ± 0.62
25	Chumphon	7.38 ± 1.11
26	Kamphaeng Phet	7.01 ± 1.10
27	Lop Buri	6.84 ± 0.09
28	Nakhon Ratchasima	5.61 ± 0.07
29	Nan	2.36 ± 0.22
	Mean	14.96 ± 1.02
	<i>P. lobata</i>	21.94 ± 0.74



Figure 23 Daidzin content in mg/100g powder of wild *P. mirifica* from 29 provinces in comparison with *P. lobata*



Table 10 Genistin content in mg/100g powder of wild *P. mirifica* from 29 provinces in comparison with *P. lobata*

No.	Province	Genistin (Mean±S.E.)
1	Kanchanaburi	85.69 ± 1.23
2	Lamphum	84.13 ± 0.54
3	Chiang Mai	58.00 ± 0.71
4	Mae Hong Son	55.44 ± 3.43
5	Sukhothai	51.43 ± 2.40
6	Ratchaburi	51.15 ± 1.75
7	Uthai Thani	50.17 ± 3.57
8	Tak	43.86 ± 1.91
9	Lop Buri	39.47 ± 1.65
10	Loei	39.11 ± 1.09
11	Saraburi	37.94 ± 3.42
12	Phetchaburi	37.56 ± 1.33
13	Chumphon	34.17 ± 4.81
14	Lampang	33.30 ± 0.08
15	Phayao	32.43 ± 1.35
16	Phrae	30.61 ± 0.81
17	Prachuap Khiri Khan	30.31 ± 1.17
18	Chiang Rai	29.58 ± 2.43
19	Chaiyaphum	29.48 ± 2.33
20	Nakhon Sawan	27.71 ± 0.75
21	Phitsanulok	26.53 ± 0.57
22	Nakhon Ratchasima	24.15 ± 1.42
23	Nong Bua Lam Phu	23.65 ± 2.14
24	Kamphaeng Phet	18.50 ± 4.45
25	Phrachin Buri	16.69 ± 0.78
26	Phetchabun	15.54 ± 1.61
27	Sakon Nakhon	14.83 ± 0.22
28	Uttharadith	10.27±0.19
29	Nan	7.62 ± 1.36
	Mean	35.84 ± 2.04
	<i>P. lobata</i>	25.63 ± 0.86

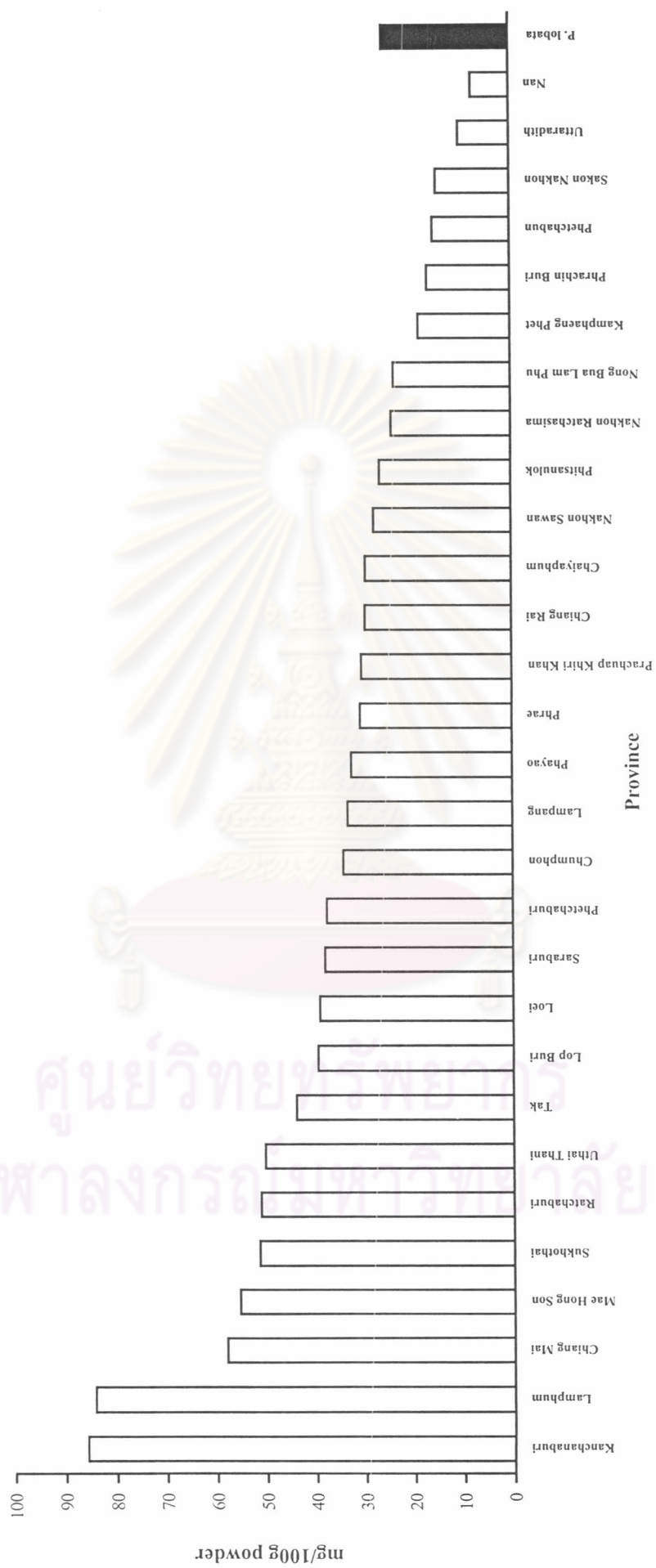


Figure 24 Genistin content in mg/100g powder of wild *P. mirifica* from 29 provinces in comparison with *P. lobata*

Table 11 Daidzein content in mg/100g powder of wild *P. mirifica* from 29 provinces in comparison with *P. lobata*

No.	Province	Daidzein (Mean±S.E.)
1	Uthai Thani	16.48 ± 1.35
2	Kanchanaburi	13.92 ± 1.26
3	Sukhothai	11.16 ± 0.85
4	Lamphum	8.59 ± 0.09
5	Chiang Mai	8.38 ± 0.22
6	Phitsanulok	8.36 ± 0.23
7	Phetchabun	8.11 ± 0.05
8	Mae Hong Son	7.52 ± 1.27
9	Nong Bua Lam Phu	7.46 ± 0.96
10	Uttharadith	7.88±0.18
11	Chaiyaphum	7.02 ± 0.89
12	Ratchaburi	6.84 ± 0.53
13	Loei	6.59 ± 0.41
14	Phetchaburi	6.00 ± 0.24
15	Lampang	5.72 ± 0.09
16	Phrae	5.45 ± 0.56
17	Saraburi	4.86 ± 0.95
18	Sakon Nakhon	4.78 ± 0.37
19	Nakhon Sawan	4.70 ± 0.37
20	Tak	4.56 ± 0.32
21	Phrachin Buri	4.28 ± 0.56
22	Nan	3.31 ± 0.31
23	Phayao	3.03 ± 0.36
24	Chumphon	2.64 ± 0.26
25	Lop Buri	2.42 ± 0.79
26	Kamphaeng Phet	2.31 ± 0.11
27	Chiang Rai	2.16 ± 0.08
28	Prachuap Khiri Khan	2.11 ± 0.36
29	Nakhon Ratchasima	1.20 ± 0.37
	Mean	6.13 ± 0.38
	<i>P. lobata</i>	10.34 ± 0.79



Figure 25 Daidzein content in mg/100g powder of wild *P. mirifica* from 29 provinces in comparison with *P. lobata*

Table 12 Genistein content in mg/100g powder of wild *P. mirifica* from 29 provinces in comparison with *P. lobata*

No.	Province	Genistein (Mean±S.E.)
1	Uthai Thani	3.66 ± 0.16
2	Kanchanaburi	3.19 ± 0.29
3	Ratchaburi	2.54 ± 0.15
4	Chiang Mai	1.93 ± 0.54
5	Nong Bua Lam Phu	1.91 ± 0.25
6	Chaiyaphum	1.89 ± 0.42
7	Phitsanulok	1.63 ± 0.05
8	Lampang	1.54 ± 0.12
9	Mae Hong Son	1.54 ± 0.08
10	Sakon Nakhon	1.42 ± 0.14
11	Phrae	1.34 ± 0.14
12	Phetchabun	1.30 ± 0.02
13	Loei	1.30 ± 0.15
14	Tak	1.15 ± 0.19
15	Phetchaburi	1.14 ± 0.04
16	Lop Buri	0.98 ± 0.09
17	Saraburi	0.87 ± 0.46
18	Uttharadith	0.87 ± 0.01
19	Lamphum	0.76 ± 0.36
20	Phayao	0.73 ± 0.30
21	Sukhothai	0.73 ± 0.23
22	Nakhon Sawan	0.72 ± 0.32
23	Prachuap Khiri Khan	0.59 ± 0.07
24	Phrachin Buri	0.51 ± 0.09
25	Chiang Rai	0.50 ± 0.28
26	Kamphaeng Phet	0.45 ± 0.08
27	Nan	0.24 ± 0.04
28	Nakhon Ratchasima	0.22 ± 0.19
29	Chumphon	0.07 ± 0.06
	Mean	1.24 ± 0.09
	<i>P. lobata</i>	0.81 ± 0.08



Figure 26 Genistein content in mg/100g powder of wild *P. mirifica* from 29 provinces in comparison with *P. lobata*

4. Analysis of the influence of different collected site on isoflavone content of *P. mirifica* collected from the same province.

The analysis of isoflavone content of the collected samples from 5 districts in Chiang Mai province revealed that all the analyzed samples showed significant different isoflavone content from each other ($p < 0.05$). The sample from Hod district exhibited the highest amount of isoflavone (140.90 mg/100g powder) as well as puerarin (39.31 mg/100g powder), daidzin (31.66 mg/100g powder), genistin (59.67 mg/100g powder), daidzein (8.77 mg/100g powder), and genistein (1.49 mg/100g powder). The sample from Chaiprakarn district exhibited the lowest amount of isoflavone (42.26 mg/100g powder) as well as puerarin (16.13 mg/100g powder), daidzin (8.01 mg/100g powder), genistin (13.92 mg/100g powder), daidzein (3.42 mg/100g powder) and genistein (0.79mg/100g powder) (Table 13, Figure 27).

The analysis of isoflavone content of the collected samples from 3 districts in Lampang province revealed that the isoflavone content is slightly different between samples from Koh Ka and Hangchat district but significantly different from Thern district. The sample from Koh Ka district showed higher level of puerarin (34.65 mg/100g powder) as compare with sample from Thern district (20.85 mg/100g powder) and Hangchat district (11.77 mg/100g powder). The sample from Hangchat showed high amount of Daidzein (19.32 mg/100g powder) as compare with the sample from Koh Ka (5.72 mg/100g powder) and Thern district (6.27 mg/100g powder, Table 14, Figure 28).

The analysis of isoflavone content of the collected samples from 3 districts in Kanchanaburi province revealed that the isoflavone content was different among samples. The sample from Srisawat district showed highest level of isoflavone content (198.29 mg/100g powder) as well as the highest amount of puerarin (45.25 mg/100g powder), daidzin (50.24 mg/100g powder), genistin (85.69 mg/100g powder), daidzein (13.92 mg/100g powder) and genistein (3.18 mg/100g powder). The sample from Sai Yoke district showed moderate level of isoflavone content (78.14 mg/100g powder) as well as the amount of puerarin (7.15 mg/100g powder), daidzin (16.61 mg/100g powder), genistin (45.93 mg/100g powder), daidzein (6.73 mg/100g powder)

and genistein (1.71 mg/100g powder). The sample from Thongphaphum district showed the lowest isoflavone content (30.67 mg/100g powder) as well as the lowest amount of puerarin (8.33 mg/100g powder), daidzin (4.01 mg/100g powder), genistin (13.69 mg/100g powder), daidzein (4.01 mg/100g powder) and genistein (0.62 mg/100g powder, Table 15, Figure 29).

The analysis of isoflavone content of the collected samples from 3 sites in 2 districts in Saraburi province revealed that the isoflavone content is different among samples. The sample from Muak Lek district site I showed highest level of isoflavone content (85.01 mg/100g powder). The sample from Phraputthabat district showed moderate level of isoflavone content (56.59 mg/100g powder). The sample from Muak Lek district site II showed the lowest isoflavone content (43.99 mg/100g powder, Table 16, Figure 30).



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Table 13 Isoflavone content in mg/100g powder of wild *P. mirifica* from 5 collected sites in Chiang Mai province

District	Puerarin (Mean±S.E.)	Daidzin (Mean±S.E.)	Genistin (Mean±S.E.)	Daidzein (Mean±S.E.)	Genistein (Mean±S.E.)	Total (Mean±S.E.)	% relative amount
Hod	39.31 ± 2.37	31.66 ± 3.10	59.67 ± 1.87	8.77 ± 0.17	1.49 ± 0.56	140.90 ± 5.67	58.46
Doi Tao	28.36 ± 3.33	28.70 ± 3.32	58.38 ± 7.96	4.05 ± 0.45	1.10 ± 0.41	120.60 ± 13.67	44.04
Doi Saket	17.17 ± 0.77	15.76 ± 0.71	44.12 ± 2.53	8.10 ± 0.53	1.66 ± 0.18	86.80 ± 1.84	46.78
Chiang Dao	10.96 ± 0.75	12.37 ± 0.58	20.99 ± 3.12	4.33 ± 0.41	1.34 ± 0.11	49.98 ± 3.80	30.83
Chaiprakarn	16.13 ± 0.96	8.01 ± 0.36	13.92 ± 2.16	3.42 ± 0.49	0.79 ± 0.32	42.26 ± 2.94	29.57
Mean	22.38 ± 2.82	19.30 ± 2.60	39.41 ± 5.29	5.73 ± 0.62	1.27 ± 0.16	88.11 ± 10.62	-

Table 14 Isoflavone content in mg/100g powder of wild *P. mirifica* from 3 collected sites Lampang province

District	Puerarin (Mean±S.E.)	Daidzin (Mean±S.E.)	Genistin (Mean±S.E.)	Daidzein (Mean±S.E.)	Genistein (Mean±S.E.)	Total (Mean±S.E.)	% relative amount
Koh Ka	34.65 ± 1.34	16.59 ± 0.07	33.30 ± 0.08	5.72 ± 0.10	1.54 ± 0.12	91.80 ± 1.72	48.41
Hangchat	11.77 ± 0.72	14.66 ± 0.70	39.91 ± 2.04	19.32 ± 2.09	2.22 ± 0.19	87.89 ± 4.36	47.05
Thern	20.85 ± 0.98	15.28 ± 1.14	29.03 ± 3.67	6.27 ± 0.68	2.54 ± 0.53	73.97 ± 5.01	43.11
Mean	22.42 ± 3.36	15.51 ± 0.48	34.08 ± 1.99	10.44 ± 2.31	2.10 ± 0.22	84.55 ± 3.35	-

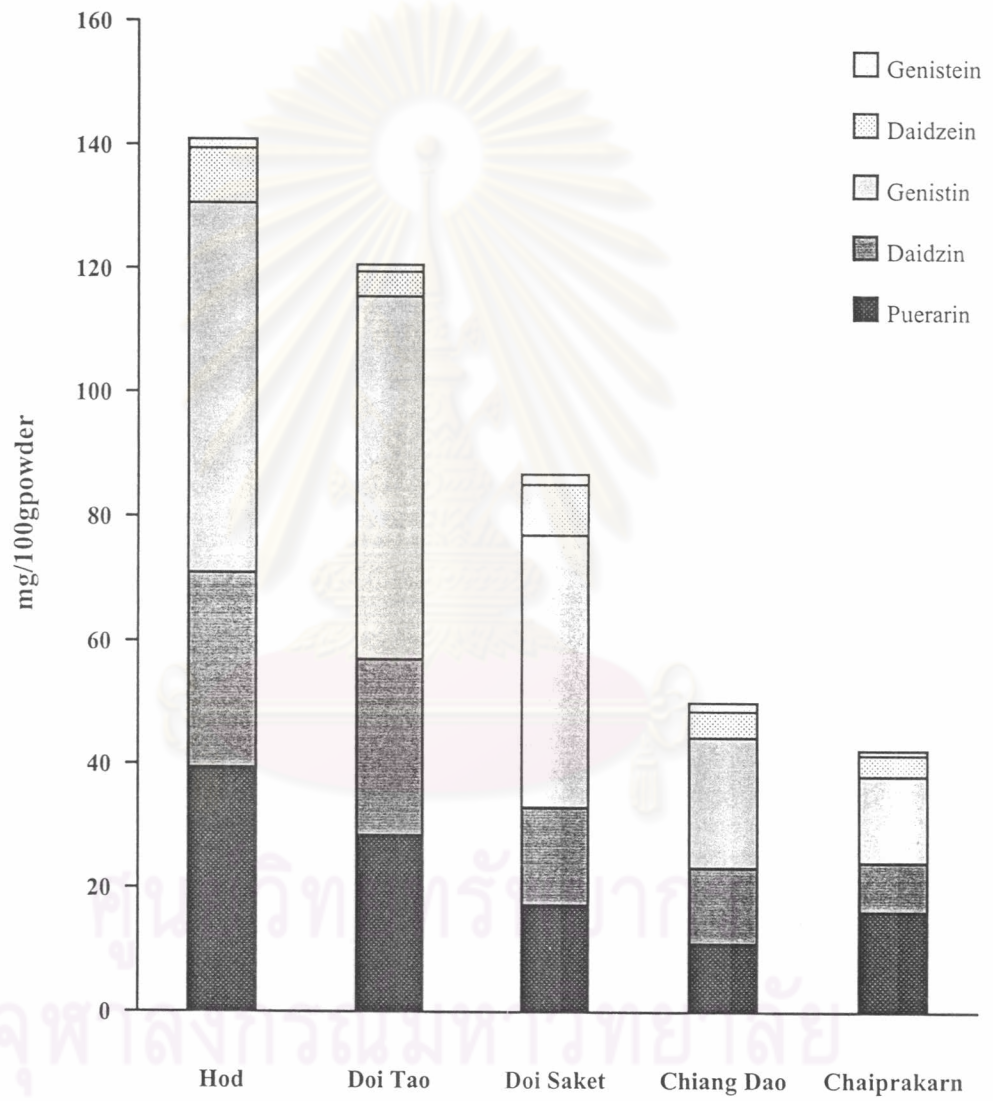


Figure 27 Isoflavone content in mg/100g powder of wild *P. mirifica* from 5 collected sites in Chiang Mai province

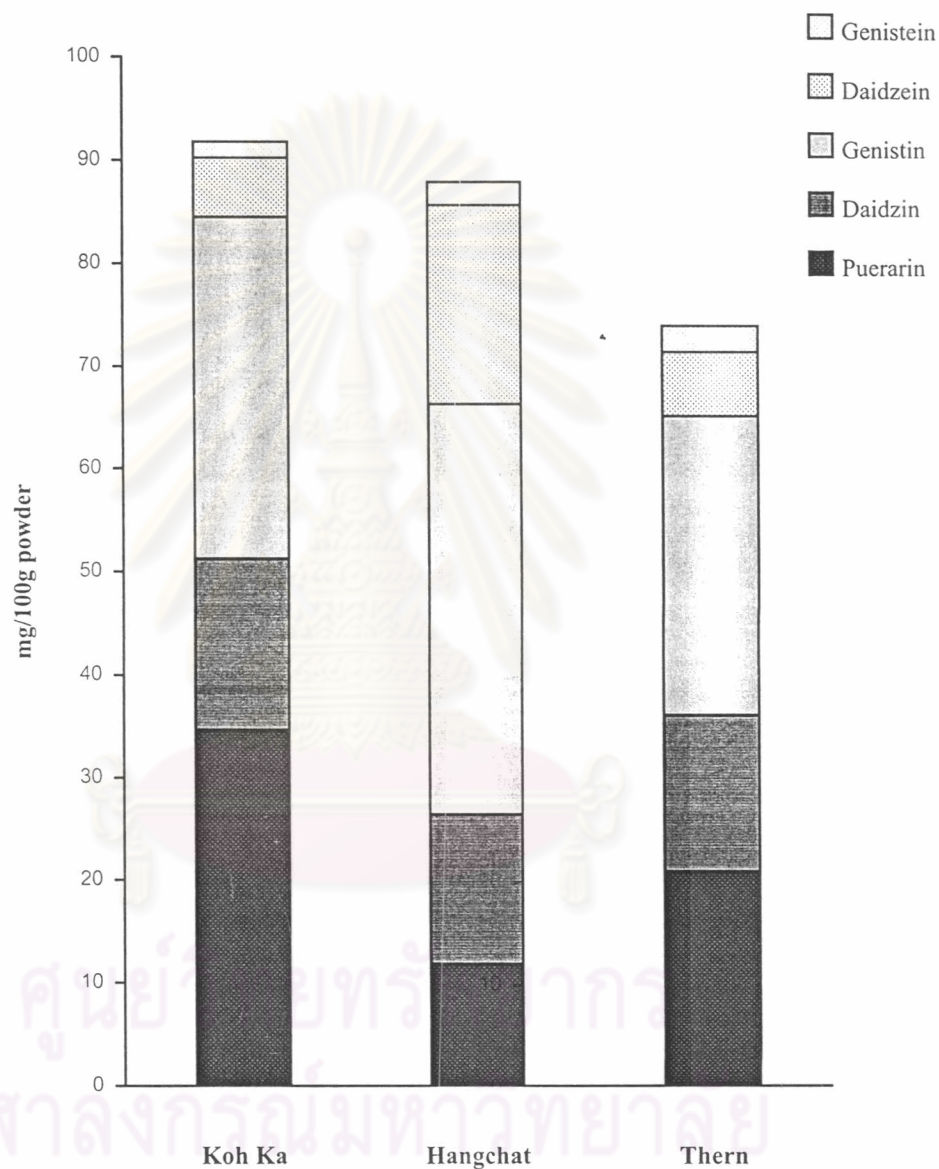


Figure 28 Isoflavone content in mg/100 g powder of wild *P. mirifica* from 3 collected sites in Lampang province

Table 15 Isoflavone content in mg/100g powder of wild *P. mirifica* from 3 collected sites in Kanchanaburi province

District	Puerarin (Mean±S.E.)	Daidzin (Mean±S.E.)	Genistin (Mean±S.E.)	Daidzein (Mean±S.E.)	Genistein (Mean±S.E.)	Total (Mean±S.E.)	% relative amount
Srisawat	45.25 ± 1.11	50.24 ± 3.23	85.69 ± 1.23	13.92 ± 1.25	3.18 ± 0.29	198.29 ± 4.61	50.55
Sai Yoke	7.15 ± 0.51	16.61 ± 0.70	45.93 ± 0.76	6.73 ± 0.32	1.71 ± 0.10	78.14 ± 0.95	34.78
Thongpha- phum	8.33 ± 0.64	4.01 ± 0.50	13.69 ± 2.85	4.01 ± 0.23	0.62 ± 0.25	30.67 ± 4.27	28.86
Mean	20.25 ± 6.27	23.62 ± 6.97	48.44 ± 10.45	8.22 ± 1.53	1.84 ± 0.39	102.37 ± 25.00	-

Table 16 Isoflavone content in mg/100g powder of wild *P. mirifica* from 3 collected sites in Saraburi province

District	Puerarin (Mean±S.E.)	Daidzin (Mean±S.E.)	Genistin (Mean±S.E.)	Daidzein (Mean±S.E.)	Genistein (Mean±S.E.)	Total (Mean±S.E.)	% relative amount
Muak Lek site I	23.42 ± 1.21	17.92 ± 0.59	37.94 ± 3.42	4.86 ± 0.95	0.88 ± 0.46	85.01 ± 4.05	41.32
Phraputthabat	10.87 ± 1.24	13.11 ± 0.23	29.03 ± 0.38	2.74 ± 0.44	0.83 ± 0.04	56.59 ± 2.33	22.94
Muak Lek site II	13.30 ± 1.90	4.84 ± 0.47	20.68 ± 1.65	3.76 ± 0.44	1.41 ± 0.38	43.99 ± 0.02	25.61
Mean	16.49 ± 2.20	11.81 ± 2.17	29.24 ± 3.09	3.92 ± 0.46	1.06 ± 0.18	62.52 ± 7.12	-

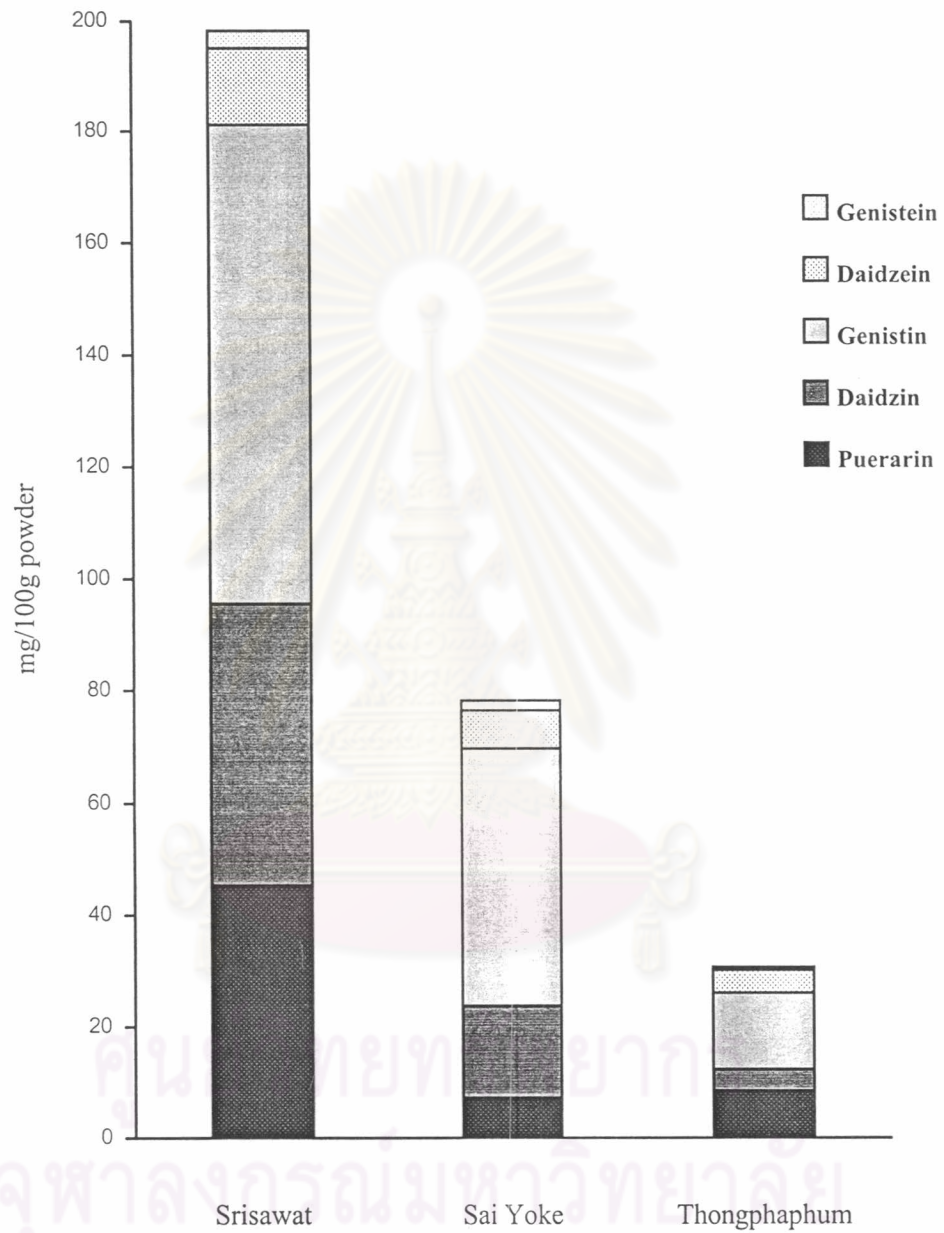


Figure 29 Isoflavone content in mg/100g powder of wild *P. mirifica* from 3 collected sites in Kanchanaburi province

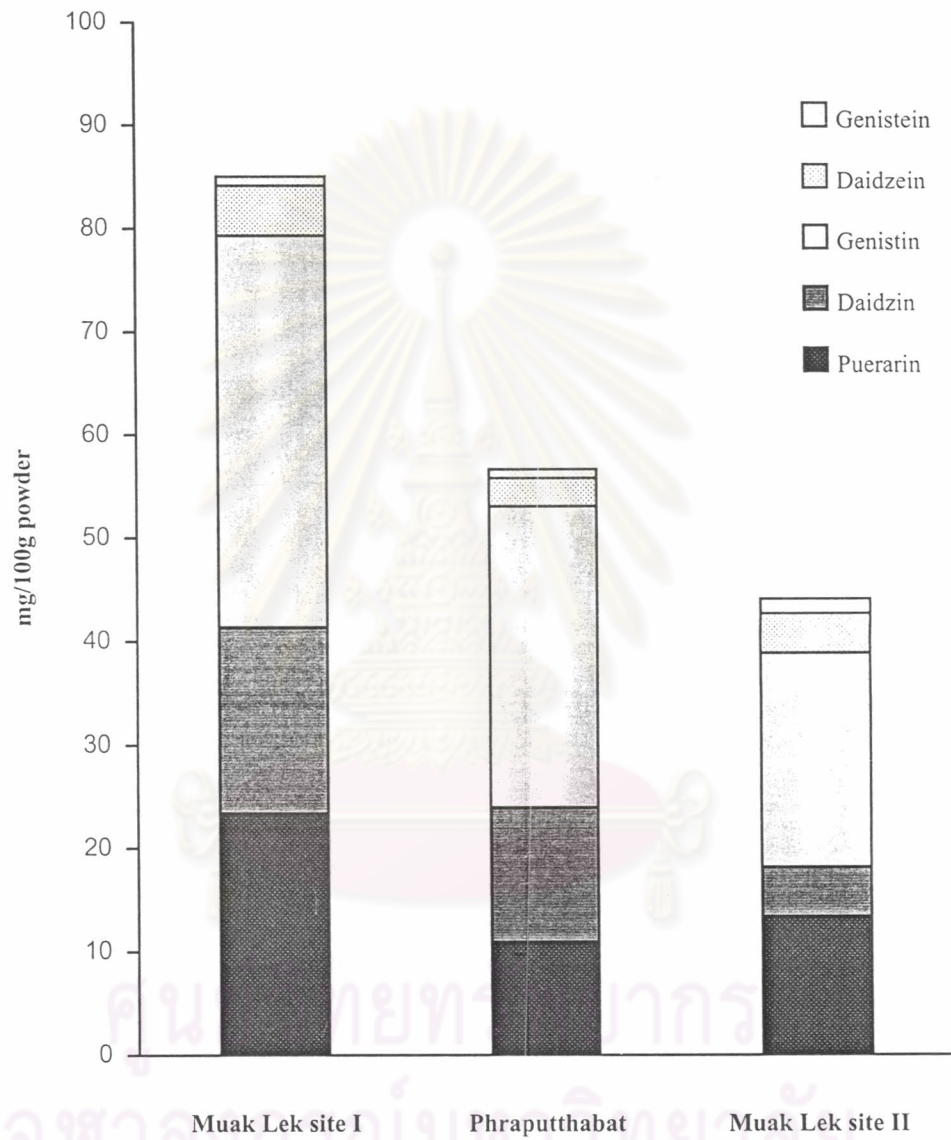


Figure 30 Isoflavone content in mg/100g powder of wild *P. mirifica* from 3 collected sites in Saraburi province

5. Analysis of the influence of field location on isoflavone content of the field grown *P. mirifica*

The analysis of isoflavone content of the collected samples in summer season from the same clone cultivated in different area from the 2 years old plants revealed that the isoflavone content was different among samples. *P. mirifica* clone Doi Tao showed highest yield in term of isoflavone from the field trial in Ratchaburi province (95.21 mg/100g powder) as compared with the field trial in Bangkok (71.09 mg/100g powder) and Chiang Rai (64.20 mg/100g powder), respectively (Table 17, Figure 31) *P. mirifica* clone Chaiprakarn showed no statistical significance difference ($p < 0.05$) of isoflavone content from the field trial plants in Ratchaburi (95.82 mg/100g powder) and Chiang Rai (90.74 mg/100g powder), respectively. (Table 18, Figure 32)



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Table 17 Isoflavone content in mg/100g powder of field grown *P. mirifica* clone Doi Tao cultivated in different location

Location	Puerarin (Mean±S.E.)	Daidzin (Mean±S.E.)	Genistin (Mean±S.E.)	Daidzein (Mean±S.E.)	Genistein (Mean±S.E.)	Total (Mean±S.E.)	% relative amount
Ratchaburi	13.97 ± 2.05	23.40 ± 2.28	43.78 ± 4.42	12.04 ± 1.62	2.02 ± 0.31	95.21 ± 8.30	41.49
Bangkok	6.95 ± 0.24	22.87 ± 2.57	35.37 ± 4.84	5.40 ± 0.34	0.49 ± 0.09	71.09 ± 6.41	48.25
Chiang Rai	5.38 ± 0.49	15.50 ± 0.76	35.87 ± 7.33	6.56 ± 0.06	0.88 ± 0.22	64.20 ± 6.37	25.29
Mean	9.74 ± 1.19	22.04 ± 1.55	39.05 ± 2.99	8.41 ± 0.98	1.20 ± 0.21	80.44 ± 5.28	-

Table 18 Isoflavone content in mg/100g powder of field grown *P. mirifica* clone Chaiprakarn cultivated in different location

Location	Puerarin (Mean±S.E.)	Daidzin (Mean±S.E.)	Genistin (Mean±S.E.)	Daidzein (Mean±S.E.)	Genistein (Mean±S.E.)	Total (Mean±S.E.)	% relative amount
Ratchaburi	5.92 ± 0.26	22.55 ± 2.49	55.95 ± 5.32	9.16 ± 0.99	2.23 ± 0.28	95.82 ± 8.07	49.59
Chiang Rai	6.31 ± 0.31	17.32 ± 1.47	45.90 ± 5.16	18.37 ± 0.99	2.83 ± 0.55	90.74 ± 5.44	39.40
Mean	6.02 ± 0.21	21.24 ± 1.98	53.44 ± 4.29	11.46 ± 1.42	2.38 ± 0.25	94.55 ± 6.11	-

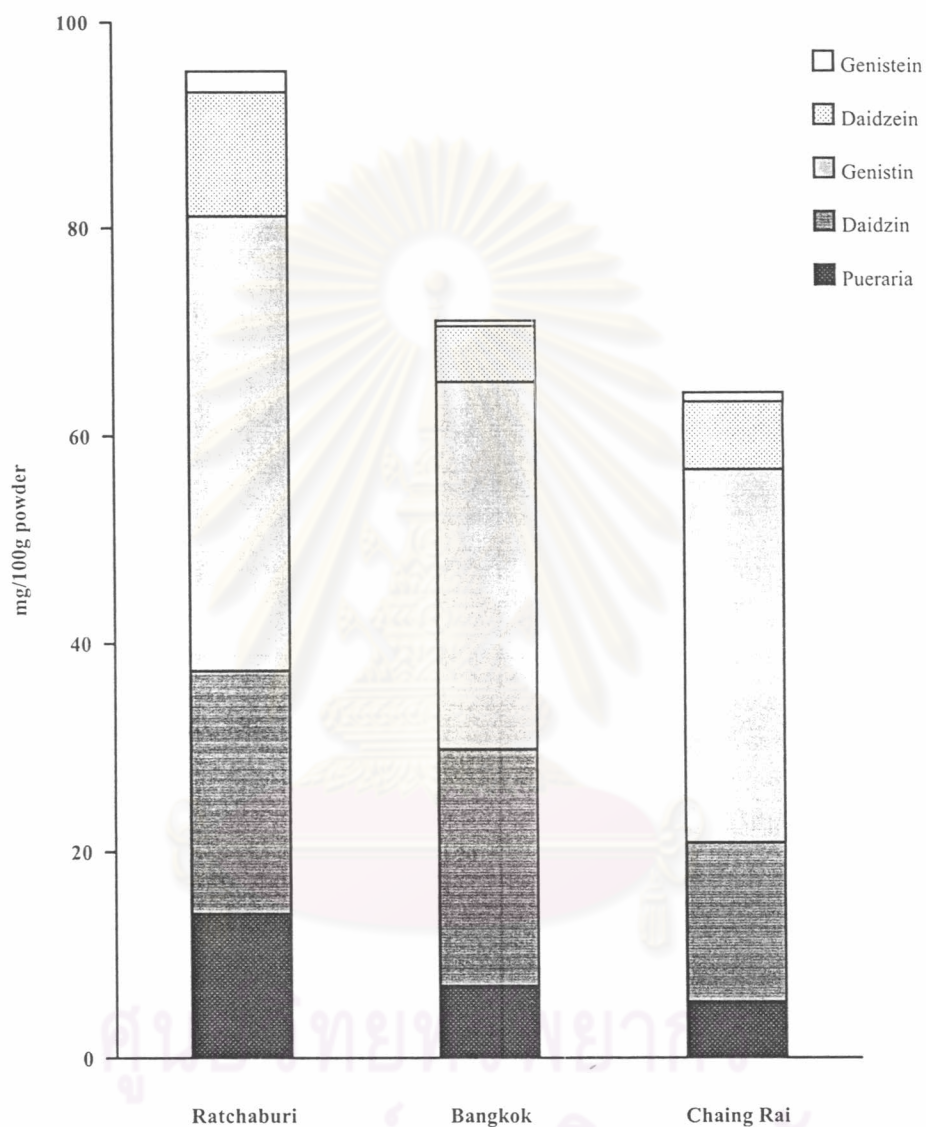


Figure 31 Isoflavone content in mg/100g powder of field grown *P. mirifica* clone Doi Tao cultivated in different location

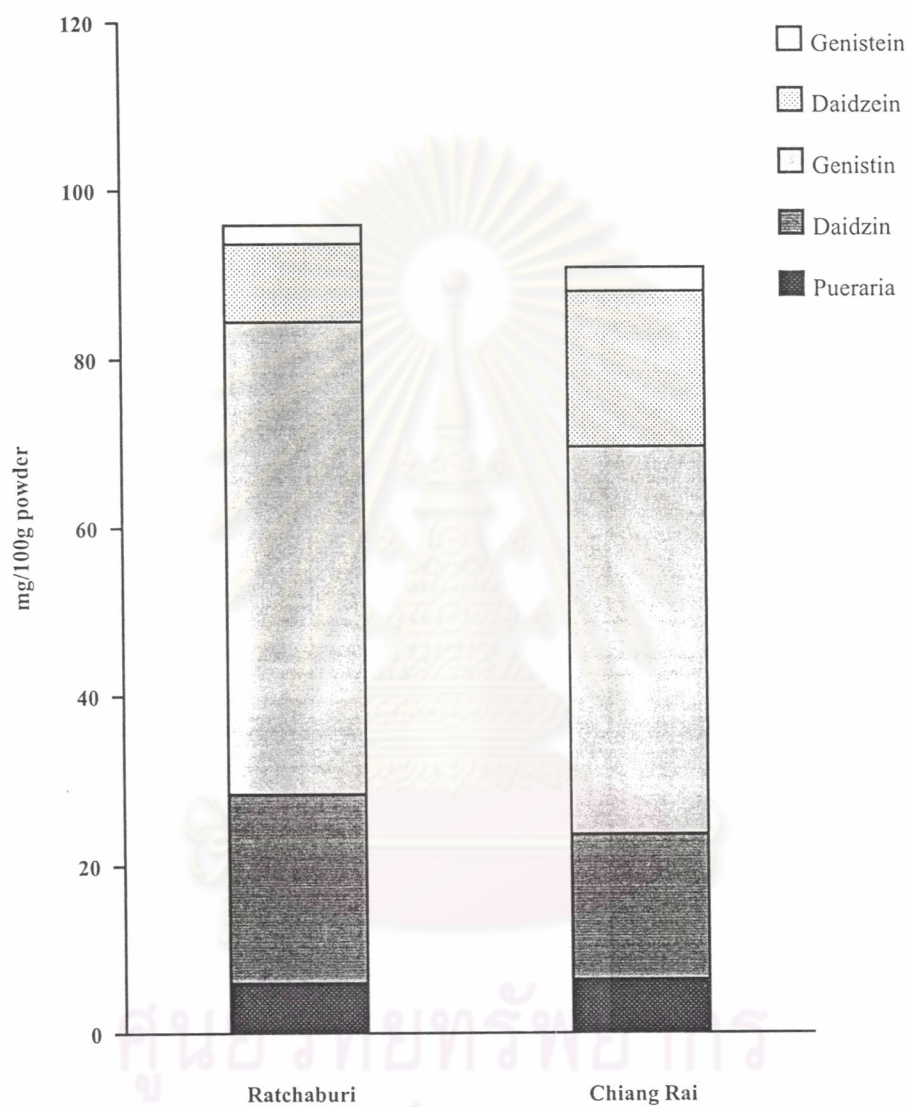


Figure 32 Isoflavone content in mg/100g powder of field grown *P. mirifica* clone Chiprakarn cultivated in different location

6. Analysis of the influence of season on isoflavone content of the field grown *P. mirifica*

The analysis of isoflavone content of the collected samples during 3 different seasons from the 2 years old plants cultivated in the same field at Ratchaburi provinces revealed that the isoflavone content was different among the samples of clone Doi Tao (Table 19, Figure 33). The sample from rainy season showed highest level of isoflavone content (113.87 mg/100g powder). The sample from winter showed moderate level of isoflavone content (106.96 mg/100g powder). The sample from season showed the lowest isoflavone content (77.31 mg/100g powder).

The analysis of isoflavone content of the collected samples during 3 different seasons from the 2 years old plants cultivated in the same area at Ratchaburi provinces revealed that the isoflavone content was different among the samples of clone Chaiprakarn (Table 20, Figure 34). The sample from winter and rainy season were no significant difference, the winter-collected samples showed highest level of isoflavone content (251.92 mg/100g powder). The samples from rainy season showed moderate level of isoflavone content (235.37 mg/100g powder). The samples from summer showed the lowest isoflavone content (204.82 mg/100g powder).

Table 19 Isoflavone content in mg/100g powder of filed grown *P. mirifica* clone Doi Tao cultivated in different season

Season	Puerarin (Mean±S.E.)	Daidzin (Mean±S.E.)	Genistin (Mean±S.E.)	Daidzein (Mean±S.E.)	Genistein (Mean±S.E.)	Total (Mean±S.E.)	% relative amount
Rainy	33.07 ± 1.97	15.29 ± 1.64	45.97 ± 2.85	18.38 ± 1.71	1.00 ± 0.22	113.87 ± 1.76	54.59
Winter	22.12 ± 2.76	18.04 ± 1.43	59.22 ± 2.48	7.16 ± 1.66	0.42 ± 0.05	106.96 ± 14.58	44.03
Summer	18.00 ± 0.49	11.73 ± 0.42	44.63 ± 3.79	2.79 ± 0.20	0.16 ± 0.01	77.31 ± 6.92	40.91
Mean	22.40 ± 2.95	15.02 ± 1.23	49.94 ± 3.31	9.44 ± 2.64	0.58 ± 0.16	99.38 ± 7.31	-

Table 20 Isoflavone content in mg/100g powder of field grown *P. mirifica* clone Chaiprakarn cultivated in different season

Season	Puerarin (Mean±S.E.)	Daidzin (Mean±S.E.)	Genistin (Mean±S.E.)	Daidzein (Mean±S.E.)	Genistein (Mean±S.E.)	Total (Mean±S.E.)	% relative amount
Rainy	39.78 ± 7.65	46.07 ± 3.50	123.46 ± 9.45	24.12 ± 1.78	1.94 ± 0.31	235.37 ± 18.67	64.42
Winter	58.17 ± 10.81	47.52 ± 3.14	128.72 ± 5.39	16.78 ± 3.41	0.73 ± 0.08	251.92 ± 15.91	58.30
Summer	71.31 ± 15.34	25.99 ± 3.02	101.75 ± 6.63	5.20 ± 0.20	0.57 ± 0.07	204.82 ± 24.77	56.06
Mean	56.42 ± 7.08	39.86 ± 2.95	117.97 ± 4.89	15.36 ± 2.24	1.08 ± 0.18	230.70 ± 11.90	-

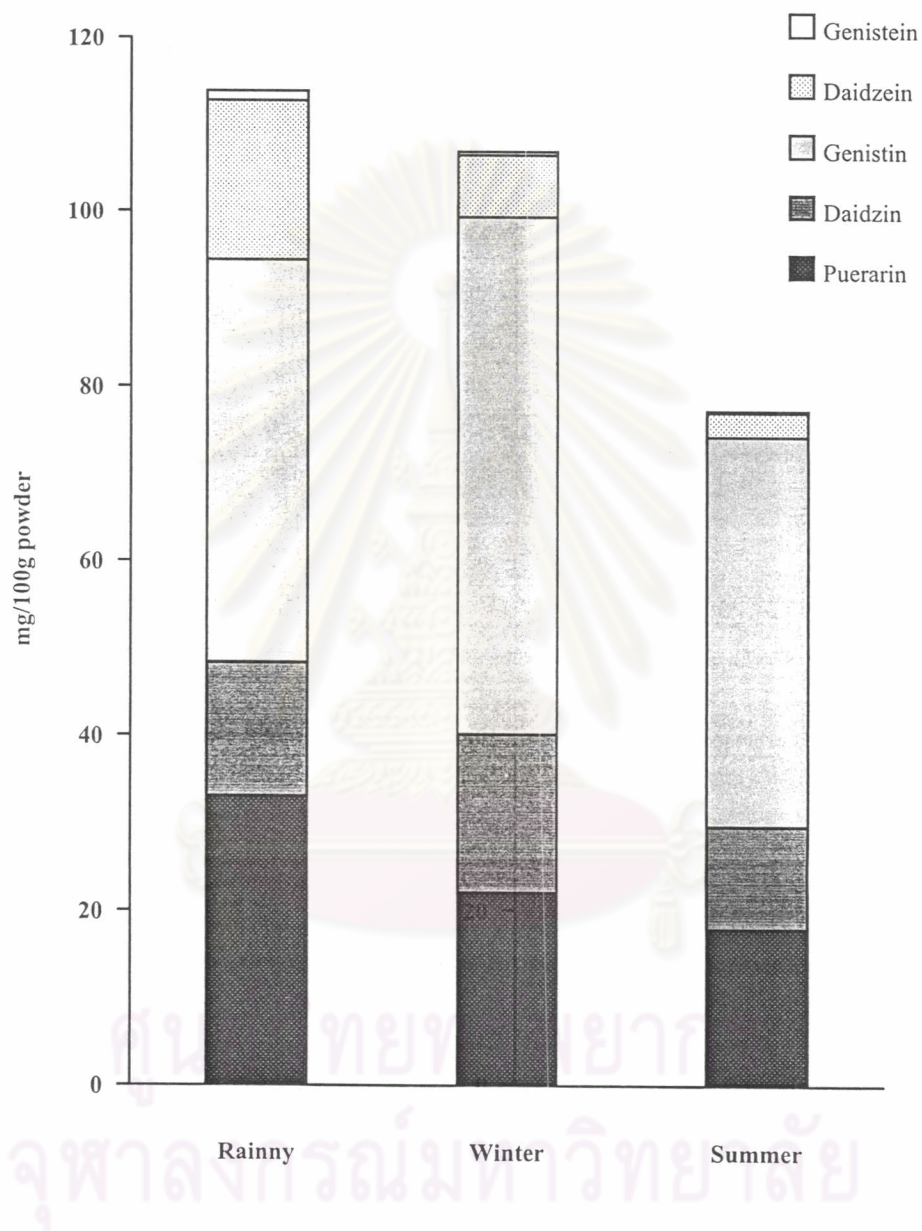


Figure 33 Isoflavone content in mg/100g powder of field grown *P. mirifica* clone Doi Tao cultivated in different season

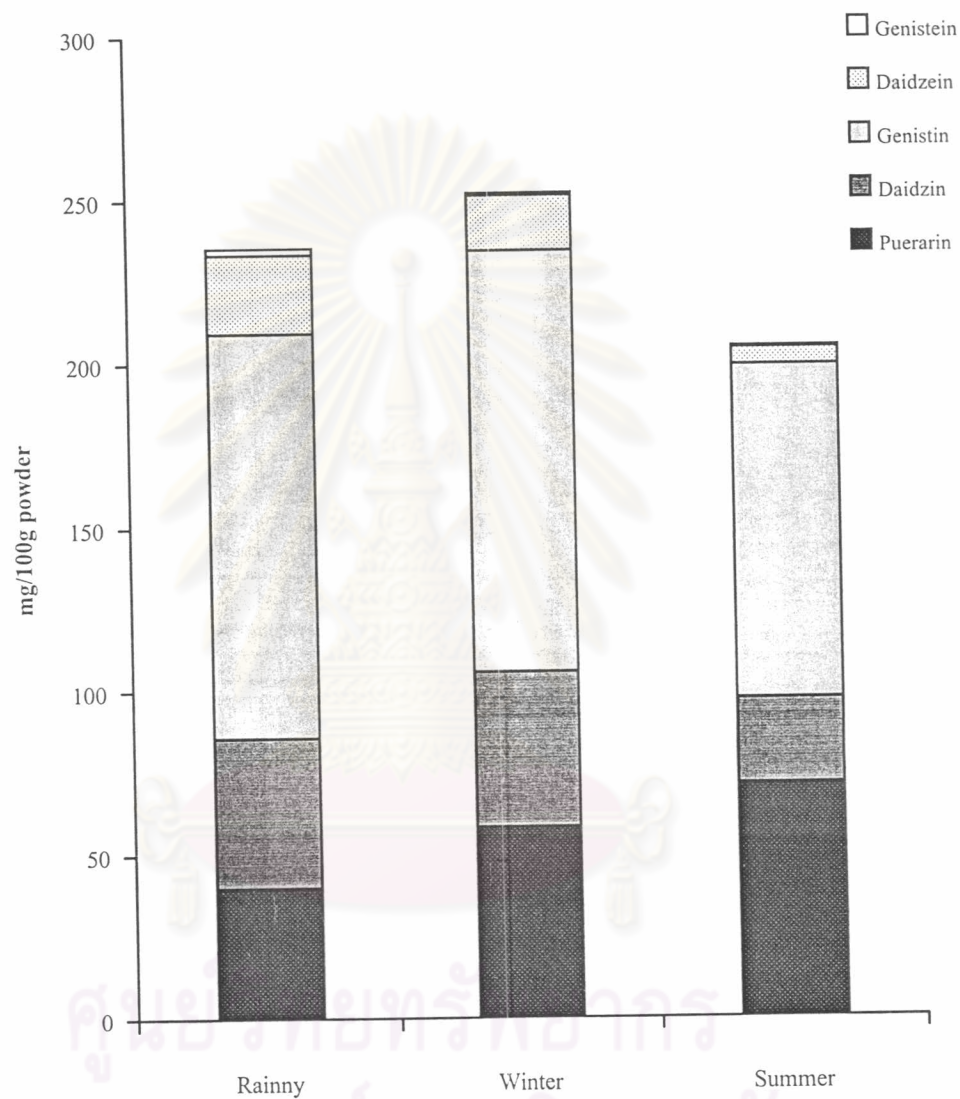


Figure 34 Isoflavone content in mg/100 g powder of field grown *P. mirifica* clone Chaiprakarn cultivated in different location

7. Analysis of the influence of genetics on isoflavone content of the field grown F₁ *P. mirifica*

The analysis of isoflavone content of the collected samples in summer from F₁ of clone Doi Tao and Sai Yoke cultivated in Ratchaburi province from the 1 year old plants revealed that the isoflavone content was different among F₁ in both clone. *P. mirifica* clone Doi Tao F₁ showed the varied amount of isoflavone content from 72.43 - 126.26 mg/100g powder (Table 21, Figure 35). *P. mirifica* clone Sai Yoke showed the varied amount of isoflavone from 115.03 - 225.78 mg/100g powder (Table 22, Figure 36). Comparison of the mean value of the 2 clones (Table 21 and 22, Figure 37) exhibited a statistical difference in which clone Sai Yoke shows higher amount of isoflavone ($p < 0.05$).



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Table 21 Isoflavone content in mg/100g powder of field grown *P. mirifica* clone Doi Tao (F₁) cultivated in Ratchaburi province

Sub-clone	Puerarin (Mean±S.E.)	Daidzin (Mean±S.E.)	Genistin (Mean±S.E.)	Daidzein (Mean±S.E.)	Genistein (Mean±S.E.)	Total (Mean±S.E.)	% relative amount
1	21.44 ± 0.49	32.38 ± 0.99	60.21 ± 3.94	10.49 ± 0.28	1.73 ± 0.52	126.26 ± 5.83	42.82
2	7.41 ± 0.29	19.16 ± 0.63	39.28 ± 1.61	18.21 ± 0.48	2.90 ± 0.41	86.95 ± 3.35	45.83
3	13.06 ± 0.56	18.65 ± 0.69	31.87 ± 0.68	7.42 ± 0.41	1.43 ± 0.34	72.43 ± 2.53	35.83
Mean	13.97 ± 2.05	23.40 ± 2.28	43.79 ± 4.42	12.04 ± 1.62	2.02 ± 0.31	95.22 ± 8.30	-

Table 22 Isoflavone content in mg/100g powder of field grown *P. mirifica* clone Sai Yoke (F₁) cultivated in Ratchaburi province

Sub-clone	Puerarin (Mean±S.E.)	Daidzin (Mean±S.E.)	Genistin (Mean±S.E.)	Daidzein (Mean±S.E.)	Genistein (Mean±S.E.)	Total (Mean±S.E.)	% relative amount
1	48.57 ± 0.63	46.83 ± 1.59	116.77 ± 7.53	10.37 ± 0.45	3.23 ± 0.09	225.78 ± 10.02	55.13
2	36.33 ± 1.65	35.27 ± 1.77	67.93 ± 3.16	14.44 ± 0.56	3.21 ± 0.28	157.17 ± 7.26	56.32
3	38.83 ± 2.00	21.93 ± 1.08	41.81 ± 3.24	10.22 ± 0.60	2.24 ± 0.29	115.03 ± 7.03	53.64
Mean	41.24 ± 2.02	34.68 ± 3.67	75.50 ± 11.27	11.67 ± 0.74	2.90 ± 0.61	165.99 ± 16.65	-

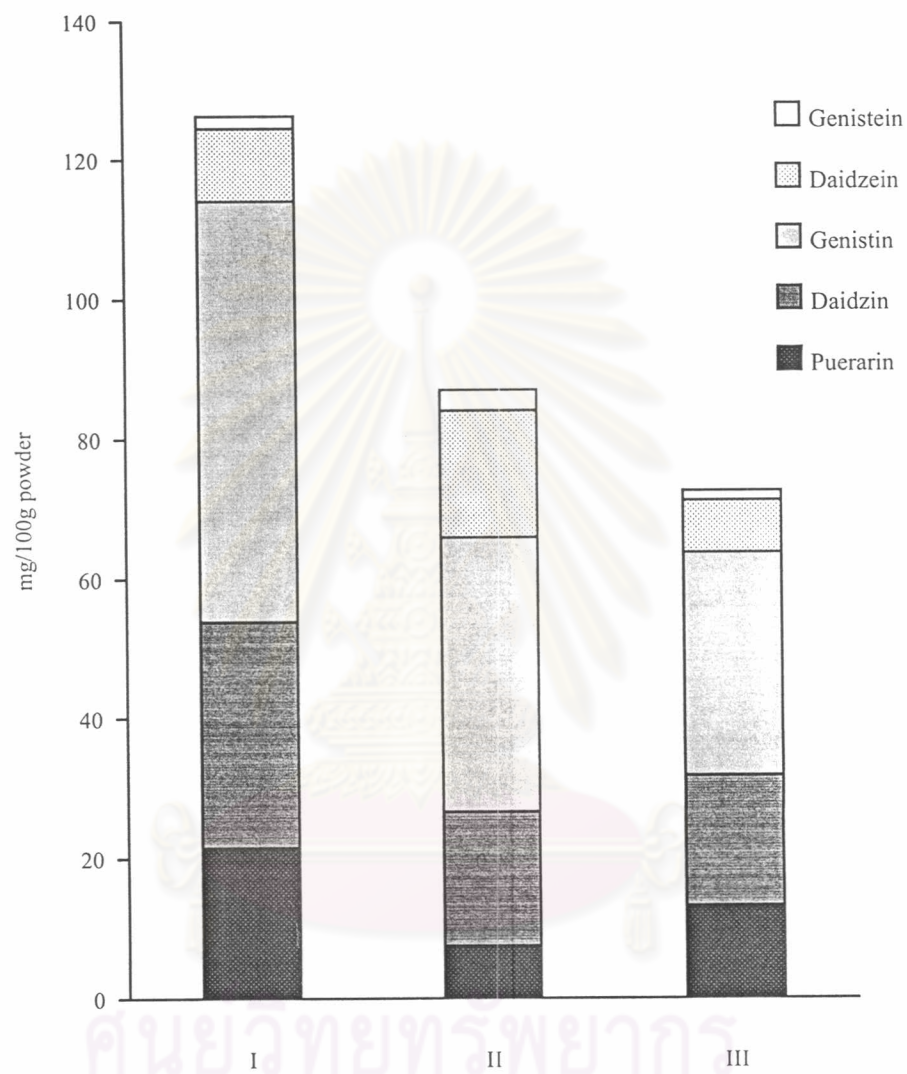


Figure 35 Isoflavone content in mg/100 g powder of field grown *P. mirifica* clone Doi Tao (F1) cultivated in Ratchaburi province

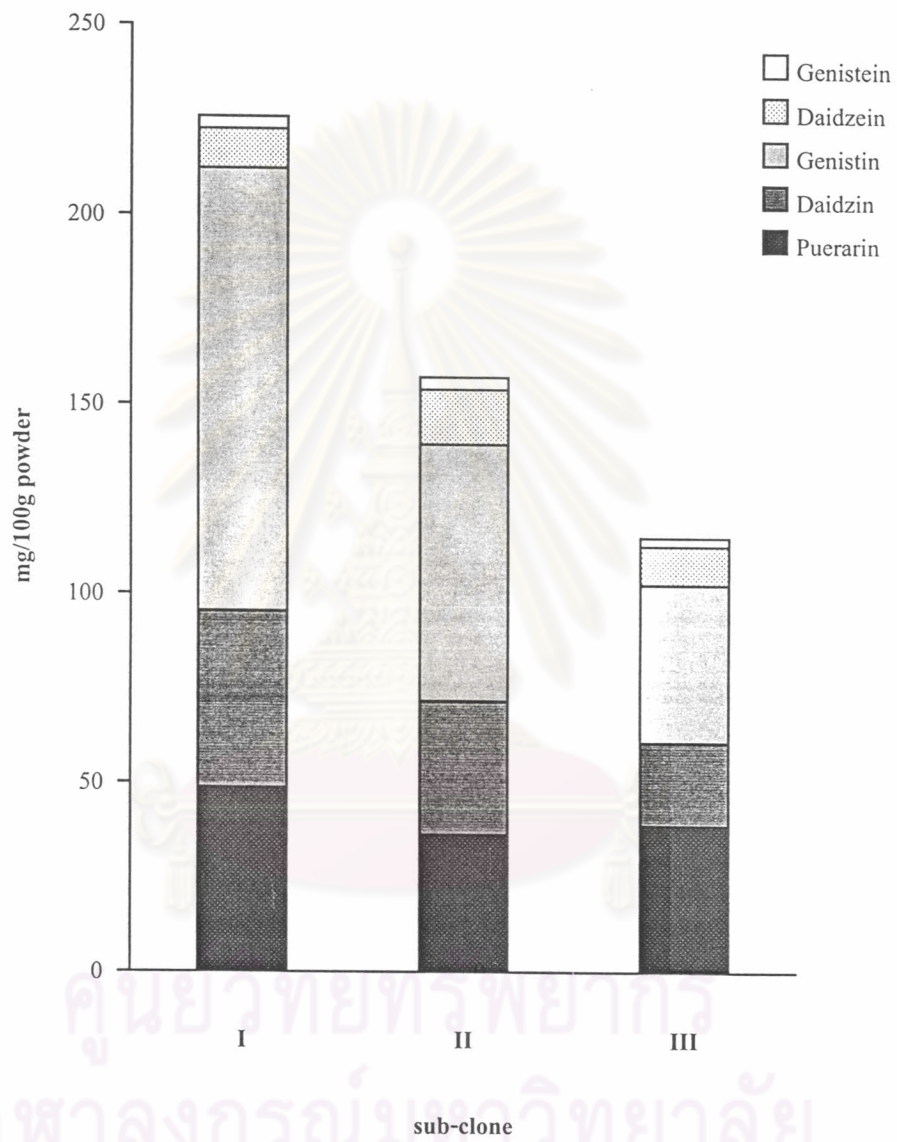


Figure 36 Isoflavone content in mg/100g powder of field grown *P.mirifica* clone Sai Yoke (F1) cultivated in Ratchaburi province

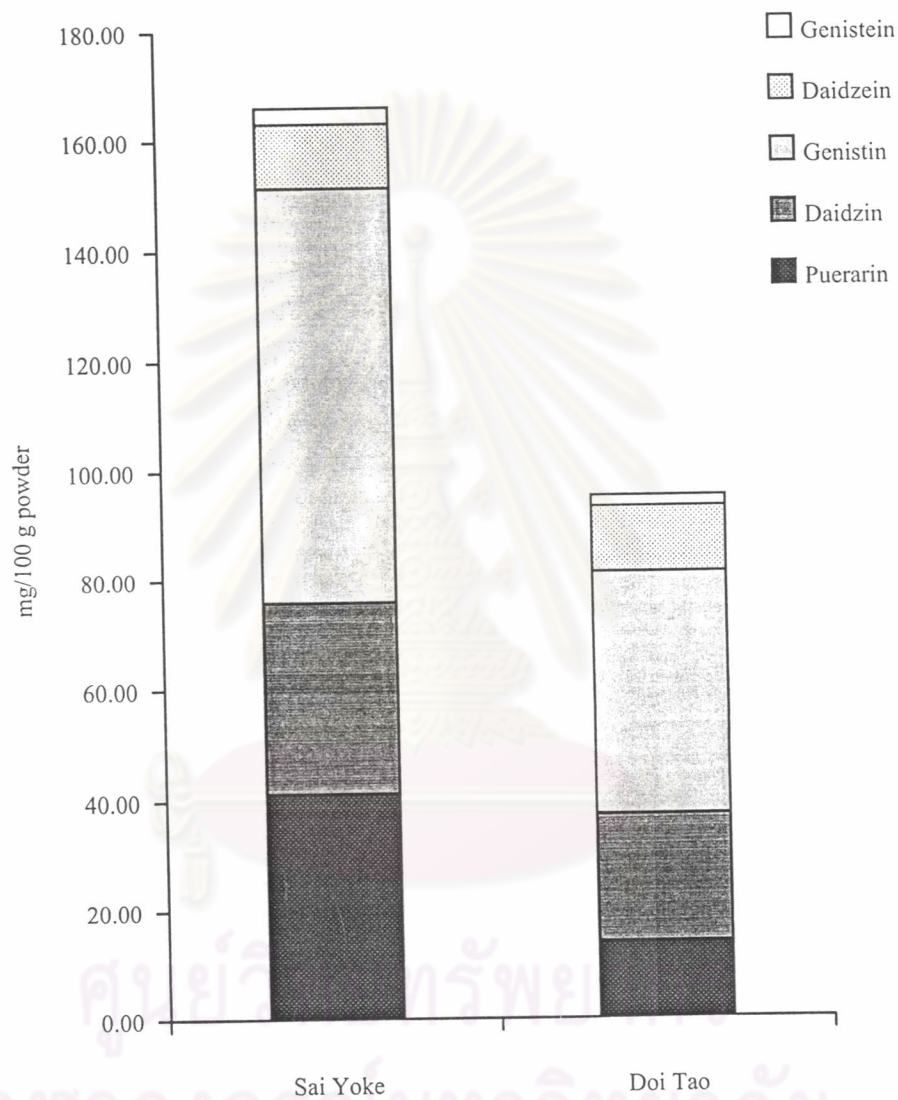


Figure 37 Isoflavone content in mg/100g powder of field grown *P. mirifica* clone Sai Yoke compare with clone Doi Tao (F1)

8. Analysis of the influence of tuber differentiation on isoflavone content of the field grown sub-clone *P. mirifica*

It was found that the individual tuber collected from the same plant, 3 sub-clone of clone Doi Tao asexually derived from the same mother plant cultivated from Ratchaburi province exhibited different in the isoflavone content. In the rainy season, sub-clone I, II, III revealed that no significant different in the isoflavone content. In the winter and summer, the plants exhibited significant different in the isoflavone content. Sub-clone Doi Tao I showed higher isoflavone content in winter than sub-clone Doi Tao II and III. Sub-clone Doi Tao I also showed less isoflavone content in summer than sub-clone Doi Tao II and III (Table 23-25, Figure 38-40).



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Table 23 Isoflavone content in mg/100g powder of field grown *P. mirifica* clone Doi Tao, cultivated in the rainy season

Sub-clone	Puerarin (Mean±S.E.)	Daidzin (Mean±S.E.)	Genistin (Mean±S.E.)	Daidzein (Mean±S.E.)	Genistein (Mean±S.E.)	Total (Mean±S.E.)	% relative amount
I	29.55 ± 0.65	16.84 ± 4.89	50.61 ± 6.69	19.35 ± 3.04	0.93 ± 0.25	117.29 ± 8.28	53.90
II	40.65 ± 1.01	16.39 ± 1.33	40.45 ± 4.82	12.96 ± 0.44	0.95 ± 0.05	111.39 ± 6.93	55.44
III	29.01 ± 1.33	12.66 ± 1.20	46.85 ± 1.78	22.83 ± 0.86	1.59 ± 0.44	112.94 ± 3.92	55.16

Table 24 Isoflavone content in mg/100g powder of field grown *P. mirifica* clone Doi Tao, cultivated in winter

Sub-clone	Puerarin (Mean±S.E.)	Daidzin (Mean±S.E.)	Genistin (Mean±S.E.)	Daidzein (Mean±S.E.)	Genistein (Mean±S.E.)	Total (Mean±S.E.)	% relative amount
I	32.88 ± 1.99	22.01 ± 2.01	66.12 ± 4.05	13.66 ± 1.17	0.60 ± 0.08	135.26 ± 7.19	52.11
II	16.92 ± 0.34	18.75 ± 0.52	58.98 ± 1.00	3.97 ± 0.08	0.32 ± 0.06	98.94 ± 1.75	39.69
III	16.56 ± 0.71	13.38 ± 1.04	52.56 ± 3.21	3.85 ± 0.25	0.34 ± 0.02	86.68 ± 4.49	40.29

Table 25 Isoflavone content in mg/100g powder of field grown *P. mirifica* clone Doi Tao, cultivated in summer

Sub-clone	Puerarin (Mean±S.E.)	Daidzin (Mean±S.E.)	Genistin (Mean±S.E.)	Daidzein (Mean±S.E.)	Genistein (Mean±S.E.)	Total (Mean±S.E.)	% relative amount
I	17.42 ± 0.59	12.40 ± 0.72	38.28 ± 2.86	3.18 ± 0.36	0.17 ± 0.02	71.44 ± 3.91	42.09
II	17.00 ± 0.60	11.33 ± 0.69	37.91 ± 4.58	3.00 ± 0.16	0.17 ± 0.02	69.40 ± 6.04	39.44
III	19.59 ± 0.49	11.46 ± 0.88	57.71 ± 3.97	2.21 ± 0.20	0.14 ± 0.01	91.11 ± 4.21	41.19

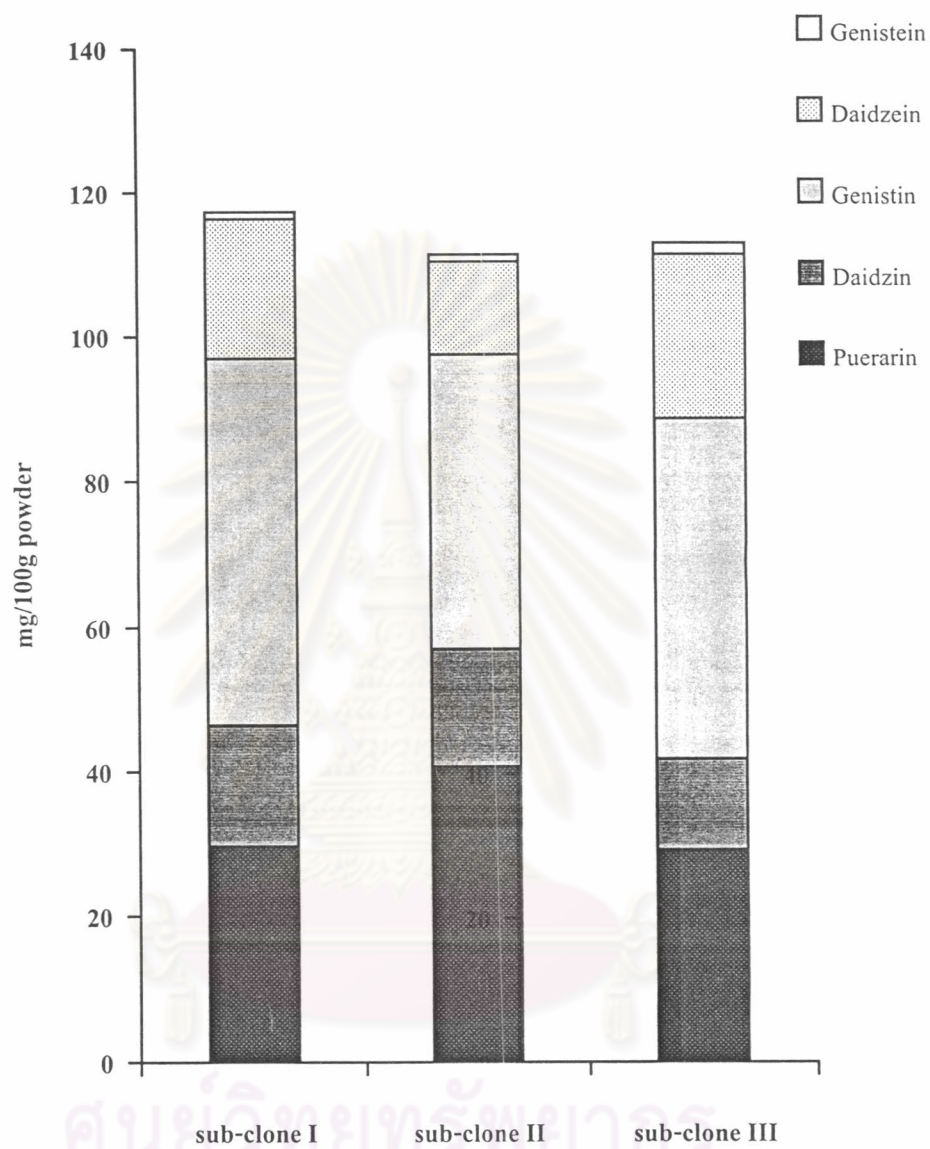


Figure 38 Isoflavone content in mg/100g powder of field grown *P. mirifica* clone Doi Tao cultivated in rainy season

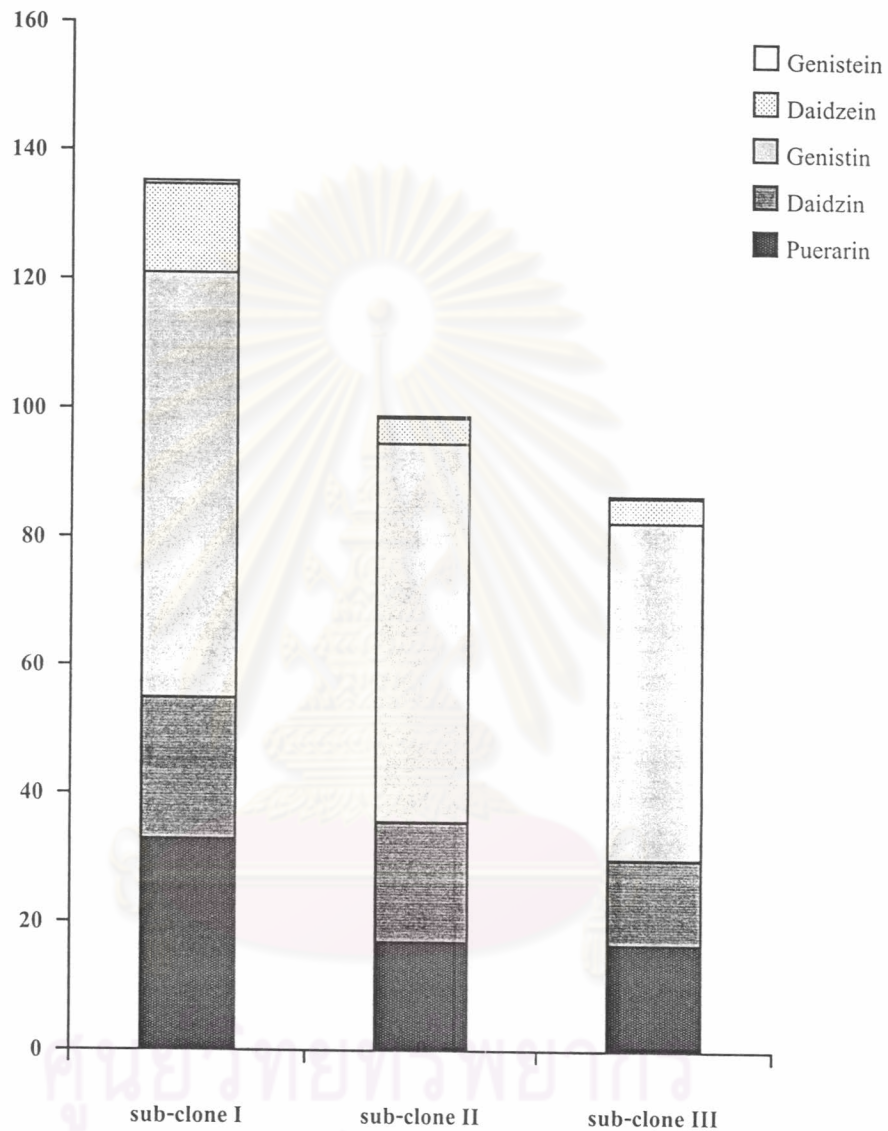


Figure 39 Isoflavone content in mg/100g powder of field grown *P. mirifica* clone Doi Tao cultivated in winter

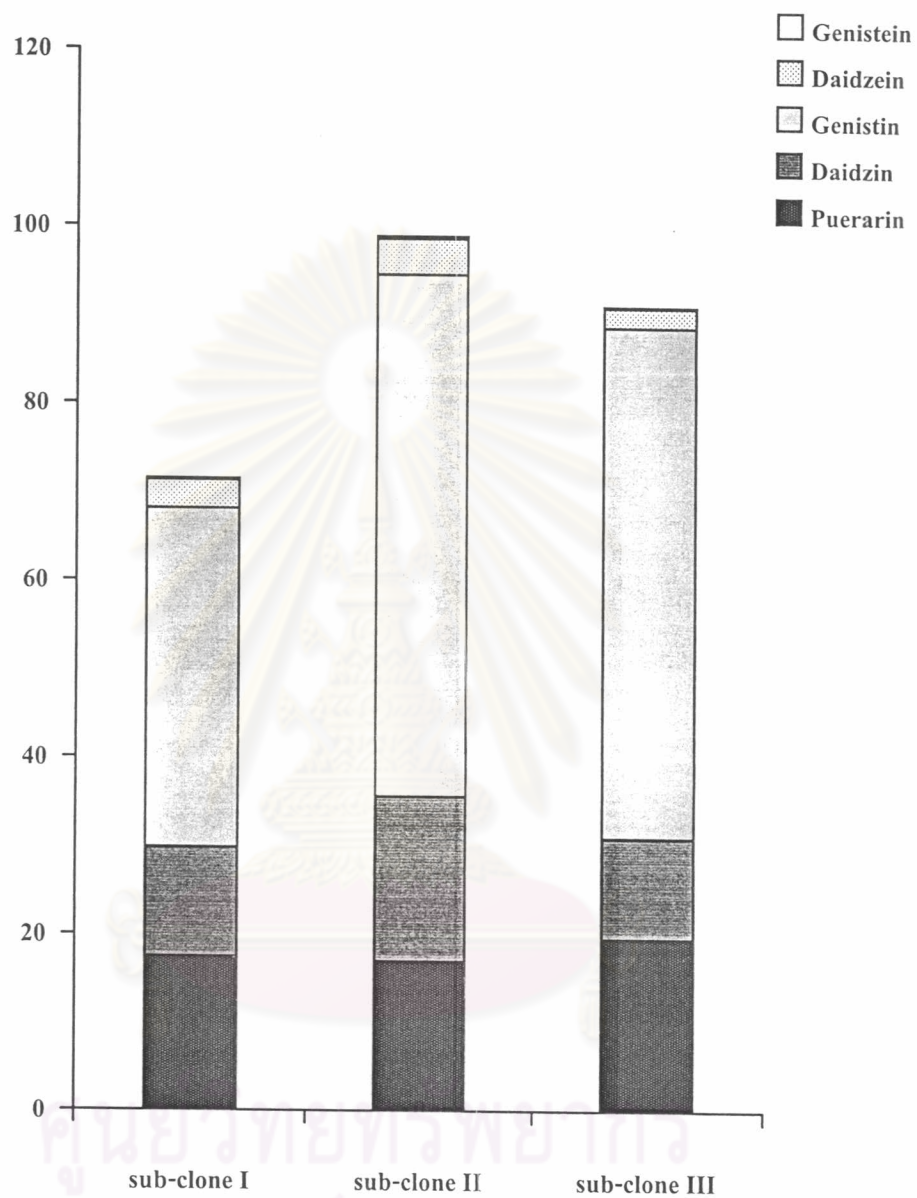


Figure 40 Isoflavone content in mg/100g powder of field grown *P. mirifica* clone Doi Tao cultivated in summer

9. HPLC fingerprint analysis of 4 selected *B. superba*

9.1 Setting up the condition for *B. superba* analysis

HPLC analysis of *B. superba* clone Lampang was started with 100 : 0 to 55 : 45 of 1.5 % (v/v) acetic acid : acetonitrile gradient system. The wavelength for analysis was performed at the wavelength of 254 nm and 270 nm for comparison of absorbance of *B. superba* extract. The absorbance at 254 nm exhibited higher resolution of total fingerprint than 270 nm (Figure 41).

9.2 HPLC fingerprint analysis of the wild *B. superba*

HPLC analysis of *B. superba* collected from Lampang, Khon Kaen, Ratchaburi and Chantaburi province revealed that the sample from Ratchaburi province showed highest total fingerprint than others.

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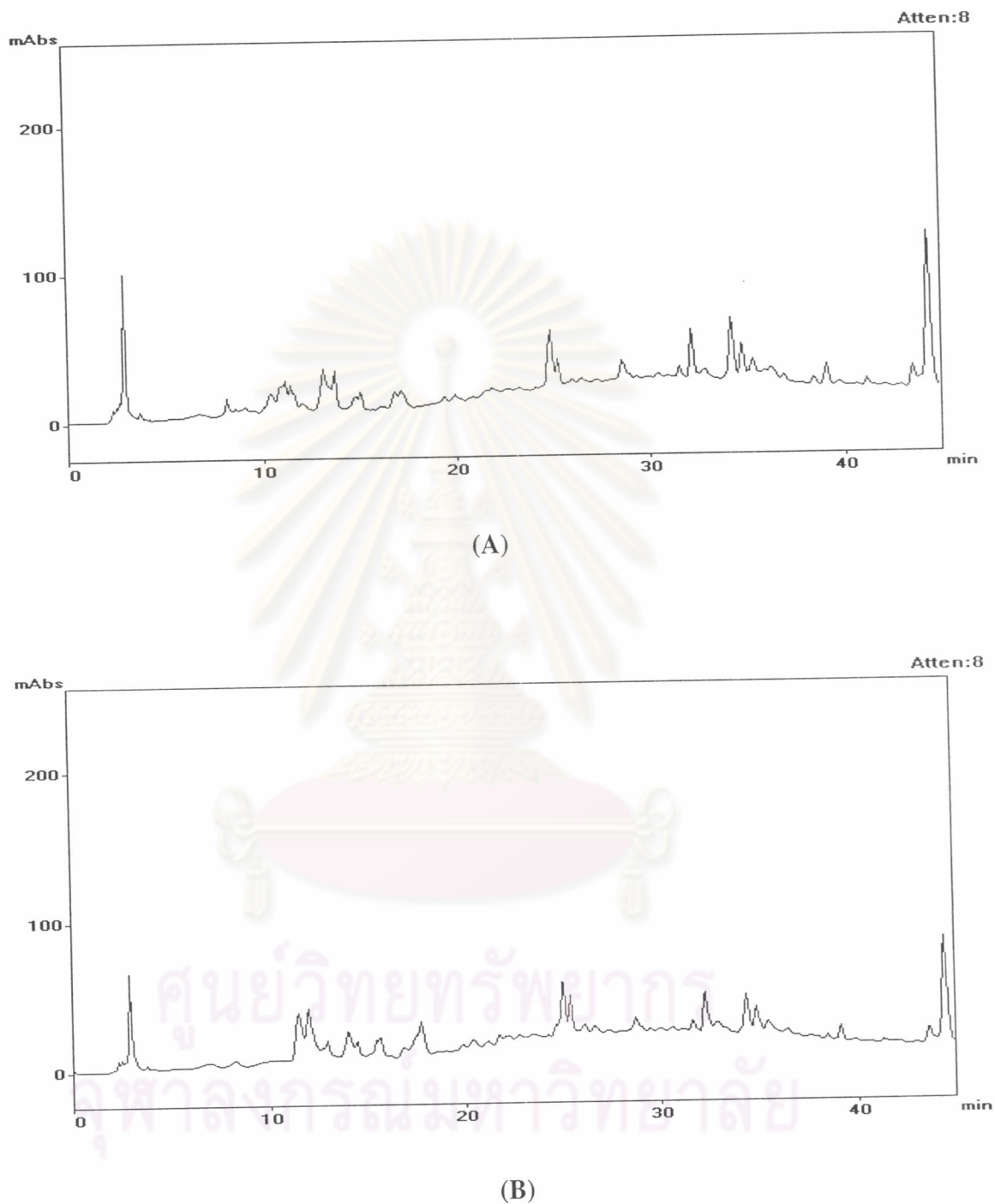


Figure 41 HPLC fingerprint of *B. superba* collected in Lampang in comparison of wavelength (A) 254 nm (B) 270 nm

10. HPLC fingerprint analysis of 3 collected *M. collettii*

10.1 Setting the condition for *M. collettii* analysis

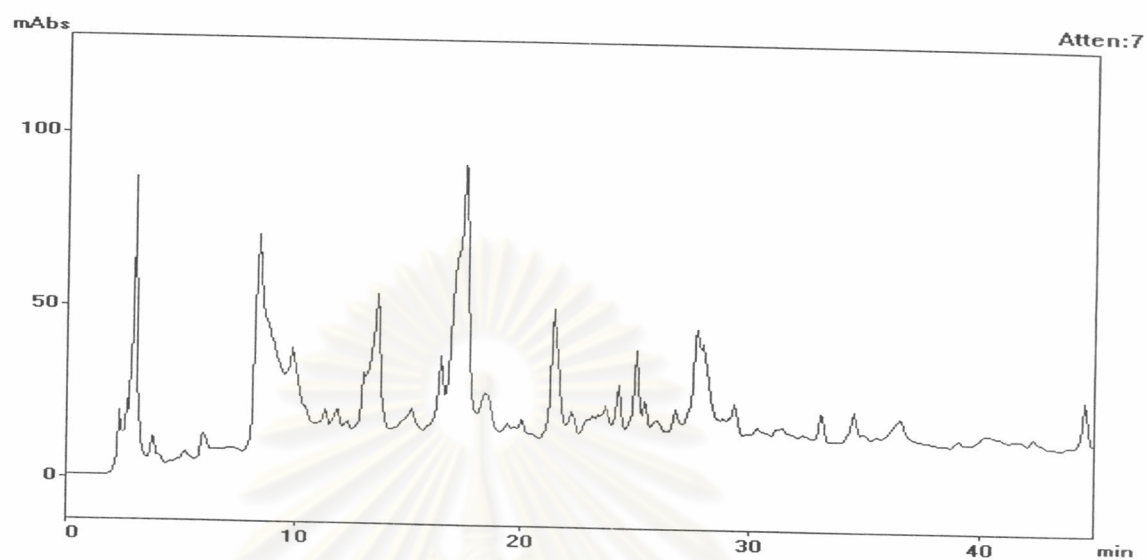
HPLC analysis of *M. collettii* clone Chiang Rai was started with 100 : 0 to 55 : 45 of 1.5 % (v/v) acetic acid : acetonitrile gradient system. The analysis was performed at the wavelength of 254 nm and 270 nm for comparison of absorbance of *M.collettii* extract. The absorbance at 270 nm exhibited higher resolution of total fingerprint than 254 nm (Figure 42).

10.2 HPLC fingerprint analysis of the wild *M. collettii*

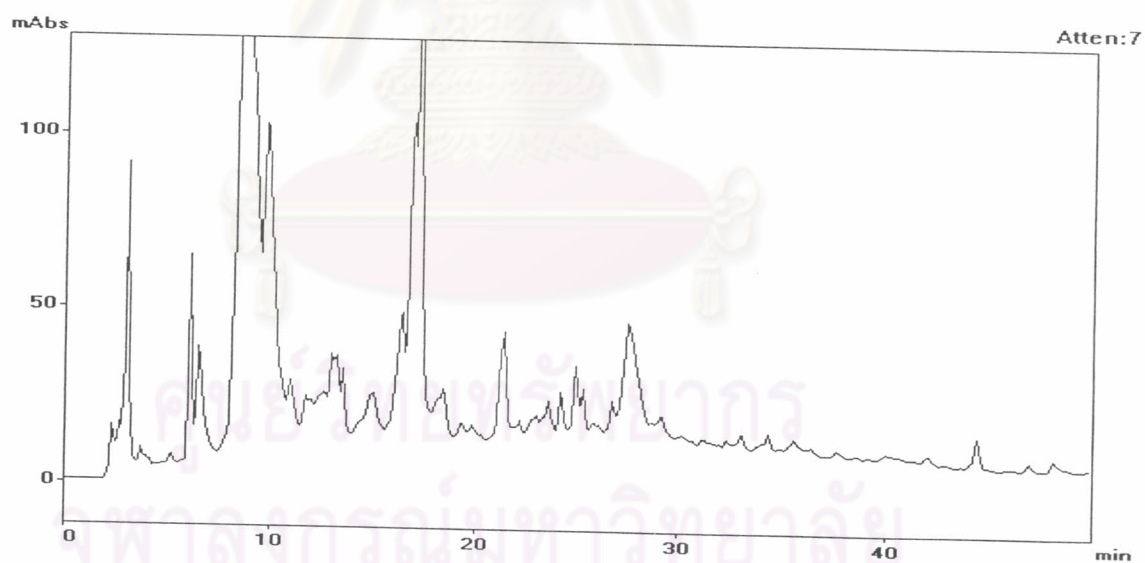
HPLC analysis of *M. collettii* collected from Chiang Rai, Lampang and Kanchanaburi province revealed that the sample from Chiang Rai province showed highest total fingerprint.



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(A)



(B)

Figure 42 HPLC fingerprint of *M. collettii* collected in Lampang in comparison of wavelength (A) 254 nm (B) 270 nm