APPENDICES

APPENDIX A

Media and reagents for viral culture

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Media and reagents for viral 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. 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.

3. Growth medium (GMEM)

MEM	90	ml
Inactivated fetal bovine serum	10	ml

4. Maintenance medium

MEM	98	ml
Inactivated fetal bovine serum	2	ml

5. Overlay medium

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

6. 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.

7. Phosphate Buffer Saline (PBS, 1X)

PBS, 10X	10	ml
Deionized water	90	ml

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

8. $NaHCO_3$ (10%)

$NaHCO_3$	10	g
Deionized water	100	ml

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

9. 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

Chemical reagents for cytotoxicity assay

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Chemical reagents for cytotoxicity assay

1. Phosphate Buffer Saline (PBS, 10X)

NaCl	40	g
KCl	1	g
Na2HPO4 (anhydrous)	5.75	g
KH2PO4	1	g
Deionized water	500	ml

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

2. Phosphate Buffer Saline (PBS, 1X)

PBS, 10X	10	ml
Deionized water	90	ml

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

3. MTT solution

MTT	2.0	mg
PBS (1X)	1.0	ml

Dissolve MTT in PBS (1X). Add filter by sterlied filter paper Ø 0.45 µm and storage at 4°C in the dark.

APPENDIX C

Chemical reagents for phytochemical group

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Chemical reagents 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. Dragendorff's reagent

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

4. Ethanol (10%)

Ethanol (95%)	10.53	ml
Deionized water	89.47	ml

5. NaOH (1N)

NaOH	4	g
Deionized water	100	ml

6. Ferric chloride in acetic acid (10%)

$FeCl_3$	10	g
Acetic acid	100	ml

7. Ferric chloride

Ferric chloride	9	g
Deionized water	100	ml

8. HCl (10%)

HCl	10	ml
Deionized water	100	ml

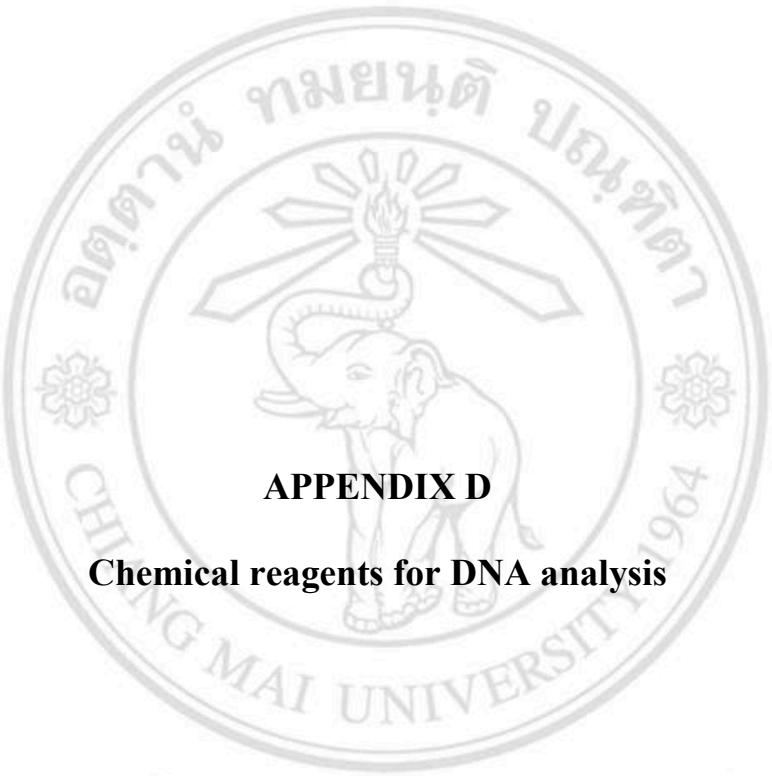
9. KOH (5%)

KOH	5	g
Deionized water	100	ml

10. 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 D

Chemical reagents for DNA analysis

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Chemical reagents for DNA analysis

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.

12. 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.

13. Loading buffer (5X)

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

Stored at room temperature.

14. 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.

15. 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.

16. TAE buffer (1X)

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

17. 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.

18. NaOH (1M)

NaOH	0.4	g
Deionized water	100	ml

APPENDIX E

Chemical reagents for protein analysis

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Chemical reagents for protein analysis

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

APS	5	g
Added distilled water to	50	ml

2. 30% (29:1) Acrylamide: Bisacrylamide

Acrylamide	29	g
Bisacrylamide	1	g

Dissolve all ingredients in steriled DI water. Store at 4°C in the dark.

3. Agarose sealing solution

Agarose	0.1	g
1X running buffer	10	ml
Bromophenol blue stock	40	µL

Dissolve agarose in 1X running buffer. The suspension was boiled until dissolve completely. Then, bromophenol blue was added and store at RT.

4. Bradford reagent

95% Ethanol	5	ml
Coomasssie G-250	0.01	g
85% Phosporic acid	10	mg

Dissolve Coomassie G-250 in 95% ethanol. Add 85% phosphoric acid and bring total volume to 100 ml with steriled DI water, Then filter by sterlied filter paper (Whatman No.1) and storage at RT in the dark.

5. 1% w/v Bromophenol blue

Bromophenol blue	0.01	g
Tris-base	0.006	g

Dissolve bromphenol blue and Tris-base in steriled DI water. Bring final volume to 1 ml and store in the dark at 4°C.

6. Bromophenol blue stock

Bromophenol blue	0.01	g
Tris-base	0.006	g

Mix all recipes in steriled DI water and bring final volume to 1 ml. Store at 4°C.

7. CBBG working solution

Colliodal CBBG dye solution	400	ml
Methanol	100	ml

Mix colliodal CBBG dye solution in methanol and store in the dark at 4°C.

8. Colloidal CBBG dye stock solution

Ammonium sulfate	50	g
85% phosphoric acid	6	ml
5% CBBG	10	ml

Dissolve all ingredients in steriled DI water. Bring final volume to 500 ml. Store in the dark at RT.

9. 5% Coomassie Brilliant Blue G-250 (CBBG) stock

CBBG	0.5	g
Steriled DI water	10	ml

Dissolve CBBG in steriled DI water. Bring final volume to 10 ml.

10. 2.5% Coomassie R-250 staining solution

Coomassie R-250	1.25	g
Methanol	250	ml
Glacial acetic acid	50	ml

Dissolve all ingredients in steriled DI water and bring final volume to 500 ml. Store at RT in the dark.

11. Destaining solution for R-250

Methanol	200	ml
Glacial acetic acid	50	ml

Dissolve all ingredients in steriled DI water and bring final volume to 500 ml. Store at RT in the dark.

12. EDTA (0.2 M) pH 8.0

EDTA	2.922	g
Steriled DI water	50	ml

Dissolve EDTA in steriled DI water and bring final volume to 50 ml. Store at 4°C.

13. Fixation solution for 2-DE

95% v/v Ethanol	150	ml
Glacial acetic acid	50	ml

Mix all ingredients in steriled DI water. Bring final volume to 500 ml. Store in the dark at RT.

14. 3X Loading Buffer

0.5 M Tris-HCl pH 6.8	3	ml
0.2 M EDTA	0.3	ml
10% SDS	3	ml
Beta-mercaptoethanol	0.3	ml
Glycerol	2.4	ml
Bromophenol blue stock	100	ml

Mix all ingredients in steriled DI water. Bring total volume to 10 ml. Store in 1 ml aliquots at -20°C.

15. Lysis Buffer

DTT	0.075	g
Urea	4.80	g
Thiourea	1.52	g
CHAPS	0.40	g
Glycerol	510	µL
Isopropanol	1	ml

Mix all ingredients above in steriled DI water. Add glycerol at the final, then adjust total volume to 10 ml with steriled DI water. The mixture was stored in 1 ml aliquots at -20°C.

16. $MgCl_2$ (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.

17. NaCl (1M)

NaCl	5.84	g
Deionized water	100	ml

18. NaOH (5 M)

NaOH	20	g
Steriled DI water	100	ml

Dissolve NaOH in steriled DI water and bring final volume to 100 ml.

19. NP-40 (10%)

NP-40	10	ml
Deionized water	100	ml

20. 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.

21. PMSF (10 mg/ml)

PMSF	50	mg
Isopropanol	5	ml

Dissolve PMSF with isopropanol and store in 1 ml aliquots at -20°C.

22. Protease Inhibitor Cocktail Set III, Animal-Free

Protease Inhibitor Cocktail	0.5	ml
Lysing solution	100	ml

23. Rehydration Buffer

DTT	0.075	g
Urea	4.80	g
Thiourea	1.52	g
CHAPS	0.40	g
Glycerol	510	µL
Isopropanol	1	ml
Bromophenol blue stock	20	µL

Mix all ingredients above in steriled DI water. Add glycerol at the final, then adjust total volume to 10 ml with steriled DI water. The mixture was stored in 1 ml aliquots at -20°C.

24. Running Buffer (10X)

Tris - base	30.3	g

Glycine	144.1	g
SDS	5	g

Disslove all ingredients in steriled DI water and bring total volume to 1,000 ml, storage at 4°C

25. SDS (10%)

SDS	10	g
Deionized water was added to	100	ml

Mixed thoroughly and kept at room temperature.

26. SDS Equilibrium Buffer

Urea	36.05	g
1.5M Tris-HCl pH 8.8	5	ml
SDS	2	g
Glycerol	34.50	ml
1% bromophenol blue	200	µL

Mix all recipes in steriled DI water and brind finol volume to 100 ml. Store in the dark at 4°C.

27. 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.

28. 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.

29. Tris-HCl pH 6.8 (1.0 M)

Tris	12.11	g

Dissolve 12.11 g Tris in steriled DI water. Then the solution was adjusted to pH 6.8 with 6M HCl. Bring final volume to 100 ml and store at 4°C.

30. Tris-HCl pH 8.8 (1.5M)

Tris	18.17	g

Mix 18.17 g Tris in steriled DI water. Then the solution was adjusted to pH 8.8 with 6M HCl. Bring final volume to 100 ml and store at 4°C.

CURRICULUM VITAE

Author's Name	Ms. Aomhatai Deethae
Date/Year of Birth	12 January 1980
Place of Birth	Uttaradit Province, Thailand
Education	2002: B.S. (Medical Technology), Department of Medical Technology, Faculty of Associated Medical Science, Chiang Mai University, Chiang Mai, Thailand. 2008: M.S. (Biology), Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.
Experience	Poster presentation in the 32th Annual Conference of Medical Technologists of Thailand (ACMTT), 7-9 May 2008 at Ambassador City Jomtien Hotel, Chonburi, Thailand. In title "Effect of Mutation in DNA Polymerase Gene of Herpes Simplex Virus Type 1 on Acyclovir resistance" Poster presentation in 5th Science Research Conference, 4-5 March 2013 at University of Payao, Payao, Thailand. In title "Effect of Mutation in DNA Polymerase Gene of Herpes Simplex Virus Type 1 on Acyclovir Resistance" Poster presentation in 6th National Conference on Algae and Plankton (NCAP 2013), 28-30 March 2013 at The Empress Chiang Mai Hotel, Chiang Mai, Thailand. In title "Anti-Herpes Simplex Virus Activity of *Spirogyra Neglecta* (Hassall) Kützing Extract" Poster presentation in the 5th Congress of European Microbiologists, 21-25 July 2013 at Leipzig Messe Congress Center, Leipzig, Germany. In title "Inhibitory effect of *Spirogyra*

neglecta (Hassall) Kützing against herpes simplex virus type 1 and 2 *in vitro* infection"

Participation in The 12th Federation of Immunological Society of Asia-Oceania (FIMSA) Advanced Training Course: Molecules and Cells of Innate Immune System, 22-25 October 2013 at The Imperial Mae Ping Hotel, Chiang Mai, Thailand.

Poster presentation in the 7th Asia Oceania Human Proteome Organization Congress and 9th International Symposium of the Protein Society of Thailand, 6-8 August 2014 at Miracle Grand Convention Hotel, Bangkok, Thailand. In title "Effect of *Spirogyra neglecta* (Hassall) Kützing extracts on herpes simplex virus proteins by proteomic analysis"

Poster presentation in the 39th Annual Conference of Medical Technologists of Thailand (ACMTT), 26-29 May 2015 at Chiang Mai International Exhibition and Convention Centre, Chiang Mai, Thailand. In title "Inhibitory efficacy of *Spirogyra neglecta* (Hassall) Kützing extracts on herpes simplex virus type 2 proteins by proteomic analysis"

Oral presentation

The 1st prize from oral presentation award hosted by the 7th National Conference on Algae and Plankton (NCAP 2015), 25-27 March 2015 at Narai Hotel, Bangkok, Thailand. In the title "Inhibitory efficacy of herpes simplex virus and development of anti-viral product from *Spirogyra neglecta* (Hassall) Kützing extract"