APPENDICES

APPENDIX A

APPENDIX A

PERCENTAGE OF YIELD AND MOISTURE CONTENT

1. Yield percent

The yield percent of the *A. incisus* extracts were calculated by using this equation:

% yield = [dryness extract wt (g) / plant material sample wt (g)] x 100

1.1 % yield of *A. incisus* ether extract

% yield = [82.50 (g) / 10 (Kg)] x 100

= 0.825

1.2 % yield of *A. incisus* methanol extract

% yield = [21.90 (g) / 2 (Kg)] x 100

= 1.095

2. Moisture content

The moisture content of the *A. incisus* extracts were measured by using SARTORIUS, model MA30 and then were calculated with this equation:

% moisture content = [(initial wt (g) – final wt (g))/ initial wt (g)] x 100

The conditions and the content of moisture are shown at the following:

Samples	Temperature (^{o}C)	Time (min)	% Moisture content
A. incisus ether extract	105	6.4	7.19±0.96
A. incisus methanol extract	104	9.2	11.36±1.18

APPENDIX B

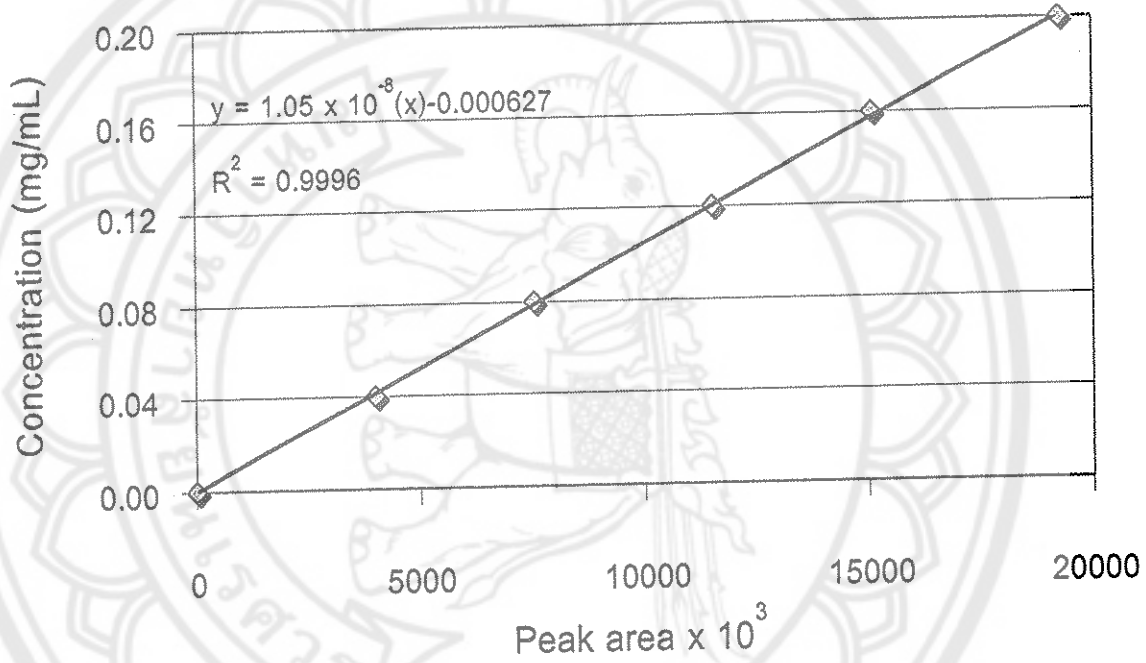
APPENDIX B

THE CALIBRATION CURVE AND CHROMATOGRAMS

1. The Peak Areas of Artocarpin at Various Concentrations of Artocarpin Standard Regarding to the Absorbance at wavelength of 282 nm

Artocarpin concentration (mg/mL)	Absorbance of Peak Areas						Mean (SD)
	Sample No. 1		Sample No. 2		Sample No. 3		
	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	
0.04	3842508	3790361	4113659	3975585	4036709	4127355	3981030 (139767)
0.08	7490936	7489955	7596750	7600830	7543871	7672599	7565824 (71314)
0.12	11273879	11260383	11565596	11765692	11558110	11861358	11547503 (246519)
0.16	14888146	15058158	15169336	15178169	14999706	15241889	15089234 (131944)
0.20	18569366	18716547	19406052	19598293	19396113	19833355	19253288 (501015)

2. The Calibration Curve of Artocarpin Standard



Where; y = Concentration (mg/mL)

x = Peak area

3. The Content of Artocarpin Contained in Ether and Methanol Extract of *A. incisus*'s Heartwood

Samples	Sample No.	Peak area	Artocarpin content (mg)	Mean of artocarpin content (mg±SD)
1.0 mg/mL of *A. incisus* ether extract	1	42785302	4.487	4.519±0.045
	2	43403436	4.552	
	3	42594857	4.473	
	4	43450667	4.562	
0.5 mg/mL of *A. incisus* methanol extract	1	9367997	1.954	1.961±0.005
	2	9427559	1.968	
	3	9403612	1.962	
	4	9381254	1.958	

4. The Calculation of Artocarpin Content of *A. incisus* Extracts

Example:

The concentration (y) can be calculated by using the equation;

$$y = 1.05 \times 10^{-8} (x) - 0.000627$$

if peak area of 1.0 mg/mL *A. incisus* ether extract is 42785302, therefore, the concentration is calculated as follow:

$$y = 1.05 \times 10^{-8} (42785302) - 0.000627$$

$$y = 0.4487$$

The method of artocarpin content calculation are the following:

- 1.0 mg/mL of the ether extract was prepared by dissolving 10.0 mg original weight of the ether extract with methanol. At this concentration, the extract provided the 0.4487 mg/mL of artocarpin.
- Then, to multiply the concentration with 10 mL, the final volume of the ether extract, therefore, the content of artocarpin contained in the ether extract of *A. incisus*'s heartwood;

  = 0.4487 mg/mL x 10 mL

  = 4.487 mg
- The obtained artocarpin was calculated in the term of percent artocarpin content;

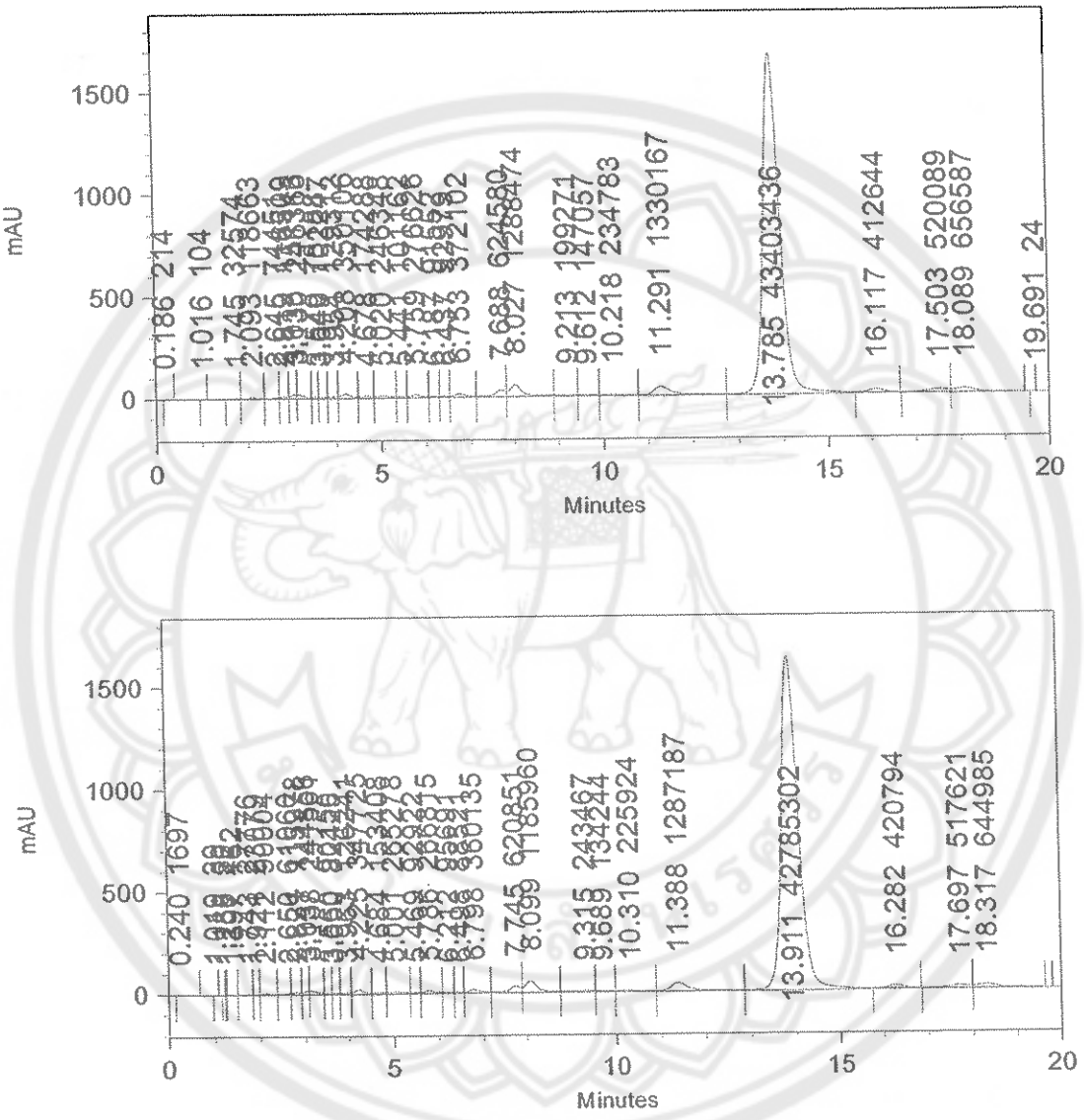
  = (4.487 mg / original weight) x 100

  = (4.487 mg / 10 mg) x 100

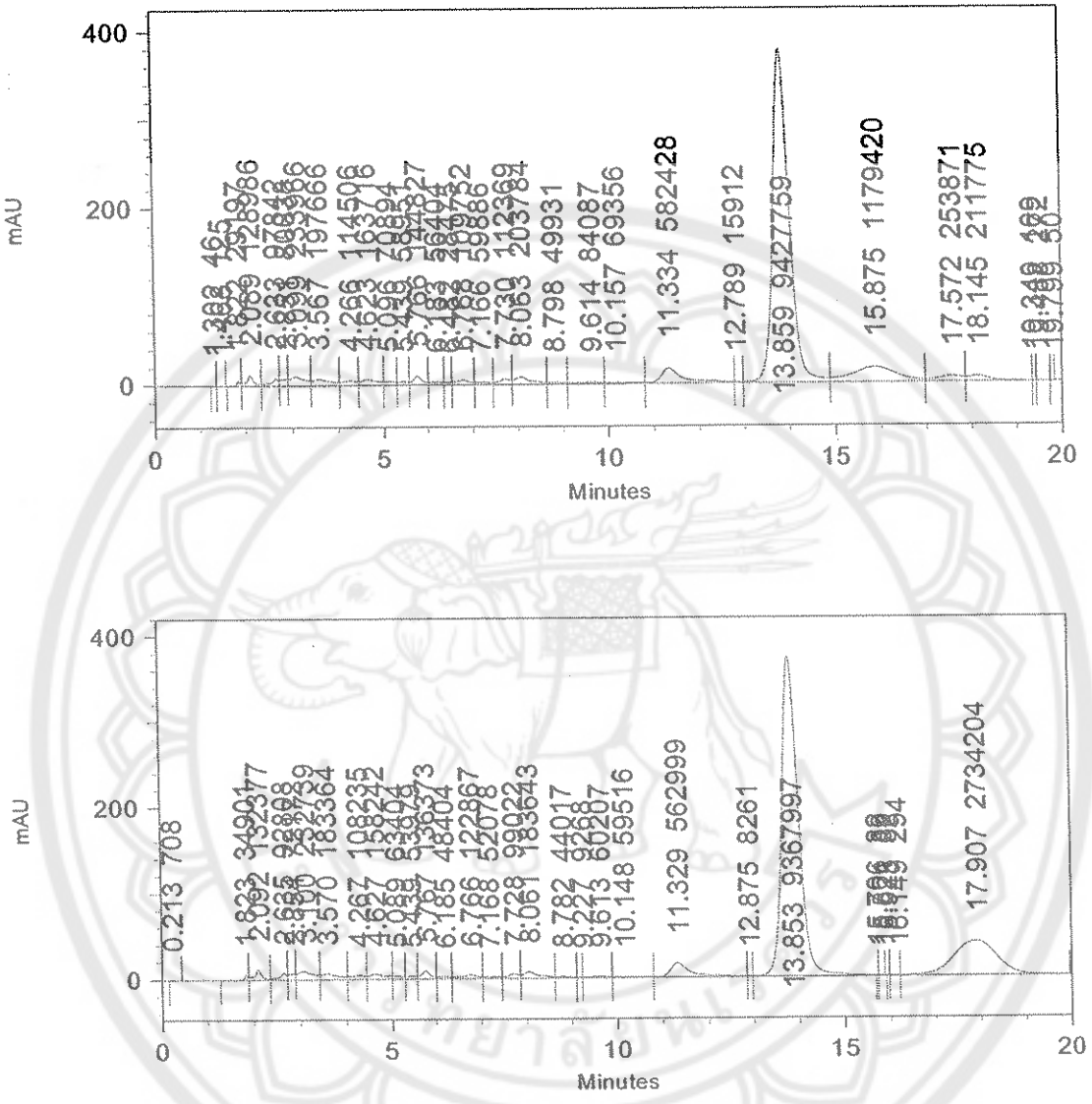
  = 44.87

5. The Chromatograms of *A. incisus* Ether Extract and Methanol Extract

5.1 HPLC Chromatogram of 1.0 mg/mL *A. incisus* Ether Extract



5.2 HPLC Chromatogram of 0.5 mg/mL *A. incisus* Methanol Extract



APPENDIX C

APPENDIX C

THE ABSORBANCES OF DOPACHROME OBTAINED FROM THE REACTION BETWEEN L-DOPA AND TYROSINASE ENZYME AT VARIOUS VOLUME RATIO

1. L-DOPA:Tyrosinase Enzyme (1:1)

1.1 0.85 mM L-DOPA : 426 units/mL tyrosinase enzyme

Time (min)	Sample	Absorbances (490 nm)				
		1	2	3	Mean (S.D.)	Δ
2	Reaction mixture	0.256	0.270	0.259	0.262 (0.007)	0.225
	L-DOPA	0.039	0.037	0.036	0.037 (0.001)	
4	Reaction mixture	0.314	0.324	0.316	0.318 (0.005)	0.280
	L-DOPA	0.039	0.037	0.038	0.038 (0001)	
6	Reaction mixture	0.347	0.355	0.349	0.350 (0.004)	0.312
	L-DOPA	0.039	0.038	0.037	0.038 (0.001)	
8	Reaction mixture	0.368	0.374	0.369	0.370 (0.003)	0.333
	L-DOPA	0.039	0.037	0.036	0.037 (0.002)	

(Cont.)

Time (min)	Sample	Absorbance (490 nm)				
		1	2	3	Mean (S.D.)	Δ
10	Reaction mixture	0.380	0.385	0.382	0.382 (0.003)	0.345
	L-DOPA	0.038	0.036	0.036	0.037 (0.001)	
12	Reaction mixture	0.392	0.393	0.392	0.392 (0.001)	0.355
	L-DOPA	0.038	0.038	0.036	0.037 (0.001)	
14	Reaction mixture	0.399	0.400	0.399	0.399 (0.001)	0.362
	L-DOPA	0.038	0.037	0.036	0.037 (0.001)	
16	Reaction mixture	0.407	0.404	0.404	0.405 (0.002)	0.368
	L-DOPA	0.038	0.037	0.036	0.037 (0.001)	
18	Reaction mixture	0.410	0.409	0.409	0.409 (0.001)	0.372
	L-DOPA	0.038	0.037	0.036	0.037 (0.001)	
20	Reaction mixture	0.424	0.413	0.413	0.417 (0.006)	0.380
	L-DOPA	0.038	0.037	0.036	0.037 (0.001)	

Note: Δ represents to the different absorbance between reaction mixture and L-DOPA

1.2 0.85 mM L-DOPA : 213 units/mL tyrosinase enzyme

Time (min)	Sample	Absorbance (490 nm)				
		1	2	3	Mean (S.D.)	Δ
2	Reaction mixture	0.129	0.129	0.125	0.128 (0.002)	0.098
	L-DOPA	0.035	0.036	0.036	0.036 (0.001)	
4	Reaction mixture	0.165	0.167	0.163	0.165 (0.002)	0.129
	L-DOPA	0.036	0.037	0.036	0.036 (0.001)	
6	Reaction mixture	0.194	0.199	0.194	0.196 (0.003)	0.160
	L-DOPA	0.036	0.036	0.036	0.036 (0.000)	
8	Reaction mixture	0.217	0.222	0.218	0.219 (0.003)	0.183
	L-DOPA	0.036	0.037	0.036	0.036 (0.001)	
10	Reaction mixture	0.235	0.240	0.236	0.237 (0.003)	0.202
	L-DOPA	0.035	0.036	0.035	0.035 (0.001)	

Note: Δ represents to the different absorbance between reaction mixture and L-DOPA

(Cont.)

Time (min)	Sample	Absorbance (490 nm)				
		1	2	3	Mean (S.D.)	Δ
12	Reaction mixture	0.250	0.255	0.252	0.252 (0.003)	0.217
	L-DOPA	0.035	0.036	0.035	0.035 (0.001)	
14	Reaction mixture	0.263	0.268	0.265	0.265 (0.003)	0.230
	L-DOPA	0.035	0.036	0.035	0.035 (0.001)	
16	Reaction mixture	0.273	0.279	0.276	0.276 (0.003)	0.241
	L-DOPA	0.035	0.036	0.035	0.035 (0.001)	
18	Reaction mixture	0.282	0.287	0.284	0.284 (0.003)	0.249
	L-DOPA	0.035	0.036	0.035	0.035 (0.001)	
20	Reaction mixture	0.288	0.293	0.291	0.291 (0.003)	0.256
	L-DOPA	0.035	0.036	0.035	0.035 (0.001)	

Note: Δ represents to the different absorbance between reaction mixture and L-DOPA

1.3 0.85 mM L-DOPA : 106.5 units/mL tyrosinase enzyme

Time (min)	Sample	Absorbance (490 nm)				
		1	2	3	Mean (S.D.)	Δ
2	Reaction mixture	0.072	0.072	0.067	0.070 (0.003)	0.032
	L-DOPA	0.036	0.040	0.039	0.038 (0.002)	
4	Reaction mixture	0.087	0.083	0.088	0.086 (0.003)	0.048
	L-DOPA	0.036	0.040	0.039	0.038 (0.002)	
6	Reaction mixture	0.099	0.100	0.097	0.099 (0.002)	0.061
	L-DOPA	0.036	0.040	0.039	0.038 (0.002)	
8	Reaction mixture	0.110	0.111	0.108	0.110 (0.002)	0.072
	L-DOPA	0.036	0.040	0.039	0.038 (0.002)	
10	Reaction mixture	0.118	0.119	0.116	0.118 (0.002)	0.080
	L-DOPA	0.036	0.039	0.038	0.038 (0.002)	

Note: Δ represents to the different absorbance between reaction mixture and L-DOPA

(Cont.)

Time (min)	Sample	Absorbance (490 nm)				
		1	2	3	Mean (S.D.)	Δ
12	Reaction mixture	0.125	0.126	0.124	0.125 (0.001)	0.087
	L-DOPA	0.036	0.039	0.038	0.038 (0.002)	
14	Reaction mixture	0.131	0.132	0.131	0.131 (0.001)	0.093
	L-DOPA	0.036	0.039	0.038	0.038 (0.002)	
16	Reaction mixture	0.137	0.137	0.136	0.137 (0.001)	0.099
	L-DOPA	0.036	0.039	0.038	0.038 (0.002)	
18	Reaction mixture	0.141	0.141	0.141	0.141 (0.001)	0.103
	L-DOPA	0.036	0.039	0.038	0.038 (0.002)	
20	Reaction mixture	0.148	0.144	0.144	0.145 (0.002)	0.107
	L-DOPA	0.036	0.039	0.038	0.038 (0.002)	

Note: Δ represents to the different absorbance between reaction mixture and L-DOPA

2. L-DOPA:Tyrosinase Enzyme (1:2)

2.1 0.85 mM L-DOPA : 436 units/mL tyrosinase enzyme

Time (min)	Sample	Absorbance (490 nm)				
		1	2	3	Mean (S.D.)	Δ
2	Reaction mixture	0.203	0.178	0.177	0.186 (0.015)	0.146
	L-DOPA	0.043	0.037	0.039	0.040 (0.003)	
4	Reaction mixture	0.23	0.197	0.196	0.208 (0.019)	0.168
	L-DOPA	0.043	0.037	0.039	0.040 (0.003)	
6	Reaction mixture	0.236	0.202	0.201	0.213 (0.020)	0.173
	L-DOPA	0.043	0.037	0.039	0.040 (0.003)	
8	Reaction mixture	0.24	0.206	0.206	0.217 (0.020)	0.178
	L-DOPA	0.043	0.037	0.039	0.040 (0.003)	
10	Reaction mixture	0.244	0.209	0.209	0.221 (0.020)	0.181
	L-DOPA	0.043	0.037	0.039	0.040 (0.003)	

Note: Δ represents to the different absorbance between reaction mixture and L-DOPA

(Cont.)

Time (min)	Sample	Absorbance (490 nm)				
		1	2	3	Mean (S.D.)	Δ
12	Reaction mixture	0.248	0.212	0.213	0.224 (0.021)	0.185
	L-DOPA	0.043	0.037	0.039	0.040 (0.003)	
14	Reaction mixture	0.25	0.215	0.216	0.227 (0.020)	0.187
	L-DOPA	0.043	0.037	0.039	0.040 (0.003)	
16	Reaction mixture	0.254	0.219	0.219	0.231 (0.020)	0.191
	L-DOPA	0.043	0.037	0.039	0.040 (0.003)	
18	Reaction mixture	0.258	0.222	0.223	0.234 (0.021)	0.195
	L-DOPA	0.043	0.037	0.039	0.040 (0.003)	
20	Reaction mixture	0.262	0.226	0.227	0.238 (0.021)	0.199
	L-DOPA	0.043	0.037	0.039	0.040 (0.003)	

Note: Δ represents to the different absorbance between reaction mixture and L-DOPA

2.2 0.85 mM L-DOPA : 213 units/mL tyrosinase enzyme

Time (min)	Sample	Absorbance (490 nm)				
		1	2	3	Mean (S.D.)	Δ
2	Reaction mixture	0.095	0.092	0.087	0.091 (0.004)	0.055
	L-DOPA	0.034	0.037	0.039	0.037 (0.003)	
4	Reaction mixture	0.135	0.133	0.130	0.133 (0.003)	0.096
	L-DOPA	0.034	0.037	0.038	0.036 (0.002)	
6	Reaction mixture	0.152	0.15	0.147	0.150 (0.003)	0.113
	L-DOPA	0.034	0.037	0.038	0.036 (0.002)	
8	Reaction mixture	0.164	0.161	0.159	0.161 (0.003)	0.125
	L-DOPA	0.034	0.037	0.038	0.036 (0.002)	
10	Reaction mixture	0.174	0.171	0.168	0.171 (0.003)	0.135
	L-DOPA	0.034	0.037	0.038	0.036 (0.002)	

Note: Δ represents to the different absorbance between reaction mixture and L-DOPA

(Cont.)

Time (min)	Sample	Absorbance (490 nm)				
		1	2	3	Mean (S.D.)	Δ
12	Reaction mixture	0.181	0.178	0.175	0.178 (0.003)	0.142
	L-DOPA	0.034	0.037	0.038	0.036 (0.002)	
14	Reaction mixture	0.186	0.183	0.181	0.183 (0.003)	0.147
	L-DOPA	0.034	0.037	0.038	0.036 (0.002)	
16	Reaction mixture	0.191	0.187	0.185	0.188 (0.003)	0.151
	L-DOPA	0.034	0.037	0.038	0.036 (0.002)	
18	Reaction mixture	0.195	0.191	0.189	0.192 (0.003)	0.155
	L-DOPA	0.034	0.037	0.038	0.036 (0.002)	
20	Reaction mixture	0.198	0.193	0.191	0.194 (0.004)	0.158
	L-DOPA	0.034	0.037	0.038	0.036 (0.003)	

Note: Δ represents to the different absorbance between reaction mixture and L-DOPA

2.3 0.85 mM L-DOPA : 106.5 units/mL tyrosinase enzyme

Time (min)	Sample	Absorbance (490 nm)				
		1	2	3	Mean (S.D.)	Δ
2	Reaction mixture	0.056	0.055	0.050	0.054 (0.003)	0.010
	L-DOPA	0.043	0.042	0.045	0.043 (0.002)	
4	Reaction mixture	0.077	0.075	0.075	0.076 (0.001)	0.032
	L-DOPA	0.045	0.045	0.042	0.044 (0.002)	
6	Reaction mixture	0.086	0.084	0.086	0.085 (0.001)	0.042
	L-DOPA	0.043	0.042	0.045	0.043 (0.002)	
8	Reaction mixture	0.094	0.093	0.090	0.092 (0.002)	0.049
	L-DOPA	0.043	0.041	0.045	0.043 (0.002)	
10	Reaction mixture	0.100	0.099	0.097	0.099 (0.002)	0.056
	L-DOPA	0.043	0.041	0.045	0.043 (0.002)	

Note: Δ represents to the different absorbance between reaction mixture and L-DOPA

(Cont.)

Time (min)	Sample	Absorbance (490 nm)				
		1	2	3	Mean (S.D.)	Δ
12	Reaction mixture	0.105	0.104	0.102	0.104 (0.002)	0.061
	L-DOPA	0.043	0.041	0.045	0.043 (0.002)	
14	Reaction mixture	0.109	0.108	0.107	0.108 (0.001)	0.065
	L-DOPA	0.043	0.041	0.045	0.043 (0.002)	
16	Reaction mixture	0.112	0.111	0.109	0.111 (0.002)	0.068
	L-DOPA	0.043	0.041	0.045	0.043 (0.002)	
18	Reaction mixture	0.115	0.113	0.111	0.113 (0.002)	0.070
	L-DOPA	0.043	0.042	0.045	0.043 (0.002)	
20	Reaction mixture	0.117	0.115	0.118	0.117 (0.002)	0.073
	L-DOPA	0.043	0.042	0.045	0.043 (0.002)	

Note: Δ represents to the different absorbance between reaction mixture and L-DOPA

3. L-DOPA:Tyrosinase Enzyme (2:1)

3.1 0.85 mM L-DOPA : 436 units/mL tyrosinase enzyme

Time (min)	Sample	Absorbance (490 nm)				
		1	2	3	Mean (S.D.)	Δ
2	Reaction mixture	0.199	0.206	0.198	0.201 (0.004)	0.170
	L-DOPA	0.038	0.041	0.041	0.040 (0.002)	
4	Reaction mixture	0.236	0.242	0.238	0.239 (0.003)	0.199
	L-DOPA	0.038	0.041	0.041	0.040 (0.002)	
6	Reaction mixture	0.267	0.273	0.268	0.269 (0.003)	0.229
	L-DOPA	0.038	0.041	0.041	0.040 (0.002)	
8	Reaction mixture	0.29	0.296	0.292	0.293 (0.003)	0.253
	L-DOPA	0.038	0.041	0.041	0.040 (0.002)	
10	Reaction mixture	0.307	0.313	0.309	0.310 (0.003)	0.270
	L-DOPA	0.038	0.041	0.040	0.040 (0.002)	

Note: Δ represents to the different absorbance between reaction mixture and L-DOPA

(Cont.)

Time (min)	Sample	Absorbance (490 nm)				
		1	2	3	Mean (S.D.)	Δ
12	Reaction mixture	0.32	0.325	0.321	0.322 (0.003)	0.282
	L-DOPA	0.038	0.04	0.041	0.040 (0.002)	
14	Reaction mixture	0.329	0.335	0.331	0.332 (0.003)	0.292
	L-DOPA	0.038	0.041	0.040	0.040 (0.002)	
16	Reaction mixture	0.337	0.342	0.338	0.339 (0.003)	0.299
	L-DOPA	0.038	0.041	0.040	0.040 (0.002)	
18	Reaction mixture	0.342	0.347	0.343	0.344 (0.003)	0.304
	L-DOPA	0.038	0.041	0.040	0.040 (0.002)	
20	Reaction mixture	0.346	0.351	0.347	0.348 (0.003)	0.308
	L-DOPA	0.038	0.041	0.041	0.040 (0.002)	

Note: Δ represents to the different absorbance between reaction mixture and L-DOPA

3.2 0.85 mM L-DOPA : 213 units/mL tyrosinase enzyme

Time (min)	Sample	Absorbance (490 nm)				
		1	2	3	Mean (S.D.)	Δ
2	Reaction mixture	0.094	0.092	0.091	0.092 (0.002)	0.053
	L-DOPA	0.036	0.038	0.042	0.039 (0.003)	
4	Reaction mixture	0.115	0.112	0.111	0.113 (0.002)	0.075
	L-DOPA	0.036	0.037	0.042	0.038 (0.003)	
6	Reaction mixture	0.130	0.131	0.128	0.130 (0.002)	0.091
	L-DOPA	0.037	0.038	0.042	0.039 (0.003)	
8	Reaction mixture	0.146	0.143	0.140	0.143 (0.003)	0.105
	L-DOPA	0.036	0.037	0.042	0.038 (0.003)	
10	Reaction mixture	0.158	0.154	0.151	0.154 (0.004)	0.115
	L-DOPA	0.036	0.038	0.042	0.039 (0.003)	

Note: Δ represents to the different absorbance between reaction mixture and L-DOPA

(Cont.)

Time (min)	Sample	Absorbance (490 nm)				
		1	2	3	Mean (S.D.)	Δ
12	Reaction mixture	0.166	0.163	0.160	0.163 (0.003)	0.124
	L-DOPA	0.036	0.038	0.042	0.039 (0.003)	
14	Reaction mixture	0.174	0.171	0.167	0.171 (0.004)	0.132
	L-DOPA	0.037	0.038	0.042	0.039 (0.003)	
16	Reaction mixture	0.180	0.177	0.172	0.176 (0.004)	0.137
	L-DOPA	0.038	0.038	0.042	0.039 (0.002)	
18	Reaction mixture	0.186	0.183	0.177	0.182 (0.005)	0.143
	L-DOPA	0.036	0.038	0.042	0.039 (0.003)	
20	Reaction mixture	0.189	0.186	0.181	0.185 (0.004)	0.146
	L-DOPA	0.038	0.038	0.042	0.039 (0.002)	

Note: Δ represents to the different absorbance between reaction mixture and L-DOPA

3.3 0.85 mM L-DOPA : 106.5 units/mL tyrosinase enzyme

Time (min)	Sample	Absorbance (490 nm)				
		1	2	3	Mean (S.D.)	Δ
2	Reaction mixture	0.054	0.051	0.05	0.052 (0.002)	0.015
	L-DOPA	0.034	0.039	0.038	0.037 (0.003)	
4	Reaction mixture	0.063	0.061	0.061	0.062 (0.001)	0.025
	L-DOPA	0.034	0.038	0.038	0.037 (0.002)	
6	Reaction mixture	0.071	0.069	0.069	0.070 (0.001)	0.033
	L-DOPA	0.034	0.038	0.038	0.037 (0.002)	
8	Reaction mixture	0.076	0.075	0.076	0.076 (0.001)	0.039
	L-DOPA	0.034	0.038	0.038	0.037 (0.002)	
10	Reaction mixture	0.080	0.08	0.081	0.080 (0.001)	0.043
	L-DOPA	0.034	0.038	0.038	0.037 (0.002)	

Note: Δ represents to the different absorbance between reaction mixture and L-DOPA

(Cont.)

Time (min)	Sample	Absorbance (490 nm)				
		1	2	3	Mean (S.D.)	Δ
12	Reaction mixture	0.085	0.084	0.086	0.085 (0.001)	0.048
	L-DOPA	0.034	0.038	0.038	0.037 (0.002)	
14	Reaction mixture	0.087	0.087	0.088	0.087 (0.001)	0.050
	L-DOPA	0.034	0.038	0.038	0.037 (0.002)	
16	Reaction mixture	0.090	0.090	0.092	0.091 (0.001)	0.054
	L-DOPA	0.034	0.038	0.038	0.037 (0.002)	
18	Reaction mixture	0.092	0.093	0.093	0.093 (0.001)	0.056
	L-DOPA	0.034	0.038	0.038	0.037 (0.002)	
20	Reaction mixture	0.094	0.093	0.095	0.094 (0.001)	0.057
	L-DOPA	0.034	0.038	0.038	0.037 (0.002)	

Note: Δ represents to the different absorbance between reaction mixture and L-DOPA

APPENDIX D

APPENDIX D

THE % TYROSINASE INHIBITION OF *A. INCISUS* EXTRACT

1. *A. incisus* Ether Extract / Propylene Glycol

Concentration (µg/ml)	Sample No.	Absorbance at 490 nm of wells				% Tyrosinase inhibition	
		A	B	C	D	[(A-B)-(C-D)] / (A-B) x 100	Mean (SD)
0.5	1	0.120	0.035	0.228	0.144	1.18	1.18
	2	0.121	0.036	0.227	0.143	1.18	
	3	0.121	0.035	0.232	0.144	-2.33	
1	1	0.120	0.036	0.225	0.143	2.38	2.77 (5.81)
	2	0.120	0.036	0.224	0.143	3.57	
	3	0.120	0.035	0.226	0.143	2.35	
5	1	0.120	0.035	0.220	0.147	14.12	13.68 (0.77)
	2	0.122	0.036	0.219	0.144	12.79	
	3	0.120	0.035	0.220	0.147	14.12	

(Cont.)

Concentration (µg/ml)	Sample No.	Absorbance at 490 nm of wells				% Tyrosinase inhibition	
		A	B	C	D	[(A-B)-(C-D)] / (A-B) x 100	Mean (SD)
10	1	0.122	0.035	0.212	0.150	28.74	28.05 (1.92)
	2	0.123	0.035	0.212	0.150	29.55	
	3	0.120	0.035	0.212	0.149	25.88	
50	1	0.121	0.035	0.193	0.154	54.65	54.48 (0.92)
	2	0.121	0.036	0.192	0.154	55.29	
	3	0.121	0.035	0.193	0.153	53.49	
100	1	0.120	0.035	0.185	0.160	70.59	74.20 (3.66)
	2	0.121	0.035	0.180	0.161	77.91	
	3	0.120	0.035	0.182	0.160	74.12	
250	1	0.120	0.035	0.181	0.158	72.94	73.83 (0.78)
	2	0.120	0.035	0.180	0.158	74.12	
	3	0.121	0.035	0.181	0.159	74.42	
500	1	0.120	0.035	0.182	0.159	73.86	74.13 (0.28)
	2	0.121	0.034	0.182	0.160	74.42	
	3	0.121	0.035	0.182	0.160	74.12	

2. *A. incisus* Ether Extract / Dimethyl Sulfoxide

Concentration (µg/ml)	Sample No.	Absorbance at 490 nm of wells				% Tyrosinase inhibition	
		A	B	C	D	[(A-B)-(C-D)] / (A-B) x 100	Mean (SD)
0.5	1	0.121	0.037	0.105	0.037	19.04	20.19 (1.39)
	2	0.122	0.037	0.106	0.037	18.82	
	3	0.124	0.035	0.106	0.036	21.34	
1	1	0.124	0.036	0.107	0.042	26.13	26.55 (1.13)
	2	0.125	0.036	0.108	0.041	24.71	
	3	0.124	0.035	0.105	0.04	26.96	
5	1	0.125	0.036	0.097	0.045	41.57	42.76 (1.26)
	2	0.124	0.036	0.096	0.045	42.04	
	3	0.126	0.035	0.097	0.046	43.95	
10	1	0.134	0.036	0.111	0.061	48.97	48.29 (0.93)
	2	0.12	0.035	0.106	0.063	49.41	
	3	0.118	0.034	0.109	0.065	47.61	

(Cont.)

Concentration (μg/ml)	Sample No.	Absorbance at 490 nm of wells				% Tyrosinase inhibition	
		A	B	C	D	[(A-B)-(C-D)] / (A-B) x 100	Mean (SD)
50	1	0.122	0.036	0.125	0.098	68.60	67.44 (2.24)
	2	0.125	0.036	0.122	0.096	70.78	
	3	0.123	0.034	0.124	0.094	66.29	
100	1	0.122	0.035	0.129	0.106	73.56	73.40 (1.42)
	2	0.123	0.036	0.128	0.107	75.86	
	3	0.122	0.036	0.128	0.105	73.25	
250	1	0.122	0.034	0.131	0.113	79.54	81.25 (1.83)
	2	0.126	0.035	0.133	0.117	82.41	
	3	0.124	0.036	0.131	0.116	82.95	
500	1	0.124	0.036	0.136	0.122	84.09	85.06 (4.24)
	2	0.122	0.036	0.139	0.12	77.90	
	3	0.122	0.036	0.138	0.126	86.04	

3. Kojic Acid / Propylene Glycol

Concentration (µg/ml)	Sample No.	Absorbance at 490 nm of wells				% Tyrosinase inhibition	
		A	B	C	D	[(A-B)-(C-D)] / (A-B) x 100	Mean (SD)
1	1	0.121	0.035	0.103	0.036	22.09	22.75 (0.73)
	2	0.120	0.036	0.100	0.035	22.62	
	3	0.120	0.035	0.099	0.034	23.53	
2	1	0.121	0.035	0.092	0.035	33.72	32.69 (1.18)
	2	0.121	0.035	0.093	0.034	31.40	
	3	0.120	0.035	0.092	0.035	32.94	
5	1	0.120	0.034	0.085	0.035	41.86	41.92 (0.58)
	2	0.122	0.035	0.085	0.035	42.53	
	3	0.121	0.034	0.086	0.035	41.38	
10	1	0.120	0.034	0.076	0.035	52.33	52.51 (0.32)
	2	0.120	0.034	0.076	0.035	52.33	
	3	0.121	0.034	0.076	0.035	52.87	

(Cont.)

Concentration (µg/ml)	Sample No.	Absorbance at 490 nm of wells				% Tyrosinase inhibition	
		A	B	C	D	[(A-B)-(C-D)] / (A-B) x 100	Mean (SD)
20	1	0.121	0.035	0.064	0.034	65.12	63.95 (1.43)
	2	0.121	0.034	0.065	0.034	64.37	
	3	0.120	0.035	0.067	0.035	62.35	
30	1	0.121	0.034	0.050	0.036	83.91	85.28 (1.61)
	2	0.120	0.034	0.048	0.035	84.88	
	3	0.120	0.035	0.046	0.035	87.06	
40	1	0.121	0.034	0.038	0.035	96.55	94.64 (1.76)
	2	0.121	0.034	0.040	0.035	94.25	
	3	0.120	0.033	0.041	0.035	93.10	
50	1	0.121	0.035	0.038	0.035	96.51	94.92 (1.38)
	2	0.120	0.035	0.040	0.035	94.12	
	3	0.120	0.035	0.040	0.035	94.12	

4. Kojic Acid / Dimethyl Sulfoxide

Concentration (µg/ml)	Sample No.	Absorbance at 490 nm of wells				% Tyrosinase inhibition	
		A	B	C	D	[(A-B)-(C-D)] / (A-B) x 100	Mean (SD)
0.1	1	0.134	0.036	0.128	0.037	7.14	7.05 (0.08)
	2	0.135	0.035	0.130	0.037	7.00	
	3	0.135	0.035	0.129	0.036	7.00	
0.5	1	0.135	0.036	0.128	0.036	7.07	7.02 (0.04)
	2	0.135	0.035	0.128	0.035	7.00	
	3	0.135	0.035	0.129	0.036	7.00	
1.0	1	0.134	0.036	0.125	0.036	9.18	9.12 (0.11)
	2	0.134	0.036	0.127	0.038	9.18	
	3	0.135	0.035	0.128	0.037	9.00	
5.0	1	0.134	0.036	0.108	0.036	26.53	26.54 (2.33)
	2	0.132	0.035	0.106	0.037	28.87	
	3	0.132	0.037	0.108	0.036	24.21	

(Cont.)

Concentration (μg/ml)	Sample No.	Absorbance at 490 nm of wells				% Tyrosinase inhibition	
		A	B	C	D	[(A-B)-(C-D)] / (A-B) x 100	Mean (SD)
10.0	1	0.132	0.036	0.070	0.038	66.67	66.79 (0.53)
	2	0.130	0.035	0.068	0.037	67.37	
	3	0.135	0.037	0.070	0.037	66.33	
25.0	1	0.125	0.036	0.055	0.036	78.65	81.94 (3.16)
	2	0.125	0.035	0.052	0.036	82.22	
	3	0.128	0.035	0.049	0.035	84.95	
250.0	1	0.136	0.034	0.040	0.036	96.08	95.59 (0.55)
	2	0.135	0.035	0.040	0.035	95.00	
	3	0.129	0.036	0.040	0.036	95.70	
500.0	1	0.135	0.036	0.040	0.036	95.96	96.08 (0.95)
	2	0.138	0.035	0.039	0.036	97.09	
	3	0.140	0.036	0.041	0.036	95.19	

APPENDIX E

APPENDIX E

DETERMINATION OF CELL VIABILITY AND MELANIN CONTENT BY USING CELL CULTURE MODEL

1. The Cell Number of Time Course Study

Time (day)	Cell number x 10^5			Mean (SD)
	Sample 1	Sample 2	Sample 3	
0	1.0	1.0	1.0	1.00 (0.00)
1	2.4	2.2	2.6	2.40 (0.20)
2	5.2	4.9	5.1	5.07 (0.15)
3	12.6	12.4	12.6	12.53 (0.12)
4	24.8	24.6	24.8	24.73 (0.12)
5	28.1	28.5	28.0	28.20 (0.26)
6	27.8	28.2	27.9	27.97 (0.21)
7	27.6	28.1	27.8	27.83 (0.25)

2. The Effect of *A. incisus* Extract and Artocarpin on Cells Number

Sample	Day 1				Day 2				Day 3			
	1	2	3	Mean (SD)	1	2	3	Mean (SD)	1	2	3	Mean (SD)
Control	2.91	2.03	2.48	2.47 (0.44)	4.87	6.73	5.79	5.80 (0.93)	18.12	17.24	17.48	17.61 (0.45)
10 µg/mL *A. incisus* extract	2.32	2.48	2.39	2.41 (0.08)	4.62	6.18	5.42	5.41 (0.78)	17.08	15.52	16.28	16.30 (0.78)
25 µg/mL *A. incisus* extract	1.62	2.61	2.90	2.12 (0.50)	4.70	5.31	4.98	4.99 (0.30)	14.08	12.92	13.65	13.51 (0.59)
100 µg/mL *A. incisus* extract	1.65	1.95	1.79	1.81 (0.15)	5.06	3.14	4.08	4.12 (0.96)	10.24	8.96	9.54	9.61 (0.64)
4.5 µg/mL Artocarpin	2.10	2.42	2.16	2.23 (0.17)	4.56	4.80	5.22	4.86 (0.33)	10.11	12.16	12.76	11.68 (1.39)

3. Melanin Content

Sample	Absorbance (490 nm)			% melanin content/cell (SD)
	1	2	3	
Control	0.122	0.127	0.114	100.00 (0.00)
100 µg/mL *A. incisus* extract	0.064	0.078	0.080	77.32 (13.87)
25 µg/mL *A. incisus* extract	0.091	0.075	0.071	73.35 (5.79)
15 µg/mL *A. incisus* extract	0.066	0.077	0.118	83.27 (9.37)
10 µg/mL *A. incisus* extract	0.110	0.109	0.091	91.24 (8.62)
5 µg/mL *A. incisus* extract	0.119	0.114	0.109	90.85 (5.04)
2 µg/mL *A. incisus* extract	0.107	0.132	0.120	94.66 (2.47)
10 µg/mL Hydroquinone	0.058	0.052	0.064	59.03 (7.62)
10 µg/mL Kojic acid	0.112	0.089	0.124	90.39 (12.92)
4.5 µg/mL Artocarpin	0.085	0.094	0.090	91.12 (2.98)
10 µg/mL Artocarpin	0.083	0.078	0.070	83.46 (5.98)

APPENDIX F

APPENDIX F

DETERMINATION OF ANTIOXIDANT ACTIVITY BY USING DPPH ASSAY

1. *A. incisus* Ether Extract

Concentrations (µg/mL)	Sample No.	Absorbance (515 nm)		
		Sample	Blank of sample	Blank (DPPH without sample)
0.5	1	1.177	0.001	1.234
	2	1.170	0.001	1.198
	3	1.173	0.001	1.218
1.0	1	1.125	0.001	1.234
	2	1.120	0.001	1.198
	3	1.127	0.001	1.218
5.0	1	1.094	0.002	1.234
	2	1.084	0.002	1.198
	3	1.092	0.002	1.218
10	1	1.060	0.002	1.234
	2	1.056	0.003	1.198
	3	1.064	0.003	1.218
25	1	1.033	0.006	1.234
	2	1.034	0.003	1.198
	3	1.036	0.004	1.218
50	1	0.991	0.006	1.234
	2	0.960	0.006	1.198
	3	0.976	0.006	1.218

(Cont.)

Concentrations (µg/mL)	Sample No.	Absorbance (515 nm)		
		Sample	Blank of sample	Blank (DPPH without sample)
100	1	0.845	0.110	1.234
	2	0.798	0.100	1.198
	3	0.836	0.140	1.218
250	1	0.662	0.205	1.234
	2	0.638	0.202	1.198
	3	0.671	0.203	1.218
500	1	0.469	0.217	1.234
	2	0.488	0.213	1.198
	3	0.482	0.202	1.218
2000	1	0.312	0.25	1.234
	2	0.324	0.24	1.198
	3	0.316	0.246	1.218

2. L-ascorbic Acid

Concentrations (µg/mL)	Sample No.	Absorbance (515 nm)		
		Sample	Blank of sample	Blank (DPPH without sample)
0.25	1	1.236	0.001	1.334
	2	1.192	0.001	1.249
	3	1.224	0.001	1.376
0.50	1	1.119	0.001	1.334
	2	1.105	0.001	1.249
	3	1.112	0.001	1.376

Concentrations (µg/mL)	Sample No.	Absorbance (515 nm)		
		Sample	Blank of sample	Blank (DPPH without sample)
2.5	1	0.825	0.001	1.334
	2	0.722	0.001	1.249
	3	0.791	0.001	1.376
5.0	1	0.409	0.001	1.334
	2	0.412	0.001	1.249
	3	0.408	0.001	1.376
25	1	0.155	0.001	1.334
	2	0.163	0.001	1.249
	3	0.156	0.001	1.376
50	1	0.082	0.001	1.334
	2	0.083	0.001	1.249
	3	0.080	0.001	1.376
125	1	0.082	0.002	1.334
	2	0.079	0.002	1.249
	3	0.081	0.001	1.376
250	1	0.082	0.002	1.334
	2	0.078	0.002	1.249
	3	0.080	0.002	1.376
1000	1	0.083	0.002	1.334
	2	0.080	0.002	1.249
	3	0.083	0.002	1.376
2500	1	0.082	0.007	1.334
	2	0.090	0.005	1.249
	3	0.083	0.004	1.376

3. Butylated Hydroxytoluene (BHT)

Concentrations (µg/mL)	Sample No.	Absorbance (515 nm)		
		Sample	Blank of sample	Blank (DPPH without sample)
0.25	1	1.217	0.006	1.390
	2	1.196	0.006	1.349
	3	1.208	0.007	1.386
0.50	1	1.198	0.008	1.390
	2	1.154	0.009	1.349
	3	1.172	0.008	1.386
2.5	1	0.679	0.069	1.390
	2	0.657	0.070	1.349
	3	0.668	0.078	1.386
5.0	1	0.372	0.104	1.390
	2	0.360	0.102	1.349
	3	0.377	0.099	1.386
25	1	0.174	0.053	1.390
	2	0.158	0.049	1.349
	3	0.143	0.060	1.386
50	1	0.105	0.031	1.390
	2	0.100	0.032	1.349
	3	0.099	0.030	1.386
125	1	0.079	0.011	1.390
	2	0.083	0.013	1.349
	3	0.081	0.013	1.386

(Cont.)

Concentrations (µg/mL)	Sample No.	Absorbance (515 nm)		
		Sample	Blank of sample	Blank (DPPH without sample)
250	1	0.088	0.040	1.390
	2	0.101	0.046	1.349
	3	0.073	0.053	1.386
1000	1	0.088	0.033	1.390
	2	0.095	0.043	1.349
	3	0.086	0.033	1.386
2500	1	0.090	0.041	1.390
	2	0.120	0.031	1.349
	3	0.100	0.029	1.386

The calculation of % free radical scavenging

Example

At the concentration of 2000 µg/mL *A. incisus* extract dissolved in DMSO, the % free radical scavenging is calculated by using the equation:

% Free radical scavenging $= [\ 1- (A_S / A_B)\ ] \times 100$

Where; A_S is an absorbance of DPPH with tested sample

A_B is an absorbance of DPPH without tested sample

Therefore, % Free radical scavenging $= [\ 1- (\ (\ 0.312-0.250) /1.234\ )\ ] \times 100$

$= [\ 1- (\ 0.062/\ 1.234\ )\ ] \times 100$

$= 94.97$