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APPENDICES

APPENDIX A

Chemical agents and instruments

1. Chemical agents

Absolute methanol GR (E. Merck, Darmstadt, Germany)

Citric acid (E. Merck, Darmstadt, Germany)

Chloroform (Sigma, MO, U.S.A.)

Crystal violet (Fluka, Switzerland)

Dimethyl sulfoxide (DMSO) (Sigma, MO, U.S.A.)

Formaldehyde 38% w/v AR (Carlo Erba, Milano, Italy)

Hydrochloric acid (E. Merck, Darmstadt, Germany)

Methylcellulose (Sigma, MO, U.S.A.)

Potassium chloride (KCl) (May & Bayer, England)

Potassium dihydrogen phosphate (KH_2PO_4) (E. Merck, Lot. NO. 547A17873, Darmstadt, Germany)

Dipotassium hydrogen phosphate (K_2HPO_4) (E. Merck, Darmstadt, Germany)

Propan-2-ol (Fisher Scientific, U.K.)

Sodium bicarbonate ($NaHCO_3$) (E. Merck, Darmstadt, Germany)

Sodium chloride (NaCl) (E. Merck, Lot. No. K27736104021, Darmstadt, Germany)

Disodium hydrogen phosphate (Na_2HPO_4) (May & Bayer, Lot. No. 50028, England)

Thiazolyl blue tetrazolium bromide (MTT) (Sigma, Lot No. 085K5304, U.S.A.)

Tragacanth (Pharmaceutical chemicals, Denmark)

Trypsin-EDTA (1X) (Gibco, Canada)

2. Instruments

Analytical balance (Satorius, Germany)

Automatic pipet (Drummond Scientific, U.S.A.)

Automatic pipet P2-20/P20-200/P100-1000 (Socorex, Switzerland)

Centrifuge (Sigma, Germany)

Hemocytometer (Boeco, Germany)

Laminar air flow (Holten, U.S.A.)

Microplate reader model 3550 (Biorad, U.S.A.)

Multichannel automatic pipette, 8 channel (Socorex, Switzerland)

Orbital Shaker SO3 (Stuart scientific, Redhill, UK)

pH meter (Beckman, U.S.A.)

Refrigerator 4°C (Sharp, Thailand)

Refrigerator 20°C (Ariston, U.S.A.)

Refrigerator -80°C (Forma Scientific, U.S.A.)

Ultrasonicator

Vortex mixer (Scientific, NY, U.S.A.)

Water bath (Thelco, U.S.A.)

3. Laboratory supplies

Cryotubes (Nunc, Denmark)

Glasswares (Pyrex, U.S.A.)

Microcentrifuge tubes

Millipore filters 0.2 μm, Acrocap filter unit (Gelman Laboratory, U.S.A.)

Pipette tips (Nunc, Denmark)

Syringe filters, Acrodisc (Pall Corporation, U.S.A.)

Tissue culture flasks (Nunc, Denmark)

Tissue culture plates (Nunc, Denmark)

APPENDIX B

Medium and Reagents

1. Growth medium

Vero cells CCL-81 were grown and maintained in Eagle's minimum essential medium (MEM). Minimum essential medium with Eagle's salts and Glutamine (Gibco, U.S.A.) without sodium bicarbonate powder 9.5 g was dissolved in deionized distilled water. 2.2g/L of sodium bicarbonate (Sigma, U.S.A.) were added to the solution. This medium solution was mixed well and adjusted pH to 7.2-7.4 with 6N HCl. Then, the solution was adjusted volume to 1,000 ml by deionized distilled water. This solution was sterilized by filtration with 0.22 μm millipore filter membrane. Before use, this MEM solution was supplemented with 10% fetal bovine serum (FBS, Gibco, U.S.A.) and 1% antibiotic-antimycotic agents (Gibco, U.S.A) which contained 10,000 units/ml of penicillin G sodium, 10,000 μg/ml of streptomycin sulfate, and 25 μg/ml of amphotericin B as fungizone in 0.85% saline.

For HeLa cells, Dulbecco's modified Eagle medium (DMEM) with high glucose, L-glutamine, pyridoxine hydrochloride and 170 mg/L sodium pyruvate (Gibco, U.S.A.), and without sodium bicarbonate was used instead of MEM in Vero cell. These DMEM powder was dissolved in deionized distilled water and 3.7 g/L of sodium bicarbonate (Sigma, U.S.A.) was added into the solution. After mixed well, the solution was adjusted pH to 7.2-7.4 with 6N HCl and adjusted volume to 1,000 ml. This solution was sterilized by filtration with 0.22 μm millipore filter membrane. 10% fetal bovine serum and 1% antibiotic-antimycotic agents were added to the medium solution before use as mentioned in MEM.

For NHDF CC-2511, growth and maintenance medium were prepared by DMEM as the same as previously mentioned in HeLa cell except for increasing fetal bovine serum supplementation to 15%.

2. 0.05% Methylene blue in distilled water

Methylene blue	5 g
Distilled water	100 ml

3. 12% Formaline in normal saline solution

38% Formaldehyde	320 ml
0.85% Normal saline (NaCl) solution	680 ml

4. Phosphate Buffer Saline Solution (PBS)

NaCl	8.00 g
KCl	0.20 g
KH_2PO_4	0.20 g
Na_2HPO_4	1.15 g
Deionized distilled water to	1,000 ml

This solution is sterilized by autoclaving for 15 minutes at 121°C, 15 lb/in^2

5. Low-pH Citrated buffer,pH 3.0

Sodium citrate	40 mM
Potassium chloride	10 mM
Sodium chloride	135 mM
Deionized distilled water to	1,000 ml

pH of this solution is adjusted to 3.0 and then the buffer is sterilized by autoclaving for 15 minutes at 121°C, 15 lb/in^2

6. Plaque overlay medium

Solution A

2x MEM with 20% FBS and 2% antibiotic-antimycotic agent

This solution was sterilized by filtration through 0.22 μm Millipore filter membrane

Solution B

Methyl Cellulose 4000 (Sigma, U.S.A.)	1.6 g
Deionized distilled water	100 ml

This solution was sterilized by autoclaving for 15 minutes at 121°C, 15 lb/in^2

The solution A and B were mixed well at a ratio of 1:1 before use

7. MTT solution

Thiazolyl blue tetrazolium bromide (MTT)	500 mg
PBS	100 ml

This solution was sterilized by filtration to remove a small amount of insoluble residues present in some batches of MTT

8. Acid-isopropanol

HCl	1.23 ml
Isopropanol	1,000 ml

Calculation of median cytotoxic concentration (CC_{50}) and effective concentration (EC_{50}) by regression formula.

CC_{50} is the concentration of lipoic acid, lipoamide, or acyclovir which exhibit 50% cytotoxicity. CC_{50} is calculated from the regression formula $Y = aX + b$ or $Y = a\log X + b$, when Y is optical density or number of viable cells, X is the concentration of lipoic acid, lipoamide, or ACV in μg/ml, a is slope, and b is an intercept or the distance between X axis and the point where the regression line come across Y axis

EC_{50} is the concentration of lipoic acid, lipoamide, or ACV which can inhibit 50% of plaque formation or optical density reduction. EC_{50} is calculated from the regression formula $Y = aX + b$ or $Y = a\log X + b$, when Y is number of plaque or optical density, X is the concentration of lipoic acid, lipoamide, or ACV in μg/ml, a is slope, and b is an intercept or the distance between X axis and the point where the regression line come across Y axis

Determination of correlation coefficient

Correlation coefficient between concentration and percent inhibition or incubation time and percent inhibition was determined using SPSS program as following example

Concentration (μg/ml)	%inhibition	Correlation coefficient
50	10.58	R = 0.95
100	27.05	
200	48.77	
400	71.01	
800	92.92	

BIOGRAPHY

Miss Pattaraporn Sasivimolphan was born on August 17, 1981 in Bangkok, Thailand. She received her Bachelor Degree of Science in Pharmacy (1st Class Honours with gold medal) in 2003 from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.