CHAPTER IV

RESULTS

4.1. Tuberous size

The tuberous size of PM-III and PM-IV is shown in Table 4.1.

Table 4.1 The annual average tuberous size of PM-III and PM-IV with parameters of circumference, height and width recorded of the largest size tuber.

PM	circum ference (cm)	height (cm)	width (cm)
PM-III	25.50±1.88	20.00±2.01*	9.00±0.77
PM-IV	38.13±3.22*	11.46±1.13	14.18±1.18*

* More than another clone at $P < 0.05$.

4.2 Tuberous weight

The annual tuberous wet weight, dry weight, and percentage of water content in the tubers of PM-III and PM-IV is shown in Table 4.2.

Table 4.2 The annual tuberous wet weight, dry weight, and percentage of water content in tuber (Mean ± S.E.M.).

Month	wet weight (g)		dry weight (g)		water content (%)	
	PM-III	PM-IV	PM-III	PM-IV	PM-III	PM-IV
March	2766.67 ± 721.88^{a}	2300.00 ± 550.76^{a}	216.32±38.95^{ab}	206.61 ± 51.81^{abc}	91.88 ± 0.77^{a}	91.00 ± 0.46^{ab}
April	3249.33 ± 423.13^{a}	5427.67 ± 1259.76^{b}	367.33±41.70^{ab}	488.00 ± 88.30^{d}	88.61 ± 0.76^{a}	90. 77 ± 0.53^{ab}
May	4150.00 ± 1905.84^{a}	3001.00 ± 745.47^{a}	408.00±152.73^{b}	302.00 ± 80.36^{abc}	89.41 ± 0.99^{a}	90.01 ± 0.22^{ab}
June	1660.00 ± 754.03^{a}	1904.33 ± 544.93^{a}	161.33±73.19^{ab}	179.33 ± 47.48^{ab}	90.23 ± 0.27^{a}	90.36 ± 0.73^{ab}
July	1202.00 ± 226.77^{a}	2207.33 ± 175.09^{a}	100.33±19.63^{a}	184.00 ± 17.62^{ab}	91.62 ± 0.48^{a}	91.68 ± 0.25^{ab}
August	1641.67 ± 704.30^{a}	3910.00 ± 390.00^{ab}	169.00±92.12^{ab}	389.33 ± 37.84^{cd*}	90.68 ± 1.16^{a}	90.00 ± 0.53^{ab}
September	1100.00 ± 404.15^{a}	3966.67 ± 145.30^{ab*}	89.67±19.23^{a}	358.00 ± 44.31^{bcd*}	90.98 ± 1.18^{a}	90.97 ± 1.04^{ab}
October	978.00 ± 472.95^{a}	2100.00 ± 702.38^{a}	113.33±69.11^{a}	216.33 ± 63.39^{abc}	90.28 ± 2.15^{a}	89.45 ± 0.55^{ab}
November	2933.33 ± 2434.02^{a}	1866.67 ± 491.03^{a}	230.67±187.20^{ab}	121.00 ± 57.49^{a}	91.19 ± 1.90^{a}	92.84 ± 2.77^{b}
December	1500.67 ± 256.04^{a}	2919.67 ± 1242.62^{a}	166.33±42.78^{ab}	276.00 ± 67.49^{abc}	88.32 ± 2.92^{a}	89.03 ± 1.88^{ab}
January	1366.67 ± 218.58^{a}	1866.67 ± 617.34^{a}	147.33±30.33^{ab}	224.33 ± 74.37^{abc}	88.82 ± 2.79^{a}	87.93 ± 1.00^{a}
February	2050.00 ± 86.60^{a}	1850.00 ± 317.54^{a}	180.50±15.30^{ab}	158.5 ± 20.50^{a}	91.23 ± 0.38^{a}	91.30 ± 0.40^{ab}
Mean ± S.E.M.	2049.86±287.69	2776.67±246.40*	195.85±25.66	258.56±22.35	90.27±0.43	90.45±0.34

* More than another clone in the same month of the collection at $P < 0.05$.

Means not sharing a common superscript letter in the same column of one collected are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

4.3 Leaf morphometry

The leaf morphometry of PM-III and PM-IV is shown in Table 4.3.

Table 4.3 Leaf morphometry of PM-III and PM-IV with parameter of leaf including, petiole length (PL), petiole diameter (PD), rachis length (RL), petiolet length (PLL), terminal leaflet length (TLL), trminal feaflet breadth (TLB), stipule length (SPL), angle of first leaf border (A^B)° and number of pairs of primary veins (NPV).

Parameter	PM-III	PM-IV
PL (cm)	25.75±1.16	32.44±0.80*
PD (cm)	0.35±0.01	0.44±0.01*
RL (cm)	5.45±0.19	7.68±0.15*
PLL (cm)	1.00±0.03*	0.93±0.02
TLL (cm)	21.53±0.36	24.34±0.40*
TLB (cm)	15.99±0.37	20.59±0.41*
SPL (cm)	0.37±0.01	0.42±0.01*
A^B (°)	29.83±1.13*	23.76±0.86
NPV (cm)	6.81±0.13	7.84±0.12*

* More than another clone at $P < 0.05$.

4.4 Pod morphometry

Pod morphometry of PM-III and PM-IV is shown in table 4.4.

Table 4.4 Pod morphometry of PM-III and PM-IV with a parameter of length, width, and number of seed per pod.

PM	length(cm)	width(cm)	seed/pod
PM-III	6.10±0.11*	0.88±0.01*	5.90±0.15*
PM-IV	4.51±0.13	0.83±0.02	3.18±0.15

* More than another clone at $P < 0.05$.

4.5. The plant crude extract weight

The plant crude extract weights are shown in table 4.5.

Table 4.5 The plant crude extract weights (g) of monthly collected *P.mirifica* since March 2005 to February 2006 derived from ethanolic extraction of 50 g powder.

month	weight (g)	
	PM-III	PM-IV
March	1.92	2.67
April	2.25	4.01
May	6.69	5.83
June	3.32	3.47
July	2.08	2.2
August	3.14	2.63
September	2.04	2.51
October	2.45	1.74
November	1.98	2.16
December	1.81	2.31
January	2.74	1.61
February	1.52	1.89
Mean±S.E.M.	2.66±0.40	2.75±0.34

The characteristic of the plants extracts are sticky wax-like material with brown color. Smell like ground peanut.

4.6. Physical factors

The two physical factors, amount of rainfall and air temperature in Ratchaburi province were monthly recorded by the Meteorological Department, Ministry of Information and Communication Technology, Thailand. In this study the period of study was divided according to the amount of rainfall and air temperature into 3 seasons of summer, (from February 2005 to April 2005), rainy season, (from May 2005 to October 2005) and winter (from November 2005 to January 2006).

Table 4.6 The monthly climate record including, maximum, minimum and mean temperature and amount of rainfall of Ratchaburi province since March 2005 to February 2006.

month	Temperature (°C)				rainfall (mm)
	maximum	minimum	mean	ΔT	
March	34.7	23.3	29.0	11.4	9.6
April	37.0	25.5	31.3	11.5	0.9
May	36.3	25.7	31.0	10.6	87.2
June	34.8	25.4	30.1	9.4	104.9
July	32.9	24.8	28.9	8.1	140.8
August	33.1	24.5	28.8	8.6	114.8
September	32.8	24.6	28.7	8.2	145.8
October	31.4	24.2	27.8	7.2	441.5
November	31.3	23.5	27.4	7.8	39.1
December	29.1	21.1	25.1	8	58.7
January	32.9	19.9	26.4	13	0.0
February	34.0	22.8	28.4	11.2	17.9

Data from Meteorological Department, Ministry of Information and Communication Technology.

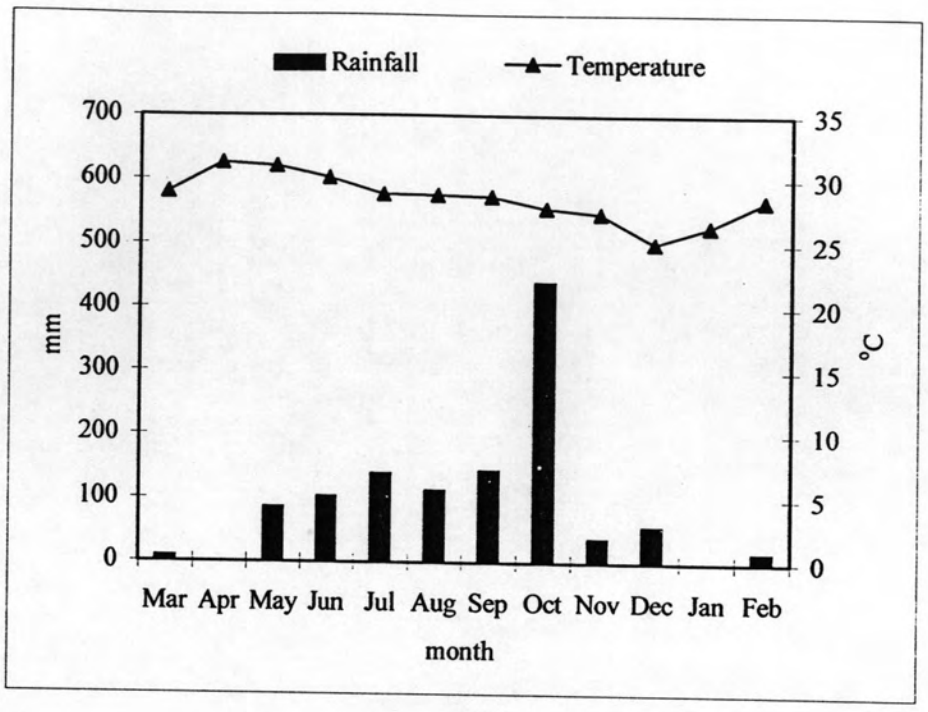

Figure 4.1 The mean temperature, and amount of rainfall in Ratchaburi province since March 2005 to February 2006 recorded by the Meteorological Department, Ministry of Information and Communication Technology, Thailand.

4.7. HPLC isoflavonoid analysis of monthly collected *P. mirifica*

HPLC analysis of the isoflavonoid contents from monthly collected PM-III since March 2005 to February 2006 exhibited a significant variation of the chemicals (Table 4.7). The maximum amount of puerarin was present in July 2005, daidzin in February 2006, genistin in June 2005, daidzein and genistein in March 2005 and total isoflavonoid in July 2005.

Table 4.7 Isoflavonoid contents in mg/100 g powder of monthly collected PM-III from Ratchaburi since March 2005 to February 2006 (Mean± S.E.M.).

month	puerarin	daidzin	genistin	daidzein	genistein	Total isoflavonoid
March	31.28±2.11^{a*}	30.36±1.86^{bcd*}	9.34±0.44^{a*}	24.65±0.78^{f}	3.85±0.31^{f}	99.48±3.99^{ab*}
April	66.74±5.05^{abc*}	42.90±0.61^{bcd*}	17.18±4.85^{bc}	9.93±0.30^{cd*}	0.49±0.15^{ab*}	137.24±10.67^{bc*}
May	73.21±8.36ab^{cdef*}	43.63±9.45^{cd*}	11.33±0.68^{a*}	9.54±0.97^{cd*}	0.12±0.04^{a*}	137.83±19.13^{bcd*}
June	98.21±2.74^{f*}	42.28±3.71^{d*}	20.68±2.06^{bc}	7.04±0.85^{bc*}	0.67±0.19^{ab*}	168.89±1.00^{de}
July	129.67±14.89^{g}	23.29±5.00^{ab*}	19.88±2.58^{bc}	11.4±1.62^{d*}	1.64±0.14^{cd*}	185.88±16.74^{e}
August	56.07±5.49^{abcd*}	35.28±0.91^{bcd*}	10.75±1.13^{a*}	4.73±0.56^{ab*}	0.68±0.07^{b*}	107.51±7.32^{b*}
September	94.34±2.38^{f*}	42.09±4.20^{cd*}	13.12±1.99^{ab*}	10.18±0.69^{cd*}	0.54±0.04^{ab*}	160.27±5.61^{cde}
October	95.02±8.45^{f*}	40.33±3.96^{bcd*}	12.72±2.06^{ab*}	3.66±0.32^{a*}	2.02±0.07^{de*}	153.76±14.38^{cde}
November	85.44±19.92^{ef*}	26.00±2.81^{abc*}	14.84±1.08^{ab*}	7.51±0.80^{bc*}	1.39±0.13^{c*}	135.17±21.35^{bcd*}
December	78.09±10.66^{def*}	35.95±5.83^{bcd*}	22.12±2.00^{c}	16.86±2.16^{e*}	2.22±0.32^{e*}	155.24±16.46^{cde}
January	38.62±4.32^{ab*}	11.62±2.25^{a*}	7.78±1.41^{a*}	3.48±0.54^{a*}	0.25±0.04^{ab*}	61.75±8.44^{a*}
February	60.46±1.92^{bcde*}	78.01±11.25^{e}	18.49±2.68^{bc}	12.81±1.78^{d*}	0.72±0.09^{b*}	170.49±13.89^{de}
Mean ± S.E.M.	73.83±5.06	37.74±2.90	14.95±0.94	10.25±1.00	1.21±0.18	137.98±6.56

* Less than the maximum amount at $P < 0.05$.

Means not sharing a common superscript letter in the same column of one collected sample are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

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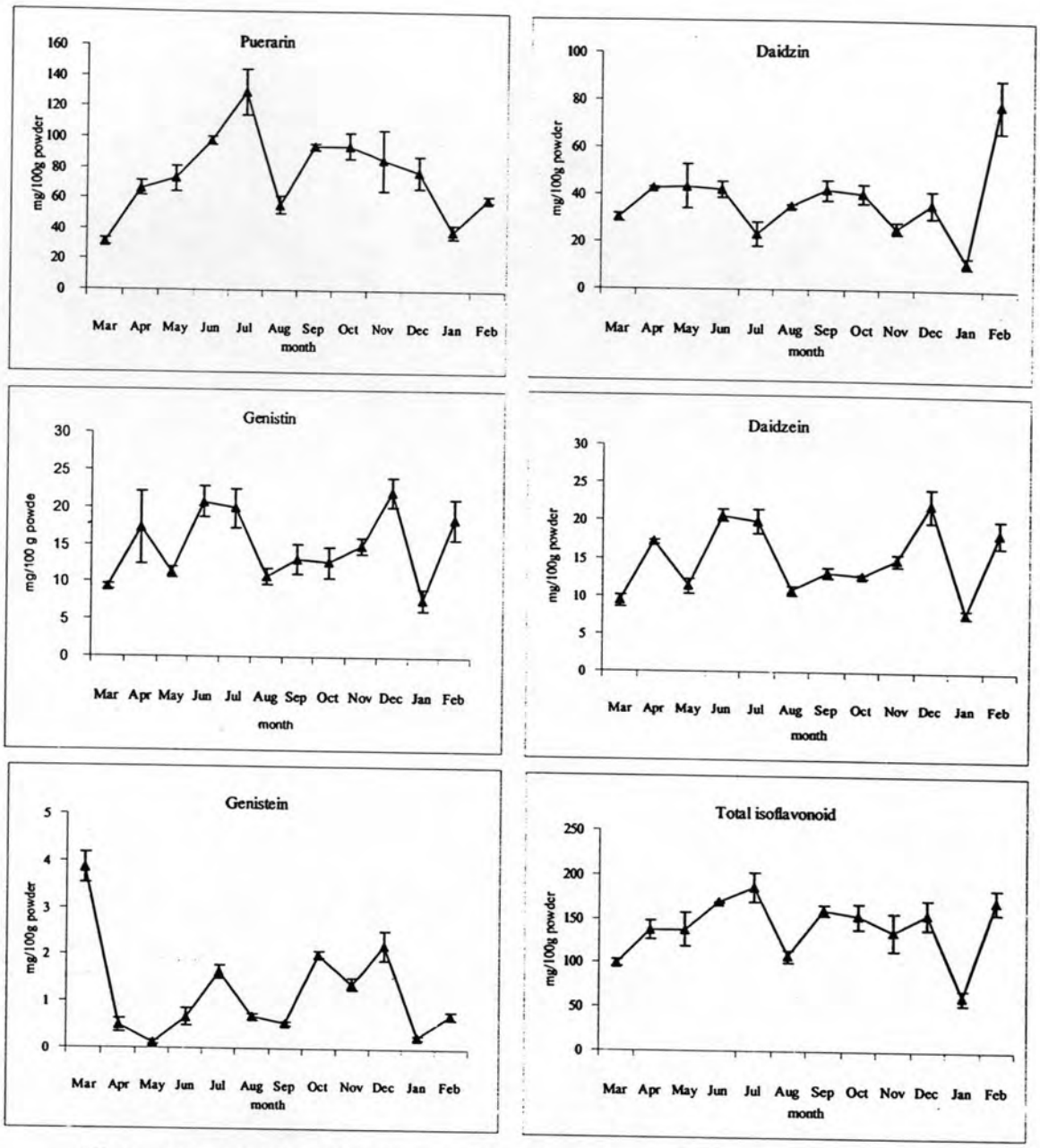


Figure 4.2 Puerarin, daidzin, genistin, daidzein, genistein and total isoflavonoid contents (mg/100g powder) in the monthly collected tubers of *P. mirifica* (PM-III) from Ratchaburi since March 2005 to February 2006.

HPLC analysis of the isoflavonoid contents from monthly collected PM-IV since March 2005 to February 2006 exhibited a significant variation of the chemicals (Table 4.8). The maximum amount of puerarin was present in March 2005, daidzin in February 2006, genistin and daidzein in March 2005, genistein in October 2006 and total isoflavonoid in March 2005.

Table 4.8 Isoflavonoid contents in mg/100 g powder of monthly collected PM-IV from Ratchaburi since March 2005 to February 2006 (Mean±S.E.M.).

month	puerarin	daidzin	genistin	daidzein	genistein	total isoflavonoid
March	187.07 ± 1.82^{e}	48.20 ± 12.54^{bc}	21.27 ± 2.75^{e}	14.26 ± 0.32^{c}	$0\pm0^{a*}$	270.79 ± 13.14^{e}
April	$55.12\pm3.45^{a*}$	$27.22\pm3.84^{a*}$	$5.36\pm1.35^{a*}$	$4.38\pm0.67^{ab*}$	$0.71\pm0.22^{bc*}$	$92.80\pm8.25^{a*}$
May	$94.88\pm31.73^{abc*}$	$25.10\pm9.37^{a*}$	$4.03\pm1.83^{a*}$	$5.45\pm1.70^{ab*}$	$0.17\pm0.09^{a*}$	$129.63\pm44.01^{abc*}$
June	$104.96\pm8.93^{abcd*}$	$33.33\pm8.60^{ab*}$	$12.43\pm3.55^{cd*}$	$6.69\pm1.17^{ab*}$	$0\pm0^{a*}$	$157.40\pm21.55^{abcd*}$
July	$64.43\pm1.34^{a*}$	$30.18\pm6.27^{ab*}$	17.83 ± 0.21^{de}	$7.59\pm0.21^{b*}$	$0.50\pm0.01^{ab*}$	$121.82\pm4.89^{ab*}$
August	$91.30\pm11.37^{abc*}$	$27.26\pm5.76^{a*}$	$7.58\pm1.06^{abc*}$	$3.69\pm0.44^{a*}$	$0.41\pm0.09^{abc*}$	$130.24\pm15.33^{abc*}$
September	$89.62\pm20.36^{abc*}$	$29.78\pm3.21^{ab*}$	$5.80\pm0.47^{ab*}$	$4.19\pm1.54^{a*}$	$0.49\pm0.04^{ab*}$	$129.89\pm24.83^{abc*}$
October	$139.37\pm14.44^{cd*}$	$35.93\pm3.67^{ab*}$	$7.97\pm0.79^{abc*}$	$4.58\pm0.37^{ab*}$	$1.79\pm0.06^{de*}$	$189.64\pm19.33^{bcd*}$
November	$92.63\pm14.98^{abc*}$	$24.05\pm2.83^{a*}$	12.34 ± 1.42^{cd}	$6.10\pm0.57^{ab*}$	$1.57\pm0.03^{d*}$	$136.69\pm17.2^{abcd*}$
December	173.66 ± 26.41^{de}	$30.66\pm3.92^{ab*}$	$14.73\pm2.84^{bcd*}$	$7.09\pm1.61^{ab*}$	2.07 ± 0.41^{e}	$228.22\pm31.49^{d*}$
January	$78.00\pm11.03^{ab*}$	$15.20\pm2.98^{a*}$	$7.89\pm1.88^{abc*}$	$4.09\pm0.82^{a*}$	$0.47\pm0.04^{ab*}$	$105.65\pm16.59^{a*}$
February	$119.61\pm1.43^{bcd*}$	56.87 ± 2.18^{c}	$11.91\pm0.95^{bcd*}$	$7.55\pm0.27^{b*}$	$1.03\pm0.51^{c*}$	$196.96\pm4.33^{cd*}$
Mean±S.E.M.	105.63±7.16	32.12±2.33	10.54±0.96	6.22±0.52	0.77±0.12	155.35±9.67

* Less than the maximum amount at $P < 0.05$.

Means not sharing a common superscript letter in the same column of one collected sample are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

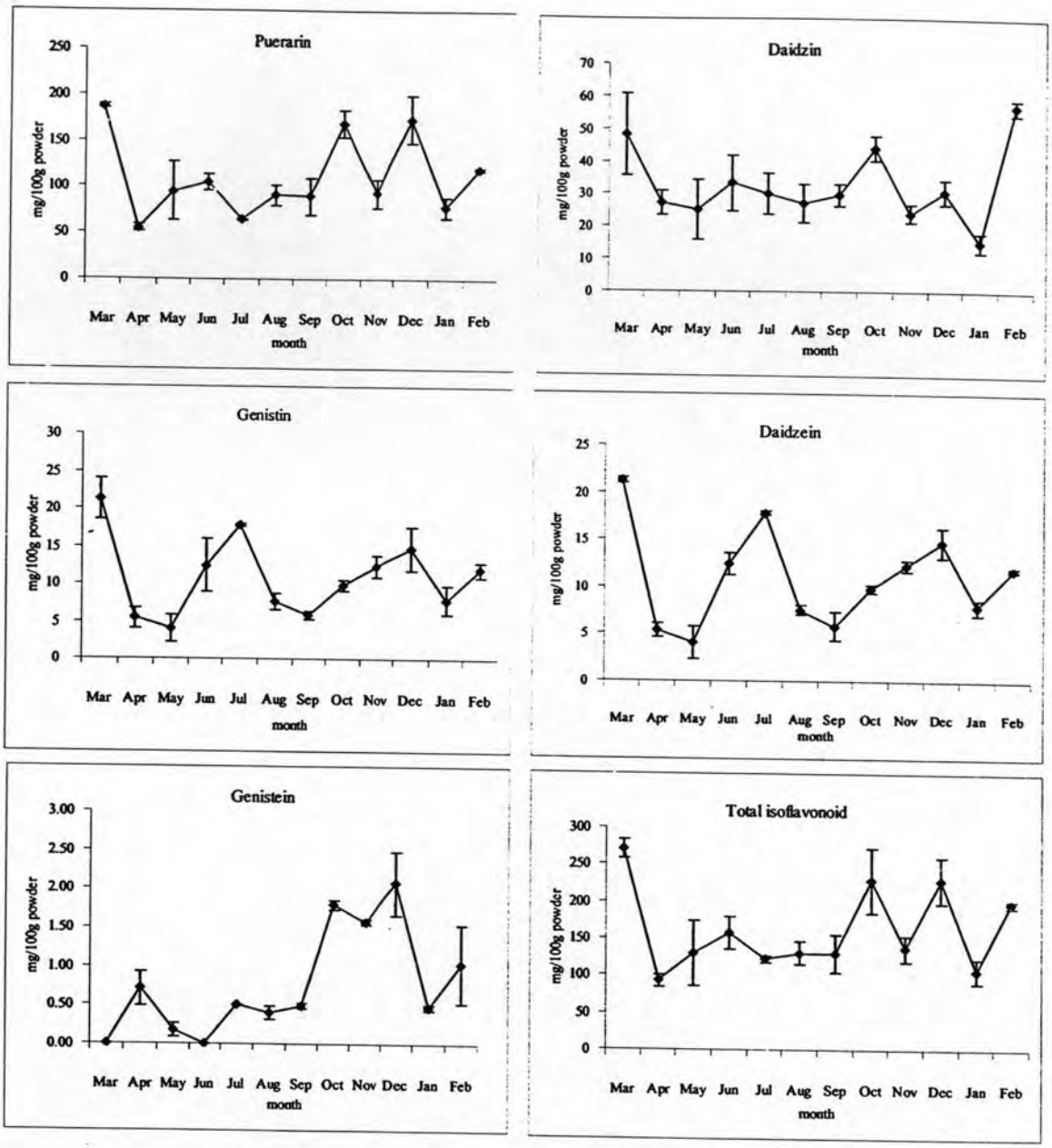


Figure 4.3 Puerarin, daidzin, genistin, daidzein, genistein and total isoflavonoid contents (mg/100g powder) in the tubers of monthly collected *P. mirifica* (PM-IV) from Ratchaburi since March 2005 to February 2006.

The isoflavonoid contents in the monthly collected PM-III since March 2005 to February 2006 exhibited a significant variation with the maximum amount of aglycoside in March 2005, glycoside in February 2006 and ratio of aglycoside and glycoside in March 2005. PM-IV showed a significant variation with the present of the maximum amount of aglycoside, glycoside and ratio of aglycoside and glycoside in March 2005.

Table 4.9 The isoflavonoid contents of PM-III compared with PM-IV in the same month of the collection.

month	puerarin		daidzin		genistin		daidzein		genistein		total isoflavonoid	
	PM-III	PM-IV	PM-III	PM-IV	PM-III	PM-IV	PM-III	PM-IV	PM-III	PM-IV	PM-III	PM-IV
March						PM-IV						
April	-	-	-	-		PM-III			-	-	-	-
May	-	-			PM-III				-	-	-	-
June	-	-	-	-	PM-III		-	-	PM-III		-	-
July	PM-III		-	-	-	-			PM-III			
August	PM-IV		-	-	-	-	-	-	-	-	-	-
September	-	-	-	-		PM-III			-	-	-	-
October	PM-IV		-	-	-	-	-	-	-	-	-	-
November	-	-	-	-	-	-	-	-	-	-	-	-
December	PM-IV		-	-		PM-III			-	-	-	-
January	PM-IV		-	-	-	-	-	-	-	-	-	-
February	PM-IV				PM-III				-	-	-	-
Mean ± S.E.M.	-	-	-	-	-	-	PM-III		-	-	-	-

The name of the clone present in table exhibits more content at $P < 0.05$.

Table 4.10 Isoflavonoid contents in glycoside form (daidzin and genistin) and aglycoside form (daidzein and genistein) in mg/100 g powder of monthly collected *P.mirifica* tubers from Ratchaburi since March 2005 to February 2006.

month	Aglycoside (mg/100 g)		Glycoside (mg/100 g)		Aglycoside/glycoside	
	PM-III	PM-IV	PM-III	PM-IV	PM-III	PM-IV
March	$28.50\pm0.47^{g\dagger}$	14.26 ± 0.32^{c}	$39.70\pm2.29^{b*}$	$69.46\pm15.29^{d\dagger}$	$0.72\pm0.03^{f\dagger}$	0.24 ± 0.06^{c}
April	$10.42\pm0.16^{de*\dagger}$	$5.10\pm0.51^{a*}$	$60.08\pm5.46^{bc*\dagger}$	$32.58\pm5.21^{ab*}$	$0.17\pm0.01^{cd*}$	0.16 ± 0.01^{ab}
May	$9.65\pm0.94^{d*\dagger}$	$5.62\pm1.78^{a*}$	$54.97\pm10.00^{bc*\dagger}$	$29.13\pm10.8^{ab*}$	$0.18\pm0.02^{bcd*}$	0.19 ± 0.01^{b}
June	$7.71\pm0.94^{bcd*}$	$6.69\pm1.17^{abc*}$	$62.96\pm4.16^{c*}$	$45.76\pm12.12^{ab*}$	$0.13\pm0.02^{ab*}$	0.15 ± 0.02^{ab}
July	$13.04\pm1.70^{e*\dagger}$	$9.39\pm1.31^{cd*}$	$43.17\pm6.17^{b*}$	$48.00\pm8.24^{abc*}$	$0.30\pm0.03^{e*\dagger}$	0.22 ± 0.05^{bc}
August	$5.41\pm0.56b^{ab*}$	$4.09\pm0.37^{a*}$	$46.03\pm2.01^{bc*}$	$34.85\pm6.77^{a*}$	$0.12\pm0.01^{a*}$	$0.12\pm0.02^{a*}$
September	$10.72\pm0.70^{de*\dagger}$	$4.68\pm1.52^{a*}$	$55.21\pm5.56^{bc*}$	$35.59\pm3.55^{ab*}$	$0.19\pm0.02^{cd*\dagger}$	$0.13\pm0.03^{ab*}$
October	$5.68\pm0.29^{abc*}$	$6.37\pm0.43^{ab*}$	$53.05\pm5.89^{bc*}$	$43.90\pm4.46^{ab*}$	$0.11\pm0.01^{a*}$	$0.12\pm0.02^{a*}$
November	$8.90\pm0.79^{cd*}$	$7.67\pm0.59^{a*}$	$40.83\pm2.85^{b*}$	$36.38\pm1.67^{ab*}$	$0.22\pm0.02^{d*}$	0.21 ± 0.01^{bc}
December	$19.08\pm1.84^{f*\dagger}$	$9.16\pm1.91^{abc*}$	$58.07\pm7.03^{bc*}$	$45.39\pm4.51^{ab*}$	$0.33\pm0.03^{e*\dagger}$	0.20 ± 0.03^{bc}
January	$3.74\pm0.57^{a*}$	$4.56\pm0.84^{a*}$	$19.40\pm3.62^{a*}$	$23.10\pm4.85^{a*}$	$0.19\pm0.01^{cd*}$	0.20 ± 0.03^{bc}
February	$13.54\pm1.88^{e*\dagger}$	$8.57\pm0.24^{bc*}$	$96.50\pm13.93^{d\dagger}$	68.78 ± 3.14^{bc}	$0.14\pm0.00^{abc*}$	$0.12\pm0.01^{a*}$
Mean±S.E.M.	11.46±1.13	7.07±0.52	52.69±3.37	42.66±2.97	0.24±0.03	$0.19\pm0.01^{\dagger}$

* Less than the maximum at $P < 0.05$.

† More than another clone at $P < 0.05$.

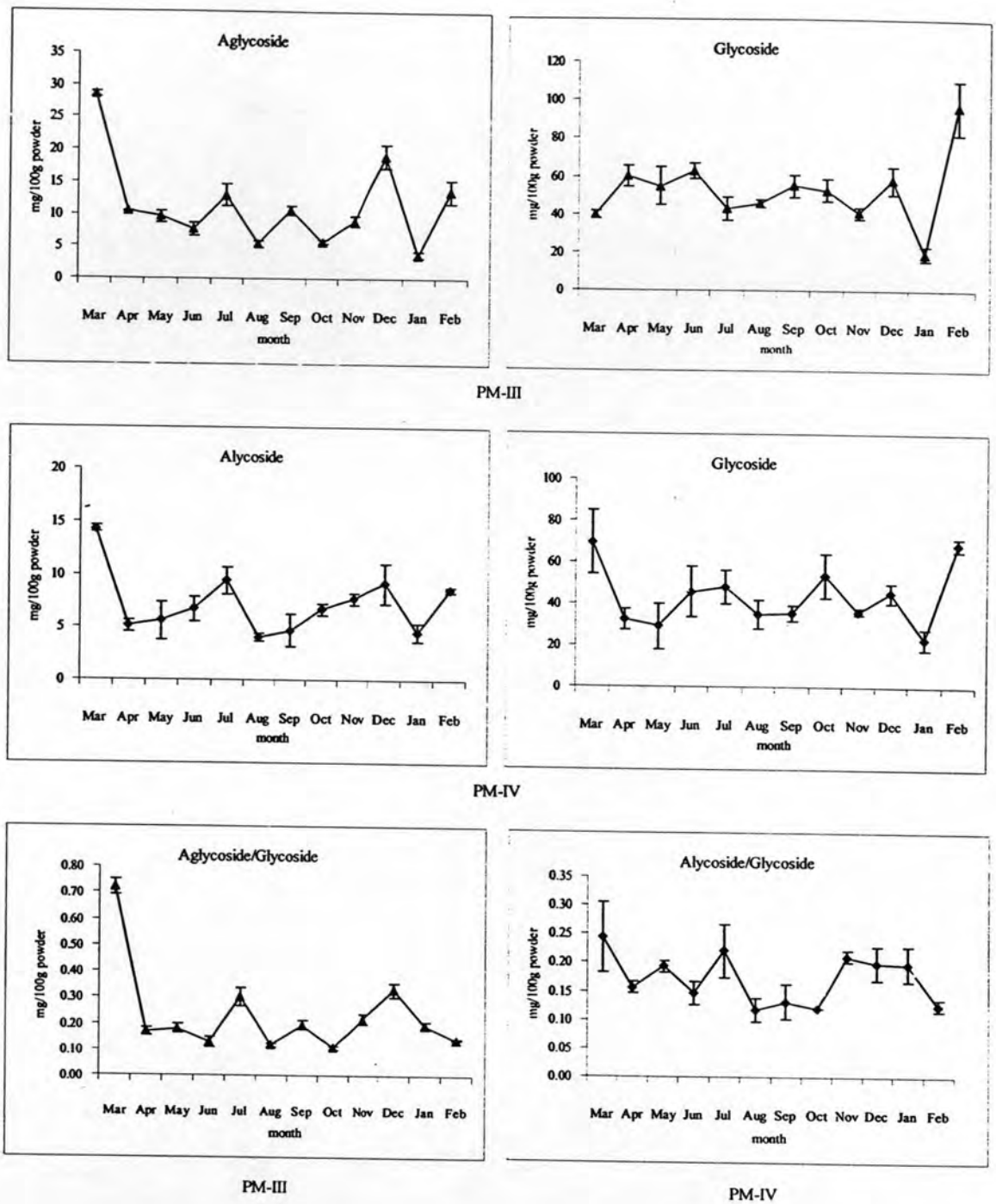


Figure 4.4 Aglycoside and glycoside contents, and ratio of aglycoside/glycoside in the monthly collected *P. mirifica* from Ratchaburi since March 2005 to February 2006.

The ratio of individual isoflavonoid against puerarin in the monthly collected PM-III and PM-IV since March 2005 to February 2006 showed a significant variation with the maximum of the ratio of daidzin/puerarin in February 2006, genistin/puerarin, daidzein/puerarin and genistein/puerarin in March 2005.

Table 4.11 The ratio of daidzein vs. daidzin and genistein vs. genistin in PM-III tubers monthly collected from Ratchaburi since March 2005 to February 2006.

month	daidzein/daidzin		genistein/genistin	
	PM-III	PM-IV	PM-III	PM-IV
March	$0.81\pm0.02^{f\dagger}$	0.35 ± 0.11^{d}	$0.41\pm0.05^{e\dagger}$	$0.00\pm0.00^{a*}$
April	$0.23\pm0.00^{cd*\dagger}$	$0.16\pm0.00^{abc*}$	$0.03\pm0.02^{abc*}$	$0.17\pm0.08^{de\dagger}$
May	$0.22\pm0.03^{bcd*\dagger}$	$0.23\pm0.02^{abcd*}$	$0.01\pm0.00^{a*}$	$0.04\pm0.00^{ab*}$
June	$0.17\pm0.02^{abc*}$	$0.21\pm0.04^{abc*}$	$0.03\pm0.01^{ab*}$	$0.00\pm0.00^{a*}$
July	$0.49\pm0.10^{e*}$	0.27 ± 0.05^{cd}	$0.08\pm0.01^{bc*}$	$0.03\pm0.00^{ab*}$
August	$0.13\pm0.01^{ab*\dagger}$	$0.14\pm0.03^{abc*}$	$0.06\pm0.01^{abc*}$	$0.05\pm0.01^{abc*}$
September	$0.24\pm0.01^{bcd*\dagger}$	$0.14\pm0.04^{abc*}$	$0.04\pm0.01^{abc*}$	$0.09\pm0.01^{abcd*}$
October	$0.09\pm0.00^{a*}$	$0.13\pm0.00^{a*}$	$0.16\pm0.03^{d*}$	0.23 ± 0.02^{e}
November	$0.29\pm0.05^{d*}$	0.26 ± 0.01^{abcd}	$0.09\pm0.01^{bc*}$	$0.13\pm0.02^{cd*}$
December	$0.47\pm0.03^{e*\dagger}$	$0.23\pm0.02^{abcd*}$	$0.10\pm0.02^{c*}$	$0.14\pm0.01^{cd*}$
January	$0.30\pm0.03^{d*\dagger}$	0.27 ± 0.03^{bcd}	$0.03\pm0.01^{abc*}$	$0.06\pm0.01^{abc*}$
February	$0.16\pm0.00^{abc*\dagger}$	$0.13\pm0.00^{ab*}$	$0.04\pm0.00^{abc*}$	$0.09\pm0.05^{bcd*}$
Mean±S.E.M.	$0.31\pm0.03^{\dagger}$	0.21±0.02	0.09±0.02	0.09±0.01

* Less than the maximum at $P < 0.05$

Means not sharing a common superscript letter in the same column of one collected sample are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

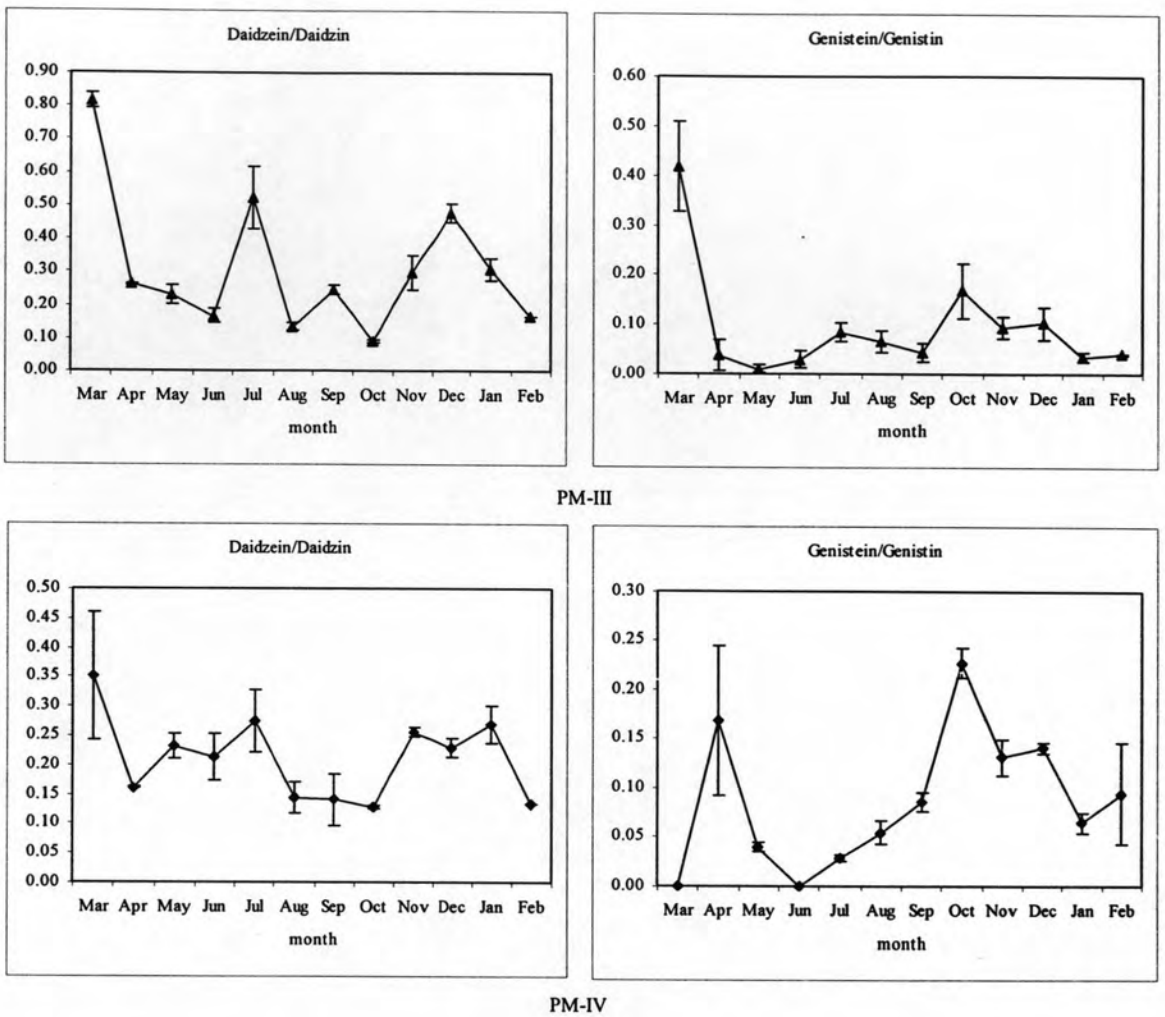


Figure 4.5 The ratio of daidzein vs. daidzin and genistein vs. genistin in monthly collected PM-III and PM-IV from Ratchaburi since March 2005 to February 2006.

Table 4.12 The ratio of individual isoflavonoid vs. puerarin in PM-III tubers monthly collected from Ratchaburi since March 2005 to February 2006.

month	daidzin/puerarin		genistin/puerarin		daidzein/puerarin		genistein/puerarin	
	PM-III	PM-IV	PM-III	PM-IV	PM-III	PM-IV	PM-III	PM-IV
March	$0.93\pm0.05^{f*}$	$0.26\pm0.07^{a*}$	0.30 ± 0.00^{bc}	$0.11\pm0.03^{ab*}$	0.68 ± 0.13^{d}	$0.08\pm0.00^{d*}$	0.20 ± 0.10^{d}	$0.00\pm0.00^{a*}$
April	$0.64\pm0.16^{ef*}$	0.49 ± 0.05^{b}	0.26 ± 0.11^{c}	$0.10\pm0.02^{ab*}$	$0.15\pm0.05^{c*}$	$0.08\pm0.01^{d*}$	$0.01\pm0.00^{ab*}$	0.01 ± 0.00^{d}
May	$0.60\pm0.06^{cd*}$	$0.26\pm0.01^{a*}$	$0.15\pm0.01^{a*}$	$0.04\pm0.02^{a*}$	$0.13\pm0.00^{b*}$	$0.06\pm0.01^{bcd*}$	$0.00\pm0.00^{a*}$	$0.00\pm0.00^{a*}$
June	$0.59\pm0.30^{bcd*}$	$0.32\pm0.05^{a*}$	$0.27\pm0.13^{ab*}$	$0.12\pm0.02^{ab*}$	$0.10\pm0.08^{ab*}$	$0.06\pm0.01^{bcd*}$	$0.01\pm0.00^{ab*}$	$0.00\pm0.00^{a*}$
July	$0.18\pm0.06^{a*}$	0.47 ± 0.11^{b}	$0.15\pm0.02^{a*}$	0.28 ± 0.05^{c}	$0.09\pm0.01^{ab*}$	0.12 ± 0.02^{e}	$0.01\pm0.00^{ab*}$	$0.01\pm0.00^{abc*}$
August	$0.63\pm0.06^{de*}$	$0.00\pm0.06^{a*}$	$0.19\pm0.02^{ab*}$	$0.08\pm0.01^{ab*}$	$0.08\pm0.01^{ab*}$	$0.04\pm0.01^{ab*}$	$0.01\pm0.00^{ab*}$	$0.00\pm0.00^{a*}$
September	$0.45\pm0.05^{abcd*}$	$0.30\pm0.05^{ab*}$	$0.14\pm0.02^{a*}$	$0.08\pm0.01^{a*}$	$0.10\pm0.01^{b*}$	$0.04\pm0.01^{abc*}$	$0.01\pm0.00^{ab*}$	$0.01\pm0.00^{abc*}$
October	$0.42\pm0.01^{abcd*}$	$0.33\pm0.00^{a*}$	$0.13\pm0.01^{a*}$	$0.06\pm0.00^{a*}$	$0.04\pm0.00^{a*}$	$0.05\pm0.00^{a*}$	$0.02\pm0.00^{bc*}$	0.01 ± 0.00^{cd}
November	$0.30\pm0.06^{abc*}$	$0.26\pm0.02^{a*}$	$0.17\pm0.06^{ab*}$	$0.13\pm0.04^{b*}$	$0.09\pm0.03^{b*}$	$0.07\pm0.01^{cd*}$	$0.02\pm0.00^{abc*}$	0.02 ± 0.00^{d}
December	$0.46\pm0.06^{bcd*}$	$0.17\pm0.01^{a*}$	0.28 ± 0.06^{bc}	$0.09\pm0.00^{ab*}$	$0.22\pm0.02^{c*}$	$0.04\pm0.00^{ab*}$	$0.03\pm0.01^{c*}$	$0.01\pm0.00^{bcd*}$
January	$0.30\pm0.03^{ab*}$	$0.19\pm0.01^{a*}$	$0.20\pm0.01^{ab*}$	$0.10\pm0.01^{ab*}$	$0.09\pm0.00^{ab*}$	$0.05\pm0.01^{abc*}$	$0.01\pm0.00^{ab*}$	$0.01\pm0.00^{bcd*}$
February	1.29 ± 0.23^{g}	0.48 ± 0.01^{b}	0.31 ± 0.05^{bc}	$0.10\pm0.01^{ab*}$	$0.21\pm0.04^{c*}$	$0.06\pm0.00^{bcd*}$	$0.01\pm0.00^{ab*}$	$0.01\pm0.00^{bcd*}$
Mean±S.E.M.	0.58±0.06	0.22±0.02	0.18±0.03	0.02±0.01	0.32±0.02	0.11±0.01	0.06±0.00	0.01±0.00

* Less than the maximum at $P < 0.05$

Means not sharing a common superscript letter in the same column of one collected sample are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

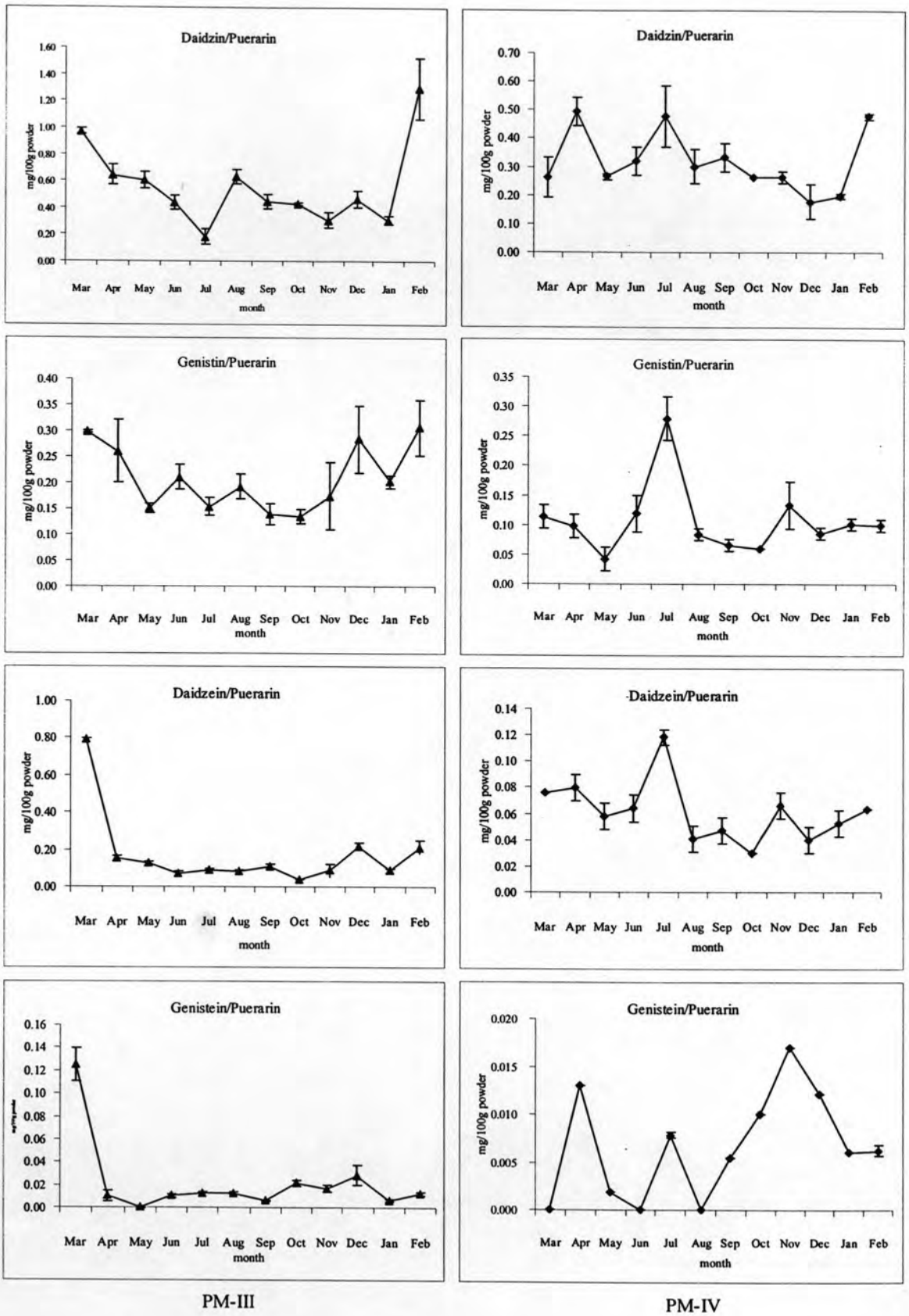


Figure 4.6 The ratio of individual isoflavonoid against puerarin in monthly collected PM-III and PM-IV from Ratchaburi since March 2005 to February 2006.

4.8 Correlation analysis of isoflavonoid content with air temperature and amount of rainfall of the field trial site

There was correlation between isoflavonoid contents and amount of rainfall but not the temperature, of Ratchaburi province followed Spearman's rho. In PM-III. It was found that puerarin and the ratio of genistin against puerarin were correlated with the amount of rainfall at $P < 0.01$. In PM-IV, genistein and ratio of daidzin against puerarin were correlated with the amount of rainfall at $P < 0.05$.

Table 4.13 Correlations coefficient of isoflavonoid contents in tubers of PM-III with temperature and amount of rainfall.

Chemical	Correlated with temperature		Correlated with amount of rainfall	
	PM-III	PM-IV	PM-III	PM-IV
puerarin	-	-	0.713**	-
daidzin	-	0.622*	-	-
genistin	-	0.755**	-	0.944**
daidzein	-	0.599*	-	-
genistein	-	0.902**	-	0.636*
total isoflavonoid	-	-	-	-
aglycoside	-	-	-	-
glycoside	-	-	-	-
aglycoside/glycoside	-	-	-	-
daidzin/puerarin	-	-	-	-
genistin/puerarin	-	-	0.732**	-
daidzein/puerarin	-	-	-	-
genistein/puerarin	-	-	-	-
daidzein/daidzin	-	-	-	-
genistein/genistin	-	-	-	-

(*), (**): Correlation is significant at the 0.05 and 0.01 level (2-tailed), respectively.

(-): No correlation

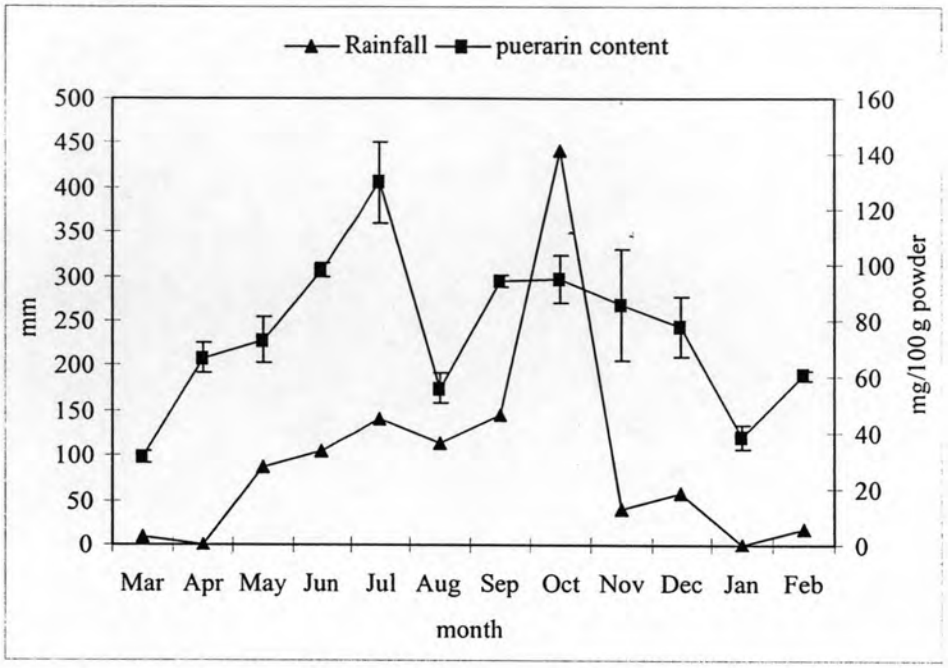


Figure 4.7 Puerarin content of PM-III in correlation with amount of rainfall during March 2005 to February 2006.

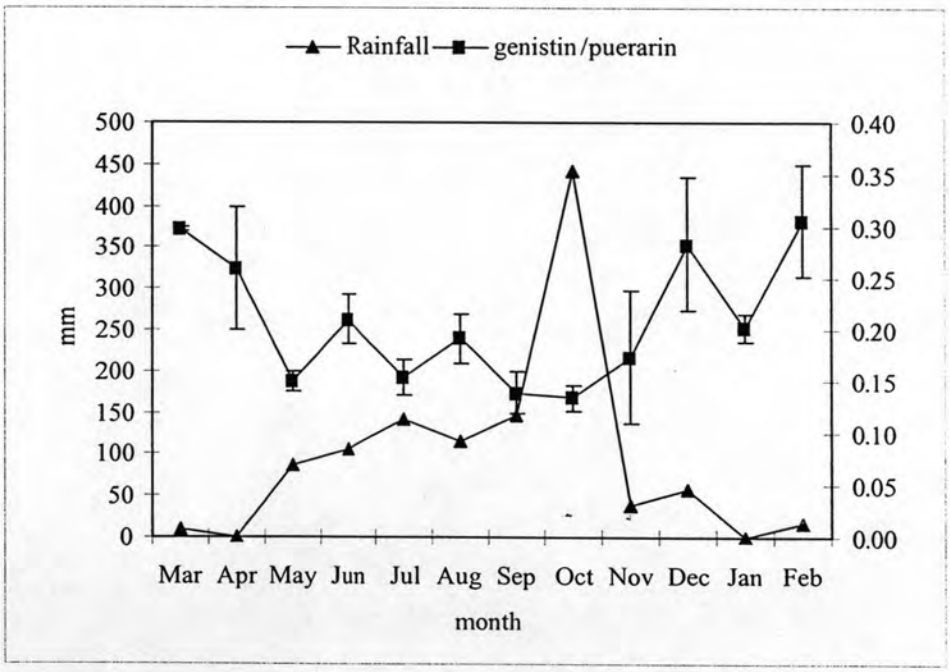


Figure 4.8 genistin/puerarin of PM-III in reverse correlation with amount of rainfall during March 2005 to February 2006.

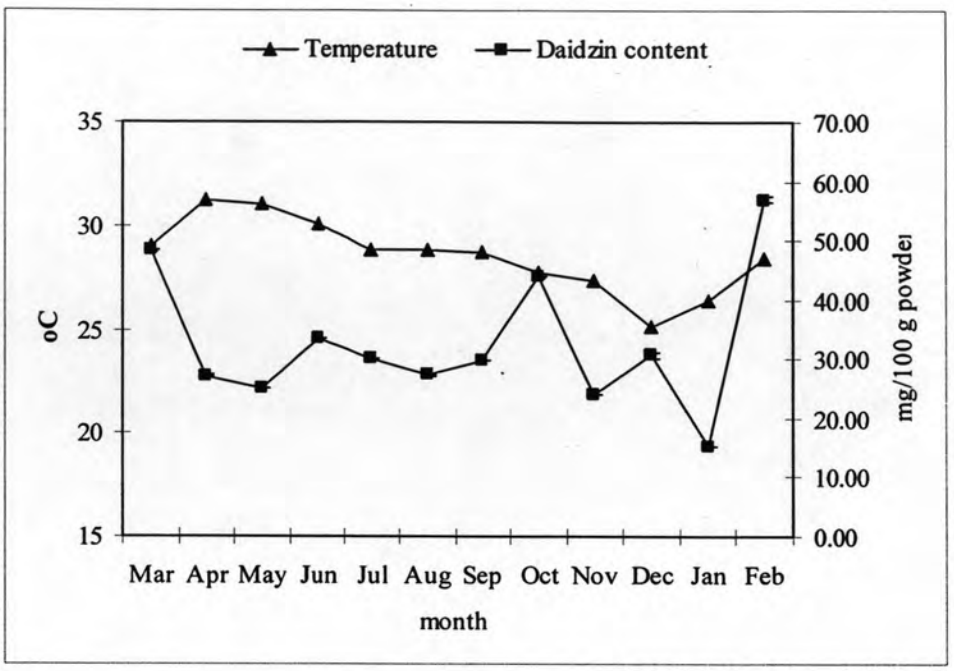


Figure 4.9 Daidzin content of PM-IV in correlation with temperature during March 2005 to February 2006.

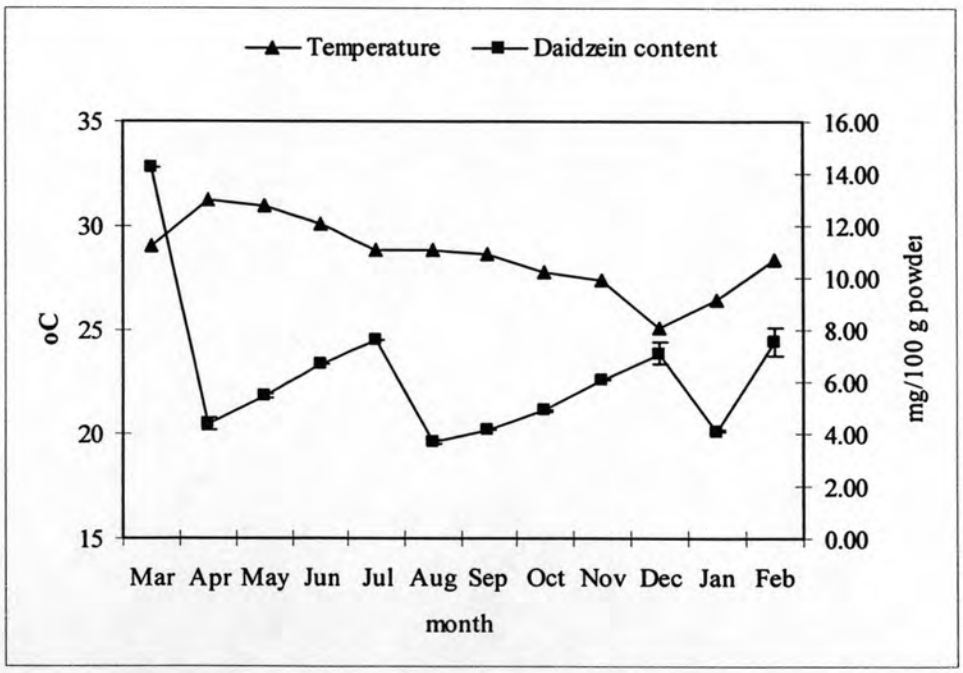


Figure 4.10 Daidzein of PM-IV in correlation with temperature during March 2005 to February 2006.

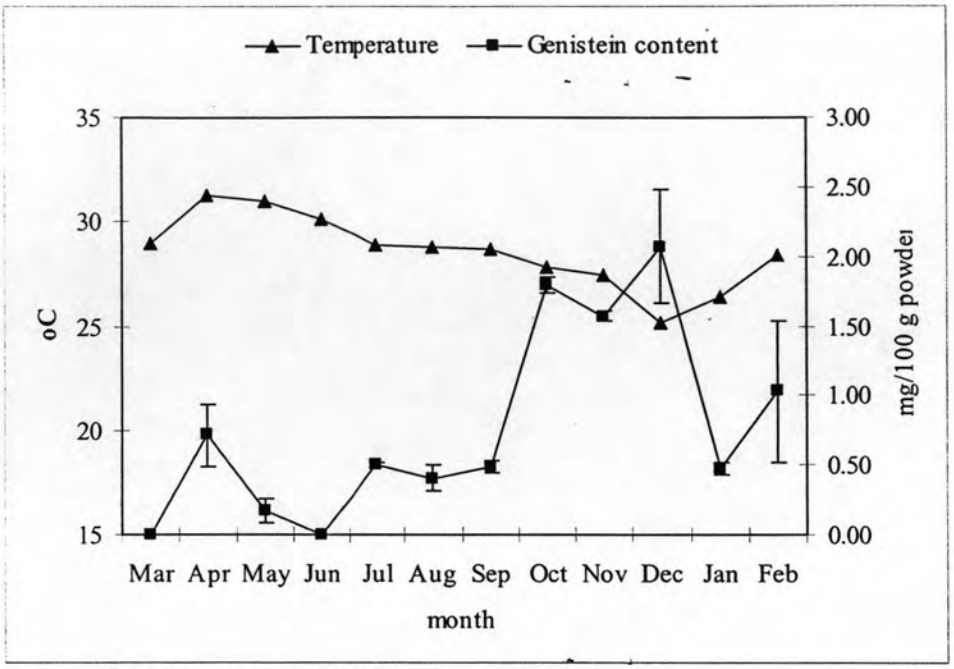


Figure 4.11 Genistein of PM-IV in correlation with temperature during March 2005 to February 2006.

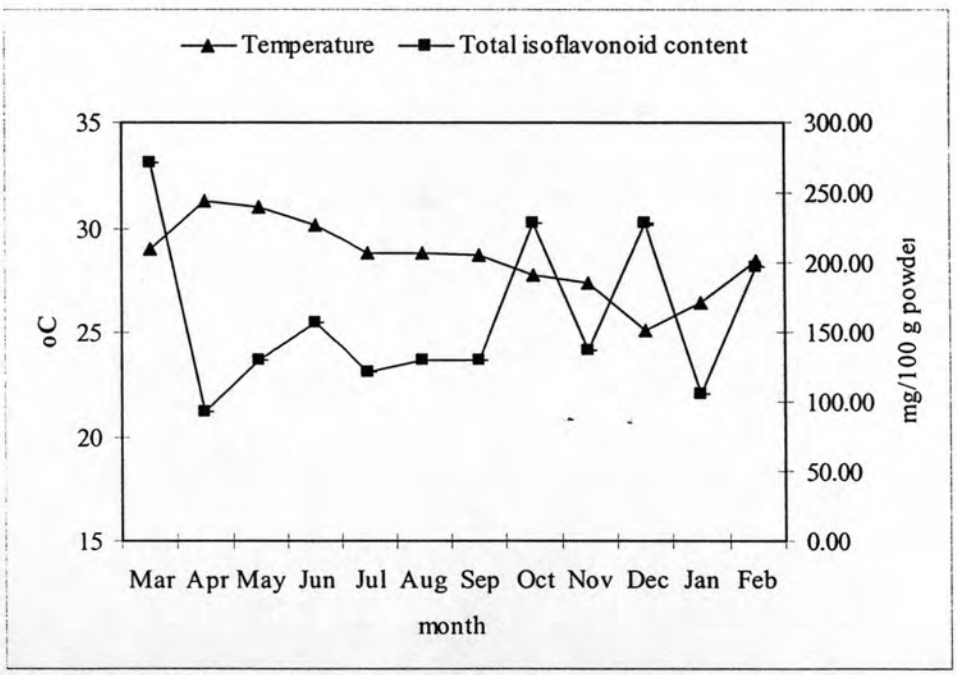


Figure 4.12 Total isoflavonoid of PM-IV in correlation with temperature during March 2005 to February 2006.

4.9. Proliferation effect of standard isoflavonoids (puerarin, daidzin, genistin, daidzein and genistein)

The growth response (Mean±S.E.M.) of MCF-7 to 5 standard isoflavonoids at the concentrations of 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11} and 10^{-12} M are compared with positive control (17 β-estradiol) and negative control (DMSO).

Table 4.14 The growth response of MCF-7 to 17 β-estradiol, puerarin, daidzin, genistin, daidzein and genistein.

Chemical	Concentrations (M)							
	10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
puerarin	216.61±9.06$^{b\Delta *}$	204.40±11.79$^{b\Delta *}$	195.57±6.62$^{b\Delta *}$	196.35±19.55^{b*}	177.65±15.10^{b*}	146.74±4.21^{a*}	155.31±2.34^{ab*}	156.87±7.05^{b*}
daidzin	230.63±8.70$^{b\Delta *}$	210.37±12.12$^{b\Delta *}$	218.42±23.47$^{b\Delta *}$	163.36±14.48^{b*}	186.22±21.54^{b*}	183.36±20.63^{b*}	175.57±19.27^{b*}	160.25±10.00^{b*}
genistin	119.47±4.00^{a}	139.21±9.16^{a*}	130.90±6.98^{a}	110.38±0.89$^{a\Delta}$	120.16±8.37$^{a\Delta}$	126.22±6.69^{a}	137.83±4.57^{ab}	121.90±3.94^{a}
daidzein	379.19±7.95$^{c\Delta *}$	415.81±19.26$^{c\Delta *}$	305.08±11.35$^{c\Delta *}$	335.56±23.06$^{c\Delta *}$	370.19±11.04$^{c\Delta *}$	357.37±6.67^{c*}	319.97±23.12^{c*}	209.16±19.72^{c*}
genistein	222.32±11.97^{b*}	162.41±5.39^{a*}	154.79±8.08^{a*}	206.39±9.38^{b*}	128.30±5.73^{a}	129.60±2.18^{a*}	127.26±8.49^{a}	129.60±4.25^{ab*}
17 β-estradiol	168.30±11.50*	149.86±16.15*	147.52±5.98*	169.60±7.98*	170.38±18.29*	-	-	-

$^{\Delta}$ Compared with positive control (17 β-estradiol) at $P < 0.05$.

* Compared with negative control (DMSO) of 100 ± 0.00 at $P < 0.05$.

Means not sharing a common superscript letter in the same row of the samples are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

The growth response percentages (Mean ± S.E.M.) of 5 standard isoflavonoids at the concentrations of 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11} and 10^{-12} M in the presence of S9 mixture are compared with positive control (17 β-estradiol) and negative control (DMSO).

Table 4.15 The growth response of MCF-7 to 17 β-estradiol, puerarin, daidzein and genistein in the presence of S9 mixture in comparison with mean of population.

Chemical	Concentrations (M)							
	10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
puerarin	$623.32\pm24.41^{c\Delta *}$	$849.02\pm38.38^{c*}$	$498.14\pm31.62^{b\Delta *}$	$558.91\pm28.63^{c\Delta *}$	$421.78\pm26.89^{ab\Delta *}$	$486.11\pm38.17^{b*}$	$444.90\pm41.34^{b*}$	$471.91\pm18.74^{a*}$
daidzin	$489.57\pm29.74^{b\Delta *}$	$549.82\pm31.72^{b\Delta *}$	$487.41\pm47.91^{b\Delta *}$	$584.63\pm17.81^{c\Delta *}$	$477.54\pm29.10^{ab*}$	$437.89\pm33.83^{ab*}$	$482.38\pm14.99^{b*}$	$424.90\pm15.29^{a*}$
genistin	$293.22\pm33.16^{a*}$	$336.86\pm40.81^{a*}$	$351.66\pm18.80^{a*}$	$547.32\pm29.11^{c\Delta *}$	$506.54\pm41.33^{b*}$	$407.50\pm29.86^{ab*}$	$345.52\pm3.18^{a*}$	$425.42\pm23.94^{a*}$
daidzein	$471.48\pm18.65^{b\Delta *}$	$379.19\pm19.77^{a*}$	$371.66\pm12.24^{a*}$	$461.52\pm14.36^{b\Delta *}$	$450.70\pm9.34^{ab\Delta *}$	$372.70\pm12.95^{a*}$	$302.83\pm33.13^{a*}$	$443.60\pm12.64^{a*}$
genistein	$330.97\pm31.85^{a*}$	$427.50\pm25.18^{a*}$	$339.63\pm30.21^{a*}$	$329.67\pm40.60^{a*}$	$385.68\pm27.71^{a\Delta *}$	$412.18\pm35.43^{ab*}$	$467.23\pm17.39^{b*}$	$553.46\pm37.42^{b*}$
17 β-estradiol	$299.11\pm34.54^{*}$	$334.00\pm28.92^{*}$	$331.49\pm37.34^{*}$	$266.30\pm13.18^{*}$	$542.73\pm31.76^{*}$	-	-	-

$^{\Delta}$ Compared with positive control (estradiol) at $P < 0.05$.

* Compared with negative control (DMSO) of 100 ± 0.00 at $P < 0.05$.

Means not sharing a common superscript letter in the same row of the samples are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

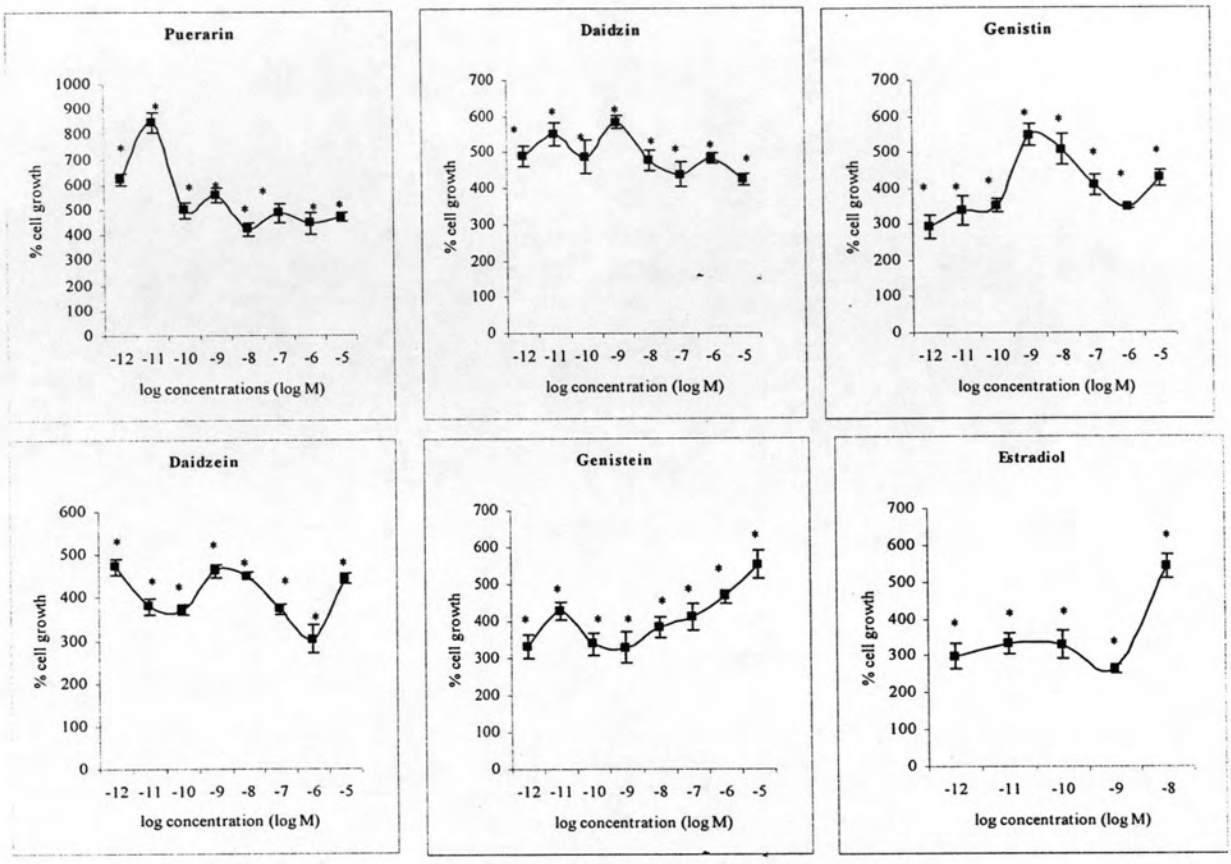


Figure 4.14 The growth response of MCF-7 to 17 β-estradiol, puerarin, daidzein and genistein in the presence of S9 mixture, in comparison with DMSO.

* Proliferation to MCF-7

The ratio of growth response of standard isoflavonoids to MCF-7 cells in the presence and absence of S9 mixture are present in Table 4.15.

Table 4.16 The ratio of growth response of MCF-7 to standard isoflavonoids in the presence and absence of S9 mixture.

Concentration (M)	Standards					
	puerarin	daidzin	genistin	daidzein	genistein	17 β-estradiol
10^{-12}	2.88±0.06^{ab}	2.12±0.08^{a}	2.46±0.27^{a}	1.25±0.08^{bc}	1.49±0.08^{a}	1.86±.38^{a}
10^{-11}	4.18±0.22^{c}	2.65±0.24^{a}	2.41±0.19^{a}	.913±0.02^{a}	2.63±0.13^{cd}	2.25±0.10^{a}
10^{-10}	2.55±0.19^{a}	2.34±0.40^{a}	2.70±0.15^{ab}	1.23±0.07^{bc}	2.24±0.27^{bc}	2.26±0.25^{a}
10^{-9}	2.91±0.24^{ab}	3.66±0.29^{b}	4.96±0.27^{e}	1.40±0.12^{c}	1.59±0.18^{ab}	1.57±0.06^{a}
10^{-8}	2.41±0.19^{a}	2.67±0.34^{a}	4.21±0.16^{d}	1.23±0.06^{bc}	3.03±0.27^{de}	3.28±0.32^{b}
10^{-7}	3.32±0.30^{b}	2.46±0.27^{a}	3.22±0.10^{bc}	1.04±0.04^{ab}	3.19±0.30^{de}	-
10^{-6}	2.88±0.30^{ab}	2.83±0.28^{a}	2.52±0.06^{a}	0.96±0.12^{ab}	3.70±0.13^{ef}	-
10^{-5}	3.04±0.22^{ab}	2.68±0.20^{a}	3.52±0.29^{c}	2.16±0.15^{d}	4.29±0.33^{f}	-

Means not sharing a common superscript letter in the same row of the samples are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

4.10. Proliferation/antiproliferation assay

P. mirifica monthly collected from Ratchaburi during March 2005 to February 2006 at the concentrations of 0.1-1000 μg/ml were tested with MCF-7. The growth response of MCF-7 to *P. mirifica* is compared with the growth of negative control (DMSO).

PM-III and PM-IV showed variation of biphasic estrogenic activity to MCF-7. The plant extracts showed proliferation effect to MCF-7 at 0.1-100 μg/ml, and antiproliferation at 1000 μg/ml (Table 4.16 and 4.17). PM-IV showed proliferation effect to MCF-7 more than PM-III (Table 4.18).

Table 4.17 The growth response of MCF-7 to PM-III in comparison with DMSO and mean of plant population.

Month	% cell growth					IC_{50}
	0.1 µg/ml	1 µg/ml	10 µg/ml	100 µg/ml	1000 µg/ml	(µg/ml)
March	93.76 ± 8.12^{b}	90.54 ± 2.48^{b}	95.59 ± 3.29^{b}	93.44 ± 3.47^{b}	$65.05\pm1.96^{a*\dagger}$	>1000
April	90.32 ± 4.04^{a}	90.8 ± 11.77^{a}	89.68 ± 5.04^{a}	89.03 ± 4.15^{a}	$80.11\pm4.10^{a\dagger}$	>1000
May	$86.70\pm2.30^{a\dagger}$	$84.83\pm2.53^{a\dagger}$	92.32 ± 2.93^{a}	91.01 ± 2.27^{a}	$85.02\pm8.08^{a\dagger}$	>1000
June	90.82 ± 5.47^{a}	89.33 ± 9.90^{a}	92.88 ± 0.75^{a}	$130.34\pm7.14^{b*\dagger}$	$81.84\pm1.31^{a\dagger}$	>1000
July	$84.08\pm4.91^{ab\dagger}$	$77.53\pm4.54^{a*\dagger}$	102.43 ± 2.39^{c}	106.37 ± 8.81^{c}	96.82 ± 4.31^{bc}	>1000
August	84.08 ± 3.56^{a}	88.76 ± 9.27^{a}	106.37 ± 8.88^{a}	101.12 ± 8.19^{a}	85.02 ± 3.20^{a}	>1000
September	$110.42\pm2.81^{c\dagger}$	102.92 ± 1.97^{bc}	95.58 ± 4.55^{b}	104.38 ± 2.96^{bc}	$78.00\pm2.93^{a\dagger}$	>1000
October	$120.33\pm9.14^{b*\dagger}$	$123.75\pm4.34^{b*\dagger}$	$131.17\pm7.71^{b*\dagger}$	111.42 ± 5.42^{b}	87.58 ± 5.12^{a}	>1000
November	109.92 ± 4.49^{a}	97.50 ± 2.90^{a}	96.33 ± 2.71^{a}	96.00 ± 5.68^{a}	94.58 ± 6.59^{a}	>1000
December	$113.92\pm4.88^{c*\dagger}$	88.50 ± 1.59^{b}	98.58 ± 2.38^{b}	90.00 ± 4.42^{b}	$72.92\pm7.40^{a\dagger}$	>1000
January	87.22 ± 4.81^{a}	$125.43\pm7.73^{bc*\dagger}$	111.85 ± 4.06^{bc}	$130.99\pm0.86^{c*\dagger}$	$106.48\pm9.51^{ab*}$	>1000
February	$80.73\pm3.43^{ab\dagger}$	$90.42\pm7.76^{b*}$	93.23 ± 4.28^{b}	$80.21\pm1.63^{ab*\dagger}$	$70.00\pm3.31^{a*\dagger}$	>1000
Mean±S.E.M.	96.03±3.95	95.86±4.26	100.50±3.34	102.03±4.58	$83.62\pm3.40^{\dagger}$	

* Different from mean at $P < 0.05$.

† Different from negative control (DMSO) at $P < 0.05$.

Means not sharing a common superscript letter in the same row of the samples are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

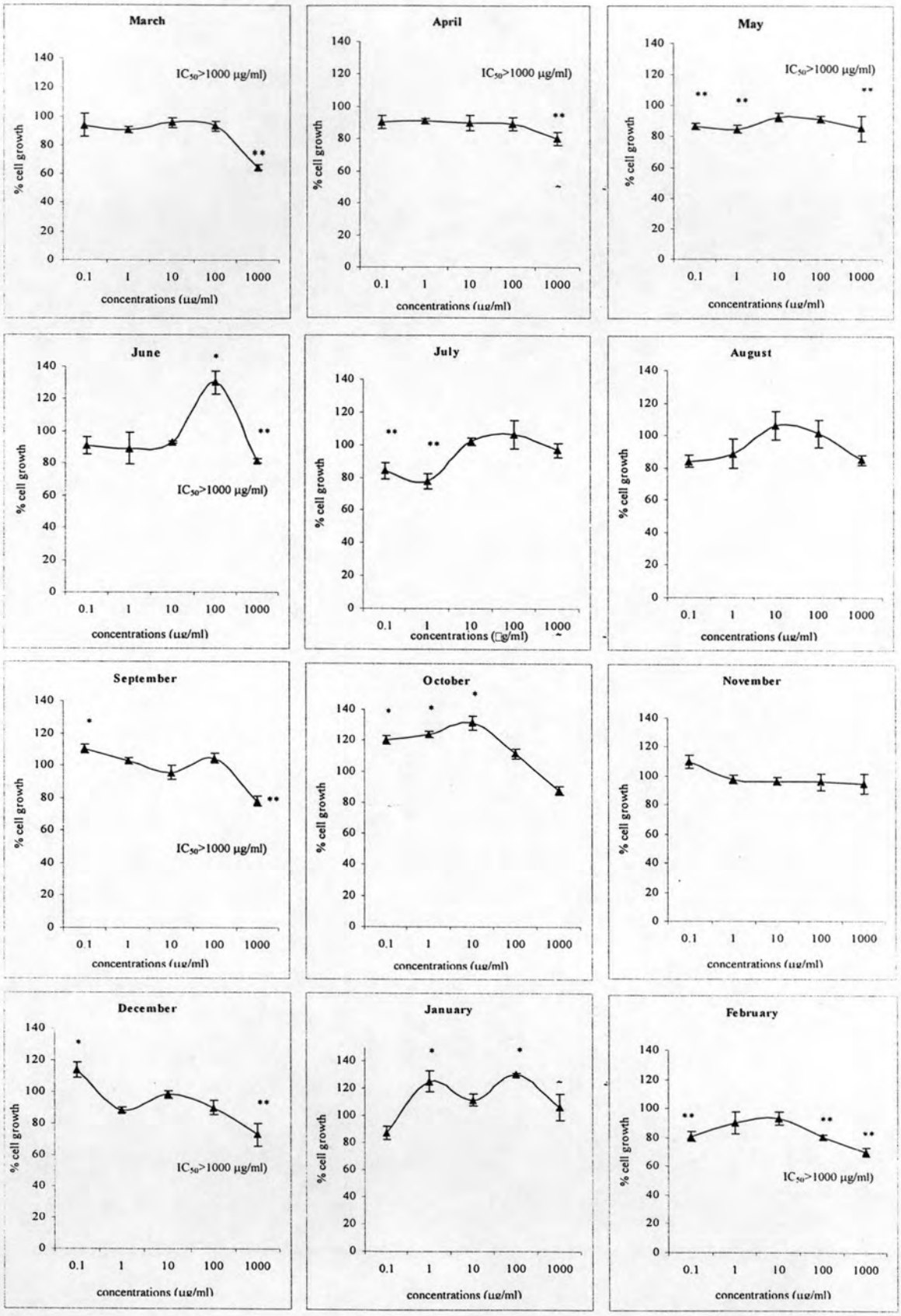


Figure 4.15 The growth response of MCF-7 to PM-III in comparison with DMSO.

* Proliferation to MCF-7; ** Antiproliferation to MCF-7

Table 4.18 The growth response of MCF-7 to PM-IV in comparison with mean of the plant population.

Month	% cell growth					IC_{50}
	0.1 µg/ml	1 µg/ml	10 µg/ml	100 µg/ml	1000 µg/ml	(µg/ml)
March	99.52 ± 4.00^{b}	$97.42\pm8.71^{ab*}$	$122.04\pm4.22^{c\dagger}$	99.57 ± 3.03^{b}	$76.24\pm10.35^{a*\dagger}$	>1000
April	107.31 ± 5.39^{ab}	$119.35\pm3.45^{c\dagger}$	$113.01\pm4.13^{ab\dagger}$	109.84 ± 0.28^{ab}	102.04 ± 3.01^{a}	>1000
May	111.05 ± 6.85^{b}	103.93 ± 4.58^{b}	104.68 ± 4.73^{b}	101.50 ± 6.34^{b}	$79.02\pm1.79^{a*\dagger}$	>1000
June	$86.14\pm5.25^{a*}$	$98.13\pm4.70^{a*}$	$93.44\pm3.12^{a*}$	$88.01\pm3.65^{a*}$	93.07 ± 11.52^{a}	>1000
July	$191.29\pm11.52^{c*\dagger}$	$155.24\pm13.86^{bc*\dagger}$	$141.95\pm15.34^{ab\dagger}$	$130.71\pm12.28^{ab*}$	108.99 ± 2.92^{a}	>1000
August	$125.65\pm4.48^{c\dagger}$	$117.98\pm0.65^{ab\dagger}$	$115.92\pm5.21^{ab\dagger}$	$120.79\pm1.62^{ab\dagger}$	$110.67\pm0.00^{a\dagger}$	>1000
September	103.83 ± 3.54^{a}	$148.25\pm1.53^{c\dagger}$	$163.00\pm4.91^{d*\dagger}$	$136.13\pm0.36^{b*\dagger}$	$135.50\pm1.88^{b*\dagger}$	>1000
October	$144.88\pm1.80^{c*\dagger}$	$143.50\pm2.31^{c*\dagger}$	$126.50\pm8.26^{b\dagger}$	110.42 ± 3.58^{a}	$112.75\pm0.87^{a\dagger}$	>1000
November	108.59 ± 4.96^{b}	$125.63\pm2.35^{c\dagger}$	$118.44\pm1.98^{bc\dagger}$	$120.63\pm4.20^{c\dagger}$	98.02 ± 1.09^{a}	>1000
December	$131.13\pm1.66^{a\dagger}$	$129.83\pm1.12^{a\dagger}$	$134.67\pm2.60^{a\dagger}$	$133.25\pm4.26^{a*\dagger}$	$127.25\pm.14^{a*\dagger}$	>1000
January	95.63 ± 2.78^{b}	$100.52\pm6.35^{b*}$	100.94 ± 9.92^{b}	$91.46\pm3.55^{b*}$	$64.90\pm5.75^{a*\dagger}$	>1000
February	91.88 ± 6.26^{a}	104.69 ± 1.41^{a},	106.67 ± 5.18^{a}	$95.21\pm4.87^{a*}$	90.73 ± 5.10^{a}	>1000
Mean±S.E.M.	$116.41\pm8.39^{\dagger}$	$120.372\pm5.89^{\dagger}$	$120.11\pm5.60^{\dagger}$	111.46 ± 4.83	99.93 ± 5.99	

* Different from mean at $P < 0.05$.

† Different from negative control (DMSO) at $P < 0.05$.

Means not sharing a common superscript letter in the same row of the samples are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

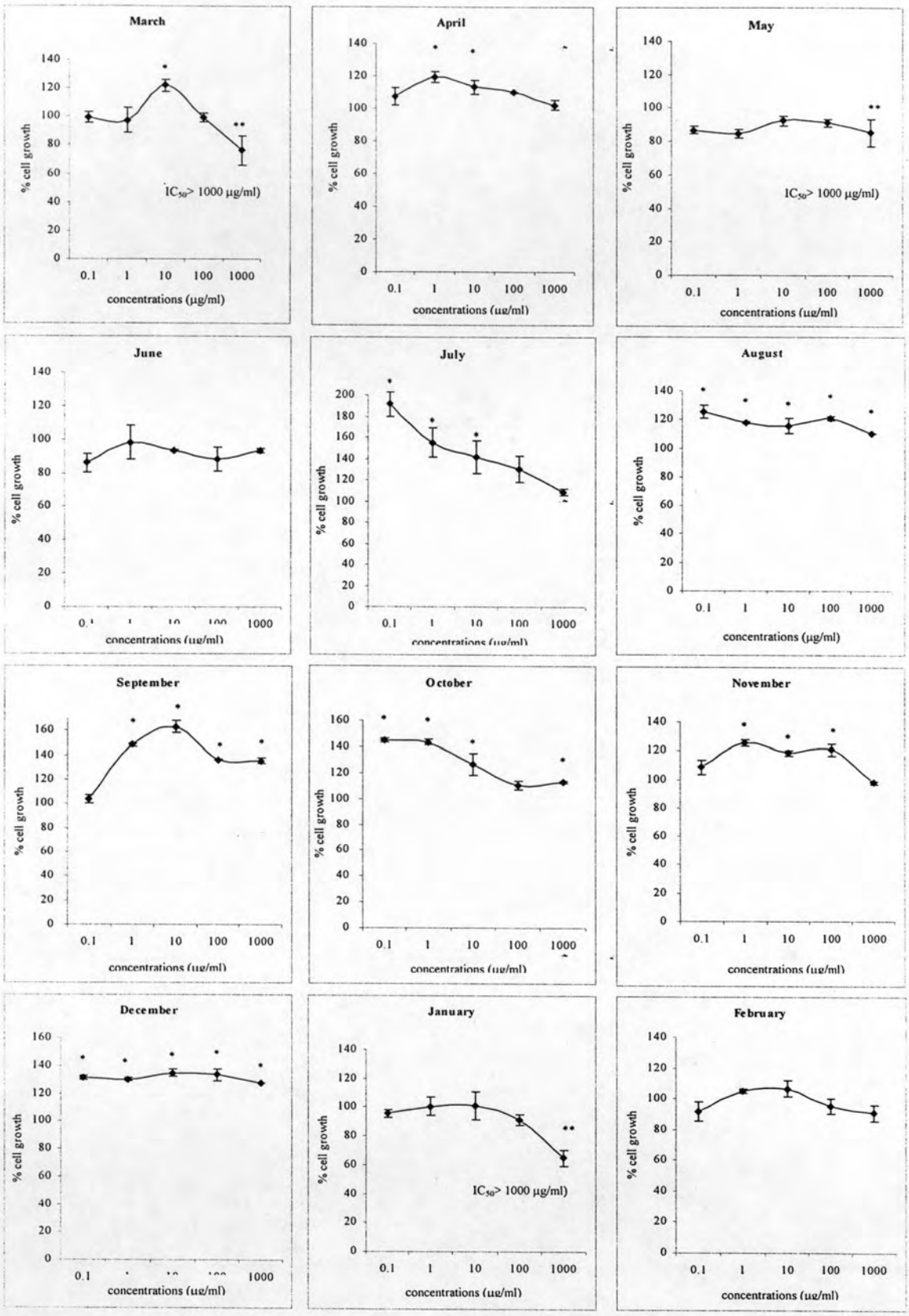


Figure 4.16 The growth response of MCF-7 to PM-IV in comparison with DMSO. (* Proliferation to MCF-7; ** Antiproliferation to MCF-7)

Table 4.19 The percentage growth of PM-III compared with PM-IV in the same concentration.

Month	Concentrations (µg/ml)									
	0.1		1		10		100		1000	
	PM-III	PM-IV	PM-III	PM-IV	PM-III	PM-IV	PM-III	PM-IV	PM-III	PM-IV
March	-	-	-	-	PM-IV		-	-	-	-
April	-	-				PM-IV				
May			PM-IV		-	-	-	-	-	-
June	-	-	-	-	-	-	PM-III		-	-
July					PM-IV				-	-
August			PM-IV		-	-		PM-IV		
September	-	-				PM-IV				
October			PM-IV		-	-	-	-	PM-IV	
November	-	-	-	-	-	-	PM-IV		-	-
December	-	-	PM-IV		-	-		PM-IV		
January	-	-	PM-III		PM-IV			PM-III		
February	-	-	-	-	-	-		PM-III		
Mean±S.E.M.	-	-	PM-IV		-	-	-	-	-	-

The name of the clone present in table exhibits more content at $P < 0.05$.

PM-III showed maximum proliferative effect and maximum antiproliferative effect in October 2005 and March 2005, respectively (Table 4.17 and Figure 4.15). PM-IV showed maximum proliferative effect and maximum antiproliferative effect in July 2005 and January 2005, respectively (Table 4.18 and Figure 4.16).

Table 4.20 The growth response percentage of PM-III extract in the presence of S9 mixture on MCF-7 cell culture in comparison with DMSO and mean of the plant population.

Month	%growth					IC_{50}
	0.1 µg/ml	1 µg/ml	10 µg/ml	100 µg/ml	1000 µg/ml	(µg/ml)
March	$374.74\pm22.99^{a\dagger}$	$513.55\pm9.84^{b*\dagger}$	$549.46\pm8.58^{b*\dagger}$	$653.19\pm31.51^{b*\dagger}$	$575.46\pm44.02^{b*\dagger}$	>1000
April	$243.87\pm4.66^{b*\dagger}$	$332.42\pm28.31^{b*\dagger}$	$83.17\pm36.22^{a*\dagger}$	$42.42\pm19.37^{a*\dagger}$	$71.18\pm23.65^{a*\dagger}$	47.62
May	$292.72\pm10.06^{b*\dagger}$	$175.19\pm20.99^{a*\dagger}$	$555.73\pm21.24^{e*\dagger}$	$438.92\pm27.06^{d\dagger}$	$364.07\pm15.02^{c\dagger}$	>1000
June	$393.28\pm26.00^{a\dagger}$	$424.16\pm25.94^{a\dagger}$	$404.37\pm16.09^{a\dagger}$	$475.97\pm66.79^{a\dagger}$	$630.93\pm11.46^{b*\dagger}$	>1000
July	$456.10\pm50.08^{a\dagger}$	$423.06\pm40.01^{a\dagger}$	$429.24\pm38.27^{a\dagger}$	$575.33\pm25.84^{b*\dagger}$	$388.92\pm21.87^{a\dagger}$	>1000
August	$167.61\pm3.19^{a*\dagger}$	$335.36\pm5.17^{b*\dagger}$	$525.25\pm10.15^{c\dagger}$	$490.00\pm34.03^{c\dagger}$	$373.02\pm26.53^{b\dagger}$	>1000
September	$209.25\pm6.78^{a*\dagger}$	$226.79\pm9.31^{ab*\dagger}$	$245.08\pm17.32^{ab*\dagger}$	$279.08\pm30.17^{b*\dagger}$	$218.75\pm7.87^{a*\dagger}$	>1000
October	$503.58\pm27.42^{c\dagger}$	$295.50\pm9.81^{a*\dagger}$	$356.29\pm7.87^{ab*\dagger}$	$388.21\pm17.68^{b\dagger}$	$390.33\pm35.27^{b\dagger}$	>1000
November	$754.70\pm66.39^{bc*\dagger}$	$637.78\pm34.61^{b*\dagger}$	$876.05\pm58.09^{c*\dagger}$	$726.77\pm33.05^{bc*\dagger}$	$431.75\pm49.74^{a\dagger}$	>1000
December	$297.00\pm3.90^{b*\dagger}$	$390.33\pm9.38^{d\dagger}$	$348.38\pm4.40^{c*\dagger}$	$343.10\pm0.55^{c*\dagger}$	$219.63\pm5.70^{a*\dagger}$	>1000
January	$721.68\pm47.12^{b*\dagger}$	$746.19\pm43.45^{b*\dagger}$	$700.77\pm32.09^{b*\dagger}$	$499.43\pm20.71^{a\dagger}$	$507.24\pm75.31^{a*\dagger}$	>1000
February	$591.36\pm10.26^{d*\dagger}$	$412.65\pm20.63^{b\dagger}$	$405.99\pm19.92^{b\dagger}$	$466.30\pm19.67^{c\dagger}$	$266.97\pm7.76^{a\dagger}$	>1000
Mean±S.E.M.	$422.76\pm30.31^{\dagger}$	$418.47\pm25.59^{\dagger}$	$476.28\pm31.14^{\dagger}$	$465.02\pm26.86^{\dagger}$	$385.72\pm24.81^{\dagger}$	

* Different from mean at $P < 0.05$.

† Different from negative control (DMSO) at $P < 0.05$.

Means not sharing a common superscript letter in the same row of the samples are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

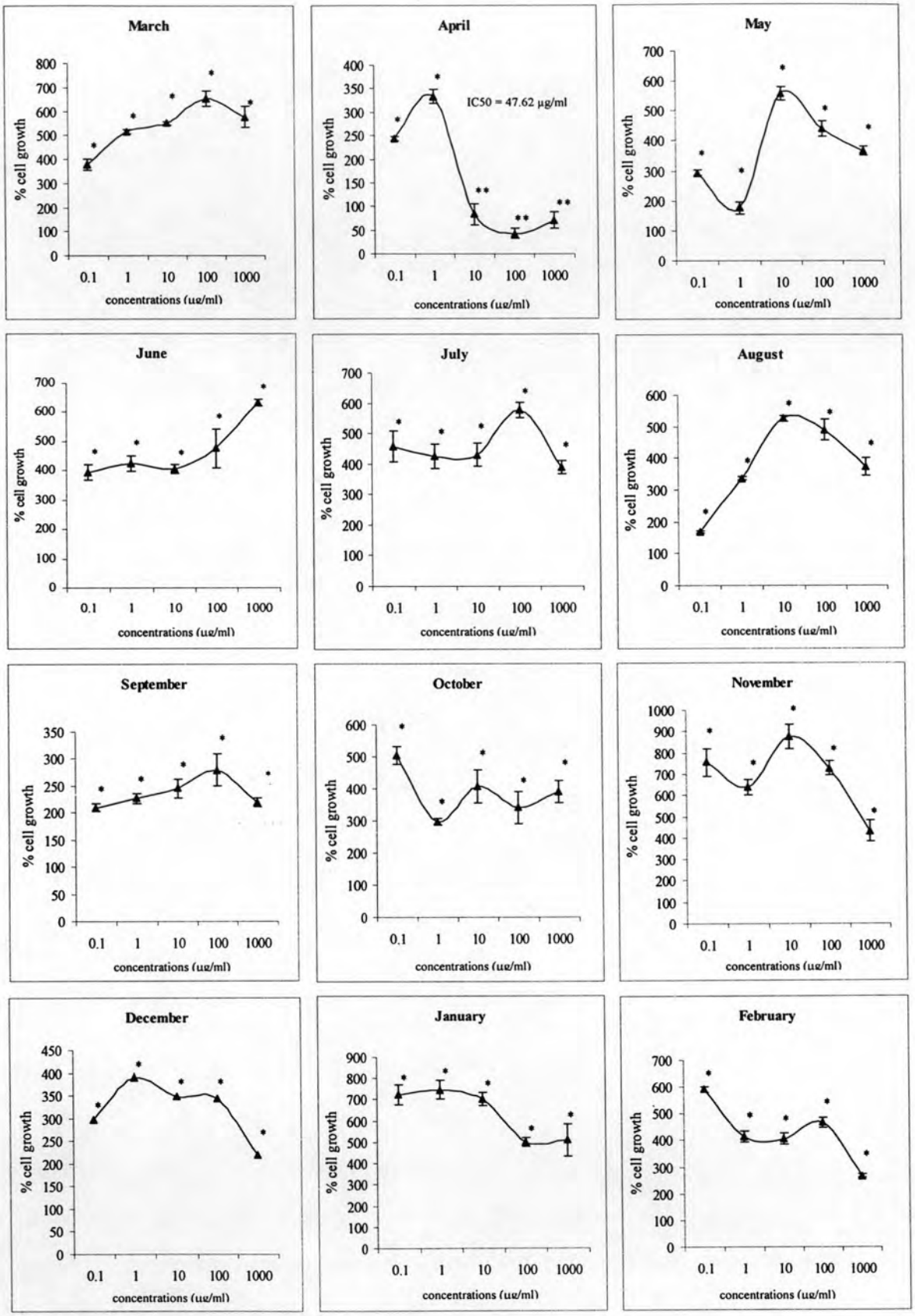


Figure 4.17 The growth response percentage of PM-III extract in the presence of S9 mixture on MCF-7 cell culture compare mean of population.

Table 4.21 The growth response percentage of PM-IV extract in the presence of S9 mixture on MCF-7 cell culture in comparison with DMSO and mean of the plant population.

Month	% cell growth					IC_{50}
	0.1 µg/ml	1 µg/ml	10 µg/ml	100 µg/ml	1000 µg/ml	(µg/ml)
March	634.99±48.78^{a*}	605.27±16.00^{ab*}	546.72±29.28^{ab*}	650.94±42.10^{ab*}	700.91±30.35^{b*}	>1000
April	613.11±13.61^{a*}	635.55±11.58^{a*}	919.38±50.78^{b*}	596.84±13.95^{a*}	669.94±9.25^{a*}	>1000
May	258.24±22.61^{ab*}	173.31±35.52^{a*}	208.24±10.42^{a*}	345.50±42.98^{b*}	336.14±33.24^{b*}	>1000
June	473.47±21.21^{b}	600.01±33.52^{c*}	473.06±23.85^{b}	531.97±17.07^{bc*}	351.05±32.24^{a*}	>1000
July	340.77±31.20^{ab}	437.81±20.99^{c}	403.13±10.46^{bc}	362.77±30.01^{bc}	261.39±42.93^{a*}	>1000
August	397.35±1.28^{bc}	323.12±20.92^{ab*}	271.91±21.47^{a*}	468.60±46.60^{b}	435.94±28.86^{b}	>1000
September	292.67±8.55^{a*}	301.42±3.64^{a*}	337.96±3.43^{b}*	301.54±4.19^{a*}	381.50±1.46^{c}	>1000
October	361.58±7.22^{a}	373.29±14.94^{a}	382.75±8.01^{a}	384.50±10.39^{a}	360.75±5.55^{a}	>1000
November	329.33±27.09^{a}	351.75±22.36^{a*}	350.75±34.51^{a*}	334.67±17.23^{a*}	383.75±13.19^{a}	>1000
December	365.17±6.08^{a}	419.79±12.63^{c}	372.38±4.55^{ab}	356.75±3.97^{a}	390.67±0.00^{b}	>1000
January	119.28±3.78^{b*}	161.51±4.49^{c*}	91.85±1.501^{a*}	102.14±10.65^{ab*}	110.52±6.25^{ab*}	>1000
February	520.32±62.31^{a*}	480.33±55.83^{a}	541.45±18.86^{a*}	522.46±34.39^{a*}	621.99±32.55^{a*}	>1000
Mean±S.E.M.	407.12±23.51†	420.75±24.47†	425.10±32.25†	428.80±23.39†	429.88±26.97†	

* Different from mean at $P < 0.05$.

† Different from DMSO at $P < 0.05$.

Means not sharing a common superscript letter in the same row of the samples are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

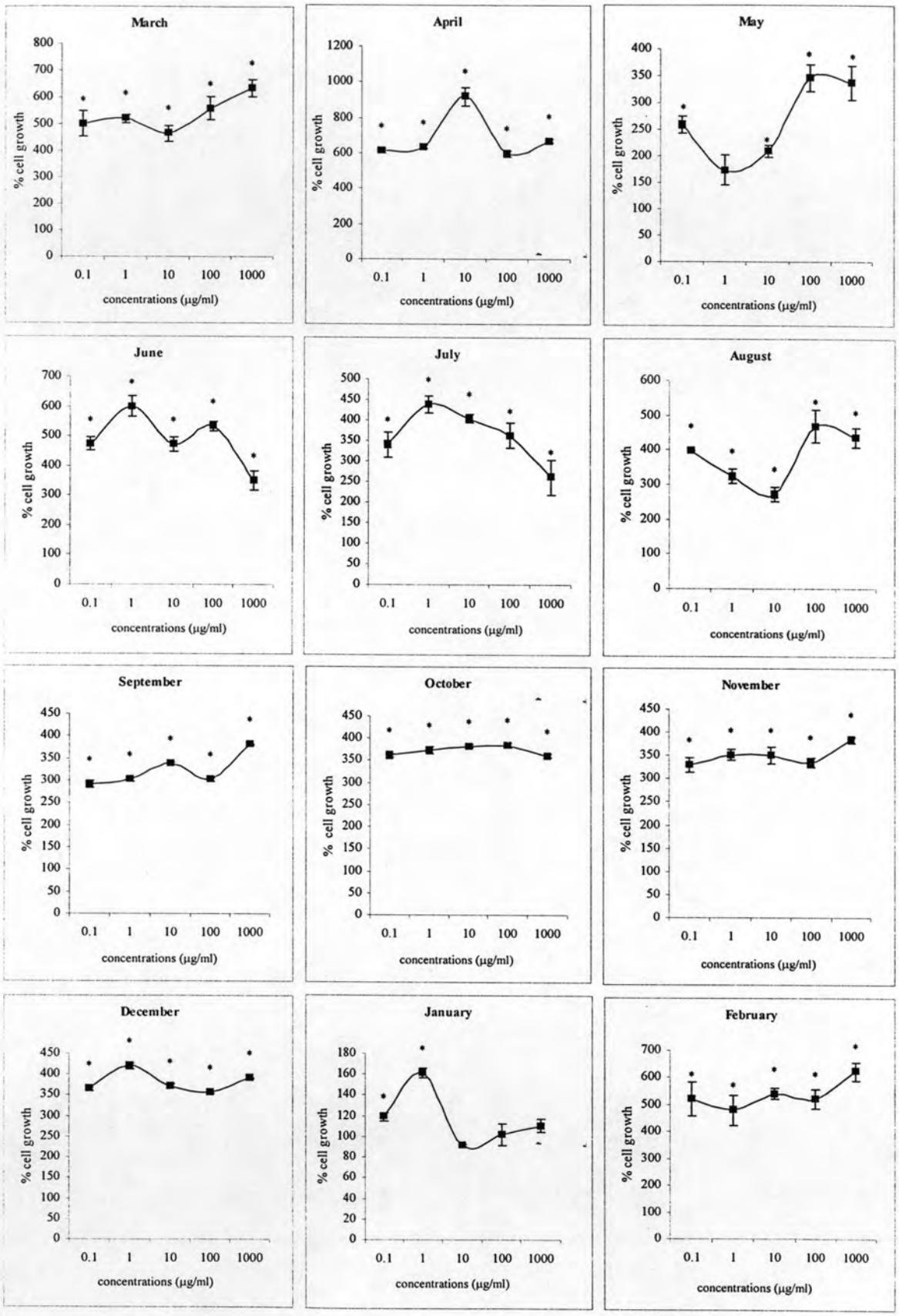
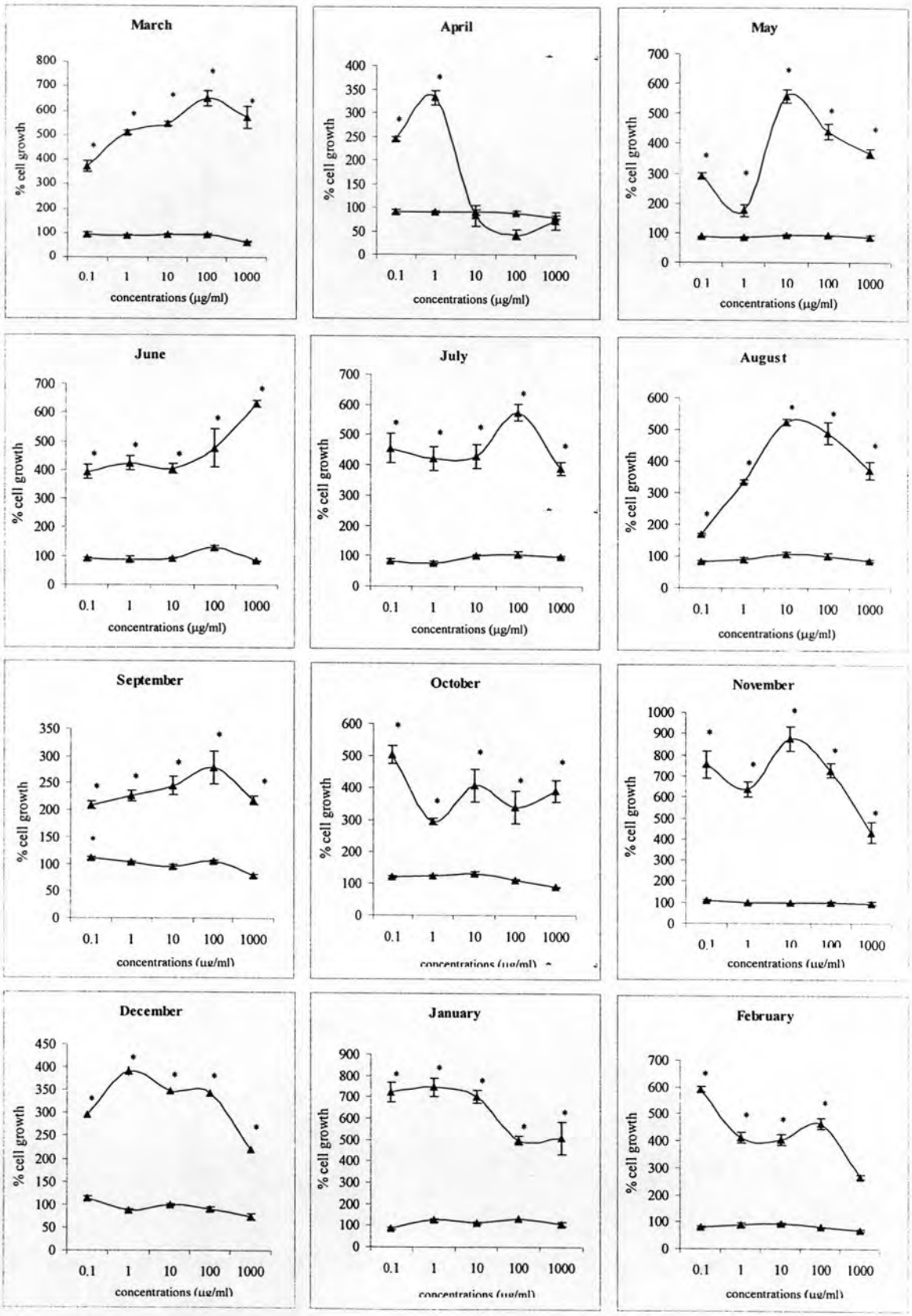


Figure 4.18 The growth response percentage of PM-IV extract in the presence of S9 mixture on MCF-7 cell culture in comparison with mean of the plant population.

Table 4.22 The ratio of growth response of PM-III and PM-IV to MCF-7 cells in the presence vs. the absence of S9 mixture.

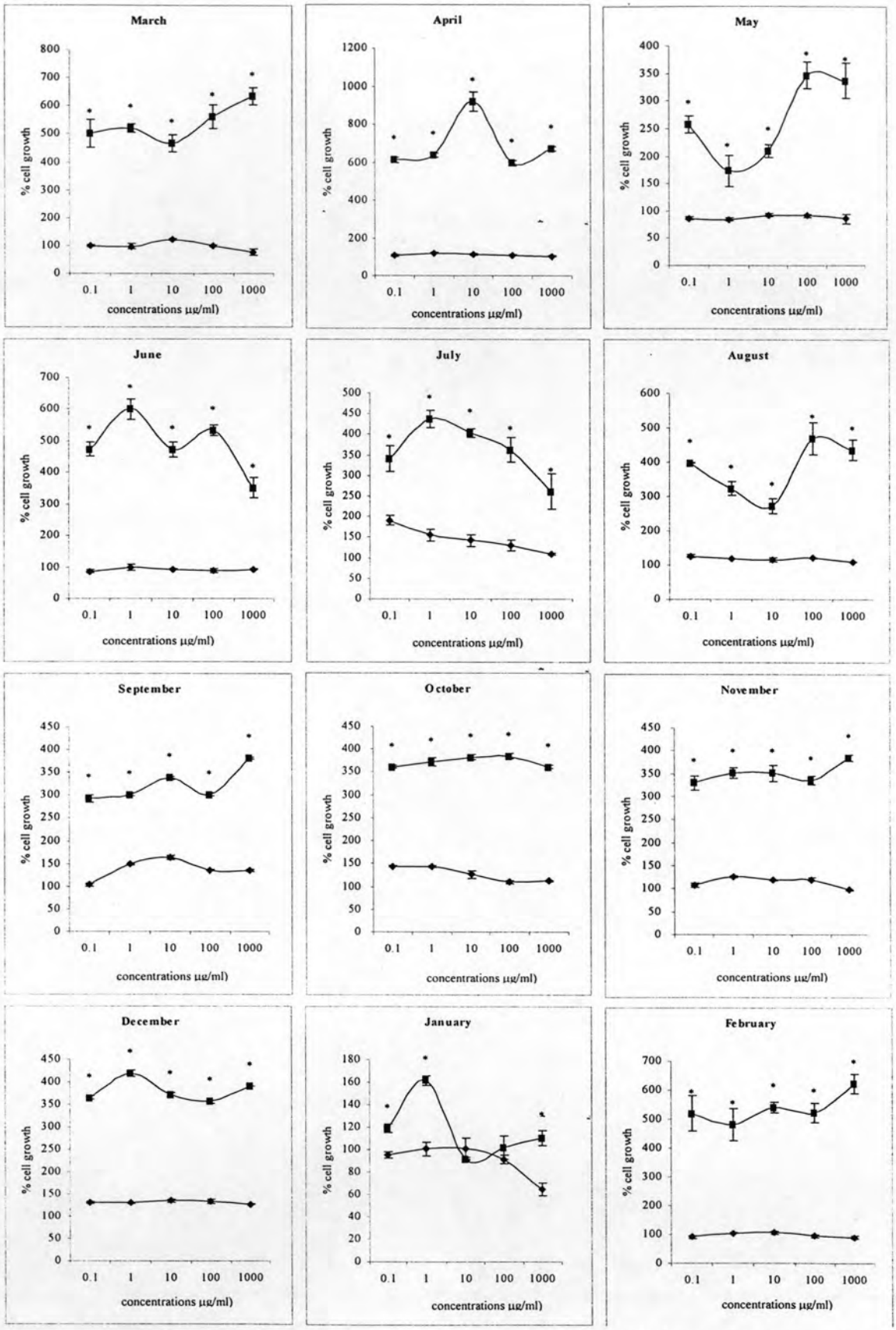
Month	Concentrations (μg/ml)									
	PM-III					PM-IV				
	0.1	1	10	100	1000	0.1	1	10	100	1000
March	4.00±0.12^{cde}	5.59±0.05^{de}	5.68±0.14^{e}	6.97±0.22^{g}	8.31±0.37^{e}	5.96±0.15^{d}	6.23±0.69^{g}	4.33±0.12^{f}	6.33±0.49^{f}	9.18±1.34^{d}
April	2.71±0.14^{abc}	3.67±0.35^{b}	0.91±0.36^{a}	0.49±0.22^{a}	0.86±0.25^{a}	5.69±0.41^{d}	5.28±0.26^{f}	7.84±0.48^{g}	5.50±0.15^{ef}	6.51±0.26^{c}
May	3.27±0.13^{bcd}	2.09±0.40^{a}	6.01±0.14^{e}	4.58±0.16^{de}	4.21±0.33^{cd}	2.37±0.33^{bc}	1.70±0.41^{ab}	2.01±0.19^{b}	3.46±0.54^{cd}	4.24±0.35^{b}
June	4.08±0.12^{de}	4.81±0.84^{bcd}	4.23±0.19^{cd}	3.26±0.43^{bc}	7.84±0.21^{e}	5.37±0.26^{d}	5.83±0.33^{fg}	4.93±0.37^{f}	5.96±0.45^{ef}	4.26±0.71^{b}
July	4.91±0.46^{e}	5.18±0.67^{cde}	4.03±0.52^{cd}	5.31±0.60^{e}	3.84±0.04^{bc}	1.68±0.23^{ab}	2.78±0.33^{cd}	2.83±0.23^{de}	3.03±0.29^{bc}	2.74±0.18^{ab}
August	2.00±0.09^{ab}	3.84±0.30^{b}	4.94±0.46^{de}	4.64±0.49^{de}	4.20±0.40^{cd}	3.17±0.11^{c}	2.62±0.19^{bcd}	2.21±0.10^{bcd}	4.25±0.23^{d}	3.71±0.18^{b}
September	1.90±0.07^{a}	2.20±0.06^{a}	2.57±0.16^{b}	2.66±0.22^{b}	2.82±0.17^{b}	2.83±0.24^{c}	2.03±0.04^{abc}	2.08±0.03^{bc}	2.22±0.05^{b}	2.82±0.05^{ab}
October	4.25±0.47^{de}	2.40±0.16^{a}	2.74±0.23^{b}	3.51±0.32^{bc}	4.51±0.60^{cd}	2.50±0.02^{bc}	2.60±0.09^{bcd}	3.05±0.20^{e}	3.49±0.12^{cd}	3.20±0.03^{ab}
November	6.52±0.81^{f}	6.25±0.16^{e}	8.65±0.75^{g}	7.28±0.36^{g}	5.09±0.47^{d}	3.05±0.30^{c}	2.80±0.13^{cd}	2.95±0.25^{de}	2.78±0.14^{bc}	3.92±0.14^{b}
December	2.61±0.08^{ab}	4.42±0.16^{bc}	3.54±0.04^{bc}	3.83±0.18^{cd}	3.07±0.27^{b}	2.79±0.08^{c}	3.23±0.11^{de}	2.77±0.09^{cde}	2.68±0.11^{bc}	3.07±0.00^{ab}
January	7.98±0.94^{g}	5.68±0.08^{de}	6.00±0.14^{e}	3.66±0.06^{bcd}	4.26±0.50^{cd}	1.25±0.07^{a}	1.61±0.06^{a}	0.93±0.08^{a}	1.11±0.09^{a}	1.72±0.13^{a}
February	7.10±0.35^{fg}	2.49±0.02^{a}	4.24±0.28^{cd}	2.68±0.10^{b}	1.12±0.05^{a}	5.41±0.57^{d}	4.10±0.30^{e}	4.94±0.29^{f}	5.22±0.45^{e}	7.24±0.45^{c}
Mean±S.E.M.	4.28±0.34	4.05±0.26	4.46±0.34	4.07±0.31	4.18±0.37	3.50±0.27	3.40±0.27	3.41±0.30	3.84±0.27	4.38±0.37

Means not sharing a common superscript letter in the same row of the samples are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.



* Difference from PM-III extract in the absence of S9 mixture.

Figure 4.19 Comparison of growth response of PM-III to MCF-7 cells in the presence and absence of S9 mixture. (▲-S9 mixture, ■ +S9 mixture)



* Difference from PM-IV extract in the absence of S9 mixture

Figure 4.20 Comparison of growth response of PM-IV to MCF-7 cells in the presence and absence of S9 mixture. (▲-S9 mixture, ■ +S9 mixture)

P. mirifica extract in the presence of S9 mixture showed more proliferative effect than *P. mirifica* in the absence of S9 mixture (Table 4.22 and Figure 4.19-4.20). Proliferative effect of S9 mixture on PM-III and PM-IV was increased 1.12 to 8.65 and 1.11 to 9.18 folds, respectively.

The interesting from this experiment is the percentage growth of the collected PM-III in April 2005 that showed antiproliferative effect at the concentrations of 10, 100 and 1000 μg/ml with IC_{50} of 47.62 μg/ml. The collected PM-III in April 2005 showed the strongest antiproferative effect, only in the presence of S9 mixture. The result of the collected PM-III in April 2005 is shown in Table 4.23.

Table 4.23 The analyzed parameters of PM-III collected in April 2005.

Physical factors		Isoflavonoid contents (mg/100 g powder)			% cell growth			
Temperature (°C)		Chemicals	PM-III	PM-IV	Conc.	-S9		+S9	
- Maximum	37.0	Puerarin	66.74±5.05	55.12±3.45	(μg/ml)	PM-III	PM-IV	PM-III	PM-IV
- Minimum	25.5	Daidzin	42.90±0.61	27.22±3.84	0.1	90.32±4.04[a]	107.31±5.39[ab]	243.87±4.66[b*]	613.11±13.61[a*]
- Mean	31.3	Genistin	17.18±4.85	5.36±1.35	1	90.8±11.77[a]	119.35±3.45[c*]	332.42±28.31[b*]	635.55±11.58[a*]
- 6T	11.5	Daidzein	9.93±0.30	4.38±0.67	10	89.68±5.04[a]	113.01±4.13[ab*]	83.17±36.22[a*]	919.38±50.78[b*]
Rainfall (mm)	0.9	Genistein	0.49±0.15	0.71±0.22	100	89.03±4.15[a]	109.84±0.28[ab]	42.42±19.37[a*]	596.84±13.95[a*]
		Total isoflavonoid	137.24±10.67	92.80±8.25	1000	80.11±4.10[a*]	102.04±3.01[a]	71.18±23.65[a*]	669.94±9.25[a*]
		Aglycoside	10.42±0.16	5.10±0.51	IC_{50}	>1000	>1000	5.19	>1000
		Glycoside	60.08±5.46	32.58±5.21	(μg/ml)				
		Aglycoside/glycoside	0.17±0.01	0.16±0.01					
		Daidzin/puerarin	0.64±0.16	0.49±0.05					
		Genistin/puerarin	0.26±0.11	0.10±0.02					
		Daidzein/puerarin	0.15±0.05	0.08±0.01					
		Genistein/puerarin	0.01±0.00	0.01±0.00					

Means not sharing a common superscript letter in the same row of the samples are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

* Different from negative control (DMSO) $P < 0.05$.

4.11. Correlation analysis of estrogenic activity with isoflavonoid contents, rainfall amount, and temperature

There were correlations between the percentage growths of MCF-7 at the concentrations of 0.1, 1, 10, 100 and 1000 μg/ml with isoflavonoid contents followed Spearman's rho. The correlations show in Table 4.23.

Table 4.24 Correlation coefficient (R^2) of proliferation/antiproliferation to MCF-7 cells of the 5 major isoflavonoids in PM-III and PM-IV

isoflavonoid contents (μg/100 g powder)	% cell growth									
	- S9 mixture					+ S9 mixture				
PM-III	0.1[b]	1[b]	10[a]	100[a]	1000[b]	0.1[a]	1[a]	10[a]	100[a]	1000[a]
puerarin	-	-	-	-	-	-	-	-	-	-
daidzin	-	-	-0.727**	-	-	-	-0.643*	-	-0.671*	-
genistin	-	-	-	-	-	-	-	-	-	-
daidzein	-	-	-	-0.657*	-0.732**	-	-	-	-	-
genistein	-	-	-	-	-	-	-	-	-	-
total isoflavonoids	-	-	-	-	-	-	-	-	-	-
aglycoside	-	-	-	-0.636*	-0.722**	-	-	-	-	-
glycoside	-	-	-0.615*	-	-	-	-	-0.699*	-0.692*	-
aglycoside/glycoside	-	-	-	-	-	-	-	-	-	-
daidzin/puerarin	-	-	-	-0.750**	-0.825**	-	-	-	-	-
genistin/puerarin	-	-	-	-	-0.619**	-	-	-	-	-
daidzein/puerarin	-	-	-	-0.775**	-0.709**	-	-	-	-	-
genistein/puerarin	-0.654*	-	-	-	-	-	-	-	-	-
daidzein/daidzin	-	-	-	-	-	-	-	-	-	-
genistein/genistin	-	-	-	-	-	-	-	-	-	-
PM-IV	0.1[b]	1[b]	10[a]	100[a]	1000[b]	0.1[a]	1[a]	10[a]	100[a]	1000[a]
puerarin	-	-	-	-	-	-	-	-	-	-
daidzin	-	-	-	-	-	0.657*	0.608*	0.650*	0.629*	-
genistin	-	-	-	-	-	-	-	-	-	-
daidzein	-	-	-	-	-	0.601*	0.601*	-	-	-
genistein	-	0.683*	-	-	-	-	-	-	-	-
total isoflavonoids	-	-	-	-	-	-	-	-	-	-
aglycoside	-	-	-	-	-	-	-	-	-	-
glycoside	-	-	-	-	-	-	0.608*	0.657*	-	-
aglycoside/glycoside	-	-	-	-	-	-	-	-	-	-
daidzin/puerarin	-	-	-	-	-	-	-	-	-	-
genistin/puerarin	-	-	-	-	-	-	-	-	-	-
daidzein/puerarin	-	-	-	-	-	-	-	-	-	-
genistein/puerarin	-	0.578*	-	-	-	-	-	-	-	-
Daidzein/daidzin	-	-	-	-	-	-	-	-	-	-
Genistein/genistin	-	-	-	-	-	-	-	-	-	-

(*), (**): Correlation is significant at $P < 0.05$ and 0.01 (2-tailed), respectively.

(-): No correlation

([a]): proliferation, ([b]): antiproliferation

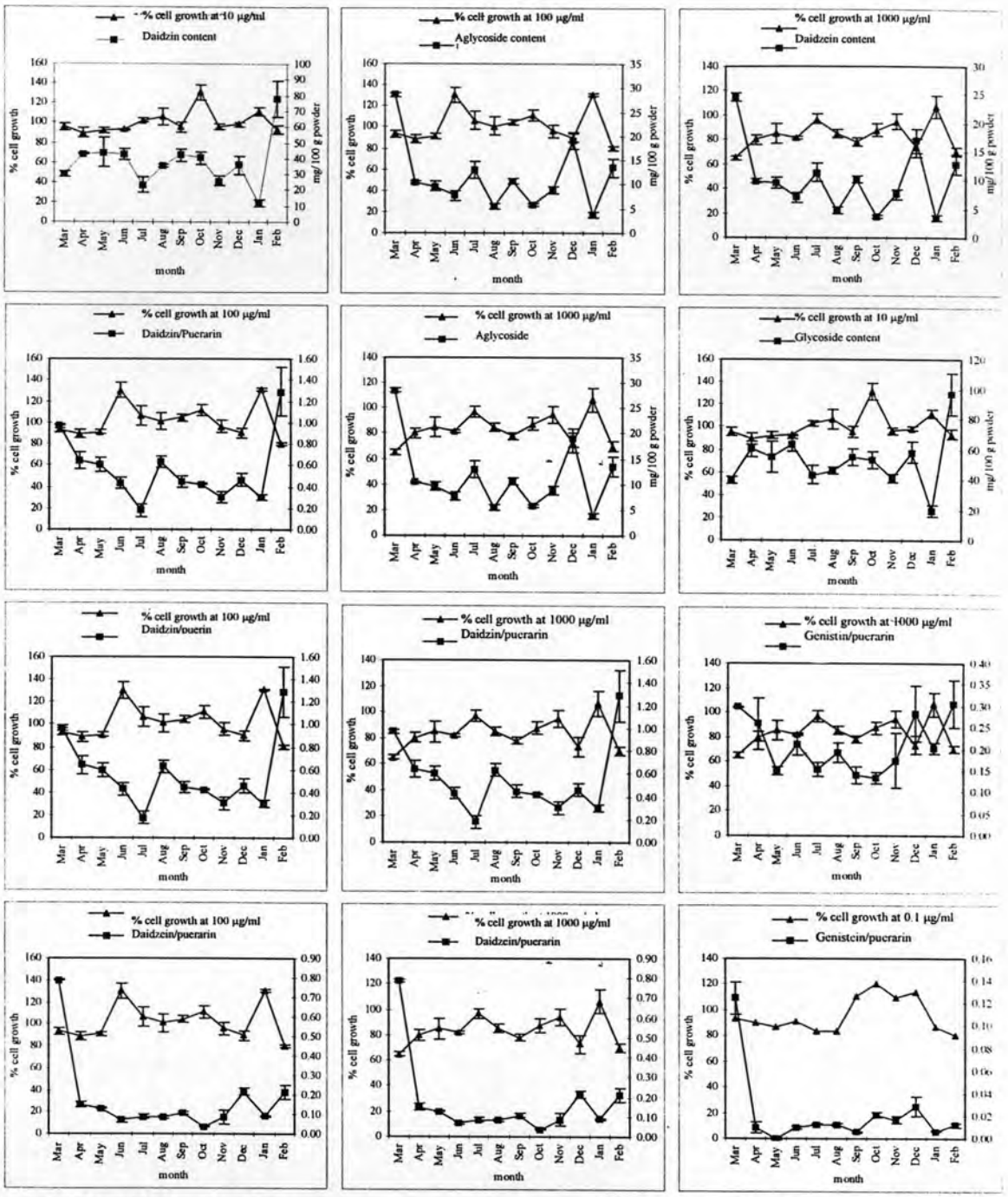


Figure 4.21 Correlation profile of percentage cell growth of MCF-7 in the absence of S9 mixture and isoflavonoid contents in PM-III.

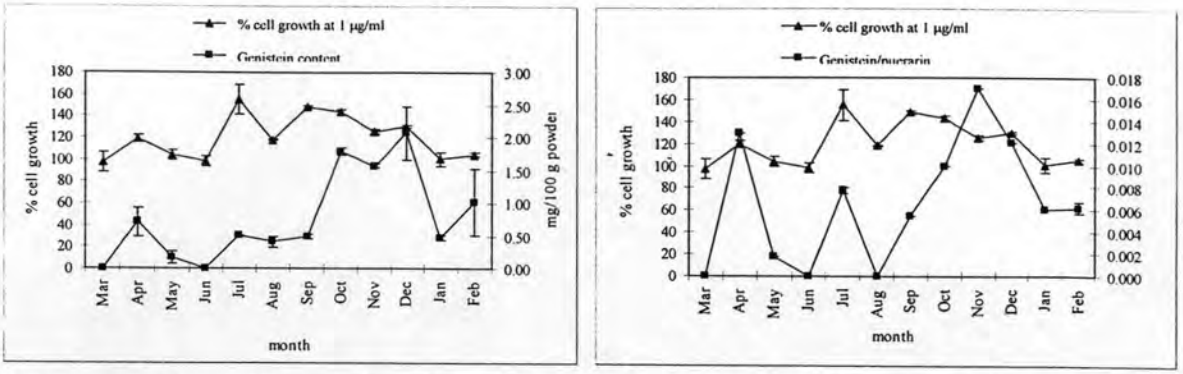


Figure 4.22 Correlation profile of percentage cell growth of MCF-7 in the absence of S9 mixture and isoflavonoid contents in PM-IV.

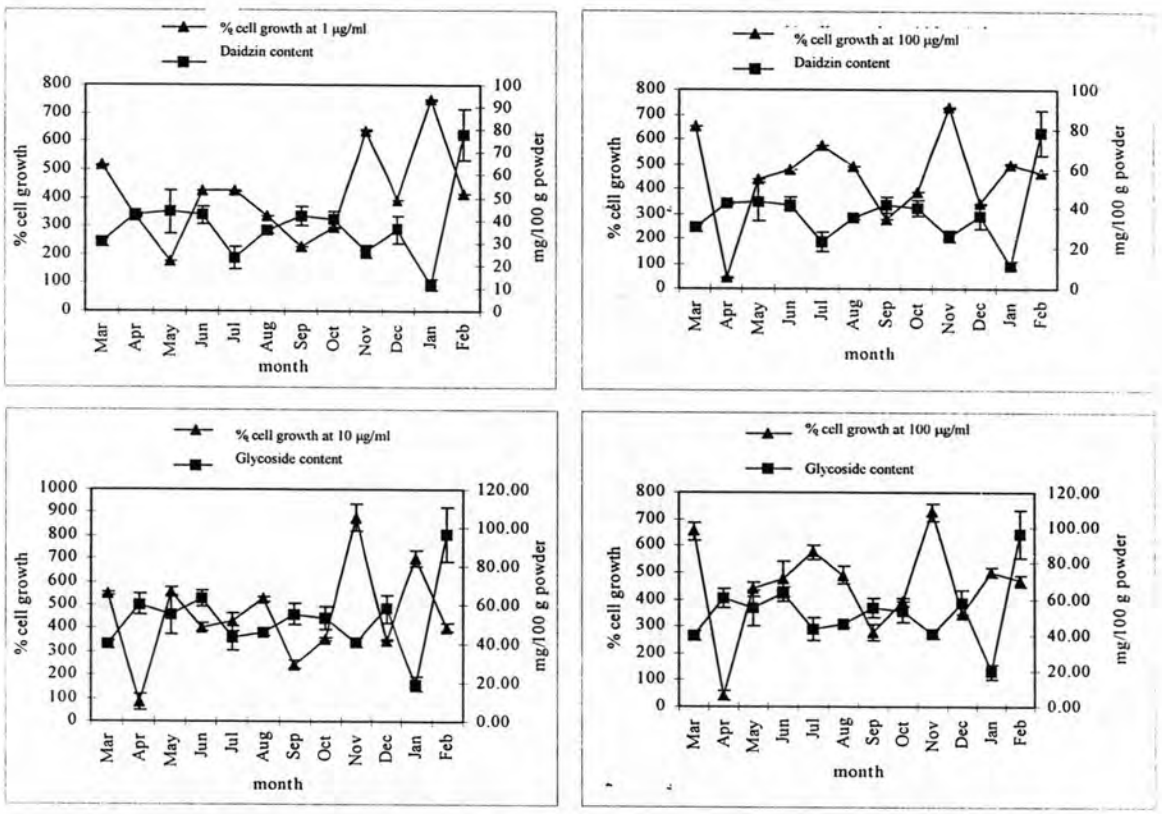


Figure 4.23 Correlation profile of percentage cell growth of MCF-7 in the presence of S9 mixture and isoflavonoid contents in PM-III.

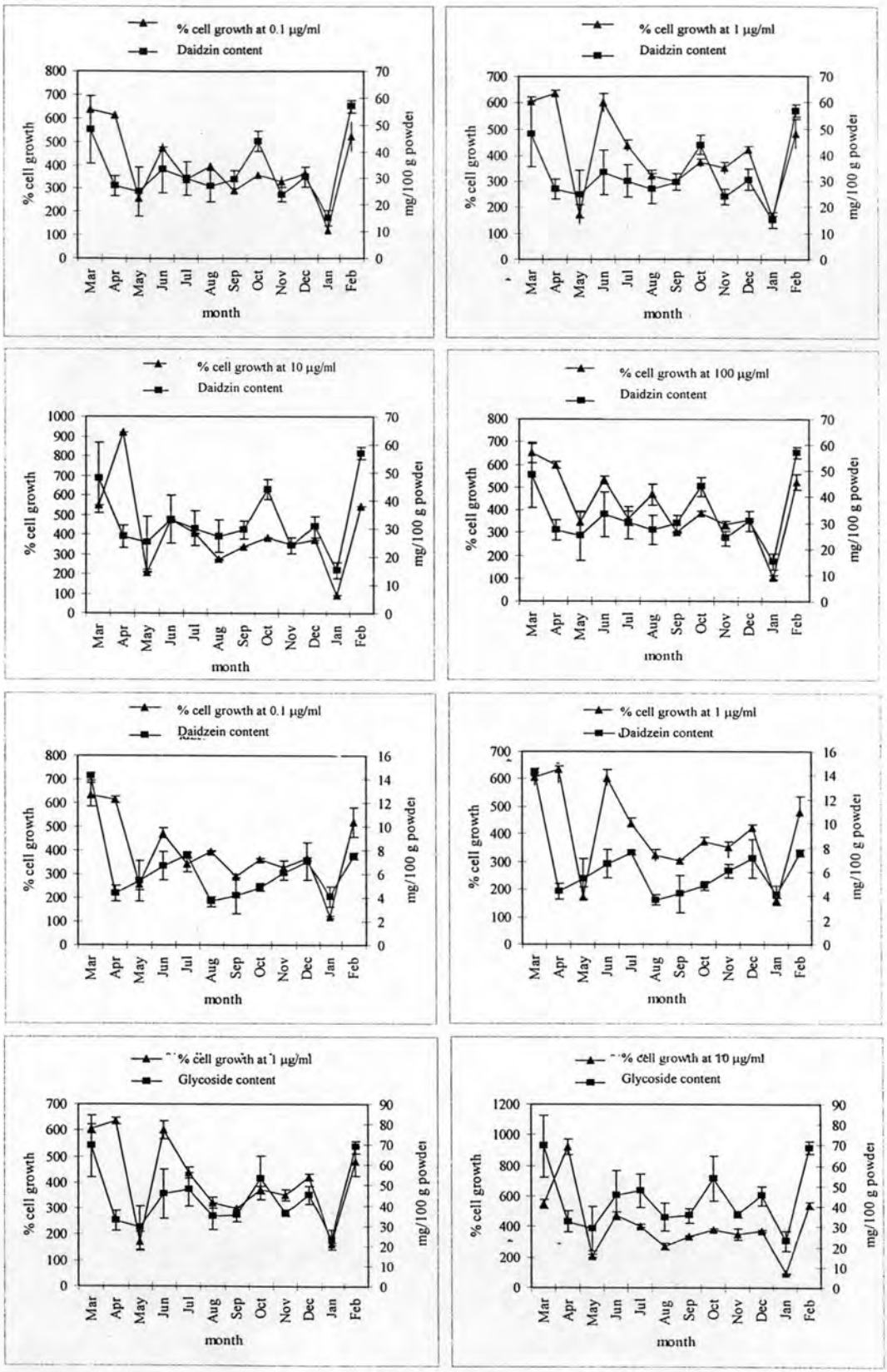


Figure 4.24 Correlation profile of percentage cell growth of MCF-7 in the presence of S9 mixture and isoflavonoid contents in PM-IV.

There were correlations between the percentage growths of MCF-7 at the concentrations of 0.1, 1, 10, 100 and 1000 μg/ml with air temperature, and amount of rainfall followed Spearman's rho. The correlations are shown in Table 4.23.

Table 4.25 Correlation coefficient (R^2) of proliferation/antiproliferation to MCF-7 cells of physical factors, including temperature and rainfall in PM-III and PM-IV.

P. mirifica	Physical factor	% cell growth									
		- S9 mixture					+ S9 mixture				
		0.1	1	10	100	1000	0.1	1	10	100	1000
PM-III	mean temperature (°C)	-	-	0.685*	-	-	-	-	-	-	-
	rainfall (mm)	-	-	-	-	-	-	-	-	-	-
PM-IV	mean temperature (°C)	-	-	-	-	-	-	-	-	-	-
	rainfall (mm)	-	0.601*	-	-	0.699*	-	-	-	-	-

(*) Correlation is significant at $P < 0.05$ (2-tailed).

(-) No correlation.

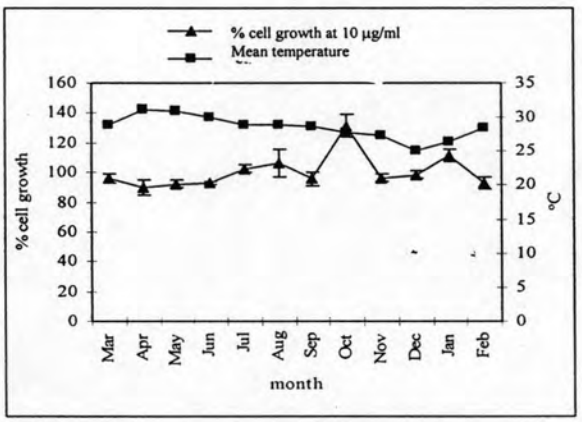


Figure 4.25 Correlation profile of percentage cell growth of MCF-7 and temperature of Ratchaburi Province during March 2005 to February 2006 in PM-III in the absence of S9 mixture.

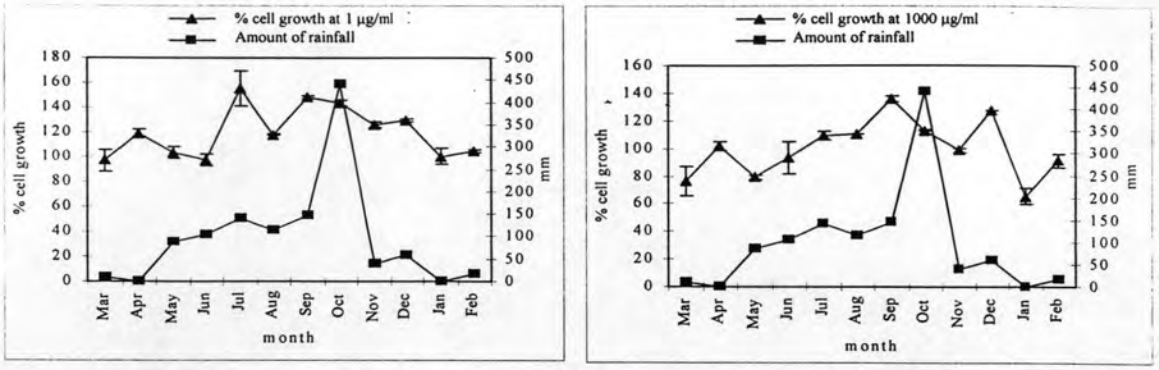


Figure 4.26 Correlation profile of percentage cell growth of MCF-7 and temperature of Ratchaburi Province during March 2005 to February 2006 in PM-III in the absence of S9 mixture.

There was no correlation between the percentage growths of MCF-7 treated with PM-III and PM-IV at the concentrations of 0.1, 1, 10, 100 and 1000 µg/ml followed Spearman's rho.

Table 4.26 Percentage growths of MCF-7 were treated with PM-III extracts in correlation with PM-IV extracts.

Correlation		PM-III	
		-S9	+S9
PM-IV	-S9	-	N.A.
	+S9	N.A.	-

(*): Correlation is significant at $P < 0.05$ (2-tailed).

(-): No correlation.

(N.A.): Non Analysis.