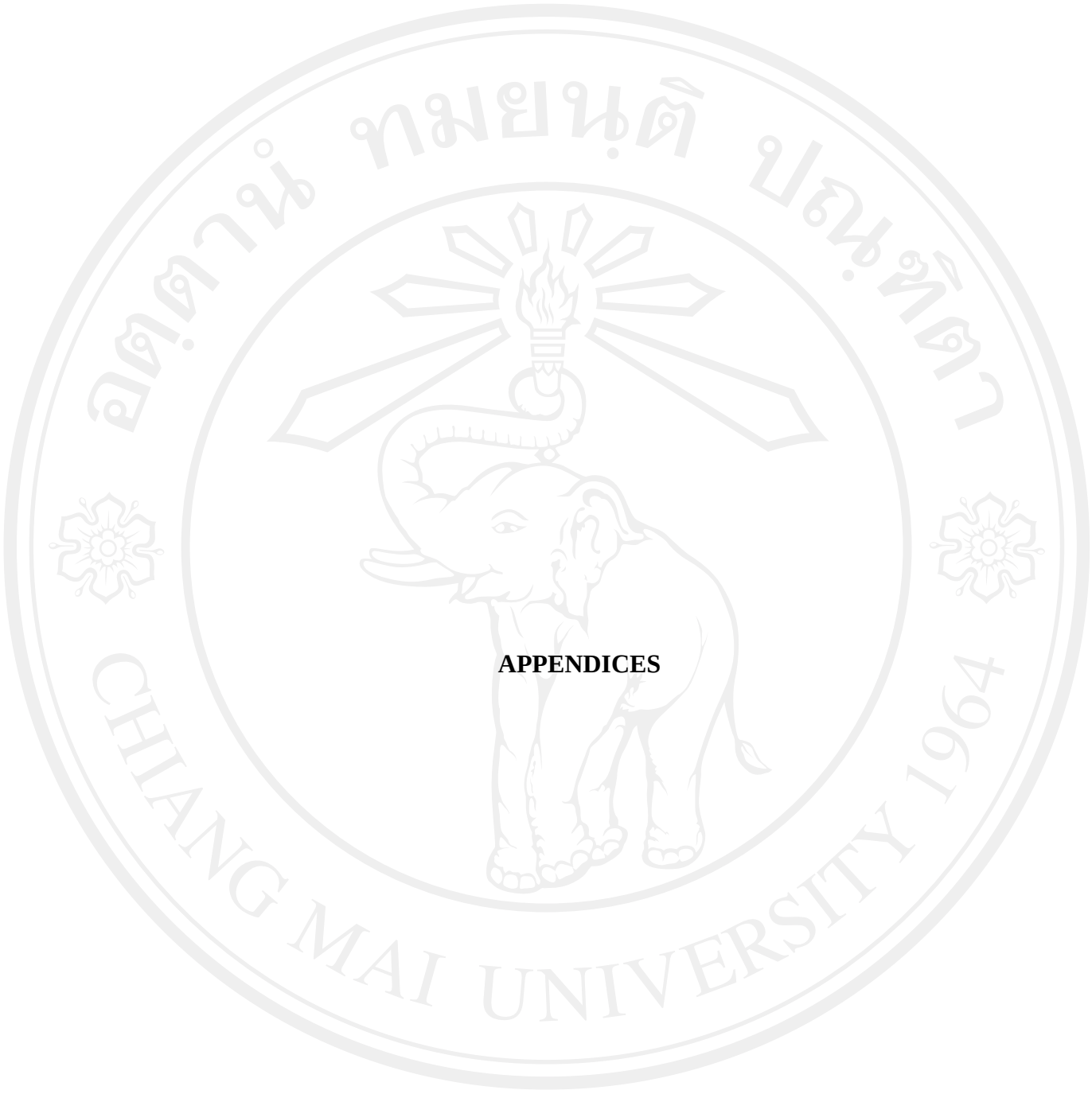


APPENDICES

APPENDIX A

Media and reagents for cells culture

APPENDIX A

Media and reagents for cells culture

1. Minimum Essential Medium (MEM)

MEM	9.6	g
$NaHCO_3$	1.8	g
Penicillin/Streptomycin (100X)	10	ml
Sterile deionized water	1000	ml

MEM powder composed of Eagle's balanced salts, L-glutamine non-essential and amino acid and was added to sterile deionized water with gentle stirring and added $NaHCO_3$ was added to adjust approximately pH 7.4. The mixture was mix until completely dissolved. After that, medium were filtered immediately through sterile 0.45 µm pore-sized cellulose acetate filter membrane, then penicillin-streptomycin, 100X was added and kept at 4ºC refrigerator.

2. Growth medium (GMEM)

MEM	90	ml
Inactivated fetal bovine serum	10	ml

3. Maintenance medium

MEM	98	ml
Inactivated fetal bovine serum	2	ml

4. Overlay medium

Sodium carboxy methylcellulose (1.5%)	3.5	ml
Growth medium	10.5	ml
$NaHCO_3$ (10%)	50	µl

5. Phosphate Buffer Saline (PBS, 10X)

NaCl	40	g
KCl	1	g
Na_2HPO_4 (anhydrous)	5.75	g
KH_2PO_4	1	g
Deionized water	500	ml

Sterilize by autoclaved at 121ºC, 15 psi for 15 minutes.

6. Phosphate Buffer Saline (PBS, 1X)

PBS, 10X	10	ml
Deionized water	90	ml

Sterilize by autoclaved at 121ºC, 15 psi for 15 minutes.

7. $NaHCO_3$ (10%)

$NaHCO_3$	10	g
Deionized water	100	ml

Sterilize by autoclaved at 121ºC, 15 psi for 15 minutes.

8. Sodium carboxy methylcellulose (1.5%)

Sodium carboxy methylcellulose	1.5	g
Deionized water	100	ml

Sterilize by autoclaved at 121ºC, 15 psi for 15 minutes.

APPENDIX B

Chemicals for cytotoxicity assay

APPENDIX B

Chemicals for cytotoxicity assay

1. Crystal violet (0.1%) in ethanol (1%)

Crystal violet	0.5	g
Ethanol, 95%	5	ml
Deionized water	495	ml

Mix thoroughly and filter through Whatman No.1. Kept in light brown bottle at room temperature.

2. Phosphate Buffer Saline (PBS, 10X)

NaCl	40	g
KCl	1	g
Na_2HPO_4 (anhydrous)	5.75	g
KH_2PO_4	1	g
Deionized water	500	ml

Sterilize by autoclaved at 121ºC, 15 psi for 15 minutes.

3. Phosphate Buffer Saline (PBS, 1X)

PBS, 10X	10	ml
Deionized water	90	ml

Sterilize by autoclaved at 121ºC, 15 psi for 15 minutes.

APPENDIX C

Chemicals for DNA extraction

APPENDIX C

Chemicals for DNA extraction

1. Lysing solution

Triton X-100 (0.25%)	0.25	ml
EDTA, 0.5M	2.0	ml
Tris-HCl, 1M	1.0	ml
Adjust volume with deionized water to	200	ml

2. EDTA (0.5M)

EDTA	16.86	g
Deionized water	100	ml

EDTA was dissolved in deionized water and NaOH, 1M was added to adjust pH 8.0. Then, volume was adjusted to 100 ml with deionized water and sterile by autoclaved at 121ºC, 15 psi for 15 minutes. The solution was stored at room temperature.

3. Tris-HCl (1M)

Tris	12.11	g
Deionized water	100	ml

The solution was adjusted pH to 8.0 with concentrated HCl and then adjusted volume to 100 ml with deionized water. Sterile by autoclaved at 121ºC, 15 psi for 15 minutes and kept at 4ºC refrigerator.

4. NaCl (5M)

NaCl	29.25	g
Deionized water	100	ml

Autoclaved at 121ºC, 15 psi for 15 minutes and kept at 4ºC refrigerator.

5. Sodium Dodecyl Sulphate (SDS, 10%)

SDS	10	g
Deionized water	100	ml

Autoclaved at 121ºC, 15 psi for 15 minutes and stored at room temperature.

6. Proteinase K (10 mg/ml)

Proteinase K	0.01	g
Added deionized water to	1	ml

Mixed thoroughly by vigorously vortexing and kept in -20ºC freezer.

7. RNase (10 mg/ml)

RNase	0.01	g
Added deionized water to	1	ml

Mixed thoroughly by vigorously vortexing and kept in -20ºC freezer.

8. NaOH (1M)

NaOH	0.4	g
Deionized water	100	ml

9. Phenol: chloroform: isoamyl alcohol (50:50:1)

Phenol	50	ml
Chloroform	50	ml
Isoamyl alcohol	1	ml

Mixed thoroughly in hood flow and kept in light brown bottle in 4ºC refrigerator.

10. Chloroform: isoamyl alcohol (50:1)

Chloroform	50	ml
Isoamyl alcohol	1	ml

Mixed thoroughly in fume hood and kept in light brown bottle in 4ºC refrigerator.

11. Sodium acetate (3M)

$NaCH_3COO.\ 3H_2O$	40.83	g

pH was adjusted with acetic acid to pH 5.5 then adjusted volume to 100 ml and autoclaved at 121ºC, 15 psi for 15 minutes. Kept at 4ºC refrigerator.

APPENDIX D

Chemicals for agarose gel electrophoresis

APPENDIX D

Chemicals for agarose gel electrophoresis

1. Tris-acetate buffer, (TAE, 50X)

Tris	242	g
EDTA (0.5M)	100	ml
Glacial acetic acid	57.1	ml
Added deionized water to	1000	ml

Autoclave at 121ºC, 15 psi for 15 minutes and kept at room temperature.

2. Loading buffer (5X)

TAE buffer (50X)	20	ml
Bromophenol blue	2	mg
Glycerol	10	ml
Deionized water	200	ml

Stored at room temperature.

3. Ethydium bromide (EtBr, 10 mg/ml)

EtBr	1	g
Sterile deionized water	100	ml

Mix well and stored in 4ºC in dark and stored at room temperature.

4. Agarose gel (0.8%)

Agarose	0.32	g
TAE buffer (1X)	40	ml

The solution was heated on hot plate until homogenized, cooled down and pour warm gel solution into gel tray.

5. TAE buffer (1X)

TAE buffer (50X)	200	ml
Sterile deionized water	800	ml

6. EDTA (0.5M)

EDTA	16.86	g
Deionized distilled water	100	ml

EDTA was dissolved in deionized water and NaOH, 1M was added to adjusted pH 8.0. Then, volume was adjusted to 100 ml with deionized water and sterile by autoclaved at 121ºC, 15 psi for 15 minutes and store at room temperature.

7. NaOH (1M)

NaOH	0.4	g
Deionized water		ml

APPENDIX E

Chemicals for cell lysates preparation

APPENDIX E

Chemicals for cell lysates preparation

1. NP-40 lysis buffer

Tris-HCl (1M)	300	µl
NaCl (1M)	300	µl
$MgCl_2$ (1M)	90	µl
NP-40 (10%)	1.5	ml
Urea (7M)	12.6	g
Thiourea (2M)	4.56	g
Deionized water was added to	30	ml

Mix thoroughly and stored in a dark bottle or a foli-wrapped clear bottle at 4ºC.

2. Tris-HCl (1.0M)

Tris	12.11	g

The solution was adjusted pH to 7.5 with concentrated HCl and then adjusted volume to 100 ml with deionized water.

3. $MgCl_2$ (1M)

$MgCl_2$. $6H_2O$	20.33	g
Deionized water	100	ml

Autoclave at 121ºC, 15 psi for 15 minutes and kept at room temperature.

4. NaCl (1M)

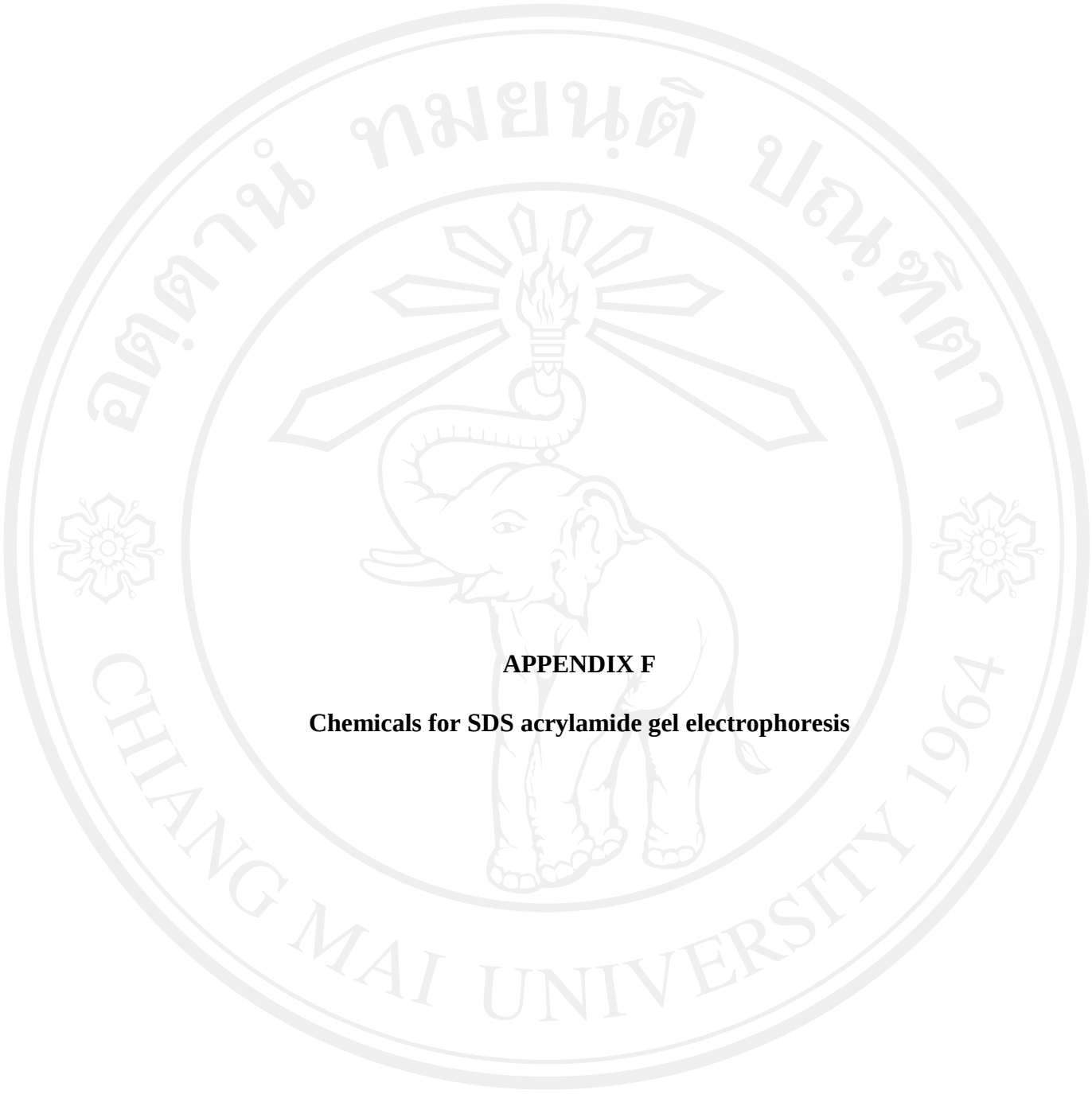
NaCl	5.84	g
Deionized water	100	ml

5. NP-40 (10%)

NP-40	10	ml
Deionized water	100	ml

6. Protease Inhibitor Cocktail Set III, Animal-Free

Protease Inhibitor Cocktail	0.5	ml
Lysing solution	100	ml



APPENDIX F

Chemicals for SDS acrylamide gel electrophoresis

APPENDIX F

Chemicals for SDS acrylamide gel electrophoresis.

1. Tris-HCl (1.5M)

Tris	18.17	g

Tris was dissolved in deionized water and adjusted pH to 8.8 with concentrated HCl and then adjusted volume to 100 ml with deionized water. Sterile by autoclaved at 121ºC, 15 psi for 15 minutes and kept at 4ºC.

2. Tris-HCl (0.5M)

Tris	6.06	g

The solution was adjusted pH to 6.8 with concentrated HCl and then adjusted volume to 100 ml with deionized water. Sterile by autoclaved at 121ºC, 15 psi for 15 minutes and kept at 4ºC.

3. SDS (10%)

SDS	10	g
Deionized water was added to	100	ml

Mixed thoroughly and kept at room temperature.

4. Amminium persulfate (APS, 10%) (prepare before use)

APS	5	g
Added distilled water to	50	ml

5. SDS-polyacrylamide gel (12.5%)

- Resolving gel (12.5%)

Deionized water	4.215	ml
Acrylamide mix (Bio-Rad, 40%)	3.13	ml
Tris-HCl (1.5M)	2.5	ml
SDS (10%)	0.1	ml
APS*(10%)	0.05	ml
TEMED*	0.005	ml

*Adding APS (10%) and TEMED into the solution after completely set the gel apparatus and other reagents.

- Stacking gel (6.51%)

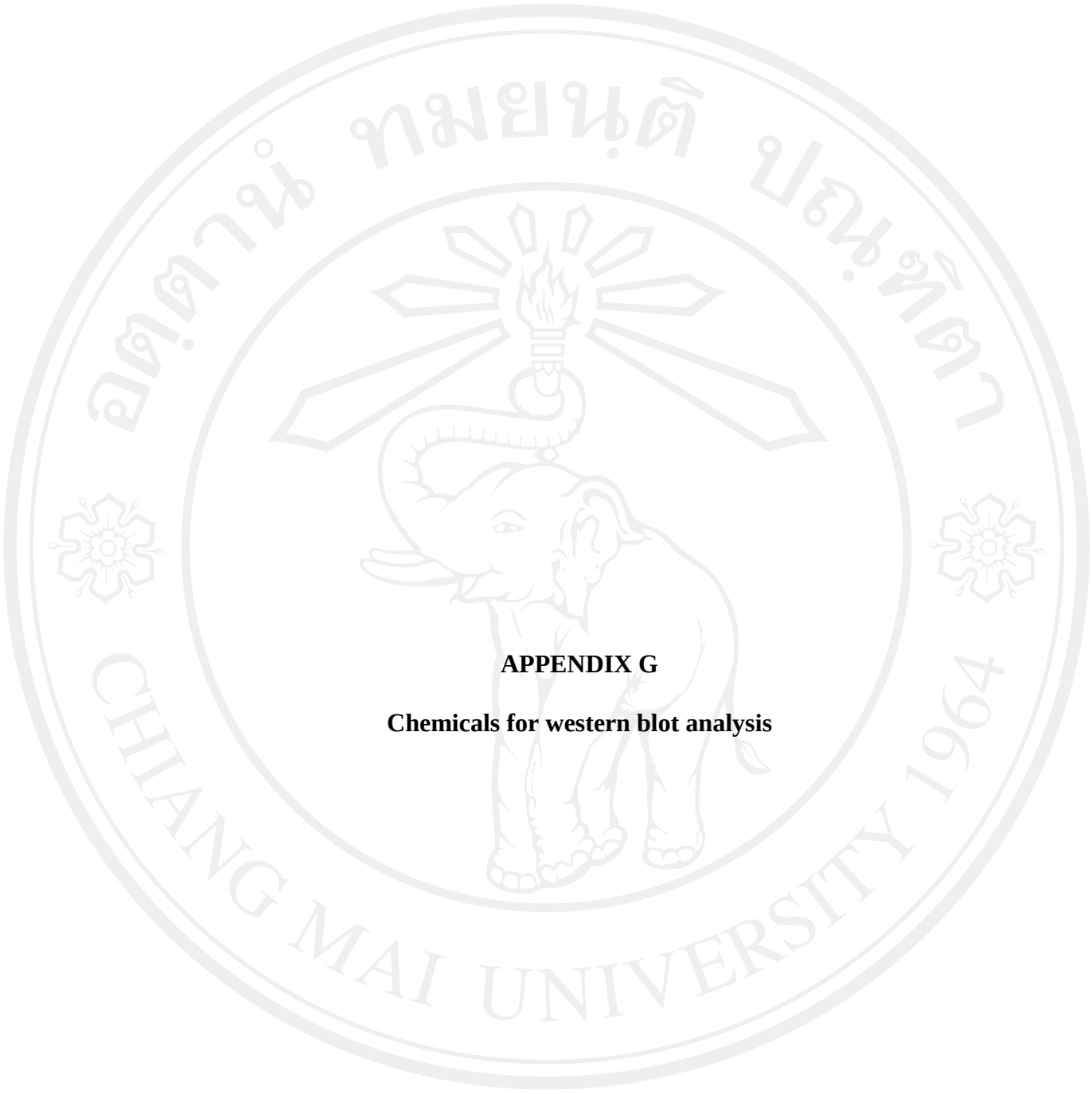
Deionized water	3.6	ml
Acrylamide mix (Bio-Rad, 30%)	1.0	ml
Tris-HCl, 1.0M	1.75	ml
SDS (10%)	0.1	ml
APS*(10%)	0.05	ml
TEMED*	0.005	ml

*Adding APS (10%) and TEMED into the solution after completely set the gel apparatus and other reagents.

6. Running buffer (5X)

Glycine	94	g
SDS (10%)	50	ml
Tris	15.1	g
Distilled water	1000	ml

Stored at room temperature



APPENDIX G

Chemicals for western blot analysis

APPENDEX G

Chemicals for Western blot analysis

1. Towbin transfer buffer (pH 8.3)

Tris (25mM)	3.03	g
Glycine (192mM)	14.4	g
Methanol (20%)	200	ml

Tris and Glycine were dissolved in 500 ml deionized water. Then, added methanol (20%) and adjusted volume to 1,000 ml with deionized water and kept at room temperature.

2. Saline

Tris	1.21	g
NaCl	9	g
Distilled water	1,000	ml

Dissolved Tris and NaCl in deionized water and adjusted pH to 7.4 with concentrated HCl and then adjusted volume to 1,000 ml with distilled water. Sterile by autoclave at 121ºC, 15 psi for 15 minutes and kept at room temperature.

3. bovine serum albumin in saline (3%)

Bovine serum albumin	0.3	g
Saline	10	ml

Mixed thoroughly. Freshly prepared daily.

4. Sample buffer (3X)

Tris-HCl (0.5M)	3	ml
EDTA (0.2M)	0.3	ml
SDS (10%)	3	ml
β-mercaptoethanol	0.3	ml
Glycerol	2.4	ml
Bromophenol blue (0.1%)	100	µl
Added distilled water to	10	ml

Mix well and stored at 4ºC. Combined with samples for electrophoresis with the ratio 3:1.

5. 4-chloro-1-naphthol (0.06%) in H_2O_2 (0.01%) in PBS

4-chloro-1-naphthol	0.06	g
H_2O_2 (30%)	0.01	ml
PBS, 1X	100	ml

Mix thoroughly prepare daily.

6. EDTA (0.2 M)

EDTA	37.22	g
Deionized water	450	ml

EDTA was dissolved in 450 ml of deionized water and adjusts pH to 8.0 with NaOH (1M). Then, bring up to 500 ml with deionized water. Sterile by autoclaved at 121ºC, 15 psi for 15 minutes and kept at room temperature.

7. Methanol (20%)

Methanol	20	ml
Deionized water	80	ml

8. Bromophenol blue (0.1%)

Bromophenol blue	0.01	g
Tris	0.006	g
Deionized water	1	ml

APPENDIX H

Chemical for phytochemical group

APPENDIX H

Chemical for phytochemical group

1. Mayer's reagent

Mercuric chloride ($HgCl_2$)	1.36	g
Potassium iodide (KI)	5.0	g
Deionized water	100	ml

2. Stock solution of Dragendorff's reagent

Solution A

Bismuth subnitrate	0.85	g
Deionized water	40	ml
Glacial acetic acid	10	ml

Solution B

Potassium iodine	8	g
Deionized water	20	ml

Solution A and Solution B use mixed to obtain stock solution and stored in dark bottle at 4ºC

3. Wagner's reagent

Iodine	1.27	g
Potassium iodide	6.0	g
Deionized water	100	ml

4. Dragendorff's reagent

Stock solution of Dragendorff's reagent	10	ml
Glacial acetic acid	20	ml
Deionized water	70	ml

5. Hager's reagent

Picric acid	1	g
Deionized water	100	ml

6. Ethanol (10%)

Ethanol (95%)	10.53	ml
Deionized water	89.47	ml

7. NaOH (1N)

NaOH	4	g
Deionized water	100	ml

8. Ferric chloride in acetic acid (10%)

$FeCl_3$	10	g
Acetic acid	100	ml

9. Ethanol (50%)

Ethanol (95%)	52.63	ml
Deionized water	47.37	ml

10. Aqueous iron (III) chloride (5%)

Iron (III) chloride	5	g
Deionized water	100	ml

11. Gelatin (1%)

Gelatin	1	g
Deionized water	100	ml

12. Ferric chloride

Ferric chloride	9	g
Deionized water	100	ml

13. Saturated lime water

$Ca(OH)_2$	0.15	g
Deionized water	100	ml

Stir vigorously and solid was settled overnight.

14. Lead acetate (PbAcO, 10%)

PbAcO	10	g
Deionized water	100	ml

15. HCl (10%)

HCl	10	ml
Deionized water	100	ml

16. KOH (5%)

KOH	5	g
Deionized water	100	ml

17. Vanilin

Vanilin	1.5	g
Sulfuric acid	2.5	ml
Ethanol (95%)	250	ml

18. Anisaldehyde-sulfuric acid

Anisaldehyde ($C_8H_8O_2$)	0.5	ml
Glacial acetic acid	50	ml
H_2SO_4	1	ml

Spray on the plate and heat at 110ºC until visualization of spots on TLC plates was present.

APPENDIX I

List of instrument

APPENDIX I

List of instrument

Names and sources of instruments were used in this present study

Instruments	Company
-20ºC refrigerator	Sanyo, SF-C991 NG, Japan
4ºC Refrigerator	LFS, Thailand
-80ºC refrigerator	Sanyo, MPF-392, Japan
Agarose gel electrophoresis chamber	Minicell Primo, E-C 320, Canada
Analytical Balance	Ohaus Adventurer, ARC 120, USA
Autoclave	Tomy, SS-325, Thaivictory Co., Ltd. Thailand
Automatic pipette 0.5-10 µl	Biohit, Finland
Automatic pipette 100-1000 µl	Biohit, Finland
Automatic pipette 20-200 µl	Biohit, Finland
Automatic pipette 5-50 µl	Biohit, Finland
Biosafety cabinet class I	Science tech Co., Ltd. Thailand
Biosafety cabinet class II	Esco, Gibthai, Thailand
CO_2 incubator	Shellab, 2323-2, USA
Compound microscope	Olympus, CX31, USA
Electrophoresis power supply	Amersham Pharmacia Biotech, EPS 301, Sweden

Instruments (continued)	**Company**
Fume hood	I-Lab fluid control Co., LTD, Thailand
Hemotocytometer	Improved Neubauer hemocytometer, Boeco, Germany
Hot air oven	Binder, ED 115, USA
Hot plate	Clitton, Cerastir, CO.,Ltd. Thailand
Incubator 25ºC	Sanyo, MIR-253, Japan
Incubator 45ºC	Gallenkamp, IPR150, USA
Inverted microscope	Olympus, CKX41, USA
Lyophilizer	Dura dry FTS systems, USA
Multichanel autopipette	Acura manual 855, Socorex, Switzerland
pH meter	Denver, UB-10, Becthai, Thailand
Pipette Aid	Tecnomara, Wallisellen, Switzerland
Refrigerated centrifuge	Sanyo, Harrier 18/80 and Hettich, EBA 12R, England
Rocking platform	ELMI, S 4, Latvia
Semi-Dry Electrophoretic Transfer Cell	Bio-Rad Laboratories, USA
Spectrophotometer	Thermo spectronic, Genesys 20, USA
Tissue culture flask	SPL life sciences, Gibthai, Thailand
Tissue culture plate	Nunc, Thermo scientific, England
Ultrasonic cleaners	Bransonic, 2210E-DTH, USA
UV transilluminator	Vilber Lourmat, V03 6685, France

Instruments (continued)	**Company**
Vaccuum rotating evaporator	BÜCHI Vacuum system B-178, Neslab, Switzerland
Vacuum pump	Rocker 400, Taiwan
Vortex mixer	Gemmy Industrial Corp., VM-300, Taiwan
Water bath incubator	Memmert, WB-10, Germany

CURRICULUM VITAE

Name Miss Pratya Chaliewchalad

Date of birth 28th December 1986

Education Background

2002-2004	High school from Sriyanusorn school, Chantraburi
2005-2008	Bachelor of Science (Microbiology) 2nd class Hons., Department of Biology, Faculty of Science, Chiang Mai University, Thailand **Title of Special Project of 4th year undergraduate student program:** Development of gel for herpes simplex virus type 2 inhibition from *Spirulina platensis* extract
2009 – present	Studying in Ph.D. (Applied Microbiology), Department of Biology, Faculty of Science, Chiang Mai University, Thailand **Title of Thesis of Ph.D. program:** Inhibition of herpes simplex virus activity *in vitro* by some medicinal plant extracts

Poster Presentation, Oral presentation, Training and Workshop, Publication

Training and Workshop

2007

Attended the seminar: “Good Manufacturing Practice (GMP)” at Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand, on August 25

2008

Attended the training: “ Clinical microbiology laboratory” , Prapokklao hospital, Chantraburi, Thailand, on March 10 - April 11

2010

Attended the seminar: “Innovation of Cell Culture for Tissue Transplantation, Tissue Engineering and Stem cell”, at Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand, on March 5

2010

Attended the workshop: “To increase teaching of teaching assistant (TA)” at Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand, on May 17-27

2010

Attended the Teaching assistant: “Virology” at Applied microbiology section, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand

2011

Attended the workshop: “The apoptosis assay using asymmetry membrane detection”, at Gibthai Training Center, Chiang Mai, Thailand, on February 9-10

2012

Attended the seminar: “Nanotechnology for health science, by Nanoscience and Nanotechnology Center” , Faculty of Science, Chiang Mai University, Chiang Mai Hill, Thailand, on February 27-29

2012

Attended the Teaching assistant: “ Virology” at Applied microbiology section, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand

2013

Attended the workshop: “ SEM workshop” at Faculty of Science, Chiang Mai University, Chiang Mai, Thailand, on September 19-20

2013

Attended the workshop: “FIMSA advanced training course 2013: Molecules and cells of innate immune system”, The Imperial Mae Ping Hotel, Chiang Mai, Thailand, on October 22-25

Poster presentation

Chaliewchalad, P and Tragoolpua, Y. 2008. Development of gel against herpes simplex virus type 2 activity by *Spirulina platensis* extract. Undergraduate Students Academic Exhibition, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand, February 27, 2008.

Chaliewchalad, P and Tragoolpua, Y. 2009. Development of gel against herpes simplex virus type 2 activity by *Spirulina platensis* extract. Industrial and Research Projects for Undergraduate Students (IRPUS)-Conference, Royal Paragon Hall, Siam Paragon, Bangkok, Thailand, March 27-29, 2009.

Chaliewchalad, P., Pheundee, C., Peerapornpisal, Y. and Tragoolpua, Y. 2009. Gel containing *S.platensis* extract inhibit herpes simplex virus. CMU Academic conference, Research Path: The Fifth Decade toward the University of Excellence, Chiang Mai University, Chiang Mai, Thailand, November 26-27, 2009.

Chaliewchalad, P. and Tragoolpua, Y. 2010 Study and development of gel product for inhibition of herpes simplex virus causing skin infection from local highland plants. Annual meeting of Highland Research and development Institute. The Empress Convention Centre, Chiang Mai, September 9-10, 2010.

Chaliewchalad, P. and Tragoolpua, Y. 2010 Anti-Herpes Simplex Virus type 2 of Activity Some Medicinal Plant Extracts. International Conference on Biotechnology for Healthy Living. The 22nd Annual Meeting of the Thai Society for Biotechnology, Prince of Songkla University, Trang Campus, Trang, Thailand, October 20-22, 2010.

Chaliewchalad, P. and Tragoolpua, Y. 2011. Study and development of inhibitory gel against herpes simplex virus infection and product for inhibition of pathogenic bacteria causing enteric disease from local highland medicinal plants. Annual meeting of Highland Research and development Institute, The Empress Convention Centre, Chiang Mai, Thailand, November 30, 2011.

Chaliewchalad, P., Wongpothi, K., Thonglem, K., Thongwai, N. and Tragoolpua, Y. 2012. Anti-herpes simplex virus type 2 activity of *Croton roxburghii* N.P. Balakr. Extract. The 38th Congress on Science and Technology of Thailand (STT38) Sciences for the Future of Mankind, The Empress Convention Centre, Chiang Mai, Thailand, October 17-19, 2012.

Chaliewchalad, P. and Tragoolpua, Y. 2012. Inhibitory effect of *Schefflera leucantha* R. Vig. extracts against herpes simplex virus type 2. The 4th International Conference on Natural Products for Health and Beauty (NATPRO4). Future Trends in Health Products: Safety and Effectiveness for All, Chiang Mai Orchid Hotel, Chiang Mai, Thailand, November 28-30, 2012.

Chaliewchalad, P. and Tragoolpua, Y. 2012. Antiviral effect of *Stemona tuberosa* L. extracts on herpes simplex virus infection. RGJ seminar series LXXXIX. Molecular Mechanisms and Technology Developments in Biomedical Researches, Kantary Hills Hotel, Chiang Mai, Thailand, August 31, 2012.

Chaliewchalad, P. and Tragoolpua, Y. 2013. Study on mode inhibition effect of *Rhinacanthus nasutus* (Linn.) Kurz. Thai medicinal plant extract against

proteins synthesis of herpes simplex virus infection 5th congress of European microbiologists, Leipzig, Germany, July 21-25, 2013.

Oral presentation

Chaliewchalad, P. and Tragoolpua., Y. 2012. Antiviral effect of *Stemona tuberosa* L. extracts on herpes simplex virus infection. The RGJ seminar series LXXXIX Molecular Mechanisms and Technology Developments in Biomedical Researches. 31 August 2012, Kantary Hills Hotel, Chiang Mai, Thailand.

Publications

Chaliewchalad, P. and Tragoolpua, Y. 2010. Anti-Herpes Simplex Virus type 2 Activity of SomeMedicinal Plant Extracts. The 22nd Annual Meeting of the Thai Society for Biotechnology "International Conference on Biotechnology for Healthy Living". Prince of Songkla University, Trang Campus, Thailand. October 20-22, 2010 page: 1396-1402.

Chaliewchalad, P., Thongwai, N. and Tragoolpua, Y. 2012. Inhibitory effect of *Rhinacanthus nasutus* (Linn.) Kurz. and *Stemona tuberosa* (Lour.) extracts on herpes simplex virus infection. *Journal of Medicinal Plants Research*, 7(2): 76-84.

Chaliewchalad, P., Chansakaow, S. and Tragoolpua, Y. 2013. Efficacy of *Houttuynia cordata* Lour extracts against herpes simplex virus infection. Chaing Mai Journal of Science. Accepted.