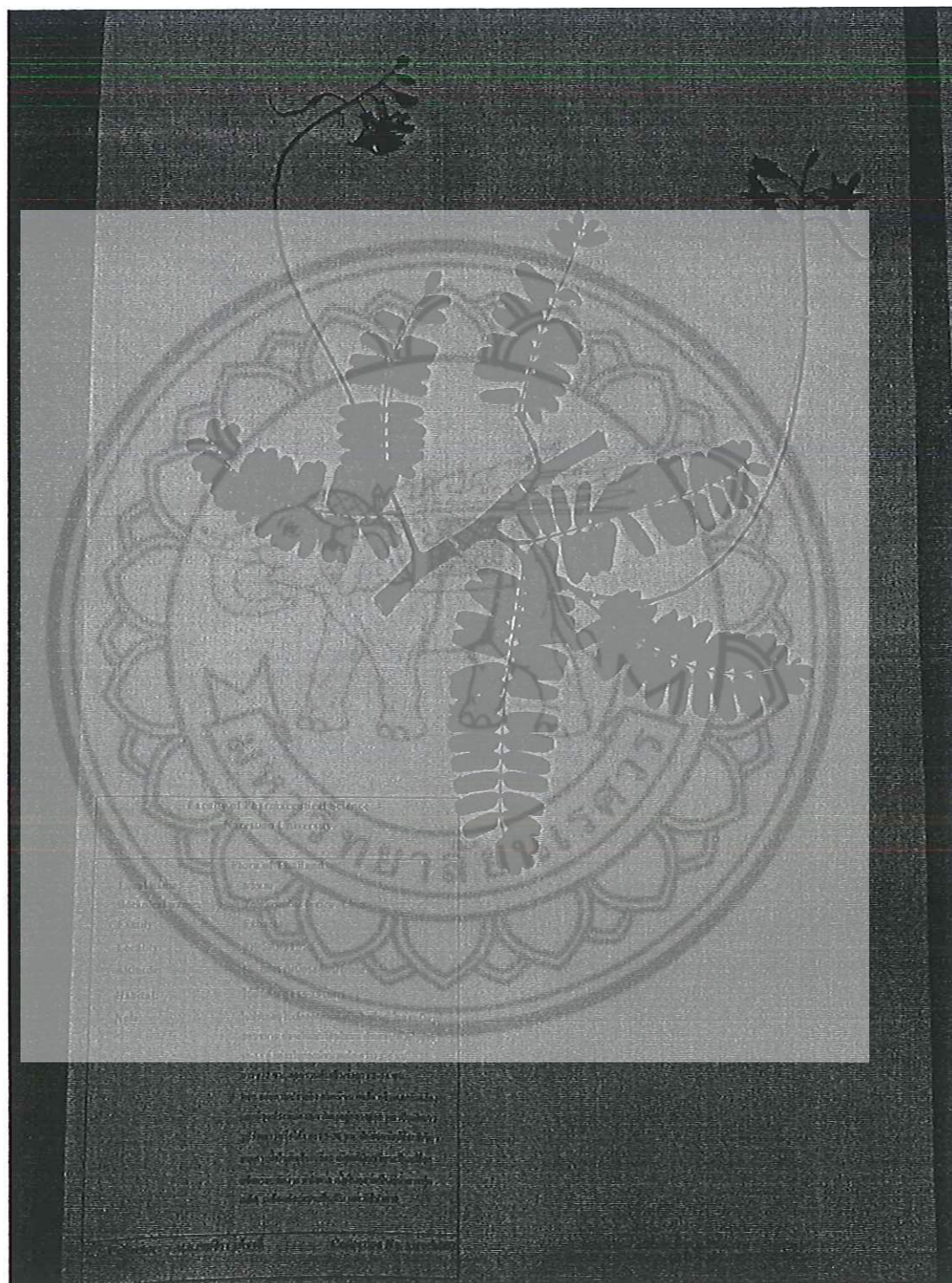




155



APPENDIX B Quantification of total phenol in the extract

Table 2 Optical density detected at 765 nm and total phenol calculation.

Sample	Absorbance at 765 nm						mean
	sample 1		sample 2		sample 3		
	1	2	1	2	1	2	
2mg/ml catechin	0.2730	0.2790	0.2550	0.2620	0.2710	0.2900	0.2717
4mg/ml catechin	0.3840	0.3950	0.3520	0.3550	0.4200	0.3930	0.3832
8 mg/ml catechin	0.6260	0.6340	0.6440	0.6410	0.6230	0.6270	0.6325
16 mg/ml catechin	0.9810	0.9730	0.9590	0.9850	0.9900	0.9740	0.9770
32 mg/ml catechin	1.5640	1.6200	1.5790	1.5720	1.5930	1.5890	1.5862
crude extract 4 mg	0.2420	0.2430	0.2450	0.2430	0.2450	0.2460	0.2440
phenol/4 mg crude	0.2963	0.3194	0.3657	0.3194	0.3657	0.3889	0.3426
phenol/1 mg crude	0.0741	0.0799	0.0914	0.0799	0.0914	0.0972	0.0856
phenol mg/g crude	74.0741	79.8611	91.4352	79.8611	91.4352	97.2222	85.6481

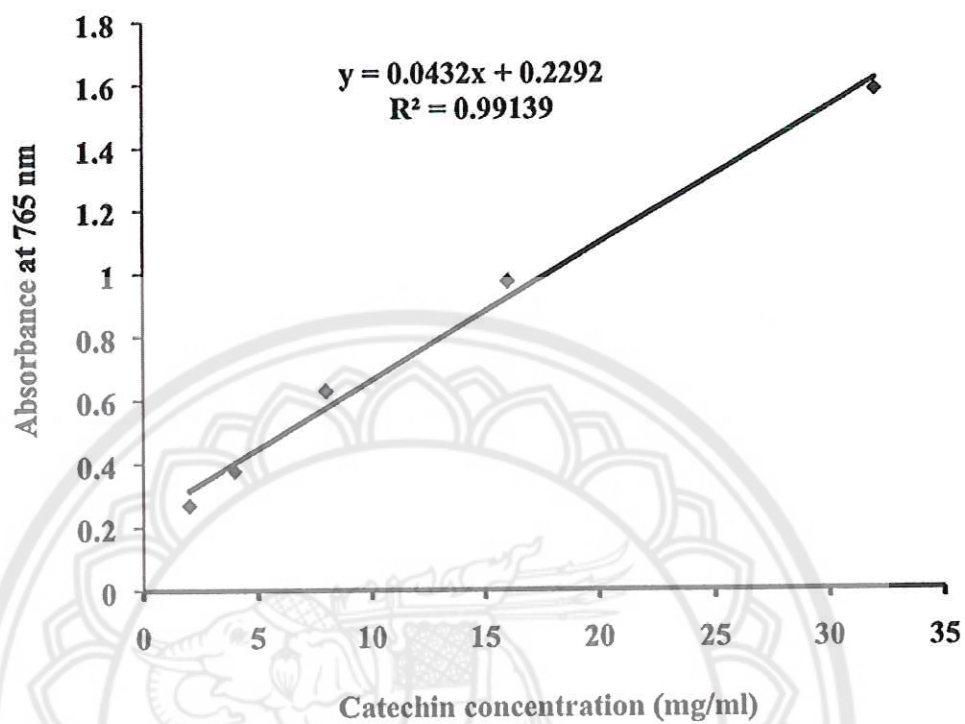


Figure 49 Standard curve of catechin

APPENDIX C Free radical scavenging activity

DPPH remained calculated as follow;

$$\%DPPH_R = [1 - [A_{(sample)}/A_{(blank)}]] \times 100$$

Table 3 Absorbance at 515 nm of DPPH assay

Sample (ug/ml)	Absorbance at 515 nm			mean	%DPPH remained
	1	2	3		
Blank	2.35	2.34	2.33	2.34	
TSE					
0.5	0.13	0.15	0.15	0.14	93.87
1	0.19	0.19	0.17	0.18	92.17
2.5	0.41	0.41	0.43	0.42	82.19
5	0.67	0.63	0.61	0.64	72.79
10	0.99	0.98	0.98	0.98	57.98
25	1.47	1.46	1.47	1.47	37.32
50	1.91	1.91	1.93	1.92	18.09
100	2.14	2.14	2.16	2.15	8.26
250	2.33	2.31	2.34	2.33	0.57
500	2.35	2.33	2.34	2.34	0.00
1000	2.34	2.33	2.35	2.34	0
L-ascobic acid					
0.5	0.11	0.12	0.14	0.12	94.73
1	0.16	0.18	0.18	0.17	92.59
2.5	0.24	0.25	0.25	0.25	89.46
5	0.49	0.51	0.49	0.50	78.77
10	0.84	0.86	0.87	0.86	63.39
25	1.19	1.18	1.18	1.18	49.43
50	1.56	1.55	1.55	1.55	33.62
100	1.76	1.77	1.77	1.77	24.50
250	2.07	2.08	2.08	2.08	11.25
500	2.23	2.24	2.22	2.23	4.70

Table 3 (Cont.)

Sample (ug/ml)	Absorbance at 515 nm			mean	%DPPH remained
	1	2	3		
Tocopherol					
0.5	0.17	0.15	0.17	0.16	93.02
1	0.19	0.19	0.2	0.19	91.74
2.5	0.26	0.26	0.27	0.26	88.75
5	0.49	0.47	0.49	0.48	79.34
10	0.79	0.78	0.78	0.78	66.52
25	1.08	1.07	1.07	1.07	54.13
50	1.35	1.36	1.37	1.36	41.88
100	1.65	1.65	1.64	1.65	29.63
250	1.97	1.99	1.98	1.98	15.38
500	2.24	2.25	2.25	2.25	3.99
1000	2.36	2.32	2.34	2.34	0

APPENDIX D Melanin content assay

Table 4 Optical density of melanin content assay in protection protocol

Protection protocol	Absorbance at 490 nm				%	SD	%melanin content
	1	2	3	%	%		
control	0.135	0.118	0.126	0.13	100	0.01	100
MSH	0.159	0.182	0.168	144.44	133.33	11.07	133.36
Tamarind seed coat							
(ug/ml)							
50	0.133	0.11	0.12	102.31	87.30	7.51	94.95
75	0.127	0.115	0.107	100.79	91.27	7.99	92.33
100	0.095	0.114	0.105	75.40	90.48	7.54	83.07
125	0.086	0.101	0.108	68.25	80.16	8.92	78.04
150	0.091	0.108	0.098	72.22	85.71	6.78	78.57
200	0.085	0.096	0.077	67.46	76.19	7.57	68.25
50 ug/ml kojic acid	0.07	0.054	0.051	55.56	42.86	8.11	46.30

Table 5 Optical density of melanin content assay in inhibition protocol

Inhibition protocol	Absorbance at 490 nm						%melanin content	
	1	2	3	%	%	%	SD	
control	0.135	0.118	0.126		0.13	100	0.01	100
MSH	0.159	0.182	0.168	125.86	144.44	133.33	9.35	134.55
Tamarind seed coatc(ug/ml)								
50	0.127	0.103	0.118	100.53	81.75	93.65	9.50	91.97
75	0.118	0.105	0.116	93.65	83.33	92.06	5.56	89.68
100	0.084	0.105	0.097	66.67	83.33	76.98	8.41	75.66
125	0.076	0.094	0.089	60.32	74.60	70.63	7.37	68.52
150	0.066	0.074	0.064	52.38	58.73	50.79	4.20	53.97
200	0.059	0.047	0.052	46.83	37.30	41.27	4.78	41.80
50 ug/ml kojic acid	0.069	0.08	0.056	54.76	63.49	44.44	9.53	54.23

APPENDIX E Effect of the extract on tyrosinase activity

Table 6 Optical density of tyrosinase activity

Sample	Absorbance at 490 nm			Mean	% Inhibition
	1	2	3		
Tamarind seed coat (ug/ml)					
0	0.126	0.093	0.104	0.108	0.00
50	0.091	0.062	0.145	0.099	7.74
75	0.06	0.109	0.076	0.082	24.15
100	0.056	0.078	0.084	0.073	32.51
125	0.039	0.075	0.053	0.056	48.30
150	0.084	0.042	0.059	0.062	42.72
200	0.025	0.067	0.033	0.042	61.30
300	0.017	0.014	0.007	0.013	88.24
400	0.006	0.011	0.019	0.012	88.85
500	0.008	0.002	0.007	0.006	94.74
Kojic acid (ug/ml)					
0	0.126	0.093	0.104	0.108	0.00
0.5	0.115	0.095	0.088	0.099	7.74
1	0.099	0.097	0.101	0.099	8.05
5	0.113	0.105	0.071	0.096	10.53
10	0.093	0.109	0.037	0.080	26.01
50	0.037	0.029	0.039	0.035	67.49
100	0.013	0.033	0.018	0.021	80.19
250	0.007	0.005	0.004	0.005	95.05
500	0.003	0.003	0.005	0.004	96.59

APPENDIX F Intracellular calcium and par-2 activity

Table 7 Optical density of fluorescence from PAR-2 activity

Sample	Fluorescence EX485 EM530			%PAR-2
	1	2	3	
control	10821	10864	10575	100.63
Tamarind seed coat extract (ug/ml)				
50	11231	9798	9919	104.44
75	10974	11932	9987	102.05
100	10242	10921	9423	95.24
125	9352	10872	10065	86.97
150	11672	10985	10043	108.54
200	9153	9913	10872	85.12
Trypsin	14593	13017	12989	135.71
				121.05
				120.79
				8.54
				125.85

APPENDIX G Prevention of h₂O₂ induced fibroblast damage

Table 8 Optical density of XTT assay

Hydrogen peroxide (umol/ml)	Absorbance at 490 nm			Viability			%Viability	%Damage	
	1	2	3	1	2	3			Mean
Untreated group									
0	0.947	0.92	0.951	100	100	100	0.939	6.875	0
100	0.359	0.418	0.289	38.21	44.49	30.76	0.355	7.801	62.17
250	0.441	0.295	0.357	46.94	31.40	38.00	0.326	1.331	65.29
500	0.252	0.275	0.255	26.82	29.27	27.14	0.265	2.533	71.79
750	0.128	0.145	0.175	13.62	15.43	18.63	0.137	2.715	85.47
1000	0.071	0.096	0.045	7.55	10.22	4.79	0.071	6.875	92.48
Treated group									
0	0.947	0.92	0.951	100	100	100	0.939	0.017	0
100	0.634	0.681	0.532	67.49	56.63	56.63	0.616	6.269	34.46
250	0.709	0.578	0.601	75.47	63.98	63.98	0.590	6.638	37.24
500	0.498	0.585	0.479	53.01	50.99	50.99	0.532	1.168	43.36
750	0.396	0.55	0.512	42.15	54.50	54.50	0.473	7.130	49.65
1000	0.392	0.581	0.425	41.73	45.24	45.24	0.466	2.028	50.39

APPENDIX H Cell cycle analysis

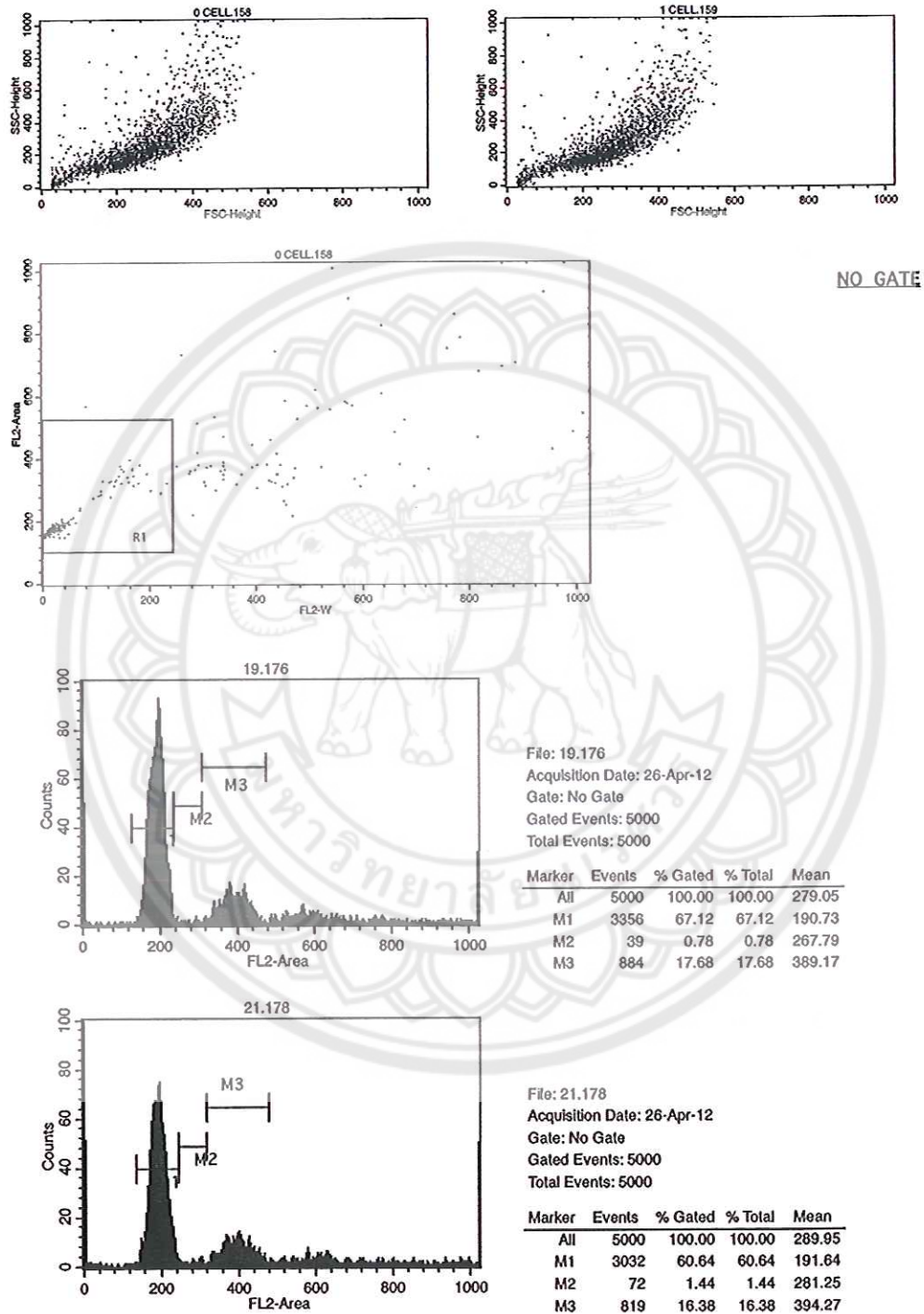
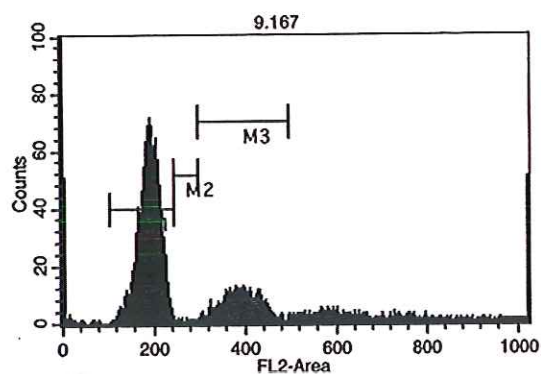
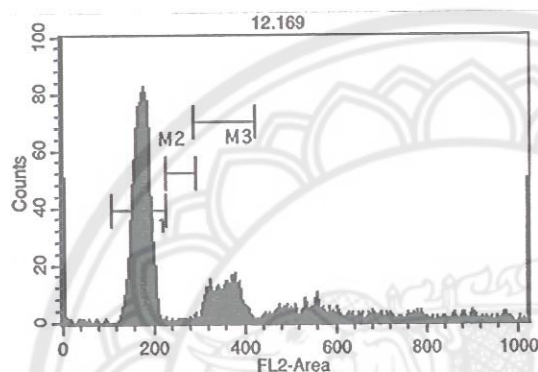


Figure 50 Sample histogram from flow cytometer analyzed by CellQuestPro software



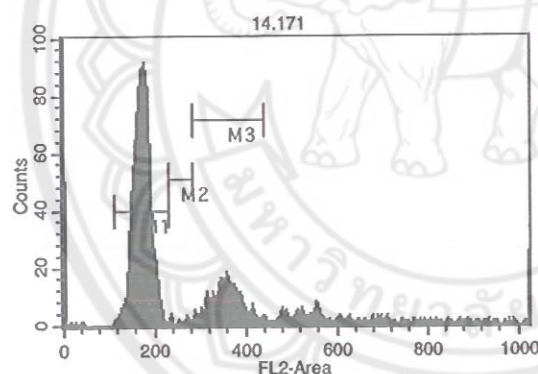
File: 9.167
Acquisition Date: 26-Apr-12
Gate: No Gate
Gated Events: 5000
Total Events: 5000

Marker	Events	% Gated	% Total	Mean
All	5000	100.00	100.00	281.94
M1	3171	63.42	63.42	188.49
M2	27	0.54	0.54	269.52
M3	914	18.28	18.28	390.39



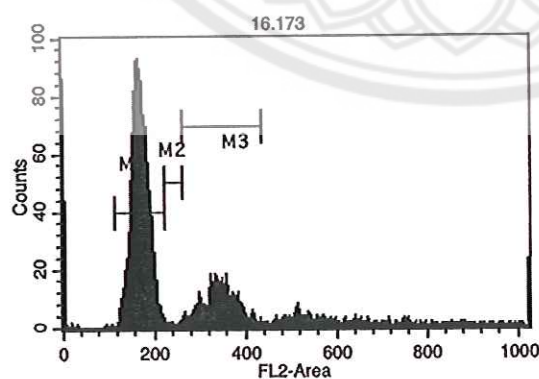
File: 12.169
Acquisition Date: 26-Apr-12
Gate: No Gate
Gated Events: 5000
Total Events: 5000

Marker	Events	% Gated	% Total	Mean
All	5000	100.00	100.00	293.85
M1	2940	58.80	58.80	172.48
M2	30	0.60	0.60	278.73
M3	877	17.54	17.54	352.79



File: 14.171
Acquisition Date: 26-Apr-12
Gate: No Gate
Gated Events: 5000
Total Events: 5000

Marker	Events	% Gated	% Total	Mean
All	5000	100.00	100.00	251.33
M1	3400	68.00	68.00	172.05
M2	28	0.56	0.56	259.21
M3	910	18.20	18.20	354.81



File: 16.173
Acquisition Date: 26-Apr-12
Gate: No Gate
Gated Events: 5000
Total Events: 5000

Marker	Events	% Gated	% Total	Mean
All	5000	100.00	100.00	254.56
M1	3297	65.94	65.94	168.67
M2	32	0.64	0.64	243.53
M3	1024	20.48	20.48	342.91

Figure 50 (Cont.)

APPENDIX I Improvement of intracellular glutathione deficiency

Table 9 Optical density of total glutathione standard

GSH (uM/ml)	Absorbance at 412 nm			Mean
	1	2	3	
0	0.0602	0.0606	0.0614	0.0607
0.39	0.1299	0.122	0.1214	0.1244
0.78	0.1822	0.1869	0.1866	0.1852
1.56	0.2532	0.2488	0.2375	0.2465
3.13	0.3031	0.3252	0.3233	0.3172
6.25	0.3976	0.3934	0.3899	0.3936
12.5	0.4732	0.471	0.4721	0.4721
25	0.5635	0.5746	0.5783	0.5721

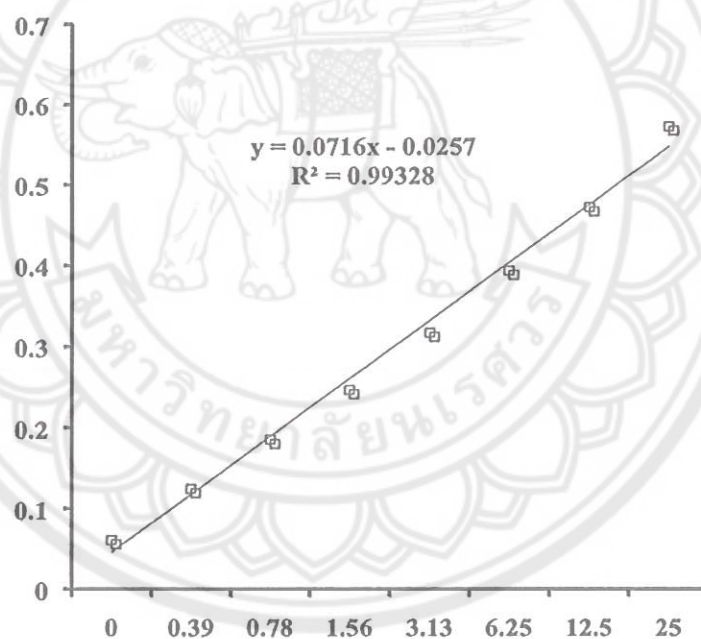


Figure 51 Total glutathione standard curve

Table 10 Optical density of total glutathione assay

Cultivation time		Absorbance at 412 nm			GSH (uM/ml)			Mean	SD
		1	2	3	1	2	3		
6h	control	0.3523	0.4129	0.3132	5.28	6.13	4.73	5.38	1.01
	UVA	0.4512	0.5135	0.4094	6.66	7.53	6.08	6.76	1.14
	TSE	0.4821	0.3498	0.5031	7.09	5.24	7.39	6.57	1.66
24h	control	0.5112	0.4978	0.5003	7.50	7.31	7.35	7.39	0.60
	UVA	0.4621	0.4902	0.4012	6.81	7.21	5.96	6.66	1.04
	UVA+TSE	0.5014	0.4999	0.5205	7.36	7.34	7.63	7.44	0.68
48h	control	0.5034	0.5123	0.5253	7.39	7.51	7.70	7.53	0.68
	UVA	0.4266	0.4198	0.4595	6.32	6.22	6.78	6.44	0.76
	UVA+TSE	0.5111	0.4977	0.5314	7.50	7.31	7.78	7.53	0.77
72h	control	0.5465	0.5024	0.5322	7.99	7.38	7.79	7.72	0.85
	UVA	0.4055	0.4203	0.3977	6.02	6.23	5.91	6.05	0.56
	UVA+TSE	0.5352	0.5125	0.5033	7.83	7.52	7.39	7.58	0.73

APPENDIX J Type-i procollagen and mmp-1 assay

Table 11 Optical density of MMP-1 standard

MMP-1 standard(pg/ml)	Absorbance at 450 nm		
	1	2	mean
0	0.1136	0.1223	0.1180
24.69	0.102	0.1091	0.1056
74.07	0.103	0.1148	0.1089
222.2	0.1129	0.1106	0.1118
666.7	0.1286	0.1244	0.1265
2000	0.1455	0.1463	0.1459
6000	0.2043	0.2114	0.2079
18000	0.4402	0.4148	0.4275

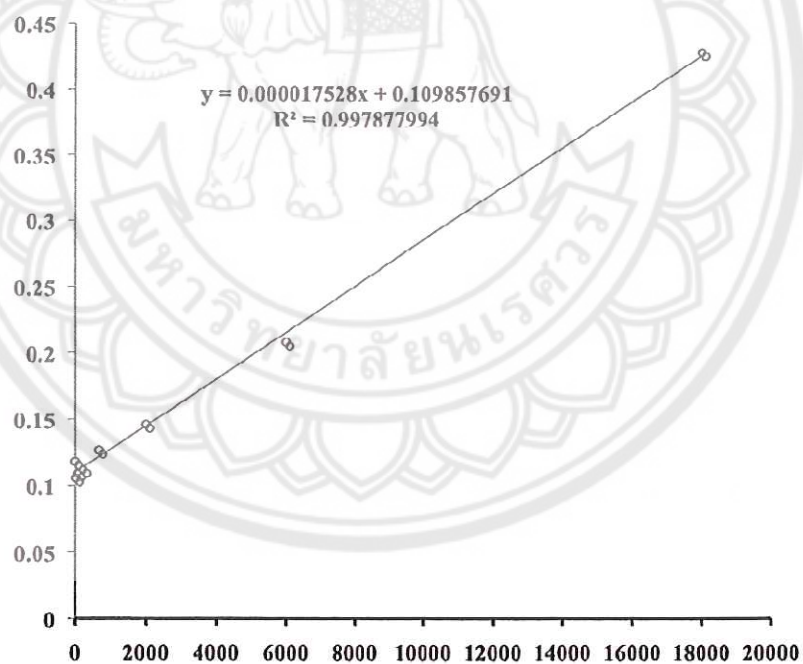


Figure 52 MMP-1 standard curve

Table 12 Optical density of MMP-1 assay

Cultivation time	Sample	Absorbance at 450 nm		MMP-1 (pg/ml)		
		1	2	1	2	Mean SD
6h	control	0.1202	0.1278	590.04	1023.63	806.84 306.60
	UVA	0.1283	0.1205	1052.16	607.16	829.66 314.66
24h	control	0.454	0.4496	292.84	254.11	273.48 27.39
	UVA	0.4521	0.4721	276.12	409.93	313.18 48.61
48	UVA+VitA	0.1222	0.1269	704.15	972.29	838.22 189.61
	UVA+TSE	0.4268	0.4341	53.39	117.66	85.53 45.44
	control	0.4496	0.4496	254.11	254.11	254.11 179.36
	UVA	0.4543	0.4543	295.49	295.49	295.49 208.62
72h	UVA+VitA	0.1222	0.402	704.15	704.15	704.15 497.62
	UVA+TSE	0.4273	0.4292	57.79	74.52	66.16 11.83
	control	0.4521	0.4673	276.12	409.93	343.02 94.62
	UVA	0.516	0.4655	838.65	394.08	615.72 399.38
	UVA+VitA	0.1272	989.41	0.13	989.41	989.41 112.67
	UVA+TSE	0.4488	0.4533	247.07	286.68	266.87 28.01

Table 13 Optical density of type-I procollagen standard

Type-1 procollagen(ng/ml)	Absorbance at 450 nm		
	1	2	mean
0	0.0483	0.0448	0.0465
10	0.1087	0.0965	0.1026
20	0.1685	0.1671	0.1678
40	0.2977	0.2558	0.2767
80	0.433	0.472	0.4525
160	0.7809	0.6976	0.7392
320	1.4799	1.3571	1.4185
640	1.8805	1.9782	1.9293

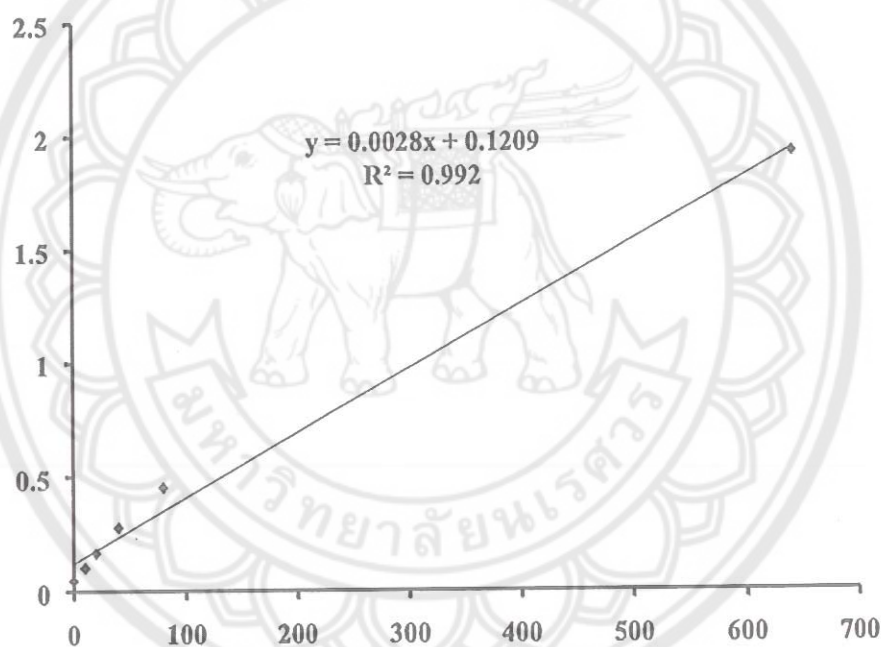


Figure 53 Type-I procollagen standard curve

Table 14 Optical density of type-I procollagen assay

Cultivation time	Sample	Absorbance at 450 nm		Type-I procollagen (ng/ml)		
		1	2	1	2	Mean SD
24h	control	1.5835	1.8712	731.75	875.60	803.68 101.72
	UVA	0.6776	0.8151	278.8	347.55	313.18 48.61
	UVA+VitC	0.9388	1.1275	409.4	503.75	456.58 66.72
	UVA+TSE	0.9459	0.8677	412.95	373.85	393.40 27.65
72h	control	1.7282	2.1154	804.1	997.70	900.90 136.90
	UVA	0.8467	0.79918	363.35	339.59	351.47 16.80
	UVA+VitC	1.0852	1.0852	482.6	482.60	482.60 66.72
	UVA+TSE	1.289	1.3161	584.5	598.05	591.28 9.58

APPENDIX K Effect of tamarind seed coat extract on contraction of fibroblast-embedded collagen lattice

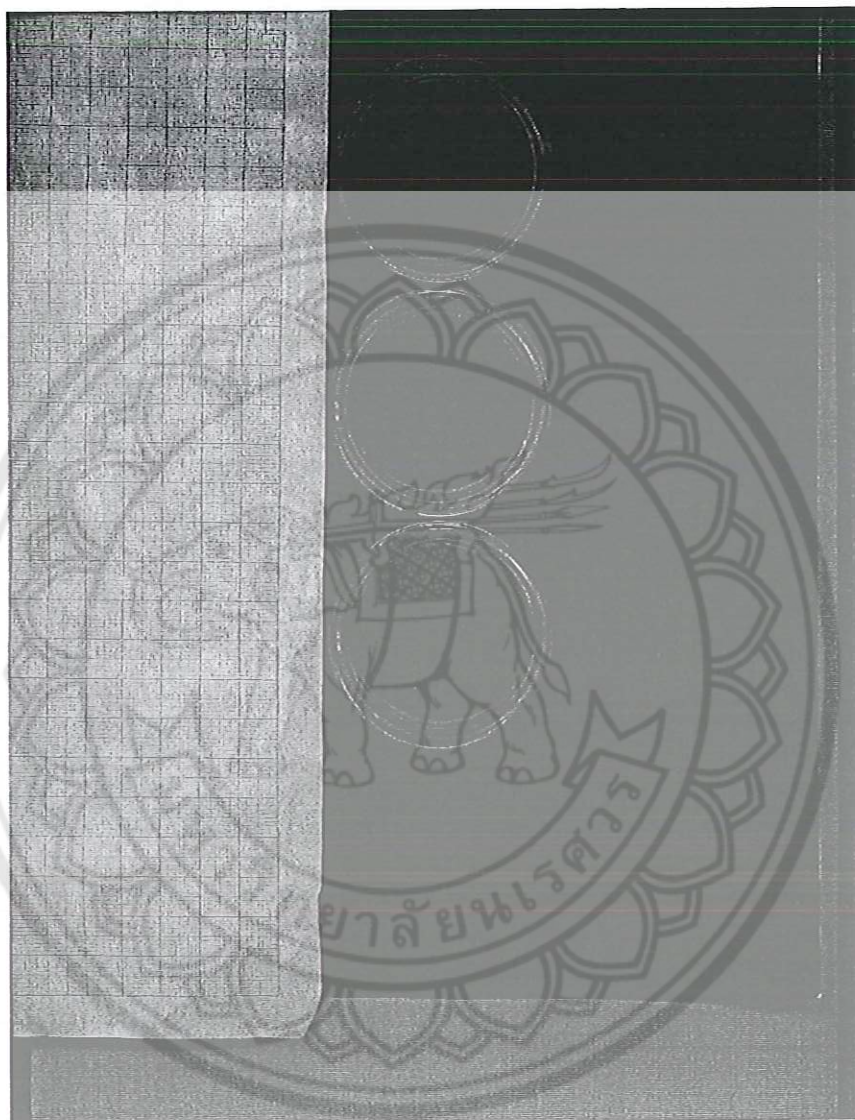


Figure 54 Sample of scanned photograph for lattices diameter measurement