



Appendix

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APPENDIX A

List of drugs and chemicals used in this study

Drugs and chemicals for phytochemical screening and antioxidant assay

Chemicals	Source/Company
2, 2'-diphenyl-1-picrylhydrazyl	Sigma Chemical Company, St. Louis, U.S.A.
3,5-dinitrobenzoic acid	Sigma Chemical Company, St. Louis, U.S.A.
Absolute ethanol	Merck, Germany
Acetic anhydride	BDH, PROLABO, France
Acetone	Merck, Germany
Ammonia solution	Merck, Germany
Bismuth nitrate	Asia Pacific Specialty Chemical limited, Australia
Chloroform	BDH, PROLABO, France
Diethyl ether	BDH, PROLABO, France
Ferric chloride	BDH, PROLABO, Belgium
Gallic acid	Sigma Chemical Company, St. Louis, U.S.A.
Gelatin powder	BDH, PROLABO, Australia
Glacial acetic acid	Merck, Germany
Hydrochloric acid	Merck, Germany
Iodine	Sigma Chemical Company, St. Louis, U.S.A.
Mercuric (II) chloride	Ajax Finechem Pty Ltd., Australia
Methanol	Merck, Germany
Nitric acid	BDH, PROLABO, France
Potassium hydroxide	Ajax Finechem Pty Ltd., Australia
Potassium iodide	BDH, PROLABO, Belgium
Sodium chloride	Merck, Germany
Sodium Hydroxide	Merck, Germany
Sulphuric acid	Merck, Germany

Drugs and chemicals for *in vitro* anti-inflammatory models

Chemicals	Source/Company
Acetic acid	Merck, Germany
Agarose	Vivantis, USA
Antibiotic antimycotic solution	Invitrogen, USA
Dimethyl sulfoxide (DMSO)	Amresco, USA
dNTP Mix	Vivantis, USA
Dulbecco's Modified Eagle's Medium (DMEM)	GIBCO, USA
Ethidium bromide	Vivantis, USA
Ethylenediaminetetraacetic acid (EDTA)	Fluka, Switzerland
Fetal bovine serum (FBS)	HyClone, USA
GeNei™ Red Dye PCR Master Mix (2X)	Fermentas, Canada
Griess reagent (modified)	Sigma-Aldrich, USA
Isopropyl alcohol	Sigma-Aldrich, USA
Lipopolysaccharides from Escherichia coli	Sigma-Aldrich, USA
Maxima™ SYBR Green qPCR Master Mix (2X)	Fermentas, Canada
Methanol	Merck, German
murine COX-2	Bio Basic Inc., Thailand
murine GAPDH	Bio Basic Inc., Thailand
murine IL-1 beta	Bio Basic Inc., Thailand
murine IL-6	Bio Basic Inc., Thailand
murine iNOS	Bio Basic Inc., Thailand
murine TNF alpha	Bio Basic Inc., Thailand
Oligo-(dT)-18 primers	Bio Basic Inc., Thailand
Precision Plus Protein™ Standards Dual Color	BIO-RAD, USA
Primers	Bio Basic Inc., Thailand
Protein loading marker	Fermentas, Canada
RevertAid M-MuLV Reverse Transcriptase	Fermentas, Canada
Ribolock Ribonuclease Inhibitor	Fermentas, Canada
RNase Away	Molecular Bio Products, USA
Sodium nitrite	Sigma-Aldrich, USA
Standard 100-base pairs DNA ladder	RBC Bioscience

Sulforhodamine B	Sigma-Aldrich, USA
Taq DNA Polymerase	Vivantis, USA
Thunderbird qPCR Master mix	Toyobo, Japan
Trichloroacetic acid	BDH chemical, England
Tris(hydroxymethyl)aminomethane	Research Organics, USA
Trisodium citrate	Merck, Germany
Trizol reagent	Invitrogen, USA
Trypan Blue	Sigma-Aldrich, USA
Trypsin	Invitrogen, USA

Drugs and chemicals for *in vivo* experimental models

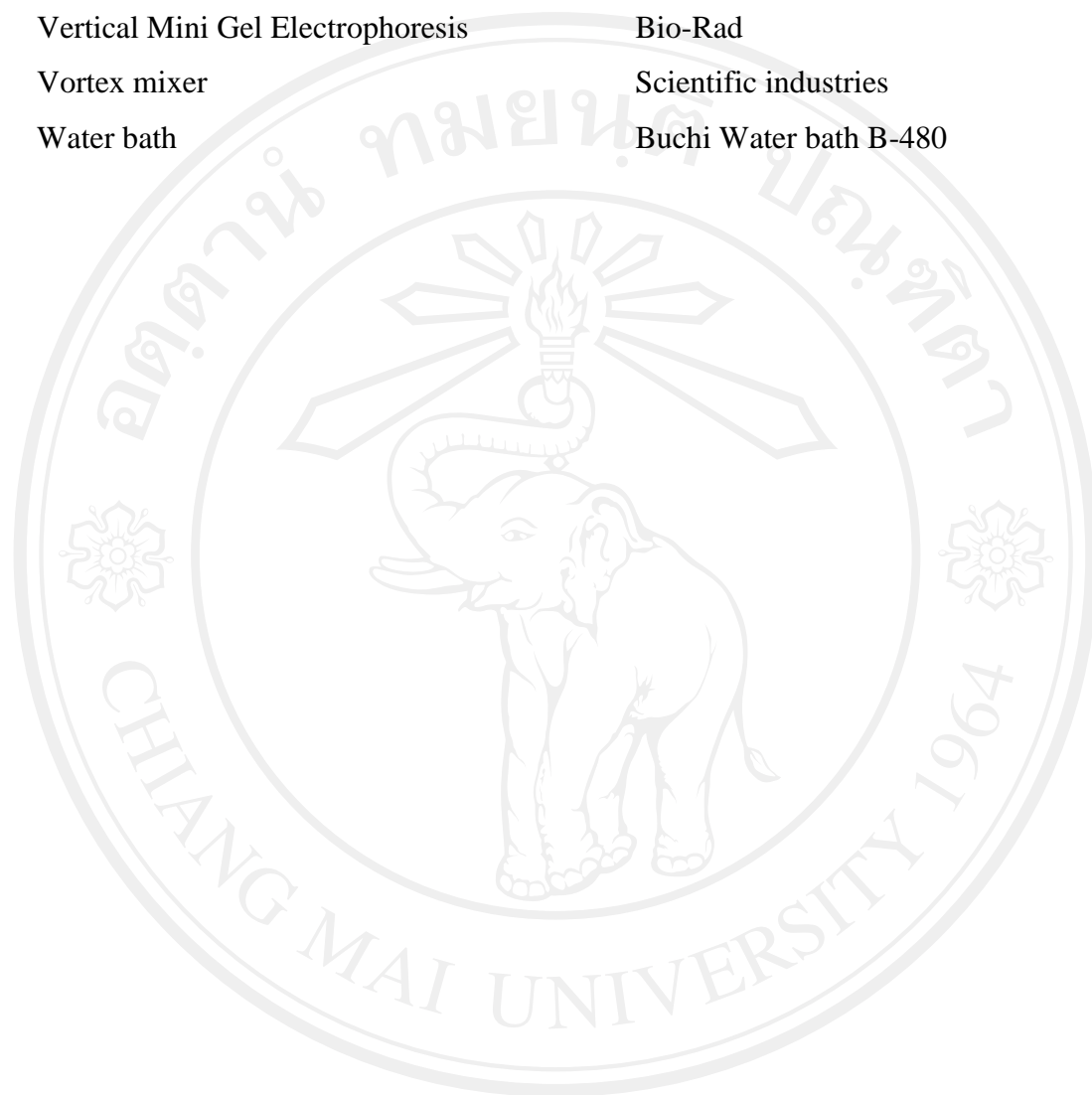
Drugs	Source/Company
Cimetidine	Sigma Chemical Company, St. Louis, U.S.A.
Indomethacin	Sigma Chemical Company, St. Louis, U.S.A.
Misoprostol	Pfizer, Thailand

Chemicals	Source/Company
Absolute ethanol	Merck, Germany
Alcian blue	Fluka Chemicals Co., Ltd., Japan
Complete Freund's adjuvant	Difco, U.S.A.
Diethyl ether	BDH, PROLABO, France
Hydrochloric acid	Merck, Germany
Magnesium chloride	Merck, Germany
Phenolphthalein	Merck, Germany
Sodium carboxymethyl cellulose	O.V. Chemical and Supplies, Thailand
Sodium chloride	Merck, Germany
Sodium Hydroxide	Merck, Germany
Sucrose	Merck, Germany
Tween ® 80	Sigma Chemical Company, St. Louis, U.S.A.

List of instruments and supplies used in this study

Instruments and supplies	Source/Company
7500 Fast Real-Time PCR System	Applied Biosystems
Analytical balance	OHAUS CORPORATION
Autoclave	Tomy autoclave SS-240
Automatic pipette	Gilson
Carbon dioxide incubator	Thermoscientific
Centrifuge	KUBOTA Corporation
Centrifuge tube 15 mL	Nunc
Centrifuge tube 50 mL	Nunc
Filter paper	Whatman International Ltd., England
Flat Cap Strips, Optically Clear	Thermo Scientific
Freezer (-20°C)	Whirlpool
Freezer (-80°C)	Forma Scientific
GeneQuant pro	Amersham Biosciences, Germany
Glassware	Pyrex and Scott duran
Hot air oven	Heraeus
iCycler thermal cycle	Bio-Rad
Inverted microscope	NIKON
Laminar flow biological cabinet	NU AIRE
Liquid nitrogen tank	International Cryogenics, Inc.
Litmus paper	Whatman International Ltd, England
Magnesium ribbon	Ajax Finechem Pty Ltd, Australia
Magnetic stirrer	Thermolyne
Microcentrifuge 5402, bench top	Eppendorf
Microcentrifuge tube	Eppendorf
Micro-plate spectrophotometer	Bio-Tek Instrument
Microwave	LG
Multi Rigid PCR 96-Well Ultra Plate	Thermo Scientific
Plethysmometer 7150	Ugo Basile, Modena, Italy
Power supply	Bio-Rad

Rotary evaporator	Buchi Rotavapor R-124
Shaker incubator	Amerex Instruments Inc.
Spectrophotometer	Shimadzu, Bara Scientific
Sterile 96-well plate	Nunc
Vertical Mini Gel Electrophoresis	Bio-Rad
Vortex mixer	Scientific industries
Water bath	Buchi Water bath B-480



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APPENDIX B

Reagent Preparation

Reagent preparation for phytochemical screening

1. 2N HCl

Diluted 16.6 mL of 37% HCl in DW and made up to 100 mL with DW.

2. Dragendorff's reagent

Bismuth nitrate	8 g
Nitric acid (30% w/v)	12 mL
KI	27.2 g

Dissolved 8 g of bismuth nitrate in 12 mL of nitric acid and dissolve 27.2 g of KI in DW, made up to 100 mL with DW.

3. Mayer's reagent

Mercuric chloride	1.36 g
KI	5.0 g

Dissolved 1.36 g of mercuric chloride and dissolve 5.0 g of KI in DW, made up to 100 mL with DW.

4. Wagner's reagent

Iodine	1.27 g
KI	2.0 g

Dissolved 1.27 g of iodine and 2.0 g of KI in DW, made up to 100 mL with DW.

5. 1% Gelatin solution

Dissolved 1.0 g of gelatin powder in DW, made up to 100 mL with DW.

6. 10% NaCl

Dissolved 10 g of NaCl in DW, made up to 100 mL with DW.

7. 1% Ferric chloride solution

Dissolved 1.0 g of ferric chloride in DW and made up to 100 mL with DW.

8. 25% Ammonia solution

Diluted 83.3 mL of 30% ammonia in DW and made up to 100 mL with DW.

9. 1N KOH in ethanol

Dissolved 5.611 g of KOH (MW = 56.11 g/mol) ethanol, made up to 100 mL with ethanol.

10. 1% 3,5-dinitrobenzoic acid in ethanol

Dissolved 1.0 g of 3,5-dinitrobenzoic acid in ethanol, made up to 100 mL with ethanol

Reagent preparation for *in vitro* anti-inflammatory models

1. 10 % TCA

Dissolved 10.0 g of TCA in DW and made up to 100 mL with DW.

2. 1% (V/V) acetic acid

Diluted 1 mL of acetic acid in DW and made up to 100 mL with DW.

3. 0.057% (W/V) SRB solution

SRB 0.057 g

1% acetic acid 100 mL

Dissolved 0.057g of SRB in 1% acetic acid and made up to 100 mL with 1% acetic acid.

4. 10 mM unbufferd tris base solution

Tris base, MW = 121.1 g/mol

Tris base 1.211 g

Dissolved 1.211 g of tris base in DW and made up to 1000 mL with DW.

5. DMEM with 10% fetal bovine serum

DMEM 89 mL

Fetal bovine serum (FBS) 10 mL

10X Antibiotic antimycotic sol. 1 mL

Diluted FBS and antibiotic in DMEM, stored at 4°C

6. DEPC treated water

Diluted 0.1 mL of DEPC in DI water and made up to 1000 mL with DI water.

7. 10X TBE buffer

Tris base [tris(hydroxymethyl)aminomethane] 108 g

Boric acid 55 g

EDTA 9.5 g

Dissolved the Tris base, boric acid and EDTA in 800 ml of DI water, made up to 1000 mL with DI water, stored at room temperature.

8. 0.5X TBE buffer

10X TBE buffer 50 mL

Diluted 50 mL of 10X TBE buffer with DI water, made up to 1000 mL, mixed well, stored at room temperature.

9. 1.0% agarose gel

Agarose gel 1.0 g

0.5X TBE buffer 100 mL

Dissolved agarose gel in TBE buffer, heat until dissolved.

10. 0.5 mg/mL ethidium bromide

10 mg/mL ethidium bromide 25 μ L

1X TBE buffer 500 mL

Diluted 25 μ L of 10 mg/mL ethidium bromide with 1X TBE buffer, mixed well, stored at room temperature.

Reagent preparation for *in vivo* experimental models

1. EtOH/HCl

37% HCl 1.7 mL

Absolute EtOH 60.0 mL

Diluted 1.7 mL of 37% HCl in DW, added 60.0 mL of absolute EtOH, made up to 100 mL with DW.

2. 0.5% CMC

Dissolved 0.5 g CMC in DW and made up to 100 mL with DW.

3. 20 mg/mL indomethacin in 0.5% CMC

indomethacin 400 mg

0.5% CMC 20 mL

Dissolved 400 mg of indomethacin in 20 mL of 0.5% CMC mixed by vortex mixer.

4. 0.1 N NaOH

Dissolved 4 g of NaOH (MW40 g/mol) in DW, made up to 1000 mL with DW.

5. 0.9% NaCl

Dissolved 9 g of NaCl in DW, made up to 1000 mL with DW.

6. 0.1 N HCl

Diluted 8.28 mL of 37% HCl in DW and made up to 1000 mL with DW.

7. 0.01 mg/mL Phenolphthalein indicator

Phenolphthalein 0.5 g

50% ethanol in DW 50 mL

Dissolved 0.5 g of phenolphthalein in 50% ethanol in DW, made up to 50 mL.

8. 0.1 % w/v alcian blue solution

Alcian blue 0.1 g

DW 100 mL

Dissolved 0.1 g of alcian blue in DW, made up to 100 mL with DW.

9. 0.5 M MgCl₂

MgCl₂·6H₂O MW = 203.3 g/mol

MgCl₂ 101.65 g

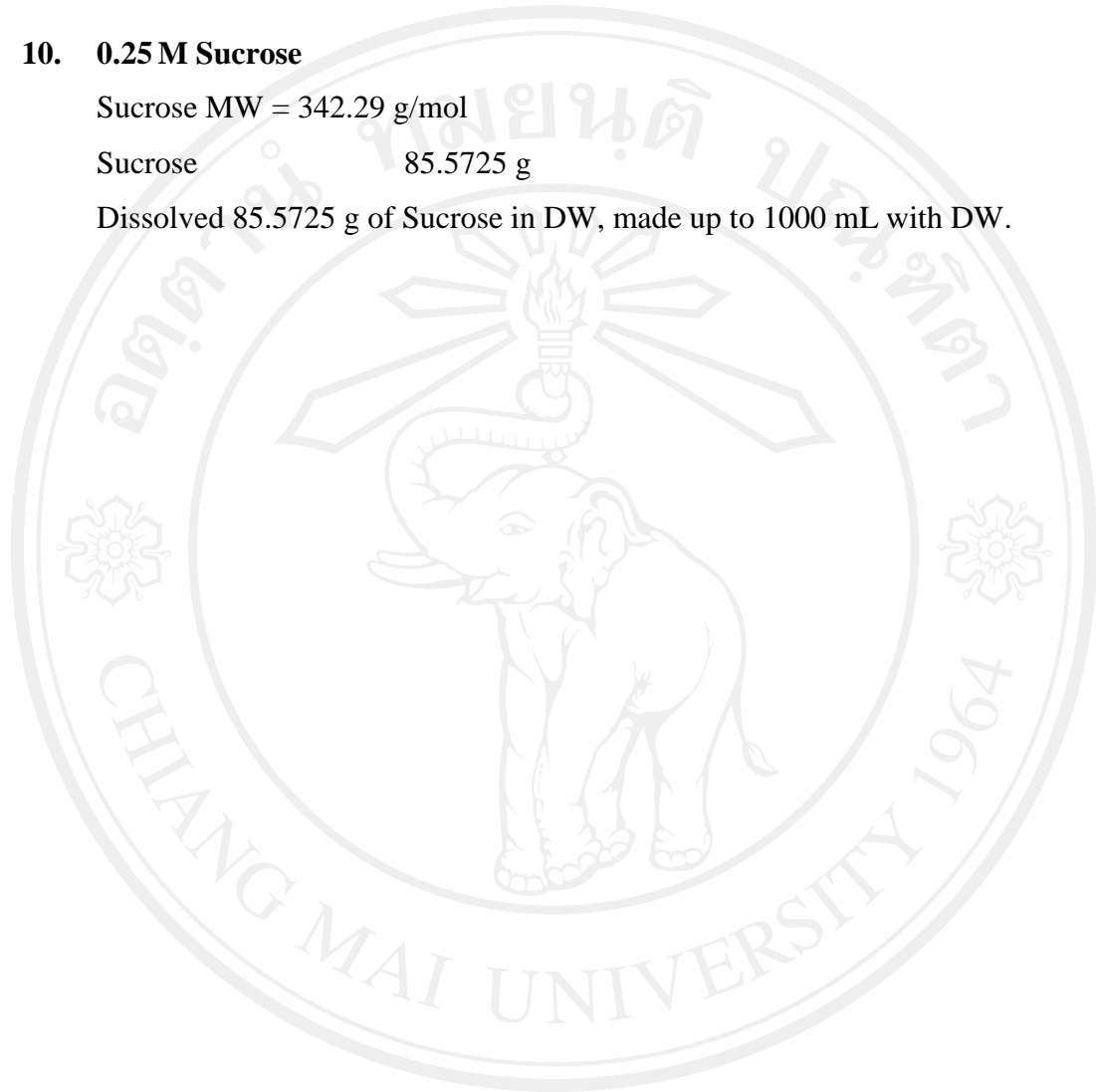
Dissolved 101.65 g of MgCl₂ in DW, made up to 1000 mL with DW.

10. 0.25 M Sucrose

Sucrose MW = 342.29 g/mol

Sucrose 85.5725 g

Dissolved 85.5725 g of Sucrose in DW, made up to 1000 mL with DW.



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