LIST OF PUBLICATIONS

1) Pukumpuang, W. and Tragoolpua, Y. Inhibition of pathogenic bacteria causing skin disease by some medicinal plant extracts. Proceeding, the 22nd Annual meeting of the Thai society for biotechnology (TSB 2010 international conferences on biotechnology for health living), Trang, Thailand, October 20-22, 2010, 1263-1268.

2) Pukumpuang, Y., Thongwai, N. and Tragoolpua, Y. 2012. Total phenolic contents, antibacterial and antioxidant activities of some Thai medicinal plant extracts. Journal of Medicinal Plants Research. 6(35); 4953-4960.

3) Pukumpuang, W., Chansakaow, S. and Tragoolpua, Y. 2014. Antioxidant activity, phenolic compound content and phytochemical constituents of *Eclipta prostrata* (Linn.) Linn. Chiang Mai Journal of Science. 41: 1-9. (Accepted September 6, 2013).

APPENDIX A

Culture Media

Brain heart infusion (BHI) agar

Beef heart	250	g
Calf brains	200	g
Dextrose	5	g
Disodium phosphate	2.5	g
Proteose peptone	10	g
Sodium chloride	5	g
Agar	15	g
Distilled water	1,000	ml

Dispensed into containers and autoclaved at 121°C for 15 minutes

Mueller Hinton agar (MHA)

Acid digest of casein	17.5	g
Beef extract	2	g
Soluble starch	1.5	g
Agar	15	g
Distilled water	1,000	ml

Dispensed into containers and autoclaved at 121°C for 15 minutes

Nutrient Agar (NA)

Beef extract	3	g
Peptone	5	g
Agar	15	g
Distilled water	1,000	ml

Dispensed into containers and autoclaved at 121°C for 15 minutes

APPENDIX B

Chemical Reagents for DNA Extraction and Agarose Gel Electrophoresis

EDTA (0.5 M)

EDTA	18.6	g
Distilled water	80	ml

The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to ≈ 8.0. Adjust the volume to 100 ml and autoclaved at 121°C for 15 minutes.

Tris-HCl (1M)

Tris base	121	g
Distilled water	800	ml

Adjusted the pH to 7.4 by adding conc. HCl and adjust the volume to 1000 ml and autoclaved at 121°C for 15 minutes.

TE buffer (10 mM Tris-Cl, pH 7.4, 1 mM EDTA)

1M Tris-Cl pH 7.4	10	ml
0.5M EDTA pH 8.0	2	ml

Adjusted the volume to 1000 ml and autoclaved at 121°C for 15 minutes.

Proteinase K (20 mg/ml)

Proteinase K	20	mg
Distilled water	1	ml

Sodium dodecyl sulfate (SDS), 10% (w/v)

SDS	10	g
Distilled water	80	ml

Adjusted the volume to 100 ml

Nacl (5M)

NaCl	292	g
Distilled water	80	ml

Adjusted the volume to 100 ml and autoclaved at 121°C for 15 minutes.

Chloroform/isoamyl alcohol (24:1)

Chloroform	24	ml
Isoamyl alcohol	1	ml

Phenol/chloroform/ isoamyl alcohol (25:24:1)

Phenol	25	ml
Chloroform	24	ml
Isoamyl alcohol	1	ml

TAE buffer (50X)

Tris base	242	g
Glacial acetic acid	57.1	ml
0.5 M EDTA pH8	100	ml

Added distilled water to 1000 ml

Ethydium bromide

5mM EtBr	60	µl
1xTAE buffer	250	µl

Mixed and kept in the dark

Loading dye (6X)

Bromophenol blue	0.25	mg
Glycerol	3	ml
5x TAE buffer	1	ml

Adjusted to volume 10 ml using distilled water, keep at -20℃

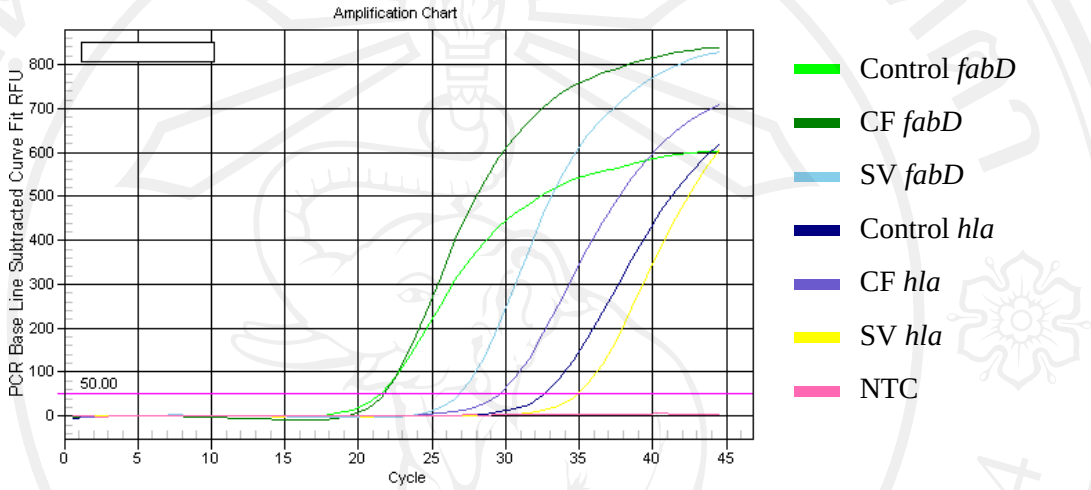
Agarose (0.8%)

Agarose	0.8	g
Distilled water	100	ml

APPENDIX C

Real Time PCR

A



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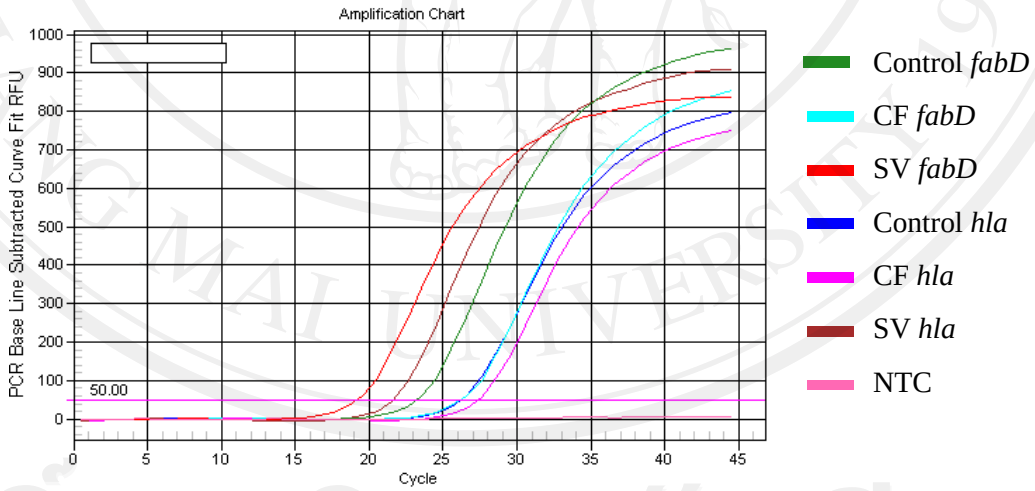
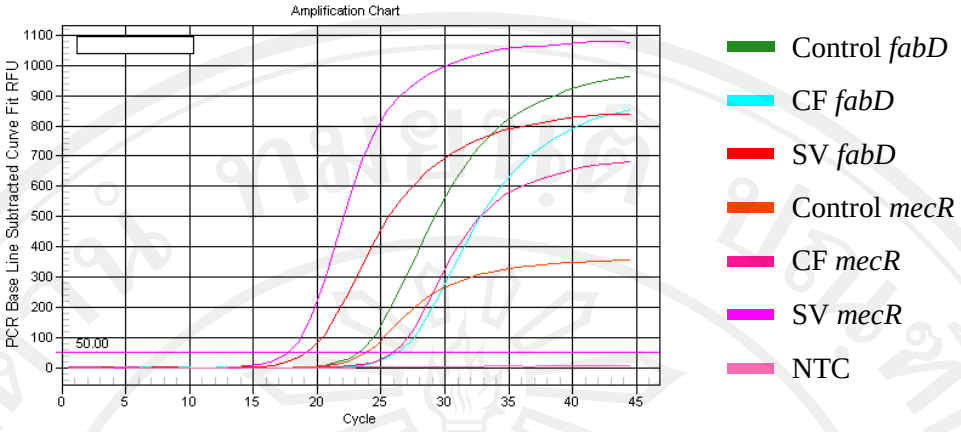


Figure C.1 Fluorescence curve from SYBR Green I detection of *hla* gene in *S. aureus* (A) and MRSA (B) after treatment with *C. fenestratum* and *S. venosa* extracts

A



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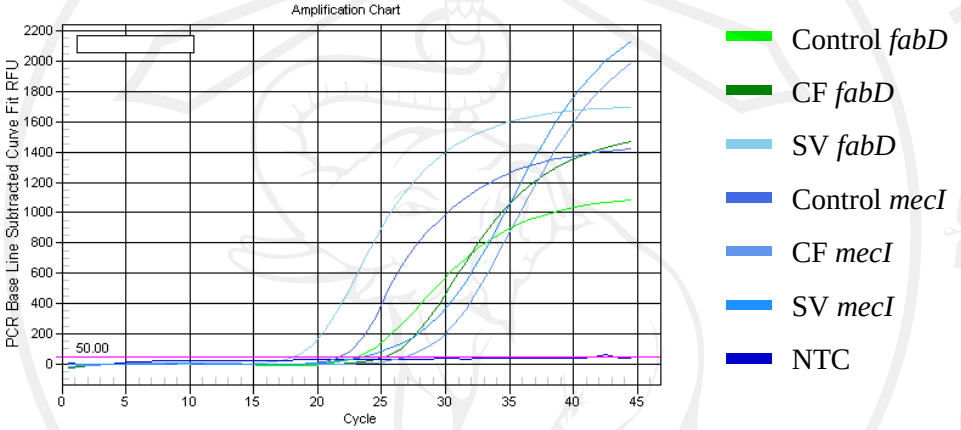

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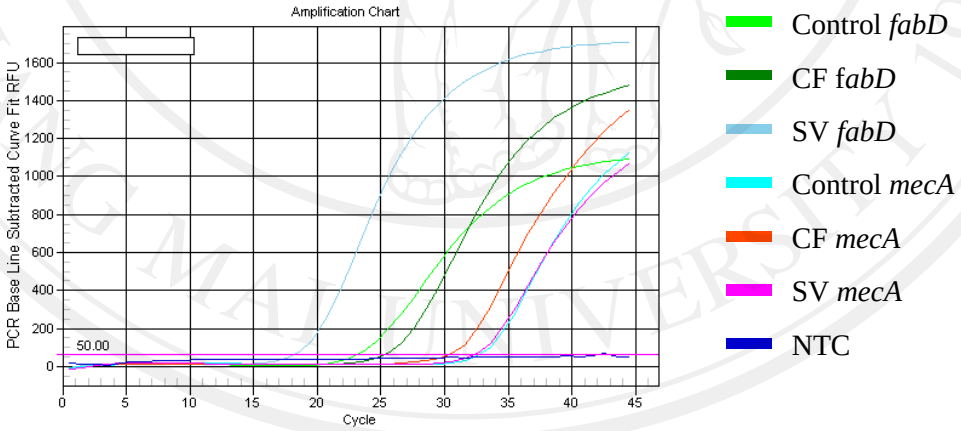
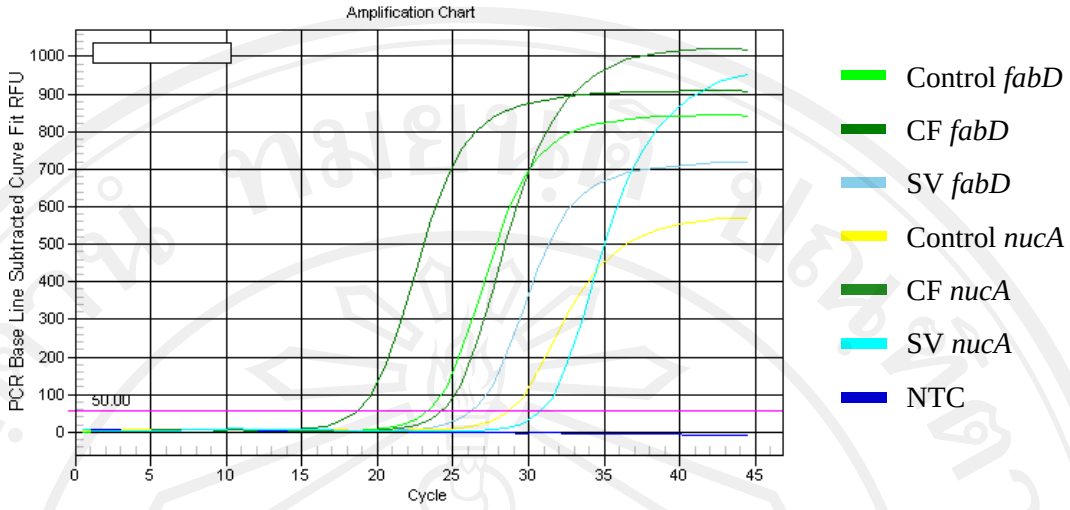


Figure C.2 Fluorescence curve from SYBR Green I detection of *mecR* (A), *mecI* (B) and *mecA* (C) gene in MRSA after treatment with *C. fenestratum* and *S. venosa* extracts

A



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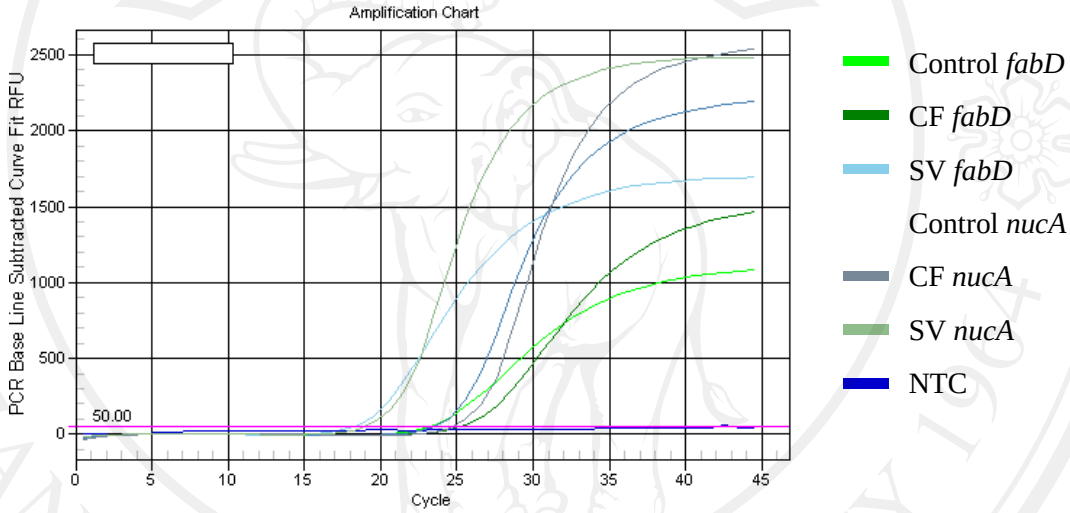

Figure C.3 Fluorescence curve from SYBR Green I detection of *nucA* gene in *S. aureus* (A) and MRSA (B) after treatment with *C. fenestratum* and *S. venosa* extracts

APPENDIX D

Chemical Reagents for Western Blotting

Tris-HCl, pH 8.8 (1.5 M)

Tris	18.17	g
SDS	0.40	g

Adjusted pH to 8.8 with Conc. HCl and then adjusted volume to 100 ml with demonized water

Tris-HCl, pH 6.8 (0.5 M)

Tris	6.06	g
SDS	0.40	g

Adjusted pH to 8.8 with Conc. HCl and then adjusted volume to 100 ml with deionized water

Running buffer (1X)

Tris base	3.02	g
Glycein	14.40	g
SDS	1	g

Adjusted volume to 1000 ml

APS (10%)

Ammonium persulfate	1	g
Deionized water	10	ml

Separating gel, pH 8.8 (10%)

40% acrylamide	2.50	ml
1.5 M Tris Cl	2.50	ml
Distilled water	4.90	ml
10% APS	50	µl
TEMED	10	µl

Stacking gel, pH 8.8 (5%)

40% acrylamide	0.375	ml
1.5 M Tris Cl	1.25	ml
Distilled water	3.35	ml
10% APS	50	µl
TEMED	5	µl

Protein staining solution

Coomassie brilliant blue R-250	1	g
Methanol	500	ml
Glacial acetic acid	74	ml

Adjust volume to 1000 ml

Destain solution

Methanol	100	ml
Glacial acetic acid	100	ml

Adjusted volume to 1000 ml

Tris buffer saline, TBS (10X)

Tris base	24.2	g
NaCl	80	g

Adjusted the pH to 7.6 by adding conc. HCl and adjusted the volume to 1000 ml and autoclaved at 121°C for 15 minutes.

Wash buffer (TBS-T) (1xTBS, 0.1% Tween20)

1xTBS	100	ml
Tween20	0.1	ml

Skim milk (5%)

TBS-T	100	ml
Skim milk	5	g

Skim milk (1%)

TBS-T	100	ml
Skim milk	1	g

Transfer buffer (Towbin buffer)

Tris base	3.03	g
Glycein	14.4	g
SDS	0.5	g
Methanol	20	ml

Adjusted volume to 100 ml

Phosphate buffer saline, PBS (10X)

NaCl	80	g
KCl	2	g
Na_2HPO_4	11.07	g
KH_2PO_4	2.40	g

Dissolved and adjusted volume to 1 L with deionized water. Autoclaved at 121 °C for 15 minutes

Bradford reagent

Comasie brilliant blue G-250	10	mg
95% ethanol	5	ml
85% phosphoric acid	10	ml

Adjusted volume to 100 ml

APPENDIX E

Chemical Reagents for Antioxidant Activity Test

1. Chemical reagents for ABTS decolorization assay

ABTS (7mM)

ABTS [2.2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)]	0.0384	g
95% ethanol	10	ml

$K_2S_2O_8$ (140 mM)

Potassium persulfate	0.3784	g
Deionized water	10	ml

Working solution (ABTS radical cation solution)

7 mM ABTS	10	ml
140 mM $K_2S_2O_8$	176	ml

Stored in dark at room temperature

2. Chemical reagents for DPPH radical scavenging assay

DPPH solution (0.1 mM)

DPPH	0.0039	g
Methanol	100	ml

3. Chemical reagents for FRAP assay

Acetate buffer, pH 3.6 (300 mM)

Sodium acetate	3.1	g
Glacial acetic acid	16	ml
Demonized water	900	ml

Check pH 3.6, adjusted volume to 1000 ml with deionized water and stored at 4 °C

HCl (40mM)

Conc. HCl	1.46	ml

Adjusted volume to 1000ml with deionized water and stored at room temperature

TPTZ solution

TPTZ	0.031	g
40mM HCl	10	ml

Dissolved at 50 °C and made fresh day of assay in new corning tube

Ferric chloride solution

$FeCl_3.6H_2O$	0.054	g
Distilled water	10	ml

FRAP reagent

Acetate buffer	100	ml
TPTZ solution	10	ml
Ferric chloride solution	10	ml
Distilled water	12	ml

Mixed and kept at 37 °C

4. Chemical reagents for total phenolic content assay

50% Folin-Ciocalteau reagent

Folin-Ciocalteau reagent	50	ml
Distilled water	50	ml

Made fresh day of assay

Sodium carbonate (5%)

Na_2CO_3	5	g
Distilled water	100	ml

5. Chemical reagents for SDS-PAGE in inhibition of oxidative protein damage by medicinal plant extracts

Stacking gel buffer (0.5 M Tris-HCl, pH 6.8)

Tris	30.275	g
SDS	2	g
Deionized water	400	ml

Adjusted pH to 6.8 with Conc. HCl and adjusted volume to 500 ml with deionized water

Separating gel buffer (1.5M Tris-HCl, pH 8.8)

Tris	90.825	g
SDS	2	g
Deionized water	400	ml

Adjusted pH to 8.8 with Conc. HCl and adjusted volume to 500 ml with deionized water

Electrophoresis buffer or tank buffer (0.025 M Tris, 0.192 M Glycine, 0.1% SDS, pH8.3)

Tris	3.02	g
Glycine	14.4	g
SDS	1	g
Deionized water	1000	ml

10% Ammonium persulfate (APS)

APS	0.1	g
Deionized water	1	ml

Protein Staining Solution (0.025% Coomassie Brilliant Blue R250, 40% (v/v) Methanol, 7% (v/v) Glacial Acetic acid)

Coomassie Brilliant Blue	0.125	g
Methanol	200	ml
Glacial Acetic acid	35	ml
Deionized water	500	ml

Filtered with Whatman No. 1 and kept in dark

Destain Solution (40% (v/v) Methanol, 7% (v/v) Glacial Acetic acid)

Methanol	200	ml
Glacial acetic acid	35	ml
Deionized water	500	ml

Adjusted volume to 500 ml with deionized water and kept at room temperature.

Sorrenson's phosphate buffer

Solution A

$Na_2HPO_4.2H_2O$	11.876	g
Deionized water	1000	ml

Solution B

KH_2PO_4	9.08	g
Deionized water	1000	ml

Sorrenson's phosphate buffer, pH 7.3 (1M)

Solution A	77.7	ml
Solution B	22.3	ml

Adjusted pH to 7.3

Sorrenson's phosphate buffer (150 M PBS)

1M Sorrenson's phosphate buffer	7.5	ml

Adjusted volume to 50 ml with deionized water

Bovine serum albumin (BSA) solution (5 mg/ml)

BSA	0.05	g
Deionized water	10	ml

$CuSO_4$ (1mM)

$CuSO_4$	0.16	g

Adjusted volume to 100 ml with deionized water and kept at 4 ºC

H_2O_2 (25M)

30% H_2O_2	285	µl

Adjusted volume to 100 ml with deionized water and kept at 4 ºC

Glutathione, 98%, Reduced form, $C_{10}H_{17}N_3O_6S$ (5 mg/ml)

GSH	0.025	g
150 mM PBS	10	ml

APPENDIX F

Standard Curves of Antioxidant Activity Test

1. Standard curve of ABTS radical scavenging activity using trolox as a standard compound

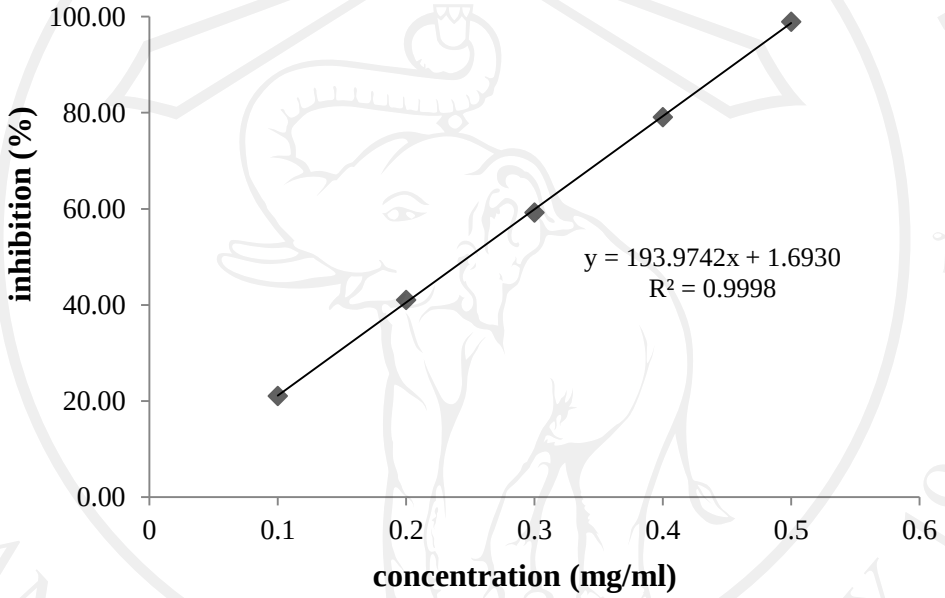

Figure F.1 The dose response curve of percentage of inhibition of radical generated from ABTS by trolox solution after measuring absorbance at 734 nm

2. Standard curve of DPPH radical scavenging activity using gallic acid as a standard compound

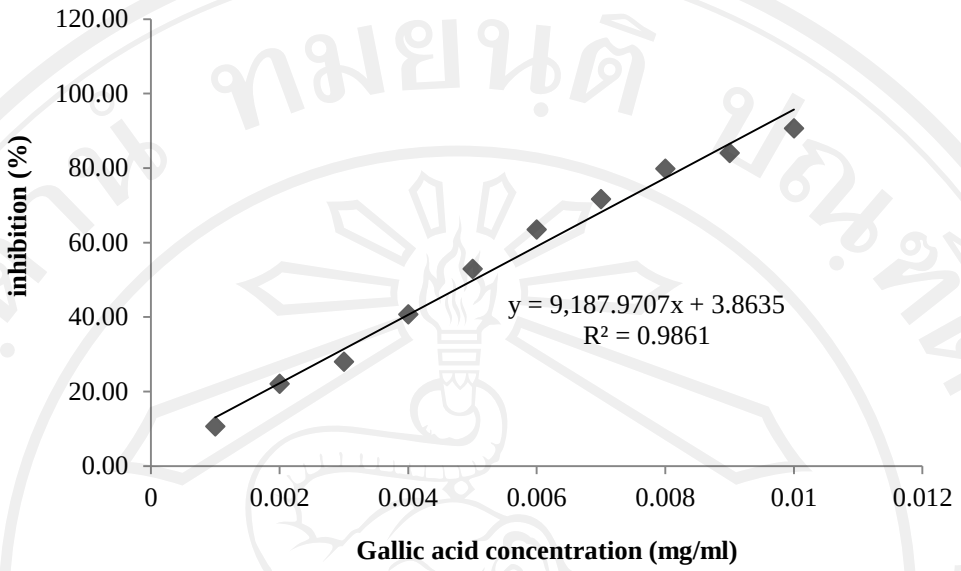

Figure F.2 The dose response curve of percentage of inhibition of radical generated from DPPH by gallic acid solution after measuring absorbance at 517 nm

3. Standard curve of ferric reducing antioxidant power (FRAP) assay using $FeSO_4$ as a standard compound

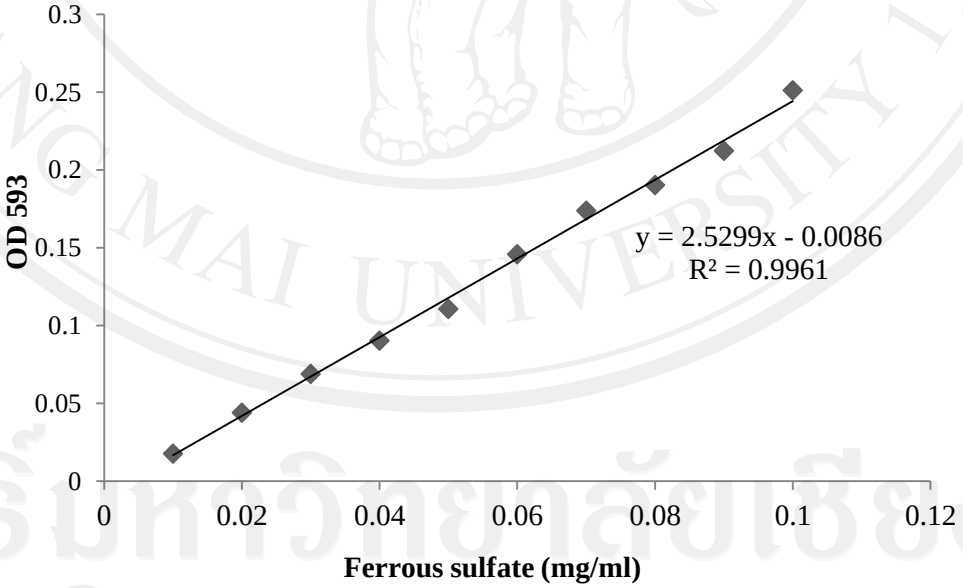

Figure F.3 Calibration curve for the absorbance at 593 nm of FRAP method as a function of concentration of ferric sulfate standard solution

4. Standard curve of total phenolic compound content test using gallic acid as a standard compound

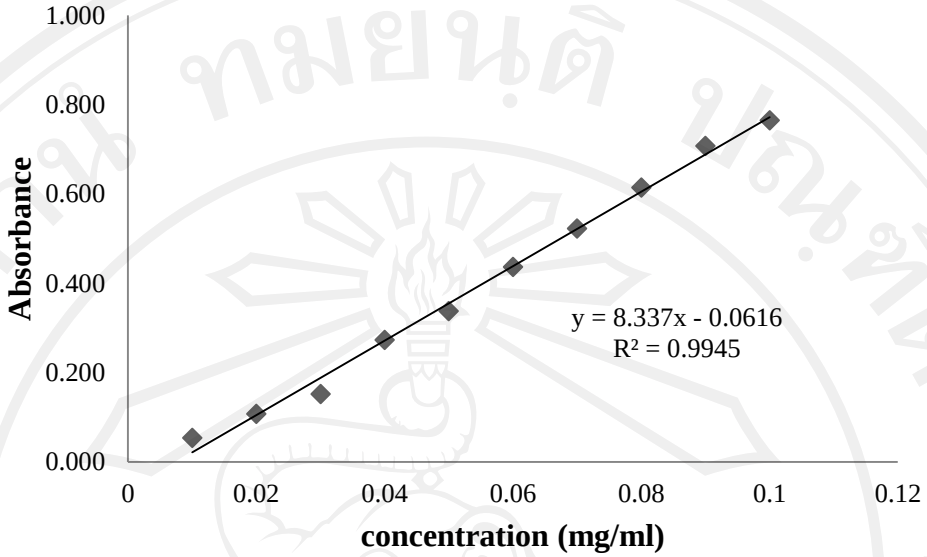

Figure F.4 Calibration curve for the absorbance at 725 nm of Folin-Ciocalteau method as a function of concentration of gallic acid standad solution

CURRICURUM VITAE

Author's Name Miss Wipawan Pukumpuang

Date/Year of Birth 7st September 1986

Place of Birth Lamphun

Education

2004	High school at Theerakarn Banhong school, Lamphun, Thailand
2008	Bachelor of Science (Biology), 1st honours, gold medal, Chiang Mai University, Chiang Mai, Thailand
2009-2014	Ph.D. student Applied Microbiology, Department of Biology Chiang Mai University, Chiang Mai, Thailand

Scholarship The research professional development project as under the Science achievement scholarship of Thailand (SAST)

Oral presentation Inhibition of some pathogenic bacteria by some medicinal plant extracts. The 38th Congress on Science and Technology of Thailand (STT38). Chiang Mai, Thailand, October 17-19, 2012.

Poster presentations Determination of steroidal saponins in some local medicinal plants. The 4th conference on science and technology for youths. Bangkok, Thailand, March 20-21, 2008.

Inhibition of pathogenic bacteria causing skin disease by some medicinal plant extracts. The 22nd Annual meeting of the Thai society for biotechnology (TSB 2010 international conferences on biotechnology for health living). Trang, Thailand, October 20-22, 2010.

Growth inhibition of pathogenic bacteria by some medicinal plant extracts. The 6th conference on science and technology for youths. Bangkok, Thailand, March 18-19, 2011.

Study and development of inhibitory gel against bacterial skin diseases from local highland medicinal plants. Annual meeting of highland research and development Institute. **The Empress Convention Centre,** Chiang Mai, Thailand, November 30, 2011.

Study of drug resistant gene in methicillin resistant *S. aureus*. Seminar in nanotechnology for health science. Faculty of Science and Nanoscience and Nanotechnology center, Chiang Mai University, February 27-29, 2012.

Inhibitory effects of Thai herbal extracts on methicillin resistant *S. aureus* (MRSA). RGJ seminar series LXXXIX, Molecular Mechanisms and Technology Developments in Biomedical Researches. August 31, 2012 **(Best poster presentation)**

Inhibitory of Pathogenic Enteric Bacteria and Anti-free radicals of vegetable extracts. The 38th Congress on Science and Technology of Thailand (STT38). Chiang Mai, Thailand, October 17-19, 2012.

Antioxidant activity and total phenolic content of Thai medicinal plants. The 4th International Conference or Natural Products for Health and Beauty (NATPRO4). Chiang Mai, Thailand, November 28-30, 2012.

Antibacterial activity and morphological alteration of pathogenic bacteria after treatment with *Coscinium fenestratum* (Gartn.) Colebr. extract. 5th congress of European Microbiologists (FEM2013). Leipzig, Germany, July 21-25, 2013.

Publications

Pukumpuang, W. and Tragoolpua, Y. Inhibition of pathogenic bacteria causing skin disease by some medicinal plant extracts. Proceeding, the 22nd Annual meeting of the Thai society for biotechnology (TSB 2010 international conferences on biotechnology for health living), Trang, Thailand, October 20-22, 2010, 1263-1268.

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