RESEARCH ARTICLE

Mutagenicity and Antimutagenicity of Hydrophilic and Lipophilic Extracts of Thai Northern Purple Rice

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Abstract

Purple rice (*Oryza sativa* L. var. *indica*) cv. Kum Doisaket is cultivated in northern Thailand. This study evaluated the mutagenic and antimutagenic properties of hydrophilic and lipophilic components of purple rice using the Ames test. The seed and hull of purple rice were extracted with hexane, methanol, ethanol, and water. The methanol extracts had the highest amounts of phenolic acids and flavonoids, while the hexane extracts contained large amount of tocols and γ-oryzanol. None of the extracts were mutagenic in *Salmonella typhimurium* strains TA98 and TA100. The hexane extract of rice hull and the methanol extract of rice seed were strongly effective against aflatoxin B1- and 2-amino-3, 4 dimethylimidazo (4, 5-f) quinoline-induced mutagenesis, while aqueous extracts showed weakly antimutagenic properties. All extracts with the exception of aqueous extracts enhanced the number of revertant colonies from benzo (a) pyrene induced-mutagenesis. None of the extracts inhibited mutagenesis induced by the direct mutagens 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide and sodium azide. The hull extracts showed more potent antimutagenicity than the seed extracts. Based on a chemical analysis, γ-oryzanol and γ-tocotrienol in the hull and cyanidin-3-glucoside and peonidin-3-glucoside in the seed are candidate antimutagens in purple rice. The antimutagenic mechanisms of purple rice might be related to either modulation of mutagen metabolizing enzymes or direct attack on electrophiles. These findings supported the use of Thai purple rice as a cancer chemopreventive agent.

Keywords: Antigenotoxicity - DNA mutation - *Oryza sativa* L. var. *indica* - *Salmonella* mutation assay

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Introduction

Cancer is a genetic disease associated with various risk factors, including direct gene mutation. Oncogenes, tumor suppressor genes, and DNA repair genes mutations can contribute to cancer, as these genes control cell growth and DNA repair. Mutations occur due to radiation, viruses, DNA replication errors, and environmental chemicals (Gasser and Raulet, 2006; Peltomaki, 2012). The *Salmonella* mutation assay is a short-term carcinogenicity test that is used to evaluate the ability of chemical compounds to induce DNA mutations in bacteria (Mortelmans and Zeiger, 2000). This test is not only used to screen for mutagenicity, but also to examine the antimutagenicity of various compounds such as phytochemicals isolated from edible plants.

Previous studies have shown that phytochemicals such as flavonoids, coumarins, quinones, and phenolic compounds possess antimutagenic activity in *Salmonella*. typhimurium (Bhattacharya, 2011). Flavonoids include strong nucleophilic compounds that may prevent DNA damage from the electrophilic metabolites of the mutagen (Marnewick et al., 2000). Oligomeric proanthocyanidins from Thai grape seeds presented antimutagenicity in TK6 cells by micronucleus and comet assays (Praphasawat et al, 2011). Anthocyanins are flavonoids that produce the colors of many fruits and vegetables. Their biological effects include antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic activities (Kong et al., 2003).

Landrace purple rice (*Oryza sativa* L. var. *indica*) cv. Kum Doisaket is a colored rice variety cultivated in Chiang Mai, Thailand. It contains high amounts of polyphenolic compounds, especially anthocyanins in both the seed and the hull. Several investigations have demonstrated that pigmented rice extract exhibits antioxidant, anti-inflammatory, and *in vitro* anticarcinogenic activities (Kong and Lee, 2010; Leardkamolkarn et al., 2010; Min et al., 2010). In addition, pigmented rice extract showed cytotoxic and apoptotic-inducing effects on human hepatocellular carcinoma HepG2 cells and also exhibited anti-metastasis activity against human breast cancer cells (Banjerdpongchai et al., 2013; Luo et al., 2014).

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Rice hull is a waste by-product obtained during the rice milling process. It contains high amounts of polyphenolic compounds that apparently function to protect the rice seed. Jeon et al. (2006) reported that phenolic compounds from methanol extract of rice hull exhibited strong antioxidant activity against reactive oxygen species induced DNA damage in human lymphocytes. In addition, Kim et al. (2007) identified momilactone B from rice hull as the active compound against human colon cancer cells. Our group found that the dichloromethane extract of purple rice hull had antimutagenic effects in the Ames test and anticlastogenic effects on diethylnitrosamine-induced micronucleus formation in rat liver (Sankam et al., 2013). Based on these observations, rice hull would appear to be a source of natural antimutagenic compounds.

Little is known concerning the mutagenic or antimutagenic properties of purple rice, as most studies have focused on its antioxidant activities. Therefore, the objectives of this study were to evaluate the *in vitro* mutagenic/antimutagenic effects of purple rice seed and hull extracted by various solvents (*n*-hexane, methanol, ethanol, and water). In addition, we determined the chemical constituents of the extracts, including γ-oryzanol, tocopherols, tocotrienols, phenolics, flavonoids, anthocyanins and anthocyanidins. The knowledge on these works contributed to the understanding on their potential antimutagenic application for the use as cancer chemoprevention.

Materials and Methods

Chemicals

Standard mutagens: aflatoxin B1 (AFB1) and benzo(a)pyrene (B(a)P), sodium azide (NaN_3) were obtained from Sigma-Aldrich Co., USA. 2-Amino-3, 4 dimethylimidazo[4,5-f]quinoline (MeIQ), 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2) and 2-aminoanthracene (2-AA) were purchased from Wako Pure Chemicals Industries Ltd., Japan. The phenolic, flavonoid, anthocyanin, anthocyanidin, γ-oryzanol, and tocols standards for chemicals analysis were high performance liquid chromatography grade. All other chemicals were analytical grade.

Preparation of purple rice extracts

Purple rice (*Oryza sativa* var. *indica*) cv. Kum Doisaket was obtained from Assoc. Prof. Dr. Dumnern Karladee, Faculty of Agriculture, Chiang Mai University. The rice was cultivated from August to November at MaeHia Agricultural Research, Demonstrative and Training Center, Chiang Mai. Purple rice grains were dehulled using a hulling machine. The seed and hull were extracted with n-hexane, absolute methanol, 95% (v/v) ethanol, and distilled water for 48h. After filtration, the solutions were evaporated under reduced pressure and lyophilized to obtain the n-hexane, methanol, ethanol and aqueous extracts.

Determination of chemical constituents

The total phenolic content was measured according to the Folin-Ciocalteu method using gallic acid as a standard and the total flavonoid content was determined via the aluminium chloride colorimetric method using catechin as a standard (Inboot et al., 2012).

Some phenolic acids and flavonoids in the purple rice seed and hull extracted with methanol, 95% ethanol, and water were measured using reverse-phased HPLC according to the method of Chen et al. (2001) with slight modification. The HPLC conditions were as follows: Column: C18 Columns; 4.6×250mm, 5μm and Eluent: 3% acetic acid in water and methanol. The wavelengths were set at 260, 280, 320, and 360nm. The quantitative determination of gallic acid, protocatechuic acid, catechin, vanillic acid, epicatechin, p-coumaric acid, ferulic acid, rutin, and quercetin were operated. The HPLC conditions for measuring anthocyanin and anthocyanidin contents were as follows: Column: RP-C18 Columns; 4.6×250 mm, 5 μm and Eluent: acetonitrile, 4% phosphoric acid in water and methanol. The wavelengths were set at 520 nm (Talavera et al., 2005). The quantitative determination of delphinidin-3-glucoside, cyanidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, delphinidin, cyanidin, pelargonidin, peonidin, and malvidin were performed.

The tocols (α, β, γ and δ forms) were analyzed using an HPLC system. The n-hexane extracts were injected through a Develosil C30 UG (250×4.6mm, 5μm). The separation was performed using the isocratic system with mobile phase conditions of methanol: distilled water (90:10). The tocols were detected via a fluorescence detector at 280 nm. The γ-oryzanol content was measured in the hexane extracts using a C18 reverse phase HPLC system. The mobile phase was composed of methanol, acetonitrile, dichloromethane, and acetic acid (50: 44: 3: 3) with a flow rate of 2mL/min. The γ-oryzanol was

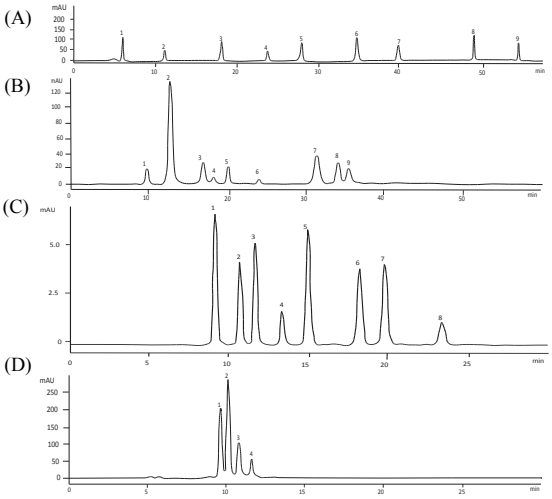

Figure 1. Chromatograms of Standard Mixtures. (A) Phenolic acids and flavonoids; 1: gallic acid, 2: protocatechuic acid, 3: catechin, 4: vanillic acid, 5: epicatechin, 6: p-coumaric acid, 7: ferulic acid, 8: rutin, and 9: quercetin; **(B)** anthocyanins and anthocyanidins; 1: delphinidin-3-glucoside, 2: cyanidin-3-O-glucoside, 3: peonidin-3-O-glucoside, 4: malvidin-3-glucoside, 5: delphinidin, 6: cyanidin, 7: pelargonidin, 8: peonidin, and 9: malvidin; **(C)** tocols; 1: δ-tocotrienol, 2: β-tocotrienol, 3: γ-tocotrienol, 4: α-tocotrienol, 5: δ-tocopherol, 6: β-tocopherol, 7: γ-tocopherol, and 8: α-tocopherol, (d) γ-oryzanol components; 1: cycloartenyl ferulate, 2: 24-methylene cycloartanyl ferulate, 3: campesteryl ferulate, and 4: β-sitosteryl ferulate

quantified using a UV detector at a wavelength of 330nm (Sankam et al., 2013). The standard chromatograms are presented in Figure 1A-1D.

Mutagenicity test

The mutagenicity test was performed in *Salmonella typhimurium* strains TA98 and TA100, with and without an exogenous metabolic activation system (S9 mix) (Maron and Ames, 1983). The S. *typhimurium* strains TA98 and TA100 were obtained from Dr. Takehiko Nohmi, National Institute of Health Science, Japan. Briefly, the tester strain was incubated with various concentrations of each extract and phosphate buffer or S9 mix. After incubation, top agar containing 0.05 mM L-histidine and 0.05 mM D-biotin was added and poured onto a minimal glucose agar plate. The plates were incubated at 37°C for 48 h and the numbers of histidine-independent revertant colonies were counted. In the absence of metabolic activation, AF-2 was used as a positive control, whereas 2-AA was used as a positive control in the presence of metabolic activation. If the revertant colonies were 2 times higher than spontaneous revertant colonies, the test sample was identified as a possible mutagen.

Antimutagenicity test

For the antimutagenicity test, the assay steps were performed as per the mutagenicity test. In the absence of S9 mix, AF-2 and NaN_3 were used as standard mutagens in strains TA98 and TA100, respectively. In the assays with S9 mix, AFB1 and MeIQ were used in strain TA98 and B(a)P was used in strain TA100. The number of histidine-independent revertant colonies was counted after incubation by comparing with the positive control. Antimutagenicity was expressed as percentage of inhibition of mutagenicity by the following formula:

$$\% \text{ Inhibition} = \{(A-B)-(C-B)/(A-B)\}(100)$$

A=number of revertants in standard mutagen plates, B=number of spontaneous revertants, C=number of revertants of test plates.

Statistical analysis

Results for chemical components were expressed as mean±SD. The results for the *Salmonella* mutation assay were expressed as mean number of His+ revertant colonies±SEM. For the antimutagenic results, the percentage inhibition was expressed as mean±SEM. Triplicate plates were tested per dose in three independent experiments.

Results and Discussion

Chemical constituents in purple rice seed and hull extracts

The major hydrophilic and lipophilic constituents present in the extracts of purple rice seed and hull are listed in Table 1. The purple rice seed and hull extracted with methanol presented the highest total phenolic and total flavonoid contents as compared to the other hydrophilic solvents. In addition, we found that purple rice hull and seed extracts contained 40-60% flavonoids in the total phenolic content. The most abundant phenolic acids contained in rice hull extracts were vanillic acid, *p*-coumaric acid, and protocatechuic acid. However, rice seed contained the highest amount of protocatechuic acid and vanillic acid, respectively. Moreover, rice hull extracts composed of epicatechin, while rice seed extract consisted of catechin. Low amounts of ferulic acid and rutin were found in rice hull extract, whereas gallic acid and quercetin were not detected in either rice seed or hull extracts.

The major anthocyanins contained in purple rice seed and hull extracts were peonidin-3-glucoside and cyanidin-3-glucoside (Table 2). Malvidin-3-glucoside was only present in purple rice hull. In addition, the anthocyanidin

Table 1. The Amounts of Phenolic and Flavonoid Compounds in Various Extracts Obtained from Seed and Hull of Purple Rice

Analytes (μg/mg extract)	Hull			Seed		
	Methanol	Ethanol	Water	Methanol	Ethanol	Water
Total phenolic contents (μg GAE/mg extract)	68.5±0.11	55.6±0.97	34.5±0.97	61.4±0.50	28.9±0.13	35.6±1.4
Total flavonoid contents (μg CE/mg extract)	27.9±0.04	25.9±1.51	9.97±0.17	37.0±1.69	21.4±1.46	18.6±0.6
Protocatechuic acid	0.57±0.01	1.14±0.02	ND	0.24±0.05	1.02±0.08	1.26±0.01
Vanillic acid	1.67±0.01	2.55±0.04	3.16±0.14	0.19±0.06	0.77±0.02	1.15±0.08
p-coumaric acid	1.21±0.02	1.33±0.03	ND	ND	ND	ND
Ferulic acid	ND	ND	0.18±0.01	ND	ND	ND
Catechin	ND	ND	ND	0.09±0.01	1.73±0.05	0.37±0.09
Epicatechin	0.46±0.06	3.00±0.45	ND	ND	ND	ND
Rutin	0.10±0.05	ND	ND	ND	ND	ND

*Values expressed as mean±SD. ND; not detected

Table 2. The Amount of Anthocyanins and Anthocyanidins in Various Extracts Obtained from Seed and Hull of Purple Rice

Analytes (μg/mg extract)	Hull			Seed		
	Methanol	Ethanol	Water	Methanol	Ethanol	Water
Cyanidin-3-glucoside	0.11±0.01	0.04±0.00	ND	0.21±0.01	0.084±0.00	ND
Peonidin-3-glucoside	0.33±0.02	0.13±0.01	ND	0.65±0.01	0.263±0.00	ND
Malvidin-3-glucoside	0.13±0.01	0.08±0.01	ND	ND	ND	ND
Delphinidin	0.04±0.01	ND	ND	0.09±0.04	ND	ND
Cyanidin	ND	ND	ND	0.131±0.01	ND	ND

*Values expressed as mean±SD. ND; not detected

content, including delphinidin and cyanidin, was highest in the methanol extract of rice seed and hull. However, delphinidin-3-glucoside, malvidin, peonidin and pelargonidin were not found in purple rice cv. Kum Doisaket. In the present study, we found that the amounts of anthocyanidins and anthocyanins in purple rice seed extracts were twice that of purple rice hull extracts.

In this study, total phenolic compounds and total flavonoids in purple rice seed and hull n-hexane extracts were not detected. The amounts of tocopherols (α, β, γ and δ forms), tocotrienols (α, β, γ and δ forms), and γ-oryzanol were determined in hexane extracts of seed and hull of purple rice (Table 3). Total vitamin E content in rice seed extracts was higher than in rice hull extracts, similar to the results for the white rice variety (Kim et al., 2012). The major vitamin E present in purple rice cv. Kum

Table 3. The Amounts of Tocotrienols, Tocopherols, and γ-oryzanol in n-hexane Extracts Obtained from Seed and Hull of Purple Rice

Analytes (μg/mg extract)	Hull-Hexane	Seed-Hexane
α-tocotrienol	ND	0.09±0.01
β-tocotrienol	ND	ND
γ-tocotrienol	0.19±0.00	0.10±0.01
δ-tocotrienol	0.02±0.00	ND
α-tocopherol	0.22±0.01	1.85±0.05
β-tocopherol	0.04±0.02	0.06±0.00
γ-tocopherol	0.15±0.01	0.25±0.00
δ-tocopherol	0.05±0.00	0.09±0.00
γ-oryzanol	17.3±0.03	3.13±0.04

*Values expressed as mean±SD. ND; not detected

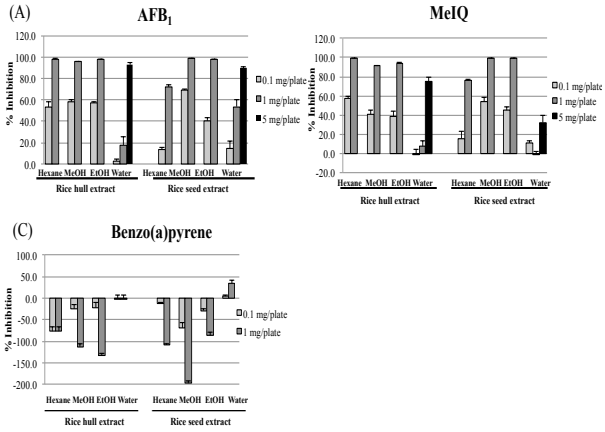

Figure 2. Antimutagenicity of Purple Rice Extracts Against the Mutagenicity of Indirect Mutagens; (A) AFB1 and **(B)** MeIQ in TA98, and **(C)** benzo(a)pyrene in TA100. Values are expressed as mean±SEM

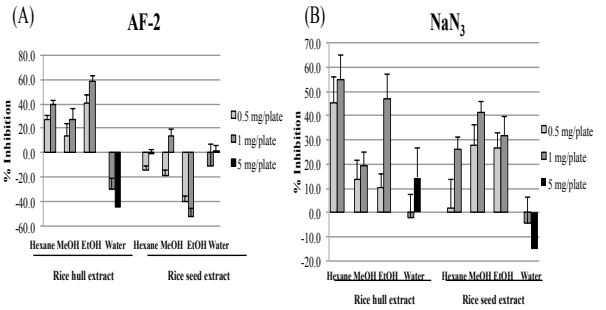

Figure 3. Effect of Purple Rice Extracts on the Mutagenicity of Direct Mutagens; (A) AF-2 in TA98 and **(B)** NaN_3 in TA100. Values are expressed as mean±SEM

Table 4. Mutagenicity of Seed and Hull Extracts of Purple Rice

Treatment	Concentration (mg/plate)	Average of His+ revertant colonies			
		TA 98		TA100	
		-S9	+S9	-S9	+S9
DMSO (50 μl/plate)		26.0±3.1	38.0±3.40	118.9±14.2	144.5±23.8
Distilled water (50 μl/plate)		27.3±1.6	34.7±3.90	110.2±9.20	166.3±18.9
2-AA (0.5μg/pl)		NA	1225±95.5	NA	940.5±41.6
AF-2 (0.1μg/pl)		445±11	NA	1058±77.5	NA
Hull-hexane	0.1	19.1±2.1	42.9±1.00	103.4±3.10	155.3±9.50
	1	23.2±2.2	37.2±2.20	86.1±6.50	90.5±10.7
	5	12.3±2.1 (K)	33.5±2.70 (K)	69.5±3.00 (K)	104.8±2.8 (K)
Hull-methanol	0.1	22.1±2.2	42.3±2.70	178.0±8.90	163.2±12.8
	1	28.9±1.2	48.9±2.80	130.7±11.9	122.5±15.9
	5	21.6±1.9(K)	62.3±5.80(K)	108.9±5.50(K)	138.8±11.0 (K)
Hull-ethanol	0.1	22.0±2.1	40.0±2.00	115.3±4.60	158.4±6.50
	1	25.8±1.7	38.6±1.60	85.6±3.30	135.0±15.1
	5	0.0±0.0 (K)	13.8±1.10 (K)	22.1±2.80 (K)	87.4±4.70 (K)
Hull-water	0.1	27.7±1.5	33.8±3.30	110.2±4.90	170.2±11.5
	1	31.1±2.6	41.2±4.00	118.9±10.0	148.2±9.90
	5	16.9±1.6	27.9±2.60	117.7±4.10	140.4±17.7
Seed-hexane	0.1	25.0±3.2	40.5±4.00	113.8±7.50	152.8±9.50
	1	23.2±0.1	36.9±2.80	111.9±15.4	121.3±14.6
	5	14.2±0.6 (K)	34.5±3.60(K)	87.9±2.00 (K)	113.3±4.80 (K)
Seed-methanol	0.1	23.7±1.6	42.8±2.60	112.5±7.00	153.8±5.60
	1	29.2±1.5	51.2±3.10	66.4±5.30	92.7±11.5
	5	32.4±1.1(K)	67.1±2.20(K)	86.6±3.80 (K)	117.0±3.90 (K)
Seed-ethanol	0.1	29.4±2.6	52.6±3.20	110.5±7.00	150.8±11.4
	1	27.6±2.3	48.6±1.70	84.7±4.80	101.5±11.3
	5	9.80±0.4(K)	58.8±1.10(K)	62.6±6.60(K)	151.0±6.90 (K)
Seed-water	0.1	31.9±0.8	35.8±1.60	114.3±6.80	170.4±8.10
	1	27.6±1.7	37.9±1.70	101.3±3.40	138.8±14.9
	5	15.1±1.6	30.2±3.00	124.2±2.30	168.3±10.5

*Values are mean±SEM. AF-2; 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide, 2-AA; 2-amino anthracine, NA; Not analyzed, K; Killing effect. The data are the mean of three independent experiments

Doisaket was α-tocopherol, while β-tocotrienol was not detected in either parts of the purple rice. Interestingly, the purple rice hull contained higher amounts of γ-oryzanol than purple rice seed.

Mutagenicity and antimutagenicity of seed and hull extracts of purple rice

The mutagenicity and antimutagenicity of purple rice seed and hull extracts were determined using S. *typhimurium* strains TA98 and TA100 in the presence and absence of metabolic activation. Purple rice hull and seed extracts did not increase the number of revertant colonies when compared to negative controls (Table 4). A constant concentration of 5mg/plate for each extract, with the exception of the aqueous extract, had a killing effect on bacteria. This may have been due to the high concentration of flavonoids with antimicrobial activity via either reducing membrane fluidity of bacterial cells or forming a complex with protein and the bacterial cell wall (Cushnie and Lamb, 2005; Salem et al., 2013). These results suggested that purple rice extracts were not mutagenic in S. *typhimurium* strains TA98 and TA100 in both presence and absence of S9 mix.

The highest amount of the purple rice extract used in the antimutagenicity assay was a non-cytotoxic dose; 5mg/plate for the aqueous extract and 1 mg/plate for other extracts. In the presence of metabolic activation, all extracts reduced the number of revertant colonies induced by AFB1 and MeIQ (Figure 2A and 2B). At the concentration of 0.1mg/plate, the methanol extract of purple rice seed moderately inhibited AFB1-induced mutagenesis (69% inhibition), while the hexane extract of hull and the methanol extract of seed inhibited MeIQ-induced mutagenesis by 58% and 54% inhibition, respectively.

Previous reports have indicated that the antimutagenic activity might be due to several mechanisms, including directly protecting DNA from the electrophilic metabolite of the mutagen and inhibiting enzymes involved in mutagen metabolism (Bhattacharya, 2011). AFB1 and MeIQ are environmental contaminants metabolized by cytochrome P450 (CYP) 1A2 and react with DNA causing mutations (Turesky and Vouros, 2004; Gross-Steinmeyer and Eaton, 2012). The purple rice extracts might suppress CYP1A2 activity, leading to diminution of AFB1- and MeIQ-induced mutagenicity. On the other hand, all extracts with the exception of the aqueous extract enhanced the number of revertant colonies induced by benzo(a)pyrene, indicating co-mutagenicity (Figure 2C). These results suggest that some phenolic compounds, including flavanoids in the organic solvent extracts of purple rice, might enhance the activity of benzo(a)pyrene activating enzymes.

In the absence of metabolic activation, the extracts had a weak inhibitory effect on direct-acting mutagens AF-2 and NaN_3 (Figure 3A and 3B). The ethanol extract of purple rice hull showed the highest inhibition rate (58% inhibition) to AF-2. However, purple rice seed extracts did not inhibit AF-2-induced mutagenesis. The hexane extract of purple rice hull had moderate antimutagenicity (55% inhibition), while the methanol extract of purple rice seed showed mild antimutagenicity (41% inhibition) against NaN_3. These results indicated that the purple rice extracts act so as to modulate xenobiotic metabolizing enzymes rather than directly react with the ultimate mutagens.

Flavonoids contain strong nucleophilic centers that can react with electrophilic compounds, leading to prevention of genotoxic damage (Marnewick et al., 2000). Moreover, the position of functional groups in flavonoids influences their antimutagenic properties (Snijman et al., 2007). Previous investigations have demonstrated that the structure of flavonoids is also associated with mutagen metabolism. For example, flavonoids containing a methoxyl group can induce CYP1A1 enzyme, which is an enzyme involved in benzo(a)pyrene metabolism (Androutsopoulos 2011). Moreover, some flavonols and flavones inhibited CYP1A2 activity, but enhanced CYP1A1 acitivity (Moon et al., 2006; Chatuphonprasert and Jarukamjorn, 2012). In addition, previous studies have emphasized that flavonoids act as AhR antagonists or agonists depending on their structure and concentration (Hodex et al., 2002). Although CYP1A1 and CYP1A2 are regulated via the aryl hydrocarbon receptor (AhR) (Delescluse et al., 2000), the CYP1A1 gene promoter is also regulated by other pathways, such as the retinoid pathway (Vecchini et al., 1994). Based on previous studies, we proposed that the flavonols and flavones in purple rice seed and hull extracts may have an effect on the induction of CYP1A1and inhibition of CYP1A2.

In the present study, the hexane extract of purple rice hull had the most potent effects concerning mutagenesis. Previous studies have found that vitamin E and γ-oryzanol exhibited antimutagenic and anticarcinogenic activities (Tavan et al., 1997; Kim et al., 2012). The hexane extract of rice hull contained more γ-oryzanol and γ-tocotrienol than hexane rice seed extract. Therefore, we propose that γ-oryzanol and γ-tocotrienol are the major antimutagenic compounds in purple rice hull.

The methanol extract of purple rice seed was the most effective antimutagen. This study has shown that the major chemical constituents in the methanol extract were phenolic acid and flavonoids. In addition, the antimutagenicity of the methanol extract of rice seed was correlated with the anthocyanin content, especially cyanidin-3-glucoside and peonidin-3-glucoside, which possess antimutagenic activity (Charoensin et al., 2012). Based on these findings, we consider the anthocyanins to be the antimutagenic compounds in purple rice seed.

Oryza sativa L. var. *indica*, Kum Doisaket, thus contains candidate antimutagenic compounds. The protective activity of the extracts of purple rice was possibly related to the enzymes involved in mutagen metabolism, via modulation of mutagen metabolizing enzymes or direct attack on mutagenic electrophiles. These findings provide valuable information for further applied research on the use of the rice products as health foods.

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References

Androutsopoulos VP, Papakyriakou A, Vourloumis D, et al (2011). Comparative CYP1A1 and CYP1B1 substrate and inhibitor profile of dietary flavonoids. *Bioorg Med Chem*, **19**, 2842-9.

Banjerdpongchai R, Wudtiwai B, Sringarm K (2013). Cytotoxic and apoptotic-inducing effects of purple rice extracts and chemotherapeutic drugs on human cancer cell lines. *Asian Pac J Cancer Prev*, **14**, 6541-8.

Bhattacharya S (2011). Natural antimutagens: A review. *Res J Med Plant,* **5**, 116-26.

Charoensin S, Taya S, Wongpornchai S, et al (2012). Assessment of genotoxicity and antigenotoxicity of and aqueous extract of cleistocalyx nervosum var. paniala in *in vitro* and *in vivo* model. *Interdiscip Toxicol*, **4**, 201-6.

Chatuphonprasert W, Jarukamjorn K (2012). Impact of six fruits-banana, guava, mangosteen, pineapple, ripe mango and ripe papaya-on murine hepatic cytochrome P450 activities. *J Appl Toxicol*, **32**, 994-1001.

Chen H, Zuo Y, Deng Y (2001). Separation and determination of flavonoids and other phenolic compounds in cranberry juice by high-performance liquid chromatography. *J Chromatography A*, **913**, 387-95.

Cushnie TP, Lamb AJ (2005). Antimicrobial activity of flavonoids. *Int J Antimicrob Agents*, **26**, 343-56.

Delescluse C, Lemaire G, Sousa G, et al (2000). Is CYP1A1 induction always related to AHR signaling pathway? *Toxicology*, **153**, 73-82.

Gasser S, Raulet D (2006). The DNA damage response, immunity and cancer. *Semin Cancer Biol*, **16**, 344-7.

Gross-Steinmeyer K, Eaton DL (2012). Dietary modulation of the biotransformation and genotoxicity of aflatoxin B1. *Toxicology*, **299**, 69-79.

Hodex P, Trefil P, Stiborova M (2002). Flavonoids-potent and versatile biologically active compounds interacting with cytochromes P450. *Chem-Biol Interact*, **139**, 1-21.

Inboot W, Taya S, Chailungka A, et al (2012). Genotoxicity and antigenotoxicity of the methanol extract of *Cleistocalyx nervosum* var. paniala seed using a *Salmonella* mutation assay and rat liver micronucleus tests. *Mol Cell Toxicol*, **8**, 19-24.

Jeon K I, Park E, Paek HR, et al (2006). Antioxidant activity of far-infrared radiated rice hull extracts on reactive oxygen species scavenging and oxidative DNA damage in human lymphocytes. *J Med Food*, **9**, 42-8.

Kim HY, Hwang IG, Kim TM, et al (2012). Chemical and functional components in different parts of rough rice (*Oryza sativa* L.) before and after germination. *Food Chem*, **134**, 288-93.

Kim SJ, Park HR, Park E, et al (2007). Cytotoxic and antitumor activity of momilactone B from rice hulls. *J Agric Food Chem*, **55**, 1702-06.

Kim SP, Kang MY, Nam SH, et al (2012). Dietary rice bran component γ-oryzanol inhibits tumor growth in tumor-bearing mice. *Mol Nutr Food Res*, **56**, 935-44.

Kong JM, Chia LS, Goh NK, et al (2003). Analysis and biological activities of anthocyanins. *Phytochemistry*, **64**, 923-33.

Kong S, Lee J (2010). Antioxidants in milling fractions of black rice cultivars. *Food Chem*, **120**, 278-81.

Leardkamolkarn V, Thongthep W, Suttiarporn P, et al (2011). Chemopreventive properties of the bran extracted from a newly-developed Thai rice: The Riceberry. *Food Chem*, **125**, 978-85.

Luo L-P, Han B, Yu X-P, et al (2014). Anti-metastasis activity of black rice anthocyanins against breast cancer: analyses using an erbb2 positive breast cancer cell line and tumoral xenograft model. *Asian Pac J Cancer Prev*, **15**, 6219-25.

Marnewick JL, Gelderblom WC, Joubert E (2000). An investigation on the antimutagenic properties of South African herbal teas. *Mutat Res*, **471**,157-66.

Maron DM, Ames BN (1983). Revised methods for the *Salmonella* mutagenicity test. *Mutat Res*, **113**, 173-215.

Min SW, Ryu SN, Kim DH (2010). Anti-inflammatory effects of black rice, cyanidin-3-O-beta-D-glycoside, and its metabolites, cyanidin and protocatechuic acid. *Int Immunopharmacol*, **10**, 959-66.

Moon YJ, Wang X, Morris ME (2006). Dietary flavonoids: Effects on xenobiotic and carcinogen metabolism. *Toxicol in Vitro*, **20**, 187-210.

Mortelmans K, Zeiger E (2000). The Ames *Salmonella*/ microsome mutagenicity assay. *Mutat Res*, **455**, 29-60.

Praphasawat R, Klungsupya P, Muangman T et al (2011). Antimutagenicity and antioxidative DNA damage properties of oligomeric proanthocyanidins from Thai grape seeds in TK6 cells. *Asian Pac J Cancer Prev*, **12**, 1317-21.

Peltomaki P (2012). Mutation and epimutations in the origin of cancer. *Exp Cell Res*, **318**, 299-310.

Salem M Z, Ali HM, El-Shanhorey NA, et al (2013). Evaluation of extracts and essential oil from *Callistemon viminalis* leaves: Antibacterial and antioxidant activities, total phenolic and flavonoid Contents. *Asian Pac J Trop Med*, **10**, 785-91.

Sankam P, Punvittayagul C, Sringam K, et al (2013). Antimutagenicity and anticlastogenicity of glutinous purple rice hull using *in vitro* and *in vivo* testing systems. *Mol Cell Tox*, **9**, 169-76.

Snijman PW, Swanevelder S, Joubert E, et al (2007). The antimutagenic activity of the major flavonoids of rooibos (*Aspalathus linearis*): some dose-response effects on mutagen activation-flavonoid interactions. *Mutat Res*, **631**, 111-23.

Talavera S, Felgines C, Texeir O, et al (2005). Anthocyanin metabolism in rats and their distribution to digestive area, kidney, and brain. *J Agric Food Chem*, **53**, 3902-8.

Tavan E, Maziere S, Narbonne JF, et al (1997). Effects of vitamins A and E on methylazoxymethanol-induced mutagenesis in *Salmonella typhimurium* strain TA100. *Mutat Res*, **377**, 231-37.

Turesky RJ, Vouros P (2004). Formation and analysis of heterocyclic aromatic amine-DNA adducts *in vitro* and *in vivo*. *J. Chormatography B*, **802**, 155-66.

Vecchini F, Lenoir-Viale MC, Cathelineau C, et al (1994). Presence of a retinoid responsive element in the promoter region of the human cytochrome P4501A1 gene. *Biochem Biophys Res Commun*, **201**, 1205-12.