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APPENDICES

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Table 4.1 Collecting times of *Centella asiatica* during January to December in 2003

Month	Source A	Source B
January	A1	B1
February	A2	B2
March	A3	B3
May	A5	B5
June	A6	B6
July	A7	B7
August	A8	B8
September	A9	B9
October	A10	B10
November	A11	B11
December	A12	B12

Tablet 4.2 Efficiency of extracting solvent for extraction of *Centella asiatica*

Extracting solvent (% methanol in water)	Percentage content			
	Madecassoside	Asiaticoside	Madecassic acid	Asiatic acid
60%	0.41	0.34	0.01	0.01
70%	0.45	0.38	0.01	0.02
80%	0.56	0.45	0.08	0.02

Table 4.3 Percentage content of extraction of *Centella asiatica* with methanol-water (80 %) at various time (refluxing time)

Refluxing time (min)	Percentage content			
	Madecassoside	Asiaticoside	Madecassic acid	Asiatic acid
15	0.204	0.147	0.003	0.008
30	0.271	0.184	0.003	0.007
45	0.327	0.218	0.014	0.023
60	0.331	0.207	0.026	0.024
90	0.415	0.256	0.025	0.028
120	0.397	0.255	0.025	0.024
180	0.416	0.273	0.023	0.027
240	0.383	0.233	0.021	0.029

Table 4.4 Effect of ratio of organic solvent in buffer on retention time and capacity factor of CA extract in HPLC chromatogram

Items	29% ACN		28% ACN	
	t _R	k'	t _R	k'
Madecassoside	3.4	1.3	4	1.7
Asiaticoside	5.4	2.6	6.8	3.5
Madecassic acid	6.3	3.2	7.5	4.0
Asiatic acid	18.2	11.1	22.0	13.7
Prednisolone	8.2	4.5	9.5	5.3

Table 4.5 Effect of pH of buffer on retention time and capacity factor of CA extract in HPLC chromatogram

Items	pH 7.1		pH 7.0	
	t _R	k'	t _R	k'
Madecassoside	3.4	1.3	3.4	1.3
Asiaticoside	5.4	2.6	5.4	2.6
Madecassic acid	6.3	3.2	7.5	4.0
Asiatic acid	18.2	11.1	21.6	13.4
Prednisolone	8.2	4.5	8.2	4.5

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Table 4.6 Accuracy test of HPLC analytical method of madecassoside, asiaticoside, madecassic acid and asiatic acid

Table 4.6.1 Accuracy test of madecassoside (MS)

Added concentration (mg/ml)	Items	Number of sample					
		1	2	3	4	5	6
0.067	%Recovery	97.59	98.41	99.56	99.15	99.96	100.06
	Average	99.12					
	%RSD	0.97					
0.133	%Recovery	97.76	97.84	99.25	102.1	101.9	97.94
	Average	99.46					
	%RSD	2.04					
0.273	%Recovery	101.2	99.18	100.9	100.0	99.18	101.0
	Average	100.2					
	%RSD	0.92					
0.333	%Recovery	102.5	102.8	102.4	102.3	102.1	101.5
	Average	102.3					
	%RSD	0.42					
0.401	%Recovery	102.3	98.85	100.1	101.2	101.3	99.52
	Average	100.5					
	%RSD	1.28					
Average of %recovery		100.2					

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Table 4.6.2 Accuracy test of asiaticoside (AS)

Added concentration (mg/ml)	Items	Number of sample					
		1	2	3	4	5	6
0.052	%Recovery	98.90	100.6	101.2	102.9	103.0	101.3
	Average	101.3					
	%RSD	1.50					
0.103	%Recovery	98.45	100.5	99.92	100.8	100.5	100.5
	Average	100.1					
	%RSD	0.86					
0.203	%Recovery	101.9	101.6	101.4	102.8	101.5	102.0
	Average	101.9					
	%RSD	0.50					
0.247	%Recovery	101.0	101.4	102.2	102.7	100.6	102.7
	Average	101.8					
	%RSD	0.86					
0.331	%Recovery	101.3	98.17	100.2	100.2	100.4	100.8
	Average	100.2					
	%RSD	1.07					
Average of %recovery		100.6					

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Table 4.6.3 Accuracy test of madecassic acid (MA)

Added concentration (mg/ml)	Items	Number of sample					
		1	2	3	4	5	6
0.008	%Recovery	99.48	100.0	100.9	97.12	100.7	97.59
	Average	99.31					
	%RSD	1.62					
0.016	%Recovery	97.81	97.25	99.16	100.2	98.42	100.9
	Average	98.97					
	%RSD	1.43					
0.033	%Recovery	98.02	99.77	101.6	99.46	99.93	101.0
	Average	99.97					
	%RSD	1.26					
0.040	%Recovery	97.39	97.18	100.810	102.8	100.9	102.0
	Average	100.2					
	%RSD	2.35					
0.048	%Recovery	97.23	97.63	97.40	98.80	101.7	101.4
	Average	99.03					
	%RSD	2.06					
Average of %recovery		99.68					

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Table 4.6.4 Accuracy test of asiatic acid (AA)

Added concentration (mg/ml)	Items	Number of sample					
		1	2	3	4	5	6
0.004	%Recovery	99.53	101.6	102.1	102.7	101.7	100.1
	Average	101.3					
	%RSD	1.21					
0.009	%Recovery	98.90	97.85	99.60	100.1	99.19	100.1
	Average	99.29					
	%RSD	0.86					
0.017	%Recovery	99.12	99.98	98.14	102.3	102.3	99.57
	Average	100.3					
	%RSD	1.71					
0.022	%Recovery	99.25	100.1	99.15	103.0	102.5	100.5
	Average	100.8					
	%RSD	1.63					
0.024	%Recovery	100.8	101.6	97.84	99.32	98.08	100.1
	Average	99.63					
	%RSD	1.51					
Average of %recovery		100.1					

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Table 4.7 Intra-day precision test of HPLC analytical method of madecassoside, asiaticoside, madecassic acid and asiatic acid

Table 4.7.1 Intra-day precision test of madecassoside

Added concentration (mg/ml)	Items	Number of sample				
		1	2	3	4	5
0.067	%Recovery	98.41	99.56	99.15	99.96	100.0
	Average	99.43				
	%RSD	0.68				
0.133	%Recovery	97.76	97.84	99.25	101.9	97.94
	Average	98.94				
	%RSD	1.79				
0.273	%Recovery	101.2	101.0	100.0	99.18	101.0
	Average	100.2				
	%RSD	0.92				
0.333	%Recovery	102.5	102.4	102.3	102.1	101.5
	Average	102.2				
	%RSD	0.38				
0.401	%Recovery	102.3	98.85	100.1	101.2	99.52
	Average	100.4				
	%RSD	1.38				
Average of %recovery		100.2				
Average of %RSD		1.60				

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Table 4.7.2 Intra-day precision test of asiaticoside

Added concentration (mg/ml)	Items	Number of sample				
		1	2	3	4	5
0.052	%Recovery	100.6	101.2	102.9	103.0	101.3
	Average	101.8				
	%RSD	1.05				
0.103	%Recovery	98.45	100.5	99.92	100.5	100.5
	Average	99.97				
	%RSD	0.89				
0.203	%Recovery	101.9	101.4	102.8	101.5	101.9
	Average	101.9				
	%RSD	0.54				
0.247	%Recovery	101.0	102.2	102.7	100.6	102.7
	Average	101.8				
	%RSD	0.94				
0.331	%Recovery	101.3	98.17	100.2	100.2	100.8
	Average	100.2				
	%RSD	1.19				
Average of %recovery		101.1				
Average of %RSD		1.21				

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Table 4.7.3 Intra-day precision test of madecassic acid

Added concentration (mg/ml)	Items	Number of sample				
		1	2	3	4	5
0.008	%Recovery	100.0	100.9	97.12	100.7	97.59
	Average	99.28				
	%RSD	1.81				
0.016	%Recovery	97.81	97.25	99.16	98.42	100.9
	Average	98.71				
	%RSD	1.43				
0.033	%Recovery	98.02	101.6	99.46	99.93	101.0
	Average	100.0				
	%RSD	1.41				
0.040	%Recovery	97.39	100.8	102.8	100.9	102.0
	Average	100.8				
	%RSD	2.04				
0.048	%Recovery	97.23	97.63	97.40	98.80	101.4
	Average	98.49				
	%RSD	1.76				
Average of %recovery		99.45				
Average of %RSD		1.75				

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Table 4.7.4 Intra-day precision test of asiatic acid

Added concentration (mg/ml)	Items	Number of sample				
		1	2	3	4	5
0.004	%Recovery	101.57	102.09	102.72	101.67	100.08
	Average	101.63				
	%RSD	0.96				
0.009	%Recovery	98.90	97.85	99.60	99.19	100.13
	Average	99.13				
	%RSD	0.86				
0.017	%Recovery	99.12	98.14	102.28	102.32	99.57
	Average	100.29				
	%RSD	1.90				
0.022	%Recovery	99.25	99.15	102.97	102.54	100.52
	Average	100.89				
	%RSD	1.78				
0.024	%Recovery	100.82	101.62	97.84	99.32	100.11
	Average	99.94				
	%RSD	1.45				
Average of %recovery		100.38				
Average of %RSD		1.51				

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Table 4.8 Inter-day precision test of HPLC analytical method of madecassoside, asiaticoside, madecassic acid and asiatic acid

Table 4.8.1 Inter-day precision test of madecassoside

Added concentration (mg/ml)	Items	Day		
		1	2	3
0.067	%Recovery	100.4	99.12	99.03
	Average	99.37		
	%RSD	0.80		
0.133	%Recovery	100.1	99.46	97.96
	Average	99.29		
	%RSD	1.12		
0.273	%Recovery	100.1	100.2	99.53
	Average	100.24		
	%RSD	0.68		
0.333	%Recovery	99.64	102.3	102.0
	Average	101.7		
	%RSD	1.42		
0.401	%Recovery	99.17	100.6	100.2
	Average	100.2		
	%RSD	0.73		
Average of %recovery		100.2		
Average of %RSD		1.51		

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Table 4.8.2 Inter-day precision test of asiaticoside

Added concentration (mg/ml)	Items	Day		
		1	2	3
0.052	%Recovery	98.42	101.3	99.09
	Average	100.3		
	%RSD	1.52		
0.103	%Recovery	99.63	100.1	99.48
	Average	99.89		
	%RSD	0.33		
0.203	%Recovery	100.7	101.9	99.60
	Average	101.2		
	%RSD	1.12		
0.247	%Recovery	99.42	101.8	101.7
	Average	101.3		
	%RSD	1.32		
0.331	%Recovery	101.7	100.2	100.6
	Average	100.6		
	%RSD	0.77		
Average of %recovery		100.6		
Average of %RSD		1.35		

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Table 4.6.3 Inter-day precision test of madecassic acid

Added concentration (mg/ml)	Items	Day		
		1	2	3
0.008	%Recovery	100.8	99.31	100.6
	Average	99.86		
	%RSD	0.80		
0.016	%Recovery	97.85	98.97	100.1
	Average	98.97		
	%RSD	1.14		
0.033	%Recovery	102.3	99.97	99.91
	Average	100.4		
	%RSD	1.31		
0.040	%Recovery	98.14	100.2	101.8
	Average	100.0		
	%RSD	1.81		
0.048	%Recovery	97.54	99.03	100.9
	Average	99.11		
	%RSD	1.71		
Average of %recovery		99.69		
Average of %RSD		1.76		

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Table 4.6.4 Inter-day precision test of asiatic acid

Added concentration (mg/ml)	Items	Day		
		1	2	3
0.004	%Recovery	100.0	101.3	102.2
	Average	101.2		
	%RSD	1.10		
0.009	%Recovery	99.92	99.29	98.65
	Average	99.29		
	%RSD	0.64		
0.017	%Recovery	98.02	100.2	99.00
	Average	99.54		
	%RSD	1.12		
0.022	%Recovery	99.45	100.8	101.3
	Average	100.6		
	%RSD	0.97		
0.024	%Recovery	100.5	99.63	100.7
	Average	100.0		
	%RSD	0.57		
Average of %recovery		100.1		
Average of %RSD		1.50		

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Table 4.9 Linearity and range test of HPLC analytical method of madecassoside, asiaticoside, madecassic acid and asiatic acid

Table 4.9.1 Linearity and range of madecassoside

Added concentration(mg/ml); x	Mean found concentration(mg/ml); y
0.067	0.067
0.133	0.132
0.273	0.274
0.333	0.339
0.401	0.402
$y = 1.001x - 0.0013, r^2 = 0.9997$	

Table 4.9.2 Linearity and range of asiaticoside

Added concentration(mg/ml); x	Mean found concentration(mg/ml); y
0.052	0.052
0.103	0.103
0.203	0.205
0.247	0.250
0.331	0.333
$y = 1.01x - 0.0005, r^2 = 1.000$	

Table 4.9.3 Linearity and range of madecassic acid

Added concentration(mg/ml); x	Mean found concentration(mg/ml); y
0.0083	0.0083
0.0165	0.0163
0.0331	0.0332
0.0395	0.0395
0.0476	0.0472
$y = 0.9957x + 2E-05, r^2 = 0.9999$	

Table 4.9.4 Linearity and range of asiatic acid

Added concentration(mg/ml); x	Mean found concentration(mg/ml); y
0.0044	0.0045
0.0087	0.0086
0.0174	0.0173
0.0217	0.0218
0.0242	0.0242
$y = x, r^2 = 0.9999$	

Tablet 4.10 System suitability test of HPLC analytical method of madecassoside, asiaticoside, madecassic acid and asiatic acid

Tablet 4.10.1 System suitability test of system precision (%RSD)

Number of injection	Peak area				
	MS	AS	MA	AA	PL
1	392181	294928	80717	29306	456684
2	388049	284954	81622	29531	452165
3	384768	288231	80418	30798	448720
4	388791	285816	82973	30346	457530
5	382528	289385	80617	30815	445992
Average of peak area	385362	287811	81356	30653	450747
%RSD	0.89	0.63	1.75	0.87	1.34

Tablet 4.10.2 System suitability test of Number of theoretical plates (N)

Number of injection	Number of theoretical plates				
	MS	AS	MA	AA	PL
1	2017.33	2786.99	4545.12	4820.77	4546.99
2	2045.44	2726.54	4493.11	4838.67	4466.47
3	2027.24	2710.31	4442.71	4617.77	4407.92
4	2022.40	2699.31	4389.68	4771.78	4394.28
5	2018.67	2662.71	4356.55	4705.78	4356.69
Average of N	2026.22	2717.31	4445.43	4750.95	4434.47
%RSD	0.56	1.67	1.71	1.91	1.67

Tablet 4.10.3 System suitability test of tailing factor

Number of injection	Tailing factor				
	MS	AS	MA	AA	PL
1	1.38	1.45	1.30	1.10	1.66
2	1.38	1.47	1.31	1.12	1.66
3	1.39	1.44	1.33	1.15	1.68
4	1.40	1.46	1.32	1.14	1.67
5	1.41	1.49	1.35	1.14	1.67
Average of tailing factor	1.39	1.46	1.32	1.13	1.67
%RSD	0.94	1.32	1.46	1.77	0.50

Tablet 4.10.4 System suitability test of Resolution (Rs)

Number of injection	Resolution				
	MS	AS	MA	AA	PL
1	3.65	5.62	2.13	11.69	3.75
2	3.65	5.63	2.11	11.29	3.91
3	3.63	5.58	2.11	11.49	3.91
4	3.63	5.58	2.11	11.15	3.87
5	3.61	5.53	2.09	11.57	3.75
Average of resolution	3.63	5.59	2.11	11.44	3.86
%RSD	0.46	0.71	0.67	1.90	1.70

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Tablet 4.11 Method validations for TLC analytical method**Tablet 4.11.1** Accuracy and precision test of TLC analytical method of madecassoside and asiaticoside**Tablet 4.11.1.1** Accuracy and precision of madecassoside

Added amount (mcg)	Items	Number of sample					
		1	2	3	4	5	6
1.08	%Recovery	98.77	99.31	101.38	99.52	99.06	99.00
	Average	99.51					
	%RSD	0.96					
2.16	%Recovery	98.42	100.3	99.22	98.49	98.96	100.2
	Average	99.25					
	%RSD	0.81					
10.80	%Recovery	100.8	100.8	100.5	100.9	102.0	99.55
	Average	100.8					
	%RSD	0.79					
16.20	%Recovery	97.79	97.63	100.6	98.36	100.2	100.7
	Average	99.23					
	%RSD	1.47					
21.60	%Recovery	98.29	97.11	100.29	98.27	98.82	97.31
	Average	98.35					
	%RSD	1.17					
Average of %recovery		99.81					
Average of %RSD		1.07					

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Tablet 4.11.1.2 Accuracy and precision of asiaticoside

Added amount (mcg)	Items	Number of sample					
		1	2	3	4	5	6
0.78	%Recovery	101.1	102.9	99.88	102.3	100.1	101.4
	Average	101.3					
	%RSD	1.18					
1.56	%Recovery	100.4	101.6	101.2	100.2	100.9	102.0
	Average	101.1					
	%RSD	0.70					
7.80	%Recovery	95.68	98.81	95.66	95.25	95.20	96.55
	Average	96.19					
	%RSD	1.43					
11.70	%Recovery	97.65	97.97	97.78	99.39	97.34	100.1
	Average	98.38					
	%RSD	1.14					
15.60	%Recovery	97.14	97.48	97.75	98.18	99.24	99.52
	Average	98.22					
	%RSD	0.98					
Average of %recovery		99.18					
Average of %RSD		1.18					

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Tablet 4.11.2 Linearity and range test of TLC analytical method of madecassoside and asiaticoside

Tablet 4.11.2.1 Linearity and range of madecassoside

Added amount (mcg)	Analytical found (mcg)
1.08	1.07
2.16	2.14
5.40	5.57
10.80	10.88
16.20	15.86
21.60	21.29
$y = 0.9809x + 0.1106, r^2 = 0.9995$	

Tablet 4.11.2.2 Linearity and range of asiaticoside

Added amount (mcg)	Analytical found (mcg)
0.78	0.79
1.56	1.58
3.90	3.92
7.80	7.54
11.70	11.5
15.60	15.20
$y = 0.9721x + 0.0604, r^2 = 0.9997$	

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Table 4.12 Percentage content of madecassoside (MS), asiaticoside (AS), madecassic acid (MA) and Asiatic acid (AA) in whole plant (a) and leaves and stems (b) of *Centella asiatica* by HPLC from 2 gardens (A and B) in annual period.

(a) Percentage content of madecassoside (MS), asiaticoside (AS), madecassic acid (MA) and Asiatic acid (AA) in whole plant of *Centella asiatica* by HPLC from 2 sources (A and B).

Month	Percentage content							
	MS		AS		MA		AA	
	A	B	A	B	A	B	A	B
Jan	1.003	0.844	1.441	0.773	0.007	0.019	0.013	0.014
	1.017	0.848	1.366	0.824	0.007	0.020	0.014	0.015
	1.049	0.849	1.483	0.775	0.007	0.020	0.012	0.014
Average	1.023	0.847	1.430	0.791	0.007	0.020	0.013	0.014
%RSD	2.30	0.31	4.14	3.65	0.00	2.94	7.69	4.03
Feb	0.315	0.142	0.290	0.180	0.407	0.252	0.157	0.160
	0.315	0.150	0.291	0.186	0.409	0.275	0.158	0.171
	0.308	0.154	0.292	0.184	0.418	0.254	0.168	0.160
Average	0.313	0.149	0.291	0.183	0.411	0.260	0.161	0.164
%RSD	1.29	4.11	0.34	1.67	1.42	4.89	3.78	3.88
Mar	1.123	1.222	0.935	0.932	0.102	0.061	0.087	0.066
	1.144	1.313	1.001	0.976	0.103	0.058	0.081	0.065
	1.180	1.274	0.947	0.978	0.109	0.061	0.089	0.064
Average	1.149	1.270	0.961	0.962	0.105	0.060	0.086	0.065
%RSD	2.51	3.60	3.66	2.70	3.62	2.89	4.86	1.54
May	1.526	1.981	1.678	1.162	0.011	0.055	0.031	0.031
	1.609	1.933	1.730	1.185	0.011	0.054	0.031	0.031
	1.600	1.910	1.819	1.151	0.012	0.052	0.032	0.032
Average	1.578	1.941	1.742	1.166	0.011	0.054	0.031	0.031
%RSD	2.89	1.87	4.09	1.49	5.09	2.85	1.84	1.84
June	1.596	1.130	0.992	1.156	0.089	0.044	0.071	0.041
	1.674	1.137	1.078	1.144	0.090	0.045	0.075	0.038
	1.721	1.175	1.040	1.144	0.093	0.045	0.074	0.039
Average	1.664	1.147	1.037	1.148	0.091	0.045	0.073	0.039
%RSD	3.80	2.11	4.16	0.60	2.30	1.29	2.84	3.88
July	0.831	1.958	0.589	1.325	0.179	0.184	0.093	0.063
	0.844	1.862	0.638	1.320	0.186	0.179	0.090	0.058
	0.839	1.919	0.617	1.365	0.180	0.188	0.094	0.059
Average	0.838	1.913	0.615	1.337	0.182	0.184	0.092	0.060
%RSD	0.78	2.52	4.00	1.85	2.08	2.46	2.25	4.41

(a) (Continue)

Month	percentage content							
	MS		AS		MA		AA	
	A	B	A	B	A	B	A	B
Aug	1.167	1.644	1.036	1.162	0.215	0.167	0.117	0.075
	1.213	1.613	1.056	1.151	0.220	0.177	0.127	0.074
	1.186	1.595	1.054	1.155	0.211	0.177	0.126	0.075
Average	1.189	1.617	1.049	1.156	0.215	0.174	0.123	0.075
%RSD	1.94	1.53	1.05	0.48	2.09	3.32	4.47	0.77
Sep	0.535	0.639	0.562	0.427	0.162	0.153	0.149	0.089
	0.574	0.625	0.572	0.401	0.158	0.157	0.151	0.091
	0.540	0.615	0.569	0.405	0.159	0.156	0.148	0.088
Average	0.550	0.626	0.568	0.411	0.160	0.155	0.149	0.089
%RSD	3.86	1.92	0.90	3.41	1.30	1.34	1.02	1.71
Oct	0.763	0.689	0.701	0.420	0.157	0.086	0.168	0.066
	0.755	0.658	0.675	0.430	0.143	0.083	0.164	0.062
	0.775	0.640	0.728	0.419	0.153	0.083	0.167	0.062
Average	0.764	0.662	0.701	0.423	0.151	0.084	0.166	0.063
%RSD	1.32	3.74	3.78	1.44	4.78	2.06	1.25	3.65
Nov	0.717	0.172	0.742	0.147	0.311	0.158	0.337	0.106
	0.763	0.165	0.725	0.135	0.289	0.146	0.324	0.095
	0.718	0.173	0.707	0.139	0.306	0.160	0.346	0.102
Average	0.733	0.170	0.725	0.140	0.302	0.155	0.336	0.101
%RSD	3.59	2.56	2.42	4.35	3.82	4.90	3.30	5.51
Dec	0.677	0.516	0.650	0.470	0.009	0.005	0.037	0.019
	0.632	0.495	0.641	0.474	0.008	0.005	0.036	0.018
	0.633	0.532	0.701	0.492	0.009	0.006	0.035	0.019
Average	0.647	0.514	0.664	0.479	0.009	0.005	0.036	0.019
%RSD	3.97	3.61	4.87	2.45	6.66	10.83	2.78	3.09
Average of percentage content	0.950	1.031	0.889	0.745	0.149	0.109	0.115	0.065

(b) Percentage content of madecassoside(MS), asiaticoside(AS), madecassic acid(MA) and Asiatic acid(AA) in leaves and stems of *Centella asiatica* by HPLC from 2 sources (A and B).

Garden	Month	Percentage content							
		MS		AS		MA		AA	
		Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Source B	Sep	1.553	0.039	1.028	0.073	0.297	0.007	0.043	0.447
		1.578	0.039	1.013	0.070	0.326	0.008	0.041	0.469
		1.524	0.038	0.977	0.073	0.305	0.007	0.040	0.448
	Average	1.552	0.039	1.006	0.072	0.309	0.007	0.041	0.455
	%RSD	1.742	1.493	2.605	2.406	4.842	7.873	3.696	2.732
	Dec	0.936	0.050	0.725	0.066	0.028	0.006	0.008	0.121
		0.911	0.050	0.701	0.068	0.027	0.007	0.008	0.125
		0.943	0.054	0.710	0.073	0.025	0.007	0.009	0.124
	Average	0.930	0.051	0.712	0.069	0.027	0.007	0.008	0.123
	%RSD	1.809	4.499	1.703	5.225	5.728	8.660	6.928	1.688
Average of percentage content		1.241	0.045	0.859	0.071	0.168	0.007	0.289	0.025
Percentage content ratio (leaf/stem)		27.58		12.10		24		11.56	
Source A	Sep	0.987	0.028	0.826	0.076	0.180	0.009	0.061	0.376
		1.040	0.029	0.874	0.078	0.188	0.009	0.056	0.362
		0.984	0.029	0.805	0.076	0.178	0.010	0.057	0.382
	Average	1.004	0.029	0.835	0.077	0.182	0.009	0.058	0.373
	%RSD	3.139	2.014	4.236	1.506	2.907	6.186	4.562	2.749
	Dec	0.936	0.061	0.759	0.095	0.029	0.010	0.011	0.124
		0.893	0.066	0.700	0.104	0.029	0.011	0.011	0.122
		0.929	0.060	0.715	0.094	0.028	0.010	0.010	0.114
	Average	0.919	0.062	0.725	0.098	0.029	0.010	0.011	0.120
	%RSD	2.510	5.157	4.232	5.639	2.014	5.587	5.413	4.410
Average of percentage content		0.962	0.046	0.780	0.087	0.105	0.010	0.247	0.034
Percentage content ratio (leaf/stem)		20.91		8.97		10.5		7.26	

Table 4.13 Percentage content of madecassoside(MS) and asiaticoside(AS) in extracted powder by HPLC.

Lot NO.	%Content		Ratio MS/AS	Average of ratio	SD of ratio
	Madecassoside	Asiaticoside			
1	44.97	31.56	1.42	1.10	0.22
2	39.66	41.41	0.96		
3	38.92	40.53	0.96		
4	47.60	38.57	1.23		
5	37.12	39.97	0.93		

Table 4.14 Stability of madecassoside and asiaticoside in extracted powder at 50 °C, 75%RH by HPLC.

Months	Lot 1		Lot 2	
	MS	AS	MS	AS
0	42.27	46.82	42.75	47.78
1	43.09	45.98	42.88	47.56
2	43.21	47.61	38.90	43.85
3	41.86	46.41	42.13	47.29

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Table 4.15 Percentage content of madecassoside(MS),and asiaticoside(AS) in whole plant of *Centella asiatica* by TLC from 2 sources (A and B) in annual period

Month	Percent content			
	MS		AS	
	A	B	A	B
Jan	0.936	0.496	1.143	0.426
	0.996	0.578	1.141	0.510
	0.982	0.607	1.114	0.493
Average	0.971	0.560	1.133	0.476
%RSD	3.232	10.274	1.430	9.324
Feb	0.332	0.284	0.237	0.176
	0.291	0.237	0.219	0.212
	0.308	0.252	0.231	0.208
Average	0.310	0.258	0.229	0.199
%RSD	6.638	9.317	4.002	9.932
Mar	0.858	1.170	0.790	0.890
	0.902	1.013	0.781	0.821
	0.858	1.075	0.725	0.827
Average	0.873	1.086	0.765	0.846
%RSD	2.911	7.281	4.602	4.518
May	1.532	1.643	1.481	1.292
	1.649	1.516	1.607	1.110
	1.622	1.777	1.536	1.262
Average	1.601	1.645	1.541	1.221
%RSD	3.828	7.932	4.098	7.989
June	1.457	1.292	0.908	0.944
	1.416	1.316	0.885	0.921
	1.341	1.181	0.844	0.837
Average	1.405	1.263	0.879	0.901
%RSD	4.188	5.705	3.688	6.233
July	0.751	1.817	0.510	1.290
	0.779	1.662	0.582	1.296
	0.716	1.781	0.511	1.349
Average	0.749	1.753	0.534	1.312
%RSD	4.216	4.611	7.726	2.454

Table 4.15 (Continue)

Month	%content			
	MS		AS	
	A	B	A	B
Aug	0.972	1.293	0.765	1.072
	0.914	1.128	0.814	0.953
	1.037	1.169	0.887	0.950
Average	0.974	1.197	0.822	0.992
%RSD	6.315	7.179	7.469	7.017
Sep	0.584	0.854	0.493	0.660
	0.637	0.997	0.585	0.676
	0.548	0.951	0.543	0.689
Average	0.590	0.934	0.540	0.675
%RSD	7.592	7.816	8.524	2.152
Oct	0.814	0.720	0.707	0.455
	0.896	0.676	0.783	0.418
	0.743	0.749	0.709	0.411
Average	0.818	0.715	0.733	0.428
%RSD	9.364	5.141	5.909	5.524
Nov	0.606	0.347	0.610	0.270
	0.593	0.335	0.572	0.255
	0.487	0.305	0.509	0.233
Average	0.562	0.329	0.564	0.253
%RSD	11.615	6.575	9.050	7.365
Dec	0.485	0.704	0.420	0.559
	0.546	0.646	0.447	0.556
	0.498	0.595	0.403	0.550
Average	0.510	0.648	0.423	0.555
%RSD	6.304	8.412	5.241	0.826
Average of annual	0.876	0.965	0.759	0.733

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Table 5.1 Chromatographic system suitability

	Retention time (t_R)	Resolution (R_s)	Tailing Factor
Madecassoside	3.4	3.63	1.39
Asiaticoside	5.4	5.58	1.46
Madecassic acid	6.3	2.11	1.32
Prednisolone	8.2	3.86	1.67
Asiatic acid	18.2	11.24	1.13

Table 5.2 Method validation test of HPLC analytical method

	Madecassoside	Asiaticoside	Madecassic acid	Asiatic acid
Accuracy (%RV)	100.16	100.64	99.68	100.14
Mean Range	97.15-102.79	97.72-100.96	97.12-102.78	97.64-102.97
Precision (%RSD)				
intra-day	1.60	1.21	1.75	1.51
inter-day	1.51	1.35	1.76	1.50
Range (mg/ml)	0.06-0.40	0.05-0.33	0.008-0.048	0.004-0.024
Linearity R^2	0.9997	1.000	0.9999	0.9999
Slope	1.011	1.010	0.9957	1.000
Intercept	-0.001	-0.005	2.00E-05	0
LOQ ($\mu\text{g/ml}$)	5.97	1.84	2.11	2.32

Tablet 5.3 Data of method validation for Thin-Layer Chromatograph

	Madecassoside	Asiaticoside
Accuracy %recovery	97.11-104.07 (99.81)	95.20-102.92 (99.18)
Precision %RSD	0.79-1.47 (1.07)	0.70-1.63(1.18)
Linearity and range		
Slope (A)	0.9809	0.9721
Intercept (B)	0.1106	0.0604
r ²	0.9995	0.9997
Range (mcg)	1.08-21.60	0.78-15.60

+

Table 5.4 Percentage content of madecassoside(MS), asiaticoside(AS), madecassic acid(MA) and Asiatic acid(AA) in whole plant (a) and leaves and stems (b) of *Centella asiatica* by HPLC in annual period

(a) Percentage content of madecassoside(MS), asiaticoside(AS), madecassic acid(MA) and Asiatic acid(AA) in whole plant of *Centella asiatica* by HPLC from 2 sources (A and B)

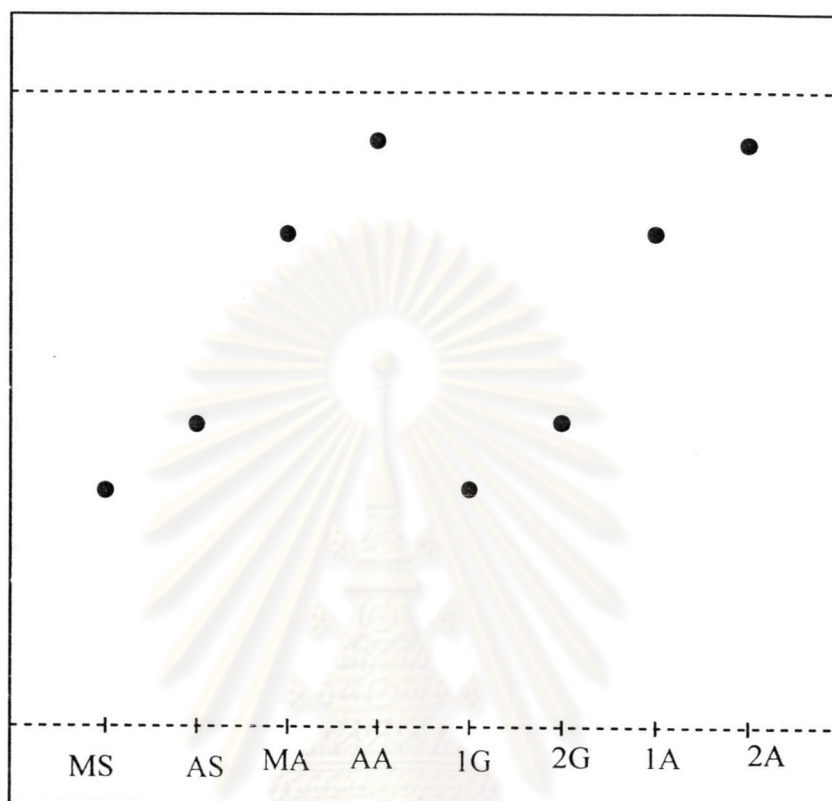
Month	Percent content							
	MS		AS		MA		AA	
	A	B	A	B	A	B	A	B
January	1.023	0.847	1.430	0.791	0.007	0.020	0.013	0.014
February	0.312	0.149	0.291	0.183	0.411	0.260	0.161	0.164
March	1.149	1.269	0.961	0.962	0.105	0.060	0.086	0.065
May	1.578	1.941	1.742	1.166	0.011	0.053	0.031	0.031
June	1.663	1.147	1.036	1.148	0.091	0.045	0.074	0.040
July	0.838	1.913	0.615	1.341	0.182	0.184	0.092	0.060
August	1.189	1.617	1.049	1.156	0.215	0.174	0.123	0.075
September	0.550	0.662	0.568	0.411	0.159	0.155	0.149	0.089
October	0.764	1.114	0.701	0.423	0.151	0.084	0.166	0.063
November	0.733	0.170	0.725	0.140	0.302	0.154	0.336	0.101
December	0.648	0.514	0.664	0.479	0.009	0.005	0.036	0.018
Average	0.956	1.033	0.909	0.745	0.159	0.112	0.124	0.069
Average of percent content	0.994		0.827		0.135		0.097	

(b) Percentage content of madecassoside(MS), asiaticoside(AS), madecassic acid(MA) and Asiatic acid(AA) in leaves and stems of *Centella asiatica* by HPLC from 2 sources (A and B)

Garden	Month	Percent content							
		MS		AS		MA		AA	
		Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Source B	9	1.552	0.037	0.993	0.072	0.309	0.007	0.445	0.043
	12	0.903	0.049	0.723	0.058	0.027	0.007	0.128	0.008
Source A	9	1.004	0.032	0.835	0.074	0.187	0.008	0.399	0.059
	12	0.845	0.064	0.725	0.096	0.023	0.011	0.126	0.010
Average		1.095	0.045	0.820	0.079	0.137	0.008	0.268	0.030
Percent content ratio (leaf/stem)		24.188		10.373		16.576		9.076	

Table 5.5 Percentage content of madecassoside(MS) and asiaticoside(AS), in whole plant of *Centella asiatica* by TLC from 2 sources (A and B) in annual period

Month	Percent content			
	MS		AS	
	A	B	A	B
January	0.971	0.643	1.133	0.547
February	0.321	0.258	0.241	0.205
March	0.838	1.026	0.695	0.800
May	1.601	1.678	1.541	1.221
June	1.660	1.478	1.052	1.058
July	0.749	1.661	0.526	1.239
August	0.953	1.132	0.793	0.939
September	0.590	0.934	0.540	0.720
October	0.818	0.763	0.765	0.464
November	0.562	0.292	0.579	0.224
December	0.568	0.747	0.486	0.647
Average	0.876	0.965	0.759	0.733
Average	0.920		0.746	

Figure 4.1 TLC chromatogram of reference standard and isolated compounds

- MS = Madecassoside standard
AS = Asiaticoside standard
MA = Madecassic acid standard
AA = Asiatic acid standard
1G = Isolated madecassoside
2G = Isolated asiaticoside
1A = Isolated madecassic acid
2A = Isolated asiatic acid

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Figure 4.2 HPLC chromatogram of reference standard and isolated compounds

Figure 4.2a HPLC chromatogram of reference standard madecassoside

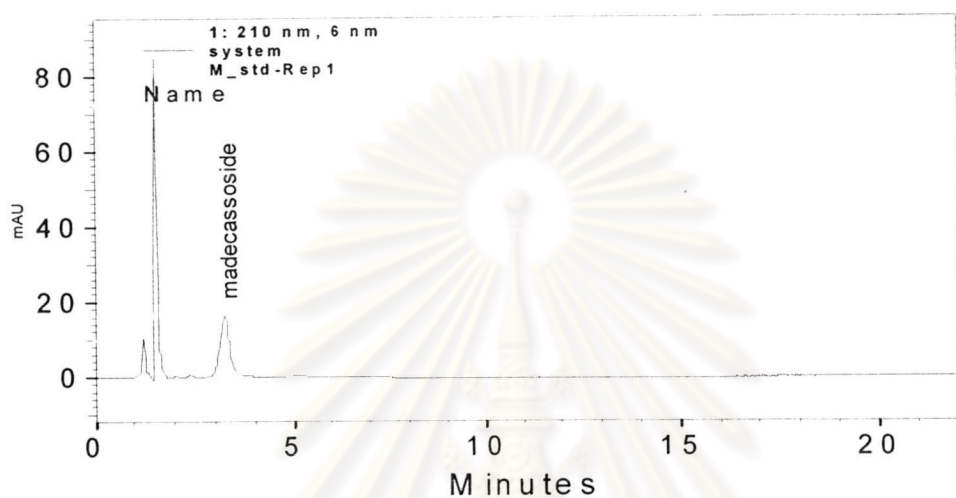


Figure 4.2b HPLC chromatogram of isolated madecassoside

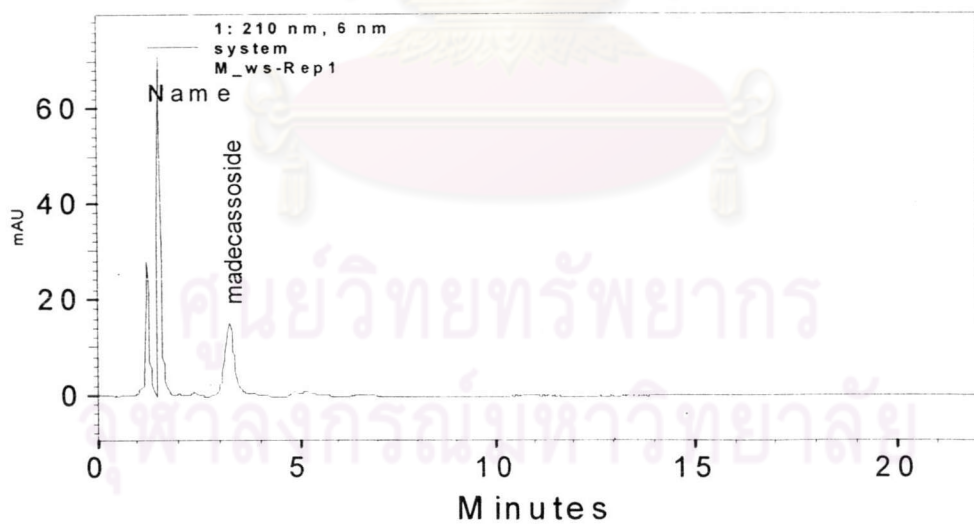


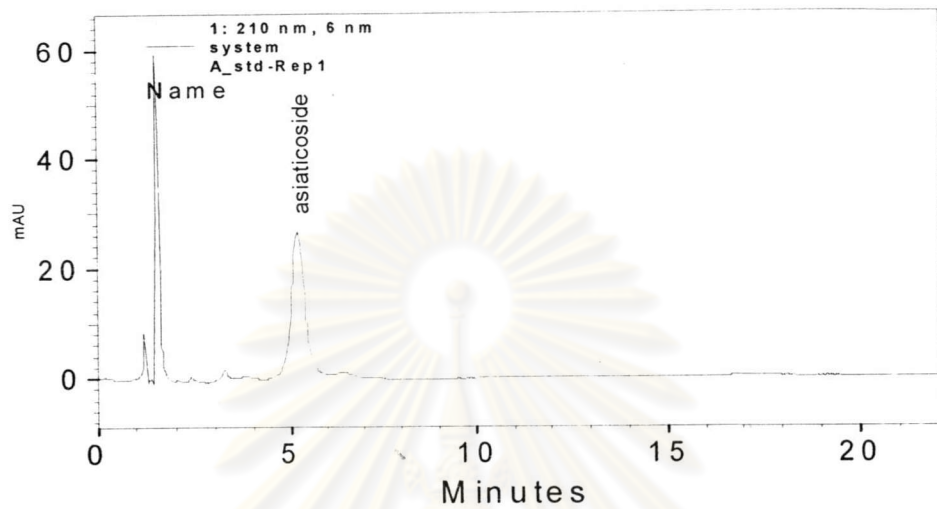
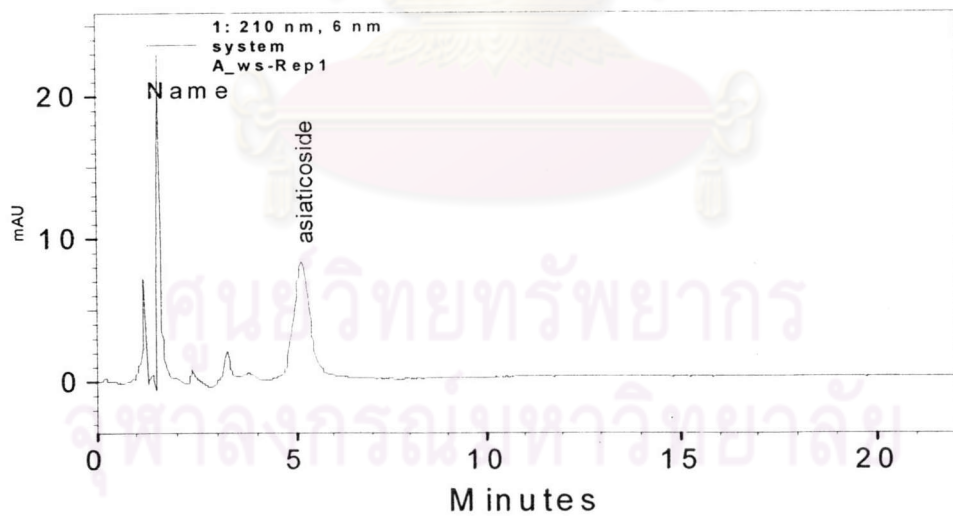
Figure 4.2c HPLC chromatogram of reference standard asiaticoside**Figure 4.2d** HPLC chromatogram of isolated asiaticoside

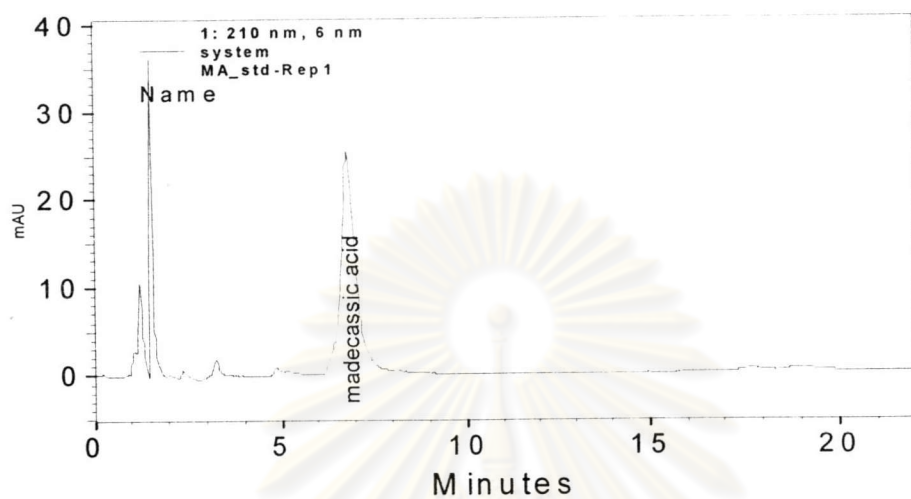
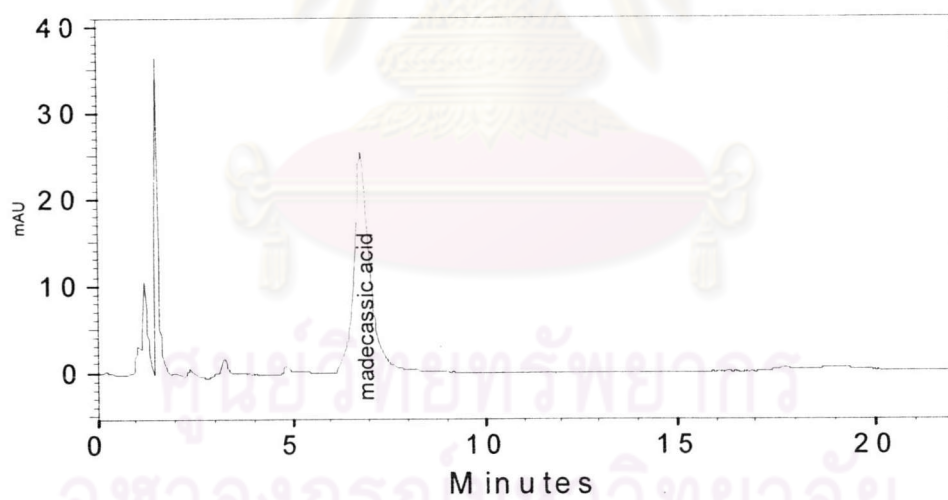
Figure 4.2e HPLC chromatogram of reference standard madecassic acid**Figure 4.2f** HPLC chromatogram of isolated madecassic acid

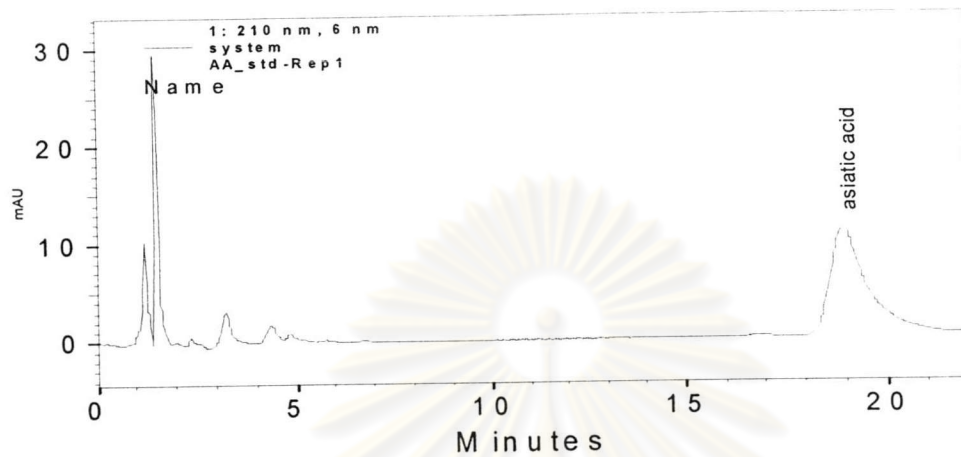
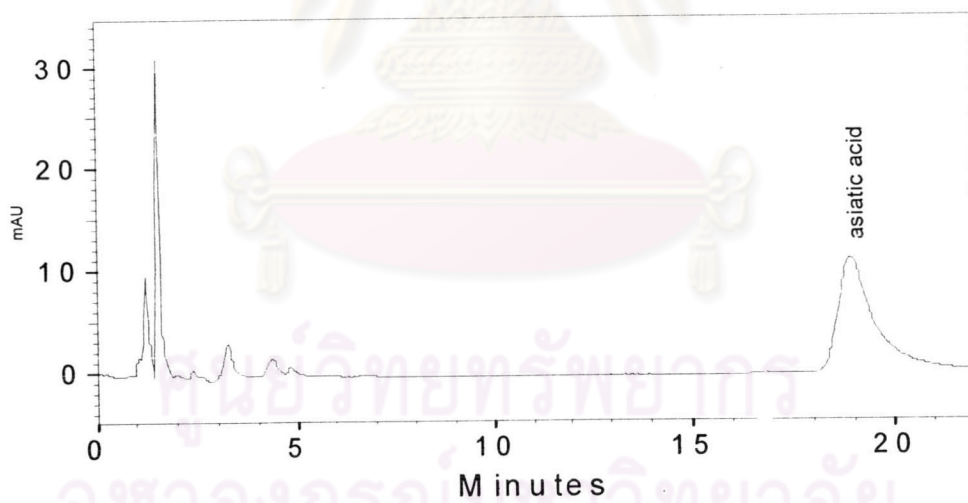
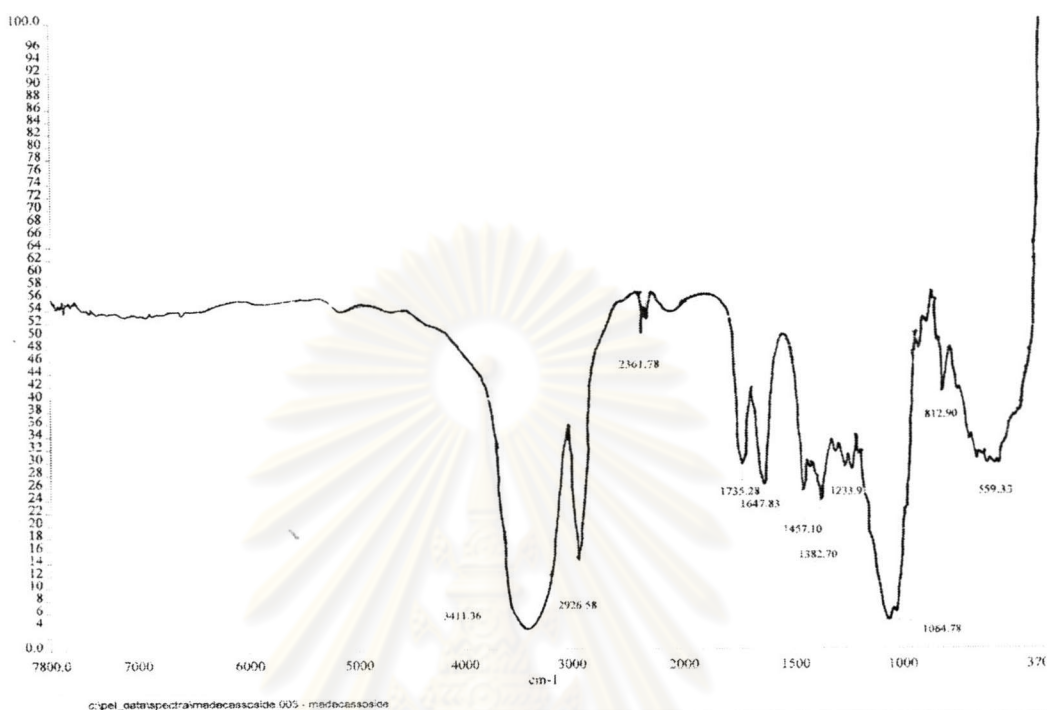
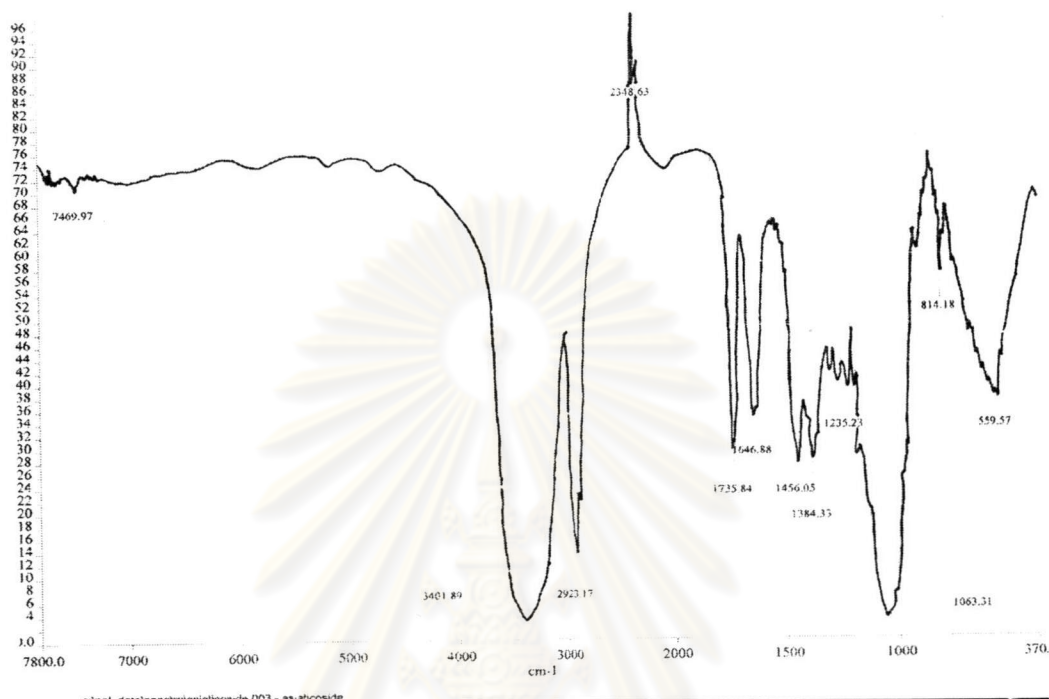
Figure 4.2g HPLC chromatogram of reference standard asiatic acid**Figure 4.2h** HPLC chromatogram of isolated asiatic acid

Figure 4.3 IR spectrum of isolated madecassoside

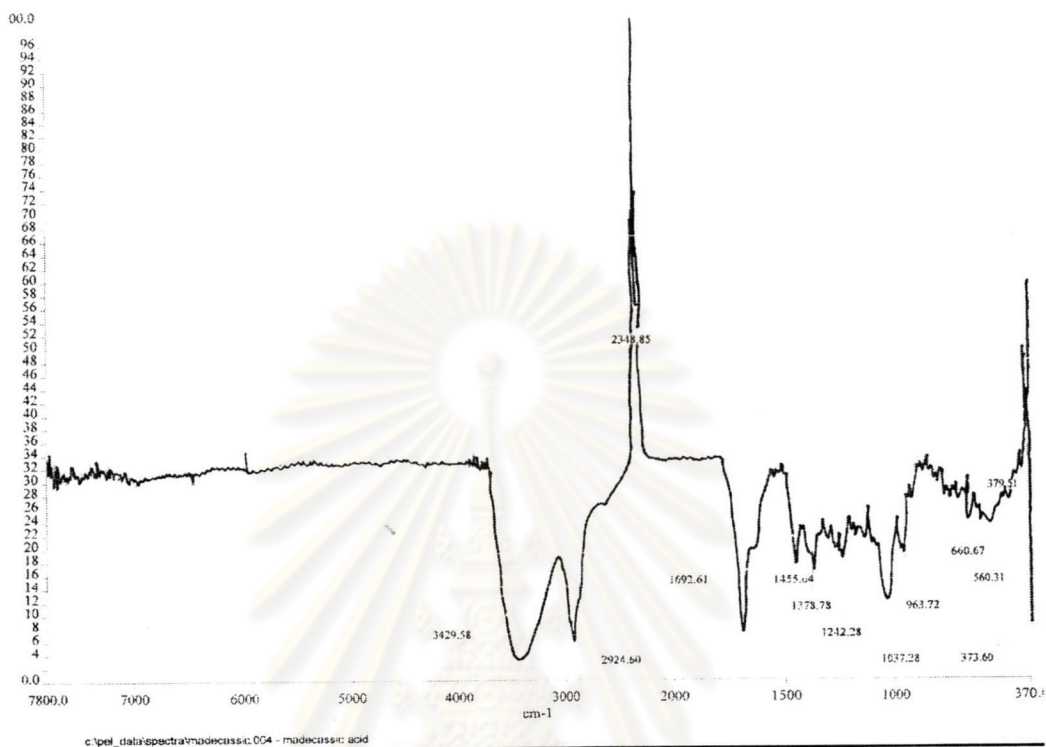
Wavenumber (cm ⁻¹)	Functional groups	Vibration
3411	-OH	OH stretching
2926	-CH ₃ , >CH ₂ and CH	CH- stretching
1735	>C=O	C=O stretching
1647	-CH ₃ and >CH ₂	CH- Bending
1064	C-OH	CO- stretching

Figure 4.4 IR spectrum of isolated asiaticoside



Wavenumber (cm ⁻¹)	Functional groups	Vibration
3401	-OH	OH stretching
2923	-CH ₃ , >CH ₂ and CH	CH- stretching
1735	>C=O	C=O stretching
1646	-CH ₃ and >CH ₂	CH- Bending
1063	C-OH	CO- stretching

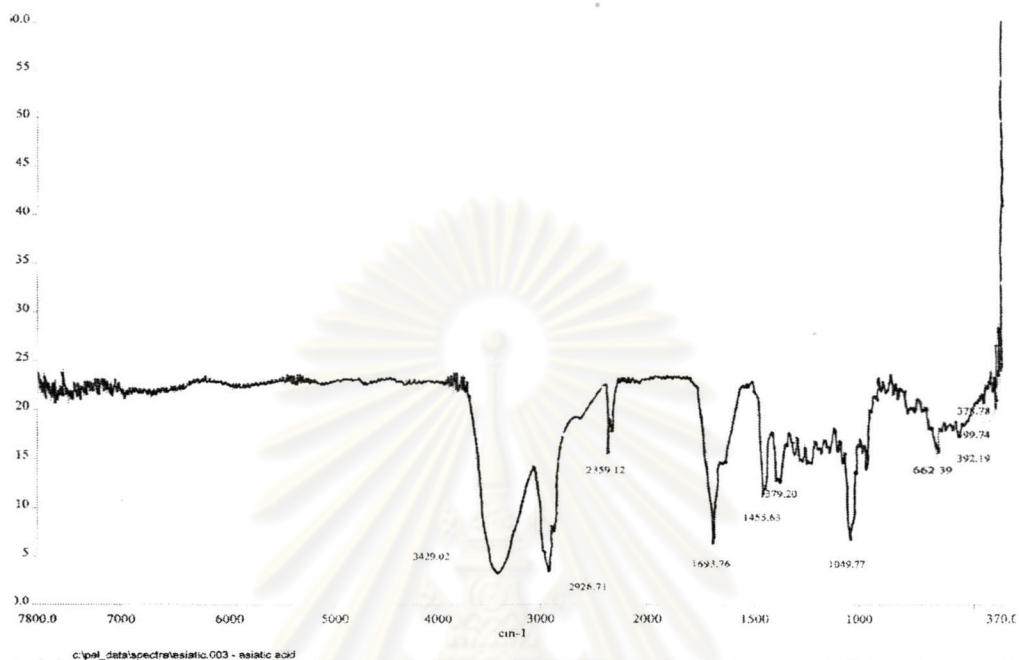
Figure 4.5 IR spectrum of isolated madecassic acid



Wavenumber (cm^{-1})	Functional groups	Vibration
3439	-OH	OH stretching
2924	-CH ₃ , >CH ₂ and CH	CH- stretching
1692	>C=O	C=O stretching
1037	C-OH	CO- stretching

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Figure 4.6 IR spectrum of isolated asiatic acid



Wavenumber (cm ⁻¹)	Functional groups	Vibration
3429	-OH	OH stretching
2926	-CH ₃ , >CH ₂ and CH	CH- stretching
1693	>C=O	C=O stretching
1049	C-OH	CO- stretching

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Figure 4.7 $^1\text{H-NMR}$ of isolated madecassoside

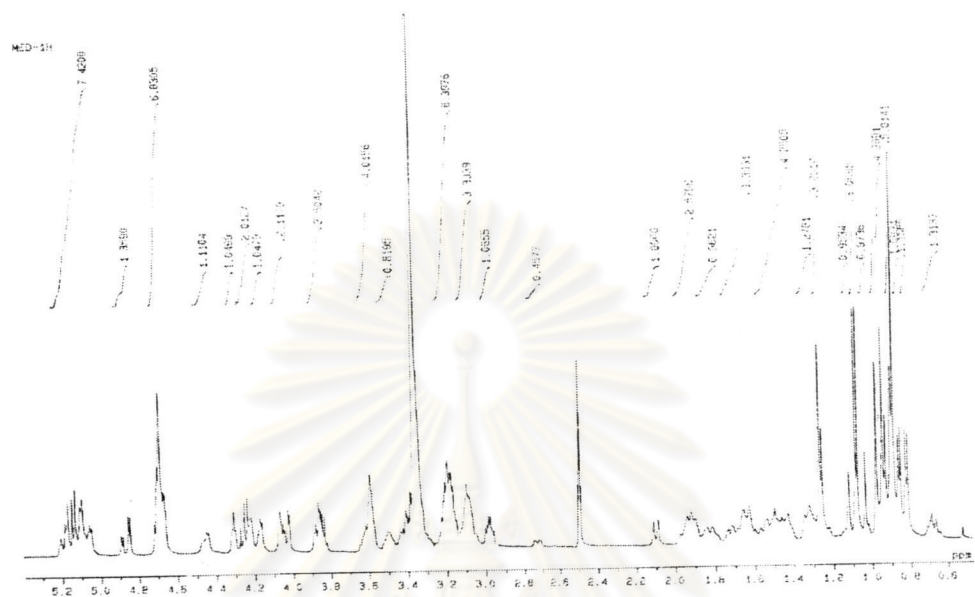


Figure 4.8 $^1\text{H-NMR}$ of isolated asiaticoside

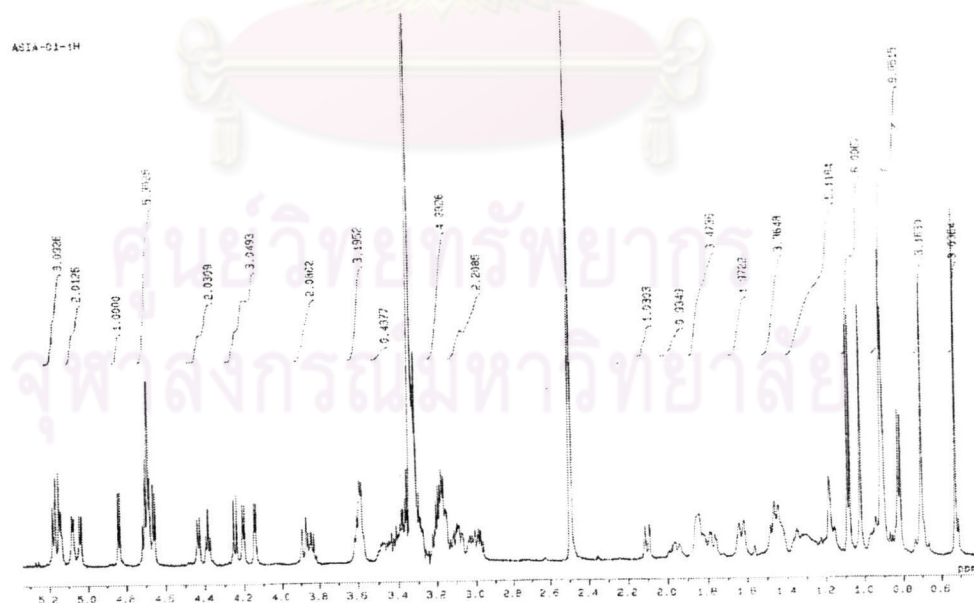


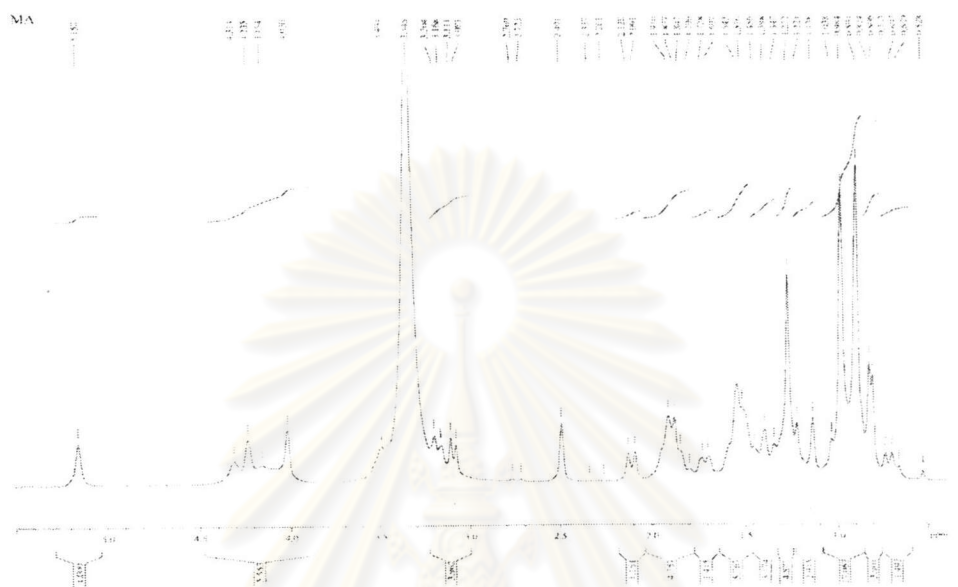
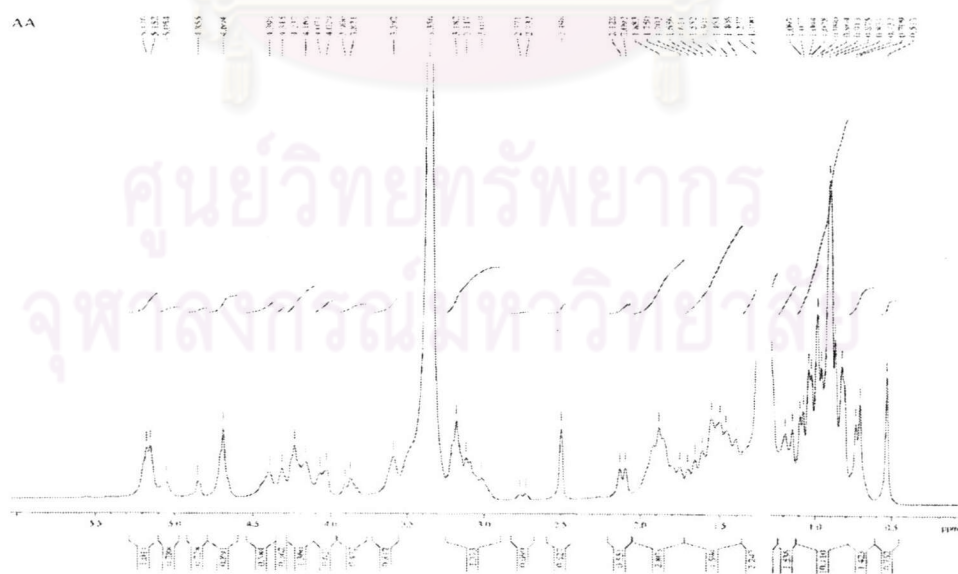
Figure 4.9 $^1\text{H-NMR}$ of isolated madecassic acid**Figure 4.10** $^1\text{H-NMR}$ of isolated asiatic acid

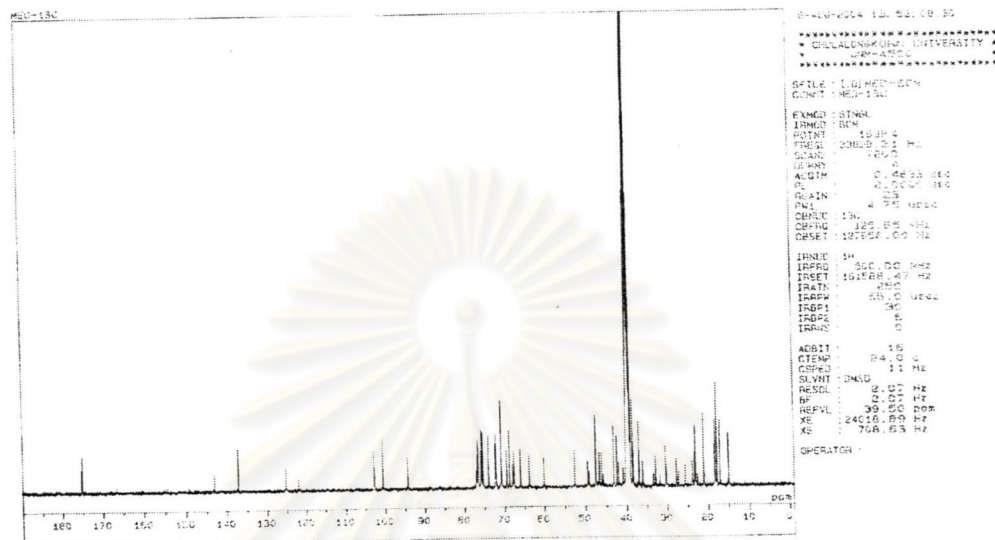
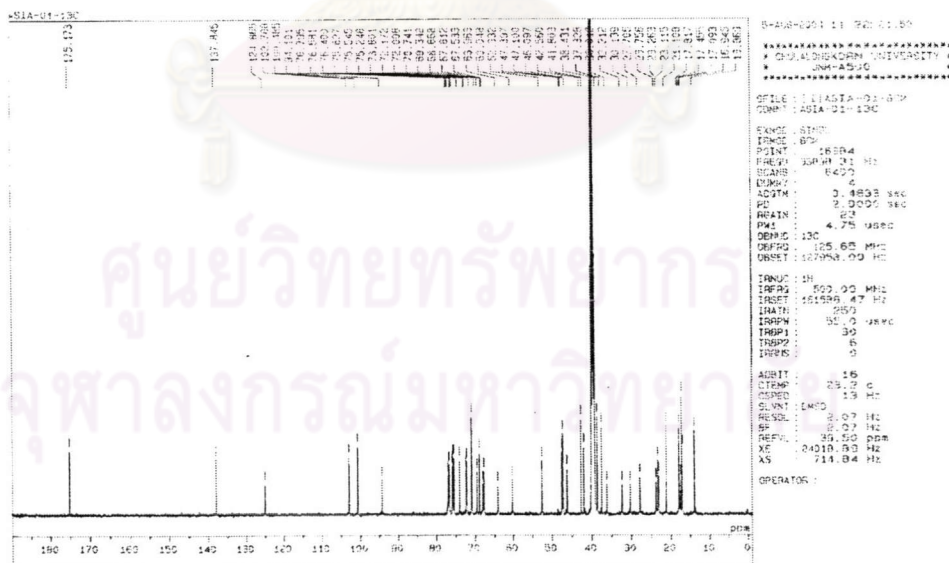
Figure 4.11 ^{13}C -NMR of isolated madecassosideFigure 4.12 ^{13}C -NMR of isolated asiaticoside

Figure 4.13 ^{13}C -NMR of isolated madecassic acid

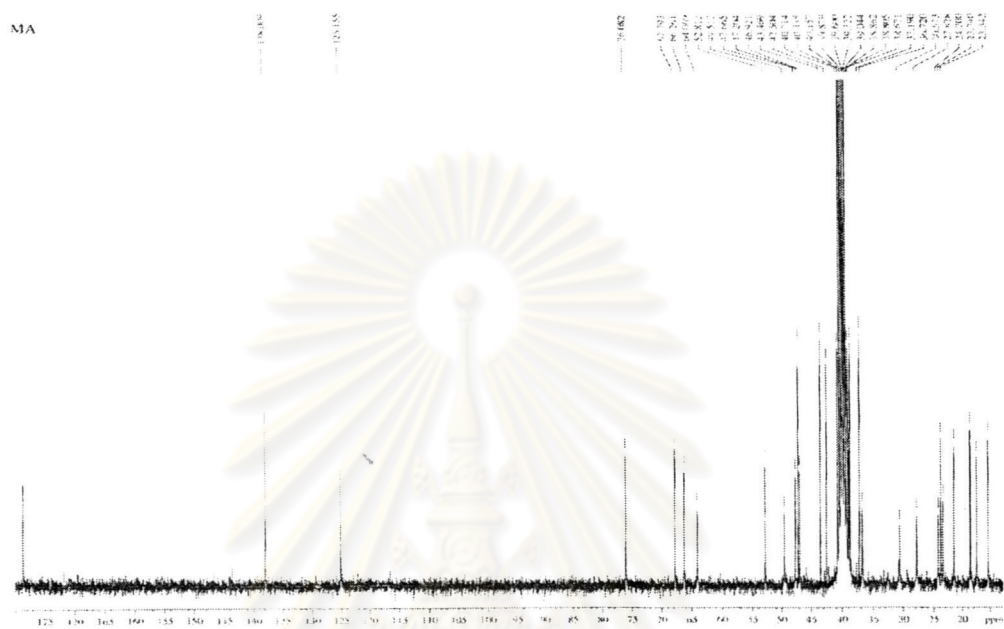


Figure 4.14 ^{13}C -NMR of isolated asiatic acid

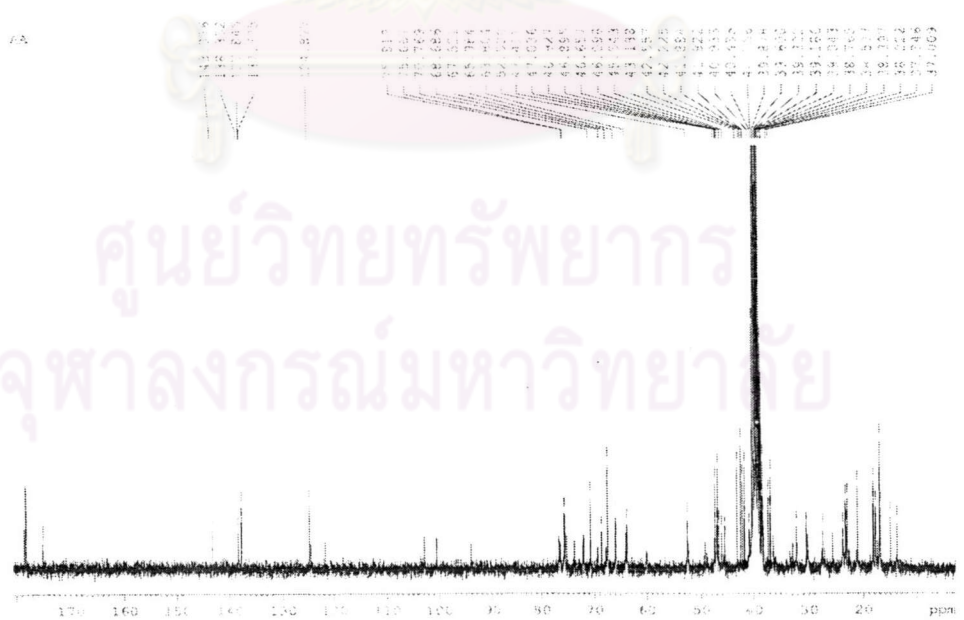
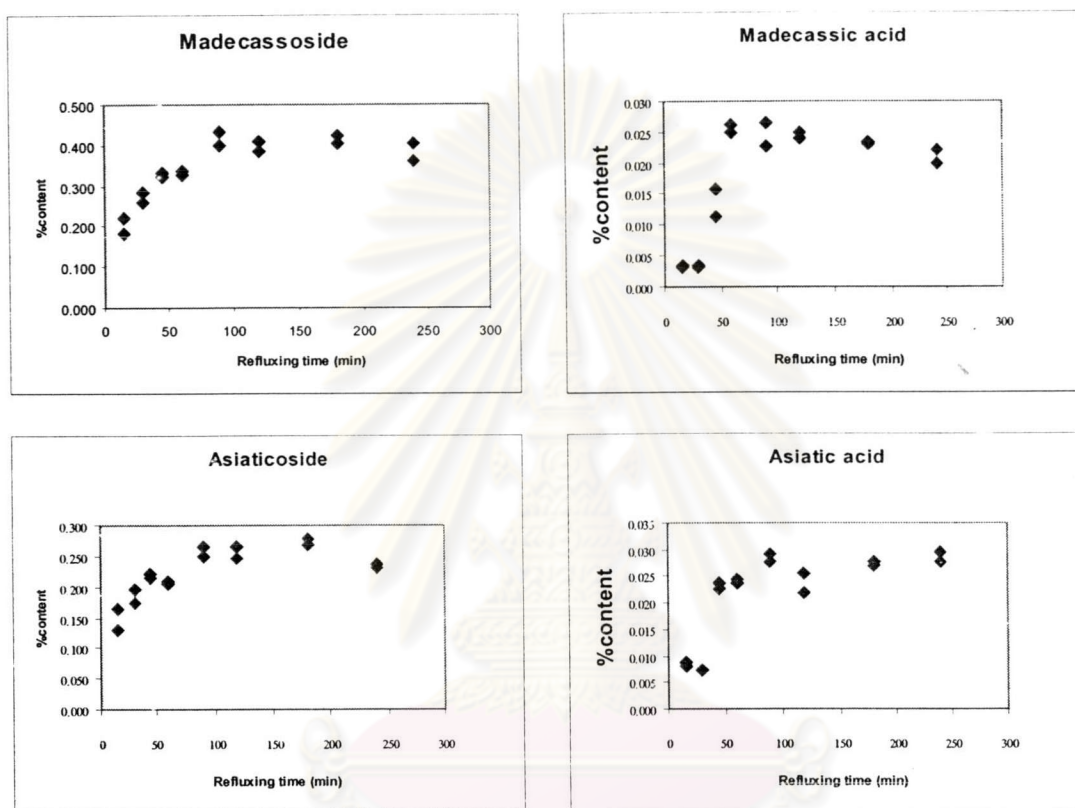


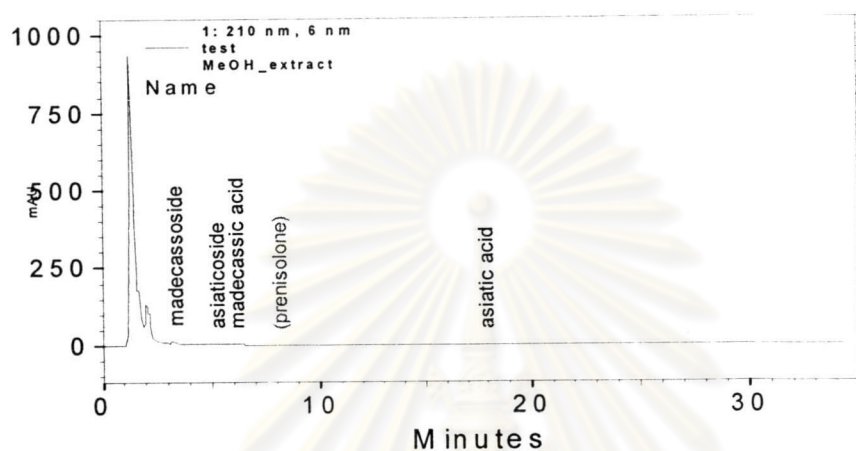
Figure 4.15 Percentage content of extraction of *Centella asiatica* with methanol-water (80 %) at various time



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Figure 4.16 HPLC chromatogram of sample with (b)/without (a) pretreatment with solid phase extraction

a HPLC chromatogram of sample without pretreatment with solid phase extraction



b HPLC chromatogram of sample after pretreated with solid phase extraction

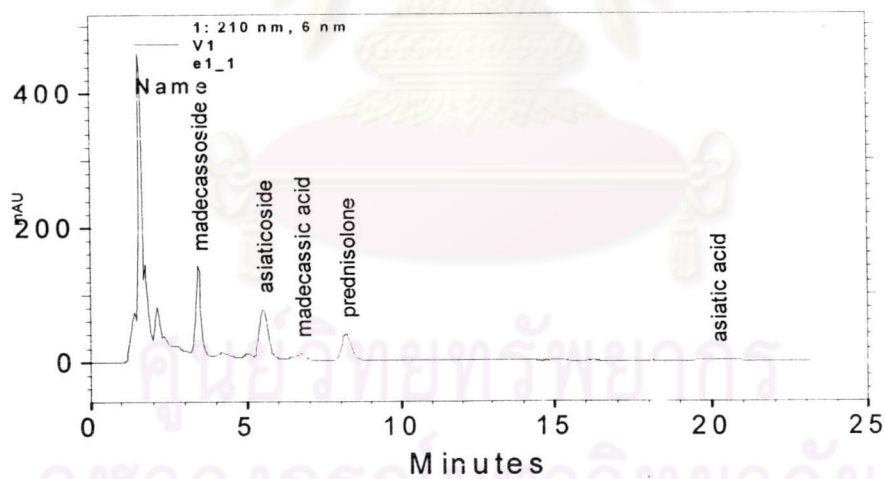
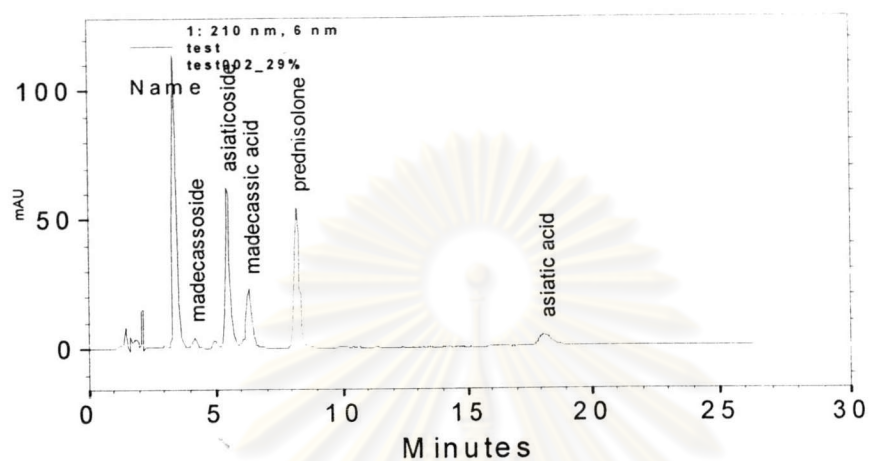
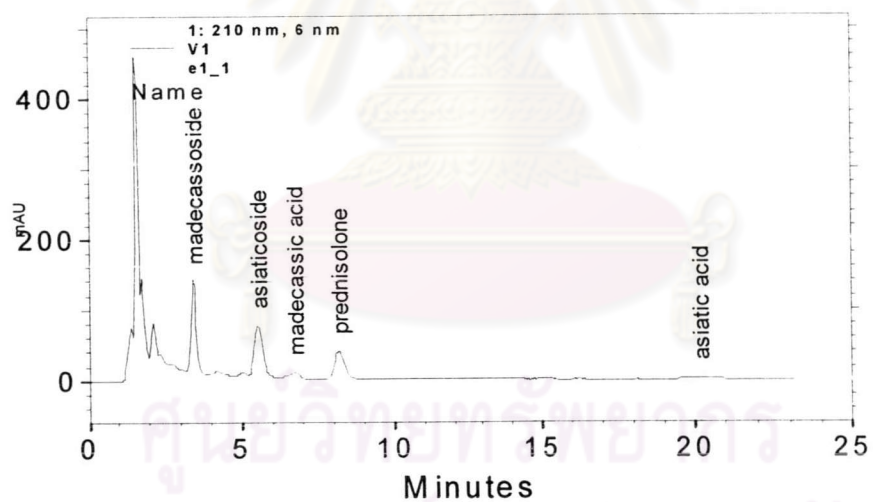
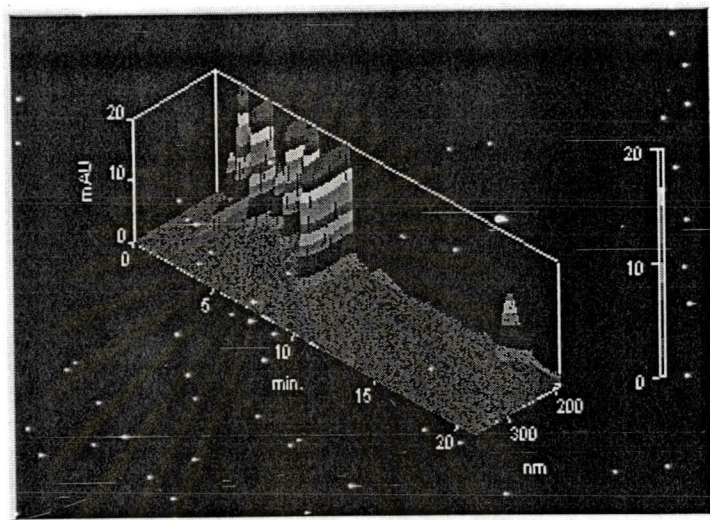


Figure 4.17 HPLC chromatogram of standard (a) and extract sample (b)**a** HPLC chromatogram of standard**b** Chromatogram of extract sample

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Figure 4.18 3D- HPLC chromatogram of standard solution (a) and sample solution (b)

a) 3D- HPLC chromatogram of standard solution



b) 3D- HPLC chromatogram of sample solution

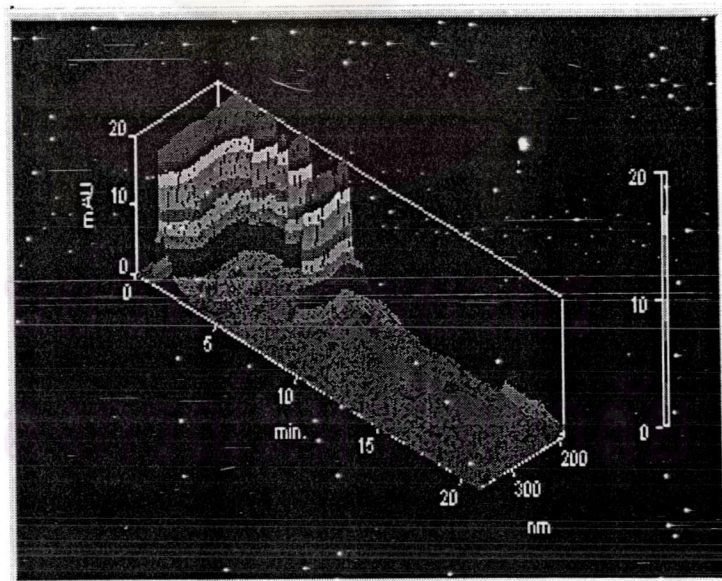
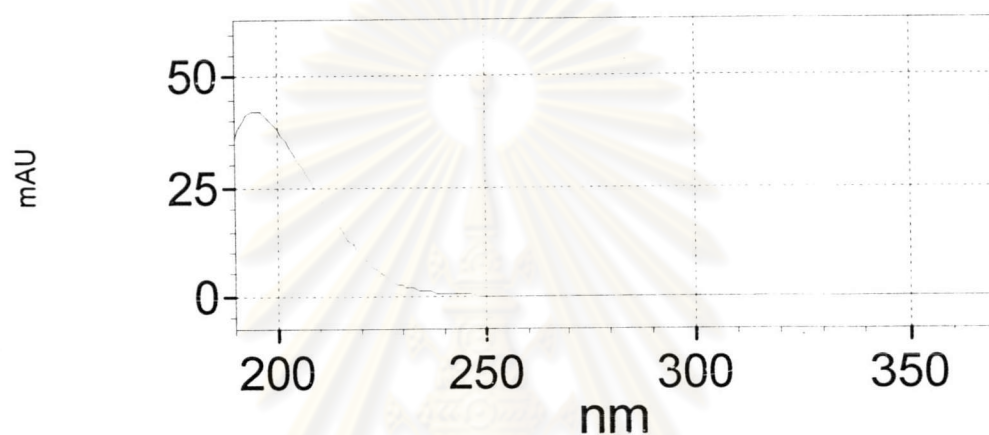


Figure 4.19 UV spectrum of each compounds in standard solution and sample solution

Figure 4.19.1 UV spectrum of madecassoside in standard solution (a) and sample solution (b)

a) UV spectrum of madecassoside in standard solution



b) UV spectrum of madecassoside in sample solution

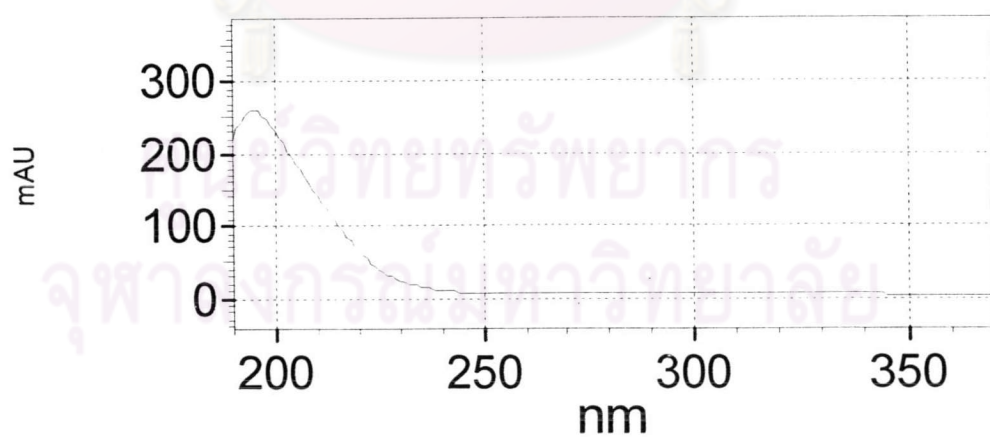
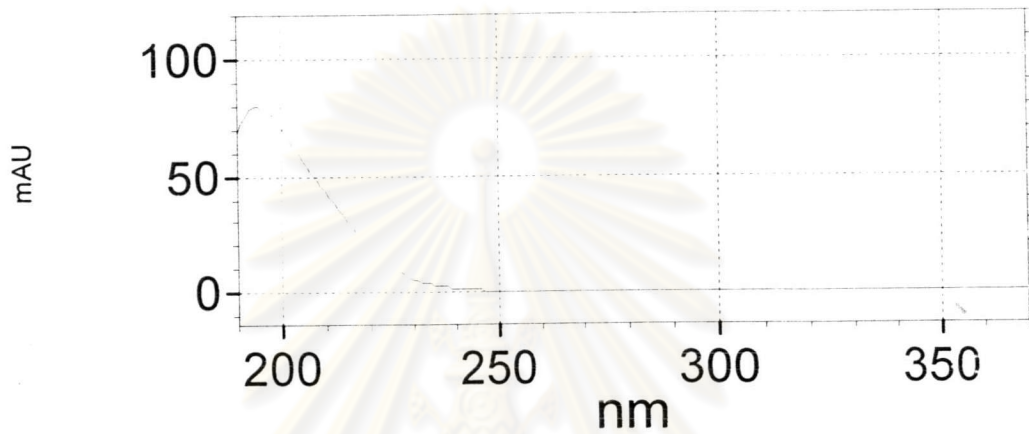


Figure 4.19.2 UV spectrum of asiaticoside in standard solution (a) and sample solution (b)

a) UV spectrum of asiaticoside in standard solution



b) UV spectrum of asiaticoside in sample solution

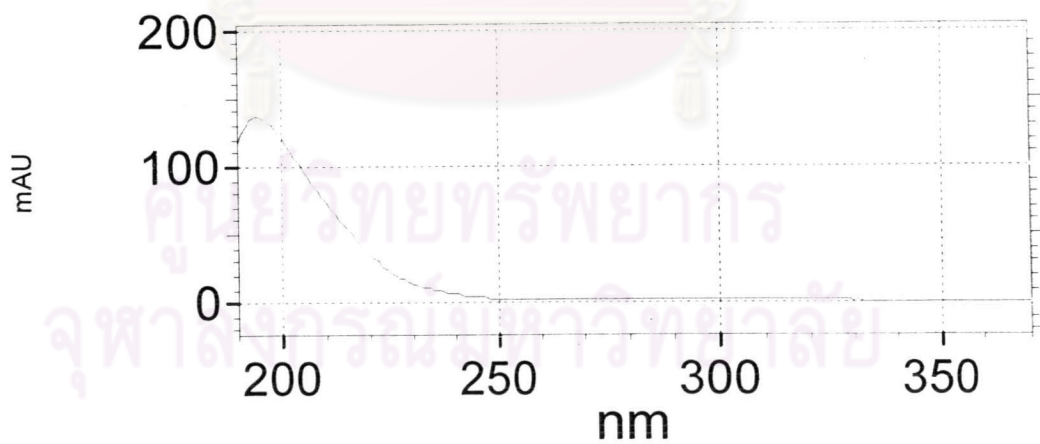
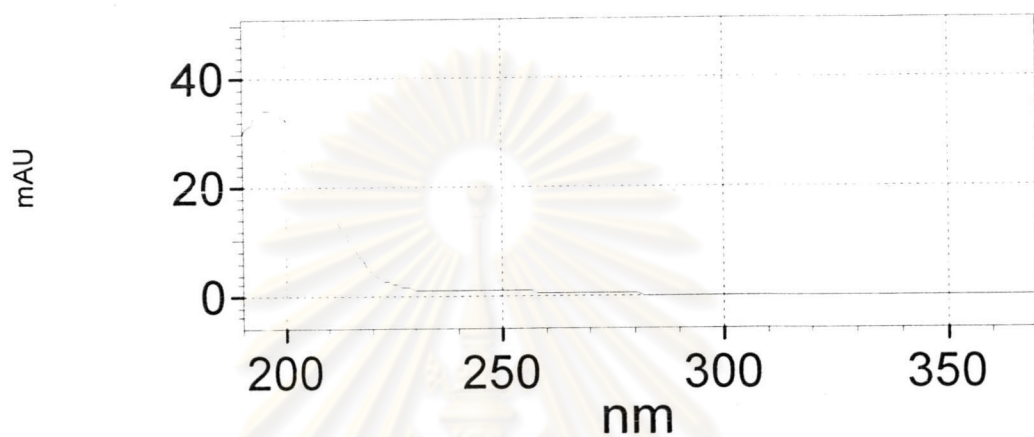


Figure 4.19.3 UV spectrum of madecassic acid in standard solution (a) and sample solution (b)

a) UV spectrum of madecassic acid in standard solution



b) UV spectrum of madecassic acid in sample solution

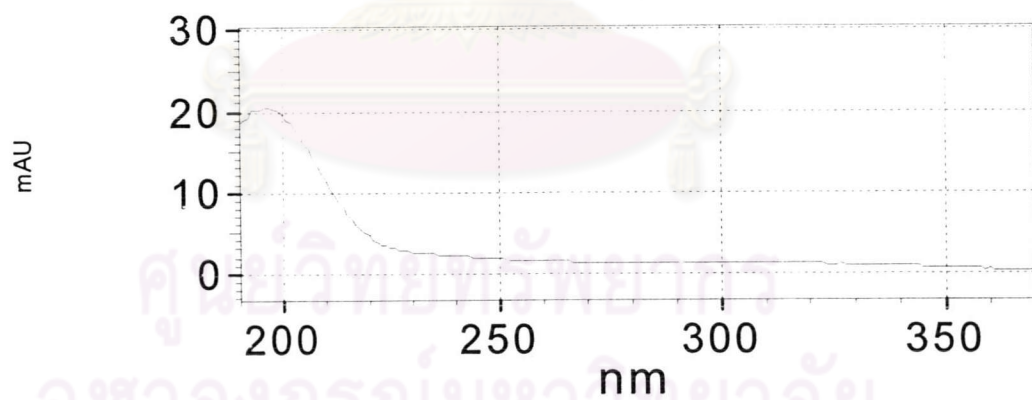
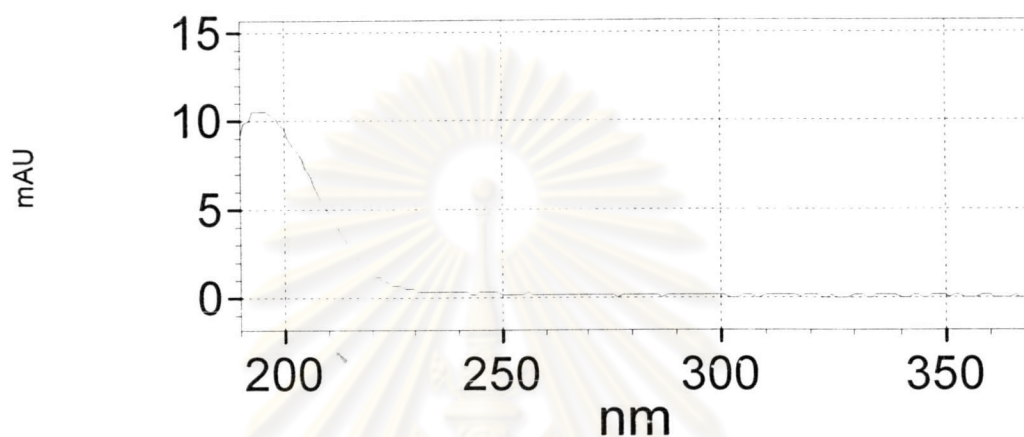


Figure 4.19.4 UV spectrum of asiatic acid in standard solution (a) and sample solution (b)

a) UV spectrum of asiatic acid in standard solution



b) UV spectrum of asiatic acid in sample solution

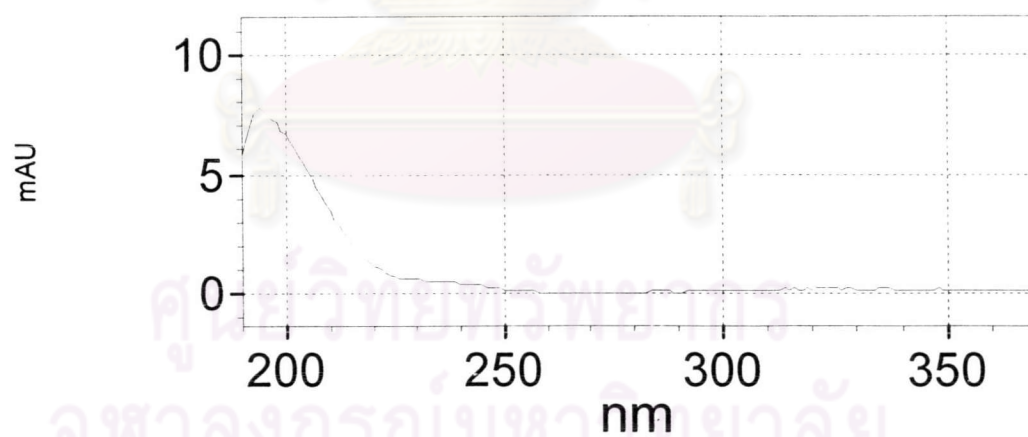
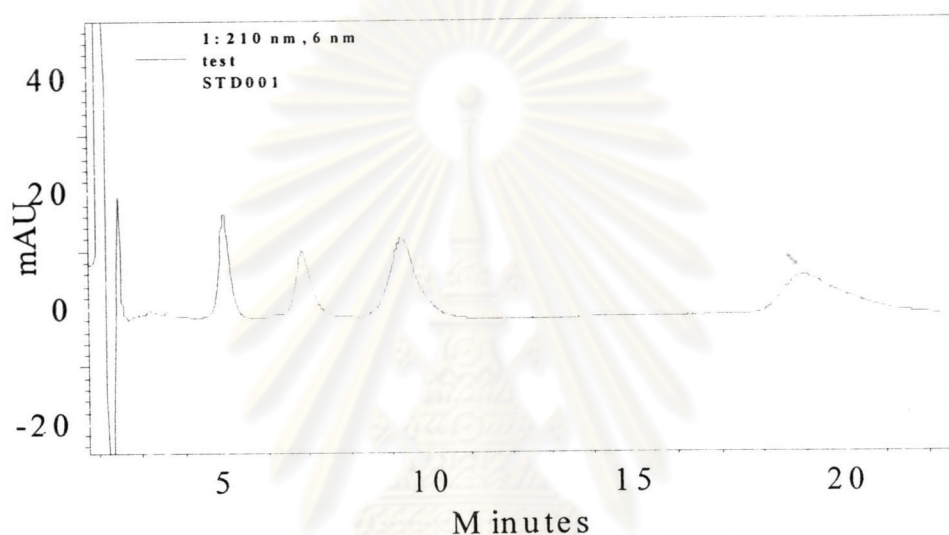


Figure 20 HPLC chromatogram of standard solution of madecassoside, asiaticoside, madecassic acid and asiatic acid by using phenyl column as stationary phase and acetonitrile-phosphate buffer (10 mM, pH 6.2)(23:77) as mobile phase



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Figure 4.21 HPLC chromatogram of standard solution in several of organic solvent in mobile phase;

Figure 4.21a HPLC chromatogram by using the mixture of acetonitrile and phosphate buffer(10mM, pH 7.1) at the proportion of 28:72 as mobile phase

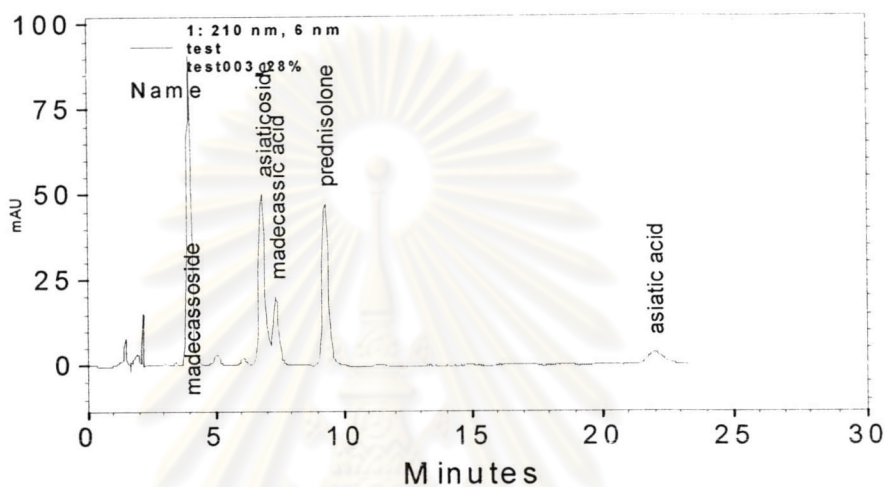


Figure 4.21b HPLC chromatogram by using the mixture of acetonitrile and phosphate buffer(10mM, pH 7.1) at the proportion of 29:71 as mobile phase

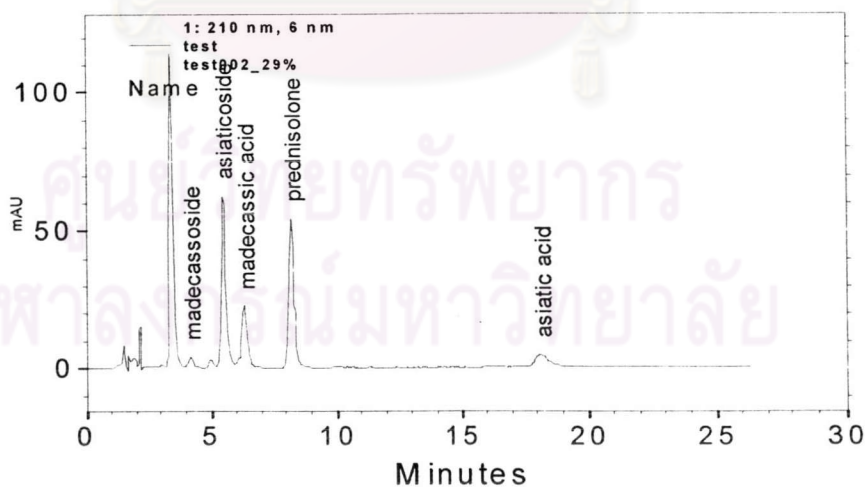
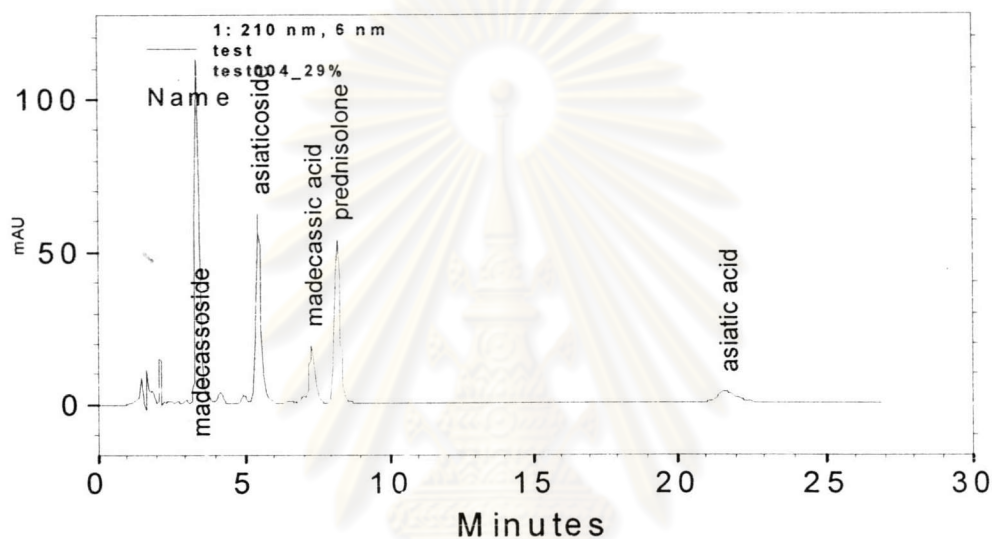


Figure 4.22 HPLC chromatogram of standard solution effected by pH of mobile phase and concentration of buffer in mobile phase

Figure 4.22.1 HPLC chromatogram of standard solution effected by pH of mobile phase; pH7.0 (a) and 7.1(b)

a) HPLC chromatogram by using the mixture of acetonitrile and phosphate buffer (10 mM) at pH 7.0 as mobile phase



b) HPLC chromatogram by using the mixture of acetonitrile and phosphate buffer (10 mM) at pH 7.1 as mobile phase

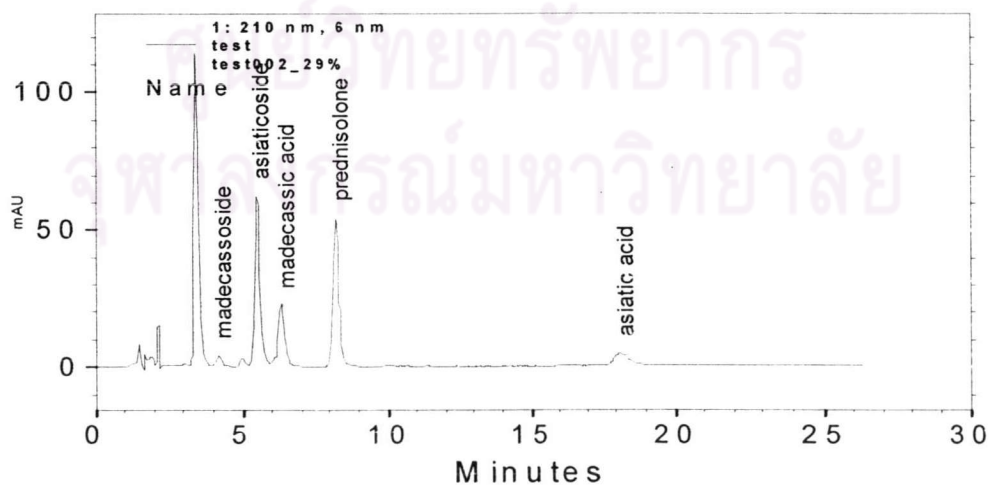
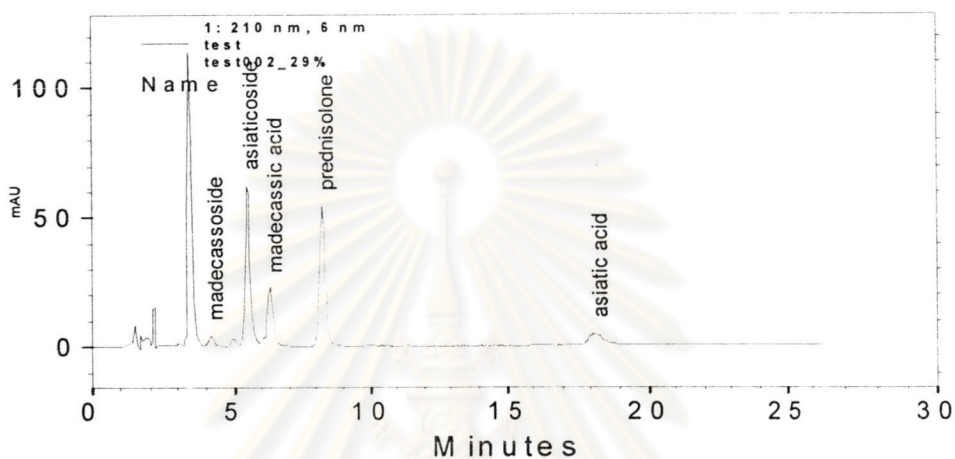


Figure 4.22.2 HPLC chromatogram of standard solution effected by concentration of buffer in mobile phase; 10mM (a) and 20mM (b)

- a) HPLC chromatogram by using the mixture of acetonitrile and phosphate buffer (10 mM) at pH 7.1 as mobile phase



- b) HPLC chromatogram by using the mixture of acetonitrile and phosphate buffer (20 mM) at pH 7.1 as mobile phase

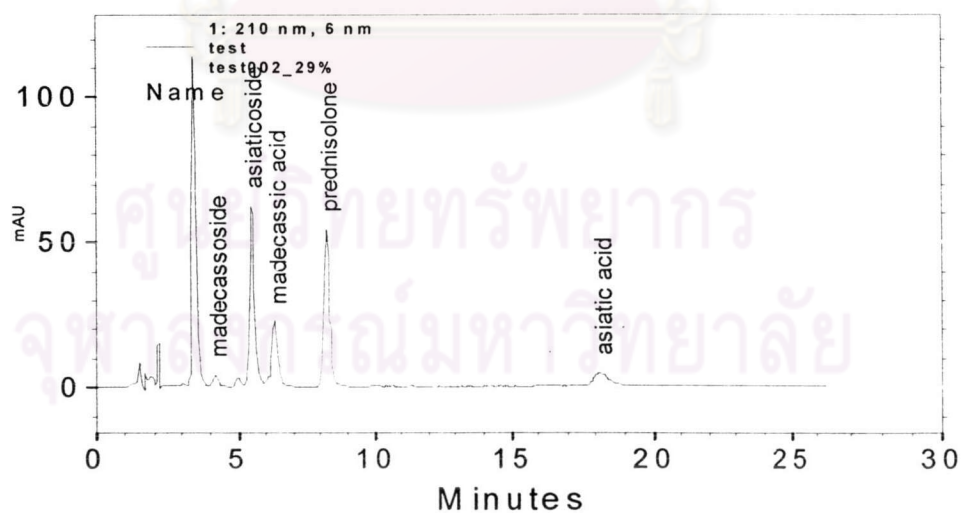
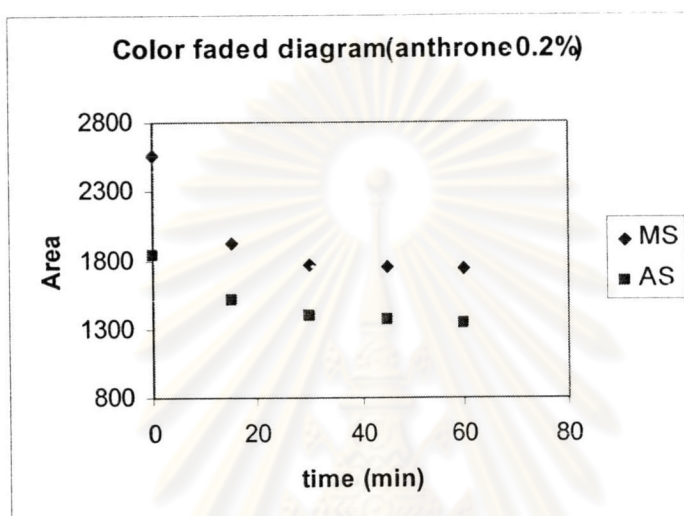


Figure 4.23 Color Faded diagram in TLC chromatogram of madecassoside (MS) and asiaticoside (AS) by using 0.2 % anthrone reagent (a) and Lieberman Burchard 's reagent (b) as spraying reagent

a) Color Faded diagram of MS and AS using 0.2% anthrone reagent



b) Color Faded diagram of MS and AS using Lieberman Burchard's reagent

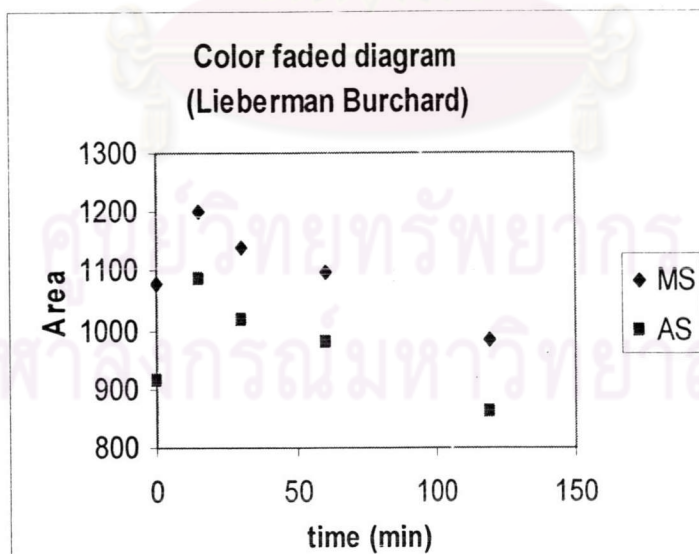
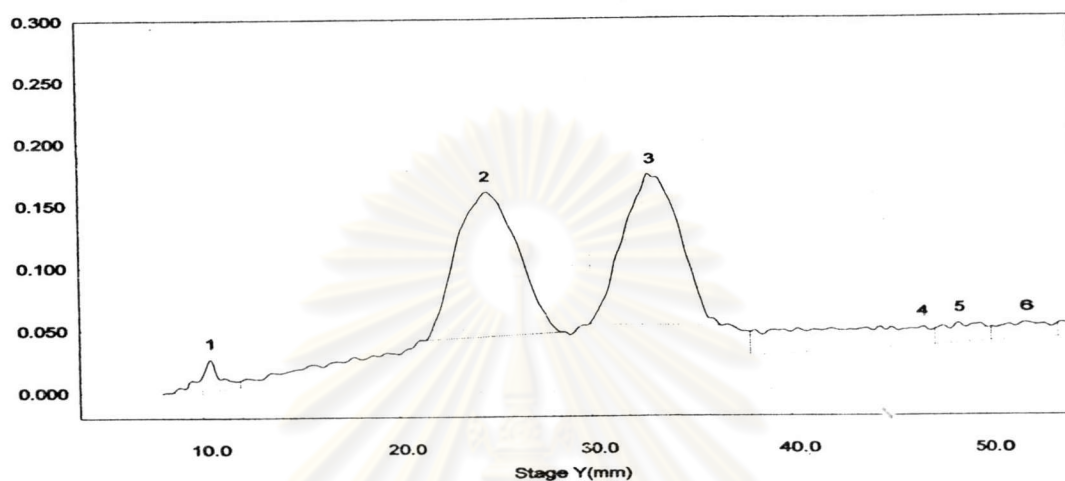


Figure 4.24 TLC chromatogram of standard (A) (MS and AS) and CA extract (B) detected by TLC- densitometer

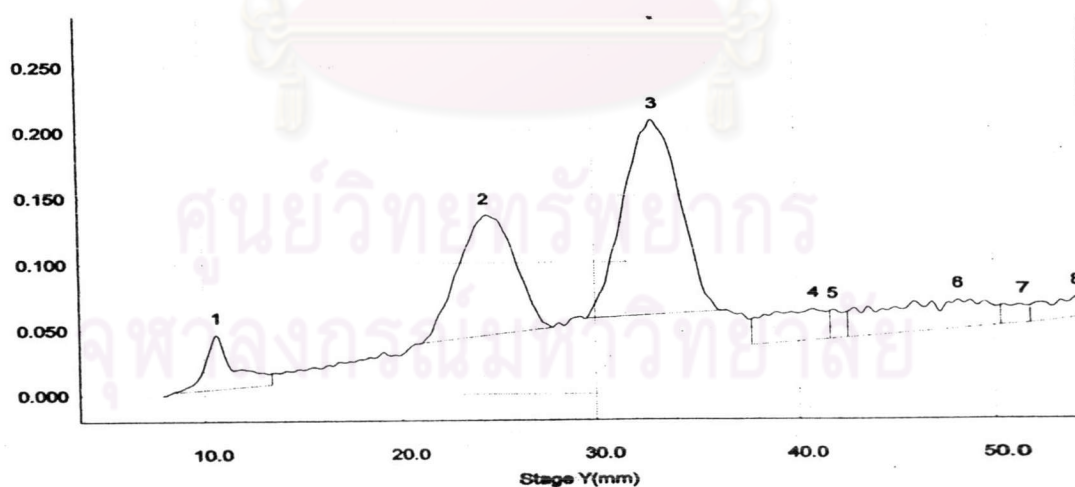
A TLC chromatogram of standard MS and AS



Peak 2 = madecassoside (MS)

3 = asiaticoside (AS)

B TLC chromatogram of CA extract



Peak 2 = madecassoside (MS)

3 = asiaticoside (AS)

Figure 4.25 Linearity and range test of HPLC and TLC analytical methods

Figure 4.25.1 Linearity and range test of HPLC analytical method of madecassoside, asiaticoside, madecassic acid and asiatic acid

Figure 4.25.1.1 Linearity test of HPLC analytical method of madecassoside

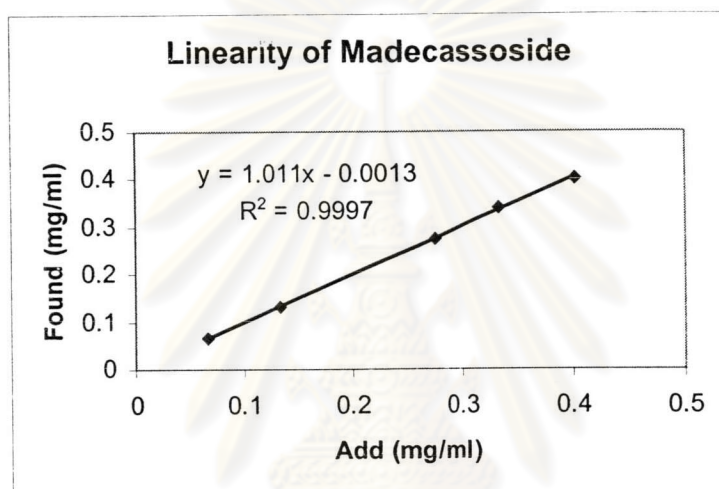


Figure 4.25.1.2 Linearity test of HPLC analytical method of asiaticoside

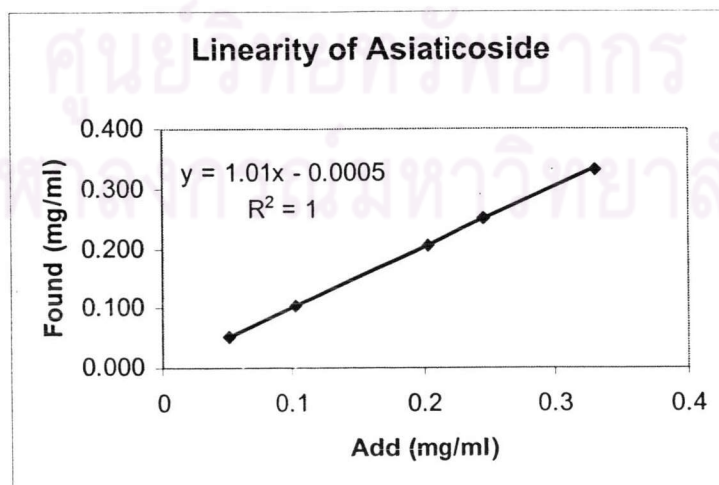


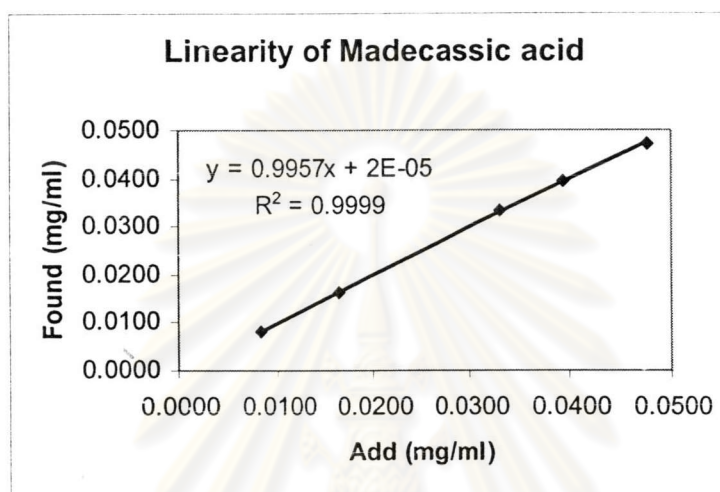
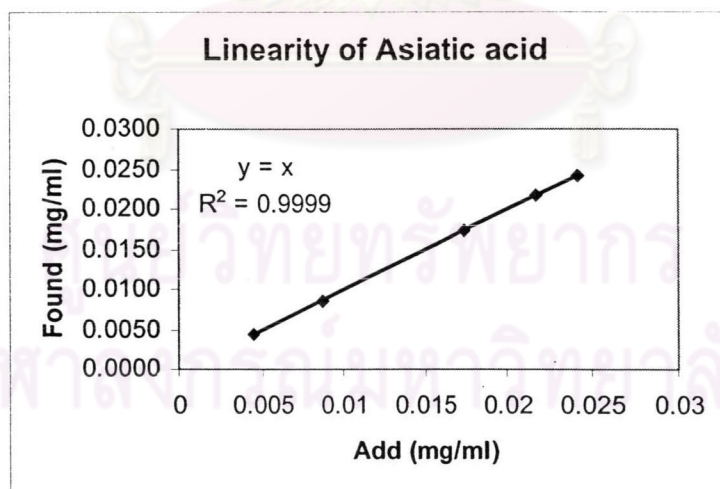
Figure 4.25.1.3 Linearity test of HPLC analytical method of madecassic acid**Figure 4.25.1.4** Linearity test of HPLC analytical method of asiatic acid

Figure 4.25.2 Linearity and range test of TLC analytical method of madecassoside and asiaticoside

Figure 4.26.2.1 Linearity test of TLC analytical method of madecassoside

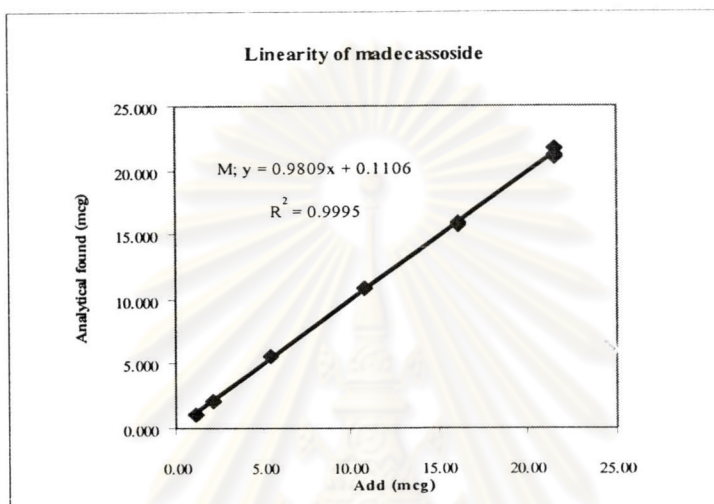


Figure 4.25.2.2 Linearity test of TLC analytical method of asiaticoside

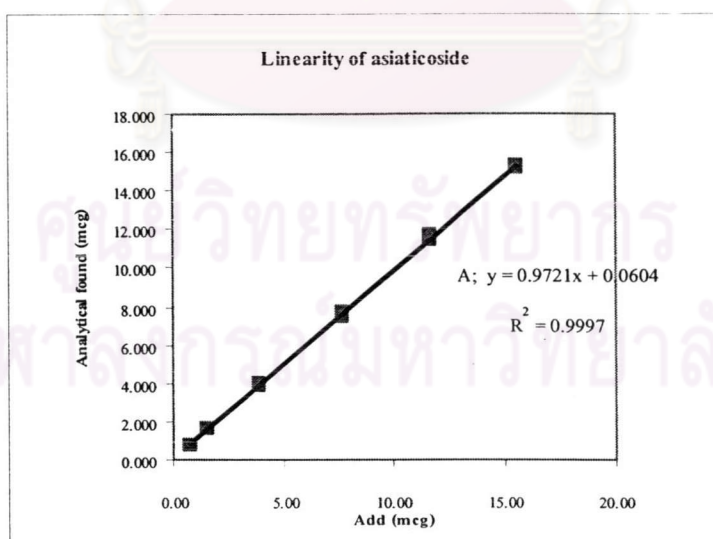
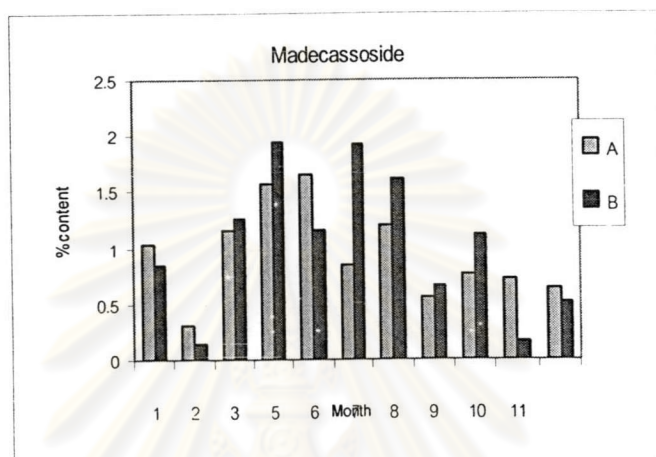
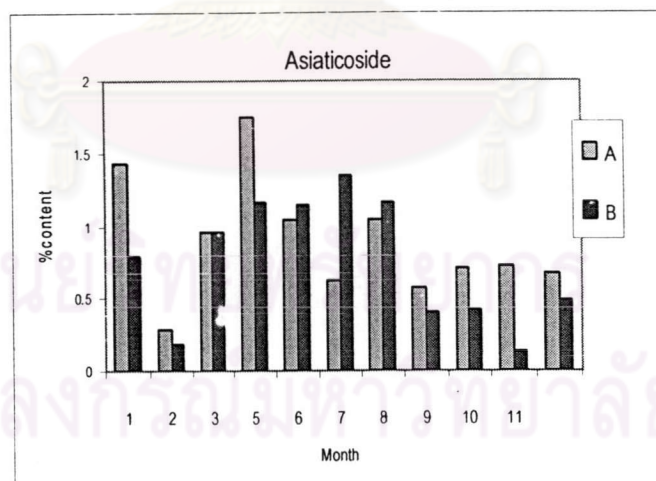


Figure 4.26 Percentage content of madecassoside(a), asiaticoside(b), madecassic acid(c) and asiatic acid(d) in *Centella asiatica* by HPLC analytical method from 2 sources (A and B) in annual period

a Percentage content of madecassoside



b Percentage content of asiaticoside



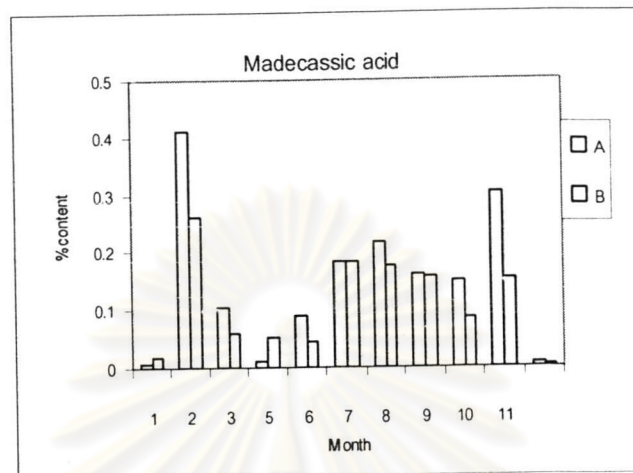
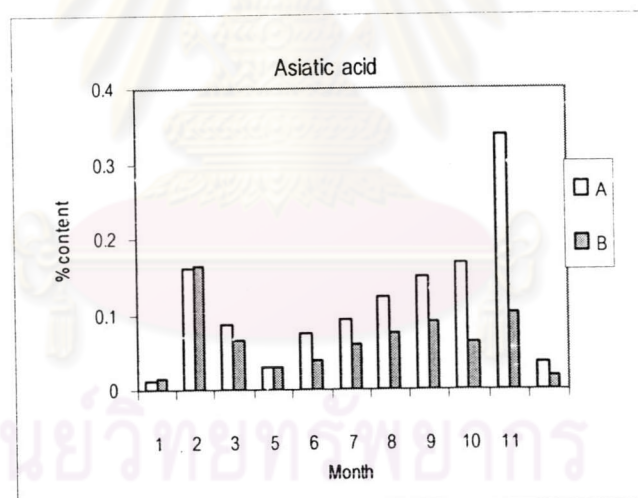
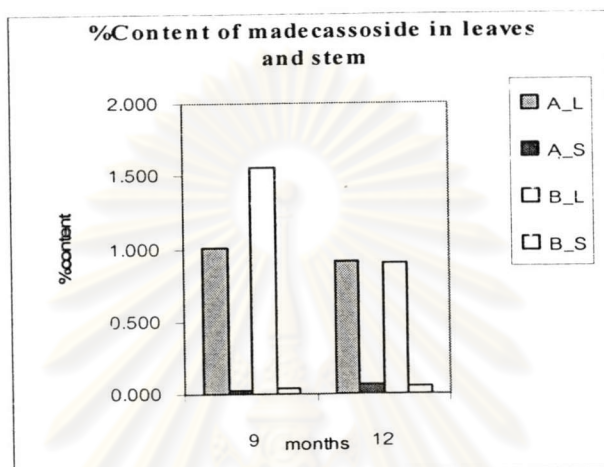
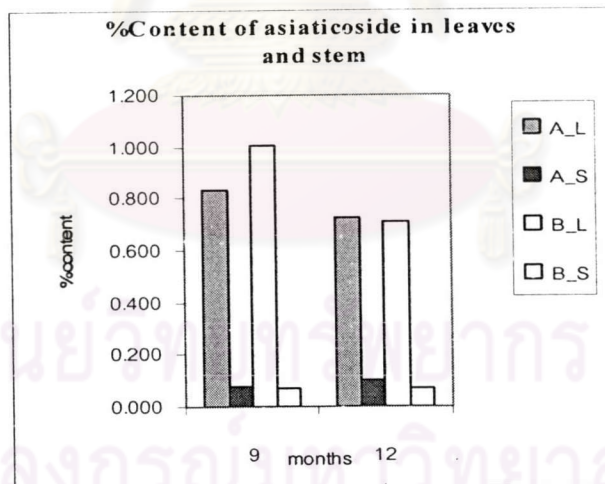
c Percentage content of madecassic acid**d** Percentage content of asiatic acid

Figure 4.27 Percentage content of madecassoside(a), asiaticoside(b), madecassic acid(c) and asiatic acid(d) in *Centella asiatica* by HPLC analytical method in part of leaves and stems from 2 sources (A and B)

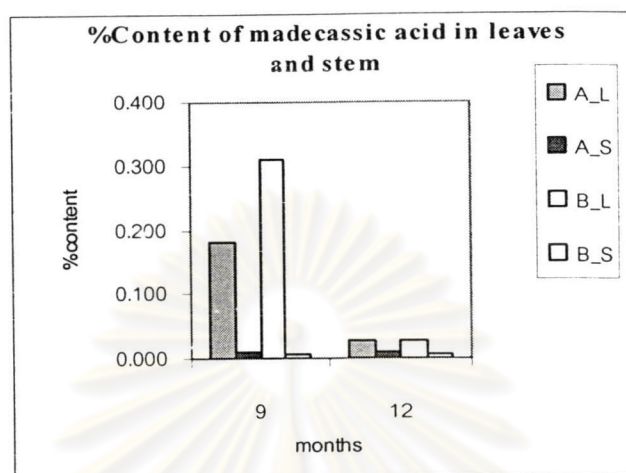
a Percentage content of madecassoside



b Percentage content of asiaticoside



c Percentage content of madecassic acid



d Percentage content of asiatic acid

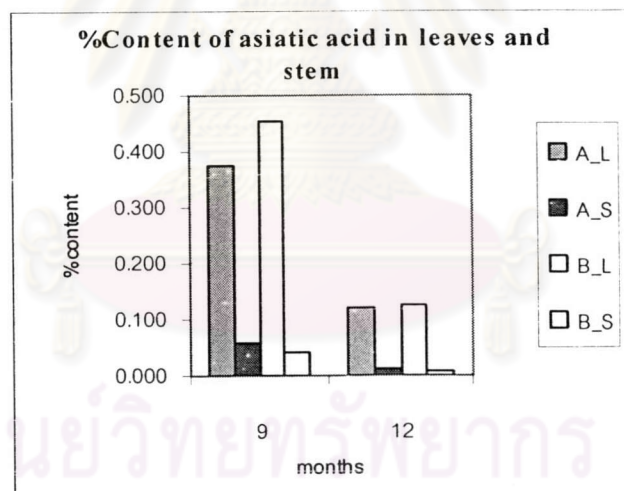
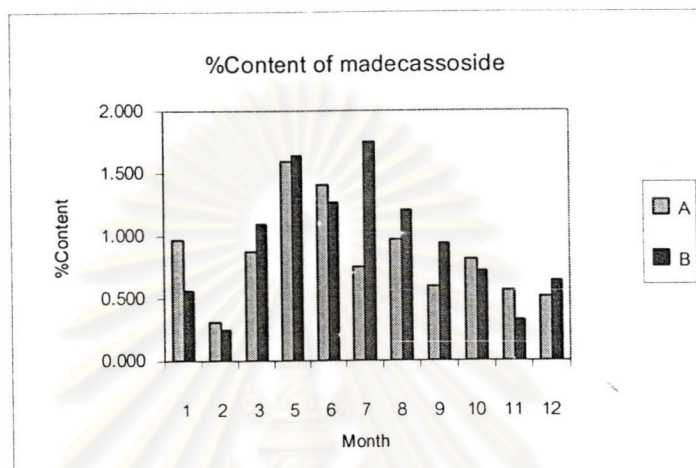


Figure 4.28 Percentage content of madecassoside (a) and asiaticoside (b) in *Centella asiatica* by TLC analytical method in annual period

a Percentage content of madecassoside



b Percentage content of asiaticoside

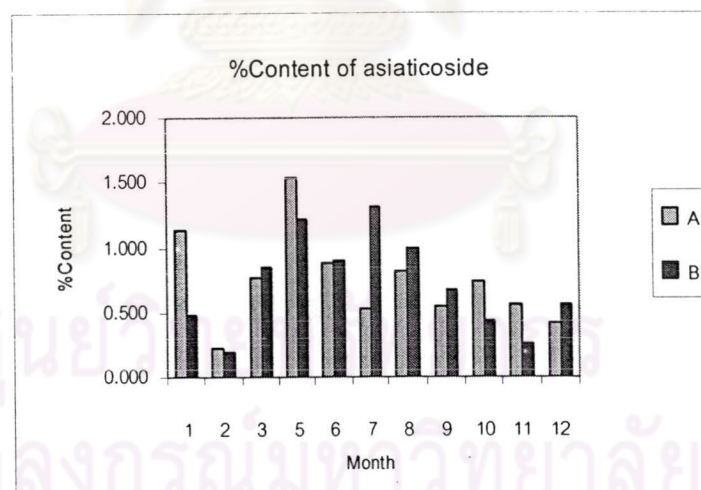
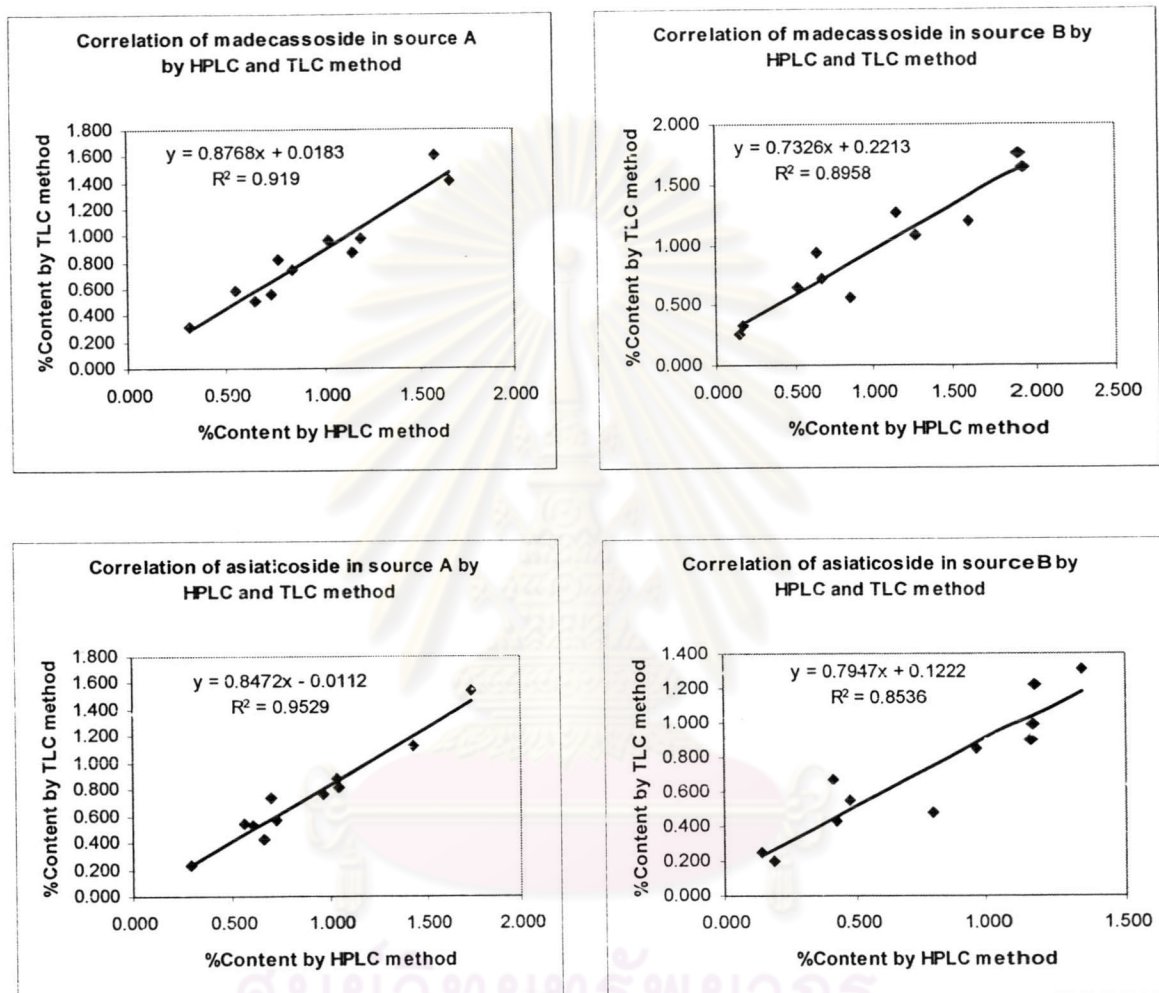


Figure 4.29 Correlation of results of determination of madecassoside and asiaticoside in *Centella asiatica* from 2 sources (A and B) by using HPLC and TLC method



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