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APPENDICES

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Appendix A

Antioxidant activities

Antioxidant activities were important properties of anthraquinones. These may be lost when extraction temperature was high or exposure when contacts to light and some organic solvents. Furthermore, antioxidant activities have related with extraction methods. Some methods may be make anthraquinones degradation. This experiment was investigated to compare the antioxidant activities at the maximum conditions in each method using DPPH method by 1, 2-diphenyl-2-picrylhydrazyl (DPPH) radical against free radicals. The color of DPPH as purple color in ethanol at absorbance 1.235 (about 110 μM) using spectrophotometer at wavelength of 517 nm as a initial reference sample. The percentage inhibition (PI%) was calculated from reduction absorbance as reaction with DPPH in series at known concentration. The anthraquinones extracts in 3 ml, known concentration, dilute in series with ethanol 1 ml and take solution 1 ml mix with 2 ml DPPH solution. Mixed solution was vortexed and kept in darkness about 2 hours at 37 $^{\circ}\text{C}$. The color of mixed solution was change when the antioxidant activities were extreme. These show in Figure A-1.

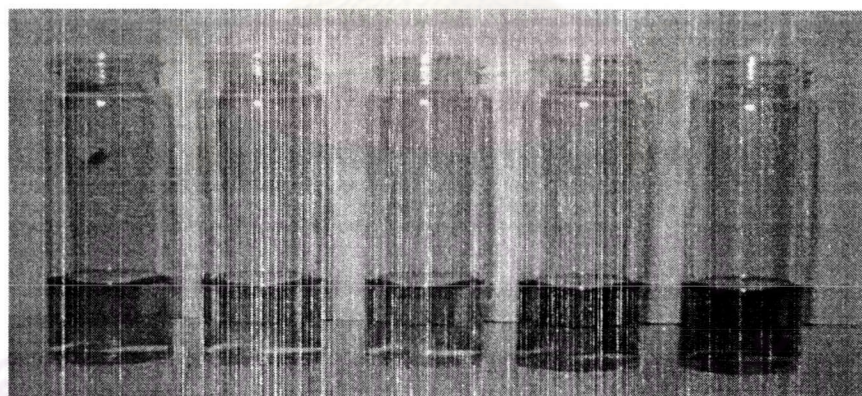


Figure A-1 Antioxidant develop for dilute sample

For example, antioxidant activity using maceration extraction

Conc. (mM)	Abs 3	PI%
0.441	0.75	60.29
0.3308	0.685	55.06
0.2481	0.654	52.57
0.186	0.611	49.12
0.1395	0.599	48.15
0.1047	0.567	45.58
0.0785	0.542	43.57
IC ₅₀ = 0.204	Ref = 1.244	

When plotting the concentration versus PI (%) was determine inhibition concentration at 50% as mean concentration of sample producing 50% reduction of the radical absorbance in Figure A-1. The plotting between PI (%) and concentration gives the fitted equation was $y = 43.884x + 41.037$ which gives IC₅₀ was 0.204 mM

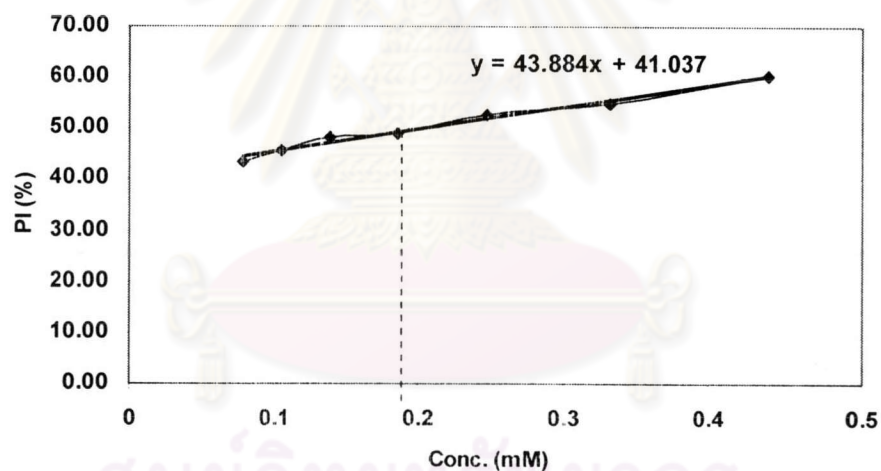


Figure A-1 Sample of IC₅₀ determination

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Appendix B

Table B-1 Standard calibration curve data of Alizarin in ethanol

Concentration of Alizarin (M)	Absorbance at 435 nm.			
	Exp.1	Exp.2	Exp.3	Average
8E-05	0.431	0.498	0.501	0.47667
6.4E-05	0.311	0.37	0.379	0.35333
5.12E-05	0.26	0.301	0.292	0.28433
4.1E-05	0.212	0.25	0.253	0.23833
3.28E-05	0.166	0.194	0.21	0.19
2.62E-05	0.137	0.157	0.155	0.14967
2.1E-05	0.12	0.129	0.123	0.124
1.68E-05	0.085	0.102	0.097	0.09467
1.34E-05	0.068	0.087	0.079	0.078
1.07E-05	0.053	0.065	0.058	0.05867
8.59E-06	0.045	0.058	0.05	0.051
6.87E-06	0.034	0.041	0.043	0.03933
5.5E-06	0.03	0.034	0.034	0.03267

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Figure B-1-1 Standard calibration curve data of Alizarin in ethanol

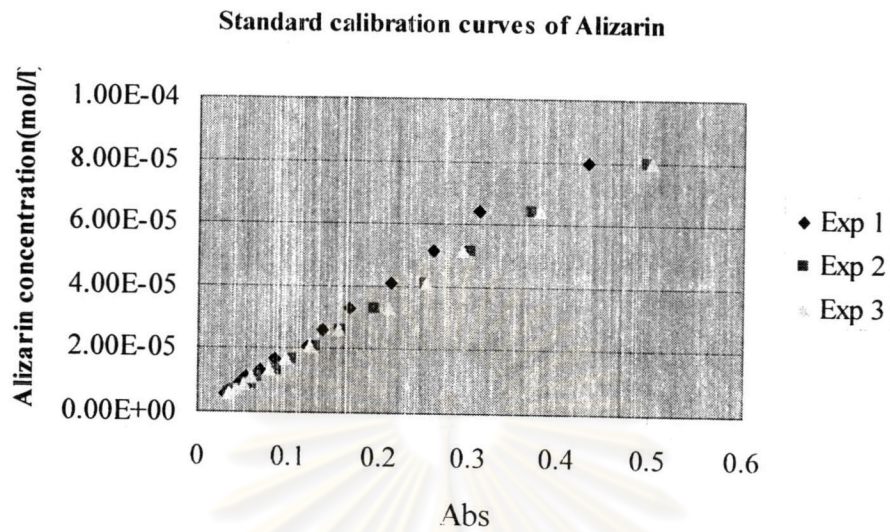


Figure B-1-2 Average standard calibration curve data of Alizarin in ethanol

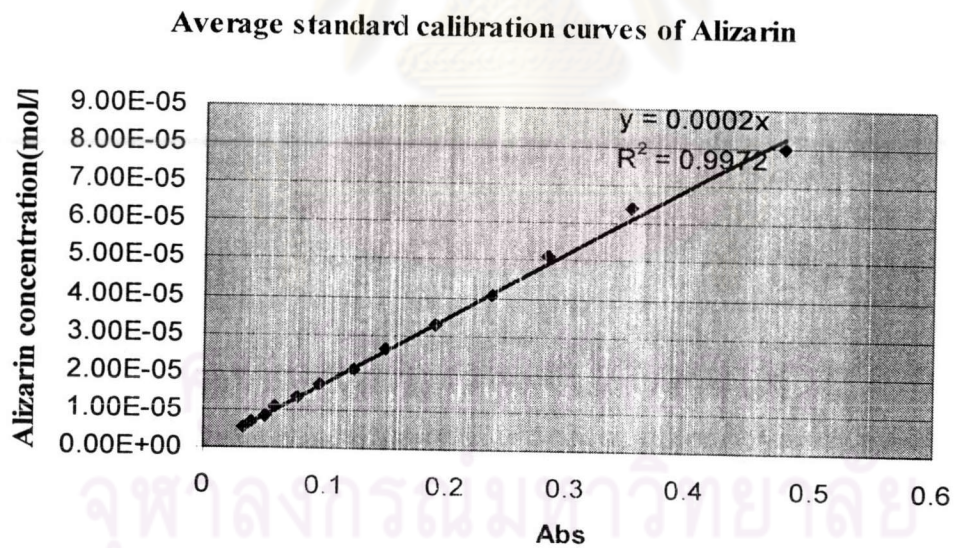


Table B-2 Standard calibration curve data of Alizarin in acetone

Concentration of Alizarin (M)	Absorbance at 435 nm.			
	Exp.1	Exp.2	Exp.3	Average
8E-05	0.412	0.397	0.4	0.403
6.4E-05	0.349	0.32	0.343	0.337
5.12E-05	0.263	0.257	0.257	0.259
4.1E-05	0.212	0.213	0.219	0.215
3.28E-05	0.171	0.169	0.173	0.171
2.62E-05	0.133	0.138	0.135	0.135
2.1E-05	0.107	0.106	0.104	0.106
1.68E-05	0.084	0.09	0.09	0.088
1.34E-05	0.069	0.071	0.065	0.068
1.07E-05	0.057	0.059	0.057	0.058

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Figure B-2-1 Standard calibration curve data of Alizarin in acetone

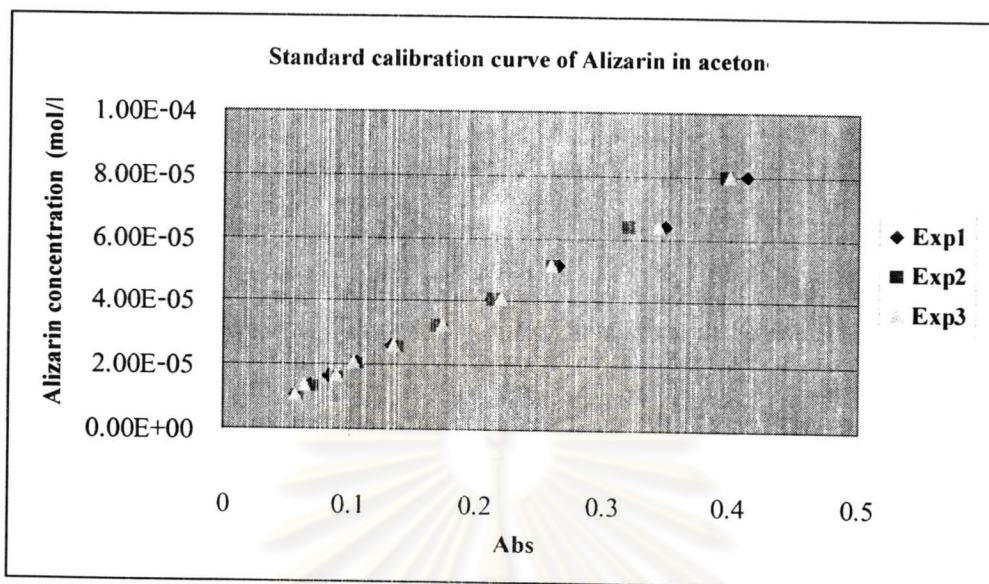


Figure B-2-2 Average standard calibration curve data of Alizarin in acetone

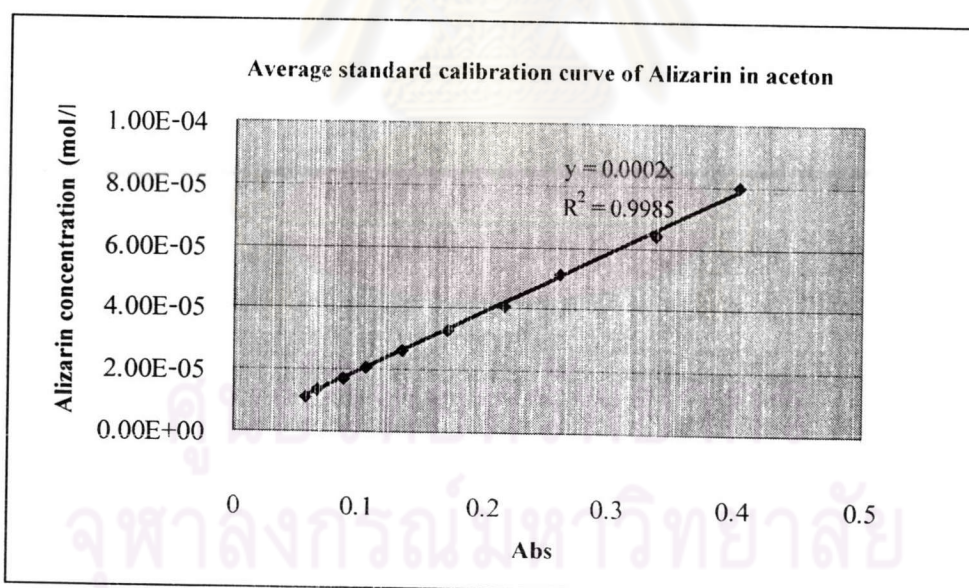


Table B-3 Standard calibration curve data of Alizarin in methanol

Concentration of Alizarin (M)	Absorbance at 435 nm.			
	Exp.1	Exp.2	Exp.3	Average
8E-05	0.435	0.47	0.484	0.463
6.4E-05	0.349	0.378	0.394	0.374
5.12E-05	0.405	0.302	0.308	0.338
4.1E-05	0.218	0.242	0.245	0.235
3.28E-05	0.169	0.19	0.197	0.185
2.62E-05	0.133	0.152	0.157	0.147
2.1E-05	0.105	0.13	0.122	0.119
1.68E-05	0.079	0.1	0.101	0.093
1.34E-05	0.067	0.079	0.076	0.074
1.07E-05	0.054	0.065	0.068	0.062

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Figure B-3-1 Standard calibration curve data of Alizarin in methanol

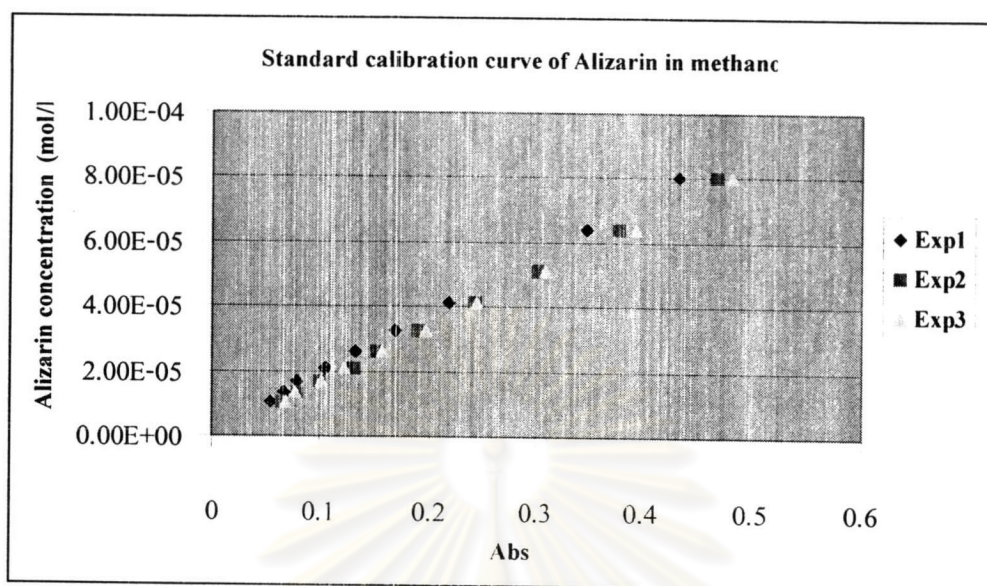


Table B-3-2 Average standard calibration curve data of Alizarin in methanol

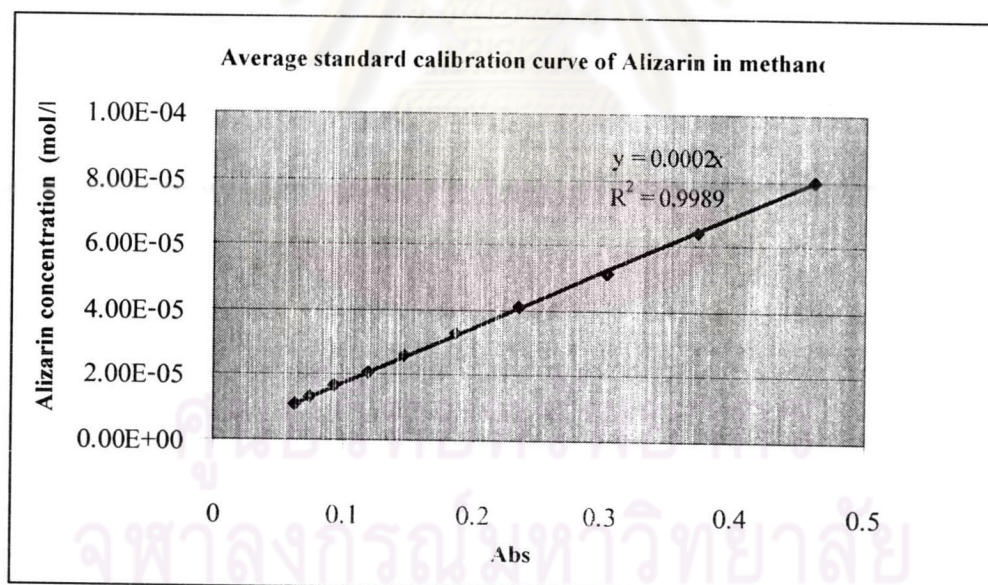


Table B-4 Standard calibration curve data of Alizarin in acetonitrile

Concentration of Alizarin (M)	Absorbance at 435 nm.			
	Exp.1	Exp.2	Exp.3	Average
8E-05	0.382	0.4	0.39	0.391
6.4E-05	0.297	0.325	0.04	0.302
5.12E-05	0.227	0.267	0.243	0.246
4.1E-05	0.188	0.201	0.195	0.195
3.28E-05	0.136	0.156	0.147	0.146
2.62E-05	0.116	0.132	0.125	0.124
2.1E-05	0.089	0.092	0.09	0.09
1.68E-05	0.062	0.07	0.058	0.063
1.34E-05	0.045	0.051	0.048	0.048

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Figure B-4-1 Standard calibration curve data of Alizarin in acetonitrile

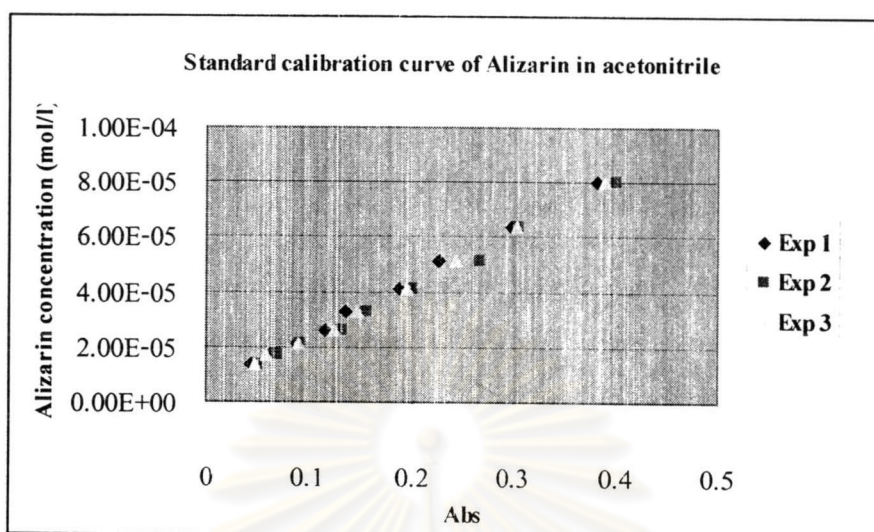


Figure B-4-2 Average standard calibration curve data of Alizarin in methanol

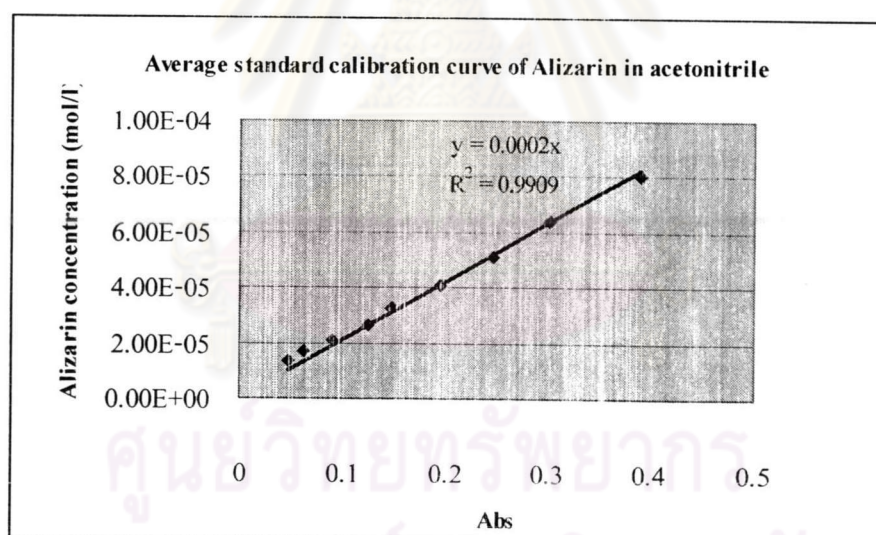


Table B-5 Standard calibration curve data of Alizarin in 80% ethanol

Concentration of Alizarin (M)	Absorbance at 435 nm.			
	Exp.1	Exp.2	Exp.3	Average
8E-05	0.361	0.4	0.38	0.38
6.4E-05	0.263	0.293	0.278	0.278
5.12E-05	0.201	0.231	0.226	0.219
4.1E-05	0.155	0.168	0.16	0.161
3.28E-05	0.115	0.14	0.131	0.129
2.62E-05	0.094	0.116	0.103	0.104
2.1E-05	0.07	0.085	0.075	0.077
1.68E-05	0.055	0.065	0.063	0.061
1.34E-05	0.046	0.052	0.052	0.05
1.07E-05	0.036	0.041	0.038	0.038

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Figure B-5-1 Standard calibration curve data of Alizarin in 80% ethanol

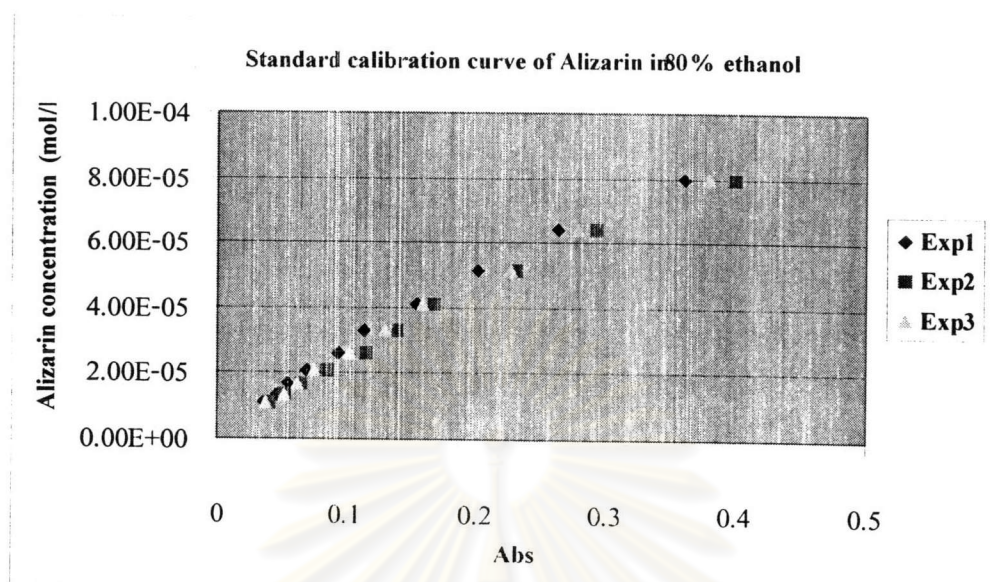


Figure B-5-2 Average standard calibration curve data of Alizarin in 80% ethanol

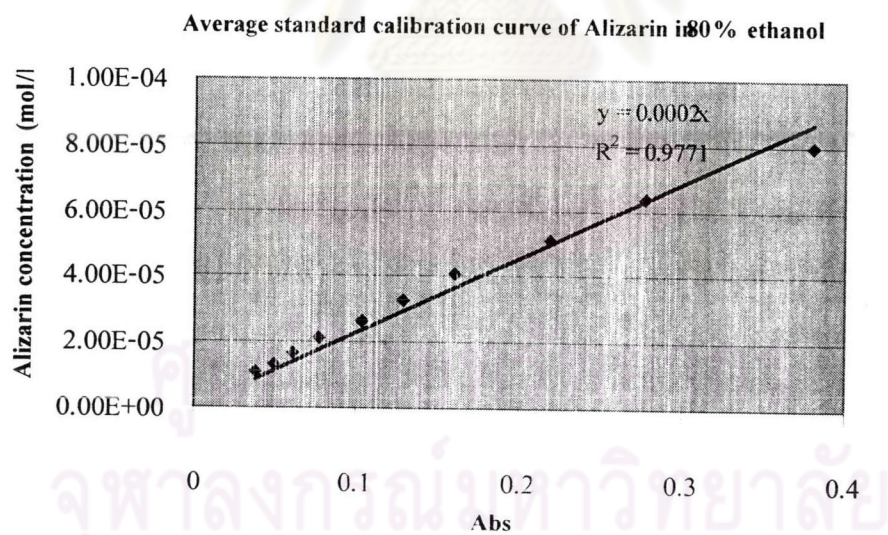


Table B-6 Standard calibration curve data of Alizarin in 50% ethanol

Concentration of Alizarin (M)	Absorbance at 435 nm.			
	Exp.1	Exp.2	Exp.3	Average
8E-05	0.324	0.309	0.315	0.316
6.4E-05	0.27	0.226	0.26	0.257
5.12E-05	0.179	0.177	0.17	0.175
4.1E-05	0.136	0.141	0.145	0.141
3.28E-05	0.102	0.107	0.103	0.104
2.62E-05	0.085	0.091	0.09	0.089
2.1E-05	0.059	0.065	0.062	0.062
1.68E-05	0.051	0.053	0.054	0.053
1.34E-05	0.036	0.045	0.04	0.04
1.07E-05	0.034	0.036	0.037	0.036

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Figure B-6-1 Standard calibration curve data of Alizarin in 50% ethanol

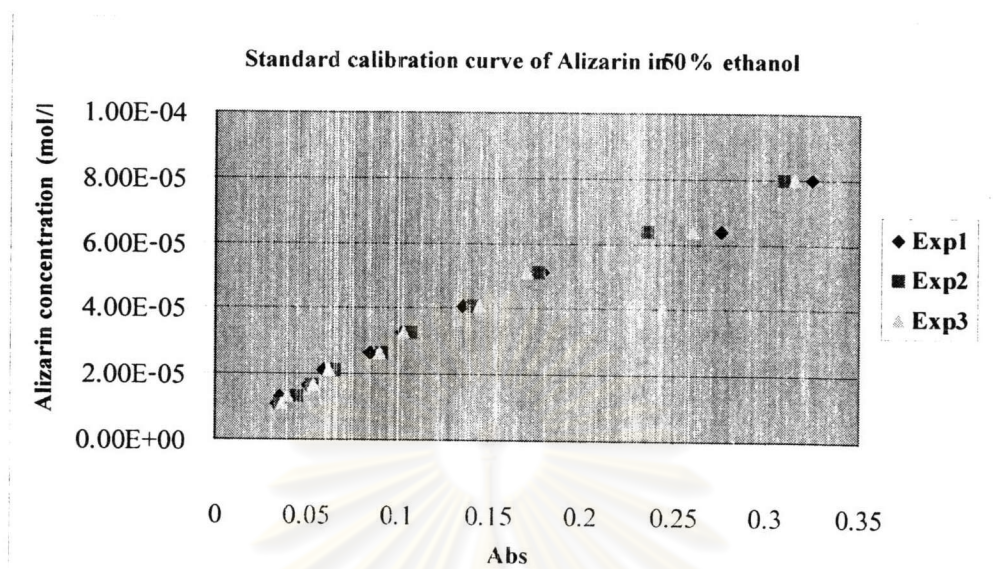


Figure B-6-2 Average standard calibration curve data of Alizarin in 50% ethanol

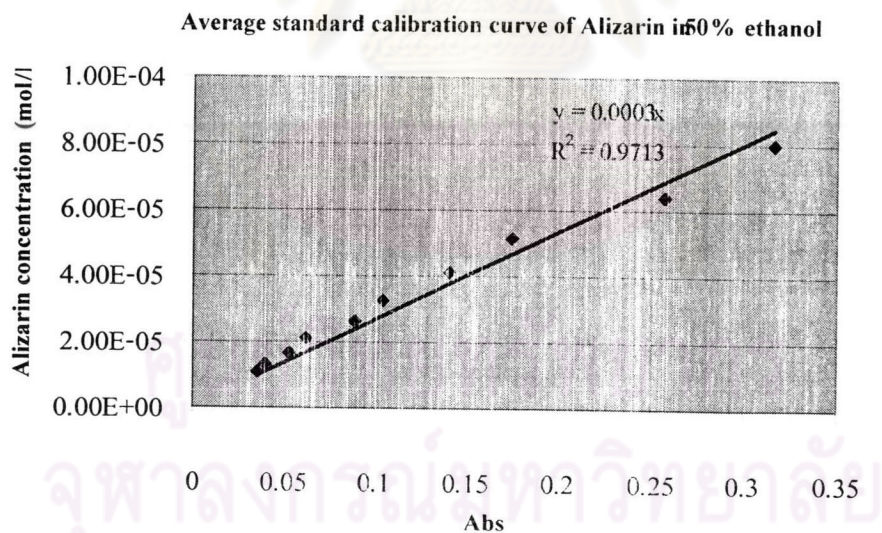


Table B-7 Standard calibration curve data of Alizarin in 20% ethanol

Concentration of Alizarin (M)	Absorbance at 435 nm.			
	Exp.1	Exp.2	Exp.3	Average
6E-05	0.145	0.141	0.134	0.14
4.5E-05	0.116	0.108	0.106	0.11
3.38E-05	0.091	0.087	0.084	0.087
2.53E-05	0.072	0.066	0.067	0.068
1.89E-05	0.057	0.049	0.053	0.053
1.42E-05	0.045	0.043	0.039	0.042
1.07E-05	0.036	0.032	0.035	0.034
8E-06	0.029	0.026	0.32	0.029
6E-06	0.025	0.023	0.023	0.024
4.5E-06	0.019	0.018	0.018	0.018

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Figure B-7-1 Standard calibration curve data of Alizarin in 20% ethanol

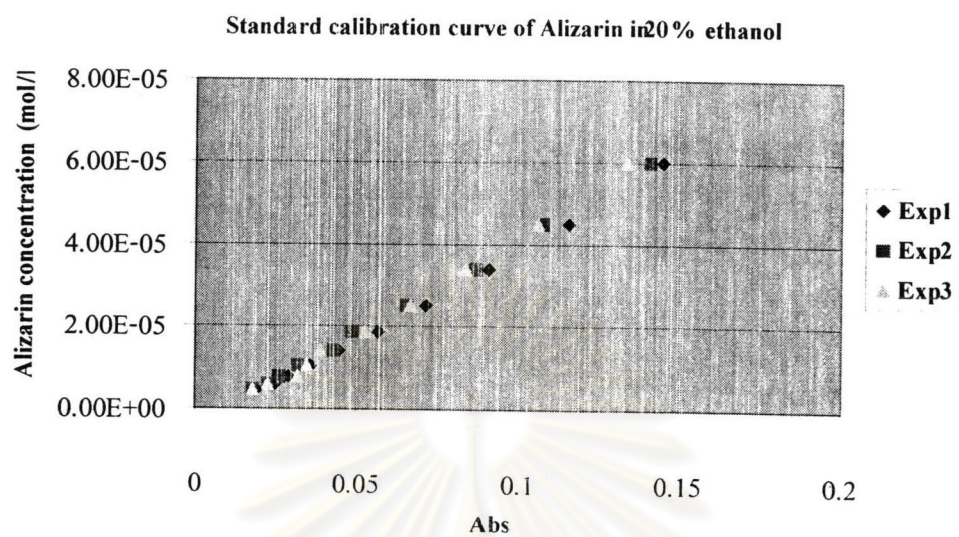
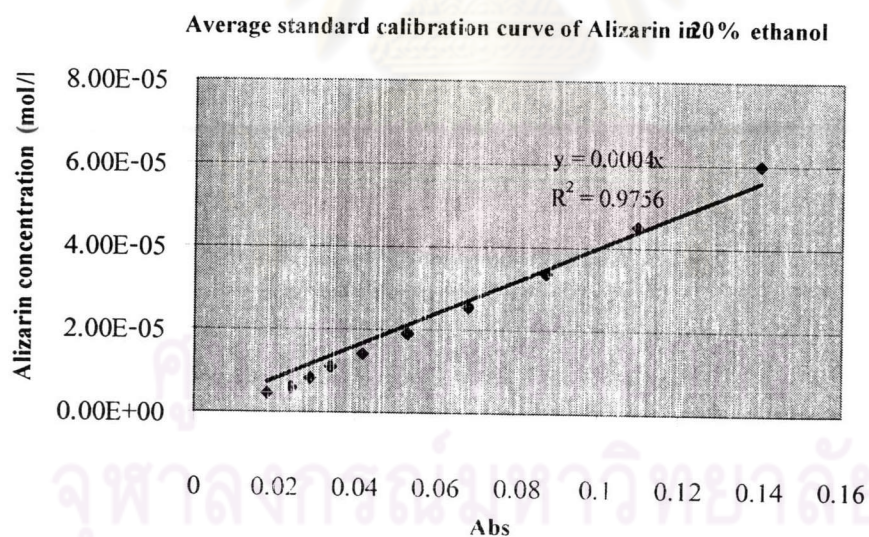


Figure B-7-2 Average standard calibration curve data of Alizarin in 20% ethanol



Appendix C

Extraction times and extraction temperatures

Maceration extraction

Table C-1 Recovery (%) of Alizarin was operating condition at ambient temperature using ethanol as solvent.

Time (min)	Recovery (%)			Avg. Recovery (%)± std.
15	26.44135	32.90891	27.18447	28.84 ± 3.539
30	31.66506	34.96358	30.37336	32.33 ± 2.367
45	34.92408	35.37415	34.14634	34.81 ± 0.621
60	40.2823	40.59869	40.60773	40.50 ± 0.185
90	43.20988	44.64809	46.28099	44.71 ± 1.537

Soxhlet extraction

Table C-2 Recovery (%) of Alizarin was operating condition at ambient temperature using ethanol as solvent.

Time (min)	Recovery (%)			Avg. Recovery (%)± std.
4 hours	97.5269	97.9672	96.8622	97.45 ± 0.556

Ultrasound assisted extraction

Table C-3 Recovery (%) of Alizarin was operating condition at extraction temperature at 25 °C and power setting 3 using ethanol as solvent

Time (min)	Recovery (%)			Avg. Recovery (%)± std.
15	34.8341	36.2851	36.4162	35.85 ± 0.878
30	36.3208	41.1765	38.4742	38.66 ± 2.433
45	42.1824	42.0601	42.2735	42.17 ± 0.107
60	42.8808	45.6522	47.469	45.33 ± 2.311
90	49.2188	51.4956	54.2636	51.66 ± 2.526

Table C-4 Recovery (%) of Alizarin was operating condition at extraction temperature at 45 °C and power setting 3 using ethanol as solvent.

Time (min)	Recovery (%)			Avg. Recovery (%)± std.
15	47.15447	44.63087	43.14559	44.98 ± 2.027
30	60.07984	58.91784	59.57447	59.52 ± 0.583
45	62.73917	61.65803	63.63636	62.68 ± 0.991
60	63.16759	65.70397	62.68657	63.85 ± 1.621
90	65.47203	67.5588	62.77022	65.27 ± 2.401

Table C-5 Recovery (%) of Alizarin was operating condition at extraction temperature at 60 °C and power setting 3 using ethanol as solvent.

Time (min)	Recovery (%)			Avg. Recovery (%)± std.
15	54.73251	55.36723	52.28216	54.13 ± 1.629
30	66.80498	61.29032	61.19536	63.1 ± 3.212
45	65.85736	63.01248	62.68657	63.85 ± 1.744
60	64.99632	64.60573	66.38358	65.33 ± 0.934
90	66.6155	65.11628	67.48279	66.4 ± 1.197

Microwave assisted extraction

Table C-6 Recovery (%) of Alizarin was operating condition at extraction temperature at 60 °C and power 60% of 1200 W using ethanol as solvent.

Time (min)	Recovery (%)			Avg. Recovery (%)± std.
5	41.80446	47.49596	46.03122	45.11 ± 2.955
10	48.19892	52.05075	51.60747	50.62 ± 2.108
15	56.11737	52.39659	53.78928	54.1 ± 1.88
20	61.65946	58.17334	57.79543	59.21 ± 2.130
30	65.81826	66.50426	65.311	65.88 ± 0.599

Table C-7 Recovery (%) of Alizarin was operating condition at extraction temperature at 80 °C and power 60% of 1200 W using ethanol as solvent.

Time (min)	Recovery (%)			Avg. Recovery (%)± std.
5	64.02439	66.91871	63.59048	64.84 ± 1.809
10	68.92093	72.91476	69.6873	70.51 ± 2.119
15	69.0298	73.37561	71.81266	71.41 ± 2.201
20	74.65438	71.45467	72.70923	72.94 ± 1.612
30	73.45409	76.5054	73.88675	74.62 ± 1.651

Table C-8 Recovery (%) of Alizarin was operating condition at extraction temperature at 100 °C and power 60% of 1200 W using ethanol as solvent.

Time (min)	Recovery (%)			Avg. Recovery (%)± std.
5	64.75771	64.86978	67.94872	65.86 ± 1.811
10	72.83237	69.18172	75.62327	72.55 ± 3.230
15	75.46012	78.99687	73.58176	76.01 ± 2.749
20	84.90276	87.71499	81.51571	84.71 ± 3.104
30	84.51071	87.47618	85.72652	85.90 ± 1.491

Table C-9 Recovery (%) of Alizarin was operating condition at extraction temperature at 120 °C and power 60% of 1200 W using ethanol as solvent.

Time (min)	Recovery (%)			Avg. Recovery (%)± std.
5	83.21784	84.393289	83.24397	83.62 ± 0.671
10	80.93135	84.343771	86.26926	83.85 ± 2.703
15	83.77004	86.675639	85.69794	85.38 ± 1.478
20	92.85714	91.224221	91.16048	91.75 ± 0.962
30	93.86397	92.320983	93.48627	93.22 ± 0.804

Type of solvents and ethanol compositions

Ultrasound assisted extraction

Table C-10 Recovery (%) of Alizarin was operating condition at extraction temperature at 25 °C, power setting 3 and extraction time 45 minutes using ultrasound assisted extraction.

Type of solvents	Recovery (%)			Avg. Recovery (%)± std.
Methanol	47.39336	49.07162	46.66667	47.71 ± 1.233
Acetone	57.33788	50.1992	50.80645	52.78 ± 3.958
Ethanol	42.18241	42.06009	42.27353	42.17 ± 0.107
Acetonitrile	50.28377	46.70992	49.76581	48.92 ± 1.931

Table C-11 Recovery (%) of Alizarin was operating condition at extraction temperature at 25 °C, power setting 3 and extraction time 45 minutes using ultrasound assisted extraction.

Ethanol compositions	Recovery (%)			Avg. Recovery (%)± std.
100 %	42.1824	42.0601	42.2735	42.17 ± 0.107
80 %	63.39455	60.29963	63.55014	62.41 ± 1.833
50 %	71.65354	68.79511	70.53345	70.33 ± 1.44
20 %	11.18881	10.90387	11.01695	11.04 ± 0.143

Microwave assisted extraction

Table C-12 Recovery (%) of Alizarin was operating condition at extraction temperature at 60 °C, power 60% of 1200 W and extraction time 15 minutes using microwave assisted extraction.

Type of solvents	Recovery (%)			Avg. Recovery (%)± std.
Methanol	88.002704	90.37117	90.322581	89.57 ± 1.354
Acetone	61.876454	64.78873	65.679838	64.12 ± 1.989
Ethanol	56.11737	52.39659	53.789279	54.1 ± 1.880
Acetonitrile	55.362493	50.86837	59.326523	55.19 ± 4.232

Table C-13 Recovery (%) of Alizarin was operating condition at extraction temperature at 60 °C, power 60% of 1200 W and extraction time 15 minutes using microwave assisted extraction.

Ethanol compositions	Recovery (%)			Avg. Recovery (%)± std.
100%	56.11737	52.39659	53.78928	54.1 ± 1.88
80%	95.71559	95.31327	96.70411	95.91 ± 0.716
50%	93.42153	93.68507	93.84131	93.64 ± 0.212
20%	66.9967	70.08453	64.42863	67.17 ± 2.832

Power

Table C-14 Recovery (%) of Alizarin was operating condition at extraction temperature at 25 °C and extraction time 45 minutes using ethanol as solvent.

Power	Recovery (%)			Avg. Recovery (%)± std.
3	42.18241	42.06009	42.27353	42.17 ± 0.107
6	47.21408	47.8022	45.94595	46.99 ± 0.949
9	50.27624	53.46062	55.75221	53.16 ± 2.75

Antioxidant activities

Table C-15 Antioxidant activities and percent inhibition of Alizarin using maceration extraction was operated at 37 °C.

Conc. (mM)	Abs 1	PI%	Conc. (mM)	Abs 2	PI%
0.451	0.759	61.01	0.3916	0.719	57.80
0.3383	0.699	56.19	0.2937	0.654	52.57
0.2537	0.666	53.54	0.2203	0.624	50.16
0.1903	0.628	50.48	0.1652	0.597	47.99
0.1427	0.605	48.63	0.1239	0.574	46.14
0.107	0.574	46.14	0.0929	0.558	44.86
0.0803	0.545	43.81	0.0697	0.54	43.41
IC ₅₀ = 0.192	Ref = 1.244		IC ₅₀ = 0.218	Ref = 1.244	

Conc. (mM)	Abs 3	PI%
0.441	0.75	60.29
0.3308	0.685	55.06
0.2481	0.654	52.57
0.186	0.611	49.12
0.1395	0.599	48.15
0.1047	0.567	45.58
0.0785	0.542	43.57
IC ₅₀ = 0.204	Ref = 1.244	

Table C-16 Antioxidant activities and percent inhibition of Alizarin using soxhlet extraction was operated at 37 °C.

Conc. (mM)	Abs 1	PI%	Conc. (mM)	Abs 2	PI%
0.2993	0.801	64.86	0.3213	0.835	67.61
0.2244	0.763	61.78	0.241	0.777	62.91
0.1683	0.698	56.52	0.1807	0.708	57.33
0.1262	0.637	51.58	0.1355	0.657	53.20
0.0947	0.614	49.72	0.1017	0.634	51.34
0.071	0.573	46.40	0.0762	0.593	48.02
0.0533	0.534	43.24	0.0572	0.561	45.43
IC ₅₀ = 0.109	Ref = 1.235		IC ₅₀ = 0.098	Ref = 1.235	

Conc. mM	Abs 3	PI%
0.3308	0.838	67.85
0.2481	0.778	63.00
0.186	0.715	57.89
0.1395	0.649	52.55
0.1047	0.623	50.45
0.0785	0.587	47.53
0.0589	0.555	44.94
IC ₅₀ = 0.106	Ref = 1.235	

Table C-17 Antioxidant activities and percent inhibition of Alizarin at 100% ethanol using ultrasonic extraction was operated at 37 °C.

Conc. (Mm)	Abs 1	PI%	Conc. mM	Abs 2	PI%
0.651	0.786	63.85	0.5334	0.786	63.44
0.4883	0.725	58.90	0.4	0.729	58.84
0.3662	0.684	55.56	0.3	0.687	55.45
0.2746	0.649	52.72	0.225	0.641	51.74
0.206	0.618	50.20	0.1688	0.605	48.83
0.1545	0.588	47.77	0.1266	0.552	44.55
0.1159	0.554	45.00	0.09493	0.527	42.53
IC ₅₀ = 0.22	Ref = 1.231		IC ₅₀ = 0.217	Ref = 1.239	

Conc. mM	Abs 2	PI%
0.5166	0.779	63.08
0.3875	0.717	58.06
0.2906	0.688	55.71
0.2179	0.637	51.58
0.1635	0.606	49.07
0.1226	0.549	44.45
0.0919	0.532	43.08
IC ₅₀ = 0.210	Ref = 1.235	

Table C-18 Antioxidant activities and percent inhibition of Alizarin at 80% ethanol using ultrasonic extraction was operated at 37 °C.

Conc. (mM)	Abs 1	PI%	Conc. (mM)	Abs 2	PI%
0.6132	0.809	65.51	0.4494	0.788	63.96
0.4599	0.783	63.40	0.3371	0.739	59.98
0.3449	0.726	58.79	0.2528	0.693	56.25
0.2587	0.681	55.14	0.1896	0.643	52.19
0.194	0.633	51.26	0.1422	0.601	48.78
0.1455	0.6	48.58	0.1066	0.589	47.81
0.1091	0.567	45.91	0.07998	0.567	46.02
IC ₅₀ = 0.1650	Ref = 1.235		IC ₅₀ = 0.151	Ref = 1.232	

Conc. (mM)	Abs 3	PI%
0.4767	0.822	66.72
0.3575	0.768	62.34
0.2681	0.718	58.28
0.2011	0.671	54.46
0.1508	0.632	51.30
0.1131	0.599	48.62
0.08484	0.575	46.67
IC ₅₀ = 0.13	Ref = 1.232	

Table C-19 Antioxidant activities and percent inhibition of Alizarin at 50% ethanol using ultrasonic extraction was operated at 37 °C.

Conc. (mM)	Abs 1	PI%	Conc. (mM)	Abs 2	PI%
0.4242	0.75	60.73	0.462	0.747	60.44
0.3182	0.705	57.09	0.3465	0.72	58.25
0.2386	0.654	52.96	0.2599	0.682	55.18
0.179	0.62	50.28	0.1949	0.644	52.10
0.1342	0.579	46.88	0.1462	0.618	50.00
0.1007	0.559	45.26	0.1096	0.592	47.90
0.0755	0.544	44.05	0.0823	0.556	44.98
IC ₅₀ = 0.189	Ref = 1.235		IC ₅₀ = 0.161	Ref = 1.236	

Conc. (mM)	Abs 2	PI%
0.4683	0.738	59.76
0.3512	0.716	57.98
0.2634	0.688	55.71
0.1976	0.647	52.39
0.1482	0.626	50.69
0.1111	0.589	47.69
0.0833	0.56	45.34
IC ₅₀ = 0.156	Ref = 1.235	

Table C-20 Antioxidant activities and percent inhibition of Alizarin at 100% ethanol using microwave extraction was operated at 37 °C.

Conc.(mM)	Abs 1	PI%	Conc.(mM)	Abs 2	PI%
0.6258	0.864	69.90	0.546	0.863	69.82
0.4693	0.817	66.10	0.4095	0.812	65.70
0.352	0.734	59.39	0.3071	0.736	59.55
0.264	0.671	54.29	0.2303	0.669	54.13
0.198	0.659	53.32	0.1728	0.642	51.94
0.1485	0.62	50.16	0.1296	0.595	48.14
0.1114	0.599	48.46	0.0972	0.586	47.41
IC ₅₀ = 0.139	Ref = 1.236		IC ₅₀ = 0.144	Ref = 1.236	

Conc. (mM)	Abs 3	PI%
0.476	0.879	71.12
0.357	0.829	67.07
0.2678	0.749	60.60
0.2008	0.681	55.10
0.1506	0.641	51.86
0.1129	0.604	48.87
0.0847	0.579	46.84
IC ₅₀ = 0.122	Ref = 1.236	

Table C-21 Antioxidant activities and percent inhibition of Alizarin at 80% ethanol using microwave extraction was operated at 37 °C.

Conc. (mM)	Abs 1	PI%	Conc. (mM)	Abs 2	PI%
0.3497	0.815	68.20	0.3591	0.808	67.62
0.2622	0.767	64.18	0.2693	0.753	63.01
0.1967	0.717	60.00	0.202	0.694	58.08
0.1475	0.645	53.97	0.1515	0.642	53.72
0.1106	0.605	50.63	0.1136	0.611	51.13
0.083	0.575	48.12	0.0852	0.575	48.12
0.0622	0.545	45.61	0.0639	0.538	45.02
IC ₅₀ = 0.101	Ref = 1.195		IC ₅₀ = 0.108	Ref = 1.195	

Conc. (mM)	Abs 3	PI%
0.3701	0.813	68.03
0.2776	0.764	63.93
0.2082	0.703	58.83
0.1561	0.645	53.97
0.1171	0.622	52.05
0.0878	0.583	48.79
0.0659	0.544	45.52
IC ₅₀ = 0.103	Ref = 1.195	

VITA

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