APPENDIX A

List of chemicals and materials used in this study

Chemicals/Materials	Source
1-Methoxy-5-methylphenazine methosulfate	AppliChem, Germany
2-NBD glucose	Invitrogen, USA
3-Isobutyl-1-methylxanthine	Sigma-Aldrich, USA
Antibiotic antimycotic solution	Invitrogen, USA
Bovine serum albumin	PAA Laboratories GmbH, Austria
Calf serum	GIBCO, USA
Cell culture 25 and 75 cm3 flask and well plates	Corning, USA
Dexamethasone	Sigma-Aldrich, China
Dimethylsulfoxide (analytical grade)	RCI Labscan Limited, Thailand
Dulbeco's Modified Eagel's Medium (DMEM)	GIBCO, USA
Fetal bovine serum (FBS)	HyClone, USA
Free Glycerol Reagent	Sigma-Aldrich, USA
HEPES	Calbiochem, Taiwan
Insulin, Human Recombinant	Sigma-Aldrich, USA

Isopropyl alcohol	BDH, England
Oil Red O	Sigma-Aldrich, USA
Oligo-(dT)-18 primers	Bio Basic Inc.,
Potassium chloride	BHD, England
Potassium dihydrogen phosphate	BHD, England
Potassium phosphate	BHD, England
Primers	WARD MADIC LTD, Thailand
Recovery™ Cell Culture Freezing Medium	GIBCO, USA
RevertAidTM First Stand cDNA Synthesis Kit	Fermentas, Canada
Ribonuclease inhibitor	Fermentas, Canada
RNase Away	Molecular Bio Products, USA
Sodium chloride	Merck, Germany
Sodium hydrogen carbonate	BDH, England
Tris	Research Organic, USA
Trizol reagent	Invitrogen, USA
Trypan Blue	Sigma-Aldrich, USA
Trypsin	Invitrogen, USA
Tumor necrosis factor (TNF-α)	Peprotech,USA
WST-1 reagent	AppliChem, German

APPENDIX B

List of instrument used in this study

Instrument	Company
Autoclave	Tomy autoclave SS-240
Automatic pipette	Thermoscientific
Carbon dioxide incubator	Thermoscientific
Centrifuge	KUBOTA CORPORATION
Micro-plate spectrophotometer	Bio-Tek Instrument
Freezer (-80^{o}C)	Forma Scientific
Freezer (-20^{o}C)	Sanyo
Glassware	Pyrex and Scott duran
Inverted microscope	NIKON
Laminar flow biological cabinet	NU AIRE
Liquid nitrogen tank	International Cryogenics, Inc.
Magnetic stirrer	Thermolyne
Nano Drop spectra	Thermoscientific
Vortex	Scientific industries
7500 Fast Real-Time PCR System	Applied Biosystems

APPENDIX C

Reagents and buffers preparation

1. Reagents for cell culture

1.1 DMEM medium

DMEM powder	13.5	g
HEPES	2.603	g
NaHCO3	3.7	g

Add DW to 1,000 ml, adjust pH 7.4 and sterile by Millipore filter membrane (0.22 µm) and stored at 4oC

1.2 Complete DMEM medium

1.2.1 Fetal calf serum medium

Calf Serum	10	mL
DMEM	88	mL
10X Antibiotic antimycotic solution	1	mL
Non-Essential amino acid	1	mL

1.2.2 MDI medium

Fetal Bovine Serum (Filter Sterilized)	10	mL
DMEM	86.8	mL
Insulin	100	µl
IBMX	1	mL
Dexamethasone	100	µl
10X Antibiotic antimycotic solution	1	mL
Non-Essential amino acid	1	mL

1.2.3 Insulin medium

Fetal Bovine Serum (Filter Sterilized)	10	mL
DMEM	87.9	mL
Insulin	100	µl
10X Antibiotic antimycotic solution	1	mL
Non-Essential amino acid	1	mL

1.2.4 FBS insulin medium

Fetal Bovine Serum (Filter Sterilized)	10	mL
DMEM	87.5	mL
Insulin	50	µl
10X Antibiotic antimycotic solution	1	mL
Non-Essential amino acid	1	mL

1.3 Phosphate buffer saline (PBS pH 7.4)

NaCl	8	g
KCl	0.2	g
Na2HPO4	1.15	g
KH2PO4	0.2	g
DW	800	mL

Adjust pH 7.4 using 1M HCl, then adjust volume to 1,000 ml

2. DEPC treated water

Deionized water	1000	mL
DEPC	0.1	mL

3. IBMX Solution (make fresh)

IBMX	0.0115	g
0.5N KOH	0.5	mL
DW	0.5	mL

Dissolve IBMX in a solution made of 0.5N KOH to a final concentration of 0.0115g/mL and then filter sterilize through a 0.22 mm syringe filter.

4. Insulin Stock Solution

Insulin	0.01	g
0.02M HCl	10	mL

167 uM (1mg/mL) in 0.02M HCl and then filter sterilized through 0.22 mm filter. Can store at -20^{o}C for long term, 4^{o}C short term.

5. Dexamethasone Stock Solution

Dexamethasone	0.0197	g
100% ethanol	5	mL

Freezer Stock: 10mM of Dex in 100% ethanol (store at -20C)

Dexamethasone	100	µl
PBS (filter)	900	µl

Working Stock: Dilute Freezer stock to 1mM in PBS.Filter sterilize and store at 4C.

6. 0.5% Oil Red O solution

Oil Red O	0.5	g
Isopropyl alcohol	100	mL

APPENDIX D

1. Chemical constituents the dichloromethane and methanol extracts of rice

Table 1 the yield of dichloromethane and methanol extracts of rice

Variety	Yield (%) Dichloromethane	Yield (%) MeOH
DSK	**2.00**	**1.39**
PYO	**1.91**	**1.15**
NAN	**1.64**	**0.95**
RD 6	**3.05**	**0.88**

Table 2 Content of γ-oryzanol in rice extracts

Variety	γ-oryzanol (mg/g)	
	Dichloromethane	**MeOH**
DSK	**36.00**	**2.18**
PYO	**44.17**	**1.62**
NAN	**42.88**	**2.98**
RD6	**10.94**	**3.03**

Table 3 Content of total anthocyanin in rice extracts

Variety	mg/g sample			
	Cyanidin-3-o-glucoside	**Cyanidin-3-o-rutinoside**	**Peonidin-3-o-glucoside**	**Total**
DSK	**2.50**	**nd**	**1.97**	**4.47**
PYO	**3.19**	**nd**	**2.60**	**5.80**
NAN	**4.40**	**nd**	**3.35**	**7.75**
RD 6	**nd**	**nd**	**nd**	**nd**

2. Phytochemical analysis by TCL

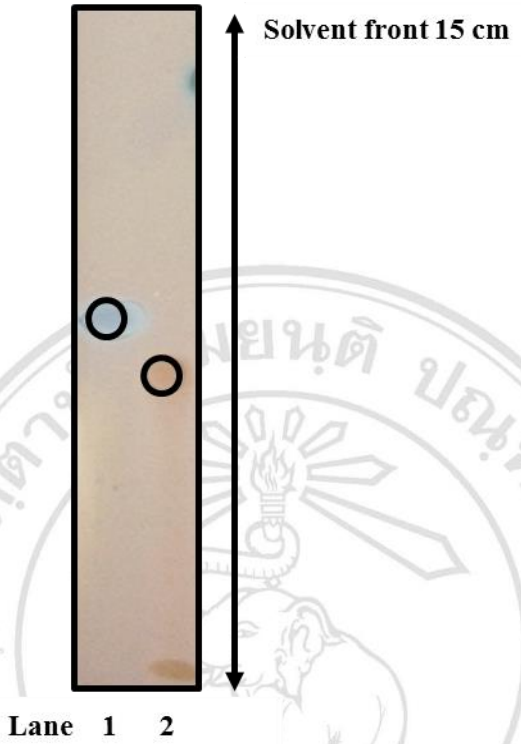

Figure 2.1 TLC Fringerprints of Standard Compounds TLC separation was run using the mixture of Chloroform:ethyl acetate:acetic acid (50:50:1) as mobile phase. The TLC plate was then dipped in anisaldehyde-sulfuric acid reagent before color developing by heat at 100°C 5 min. Lane 1-3 are standard compouds; ferulic acid and quercetin, respectively.

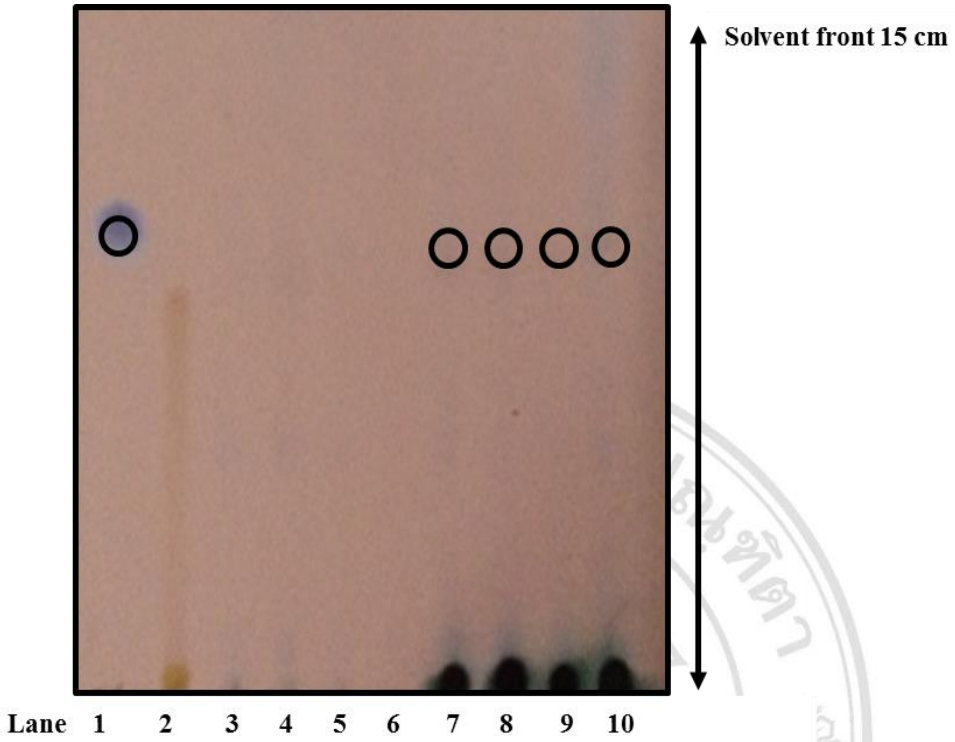

Figure 2.2 TLC Fringerprints of Rice Extract. TLC separation was run using the mixture of Chloroform:ethyl acetate:acetic acid (50:50:1) as mobile phase. The TLC plate was then dipped in anisaldehyde-sulfuric acid reagent before color developing by heat at 100°C 5 min. Lane 1-2 are ferulic acid and quercetin. Lane 3-6 is Dichloromethane extract of DSK, PYO, NAN and RD6, respectively. Lane 7-10 is Methanolic extract of DSK, PYO, NAN and RD6, respectively

CURRICULUM VITAE

Name Miss Nareeya Waloh

Date of birth May 16, 1989

Place of Birth Pattani Province, Thailand

Education 2006, Certificate of High School
Dechapattanayanukul School, Pattani, Thailand
2010, Bachelor of Science,
Faculty of Science, Kasetsart University, Bangkok, Thailand

Publication

Waloh N, Wongnoppavich A, Bonness A. Effects of Purple Rice (*Oriza sativa* L.) on Adipogenesis and Inflammation-induced Insulin Resistance in Adipocyte. The 4th International Biochemistry and Molecular Biology Conference. 2-3 April, 2014, 173. Bangkok, Thailand. **(**Proceeding and poster presentation)