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APPENDICES

APPENDIX I

UV spectrophotometer results

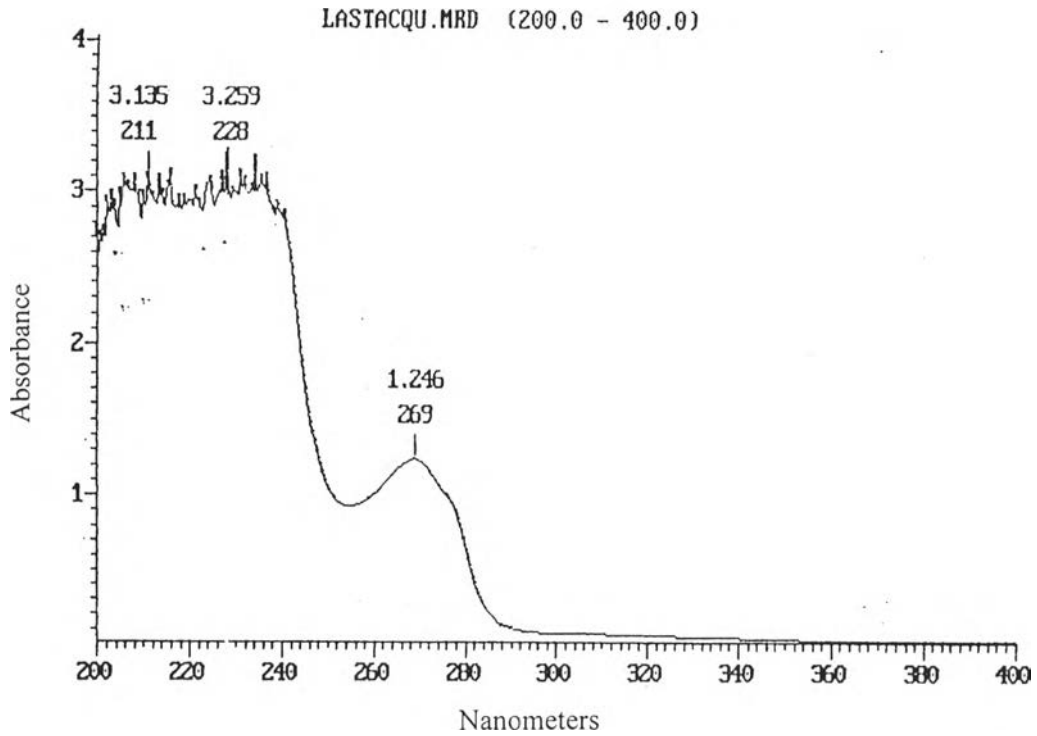

Figure 20. The UV absorption spectrum of Epigallocatechin (EGC)

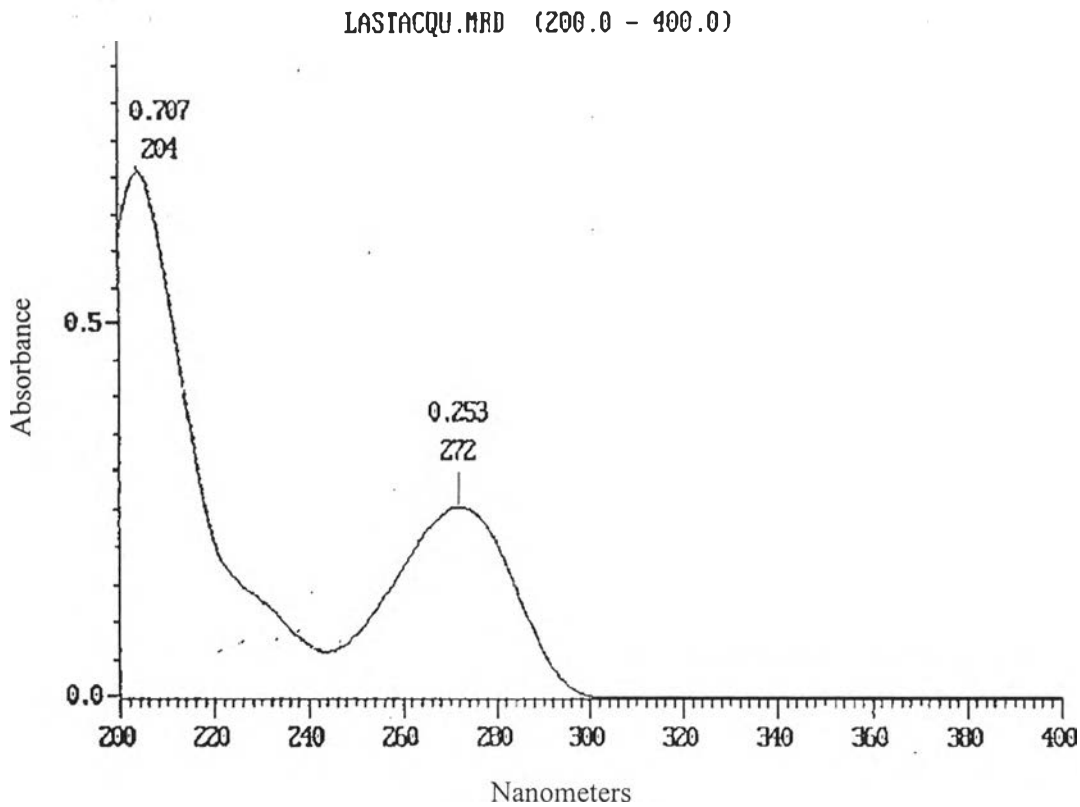

Figure 21. The UV absorption spectrum of caffeine

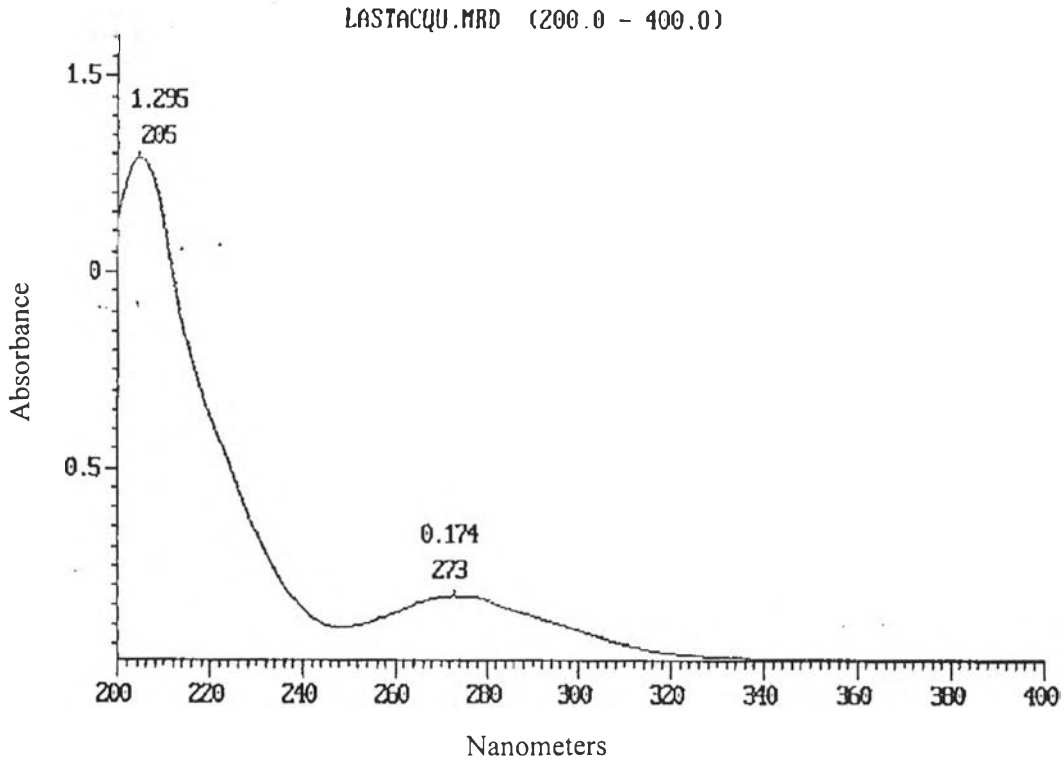

Figure 22. The UV absorption spectrum of Epigallocatechin gallate (EGCG)

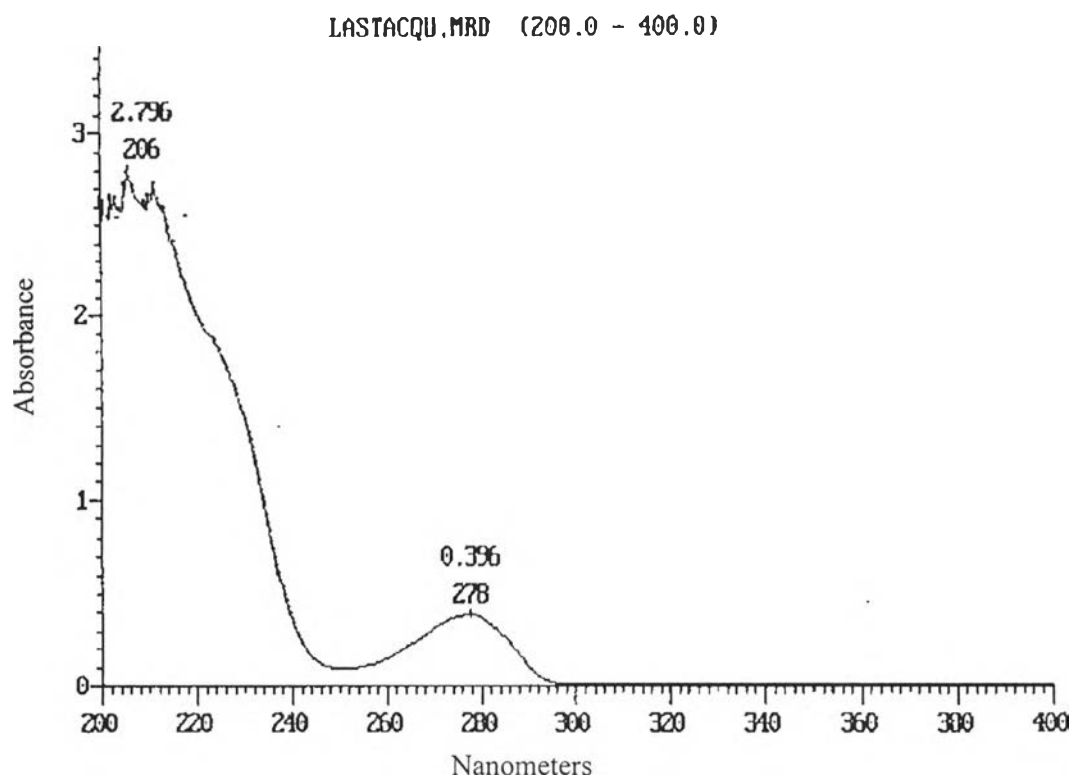

Figure 23. The UV absorption spectrum of Epicatechin (EC)

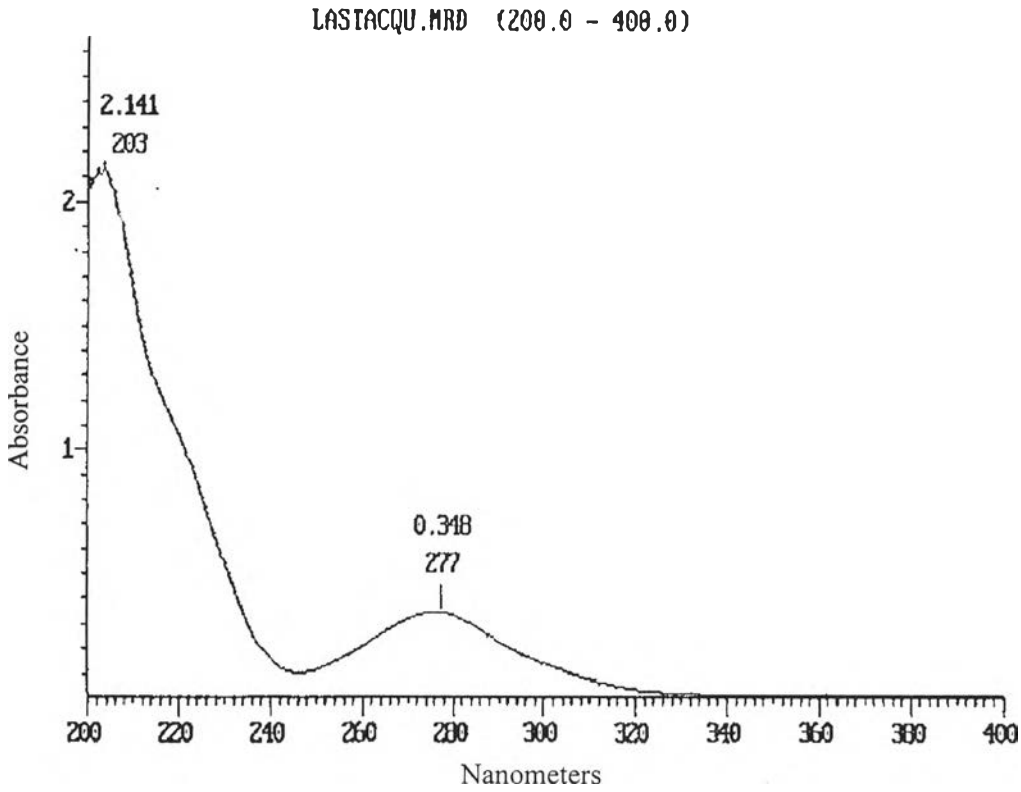

Figure 24. The UV absorption spectrum of Epicatechin gallate (ECG)

APPENDIX II

High performance liquid chromatograms of green tea extracts

Figure 25. High-performance liquid chromatogram of Japanese green tea extract. EGC = (-)-epigallocatechin, Caffeine, EGCG = (-)-epigallocatechin 3-*O*-gallate, EC = (-)-epicatechin, ECG = (-)-epicatechin 3-*O*-gallate.

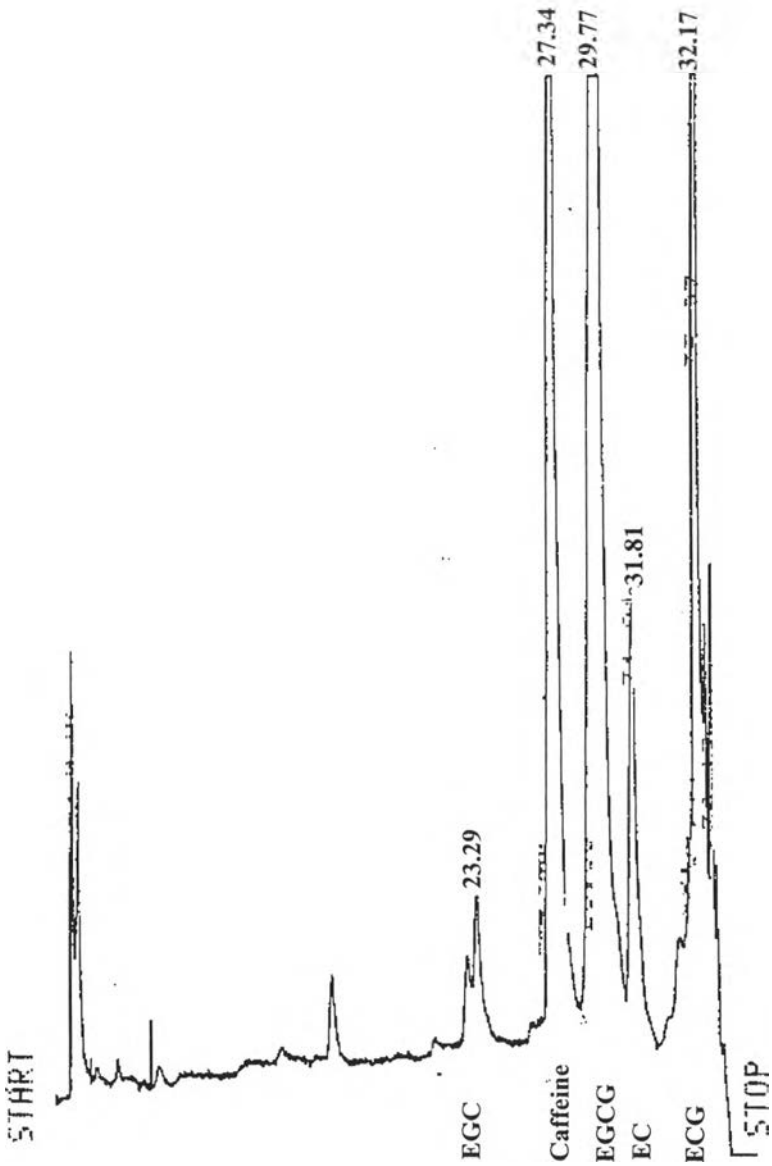

Figure 26. High-performance liquid chromatogram of Myanmar green tea extract. EGC = (-)-epigallocatechin, Caffeine, EGCG = (-)-epigallocatechin 3-*O*-gallate, EC = (-)-epicatechin, ECG = (-)-epicatechin 3-*O*-gallate.

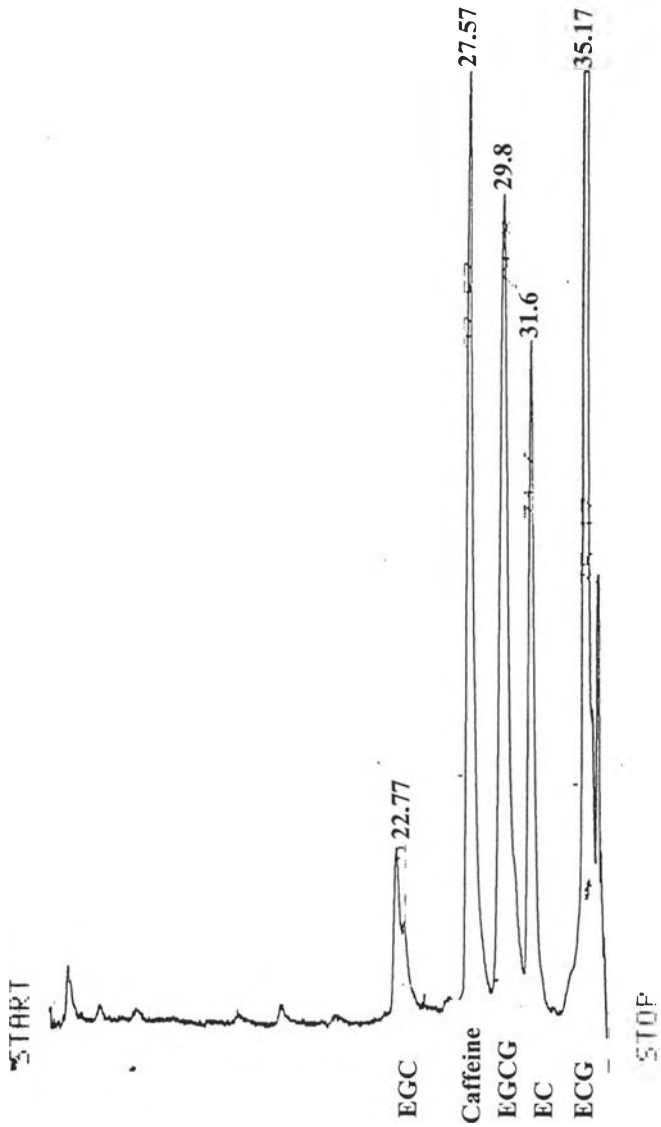

Figure 27. High-performance liquid chromatogram of Thai green tea extract. EGC = (-)-epigallocatechin, Caffeine, EGCG = (-)-epigallocatechin 3-*O*-gallate, EC = (-)-epicatechin, ECG = (-)-epicatechin 3-*O*-gallate.

Appendix III

HPLC validation

1. HPLC assay for EGC analysis

The analytical method for EGC was performed and validated for its accuracy, precision, specificity and linearity.

1.1 **Accuracy**

Initial amount (μg)	Analytical amount	% Recovery
1	0.99	99
1	0.99	99
1	1	100
		Mean = 99.33
		SD =0.58

1.2 Precision

1.2.1 Within run precision data

Amount (μg)	Peak area			Mean	SD	%CV
	N_1	N_2	N_1			
1	3025	3094	2981	3033	56.96	1.88
3	19392	18976	19561	19310	301.06	1.56
5	30611	30942	31074	30876	238.52	0.77
7	49375	48699	49944	49339	623.26	1.26

1.2.2 Between run precision data

Amount (μg)	Peak area			Mean	SD	%CV
	D_1	D_2	D_3			
1	3025	2976	3084	3028	54.08	1.78
3	19392	18772	19413	19192	364.17	1.90
5	30611	31119	30438	30722	353.97	1.15
7	49375	48654	49981	49336	664.33	1.35

1.3 Specificity

Figure 6. shows the chromatogram of the standard catechins and caffeine mixture.

1.4 Linearity

Table 1 shows the peak area of the standard EGC solution and Figure 7. shows the calibration curve of the standard EGC.

2. HPLC assay for caffeine analysis

The analytical method for caffeine was performed and validated for its accuracy, precision, specificity and linearity.

2.1 Accuracy

Initial amount (μg)	Analytical amount	% Recovery
1.77	1.78	100.56
1.77	1.78	100.56
1.77	1.77	100
		Mean = 100.38
		SD =0.33

2.2 Precision

2.2.1Within run precision data

Amount (μg)	Peak area			Mean	SD	%CV
	N_1	N_2	N_1			
0.59	51329	51287	51122	51216	84.87	0.16
1.47	166711	166650	166271	166544	238.38	0.14
1.77	165032	165279	164798	165036	240.53	0.14
2.36	216126	217159	216235	216507	567.56	0.26

2.2.2 Between run precision data

Amount (μg)	Peak area			Mean	SD	%CV
	D_1	D_2	D_3			
0.59	51329	51419	51278	51342	71.39	0.14
1.47	166711	163200	165431	165114	1776.84	1.08
1.77	165032	163496	166201	164909	1356.64	0.82
2.36	216126	219299	217584	217669	1588.23	0.73

2.3 Specificity

Figure 6. shows the chromatogram of the standard catechins and caffeine mixture.

2.4 Linearity

Table 2 shows the peak area of the standard caffeine solution and Figure 8. shows the calibration curve of the standard caffeine.

3. HPLC assay for EC analysis

The analytical method for EC was performed and validated for its accuracy, precision, specificity and linearity.

3.1 Accuracy

Initial amount μg	Analytical amount	% Recovery
1.5	1.54	102.67
1.5	1.49	99.33
1.5	1.54	102.67
		Mean = 101.56
		SD =1.92

3.2 Precision

3.2.1. Within run precision data

Amount (μg)	Peak area			Mean	SD	%CV
	N_1	N_2	N_1			
0.5	12280	12158	12397	12278	119.51	0.97
1.5	56565	54442	56147	55718	1124.64	2.02
2.5	94795	94012	95118	94642	568.72	0.600
3.5	138334	135774	133690	135933	2326.06	1.71

3.2.2. Between run precision data

Amount (μg)	Peak area			Mean	SD	%CV
	D_1	D_2	D_3			
0.5	12280	12086	12475	12280	194.50	1.58
1.5	56565	54391	55876	55611	1111.02	1.99
2.5	94795	94551	95468	94938	474.93	0.50
3.5	138334	137864	139426	138541	801.37	0.58

3.3. Specificity

Figure 6. shows the chromatogram of the standard catechins and caffeine mixture.

3.4. Linearity

Table 4 shows the peak area of the standard EC solution and Figure 10. shows the calibration curve of the standard EC.

4. HPLC assay for EGCG analysis

The analytical method for EGCG was performed and validated for its accuracy, precision, specificity and linearity.

4.1 Accuracy

Initial amount μg	Analytical amount	% Recovery
6.15	6.08	98.86
6.15	6.12	99.51
6.15	6.09	99.02
		Mean = 99.13
		SD =0.34

4.2 Precision

4.2.1. Within run precision data

Amount (μg)	Peak area			Mean	SD	%CV
	N_1	N_2	N_1			
4.1	67675	68461	67132	67756	668.19	0.99
6.15	131020	132180	131318	131506	602.42	0.46
10.25	275383	273495	275884	274921	1259.82	0.46
16.4	546619	557631	549996	551415	5641.54	1.02

4.2.2. Between run precision data

Amount (μg)	Peak area			Mean	SD	%CV
	D_1	D_2	D_3			
4.1	67675	68543	65966	67395	1311.17	1.95
6.15	131020	130487	131159	130889	354.73	0.27
10.25	275383	285467	275113	278654	5901.49	2.12
16.4	546619	528764	539466	538283	8986.09	1.67

4.3. Specificity

Figure 6. shows the chromatogram of the standard catechins and caffeine mixture.

4.4. Linearity

Table 3 shows the peak area of the standard EGCG solution and Figure 9. shows the calibration curve of the standard EGCG.

5. HPLC assay for ECG analysis

The analytical method for ECG was performed and validated for its accuracy, precision, specificity and linearity.

5.1. Accuracy

Initial amount (μg)	Analytical amount	% Recovery
4.35	4.35	100
4.35	4.46	102.52
4.35	4.33	99.54
		Mean = 100.69
		SD =1.61

5.2 Precision

5.2.1 Within run precision data

Amount (μg)	Peak area			Mean	SD	%CV
	N_1	N_2	N_1			
1.45	69608	68756	69777	69380	547.25	0.79
2.9	153059	164321	153779	157053	6304.56	4.01
4.35	276858	284366	274844	278689	5018.21	1.80
5.8	389712	386651	375963	384109	7218.47	1.88

5.2.2 Between run precision data

Amount (μg)	Peak area			Mean	SD	%CV
	D_1	D_2	D_3			
1.45	69608	69445	68653	69235	510.86	0.74
2.9	153059	151746	154819	153208	1541.91	1.00
4.35	276858	276654	284132	279215	4259.76	1.53
5.8	389712	381136	387354	386067	4430.42	1.15

5.3. Specificity

Figure 6. shows the chromatogram of the standard catechins and caffeine mixture.

5.4. Linearity

Table 5 shows the peak area of the standard ECG solution and Figure 11. shows the calibration curve of the standard ECG.

Appendix IV

Antioxidant activity study

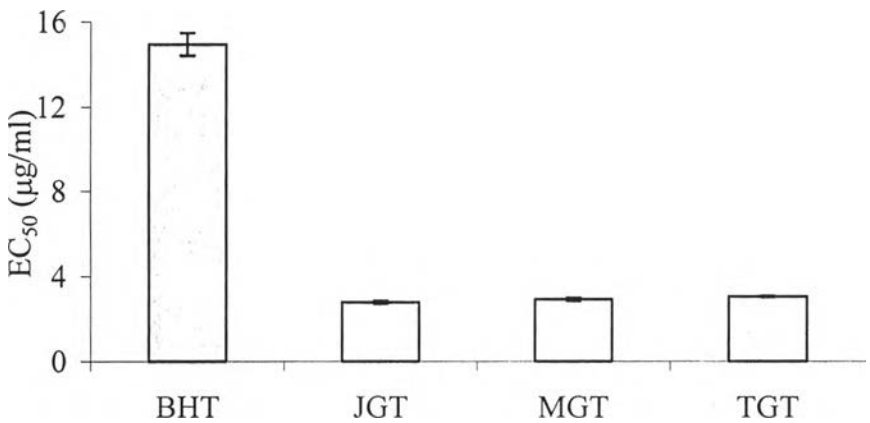

Figure 28. Comparison of the radical scavenging activity of green tea extracts at EC_{50} studied by DPPH assay

The significance difference was observed in comparison between BHT with green tea extracts and also in comparison among three green tea extracts ($p<0.05$)

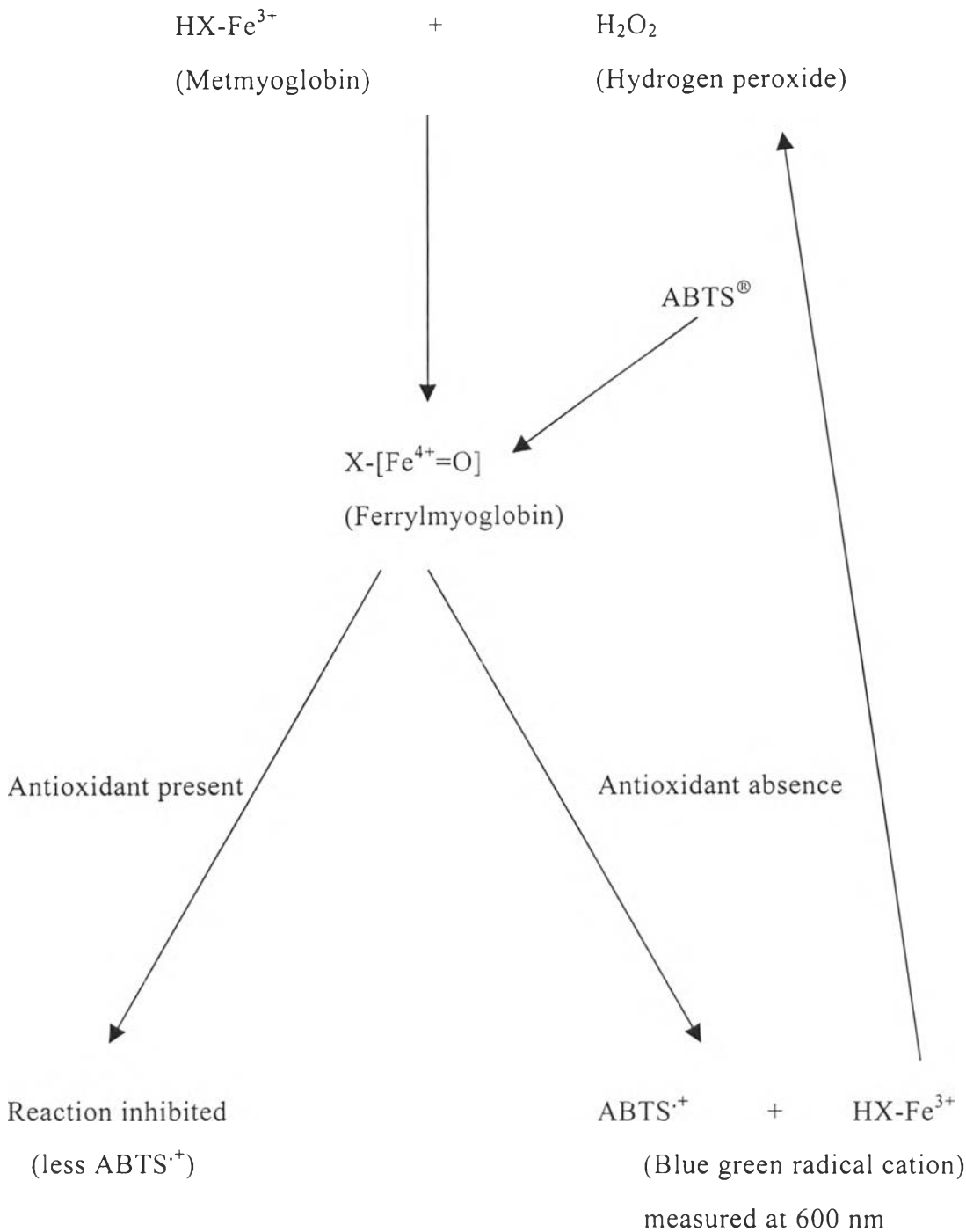

Figure 29. Diagram showing the reaction of total antioxidant status assay (Randox® kit)

Appendix V

Cell culture preparation

1. Melanoma Cell line (A375)

Organism	:	*Homo Sapiens* (human)
Morphology	:	Epithelial
Tissue type	:	skin, malignant melanoma
Growth properties	:	adherent type

2. Cell culture medium

RPMI medium 1640	16.2 g / packet
Antibiotic (Penicillin + Streptomycin) solution	20 ml
Sodium bicarbonate	2 g
Distilled water up to	1 L

16.2 g of RPMI mediumn 1640 powder was dissolved in 750 ml of deionized water by stirring gently at room temperature. Then 2 g of $NaHCO_3$ and 20 ml of the antibiotic mixture, which contain penicillin and streptomycin, were added and were stirred by magnetic stirrer until dissolved. After that it was made up to 1 L volume by adding more distilled water. The final pH of the medium (7.2) was adjusted by adding slowly with 1 molar NaOH or 1 molar HCl.

Culture medium was sterilized immediately through filtration at 0.22 micro size membrane in laminar airflow hood, sealed and kept at 4°C in the refrigerator. After inactivation of fetal bovine serum at 56°C for 30 min., it was added into the culture medium just before use.

3. MTT solubilization medium

Triton	20 g
HCl	1.72 ml
Isopropanol to	200 ml

20 g of triton was weighted and mixed with one third of isopropanol. Then 1.72 ml of HCl was added drop by drop and the mixture was shaked thoroughly. Then it was made up to 200 ml volume by adding more isopropanol into the volumetric flask.

Appendix VI

Statistical data for comparison among green tea extracts

1. Comparison on the total polyphenol content

Table 17. The statistical data for the comparison of total polyphenol content

Extract	Total polyphenol content		
	N_1	N_2	N_3
JGT	55.95	57.34	59.18
MGT	56.28	55.90	52.35
TGT	51.14	53.75	55.60

Analysis of Variance Procedure

1. Dependent Variable: CONTENT

Source	DF	Sum of Squares	Mean Square	F value	Pr>F
Model	2	24.76506667	12.382553333	3.01	0.1244
Error	6	24.68953333	4.11492222		
Corrected Total	8	49.45460000			

R-Square	C.V.	Root MSE	CONTENT Mean
0.500764	3.669771	2.02852711	55.27666667

Source	DF	Anova SS	Mean Square	F Value	Pr>F
EXTRACT	2	24.76506667	12.38253333	3.01	0.1244

2. T tests (LSD) for variable : CONTENT

NOTE :This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha = 0.05 df = 6 MSE = 4.114922

Critical Value of T = 2.45

Least Significant Difference = 4.0528

Means with the same letter are not significantly different.

T Grouping	Mean	N	EXTRACT
A	57.490	3	J
A	54.843	3	M
A	53.497	3	T

2. Comparison on the radical scavenging activity of green tea extracts on DPPH radical

Table 18. The statistical data for the comparison of EC_{50} on DPPH radical

Extract	EC_{50} (μg/ml)		
	N_1	N_2	N_3
JGT	2.72	2.83	2.85
MGT	2.88	2.92	3.02
TGT	3.04	3.09	3.08

Analysis of Variance Procedure

1. Dependent Variable: EC_{50}

Source	DF	Sum of Squares	Mean Square	F value	Pr>F
Model	2	0.10940000	0.05470000	15.19	0.0045
Error	6	0.02160000	0.00360000		
Corrected Total	8	0.13100000			

R-Square	C.V.	Root MSE	EC_{50} Mean
0.835115	2.043133	0.06000000	2.93666667

Source	DF	Anova SS	Mean Square	F Value	Pr>F
EXTRACT	2	0.10940000	0.05470000	15.19	0.0045

2. T tests (LSD) for variable : EC_{50}

NOTE : This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha = 0.05 df = 6 MSE = 0.0036

Critical Value of T = 2.45

Least Significant Difference = 0.1199

Means with the same letter are not significantly different.

T Grouping	Mean	N	EXTRACT
A	3.07000	3	T
B	2.94000	3	M
C	2.80000	3	J

3. Comparison on the radical scavenging activity of BHT and green tea extracts on DPPH radical

Table 19. The statistical data for the comparison of EC_{50} on DPPH radical

Extract	EC_{50} (μg/ml)		
	N_1	N_2	N_3
BHT	15.49	14.92	14.44
JGT	2.72	2.83	2.85
MGT	2.88	2.92	3.02
TGT	3.04	3.09	3.08

Analysis of Variance Procedure

1. Dependent Variable: EC_{50}

Source	DF	Sum of Squares	Mean Square	F value	Pr>F
Model	3	324.82980000	108.27660000	1508.56	0.0001
Error	8	0.57420000	0.07177500		
Corrected Total	11	325.40400000			

R-Square	C.V.	Root MSE	EC_{50} Mean
0.998235	4.510245	0.26790857	5.94000000

Source	DF	Anova SS	Mean Square	F Value	Pr>F
EXTRACT	3	324.82980000	108.27990000	1508.56	0.0001

2. T tests (LSD) for variable : EC_{50}

NOTE : This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha = 0.05 df = 8 MSE = 0.071775

Critical Value of T = 2.31

Least Significant Difference = 0.5044

Means with the same letter are not significantly different.

T Grouping	Mean	N	EXTRACT
A	14.9500	3	B
B	3.0700	3	T
B	2.9400	3	M
B	2.8000	3	J

4. Comparison of the total antioxidant activity

Table 20. The statistical data for the comparison of the total antioxidant activity

Extract	Total antioxidant activity (mmol/l)		
	N_1	N_2	N_3
JGT	1.02	0.87	0.95
MGT	0.99	0.76	0.84
TGT	0.72	0.87	0.91

Analysis of Variance Procedure

1. Dependent Variable: TOTAL

Source	DF	Sum of Squares	Mean Square	F value	Pr>F
Model	2	0.02068889	0.01034444	1.06	0.4037
Error	6	0.05860000	0.00976667		
Corrected Total	8	0.07928889			

R-Square	C.V.	Root MSE	TOTAL Mean
0.259313	11.21612	0.09882645	0.88111111

Source	DF	Anova SS	Mean Square	F Value	Pr>F
EXTRACT	2	0.02068889	0.01034444	1.06	0.4037

2. T tests (LSD) for variable : TOTAL

NOTE : This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha = 0.05 df = 6 MSE = 0.009767

Critical Value of T = 2.45

Least Significant Difference = 0.1974

Means with the same letter are not significantly different.

T Grouping	Mean	N	EXTRACT
A	0.94667	3	J
A	0.86333	3	M
A	0.83333	3	T

5. Comparison on the cytotoxicity assay

Table 21. The statistical data for the comparison of EC_{50} on melanoma cell line by MTT assay

Extract	EC_{50} (μg/ml)		
	N_1	N_2	N_3
JGT	171	173	175
MGT	199	195	200
TGT	203	206	209

Analysis of Variance Procedure

1. Dependent Variable: EC_{50}

Source	DF	Sum of Squares	Mean Square	F value	Pr>F
Model	2	1778.00000000	889.00000000	133.35	0.0001
Error	6	40.00000000	6.66666667		
Corrected Total	8	1818.00000000			

	R-Square	C.V.	Root MSE	EC_{50}
Mean	0.977998	1.342455	2.58198890	192.33333333

Source	DF	Anova SS	Mean Square	F Value	Pr>F
EXTRACT	2	1778.000000000	889.00000000	133.35	0.0001

2. T tests (LSD) for variable : EC_{50}

NOTE : This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha = 0.05 df = 6 MSE = 6.666667

Critical Value of T = 2.45

Least Significant Difference = 5.1585

Means with the same letter are not significantly different.

T Grouping	Mean	N	EXTRACT
A	206.000	3	T
B	198.000	3	M
C	173.000	3	J

BIOGRAPHY

Miss Cho Sanda Aung was born on 3rd November, 1975 in Yangon, Myanmar. She received her Bachelor of Pharmacy (B.Pharm.) from the Institute of Pharmacy, Ministry of Health, Yangon, Myanmar in 2000.