

Chapter IV

Results

1 The plant extraction

Table 5 Weight of crude extracts of *P. mirifica*, *B. superba* and *M. collettii*

Provinces	Weight extract of <i>P. mirifica</i> (g/50g powder)	Weight extract of <i>B. superba</i> (g/50g powder)	Weight extract of <i>M. collettii</i> (g/50g powder)
Chiang Rai	3.84	2.67	3.19
Chiang Mai	1.88	3.87	2.89
Mae Hong Son	2.31	2.32	-
Phayao	1.85	-	-
Nan	1.39	-	-
Lampang	3.75	2.64	2.06
Phrae	2.13	-	-
Lamphun	2.37	-	-
Uttharadith	2.17	2.22	-
Sukhothai	2.2	-	-
Tak	2.45	2.22	-
Phitsanulok	2.38	3.79	-
Phetchabun	3.24	2.7	-
kamphaeng phet	2.68	-	-
Nakhon Sawan	3.01	2.7	-
Uthai Thani	2.88	-	-
Loei	-	2.82	-
Sakon Nakhon	3.22	2.07	-
Nong Bua Lam Phu	2.13	2.98	-
Srisaket	-	2.74	-
Khon Kaen	-	3.06	-
Chaiyaphum	2.03	1.29	-
Nakhon Ratchasima	2.82	1.12	-
Saraburi	2.65	2.18	-
Lop Buri	2.85	2.33	-
Kanchanaburi	2.94	2.61	2.68
Phrachin Buri	1.38	2.3	-
Ratchaburi	2.43	2.6	-
Phetchaburi	2.29	-	-
Chonburi	-	3.69	-
Chantaburi	-	2.67	-
Chachoengsoa	-	3.83	-
Prachuap Khiri Khan	3.96	-	-
Chumphon	1.97	-	-

The characteristic of the plants extracts are summarized in the **Table 6**

Table 6. The characteristic of the plant extracts

Plants	Extract characteristic
<i>P. mirifica</i>	Sticky wax-like material with brown color. Smell-like ground peanut.
<i>B. superba</i>	Sticky wax-like material with dark brown color. Smell-like ground peanut
<i>M. collettii</i>	Sticky wax-like crystal with black color. Very strong smell.

2. Proliferative effect of puerarin

Puerarin at the concentration of 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , 10^{-10} , 10^{-11} , 10^{-12} , 10^{-13} M. was compared with Estradiol at 10^{-10} , 10^{-11} , 10^{-12} M. in the incubation test with MCF-7 cells. Puerarin at the concentration of 10^{-6} M. and Estradiol at the concentration of 10^{-10} M showed proliferative effect to MCF-7 cells.

Table 7 The percentage growth of Puerarin and Estradiol on MCF-7 cells

concentration	Estradiol (%growth of MCF-7)	Puerarin(%growth of MCF-7)
10^{-6} M	-	130 ^a
10^{-7} M	-	124±16.04
10^{-8} M	-	124±12.77
10^{-9} M	-	118±19.40
10^{-10} M	146±5.81*	122±8.19
10^{-11} M	142±36.10	112±4.06
10^{-12} M	130±15.25	106±14.25
10^{-13} M	-	106±8.08

a: n=1, others n=3

* significant , $p < 0.05$

3. Cytotoxicity test

3.1 *Pueraria mirifica*

The wild *P. mirifica* collected from 28 provinces in Thailand showed variation of estrogenic activity. *P. mirifica* extracts show clearly biphasic effect with MCF-7 cell culture. The extract stimulated the cellular proliferation at the concentration of 1 µg/ml ($p < 0.05$) and partially inhibited the cellular proliferation at the concentration of 1,000 µg/ml. (Table 18 and Figure 7). There were 16 samples, from the higher- to the lower-order of proliferative effect at the concentration of 1 µg/ml, including Phitsanuloke, Nakhon Sawan, Phetchabun, Sukhothai, Nan, Chiang Rai, Chiang Mai, Prachuap Khiri Khan, Ratchaburi, Mae Hong Son, Lamphun, Chumphon, Phrae, Chaiyaphum, Lop Buri and Prachinburi exhibited significant proliferation effect. Interestingly, the crude extract collected from Phitsanuloke at the concentration of 1 µg/ml showed proliferative effect ($145.70 \pm 8.93\%$), the same as estradiol at the concentration of 10^{-10} M. ($146 \pm 5.81\%$).

The mean values of proliferative effect dose (1 µg/ml) of each samples were compared with the mean value of population. Phitsanulok and Nakhon Sawan samples exhibited significant higher value than the mean value of the population ($121.31 \pm 1.56\%$) (Table 17).

The analysis of the collected samples during 3 different seasons from the 2 years old plants cultivated in the same field at Ratchaburi province confirmed the variation of anti-proliferative effect (Table 19, Figure 8). The sample from Chaiprakan collected in rainy season showed the highest anti-proliferative effect.

3.2 *Butea superba*

The wild *B. superba* collected from 24 provinces in Thailand showed variation of proliferative- and anti-proliferative effect. We could divide *B. superba* into 2 groups. The first group, comprise only Mae Hong Son, stimulated the cellular proliferation at the concentration of 0.1 µg/ml ($p < 0.05$) (Table 21 and Figure

9). The other samples showed no proliferative effect. There was only Prachinburi ($78.55 \pm 4.65\%$) exhibited significant lower than mean. (Table 20)

B. superba inhibited the cellular anti-proliferation at the concentrations of 100-1,000 $\mu\text{g/ml}$. There were 10 samples, including sample collected from Kanchanaburi (IC_{50} 76.65), Ratchaburi (IC_{50} 109.82), Tak (IC_{50} 184.35), Phitsanulok (IC_{50} 344.12), Srisaket (IC_{50} 381.97), Khon Kaen (IC_{50} 545.29), Nakhon Ratchasima (IC_{50} 546.23), Chachoengsoa (IC_{50} 606.58), Petchabun (IC_{50} 683.87), and Uttharadith (IC_{50} 733.87) exhibited IC_{50} value lower than 1,000 $\mu\text{g/ml}$.

3.3 *Mucuna collettii*

M. collettii collected from 4 provinces showed no proliferative effect. The extracts inhibited the cellular proliferation at the concentrations of 10-1,000 $\mu\text{g/ml}$ ($p < 0.05$). (Table 22 and Figure 10) All collected samples of *M. collettii* exhibited IC_{50} value lower than 1,000 $\mu\text{g/ml}$. Only the sample collected from Kanchanaburi showed significant stronger IC_{50} than the mean value of the population ($31.87 \pm 3.94 \mu\text{g/ml}$)

3.4 Correlation of isoflavone content in *P. mirifica* collected from 28 provinces with proliferative effect

I. Direct assay with puerarin-
-estradiol and crude extracts of *P. mirifica* exhibited stronger proliferative effect than puerarin.

II. Indirect assay with isoflavone content (Subtang, 2002). It was convinced that the proliferative effects might relate with daizein. Daizein contents of the first 5 highest proliferative effects were significantly ($p < 0.05$) higher than the left, 23 samples. Interestingly, aglycoside isoflavone, daizein and genistein contents of the first 5 highest proliferative effects were significantly ($p < 0.05$) higher than the left. Genistein of the first 5 highest proliferative effects exhibited no significant ($p < 0.05$).

Table 8 The percentage growth of MCF-7 of the first 5 highest proliferation effect in correlation with isoflavone content (%)

No.	Source	Proliferation effect(1 μ g/ml)	Puerarin (%)	Daidzin (%)	Genistin (%)	Daidzein (%)	Genistein (%)
1	Phitsanulok	145.70 \pm 8.93	41.94 \pm 0.34	14.60 \pm 0.21	31.58 \pm 0.23	9.95 \pm 0.06	1.94 \pm 0.02
2	Nakhon Sawan	142.99 \pm 11.84	21.15 \pm 1.55	25.81 \pm 1.37	44.41 \pm 2.76	7.46 \pm 0.27	1.15 \pm 0.50
3	Phetchabun	135.67 \pm 2.11	20.96 \pm 0.42	23.46 \pm 1.78	34.52 \pm 2.44	18.13 \pm 0.72	2.90 \pm 0.11
4	Sukhothai	134.73 \pm 11.90	13.76 \pm 0.51	24.45 \pm 0.22	50.20 \pm 0.71	10.86 \pm 0.30	0.72 \pm 0.25
5	Nan	128.53 \pm 3.26	28.37 \pm 1.54	12.61 \pm 0.92	40.04 \pm 4.17	17.69 \pm 1.47	1.31 \pm 0.25
Mean+S.E.		137.52 \pm 3.61	25.24 \pm 4.77	20.19 \pm 2.73	40.15 \pm 3.35	12.82 \pm 2.15	2.65 \pm 0.41



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Table 9 The isoflavone content (%) of 23 *P. mirifica* samples with lower proliferation effect than the first 5 highest proliferation effect

No.	Source	Proliferation effect (1 µg/ml)	Puerarin (%)	Daidzin (%)	Genistin (%)	Daidzein (%)	Genistein (%)
6	Chiang Rai	128.43±5.89	32.89±1.42	14.19±1.97	48.58±3.06	3.55±0.07	0.79±0.44
7	Chiang Mai	128.43±8.13	21.51±1.26	22.77±1.40	45.17±3.60	8.06±1.21	2.48±0.26
8	Prachuap Khiri Khan	127.71±8.66	23.57±0.76	20.97±1.64	44.48±0.59	9.05±0.39	1.92±0.31
9	Ratchaburi	127.12±4.56	10.43±0.10	18.14±0.44	60.32±0.27	8.08±0.66	3.01±0.28
10	Mae Hong Son	127.04±8.81	26.80±0.90	20.15±0.81	33.52±1.53	16.27±0.68	3.23±0.10
11	Lamphun	125.83±3.26	21.40±0.59	18.28±0.27	54.28±0.37	5.54±0.02	0.49±0.22
12	Chumphon	123.69±7.05	16.15±1.09	14.13±1.09	63.93±2.43	4.98±0.03	0.80±0.28
13	Kanchanaburi	123.67±12.49	9.14±0.54	21.26±0.89	58.79±1.24	8.61±0.33	2.19±0.09
14	Phrae	122.97±4.37	38.46±1.59	11.57±0.67	42.99±3.02	5.24±1.24	1.73±0.18
15	Chaiyaphum	120.62±2.74	23.32±1.97	19.15±0.69	43.95±1.31	10.64±1.83	2.92±0.85
16	Lop Buri	120.07±7.16	28.13±0.78	9.94±0.56	57.09±0.73	3.41±1.01	1.41±0.06
17	Prachin Buri	119.69±10.27	26.48±0.79	27.80±1.30	35.51±0.86	9.12±1.21	1.07±0.18
18	Nong Bua Lam Phu	118.46±7.25	21.74±3.30	20.25±4.31	41.51±4.95	13.10±1.96	3.38±0.60
19	Phayao	117.55±5.38	22.41±1.14	14.68±0.57	56.41±1.11	5.25±0.45	1.25±0.49
20	Tak	115.13±6.51	20.35±0.97	13.89±0.30	48.18±1.23	15.65±0.69	1.93±0.13
21	Sakon Nakhon	114.31±12.12	72.80±0.29	9.56±0.07	12.44±0.16	4.00±0.29	1.19±0.10
22	Uttaradith	112.92±3.77	48.71±0.09	22.04±0.03	16.54±0.04	12.69±0.08	0.00±0.00
23	Nakhon Ratchasima	112.68±4.87	29.72±2.67	12.69±0.22	54.46±1.68	2.66±0.79	0.46±0.42
24	Kamphaeng Phet	111.89±4.05	35.66±2.98	16.65±4.03	41.26±7.47	5.33±0.26	1.08±0.30
25	Lampang	111.61±5.45	37.71±0.77	18.09±0.26	36.29±0.60	6.23±0.01	1.67±0.10
26	Phetchaburi	105.92±4.99	16.76±0.13	26.37±1.32	47.78±1.44	7.64±0.36	1.44±0.02
27	Saraburi	105.40±4.07	14.80±4.42	35.76±12.50	42.95±8.44	4.56±0.42	1.89±0.42
28	Uthai thani	102.35±7.00	10.53±0.55	21.17±0.66	48.73±0.77	15.99±0.49	3.57±0.06
Mean± S.E.		115.13±2.62	26.50±2.93	18.67±1.27	59.35±14.19	8.07±0.87	1.51±0.18

Table 10 Mean of isoflavone content (%) of the first 5 highest proliferation effect compared with 23 *P. mirifica* samples.

Group	Proliferation effect(1 μ g/ml)	Puerarin (%)	Daidzin (%)	Genistin (%)	Daidzein (%)	Genistein (%)
Mean \pm S.E. of 5 PM	137.52 \pm 3.61	25.24 \pm 4.77	20.19 \pm 2.73	40.15 \pm 3.35	12.82 \pm 2.15*	2.65 \pm 0.41
Mean \pm S.E. of 23 PM	115.13 \pm 2.62	26.50 \pm 2.93	18.67 \pm 1.27	59.35 \pm 14.19	8.07 \pm 0.87	1.51 \pm 0.18

5 PM; the first 5 highest proliferation effect of *P. mirifica*

22 PM; the left 22 *P. mirifica* with lower proliferation effect

Table 11 Isoflavone glycoside and aglycoside content (%) of the first 5 highest proliferation effect of *P. mirifica*

No.	Source	Proliferation effect (1 μ g/ml)	Isoflavone glycoside(%)	Isoflavone aglycoside(%)
1	Phitsanulok	145.70 \pm 8.93	79.53 \pm 0.48	20.46 \pm 0.26
2	Nakhon Sawan	142.99 \pm 11.84	89.06 \pm 0.39	10.93 \pm 0.39
3	Phetchabun	135.67 \pm 2.11	73.38 \pm 0.97	26.61 \pm 0.97
4	Sukhothai	134.73 \pm 11.90	86.56 \pm 0.14	13.43 \pm 0.14
5	Nan	128.53 \pm 3.26	73.34 \pm 3.02	26.65 \pm 3.02
	Mean \pm S.E.	137.52 \pm 3.61	80.37 \pm 3.26	19.62 \pm 3.26

Table 12 Isoflavone glycoside and aglycoside content (%) in 23 *P. mirifica* samples with lower proliferation effect than the first 5 highest proliferation effect

No.	Source	Proliferation effect(1 μ g/ml)	Isoflavone glycoside(%)	Isoflavone aglycoside(%)
6	Chiang Rai	128.43 \pm 5.89	93.54 \pm 0.47	6.46 \pm 0.47
7	Chiang Mai	128.43 \pm 8.13	86.51 \pm 2.06	13.48 \pm 2.06
8	Prachuap Khiri Khan	127.71 \pm 8.66	85.62 \pm 1.04	14.37 \pm 1.04
9	Ratchaburi	127.12 \pm 4.56	87.61 \pm 0.84	12.39 \pm 0.84
10	Mae Hong Son	127.04 \pm 8.81	73.34 \pm 0.89	26.65 \pm 0.89
11	Lamphun	125.83 \pm 3.26	92.33 \pm 0.26	7.67 \pm 0.26
12	Chumphon	123.69 \pm 7.05	93.09 \pm 0.43	6.91 \pm 0.43
13	Kanchanaburi	123.67 \pm 12.49	88.11 \pm 0.50	11.89 \pm 0.50
14	Phrae	122.97 \pm 4.37	88.54 \pm 2.36	11.46 \pm 2.36
15	Chaiyaphum	120.62 \pm 2.74	82.45 \pm 2.95	17.54 \pm 2.95
16	Lop Buri	120.07 \pm 7.16	93.29 \pm 1.42	6.71 \pm 1.42
17	Prachin Buri	119.69 \pm 10.27	86.09 \pm 1.61	13.90 \pm 1.61
18	Nong Bua Lam Phu	118.46 \pm 7.25	79.14 \pm 2.46	20.86 \pm 2.46
19	Phayoa	117.55 \pm 5.38	91.64 \pm 1.13	8.35 \pm 1.13
20	Tak	115.13 \pm 6.51	77.93 \pm 0.81	22.06 \pm 0.81
21	Sakon Nakhon	114.31 \pm 12.12	80.95 \pm 1.19	19.04 \pm 1.19
22	Uttharadith	112.92 \pm 3.77	75.25 \pm 0.12	24.75 \pm 0.12
23	Nakhon Ratchasima	112.68 \pm 4.87	95.69 \pm 1.62	4.31 \pm 1.62
24	Kamphaeng Phet	111.89 \pm 4.05	89.89 \pm 1.37	10.10 \pm 1.37
25	Lampang	111.61 \pm 5.45	87.30 \pm 0.30	12.69 \pm 0.30
26	Phetchaburi	105.92 \pm 4.99	89.08 \pm 0.48	10.91 \pm 0.48
27	Saraburi	105.40 \pm 4.07	92.38 \pm 0.53	7.61 \pm 0.53
28	Uthai thani	102.35 \pm 7.00	78.12 \pm 0.65	21.87 \pm 0.65
	Mean \pm S.E.	115.13 \pm 2.62	86.43 \pm 1.33	13.56 \pm 1.33

Table 13 Mean of isoflavone content (%) in first 5 highest proliferation effect compared with 23 *P. mirifica* samples.

No.	Group	Proliferation effect (1 μ g/ml)	Isoflavone glycoside(%)	Isoflavone aglycoside(%)
1	Mean \pm S.E. of 5 PM	137.52 \pm 3.61	80.37 \pm 3.26	19.62 \pm 3.26*
2	Mean \pm S.E. of 22 PM	115.13 \pm 2.62	86.43 \pm 1.33	13.56 \pm 1.33

5 PM; the first 5 highest proliferation effect of *P. mirifica*

22 PM; the left 23 *P. mirifica* with lower proliferation effect

Table 14 The ranked *P. mirifica* samples according to the amount of isoflavone; puerarin, daidzin, genistin, daidzein and genistein contents.

Proliferation effect(1 μ g/ml)	Puerarin (%)	Daidzin (%)	Genistin (%)	Daidzein (%)	Genistein (%)	Total Isoflavone
1 st Phitsanulok	3 rd Phitsanulok	20 th Phitsanulok	26 th Phitsanulok	10 th Phitsanulok	1 st Phitsanulok	7 th Phitsanulok
2 nd Nakon Sawan	8 th Nakon Sawan	4 th Nakon Sawan	15 th Nakon Sawan	17 th Nakon Sawan	2 nd Nakon Sawan	12 nd Nakon Sawan
3 rd Phetchabun	5 th Phetchabun	6 th Phetchabun	24 th Phetchabun	1 st Phetchabun	3 rd Phetchabun	27 th Phetchabun
4 th Sukhothai	2 nd Sukhothai	5 th Sukhothai	8 th Sukhothai	8 th Sukhothai	4 th Sukhothai	4 th Sukhothai
5 th Nan	10 th Nan	25 th Nan	21 st Nan	2 nd Nan	5 th Nan	28 th Nan
Mapping frequency	3/6	1/6	0/6	2/6	5/6	1/6

Table 15 Isoflavone content in mg/100g powder of filed grown *P. mirifica* clone Doi Tao cultivated in different season (Subtang, 2002)

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 16 Isoflavone content in mg/100g powder of field grown *P. mirifica* clone Chaiprakarn cultivated in different season (Subtang, 2002)

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	45.07 ± 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	-

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Table 17 The growth response percentage of *P. mirifica* extract on MCF-7 cell culture compare mean of population.

Provinces	0.1 $\mu\text{g/ml}$	1 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$	1000 $\mu\text{g/ml}$	IC ₅₀ ($\mu\text{g/ml}$)
Control \pm S.E.	100 \pm 3.19	100 \pm 3.19	100 \pm 3.19	100 \pm 3.19	100 \pm 3.19	-
Phitsanulok	113.71 \pm 1.70	145.70 \pm 8.93*	73.99 \pm 3.64	55.50 \pm 8.27*	50.30 \pm 4.22	>1000
Nakhon Sawan	113.77 \pm 10.35	142.99 \pm 11.84*	91.65 \pm 7.39	75.76 \pm 8.53	61.48 \pm 10.40	>1000
Phetchabun	116.51 \pm 12.81	135.67 \pm 2.11	73.45 \pm 6.18	68.03 \pm 8.26	49.11 \pm 4.55*	>1000
Sukhothai	105.95 \pm 9.97	134.73 \pm 11.90	80.65 \pm 3.93	74.99 \pm 10.42	61.46 \pm 2.62	>1000
Nan	105.78 \pm 4.92	128.53 \pm 3.26	83.13 \pm 2.48	59.41 \pm 2.00	54.55 \pm 5.56	>1000
Chiang Rai	113.74 \pm 12.33	128.43 \pm 5.89	81.69 \pm 5.13	81.45 \pm 15.91	70.01 \pm 8.80	>1000
Chiang Mai	100.49 \pm 1.19	128.43 \pm 8.13	92.46 \pm 5.04	80.02 \pm 0.41	73.66 \pm 5.34	>1000
Prachuap Khiri Khan	111.65 \pm 5.29	127.71 \pm 8.66	94.79 \pm 5.43	74.46 \pm 1.34	71.22 \pm 5.80	>1000
Ratchaburi	116.80 \pm 2.17	127.12 \pm 4.56	106.06 \pm 6.06	91.14 \pm 1.13	55.71 \pm 3.22	>1000
Mae Hong Son	102.91 \pm 10.02	127.04 \pm 8.81	97.00 \pm 7.39	87.32 \pm 3.60	57.37 \pm 1.77	>1000
Lamphun	105.73 \pm 2.86	125.83 \pm 3.26	90.35 \pm 1.88	85.78 \pm 3.81	74.10 \pm 3.54	>1000
Chumphon	102.75 \pm 13.33	123.69 \pm 7.05	106.38 \pm 14.27*	100.69 \pm 8.06*	82.44 \pm 5.36	>1000
Kanchanaburi	101.37 \pm 1.37	123.67 \pm 12.49	82.27 \pm 5.66	77.27 \pm 3.38	69.74 \pm 5.02	>1000
Phrae	108.44 \pm 4.41	122.97 \pm 4.37	73.08 \pm 3.01	60.30 \pm 7.79	55.26 \pm 3.02	>1000
Chaiyaphum	105.92 \pm 3.06	120.62 \pm 2.74	96.60 \pm 5.92	87.14 \pm 2.33	75.78 \pm 2.15	>1000

Table 17 (Continued) The growth response percentage of *P. mirifica* extract on MCF-7 cell culture compare mean of population.

Provinces	0.1 µg/ml	1 µg/ml	10 µg/ml	100 µg/ml	1000 µg/ml	IC ₅₀ (µg/ml)
Lop Buri	102.86±0.14	120.07±7.16	83.76±5.82	79.54±6.19	72.14±4.07	>1000
Phrachin Buri	114.13±3.88	119.69±10.27	107.19±4.13	93.35±6.65	78.25±5.17	>1000
Nong Bua Lam Phu	109.90±15.06	118.46±7.25	94.20±7.37	80.46±10.39	56.34±6.83	>1000
Phayoa	114.63±6.35	117.55±5.38	91.55±4.81	92.87±2.68	72.44±5.30	>1000
Tak	99.82±5.02	115.13±6.51	74.54±14.79	60.31±14.8	55.85±7.80	>1000
Sakon Nakhon	111.31±2.68	114.31±12.12	84.49±5.87	73.86±2.56	64.33±7.46	>1000
Uttharadith	100.25±2.80	112.92±3.77	85.61±14.22	63.84±7.04	62.88±0.89	>1000
Nakhon Ratchasima	100.79±4.31	112.68±4.87	73.28±13.04	65.00±14.63	46.77±9.85*	1210.02
Kamphaeng Phet	104.74±4.38	111.89±4.05	96.39±7.38	91.75±6.03	75.35±3.64	>1000
Lampang	105.11±2.31	111.61±5.45	93.51±3.03	80.50±4.77	66.93±6.95	>1000
Phetchaburi	100.03±4.8	105.92±4.99	81.07±6.12	68.02±6.02	51.34±6.99	>1000
Saraburi	100.29±9.02	105.4±4.07	74.74±9.69	70.86±7.40	50.19±2.73*	>1000
Uthai Thani	99.26±1.46	102.35±7.00	92.12±5.52	78.71±6.98	76.06±3.62	>1000
Means±S.E.	105.66±1.14	121.07±1.56	86.62±1.49	76.95±1.65	64.36±1.49	>1000

* significant at $p < 0.05$, $n=3$

Table 18 The growth response percentage of *P. mirifica* extract on MCF-7 cell culture compare with control.

Provinces	0.1 µg/ml	1 µg/ml	10 µg/ml	100 µg/ml	1000 µg/ml	IC ₅₀ (µg/ml)
Control±S.E.	100±3.19	100±3.19	100±3.19	100±3.19	100±3.19	-
Phitsanulok	113.71±1.70	145.70±8.93*	73.99±3.64*	55.50±8.27*	50.30±4.22*	>1000
Nakhon Sawan	113.77±10.35	142.99±11.84*	91.65±7.39	75.76±8.53*	61.48±10.40*	>1000
Phetchabun	116.51±12.81	135.67±2.11*	73.45±6.18*	68.03±8.26*	49.11±4.55*	>1000
Sukhothai	105.95±9.97	134.73±11.90*	80.65±3.93	74.99±10.42*	61.46±2.62*	>1000
Nan	105.78±4.92	128.53±3.26*	83.13±2.48	59.41±2.00*	54.55±5.56*	>1000
Chiang Rai	113.74±12.33	128.43±5.89*	81.69±5.13	81.45±15.91	70.01±8.80*	>1000
Chiang Mai	100.49±1.19	128.43±8.13*	92.46±5.04	80.02±0.41*	73.66±5.34*	>1000
Prachuap Khiri Khan	111.65±5.29	127.71±8.66*	94.79±5.43	74.46±1.34*	71.22±5.80*	>1000
Ratchaburi	116.80±2.17	127.12±4.56*	106.06±6.06	91.14±1.13	55.71±3.22*	>1000
Mae Hong Son	102.91±10.02	127.04±8.81*	97.00±7.39	87.32±3.60	57.37±1.77*	>1000
Lamphun	105.73±2.86	125.83±3.26*	90.35±1.88*	85.78±3.81	74.10±3.54*	>1000
Chumphon	102.75±13.33	123.69±7.05*	106.38±14.27	100.69±8.06	82.44±5.36*	>1000
Kanchanaburi	101.37±1.37	123.67±12.49	82.27±5.66	77.27±3.38*	69.74±5.02*	>1000
Phrae	108.44±4.41	122.97±4.37*	73.08±3.01*	60.30±7.79*	55.26±3.02*	>1000
Chaiyaphum	105.92±3.06	120.62±2.74*	96.60±5.92	87.14±2.33	75.78±2.15*	>1000

* Significant at $p < 0.05$, n= 3

Table 18 (Continued) The growth response percentage of *P. mirifica* extract on MCF-7 cell culture compare with control.

Provinces	0.1 $\mu\text{g/ml}$	1 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$	1000 $\mu\text{g/ml}$	IC ₅₀ ($\mu\text{g/ml}$)
Lop Buri	102.86 \pm 0.14	120.07 \pm 7.16*	83.76 \pm 5.82	79.54 \pm 6.19*	72.14 \pm 4.07*	>1000
Phrachin Buri	114.13 \pm 3.88	119.69 \pm 10.27*	107.19 \pm 4.13*	93.35 \pm 6.65*	78.25 \pm 5.17*	>1000
Nong Bua Lam Phu	109.90 \pm 15.06	118.46 \pm 7.25	94.20 \pm 7.37	80.46 \pm 10.39	56.34 \pm 6.83*	>1000
Phayoa	114.63 \pm 6.35	117.55 \pm 5.38	91.55 \pm 4.81	92.87 \pm 2.68	72.44 \pm 5.30*	>1000
Tak	99.82 \pm 5.02	115.13 \pm 6.51	74.54 \pm 14.79*	60.31 \pm 14.8*	55.85 \pm 7.80*	>1000
Sakon Nakhon	111.31 \pm 2.68	114.31 \pm 12.12	84.49 \pm 5.87	73.86 \pm 2.56*	64.33 \pm 7.46*	>1000
Uttharadith	100.25 \pm 2.80	112.92 \pm 3.77	85.61 \pm 14.22	63.84 \pm 7.04*	62.88 \pm 0.89*	>1000
Nakhon Ratchasima	100.79 \pm 4.31	112.68 \pm 4.87	73.28 \pm 13.04*	65.00 \pm 14.63*	46.77 \pm 9.85*	1210.02
kamphaeng phet	104.74 \pm 4.38	111.89 \pm 4.05	96.39 \pm 7.38	91.75 \pm 6.03	75.35 \pm 3.64*	>1000
Lampang	105.11 \pm 2.31	111.61 \pm 5.45	93.51 \pm 3.03	80.50 \pm 4.77*	66.93 \pm 6.95*	>1000
Phetchaburi	100.03 \pm 4.8	105.92 \pm 4.99	81.07 \pm 6.12	68.02 \pm 6.02*	51.34 \pm 6.99*	>1000
Saraburi	100.29 \pm 9.02	105.4 \pm 4.07	74.74 \pm 9.69*	70.86 \pm 7.40*	50.19 \pm 2.73*	>1000
Uthai Thani	99.26 \pm 1.46	102.35 \pm 7.00	92.12 \pm 5.52	78.71 \pm 6.98	76.06 \pm 3.62*	>1000
Means \pm S.E.	105.66 \pm 1.14	121.07 \pm 1.56	86.62 \pm 1.49	76.95 \pm 1.65	64.36 \pm 1.49	>1000

* Significant at $p < 0.05$, $n=3$

Figure 7 Effect of *P. mirifica* extract on the growth of MCF-7 cell culture

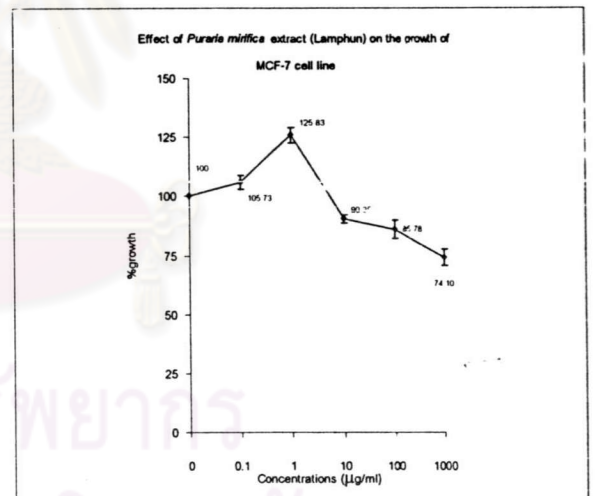
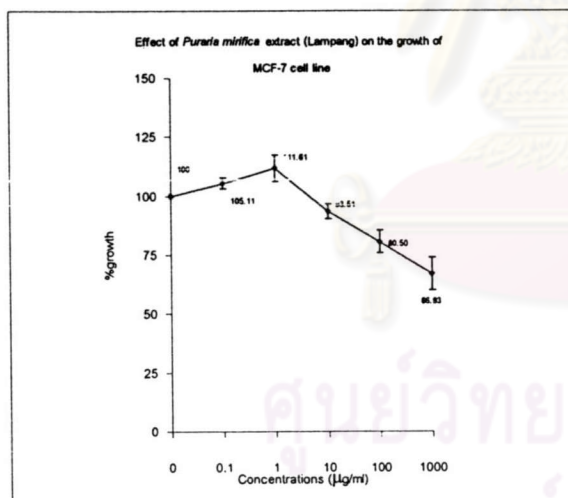
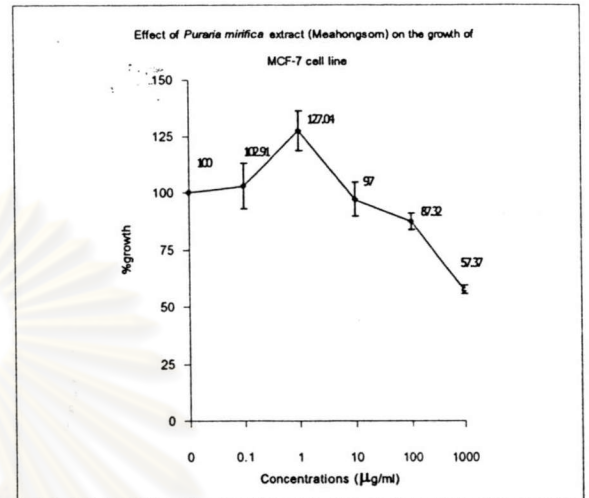
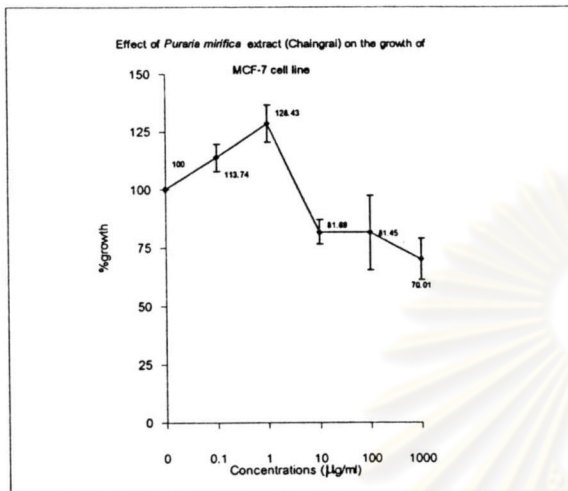


Figure 7 (Continued) Effect of *P. mirifica* extract on the growth of MCF-7 cell culture

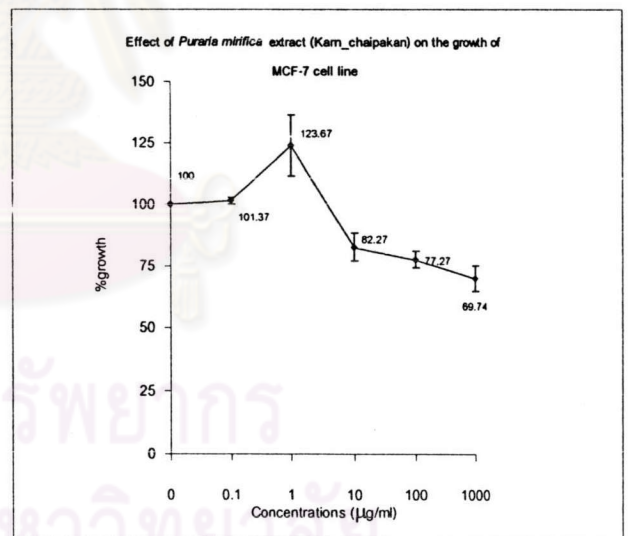
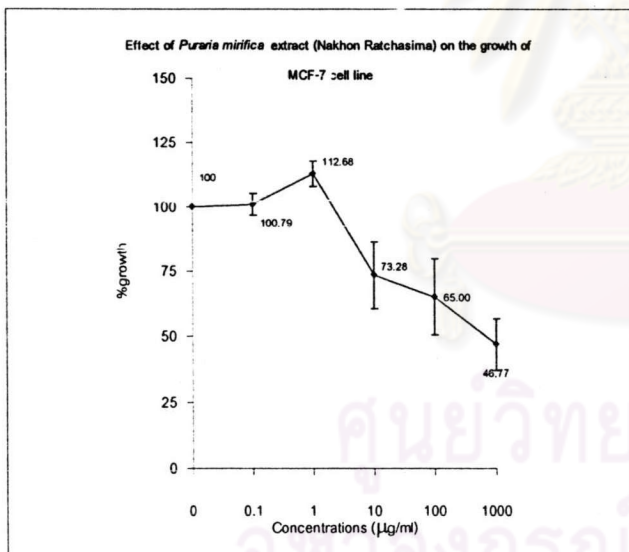
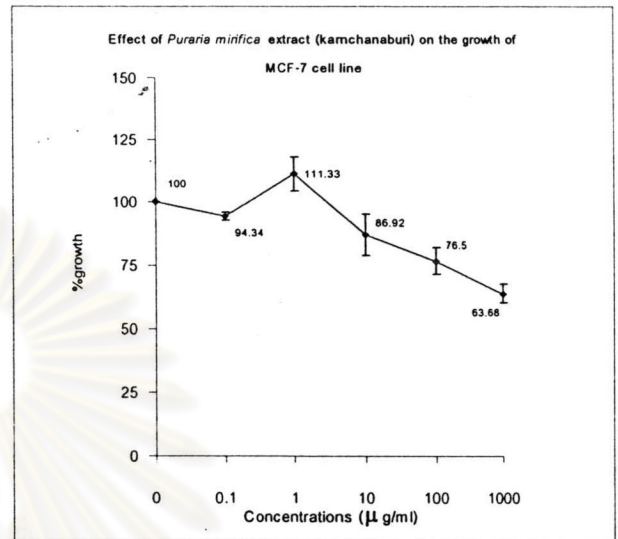
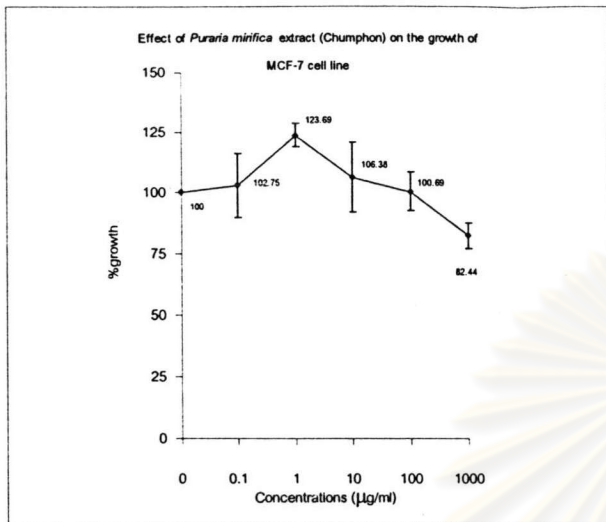


Figure 7 (Continued) Effect of *P. mirifica* extract on the growth of MCF-7 cell culture

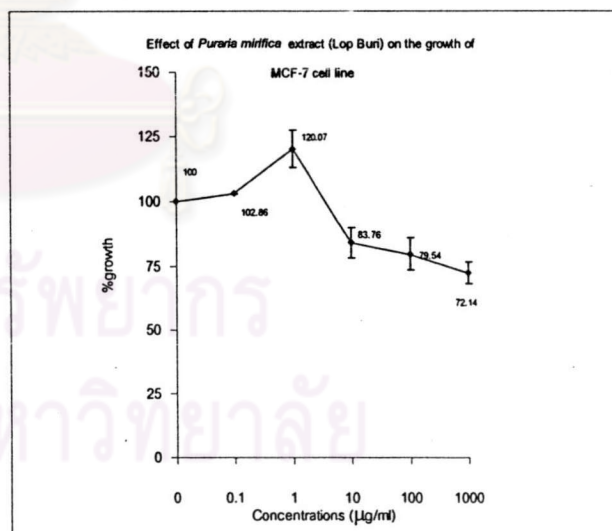
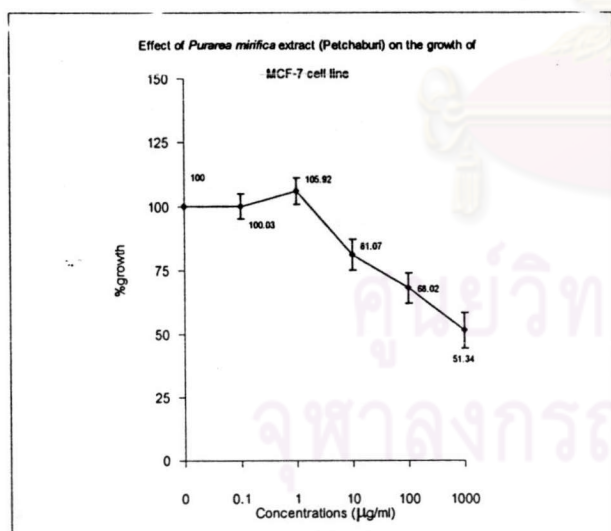
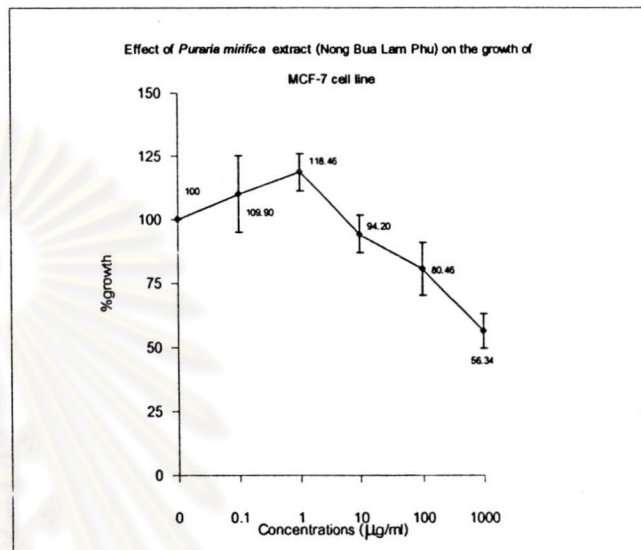
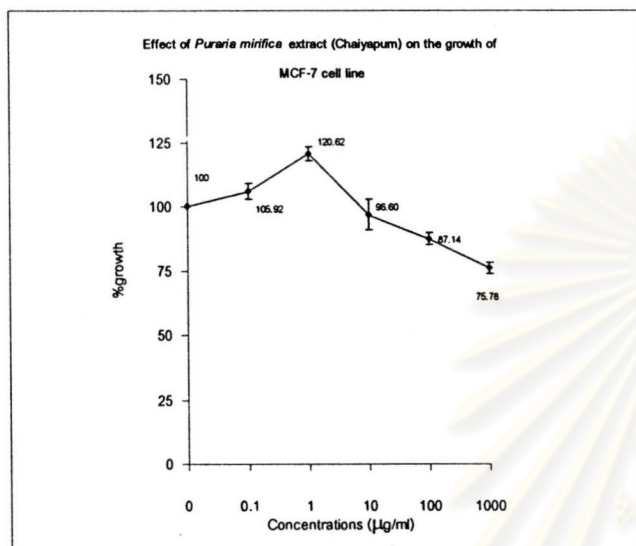


Figure 7 (Continued) Effect of *P. mirifica* extract on the growth of MCF-7 cell culture

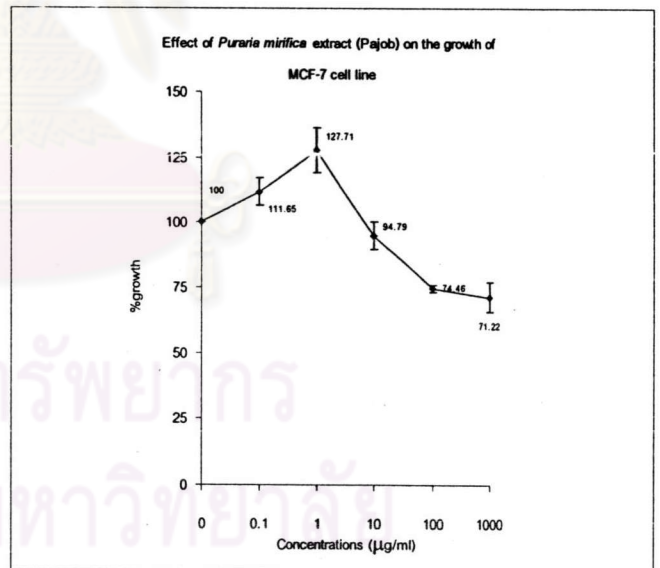
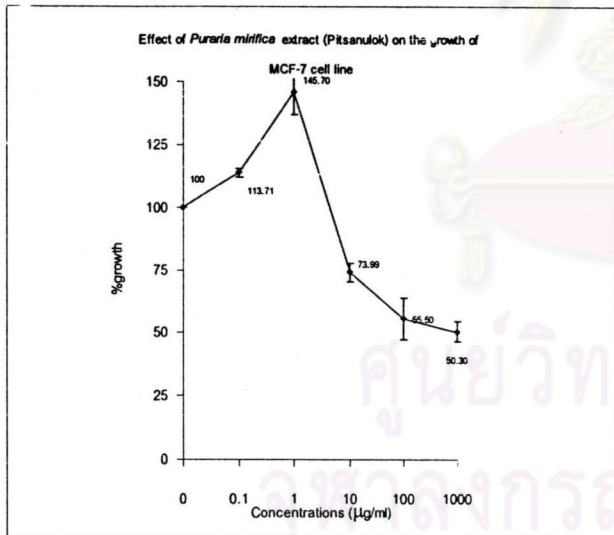
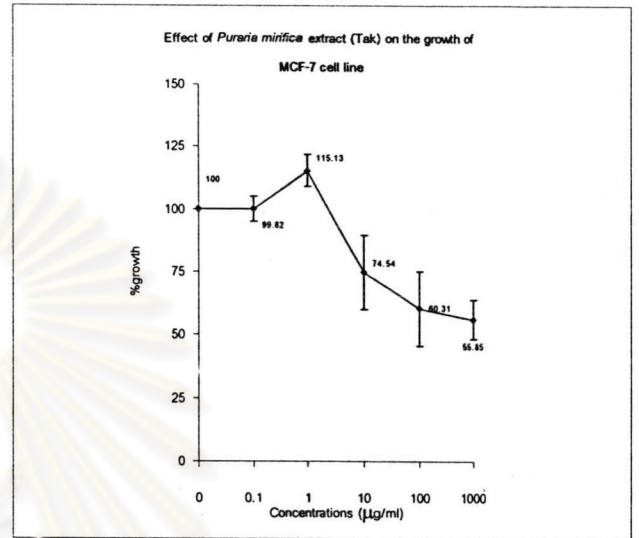
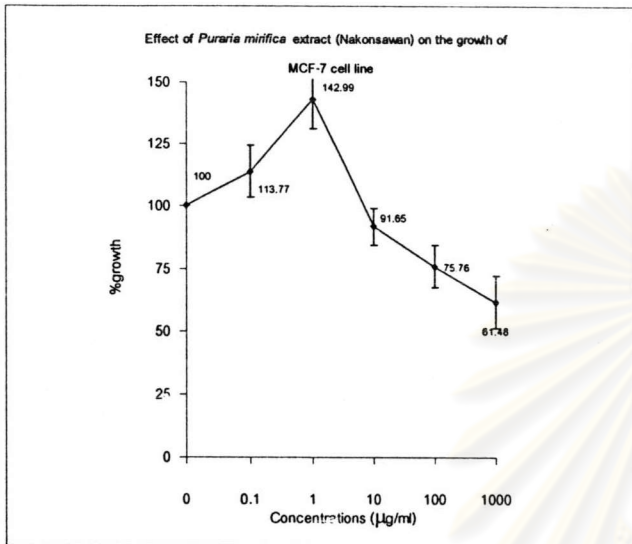


Figure 7 (Continued) Effect of *P. mirifica* extract on the growth of MCF-7 cell culture

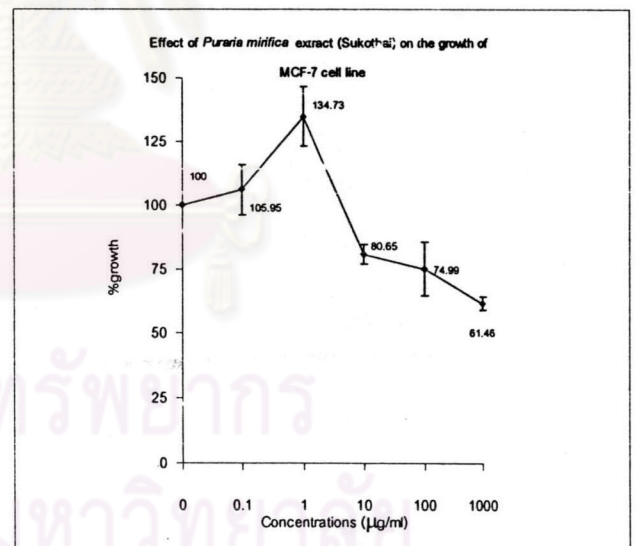
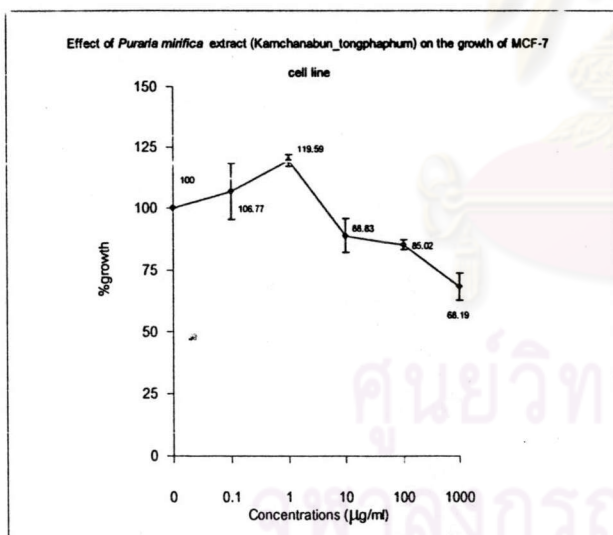
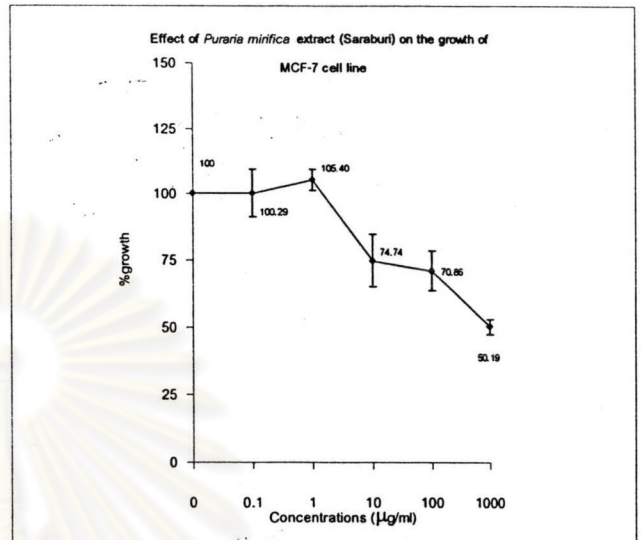
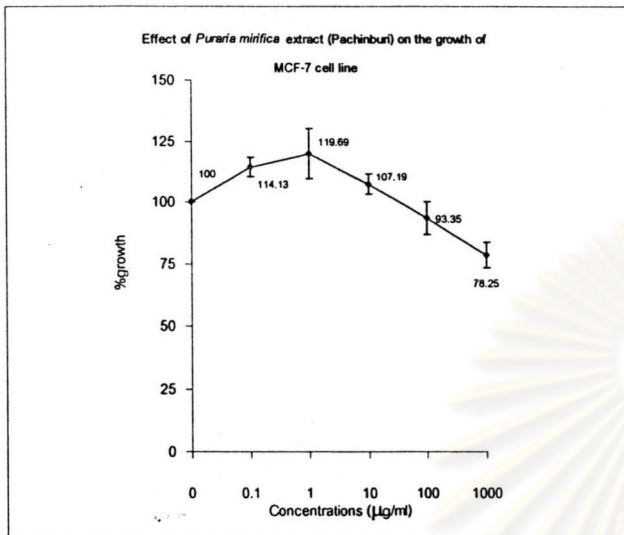


Figure 7 (Continued) Effect of *P. mirifica* extract on the growth of MCF-7 cell culture

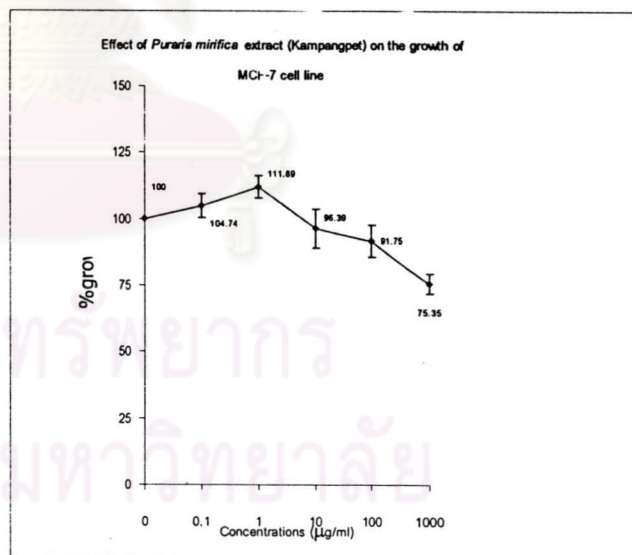
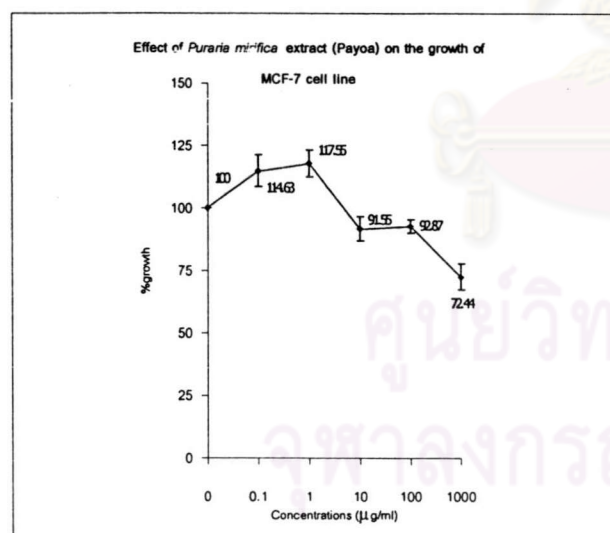
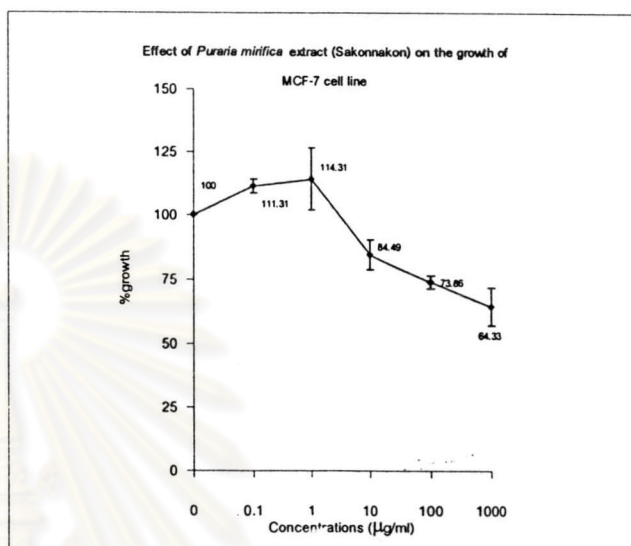
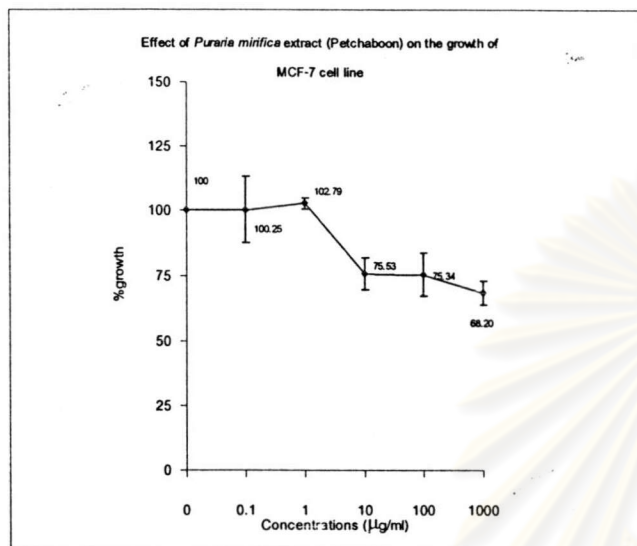


Figure 7 (Continued) Effect of *P. mirifica* extract on the growth of MCF-7 cell culture

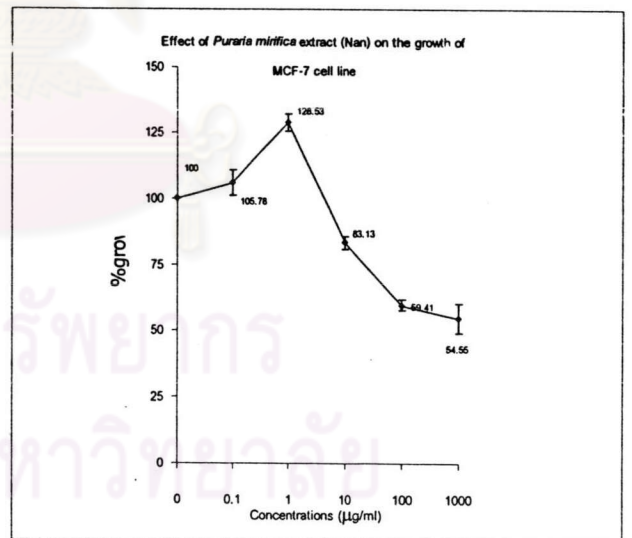
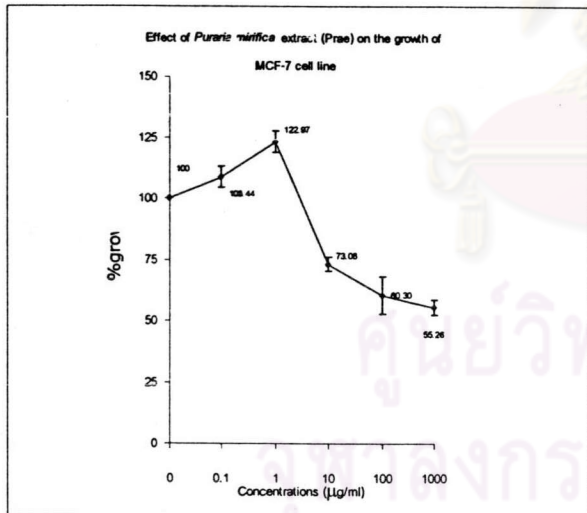
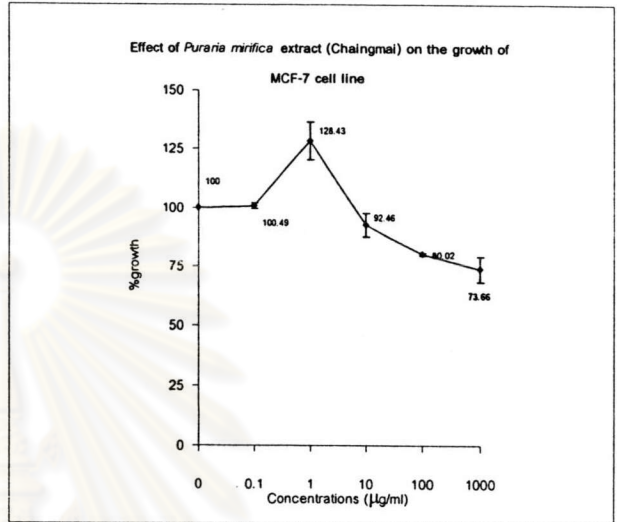
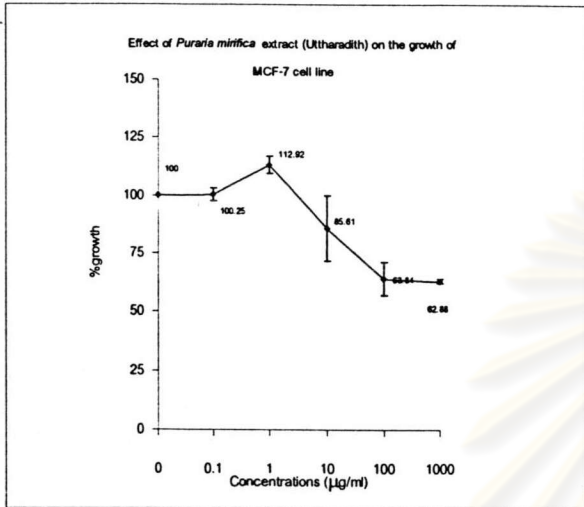
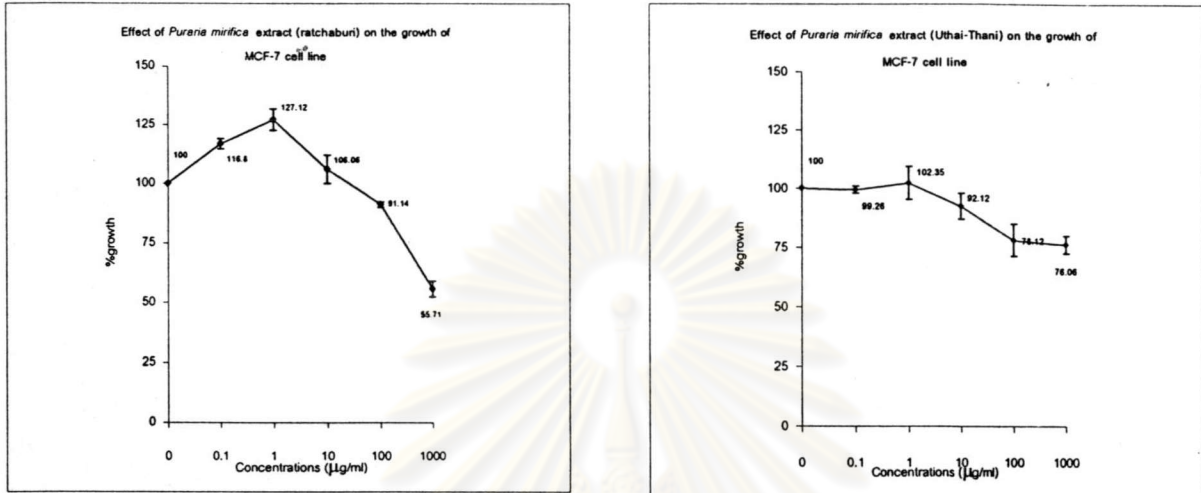


Figure 7 (Continued) Effect of *P. mirifica* extract on the growth of MCF-7 cell culture



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Figure 7 Effect of *P. mirifica* extract on the growth of MCF-7 cell culture

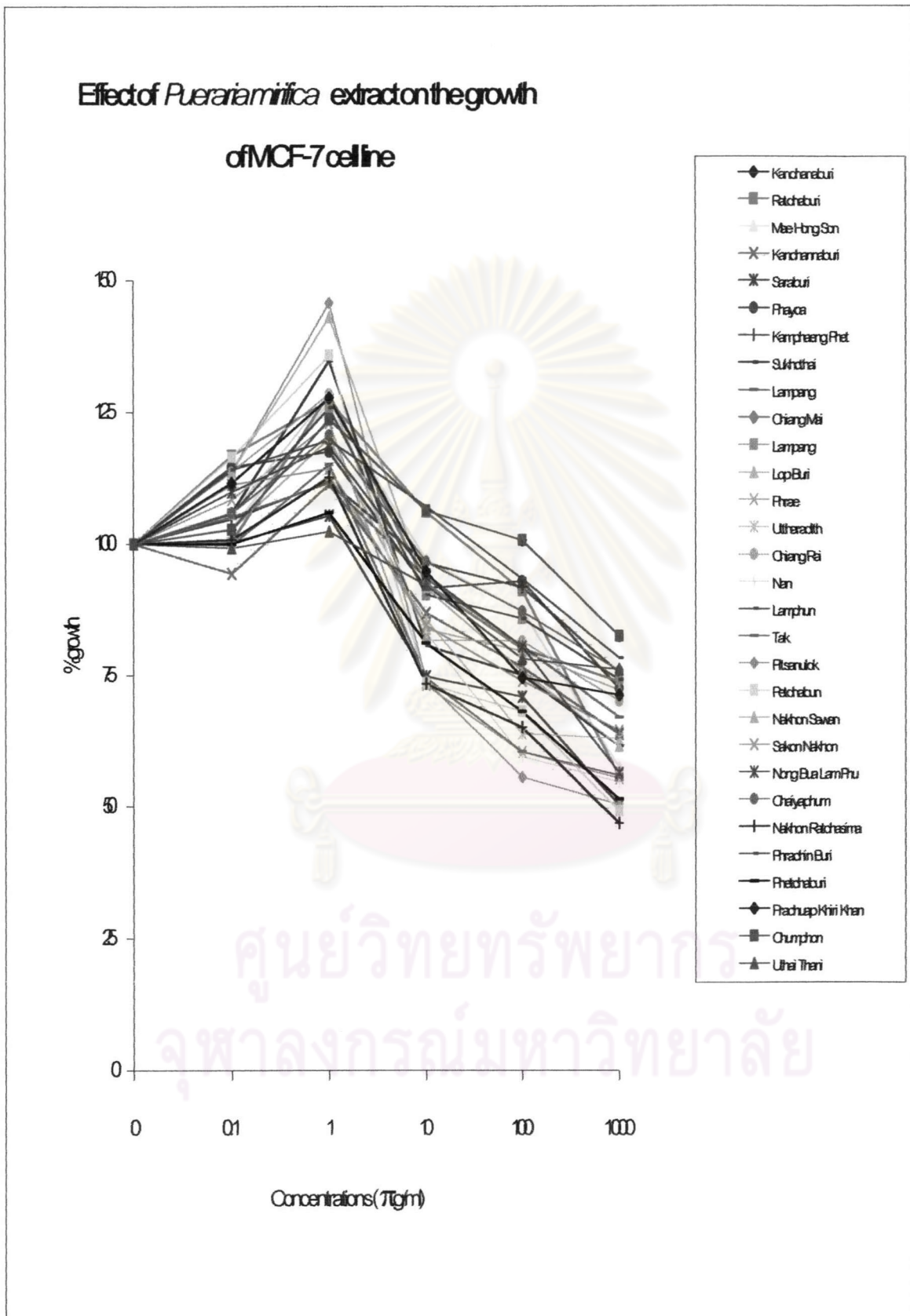


Table 19 The growth response percentage of MCF-7 cell culture of *P. mirifica* extract different season ($p < 0.05$)

cultivar	0.1 $\mu\text{g/ml}$	1 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$	1000 $\mu\text{g/ml}$	IC ₅₀
Chaiprakarn-rainny	106.72 \pm 1.97	107.94 \pm 3.58	94.65 \pm 1.41	82.82 \pm 2.61	51.57 \pm 1.52*	> 1000
Chaiprakarn-winter	105.96 \pm 4.35	119.26 \pm 3.33	107.68 \pm 2.78	88.67 \pm 7.56	84.25 \pm 0.89	> 1000
Chaiprakarn-summer	114.13 \pm 3.88	119.69 \pm 10.27	107.19 \pm 4.13* ¹	93.35 \pm 6.65	78.25 \pm 5.17	> 1000
Doi Tao-rainny	103.34 \pm 10.17	111.94 \pm 2.67	83.98 \pm 11.46	81.72 \pm 6.29	71.04 \pm 2.77	> 1000
Doi Tao-winter	104.39 \pm 5.43	115.57 \pm 8.39	99.85 \pm 3.05	95.69 \pm 4.64	72.96 \pm 9.62	> 1000
Doi Tao-summer	104.93 \pm 7.75	109.81 \pm 5.18	95.01 \pm 10.33	84.69 \pm 7.06	76.54 \pm 13.53	> 1000

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Figure 8 Effect of *P. mirifica* extract (Chaiprakam) on the growth of MCF-7 cell culture of different season

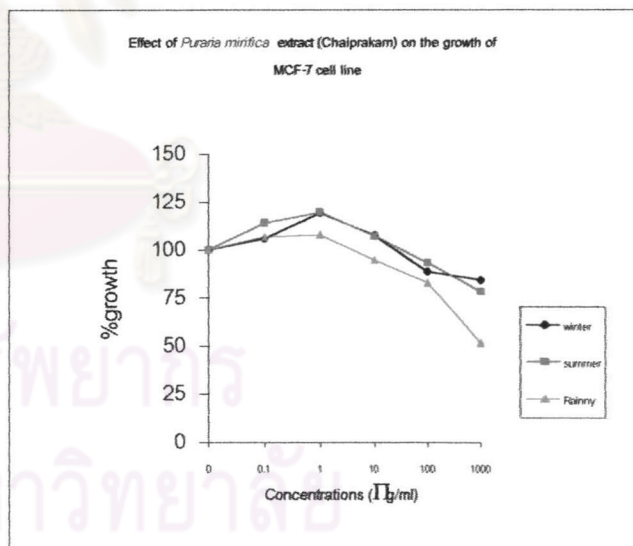
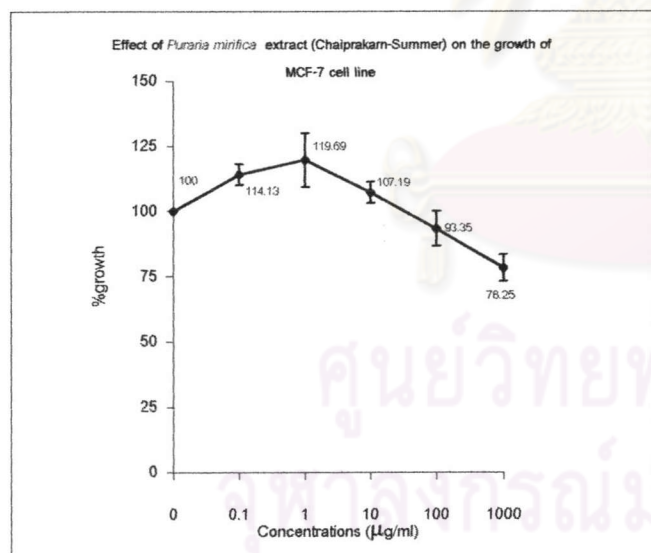
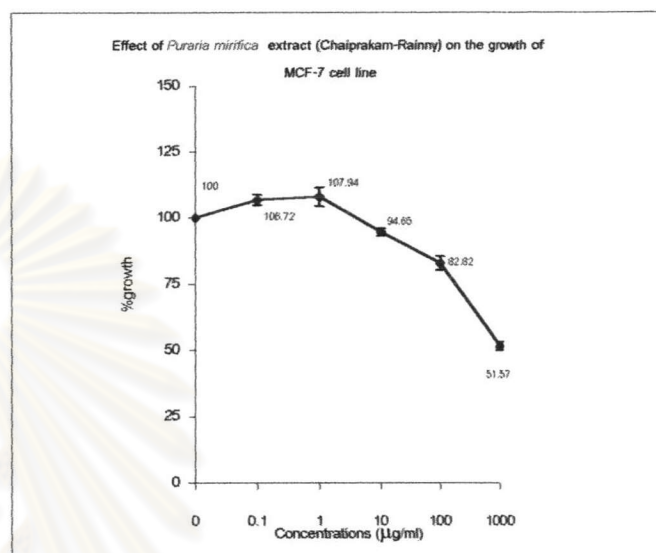
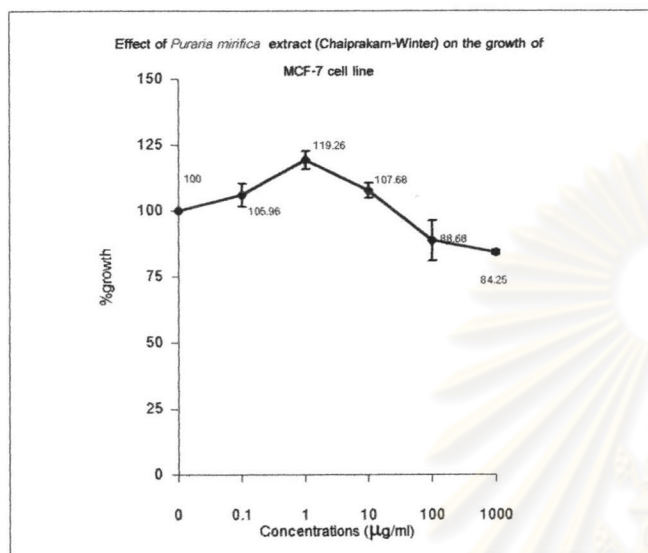


Figure 9 Effect of *P. mirifica* extract (Doi Tao) on the growth of MCF-7 cell culture of different season

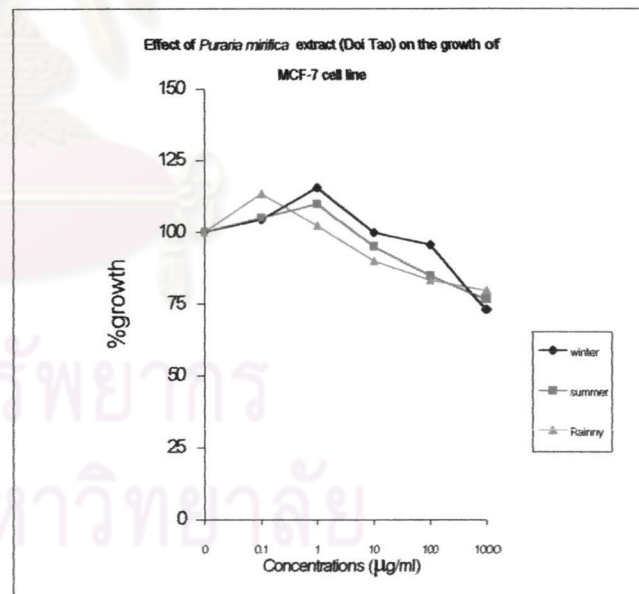
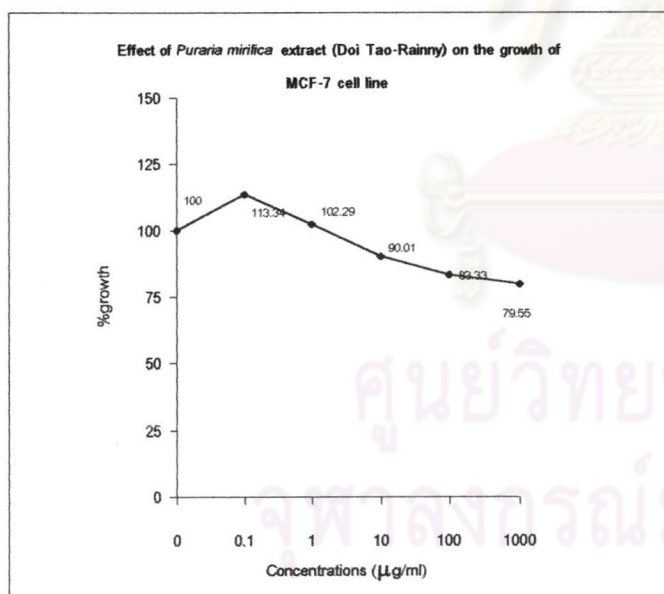
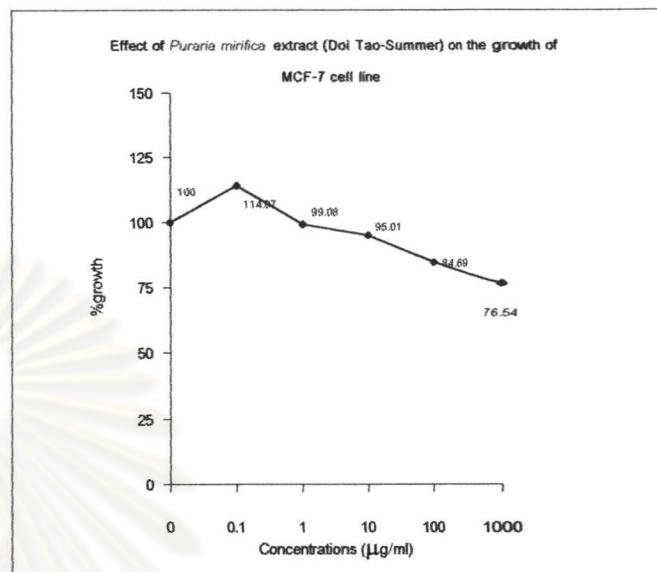
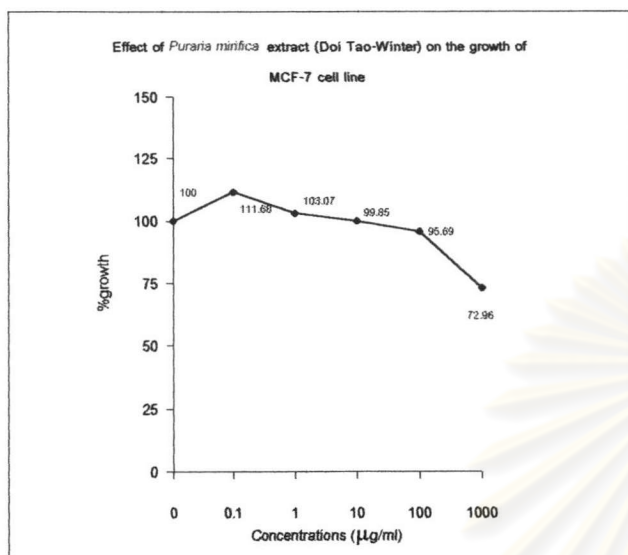


Table 20 The growth response percentage of *B. superba* extract on MCF-7 cell culture compare with mean of population.

Provinces	0.1 $\mu\text{g/ml}$	1 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$	1000 $\mu\text{g/ml}$	IC ₅₀ ($\mu\text{g/ml}$)
Control \pm S.E.	100 \pm 7.09	100 \pm 7.09	100 \pm 7.09	100 \pm 7.09	100 \pm 7.09	-
Kanchanaburi	97.88 \pm 5.38	81.73 \pm 8.97	64.4 \pm 2.29	50.46 \pm 1.11	34.13 \pm 2.58	76.65
Ratchaburi	96.86 \pm 10.29	61.77 \pm 5.98	51.14 \pm 3.64*	55.12 \pm 14.77	40.41 \pm 11.21	109.82
Tak	86.74 \pm 9.95	73.54 \pm 3.77	70.42 \pm 5.11	54.51 \pm 8.34	32.58 \pm 1.61	184.35
Phitsanulok	103.02 \pm 12.78	91.93 \pm 11.31	75.13 \pm 7.50	55.98 \pm 12.53	40.56 \pm 2.62	344.12
Srisaket	100.49 \pm 4.43	75.46 \pm 6.34	61.80 \pm 10.36	51.74 \pm 9.53	41.15 \pm 5.65	381.97
Khon Kaen	103.15 \pm 11.18	83.06 \pm 4.69	83.57 \pm 4.77	73.95 \pm 14.26	30.83 \pm 2.97*	545.29
Nakhon Ratchasima	100.40 \pm 10.39	100.31 \pm 9.2	79.33 \pm 6.64	69.63 \pm 5.93	24.01 \pm 1.09*	546.23
Chachoengsoa	98.34 \pm 11.56	79.45 \pm 2.65	67.06 \pm 5.75	53.03 \pm 4.76	43.26 \pm 6.23	606.58
Phetchabun	105.75 \pm 11.2	80.41 \pm 12.24	69.94 \pm 10.47	62.71 \pm 6.06	39.18 \pm 9.15	683.87
Uttharadith	79.82 \pm 7.23	73.99 \pm 9.17	66.69 \pm 9.69	65.87 \pm 6.36	41.5 \pm 1.83	733.87
Chantaburi	114.86 \pm 4.53	103.15 \pm 1.00*	85.28 \pm 6.12	62.67 \pm 6.97	43.7 \pm 0.84	934.73
Lampang	120.7 \pm 12.57	87.04 \pm 8.66	66.73 \pm 6.39	59.29 \pm 8.19	49.35 \pm 7.27	980.3
Mae Hong Son	133.4 \pm 12.24*	110.7 \pm 16.68	70.02 \pm 11.09	57.92 \pm 5.18	48.62 \pm 1.76	1005.17
Saraburi	95.60 \pm 12.25	93.60 \pm 10.27	63.34 \pm 8.17	52.84 \pm 12.09	40.88 \pm 4.89	1117.54
Chiang Rai	115.66 \pm 5.91	88.01 \pm 7.71	74.45 \pm 4.62	72.66 \pm 7.42	49.91 \pm 10.39	1221.01
Prachinburi	78.55 \pm 4.65*	72.84 \pm 6.04	71.15 \pm 7.98	65.87 \pm 2.34	47.55 \pm 5.35	1261.73
Nakhon Sawan	111.45 \pm 12.79	104.85 \pm 8.31	76.60 \pm 9.57	71.35 \pm 6.19	47.52 \pm 6.82	1281.10
Sakon Nakhon	95.39 \pm 13.83	86.80 \pm 2.21	85.66 \pm 13.85	79.98 \pm 11.29	50.19 \pm 4.59	>1000
Chiang Mai	108.4 \pm 2.49	84.69 \pm 6.36	79.88 \pm 4.23	62.16 \pm 0.84	53.24 \pm 5.56	>1000
Nong Bua Lam Phu	100.70 \pm 4.28	92.48 \pm 8.20	90.59 \pm 7.00	83.08 \pm 10.84	54.01 \pm 3.71	>1000
Loei	97.86 \pm 10.23	88.85 \pm 9.25	82.48 \pm 2.52	76.01 \pm 2.63	54.43 \pm 10.00	>1000
Chonburi	95.33 \pm 6.39	91.29 \pm 11.69	87.07 \pm 4.60	59.34 \pm 4.74	57.53 \pm 9.19	>1000
Lop Buri	98.32 \pm 11.67	97.30 \pm 9.81	93.53 \pm 7.78	83.92 \pm 6.65	59.57 \pm 12.89	>1000
Chaiyaphum	97.81 \pm 12.28	95.56 \pm 3.85	89.96 \pm 1.66	88.60 \pm 4.96*	78.84 \pm 1.62*	>1000
Means \pm SEM	101.45 \pm 2.04	88.40 \pm 2.02	76.25 \pm 1.79	66.75 \pm 1.99	48.07 \pm 2.03	865.61

* Significant at $p < 0.05$, $n=3$

Table 21 The growth response percentage of *B. superba* extract on MCF-7 cell culture compare with control

Provinces	0.1 µg/ml	1 µg/ml	10 µg/ml	100 µg/ml	1000 µg/ml	IC ₅₀ (µg/ml)
Control±S.E.	100±7.09	100±7.09	100±7.09	100±7.09	100±7.09	-
Mae Hong Son	133.4±12.24*	110.7±16.68	70.02±11.09*	57.92±5.18*	48.62±1.76*	1005.17
Lampang	120.7±12.57	87.04±8.66	66.73±6.39*	59.29±8.19*	49.35±7.27*	980.3
Chiang Rai	115.66±5.91	88.01±7.71	74.45±4.62*	72.66±7.42*	49.91±10.39*	1221.01
Chantaburi	114.86±4.53	103.15±1.00	85.28±6.12	62.67±6.97*	43.7±0.84*	934.73
Nakhon Sawan	111.45±12.79	104.85±8.31	76.60±9.57*	71.35±6.19*	47.52±6.82*	1281.10
Chiang Mai	108.4±2.49	84.69±6.36	79.88±4.23*	62.16±0.84*	53.24±5.56*	>1000
Phetchabun	105.75±11.2	80.41±12.24	69.94±10.47*	62.71±6.06*	39.18±9.15*	683.87
Khon Kaen	103.15±11.18	83.06±4.69	83.57±4.77	73.95±14.26*	30.83±2.97*	545.29
Phitsanulok	103.02±12.78	91.93±11.31	75.13±7.50*	55.98±12.53*	40.56±2.62*	344.12
NongBua LamPhu	100.70±4.28	92.48±8.20	90.59±7.00	83.08±10.84	54.01±3.71*	>1000
Srisaket	100.49±4.43	75.46±6.34	61.80±10.36*	51.74±9.53*	41.15±5.65*	381.97
NakhonRatchasima	100.40±10.39	100.31±9.2	79.33±6.64*	69.63±5.93*	24.01±1.09*	546.23
Chachoengsoa	98.34±11.56	79.45±2.65	67.06±5.75*	53.03±4.76*	43.26±6.23*	606.58
Lop Buri	98.32±11.67	97.30±9.81	93.53±7.78	83.92±6.65	59.57±12.89*	>1000
Kanchanaburi	97.88±5.38	81.73±8.97	64.4±2.29*	50.46±1.11*	34.13±2.58*	76.65
Loei	97.86±10.23	88.85±9.25	82.48±2.52	76.01±2.63*	54.43±10.00*	>1000
Chaiyaphum	97.81±12.28	95.56±3.85	89.96±1.66	88.60±4.96	78.84±1.62*	>1000
Ratchaburi	96.86±10.29	61.77±5.98*	51.14±3.64*	55.12±14.77*	40.41±11.21*	109.82
Saraburi	95.60±12.25	93.60±10.27	63.34±8.17*	52.84±12.09*	40.88±4.89*	1117.54
Sakon Nakhon	95.39±13.83	86.80±2.21	85.66±13.85	79.98±11.29	50.19±4.59*	>1000
Chonburi	95.33±6.39	91.29±11.69	87.07±4.60	59.34±4.74*	57.53±9.19*	>1000
Tak	86.74±9.95	73.54±3.77	70.42±5.11*	54.51±8.34*	32.58±1.61*	184.35
Uttharadith	79.82±7.23	73.99±9.17	66.69±9.69*	65.87±6.36*	41.5±1.83*	733.87
Prachinburi	78.55±4.65	72.84±6.04	71.15±7.98*	65.87±2.34*	47.55±5.35*	1261.73
Means±S.E.	101.45±2.04	88.40±2.02	76.25±1.79	66.75±1.99	48.07±2.03	865.61

* Significant at $p < 0.05$, $n=3$

Figure 10 Effect of *B. superba* extract of the growth on MCF-7 cell culture

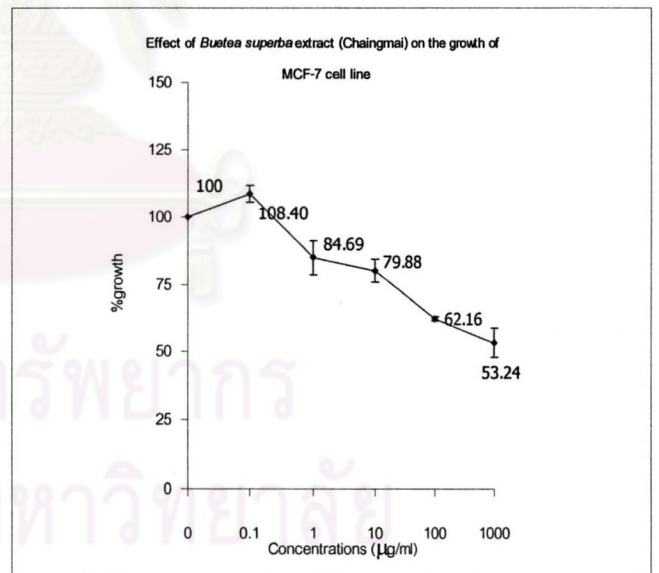
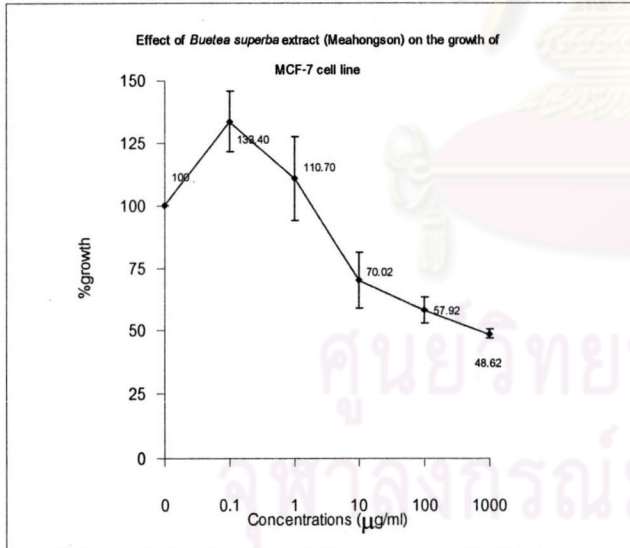
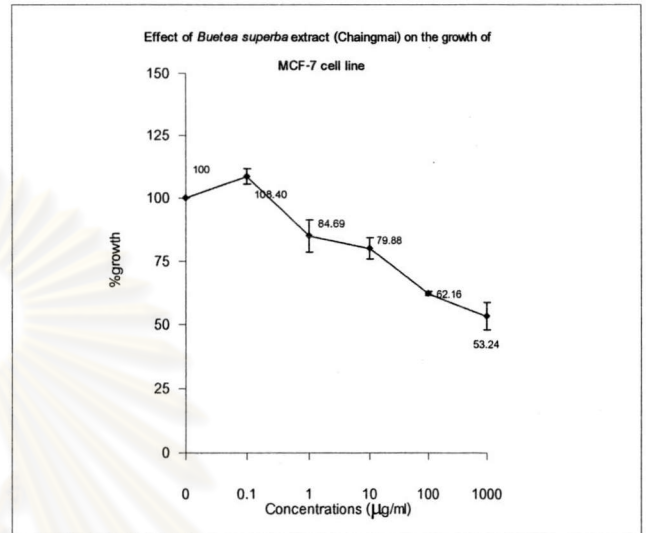
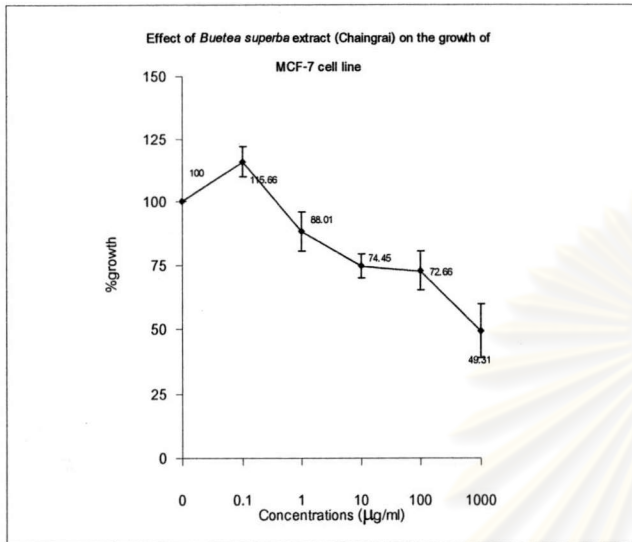


Figure 10 (Continued) Effect of *B. superba* extract of the growth on MCF-7 cell culture

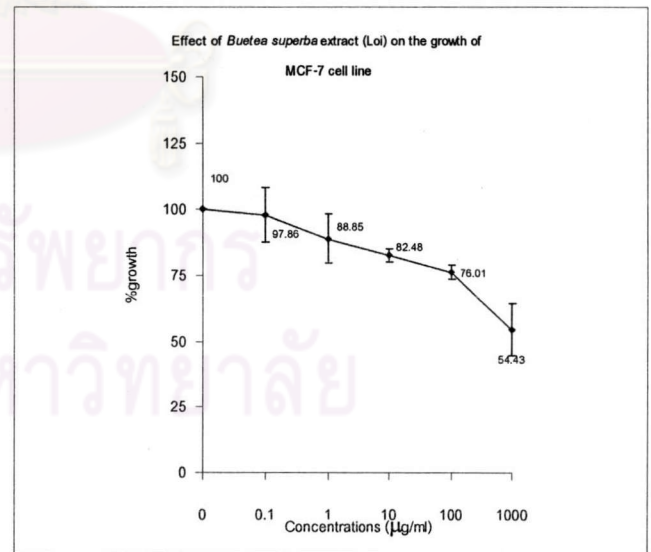
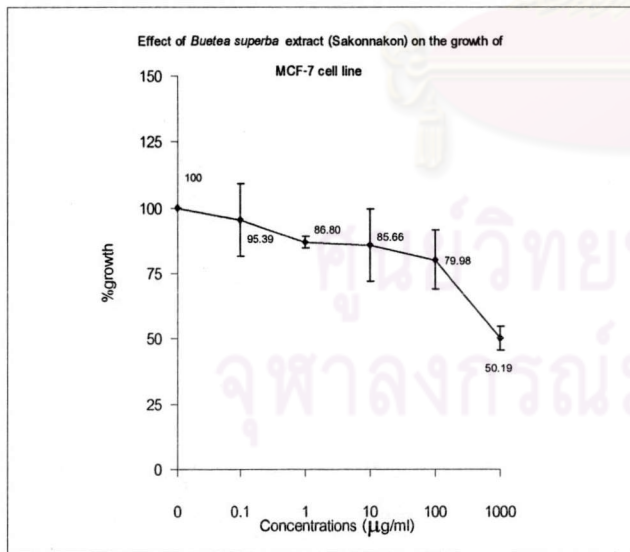
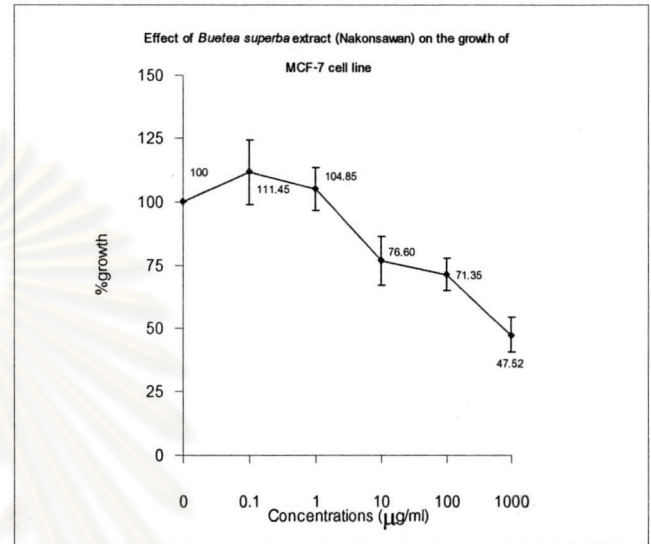
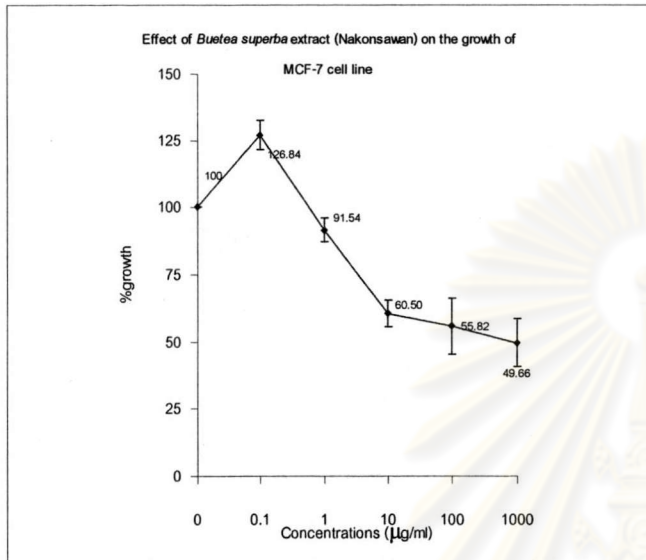


Figure 10 (Continued) Effect of *B. superba* extract of the growth on MCF-7 cell culture

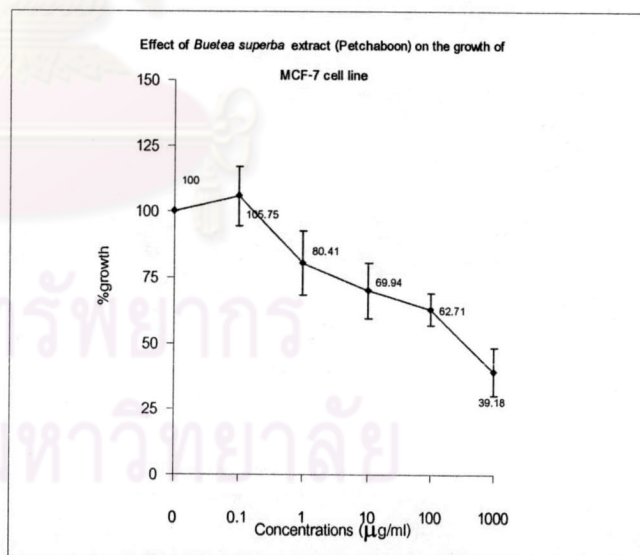
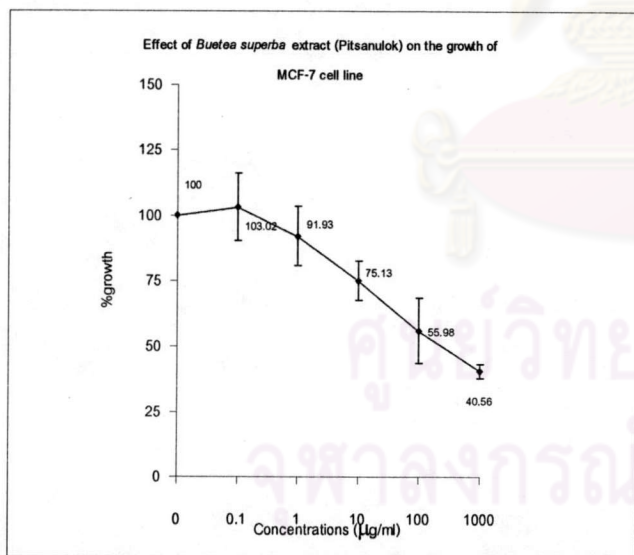
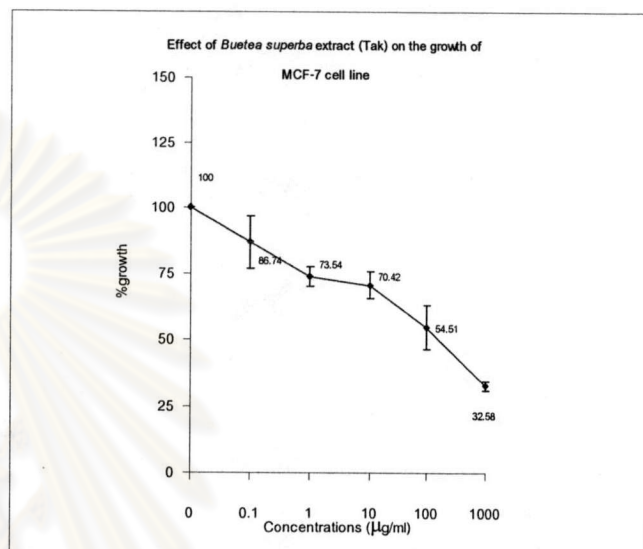
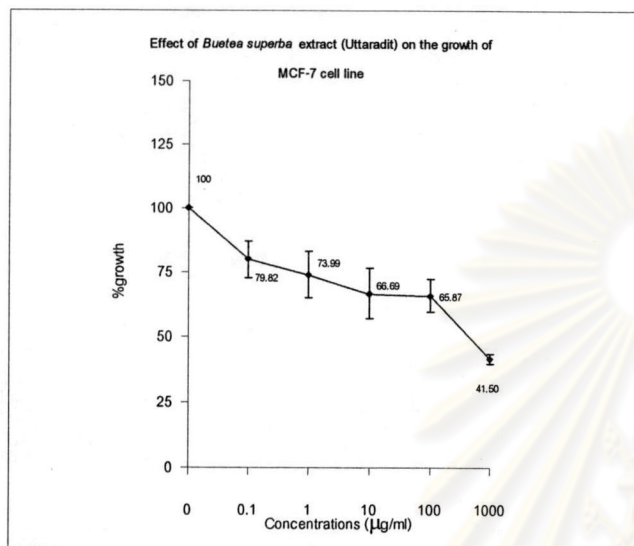


Figure 10 (Continued) Effect of *B. superba* extract of the growth on MCF-7 cell culture

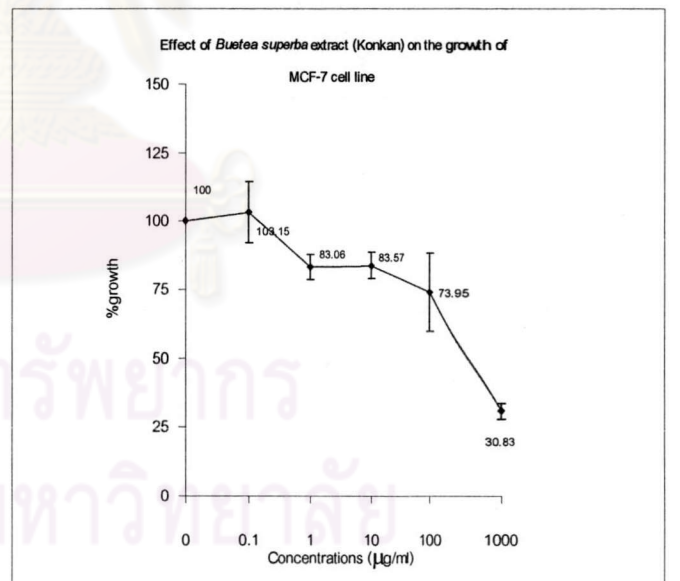
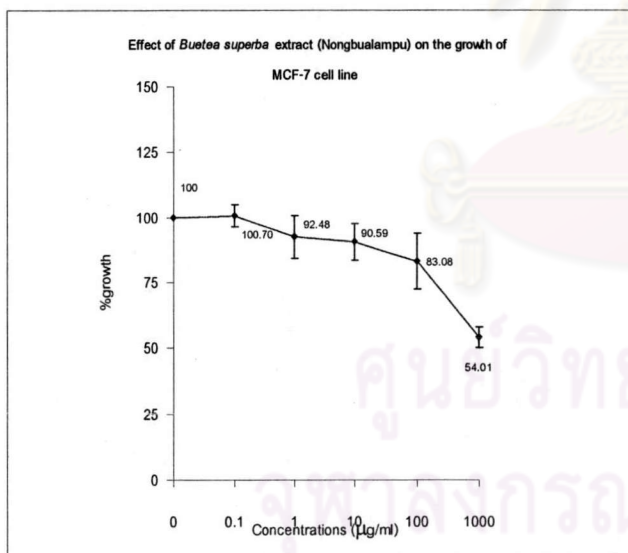
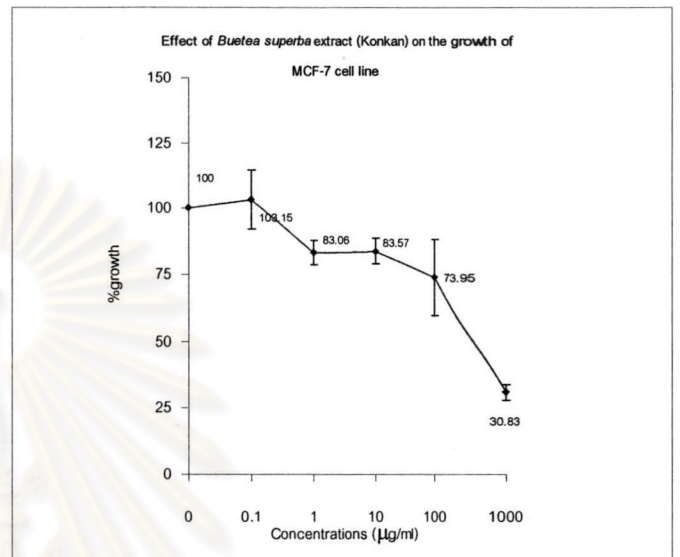
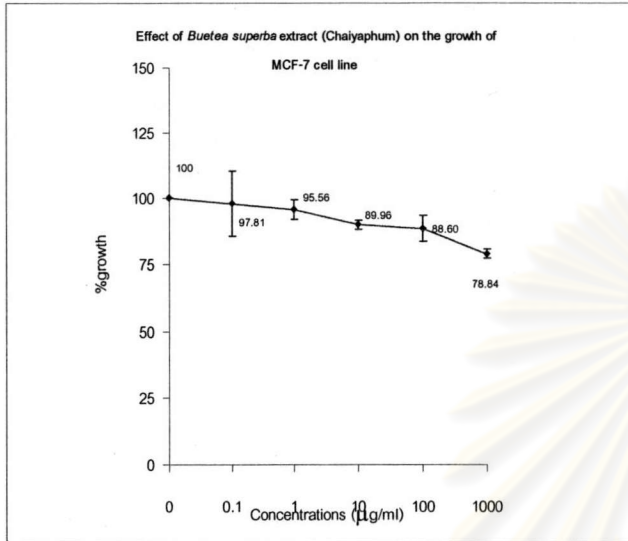


Figure 10 (Continued) Effect of *B. superba* extract on the growth of MCF-7 cell culture

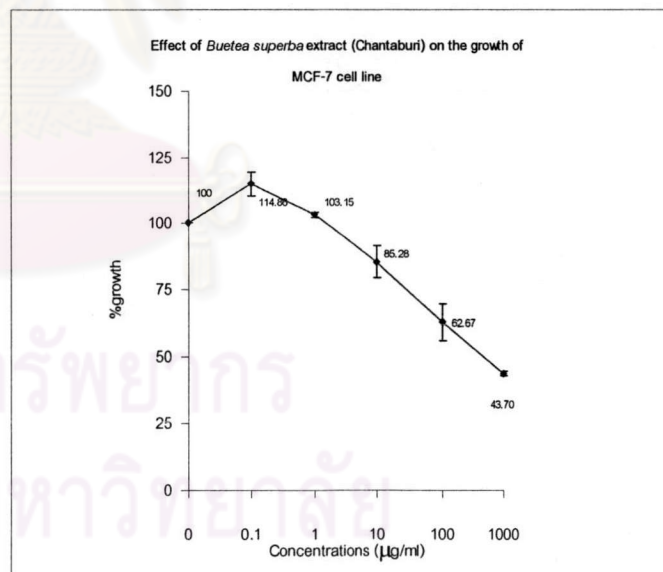
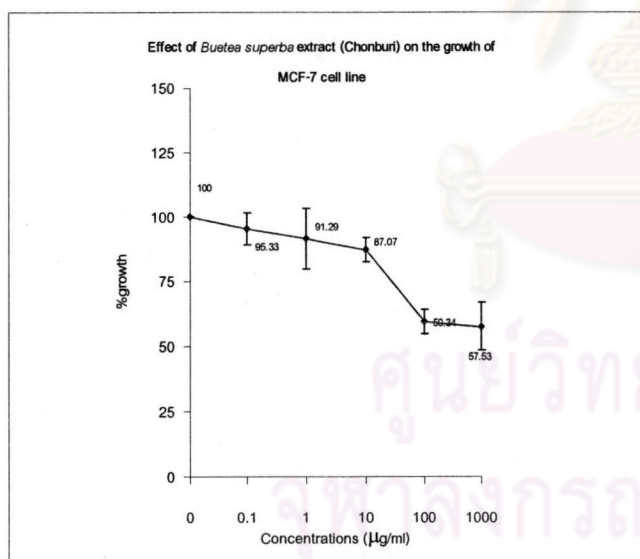
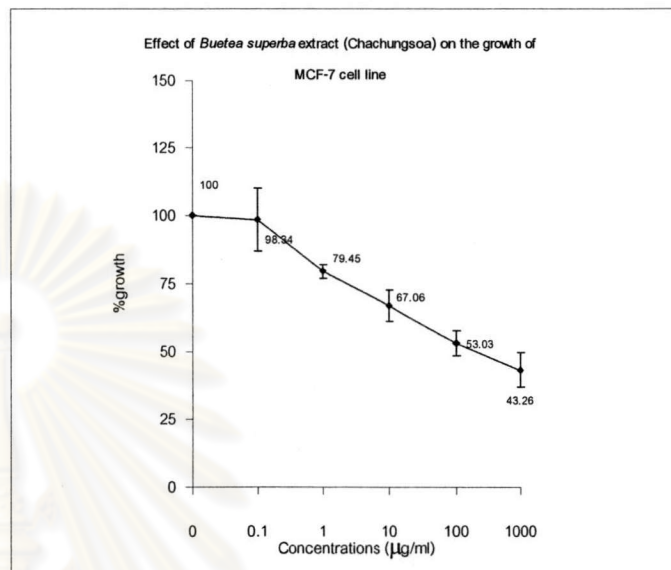
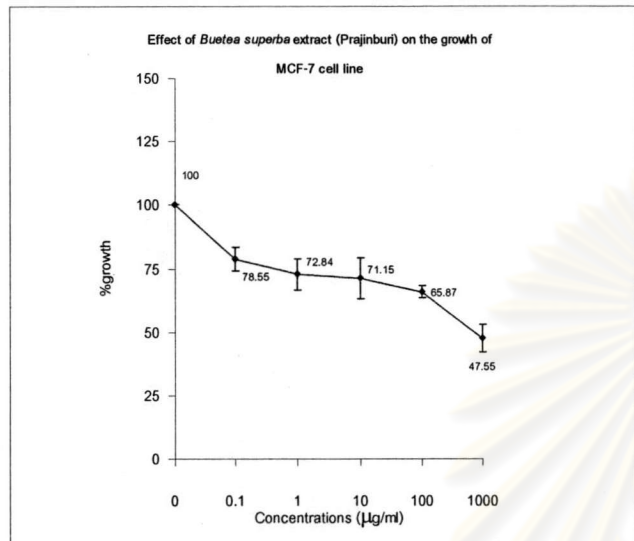


Figure 10 (Continued) Effect of *B. superba* extract of the growth on MCF-7 cell culture

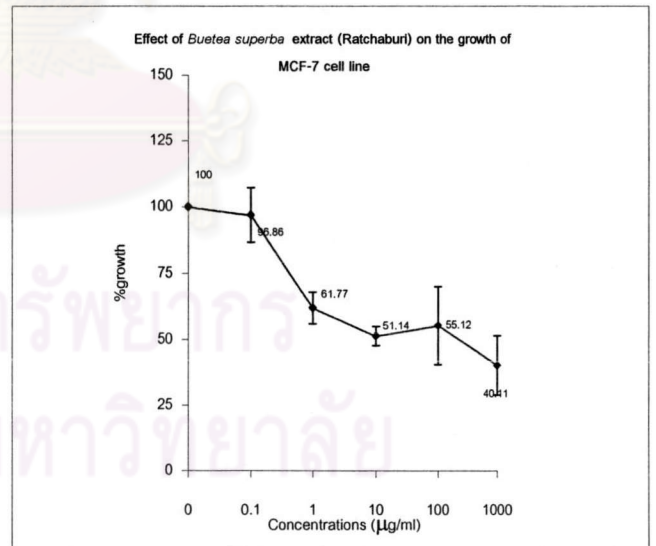
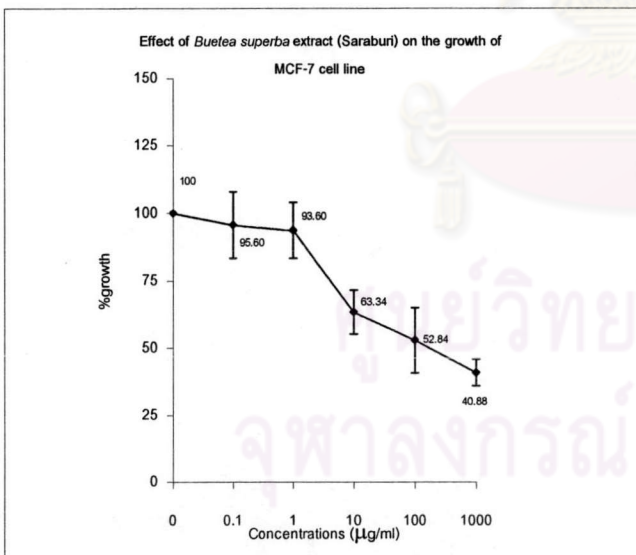
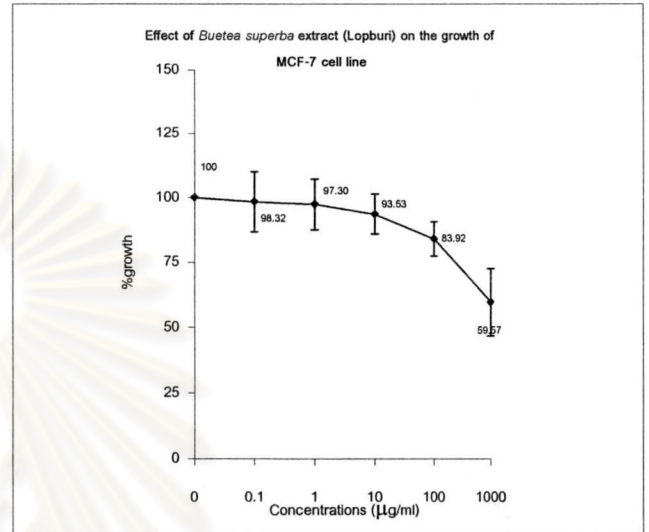
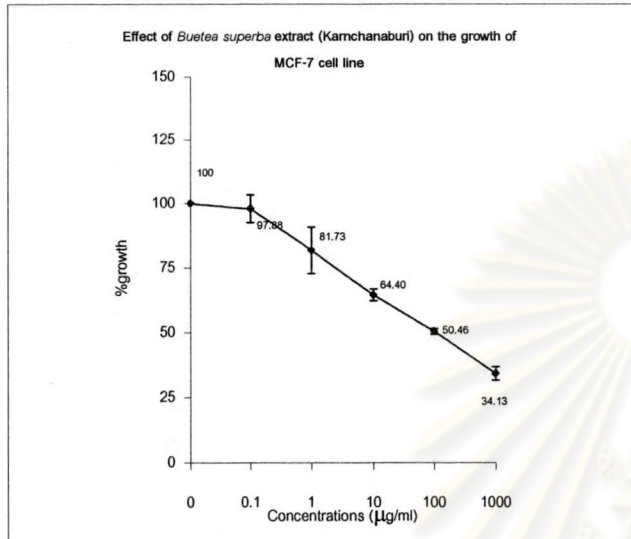


Figure 10 Effect of *B. superba* extract of the growth on MCF-7 cell culture

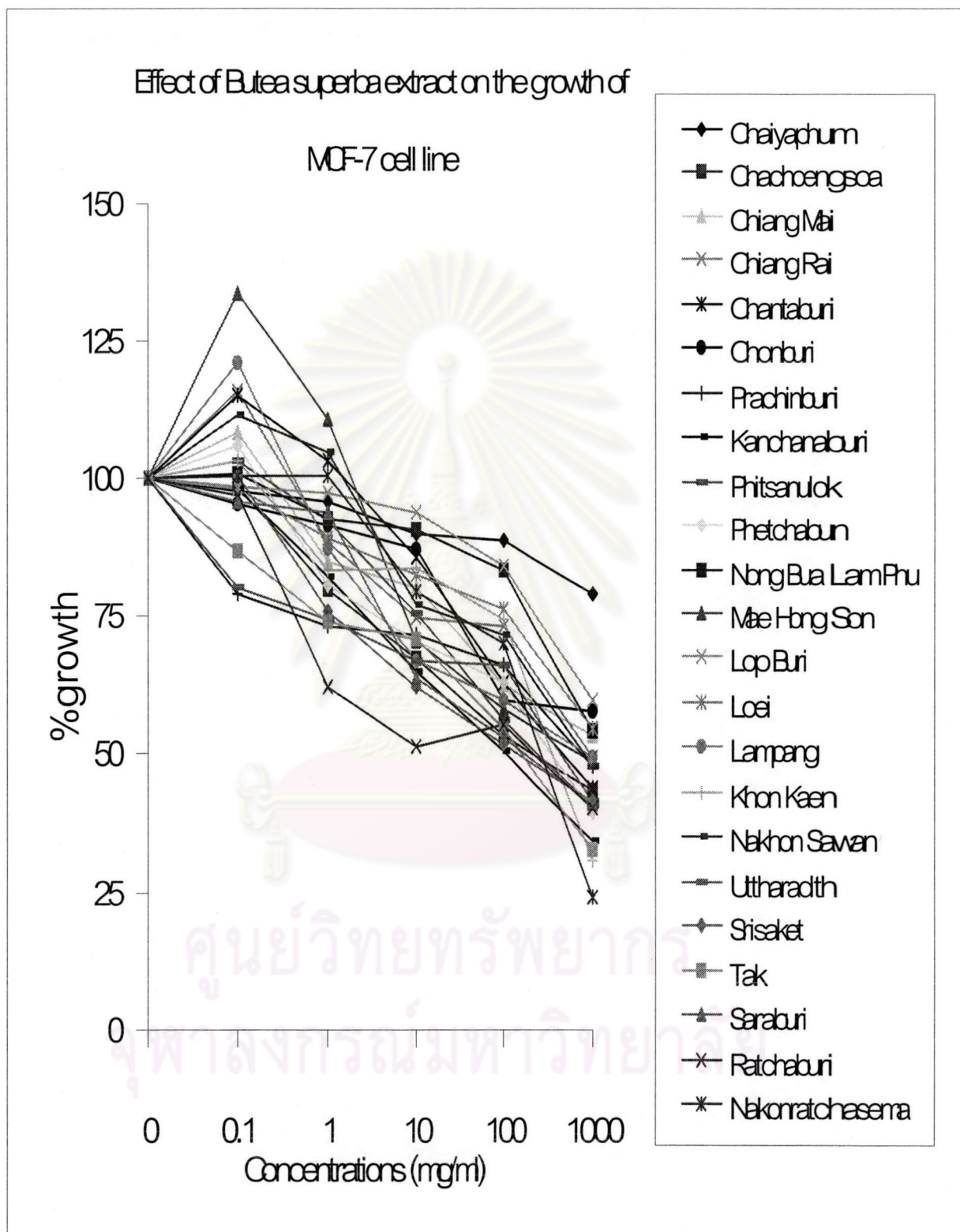


Figure 10 Effect of *B. superba* extract of the growth on MCF-7 cell culture

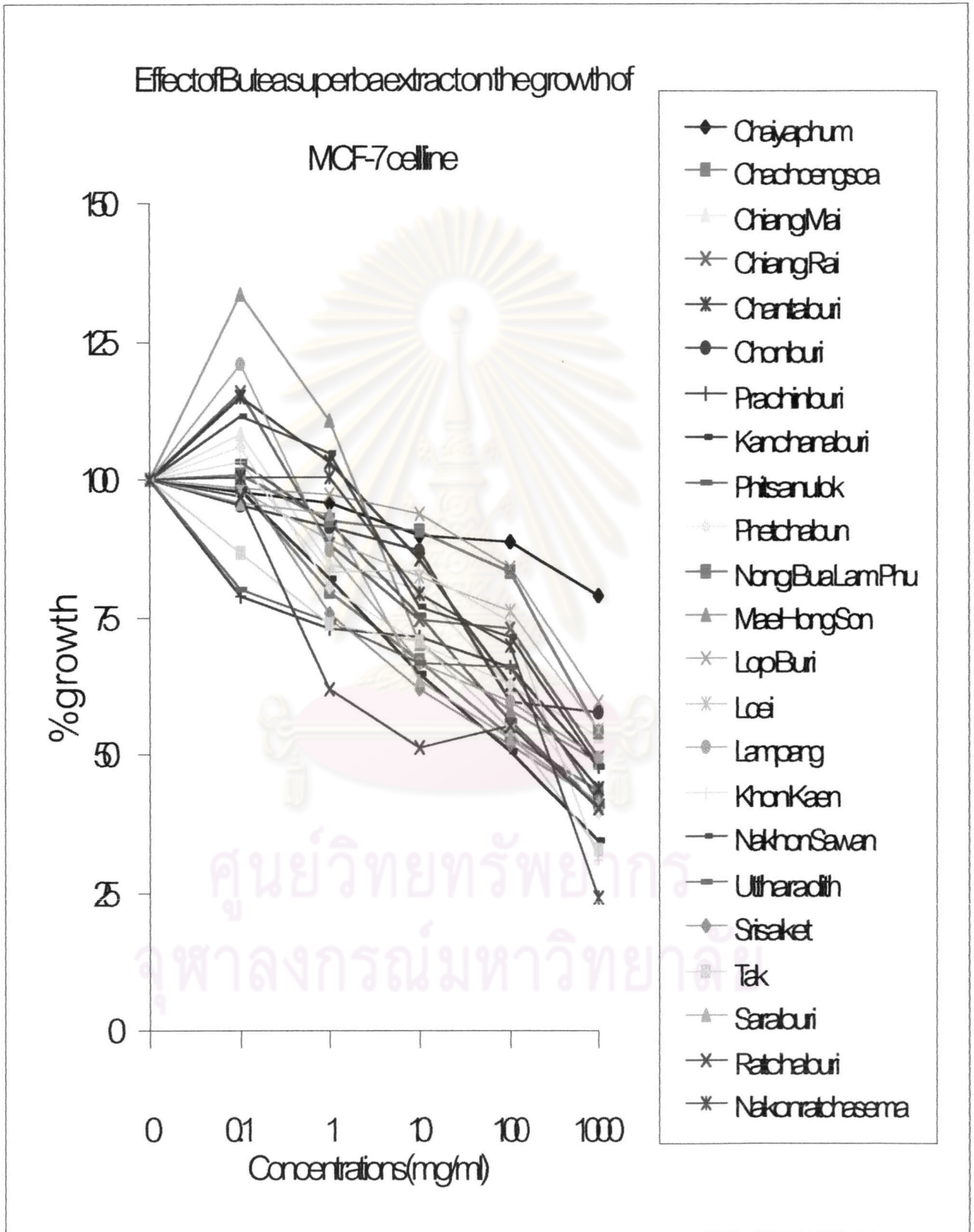


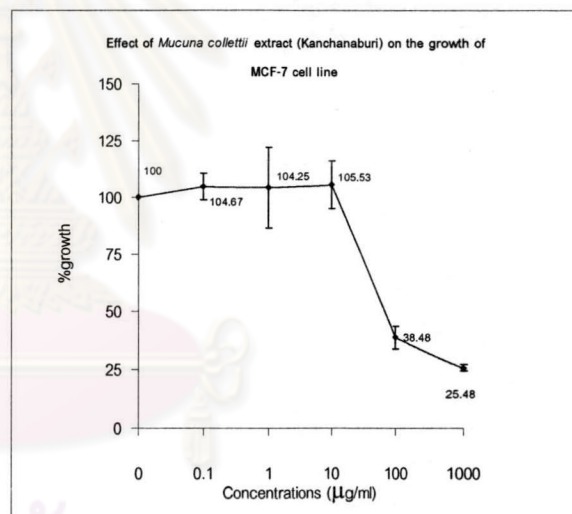
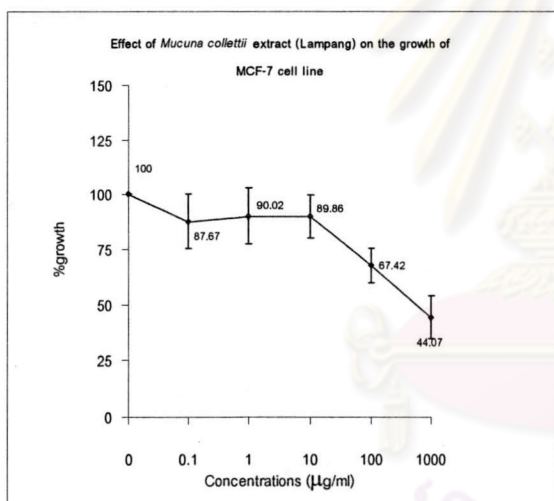
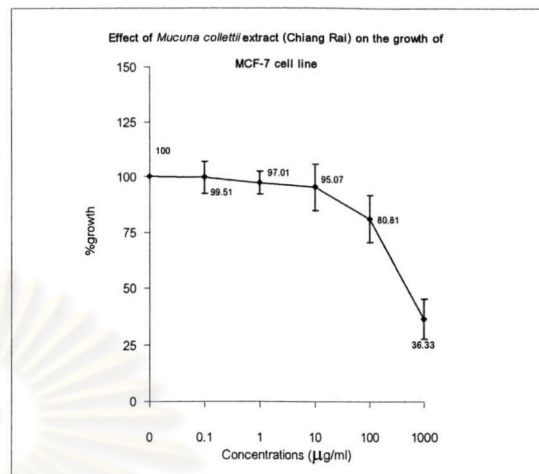
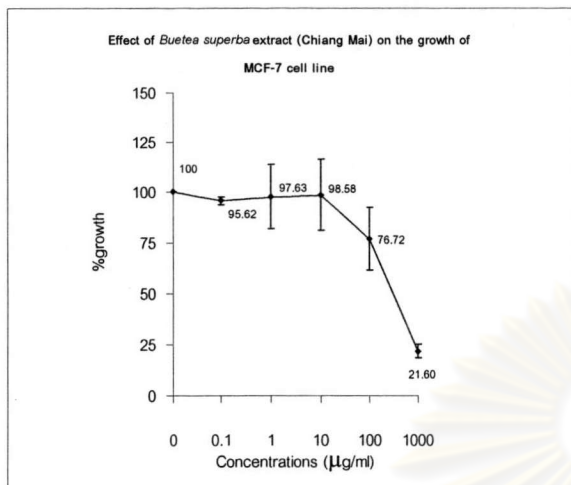
Table 22 The growth response percentage of *M. collettii* extract on MCF-7 cell culture.

Provinces	0.1 µg/ml	1 µg/ml	10 µg/ml	100 µg/ml	1000 µg/ml	IC ₅₀
Control±SE	100±2.27	100±2.27	100±2.27	100±2.27	100±2.27	-
Kanchanaburi	104.67±5.87	104.25±17.76	105.53±10.57	38.48±4.86*	25.48±1.29*	114.90
Chiang Mai	95.62±2.06	97.63±15.95	98.58±17.58	76.72±15.65	21.60±3.36	254.24
Chiang Rai	99.51±7.14	97.01±5.11	95.07±10.64	80.81±10.61	36.33±8.81	522.11
Lampang	87.67±12.32	90.02±12.81	89.86±9.74	50.30±2.46*	44.07±9.70	555.38
Means±SE	96.87±3.80	97.23±6.13	97.26±5.26	61.57±6.80	31.87±3.94	361.66

*Significant at $p < 0.05$, $n=3$ (compare with mean of population)



Figure 11 Effect of *M. collettii* extract of the growth on MCF-7 cell culture



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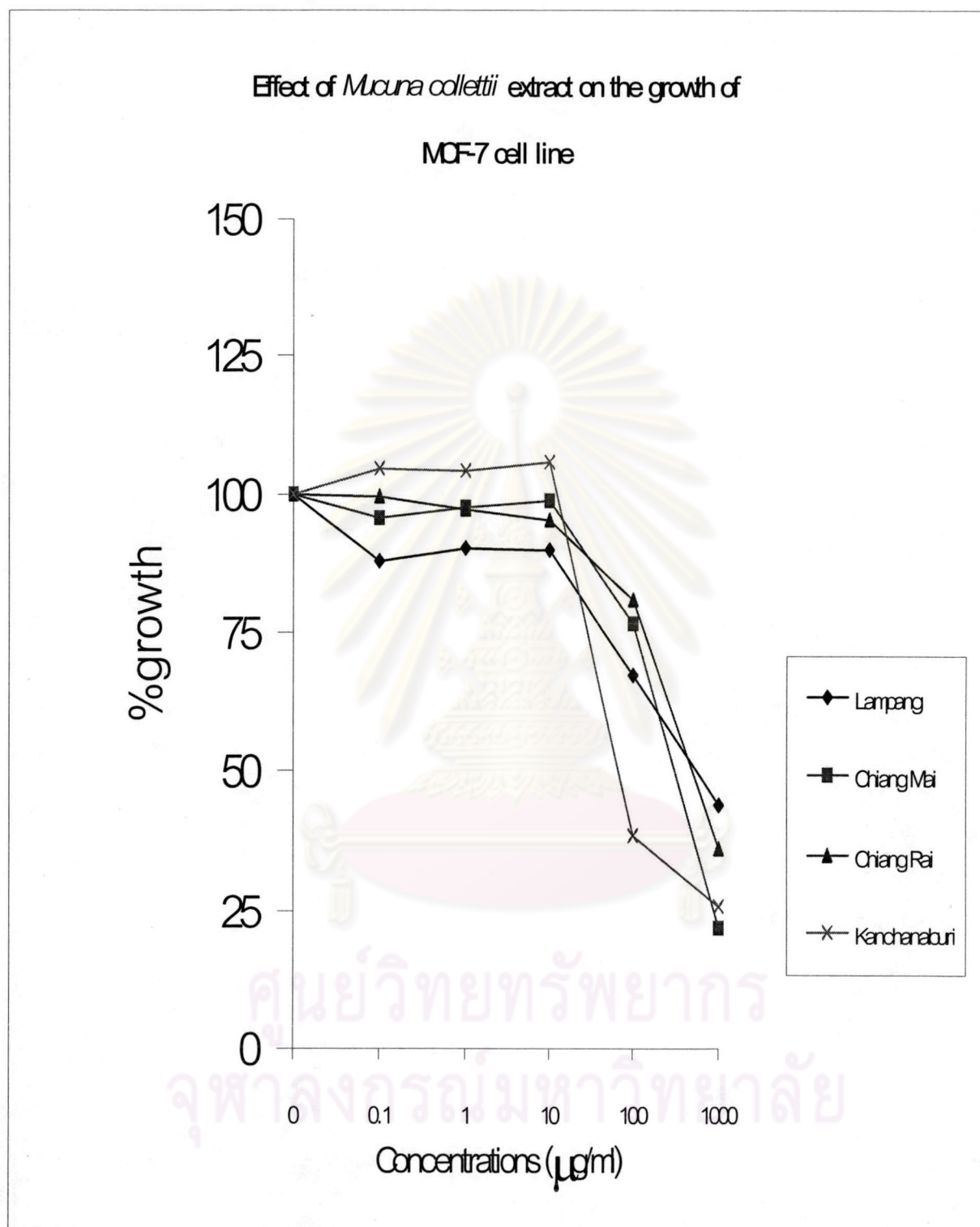
Figure 11 Effect of *M. collettii* extract of the growth on MCF-7 cell culture

Figure 11 Effect of *M. collettii* extract of the growth on MCF-7 cell culture