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Antioxidant and anti-inflammatory activities of gamma-oryzanol rich extracts from Thai purple rice bran

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This study investigated the antioxidant and anti-inflammatory activities of γ -oryzanol rich extracts from Thai glutinous purple rice bran. Five purple rice bran samples, *PAH E-KAW*, *GAM PEUAK*, *NIAW DAM*, *GAM THOR* and *GAM BOUNG* were obtained from local glutinous cultivars in Northern Thailand. The γ -oryzanol rich extracts were prepared by soxhlet extraction and semi-purified by column chromatography. The extracts contained γ -oryzanol in the range of 1.23 to 9.14% w/w. The extracts containing more than 5% w/w of γ -oryzanol (*PAH E-KAW*, *GAM THOR* and *GAM BOUNG*) were selected for investigation of antioxidant and anti-inflammatory activities; and first standardized by adding the equivalent amount of each extract to give 10% w/v γ -oryzanol. All of extracts exerted moderate ABTS^{••} and superoxide anion scavenging activity. *GAM BOUNG* exhibited the highest ABTS^{••} scavenging activity with VCEAC value of 201.3 ± 1.84 mg L-ascorbic acid/ gram extract, TEAC value of 267.2 ± 2.96 mg Trolox/ gram extract and QEAC value of 137.4 ± 3.41 mg Quercetin/ gram extract followed by *PAH E-KAW* and *GAM THOR*, respectively. *GAM BOUNG* also showed the highest activity on superoxide anion scavenging at $IC_{50} = 12.56 \pm 0.13$ μ g/ml followed by *GAM THOR* and *PAH E-KAW*. Furthermore, *GAM BOUNG* exhibited a strong inhibition effect on linoleic acid peroxidation ($IC_{50} = 36.79 \pm 2.97$ μ g/ml). Additionally, all of the extracts showed a strong anti-inflammatory activity through inhibitory effect on nitric oxide (NO) production in combined LPS-IFN- γ -activated RAW 264.7 murine macrophage cells. *GAM BOUNG* exhibited the highest inhibitory effects on NO production ($IC_{50} = 29.32 \pm 2.21$ μ g/ml), without exerting cytotoxicity followed by *GAM THOR* and *PAH E-KAW*. These results indicate that γ -oryzanol rich extracts from Thai glutinous purple rice bran are acting as a lipophilic radical scavenger better than a hydrophilic radical scavenger. Moreover, these extracts exert a potent anti-inflammatory activity.

Key words: Purple rice bran, γ -oryzanol, antioxidation, anti-inflammatory.

INTRODUCTION

Overproduction of free radicals and/or oxidants can cause oxidative stress and oxidative damage to biological

macromolecules including lipid, protein and nucleic acid, which are associated with chronic diseases such as cancer, cardiovascular disease and neurological disease (Pratico and Delanty, 2000). Free radicals such as superoxide anion, (NO) and peroxy nitrite play important roles in the inflammatory process. The recognition of NO production by activated macrophages as a part of the

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inflammatory process is an important milestone in assessing the biological production of NO (Perkins et al., 1999). NO can induce cyclooxygenase, the rate limiting step enzyme for the inflammation process, and increase the production of interleukin-1 (IL-1) and tumor necrosis factor (TNF) (Jang and Murrell, 1998).

Rice bran is one of the valuable by products of the rice milling process, particularly rich in dietary fibers and contains significant quantities of starch, protein, vitamins and dietary minerals. Rice bran oil (RBO), the oil extracted from the germ and inner husk of rice, is rich in gamma-oryzanol, essential vitamin E complex, tocotrienols and β -sitosterol (Chotimakorn et al., 2008). Gamma-oryzanol (γ -oryzanol) is a mixture of phytosteryl ferulate esters which exist in the RBO (Scavariello and Arellano, 1998). Initially, γ -oryzanol was thought to be a single compound. Recently, however, it has been shown to be a mixture of 10 phytosteryl ferulate esters. Three of these, cycloartenyl ferulate, 24-methylenecycloartanyl ferulate and campesterol ferulate, have been identified as the major components of γ -oryzanol (Xu and Godber, 1999). γ -Oryzanol has been suggested to have properties beneficial to health, including: improving the plasma lipid profile, reducing total plasma cholesterol, increasing HDL cholesterol levels and inhibiting platelet aggregation (Cicero and Gaddi, 2001). Moreover, it was reported that γ -oryzanol exhibited antioxidant properties (Kim et al., 1995; Hiramitsu and Armstrong, 1991; Xu et al., 2001; Juliano et al., 2005; Sugano and Tsuji, 1997; Vorarat et al., 2010).

In Thailand, rice exhibits many colors, such as black, purple or red, depending on the pigments accumulated in the pericarp and bran layer of the rice kernels. Thai purple rice varieties, known as *KHAO' NIAW DAM* or *KHAO' GAM*, are common throughout the country, but especially in the North. Thai purple rice is usually cultivated in highland or mountainous areas by ethnic minorities. In addition to using a food source, the ethnic minorities use the rice in holy ceremonies and traditional medicine. In Thailand, Thai purple rice has also been popular in maintaining one's health and disease prevention. Thai purple rice continues to attract attention as a high value-added crop and has become a driving force in the local economic development of the Thai countryside.

Many studies have reported that purple and black rice contains two to three times the amount of anthocyanin, γ -oryzanol and phenolic compounds than white rice (Ryu et al., 1998; Boonsit et al., 2010). Furthermore, it has been reported that colored rice bran contains more phenolic compounds and exhibits more antioxidant activity than white rice bran (Fujita et al., 2010; Muntana and Prasong, 2010; Sompong et al., 2011). Dietary pigmented rice protects lipid peroxidation in rat kidneys (Toyokuni et al., 2002) and suppressed ROS in an *in vitro* assay

(Hu et al., 2003). Given the potential health benefits of Thai purple rice and its importance in local agriculture, studies of the active component and biological activity of Thai purple rice and rice bran are warranted.

The present study was carried out to measure the γ -oryzanol content and to investigate the antioxidant and anti-inflammatory activities of standardized γ -oryzanol rich extract from Thai glutinous purple rice bran. Results from this study may provide the preliminary *in vitro* data to support the use of standardized γ -oryzanol rich extract from Thai glutinous purple rice bran as an active ingredient in nutraceuticals or cosmeceuticals.

MATERIALS AND METHODS

Chemicals and materials

γ -Oryzanol was purchased from Wako Pure Chemical Industries (Japan). L-ascorbic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), quercetin, rutin and curcumin were purchased from Sigma Chemical Company (St. Louis, MO). All solvents and chemicals used were either high performance liquid chromatography (HPLC) grade or analytical grade and were purchased commercially from Sigma Chemical Company (St. Louis, MO), Fluka Chemical Company. (Switzerland), Merck (Darmstadt, Germany), Invitrogen (USA) and Roche (Germany). RAW 264.7 murine macrophage cell lines were purchased from American Cell Culture Collection (Bethesda, MD, USA). Five local glutinous purple rice cultivars (*Oryza sativa* L.) were collected from Northern Thailand, *NIAW DAM*, *GAM THOR* and *GAM BOUNG* (Phob Pra Agricultural Extension Office, Tak Province), *PAH E-KAW* (Mae Hong Son Rice Research Center, Mae Hong Son Province), and *GAM PEUAK* (Phrae Rice Seed Center, Phrae Province) in December, 2009. The samples were collected from two different climatic/geographic zones. Group I, *PAH E-KAW*, *GAM THOR* and *GAM BOUNG*, were grown in the mountains. While Group II, *NIAW DAM* and *GAM PEUAK*, were grown on the plains. All samples were dried in a hot air oven at 60°C for 48 h. Then, fresh rice bran was obtained from the rice milling process and sieved through a 60-mesh stainer and stored at -20°C until use.

Preparation of γ -oryzanol rich extract

The extraction and semi-purification of γ -oryzanol rich extract were carried out using an improved method with some modifications (Xu and Godber, 1999). First, 50 g of rice bran was extracted with a 100 ml hexane and ethyl acetate solution (7:3) in a Soxhlet extractor for 3 h. Then, the organic solvent layer was evaporated under reduced pressure to obtain crude RBO.

Subsequently, crude RBO was semi-purified by column chromatography packed with silica gel (grade 60) to remove triglyceride and lipids. For flushing through the column, the crude RBO was first dissolved in hexane/ethyl acetate (9:1). Then, hexane/ethyl acetate (7:3) was allowed to flow through the column and the eluent was collected. Finally, the column was washed with hexane/ethyl acetate (1:1). The extract was concentrated under reduced pressure by using a rotary evaporator to obtain the semi-purified γ -oryzanol rich extract.

Determination of γ -oryzanol content and standardization of the extracts

The γ -oryzanol content of the extracts was determined according to the improved method of Xu and Godber (1999). Reversed-phase HPLC was performed using the Shimadzu system (Japan), including LC-10AV VP pumps and SPD-10AV VP with UV detector. The 250 \times 4.6 mm diameter Alltima C18 column was obtained from Alltech Company Limited. The mobile phase consisted of methanol, acetonitrile, dichloromethane, and acetic acid (50, 44, 3 and 3%, respectively). The wavelength of the detector was set at 330 nm and the flow rate was 1.4 ml/min. All samples were tested in triplicate. Finally, the extracts containing more than 5% w/w of γ -oryzanol were selected to standardize by adding the equivalent amount of the extracts to give 10% w/v γ -oryzanol before being investigated for antioxidant and anti-inflammatory activities.

Antioxidant activity determination

ABTS assay

2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical cation decolorization assay was carried out using an improved method with some modifications (Re et al., 1999). ABTS^{•+} was generated by oxidation of 7.0 mM ABTS with 2.5 mM potassium persulfate for 16 h in the dark at room temperature (stock solution). Then, the ABTS^{•+} stock solution was diluted with absolute ethanol to give an absorbance to 0.7 ± 0.2 at 734 nm before being used (ABTS^{•+} working solution). Different concentrations of the extract or control γ -oryzanol, along with the standard L-ascorbic acid, Trolox and quercetin were mixed to ABTS^{•+} working solution. The higher the degree of decoloration of ABTS^{•+} solution, the more efficient the scavenging ability of the tested compounds. The decrease of absorbance was measured after 3 min of incubation in the dark at room temperature. All determinations were carried out in triplicate. The results are expressed as Vitamin C Equivalent Antioxidant Capacity (VCEAC), Trolox Equivalent Antioxidant Capacity (TEAC) and Quercetin Equivalent Antioxidant Capacity (QEAC).

Scavenging effects on superoxide anion

The scavenging effects on superoxide anion of γ -oryzanol rich extracts were assayed according to the method of Yanping et al. (2004). Superoxide anion radicals were generated in a phenazine methosulfate (PMS) - β -nicotinamide adenine dinucleotide (NADH) system by oxidation of NADH and analyzed by the reduction of nitroblue tetrazolium (NBT). The reaction mixture was performed in 200 μ L phosphate buffer saline (PBS) buffer (pH 7.4), which contained 78 μ M of NADH, 25 μ M of NBT and 45 μ M of ethylene diamine tetraacetic acid (EDTA) in a 96-well plate, with different concentrations of the tested sample. PMS was added to initiate the reaction. After 5 min of incubation in the dark at room temperature, the absorbance was measured at 560 nm using a Beckman Coulter microplate reader. L-ascorbic acid was used as positive control. All samples were tested in triplicate. The results are expressed as 50% inhibition concentration value (IC_{50} , μ g/ml).

Linoleic acid peroxidation assay

Linoleic acid peroxidation was assayed according to the method of

Choi et al. (2002) with slight modifications. The linoleic acid emulsion was prepared in phosphate buffer saline (PBS, pH 7.0) by using Tween 20 as emulsifier. The reaction mixture contained 20 mM linoleic acid emulsion, 100 mM Tris- hydrochloric acid (HCl), 20 mM ascorbic acid and different concentrations of samples or control γ -oryzanol, along with the standard rutin and catechin. Then, 40 mM $FeSO_4 \cdot H_2O$ was added to initiate the reaction. The reaction tubes were incubated at 37°C in the dark for 30 min and terminated by adding 103.5 μ L trichloroacetic acid (40% v/v). Subsequently, 1% w/v thiobarbituric acid in 50 mM sodium hydroxide solution was added to the reaction mixture, followed by heating it at 95°C for 10 min and then centrifuged at 3000 rpm for 10 min. The absorbance of thiobarbituric acid-reacting substance (TBARS) in the supernatant was measured at 532 nm. All samples were tested in triplicates. The results are expressed as 50% inhibition concentration value (IC_{50} , μ g/ml).

Anti-inflammatory activity determination

Inhibition of NO production

The inhibition of NO production was assayed using the method of Tuntipopipat et al. (2009) with slight modifications. RAW 264.7 murine macrophage cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 units/ml penicillin and 100 μ g/ml streptomycin. To determine the inhibitory activity on NO production, the cells (2×10^5 cells/well) were pre-incubated in 24-well plates for 24 h. Then cells were replaced with a new medium with combined lipopolysaccharide (LPS, final concentration 2 ng/ml) - interferon- γ (IFN- γ , final concentration 50 pg/ml) and various concentrations of tested samples (10 to 100 μ g/ml). After 24 h incubation, the media was removed and analyzed for NO production. The quantity of nitrite in the culture medium was measured as an indicator of NO production. The amount of nitrite, a stable form of NO, was measured using Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2% phosphoric acid). Fresh culture medium was used as a blank. Finally, the absorbance was measured at 540 nm against a standard curve of potassium nitrite. Concurrently, the viability of RAW 264.7 cells was assayed according to the method of Jomha et al. (2006). Cell viability of each extract was assayed after stimulation with LPS-IFN- γ in the absence or presence of tested samples for 24 h by cell proliferation reagent WST-1

Statistical analysis

All results were expressed as a mean of three replicate \pm standard deviations (SD). All statistical analysis was conducted using SPSS (version 16). *P* values < 0.01 were considered to be significant.

RESULTS

Extract yield and γ -oryzanol content

The γ -oryzanol content of γ -oryzanol rich extracts from Thai glutinous purple rice bran was determined using reversed-phase HPLC. As shown in Figure 1, standard γ -oryzanol is composed of three major peaks. Similarly, the γ -oryzanol rich extracts are composed of three major

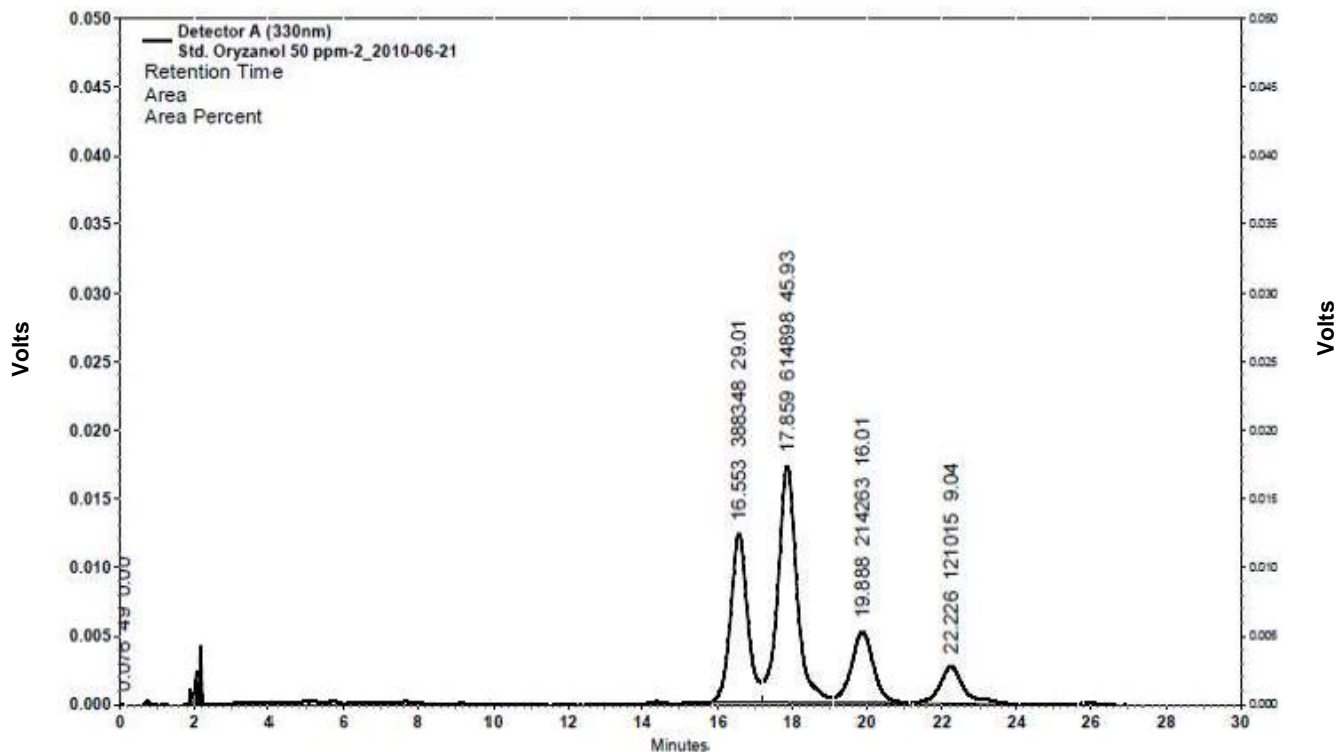


Figure 1. HPLC chromatogram of control γ -oryzanol.

peaks, but also include an additional five minor peaks (Figure 2). The γ -oryzanol rich extracts might be composed of three major components similar to standard γ -oryzanol and an additional five minor components. The results in Table 1 indicated that the γ -oryzanol content of γ -oryzanol rich extracts from different cultivars were in the range of 1.23 to 9.14% w/w. The γ -oryzanol rich extract from *PAH E-KAW* exhibited the highest γ -oryzanol content with $9.14 \pm 0.09\%$ w/w, followed by *GAM THOR*, *GAM BOUNG*, *GAM PEUAK*, and *NI AW DAM*, respectively. The γ -oryzanol rich extracts which contained γ -oryzanol more than 5% w/w (*PAH E-KAW*, *GAM THOR* and *GAM BOUNG*) were selected to standardize by adding the equivalent amount of each extract to give 10% w/v γ -oryzanol before being investigated for antioxidant and anti-inflammatory activities.

Antioxidant activity of γ -oryzanol rich extract

Three antioxidant assays were utilized to evaluate the free radical scavenging and antioxidant activities of the γ -oryzanol rich extracts. The standardized γ -oryzanol rich extracts exhibited the scavenging activity on ABTS^{•+} and superoxide anion in a dose-dependent manner. *GAM*

BOUNG exhibited the highest ABTS^{•+} scavenging activity with a VCEAC value of 201.3 ± 1.84 mg L-ascorbic acid/gram extract, TEAC value of 267.2 ± 2.96 mg Trolox/gram extract and QEAC value of 137.4 ± 3.41 mg Quercetin/gram extract followed by *PAH E-KAW* and *GAM THOR*, respectively. However, the scavenging activity of all extracts was lower than control γ -oryzanol (Figure 3). *GAM BOUNG* also exhibited the highest scavenging effects on superoxide anion with an IC₅₀ value of 12.56 ± 0.13 μ g/ml, followed by *GAM THOR* and *PAH E-KAW*, respectively. The superoxide scavenging activity of all extracts was stronger than control γ -oryzanol, but lower than standard L-ascorbic acid (Table 2) *GAM BOUNG*, *GAM THOR* and control γ -oryzanol showed the highest inhibitory effect on linoleic acid peroxidation followed by *PAH E-KAW*, rutin and catechin, respectively (Table 2). The γ -oryzanol rich extract from *GAM BOUNG*, *GAM THOR* and standard γ -oryzanol displayed similar statistical inhibition on linoleic acid peroxidation.

Anti-inflammatory activity of γ -oryzanol rich extract

Anti-inflammatory activity of γ -oryzanol rich extract was

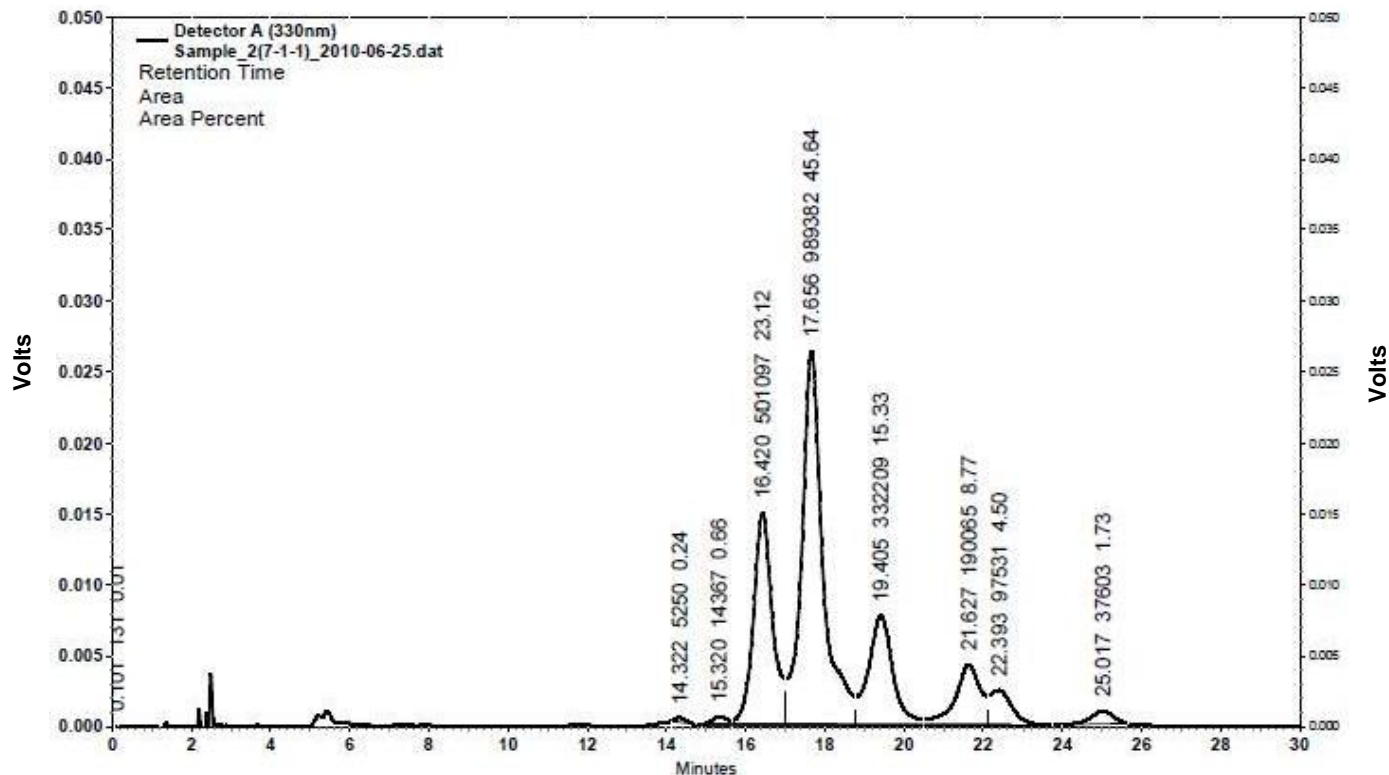


Figure 2. HPLC chromatogram of γ -oryzanol rich extract from *GAM THOR*.

Table 1. Yield and γ -oryzanol content of purple rice bran extract.

Rice cultivar	Extract yield (% w/w)	γ -oryzanol content (% w/w extract)	γ -oryzanol content (mg/ 100 g DW)
<i>NIAW DAM</i>	1.19 ^d	1.23 \pm 0.08 ^e	12.21 \pm 0.25 ^e
<i>GAM BOUNG</i>	1.45 ^a	6.17 \pm 0.09 ^c	120.60 \pm 1.72 ^a
<i>GAM THOR</i>	1.35 ^b	7.47 \pm 0.26 ^b	107.49 \pm 1.21 ^b
<i>PAH E-KAW</i>	1.23 ^c	9.14 \pm 0.09 ^a	94.09 \pm 0.61 ^c
<i>GAM PEUAK</i>	1.06 ^e	2.25 \pm 0.13 ^d	30.35 \pm 0.44 ^d

DW: dry weight, Mean values within a column superscripted by the same letter are not significantly different at $p < 0.01$.

determined by using RAW 264.7 murine macrophage cell line. Inhibition of combined LPS-IFN- γ -mediated NO production in RAW 264.7 was determined via Griess reaction and a spectrophotometric determination for nitrite was carried out to quantify the nitrite level in the cultured medium. Interestingly, γ -oryzanol rich extracts from Thai glutinous purple rice bran were effective in inhibiting the combined LPS-IFN- γ -mediated induction of NO production, which was comparable to standard curcumin (Table 3). The extract from *GAM BOUNG* also exerted the highest inhibitory activity on NO production from RAW 264.7 murine macrophage cell line followed by

GAM THOR, *PAH E-KAW* and control γ -oryzanol, respectively.

DISCUSSION

γ -Oryzanol rich extracts from Thai glutinous purple rice bran are comprised of three major components and five minor components. The results indicated that the γ -oryzanol content of γ -oryzanol rich extracts were in the range of 1.23 to 9.14% w/w, varying by cultivar, topography and environment. The Thai glutinous purple

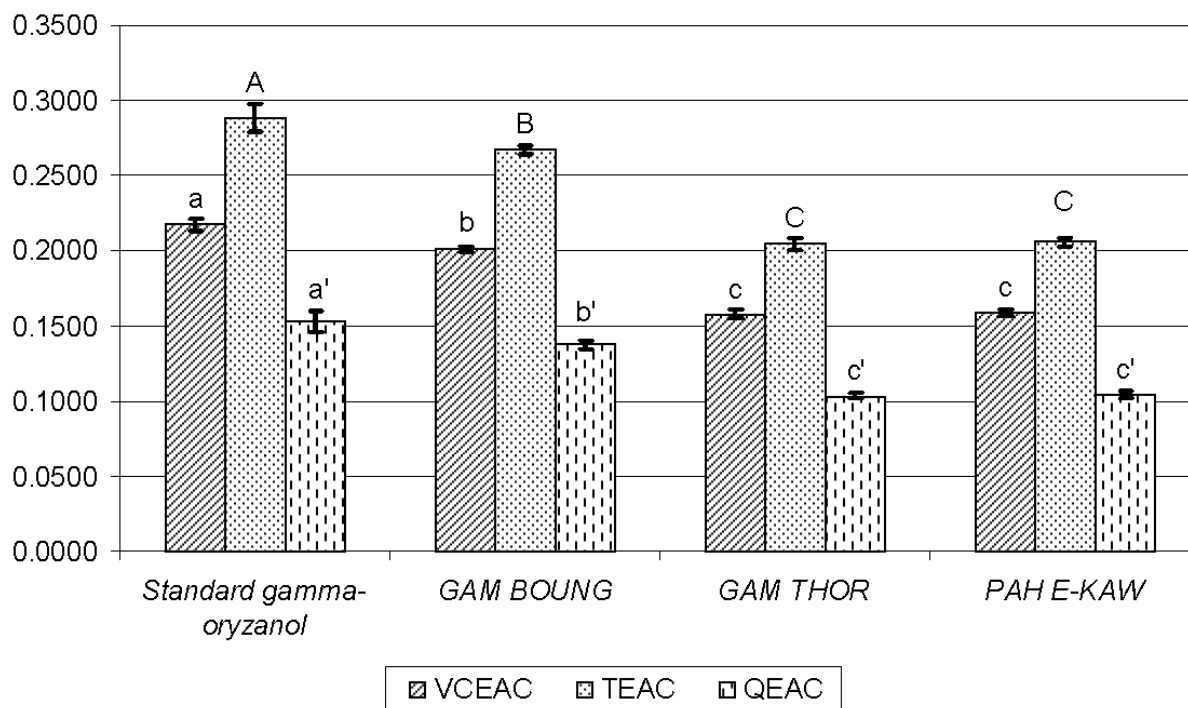


Figure 3. ABTS** scavenging activity of standardized γ -oryzanol rich extracts. Data was expressed as mean \pm SD. $n=3$, statistically significant at $p < 0.01$.

Table 2. 50% inhibition concentration of standardized γ -oryzanol rich extracts for linoleic acid peroxidation and scavenging effects on superoxide anion.

Sample	IC ₅₀ (μ g/ml)	
	Inhibition of linoleic acid peroxidation	Scavenging effects on superoxide anion
GAM BOUNG	36.79 \pm 2.07 ^a	12.56 \pm 0.13 ^b
GAM THOR	37.84 \pm 2.29 ^a	14.04 \pm 0.89 ^b
PAH E-KAW	12.56 \pm 0.13 ^b	17.00 \pm 0.46 ^c
γ -oryzanol	39.84 \pm 0.43 ^a	18.53 \pm 0.72 ^c
L-ascorbic acid	-	7.35 \pm 0.27 ^a
Rutin	62.33 \pm 0.89 ^c	-
Catechin	65.89 \pm 0.76 ^c	-

Data was expressed as mean \pm SD. $n=3$. Mean values within a column superscripted by the same letter are not significantly different at $p < 0.01$.

rice bran collected from the mountains (GAM BOUNG, GAM THOR and PAH E-KAW) contained higher γ -oryzanol content than the purple rice bran collected from the plains (NIAW DAM and GAM PEUAK). The extracts containing γ -oryzanol of more than 5% w/w, GAM BOUNG, GAM THOR and PAH E-KAW, were selected for further investigation for anti-inflammatory and antioxidant activities.

Three *in vitro* antioxidant assays were utilized to evaluate free radical scavenging and antioxidant activities of γ -oryzanol rich extracts. ABTS assay is one of the methods generally used to test the preliminary radical scavenging activity of a compound or plant extract. The ABTS**, generated from oxidation of ABTS by potassium persulfate, is presented as an excellent tool for determining the antioxidant activity of hydrogen-donating

Table 3. 50% inhibition concentration of standardized γ -oryzanol rich extracts on NO production.

Sample	IC ₅₀ (μ g/ml)
<i>GAM BOUNG</i>	29.32 \pm 2.21 ^b
<i>GAM THOR</i>	34.48 \pm 2.19 ^c
<i>PAH E-KAW</i>	38.41 \pm 3.16 ^d
Standard γ -oryzanol	43.31 \pm 3.32 ^d
Curcumin	22.67 \pm 1.98 ^a

Data was expressed as mean \pm SD. $n=3$. Mean values within a column superscripted by the same letter are not significantly different at $p < 0.01$.

mechanisms and of chain-breaking antioxidants (Leong and Shui, 2002).

In the present study, γ -oryzanol rich extracts exhibited moderate activity on ABTS radical scavenging, which is comparable to standard L-ascorbic acid, Trolox and quercetin. All samples showed a dose-dependence in ABTS radical scavenging. Quercetin was the most effective on ABTS radical scavenging because it possesses 5 hydroxyl groups followed by L-ascorbic acid, Trolox, control γ -oryzanol and γ -oryzanol rich extracts. This may be attributed to the fact that the hydroxyl group on the aromatic ring of γ -oryzanol could not easily donate the hydrogen atom due to steric hindrance of the complex ester structure.

Superoxide anion scavenging activity of the extracts demonstrated in the following order: *GAM BOUNG* > *GAM THOR* > *PAH E-KAW* > control γ -oryzanol. These results revealed that γ -oryzanol rich extracts from Thai glutinous purple rice bran were an efficient scavenger of superoxide anion radical. However, standard L-ascorbic acid showed greater activity. Our result was different from those reported by Juliano et al. (2005), who reported that γ -oryzanol was unable to scavenge superoxide anion radicals, which were generated during the autoxidation of FeCl₂ in MOPS buffer (5 mM, pH 7.5).

Lipid peroxidation is a well-established mechanism for cellular injury in animals and is used as an indicator of oxidative stress in cells and tissues. In this study, linoleic acid was used as the substrate of lipid peroxidation. All of the samples exhibited the inhibition effect on linoleic acid peroxidation in a dose-dependent manner. Table 2 demonstrated γ -oryzanol rich extract from *GAM BOUNG* exhibited the highest inhibition activity on linoleic acid peroxidation with the lowest IC₅₀ value. Interestingly, the extracts from *GAM BOUNG* and *GAM THOR* were as effective as those of control γ -oryzanol in inhibition of linoleic peroxidation. Furthermore, all of the γ -oryzanol rich extracts and the standard γ -oryzanol exhibited potent inhibition activity on linoleic acid peroxidation, which were higher than standard rutin and catechin. The present results from three different *in vitro* antioxidant assays

indicate that γ -oryzanol rich extracts from Thai glutinous black rice bran are acting as a lipophilic radical scavenger better than a hydrophilic radical scavenger. In this investigation, γ -oryzanol rich extracts inhibit NO production from combined LPS-IFN- γ -activated RAW 264.7 mouse macrophage cell lines in a dose-dependent manner. The inhibition activity on nitric oxide production from RAW 264.7 mouse macrophage cell line occurred in the following order: *GAM BOUNG* > *GAM THOR* > *PAH E-KAW* > control γ -oryzanol. It is proposed here that the strong anti-inflammatory property is mainly due to the high concentration γ -oryzanol in the extracts. However, the inhibition activity of all of the extracts was less than the standard curcumin, a potent anti-inflammatory. It is possible that γ -oryzanol may penetrate through the cell membrane due to its lipophilicity property. The results here correspond to the studies that found that sterol ferulates exhibit anti-inflammatory activity against 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation in mice (Akihisa et al., 2000). Moreover, black rice bran extract significantly suppressed TPA-induced inflammation and decreased the production of TNF- α , IL-1 β , IL-6 and LTB4 (Choi et al., 2010).

In conclusion, Thai glutinous purple rice bran collected from the mountain (*GAM BOUNG*, *GAM THOR* and *PAH E-KAW*) contained a higher amount of γ -oryzanol. The standardized γ -oryzanol rich extracts from *GAM BOUNG*, *GAM THOR* and *PAH E-KAW* were found to be the moderate radical scavengers and showed strong inhibition activity on linoleic acid peroxidation. Furthermore, the γ -oryzanol rich extracts were found to be a potent anti-inflammatory through the inhibition of NO production from RAW 264.7 mouse macrophage cell line.

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REFERENCES

- Akihisa T, Yasukawa K, Yamaura M, Ukiya M, Kimura Y, Shimizu N, Arai K (2000). Triterpene alcohol and sterol ferulates from rice bran and their an-inflammatory effects. *J. Agric. Food Chem.*, 48: 2313-2319.
- Boonsit P, Pongpiachan P, Julsrigival S, Karladee D (2010). Gamma oryzanol content in glutinous purple rice landrace varieties. *C.M.U. J. Nat. Sci.*, 9(1): 151-157.
- Choi CW, Kim SC, Hwang SS, Choi BK, Ahn HJ, Lee MY (2002). Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant Sci.*, 163: 1161-1168.
- Choi SP, Kim SP, Kang MY, Nam SH, Friedman M (2010). Protective effects of black rice bran against chemical-induced inflammation of mouse skin. *J. Agric. Food Chem.*, 58(18): 10007-10015.
- Chotimakorn C, Benjakul S, Silalai N (2008). Antioxidant components and properties of five long-grained rice bran extracts from commercial available cultivars in Thailand. *Food Chem.*, 111: 636-641.
- Cicero AFG, Gaddi A (2001). Rice bran oil and γ -oryzanol in the treatment of hyperlipoproteinemias and other conditions. *Phytother. Res.*, 15: 277-289.
- Fujita A, Fujitake H, Kawakami K, Nomura M (2010). Antioxidant activity of colored rice bran obtained at different milling yields. *J. Oleo Sci.*, 59(10): 563-568.
- Hiramitsu T, Armstrong D (1991). Preventive effect of antioxidants on lipid peroxidation in the retina. *Ophthalmic Res.*, 23: 196-203.
- Hu C, Zawistowski J, Ling W, Kitts DD (2003). Black rice (*Oryza sativa* L. *indica*) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. *J. Agric. Food Chem.*, 51: 5271-5277.
- Jang D, Murrell G (1998). Nitric oxide in arthritis. *Free Radical Biol. Med.*, 24: 1511-1519.
- Jomha NM, Elliott JAW, Law GK, McGann LE (2006). Evaluation of chondrocyte survival in situ using WST-1 and membrane integrity stains. *Cell Tissue Bank*, 8: 179-186.
- Juliano C, Cossu M, Alamanni MC, Piu L (2005). Antioxidant activity of gamma-oryzanol: Mechanism of action and its effect on oxidative stability of pharmaceutical oils. *Int. J. Pharm.*, 299: 146-154.
- Kim JS, Han D, Moon KD, Rhee JS (1995). Measurement of superoxide dismutase-like activity of natural antioxidants. *Biosci. Biotechnol. Biochem.*, 59: 822-826.
- Leong LP, Shui G (2002). An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chem.*, 76: 69-75.
- Muntana N, Prasong S (2010). Study on total phenolic contents and their antioxidant activities of Thai white, red and black rice bran extracts. *Pak. J. Biol. Sci.*, 13: 170-274.
- Perkins DJ, Kreamsner PG, Schmid D, Misukonis MA, Kelly MA, Weinberg JB (1999). Blood mononuclear cell nitric oxide production and plasma cytokine levels in healthy Gabonese children with prior mild or severe malaria. *Infect. Immun.*, 67(9): 4977-4981.
- Pratico D, Delanty N (2000). Oxidative injury in disease of the central nervous system: focus on Alzheimer's disease. *Am. J. Med.*, 109: 577-585.
- Re R, Pellegrini N, Proteggemnte A, Pannala A, Yang A, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.*, 26: 1231-1237.
- Ryu SN, Park SZ, Ho CT (1998). High performance liquid chromatographic determination of anthocyanin pigments in some varieties of black rice. *J. Food Drug Anal.*, 6: 729-736.
- Scavariello EM, Arellano DB (1998). Gamma-oryzanol: an important component in rice bran oil. *Arch. Latinoam. Nutr.*, 48(1): 7-12.
- Sompong R, Siebenhandl-Ehn S, Linsberger-Martin G, Berghofer E (2011). Phytochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. *Food Chem.*, 124: 132-140.
- Sugano M, Tsuji E (1997). Rice bran oil and cholesterol metabolism. *J. Nutr.*, 127: 521S-524S.
- Toyokuni S, Itani T, Morimitsu Y, Okada K, Ozeki M, Kondo S, Uchida K, Osawa T, Hiai H, Tashiro T (2002). Protective effect of colored rice over white rice on Fenton reaction-based renal lipid peroxidation in rats. *Free Radical Res.*, 36: 583-592.
- Tuntipopipat S, Muangnoi C, Failla ML (2009). Anti-inflammatory activities of extracts of Thai spice and herbs with lipopolysaccharide-activated RAW 264.7 murine macrophage. *J. Med. Food*, 12(6): 1213-1220.
- Vorarat S, Managit C, Iamthanakul L, Soparat W, Kamkaen N (2010). Examination of antioxidant activity and development of rice bran oil and gamma-oryzanol microemulsion. *J. Health Res.*, 24(2): 67-72.
- Xu Z, Godber JS (1999). Purification and identification of components of γ -oryzanol in rice bran. *J. Agric. Food Chem.*, 47: 2724-2728.
- Xu Z, Hua N, Godber JS (2001). Antioxidant activity of tocopherol, tocotrienols, and gamma-oryzanol components from rice bran against cholesterol oxidation accelerated by 2,2'-azobis(2-methylpropionamide) dihydrochloride. *J. Agric. Food Chem.*, 49: 2077-2081.
- Yanping L, Yanhua L, Dongzhi W (2004). Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. *in vitro*. *J. Agric. Food Chem.*, 52: 5032-5029.