

Phenolic and Flavonoid Contents of Thai Rice Extracts and Their Correlation with Antioxidant Activities using Chemical and Cell Assays

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Background: Free radicals induce oxidative stress in various cell components, leading to certain diseases. Plant-derived antioxidants have become a profitable alternative to prevent oxidative stress in cells due to adverse effects of some synthetic antioxidants.

Objective: To determine the total phenolic and flavonoid contents and to evaluate the correlation between these two compounds and their antioxidant properties in the ethanolic extracts of brown rice and rice bran from Thai rice cultivars: Sangyod red rice and Dawk Mali 105 white rice using the chemical and cell assays.

Material and Method: Total phenolic and flavonoid contents in all of the rice ethanolic extracts were determined using the colorimetric assays, as well as their antioxidant activity was analyzed through two chemical assays: DPPH radical-scavenging and inhibition of lipid peroxidation assays, as well as through a cell-based assay: scavenging capacity against intracellular superoxide in cells using DCF.

Results: All the rice extracts displayed their antioxidant activities in a dose dependent manner through different assays, which were expressed as EC_{50} values. The DPPH scavenging assay revealed very high scavenging activity in both Sangyod brown rice and rice bran extracts. Positive correlations between this activity and total phenolic and flavonoid contents suggest the major free radical scavenging activity of such compounds. In contrast, the ethanolic extract of Sangyod rice bran exhibited non-significant anti-lipid peroxidation activity relative to that of Sangyod brown rice and Dawk Mali 105 rice bran. Phenolic content was correlated to some extent with anti-lipid peroxidation activity, whereas flavonoid content and such activity showed a relatively weak correlation. Importantly, the cell-based assay also detected potent scavenging activity against superoxide production in HL-60 cells pretreated with Sangyod extracts. The content of phenolics was a major contributor to this scavenging activity including that of flavonoids but to a lesser extent.

Conclusion: These findings suggest that ethanolic extracts of brown rice and rice bran of Sangyod red rice can be promising sources of potential natural antioxidants.

Keywords: Antioxidant activity, Pigmented rice, Brown rice, Rice bran, Ethanolic extract

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Free radicals have been claimed to induce oxidative stress in various cell components including proteins, lipids and DNA, ultimately leading to some certain diseases such as cancer, cardiovascular

diseases, ageing, and inflammatory diseases⁽¹⁾. Antioxidants from natural sources such as vegetables, fruits and cereals have become a profitable alternative to prevent oxidative stress due to adverse effects of some synthetic antioxidants^(2,3).

Rice bran is also a rich source of natural antioxidants such as phenols and polyphenolic compounds, especially flavonoids, a class of water-soluble plant pigments with many colors. Both phenolic and flavonoid compounds are potent antioxidants that can act as free radical scavengers⁽⁴⁾, reducing agents,

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and/or metal ion chelators⁽⁵⁾, thus providing various human health benefits⁽⁶⁾. Bran of red and black rice cultivars have previously been shown to exhibit greater antioxidant activity and phenolic content than bran of nonpigmented rice^(7,8). In addition, these pigmented rice extracts effectively decrease oxidative stress and inflammation as well as atherosclerotic lesions^(9,10). Many varieties of colored rice are mainly produced in Southeast Asian countries. Among these, Sangyod is a red rice cultivar, typically grown in Southern Thailand.

The total phenolic contents and antioxidant properties have been studied in some Thai nonpigmented⁽⁵⁾ and colored rice cultivars^(7,11), but are limited, especially concerning the relationship between their phenolic content and antioxidant capacity⁽¹¹⁾. Therefore, the present study aimed to determine the total phenolic and flavonoid contents and to evaluate the correlation between these two compounds and their antioxidant properties in the ethanolic extracts of brown rice and rice bran from Sangyod using chemical and cell assays as compared to those of Thai nonpigmented rice cultivar.

Material and Method

Folin-Ciocalteu reagent, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH[•]), gallic acid, vitamin E, catechin, brain extract from bovine brain and Hanks' balanced salt solution (HBSS) were purchased from Sigma, Germany. Phorbol 12-myristate 13-acetate (PMA) was purchased from Sigma, USA. Rutin trihydrate, sodium carbonated anhydrous, thiobarbituric acid and 2, 6-Di-tert-butyl-4-methylphenol (BHT) were purchased from Fluka, Spain. Dichlorofluorescein diacetate (DCF-DA) was purchased from Invitrogen, USA. The analytical grade methanol and other organic solvents were purchased from Merck, Germany. Other chemicals used in the present study were of analytical grade.

Preparation of ethanolic extracts of brown rice and rice bran

Brown rice and rice bran powder of Dawk Mali 105 and Sangyod (1,000 g) were macerated in 95% ethanol for 3 days and filtered through Whatman No. 1 filter paper to obtain uniform fractions of brown rice and rice bran. The ethanol was then removed from the extract by a rotary evaporator. For Dawk Mali 105, the percentage yield of the ethanolic extracts of brown rice and rice bran is 1.44% and 4.6%, respectively. For Sangyod, the percentage yield of the ethanolic extracts of brown rice and rice bran is 2.01% and 9.8%, respectively.

Cell culture and differentiation induction

Human promyelocytic leukemia cell line (HL-60) was purchased from the American Type Culture Collection (ATCC, USA) and cultured in RPMI 1640 medium (HyClone, UK) supplemented with 15% heat-inactivated fetal bovine serum (FBS) (HyClone, UK), 100 units/ml penicillin and 0.1 mg/ml streptomycin (Gibco, USA) at 37°C and 5% CO₂ atmosphere. To induce myeloid differentiation, HL-60 cells (5 x 10⁵ cells/ml) were cultivated for 6-8 days in RPMI 1640 containing 1.3% DMSO. HL-60 cells differentiated into neutrophils and monocytes had smaller cell size with increased expression of the cell surface antigen, β_2 -integrin CD11b in plasma membrane, which was monitored by the FACSCalibur flow cytometer (Becton Dickinson) using PE-conjugated mouse monoclonal (IgG_{2a}, kappa) anti-human CD11b antibody (clone D12, Becton Dickinson, BD Biosciences, USA). Cell numbers were counted using a hemocytometer and cell viability was determined by the trypan blue exclusion test.

Determination of total phenolic content

Total phenolic content was determined using the Folin-Ciocalteu reagent according to the method of Amin et al⁽¹²⁾ with some modifications. In the reaction mixture, an appropriate dilution of the rice extract dissolved in methanol (14 μ l) was reacted with 105 μ l of freshly prepared diluted Folin Ciocalteu reagent, and then neutralized with 105 μ l of 7.5 % sodium carbonate. After incubation for 1 h at room temperature, the absorbance of the resulting solution was measured at 725 nm using a microplate reader (PowerWave XS, BioTek). Gallic acid was used as a standard and the results of total phenolic content were expressed as mg of gallic acid equivalent/g of rice extract.

Determination of total flavonoid content

Total flavonoid content was determined according to the method of Jia et al⁽¹³⁾ with some modifications. Briefly, an appropriate dilution of the rice extract dissolved in methanol (500 μ l) was reacted with 150 μ l of 5% NaNO₂. After incubation for 5 min at room temperature, 300 μ l of 10 % AlCl₃.6H₂O was added. After further incubation for 5 min at room temperature, the reaction mixture was treated with 1 ml of 1 M NaOH and was then centrifuged at 4,750 rpm for 10 min. The absorbance of the supernatant (200 μ l) was measured at 415 nm using a microplate reader. Rutin hydrate was used as a standard and the results of total flavonoid content were expressed as mg of rutin hydrate equivalent/g of rice extract.

Determination of DPPH radical-scavenging activity

The radical-scavenging activity of rice extracts was determined by the method of Brand-Williams et al⁽¹⁴⁾. Briefly, 100 µl of 1 mg% DPPH solution in ethanol was added to 100 µl of different dilutions of the rice extracts (1 µg/ml to 1,000 µg/ml) in ethanol. The mixtures were shaken and allowed to stand for 30 min in the dark at room temperature. A decrease in absorbance of these extracts was measured at 517 nm using a microplate reader and compared to that of the control (without the extract). DPPH free radical scavenging ability (%) of each concentration of the rice extract was calculated by the following formula: % scavenging ability = $100 \times [A_{517 \text{ nm of control}} - A_{517 \text{ nm of sample}}] / A_{517 \text{ nm of control}}$. The scavenging activity was expressed as 50% effective concentration, EC₅₀ (µg/ml) described in Statistical Analyses. Catechin was used as a positive control.

Determination of anti-lipid peroxidation activity

The inhibitory capacity of rice extracts against lipid peroxidation of liposome was measured according to the method of Conforti et al⁽¹⁵⁾. Lipid peroxidation was induced in liposomal membrane prepared from bovine brain extract in phosphate buffered saline (5 mg/ml) by adding 1 mM FeCl₃ and 1 mM ascorbic acid as a pro-oxidant in the presence of Fe, followed by incubation at 37°C for 30 min, leading to the formation of free malondialdehyde (MDA). Different concentrations of the extract (100 µg/ml to 10 mg/ml) were also added to test its inhibitory activity against lipid peroxidation. Thiobarbituric acid (TBA) (1%), BHT (2%), and HCl (1%) was added and the mixture was heated at 95°C for 30 min to yield the red TBA-MDA complex. After cooling, 2.1 ml of n-butanol was used to extract these color products, which were measured calorimetrically at 532 nm using a microplate reader and compared to that of the control (without the extract). The inhibition of lipid peroxidation (%) was calculated by the following formula: % inhibition = $100 \times [(A_{532 \text{ nm of control}}) - (A_{532 \text{ nm of sample}} - A_{532 \text{ nm of sample alone}})] / A_{532 \text{ nm of control}}$. The inhibition was expressed as EC₅₀ (mg/ml) described in Statistical Analyses. Vitamin E was used as a positive control.

Determination of intracellular superoxide scavenging capacity

The inhibition of hydrogen peroxide and superoxide production by rice extracts was carried out according to the method of Lin et al⁽¹⁶⁾. DCF-DA, a nonfluorescence probe was used to determine the

intracellular superoxide level since it becomes fluorescent following oxidation by superoxide produced during respiratory burst. HL-60 cells (1×10^6) were incubated in 1 µM DCF-DA and in different concentrations of the extract (100 µg/ml to 1,500 µg/ml) at 37°C for 15 min, then stimulated with 100 ng/ml PMA, and incubated for another 15 min. Flow cytometric analysis of DCF fluorescence intensity was then performed to determine the remaining superoxide level in HL-60 cells using the FACSCalibur flow cytometer (Becton Dickinson) and CellQuest 3.0.1 software (Becton Dickinson). The mean fluorescence intensity (MFI) of treated cells ($> 1 \times 10^4$) relative to that of non-treated cells (without the extract) as a control was analyzed for generated hydrogen peroxide and superoxide. The inhibition of superoxide production (%) was calculated by the following formula: % inhibition of O₂⁻ production = $100 \times [(MFI_{\text{of control}} - MFI_{\text{of background}}) - (MFI_{\text{of sample}} - MFI_{\text{of background}})] / (MFI_{\text{of control}} - MFI_{\text{of background}})$. The background absorbance was determined by incubating cells without PMA activation. The inhibition was expressed as EC₅₀ (µg/ml) described in Statistical Analyses. Catechin was used as a positive control.

Statistical analyses

The EC₅₀ (effective concentration providing 50% inhibition) was calculated from a dose-response curve obtained by plotting inhibition percentage versus the corresponding extract concentration (four to six different concentrations for each extract) using GraphPad Prism software and cubic spine interpolation. The results were expressed as mean ± SD from at least three separate experiments. All statistical analyses were carried out using SPSS for Windows. Significant differences between means were determined by one way analysis of variance (ANOVA) followed by LSD or Dunnett's T3 for multiple comparisons. Differences were considered significant at p-value < 0.05.

Results

Total phenolic content of rice extracts

The total phenolic content of the ethanolic extract of brown rice and rice bran from Dawk Mali 105 and Sangyod fell in the range of 18.97 to 189.20 mg gallic acid equivalent per gram rice extract (Table 1). Moreover, the ethanolic extract of Sangyod rice bran contained the highest phenolic content with approximately 3, 8, 10 times higher than that of Sangyod brown rice as well as rice bran and brown rice from Dawk Mali 105, respectively. Significant differences in

phenolic contents were observed between the two rice cultivars ($p < 0.05$).

Total flavonoid content of rice extracts

The total flavonoid content of the ethanolic extract of brown rice and rice bran from Dawk Mali 105 and Sangyod was in the range of 18.75 to 46.13 mg rutin hydrate equivalent per gram rice extract (Table 1). The ethanolic extract of Sangyod rice bran contained the highest flavonoid content with approximately 2.5, 2.3, 1.8 times higher than that of Sangyod brown rice as well as brown rice and rice bran from Dawk Mali 105, respectively. There was no significant difference ($p < 0.05$) between total flavonoid content of Sangyod brown rice and Dawk Mali 105 brown rice (Table 1).

DPPH radical-scavenging activity of rice extracts

The DPPH radical-scavenging assay is widely used to evaluate the ability of plant extracts to act as free radical scavengers or hydrogen donors. Antioxidants in the extract react with stable DPPH radical and convert it to a non-radical form. Decreased extent of absorption of radical DPPH at 517 nm indicates radical scavenging activity of extracts. Using this assay, all ethanolic extracts tested exhibited a dose-dependent DPPH radical scavenging activity (data not shown) and showed 50% radical scavenging activity (EC_{50}) in the range of 3.54 to 230.71 $\mu\text{g/ml}$ (Fig. 1A). The lower the EC_{50} value, the higher the antioxidant activity. The ethanolic extract of Sangyod rice bran significantly exhibited the highest inhibition effect (EC_{50} 3.54 $\mu\text{g/ml}$) with approximately 3-, 44-, 65-fold difference compared to that of Sangyod brown rice as well as rice bran and brown rice from Dawk Mali 105, respectively.

Furthermore, the correlation coefficient (R) between antioxidant activities and phenolic or flavonoid content in the four rice extracts was determined.

Correlation graphs were plotted between $1/EC_{50}$ values (including of DPPH scavenging activity, anti-lipid peroxidation activity, and superoxide anion scavenging activity) and phenolic and flavonoid contents. Only two correlation graphs (*i.e.*, DPPH vs. phenolic, DPPH vs. flavonoid) are shown in Fig. 2 as representatives of all the six graphs, while Table 2 shows the correlation coefficient (R) between the three antioxidant assays

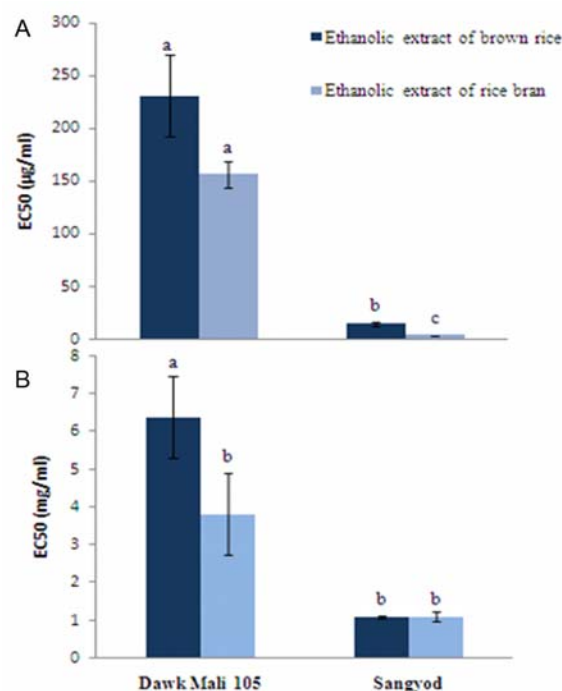


Fig. 1 EC_{50} values \pm standard deviation ($n = 3-8$) of the ethanolic extracts from Dawk Mali 105 and Sangyod rice cultivars in scavenging stable free DPPH radical (A), and in inhibiting lipid peroxidation (B). Bars marked by different letters are significantly different ($p < 0.05$)

Table 1. Total phenolic and flavonoid contents of the ethanolic extracts from Dawk Mali 105 and Sangyod rice cultivars

Rice extract	Total phenolic content (mg gallic acid eq/g rice extract)	Total flavonoid content (mg rutin hydrate eq/g rice extract)
Dawk Mali 105		
Ethanolic extract of brown rice	18.97 \pm 1.64a	19.73 \pm 1.04a
Ethanolic extract of rice bran	23.27 \pm 1.88a	26.01 \pm 1.30b
Sangyod		
Ethanolic extract of brown rice	64.49 \pm 4.97b	18.75 \pm 0.80a
Ethanolic extract of rice bran	189.20 \pm 6.74c	46.13 \pm 0.63c

Results represent means \pm standard deviation ($n = 4$). In each column, different letters mean significant differences ($p < 0.05$)

Table 2. The r-values (correlation coefficients) and the p-values between antioxidant activities ($1/EC_{50}$) and phenolic or flavonoid content

	DPPH scavenging activity		Anti- Lipid peroxidation activity		Superoxide scavenging activity	
	r	p	r	p	r	p
Phenolic content	0.999	0.001	0.753	0.247	0.987	0.013
Flavonoid content	0.909	0.091	0.435	0.565	0.822	0.178

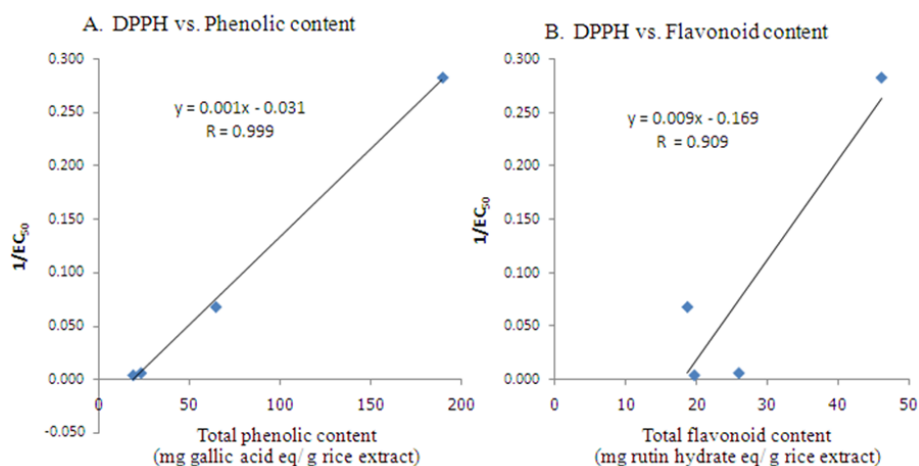


Fig. 2 Correlation graphs for DPPH $1/EC_{50}$ values and total phenolic content (A), flavonoid content in the four rice extracts (B)

used in this study and phenolic and flavonoid contents. In the case of DPPH scavenging activity, significant positive correlations were observed between $1/EC_{50}$ values for DPPH and the total phenolic content ($R = 0.999$) and the flavonoid content ($R = 0.909$), indicating the major contribution of both compounds to this antioxidant activity. Therefore, very high DPPH radical scavenging activity of Sangyod rice bran relative to the activity of Dawk Mali 105 extracts can be attributed to the presence of 8 to 10 times more phenolic content. Furthermore, DPPH radical scavenging activity of all the rice extracts was lower than that of catechin (EC_{50} 1.31 ± 0.11 μ g/ml), except for the bran extract of Sangyod having an EC_{50} close to that of the control.

Inhibition of lipid peroxidation by rice extracts

Lipid peroxidation involves the direct reaction of oxygen radicals with polyunsaturated fatty acids in cell membrane through radical-induced chain reaction mechanisms, leading to membrane damage. The lipid peroxidation assay is used to test the capacity of plant

extracts to act as chain-breaking antioxidants against this lipid peroxidation, resulting in a decrease in the level of oxidation product (MDA). Using this assay, all the rice extracts showed dose-dependent inhibitory activity against liposome oxidation (data not shown) with the EC_{50} in the range of 1.09 to 6.39 mg/ml (Fig. 1B). Despite possessing the highest scavenging activity, the ethanolic extract of Sangyod rice bran exhibited non-significant anti-lipid peroxidation activity relative to that of Sangyod brown rice and Dawk Mali 105 rice bran (Fig. 1B).

As shown in Table 2, Phenolic content was correlated to some extent with anti-lipid peroxidation activity ($R = 0.753$), whereas flavonoid content and such activity showed a relatively weak correlation ($R = 0.435$). This result suggested that flavonoid compounds obtained from Sangyod rice extract exert less inhibitory effect on lipid peroxidation than phenolic compounds. Moreover, all the extracts demonstrated very poorly anti-lipid peroxidation activity as compared to vitamin E used as a positive control (EC_{50} 3.24 ± 1.91 μ g/ml)

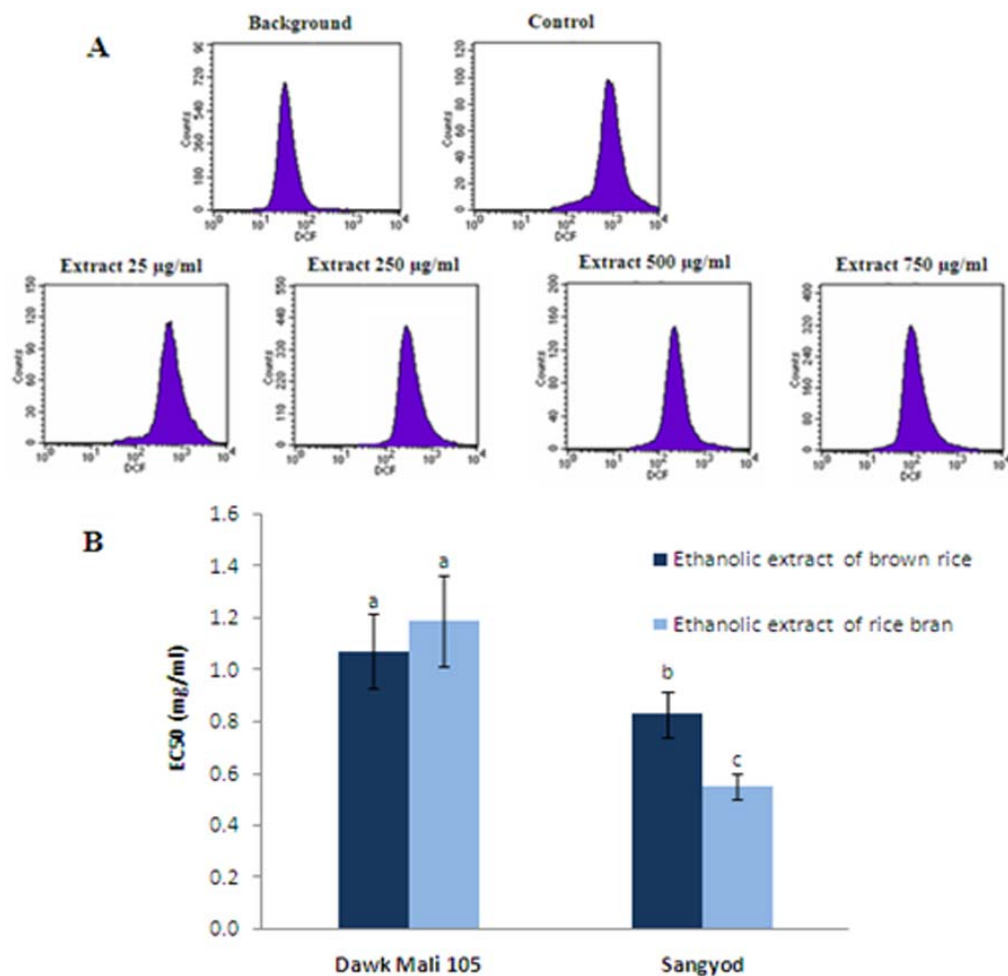


Fig. 3 The histograms of the remaining mean fluorescence intensity in HL-60 cells treated with different concentrations of the ethanolic extract of Sangyod rice bran were shown as representatives in comparison to the background and the control (A). EC₅₀ values of the ethanolic extracts from Dawk Mali 105 and Sangyod rice cultivars in scavenging PMA-stimulated superoxide production in HL-60 cells measured by DCF fluorescence intensity (B). Bars marked by different letters are significantly different ($p < 0.05$)

(Fig. 1B).

Inhibition of superoxide production by DCF assay

Besides the two chemical-based assays, the cell-based DCF assay was used to evaluate the scavenging activity of all the rice extracts against PMA-induced superoxide production in differentiated HL-60 cells. After PMA stimulation, DCF fluorescence intensity was quantified to determine the remaining superoxide level in pretreated cells. Fig. 3A illustrates the histograms of DCF fluorescence intensity in cells pre-treated with different dilutions of Sangyod rice bran extract as representatives. In comparison with the background and the control, a histogram shifts to the left with various degrees over increasing extract

concentrations, indicating that the extract scavenged superoxide produced in the cells in a dose-dependent manner, resulting in decreased fluorescence intensity of the DCF. The actual antioxidant potency of those concentrations was confirmed by determining the percentage of cell death using Propidium Iodide (PI) staining followed by FACS analysis and less than 25% cell death was obtained.

All the rice extracts showed dose-dependent scavenging activity on superoxide production (data not shown) with the EC₅₀ in the range of 0.55 to 1.19 mg/ml (Fig. 3B). The ethanolic extract of Sangyod rice bran significantly exhibited the highest inhibitory effect (EC₅₀ 0.55 mg/ml) with only 1.5- to 2.2-fold difference as compared to the other extracts. Minor differences in

this scavenging activity in the cell assay could be due to the decreased solubility of most ethanolic extracts containing more non-polar compounds in aqueous buffer solutions used in the assay, leading to a decrease in the amount of bioactive compounds. Positive correlations were observed between the scavenging activity against superoxide production and the contents of phenolic compounds ($R = 0.987$) and flavonoid compounds, but to a lesser extent ($R = 0.822$) Table 2. In addition, all tested extracts had much lower scavenging activity compared to that of catechin as a positive control ($EC_{50} 45.97 \pm 9.74 \mu\text{g/ml}$) (Fig. 3).

Discussion

Many studies have examined the use of different solvent systems to extract rice bran such as hexane⁽¹⁷⁾, methanol^(5,18), ethanol-water (70:30 v/v)⁽¹⁹⁾, and water at room temperature⁽²⁰⁾. In our previous study, the two water-extraction methods were demonstrated to be effective for extracting water-soluble substances containing phenolic and flavonoid compounds from Sangyod red rice cultivar⁽²¹⁾. In the present study, brown rice and rice bran of Sangyod red rice and Dawk Mali 105 white rice were extracted with 95% ethanol. All the extracts showed over 100-fold greater phenolic and flavonoid compounds contents than did their water extract⁽²¹⁾. Among these extracts, Sangyod extracts demonstrated high levels of such compounds as compared to the nonpigmented rice extracts, which are in agreement with other pigmented rice varieties in other previous studies^(7,8).

The antioxidant activity of Sangyod extracts relative to that of white rice extracts were analyzed using both chemical and cell assays. It is important to use various assays to prove this activity since natural antioxidants from plant materials have different antioxidant capacities, which do not necessarily show potent activity with any one particular assay. Among the four rice extracts, both brown rice and rice bran extracts from Sangyod red rice exhibited potent inhibitory actions using the three antioxidant assays (*i.e.*, The DPPH scavenging assay, the lipid peroxidation assay, and the cell-based method using DCF). Although the antioxidant capacities of the extracts were significantly lower than those of catechin and vitamin E, it was evident that these extracts did show different antioxidant abilities. The higher R values of phenolic compounds in all the three assays suggested that such compounds exert more inhibitory effect on DPPH radical, lipid peroxidation, and superoxide anions than flavonoid in the rice extracts. No significant antioxidant

effects of flavonoid compound were observed in all the three assays. Only specific structures of phenolic compounds possess potent anti-lipid peroxidation activity⁽²²⁾. Moreover, the R values of phenolic compounds in all the three antioxidant activities indicated that such compounds can act as potent DPPH radical and superoxide scavengers rather than an inhibitor of lipid peroxidation. Importantly, the positive results from the cell-based method suggest that both brown rice and rice bran extracts of Sangyod red rice are powerful to prevent or delay cell oxidative damages caused by free radicals. These results offer some vital information since the assay tends to more closely reflect antioxidant effects *in vivo* than do other chemical assays despite having some limits. In addition, it is important to note that the ethanolic extracts of Sangyod extracts were shown to be more effective than their water extracts⁽²¹⁾. The present study is one of a few reports that perform a correlation analysis between antioxidant activity and total phenolic and flavonoid compounds in Thai rice varieties⁽¹¹⁾.

In conclusion, the findings of the present study suggest that the brown rice and rice bran extracts of Sangyod pigmented rice possess high antioxidant potential and could thus be potential sources of natural antioxidants, especially phenolic and flavonoid compounds. The present study provides information on their nutritional values, thereby increasing the consumption of such pigmented rice cultivar for protective effect against some diseases.

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Potential conflicts of interest

None.

References

1. Auten RL, Davis JM. Oxygen toxicity and reactive oxygen species: the devil is in the details. *Pediatr Res* 2009; 66: 121-7.
2. Ito N, Hirose M, Fukushima S, Tsuda H, Shirai T, Tatematsu M. Studies on antioxidants: their carcinogenic and modifying effects on chemical carcinogenesis. *Food Chem Toxicol* 1986; 24: 1071-82.
3. Ito N, Fukushima S, Tsuda H. Carcinogenicity and modification of the carcinogenic response by BHA, BHT, and other antioxidants. *Crit Rev Toxicol* 1985; 15: 109-50.

4. Okai Y, Higashi-Okai K. Radical-scavenging activity of hot water extract of Japanese rice bran—association with phenolic acids. *J UOEH* 2006; 28: 1-12.
5. Chotimarkorn C, Benjakul S, Silalai N. Antioxidant components and properties of five long-grained rice bran extracts from commercial available cultivars in Thailand. *Food Chem* 2008; 111: 636-41.
6. Shahidi F, Wanasundara PK. Phenolic antioxidants. *Crit Rev Food Sci Nutr* 1992; 32: 67-103.
7. Muntana N, Prasong S. Study on total phenolic contents and their antioxidant activities of Thai white, red and black rice bran extracts. *Pak J Biol Sci* 2010; 13: 170-4.
8. Higashi-Okai K, Ishida E, Nakamura Y, Fujiwara S, Okai Y. Potent antioxidant and radical-scavenging activities of traditional Japanese cereal grains. *J UOEH* 2008; 30: 375-89.
9. Xia M, Ling WH, Ma J, Kitts DD, Zawistowski J. Supplementation of diets with the black rice pigment fraction attenuates atherosclerotic plaque formation in apolipoprotein e deficient mice. *J Nutr* 2003; 133: 744-51.
10. Ling WH, Cheng QX, Ma J, Wang T. Red and black rice decrease atherosclerotic plaque formation and increase antioxidant status in rabbits. *J Nutr* 2001; 131: 1421-6.
11. Butsat S, Siriamornpun S. Phenolic acids and antioxidant activities in husk of different thai rice varieties. *Food Sci Technol Int* 2010; 16: 329-36.
12. Amin I, Norazaidah Y, Emmy Hainida KI. Antioxidant activity and phenolic content of raw and balanced *Amaranthus* species. *Food Chem* 2006; 94: 47-52.
13. Jia Z, Tang M, Wu J. The determination of flavonoid contents in mulberry and their scavenging effects on superoxides radicals. *Food Chem* 1998; 64: 555-9.
14. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *Lebenson Wiss Technol* 1995; 28: 25-30.
15. Conforti F, Statti GA, Tundis R, Menichini F, Houghton P. Antioxidant activity of methanolic extract of *Hypericum triquetrifolium* Turra aerial part. *Fitoterapia* 2002; 73: 479-83.
16. Lin JK, Chen PC, Ho CT, Lin-Shiau SY. Inhibition of xanthine oxidase and suppression of intracellular reactive oxygen species in HL-60 cells by theaflavin-3,3'-digallate, (-)-epigallocatechin-3-gallate, and propyl gallate. *J Agric Food Chem* 2000; 48: 2736-43.
17. Jang S, Xu Z. Lipophilic and hydrophilic antioxidants and their antioxidant activities in purple rice bran. *J Agric Food Chem* 2009; 57: 858-62.
18. Hu C, Zawistowski J, Ling W, Kitts DD. 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* 2003; 51: 5271-7.
19. Nam SH, Choi SP, Kang MY, Kozukue N, Friedman M. Antioxidative, antimutagenic and anticarcinogenic activities of rice bran extracts in chemical and cell assays. *J Agric Food Chem* 2005; 53: 816-22.
20. Higashi-Okai K, Kanbara K, Amano K, Hagiwara A, Sugita C, Matsumoto N et al. Potent antioxidative and antigenotoxic activity in aqueous extract of Japanese rice bran—association with peroxidase activity. *Phytother Res* 2004; 18: 628-33.
21. Srisawat U, Panunto W, Kaendee N, Tanuchit S, Itharat A, Lerdvuthisophon N, et al. Determination of phenolic compounds, flavonoids, and antioxidant activities in water extracts of Thai red and white rice cultivars. *J Med Assoc Thai* 2010; 93 (Suppl 7): S83-91.
22. Cotellet N, Bernier JL, Catteau JP, Pommery J, Wallet JC, Gaydou EM. Antioxidant properties of hydroxy-flavones. *Free Radic Biol Med* 1996; 20: 35-43.

ปริมาณสารฟีนอลิกและฟลาโวนอยด์ของสารสกัดจากข้าวไทยและความสัมพันธ์ของสารกับฤทธิ์ต้านอนุมูลอิสระที่วิเคราะห์โดยวิธีทางเคมีและเซลล์เพาะเลี้ยง

พินทุสร หาญสกุล, อัมรัตน์ ศรีสวัสดิ์, อรุณพร อธิรัตน์, นุชสิริ เลิศคุณิโสภณ

ภูมิหลัง: สารอนุมูลอิสระทำลายดีเอ็นเอและองค์ประกอบอื่นๆ ของเซลล์ ก่อให้เกิดโรคต่างๆ มากมาย ข้าว ซึ่งเป็นอาหารหลักของชาวเอเชีย เป็นแหล่งสารต้านอนุมูลอิสระสำคัญแหล่งหนึ่งที่สามารถป้องกันและยับยั้งปฏิกิริยาของสารอนุมูลอิสระที่เกิดขึ้นภายในเซลล์

วัตถุประสงค์: เพื่อวิเคราะห์ปริมาณสารฟีนอลิกและฟลาโวนอยด์ และความสัมพันธ์ของสารกับฤทธิ์ในการต้านอนุมูลอิสระของสารสกัดเอทานอลจากข้าวกล้องและรำข้าวของข้าวไทยสังข์หยดและดอกมะลิ 105 โดยทำการทดสอบฤทธิ์ต้านอนุมูลอิสระด้วยระบบสารเคมีและระบบใช้เซลล์เพาะเลี้ยง

วัสดุและวิธีการ: วิเคราะห์หาปริมาณสารฟีนอลิกและฟลาโวนอยด์ในสารสกัดเอทานอลจากข้าวทั้งสองสายพันธุ์โดย colorimetric assay และวิเคราะห์หาฤทธิ์ต้านอนุมูลอิสระโดยใช้ระบบสารเคมี ได้แก่ DPPH radical-scavenging assay และ lipid peroxidation inhibition assay นอกจากนี้ยังได้ทดสอบฤทธิ์ต้านอนุมูลอิสระโดยใช้ระบบเซลล์ ซึ่งเป็นการทดสอบฤทธิ์ scavenging activity ของสารสกัดในการยับยั้งการสร้าง superoxide ในเซลล์ซึ่งตรวจวัดได้โดย DCF assay

ผลการวิจัย: จากการทดสอบสารสกัดเอทานอล โดยวิธีทดสอบทั้งสองระบบพบว่าสารสกัดมีฤทธิ์ต้านอนุมูลอิสระโดยขึ้นอยู่กับความเข้มข้นของสาร ซึ่งแสดงโดยค่า EC_{50} , การใช้ DPPH radical-scavenging assay ตรวจสอบฤทธิ์สารสกัด พบว่าสารสกัดเอทานอลของข้าวสังข์หยด ทั้งส่วนข้าวกล้องและรำข้าวมีฤทธิ์ยับยั้ง DPPH ในรูปอนุมูลอิสระ โดยมีฤทธิ์สอดคล้องกับปริมาณของสารฟีนอลิกและสารฟลาโวนอยด์อย่างชัดเจน แตกต่างจากผลการทดสอบ ด้วย lipid peroxidation assay ซึ่งพบว่ามีฤทธิ์ต่ำและไม่แตกต่างอย่างมีนัยสำคัญกับรำข้าวดอกมะลิ 105 และที่สำคัญพบว่าสารสกัดเอทานอลของข้าวสังข์หยดมีฤทธิ์ในการกำจัด superoxide ภายในเซลล์ โดยมีสารฟีนอลิกเป็นสารออกฤทธิ์หลัก

สรุป: จากผลการศึกษาชี้ให้เห็นว่าสารสกัดน้ำจากข้าวสังข์หยดซึ่งเป็นข้าวมีสี เป็นแหล่งของสารต้านอนุมูลอิสระจากธรรมชาติที่มีศักยภาพในการนำไปใช้ประโยชน์ในด้านต่างๆ
