CHAPTER IV

RESULTS

4.1. Plant morphometry

4.1.1. Leaf morphometry

The leaf morphometry of PM-I, PM-II, PM-III, PM-IV and PM-V is shown in Table 4.1.

Table 4.1 Leaf morphometry analysis of 5 clones of *P. mirifica* with parameter of leaf including, petiole length (PL), petiole diameter (PD), rachis length (RL), petiolet length (PLL), terminal leaflet length (TLL), terminal leaflet breadth (TLB), stipule length (SPL), angle of first leaf border $(A^\wedge B)^o$ and number of pairs of primary veins (NPV) (Mean±S.E.M.).

PM	Leaf								
	PL (cm)	PD (cm)	RL (cm)	PLL (cm)	TLL (cm)	TLB (cm)	SPL (cm)	A^B (^{o})	NPV
PM-I	18.24 ± 0.62^{a}	0.36 ± 0.047^{ab}	4.84 ± 0.13^{ab}	0.70 ± 0.014^{a}	23.05 ± 0.31^{bc}	14.51 ± 0.22^{a}	0.36 ± 0.006^{b}	37.53 ± 0.93^{c}	6.33 ± 0.11^{a}
PM-II	21.18 ± 0.61^{ab}	0.338 ± 0.011^{ab}	6.24 ± 0.13^{d}	0.93 ± 0.023^{bc}	22.54 ± 0.43^{bc}	18.29 ± 0.41^{c}	0.37 ± 0.009^{b}	38.96 ± 0.68^{c}	6.94 ± 0.10^{a}
PM-III	25.75 ± 1.16^{c}	0.345 ± 0.009^{b}	5.45 ± 0.19^{bc}	0.99 ± 0.029^{c}	21.53 ± 0.36^{b}	16.00 ± 0.37^{ab}	0.37 ± 0.01^{b}	29.83 ± 1.13^{b}	6.81 ± 0.13^{a}
PM-IV	32.44 ± 0.80^{d}	0.44 ± 0.006^{b}	7.68 ± 0.15^{e}	0.93 ± 0.016^{bc}	24.34 ± 0.39^{c}	20.59 ± 0.41^{d}	0.42 ± 0.01^{c}	23.76 ± 0.86^{a}	7.84 ± 0.12^{b}
PM-V	17.64 ± 0.33^{a}	0.28 ± 0.004^{a}	4.32 ± 0.07^{a}	0.61 ± 0.012^{a}	15.39 ± 0.19^{a}	14.82 ± 0.21^{a}	0.13 ± 0.007^{a}	21.18 ± 0.59^{a}	6.80 ± 0.07^{a}
Mean±S.E.M.	23.05 ± 0.48^{bc}	0.35 ± 0.10^{ab}	5.71 ± 0.10^{cd}	0.83 ± 0.13^{b}	21.37 ± 0.25^{b}	16.84 ± 0.21^{bc}	0.33 ± 0.008^{b}	30.25 ± 0.59^{b}	6.94 ± 0.06^{a}

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

4.1.2. Pod

Pod morphometry of PM-I, PM-II, PM-III, PM-IV and PM-V is shown in table 4.2.

Table 4.2 Pod morphometry of 5 cultivars of *P. mirifica* (Mean±S.E.M.).

PM	Pod		
	length (cm)	width (cm)	seed/pod
PM-I	5.71 ± 0.25^{d}	0.79 ± 0.008^{bc}	2.96 ± 0.19^{b}
PM-II	2.97 ± 0.11^{a}	0.57 ± 0.012^{a}	2.32 ± 0.14^{ab}
PM-III	6.10 ± 0.11^{d}	0.88 ± 0.014^{c}	5.90 ± 0.16^{c}
PM-IV	4.31 ± 0.13^{c}	0.85 ± 0.021^{c}	2.82 ± 0.14^{ab}
PM-V	3.33 ± 0.12^{ab}	0.62 ± 0.008^{a}	1.98 ± 0.13^{a}
Mean±S.E.M.	4.10 ± 0.10^{bc}	0.72 ± 0.009^{b}	2.59 ± 0.08^{ab}

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

4.1.3. Tuber

The tuberous fresh weight, dry weight and percentage of water content in the tubers of PM-I, PM-II, PM-III, PM-IV and PM-V are shown in Table 4.3.

Table 4.3 The tuberous fresh weight, dry weight and percentage of water content in the tubers (Mean±S.E.M.)

Season	Clone	Fresh weight (g)	Dry weight (g)	% water
Summer	PM-I	912.90±150.64^{a}	84.13±8.17^{a}	90.50±0.96^{ab}
	PM-II	1045.61±742.91^{a}	95.88±66.27^{a}	90.09±1.15^{ab}
	PM-III	1421.89±310.14^{a}	137.17±31.56^{a}	90.37±0.38^{ab}
	PM-IV	1670.61±266.09^{a}	184.37±22.54^{a}	88.77±1.00^{a}
	PM-V	2046.52±1477.67^{a}	99.83±50.39^{a}	92.93±1.62^{b}
	Mean±SEM	1419.51±309.44^{a}	120.27±18.41^{a}	90.53±0.55^{ab}
Rainy season	PM-I	997.13±212.73^{a}	91.57±21.39^{a}	90.38±1.97^{ab}
	PM-II	1356.20±145.06^{a}	101.37±10.97^{a}	92.13±1.84^{ab}
	PM-III	1201.63±226.87^{a}	100.36±19.61^{a}	91.61±0.50^{ab}
	PM-IV	2401.44±749.46^{a}	252.24±63.32^{b}	89.06±1.46^{a}
	PM-V	1746.21±609.97^{a}	103.56±35.89^{a}	93.90±0.42^{b}
	Mean±SEM	1540.52±218.13^{a}	129.82±21.16^{a}	91.42±0.69^{ab}
Winter	PM-I	1096.87±138.95^{a}	120.93±6.88^{a}	88.77±0.81^{a}
	PM-II	1564.46±125.64^{ab}	162.95±24.52^{ab}	89.67±0.83^{a}
	PM-III	1500.67±256.04^{ab}	166.33±42.78^{ab}	88.32±2.92^{a}
	PM-IV	2210.43±210.16^{b}	243.09±35.84^{b}	89.03±1.14^{a}
	PM-V	1594.96±281.32^{ab}	156.61±11.6^{ab}	89.61±1.75^{a}
	Mean±SEM	1593.48±124.48^{ab}	169.98±15.02^{ab}	89.08±0.65^{a}

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

4.1.4. The plant crude extracts weight

The plant crude extract weights are shown in Table 4.4.

Table 4.4 The plant crude extract weights (g) of seasonal collected *P. mirifica* in 2005 derived from ethanolic extraction of 50 g powder.

PM	Weight (g)		
	Summer	Rainy season	Winter
PM-I	2.08	2.04	0.68
PM-II	2.17	1.71	1.12
PM-III	1.09	1.62	1.10
PM-IV	4.01	2.63	2.31
PM-V	1.53	1.52	1.23
Mean±S.E.M.	2.18±0.50	1.90±0.20	1.28±0.27

Table 4.5 The percentage of relative plant crude extract weights derived from ethanolic extraction of 50 g powder.

PM	% relative crude extract		
	Summer	Rainy season	Winter
PM-I	4.16	4.08	1.36
PM-II	4.34	3.42	2.24
PM-III	2.18	3.24	2.20
PM-IV	8.02	5.26	4.62
PM-V	3.06	3.04	2.46
Mean±S.E.M.	4.35±1.00	3.81±0.40	2.58±0.54

The characteristic of the plant extracts is sticky wax-like material with brown color. Smell likes ground peanut.

4.2. Physical factors

The two physical factors, amount of rainfall and temperature in Ratchaburi Province were monthly recorded by the Meteorological Department, Ministry of Information and Communication Technology. The study period was described according to the rainfall and temperature into 3 seasons as follow; summer (February-May), rainy season (June-October) and winter (November-January).

Table 4.6 The monthly climate record including, maximum, minimum and mean temperature, and amount of rainfall in Ratchaburi Province in 2005.

Month	Temperature (°C)				rainfall (mm)
	maximum	minimum	mean	ΔT	
January	32.9	26.4	26.4	6.5	6.5
February	35.4	29.1	29.1	6.3	0.0
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	33.0	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	8.0	58.7

Data from Meteorological Department, Ministry of Information and Communication Technology

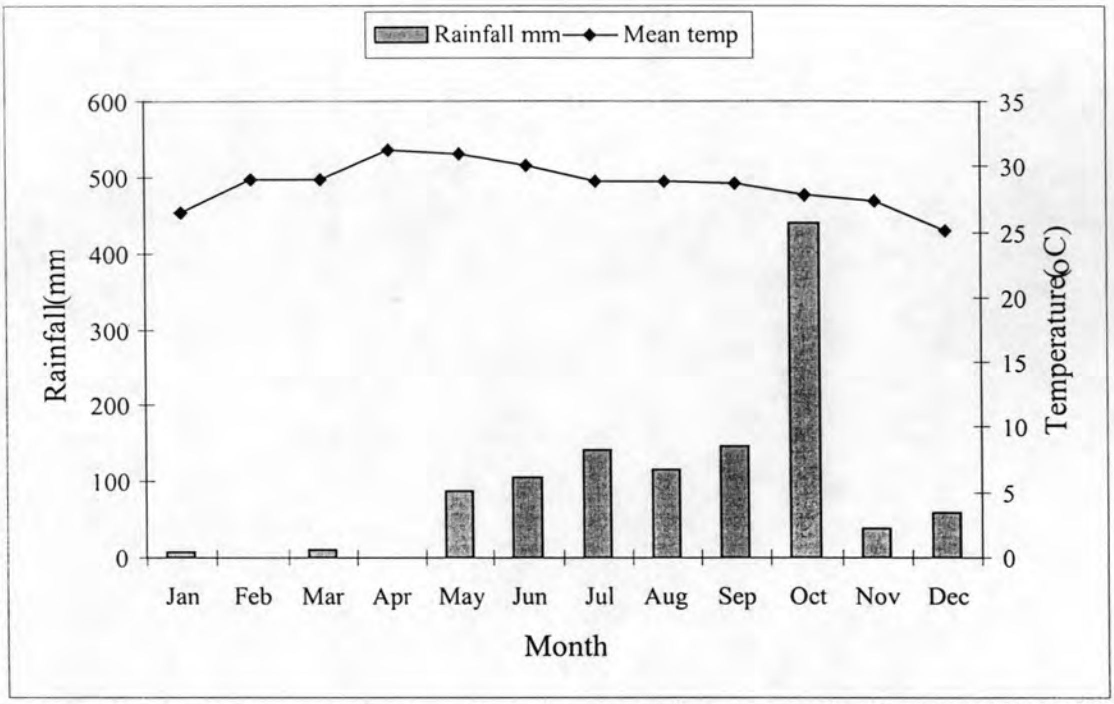


Figure 4.1 Climatic conditions during the experiment. In April 2005, the mean temperature was 31.3 °C, and the amount of rainfall was 0.9 mm. In August 2005, the mean temperature was 28.7 °C, and the amount of rainfall was 114.8 mm. In December 2005, the mean temperature was 25.1 °C, and the amount of rainfall was 58.7 mm.

4.3. Isoflavonoid HPLC fingerprints analysis of seasonal collected of *P. mirifica* in 2005

HPLC analysis of isoflavonoid contents of 5 clones of *P. mirifica* in three seasons collected in 2005 exhibited significant variation of the chemicals. In summer the maximum amount of total isoflavonoid and puerarin was found in PM-IV, maximum daidzin and genistin was found in PM-II, and maximum daidzein and genistein was found in PM-III. In rainy season the maximum amount of total isoflavonoid and puerarin was found in PM-III, maximum daidzin was found in PM-V, maximum genistin and daidzein was found in PM-II and maximum genistein was found in PM-I. In winter the maximum total isoflavonoid, puerarin, daidzin and genistin were found in PM-IV, maximum daidzein was found in PM-I and maximum genistein was found in PM-V.

Table 4.7 Isoflavonoid contents in mg/100g of *P. mirifica* tuberous powder cultivated in the same place during summer, rainy season and winter (Mean ± S.E.M.)

Summer 2005

PM	Puerarin	Daidzin	Genistin	Daidzein	Genistein	Total
PM-I	56.50±14.79^{ab}	26.30±2.56^{ab}	5.66±2.24^{a}	16.09±8.52^{a}	3.74±2.49^{ab}	108.30±21.52^{a}
PM-II	90.42±24.87^{bc}	54.22±26.46^{b}	18.97±11.97^{a}	14.26±5.79^{a}	1.88±1.88^{ab}	179.74±66.86^{a}
PM-III	28.30±3.22^{a}	26.34±4.43^{ab}	12.41±3.10^{a}	19.36±5.35^{a}	5.61±1.79^{b}	92.03±8.45^{a}
PM-IV	127.22±16.47^{c}	37.14±5.40^{ab}	10.48±1.39^{a}	6.42±0.73^{a}	0.00±0.00^{a}	181.26±22.24^{a}
PM-V	52.92±22.41^{ab}	12.22±1.86^{a}	5.42±2.13^{a}	9.78±3.09^{a}	0.35±0.35^{a}	80.69±22.38^{a}
Mean ± S.E.M.	71.07±11.43$^{a'}$	31.24±5.96$^{a'}$	10.59±2.54$^{a'}$	13.18±2.37$^{a'}$	2.32±0.83$^{a'}$	128.40±17.49$^{a'}$

Rainy season 2005

PM	Puerarin	Daidzin	Genistin	Daidzein	Genistein	Total
PM-I	91.26±15.29^{ab}	35.48±6.84^{a}	17.81±3.49^{a}	14.13±3.00^{a}	15.23±3.66^{b}	173.90±19.67^{a}
PM-II	76.59±14.70^{a}	32.08±9.03^{a}	26.40±9.23^{a}	16.70±4.70^{a}	4.96±3.55^{a}	156.73±40.95^{a}
PM-III	132.05±13.79^{b}	28.03±3.25^{a}	16.57±1.35^{a}	12.12±1.77^{a}	2.38±0.07^{a}	191.16±10.21^{a}
PM-IV	131.11±18.20^{b}	29.48±6.19^{a}	17.02±1.72^{a}	8.66±0.54^{a}	2.36±2.36^{a}	188.64±20.81^{a}
PM-V	77.75±22.35^{a}	38.01±2.26^{a}	15.68±2.16^{a}	8.57±3.32^{a}	0.00±0.00^{a}	140.01±21.59^{a}
Mean ± S.E.M.	101.75±9.29$^{b'}$	32.62±2.49$^{a'}$	18.70±2.04$^{c'}$	12.04±1.42$^{a'}$	4.99±1.72$^{b'}$	170.09±10.70$^{b'}$

Winter 2005

PM	Puerarin	Daidzin	Genistin	Daidzein	Genistein	Total
PM-I	91.73±23.29^{a}	30.05±4.38^{a}	12.81 ±0.98^{ab}	14.58±1.46^{b}	0.77±0.26^{a}	149.93±23.21^{ab}
PM-II	77.64±20.38^{a}	26.96±2.88^{a}	11.09±1.05^{a}	10.72±2.59^{ab}	1.51±0.51^{a}	127.92±20.57^{a}
PM-III	87.96±20.78^{a}	35.72±4.33^{ab}	15.05±1.11^{bc}	7.73±0.79^{a}	1.96±0.71^{a}	148.42±23.51^{ab}
PM-IV	168.69±48.13^{a}	50.09±11.13^{b}	16.61±1.35^{c}	11.40±2.33^{ab}	2.51±1.94^{a}	249.31±60.63^{b}
PM-V	116.73±28.06^{a}	42.66±5.66^{ab}	13.53±1.49^{abc}	9.51±1.13^{ab}	2.61±1.77^{a}	185.04±32.21^{ab}
Mean ± S.E.M.	108.55 ±14.31$^{b'}$	37.10±3.29$^{a'}$	13.82±0.68$^{b'}$	10.79±0.91$^{a'}$	1.87 ±0.50$^{a'}$	172.13±17.56$^{b'}$

Means not sharing a common superscript letter with or without prime in the same column are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

Table 4.8 Isoflavonoid contents in mg/100g of *P. mirifica* tuberous powder collected from five clones cultivated in the same place during summer, rainy season and winter (Mean ± S.E.M.)

		Puerarin	Daidzin	Genistin	Daidzein	Genistein	Total isoflavonoid
PM-I	Summer	56.50±14.79^{a}	26.30±2.56^{a}	5.66±2.24^{a}	16.09±8.52^{a}	3.74±2.49^{a}	108.30±21.52^{a}
	Rainy season	91.26±15.29^{a}	35.48±6.84^{a}	17.81±3.49^{b}	14.13±3.00^{a}	15.23±3.66^{b}	173.90±19.67^{a}
	Winter	91.73±23.29^{a}	30.05±4.38^{a}	12.81 ±0.98^{ab}	14.58±1.46^{a}	0.77±0.26^{a}	149.93±23.21^{a}
	Mean± S.E.M.	79.83±10.81$^{a'}$	30.61±2.80$^{a'}$	12.09±2.15$^{a'}$	14.93±2.66$^{b'}$	6.58±2.55$^{b'}$	144.05±14.01$^{a'}$
PM-II	Summer	90.42±24.87^{a}	54.22±26.46^{a}	18.97±11.97^{a}	14.26±5.79^{a}	1.88±1.88^{a}	179.74±66.86^{a}
	Rainy season	76.59±14.70^{a}	32.08±9.03^{a}	26.40±9.23^{a}	16.70±4.70^{a}	4.96±3.55^{a}	156.73±40.95^{a}
	Winter	77.64±20.38^{a}	26.96±2.88^{a}	11.09±1.05^{a}	10.72±2.59^{a}	1.51±0.51^{a}	127.92±20.57^{a}
	Mean± S.E.M.	81.55±10.44$^{a'}$	37.75±9.13$^{a'}$	18.82±4.90$^{b'}$	13.89±2.44$^{b'}$	2.78±1.29$^{a'}$	154.80±24.57$^{a'}$
PM-III	Summer	28.30±3.22^{a}	26.34±4.43^{a}	12.41±3.10^{a}	19.36±5.35^{a}	5.61±1.79^{a}	92.03±8.45^{a}
	Rainy season	132.05±13.79^{b}	28.03±3.25^{a}	16.57±1.35^{a}	12.12±1.77^{a}	2.38±0.07^{a}	191.16±10.21^{b}
	Winter	87.96±20.78^{b}	35.72±4.33^{a}	15.05±1.11^{a}	7.73±0.79^{a}	1.96±0.71^{a}	148.42±23.51^{b}
	Mean± S.E.M.	82.77±16.69$^{a'}$	30.03±2.48$^{a'}$	14.68±1.19$^{ab'}$	13.07±2.36$^{b'}$	3.32±0.80$^{a'}$	143.87±16.33$^{a'}$
PM-IV	Summer	127.22±16.47^{a}	37.14±5.40^{a}	10.48±1.39^{a}	6.42±0.73^{a}	0.00±0.00^{a}	181.26±22.24^{a}
	Rainy season	131.11±18.20^{a}	29.48±6.19^{a}	17.02±1.72^{b}	8.66±0.54^{a}	2.36±2.36^{a}	188.64±20.81^{a}
	Winter	168.69±48.13^{a}	50.09±11.13^{a}	16.61±1.35^{b}	11.40±2.33^{a}	2.51±1.94^{a}	249.31±60.63^{a}
	Mean± S.E.M.	142.34±16.94$^{b'}$	38.90±4.50$^{a'}$	14.71±1.30$^{ab'}$	8.83±1.02$^{a'}$	1.62±0.97$^{a'}$	206.40±22.36$^{b'}$
PM-V	Summer	52.92±22.41^{a}	12.22±1.86^{a}	5.42±2.13^{a}	9.78±3.09^{a}	0.35±0.35^{a}	80.69±22.38^{a}
	Rainy season	77.75±22.35^{a}	38.01±2.26^{b}	15.68±2.16^{b}	8.57±3.32^{a}	0.00±0.00^{a}	140.01±21.59^{ab}
	Winter	116.73±28.06^{a}	42.66±5.66^{b}	13.53±1.49^{b}	9.51±1.13^{b}	2.61±1.77^{a}	185.04±32.21^{b}
	Mean± S.E.M.	82.46±15.34$^{a'}$	30.96±5.08$^{a'}$	11.54±1.84$^{a'}$	9.29±1.36$^{a'}$	0.99±0.66$^{a'}$	135.25±19.88$^{a'}$

Means not sharing a common superscript letter with or without prime in the same column are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

Table 4.9 The isoflavonoid contents of *P. mirifica* collected in three seasons showed significant variation with maximum amount of aglycoside (daidzein and genistein) and glycoside (daidzin and genistin)

Summer	aglycoside	glycoside	aglycoside/glycoside	glycoside/aglycoside	glycoside+aglycoside/puerarin
PM-I	19.83 ± 11.0^{a}	31.97 ± 1.20^{a}	0.60 ± 0.32^{a}	3.42 ± 1.93^{ab}	1.00 ± 0.23^{a}
PM-II	16.14 ± 7.46^{a}	73.19 ± 38.42^{a}	0.25 ± 0.10^{a}	5.48 ± 2.04^{ab}	0.95 ± 0.27^{a}
PM-III	24.97 ± 3.56^{a}	38.75 ± 2.48^{a}	0.64 ± 0.08^{a}	1.61 ± 0.23^{a}	2.27 ± 0.09^{b}
PM-IV	6.42 ± 0.73^{a}	47.62 ± 5.69^{a}	0.14 ± 0.001^{a}	7.40 ± 0.07^{b}	0.43 ± 0.02^{a}
PM-V	10.13 ± 3.44^{a}	17.64 ± 3.41^{a}	0.60 ± 0.17^{a}	2.10 ± 0.78^{a}	0.73 ± 0.33^{a}
Mean ± S.E.M	$15.50\pm2.98^{ab'}$	$41.83\pm8.25^{a'}$	$0.45\pm0.09^{b'}$	$4.00\pm0.76^{a'}$	$1.08\pm0.19^{b'}$

Rainy season	aglycoside	glycoside	aglycoside/glycoside	glycoside/aglycoside	glycoside+aglycoside/puerarin
PM-I	29.36 ± 6.51^{b}	53.29 ± 9.72^{a}	0.62 ± 0.21^{b}	2.19 ± 0.90^{a}	0.94 ± 0.14^{a}
PM-II	21.66 ± 8.24^{ab}	58.48 ± 18.10^{a}	0.35 ± 0.03^{ab}	2.85 ± 0.20^{a}	0.99 ± 0.14^{a}
PM-III	14.51 ± 1.83^{ab}	44.60 ± 4.52^{a}	0.34 ± 0.07^{ab}	3.21 ± 0.57^{a}	0.46 ± 0.08^{a}
PM-IV	11.03 ± 1.93^{a}	46.50 ± 4.48^{a}	0.25 ± 0.07^{a}	4.55 ± 0.97^{a}	0.45 ± 0.05^{a}
PM-V	8.57 ± 3.32^{a}	53.69 ± 3.77^{a}	0.17 ± 0.07^{a}	13.34 ± 9.13^{a}	0.97 ± 0.30^{a}
Mean ± S.E.M	$17.02\pm2.79^{b'}$	$51.31\pm3.93^{a'}$	$0.35\pm0.06^{ab'}$	$5.23\pm1.91^{a'}$	$0.76\pm0.09^{a'}$

Winter	aglycoside	glycoside	aglycoside/glycoside	glycoside/aglycoside	glycoside+aglycoside/puerarin
PM-I	15.35 ± 1.71^{a}	42.86 ± 4.13^{ab}	0.36 ± 0.05^{a}	2.86 ± 0.41^{a}	0.77 ± 0.28^{a}
PM-II	12.23 ± 2.12^{a}	38.05 ± 3.51^{a}	0.33 ± 0.08^{a}	3.33 ± 0.67^{a}	0.73 ± 0.16^{a}
PM-III	9.69 ± 1.33^{a}	50.77 ± 4.60^{ab}	0.19 ± 0.02^{a}	5.37 ± 0.64^{a}	0.77 ± 0.17^{a}
PM-IV	13.92 ± 3.52^{a}	66.70 ± 12.41^{b}	0.22 ± 0.08^{a}	5.43 ± 1.40^{a}	0.51 ± 0.06^{a}
PM-V	12.12 ± 2.85^{a}	56.19 ± 6.79^{ab}	0.22 ± 0.06^{a}	5.11 ± 1.18^{a}	0.64 ± 0.11^{a}
Mean ± S.E.M	$12.66\pm1.05^{a'}$	$50.91\pm3.80^{a'}$	$0.267\pm0.03^{a'}$	$4.42\pm0.46^{a'}$	$0.68\pm0.07^{a'}$

Means not sharing a common superscript letter with or without prime in the same column are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

Table 4.10 The isoflavonoid contents of five clones of *P. mirifica* collected in three seasons showed significant variation with maximum amount of aglycoside (daidzein and genistein) and glycoside (daidzin and genistin)

		aglycoside	glycoside	aglycoside/glycoside	glycoside/aglycoside	glycoside+aglycoside/puerarin
PM-I	Summer	19.83 ± 11.0^{a}	31.97 ± 1.20^{a}	0.60 ± 0.32^{a}	3.42 ± 1.93^{a}	1.00 ± 0.23^{a}
	Rainy season	29.36 ± 6.51^{a}	53.29 ± 9.72^{a}	0.62 ± 0.21^{a}	2.19 ± 0.90^{a}	0.94 ± 0.14^{a}
	Winter	15.35 ± 1.71^{a}	42.86 ± 4.13^{a}	0.36 ± 0.05^{a}	2.86 ± 0.41^{a}	0.77 ± 0.28^{a}
	Mean± S.E.M.	$21.51\pm4.26^{b'}$	$42.71\pm4.35^{a'}$	$0.53\pm0.12^{c'}$	$2.82\pm0.65^{a'}$	$0.90\pm0.12^{b'}$
PM-II	Summer	16.14 ± 7.46^{a}	73.19 ± 38.42^{a}	0.25 ± 0.10^{a}	5.48 ± 2.04^{a}	0.95 ± 0.27^{a}
	Rainy season	21.66 ± 8.24^{a}	58.48 ± 18.10^{a}	0.35 ± 0.03^{a}	2.85 ± 0.20^{a}	0.99 ± 0.14^{a}
	Winter	12.23 ± 2.12^{a}	38.05 ± 3.51^{a}	0.33 ± 0.08^{a}	3.33 ± 0.67^{a}	0.73 ± 0.16^{a}
	Mean± S.E.M.	$16.68\pm3.54^{b'}$	$56.57\pm13.31^{a'}$	$0.31\pm0.04^{ab'}$	$3.89\pm0.74^{ab'}$	$0.89\pm0.11^{b'}$
PM-III	Summer	24.97 ± 3.56^{b}	38.75 ± 2.48^{a}	0.64 ± 0.08^{b}	1.61 ± 0.23^{a}	2.27 ± 0.09^{c}
	Rainy season	14.51 ± 1.83^{a}	44.60 ± 4.52^{a}	0.34 ± 0.07^{a}	3.21 ± 0.57^{b}	0.46 ± 0.08^{a}
	Winter	9.69 ± 1.33^{a}	50.77 ± 4.60^{a}	0.19 ± 0.02^{a}	5.37 ± 0.64^{c}	0.77 ± 0.17^{ab}
	Mean± S.E.M.	$16.39\pm2.56^{b'}$	$44.71\pm2.64^{a'}$	$0.39\pm0.07^{bc'}$	$3.39\pm0.60^{ab'}$	$1.17\pm0.29^{b'}$
PM-IV	Summer	6.42 ± 0.73^{a}	47.62 ± 5.69^{a}	0.14 ± 0.001^{a}	7.40 ± 0.07^{a}	0.43 ± 0.02^{a}
	Rainy season	11.03 ± 1.93^{a}	46.50 ± 4.48^{a}	0.25 ± 0.07^{a}	4.55 ± 0.97^{a}	0.45 ± 0.05^{a}
	Winter	13.92 ± 3.52^{a}	66.70 ± 12.41^{a}	0.22 ± 0.08^{a}	5.43 ± 1.40^{a}	0.51 ± 0.06^{a}
	Mean± S.E.M.	$10.45\pm1.61^{a'}$	$53.61\pm5.29^{a'}$	$0.20\pm0.03^{a'}$	$5.80\pm0.65^{bc'}$	$0.46\pm0.30^{a'}$
PM-V	Summer	10.13 ± 3.44^{a}	17.64 ± 3.41^{a}	0.60 ± 0.17^{b}	2.10 ± 0.78^{a}	0.73 ± 0.33^{a}
	Rainy season	8.57 ± 3.32^{a}	53.69 ± 3.77^{b}	0.17 ± 0.07^{a}	13.34 ± 9.13^{a}	0.97 ± 0.30^{a}
	Winter	12.12 ± 2.85^{a}	56.19 ± 6.79^{b}	0.22 ± 0.06^{a}	5.11 ± 1.18^{a}	0.64 ± 0.11^{a}
	Mean± S.E.M.	$10.27\pm1.69^{a'}$	$42.51\pm6.69^{a'}$	$0.33\pm0.09^{ab'}$	$6.85\pm3.15^{c'}$	$0.78\pm0.14^{ab'}$

Means not sharing a common superscript letter with or without prime in the same column are significantly different ($P<0.05$) as determine by Duncan's multiple range test.

Summer

Rainy

Winter

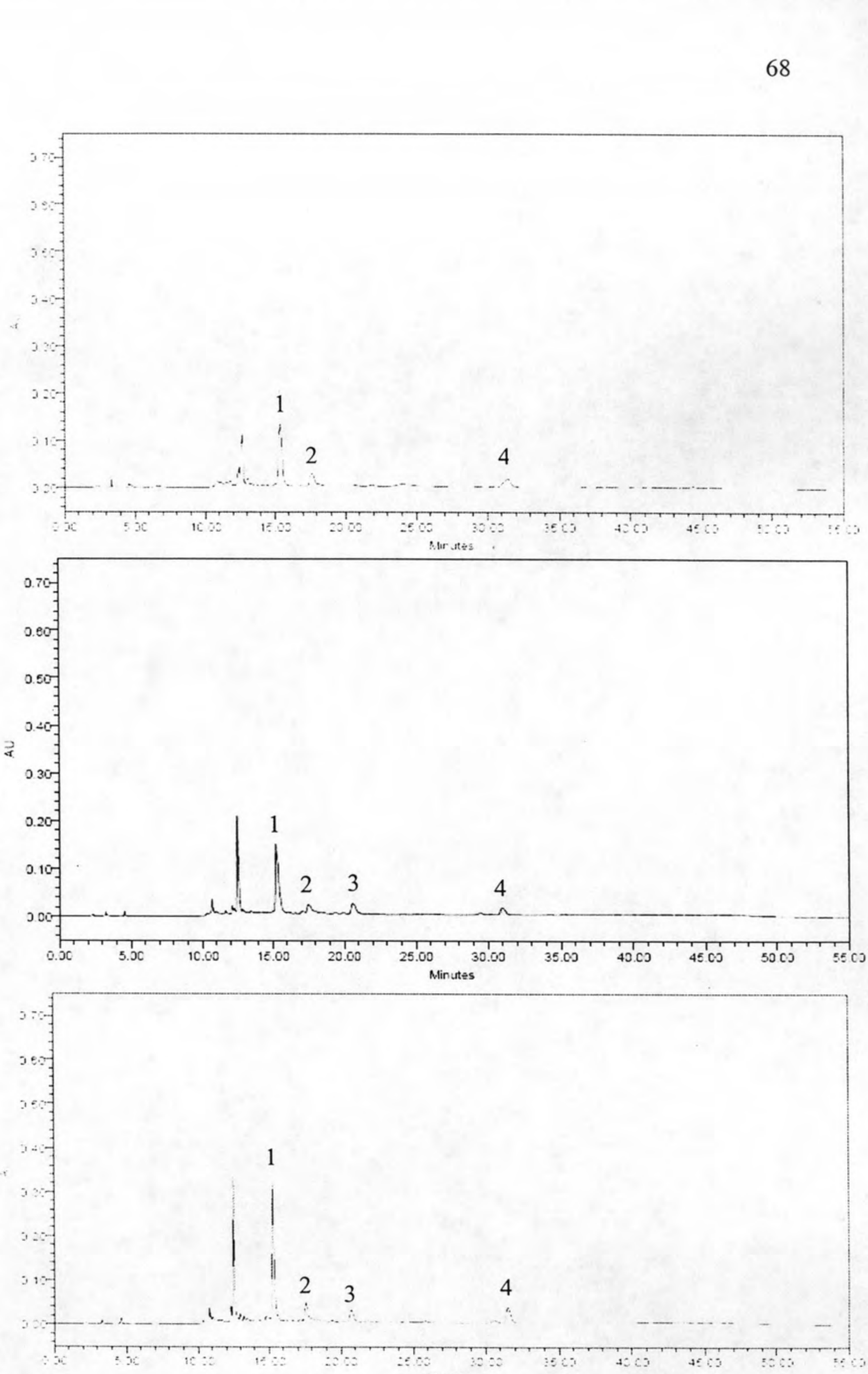


Figure 4.2 HPLC isoflavonoid fingerprint of *Pueraria mirifica* tubers PM-I in summer, rainy season and winter. Legend of figure: 1; puerarin, 2; daidzin, 3; genistin, 4; daidzein, 5; genistein

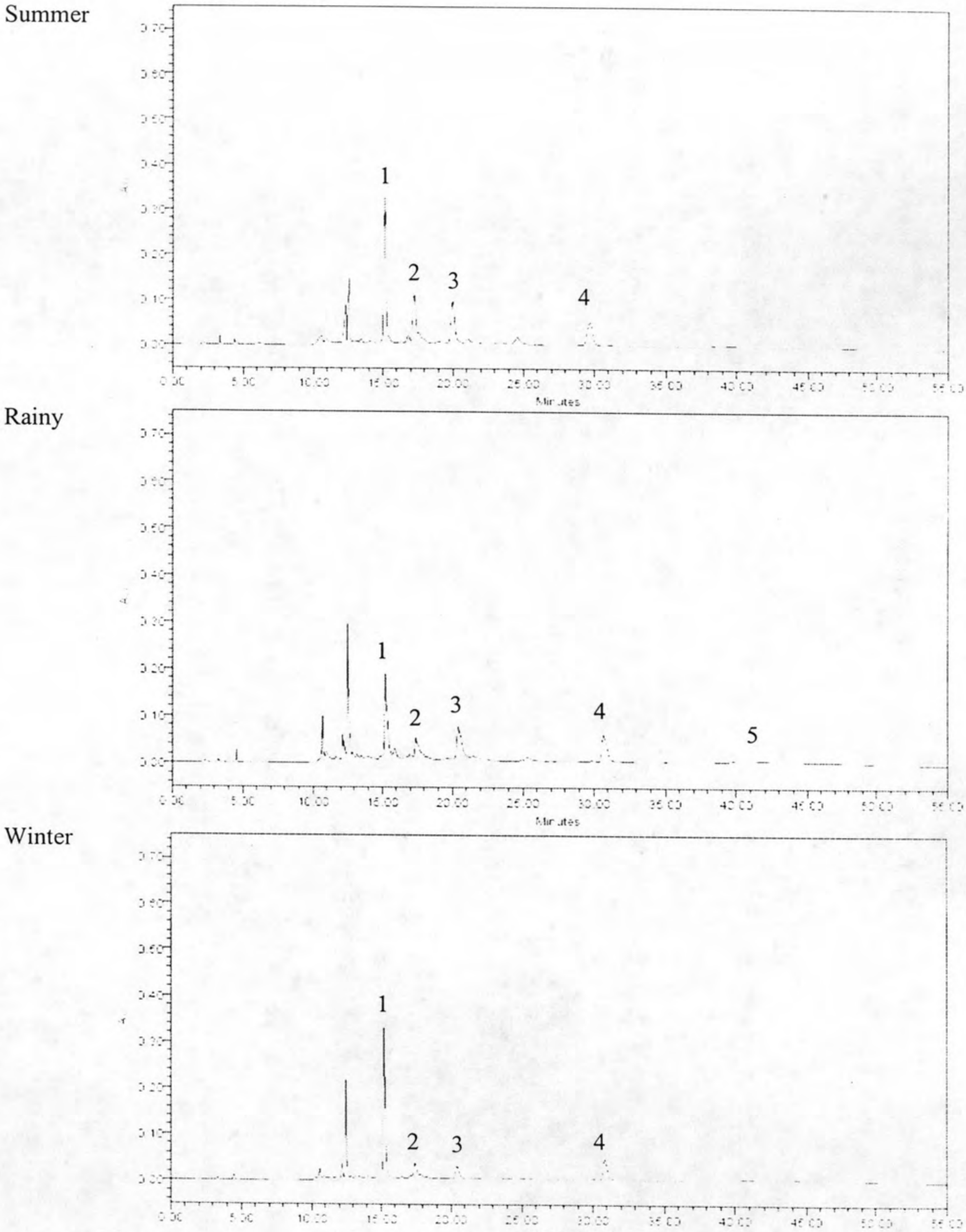


Figure 4.3 HPLC isoflavonoid fingerprint of *Pueraria mirifica* tubers PM-II in summer, rainy season and winter. Legend of figure: 1; puerarin, 2; daidzin, 3; genistin, 4; daidzein, 5; genistein

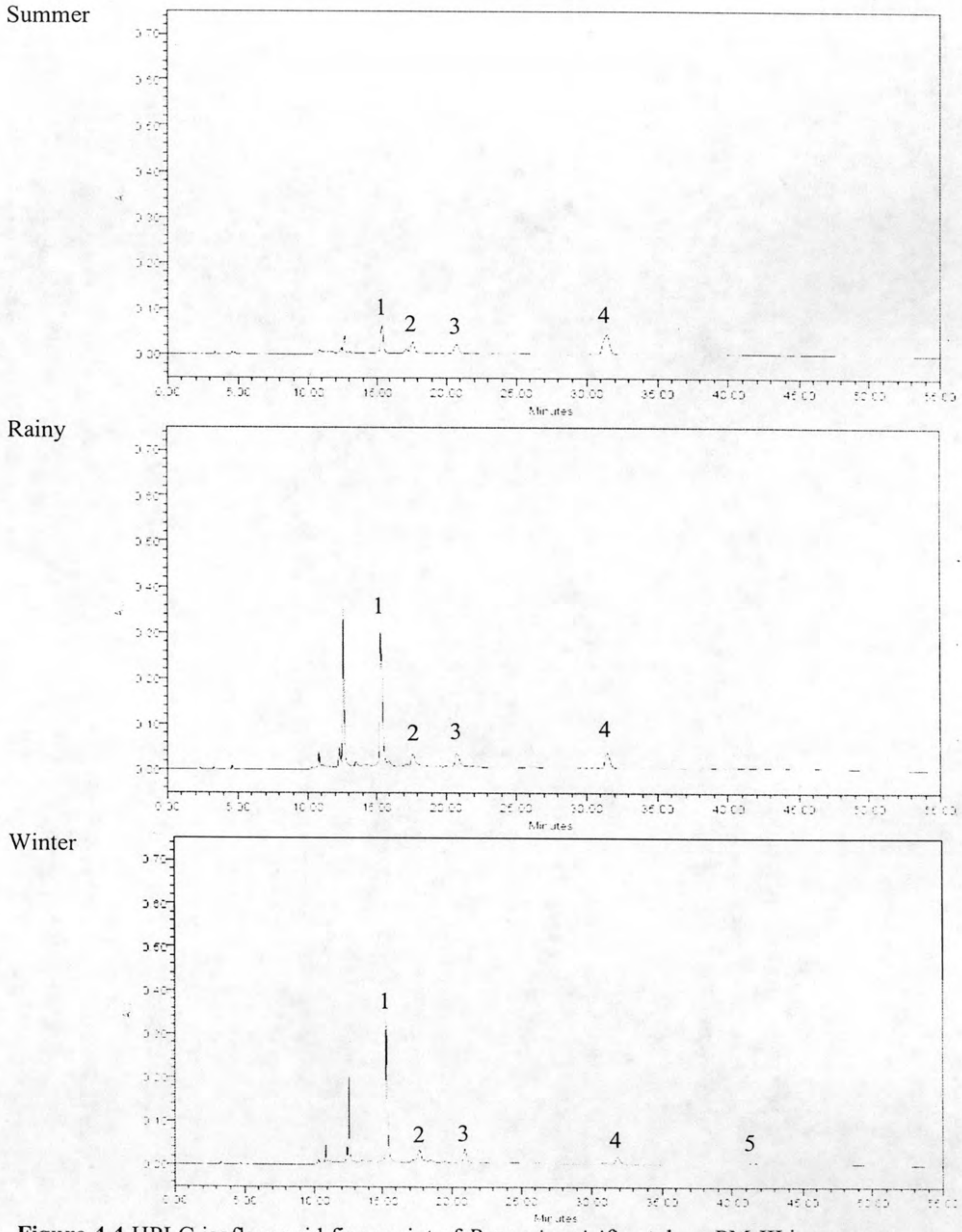


Figure 4.4 HPLC isoflavonoid fingerprint of *Pueraria mirifica* tubers PM-III in summer, rainy season and winter. Legend of figure: 1; puerarin, 2; daidzin, 3; genistin, 4; daidzein, 5; genistein

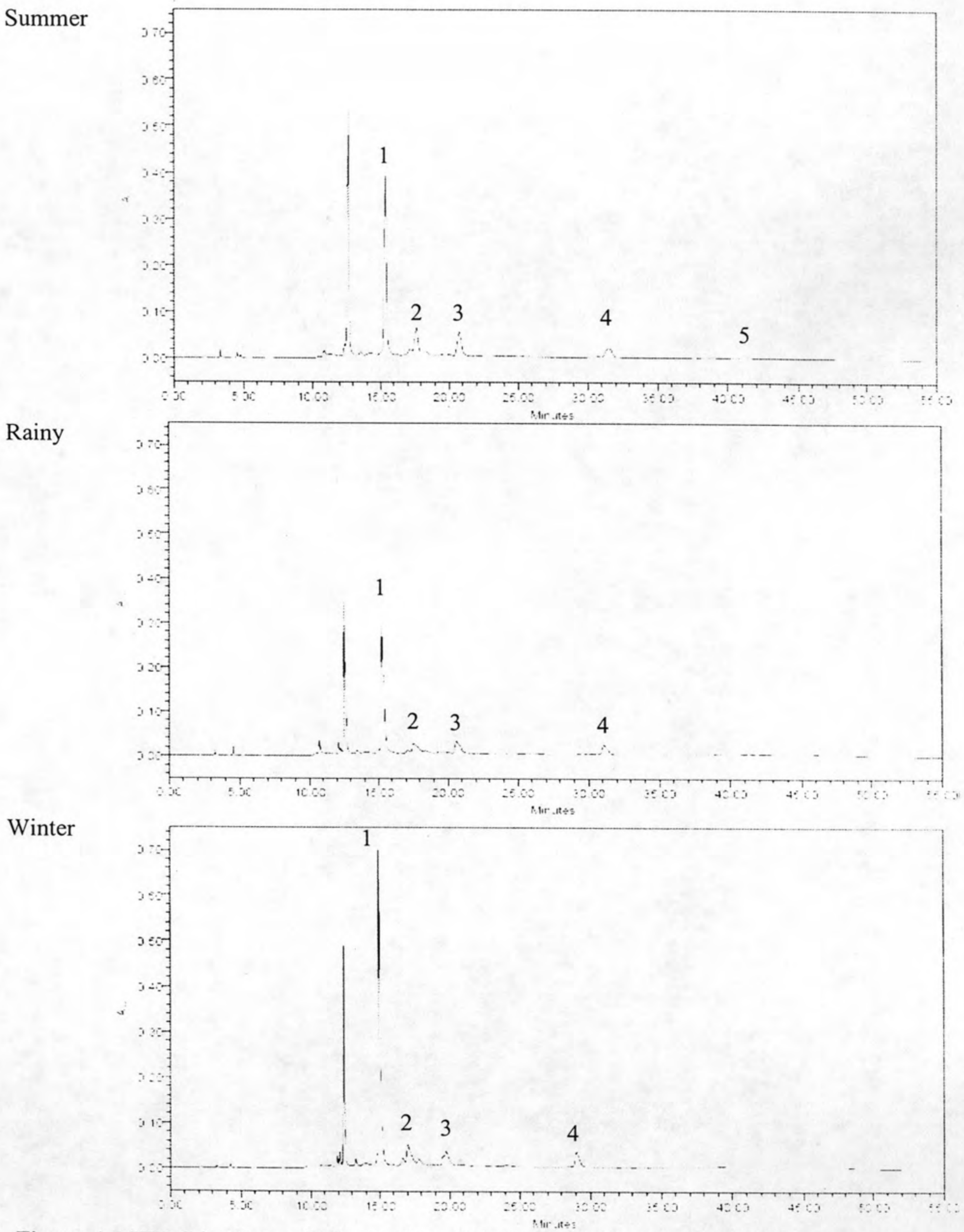


Figure 4.5 HPLC isoflavonoid fingerprint of *Pueraria mirifica* tubers PM-IV in summer, rainy season and winter. Legend of figure: 1; puerarin, 2; daidzin, 3; genistin, 4; daidzein, 5; genistein

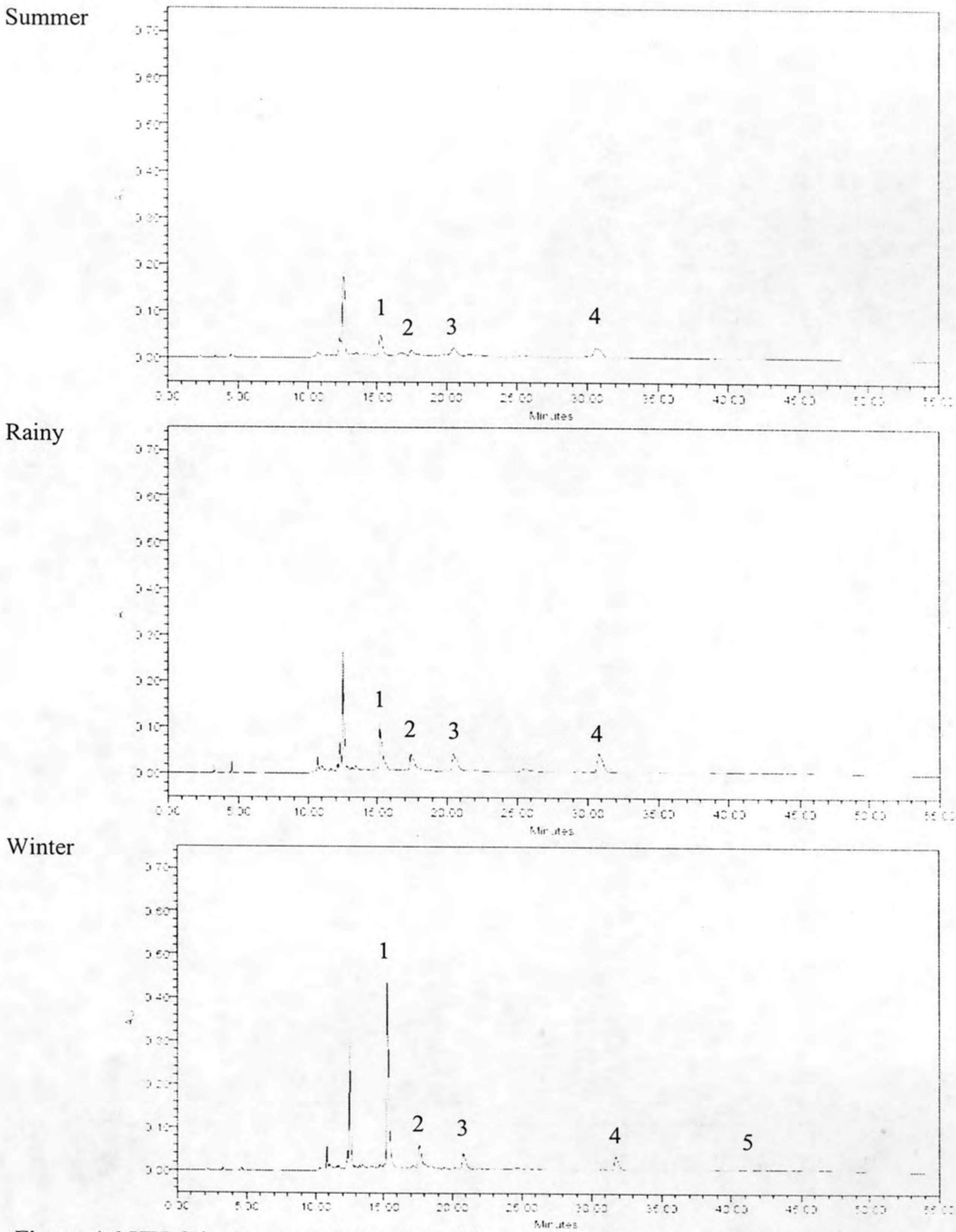


Figure 4.6 HPLC isoflavonoid fingerprint of *Pueraria mirifica* tubers PM-V in summer, rainy season and winter. Legend of figure: 1; puerarin, 2; daidzin, 3; genistin, 4; daidzein, 5; geniste

4.4 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 rainy season, genistin and ratio of glycoside against aglycoside were correlated with the amount of rainfall at $P < 0.01$ and ratio of aglycoside against glycoside was reverse correlated with the amount of rainfall at $P < 0.05$. In winter, the ratio of aglycoside and glycoside against puerarin were correlated with the amount of rainfall at $P < 0.01$.

Table 4.11 Correlations analysis of isoflavonoids contents in tubers of *P. mirifica* with temperature and amount of rainfall.

Isoflavonoid contents (μg/100 g powder)	Summer		Rainy season		Winter	
	Temp	Rainfall	Temp	Rainfall	Temp	Rainfall
Puerarin	-	-	-	-	-	-
Daidzin	-	-	-	-	-	-
Genistin	-	-	-	**	-	-
Daidzein	-	-	-	-	-	-
Genistein	-	-	-	-	-	-
Total isoflavonoid	-	-	-	-	-	-
aglycoside	-	-	-	-	-	-
glycoside	-	-	-	-	-	-
aglycoside/glycoside	-	-	-	†	-	-
glycoside/aglycoside	-	-	-	**	-	-
(aglycoside+glycoside)/puerarin	-	-	-	-	-	**

** : Correlation is significant at the 0.01 level (2-tailed)

† : Negative correlation is significant at the 0.05 level (2-tailed)

4.5. Bioassay

4.5.1. Percent scavenging of the free radicals of *P. mirifica*

The percent scavenging was calculated for scavenge the free radicals of 5 clones of *P. mirifica*. The effective concentrations were in the range of 375-6000 μg/ml. The results revealed that *P. mirifica* was able to inhibit DPPH free radicals. The *P. mirifica* extract at dose of 6000 μg/ml was evaluated for the highest percent scavenging. In summer and winter, PM-I showed the highest percent scavenging but in rainy season PM-II showed the highest percent scavenging.

There was correlation between isoflavonoid contents and percentage of scavenging. In summer, it was found that aglycoside contents were correlated with percentage of scavenging at $P < 0.05$ (Table 4.12).

Table 4.12 Percentage of scavenging of α-tocopherol

	Concentration (μM)					IC_{50} (μg/ml)
	6.25	12.5	25	50	100	
α-tocopherol	41.33	47.95	51.07	62.57	81.29	15.23

Table 4.13 Percentage of scavenging of *P. mirifica* collected in three seasons.

		Concentration (μg/ml)					IC_{50}
		375	750	1500	3000	6000	(μg/ml)
Summer	PM-I	5.46±2.14 ^{a}	11.76±3.86 ^{a}	15.59±7.33 ^{a}	26.90±10.27 ^{a}	33.66±3.41 ^{b}	7280.24
	PM-II	4.87±2.62 ^{a}	6.50±2.57 ^{a}	6.82±2.90 ^{a}	11.96±0.80 ^{a}	13.52±3.48 ^{a}	13868.13
	PM-III	4.29±1.79 ^{a}	4.48±2.16 ^{a}	8.38±3.24 ^{a}	11.43±1.63 ^{a}	12.93±1.87 ^{a}	13982.37
	PM-IV	5.09±3.99 ^{a}	7.36±4.28 ^{a}	13.58±3.09 ^{a}	14.94±3.48 ^{a}	17.07±8.10 ^{ab}	12189.92
	PM-V	5.85±3.81 ^{a}	6.63±0.23 ^{a}	13.13±1.23 ^{a}	18.91±2.40 ^{a}	20.08±6.42 ^{ab}	10436.95
	Mean± S.E.M.	5.11±1.14 $^{a'}$	7.33±1.43 $^{a'}$	11.81±1.78 $^{a'}$	16.83±3.83 $^{a'}$	19.45±2.81 $^{a'}$	10774.39
Rainy season	PM-I	6.63±3.64 ^{a}	7.60±3.79 ^{a}	10.53±3.12 ^{a}	14.81±7.23 ^{a}	15.66±7.38 ^{a}	13465.99
	PM-II	1.95±0.92 ^{a}	3.90±2.22 ^{a}	8.51±2.96 ^{a}	15.59±2.60 ^{a}	19.30±3.94 ^{a}	9730.73
	PM-III	4.87±1.28 ^{a}	7.04±4.25 ^{a}	9.75±2.65 ^{a}	10.72±1.96 ^{a}	15.59±2.06 ^{a}	13248.05
	PM-IV	9.94±4.31 ^{a}	10.46±2.11 ^{a}	11.37±4.16 ^{a}	14.68±0.23 ^{a}	15.14±4.62 ^{a}	16279.04
	PM-V	4.09±2.60 ^{a}	5.22±2.44 ^{a}	8.51±1.79 ^{a}	12.74±1.24 ^{a}	15.79±1.75 ^{a}	11967.38
	Mean± S.E.M.	5.50±1.68 $^{a'}$	6.90±1.46 $^{a'}$	9.79±1.19 $^{a'}$	13.76±1.44 $^{a'}$	16.22±1.73 $^{a'}$	12514.49
Winter	PM-I	9.23±4.91 ^{a}	10.07±4.75 ^{a}	17.35±3.38 ^{a}	22.03±2.89 ^{a}	25.21±6.38 ^{a}	9478.81
	PM-II	1.69±0.57 ^{a}	4.09±2.98 ^{a}	4.22±2.06 ^{a}	13.19±1.06 ^{a}	20.03±9.10 ^{a}	9644.80
	PM-III	7.04±4.45 ^{a}	8.51±4.56 ^{a}	11.44±4.04 ^{a}	14.62±6.01 ^{a}	17.15±6.44 ^{a}	12878.65
	PM-IV	6.50±3.59 ^{a}	7.52±4.00 ^{a}	9.42±4.91 ^{a}	14.04±3.28 ^{a}	14.75±3.48 ^{a}	14045.23
	PM-V	2.99±1.84 ^{a}	8.97±3.14 ^{a}	14.17±3.19 ^{a}	16.70±1.60 ^{a}	20.64±8.77 ^{a}	10324.55
	Mean± S.E.M.	5.64±2.09 $^{a'}$	7.94±1.59 $^{a'}$	11.68±1.89 $^{a'}$	15.93±1.55 $^{a'}$	19.57±3.02 $^{a'}$	11010.08

Means not sharing a common superscript letter with or without prime in the same column are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

Table 4.14 Percentage of scavenging of five clones of *P. mirifica.*

		Concentration (μg/ml)				
		375	750	1500	3000	6000
PM-I	Summer	5.46±2.14 ^{a}	11.76±3.86 ^{a}	15.59±7.33 ^{a}	26.90±10.27 ^{a}	33.66±3.41 ^{a}
	Rainy season	6.63±3.64 ^{a}	7.60±3.79 ^{a}	10.53±3.12 ^{a}	14.81±7.23 ^{a}	15.66±7.38 ^{a}
	Winter	9.23±4.91 ^{a}	10.07±4.75 ^{a}	17.35±3.38 ^{a}	22.03±2.89 ^{a}	25.21±6.38 ^{a}
	Mean± S.E.M.	7.10±1.95 $^{b'}$	9.81±2.16 $^{b'}$	14.49±2.70 $^{c'}$	21.25±6.51 $^{b'}$	24.84±3.96 $^{b'}$
PM-II	Summer	4.87±2.62 ^{a}	6.50±2.57 ^{a}	6.82±2.90 ^{a}	11.96±0.80 ^{a}	13.52±3.48 ^{a}
	Rainy season	1.95±0.92 ^{a}	3.90±2.22 ^{a}	8.51±2.96 ^{a}	15.59±2.60 ^{a}	19.30±3.94 ^{a}
	Winter	1.69±0.57 ^{a}	4.09±2.98 ^{a}	4.22±2.06 ^{a}	13.19±1.06 ^{a}	20.03±9.10 ^{a}
	Mean± S.E.M.	2.84±1.30 $^{a'}$	4.83±1.71 $^{a'}$	6.52±1.47 $^{a'}$	13.58±1.00 $^{a'}$	17.62±3.45 $^{a'}$
PM-III	Summer	4.29±1.79 ^{a}	4.48±2.16 ^{a}	8.38±3.24 ^{a}	11.43±1.63 ^{a}	12.93±1.87 ^{a}
	Rainy season	4.87±1.28 ^{a}	7.04±4.25 ^{a}	9.75±2.65 ^{a}	10.72±1.96 ^{a}	15.59±2.06 ^{a}
	Winter	7.04±4.45 ^{a}	8.51±4.56 ^{a}	11.44±4.04 ^{a}	14.62±6.01 ^{a}	17.15±6.44 ^{a}
	Mean± S.E.M.	5.40±1.49 $^{ab'}$	6.68±2.27 $^{ab'}$	9.85±1.74 $^{ab'}$	12.25±1.98 $^{a'}$	15.23±2.12 $^{a'}$
PM-IV	Summer	5.09±3.99 ^{a}	7.36±4.28 ^{a}	13.58±3.09 ^{a}	14.94±3.48 ^{a}	17.07±8.10 ^{a}
	Rainy season	9.94±4.31 ^{a}	10.46±2.11 ^{a}	11.37±4.16 ^{a}	14.68±0.23 ^{a}	15.14±4.62 ^{a}
	Winter	6.50±3.59 ^{a}	7.52±4.00 ^{a}	9.42±4.91 ^{a}	14.04±3.28 ^{a}	14.75±3.48 ^{a}
	Mean± S.E.M.	7.18±2.74 $^{b'}$	8.45±1.87 $^{ab'}$	11.46±2.35 $^{bc'}$	14.55±1.39 $^{a'}$	15.65±2.90 $^{a'}$
PM-V	Summer	5.85±3.81 ^{a}	6.63±0.23 ^{a}	13.13±1.23 ^{a}	18.91±2.40 ^{a}	20.08±6.42 ^{a}
	Rainy season	4.09±2.60 ^{a}	5.22±2.44 ^{a}	8.51±1.79 ^{a}	12.74±1.24 ^{a}	15.79±1.75 ^{a}
	Winter	2.99±1.84 ^{a}	8.97±3.14 ^{a}	14.17±3.19 ^{a}	16.70±1.60 ^{a}	20.64±8.77 ^{a}
	Mean± S.E.M.	4.31±2.80 $^{ab'}$	6.94±1.27 $^{ab'}$	11.93±1.41 $^{bc'}$	16.11±1.28 $^{ab'}$	18.84±3.27 $^{a'}$

Means not sharing a common superscript letter with or without prime in the same column are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

Table 4.15 Correlations analysis of percentage of scavenging with the major isoflavonoids in *P. mirifica*.

Season	Isoflavonoid contents	% Scavenging				
	(μg/100 g powder)	375	750	1500	3000	6000
Summer	Puerarin	-	-	-	-	-
	Daidzin	-	-	-	-	-
	Genistin	-	-	-	-	-
	Daidzein	-	-	-	-	-
	Genistein	-	-	-	-	-
	Total isoflavonoid	-	-	-	-	-
	aglycoside	-	*	-	-	-
	aglycoside/glycoside	-	-	-	-	-
Rainy season	Puerarin	-	-	-	-	-
	Daidzin	-	-	-	-	-
	Genistin	-	-	-	-	-
	Daidzein	-	-	-	-	-
	Genistein	-	-	-	-	-
	Total isoflavonoid	-	-	-	-	-
	aglycoside	-	-	-	-	-
	aglycoside/glycoside	-	-	-	-	-
Winter	Puerarin	-	-	-	-	-
	Daidzin	-	-	-	-	-
	Genistin	-	-	-	-	-
	Daidzein	-	-	-	-	-
	Genistein	-	-	-	-	-
	Total isoflavonoid	-	-	-	-	-
	aglycoside	-	-	-	-	-
	aglycoside/glycoside	-	-	-	-	-

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

(-) : No correlation

4.5.2. Proliferation/antiproliferation assay on MCF-7 cells

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

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

Table 4.16 The growth response percentage of 17β-estradiol, puerarin, daidzin, genistin, daidzein and genistein in the absence of S9 mixture on MCF-7 cell culture.

Standard	Concentration (M)							
	10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
Puerarin	216.61±9.06^{d}	204.40±11.79^{c}	195.57±6.62^{cd}	196.35±19.55^{b}	177.65±15.10^{bc}	146.74±4.21^{b}	155.31±2.34^{bc}	156.87±7.05^{cd}
daidzin	230.63±8.70^{d}	210.37±12.12^{c}	218.42±23.47^{c}	163.36±14.48^{ab}	186.22±21.54^{c}	183.36±20.63^{c}	175.57±19.27^{c}	160.25±10.0^{d}
genistin	119.47±4.0^{a}	139.21±9.17^{b}	154.79±24.89^{bc}	174.27±37.78^{ab}	147.99±29.06^{abc}	126.22±6.69^{ab}	152.45±15.33^{bc}	138.17±16.75^{cd}
daidzein	134.27±9.62^{ab}	131.16±11.76^{ab}	134.01±13.96^{ab}	193.75±36.97^{b}	127.26±13.93^{ab}	112.98±4.72^{a}	109.60±4.81^{a}	103.11±7.21^{ab}
genistein	197.91±27.19^{cd}	161.03±5.56^{b}	154.79±8.08^{bc}	163.10±18.52^{ab}	128.30±5.73^{ab}	129.60±2.18^{ab}	127.26±8.49^{ab}	129.60±4.25^{bc}
17β-estradiol	168.30±11.50^{bc}	149.86±16.15^{b}	147.52±5.98^{b}	169.60±7.98^{ab}	170.38±18.29^{bc}	-	-	-
DMSO	100±0.00^{a}							

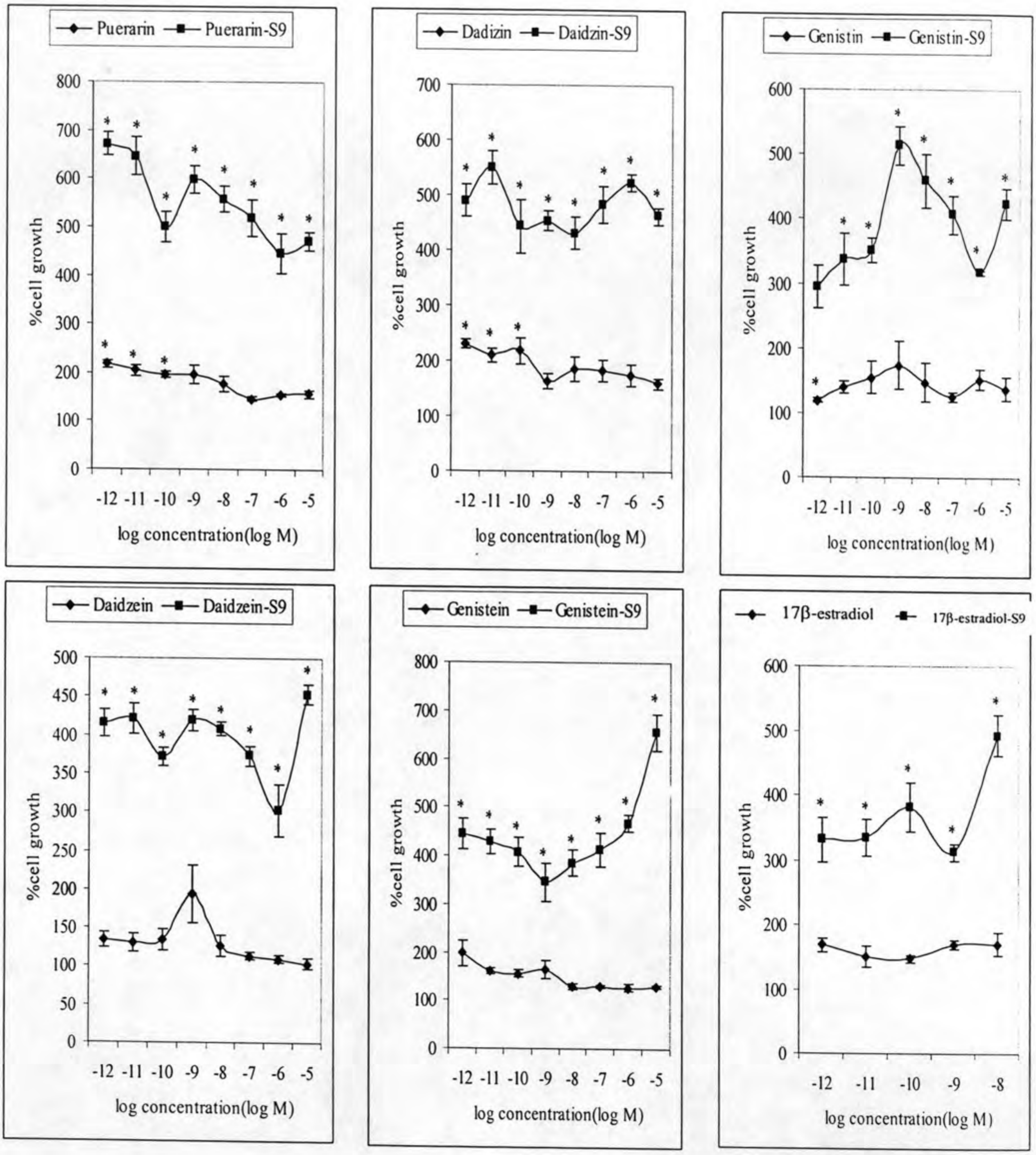
Means not sharing a common superscript letter in the same row of the samples are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

The growth response percentages (Mean ± S.E.M) of 5 standard isoflavonoids at the concentration 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 were compared with positive control (17β-estradiol) and negative control (DMSO).

Table 4.17 The growth response percentage of 17β-estradiol, puerarin, daidzin, genistin, daidzein and genistein in the presence of S9 mixture on MCF-7 cell culture.

Standard	Concentration (M)							
	10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
puerarin	671.63±24.41^{c}	646.44±38.38^{d}	513.72±31.62^{b}	598.39±28.63^{d}	557.36±26.89^{b}	518.92±38.17^{c}	444.90±41.34^{c}	471.91±18.74^{b}
daidzin	489.57±29.74^{bc}	549.82±31.72^{cd}	443.34±47.91^{b}	453.99±17.81^{bcd}	431.39±29.10^{b}	484.12±33.83^{bc}	523.33±14.99^{c}	462.82±15.29^{b}
genistin	293.22±33.16^{ab}	336.86±40.81^{b}	351.66±18.80^{b}	513.46±29.11^{cd}	459.18±41.33^{b}	407.50±29.86^{bc}	317.12±3.18^{b}	425.42±23.94^{b}
daidzein	415.03±18.65^{b}	421.26±19.77^{bc}	377.37±12.24^{b}	419.19±14.36^{bcd}	408.02±9.34^{b}	372.70±12.95^{b}	302.83±33.13^{b}	453.21±12.64^{b}
genistein	445.16±31.85^{bc}	427.50±25.18^{bc}	406.98±30.21^{b}	344.91±40.60^{bc}	385.68±27.71^{b}	412.17±35.43^{bc}	467.23±17.39^{c}	655.01±37.42^{c}
17β-estradiol	330.62±34.54^{ab}	334.0±28.92^{b}	381.79±37.34^{b}	311.92±13.18^{b}	492.69±31.76^{b}	-	-	-
DMSO	100±0.00^{a}							

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.



*Proliferation to MCF-7 cell

Figure 4.7 The growth response percentage of 17β-estradiol, puerarin, daidzin, genistin, daidzein and genistein on MCF-7 cell culture in the absence and presence of S9 mixture in comparison with DMSO.

The ratio of growth response of standard isoflavonoids to MCF-7 cells in the presence and absence of S9 mixture were present in Table 4.18.

Table 4.18 The ratio of growth response of standard isoflavonoids to MCF-7 cells in the presence and absence of S9 mixture.

Standard	Concentration (M)							
	10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
Puerarin	3.10	3.16	2.63	3.05	3.14	3.54	2.86	3.01
Daidzin	2.12	2.61	2.03	2.78	2.32	2.64	2.98	2.89
Genistin	2.45	2.42	2.23	2.95	3.10	3.23	2.08	3.08
Daidzein	3.09	3.21	2.82	2.16	3.21	3.30	2.76	4.40
Genistein	2.25	2.65	2.63	2.11	3.01	3.18	3.67	5.13
17 β-estradiol	2.21	2.23	2.59	1.84	2.89	-	-	-

4.5.2.2. Proliferation/antiproliferation assay

P. mirifica collected from Ratchaburi in 2005 at the concentrations of 0.1-1000 μg/ml were tested with MCF-7. The growth response of MCF-7 cells to *P. mirifica* was compared with the growth of negative control (DMSO).

PM-I, PM-II, PM-III, PM-IV and PM-V showed variation of biphasic estrogenic activity to MCF-7. The plant extracts showed proliferative effect to MCF-7 cells at 10-100 μg/ml, and antiproliferation at 1000 μg/ml (Table 4.19-4.20).

Table 4.19 The growth response of MCF-7 of plant extracts collected in three seasons in the absence of S9 mixture.

Season	PM (μg/ml)	% cell growth 0.1	1	10	100	1000	IC_{50} (μg/ml)
Summer	PM-I	103.51 ± 0.977^{a}	105.09 ± 1.50^{ab}	126.49 ± 4.57^{abc}	144.21 ± 6.37^{b}	124.21 ± 18.16^{b}	-
	PM-II	105.67 ± 16.71^{a}	108.33 ± 6.84^{ab}	159.00 ± 7.81^{c}	96.33 ± 6.36^{a}	75.00 ± 1.53^{a}	1435.61
	PM-III	108.04 ± 2.16^{a}	117.39 ± 5.72^{b}	131.24 ± 18.64^{abc}	113.93 ± 1.39^{ab}	89.49 ± 10.32^{ab}	-
	PM-IV	95.26 ± 13.55^{a}	96.67 ± 10.43^{a}	109.12 ± 7.77^{ab}	122.98 ± 27.45^{ab}	75.09 ± 20.09^{a}	1541.10
	PM-V	83.98 ± 6.21^{a}	101.81 ± 4.13^{ab}	147.80 ± 29.0^{bc}	93.80 ± 3.82^{a}	86.82 ± 6.77^{ab}	4439.82
	Mean±S.E.M.	$99.41\pm3.70^{b'}$	$104.88\pm2.59^{a'}$	$128.94\pm7.12^{b'}$	$111.88\pm5.93^{a'}$	$91.77\pm5.85^{a'}$	2019.17
	DMSO	100.00 ± 0.00^{a}	100.00 ± 0.00^{ab}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{ab}	
Rainy season	PM-I	87.54 ± 0.63^{a}	118.60 ± 7.04^{a}	127.89 ± 3.68^{a}	104.74 ± 13.21^{a}	90.00 ± 1.10^{a}	6039.86
	PM-II	92.11 ± 2.11^{a}	114.97 ± 5.41^{a}	120.16 ± 12.67^{a}	147.52 ± 27.67^{a}	116.01 ± 8.49^{a}	-
	PM-III	90.96 ± 10.35^{a}	112.92 ± 20.49^{a}	140.31 ± 42.15^{a}	143.67 ± 23.51^{a}	144.44 ± 49.88^{a}	-
	PM-IV	96.67 ± 5.06^{a}	97.02 ± 6.76^{a}	92.46 ± 0.35^{a}	100.88 ± 7.21^{a}	90.35 ± 1.53^{a}	3500.67
	PM-V	86.85 ± 2.39^{a}	88.07 ± 6.51^{a}	98.78 ± 3.920^{a}	96.79 ± 3.21^{a}	93.88 ± 3.72^{a}	-
	Mean±S.E.M.	$92.36\pm2.03^{a'}$	$105.29\pm4.31^{a'}$	$113.27\pm7.50^{a'}$	$115.60\pm7.57^{a'}$	$105.78\pm8.53^{b'}$	-
	DMSO	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	
Winter	PM-I	97.54 ± 2.30^{abc}	102.63 ± 0.61^{ab}	114.91 ± 19.67^{a}	118.60 ± 8.36^{bc}	102.63 ± 14.03^{b}	-
	PM-II	90.00 ± 1.10^{a}	114.74 ± 14.79^{b}	106.67 ± 14.39^{a}	94.21 ± 1.10^{a}	65.61 ± 9.91^{a}	1320.16
	PM-III	105.97 ± 6.49^{c}	122.24 ± 4.54^{b}	147.52 ± 37.40^{a}	129.86 ± 9.60^{c}	110.47 ± 0.69^{b}	-
	PM-IV	90.20 ± 0.98^{a}	100.00 ± 13.17^{ab}	150.98 ± 14.75^{a}	120.92 ± 8.50^{bc}	99.02 ± 6.86^{b}	-
	PM-V	90.20 ± 2.24^{a}	82.91 ± 6.07^{a}	95.24 ± 17.52^{a}	103.36 ± 7.00^{ab}	89.92 ± 3.03^{b}	10637.90
	Mean±S.E.M.	$95.68\pm1.80^{ab'}$	$103.75\pm4.23^{a'}$	$119.22\pm8.84^{ab'}$	$111.16\pm3.89^{a'}$	$94.61\pm4.35^{ab'}$	4887.42
	DMSO	100.00 ± 0.00^{bc}	100.00 ± 0.00^{ab}	100.00 ± 0.00^{a}	100.00 ± 0.00^{ab}	100.00 ± 0.00^{b}	

Means not sharing a common superscript letter with or without prime in the same column are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

Table 4.20 The growth response of MCF-7 of five plant extracts in the absence of S9 mixture.

		% cell growth				
		0.1	1	10	100	1000
PM-I	Summer	103.51 ± 0.977^{c}	105.09 ± 1.50^{ab}	126.49 ± 4.57^{a}	144.21 ± 6.37^{b}	124.21 ± 18.16^{a}
	Rainy season	87.54 ± 0.63^{a}	118.60 ± 7.04^{b}	127.89 ± 3.68^{a}	104.74 ± 13.21^{a}	90.00 ± 1.10^{a}
	Winter	97.54 ± 2.30^{b}	102.63 ± 0.61^{a}	114.91 ± 19.67^{a}	118.60 ± 8.36^{ab}	102.63 ± 14.03^{a}
	Mean± S.E.M.	$96.20\pm2.44^{b'}$	$108.77\pm3.24^{b'}$	$123.10\pm6.27^{ab'}$	$122.51\pm7.56^{b'}$	$105.61\pm8.30^{ab'}$
PM-II	Summer	105.67 ± 16.71^{a}	108.33 ± 6.84^{a}	159.00 ± 7.81^{b}	96.33 ± 6.36^{a}	75.00 ± 1.53^{a}
	Rainy season	92.11 ± 2.11^{a}	114.97 ± 5.41^{a}	120.16 ± 12.67^{ab}	147.52 ± 27.67^{a}	116.01 ± 8.49^{b}
	Winter	90.00 ± 1.10^{a}	114.74 ± 14.79^{a}	106.67 ± 14.39^{a}	94.21 ± 1.10^{a}	65.61 ± 9.91^{a}
	Mean± S.E.M.	$95.93\pm5.45^{b'}$	$112.68\pm5.07^{b'}$	$128.61\pm9.86^{ab'}$	$112.69\pm11.97^{ab'}$	$85.54\pm8.62^{a'}$
PM-III	Summer	108.04 ± 2.16^{a}	117.39 ± 5.72^{a}	131.24 ± 18.64^{a}	113.93 ± 1.39^{a}	89.49 ± 10.32^{a}
	Rainy season	90.96 ± 10.35^{a}	112.92 ± 20.49^{a}	140.31 ± 42.15^{a}	143.67 ± 23.51^{a}	144.44 ± 49.88^{a}
	Winter	105.97 ± 6.49^{a}	122.24 ± 4.54^{a}	147.52 ± 37.40^{a}	129.86 ± 9.60^{a}	110.47 ± 0.69^{a}
	Mean± S.E.M.	$101.65\pm4.48^{b'}$	$117.52\pm6.42^{b'}$	$139.69\pm17.29^{b'}$	$129.15\pm8.51^{b'}$	$114.80\pm16.74^{b'}$
PM-IV	Summer	95.26 ± 13.55^{a}	96.67 ± 10.43^{a}	109.12 ± 7.77^{a}	122.98 ± 27.45^{a}	75.09 ± 20.09^{a}
	Rainy season	96.67 ± 5.06^{a}	97.02 ± 6.76^{a}	92.46 ± 0.35^{a}	100.88 ± 7.21^{a}	90.35 ± 1.53^{a}
	Winter	90.20 ± 0.98^{a}	100.00 ± 13.17^{a}	150.98 ± 14.75^{b}	120.92 ± 8.50^{a}	99.02 ± 6.86^{a}
	Mean± S.E.M.	$94.04\pm4.30^{ab'}$	$97.89\pm5.25^{a'}$	$117.52\pm9.95^{ab'}$	$114.92\pm9.25^{b'}$	$88.15\pm7.07^{ab'}$
PM-V	Summer	83.98 ± 6.21^{a}	101.81 ± 4.13^{a}	147.80 ± 29.0^{a}	93.80 ± 3.82^{a}	86.82 ± 6.77^{a}
	Rainy season	86.85 ± 2.39^{a}	88.07 ± 6.51^{a}	98.78 ± 3.90^{a}	96.79 ± 3.21^{a}	93.88 ± 3.72^{a}
	Winter	90.20 ± 2.24^{a}	82.91 ± 6.07^{a}	95.24 ± 17.52^{a}	103.36 ± 7.00^{a}	89.92 ± 3.03^{a}
	Mean± S.E.M.	$87.01\pm2.22^{a'}$	$90.93\pm4.00^{a'}$	$113.94\pm13.00^{a'}$	$97.98\pm2.86^{a'}$	$90.21\pm2.60^{ab'}$

Means not sharing a common superscript letter with or without prime in the same column are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

From Table 4.19, PM-II, PM-V and PM-I collected in summer and PM-III collected in winter exhibited proliferative effect at the concentration 10 and 100 μg/ml. PM-II collected in summer showed the maximum proliferative effect (159.0% at 10 μg/ml). PM-II collected in winter exhibited anti-proliferative effect at the concentration of 1000 μg/ml.

Table 4.21 The growth response of MCF-7 of plant extracts collected in three seasons in the presence of S9 mixture.

Season	PM (μg/ml)	% cell growth 0.1	1	10	100	1000	IC_{50} (μg/ml)
Summer	PM-I	599.43±38.01^{c}	602.89±51.11^{c}	684.96±46.77^{d}	715.44±65.15^{b}	343.17±34.31^{b}	>1000
	PM-II	441.18±60.76^{bc}	457.10±16.83^{b}	622.98±41.23^{cd}	679.77±61.71^{b}	765.30±83.20^{c}	>1000
	PM-III	337.65±73.16^{b}	465.95±40.52^{bc}	466.91±69.62^{b}	534.05±32.20^{b}	759.71±29.71^{c}	>1000
	PM-IV	530.94±38.70^{bc}	548.68±42.00^{bc}	561.63±45.63^{bcd}	572.42±44.85^{b}	382.73±11.06^{b}	>1000
	PM-V	351.56±132.60^{b}	424.94±79.54^{b}	493.29±58.26^{bc}	704.32±106.71^{b}	440.53±20.15^{b}	>1000
	DMSO			100.00±0.00^{a}			
	Mean±S.E.M.	393.46±45.78$^{b'}$	433.26±42.02$^{ab'}$	488.29±48.70$^{ab'}$	551.00±55.68$^{b'}$	465.24±58.70$^{b'}$	
Rainy season	PM-I	675.96±181.43^{b}	717.86±122.07^{b}	748.68±43.64^{b}	621.59±125.38^{b}	242.75±121.73^{ab}	>1000
	PM-II	579.35±105.70^{b}	582.81±187.72^{b}	666.61±309.80^{b}	666.61±224.55^{b}	408.28±131.96^{bc}	>1000
	PM-III	451.56±102.67^{ab}	387.29±6.90^{ab}	394.96±105.92^{ab}	440.77±19.60^{b}	481.06±20.63^{c}	>1000
	PM-IV	571.22±153.91^{b}	627.58±107.15^{b}	772.42±55.66^{b}	660.91±23.50^{b}	347.48±37.47^{bc}	>1000
	PM-V	391.85±108.37^{ab}	625.90±78.32^{b}	620.14±86.53^{b}	618.47±24.9^{b}	471.22±14.29^{bc}	>1000
	DMSO			100.00±0.00^{a}			
	Mean±S.E.M.	461.66±61.65$^{b'}$	506.91±62.19$^{b'}$	550.47±74.97$^{b'}$	518.06±60.99$^{ab'}$	341.22±41.70$^{a'}$	
Winter	PM-I	462.83±83.11^{c}	715.11±26.15^{d}	809.35±118.23^{d}	735.49±14.60^{e}	260.91±55.95^{ab}	>1000
	PM-II	151.05±25.06^{ab}	210.00±3.22^{b}	259.82±23.21^{ab}	284.56±52.21^{b}	394.04±19.15^{bc}	>1000
	PM-III	351.80±94.67^{c}	432.37±37.61^{c}	443.41±60.40^{bc}	626.86±39.64^{d}	467.63±89.57^{c}	>1000
	PM-IV	375.54±28.79^{c}	435.01±42.09^{c}	552.52±78.74^{c}	522.30±38.00^{cd}	399.28±72.32^{bc}	>1000
	PM-V	335.49±27.02^{c}	386.09±36.55^{c}	462.11±28.65^{c}	423.74±44.05^{c}	361.87±52.75^{bc}	>1000
	DMSO			100.00±0.00^{a}			
	Mean±S.E.M.	296.12±36.30$^{a'}$	379.76±48.08$^{a'}$	437.87±58.41$^{a'}$	448.83±52.80$^{a'}$	330.62±35.09$^{a'}$	

Means not sharing a common superscript letter with or without prime in the same column are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

Table 4.22 The growth response of MCF-7 of five plant extracts in the presence of S9 mixture.

		% cell growth				
		0.1	1	10	100	1000
PM-I	Summer	599.43±38.01^{a}	602.89±51.11^{a}	684.96±46.77^{a}	715.44±65.15^{a}	343.17±34.31^{a}
	Rainy season	675.96±181.43^{a}	717.86±122.07^{a}	748.68±43.64^{a}	621.59±125.38^{a}	242.75±121.73^{a}
	Winter	462.83±83.11^{a}	715.11±26.15^{a}	809.35±118.23^{a}	735.49±14.60^{a}	260.91±55.95^{a}
	Mean± S.E.M.	579.41±66.41$^{c'}$	678.62±43.30$^{c'}$	747.67±42.76$^{c'}$	690.84±44.60$^{b'}$	282.28±42.81$^{a'}$
PM-II	Summer	441.18±60.76^{b}	457.10±16.83^{a}	622.98±41.23^{a}	679.77±61.71^{a}	765.30±83.20^{b}
	Rainy season	579.35±105.70^{b}	582.81±187.72^{a}	666.61±309.80^{a}	666.61±224.55^{a}	408.28±131.96^{a}
	Winter	151.05±25.06^{a}	210.00±3.22^{a}	259.82±23.21^{a}	284.56±52.21^{a}	394.04±19.15^{a}
	Mean± S.E.M.	390.52±72.61$^{ab'}$	416.64±77.19$^{a'}$	516.47±111.09$^{ab'}$	543.65±94.58$^{a'}$	522.54±75.80$^{c'}$
PM-III	Summer	337.65±73.16^{a}	465.95±40.52^{a}	466.91±69.62^{a}	534.05±32.20^{ab}	759.71±29.71^{b}
	Rainy season	451.56±102.67^{a}	387.29±6.90^{a}	394.96±105.92^{a}	440.77±19.60^{a}	481.06±20.63^{a}
	Winter	351.80±94.67^{a}	432.37±37.61^{a}	443.41±60.40^{a}	626.86±39.64^{b}	467.63±89.57^{a}
	Mean± S.E.M.	380.34±48.91$^{ab'}$	428.54±19.71$^{a'}$	435.09±41.89$^{a'}$	533.89±31.16$^{a'}$	569.46±55.17$^{c'}$
PM-IV	Summer	530.94±38.70^{a}	548.68±42.00^{a}	561.63±45.63^{a}	572.42±44.85^{ab}	382.73±11.06^{a}
	Rainy season	571.22±153.91^{a}	627.58±107.15^{a}	772.42±55.66^{a}	660.91±23.50^{b}	347.48±37.47^{a}
	Winter	375.54±28.79^{a}	435.01±42.09^{a}	552.52±78.74^{a}	522.30±38.00^{a}	399.28±72.32^{a}
	Mean± S.E.M.	492.57±55.30$^{bc'}$	537.09±45.08$^{b'}$	628.86±47.31$^{b'}$	585.21±27.28$^{a'}$	376.50±24.93$^{b'}$
PM-V	Summer	351.56±132.60^{a}	424.94±79.54^{a}	493.29±58.26^{a}	704.32±106.71^{b}	440.53±20.15^{a}
	Rainy season	391.85±108.37^{a}	625.90±78.32^{a}	620.14±86.53^{a}	618.47±24.9^{ab}	471.22±14.29^{a}
	Winter	335.49±27.02^{a}	386.09±36.55^{a}	462.11±28.65^{a}	423.74±44.05^{a}	361.87±52.75^{a}
	Mean± S.E.M.	359.63±50.74$^{a'}$	478.98±50.30$^{ab'}$	525.18±39.49$^{ab'}$	582.17±53.71$^{a'}$	424.54±23.41$^{b'}$

Means not sharing a common superscript letter with or without prime in the same column are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

Table 4.23 The ratio of growth response of PM-I, PM-II, PM-III, PM-IV and PM-V to MCF-7 cells in the presence and absence of S9 mixture.

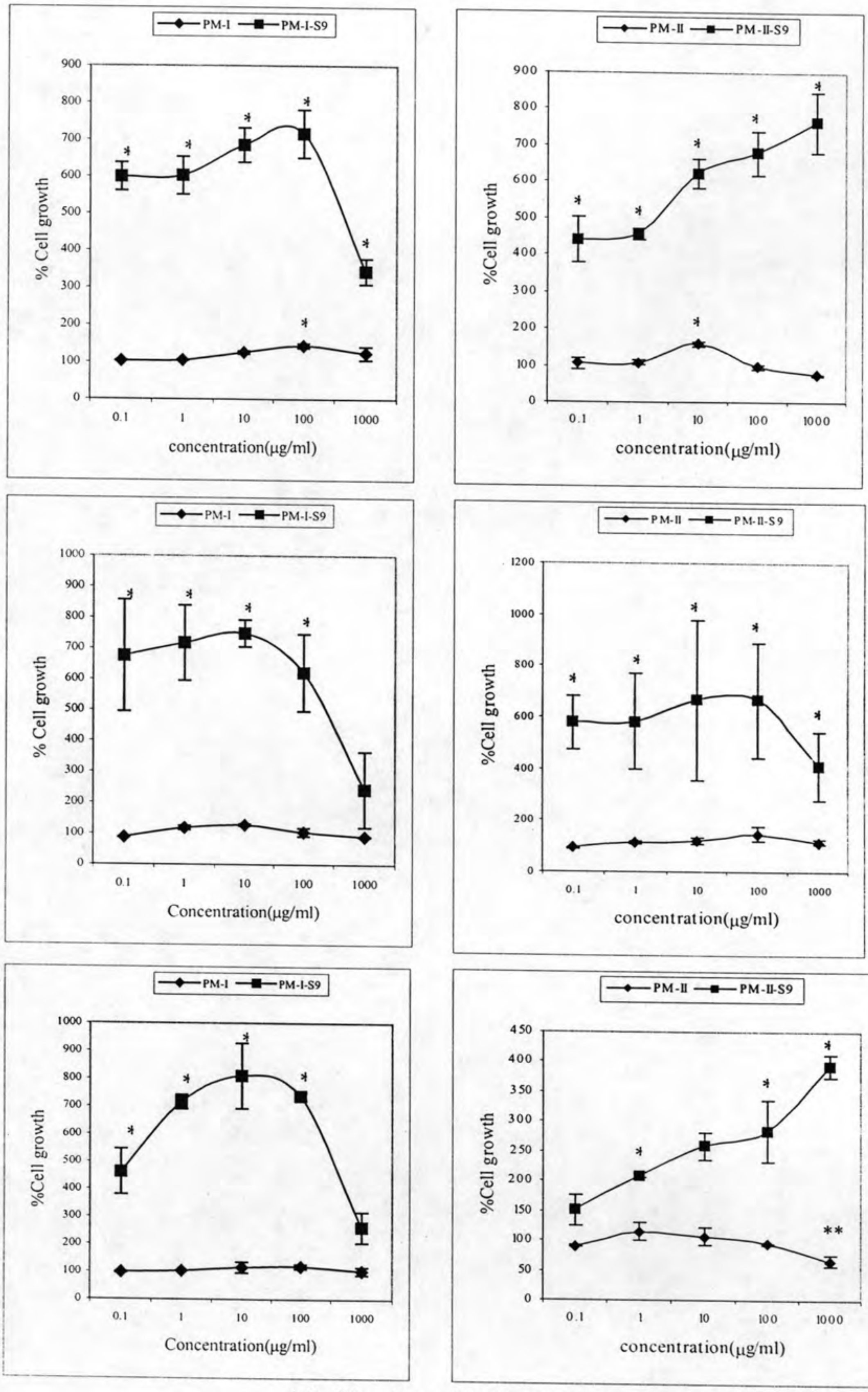
Season	PM	Concentration (μg/ml)				
		0.1	1	10	100	1000
Summer	PM-I	5.79	5.74	5.42	4.96	2.76
	PM-II	4.18	4.22	3.92	7.06	10.20
	PM-III	3.13	3.97	3.56	4.69	8.49
	PM-IV	5.57	5.68	5.15	4.65	5.10
	PM-V	4.19	4.17	3.34	7.51	5.07
Rainy	PM-I	7.72	6.05	5.85	5.93	2.70
	PM-II	6.29	5.07	5.55	4.52	3.52
	PM-III	4.96	3.43	2.81	3.07	3.33
	PM-IV	5.91	6.47	8.35	6.55	3.85
	PM-V	4.51	7.10	6.28	6.39	5.02
Winter	PM-I	4.75	6.97	7.04	6.20	2.54
	PM-II	1.68	1.83	2.44	3.02	6.01
	PM-III	3.32	3.53	3.01	4.83	4.23
	PM-IV	4.16	4.35	3.66	4.32	4.03
	PM-V	3.72	4.66	4.85	4.10	4.02

P. mirifica extract in the presence of S9 mixture showed more proliferative effect than *P. mirifica* in the absence of S9 mixture (Table 4.21-4.22 and Figure4.8-4.10). Proliferative effect of S9 mixture on PM-I, PM-II, PM-III, PM-IV and PM-V was increased 2.76 to 5.79, 3.92 to 10.20, 3.13 to 8.49, 4.65 to 5.68 and 3.34 to 7.51 folds in summer, 2.70 to 7.72, 3.52 to 6.29, 2.81 to 4.96, 3.85 to 8.35 and 4.51 to 7.10 folds in rainy season, 2.54 to 7.04, 1.68 to 6.01, 3.01 to 4.83, 3.66 to 4.35 and 3.72 to 4.85 folds in winter (Table 4.23).

Summer

Rainy

Winter



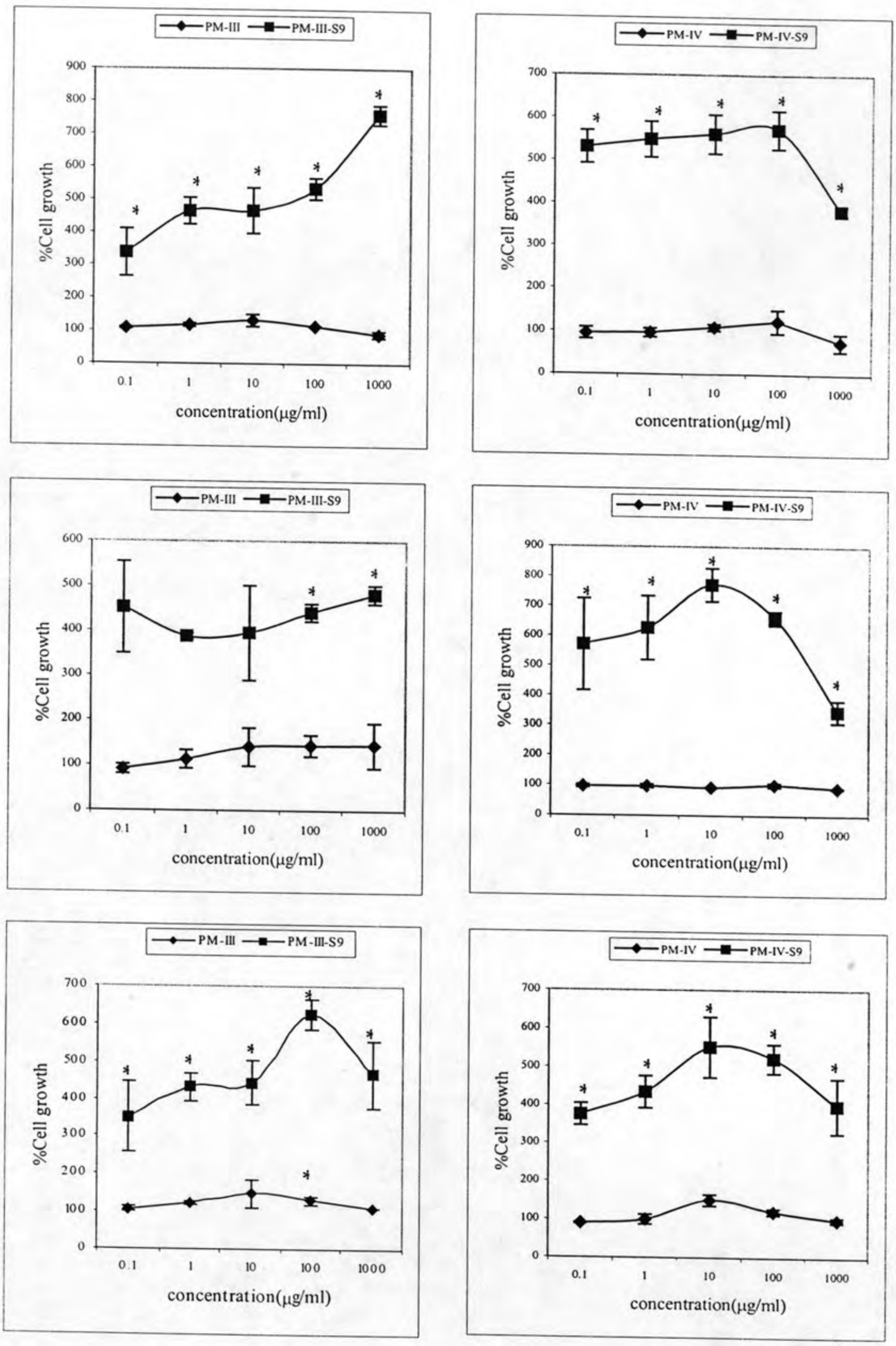
* Proliferation on MCF-7 cell, **Antiproliferation on MCF-7 cell

Figure 4.8 Comparison of growth response of PM-I and PM-II to MCF-7 cells in the presence and absence of S9 mixture. (♦ -S9 mixture, ■ +S9 mixture)

Summer

Rainy

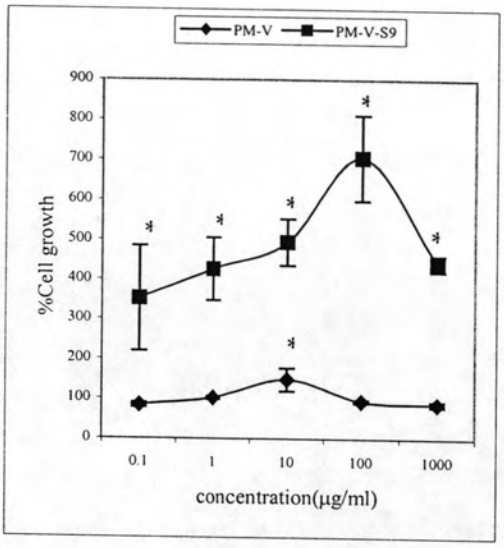
Winter



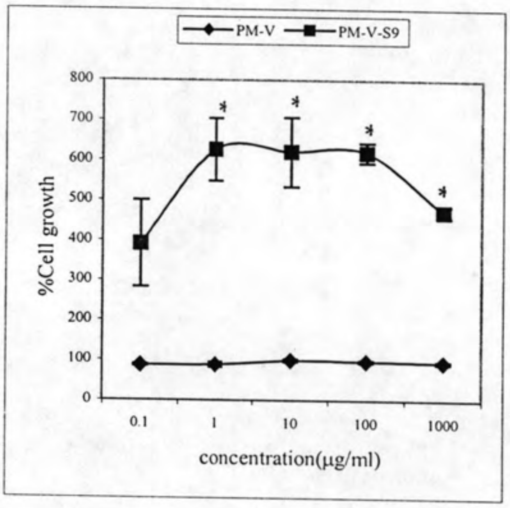
* Proliferation on MCF-7 cell

Figure 4.9 Comparison of growth response of PM-III and PM-IV to MCF-7 cells in the presence and absence of S9 mixture. (♦ -S9 mixture, ■ +S9 mixture)

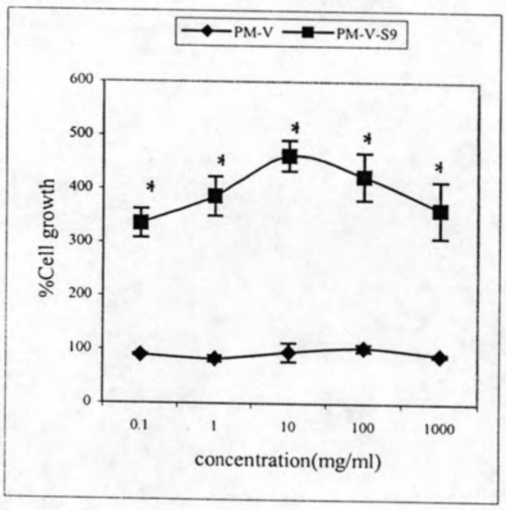
Summer



Rainy



Winter



* Proliferation on MCF-7 cell

Figure 4.10 Comparison of growth response of PM-V to MCF-7 cells in the presence and absence of S9 mixture. (♦ -S9 mixture, ■ +S9 mixture)

There were correlations between the percentage cell 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 correlation showed in Table 4.24.

Table 4.24 Correlations analysis of proliferation to MCF-7 cells with the major isoflavonoids

Season	Isoflavonoid contents (μg/100 g powder)	% Cell growth									
		-S9 mixture					+S9 mixture				
		0.1	1	10	100	1000	0.1	1	10	100	1000
Summer	Puerarin	-	-	-	-	-	-	-	-	-	-
	Daidzin	-	-	-	-	-	-	-	-	-	-
	Genistin	-	-	-	-	-	-	-	-	-	-
	Daidzein	-	*	-	-	-	-	-	-	-	-
	Genistein	-	*	-	-	-	-	-	-	-	-
	Total isoflavonoid	-	-	-	-	-	-	-	-	-	-
	aglycoside	-	-	-	-	-	**	-	*	-	-
	aglycoside/glycoside	-	-	-	-	-	-	-	**	-	-
Rainy season	Puerarin	-	-	-	-	-	-	-	-	-	-
	Daidzin	-	-	-	-	-	-	-	-	-	-
	Genistin	-	-	-	-	-	*	-	-	-	-
	Daidzein	-	*	-	*	-	-	-	-	-	-
	Genistein	-	**	-	-	-	*	-	-	-	-
	Total isoflavonoid	-	-	-	-	-	-	-	-	-	-
	aglycoside	-	-	**	-	-	-	-	-	-	-
	aglvcoside/glycoside	-	-	-	-	-	-	-	-	-	-
Winter	Puerarin	-	-	-	-	-	-	-	-	-	-
	Daidzin	-	-	-	-	-	-	-	-	-	-
	Genistin	-	-	-	-	-	-	-	-	-	-
	Daidzein	-	-	-	-	-	-	-	-	-	-
	Genistein	-	-	-	-	-	-	-	-	-	-
	Total isoflavonoid	-	-	-	-	-	-	-	-	-	-
	aglycoside	-	-	-	-	-	-	-	-	-	-
	aglycoside/glycoside	-	-	-	-	-	-	-	-	-	-

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

(-): No correlation

4.5.3. Effect of *P. mirifica* on vaginal cornification in ovariectomized rats

At the end of experiment, day 14^{th} of ovariectomy, the rat exhibited only leucocyte cell (L-type). It confirmed the completely disappear of ovaries and no endogenous ovarian estrogen produced.

4.5.3.1. Control groups

Negative control; the administration of distilled water did not influence the vaginal epithelium differentiation, only L-type cells were found (Figure 4.11)

Positive control ; the daily subcutaneous injection of 200 μg/100g BW/day of 17β-estradiol for 14 days during the treatment period induced cornification of the vaginal epithelium cells as early as the next day of the treatment (D_2) , and kept the Co-type cells until 6 days after the cessation of 17β-estradiol treatment (Figure 4.12)

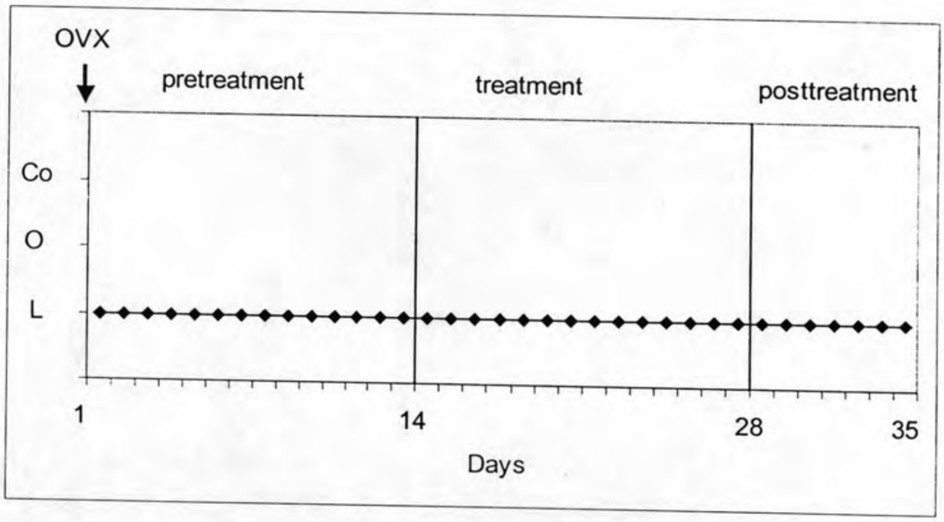


Figure 4.11 Differentiation of vaginal epithelium cells in rats treated with distilled water (Co; cornified cells, O; nucleated cells, L; leucocyte cells, OVX; ovariectomy)

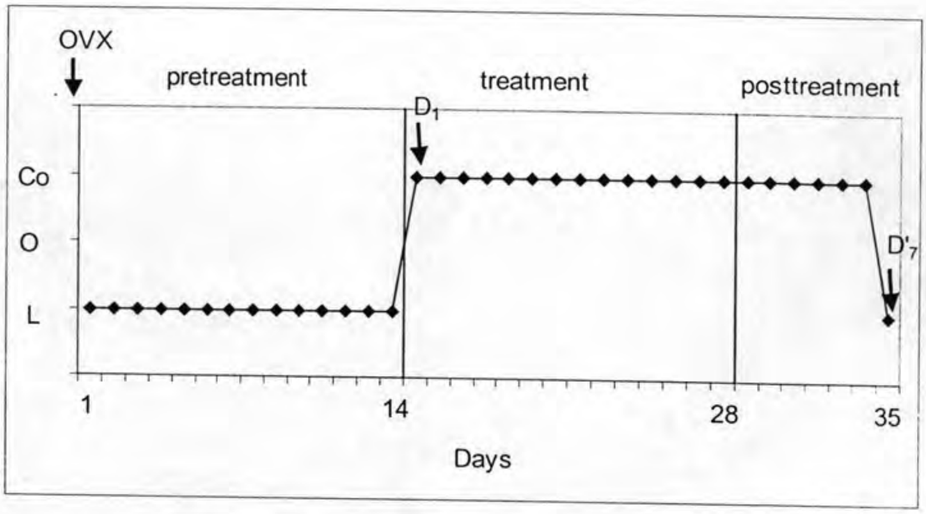


Figure 4.12 Differentiation of vaginal epithelium cells in rats treated with 200 μg/100g BW of 17β-estradiol (Co; cornified cells, O; nucleated cells, L; leucocyte cells, D; Day with appearance of cornified cells, D'; Day with appearance of leucocyte cells, OVX; ovariectomy)

4.5.3.2. *P. mirifica* treatment groups

The results were presented in Figure 4.13 to 4.17 and were described in term of collected seasons as follows;

Summer : The study of plant sample collected in April

Rat treated with PM-I, PM-III and PM-IV sample at the dosage of 100 mg/kg BW, the cell type was change from L to Co within 5 days of the 14-day treatment period and reverse to L-type cell within 3 days after cessation of the treatment. Rats treated with PM-II at the same dosage, the cell type was changed from L to Co within 5 days of 14-day treatment period and reversed to L-type cells within 2 days after cessation of the treatment. Rats treated with PM-V at the same dosage, the cell type was changed from L to Co within 4 days of 14-day treatment period and reversed to L-type cells within 2 days after cessation of the treatment. Rat treated with PM-I and PM-II sample at the dosage 1000 mg/kg BW, the cell type was changed from L-type cells to Co-type cell within 4 days of the 14-day treatment period and reversed to L-type cells within 3 days after cessation of the treatment. Rat treated with PM-III sample at the same dosage, the cell type was changed from L-type cells to Co-type cells within 3 days of the 14-day treatment period and recovered within 6 days after cessation of the treatment. Rat treated with PM-IV and PM-V sample at the same dosage, the cell type was changed from L-type cells to Co-type cells within 4 days of the 14-day treatment period and recovered within 4 days after cessation of the treatment (Figure 4.13 - 4.17)

Rainy season : The study of plant sample collected in August

Rat treated with PM-I sample at the dosage of 100 mg/kg BW, the cell type was change from L to Co within 7 days of the 14-day treatment period and reverse to L-type cell within 4 days after cessation of the treatment. Rats treated with PM-III and PM-IV sample at the dosage of 100 mg/kg BW did not have any effect on vaginal epithelium, an only L-type cell was found. Rats treated with PM-II and PM-V at the same dosage, the cell type was changed from L to Co within 9 days of 14-day treatment period and reversed to L-type cells within 4 days after cessation of the treatment. Rat treated with PM-I sample at the dosage 1000 mg/kg BW, the cell type was changed from L-type cells to Co-type cell within 4 days of the 14-day treatment period and reversed to L-type cells within 2 days after cessation of the treatment. Rat treated with PM-II sample at the same dosage, the cell type was changed from L-type cells to Co-type cells within 4 days of the 14-day treatment period and recovered within 4 days after cessation of the treatment Rats treated with PM-III at the same dosage, the cell type was changed from L to Co within 3 days of 14-day treatment period and reversed to L-type cells within 3 days after cessation of the treatment. Rats treated with PM-IV and PM-V at the same dosage, the cell type was changed from L to Co within 4 days of 14-day treatment period and reversed to L-type cells within 3 days after cessation of the treatment. (Figure 4.13 – 4.17)

Winter : The study of plant sample collected in December

Rat treated with PM-I sample at the dosage of 100 mg/kg BW, the cell type was change from L to Co within 5 days of the 14-day treatment period and reverse to L-type cell within 2 days after cessation of the treatment. Rats treated with PM-II sample at the same dosage, the cell type was change from L to Co within 6 days of the 14-day treatment period and reverse to L-type cell within 3 days after cessation of the treatment. Rat treated with PM-III and PM-IV sample at the same dosage, the cell type was change from L to Co within 5 days of the 14-day treatment period and reverse to L-type cell within 3 days after cessation of the treatment. Rats treated with PM-V at the same dosage, the cell type was changed from

L to Co within 7 days of 14-day treatment period and reversed to L-type cells within 3 days after cessation of the treatment. Rat treated with PM-I and PM-III sample at the dosage 1000 mg/kg BW, the cell type was changed from L-type cells to Co-type cell within 3 days of the 14-day treatment period and reversed to L-type cells within 5 days after cessation of the treatment. Rat treated with PM-II, PM-IV and PM-V sample at the same dosage, the cell type was changed from L-type cells to Co-type cells within 3 days of the 14-day treatment period and recovered within 4 days after cessation of the treatment (Figure 4.13 – 4.17)

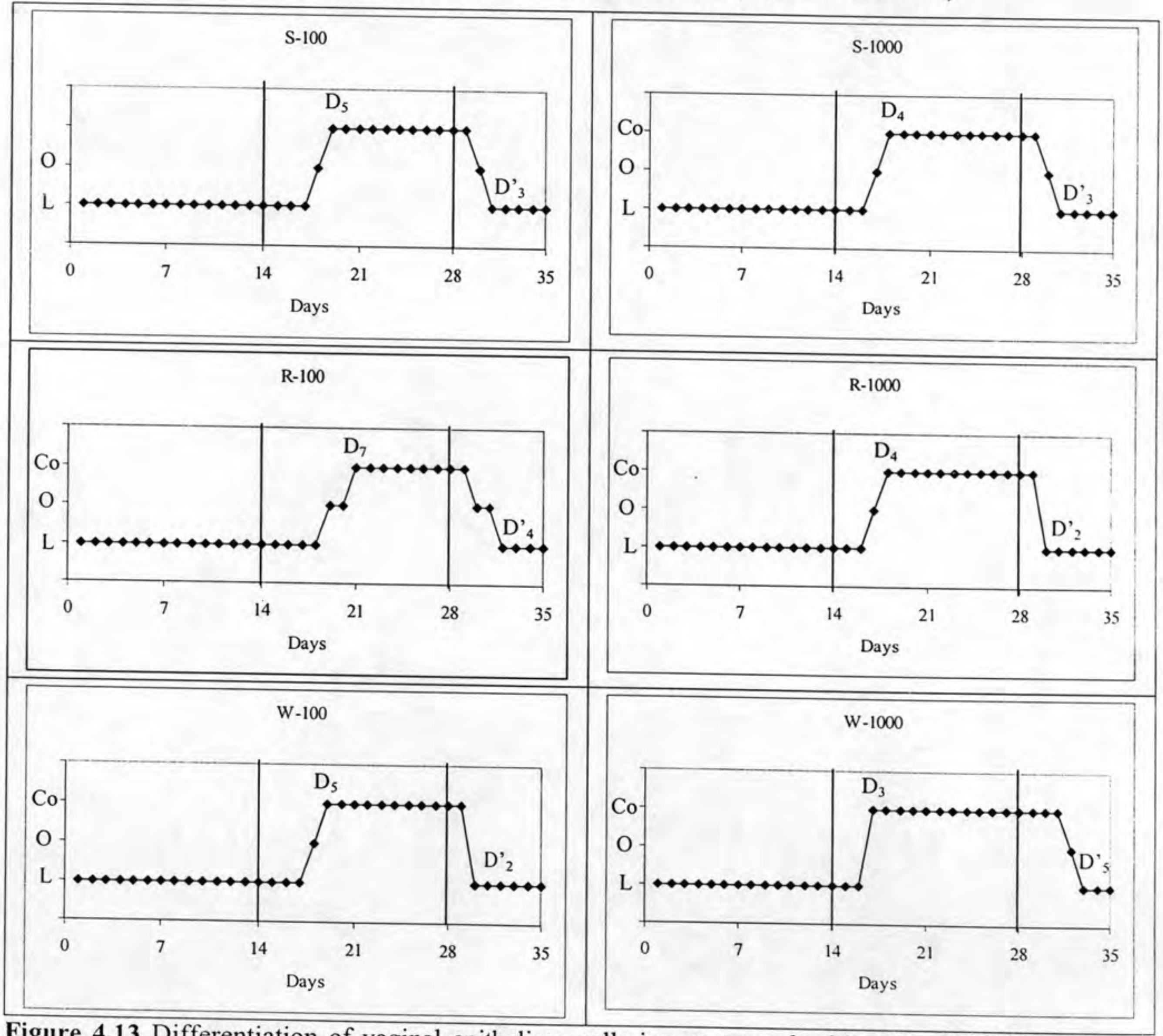


Figure 4.13 Differentiation of vaginal epithelium cells in rats treated with 100 and 1000 mg/kg BW of PM-I powder derived from tubers collected in three seasons ; S-Summer, R-Rainy, W-Winter (Co; cornified cells, O; nucleated cells, L; leucocyte cell, D; Day with appearance of cornified cells, D' ; Day with appearance of leucocyte cells).

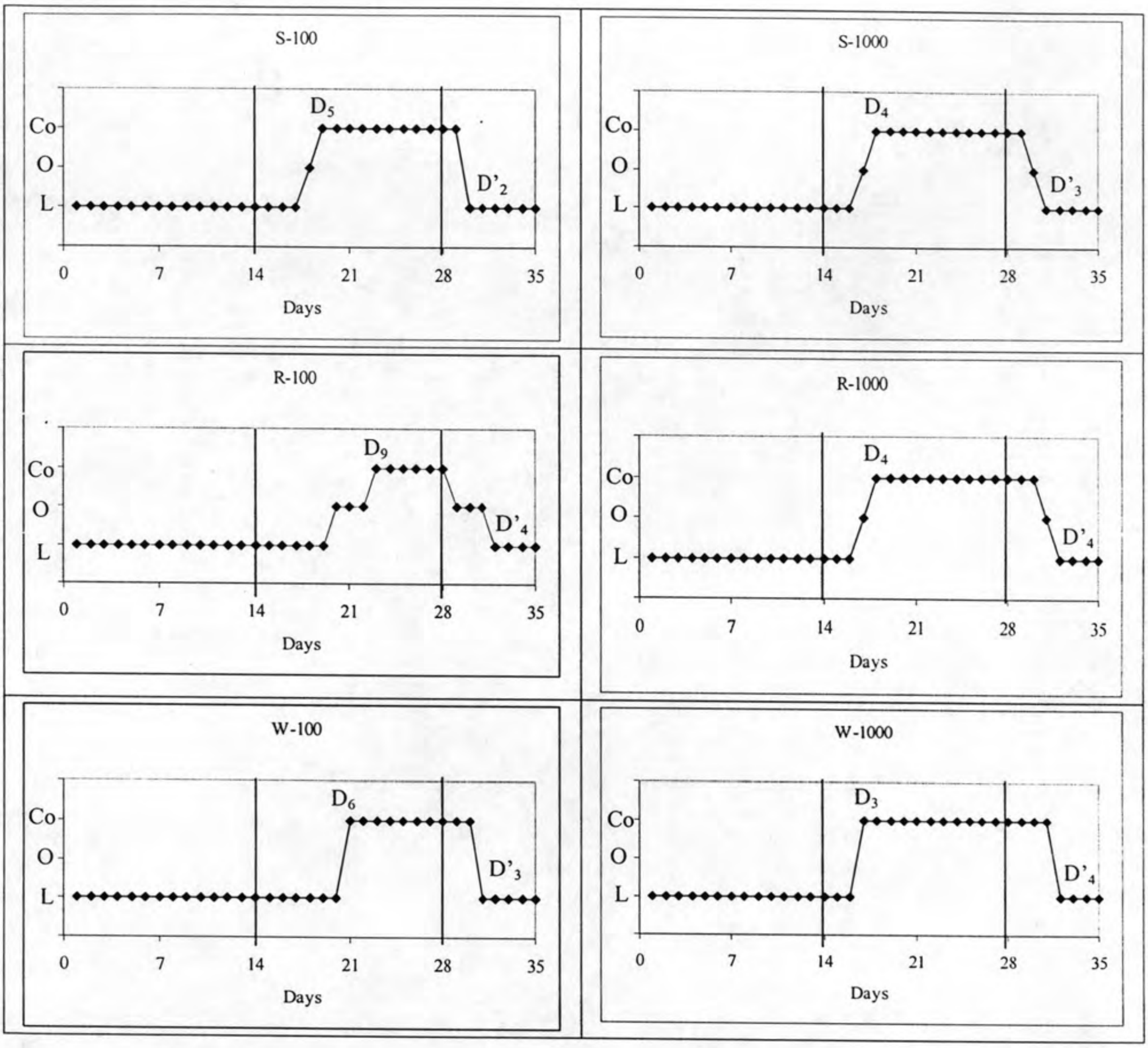


Figure 4.14 Differentiation of vaginal epithelium cells in rats treated with 100 and 1000 mg/kg BW of PM-II powder derived from tuber in three seasons ; S-Summer, R-Rainy, W-Winter (Co; cornified cells, O; nucleated cells, L; leucocyte cell, D; Day with appearance of cornified cells, D' ; Day with appearance of leucocyte cells).

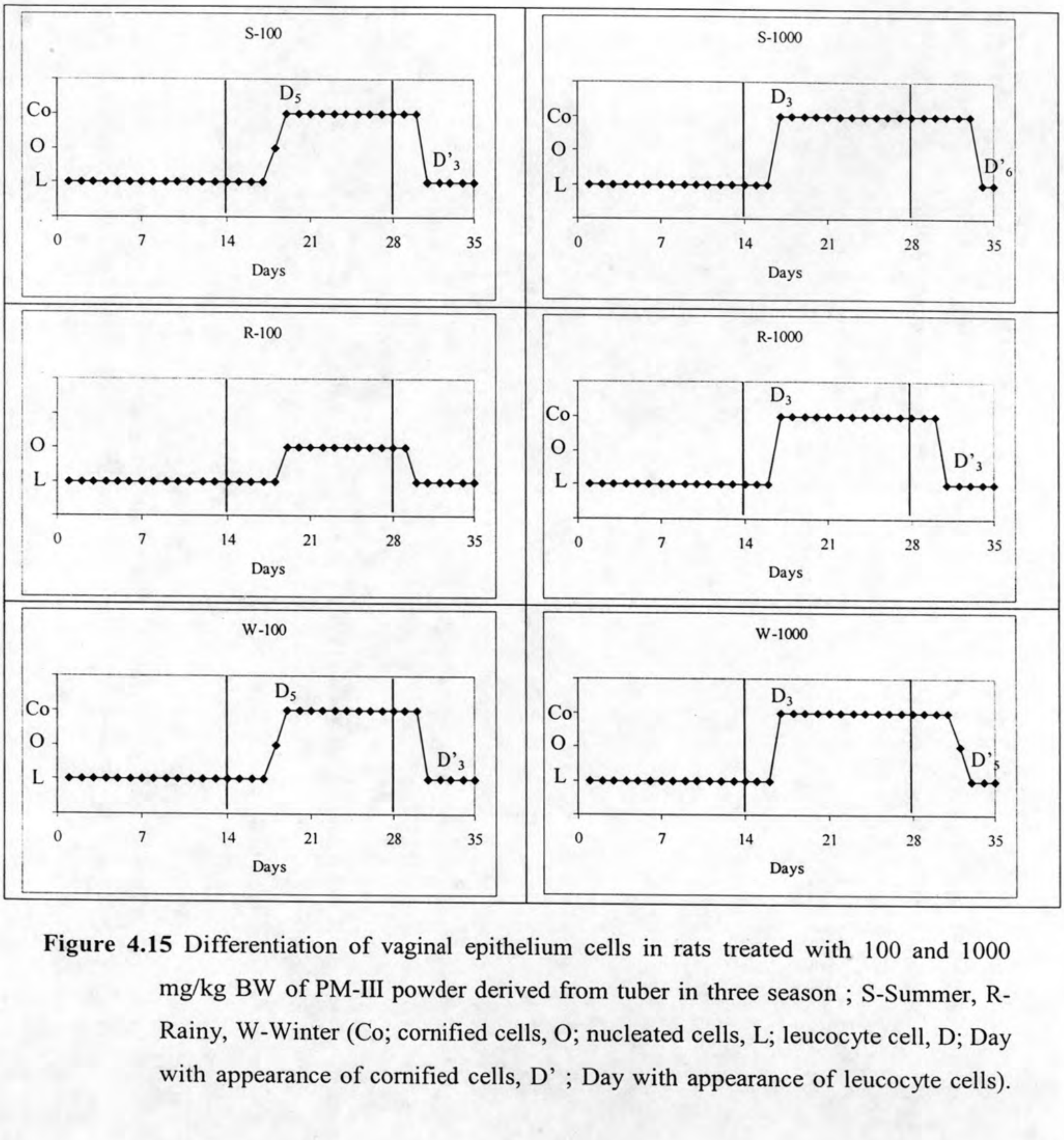


Figure 4.15 Differentiation of vaginal epithelium cells in rats treated with 100 and 1000 mg/kg BW of PM-III powder derived from tuber in three season ; S-Summer, R-Rainy, W-Winter (Co; cornified cells, O; nucleated cells, L; leucocyte cell, D; Day with appearance of cornified cells, D' ; Day with appearance of leucocyte cells).

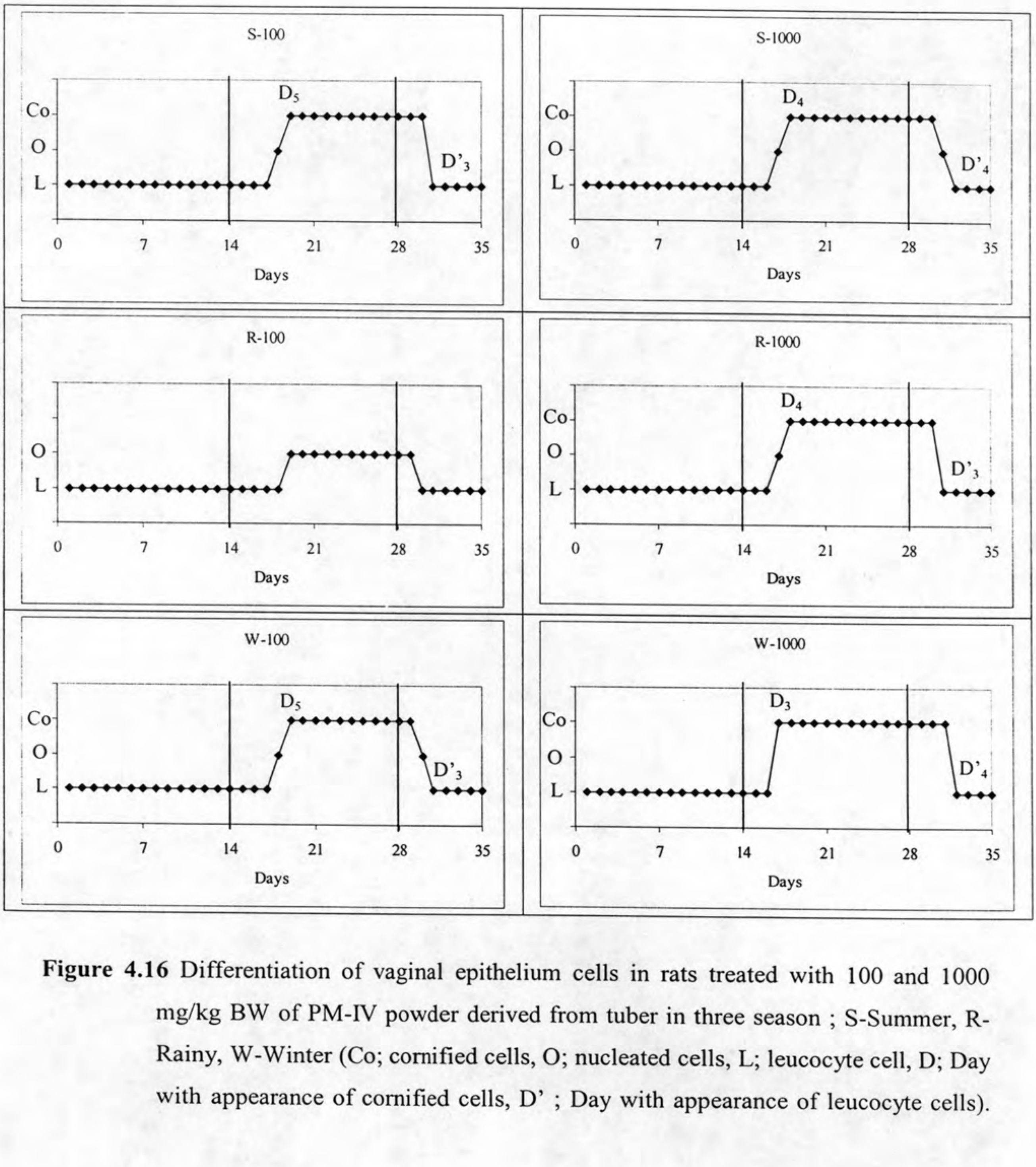


Figure 4.16 Differentiation of vaginal epithelium cells in rats treated with 100 and 1000 mg/kg BW of PM-IV powder derived from tuber in three season ; S-Summer, R-Rainy, W-Winter (Co; cornified cells, O; nucleated cells, L; leucocyte cell, D; Day with appearance of cornified cells, D' ; Day with appearance of leucocyte cells).

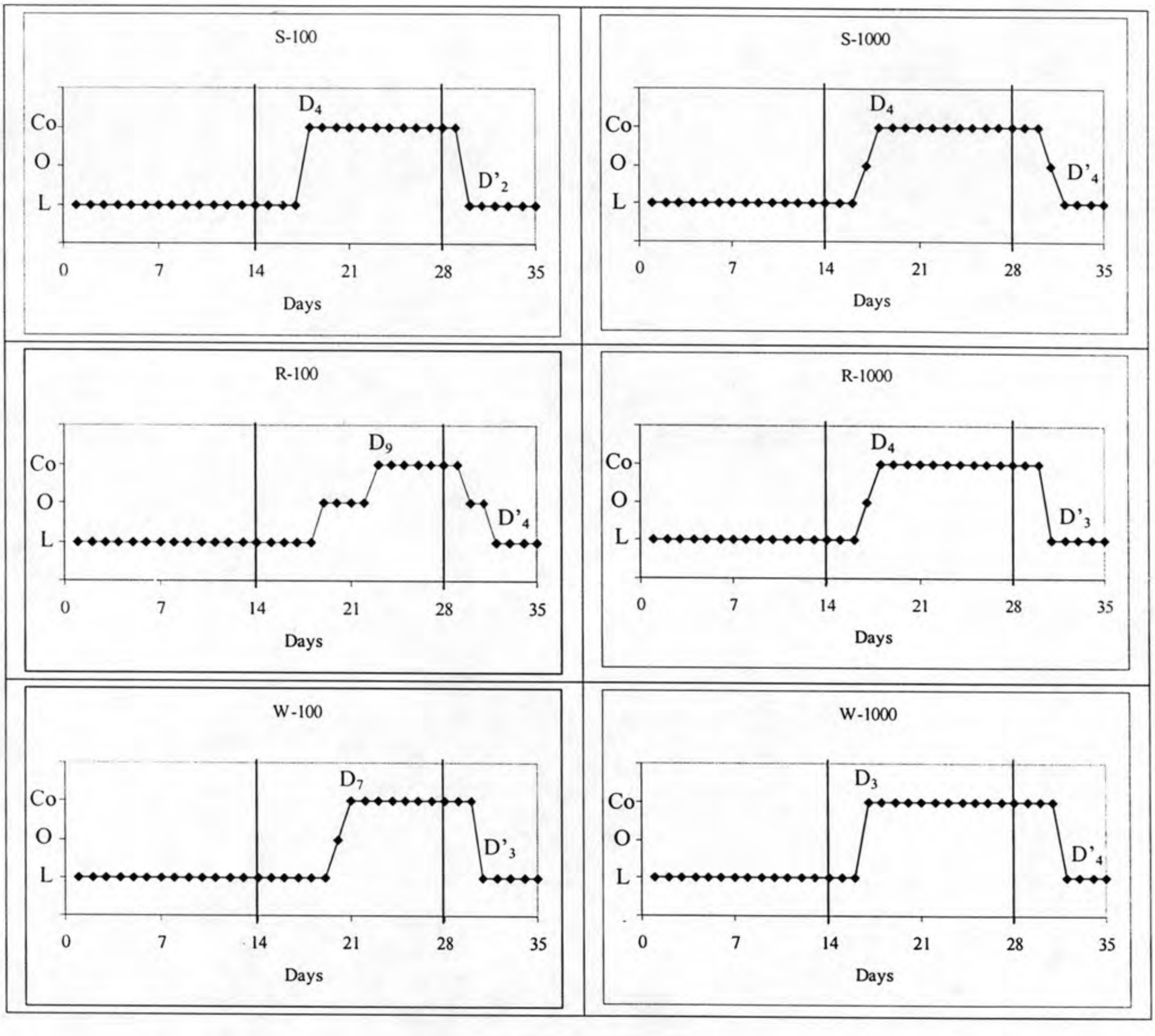


Figure 4.17 Differentiation of vaginal epithelium cells in rats treated with 100 and 1000 mg/kg BW of PM-V powder derived from tuber in three season ; S-Summer, R-Rainy, W-Winter (Co; cornified cells, O; nucleated cells, L; leucocyte cell, D; Day with appearance of cornified cells, D' ; Day with appearance of leucocyte cells).

From the changes of vaginal epithelium cells in rats treated with 100 and 1000 mg/kg BW of *P. mirifica* powder (PM-I, PM-II, PM-III, PM-IV and PM-V), there were differences in the first day of appearance of cornified cell during treatment period. It could be summarized in Table 4.25.

Table 4.25 First day of appearance of cornified cells in rats after treated with *Pueraria mifica*, distilled water and 17β-estradiol, during treatment period (N^L; No cornified cells, and leucocyte cells were found throughout the experiment period and 14 days were used as a number for statistical analysis).

Season		Dose 100 mg/kg BW	Dose 1000 mg/kg BW
Summer	PM-I	5	4
	PM-II	5	4
	PM-III	5	3
	PM-IV	5	4
	PM-V	4	4
Mean±S.E.M.		4.80 ± 0.20^b	3.80 ± 0.20^c
Rainy season	PM-I	7	4
	PM-II	9	4
	PM-III	N^L	3
	PM-IV	N^L	4
	PM-V	9	4
Mean±S.E.M.		10.60 ± 1.44^c	3.80 ± 0.20^c
Winter	PM-I	5	3
	PM-II	6	3
	PM-III	5	3
	PM-IV	5	3
	PM-V	7	3
Mean±S.E.M.		5.60 ± 0.40^b	3.00 ± 0.00^b
Control groups	DW	N^L	
	17β-estradiol	$1.00 \pm 0.00^{a/a}$	

Means not sharing a common superscript letter are significantly different (P < 0.05) as determined by Duncan's multiple range test.

From the statistical analysis of the period (day) of appearance of cornified cells among 3 seasons; summer, rainy season and winter in Table 4.25 it was found that PM-III and PM-IV collected in rainy season did not show vaginal epithelium cornification at the dosage of 100 mg/kg BW. Samples collected in rainy season were statistical differences among those 3 seasons. The dosage of 1000 mg/kg BW of PM collected in winter tended to exhibit higher estrogenic activity (sooner of first day of appearance of cornified cells) than summer and rainy season.

Table 4.26 First day of appearance of leucocyte cells in rats after treated with *Pueraria mifica*, distilled water and 17β-estradiol during post-treatment period (N^L; No cornified cells and leucocyte cells were found throughout the experiment period).

Season		Dose 100 mg/kg BW	Dose 1000 mg/kg BW
Summer	PM-I	3	3
	PM-II	2	3
	PM-III	3	6
	PM-IV	3	4
	PM-V	2	4
Mean±S.E.M.		2.60 ± 0.24^{b}	4.00 ± 0.55^{bc}
Rainy season	PM-I	4	2
	PM-II	4	4
	PM-III	2	3
	PM-IV	2	3
	PM-V	4	3
Mean±S.E.M.		3.20 ± 0.24^{b}	3.00 ± 0.32^{b}
Winter	PM-I	2	5
	PM-II	3	4
	PM-III	3	5
	PM-IV	3	4
	PM-V	3	4
Mean±S.E.M.		2.80 ± 0.20^{b}	4.40 ± 0.24^{c}
Control groups	DW	$1.00 \pm 0.00^{a/a}$	
	17β-estradiol	$7.00 \pm 0.00^{d/d}$	

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

From the statistical analysis of the first day of appearance of leucocytes cells among 3 seasons; summer, rainy season and winter in Table 4.21, *P. mirifica* collected in rainy season tended to exhibit weaker estrogenic activity (shorter day of leucocyte apperrances) than the other seasons. The dosage of 1000 mg/kg BW of *P. mirifica* collected in winter tended to exhibit stronger estrogenic activity (longer day of leucocyte appearances) than summer and rainy season.

When the data of Table 4.20 and 4.21 were taken into account and calculated for the duration of the appearance of cornified cells during the treatment and post-treatment periods, the estrogenic activity of *P. mirifica* collected in 3 seasons could be ranked as shown in Table 4.27 and Figure 4.13 - 4.17.

Table 4.27 The duration of appearance of cornified cells during treatment and post treatment periods.

Season		Dose 100 mg/kg BW/day	Dose 1000 mg/kg BW/day
Summer	PM-I	11	12
	PM-II	11	12
	PM-III	12	16
	PM-IV	12	13
	PM-V	12	13
Mean±S.E.M.		11.60 ± 0.24^{c}	13.20 ± 0.73^{bc}
Rainy season	PM-I	9	12
	PM-II	6	13
	PM-III	0	14
	PM-IV	0	13
	PM-V	7	13
Mean±S.E.M.		4.40 ± 1.86^{b}	13.00 ± 0.32^{bc}
Winter	PM-I	11	15
	PM-II	10	15
	PM-III	12	15
	PM-IV	11	15
	PM-V	10	15
Mean±S.E.M.		10.80 ± 0.37^{c}	15.00 ± 0.00^{c}
Control groups	DW	$0.00 \pm 0.00^{a/a}$	
	17β-estradiol	$20.00 \pm 0.00^{d/d}$	

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

To compare the estrogenic activity of *P. mirifica* of the five clones (PM-I, PM-II, PM-III, PM-IV and PM-V), the length of the appearance of cornified cells after *P. mirifica* treatment and post-treatment were compared. Sample at dose 1000 mg/kg BW collected in winter were statistical higher than the others. The dose 100 and 1000 mg/kg BW of *P. mirifica* collected in rainy season tended to show the lowest estrogenic activity (the shortest duration of appearances of cornified cells).

To compare the estrogenic activity of *P. mirifica* of the two doses, the length of the appearance of cornified cells after *P. mirifica* treatment and post-treatment were compared. At the dosage of 100 mg/kg BW, it was significant longer than the negative control and significantly shorter than the positive control. At the dose of 1000 mg/kg BW, the rats exhibited the hight estrogenic activity but were still significantly lower than the positive control ($P < 0.05$) (Figure 4.18).

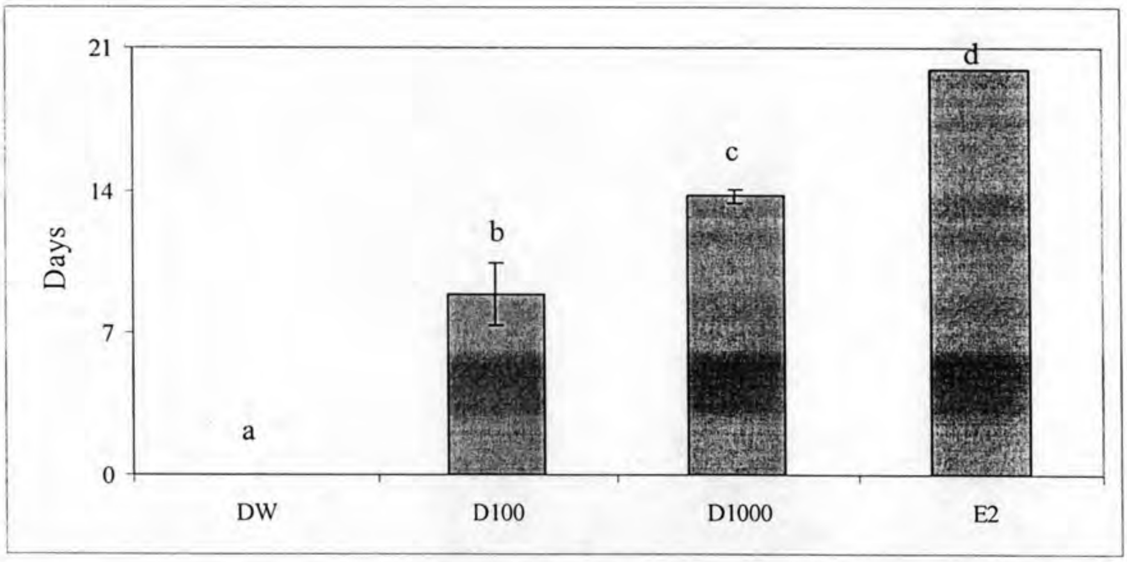


Figure 4.18 The mean ± S.E.M. of the length of the appearance of cornified cells during 21-day period of *P. mirifica* treatment and post-treatment.
Means not sharing a common superscript letter are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Table 4.28 Correlations analysis of the length of the appearance of cornified cells during treatment and post-treatment period of *P. mirifica* with the major isoflavonoids in *P. mirifica*.

Season	Isoflavonoid	The length of the appearance of cornified cells	
		100 mg/kg BW/day	1000 mg/kg BW/day
Summer	Puerarin	-	-
	Daidzin	-	-
	Genistin	-	-
	Daidzein	-	-
	Genistein	-	-
	Total isoflavonoid	-	-
	aglycoside	-	-
	aglycoside/glycoside	†	††
Rainy season	Puerarin	-	-
	Daidzin	-	-
	Genistin	-	-
	Daidzein	-	-
	Genistein	-	-
	Total isoflavonoid	-	-
	aglycoside	†	-
	aglycoside/glycoside	†	-
Winter	Puerarin	-	-
	Daidzin	-	-
	Genistin	-	-
	Daidzein	-	-
	Genistein	-	-
	Total isoflavonoid	-	-
	aglycoside	-	-
	aglycoside/glycoside	-	-

†† : Negative correlation is significant at the 0.01 level (2-tailed)

† : Negative correlation is significant at the 0.05 level (2-tailed)

\- : No correlation

4.5.4. Effect of *P. mirifica* on the percentage of cornified cells in ovariectomized rats

From the result of vaginal cornification in rats after treated with 100 mg/kg BW of *P. mirifica*, it was found that the earliest response is on D_3 after treated with PM-III collected in winter. The earliest response is on D_3 after treated with PM-III collected in summer and rainy, all PM collected in winter after treated with 1000 mg/kg BW. These results agreed with the duration of appearance of cornified cells during both of treatment and post treatment periods.

The vaginal smear cells were counted and calculated for the percentage of cornified cells. One-hundred vaginal smear cells were randomly counted for all of Co, O and L cell types.

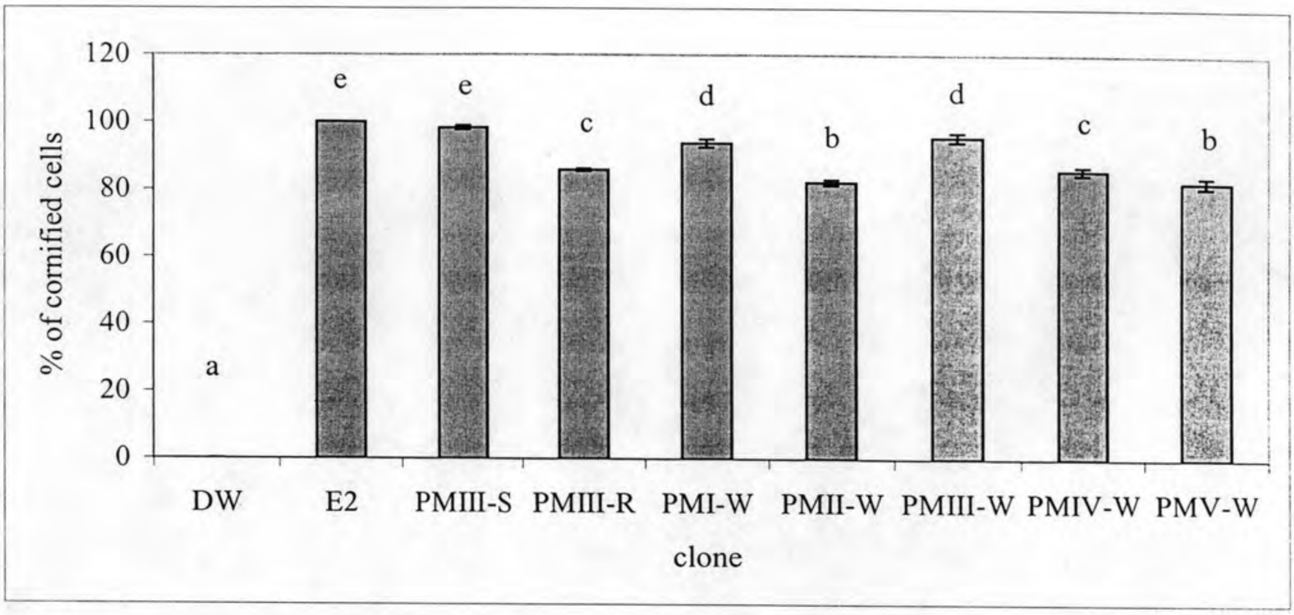


Figure 4.19 The percentage of rat cornified cells counts on D_3 after feeding of *P. mirifica* at dose of 1000 mg/kg BW, distilled water and 17β-estradiol.

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

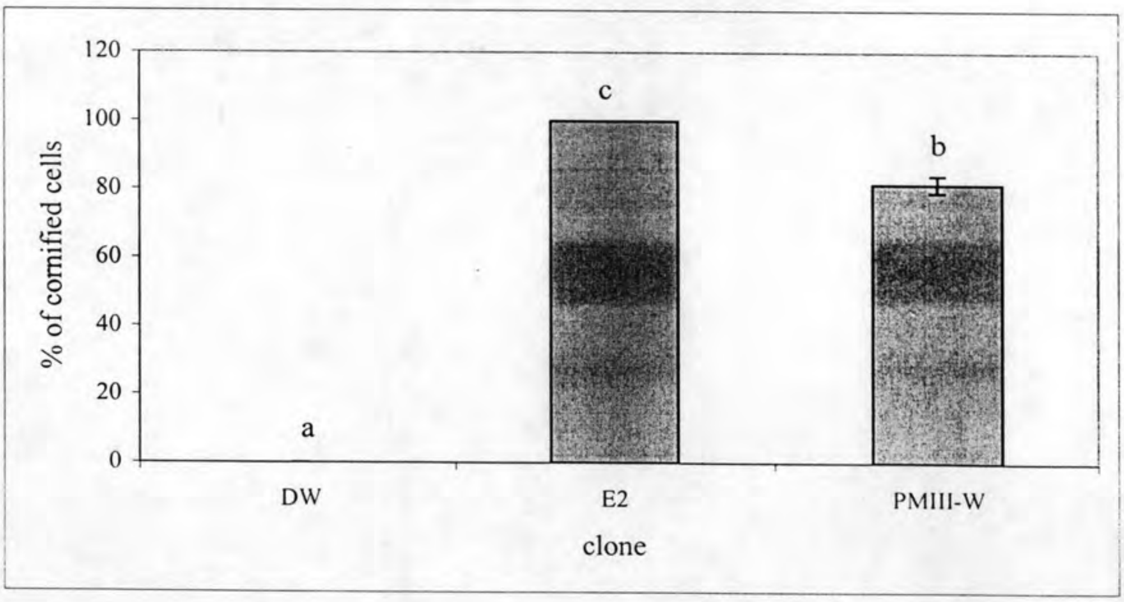


Figure 4.20 The percentage of rat cornified cells counts on D_5 after feeding of *P. mirifica* at dose of 100 mg/kg BW, distilled water and 17β-estradiol.

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

4.5.5. Effect of *P. mirifica* on body weight of ovariectomized rats

The rat body weights in all groups were not significant difference during the pretreatment period (day 1^{st} and 7^{th}) compared to the negative control group. During the *P. mirifica* treatment, the rat body weight was decreased in a dose-dependent manner. The body weight changes in each group are as follows;

4.5.5.1 Control groups

Negative control: The body weight of rats in the negative control group was significantly increased from day 1 presented in Figure 4.21.

Positive control (17β-estradiol): The body weight of rats in the positive control group compared to the negative control group was significantly increased as presented in Figure 4.21.

4.5.5.2 *P. mirifica* treatement groups

Dose 100 mg/kg BW/day

The body weights were significant decreased from the control group in the day 7, 14, 21, 28, 35 ($P < 0.05$), (Figure 4.21).

Dose 1,000 mg/kg BW/day

The body weights were significant decreased from the control group in the day 7, 14, 21, 28, 35 ($P < 0.05$), (Figure 4.21).

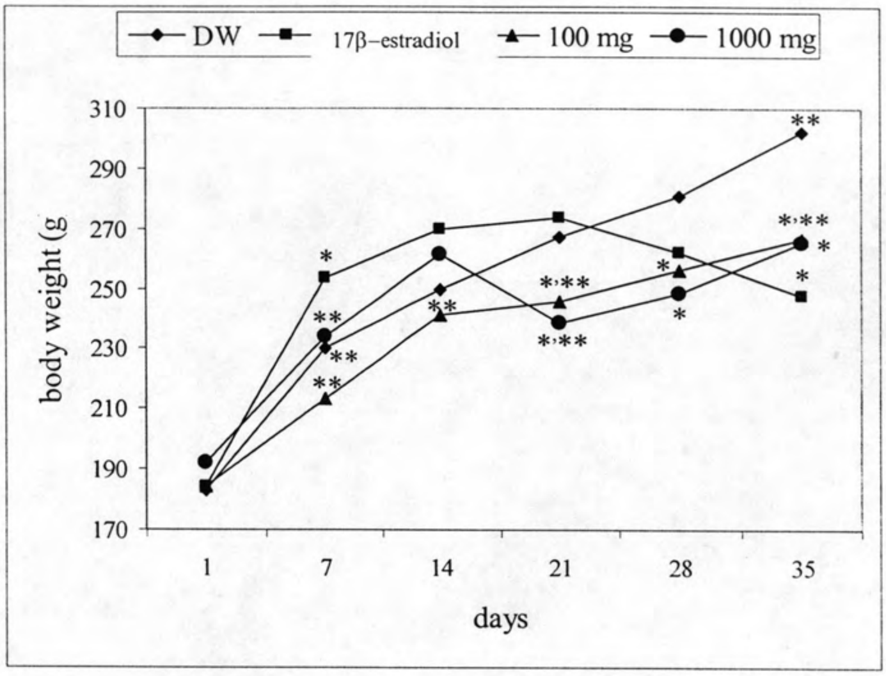


Figure 4.21 The body weight of rat treated with distilled water, 17β-estradiol, *P. mirifica* at the dose of 100 and 1000 mg/kg BW on day 1, 7, 14, 21, 28, 35.

* : significant difference from negative control

** : significant difference from positive control

4.5.6. Effect of *P. mirifica* on uterine weights

The result of uterine weight in rats treated with *P. mirifica* (PM-I, PM-II, PM-III, PM-IV and PM-V) 17β-estradiol and distilled water are presented in Table 4.29 and 4.30.

However, there were no significant differences of uterine weights in rats treated with 1000 mg/kg BW of the three seasons (Table 4.29).

When the data of uterine weights of rats treated with PM collected in three seasons were pooled and compared between doses, the uterine weight was increased in a dose dependent manner as presented in Figure 4.22. The uterine weight of rats treated with 100 and 1000 mg/kg BW of *P. mirifica* showed significantly greater than the negative control but less than the positive control.

Table 4.29 The uterine weights (g) of rat treated with 100 mg/kg BW of 5 clones of *P. mirifica* at the end of post-treatment period (day 35th).

clone	100 mg/kg BW		
	Summer	Rainy	Winter
PM-I	0.145 ± 0.047^{a}	0.166 ± 0.048^{a}	0.173 ± 0.01^{b}
PM-II	0.133 ± 0.013^{a}	0.179 ± 0.032^{a}	0.175 ± 0.009^{b}
PM-III	0.198 ± 0.005^{a}	0.178 ± 0.007^{a}	0.161 ± 0.007^{ab}
PM-IV	0.171 ± 0.044^{a}	0.150 ± 0.011^{a}	0.159 ± 0.007^{ab}
PM-V	0.177 ± 0.019^{a}	0.170 ± 0.019^{a}	0.170 ± 0.016^{b}
Mean	$0.165 \pm 0.013^{a'}$	$0.169 \pm 0.011^{a'}$	$0.168 \pm 0.004^{a'}$
17β-estradiol	0.537 ± 0.0142^{b}	0.537 ± 0.0142^{b}	0.537 ± 0.0142^{c}
DW		0.133 ± 0.0014^{a}	

Means not sharing a common superscript letter with or without prime in the same column are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

Table 4.30 The uterine weights of rat treated with 1000 mg/kg BW of 5 clones of *P. mirifica* at the end of treatment period (day 28^{th})

clone	Treatment period (1000 mg/kg BW)		
	Summer	Rainy	Winter
PM-I	0.263 ± 0.009^{b}	0.334 ± 0.033^{b}	0.314 ± 0.041^{b}
PM-II	0.335 ± 0.023^{bc}	0.369 ± 0.014^{b}	0.389 ± 0.02^{b}
PM-III	0.537 ± 0.031^{d}	0.478 ± 0.032^{c}	0.324 ± 0.043^{b}
PM-IV	0.360 ± 0.032^{c}	0.336 ± 0.052^{b}	0.363 ± 0.034^{b}
PM-V	0.365 ± 0.054^{c}	0.387 ± 0.042^{bc}	0.394 ± 0.026^{b}
Mean	$0.372 \pm 0.023^{a'}$	$0.381 \pm 0.018^{a'}$	$0.357 \pm 0.015^{a'}$
17β-estradiol	1.152 ± 0.019^{e}	1.152 ± 0.019^{d}	1.152 ± 0.019^{c}
DW		0.137 ± 0.001^{a}	

Means not sharing a common superscript letter with or without prime in the same column are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

Table 4.31 The uterine weights of rat treated with 1000 mg/kg BW of 5 clones of *P. mirifica* at the post-treatment period (day 35^{th})

clone	Post-treatment period (1000 mg/kg BW)		
	Summer	Rainy	Winter
PM-I	0.175 ± 0.022^{ab}	0.198 ± 0.009^{ab}	0.224 ± 0.016^{b}
PM-II	0.218 ± 0.038^{bc}	0.269 ± 0.028^{b}	0.232 ± 0.006^{b}
PM-III	0.471 ± 0.011^{d}	0.227 ± 0.005^{b}	0.231 ± 0.009^{b}
PM-IV	0.211 ± 0.017^{bc}	0.231 ± 0.052^{b}	0.228 ± 0.013^{b}
PM-V	0.208 ± 0.034^{bc}	0.245 ± 0.042^{b}	0.233 ± 0.011^{b}
Mean	$0.257 \pm 0.025^{a'}$	$0.234 \pm 0.014^{a'}$	$0.229 \pm 0.005^{a'}$
17β-estradiol	0.537 ± 0.001^{d}	0.537 ± 0.001^{c}	0.537 ± 0.001^{c}
DW		0.133 ± 0.014^{a}	

Means not sharing a common superscript letter with or without prime in the same column are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

Table 4.32 The relative percentage of uterine weights and body weight of rat treated with 1000 mg/kg BW of 5 clones of *P. mirifica* at the end of treatment period (day 28th)

clone	% relative weight in treatment period		
	Summer	Rainy	Winter
PM-I	0.108	0.146	0.117
PM-II	0.136	0.165	0.153
PM-III	0.222	0.203	0.128
PM-IV	0.148	0.154	0.142
PM-V	0.154	0.175	0.163
Mean	0.154	0.169	0.141
17β-estradiol	0.441	0.441	0.441
DW		0.049	

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

Table 4.33 The relative percentage of uterine weights and body weight of rat treated with 1000 mg/kg BW of 5 clones of *P. mirifica* at the post-treatment period (day 35th)

clone	% relative weight in post-treatment period		
	Summer	Rainy	Winter
PM-I	0.067	0.080	0.079
PM-II	0.083	0.110	0.088
PM-III	0.185	0.090	0.085
PM-IV	0.083	0.096	0.085
PM-V	0.080	0.102	0.093
Mean	0.100	0.095	0.086
17β-estradiol	0.217	0.217	0.217
DW		0.045	

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

Table 4.34 The uterine weights of rat treated with 100 mg/kg BW and 1000 mg/kg BW of 5 clones of *P. mirifica* at the end of treatment period (day 28th) and post-treatment period (day 35th)

		Treatment period		Post-treatment period
		100 mg/kg BW	1000 mg/kg BW	1000 mg/kg BW
PM-I	Summer	0.145 ± 0.047^{a}	0.263±0.009^{a}	0.175±0.022^{a}
	Rainy season	0.166 ± 0.048^{a}	0.334±0.033^{a}	0.198±0.009^{a}
	Winter	0.173 ± 0.010^{a}	0.314±0.041^{a}	0.224±0.016^{a}
	Mean± S.E.M.	0.161±0.021$^{a'}$	0.304±0.018$^{a'}$	0.199±0.010$^{a'}$
PM-II	Summer	0.133±0.013^{a}	0.335±0.023^{a}	0.218±0.038^{a}
	Rainy season	0.179±0.032^{a}	0.369±0.014^{a}	0.269±0.028^{a}
	Winter	0.175±0.009^{a}	0.389±0.020^{a}	0.232±0.006^{a}
	Mean± S.E.M.	0.162±0.012$^{a'}$	0.364±0.012$^{b'}$	0.240±0.016$^{b'}$
PM-III	Summer	0.198±0.005^{b}	0.537±0.031^{b}	0.471±0.011^{c}
	Rainy season	0.178±0.007^{ab}	0.478±0.032^{b}	0.227±0.005^{a}
	Winter	0.161±0.007^{a}	0.324±0.043^{a}	0.231±0.009^{a}
	Mean± S.E.M.	0.179±0.005$^{a'}$	0.447±0.031$^{c'}$	0.310±0.031$^{c'}$
PM-IV	Summer	0.171±0.044^{a}	0.360±0.032^{a}	0.211±0.017^{a}
	Rainy season	0.150±0.011^{a}	0.336±0.052^{a}	0.231±0.052^{a}
	Winter	0.159±0.007^{a}	0.363±0.034^{a}	0.228±0.013^{a}
	Mean± S.E.M.	0.160±0.014$^{a'}$	0.353±0.022$^{b'}$	0.223±0.018$^{ab'}$
PM-V	Summer	0.177±0.019^{a}	0.365±0.054^{a}	0.208±0.034^{a}
	Rainy season	0.170±0.019^{a}	0.387±0.042^{a}	0.245±0.042^{a}
	Winter	0.170±0.016^{a}	0.394±0.026^{a}	0.233±0.011^{a}
	Mean± S.E.M.	0.172±0.009$^{a'}$	0.382±0.023$^{b'}$	0.229±0.017$^{ab'}$

Means not sharing a common superscript letter with or without prime in the same column are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

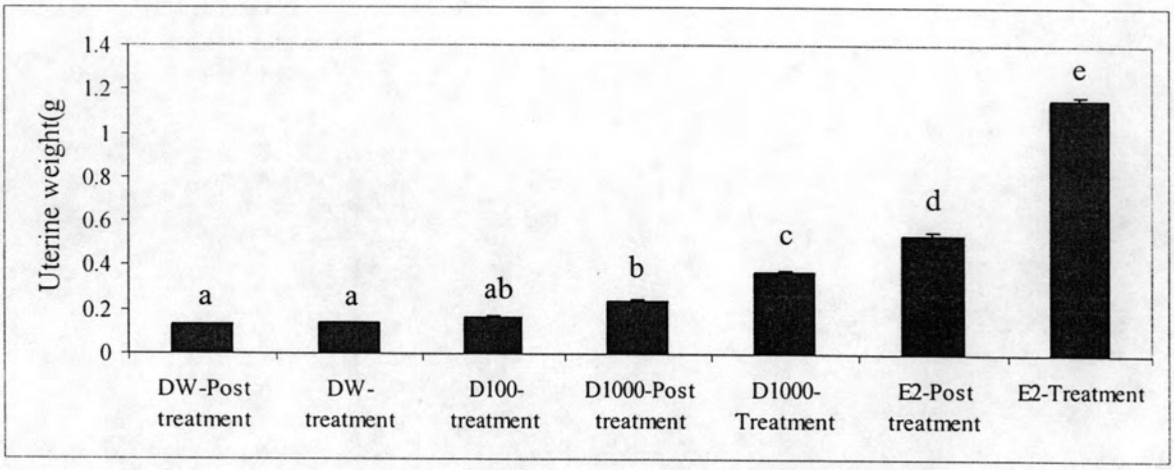


Figure 4.22 The rat uterine weight in the control, treatment and post-treatment group

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

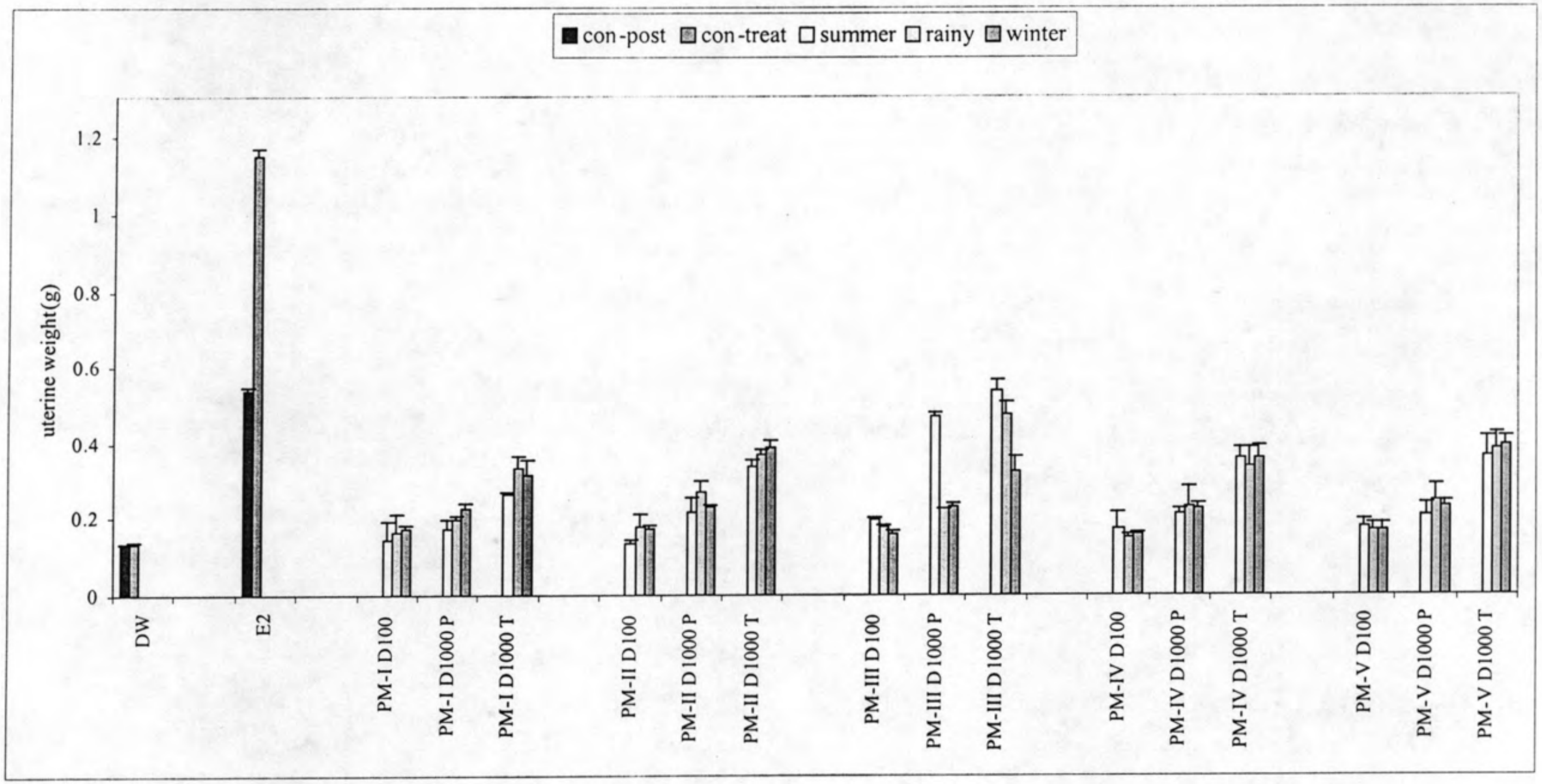


Figure 4.23 The rat uterine weight in the control and *P. mirifica* in treatment and post-treatment groups

Table 4.35 Correlations analysis of uterine weight with the major isoflavonoids in *P. mirifica*.

Season	Isoflavonoid	Uterine weight		
		Treatment (1000 mg/kg BW)	Post treatment (1000 mg/kg BW)	Post treatment (100 mg/kg BW)
Summer	Puerarin	-	-	-
	Daidzin	-	-	-
	Genistin	-	-	-
	Daidzein	-	-	-
	Genistein	-	-	-
	Total isoflavonoid	-	-	-
	aglycoside	†	-	-
	aglycoside/glycoside	††	-	-
Rainy season	Puerarin	-	-	-
	Daidzin	-	-	-
	Genistin	-	-	-
	Daidzein	-	-	-
	Genistein	-	-	-
	Total isoflavonoid	-	-	-
	aglycoside	-	-	-
	aglycoside/glycoside	-	-	-
Winter	Puerarin	-	-	-
	Daidzin	-	-	†
	Genistin	-	-	††
	Daidzein	-	-	-
	Genistein	-	-	-
	Total isoflavonoid	-	-	-
	aglycoside	-	-	-
	aglycoside/glycoside	-	-	-

† : Negative correlation is significant at the 0.05 level (2-tailed)
†† : Negative correlation is significant at the 0.01 level (2-tailed)
\- : No correlation

4.5.7. Effect of *P. mirifica* on uterine gland number of ovariectomized rats

Uterus tissue was submitted to histology preparation and analysis. The key parameter is uterine gland number. The result of uterine gland number in rats treated with *P. mirifica* (PM-I, PM-II, PM-III, PM-IV and PM-V), 17β-estradiol and distilled water are presented in Table 4.36 and Figure 4.24. The increment of uterine gland number at the end of the treatment period (day 28th) was agreed with changes of vaginal epithelial cells in PM-III collected in rainy season and winter. The uterine gland number in the uterine tissue of rat treated with 1000 mg/kg BW of PM-I and PM-IV collected in summer, PM-III collected in rainy season and winter were significantly higher than others (Table 4.36).

The uterine gland number in the uterine tissue of rat treated with 1000 mg/kg BW collected in summer were as follows; PM-I > PM-IV > PM-III ≥PM-V > PM-II, rainy season were as follows; PM-III > PM-IV > PM-I > PM-V > PM-II, and winter were as follows; PM-III ≥PM-IV > PM-I ≥PM-II > PM-V. However, there were no significant differences of uterine gland number in uterine tissue of rats treated with 1000 mg/kg BW of PM-V collected in three seasons (Figure 4.24).

The increment of uterine glands also depended on the clones of *P. mirifica*. The uterine glands in uterine tissue of rats treated with 1000 mg/kg BW of *P. mirifica* clone PM-I in summer and PM-III in rainy season and winter were more than others but PM-II and PM-V were lower than others in every season (Table 4.36).

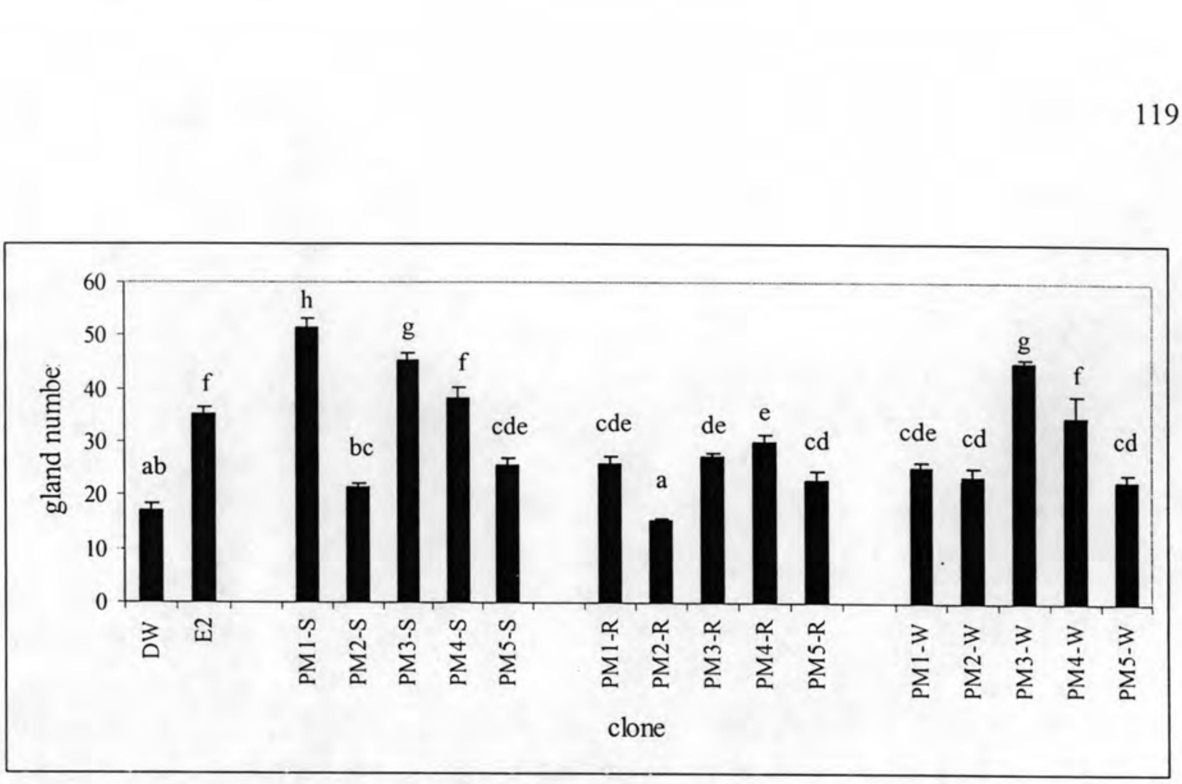


Figure 4.24 The number of uterine gland at the dose of 1000 mg/kg BW of PM. Means not sharing a common superscript letter are significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

Table 4.36 The uterine gland number of rats treated with 1000 mg/kg BW of *P. mirifica* at the end of treatment period (day 28th)

clone	Gland number		
	Summer	Rainy season	Winter
PM-I	51.33 ± 1.80^{e}	25.96 ± 1.32^{bc}	25.33 ± 0.93^{ab}
PM-II	21.42 ± 0.74^{ab}	15.21 ± 0.55^{a}	23.46 ± 1.76^{ab}
PM-III	45.44 ± 1.14^{d}	27.33 ± 0.65^{bc}	44.83 ± 0.70^{d}
PM-IV	38.30 ± 1.87^{c}	29.85 ± 1.62^{c}	34.83 ± 3.99^{c}
PM-V	25.57 ± 1.31^{b}	22.75 ± 1.63^{b}	22.73 ± 1.45^{ab}
Mean	$36.01\pm1.27^{c'}$	$23.85\pm0.74^{a'}$	$29.53\pm1.24^{ab'}$
DW	17.00 ± 1.33^{a}	17.00 ± 1.33^{a}	17.00 ± 1.33^{a}
17β-estradiol	35.17 ± 1.21^{c}	35.17 ± 1.21^{d}	35.17 ± 1.21^{c}

Means not sharing a common superscript letter with or without prime in the same column are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

4.5.8 Effect of *P. mirifica* on the increment of uterine tissue of ovariectomized rats

Qualitative analysis is submitted to analyze the cross section area of uterine tissue is expressed into 3 parts of the uterus, including myometrium, endometrium and lumen (Table 4.37-4.38, Figure 4.25). Myometrium and endometrium treated with PM-I collected in summer, PM-III collected in rainy season and PM-I, PM-II and PM-IV collected in winter was thicker than the others. Lumen in rats treated with PM-III collected in summer and rainy season was larger than the others but smaller than rats treated with 17β-estradiol. In winter there was no difference in cross section area of lumen. In PM-I collected in winter, myometrium, endometrium and lumen was thiner than in summer and rainy season. In PM-II collected in winter, myometrium and endometrium was thicker in winter than in summer and rainy season. In PM-III collected in rainy seasons, myometrium and endometrium was thicker than in summer and winter. In PM-V collected in summer, myometrium, endometrium and lumen was thicker than in rainy season and winter (Table 4.37). There was thus a correlation change between myometrium, endometrium and lumen (Table 4.39).

Table 4.37 The cross section area of uterine tissue of rats treated with 1000mg/kg BW of *P.mirifica* at the end of treatment peroid (Day 28th).

Summer

	Myometrium (mm^2)	Endometrium (mm^2)	Lumen (mm^2)
PM-I	0.0316 ± 0.00080^{f}	0.0295 ± 0.00051^{f}	0.00115 ± 0.000055^{a}
PM-II	0.0207 ± 0.00030^{c}	0.0167 ± 0.00038^{c}	0.00299 ± 0.000346^{ab}
PM-III	0.0242 ± 0.00075^{d}	0.0178 ± 0.00118^{cd}	0.00724 ± 0.002347^{b}
PM-IV	0.0279 ± 0.00063^{e}	0.0178 ± 0.00025^{cd}	0.00111 ± 0.000080^{a}
PM-V	0.0264 ± 0.00070^{ef}	0.0199 ± 0.00039^{de}	0.00335 ± 0.000175^{ab}
Mean	$0.0263\pm0.00046^{c'}$	$0.0205\pm0.00052^{c'}$	$0.003\pm0.000427^{b'}$
E2	0.0160 ± 0.00093^{b}	0.0129 ± 0.00075^{b}	0.03713 ± 0.002316^{c}
H_2O	0.0071 ± 0.00037^{a}	0.0039 ± 0.00024^{a}	0.00019 ± 0.000015^{a}

Rainy season

	Myometrium (mm^2)	Endometrium (mm^2)	Lumen (mm^2)
PM-I	0.0304 ± 0.00025^{f}	0.0246 ± 0.00021^{g}	0.00275 ± 0.000061^{ab}
PM-II	0.0140 ± 0.00038^{b}	0.0085 ± 0.00012^{b}	0.00090 ± 0.000074^{a}
PM-III	0.0362 ± 0.0010^{g}	0.0256 ± 0.00049^{g}	0.00421 ± 0.000128^{b}
PM-IV	0.0275 ± 0.00093^{e}	0.0199 ± 0.00093^{f}	0.00125 ± 0.000193^{ab}
PM-V	0.0169 ± 0.00026^{c}	0.0108 ± 0.00022^{c}	0.00115 ± 0.000053^{ab}
Mean	$0.0245\pm0.00085^{b'}$	$0.0175\pm0.00071^{a'}$	$0.00201\pm0.000127^{a'}$
E2	0.0160 ± 0.00093^{bc}	0.0129 ± 0.00075^{d}	0.03713 ± 0.002316^{c}
H_2O	0.0071 ± 0.00037^{a}	0.0039 ± 0.00024^{a}	0.00019 ± 0.000015^{a}

Winter

	Myometrium (mm^2)	Endometrium (mm^2)	Lumen (mm^2)
PM-I	0.0247 ± 0.00094^{d}	0.0171 ± 0.00041^{cd}	0.00093 ± 0.000100^{a}
PM-II	0.0231 ± 0.00149^{d}	0.0210 ± 0.00177^{d}	0.00171 ± 0.000233^{a}
PM-III	0.0187 ± 0.00074^{bc}	0.0147 ± 0.00028^{bc}	0.00104 ± 0.000040^{a}
PM-IV	0.0234 ± 0.0012^{d}	0.0194 ± 0.00147^{d}	0.00293 ± 0.000434^{a}
PM-V	0.0223 ± 0.00036^{cd}	0.0196 ± 0.00071^{d}	0.00219 ± 0.000304^{a}
Mean	$0.0226\pm0.00050^{a'}$	$0.0185\pm0.00055^{ab'}$	$0.00180\pm0.000140^{a'}$
E2	0.0160 ± 0.00093^{b}	0.0129 ± 0.00075^{b}	0.03713 ± 0.002316^{b}
H_2O	0.0071 ± 0.00037^{a}	0.0039 ± 0.00024^{a}	0.00019 ± 0.000015^{a}

Means not sharing a common superscript letter with or without prime in the same column are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

Table 4.38 The cross section area of uterine tissue of rats treated with 1000mg/kg BW of five clones of *P.mirifica* at the end of treatment peroid (Day 28th).

		Cross section aera (mm^2)			gland
		myometrium	endometrium	lumen	number
PM-I	Summer	0.0316±0.00080^b	0.0295±0.00051^c	0.00115±0.000055^a	51.33±1.80^c
	Rainy season	0.0304±0.00025^b	0.0246±0.00021^b	0.00275±0.000061^c	25.96±1.32^a
	Winter	0.0247±0.00094^a	0.0171±0.00041^a	0.00093±0.000100^a	25.33±0.93^a
	Mean± S.E.M.	0.0289±0.00055$^{d'}$	0.0237±0.00065$^{d'}$	0.00161±0.000105$^{a'}$	34.21±1.64$^{c'}$
PM-II	Summer	0.0207±0.00030^b	0.0167±0.00038^{bc}	0.00299±0.000346^b	21.42±0.74^b
	Rainy season	0.0140±0.00038^a	0.0085±0.00012^a	0.00090±0.000074^a	15.21±0.55^a
	Winter	0.0231±0.00149^b	0.0210±0.00177^c	0.00171±0.000233^a	23.46±1.76^b
	Mean± S.E.M.	0.0193±0.00069$^{a'}$	0.0154±0.00086$^{a'}$	0.00187±0.000173$^{ab'}$	20.03±0.77$^{a'}$
PM-III	Summer	0.0242±0.00075^b	0.0178±0.00118^b	0.00724±0.002347^c	45.44±1.14^c
	Rainy season	0.0362±0.0010^c	0.0256±0.00049^c	0.00421±0.000128^b	27.33±0.65^a
	Winter	0.0187±0.00074^a	0.0147±0.00028^a	0.00104±0.000040^a	44.83±0.70^c
	Mean± S.E.M.	0.0264±0.00046$^{c'}$	0.0194±0.00052$^{c'}$	0.00416±0.000427$^{c'}$	39.20±1.25$^{d'}$
PM-IV	Summer	0.0279±0.00063^b	0.0178±0.00025^a	0.00111±0.000080^a	38.30±1.87^a
	Rainy season	0.0275±0.00093^b	0.0199±0.00093^a	0.00125±0.000193^a	29.85±1.62^a
	Winter	0.0234±0.0012^a	0.0194±0.00147^a	0.00293±0.000434^b	34.83±3.99^a
	Mean± S.E.M.	0.0263±0.00042$^{c'}$	0.0190±0.00048$^{c'}$	0.00176±0.000427$^{ab'}$	34.54±1.67$^{c'}$
PM-V	Summer	0.0264±0.00070^c	0.0199±0.00039^c	0.00335±0.000175^c	25.57±1.31^a
	Rainy season	0.0169±0.00026^a	0.0108±0.00022^a	0.00115±0.000053^a	22.75±1.63^a
	Winter	0.0223±0.00036^b	0.0196±0.00071^c	0.00219±0.000304^b	22.73±1.45^a
	Mean± S.E.M.	0.0218±0.00054$^{b'}$	0.0167±0.00058$^{b'}$	0.00222±0.000158$^{b'}$	23.67±0.85$^{b'}$

Means not sharing a common superscript letter with or without prime in the same column are significantly different (P<0.05) as determined by Duncan's multiple range test.

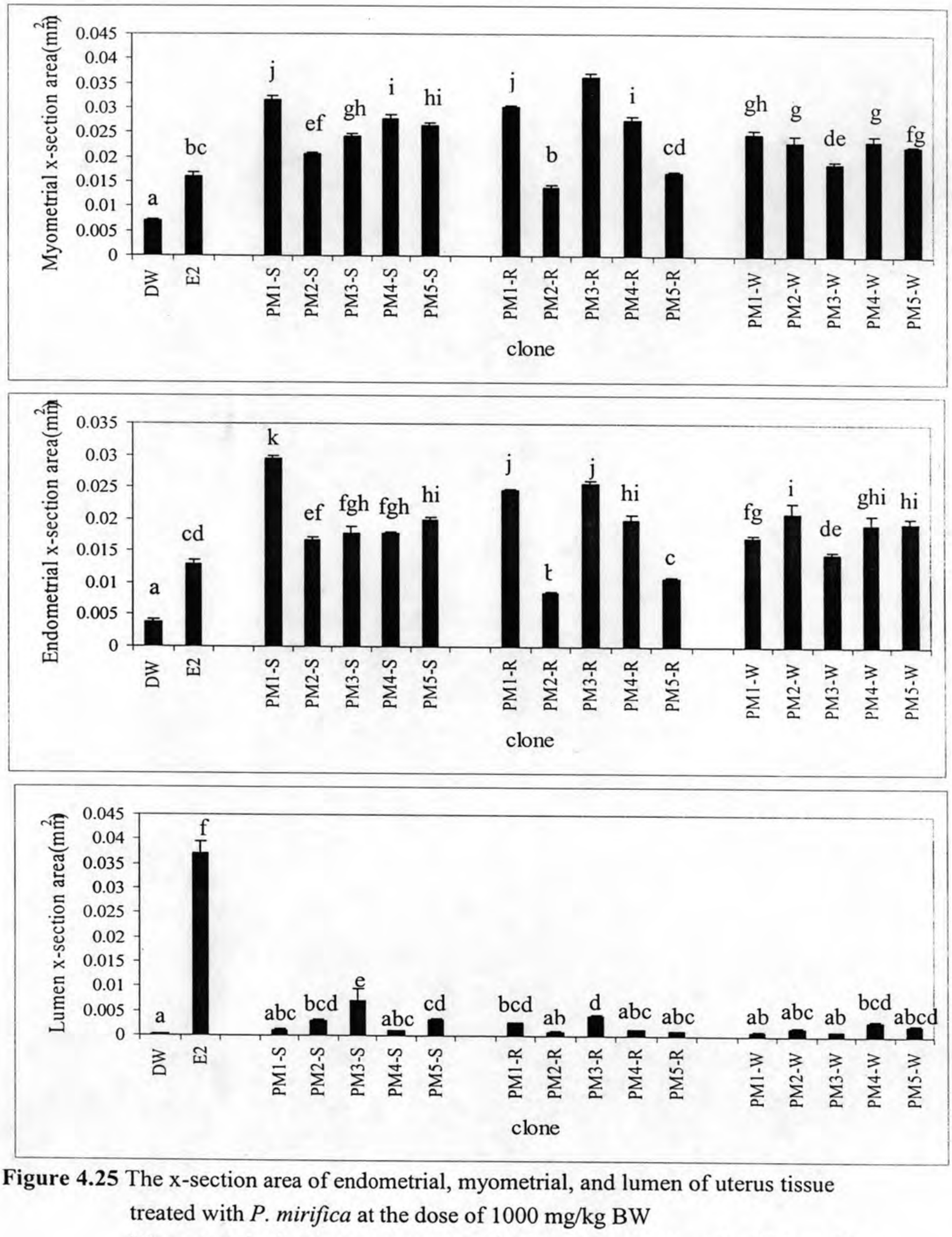


Figure 4.25 The x-section area of endometrial, myometrial, and lumen of uterus tissue treated with *P. mirifica* at the dose of 1000 mg/kg BW

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

Table 4.39 Correlations analysis of cross section area of uterine tissue and number of uterine gland with the major isoflavonoids in *P. mirifica*.

Season	Isoflavonoid	Cross section area			Gland number
		Myometrium	Endometrium	Lumen	
Summer	Puerarin	-	-	†	-
	Daidzin	-	-	-	-
	Genistin	-	-	-	-
	Daidzein	-	-	-	-
	Genistein	-	-	-	-
	Total isoflavonoid	-	-	-	-
	aglycoside	-	-	†	-
	aglycoside/glycoside	-	-	-	-
Rainy season	Puerarin	-	-	-	*
	Daidzin	-	-	-	-
	Genistin	-	-	-	-
	Daidzein	-	-	-	-
	Genistein	-	-	-	-
	Total isoflavonoid	-	-	-	-
	glycoside	-	-	-	-
	aglycoside/glycoside	-	-	-	-
Winter	Puerarin	-	-	-	-
	Daidzin	-	-	-	-
	Genistin	-	-	-	-
	Daidzein	**	-	-	-
	Genistein	-	-	-	-
	Total isoflavonoid	-	-	-	-
	aglycoside	-	-	-	-
	aglycoside/glycoside	-	-	-	-

* : Correlation is significant at the 0.05 level (2-tailed)

** : Correlation is significant at the 0.01 level (2-tailed)

† : Negative correlation is significant at the 0.05 level (2-tailed)

\- : No correlation

The result of estrogenic activity of *P. mirifica* was determined by the cross section area of uterine tissue assay and the uterine gland number assay and the isoflavone contents of PM-I, PM-II, PM-III, PM-IV and PM-V. In summer, the cross section area of lumen is negative correlated with puerarin and aglycoside content ($P < 0.05$) (Table 4.37). In rainy season, the cross section area of myometrium, endometrium and lumen are correlated with daidzin ($P < 0.05$). In winter, the cross section area of myometrium is correlated with daidzein ($P < 0.01$). Uterine gland number was correlated with puerarin in rainy season.