APPENDICES

APPENDIX A

STANDARD CURVE OF ANTIOXIDANT ACTIVITY

Standard curve of Trolox for ABTS assay

The preparation of 500 µM Trolox stock solution by weighed Trolox 0.0125 g, dissolved in absolute ethanol and adjusted to 100 ml in volumetric flask. Trolox stock solution was dilute with absolute ethanol to obtain the series concentrations which presented in Table A.1.

Table A.1 Preparation of the series Trolox stock solutions

Concentration of Trolox (µM)	Volume of Trolox stock solution (µl)	Volume of absolute ethanol (µl)
10	50	950
50	100	900
100	200	800
200	400	600
300	600	400
400	800	200
500	1000	0

The entire solutions were reacted with $ABTS^{\bullet+}$ working solution and measured for their visible absorbance by using microtitre plate reader at 750 nm for 5 min. All measurements were carried out in triplicate. The % inhibition of the solutions were

calculated (Table A.2) and plotted as calibration curve of standard Trolox (Figure A.1). The representative regression coefficient (r^2) was 0.9986 and the linear regression equation was y = 1.9913x - 0.8864.

Table A.2 Absorbance value and %inhibition of standard Trolox

Final conc. (μM)	Absorbance at 750 nm for 5 min			% inhibition			
	1	2	3	1	2	3	Mean ± SD
Control	0.830	0.835	0.836				
1	0.804	0.804	0.807	3.13	3.71	3.47	3.44 ± 0.29
5	0.758	0.766	0.768	8.67	8.26	8.13	8.36 ± 0.28
10	0.677	0.688	0.696	18.43	17.60	16.75	17.59 ± 0.84
20	0.504	0.512	0.507	39.28	38.68	39.35	39.10 ± 0.37
30	0.347	0.347	0.367	58.19	58.44	56.10	57.58 ± 1.29
40	0.183	0.171	0.188	77.95	79.52	77.51	78.33 ± 1.06
50	-0.006	0.001	0.004	100.72	99.88	99.52	100.04 ± 0.62

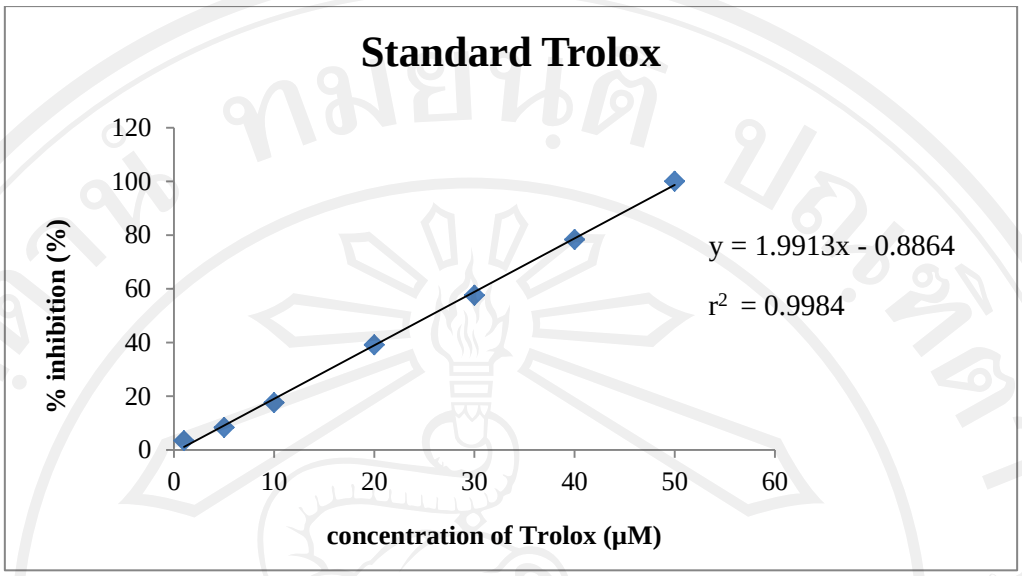

Figure A.1 Standard curve of Trolox

Standard curve of ferrous sulphate ($FeSO_4.7H_20$) for FRAP assay

The preparation of 10 mM $FeSO_4.7H_20$ stock solution by weighed $FeSO_4.7H_20$ 0.2752 g, dissolved in absolute ethanol and adjusted to 100 ml in volumetric flask. $FeSO_4.7H_20$ stock solution was dilute with absolute ethanol to obtain the series concentrations which presented in Table A.3.

Table A.3 Preparation of the series $FeSO_4.7H_20$ stock solutions

Concentration of $FeSO_4.7H_20$ (µM)	Volume of $FeSO_4.7H_20$ stock solution (µl)	Volume of absolute ethanol (µl)
500	50	950
1000	100	900
2000	200	800
3000	300	700
4000	400	600
5000	500	500
6000	600	400

The entire solutions were reacted with FRAP working solution and measured for their visible absorbance by using microtitre plate reader at 595 nm for 5 min. All measurements were carried out in triplicate. The absorbance of the solutions (Table A.4) was plotted as calibration curve of standard $FeSO_4.7H_20$ (Figure A.2). The representative regression coefficient (r^2) was 0.9991 and the linear regression equation was $y = 0.002x + 0.0035$.

Table A.4 Absorbance value of $FeSO_4.7H_20$ standard

Final conc. (µM)	Absorbance of $FeSO_4.7H_20$ standard at 595 nm for 5 min			
	1	2	3	Mean ± SD
50	0.128	0.111	0.108	0.116 ± 0.111
100	0.199	0.2	0.181	0.193 ± 0.111
200	0.429	0.427	0.393	0.416 ± 0.020
300	0.624	0.598	0.568	0.597 ± 0.028
400	0.798	0.809	0.784	0.797 ± 0.013
500	1.107	0.971	0.916	0.998 ± 0.098
600	1.258	1.214	1.199	1.224 ±0.031

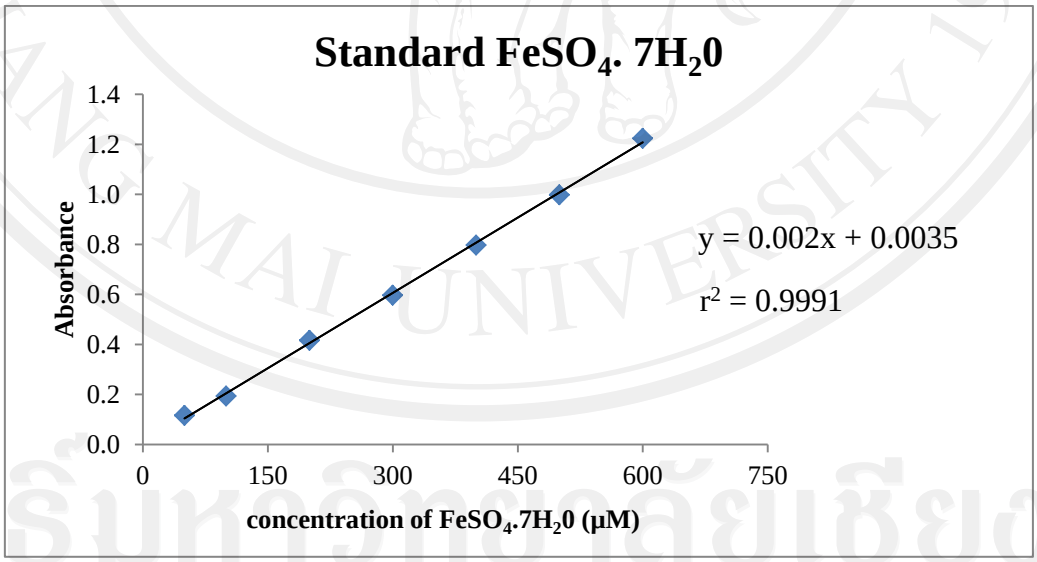

Figure A.2 Standard curve of $FeSO_4.7H_20$

Standard curve of gallic acid for folin-ciocalteu assay

The preparation of 1 mg/ml gallic acid stock solution by weighed gallic acid 0.0100 g, dissolved in absolute ethanol and adjusted to 10 ml in volumetric flask. Gallic acid stock solution was dilute with absolute ethanol to obtain the series concentrations which presented in Table A.5.

Table A.5 Preparation of the series gallic acid stock solutions

Concentration of gallic acid (µg/ml)	Volume of gallic acid stock solution (µl)	Volume of absolute ethanol (µl)
10	10	990
50	50	950
100	100	900
200	200	800
300	300	700
400	400	600
500	500	500

The entire solutions were reacted with folin-ciocalteu reagent and 2 % $NaCO_3$ solution and measured for their visible absorbance by using microtitre plate reader at 750 nm for 2 hours. All measurements were carried out in triplicate. The absorbance of the solutions (Table A.6) was plotted as calibration curve of standard gallic acid (Figure A.3). The representative regression coefficient (r^2) was 0.9992 and the linear regression equation was $y = 0.0317x + 0.0171$.

Table A.6 Absorbance value of gallic acid standard

Final conc. (µM)	Absorbance of gallic acid standard at 750 nm for 2 hours			
	1	2	3	Mean ± SD
1	0.165	0.168	0.166	0.166 ± 0.005
5	0.360	0.352	0.351	0.354 ± 0.002
10	0.674	0.668	0.673	0.672 ± 0.005
20	0.951	0.945	0.954	0.950 ± 0.003
30	1.285	1.278	1.282	1.282 ± 0.004
40	1.537	1.494	1.558	1.530 ± 0.033
50	1.866	1.844	1.892	1.867 ± 0.024

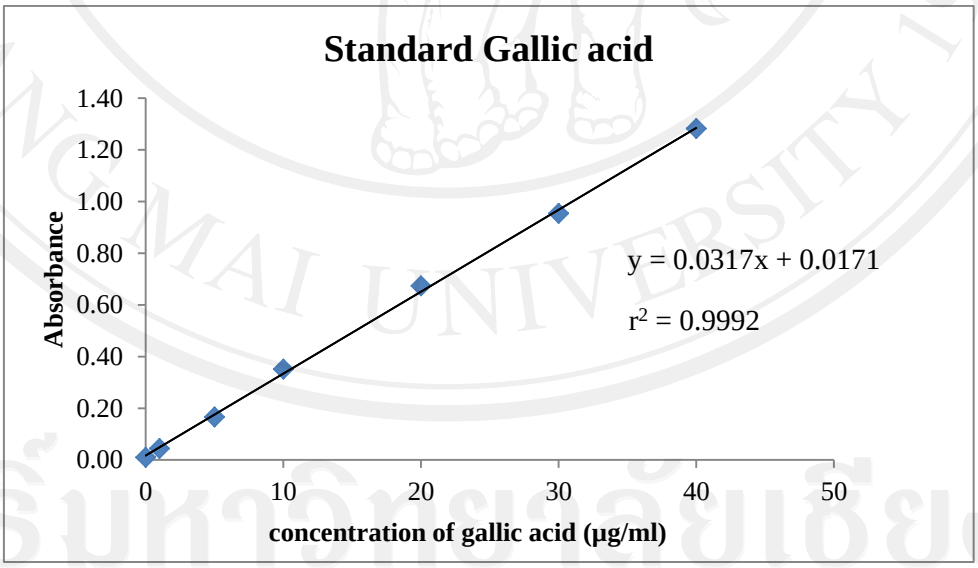

Figure A.3 standard curve of gallic acid

Standard curve of quercetin for aluminium chloride colorimetric assay

The preparation of 200 µg/ml gallic acid stock solution by weighed quercetin 0.0020 g, dissolved in absolute ethanol and adjusted to 10 ml in volumetric flask. quercetin stock solution was dilute with absolute ethanol to obtain the series concentrations which presented in Table A.7.

Table A.7 Preparation of the series quercetin stock solutions

Concentration of quercetin (µg/ml)	Volume of quercetin stock solution (µl)	Volume of absolute ethanol (µl)
1	5	995
5	25	975
10	50	950
20	100	900
40	200	800
60	300	700
80	400	600
100	500	500
200	200	0

The entire solutions were reacted with 10% $AlCl_3$, 1 M CH_3COOK, and DI water. They measured for their visible absorbance by using UV-vis spectrophotometer at 415 nm for 30 min. All measurements were carried out in triplicate. The absorbance of the solutions (Table A.8) was plotted as calibration curve of standard quercetin

(Figure A.4). The representative regression coefficient (r^2) was 0.9996 and the linear regression equation was y = 0.0596x + 0.0069.

Table A.8 Absorbance value of quercetin standard

Final conc. (µM)	Absorbance of quercetin standard at 415 nm for 30 min			
	1	2	3	Mean ± SD
0.1	0.011	0.011	0.011	0.011 ± 0.000
0.5	0.041	0.042	0.042	0.042 ± 0.001
1	0.070	0.069	0.069	0.069 ± 0.001
2	0.121	0.121	0.121	0.121 ±0.000
4	0.243	0.243	0.243	0.243 ±0.000
6	0.361	0.36	0.36	0.360 ± 0.001
8	0.499	0.498	0.498	0.498 ± 0.001
10	0.593	0.593	0.592	0.593 ± 0.001
20	1.200	1.200	1.200	1.200 ± 0.000

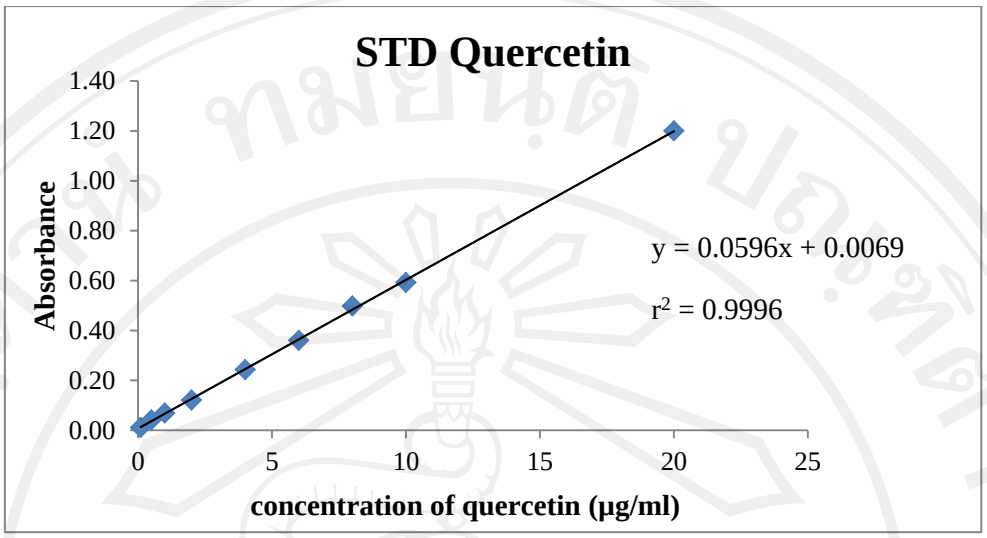

Figure A.4 Standard curve of quercetin

APPENDIX B

EXAMPLE OF CALCULATION IN ANTIOXIDANT ACTIVITY

TEAC value from ABTS assay

Sample = 1 mg/ml, Final concentration = 0.1 mg/ml

Abs_{sample} = 0.482, $Abs_{control}$ = 0.875

From equation:

$$\% \text{ inhibition} = \frac{(Abs_{control} - Abs_{sample}) \times 100}{Abs_{control}}$$

$$\% \text{ inhibition} = \frac{(0.875-0.482) \times 100}{0.875}$$

$$= 44.91\ \%$$

Weight of sample in 200 µl:

The solution 1000 µl contained the sample 0.1 mg

The solution 200 µl contained the sample 0.02 mg

% inhibition/ 1 mg of sample:

The sample 0.02 mg has % inhibition 41.91 %

The sample 1 mg has % inhibition 2095.5 %

TEAC value (mM/mg extract):

Replaced % inhibition/ 1 mg of sample as y variable in equation from calibration curve of Trolox:

From equation y = 1.9913x - 0.8864

2095.5 = 1.9913x - 0.8864

x = 1052.77 µM/mg extract

x = 1.053 mM/mg extract

EC value from FRAP assay

Sample = 1 mg/ml, Final concentration = 0.1 mg/ml

Abs_{sample} = 0.482

Weight of sample in 200 µl:

The solution 1000 µl contained the sample 0.1 mg

The solution 200 µl contained the sample 0.02 mg

Absorbance/ 1 mg of sample:

The sample 0.02 mg has absorbance 0.482

The sample 1 mg has absorbance 24.1

EC value (mM/mg extract):

Replaced absorbance/ 1 mg of sample as y variable in equation from calibration curve of $FeSO_4.7H_20$:

From equation y = 0.002x + 0.0035

24.1 = 0.002x + 0.0035

x = 12048.25 µM/mg extract

x = 12.048 mM/mg extract

GAE value from Folin-ciocalteu assay and QE value from aluminium chloride colorimetric assay

For example:

Sample = 1 mg/ml, Final concentration = 0.1 mg/ml

Abs_{sample} = 0.482

Weight of sample in 200 µl:

The solution 1000 µl contained the sample 0.1 mg

The solution 200 µl contained the sample 0.02 mg

GAE value (mg/mg extract):

Replaced absorbance of sample as y variable in equation from calibration curve of Gallic acid:

From equation y = 0.0317x + 0.0171

0.482 = 0.002x + 0.0035

x = 239.25 µg/ml

Calculated GAE value as weight:

The solution 1000 µl contained the sample 239.25 µg

The solution 200 µl contained the sample 47.85 µg

Calculated GAE value as mg/ g extract:

$$\text{There is GAE value} = \frac{47.85\ \mu g}{0.002\ g}$$

$$= 21425\ \mu g/\ g\ \text{extract}$$

$$= 21.23\ \mu g/\ g\ \text{extract}$$

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Oral presentation	**Rinrampai Puttipan**, Sunee Chansakaow, Malyn Chulasiri, Siriporn Okonogi. *In Vitro* Antioxidant Activity of Selected Thai Medicinal Plant Extracts. Oral presentation at 1st Asian Plus Three Graduate Research Congress, March 1-2, 2012. The Empress Hotel, Chiang Mai, Thailand.