

APPENDIX A

List of chemicals and materials used in this study

Name of chemicals	Sources
Acrylamide	Sigma-Aldrich, USA
Ammonium per sulfate	Sigma-Aldrich, USA
Bovine serum albumin	Sigma-Aldrich, USA
Bradford protein assay	Bio-Rad Laboratories, USA
Crystal violet	Sigma-Aldrich, USA
Commasie brilliant blue R-250	Bio-Rad Laboratories, USA
Commasie Plus™ Protein Assay Reagent	Thermo scientific, USA
Dibasic sodium phosphate	Sigma-Aldrich, USA
Dimethyl sulfoxide (DMSO)	E.Merck, Germany
Dulbeco's modified eagle's medium	Gibco, USA
Dulbeco's modified eagle's medium (without phenol red)	Gibco, USA
ECL reagent	GE Healthcare, UK
Ethanol	E.Merck, Germany
Ethidium bromide	Bio-Rad Laboratories, USA
EDTA	Sigma-Aldrich, USA
Fetal bovine serum	Hyclone, USA
Glycine	Sigma-Aldrich, USA
Guava nexin reagent for apoptotic assay	E.Merck, Germany
HEPES	Sigma-Aldrich, USA
High range molecular weight marker	Bio-Rad Laboratories, USA
Hydrochloric acid	E.Merck, Germany
L-glutamine	Gibco, USA

Name of chemicals	Sources
Mercaptoethanol	Sigma-Aldrich, USA
Methanol	E.Merck, Germany
Monobasic sodium phosphate	Sigma-Aldrich, USA
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)	USB, USA
Non-fat dry milk	Difco, USA
Nitrocellulose membrane	GE Healthcare, UK
Penicillin-streptomycin	Gibco, USA
Phenol	E.Merck, Germany
Potassium chloride	E.Merck, Germany
Potassium dihydrogen phosphate	E.Merck, Germany
Potassium phosphate	E.Merck, Germany
Protease inhibitor cocktail kit	Thermo scientific, USA
Restore™ Western Blot stripping buffer	Thermo scientific, USA
RIPA buffer	Thermo scientific, USA
Sodium acetate	E.Merck, Germany
Sodium chloride	E.Merck, Germany
Sodium dodecyl sulfate	Sigma-Aldrich, USA
Sodium hydroxide	E.Merck, Germany
Standard albumin	Thermo scientific, USA
Trichloroacetic acid (TCA)	E.Merck, Germany
Tris-base	Sigma-Aldrich, USA
Triton X-100	Sigma-Aldrich, USA
Trypsin-EDTA	Gibco, USA
Tween 20	Sigma-Aldrich, USA
Filter paper, 24.0 cm	Whatman, UK

APPENDIX B

List of instruments used in this study

Instruments	Sources
Amicon filtration device, 30 kDa molecular weight cut off membrane	E.Merck, Germany
Analytical balance	Mettler Toledo ME54, UK
Autoclave	Tomy autoclave SS-240, USA
Automatic pipette	Gilson Scientific, UK
Carbondioxide incubator	Forma Scientific, USA
Centrifugation	Eppendorf 5702R, Germany
Deionized water machine	Barnstead, Thermo scientific, USA
Distilled water machine	Hamilton, UK
Freezer (-80 °C)	Forma Scientific, USA
Freezer (-20 °C)	Sanyo, Japan
Glassware	Pyrex, Thailand
Guava easyCyte HT flow cytometer	Merck Millipore Corporation, USA
Hot air oven	Haraeus, Thermo Scientific, USA
Inverted microscope	Nikon, Japan
Laminar flow biological cabinet	AIR2000 Fembrook Lane, Plymouth, MN55447, USA
Light microscope	Olympia Tokyo, Japan
Liquid nitrogen tank	Taylor-wharton , UK
Lyophilizer	Christ Alpha1-4, Germany
Magnetic stirrer	Sybron / Thermolyne, Thermo Scientific, USA

Instruments

Sources

Microcentrifuge, bench-topped	BD Clay Adams, Thermo Scientific, USA
Microplate reader	Microplate autoreader EL311s, Bio-Tek Instruments, UK
Pasture pipette	Pyrex, Thailand
pH meter	SP-2100, Suntex, Taiwan
Polystyrene FACS tube (10 x75 mm)	Falcon, BD Biosciences, USA
Refrigerator	Hitachi, Japan
Shaking water bath	Unitronic 320 OR, Thermo Scientific, USA
25 or 75 cm ³ T-flask	Nunc, USA
6 or 24 or 24 well plate	Nunc, USA
Trans-blot® electrophoretic transfer cell	Bio-Rad, Thailand
Vortex	Scientific industries, USA
Water bath	GFL 1083, Thermo Scientific, USA

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APPENDIX C

Preparation of some reagents and buffers

Cell culture

1. Incomplete DMEM medium with phenol red

DMEM	1	package (13.5 g)
HEPES	2.603	g
NaHCO ₃	3.7	g
Deionized distilled water	800	mL

Adjust pH to 7.4 then add volume with deionized water to 1,000 ml and sterile by suction filter (membrane pore size 0.2 μ m) and store at 4 °C.

2. Complete culture media

Incomplete DMEM	445	mL
Fetal bovine serum	50	mL
Penicillin-streptomycin	5	mL

Stored at 4°C

3. Freezing solution

Incomplete DMEM	7	mL
Fetal bovine serum	2	mL
DMSO	1	mL

Stored at 4°C

4. Tris-EDTA (pH 7.4)

NaCl	8.6	g
KCl	0.38	g
Na ₂ HPO ₄	3.7	g
Tris-base	3.02	g
EDTA	0.038	g
Deionize distilled water	800	mL

Adjust pH to 7.4 then add volume with deionized water to 1,000 ml and sterile by autoclave or suction filter (membrane pore size 0.2 μ m)

5. 0.25% Trypsin

2.5% Trypsin	10	mL
Tris-EDTA (sterile)	90	mL

Measurement of cell survival

1. Phosphate buffer saline (PBS) pH 7.4

NaCl	8	g
KCl	0.25	g
Na ₂ HPO ₄	1.44	g
KH ₂ PO ₄	0.25	g
Deionized distilled water	800	mL

Adjust pH to 7.4 then add volume with deionized water to 1,000 ml and sterile by suction filter (membrane pore size 0.2 μ m)

2. MTT stock dye solution

MTT dye	1	g
PBS pH 7.4	200	mL

Filter with membrane filter pore size 0.2 μ m and collect in dark container.

Western blot analysis

1. RIPA contains proteinase inhibitor (prepare before used)

Protease inhibitor cocktail kit	1	tablet
RIPA buffer	10	mL

2. Tris Buffered Saline (TBS)

Tris-base	2.42	g
NaCl	8	g
Deionized distilled water	800	mL

Adjust pH to 7.4 then adjust volume with deionized water to 1000 ml

3. 10% SDS

SDS	10	g
Deionize distilled water	100	mL

4. 10% Ammonium persulfate

APS	1	g
Deionize distilled water	10	mL

5. Separating gel

5.1 Separating gel 8% SDS-PAGE

Deionized water	4.6	mL
30% Acrylamide	2.67	mL
Tris buffer (pH 8.8)	2.5	mL
10% SDS	100	μ L
10% APS	100	μ L
TEMED	10	μ L

5.2 Separating gel 10% SDS-PAGE

Deionized water	3.9	mL
30% Acrylaamide	3.33	mL
Tris buffer (pH 8.8)	2.5	mL
10% SDS	100	μ L
10% APS	100	μ L
TEMED	10	μ L

5.3 Separating gel 12% SDS-PAGE

Deionized water	3.3	mL
30% Acrylaamide	4	mL
Tris buffer (pH 8.8)	2.5	mL
10% SDS	100	μ L
10% APS	100	μ L
TEMED	10	μ L

6. Stacking gel (4%)

Deionized water	2.3	mL
30% Acrylaamide	0.67	mL
Tris buffer (pH 6.8)	1	mL
10% SDS	40	μ L
10% APS	40	μ L
TEMED	4	μ L

7. Running buffer

Tris-base	3.04	g
Glycine	14.4	g
10% SDS	10	mL
Deionized distilled water	1,000	mL

8. Blotting buffer

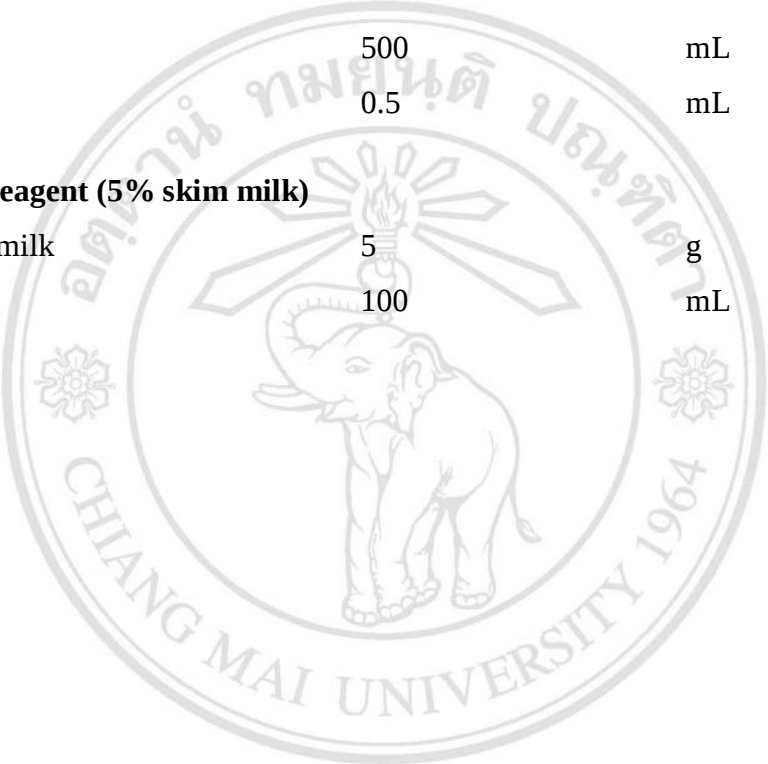
Tris-base	3.04	g
Glycine	14.4	g
Methanol	200	mL
Deionized distilled water	800	mL

9. TBS-T (0.1% v/v Tween)

TBS	500	mL
Tween	0.5	mL

10. Blocking reagent (5% skim milk)

Non-fat dry milk	5	g
TBS-T	100	mL



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CURRICULUM VITAE

Name	Mrs. May Thuu Mon
Date of Birth	June 15, 1982
Place of Birth	Yangon, Myanmar
Nationality	Myanmar
Education	2000 – 2006 M.B., B.S. (Bachelor of Medicine, Bachelor of Surgery) University of Medicine 2, Yangon, Myanmar 2010 – 2013 M. Med. Sc (Biochemistry) University of Medicine 2, Yangon, Myanmar 2014 – 2018 Ph.D. (Biochemistry) Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
Scholarships	2014 – 2018 Faculty of Medicine Research Fund, Chiang Mai University, Chiang Mai, Thailand
Experiences	2008 – 2013 Demonstrator, Department of Biochemistry, University of Medicine 2, Yangon, Myanmar 2013 – present Assistant Lecturer, Department of Biochemistry, University of Medicine 2, Yangon, Myanmar

Presentations

Mon MT, Yodkeeree S and Limtrakul P. Cytokines production and drug sensitivity profiles in human ovarian cancer cells. The 15th Annual Biochemical Research Meeting, July 26-27, 2015, Department of Biochemistry, Chiang Mai University, Chiang Mai, Thailand (Oral presentation).

Mon MT, Yodkeeree S, Pitchakan P and Limtrakul P. Crebanine potentiates the anticancer effect of carboplatin in ovarian cancer cells. The 5th International Biochemistry and Molecular Biology Conference, May 26-27, 2016, The Samila Beach Hotel, Songkla, Hat Yai, Thailand (Poster presentation).

Mon MT, Yodkeeree S and Limtrakul P. Crebanine enhances cisplatin sensitivity in ovarian cancer cells via apoptotic pathway. The 16th Annual Biochemical Research Meeting, July 28-29, 2016, Department of Biochemistry, Chiang Mai University, Chiang Mai, Thailand (Oral presentation).

Mon MT, Yodkeeree S and Limtrakul P. Inhibition of IL-6/STAT3 axis by crebanine decrease ovarian tumor aggressiveness. The 17th Annual Biochemical Research Meeting, July 25-27, 2017, Department of Biochemistry, Chiang Mai University, Chiang Mai, Thailand (Oral presentation).

Publications

Mon MT, Yodkeeree S, Punfa W, Umsumarng S, Lekwanavijit S, Siriaunkgul S, Suprasert P and Limtrakul P. Relationships of *ex-vivo* drug resistance assay and cytokine production with clinicopathological features in the primary cell culture of Thai ovarian and fallopian tube cancer patients. *Asian Pac J Cancer Prev*. 2017;18(11):3063-3071.

Mon MT, Yodkeeree S, Punfa W, Pompimon W and Limtrakul P. Alkaloids from *Stephania venosa* as chemo-sensitizers in SKOV3 ovarian cancer cells via Akt/NF- κ B signaling. *Chem Pharm Bull (Tokyo)*. 2018;66(2).

Trainings and Workshops

Basic Biostatistics Postgraduate Refresher Course, Preventive and Social Medicine Society, Myanmar Medical Association (2009)

Basic Medical Education Course, Medical Education Unit, University of Medicine 2 Yangon, Myanmar (2010)

International Workshop on Protein Expression and Purification Strategies at Research Division, Faculty of Medicine, Chulalongkorn University, Thailand (28 October 2013 - 1 November 2013)

Scientific skills

1. Cell line culture
2. Primary cell culture from solid tissues of patients
3. Basic molecular techniques such as protein extractions, DNA/RNA extraction, Western blot analysis, zymography, ELISA, flow cytometry and PCR techniques



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