APPENDIX A

List of chemicals and materials used in the study

Chemicals/materials	Source
Agarose	Vivantis, USA
Absolute ethanol	Liquor distillery organization excise department, Thailand
Acryl/Bis solution	Amresco, USA
Ammonium persulfate	Amresco, USA
Bicalutamide	AstraZeneca UK Limited, UK
Bromophenol blue	USB Corporation Cleveland, USA
Corn oil	Sigma-Aldrich, USA
Developer and Replenisher	Carestream, Malaysia
Diethyl ether	RCI Labscan, Thailand
Dimethyl sulfoxide (DMSO)	RCI Labscan, Thailand
3-(4,5-Dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT)	Bio Basic Inc., Canada
ECL substrate buffer	Bio-Rad Laboratories, USA
Enrofloxacin	General drugs house, Thailand
Ethanol	RCI Labscan, Thailand
Fetal bovine serum	HyClone, UK

Chemicals/materials	Source
Finasteride	MSD International GmbH, USA
Fixer and Replenisher	Kodak, Malaysia
Formaldehyde	RCI Labscan, Thailand
Glycerol	USB Corporation Cleveland, USA
Glycerol gel loading dye	Amresco, USA
Glycine	Amresco, USA
HybondTM-ECL nitrocellulose membrane	GE Healthcare life sciences, Germany
Ladder	Bioline USA Inc., USA
Mercaptoethanol	Pharmacia Biotech, Sweden
Methanol	RCI Labscan, Thailand
Penicillin Streptomycin	Life Technologies, USA
Protease inhibitor Cocktail	Sigma-Aldrich, USA
RPMI medium 1640	Life Technologies, USA
Tetramethylethylenediamine (TEMED)	USB Corporation Cleveland, USA
Testosterone propionate	Wako, Japan
Tissue lysis buffer	Bio Basic Inc., Canada
Triton X-100	Sigma-Aldrich, USA
Tris	Amresco, USA
Trypsin-EDTA	Life Technologies, Canada
Tween 20	Sigma-Aldrich, USA

Chemicals/materials	Source
Skim milk	HiMedia Laboratories, India
Sodium deoxycholate	Sigma-Aldrich, USA
Sodium dodecyl sulfate	USB Corporation Cleveland, USA
Sodium chloride	Merck, Denmark
Zolentil 100	Virbac, Thailand
X-ray film	Kodak, USA

APPENDIX B

List of instrument used in the study

Instrument	Model	Source
Autoclave	SS-245	TOMY, Japan
Centrifuge		TOMY, Japan
CO_2 incubator	BL-1600	ASTEC Co., Ltd., Japan
Evaporator		BUCHI, Switzerland
Freezer (-20 ºC)		Nihong, Japan
Freezer (-80 ºC)		NUAIRE, USA
Laminar air flow	S-2010	Heto-Holten, Denmark
Light microscope	CH112	Olympus , Japan
Lyophilizer		Thermo Electron Corporation, USA
pH meter	pHTestr®	Oakton®
Power supply	PowerPac HC	Bio-Rad, USA
Refrigerator		LG, Thailand
Semi dry blotter	SD10	Cleaver scientific, UK
Super speed centrifuge		Eppendorf, Germany
Water bath	OLS200	Grant, UK

APPENDIX C

Reagents and buffers preparation

1. Reagent for determination of phenolic content

1.1 7% Na_2CO_3

Na_2Co_3	7	g
DI	100	ml

1.2 Folin-Ciocalteu's phenol reagent

Dilute before use with 1:1	100	ml

2. Reagent for DPPH assay (0.2 mM DPPH)

DPPH	0.8	mg
Absolute MeOH	10	ml

3. Reagent for determination for prostatic hyperplasia in rat

3.1 1M Phosphate buffer saline (PBS) pH 7.4

NaCl	40	g
KCl	1.0	g
Na_2HPO_4	5.75	g
KH_2PO_4	1.0	g
DI	350	ml

3.2 10% formaldehyde in PBS

PBS	900	ml
Formaldehyde	100	ml

4. Reagent for Western blot

4.1 RIPA buffer (1 M Tris pH 7.4, 5 M NaCl, 10% triton X-100, 1% sodium deoxycholate, 0.5 M EDTA)

5 M NaCl	3	ml
1 M Tris pH 7.4	5	ml
0.5 M EDTA	0.2	ml
10% Triton x-100	10	ml
Sodium deoxycholate	1	g
Sterilized DI	81.8	ml
Total	100	ml

Protease inhibitor cocktail (add before use)

4.2 30% Acrylamide/Bis Solution

40% Acrylamide/Bis Solution	7.5	ml
DI	2.5	ml

4.3 0.5 M Tris-HCI pH 6.8

Tris	6.0	g
DI	80	ml

Adjust pH to 6.8 with HCI then adjust volume to 100 ml and kept at 4 ºC

4.4 1.5 M Tris-HCI pH 8.8

Tris	18.15	g
DI	80	ml

Adjust pH to 8.8 with HCI then adjust volume to 100 ml and kept at 4 ºC

4.5 10% Sodium dodecyl sulfate (SDS)

SDS	10	g
DI	100	ml

Keep at room temperature

4.6 10% ammonium persulfate (APS) (preparation before use)

APS	0.1	g
DI	1	ml

4.7 Separating gel preparation (12% gel)

DI	3.4	ml
1.5 M Tris-HCI pH 8.8	2.5	ml
10% SDS	100	µl
30% Acrylamide/Bis Solution	4.0	ml
10% APS	100	µl
TEMED	10	µl

Add APS and TEMED into the solution after completely set the gel apparatus

4.8 Separating gel preparation (8% gel)

DI	4.7	ml
1.5 M Tris-HCI pH 8.8	2.5	ml
10% SDS	100	µl
30% Acrylamide/Bis Solution	2.7	ml
10% APS	100	µl
TEMED	10	µl

Add APS and TEMED into the solution after completely set the gel apparatus

4.9 Separating gel preparation (4% gel)

DI	3.05	ml
0.5 M Tris-HCI pH 6.8	1.25	ml
10% SDS	50	µl
30% Acrylamide/Bis Solution	0.65	ml
10% APS	50	µl
TEMED	5	µl

Add APS and TEMED into the solution after completely set the gel apparatus

4.10 0.5% Bromophenol blue

Bromophenol blue	0.05	g
DI	10	ml

4.11 2X Stock sample buffer

0.5 M Tris-HCI pH 6.8	250	µl
10% SDS	400	µl
Glycerol	200	µl
0.5% BPB	50	µl
DI	100	ml

4.12 SDS reducing buffer

Stock sample buffer	950	µl
2-mercaptoethanol	50	µl

(add immediately before use)

4.13 10X Running buffer

Tris-base	30	g
Glycine	144	g
10% SDS	10	ml

DW to 1000 ml

Keep at 4 ºC

4.14 1X Running buffer

10X	100	ml
DI	900	ml

4.15 1X Blotting buffer

Tris-base	3	g
Glycine	14.4	g
DI	800	ml

Add Methanol to adjust volume to 1000 ml

4.16 Tris-buffered saline (TBS) pH 7.5

Tris	24.2	g
NaCI	80	g
Distilled water	800	g

Adjust pH to 7.5 then adjust volume to 1000 ml keep at 4 ºC

4.17 Washing buffer (0.05% Tween 20)

1X TBS	99.95	ml
Tween 20	0.05	ml

Keep at 4 ºC

4.18 Washing buffer (1% Tween 20)

1X TBS	99	ml
Tween 20	1	ml

Keep at 4 ºC

4.19 Blocking buffer (5% Skim milk prepare before use)

Skim milk	1	g
Washing buffer (0.05% Tween 20)	20	ml

4.20 Stripping buffer

Glycine	15	g
SDS	1	g
Tween 20	10	ml
DI	800	ml

Adjust pH to 2.2 then adjust volume to 1000 ml keep at 4 ºC

5. Reagent for cell culture

5.1 RPMI medium 1640

RPMI powder	10.4	g
$NaHCO_3$	2.0	g
100X Penicillin Streptomycin	10.0	ml
DW	800	ml

Adjust pH to 7.0-7.4 then adjust volume to 1000 ml keep at 4 ºC

5.2 Complete RPMI medium

RPMI	90	ml
Fetal bovine serum	10	ml

6. Reagent for cytotoxicity by MTT assay (12mM MTT)

MTT	5	mg
Sterile PBS	1	ml

Filtrate through 0.45 µM filter and keep at 4 ºC (within 1 month)

7. Reagent for RT-PCR

7.1 0.5 M EDTA (pH 8.0)

EDTA	186.1	g
DI	800	ml

Add NaOH solution to adjust pH to 8.0 then adjust volume to 1000 ml and sterilize in an autoclave

7.2 50X TAE Electrophoresis Buffer

Tris-base	242	g
Glacial Acetic Acid	57.1	ml
0.5 M EDTA (pH 8.0)	100	ml
DI	600	ml

Adjust to 1000 ml

7.2 0.5X TAE Electrophoresis Buffer

50 X TAE	10	ml
DI	990	ml

7.3 2% Agarose gel

Agarose	2	g
0.5 X TAE	100	ml

Microwave for 1-3 minute then add ethidium bromide (EtBr) 5 µl

APPENDIX D

Certificate of Approval

For Use of Animals

Faculty of Medicine, Chiang Mai University

Protocol Number: **19/2557**

Title of project: **The study of effects of purple rice extract on testosterone-induced benign prostatic hyperplasia**

Principal investigator: **Assistant Professor Teera Chewonarin, Ph.D.**

Affiliation: **Department of Biochemistry**

The Faculty of Medicine, Chiang Mai University, supported by the results of Animal Ethics committee review, that the use of animals in the project conforms with international and national guidelines for ethical conduct on the care and use of animals.

Hereby approves the research proposal to be conducted under its proposed scheme. The approval is effective from **2 September 2014**

Kom Sukontason

Kom Sukontason, M.D., Ph.D.

Professor

Chair

Date 9 September 2014

W.N

Wattana Navacharoen, M.D.

Associate Professor

Dean

Date 9 September 2014

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	Kiriya, C., Summart, R., Chewonarin, T., (2016). Effects of purple rice (*Oryza sativa* L. *indica*) extract on testosterone-induced prostatic hyperplasia in rats and on growth inhibition against human prostate cancer cell line. The 14th Annual Biochemistry Research Meeting 2016, 28th-29th July, Chiang Mai, Thailand

Proceeding

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