APPENDIX A

Medium and chemical solution

1. Rose Bengal agar (Atlas, 2010)

Agar	15.0 g
Glucose	10.0 g
Malt extract agar	20.0 g
Rose Bengal	0.05 g
Chloramphenicol	10.0 ml

Preparation of chloramphenicol solution

Add 0.10 g of chloramphenicol to distilled water and bring volume to 10.0 ml. Mix thoroughly and filter sterilize.

Preparation of medium

Add components, except chloramphenicol solution, to distilled water and bring the volume to 990 ml and sterilized in autoclave at 121°C for 15 min. Cool to 45−50°C and aseptically add sterile the chloramphenicol solution.

2. 0.004% DPPH solution (Molyneux, 2009)

0.004% DPPH solution was prepared in methanol by dissolving 4 mg of DPPH in methanol and made up to 100 ml with ethanol. The solution was kept in darkness.

APPENDIX B

Calculation

1. Percentage yield of RMR

$$\% \text{ Yield of RMR} = \frac{\text{Weight of RMR after dried (g)}}{\text{Weight of rice before fermentation (g)}} \text{ x } 100\ \%$$

For example

Before SSF	After SSF	After dried
20.00 g	15.38 g	5.71 g

$$\% \text{ Yield of RMR} = \frac{5.71\ (g)}{20.00\ (g)} \text{ x } 100\ \% = 28.55\%$$

2. Monacolin K determination

2.1 Preparation of standard calibration curve

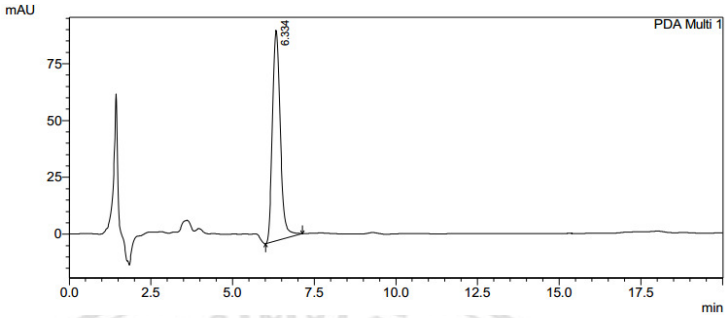
Monacolin K standard was dissolved in 75% ethanol. The initial concentration of standard was 100 ppm and reduced the concentration of a solution by 2-fold dilution (100, 50, 25, 12.5 and 6.25 ppm). All of standard was injected in triplicates time (B-1).

2.2 HPLC Condition

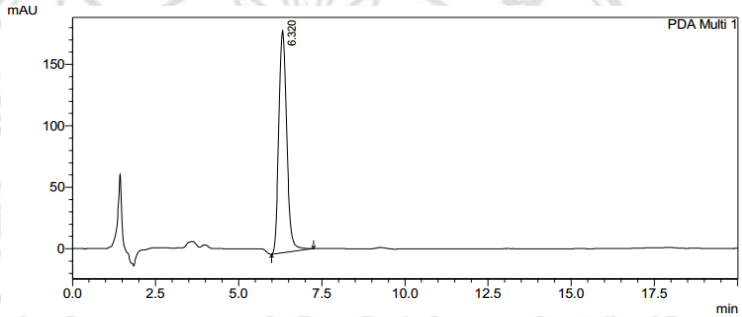
Column : RP-C18 [150 - 2.0 (5µm)]
Mobile phase : Acetonitrile: 0.5% phosphoric acid (65:35, v/v)
Flow rate : 0.7 ml/min
Detector : UV detection at 238 nm
Injection volume : 20 µl

2.3 Chromatogram

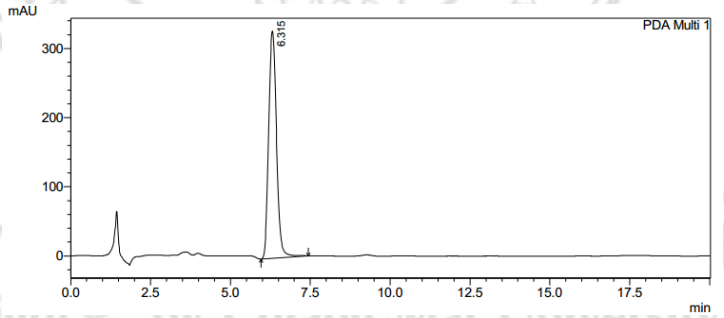
Monacolin K 6.25 ppm



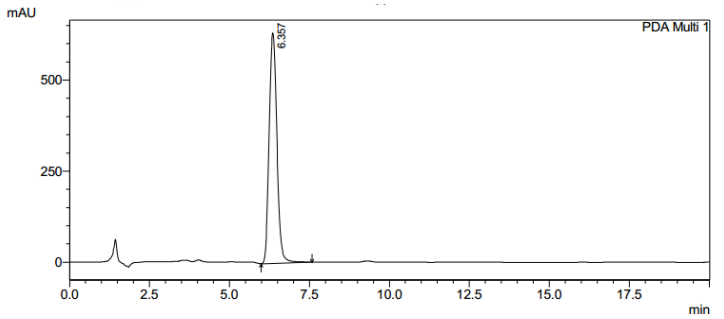
Monacolin K 12.5 ppm



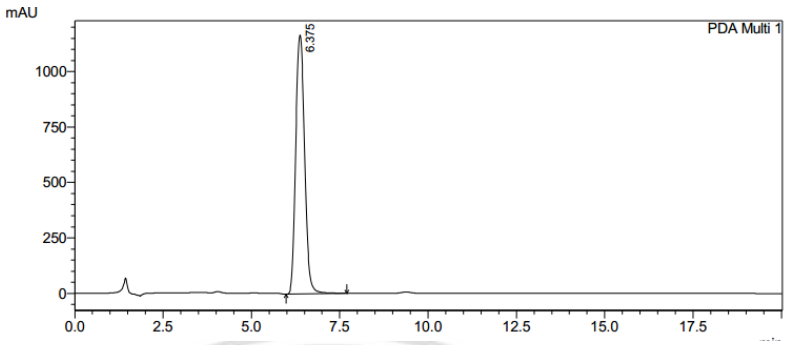
Monacolin K 25 ppm



Monacolin K 50 ppm

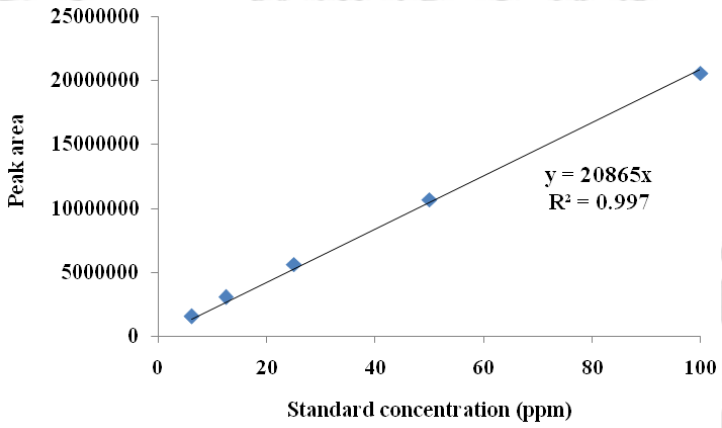


Monacolin K 100 ppm



B-1 The peak area of monacolin K standard

Standard concentration (ppm)	Peak area
6.25	1564334
12.5	3030984
25	5613199
50	10686053
100	20569852

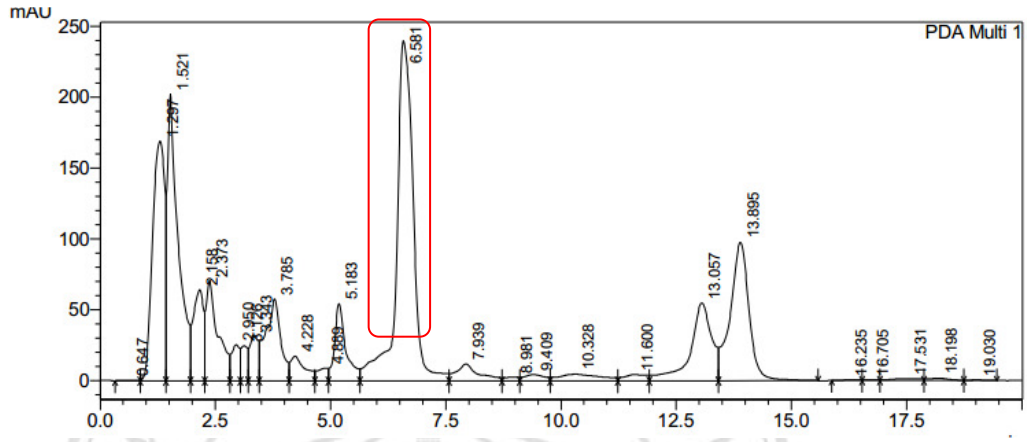


B-2 Linear equation from standard calibration curve for monacolin k determination

Determination of the MK content in the products was carried out by comparing the peak area with the standard peak area that obtained by HPLC at the same condition. From the B-2 showed the linear equation was Y = 20865X, where X was monacolin K content (ppm) and Y was the peak area.

$$\text{Monacolin K (ppm)} = \frac{\text{Peak area}}{20865}$$

For example



This figure showed the chromatogram of monacolin K in RMR product. Peak area of sample was **6023806**, therefore

Monacolin K content $= \frac{\text{Peak area}}{20865}$

$= \frac{6023806}{20865}$

= 288.70 ppm

1000 ml of extracts had MK = 288.70 mg

1 ml of extracts had MK $= \frac{288.70}{1000}$ = 0.2887 mg

50 ml of extracts had MK = 0.2887 X 50 = 14.435 mg

0.5 g of sample had MK = 14.435 mg

1.0 g of sample had MK $= \frac{14.435}{0.5}$ = 28.87 mg

Sample had MK content =28.87 mg/g or 28870 ppm

3. Citrinin determination

3.1 Preparation of standard calibration curve

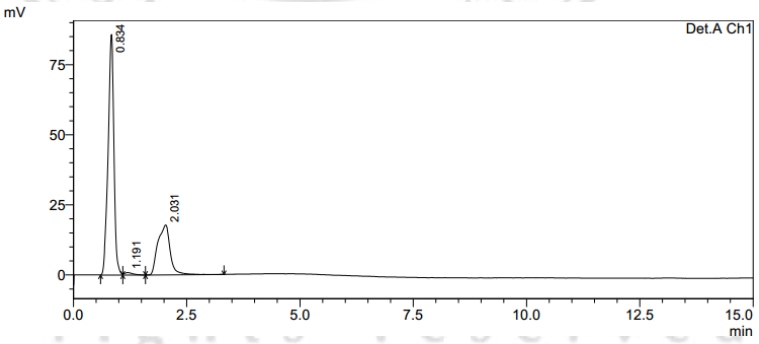
Citrinin standard was dissolved in 75% ethanol. The initial concentration of standard was 100 ppm and reduced the concentration of a solution by 2-fold dilution (100, 50, 25, 12.5 and 6.25 ppm). All of standard was injected in triplicates time (B-3).

3.2 HPLC Condition

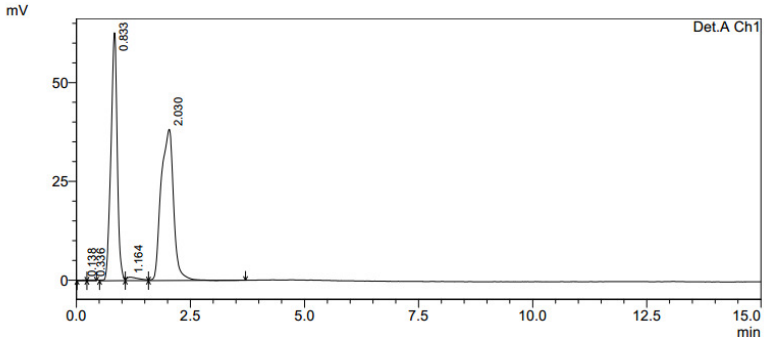
Column : RP-C18 [150 - 2.0 (5µm)]
Mobile phase : Acetonitrile: Water: Triflouacetate (550:450:0.5 v/v/v)
Flow rate : 0.6 ml/min
Detector : Fluorescent detection excitation at 330 nm emission at 500 nm
Injection volume : 20 µl

3.3 Chromatogram

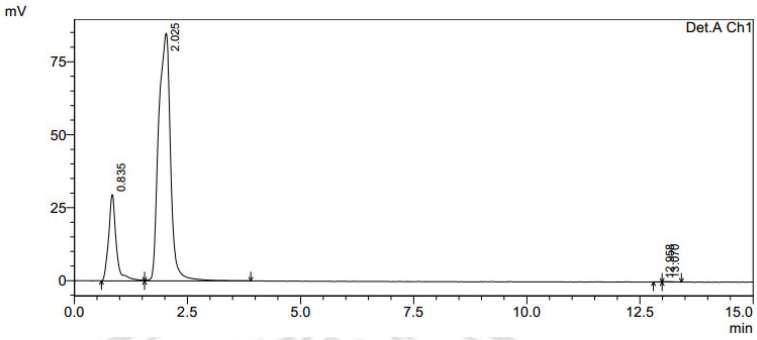
Citrinin 125 ppb



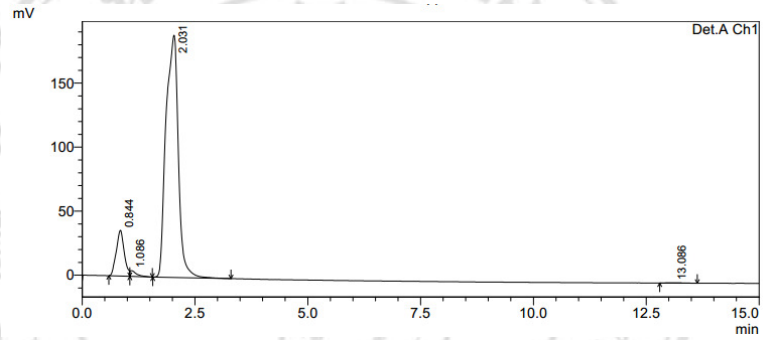
Citrinin 250 ppb



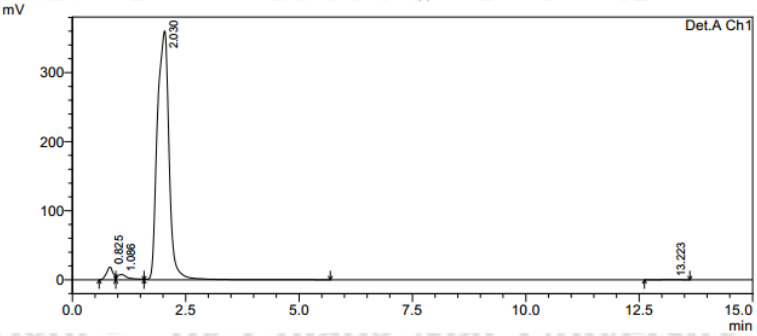
Citrinin 500 ppb



Citrinin 1000 ppb

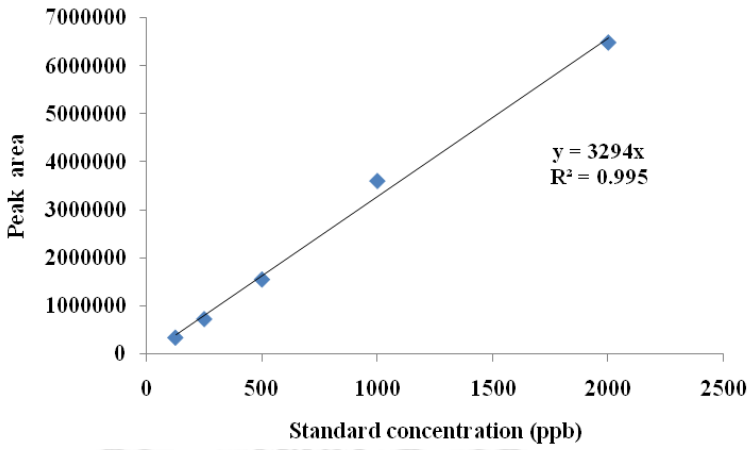


Citrinin 2000 ppb



B-3 The peak area of citrinin standard

Standard concentration (ppb)	Average peak area
125	334976
250	721635
500	1545470
1000	3596706
2000	6479671

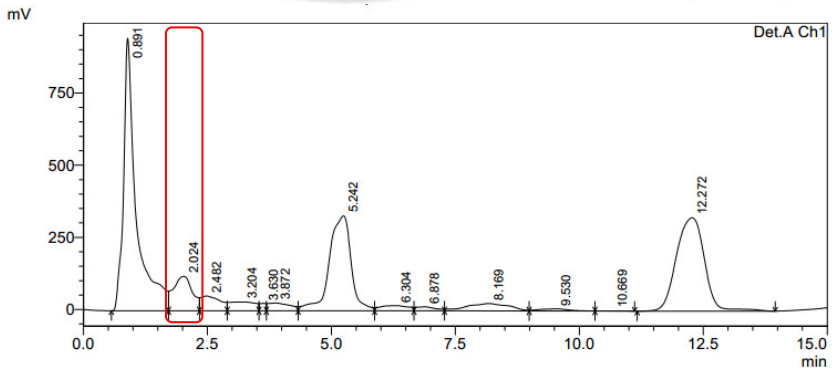


B-4 Linear equation from standard calibration curve for citrinin determination

Determination of the citrinin content in the products was carried out by comparing the peak area with the standard peak area that obtained by HPLC at the same condition. From the B-4 showed the linear equation was Y = 3294X, where X was citrinin content (ppb) and Y was the peak area.

$$\text{Citrinin (ppb)} = \frac{\text{Peak area}}{3294}$$

For example



This figure showed the chromatogram of citrinin in RMR product. Peak area of sample was **3163258**, therefore

$$\text{Citrinin content} = \frac{\text{Peak area}}{3294}$$

$= \frac{3163258}{3294}$

= 960.30 ppb

1000 ml of extracts had citrinin = 960.30 mg

1 ml of extracts had citrinin $= \frac{960.30}{1000}$ = 0.9603 mg

1.0 g of sample had citrinin = 0.9603 mg/g

Sample had citrinin content =0.9603 ppm or 960.3 ppb

4. Percentage of antioxidant activity calculation

$$\% \text{ Antioxidant activity} = 100 - \left[\frac{Abs_{sample} - Abs_{blank}}{Abs_{control}}\right] \times 100\%$$

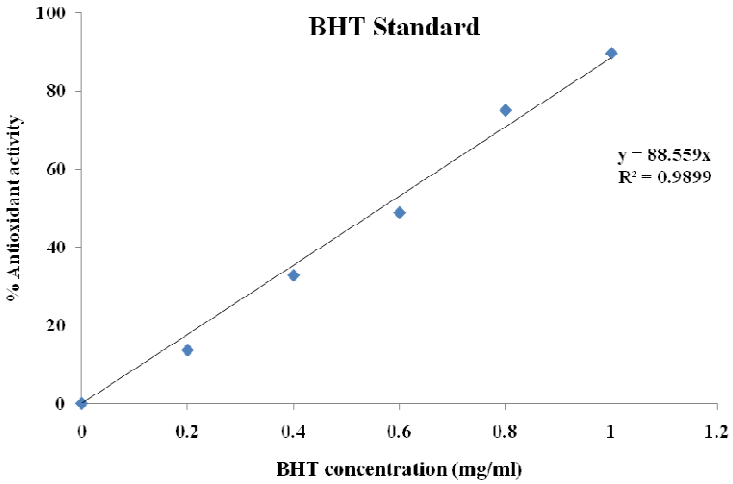
$Abs_{control}$ = Absorbance of control (DPPH 2ml + methanol 1ml)

Abs_{sample} = Absorbance of sample (DPPH 2ml + extracts 1 ml)

Abs_{blank} = Absorbance of extract (methanol 2ml + extracts 1 ml)

B-5 Percentage of antioxidant activity of BHT

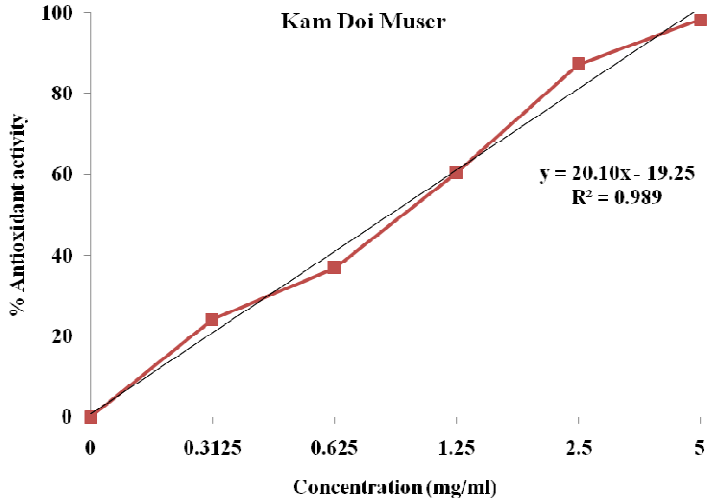
Concentration (mg/ml)	% Antioxidant activity
0	0
0.2	13.61
0.4	32.77
0.6	48.84
0.8	75.02
1	89.68

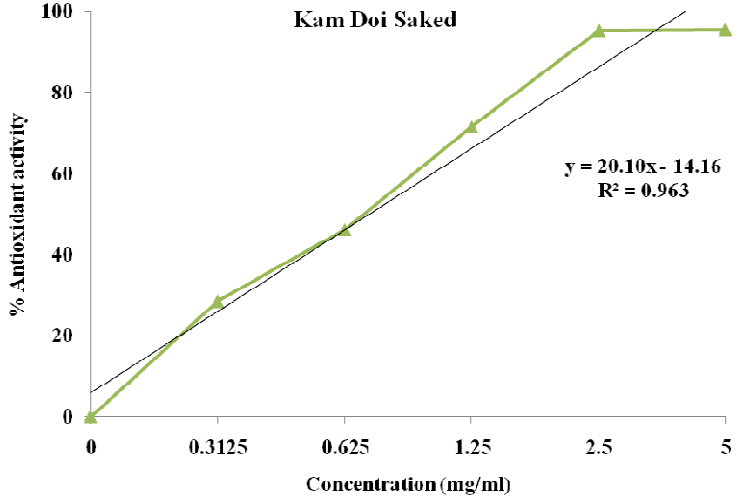


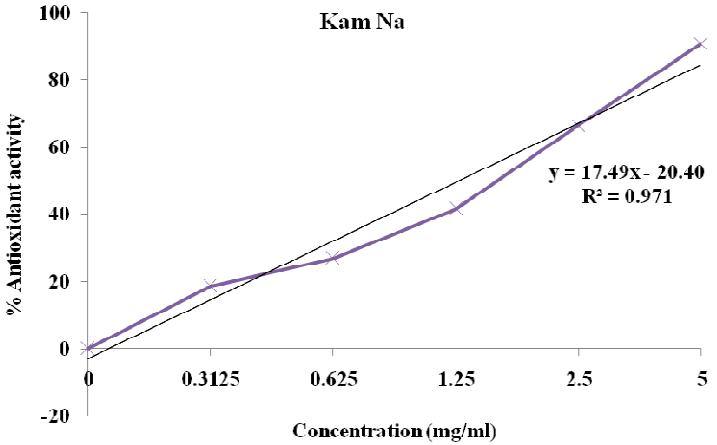
B-6 Linear equation from standard calibration curve between the concentration of BHT and % antioxidant activity.

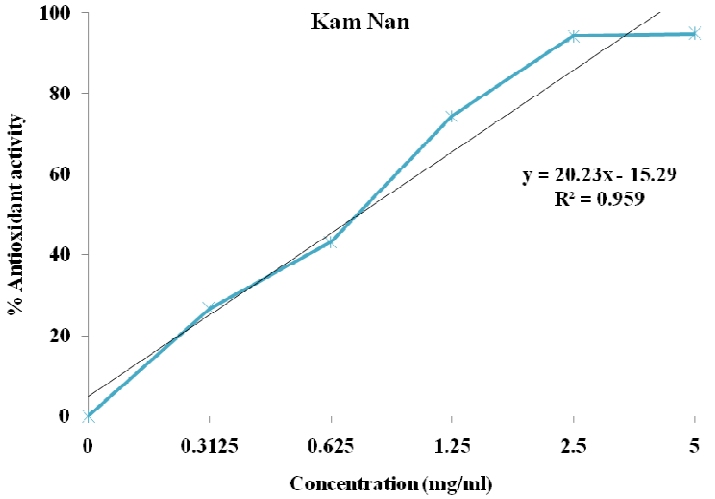
Table 4 Percentage of antioxidant activity of RMR

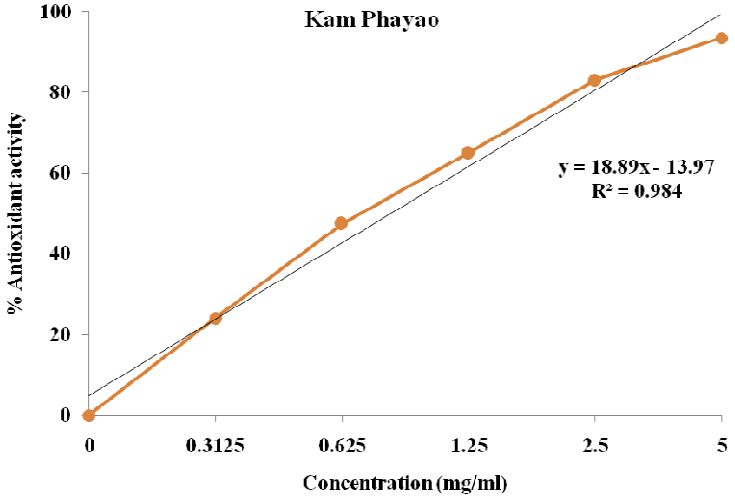
Concentration (mg/ml)	% Antioxidant activity					
	Doi Muser	Doi Saked	Na	Nan	Phayao	Hom CMU
0	0.00	0.00	0.00	0.00	0.00	0.00
0.3125	24.08	28.53	18.82	26.61	24.09	25.39
0.625	36.92	46.23	27.01	43.20	47.45	39.15
1.25	60.34	71.57	41.68	74.30	64.92	60.34
2.5	87.15	95.35	66.51	94.28	83.00	87.10
5	98.23	95.60	90.90	94.84	93.40	96.16

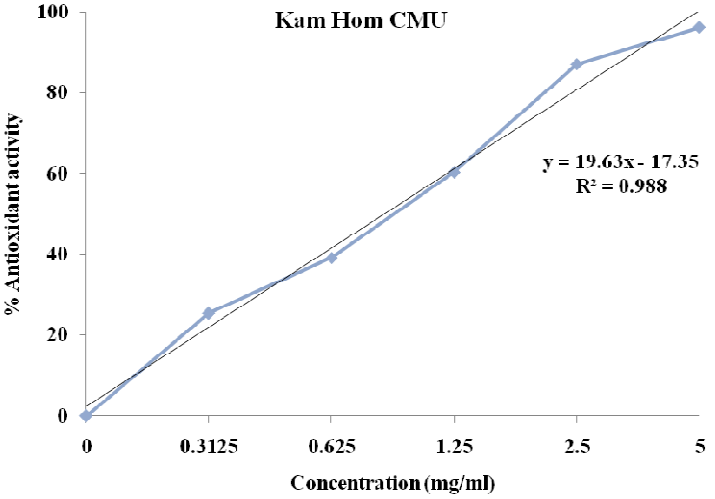





Kam Na
% Antioxidant activity
Concentration (mg/ml)
y = 17.49x - 20.40
R² = 0.971
100
80
60
40
20
0
-20
0
0.3125
0.625
1.25
2.5
5


Kam Nan
% Antioxidant activity
Concentration (mg/ml)
y = 20.23x - 15.29
R² = 0.959
100
80
60
40
20
0
0
0.3125
0.625
1.25
2.5
5


Kam Phayao
% Antioxidant activity
Concentration (mg/ml)
y = 18.89x - 13.97
R² = 0.984
100
80
60
40
20
0
0
0.3125
0.625
1.25
2.5
5


Kam Hom CMU
% Antioxidant activity
Concentration (mg/ml)
y = 19.63x - 17.35
R² = 0.988
100
80
60
40
20
0
0
0.3125
0.625
1.25
2.5
5

5. Cholesterol quantification

5.1 Cholesterol standard preparation

Dilute the Cholesterol Standard to a concentration of 0.5 µg/µl by adding 20 µl Cholesterol Standard to 180 µl Cholesterol Reaction Buffer; mix well. Add the indicated volumes of diluted Cholesterol Standard and Cholesterol Reaction Buffer (Table 5) to individual designated standard wells.

Table 5. Cholesterol standard preparation

Volume of 0.5 µg/µl Diluted Cholesterol Standard	Volume of Cholesterol Reaction Buffer	Final Concentration of Cholesterol
0 µl	50 µl	0 µg
4 µl	46 µl	2 µg
8 µl	42 µl	4 µg
12 µl	38 µl	6 µg
16 µl	34 µl	8 µg
20 µl	30 µl	10 µg

5.2 Sample preparation

Egg yolks were completely separated from the albumen. 25 µL of yolk was mixed using vortex in 475 µL of buffer (chloroform: isopropanol: NP40; 7: 11: 0.1) and centrifuged at 12000 rpm for 10 min. Transfer all of the liquid (organic phase) avoiding the pellet, to a new tube and dry at 50°C to remove chloroform.

5.3 Reaction mix preparation and calculation

The total volume of reaction mix in each well is 50 µl.

- 44 µl Cholesterol Reaction Buffer
- 2 µl Cholesterol Probe
- 2 µl Enzyme Mix
- 2 µl Cholesterol Esterase

1. Add 50 µl standards and samples (as prepared above) to designated wells.
2. Add 50 µl Reaction Mix to each well and incubate for 1 h at 37°C. Protect the plate from light during the incubation.
3. Measure the absorbance at 570 nm for colorimetric detection (Table 6).

Table 6. The absorbance at 570 nm

Concentration of Cholesterol	Average absorbance at 570 nm
0 µg	0.000
2 µg	0.188
4 µg	0.357
6 µg	0.505
8 µg	0.610
10 µg	0.772

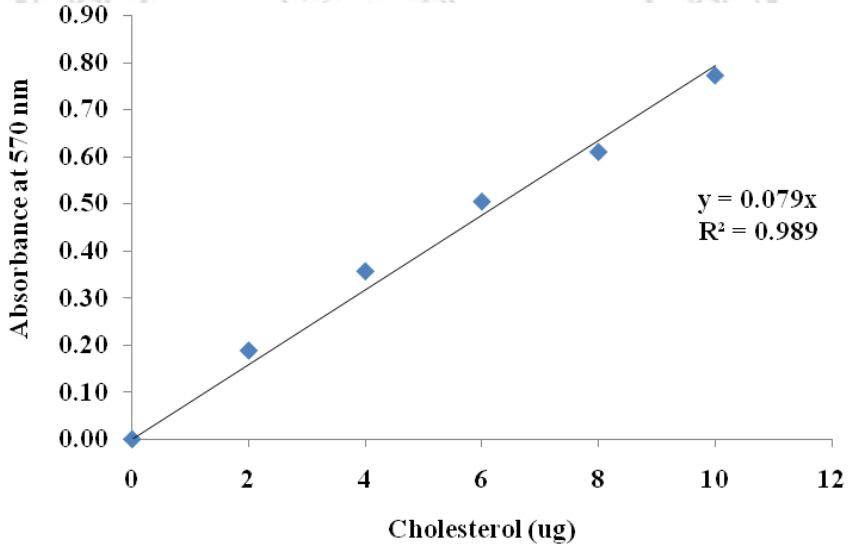


Figure 6. Linear equation from standard calibration curve between the concentration of cholesterol and absorbance at 570 nm.

From the Figure 6 showed the linear equation was Y = 0.079X, where X was cholesterol content (µg) and Y was the absorbance at 570 nm

$$\text{Cholesterol content (µg)} = \frac{\text{absorbance}}{0.079}$$

For example

Sample O.D. = 0.868

Dilution 4X

$$Y = 0.079X$$

$$\text{Cholesterol content} = \frac{0.868}{0.079} \times 4$$

Cholesterol content = 43.95 µg

50 µl of sample had 2.5 µl of yolk

2.5 µl of yolk sample had cholesterol = 43.95 µg

$$\text{1000 µl of yolk sample had cholesterol} = \frac{1000}{2.5} \times 43.95 = 17579 \text{ µg}$$

1.0 g yolk sample had cholesterol = 17579 µg

Sample had cholesterol content = 17579.2 µg/g or 17.58 mg/g

CURRICULUM VITAE

Author's Name	Miss Prodpran Pengnoi
Date of birth	July 19th, 1988
Place of birth	Nakhonsawan Province, Thailand
Education	**2003-2005** Secondary high school, Lasalle Chotiravi Nakhonsawan**,** Nakhonsawan Thailand
	2006-2009 Bachelor of Science (Microbiology) 1st Class Honor, Faculty of Science, Chiang Mai University, Thailand
	2010-2016 Doctor of Philosophy (Applied microbiology) Faculty of Science, Chiang Mai University, Thailand
Scholarship	**2006-2009** Petch Thong Guaw Scholarship, Faculty of Science, Chiang Mai University, Thailand
	2009 Industrial and Research Projects for Undergraduate Students (IRPUS)
	2010-2016 The Royal Golden Jubilee Ph.D. Program
	2011 Japan Society for the Promotion of Science, Japan
Experience	**March- May 2009** Work training in Intellectual Property section**,** Institute of Food Research and Product Development