APPENDICES

APPENDIX A

List of the chemicals and materials used in the study

Chemicals and materials	Source
β-Actin antibody	Abcam, USA
β-NADPH	Oriental Yeast, Japan
1-Chloro-2, 4-dinitrobenzene	Fluka A.G., Switzerland
2-Mercaptoethanol	Sigma-Aldrich, USA
4-Para nitrophenol	Fisons Scientific, England
4´, 6-Diamidino-2-phenylindole dihydrochloride	Invitrogen, USA
Absolute ethanol	BDH, England
Acrylamide	Biorad, USA
Aluminium chloride hexahydrate	Merck A.G., Germany
Ammonium persulfate	Carlo-Erba, Italy
Bis-acrylamide	Biorad, USA
Bovine serum albumin	Sigma-Aldrich, USA
Bovine serum albumin standard	Thermo Fisher Scientific, USA
Bromophenol blue	Sigma-Aldrich, USA
Calcium chloride dihydrate	Merck A.G., Germany
Catechin	Sigma-Aldrich, USA
Chemiluminescent film	Amersham, England

Chemicals and materials	Source
Chloroform	LAB-SCAN, Thailand
Collagenase type IV	Invitrogen, USA
Copper sulfate	Merck A.G., Germany
Cytochrome c Type VI	Sigma-Aldrich, USA
Dichloromethane	BDH, England
Dichlorophenolindophenol	Sigma Chemical, USA
Diethyl ether	BDH, England
Diethylnitrosamine	Sigma Chemical, USA
Dimethyl sulfoxide	BDH, England
Dipotassium hydrogen phosphate (anhydrous)	BDH, England
Disodium hydrogen orthophosphate	BDH, England
Dithiothretiol	Sigma Chemical, USA
Enhanced chemiluminescence	Thermo Fisher Scientific, USA
Ethylenediaminetetraacetic acid	Sigma Chemical, USA
Flavin adenine dinucleotide	Sigma Chemical, USA
Folin & Ciocalteu' s phenol reagent	Fluka A.G., Switzerland
Formalin	BDH, England
Gallic acid	Sigma-Aldrich, USA
Glucose-6-phosphate	Sigma-Aldrich, USA
Glucose-6-phosphate dehydrogenase	Sigma-Aldrich, USA
Glutathione (Reduced form)	Wako, Japan
Glycerol	Sigma-Aldrich, USA
GST Alpha antibody	Diagnostic international, USA

Chemicals and materials	Source
Goat anti-rabbit IgG peroxidase conjugate	Biorad, USA
Hemin	Sigma-Aldrich, USA
Hemeoxygenase-1 antibody	Stressgen, USA
HEPES, free acid	Amresco, USA
Hydrochloric acid	BDH, England
Isopropanol	Sigma-Aldrich, USA
Methanol	BDH, England
Millipore membrane	Nihon Millipore, Japan
Magnesium chloride	APS Finechem, Australia
Phenol red sodium salt	Amresco, USA
Phenylmethylsulfonylfluoride	Sigma-Aldrich, USA
Potassium chloride	Carlo-Erba, Italy
Potassium cyanide	Merck A.G., Germany
Potassium hydroxide	Carlo-Erba, Italy
Potassium dihydrogen phosphate	May and Baker, England
Potassium phosphate	Merck A.G., Germany
Skimmed milk	Merck A.G., Germany
Sodium bicarbonate	BDH, England
Sodium chloride	BDH, England
Sodium dihydrogen phosphate	BDH, England
Sodium dodecyl sulfate	Biorad, USA
Sodium hydrogen carbonate	BDH, England

Chemicals and materials	Source
Sodium hydroxide	BDH, England
Sodium nitrite	AJAX chemicals, Australia
Sodium potassium tartrate	Mallinckrodt chemilcal work, USA
TEMED	USB, USA
Trichloroacetic acid	BDH, England
Tris base	Vivantis, Malaysia
Tween 20	USB, USA
Tween 80	BDH, England
UDP-glucuronic acid	Sigma-Aldrich, USA
X-ray film	Kodak, USA

APPENDIX B

List of the instruments used in the study

Instrument	Model	Source
Blotting apparatus	Trans-blot SD cell	Biorad, USA
Centrifugator	PMC-060	Tomy Seiko, Japan
	22R D-78532	Mikro, Germany
Electrophoretic apparatus	AE-6500	Atto Corp., Japan
Film cassette	RPN 11649	Amersham, England
Freezer (-86^{o}C)	0838	Foema Scientific, USA
Fluorescent microscope	AX-70	Olympus, Japan
Homogenizer	HS-30E	Daihan, Korea
Hotplate/stirrer	HPMS	Whatman, USA
Microplate reader	MCC/340	ICN, Flow, USA
pH meter	320	Mettler Toledo, USA
Peristalic pump	MP-100	Tokyo Rikakikai, Japan
Refrigerator	SR-F511	Sanyo, Thailand
Ultracentrifugator	L-100 XP	Beckman Coulter
UV-Vis Spectrophotometer	UV-1700	Shimazu, Japan
Vortex	G-560E	Scientific industries, USA
Water bath	W-350	Mammert, Germany

Instrumen	**Model**	**Source**
Water bath shaker	N1-13S	Hangzhou Bioer Technology, China
Incubator	Heraeus B 5060	Burladingen, Germany
Dry bath incubator	EL-02-220	Major Science, USA

APPENDIX C

Reagent and buffers preparation

1. Preparation of mediums for liver-cell suspension

1.1 Preperfusion medium

NaCl	8	g
KCl	0.4	g
KH_2PO_4	0.06	g
Na_2HPO_4	0.09	g
EGTA	0.195	g
HEPES	2.39	g
$NaHCO_3$	0.35	g

After dissolve in distilled water, adjust pH to 7.4 with 1N NaOH and bring to 1 L with distilled water

1.2 Collagenase medium

NaCl	8	g
KCl	0.4	g
KH_2PO_4	0.06	g
Na_2HPO_4	0.09	g
Phenol red	0.01	g
$CaCl_2 \cdot 2H_2O$	0.56	g
HEPES	2.39	g

$NaHCO_3$	0.35	g
Collagenase type IV	0.5	g

After completely dissolve in 1 liter of distilled water, adjust pH to 7.4 with 1 N NaOH

1.3 Phosphate buffer saline ($Mg^{2+,}$ $Ca^{2+,}$ free)

NaCl	8	g
KCl	0.2	g
KH_2PO_4	0.2	g
Na_2HPO_4	1.15	g

After dissolve in distilled water,adjust pH to 7.4 with 1N NaOH and bring to 1 L with distilled water

2. Preparation of buffers for microsome fraction

2.1 Homogenizing buffer

Dissolve 11.5 g of KCl and 0.37 g of EDTA in 1000 ml of deionized water, add 1 ml of 0.25 M PMSF (in ethanol) and adjust pH to 7.4 with 1 N NaOH.

2.2 Microsome suspension buffer

KH_2PO_4	0.14	g
EDTA	0.004	g
DTT	0.002	g
Glycerol	3	ml
Deionized water	10	ml

Dissolve all ingredients in deionized water. Then adjust of solution to 7.4 with conc. KOH.

3. Preparation of reagent for total protein assay by Lowry method

3.1. 2% Na_2CO_3 in 0.1 N NaOH (Reagent A)

Dissolve 10 g of Na_2CO_3 and 2 g of NaOH in deionized water. Add NaOH and adjust pH to 8.0 with 2 N HCl and adjust volume to 500 ml.

3.2. 0.5% $CuSO_4.5H_2O$ (Reagent B)

Dissolve 1 g of $CuSO_4$ in 200 ml of distilled water.

3.3. 1% $KNaC_4H_4O_6.4H_2O$ (Reagent C)

Dissolve 1 g of $KNaC_4H_4O_6$ in 100 ml of distilled water.

3.4. Carbonate-copper solution (Reagent D)

Prepare Carbonate-copper solution by mixing reagent A, B and C at ratio 50:1:1.

3.5. Diluted Folin reagent

Prepare Folin reagent by diluting Folin-Ciocalteu' phenol reagent in water at ratio 1:1.

4. Preparation of SDS-PAGE reagents and buffers

4.1. 30% Acrylamide

Dissolve 30 g of acrylamide in 100 ml of deionized water.

4.2. 0.8% Bisacrylamide

Dissolve 0.8 g of acrylamide in 100 ml of deionized water

4.3. *Stacking gel buffer pH 6.8*

Dissolve 60.57 g of Tris base and 8 g of sodium dodecyl sulfate in deionized water. Adjust pH and total volume to 7.4 with conc. NaOH and to 1000 ml, respectively

4.4. 10% Ammonium persulfate

Dissolve 10 g of ammonium persulfate in 100 ml of deionized water.

4.5. Sample buffer pH 6.8

Tris base	0.15	g
Sodium dodecyl sulfate	0.40	g
Glycerol	2	g
2-mercaptophenol	1	g
0.002% Bromophenol blue	0.005	ml
Deionized water	1	L

Dissolve all ingredients in deionized water. Then, adjust pH and total volume to 6.8 with conc. HCl.

4.6. Electrode buffer

Tris base	3.03	g
Glycine	4.41	g
Sodium dodecyl sulfate	1	g
Deionized water	1	L

Dissolve all ingredients in deionized water and adjust volume to 1000 ml

4.7. Blotting buffer

Tris base	12.11	g
Glycine	14.40	g

Dissolve 12.11 g of Tris base and 14.4 g of glycine in deionized water. Then, add 200 ml of methanol and adjust total volume to 1 L.

5. Preparation of immunostaining buffer

5.1. Phosphate buffer saline pH 7.5

Dissolve 1.56 g of NaH_2PO_4 and 9 g of NaCl in deionized water. Then, adjust pH and total volume to 7.5 with conc. NaOH and to 1 L, respectively.

5.2. Tween-phosphate buffer saline (TPBS) pH 7.5

$NaH_2PO_4.2H_2O$	1.56	g
NaCl	9	g
Tween 20	0.5	ml
Deionized water	1	L

Dissolve $NaH_2PO_4.2H_2O$ and NaCl in deionized water. Adjust pH and total volume to 6.8 with conc. HCl. Finally, add 0.5 ml of Tween 20.

5.3. 5% Skimmed milk

Dissolve 5 g of non- fat dried milk in 100 ml of TPBS.

5.4. 10% BSA

Dissolve 10 g of BSA in 100 ml of TPBS.

5.5. TPBS-0.2% BSA

Add 2 ml of 10% BSA into 100 ml of TPBS.

6. Preparation of SDS-PAGE gel

6.1. *Separating gel/ 2 gels*

30% Acrylamide	5	ml
0.8% Bisacrylamide	3.75	ml
Deionized water	6.25	ml
TEMED	10	µl
10% APS	100	µl

6.2. Stacking gel/ 2 gels

30% Acrylamide	0.56	ml
Stacking gel buffer pH 6.8	1.2	ml
Deionized water	6.25	ml
TEMED	10	µl
10% APS	100	µl

APPENDIX D

Morphology of hepatocytes

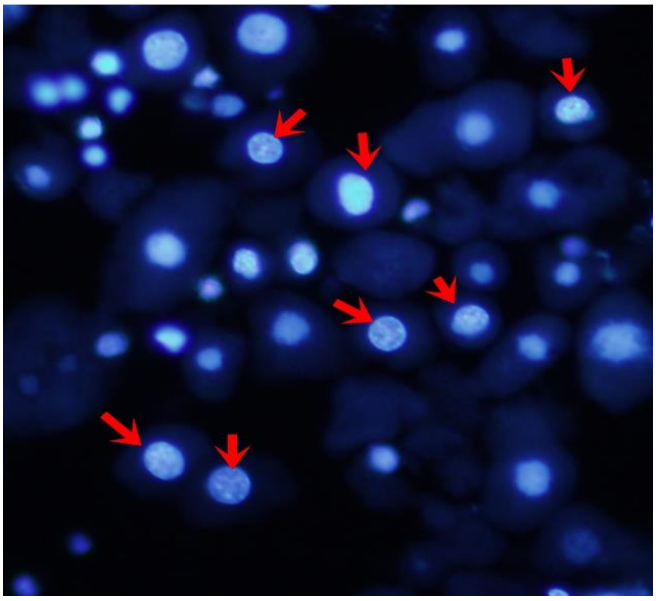

Figure S-1 Normal hepatocytes

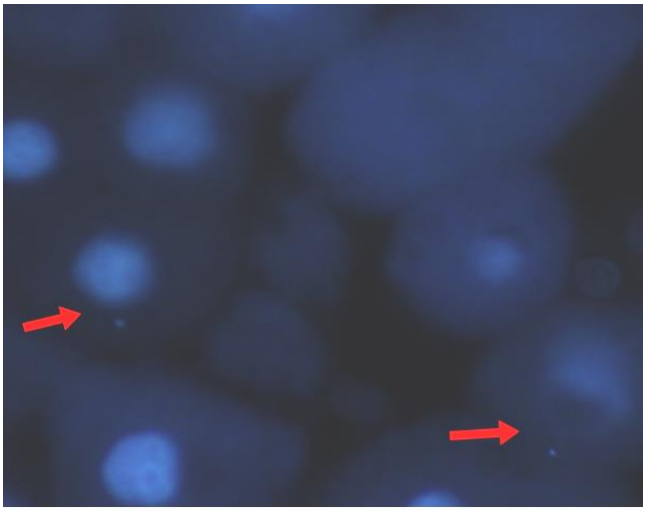

Figure S-2 Mono-micronucleus hepatocytes

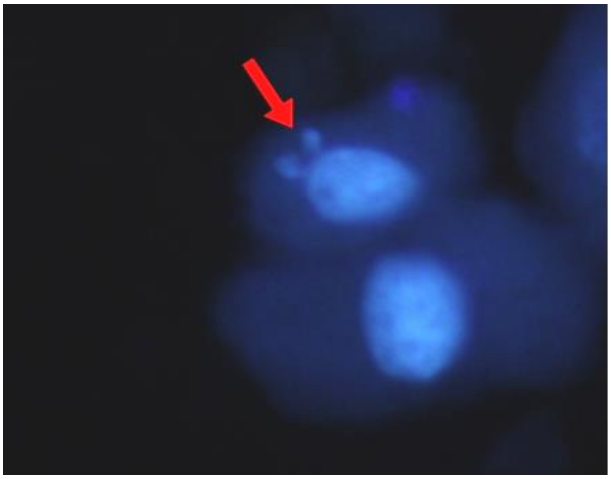

Figure S-3 Bi-micronucleus hepatocyte

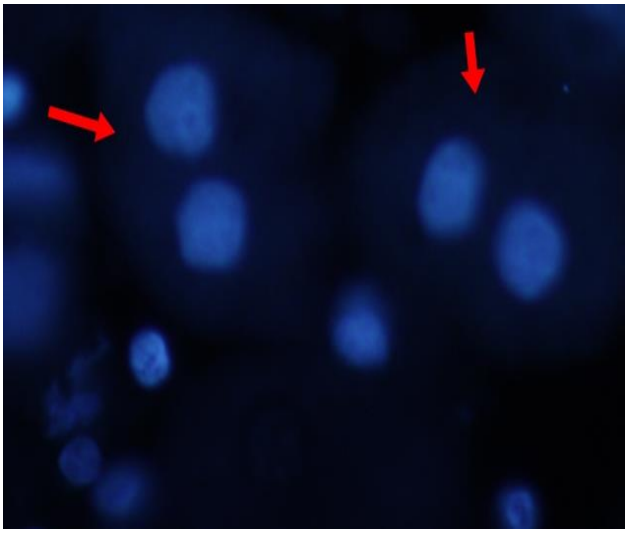

Figure S-4 Binucleated hepatocytes

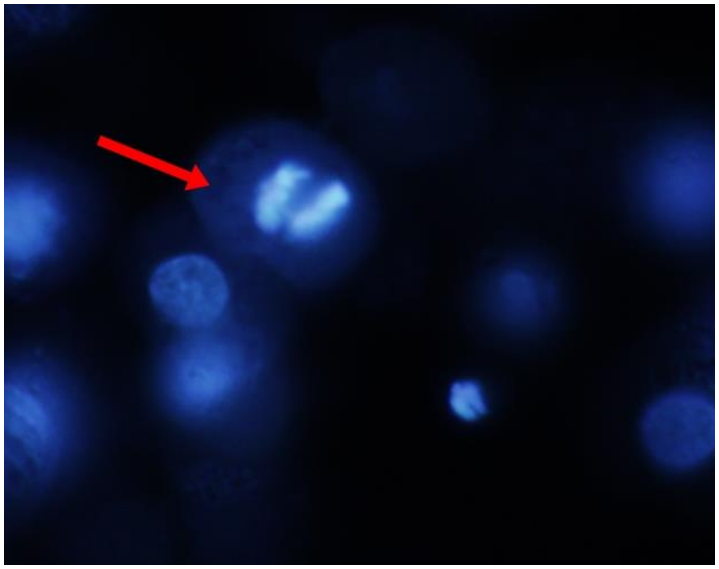

Figure S-5 Anaphase hepatocyte

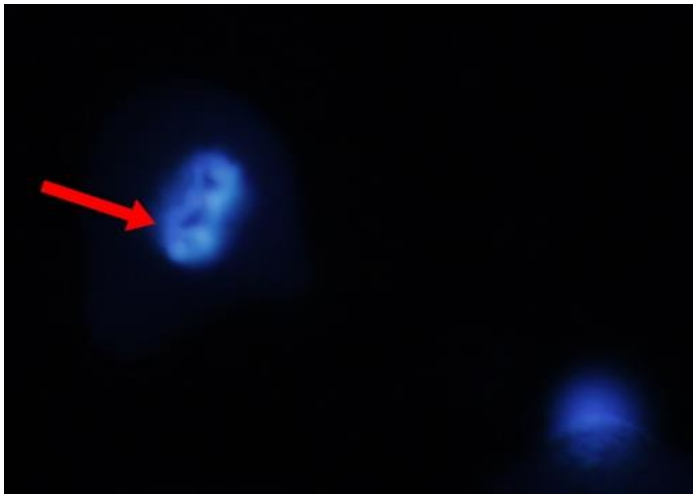

Figure S-6 Metaphase hepatocyte

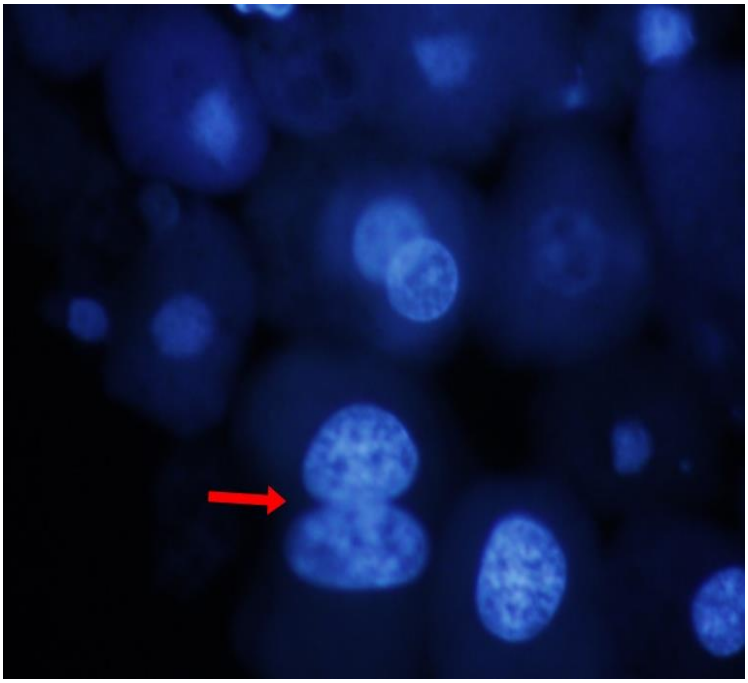

Figure S-7 Telophase hepatocyte

APPENDIX E

Chemical constituents the dichloromethane extract of glutinous purple rice hull

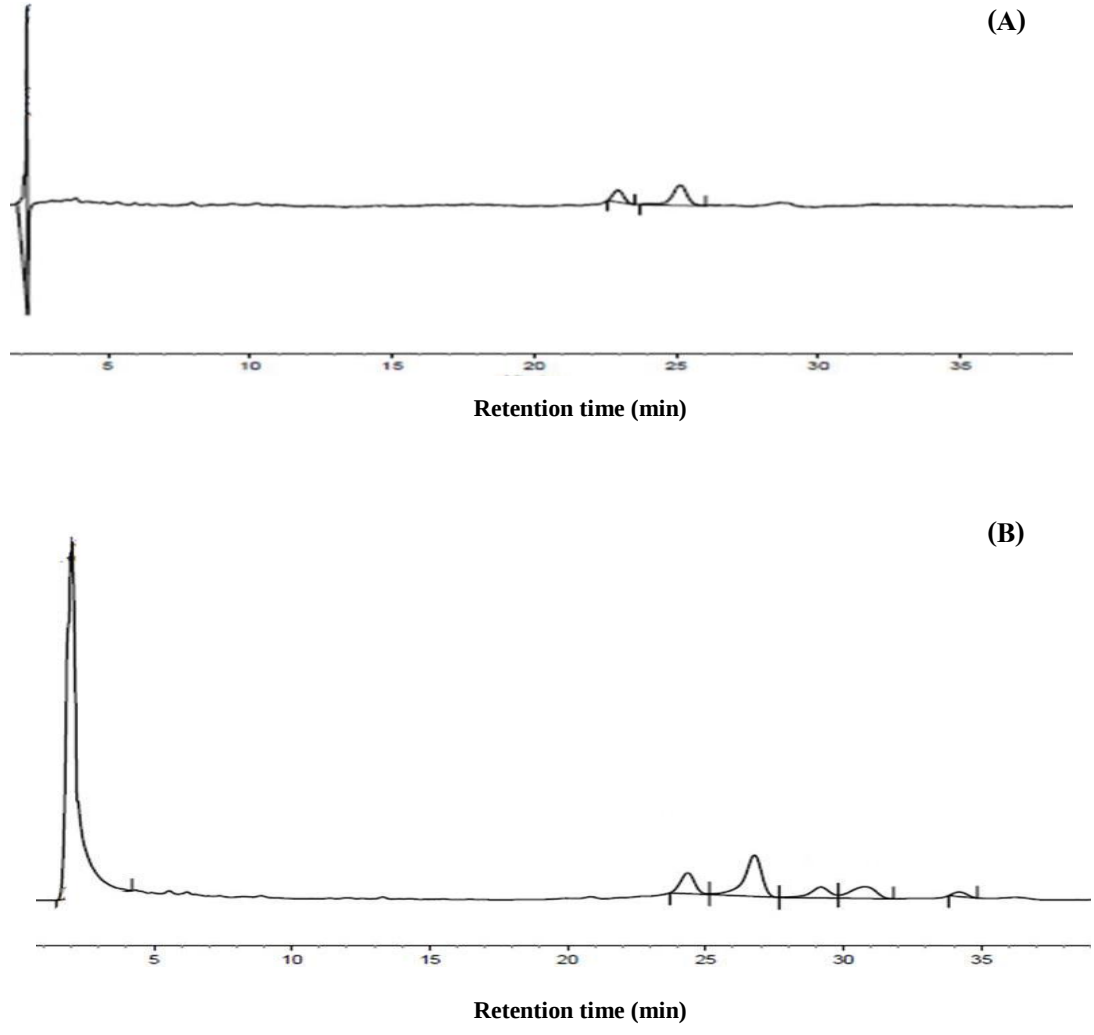

Figure S-8 Chromatograms of standard gamma oryzanol (A) and dichloromethane extract of purple rice hull (B)

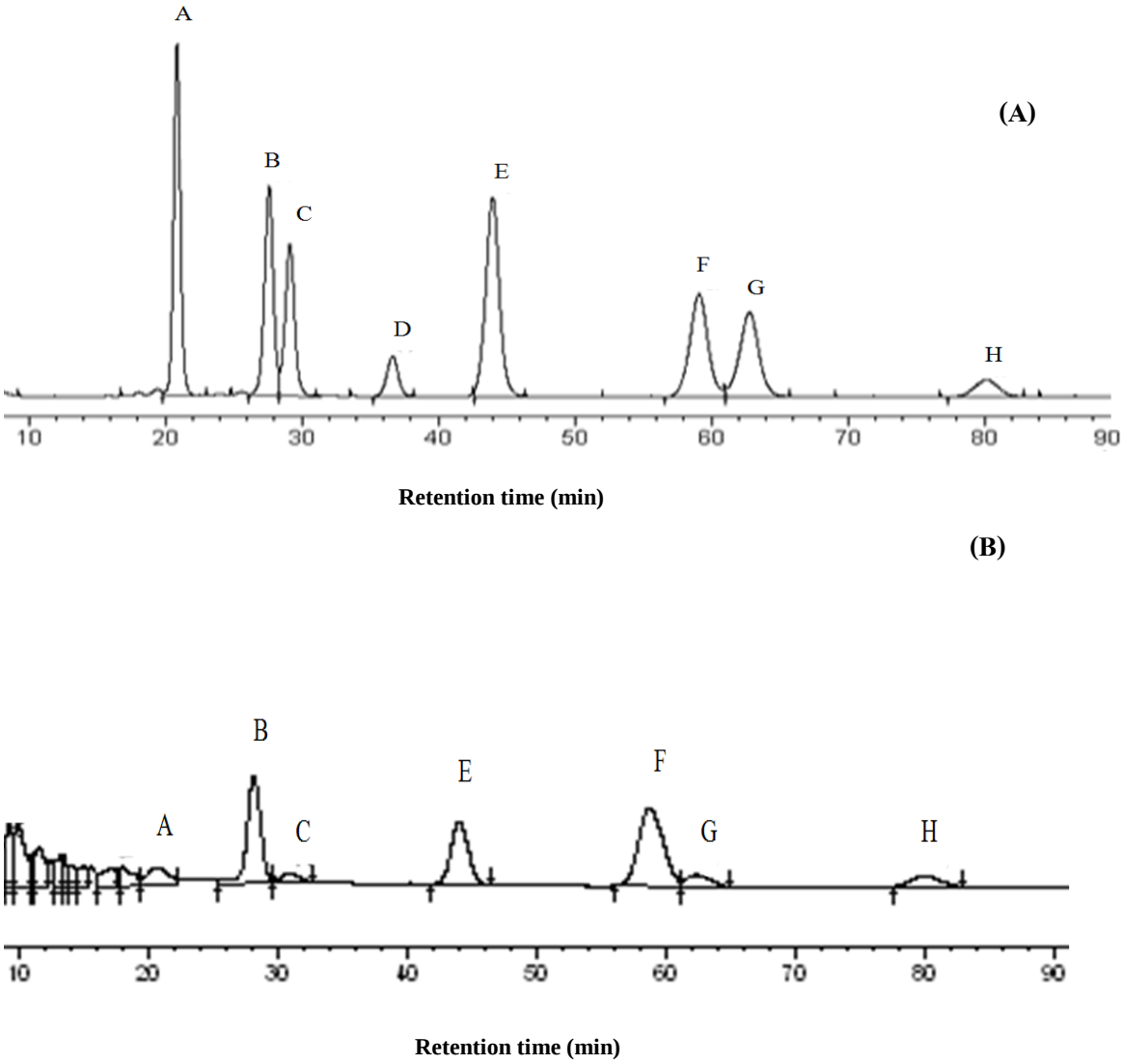

Figure S-9 Chromatograms of standard tocols (A) and dichloromethane extract of purple rice hull (B). Peaks A, B, C and D are δ-, γ–, β– and α– tocotrienols are A, B, C and D, respectively. Peaks E, F, G and H are δ–, γ–, β–, α– tocopherols

Table S-1 Chemical constituents in the dichloromethane extract of glutinous purple rice hull

Parameters	Chemical constituents
γ-oryzanol (mg /g extract)	23.62 ± 0.07
α- Tocopherol (μg/g extract)	65.32 ± 0.87
β- Tocopherol (μg/g extract)	14.15 ± 0.30
δ- Tocopherol (μg/g extract)	87.45 ± 0.15
γ- Tocopherol (μg/g extract)	44.08 ± 0.34
α- Tocotrienol (μg/g extract)	N. D.
β- Tocotrienol (μg/g extract)	10.93 ± 0.16
δ- Tocotrienol (μg/g extract)	8.64 ± 0.05
γ- Tocotrienol (μg/g extract)	67.38 ± 0.73

Values are expressed as mean ± standard deviation.

N.D.: not determine

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Publication

1. Sankam P., Punvittayagul C., Sringam K., Chaiyasut C. and Wongpoomchai R. (2013). Antimutagenicity and anticlastogenicity of glutinous purple rice hull. *Mol Cell Toxicol*. 9(2): 169-176.

Presentations

1. Sankam P., Punvittayagul C., Sringam K. and Wongpoomchai R. Clastogenicity and anticlastogenicity of purple rice hull (*Oryza sativa* L.var. *indica*) extract in rats liver. Poster presentation at the 8th Congress of Toxicology in Developing Countries (8CTDC). September 10-13, 2012, Centara grand at Central Ladprao, Bangkok, Thailand, page 88.

2. Sankam P., Punvittayagul C., Sringam K. and Wongpoomchai R. Antimutagenicity and anticlastogenicity of glutinous purple rice hull. Poster presentation at the 7th Princess Chulabhorn International Science Congress. November 29 - December 3, 2012, Shangri-la hotel, Bangkok, Thailand.

3. Sankam P., Punvittayagul C., Sringam K. and Wongpoomchai R. Mechanistic studies of purple glutinous rice extracts (*Oryza Sativa* L.var. *indica*) on mutagenesis induced by diethylnitrosamine in rats. Oral presentation at 10th Biochemical Research Meeting, 16-17 May, 2012, Faculty of Medicine, Chiang Mai University, page 12.

4. Sankam P., Punvittayagul C., Sringam K. and Wongpoomchai R. Inhibitory mechanism of glutinous purple rice on the initiation stage of hepatocarcinogenesis. Oral presentation at 11th Biochemical Research Meeting, 16-17 May, 2013, Faculty of Medicine, Chiang Mai University, page 17.