APPENDIX A

List of the chemicals and materials used in the study

Chemicals/Materials	Source
α-tocopherol	Sigma-Aldrich, USA
β-actin antibody	Abcam, USA
β-NADPH	Oriental yeast, Japan
γ-oryzanol	Sigma-Aldrich, USA
1-Chloro-2, 4-dinitrobenzene	Fluka, Switzerland
2-Mercaptoethanol	Sigma-Aldrich, USA
4', 6-Diamidino-2-phenylindole dihydrochloride	Invitrogen, USA
Acetic acid	BDH ProLabo, England
Acrylamide	Biorad, USA
Aflatoxin B_1	Sigma-Aldrich, USA
$AlCl_3$	BDH ProLabo, England
Ammonium acetate	Merck, Germany
Bis-acrylamide	Biorad, USA
Bovine serum albumin	Thermo Fisher Scientific, USA
Bromophenol blue	Sigma-Aldrich, USA
Catechin	Sigma-Aldrich, USA
Collagenase type IV	Gibco, USA
Cytochrome c type VI	Sigma-Aldrich, USA
Dichloromethane	RCI Labscan, Thailand
Diethyl ether	RCI Labscan, Thailand
Dimethylsulfoxide	RCI Labscan, Thailand
EGTA	Amresco, USA

Enhanced chemiluminescence	Thermo Fisher Scientific, USA
Erythromycin	Sigma-Aldrich, USA
Ethoxyresorufin	Sigma-Aldrich, USA
Folin & Ciocalteu's phenol reagent	BDH ProLabo, England
Formaldehyde	RCI Labscan, Thailand
Formalin	BDH ProLabo, England
Gallic acid	Sigma-Aldrich, USA
Goat anti-rabit IgG peroxidase conjugate	Biorad, USA
GST Alpha antibody	Diagnostic international, USA
HEPES, free acid	Amresco, USA
K_2HPO_4	BDH ProLabo, England
KCl	BDH ProLabo, England
KCN	BDH ProLabo, England
Methanol	RCI Labscan, Thailand
Methoxyresorufin	Sigma-Aldrich, USA
Millipore membrane	APS Finechem, Australia
$MgCl_2$	Univar, USA
Na_2CO_3	BDH ProLabo, England
$NaNO_2$	BDH ProLabo, England
NaOH	BDH ProLabo, England
Phenol red sodium salt	Amresco, USA
PNP	Thermo Fisher Scientific, USA
Prestained Protein Marker	BioLabs, England
Resorufin standard	Sigma-Aldrich, USA
Skim milk	Merck, Germany
TEMED	USB, USA
Trichloroacetic acid	Merck, Germany
Tris	Vivantis, Malaysia
Tween-80	Merck, Germany

UDP-glucuronic acid	Sigma-Aldrich, USA
UGT 1A antibody	Cell Signaling, USA

APPENDIX B

List of the instruments used in the study

Instruments	Model	Source
Analytical balance	PA214	Ohaus, USA
Blotting apparatus	AE-6687	Atto Corp., Japan
Centrifuge	MX-305	Tomy Seiko, Japan
Dry bath incubator	EL-02-220	Major Science, USA
Evaporator	MX07R-20-HD2E	Heidolph, Germany
Film cassette	RPN11649	Amersham, England
Fluorescent microscopy	AX-70	Olympus, Japan
Freezer (-80^{o}C)	OA2694	Ogawa, Japan
Homogenizer	HS-30E	Daihan, Korea
Hot plate/stirrer	C-MAG HS7	IKA, Germany
Microplate reader	MCC/340	ICN, Flow, England
Peristaltic pump	400	Rocker, Germany
pH meter	PH500B	Clean, USA
Refrigerator	R-S600P2TH	Hitachi, Japan
UV-Spectrophotometer	UV-1700	Shimadzu, Japan
Vortex	VM-10	Wisemix, Germany
Water bath	YCW-04M	Gemmy, Taiwan

APPENDIX C

Reagents and buffers preparation

1. Preparation of media for liver-cell suspension

1.1 Preperfusion medium

NaCl	8.00	g
KCl	0.40	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 completely dissolve in distilled water, adjust pH to 7.4 with 1 N NaOH and fill up to 1 liter.

1.2 Collagenase medium

NaCl	8.00	g
KCl	0.40	g
KH_2PO_4	0.06	g
Na_2HPO_4	0.09	g
HEPES	2.39	g
$NaHCO_3$	0.35	g
$CaCl_2 \cdot 2H_2O$	0.56	g
Phenol red	0.01	g
Collagenase IV	0.50	g

After completely dissolve in deionized water, adjust pH to 7.4 with 1 N NaOH and fill up to 1 liter.

1.3 Phosphate-buffered saline (Mg^{2+}, Ca^{2+} free)

NaCl	8.00	g
KCl	0.20	g
KH_2PO_4	0.20	g
Na_2HPO_4	1.15	g

After completely dissolve in distilled water, adjust pH to 7.4 with 1 N NaOH and fill up to 1 liter.

1.4 Buffer formalin pH 7.4

Formalin	100	ml
NaH_2PO_4	4.00	g
Na_2HPO_4	6.50	g

After completely dissolve in distilled water, adjust pH to 7.4 with 1 N NaOH and fill up to 1 liter.

2. Preparation of buffers for microsome fraction

2.1 Homogenizing buffer

KCl	6.90	g
EDTA·2Na	0.22335	g

After completely dissolve in deionized water, add 0.6 ml of 0.25 M PMSF and fill up to 600 ml.

2.2 Microsome suspension buffer

KH_2PO_4	0.68045	g
EDTA·2Na	0.01861	g
DTT	7.7125	mg
Glycerol	15.00	ml

After completely dissolve in deionized water, adjust pH to 7.4 with conc. KOH and fill up to 50 ml.

3. Preparation of reagent for micronucleus staining

Dissolve 4',6-diamidino-2-phenylindole (DAPI) 20 µl in 1 ml of deionized water.

4. Preparation of reagents and buffer for total protein assay by Lowry method

4.1 2% Na_2CO_3 in 0.1 N NaOH (Reagent A)

Dissolve 10 g of Na_2CO_3 and 2.0 g of NaOH in deionized water and fill up to 500 ml.

4.2 0.5% $CuSO_4 \cdot H_2O$ (Reagent B)

Dissolve 1.0 g of $CuSO_4 \cdot H_2O$ in deionized water and fill up to 200 ml.

4.3 1% potassium sodium tartrate ($KNaC_4H_4O \cdot 4H_2O$) (Reagent C)

Dissolve 1.0 g of $KNaC_4H_4O \cdot 4H_2O$ in deionized water and fill up to 100 ml.

4.4 Carbonate-copper solution (Reagent D)

Mix reagent A, B and C at ratio 50:1:1.

4.5 Diluted Folin reagent

Dilute Folin-Ciocalteu's phenol reagent in deionized water at ratio 1:1.

5. Preparation of reagent and buffer for CYP 1A activity assay

5.1 0.01 M Tris pH 7.8

Dissolve 1.2114 g of Tris in deionized water, adjust pH to 7.8 with conc. KOH and fill up to 100 ml.

5.2 10 mM NADPH

Dissolve 7.44 mg of NADPH in 1.0 ml deionized water and dilute to 0.5 mM NADPH.

6. Preparation of reagent and buffer for CYP 3A activity assay

6.1 Standard formaldehyde

Mix 7.9 µl of 38% formaldehyde and 92.1 µl of distilled water.

6.2 40% TCA

Dissolve 80 mg of TCA in 200 ml distilled water and dilute to 12.5% TCA.

6.3 10 mM Erythromycin

Dissolve 36.7 mg of erythromycin in 5 ml of DMSO.

6.4 10 X Phosphate-buffered saline (PBS)

Dissolve 8.0 g of NaCl, 1.15 g of Na_2HPO_4, 0.2 g of KCl and 0.2 g of KH_2PO_4 in 100 ml distilled water.

6.5 150 mM $MgCl_2$

Dissolve 0.304 g of $MgCl_2$ in 10 ml of distilled water.

6.6 10 mM NADPH

Dissolve 14.88 mg of NADPH in 2 ml of deionized water and dilute to 5 mM NADPH.

6.7 Nash reagent

Dissolve 2.25 g of ammonium acetate, 45 µl of acetic acid, 30 µl of acetylacetone in 7.5 ml of distilled water.

7. Preparation of reagents and buffer for NADPH-P450 reductase activity assay

7.1 0.3 M potassium phosphate buffer pH 7.5

Dissolve 4.0827 g of KH_2PO_4 in deionized water, adjust pH to 7.5 with conc. KOH and fill to 100 ml.

7.2 1 mM cytochrome c Type VI (Horse Heart Type VI)

Dissolve 10 ml of cytochrome c in 1 ml of deionized water.

7.3 50 mM KCN

Dissolve 0.3256 g of KCN in 1 ml of deionized water.

8. Preparation of reagents and buffer for glutathione-*S* transferase activity assay

8.1 10 mM reduced glutathione

Dissolve 30.7 g of GSH in 10 ml of deionized water

8.2 10 mM 1-chloro-2, 4-dinitrobenzene

Dissolve 102 mg of CDNB in 20 ml of absolute ethanol and adjust volume to 50 ml by deionized water.

8.3 0.2 M potassium phosphate buffer pH 6.5

Dissolve 2.722 g of KH_2PO_4 in deionized water, adjust pH to 6.5 with conc. KOH and fill up to 80 ml.

9. Preparation of reagents and buffer for UDP-glucuronyl transferase activity assay

9.1 200 mM Tris buffer pH 8.0

Dissolve 2.4228 g of Tris in deionized water, adjust pH to 8.5 with conc. HCl and fill up to 100 ml.

9.2 5 mM p-nitrophenol

Dissolve 70 mg of PNP in 100 ml of deionized water.

9.3 40 mM $MgCl_2$

Dissolve 813.24 mg of $MgCl_2$ in 100 ml of deionized water.

9.4 20 mM UDP-GA

Dissolve 64.63 mg of UDP-GA in 10 ml of deionized water.

9.5 10% TCA

Dissolve 10 g of TCA in 100 ml of deionized water.

9.6 1 mM NaOH

Dissolve 4 g of NaOH in 100 ml of deionized water.

10. Preparation of SDS-PAGE reagent and buffer

10.1 30% Acrylamide

Dissolve 30 g of 30% acrylamide and 0.8 g of 0.8% bis-acrylamide in deionized water and fill up to 100 ml.

10.2 Separating gel buffer pH 8.8

Dissolve 18.171 g of 0.5 M Tris and 0.4 g of 0.4% SDS in deionized water, adjust pH to 8.8 with conc. HCl and fill up to 100 ml.

10.3 Stacking gel buffer pH 6.8

Dissolve 6.057 g of 0.5 M Tris and 0.8 g of 0.4% SDS in deionized water, adjust pH to 6.8 with conc. HCl and fill up to 100 ml.

10.4 10% Ammonium persulphate

Dissolve 1.0 g of ammonium persulfate in 10 ml of deionized water.

10.5 Sample solution pH 6.8

0.125 M Tris	1.51 g
4% SDS	4.00 g
20% Glycerol	20.00 ml
10% Mercaptoethanol	10.00 ml
0.002% Bromphenol blue	0.002 g

After completely dissolve in deionized water, adjust pH to 6.8 with conc. HCl and fill up to 100 ml.

10.6 Electrode buffer

0.25 M Tris	15.145 g
0.92 M Glycine	22.065 g
1% SDS	5.00 g

Dissolve in 500 ml of deionized water.

10.7 Blotting buffer

Dissolve 60.55 g of Tris and 72.0 g of glycine in 800 ml of deionized water

11. Preparation of immunostaining buffer

11.1 Phosphate-buffered saline

$NaH_2PO_4 \cdot 2H_2O$	15.601 g
NaCl	90.00 g

After completely dissolve in deionized water, adjust pH to 7.5 with conc. NaOH and fill up to 1000 ml.

11.2 Tween-phosphate buffer saline (TPBS)

$NaH_2PO_4 \cdot 2H_2O$	15.601 g
NaCl	90.00 g
Tween 20	5.00 ml

After completely dissolve in deionized water, adjust pH to 7.5 with conc. NaOH and fill up to 1000 ml.

11.3 5% skim milk

Dissolve 800 mg of skim milk in 16 ml of TPBS.

11.4 10% BSA

Dissolve 1.0 g of BSA in 10 ml of deionized water.

11.5 TPBS-0.2 with BSA

Dissolve 2 ml of 10% BSA in 100 ml of TPBS.

APPENDIX D

Extraction and isolation of purple rice bran

The purple rice bran was soaked in dark with absoluted methanol for 72 hr. The solvent was vacuum filtrated using Whatman filter sheet No. 1. Then, the residues were soaked in dichloromethane for 72 hr in dark and filtration. After concentrated and dehydrated processes, each extract was evaluated for the percent yield and amount of total phenolic compounds and flavonoids.

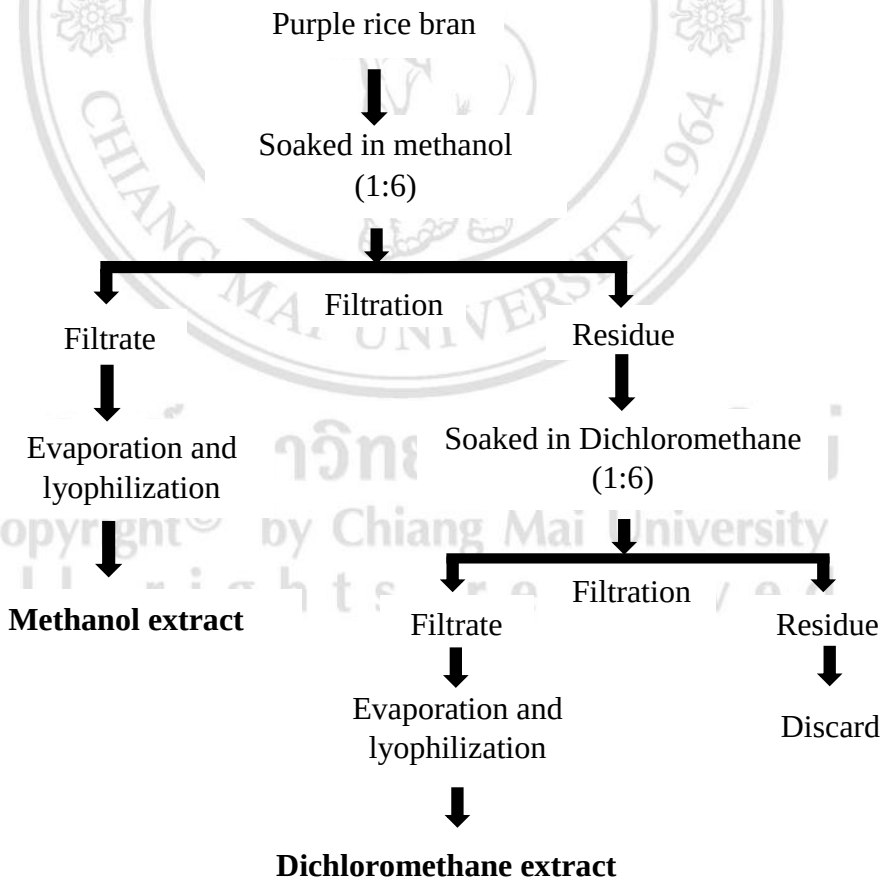

Figure 46. The alternative extraction of purple rice bran

Table 11. Total phenolic compounds and flavonoids in purple rice bran extracts

Compounds	Contents (mg/g extract)	
	Methanol extract	**Dichloromethane extract**
Yield (%)	8.64 ± 0.00	3.28 ± 0.00
Total phenolic compounds	29.93 ± 0.80	0.56 ± 0.44
Total flavonoids	38.30 ± 1.48	6.65 ± 2.47

Values are expressed as mean ± SD

CURRICULUM VITAE

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Publication	Suwannakul N, Punvittayagul C, Jarukamjorn K, Wongpoomchai R. Purple rice bran extract attenuates aflatoxin B_1-induced initiation stage of hepatocarcinogenesis by alteration of xenobiotic metabolizing enzymes. *Asian Pac J Cancer Prev*. 2015, 16(8), 3371-3376.
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